**Spatial and temporal analyses of Atlantic common bottlenose dolphin (*Tursiops truncatus*) vocalizations and distribution patterns in Charleston Harbor, South Carolina**

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**ABSTRACT**

Common bottlenose dolphins (*Tursiops truncatus*) rely on a range of vocalizations for social interactions, navigating complex environments, and acquisition of prey. However, there is increasing evidence that anthropogenic noise may alter acoustic behavior. Long-term passive acoustic monitoring (PAM) of dolphin vocalizations coupled with photo-ID visual surveys in the May River estuary, South Carolina identified dolphin sighting abundance peaks in late summer, whereas vocalizations peaked in the winter. The Charleston Harbor, similar in salt marsh habitat to the May, is a deep inlet that experiences high vessel traffic. Spatial analyses of historical Charleston photo-ID surveys identified multiple core use areas and seasonal shifts in dolphin abundance. This study utilizes PAM, visual survey, and prey abundance data collected in Charleston Harbor from December 2017 to June 2019 to determine how: i) temporal, spatial, environmental, and anthropogenic factors influence the acoustic repertoire of dolphins and ii) acoustic and visual detections correlate across space and time. Dolphin vocalizations display spatial variation in the harbor; vocalizations peaked in fall and winter following decreases in water temperature and daylight hours. Sighting abundance varied spatially with highest abundance (N=272) and vocalizations (N=43,967) near the South Carolina Aquarium, and lowest abundance (N=72) and vocalizations (N=10,979) in the upper Ashley River.

**INTRODUCTION**

Acoustic surveying of marine environments has shown to be an effective, indirect method for understanding the occurrence of marine mammal species over spatial and temporal scales (Rogers et al., 2013, Monczak et al., 2019). While visual survey methods (i.e., boat-based or aerial) rely on suitable weather conditions, daylight hours, and above-water animal observations to estimate abundance and distribution, a strong advantage of passive acoustic monitoring (PAM) is that it offers greater temporal resolution and data collection in adverse conditions. One constraint with PAM is that vocalization detections provide a general count of acoustic activity but differentiating individual cetaceans and extrapolating to abundance estimates have proven challenging (Marques et al., 2010; Mellinger et al., 2007; Simard et al., 2015). Additional bias can be associated with animals that are present but not vocalizing. For these reasons, PAM is used to estimate relative rather than absolute densities of animal abundance (Marques et al., 2010; Rogers et al., 2013) whereas visual surveys offer specific measures through individual counts of cetacean species (Zolman, 2002; Speakman et al., 2006; Speakman et al., 2010; Balmer et al., 2012; Waring et al., 2016; Balmer et al., 2018). The application of long-term acoustic monitoring can bolster understanding of the abundance, distribution, and behavior of cetacean species, including common bottlenose dolphins (*Tursiops truncatus*) (Castellote et al., 2015).

The Atlantic common bottlenose dolphin is a soniferous species that uses a range of vocalizations (i.e., whistles, clicks, and burst pulses) for communicating, navigating, and foraging. Whistles are omnidirectional, frequency modulated calls important in a variety of social contexts, with remarkable signal plasticity that can respond to background noise (Herzing, 1996; King and Janik, 2013; van Ginkel et al., 2017). Echolocation click trains are directional, bio-sonar signals important for navigation, prey detection, and prey capture. Burst pulses consist of a variety of pulsed signals with high repetition rate and short inter-click intervals. These signals have yet to be fully described and the functional significance of burst pulses is not well understood. For this reason, most studies on dolphin acoustic repertoire focus on whistles and echolocation bouts (Luis et al., 2016). Since acoustics play an important role in feeding events, PAM offers non-invasive approaches for understanding cetacean foraging activity (Pirotta et al., 2015; Castellote et al., 2020). Echolocation processes related to feeding have been determined with the assessment of inter-click intervals (ICI), the amount of time between clicks within an echolocation bout (Madsen and Surlykke, 2013). Click trains of very short ICIs (less than 10 ms) have been classified as foraging buzzes, associated with attempted and/or successful prey captures (Simon et al., 2010; Nuuttila et al., 2013; Miller et al., 2004; Pirotta et al., 2015). Acevedo-Gutiérrez and Stienessen (2004) found wild bottlenose dolphins near Isla del Coco, Costa Rica, whistled at higher rates when in feeding groups versus when in non-feeding groups. PAM can provide important insight on behavioral processes associated with social and foraging vocalizations to better understand habitat use areas identified by visual surveys.

The Marine Sensory and Neurobiology Lab led by Dr. Eric Montie at University of South Carolina Beaufort (USCB) is using long-term PAM to understand the spatial and temporal rhythms of the soundscape of the May River estuary, Bluffton, South Carolina. The major biophonic contributors in this estuarine system are snapping shrimp *(Alpheus heterochaelis and A. anagulosus)*, soniferous fish (spp. belonging to family Sciaenidae), and bottlenose dolphins (*T. truncatus*). Snap rates, fish calls, and dolphin vocalizations were found in lowest abundance near the headwaters and highest towards the mouth. Fish chorusing aggregations of silver perch (*Bairdiella chrysoura*), spotted seatrout (*Cynoscion nebulosus*)*,* and red drum (*Sciaenops ocellatus*) were found to occur more frequently at the mouth, indicating this area in the May River is an important spawning ground. It has been hypothesized that increased depth, higher salinities, and higher dissolved oxygen levels at the mouth of the river promote greater secondary production and spawning aggregations (Monczak et al., 2017, 2019, and 2020). Recent studies further assessing the long-term PAM and visual monitoring of dolphins in the May River have described spatial and temporal patterns in vocalizations, dolphin abundance, and residency patterns. Marian (2020) identified a significant positive correlation between number of dolphins present and number of vocalizations detected at the mouth. This correlation was not apparent in the winter, however, when vocalizations (predominantly echolocation) peaked and visual sightings decreased. This shift may be associated with prey movements causing resident dolphins to move towards the mouth where prey resources are greater. In addition, with overall prey abundance decreasing in the winter, it is suspected that dolphins must echolocate more and cannot rely on passive listening to detect prey during summer months when abundance is highest and spawning soniferous fish are aggregating (Marian, 2020; McCabe et al., 2010). Stomach content analyses from stranded dolphins in the waters around Charleston identified that fish species in the Family Sciaenidae were found to be a prominent portion of dolphin diet composition (Pate and McFee, 2012).

Common bottlenose dolphins are ubiquitous throughout the coastal and offshore waters of the United States (Bearzi et al., 2009). In South Carolina, the National Marine Fisheries Service (NMFS) has defined three bay, sound, and estuary (BSE) stocks (i.e., the Northern South Carolina Estuarine System Stock, the Charleston Estuarine System Stock, and the Northern Georgia/Southern South Carolina Estuarine System Stock) and two coastal stocks (i.e., the South Carolina/Georgia Coastal Stock and the Southern Migratory Coastal Stock) in which there are varying degrees of spatial overlap (Waring et al., 2016). For the Charleston Estuarine System (CES) Stock, photo-ID surveys were initiated in 1994 for a subset (Stono River Estuary and Charleston Harbor) of this stock’s boundaries and the results of this study identified three different site fidelity patterns for individual dolphins: annual residents, seasonal residents, or coastal transients (Zolman 2002). Additional photo-ID effort across extended temporal (i.e., 1994-2003) and spatial (i.e., Stono River Estuary, Charleston Harbor, Ashley River, Cooper River, Wando River, and adjacent coastal waters) scales identified 839 discrete individuals (Speakman et al., 2006). Systematic surveys were conducted from 2004-2006 to estimate abundance of the CES Stock and also identified dolphin abundance to peak during the summer. This increase in abundance was attributed in part to seasonal residents and transients that were sighted in the mouth of Charleston Harbor (Speakman et al., 2010). Continued photo-ID survey effort from 2004-2009, using ArcGIS spatial analyses of sightings, identified multiple core use areas of the CES Stock (i.e., at the mouth of the harbor, near Drum Island, in the Ashley and Wando river systems) by determining kernel density estimates for dolphins sighted more than 11 times and across all seasons (Bouchillon et al., 2019). Seasonal prey abundance and distribution throughout the harbor may influence use of the core areas identified.

The Ashley, Cooper, and Wando Rivers empty into Charleston Harbor and this region has been identified as important nursery and year-round habitat for various offshore and inshore fish species, respectively (Wenner et al., 1984; SCDNR SEAMAP, 2000; Arnott, 2013). Trawling surveys conducted in the harbor have identified overall fish abundance to vary seasonally, with catch rates greatest in the summer and lowest in the winter (Arnott, 2013). Bouchillon et al. (2019) determined that dolphin distribution was highest at the entrance to Charleston Harbor during summer and fall. In contrast, dolphin distribution shifted seasonally with more individuals being sighted farther inside Charleston Harbor as well as into the Ashley and Wando Rivers during spring and winter. Seasonality in prey availability and water temperature have been predicted to be primary factors in dolphin seasonal distribution patterns (Barco, 1999; Torres et al., 2005; Toth et al., 2011). Similar to the May River, the mouth of Charleston Harbor likely supports higher secondary production, which is higher during summer and fall. In winter, dolphin diet composition may be supplied by larger, lesser abundant prey within the inner portions of Charleston Harbor and adjacent rivers (Bouchillon et al. 2019). Characterizing the spatial and temporal vocalization behavior of dolphins within Charleston Harbor will provide insight into these observed shifts in seasonal distribution of dolphins and their prey.

In a highly developed estuarine ecosystem, PAM and visual survey methods together can provide a more detailed picture for understanding how vessel noise influences bottlenose dolphin behavior. From December 2017 to June 2019, continuous PAM and water quality measurements (e.g. water temperature and depth) were recorded in addition to bimonthly photo-ID surveys in six of the core use areas determined by Bouchillon et al. (2019) within Charleston Harbor and surrounding waters. During this time period, the South Carolina Department of Natural Resources (SCDNR) also conducted trammel net and estuarine trawl surveys to monitor fish species diversity and abundance. The proposed study aims to conduct a multi-faceted approach using the data described above to characterize bottlenose dolphin acoustic vocalizations and visual sightings within Charleston Harbor on a spatial and temporal scale. There will be two specific goals for this study: i) Determine how environmental and anthropogenic factors, as well as prey abundance, influence dolphin vocalizations and distribution patterns, and ii) Compare dolphin acoustic repertoire between the highly industrialized Charleston Harbor and a reference habitat, the May River.

**METHODS**

***Study Areas***

The Charleston Harbor (32˚40’N, 79˚55’W) is an inlet that is 3.7 km in width and 7.56 km long from the mouth of the harbor to the eastern edge of downtown Charleston proper (Fig. 1). The Ashley, Cooper, and Wando River are tributaries that empty into the Harbor, with the Wando terminal the major port of Charleston. Placement of recording stations within Charleston Harbor was determined based on core use areas of the CES Stock using kernel density estimate (KDE) analyses on photo-ID surveys conducted from 2004-2009 (Bouchillon, 2016; Bouchillon et al., 2019). Long-term PAM, environmental, and visual abundance data collection began in June 2017. Due to technical issues during the first recorder deployment, the present study will focus on data collected from December 12th, 2017 to June 3rd, 2019.

***Passive Acoustic and Environmental Data Collection***

In Charleston Harbor, DSG-ST passive acoustic recorders were mounted within custom built instrument frames with attached water temperature (HOBO Water Temperature Pro v2 U22-001) and water level loggers (HOBO 100-Foot Depth Water Level Data Logger U20-001-02-Ti). Prior to deployments, recorders and frames are painted with anti-fouling paint to minimize damage to recorders. From December 2017 to June 2019, recorders were deployed at 6 locations in the harbor (A, B, C, D, E, F) set to record for 2 minutes every 20 minutes at a sampling rate of 96 kHz (Table 1). From November 2019 to 2020, DSG-Ocean and LS1 recorders were deployed at 3 stations in the May River and Charleston Harbor respectively, set to record at the same duty cycle and sampling rate during the other time series (Table 2). Following each deployment, wav files are downloaded for manual analysis in Adobe Audition CS5.5 Software. Rainfall data will be provided by the National Oceanic and Atmospheric Administration (NOAA) rain gauges that are located within Charleston Harbor and the May River. National Estuarine Research Reserve (NERR) Centralized Data Management Office (CDMO) will provide dissolved oxygen, salinity, specific conductivity, turbidity, pH, and chlorophyll measurements (duty cycle every 15 min) near the mouth of Charleston Harbor (close to station D).

***Visual Surveys***

Small vessel-based photo-ID surveys in Charleston Harbor began in June 2017 and were conducted bimonthly within a designated 2 km radius around each recording station. Once dolphin(s) were observed, sighting data collected included GPS location, start and end times of sighting, group size estimates for dolphins, calves, and neonates (i.e., minimum, maximum, and best estimate), weather conditions, water depth (m), and water quality measurements using a YSI Handheld Multiparameter Instrument. Water quality measures included water temperature, salinity, dissolved oxygen, and turbidity. These vessel-based surveys were conducted under General Authorization for Scientific Research Letter of Confirmation No. 18859 issued by NMFS to Dr. Patricia Fair. Best estimates of group size across sightings for total dolphins and total calves will be assessed temporally (i.e. by season, year, and entire study). Photographs were taken of the dorsal fin of each group member to later use photo-ID methods (Speakman et al., 2010; Balmer et al., 2012; Balmer et al., 2018) to identify individual dolphins.

***Analysis of Acoustic Files***

Acoustic data collected from December 2017 to June 2019 in Charleston Harbor were manually analyzed on the hour using Adobe Audition CS5.5 software. Spectrograms are visualized using a spectral resolution of 2048 and a 10 s time window. In each file, bottlenose dolphin whistles, burst pulses, and echolocation bouts were identified and counted. Additionally, all biological sounds (e.g. fish calls and/or choruses, manatees, right whales), physical sounds (waves and rain), deployment artifacts (e.g. chain scrapes or knocks), and anthropogenic noise presence (e.g. vessels and dredging) were recorded.

***Data and Statistical Analysis***

Statistical analysis was performed using R software version 3.6.1. For all sightings recorded within each survey area surrounding a recording station, acoustic files recorded closest in time (within 10 minutes) of the start or end times of sightings were used to match vocalization detections with sighting abundance counts. Acoustic data included total number of vocalizations, total number of echolocation bouts, total number of burst pulses, total number of whistles, fish calling scores for the four main chorusing soniferous fish species in our estuaries (Oyster Toadfish, Silver Perch, Red Drum, and Spotted Seatrout; Monczak et al. 2019), and anthropogenic noise presence/absence (0/1 score). To account for occurrences when no dolphins were sighted in a particular site, these zeros in dolphins sighted were matched to acoustics on the hour falling within and closest to the 50 minute visual survey period. Data was then summed bimonthly (across surveys) at each survey site. For anthropogenic noise, rather than summing these data a new factor named ‘Noise Ratio’ was created which represented the percent of files with noise marked as present out of all the files assessed. To assess the influence calf presence may have on vocalization frequency, total dolphins was broken down into two new variables, ‘Adult’ and ‘Calf’. Calf was found to be insignificant in all models tested and final models were run looking at total dolphins rather than these age class specific variables.

Generalized linear models (GLMs) were used to assess the influence of the total number of dolphins sighted on the total number of dolphin vocalizations, total echolocation bouts, total burst pulses, and total whistles in four separate generalized linear models. Model selection was based on a forward stepwise selection process and used Akaike information criterion (AIC) for model comparison. In each model, multiple factors (location/survey site, season, fish calling score sum, and noise ratio) were included to check for spatial, seasonal, environmental, and/or anthropogenic variation in vocalizations and sightings. Surveys that happen to fall on days in between deployments when the recorders were out of the water were excluded in analysis. Assessed multiple GLMs with Gaussian, Poisson, and Negative Binomial distributions and found a Gaussian distribution with a Log+1 transformation of vocalizations fit best. To understand the importance of factors tested in the model on vocalizations, stepwise model selection was conducted using the stepAIC() function in R and using AIC criterion kept or removed certain factors from the model. All models displayed AICs lower by 2 points with oyster toadfish calling and noise ratio variables removed. For the models selected, likelihood-ratio chi-square tests were conducted to further understand the importance and effect size of each factor in the model. Tukey Kramer HSD multiple comparison of means post hoc tests were conducted for each selected model to define differences between group means within each of the factors included.

**RESULTS**

***Acoustic and Visual Detections***

***Models***

***Spatial Patterns***

***Temporal Patterns***

***Environmental and Anthropogenic Factors***