5-8-2020

# Testing the Potential of Environmental DNA: Genetic Monitoring of Estuarine and Coastal Bottlenose Dolphin (Tursiops truncatus) Stocks in South Carolina

Kathryn Greiner-Ferris
*Coastal Carolina University*

# Testing the Potential of Environmental DNA: Genetic Monitoring of Estuarine and Coastal Bottlenose Dolphin (*Tursiops truncatus*) Stocks in South Carolina

By

Kathryn Greiner-Ferris

---

Submitted in Partial Fulfillment of the

Requirements for the Degree of Master of Science in

Coastal Marine and Wetland Studies in the

School of the Coastal Environment

Coastal Carolina University

2020

| | |
|---|---|
| Dr. Robert Young, Major Professor | Dr. Patricia Rosel, committee member |
| Dr. Erin Burge, committee member | Dr. Richard Viso, SCMSS Director |
| Dr. John Hutchens, committee member | Dr. Michael Roberts, Dean |

**ABSTRACT**

Environmental DNA (eDNA) analysis is a non-invasive monitoring technique that can detect and potentially monitor elusive marine mammals. To date, the majority of eDNA studies have been performed in freshwater environments, partially due to methodological challenges posed by higher salinities and increased dilution effects of large water masses in marine environments. The objective of this study was to design and optimize species-specific oligonucleotide PCR primers to accurately detect and quantify common bottlenose dolphin (*Tursiops truncatus*) eDNA collected from the marine environment and to evaluate potential trends between eDNA concentration and dolphin abundance and seasonality. Primer pairs were designed to target 159 and 92 base pair (bp) fragments of the mitochondrial DNA (mtDNA) control region and Cytochrome *b* gene (*cytb*), respectively. Common bottlenose dolphin eDNA was analyzed from water samples collected in two estuarine salt marshes (North Inlet and Cape Romain) and the coastal ocean in South Carolina, USA. A total of 176 water samples were analyzed, including 132 from predetermined survey locations and 44 collected directly in the wake of dolphins. Relationships were observed between (1) location and number of positive eDNA detections per survey, (2) mean concentration of positive eDNA detections and dolphin sightings per survey, and (3) dolphin group size and the concentration of eDNA in water samples collected in the group's wake in salt marsh systems. Results provide evidence for the utility of eDNA techniques in examining the presence, relative abundance, and distribution of common bottlenose dolphins. This study highlights the challenges and implications of eDNA detection in the marine environment.

**TABLE OF CONTENTS**

## LIST OF TABLES

**LIST OF FIGURES**

## INTRODUCTION

Advances in genetic monitoring have led to methods to utilize cellular material shed from the bodies of organisms in aquatic environments (Foote et al., 2012). Genetic material is deposited into the environment by organisms via sloughing of skin, urination, defecation, gamete emission, and saliva (Foote et al., 2012) and is collectively referred to as environmental DNA (eDNA; Kelly et al., 2014a; Baker et al., 2018). eDNA sampling is a cost effective, non-invasive sampling method that can detect multiple organisms down to the species-level from just a single sample of water (Sawaya et al., 2019). Given an increase in the use of eDNA for monitoring biodiversity in freshwater environments, and more recently in marine environments, it is important to consider both the advantages and limitations of these methods. With further research, novel applications for genetic information have the potential to generate key data to guide the conservation and management of marine mammals (Sigsgaard et al., 2016; Parsons et al., 2018).

### *Uses and efficacy of eDNA*

Similar to how a track print indicates the presence of terrestrial species, eDNA serves as a reliable indicator of the presence or absence of species in aquatic environments (Cristescu and Hebert, 2018). With the potential to assess large scale biodiversity, eDNA has been used to distinguish different habitat types based on rises in unique taxonomic eDNA concentration known to occupy specific habitats (Port et al., 2016). Studies have also suggested that eDNA sampling may be more efficient at detecting species than visual surveys (Yamamoto et al., 2017). This was supported by a single 6 h eDNA survey where the number of species detected was comparable to the number of species detected from 14 rounds of underwater visual census surveys

(Yamamoto et al., 2017). eDNA analysis has been successful in detecting endangered or undetected invasive species that may be overlooked by visual surveys (Bohmann, 2014). Investigating a species of conservation concern, Olson et al. (2012) used eDNA to detect a subspecies of the hellbender salamander (*Cryptobranchus a. alleganiensis*) from samples of river water where species abundance was very low (Olson et al., 2012). As eDNA research expands, ability to use eDNA for monitoring of biodiversity beyond species presence could be a powerful means of surveying large portions of an environment (Kelly et al., 2014b).

To date, the majority of eDNA studies have been performed in freshwater environments, partially due to methodological challenges posed by higher salinities and increased dilution effects of large water masses in marine environments (Harper et al., 2020). Marine mammal detection by eDNA was first investigated by Foote et al. (2012), who isolated harbor porpoise (*Phocoena phocoena*) eDNA from water collected in close proximity to a sea pen containing four individuals. Target eDNA was consistently detected within 10 m from the sea pen. Detection of eDNA decreased further from the sea pen due to dilution from tidal water movements. The number of studies using eDNA to detect marine mammals is increasing, including the Yangtze finless porpoise (*Neophocaena asiaeorientalis;* Stewart et al., 2017), killer whales (*Orcinus orca;* Baker et al., 2018; Pinfield et al., 2019), and West Indian manatees (*Trichechus manatus*; Hunter et al., 2018). Recent work reported a considerable persistence of eDNA in samples following an encounter with killer whales in the coastal waters around the San Juan Islands (Baker et al., 2018). Water collections occurred every 15 min from the water mass where the whales had passed through, which drifted more than 4 km due to tidal

currents. Despite the dynamics of sampling in the marine environment, the ability to detect target eDNA persisted for at least an hour after five different encounters and up to two hours in one encounter (Baker et al., 2018). The results of this serial sampling method provided insight into the dispersal and longevity of eDNA in a marine environment over a prolonged period of time (Baker et al., 2018).

A number of studies have investigated eDNA as a means to estimate relative abundance and/or biomass of both fish and marine mammals (Kelly et al., 2014b; Baldigo et al., 2017; Hunter et al., 2017; Stewart et al., 2017). The presence of eDNA in a sample is typically assessed by amplifying short fragments of eDNA using polymerase chain reaction (PCR) chemistry. Real-time quantitative polymerase chain reaction (qPCR) can be used to calculate the concentration of eDNA in a reaction with a regression model to infer the relationship between individuals and eDNA concentration (Baldigo et al., 2017; Hunter et al., 2017; Stewart et al., 2017). While these studies show strong correlations between target eDNA concentrations and species density and/or biomass, they also acknowledge the numerous factors that affect eDNA distribution and degradation. Cases where eDNA is detected in the environment in the absence of target organisms is considered a false positive (Ficetola et al. 2008; Stoeckle et al. 2016; Beng and Cortlett, 2020). When eDNA is not detected but the target organism is present, it is considered a false negative (Ficetola et al. 2008; Schmidt et al. 2013; Beng and Cortlett, 2020). When eDNA is released from an organism it is subject to factors such as UV exposure, pH, temperature and flow rate which degrade and impact eDNA concentration (Dejean et al., 2011; Barnes et al., 2014; Parsons et al., 2018). Dispersion of target eDNA molecules can vary under different environmental conditions (Dıaz-Ferguson et al., 2014;

Moyer et al., 2014; Furlan et al., 2016) which can cause patchy distribution and highly variable concentrations of target eDNA (Nathan et al., 2014; Turner et al., 2015; Hunter et al., 2015; Hinlo et al., 2016). A positive detection may be the result of water transport containing target eDNA from other locations, predator excrement, or even deceased organisms and therefore does not always imply the presence of the target species (Hinlo et al., 2017; Bylemans et al., 2018).

*Conservation potential*

Traditional methods to examine population structure and abundance of marine mammals include photo-identification (mark-recapture) and aerial or boat line-transect surveys (Hunter et al., 2018). Visual identification can be restricted by adverse survey conditions caused by weather, time of day, and visibility (Dizon et al., 1992; Hupman et al., 2018). Individuals that are present can easily be missed if they are temporarily submerged (Hupman et al., 2018). Because eDNA can detect a species without the need for visual observation, it can potentially account for false negative errors in detection (Lodge et al., 2012; Minamoto et al., 2012). Detecting rare, elusive, or vulnerable cetacean species with eDNA may be more efficient, particularly in remote or otherwise difficult to survey locations where opportunities to identify species are limited due to turbidity or sea state (Hunter et al., 2018). Monitoring eDNA concentration may also be applicable as an indirect indicator of a species' natural behavior (Hanson et al., 2018). For example, spawning events are likely to cause an increase in eDNA concentration due to communal release of gametes (Hanson et al., 2018). Similarly, if eDNA concentration is altered by large gatherings of individuals, then species-specific eDNA concentrations may have the potential to serve as a proxy for migration patterns of some marine

mammals (Hanson et al., 2018). eDNA methods provide an additional detection opportunity and offer a potentially broader ecological understanding of the marine environment.

Reliable assessments of species presence, distribution, and abundance are critical to inform conservation management (Furlan et al., 2016). Common bottlenose dolphins (*Tursiops truncatus)* are abundant in the estuarine and coastal environments of South Carolina (SC), United States (US) and are directly exposed to anthropogenic influences (Rice, 1998; Gubbins, 2002; Rosel et al., 2009; Rosel et al., 2017). The area's extensive salt marshes, tidal creeks, and multiple riverine inputs (Dame et al., 2000) are vulnerable to increased pollutant loads and habitat degradation and loss (Díaz-Ferguson and Moyer, 2014). The status of estuarine and coastal migratory common bottlenose dolphin stocks along the US East Coast are identified and assessed by National Oceanic and Atmospheric Administration/National Marine Fisheries Service (NOAA/NMFS) for conservation and management (Waring et al., 2014; Waring et al., 2015). Implementation of eDNA methods could supplement or possibly even reduce the time and effort spent on visual surveys. Cetaceans such as common bottlenose dolphins may serve as a model species to explore the potential of eDNA as a tool for conservation due to their consistent release of DNA into the marine environment through high rates of dermal cell turnover (Hicks et al., 1985). The applicability and limitations of eDNA for common bottlenose dolphins have never been evaluated, therefore species-specific PCR primers need to be optimized for eDNA detection of common bottlenose dolphins. Potentially, eDNA analyses will be used detect common bottlenose dolphins in other estuarine and coastal systems outside of the SC and as a proxy for population abundance estimates.

*Objective and Predictions:*

The objective of this study was to design and optimize species-specific PCR primers to accurately detect and quantify common bottlenose dolphin eDNA collected from the marine environment and to evaluate potential trends between eDNA concentration and abundance and seasonality. I predict that:

1. eDNA released in the wake of wild common bottlenose dolphins can be collected from water, extracted for genetic material, and the concentration of extracted eDNA can be measured with qPCR.
2. The concentration of eDNA in water samples collected in the wake of common bottlenose dolphin groups correlates with the number of individuals that are present in concurrent visual surveys, and the relationship does not vary between salt marsh estuarine systems and the adjacent coastal ocean.
3. In a comparison of two SC salt marsh systems with historical differences in common bottlenose dolphin abundances, dissimilarities in mean dolphin eDNA concentrations will exhibit similar trends to current and historical abundance surveys in these systems.
4. Seasonal changes (warm to cold) in mean bottlenose dolphin eDNA concentration in South Carolina salt marsh systems exhibit similar trends to current in these systems.

## MATERIALS AND METHODS

### *Study Area*

eDNA collection surveys, hereafter referred to as surveys, were performed in three different locations: two SC salt marsh systems, North Inlet and Cape Romain, and the coastal Atlantic Ocean near Murrells Inlet, SC (Figure 1). North Inlet is a 32 $km^2$ tidally driven salt marsh estuary composed of protected marshes and salt marsh creeks (Dame et al., 1986). The Cape Romain system is a much larger salt marsh system with an area at least four times that of North Inlet (Sloan, 2006; Google earth, n.d.). These two marshes facilitate productivity in the estuaries and the adjacent coastal ocean ecosystem by filtering and processing suspended particulate and dissolved materials from tidal waters (Dame et al., 1986; Dame et al., 1989). Northern SC Estuarine System Stock (NSCSS) resident common bottlenose dolphins are found year-round in both Cape Romain and North Inlet, with a higher abundance in Cape Romain (Young and Phillips, 2002; Sloan, 2006; Brusa et al., 2016; Silva et al., 2020). Estuarine common bottlenose dolphin abundance in SC and southern North Carolina (NC) is highest during an extended warm season (May–late October) and declines during the cold season (November–April; Speakman et al., 2010) when prey species move offshore and estuarine dolphins spend more time in the coastal ocean (Brusa et al., 2016; Silva et al., 2020). The northern coast of SC is characterized by a gentle sloping continental shelf (Taylor et al. 2008; Silva, 2016) with both sand and hard bottom substrates of variable extent (Ojeda et al., 2004). Two coastal stocks of common bottlenose dolphins inhabit coastal waters of SC: The Southern Migratory Coastal Stock (SM), and the South Carolina/Georgia Coastal Stock (SC/GA). Most sightings of common bottlenose dolphins

in the coastal waters of northern SC occur in late fall (October/November), due to a migratory pulse of SM stock dolphins, and are primarily located within a few km from shore (Silva, 2016).

*Dolphin surveys and eDNA water sample collections*

Four winter salt marsh surveys were completed in North Inlet from January through early March of 2019 and eight warm season salt marsh surveys (four in each location) were completed from May 2019 until mid July of 2019. This enabled between-season comparisons for North Inlet surveys and between-system comparisons during the warm season. Two coastal surveys were conducted in November 2019, when dolphin sightings were more reliable due to the fall migratory peak (Silva, 2016). Salt marsh surveys in North Inlet and Cape Romain each followed a consistent 30 km transect with a crew of 3 in a 5.5 m aluminum skiff (Figure 2; Figure 3). The coastal surveys also followed a 30 km transect that consisted of a 15 km transect to the north of Murrells Inlet at a distance of 0.5 km offshore and a 15 km returning transect at a distance of 1.5 km offshore, similar to a survey protocol used by Silva (2016) (Figure 4). A 5.5 m Rigid Hull Inflatable Boat (RHIB) was used for the coastal surveys.

Two categories of 1 L water samples were collected during each survey: interval samples and wake samples. Twelve interval samples were collected every 2.5 km during each 30 km survey in all locations. Time of interval sample, GPS location, water temperature, and salinity were recorded for each interval sample. The mean concentration of target eDNA in the twelve interval samples was used to determine the mean concentration of common bottlenose dolphin eDNA for each survey. Wake samples were collected when dolphins were encountered on a survey. Each encounter with an

individual or group of dolphins was considered an event. A group was defined as all dolphins within 10 m of another individual using the conservative 10 m chain rule (Quintana-Rizzo and Wells, 2001; Gibson and Mann, 2009). The vessel was positioned in line with the estimated lateral center of the group wake while remaining approximately 30 m behind a line defined by the last trailing individual in the group. Wake samples were collected at the bow of the boat, from the air/surface interface of the water while at idle speed or in neutral. Three triplicate 1 L water samples were collected from the area of the surface print left by surfacing individuals, generally within 30 seconds of surfacing. For each event, we documented number of individuals, sub-group size (a subset of the group interacting more closely with one another), behavior of individuals in the group, estimated lateral distance/spacing between individuals, and cohesiveness of the group (distance and orientation of individuals). Physical data collected included time of wake sample, GPS location, water temperature, salinity, heading, tidal stage, and tidal current direction (relative to heading). In most cases, standard photo-identification techniques were employed, though not required for this study. When possible, the dorsal fins of every member of the group were photographed from a perpendicular angle (Speakman et al. 2010) using a Canon Digital SLR camera. Photographs will later be organized into Finbase, a database for dolphin photo-identification data for researchers to identify individuals based on their markings (Speakman et al., 2010).

Water sample collection bottles and coolers were cleaned with 20% bleach in order to remove any traces of cetacean DNA prior to sampling (Ma et al., 2016). A sterilized collection bottle filled with distilled water was kept in the cooler to monitor for contamination between water samples during surveys. Water samples were placed

directly in a cooler with ice and transported back to the laboratory to be filtered within 24 h through a 47 mm mixed cellulose ester (MCE) filter paper with 0.45 μm pore size (Ma et al., 2016). The filtration system was rinsed with 20% bleach between each sample. To monitor for cross contamination, 1 L of deionized water was filtered through the filtration system after each sterilization. Filters were later tested for potential lab contamination. Control and experimental filters were carefully removed from the filter unit, folded, and placed into a 2 ml LoBind tube (Ma et al., 2016) and stored at -20 °C until eDNA extraction was performed (Majaneva et al., 2018).

*eDNA extraction*

Extraction was performed with the DNeasy Blood and Tissue kit (Qiagen) reagents using modified protocols (Ma et al., 2016; Majeneva et al., 2018). Initially, 50 μl of proteinase K and 500 μl ATL buffer were added to 2 mL LoBind tubes containing filters (Majeneva et al., 2018) and samples incubated overnight at 37°C on a rocking platform set at 200 rpm (Majeneva et al., 2018). Extraction methods then followed manufacturer's protocol. The extraction protocol was modified after initial DNA extractions showed signs of inhibition in PCR. Volumes of ATL buffer and proteinase K were increased to 600 μl and 60 μl, respectively. After incubating at 37°C for 24 h, samples were vortexed for 15 s and spun down to separate out excess debris. All liquid was transferred to a new 2 ml LoBind tube containing equal parts AL buffer and 99% ethanol. The mixture was vortexed and transferred to a DNeasy spin column. The final product consisted of 100 μl of elution buffer warmed to 70 °C which had been added to the membrane and left at room temperature for 10 min.

*Quantitative PCR assay development*

Two sets of common bottlenose dolphin oligonucleotide PCR primers were designed using the mitochondrial DNA (mtDNA) cytochrome b (*cytB*; NCBI accession number EU557093; Xiong et al., 2009) and control region reference sequence (NCBI accession number NC01205; Xiong et al., 2009). Alignments with other closely related delphinid species and local non-target species were created with Clustal Omega (Table 1; Madeira et al., 2019). Variable regions of the genes were isolated by eye and primer pairs were designed using Primer3 to target segments with 65–200 base pairs (bp; Beauclerc et al., 2017). Primer pair parameters required 2 to 3 mismatches with local non-target species, a G-C content between 40-60% and, melting temperatures ($T_m$) between 52-65°C with both primers differing no more than 5 °C (Lorenz et al., 2012). BLAST searches (Ye et al., 2012) compared primer sequences to all available sequence data on the NCBI genetic sequence database to confirm that primer pairs did not match non-target sequences (Jerde et al., 2011; Thomsen et al., 2012). The software's default primer specificity stringency parameters were used to retrieve the template and specificity information. The primer pairs were considered species specific if there were at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps at the 3' end of the primers (Ye et al., 2012). Genomic DNA from Atlantic spotted dolphin (*Stenella frontalis*), pygmy sperm whale (*Kogia breviceps*), and sandbar shark (*Carcharhinus plumbeus*) were selected to determine species specificity and ensure that the primers did not amplify non-target species (Davy et al., 2015). The Atlantic spotted dolphin is a closely related Delphinid species (Leduc, 2009) and was used to determine species specificity, while the pygmy sperm whale is a distantly related odontocete family (Hooker, 2009) and was used to determine family level or sub-order specificity.

Common bottlenose dolphins are the only common inshore dolphin reported in SC, but Atlantic spotted dolphins can be found as close as 10-15 km offshore in the summer (Adams and Rosel, 2005; Santos-Neto *et al*., 2014). From 2009 through 2018, SC only had 0.3 spotted dolphin strandings per year (McFee, W., unpublished data). The pygmy sperm whale is also an offshore, primarily shelf edge species (Santos et al., 2006), but it is the second most common stranding in SC, averaging 3.6 strandings per year over the same period (McFee, W., unpublished data) Water samples collected from the Georgia Aquarium common bottlenose dolphin tank and genomic DNA extracted from common bottlenose dolphin skin biopsies archived from SC strandings, provided by Wayne McFee (NOAA/CCEHBR), were used as positive controls. Genomic DNA concentrations were measured with a Qubit Fluorometer and a double-stranded DNA (dsDNA) quantification assay (Invitrogen).

Two primer pairs were developed to amplify different regions of genes (Davy et al., 2015; Freeland and Joanna, 2017) that varied in length. Primers TtDloopF (5'-CACACGTGCATGCTAATATTTAG-3') and TtDloopR (5'-GAGTGACCATAGGATATATGGAGA-3') were used to amplify a 159 bp region of the mtDNA control region and primers TtCytbF (5-'CGAGTGAATCTGAGGTGGATTT-3') and TtCytbR (5'-CAATGCTGTGATGATGAATGGAAGA-3') were used to amplify a 92 bp region of the *cytb* gene (Table 2). All PCR preparations were performed in a room separate from PCR machines and post-PCR products. Preliminary screening of primer pairs was performed on a C1000 Touch Thermal Cycler (Bio Rad). Optimal annealing temperature ($T_a$) of successful primers (Table 2) was determined by running a gradient PCR with an $T_a$ range of 54-70°C (Lorenz, 2012) in 50 µl reactions containing 5 ng

DNA, 0.5 µl Platinum Taq DNA polymerase (5 Units/ul; Thermofisher Scientific), 10× PCR Buffer (200mM Tris-HCl (pH 8.4), 500 mM KCl), 1.5 µl $MgCl_2$ (1.5 mM), 1 µl dNTP (0.2 mM), 1 µl of each primer diluted to 10 µM, and deionized water. The thermal cycling profile included an initial denaturation step at 94°C for 2 min, followed by 40 cycles of 94°C for 30 s, 55°C (TtDloopF/ TtDloopR) and 58°C (TtCytbF/ TtCytbR) for 30 s, 72°C for 30 s, and one final extension step at 72°C for 5 min. Five µl of PCR products were visualized on a 1.5% agarose gel stained with SYBR Safe DNA Gel Stain (Thermofisher Scientific; Weber et al., 2007). Amplification was considered successful when a single band was observed at the expected fragment size (Beauclerc et al., 2018). Genomic DNA extracted from dolphin skin biopsies was diluted from 10 ng to 0.001 pg (Baker et al., 2018) and used to define the limits of detection (LOD) and limits of quantification (LOQ) for the qPCR assay (Salter et al., 2019). Quantitative PCR was performed on a Stratagene Mx3005P (Thermofisher Scientific) in a total reaction volume of 20 µl, containing 2 µL of template DNA (standards ranged from 10 to 0.01 pg/reaction and the concentration of DNA extracted from the filters was unknown), 10 µl 2X Power SYBR Green Master Mix (Fisher Scientific Inc) with ROX reference dye, 1 µl of each primer diluted to 10 µM and, 6 µl of deionized water. Experimentation with Bovine Serum Albumin (BSA; Thermofisher) was conducted to reduce the risk of PCR inhibition. Reactions were spiked with various BSA (20mg/ml) volumes (0.05 µl, 0.1 µl, 0.15 µl, and 0.2 µl), but this was unsuccessful in reducing inhibition. Final extractions were diluted with deionized water 1:50 to lower the risk of PCR inhibition (Majaneva et al., 2018). Quantitative cycling conditions were as follows: an initial incubation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C

(TtDloopF/ TtDloopR) and 58°C, and extension at 74°C for 15 s. Each qPCR reaction was run in triplicate along with standards, no template controls, positive DNA control (1 ng/µl common bottlenose dolphin genomic DNA), negative DNA control (1 ng/µl; Atlantic spotted dolphin or pygmy sperm whale) and, field and laboratory controls (Pinfield et al, 2019). Non-specific amplification and primer dimers were ruled out with a melt curve analysis at the end of each run (Lorenz, 2012). A qPCR reaction was considered positive for eDNA if one of the three qPCR replicates amplified (Tingley et al., 2019) within the threshold defined by the LOD and LOQ. To distinguish between water sample extracts that truly do not contain target eDNA and PCR inhibition, all potentially negative qPCR reactions were spiked with a known concentration of common bottlenose dolphin DNA. A quantification cycle (Cq; the cycle at which the arch of the amplification curve is greatest; Bustin et al., 2009) shift ≥ 3 cycles was considered as total inhibition and the reaction was not considered for analysis. (Salter et al., 2019) At the end of each run, the Applied Biosystems QuantStudio Software v1.4 provided the DNA concentration for the measured Cq values calculated from the standard curve.

*Data analysis*

Occupancy analysis was performed in RStudio Desktop version 1.2.1335. The probability of species occurrence at a location, the conditional probability of species occurrence in a sample, and the conditional probability of species detection in a PCR replicate were modeled using the R package EDNAOCCUPANCY (Dorazio and Erickson, 2018). The hierarchal model consisted of three levels, including: sample locations (Cape Romain, coastal ocean, and North Inlet in the warm and cold season), interval samples (replicate

water samples collected at each location), and sub-sample (qPCR replicates for each interval sample). Interval sample concentrations and PCR replicates were treated as binary variables (1 = presence and 0 = absence). Picogram per liter was calculated according to the equation: pg/ L= pg/L reaction × [($E_{vol}$/$R_{vol}$)/$S_{vol}$] × dilution factor, where $E_{vol}$ and $R_{vol}$ are the extraction volume and qPCR reaction volume (µl) and $S_{vol}$ is the filtered sample volume (L) (Salter et al., 2019). Statistical analysis was performed in IBM SPSS Statistics Version 25. A Shapiro-Wilk test was used to test for normal distribution and goodness of fit. Positive wake samples were $\log_{10}$ transformed to satisfy normality assumptions. A linear regression model was used to test the correlation between the concentration of eDNA in water samples collected in the wake of a group and the number of individuals the group. Average eDNA concentration of all interval samples was calculated for all three locations: North Inlet (warm and cold season), Cape Romain, and the coastal ocean. A parametric multivariate analysis (Pearson correlation) was performed to test the significance ($p<0.01$) of survey locations (Cape Romain, coastal ocean, and North Inlet in the warm and cold season), concentration of positive interval samples, percent of positive detections, and dolphin sightings. The same parameters were also applied to test the significance of abiotic factors (temperature and salinity) on coastal and estuarine interval sample concentrations and between group size, abiotic factors (temperature, salinity and current relative to group direction) and wake sample concentration in estuarine environments. Direction of current was not recorded in the coastal environment, so coastal wake samples were not included in this analysis.

Detection rates for each survey location were calculated as the percent of all interval samples from all surveys that tested positive for eDNA. Samples affected by

inhibition were not included in the calculation. The spatial distribution of positive eDNA detections from interval samples, as well as the distribution and number of visual dolphin sightings, were graphically displayed using GPS Visualizer (Schneider, 2013). For this map, the percent of positive detections was calculated for each interval sampling site. Samples affected by inhibition were not included in the calculation, and no site had more than one inhibited sample. Visual sightings and eDNA detections were mapped according to coordinates recorded at the detection site.

## RESULTS

### *Specificity and validation of eDNA assay*

Two primer pairs were developed to amplify different regions of genes (Davy et al., 2015; Freeland and Joanna, 2017) that vary in length. Primers TtDloopF (5'-CACACGTGCATGCTAATATTTAG-3') and TtDloopR (5'-GAGTGACCATAGGATATATGGAGA-3') were used to amplify a 159 bp region of the mtDNA control region and primers TtCytbF (5-'CGAGTGAATCTGAGGTGGATTT-3') and TtCytbR (5'-CAATGCTGTGATGATGAATGGAAGA-3') were used to amplify a 92 bp region of the *cytb* gene (Table 2). Both primer pairs successfully amplified DNA of the expected fragment lengths using common bottlenose dolphin genomic DNA extracted from skin biopsies and aquarium water samples. Gel electrophoresis confirmed species-specificity of TtDloopF/ TtDloopR as it did not amplify Atlantic spotted dolphin DNA (Figure 5). Conversely, TtCytbF/ TtCytbR did amplify Atlantic spotted dolphin DNA, though it did not generate amplification for all other non-target DNA controls, suggesting

that it may be delphinid specific (Figure 6). TtDloopR/TtDloopF was found to only amplify common bottlenose dolphin DNA extracted from tissue biopsies and aquarium controls, indicating that it targeted a larger fragment size than what was present in the water samples collected in North Inlet, Cape Romain, and the coastal ocean. TtCytbR/TtCytbF, which amplified a smaller DNA fragment size, exhibited greater sensitivity to amplifying fragmented eDNA. Given the survey seasons and locations, the chances of detecting another delphinid species was negligible, therefore TtCytbF/TtCytbR was selected for this project due to small fragment size. The resulting average qPCR LOD was a $C_t$ of 35, and a concentration of 0.01 pg/reaction. Samples that did not meet this threshold were considered negative.

*Wake and interval sample collections*

A total of 16 surveys were completed in this study, however 3 were removed from analysis due to substantial PCR inhibitors in the water samples (Appendix Table 1-2). Modified extraction protocols reduced, although likely did not eliminate, PCR inhibitors in the remaining survey data. All field sampling blanks resulted in no amplification. Three wake samples collected from the coastal environment were removed from analysis due to evidence of contamination in the filtration control. There were 79 samples that did not amplify and were tested for inhibition. Of those 79 samples, total inhibition resulted in the removal of 9 out 132 interval samples (Appendix Table 1) and 2 out of 44 wakes samples (Appendix Table 2).

The number of dolphins sighted per survey ranged from 1-87 individuals. Mean eDNA concentration of interval samples per survey, including negative detections (0 pg/L) ranged from 30.51 to 143.09 pg/L (Figure 7). Two interval samples collected in

North Inlet (#46 and #101; Appendix Table 1) were nearly 400 times greater than the other interval samples. These interval samples were excluded from mean analyses to eliminate statistical skewness of the data set, similar to the actions of Baker et al. (2018) who also eliminated water samples with elevated eDNA concentrations, suspecting they were spiked with large amounts of fecal matter. Common bottlenose dolphin eDNA was detected in 67 of 123 interval samples (Table 3). Detection rates (percent of positive interval samples) per location ranged from 31% to 77% (Table 3).

Behavior of individuals varied with group size and location (Appendix Table 4). In Cape Romain, groups ranging from 7-15 were observed physically interacting, tail slapping, jumping, active surfacing, and porpoising in the wake of a shrimp boat. The largest group observed in North Inlet was estimated to consist of nine individuals. This group did not exhibit any of the behaviors observed in Cape Romain. In addition, estimating the number of dolphins in a large group was more difficult due to individuals joining and dispersing while smaller groups of dolphins were more cohesive. Smaller groups of dolphins observed on all salt marsh surveys were typically observed passively milling or traveling slowly.

*eDNA concentration*

Temperature and salinity values from all surveys can be found in Appendix Table 3. Interval sample and wake sample concentrations were not significantly related to temperature or salinity (Table 4). In addition, whether the dolphins were moving with or against the currents, which could potentially impact the duration of their exposure to a water mass, did not significantly impact the wake sample concentrations (Table 5; Appendix Table 4). Mean common bottlenose dolphin eDNA concentrations per location

(Table 6) did not follow the visual survey abundance trends. The mean eDNA concentrations of Cape Romain, North Inlet (cold season), North Inlet (warm season), and the coastal environment were not significantly different, therefore did not reflect dolphin abundance. However, correlations were observed between (1) location and number of positive interval samples per survey ($p=0.0001$; Table 7) and (2) concentrations of positive interval samples and dolphin sightings per survey ($p=0.003$; Table 7).

Group sizes ranged from 1-15 individuals (Appendix Table 2). Because there was not a significant relationship between seasonality and mean eDNA concertation per location, both warm and cold season wake samples were analyzed together. A linear regression model revealed a positive correlation in the estuarine environment between number of individuals in a group and the eDNA concentration of concurrent wake ($n=24$; $R_2=0.32$; $P=0.004$; $y= 0.0397x + 1.7879$; Figure 8). eDNA concentrations of coastal wake samples were consistently lower than estuarine samples collected in the wake of similar group sizes. The correlation between number of individuals in a group and the eDNA concentration in coastal waters was not significant, but it did exhibit a positive trend ($n=11$; $R^2 = 0.3604$; $y = 0.0407x + 1.3452$, $P=.21$; Figure 8). Two coastal surveys resulted in only 6 out of 11 wake samples that were positive for eDNA. If an additional survey had been performed in the coastal environment, there may have been more positive wake samples to include in the analysis. Therefore, it is likely that regression analysis of coastal samples was impacted by this small sample size.

Mean eDNA concentration was not significantly different between locations and season; therefore, only the detection rates (and not the eDNA concentrations) at

predetermined interval samples were mapped and compared with sightings of common bottlenose dolphins per location (Figures 9-12). In Cape Romain, areas with a high abundance of individuals sighted is comparable to areas with a high rate of detection (Figure 9). Similar trends are observed in North Inlet in the warms season, but to a lesser extent (Figure 10). In addition, North Inlet areas with a high rate of detection in the warm season appeared to be retained in North Inlet into the cold season (Figures 11). This is consistent with Brusa et al. (2016) who found that dolphin abundance in North Inlet was higher during the warm season. In addition, 11 individuals were consistently spotted in North Inlet year-round, which further supports the present studies' conclusion that dolphins are still present in North Inlet during the cold season, but spotted less frequently. Coastal surveys showed little agreement between detection of interval samples and sightings. Eleven out of the 14 positive interval samples were detected 1.5 km from shore whereas all visual detections were closer to 0.5 km from shore (Figure 12).

*Probabilities of eDNA occupancy*

A multi-scale occupancy model was used to estimate site occupancy probability ($\psi$) median occupancy in a single water sample, given site presence ($\theta$), and median detection probability at the qPCR level ($\rho$; Table 8). At each of the 4 locations there was at least one interval sample containing common bottlenose dolphin eDNA, therefore $\theta = 1$ across all locations. The occupancy model fit with the presence of dolphins observed in the estuaries, with Cape Romain having the highest probability of detection in a single sample ($\theta = 0.75$) and North Inlet having the lowest ($\theta = 0.51$) during the cold season. Despite encountering the second greatest number of individuals on coastal surveys, the

occupancy model revealed a lower than expected sample occupancy estimate for the coastal ocean ($\theta$ =0.56).

## DISCUSSION

I was successful in designing a novel oligonucleotide PCR primer pair to quantify common bottlenose dolphin eDNA. Common bottlenose dolphins were detected in all three study locations. eDNA analysis suggested a correlation between eDNA concentration and dolphin abundance. Furthermore, certain abiotic factors were identified as influencing quantification of eDNA. Modifications and suggestions for future studies were explored to overcome challenges for sampling in the marine environment.

### *Primer Specificity*

In designing primers, targeting short fragments increases the likelihood of amplifying degraded eDNA (Axtner et al., 2018), particularly in environments with highly fragmented eDNA. Conversely, targeting longer fragments offers greater taxonomic resolution and flexibility to target regions of high genetic variability (Bylemans et al., 2018) In order to achieve species-specificity and small fragment size, TtDloopR/TtDloopF was designed to target a longer species-specific (159 bp) fragment of the mtDNA control region and TtCytBR/TtCytBF was designed to amplify a shorter (92 bp) fragment of the mtDNA *cytb* gene. Both primer pairs successfully amplified common bottlenose dolphin eDNA extracted from aquarium samples, but TtCytBR/TtCytBF provided superior amplification metrics on environmental samples than TtDloopR/TtDloopF, suggesting that fragments of eDNA in estuarine salt marsh and

coastal systems were likely shorter than 159 bp. This is consistent with other marine mammal eDNA studies. Hunter et al. (2018) and Stewart et al. (2017) designed primers to amplify eDNA fragments less than 100 bp to increase the detection of the West Indian manatee and the Yangtze finless porpoise, respectively. The current study supports that conclusion that that primer pair sensitivity estuarine and coastal environments decreases with increasing target fragment length (Deiner et al., 2017; Axtner et al., 2018).

*Understanding eDNA in the Marine Environment*

Probability estimates of species occurrence in a single sample corresponded with the number of sightings per location in Cape Romain and North Inlet. The model provided the highest estimate of sample occupancy in Cape Romain where the greatest number of dolphins was sighted, followed by North Inlet in the warm season, and North Inlet in the cold season. A greater number of dolphins were sighted in the coastal environment than North Inlet, yet the model reported a sample occurrence probability of 56% and 69%, respectively. This implies that North Inlet had a greater probability of generating a positive detection from a single water sample than the coastal environment. The two coastal surveys likely did not provide enough data to provide an accurate estimate of sample occupancy in the coastal environment (Schmelzle and Kinzinger, 2016). This model can be used to assess survey designs and experiments to ensure that there are enough replicates needed to produce reliable estimates.

The positive correlation between number of individuals in a group and the concentration of eDNA in wake samples suggest that a larger group of dolphins releases more genetic material in their wake. However, there may be other factors aside from the number of individuals that influence the eDNA concentration of wake samples. Large

groups of dolphins exhibited more active behavior compared to groups made up of only a few individuals. Certain behaviors that were only observed in large groups included physical interaction, porpoising, tail slapping, jumping, and active surfacing. These actions are likely to be a key driver behind eDNA concentrations of wake sample and group size.

Quantitative data was mapped to illustrate eDNA detection hot spots based on the frequency of eDNA detections from interval samples and sightings per survey. Both Cape Romain and North Inlet exhibited eDNA detection hot spots which coincided with large group events. Conversely, there were also eDNA detection hot spots that did not correspond with an event. It is unclear if a positive interval sample is an indicator of a missed individual or if it is an artifact of eDNA transport due to tidal currents. Without a strong understanding of the water movements and the degradation rate of the eDNA, interpreting positive eDNA data can be challenging. eDNA detection hotspots from coastal surveys did not appear to correspond with areas where large groups of individuals were sighted. The majority of positive interval samples were collected roughly 1.5 km from shore while most dolphin sightings occurred roughly 0.5 km from shore. In open water systems, such as the coastal ocean, eDNA may be quickly transported from the site of release. eDNA dispersion may be more rapid due to large water masses exchanging at higher flows than in an estuarine system (Kelly et al., 2019; Cristescu and Hebert et al., 2018). Both coastal surveys were conducted an hour after low tide and the sea state was choppier than estuarine environments (Beaufort 2-3). Under these circumstances, it is possible that sea state contributed to the dispersion of eDNA. It is also likely that some visual sightings of dolphins were missed due to these conditions, which could partially

explain the poor agreement between the locations of visual sightings and the location of eDNA detections.

There are several factors that may contribute to why methods for targeting eDNA in one ecosystem may not be applicable to another. Two studies reported surprisingly different results on killer whale eDNA detections in a brackish sound vs the open ocean (Baker et al., 2018; Pinfield et al., 2019). Researches successfully detected killer whale eDNA in the US waters of Puget Sound despite substantial drifting from the initial site where the whales had passed (Baker et al., 2018). In contrast, a study conducted in pelagic waters off Iceland was unable to conclusively amplify killer whale eDNA despite sampling in calm waters and within 10 m of the target species (Pinfield et al., 2019). The authors considered numerous factors that may have affected their results. An increase in wind and wave action can cause intact genomic DNA within living cells to transform into extracellular fragments that are too small to be detected. This may explain why detection rates in coastal wake samples was only 54%. In addition, cold seawater temperatures may cause lower skin shedding rates of some marine mammals (Pinfield et al., 2019). Colder water temperature has been suggested to slow the rate of skin shedding of humpback whales (*Megaptera novaeangliae)* and sperm whales (*Physeter microcephalus*; Pinfield et al., 2019). In the present study, the mean temperature of coastal waters compared to the estuarine waters sampled in the same season ranged from 14.1 to 19.8 °C compared to 23.2 to 28.8 °C, respectively. Consequently, wake concentrations may have been lower in coastal waters than in the estuaries due to reduced rate of skin shedding in the colder environment (Dejean et al., 2011; Barnes et al., 2014; Parsons et al., 2018). Because of the variability in shedding rates of a species, temperature and other abiotic factors should

be considered as critical covariates to accommodate for location specific variation in eDNA dynamics.

*PCR Inhibition*

The color of a water sample was usually a good indicator that the water sample contained a large concentration of PCR inhibitors. This is because inhibition is caused by particles containing inhibitory compounds in the water that interfere with PCR amplification and limit or completely mask the detection of target DNA (Cao et al., 2015; Jane et al., 2015). Algae blooms have been reported to inhibit PCR in environmental samples and was likely a cause of PCR inhibition in this study (Schrader et al., 2012). Similarly, water samples collected from brackish-water sites in the Florida Panhandle confirmed that high level of inhibition was caused by tannins produced by vegetation that surrounds the bodies of water (Hunter et al., 2018). Tidal diffusion is a dominant factor that flushes out PCR inhibitors in a tidal salt marsh system (Kjerfve et al., 1991). We observed the highest level of inhibition in the salt marshes during the warm season in North Inlet and the least amount in Cape Romain. Cape Romain has no riverine input and is solely influenced by the adjacent coastal ocean while North Inlet has partial connections to Winyah Bay (Kjerfve et al., 1991). The turbidity of North Inlet may be in part due to sediment discharge from the Waccamaw, Black, and Sampit rivers carrying a high concentration of organic material (Goñi et al., 2003; Goodman, 2013). Increased water volume and higher flows in open water systems dilutes inhibitory particles and lowers the chance of PCR inhibition limiting eDNA detection (Jane et al., 2015; Roussel et al., 2015). These factors were likely contributors to the coastal ocean surveys resulting in zero evidence of inhibition (Jane et al., 2015).

All methods to reduce PCR inhibitors from environmental samples can add to measurement errors and increase the chance of contamination (Cao et al., 2015). However, if inhibitors are not effectively removed from waters samples, interference with PCR amplification can profoundly affect detectability and lead to target underestimation (Cao et al., 2015). Methods to remove inhibitors include DNA purification kits such as a OneStep PCR Inhibitor Removal Kit and Bovine Serum Albumin (BSA; Hunter et al., 2017; Strand et al., 2011; Jane et al., 2015). Inhibited samples in this study did not improve with the addition of BSA and instead were diluted 1:50 in deionized water. Despite this effort, the presence of PCR inhibitors resulted in no amplification in many of the qPCR reactions in this study. Because all eDNA extractions were diluted, it is possible that water samples with a low yet detectable concentration were diluted below the detection limit and recorded as containing no eDNA (Baker et al., 2018; Majaneva et al., 2018; Jane et al., 2015). Inhibition is an occurrence that arises in most eDNA studies and yet is not commonly regarded as a critical factor that can lead to underestimates or false negatives (Jane et al., 2015; Baldigo et al., 2017). However, false negatives are inevitable in presence/absences surveys. For example, visual surveys are often repeated in a single location to account for individuals that may have been missed in previous surveys. Using eDNA in combination with visual surveys can potentially account for false negative errors in detection that occur in both methods (Lodge et al., 2012; Minamoto et al., 2012).

*Practical applications of eDNA*

Detection and monitoring of species may be more efficient with eDNA compared to traditional methods that often involve large amounts of time and effort (Davy et al.,

2015; Beng and Corlett, 2020). Studies comparing the cost-efficiency of eDNA to traditional methods have reported that eDNA sampling is relatively cheaper than aerial and vessel-based surveys (Davy et al., 2015, Sigsgaard et al., 2015, Stewart et al., 2017; Beng and Corlett, 2020). Before implementing expensive, labor intensive visual surveys (Balmer et al., 2014), eDNA can gather baseline data on species abundance (Beng and Corlett, 2020). For example, similar to the utilization in this study, eDNA can be used as a preliminary tool to observe mean eDNA concentrations of a system and monitor changes over time to infer abundance estimates. This could be applied when starting a habitat utilization study or an assessment of seasonal distribution. However, it is clear from the results that this use of eDNA may not be applicable in open water systems. In addition, eDNA may be a preferred method to achieve detection probabilities for rare, cryptic, and elusive species. Using an automatic sampling technique, eDNA can provide biodiversity assessments with limited anthropogenic influences to the target species or its habitats (Beng and Corlett, 2020). However, eDNA and traditional survey methods can provide different information and should not be considered as alternative methods for assessing and monitoring biodiversity (Beng and Corlett, 2020). While eDNA is not yet successful at determining exact abundance estimates of a species, the present study suggests that eDNA may be used as a tool to examine basic measures of relative abundance and distribution of marine mammals. In combination with historical data, eDNA can be a useful tool to measure the effectiveness of protected areas. Integrating the data of two different techniques provides an efficient method to optimize the deployment of management resources (Beng and Corlett, 2020).

*Conclusion*

This study provides evidence supporting eDNA detection as a method for assessing the presence of marine mammals and supports quantitative PCR as a highly sensitive method to detect and identify common bottlenose dolphin eDNA in estuarine and coastal systems. Optimized primer pairs detected 92 bp fragments of target eDNA in all three survey locations. While eDNA concentration cannot be used to quantify exact number dolphins in a location, results suggest that eDNA may be used as a proxy for species abundance estimations. The wake sample concentrations provided in insight into how much eDNA dolphins shed. Quantifying the amount of eDNA released from an individual is an important variable that can impact eDNA abundance estimations and should be further studied.

The results of this study contribute to the general knowledge and design of eDNA methodology in the marine environment. This study also highlights the challenges of using the same eDNA methodology across different systems. Dispersal, degradation, and inhibition of eDNA are all likely to differ among environments. Future studies should consider optimizing habitat specific eDNA methodologies. Continued contributions from other eDNA studies will inevitably result in a reliable, non-invasive, genetic monitoring technique that can be applied to marine environments.

## TABLES

**Table 1.** Accession numbers for mtDNA sequences of non-target organisms used to design environmental DNA (eDNA) primers for the common bottlenose dolphin.

| Non-target species | GenBank accession number |
|---|---|
| *Carcharhinus plumbeus* | NC_024596.1 |
| *Globicephala macrorhynchus* | NC_019578.2 |
| *Kogia breviceps* | NC_005272.1 |
| *Orcinus orca* | NC_023889.1 |
| *Sciaenops ocellatus* | JQ286004.1 |
| *Stenella attenuata* | NC_012051.1 |
| *Stenella frontalis* | AF084089.1<br>EF090645.1<br>EF682658.1 |

**Table 2.** PCR primers used for amplification of targeted sequences of common bottlenose dolphins.

| Primer | Primer sequence (5′–3′) | $T_m$ (°C) | GC % | $T_a$ (°C) | Product size (bp) |
|---|---|---|---|---|---|
| TtDloopF | CACACGTGCATGCTAATATTTAG | 57.1 | 39.1 | 55 | 159 |
| TtDloopR | GAGTGACCATAGGATATATGGAGA | 59.7 | 40 | | |
| TtCytbF | CGAGTGAATCTGAGGTGGATTT | 58.4 | 45.5 | 58 | 92 |
| TtCytbR | CAATGCTGTGATGATGAATGGAAGA | 59.7 | 40 | | |

**Table 3.** eDNA results from interval water samples. Results for each location are calculated from all the surveys completed in a location.

| Location | Season | Detection/total samples (% positive) | Mean ± standard deviation concentration (pg/L) | Min/max concentration (pg/L) | Mean ± Standard deviation of dolphin abundance |
|---|---|---|---|---|---|
| Cape Romain | warm | 27/35 (77) | 83.60 ± 143.09 | 25.23/826.5 | 46 ± 36.51 |
| North Inlet | cold | 11/36 (31) | 21.05 ± 36.15 | 25/107.625 | 3 ± 3 |
| North Inlet | warm | 15/30 (50) | 39.09 ± 36.64 | 31.7/123.08 | 10.33 ± 7.77 |
| Coastal | warm | 14/24 (58) | 30.51 ± 30.61 | 28.13/96.43 | 33 ± 2.83 |

**Table 4.** Multivariate analysis testing the relationship between abiotic factors (temperature and salinity) and eDNA concentration from coastal and estuarine interval samples

| Predictor | Standard Error | t-statistic | p-value |
|---|---|---|---|
| Temperature | 0.1626 | 0.7966 | 0.4301 |
| Salinity | 0.0396 | -0.6181 | 0.5397 |

**Table 5.** Multivariate analysis measuring the effect of dolphin group size, abiotic factors (temperature, salinity and current direction relative to group direction) and wake sample eDNA concentration in estuarine environments. Direction of current was not recorded in the coastal environment, so coastal wake samples were not included in this analysis.

| Predictor | Standard Error | t-statistic | p-value |
|---|---|---|---|
| Temperature | 0.027 | 0.723 | 0.4796 |
| Salinity | 0.0106 | -1.8211 | 0.0873 |
| Group traveling with current | 0.1084 | -0.92 | 0.3713 |
| Group size | 0.28 | 2.9 | 0.004 |

**Table 6.** eDNA detections and concentration from water samples collected in the wake of dolphins, calculated for all surveys completed in each location.

| Location | Season | Mean ± standard deviation of group size | Detections/total wake samples (% positive) | Mean ± standard deviation concentration (pg/L) |
|---|---|---|---|---|
| Cape Romain | warm | 5.39 ± 3.72 | 16/18 (88) | 133.19 ± 102.45 |
| North Inlet | cold | 1.8 ± 0.84 | 3/5 (40) | 76.01 ± 21.05 |
| North Inlet | warm | 3.1 ± 2.92 | 5/10 (50) | 90.04 ± 32.21 |
| Coastal | warm | 6 ± 3.97 | 6/11 (54) | 57.45 ± 42.82 |

**Table 7.** Pearson test of significance for correlation between variables survey locations (Cape Romain, coastal ocean, and North Inlet in the warm and cold season), eDNA concentration of positive interval samples, percent of positive detections, and dolphin sightings per survey ($p<.01$).

| Variables | Pearson correlation | Significance (2-tailed) |
|---|---|---|
| Location & positive detection concentrations per survey | -0.175 | 0.157 |
| Location & percent positive per location | -0.582** | 0.000 |
| Location & sightings | -0.343** | 0.004 |
| Positive detection concentrations per survey & percent positive | 0.190 | 0.123 |
| Positive detection concentrations per survey & sightings | 0.360** | 0.003 |
| Percent positive per location & sightings | 0.554** | 0.000 |

**. Correlation is significant at the 0.01 level (2-tailed).

**Table 8.** Common bottlenose dolphin eDNA Bayesian estimates of occupancy probability (ψ) median occupancy in a single water sample, given site presence (θ), and median detection probability at the qPCR level (ρ). 95% confidence interval (CI) are given for each parameter of the occupancy model.

| Site | Season | Site occurrence probability (ψ) (95% CI) | Single sample occurrence probability (θ) (95% CI) | qPCR replicate occurrence probability (ρ) (95% CI) |
|---|---|---|---|---|
| Cape Romain | warm | 1 | 0.75 (0.62-0.85) | 0.76 (0.67-0.83) |
| North Inlet | cold | 1 | 0.69 (0.58-0.79) | 0.73 (0.62-0.82) |
| North Inlet | warm | 1 | 0.51 (0.38-0.63) | 0.76 (0.68-0.82) |
| Coastal | warm | 1 | 0.56 (0.39-0.71) | 0.75 (0.61-0.86) |

## FIGURES

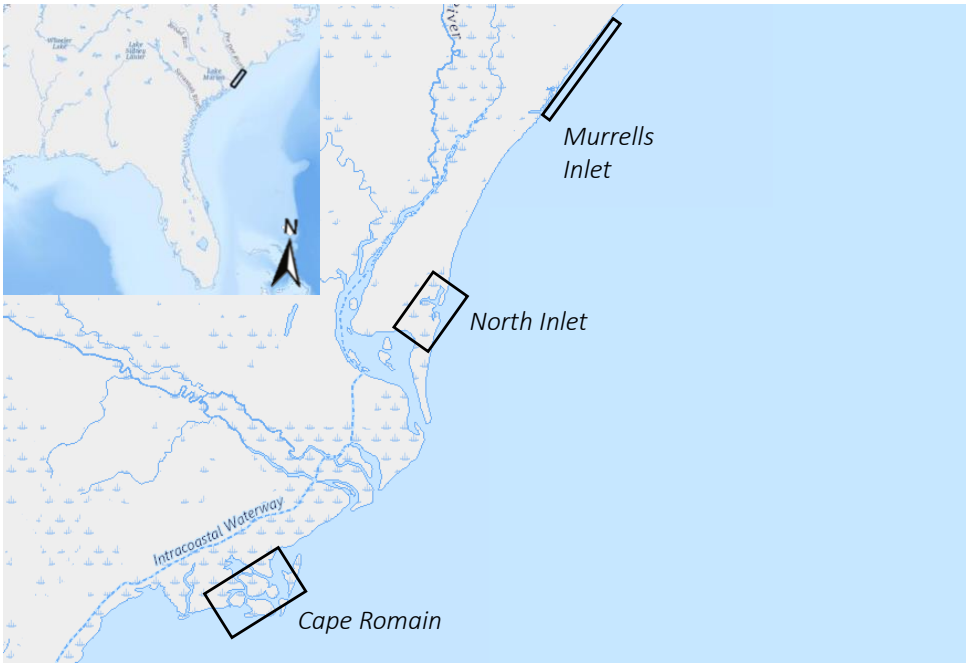


**Figure 1.** Survey locations, including salt marsh estuarine systems of North Inlet and Cape Romain and the coastal waters near Murrells Inlet.

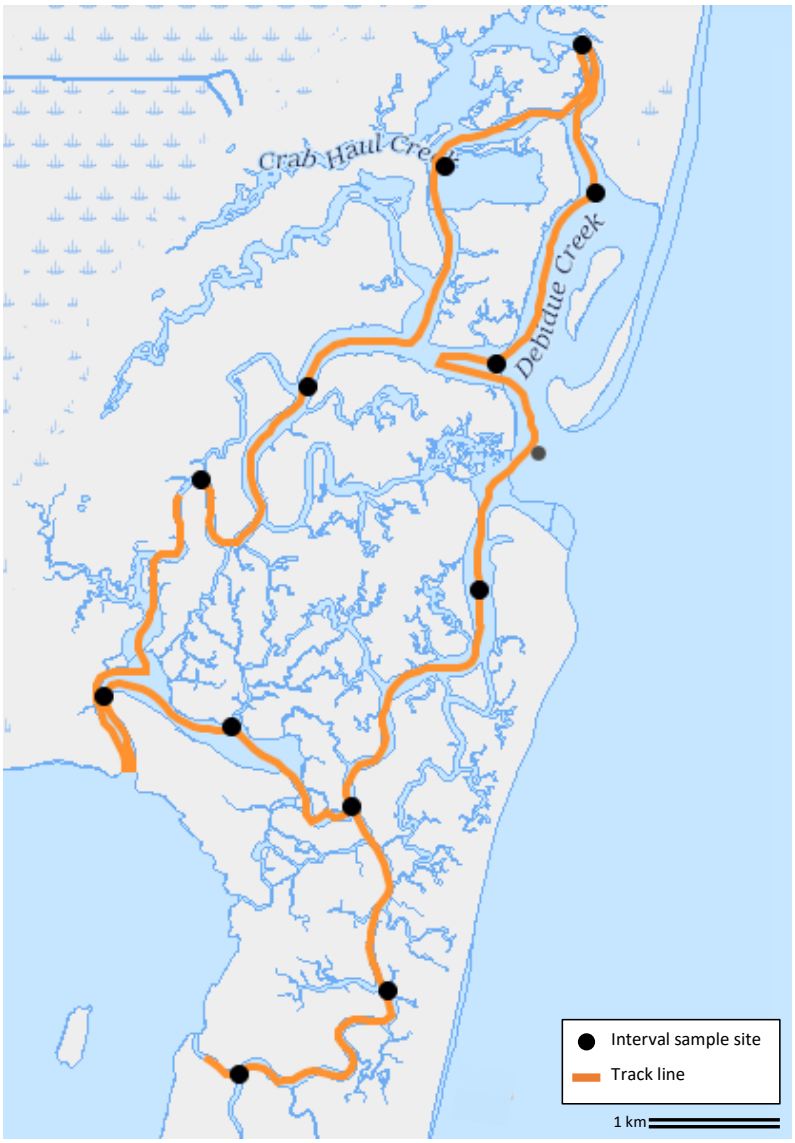


**Figure 2.** North Inlet 30 km transect survey track with 12 interval sample sites.

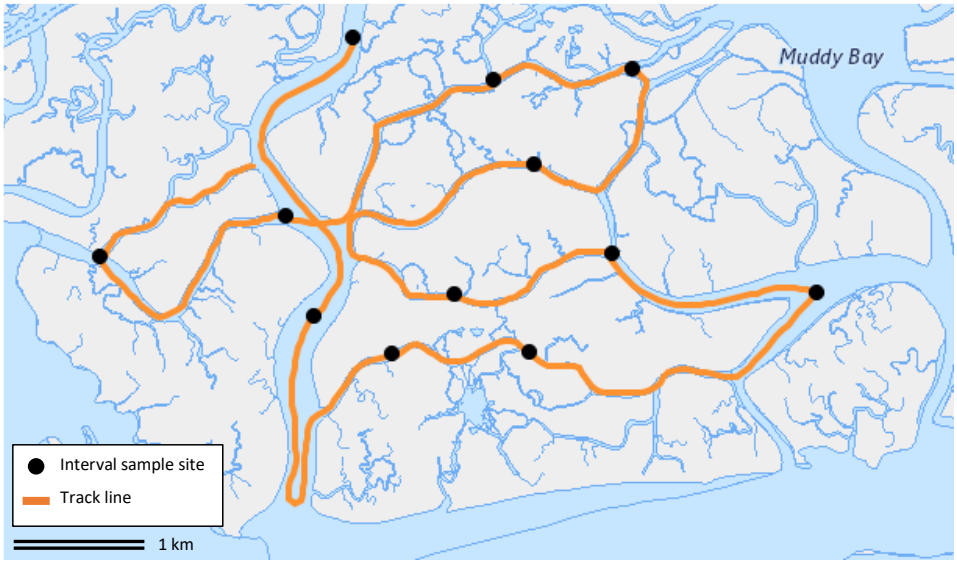


**Figure 3.** Cape Romain 30 km transect survey track with 12 interval sample sites.

.

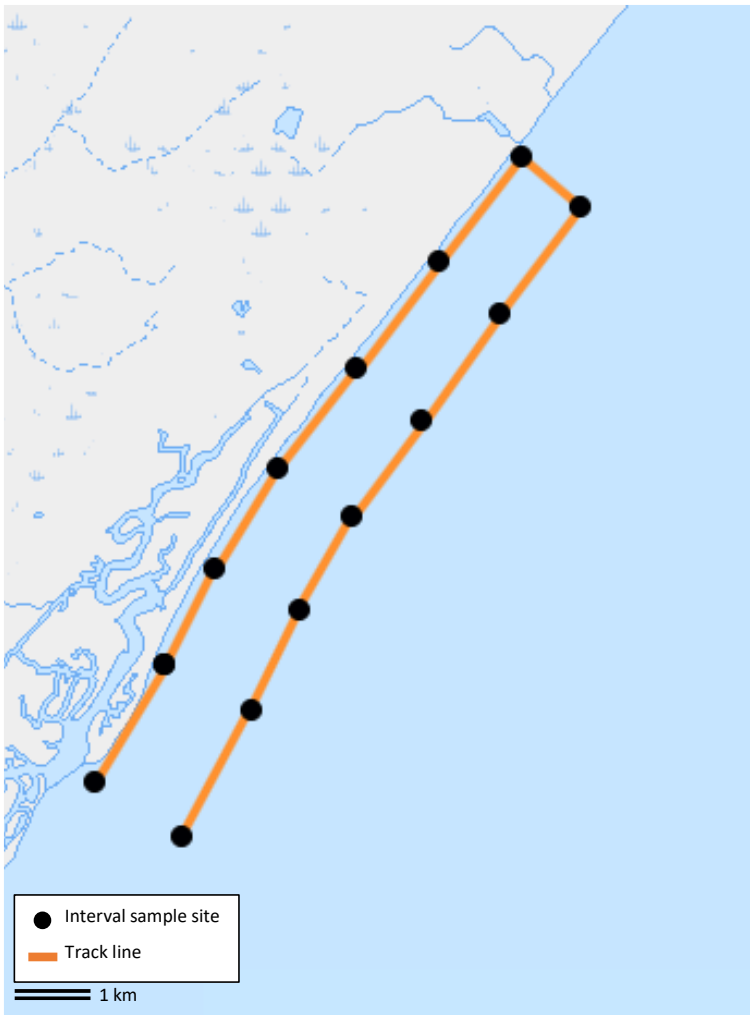


**Figure 4.** Coastal ocean 30 km transect survey track 12 interval sample sites.

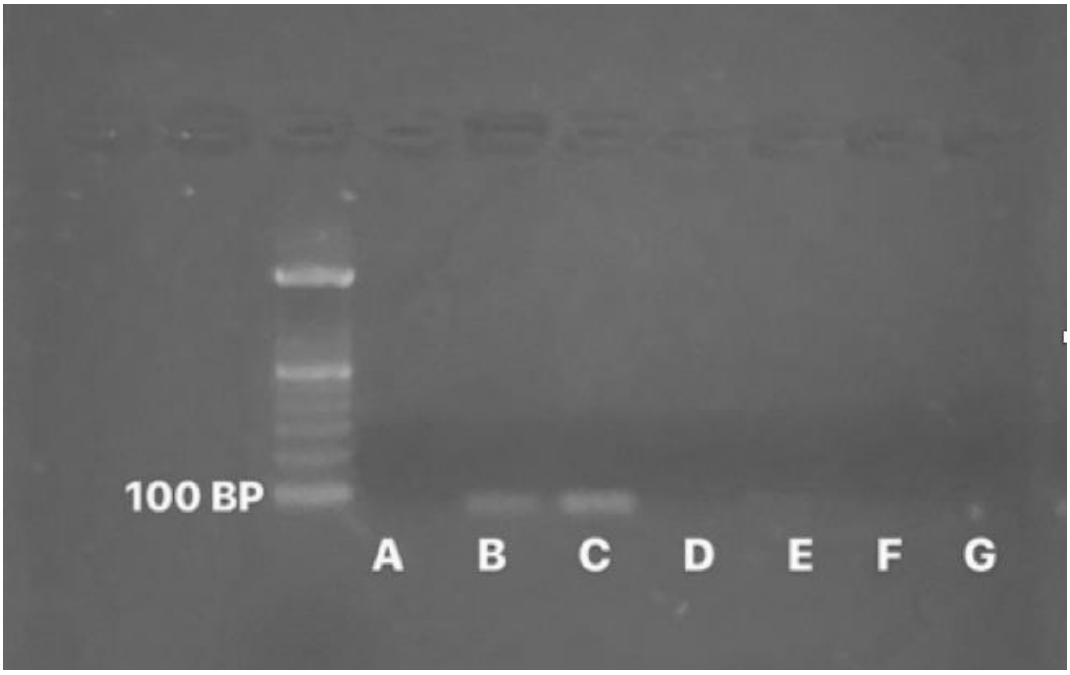


**Figure 5.** 159 bp region of the control region; A) Atlantic spotted dolphin, (B) aquarium water sample, (C) common bottlenose dolphin, (D) no template control, (E) freshwater, (F) pygmy sperm whale (G) sandbar shark.


**Figure 6**. 92 bp region of the *cytb* gene; (A) Common bottlenose dolphin, (B) Aquarium dolphin water sample, (C) pygmy sperm whale, (D) no template control, (E) freshwater, (F) Atlantic spotted dolphin, (G) sandbar shark.

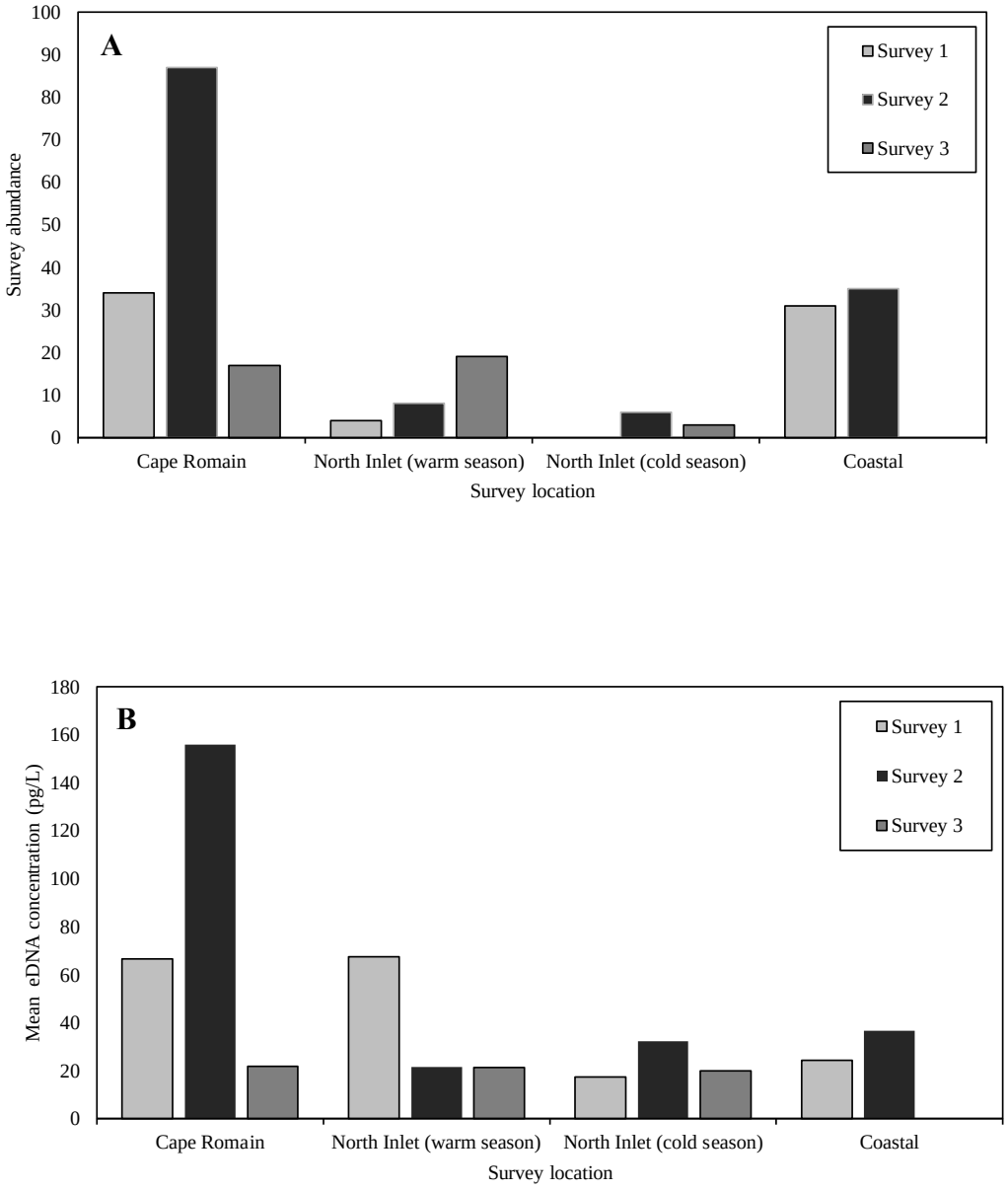


**Figure 7. (**A) Bar graph demonstrating the survey abundance variability per survey in four eDNA survey locations. (B) Bar graph demonstrating the mean eDNA concentration calculated from positive interval samples in four survey locations.

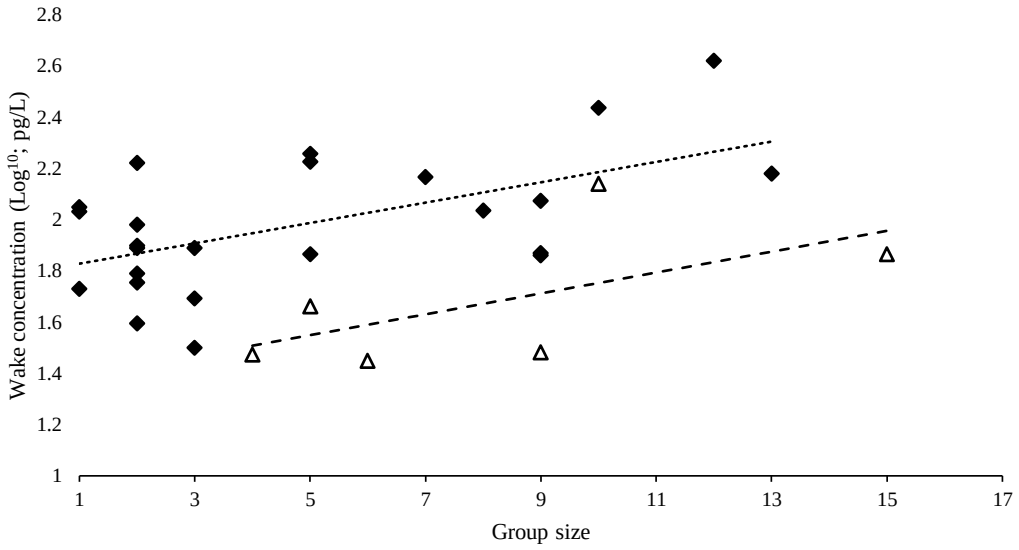


**Figure 8.** Correlation between wake sample eDNA concentration (expressed in $Log_{10}$ of estimated copies/L) and group size in salt marsh ($R_2$=0.32; y = 0.0397x + 1.787; ◆) and coastal environments ($R^2$ = 0.3604; y = 0.0407x + 1.3452; △). The relationship is significant in the estuarine environment (p=0.004) but not in the coastal environment (p=0.21).

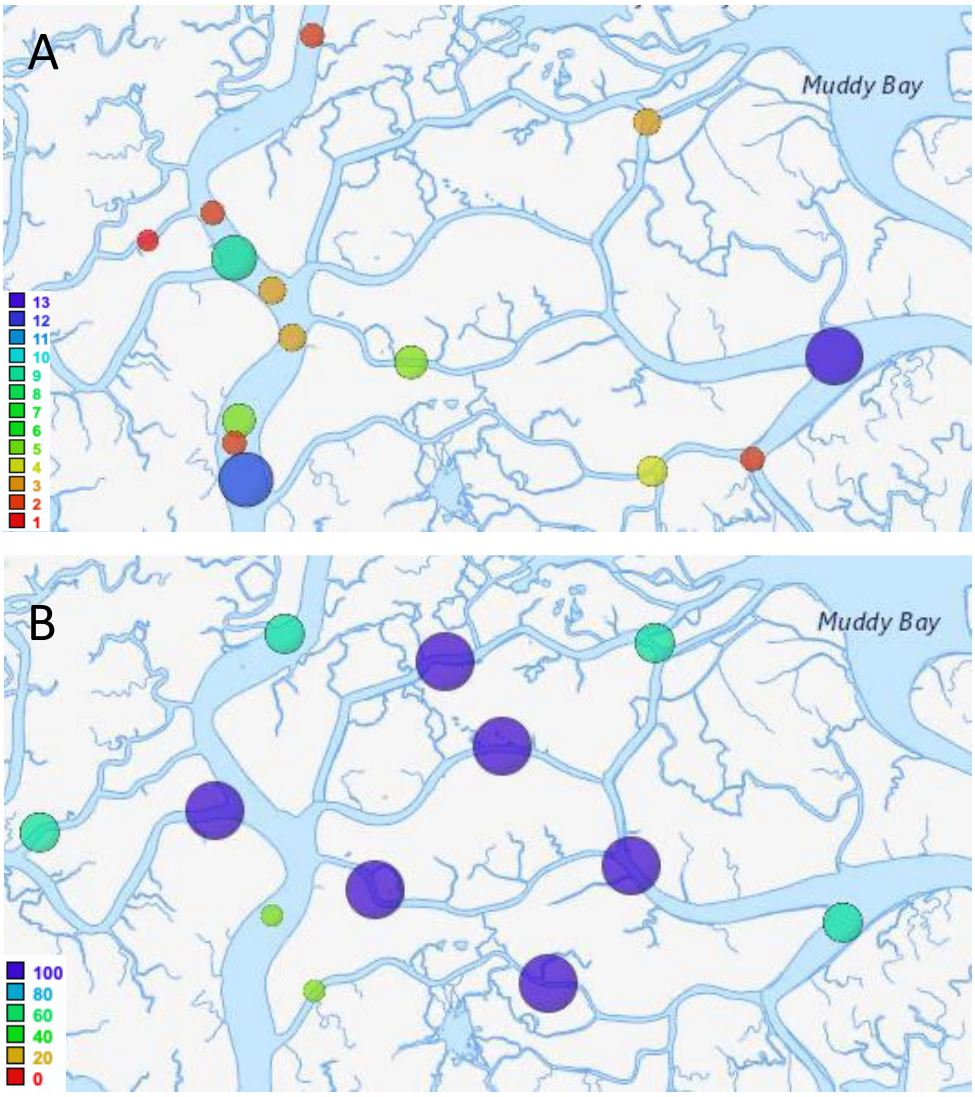


**Figure 9.** (A) Common bottlenose dolphin sightings in Cape Romain during the warm season. Dolphin sighting locations are represented by the colored dots, and the size and color of the dots reflects group size. (B) Positive eDNA detection rates of predetermined interval sites in Cape Romain during the warm season. Detection rates are represented by the size and color of circles.

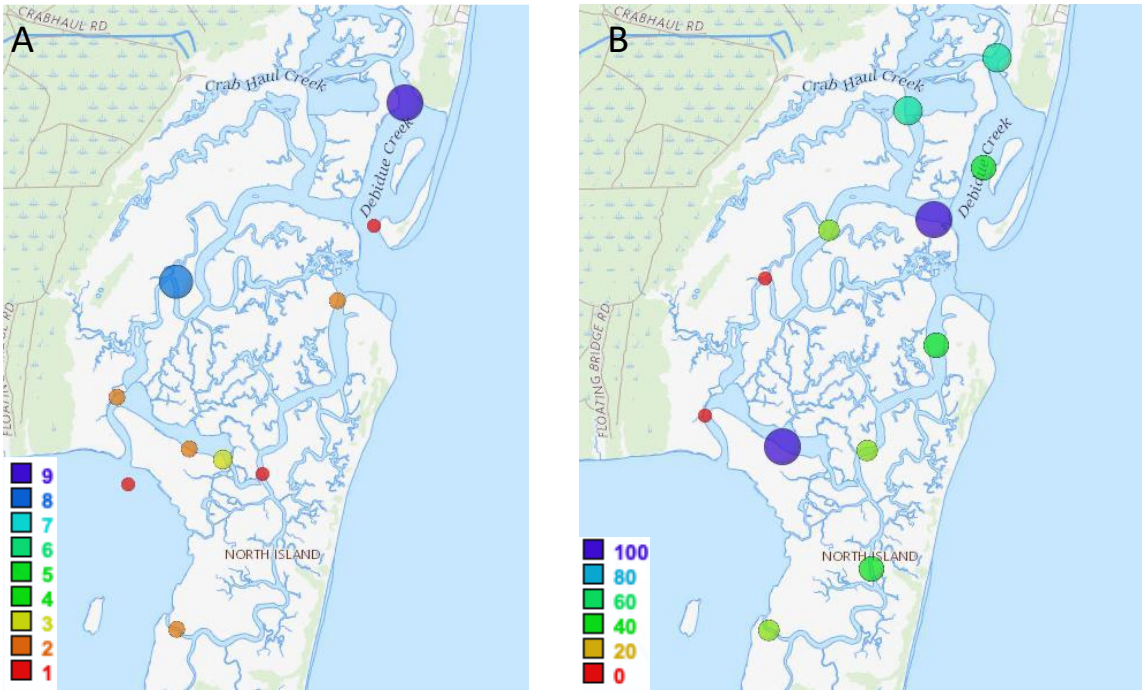


**Figure 10.** (A) Common bottlenose dolphin sightings in North Inlet during the warm season. Dolphin sighting locations are represented by the colored dots, and the size and color of the dots reflects group size. (B) Positive eDNA detection rates of predetermined interval sites in North Inlet during the warm season. Detection rates are represented by the size and color of circles.

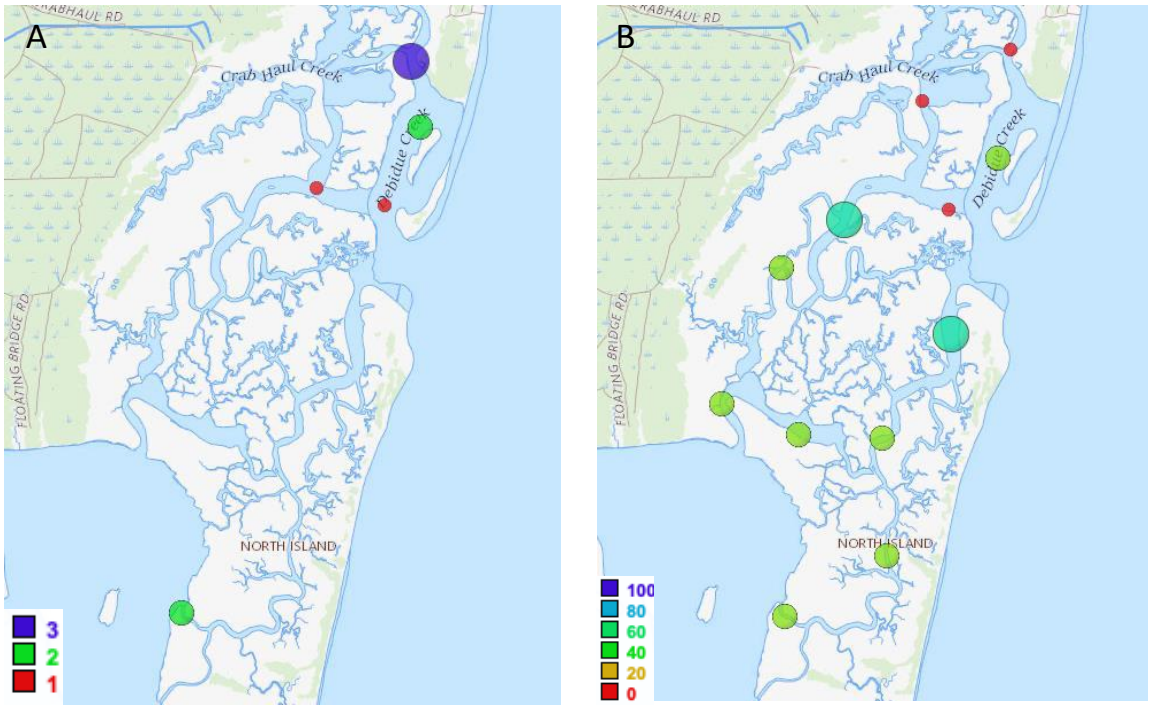


**Figure 11.** (A) Common bottlenose dolphin sightings in North Inlet during the cold season. Dolphin sighting locations are represented by the colored dots, and the size and color of the dots reflects group size. (B) Positive eDNA detection rates of predetermined interval sites in North Inlet during the cold season. Detection rates are represented by the size and color of circles.

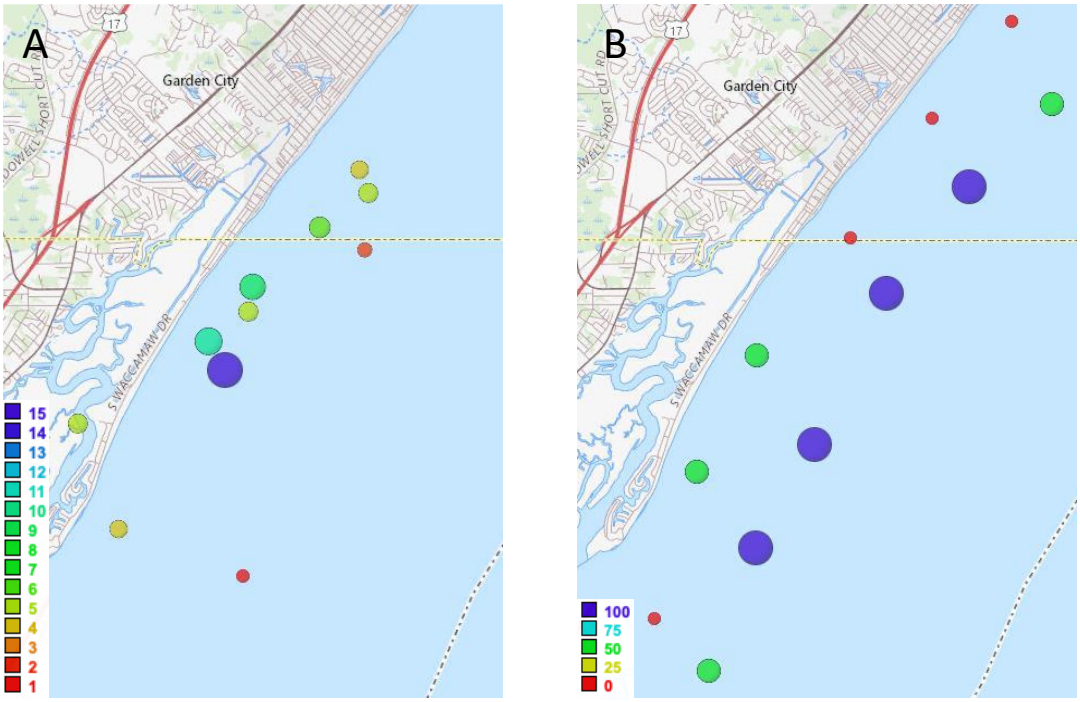


**Figure 12.** (A) Common bottlenose dolphin sightings in the coastal environment. Dolphin sighting locations are represented by the colored dots, and the size and color of the dots reflects group size. Predetermined interval sample sites are represented by gray dots. (B) Positive eDNA detection rates of predetermined interval sites in coastal environment. Detection rates are represented by the size and color of circles.

## REFERENCES


Adams, L. D., & Rosel, P. E. (2006). Population differentiation of the Atlantic spotted dolphin (*Stenella frontalis*) in the western North Atlantic, including the Gulf of Mexico. *Marine Biology*, *148*(3), 671-681.

Axtner, J., Crampton-Platt, A., Hörig, L. A., Mohamed, A., Xu, C. C., Yu, D. W., & Wilting, A. (2019). An efficient and robust laboratory workflow and tetrapod database for larger scale environmental DNA studies. *GigaScience*, *8*(4), giz029.

Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J. R., Maciejewski, A., & Wishart, D. S. (2016). Heatmapper: web-enabled heat mapping for all. *Nucleic acids research*, *44*(W1), W147-W153.

Baker, C. S., Steel, D., Nieukirk, S., & Klinck, H. (2018). Environmental DNA (eDNA) from the wake of the whales: droplet digital PCR for detection and species identification. *Frontiers in Marine Science*, *5*, 133.

Baldigo, B. P., Sporn, L. A., George, S. D., & Ball, J. A. (2017). Efficacy of environmental DNA to detect and quantify brook trout populations in headwater streams of the Adirondack Mountains, New York. *Transactions of the American Fisheries Society*, *146*(1), 99-111.

Balmer, B. C., Wells, R. S., Schwacke, L. H., Schwacke, J. H., Danielson, B., George, R. C., & Speakman, T. R. (2014). Integrating multiple techniques to identify stock boundaries of common bottlenose dolphins (*Tursiops truncatus*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(4), 511-521.

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1-17.

Beauclerc, K., Wozney, K., Smith, C., & Wilson, C. (2019). Development of quantitative PCR primers and probes for environmental DNA detection of amphibians in Ontario. *Conservation genetics resources*, *11*(1), 43-46.

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Douglas, W.Y.& De Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in ecology & evolution*, *29*(6), 358-367.

Brusa, J. L., Young, R. F., & Swanson, T. (2016). Abundance, ranging patterns, and social behavior of bottlenose dolphins (*Tursiops truncatus*) in an estuarine terminus. *Aquatic Mammals*, *42*(1), 109.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., & Vandesompele, J. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments.

Bylemans, J., Furlan, E. M., Gleeson, D. M., Hardy, C. M., & Duncan, R. P. (2018). Does size matter? An experimental evaluation of the relative abundance and decay rates of aquatic environmental DNA. *Environmental science & technology*, *52*(11), 6408-6416.

Cao, Y., Griffith, J. F., Dorevitch, S., & Weisberg, S. B. (2012). Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring Enterococcus in environmental waters. *Journal of applied microbiology*, *113*(1), 66-75.

Cristescu, M. E., & Hebert, P. D. (2018). Uses and misuses of environmental DNA in biodiversity science and conservation. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 209-230.

Dame, R. F., Spurrier, J. D., & Wolaver, T. G. (1989). Carbon, nitrogen and phosphorus processing by an oyster reef. *Marine Ecology Progress Series*, 249-256.

Dame, R., Alber, M., Allen, D., Mallin, M., Montague, C., Lewitus, A., Chalmers, A., Gardner, R., Gilman, C., Kjerfve, B., & Pinckney, J. (2000). Estuaries of the south Atlantic coast of North America: their geographical signatures. *Estuaries*, *23*(6), 793-819.

Davy, C. M., Kidd, A. G., & Wilson, C. C. (2015). Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. *PloS one*, *10*(7).

Deiner, K., Walser, J. C., Mächler, E., & Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*, *183*, 53-63.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., & Miaud, C. (2011). Persistence of environmental DNA in freshwater ecosystems. *PloS one*, *6*(8).

Díaz-Ferguson, E. E., & Moyer, G. R. (2014). History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. *Revista de biologia tropical*, *62*(4), 1273-1284.

Dizon, A. E., Lockyer, C., Perrin, W. F., Demaster, D. P., & Sisson, J. (1992). Rethinking the stock concept: a phylogeographic approach. *Conservation Biology*, *6*(1), 24-36.

Foote, A. D., Thomsen, P. F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L. A., Salling,A.B., Galatius, A., Orlando, L., & Gilbert, M. T. P. (2012). Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PloS one*, *7*(8).

Freeland, J. R. (2017). The importance of molecular markers and primer design when characterizing biodiversity from environmental DNA. *Genome*, *60*(4), 358-374.

Furlan, E. M., Gleeson, D., Hardy, C. M., & Duncan, R. P. (2016). A framework for estimating the sensitivity of eDNA surveys. *Molecular ecology resources*, *16*(3), 641-654.

Gibson, Q. A., & Mann, J. (2009). Do sampling method and sample size affect basic measures of dolphin sociality?. *Marine Mammal Science*, *25*(1), 187-198.

Gubbins, C. (2002). Use of home ranges by resident bottlenose dolphins (Tursiops truncatus) in a South Carolina estuary. *Journal of Mammalogy*, *83*(1), 178-187.

Harper, L. R., Griffiths, N. P., Lawson Handley, L., Sayer, C. D., Read, D. S., Harper, K. J., Blackman, R.C., Li, J., & Hänfling, B. (2019). Development and application of environmental DNA surveillance for the threatened crucian carp (*Carassius carassius*). *Freshwater biology*, *64*(1), 93-107.

Hicks, B. D., Aubin, D. J. S., Geraci, J. R., & Brown, W. R. (1985). Epidermal growth in the bottlenose dolphin, *Tursiops truncatus. Journal of Investigative Dermatology*, *85*(1), 60-63.

Hinlo, R., Furlan, E., Suitor, L., & Gleeson, D. (2017). Environmental DNA monitoring and management of invasive fish: comparison of eDNA and fyke netting. *Management of Biological Invasions*, *8*(1), 89.

Hooker, S. K. (2009). Toothed whales, overview. In *Encyclopedia of marine mammals* (pp. 1173-1179). Academic Press.

Hunter, M. E., Oyler-McCance, S. J., Dorazio, R. M., Fike, J. A., Smith, B. J., Hunter, C. T., Reed, R.N., & Hart, K. M. (2015). Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive Burmese pythons. *PloS one*, *10*(4).

Hunter, M. E., Dorazio, R. M., Butterfield, J. S., Meigs-Friend, G., Nico, L. G., & Ferrante, J. A. (2017). Detection limits of quantitative and digital PCR assays and their influence in presence–absence surveys of environmental DNA. *Molecular ecology resources*, *17*(2), 221-229.

Hunter, M. E., Meigs-Friend, G., Ferrante, J. A., Kamla, A. T., Dorazio, R. M., Diagne, L. K., Luna, F., Lanyon, J.M., & Reid, J. P. (2018). Surveys of environmental DNA (eDNA): a new approach to estimate occurrence in Vulnerable manatee populations. *Endangered Species Research*, *35*, 101-111.

Hupman, K., Stockin, K. A., Pollock, K., Pawley, M. D., Dwyer, S. L., Lea, C., & Tezanos-Pinto, G. (2018). Correction: Challenges of implementing Mark-recapture studies on poorly marked gregarious delphinids. *PloS one*, *13*(8), e0203356.

Irvine, A. B., Scott, M. D., Wells, R. S., & Kaufmann, J. H. (1981). Movements and activities of the Atlantic bottlenose dolphin, *Tursiops truncatus*, near Sarasota, Florida. *Fishery bulletin*, *79*(4), 671-688.

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B.H., & Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular ecology resources*, *15*(1), 216-227.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150-157.

Juen, A., & Traugott, M. (2006). Amplification facilitators and multiplex PCR: Tools to overcome PCR-inhibition in DNA-gut-content analysis of soil-living invertebrates. *Soil Biology and Biochemistry*, *38*(7), 1872-1879.

Kelly, R. P., Port, J. A., Yamahara, K. M., & Crowder, L. B. (2014a). Using environmental DNA to census marine fishes in a large mesocosm. *PloS one*, *9*(1).

Kelly, R. P., Port, J. A., Yamahara, K. M., Martone, R. G., Lowell, N., Thomsen, P. F., Mach, M.E., Bennett, M., Prahler, E., Caldwell, M.R., & Crowder, L. B. (2014b). Harnessing DNA to improve environmental management. *Science*, *344*(6191), 1455-1456.

Kelly, R. P., Shelton, A. O., & Gallego, R. (2019). Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. *Scientific reports*, *9*(1), 1-14.

Kjerfve, B., Miranda, L. B., & Wolanski, E. (1991). Modelling water circulation in an estuary and intertidal salt marsh system. *Netherlands Journal of Sea Research*, *28*(3), 141-147.

Kucklick, J., Schwacke, L., Wells, R., Hohn, A., Guichard, A., Yordy, J., Hansen, L., Zolman, E., Wilson, R., Litz, J., & Nowacek, D. (2011). Bottlenose dolphins as indicators of persistent organic pollutants in the western North Atlantic Ocean

and northern Gulf of Mexico. *Environmental science & technology*, *45*(10), 4270-4277.

Laska, D., Speakman, T., & Fair, P. A. (2011). Community overlap of bottlenose dolphins (*Tursiops truncatus*) found in coastal waters near Charleston, South Carolina. *J Mar Anim Ecol*, *4*, 10-18.

LeDuc, R. (2009). Delphinids, overview. In *Encyclopedia of marine mammals* (pp. 298-302). Academic Press.

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., Feder, J.L., Mahon, A.R., & Pfrender, M. E. (2012). Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. *Molecular ecology*, *21*(11), 2555-2558.

Lorenz, T. C. (2012). Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *JoVE (Journal of Visualized Experiments)*, (63), e3998.

Ma, H., Stewart, K., Lougheed, S., Zheng, J., Wang, Y., & Zhao, J. (2016). Characterization, optimization, and validation of environmental DNA (eDNA) markers to detect an endangered aquatic mammal. *Conservation Genetics Resources*, *8*(4), 561-568.

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., & Lopez, R. (2019). The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic acids research*, *47*(W1), W636-W641.

Majaneva, M., Diserud, O. H., Eagle, S. H., Boström, E., Hajibabaei, M., & Ekrem, T. (2018). Environmental DNA filtration techniques affect recovered biodiversity. *Scientific reports*, *8*(1), 1-11.

Minamoto, T., Honjo, M. N., Yamanaka, H., Uchii, K., & Kawabata, Z. I. (2012). Nationwide Cyprinid herpesvirus 3 contamination in natural rivers of Japan. *Research in veterinary science*, *93*(1), 508-514.

Moyer, G. R., Diaz-Ferguson, E., Hill, J. E., & Shea, C. (2014). Assessing environmental DNA detection in controlled lentic systems. *PloS one*, *9*(7).

Nathan, L. M., Simmons, M., Wegleitner, B. J., Jerde, C. L., & Mahon, A. R. (2014). Quantifying environmental DNA signals for aquatic invasive species across multiple detection platforms. *Environmental science & technology*, *48*(21), 12800-12806.

Noren, D. P., & Mocklin, J. A. (2012). Review of cetacean biopsy techniques: factors contributing to successful sample collection and physiological and behavioral impacts. *Marine Mammal Science*, *28*(1), 154-199.

Ojeda, G. Y., Gayes, P. T., Van Dolah, R. F., & Schwab, W. C. (2004). Spatially quantitative seafloor habitat mapping: example from the northern South Carolina inner continental shelf. *Estuarine, Coastal and Shelf Science*, *59*(3), 399-416.

Olson, Z. H., Briggler, J. T., & Williams, R. N. (2012). An eDNA approach to detect eastern hellbenders (*Cryptobranchus alleganiensis*) using samples of water. *Wildlife Research*, *39*(7), 629-636.

Parsons, K. M., Everett, M., Dahlheim, M., & Park, L. (2018). Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. *Royal Society open science*, *5*(8), 180537.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(8), 1123-1130.

Pinfield, R., Dillane, E., Runge, A. K. W., Evans, A., Mirimin, L., Niemann, J., Reed, T.E., Reid, D.G., Rogan, E., Samarra, F.I., & Sigsgaard, E. E. (2019). False-negative detections from environmental DNA collected in the presence of large numbers of killer whales (*Orcinus orca*). *Environmental DNA*, *1*(4), 316-328.

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., Yamahara, K.M., & Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular ecology*, *25*(2), 527-541.

Quintana-Rizzo, E., & Wells, R. S. (2001). Resighting and association patterns of bottlenose dolphins (Tursiops truncatus) in the Cedar Keys, Florida: insights into social organization. *Canadian Journal of Zoology*, *79*(3), 447-456.

Read, A. J., Urian, K. W., Wilson, B., & Waples, D. M. (2003). Abundance of bottlenose dolphins in the bays, sounds, and estuaries of North Carolina. *Marine Mammal Science*, *19*(1), 59-073.

Rice, D. W. (1998). Marine mammals of the world. *Systematics and distribution*.

Rosel, P. E., Hansen, L., & Hohn, A. A. (2009). Restricted dispersal in a continuously distributed marine species: common bottlenose dolphins *Tursiops truncatus* in coastal waters of the western North Atlantic. *Molecular Ecology*, *18*(24), 5030-5045.

Rosel, P. E., Wilcox, L. A., Sinclair, C., Speakman, T. R., Tumlin, M. C., Litz, J. A., & Zolman, E. S. (2017). Genetic assignment to stock of stranded common

bottlenose dolphins in southeastern Louisiana after the Deepwater Horizon oil spill. *Endangered Species Research, 33,* 221-234.

Roussel, J. M., Paillisson, J. M., Treguier, A., & Petit, E. (2015). The downside of eDNA as a survey tool in water bodies. *Journal of Applied Ecology, 52*(4), 823-826.

Salter, I., Joensen, M., Kristiansen, R., Steingrund, P., & Vestergaard, P. (2019). Environmental DNA concentrations are correlated with regional biomass of Atlantic cod in oceanic waters. *Communications biology, 2*(1), 1-9.

Santos, M. B., Pierce, G. J., Lopez, A., Reid, R. J., Ridoux, V., & Mente, E. (2006). Pygmy sperm whales *Kogia breviceps* in the Northeast Atlantic: New information on stomach contents and strandings. *Marine Mammal Science*, *22*(3), 600-616.

Santos-Neto, E. B., Azevedo-Silva, C. E., Bisi, T. L., Santos, J., Meirelles, A. C. O., Carvalho, V. L., Azevedo, A.F., Guimarães, J.E., & Lailson-Brito, J. (2014). Organochlorine concentrations (PCBs, DDTs, HCHs, HCB and MIREX) in delphinids stranded at the northeastern Brazil. *Science of the total environment*, *472*, 194-203.

Sawaya, N. A., Djurhuus, A., Closek, C. J., Hepner, M., Olesin, E., Visser, L., Kelble, C., Hubbard, K., & Breitbart, M. (2019). Assessing eukaryotic biodiversity in the Florida Keys National Marine Sanctuary through environmental DNA metabarcoding. *Ecology and evolution*, *9*(3), 1029-1040.

Schneider, A. (2013). GPS visualizer. *GPS Visualizer*

Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012). PCR inhibitors–occurrence, properties and removal. *Journal of applied microbiology, 113*(5), 1014-1026.

Schmelzle, M.C., & Kinziger, A. P. (2016). Using occupancy modelling to compare environmental DNA to traditional field methods for regional-scale monitoring of endangered aquatic species. *Molecular Ecology Resources,* (16(4), 895-908.

Sigsgaard, E. E., Nielsen, I. B., Bach, S. S., Lorenzen, E. D., Robinson, D. P., Knudsen, S. W., Pedersen, M.W., Al Jaidah, M., Orlando, L., Willerslev, E., & Møller, P. R. (2017). Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature ecology & evolution, 1*(1), 0004.

Silva, D. C. (2016). *Use of photo-identification and mark-recapture techniques to identify characteristics of the stock structure of coastal bottlenose dolphins, Tursiops truncatus off northern South Carolina* (Master's thesis, Coastal Carolina University).

Silva, D.C., Young, R.F., Lavin, A., O'Shea, CR.., & Murray, E. (2020). Abundance and seasonal distribution of the Southern North Carolina Estuarine System Stock of common bottlenose dolphins (*Tursiops truncatus*). *Journal of Cetacean Research and Management, 21,* 33-43.

Sloan, P. E. (2006). *Residency patterns, seasonality and habitat use among bottlenose dolphins, Tursiops truncatus, in the Cape Romain National Wildlife Refuge, SC* (Doctoral dissertation, University of North Carolina at Wilmington).

Speakman, T. R., Lane, S. M., Schwacke, L. H., Fair, P. A., & Zolman, E. S. (2010). Mark- recapture estimates of seasonal abundance and survivorship for bottlenose dolphins (*Tursiops truncatus*) near Charleston, South Carolina, USA. *Journal of Cetacean Research and Management, 11*(2), 153-162.

Stat, M., John, J., DiBattista, J. D., Newman, S. J., Bunce, M., & Harvey, E. S. (2019). Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology, 33*(1), 196-205.

Stewart, K., Ma, H., Zheng, J., & Zhao, J. (2017). Using environmental DNA to assess population-wide spatiotemporal reserve use. *Conservation Biology, 31*(5), 1173-1182.

Stoeckle, M. Y., Soboleva, L., & Charlop-Powers, Z. (2017). Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. *PloS one, 12*(4).

Strand, D. A., Holst-Jensen, A., Viljugrein, H., Edvardsen, B., Klaveness, D., Jussila, J., & Vrålstad, T. (2011). Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments. *Diseases of aquatic organisms, 95*(1), 9-17.

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation, 183*, 85-92.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. I. (2012). Estimation of fish biomass using environmental DNA. *PloS one, 7*(4).

Taylor, L. A., Eakins, B. W., Warnken, R. R., Carignan, K. S., Sharman, G. F., Schoolcraft, D. C., & Sloss, P. W. (2008). Digital elevation models of Myrtle Beach, South Carolina: procedures, data sources and analysis.

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS one, 7*(8).

Tingley, R., Greenlees, M., Oertel, S., van Rooyen, A. R., & Weeks, A. R. (2019). Environmental DNA sampling as a surveillance tool for cane toad Rhinella marina introductions on offshore islands. *Biological invasions, 21*(1), 1-6.

Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation, 183*, 93-102.

Waring, G.T., E. Josephson, K. Maze-Foley and P.E. Rosel. 2014. US Atlantic and Gulf of Mexico Marine Mammal Stock Assessments – 2013. NOAA Technical Memorandum NMFS-NE-228. 464 pp.

Waring, G.T., E. Josephson, K. Maze-Foley and P.E. Rosel. 2015. US Atlantic and Gulf of Mexico Marine Mammal Stock Assessments – 2014. NOAA Technical Memorandum NMFS-NE-231. 361 pp. doi:10.7289/V5TQ5ZH0.

Weber, L. I., Luca, M. J. D., Barreto, A. S., & Souza, T. T. D. (2007). Successful amplification of mitochondrial DNA from dentin of the bottlenose dolphin *Tursiops truncatus*. *Brazilian Archives of Biology and Technology*, *50*(1), 11-19.

Würsig, B., Jefferson, T. A., Hammond, P. S., Mizroch, S. A., & Donovan, G. P. (1990). Individual recognition of cetaceans: use of photo-identification and other techniques to estimate population parameters. *Report of the International Whaling Commission*, Special Issue 12, Cambridge.

Xiong, Y., Brandley, M. C., Xu, S., Zhou, K., & Yang, G. (2009). Seven new dolphin mitochondrial genomes and a time-calibrated phylogeny of whales. *BMC Evolutionary Biology*, *9*(1), 20.

Yamamoto, S., Minami, K., Fukaya, K., Takahashi, K., Sawada, H., Murakami, H., Tsuji, S., Hashizume, H., Kubonaga, S., Horiuchi, T., & Hongo, M. (2016). Environmental DNA as a 'snapshot'of fish distribution: A case study of Japanese jack mackerel in Maizuru Bay, Sea of Japan. *PLoS One, 11*(3).

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC bioinformatics, 13*(1), 134.

Young, R. F., & Phillips, H. D. (2002). Primary production required to support bottlenose dolphins in a salt marsh estuarine creek system. *Marine Mammal Science, 18*(2), 358-373.

## APPENDIX

**Table** 1. Interval sample concentration survey data. Reactions were considered negative (N) if concentrations are <0.001/reaction or inhibited (n/a).

| Location | Date | Total | Sample number | Interval sample | Latitude | Longitude | Reaction Concentration (pg) | Detection (Y/N) |
|---|---|---|---|---|---|---|---|---|
| North Inlet | 1/6/19 | 0 | 1 | 1 | 33.332 | -79.187 | 0 | N |
| North Inlet | 1/6/19 | 0 | 2 | 2 | 33.346 | -79.176 | 1.23E-02 | Y |
| North Inlet | 1/6/19 | 0 | 3 | 3 | 33.353 | -79.163 | 0 | N |
| North Inlet | 1/6/19 | 0 | 4 | 4 | 33.338 | -79.165 | 0 | N |
| North Inlet | 1/6/19 | 0 | 5 | 5 | 33.334 | -79.171 | 0 | N |
| North Inlet | 1/6/19 | 0 | 6 | 6 | 33.317 | -79.171 | 0 | N |
| North Inlet | 1/6/19 | 0 | 7 | 7 | 33.305 | -79.180 | 1.01E-02 | Y |
| North Inlet | 1/6/19 | 0 | 8 | 8 | 33.289 | -79.181 | 0 | N |
| North Inlet | 1/6/19 | 0 | 9 | 9 | 33.282 | -79.196 | 2.65E-02 | Y |
| North Inlet | 1/6/19 | 0 | 10 | 10 | 33.305 | -79.195 | 3.75E-02 | Y |
| North Inlet | 1/6/19 | 0 | 11 | 11 | 33.309 | -79.206 | 0 | N |
| North Inlet | 1/6/19 | 0 | 12 | 12 | 33.324 | -79.199 | 0 | N |
| North Inlet | 1/7/19 | 6 | 13 | 1 | 33.282 | -79.196 | 0 | N |
| North Inlet | 1/7/19 | 6 | 14 | 2 | 33.332 | -79.188 | 0 | N |
| North Inlet | 1/7/19 | 6 | 15 | 3 | 33.324 | -79.199 | 0 | N |
| North Inlet | 1/7/19 | 6 | 16 | 4 | 33.308 | -79.206 | 8.12E-02 | Y |
| North Inlet | 1/7/19 | 6 | 17 | 5 | 33.305 | -79.195 | 0 | N |
| North Inlet | 1/7/19 | 6 | 18 | 6 | 33.289 | -79.181 | 4.31E-02 | Y |
| North Inlet | 1/7/19 | 6 | 19 | 7 | 33.305 | -79.180 | 0 | N |
| North Inlet | 1/7/19 | 6 | 20 | 8 | 33.317 | -79.171 | 0 | N |
| North Inlet | 1/7/19 | 6 | 21 | 9 | 33.333 | -79.172 | 0 | N |
| North Inlet | 1/7/19 | 6 | 22 | 10 | 33.338 | -79.165 | 0 | N |
| North Inlet | 1/7/19 | 6 | 23 | 11 | 33.353 | -79.163 | 1.00E-02 | Y |
| North Inlet | 1/7/19 | 6 | 24 | 12 | 33.346 | -79.176 | 2.00E-02 | Y |
| North Inlet | 3/3/19 | 3 | 25 | 1 | 33.304 | -79.181 | 0 | N |
| North Inlet | 3/3/19 | 3 | 26 | 2 | 33.332 | -79.187 | 1.99E-02 | Y |
| North Inlet | 3/3/19 | 3 | 27 | 3 | 33.345 | -79.176 | 0 | N |
| North Inlet | 3/3/19 | 3 | 28 | 4 | 33.353 | -79.162 | 0 | N |
| North Inlet | 3/3/19 | 3 | 29 | 5 | 33.338 | -79.165 | 0 | N |
| North Inlet | 3/3/19 | 3 | 30 | 6 | 33.333 | -79.173 | 3.38E-02 | Y |
| North Inlet | 3/3/19 | 3 | 31 | 7 | 33.317 | -79.171 | 0 | N |
| North Inlet | 3/3/19 | 3 | 32 | 8 | 33.289 | -79.181 | 4.23E-02 | Y |
| North Inlet | 3/3/19 | 3 | 33 | 9 | 33.282 | -79.196 | 0 | N |
| North Inlet | 3/3/19 | 3 | 34 | 10 | 33.305 | -79.195 | 0 | N |
| North Inlet | 3/3/19 | 3 | 35 | 11 | 33.308 | -79.206 | 0 | N |
| North Inlet | 3/3/19 | 3 | 36 | 12 | 33.325 | -79.197 | 0 | N |
| North Inlet | 5/16/19 | 4 | 37 | 1 | 33.332 | -79.187 | 0 | N |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| North Inlet | 5/16/19 | 4 | 38 | 2 | 33.346 | -79.176 | 0 | N |
| North Inlet | 5/16/19 | 4 | 39 | 3 | 33.353 | -79.163 | 2.33E-02 | Y |
| North Inlet | 5/16/19 | 4 | 40 | 4 | 33.339 | -79.165 | 3.08E-02 | Y |
| North Inlet | 5/16/19 | 4 | 41 | 5 | 33.333 | -79.172 | 4.31E-02 | Y |
| North Inlet | 5/16/19 | 4 | 42 | 6 | 33.317 | -79.171 | 1.27E-02 | Y |
| North Inlet | 5/16/19 | 4 | 43 | 7 | 33.304 | -79.181 | 2.99E-02 | Y |
| North Inlet | 5/16/19 | 4 | 44 | 8 | 33.289 | -79.181 | 4.92E-02 | Y |
| North Inlet | 5/16/19 | 4 | 45 | 9 | 33.282 | -79.196 | 0 | N |
| North Inlet | 5/16/19 | 4 | 46 | 10 | 33.304 | -79.194 | 2.30E+00 | Y |
| North Inlet | 5/16/19 | 4 | 47 | 11 | 33.308 | -79.206 | 0 | N |
| North Inlet | 5/16/19 | 4 | 48 | 12 | 33.325 | -79.198 | 0 | N |
| Cape Romain | 6/7/19 | 34 | 49 | 1 | 33.052 | -79.473 | 3.14E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 50 | 2 | 33.030 | -79.474 | n/a | N |
| Cape Romain | 6/7/19 | 34 | 51 | 3 | 33.024 | -79.470 | 3.05E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 52 | 4 | 33.025 | -79.448 | 3.02E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 53 | 5 | 33.030 | -79.421 | 3.04E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 54 | 6 | 33.034 | -79.440 | 2.00E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 55 | 7 | 33.032 | -79.464 | 4.00E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 56 | 8 | 33.044 | -79.452 | 2.84E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 57 | 9 | 33.052 | -79.438 | 1.23E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 58 | 10 | 33.050 | -79.458 | 1.16E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 59 | 11 | 33.039 | -79.479 | 4.27E-02 | Y |
| Cape Romain | 6/7/19 | 34 | 60 | 12 | 33.037 | -79.496 | 1.60E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 61 | 1 | 33.030 | -79.474 | 1.05E-01 | Y |
| Cape Romain | 6/14/19 | 87 | 62 | 2 | 33.025 | -79.469 | 3.20E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 63 | 3 | 33.025 | -79.446 | 9.01E-03 | N |
| Cape Romain | 6/14/19 | 87 | 64 | 4 | 33.024 | -79.428 | 3.69E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 65 | 5 | 33.028 | -79.422 | 5.29E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 66 | 6 | 33.034 | -79.440 | 1.48E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 67 | 7 | 33.032 | -79.464 | 3.19E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 68 | 8 | 33.043 | -79.450 | 3.21E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 69 | 9 | 33.052 | -79.438 | 1.75E-03 | N |
| Cape Romain | 6/14/19 | 87 | 70 | 10 | 33.049 | -79.462 | 2.57E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 71 | 11 | 33.037 | -79.485 | 8.64E-02 | Y |
| Cape Romain | 6/14/19 | 87 | 72 | 12 | 33.040 | -79.490 | 3.31E-01 | Y |
| Cape Romain | 6/18/19 | 17 | 73 | 1 | 33.051 | -79.473 | 0 | N |
| Cape Romain | 6/18/19 | 17 | 74 | 2 | 33.031 | -79.473 | 0 | N |
| Cape Romain | 6/18/19 | 17 | 75 | 3 | 33.025 | -79.469 | 0 | N |
| Cape Romain | 6/18/19 | 17 | 76 | 4 | 33.025 | -79.445 | 1.62E-02 | Y |
| Cape Romain | 6/18/19 | 17 | 77 | 5 | 33.028 | -79.422 | 0 | N |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cape Romain | 6/18/19 | 17 | 78 | 6 | 33.034 | -79.440 | 1.01E-02 | Y |
| Cape Romain | 6/18/19 | 17 | 79 | 7 | 33.031 | -79.456 | 1.13E-02 | Y |
| Cape Romain | 6/18/19 | 17 | 80 | 8 | 33.044 | -79.453 | 1.87E-02 | Y |
| Cape Romain | 6/18/19 | 17 | 81 | 9 | 33.052 | -79.438 | 1.20E-02 | Y |
| Cape Romain | 6/18/19 | 17 | 82 | 10 | 33.049 | -79.462 | 1.43E-02 | Y |
| Cape Romain | 6/18/19 | 17 | 83 | 11 | 33.038 | -79.482 | 1.32E-02 | Y |
| Cape Romain | 6/18/19 | 17 | 84 | 12 | 33.038 | -79.491 | 0 | N |
| North Inlet | 6/21/19 | 8 | 85 | 1 | 33.332 | -79.187 | 2.53E-02 | Y |
| North Inlet | 6/21/19 | 8 | 86 | 2 | 33.346 | -79.176 | 0 | N |
| North Inlet | 6/21/19 | 8 | 87 | 3 | 33.353 | -79.163 | 0 | N |
| North Inlet | 6/21/19 | 8 | 88 | 4 | 33.339 | -79.164 | n/a | N |
| North Inlet | 6/21/19 | 8 | 89 | 5 | 33.318 | -79.171 | n/a | N |
| North Inlet | 6/21/19 | 8 | 90 | 6 | 33.333 | -79.173 | n/a | N |
| North Inlet | 6/21/19 | 8 | 91 | 7 | 33.304 | -79.181 | 0 | N |
| North Inlet | 6/21/19 | 8 | 92 | 8 | 33.289 | -79.181 | 0 | N |
| North Inlet | 6/21/19 | 8 | 93 | 9 | 33.282 | -79.196 | 2.03E-02 | Y |
| North Inlet | 6/21/19 | 8 | 94 | 10 | 33.305 | -79.195 | 2.30E-02 | Y |
| North Inlet | 6/21/19 | 8 | 95 | 11 | 33.305 | -79.203 | 0 | N |
| North Inlet | 6/21/19 | 8 | 96 | 12 | 33.324 | -79.199 | 0 | N |
| North Inlet | 7/12/19 | 19 | 97 | 1 | 33.332 | -79.188 | 1.78E-02 | Y |
| North Inlet | 7/12/19 | 19 | 98 | 2 | 33.346 | -79.176 | 2.01E-02 | Y |
| North Inlet | 7/12/19 | 19 | 99 | 3 | 33.353 | -79.162 | 1.60E-02 | Y |
| North Inlet | 7/12/19 | 19 | 100 | 4 | 33.339 | -79.165 | 0 | N |
| North Inlet | 7/12/19 | 19 | 101 | 5 | 33.333 | -79.172 | 7.39E+00 | Y |
| North Inlet | 7/12/19 | 19 | 102 | 6 | 33.318 | -79.171 | 0 | N |
| North Inlet | 7/12/19 | 19 | 103 | 7 | 33.304 | -79.181 | 0 | N |
| North Inlet | 7/12/19 | 19 | 104 | 8 | 33.289 | -79.181 | n/a | N |
| North Inlet | 7/12/19 | 19 | 105 | 9 | 33.281 | -79.196 | 0 | N |
| North Inlet | 7/12/19 | 19 | 106 | 10 | 33.305 | -79.195 | 1.43E-02 | Y |
| North Inlet | 7/12/19 | 19 | 107 | 11 | 33.308 | -79.206 | n/a | N |
| North Inlet | 7/12/19 | 19 | 108 | 12 | 33.324 | -79.199 | n/a | N |
| Coastal | 11/5/19 | 35 | 109 | 1 | 33.522 | -79.024 | 1.44E-02 | Y |
| Coastal | 11/5/19 | 35 | 110 | 2 | 33.523 | -79.024 | 0 | N |
| Coastal | 11/5/19 | 35 | 111 | 3 | 33.542 | -79.017 | 0 | N |
| Coastal | 11/5/19 | 35 | 112 | 4 | 33.558 | -79.008 | 0 | N |
| Coastal | 11/5/19 | 35 | 113 | 5 | 33.573 | -78.993 | 0 | N |
| Coastal | 11/5/19 | 35 | 114 | 6 | 33.589 | -78.980 | 0 | N |
| Coastal | 11/5/19 | 35 | 115 | 7 | 33.601 | -78.967 | 1.17E-02 | Y |
| Coastal | 11/5/19 | 35 | 116 | 8 | 33.591 | -78.961 | 1.22E-02 | Y |
| Coastal | 11/5/19 | 35 | 117 | 9 | 33.580 | -78.974 | 2.19E-02 | Y |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Coastal | 11/5/19 | 35 | 118 | 10 | 33.566 | -78.987 | 1.38E-02 | Y |
| Coastal | 11/5/19 | 35 | 119 | 11 | 33.546 | -78.999 | 2.37E-02 | Y |
| Coastal | 11/5/19 | 35 | 120 | 12 | 33.532 | -79.008 | 1.97E-02 | Y |
| Coastal | 11/21/19 | 31 | 121 | 1 | 33.516 | -79.015 | 0 | N |
| Coastal | 11/21/19 | 31 | 122 | 2 | 33.535 | -79.020 | 0 | N |
| Coastal | 11/21/19 | 31 | 123 | 3 | 33.546 | -79.011 | 2.06E-02 | Y |
| Coastal | 11/21/19 | 31 | 124 | 4 | 33.565 | -78.998 | 3.86E-02 | Y |
| Coastal | 11/21/19 | 31 | 125 | 5 | 33.577 | -78.985 | 0 | N |
| Coastal | 11/21/19 | 31 | 126 | 6 | 33.593 | -78.971 | 0 | N |
| Coastal | 11/21/19 | 31 | 127 | 7 | 33.590 | -78.953 | 0 | N |
| Coastal | 11/21/19 | 31 | 128 | 8 | 33.576 | -78.966 | 3.75E-02 | Y |
| Coastal | 11/21/19 | 31 | 129 | 9 | 33.558 | -78.979 | 1.44E-02 | Y |
| Coastal | 11/21/19 | 31 | 130 | 10 | 33.539 | -78.994 | 2.18E-02 | Y |
| Coastal | 11/21/19 | 31 | 131 | 11 | 33.522 | -79.005 | 2.15E-02 | Y |
| Coastal | 11/21/19 | 31 | 132 | 12 | 33.509 | -79.020 | 2.11E-02 | Y |

**Table 2**. Wake concentration survey data. Reactions were considered negative (N) if concentrations are <.001/reaction or inhibited (n/a).

| Location | Date | Sample number | Latitude | Longitude | Group size estimate | Reaction Concentration (pg) | Detection (Y/N) |
|---|---|---|---|---|---|---|---|
| North Inlet | 1/7/19 | 1 | 33.334 | -79.166 | 1 | 2.15E-02 | Y |
| North Inlet | 1/7/19 | 2 | 33.343 | -79.162 | 2 | 3.16E-02 | Y |
| North Inlet | 1/7/19 | 3 | 33.351 | -79.163 | 3 | 0 | N |
| North Inlet | 3/3/19 | 4 | 33.336 | -79.177 | 1 | 0 | N |
| North Inlet | 3/3/19 | 5 | 33.283 | -79.197 | 2 | 3.82E-02 | Y |
| North Inlet | 5/16/19 | 6 | 33.323 | -79.172 | 2 | 1.57E-02 | Y |
| North Inlet | 5/16/19 | 7 | 33.282 | -79.196 | 2 | 3.09E-02 | Y |
| Cape Romain | 6/7/19 | 8 | 33.041 | -79.546 | 2 | 2.27E-02 | Y |
| Cape Romain | 6/7/19 | 9 | 33.043 | -79.479 | 2 | 2.47E-02 | Y |
| Cape Romain | 6/7/19 | 10 | 33.040 | -79.477 | 9 | 2.96E-02 | Y |
| Cape Romain | 6/7/19 | 11 | 33.038 | -79.476 | 9 | 2.90E-02 | Y |
| Cape Romain | 6/7/19 | 12 | 33.033 | -79.471 | 3 | 1.26E-02 | Y |
| Cape Romain | 6/7/19 | 13 | 33.027 | -79.476 | 5 | 2.93E-02 | Y |
| Cape Romain | 6/7/19 | 14 | 33.050 | -79.438 | 3 | 1.97E-02 | Y |
| Cape Romain | 6/14/19 | 15 | 33.022 | -79.476 | 5 | 7.22E-02 | Y |
| Cape Romain | 6/14/19 | 16 | 33.018 | -79.475 | 12 | 1.67E-01 | Y |
| Cape Romain | 6/14/19 | 17 | 33.024 | -79.428 | 2 | 3.23E-03 | N |
| Cape Romain | 6/14/19 | 18 | 33.032 | -79.420 | 13 | 6.03E-02 | Y |
| Cape Romain | 6/14/19 | 19 | 33.031 | -79.426 | 10 | 1.09E-01 | Y |
| Cape Romain | 6/14/19 | 20 | 33.031 | -79.460 | 5 | 6.72E-02 | Y |
| Cape Romain | 6/14/19 | 21 | 33.037 | -79.473 | 3 | 3.09E-02 | Y |
| Cape Romain | 6/18/19 | 22 | 33.057 | -79.470 | 2 | 6.66E-02 | Y |
| Cape Romain | 6/18/19 | 23 | 33.025 | -79.477 | 7 | 5.87E-02 | Y |
| Cape Romain | 6/18/19 | 24 | 33.023 | -79.437 | 4 | 9.30E-03 | N |
| Cape Romain | 6/18/19 | 25 | 33.041 | -79.485 | 1 | 4.47E-02 | Y |
| North Inlet | 6/21/19 | 26 | 33.300 | -79.203 | 1 | 8.47E-03 | N |
| North Inlet | 6/21/19 | 27 | 33.311 | -79.205 | 2 | n/a | n/a |
| North Inlet | 6/21/19 | 28 | 33.304 | -79.194 | 2 | n/a | n/a |
| North Inlet | 6/21/19 | 29 | 33.303 | -79.189 | 3 | 3.77E-03 | N |
| North Inlet | 7/12/19 | 30 | 33.332 | -79.166 | 1 | 4.29E-02 | Y |
| North Inlet | 7/12/19 | 31 | 33.347 | -79.162 | 9 | 4.72E-02 | Y |

**Table 3.** Physical data (temperature, salinity) for interval sample collections. N/A indicates that the data were not recorded for an event.

| Location | Date | Interval sample | Temperature °C | Salinity |
|---|---|---|---|---|
| North Inlet | 1/6/19 | 1 | 13 | 16.8 |
| North Inlet | 1/6/19 | 2 | 13.4 | 23.8 |
| North Inlet | 1/6/19 | 3 | 13.3 | 20.8 |
| North Inlet | 1/6/19 | 4 | 13.3 | 24.6 |
| North Inlet | 1/6/19 | 5 | 13.3 | 24.5 |
| North Inlet | 1/6/19 | 6 | 13.2 | 15.6 |
| North Inlet | 1/6/19 | 7 | 13.3 | 8.1 |
| North Inlet | 1/6/19 | 8 | 13.4 | 1 |
| North Inlet | 1/6/19 | 9 | 12.9 | 0.3 |
| North Inlet | 1/6/19 | 10 | 13.5 | 1.3 |
| North Inlet | 1/6/19 | 11 | 12.7 | 1.8 |
| North Inlet | 1/6/19 | 12 | 13.7 | 2.1 |
| North Inlet | 1/7/19 | 1 | n/a | n/a |
| North Inlet | 1/7/19 | 2 | n/a | n/a |
| North Inlet | 1/7/19 | 3 | n/a | n/a |
| North Inlet | 1/7/19 | 4 | n/a | n/a |
| North Inlet | 1/7/19 | 5 | n/a | n/a |
| North Inlet | 1/7/19 | 6 | n/a | n/a |
| North Inlet | 1/7/19 | 7 | n/a | n/a |
| North Inlet | 1/7/19 | 8 | n/a | n/a |
| North Inlet | 1/7/19 | 9 | n/a | n/a |
| North Inlet | 1/7/19 | 10 | n/a | n/a |
| North Inlet | 1/7/19 | 11 | n/a | n/a |
| North Inlet | 1/7/19 | 12 | n/a | n/a |
| North Inlet | 3/3/19 | 1 | 15.7 | 28.4 |
| North Inlet | 3/3/19 | 2 | 15.9 | 29.9 |
| North Inlet | 3/3/19 | 3 | 16.3 | 29.4 |
| North Inlet | 3/3/19 | 4 | 15.7 | 30.5 |
| North Inlet | 3/3/19 | 5 | 15.6 | 29.5 |
| North Inlet | 3/3/19 | 6 | 15.8 | 25.3 |
| North Inlet | 3/3/19 | 7 | 16.4 | 19.3 |
| North Inlet | 3/3/19 | 8 | 17 | 11.8 |
| North Inlet | 3/3/19 | 9 | 16.2 | 6.6 |
| North Inlet | 3/3/19 | 10 | 17 | 10.8 |
| North Inlet | 3/3/19 | 11 | 16.8 | 9 |
| North Inlet | 3/3/19 | 12 | 17.1 | 12.2 |
| North Inlet | 5/16/19 | 1 | 22.59 | 33 |
| North Inlet | 5/16/19 | 2 | 22.7 | 33.8 |
| North Inlet | 5/16/19 | 3 | 22.88 | 33.44 |

| | | | | |
|---|---|---|---|---|
| Cape Romain | 6/18/19 | 8 | 28.9 | 28.52 |
| Cape Romain | 6/18/19 | 9 | 29.27 | 27.6 |
| Cape Romain | 6/18/19 | 10 | 29.22 | 28.08 |
| Cape Romain | 6/18/19 | 11 | 29.21 | 29.54 |
| Cape Romain | 6/18/19 | 12 | 29.22 | 28.53 |
| North Inlet | 6/21/19 | 1 | 27.29 | 27.45 |
| North Inlet | 6/21/19 | 2 | 26.68 | 32.15 |
| North Inlet | 6/21/19 | 3 | 26.93 | 31.45 |
| North Inlet | 6/21/19 | 4 | 26.69 | 34.64 |
| North Inlet | 6/21/19 | 5 | 26.4 | 34.83 |
| North Inlet | 6/21/19 | 6 | 26.49 | 35 |
| North Inlet | 6/21/19 | 7 | 26.58 | 32.99 |
| North Inlet | 6/21/19 | 8 | 27.34 | 25.29 |
| North Inlet | 6/21/19 | 9 | 26.79 | 5.39 |
| North Inlet | 6/21/19 | 10 | 26.79 | 5.39 |
| North Inlet | 6/21/19 | 11 | 27.87 | 4.36 |
| North Inlet | 6/21/19 | 12 | 27.66 | 30.95 |
| North Inlet | 7/12/19 | 1 | 28.9 | 34.58 |
| North Inlet | 7/12/19 | 2 | 28.51 | 34.82 |
| North Inlet | 7/12/19 | 3 | 29.01 | 33.5 |
| North Inlet | 7/12/19 | 4 | 28.91 | 34.48 |
| North Inlet | 7/12/19 | 5 | 29.61 | 34.55 |
| North Inlet | 7/12/19 | 6 | 28.91 | 33 |
| North Inlet | 7/12/19 | 7 | 29.01 | 28.06 |
| North Inlet | 7/12/19 | 8 | 29.67 | 23.51 |
| North Inlet | 7/12/19 | 9 | 34.55 | 15.38 |
| North Inlet | 7/12/19 | 10 | 30.36 | 24.06 |
| North Inlet | 7/12/19 | 11 | 30.65 | 12.93 |
| North Inlet | 7/12/19 | 12 | 30.63 | 21.57 |
| Coastal | 11/5/19 | 1 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 2 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 3 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 4 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 5 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 6 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 7 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 8 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 9 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 10 | 19.88 | 35.26 |
| Coastal | 11/5/19 | 11 | 19.88 | 35.26 |

| | | | | |
|---|---|---|---|---|
| Coastal | 11/5/19 | 12 | 19.88 | 35.26 |
| Coastal | 11/21/19 | 1 | 13.79 | 35.32 |
| Coastal | 11/21/19 | 2 | 13.64 | 35.29 |
| Coastal | 11/21/19 | 3 | 13.79 | 35.27 |
| Coastal | 11/21/19 | 4 | 13.76 | 35.25 |
| Coastal | 11/21/19 | 5 | 13.7 | 35.25 |
| Coastal | 11/21/19 | 6 | 13.99 | 35.18 |
| Coastal | 11/21/19 | 7 | 14.09 | 35.25 |
| Coastal | 11/21/19 | 8 | 14.18 | 35.15 |
| Coastal | 11/21/19 | 9 | 14.29 | 34.87 |
| Coastal | 11/21/19 | 10 | 14.21 | 35.14 |
| Coastal | 11/21/19 | 11 | 14.42 | 35.16 |
| Coastal | 11/21/19 | 12 | 13.85 | 35.15 |

**Table 4.** Physical data (temperature, salinity, and whether the group was traveling with the current) and behavior observations for wake sample collections. N/A indicates that the data was not recorded for an eve

| Location | Date | Sample number | Group size estimate | Temperature (C) | Salinity | Behavior | Travelir current |
|---|---|---|---|---|---|---|---|
| North Inlet | 1/7/19 | 1 | 1 | n/a | n/a | | Y |
| North Inlet | 1/7/19 | 2 | 2 | n/a | n/a | | N |
| North Inlet | 1/7/19 | 3 | 3 | n/a | n/a | | N |
| North Inlet | 3/3/19 | 4 | 1 | 15.4 | 30.2 | | Y |
| North Inlet | 3/3/19 | 5 | 2 | 16.2 | 6.6 | | N |
| North Inlet | 5/16/19 | 6 | 2 | 22.81 | 33.7 | | Y |
| North Inlet | 5/16/19 | 7 | 2 | 22.95 | 16.94 | | Y |
| Cape Romain | 6/7/19 | 8 | 2 | 26.84 | 35.4 | | N |
| Cape Romain | 6/7/19 | 9 | 2 | 26.1 | 35.33 | | N |
| Cape Romain | 6/7/19 | 10 | 9 | 26.7 | 35.3 | surface active | N |
| Cape Romain | 6/7/19 | 11 | 9 | 26.7 | 35.3 | Physical interaction | Y |
| Cape Romain | 6/7/19 | 12 | 3 | 26.83 | 35.3 | | N |
| Cape Romain | 6/7/19 | 13 | 5 | 26.7 | 35.3 | | N/A |
| Cape Romain | 6/7/19 | 14 | 3 | 27.05 | 35.19 | | N/A |
| Cape Romain | 6/14/19 | 15 | 5 | 25.48 | 29.54 | | Y |
| Cape Romain | 6/14/19 | 16 | 12 | 25.48 | 29.54 | porpoising | Y |
| Cape Romain | 6/14/19 | 17 | 2 | 25.46 | 31.17 | | N |
| Cape Romain | 6/14/19 | 18 | 3 | 25.6 | 29.33 | | Y |
| Cape Romain | 6/14/19 | 19 | 10 | 25.6 | 29.33 | surface active | N/A |
| Cape Romain | 6/14/19 | 20 | 5 | 25.9 | 29.34 | very evasive | N/A |
| Cape Romain | 6/14/19 | 21 | 3 | 26.46 | 23.18 | fin slapping | N/A |
| Cape Romain | 6/18/19 | 22 | 2 | 28.35 | 30.87 | | Y |
| Cape Romain | 6/18/19 | 23 | 7 | 28.47 | 31.86 | surface active | Y |
| Cape Romain | 6/18/19 | 24 | 4 | 29.1 | 30.3 | | N |
| Cape Romain | 6/18/19 | 25 | 1 | 29.29 | 27.95 | | Y |
| North Inlet | 6/21/19 | 26 | 1 | 27.57 | 2.99 | | N |
| North Inlet | 6/21/19 | 27 | 2 | 28.34 | 12.38 | | Y |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| North Inlet | 6/21/19 | 28 | 2 | 29.26 | 18.07 | | Y |
| North Inlet | 6/21/19 | 29 | 3 | 29.26 | 18.07 | | N |
| North Inlet | 7/12/19 | 30 | 1 | 28.78 | 34.86 | | N/A |
| North Inlet | 7/12/19 | 31 | 9 | 24.08 | 32.9 | foraging | N/A |
| North Inlet | 7/12/19 | 32 | 1 | 29.25 | 26.57 | | N |
| North Inlet | 7/12/19 | 33 | 8 | 30.64 | 21.8 | | Y |
| Coastal | 11/5/19 | 34 | 1 | 19.88 | 35.26 | | N/A |
| Coastal | 11/5/19 | 35 | 4 | 19.88 | 35.26 | | N/A |
| Coastal | 11/5/19 | 36 | 10 | 19.88 | 35.26 | spread out | N/A |
| Coastal | 11/5/19 | 37 | 15 | 19.88 | 35.26 | spread out | N/A |
| Coastal | 11/5/19 | 38 | 5 | 19.88 | 35.26 | | N/A |
| Coastal | 11/21/19 | 39 | 2 | 12.92 | 34.65 | | N/A |
| Coastal | 11/21/19 | 40 | 5 | 14.52 | 35.25 | | N/A |
| Coastal | 11/21/19 | 41 | 6 | 14.4 | 35.25 | | N/A |
| Coastal | 11/21/19 | 42 | 4 | 14.37 | 35.15 | | N/A |
| Coastal | 11/21/19 | 43 | 5 | 14.37 | 35.15 | | N/A |
| Coastal | 11/21/19 | 44 | 9 | 14.15 | 35.24 | physical interaction | N/A |

**Table 5.** Model summary regression analysis of number of dolphins in a group and concentration of eDNA in the wake of the group. eDNA concentrations in the wake of dolphins is moderately correlated by the number of dolphins in the group

**Table 6.** Shapiro-Wilk normality test of normality $\log_{10}$ transformed eDNA concentrations group by location

| Location | Statistic | Degrees of freedom | Significance |
|---|---|---|---|
| Cape Romain | 0.898 | 44 | 0.003 |
| North Inlet (warm season) | 0.932 | 18 | 0.610 |
| North Inlet (cold season) | 0.949 | 14 | 0.629 |
| Coastal | 0.912 | 20 | 0.167 |