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Site Fidelity of Bottlenose Dolphins (Tursiops truncatus) Using Elemental Analyses and Photo-Identification

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SITE FIDELITY OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) USING ELEMENTAL ANALYSES AND PHOTO-IDENTIFICATION

A Thesis

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by
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LIST OF SYMBOLS AND ABBREVIATIONS

AIC: Akaike information criterion

ANOVA: Analysis of variance

As: Arsenic

Ba: Barium

Cd: Cadmium

Co: Cobalt

Cr: Chromium

Cu: Copper

δ: Delta

EPA: Environmental Protection Agency

EMSS: Eastern Mississippi Sound

Fe: Iron

HWI: Half weight index

IACUC: Institutional Animal Care and Use Committee

ICW: Intercoastal Waterway

LA-ICP-MS: Laser ablation inductively coupled plasma mass spectrometry

LOD: Limit of detection

MCLG: Maximum contaminant level goal

NIST: The National Institute of Science and Technology

NMFS: National Marine Fisheries Service

p: p value

Pb: Lead

Photo-ID: Photo-identification

r: Correlational coefficient

RSD: Relative standard deviations

S: Social differentiation estimate

SD: Standard deviation

SEA: Standard ellipse area

SEAc: Standard ellipse area adjusted for small sample sizes

SIBER: Stable Isotope Bayesian Ellipses in R

Sr: Strontium

Sr:Ba: Strontium to barium ratios

TA: Total area

VIF: Variance inflation factor

w: Akaike weight

ABSTRACT

Clance, Lauren, R., M. S., University of South Alabama, May 2022 Site fidelity of bottlenose dolphins (*Tursiops truncatus*) using elemental analyses and photo-identification. Chair of Committee: Ruth, Carmichael, Ph.D.

The objective of this study was to determine if bottlenose dolphins (*Tursiops truncatus*) in Alabama waters acquire location-specific elemental signatures indicative of site fidelity. I measured trace metal concentrations (Chapter II) and stable isotope ratios (Chapter III) in skin from free-ranging dolphins remotely biopsied in Mobile Bay, the northern Gulf of Mexico (Gulf) and eastern Mississippi Sound (EMSS). Elemental findings were corroborated with photo-identification data. I found that dolphins acquired some location-specific elements indicative of site fidelity, with the greatest differences at geographic extremes, particularly relative to salinity. For trace metals, highest concentrations were found in winter, consistent with higher riverine discharge, while stable isotope ratios did not differ between seasons. Photo-identification data supported fidelity patterns indicated by elements, showing higher connectivity within Mobile Bay than between Mobile Bay and the Gulf or EMSS. I found little or no evidence of site fidelity by dolphin groups, likely due to limited data. This multi-disciplinary study established baseline data for the understudied dolphin population in Alabama waters.

GENERAL INTRODUCTION

The common bottlenose dolphin (*Tursiops truncatus*) is a globally distributed species that is known to inhabit diverse bodies of coastal and offshore waters and serve as an environmental sentinel (Fair & Becker 2000, Wells et al. 2004). In many areas, bottlenose dolphins exhibit high site fidelity (Mazzoil et al. 2005, Bassos-Hull et al. 2013, Passadore et al. 2018a) and have been shown not to travel outside their home ranges, even if conditions are highly unfavorable (Mazzoil et al. 2008). Generally, site fidelity, is defined as an animal repeatedly returning to a location (Switzer 1997), and thus, repeatedly exposed to the local environmental conditions and diet (Shane et al. 1986). As a result, site fidelity patterns can mediate exposure to factors such as freshwater and anthropogenic contaminants, which may be harmful to dolphins (Smultea & Würsig 1995, Matkin et al. 2008, Mazzoil et al. 2008, Dias et al. 2017, McBride-Kerbet & Toms 2021, Takeshita et al. 2021). Dolphins can also form long term social bonds (Maze-Foley & Würsig 2002, Bouveroux & Mallefet 2010, Wells 2013) where they may travel and feed in the same locations with the same associates frequently. Because of these behaviors, measuring site fidelity of both individuals and groups is important, especially if contaminants are present in the environment to which dolphins repeatedly return.

There is currently a gap in knowledge of dolphin movements in many regions of the world, including the northern Gulf of Mexico (Hubard et al. 2004, Bouveroux et al. 2014, Balmer et al. 2018). This data gap is particularly large in Alabama, where few studies on free-ranging populations have been conducted, and they have not covered the entire region (Cloyed et al. 2021b, Cloyed et al. 2021c). Coastal Alabama, like many coastal areas globally, is characterized by high freshwater discharge that can deliver excess nutrients, sediments, and anthropogenic contaminants such as metals (Pennock et al. 1999, Coogan & Dzwonkowski 2018, Mobile Bay NEP 2019), which originate from residential, agricultural and industrial practices (Brady 1979, Mobile NEP 2008, Ellis et al. 2011, Montiel et al. 2019) and can be heightened during periods of excess discharge that typically occur seasonally (Schroeder & Wiseman 1988, Pennock et al. 1999). Understanding dolphin movement patterns is essential to understanding how these patterns may mediate exposure to anthropogenic and other environmental influences in Alabama and elsewhere, and therefore, managing ecosystem and dolphin health.

Photo-identification (photo-ID), which is commonly used to assess dolphin movements, cannot characterize movements relative to habitat use and diet that are needed to fully define site fidelity (Kiszka et al. 2011, Wilson et al. 2012). Specifically, photo-ID is limited to defining dolphin movements during the discrete period of study and may not detect the full range of habitat use or associations within or among dolphin groups (Balmer et al. 2013, Vermeulen et al. 2017, Sinclar et al. 2017). Elements in marine mammal tissues such as stable isotope ratios (Olin et al. 2012, Smith et al. 2021) and trace metals (Kunito et al. 2002, Stavros et al. 2007, Botta et al. 2015) tend to vary with diet and environmental conditions, providing additional information to supplement photo-ID and define site fidelity. Elemental ratios and concentrations additionally allow inferences about freshwater and contaminant exposures not provided by photo-ID alone (Rubenstein & Hobson 2004, Fry 2006, Christiansen et al. 2015). Used in combination, these techniques may provide a powerful but as of yet poorly tested tool to detect site fidelity and determine if fidelity drives differences in diet or risk associated with use of some habitats.

The main objective of this thesis was to determine if bottlenose dolphins (*Tursiops truncatus*) in Mobile Bay, Alabama and the nearby waters of the Gulf of Mexico and Eastern Mississippi Sound (EMSS) acquired location-specific trace metal concentrations (Chapter II) and stable isotope ratios (Chapter III) that indicated site fidelity. I obtained dolphin skin by remote biopsy and measured elemental values by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) for trace metals (As, Ba, Cd, Co, Cr, Cu, Fe, Pb, Sr) and isotope ratio mass spectrometry (IRMS) for stable isotopes ($\delta^{13}C$, $\delta^{15}N$). I compared elemental values in skin among dolphins from different locations within Mobile Bay and adjacent waters to seasonal environmental conditions. Movement patterns of individuals and groups were corroborated using photo-ID data. This approach provides more sufficient data to understand how movement patterns can affect incorporation of location-specific elements into tissues and potentially mediate exposure to environmental stressors and contaminants. Overall, this study fills baseline data gaps about dolphin movements, diet and habitat use in the northern Gulf of Mexico using a novel multi-disciplinary approach to define site fidelity that can be applied to terrestrial, aquatic, and marine animals worldwide. Of note, this study is the first to use LA-ICP-MS to analyze trace metals in dolphin skin, demonstrating the potential of this method for use in future studies.

CHAPTER II

TRACE METALS IN DOLPHIN SKIN PROVIDE EVIDENCE FOR SITE FIDELITY OF BOTTLENOSE DOLPHINS IN THE NORTHCENTRAL GULF OF MEXICO

Abstract

Trace metals have been used to define habitat use for many species but are infrequently paired with observational or photographic data to define site fidelity. I investigated seasonal patterns of site fidelity for individual and groups of resident dolphins in Mobile Bay, Alabama (MOB) and nearby waters of the northern Gulf of Mexico (Gulf) and Eastern Mississippi Sound (EMSS), regions that are highly influenced by freshwater and anthropogenic sources of trace metals. I determined trace metal concentrations (As, Ba, Cd, Co, Cr, Cu, Fe, Pb, Sr) in skin of remotely biopsied dolphins using a combination of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and photo-identification. Photo-identification was also used to define dolphin social groups and link identified individuals to specific habitat areas for comparison of trace metal concentrations among habitat regions and between seasons. For individual dolphins, concentrations of Fe, Pb and Cd were higher in winter, coincidental with periods of higher riverine discharge through the MOB estuary. Regional differences in Fe (in summer), As, Cu, and Co in dolphin skin, indicated some dolphins had sufficient site fidelity to acquire region-specific metal concentrations. Arsenic was higher in Gulf samples compared to other regions, indicating that this metal may be a good indicator of Gulf habitat use. Overall, these data suggested varying degrees of site fidelity among individual dolphins, with higher movement within Mobile Bay and less between MOB and Gulf or EMSS. For groups of dolphins, site fidelity patterns were not clearly defined with the available data. This study is an important first step to inform future demographic studies that link movement and habitat use to potential drivers of dolphin abundance and health. Furthermore, these findings define baseline values for trace metals in resident dolphins in the northcentral Gulf of Mexico and documents the first use of LA-ICP-MS methods for dolphin skin.

Introduction

Bottlenose dolphins (*Tursiops truncatus*) are a globally distributed species and are known to inhabit diverse bodies of coastal and offshore waters, many of which are highly impacted by anthropogenic influences (Fair & Becker 2000, Wells et al. 2004). Bottlenose dolphins also often exhibit high site fidelity (Mazzoil et al. 2005, Bassos-Hull et al. 2013, Passadore et al. 2018a) and may repeatedly return to areas with potentially unfavorable conditions such as contaminated or low salinity waters due to high freshwater inputs (Smultea & Würsig 1995, Matkin et al. 2008, Mazzoil et al. 2008, Dias et al. 2017, Cloyed et al. 2021b, McBride-Kerbet & Toms 2021, Takeshita et al. 2021).

Because dolphins travel and feed alone and in groups (Barros 1993, Barros & Wells 1998, Gazda et al. 2005, Heithaus & Dill 2009), site fidelity may mediate exposure to contaminants for dolphins at individual and group or population levels.

Photo-ID studies on common bottlenose dolphins have been conducted around the world in rivers, bays, sounds and offshore to help define movement patterns, including site fidelity (Elfes et al. 2010, Gonzalvo et al. 2014, Wells 2014, Meager et al. 2018, Balmer et al. 2019). Although observational methods such as photo-ID are useful to define site fidelity patterns, they cannot contribute to understanding habitat use beyond the discrete observation periods because animals cannot be tracked without being directly observed via this method (Würsig & Würsig 1977, Würsig & Jefferson 1990, Urian 1999). Furthermore, photo-ID alone has limited ability to link geographic locations to environmental attributes that have implications for dolphin health. Hence, integrating additional sampling methods with photo-ID is necessary to understand how movement patterns may impact exposure to anthropogenic pollutants or other environmental risks and better inform dolphin conservation.

Trace metal concentrations in the environment vary spatially relative to their sources and environmental attributes. Metals can originate from natural or anthropogenic sources (Law 1996) and are assimilated in resident dolphins relative to ratios in the environment, primarily through diet (Monaci et al. 1998, Das et al. 2003, Brookens et al. 2007). For example, barium and strontium can be indicators of freshwater and marine influence (Deming et al. 2020, Nelson & Powers 2020), respectively, though their ratios vary by location and with processes such as sediment supply, turbidity, and water chemistry (Coffey et al. 1997, Elsdon & Gillanders 2002, Elsdon & Gillanders 2004).

High amounts of arsenic, chromium, cobalt and lead are often associated with anthropogenic contaminants from industrial outputs and shipping, while high amounts of cobalt, lead, and cadmium are typically associated with oil-derived materials (Takahashi 2009, Khan et al. 2014). Trace metal concentrations usually decrease with distance from their source (Ip et al. 2007), potentially providing traceable variation at a local scale. Metal concentrations also may vary seasonally with river discharge (Shiller & Boyle 1991, Olivie-Lauquet et al. 2001, Reiman et al. 2018), which is known to convey excess nutrients, sediments, and anthropogenic contaminants to nearshore waters that comprise home ranges of bottlenose dolphin populations globally (Schroeder & Wiseman 1988, Pennock et al. 1999, Fair & Becker 2000). Because of these trends, trace metals have been successfully used as geographic and temporal indicators of habitat use, inferring exposure to contaminants and freshwater sources (Hui et al. 2001, Belpaire & Goemans 2007, Waltham et al. 2011, Komoroske et al. 2012, Barraza et al. 2019).

While many metals can be toxic in high concentrations, trace metals can have non-lethal and lethal effects on wildlife health. Effects of metals on wildlife include immunosuppression, endocrine disruption and failed or impaired reproduction (e.g., De Swart et al. 1996, Reijnders et al. 2009, Schwacke et al. 2011, Galligan et al. 2019, Sonne et al. 2020). Studies on wild dolphins have linked contaminant exposures to habitat use (Kunito et al. 2002, Stavros et al. 2007, Botta et al. 2015, Deming et al. 2020), demonstrating the potential for animals to acquire location-specific metal concentrations in their tissues. Spatial and seasonal variation of trace metals in dolphin tissues, therefore, may provide evidence of dolphin site fidelity relative to anthropogenic contaminant exposure and inform spatial and temporal scales for managing pollution and monitoring health of different populations.

The objective of this study was to determine if bottlenose dolphins acquired location-specific trace metal (As, Ba, Cd, Co, Cr, Cu, Fe, Pb, Sr) concentrations indicative of site fidelity and potential contaminant exposure risk. To define trace metal concentrations across the range of local environmental gradients, including salinity and anthropogenic inputs, I analyzed skin from remotely biopsied dolphins in Mobile Bay, Alabama and adjacent waters of the northern Gulf of Mexico and Eastern Mississippi Sound. Samples were collected during summer and winter in Mobile Bay to test for seasonal variation. To corroborate trace metal-based site fidelity, biopsied dolphins were individually photo-identified and compared to images from ongoing capture-mark-recapture surveys in the study area. Resighting data were used to document the number and location of sightings, determine the influence of movement on variation in trace metal values, and define social clusters for analysis of site fidelity of groups. This study provides baseline data on relationships between dolphin locations and trace metal concentrations in an urbanized, freshwater dominated estuary and demonstrates the first use of LA-ICP-MS to determine metal concentrations in dolphin skin. These data will be useful to assess future changes in habitat use and potential contaminant exposure in Alabama waters and inform management and conservation strategies for bottlenose dolphin populations in similar habitats worldwide.

Methods

Study sites

Mobile Bay is a microtidal, freshwater-dominated estuary located in Alabama on the northern Gulf of Mexico. More than 50 billion m^3 y^{-1} of freshwater flows through Mobile Bay, peaking in winter and spring when salinity stratification is highest (Coogan & Dzwonkowski 2018, Mobile Bay NEP 2019). Industrial centers and ports are concentrated in the North and Central regions of Mobile Bay (Figure 1.1; Mobile NEP 2008). Although ~3 m deep on average, the bay has a ship channel (~14 m deep) that runs mostly north-south (Figure 1.1), the Intercoastal Waterway (ICW) with an east-west orientation, and shallower channels (Schroeder et al. 1990, Mobile NEP 2008). Deeper channels are used by large shipping traffic, while the ICW and shallower channels are mostly used by smaller boat traffic. Land surrounding Mobile Bay is urbanized and has large agricultural areas, particularly east of the bay (Ellis et al. 2011, Montiel et al. 2019). The City of Mobile and associated suburban centers are located northwest of the bay, comprising a historically known but poorly documented source of point and non-point contaminants to the bay (Brady 1979).

Water from Mobile Bay drains west into eastern Mississippi Sound (EMSS) and south into the adjacent waters of the northern Gulf of Mexico (hereafter referred to as Gulf), bringing freshwater and potential contaminants to these areas (U.S. Army Corps of Engineers 2018). EMSS is characterized by shallow waters, deep ship channels and riverine influences from Mobile Bay and other river systems (Eleuterius 1976). The Gulf is characterized by deep waters with ship channels and fairways, which have large vessel traffic. EMSS and the Gulf act as geographic endpoints in this study because of their potential connectivity to Mobile Bay, as well as their salinity regimes in connection with Mobile Bay flow patterns. Salinity is higher in the Gulf compared to Mobile Bay and increases with distance from the mouth of Mobile Bay (Coogan & Dzwonkowski 2019). Both Mobile Bay and the Gulf have a history of construction, use, and removal of oil and natural gas rigs (Wang et al. 2014, Meng 2016, BOEM 2021). Accidental and intentional release of contaminants, including those containing metals, into the environment has occurred (Ward & Tunnell 2017).

Data Collection

Sample collection.

Remote biopsy sampling surveys occurred during winter (December 2019 to February 2020) and summer (August -September 2020) in Mobile Bay, AL; August to October 2019 in EMSS; and winter (December 2019 to February 2020) in the Gulf. Samples were not collected seasonally in EMSS and Gulf due to logistics, including COVID-19 pandemic limitations and weather, but were included to provide western and southern geographic endpoints for spatial comparisons. Skin and blubber samples (N=115) were obtained from live dolphins (1-2 samples per dolphin group) with a specialized crossbow (Barnett Panzer V, 68 kg draw weight) using a 10 x 25 mm or 10 x 30 mm ethanol sterilized dart according to established boat-based methods, including the limitation of sample collection to large juveniles or adults, in groups with no neonates and few to no calves present (Krützen et al. 2002, Sinclair et al. 2015). The target area on dolphins for sample collection was above the midline and below the dorsal fin (Gorgone et al. 2008). Samples were collected under the National Marine Fisheries Service (NMFS), Marine Mammal Protection Act and Endangered Species Act Permit # 21938 and NMFS Atlantic Institutional Animal Care and Use Committee (IACUC) authorizations 2017-001 and 2020-002.

Photo-identification.

To identify dolphins that were biopsied and continue to track their movements, animals were photographed during biopsy surveys and photographs were compared to images from capture-mark-recapture surveys conducted during the same seasons as biopsy sampling and to photographs from other biopsy surveys. In all cases, high-resolution digital cameras (Canon 7D and 90D) fitted with 100-400mm lenses were used to photograph the dorsal fins of individual dolphins encountered in the study area, identified by natural and long-lasting markings (Würrsig & Würrsig 1977, Würrsig & Jefferson 1990, Urian 1999). All photographs were taken in suitable sighting conditions (< Beaufort Sea State 3). Capture-mark-recapture surveys were conducted according to established protocols on pre-determined track lines (Rosel et al. 2011), covering the entire study area a total of three times. Surveys were completed within the shortest time possible to accurately sight the population using the study area within the study time frame (Rosel et al. 2011). To identify unique individuals, document resightings, and define associates sighted in groups for elemental comparisons, photos were organized and sorted following Melancon et al. (2011), including matching of fins with FinBase (the photo-ID database that contains the catalog of images of identified dorsal fins, Adams et al. 2006) and FinFindR. FinFindR traced the leading edge of dorsal fins and compared the trace to all images in the catalog, providing the top 50 closest matches (Thompson et al. 2022). The "primary matcher" examined the top 50 possibilities. If a matching ID was found it was considered a resighting, and if a match was not found, the dolphin was considered a new ID. This process was replicated by a "secondary matcher" for quality assurance and quality control, and if the same result was found, the fin was added to the dorsal fin catalog in FinBase. If the same result was not found, the final decision was made by an independent third party. (Melancon et al. 2011).

Trace metals analysis.

Skin was removed from the blubber in the field and frozen in a liquid nitrogen dewar before storage at -80°C until analysis. Tissue samples were rinsed thoroughly with ultrapure water and dried at 60°C to a constant weight, typically for 24–48 hours. Trace metal determination was performed by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) using a laser ablation ESI NWR213 (Elemental Scientific Lasers, Bozeman, MT) coupled to an Agilent 7700 series ICP-MS (Agilent Technologies, Santa Clara, CA) for the following metals: Arsenic (^{75}As), Barium (^{137}Ba), Cadmium (^{111}Cd), Cobalt (^{59}Co), Chromium (^{52}Cr), Copper (^{63}Cu), Iron (^{56}Fe), Lead (^{208}Pb), and Strontium (^{88}Sr). These metals were chosen because of their potential for geographic distinction in the study area (Brady 1979, Ellis et al. 2011, Montiel et al. 2019, Deming et al. 2020, Nelson & Powers 2020). Pre-ablation was run with an 80 µm spot size at a rate of 5 Hz with a 10 sec dwell time, 5 µm depth, and 30% output. Ablation was run with a 20 µm spot size at a rate of 20 Hz with a 30 sec dwell time, 5 µm depth, and 40% output.

Each sample was ablated with three spots. There was a 20 sec warmup and 10 sec washout for both the pre-ablation and ablation. The National Institute of Science and Technology (NIST) SRM 612 glass standard was used to tune the instrument and was the within-run reference material (Pearce et al. 1997), in the absence of a matrix-matched reference material (Günther et al. 2001, Sylvester 2008).

Data Analysis

Calculating trace metal concentrations.

Trace metal concentrations in dolphin skin were calculated using *iolite* (version 4) data processing and visualization software. One sample was excluded from subsequent analysis because no counts were detected during laser ablation. Background (baseline) counts for each element were subtracted from sample concentrations, and the concentration output was then calculated relative to concentrations of the NIST 612 standard (Longerich et al. 1996). Any values beyond 2 standard deviations from the mean were deemed outliers and removed by results processing in *iolite*. An additional control module was run in *iolite* using the NIST 612 standard with default alarm, warning, variation alarm and variation warning values of 20%, 15%, 30% and 25% of relative standard deviation (RSD), respectively. This secondary check measured the precision of the LA-ICP-MS by comparing the mean measured value in samples to the accepted value based on the NIST 612 standard and identified variance within the data set.

Data were exported from *iolite*, including concentrations for the three spots on each sample, the standard deviation, and the number of data points. Limits of detection (LOD) were established using previous literature methods built into *iolite*, and these values were reported as “Below LOD” (Longerich et al. 1997, Pettke et al. 2012, Howell et al. 2013). After export, the concentrations in each sample were determined by averaging the concentrations of the three spots for each metal. To capture the best possible mean values for individual animals, any final values beyond 2 standard deviations from the mean of all dolphins for each metal were deemed outliers. In these cases, values were multiple orders of magnitude higher than the mean and thought to not accurately represent metal values in dolphin skin. For example, the raw average of Fe concentrations for all sampled dolphins was ~500 ppm, and an animal with a value of ~30,000 ppm was discarded as an outlier. For subsequent analyses, values that were below LOD were designated as 0, and Sr to Ba ratios were calculated only for samples with Ba concentrations > 0.

Trace metal based site fidelity.

To determine if trace metal values in dolphin skin varied by season or geographic location, each sampled dolphin was assigned a region based on their biopsy location and local salinity regimes (North, Central, Southwest, Southeast, EMSS, or Gulf; Figure 1.1; Appendix A, Figure A1). A one-way analysis of variance (ANOVA) was used in R (Version 1.4.1717; R Core Team 2009) to make comparisons between seasons (summer and winter) and among regions. Seasonal comparisons were limited to samples collected in Mobile Bay, where data were collected for both seasons. I further determined if trace metal concentrations in dolphin skin varied continuously by geographic location by comparing metal concentrations in dolphin skin to latitude, longitude and salinity (an environmental proxy for location down bay) as explanatory variables. I used general linear models with a gaussian distribution and *identity* link in R. I constructed a global model with each trace metal as a response variable and latitude, longitude and salinity as explanatory variables. I conducted backward selection, where the global model was run first, followed by all other model combinations in order of decreasing complexity, with a null model run last. To identify the best fitting model for my data and to measure the relative strength of each model, Akaike Information Criterion (AIC) values and Akaike weights (*w*) were calculated. The model with the highest weight (and therefore the highest probability of fit to the data) was considered the strongest, best fitting model, and models with weights of greater than 0.1 indicated that they were weaker but still worthy of consideration (Burnham et al. 2011). Variance inflation factors (VIF's) and correlational tests were conducted to test for relatedness among explanatory variables. Season could not be included in the models because it was closely correlated with other explanatory variables and caused high VIF's. Hence, the models were run separately for each season when metals differed in concentration between seasons.

Photo-identification based site fidelity.

To corroborate site fidelity for individual dolphins in the study area, I tested the effect of movement observed by photo-ID on variation in stable isotope ratios. To do this, I plotted the location of each unique sighting and resighting of biopsied animals (photographed during both capture-mark-recapture and biopsy surveys) and calculated the average displacement between sightings. The average displacement was calculated for each biopsied animal sighted 3 or more times, excluding a single ID that was darted twice and the animal for which trace metal concentrations could not be measured. This sighting frequency threshold was chosen because it was higher than the median sighting frequency (N= 2), and therefore, would more likely include resident dolphins in the analyses. I then calculated the absolute difference between each dolphin's trace metal concentration and the mean value for the region and plotted it against their average displacement. I conducted a linear regression and F test to identify trends between metal variation and average displacement. All map plotting was done in QGIS (version 3.16), and graphing was done in R (Version 1.4.1717; R Core Team 2009).

To determine if individual site fidelity could be indicative of group fidelity, I conducted an association analysis between individual biopsied dolphins and all dolphins resighted during capture-mark-recapture and biopsy surveys. Association analysis was done using SOCPROG version 2.9 (Whitehead 2009) and associations quantified using a daily sampling period. Restrictions were set to include animals that had been sighted 4 or more times to provide sufficient data for analysis and ideally include dolphins more likely to be residents, because social interactions can be complex and higher sighting frequencies can better capture these patterns (Whitehead 2008). This analysis uses a half weight index (HWI) to calculate association patterns or indices ranging from 0 to 1, where 0 indicates dolphins were never sighted together and 1 indicates dolphins were always sighted together. I also calculated a social differentiation estimate (S), which indicates the level of populational organization, where individuals associate with dolphins in their own (homogenous) or other (heterogenous) clusters. S values greater than 2.0 indicate extremely differentiated populations, where social clusters do not interact, while values greater than 0.5 indicate heterogenous populations, and values less than 0.3 indicate homogenous populations, where social clusters often interact.

Correlation analysis (r values) was used to test how well S values represented social patterns in the population, where r values greater than 0.4 indicate confidence that the S value represents homogeneity of the population (Whitehead 2008, Whitehead 2009). To determine if social groups identified by the association analysis had location-specific trace metal values, comparisons among social groups were made using a one-way analysis of variance (ANOVA) and a Tukey post-hoc test in R. Sighting locations for all members of a social group were then compared spatially to relate metal differences to geographic locations.

Results

Dolphins sampled

A total of 114 dolphin skin samples were analyzed from remotely biopsied dolphins during the study period (80 from Mobile Bay, 28 from the Gulf, and 6 from EMSS; Table 1.1). Of the samples collected in Mobile Bay, 40 were collected in summer and 40 in winter (Figure 1.1). Ninety-seven percent of dolphin skin samples had detectable concentrations of at least one trace metal, with 65% having three or more detectable metals, and 14% having all metals above the limit of detection on average (Appendix A, Table A1). All elements had at least one outlier removed (Appendix A, Table A2). Overall, concentrations in dolphin skin were highest for Fe, followed by As, Cu, Sr, Cr, Ba, Cd, Pb and Co (Appendix A, Table A1).

Trace metal-based site fidelity.

Concentrations of some metals varied between seasons for dolphins sampled in Mobile Bay regions (North, Central, Southwest and Southeast). Maximum concentrations of all metals in dolphin skin were higher in winter compared to summer, with mean seasonal differences for Fe, Cd and Pb in Mobile Bay animals (Figure 1.2; Table 1.2). Of these metals, Fe also varied among regions, with higher concentrations in the Southwest compared to the Southeast in summer (Figure 1.2; Appendix A, Table A3). EMSS and Gulf regions were not included in the subsequent analyses for Fe, Cd and Pb because seasonal samples were not available from those regions, and when they were included as geographic endpoints regardless of season, there were no changes in statistical relationships (Figure 1.2).

For metals that did not show seasonal differences, As, Cu, and Co varied among regions within Mobile Bay and when compared to geographic endpoints in EMSS and Gulf (Figure 1.3; Appendix A, Table A3). For As, skin samples collected in the Gulf were higher than all other regions, while Co was higher in the Central region compared to the Gulf (Figure 1.3). For Cu, there were differences among sites, but the pair-wise comparisons with the lowest p value (0.12) occurred between the Southwest and Southeast regions, where Cu was higher in the Southwest (Table 1.2; Appendix A, Table A3). Although not significant, median Sr values were most different between the North region, and EMSS (lowest) and the Gulf (highest) values. Median Sr:Ba ratios were most different between the North region, where they were the lowest, and the Gulf, where they were the highest (Figure 1.3; Appendix A, Table A3).

Some metal concentrations varied with latitude, longitude, and salinity within Mobile Bay during the sampling period. The best fitting models showed Fe decreased with longitude and increased with salinity during winter, but only salinity was significant (Figure 1.4; Appendix A, Table A4; $w=0.23$, $p=0.02$). Three additional models had weights greater than 0.1, including latitude and salinity ($w=0.23$), salinity alone ($w=0.20$) and latitude, longitude, and salinity ($w=0.19$). There was no effect of these factors on Fe concentrations during summer. Pb concentrations during summer decreased with latitude and salinity, but only salinity was significant (Figure 1.4; Appendix A, Table A4; $w=0.29$, $p=0.03$). Three additional models had weights greater than 0.1, including the null model ($w=0.18$), salinity alone ($w=0.16$) and latitude, longitude, and salinity ($w=0.11$). There was no effect of these factors on Pb concentrations during winter. Cu also varied with longitude and salinity, but only salinity was significant (Figure 1.4; Appendix A, Table A4; $w=0.30$, $p<0.001$). Three additional models for Cu had weights greater than 0.1, including latitude, longitude and salinity ($w=0.26$), latitude and salinity ($w=0.26$) and salinity alone ($w=0.17$). The best fitting model to explain geographic patterns in Sr concentrations contained only salinity (Figure 1.4; Appendix A, Table A4; $w=0.40$, $p<0.001$). Three additional models had weights greater than 0.1, including latitude and salinity ($w=0.25$), longitude and salinity ($w=0.20$) and latitude, longitude and salinity ($w=0.11$). The best fitting model to explain patterns in Sr:Ba contained longitude and salinity, but only longitude was significant (Figure 1.4; Appendix A, Table A4; w=0.25, $p=0.03$). Three additional models had weights greater than 0.1, including longitude only ($w=0.22$), latitude and longitude ($w=0.19$) and latitude, longitude and salinity ($w=0.11$). No other elements had significant variables in their best fitting models.

Corroboration of site fidelity using photo-identification

Site fidelity of individuals.

Thirty-five dolphins (31% of those biopsied) were sighted 3 or more times and were used to corroborate site fidelity with photo-ID resighting metrics. Of these dolphins, some (N=11, 31%), were sighted in their respective biopsy region. Others were seen in two regions (N=18, 51%) and three regions (N=6, 17%). The number of regions that dolphins were sighted in varied on an individual scale. For example, ID 353 was sighted 7 times, always in the region in which it was biopsied, but ID 765 was sighted 4 times in 3 different regions (Appendix A, Figure A2).

Dolphins biopsied in the Central and Southeast regions traveled the farthest distances between sightings and had the most variation in displacement (Figure 1.5). Average displacement per sighting for biopsied dolphins was 3.68 ± 0.73 km in the North region, 4.58 ± 0.70 km in the Central region, 2.98 ± 0.39 km in the Southeast region, and 0.72 km in the Gulf region (a single animal). No dolphins biopsied in the Southwest or EMSS were sighted frequently enough to be used in site fidelity analyses.

For individual dolphins, variation in Cd concentration in skin increased with increasing displacement between sightings (Figure 1.5; $y=0.093x+0.42$, $R^2=0.13$, $F_{reg32}=4.47$, $p=0.04$), and the minimum values for variation increased with displacement for Sr (Figure 1.5). Variation in concentration was not significant with displacement for other metals. In general, dolphins biopsied in the Southeast region had the largest trace metal variation, while dolphins biopsied in the North had the least compared to other regions.

Site fidelity of groups.

The association analysis identified 11 clusters of social groups from biopsied dolphins sighted 4 or more times and their associates. These clusters were condensed into 9 broader clusters (designated A-H) so that each cluster contained at least one biopsied individual. The cluster analysis also identified a dolphin duo (designated G) and a lone individual (designated H). Association indices ranged from 0.1-0.65, with the overall mean at 0.32 (SD=0.33). The social differentiation estimate (S) was 0.679, and the R value was 0.185, below the threshold (0.4) for confidently representing the population. Some clusters (A, B, E, F) contained very high levels of associations (i.e., some individuals had HWI values of ≥ 0.8) (Appendix A, Figure A3). The number of total individuals within clusters ranged from 1 to 21, and the number of biopsied individuals within clusters ranged from 1-7, with two clusters (E and H) containing a single darted animal (Appendix A, Table A5).

For groups of dolphins, the mean Cd concentration in skin of dolphins in cluster A was higher than cluster E (Figure 1.6; one-way ANOVA: F_{reg7}=3.12, *p*=0.03; Appendix A, Tables A5 and A6; Tukey post-hoc test: *p*=0.05; Appendix A, Table A7). Dolphins in cluster A used the Central region of Mobile Bay whereas dolphins in cluster E did not (Figure 1.6). No other metals varied by cluster (Appendix A, Table A6, Table A7).

Discussion

Dolphins in the Mobile Bay estuary system acquired some regionally specific trace metal values, indicating the successful use of this method as a geographic tracer with select elements. Metals showed the greatest promise as regional indicators of site fidelity at geographic extremes. For example, concentrations were most different between the Gulf region and all other regions for As and between the Gulf and Central region for Co. There were also regional differences in Fe, with concentrations showing the greatest difference between the Southwest and Southeast regions during the summer. Some regional differences were linked to variation in salinity. Although not significant, Sr:Ba, which is an indicator of relative marine to freshwater influence, generally increased from north to south in Mobile Bay with the known salinity gradient (Deming et al. 2020, Nelson & Powers 2020), having the greatest differences at geographic extremes. Accordingly, Sr alone increased with salinity. Observed movement by photo-ID corroborated trace metal-based evidence of site fidelity, where movement of dolphins was greater among regions within Mobile Bay than between Mobile Bay and the Gulf or EMSS. Low observed movement of animals between Mobile Bay and adjacent waters or among areas more geographically distant can explain the observed regional differences in Co and As and suggest these elements may be particularly useful to differentiating dolphins that use these different habitats.

For dolphin groups, association and cluster analyses revealed that although there was little direct interaction among social clusters, sampled dolphins likely traveled and foraged in the same general regions, or along similar environmental gradients, due to similar trace metal concentrations. Statistics from the association analysis, however, indicated that these results may not be fully representative of the social structure in our region. Previous studies in the Gulf of Mexico have found higher association indices than this study (Bouveroux & Mallefet 2010, Wells et al. 2013), but were temporally longer studies. Although there was not enough photographic data to fully corroborate site fidelity patterns of groups for comparison to trace metal concentrations, additional resightings will enhance this dataset and allow these comparisons to be made in the future using data from this study or others.

Some metals in dolphin skin also varied seasonally, potentially driven by increased riverine discharge during winter and seasonal variation in water temperature. Maximum concentrations of all metals occurred in the winter season when riverine discharge is highest and salinity lowest for Mobile Bay (Coogan & Dzwonkowski 2018), suggesting that river discharge is a likely source of some trace metals that are subsequently assimilated into dolphin skin from their diet (Monaci et al. 1998, Das et al. 2003, Brookens et al. 2007). Storm water, agricultural runoff and industrial inputs are known to be transported from upstream sources (Isphording & Flowers 1990, Mobile NEP 2008), and increased flow may increase concentrations of pollutants from these anthropogenic sources on the watershed (Schroeder & Wiseman 1988, Pennock et al. 1999). For example, in this study, Pb concentrations decreased with increasing salinity during the summer, when riverine discharge would be lower. Previous studies have also found links between seasonal trace metal concentrations and riverine discharge or between wet and dry periods (Shiller & Boyle 1991, Olivie-Lauquet et al. 2001, Ebrahimpour & Musrifah 2010, Reiman et al. 2018). Metal accumulation in fish (Somero et al. 1977, Blanchard & Grosell 2005) and bivalves (Jackim et al. 1977, Wright

& Zamuda 1987) is generally higher in lower salinity conditions, though accumulation can be highly variable depending on species-specific tissue turnover rates and environmental conditions such as temperature (Jackim et al. 1977, Kidwell et al. 1995, Jezierska & Witeska 2006, Bonsignore et al. 2018). Studies on fish (Feldhausen & Johnson 1983, Ebrahimpour & Musrifah 2010, Mendil et al. 2010), oyster (Chan & Wang 2018, Peter 2020) and cetacean tissues (Bryan et al. 2007, Rioux et al. 2012) also found seasonal trends in concentrations of trace metals. These seasonal spatial patterns highlight the importance of collecting data during periods that reflect the range of environmental conditions in a system.

Elevated levels of some metals in dolphins biopsied during this study could be related to location-specific diet. Previous studies have found that cephalopods and crustaceans may have higher concentrations of As and Cd in their tissues (Penicaud et al. 2017, Lischka et al. 2018, Ramon et al. 2021) and cetaceans that feed mainly on those groups may also have higher concentrations in their tissues (Kubota et al. 2001, Page-Karjian et al. 2020). Dolphins in the study area are known to consume cephalopods, which may be among their dominant prey sources (Cloyed et al. 2021b, Clance unpublished data), potentially explaining this pattern. Prey sampling during the study period did not find cephalopods in the North and Central regions (Chapter III), but cephalopods were present in the Southwest, Southeast and Gulf regions. This finding could mean that cephalopods and/or another source of As is primarily derived from the Gulf region. Therefore, these location-specific concentrations of at least As in dolphin tissues could indicate a link to dietary differences between dolphins in North and Central Mobile Bay and the Gulf.

While observed movement could be related to trace metal concentrations, displacement from the biopsy location was a poor predictor of metal variation. Variation in movement of individual dolphins and groups contributed to some variation in Cd, but not other trace metal concentrations. For individual dolphins, the increase in Cd variation with displacement length could not be explained by the spatio-temporal factors (season, latitude, longitude, salinity) considered in this study. The metric we used for quantifying displacement did not account for the direction dolphins traveled or the absolute magnitude of elemental variation in the environmental along the axes traveled. This finding emphasizes that the spatial scale at which a geographic tracer varies is important to interpreting relationships to movement. For example, dolphins may travel short distances but show high elemental variation if the gradient of metal concentrations over that distance is high, and they may have lower elemental variation despite traveling longer distances when the concentration gradient is low across the distance traveled. Additionally, because most dolphins used multiple regions, average metal concentrations for any biopsy region may not be sufficiently discrete to detect variation with distance between adjacent regions, with a limited sample size. Unobserved dolphin movement outside of our survey period or area also could have occurred and would not be reflected in the sighting data.

Concentrations of metals in dolphin skin for this study are similar to or higher than previously reported studies and are above established safety thresholds for marine life and human health, indicating the potential risk to dolphin health in Alabama waters. My data indicate risks could be exacerbated during periods of high discharge and variable salinity regimes. Most of the average metal concentrations in dolphin skin in every region of this study were above the Environmental Protection Agency's (US EPA) National Recommended Water Quality Criteria for Saltwater Aquatic Life for both acute and chronic exposure (US EPA 2021b), including in summer when maximum metal concentrations were lower. Most values were also above the EPA's Maximum Contaminant Level Goal (MCLG), that defines the threshold below which there is no known risk to human health, as described in their National Primary Drinking Water Regulations (EPA 2021a). Though these thresholds apply to humans and saltwater organisms, there are currently no specific health thresholds for metal concentrations in dolphin skin. Although metals such as Co, Cu, and Fe can be essential for biological processes, they can be toxic in higher amounts and along with As, Pb, and Cd they can be associated with anthropogenic contaminants from industrial centers, shipping, and oil derived materials (Takahashi 2009, Khan et al. 2014). Heavy metals including Cd, Pb, and Co have been found in fish tissues in the northern Gulf of Mexico with anthropogenic influences as suspect sources (Feldhausen & Johnson 1983, Hanson 1997, Ploetz et al. 2007), corroborating accumulation in dolphin tissues through diet (Thompson 1990). It is also promising that the metal values in dolphin skin samples from this study were similar to or higher than previously reported (Stavros et al. 2007, Bryan et al. 2007, Stavros et al. 2011, Aubail et al. 2013) because this study used different methodology from previous studies. Many previous studies report metal concentrations in terms of wet weight, as opposed to dry weight, which was used in this study, making direct comparisons to past studies difficult. This study is also the first to document use of LA-ICP-MS to quantify trace metal concentrations in dolphin skin. This method should be considered for future studies because laser ablation is non-destructive and tissue samples from live dolphins are usually small and difficult to obtain, limiting the number of chemical analyses that can be conducted on samples.

This study lays the groundwork for several areas of future research. For example, the number of biopsied individuals within clusters defined by photo-ID was small due to the limited number of biopsied and non-biopsied animals that were resighted. Future resightings will contribute to this dataset and allow for a better characterization of associations among individuals throughout the study area to better corroborate group fidelity, so this analysis should be repeated when more data are available. Presence of mom-calf pairs within clusters likely limited the ability for biopsy sample collection, also contributing to low sample size. Metal concentrations also may vary with dolphin size, age, sex, and reproductive status (Bryan et al. 2007, Stavros et al. 2007, Yordy et al. 2010, Peterson et al. 2014), which this study minimized by targeting adult animals. This study could not fully consider these demographic factors due to logistics and remote sampling methodology, but they could be incorporated into future studies. Prey species and water samples also were not analyzed for trace metals, limiting our ability to extend our findings to fully understand relationships to metal bioavailability. This process is complex, and many factors that affect metal delivery and dolphin status such as freshwater discharge (salinity) and season (temperature) also affect bioavailability (Luoma 1983, McGeer 2004, Ahlf et al. 2009) and merit future study. I demonstrated the potential for the multidisciplinary approach used in this study to be applied to address these types of questions in the future.

Estimates of site fidelity using only observational methods requires long term study and is limited in ability to link movements to aspects of habitat use that may be necessary to fully define conservation risks for mobile predators such as bottlenose dolphins. This study demonstrates the merits of using elemental analyses in tandem with photo-ID. Potential toxicity of metals in combination with observed seasonal trends suggest dolphins in the northcentral Gulf of Mexico could be at higher risk for contaminant exposure during winter months or during periods of excess freshwater discharge. Currently, it is known that prolonged freshwater exposure can lead to skin lesions and even mortality in dolphins, but the exact cause of these health issues is unknown (Deming et al. 2020, McClain et al. 2020). My data could contribute to understanding this phenomenon to determine how low salinity environments and co-variates such as metals may affect dolphin health. These data begin to fill current gaps regarding how site fidelity patterns may expose dolphins to environmental risks. This information is needed to establish baseline levels of contaminants in dolphin skin and set relevant spatio-temporal scales for conservation and management strategies. These data could be used to help identify non-lethal versus lethal levels of metals in dolphin skin and further characterize contaminant exposure in free-ranging populations, which could also provide better information to stranding networks about dolphin mortality.

Tables

Table 1.1. Number (N) of collected dolphin biopsy samples analyzed for trace metals by regions in Figure 1.1 (North, Central, Southwest, Southeast, EMSS and Gulf) and season (winter, summer).

Region	N winter	N summer	N total
North	2	5	7
Central	7	13	20
Southwest	4	4	8
Southeast	28	17	45
EMSS	0	6	6
Gulf	24	4	28
			114

Table 1.2. Results of one-way ANOVA comparing elemental concentrations in dolphin skin between seasons in Mobile Bay and among all regions in Figure 1.1. N = number of samples, F = F statistic, df = degrees of freedom and p = p-value. Significant p-values are in bold.

	Season				Region			
	N	F	df	p	N	F	df	p
As	79	0.001	1	0.98	110	4.64	5	**<0.001**
Ba	78	0.18	1	0.68	112	0.77	5	0.57
Cd	79	7.17	1	**<0.01**	112	0.86	5	0.51
Co	77	0.70	1	0.41	111	2.96	5	**0.015**
Cr	78	1.43	1	0.23	112	0.27	5	0.93
Cu	78	1.86	1	0.18	110	2.54	5	**0.033**
Fe	79	6.95	1	**0.010**	113	2.57	5	**0.031**
Pb	77	4.92	1	**0.030**	111	0.085	5	0.99
Sr	78	0.20	1	0.65	112	1.61	5	0.16
Sr:Ba	59	3.20	1	0.079	74	1.12	5	0.36

Figures

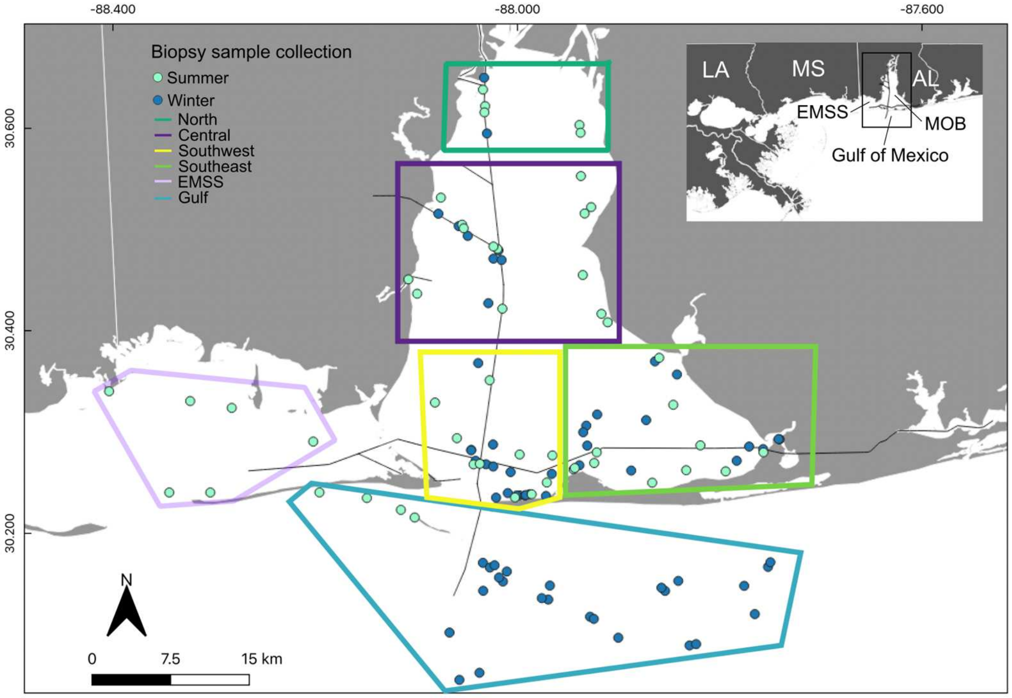

Figure 1.1. Locations of seasonal remote biopsy in Mobile Bay, AL (MOB) and adjacent waters of eastern Mississippi Sound (EMSS) and the northern Gulf of Mexico (Gulf). Colored polygons indicate regions (North, Central, Southwest, Southeast, EMSS, Gulf) used for testing site fidelity. Black lines indicate ship channels.

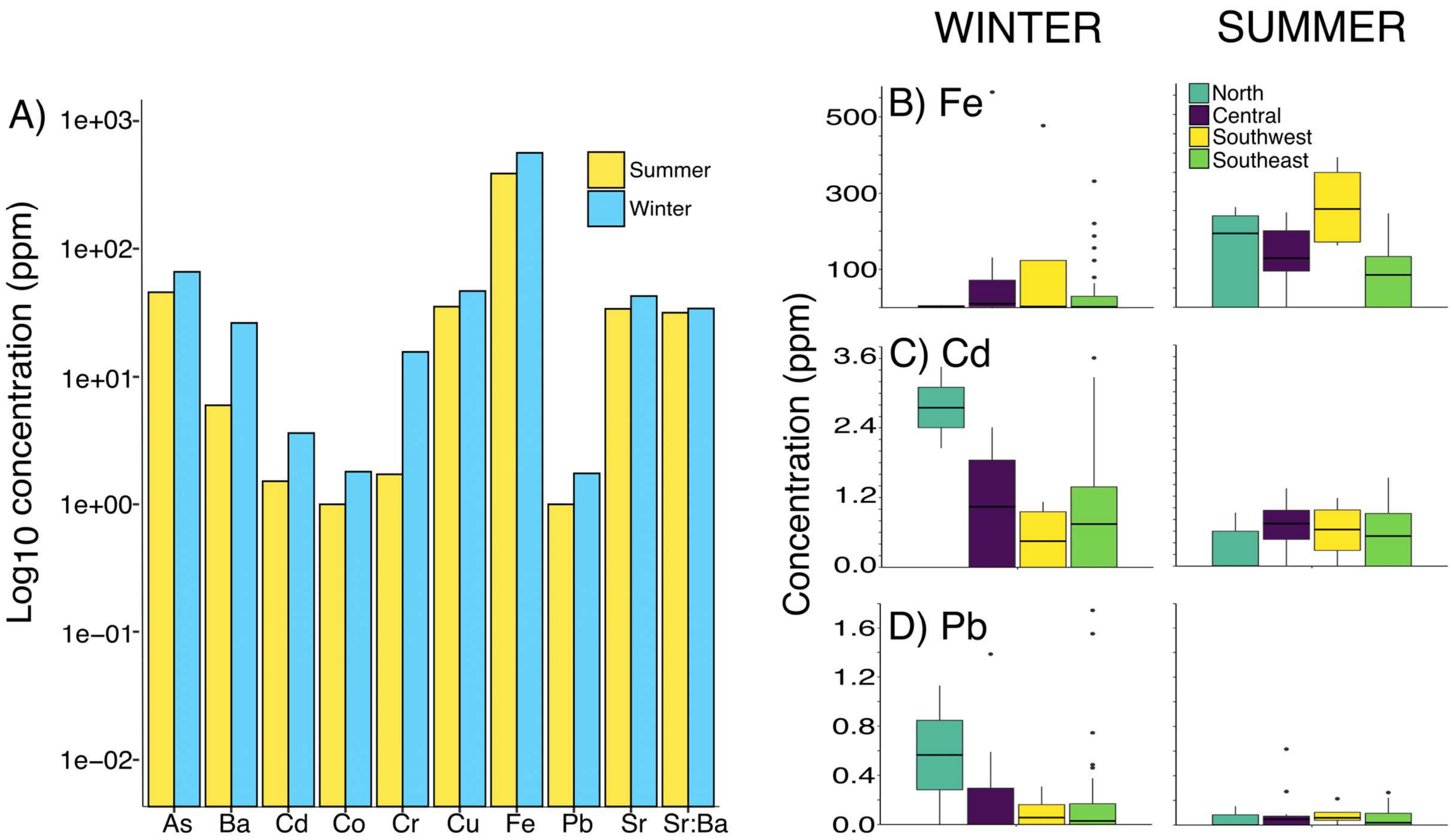

Figure 1.2. Maximum trace metal concentrations in parts per million (ppm) in dolphin skin (panel A). Trace metal concentrations that showed seasonal differences separated by region for Fe (panel B), Cd (panel C) and Pb (panel D) that. North (N), Central (C), Southwest (SW), and Southeast (SE). The boxes represent the interquartile range, the black bars represent the median, and the dots outside the boxes represent values > 1.5 < 3 times outside the interquartile range.

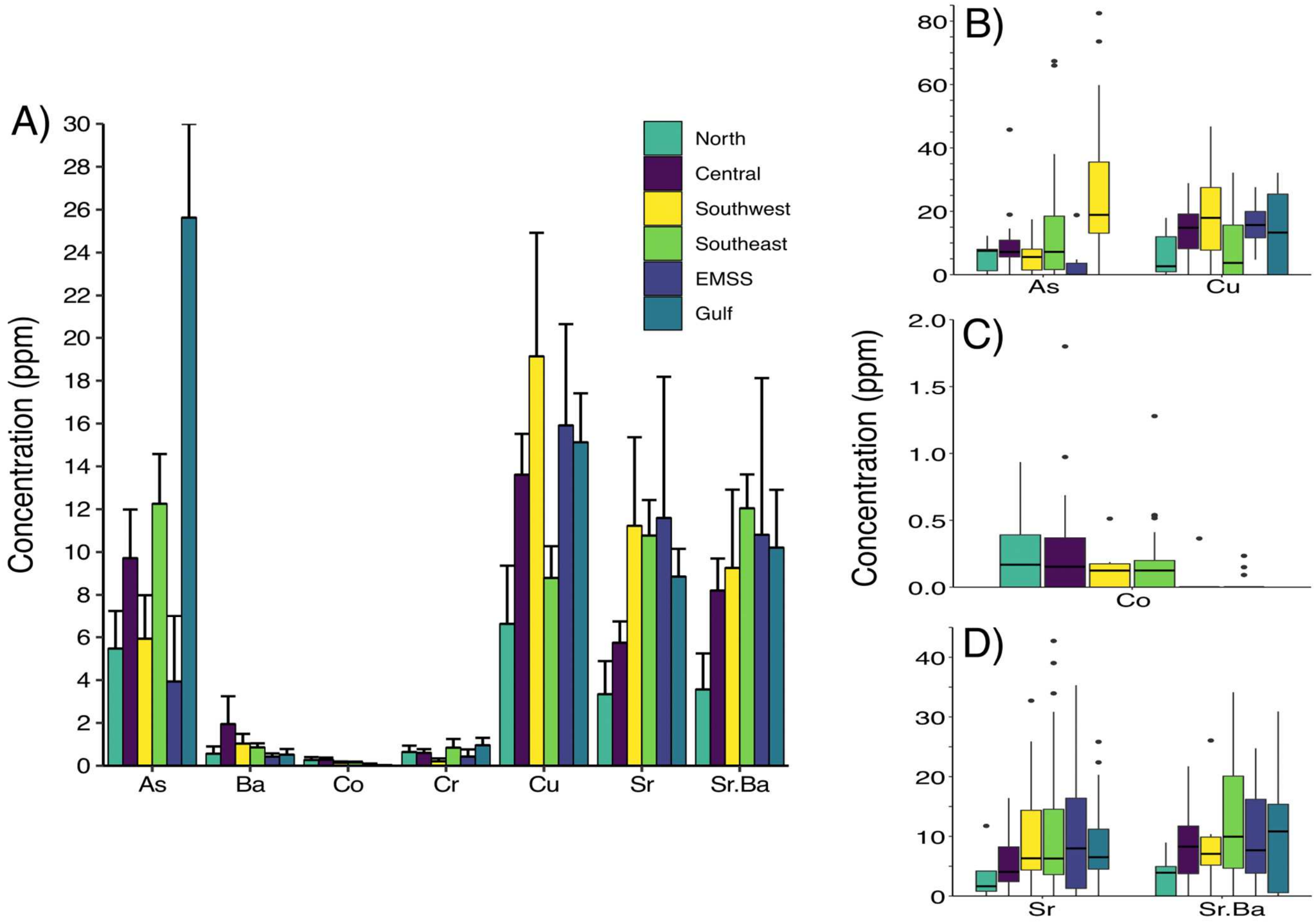

Figure 1.3. Average trace metal concentrations in parts per million (ppm) in dolphin skin, separated by region for all metals that did not show seasonal differences (A; Arsenic (As), Barium (Ba), Cobalt (Co), Chromium (Cr), Copper (Cu), Strontium (Sr) and Strontium to Barium ratios (Sr:Ba), with metals that differed by region also shown as box plots, rescaled for clarity (B-D). North (N), Central (C), Southwest (SW), Southeast (SE), EMSS (MS) and Gulf (G). The boxes represent the interquartile range, the black bars represent the median, and the dots outside the boxes represent values > 1.5 < 3 times outside the interquartile range.

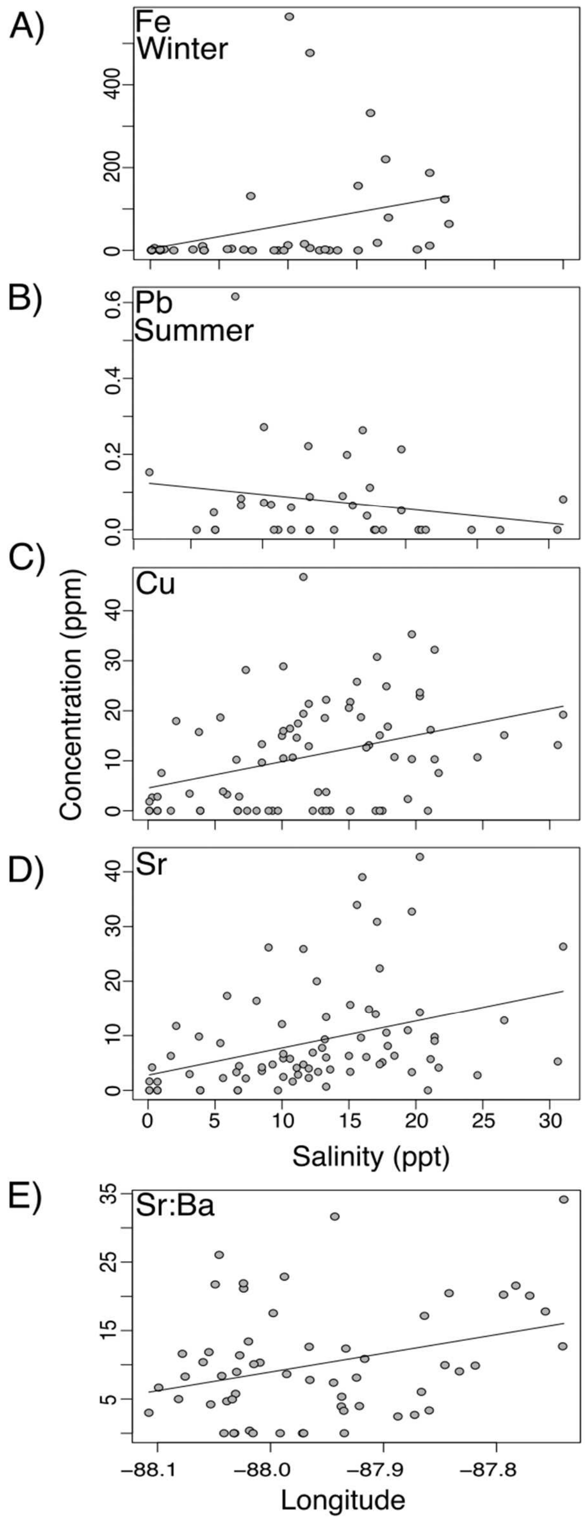

Figure 1.4. Metal concentrations in dolphin skin compared to salinity (A; Fe in winter: $y = 6.86x-27208.00$; B; Pb in summer: $y = -0.010x+13.12$; C; Cu: $y = 0.58x-1902.14$; D; Sr: $y = 0.49x+2.77$) and longitude (E; Sr:Ba: $y = 23.72x + 2093.37$) among animals sampled in Mobile Bay.

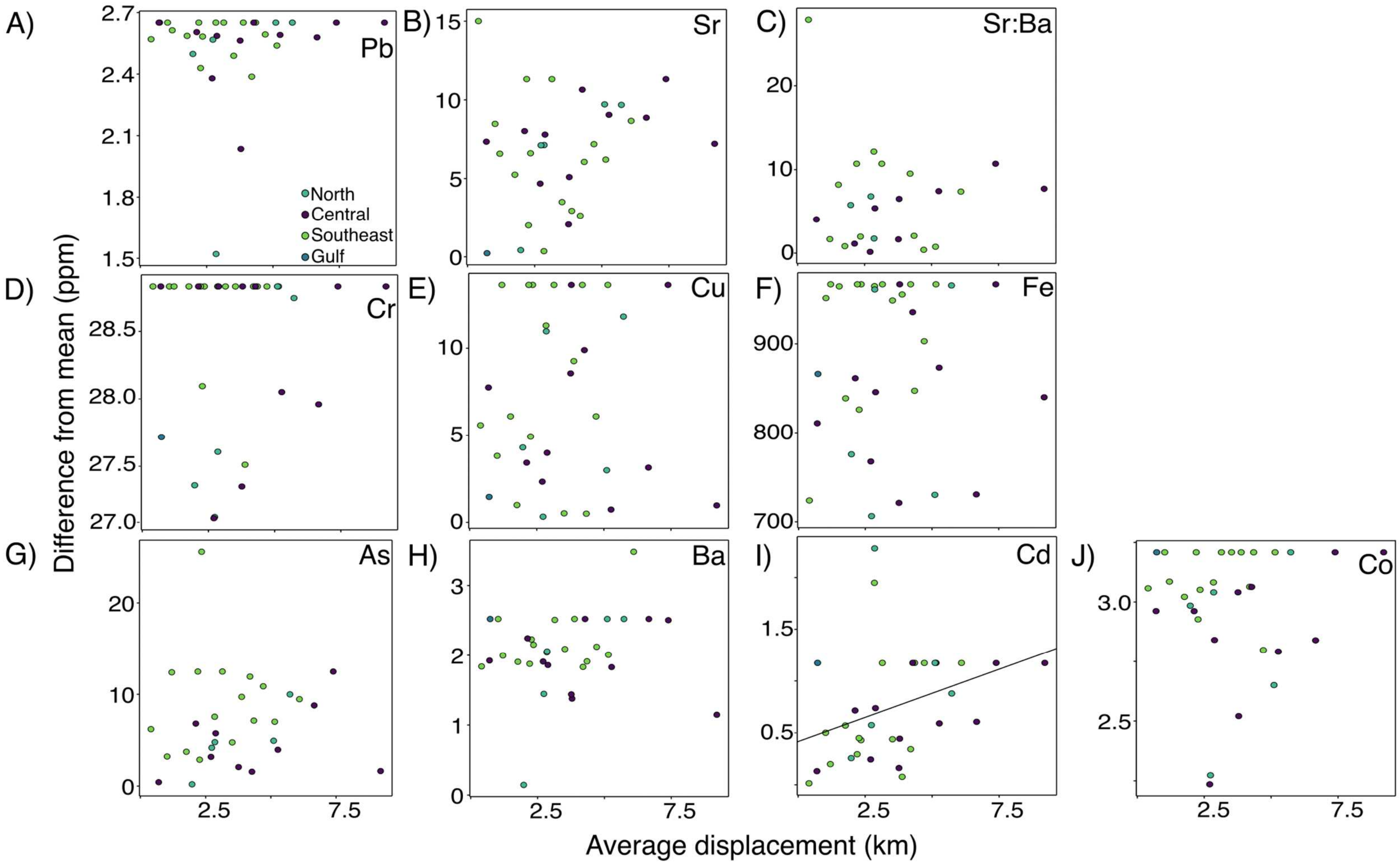

Figure 1.5. Variation (defined as the absolute difference from the mean) for As (G), Ba (H), Cd (I), Co (J), Cr (D), Cu (E), Fe (F), Pb (A), and Sr (B) and Sr:Ba (C) values in dolphin skin compared to the average displacement, for dolphins sighted ≥ 3 times. One point for Cr (1.5, 59.37) and Pb (6.09, 19.68) is not shown for clarity.

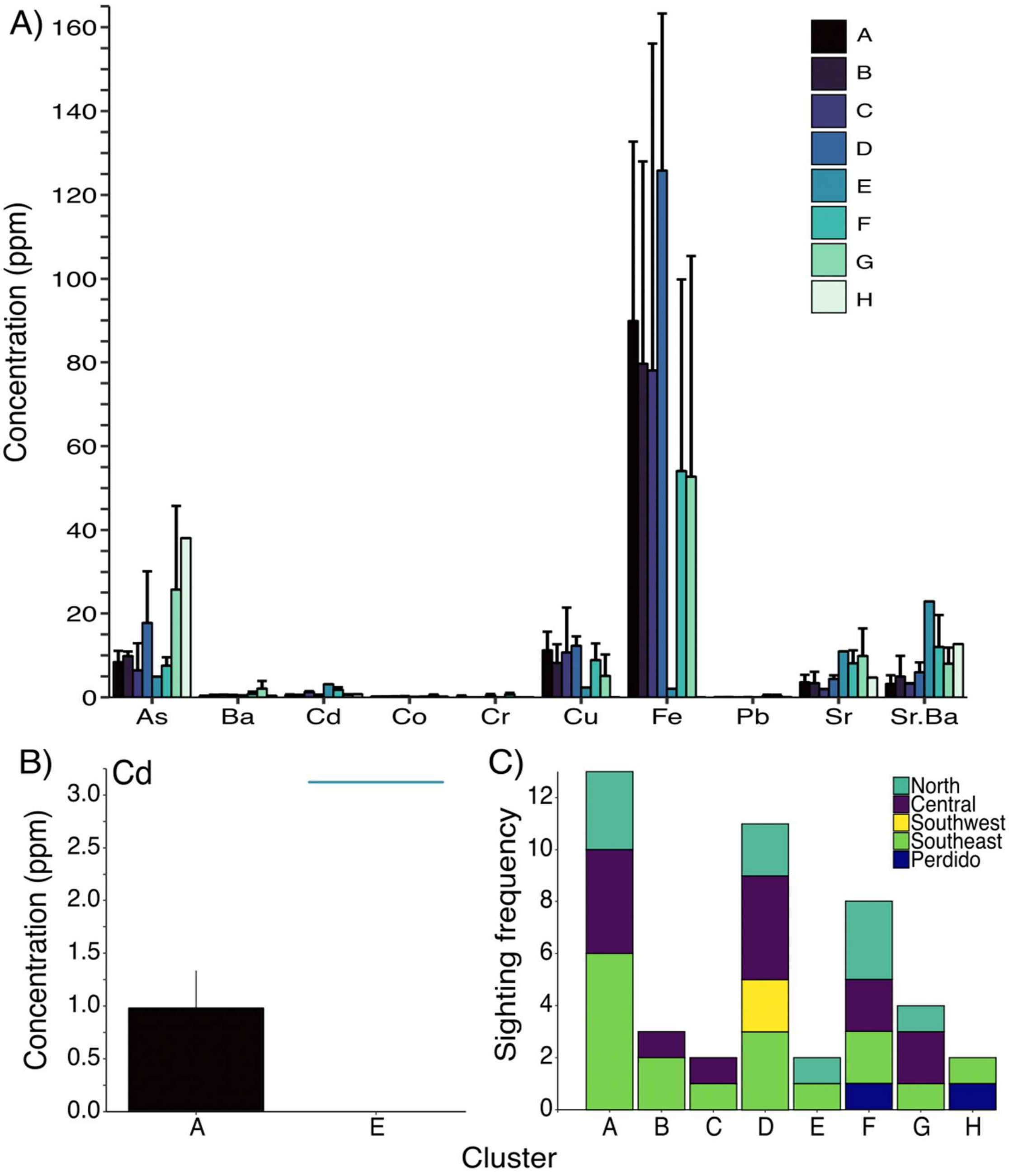

Figure 1.6. Trace element concentrations (A) of Arsenic (As), Barium (Ba), Cadmium (Cd; B) Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe) , Lead (Pb), Strontium (Sr) and Strontium to Barium ratios (Sr:Ba) in parts per million (ppm) in dolphin skin for different social groups (A-H) in Mobile Bay and nearby Perdido Bay, AL, defined by cluster analysis for individual dolphins with a minimum of 3 sightings (C), with Cd shown rescaled for clarity (bottom left) and sighting frequency and location shown for each cluster (bottom right), separated by regions defined in Figure 1.1.

CHAPTER III

SITE FIDELITY OF INDIVIDUALS AND GROUPS OF BOTTLENOSE DOLPHINS USING STABLE ISOTOPE ANALYSIS

Abstract

Little is known about movements and habitat use of bottlenose dolphins (*Tursiops truncatus*) in the northcentral Gulf of Mexico. In this study, I defined seasonal patterns of site fidelity of dolphins in Mobile Bay, Alabama and nearby waters of the Gulf of Mexico (Gulf) and Eastern Mississippi Sound (EMSS) using a combination of stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) in dolphin skin and direct observation by photo-identification. Skin samples were collected by remote biopsy darting during summer (June-August) and winter (December-February). To link site fidelity to diet, stable isotope ratios in dolphin skin were compared to ratios in prey species collected in the same season and location as dolphin samples. Dolphins acquired some location-specific stable isotope ratios indicative of site fidelity. $\delta^{13}C$ values increased with increasing salinity, with the most isotopic distinction at geographic (latitudinal) extremes. $\delta^{15}N$ values varied with longitude but not latitude or salinity. Accordingly, the isotopic niche of dolphins showed the greatest difference between animals sampled in North Mobile Bay and the Gulf or EMSS. Photo-identification data corroborated greater movement of individual dolphins within Mobile Bay than between the bay and Gulf or EMSS. These data demonstrate the usefulness of stable isotope data to complement traditional photo-identification methods

to define site fidelity and relationships between habitat use and location-specific diet. This approach can be applied to define spatial and temporal scales for habitat management and conservation strategies for dolphin or other cetacean populations throughout their range.

Introduction

The bottlenose dolphin (*Tursiops truncatus*) is a highly mobile, globally distributed species (Fair & Becker 2000, Wells et al. 2004). In many inshore areas, bottlenose dolphins exhibit high site fidelity (Mazzoil et al. 2005, Bassos-Hull et al. 2013, Passadore et al. 2018a, Cloyed et al. 2021b, Cloyed et al. 2021c), even to locations with unfavorable conditions such as low salinity waters (Mazzoil et al. 2008, McBride-Kerbet & Toms 2021, Takeshita et al. 2021). Because movements may be driven, in part, by food sources and availability, there can be tight links between site fidelity and dolphin diet (Shane et al. 1986, Neumann 2001, Santos-Carvallo et al. 2018, Haughey et al. 2020). Although dolphin diet can be diverse, including fish, crustaceans, and cephalopods (Shane et al. 1986, Barros 1990), individuals may feed within a relatively narrow habitat range (Cloyed et al. 2021a). Bottlenose dolphins are also known to form long-term social bonds (Maze-Foley & Würsig 2002, Bouveroux & Mallefet 2010, Wells et al. 2013) and may travel and feed in groups (Barros & Wells 1998, Gazda et al. 2005, Heithaus & Dill 2009) or alone (Barros et al. 1993, Barros & Wells 1998). Furthermore, there is evidence at least some populations exhibit individual variation in foraging and habitat use within groups or populations (Nowacek 2002, Sargeant et al. 2007, Rossman et al. 2015, Cloyed et al. 2021c). There is a need, therefore, to study site fidelity and diet at multiple spatio-temporal and population scales to understand relationships between dolphins and their habitat for conservation and management (e.g., Nowacek et al. 2016, Passadore et al. 2018b, McBride-Kebert & Toms 2021).

Observational methods, such as photo-ID, have been successfully used to track movement of individuals (Ballance 1992, Swingle et al. 1993, Saulitis et al. 2000), but are limited in application for understanding habitat use. Photo-ID is useful to track dolphins over long time frames because it relies on long-lasting markings such as notches and scars present on dorsal fins and bodies (Würsig & Würsig 1977, Würsig & Jefferson 1990, Urian 1999). Because dolphins are highly mobile, movements to and use of habitats or food resources outside locations and time periods of direct observation will be missed by photo-ID alone (Balmer et al. 2008, Haughey et al. 2020). Stable isotope ratios have been used to successfully define movement patterns and resource use within and among populations through time (Fry 2006). For example, stable isotope ratios can vary geospatially, with $\delta^{13}C$ generally increasing with salinity as organic matter inputs transition from terrestrial to marine sources (Fry 2002, Fry 2006, Abrantes et al. 2013). $\delta^{15}N$ may also vary along salinity gradients and with urbanization or type of land-derived wastewater influences to local waters (McClelland et al. 1997, Kovacs et al. 2010, Darrow et al. 2017). Stable isotope ratios also vary with trophic position, with $\delta^{13}C$ tending to conservatively reflect the base of the food web and $\delta^{15}N$ increasing 2-4‰ per trophic level (DeNiro & Epstein 1981, Rau 1981, Minagawa & Wada 1984). In marine mammals, carbon and nitrogen isotopic values have been found to vary seasonally, potentially due to shifts in feeding habitat, environmental conditions, or prey availability

(Olin et al. 2012, Guerra et al. 2020, Peters et al. 2020). Environmental conditions and prey availability together may determine stable isotope ratios in cetaceans at a given location (Newsome et al. 2007, Wilson et al. 2012, Wells et al. 2013), and therefore, they are promising as geographic and temporal tracers that can link diet and habitat use.

Previous studies indicate understanding links between diet and habitat use are likely to be key to defining site fidelity for many species. Across many mobile species, such as fish (Green et al. 2012, Vinagre et al. 2016), birds (Yohannes et al. 2007, Wakefield et al. 2015), turtles (Pajuelo et al. 2012, Hancock et al. 2018) and mammals (Olin et al. 2012, Smith et al. 2021) stable isotopes can identify subpopulations, characterize population connectivity, define site fidelity, and determine diet on finer scales that may not be captured by tracking or observational data alone. For example, live bottlenose dolphins show differences in stable isotope ratios in skin among bays in Louisiana, Alabama, and Florida, with location-specific variance within individual embayments (Cloyed et al. 2021b). Similarly, stable isotope values in sharks have demonstrated discrete habitat use and associated diets across a relatively small spatial scale in Mobile Bay, Alabama (Drymon et al. 2012). These differences within populations were driven by fluxes in environmental conditions and prey availability among sites, indicating embayment-level distinctions are possible even in highly mobile species.

The major objective of this study is to determine if dolphins acquire location-specific stable isotope ratios (δ^{13}C‰, δ^{15}N‰) indicative of site fidelity. To define stable isotope ratios across the range of local habitats and food resources, including along a salinity gradient, I analyzed skin from remotely biopsied dolphins and their prey species in Mobile Bay, Alabama and adjacent waters of the northern Gulf of Mexico and in dolphins from Eastern Mississippi Sound. Samples were collected during summer and winter in Mobile Bay to account for seasonal variation. To corroborate isotope-based site fidelity, biopsied dolphins were individually photo-identified and compared to images from ongoing capture-mark-recapture surveys in the study area. Resighting data were used to document the number and location of sightings, determine the influence of movement on variation in stable isotope values, and define social clusters for group site fidelity analysis. This study provides baseline data on dolphin movements, diet, and habitat use that are needed to inform spatio-temporal and population level scales for management and conservation strategies of dolphin populations in the study area and elsewhere.

Methods

Study site

Mobile Bay is a freshwater dominated estuary located in Alabama in the northern Gulf of Mexico, with a total area of 1070 km^2 (Mobile Bay NEP 2019; ~51 km long by ~37 km wide at maximum width). The bay is mostly ~3 m deep with a ship channel (~14 m deep) that runs mostly north-south (Figure 2.1), the Intercoastal Waterway (ICW) with an east-west orientation, and shallower channels (Schroeder et al. 1990, Mobile NEP 2008). Deeper channels are used by large shipping traffic, while the ICW and shallower channels are mostly used by smaller boat traffic More than 50 billion m^3/year of freshwater passes through the bay from the Mobile Bay watershed, with the highest discharges occurring in winter and spring, resulting in gradient of increasing salinity from north to south down the bay (Coogan & Dzwonkowski 2018, US Army Corps of Engineers 2018, Mobile Bay NEP 2019). Water from Mobile Bay drains west into eastern Mississippi Sound (EMSS; Eleuterius 1976) and south into the Gulf of Mexico (hereafter referred to as Gulf), widening influences of freshwater discharge into these regions (U.S. Army Corps of Engineers 2018). EMSS and the Gulf act as geographic endpoints in this study because of their potential connectivity to Mobile Bay, as well as their salinity regimes in connection with Mobile Bay flow patterns. Salinity is higher in the Gulf compared to Mobile Bay and increases with distance from the mouth of Mobile Bay (Coogan & Dzwonkowski 2019).

The current bottlenose dolphin population in Alabama waters is poorly documented in existing literature. Although some information is known about dolphin distribution and ranges (Goodwin 1985, Cloyed et al. 2021c), demographics and movement patterns of populations is more unknown. A diverse array of prey items are available to dolphins in Mobile Bay, including crustaceans (such as crabs and shrimp), cephalopods (such as squid) and many fish species (about 140). Fish species are mainly comprised of Spot, Atlantic Croaker, Gulf Menhaden, Bay Anchovy, Threadfin Shad and Blue Catfish in Mobile Bay and in the Gulf, Red Snapper (Beane et al. 2020).

Data Collection

Sample collection.

Dolphin skin samples were collected by remote biopsy sampling during winter (December 2019 to February 2020) and summer (August -September 2020) in Mobile Bay, AL; August to October 2019 in EMSS; and December 2019 to February 2020 in the Gulf. Samples were collected in one season for EMSS and Gulf due to logistics, including weather and COVID-19 pandemic limitations. These samples were not used for seasonal comparisons, but they were included to provide western and southern geographic endpoints for regional comparisons. Biopsies (N=115) were obtained from live dolphins with a specialized crossbow (Barnett Panzer V, 68 kg draw weight), following Sinclair et al. (2015) and as described in Chapter 1. Samples were collected under the National Marine Fisheries Service (NMFS), Marine Mammal Protection Act and Endangered Species Act Permit # 21938 and NMFS Atlantic Institutional Animal Care and Use Committee (IACUC) authorizations 2017-001 and 2020-002.

Prey species were collected concurrently with biopsy surveys in Mobile Bay and the Gulf. Prey sampling was conducted near the time and locations of biopsy surveys to broadly capture available prey species that may contribute to dolphin diet. Samples were collected by trawling for 20 minutes with a 16-foot otter trawl (7.6 m wide, 3.8 cm mesh) at six sites (Figure 2.1), corresponding to regions of Mobile Bay (North, Central, Southwest, Southeast) and in the Gulf, where samples from two subsites combined. Samples were sorted by species and size, and muscle tissue from up to six individuals per species per site was dissected from the whole specimen and stored at -20°C until sample processing. All fish, crustaceans, and cephalopods that may be prey of dolphins were used for analysis (Barros 1990, Bowen-Stevens et al. 2021), with less common species such as eels, sea pansy, and spadefish excluded. Prey samples were not available for EMSS during the biopsy period, and that area is excluded from prey analyses.

Stable isotope sample analyses.

Dolphin skin was prepared for analysis as described in Chapter 1, using the same sample that was non-destructively analyzed for trace metals analysis. Prey samples were rinsed thoroughly with ultrapure water and dried at 60°C for 24–48 hours, to a constant weight. Both dolphin and prey tissues were ground with a Beadbug 6 Microtube Homogenizer at 5000 rpm, 45s, for 5 cycles. All samples were packed in tin capsules and sent to the University of California Davis Stable Isotope Facility (Davis, CA, USA) where carbon and nitrogen stable isotope ratios were determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS; Sercon Ltd., Cheshire, UK). As internal controls, tins were analyzed along with an acetanilide standard (Costech) of known isotope ratio and pseudo-replicates of randomly chosen samples, representing ~10% of the total sample number to account for variation due to sample handling and instrument reproducibility. Repeated analysis of in-house reference materials showed that precision (SD) was ±0.08‰ and 0.09‰ for carbon and ±0.05‰ and 0.08‰ for nitrogen, for dolphin and prey samples respectively, which are below the long-term values for instrument reproducibility (https://stableisotopefacility.ucdavis.edu). Average differences in pseudoreplicates (±SE) were 0.39±0.46‰ and 0.10±0.09‰ for carbon and 0.19±0.20‰ and 0.07±0.05‰ for nitrogen for dolphin and prey samples respectively. Because dolphin skin samples obtained by remote biopsy were too small for lipid extraction, I applied a mass balance lipid-correction model, established with dolphins in the study area (Cloyed et al. 2020).

Photo-identification.

To identify dolphins that were biopsied and continue to track their movements, darted animals were photographed, and these photographs were compared to images from capture-mark-recapture surveys conducted during the same time period as biopsy sampling and from biopsy surveys. High-resolution digital cameras (Canon 7D and 90D) fitted with 100-400mm lenses were used to photograph the dorsal fins of individual dolphins in the study area, and photographs were processed using FinBase and FinFindR (Adams et al. 2006, Melancon et al. 2011, Thompson et al. 2022) as described in Chapter II.

Data Analysis

Isotope-based site fidelity.

To determine if individual dolphin stable isotope values (δ^{13}C‰ and δ^{15}N‰) varied by geographic location in each season, I used general linear models with a gaussian distribution and *identity* link using R. I first made seasonal comparisons among regions in Mobile Bay. When seasonal differences in stable isotope ratios were not found, data for summer and winter were combined for subsequent analyses and Gulf samples were added for comparisons as a geographic endpoint. EMSS samples were not included due to the limited sample size and time of collection. A global model was constructed with each isotope ratio as a response variable and latitude, longitude, salinity, and season as explanatory variables. The model also included interactions between season and salinity, latitude, and longitude. All subsequent methods for general linear models, including the use of Akaike Information Criterion (AIC) values and Akaike weights (*w*) to identify the best fitting model, were applied as described in Chapter 1.

To compare stable isotope ratios in individual dolphin tissues among assigned regions (North, Central, Southwest, Southeast, EMSS, or Gulf; Figure 2.1), I conducted Stable Isotope Bayesian Ellipses in R (SIBER; Jackson et al. 2011) using $\delta^{13}C$‰ and $\delta^{15}N$‰ values of dolphin skin (N=115) and prey (N=283) samples. SIBER calculated the convex hull total area (TA), which encompasses all data points, standard ellipse area (SEA) and standard ellipse area adjusted for small sample sizes (SEAc) for each region, where the ellipses represented 95% credible intervals. SIBER also calculated the Bayesian overlap of each ellipse (one ellipse per region) to all other ellipses. Ellipses that have more overlap are considered closer isotopically, while ellipses that have less overlap are considered less similar (Layman et al. 2007, Jackson et al. 2011).

To define relationships between diet and site fidelity, stable isotope ratios in prey species and dolphins were plotted together using R. To control for variation in species composition among sites, in cases where prey species were not found at all sites, prey species were categorized into taxonomic groups for comparisons (Appendix B, Table B2; Anchovy, Ariidae, Cephalopoda, non-anchovy Clupeiformes (hereafter referred to as Clupeiformes), Decapoda, or Perciformes).

Photo-identification based site fidelity.

To corroborate site fidelity for individual dolphins in the study area using photo-ID data, I tested the effect of observed movement on variation in stable isotope ratios. To do this, I calculated the average displacement per sighting for each individual dolphin by plotting the location of all sightings of identified biopsied animals (QGIS version 3.16), and divided the average displacement between sightings by the number of sightings. The average displacement was calculated for each biopsied animal sighted 3 or more times, except for a single animal that was darted twice. I then calculated individual isotopic variation from each regional mean as the absolute difference between each dolphin's stable isotope ratio and the mean stable isotope value for the region in which the animal was biopsied and compared this difference to the average displacement. I conducted a linear regression and F test to identify relationships between stable isotope variation and average displacement in R (Version 1.4.1717; R Core Team 2009).

To determine site fidelity of dolphin groups, I conducted an association analysis of individual biopsied dolphins and all dolphins resighted during capture-mark-recapture and biopsy surveys using SOCPROG version 2.9 (Whitehead 2009), as described in Chapter 1. To determine if social groups identified by the association analysis had location-specific stable isotope ratios, among group comparisons were made using a one-way ANOVA and a Tukey post-hoc test in R (Version 1.4.1717; R Core Team 2009).

Results

A total of 115 dolphin skin samples were collected from remotely biopsied dolphins during the study period (Appendix B, Table B1; 80 from Mobile Bay, 29 from the Gulf, and 6 from EMSS). Of these samples, 105 were analyzed for seasonal and environmental comparisons and 115 were analyzed for regional niche comparisons. Of the samples collected in Mobile Bay, 40 were collected in summer and 40 in winter (Figure 2.1). A total of 238 prey samples were analyzed (ranging from 2-19 cm in total length, depending on species) with N=187 (15 species) for winter and N=51(13 species) for summer (Appendix B, Table B2).

Stable isotope-based site fidelity

Stable isotope ratios in dolphins and prey.

Stable isotope ratios in dolphin skin ranged from -24.19 to -16.93 ‰ for δ^{13}C‰ and 13.46 to 16.74 ‰ for δ^{15}N‰ (Figure 2.2). Regionally, average δ^{13}C‰ values in dolphin skin were slightly lower in North and Central regions (ranging -19 to -21 ‰) than in southern and Gulf regions (-22 ‰), while average δ^{15}N‰ values were 15 to 16 ‰ in dolphins from all regions (Figure 2.2, Appendix B, Table B1). δ^{13}C‰ values in prey were also lower in the North and Central regions (ranging -24 to -27 ‰) compared to southern and Gulf regions (-19 to -25 ‰), and δ^{15}N‰ values were 12 to 16 ‰ across regions (Figure 2.2; Appendix B, Table B2). The range of dolphin and prey carbon values were most constrained in the North region and widest in the Southwest and Southeast regions (Figure 2.2).

Seasonal and environmental comparisons.

Season had no effect on δ^{13}C‰ or δ^{15}N‰ values in dolphin skin. δ^{13}C‰ values in dolphin skin increased with salinity and marginally decreased with latitude (Figure 2.3). The best fitting model to explain patterns of δ^{13}C‰ in dolphin skin contained salinity, latitude, and longitude as variables, but only salinity was significant (w=0.32, salinity: p<0.001; Appendix B, Table B3). Latitude was included in the second-best fitting model with salinity (w=0.27, salinity: p<0.001, latitude: p<0.06; Appendix B, Table B3). Two additional models had Akaike weights > 0.1, salinity and longitude (w=0.18) and salinity alone (w=0.11). There was no significant effect of latitude or longitude alone on δ^{13}C‰ (Figure 2.3). δ^{15}N‰ values in dolphin skin decreased with longitude, and the best fitting model to explain patterns of δ^{15}N‰ in dolphin skin included longitude alone (Appendix B, Table B3; w=0.37, p<0.001). Three additional models had weights > 0.1, including latitude and longitude (w=0.19), salinity and longitude (w=0.16) and salinity, latitude, and longitude (w=0.14), but there were no significant effects of latitude, salinity, or season alone on δ^{15}N‰ (Figure 2.3).

Regional niche comparisons.

SIBER models indicated some isotopic distinction between regions that were most geographically separated (Figure 2.4). The North and Gulf regions had the smallest adjusted ellipse areas and smallest standard ellipse areas (excluding EMSS) for dolphin and prey species, respectively (Figure 2.4, Table 2.1). There was high niche overlap among regions for dolphins (>25%) and prey (>51%), and overlap generally decreased with increasing distance between regions (Figure 2.4, Table 2.2). As a result, ellipses for the North and Gulf regions overlapped the least and the North overlapped the most with nearby regions like Central and Southwest (Table 2.2). EMSS also had less overlap with other regions, excluding the Southeast. Despite overall high overlap (>51%) among regions for prey species, overlap among regions had a similar pattern to dolphins (Figure 2.4; Table 2.2).

Corroboration of site fidelity using photo-identification

Site fidelity of individuals.

Thirty-five dolphins were sighted 3 times or more and were used to corroborate site fidelity with resighting data (see Chapter 1). Some (N=11, 31%), were sighted in their respective biopsied region and did not travel outside of this region. Others were seen in two (N=18, 51%) and three (N=6, 17%) regions, as described in Chapter 1. There was no relationship between variation in stable isotope ratios and average displacement between sightings for δ^{13}C‰ (Figure 2.5; $F_{\text{reg}34}=0.093$, $p=0.76$). Variation δ^{15}N‰ values decreased with displacement (Figure 2.5; $y=-0.08x+0.78$, $R^2=0.23$, $F_{\text{reg}34}=9.90$, $p<0.01$). For both δ^{13}C‰ and δ^{15}N‰, dolphins biopsied in the Southeast region had the largest isotope variation from the mean of the region in which they were biopsied, while dolphins biopsied in the North had the least in comparison to other regions. Dolphins biopsied in the Central region had the most variation in displacement, on average traveling the longest distances between sightings (Figure 2.5).

Site fidelity of groups.

The association analysis revealed 8 clusters of social groups (designated A-H) amongst biopsied dolphins sighted 4 or more times and their associates, with the overall mean association index at 0.32 (SD=0.33). The social differentiation estimate (S) was 0.679, and the R value was 0.185, below the threshold (0.4) for confidently representing the population. Some clusters (A, B, E, and F) contained very high levels of associations (i.e., some individuals had HWI values of ≥ 0.8) (Appendix A, Figure A3). The number of individuals within clusters ranged from 1 to 21, with 2 clusters having a single individual.

For $\delta^{13}C$‰, there were no differences among clusters (Figure 6; one way ANOVA, F=0.65, df=7, *p*=0.71). For $\delta^{15}N$‰ there was a difference between clusters H and G (Figure 2.6; one way ANOVA, F_{reg8}=2.76, *p*=0.04; Tukey post hoc test: *p*=0.02; Appendix B, Tables B4 and B5). Members of each of the 8 clusters were sighted in multiple regions, but primarily in the North, Central and Southeast (Figure 2.6).

Discussion

I found that dolphins acquired location-specific stable isotope ratios indicative of site fidelity, particularly with increasing geographic distance between sites. For carbon, regional isotopic variation was primarily driven by salinity. $\delta^{13}C$‰ values increased from north to south (with decreasing latitude), where salinity typically increased in the same direction. Differences were greatest when comparing Mobile Bay regions as a whole to EMSS and the Gulf. These findings align with previous studies that have cited salinity as a potential driver of isotope values in systems that have high variation in salinity or when comparing geographic extremes (Browning 2013, Genoves et al. 2020, Cloyed et al. 2021b). Accordingly, the greatest overlap of isotopic niche space for prey and dolphins was among regions within Mobile Bay, with the least overlap to EMSS and the Gulf. During the study period in 2020, a record volume of freshwater was discharged through the Mobile-Tensaw river basin and into Mobile Bay (Committee on Commerce, Science and Transportation 2020), drastically decreasing salinity values. These conditions likely contributed to some homogenizing of salinity conditions (and likely prey resources) in Mobile Bay prior to and during the winter sampling period. Hence, it is particularly noteworthy that dolphins still acquired location-specific isotope ratios relative to salinity inputs, and these conditions may explain why δ^{13}C‰ values were less tightly linked to latitude than salinity and most different at larger spatial scales.

In contrast, δ^{15}N‰ values varied with longitude rather than latitude, potentially due to a combination of factors. The observed decrease in δ^{15}N‰ values with longitude were driven by a discrete subset of animals (N =3) darted in the Southeast region of Mobile Bay that had depleted δ^{15}N‰ values. Taken alone, these isotope data could indicate that dolphins farther east were eating at lower trophic levels or that sources of dissolved inorganic nitrogen that support the base of the food web were different or isotopically depleted in the Southeast region compared to the rest of Mobile Bay. Previous studies have shown longitudinal trends in δ^{15}N‰ values (Kurle & Worthy 2002, Miyajima et al. 2009, Cardona-Marek et al. 2009, Laakman & Agul 2010), including among stranded dolphins, where lower δ^{15}N‰ values were found in dolphins from Perdido compared to those from adjacent waters (Murray et al. unpublished, Cloyed et al.

2021c). Because $\delta^{15}N‰$ values in dolphin skin responded to different spatially discrete environmental factors than $\delta^{13}C‰$, these findings emphasize the importance of using multiple indicators to define geospatial relationships.

The resolution of dolphin movement in this study was much improved with the addition of photographic data. Mapping of animal sighting locations confirmed that there was some high movement and overlap within regions of Mobile Bay, especially at mid-latitudes, but little to no observed connectivity between Mobile Bay, the Gulf and EMSS. Previous data from dolphins tagged around Dauphin Island showed that although there is some connectivity of dolphins between EMSS and Mobile Bay, this is mostly limited to the western side of the bay (Cloyed et al. 2021b). Other studies have found varying levels of overlapping communities within dolphin populations, ranging from high (Gubbins 2002, Fazioli et al. 2006, Laska et al. 2011) to little or no overlap (Urian et al. 2009, Tyson et al. 2011). In these cases, most communities that overlapped highly did so on smaller spatial scales than this study, and those that overlapped less were still studied on similar but smaller spatial scales. Mapping of photographic data also revealed that half of the samples with low nitrogen values in Mobile Bay were from animals that also use neighboring Perdido Bay. Perdido Bay is located to the east and hydrologically connected to Mobile Bay by the intracoastal waterway. Dolphins in Perdido Bay were not analyzed as part of this study, but data were available as part of a larger photo-ID catalog for the area. Other individuals with low $\delta^{15}N‰$ values were either not photographed when darted and could not be tracked or had resighting frequency that was too low to corroborate repeated use of southeast Mobile Bay or Perdido. Dedicated photo-ID efforts in the Gulf or EMSS would likewise enhance our investigation of movement patterns of dolphins to and from these regions.

Regional variation of stable isotope ratios in dolphins did not strictly follow patterns observed in prey. Prey species showed greater mean isotopic differences among regions than dolphins but had broader niche space and overlap. Dolphins are known to consume a mixture of the various prey groups analyzed in this study (Barros & Wells 1998, Bowen-Stevens et al. 2021, Cloyed et al. 2021c). These findings could result from dolphins selectively feeding among available prey but not consuming all available prey or the same quantities or types of prey among locations. Previous findings for dolphin diet in the northern Gulf of Mexico and elsewhere indicate that variances in stable isotope ratios may be explained by interannual variation in prey availability and environmental gradients, particularly salinity, as observed during this study (Barros 1990, Berrens McCabe et al. 2010, Wilson et al. 2012, Wells et al. 2013, Wilson et al. 2017, Cloyed et al. 2021b). Bottlenose dolphins have been previously designated as flexible generalists and could potentially adjust to changes in environmental conditions by shifting diet if necessary (Cloyed et al. 2021c), making them more resilient if prey availability and abundance change. Furthermore, these findings could show evidence for site-specific isotopic ratios even as preferred prey items shift, indicating that this method can be used in a dynamic system such as Mobile Bay.

Observed movement from the biopsy location did not appear to contribute to variation in stable isotope ratios of carbon but did for nitrogen. Variation in δ^{15}N‰ values decreased with displacement, suggesting that dolphins in this study acquired more consistent stable isotope ratios in skin when they traveled greater distances. These findings are counter to our expectations if mean stable isotope ratios differ among regions, and biopsy locations represent the region where animals spent the majority of time. For example, in this study dolphins that moved less across a stronger $\delta^{15}N‰$ gradient (such as with animals darted in the Southeast) had values that deviated more from the overall mean than those that moved more across a weaker $\delta^{15}N‰$ gradient (such as with animals darted in the Central region). This analysis, however, did not account for which direction the displacement occurred, meaning that if it did not occur along the environmental gradient that mediated isotopic variation, trends may not be detectable. Lack of strong relationships between average displacement and isotope variation could also be due to dolphins moving to an area but not necessarily feeding in that region. This analysis indicates that absolute displacement alone cannot be used as a proxy for variation in elemental ratios and that the spatial gradient of elemental ratios must be known.

Association and cluster analyses revealed that social clusters had similar isotopic ratios indicating that individuals within clusters could be foraging in the same general regions or regions of similar environmental gradients. No clusters were different in terms of $\delta^{13}C‰$, but two clusters were different in terms of $\delta^{15}N‰$, likely because an individual within one of the clusters used Perdido Bay, a more remote foraging location that likely has a lower $\delta^{15}N‰$ baseline compared to Mobile Bay. This pattern is consistent with the finding that $\delta^{15}N‰$ was affected by longitude. Notably, of these clusters, one cluster contained two darted individuals, and the other a single individual, meaning that these findings are based off a low sample size. Additionally, the statistics from the association analysis indicate that these results may not be representative of the true social structure in our study area (R value < 0.4) and could change with additional data. Previous studies in the Gulf of Mexico have found higher association indices than my study (Bouveroux & Mallefet 2010, Wells 2013), but were long term studies with larger numbers of resightings per individual. Additional resightings will contribute to this dataset and allow defining stronger associations among individuals throughout the study area to better corroborate group fidelity in the future.

Defining site fidelity using only observational methods requires long term study and is not always feasible. This study demonstrates the benefits of combining photo-ID data with stable isotope data to better understand relationships between animal movement and habitat use. Stable isotope analysis can be used to estimate diet and nutrition, even when study animals are not being directly observed, and can account for movement outside study regions, which is key when working with highly mobile species such as marine mammals. These multi-disciplinary methods can be applied to other mobile species, not limited to cetaceans, and species in other systems to characterize site fidelity patterns. The data from this study indicate that individual dolphins show sufficient site fidelity to acquire location-specific stable isotope ratios relative to local diet and environmental attributes. The spatial scale at which site fidelity was detectable depended on the scale of isotopic variation across locations. Studying the overlap of individuals and groups of dolphins is important to understanding the scales at which populations become discrete and may use different resources or need to be managed differently. The limited apparent overlap between dolphins in within Mobile Bay and adjacent areas in the Gulf and EMSS may mean these habitats and potential resident dolphin populations will benefit from location-specific management. These data further establish baseline stable isotope ratios in free-ranging dolphins in Alabama waters and define site fidelity parameters that can be monitored in the future for changes in conjunction with environmental shifts that may have implications for dolphin habitat and health.

Tables

Table 2.1. Total area of convex hull (TA), standard ellipse area (SEA) and standard ellipse area adjusted for small sample sizes (SEAc) for dolphin and prey isotopes ($\delta^{13}C$ ‰, $\delta^{15}N$ ‰) from each region shown in Figure 2.1.

Source	**Region**	**TA**	**SEA**	**SEAc**
Dolphin	North	1.69	1.15	1.39
	Central	5.70	1.70	1.79
	Southwest	2.56	1.43	1.66
	Southeast	15.06	3.78	3.87
	EMSS	1.61	1.37	1.71
	GOM	4.92	1.50	1.55
Prey	North	38.86	11.92	12.30
	Central	38.08	9.69	9.97
	Southwest	47.31	12.21	12.39
	Southeast	45.07	11.60	11.94
	GOM	51.34	11.43	11.53

Table 2.2. Bayesian overlap of regions (shown in Figure 2.1) from dolphin and prey stable isotope ($\delta^{13}C$ ‰, $\delta^{15}N$ ‰) values. Regions that had the least overlap are shown in bold. Prey species were not available for EMSS. The reference region is the region at the top of column (for example, the top-right value, 35.4, is the percentage of the GOM ellipse that overlaps with the ellipse, whereas the bottom-left value, 25.3, is the proportion of the North ellipse that overlaps with the GOM ellipse).

Source	Region	North	Central	Southwest	Southeast	EMSS	GOM
Dolphin	North	-	78.8	57.3	97.6	39.5	35.4
	Central	48.4	-	54.7	90.9	36.3	56.8
	Southwest	33.5	52.1	-	71	32.2	40.8
	Southeast	**27.8**	42.1	34.5	-	**29.8**	33.8
	EMSS	**29.3**	43.8	40.8	77.5	-	38.2
	GOM	**25.3**	50.8	37.3	84.9	36.8	-
Prey	North	-	57.1	64.4	66.4	NA	55.0
	Central	71.1	-	73.8	77.3	NA	67.8
	Southwest	63.9	58.7	-	77.5	NA	85.4
	Southeast	75.6	70.7	89.1	-	NA	75.8
	GOM	51.1	57.2	83.8	75.8	NA	-

Figures

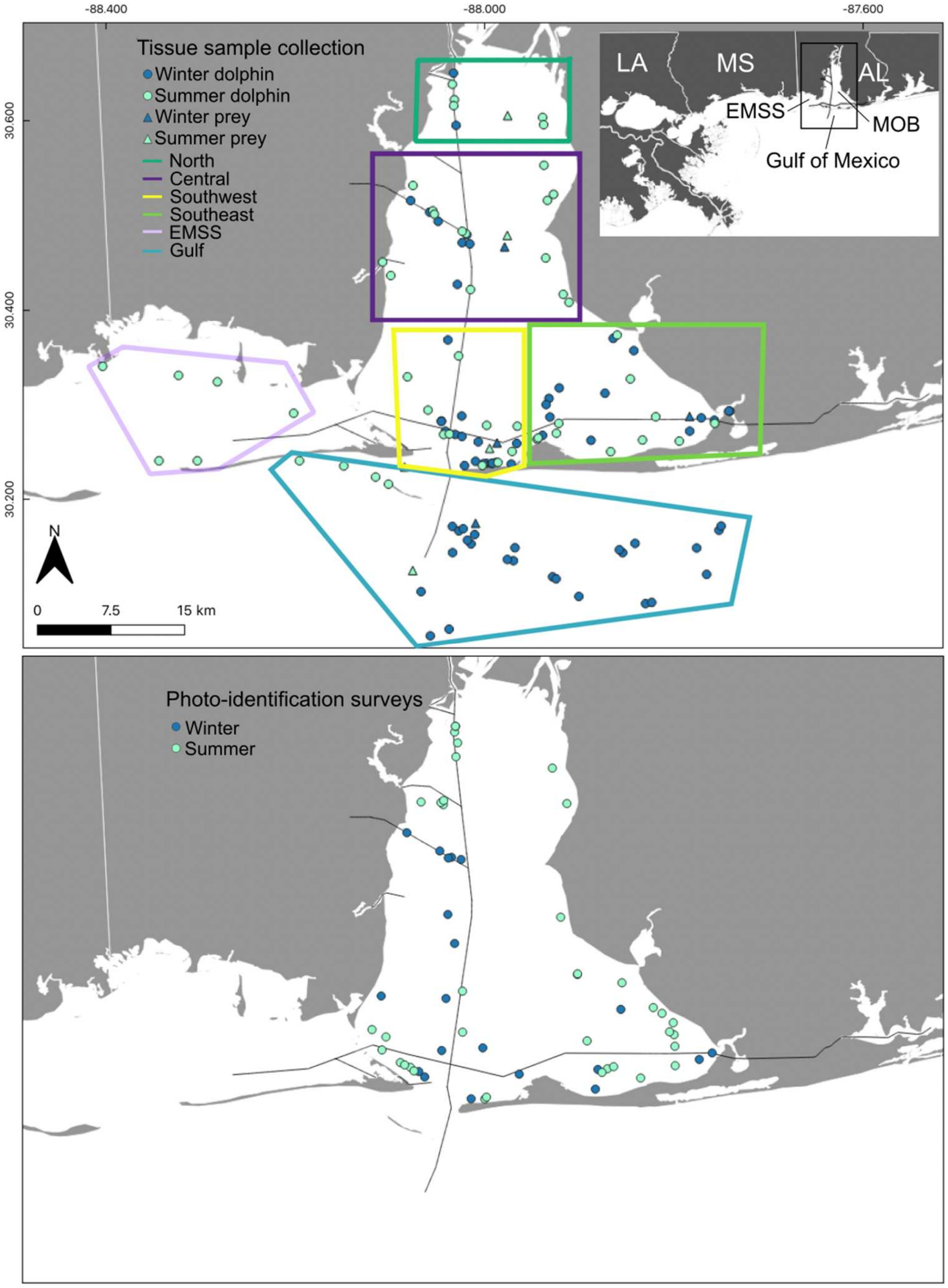

Figure 2.1. Locations of seasonal remote biopsy and prey species sampling (top) and photo-ID capture-mark-recapture surveys where biopsied individuals were sighted (bottom) in Mobile Bay, AL (MOB) and adjacent waters of eastern Mississippi Sound (EMSS) and the Gulf. Colored polygons indicate regions (North, Central, Southwest, Southeast, EMSS, Gulf) used for testing site fidelity. Black lines indicate ship channels.

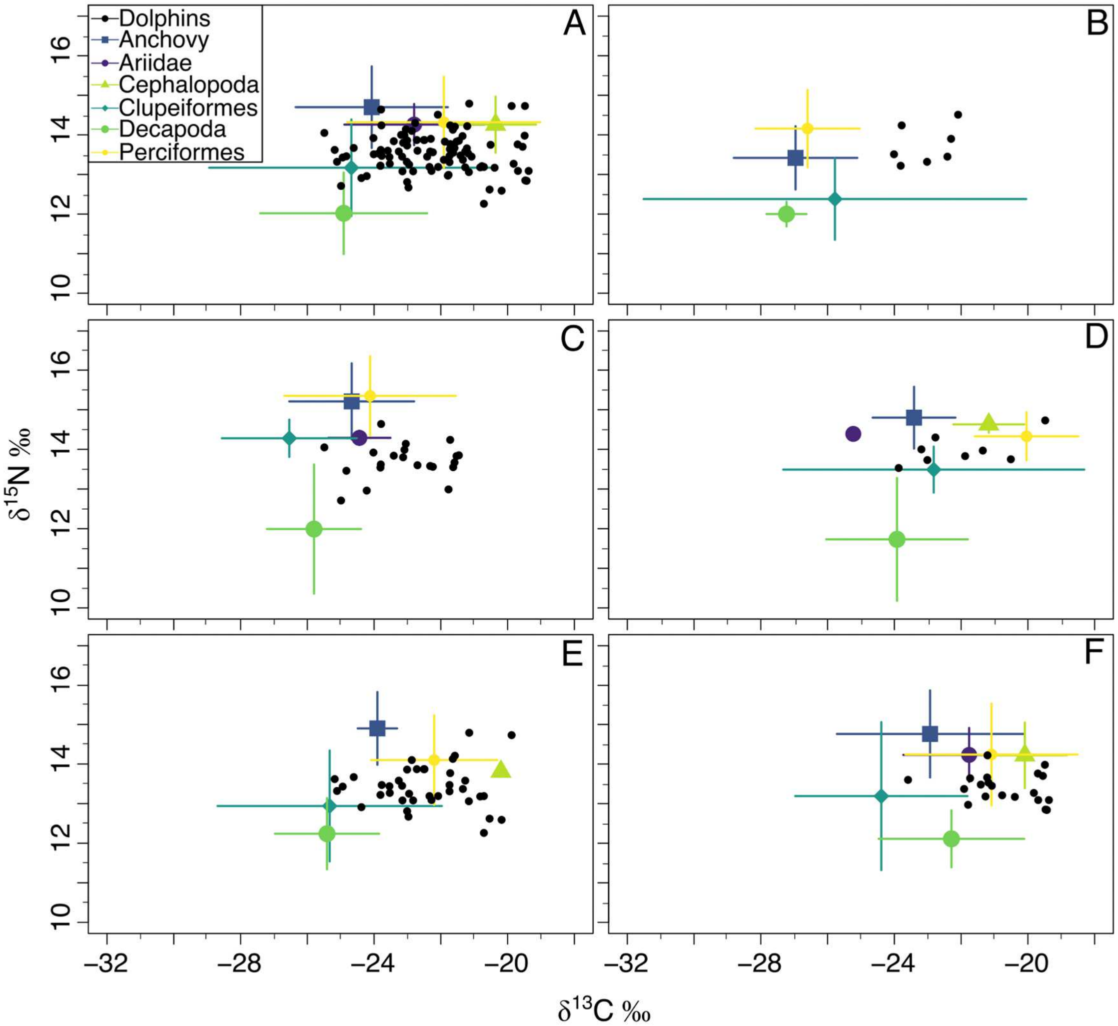

Figure 2.2. δ^{13}C ‰ and δ^{15}N ‰ in dolphin skin and prey muscle (Anchovy, Ariidae, Cephalopoda, Clupeiformes, Decapoda and Perciformes) from all regions combined (A), North (B), Central (C), Southwest (D), Southeast (E), and Gulf (F).

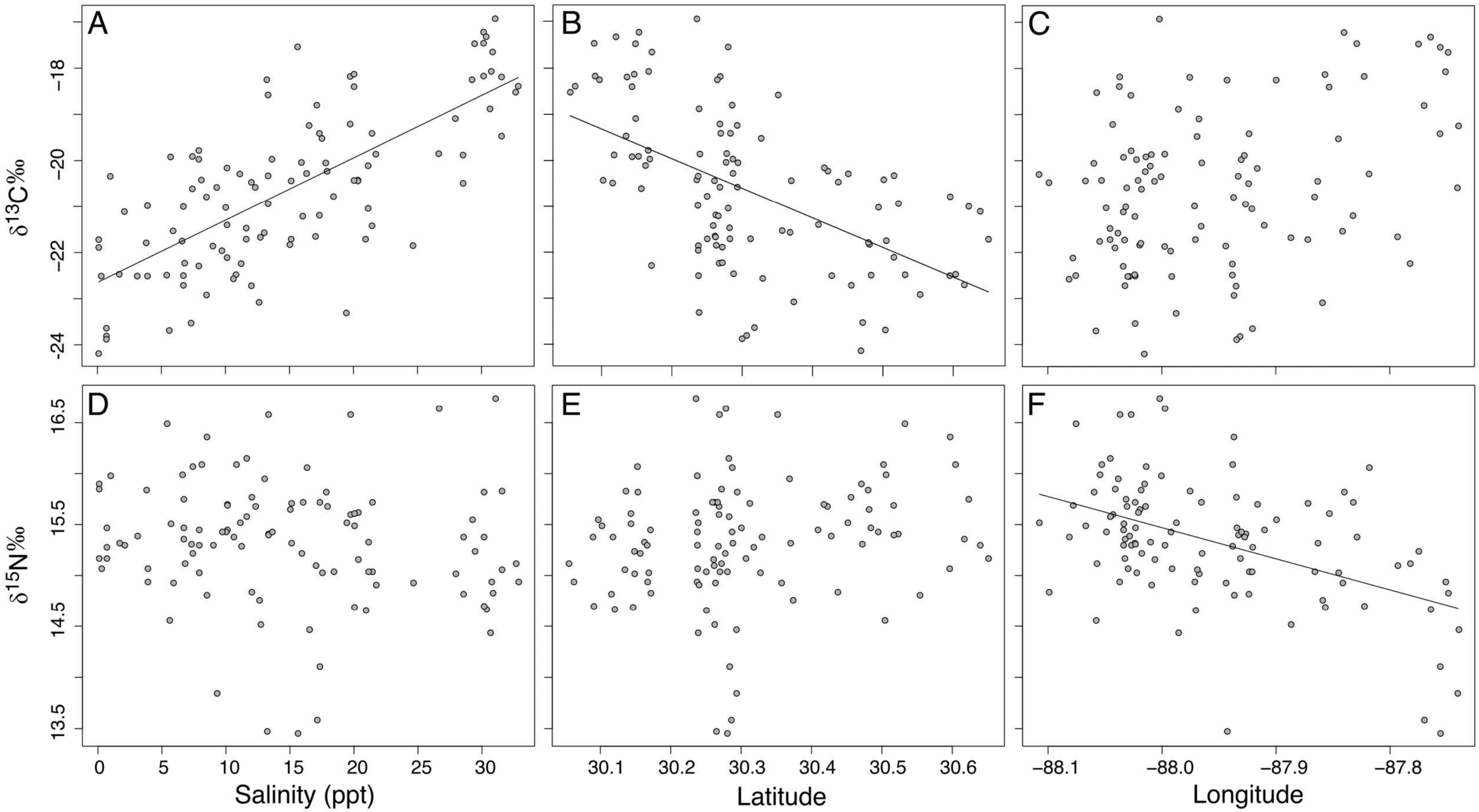

Figure 2.3. δ^{13}C ‰ (top) and δ^{15}N ‰ (bottom) in dolphin skin compared to salinity (A,D), latitude (B,E) and longitude (C,F) in Mobile Bay and Gulf regions for all seasons combined. Graphs with lines (panels A; y=0.11x+204.35, and F; y=-3.05x-253.39) represent significant relationships between isotope and factor, while panel B (y=1.97x+204.35) represents a marginally significant relationship between carbon and latitude.

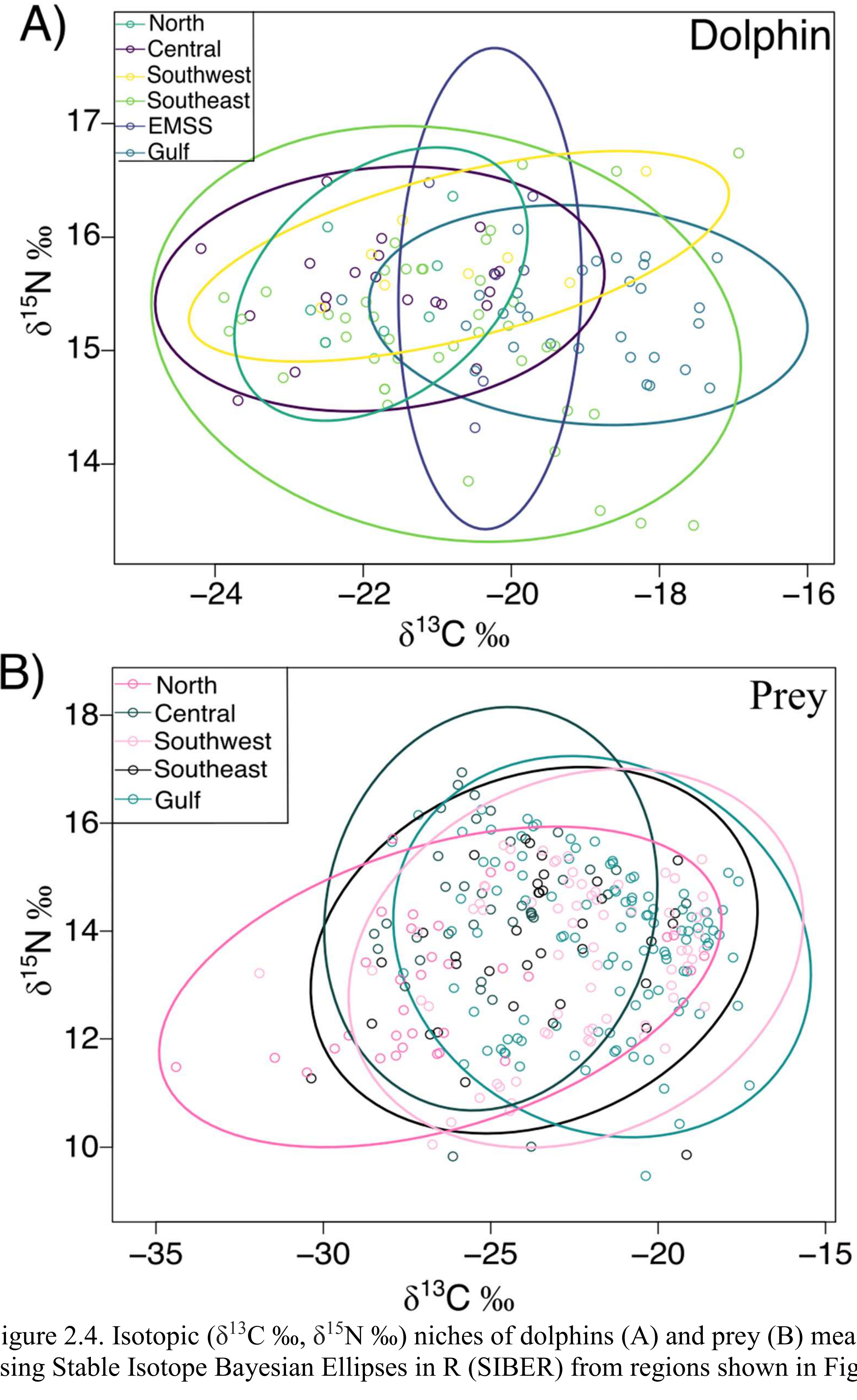

Figure 2.4. Isotopic ($\delta^{13}C$ ‰, $\delta^{15}N$ ‰) niches of dolphins (A) and prey (B) measured using Stable Isotope Bayesian Ellipses in R (SIBER) from regions shown in Figure 2.1.

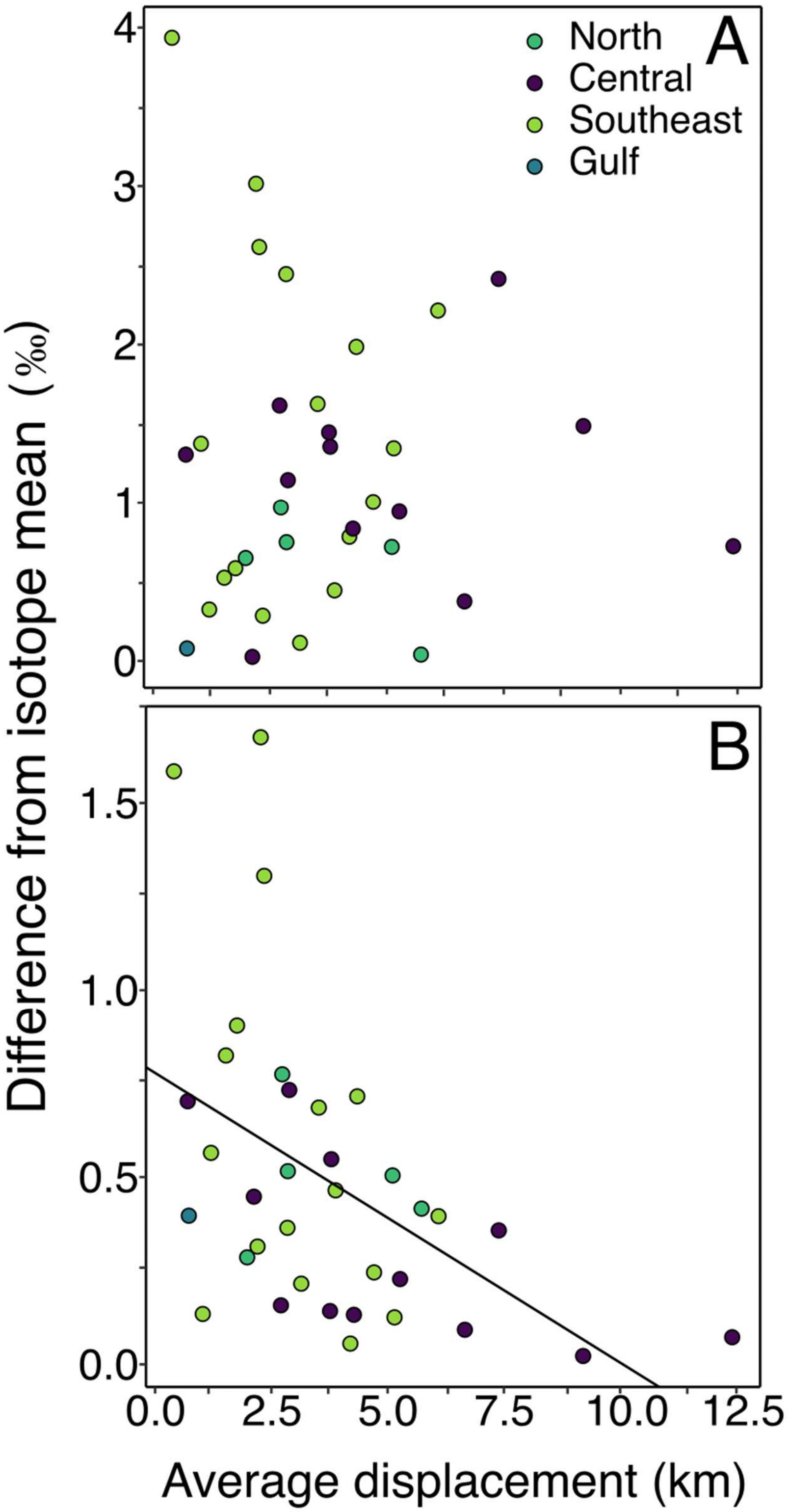

Figure 2.5. Isotopic variation defined as the absolute difference from the regional mean for $\delta^{13}C$‰ (A) and $\delta^{15}N$‰ (B) values in skin compared to the average displacement per sighting for individual dolphins sighted 3 or more times.

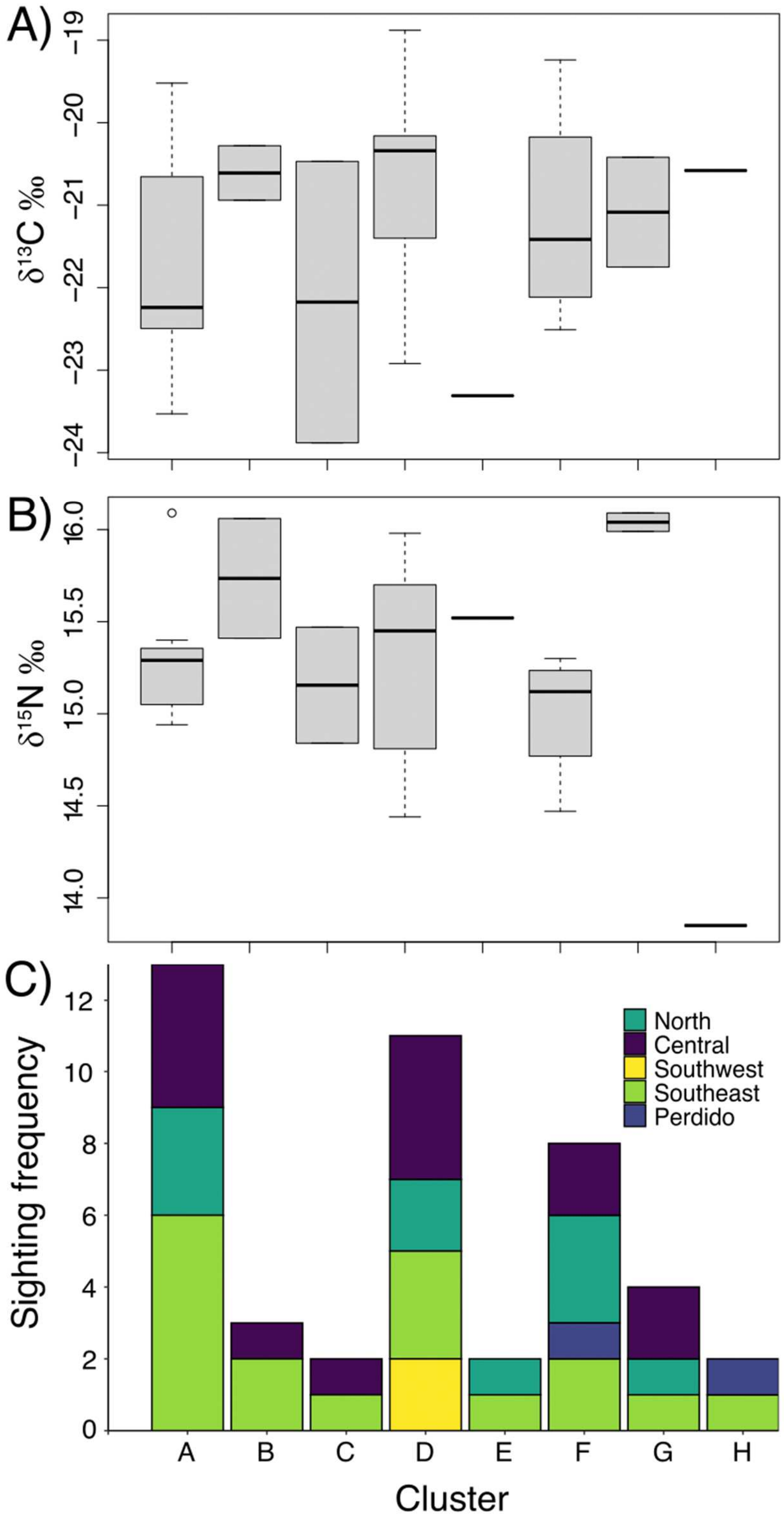

Figure 2.6. Median $\delta^{13}C$‰ (A) and $\delta^{15}N$‰ (B) stable isotope ratios in dolphin skin for different social groups (A-H) in Mobile Bay, AL defined by cluster analysis for individual dolphins with a minimum of 3 sightings. Sighting frequency and location (C) for each cluster, separated by regions defined in Figure 2.1.

CONCLUSION

The combination of two independent elemental analyses with photo-ID data allowed for a better understanding of dolphin site fidelity patterns. Individual bottlenose dolphins in this study exhibited varying degrees of site fidelity, with elemental differences more evident at larger spatial scales. Trace metal concentrations and stable isotope ratios in dolphin skin were most similar among regions within Mobile Bay than between Mobile Bay and the Gulf or EMSS regions. Most notably, As concentrations were considerably higher in the Gulf than all other regions, and Co concentrations were highest in the North and Central regions of Mobile Bay, and much lower in the Gulf and EMSS. Trace metal concentrations were highest in the winter, likely due to riverine discharge, and several elements (Fe, Pb, Sr, Cu) had additional relationships to salinity. $\delta^{13}C$ values increased with salinity, relative to latitude in Mobile Bay, while $\delta^{15}N$ values varied with longitude, driven by values in dolphins that also used adjacent Perdido Bay (an area known to have depleted nitrogen values). Stable isotope ratios did not differ between seasons. Photo-ID evidence corroborated element-based site fidelity, indicating that some dolphins within Mobile Bay were highly mobile among regions (North, Central, Southwest, Southeast), but there was little movement and connectivity between the bay, Gulf and EMSS (Appendix C, Figure C1). Additional resightings will improve assessments of site fidelity of groups.

Because it is not feasible to directly observe dolphins at all times and because they are highly mobile, elemental data were useful to fill gaps with time-integrated biological samples that could somewhat account for movement and resource use outside of the study area. Trace metals and stable isotope ratios did not vary with the same environmental factors, providing multiple lines of evidence about dolphin movement and space use. Both trace metal and stable isotope data provided information on salinity conditions and influence of freshwater discharge on habitats used by dolphins, highlighting the importance this environmental attribute to dolphin ecology in the study system. Each analysis also contributed unique data that cannot be obtained from the other approach. For example, trace metal data can indicate exposure to anthropogenic contaminants, and stable isotope data can be applied to characterize diet and its nutritionally important components. Overall, this study demonstrates the combination of elemental analyses and photo-ID can provide data needed to define site fidelity and better understand relationships between animal movements and habitat use.

By using LA-ICP-MS, a non-destructive method for determining trace metal concentrations, I was then able to perform trace metal and stable isotope analyses on the same tissue. Due to the small size of skin samples, without using this method, only one kind of analysis could have been performed on a given sample or samples would have been divided between the analyses, using different animals for each analysis and limiting the power of the results. To my knowledge, no other studies have measured trace metal concentrations using LA-ICP-MS in dolphin skin. This method can be useful for collaborative studies where samples are divided amongst several different groups or where samples are hard to collect and multiple chemical analyses are desired. The use of three methods together was a powerful analytical approach that is not often employed but provides more sufficient data to understand how movement patterns can mediate exposure to environmental stressors such as low salinity water or contaminants.

This study has implications for dolphin exposure to harmful conditions that could be worsened by factors such as climate change and urbanization. Climate change could increase risks of freshwater exposure for dolphins by increasing rain or storm events, subsequently increasing riverine discharge and further contributing to low salinity conditions in the region (Zimmerman et al. 2001, Palmer et al. 2008, Xu & Luo 2015, Sharafati et al. 2020). Because riverine discharge can be associated with increased contaminant loads (Schroeder & Wiseman 1988, Pennock et al. 1999), and in this study coincided with peak metal concentrations, dolphins in my study region and others in estuaries worldwide may have increased health risks as climate change intensifies. Ongoing and increasing urbanization in the region could also increase health risks by increasing contaminant loads into estuarine waters and affecting both dolphins and their prey (Kennish 2002, Van Dolah et al. 2008, Freeman et al. 2019). These changes elevate future risk levels for dolphins in estuaries and other coastal locations, potentially leading to harmful impacts and higher rates in mortality. The novel method used in this study provides a method for testing these relationships in the future.

Finally, the methods used in this study are transferrable to other species in other systems, but the scales that are considered may vary among study locations. For this approach to be applied elsewhere, the gradients of the intended tracers must be known in the target system. Although some tracers in this study were more useful for regional distinction than others, those same tracers may not necessarily be consistently useful in other systems. The general methodology, however, can still be applied and locally calibrated. This study, however, demonstrates that this multi-disciplinary approach can also be affective in an environmentally dynamic and hydrologically complex systems, with rapidly changing riverine discharge and salinity regimes. The approach may be even more affective in systems that are more static, where environmental gradients may be more distinctive and stable year-round or more seasonally and spatially predictable. Importantly, this study provides an approach to determine the spatial and temporal scale of site fidelity for a highly mobile predator and environmental sentinel for which investigation is needed to support management evaluations and conservation decisions.

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Appendix A: Chapter II supplemental tables and figures

Table A1. Trace element concentrations of Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), and Strontium (Sr) and Strontium to Barium ratios (Sr:Ba) in parts per million (ppm) in skin of individual dolphins (individual identification number = ID), season, and region. For Sr:Ba, NA indicates an outlier or an undefined ratio in cases where Ba was below detection. For all other elements, NA indicates a removed outlier. Standard error values are in parentheses.

ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
540	Winter	North	7.69 (0.58)	0.47 (0.13)	3.46 (0.42)	0.17 (0.05)	1.23 (0.51)	2.66 (0.43)	5.78 (3.35)	1.13 (0.52)	4.19 (0.70)	8.96
575	Winter	North	2.49 (0.10)	0	2.05	0	0.09	1.82 (0.14)	1.27 (0.21)	0	1.64 (0.10)	NA
548	Summer	North	12.28 (2.36)	2.38 (0.62)	0.92 (0.06)	0.22 (0.04)	1.47 (0.32)	17.94 (0.51)	191.05 (30.90)	0.15	11.75 (1.78)	4.94
610	Summer	North	8.30 (0.98)	1.08 (0.14)	0.60	0.94 (0.07)	1.71 (0.20)	13.31 (1.13)	260.43 (44.18)	0.08 (0.01)	4.21 (0.20)	3.91
765	Summer	North	7.54 (0.30)	0	0	0.56 (0.04)	0	10.65 (0.68)	236.58 (47.26)	0	1.61 (0.07)	NA
809	Summer	North	0	0.01	0	0	0	0	0	0	0	0
1118	Summer	North	0	0.02 (0)	0	0	0	0	0	0	0	0
167	Winter	Central	7.87 (0.29)	0.00	1.29 (0.06)	0.00	0.00	28.15 (1.52)	131.15 (10.66)	0.00	2.18 (0.14)	NA
171	Summer	Central	10.84 (3.28)	1.37 (0.31)	0.00	0.00	0.00	14.61 (0.94)	126.77 (13.96)	0.00	4.11 (0.65)	2.99

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	179	Winter	Central	6.21 (0.48)	0.50	1.04 (0.03)	1.80 (0.11)	2.65 (0.25)	28.88 (1.57)	564.68 (166.93)	1.39 (0.51)	5.82 (1.14)	11.61
	343	Winter	Central	0.00	0.56	0.00	0.00	0.00	14.98 (1.34)	12.43 (2.07)	0.00	12.08 (0.22)	21.74
	353	Summer	Central	12.90 (3.02)	0.60 (0.25)	1.04 (0.01)	0.25	0.00	21.38 (3.22)	156.09 (51.82)	0.00	3.98 (1.45)	6.67
	418	Winter	Central	NA	26.33 (0.13)	0.00	0.00	1.19 (0.08)	15.74 (0.61)	10.25 (0.94)	0.59 (0.02)	9.81 (2.34)	0.37
	424	Summer	Central	3.69 (0.57)	0.00	0.57	0.37 (0.02)	0.87 (0.21)	10.49 (0.74)	236.11 (3.32)	0.07 (0.01)	2.45 (0.14)	NA
	602	Summer	Central	6.73 (0.64)	0.66 (0.17)	0.44	0.37 (0.02)	0.00	9.64 (0.81)	121.09 (23.23)	0.06	3.53 (0.29)	5.33
	633	Summer	Central	45.74 (1.59)	3.90 (0.07)	0.73 (0.04)	0.69 (0.04)	0.00	0.00	0.00	0.62 (0.07)	16.42 (0.70)	4.21
	727	Winter	Central	7.16 (0.63)	0.00	2.40 (0.14)	0.11 (0.01)	0.00	3.42 (0.10)	2.05 (0.36)	0.00	2.95 (0.69)	NA
	743	Winter	Central	0.00	0.02 (0)	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	930	Summer	Central	10.92 (1.83)	0.00	0.00	0.15	0.00	3.74 (0.36)	31.26 (2.04)	0.00	0.67 (0.11)	NA
	959	Summer	Central	5.67 (0.74)	0.28 (0.01)	0.46	0.25 (0.02)	0.00	10.21 (0.78)	105.45 (18.61)	0.05	3.30 (0.15)	11.85
	1040	Summer	Central	8.52 (0.18)	0.69 (0.13)	0.58	0.42 (0.03)	0.78 (0.06)	12.90 (0.69)	93.55 (14.99)	0.06	2.27 (0.25)	3.28

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	1177	Summer	Central	14.55 (1.13)	1.08 (0.11)	1.33 (0.05)	0.17 (0.01)	1.48 (0.17)	22.19 (1.45)	245.61 (10.891)	0.09 (0)	13.40 (1.91)	12.37
	1205	Summer	Central	18.94 (0.87)	0.80 (0.09)	1.07 (0.02)	0.16	0.57	16.82 (1.04)	197.77 (16.64)	0.00	8.10 (0.91)	10.09
	1233	Summer	Central	5.55 (0.34)	0.47	0.87	0.00	1.10	20.57 (1.57)	150.06 (11.25)	0.00	6.29 (1.21)	13.40
	1235	Summer	Central	9.30 (0.74)	0.61 (0.04)	0.93	0.97 (0.05)	1.72 (0.05)	15.97 (0.99)	199.11 (14.91)	0.27 (0.05)	6.65 (0.05)	10.86
	1292	Summer	Central	6.70 (1.54)	1.04 (0.09)	0.96 (0)	0.00	1.65 (0.09)	18.65 (0.77)	81.96 (1.61)	0.00	8.61 (1.13)	8.27
	NA	Winter	Central	3.33 (0.16)	0.00	2.41 (0.40)	0.00	0.00	3.85 (0.20)	2.88	0.00	2.24 (0.19)	NA
	231	Winter	Southwest	0.12 (0)	1.19 (0.35)	0.90 (0.05)	0.10 (0.01)	0.00	0.00	0.00	0.31 (0.19)	6.88 (0.73)	5.76
	265	Winter	Southwest	6.95 (0.56)	0.99 (0.08)	1.13 (0.02)	0.14 (0.02)	0.81	46.76 (4.01)	477.04 (76.18)	0.11 (0.01)	25.90 (2.19)	26.06
	266	Winter	Southwest	1.92	0.00	0.00	0.00	0.00	19.40 (11.20)	5.56 (0.27)	0.00	4.71 (0.49)	NA
	825	Winter	Southwest	0.00	0.01 (0)	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	1110	Summer	Southwest	7.68 (0.09)	1.01 (0.25)	0.90 (0.14)	0.00	0.00	24.88 (0.25)	389.54 (40.95)	0.00	10.53 (1.09)	10.38
	1134	Summer	Southwest	17.46 (3.29)	3.91 (1.01)	1.17 (0.10)	0.51 (0.06)	0.00	35.29 (8.57)	336.80 (93.11)	0.21 (0.05)	32.73 (6.37)	8.37

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	1210	Summer	Southwest	4.19 (0.13)	0.00	0.36	0.19 (0.01)	0.00	10.32 (0.34)	171.63 (5.55)	0.05	3.31 (0.31)	NA
	1265	Summer	Southwest	9.14 (0.81)	1.15 (0.04)	0.00	0.17 (0.02)	0.85	16.43 (1.30)	159.82 (6.93)	0.07	5.75 (0.46)	4.99
	15	Winter	Southeast	35.19 (0.33)	NA	0.00	NA	0.00	0.00	0.00	NA	NA	1.94
	167	Winter	Southeast	0.00	0.01 (0)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	187	Winter	Southeast	0.39 (0.02)	1.66 (0.14)	2.71 (0.11)	0.54 (0.03)	0.00	0.00	0.00	0.38 (0.01)	7.75 (0.28)	4.67
	227	Winter	Southeast	0.07 (0)	0.20 (0.03)	1.15 (0.67)	0.00	0.00	0.00	0.00	0.02	3.37 (0.17)	17.15
	236	Winter	Southeast	65.97 (2.02)	5.85 (0.73)	1.30	0.33 (0.04)	2.62 (0.45)	21.75 (3.20)	155.97 (21.80)	0.49 (0.08)	15.66 (3.86)	2.67
	240	Winter	Southeast	7.72 (0.08)	0.43 (0.04)	0.74 (0.02)	0.00	0.00	13.12 (1.01)	18.12 (2.27)	0.16 (0.03)	14.82 (1.36)	34.14
	245	Winter	Southeast	0.12 (0.01)	0.00	0.79 (0.01)	0.12 (0.01)	0.00	0.00	0.00	0.04 (0.01)	3.80 (0.37)	NA
	256	Winter	Southeast	2.76 (0.15)	0.00	1.10 (0.07)	0.00	1.32 (0.07)	22.90 (0.57)	11.38 (0.21)	0.00	14.26 (0.34)	NA
	257	Winter	Southeast	4.93 (0.31)	0.48 (0.05)	3.12 (0.11)	0.13 (0.02)	0.00	2.33 (0.14)	2.05 (0.37)	0.00	10.96 (0.25)	22.87
	259	Winter	Southeast	15.75 (0.56)	1.07 (0.52)	0.69 (0.02)	0.52 (0.15)	15.66 (7.50)	23.66 (10.31)	187.17 (92.24)	1.75 (0.24)	42.73 (17.30)	NA

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	264	Winter	Southeast	67.38 (3.09)	NA	NA	NA	NA	7.55 (0.27)	2.30 (0.09)	NA	NA	2.51
	302	Winter	Southeast	29.29 (5.08)	1.85 (0.58)	1.48 (0.24)	0.53 (0.12)	5.89 (0.68)	NA	331.80 (78.64)	0.46 (0.10)	39.03 (5.950	21.13
	356	Winter	Southeast	12.22	0.77 (0.10)	0.00	0.00	0.00	32.20 (3.07)	123.25 (39.14)	0.00	9.75 (1.23)	12.63
	358	Winter	Southeast	9.25 (0.15)	0.00	0.67 (0.02)	0.00	0.00	17.45 (0.98)	15.48 (7.09)	0.00	2.85 (0.34)	NA
	359	Winter	Southeast	8.18 (0.21)	1.26 (0.06)	1.09 (0.42)	0.00	5.66 (1.55)	15.09 (5.29)	79.17 (23.20)	1.55 (0.42)	22.34 (3.93)	17.78
	372	Winter	Southeast	1.61 (0.01)	0.40	0.00	0.41 (0.02)	0.00	7.55 (0.31)	63.86 (0.27)	0.06	4.14 (0.73)	10.31
	374	Winter	Southeast	25.88 (1.38)	0.29 (0.03)	0.71 (0.20)	1.28 (0.03)	0.00	0.00	0.00	0.75 (0.22)	6.28 (0.25)	21.90
	378	Winter	Southeast	38.07 (3.10)	0.37	0.74 (0.09)	0.16 (0.01)	0.00	0.00	0.00	0.07	4.71 (0.46)	12.70
	532	Winter	Southeast	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	609	Winter	Southeast	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA
	690	Winter	Southeast	21.67 (4.41)	0.45	0.00	0.00	0.00	0.00	0.00	0.00	26.17 (6.98)	NA
	709	Winter	Southeast	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA
	712	Winter	Southeast	7.07 (0.27)	0.21	3.21 (0.13)	0.13 (0.01)	0.00	2.83 (0.10)	1.8 (0.39)	0.08	4.43 (0.25)	21.56

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	713	Winter	Southeast	6.62 (0.63)	1.38 (0.33)	3.61 (0.18)	0.00	0.00	3.71 (0.17)	2.21 (0.46)	0.00	3.37 (1.95)	2.45
	747	Winter	Southeast	2.76	0.85	0.00	0.00	0.72	3.26	3.98	0.17	17.33	20.46
	763	Winter	Southeast	0.00	0.64	1.47 (0.20)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	NA	Winter	Southeast	5.19 (0.27)	0.00	3.28 (0.12)	0.13 (0.01)	0.00	2.80 (0.21)	2.48 (0.42)	0.00	1.58 (0.42)	NA
	NA	Winter	Southeast	6.06 (1.76)	1.54 (0.35)	0.88 (0.15)	0.00	0.78	30.76 (0.67)	220.01 (40.25)	0.08	30.86 (6.27)	20.09
	301	Summer	Southeast	0.55 (0.06)	0.69 (0.24)	1.52 (0.14)	0.14 (0.03)	0.00	0.00	0.00	0.26 (0.23)	13.95 (2.22)	20.24
	392	Summer	Southeast	28.81 (3.84)	0.77 0.15)	1.41 (0.05)	0.13	0.77	25.79 (1.50)	37.87 (5.38)	0.09 (0.01)	33.95 (8.17)	NA
	511	Summer	Southeast	19.48 (4.33)	0.51 (0)	0.00	0.00	0.00	0.00	0.00	0.11 (0.01)	5.12 (0.93)	9.95
	587	Summer	Southeast	5.35 (0.65)	0.61	0.00	0.00	0.00	13.14 (1.17)	119.50 (39.01)	0.00	5.26 (0.55)	8.64
	796	Summer	Southeast	9.61 (0.43)	0.29	0.72 (0.12)	0.28 (0.04)	0.74	18.56 (1.64)	140.77 (21.21)	0.22 (0.06)	9.31 (1.18)	31.66
	978	Summer	Southeast	0.08 (0.01)	0.53 (0.06)	0.97 (0.12)	0.12 (0.02)	0.00	0.00	0.00	0.04 (0)	4.74 (0.58)	9.03
	979	Summer	Southeast	8.74 (0.96)	0.62 (0.06)	0.60 (0.09)	0.19 (0.03)	0.00	12.64 (0.53)	128.00 (3.66)	0.06	6.08 (0.59)	9.88

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	1029	Summer	Southeast	18.67 (4.49)	0.68 (0.21)	1.16 (0.26)	0.15 (0.02)	0.00	19.21 (4.68)	242.89 (90.69)	0.08 (0.01)	26.33 (7.53)	NA
	1090	Summer	Southeast	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1096	Summer	Southeast	11.04 (0.20)	1.43 (0.40)	0.00	0.00	0.00	16.18 (0.82)	85.16 (9.54)	0.00	5.69 (0.42)	3.96
	1135	Summer	Southeast	5.59 (0.45)	0.37 (0.08)	0.51	0.26 (0.04)	0.00	10.68 (0.08)	81.12 (3.25)	0.00	2.76 (0.14)	7.38
	1138	Summer	Southeast	7.58 (0.39)	1.11 (0.16)	0.00	0.14 (0.02)	0.00	10.29 (0.53)	82.00 (5.70)	0.00	9.02 (0.83)	8.12
	1139	Summer	Southeast	8.89 (0.22)	1.04 (0.06)	0.52 (0.08)	0.18 (0.01)	0.00	10.73 (0.18)	153.40 (9.01)	0.00	6.31 (0.43)	6.04
	1162	Summer	Southeast	7.19 (1.15)	0.73	0.00	0.20	0.84	15.11 (2.56)	116.72 (21.04)	0.00	12.79 (3.80)	17.54
	1241	Summer	Southeast	3.02 (0.46)	6.00 (0.62)	0.00	NA	NA	NA	NA	NA	19.99 (2.14)	3.33
	1252	Summer	Southeast	18.48 (2.30)	1.24 (0.21)	0.90 (0.16)	0.26	1.38 (0.38)	18.71 (1.48)	179.00 (44.49)	0.20	9.62 (1.06)	7.78
	1291	Summer	Southeast	24.22 (3.48)	0.53 (0.09)	0.52 (0.07)	0.23 (0.13)	0.00	0.00	0.00	0.00	5.99 (3.46)	11.38
	124	Summer	EMSS	18.79 (1.16)	0.44 (0.02)	0.64 (0.06)	0.36 (0.01)	0.54	17.38 (1.03)	35.03 (6.13)	0.10 (0.01)	10.83 (1.72)	24.75
	283	Summer	EMSS	0.00	0.00	1.76 (0.15)	0.00	0.00	27.62 (1.91)	236.41 (57.05)	0.07 (0.01)	18.26 (2.15)	NA

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	290	Summer	EMSS	0.00	0.00	NA	0.00	0.00	NA	0.00	0.00	0.00	NA
	312	Summer	EMSS	0.00	0.55	0.00	0.00	2.03 (0.25)	4.72 (3.23)	110.53	0.57 (0.13)	35.32 (3.09)	NA
	387	Summer	EMSS	0.00	0.91	4.48 (0.51)	0.00	0.00	NA	0.00	0.12 (0.02)	0.00	0.00
	NA	Summer	EMSS	4.81 (0.20)	0.67 (0.01)	0.91	0.00	0.00	13.93 (0.60)	143.61 (31.02)	0.11 (0.01)	5.14 (0.20)	7.66
	58	Winter	Gulf	35.54 (1.65)	0.00	1.21 (0.08)	0.00	0.00	12.66 (1.05)	7.14 (0.19)	0.00	4.24 (0.35)	NA
	143	Winter	Gulf	22.21 (0.76)	0.00	1.47 (0.17)	0.00	4.55 (0.88)	32.14 (1.87)	227.70 (45.38)	0.06 (0)	18.90 (0.34)	NA
	154	Winter	Gulf	18.31 (4.63)	0.00	1.37 (0.25)	0.00	0.00	25.00 (4.71)	66.86 (20.07)	0.00	6.31 (1.48)	NA
	191	Winter	Gulf	8.87 (0.63)	0.00	1.59 (0.25)	0.00	1.00	31.47 (2.42)	95.73 (22.93)	0.00	20.32 (0.40)	NA
	197	Winter	Gulf	14.76 (0.29)	0.59 (0.10)	0.00	0.00	0.00	29.59 (1.86)	103.44 (11.92)	0.00	4.49 (0.63)	7.58
	198	Winter	Gulf	17.00 (2.19)	0.66 (0.18)	1.14 (0.15)	0.00	0.91 (0.08)	21.01 (2.19)	109.60 (36.77)	0.13 (0)	25.83 (1.99)	NA
	205	Winter	Gulf	47.33 (2.69)	0.00	1.21 (0.07)	0.00	0.00	30.74 (0.51)	114.60 (11.68)	0.10 (0.01)	22.39 (1.70)	NA
	206	Winter	Gulf	18.87 (3.80)	0.51	0.00	0.00	6.49 (4.64)	3.89 (0.32)	47.74 (5.02)	0.37 (0.03)	8.49 (1.79)	16.79

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	218	Winter	Gulf	4.58 (1.02)	0.00	0.00	0.23 (0.01)	1.64 (0.18)	13.15 (0.52)	72.08 (3.83)	0.17 (0.02)	9.90 (0.26)	NA
	313	Winter	Gulf	17.55 (0.55)	0.00	1.48 (0.13)	0.00	0.00	31.59 (1.09)	131.94 (24.77)	0.00	8.83 (1.16)	NA
	334	Winter	Gulf	19.21 (2.43)	0.00	0.56	0.00	0.00	18.95 (0.99)	49.24 (1.41)	0.00	5.57 (0.83)	NA
	639	Winter	Gulf	45.56 (4.60)	0.36 (0.03)	0.61 (0.03)	0.00	0.00	0.00	0.00	0.00	5.30 (0.73)	14.91
	646	Winter	Gulf	39.29 (2.34)	0.96 (0.38)	1.06 (0.05)	0.00	4.48 (0.86)	13.41 (0.73)	80.04 (35.86)	0.24 (0.14)	11.56 (6.67)	12.07
	674	Winter	Gulf	73.56 (4.70)	0.00	0.56	0.00	0.00	0.00	0.00	0.07	4.85 (1.32)	NA
	675	Winter	Gulf	NA	0.00	0.66 (0.08)	0.00	0.00	0.00	0.00	0.00	3.17 (0.17)	NA
	677	Winter	Gulf	0.00	0.01	1.38 (0.26)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	684	Winter	Gulf	NA	0.00	0.58	0.15 (0.02)	0.00	0.00	0.00	0.07 (0.01)	4.51 (0.61)	NA
	813	Winter	Gulf	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	NA	Winter	Gulf	0.00	0.31	0.94 (0.06)	0.091216491 (0)	0.00	0.00	0.00	0.13	0.00	0.00
	NA	Winter	Gulf	NA	0.70 (0.08)	0.81 (0.07)	0.00	0.00	0.00	0.00	0.06	6.70 (0.23)	9.56

Cont.	ID	Season	Region	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	NA	Winter	Gulf	82.48 (28.79)	7.49 (2.04)	1.18 (0.36)	0.00	0.00	30.31 (5.72)	220.04	1.58 (1.03)	5.61 (1.21)	0.75
	NA	Winter	Gulf	23.91 (2.69)	0.00	0.90	0.00	0.00	26.72 (2.80)	195.17 (50.73)	0.10	10.87 (1.38)	NA
	NA	Winter	Gulf	28.32 (1.54)	0.00	1.58	0.00	1.71 (0.03)	24.82 (2.91)	75.29 (31.36)	0.12 (0)	14.46 (2.58)	NA
	NA	Winter	Gulf	17.59 (2.29)	0.00	0.87	0.00	0.00	11.46 (0.75)	20.43 (3.62)	0.00	3.15 (0.41)	NA
	28	Summer	Gulf	59.83 (40.93)	0.00	0.00	0.00	1.12 (0.06)	12.18 (1.41)	100.58 (15.77)	0.00	11.09 (2.26)	NA
	140	Summer	Gulf	13.10 (1.01)	1.05 (0.18)	1.25	0.00	4.96 (0.75)	18.54 (3.33)	445.42 (86.09)	0.92 (0.17)	17.85 (2.54)	16.99
	143	Summer	Gulf	23.90 (2.58)	0.61 (0.11)	1.05 (0.05)	0.00	0.00	22.72 (1.62)	29.83 (0.43)	0.05	7.89 (1.01)	12.91
	NA	Summer	Gulf	8.95 (0.55)	0	0.80 (0.04)	0	0	13.14 (0.23)	68.99 (2.98)	0	5.66 (0.38)	NA

Table A2. Number of samples (N) used in analysis for each metal after outliers were removed, relative to N=114 before outliers were removed.

Metal	N
As	110
Ba	112
Cd	112
Co	111
Cr	112
Cu	110
Fe	113
Pb	111
Sr	112

Table A3. P values for regional comparisons of Mobile Bay regions (North (N), Central (C), Southwest (SW), Southeast (SE)) from Tukey post-hoc tests. #= sample size. Significant p values are in bold.

	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
#	110	112	112	112	112	110	113	111	112	74
N-C	0.99	0.85	0.99	0.99	1.00	0.67	0.99	0.99	0.99	0.89
SE-C	0.99	0.67	0.99	0.46	0.93	0.55	0.25	0.99	0.31	0.69
SW-C	0.99	0.96	0.98	0.77	0.99	0.81	0.65	0.99	0.69	0.99
SE-N	0.88	0.99	0.99	0.89	0.99	0.99	0.94	0.99	0.34	0.30
SW-N	0.99	0.99	0.92	0.93	0.99	0.21	0.56	0.99	0.54	0.87
SW-SE	0.89	0.99	0.95	0.99	0.97	0.12	**0.022**	0.99	0.99	0.98

Table A4. Statistics for the parameters of models for Cu, Sr, Sr:Ba, Fe and Pb. Estimates, t-values (t) and p values (p) are reported for the best fitting model for each isotope, indicated by “*”. Akaike weights (w), Akaike information criterion (AIC) and delta Akaike information criterion (ΔAIC) were reported for each model in the table.

	Element	Model	Parameter	Estimate (∓SE)	t value	p	w	AIC	ΔAIC
	Cu	Model 4	Intercept	-1902.14 (1099.89)	-1.73	0.09	0.30	581.50	0.00
			Salinity	0.58 (0.15)	-1.73	0.09			
			Longitude	-21.67 (12.50)	3.73	<0.001			
		Model 2	Intercept	-497.29 (301.45)	-1.65	0.10	0.26	581.73	0.23
			Salinity	0.65 (0.17)	3.83	<0.001			
			Latitude	16.48 (9.90)	1.66	0.10			
		Global	Intercept	-1944.02 (1095.59)	-1.77	0.08	0.26	581.77	0.27
			Salinity	0.67 (0.17)	3.95	<0.001			
			Latitude	13.07 (10.15)	1.29	0.20			
			Longitude	-17.62 (12.83)	-1.37	0.17			
		Model 7	Intercept	4.54 (2.17)	2.10	0.04	0.17	582.56	1.06
			Salinity	0.53 (0.15)	3.41	0.001			
		Null	Intercept	10.89 (1.19)	9.13	<0.001	1.82e-2	591.68	10.19
		Model 5	Intercept	-1074.76 (1165.50)	-0.92	0.36	1.04e-2	592.80	11.30
			Longitude	-12.34 (13.25)	-0.93	0.35			

	Model 3	Intercept	-1097.36 (1174.25)	-0.93	0.35	4.08e-4	594.68	13.18
		Latitude	-3.42 (10.11)	-0.34	0.74			
		Longitude	-13.78 (13.99)	-0.98	0.33			
Sr	Model 7	Intercept	2.77 (2.02)	1.37	0.17	0.40	566.77	0.00
		Salinity	0.50 (0.14)	3.49	<0.001			
	Model 2	Intercept	297.48 (291.37)	1.02	0.31	0.25	567.71	0.94
		Salinity	0.41 (0.16)	2.51	0.01			
		Latitude	-9.67 (9.56	-1.01	0.31			
	Model 4	Intercept	787.60 (998.28)	0.79	0.43	0.20	568.12	1.36
		Salinity	0.47 (0.14)	3.28	<0.01			
		Longitude	8.92 (11.34)	0.79	0.43			
	Global	Intercept	812.42 (1000.82)	0.81	0.42	0.11	569.40	2.64
		Salinity	0.41 (0.16)	2.48	0.01			
		Latitude	-8.26 (9.96)	-0.83	0.41			
		Longitude	6.34 (11.79)	0.54	0.59			
	Model 3	Intercept	1255.43 (1018.21)	1.23	0.22	0.029	573.65	6.89
		Latitude	-20.02 (9.06)	-2.21	0.03			
		Longitude	7.26 (12.18)	0.60	0.55			
	Null	Intercept	8.86 (1.08)	8.19	<0.001	0.013	576.38	9.61
	Model 5	Intercept	1397.75 (1041.81)	1.34	0.18	2.94e-3	576.57	9.81

		Longitude	15.79 (11.84)	1.33	0.19			
Sr:Ba	Model 4	Intercept	2093.37 (954.01)	2.19	0.03	0.25	414.94	0.00
		Salinity	0.22 (0.15)	1.45	0.15			
		Longitude	23.72 (10.84)	2.19	0.03			
	Model 5	Intercept	2413.11 (937.31)	2.58	0.01	0.22	415.12	0.18
		Longitude	27.32 (10.66)	2.56	0.01			
	Model 3	Intercept	2284.45 (937.52)	2.44	0.02	0.19	415.42	0.48
		Latitude	-11.81 (9.23)	-1.28	0.21			
		Longitude	21.78 (11.45)	1.90	0.06			
	Global	Intercept	2096 (958.70)	2.19	0.03	0.11	416.45	1.51
		Salinity	0.16 (0.17)	0.95	0.34			
		Latitude	-7.07 (10.49)	-0.67	0.50			
		Longitude	21.30 (11.47	1.86	0.07			
	Model 7	Intercept	6.32 (2.19)	2.88	<0.01	0.060	417.78	2.84
		Salinity	0.30 (0.15)	1.94	0.06			
	Model 2	Intercept	407.27 (310.20)	1.31	0.19	0.052	418.04	3.10
		Salinity	0.18 (0.18)	1.01	0.31			
		Latitude	-13.16 (10.18)	-1.29	0.20			
	Null	Intercept	10.06 (1.07)	9.36	<0.001	0.024	419.56	4.62

Fe	Model 4	Intercept	-27208.00 (18410.24)	-1.48	0.15	0.23	515.27	0.00
		Salinity	6.86 (2.92)	2.35	0.02			
		Longitude	-309.25 (209.22)	-1.48	0.15			
	Model 2	Intercept	-8503.35 (5781.46)	-1.47	0.15	0.23	515.29	0.02
		Salinity	7.68 (3.09)	2.48	0.02			
		Latitude	279.95 (190.25)	-1.47	0.15			
	Model 7	Intercept	3.82 (33.42)	0.11	0.91	0.20	515.56	0.29
		Salinity	5.88 (2.88)	2.04	0.05			
	Global	Intercept	-29632.96 (18415.84)	-1.61	0.12	0.19	515.71	0.43
		Salinity	8.19 (3.10)	2.64	0.01			
		Latitude	231.89 (193.26)	1.20	0.24			
		Longitude	-256.73 (212.58)	-1.21	0.23			
	Null	Intercept	59.37 (20.12)	2.95	<0.01	0.068	517.72	2.45
	Model 5	Intercept	-17301.10 (18934.50)	-0.91	0.37	0.039	518.84	3.57
		Longitude	-197.40 (215.3)	-0.92	0.36			
	Model 3	Intercept	-17411.66 (19169.89)	-0.91	0.37	0.015	520.77	5.40
		Latitude	50.32 (194.22)	0.26	0.80			
		Longitude	-181.27 (226.56)	0.43	0.43			

Pb	Model 2	Intercept	13.12 (7.39)	1.78	0.08	0.29	-51.11	0.00
		Salinity	-0.010 (0.0048)	-2.20	0.03			
		Latitude	-0.42 (0.24)	-1.76	0.09			
	Null	Intercept	0.075 (0.019)	3.85	<0.001	0.18	-50.16	0.94
	Model 7	Intercept	0.13 (0.048)	2.76	<0.01	0.16	-49.89	1.21
		Salinity	-0.0038 (0.0029)	-1.29	0.20			
	Global	Intercept	19.60 (20.23)	0.97	0.34	0.11	-49.24	1.87
		Salinity	-0.010 (0.0049)	-2.14	0.04			
		Latitude	-0.40 (0.25)	-1.58	0.12			
		Longitude	0.082 (0.24)	0.34	0.73			
	Model 4	Intercept	16.00 (20.53)	0.78	0.44	0.080	-48.53	2.57
		Salinity	-0.0043 (0.0030)	-1.42	0.16			
		Longitude	0.18 (0.23)	0.77	0.44			
	Model 5	Intercept	9.60 (20.32)	0.47	0.64	0.075	-48.39	2.71
		Longitude	0.11 (0.23)	0.47	0.64			
	Model 3	Intercept	10.01 (20.72)	0.48	0.63	0.028	-46.43	4.67
		Latitude	0.030 (0.16)	0.19	0.85			
		Longitude	0.12 (0.25)	0.50	0.62			

Table A5. Number of individuals (N) in each cluster, with the number of darted dolphins in parentheses and mean trace element values of Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), and Strontium (Sr) and Strontium to Barium ratios (Sr:Ba) with standard error (±SE) in parentheses for defined clusters (A-H).

Cluster	N (darted)	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
A	21 (7)	8.38 (7.11)	0.23 (0.42)	0.47 (0.62)	0.10 (0.21)	0.21 (0.56)	11.21 (11.78)	89.83 (113.50)	0.03 (0.049)	3.59 (4.67)	3.19 (5.49)
B	8 (2)	9.83 (1.54)	0.31 (0.43)	0.30 (0.43)	0.17 (0.030)	0	8.19 (6.29)	79.63 (68.40)	0.03 (0.046)	3.37 (3.82)	4.94 (6.98)
C	5 (2)	6.45 (9.01)	0.62 (0.033)	1.25 (0.30)	0.12 (0.17)	0	10.6 (15.13)	78.05 (110.37)	0	1.99 (2.81)	3.33 (4.71)
D	9 (5)	17.74 (27.77)	0.44 (0.30)	0.47 (0.36)	0.18 (0.21)	0.39 (0.55)	12.28 (5.05)	135.62 (89.97)	0.03 (0.039)	4.38 (1.72)	5.98 (5.25)
E	3 (1)	4.93	0.48	3.12	0.13	0	2.33	2.05	0	10.96	22.87
F	8 (4)	7.55 (4.00)	0.82 (1.06)	1.79 (1.25)	0.10 (0.11)	0.70 (0.76)	8.88 (7.93)	54.06 (91.60)	0.36 (0.52)	8.10 (6.21)	12.0 (15.20)
G	2 (2)	25.71 (28.00)	2.09 (2.56)	0.59 (0.19)	0.47 (0.31)	0	5.10 (7.22)	52.73 (74.57)	0.33 (0.40)	9.86 (9.27)	8.03 (5.40)
H	1 (1)	38.07	0.37	0.74	0.16	0	0	0	0.07	4.71	12.70

Table A6. Results of one-way ANOVA comparing elemental concentrations in dolphin social clusters. *F* = F statistic, df = degrees of freedom and p = p-value. Significant p-value is shown in bold.

Element	*F*	df	p
As	0.79	7	0.60
Ba	1.11	7	0.41
Cd	3.12	7	**0.03**
Co	0.88	7	0.55
Cr	0.67	7	0.70
Cu	0.39	7	0.89
Fe	0.48	7	0.84
Pb	1.02	7	0.46
Sr	0.93	7	0.51
Sr:Ba	1.11	7	0.40

Table A7. P values of cluster comparisons (A-H) from Tukey post-hoc tests. Marginal p-value is shown in bold.

Cluster	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
B-A	1.00	1.00	0.99	0.99	0.99	0.99	1.00	1.00	1.00	0.99
C-A	0.99	0.99	0.86	1.00	0.99	1.00	0.99	0.99	0.99	1.00
D-A	0.97	0.99	1.00	0.99	0.99	0.99	0.99	1.00	0.99	0.99
E-A	0.99	0.99	**0.05**	1.00	0.99	0.99	0.99	1.00	0.84	0.38
F-A	1.00	0.95	0.13	1.00	0.84	0.99	0.99	0.47	0.81	0.68
G-A	0.88	0.21	0.99	0.34	0.99	0.99	0.99	0.81	0.75	0.99
H-A	0.69	0.99	0.99	0.99	0.99	0.95	0.99	0.99	0.99	0.95
C-B	0.99	0.99	0.87	0.99	1.00	0.99	1.00	1.00	0.99	0.99
D-B	0.99	0.99	0.99	1.00	0.99	0.99	0.99	1.00	0.99	0.99
E-B	0.99	0.99	0.08	0.99	1.00	0.99	0.99	1.00	0.90	0.64
F-B	0.99	0.99	0.31	0.99	0.81	1.00	0.99	0.81	0.94	0.97
G-B	0.97	0.49	0.99	0.77	1.00	0.99	0.99	0.93	0.88	0.99
H-B	0.84	1.00	0.99	1.00	1.00	0.99	0.99	1.00	0.99	0.99
D-C	0.99	0.99	0.90	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Cont.	Cluster	As	Ba	Cd	Co	Cr	Cu	Fe	Pb	Sr	Sr:Ba
	E-C	1.00	0.99	0.44	1.00	1.00	0.99	0.99	1.00	0.80	0.55
	F-C	1.00	0.99	0.98	0.99	0.81	0.99	0.99	0.73	0.83	0.91
	G-C	0.93	0.70	0.98	0.65	1.00	0.99	0.99	0.89	0.74	0.99
	H-C	0.76	0.99	0.99	0.99	1.00	0.98	0.99	0.99	0.99	0.98
	E-D	0.99	1.00	0.07	0.99	0.99	0.98	0.91	1.00	0.92	0.58
	F-D	0.97	0.99	0.23	0.99	0.99	0.99	0.91	0.87	0.95	0.95
	G-D	0.99	0.41	0.99	0.70	0.99	0.98	0.97	1.00	0.89	0.99
	H-D	0.94	1.00	0.99	1.00	0.99	0.92	0.90	0.90	1.00	0.99
	F-E	0.99	0.99	0.71	0.99	0.94	0.99	0.99	0.96	0.99	0.93
	G-E	0.96	0.79	0.15	0.83	1.00	0.99	0.99	0.99	0.99	0.81
	H-E	0.83	1.00	0.33	1.00	1.00	0.99	1.00	0.99	0.98	0.98
	G-F	0.89	0.70	0.56	0.41	0.81	0.99	1.00	0.99	0.99	0.99
	H-F	0.71	0.99	0.88	0.99	0.94	0.99	0.99	0.96	0.99	1.00
	H-G	0.99	0.74	0.99	0.89	1.00	0.99	0.99	0.99	0.99	0.99

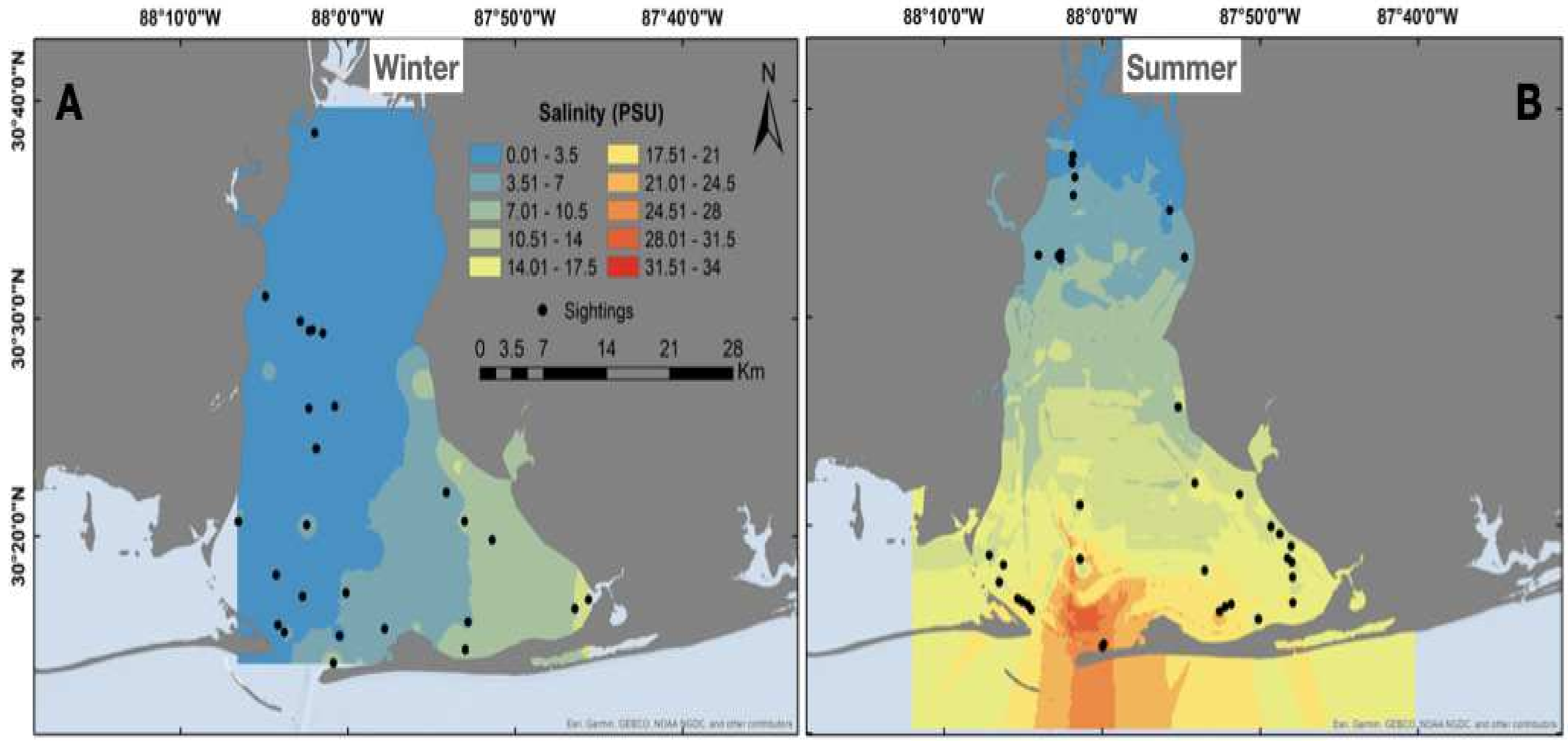

Figure A1. Interpolated surface salinity data collected during photo-ID surveys in Mobile Bay. (A) Salinity values collected in January 2020 using a YSI (n = 149); (B) Salinity values collected in June 2020 using a dataflow continuous data logger (n =44,984).

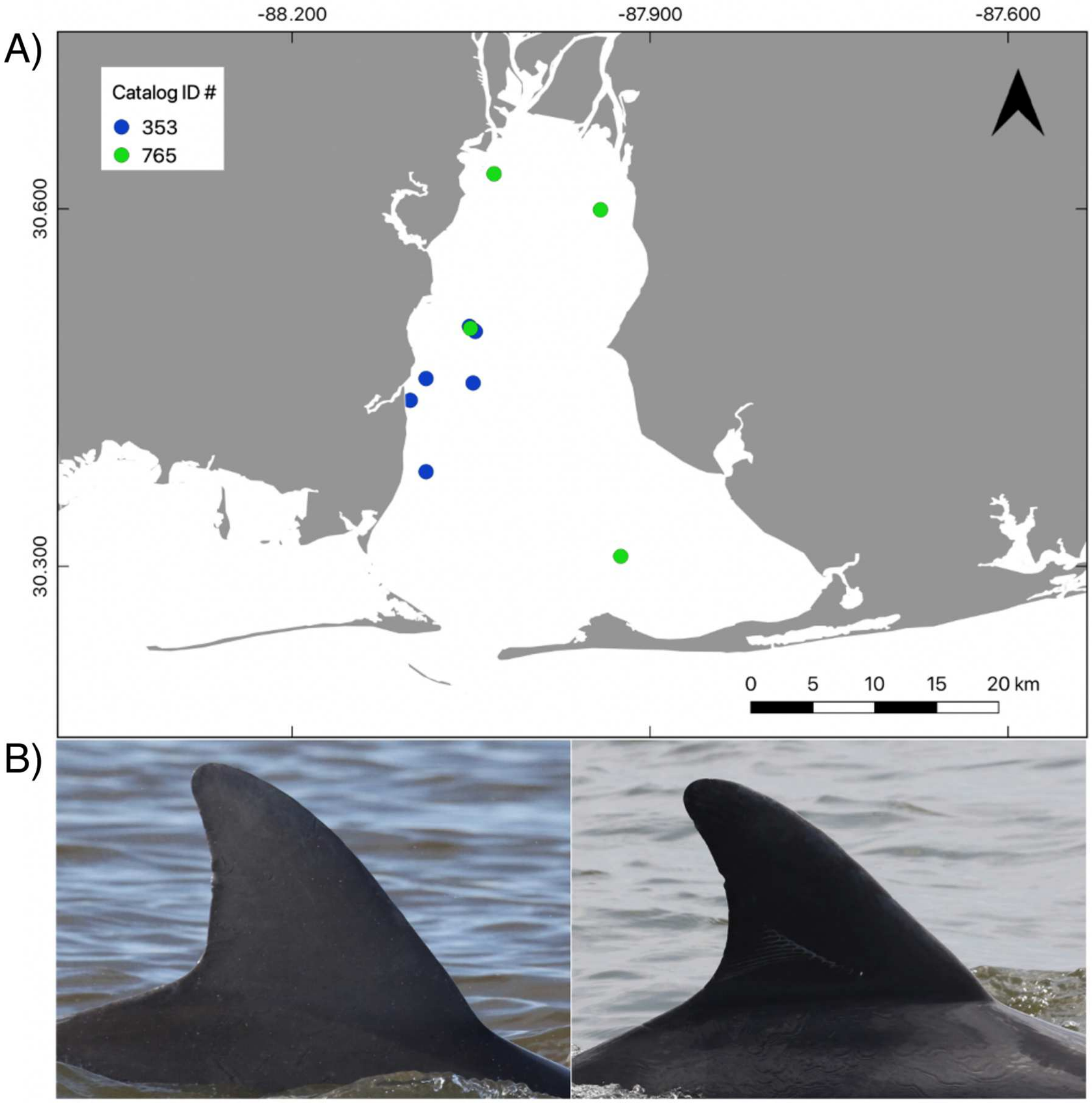

Figure A2. Sighting locations (A) and dorsal fins (B) of ID 353 (left) and ID 765 (right) as examples to demonstrate individual variation in movement patterns.

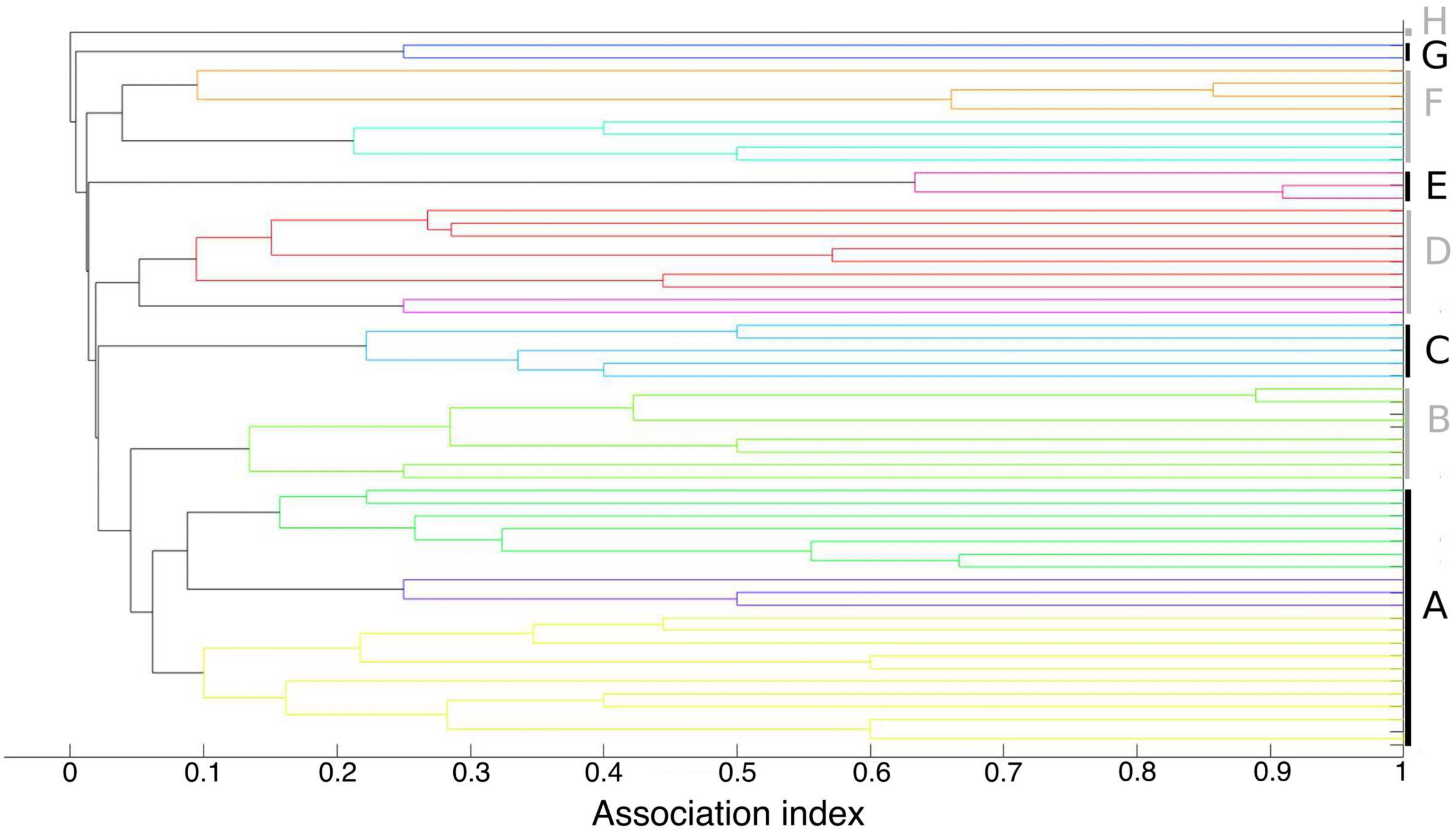

Figure A3. Cluster diagram of association indices between biopsied individuals and their associates sighted four or more times. Each of the clusters is designated by a different assigned letter A-H. Each tip refers to a single individual. Short black lines at the right of the figure (for example in cluster B) indicate that dolphins were always sighted together.

Appendix B: Chapter III supplemental tables

Table B1. Number of dolphin biopsy samples collected during winter and summer in regions of Mobile Bay, Alabama (North, Central, Southwest, Southeast), EMSS and Gulf and corresponding mean (± SE) stable isotope ratios in dolphin skin.

	Number				
Region	Winter	Summer	Total	$\delta^{13}C$‰	$\delta^{15}N$‰
North	2	5	7	-21.76 ± 0.31	15.59 (0.18)
Central	6	15	21	-21.78 ± 0.27	15.54 (0.10)
Southwest	4	4	8	-20.71 ± 0.53	15.83 (0.13)
Southeast	27	17	44	-20.87 ± 0.24	15.16 (0.11)
EMSS	0	6	6	-20.29 ± 0.20	15.55 (0.35)
Gulf	25	4	29	-18.96 ± 0.22	15.31 (0.07)

Table B2. Prey species, categorized by taxonomic group, analyzed for stable isotopes (δ^{13}C‰ and δ^{15}N‰) from regions in Figure 2.1 (North, Central, Southeast, Southwest and GOM – one inshore and one offshore site combined. (n) indicates the number of samples analyzed for each taxonomic group.

Season	Site	Taxonomic group	n	Carbon± (SE)	Nitrogen± (SE)	Species
Winter	Central	Anchovy	6	-24.98 (0.49)	15.95 (0.17)	*Anchoa hepsetus*
		Ariidae	1	-25.51	14.12	*Ariopsis felis*
		Clupeiformes	6	-26.54 (0.83)	14.28 (0.19)	*Dorosoma petenense*
		Decapoda	7	-25.81 (0.58)	12.00 (0.66)	*Penaeus aztecus*
		Perciformes	9	-24.12 (0.86)	15.35 (0.33)	*Micropogonias undulatus* *Cynoscion arenarius*
	Southwest	Anchovy	6	-24.07 (0.44)	15.37 (0.14)	*Anchoa hepsetus*
		Ariidae	1	-25.23	14.39	*Ariopsis felis*
		Cephalopoda	1	-19.67	14.34	*Lolliguncula brevis*
		Clupeiformes	6	-26.46 (1.46)	13.80 (0.24)	*Dorosoma petenense*
		Decapoda	12	-24.34 (0.84)	11.79 (0.59)	*Callinectes sapidus* *Penaeus aztecus*
		Perciformes	5	-21.12 (0.85)	14.39 (0.39)	*Leiostomus xanthrus*
	Southeast	Anchovy	6	-23.90 (0.24)	14.90 (0.37)	*Anchoa hepsetus*
		Cephalopoda	1	-20.20	13.81	*Lolliguncula brevis*
		Clupeiformes	10	-25.33 (1.06)	12.94 (0.44)	*Brevoortia patronus* *Dorosoma petenense*
		Decapoda	5	-25.41 (0.78)	12.24 (0.45)	*Penaeus aztecus*
		Perciformes	14	-22.20 (0.52)	14.10 (0.31)	*Cynoscion nebulosus* *Leiostomus xanthrus* *Micropogonias undulatus*
	GOM	Anchovy	12	-22.93 (0.84)	14.76 (0.33)	*Anchoa hepsetus*
		Ariidae	7	-21.76 (0.70)	14.23 (0.24)	*Ariopsis felis*
		Cephalopoda	7	-21.16 (0.33)	14.80 (0.19)	*Lolliguncula brevis*
		Clupeiformes	6	-24.39 (1.05)	13.19 (0.76)	*Dorosoma petenense*
		Decapoda	16	-22.59 (0.67)	12.25 (0.21)	*Callinectes sapidus*

Cont.	Season	Site	Taxonomic group	n	Carbon± (SE)	Nitrogen± (SE)	Species
							Penaeus aztecus
							Portunus gibbesii
			Perciformes	43	-21.10 (0.40)	14.24 (0.20)	*Cynoscion arenarius*
							Lagodon rhomboides
							Leiostomus xanthrus
							Micropogonias undulatus
							Selene setapinnis
	Summer	North	Anchovy	6	-26.96 (0.75)	13.42 (0.33)	*Anchoa hepsetus*
			Clupeiformes	11	-25.78 (1.73)	12.39 (0.31)	*Brevoortia patronus*
							Dorosoma petenense
							Harengula jaguana
			Decapoda	7	-27.23 (0.24)	12.00 (0.13)	*Penaeus aztecus*
			Perciformes	10	-26.60 (0.52)	14.16 (0.33)	*Cynoscion spp*
							Lagodon rhomboides
							Micropogonias undulatus
		Central	Anchovy	5	-23.91 (0.16)	14.38 (0.09)	*Anchoa hepsetus*
			Ariidae	3	-24.31 (1.15)	14.32 (0.23)	*Ariopsis felis*
		Southwest	Anchovy	6	-22.86 (0.50)	14.34 (0.31)	*Anchoa hepsetus*
			Cephalopoda	4	-21.68 (0.24)	14.72 (0.07)	*Lolliguncula brevis*
			Clupeiformes	7	-19.18 (0.15)	13.19 (0.17)	*Harengula jaguana*
			Decapoda	7	-23.34 (0.74)	11.63 (0.25)	*Penaeus aztecus*
			Perciformes	10	-19.50 (0.35)	14.30 (0.15)	*Chloroscombus chrysurus*
		GOM	Cephalopoda	7	-19.02 (0.12)	13.64 (0.28)	*Lolliguncula brevis*
			Decapoda	8	-21.79 (0.85)	11.72 (0.22)	*Penaeus aztecus*

Table B3. Statistics for the parameters of models for δ^{13}C‰ and δ^{15}N‰. Estimates, t-values (t) and p values (p) are reported for the best fitting model for each isotope, indicated by "*". Akaike weights (w), Akaike information criterion (AIC) and delta Akaike information criterion (ΔAIC) were reported for each model in the table.

Isotope	Model	Parameter	Estimate (∓SE)	t	p	w	AIC	ΔAIC
δ^{13}C	Model 13*	Intercept	204.35 (114.26)	1.79	0.08	0.32	337.11	0.00
		Salinity	0.11 (0.01)	6.87	<0.001			
		Latitude	-1.76 (1.00)	-1.76	0.08			
		Longitude	1.97 (1.29)	1.53	0.13			
	Model 3	Salinity	0.12 (0.016)	7.31	<0.001	0.27	337.50	0.39
		Latitude	-1.93 (1.00)	-1.92	0.06			
	Model 9	Salinity	0.13 (0.013)	9.53	0.09	0.18	338.27	1.16
		Longitude	2.22 (1.29)	1.71	<0.001			
	Model 7	Salinity	0.13 (0.013)	10.63	<0.001	0.11	339.25	2.14
	Model 12	Latitude	-5.72 (0.99)	-5.78	<0.001	1.57e-09	375.40	38.29
		Longitude	3.83 (1.52)	2.52	0.013			
	Model 10	Longitude	6.36 (1.67)	3.81	<0.001	1.48e-15	403.14	66.03
	Model 16	null	-20.65 (0.17)	-123	<0.001	3.94e-18	415.00	77.90
δ^{15}N	Model 10*	Intercept	-253.39 (48.75)	-5.20	<0.001	0.37	171.62	0.00
		Longitude	-3.06 (0.55)	-5.51	<0.001			
	Model 12	Latitude	0.29 (0.38)	0.77	0.44	0.19	173.00	1.38
		Longitude	-2.92 (0.58)	-5.04	<0.001			
	Model 9	Salinity	3.13e-03 (5.91e-01)	0.51	0.61	0.16	173.35	1.73
		Longitude	-3.16 (5.91e-01)	-5.35	<0.001			
	Model 13	Salinity	8.73e-03 (7.44e-03)	1.17	0.24	0.14	173.58	1.96
		Latitude	6.03e-01 (4.61e-01)	1.31	0.19			
		Longitude	-3.07 (5.92e-01)	-5.19	<0.001			
	Model 3	Salinity	0.00065 (0.0081)	0.079	0.94	1.57e-06	196.37	24.75
		Latitude	0.87 (0.51)	1.69	0.093			
	Model 16	null	15.33 (0.059)	258.1	<0.001	1.29e-06	196.76	25.14
	Model 7	Salinity	-0.0078 (0.0065)	-1.21	0.23	9.98e-07	197.28	25.66

Table B4. Number of individuals (N) in each cluster, from Figure A3, with the number of darted dolphins in parentheses and mean isotope values ($\delta^{13}C$‰ and $\delta^{15}N$‰) with standard deviations (SD) in parentheses for defined clusters (A-H).

Cluster	N (darted)	Carbon±(SD)	Nitrogen± (SD)
A	21 (7)	-21.66 (1.42)	15.30 (0.38)
B	8 (2)	-20.61 (0.47)	25.73 (0.46)
C	5 (2)	-22.17 (2.41)	15.15 (0.44)
D	9 (5)	-20.74 (1.51)	15.28 (0.64)
E	3 (1)	-23.31	15.52
F	8 (4)	-21.14 (1.39)	15.00 (0.37)
G	2 (2)	-21.09 (0.94)	16.04 (0.07)
H	1 (1)	-21.08	13.85

Table B5. Resulting *p* values from the Tukey Post Hoc test conducted to determine isotopic differences in δ^{15}N‰ between clusters, with statistically significant pairings in bold.

Cluster pairing	*p* value
B-A	0.93
C-A	0.99
D-A	1.00
E-A	0.99
F-A	0.96
G-A	0.50
H-A	0.12
C-B	0.90
D-B	0.92
E-B	0.99
F-B	0.60
G-B	0.99
H-B	0.06
D-C	0.99
E-C	0.99
F-C	0.99
G-C	0.55
H-C	0.33
E-D	0.99
F-D	0.98

Cont.	Cluster pairing	*p* value
	G-D	0.51
	H-D	0.15
	F-E	0.97
	G-E	0.98
	H-E	0.23
	G-F	0.22
	H-F	0.37
	H-G	**0.02**

Appendix C: Conclusion supplemental figure

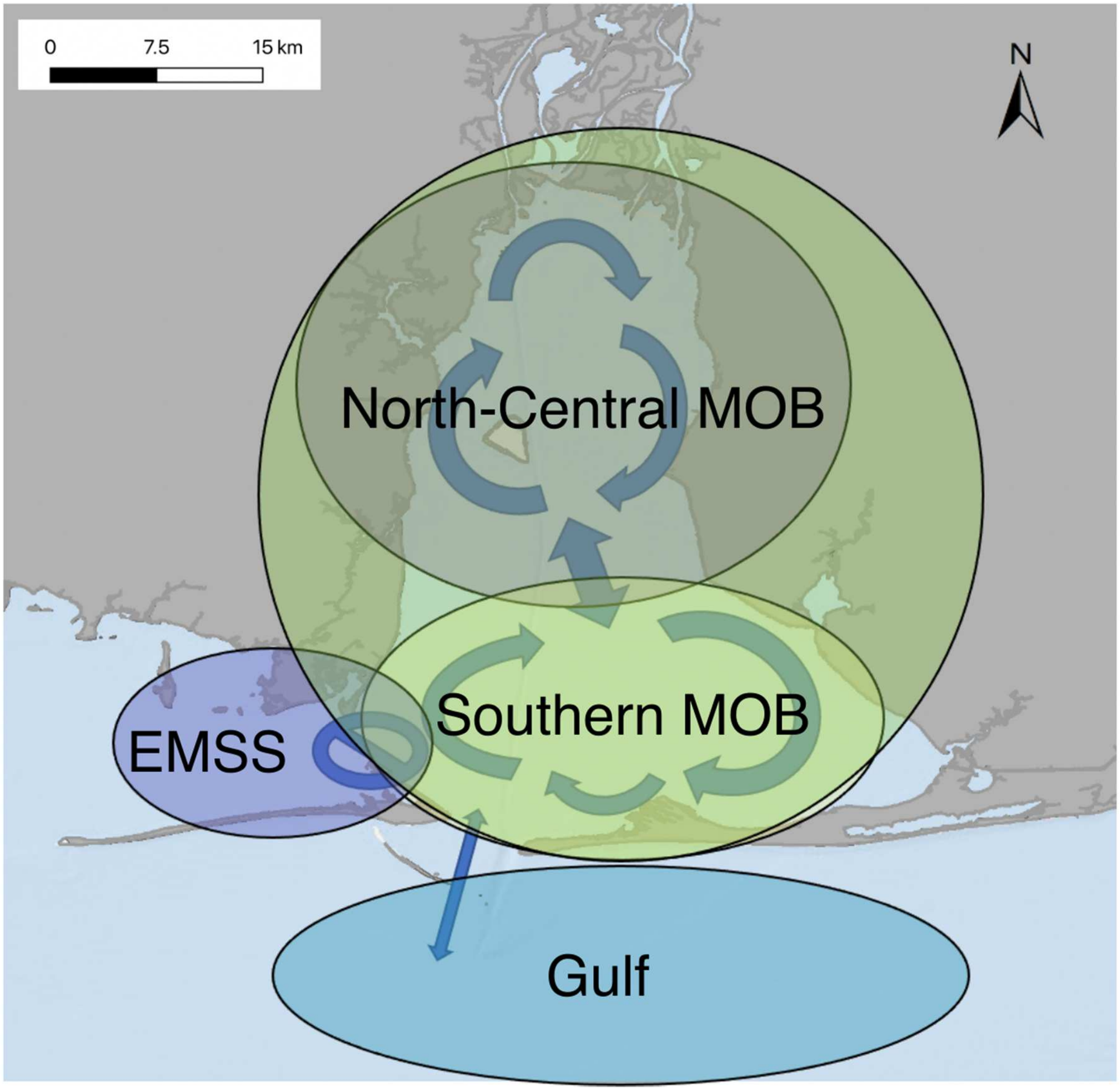

Figure C1. Dolphin movement (shown by arrows) among and between MOB, EMSS and Gulf regions. Scales of more distinct geographic comparisons (shown by colored circles) for geographic tracers.

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