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Using Photo-Identification and Genetic Data to Examine Fine-Scale Population Structure of Common Bottlenose Dolphins (*Tursiops truncatus*) in the Estuarine Waters Surrounding Savannah, Georgia

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Abstract

Many marine mammal species exhibit complex patterns of population structure. Specifically, common bottlenose dolphin (*Tursiops truncatus*) populations in the southeastern United States display varying degrees of spatial overlap and residency, including some that have year-round site fidelity to localized bays, sounds, and estuaries. Evidence of resident estuarine animals along the U.S. Atlantic and Gulf of Mexico coastlines is supported through photo-identification and genetic studies, although, currently, few studies have integrated both methods. The purpose of this project was to couple long-term photo-identification data with spatial and genetic analyses to examine population structure of common bottlenose dolphins in northern Georgia. Genomic DNA was extracted from skin samples (n = 69) collected in the Northern Georgia/Southern South Carolina Estuarine System Stock, and a portion of the mitochondrial DNA control region was sequenced. To determine potential fine-scale geographic delineations within this stock, the study area was split into three regions: (1) north, (2) buffer, and (3) south. No significant genetic differentiation was found when regions were compared by only sample collection location. The sighting locations of sampled dolphins that had been seen ≥ 10 times between 2009 and 2017 (n = 45) were mapped in *ArcMap*, Version 10.2. In an analysis of dolphins with ≥ 10 sightings only, a significant difference in F_{ST} was found between the north vs buffer area (p = 0.0147). When dolphins with ≥ 10 sightings (n = 45) were assigned to the region where ≥ 50% of their sightings were located, a significant difference in F_{ST} was found between the north vs buffer and north vs south regions (p = 0.0018 and p = 0.0164, respectively). The addition of spatial data revealed genetic division among the three regions. Thus, the combination of photo-identification and genetic analyses used in this study may be useful in determining population structure in the future.

Key Words: common bottlenose dolphin, *Tursiops truncatus*, population structure, mitochondrial DNA, photo-identification

Introduction

Marine mammals can exhibit broad distributions and ranging patterns over extended distances resulting in complex patterns of population structure (Hoelzel et al., 1998). Despite their extensive ranging capabilities, many marine mammal species display population structure on a much finer spatial scale than their full distribution might suggest (Norris, 2000). Understanding aspects of a population, such as geographic range, abundance, and demographic trends, is necessary for proper conservation and management (Wade & Angliss, 1997; Hoelzel et al., 1998), which relies on correctly identifying population units (Baird et al., 2009). The U.S. Marine Mammal Protection Act (MMPA) of 1972 defines a stock as "a group of marine mammals of the same species or smaller taxa in a common spatial arrangement that interbreed when mature." Stocks should be, under most circumstances, demographically independent populations, and the primary goal of the MMPA is to conserve

stocks as a significant functioning element of the ecosystem, which includes preventing any marine mammal stock from dropping below its optimum sustainable population level (Wade & Angliss, 1997). Therefore, accurately identifying demographically independent populations is a critical task for marine mammal management (Wade & Angliss, 1997).

An integrative approach that combines genetics with spatial data collected through photo-identification can provide additional insight into population structure. Evidence of such an approach has been exhibited in several marine mammal species, including the short-finned pilot whale (*Globicephela macrorhynchus*; Alves et al., 2013) and the southern right whale (*Eubalaena australis*; Carroll et al., 2011). Torres et al. (2003) examined the distribution and overlap of two common bottlenose dolphin (*Tursiops truncatus*) ecotypes and found that combining spatial data and genetics can provide insight into the distribution of overlapping populations. Areas utilized only by the coastal ecotype or only by the offshore ecotype of common bottlenose dolphins were identified using spatial and molecular analyses (Torres et al., 2003). Furthermore, evidence of habitat partitioning by common bottlenose dolphins between estuarine and bordering coastal waters has been found through photo-identification (Fazioli et al., 2006) and genetic studies (Sellas et al., 2005) in the northern Gulf of Mexico. Consequently, the combination of spatial analyses with genetic data can provide evidence of multiple, demographically independent populations of common bottlenose dolphins (Rosel et al., 2017a).

Common bottlenose dolphins in the southeastern U.S. exhibit varying degrees of residency, including small, localized bay, sound, and estuary (BSE) populations (Shane, 1980; Wells et al., 1987; Scott et al., 1990). Evidence of residency in estuarine populations along the U.S. Atlantic and Gulf of Mexico coastlines is supported through photo-identification (Wells et al., 1980; Zolman, 2002; Mazzoil et al., 2005; Balmer et al., 2008, 2014), genetic (Sellas et al., 2005; Rosel et al., 2009), and telemetry (Wells et al., 2017; Balmer et al., 2018a, 2018b) studies. Multiple populations of BSE common bottlenose dolphins exist within the complex system of salt marshes and bays along the eastern coast of the U.S. (Hayes et al., 2017). The National Marine Fisheries Service (NMFS) has defined the Northern Georgia/Southern South Carolina Estuarine System (NGSSCES) Stock of common bottlenose dolphins as residing in the estuarine waters between the North Edisto River in South Carolina and northern Ossabaw Sound in Georgia. NMFS considers the NGSSCES Stock of common bottlenose dolphins to be a strategic stock because the estimated abundance of the stock is small, and few mortalities would surpass the potential biological removal level (Hayes et al., 2017). Based on recent mark-recapture data, it has been postulated that the current southern boundary of the NGSSCES Stock needs to be modified, and animals residing from the Savannah River to the Wilmington River may be distinct from the animals residing between the Wilmington River and northern Ossabaw Sound (Thompson et al., 2017). The incomplete understanding of the region inhabited by the NGSSCES Stock and the movement patterns of these dolphins make it difficult to implement long-term sustainability plans for this protected species and strategic stock.

Many BSE populations in the southeastern U.S. have minimal to no genetic and photo-identification data, which are the primary and supplemental type of data, respectively, used to identify population structure (Sellas et al., 2005; Rosel et al., 2009, 2017a; Litz et al., 2012). Using the geographic location where the sample was collected is the most conservative method of estimating where an individual dolphin may reside and/or identifying with which population the animal is associated. However, incorporation of long-term sighting histories into sampling design for genetic studies is a unique method and allows for the integration of multiple types of data into population structure analyses. Combining multiple methods to investigate population structure has been found to be fruitful (Torres et al., 2003; Balmer et al., 2011). A study investigating residency patterns of common bottlenose dolphins in the Stono River estuary in South Carolina via photo-identification has been used to suggest that incorporating genetics could clarify population discreteness in future studies (Zolman, 2002). However, there have been few published studies combining long-term sighting histories and genetic data to examine population structure (Carroll et al., 2011; Alves et al., 2013). The ability to integrate long-term sighting data with genetic information is a novel approach that may allow for a more comprehensive assessment of common bottlenose dolphin population structure in the southeastern U.S. and offers potential applications for other marine mammal populations globally. The purpose of this study was to integrate multiple tools—long-term photo-identification and genetic data—to examine population structure of common bottlenose dolphins in the estuarine waters surrounding Savannah, Georgia.

Methods

The study area consisted of the estuarine waters south of Savannah, Georgia, from the southern Savannah River channel to northern Ossabaw Sound. This area comprised the southern range of the NGSSCES Stock extending into the

northernmost region of the Central Georgia Estuarine System (CGES) Stock (Hayes et al., 2017). It covered approximately 400 km^2 of small creeks, salt marshes, larger rivers, and sounds (Figure 1; defined in Perrtree et al., 2014). Photo-identification surveys were conducted from April 2009 to June 2015. Surveys were conducted at an on-effort speed of 33 to 40 km/h along previously determined transects (Figure 2; methods reviewed in Perrtree et al., 2014). The dorsal fin of each dolphin within a group was photographed using digital single lens reflex (DSLR) cameras (Cannon EOS 40D and Nikon D90) with 70-300 mm or 70-400 mm zoom lenses. Dolphins were considered a group if they were within 100 m of each other, moving in the same direction, and engaging in similar behaviors (Shane, 1990). Images were matched to images collected from previous survey efforts, and a catalog was created for the Savannah area. All dolphins entered into the Savannah area catalog were given a unique numerical identification code. There are a total of 508 cataloged individuals in this database.

To identify animals to target for remote biopsy, sighting history data for each individual dolphin were examined from the long-term photo-identification database for this study area. Cataloged individuals with ≥ 10 sightings were identified as high priority biopsy targets based upon the minimum number of sightings required for home range estimation by Urian et al. (2009). Individuals with < 10 sightings were considered moderate priority targets. Neonates, young calves, and their mothers were not considered for biopsy. Individuals were identified as neonates if fetal folds or fetal lines were present, if the individual was less than half the size of an adult, or if the individual was observed swimming in the echelon position (Shane, 1990; Mann & Smuts, 1999; Thayer et al., 2003; McFee et al., 2014). Dolphins with nondistinctive fins were considered low priority targets because there were less

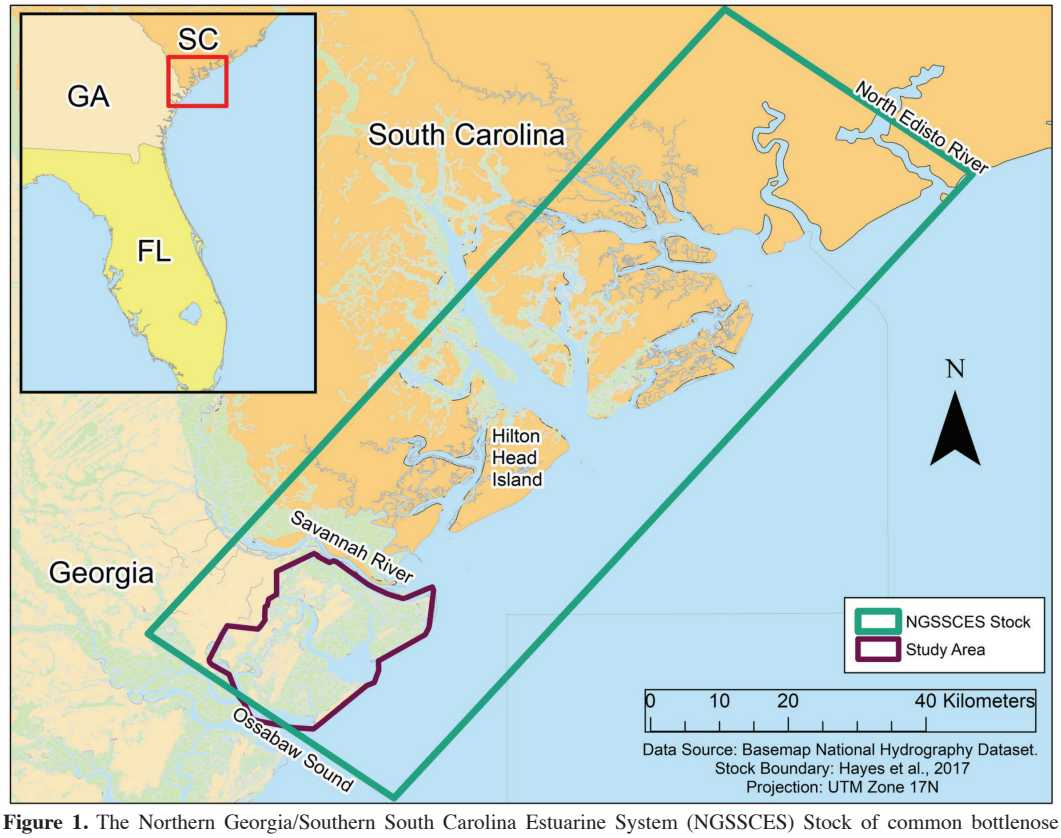

Figure 1. The Northern Georgia/Southern South Carolina Estuarine System (NGSSCES) Stock of common bottlenose dolphins (*Tursiops truncatus*) on the eastern coast of the United States and in the Savannah, Georgia, study area. The NGSSCES Stock is continuous in the estuarine waters from the North Edisto River, South Carolina, to northern Ossabaw Sound, Georgia (Hayes et al., 2017). The Savannah study area includes the inshore waters of Savannah from the Savannah River to northern Ossabaw Sound.

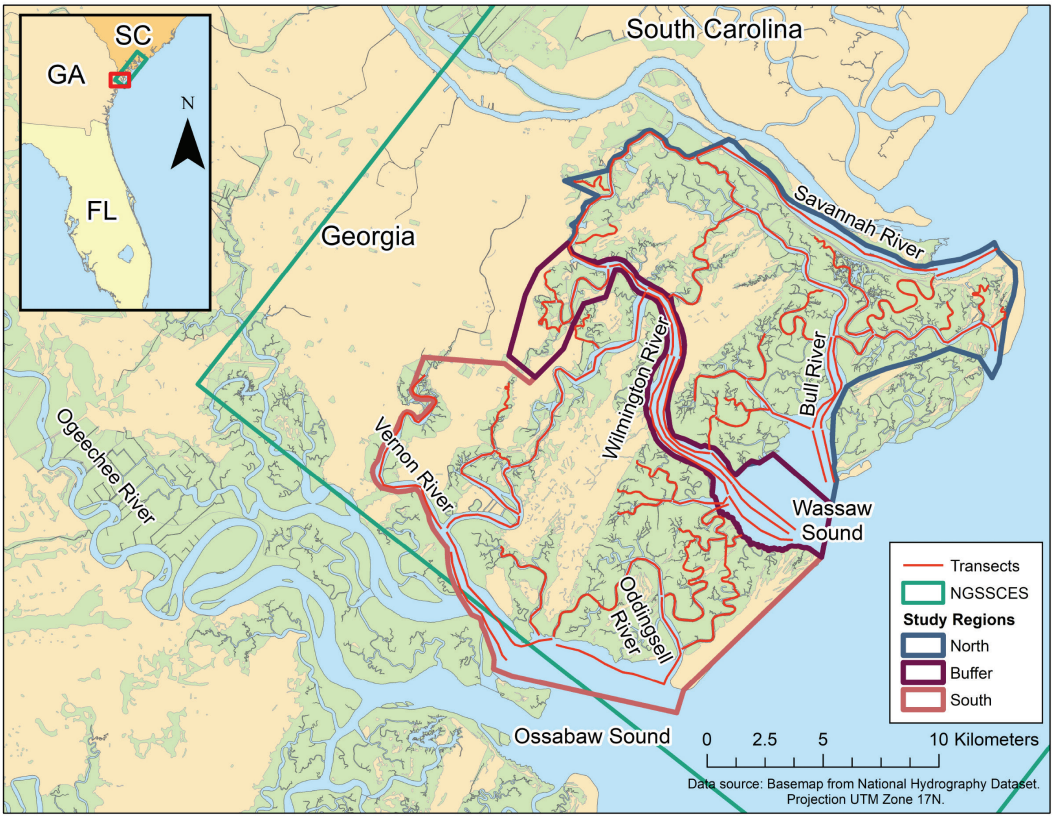

Figure 2. The Savannah, Georgia, study area was categorized into three segments: (1) a north region that consisted of the estuarine waters between the Savannah River and the Wilmington River, (2) a buffer region that included the Wilmington River and Wassaw Sound, and (3) a south region that included the area between the Wilmington River and northern Ossabaw Sound. Photo-identification survey transects are in red.

sighting history data available for these individuals. Sighting locations for each dolphin with ≥ 10 sightings were mapped in *ArcGIS*, Version 10.2 (ESRI, Redlands, CA, USA). The study area was categorized into three regions: (1) a northern region that consisted of the estuarine waters from the Savannah River to the Wilmington River, (2) a buffer region that included the Wilmington River and Wassaw Sound, and (3) a southern region that included the area between the Wilmington River and northern Ossabaw Sound (Figure 2). The regions were categorized based on previous photo-identification research (Thompson et al., 2017), which indicated that these regions could contain two separate populations with the buffer region acting as an area of overlap. The number of sightings in the north and south regions as well as the number of sightings within small creeks were counted and recorded for every individual with ≥ 10 sightings. Small creeks included all creeks within the study area, excluding Wassaw and Ossabaw Sounds, the Savannah River, the Wilmington River, the Vernon River, and portions of the Bull and Oddingsell Rivers (Figure 2).

To properly examine BSE populations only, it was necessary to exclude coastal animals—animals that live primarily in coastal waters and not the BSE waters—from the dataset. Thus, criteria were developed to ensure individuals biopsied were likely from the resident NGSSCES Stock and not the adjacent coastal stock, whose members are known to make short-term (transient) forays into estuarine waters, particularly at the larger openings of the bays to coastal waters (Balmer et al., 2018b). Of the dolphins with ≥ 10 sightings, animals with ≥ 50% of their sightings within small creeks were identified. Identifying animals with a majority of their sightings in small creeks provided a greater chance that the target animals were truly estuarine dolphins. In addition, common bottlenose dolphins residing outside of estuarine environments are thought to enter estuaries primarily during warm summer months (Speakman et al.,

2010; Balmer et al., 2013); thus, samples were collected in non-summer months: February, March, and September. Furthermore, dolphins with ≥ 50% of their sightings occurring in the north or south regions and ≤ 15% occurring in the opposite region were identified as target animals for biopsy. These metrics were formulated with the goal of maximizing the percentage of sightings in the target area while minimizing the percentage of sightings in the nontarget area, while also isolating small creek animals from coastal animals.

Remote biopsy sampling is a common tool for collecting tissue samples for genetic analyses (Sinclair et al., 2015). Remote biopsy sampling was conducted in September 2015 and February and March 2017. A total of 50 skin samples were collected using a crossbow and dart fitted with a 10 × 25 mm stainless steel sampling tip. The sampling tip contained a sharpened cutting edge angled inward to hold the sample in place. The size and type of sampling tip was chosen based on methods described in Sinclair et al. (2015). The crossbow was equipped with a retainer to hold the dart in place and a battery-powered sight to increase accuracy. The same digital DSLR cameras utilized during photo-identification surveys were used during remote biopsy sampling. When a group of dolphins was sighted, the group was approached to determine if an animal suitable for biopsy was present. Known target individuals identified using an onboard photo-identification catalog were prioritized for sampling. However, if no known dolphins were within the group, dolphins with unidentified yet distinctive fins were considered for biopsy. Dolphins with unidentified fins were only sampled if found in a small creek away from the open ocean to improve the chances that the sample was from an estuarine dolphin.

Sampling was attempted when the dolphin was perpendicular to the sampler and 4 to 10 m from the boat (Krützen et al., 2002). The sample was collected from below the dorsal fin on either side of the animal. After the dart came into contact with the animal and a sample was obtained, the dart floated near the surface and was retrieved. The sample was then removed from the sampling tip and cut into five separate pieces. One section—half of the skin—was placed directly into a 5 mL 20% DMSO/saturated NaCl vial for later genetic analysis and was stored at room temperature. Additionally, skin samples from stranded animals (n = 19) were provided by the Georgia Department of Natural Resources. These samples were collected using a scalpel blade to remove a small portion of skin from the body of the animal below the dorsal fin, placed into 5 mL 20% DMSO/saturated NaCl vials, and stored at room temperature. Stranding samples were collected from April 2008 to May 2017.

Genomic DNA was extracted from the skin samples using a Qiagen DNeasy Blood and Tissue extraction kit. Upon completion of extraction, the DNA was stored at 4°C. The quality of the extracted DNA was examined by gel electrophoresis. The quantity of DNA was measured via fluorometry on a Hoefer DyNA Quant 200 fluorometer. Extracted DNA was diluted to 25 ng/µl, but samples with < 30 ng/µl DNA were not diluted. A 500 bp fragment of the 5' end of the mitochondrial DNA (mtDNA) control region and adjacent tRNAs were amplified using the polymerase chain reaction (PCR) with the primers L15824 (5'-CCTCACTCCTCCCTAAGACT-3') (Rosel et al., 1999) and H16498 (5'-CCTGAA GTAAGAACCA GATG-3') (Rosel et al., 1994). Extracted genomic DNA (25 ng) was added to a 24 µl PCR reaction mix that included 20 mM Tris HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.3 µM of each primer, 150 µM dNTPs, and 5 U Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA). For samples with a DNA concentration < 25 ng/µl, 0.24 mg ml^{-1} of Bovine Serum Albumin (BSA) was added. The cycling profile was an initial cycle at 95°C for 30 s, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. After 30 cycles, the PCR continued at 72°C for 7 min and then was held at 4°C. PCR product was run on a 0.8% agarose gel to test the success of the reaction and, if successful, the remaining product was purified using a 0.8% low melting point agarose gel. Each band was excised from the gel under UV light with a razor blade, placed in a labeled 1.5 ml tube, digested using 2.5 U of agarase (Thermo Fisher Scientific), and held at 40°C overnight. A cycle sequencing PCR reaction was then prepared using the original primers, 5X buffer, and BigDye, Version 1.1, dye terminator mix (Applied Biosystems, Foster City, CA, USA). The cycling profile was an initial 96°C for 1 min, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, 60°C for 4 min, and then held at 4°C. The cycle sequencing products were cleaned with Sephadex®, completely dried, and then stored at -20°C. The final sequencing products were loaded on an ABI 3130 Genetic Analyzer. All samples were sequenced in both forward and reverse directions. Sequences were edited and aligned creating consensus sequences using *Geneious*, Version 10.2.3. Consensus sequences were aligned with a master alignment to determine haplotypes.

Nucleotide diversity (π) and haplotype diversity (h; Nei, 1987), which measure the degree of polymorphism within a population, were estimated using *ARLEQUIN*, Version 3.5.2.2 (Excoffier & Lischer, 2010). Population differentiation, F_{ST} and Φ_{ST}, were estimated in *ARLEQUIN* using an analysis of molecular variance (AMOVA) framework. Significance values were determined using 10,000

permutations. The program jModelTest (Guindon & Gascuel, 2003; Darriba et al., 2012) was used to determine the best evolutionary model for estimating Φ_{ST}. The Tamura & Nei (1993) model with a gamma correction ($\alpha = 0.5$) was used for the distance matrix in the analyses. F_{ST} and Φ_{ST} were calculated for regions north vs buffer, north vs south, and buffer vs south. The Bonferroni false discovery test was used to correct probability values for multiple comparisons.

Data were first analyzed based on the location where each sample was collected; F_{ST} and Φ_{ST} values were calculated comparing all samples collected in the north, buffer, or south region. Then, data were analyzed, and F_{ST} and Φ_{ST} values were calculated based on sample collection location for only animals with ≥ 10 sightings. Lastly, based on sighting history data, samples from dolphins with ≥ 10 sightings were assigned to the region where ≥ 50% of the animal's sightings were located, and F_{ST} and Φ_{ST} values were calculated. In summary, analyses were first conducted on all the animals based on sample collection location and then with the inclusion of sighting history data to observe how the addition of photo-identification data may have helped characterize population structure.

Results

A total of 69 skin samples for genetic analysis were collected in the estuarine waters surrounding Savannah, Georgia. Fifty samples were collected through biopsy efforts, and 19 samples were obtained from stranded animals. Twenty-one samples were collected from the north region, 14 samples were collected from the buffer region, and 28 samples were collected from the south region. Six samples were collected outside of the study area on Tybee Beach and upstream from the Savannah and Ogeechee Rivers. Samples collected outside of the study area were obtained from stranded individuals and were included into the closest study region from which the sample was collected for analyses. Fifty-eight samples were matched to known dolphins in the Savannah study area catalog, and 45 of those had ≥ 10 sightings.

Five haplotypes were identified from all 69 samples. All haplotypes were associated with the western North Atlantic coastal ecotype (Rosel et al., 2009). Four animals were identified as Ttr1, four were Ttr4, two were Ttr5, 43 were Ttr6, and 16 were Ttr9 (haplotype names from Rosel et al., 2009; Table 1 & Figure 3). The most common haplotype, Ttr6, was found in all three study regions. The next most frequent haplotype, Ttr9, was also found in all three regions. Haplotype Ttr1 was found only in the north and south regions; Ttr4 was found only in the north region; and Ttr5 was found only in the buffer and south regions. The distribution of each haplotype is shown in Table 1. Haplotype and nucleotide diversity for all 69 samples based on sample collection location is shown in Table 2a; animals with ≥ 10 sightings based on sample collection location are shown in Table 2b; and animals with ≥ 10 sightings assigned to the location where ≥ 50% of their sightings were located are found in Table 2c.

Genetic differentiation (F_{ST} and Φ_{ST}) was estimated for regions north vs south, north vs buffer, and buffer vs south utilizing three different data analyses. First, F_{ST} and Φ_{ST} were estimated for the sample collection location of all 69 samples, with no significant differences observed for any comparison after Bonferroni correction (Table 3a). Second, F_{ST} and Φ_{ST} were estimated based on the sampling collection location of only animals with ≥ 10 sightings ($n = 45$); only the estimate of F_{ST} between the north and buffer regions differed significantly from zero (Table 3b). A significant difference was found for F_{ST} between the north vs buffer and north vs south regions when the samples were assigned to location by sighting history rather than sample collection location (Table 3c). In this case, the percentage of sightings in each of

Table 1. Mitochondrial DNA control region haplotypes found among 69 common bottlenose dolphins (*Tursiops truncatus*) sampled in the estuarine waters of Savannah, Georgia. Total number of individuals with each haplotype, the percentage of each haplotype in the sample population, and the number of individuals with each haplotype exhibited in the north, buffer, and south regions are included.

Haplotype	Number of individuals	Percentage of total sample population	Number of individuals in the north region	Number of individuals in the buffer region	Number of individuals in the south region
Ttr1	4	0.6	2	0	2
Ttr4	4	0.6	4	0	0
Ttr5	2	0.3	0	1	1
Ttr6	43	62.0	13	12	18
Ttr9	16	23.0	7	1	8
Total	69		26	14	29

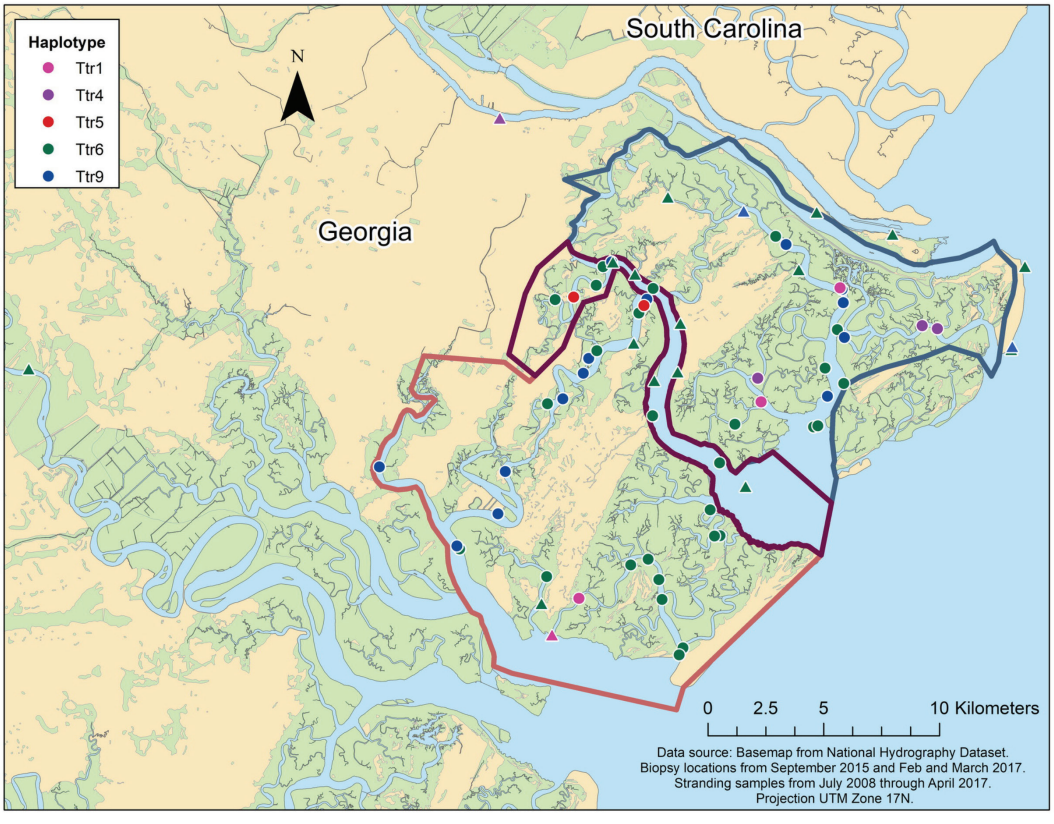

Figure 3. Biopsy (circles) and stranding (triangles) samples collected from common bottlenose dolphins in Savannah, Georgia, from the Savannah River to northern Ossabaw Sound. A total of 69 samples were collected. Biopsy samples (n = 50) were collected in September 2015 and February and March 2017. Stranding samples (n = 19) were collected from July 2008 through April 2017. Sampling locations are color coded to match identified mitochondrial control region haplotypes.

the three regions was calculated for each of the 45 individuals, and they were assigned to the region where 50% or more of their sightings were found.

Discussion

In contrast to using sampling collection location alone as a proxy for regional affiliation where no significant differences were seen among regions, a significant difference in F_{ST} was found between the north and the buffer regions when using animals with ≥ 10 sightings in the analyses, and a significant difference was found between the north and the south regions with the inclusion of sighting history information. Thus, the addition of photo-identification data revealed a significant difference between the north and buffer regions and the north and south regions that was not identified using sample collection location alone. However, no difference was detected between the buffer and south regions with any of the three data partitions. It is possible that instead of the Wilmington River being a buffer region, the area is alternatively a part of the south region and both are separate from the north region, thus creating two study regions instead of three. If true, the Wilmington River might be a more appropriate border for the NGSSCES and CGES Stocks. Also, the sample size from the buffer region was the lowest, and this may have had an effect on the power to detect significant differences.

Another possible interpretation of the results from this study is that the larger tributaries, such as the Wilmington River (buffer region), are an area of BSE and coastal population overlap. An overlap of populations and sampling in these areas may explain why no genetic differentiation was found without the inclusion of photo-identification data. Balmer et al. (2013) suggested that based on photo-identification data from southern Georgia, dolphins from the western North Atlantic South Carolina/Georgia Coastal Stock were sighted primarily in larger tributaries, and these were potentially areas of shared habitat between the BSE and adjacent coastal population(s). This hypothesis was further

Table 2. Genetic diversity estimates in mtDNA control region sequences including number of haplotypes (H), mean haplotype diversity (h), and nucleotide diversity (π) based on sample collection location of all 69 samples (a), sample collection location of dolphins with ≥ 10 sightings (b), and sighting history data (c). Samples from dolphins with ≥ 10 sightings were assigned to the region where ≥ 50% of the animal's sightings were located. *n* = sample size; SE = standard error.

		n	H	h (SE)	π (SE)
(a)	All	69	5	0.6905 (0.5306)	0.0019 (0.0016)
	North	26	4	0.8276 (0.6108)	0.0023 (0.0019)
	Buffer	14	3	0.4285 (0.4107)	0.0012 (0.0013)
	South	29	4	0.6748 (0.5313)	0.0019 (0.0016)
(b)	All	45	5	0.6767 (0.5270)	0.0019 (0.0016)
	North	16	4	1.0166 (0.7182)	0.0028 (0.0022)
	Buffer	11	2	0.1818 (0.2534)	0.0005 (0.0008)
	South	18	3	0.7134 (0.5594)	0.0020 (0.0017)
(c)	All	45	5	0.6767 (0.5270)	0.0019 (0.0016)
	North	18	4	1.0065 (0.7091)	0.0028 (0.0022)
	Buffer	13	2	0.1538 (0.2286)	0.0043 (0.0007)
	South	14	3	0.5494 (0.4796)	0.0015 (0.0015)

Table 3. Pairwise estimates of genetic differentiation between the north, buffer, and south regions in the Savannah study area based on sample collection location of all 69 samples (a), sample collection location of dolphins with ≥ 10 sightings (b), and sighting history data (c). Samples from dolphins with ≥ 10 sightings were assigned to the region where ≥ 50% of the animal's sightings were located. F_{ST} and Φ_{ST} were estimated among regions in *ARLEQUIN*, Version 3.5.2.2. F_{ST} values are reported below the diagonal, and Φ_{ST} values are reported above. * = significant difference after Bonferroni correction for multiple comparisons.

		n	North	Buffer	South
(a)	North	26	--	0.0404 (p = 0.1433)	-0.0020 (p = 0.4069)
	Buffer	14	0.1169 (p = 0.0303)	--	0.0307 (p = 0.2401)
	South	29	-0.0040 (p = 0.4219)	0.0556 (p = 0.1341)	--
(b)	North	16	--	0.0634 (p = 0.0978)	-0.0101 (p = 0.5072)
	Buffer	11	0.2237 (p = 0.0147)*	--	0.0168 (p = 0.2844)
	South	18	0.0507 (p = 0.1279)	0.0500 (p = 0.2036)	--
(c)	North	18	--	0.1241 (p = 0.0407)	0.0528 (p = 0.1371)
	Buffer	13	0.2969 (p = 0.0018)*	--	-0.0430 (p = 0.9999)
	South	14	0.1678 (p = 0.0164)*	-0.0229 (p = 0.5944)	--

strengthened by satellite telemetry data which demonstrated higher overlap between BSE and coastal dolphins in the larger tributaries and sounds of southern Georgia (Balmer et al., 2018b).

Additionally, some common bottlenose dolphin populations are known to exhibit seasonal changes in distribution (Speakman et al., 2010). In some locations, coastal animals travel into bays, sounds, and estuaries in the warmer months (Speakman et al., 2010; Balmer et al., 2013). Remote biopsy sampling was conducted in February, March, and September, which are considered part of the winter and fall seasons; and samples collected from stranded animals were collected year-round. The animals biopsied in the buffer region may actually be coastal animals that had traveled inshore, or they may be a mixture of coastal animals and estuarine residents. However, this would indicate that coastal animals travel into the bays in the cooler seasons as well which is in contrast to the findings of Speakman et al. (2010) and Balmer et al. (2013). The inclusion of coastal animals provides a possible explanation for the lack of a significant difference found using only sample collection location. Without the inclusion of photo-identification data, a mixture of coastal and estuarine animals may have been used in the analysis. Genetic analyses that include animals from the coastal stock may help sort out these different hypotheses.

In addition, to fully compare the genetic differences among locations examined in this study, nuclear DNA analysis, such as microsatellites, should be included. Unlike mtDNA, microsatellites are inherited from both parents and may provide a more thorough examination of relatedness and gene flow (Rosel et al., 2017b). Future studies should include microsatellite data or other nuclear data in addition to mtDNA to acquire a more comprehensive assessment of population structure and to determine whether there are multiple demographically independent populations within the NGSSCES Stock. Nuclear microsatellite data would also allow examination of relatedness among the samples and would ensure estimates of differentiation among regions are not influenced by groups of highly related individuals.

The dolphins in the Savannah area are known to interact with recreational and commercial fishing vessels (Perrtree et al., 2014). These human-interaction behaviors in the Savannah area were found at a much higher rate than in other areas with known human-interaction behaviors (Perrtree et al., 2014). Interactions between dolphins and humans can lead to serious injury (Powell et al., 2018; Balmer et al., 2019), mortality (McFee et al., 2006; Donaldson et al., 2010), and behavioral changes (Mann & Smuts, 1999; Orams, 2002; Hazelkorn et al., 2016). There is currently no abundance estimate nor estimate of potential biological removal (PBR) for the NGSSCES Stock, yet a recent Atlantic Bottlenose Dolphin Take Reduction Team noted that the common bottlenose dolphins in the Savannah area may be close to exceeding PBR due to crab pot entanglements. With little known about the dolphins in the Savannah area coupled with the threat of cumulative anthropogenic impacts, it is imperative to use a multifaceted approach, such as the combination of genetics and photo-identification, to better understand the population structure and, thus, the stock delineations of the NGSSCES. The inclusion of spatial data may give a more accurate representation of population structure and genetic make-up of a population. Therefore, methods in this study could be utilized by future studies in which long-term photo-identification data are available to improve our understanding of the complex population structure of common bottlenose dolphins and other marine mammals.

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