OCS Study
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Bottlenose Dolphin Stock Structure in the Gulf of Mexico

US Department of the Interior
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DISCLAIMER

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ABOUT THE COVER

BOEM photograph of bottlenose dolphins.

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List of Abbreviations and Acronyms

Short form	Long form
BSE	Bays, sounds, and estuaries
CC	Corpus Christi Bay
FB	freeze-banded
GAMMS	Guidelines for Assessing Marine Mammal Stocks
ICW	Intracoastal Waterway
MG	Matagorda Bay
MMPA	Marine Mammal Protection Act
MSS	Mississippi Sound
SAB	San Antonio Bay
TCB	Texas Coastal bend
UME	Unusual Mortality Event

1 Introduction

1.1 Bottlenose Dolphin Stock Structure

The common bottlenose dolphin, *Tursiops truncatus*, is ubiquitous in the estuarine and nearshore coastal waters of the northern Gulf of Mexico. Bottlenose dolphins occur in a diverse range of habitats including within estuaries, shallow Continental Shelf waters, Outer Continental Shelf waters, and deep waters over the inner continental slope. In addition, two genetically and morphologically distinct ecotypes occur within U.S. waters. The larger, more robust "offshore" ecotype tends to occupy waters of the outer continental shelf and inner slope, while the more slender "coastal" ecotype occurs in waters closer to shore and within estuaries and embayments (Hoelzel et al. 1998; Mead and Potter 1995; Rosel et al. 2009; Vollmer 2011).

Associated with this diverse range of habitats, there is a diverse range of residency and movement patterns observed among sympatric bottlenose dolphin populations. Some populations demonstrate large scale seasonal movements, most notably the "Northern Migratory Stock" of bottlenose dolphins along the U.S. East Coast that demonstrates northern movements during summer months to waters off the coast of New Jersey and a more southern distribution during cooler months occurring off the coast of North Carolina (Waring et al. 2015). In contrast, populations residing within estuarine waters demonstrate long-term residency with relatively localized movements and limited migration. The most well documented resident estuarine stock is the Sarasota Bay population on the southwest coast of Florida which has documented individuals inhabiting this relatively small embayment for over 40 years and multiple generations (Irvine et al. 1981, Wells 2003). Similar long-term residency patterns have been observed in multiple embayments along the Atlantic coast including Pamlico Sound, North Carolina (Read et al. 2003), Charleston, South Carolina (Zolman 2002, Speakman et al. 2006), Indian River Lagoon, Florida (Mazzoil et al. 2005) and Biscayne Bay, Florida (Litz 2007). Long-term residency has been documented in several estuarine systems along the Gulf coast including within St. Joseph's Bay, Florida (Balmer et al. 2008), Mississippi Sound, Mississippi (Hubard et al. 2004), and Texas (Fertl 1994, Lynn 1995).

While estuarine habitats clearly support long-term resident bottlenose dolphin populations, the spatial extent and seasonal movements of these populations are generally poorly understood. In several cases, there is demonstrated use of nearshore (generally <2 km from shore) coastal waters or barrier islands by resident "estuarine" bottlenose dolphins. This is the case, for example, in the relatively large and open Mississippi Sound embayment where estuarine residents are frequently observed near and around barrier islands that border coastal waters (Hubard et al. 2004, Mackey 2010). Similarly, resident animals that occur within Pamlico Sound, North Carolina during summer months use nearshore coastal waters near inlets and seasonally may move offshore into warmer coastal waters during winter months (Read et al. 2003). In addition, "transient" animals are frequently observed within estuaries that may indicate either a seasonal or intermittent movement of individuals from nearby coastal habitats into estuarine waters. For example, "transient" (i.e., non-resident) animals were observed within a photo-identification study of Choctawhatchee Bay, Florida (Conn et al. 2011), and a seasonal influx of presumably coastal animals was observed in St. Joseph's Bay, Florida (Balmer et al., 2008). Thus, while it is clear that estuarine habitats support localized and resident bottlenose dolphins, the degree to which these groups interact with adjacent coastal populations and/or the specific geographic boundaries between putative populations is often unclear. Directed sampling and analysis of genetic population structure are required to clearly establish genetic differentiation and spatial boundaries for these populations.

An analysis of genetic population structure in bottlenose dolphins along the U.S. East Coast indicated significant genetic differentiation among five potential populations including significant differences both between animals occupying different estuaries and between estuarine and coastal populations (Rosel et al., 2009). In both the mitochondrial and nuclear genome, there were significant differences indicating demographic isolation between resident animals in estuaries between Charleston, South Carolina, southern Georgia, and Jacksonville, Florida. This study also included samples from the Gulf of Mexico (collected in the Florida Panhandle) and found strong differences with those populations occurring within the western North Atlantic. A similar study found significant genetic differences between resident animals within Biscayne Bay, Florida and those within Florida Bay, Florida indicating limited gene flow between these adjacent estuaries (Litz et al. 2012).

Within the Gulf of Mexico, there have been few direct studies of genetic differentiation among resident estuarine dolphin groups. The majority of available studies examined differentiation between Gulf of Mexico and western North Atlantic populations within the framework of evaluating basin-scale population structure within bottlenose dolphins (reviewed in Vollmer and Rosel 2013). These findings agree with the results of the Rosel et al. (2009) study indicating significant differentiation between western North Atlantic and Gulf of Mexico bottlenose dolphins. A study by Sellas et al. (2005) examined genetic differences among estuaries and adjacent coastal waters along the west coast of Florida and included samples from the western Gulf of Mexico. Microsatellite analyses demonstrated significant differences between all of these estuarine populations and differentiation between coastal and inshore populations (Sellas et al. 2005). These findings suggest significant fine-scale separation between adjacent populations despite a lack of geographic barriers to gene flow. This may be related to residence within localized habitats and limited dispersal of individuals documented in long term studies of animal movements and residency within estuaries.

The unit of management mandated by the U.S. Marine Mammal Protection Act (MMPA) is the “population stock” which is defined as a group of animals that occupy the same habitat and interbreed when mature. It has been further clarified by through the National Marine Fisheries Services Guidelines for Assessing Marine Mammal Stocks (GAMMS) as a demographically isolated group where internal processes (i.e., births and deaths) are much more important than external processes (i.e., immigration and emigration) for controlling population dynamics (Wade and Angliss 1997). Functionally, delineation of stocks can be accomplished through a variety of tools that quantify the degree of demographic isolation between groups of dolphins occupying different habitats. However, genetic evidence for differentiation is the strongest available indicator of demographic isolation since even very low rates of interbreeding between groups will be sufficient to cause homogeneity. Statistically significant differences in mitochondrial and/or nuclear allele frequencies are an indicator of long term, stable demographic isolation between groups of dolphins. Therefore, genetic data provides sufficient evidence to delineate stocks as defined under the MMPA (Wade and Angliss 1997).

In the Gulf of Mexico, there are 31 defined stocks of bottlenose dolphins within Bays, Sounds and Estuaries (BSE), 3 stocks in nearshore coastal waters (<20 m depth), a Gulf-wide continental shelf stock (waters < 200 m depth), and an oceanic stock occupying waters deeper than 200 m (Waring et al. 2015, Figure 1).

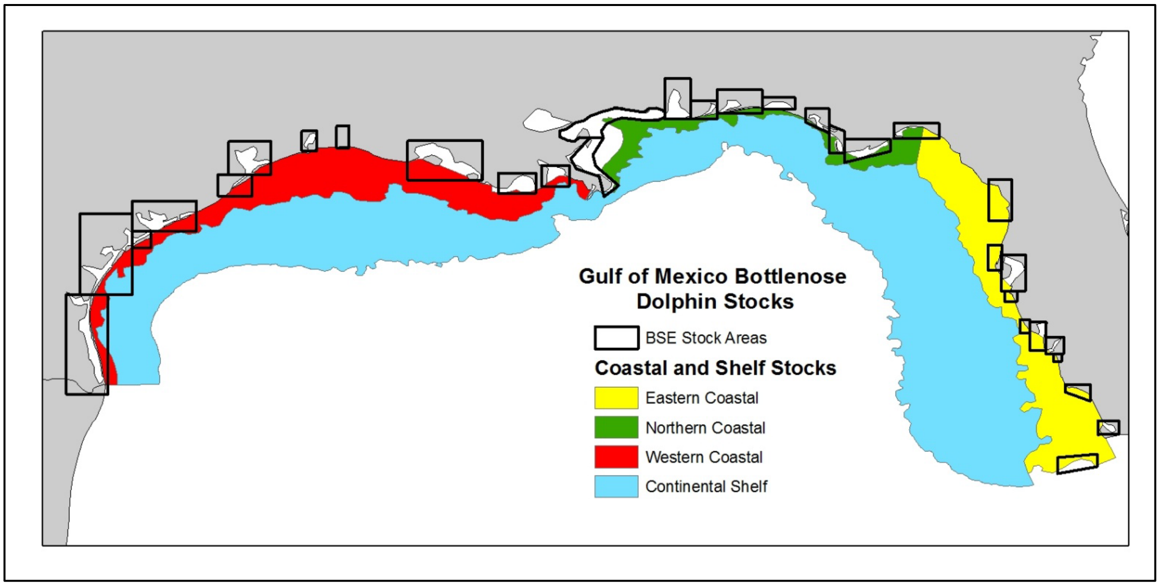

Figure 1. Bottlenose dolphin stocks in the Gulf of Mexico.

The BSE stocks, and their boundaries, were defined based upon the evidence of year-round residence of small local populations rather than directed studies of the degree of demographic isolation between stocks. With the exception of the Sellas et al. (2005) study, there has been no direct examination of the appropriate delineation amongst these defined stocks. Similarly, the boundaries for the three coastal stocks (Western, Northern, and Eastern) and the continental shelf stock were based upon presumed biogeographic breaks rather than direct examination of demographic structure. A more recent analysis of available genetic data suggests a significantly more complex structure even in coastal and continental shelf waters (Vollmer 2011). Therefore, studies of population structure among the BSE stocks of the northern Gulf of Mexico are critically needed to improve their management and conservation.

1.2 Bottlenose Dolphin Stocks on the Texas Coast

Seven defined bottlenose dolphin stocks occupy the bays, sounds, and estuaries along the Texas coast. In addition, the Western Coastal stock occurs along the Texas and Louisiana coasts extending east to the mouth of the Mississippi River in nearshore coastal waters in depths less than 20m (Waring et al. 2015). The estuarine stocks occur in all the major embayments along the coast and include: Sabine Lake, Galveston Bay, West Bay, Matagorda Bay, Copano Bay/Aransas Bay/San Antonio Bay/Espiritu Santo Bay, Corpus Christi Bay, and Laguna Madre (Waring et al. 2015; Phillips and Rosel 2014). Some of the dolphins occurring in the Matagorda-Espiritu Santo Bay area (Lynn and Würsig 2002), Aransas Pass (Shane 1977, Weller 1998), San Luis Pass (Maze and Würsig 1999; Irwin and Würsig 2004), and Galveston Bay (Fertl 1994) have been reported as long-term residents. However, there have been no directed genetic studies to date to verify the boundaries between these stocks or the degree to which they are distinct from animals in coastal waters. However, there have been studies in several of these estuaries that have examined ranging patterns and residency of individuals.

Limited radio tracking studies conducted in the early 1990s demonstrate movement of individuals between adjacent estuaries along the Texas coast. For example, a study of 35 bottlenose dolphins captured in Matagorda Bay or Espiritu Santo Bay during 1992 and 1993 showed movements of some individuals into San Antonio Bay and spending days or weeks in this region before moving back into Matagorda Bay (Lynn and Würsig 2002). Radio tagged animals from this study also left the Bay and moved into nearshore waters of the Gulf of Mexico. The estimated distances from shore were typically less than 1km; however, uncertainty in locations from the radio tags makes assessment of the use of coastal habitats difficult (Lynn 1995). Ten of the animals freeze-branded (FB) during this study were never re-sighted in follow up surveys. However, one of these animals was seen after the survey offshore of Galveston and another was re-sighted near the Corpus Christi Ship Channel (Lynn 1995). These data suggest a degree of movement of individuals between adjacent estuaries and coastal waters. It is possible that some of the animals captured and FB in Matagorda Bay were actually coastal stock animals that had larger ranging patterns than true estuarine residents.

There are varying degrees of information about residency and seasonal movements within the different estuaries of the Texas coast. Past photo-identification studies have documented seasonal movement patterns within estuaries and multiple patterns of residency. For example, in Copano Bay-Aransas Bay there appeared to be spring/summer, fall/winter, and year-round resident animals (Shane 1980). In Matagorda Bay, Gruber (1981) indicated that dolphins moved seasonally in and out of the Bay, and that there was seasonal variation in movement patterns within the Bay. Surveys indicated that dolphins more frequently occurred along the western edges of Matagorda Bay rather than the middle portion of the Bay during winter months (Barham et al. 1980). Photo-identification studies in Galveston Bay and coastal Gulf waters documented over 1,000 unique individuals. However, most of these animals were sighted only once and associations between individuals were weak with frequent exchanges of members between groups. Only 200 of these animals were identified as long-term residents with the remainder potentially traveling through the area (Bräger et al. 1994).

Overall, there has been relatively limited study of the stock structure, abundance, and ecology of bottlenose dolphins inhabiting estuarine waters along the Texas coast. Initial delineations between the stocks were described based upon strata used during aerial surveys conducted during the early 1990s and limited information on residency and movement patterns. None of these stocks has a recent abundance estimate (Waring et al. 2015). A review of available information on anthropogenic and natural stressors for these stocks demonstrates a number of potential sources of mortality. Many of the Texas stocks are likely exposed to impacts from pollution associated with oil and gas activities, dredging and construction, vessel traffic, and algal blooms (Phillips and Rosel 2014). There have also been several large scale Unusual Mortality Events (UMEs) along the Texas coast during the last two decades that have impacted these stocks (Litz et al. 2014). The overall lack of information combined with the prevalence of multiple sources of potential mortality make these stocks a high priority for data collection to better inform management and conservation actions (Phillips and Rosel 2014).

1.3 Bottlenose Dolphins in Mississippi Sound

Mississippi Sound is a large embayment extending along the coasts of Alabama, Mississippi, and Northern Louisiana. It is open to the coastal waters of the Gulf of Mexico through large passes between several barrier islands. As a result, it is probable that there is significant exchange between resident bottlenose dolphins that occupy nearshore estuarine waters and waters around the barrier islands and animals from the adjacent Northern Coastal stock. The stock area as currently defined includes Lake Borgne and Bay Boudreau at the western end of Mississippi Sound. The inclusion of these areas results from management surrounding a bottlenose dolphin live-capture fishery that was conducted during 1973–1988 (Scott 1990). During the period when this fishery was active, 202 bottlenose dolphins were removed from waters of Mississippi Sound and adjacent areas out of a total of 533 bottlenose dolphins collected in the southeastern United States (Scott 1990).

Photo-identification studies conducted in 1995–1996 identified long-term resident animals based upon re-sights of individuals first seen in 1991 and animals that had been FB during live captures in the early 1980s (Hubard et al. 2004). Bottlenose dolphins displayed varying degrees of site fidelity with some animals showing preferences for different habitats such as barrier islands, channels, or the mainland coastline (Hubard et al. 2004). Mackey (2010) examined seasonal residence and site fidelity during surveys conducted from 2004 to 2007. Mackey identified 678 animals and classified 71 of these as year-round residents, 109 as seasonal residents, and 498 as transients. The transient animals are likely to occur near or around barrier islands and may represent the occurrence of animals from the Northern coastal stock occurring within the surveyed area. Both of these studies documented numerous animals seen only on one occasion and suggested that animals may move out of the Sound into nearshore coastal waters during winter months (Mackey 2010, Hubard et al. 2004).

Density and abundance estimates for the Mississippi Sound stock have been derived from both small vessel and aerial line transect surveys. Hubard et al. (2004) estimated a density of 1.3 animals km^2 during peak densities in the summer months. Densities were lower during winter months with the total estimated abundance declining by approximately 50%. Miller et al. (2013) conducted a small-boat line transect study of the western half of Mississippi Sound including regions around the barrier islands and offshore. This study found a similar estimate of density during summer to that of Hubard et al. (2004) at 1.1 animals km^2 during summer months and a lower estimate of density (0.7 animals km^2) during winter months. These data collectively suggest movement of transients into nearshore waters of the Gulf or further offshore during colder months, perhaps associated with changes in prey resources or habitat characteristics. The most recent abundance estimates used in MMPA stock assessment reports for this stock are based upon aerial surveys conducted during 2011–2012 that covered the entire bay during all four seasons. The abundance of dolphins in the Mississippi Sound area was highest during warmer seasons (spring-fall) and lowest during winter months. The current abundance estimate for the stock is based upon the winter abundance estimate which is assumed to represent resident dolphins (Waring et al. 2015).

Assessments of the status of the Mississippi Sound stock are complicated by the uncertainty surrounding the seasonal exchange of transient animals in nearshore coastal waters and variable habitat preferences for potentially resident animals. Both the Mackey (2010) and Hubard et al. (2004) studies suggest differences in habitat use within the "resident" animals with some preferring nearshore habitats and others preferring habitats around the barrier islands. In addition, the existing photo-identification studies have not covered the entire east-west range of the stock, and it is possible that there may be genetic structure within Mississippi Sound. Finally, the relationships between the Bay Boudreau portion of the stock area and adjacent areas of Chandeleur Sound are unknown. The Mississippi Sound stock experiences incidental mortality in several commercial fisheries and has experienced several significant UMEs. Most notably, the stock was impacted by the 2010–2015 Northern Gulf of Mexico UME (Litz et al. 2015) and experienced exposure to oil from the *Deepwater Horizon* oil spill (Venn-Watson et al. 2015). The assessment of injury to the Mississippi Sound stock due to the *Deepwater Horizon* event included additional studies of residency and movement patterns, density estimation, and genetic evaluation of population structure through biopsy sampling.

1.4 Study Objectives

The objective of this study was to conduct sampling of bottlenose dolphins within estuaries and nearshore coastal waters of the western Gulf of Mexico to elucidate population structure amongst adjacent groups of bottlenose dolphins. The primary sampling effort focused on the estuaries of the "Texas Coastal Bend" ranging between Corpus Christi Bay and Matagorda Bay (Figure 2). This region was selected due to the high degree of uncertainty associated with the established stock boundaries along with evidence for anthropogenic and natural stressors occurring in the area (Phillips and Rosel 2014). In addition to genetic sampling, biopsy samples were processed and stored for later stable isotope and contaminant analyses. Photo-identification images of encountered individuals were collected to evaluate movement and re-sight patterns. Sampling was conducted over three seasons (summer months during 2012-2014) in estuaries of the Texas coastal bend and adjacent coastal waters. In addition, sampling was conducted in western Mississippi Sound during the summer and winter of 2013. Samples collected from this study will be integrated with samples from the eastern Mississippi Sound, northern Chandeleur Sound and coastal waters to help elucidate bottlenose dolphin stock structure. Initial analyses of genetic differentiation between the estuarine systems of the Texas Bend are presented here. Additional analyses are ongoing as part of a larger project to assess Gulf of Mexico BSE bottlenose dolphin population structure on a larger scale.

2 Field Sampling

2.1 Survey Areas

2.1.1 Texas Coastal Bend

The survey area for this project was the central Texas coast, locally referred to as the Texas Coastal Bend (TCB) (Figure 2). The large survey area was split into two broad categories. The southern portion was Corpus Christi (CC) and included Upper Laguna Madre, Corpus Christi Bay, Redfish Bay, Aransas Bay, Copano Bay and Gulf of Mexico coastal waters directly adjacent to these bays out to 20m depth. The northern portion of the TCB was referred to as the Matagorda (MG) and included San Antonio Bay, Espiritu Santo Bay, Lavaca Bay, Matagorda Bay, Tres Palacios Bay, and the coastal waters directly adjacent to these bays out to 20 m depth.

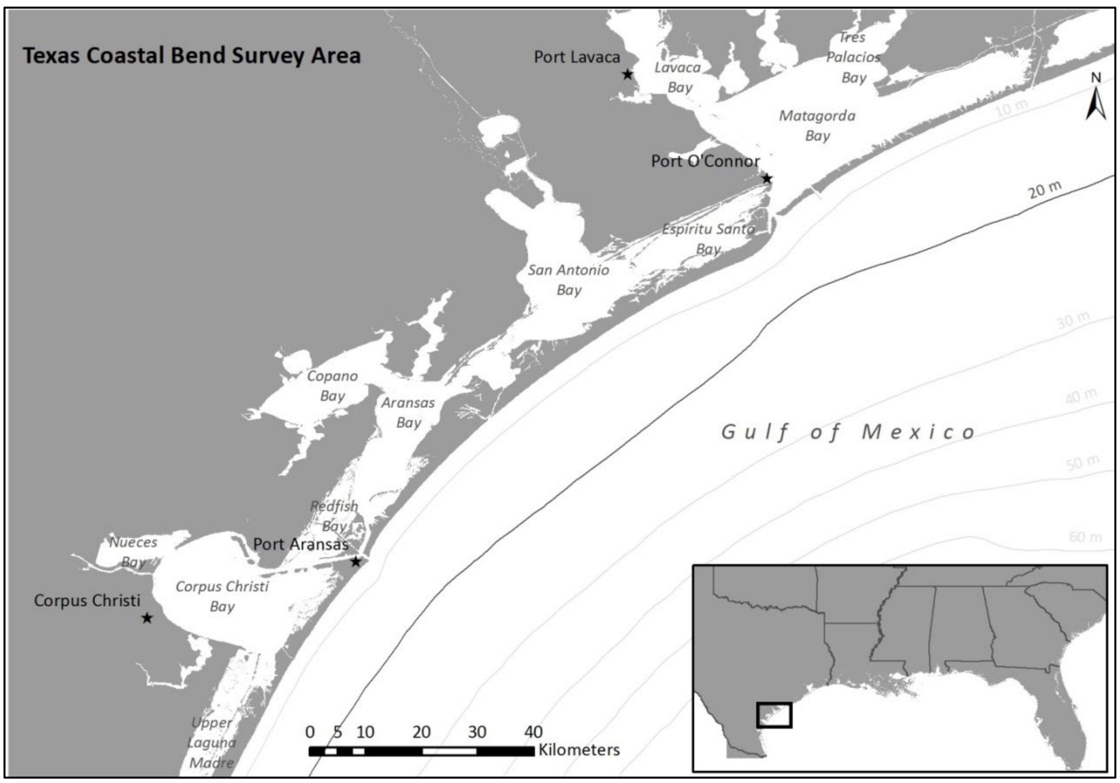

Figure 2. Sampling region in the Texas Coastal Bend.

2.1.2 Western Mississippi Sound

The survey area for this project was the western half of Mississippi Sound, Mississippi (MSS) (Figure 3). MSS waters from the mainland shore to roughly halfway to the barrier islands (approximately 4 to 6 km from the mainland) were surveyed. Long Beach, Mississippi served as the base of operations. The research vessels were kept at the Long Beach City Harbor, in Long Beach, Mississippi.

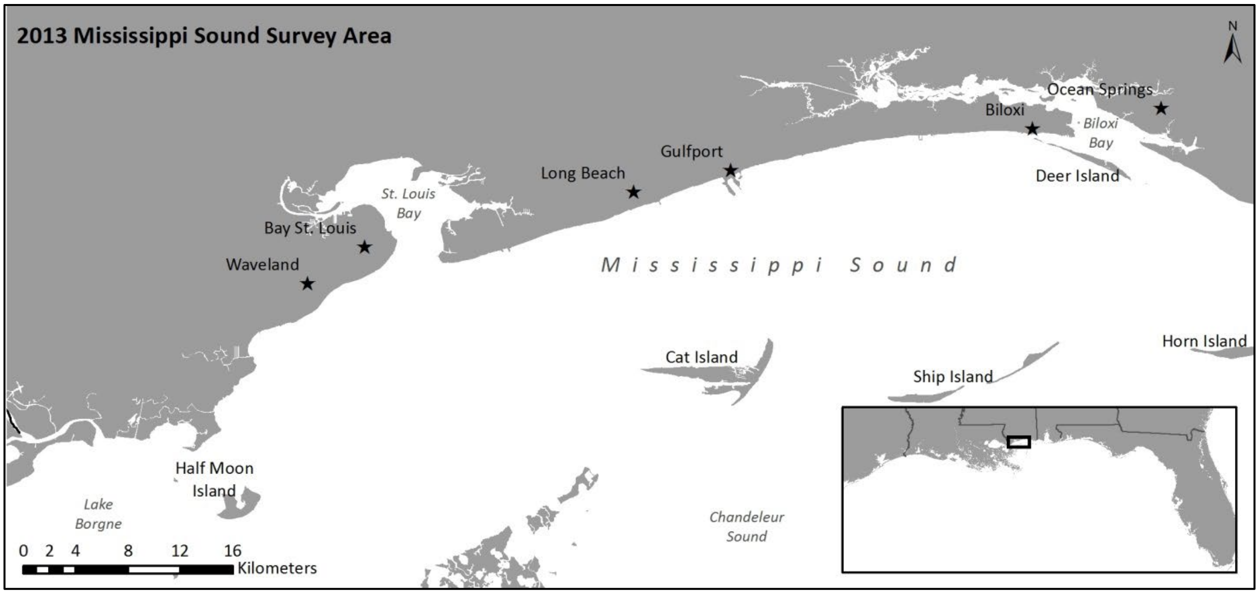

Figure 3. Sampling area in Western Mississippi Sound.

2.2 Photo Identification Field Methods

Through the course of the surveys, several small boats were used: the 6.4-m NOAA *R/V Trailing Edge* (TE), the 7.5-m NOAA *R/V R2*, the 7.3-m NOAA *R/V Top Notch* (TN), the 7.3-m *R/V R3*. Survey effort was largely ad hoc; for example, if the research team sampled in a particular bay or area on day 1, they would make an effort to sample in a different bay or area on day 2, etc. For TCB coastal and MSS surveys, tracklines were delineated to facilitate full-coverage of the survey area. For each boat, the survey team consisted of three or four scientists. Operational duties included driving, photographing, data recording, and biopsy sampling. The survey teams searched for dolphins visually in Beaufort Sea State 4 or less. A survey effort log (e.g., start and end time, weather conditions) was completed for each survey and each vessel's trackline was recorded on a handheld global positioning system (GPS) and downloaded at the end of each survey day as described in Melancon et al. (2011).

When a dolphin group was sighted, it was approached for data collection similar to photo-ID methods described by Melancon et al. (2011). Sighting data collected include date, start and end time, GPS location, environmental conditions, group size and composition, behavioral observations, and general notes. Digital photographs of dorsal fins were captured with high speed and high resolution (18 megapixel) SLR digital cameras equipped with 100–400 mm zoom lenses. However, since the focus of the project was biopsy collection, total photographic coverage of all dolphins present was not always completed.

2.3 Biopsy Sample Collection Methods

If conditions warranted biopsy collection, sampling attempts were made. Circumstances that would potentially eliminate a group from sampling consideration include: presence of one or more neonates or very young (<1 year old) calves; prolonged stress reactions by one or more dolphins; or groups primarily composed of previously sampled dolphins.

Dolphin biopsy samples were collected and processed similar to the description given in Sinclair et al. (2015) with a crossbow or a rifle and a specialized dart fitted with custom designed biopsy tip that extracted a small plug of skin tissue and underlying blubber from the animal. A Finn Larsen 10x25mm tip was used for these projects (Ceta-Dart, Copenhagen, Denmark). Data relevant to each sampling attempt were recorded and included GPS location, time, date, sampler and recorder name, species, body location struck, and behavioral reaction, regardless if a sample was collected. A complete log of the biopsy data is maintained at the NOAA Fisheries Mississippi Laboratory.

When a full-depth biopsy sample was collected, each sample was sectioned into six subsamples for genetics, stable isotopes, reproductive hormones, contaminants, genomics and histopathology studies. For samples that were not full-depth but contained blubber, the contaminant and hormone subsamples were combined and stored consistent with contaminant protocols; this yielded five subsamples. If the sample was skin only, genetics was given priority; if enough skin was present (i.e., skin from a glancing shot with a lot of skin) the sample was split into two subsamples for genetics and stable isotopes.

2.4 Survey Effort and Dolphin Sightings

2.4.1 Texas Coastal Bend

A total of 128 surveys covering 14,181km of survey track distance were conducted during the 3-year project (Table 1, Figure 4). All surveys were conducted in warm-water months each year (May, June, July, August, or September), although the survey effort year to year did not occur during the same dates.

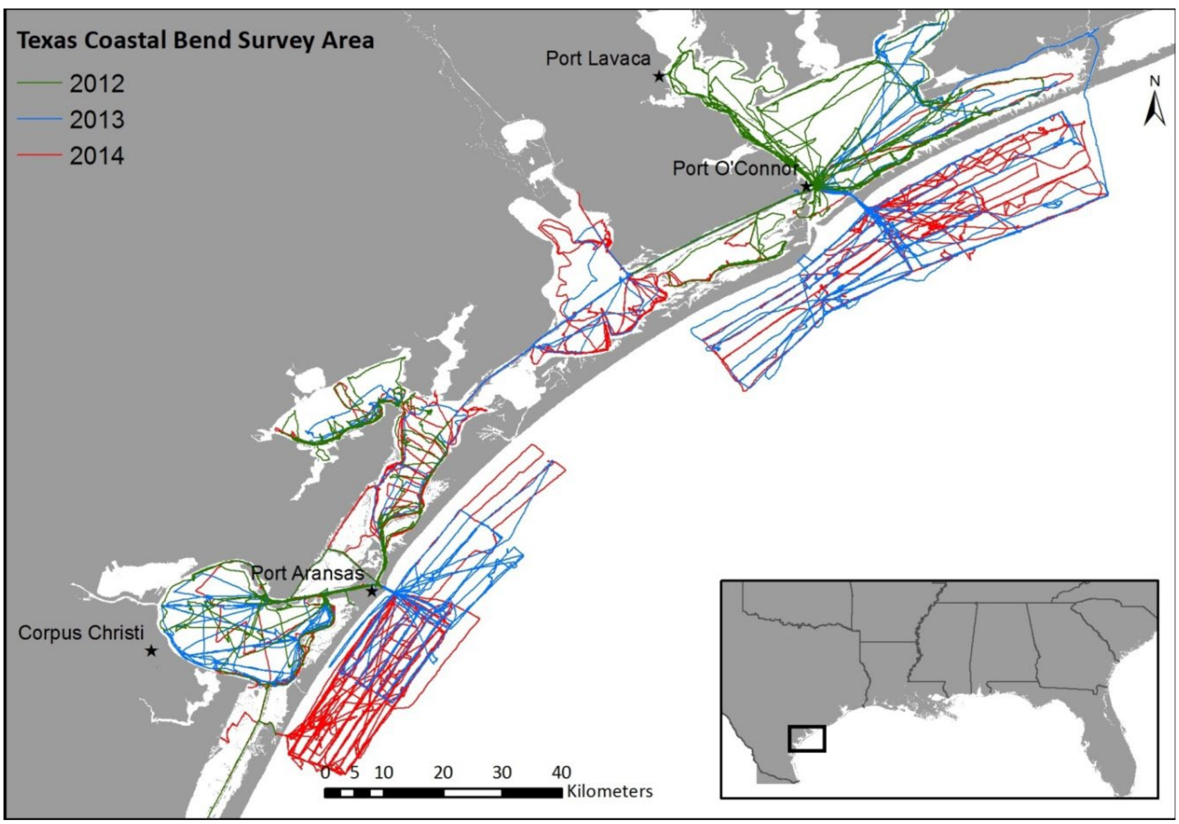

Figure 4. Survey effort in the Texas Coastal Bend region.

Table 1. Summary of survey effort completed during the Texas Coastal Bend Biopsy Projects from 2012 to 2014.

Survey Year	Number of Surveys	Survey Distance (km)	Average Sea State
2012	35	2,868	2.4
2013	40	4,851	2.8
2014	53	6,462	3.4
Total	128	14,181	2.9

Surveys were conducted in the CC survey area (n = 18) from 15 June 2012 to 8 July 2012 and the MG survey area (n = 17) from 25 July 2012 to 18 August 2012. Surveys conducted during 2012 utilized one survey vessel due to limited personnel. For the 2013 sampling period, more personnel were available, two survey vessels were used and sampling co-occurred in each survey area. Biopsy surveys (CC n = 20; MG n = 20) were conducted from 1–28 June 2013. The final sampling period in 2014 was comprised of two parts. Part I was similar to the 2013 sampling period and two vessels were used. Forty-three surveys (CC n = 20; MG n = 23) were completed from 25 May to 23 June 2014. Ten surveys (CC n = 6; MG n = 4) were conducted during Part II from 5–24 September 2014.

During the 3-year study, 506 dolphin groups were encountered (2012 n = 210; 2013 n = 144; 2014 n = 152) composed of 4,179 dolphins (Figure 5). The number of dolphin groups sighted per day was variable and ranged from 1–12 in 2012, 1–8 in 2013, and 1–6 in 2014. Calves and/or neonates were present in 45% (n = 227) of the total sightings. Calves composed 10% (n = 415) and neonates <1% (n = 36) of the total number of dolphins encountered. Group size ranged from 1–40 in the BSEs to 1–65 on the coast. Mean group size in the BSE portions of CC and MG was 7.8 ± 7.25 (n = 408) and the coastal portions were 10.2 ± 9.97 (n = 98, Table 2).

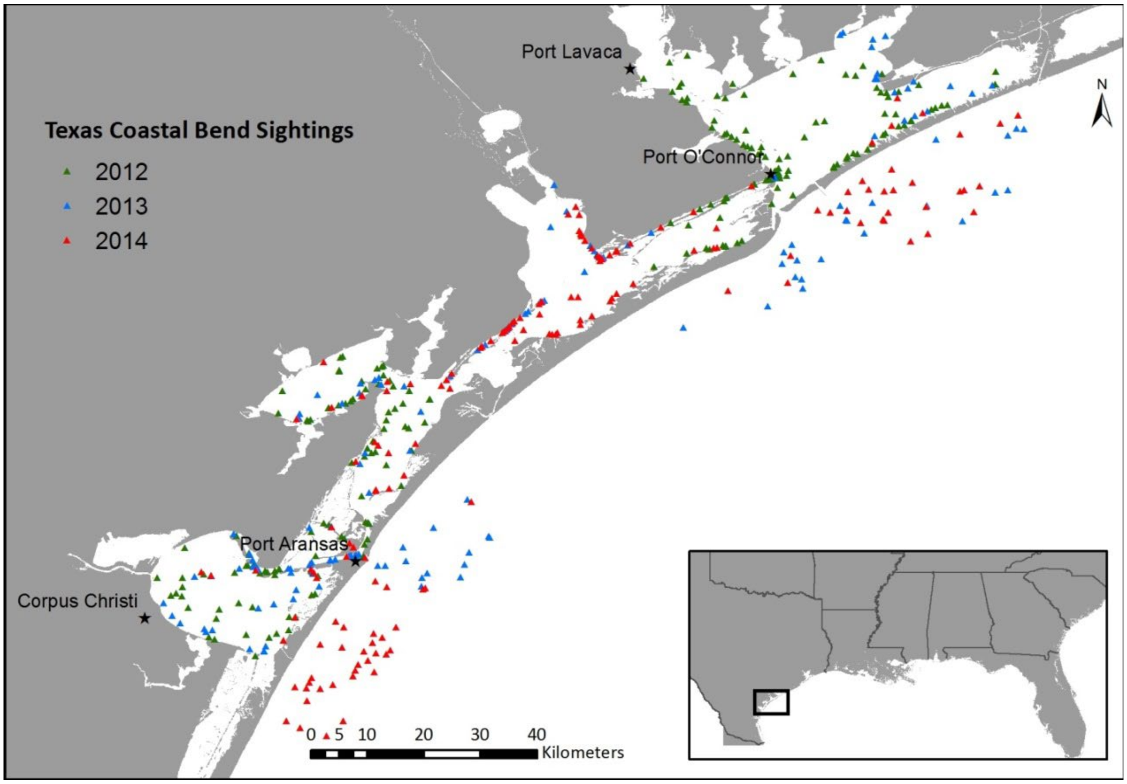

Figure 1. Locations of bottlenose dolphin (*Tursiops truncatus*) groups encountered in 2012, 2013, and 2014 during the Texas Coastal Bend Biopsy Project.

Table 2. Sightings data from bottlenose dolphin groups encountered during the Texas Coastal Bend in bay, sound and estuarine (BSE) and Coastal portions of the Corpus Christi and Matagorda survey areas.

(SD = Standard Deviation).

Survey Area	Number of Sightings (*n*)	Number of Dolphins Encountered	Max Number of Sightings per Day	Group Size: Range	Group Size: Mean (SD)
BSE	408	3,182	12	1–40	7.8 (7.25)
Coastal	98	997	4	1–65	10.2 (9.97)

2.4.2 Western Mississippi Sound

In 2013, 47 surveys were conducted in MSS (Winter n = 24; Summer n = 23) covering 3,991km of survey tracks (Table 3, Figure 6). Two research vessels were utilized for both sampling periods. Survey dates were 7–24 January 2013 and 12–29 August 2013 for the winter and summer sampling periods, respectively.

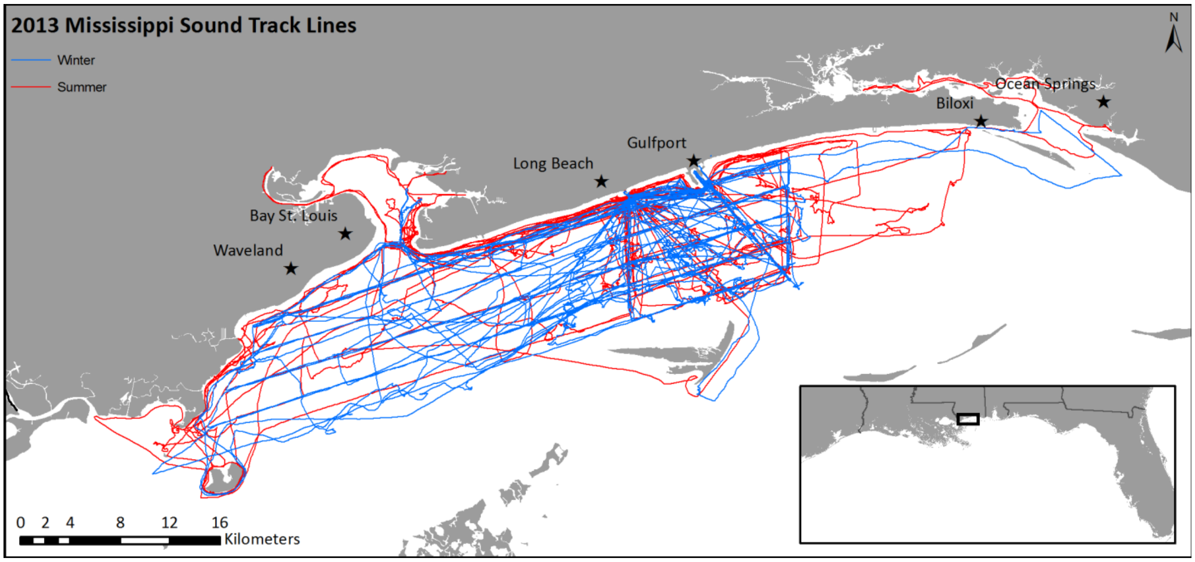

Figure 2. Survey track lines completed during western Mississippi Sound Biopsy Projects in 2013.

Table 3. Summary of survey effort completed during the western Mississippi Sound Biopsy Projects in 2013.

Survey Season	Number of Surveys	Survey Effort (km)	Average Sea State
Winter	24	2,088	2.5
Summer	23	1,903	2.1
Total	47	3,991	2.3

A total of 155 dolphin groups were encountered (Winter *n* = 71; Summer *n* = 84) composed of 1,234 individual dolphins (Figure 7). The number of groups sighted per day was variable and ranged from 0–8 in Winter and 1–7 in Summer. Calves were present in 43% (*n* = 66) of the total sightings and represented 8% of the total number of dolphins encountered. No neonates were observed in either survey period. Group size ranged from 1–18 animals in the Winter to 1–45 animals in the Summer. Mean group size in was 5.2 ± 4.05 in Winter and 10.3 ± 7.27 in Summer (Table 4).

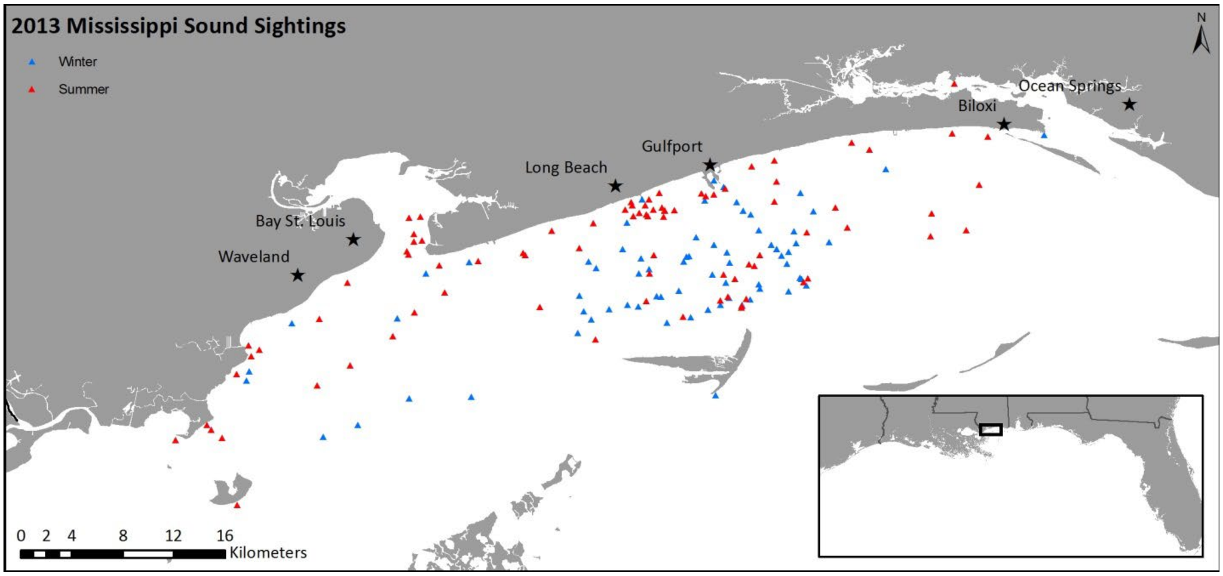

Figure 3. Locations of bottlenose dolphins sighted during Winter and Summer 2013 in Mississippi Sound.

Table 4. Sightings data from bottlenose dolphin groups encountered during the Mississippi Sound Biopsy Project in 2013.

(SD = Standard Deviation).

Survey Area	Number of Sightings (*n*)	Number of Dolphins Encountered	Max Number of Sightings per Day	Group Size: Range	Group Size: Mean (SD)
Winter	71	368	8	1–18	5.2 (4.05)
Summer	84	866	7	1–45	10.3 (7.27)

2.5 Biopsy Samples Collected

2.5.1 Texas Coastal Bend

A total of 247 biopsy samples were collected during the course of the study, yielding 1,355 tissue sub-samples (Table 5). In 2012, the CC sampling effort was concentrated in Corpus Christi Bay, Redfish Bay, Aransas Bay, and Copano Bay. The 2012 MG sampling effort was concentrated in Matagorda Bay, Lavaca Bay, and Espiritu Santo Bay. The following year sampling was expanded to the Gulf of Mexico coastal waters adjacent to the two survey areas, as well as San Antonio Bay and Tres Palacios Bay. In 2014, all bay and coastal areas were sampled again. Coastal effort in 2014 was concentrated to the southeastern end of the CC survey area, whereas MG survey area effort was evenly spread (Figure 8).

Table 5. Summary of biopsy sub-samples collected during the Texas Coastal Bend Biopsy Projects from 2012–2014.

Survey Year	Genetics	Stable Isotopes	Reproductive Hormones	Contami-nants	Genomics	Histo-pathology	Number of Sub-samples
2012	85	81	74	78	79	79	475
2013	91	88	79	85	77	77	497
2014	71	67	61	62	61	61	383
Total	247	236	214	225	217	217	1,355

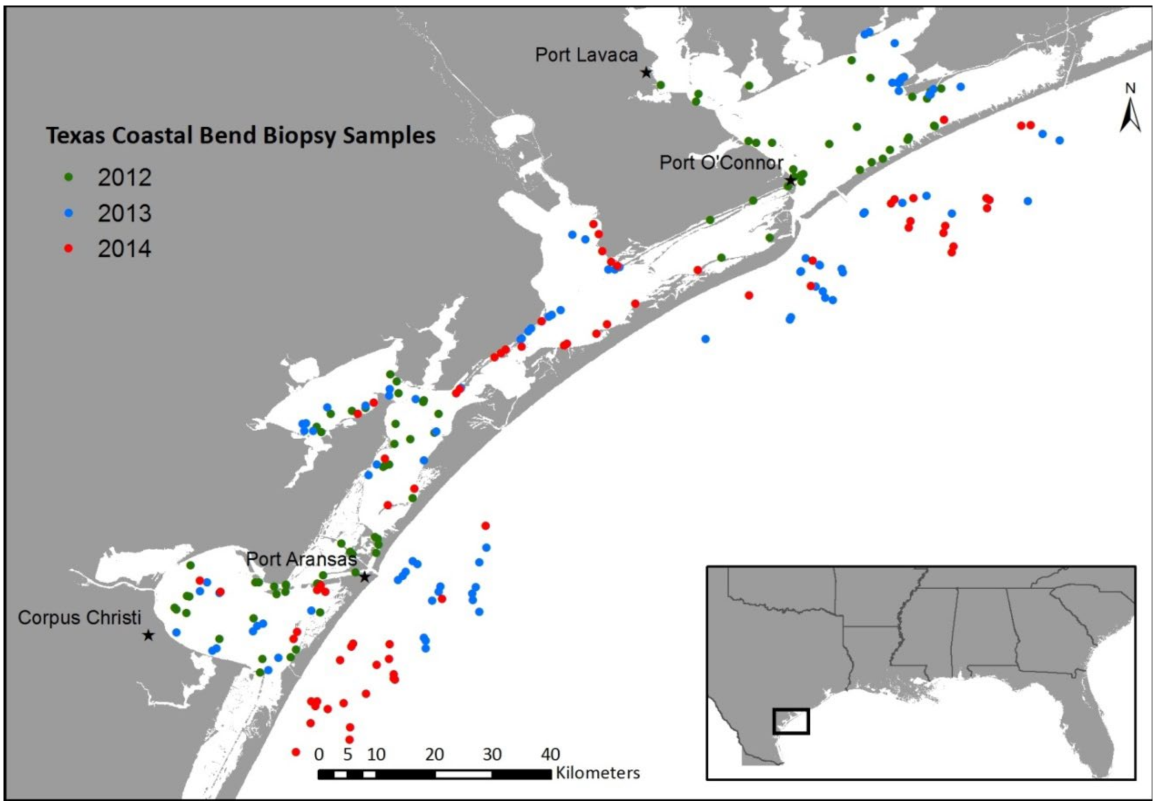

Figure 4. Locations of biopsy samples collected during Texas coastal Bend biopsy projects in 2012, 2013, and 2014.

2.5.2 Western Mississippi Sound

A total of 81 biopsies (Winter n = 31; Summer n = 50) were collected in 2013 from western MSS, yielding 455 tissue sub-samples (Table 6). Winter samples were concentrated in the center of the study area, and farther away from the mainland. Summer samples were more evenly distributed throughout the survey area (Figure 9).

Table 6. Summary of biopsy sub-samples collected during the western Mississippi Sound Biopsy Projects in 2013.

Survey Year	Genetics	Stable Isotopes	Reproductive Hormones	Contam-inants	Genomics	Histo-pathology	Number of Sub-samples
Winter	31	28	28	29	28	28	172
Summer	50	48	45	48	16	16	283
Total	81	76	73	77	74	74	455

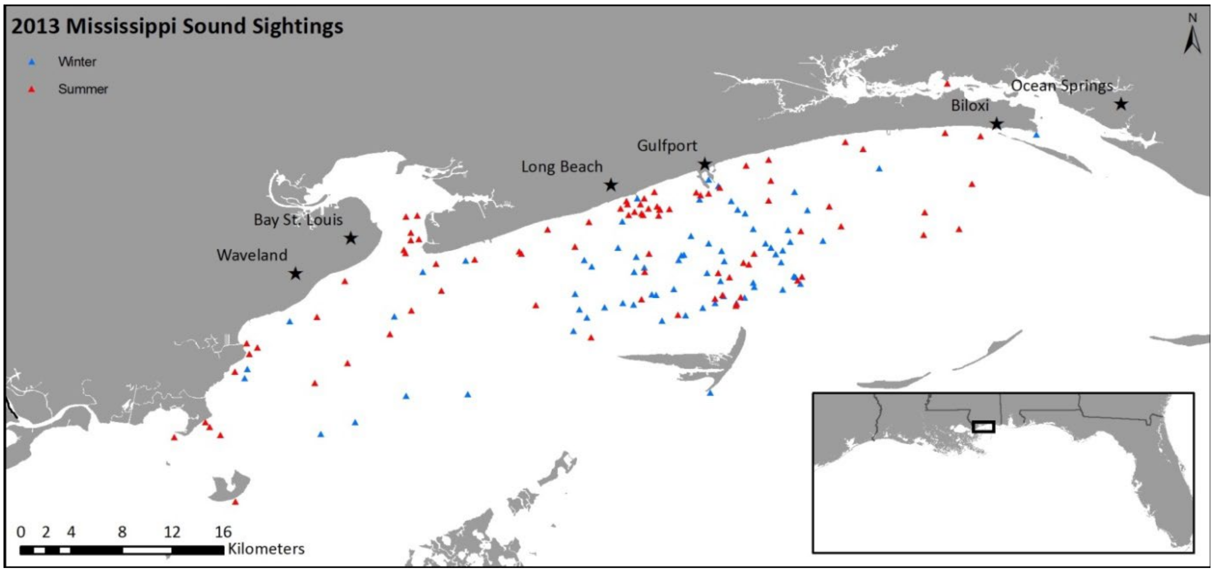

Figure 5. Locations of bottlenose dolphins sighted during Winter and Summer 2013 in Mississippi Sound.

3 Photo-Identification Results

3.1 Texas Coastal Bend

Photographs for individual dolphin identification were collected for 96% (n = 485) of dolphin groups sighted (n = 506). A total of 28,940 digital photographs were collected over the 3-year survey period (CC n = 14,516; MG n = 14,424). From these, a total of 1,797 distinct individuals were identified and cataloged from the BSE and Coastal portions of the survey area (CC n = 966; MG n= 791). All matches from 2012-2014 have been verified. Tentative new fins from 2014 are pending verification.

Coastal dolphins identified constituted 283 individuals in CC and 167 individuals in MG. The majority of the coastal dolphins were only seen on one occasion (CC 93% n = 263; MG 95% n = 159). The majority of BSE distinct dolphins were also seen only once (CC 70% n = 480; MG 67% n = 420), and almost all BSE dolphins had three or fewer sightings (CC 98% n = 670; MG 97% n = 606) (Figure 10).

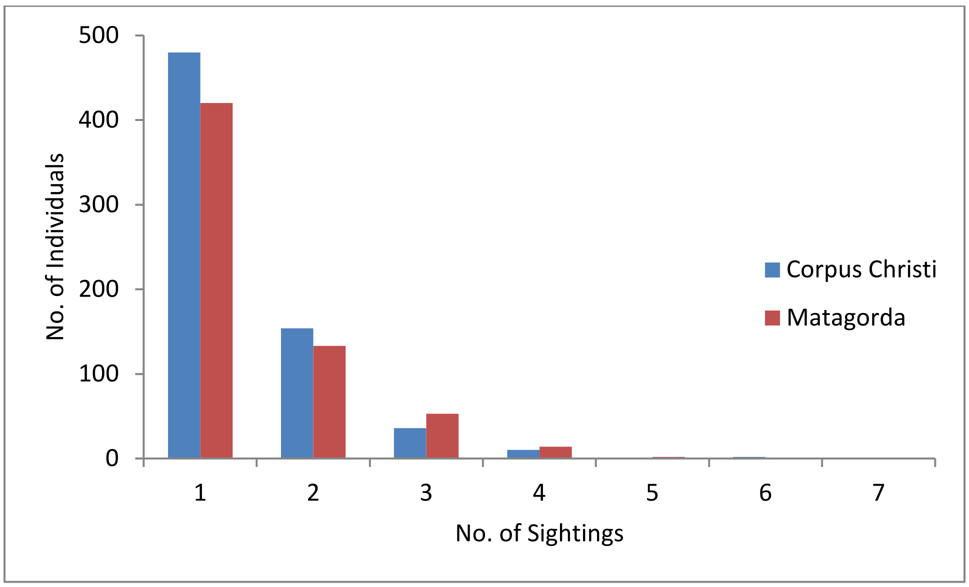

Figure 6. Frequency of re-sighting for distinct bottlenose dolphins in the BSE portion of the Corpus Christi (n = 683) and Matagorda (n = 624) survey areas.

The most frequently sighted dolphins in CC and MG were seen six and seven times, respectively. A high number of new dolphins were identified and added to the catalog (excluding coastal animals) each year the survey was conducted (Figure 11).

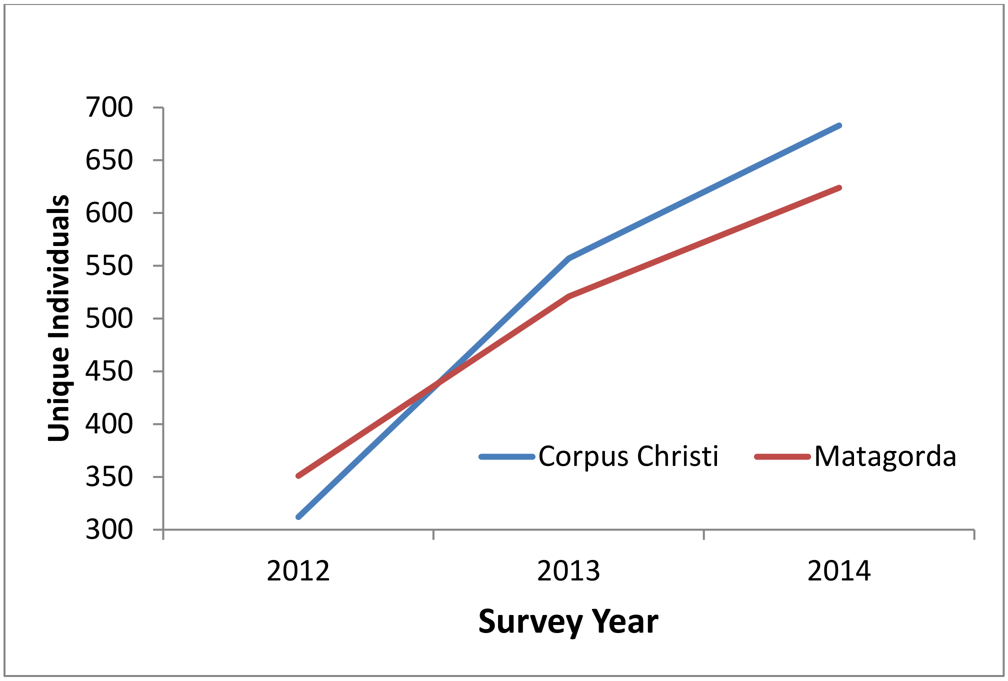

Figure 7. Discovery curve of new dolphins (coastal excluded) added to the Texas Coastal Bend Bottlenose Dolphins Catalog from 2012 to 2014.

Eight animals with higher numbers of sightings (≥5) displayed different spatial sightings patterns. Individuals 8000 and 2002 were only seen in the CC survey in the CC channel, the Lydia Anne Channel or Aransas Pass. Individual 7012 was seen exclusively in Aransas Bay. Three animals (6457, 6495 and 1012) were seen in the Intracoastal Waterway (ICW) on either side of San Antonio Bay (SAB) (with the exception of one sighting of 6457 in the Victoria Barge Canal). Individual 7149 was seen multiple times at the intersection of the ICW, Victoria Barge Canal and SAB with an additional two sightings in Espiritu Santo Bay. Finally, individual 7185 was seen exclusively in the northeastern portion of Matagorda Bay (Figure 12).

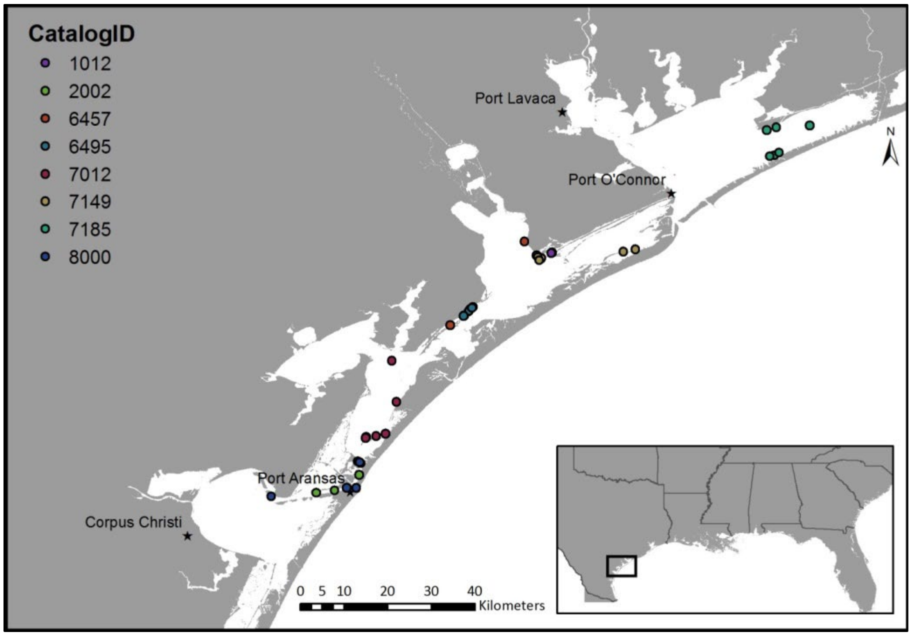

Figure 8. Sighting locations of distinct dolphins with five or more sightings in the Texas Coastal Bend Survey area from 2012 to 2014.

Five distinct individuals were seen between the CC and MG survey areas. Four animals (Catalog IDs 3036, 6286, 7241, and 7261) ranges were limited to the Coastal portions of the survey areas. One animal (Catalog ID 7092) was seen once in each survey area (Figure 13).

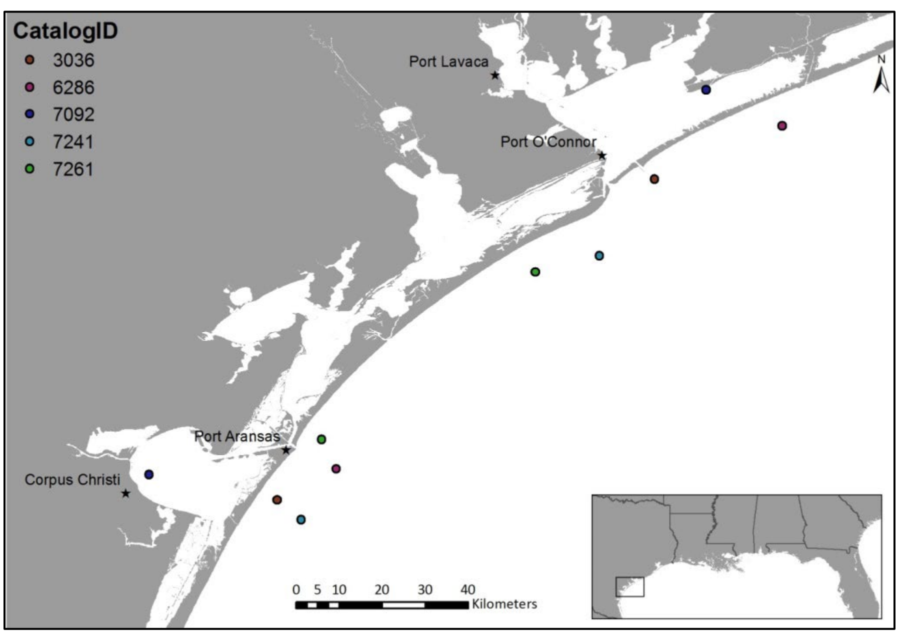

Figure 9. Sighting locations of bottlenose dolphins seen in both Corpus Christi and Matagorda survey areas.

Finally, three individuals that were FB during live capture-release health assessments in 1992 (Würsig and Lynn 1996) were seen in Matagorda Bay during the 2012 survey season (Figure 14). FB 534 (♂, estimated 9 years old in 1992) was seen once on 31 July 2012 in the ICW, roughly 9km southwest of the Port O'Connor (POC) jetty (Figure 15). FB 515 (♀, estimated 12 years old in 1992) was seen three times, all in relatively close vicinity to her capture location in 1992 (Figure 16). During two of the sightings (28 July and 8 August 2012) she was recorded with a calf (she was also captured with a calf in 1992). The ICW sighting (12 August 2012) had a relatively high group size estimate (n = 30) and many calves (n = 8) were recorded. Due to the large number of animals in tight proximity, a calf was not specifically assigned to her on that occasion. A single photograph of a third FB (possibly 522 or 523) was also collected on 18 August 2012, but photographic quality precluded definitive identification (Figure 17).

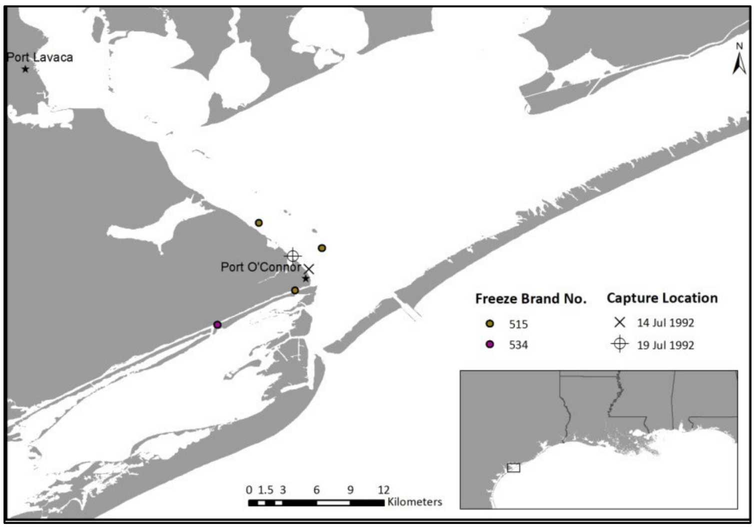

Figure 10. Sighting locations of two FB animals seen during August 2012 Texas Coastal Bend surveys in Matagorda Bay, Texas.

Figure 11. FB 534 sighted 19 July 2012 during Texas Coastal Bend Surveys

Figure 12. FB 515 sighted 28 July, 8 and 12 August 2012 during the Texas Coastal Bend surveys.

Figure 13. Undetermined FB animal sighted 18 August 2012 during Texas Coastal Bend surveys.

3.2 Western Mississippi Sound

Photographs were collected for 96% (n = 149) of groups encountered during these surveys. A total of 10,479 digital photographs were collected in the two survey seasons (Winter n = 3,021; Summer n = 7,458). Photographs for dolphin identification are undergoing analysis and comparison to the NMFS Mississippi Sound Bottlenose Dolphin Catalog that includes animals seen as far back as 1995 (Hubard et al. 2004).

4 Genetic Analysis of Population Structure

4.1 Analysis Methods

4.1.1 Sample Collection

Tissue sample biopsies were collected from 244 *T. truncatus* in Texas between 2012 and 2014 via skin biopsy dart gun or cross bow and preserved in 20% dimethyl sulfoxide saturated with sodium chloride. The samples were collected from the following currently recognized Texas BSE stock areas: Corpus Christi Bay ($N = 42$), Aransas and Copano Bays ($N = 40$), Aransas Pass ($N = 9$), the San Antonio Bay, Espiritu Santo Bay and Port O'Connor area ($N = 42$), and Matagorda Bay ($N = 34$), as well as the coastal waters adjacent to Corpus Christi Bay ($N = 40$) and coastal waters adjacent to Matagorda Bay ($N = 37$) (Figure 18). The samples from dolphins from the Port O'Connor area were grouped with San Antonio Bay and Espiritu Santo Bay because data from a radio tracking study found that dolphins from Port O'Connor spent a substantial amount of time in these bays (Würsig and Lynn 1996). Due to the uncertainty of whether the samples collected from *T. truncatus* in Aransas Pass were from Corpus Christi Bay, Aransas Bay or the adjacent coastal waters, these samples were omitted from all data analyses. The analysis was augmented by incorporating previously collected samples (1992) from Espiritu Santo Bay or Port O'Connor ($N = 19$) and Matagorda Bay ($N = 11$) (excluding calves from mom/calf pairs) (Sellas et al. 2005) after preliminary analyses indicated there were no significant differences in the allele or mtDNA haplotype frequencies between the 1992 and 2012–2014 samples.

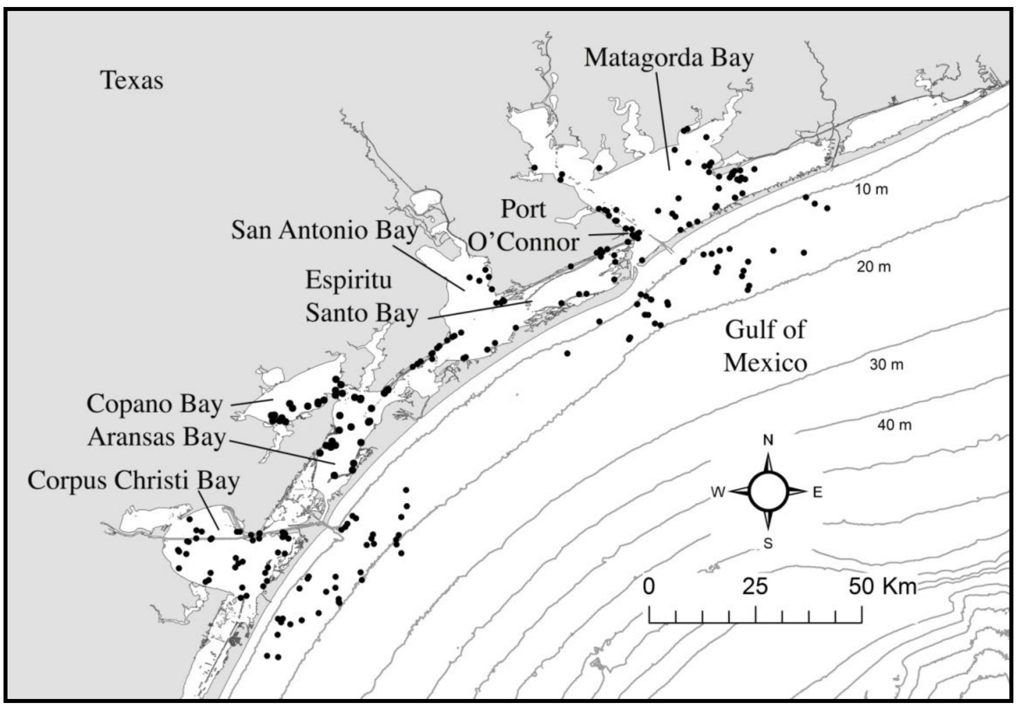

Figure 18. Samples of *Tursiops truncatus* in the bays, sounds, and estuaries (BSEs) and coastal waters in Texas used in the genetic analysis.

4.1.2 Genetic Methods

DNA extraction, sexing and DNA sequencing

Total genomic DNA was extracted from ~15–25 mg of skin using standard proteinase K digestion and phenol-chloroform organic extraction protocols, as described in Rosel and Block (1996). DNA quality was assessed via gel electrophoresis and DNA quantity determined by fluorometry. Sex of the biopsied individuals was determined via polymerase chain reaction (PCR) using the primers ZFX0582F and ZFX0923R to target the ZFX locus and PMSRYF and TtSRYR to target the SRY locus following the method of Rosel (2003).

A ~500 base pair (bp) portion of the mitochondrial (mtDNA) control region was amplified via PCR using the primers L15824 and H16498 (Rosel et al. 1999) and a PCR protocol modified slightly from that of (Sellas et al. 2005). PCR amplification was performed in a reaction mixture containing ~25 - 50 ng of DNA, 20mM Tris-HCL pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.3 µM of each primer, 0.15 mM dNTP's and 1U of *Taq* DNA polymerase. The control region sequence was amplified using an initial denaturation at 95° C for 30s, followed by 30 cycles of 95° C for 30s, 55° C for 30s and 72° C for 30s, and a final extension at 72° C for 7 min. PCR products were purified and sequenced as described in (Sellas et al. 2005) on an ABI 3130 Genetic Analyzer. Forward and reverse sequences were edited in Sequencher v.5.3 and used to create a consensus sequence. Consensus sequences were aligned by eye in Sequencher v.5.3, trimmed to 354-bp and MACCLADE 4.08a (Maddison & Maddison 2000) was used to identify individuals with the same haplotype.

Microsatellite genotyping

Data were generated at 19 dinucleotide microsatellite loci for each individual: Ttr04, Ttr11, Ttr19, Ttr34, Ttr48, Ttr58, Ttr63, FF6 (Rosel et al. 2005), MK5, MK6, MK8, MK9 (Krützen et al. 2001), TxVt5, TxVt5 (Rooney et al. 1999), EV14, EV37, EV94 (Valsecchi & Amos 1996), KWM12a (Hoelzel et al. 1998), and Ppho130 (Rosel et al. 1999). The primers for EV94 were modified (F: 5'-ACATGGCCATCGCTCTTAAC-3' and R: 5'-GTTTATAAGGGTGAATTTTATGG-3') from that of (Valsecchi & Amos 1996) after (Rosel et al. 2009) found evidence of null alleles at this locus. Forward primers were fluorescently labeled and a pig-tail sequence (GTTT) was added to reverse primers for FF6, MK5, MK8, MK9, KWM12a, and Ppho130 to facilitate adenylation by Taq DNA polymerase (Brownstein et al. 1996). PCR reaction mixtures and cycle profiles were as described in (Rosel et al. 2009) with minor modifications. PCR amplification was performed in a 20µl reaction mixture containing 25 - 50ng DNA, 20mM Tris-HCl, pH 8.4, 50mM KCl, 1.5mM $MgCl_2$, 0.15mM dNTPs, 0.6U of *Taq* polymerase (Invitrogen) and forward and reverse primers. PCR products were run on an ABI 3130 Genetic Analyzer with LIZ-500 size standard. The sizes of alleles at each locus were scored using the software GENEMAPPER 5 (Applied Biosystems).

4.1.3 Preliminary Genetic Data analysis

The Microsoft© Excel™ add-in Microsatellite Toolkit (Park 2001) was used to identify duplicate samples in the dataset, using data from the 19 microsatellite loci. Relatedness between individuals was estimated to reduce any potential bias in sampling close relatives (i.e., parent/offspring) within populations. Estimates of relatedness were performed using the program RE-RAT and the method by (Queller & Goodnight 1989), with 100 simulations (Schwacke et al. 2005). When individuals were likely to be close relatives ($R \geq 0.45$), i.e., parent/offspring, and were sampled from the same group of dolphins, one of the individuals in the related pair was dropped from the analyses.

4.1.3.1 mtDNA data analysis

Levels of genetic diversity were estimated in terms of the number of haplotypes, haplotype (*h*) diversity and nucleotide (π) diversity, in ARLEQUIN version 3.5.1.2 (Excoffier & Lischer 2010). Haplotype diversity represents the probability that two randomly selected individuals exhibit different haplotypes (Nei 1987). Values of *h* range from 0, where all individuals have the same haplotype to 1, where all individuals have different haplotypes. Nucleotide diversity is the probability that two randomly selected homologous nucleotides are different, providing a measure of the extent of genetic differences between individuals in a population (Nei 1987). Nucleotide diversity was estimated using the Tamura and Nei (1993) substitution model and a gamma correction of 0.444; this model was determined to be the best model given the data via comparisons with other models using JMODELTEST2 (Darriba et al. 2012; Guindon & Gascuel 2003).

To assess genetic differentiation in populations of *T. truncatus* from Texas BSE and coastal waters, F_{ST} and Φ_{ST} were estimated for pairwise comparisons and the statistical significance of the values was assessed with 10 000 permutations in ARLEQUIN ver 3.5.1.2 (Excoffier & Lischer 2010). A sequential Bonferroni correction for multiple tests was applied to the F_{ST} *P*-values (Holm 1979).

4.1.3.2 Microsatellite data analysis

Levels of genetic diversity in selected populations of *T. truncatus* were assessed in terms of the number of alleles and the observed heterozygosity. The observed heterozygosity is the proportion of the population that is heterozygous (two different alleles) at a given locus. F_{ST} was estimated for pairwise comparisons of populations and statistical significance of the values was assessed with 10 000 permutations in FSTAT 2.9.3 (Goudet 1995). A Bonferroni correction for multiple tests was applied to the F_{ST} *P* values (Holm 1979).

4.2 Results

Of the 244 samples collected in Texas between 2012 and 2014, all but one were sexed, DNA sequenced and genotyped at 19 microsatellite loci. One sample collected from the coastal waters adjacent to Corpus Christi was a skin fleck and alleles could not reliably be amplified at most of the microsatellite loci attempted, and therefore, was dropped from the analysis.

Of the 243 samples that were successfully sexed, DNA sequenced and genotyped at 19 microsatellite loci, six duplicate samples were identified; one pair in Corpus Christi Bay, one pair in Aransas and Copano Bays, one pair in San Antonio Bay, two pairs in Matagorda Bay, and one pair in coastal waters in the Gulf of Mexico. The analysis of relatedness for all samples, including those collected in 1992, identified two pairs of close relatives ($R \geq 0.45$) that were also from the same group of dolphins or, in the case of the samples from 1992, the same net set; one pair from coastal waters adjacent to Matagorda Bay and one pair in Matagorda Bay (from the 1992 dataset). One individual of each of these pairs was dropped from the dataset to avoid any potential bias in sampling close relatives. Four individuals sampled in coastal waters in the Gulf of Mexico had mtDNA haplotypes characteristic of the offshore morphotype and were dropped from the dataset. Finally, the nine samples from Aransas Pass were excluded from further analysis, though they were extracted, sexed, sequenced and genotyped, because of uncertainties regarding whether the *T. truncatus* in Aransas Pass were from Corpus Christi Bay, Aransas Bay or the adjacent coastal waters.

Ultimately, data analyses were conducted with 252 individuals, 233 from the BOEM-supported biopsy effort and 19 from the 1992 sampling effort: 41 from Corpus Christi Bay, 39 from Aransas and Copano Bays, 60 from San Antonio Bay/Espiritu Santo Bay and Port O'Connor area (19 from 1992), 42 from Matagorda Bay (10 from 1992), 36 from the coastal waters adjacent to Corpus Christi Bay, and 34 in the coastal waters adjacent to Matagorda Bay (Figure 18). The samples from the two coastal locations in the Gulf of Mexico were not genetically differentiated based on preliminary *F*-statistics (data not shown), therefore, these samples were pooled in analyses.

Samples were collected from more males than females in most locations. The sex ratio for collected samples was ~3 males to 1 female in Corpus Christi Bay, 2 to one in Matagorda Bay and ~1.6-1.7 to 1 in the San Antonio Bay to Port O'Connor area. The coastal samples overall had roughly an equivalent number of males and females. The result of generally sampling more males than females is commonly seen in biopsy effort for estuarine bottlenose dolphins and is not surprising as females with calves were purposely not sampled.

4.2.1 Genetic diversity

The levels of mtDNA diversity in each population of *T. truncatus* in terms of haplotype and nucleotide diversities were moderate and low, respectively (Table 7). Haplotype diversity was highest and fairly similar in Copano and Aransas Bays, Matagorda Bay, and the coastal population in the Gulf of Mexico. Haplotype diversity was lowest (and fairly similar) in the Corpus Christi Bay and San Antonio to Port O'Connor populations. Nucleotide diversity was highest in the coastal population in the Gulf of Mexico, and lower in the BSEs (Table 7).

The average number of alleles per microsatellite locus was also highest in the coastal population in the Gulf of Mexico, which is not entirely unexpected since this population also had the largest sample size and probably has the highest abundance as well. Levels of observed heterozygosity were moderate and similar across all populations.

Table 7. Summary statistics of levels of genetic diversity in *Tursiops truncatus*.

N = number of samples, H = number of haplotypes, h = haplotype diversity, π = nucleotide diversity, k = number of alleles averaged over all loci, HO = observed heterozygosity averaged over all loci.

Survey Year	*N*	*H*	mtDNA *h*	*π*	*N*	Microsattel ites *k*	*Ho*
CCB	41	4	0.500 ± 0.080	0.0016 ± 0.0015	41	5.895	0.617
CB/AB	39	8	0.775 ± 0.048	0.0032 ± 0.0024	39	5.895	0.598
SAB/ESB/PO	60	6	0.052 ± 0.060	0.0016 ± 0.0014	60	5.842	0.610
MB	42	7	0.740 ± 0.042	0.0030 ±0.0022	42	6.263	0.648
Coastal TX	70	12	0.709 ± 0.036	0.0082 ± 0.0048	70	7.579	0.643

Notes: CCB = Corpus Christi Bay, CB/AB = Copano and Aransas Bays, SAB/ESB/PO = San Antonio Bay, Espiritu Santo Bay and Port O'Connor, MB = Matagorda Bay, Coastal TX = adjacent coastal Texas waters.

4.2.2 Population structure

Significant genetic differentiation was observed between the *T. truncatus* BSE populations and the adjacent coastal population in the Gulf of Mexico in Texas, using data from both the mtDNA and microsatellite markers (Tables 3 and 4).

Significant genetic structure was also evident for both datasets at surprisingly small spatial scales in Texas estuarine waters. The results of the analysis of population structure using data from microsatellite loci suggests that each bay, e.g., Corpus Christi Bay, Aransas/Copano Bays, San Antonio/Espiritu Santo Bays/Port O'Connor and Matagorda Bay contain a genetically differentiated stock (Table 4). Somewhat similar results were obtained from pairwise values of F_{ST} and Φ_{ST} for mtDNA, except that the values of F_{ST} and Φ_{ST} for the Aransas and Copano Bays versus Matagorda Bay comparison were not statistically significant (Table 3).

Table 8. Pairwise FST (below diagonal) and ΦST (above diagonal) and the associated P-values.

Statistically significant values of FST and ΦST after a sequential Bonferroni correction (P < 0.025) (Holm 1979) are indicated in bold. Location abbreviations as in Table 7.

Survey Year	CCB	CB/AB	SAB/ESB/SWMB	MB	Coastal TX
CCB		**0.1259** (0.000)	**0.2972** (0.000)	**0.1152** (0.001)	**0.2810** (0.000)
CB/AB	**0.0776** (0.006)		**0.4082** (0.000)	-0.0073 (0.541)	**0.2759** (0.000)
SAB/ESB/PO	**0.2691** (0.000)	**0.2510** (0.000)		**0.3804** (0.000)	**0.4197** (0.000)
MB	**0.1182** (0.001)	0.0219 (0.102)	**0.2337** (0.000)		**0.2904** (0.000)
Coastal TX	**0.1503** (0.000)	**0.1083** (0.000)	**0.3119** (0.000)	**0.1509** (0.000)	

Table 9. Pairwise FST (below diagonal) and P-values (above diagonal).

Statistically significant values of FST after a Bonferroni correction (P < 0.005) indicated in bold. Location abbreviations as in Table 7.

Survey Year	CCB	CB/AB	SAB/ESB/SWMB	MB	Coastal TX
CCB		0.000	0.000	0.000	0.000
CB/AB	**0.0192**		0.000	0.000	0.000
SAB/ESB/PO	**0.0277**	**0.0277**		0.000	0.000
MB	**0.0151**	**0.0242**	**0.0270**		0.000
Coastal TX	**0.0164**	**0.0284**	**0.0329**	**0.0187**	

4.3 Initial Findings of Genetic Study

The data acquired from the two types of genetic markers, mtDNA and microsatellite loci, in this study suggests that *T. truncatus* in the coastal waters of Texas are genetically differentiated from those in the Texas BSEs, and therefore belong to a separate stock. Further, the presence of significant genetic structure among the sampled bays in Texas suggests that population structure can be found over relatively small spatial scales in BSE *T. truncatus*. The slight discrepancy in the patterns of genetic structure in the BSEs, depending on whether mtDNA or microsatellite data were used, could be due to a lack of power in the mtDNA data, as it is only a single locus, and given that the value of F_{ST} for this comparison using 19 microsatellite loci was statistically significant (Table 9). It is also possible that there is occasional gene flow or movements of *T. truncatus* between the BSEs, making it difficult to detect genetic differences with a single locus. In order to more fully understand movements and gene flow between the BSE populations in Texas additional analyses are currently underway. The results of these additional analyses will examine stock boundaries more closely and determine whether the current *T. truncatus* BSE stock designations need to be re-evaluated.

5 Discussion

In the Texas Coastal Bend area, bottlenose dolphins were encountered in relatively high densities throughout the three summer surveys. A total of 506 dolphin groups were encountered totaling 4,179 individuals. Dolphins were also frequently encountered in adjacent coastal waters, and there were significant differences in the group sizes between estuarine and coastal bottlenose dolphins with larger mean group sizes in coastal waters. Dolphins were sighted throughout the estuarine systems sampled with no evident breaks in distribution between adjacent estuaries. Dolphins were generally observed more frequently around the edges of the bays rather than in the middle of the bays in more open waters. In addition, there was a relatively high concentration of dolphins in and around shipping channels, particularly within the channel entering Corpus Christi Bay (Figure 5). The association of animals with habitats along the edges of the embayments is consistent with the observations of Barham et al. (1980) in Matagorda Bay.

In coastal waters along the Texas coast, bottlenose dolphins were distributed somewhat uniformly in the sampled areas. Sampling was not conducted in waters < 2km from shore to avoid the possibility of collecting samples from estuarine animals that were using coastal habitats as opposed to the targeted coastal stocks. The latitudinal gap in distribution was also a result of sampling design since the vessels were not capable of covering the entire latitudinal range in one day (Figure 5). The sampled animals are from the Western Coastal stock of bottlenose dolphins which ranges between Brownsville, Texas and the Mississippi River Delta in Louisiana (Waring et al. 2015). The seasonal movement patterns of this stock are unknown, and therefore it is not clear what proportion of the stock was available to be sampled during the current study.

Within the estuarine waters of the Corpus Christi and Matagorda Bay areas, a total of 1,347 distinct individual dolphins have been identified. Because the primary goal of this study was the collection of biopsy samples, it is unlikely that this represents all of the unique individuals encountered during the surveys. The vast majority of animals were sighted only one time during the study despite repeated sampling within discrete areas. The discovery curve (Figure 11) for new dolphins in both regions was approximately linear, indicating that new dolphins remained to be sampled and suggesting that we identified only a portion of the bottlenose dolphins occupying these embayments. Likewise, only five duplicate pairs of biopsy samples were identified within estuarine waters, again indicating that the probability of multiple encounters with the same animals was relatively low. Collectively, these data suggest that there is a fairly large population of bottlenose dolphins (exceeding 1,300 animals) occupying the waters of the Texas estuaries during summer months.

The few animals that were re-sighted multiple times in estuarine waters were observed within relatively restricted areas. The re-sighted animals were generally restricted to one particular embayment, though at least one individual was also seen moving within the ICW on either side of San Antonio Bay (Figure 12). Only one individual occurring in estuarine waters was seen in different areas; this animal was observed once in Corpus Christi Bay and once in Matagorda Bay. In contrast, four animals were seen in both the northern and southern coastal areas, suggesting greater movement of coastal animals between these regions. Overall, these sighting patterns are consistent with limited ranging patterns and residency within individual estuaries. These restricted movement patterns are typical of bottlenose dolphins occupying estuarine systems in other areas of the Gulf of Mexico (e.g., Sellas et al. 2005) and the limited previous studies of Texas estuaries indicating resident animals. The encounter of three animals in Matagorda Bay that were FB in the early 1990s also provides additional evidence for long-term residency within these estuaries.

The initial analysis of genetic results demonstrated significant partitioning of habitats and population structure within the estuaries of the Texas Coastal Bend. Animals occupying estuarine waters were genetically distinct from those occupying adjacent coastal waters. Within coastal waters, there was no distinction between animals within the northern and southern sampling areas. This is consistent with the hypothesis of a single coastal stock occupying these waters during the summer months. Interestingly, four animals with mtDNA haplotypes consistent with the offshore morphotype were sampled within coastal waters. Sampling was restricted to waters less than 2 km from shore to avoid the potential for overlap with either continental shelf stock animals or offshore animals. However, it is apparent that at least some offshore animals do approach fairly close to shore and overlap with both the shelf and coastal stocks.

All pair-wise comparisons of population structure between the four major estuaries within the Texas Coastal Bend area were highly significant using data from microsatellite loci indicating limited gene flow between these adjacent and connected areas and genetically distinct stocks. This high degree of differentiation is consistent with the findings of Sellas et al. (2005) and Litz et al. (2012) that demonstrated significant differentiation and limited gene flow among dolphins occupying adjacent estuaries. Interestingly, there is a greater degree of differentiation than is currently captured in the defined stock structure. Copano Bay, Aransas Bay, San Antonio Bay, and Espiritu Santo Bay are currently contained within one stock boundary (Waring et al. 2015). However, the current analysis demonstrates significant genetic differences between the Copano/Aransas Bays and the San Antonio/Espiritu Santo Bays. Additional genetic analysis is ongoing to verify appropriate boundaries between these potential stocks; however, the findings to date indicate significant levels of genetic structure even at relatively small spatial scales. As noted above, the photo-identification data indicate a relatively large abundance of dolphins within these sampled areas. However, this large number of animals appears to be split among multiple smaller stocks, each of which may experience impacts from differing anthropogenic and natural stressors.

The accurate identification and delineation of stocks is a critical step in the effective management and conservation of bottlenose stocks in the Gulf of Mexico. In the absence of accurate stock delineations, it is impossible to correctly estimate the abundance of a stock or to accurately quantify the impacts of human caused mortality as required under the MMPA. As reviewed in Phillips and Rosel (2014), the stocks occupying estuarine waters along the Texas coast are exposed to a broad range of stressors that have the potential to impact population dynamics. The current study is the first to directly examine population structure for these estuarine stocks. Once accurate stock boundaries are established, follow on studies will be able to quantify abundance and thereby accurately assess the status of these stocks.

6 Conclusions

- A total of 247 biopsy samples were collected from bottlenose dolphins during summer months in estuaries along the Texas yielding 1,355 tissue sub-samples. Sub-samples include storage for stable isotope analysis, hormone analysis, and contaminant analysis at a future date.

- Eighty-one (81) biopsy samples were collected from western Mississippi Sound during the summer and winter of 2013 yielding 455 subsamples. These samples will be integrated with samples collected from other portions of Mississippi Sound and adjacent coastal waters to help elucidate population structure in this region.

- Photo-identification analysis in the Texas Coastal Bend indicated the encounter of at least 1,347 individuals in estuarine waters and 450 individuals in coastal waters. The low resight rate and the linear shape of the discovery curve in estuarine waters indicates a relatively high abundance of dolphins within the surveyed areas.

- Animals sighted multiple times within estuarine waters had a relatively restricted range, and only one animal was seen in two different estuaries. In addition, three animals FB in Matagorda Bay during the early 1990s were encountered during the current surveys. These data provide evidence for both restricted movement and long-term residence within these estuaries.

- Genetic analysis of samples from the Texas Coastal Bend estuaries indicate significant population structure with genetically distinct stocks occupying each of the tested areas. The northern and southern coastal areas did not indicate genetic differences; however, they were distinct from the estuarine populations.

- The areas tested include estuaries that are currently combined in one stock. Ongoing data analysis will help to delineate appropriate boundaries between stocks and determine if the current stock structure requires revision.

- This study is the first to examine population structure within the estuaries along the Texas coast and demonstrates a high degree of genetic differentiation among adjacent stocks. This supports the general finding that bottlenose dolphin stocks may be highly localized and demographically isolated over relatively small spatial scales.

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