FINAL REPORT

Impacts of the 2010 Deepwater Horizon Oil Spill on Estuarine Bottlenose Dolphin populations in the West Florida Panhandle

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Contract number: 4710-1101-00-D

January 2013

Abstract

As apex predators, bottlenose dolphins serve as key sentinel species for monitoring ocean and human health. Their positions in oceanic and coastal ecosystems emphasize their relevance for monitoring the potential impacts of oil and oil dispersants on these fragile systems over both the short term and long term. The Deepwater Horizon (MC-252) oil spill event of April-July 2010 resulted in sporadic fouling of northern Gulf of Mexico shorelines. High levels of wildlife mortality were reported for each of the coastal states where the residual spill products landed, and concern mounted that measureable changes would occur in marine mammal feeding ecology, habitat utilization, abundance and survival over time. Residual oil reached most of the Northern Gulf beaches and weathered oil began washing into Northwest Florida in June as slicks entered the Pensacola Bay estuary during incoming tides. Over the next year the post-spill mortality of dolphins in the Northern Gulf reached unprecedented numbers resulting in a federally declared Unusual Mortality Event for this coast. Marine Mammal Stranding Network members conducted enhanced data collection protocols and necropsy procedures for deceased animals, allowing the collection of important tissues and life history data to assist in understanding the impacts of the spill event coincident with the die-off. Lacking was a program to conduct ongoing monitoring of the live dolphin populations in the margin zones of the spill, namely the westernmost estuaries of the Florida Panhandle.

Our UCF team in partnership with staff of the Florida Fish and Wildlife Research Institute (FWRI) and the Sarasota Dolphin Research Program (SDRP) at Mote Marine Laboratory provided a rapid response study to evaluate the local bottlenose dolphin status in this region. We expanded on previous research that had been conducted in Choctawhatchee Bay to incorporate the Pensacola Bay segment of the area, and conducted a comprehensive Mark-Recapture effort over an 18 month period to create a photo-id catalog of individual dolphins for estimating dolphin abundance, habitat use, site fidelity, grand scale movement, and foraging patterns. In addition, we collected remote dart-biopsy samples from free swimming dolphins inhabiting discrete segments of the habitat in order to elucidate foraging dynamics and genetic structure. Collections of putative prey species allowed analyses to be made of nutritional characteristics that would lead to a predictive model of diet composition of the apex predators (*e.g.* dolphins) and therefore potential food chain effects on their health.

This is the first structured long-term study to ever provide a baseline abundance estimate of bottlenose dolphins in the Pensacola Bay region, including western Santa Rosa Sound and Big Lagoon. Survey data from January 2010 through August 2011 indicate a super-population of dolphins in these bays in excess of 600 animals. We found that approximately 67% of these were seasonal residents of the bays, and that others belong to a transient community that migrated along the coastline. Dolphins around Destin Pass demonstrated high site fidelity, while those in the inshore bays made frequent movements throughout the estuary from the Choctawhatchee River across Santa Rosa Sound to western Pensacola Bay. This indicates that dolphin inhabitants of Choctawhatchee Bay, which was only lightly impacted by oil sheens, could have had greater contaminant exposure during travels into Lower Pensacola Bay.

A large number of studies have been undertaken in recent years using stable isotopes to better understand and explore distributional patterns of marine mammals but many suffer from a lack of knowledge of the specific local prey base or the movement patterns of the consumer. Isotopic niche analysis allows for a clearer understanding of ecosystem dynamics and energy flow and

because tissues of animals differ in isotopic composition, as a result of differences in their diet, isotopic niche is a reflection of ecological niche. Our isotopic assessment of bottlenose dolphin communities in this Bay complex allowed us to better interpret their ecological niches and therefore have a better understanding of potential oil exposure through their food chain. Our preliminary assessment of the prey base for bottlenose dolphins in this area indicates significant species differences, both temporal and regional, within the Bay complex. Many species of fish collected in eastern Choctawhatchee Bay had significantly different $\delta^{13}C$ signatures consistent with the influence of the freshwater influx from the Choctawhatchee River. These signatures were clearly reflected in the isotopic signatures of dolphins residing in this area. We ultimately identified a group of dolphins that exploits the central and eastern reaches of Choctawhatchee Bay, a second group of dolphins inhabiting western Choctawhatchee Bay and Santa Rosa Sound and a third group of dolphins that spends a great deal of time exploiting the inlet areas of lower Pensacola Bay and Destin Pass. Consistent with photo-id evidence, some dolphins move around parts of the system while others display strong site fidelity. Ultimately their isotopic signatures allow us to assign them to different residency groups and better understand their habitat use, feeding preferences and potential exposure threats to oil. Isotopic data also supports the conclusion that during the winter dolphins aggregate into areas around the passes and in deeper waters possibly avoiding the shallower areas of east Choctawhatchee Bay and the Santa Rosa Sound.

Many of the prey species we examined in the present study are not year-round residents of these bays but actually spawn in the Gulf of Mexico. They are therefore potential reservoirs of incidental contaminants from the Gulf. Additionally, the energetic and/or nutritional value, health, or size of these fish populations could be seriously impacted by changes in ecosystem dynamics at a lower trophic level outside of our study area. Numerous studies are documenting these types of impacts by examining microbes, plankton, crustaceans and fishes. Over time, changes in the quality and/or quantity of the prey base exploited by apex predators could lead to direct changes in their foraging habits and nutritional status or to indirect changes in their health status. Even year-round residents of coastal bays and estuaries that rarely, if ever, venture into Gulf waters could be seriously impacted. Pre- and post-spill knowledge of the spatial and temporal scales of the movements of our resident dolphins, their population structure, specific habitat utilization and feeding preferences is critical to the eventual interpretation of toxicological and health status. Our results will enable resource managers to begin to develop predictive models that evaluate response strategies and to integrate the impacts of stressors at all levels of the ecosystem but ultimately, potential long-term impacts facing these dolphins are largely unknown and continued attention and monitoring is critical.

INTRODUCTION

The Deepwater Horizon (MC-252) oil spill event of April-July 2010 resulted in sporadic fouling of northern Gulf of Mexico shorelines from the release of 4.8 million barrels of crude oil, dispersants, and residual products (National Commission 2011). Over 650 miles of Gulf coastal habitat were oiled; more than 130 miles of which were designated as moderately to heavily oiled (National Commission 2011). The majority of the oil landed in the Louisiana delta and on the Mississippi barrier islands, with lesser impacts to shorelines eastward in Alabama and Northwest Florida. The entire 4-state region was included in the impact response zone and concern over wildlife mortality was high throughout the affected areas (National Commission 2011, NRDA 2012).

By August 2010, residual oil had reached most northern Gulf beaches. Weathered oil first began washing onto Northwest Florida shorelines in June and slicks were detected entering Perdido and Pensacola Bays during incoming tides (National Commission 2011). Sporadic tarmats had accumulated on estuarine shorelines inside of Perdido Bay, Pensacola Bay, and western Santa Rosa Sound near Gulf Breeze and Pensacola Beach, FL (Griggs, 2010). By December 2011, over 1.27 million kg of oiled material had been collected off beaches in Northwest Florida, 99% of which was on Perdido Key and Santa Rosa Island in the westernmost Panhandle counties encompassing the Perdido-Pensacola-Choctawhatchee Bay complex (FDEP 2011).

High levels of wildlife mortality were reported for each of the coastal states where the residual spill products landed, with particular emphasis on avian, sea turtle, and marine mammal species (NOAA, 2010). Given the potential for the oil spill affecting prey organisms at lower trophic levels (Mitra *et al.* 2012) and the likelihood that upper level consumers (*e.g.* marine mammals) in the coastal regions directly encountered slicks of oil and dispersants, there was anticipation that measureable changes would occur in marine mammal feeding ecology, habitat utilization, abundance and survival over time (*e.g.*, Loughlin 1994, Gannon and Waples 2004, Bowen and Cox 2009, NRDA 2012).

Indeed, by November 2010, at the end of the immediate spill response phase, 109 stranded marine mammals had been collected in the region. The majority of these were bottlenose dolphins (*Tursiops truncatus*) but there were also offshore species such as *Stenella* sp. and *Kogia* sp (Figure 1.1). Over the next year the post-spill mortality of dolphins in the northern Gulf of Mexico reached unprecedented numbers (Litz *et al.* 2011). The Natural Resource Damage Assessment Status report (NRDA 2012, pg 54) stated:

> "In early 2011, NOAA declared an Unusual Mortality Event (UME) for cetaceans (whales and dolphins) in the northern Gulf of Mexico from February 2010 through the present. Under the Marine Mammal Protection Act of 1991, a UME is defined as 'a stranding that is unexpected, involves a significant die-off of any marine mammal population and demands immediate response.' The impetus for the declaration was the sharp increase in the discovery of premature, stillborn or neonatal bottlenose dolphin strandings in the region beginning in February 2010. From February through April 2010, 114 cetacean strandings were documented; in the six months between May and the beginning of November 2010, 122 cetaceans were documented as stranded or reported dead in the offshore. Since then, the stranding rate continued to be well above historical averages. In 2011, there were

356 strandings compared to a historical average of 74. Of specific concern is the increase in the number of premature, stillborn or neonatal stranded bottlenose dolphins documented in February and March 2011. In February 2011, stranding was documented for 34 neonatal bottlenose dolphins compared with only one documented neonatal stranding in 2010 (and an average of two documented neonatal strandings for the years 2002-2007)."

NOAA's Working Group on Marine Mammal Unusual Mortality Events actually began consulting with northern Gulf stranding network partners in February 2010, prior to the MC-252 event, due to the observed spike in dolphin mortalities (Figure 1.1) (NMFS 2010, Litz 2012). Because of the complicating factors involving the MC-252 response, NOAA delayed declaration of the UME until Dec 2010, ultimately made retroactive to February of 2010 (Litz *et al.* 2011). The declaration advised stranding network members to conduct enhanced data collection protocols and necropsy procedures for deceased animals (Geraci and Lounsbury 1993, Galloway and Ahlquist 1997, Rowles *et al.* 2001, Johnson and Zaccardi 2006, NMFS 2010, Litz 2012, NRDA 2012).

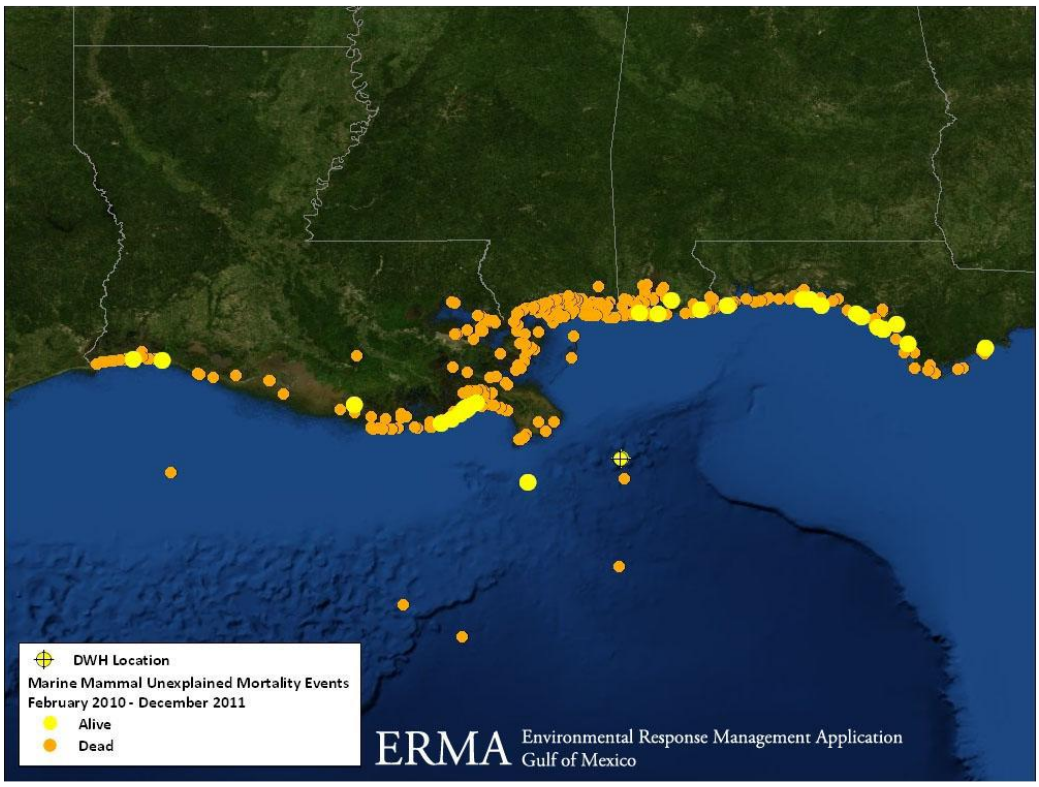

Figure 1.1: Locations of recovered stranded marine mammals (source NOAA/NRDA 2012).

In the early part of 2011 there was an unusual number of perinatal (near term to neonatal) bottlenose dolphin mortalities in the northern Gulf of Mexico. Between 1 January and 30 April 2011, 184 bottlenose dolphins, including 84 (46%) perinatal calves (< 115 cm), washed ashore from Louisiana to northwestern Florida. While the majority of carcasses were discovered on the coast of Louisiana and Mississippi, the proportion of stranded perinatal dolphins was highest on the Mississippi-Alabama coast (Carmichael *et al.* 2012). The timing of this event early in the first peak calving season after the MC252 oil spill raised concerns that these mortalities were potentially a result of exposure to oil or dispersant-derived contaminants (Gutman 2011, Herrmann 2011, Semansky 2011, Carmichael *et al.* 2012). The National Marine Fisheries Service (NMFS) has considered these mortalities to be part of the previously declared longer-term UME, pending availability of additional data that could allow determination of multiple UMEs in this region. Preliminary findings indicate that marine *Brucellosis* played a role in a number of infant mortalities and NOAA scientists are investigating the potential link between bacteria, along with other pathogens, to the oil spill event (www.nmfs.noaa.gov/pr/pdfs/health/brucella_infection.pdf).

Comprehensive health assessments of 32 live dolphins from Barataria Bay in the summer of 2011 indicated that many of those dolphins were underweight, anemic, had low blood sugar and/or some symptoms of liver and lung disease (NOAA 2012). Nearly half also had abnormally low levels of hormones responsible for stress response, metabolism, and immune function. Based on the timing, Carmichael *et al.* (2012) suggested that these dolphins were in poor condition as a result of potentially compromised food resources, possibly related to the extended duration of cold weather for two winters (2010 – 2011) or effects of the MC-252 oil spill.

The northwest Florida coastline is well recognized for the many pristine estuaries hosting an abundance of fishery resources and ecotourism opportunities (Blaylock 1983, Livingston 1986, Beck *et al.* 2000, Beck *et al.* 2003, Ruth and Handley 2007). Bottlenose dolphin populations are an important economic resource for the wildlife ecotourism sector (Bejder and Samuels 2004), a reality which prompted NOAA in 2008 to implement a training and certification program (www.dolphinsmart.org/dsrefreshertraining/index.php/main/nav/11) for dolphin ecotourism operators along the Gulf Coast. Dolphin watching is a major tourism enterprise along the Northwest Florida coast, and dolphin watch ecotourism in Gulf Shores and Orange Beach, Alabama, has been identified as a major component of the region's $2.6 billion tourism market (McDonough 2008, Malone 2012).

Presently, dolphin stocks in Gulf coast bays are considered to be independent populations (Blaylock and Hoggard, 1994; Waring *et al.*, 2011) but there is evidence to suggest that animals move between estuaries in Northwest Florida (Balmer *et al.*, 2008, Shippee 2010, Wilson *et al.* 2012). Due to the uncertainty of the status of estuarine dolphins in the Florida Panhandle, NMFS lists the dolphin populations in this region as "strategic" stocks (Waring *et al.* 2011).

A limited mark-recapture study in Choctawhatchee Bay using photo-identification of dolphins was conducted in 1989-92 that catalogued 71 individual animals in the bay and adjacent Gulf (M. Townsend, unpublished data). An aerial line-transect survey for this region in 1993 arrived at an estimate of 242 animals for Choctawhatchee Bay and 33 for adjacent Pensacola Bay (Blaylock and Hoggard 1994, Waring *et al.* 2011). Since then, two Unusual Mortality Events resulted in significant losses of animals in the area: over 100 dolphins died in 1999-2000 between eastern Choctawhatchee Bay and Lower Pensacola Bay, and 50 dolphins died in a UME

during 2005-06 within Choctawhatchee Bay and the adjacent Gulf shoreline (Bowen 2006, Shippee 2010). Considering the background mortality rate of 13.4 (±2.7 SE, n=27) dolphins per year dating back to 1990, these UMEs may have resulted in a significant reduction of the resident dolphin population in the Choctawhatchee Bay area (Waring *et al.* 2011). A follow-up mark-recapture study was conducted by NMFS researchers in summer 2007 to develop a population estimate for Choctawhatchee Bay (Conn *et al.* 2011), which suggested a resident population of 176 dolphins. In 2008, Pabody completed a 16-month photo-id study that created a catalogue of 88 distinct dolphins seen frequently in the Perdido Bay estuary. There is no historical estimate of dolphin abundance for Pensacola Bay, Big Lagoon, or Santa Rosa Sound with the exception of the single 1993 count (Waring *et al.* 2011).

Continuation of longitudinal studies in multiple seasons over several years allows evaluating shifts in dolphin habitat use, movement patterns, and home range expansion and contraction (Balmer *et al.* 2008, Mazzoil *et al.* 2005, Odell and Asper 1990, O'Shea and Odell 2008, Scott *et al.* 1990). Since 2006, a long-term photo-id study has been underway in the Choctawhatchee-Pensacola Bay region as a part of a doctoral dissertation (S. Shippee, Dept of Biology, UCF). This project provides a baseline catalog of individual dolphins that were present prior to and during the MC252 event, which can be used to compare future population changes in this region. A better understanding of dolphin movements throughout the connected estuaries in the region will provide a measure of individual dolphins' potential contaminant exposure resulting from the MC252 event and possible predictions of long-term toxicity or reproductive decline. Further, analysis of tissues acquired from free swimming and stranded dolphins in partnership with the Marine Mammal Stranding Network is useful in determining changes in prey composition and nutritional status over time (Loughlin 1994, Worthy 2001, Gannon and Waples 2004, Bowen and Cox 2009, Worthy and Worthy 2011).

Understanding material flows and nutrient cycling pathways is a fundamental component of ecosystem research and considerable effort has been invested in trying to trace those broad scale dynamics and predator-prey relationships. Understanding these relationships can also give better insights into potential issues relating to habitat quality, pollutant loading, and/or general health status of systems. The use of naturally occurring carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes has been developed as a technique to trace diet through the carbon and nitrogen pathways (DeNiro and Epstein 1978, 1981, Pauly *et al.*1998). Several factors can influence the isotopic ratio of predators living in different marine regions, including differences due to oceanographic factors in a given area and variation in feeding habits of the prey species consumed. Isotope ratios are ultimately determined by the general type of food (*i.e.*, original method of carbon fixation, number of trophic levels, *etc*.) that has been incorporated into the animal over the past several weeks or months and can provide an overall portrait of an average diet. With multiple types of food generally available, isotope ratios can indicate, but not prove, that a certain type of food was consumed. Isotopic analysis of animal tissues can be used to reconstruct the diet when food sources have different $\delta^{15}N$ values. The ^{15}N enrichment between trophic levels ranges from 1.3 to 5.3‰, averaging 3.4 ± 1.1‰ (DeNiro and Epstein 1978, 1981). On the other hand, the $\delta^{13}C$ values of animal tissue are very close to those in their diet, and only a small increase in $\delta^{13}C$ content (about 1‰ enrichment) occurs with increasing trophic level. The primary theoretical basis of using $\delta^{13}C$ as a tracer is that the characteristic ratios of different sources are preserved as the carbon is cycled through organisms and detritus. Consequently, differences in $\delta^{13}C$ values have been used for prey selection analysis of animals in an ecosystem.

Geographic location has been shown to affect the carbon isotope (*e.g.* Abend and Smith 1995, Jennings *et al.* 1997, Boyce *et al.* 2001, Guest and Connolly 2004, Gerard and Muhling 2010, Guest *et al.* 2010) and nitrogen isotope (*e.g.* Abend and Smith 1995, Jennings *et al.* 1997, Schmutz and Hobson 1998, Guest *et al.* 2010) of both aquatic and terrestrial taxa. Despite long-held theories of large-scale movement and assimilation of nutrients in estuaries, recent evidence suggests that in some estuaries movements of nutrients occurs at a much finer scale than previously considered, in some cases over meters, and that a much more limited exchange occurs (Guest and Connolly 2004, Adams and Paperno 2012). Recent isotope studies on invertebrates and fish (*e.g.*, Murphy and Abrajano 1994, Guest and Connolly 2004) have indicated large differences in isotope ratios over limited geographic areas. It has also been shown that terrestrial anthropogenic influences can cause stable isotope signature changes in aquatic species over an extended period of time (Hyodo *et al.* 2008).

In a system with many parallels to the Pensacola Bay – Choctawhatchee Bay system, Adams and Paperno (2012) conducted a study in three different sub-basins of the Indian River Lagoon (IRL) and determined that spotted seatrout (*Cynoscion nebulosus*) showed unique stable isotope ratios within these lagoon sub-basins. They theorized that the hydrology, nutrient inputs and prey assemblages within each sub-basin, along with differences in the habitat, were responsible for significant difference between the isotopes signatures. Additional studies have indicated that conspecifics of striped mullet, pinfish and spotted seatrout located 30 km apart have distinct isotopic signatures (Fletcher-Odom 2012, Fletcher-Odom and Worthy unpubl. data). The ability to discern small-scale differences in prey suggests that similar differences could be present in the apex consumers as was the case with IRL bottlenose dolphin populations (Worthy and Worthy 2011).

The current study was undertaken to address potential long term impacts to dolphin stock structure in this region. Building on existing information for bottlenose dolphins in these interconnected estuaries and the adjacent nearshore Gulf of Mexico waters, we conducted a 12 month comprehensive project to 1) identify dolphin habitat use patterns, site fidelity, and derive data for estimating abundance and population status and 2) explore foraging dynamics, in conjunction with samples acquired from putative prey fish species and independently assess regional discrimination of dolphin groups using stable isotopes. The current work expanded the original area of focus to include the entire distance of Santa Rosa Sound, which previously had only been limited to the eastern portion. We also added a focus on the lower portion of Pensacola Bay and Big Lagoon, which we suspected to be an important habitat for dolphins owing to the large seagrass expanses. This project was further enhanced by providing an avenue for acquiring tissue samples via remote dart biopsy of free swimming dolphins, and from recovery of deceased animals on local shorelines. By elucidating the movements and foraging habitats of the sampled animals from the resighting data, it became possible to assign them to specific estuarine segments that would assist in understanding their foraging patterns. Other samples were collected and archived to support future research on genetic structure, contaminant loads, and fatty acid signature analysis. The ultimate goal of this study was to provide management with information on the status of bottlenose dolphin stocks that inhabited the estuaries in this study, which were not well studied previously.

MATERIALS AND METHODS:
Part 1 – Abundance, site fidelity, and habitat use

Dolphin Mark-Recapture effort: Many longitudinal studies have been done using surveys of estuarine and near-shore regions from small boats to monitor bottlenose dolphin populations and estimate abundance levels (Wells and Scott, 1990, Zolman 2002, Hansen *et al.* 2004, Mazzoil *et al.* 2004, Sellas *et al.* 2005, Adams *et al.* 2008, Balmer *et al.* 2011, Conn *et al.* 2011, Wilson *et al.* 2012). Low-level surveys are conducted to determine the dolphin communities and identify individuals for analysis of minimum resident population size, distribution, and habitat selection. Since portions of our study area had existing identification catalogs dating back to 2006, our current effort was enhanced by having a baseline for comparison (Shippee 2010, Conn *et al,* 2011).

The study area for this project was divided into six segments of the estuarine and near-shore Gulf of Mexico between east Choctawhatchee Bay and Big Lagoon (Figure 1.2). GIS shapefiles of the relevant bay contours were downloaded from the Florida Geographic Data Library (FGDL.com), processed, and segments were drawn using ArcMap 10 (Environmental Systems Research Inst., Redlands CA).

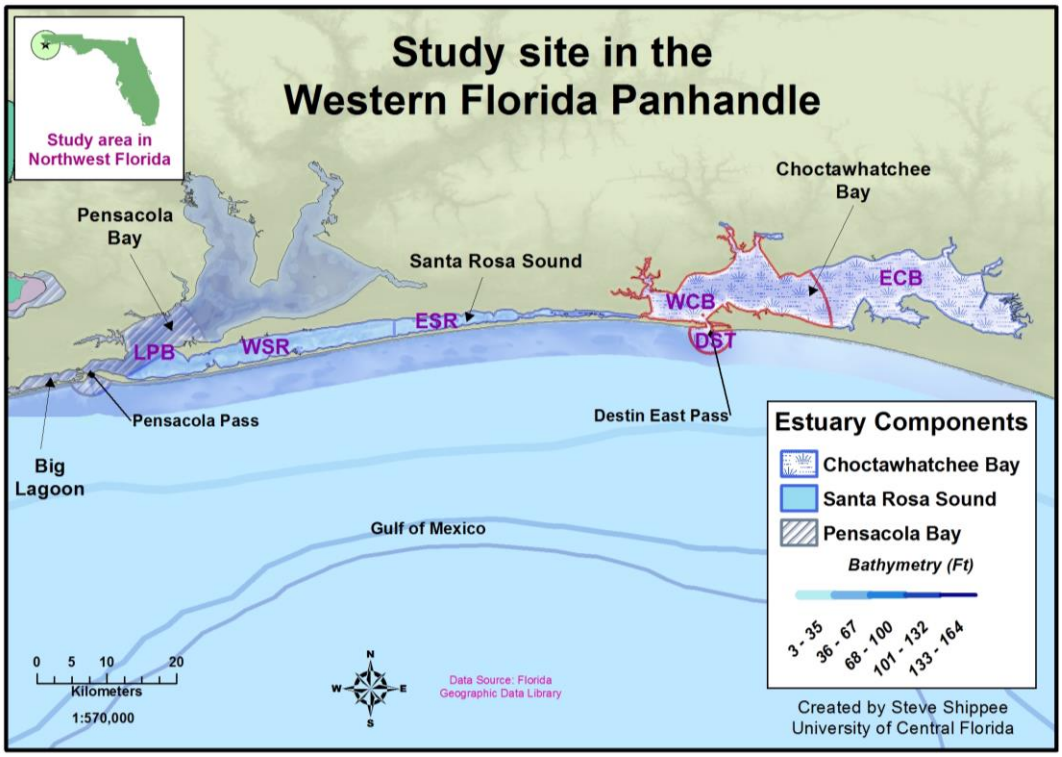

Figure 1.2: Map of Choctawhatchee and Pensacola Bay region of NW Florida. LPB = Lower Pensacola Bay; WSR and ESR = Western and Eastern Santa Rosa Sound; WCB and ECB = Western and Eastern Choctawhatchee Bay; DST = Destin inlet

Only the lower portion of Pensacola Bay (LPB) was surveyed in this study due to the expansiveness of upper Escambia and East Bays, and because they are not directly continuous with Santa Rosa Sound. The LPB segment was bounded at the eastern edge by a N-S line from Emanuel Point in Pensacola to Butcherpen Cove in Gulf Breeze, extending west across the bay including each bayou, Pensacola Pass, and Big Lagoon west to Langley Point. Santa Rosa Sound (SRS) was divided into two segments with eastern (ESR) and western (WSR) portions divided by a N-S line at the end of Gulf Islands Seashores near Navarre Beach. Choctawhatchee Bay was divided into three segments: the west portion of the bay (WCB) from Fort Walton Beach and including all bayous extending eastward to a N-S line drawn from Stake Point; all eastern portions of the bay (ECB) including bayous from that line to the Choctawhatchee River Delta; and the area surrounding Destin East Pass (DST) demarked to the north by an arc from Marler Bayou on the east to the US Coast Guard station at the west. Both the LPB and DST segments included the areas on the outside of the inlets extending approximately 2 km southward into the Gulf and 6 km distance E-W along the nearby Gulf shoreline.

Boat survey routes were drawn using GPS software (Mapsource ver 6.15, Garmin International, Inc, Olathe, KS) and downloaded to the GPS unit used on the search vessel. Each segment was of sufficiently small area to allow completion of boat-based surveys in one to two day's effort (Table 1.1); the eastern SRS component was very narrow and could be easily searched in less than one day. ECB constituted the largest area at ~200 km^2, but the open water mid-bay portion west of Four Mile Point was difficult to transect and thus excluded from most search efforts.

Table 1.1: Survey segments and search track line characteristics.

Site	Area (km^2)	Search Length (km)	Survey Effort (Days each)
LPB	100.0	145	2.0
WSR	75.0	103	1.5
ESR	35.0	95	1.5
WCB	125.0	148	2.0
DST	50.0	57	1.0
ECB	200.0	155	2.0

LPB - Pensacola Bay, SRS - Santa Rosa Sound (east and west), DST - Destin Pass, WCB - Choctawhatchee Bay (east and west)

Track lines served as navigation guides for each segment (Figure 1.3), which varied in separation distance and heading to best conform with the search areas: LPB transects were spaced at 2.0 km intervals heading NW-SE; zigzag lines were used to navigate through WSR; contour lines guided surveys through ESR, all narrows, bayous, and Big Lagoon; N-S lines with 2.0 km spacing guided surveys in all of WCB and ECB. The DST and LPB inlet surveys followed the channels and coastline contours. The Gulf shoreline surveys at Destin Pass spanned up to 8 km west because of the predictable movement of dolphins in that region. Navigation of the survey segments were adjusted each trip to optimize sighting probabilities for actual weather conditions.

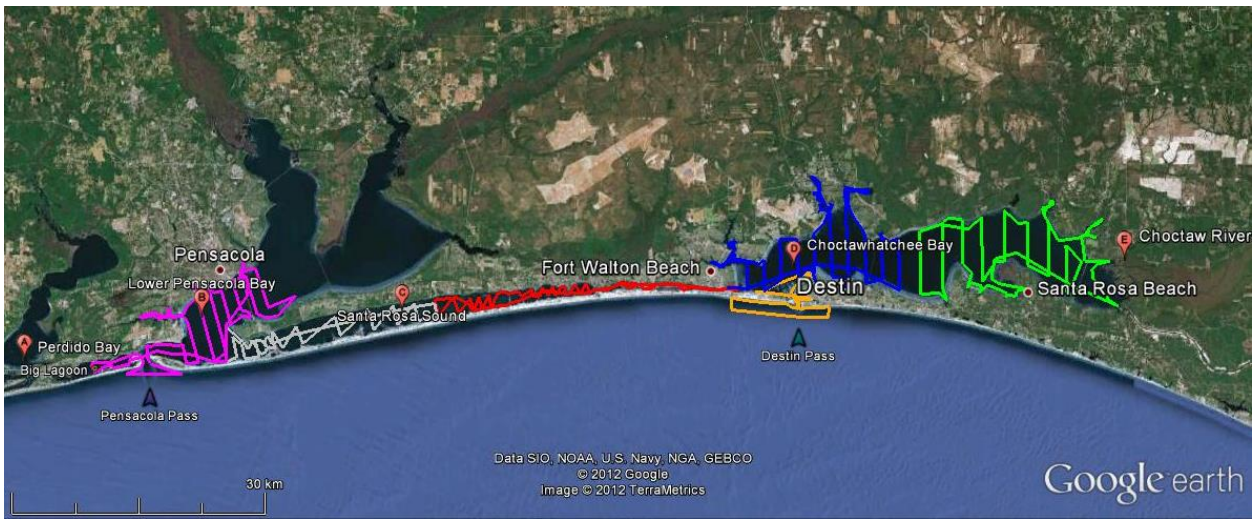

Figure 1.3: Survey tacks of boat transects in each of the six estuarine segments (denoted by color).

Quarterly surveys were planned to occur in as short a time period as possible to allow "capture" of individuals present in each segment while minimizing the effects of immigration, emigration, births, and mortality. Surveys of all planned track lines could be completed within a 10 day interval under normal seasonal weather conditions; quicker completion could be accomplished in ideal conditions. Partial surveys were allowed under difficult weather conditions as long as a reasonable assumption of detection was maintained; these typically involved searching protected waters such as bayous and the narrow waterways of ESR.

Survey and sighting techniques: The following periods were used to define seasons: December-February = Winter; March-May = Spring; June-August = Summer; September-November = Fall. Since dolphin birthing season is typically in the late winter through spring in this region (Urian *et al.* 1996), encounters in the study area during spring 2010 were included in the analysis to facilitate identification of juveniles with known maternity existing just prior to the MC252 event. Multiple focused surveys were conducted from January-September 2010 at DST, WCB, ESR, and random trips were made in WSR and LPB. After the official establishment of this project in August 2010, comprehensive surveys were started. Mark-Recapture analysis began with the initial "mark" session in October 2010, which covered all the planned survey areas in each bay segment. Follow-up surveys were then conducted each subsequent season to acquire "recapture" datasets. Surveys in November 2010 and April 2011 as a part of the biopsy collection effort (see below) followed methods that would allow sightings to be included in the recapture dataset.

All small boat surveys were conducted in accordance with NOAA Scientific Research Permit No. 522-1785 issued to co-PI Wells, and under UCF - IACUC protocol 08-21W. Observers typically conducted surveys from a 5.5m center console outboard boat with 90 HP motor. A few short opportunistic trips were made aboard commercial dolphin-watch vessels. We used

standardized photo-id protocols (SDRP 2006) and collected concurrent environmental and habitat data. Daily survey tracks and sighting locations were recorded with a GPSMap-176 unit (Garmin Ltd, Olathe, KS). Water depths were taken from the on-board bottom sounder. Water parameters for clarity, salinity, temperature, and dissolved oxygen were measured using a 20 cm secchi disk and YSI-85 probe (YSI Inc, Yellow Springs, OH).

Surveys typically followed the pre-plotted tracklines for each bay segment although the boat was allowed to deviate to explore off-track sightings or to avoid long open-water routes in poor environmental conditions. Boat speed was maintained just enough to keep the hull on plane, usually at 28-35 kph. Two experienced observers watched ahead for dolphins as the vessel progressed along the track. Effort was differentiated between searching open water transects, searching bayous/sheltered waters, making observations, and when sighting probability was low. Effort type and time were manually recorded for each survey leg along with sighting conditions. Survey effort was stopped whenever conditions reduced sighting probability below a reasonable likelihood of detection within 150 m of either side of the bow. Under normal conditions, probability of detection exceeded 250 m to either side. Sea state conditions above Beaufort 3 were considered too high for good sighting probability, although chance encounters were prosecuted and allowed to be included in analysis.

Whenever dolphins were encountered, the boat was maneuvered to within 50 m and the crew collected digital photographs as described below. Notes were recorded on group size, estimated numbers of adults, juveniles and young-of-the-year, travel direction, behavioral activity, presence of *Xenobalanus* (a cetacean-specific barnacle), and any apparent injuries or entanglement scars on individual dolphins. After sufficient photos were acquired for identification of group members and related observational data were completed, the boat would resume on the planned survey track. A typical sighting took 15 to 45 min to prosecute. Each encounter was recorded as a distinct sighting, even if groups were re-sighted later in the same day. Sighting data were recorded on paper datasheets in the field, which were subsequently transferred into MS Excel spreadsheets at the office.

Photo-identification of dolphins to develop a catalogue of individuals. As frequently as possible, all dolphin dorsal fins and other identifying features were photographed during sightings. Photos were taken using digital SLR cameras (Nikon D300, D300s, or D70 with 70-300 mm VR-II zoom lenses; Nikon Inc, Melville, NY). To control file size, the cameras were usually set for optimized jpeg compression in "Large-Fine" format. Aperture priority with ISO sensitivity auto control was selected for fastest shutter speed to reduce motion blur. Cameras were date and time-synched with the on-board GPS device before each use to insure corroborating time stamps within the photo EXIF data. Images were recorded on Compact Flash cards, which were downloaded and saved in original format to secure hard drives after each day of observations. Photo analysis was made on MS Windows computer platforms with ACDSee software (ACD Systems, Saanichton, BC, Canada). Editing was always done on duplicate sets of images made from the archive of stored original images.

We followed identification protocols developed by Sarasota Dolphin Research Program (SDRP 2006) to establish a catalog of observed individuals (Scott *et al.* 1990, Wells 2002). Dolphins were identified by visually matching images within ACDSee. To confirm matches, images were transferred to Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA) and placed in transparent layers for overlaying and comparing identifying features. Field data and notes that

complemented the photographs, along with location coordinates, were combined with the photo-id catalog for final analysis. Trip and analysis data were maintained in MS Excel and Access database applications, while the ACDSee software database was used to create and organize a searchable photo catalog of edited images.

Best quality photos from each day were sorted and selected for analysis. Dolphin identifications were categorized into four levels of distinctiveness of markings: high, medium, low, and non-distinct. Images of dolphins with low distinctiveness were included in the daily edited photos if they were unique from all other dolphins in the sighting, although they might not be identifiable. Calves were defined as young animals visually distinguishable in size from the adults, especially when seen in calf position next to the presumed mother dolphin. Very young animals that could be assigned to a mother were given identifications if they had consistent markings that could be used over subsequent sightings. Unmarked dolphins or those with very low distinctiveness were counted as individuals present in a sighting but were not uniquely identified. Analysis of individual identifications and group composition (*e.g.* number of known marked animals to unmarked animals) was made for each sighting on each survey day. Catalog names for every distinctly identified dolphin consisted of an alphanumeric ID that was entered into the master datasheet that could be readily queried by date, sighting, location, and season. Newly photographed dolphins were compared against existing catalogs previously compiled for Choctawhatchee Bay (A. Gorgone and L. Hansen, pers. comm. 2008); Choctawhatchee Bay and Destin 2006-09 (Shippee 2010); offshore Gulf of Mexico near Destin and Orange Beach; and Perdido Bay (Shippee *et al.* 2011). Matches were given the same names as the earlier catalogs to maintain long-term consistency; new identifications were named sequentially following the convention of "Site+ID Number" where site was specific to the location of first sighting (i.e. CB=Choctawhatchee Bay; EB=Escambia Bay; PD=Perdido Bay; GM=Gulf of Mexico).

Construction of a photo-id catalog is a time-intensive process of visually matching images on computer monitors. Typically, this process required one to three days of analysis per survey day depending on the number of photos collected. Animals of medium and high distinctiveness were easily matched, but most non-distinct fins present in the population required more detailed visual examination. Although several computer assisted programs exist to facilitate matching fins (*e.g.* Darwin, Eckerd College Dolphin Research Group; and Finscan, Texas A&M University), they were found to be of limited use in this study because of the high proportion of minimally distinct calf fins that were identified primarily using mother-calf affiliations and the frequent appearance of *Xenobalanus* barnacles on inlet-associated dolphins that obscured fin features.

Remote Dart Biopsy surveys: Acquisition of epidermis and blubber tissue via remote dart biopsy is a commonly used technique to evaluate free swimming bottlenose dolphins' health, genetics, nutritional status, foraging dynamics, and contaminant load (Hansen *et al.* 2004, Sellas *et al.* 2005, Balmer *et al.* 2011, Wilson *et al.* 2012). Team members from SDRP and UCF collaborated during November 2010 and April 2011 on vessel-based, remote biopsy surveys. These trips were intended to maximize dolphin encounters in order to facilitate sample collection; surveys were conducted in each study segment for one or two days in areas of previously high dolphin occurrence. All remote biopsy surveys were conducted in accordance with NOAA Scientific Research Permit No. 522-1785 issued to co-PI R.S. Wells, and performed by SDRP staff. Remote biopsy protocols used in this project are described in detail in other projects (Hanson *et al.* 2004, Balmer *et al.* 2011, Wenzel *et al.* 2010) and are specifically described in Part 2 (below). Surveys were conducted from the 5.5 m outboard boat used in the

mark-recapture sessions. The boat was driven at the normal survey speed through the search segment until dolphins were sighted, at which point the animals were photographed and observed to determine eligibility for sampling.

In contrast to mark-recapture surveys in which sightings were recorded for any dolphins observed, remote biopsy surveys were more selective. Once a dolphin group was identified for remote biopsy sampling, a sighting was recorded. Photos of as many dolphins as possible were taken along with those that were targeted for sampling. Data collected included GPS locations, boat tracks, and environmental/water parameters. The crew usually continued to follow darted dolphins for a brief period to monitor behavioral reactions to sampling, and recorded notes on observations. Remote-dart biopsy sampling typically required 15 to 30 mins of observation and close following, although some attempts lasted for longer periods of time. After completion of the dart sampling in a dolphin encounter, the boat resumed driving through the planned survey area at search speed. Daily biopsy surveys were completed when either sufficient samples were acquired, or the maximum day length was reached.

Sighting and photo-identification analyses: Datasets were analyzed to create a series of preliminary summaries including an overall discovery curve, sighting frequency tables, number of individuals sighted in each bay segment and by season, distribution pattern tables, and ultimately dolphin community composition. Graphs and charts were created in MS Excel to visually portray these summaries. A minimum population estimate for resident dolphins in each community was derived from the resighting data defined by number of individual dolphins assigned to each bay segment seen more than once during the year. Estimates of number of transients dolphins encountered were constrained to those that were only seen a single time during the study period, unless they had a sighting history prior to August 2010. For this analysis, those dolphins sighted at least once in prior years were considered seasonal residents rather than transients. Subarea site fidelity was determined by the frequency that individuals were sighted in the same segment across the study period: *e.g.* those with the highest site fidelity were consistently seen in the same segment on all sightings; those with weak site fidelity were seen multiple times but in more than one segment.

Dolphins that were sampled via remote dart biopsy were identified from the photographs and assigned to the correlated sample numbers. All sighting information for each dolphin throughout the period of this study, as well as existing sighting data from prior observations since 2006, were used to establish their home ranging and site fidelity affiliations. Dolphins that were sighted across multiple segments were assigned to the segment where most frequently sighted; those with only a single sighting were assigned to the location where sampled. The subsequent resighting table and site affiliations were used to place individual dolphins into groups that were necessary for defining the resulting data from the stable isotope analysis.

Initial Mark and Recapture histories were prepared for export into MARK, a program used to model various parameter estimates from marked animals based on recaptures (White *et al.* 2001). Based on findings from a similar study regarding closure and equal-catchability, Pollock's robust design model (Pollock 1982) can be applied to the mark-recapture data to estimate abundance and survival rates (Conn *et al.* 2011). This analysis cannot be completed until the full dataset of photo-id images is cataloged for the study period across all four seasons (currently still in process as of time of this report in January 2013).

Part 2 – Isotopic signatures of putative prey and dolphins, site fidelity and feeding habits

1) a) ***Fish Collections:***
The FWC/FWRI Fisheries Independent Monitoring (FIM) Program at the Apalachicola Field Lab conducted directed/targeted sampling for putative dolphin prey items in the shallow water habitats in Pensacola Bay and Choctawhatchee Bay during the periods November 23-24, 2010, February 8-9, 2011, April 19-21, 2011, and July 20-22, 2011. Sampling took place using a 183-m haul seine (up to six sets/day) with supplemental cast netting. Apalachicola Field Lab personnel collected fishes and macroinvertebrates and recorded species name, length, and abundance (Tables 1 and 2). Standard water quality parameters (dissolved oxygen, temperature, salinity, secchi disk) were assessed at each collection site. When possible, ten to thirty individuals, of 75-200 mm SL, were retained of each following species: *Brevoortia spp* (menhaden); *Eucinostomus gula* (common mojarra); *Eucinostimus harengulus* (Tidewater mojarra); *Leiostomus xanthurus* (spot); *Micropogonius undulatus* (Atlantic croaker); *Bairdiella chrysoura* (silver perch); *Cynoscion nebulosus* (spotted seatrout); *Cynoscion arenarius* (sand seatrout); *Mugil cephalus* (striped mullet); *Mugil curema* (white mullet); *Orthopristis chrysoptera* (pigfish); *Lagodon rhomboides* (pinfish); *Elops saurus* (ladyfish); *Strongylura marina* (Atlantic needlefish), as well as other common fish in the area. All fish samples were sent to the University of Central Florida for processing and analysis (see below for methodology).

b) ***Remote Dart Biopsy tissue sampling:*** The sampling team consisted of a four person crew working from a 5.5 m outboard vessel (described above), with the sampler (rifleman or arbalester) positioned on the bow when approaching dolphins. To optimize remote-biopsy sampling success, slow travelling dolphin groups surfacing multiple times sequentially were the primary targets for sampling. Those with distinct fins or identifying marks were preferred; females with dependent calves, very young animals, and animals already sampled were avoided. Dolphins were selected for sampling based on ease of approach, size, recognizable features (marks), independence from offspring/mothers, and general appearance of good health. Once a dolphin was selected, the boat was maneuvered to within 5m with the darter and photographer at the bow in position for firing a dart.

All shots of biopsy darts are directed away from the vessel at no more than a 90 degree angle off the bow. Shots are taken only when dolphins were within 2 and 10 m of the boat and the animal is surfacing predictably by itself. All shots were photographed by the cameraman working in concert with the darter to acquire images of the target dolphin.

The dart consisted of a 0.3 m carbon-fiber bolt holding a 25 x 10 mm stainless cutter head with a beveled, leading edge and rear facing prongs. Two methods were used to propel the dart at the target dolphins: a 0.22 blank charge fired from a modified 0.22 caliber rifle; or a recurve crossbow with a draw weight of 68 kg (Barnett Outdoors LLC, Tarpon Springs FL). Sampling location was typically just under or anterior to the dorsal fin on the body flank and penetrated through the epidermis to a depth of 15-20 mm. The darts are designed to rebound off the flank after penetration, holding the epidermis/blubber sample core afloat in the water column for easy retrieval by the boat crew. Following recovery of darts, the boat would continue to track the target dolphin for 15 to 30 mins to observe post-biopsy behavior and acquire additional photos. Additional biopsy attempts within the same dolphin group were permitted if the previous

sampled individual could be identified and the group was not showing adverse reaction to subsequent approaches.

Notes were kept on the body sampling site, conditions of shot, if sample was collected, length of sample, dolphin reaction, and shot distance. Samples were handled using sterile techniques after recovery and processed immediately on board. Epidermis and blubber was sectioned into four longitudinal quarters using a sterile blade and forceps and then placed into containers for preservation: 1) one skin section in 20% buffered DMSO vials for genetics; 2) one skin section into a cryovial for stable isotope analysis; 3) one blubber section into a cryovial for fatty acid signature analysis; and 4) one blubber section into a Teflon jar for contaminant analysis. DMSO vials were stored at ambient temperature; all other sample containers were immediately frozen at -80^{o}C in a liquid N_2 dry shipper onboard the boat. All samples were transferred from the boat to storage containers (dry box or liquid N_2 Dewar) at the completion of each day, and subsequently express shipped to labs for analysis at session's end. Genetics samples were stored in DMSO vials until shipped. SI vials were shipped on dry ice to Dr. P. Ostrom at Michigan State University. Genetic samples were sent to Dr. P. Rosel at the NOAA Fisheries Science Center in Lafayette LA. PAH/toxin samples went to NIST in Charleston SC.

c) Stranded animal investigation and sampling: Our research team was engaged in marine mammal stranding response since 2008 in partnership with Emerald Coast Wildlife Refuge (ECWR) (ecwildliferefuge.com/ ecwr/strandingcenter), therefore being well positioned to record and evaluate bottlenose dolphin mortalities during the study period. The ECWR stranding response area included the entire region from Choctawhatchee Bay through Perdido Bay and all dolphin strandings were responded to as quickly as possible. Trained investigators always made field observations or examined deceased dolphins. Whenever possible, photos were taken of the fins and any distinct markings that would allow matching stranded dolphins to the Choctawhatchee-Pensacola photo-id catalog.

Participants in this study worked under letter of authorization through the NOAA Marine Mammal Health and Stranding Response Program (MMHSRP) to ECWR in Fort Walton Beach, FL to conduct response and examination of stranded marine mammals. All stranded animals discovered in the study region that could be accessed were examined for cause of stranding, and were handled following the protocols disseminated by the MMHSRP. Beach-cast carcasses were examined and sampled by members of the ECWR team. Select tissues were collected for use in this study: skin for both genetic analysis and stable isotope analysis. Other information collected in the course of the exam was gathered from the final necropsy report, including possible human interaction, stomach contents and potential algal/phyto-toxin exposure. Enhanced necropsy exams resulted in organ tissue samples for nutritional, chemical, viral, bacterial, and life history studies, as directed by the NOAA regional stranding coordinator, which were archived for eventual analysis by the UME investigative team.

2) *Stable Isotope Preparation and Analysis:*

Carbon and nitrogen naturally occur in two stable forms. Lighter forms, ^{14}N and ^{12}C, are more abundant than the heavier forms, ^{15}N and ^{13}C. The common vernacular is to refer to the heavier isotope concentration as a ratio in δ notation (‰) as determined from:

$$\delta X = [(R_{sample}/R_{standard})-1] \times 1000$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Isotopic analysis requires approximately 1 mg of dry sample.

Lipids are depleted in ^{13}C relative to lean tissue and therefore all fish were lipid-extracted using petroleum ether prior to isotope analysis (Schlechtriem *et al.* 2003, Post *et al.* 2007). Freeze-dried, lipid-extracted fish were ground to a fine powder using a Wig-L-Bug Amalgamator (Crescent Dental Manufacturing) and aliquots (0.9-1.2 mg) sealed in 5 x 9 mm tin capsules. Samples were analyzed by mass spectrometry (Thermo Finnigan DELTAplus and DELTA C). Standard reference materials for ^{15}N and ^{13}C included atmospheric N_2 and Pee Dee Belemnite, respectively. Analytical errors were ±0.01 SE for both test standards (bovine tissue). Quality assurance of stable isotope ratios was tested by running one known standard sample for every 12 unknown samples.

Dolphin skin was ground to a fine powder using a ball and capsule amalgamator (Cresent Industries), freeze-dried, and lipid extracted via soxhlet extraction using an azeotropic mixture of chloroform and methanol. The stable carbon and nitrogen isotopic composition of a ~1.0 mg aliquot of powder was determined using an elemental analyzer (Eurovector) interfaced to an Isoprime mass spectrometer. Isotope values are expressed as: $\delta X = \{(R_{sample} / R_{standard}) - 1\} \times 1{,}000$, where X represents ^{13}C or ^{15}N, and R represents $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ and $\delta^{15}N$, respectively. In-house standards for $\delta^{13}C$ and $\delta^{15}N$ were calibrated against V-PDB standard and air, respectively.

3) *Statistical Analyses and Data Modeling:*
Statistical analyses were performed using SPSS Statistics (version 19.0), PC-ORD (Version 5.33, MjM Software Design) and S-Plus (Professional Edition, Version 6.2.1, Insightful Corporation) and plotted using SigmaPlot (Version 10.0, Systat Software). Normality and homogeneity of variance assumptions were verified using Kolmogorov-Smirnov and Bartlett tests, respectively. A General Linear Model MANOVA (SPSS) was used to detect differences in isotopic signatures for each time interval for each species. Tukey post-hoc comparisons were carried out when species differed significantly from each other. The level of statistical significance was set at $p=0.05$. Mean values presented in the text are ± SD (except where noted).

To begin to describe trophic structure of the system we applied the approach of Jackson *et al.* (2011) and Jackson *et al.* (2012) where they attempted to improve on the use of convex hulls to describe population niche. The Layman metric of convex hull area (TA) can be converted directly to a measure of population niche area but it is highly sensitive to sample size and as a result its value increases with sample size. In contrast the standard ellipse area (SEA) asymptotes quickly at around n=30. While convex hulls increase as more samples are added, a standard ellipse contains 40% of the data regardless of sample size. The corrected SEA_c provides a highly satisfactory correction for all sample sizes (Jackson *et al.* 2011, Jackson *et al.* 2012). We use SEA_c as a measure of the mean core population isotopic niche. This area is to bivariate data as standard deviation is to univariate data. One approach to calculating the isotopic δ-space is to use a Bayesian approach as suggested by Jackson *et al.* (2011). They recommend a minimum of 10 samples per group. All metrics were bootstrapped (n=10,000).

Other metrics include NR (nitrogen range) providing information the trophic length of the community, CR (carbon range) giving an estimate of the diversity of basal resources, total area

of the convex hull giving an indication of niche width, mean distance to centroid (CD) which indicates the diversity of diet or population trophic diversity, mean nearest neighbor distance (MNND) measuring the density and clustering of species in the community and standard deviation of nearest neighbor distance (SDNND) which measures the spread of individuals within isotopic space or population trophic evenness (Jackson *et al.* 2011).

Lastly we used the Bayesian mixing model SIAR (Stable Isotope Analysis in R) to provide an estimate of the relative contributions of various resources assimilated by the different bottlenose dolphin groups (Parnell *et al.* 2010) (http://cran.r-project.org/web/packages/siar/index.html). SIAR is a package designed to solve mixing models for stable isotope data within a Bayesian framework. This model integrates variability in resource and consumer isotope values, providing a distinct advantage over other mixing models. This approach also allows for the integration of species-specific diet-tissue discrimination values. The caveat is that SIAR will try to fit a model even if the data are inappropriate and therefore care needs to be exerted when choosing potential prey.

SIAR analysis requires knowledge of diet-tissue fractionation values (Δ) for the consumer. We recently determined diet-tissue discrimination values and turnover time for bottlenose dolphin skin (Browning and Worthy unpubl.data). Mean $\Delta^{15}N$ value for bottlenose dolphin skin was $2.14 \pm 0.85‰$ and overall mean $\Delta^{13}C$ value for dolphin skin was $1.67 \pm 0.32‰$. Calculated half-lives of $\delta^{15}N$ ranged from 14 to 23 days with a mean half-life of 15.3 ± 1.7 days, while half-lives of $\delta^{13}C$ ranged from 11 to 22 days with a mean of 13.9 ± 4.8 days. These half-lives mean that dolphin skin isotopic values reflect prey consumption over the previous 6-8 weeks (given that it takes approximately 3-4 half-lives for complete transition).

Results

Part 1 – Abundance, site fidelity, and habitat use

Dolphin Mark-Recapture surveys:

a) Survey effort and dolphin sightings:

Prior to initiation of the current study, the UCF team had been conducting periodic surveys in the segments of WCB, DST, ESR, and LPB. Between January and August 2010, there were 54 surveys involving 82.5 hours of search effort. These were a component of a study to identify inlet-associated dolphins in those regions (Shippee *et al.* 2011). Since this created a baseline catalog of dolphins including young-of-the-year existing at the beginning of the MC-252 event, it was added for reference in the present study. The majority of the effort was spent in the areas closest to Destin Pass; there were no trips made to the eastern or middle part of Choctawhatchee Bay during this period and only one short run into WSR near Gulf Breeze. Two brief trips were made in LPB near Pensacola Pass.

To start the present study, eight limited searches were made in western Choctawhatchee Bay and Destin during September 2010. Initial 'Mark' sessions were then conducted in October across all study segments with subsequent 'Recapture' sessions each season. Biopsy trips were made during 6 days in November 2010 and 5 days in April 2011; these were added to the recapture surveys. Three more recapture surveys were conducted in February, March, and August 2011. Intermittent observational trips occurred when possible to document healing of biopsy wounds and to watch for new born calves. The project portion of this study encompassed 125 surveys completed across the separate segments.

In total, there were 179 segment surveys over 90 separate days from Jan 2010 through Aug 2011 covering 6906 km distance during 530 hours on the water (Table 1.1). The total distance and time spent 'searching' was reduced by 727 km for 127.5 h of effort spent observing dolphins during sightings. Several segments were usually transited per day and dolphins were sighted on 88 of the 90 survey days; there were 31 segment searches with no dolphin sightings (17.3%). Overall, 2295 dolphins were encountered from field estimates; this includes individuals that were repeatedly sighted and therefore counted multiple times (Table 1.1). During the entire period, over 52,000 identification photos were taken (Table 1.1).

During the pre-project phase, effort was concentrated on the calving months during spring and early summer. After initiation of the project (Sep 2010 – Aug 2011), effort was more uniformly distributed across all seasons although slightly greater in the spring and fall (Table 1.2). Overall, the pooled search effort was highest in spring seasons, and lowest in winter months when weather conditions were less favorable for making observations (Table 1.2).

A simplistic measure of the density of dolphins encountered in a location or season is given by the calculation of dolphins/km searched. This includes encounters with the same animals on multiple days and thus does not indicate population size; rather it reflects the presence of dolphins spatially and temporally. The highest density of dolphins was in DST and lowest in ESR (Figure 1.4, Figure 1.5). Dolphin density was consistently higher in the winter (Figure 1.5), which was attributed to the movement of dolphins toward the more tidally influenced parts of the bays (DST and LPB). During spring and summer, dolphins were more widely dispersed and the observed density was lower (Figure 1.5). Direct comparisons of the pre-project and project

phases cannot be made due to the limited range and effort of the former, although it is noteworthy that dolphin density was approximately equal in the winter for both phases (Figure 1.4, Figure 1.5).

Sightings occurred over shorter search distances in DST (8 km) and ECB (13 km) than other areas (Figure 1.6A). ESR had the greatest average search distance (36 km) between sightings. Seasonally, the likelihood of encountering dolphins varied from one sighting per 16 km searched in the fall, to one sighting per 22 km in the spring (Figure 1.6B). Dolphin group size (average number of animals per sighting) varied widely across locations, with larger groups commonly seen in DST, WSR, and LPB (Figure 1.7A). Numerous encounters of large groups of dolphins occurred at DST and WSR/LPB with the highest being 48 animals in one pod. The average group size was larger in winter months and smaller in the fall (Figure 1.7B).

b. Photo-identification catalogue:

As of December 2012, survey photos have been analyzed from January 2010 through May 2011; 14 survey days remain to complete the analysis through Aug 2011. In this period, 633 individual dolphins were identified from the photos. Dorsal fin marks and body scars were used as the primary distinguishing features; indistinct young calves consistently seen with recognizable females were assigned provisional identifications. Of the 633 dolphins, 3% were not distinct, 25% were of low distinctiveness, and 72% were of medium and high distinctiveness. 366 dolphins (57.8%) were resighted during the period (Table 1.3): 195 (30.8%) were seen on 3 or more days and 23 dolphins were sighted ten or more times, with two dolphins resighted 14 times (Figure 1.8).

The rate of discovery of new fins decreased consistently over time and the curve becomes relatively flat by April 2011 (Figure 1.9). Separate catalogs were created for three general locations: Choctawhatchee Bay and Destin containing 390 dolphins; Lower Pensacola Bay containing 170 dolphins; and alongshore Gulf of Mexico with 73 dolphins. The latter were separately defined since they were seen in the Gulf between Destin and Pensacola but never sighted inside the estuaries. Dolphins seen in Santa Rosa Sound were placed in either of the estuarine catalogs depending on location where first seen: ESR dolphins were typically added to Choctawhatchee and WSR placed in Pensacola. Identifications of resident animals seen in both bays were kept consistent regardless of where they had greatest site fidelity to avoid duplication since 39% of them were seen at multiple sites. By comparison, only 27% showed strong site fidelity to any single bay or inlet segment.

c) Dolphin abundance and distribution:

Conn *et al.* (2011) studied bottlenose dolphins in Choctawhatchee Bay in summer of 2007 and estimated a superpopulation of 232 (±13 SE) with a resident population size of 179 animals. We resighted 153 (65.9%) of the fins contained in the Conn *et al.* catalog during our surveys from January 2010 through May 2011. During the pre-project surveys undertaken during January-August 2010, we identified 183 dolphins: 163 in DST-WCB-ESR and 20 in LPB-WSR (Table 1.4). Of those, 47% were previously identified in the 2007 study. Thirty dolphins (16.4%) sighted in spring-summer of 2010 were determined to be dependent young calves or yearlings born since early 2009. This pre-project count is a representative sample of animals likely exposed to oil contaminants during the peak of the MC-252 spill event when residual oil and contaminants were washing up on area shorelines, especially since they were sighted near the inlet-associated locations. 85% (155) of the dolphins sighted in the pre-project phase were

resighted during the project surveys; many of the others may have been resighted but were simply not identifiable due to low distinctiveness. 83% of the young animals that were noted in the pre-project phase were resighted at least once during the comprehensive surveys from September 2010 through May 2011.

Sightings of dolphins occurred in all study segments during all seasons, although as noted earlier there were fewer sightings in ESR than other areas (Figure 1.10). Dolphins associated with inlets at Destin made frequent movements in and out of the bay to the nearshore Gulf (Figure 1.10). We found that resident dolphins at DST made Gulf-Bay excursions on a daily basis year round. Because the topography and expanse of Pensacola inlet is much different than Destin (max depth 18 m at Pensacola Pass compared to 6 m depth at Destin East Pass), good photos of dolphins in that region were difficult to obtain due to increased dive times and unpredictable surfacings. We could not document daily dolphin movements from LPB to the nearshore Gulf other than on a few occasions. During the peak of the spill event in summer 2010 there was floating oil residue inside LPB, and submerged tar mat deposits were found at numerous sites through the spring of 2011, especially around Pensacola Pass, on Perdido Key, and in Big Lagoon (Griggs 2010, FDEP, 2011). Owing to the openness of the inlet at LPB, dolphins in that segment should have had the greatest exposures to MC-252 contaminants, although resident dolphins at DST would also have been exposed during their frequent Gulf excursions.

Resightings of individual dolphins were scored by segment (Figure 1.11). Each dolphin was placed on the matrix once in a single segment category. Of those, 33.8% were sighted one time and labeled as 'transient'; 27.5% were resighted only in a single segment; and the remaining 38.7% travelled between segments. A total of 229 dolphins were sighted only around the inlets at Destin and Pensacola, of which 73 were sighted exclusively in the Gulf. 404 dolphins (63.8%) were found inside the estuaries, either in only one segment or travelling between segments. This expands the findings of our prior study of dolphins in Choctawhatchee Bay during 2008-09 (Shippee 2010) where a number of dolphins were known to travel between bays through Santa Rosa Sound.

The distribution of dolphins across the study area segments is presented in a map view in Figure 1.12. Of particular note is the number of dolphins that were seen ranging great distances across the study area. There were 23 animals seen at both Destin inlet and Pensacola Bay; most of these were never seen in SRS and periodically had multiple clusters of *Xenobalanus* barnacles on their fins indicating their affinity to Gulf shoreline habitat. This observation is consistent with the finding that dolphins transit between inlets along the nearshore beachfront (Shippee 2010), which potentially exposed these particular animals to higher levels of MC-252 contaminants than the full-time estuarine residents.

Seasonal distribution of dolphins across the study area was calculated by pooling all sighting data between Jan 2010-May 2011 (Figure 1.13). The highest count of dolphins occurred in the fall (433 total); winter was second highest at 307 dolphins; and spring had 251 pooled across both years. Summer 2011 sighting analysis remains to be completed. The greatest concentration of dolphins was near Destin, although this includes sightings of transients and Gulf shoreline dolphins as well as those within the estuary that were also sighted in other segments in the same season.

Part 2 – Isotopic signatures of putative prey and dolphins, site fidelity, and feeding habits

1. Prey Collections:

A total of 985 fish, representing thirty-one species were collected (Table 2.1, Table 2.2, Figure 2.1). Fish were individually analyzed for basic proximate composition (n=985) with a subset analyzed for stable isotope concentrations (n=436). Some species were not available in all sampling periods and other species were only collected occasionally.

Salinity was generally consistent over sampling areas and collection periods ranging from 23.8‰ to 27.2‰ (Figure 2.2 and 2.3). The exception was the sampling area in ECB during July 2011 when salinity was 11.0‰ (Figure 2.2). Water temperatures varied seasonally but were generally consistent within a sampling period over the different collection areas (Figure 2.2 and 2.3). Temperature varied from a low of 10.4°C in PB during February 2011to a high of 32.5°C in CB during July 2011 (Figure 2.2 and 2.3). Dissolved oxygen was good ranging from 5.9 to 10.1 mg L^{-1} (Figure 2.2 and 2.3).

In November 2010, 683 individuals of 26 species were collected (Table 2.1) and 125 individuals of 6 species were retained (Table 2.3) in WCB and 1465 individuals of 23 species were collected (Table 2.2) and 157 individuals of 8 species were retained (Table 2.4) in PB. In February 2011, 113 individuals of 4 species were collected (Table 2.1) and 45 individuals of 3 species (Table 2.3) were retained in WCB and 17 individuals of 5 species were collected (Table 2.2) and 15 individuals of 4 species were retained (Table 2.4) in PB. During April 2011, 321 individuals of 18 species were collected (Table 2.1) and 73 individuals of 8 species were retained (Table 2.3) in WCB and 549 individuals of 15 species were collected (Table 2.2) and 59 individuals of 4 species were retained (Table 2.4) in PB. During July 2011, 479 individuals of 14 species were collected (Table 2.1) and 211 individuals of 12 species were retained (Table 2.3) in ECB and 317 individuals of 24 species were collected (Table 2.2) and 109 individuals of 18 species were retained (Table 2.4) in PB. To assess isotopic signatures of fish in ECB, sampling sites were shifted to locations with lower salinities (Figure 2.2 and 2.3) in the eastern portion of the bay during July 2011.

Additional samples were collected opportunistically in WCB on February 9, 2011 but catches were small and species diversity low and only 4 individual *Cynoscion nebulosus* (average SL=316.3 mm; 240-480 mm) were collected. On March 5, 2011, 139 individuals of 12 species were collected in SRS, 8 individuals of 2 species were collected on May 5, 2011 in SRS and 23 individuals of 3 species were collected on July 14, 2011 in SRS with all individuals being retained for analyses (Table 2.5).

2. Stable Isotope Analysis for Fish:

Overall, $\delta^{13}C$ values of whole fish ranged from -25.8 ± 0.02‰ (± SD) (n=3) (sheepshead in ECB in July 2011) to -12.4 ± 1.2‰ (n=5) (mojarra in WCB in November 2010), while $\delta^{15}N$ values ranged from 7.1 ± 0.8‰ (n=14) (striped mullet in Pensacola Bay in November 2010) to 13.8 ± 0.6‰ (n=5) (red drum in ECB in July 2011) (Table 2.6). When adequate sample sizes were available (n>10), individual species were examined using a MANOVA to determine if there

were significant intra-specific or intra-specific differences between sampling periods and/or Bays in $\delta^{13}C$ or $\delta^{15}N$.

a) Within Season Inter-specific Comparisons:

i) November 2010:

Atlantic needlefish (n=5), pigfish (n=6), pinfish (n=12), silver perch (n=14), spot (n=10), spotted seatrout (n=5), striped mullet (n=13) and white mullet (n=2) were all collected from PB, while Atlantic needlefish (n=4), pigfish (n=4), pinfish (n=10), silver perch (n=12), spotted seatrout (n=5) and mojarra (n=5) were collected from WCB (Figure 2.4, Table 2.6). Isotopic signatures ranged from mullet ($\delta^{15}N$ – 7.05 + 0.8‰; $\delta^{13}C$ – -13.1 + 0.7‰) to needlefish ($\delta^{15}N$ – 13.8 + 0.7‰; $\delta^{13}C$ – -17.9 + 0.9‰). Mojarra was a species that was collected irregularly and had an isotopic signature of $\delta^{15}N$ – 8.9 + 0.5‰ and $\delta^{13}C$ – -12.4 + 1.2‰ (Figure 2.4, Table 2.6).

ii) February – March 2011:

Pigfish (n=2), pinfish (n=1), silver perch (n=1), spot (n=13), striped mullet (n=1), white mullet (n=1), white trout (n=4) and Atlantic croaker (n=1) were collected in PB. Pinfish (n=10) and spotted seatrout (n=4) were collected from WCB. Catches were small and species diversity low due to the cold inshore water temperatures (10.3-10.7°C) (Figure 2.2). Isotopic signatures ranged from mullet ($\delta^{15}N$ – 9.48‰; $\delta^{13}C$ – -11.7‰) to spotted seatrout ($\delta^{15}N$ – 12.8 + 0.4‰; $\delta^{13}C$ – -16.4 + 0.9‰) (Figure 2.5, Table 2.6). White trout ($\delta^{15}N$ – 13.1 + 0.7‰; $\delta^{13}C$ – -18.9 + 0.8‰) and Atlantic croaker ($\delta^{15}N$ – 11.9‰; $\delta^{13}C$ – -19.8‰) were uncommon catches (Figure 2.5, Table 2.6).

To supplement these catches, additional collections were undertaken in SRS during early March. As a result, bay squid (n=5), narrow squid (n=7), brown shrimp (n=2), Gulf butterfish (n=5), hardhead catfish (n=1), inshore lizardfish (n=1), sand perch (n=3) and southern hake (n=5) were also analyzed (Table 2.5). Sample sizes were small and therefore statistical analyses were not undertaken but collectively these species had $\delta^{13}C$ signatures that were lower than seen either PB or CB but not as low as ECB (Table 2.7, Figure 2.5). Narrow squid ($\delta^{15}N$ – 12.5 + 0.2‰; $\delta^{13}C$ – -17.7 + 0.2‰) and bay squid ($\delta^{15}N$ – 12.9 + 0.5‰; $\delta^{13}C$ – -18.4 + 0.6‰) had similar isotopic signatures and fell in the same range as spotted seatrout (Table 2.7, Figure 2.5). Brown shrimp ($\delta^{15}N$ – 9.0 + 0.2‰; $\delta^{13}C$ – -18.6 + 2.5‰) were similar to mullet (Table 2.7, Figure 2.5). Other species sampled in SRS had $\delta^{15}N$ values ranging from 11.9‰ to 13.1‰ and $\delta^{13}C$ values ranging from -19.1‰ to -15.7‰ (Table 2.7, Figure 2.5).

iii) April 2011:

Pinfish (n=10), spotted seatrout (n=1), striped mullet (n=14), white mullet (n=5) and re drum (n=6) were collected in Pensacola Bay while Atlantic needlefish (n=3), spot (n=2) spotted seatrout (n=4), striped mullet (n=11), mojarra (n=4), red drum (n=5) and American halfbeak (n=1) were collected in WCB (Table 2.8, Figure 2.6). Isotopic signatures ranged from striped mullet ($\delta^{15}N$ – 7.7 + 0.9‰; $\delta^{13}C$ – -13.7 + 0.6‰) to Atlantic needlefish ($\delta^{15}N$ – 12.7 + 0.6‰; $\delta^{13}C$ – -17.1 + 0.1‰) (Table 2.8, Figure 2.6). Red drum did not differ between PB and WCB ($\delta^{15}N$ – 11.0 + 0.7‰; $\delta^{13}C$ – -16.0 + 0.6‰ vs $\delta^{15}N$ – 11.0 + 0.3‰; $\delta^{13}C$ – -15.9 + 0.5‰ respectively). Mojarra was similar to pinfish with a signature of $\delta^{15}N$ – 10.3 + 0.6‰; $\delta^{13}C$ – -13.6 + 1.2‰ (Table 2.8, Figure 2.6).

iv) July 2011:
The locations of sampling in Choctawhatchee Bay were changed during July 2011 to sample sites with lower salinities (Figure 2.2) in the eastern end of the bay (ECB) where samples were also being collected from resident dolphins. Atlantic needlefish (n=1), pigfish (n=10), pinfish (n=11), silver perch (n=2), spot (n=4), spotted seatrout (n=1), striped mullet (n=5), red drum (n=5 and Atlantic croaker (n=10) were all collected from PB, while pigfish (n=5), pinfish (n=10), silver perch (n=10), spot (n=10), spotted seatrout (n=1), striped mullet (n=13), red drum (n=5) and Atlantic croaker (n=10) were collected from ECB (Table 2.3 and 2.4, Figure 2.7). As described previously, some of these species from ECB showed significantly depleted $\delta^{13}C$ signatures relative to fish sampled in other locations.

A number of additional species were also collected during these sampling sessions. These included Florida pompano (n=2, PB), Gulf toadfish (n=3, PB), inshore lizardfish (n=5, PB; n=3, ECB), ladyfish (n=1, PB; n=6, ECB), leatherjacket (n=1, PB), longnose killifish (n=5, PB), Tidewater mojarra (n=2, PB; n=3, ECB), sheepshead (n=1, PB) and yellowfin menhaden (n=7, ECB) (Table 2.8). Although sample sizes were too small for statistical analyses, most species collected in ECB had significantly more depleted $\delta^{13}C$ signatures relative to conspecifics sampled elsewhere – generally averaging between -23‰ and -26‰. These included ladyfish, Tidewater mojarra, sheepshead, and yellowfin menhaden (Table 2.8, Figure 2.7).

b) Intra-Specific and Inter-Specific Regional/Temporal Comparisons:
Not all fish/invertebrate species were collected in all seasons/locations and therefore temporal and spatial comparisons were not feasible in all cases. Pinfish, spotted seatrout, spot and silver perch were available with adequate sample sizes to begin to explore potential temporal and spatial differences. Species differed in terms of their average location in isotopic niche space as a result of differences in $\delta^{13}C$ and/or $\delta^{15}N$.

Pinfish were available from PB in November 2010, April 2011, and July 2011 and from WCB in November 2010, February 2011, and July 2011. Pinfish collected from PB and WCB in November did not differ from each other in $\delta^{15}N$ (9.7 and 9.9‰ respectively) but had significantly lower $\delta^{15}N$ ($F_{(5,63)}$=9.708, $p<0.0001$) than other samples (Table 2.6). $\delta^{15}N$ values of PB pinfish collected in April (10.3 + 0.4‰) and July (10.8 + 0.6‰) were not different from WCB fish collected in February (10.8 + 0.4‰) or ECB in July (10.9 + 0.6‰) (Table 2.6). Pinfish collected in WCB in February and November (-18.23 + and -17.96 + 1.1‰) were significantly more depleted in $\delta^{13}C$ than any other region or time ($F_{(5,63)}$=31.109, $p<0.001$). $\delta^{13}C$ values in pinfish collected in ECB in July (-15.5 + 0.4‰) did not differ from PB collected in November (-15.5 + 0.9‰), April (-16.0 + 0.3‰) or July (-15.0 + 0.4‰) and PB did not differ significantly across those same collection periods (Table 2.6).

Striped mullet were available from PB in November 2010, April 2011, and July 2011 and from WCB in April 2011 and ECB in July 2011. WCB mullet collected in April (8.7 +0.8‰) and ECB fish collected in July (8.0 + 1.1‰) were significantly higher in $\delta^{15}N$ than PB seatrout in July (7.2 + 1.9‰) or November (7.0 + 0.8‰) ($F_{(4,52)}$=4.657, $p<0.003$) (Table 2.6). ECB fish collected in July (-20.5 + 1.5‰) were significantly more depleted in $\delta^{13}C$ than WCB mullet collected in April (-13.7 + 1.4‰) or PB mullet at any time of year ($F_{(4,57)}$=92.519, $p<0.0001$) (Table 2.6).

Spot were available from PB in November 2010, February 2011, and July 2011 and from ECB in July 2011. Spot showed significant seasonal differences in $\delta^{15}N$ over the course of the year in PB in November (9.5 + 0.4‰), February (10.9 + 1.0‰), and July (7.8 + 0.6‰), as well as with ECB in July (12.2 + 0.6‰) ($F_{(3,37)}$=43.884, p<0.0001) (Table 2.6). Spot showed no significant differences in $\delta^{13}C$ within PB between July (-12.8 + 0.2‰) and November (-13.6 + 0.4‰) but was significantly more depleted in PB in February (-16.8 + 1.0‰) ($F_{(3,37)}$=183.156, p<0.0001) (Table 2.6). ECB spot sampled in July were the most depleted in $\delta^{13}C$ (-21.0 + 0.8‰) (Table 2.6).

Silver perch were collected from PB in November 2010, February 2011, and July 2011 and from ECB in July 2011. During these time periods there were no significant differences in $\delta^{15}N$ ($F_{(3,36)}$=1.480, p=0.238) but there were significant differences in $\delta^{13}C$ with PB (February and November) (-18.1‰ and -17.5 + 0.8‰ respectively) differing significantly from WCB and ECB (February and July) (-16.2 + 1.0‰ and -15.6 + 0.4‰ respectively) ($F_{(3,33)}$=14.685, p<0.0001) (Table 2.6). No within Bay differences were noted.

c) Regional Comparisons:

There were significant differences between species and regions (Table 2.6) ($\delta^{15}N$ - $F_{(15,299)}$ = 56.554, p<0.0001; $\delta^{13}C$ - $F_{(15,299)}$ = 70.07, p<0.0001) (Table 2.9). Intra-specific comparisons between fish caught in ECB with fish of the same species collected elsewhere indicated that some species collected in ECB had significantly depleted $\delta^{13}C$ values (Table 2.9). Other species showed no differences. Spot, striped mullet, Atlantic croaker and red drum collected in ECB had significantly depleted $\delta^{13}C$ values compared to conspecifics collected elsewhere (Table 2.9). Similarly the single spotted seatrout collected in ECB was considerably more depleted than seatrout collected elsewhere. Silver perch and pinfish in ECB had significantly more enriched $\delta^{13}C$ values than these latter species but did not differ significantly from their conspecifics (Table 2.9). Pigfish collected in ECB had the most enriched $\delta^{13}C$ of any species and were significantly different from pigfish collected elsewhere (Table 2.9).

Striped mullet, perch and pinfish collected in ECB were not significantly different in $\delta^{15}N$ from conspecifics collected elsewhere. ECB spot, croaker, and red drum all exhibited $\delta^{15}N$ values that were significantly higher than conspecifics, whereas ECB pigfish had significantly lower $\delta^{15}N$ levels. The single spotted seatrout collected in ECB was considerably more enriched in $\delta^{15}N$ than seatrout collected elsewhere.

A summary of fish collected in ECB versus pooled data for conspecifics collected in western CB and PB over all other seasons revealed several clusters of species (Figure 2.8, Table 2.9). With carbon signatures of less than -19‰, ECB red drum and the single ECB spotted sea trout clustered together with high nitrogen signatures, ECB spot and ECB Atlantic croaker clustered together with nitrogen signatures of approximately 12.5‰, and ECB striped mullet had a nitrogen signature of approximately 8‰ (Figure 2.8, Table 2.9). ECB pigfish were isotopically similar to non-ECB striped mullet (Figure 2.8, Table 2.9) and non-ECB spotted seatrout were significantly from all other species (Figure 2.8, Table 2.9). Silver perch (ECB and non-ECB), pinfish (ECB and non-ECB), non-ECB Atlantic croaker, non-ECB pigfish, non-ECB spot and non-ECB red drum all clustered together and were not distinguishable (Figure 2.8, Table 2.9).

c) SIBER Analysis of Fish:
A Stable Isotope Bayesian Ellipse in R (SIBER) (Jackson *et al.* 2011) analysis of the non-ECB fish isotope data gave near identical results as the isotopic biplots indicated (Figure 2.9, Figure 2.10). This analysis gives an indication of significant niche overlap between species but consistently shows many ECB species differing from conspecifics found elsewhere.

3. Dolphin Isotope Analyses
Epidermis samples acquired from both the biopsy darted dolphins and from stranded bottlenose dolphins found in the study area were used in the stable isotope analysis. A total of 63 darted and 13 stranded dolphin samples were analyzed (Figure 2.11). Resighting data for 55 of the sampled dolphins were available (Table 2.11); 25 of the dolphins were sighted 3 or more times during January 2010-May 2011 and one dolphin was sighted 14 times. For those with resighting information, home range and site affiliations were assigned to group samples into the study site segments, while dolphins with only a single sighting were provisionally assigned to the location where darted. Unfamiliar stranded dolphins were assigned to the locations where found but these were plotted independently from the known dolphin samples. None of the sampled dolphins were found to be specific to ESR. Five dolphins that were sampled in April 2011 were sighted at multiple locations around Choctawhatchee Bay spanning both east and west segments and these individuals were assigned to a new group named "Middle Choctawhatchee Bay" (MCB) for that season. All dolphins in the November sample group were readily assigned to the previously defined site affiliations.

***a) Biopsied dolphin samples*:**
A total of 6 dolphin groups were identified, based on residency patterns and movement habits (see previous section), including east Choctawhatchee Bay (ECB), mid-Choctawhatchee Bay (MCB), west Choctawhatchee Bay (WCB), Destin Pass (DP), Santa Rosa Sound (SRS) and lower Pensacola Bay (PB). These dolphin groups differed in terms of their average location in isotopic niche space as a result of differences in $\delta^{13}C$ and/or $\delta^{15}N$.

Biopsy sessions occurred on 8-13 Nov 2010, and five months later during 18-22 April 2011. Biopsy samples were acquired from 32 dolphins in the fall and 34 in the spring, for a total of 66 samples, with one dolphin being resampled in the spring sessions. Sex was known or presumed for 34 of the animals; males represented 36% of samples and females 15%, while 47% were of unknown sex (genetic/sex analyses are pending as of 07 Dec 2012; P. Rosel, pers. comm.).

Darting occurred across all study segments in both sessions (Table 2.10). 58% of November samples were acquired from dolphins near DST and WSR, while half of the April samples were taken in WCB and DST (Table 2.10). Sighting histories existed in the photo-id catalog for 55 of the biopsied dolphins, while 10 were individuals that were never resighted after being darted. Known locations of biopsy sampling as well as any sighting history data was used to construct the site affiliation dataset discussed above.

Not all samples were complete blubber depth: two in November and one in April were partial and could only be used for genetic analysis. Samples were shipped for analysis within 5 days of each session: genetics sent to NMFS SEFSC in Lafayette LA; blubber contaminants to NIST lab in Charleston SC; and stable isotope to MSU. Results from genetic and contaminant analysis are pending as of December 2012.

b) *Isotopic Signatures of biopsied dolphins*:
Dolphins sampled in ECB in November had $\delta^{15}N$ values of 14.4 ± 0.2‰ (SD) (14.2 to 14.6‰) and $\delta^{13}C$ values of -19.7 ± 1.2‰ (-21.2 to -18.7‰) (Table 2.12, Figure 2.12). In April, ECB dolphins had $\delta^{15}N$ values of 14.0 ± 0.2‰ (SD) (13.7 to 14.1‰) and $\delta^{13}C$ values of -18.7 ± 0.3‰ (-19.0 to -18.3‰) (Table 2.12, Figure 2.13). There were significant changes in both $\delta^{15}N$ and $\delta^{13}C$ ($F=_{(10,66)}=9.851$, $p<0.01$ and $F=_{(10,66)}=5.264$, $p<0.03$ respectively). Dolphins in the MCB were only sampled in April (Table 2.12, Figure 2.13). These latter dolphins had $\delta^{15}N$ values of 13.7 ± 0.1‰ (SD) (13.6 to 13.8‰) and $\delta^{13}C$ values of -18.7 ± 0.3‰ (-19.2 to -18.6‰) and were not significantly different from ECB dolphins. WCB dolphins sampled in November had $\delta^{15}N$ values of 12.7 ± 0.5‰ (SD) (12.0 to 13.3‰) and $\delta^{13}C$ values of -17.3 ± 0.9‰ (-18.6 to -16.4‰) (Table 2.12, Figure 2.12). WCB dolphins sampled in November were significantly different from ECB dolphins in both $\delta^{15}N$ and $\delta^{13}C$ ($F=_{(4,32)}=16.34$, $p<0.0001$ and $F=_{(4,32)}=4.468$, $p<0.007$ respectively). WCB dolphins sampled in April had $\delta^{15}N$ values of 13.5 ± 0.2‰ (SD) (13.2 to 13.8‰) and $\delta^{13}C$ values of -17.6 ± 0.3‰ (-17.9 to -17.2‰) (Table 2.12, Figure 2.13). WCB dolphins in April were not significantly different from ECB or MCB dolphins in $\delta^{15}N$ but had significantly higher $\delta^{13}C$ values ($F=_{(5,34)}=23.343$ $p<0.0001$).

DP dolphins sampled in November had $\delta^{15}N$ values of 14.0 ± 0.4‰ (SD) (13.6 to 14.5‰) and $\delta^{13}C$ values of -17.6 ± 0.8‰ (-19.0 to -16.1‰) and were not significantly different from WCB dolphins for either $\delta^{15}N$ or $\delta^{13}C$ but were significantly different from ECB dolphins for both isotopes ($F=_{(4,32)}=16.34$, $p<0.0001$ and $F=_{(4,32)}=4.468$, $p<0.007$ respectively) (Table 2.12, Figure 2.12). In April, these DP dolphins had not changed significantly from their November signatures and had $\delta^{15}N$ values of 14.4 ± 0.5‰ (SD) (13.7 to 15.2‰) and $\delta^{13}C$ values of -17.2 ± 0.3‰ (-17.5 to -16.6‰) (Table 2.12, Figure 2.13). At this time, DP dolphins were not significantly different from WCB dolphins for $\delta^{13}C$ but were significantly different from ECB and MCB dolphins ($F=_{(5,34)}=23.343$ $p<0.0001$).

SRS dolphins exhibited no significant seasonal changes in their isotopic signatures. SRS dolphins in November had $\delta^{15}N$ values of 13.4 ± 0.4‰ (SD) (12.4 to 14.0‰) and $\delta^{13}C$ values of -18.0 ± 1.2‰ (-19.3 to -15.6‰) (Table 2.12, Figure 2.12). In April these were essentially unchanged with $\delta^{15}N$ values of 13.5 ± 0.5‰ (SD) (12.7 to 14.1‰) and $\delta^{13}C$ values of -17.7 ± 0.3‰ (-18.1 to -17.2‰) (Table 2.12, Figure 2.13). SRS dolphins were only significantly different from DP dolphins with respect to $\delta^{15}N$ during April ($F=_{(5,34)}=5.983$ $p<0.001$).

PB dolphins in November had $\delta^{15}N$ values of 14.2 ± 0.1‰ (SD) (14.1 to 14.4‰) and $\delta^{13}C$ values of -17.7 ± 0.5‰ (-18.1 to -17.2‰) (Table 2.12, Figure 2.12). These were unchanged in April with $\delta^{15}N$ values of 14.1 ± 0.4‰ (SD) (13.6 to 14.6‰) and $\delta^{13}C$ values of -17.7 ± 0.1‰ (-17.8 to -17.5‰) (Table 2.12, Figure 2.13). PB dolphins were not significantly different from SRS dolphins except during November when they had significantly higher $\delta^{15}N$ values ($F=_{(4,32)}=16.34$, $p<0.0001$).

c) *Isotopic Signatures of stranded dolphins*:
During the period of this study, a total of eight adult bottlenose dolphins stranded on shorelines within the study area and were sampled (Figure 2.14). Five of those were recovered in the spring of 2010 prior to the oil spill event. In addition, 5 perinatal *Tursiops* carcasses were also recovered in spring 2011 and sampled. Sighting histories were available for three of the adults but none of the others were known in the inshore or Gulf catalogs. Stranded dolphin samples were archived for future study, with the exception that stable isotope signatures were assessed.

Cause of stranding for all of the dolphins remains under investigation and they have not been directly linked to the MC-252 event; two animals were net entangled and drowned. None of the bottlenose dolphins that stranded in the study area had visible evidence of oil residue on skin or mucosa.

Dolphins that, based on the location of their discovery, were tentatively assigned to one of the defined dolphin groups were generally similar in their isotopic signature to dolphins normally residing in that region (Figure 2.15). Dolphins presumed to belong to the PB, ECB, and DP groups were consistent with other members of those groups (Figure 2.15). Dolphins retrieved from the SRS region showed considerable variability in their signatures with some dolphins resembling SRS dolphins, while others mirrored the signatures of ECB, DP or PB animals. This is not inconsistent with the SRS area being a transiting region for dolphins moving between other areas.

d) SIBER Analysis of Dolphin Groups:
There was no seasonal change in overall NR (November - 2.56‰ vs April - 2.53‰) but there was a decrease in CR dropping from 5.44‰ in November to 2.65‰ in April consistent with an aggregation of dolphins in limited areas, specifically avoiding ECB (Table 2.14).

Consistent with changes in NR and CR, SEA_c decreased for all dolphin groups between seasons with the lowest values occurring during April (Table 2.14). Most changes were on the order of a 3 to 4-fold decrease, while dolphins in WCB exhibited an 8-fold decrease. CD was also lowest in April in all cases mirroring potentially reduced prey diversity during the winter months. The largest values of CD occurred during November in the ECB and SRS suggesting a wide diversity of prey diversity in those areas. PB showed the smallest seasonal change ranging from 0.39 (November) to 0.30 (April) (Table 2.14).

Mean nearest neighbor distance (MNND) measures the density and clustering of species in the community and standard deviation of nearest neighbor distance (SDNND) measures the spread of individuals within isotopic space or population trophic evenness. Overall MNND changed from 0.35 to 0.19 between November and April and SDNND dropped from 0.25 to 0.16 over the same time period (Table 14). Decreases in CD, SEA_c, CR, MNND and SDNND are all consistent with dolphins exploiting less diverse prey items and a reduced geographic area (Table 2.14).

SEA_c can also be used to visualize niche overlap between the different groups and seasonal changes (Figure 2.16 and 2.17). The SEA_c of the dolphin groups show clear separation of ECB dolphins from other dolphins during November and April and a high degree of similarity but not overlap with MCB dolphins in April. The high degree of overlap between animals living in the passes, DP and SRS dolphins, is more evident in November than April but they are clearly different from SRS and WCB dolphins. A merging of SRS and WCB dolphins clearly occurs between November and April.

DISCUSSION

The bottlenose dolphin has served as a sentinel species in numerous studies to determine health of estuarine ecosystems impacted by contaminants or environmental catastrophes (Hansen *et al.* 2004, Wells *et al.* 2004, Durden *et al.* 2007, Adams *et al.* 2008, Fair *et al.* 2010, Yordy *et al.* 2010, Balmer *et al.* 2011, Stavros *et al.* 2011, Wilson *et al.* 2012). As apex predators, estuarine dolphin abundance and spatial distribution can also serve as an indicator for a variety of prey species (Wells *et al.* 1980, Barros and Odell 1990, Barros and Wells 1998, Gannon *et al.* 2004, McCabe *et al.* 2010). Long-term changes to trophic webs may take several seasons to affect upper level organisms, eventually being evidenced by declines in general health, nutritional status, fecundity, and/or juvenile survival of apex predators (Geraci and St. Aubin 1988, 1990, Hellou *et al.* 1990, Loughlin 1994, Fair and Becker 2000, O'Shea and Odell 2008). Changes in resident population size of cetaceans following catastrophic events have been documented in killer whales (*Orcinus orca*) and bottlenose dolphins (Dahlheim and Matkin 1994, Matkin *et al.* 1997, Mazzoil *et al.*, 2005, Durden *et al.* 2007, Balmer *et al.* 2008, Stolen *et al.* 2007, Stavros *et al.* 2011). Monitoring for changes in cetacean abundance and distribution following the Deepwater Horizon spill in combination with the 2010 northern Gulf of Mexico UME event was prescribed in the ongoing investigations by NOAA/NMFS and the Gulf Ecosystems Restoration Task Force (GCERTF 2011, NRDA 2012, Walker *et al.* 2012).

Dolphins are known to forage in seagrass habitats in well-studied estuaries (*e.g.*, Wells *et al.*1980, Barros and Odell 1990, Barros and Wells 1998, Gannon and Waples 2004, McCabe *et al.* 2010). Research on dolphin foraging ecology has clearly demonstrated the connection of female-calf groups with sheltered seagrass meadows in the early neonatal period, implying that such habitats are essential nurseries for juveniles (Waples 1995). Dolphins prey primarily on sciaenid fishes, sparids, carangid fishes, and mullets (Odell and Asper 1990, Barros and Wells 1998, Gannon and Waples 2004, Bowen and Cox 2009) and Choctawhatchee and Pensacola Bays are well known seasonal habitat for spotted seatrout, red drum, sheepshead, croaker, pigfish, pinfish, and jack crevalle. These species depend on a variety of prey associated with seagrasses/marshes, with finger mullet, pinfish, menhaden, pilchards, shrimp, crabs and sandfleas being the most important food items (Settoon, 2001). In addition, the various bays in NW Florida Panhandle have long been important as a productive commercial mullet fishery (Mahmoudi 2000).

Assessing dolphin abundance and population stability in the NW Florida Panhandle has been the subject of recent research following several UME events, some associated with harmful algal blooms (*e.g.*, NMFS 2004, Bowen 2006, Waring *et al.* 2011). Balmer *et al.* (2008) began long-term studies in St. Joeseph Bay in 2004, and additional work started in Apalachicola Bay in 2005 (Rycyk and Nowacek 2005). Research had previously been conducted on dolphins in the Panama City region (Colburn 1999; Samuels and Bejder 2004, Stewart 2006) and a photo-id catalog was created by Bourveroux and Mallefet (2010). Less well described is the dolphin population that inhabits Choctawhatchee Bay to Perdido Bays, including the inlets, extensive bayous, and rivers deltas throughout the connected inshore estuaries (Figure 1.2).

Choctawhatchee Bay encompasses 334 km^2 and connects with Santa Rosa Sound, a natural inshore waterway that runs 60 km west to Pensacola Bay and which is bounded on the south by Santa Rosa Island, a 75 km long barrier island. A single opening to the Gulf of Mexico is found at East Pass near Destin where a sheltered harbor is situated. At the eastern extreme of

Choctawhatchee Bay, the Intracoastal Waterway connects via a 31 km dredged barge canal with West Bay in Panama City. Freshwater flow to Choctawhatchee Bay comes from the 13,856 km^2 watershed which includes the Choctawhatchee River system, along with many lesser creeks that feed into the bay. Typical undeveloped coastlines of the bay are fringed by salt marshes. Choctawhatchee Bay has a salinity of 15-28‰ (although the eastern reaches can get considerably less saline), a max depth of 10.9 m (mean = 5.0 m) with extensive shallows, a yearly temperature range of 10˚ to 30˚C and once-daily tides of 0.25-0.80 m. Strong tidal flows result in large shifts in salinity that limit seagrasses mostly to the western portions of the bay (*e.g.*, Ruth and Handley 2007, Lazzarino 2010, Yarbro and Carlson 2011). Owing to the shape of the basin and the inflow of fresh water primarily from the east, Choctawhatchee Bay has been shown to consist of three habitat zones (Figure 2.18) based on eutrophication parameters and water chemistry profiles ranging from a river influenced region to one dominated by Gulf tidal influences (Beauregard 2010).

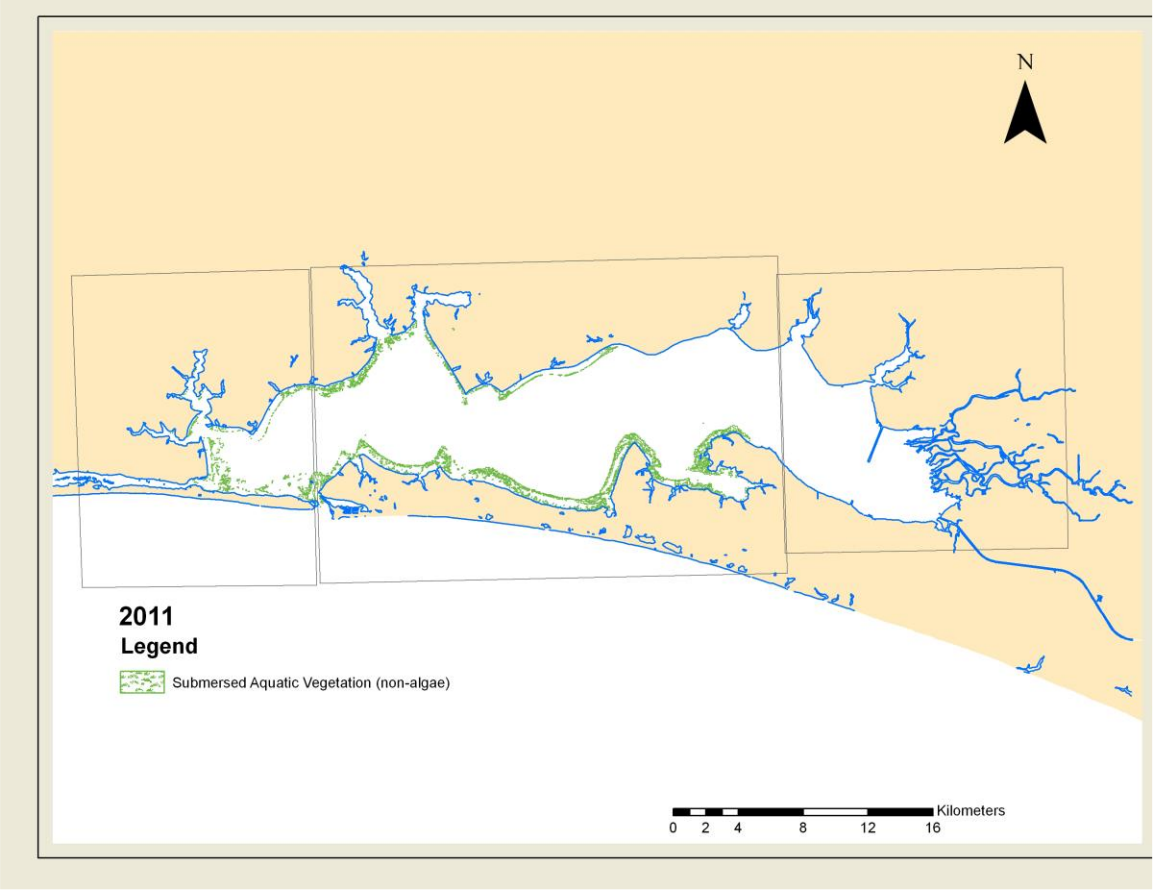

Figure 2.18: ChoctawhatcheeBay can be divided into 3 regions – East Choctawhatchee Bay (ECB), middle Choctawhatchee Bay (MCB) and West Choctawhatchee Bay (WCB) – based on SAV and water quality characteristics. (Ruth and Handley 2006)
Map source: http://www.basinalliance.org/page.cfm?articleID=13

Pensacola Bay is an extensive estuary consisting of open surface waters of about 373 km^2 and is divided into five segments: Escambia Bay, East Bay, Lower Pensacola Bay, Big Lagoon, and Santa Rosa Sound. Four rivers drain into the bay system and the watershed covers nearly 18,130 km^2. All bay segments eventually drain into Lower Pensacola Bay which has a natural deep water opening to the Gulf of Mexico between the western end of Santa Rosa Island and Perdido Key. The upper reaches of the estuary are primarily river dominated; the lower portion is tidally influenced by the strong daily ebb and flow of salt water from the Gulf. Regions of the bay surrounding the port of Pensacola and the Naval Air Station have been heavily dredged, resulting in the loss of the majority of the historical seagrass beds that once dominated the system (Handley *et al.* 2007). Seagrass losses are less significant in the Santa Rosa Sound and Big Lagoon segments of the bay which are fringed by marsh vegetation and retain natural depths except for the navigation channel in the Intracoastal Waterway.

The Intracoastal Waterway (ICW) running between Pensacola Bay and Choctawhatchee Bays has a channel depth maintained to 4.5 m. The average water depth of Santa Rosa Sound outside of the ICW is typically less than 1 m and along the southern side is generally less than 0.5 m within 100 m of shore. Extensive grassbeds line the south shore primarily at the western end of the sound, but can also be found along stretches of shallow waters behind protected spoil islands near the Fort Walton Beach area. Mesohaline marshes of cordgrass, juncus, and rush line the sound along much of its length although development on the northern shore has caused the loss of significant portions of this habitat feature. Numerous small tributaries and drainages empty into the sound, but there are no major sources of fresh water input. The flow of water is tidally driven from the bays at each end, but the Sound has relatively low salinity and temperature during the winter due to localized freshwater runoff. Summer water temperatures in the Sound can reach 35°C but salinity rarely exceeds 25‰ and can range as low as 10‰ after heavy rains (Aqualab Database, EPA Storet Station 320100A5). Isotopic evidence suggests that such waterways may make important contributions to estuarine food webs, and prey from these habitats is found in the guts of many transient marine fishes (Gillanders 2003).

The full sighting dataset for dolphin across Fall 2010 through Summer 2011 has not yet been completely analyzed, and therefore this report does not include a population estimate based on a mark-recapture analysis. This product will be in a manuscript planned for 2013 describing the findings of the analysis. Despite the incomplete analysis, the sighting histories and discovery curve presented in our results shows that a superpopulation of at least 633 dolphins inhabited the Choctawhatchee Bay and Pensacola Bay estuaries during the study period. Of that total, 33.8% were seen only a single time and therefore defined as transient (per Conn *et al.* 2011). We defined the remaining 419 of these dolphins as resident for at least a portion of the year. There is clearly a sizeable resident population of dolphins at both inlets, with the greatest amount of site fidelity found around the Destin Pass. Our analysis to date suggests that dolphins in this region can be placed into at least three general communities: inshore estuarine, inlet associated, and nearshore Gulf coastal. A small portion of the population maintains site fidelity to the riverine dominated region of eastern Choctawhatchee Bay.

This project provides the first reliable dataset of bottlenose dolphin abundance and distribution in the Western Santa Rosa Sound and Pensacola Bay estuaries. Prior work conducted in 2008-2009 indicated that movement of dolphins between here and Choctawhatchee Bay should be expected (Shippee 2010). In addition to photo-id observations of dolphins during boat surveys, there were also findings of stranded animals in LPB and WSR that had previously been identified in the

2007 Choctawhatchee Bay photo-id catalog. In the 2008-09 study, a reduction in dolphin sightings in Choctawhatchee Bay, compared to 2007, was coincident with high rainfall causing a decreased overall estuarine salinity, and associated declines in commercial mullet and shrimp harvests in the bay (Shippee 2010). This latter study was conducted just after red tide blooms that resulted in major fish kills in Choctawhatchee Bay during 2006 and 2007 and Shippee concluded that dolphins may have moved between bays in response to variable prey fish abundance.

Our current observations indicate that dolphins made frequent excursions between Pensacola Bay and Choctawhatchee Bay/Destin inlet during 2010-11. The 419 'resident' dolphins were split between those that had site fidelity to only one estuary segment (174) and those seen travelling between segments (245). We found that 81 (33%) of those transited between the Pensacola bay region and Choctawhatchee Bay. Further, at least 23 dolphins made this trip along the Gulf shoreline where potential direct exposure to contaminants from the Deepwater Horizon event was likely. Given the accumulation of oil products along inshore reaches of the Pensacola Bay estuary (Griggs 2010, National Commission 2011), it is assumed that dolphins frequenting the inlet section of Lower Pensacola Bay also had potential exposure to Deepwater Horizon event residuals.

A separate community of dolphins was identified along the nearshore Gulf between the Destin and Pensacola inlet, consisting of at least 73 individuals that were never sighted inside the estuaries, 28.8% of which were sighted more than once. These dolphins were within 2 km of the beachfront and often were seen in association with individuals that were known to travel into the inlets. These observations agree with a prior finding (Shippee *et al.* 2011) that there is a probable overlap of foraging patterns and genetic characteristics for inlet associated and coastal dolphins. This indicates that inlet associated dolphins at both Pensacola and Destin Pass had the highest probable direct exposure to Deepwater Horizon event contaminants, and would have been indirectly impacted by changes in prey base that resulted from lower trophic level disruptions.

We also found that some dolphins affiliate strongly with the eastern portion of Choctawhatchee Bay near the river mouth and maintained seasonal residency. The present study did not investigate habitat use in the upper reaches of the Pensacola estuary in East Bay or near the Escambia River delta and there may be similar populations of dolphins in those areas. The river influenced portions of the bays have very distinct habitat characteristics compared to the tidally influenced regions, primarily the lack of seagrass communities and presence of stenohaline fish assemblages that migrate from the river tributaries during low salinity conditions (*e.g.,* Lazzarino 2010, Ruth and Handley 2007, Yarbro and Carlson 2011). Dolphins that inhabited the river-influenced portions of Choctawhatchee Bay during 2010-11 had the least likely exposure to contaminants from the Deepwater Horizon event. There remains the possibility that foraging patterns changed since the dolphins inhabiting the inner estuary made seasonal movements toward the deeper middle portion of the bay where greater tidal exchange occurs with Gulf water through Destin Pass (Ruth and Handley 2007, Shippee 2010).

Over the course of the year, all dolphin communities exhibited the greatest number of individuals during the fall and winter months, even when corrected for survey effort (Figure 1.5 and 1.6b). Observations of inshore dolphins during winter months increased in the region closer to the inlet (middle and western Choctawhatchee Bay) and decreased in Santa Rosa Sound. Likewise,

fishery records reflect that some putative prey species also moved toward deeper water during these periods of the year. The migration of numerous fish species (Mugilidae, Clupeidae) toward Gulf waters in fall and winter months for spawning may explain, at least partially, corresponding dolphin movements as they pursue this prey resource.

Historically, feeding habits of dolphin populations have been determined through examination of stomach contents of dead, stranded animals (Barros and Odell 1990, Barros 1993). These studies have identified several taxa of fish that are consistently important, specifically spotted seatrout, silver perch, striped mullet, Atlantic croaker, and oyster toadfish (*e.g.,* Barros and Odell 1990, Barros 1993). Also identified in these studies but of lesser importance were pinfish, pigfish, spot, weakfishes (*Cynoscion arenarius* or possibly *C. regalis*; see Tringali *et al.* 2011), and southern kingfish. A large number of studies have been undertaken in recent years using stable isotopes to better understand and explore distributional patterns of marine mammals (*e.g.,* Barros *et al.* 2010, McCabe *et al*. 2010, Botta *et al.* 2011, Gibbs *et al.* 2011, Lowther and Goldsworthy 2011, Mèndez-Fernandez *et al.* 2012, Ruiz-Cooley *et al.* 2012, Rioux *et al.* 2012, Wilson *et al.* 2012). Gibbs *et al.* (2011) found distinct differences between bottlenose dolphins living in coastal and offshore habitats in terms of $\delta^{13}C$ and $\delta^{15}N$ and corroborated their findings with stomach content analysis. Isotopic niche analysis (*e.g.,* Newsome *et al.* 2007) allows for a clearer understanding of ecosystem dynamics and energy flow. Because tissues of animals differ in isotopic composition as a result of differences in their diet, isotopic niche is a reflection of ecological niche.

Despite the diversity and abundance of the fish community of Choctawhatchee and Pensacola Bays, many species appear to occupy similar trophic positions comparable to that seen in other systems such as the Indian River Lagoon (*e.g.,* Paperno *et al.* 2006, Fletcher-Odom 2012) and in fact, most species occupied very little unique isotopic niche space, although mean isotopic values did differ between species. Many species have been shown to exhibit seasonal changes in signatures over the course of the year, presumably related to migration, ontogenetic changes, shifting feeding habits and/or physiological stresses (*e.g.,* Tamelander *et al.* 2006, Ferraton *et al.* 2007, Worthy and Worthy 2011).

In our study area, striped mullet are considered to be primary consumers and benthic detritivores. This species showed significant differences in isotopic signatures between ECB and the rest of the study area. Adult striped mullet feed on a variety of food sources such as surface bacterial scum, sediment particles, detritus, diatoms, green algae, and blue-green algae (Collins 1985a, Collins 1985b, Phillips *et al.* 1989). Differences in salinity and SAV between ECB and the rest of the system have resulted in local mullet exhibiting significant differences in their source isotopes, consistent with previous results for mullet in the Indian River Lagoon (Fletcher-Odom 2012, Fletcher-Odom and Worthy unpubl. data,)

Similar isotopic variation was observed in red drum, spot, pigfish, Atlantic croaker, and spotted seatrout, but not in silver perch or pinfish. These species differed significantly in their carbon signatures indicating a strong faithfulness to the ECB. Other species that were opportunistically collected from the ECB showed a similar tendency to depleted carbon values. Spotted sea trout are opportunistic carnivores feed primarily on fish (79%) and macroinvertebrates (13%) (Darnell 1958, Lassuy 1983, McMichael and Peters 1989, Patillo *et al.* 1997). Adult spot which are typically described as opportunistic bottom-feeders, eat polychaetes, copepods, and diatoms by scooping up benthic sediments (Phillips *et al.* 1989). Adult Atlantic croaker are typically

described as carnivores feeding primarily on crustaceans, mollusks, and fish (Bowman *et al.* 2000) and occupied a similar trophic position as silver perch and spotted seatrout. Darnell (1958) found a reasonable amount of dietary overlap between spotted seatrout and Atlantic croaker, but with the latter more likely to emphasize larger food items.

Pigfish, another carnivore, also vary their feeding habits with age, but are primarily benthic carnivores as adults. Feeding habits of pigfish vary with growth stage but adult pigfish, which are generally found over mud bottoms and occasionally over sandy vegetated areas, are primarily benthic carnivores (Patillo *et al.* 1997) consuming polychaetes, shrimps, mollusks, crabs, amphipods and insects (Sutter and McIlwain 1987).

Pinfish, which are omnivores, have a diverse diet consuming minnows, crustaceans, amphipods, shrimps, and molluscs, and occasionally eating seaweed and organic debris. Pinfish are capable of prey switching (Muncy 1984, Luczkovich *et al.* 1995) and may be prey-selective at certain times, in certain areas or at certain stages of growth (Darcy 1985). Stoner (1980) suggested variation in pinfish diets among sampling locations was due in part to variation in macrophyte abundance, with increased carnivory demonstrated at relatively unvegetated sites. While pinfish which are typically found in association with seagrass beds (Hansen 1969, Muncy 1984, Stoner 1980, Luczkovich *et al.* 1995), the seagrass beds vary in abundance and extent on a seasonal basis perhaps necessitating prey switching.

We found that many dolphins routinely moved between Destin and Pensacola Pass, as well as between Choctawhatchee and Pensacola Bay through Santa Rosa Sound. Yet ECB dolphins were rarely found outside their home area. The consistently significant difference in stable isotope values between these and other dolphins is reflected in their site fidelity to that part of the bay, which has a high riverine influence. ECB is very different from the rest of the region due to lack of seagrass and the higher amount of inflow of nutrients from the river delta (Beauregard 2010). Published $\delta^{13}C$ ratios for fish in the Suwanee River average -26‰ (Gu *et al.* 2001) similar to our values for the ECB and are consistent with ECB dolphins consuming a significant fraction of their prey from a freshwater origin given the diet-tissue discrimination factor for bottlenose dolphins (Browning *et al.* 2010). During the winter, SRS is a lot like ECB – low salinity, not much seagrass, and nutrients derived from upland runoff after rainfall. But SRS has a good tidal exchange most of the time from Pensacola direction and these impacts may be moderated.

Dolphins are known to move around this Bay complex. At least two biopsied dolphins that we know from DP were also seen in PB. Dolphins from WCB frequently range into SRS and also to DP, although they tend to stay inside the bay. Seven biopsied dolphins were seen in WCB and inside DP, while five other biopsied dolphins were seen in WCB and SRS. Four biopsied dolphins were seen in both Lower PB and SRS, while two others were seen at opposite ends of SRS. "Inlet" dolphins (DP and PB) generally do not go deep into the bays and spend the majority of their time outside the pass along the beachfront and would therefore be expected to have isotopic values different from bay residents. Consistently “inlet” dolphins (PB and DP) were not significantly different in their isotopic signatures.

In November, mullet are heading out the Destin pass to spawn in the Gulf and dolphins are likely pursuing this prey base. WCB dolphins, in November, were observed feeding on mature mullet heading out into the Gulf to spawn. SRS and WCB dolphins were not significantly different

from each other during either season, but WCB dolphins had significantly lower $\delta^{15}N$ values in November consistent with these dolphins exploiting lower trophic level. During April, WCB dolphins had the same isotopic values as PB, SRS and DP implying a seasonal shift in the WCB dolphin feeding habits.

In April, it's possible that most of the dolphins are still spending more time near the inlets or in the middle of the bays (WCB or LPB) perhaps because their prey aren't spending as much time in the shallower bayou and riverine zones. There are fewer dolphins in SRS, ECB, and the northern part of WCB in the winter and spring consistent with those habitats being less productive in the winter months. That would change quickly with seasonal temperature and rainfall variations and you see a shift in the presumed diets of ECB and MCB dolphins during the summer with an increased dependence on needlefish, seatrout and red drum and less dependence on mullet.

On a wider scale, isotopic signatures could potentially assign unknown dolphins to home Bay systems. Barros *et al.* (2010) and Gibbs *et al.* (2012) have distinguished coastal/bay resident dolphins from offshore populations using stable isotopes. Recently, Wilson *et al.* (2012) examined dolphins living in 3 Florida Bay systems. They examined 3 groups of Florida Gulf coast dolphins and by using priority organic pollutants and stable isotope ratios (^{34}S, ^{13}C, and ^{15}N), they distinguished 2 different groups: St. George Sound and St. Andrews/St. Josephs Bays (Wilson *et al.* 2012). Despite their close geographic proximity, sighting and tracking data were consistent with the isotopic data indicating limited movements of dolphins between St. Joseph Bay and St. George Sound (Nowacek 2008, Balmer *et al.*, 2008). Comparing isotopic signatures of our inlet dolphins (PB and DP) with these other Gulf coast populations, show they also have a distinctly different signature (Figure 2.19). Application of isotopic signatures over a wider scale could ultimately resolve some questions relating to the population structure of the northern Gulf of Mexico and ultimately, could allow for better understanding of the roles of these dolphins as apex estuarine predators and a more effective management of the ecosystems in which they live.

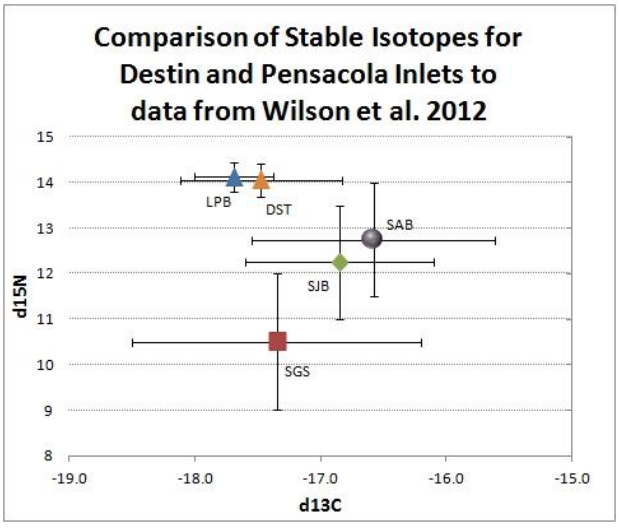

Figure 2.19: PB and DP dolphins from the present study compared to dolphin found in St Andrew Bay (SAB), St Josephs Bay (SJB) and St Georges Sound (SGS) Florida (data derived from Wilson *et al.* 2012).

Despite similarities and overlap in feeding habits, recognizable groups of bottlenose dolphins were distinguishable using stable isotopes and confirmed with photo-id analysis. Assignment of dolphins to home range areas using stable isotope data also agree with observations of dolphin movements. In light of our findings that dolphins exhibit the greatest site fidelity to the inlet regions, it is noteworthy to point out the economic importance of these animals to the ecotourism industry. The main locations for dolphin-watching enterprises within our study area are at Destin harbor and at Pensacola Beach, but other cities in the area have significant ecotourism (*e.g.*, Panama City FL and Orange Beach AL). These operations depend on the regular daily sighting of dolphins in the waters surrounding the inlets and would be harmed if a decline occurred in resident dolphin abundance potentially having a huge local economic impact.

The present study demonstrates the overlap of the dolphin communities seasonally and spatially, and indicates that Pensacola and Choctawhatchee Bays likely comprise a sympatric population rather than independent stocks of dolphins, as currently defined in the stock assessment (Waring *et al.* 2011). At the same time there is a level of separation that puts portions of the population at greater risk than others for potential oil exposure. Adverse impacts on the dolphin communities in either of the estuaries could have a deleterious effect to the overall population structure. This calls for the need to conduct follow-up monitoring in future years to compare long-term changes in habitat use, foraging patterns, and community structure to determine the potential long-term effects resulting from the Deepwater Horizon event in the Northwest Florida region.

In order to examine feeding habits of our resident dolphins, we focused on fish collected within the Bay complex. However, many of these species spawn in the Gulf of Mexico and are therefore potentially reservoirs of incidental contaminants derived from the Gulf. The nutritional value or availability of these fish populations could therefore be impacted by changes in ecosystem dynamics in regions far distant from where the dolphins reside. Over time, changes in the quality and/or quantity of the prey base exploited by apex predators could lead to direct changes in their foraging habits and nutritional condition or to indirect changes in their health status. Even the year-round residents of coastal bays and estuaries that rarely, if ever, venture into Gulf waters, could be seriously impacted. Carmichael *et al.* (2012) suggested that we may have already seen the results of these indirect ecosystem level effects on coastal bottlenose dolphins resulting in the 2011 die-off of neonates in the northern Gulf. Numerous studies have been published over the past two years discussing various documented impacts of the Deepwater Horizon oil spill ranging from effects on coastal marshes, to observations of diseased fish and the infiltration of the planktonic food web (*e.g.*, Whitehead *et al.* 2011, Hicken *et al.* 2011, Mitra *et al.* 2012, Chanton *et al.* 2012). Others (*e.g.*, Fodrie and Heck 2011) have concluded that immediate, catastrophic losses of 2010 cohorts were largely avoided and that no acute shifts in species composition occurred following the spill. Despite the range of conclusions, all studies come to the same recommendation – potential long-term impacts facing these species as a result of chronic exposure and potential delayed indirect effects require continuing attention and monitoring and that special focus needs to be paid to our near-shore areas.

ACKNOWLEDGEMENTS

Projects of this type are not possible without a great deal of assistance in all aspects of field surveys, stranding response, and biopsy cruises. We especially thank Jenny Litz, Elizabeth Stratton, and Ruth Ewing (National Marine Fisheries Service), Aaron Barleycorn and Jason Allen (SDRP, Mote Marine Lab), Tara Kirby (Ship Ahoy Assoc.), Christina Toms (UCF Biology and USM Hattiesburg), Courtney Smith (USM Hattiesburg), Monica Parries (Univ. West Florida), Amanda Wilkerson, Stephanie Kadletz, Deb Edwards, Cathy Holmes, Jeff Skipper, and Michelle Gonzales (Emerald Coast Wildlife Refuge), Sarah Kalinoski and Julia Terrell (Choctawhatchee Basin Alliance of NW FL State College) and Gary Parsons (Choctawhatchee Audubon Society). Sample preparation and analyses of fish and dolphin samples were undertaken by a dedicated crew at UCF including Tamara Worthy (PEBL) and a hard working group of undergraduate assistants including Christine Hollis, Melyssa Allen, Victoria Davis, Jessica Serrano, Christina Nagy, Caitlin Stevenson, Kelly Halpin, and Davi Foreman. Dolphin skin isotope analyses were undertaken by Hasand Gandhi in the lab of Dr. Peggy Ostrom, Michigan State University. This research was made possible by a grant from The Gulf of Mexico Research Initiative through the Florida Institute of Oceanography.

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Figure and Tables

Part 1 – Abundance, site fidelity, and habitat use

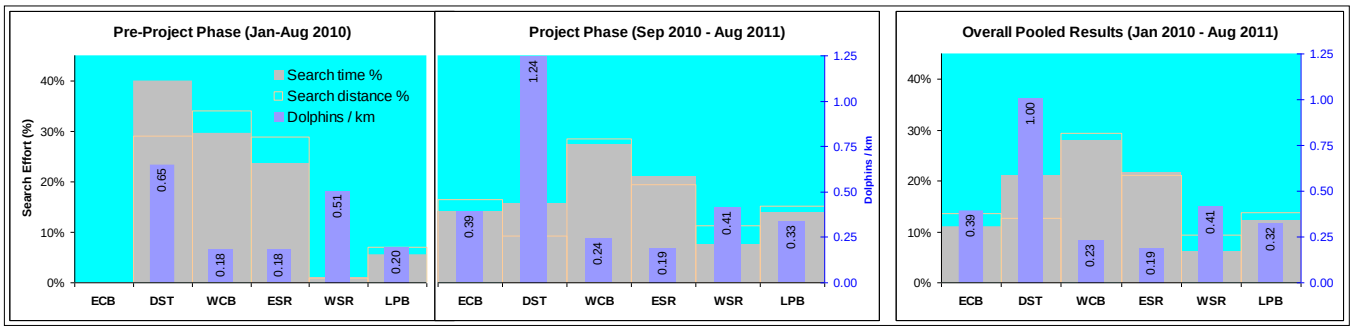

Figure 1.4: Dolphin density by segment based on encounter frequencies during surveys. Segment locations are defined as LPB – Lower Pensacola Bay, WSR – West Santa Rosa Sound, ESR – East Santa Rosa Sound, WCB West Choctawhatchee Bay, DST – Destin, and ECB – East Choctawhatchee Bay.

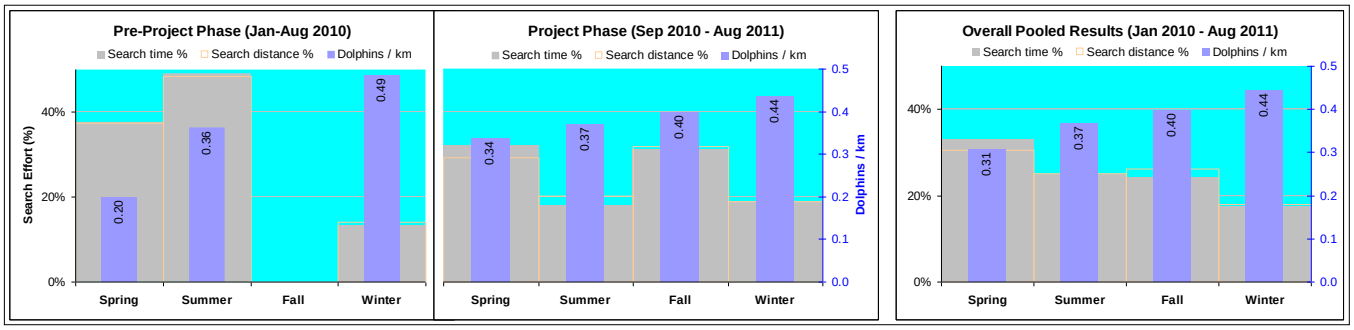

Figure 1.5: Dolphin density by season based on encounter frequencies during surveys. The following periods were used to define seasons: December-February = Winter; March-May = Spring; June-August = Summer; September-November = Fall.

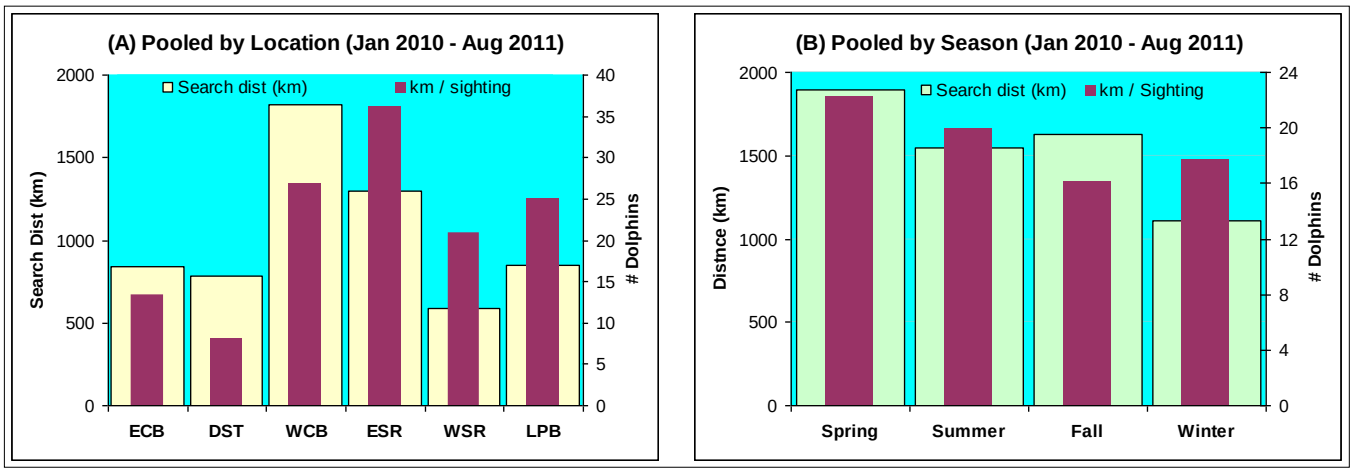

Figure 1.6: Search distance covered and average distance travelled per dolphin sighting by (A) study segment and (B) by season. Segment locations are defined as LPB – Lower Pensacola Bay, WSR – West Santa Rosa Sound, ESR – East Santa Rosa Sound, WCB West Choctawhatchee Bay, DST – Destin, and ECB – East Choctawhatchee Bay. The following periods were used to define seasons: December-February = Winter; March-May = Spring; June-August = Summer; September-November = Fall.

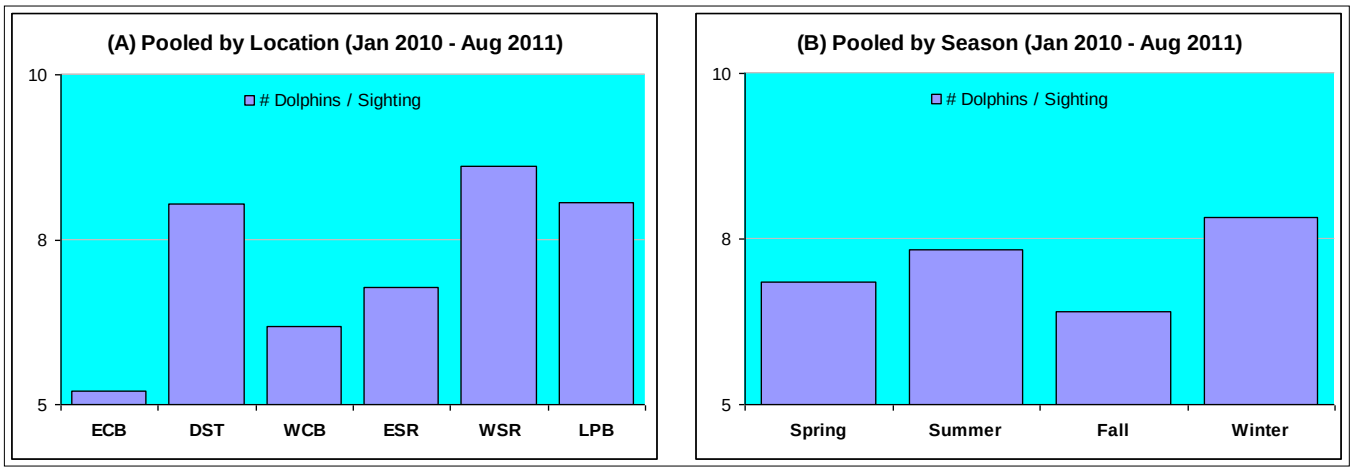

Figure 1.7: Average dolphin group size by (A) study segment and (B) by season. Segment locations are defined as LPB – Lower Pensacola Bay, WSR – West Santa Rosa Sound, ESR – East Santa Rosa Sound, WCB West Choctawhatchee Bay, DST – Destin, and ECB – East Choctawhatchee Bay. The following periods were used to define seasons: December-February = Winter; March-May = Spring; June-August = Summer; September-November = Fall.

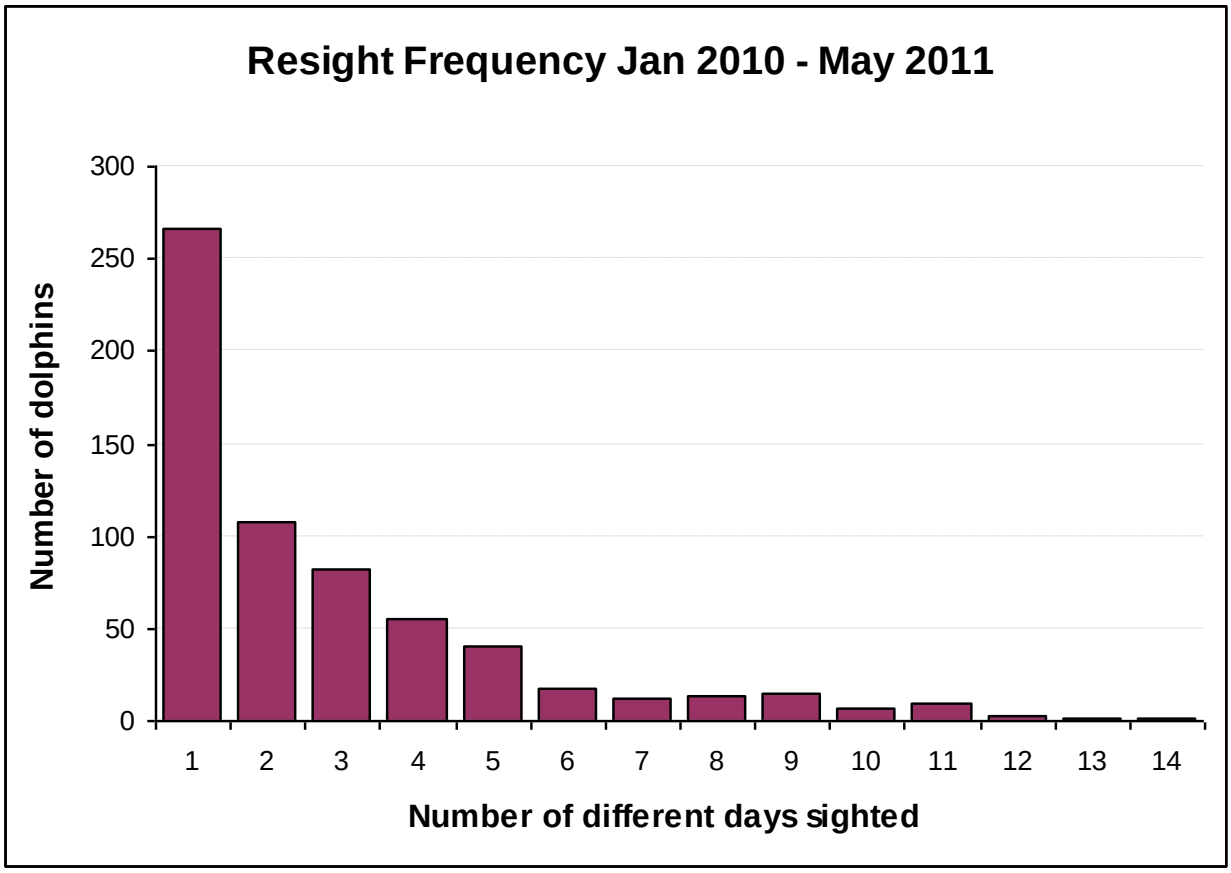

Figure 1.8. Frequency of dolphin resightings from photo-id analysis

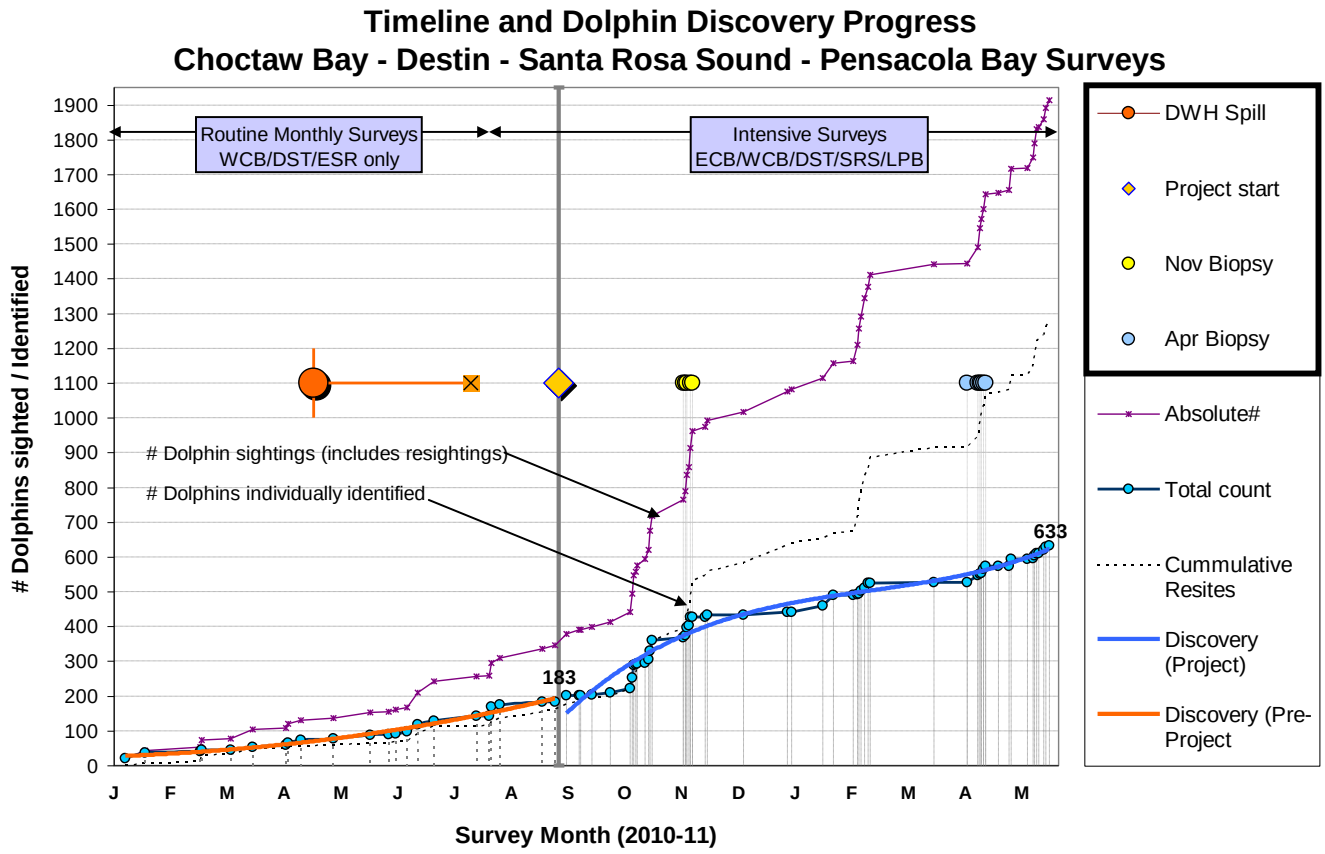

Figure 1.9. Discovery curve Jan 2010 – June 2011 from photo-id analysis. Segment locations are defined as LPB – Lower Pensacola Bay, WSR – West Santa Rosa Sound, ESR – East Santa Rosa Sound, SRS – combined Santa Rosa Sound, WCB West Choctawhatchee Bay, DST – Destin, and ECB – East Choctawhatchee Bay.

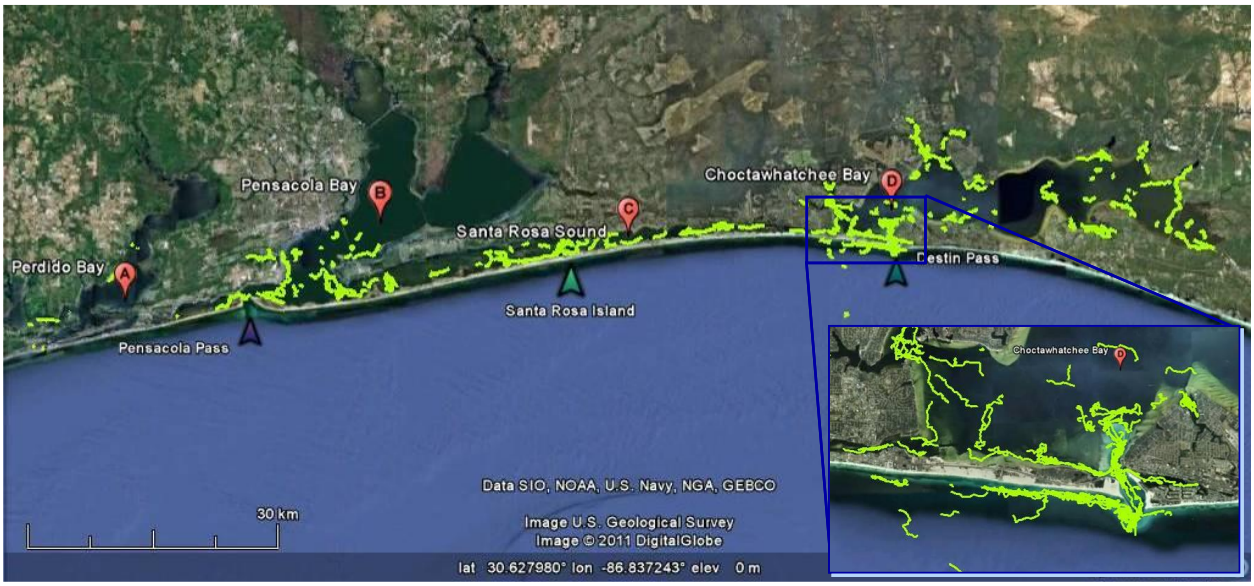

Figure 1.10. Boat tracks (in yellow) where sightings occurred. Inset shows dolphin encounters around DST site.

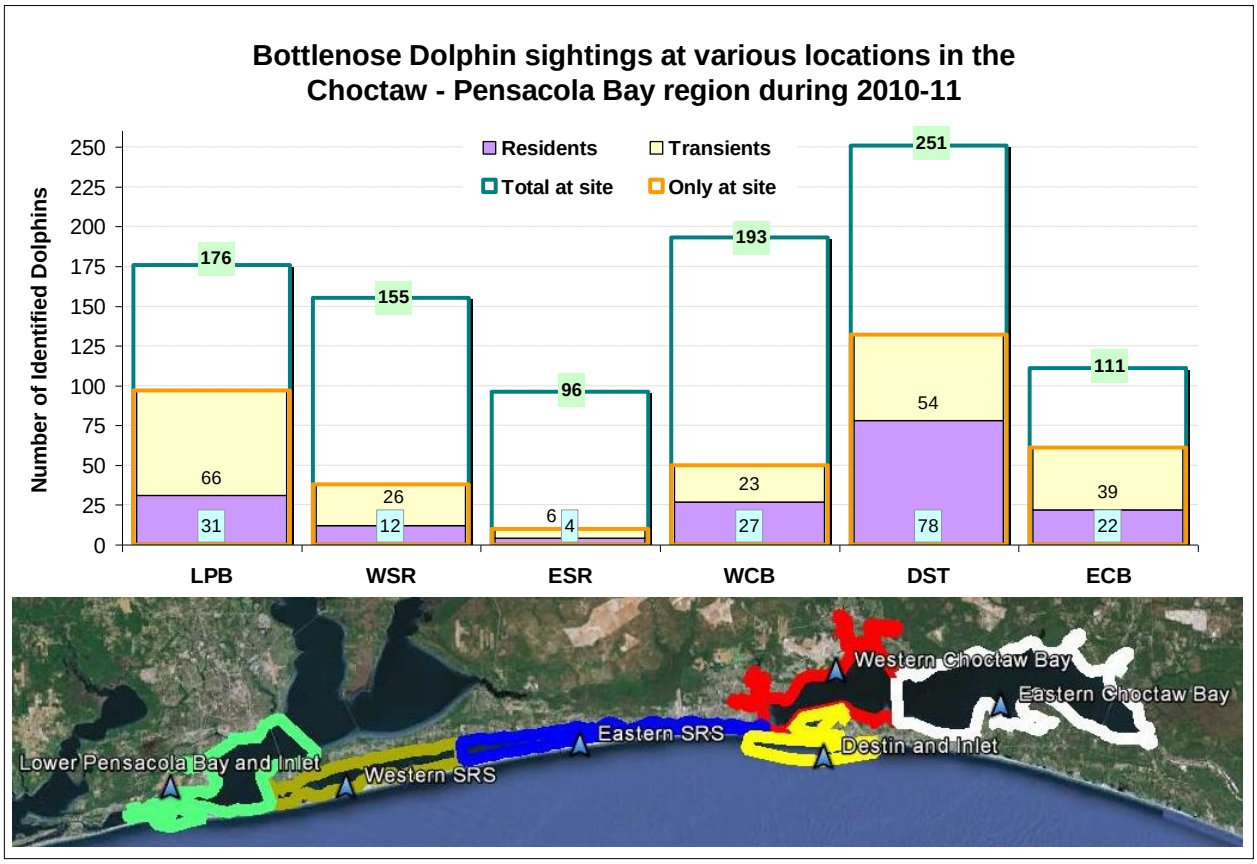

Figure 1.11. Dolphin sightings by location, Jan 2010 through May 2011. Residents were dolphins that were only seen in their home segment, transients were animals that were only seen one occasion and travelers were seen in multiple segments. Segment locations are defined as LPB – Lower Pensacola Bay, WSR – West Santa Rosa Sound, ESR – East Santa Rosa Sound, WCB West Choctawhatchee Bay, DST – Destin, and ECB – East Choctawhatchee Bay.

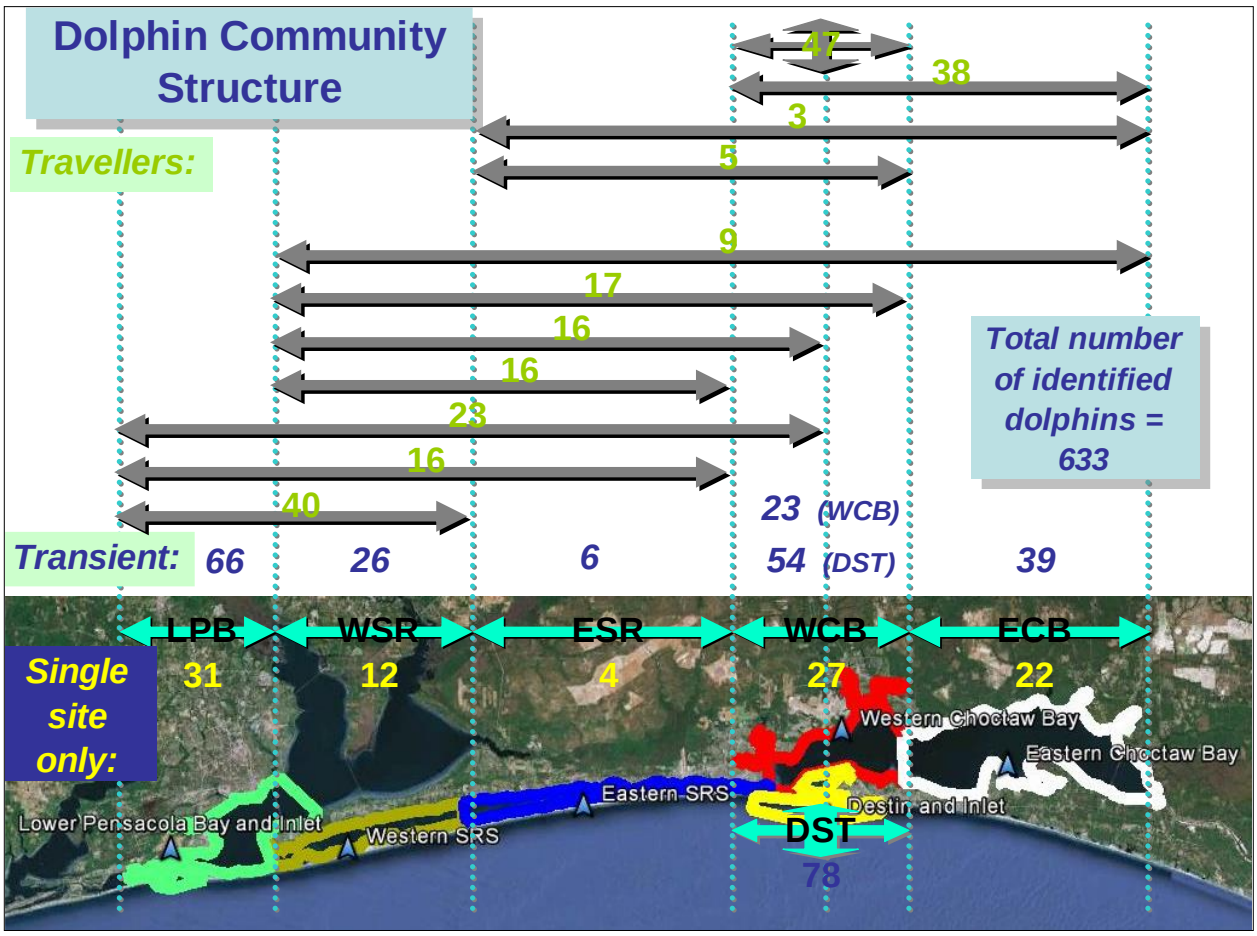

Figure 1.12. Dolphin community composition by locations sighted. Numbers indicate count of dolphins in each segment; Transients were animals that were only seen one occasion; Travelers were seen in multiple segments. Segment locations are defined as LPB – Lower Pensacola Bay, WSR – West Santa Rosa Sound, ESR – East Santa Rosa Sound, WCB West Choctawhatchee Bay, DST – Destin, and ECB – East Choctawhatchee Bay.

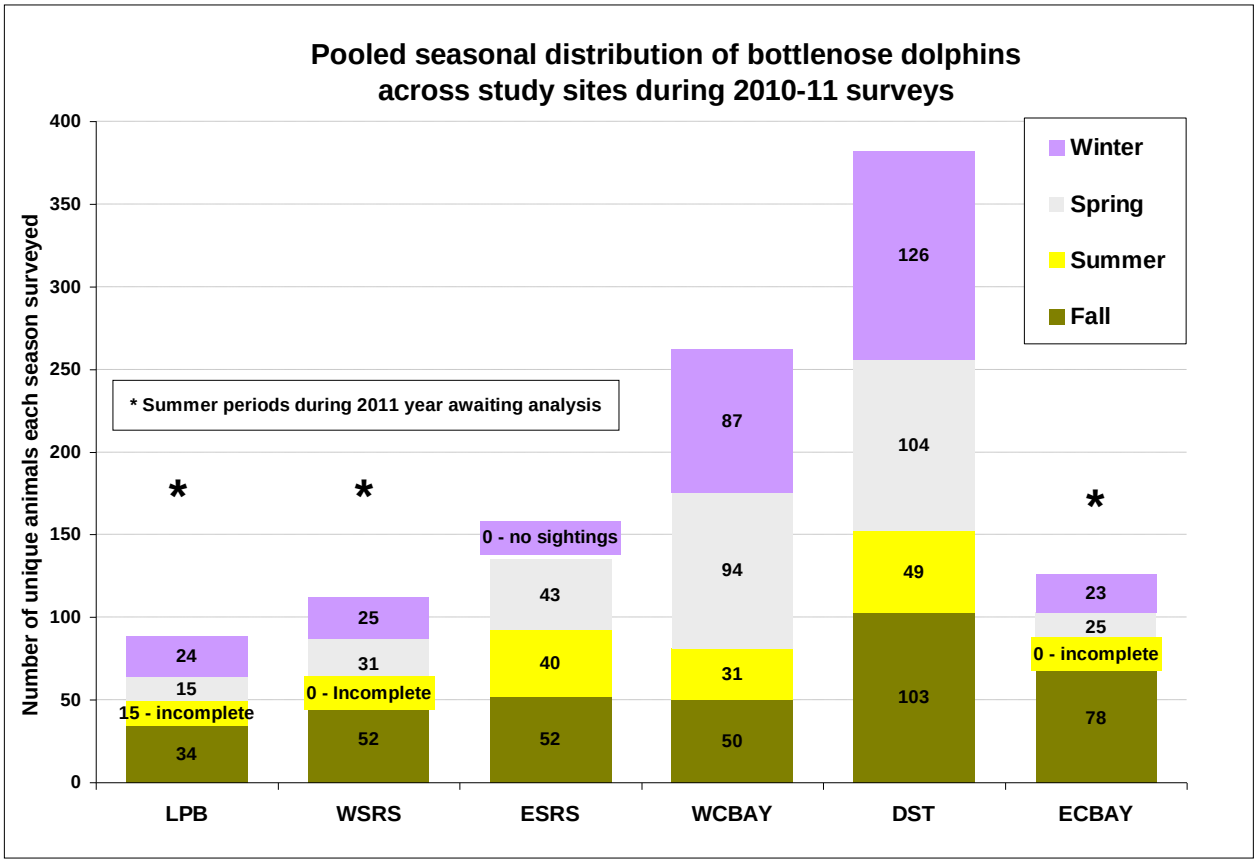

Figure 1.13. Seasonal occurrence of dolphins across segments. The following periods were used to define seasons: December-February = Winter; March-May = Spring; June-August = Summer; September-November = Fall.

Table 1.1: Summary of boat surveys in the study area segments.

Segment / Phase	# Surveys	# Sightings	# Dolphins Encounterd	Search dist (km)	Search time (hr:mm:ss)	Search effort (%)	Dolphins / km	Sightings / km
Pre-Project: Jan - Aug 2010								
ECB	0	0			0:00:00	0%		
DST	18	30	203	313	32:59:54	40%	0.648	0.096
WCB	16	12	66	367	24:31:29	30%	0.180	0.033
ESR	17	7	56	311	19:37:39	24%	0.180	0.022
WSR	1	2	5	10	0:48:00	1%	0.505	0.202
LPB	2	4	15	76	4:38:02	6%	0.196	0.052
TOTAL:	**54**	**55**	**345**	**1078**	**82:35:04**	<>	**0.320**	**0.051**
Project: Sep 2010 - Aug 2011								
ECB	11	63	328	840	41:15:00	14%	0.391	0.075
DST	27	68	585	471	46:08:23	16%	1.243	0.144
WCB	34	56	354	1453	80:07:50	27%	0.244	0.039
ESR	32	29	188	989	61:25:33	21%	0.190	0.029
WSR	9	26	236	574	22:05:30	8%	0.411	0.045
LPB	12	30	259	774	40:46:43	14%	0.334	0.039
TOTAL:	**125**	**272**	**1950**	**5101**	**291:48:59**	<>	**0.382**	**0.053**
Overall Survey Effort 2010-11								
ECB	11	63	328	840	41:15:00	11%	0.391	0.075
DST	45	98	788	784	79:08:17	21%	1.005	0.125
WCB	50	68	420	1820	104:39:19	28%	0.231	0.037
ESR	49	36	244	1301	81:03:12	22%	0.188	0.028
WSR	10	28	241	584	22:53:30	6%	0.413	0.048
LPB	14	34	274	851	45:24:45	12%	0.322	0.040
TOTAL:	**179**	**327**	**2295**	**6179**	**374:24:03**	<>	**0.371**	**0.053**

Table 1.2: Seasonal distribution of survey effort.

Segment / Phase	# Surveys	# Sightings	# Dolphins Encntrd	Search dist (km)	Search time (hr:mm:ss)	Search effort (%)	Dolphins / km	Sightings / km
Pre-Project: Jan - Aug 2010								
Spring	20	22	81	404	31:05:05	38%	0.201	0.055
Summer	24	24	190	522	40:32:33	49%	0.364	0.046
Fall	0	0						
Winter	10	9	74	152	10:57:26	13%	0.487	0.059
TOTAL:	**54**	**55**	**345**	**1078**	**82:35:04**	**<>**	**0.320**	**0.051**
Project: Sep 2010 - Aug 2011								
Spring	35	63	502	1488	93:10:08	30%	0.337	0.042
Summer	27	54	382	1028	52:45:46	18%	0.372	0.053
Fall	38	101	647	1624	90:52:30	34%	0.398	0.062
Winter	25	54	419	960	55:00:35	18%	0.436	0.056
TOTAL:	**125**	**272**	**1950**	**5101**	**291:48:59**	**<>**	**0.382**	**0.053**
Overall Survey Effort 2010-11								
Spring	55	85	583	1891	124:15:13	33%	0.308	0.045
Summer	51	78	572	1550	93:18:19	25%	0.369	0.050
Fall	38	101	647	1624	90:52:30	24%	0.398	0.062
Winter	35	63	493	1112	65:58:01	18%	0.443	0.057
TOTAL:	**179**	**327**	**2295**	**6179**	**374:24:03**	**<>**	**0.371**	**0.053**

Table 1.3: Between January 2010 and May 2011, 633 individual dolphins were identified from photos. Of these dolphins, 3% were not distinct, 25% were of low distinctiveness, and 72% were of medium and high distinctiveness. 366 dolphins (57.8%) were resighted during the period: 195 (30.8%) were seen on 3 or more days and 23 dolphins were sighted ten or more times, with two dolphins resighted 14 times.

Sighting Frequency (# of days)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Dolphin Count	267	107	82	55	40	18	12	14	15	7	10	3	1	2
% of total	16.9	13.1	8.9	6.5	3.0	2.0	2.3	2.5	1.2	1.7	0.5	0.2	0.4	0.3

Table 1.4: Dolphins identified in the study area during the Pre-Project Phase (January through August 2010).

(DST-Destin Pass, WCB – West Choctawhatchee Bay, ESR – East Santa Rosa Sound, WSR – West Santa Rosa Sound, PBP – Lower Pensacola Bay)

Jan-Aug 2010	**DST**	**WCB**	**ESR**	**WSR**	**LPB**	**Total**
# Sighted by location	100	29	34	5	15	183
DST-WCB-ESR	163					
LPB-WSR				20		
# Calves and yearlings = 30				# Known (NMFS 2007) = 86		

Part 2 – Isotopic signatures of putative prey and dolphins, site fidelity and feeding habits

a.

b.

c.

d.

Figure 2.1: Collection sites for putative prey of bottlenose dolphins during a) November 2010, b) February-March 2011, c) April 2011 and d) July 2011.

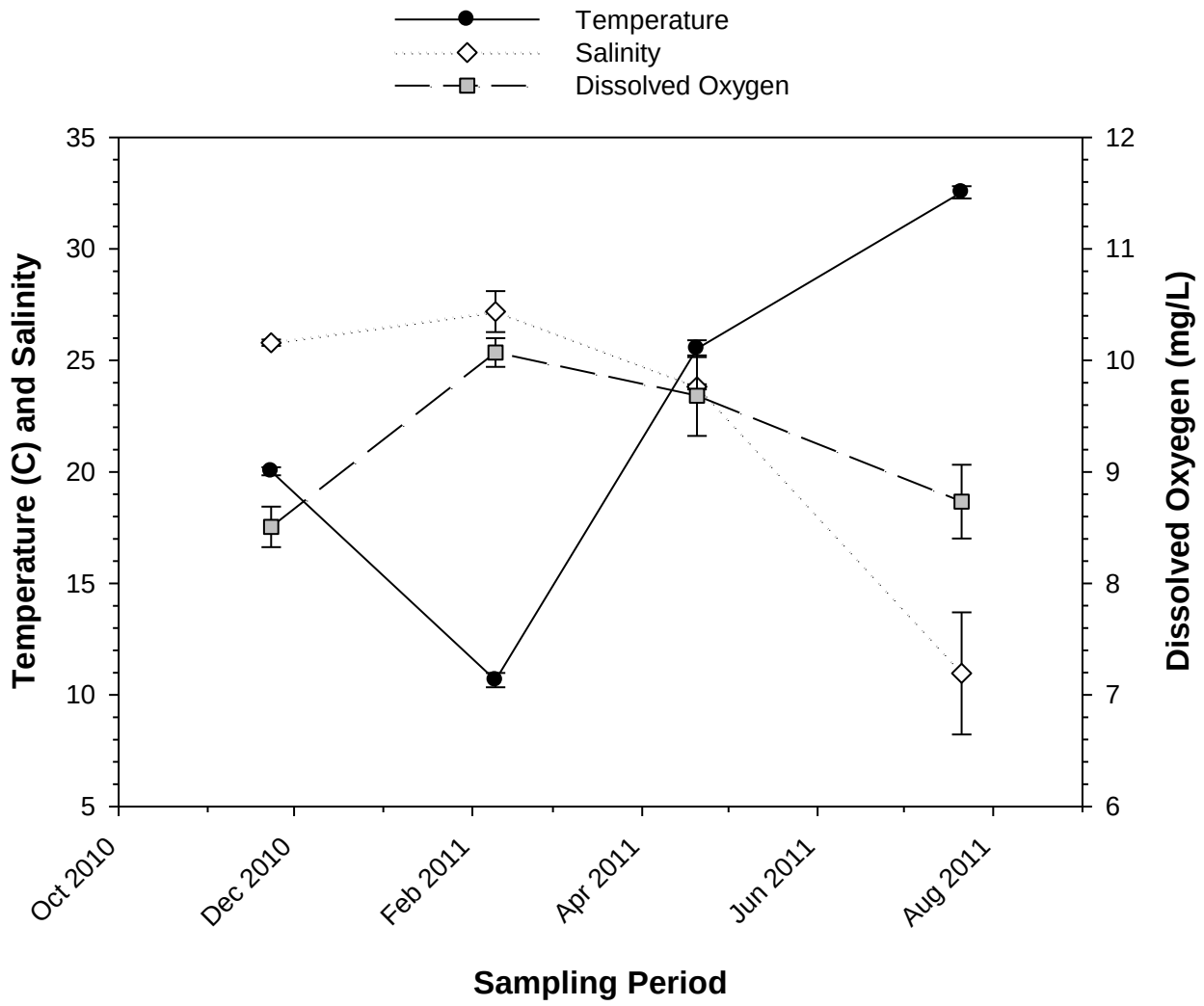

Figure 2.2. Water temperature (°C), salinity (‰), and dissolved oxygen (mgL^{-1}) (mean ± SE) for sample sites in Choctawhatchee Bay. Average salinity of east Choctawhatchee Bay was 8.0 ± 1.7‰ during July 2011.

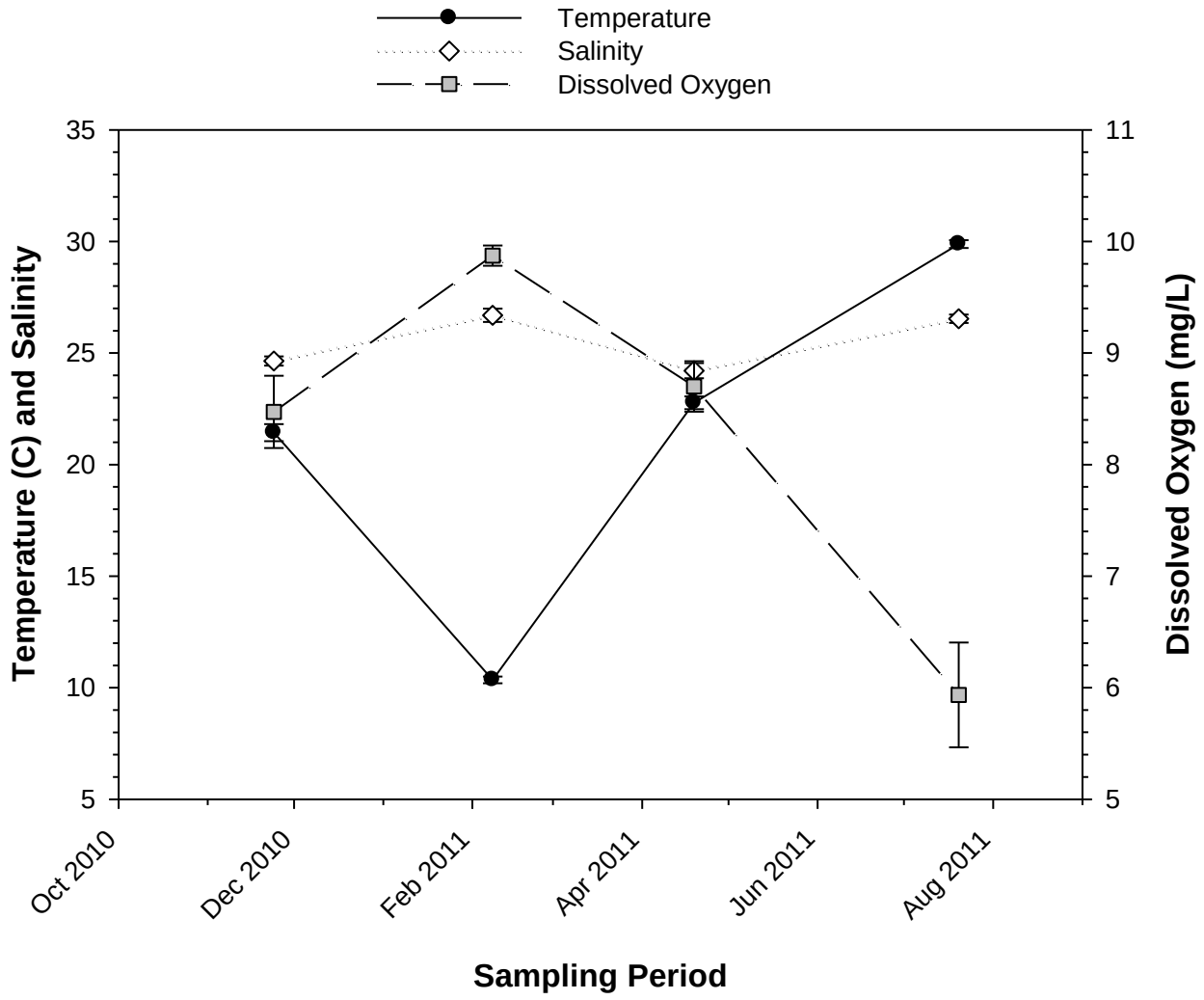

Figure 2.3. Water temperature (°C), salinity (‰), and dissolved oxygen (mgL^{-1}) (mean ± SE) for sample sites in Pensacola Bay.

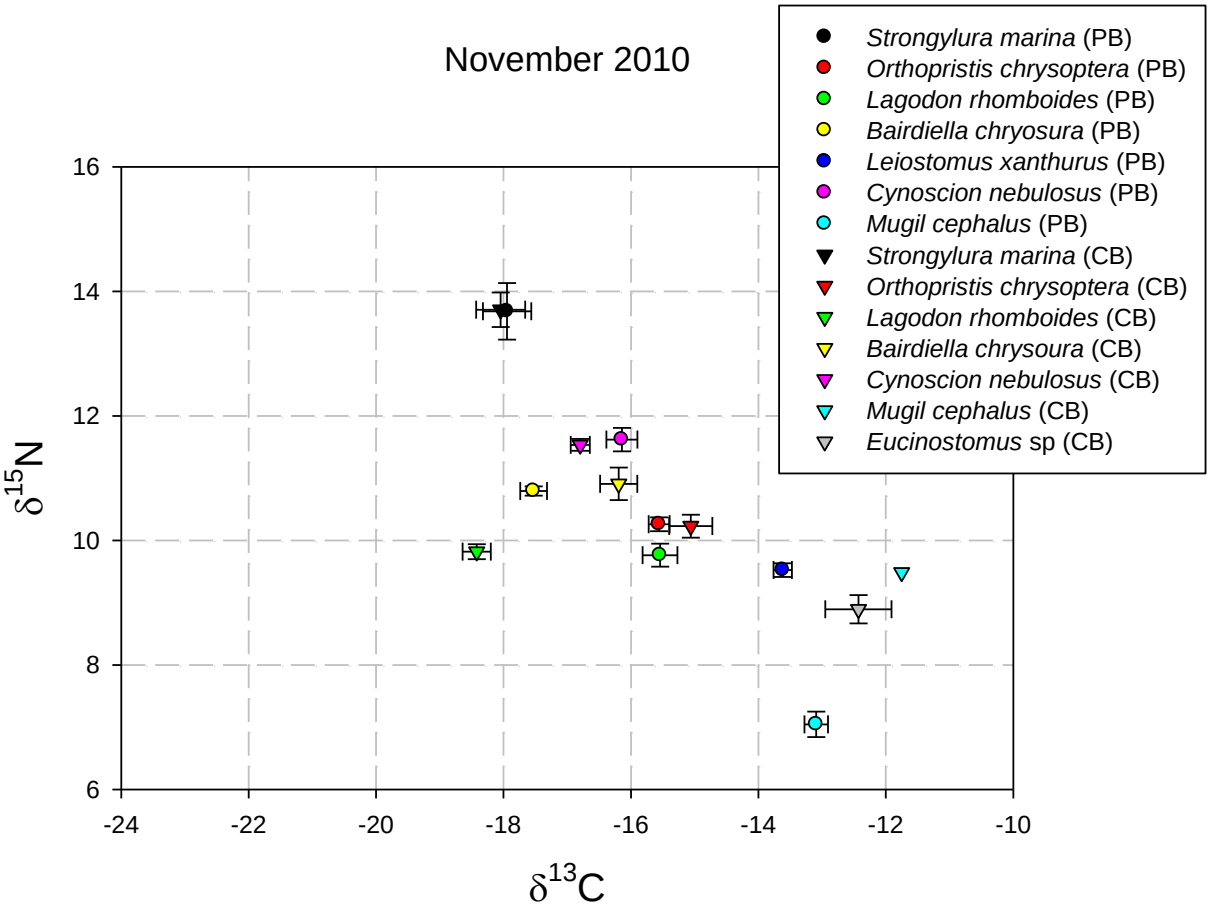

Figure 2.4: Isotopic signatures (‰) of fish collected during November 2010 over all regions. Fish were either collected in Pensacola Bay (PB) or Choctawhatchee Bay (CB). All values are mean ± SD. Common names are outlined in Table 2.3.

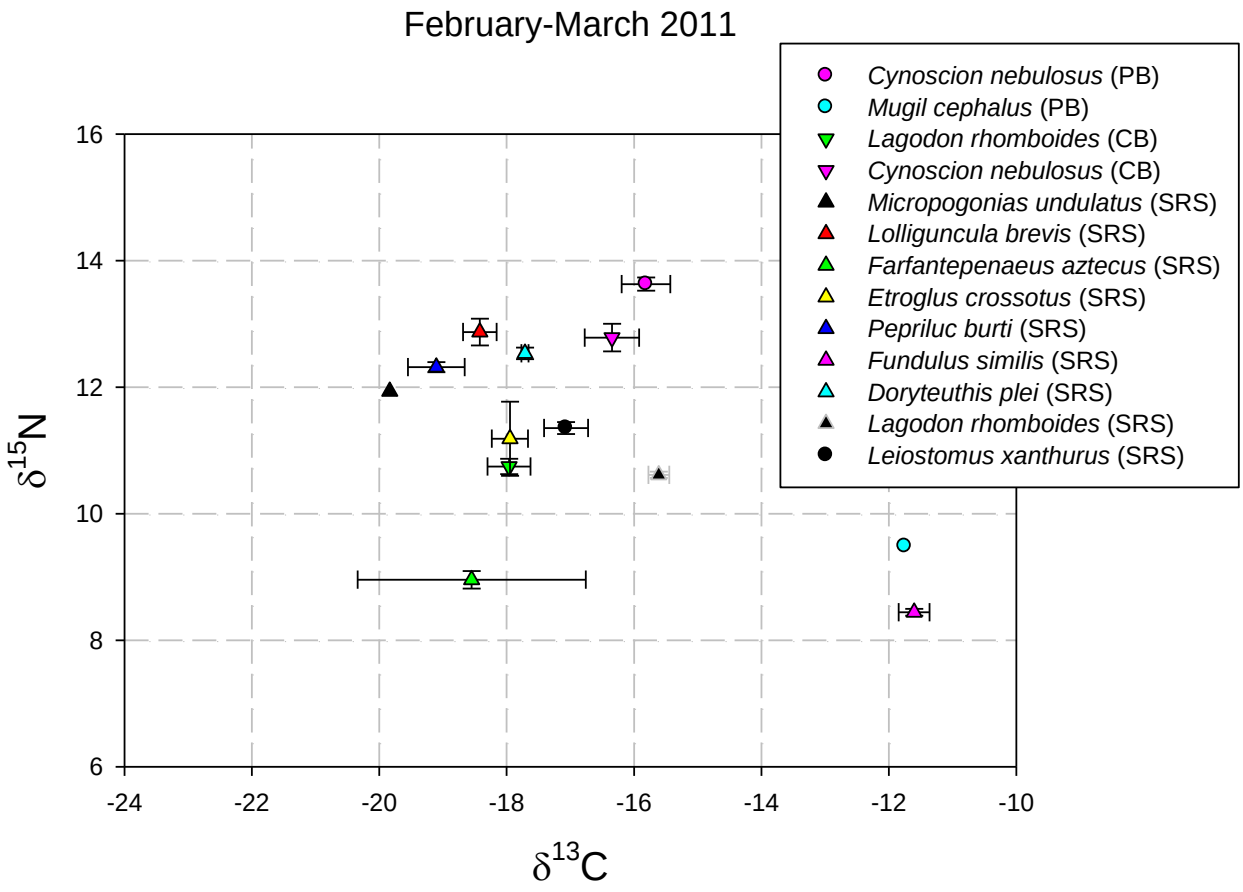

Figure 2.5: Isotopic signatures (‰) of fish collected during February-March 2011 over all regions. Fish were either collected in Pensacola Bay (PB)., Choctawhatchee Bay (CB) or Santa Rosa Sound (SRS). All values are mean ± SD. Common names are outlined in Table 2.3.

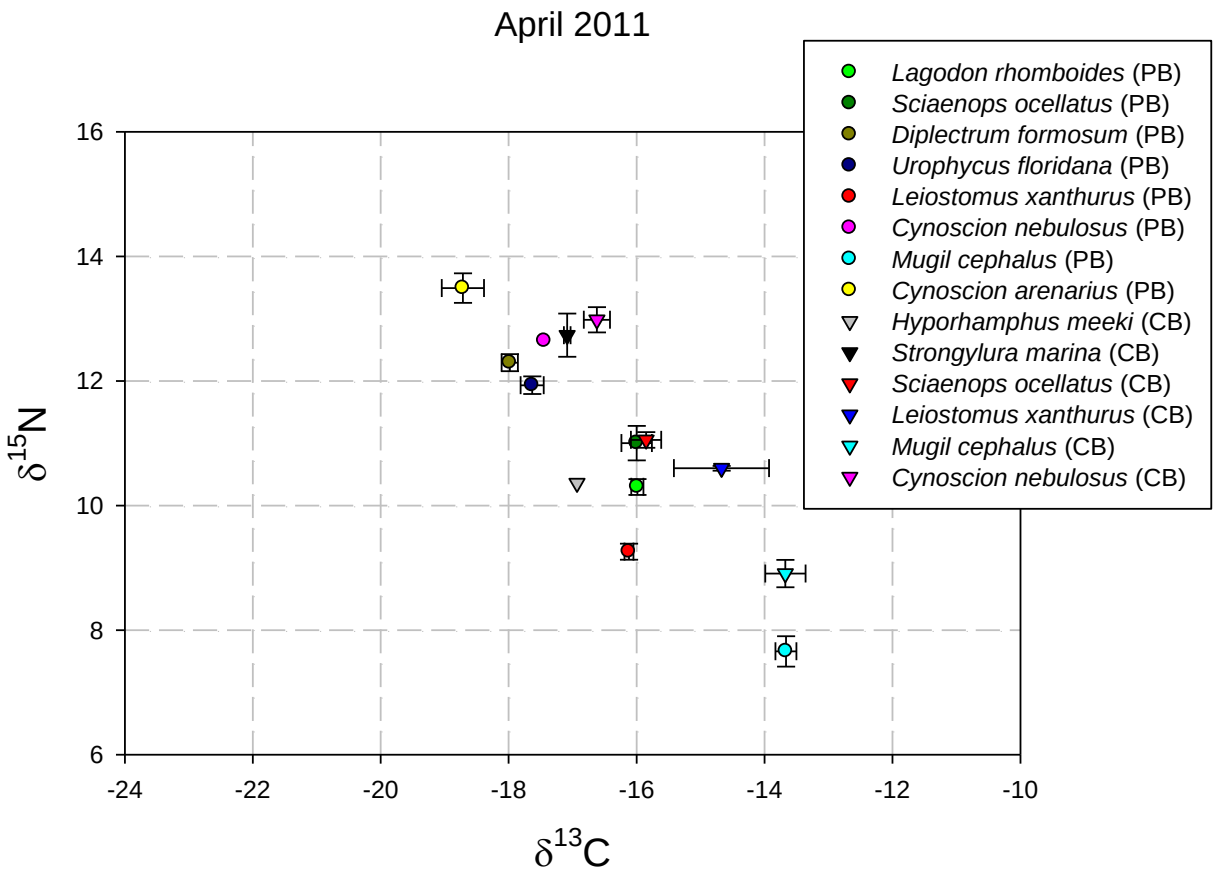

Figure 2.6: Isotopic signatures (‰) of fish collected during April 2011 over all regions. Fish were either collected in Pensacola Bay (PB) or Choctawhatchee Bay (CB). All values are mean ± SD. Common names are outlined in Table 2.3.

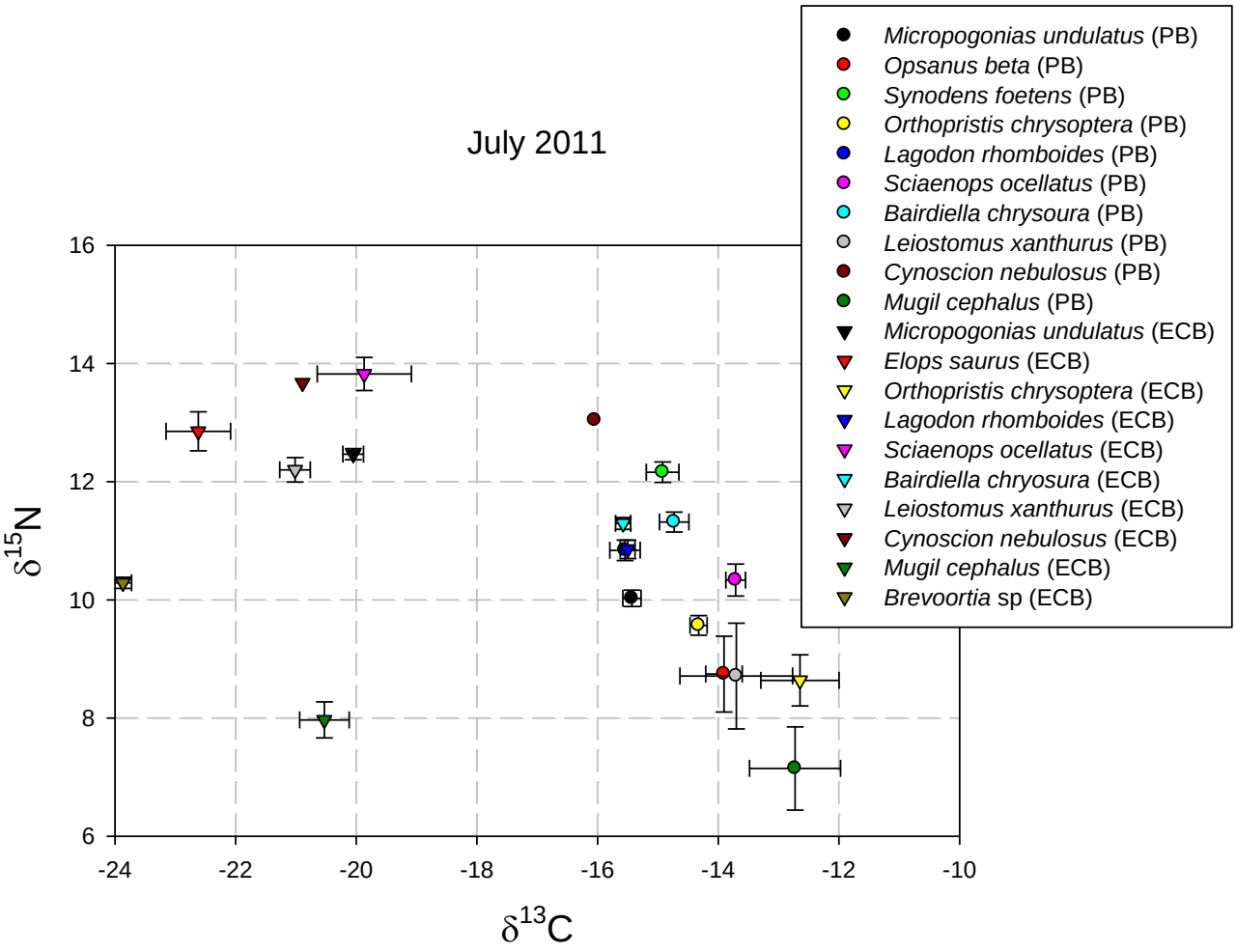

Figure 2.7: Isotopic signatures (‰) of fish collected during July 2011 over all regions. Fish were either collected in Pensacola Bay (PB) or Choctawhatchee Bay (CB). All values are mean ± SD. Common names are outlined in Table 2.3.

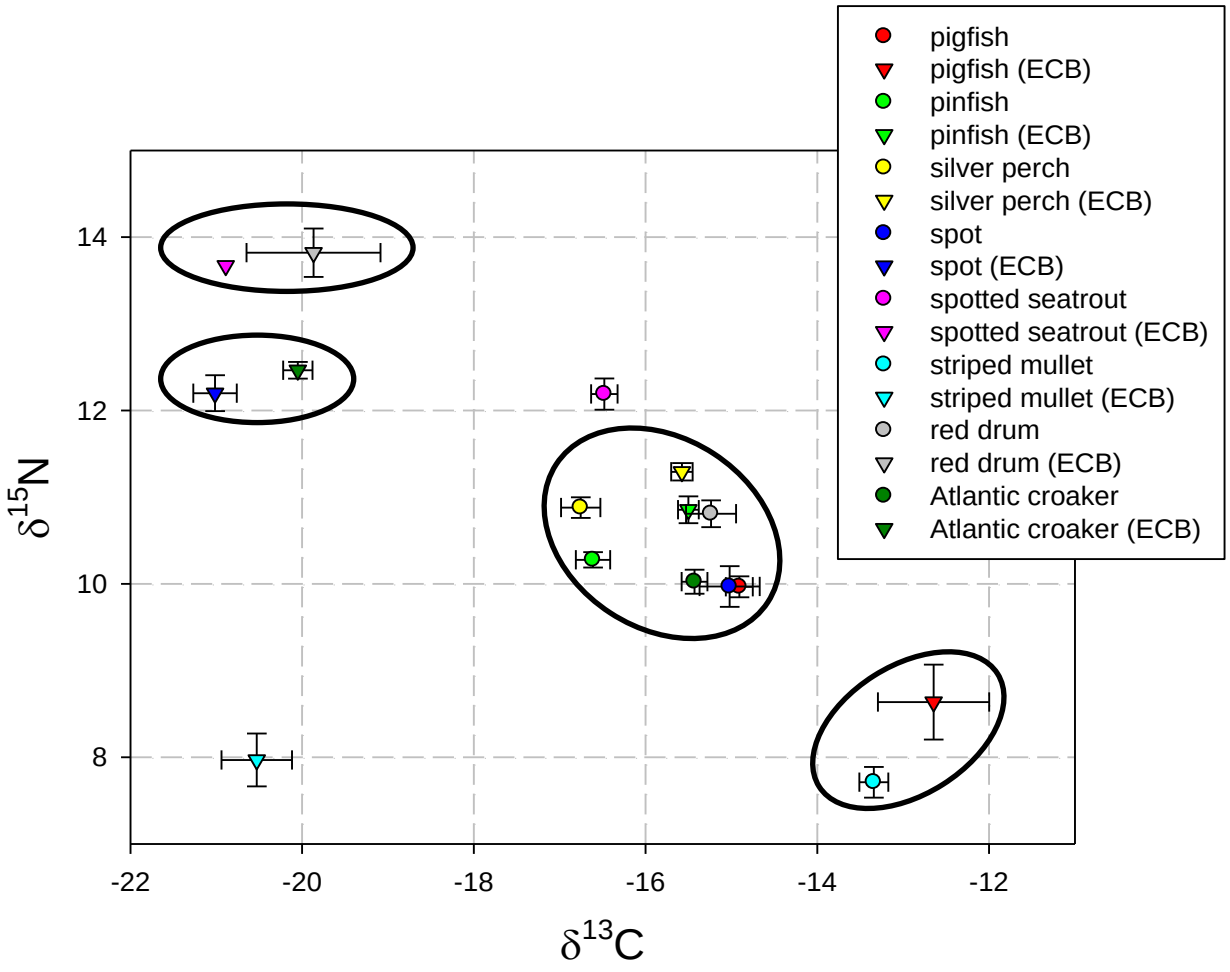

Figure 2.8: Fish data used in SIAR analyses of dolphin diets. All values are mean ± SD. Values are either averaged over all areas or are specific to east Choctawhatchee Bay (ECB). Circled points are not significantly different (see text).

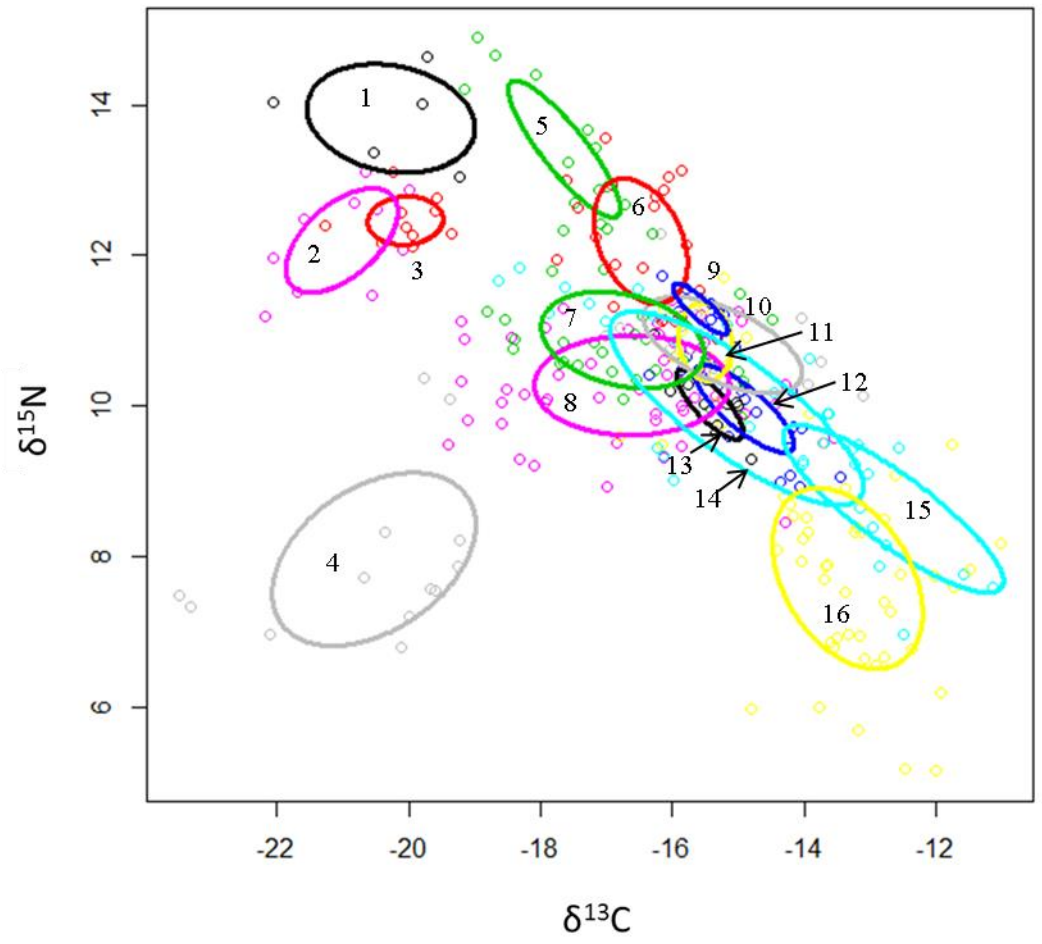

Figure 2.9: Circles enclose the standard ellipse area (SEA) for each fish species, including samples collected in the ECB.

1. ECB red drum, 2. ECB spot, 3. ECB Atlantic croaker, 4. ECB striped mullet, 5. spotted seatrout, 6. ECB silver perch, 7. silver perch, 8. pinfish, 9. ECB silver perch, 10. ECB pinfish, 11. red drum, 12. pigfish, 13. spot, 14. Atlantic croaker, 15. ECB pinfish, and 16. striped mullet.

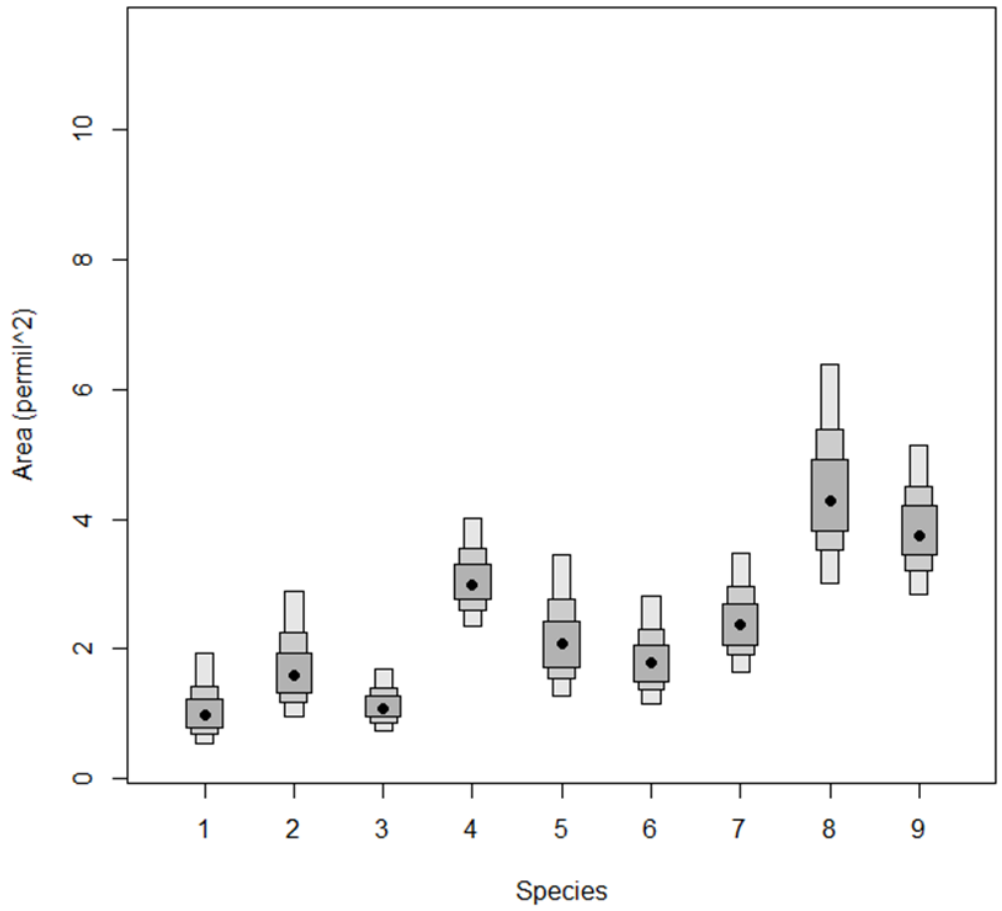

Figure 2.10. Density plot showing the confidence intervals for the standard ellipse areas. The black points correspond to the mean SEA for each fish species while the grey and white boxed areas reflect the 95, 75 and 50% confidence intervals, respectively.

Species are: 1. Atlantic needlefish, 2. pigfish, 3. pinfish, 4. silver perch, 5. Spot, 6. seatrout, 7. striped mullet, 8. red drum, and 9. Atlantic croaker.

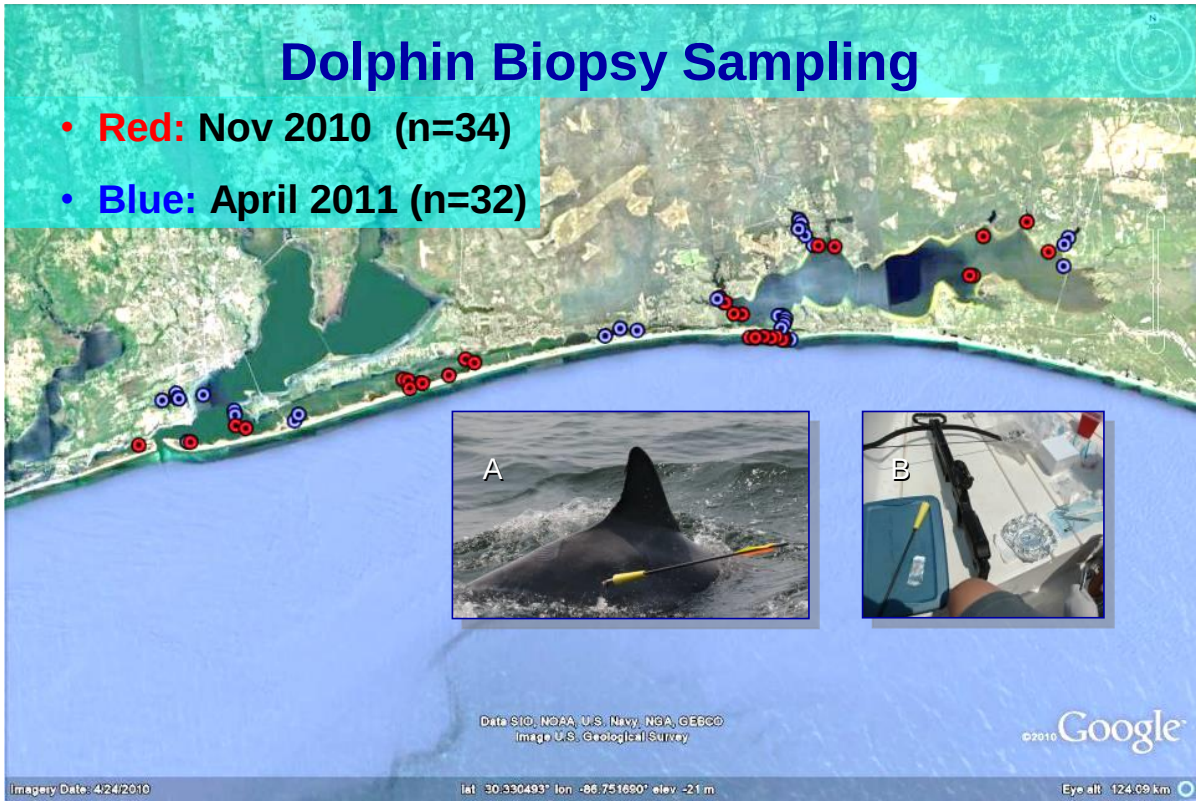

Figure 2.11: Remote biopsy darting sample locations for November 2010 and April 2011. (A) shows dart reflecting off dolphin; (B) shows crossbow and sample preparation area on boat.

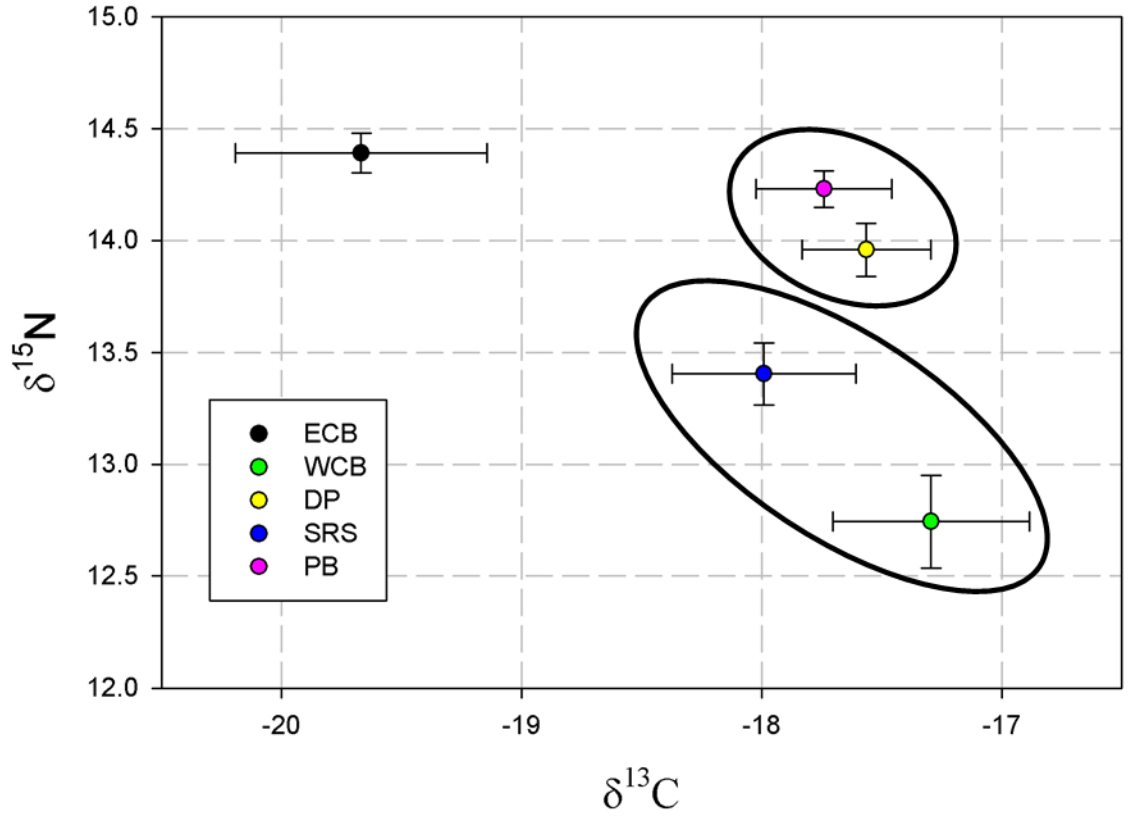

Figure 2.12. Isotopic signatures (‰) of dolphins collected during November 2010 over all regions. Dolphins were sampled in Pensacola Bay (PB), Santa Rosa Sound (SRS), Destin Pass (DP) or east (ECB) or west Choctawhatchee Bay (WCB). All values are mean ± SD. Circled points are not significantly different (see text).

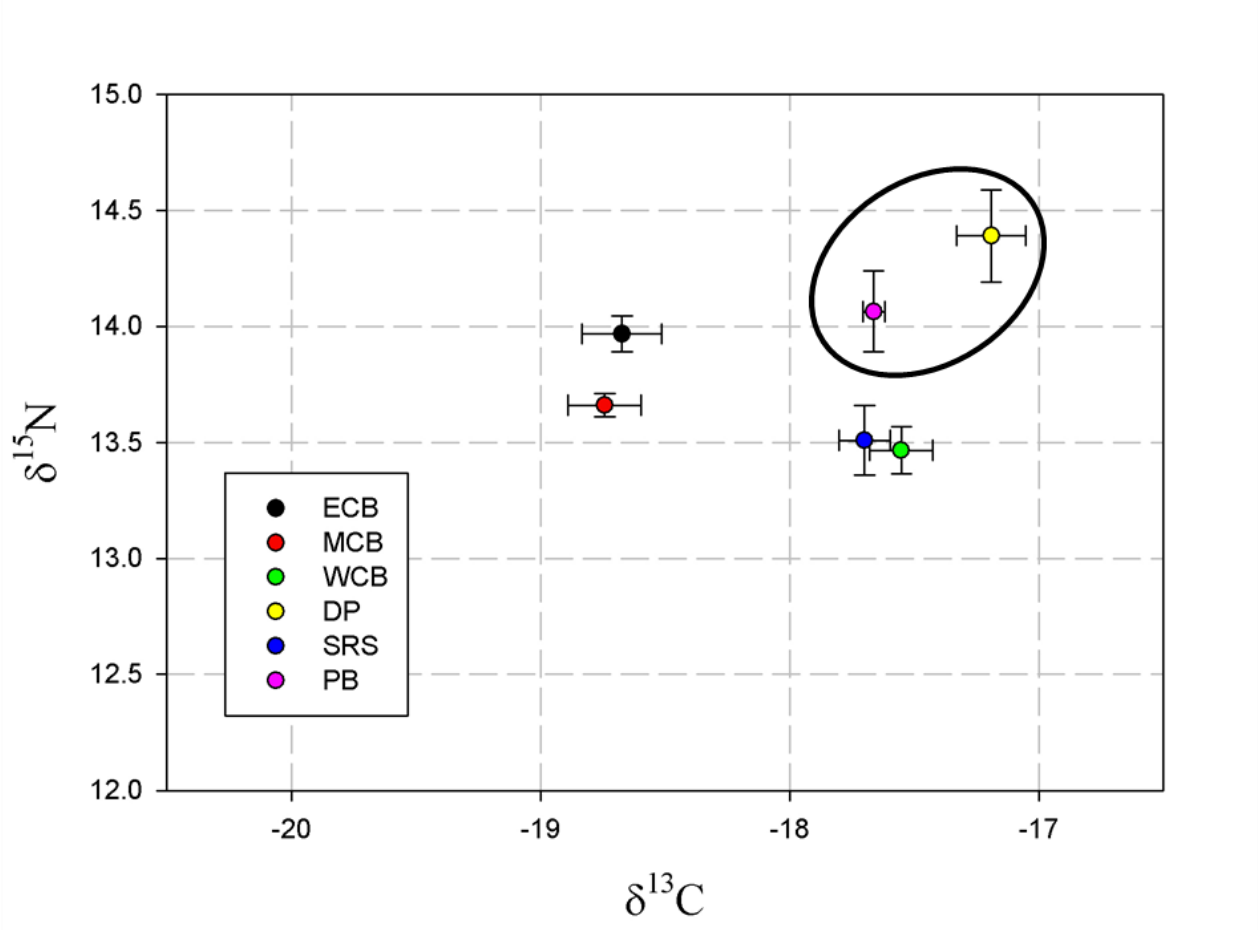

Figure 2.13. Isotopic signatures (‰) of dolphins collected during April 2011 over all regions. Dolphins were sampled in Pensacola Bay (PB), Santa Rosa Sound (SRS), Destin Pass (DP) or east (ECB), mid- (MCB) or west Choctawhatchee Bay (WCB). All values are mean ± SD. Circled points are not significantly different (see text).

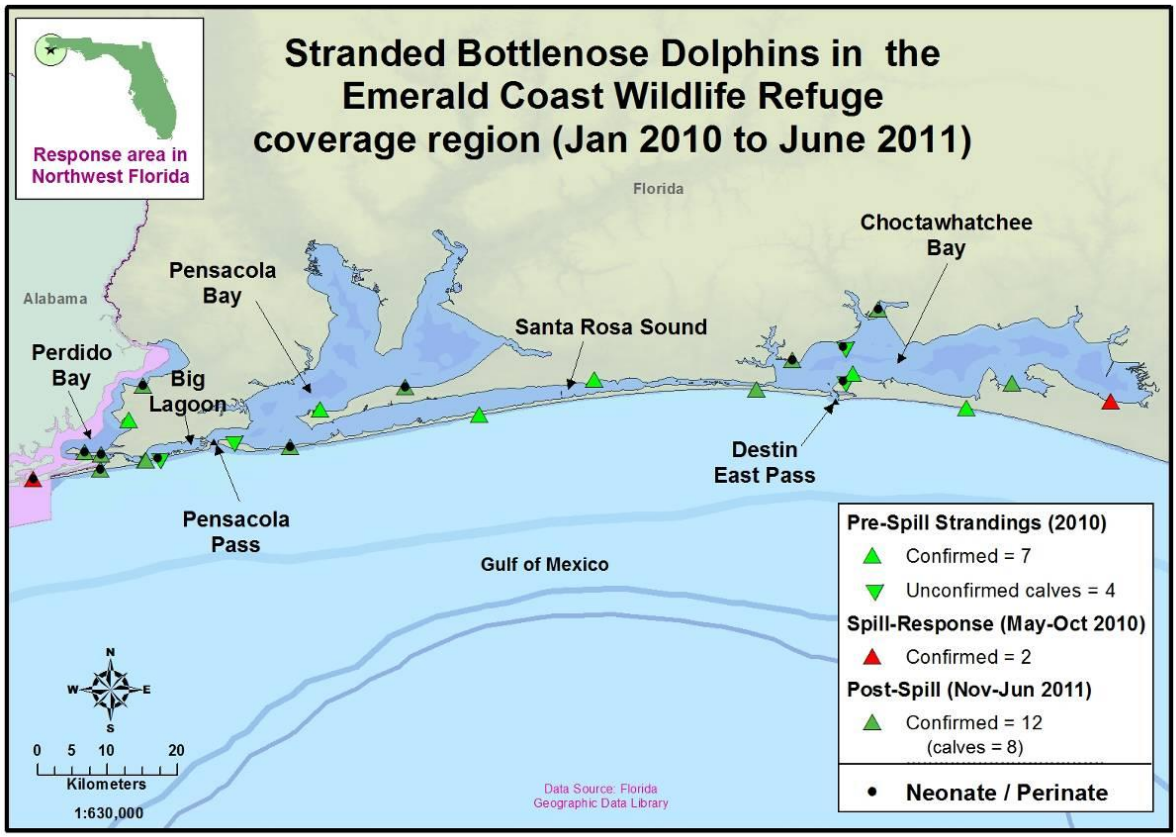

Figure 2.14. Stranded dolphins recovered in the NW Florida region by ECWR between 2010 and 2011 (see Table 2.13 for stranding details).

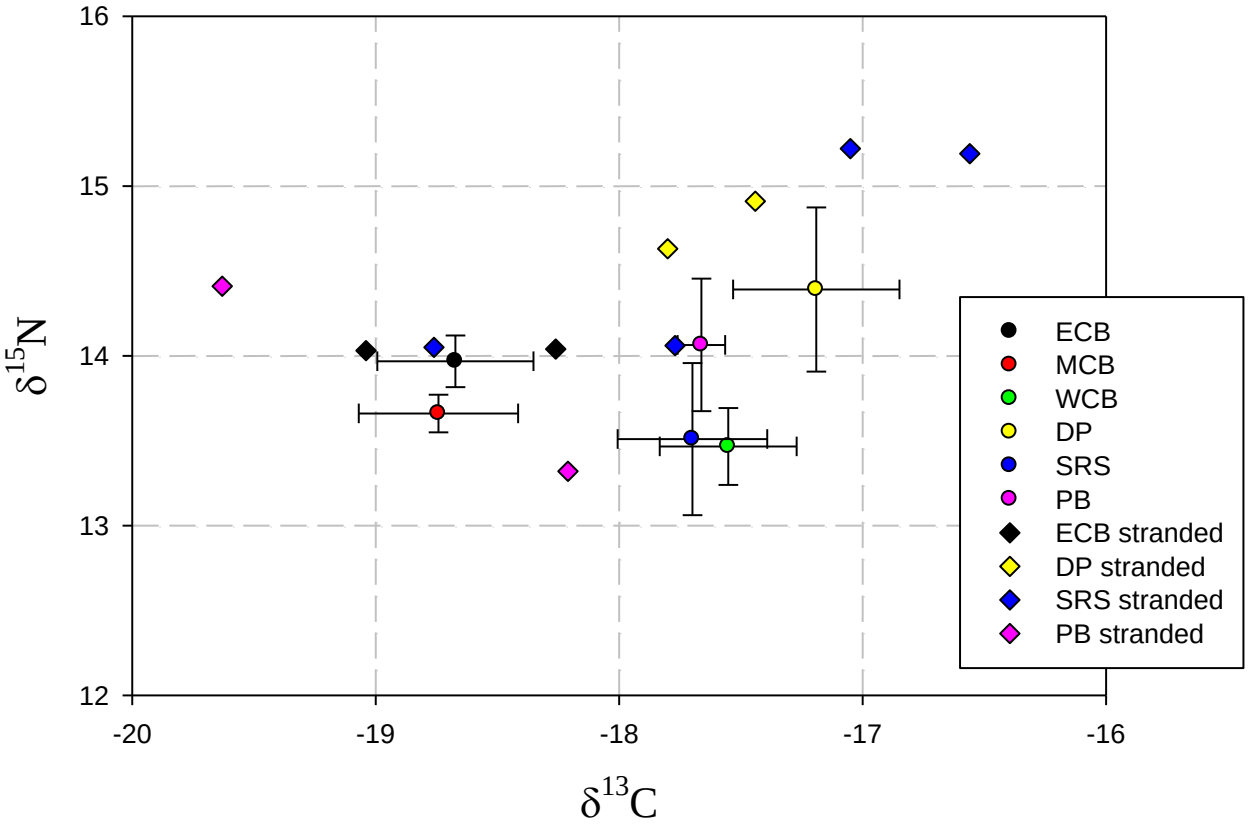

Figure 2.15. Isotopic signatures (‰) of stranded dolphins alongside data for biopsied dolphins collected during April 2011. Stranded dolphins were tentatively assigned to a region based on stranding location (Table 2.13, Figure 2.14). Recognizing that dead dolphins might drift prior to discovery or that sick dolphins could swim away from their normal environs means that these group assignments are speculative. Live dolphins were remote dart biopsy sampled in Pensacola Bay (PB), Santa Rosa Sound (SRS), Destin Pass (DP), east Choctawhatchee Bay (ECB), mid-Choctawhatchee Bay (MCB) or west Choctawhatchee Bay (WCB). All values are mean ± SD.

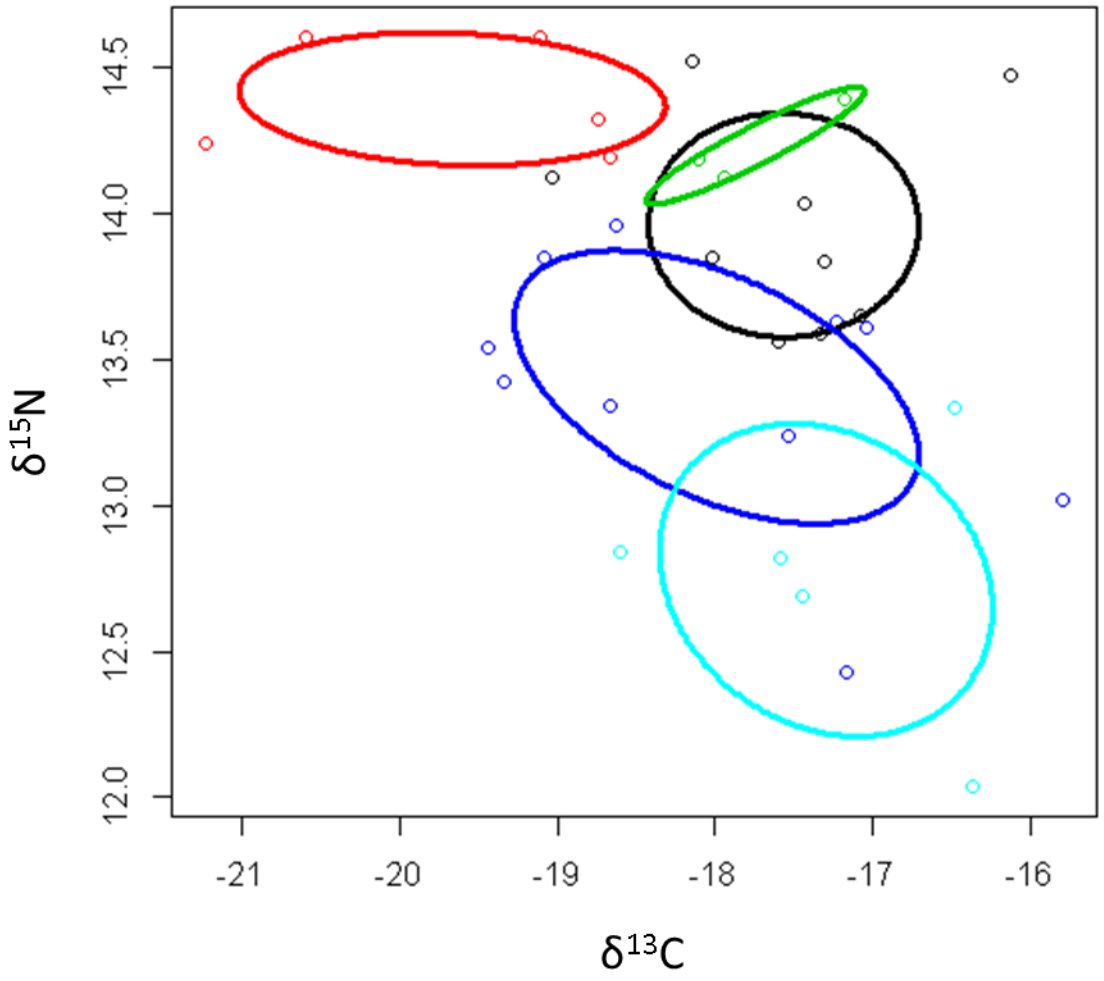

Figure 2.16: Circles enclose the standard ellipse area (SEA) for November dolphins indicating ecological niche area.

red=ECB, purple = SRS, cyan = WCB, green = LPB, black = Destin

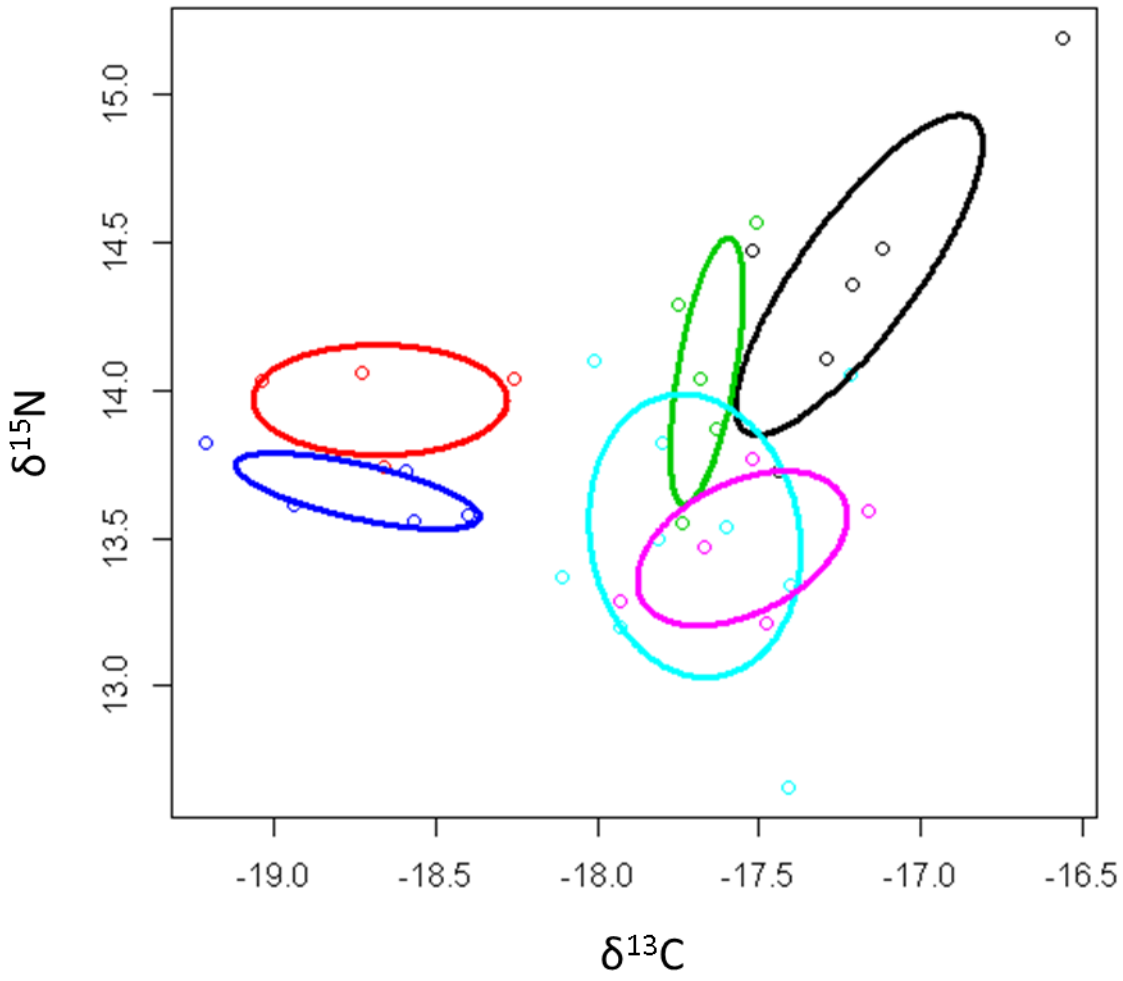

Figure 2.17: Circles enclose the standard ellipse area (SEA) for April dolphins indicating ecological niche area.

red=ECB, purple = MCB, cyan = WCB, pink = SRS, green = LPB, black = Destin

Table 2.1. List of species collected in Choctawhatchee Bay with total number of individual fish collected and their standard lengths (SL) for the four sampling periods.

Sampling Period	Species	Number Collected	Avg SL (mm)	SL (min)	SL (max)
11/23/2010	*Lagodon rhomboides*	404	100.7	70	171
11/23/2010	*Eucinostomus gula*	80	77.6	62	98
11/23/2010	*Cynoscion nebulosus*	42	177.5	64	375
11/23/2010	*Strongylura marina*	35	417.8	350	472
11/23/2010	*Bairdiella chrysoura*	31	94.3	70	133
11/23/2010	*Archosargus probatocephalus*	16	198.0	52	360
11/23/2010	*Ariopsis felis*	13	330.8	300	383
11/23/2010	*Eucinostomus harengulus*	8	93.5	92	95
11/23/2010	*Sphoeroides nephalus*	8	190.9	163	216
11/23/2010	*Orthopristis chrysoptera*	7	108.7	95	125
11/23/2010	*Farfantepenaeus duorarum*	6	20.7	17	26
11/23/2010	*Callinectes sapidus*	5	138.5	119	155
11/23/2010	*Dasyatis sabina*	4	159.3	144	172
11/23/2010	*Leiostomus xanthurus*	4	79.8	76	83
11/23/2010	*Sciaenops ocellatus*	4	301.0	290	312
11/23/2010	*Chilomycterus schoepfii*	3	229.7	210	259
11/23/2010	*Sphyraena barracuda*	3	234.3	179	332
11/23/2010	*Chasmodes saburrae*	2	59.5	49	70
11/23/2010	*Opsanus beta*	2	101.0	80	122
11/23/2010	*Achirus lineatus*	1	43.0	43	43
11/23/2010	*Centropristis striata*	1	78.0	78	78
11/23/2010	*Citharichthys macrops*	1	63.0	63	63
11/23/2010	*Diplectrum formosum*	1	78.0	78	78
11/23/2010	*Eucinostomus spp.*	1	57.0	57	57
11/23/2010	*Paralichthys abligutta*	1	242.0	242	242
11/23/2010	*Trachinotus carolinus*	1	387.0	387	387
2/9/2011	*Lagodon rhomboides*	107	80.1	58	101
2/9/2011	*Cynoscion nebulosus*	4	415.8	365	451
2/9/2011	*Paralichthys abligutta*	1	270.0	270	270
2/9/2011	*Prionotus tribulus*	1	214.0	214	214
4/20/2011	*Lagodon rhomboides*	204	110.9	65	187
4/20/2011	*Sciaenops ocellatus*	57	197.4	93	510
4/20/2011	*Callinectes sapidus*	16	116.9	46	174
4/20/2011	*Leiostomus xanthurus*	10	299.5	148	413
4/20/2011	*Mugil cephalus*	8	331.4	148	413
4/20/2011	*Eucinostomus spp.*	6	127.3	113	135
4/20/2011	*Ariopsis felis*	4	336.5	255	383

4/20/2011	*Cynoscion nebulosus*	4	211.8	140	350
4/20/2011	*Archosargus probatocephalus*	2	273.0	242	304
4/20/2011	*Strongylura marina*	2	455.0	440	470
4/20/2011	*Chasmodes saburrae*	1	62.0	62	62
4/20/2011	*Chilomycterus schoepfii*	1	104.0	104	104
4/20/2011	*Dasyatis americana*	1	689.0	689	689
4/20/2011	*Dasyatis sabina*	1	196.0	196	196
4/20/2011	*Farfantepenaeus duorarum*	1	23.0	23	23
4/20/2011	*Hyporhamphus meeki*	1	253.0	253	253
4/20/2011	*Opsanus beta*	1	85.0	85	85
4/20/2011	*Sphoeroides nephalus*	1	104.0	104	104
7/21/2011	*Lagodon rhomboides*	198	110.2	74	180
7/21/2011	*Micropogonias undulatus*	73	171.3	140	205
7/21/2011	*Brevoortia spp.*	68	75.7	67	95
7/21/2011	*Bairdiella chrysoura*	30	129.8	112	145
7/21/2011	*Mugil cephalus*	29	208.2	120	366
7/21/2011	*Elops saurus*	26	263.8	85	404
7/21/2011	*Sciaenops ocellatus*	23	269.0	172	546
7/21/2011	*Leiostomus xanthurus*	12	137.9	107	165
7/21/2011	*Orthopristis chrysoptera*	7	145.9	95	191
7/21/2011	*Archosargus probatocephalus*	5	335.6	300	379
7/21/2011	*Ariopsis felis*	3	310.3	299	322
7/21/2011	*Synodus foetens*	3	219.0	215	223
7/21/2011	*Callinectes sapidus*	1	104.0	104	104
7/21/2011	*Cynoscion nebulosus*	1	138.0	138	138

Table 2.2. List of species collected in Pensacola Bay with total number of individual fish collected and their standard lengths (SL - mm) for the four sampling periods.

Sampling Period	Species	Number Collected	SL (mean)	SL (min)	SL (max)
11/24/2010	*Lagodon rhomboides*	804	80.1	64	112
11/24/2010	*Bairdiella chrysoura*	136	97.6	78	133
11/24/2010	*Leiostomus xanthurus*	54	81.0	72	117
11/24/2010	*Strongylura marina*	54	418.5	342	529
11/24/2010	*Sciaenops ocellatus*	41	272.0	165	545
11/24/2010	*Orthopristis chrysoptera*	36	89.6	79	111
11/24/2010	*Cynoscion nebulosus*	30	160.6	53	448
11/24/2010	*Mugil cephalus*	25	224.1	95	405
11/24/2010	*Opsanus beta*	9	124.9	97	172
11/24/2010	*Dasyatis sabina*	7	239.9	168	302
11/24/2010	*Archosargus probatocephalus*	6	270.7	75	385
11/24/2010	*Eucinostomus harengulus*	5	100.0	93	110
11/24/2010	*Callinectes sapidus*	4	102.0	48	143
11/24/2010	*Peprilus burti*	3	41.0	30	48
11/24/2010	*Mugil curema*	2	154.5	142	167
11/24/2010	*Achirus lineatus*	1	44.0	44	44
11/24/2010	*Chilomycterus schoepfii*	1	115.0	50	180
11/24/2010	*Dasyatis say*	1	248.0	248	248
11/24/2010	*Eucinostomus gula*	1	65.0	65	65
11/24/2010	*Farfantepenaeus duorarum*	1	16.0	16	16
11/24/2010	*Sphyraena barracuda*	1	310.0	310	310
11/24/2010	*Sphoeroides nephalus*	1	162.0	162	162
2/8/2011	*Archosargus probatocephalus*	9	374.9	337	460
2/8/2011	*Cynoscion nebulosus*	4	527.5	485	594
2/8/2011	*Sciaenops ocellatus*	2	793.5	742	845
2/8/2011	*Mugil cephalus*	1	362.0	362	362
2/8/2011	*Mugil curema*	1	155.0	155	155
4/19/2011	*Lagodon rhombiodes*	418	103.2	75	156
4/19/2011	*Fundulus similis*	46	119.0	104	135
4/19/2011	*Sciaenops ocellatus*	46	185.6	109	556
4/19/2011	*Mugil cephalus*	16	197.3	113	342
4/19/2011	*Callinectes sapidus*	7	113.0	72	155
4/19/2011	*Archosargus probatocephalus*	3	267.7	262	276
4/19/2011	*Leiostomus xanthurus*	3	94.0	62	128
4/19/2011	*Dasyatis sabina*	2	278.0	244	312
4/19/2011	*Fundulus grandis*	2	128.5	125	132

4/19/2011	*Ancylopsetta quadrocellata*	1	98.0	98	98
4/19/2011	*Ariopsis felis*	1	305.0	305	305
4/19/2011	*Astroscopus y-graecum*	1	78.0	78	78
4/19/2011	*Chilomycterus schoepfii*	1	194.0	194	194
4/19/2011	*Cynoscion nebulosus*	1	144.0	144	144
4/19/2011	*Rhinoptera bonasus*	1	800.0	800	800
7/20/2011	*Lagodon rhomboides*	142	100.8	72	149
7/20/2011	*Orthopristis chrysoptera*	56	114.0	72	213
7/20/2011	*Callinectes sapidus*	22	93.9	55	166
7/20/2011	*Chilomycterus schoepfii*	19	125.4	50	192
7/20/2011	*Leiostomus xanthurus*	12	107.0	87	197
7/20/2011	*Synodens foetens*	12	197.7	179	223
7/20/2011	*Mugil cephalus*	9	256.1	109	400
7/20/2011	*Sciaenops ocellatus*	9	166.0	127	237
7/20/2011	*Fundulus similis*	6	127.7	120	133
7/20/2011	*Bairdiella chrysoura*	4	127.5	113	135
7/20/2011	*Opsanus beta*	4	156.0	124	178
7/20/2011	*Trachinotus carolinus*	4	90.5	82	100
7/20/2011	*Acanthostracion quadricornis*	2	130.5	129	132
7/20/2011	*Chloroscombrus chrysurus*	2	184.5	180	189
7/20/2011	*Eucinostomus harengulus*	2	131.5	124	139
7/20/2011	*Priontus scitulus*	2	167.5	132	203
7/20/2011	*Sphoeroides nephalus*	2	157.5	105	210
7/20/2011	*Archosargus probatocephalus*	1	260.0	260	260
7/20/2011	*Brevoortia spp.*	1	98.0	98	98
7/20/2011	*Cynoscion nebulosus*	1	361.0	361	361
7/20/2011	*Dasyatis say*	1	452.0	452	452
7/20/2011	*Elops saurus*	1	428.0	428	428
7/20/2011	*Oligoplites saurus*	1	183.0	183	183
7/20/2011	*Rachycentron canadum*	1	224.0	224	224
7/20/2011	*Strongylura marina*	1	523.0	523	523

Table 2.3. Principal species examined in the present study. Species are listed alphabetically within family. Individual fish collected in Choctawhatchee Bay and retained for analyses in each sampling period.

Family	Species	Common Name
Clupeidae	*Brevoortia* spp.	menhaden
Mugilidae	*Mugil cephalus*	striped mullet
Belondiae	*Strongylura marina*	needlefish
Haemulidae	*Orthopristis chrysoptera*	pigfish
Sparidae	*Lagodon rhomboides*	pinfish
Sciaenidae	*Bairdiella chrysoura*	silver perch
	Cynoscion nebulosus	spotted seatrout
	Leiostomus xanthurus	spot
	Micropogonias undulatus	Atlantic croaker
	Sciaenops ocellatus	red drum

Sampling Period	Species	Number Sampled	Total Sampled
11/23/2010	*Bairdiella chrysoura*	23	
11/24/2010	*Cynoscion nebulosus*	28	
11/25/2010	*Eucinostomus gula*	25	
11/26/2010	*Lagodon rhomboides*	22	
11/27/2010	*Orthopristis chrysoptera*	7	
11/28/2010	*Strongylura marina*	20	125
2/9/2011	*Cynoscion nebulosus*	4	
2/9/2011	*Lagodon rhomboides*	40	
2/9/2011	*Paralichthys abligutta*	1	45
4/20/2011	*Cynoscion nebulosus*	4	
4/20/2011	*Eucinostomous spp.*	6	
4/20/2011	*Hyporhamphus meeki*	1	
4/20/2011	*Lagodon rhomboides*	20	
4/20/2011	*Leiostomus xanthurus*	10	
4/20/2011	*Mugil cephalus*	8	
4/20/2011	*Sciaenops ocellatus*	22	
4/20/2011	*Strongylura marina*	29	73
7/21/2011	*Archosargus probatocephalus*	5	
7/21/2011	*Bairdiella chrysoura*	20	
7/21/2011	*Brevoortia spp.*	30	
7/21/2011	*Cynoscion nebulosus*	1	
7/21/2011	*Elops saurus*	25	
7/21/2011	*Lagodon rhomboides*	40	

7/21/2011	*Leiostomus xanthurus*	12	
7/21/2011	*Micropogonias undulatus*	20	
7/21/2011	*Mugil cephalus*	25	
7/21/2011	*Orthopristis chrysoptera*	7	
7/21/2011	*Sciaenops ocellatus*	23	
7/21/2011	*Synodus foetens*	3	211

Table 2.4. Individual fish collected in Pensacola Bay and retained for analyses in each sampling period.

Sampling Period	Species	Number Sampled	Total Sampled
11/24/2010	*Bairdiella chrysoura*	25	
11/24/2010	*Cynoscion nebulosus*	21	
11/24/2010	*Lagodon rhomboides*	22	
11/24/2010	*Leiostomus xanthurus*	23	
11/24/2010	*Mugil cephalus*	20	
11/24/2010	*Mugil curema*	1	
11/24/2010	*Orthopristis chrysoptera*	25	
11/25/2010	*Strongylura marina*	20	157
2/8/2011	*Cynoscion nebulosus*	4	
2/8/2011	*Archosargus probatocephalus*	9	
2/8/2011	*Mugil cephalus*	1	
2/8/2011	*Mugil curema*	1	15
4/19/2011	*Cynoscion nebulosus*	1	
4/19/2011	*Lagodon rhomboides*	21	
4/19/2011	*Mugil cephalus*	16	
4/19/2011	*Sciaenops ocellatus*	21	59
7/20/2011	*Archosargus probatocephalus*	1	
7/20/2011	*Bairdiella chrysoura*	4	
7/20/2011	*Brevoortia spp.*	1	
7/20/2011	*Chloroscombrus chrysurus*	2	
7/20/2011	*Cynoscion nebulosus*	1	
7/20/2011	*Elops saurus*	1	
7/20/2011	*Eucinostomus harengulus*	2	
7/20/2011	*Lagodon rhomboides*	21	
7/20/2011	*Leiostomus xanthurus*	12	
7/20/2011	*Mugil cephalus*	1	
7/20/2011	*Oligoplites saurus*	1	
7/20/2011	*Opsanus beta*	4	
7/20/2011	*Orthopristis chrysoptera*	23	
7/20/2011	*Rachycentron canadum*	1	
7/20/2011	*Sciaenops ocellatus*	9	
7/20/2011	*Strongylura marina*	1	
7/20/2011	*Synodus foetens*	12	
7/20/2011	*Trachinotus carolinus*	4	109

Table 2.5: Species collected and retained for analyses from Santa Rosa Sound with total number of individual fish collected and their standard lengths (SL - mm).

Sampling Period	Species	Number Collected	SL (mean)	SL (min)	SL (max)
3/5/2011	*Lagodon rhomboides*	6	86.5	80	94
3/5/2011	*Orthopristis chrysoptera*	4	115.0	97	126
3/5/2011	*Leiostomus xanthurus*	53	126.1	105	153
3/5/2011	*Diplectrum formosum*	5	107.8	96	120
3/5/2011	*Peprilus burti*	9	92.3	85	105
3/5/2011	*Lolliguncula brevis*	17	108.9	65	180
3/5/2011	*Doryteuthis plei*	14	127.1	98	160
3/5/2011	*Farfantepenaeus aztecus*	4	135.3	120	156
3/5/2011	*Etroglus crossotus*	5	96.0	87	102
3/5/2011	*Cynoscion arenarius*	5	201.6	184	236
3/5/2011	*Urophycis floridana*	15	137.6	85	180
3/5/2011	*Ariopsis felis*	2	337.0	314	360
5/5/2011	*Mugil cephalus*	3	291.7	230	330
5/5/2011	*Mugil curema*	5	218.2	210	221
7/14/2011	*Micropogonias undulatus*	16	218.1	149	235
7/14/2011	*Lagodon rhomboides*	3	145.3	137	151
7/19/2011	*Orthopristis chrysoptera*	4	183.0	176	189

Table 2.6: Isotopic values of fish ($\delta^{15}N$, $\delta^{13}C$, n) collected in Pensacola and Choctawhatchee Bays, 2010-2011. Fish samples collected in Choctawhatchee Bay during were collected in the eastern portion of the Bay (ECB) whereas all other samples were collected in the western area (WCB).

	November 2010		February 2011		April 2011		July 2011	
Species	**Pensacola**	**Choctawhatchee**	**Pensacola**	**Choctawhatchee**	**Pensacola**	**Choctawhatchee**	**Pensacola**	**Choctawhatchee**
Atlantic needlefish	13.68 ± 1.02 -17.94 ± 0.85 (5)	13.78 ± 0.68 -17.91 ± 0.93 (4)				12.73 ± 0.60 -17.08 ± 0.09 (3)	12.67 -16.73 (1)	
pigfish	10.24 ± 0.37 -15.54 ± 0.51 (6)	10.23 ± 0.37 -15.06 ± 0.68 (4)	10.61 ± 0.07 -15.61 ± 0.23 (2)				9.57 ± 0.53 -14.33 ± 0.45 (10)	8.64 ± 0.97 -12.65 ± 1.45 (5)
pinfish	9.76 ± 0.64 -15.54 ± 0.94 (12)	9.82 ± 0.38 -18.42 ± 0.70 (10)	10 -15.87 (1)	10.75 ± 0.39 -17.96 ± 1.07 (10)	10.30 ± 0.40 -15.99 ± 0.30 (10)		10.84 ± 0.57 -15.99 ± 0.83 (11)	10.86 ±0.49 -15.50 ± 0.38 (10)
silver perch	10.79 ± 0.28 -17.53 ± 0.78 (14)	10.91 ± 0.91 -16.19 ± 1.01 (12)	12.67 -18.18 (1)				11.32 ± 0.24 -14.73 ± 0.34 (2)	11.29 ± 0.32 -15.58 ± 0.39 (10)
spot	9.52 ± 0.35 -13.62 ± 0.45 (10)		10.87 ±0.96 -16.85 ± 1.04 (13)			10.60 ± 0.05 -14.67 ± 1.05 (2)	7.85 ± 0.62 -12.77 ± 0.21 (4)	12.20 ± 0.65 -21.02 ±0.80 (10)
spotted seatrout	11.53 ± 0.54 -16.09 ± 0.74 (5)	11.49 ± 0.40 -16.76 ± 0.67 (5)		12.78 ± 0.44 -16.35 ± 0.85 (4)	12.64 -17.44 (1)	12.98 ± 0.41 -16.62 ± 0.41 (4)	13.04 -16.05 (1)	13.67 -20.89 (1)
striped mullet	7.05 ± 0.77 -13.09 ± 0.69 (14)		9.48 -11.75 (1)		7.66 ± 0.91 -13.67 ± 0.61 (14)	8.71 ± 0.82 -13.72 ± 1.37 (11)	7.17 ± 1.93 -12.62 ± 2.04 (5)	7.97 ± 1.10 -20.53 ± 1.48 (13)
white mullet	8.91 ± 1.12 -15.09 ± 1.33 (2)		8.33 -15.5 (1)		7.67 ± 1.35 -13.54 ± 1.00 (5)			
white trout			13.10 ± 0.72 -18.88 ± 0.79 (4)					
mojarra		8.89 ± 0.51 -12.43 ± 1.16 (5)				10.28 ± 0.55 -13.60 ± 1.18 (4)		
red drum					11.00 ± 0.68 -16.00 ± 0.59 (6)	11.05 ± 0.28 -15.85 ± 0.53 (5)	10.33 ± 0.60 -13.71 ± 0.36 (5)	13.82 ± 0.63 -19.87 ± 1.75 (5)
Atlantic croaker			11.93 -19.84 (1)				10.03 ± 0.44 -15.43 ± 0.48 (10)	12.46 ± 0.30 -20.05 ± 0.54 (10)
cobia							9.48 -16.82 (1)	
American halfbeak						10.36 -16.93 (1)		

Table 2.7: Isotopic values ($\delta^{15}N$, $\delta^{13}C$, n) of fish collected in Santa Rosa Sound during March 2010.

Species	**Santa Rosa Sound**
bay squid	12.87 ± 0.47 -18.42 ± 0.59 (5)
narrow squid	12.53 ± 0.24 -17.71 ± 0.15 (7)
brown shrimp	8.96 ± 0.20 -18.55 ± 2.53 (2)
Gulf butterfish	12.31 ± 0.18 -19.10 ± 1.00 (5)
hardhead catfish	13.02 -18.08 (1)
inshore lizardfish	12.05 -15.70 (1)
sand perch	12.17 ± 0.14 -17.92 ± 0.27 (3)
southern hake	11.93 ± 0.32 -17.63 ± 0.41 (5)

Table 2.8: Isotopic values of fish ($\delta^{15}N$, $\delta^{13}C$, n) collected in Pensacola and Choctawhatchee bays during July 2011.

Species	**Pensacola Bay**	**Choctawhatchee Bay**
Florida pompano	6.27 ± 0.31 -12.45 ± 0.24 (2)	
Gulf toadfish	8.74 ± 1.11 -13.90 ± 0.52 (3)	
inshore lizardfish	12.16 ± 0.39 -14.92 ± 0.60 (5)	10.85 ± 0.33 -14.32 ± 0.43 (3)
ladyfish	11.54 -15.58 (1)	12.85 ± 0.81 -22.62 ± 1.31 (6)
leatherjacket	12.46 -17.55 (1)	
longnose killifish	8.44 ± 0.12 -11.60 ± 0.54 (5)	
Tidewater mojarra	11.21 ± 1.45 -15.62 ± 0.58 (2)	10.29 ± 0.25 -24.34 ± 0.02 (3)
sheepshead	10.74 -17.72 (1)	12.57 ± 0.05 -25.78 ± 0.43 (2)
yellowfin menhaden		10.31 ± 0.34 -23.81 ± 0.43 (7)

Table 2.9: Comparisons of pooled isotopic data for all species versus ECB samples (July). Data are presented in Figure 2.8 and groups with similar letters are not significantly different from each other (Tukey's post-hoc test).

	$\delta^{13}C$	$\delta^{15}N$
spot (ECB)	a	d
striped mullet (ECB)	a	a
Atlantic croaker (ECB)	a	d, e
red drum (ECB)	a	f
Atlantic needlefish	b	e, f
silver perch	b, c	b, c
pinfish	b, c, d	b, c
spotted seatrout	b, c, d	d
silver perch (ECB)	c, d	c, d
pinfish (ECB)	c, d	b, c
Atlantic croaker	c, d	b
red drum	c, d	b, c
spot	d, e	b
pigfish	d, e	b
striped mullet	e, f	a
pigfish (ECB)	f	a

Table 2.10. Proportions of bottlenose dolphins sampled by remote dart biopsy sampling as a function of season and location.

Location	Total	Fall	% Fall	Spring	% Spring
East CBAY (ECB)	9	6	9.1%	3	4.5%
Destin (DP)	17	10	15.2%	7	10.6%
West CBAY (WCB	15	6	9.1%	9	13.6%
East SRS (SRS)	3	0	0.0%	3	4.5%
West SRS (SRS)	14	10	15.2%	4	6.1%
Pensacola Bay (PB)	8	2	3.0%	6	9.1%
TOTAL	**66**	**34**	**51.5%**	**32**	**48.5%**

Table 2.11. Sample summary and resighting histories of dolphins used in stable isotope analysis.

Sighting Frequency	Dolphin Count	% of total
1 time	18	24.7%
2 times	11	15.1%
3 times	19	26.0%
4 times	5	6.8%
5 times	7	9.6%
6 times	5	6.8%
7 times	1	1.4%
8 times	1	1.4%
9 times	4	5.5%
11 times	1	1.4%
14 times	1	1.4%
Total Sightings	**73**	

Resighting Summary	Number of dolphins	% of total
# seen >1 time	55	75.3%
# seen >2 times	43	58.9%
# seen >3 times	25	34.2%

# Individual dolphins	63
# Dart biopsy samples	64
# Stranded samples	13
Total # samples	**77**
Adult and Subadults	69
Yearlings	3
Perinates	5

Table 2.12

a) Dolphin isotope comparisons between regions in each of the collection periods. There were significant differences between groups in each of November 2010 ($\delta^{15}N$ - $F_{(4,32)}=16.34$ ($p<0.0001$)), $\delta^{13}C$ - $F_{(4,32)}=4.468$ ($p<0.007$) and April 2011($\delta^{15}N$ - $F_{(5,34)}=5.983$ ($p<0.001$), $\delta^{13}C$ - $F_{(5,34)}=23.343$ ($p<0.0001$)). Data are plotted graphically in Figures 2.12 and 2.13.

Group	November		April	
	$\delta^{15}N$	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**
ECB	14.4 + 0.2 (5)	-19.7 + 1.2 (5)	14.0 + 0.2 (3)	-18.7 + 0.3 (3)
MCB			13.7 + 0.1 (5)	-18.7 + 0.3 (5)
WCB	12.7 + 0.5 (5)	-17.3 + 0.9 (5)	13.5 + 0.2 (5)	-17.6 + 0.3 (5)
DP	14.0 + 0.4 (9)	-17.6 + 0.8 (9)	14.4 + 0.5 (5)	-17.2 + 0.3 (5)
SRS	13.4 + 0.4 (10)	-18.0 + 1.2 (10)	13.5 + 0.5 (9)	-17.7 + 0.3 (9)
PB	14.2 + 0.1 (3)	-17.7 + 0.5 (3)	14.1 + 0.4 (5)	-17.7 + 0.1 (5)

b) Tukeys post-hoc tests revealed that in November, $\delta^{13}C$ values of ECB dolphins differed from all other groups except SRS, that $\delta^{15}N$ values of WCB and SRS were similar, that DP and SRS were the same, and that there were no significant differences between DP and PB. In April, ECB and MCB separated from all other dolphins in terms of $\delta^{13}C$ values while only DP differed in $\delta^{15}N$.

	November 2010		April 2011	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
ECB	a	c	a	a, b
MCB			a	a
WCB	b	a	b	a
DP	b	b, c	b	b
SRS	a, b	a, b	b	a
PB	b	c	b	a, b

Table 2.13: Collection information, gender and age class of dolphins recovered through the activities of the ECWR stranding response program. Dolphins were assigned to a tentative dolphin group based on stranding location (Figure 2.14). Isotopic data ($\delta^{15}N$, $\delta^{13}C$) were compared to free-ranging dolphins sampled by remote dart biopsy see Figure 2.15.

Date	ID #	dolphin group	latitude	longitude	$\delta^{13}C$	$\delta^{15}N$	Sex	Age Class
4/23/2009	FLGM042309-06	PB	30.4635	-87.1622	-18.21	13.32	M	adult
3/3/2010	ECWR030310-04	SRS	30.4078	-86.8199	-18.76	14.05	M	adult
3/8/2010	ECWR030810-05	DP	30.3711	-86.3424	-17.80	14.63	M	adult
3/29/2010	ECWR032910-08	PB	30.3689	-87.1722	-19.63	14.41	F	adult
4/9/2010	ECWR040910-09	DP	30.4153	-86.4884	-17.44	14.91	M	adult
4/14/2010	ECWR041410-10	SRS	30.3626	-86.9684	-17.05	15.22	M	yearling
12/19/2010	ECWR121910-17	SRS	30.3045	-87.3958	-17.18	14.39	M	yearling
1/5/2011	ECWR010511-01	SRS	30.3981	-87.0635	-17.66	16.30	M	calf
1/31/2011	ECWR013111-02	SRS	30.3216	-87.2105	-16.45	17.26	F	calf
3/1/2011	ECWR030111-04	PB	30.2923	-87.4545	-17.85	16.83	M	calf
03/05/11	ECWR030511-05	SRS	30.3956	-86.6124	-16.56	15.19	M	adult
03/17/11	ECWR031711-06	DP	30.4976	-86.4559	-18.01	15.54	M	calf
03/20/11	ECWR032011-07	PB	30.3121	-87.4540	-17.99	14.78	M	calf
04/07/11	ECWR040711-11	SRS	30.3724	-86.9090	-17.77	14.06	F	adult
04/12/11	ECWR041211-12	ECB	30.4028	-86.2841	-19.04	14.03	M	subadult
04/12/11	ECWR041211-13	ECB	30.4027	-86.2840	-18.26	14.04	F	subadult

Table 2.14: Population metrics for dolphins sampled in different regions during November 2010 and April 2011. November samples are representative of summer feeding while April samples represent winter/spring feeding.

Group	Date	SEA	SEA_c	TA	NR	CR	CD	MNND	SDNND
combined	November				2.56	5.44	1.17	0.35	0.25
	April				2.53	2.65	0.68	0.19	0.16
ECB	November	0.72	0.96	0.80	0.41	2.57	1.02	0.44	0.29
	April	0.15	0.23	0.13	0.32	0.78	0.28	0.36	0.08
MCB	April	0.08	0.11	0.10	0.26	0.81	0.28	0.24	0.09
WCB	November	1.37	1.74	1.40	1.29	2.24	0.79	0.76	0.52
	April	0.18	0.24	0.22	0.56	0.77	0.30	0.39	0.04
DST	November	0.90	1.03	1.67	0.96	2.91	0.68	0.52	0.38
	April	0.30	0.37	0.38	1.46	0.96	0.42	0.37	0.28
SRS	November	1.46	1.68	2.79	1.53	3.65	1.13	0.50	0.40
	April	0.43	0.49	0.85	1.44	0.89	0.44	0.35	0.18
LPB	November	0.08	0.16	0.44	0.27	0.92	0.39	0.38	0.37
	April	0.10	0.13	0.11	1.02	0.24	0.30	0.26	0.09

SEA = standard ellipse area; SEA_c = corrected standard ellipse area; TA = area of convex hull; NR = $\delta^{15}N$ range; CR = $\delta^{13}C$ range; CD = mean distance to centroid; MNND = mean nearest neighbor distance; SDNND = standard deviation of mean nearest neighbor distance.