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HABITAT USE AND FEEDING ECOLOGY OF DELPHINIDS INFERRED FROM STABLE
ISOTOPES AND FATTY ACID SIGNATURES

by

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A dissertation submitted in partial fulfillment of the requirements
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ABSTRACT

Prey availability, directly or indirectly, affects all aspects of a predator's life history and is a primary factor influencing habitat selection and movements. This is especially true for delphinid species where it has been documented that the behaviors and movements of dolphins are strongly influenced by food availability. Unfortunately, the feeding ecology and habitat use patterns of many of these species are poorly understood. Many methodologies that have been employed to explore these facets of dolphin ecology have limitations and constraints or are logistically infeasible. Stable isotope and fatty acid signature analyses have been used extensively on a wide variety of species and have been shown to be methodologies that overcome some of these limitations. These approaches can provide information on feeding habits and the geographic origin of the prey thereby giving tremendous insight into habitat usage patterns. The present study applied stable isotope and fatty acid signature methodologies to gain insight into the feeding ecology and habitat usage of various dolphin species to improve upon our understanding of these important facets of their life histories.

The application of stable isotope analysis in ecological studies relies on both species and tissue specific measurements of parameters such as diet-tissue discrimination factors, the difference in stable isotope ratio between a consumer and its prey, and turnover rates, the change in tissue isotopic composition attributable to growth and tissue replacement. Initially, controlled studies were conducted and animals were switched from one isotopically distinct diet to another which allowed for the calculation of these values in bottlenose dolphin skin. Diet-tissue discrimination factors for dolphin skin averaged 2.20‰ for nitrogen and 0.82‰ for carbon. Average turnover rates (expressed in half-lives) in dolphin skin were 17 days for nitrogen and

16.5 days for carbon. The present study represents the first reported diet-tissue discrimination factors and turnover rates for carbon and nitrogen in the skin of any cetacean.

Next, skin samples were collected from net-entangled and free-ranging dolphin species off the coast of South Africa and analyzed for stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios. The Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), the common dolphin (*Delphinus capensis*), the striped dolphin (*Stenella coeruleoalba*), and the humpback dolphin (*Sousa chinensis*) all occur off the southeastern coast of South Africa with overlapping distributions. Isotopic signatures revealed resource partitioning among these four species of dolphins with differences in diets, as well as differences in the use of habitat. Mean values for $\delta^{15}\text{N}$ ranged from $11.92 \pm 0.11\text{‰}$ (n=3) for striped dolphins to $14.95 \pm 0.19\text{‰}$ (n=27) for humpback dolphins, indicating that these species are feeding at different trophic levels. Striped dolphin carbon isotope signatures were consistent with evidence that they typically forage further offshore ($-17.94 \pm 0.14\text{‰}$) and the carbon isotope values of the humpback dolphins reflected their use of inshore habitats by comparison ($-15.16 \pm 0.12\text{‰}$). Common and bottlenose dolphins for nitrogen ($13.66 \pm 0.08\text{‰}$, $14.35 \pm 0.07\text{‰}$ respectively) and carbon ($-15.48 \pm 0.07\text{‰}$, $-15.76 \pm 0.06\text{‰}$ respectively) fell in between these two extremes. Analyses also revealed that males and females have differences in their diets. On average, males were enriched in $\delta^{15}\text{N}$ by 0.74‰ compared to females suggesting some dietary differences in prey composition. Isotopic niche width has been compared to traditional measures of niches used by ecologists and was measured for these South African dolphins. Humpback and bottlenose dolphins had the largest standard elliptical area (SEA), striped dolphins had the smallest SEA, and the SEA for common dolphins was intermediate. Larger SEA values reflect a broader trophic diversity, while smaller SEA values reflect a narrower trophic diversity or a more specialized niche.

Finally, a resident group of bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon (IRL) in east central Florida were sampled and explored for differences in isotopic signatures based on sex, age category, season, and location within the IRL. In addition to stable isotope analysis, fatty acid analysis was also used to compare and contrast the findings between the two techniques. Comparison of stable isotopic signatures revealed differences among age categories and among locations. Fatty acid analysis was able to discern further and found differences in the signatures between male and female dolphins. The combination of both techniques allowed for an extensive examination into the feeding ecology and habitat utilization of these resident dolphins. The Bayesian mixing model (Stable Isotope Analysis in R- SIAR) was validated using controlled study data and was found to be accurate when inputting isotopically distinct prey items (sources). The mixing model was then used to estimate the proportions of prey items that make up the diet of Indian River Lagoon bottlenose dolphins. Two models were run in which dolphins were grouped together (model 1) and dolphins were separated by year (model 2). Results of the model reaffirm stomach content analysis results previously obtained.

Stable isotope techniques were applied to various dolphin species to gain better understanding of their feeding ecology and habitat utilization. Resource partitioning was suggested for four South African dolphin species which gives crucial insight into the ecology of both at-risk and data-deficient species. These discernments will provide much needed data to conservationists and managers and contributes to our general understanding of these species. This is the first study of its kind to undertake controlled diet studies with bottlenose dolphins which determined diet-tissue discrimination values and turnover rates for carbon and nitrogen isotopes in the skin of any cetacean. The current study is also the first of its kind to attempt to

model bottlenose dolphin diet in the Indian River using stable isotopes. Food, being a primary driver for many species, can lend explanation of things like movement patterns, habitat usage, competition, reproductive success, survival, and the spread of diseases, which has been an issue in Indian River dolphins in recent years. Dietary information modeled in this study provided new data for the relative contribution of a suite of potential prey to an apex predator in the Indian River. Data produced through the current study contributes towards a large, unprecedented step forward in understanding dolphin ecology and the roll of cetacean stable isotope ecology.

This dissertation is dedicated to my family who are so proud and supportive and who encouraged and inspired me to work tirelessly to reach this goal. And in memory of the best dad a girl could have. I miss you dad.

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CHAPTER 1: INTRODUCTION

An animal's reproductive success and ability to survive is tightly linked to its habitat and associated resources (Gunnarsson *et al.* 2005). Habitat selection dictates quality of refuge, mate availability, and both quantity and quality of food resources. Prey availability affects many aspects of a predator's life history and is considered a primary factor influencing habitat selection and movements, whether they are small scale movements within a habitat or large scale movements like the migration patterns of many birds and whales (Hobson 1999, Kenney *et al.* 2001, Witteveen *et al.* 2009, Boyle *et al.* 2011, Wasko and Sasa 2012). Boyle *et al.* (2011) explored some of the underlying mechanisms for tropical bird migration and concluded that limitation of food resources is one of the most influential factors in the evolution of the movements of these birds. Similarly, Kenney *et al.* (2001) examined migratory patterns and prey abundance detection of right whales and concluded that the movement of these animals is reflective of prey abundance and distribution. This theory also holds true for delphinid species where it has been documented that the behaviors and movements of dolphins are strongly influenced by food availability (e.g., Shane 1977, Hanson and Defran 1993).

The Family Delphinidae (Order Cetacea) includes 17 genera and over 30 species of dolphins found in all of the world's oceans, intertidal waterways and seas, and even in some rivers and estuaries. Some dolphins travel alone or in small groups whereas others have been observed in pods numbering in the thousands. They range in body size from approximately 1.5 m in length (50 kg) to up to 10 m (7000 kg) and males are typically larger than females. The lifespan of most delphinids averages 25 years, though they tend to live longer in captivity.

Sexual maturity is achieved between five and 13 years of age depending on species and geographic location. Gestation lasts approximately 10 to 12 months and generally results in a single calf. Weaning is typically initiated at approximately one year and calves will start to incorporate some fish into their diet at this point. Weaning is typically complete by 3 – 8 years, although this is highly variable (Mann *et al.* 2000).

Dolphins are considered apex predators and prey primarily on fish, squid, and crustaceans, however, killer whales (*Orcinus orca*) also prey on other marine mammals and birds. Collectively they employ a variety of hunting strategies and, like other odontocetes, dolphins echolocate and may even use these pulses of sound to stun prey. In many cases, several species of dolphins can co-exist in a given region or habitat and have been observed in mixed feeding aggregates (Saayman 1972).

Conservation concerns with delphinids vary with some species and/or populations considered stable, while others have been reported as “data deficient” by the IUCN, and still others are listed as vulnerable, threatened, or endangered (IUCN 2008). Some considerable threats to the conservation and management of delphinids are pollution, habitat destruction, and accidental catch. These threats reflect the inshore nature of a lot of dolphin species where interactions with humans can be an issue. Some species that are considered to be shallow water coastal dolphins, such as the humpback dolphin (*Sousa chinensis*), have been shown to have very high levels of pollutants, such as chlorinated hydrocarbons, in their blubber (Cockcroft *et al.* 1991).

Understanding the feeding ecology of dolphins is critical to better understand their roles as consumers in their marine environment and therefore the threats that they face. However, studying foraging behaviors of a marine animal presents serious logistical challenges. Marine

mammals undertake the majority of their feeding underwater and/or far out to sea where direct observations are difficult if not impossible. They also tend to be fast moving and can travel over large distances making observations and quantification extremely difficult. Historically, research into the feeding ecology of marine mammals has been approached in a variety of ways, including anecdotal observations (e.g., Caldwell and Caldwell 1972, Irvine *et al.* 1981, Shane 1990), fecal analysis of hard remains (e.g., Sinclair and Zeppelin 2002), DNA analysis of feces (e.g., Meekan *et al.* 2009), examination of stomach contents (e.g., Gunter 1942, Barros and Odell 1990, Mead and Potter 1990, Barros 1993, Barros and Wells 1998, Barros *et al.* 2000), telemetry studies (e.g., Shippee *et al.* 2005), and fatty acid signature analysis (e.g., Iverson *et al.* 1997, Samuel and Worthy 2004, Walton *et al.* 2007). While all of these methodologies have greatly contributed to the data currently available on dolphin feeding ecology, each presents limitations. Collection of fecal matter and identifying the source animal presents challenges given the aquatic environment and the reality that animals are often submerged. Collecting stomach contents from live animals (lavage) is invasive and identification of stomach contents either from live or dead animals may be difficult due to erosion of hard parts. Stomach contents may also represent only part of the diet since prey lacking hard parts will not be retained in the gut of the predator and some hard parts (such as squid beaks) may be retained in the stomach and therefore skew interpretation of relative importance of various prey species. Dead animals may have been unhealthy and therefore, not be representative of the population at large as far as stomach contents are concerned. In addition, stomach content analysis typically will only reveal information about the last meal or two, rather than representing long-term feeding history. Telemetry studies are incredibly expensive and still depend on direct observation to identify prey taken.

Fatty acid analysis has become a frequently used indirect measurement of feeding ecology among marine researchers, although some concerns have been expressed recently (e.g., Rosen and Tollit 2012). In marine food webs, long chain fatty acids pass from prey to carnivorous consumers relatively unchanged allowing for the reflection of a prey's fatty acid profile to be seen in the fat or blubber of the consumer (Ackman 1980). Relatively few fatty acids are biosynthesized in carnivores therefore making it possible to identify those fatty acids derived from the diet. These are taken up by the consumer during digestion and although there is slight modification of these dietary fatty acids due to metabolism such that the composition of consumer tissue does not exactly match that of the prey, they are laid down in a predictable way (Iverson *et al.* 1997, Budge *et al.* 2006). Fatty acid analysis has been used in many feeding ecology studies to potentially discern dietary choices, however, to properly interpret the fatty acid signature, correction factors need to be developed and these are still unavailable for cetaceans (e.g., Budge *et al.* 2006, Rosen and Tollit 2012). This technique also requires that samples include the entire depth of the blubber layer thereby requiring deep biopsy sampling which is invasive and logistically challenging. Despite these limitations, fatty acid analysis can be used to discriminate between different subpopulations of animals or individuals within a population that are exhibiting different feeding habits (Iverson *et al.* 1997).

In recent years, stable isotope analysis has increasingly been used to gain a better understanding of the feeding ecology of many species, including marine mammals (e.g., Ramsay and Hobson 1991; Ames *et al.* 1996; Walker *et al.* 1999; Kurle and Worthy 2002; Yamamuro *et al.* 2004; Lee *et al.* 2005; Newsome *et al.* 2006; Reich and Worthy 2006; Alves-Stanley and Worthy 2009; Witteveen *et al.* 2011). Stable isotopes are elements that share the same number of protons but differ in the number of neutrons, hence resulting in different atomic weights.

Stable isotope analysis is based on the principle that the isotopic make-up of consumer tissues reflects that of their diet (e.g., Deniro and Epstein 1978, Deniro and Epstein 1981, Fry 1988).

The stable isotope ratio in the tissues of a consumer reflects its diet over some period of time, not just the most recent prey eaten thereby giving a better assessment of feeding history. Sampling for this technique is non-lethal, allowing for repeated sampling, and can be done on free-ranging animals using a superficial biopsy dart or through the collection of sloughed skin.

In feeding ecology studies the most commonly used stable isotopes are carbon and nitrogen. The lighter forms of these two isotopes, ^{12}C and ^{14}N , occur more frequently than their heavier counterparts, ^{13}C and ^{15}N . Diet-tissue discrimination (also known as fractionation) results in the tissues of consumers having a different ratio of heavy to light isotopes than that of the tissues of its prey. This is due to certain isotopes being preferentially selected in certain biochemical processes. Most enzymes, for example, show an affinity for the lighter forms of carbon and nitrogen isotopes. This results in a step-wise enrichment where the heavier isotope increases in concentration relative to the standard for that particular element and ultimately allows for the measurement of ratios of stable isotopes. In marine ecosystems, nitrogen isotope ratios are typically used to reflect trophic level, whereas carbon isotopic ratios are more indicative of sources of primary production (Rau *et al.* 1983, Fry 1988). Differing affinities for the heavier and lighter isotopes result in the consumer tissue being more enriched in ^{13}C , compared to the diet, by approximately 1‰ (Deniro and Epstein 1978) whereas the diet-tissue discrimination for ^{15}N averages 2-5‰ (Rau *et al.* 1983, Minagawa and Wada 1984, Hobson *et al.* 1994). It is this latter shift that is generally used to assess step-wise trophic level changes and is what allows for the potential interpretation of feeding history by making comparisons of which trophic level a predator is feeding at (Deniro and Epstein 1981, Peterson and Fry 1987).

Because the isotopic ratio of nitrogen can indicate trophic level, it has also been used to discern weaning in a variety of mammals. When mammals are nursing, their tissues will be isotopically enriched in nitrogen when compared to that of older, weaned individuals. Because they are consuming milk which is essentially isotopically the same as the tissue of the mother, they are feeding at a higher trophic level than that of adult dolphins feeding primarily on fish resulting in enriched nitrogen values (Knoff *et al.* 2007). This allows for clear isotopic distinction among age classes. This has been observed in northern fur seals (Hobson *et al.* 1997), in bottlenose dolphins (Knoff *et al.* 2007), and bowhead whales (Hobson and Schell 1998), among many other mammalian species. Similarly, animals undergoing a fast will also show enriched nitrogen values because they are, in essence, feeding on themselves (Gannes *et al.* 1998, Cherel *et al.* 2005).

Diet-tissue discrimination values can vary dramatically depending on the tissue being sampled and the species in question. In order to more accurately evaluate stable isotope data, specific discrimination values need to be determined for all species and tissues being studied using this methodology. This requires controlled studies where animals are fed an isotopically known diet for a period of time and then sampled and different tissues measured for their carbon and nitrogen signatures. Despite the growing popularity of using stable isotope analysis to evaluate feeding ecology in marine mammals, there are currently only two published studies on cetacean diet-tissue discrimination values for skin tissue (the most commonly available tissue). One of those studies presents opportunistically obtained values from a single killer whale and neither was associated with any controlled diet (Caut *et al.* 2011, Borrell *et al.* 2012). Marine biologists commonly use diet-tissue discrimination values measured from birds and small

mammals such as gerbils and apply these to marine mammal stable isotope data. Clearly, there is a need to establish appropriate values for proper interpretation in these types of studies.

To interpret temporal changes in the isotopic signature of consumer tissues as diet composition changes, turnover rates of those tissues must be known (Bosley *et al.* 2002). If turnover rate and diet-tissue fractionation are known, one can ultimately make inferences about changes in habitat, diet, and/or migratory patterns (Mitani *et al.* 2006; Marcoux *et al.* 2007; Witteveen *et al.* 2011). Turnover rates of stable isotopes will also vary depending on the species involved and type of tissue being sampled. For example, tissues such as plasma or liver tissue will have fast turnover rates and provide information on recently consumed diets, whereas, bone tissue will have a slower turnover time and provide information over a longer dietary history (Tieszan *et al.* 1983, Ogden *et al.* 2004). Data on turnover rates for differing tissues and species are scarce in the literature, and there are no data available for turnover rates in cetacean skin, the most accessible tissue, with the only marine mammal study having been undertaken on skin turnover rates in manatees (Alves-Stanley and Worthy 2009). Controlled studies that involve switching animals from one isotopically distinct diet to another are needed to start to fill in the vast gap in our knowledge and abilities to utilize isotope data to its fullest.

In studies where data on the isotopic signatures of potential prey are available in addition to values for the consumer's tissue, mass-balance equations (mixing models) can be used to determine the relative contribution of prey species to the consumer's diet (e.g., Gomez-Campos *et al.* 2011, Wai *et al.* 2011). Over the last decade or so, various models have been developed for this purpose with differing capabilities. Originally, models were limited in the number of sources (prey items) that could be inputted into the model to $n + 1$, where n equals the number of isotopes being measured. More recent Bayesian approaches have overcome this limitation,

allowing uncapped numbers of sources, as well as incorporating various sources of variation into the model. Variance in the isotopic signatures of prey as well as variance in the diet-tissue discrimination values can be accounted for in models such as Stable Isotope Analysis in R (SIAR), making for a more robust model. These mixing models potentially allow researchers the ability to estimate relative contribution of sources to a predator's diet.

The goal of this dissertation was to increase our understanding of delphinid feeding ecology and habitat utilization through analysis of stable carbon and nitrogen isotope ratios and fatty acid signatures. The specific objectives were 1) to perform controlled feeding studies of bottlenose dolphins in order to calculate stable isotope diet-tissue discrimination factors and turnover rates in dolphin skin (chapter 2), 2) to explore resource partitioning of South African delphinid species using carbon and nitrogen stable isotopic signatures (chapter 3), and 3) to assess habitat usage and feeding ecology of bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida through stable isotope and fatty acid analyses as well as to use a Bayesian mixing model to estimate relative proportions of diet items for this population (chapter 4).

In order to assess diet-tissue discrimination and turnover times (Chapter 2 – “Controlled Feeding Studies in Bottlenose Dolphins (*Tursiops truncatus*): Calculation of Stable Isotope Diet-Tissue Discrimination Factors and Turnover Rates in Dolphin Skin.”), controlled diet switching experiments involving animals held at Farglory Ocean Park Hualien, Taiwan and SeaWorld Orlando, Florida were undertaken. Diet-tissue discrimination factors were calculated for bottlenose dolphin skin as were turnover rates of carbon and nitrogen. This study is the first of its kind to calculate these values in skin of bottlenose dolphins.

Inter and intra-specific differences in isotopic signatures were explored to determine if resource partitioning was occurring among four delphinid species with overlapping home ranges (Chapter 3 – “Assessing Resource Partitioning in South African Delphinids Through Stable Isotope Analysis.”). This chapter explores carbon and nitrogen isotopic signatures of four delphinid species: Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), common dolphins (*Delphinus capensis*), striped dolphins (*Stenella coeruleoalba*), and humpback dolphins (*Sousa chinensis*), off the coast of South Africa over several decades to assess whether resource partitioning is occurring among these species with overlapping home ranges.

A detailed examination of bottlenose dolphins in east-central Florida (Chapter 4 – “Examining Feeding Ecology and Habitat Usage of Bottlenose Dolphins in the Indian River Lagoon Through Stable Isotope and Fatty Acid Analyses.”) investigates differences in both stable isotope and fatty acid signatures of a resident population of dolphins. Factors examined were differences among locations within the Indian River Lagoon, differences among age classes of dolphins, among collection years, and between sexes to determine if individuals of this population are employing different strategies in feeding or utilizing the habitat differently. In addition, use of the Bayesian mixing model, SIAR was made to model feeding habits of IRL dolphins. A suite of potential prey caught in the same location and timeframe for another study were used as source input for the model and predictions of relative contributions of prey to the dolphins’ diet were made. This chapter also attempts to use data from controlled feeding studies to validate model accuracy.

The studies described in the chapters of this dissertation demonstrate an application of stable isotope ecology on both a fine and broad scale. Results from each chapter present novel findings and contribute to the understanding of delphinid feeding ecology and habitat utilization,

as well as being the first to present important and needed stable isotope values and validating frequently used mixing models.

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CHAPTER 2: CONTROLLED FEEDING STUDIES IN BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS): CALCULATION OF STABLE ISOTOPE DIET-TISSUE DISCRIMINATION FACTORS AND TURNOVER RATES IN DOLPHIN SKIN

Introduction

In recent years, stable isotope analysis has been effectively used to gain a better understanding of the feeding ecology of many species, including marine mammals (e.g., Ramsay and Hobson 1991; Ames *et al.* 1996; Walker *et al.* 1999; Kurle and Worthy 2002; Yamamuro *et al.* 2004; Lee *et al.* 2005; Newsome *et al.* 2006; Reich and Worthy 2006; Alves-Stanley and Worthy 2009; Witteveen *et al.* 2011). Stable isotope analysis utilizes naturally occurring isotopes, such as those of nitrogen and carbon, which are passed from prey to consumer and are reflected in the consumer's tissues. The stable isotope ratio in the tissues of a consumer reflects its diet over some period of time, not just the most recent prey eaten. Sampling for this technique is non-lethal, allowing for repeated sampling, and can be safely done on free-ranging animals using a biopsy dart.

Although stable isotope analysis is a powerful and important tool to gain insight into the ecology of animals, there are additional values that are imperative to the proper interpretation of such data. Many assumptions that are critical to applying these approaches in the field have not been assessed using controlled feeding studies. In order to accurately interpret and apply stable isotope analysis to understanding feeding ecology of wild marine mammals, diet-tissue discrimination factors and turnover rates of these isotopes must first be determined (Tieszen *et al.* 1983; Hobson *et al.* 1996; Seminoff *et al.* 2007), perhaps even at a species-specific level (e.g., DeMots *et al.* 2010, Wyatt *et al.* 2010). The stable isotope ratio of a consumer will differ

slightly from that of its prey. This shift is referred to as diet-tissue discrimination (or fractionation) and occurs because even though isotopes of a given element undergo the same biochemical processes in the body, they react differently as a result of differences in their atomic weights. The different affinities for the heavier and lighter isotopes result in the consumer tissue being more enriched in ^{13}C , compared to the diet, by an average of approximately 1‰ (Deniro and Epstein 1978) and the diet-tissue discrimination for ^{15}N averages 2-5‰ (Rau *et al.* 1983, Minagawa and Wada 1984, Hobson *et al.* 1994). It is this latter shift that is generally used to assess step-wise trophic level changes and is what allows for the potential interpretation of feeding history (Deniro and Epstein 1981, Peterson and Fry 1987). Discrimination values can vary dramatically depending on the tissue being sampled and the species in question. In order to more accurately evaluate stable isotope data, tissue-specific diet-tissue discrimination values need to be determined for cetaceans and specifically for skin, being the tissue that is most easily obtained. There are currently only two published studies on cetacean diet-tissue discrimination values for skin tissue and one of those studies presents values from a single killer whale (Caut *et al.* 2011, Borrell *et al.* 2012).

To interpret temporal changes in the isotopic signature of consumer tissues as diet composition changes, turnover rates of those tissues must be known (Bosley *et al.* 2002). If turnover rate and diet-tissue discrimination are known, one can ultimately infer changes in habitat, diet, and/or migratory patterns (Mitani *et al.* 2006; Marcoux *et al.* 2007; Witteveen *et al.* 2011). Turnover rates of stable isotopes will vary depending on the species involved and type of tissue being sampled. Tissues such as plasma or liver tissue will have fast turnover rates and provide information on recently consumed diets, whereas, bone tissue will have a slower turnover time and provide information over a longer dietary history (Tieszan *et al.* 1983, Ogden

et al. 2004). As such, different tissues occurring in differing species will have different turnover rates. Things such as growth, nutritional status, and diet composition can also influence turnover rates and must be taken into account. In order to appropriately use stable isotope data to answer ecological questions, turnover rates first need to be established.

Studies undertaken under controlled conditions are critical to establish discrimination values and turnover rates because the assessment of these values requires an opportunity to experimentally switch an animal from one isotopically distinct diet to another isotopically distinct diet under controlled conditions. Despite the large number of field studies that have applied stable isotope analysis to marine mammals (e.g., Ramsay and Hobson 1991; Ames *et al.* 1996; Walker *et al.* 1999; Kurle and Worthy 2002; Yamamuro *et al.* 2004; Lee *et al.* 2005; Newsome *et al.* 2006; Reich and Worthy 2006; Alves-Stanley and Worthy 2009; Witteveen *et al.* 2011) there are no turnover rates available for skin (the most commonly available tissue) for any cetacean species and only one for any marine mammal (manatee skin: Alves-Stanley and Worthy 2009). Because turnover rates and diet-tissue discrimination values often differ between tissues and species, it is crucial to broaden marine mammal isotope turnover research in order to accurately interpret stable isotope data. Having these critical values will also allow researchers to go back and more accurately interpret historical data.

Data for carbon and nitrogen turnover rates are scarce in the literature and most studies have been performed on birds and small mammals (e.g., Teiszan *et al.* 1983, Voigt *et al.* 2003, Ogden *et al.* 2004). Other studies that have reported turnover values for larger mammals include alpacas, bears, horses, seals, and cattle, however, none of these studies measured turnover rates of carbon and nitrogen in skin (e.g., Hilderbrand *et al.* 1996, Zhao and Schell 2004, Sponheimer *et al.* 2006). The quality and quantity of protein in a consumer's diet can affect turnover rate of

proteins in given tissues, hence also affecting carbon and nitrogen turnover rates (Lobley 2003, Zhao *et al.* 2006). Cetacean skin is made up of a thick partially keratinized epidermal layer and an underlying dermal layer made up of fibroelastin tissue (Reeb *et al.* 2007). In a study where large biopsy samples (3-5 cm in diameter and 1 cm deep) were surgically removed from bottlenose dolphins there was almost total healing after 42 days with only a slight discoloration left on the surface of the skin (Weller *et al.* 1997). Isotopic turnover rates can vary among tissue types, species, body sizes, nutritional states, and protein turnover rates which in turn can be influenced by diet quality (Martinez del Rio *et al.* 2009, Newsome *et al.* 2010) making comparisons among studies difficult.

Knowledge of turnover rates and diet-tissue discrimination values are critical in interpreting isotope-based feeding ecology studies involving free-ranging dolphins in order to assess feeding habits and to evaluate critical habitat needs. Stable isotope data not only provides important insight into dietary habits, but it also potentially allows for identification of habitat preferences and gives insight into habitat usage. By establishing these much needed values for delphinids, stable isotope data will be better used to evaluate important aspects of the ecology of these animals and scientists, ultimately, will be better armed to make important conservation decisions for these species. Published rates of wound healing lend insight into potential turnover rates for dolphin integument and in the absence of empirical data for these species, we hypothesize that turnover rates in cetaceans will be considerably faster than would be suggested from literature values. The goal of the present study was therefore to assess these values in the skin of *ex situ* bottlenose dolphins (*Tursiops truncatus*).

Methods

Animals and Sample Collection

Whole frozen individual herring (*Clupea harengus*), capelin (*Mallotus villosus*), and “whitebait” were collected at Sea World of Florida (SWF) and processed at the University of Central Florida (UCF). Initially mass and standard lengths were obtained and then individual fish were homogenized in a blender. Ground fish were then freeze dried individually and stored in plastic Whirl-Pak bags until further processing. Isotope samples of SWF fish were prepared at UCF and subsequently analyzed at the University of Georgia (see below).

Dietary samples of species fed to dolphins were collected throughout the study at Far Glory Ocean Park (FGOP), Taiwan to monitor potential changes in dietary isotopic composition and to serve as a comparison to the isotopic signature of the skin for calculation of diet-tissue discrimination. Preliminary processing of FGOP fish samples occurred at National Sun Yat-Sen University, Kaohsiung, Taiwan. Standard length and body mass were obtained for intact frozen fish which were then individually ground into a homogenous sample using blenders. Homogenized samples were then freeze dried and stored in Whirl-Pak bags for shipment to National Taiwan University, Taipei, Taiwan for further processing and isotopic analyses (see below).

Water content of each individually homogenized fish was determined gravimetrically by drying in a lyophilizer (LabConco) for 96 hours. Gross lipid content was determined gravimetrically by extracting with petroleum ether in a Soxhlet extractor for 24 hours. Samples were then placed in an oven at 60°C for 24 hours to remove any remaining solvent. Ash content was determined by heating at 100°C for 2 hours and then 550°C for 24 hours. Carbohydrate

content was not measured because that component is generally low in marine fish species and its contribution to total energy content is close to zero (e.g., Craig *et al.* 1978; Anthony *et al.* 2000; Ball *et al.* 2007). “Protein” content was estimated to be the remaining mass. Energy density was calculated using conversion factors for lipid (39.3 MJ kg⁻¹) and protein/carbohydrate (18.0 MJ kg⁻¹) (Craig *et al.* 1978; Anthony *et al.* 2000; Ball *et al.* 2007).

A total of 27 bottlenose dolphins were measured at SWF. The first group (SWF-1) were all adult males (n=8), while the second group (SWF-2) consisted of adult males (n = 9), adult females (n = 3), pregnant females (n = 2), and juveniles (females, n = 4; male, n=1). All dolphins (SWF-1 and SWF-2) were fed a mixture of herring and capelin at consistent proportions for a period of two months prior to sample collection. SWF-2 animals, however, also received a portion of their diet (capelin and whitebait) from the general public. Amounts consumed were estimated by animal care staff who observed interactions with the public.

Small samples of skin (approx. 20 mg) were collected from each dolphin at SWF on a single occasion using 7 mm diameter disposable dermal curettes (Miltex). These samples were used only in determining diet-tissue discrimination factors. The curette was dragged across the surface of dolphin skin, which had been patted dry, with superficial skin being collected in the loop of the curette. Skin was transferred to plastic microcentrifuge vials (Novatech, model D1010) and kept at -20°C until further processing.

Adult female bottlenose dolphins at FGOP were divided into two groups (FGOP-1, n=4; FGOP-2, n=2) each of which underwent two diet shifts. Initial diets (duration 8 weeks) served to establish an isotopically known baseline diet and calculate diet-tissue discrimination; while the second diet shift (duration 9 weeks) was utilized to calculate both isotope turnover rates and

another value for diet-tissue discrimination. Small samples of skin (*ca.* 20 mg) were collected weekly using the same approach as above and kept at -20°C until further processing.

Prior to the start of the current study, FGOP dolphins were being fed a mixture of four species of fish and two species of squid in varying amounts. Based on availability of prey items and stable isotopic values for each item, two isotopically distinct diets, each consisting of two species of fish, were derived from food items that dolphins were already consuming. To develop these two experimental diets, samples of whole prey items were taken and analyzed to determine isotopic signatures. Diet A consisted of 80% (by mass) *Cololabis sarira* and 20% *Scomber scombrus*, while Diet B consisted of 80% *Mallotus villosus* and 20% *Trachurus japonicas*. FGOP-1 dolphins were fed Diet A for 8 weeks to establish a baseline isotope signature and then switched to Diet B for 9 weeks. Assessment of turnover time and diet-tissue discrimination factors started as of the day they commenced consuming Diet B. FGOP-2 dolphins were initially offered Diet B for 8 weeks to establish a baseline and then transitioned to Diet A for 9 weeks. Assessment of turnover time and diet-tissue discrimination factors started on the first day of consuming Diet A. Skin samples were collected weekly from each animal for 2 weeks prior to the start of the study, throughout the duration of transitioning animals onto the first isotopically known diet, and then for the duration of the shift to the second diet. There were five occasions when samples were not collected from individual dolphins due to them being uncooperative. Dolphin skin samples were sent to National Taiwan University, Taipei, Taiwan for sample preparation and isotopic analysis (see below).

Stable Isotope Analysis

Dolphin skin samples were placed in a drying oven at 60°C for 24 hours. Skin samples and freeze dried ground fish (see above) were then wrapped in glass microfilter paper (Whatman, GF/A), placed in cellulose thimbles (Advantec, grade 84), and lipid extracted for 24 hours using petroleum ether in a Soxhlet extractor. Samples were then placed in a drying oven at 60°C for 24 hours to remove any remaining solvent. Samples were re-ground into a fine powder using a Wig-L-Bug amalgamator (Dentsply, model MSD). Aliquots of skin and fish (0.9-1.5 mg) sampled in Taiwan were sealed into 6 mm by 4 mm tin capsules (Elemental Microanalysis, model D1006) and analyzed for carbon and nitrogen stable isotopes using a stable isotope ratio mass spectrometer at National Taiwan University (Thermo Scientific Delta V Advantage IRMS). Aliquots of fish and skin samples collected in the USA (0.9-1.5 mg) were sealed into 5 mm by 9 mm tin capsules and analyzed by IRMS at the University of Georgia (Finnigan MAT Delta Plus XL). Available sample mass was too small for detection in the case of six FGOP skin samples. Data is expressed in delta notation (δ):

$$\delta X (\text{‰}) = \left(\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right) \times 1000 \quad (1)$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$. Standard reference materials were carbon from PeeDee Belemnite limestone and atmospheric nitrogen gas. To assess quality control in sample analysis of stable isotope ratios, a known standard sample (fish muscle at National Taiwan University and bovine tissue at the University of Georgia) was run periodically after unknown samples. Analytical errors for the standard samples (n=38) were $\pm 0.06\text{‰}$ (SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at National Taiwan University and were $\pm 0.01\text{‰}$ (SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ when running the University of Georgia standard samples (n = 10).

Diet-tissue discrimination values for each isotope were calculated by assessing the difference in delta values between FGOP dolphin skin (y) (on days 0 and 63) and the fish diet (w) using the $\Delta_{\text{tissue-diet}}$ notation (Caut *et al.* 2011):

$$\Delta_{\text{tissue-diet}} = \delta_y - \delta_w \quad (2)$$

Turnover rates of carbon and nitrogen in FGOP dolphin skin were calculated using the exponential model used by Ogden *et al.* (2004):

$$Y(t) = y_a + ae^{-bt} \quad (3)$$

in which $Y(t)$ is the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ value of dolphin skin at time t , y_a is the value approached asymptotically, a is the absolute change in value after diet switch, b is turnover rate of carbon or nitrogen in dolphin skin, and t is time (days) since diet switch. Exponential decay curves were fit with y_a set to the expected skin isotopic value based on diet and the individually calculated diet-specific value for both $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$. Turnover rate was expressed in terms of half-life, the time (b) it takes for the isotopic composition of the skin to reach halfway between the initial and final values:

$$\frac{-\ln(0.5)}{b} \quad (4)$$

where b is turnover rate of carbon or nitrogen in dolphin skin.

Statistical Analyses

Stable isotope data were tested for normality using Shapiro-Wilk's and tested for homogeneity of variance using Levene's test. Differences in stable isotope signatures for fish

species were explored for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ using GLM multivariate analysis. Tukey's HSD post hoc tests were performed to determine homogeneous subsets. All statistical analyses were conducted using SPSS 20.0 with a critical value of $\alpha = 0.05$ and plotted using SigmaPlot (Version 10.0, Systat Software). Values reported are mean \pm SE.

Results

Diet

A total of 82 fish from three species were analyzed at SWF (Table 2.1). Isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were normally distributed for all species (S-W; $p \geq 0.186$) except for capelin ($\delta^{13}\text{C}$) (S-W = 0.85, $p = 0.04$). Fish species were found to be significantly different from each other (Wilks' Lambda: $F_{4,156} = 170.64$, $p < 0.001$) for both $\delta^{15}\text{N}$ ($F_{2,79} = 162.91$, $p < 0.001$) and ^{13}C ($F_{4,156} = 701.51$, $p < 0.001$) (Table 2.1). Herring ($11.52 \pm 0.09\text{‰}$), capelin ($11.89 \pm 0.06\text{‰}$), and whitebait ($13.45 \pm 0.04\text{‰}$), at SWF were significantly different from one another with respect to $\delta^{15}\text{N}$ (Tukey's HSD, $p \leq 0.01$). Whitebait ($-15.67 \pm 0.08\text{‰}$) was significantly different from both herring ($-19.56 \pm 0.09\text{‰}$) and capelin ($-19.71 \pm 0.06\text{‰}$) with respect to $\delta^{13}\text{C}$ (Tukey's HSD, $p < 0.001$), while capelin and herring were not significantly different from each other (Tukey's HSD, $p = 0.297$).

SWF-1 dolphins received 60% (by mass) capelin and 40% herring resulting in a combined $\delta^{15}\text{N}$ signature of 11.70‰ and $\delta^{13}\text{C}$ signature of -19.42‰ (Table 2.2). Proportions of each species of fish fed to individual SWF-2 dolphins varied according to age and reproductive status and as a result combined signatures of diets for these individuals ranged between 12.04‰ and 12.46‰ for $\delta^{15}\text{N}$ and between -18.19‰ and -19.27‰ for $\delta^{13}\text{C}$ (Table 2.2).

A total of 56 fish from four species were analyzed at FGOP (Table 2.1). Isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were normally distributed for all species (S-W; $p \geq 0.09$) except for $\delta^{15}\text{N}$ in *Cololabis sarira* (S-W = 0.85, $p = 0.02$). Fish species were found to be significantly different for both $\delta^{15}\text{N}$ ($F_{3,52} = 477.91$, $p < 0.001$) and $\delta^{13}\text{C}$ ($F_{3,52} = 202.48$, $p < 0.001$). *Cololabis sarira* ($7.55 \pm 0.11\text{‰}$) and *Mallotus villosus* ($12.83 \pm 0.06\text{‰}$) were significantly different (Tukey's HSD, $p < 0.05$) from other species for $\delta^{15}\text{N}$ and *Scomber scombrus* ($11.22 \pm 0.14\text{‰}$) and *Trachurus japonicas* ($11.38 \pm 0.09\text{‰}$) were not significantly different from each other (Tukey's HSD, $p > 0.05$) (Table 2.2). *Cololabis sarira* ($-21.39 \pm 0.09\text{‰}$) and *Mallotus villosus* ($-19.73 \pm 0.05\text{‰}$) were significantly different from other species for $\delta^{13}\text{C}$ (Tukey's HSD, $p < 0.05$) and *Scomber scombrus* ($-16.87 \pm 0.28\text{‰}$) and *Trachurus japonicas* ($-17.02 \pm 0.06\text{‰}$) were not significantly different from each other (Tukey's HSD, $p > 0.05$) (Table 2.2).

At FGOP, Diet A (80% *Cololabis sarira*, 20% *Scomber scombrus*) had an overall nitrogen signature of 8.30‰ and carbon signature of -20.48‰ (Table 2.2). Diet B (80% *Mallotus villosus*, 20% *Trachurus japonicas*) had an overall nitrogen signature of 12.54‰ and carbon signature of -19.19‰ (Table 2.2).

Dolphin Isotopic Values and Diet-Tissue Discrimination

Overall, dolphin skin differed significantly in both $\delta^{15}\text{N}$ ($F_{4,44} = 14.90$, $p < 0.001$) and $\delta^{13}\text{C}$ ($F_{4,44} = 3.89$, $p < 0.009$) between different groups (Table 2.3). There were no significant differences between SWF dolphins regardless of treatment in either $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ (Tukey's HSD, $p > 0.05$) (Table 2.3, Figure 2.1). FGOP dolphins consuming the pre-experiment diet were not significantly different from diet A ($\delta^{15}\text{N}$) ($p = 0.34$) or diet B (Tukey's HSD, $p > 0.05$) (Table 2.3,

Figure 2.1), but Diets A and B differed significantly in $\delta^{15}\text{N}$ (Tukey's HSD, $p < 0.05$). FGOP dolphins consuming the pre-experiment diet had more enriched $\delta^{13}\text{C}$ values than dolphins consuming diet A (Tukey's HSD, $p < 0.05$), but were not significantly different from dolphins consuming diet B ($p = 0.66$) (Tukey's HSD, $p > 0.05$) (Table 2.3, Figure 2.1).

Diet-tissue discrimination values were significantly different for both $\Delta^{15}\text{N}$ ($F_{3,38} = 19.98$, $p < 0.001$) and $\Delta^{13}\text{C}$ ($F_{3,38} = 20.333$, $p < 0.001$) between groups. SWF-1 ($2.09 \pm 0.07\text{‰}$) and SWF-2 ($1.90 \pm 0.08\text{‰}$) adults did not differ in $\Delta^{15}\text{N}$ (Tukey's, $p > 0.05$), but SWF-2 juveniles ($2.61 \pm 0.14\text{‰}$) had a significantly greater $\Delta^{15}\text{N}$ (Tukey's, $p < 0.05$) (Table 2.3, Figure 2.2). FGOP dolphins consuming Diet B ($1.68 \pm 0.11\text{‰}$) had a significantly lower $\Delta^{15}\text{N}$ than animals consuming Diet A ($2.96 \pm 0.12\text{‰}$) (Tukey's, $p < 0.05$) but were not significantly different from SWF-1 or SWF-2 adults (Tukey's, $p > 0.05$). SWF-2 juveniles were not significantly different from FGOP – Diet A dolphins (Tukey's, $p > 0.05$) (Table 2.3, Figure 2.2).

SWF-2 juvenile ($0.53 \pm 0.19\text{‰}$) and adult SWF-2 ($0.66 \pm 0.07\text{‰}$) dolphins did not differ significantly from each other in $\Delta^{13}\text{C}$ (Tukey's, $p > 0.05$) but had significantly lower $\Delta^{13}\text{C}$ than all other dolphin groups (Tukey's, $p < 0.05$). SWF-1 adults ($1.28 \pm 0.16\text{‰}$) were not significantly different from FGOP – Diet B ($1.60 \pm 0.11\text{‰}$) dolphins (Tukey's, $p > 0.05$) (Table 2.3, Figure 2.2) and there were no significant differences between FGOP dolphins consuming Diet A ($2.04 \pm 0.14\text{‰}$) and Diet B (Tukey's, $p > 0.05$) ($p = 0.127$) (Table 2.3, Figure 2.2).

Diet-tissue discrimination values for nitrogen ($\Delta^{15}\text{N}$) were not significantly different between SWF-1 adults, SWF-2 adults, and FGOP dolphins consuming diet B (Tukey's, $p > 0.05$) (Table 2.3). SWF-2 juveniles and FGOP dolphins consuming diet A were also not significantly different (Tukey's, $p > 0.05$) but were significantly different from other groups (Table 2.3).

Diet-tissue discrimination values for nitrogen ($\Delta^{15}\text{N}$) were inversely related to dietary $\delta^{15}\text{N}$ (Table 2.3) ($F = 39.884$, $p < 0.001$) (Figure 2.3): $\Delta^{15}\text{N} = -0.288 (\delta^{15}\text{N}) + 5.371$ ($R^2 = 0.52$).

Diet-tissue discrimination values for carbon ($\Delta^{13}\text{C}$) were not significantly different for SWF-2 adults and SWF-2 juveniles (Tukey's, $p > 0.05$), but those dolphins were significantly different from SWF-1 adults (Tukey's, $p < 0.05$). SWF-1 adults were not significantly different from FGOP dolphins consuming diet B (Tukey's, $p > 0.05$), which in turn were not significantly different from FGOP dolphins consuming diet A (Tukey's, $p < 0.05$). Diet-tissue discrimination values for carbon ($\Delta^{13}\text{C}$) were inversely related to dietary $\delta^{13}\text{C}$ ($F=15.278$, $p < 0.001$) (Figure 2.3) with: $\Delta^{13}\text{C} = -0.833 (\delta^{13}\text{C}) - 15.17$ ($R^2 = 0.29$).

Isotopic Half-Life

Calculated half-lives for $\delta^{15}\text{N}$ ranged from 14 to 23 days (Table 2.4, Figure 2.4). Mean half-life for diet B was 18.2 ± 3.6 days ($n=4$) and was not significantly different from diet A which had a mean half-life of 15.3 ± 1.7 days ($n=2$) ($t=-1.33$, $df= 3$, $p=0.256$). Combined half-life for $\delta^{15}\text{N}$ was 17.2 ± 1.3 days ($n=6$). Half-lives for $\delta^{13}\text{C}$ ranged from 11 to 23 days (Table 2.4, Figure 2.4). Diet B had a mean half-life of 13.9 ± 4.8 days ($n=4$) which was significantly shorter than 22.0 ± 0.5 days ($n=2$) ($t=3.39$, $df= 3$, $p<0.04$) for diet A.

Discussion

This is the first time that diet-tissue discrimination values and turnover times have been assessed for the skin of any cetacean using a controlled feeding experiment. Nitrogen stable isotopes are frequently used in ecological studies to estimate trophic position and interpret

feeding habits. Knowledge of diet-tissue fractionation and turnover times are critical for accurate interpretation of isotope data because they help define the delay needed for the consumer tissues to reach equilibrium with the food source. Defined as the change in tissue isotopic composition attributable to growth and tissue replacement, there are few studies that provide data on fractionation and none that address turnover rates. Historically, isotope turnover studies have been limited, in part, because they generally require access to captive animals that can be exposed to a diet shift.

Discrimination factors used in isotope studies are typically assumed to range from 2-5‰ for nitrogen and 1-2‰ for carbon (DeNiro and Epstein 1981, Das *et al.* 2000, Kelly 2000, Herman *et al.* 2005). Diet-tissue discrimination values for nitrogen, calculated in the present study, fell in the low end of this range with evidence of significant differences based on the quality of the diet (lipid content) being consumed and maturity of the dolphin sampled. Juvenile SWF dolphins had a significantly higher $\Delta^{15}\text{N}$ ($2.46 \pm 0.17\text{‰}$) than SWF adults consuming the same diet ($1.92 \pm 0.14\text{‰}$) but did not differ significantly in $\Delta^{13}\text{C}$ ($0.53 \pm 0.17\text{‰}$ versus $0.84 \pm 0.18\text{‰}$). FGOP dolphins consuming diet B (low fat) had significantly lower $\Delta^{15}\text{N}$ ($1.68 \pm 0.11\text{‰}$) than FGOP dolphins consuming diet A (high fat) ($2.96 \pm 0.12\text{‰}$) but both diets resulted in similar $\Delta^{13}\text{C}$ ($1.6 \pm 0.09\text{‰}$ versus $2.04 \pm 0.14\text{‰}$).

Turnover rates of stable isotopes quantify the period of time it takes for the isotopic signature of consumer tissues to reflect a new diet. It has been estimated that it takes approximately 2-3 half-lives for the complete integration of a new isotopic signature into consumer tissues (Hobson and Clark 1992). In the present study, nitrogen had a half-life, depending on diet, of 17.2 ± 1.3 days and carbon had a comparable half-life of between 13.9 ± 4.8 (diet B) and 22.1 ± 0.5 days (diet A). The ability to use stable isotopes to quantify dietary

preferences over these relatively short temporal periods facilitates the greater resolution of potential prey switching, seasonal changes in foraging behavior, or small spatial scale differences in feeding habits.

At present, there are few studies that have measured discrimination factors for marine mammal skin. Alves-Stanley and Worthy (2009) calculated discrimination values in the skin of *ex situ* manatees to be 2.8‰ for carbon, lower than the 4.1‰ carbon discrimination reported by Ames *et al.* (1996). Hobson *et al.* (1996) found skin of *ex situ* harbor seals to be enriched by 2.3‰ for nitrogen and 2.8‰ for carbon. More recently, Caut *et al.* (2011) was able to calculate discrimination factors using skin from a single killer whale and found $\Delta^{15}\text{N}$ to be 3.2‰ and $\Delta^{13}\text{C}$ to be 2.4‰ and Borrell *et al.* (2012) estimated nitrogen and carbon discrimination factors for skin of *in situ* fin whales to be 2.8‰ and 1.3‰ respectively, similar to measured values in the present study.

Diet-tissue discrimination factors are thought to be influenced by a variety of variables such as metabolic rate of the animal and/or tissue being studied, age of the animal, nutritional quality of the diet, tissue being sampled, and the taxon being investigated (Hobson and Clark 1992, Caut *et al.* 2009, Martinez del Rio *et al.* 2009, Newsome *et al.* 2010, Borrell *et al.* 2012). Tissues of the integumentary system generally tend to have higher carbon discrimination values compared to those of other tissues in mammals as they are comprised of mostly keratin (Borrell *et al.* 2012). Tieszan *et al.* (1983) and Hobson *et al.* (1996) found hair to have the highest carbon discrimination values compared to other types of tissues. Differing food composition can also result in differential rates of assimilation and storage of nutrients. Howland *et al.* (2003) reported that pigs fed diets with differing amino acid composition, exhibited diet-tissue fractionation factors for bone collagen ranging from 0.5‰ to 6.1‰.

It has been previously noted that diet-tissue discrimination factors differ between different diets and between tissues (e.g. Roth and Hobson 2000, Pearson *et al.* 2003, Miron *et al.* 2006). Some have suggested that $\Delta^{15}\text{N}$ should increase as protein content (%N) increases in the diet (quantity hypothesis) (e.g., Pearson *et al.* 2003, Martinez del Rio *et al.* 2009) while others have suggested that $\Delta^{15}\text{N}$ should decrease as dietary protein quality increases (quality hypothesis) (e.g., Roth and Hobson 2000, Robbins *et al.* 2010). Florin *et al.* (2011) found that neither of these hypotheses alone can fully explain variability in $\Delta^{15}\text{N}$. They found that it was likely a combination of protein quality and quantity based on the assessment that low protein quality and high protein content have the potential to increase $\Delta^{15}\text{N}$ by increasing protein turnover rates.

Dolphins in the present study were consuming protein of similar biological value (fish) and therefore interpretation of our data relates to the absolute quantity of protein consumed. Dolphins consuming isocaloric diets which vary in fat content will consume different absolute masses of protein. In the present study, dolphins consuming a high fat diet (14.2%), and therefore relatively low protein intake, had significantly greater diet-tissue discrimination factors for both nitrogen and carbon than dolphins consuming diets with lower fat (2-6%)(i.e., high protein) content, consistent with the quality hypothesis (Pearson *et al.* 2003).

Another, often overlooked, factor impacting discrimination factors is the absolute isotopic value of the diet itself (see Caut *et al.* 2009). In their review they determined that there are significant relationships between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of diets and the resultant discrimination values for those consumers. In the present study, we determined that there was a significant negative relationship for ^{15}N and a weaker relationship for ^{13}C similar to the findings of Caut *et al.* (2009).

Caut *et al.* (2011) had variable results when calculating half-lives for blood plasma and red blood cells in *ex situ* bottlenose dolphins and killer whales. They reported carbon and nitrogen half-life values of bottlenose dolphin red blood cells as 27.5 (12.5 to 46 days) and 127 days respectively. Killer whales also showed a large range in carbon half-lives when measured in red blood cells (13.5 – 60 days). The authors attributed this large variation to a low isotopic shift between test diets. The only previous study in the literature that has investigated turnover rates in marine mammal skin was done with manatees (Alves-Stanley and Worthy 2009). This study investigated half-lives of carbon and nitrogen in the skin of both coastal (marine environment) and riverine (freshwater environment) manatees. Coastal manatees had a mean carbon half-life of 53 days and nitrogen half-life of 27 days, while riverine manatees had a half-life for carbon of 59 days and nitrogen of 58 days. Turnover rates may be slower in manatees than in bottlenose dolphins in part, due to the difference in body size (1000 kg versus 260 kg respectively) (U.S. Fish and Wildlife Service 2001, Kastelein *et al.* 2002), but also the lower metabolic rate of manatees (Irvine 1983). Martinez del Rio *et al.* (2009) cautioned that incorporation rates cannot be compared among animals of differing body sizes without risking integrating large error into the interpretation of results. Carleton and Martinez del Rio (2005) summarized published values for carbon half-lives in avian blood which display a trend of increasing turnover time with body mass. Body size differences were also correlated with turnover rates seen between blood components of *ex situ* killer whales and bottlenose dolphins (Caut *et al.* 2011).

Stable isotope analysis has become increasingly popular in ecological studies to trace movement, analyze habitat usage, to compare trophic levels of organisms and to recreate food webs. Gannes *et al.* (1997) predicted an “explosion” in the usage of stable isotopes and argued

that controlled studies are needed to establish species and tissue specific baseline values that can be applied in field studies. Despite its wide-spread application, the literature is still lacking information on discrimination values for most cetacean tissue types and turnover rates are virtually non-existent. Over the past few years, there have been repeated calls for direct measurements of the basic assumptions and parameters involved in stable isotope ecology (e.g., Gannes *et al.* 1997; Caut *et al.* 2009, Martinez del Rio *et al.* 2009, Wolf *et al.* 2009, Wyatt *et al.* 2010). Tissue specific discrimination values, especially skin, allow ecologists to establish trophic levels for organisms and turnover rate data is necessary for interpreting temporal scales. Both of these values are necessary when trying to recreate dietary history and discrimination factors are critical for use in mixing models (e.g., Phillips and Gregg 2001, Phillips and Gregg 2003, Parnell *et al.* 2010). Indeed many of these mixing models have been shown to be very sensitive to variation in discrimination factors (e.g., Bond and Diamond 2011). To date, cetacean researchers have been relying on published values from organisms ranging from pinnipeds to gerbils and birds.

Collectively, our results suggest that prey composition could be a significant factor to consider when applying discrimination factors to field studies. Frequently, in the absence of species-specific data, average discrimination factors ($\Delta^{15}\text{N} \sim 3.4\text{‰}$ and $\Delta^{13}\text{C} \sim 1\text{‰}$ C) are applied in interpreting field studies. Our results suggest that while diet-tissue discrimination factors for $\Delta^{13}\text{C}$ may be approximately 1‰ in odontocetes, $\Delta^{15}\text{N}$ may be closer to 2.0‰ and may show significant variability as dietary quality changes on a seasonal basis, but also as a function of the trophic level of the prey being consumed. Therefore, relying on the frequently cited average discrimination factors from the literature might lead to misinterpretation of delphinid isotope data.

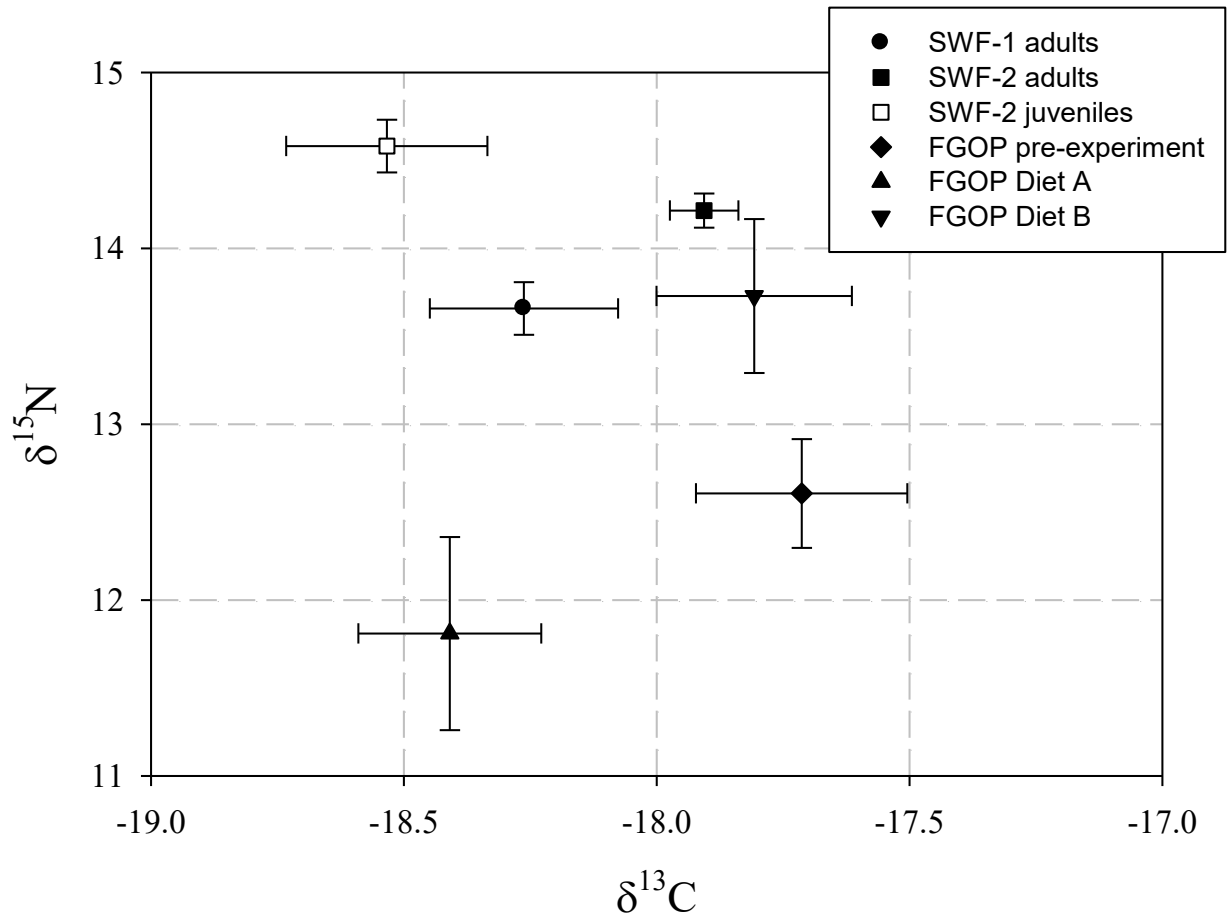


Figure 2.1: Carbon and nitrogen isotope values (mean \pm SE) of epidermal tissue of bottlenose dolphins at Sea World of Florida and Far Glory Ocean Park. Categories are as described in Materials and Methods.

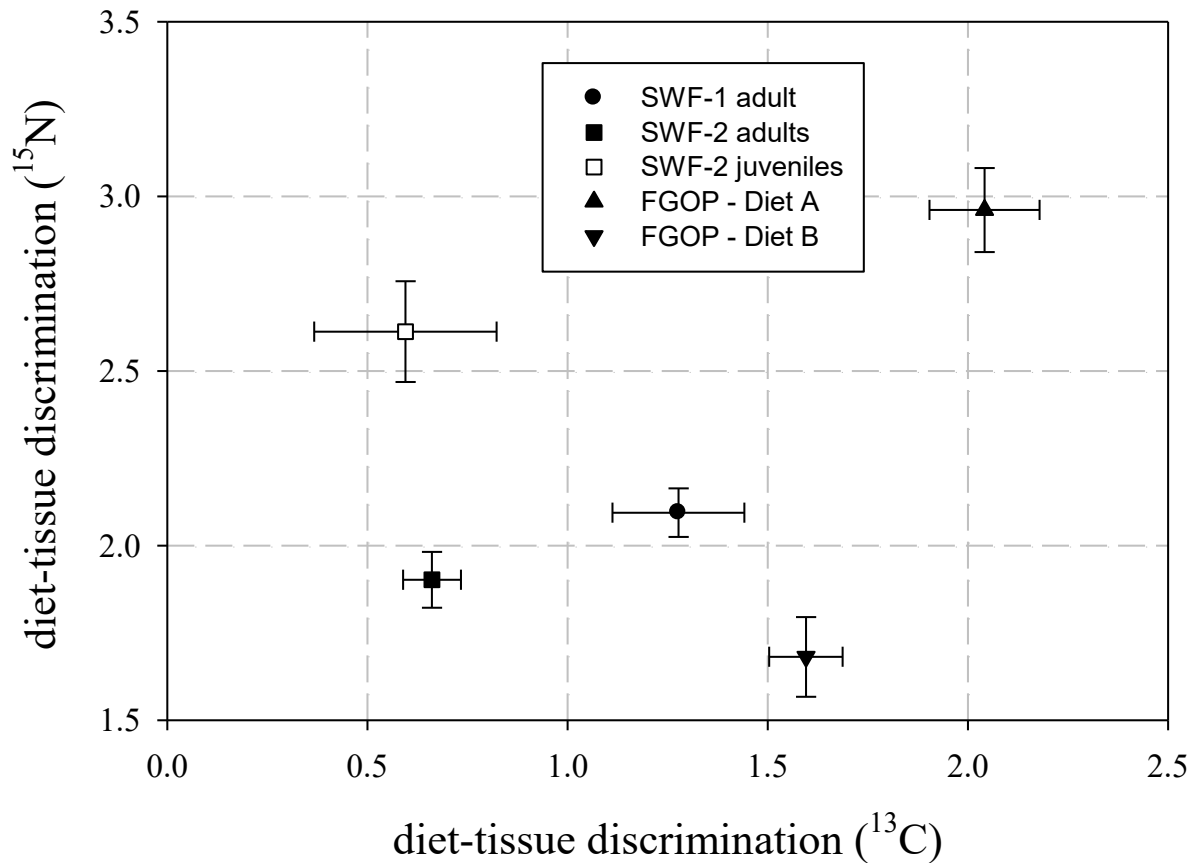


Figure 2.2: Diet-tissue discrimination values were significantly different for both $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$. SWF-1 and SWF-2 adults did not differ in $\Delta^{15}\text{N}$ ($p=0.952$), but SWF-2 juveniles had a significantly greater $\Delta^{15}\text{N}$. FGOP dolphins consuming Diet B had a significantly lower $\Delta^{15}\text{N}$ than animals consuming Diet A but were not significantly different from SWF-1 or SWF-2 adults. SWF-2 juveniles were not significantly different from FGOP – Diet A dolphins. SWF-2 juvenile and adult dolphins did not differ significantly from each other in $\Delta^{13}\text{C}$ but had significantly lower $\Delta^{13}\text{C}$ than all other dolphin groups. SWF-1 adults were not different from FGOP – Diet B dolphins. There were no significant differences between FGOP dolphins consuming Diet A and Diet B.

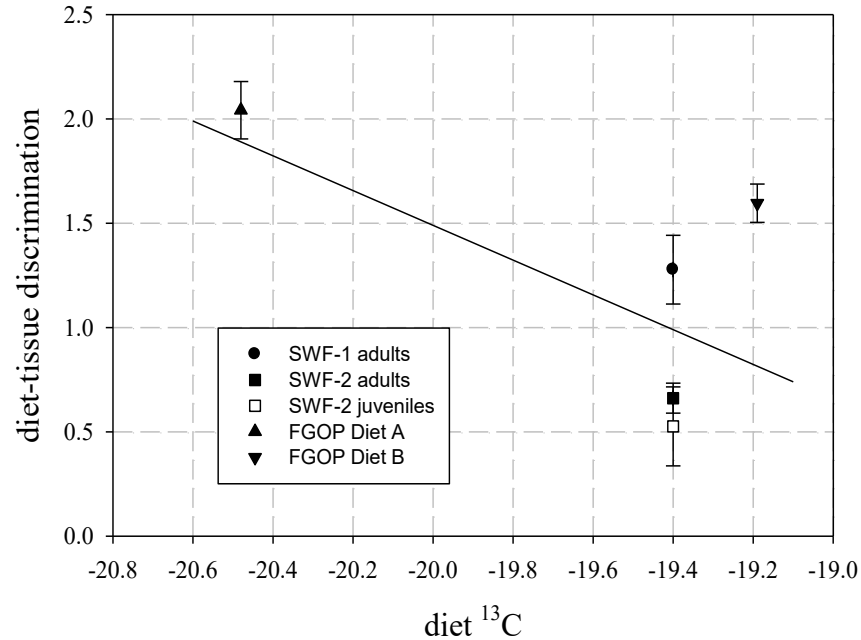
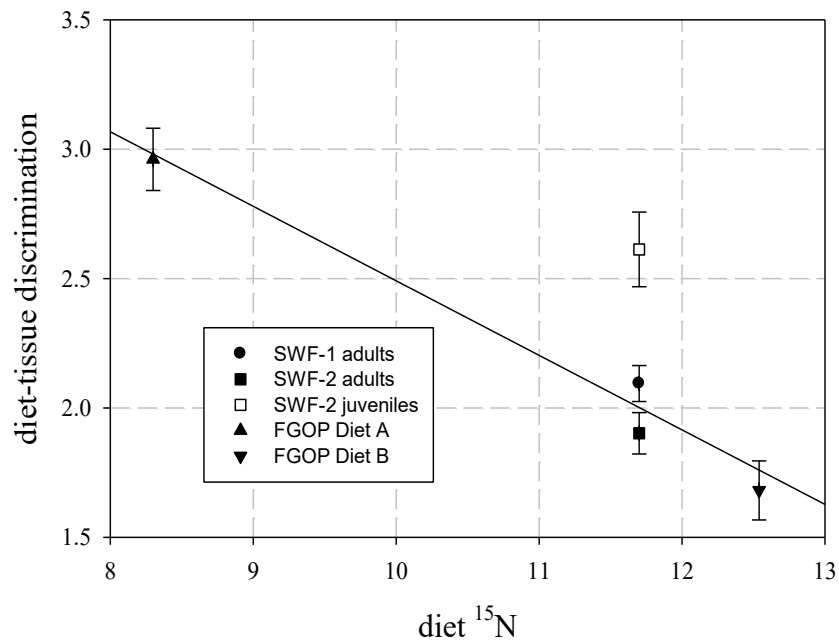


Figure 2.3: Diet-tissue discrimination for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰) ($\pm\text{SD}$) as a function of dietary isotope values (‰). Only adult data points were used in generating regressions. $\Delta^{15}\text{N} = -0.288 (\delta^{15}\text{N}) + 5.371 (R^2 = 0.52)$ and $\Delta^{13}\text{C} = -0.833 (\delta^{13}\text{C}) - 15.17 (R^2 = 0.29)$.

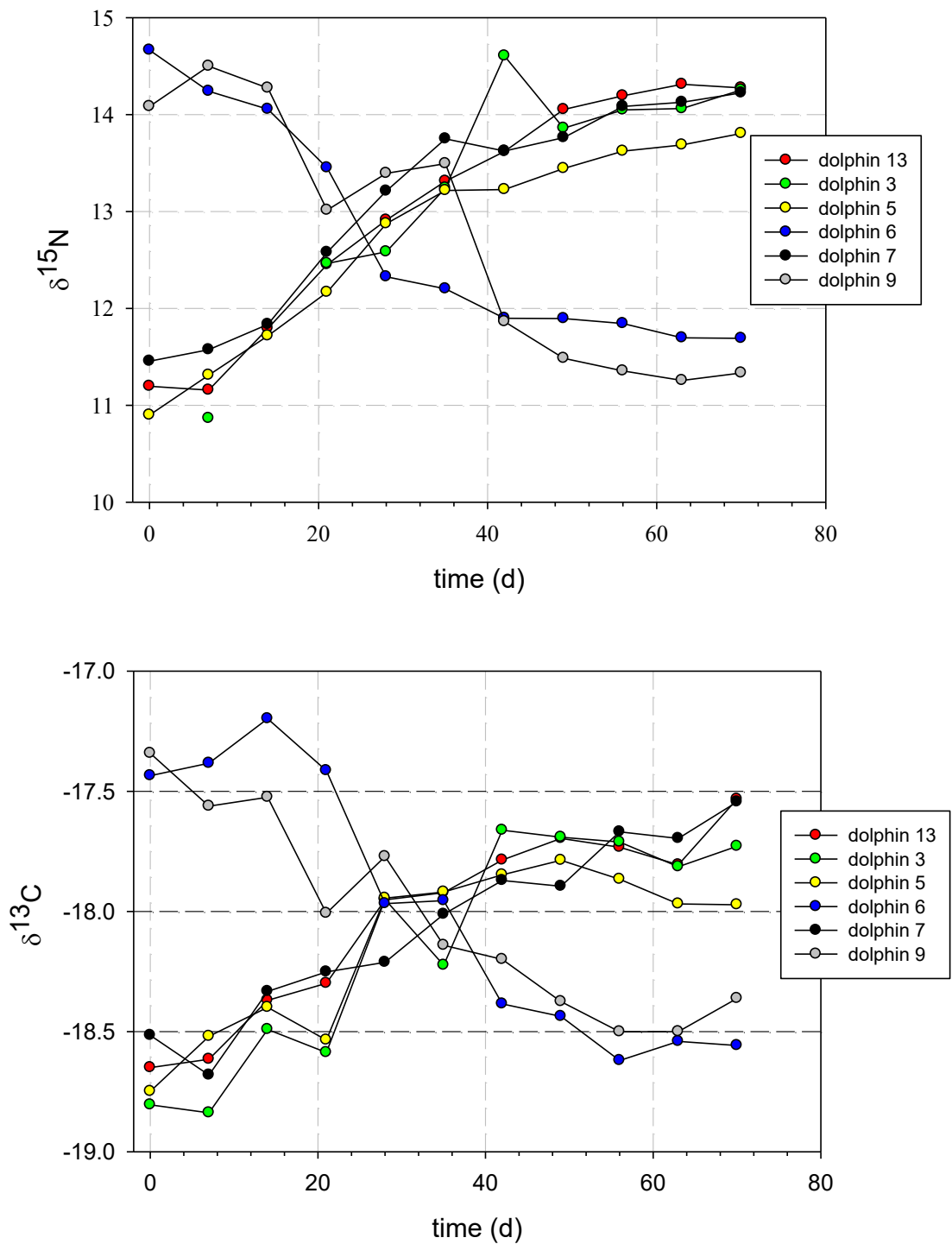


Figure 2.4: Nitrogen ($\delta^{15}\text{N}$ - top) and carbon ($\delta^{13}\text{C}$ - bottom) isotope values in bottlenose dolphin skin (FGOP) as a function of days after diet switch.

Table 2.1: Mass, total length, and nutritional value (\pm SD) of fish utilized in diets of *ex situ* bottlenose dolphins.

Species	Mass (g)	Total length (mm)	Lipid (%)	Water (%)
SeaWorld of Florida				
<i>Clupea harengus</i>	172.8 \pm 40.6	257.7 \pm 37.0	7.3 \pm 0.8%	70.6 \pm 0.6%
<i>Mallotus villosus</i>	28.9 \pm 2.4	161.9 \pm 5.2	5.2 \pm 1.1%	77.9 \pm 0.3%
“whitebait”	9.7 \pm 1.4	98.6 \pm 4.6	2.3 \pm 0.9%	82.4 \pm 0.9%
Far Glory Ocean Park				
<i>Cololabis sarira</i>	66.5 \pm 7.0	274.6 \pm 8.2	17.0 \pm 0.9%	68.9 \pm 1.2%
<i>Scomber japonicus</i>	121.1 \pm 18.3	239.9 \pm 14.7	3.4 \pm 0.4%	78.5 \pm 0.8%
<i>Mallotus villosus</i>	21.9 \pm 2.0	151.6 \pm 4.9	1.5 \pm 0.9%	82.2 \pm 1.2%
<i>Trachurus japonicus</i>	115.4 \pm 18.5	234.6 \pm 16.2	6.7 \pm 0.8%	87.3 \pm 0.7%

Table 2.2: Isotopic composition (\pm SE) of prey items offered to *ex situ* dolphins and overall isotopic content of diets used in the present study

Diet	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
SeaWorld		
<i>Clupea harengus</i>	$11.87 \pm 0.09\text{‰}$	$-19.86 \pm 0.09\text{‰}$
<i>Mallotus villosus</i>	$11.74 \pm 0.08\text{‰}$	$-19.87 \pm 0.05\text{‰}$
“whitebait”	$13.45 \pm 0.04\text{‰}$	$-15.67 \pm 0.08\text{‰}$
SWF-1 diet (60% <i>Mallotus villosus</i> , 40% <i>Clupea harengus</i>)	11.70‰	-19.42‰
SWF-2 diet (variable amounts of <i>Mallotus villosus</i> , <i>Clupea harengus</i> , and whitebait)	$12.04 - 12.46\text{‰}$	$-18.19\text{‰} - -19.27\text{‰}$
Far Glory Ocean Park		
<i>Cololabis sarira</i>	$7.55 \pm 0.11\text{‰}$	$-21.39 \pm 0.09\text{‰}$
<i>Scomber scombrus</i>	$11.22 \pm 0.14\text{‰}$	$-16.87 \pm 0.28\text{‰}$
<i>Mallotus villosus</i>	$12.83 \pm 0.06\text{‰}$	$-19.73 \pm 0.05\text{‰}$
<i>Trachurus japonicas</i>	$11.38 \pm 0.09\text{‰}$	$-17.02 \pm 0.06\text{‰}$
Diet A (80% <i>Cololabis sarira</i> , 20% <i>Scomber scombrus</i>)	8.30‰	-20.48‰
Diet B (80% <i>Mallotus villosus</i> , 20% <i>Trachurus japonicas</i>)	12.54‰	-19.19‰

Table 2.3: Isotope values (top) (\pm SE) and diet-tissue discrimination values (bottom) for SWF and FGOP dolphins. FGOP dolphins were sampled after being on either diet A or B for 7-8 weeks. Superscript letters denote a lack of significant difference.

Group	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
SWF-1 – adults	13.66 ± 0.15 (n=8) ^{b,c}	-18.26 ± 0.19 (n=8) ^{a,b,c}
SWF-2 – adults	14.21 ± 0.10 (n=14) ^c	-17.91 ± 0.68 (n=14) ^{a,b,c}
SWF-2 – juveniles	14.58 ± 0.15 (n=5) ^c	-18.53 ± 0.20 (n=5) ^a
FGOP – pre-experiment	12.61 ± 0.31 (n=12) ^{a,b}	-17.71 ± 0.21 (n=12) ^c
FGOP – diet A	11.81 ± 0.55 (n=6) ^a	-18.41 ± 0.18 (n=6) ^a
FGOP – diet B	13.73 ± 0.44 (n=6) ^{b,c}	-17.81 ± 0.19 (n=6) ^{b,c}

Group	$\Delta^{15}\text{N}$ (‰)	$\Delta^{13}\text{C}$ (‰)
SWF-1 – adults	2.09 ± 0.07 (n=8) ^a	1.28 ± 0.16 (n=8) ^b
SWF-2 – adults	1.90 ± 0.08 (n=14) ^a	0.66 ± 0.07 (n=14) ^a
SWF-2 – juveniles	2.61 ± 0.14 (n=5) ^b	0.53 ± 0.19 (n=5) ^a
FGOP – diet A	2.96 ± 0.12 (n=6) ^b	2.04 ± 0.14 (n=6) ^c
FGOP – diet B	1.68 ± 0.11 (n=6) ^a	1.60 ± 0.09 (n=6) ^{b,c}

Table 2.4: Exponential decay equations and half-lives for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ turnover in epidermal tissue of *ex situ* dolphins at FGOP

Animal ID	Diet	Equation	R ²	Half-life (days)	$\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ on day 0 (‰)
$\delta^{15}\text{N}$					
Dolphin 13	B	$y = 14.64 + (-3.63) e^{-0.043t}$	0.93	16.1	11.16
Dolphin 3	B	$y = 14.29 + (-3.08) e^{-0.048t}$	0.70	14.4	10.87
Dolphin 5	B	$y = 14.13 + (-2.93) e^{-0.035t}$	0.98	19.8	11.31
Dolphin 7	B	$y = 14.68 + (-3.03) e^{-0.031t}$	0.99	22.4	11.46
		mean		18.2 ± 3.6	11.2 ± 0.2
Dolphin 6	A	$y = 11.36 + (3.13) e^{-0.042t}$	0.94	16.5	14.24
Dolphin 9	A	$y = 11.06 + (3.89) e^{-0.049t}$	0.99	14.1	14.08
		mean		15.3 ± 1.7	14.2 ± 0.1
$\delta^{13}\text{C}$					
Dolphin 13	B	$y = -17.63 + (-1.06) e^{-0.063t}$	0.83	11.0	-18.61
Dolphin 3	B	$y = -17.70 + (-1.14) e^{-0.059t}$	0.87	11.7	-18.84
Dolphin 5	B	$y = -17.86 + (-0.76) e^{-0.059t}$	0.77	11.7	-18.52
Dolphin 7	B	$y = -17.52 + (-1.13) e^{-0.033t}$	0.95	21.0	-18.68
		mean		13.9 ± 4.8	-18.7 ± 0.1
Dolphin 6	A	$y = -18.81 + (1.70) e^{-0.031t}$	0.90	22.4	-17.38
Dolphin 9	A	$y = -18.59 + (1.11) e^{-0.032t}$	0.87	21.7	-17.48
		mean		22.1 ± 0.5	-17.4 ± 0.2

Exponential decay curves ($y(t) = y_a + ae^{-bt}$) were fit with y_a set to the expected value based on diet and the calculated diet-specific value for $\Delta^{15}\text{N}$ or $\Delta^{13}\text{C}$ as appropriate.

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CHAPTER 3: ASSESSING RESOURCE PARTITIONING OF SOUTH AFRICAN DELPHINIDS THROUGH STABLE ISOTOPE ANALYSIS

Introduction

Members of the family Delphinidae are found globally in all types of aquatic habitats with some having broad ranges and others having more restricted distributions. Many species of delphinids co-exist in the same geographic region or have overlapping distributions. For example, near-shore delphinids found in the Indian Ocean off the south-eastern coast of South Africa include the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), the common dolphin (*Delphinus capensis*), the striped dolphin (*Stenella coeruleoalba*), and the humpback dolphin (*Sousa chinensis*). All of these species, particularly the inshore inhabitants, face conservation threats such as incidental catch in fisheries' nets (e.g., Cockcroft and Krohn 1994, Amir *et al.* 2005) as well as shark nets (e.g., Cockcroft and Ross 1990, Peddemors *et al.* 1990, Meyer *et al.* 2011), habitat loss (e.g., Kiszka *et al.* 2009), and the overfishing of prey species (e.g., Sekiguchi *et al.* 1992, Roy *et al.* 2007).

Relatively little is known about the feeding ecology of these species and several are considered to be at risk. The Indo-Pacific bottlenose dolphin has been designated as “data deficient” by the International Union for Conservation of Nature (IUCN 2008) because it is a coastal species, potentially impacted by habitat degradation that has yet to be quantified. These dolphins are typically found in groups averaging 60 individuals off of South Africa but numbers can vary with locale and have been observed in mixed groups with other delphinid species, both close to shore and in deeper waters (Saayman 1972). Observational studies suggest that bottlenose dolphins typically aren't found in waters deeper than 30 m off the South African coast

(Cockcroft pers. comm.). Common dolphins are also listed as “data deficient” by the IUCN (2008) due to a lack of information on how incidental and direct takes has affected their populations. This species frequently travels in large groups averaging around 250 individuals, but can reach numbers in the thousands. Striped dolphins are listed as being of “least concern” due to a relatively large population size, whereas the humpback dolphin is most at risk and has been designated as “near threatened” (IUCN 2008). Being a coastal dolphin, humpback dolphins suffer from habitat destruction and both incidental and direct takes in fisheries (e.g., Karczmarski 2000). Humpback dolphins are typically found close to shore in small groups of typically less than ten individuals. The entire South African *Sousa* population has been estimated to be 1000 animals or less (Karczmarski 1996). Coastal pollution is another threat to this species and it has been found that humpback dolphins have the highest levels of chlorinated hydrocarbons (PCBs, DDT and dieldrin) compared to other marine mammals in the region of the Kwazulu-Natal coast (Cockcroft *et al.* 1991).

Collectively, these four species have been observed in the same geographic areas, and in some cases, have been observed occurring in mixed-species groupings, suggesting that there is either interspecific competition for resources, or that these sympatric dolphins are partitioning resources. Given that there are several known conservation pressures that may negatively impact these populations, it is critical to investigate whether there are additional pressures being exerted through competition for resources. Additionally, insight into the ecology of these four sympatric species will allow for the development of appropriate policies and management efforts to conserve and protect them.

Historically, research into the feeding ecology of marine mammals has been approached in a variety of ways, including anecdotal observations (e.g., Caldwell and Caldwell 1972, Irvine

et al. 1981, Shane 1990), fecal analysis of hard remains (e.g., Sinclair and Zeppelin 2002), DNA analysis of feces (e.g., Meekan *et al.* 2009), examination of stomach contents of dead stranded animals (e.g., Gunter 1942, Barros and Odell 1990, Mead and Potter 1990, Barros 1993, Barros and Wells 1998, Barros *et al.* 2000), and stomach lavage (Antonelis *et al.* 1987). More recently indirect assessments of feeding habits using stable isotopes and fatty acid signature analysis have been added to gut content and fecal analysis (Wang *et al.* 2002, Bearzi 2005, Gross *et al.* 2009, Loseto *et al.* 2009). While all are useful techniques, each has limitations. Observing feeding habits of aquatic animals is logistically challenging and unpredictable since a large proportion of time is spent underwater. Collection of fecal matter and identifying source animals presents challenges with this type of analysis given the fluid environment and that animals are often submerged. Collecting stomach contents from live animals (lavage) is highly invasive and identification of stomach contents either from live or dead animals may be difficult due to erosion of the hard parts and may only represent part of the diet as prey lacking hard parts will not be retained in the gut of the predator and in other cases some hard parts, such as squid beaks, can be retained in the stomach and thereby overestimate the dietary significance of some species.

Stable isotope analysis can overcome some of these limitations. Stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) have become a common tool in many ecological studies, including marine mammal feeding ecology (e.g., Barros *et al.* 1995, Das 2000, Ruiz-Cooley *et al.* 2004, Alves 2007, Newsome *et al.* 2009, Witteveen *et al.* 2009). Stable isotope signatures in consumer tissues reflect that of the isotopic ratio in the diet (e.g., Deniro and Epstein 1978, Deniro and Epstein 1981, Fry 1988). Lighter forms of isotopes, ^{12}C and ^{14}N , occur more frequently than their heavier counterparts, ^{13}C and ^{15}N , in consumer tissues because lighter isotopes are preferentially selected in certain biochemical processes (Caut *et al.* 2009). This bias

to lighter isotopes is called diet-tissue discrimination (or as fractionation) and causes consumer tissues to have a different isotope ratio than that of prey tissues. This results in a step-wise enrichment where the heavier isotope increases in concentration relative to the standard for that particular element and ultimately allows for the measurement of ratios of stable isotopes.

Nitrogen isotope ratios indicate trophic level, whereas carbon isotopic ratios indicate sources of primary production (Rau *et al.* 1983, Fry 1988). Nitrogen isotope ratios can be used as an indicator of trophic level due to a predictable step-wise increase with each trophic level within a food chain, while carbon values reflect the contributions of primary producers, hence allowing distinction between terrestrial, freshwater or marine sources, and within the latter, offshore versus inshore (Rau *et al.* 1983, Fry 1988).

Using stable isotopes to determine specific prey species consumed by a predator that has many contributing sources to its diet, including those with similar isotopic signatures, proves challenging although there are a number of modeling approaches that have been developed in recent years to attempt to do so including Bayesian modeling approaches such as SIAR (Stable Isotope Analysis in R) (e.g., Gomez-Campos *et al.* 2011b, Witteveen *et al.* 2012). Even more recently, metrics have been developed for calculating isotopic niche width using SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson *et al.* 2011). This latter approach allows for prediction of trophic diversity with a larger niche width (elliptical area), indicating larger trophic diversity (more of a generalist consumer) and a smaller niche width (elliptical area) indicating a smaller trophic diversity, indicating more of a specialist consumer (Thomson *et al.* 2012). In spite of these new developments, teasing apart prey items that have overlapping isotopic signatures is impossible, however, basic analysis of these isotopes does allow for inter- and intra- specific comparisons of trophic levels, niche width and habitat utilization.

Based on observational reports in the literature, there is an indication that these four species are, to some extent, utilizing their habitat in varying ways. Saayman (1972) witnessed some of these species in mixed aggregates but also noted that striped dolphins tend to stay further offshore and humpback dolphins tended to stay very close to the shoreline, indicating the partitioning of spatial resources. Stomach content studies are limited on these four species but what has been reported, again, indicates some degree of partitioning of prey resources. Cockcroft and Ross (1990) found differences among prey species found in the guts of bottlenose and humpback dolphins and also found different diet compositions between male and female bottlenose dolphins. When looking at stomach content studies of striped dolphins, they tend to eat mostly deep water, offshore species and when diversity of prey items consumed were compared to that of common and bottlenose dolphins, they had a much lower dietary range (Sekiguchi *et al.* 1992).

These sympatric delphinid species may partition resources through variations in habitat use, temporal activity, and/or dietary preferences with the result that coexistence is possible and competition is reduced (Saayman and Taylor 1973, Baird and Whitehead 2000, Parra 2006, Spitz *et al.* 2011, Wang *et al.* 2012). Of these, feeding habits are believed to be the largest driving force in niche differentiation (Wang *et al.* 2012), thus, an understanding of species-specific habitat utilization and inter-specific trophic relationships is fundamental to making appropriate conservation and management decisions for cetaceans. There are suggestions in the literature that these four species of dolphins are partitioning resources off the coast of South Africa. Observational studies indicate that there is a gradient in the use of inshore and offshore waters among these species, implying the partitioning of habitat resources (Saayman *et al.* 1972). Limited stomach content studies also suggest that there may be some partitioning of prey

resources. Cockcroft and Ross (1990) examined stomach contents of both South African bottlenose and humpback dolphins and indicated that although there was some overlap of prey species found in stomachs of both dolphin species, half of the items found in the guts of humpback dolphins were not present in bottlenose stomachs. Prey species that were most important to common dolphins differed yet again with a heavy dependence on large schooling fish species such as anchovies and sardines (Young and Cockcroft 1994), whereas striped dolphins foraged mostly on pelagic, offshore species such as hake and young chokka squid (Ross 1984, Sekiguchi *et al.* 1992). Based on these observations, it was hypothesized that these South African delphinids would exhibit interspecific and intraspecific partitioning of both habitat and prey resources and will exhibit differences in isotopic niche width, all allowing for a reduction of competition for resources among these broad sympatric species. Based on these suggestions, the goal of the present study was to use carbon and nitrogen stable isotope signatures to determine any potential inter- and intra-specific resource partitioning among the four delphinid species residing off the coast of South Africa and to measure isotopic niche width for each of these species.

Methods

Sample Collection

Skin samples were collected opportunistically between 1995 and 2005 from dead dolphins that became entangled in shark nets deployed off the coast of Kwazulu-Natal, South Africa (Figure 3.1) as a means to protect popular swimming beaches. These nets were deployed and monitored by the Kwazulu-Natal Sharks Board and were typically placed approximately 400

m offshore in water depths of 10-14 m. Forty five km of nets were set in 1995 off of the Natal coast but net coverage was gradually reduced to about 40 km by 2005. Nets were checked daily for entangled animals by the staff of the Sharks Board, weather permitting. Entangled dolphins were collected and frozen at -20°C until sample harvesting. During necropsies sex was determined, morphometric measures measured, and skin samples collected. Frozen samples were then transported and held at the Port Elizabeth Museum, Port Elizabeth, South Africa, and stored at -20°C. A total of 227 skin samples (approximately 3g each) were collected from adult bottlenose dolphins (*Tursiops aduncus*, n=119), common dolphins (*Delphinus capensis*, n=78), humpback dolphins (*Sousa chinensis*, n=27), and striped dolphins (*Stenella coeruleoalba*, n=3).

An additional 13 skin samples were collected in 2008 from live adult free-ranging bottlenose dolphins off the south-central coast of South Africa in the waters of Plettenberg Bay (Figure 3.2). Biopsy samples were collected using a corer-tipped bolt launched from a crossbow (150 lb. pull) (Figure 3.3). The bolt had a ring at the base of the tip to prevent the dart from becoming embedded and to help the dart bounce back out of the animal. The modified tip contained a barb to retain the sample (skin and part of the blubber layer) and the bolt had floats attached to the shaft, allowing for its retrieval using a dip net. Samples were immediately removed from the barb using sterilized forceps, placed into a vial labeled with the date and sample identification number, and stored on ice until return to shore. Additional information gathered at each event was sampling location (longitude and latitude), sea conditions, whether the target individual was traveling alone or in a group, estimated body length, and behavior pre- and post-darting. Photographs of dorsal fins were also taken as part of a separate, on-going study to identify individual dolphins using dorsal fin characteristics. Upon return to shore, skin was separated from the underlying blubber layer and kept frozen at -20°C until further processing.

Sample Preparation and Stable Isotope Analysis

Skin collected from biopsies was initially placed in a drying oven at 60°C for 24 hours to remove water, and then lipid extracted using petroleum ether in a Soxhlet extractor for 24 hours. Lipid-extracted samples were again placed in a drying oven for 24 hours to remove any remaining solvent. Dried, lipid-extracted samples were then ground, using a Wig-L-Bug grinder (Crescent, model MSD), into a homogeneous powder. Aliquots (0.9-1.5 mg) were then sealed into 5 mm by 9 mm tin capsules and analyzed at the University of Georgia, Institute of Ecology Stable Isotope Laboratory using a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer (IRMS). Data were expressed and reported in parts per thousand (‰) using delta notation (δ):

$$X(\text{‰}) = \left(\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right) \times 1000 \quad (5)$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$. Standard reference materials were carbon from PeeDee Belemnite limestone and atmospheric nitrogen gas. To assure quality control in sample analysis of stable isotope ratios, a known standard sample (bovine tissue) was run after every 12 unknown samples (dolphin skin). Analytical errors for the standard samples (n=20) were $\pm 0.01\text{‰}$ (SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Statistical Analyses

Data were tested for normality using Shapiro-Wilks (n < 50) and Kolmogorov-Smirnov tests (n > 50), and tested for homogeneity of variance using Levene's test. Differences among species, sex, location and season were explored separately for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ using general

linear model (GLM) univariate analysis. The sex variable was divided into three categories: male, female, and unknown, with the latter grouping included to account for samples collected through biopsy darting of live animals. Tukey's HSD post hoc tests were performed to determine homogeneous subsets. Raw data were analyzed in general linear models because: transformations failed to improve the few non-normal data; visual inspection of normal Q-Q plots and histograms indicated normality; and general linear models are considered robust to deviations from normality (Field 2005). Statistical analyses were conducted using SPSS 20.0 with a critical value of $\alpha = 0.05$. Values reported are mean \pm SE. In addition, niche width was explored for each species using the SIBER function in the SIAR package and reported as standard ellipse area (SEA)(%²).

Results

Stable isotope ratios were analyzed for 240 dolphin skin samples (Figure 3.4). Isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were normally distributed for all species except bottlenose dolphins (K-S = 0.079, $p = 0.043$). Females, males and, animals of unknown sex were each distributed normally for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. However, seasonal data were significantly non-normal for winter (K-S = 0.081, $p = 0.011$ for $\delta^{15}\text{N}$ and K-S = 0.075, $p = 0.027$ for $\delta^{13}\text{C}$) and results were mixed for the summer season (K-S = 0.120, $p = 0.007$ for $\delta^{15}\text{N}$ and K-S = 0.068, $p = 0.200$ for $\delta^{13}\text{C}$).

For all data combined, the mean $\delta^{15}\text{N}$ was 14.16 ± 0.06 and the mean $\delta^{13}\text{C}$ was -15.62 ± 0.05 . Mean values for $\delta^{15}\text{N}$ ranged from 11.92 ± 0.11 (n=3) for striped dolphins to 14.95 ± 0.19 (n=27) for humpback dolphins. The same trend was seen in mean $\delta^{13}\text{C}$ values with $-16.97 \pm$

0.14 for striped dolphins and a high mean value of -15.16 ± 0.12 for humpback dolphins (Table 3.1).

Species ($F_{3,228} = 24.605$, $p < 0.001$) and sexes ($F_{2,228} = 13.919$, $p < 0.001$) were significant for $\delta^{15}\text{N}$ values, though seasons were not ($F_{1,228} = 1.852$, $p = 0.175$), thus making interactions of species and sex with season insignificant. Data for $\delta^{13}\text{C}$ in dolphin skin were significant among species ($F_{3,228} = 13.970$, $p < 0.001$) and thus most interactions including species were all significant as well. Remaining factors were not found to be significant: sex ($F_{2,228} = 1.641$, $p = 0.196$), season ($F_{1,228} = 3.158$, $p = 0.077$) and the interaction of species and sex ($F_{2,228} = 0.045$, $p = 0.956$).

Locational effects were tested for bottlenose dolphins between the Kwazulu-Natal shark net caught animals and the biopsied Plettenberg Bay animals. There were no significant differences found between bottlenose dolphins sampled in Durban shark nets or those biopsied in Plettenberg Bay for either nitrogen or carbon ($F_{1,130} = 0.903$, $p = 0.344$ for ^{15}N , and $F_{1,130} = 1.493$, $p = 0.224$ for ^{13}C) (Figure 3.5) and therefore bottlenose dolphins were considered to be a single group.

Tukey's HSD post hoc tests revealed that bottlenose, common, humpback and striped dolphins were all significantly different from one another for $\delta^{15}\text{N}$ (all p values ≤ 0.01 ; *Stenella coeruleoalba* 11.92 ± 0.11 , *Delphinus capensis* 13.66 ± 0.08 , *Tursiops aduncus* 14.35 ± 0.07 , *Sousa chinensis* 14.95 ± 0.19) (Figure 3.6). Both bottlenose and striped dolphins were significantly different from humpback and common dolphins (all p values ≤ 0.018) for $\delta^{13}\text{C}$ values, while the latter species were not significantly different from each other ($p = 0.131$) (Figure 3.6). Carbon signatures were significantly lower for striped (-16.97 ± 0.14) and bottlenose (-15.76 ± 0.06) dolphins, when compared with common (-15.48 ± 0.07) and

humpback (-15.16 ± 0.12) dolphins. When examining post hoc test results among categories of sex for $\delta^{15}\text{N}$ values, males and females were significantly different ($p < 0.001$) from one another but males and those of unknown sex were not significantly different ($p = 1.000$) (Figure 3.7).

The standard ellipse area (SEA) for common dolphins was 1.233‰^2 , for humpback dolphins it was 1.823‰^2 , for striped dolphins it was 0.029‰^2 , and finally, for bottlenose dolphins the SEA was 1.765‰^2 (Figure 3.8).

Discussion

The objective of the current study was to investigate potential trophic partitioning, based on stable isotope signatures, among and within four sympatric delphinid species inhabiting waters off the South Africa coast. Differences among species were significant for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and differences between sexes were also significant for $\delta^{15}\text{N}$, but not for $\delta^{13}\text{C}$. There were no significant differences observed between the two sampling locations (Natal and Plettenberg Bay) for bottlenose dolphins.

Stable isotope ratios of carbon primarily reflect the source of primary productivity and therefore can lend information about the habitat in which the predator has been foraging (e.g., Wada *et al.* 1987, Kurle and Worthy 2002). In aquatic ecosystems, carbon isotope ratios reflect differences between freshwater and marine sources as well as offshore/pelagic habitats versus inshore/benthic sources. Carbon isotope values tend to become more enriched from offshore to inshore locations, thus serving as a potential indicator of habitat usage (Gomez-Campos 2011a). Although there is some degree of overlap in the ranges of the four species measured in the current study, there are some known spatial differences.

Striped dolphin carbon isotope signatures were consistent with evidence that they typically forage further offshore (>500m depth; Saayman *et al.* 1972, Ross 1984, Findlay *et al.* 1992) and that they differed from the other three species (common, humpback, and bottlenose dolphins). Published stomach content analyses confirm this with the prevalence (over 80%) of prey items having luminous organs (Ross 1984). Although the interpretation of stable isotope results in the current study are consistent with that in the literature, caution is warranted due to low sample sizes.

Groups of common dolphins average 250 individuals but have shown variation with pods containing thousands of individuals (Findlay *et al.* 1992). They are typically found offshore in deeper, warmer waters, however, individuals off the Kwazulu-Natal coast come closer inshore once a year in response to the movement of sardines up the coast (Peddemors 1999). Prey found in stomach content analyses are typically of pelagic origin, once again echoing their offshore preference (Young and Cockcroft 1994).

It is estimated that there are approximately 900 resident bottlenose dolphins off the coast of Kwazulu-Natal with an average group size of 67 individuals (Cockcroft and Ross 1990). This species seems to be an opportunistic feeder with diets being comprised of over 72 species, although 6 species accounted for approximately 60% of the diet by mass (Cockcroft and Ross 1990).

Finally, estimates of abundance for the Kwazulu-Natal humpback dolphin population suggest that there are approximately 160 individuals (Durham 1994) with an average group size of 7 animals (Karczmarski 1996). These are found in shallow, inshore waters including estuaries and the mouths of rivers and enclosed bays (Karczmarski 1996) and the observation of demersal

inshore fish species in their stomach contents is consistent with their use of these inshore habitats (Barros and Cockcroft 1991).

In the present study, humpback dolphins had the most enriched $\delta^{13}\text{C}$ values while striped dolphins were most depleted. Common dolphins were not significantly different from humpback dolphins although on average, had slightly lower $\delta^{13}\text{C}$ values. Values for bottlenose dolphins fell between common and striped dolphins. These findings suggest some spatial separation when it comes to foraging, despite overlapping ranges. Based on carbon values in the current study, humpback and common dolphins are foraging closest inshore, followed by bottlenose dolphins and then finally striped dolphins which would be feeding in more offshore waters. Stomach content analysis of stranded humpback dolphins identified fish species associated with inshore and estuarine habitats, consistent with suggestions that humpback dolphins utilize inshore waters (Barros and Cockcroft 1991). An observational study by Saayman *et al.* (1972) confirmed that South African humpback dolphins remained near shore (within 250 m), foraging on reefs in turbid waters along rocky coastlines. They also classified striped dolphins as pelagic, feeding offshore in deeper waters, although occasionally coming in as close as 1 km to shore. Contrary to the findings of the current study that indicate humpback and common dolphins forage in similar ranges, Saayman *et al.* (1972) observed bottlenose dolphins more often in close vicinity to humpback dolphins than common dolphins. Bottlenose dolphins were observed following similar inshore routes as humpback dolphins and on occasion were seen in mixed species groups with humpbacks. It was also noted by Saayman *et al.* (1972), however, that bottlenose dolphins spent similar amounts of time in shallow inshore waters as they did in deeper waters. The stable isotope data reported here would have been influenced if bottlenose dolphins frequented offshore waters as often as inshore and as a result, $\delta^{13}\text{C}$ values would be the average of these two habitats.

Another plausible explanation for the unexpected placement of bottlenose and common dolphins with respect to their carbon isotope values could be related to the activities of the common dolphins. Although typically offshore animals, it has been observed that common dolphins will come inshore in response to the sardine migration which occurs in the 200 m isobath (Crawford 1981, Peddemors 1999). Stomach content studies of stranded and shark net entangled animals reveal sardines as part of their diet (Sekiguchi *et al.* 1992, Young and Cockcroft 1994). If common dolphins are traveling near shore to feed on sardines, it would be expected for their carbon signature to become more enriched as inshore habitats tend to be more carbon enriched than offshore (Hobson *et al.* 1994).

According to Saayman *et al.* (1972), these four species of dolphins would generally occur from inshore to offshore in the following order: humpback, bottlenose, common and striped dolphins. Sekiguchi *et al.* 1992 used stomach content analysis to also classify Indo-Pacific bottlenose dolphins as feeding inshore, followed by common dolphins feeding in the upper column of deeper waters and then striped dolphins in even deeper, offshore waters.

Nitrogen isotopic signatures indicate differences in the trophic levels of consumers. Clear distinctions in nitrogen signatures were observed among the four species. This indicates differences in the prey types or proportions of prey taken in by these delphinids and illustrates some degree of resource partitioning. Humpback dolphins were most enriched in $\delta^{15}\text{N}$ and averaged 3‰ greater than the most nitrogen depleted of the four species, the striped dolphin. This difference suggests that humpback dolphins forage approximately one trophic level above striped dolphins in this ecosystem (Rau *et al.* 1983). In marine mammals, the accepted $\delta^{15}\text{N}$ enrichment value between trophic levels is between 2 and 5‰ depending on the species studied (Fry 1988, Hobson *et al.* 1994, Hobson *et al.* 1996, Gomez-Campos 2011a , Chapter “2” present

study). Bottlenose and common dolphins were intermediate in ^{15}N but closer to humpbacks than to striped dolphins. The results reported here are generally consistent with reported analyses of stomach contents. Cockcroft and Ross (1983) and Young and Cockcroft (1994) examined stomach contents of South African delphinids and found that common dolphins preyed primarily on pilchard (*Sardinops ocellatus*), which are offshore pelagic fish that are filter feeders, whereas bottlenose and humpback dolphins fed mostly on inshore species. The prey of importance to bottlenose dolphins were piggies (*Pomadasys olivaceum*), a benthic predator that feeds on prey such as small shrimp and flatworms, and cuttlefish (*Sepia spp.*), while the species that were most commonly found in the stomachs of humpback dolphins were glassnoses (*Thrissa vitrirostris*), striped grunter (*Pomadasys striatum*) and cuttlefish (*Sepia spp.*). Glassnoses are known to be filter feeders, eating plankton and fish larvae and are primarily found off the South African coast in estuaries and bays and striped grunters can also be found in bays and along shallow, rocky coasts eating small crustaceans (Van Der Elst 1993). Barros and Cockcroft (1999) also looked at stomach contents of bottlenose and humpback dolphins and found that of the 64 species of teleosts and cephalopods consumed by humpback dolphins, only 31 were also found in the stomachs of bottlenose dolphins indicating some differences in the utilization of food resources. Because a majority of the species found in humpback dolphin stomachs were sound-producing fish, Barros and Cockcroft (1999) hypothesized that these species forage in the shallow, turbid waters of estuaries, whereas bottlenose dolphins also hunted in clearer, deeper waters. Young and Cockcroft (1994) found pilchard to be an important prey species to common dolphins, among other small pelagic fish. Sekiguchi *et al.* (1992) examined stomach contents of 20 small African odontocetes of which included bottlenose, common and striped dolphins. Their results echoed the findings of previously mentioned studies and they concluded that bottlenose dolphins

fed near the coast on species such as chokka squid, mullet (*Mugil sp.*), a benthic grazer, and some species that were found in offshore waters such as horse mackerel (*Trachurus capensis*) that feed on other small fish and crustaceans. They also found common dolphins were feeding on pelagic schooling fish with the most common prey species being pilchard. Finally, striped dolphins seemed to prey on deeper water species such as young chokka squid and hake (*Merluccius sp.*) and stomach content analysis revealed that at least 80% of prey had luminous organs, indicating a deep water habitat (Ross 1984, Sekiguchi *et al.* 1992). Hake are known to feed on juvenile hake as well as horse mackerel (Van Der Elst 1993). Nam *et al.* (2011) calculated nitrogen isotope ratios for cuttlefish to be approximately 8‰, considerably lower than that of most prey items of both bottlenose (horse mackerel 13‰) and striped dolphins (hake 12.5‰) as reported by Iitembu *et al.* (2012). Nitrogen stable isotope values for these prey items are scarce in the literature, however, the works discussed above support the findings in the current study. Examining the natural history of these prey species also supports our findings of differences in both carbon and nitrogen signatures of their dolphin consumers. The $\delta^{15}\text{N}$ findings in the present study confirm what has previously been suggested by stomach content analysis studies, which is that these four species of South African dolphins are feeding on different prey items or differing proportions of prey, and as a result, have significantly different nitrogen signatures.

Male bottlenose dolphins were not significantly different from animals of “unknown sex”, suggesting that darted animals were likely male. Males and individuals of “unknown sex” were significantly more enriched in $\delta^{15}\text{N}$ than females, suggesting possible differences in prey selection between male and female dolphins. On average, males were enriched in $\delta^{15}\text{N}$ by 0.74‰ compared to females suggesting some dietary differences in prey composition.

Differences in foraging between male and female common dolphins has been documented (e.g., Young and Cockcroft 1994, Chou *et al.* 1995) and it has been shown that mature females had a significantly higher proportion of cephalopods in their stomachs compared to mature males (Silva 1999). Cockcroft and Ross (1990b) examined feeding in the Indian Ocean bottlenose dolphins off the coast of Natal, South Africa and found significant differences in male and female diets. In contrast, however, Amir *et al.* (2005), found no significant differences in the prey composition between male and female dolphins when examining stomach contents of Indo-Pacific bottlenose dolphins.

Pods of dolphins can vary dramatically in size, from a few to thousands of individuals, and can range from being extremely fluid with individuals coming and going constantly, to a much more rigid structure (Moeller 2012). When considering the composition of social groupings of most large delphinid pods however, they include mature males and females as well as immature individuals; therefore, it is not surprising that there would be some overlap in prey species taken. However, most of these groups retain a fission-fusion nature with an open social network where individuals move in and out due to the low energetic cost of locomotion (Randic *et al.* 2012). In mammals, female lifetime reproductive success is tied to access to resources such as food, while in males it is limited to access to receptive mates (Moeller 2012). This can result in males moving over larger areas than that of female dolphins, hence giving males access to differing prey items. Mature females may also be restricted in range and diving depths because of accompanying nursing calves and immatures that are limited in their distribution (Ringelstein *et al.* 2006). These factors may lend explanation for the significant differences in $\delta^{15}\text{N}$ between sexes seen in the current study, however, it must be kept in mind that differences among sexes may be an artifact of differences among species.

Isotopic niche width has been compared to traditional measures of niches used by ecologists. Because stable isotope measurements are influenced directly by what an animal consumes and its habitat, they are indicators of isotopic niche (Newsome *et al.* 2007). In the current study, humpback and bottlenose dolphins had the largest SEA, striped dolphins had the smallest SEA, and the SEA for common dolphins was intermediate. Larger SEA values reflect a broader trophic diversity, while smaller SEA values reflect a narrower trophic diversity or a more specialized niche. In general, delphinids are thought to be generalist consumers, although, some studies indicate that some species may be more selective in their prey choices such as targeting soniferous fishes (Gannon *et al.* 2005). The niche width of striped dolphins was considerably lower than the other three dolphin species indicating a lower trophic diversity. Stomach content analysis of striped dolphins revealed a heavier dependence on cephalopods over fish and striped dolphins had a lower measure of dietary diversity (measured by the Shannon-Wiener Information Measure) when compared to bottlenose and common dolphins (Sekiguchi *et al.* 1992). Although SIBER is robust with respect to small sample sizes and differences among sample sizes, it should be noted that the sample size for striped dolphins was much smaller than that of the other dolphin species.

In summary, this study supports evidence for resource partitioning in four sympatric South African delphinid species. Striped dolphins were most different from the other three species (common, bottlenose, and humpback), though more striped dolphin individuals need to be darted in subsequent work to confirm this pattern. Carbon isotope signatures indicated some differences in the way the habitat was utilized among the four species. Sympatric species are able to coexist by reducing competitive pressures through resource partitioning in three primary ways: temporal variation, diet composition, and habitat utilization (Friedlaender *et al.* 2009,

Wang *et al.* 2012). This study was the first of its kind to indicate that South African common, bottlenose, humpback, and striped dolphins partition foraging space and diet. All species showed a partitioning of diet, partly related to spatial differences in foraging also indicated. Studies such as these that reveal information on the ecology of apex predators like cetaceans are necessary to facilitate the proper management and conservation of these species. This is of particular importance when dealing with those at-risk species such as the humpback dolphin where conservation of its resources is imperative to the future success of its populations. For future stable isotope studies, it would be of value to incorporate the use of fatty acid signature analysis which is potentially able to tease apart differences in feeding ecology with finer resolution (see chapter “4”). In regards to the current study, perhaps this additional depth of detail might further reveal elements of resource usage by the South African dolphin species and could be used to evaluate any interactions with local fisheries.

Table 3.1: Mean values (\pm SE) of $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) by species.

Species	Sample Size (n)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
humpback dolphin (<i>Sousa chinensis</i>)	27	-15.16 ± 0.12	14.95 ± 0.19
common dolphin) (<i>Delphinus capensis</i>)	78	-15.48 ± 0.07	13.66 ± 0.08
bottlenose dolphin (<i>Tursiops aduncus</i>)	132	-15.76 ± 0.06	14.35 ± 0.07
striped dolphin (<i>Stenella coeruleoalba</i>)	3	-16.97 ± 0.14	11.92 ± 0.11

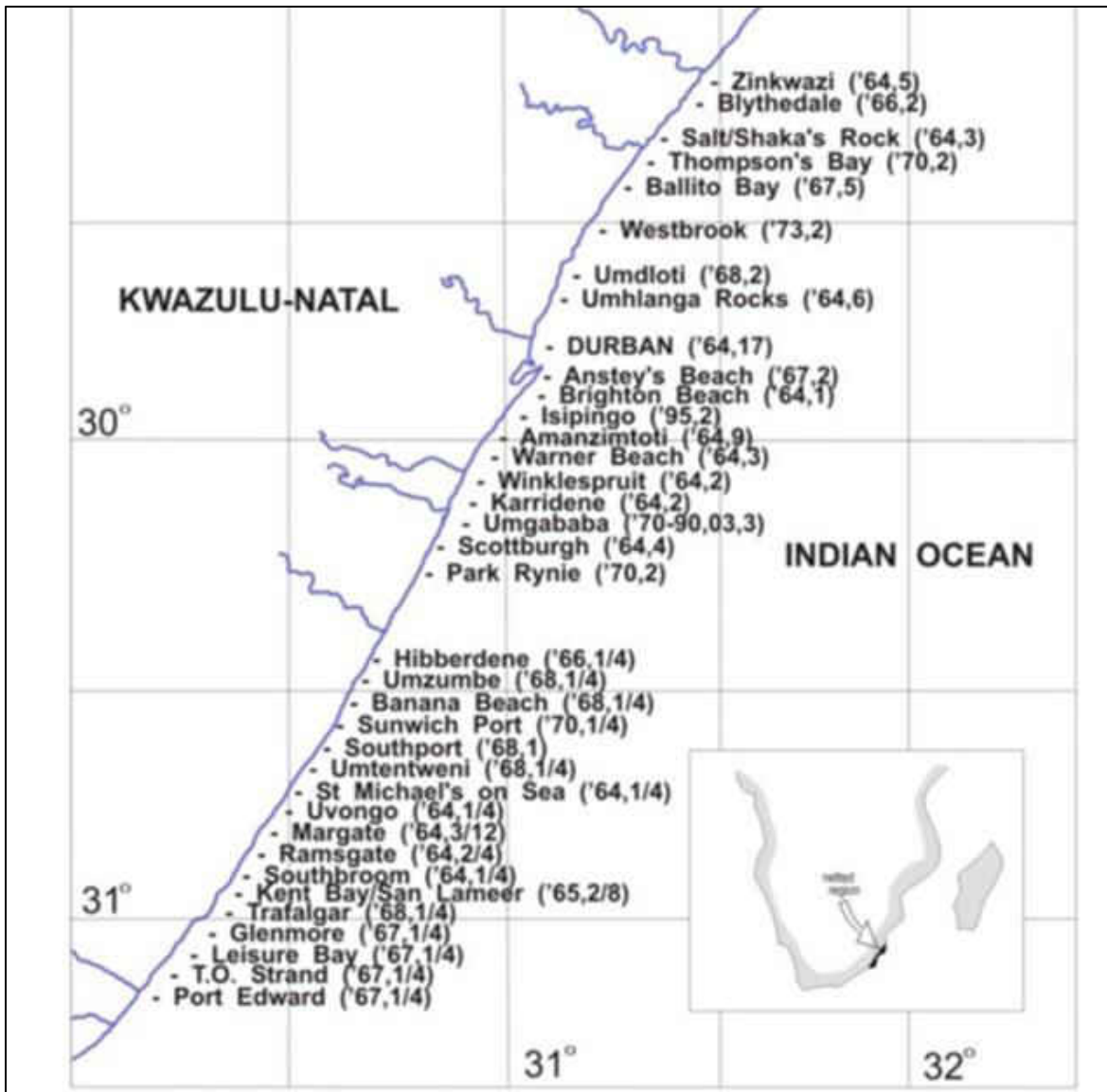


Figure 3.1: Map depicting shark nets deployed off the coast of Kwazulu-Natal. The first number found in the brackets indicates the year the nets were initially deployed and the second/third indicates the number of nets/drumlines used (As adapted by Kwazulu-Natal Sharks Board, 2011).

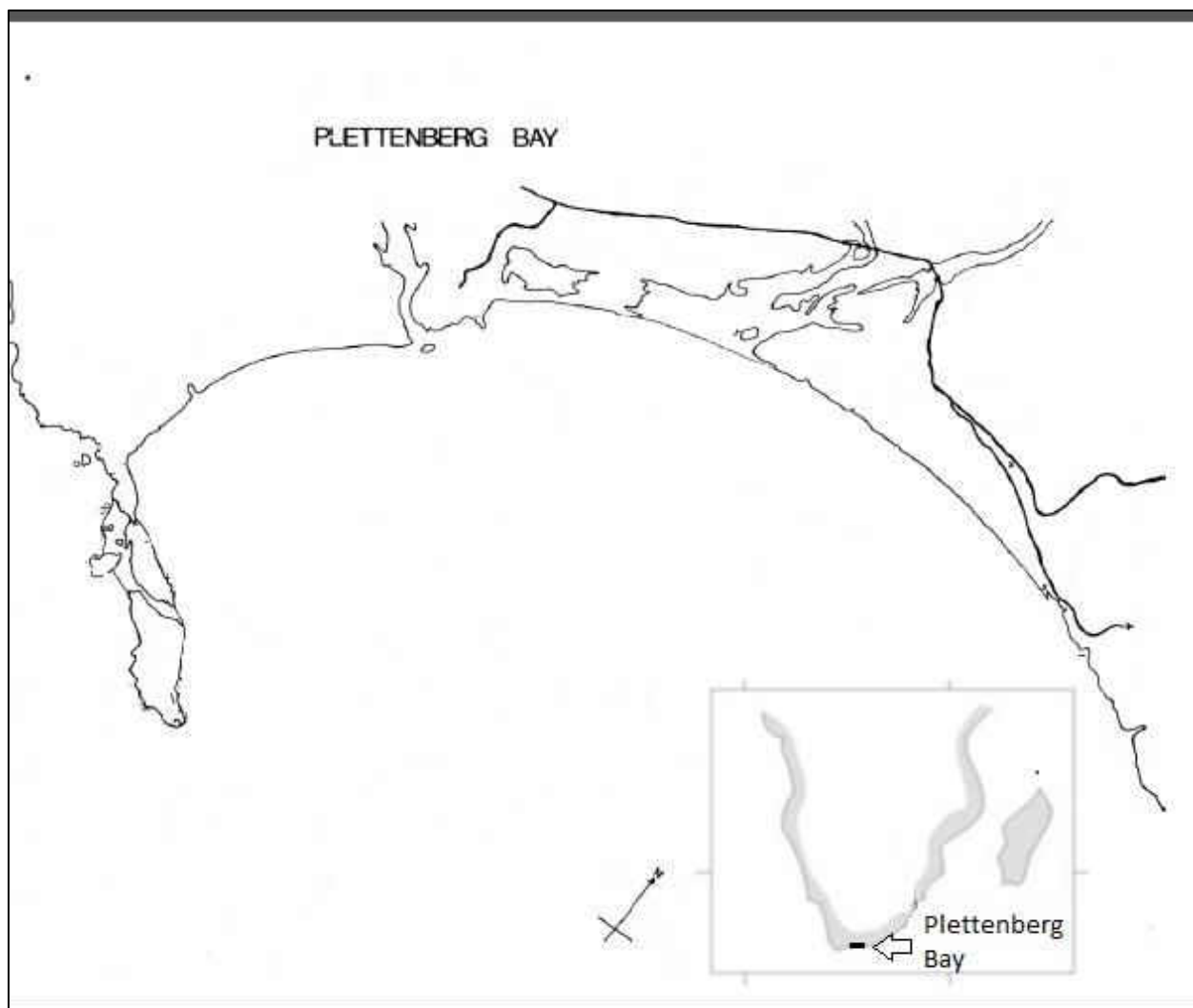


Figure 3.2: Map of Plettenberg Bay, South Africa with insert showing the location within the country.



Figure 3.3: Corer-tipped bolt used for biopsy sampling of South African delphinids.

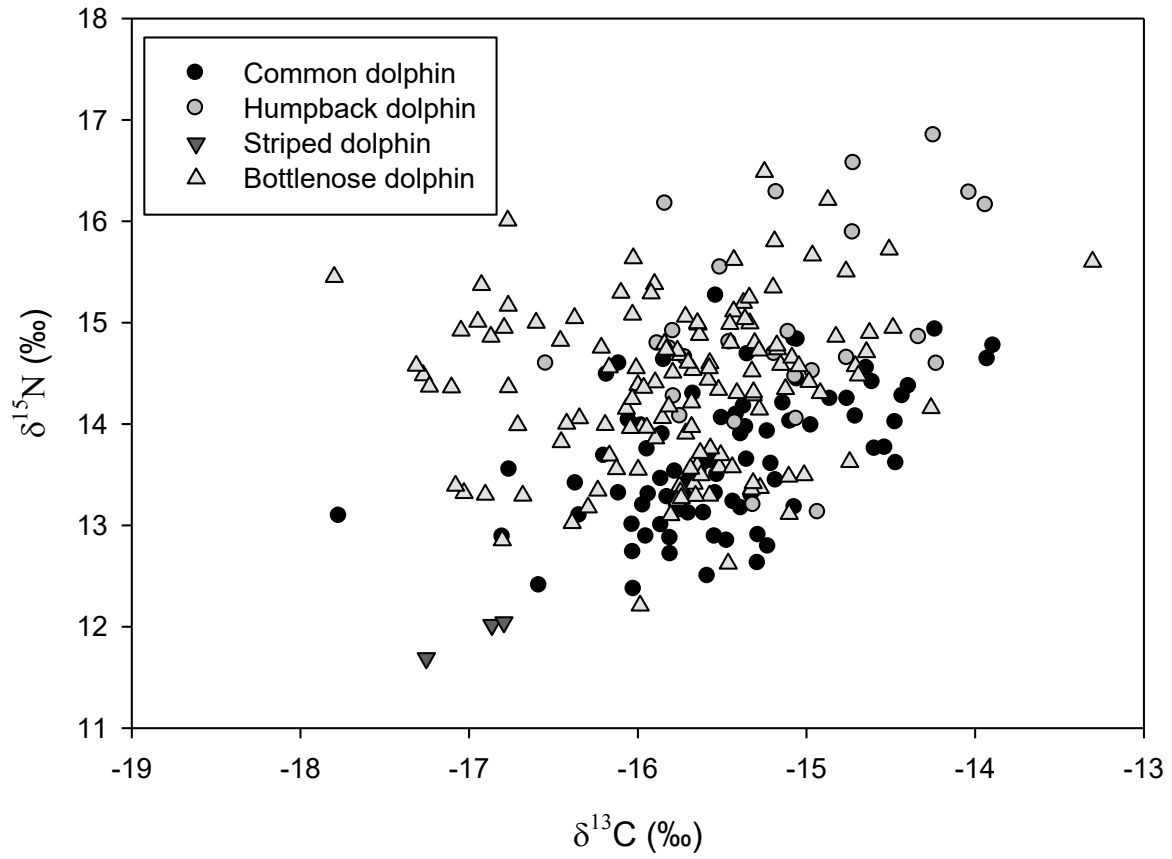


Figure 3.4: Stable isotope data for South African dolphin skin samples separated by dolphin species.

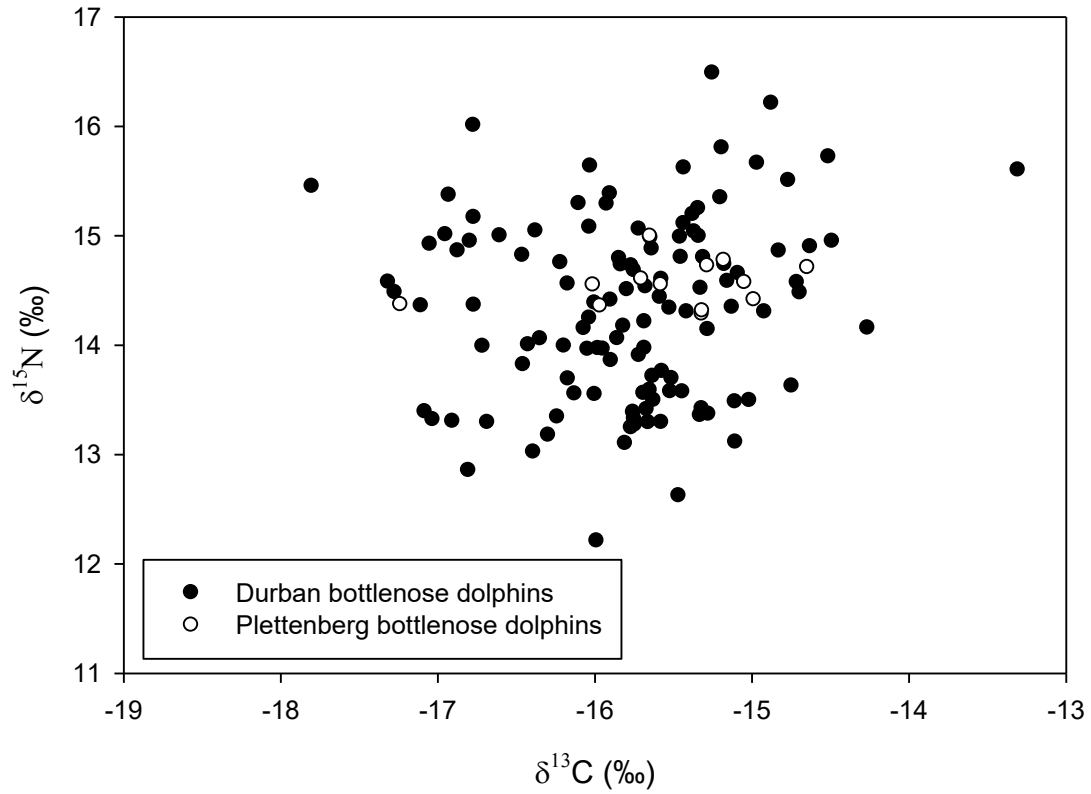


Figure 3.5: Stable carbon and nitrogen isotope values of South African bottlenose dolphin skin samples separated by collection location.

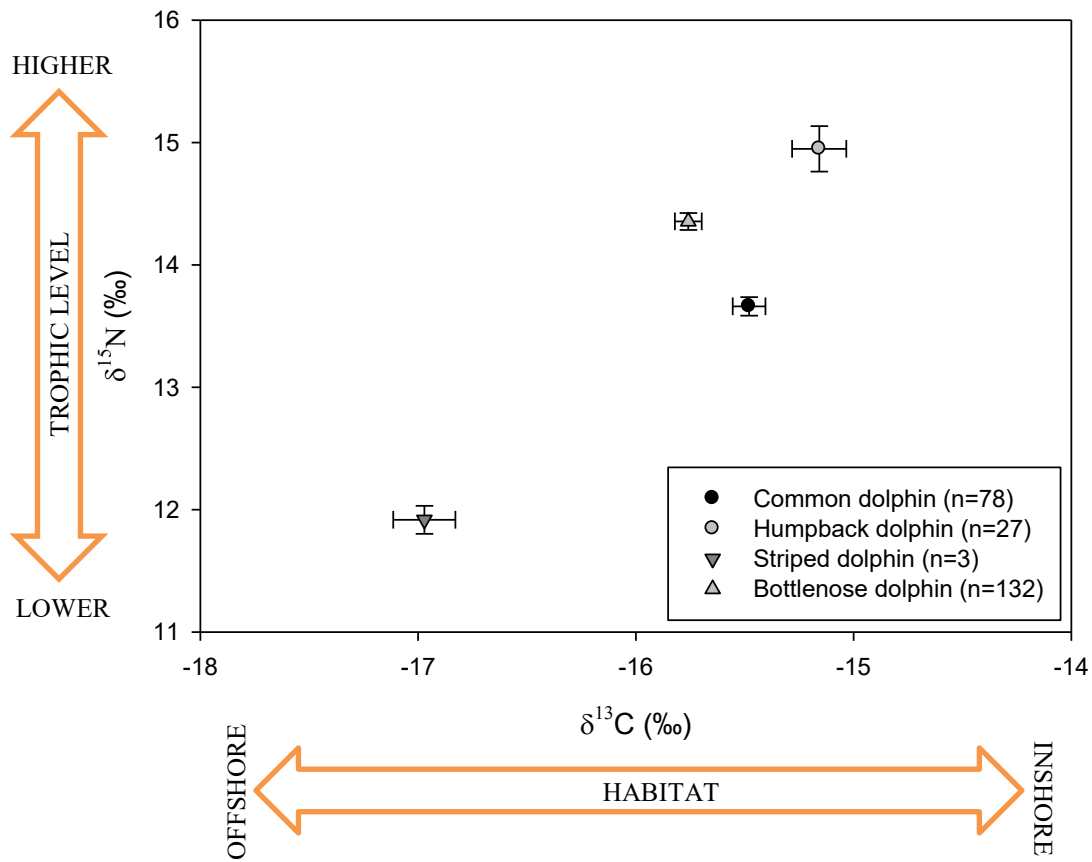


Figure 3.6: Carbon and nitrogen values of South African dolphin skin expressed as mean \pm SE.

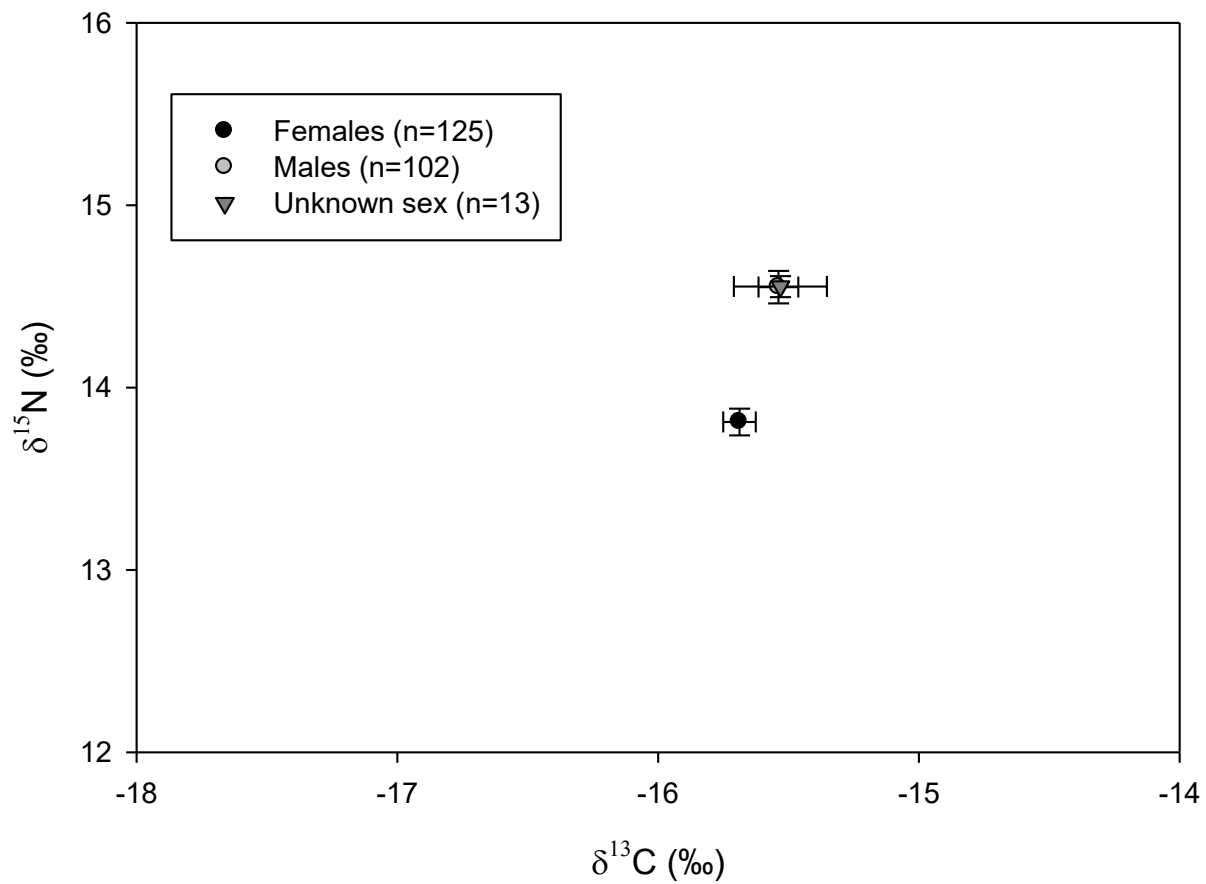


Figure 3.7: Combined stable carbon and nitrogen isotope values for males, females and animals of unknown sex for the four species of South African dolphins

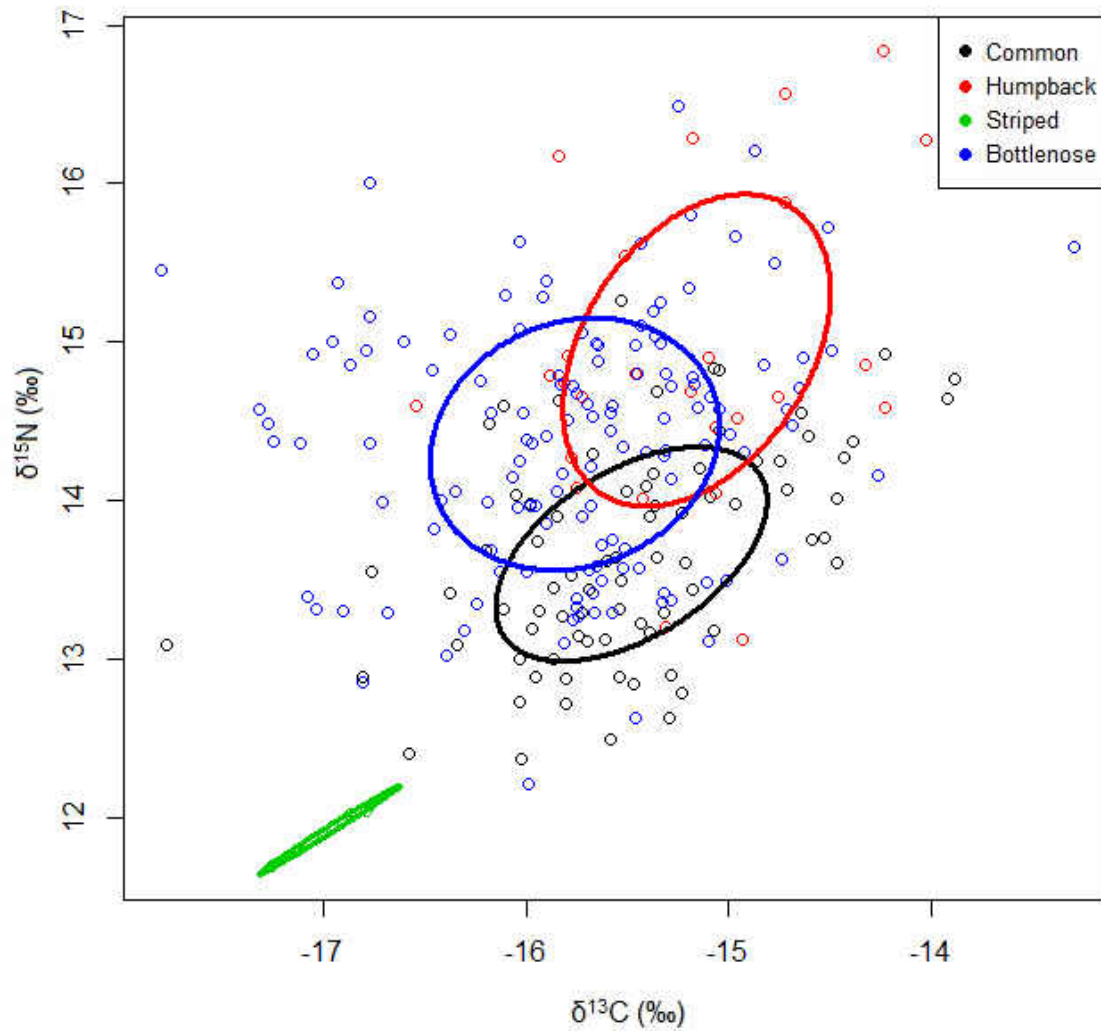


Figure 3.8: Biplot showing the isotopic signature of South African delphinids by species with standard ellipses as calculated using SIBER

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CHAPTER 4: EXAMINING FEEDING ECOLOGY AND HABITAT USAGE OF BOTTLENOSE DOLPHINS IN THE INDIAN RIVER LAGOON THROUGH STABLE ISOTOPE AND FATTY ACID ANALYSES

Introduction

Understanding an organism's feeding ecology and habitat usage provides important insight into that animal's biology and can allow scientists to make appropriate decisions regarding conservation efforts. For some species that are logistically difficult to observe, this can prove to be a challenge for researchers. Included in this category are marine mammals which do the majority of their feeding underwater and/or far out to sea where direct observations are difficult if not impossible. They also tend to be fast moving and can travel over large distances. To overcome these collective challenges, a variety of techniques have been used to indirectly study the feeding ecology of marine mammals such as fecal analysis (e.g., Sinclair and Zeppelin 2002), telemetry studies (e.g., Shippee *et al.* 2005), and analysis of stomach contents (e.g., Young and Cockroft 1994). While these methodologies have provided much of the information that contributes to our understanding of marine mammal feeding ecology, each has substantial limitations. Fecal analysis is obviously a challenge when dealing with species that spend their entire lives in the water where it is difficult to collect samples for analyses and to assign those samples to specific source animals when samples can be retrieved. Telemetry studies offer useful information regarding habit usage in the form of movement patterns and diving data; however, these studies are very costly and only provide information on a few individuals over a relatively short period of time (e.g., Shippee *et al.* 2005). Stomach content analysis is a rather invasive technique when done on live animals (stomach lavage), which only

provides information on the last meal ingested by that animal. In addition, stomach content analysis can be biased towards prey items that contain digestible resistant parts, thus potentially minimizing the importance of soft bodied species. Identification of prey items is limited to hard parts such as otoliths and squid beaks which themselves are vulnerable to erosion, making it difficult to properly identify species or accurately determine size of the diet items (Barros and Odell 1990). When this technique is generally applied to dead individuals, there is the potential that the animal died due to disease and therefore, its stomach contents may not accurately reflect the dietary preferences of a healthy member of the population. Because of these short-comings, researchers have increasingly turned to indirect techniques such as stable isotope analysis and fatty acid analysis. Often, these two techniques are used in conjunction to unveil different information about diet and feeding habits.

Feeding in bottlenose dolphins (*Tursiops truncatus*) has been studied in a variety of ways, including anecdotal observations of dolphins capturing prey (e.g., Caldwell and Caldwell 1972, Irvine *et al.* 1981, Shane 1990), examinations of stomach contents of dead stranded dolphins (e.g., Gunter 1942, Barros and Odell 1990, Mead and Potter 1990, Barros 1993, Barros and Wells 1998, Barros *et al.* 2000), the use of stable isotopes (e.g., Barros *et al.* 1995, Barros *et al.* 2010, Fernandez *et al.* 2011), and fatty acid signatures (e.g., Samuel and Worthy 2004, Walton *et al.* 2007).

Stable isotope analysis is based on the principle that the isotopic composition of the tissues of a consumer reflect that of the diet it has consumed (e.g., Deniro and Epstein 1978, Deniro and Epstein 1981, Fry 1988). Stable isotopes are variations of an element with differing numbers of neutrons resulting in different atomic weights. In ecological studies, two of the most commonly used isotopes are carbon and nitrogen. The lighter forms of these two isotopes, ^{12}C

and ^{14}N , occur more frequently than their heavier counterparts, ^{13}C and ^{15}N . Diet-tissue discrimination (also known as fractionation) results in tissues of consumers having a different ratio of heavy to light isotopes than those of prey tissues. This is due to certain isotopes being preferentially selected in certain biochemical processes. Most enzymes, for example, show an affinity for the lighter forms of carbon and nitrogen isotopes. This results in a step-wise enrichment where the heavier isotope increases in concentration relative to the standard for that particular element and ultimately allows for the measurement of ratios of stable isotopes. In marine ecosystems, nitrogen isotope ratios are typically used to reflect trophic level, whereas carbon isotope ratios are more indicative of sources of primary production (Fry 1988, Rau *et al.* 1983). Stable isotope analysis has been used to describe the ecology of many species as well as being used as an important tool in feeding ecology studies. Because a variety of tissues, such as whiskers, hair, blood, and skin can be sampled for stable isotope analysis, it serves as a versatile and relatively non-invasive means of learning about a species' biology and has been used to study many marine mammal species (e.g., Ruiz-Cooley 2004, Tucker *et al.* 2007, Witteveen *et al.* 2009, Alves-Stanley and Worthy 2010, Barros *et al.* 2010, Fernandez *et al.* 2011, Rioux *et al.* 2012). The distribution of many cetaceans is closely linked to the distribution of their prey. For example, the seasonal distribution of minke whales (*Balaenoptera acutorostrata*) off the coast of Scotland was linked to distribution and movement of their prey species (Macleod *et al.* 2004). Similarly, the movement and distribution of common dolphins (*Delphinus capensis*) in South Africa was linked to the seasonal migration of sardine (Young and Cockcroft 1994). It is therefore imperative to understand cetacean feeding ecology as it lends insight into other aspects of their ecology such as distribution, habitat usage, migration and niche differentiation. In studies where a suite of potential prey are available in addition to the consumers tissue, mass-

balance equations (mixing models) can be used to determine the relative contribution of prey species to the consumer's diet (Gomez-Campos *et al.* 2011, Wai *et al.* 2011). Bayesian mixing models, in particular, allow for the input of diet-tissue discrimination factors and also account for variability in these factors as well as that in dietary sources (Phillips 2012). They also allow for inclusion of a larger number of dietary sources than some of the older models that were restricted to $n + 1$, where n represents the number of isotopes being measured (Phillips and Gregg 2003). Ground-truthing of these mixing models using data collected from controlled diet studies is needed to ascertain their degree of certainty when predicting diet composition.

While stable isotope analysis can provide information on trophic level and sources of primary production, fatty acid analysis can give insight into a consumer's diet on a finer scale. In marine food webs, long chain fatty acids pass from prey to carnivore consumers relatively unchanged allowing for the reflection of a prey's fatty acid profile to be seen in the fat or blubber of the consumer (Ackman 1980). Relatively few fatty acids are biosynthesized in animals therefore making it possible to identify dietary fatty acids. These are taken up by the consumer during digestion and although there is slight modification of these dietary fatty acids due to metabolism such that the composition of consumer tissue does not exactly match that of the prey, they are laid down in a predictable way (Iverson *et al.* 1997). Fatty acid analysis has been used in many feeding ecology studies to potentially discern dietary choices, however, to properly interpret the fatty acid signature, you need to develop correction factors and these are still unavailable for cetaceans (e.g., Budge *et al.* 2006). Despite this limitation, fatty acid analysis can also be used to discriminate between different subpopulations of animals that are exhibiting different feeding habits (Iverson *et al.* 1997).

The combination of stable isotope and fatty acid analyses can facilitate a better understanding of marine mammal feeding ecology and habitat utilization. When studying a top predator of an ecosystem, such as a marine mammal, these methods can be used to compare and contrast different individuals in a population (i.e. males and females or individuals of different age categories), individuals among different locations, or to track changes over time. The incorporation of fatty acid analysis increases the potential of doing this with a finer resolution due to the large number of fatty acids available to compare (up to 70 fatty acids) (Fletcher-Odom 2012).

The Indian River Lagoon (IRL) is a 250 km long estuary comprised of four distinct bodies of water running along the east coast of Florida including Mosquito Lagoon, Indian River, Banana River and the St. Lucie Estuary (Figure 4.1). There are five inlets and one lock (Cape Canaveral lock) connecting the IRL to the Atlantic Ocean. The estuary ranges in width from a few hundred meters to 9 km and averages in depth at approximately 1.5 m with maximum depths of around 4 m. In 1990 the United States Environmental Protection Agency designated the IRL as an Estuary of National Significance to help preserve one of the most biodiverse estuaries in North America. Pressures from human population growth, overfishing, habitat degradation and inflows of pollutants, nutrients and freshwater made it necessary to put conservation efforts into force. It is estimated that there are approximately 800 resident dolphins in the IRL that rarely, if ever, leave the lagoon (Mazzoil *et al.* 2008a). Bottlenose dolphins in the IRL show strong site fidelity to specific areas in either northern or southern portions of the system (Mazzoil *et al.* 2008a, Mazzoil *et al.* 2008b) and a number of recent studies have noted significant health issues related to these distribution patterns, with northern IRL dolphins exhibiting significantly greater

health issues than southern IRL animals (e.g., Bossart *et al.* 2003, Bossart *et al.* 2006, Goldstein *et al.* 2006, Reif *et al.* 2006, Greig *et al.* 2007, Durden *et al.* 2007).

Most studies on small cetaceans have described the social structure of a particular population, but have not examined the ecological forces that shape that structure. Shane *et al.* (1986) determined that food resources are one of the most important factors affecting bottlenose dolphin movements. Many studies examining the movement patterns and strategies employed by dolphins have concluded that they are primarily tracking prey movements (e.g., Shane 1977, Hanson and Defran 1993, Waples *et al.* 1995, Hart 1997). Waples *et al.* (1995) determined that habitat usage of bottlenose dolphins in Sarasota Bay, FL was dependent on season, with dolphins frequenting grass flats during the summer and deep-water passes and coastal Gulf waters in the winter. These changes were directly attributable to changes in prey distribution. Clearly understanding what these animals eat is critical to understanding how they are using their environment.

The objectives of the current study were to 1) better understand regional site fidelity and feeding habits of the bottlenose dolphins within the IRL based on carbon and nitrogen isotopic ratios and fatty acid signatures, 2) to validate a Bayesian mixing model (SIAR) with stable isotope data collected from controlled groups of dolphins housed at SeaWorld Orlando, Florida and 3) to utilize this mixing model using dolphin skin and a suite of potential prey to predict diet composition for the IRL bottlenose dolphin, all with the larger goal of supporting conservation and management decisions.

Methods

In Situ Dolphins

Stranded IRL Dolphins

A total of 54 skin samples were collected opportunistically from dead, stranded bottlenose dolphins in the IRL during 1994-2004. Standard length of animals was measured and sex was determined. Standard length was used as a gross estimate of age based on growth equations (Stolen *et al.* 2002) and weaning age estimates (Mann *et al.* 2000). Mann *et al.* (2000) reported weaning ages ranging from 2.7 to 8 years with 67% weaned by 4 years old. In the present study, dolphins were grouped into three age classes: calves, sub-adults, and adults. As a conservative estimate (due to the variability in growth rates between individuals), calves were considered to be less than one year old since it is anticipated that they would not be weaned and that the majority of their diet consisted of milk. Sub-adult animals were between 1 and 3.5 years, and may have included nursing animals as well as those beginning to incorporate fish into their diet. Adults were considered greater than or equal to four years old. Samples of skin (approx. 5 g) were collected, placed in Teflon bags and kept on ice until return to the lab at which time they were placed in freezers and kept at -20°C.

Live Captured IRL Dolphins:

A total of 133 skin (2003-2007, 2010, 2011) and 65 blubber samples (2003-2005) were collected from live dolphins that were captured as part of a population health assessment study by a research team led by personnel from Harbor Branch Oceanographic Institute under the

direction of the National Ocean Service, NOAA (NMFS permit number 998-1678-00). Samples were collected during summer months (June and July) from two separate areas in the IRL. The northern capture area included the Mosquito Lagoon, and portions of the Indian River and Banana River north of latitude 28°15'N. The southern portion of the lagoon included the St. Lucie inlet, the north and south forks of the St. Lucie River, as well as the IRL south of latitude 27°25'N.

Bottlenose dolphins were captured, examined, sampled, marked, and safely released in June or July of 2003 through 2007, 2010, and 2011. Dolphins were collected by encircling them with a large mesh seine net (366 m, 6 m strike depth, and 8.9 cm twine mesh double lead line, and double floats net) in water depths of approximately 2 m or less. Once encircled, small boats with experienced animal handlers surrounded the net circumference to restrain and support the animal(s) after striking the collection net perimeter. Once the dolphin had struck the net and was encircled, experienced animal handlers restrained and secured the dolphin for retrieval of samples. Dolphins were examined, freeze-branded, weighed, standard lengths were measured, sex was determined, blood was drawn, and finally skin and blubber samples were taken. Prior to biopsying, the area was cleaned and disinfected using an antiseptic iodine scrub and further prepared with a local anesthetic (2% lidocaine). Wedge biopsies of skin and full blubber depth of approximately 10 g was taken from the animals along the mid lateral line below the dorsal fin. Samples were placed in Teflon bags and placed in a nitrogen vapor Dewar until returning to the lab where they were stored at -80°C until further analyses. After a short period of observation, dolphins were safely removed from the net enclosure and released.

Ex situ Dolphins

SeaWorld

A total of 26 animals held in two separate exhibits, “Key West” (n=18) and “Whales and Dolphins” (n=8) at SeaWorld Orlando, Florida were utilized in this study. Animals and sample collections were described in detail in Chapter 2. Briefly, dolphins were fed capelin and herring (“Whales and Dolphins”) or capelin, herring, and whitebait (“Key West”). Adult male dolphins held at the “Whales and Dolphins” exhibit were fed similar proportions of each fish species for approximately two months prior to sample collection. Dolphins housed at Sea World’s “Key West” exhibit consisted of adult males (n = 9), adult females (n = 3), pregnant females (n = 2), and juveniles (females, n = 4) and were fed consistent proportions for approximately one month prior to sample collections.

Samples of skin (approx. 20 mg) were collected from each dolphin, on a single occasion, using 7 mm diameter disposable dermal curettes (Miltex). Skin was transferred to plastic microcentrifuge vials (Novatech, model D1010) and kept at -20°C until further processing. Frozen skin samples were placed in a drying oven at 60°C for 24 hours to remove water prior to further processing for stable isotope analysis. Whole frozen samples of herring (n = 15 from “Whales and Dolphins” and n = 15 from “Key West”), capelin (n = 15 from “Whales and Dolphins” and n = 17 from “Key West”), and whitebait (n = 20 from “Key West”) were collected and processed at UCF. Initially mass and standard lengths were obtained and then individual fish were homogenized in a blender. Ground fish were then freeze dried individually and stored in plastic Whirl-Pak bags until further processing. Isotope samples were prepared at UCF and subsequently analyzed at the University of Georgia (see below).

IRL Fish

As part of a separate, ongoing study, a variety of fish species were collected from the central portion of the Indian River for analyses of stable isotopes and fatty acid signatures. In the current study, seven species of fish that were collected from 2004 through 2007 were utilized as source input for the dietary mixing model. Due to an approximate turnover time of one month in the skin of dolphins (see chapter 2), only stable isotope values of fish caught in the month of May each sampling year were utilized. A total of 187 fish were analyzed: n = 9 pigfish (*Orthopristis chrysoptera*), n = 23 pinfish (*Lagodon rhomboids*), n = 43 silver perch (*Bairdiella chrysoura*), n = 11 spot (*Leiostomus xanthurus*), n = 25 spotted seatrout (*Cynoscion nebulosus*), n = 31 striped mullet (*Mugil cephalus*), and n = 45 white mullet (*Mugil curema*). Methodology was as described in Worthy and Worthy (2011) but briefly, fish were caught with a 183 m (37.5 mm stretched mesh) center-bag seine and were kept frozen (-20 °C) until processing. Individual fish were weighed (g), measured for standard length (mm), ground individually into a homogeneous consistency, and were then freeze dried for 48 hours.

Sample Preparation and Stable Isotope Analysis

Skin collected from live biopsies (n=133), stranded dolphins (n=54), and captive dolphins (n=26) were initially placed in a drying oven at 60°C for 24 hours to remove water, and then skin and fish samples were lipid extracted using petroleum ether in a Soxhlet extractor for 24 hours. Lipids are depleted in ¹³C relative to lean tissue and therefore all samples were lipid-extracted using petroleum ether prior to isotope analysis (Schlechtriem *et al.* 2003, Post *et al.* 2007).

Lipid-extracted samples were again placed in a drying oven for 24 hours to remove any remaining solvent. Dried, lipid-extracted samples were then ground, using a Wig-L-Bug grinder (Crescent, model MSD), into a homogeneous powder. Aliquots (0.9-1.5 mg) were then sealed into 5 mm by 9 mm tin capsules and analyzed at the University of Georgia, Institute of Ecology Stable Isotope Laboratory using a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer (IRMS). Data were expressed and reported as per mil (‰) using delta notation (δ):

$$X(\text{‰}) = \left(\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right) \times 1000 \quad (6)$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$. Standard reference materials were carbon from PeeDee Belemnite limestone and atmospheric nitrogen gas. To assure quality control in sample analysis of stable isotope ratios, a known standard sample (bovine tissue) was run after every 12 unknown samples (dolphin skin and fish). Analytical errors for the standard samples ($n=37$) were $\pm 0.01\%$ (SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Sample Preparation and Fatty Acid Analysis

Lipid analyses were performed following methodologies in Iverson (1993) and Iverson *et al.* (1997), and further modified as per Samuel and Worthy (2004). Lipids were extracted from blubber samples ($n = 65$) using a solution of 2:1 chloroform/methanol. Fatty acid methyl esters (FAMES) were prepared from the extracted lipid by adding 0.5 N sulfuric acid in methanol and dichloromethane. This solution was then placed in the dark for 72-96 h. FAMES were then analyzed using a gas-liquid chromatograph (Perkin-Elmer Autosystem XL) using appropriate software on a connected laptop (Totalchrom version 6.3.1, Perkin Elmer). Resultant chromatograms were calibrated by comparing to known standard mixtures (Nu-Chek Prep,

Elysian MN) and secondary external reference standards to determine fatty acid composition. Fatty acids were named using International Union of Pure and Applied Chemistry nomenclature as per the following format: carbon length: number of double bonds and location (n-x) of the first double bond in relation to the terminal methyl end of the fatty acid. Fatty acids were converted to percent amount of the total sample, and standardized by dividing each fatty acid detected by the total percent amount of all identified fatty acids. The subset of the total assessed fatty acids referred to as the “extended dietary fatty acids” (Iverson *et al.* 2004) was used in statistical analysis (> 0.5%).

Statistical Analyses

Stable isotope data were tested for normality using Shapiro-Wilks ($n < 50$) and Kolmogorov-Smirnov tests ($n > 50$), and tested for homogeneity of variance using Levene’s test. Due to a difference in sampling period and methodology, samples collected from stranded IRL dolphins were evaluated separately from those of the live captured dolphins and comparisons between the two was not undertaken due to small sample sizes. For stranded animals, differences among age class, sex, season, and year were explored for ^{15}N and ^{13}C using GLM multivariate analysis. For live caught animals, differences among location within the IRL, between sexes, and among collection years were explored for ^{15}N and ^{13}C using GLM multivariate analysis. For IRL fish, differences among species and year were explored for ^{15}N and ^{13}C using GLM multivariate analysis. Data were analyzed in general linear models because: transformations failed to improve the few non-normal data; visual inspection of normal Q-Q plots and histograms indicated normality; and general linear models are considered robust to

deviations from normality (Field 2005). Tukey's HSD post hoc tests were performed to determine homogeneous subsets. Fish not shown to be significantly different through homogenous subsets were grouped into prey categories for input into diet modeling (Phillips *et al.* 2005). Regression analyses were done between stable isotope data and salinity, as well as between isotope data and water temperatures. Salinity and water temperatures from 2002 to 2011 were determined from historical data collected by the St. Johns Water Management District. All statistical analyses were conducted using SPSS 20.0 with a critical value of $\alpha = 0.05$ and plotted using SigmaPlot (Version 10.0, Systat Software). Values reported are mean \pm SE.

Classification and Regression Tree (CART) analysis (S-Plus - Professional Edition, Version 6.2.1, Insightful Corporation) was used to analyze fatty acid data. CART trees are grown by repeatedly splitting the data via algorithms that partition the data into mutually exclusive groups (Breiman *et al.* 1984, De'ath and Fabricius 2000) ultimately dividing samples into a series of sequential dichotomous groups based on an individual fatty acid with the greatest deviance in concentration. CART is a non-parametric method that does not limit the number of variables due to small sample size and variables need not be normally distributed, so that untransformed data may be analyzed with this method. Multi-Response Permutation Procedure (MRPP) (PC-ORD 5) is another non-parametric test to examine differences between or among groups and was used to verify CART findings.

Diet Modeling

Relative contribution of assimilated diet sources to dolphin skin was determined using the Bayesian mixing model, SIAR, with isotopic values of dolphin skin (consumer), fish (sources),

and diet-tissue fractionation factors as input parameters. SIAR overcomes some of the limitations of previous models by allowing an uncapped number of dietary sources to be inputted, as well as accounting for variation in both the isotope values and the diet-tissue fractionation factors, making it a more rigorous model (Parnell *et al.* 2008, Drago *et al.* 2010, Parnell *et al.* 2010).

Model Validation using SeaWorld dolphins

Carbon and nitrogen values were entered individually for each SeaWorld dolphin, while fish values were entered as mean \pm SD for each prey group. Diet-tissue discrimination factors that were previously calculated for these SeaWorld bottlenose dolphins (see chapter 2) were entered into the models as $2.20 \pm 0.10\%$ for $\delta^{15}\text{N}$ and $0.82 \pm 0.14\%$ for $\delta^{13}\text{C}$. Two models were run separately for the animals in each exhibit (model 1: “Key West” and model 2: “Whales and Dolphins”).

In model 1, dolphins were separated into categories depending on their reproductive status: adult males ($n = 9$), adult females ($n = 3$), pregnant females ($n = 2$), and female juveniles ($n = 4$). These dolphins were being fed various proportions of three fish species: herring ($\delta^{15}\text{N}$: 11.87 ± 0.40 , $\delta^{13}\text{C}$: -19.86 ± 0.31), capelin ($\delta^{15}\text{N}$: 11.74 ± 0.33 , $\delta^{13}\text{C}$: -19.87 ± 0.20), and whitebait ($\delta^{15}\text{N}$: 13.45 ± 0.20 , $\delta^{13}\text{C}$: -15.67 ± 0.34). Capelin and herring were combined into one prey group for input into the model due to similar nitrogen and carbon signatures. Results are presented as 95% credibility intervals (Bayesian confidence interval).

In model 2, all dolphins held in the “Whales and Dolphin” exhibit were adult males and therefore categorized in a single group for model input. These dolphins were fed a mixture of

60% (by mass) capelin ($\delta^{15}\text{N}$: 12.06 ± 0.31 , $\delta^{13}\text{C}$: -19.53 ± 0.41) and 40% herring ($\delta^{15}\text{N}$: 11.16 ± 0.25 , $\delta^{13}\text{C}$: -19.25 ± 0.47). Results are presented as 95% credibility intervals (Bayesian confidence interval).

Model Application to IRL Dolphins

Carbon and nitrogen values from live biopsied dolphins from 2004-2007 (n=35) were entered individually for each dolphin, while fish values were entered as mean \pm SD for each prey group. Diet-tissue discrimination factors for adult bottlenose dolphins were entered into the model as (see chapter 2). Potential diets of bottlenose dolphins were explored with all dolphins grouped together (model 1) and then for individual dolphin sampling years (model 2). Results are presented as 95% credibility intervals (Bayesian confidence interval).

Results

Stable Isotope Analysis

Stranded IRL Dolphins

Stable isotope ratios were analyzed for 54 samples taken from dead, stranded IRL dolphins between 1994 and 2004. Isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were normally distributed for all age classes, both sexes, seasons, and all collection years. MANOVA was run with the following main effects: age class, sex, season, and year. The Interactions of main effects were also explored. Age class had a significant effect (Wilks' Lambda: $F_{4, 38} = 3.933$, $p = 0.008$) as did year (Wilks' Lambda: $F_{14, 38} = 3.000$, $p = 0.004$) but no other effects were significant. The $\delta^{13}\text{C}$

values did not differ among sampling years ($F_{7, 20} = 2.106$, $p = 0.091$), however $\delta^{15}\text{N}$ values did ($F_{7, 20} = 2.930$, $p = 0.028$) (Figure 4.2). Further exploration using Tukey's HSD post hoc tests indicated that nitrogen values for the sampling year 1998 were not significantly different from sampling years 1994 ($p = 0.267$) or 1995 ($p = 0.197$) but did differ significantly from all other sampling years (all p values ≤ 0.037). Sampling year 1998 had the most enriched nitrogen values ($14.84 \pm 0.95\text{‰}$) while 2000 had the most depleted ($12.27 \pm 0.42\text{‰}$). The $\delta^{13}\text{C}$ values did not differ between age classes ($F_{2, 20} = 0.764$, $p = 0.479$), however $\delta^{15}\text{N}$ values did ($F_{2, 20} = 7.780$, $p = 0.003$). There were no significant relationships between salinity ($R^2 = 0.12$, $p = 0.348$) or water temperature ($R^2 = 0.14$, $p = 0.313$) to carbon or nitrogen isotope ratios. Tukey's HSD post hoc tests revealed that all three age groups were significantly different from each other (all p values ≤ 0.028) (Figure 4.3). Skin from calves was the most enriched in $\delta^{15}\text{N}$ ($14.46 \pm 0.33\text{‰}$), followed by sub-adults ($13.61 \pm 0.26\text{‰}$), and adults were most depleted in $\delta^{15}\text{N}$ ($12.70 \pm 0.13\text{‰}$). Skin from calves was enriched in $\delta^{15}\text{N}$ by an average of 1.80‰ compared to that of adults.

Live Captured IRL Dolphins

Stable isotope ratios were analyzed for 133 samples taken from live captured dolphins in the IRL. Isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were normally distributed for all locations. Data for females were distributed normally for $\delta^{15}\text{N}$ (S-W = 0.966, $p = 0.336$), however, data for males were not (K-S = 0.119, $p = 0.002$). Neither female nor male data were distributed normally for $\delta^{13}\text{C}$ (females: S-W = 0.788, $p < 0.001$; males: K-S = 0.145, $p < 0.001$). Finally, results varied for sampling years with years 2006, 2007, 2010 and 2011 being normally distributed for both

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (all p values ≥ 0.062), whereas, the sampling years 2003 and 2005 were not distributed normally for either $\delta^{15}\text{N}$ (2003: S-W = 0.920, $p = 0.014$; 2005: S-W = 0.840, $p = 0.010$) and $\delta^{13}\text{C}$ (2003: S-W = 0.893, $p = 0.003$; 2005: S-W = 0.869, $p = 0.027$). Results were mixed for the sampling year 2004 which was distributed normally for $\delta^{15}\text{N}$ ($p = 0.105$) but not for $\delta^{13}\text{C}$ (S-W = 0.887, $p = 0.007$).

Data for dolphin skin were not significantly different for sex (Wilks' Lambda: $F_{2, 93} = 0.859$, $p = 0.427$) but were for location within the IRL (Wilks' Lambda: $F_{8, 186} = 8.491$, $p < 0.001$) and for sampling year (Wilks' Lambda: $F_{12, 186} = 4.393$, $p < 0.001$). Location within the IRL was significant for both ^{15}N ($F_{4, 94} = 5.720$, $p < 0.001$) and ^{13}C ($F_{4, 94} = 17.841$, $p < 0.001$) as was sampling year for both ^{15}N ($F_{6, 94} = 7.117$, $p < 0.001$) and ^{13}C ($F_{6, 94} = 3.730$, $p = 0.002$).

Tukey's HSD post hoc tests reveal that sampling year 2005 was significantly more depleted in carbon ($-17.25 \pm 0.61\%$) than all other sampling years (all p values ≤ 0.003). Sampling year 2011 had the most enriched carbon value ($-14.39 \pm 0.23\%$) and was significantly different from all other sampling years (all p values < 0.014) aside from 2007 ($p = 0.981$). For nitrogen, sampling year 2005 had the most enriched nitrogen value ($12.56 \pm 0.19\%$) while 2011 was the most depleted ($10.98 \pm 0.19\%$). Tukey's HSD post hoc tests indicated that sampling year 2011 was not significantly different in nitrogen values from that of 2004 ($p = 0.165$) or 2006 ($p = 0.084$) but was significantly different for the remaining sampling years (all p values ≤ 0.004). Post hoc tests also indicated that sampling year 2005 was not significantly different in nitrogen values from that of years 2003, 2007, and 2010, but did significantly differ from remaining collection years (all p values ≤ 0.010). There were no significant relationships between salinity ($R^2 = 0.19$, $p = 0.307$) or water temperature ($R^2 = 0.11$, $p = 0.378$) to carbon or nitrogen isotope ratios.

Tukey's HSD post hoc tests indicated that for $\delta^{13}\text{C}$, St. Lucie Estuary was significantly different than all other locations (all p values < 0.001), while Mosquito Lagoon was significantly different from both North and South Indian River (p = 0.025, p = 0.003 respectively). Banana River, North Indian River and South Indian River were not significantly different from one another (all p values \geq 0.334). Finally, Banana River and Mosquito lagoon were not significantly different from each other (p = 0.572). Mosquito Lagoon was the most carbon enriched location ($-14.33 \pm 0.23\text{‰}$), followed by Banana River ($-14.93 \pm 0.28\text{‰}$), North Indian River ($-15.32 \pm 0.19\text{‰}$), South Indian River ($-15.59 \pm 0.14\text{‰}$), and finally St. Lucie Estuary ($-18.70 \pm 0.54\text{‰}$). Tukey's HSD post hoc tests indicated that $\delta^{15}\text{N}$ values for St. Lucie were significantly different than all other locations (all p values \leq 0.002). Banana River was not significantly different than that of Mosquito lagoon (p = 0.996) but was significantly different to the remaining locations (all p values \leq 0.021), while Mosquito Lagoon, North Indian River, and South Indian River were not significantly different from one another (p \geq 0.057). St. Lucie Estuary was the most nitrogen enriched location ($12.81 \pm 0.13\text{‰}$), followed by South Indian River ($12.03 \pm 0.11\text{‰}$), North Indian River ($12.02 \pm 0.17\text{‰}$), Mosquito Lagoon ($11.45 \pm 0.29\text{‰}$), and finally with the most nitrogen depleted signature, Banana River ($11.35 \pm 0.33\text{‰}$) (Figure 4.4).

IRL Potential Prey Items (Fish)

A total of 187 fish samples were analyzed. Isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were normally distributed for all collection years (S-W; p \geq 0.092). Fish species were distributed normally for $\delta^{15}\text{N}$ for all species (S-W; p \geq 0.054) except for spot (S-W = 0.772, p = 0.004) and white mullet

(S-W = 0.936, $p = 0.016$). They were also distributed normally for $\delta^{13}\text{C}$ (S-W; $p \geq 0.60$) with the exception of striped mullet (S-W = 0.893, $p = 0.005$). The fish species most enriched in regards to $\delta^{15}\text{N}$ was seatrout ($13.36 \pm 0.99\text{‰}$) and the most depleted in nitrogen was white mullet ($8.16 \pm 0.97\text{‰}$) (Figure 4.5). For $\delta^{13}\text{C}$ the most enriched fish species was white mullet ($-14.05 \pm 1.51\text{‰}$) and the most depleted was spotted seatrout ($-18.22 \pm 0.76\text{‰}$). Fish values were not significantly different based on year (Wilks' Lambda: $F_{6, 326} = 1.539$, $p = 0.234$) but were significantly different in terms of species (Wilks' Lambda: $F_{12, 326} = 40.743$, $p \leq 0.001$). Species was significant for both $\delta^{15}\text{N}$ ($F_{6, 164} = 106.666$, $p \leq 0.001$) and for $\delta^{13}\text{C}$ ($F_{6, 164} = 21.485$, $p \leq 0.001$).

Post hoc tests indicated that spotted seatrout and silver perch were significantly different than all other species in regards to nitrogen. Pigfish, pinfish and spot were not significantly different from each other but were different from the homogenous subset of white mullet and striped mullet. In regards to carbon, there were three homogenous subsets with white mullet and pigfish in one which was significantly different from all other species. Spotted seatrout and pinfish were in another subset and finally, silver perch, striped mullet and perch were in the third homogenous subset. Because pinfish and spot were the only species that were not significantly different from each other for both carbon and nitrogen, they were grouped together into "pinspot" for input into the mixing model as a dietary source (Phillips *et al.* 2005). All of the remaining fish were kept as independent source groups (Table 4.1).

Fatty Acid Signature Analysis

CART analysis was performed on 65 blubber samples collected between 2003 and 2005. Comparisons of collection years indicated a significant difference (overall MR = 3/65, $p =$

0.046). Two samples collected in 2004 were misclassified as 2005 and one sample collected in 2005 was misclassified as 2004 (Figure 4.6). MRPP confirmed these findings ($p < 0.001$). Males and females also showed significant differences with only two animals being misclassified ($p = 0.031$) (Figure 4.7) and the results of MRPP were consistent ($p < 0.001$). Finally, locations within the IRL were also significantly different from one another (CART: $p = 0.046$; MRPP: $p < 0.001$) resulting in the separation of dolphins into Mosquito Lagoon, northern IRL, Banana River, southern IRL and St Lucie Estuary segments (Figure 4.8). In general, dolphins from the northern segments of the IRL separated (MR = 1/42, $p = 0.024$) collectively from dolphins from the southerly segments of the IRL (MR = 0/23, $p = 0.0$) using 20:4 n-6 and 18:1 n-7. Mosquito Lagoon dolphins separated from other northern IRL dolphins using 18:1 n-7 (MR=1/11, $p = 0.091$) with one misclassified in the southern IRL group. Within this northern group, dolphins sampled in the Banana River could be further distinguished with a MR = 2/13 ($p = 0.154$) (using 16:3 n-4). The two misclassified Banana River animals were placed in the northern IRL group. Dolphins from the southern regions could be subdivided into southern IRL animals and St Lucie Estuary dolphins using 18:2 n-6 (MR= 0/23, $p = 0$).

Model Validation

Ex Situ Dolphins

Carbon and nitrogen isotope ratios of the skin of captive bottlenose dolphins held at the “Key West” exhibit (SWF-2) at SeaWorld, Orlando, Florida and three prey fish species were inputted into SIAR to validate the predictability of dietary proportions estimated by the mixing model. Dolphins were separated into categories depending on their reproductive status and

capelin and herring were grouped into one prey category for model input (Figure 4.9). The actual proportions that adult female dolphins were fed for one month prior to sampling were 73% capelin/herring and 27% whitebait. SIAR's predictions for the relative contributions to diet of this category of dolphins were very close to actual values (mean: 77.5%, 95% credibility interval: 63%-93%; 22.5%, 6.5%-37% respectively) (Figure 4.10). For adult male dolphins, actual diet fed was 69% capelin/herring and 31% whitebait. SIAR was even more accurate with the predictive proportions of this group's diet: mean: 71%, 95% credibility interval: 65%-77.5%; 29%, 22.5%-35% respectively (Figure 4.10). Pregnant females were fed 61% capelin/herring and 39% whitebait and this was well predicted by SIAR (mean: 59%, 95% credibility interval: 34%-83%; 41%, 17%-66% respectively) (Figure 4.10). Finally, juvenile females were fed a mixture of 80% capelin/herring and 20% whitebait. SIAR was again able to reasonably predict relative contribution to diet (mean: 77%, 95% credibility interval: 56%-94%; 23%, 6%-44% respectively) (Figure 4.10).

Carbon and nitrogen isotope ratios of the skin of captive bottlenose dolphins held at the "Whales and Dolphins" exhibit (SWF-1) at SeaWorld, Orlando, Florida and two prey fish species were inputted into SIAR to validate the predictability of dietary proportions estimated by the mixing model. Dolphins were entered into the model in one group and the two prey species were entered individually. The actual diet that these captive dolphins were fed for two months prior to sampling was a mixture of 60% capelin and 40% herring. Although these two prey sources were very similar in their isotopic signatures, SIAR was still able to discriminate between them to a degree (mean: 53%, 95% credibility interval: 22%-84%; 47%, 16%-78% respectively) (Figure 4.11).

In Situ Dolphins - Indian River Lagoon

Carbon and nitrogen isotope ratios of the skin of Indian River bottlenose dolphins and seven species of potential prey were input into SIAR to predict relative contributions to dolphin diet. Two models were run (model 1 and model 2) to model potential contributions of source groups to all samples combined (Figure 4.12) and then each sampling year separately (Figure 4.13).

In model 1, where all bottlenose samples were combined, striped mullet, “pinspot” and spotted seatrout were the predominant contributors to dolphin diet (mean: 29%, 95% credibility interval: 12%-45%; 26%, 2%-48%; 21%, 8%-33% respectively). Silver perch also made a significant contribution to diet (13%, 0%-31%) and white mullet (6%, 0%-15%) and pigfish (4%, 0%-11%) less so (Figure 4.14).

When dolphins were grouped according to sample collection year (model 2), there were differences in prey contribution temporally. Collection year 2004 followed the general trend of grouped animals with spotted seatrout (24%, 8%-39%), pinspot (20%, 0%-38%) and striped mullet (18%, 0%-34%) being the predominant contributors. Silver perch (17%, 0%-34%) was the next most significant source group followed by equal contributions by white mullet (11%, 0%-26%) and pigfish (11%, 0%-26%) (Figure 4.15). Spotted seatrout (30%, 16%-43%) and pinspot (20%, 1%-37%) remained as top contributing source groups in 2005 and silver perch (18%, 0%-34%) was the third most abundant. Striped mullet dropped in importance slightly as a diet source (16%, 0%-30%) and again, white mullet (8%, 0%-19%) and pigfish (8%, 0%-20%) were least important contributors (Figure 4.15). Again, in 2006, pinspot (25%, 3%-45%) was one of the most important source groups, along with striped mullet (30%, 13%-46%) and silver perch (15%, 0%-31%). White mullet (13%, 0%-27%) was of next importance and spotted

seatrout (11%, 0%, 22%) dropped in contribution amount from previous years. Pigfish (7%, 0%-17%) was again, the least important contributing source group (Figure 4.15). In 2007, spotted seatrout (19%, 6%-31%) again became one of the most important sources and pigfish (19%, 1%-34%) was predicted to be equally as important. The remaining four source groups, silver perch (17%, 0%-32%), white mullet (16%, 0%-29%), pinspot (15%, 0%-30%), and striped mullet (13%, 0%-27%) were all relatively similar in their contributions (Figure 4.15).

Discussion

Stable isotope analyses of skin from stranded IRL dolphins indicated differences among collection years and among age class for $\delta^{15}\text{N}$ values. Calves, sub-adults, and adults were all distinguishable from one another with calves being the most enriched and adults being the most depleted. Stable isotope analysis of samples from live IRL dolphins indicated differences among collection years and regional differentiation of dolphins within the IRL into Mosquito Lagoon and Banana River subpopulations in the north, and a St Lucie Estuary subpopulation in the south, but was unable to significantly distinguish northern and southern Indian River subpopulations. There is, however, a trend of decreasing carbon values from north to south in the IRL and the opposing trend for nitrogen with the most enriched values being in the south (St. Lucie Estuary) and becoming more depleted traveling north in the IRL. Application of fatty acid analysis allowed for finer resolution of dolphins into a Mosquito Lagoon subpopulation and a Banana River subpopulation distinguished in the north, a separation of northern and southern Indian River subpopulations, and a St Lucie Estuary subpopulation. Dolphins were grouped into their respective sub-populations regardless of sex or year of sampling. Fatty acid analysis also

revealed a significant difference between male and female dolphins that was not revealed with stable isotope analysis.

The IRL is the ideal location for undertaking a study of the feeding habits of a top predator, such as the bottlenose dolphin, because it is a semi-closed lagoon environment that supports a year-round resident population of bottlenose dolphins (Leatherwood 1979, Odell and Asper 1990, Rudin 1991, Spellman 1991, Booth 1993, Fick 1995). Preferred prey species for this population have previously been quantified (Barros 1993, Barros and Odell 1995) and prey distribution and behavior have been extensively studied (Gilmore 1977, Mok and Gilmore 1985, Mulligan and Snelson 1983, Snelson 1983, Gilmore 1988, Brown-Peterson and Eames 1990, Tremain and Adams 1995) aiding in the interpretation of the interaction between predator and prey. It is also one of the most biologically diverse estuaries in North America having been designated as an “Estuary of National Significance” by the National Estuary Program.

Differences between sexes discovered in the present study through fatty acid analysis suggest a difference in feeding ecology. The literature shows mixed conclusions when it comes to potential differences in prey choice between male and female delphinids. Differences in foraging between male and female common dolphins has been documented (e.g., Young and Cockcroft 1994, Chou *et al.* 1995) and in regards to stomach contents of this species, it has been shown that mature females had a significantly higher proportion of cephalopods in their diet compared to mature males (Silva 1999). However, when examining stomach contents of Indo-Pacific bottlenose dolphins, Amir *et al.* 2005, found no significant differences in prey preferences between male and female dolphins, whereas Cockcroft and Ross (1990) found sex-specific differences in prey choice. Samuel and Worthy (2005) also showed differences in the fatty acid profiles of males and female bottlenose dolphins that had stranded along the Texas and

Louisiana coasts. Most dolphin groups retain a fission-fusion nature with an open social network where individuals move in and out due to the low energetic cost of locomotion (Randic *et al.* 2012). In mammals, female lifetime reproductive success is tied to access to resources such as food, while in males it is limited to access to receptive mates (Moeller 2012). This can result in males moving over larger areas than that of female dolphins, hence giving males access to differing prey items. Mature females may also be restricted in range and diving depths because of accompanying nursing calves and immatures that are limited in their distribution (Ringelstein *et al.* 2006) which has been observed along the Gulf Coast of the United States (Barros and Odell 1990). Bottlenose dolphins feeding on different prey items, as well as on different size classes within each prey species, would exhibit differences in blubber fatty acid composition (Iverson *et al.* 1997). Stable isotope analysis did not detect significant differences between sexes in the current study, whereas fatty acid analysis did. This suggests that male and female dolphins within the IRL are feeding at the same trophic level and in the same vicinity but are choosing different prey species or differing in the proportions of prey they ingest. A separate, on-going study that evaluates the stable isotope and fatty acid signatures of a suite of fish species collected from the IRL, indicates that potential dolphin prey species can readily be distinguished from one another using fatty acid signatures (Worthy *et al.* submitted MS). This degree of separation in IRL fish fatty acids supports the notion that fatty acid signature analysis can be utilized to assess differences in the feeding ecology of an apex predator such as the bottlenose dolphin.

In the present study, age classes were readily distinguished from one another using stable isotope analysis. Calves were classified as individuals under the age of one year which means their diet would consist mostly, if not at all, of milk. Sub-adults were individuals between one and three and a half years of age and could include individuals that were still consuming milk,

but that also incorporated fish into their diet, and fully weaned individuals. Finally, adults were over the age of four and would be fully weaned and foraging mostly on fish species. When mammals are nursing, their tissues will be isotopically enriched in nitrogen when compared to that of older, weaned individuals. Because they are consuming milk which is essentially isotopically the same as the tissue of the mother, they are feeding at a higher trophic level than that of adult dolphins feeding primarily on fish resulting in enriched nitrogen values (Knoff *et al.* 2007). This allows for clear isotopic distinction among age classes. This has been observed in northern fur seals (Hobson *et al.* 1997), in bottlenose dolphins (Knoff *et al.* 2007), and bowhead whales (Hobson and Schell 1998), among many other mammalian species. As expected, in the current study, calves were the most enriched in nitrogen, reflecting their higher trophic level milk diet, sub-adults were the second most enriched, reflecting a mixed diet of milk and fish, and finally adults were most depleted by comparison. In some species of delphinids, age of weaning is still unknown and analysis of nitrogen isotope values may lend some insight.

It has been previously documented that bottlenose dolphins in the IRL have a high level of site fidelity (e.g., Mazzoil *et al.* 2005, Mazzoil *et al.* 2008a, Odell and Asper 1990). Regional differentiation of isotopic signatures of dolphin skin and fatty acids noted in the present study could indicate regional differences in feeding habits or be consistent with regional differentiation of the prey (Fletcher-Odom 2012). There is evidence of significant differences in the stable isotope and fatty acid signatures of white mullet (*Mugil curema*), pinfish (*Lagodon rhomboides*) and spotted seatrout (*Cynoscion nebulosus*) over distances of 10-15 miles separation in the IRL (Fletcher-Odom 2012) which would support the regional differentiation of bottlenose dolphins. Consistent with this were the observations of Mazzoil *et al.* (2008b) when they radio-tracked two male dolphins in the IRL and observed fidelity to relatively restricted regions. St. Lucie Estuary

dolphins showed significantly different carbon and fatty acid signatures and the significant difference in stable isotope and fatty acid values between these and other IRL dolphins is probably due to their site fidelity to an area where there is a higher riverine influence. The St. Lucie estuary segment has a high influx of fresh water from rivers, lakes and feed from drainage canals. Freshwater sources generally have more depleted carbon signatures compared to that of brackish or marine sources. Primary producers incorporate carbon from the dissolved inorganic carbon in the surrounding water into their tissues. This carbon source is depleted in ^{13}C compared to that of saltwater because of preferential isotopic selection during the biochemical process in which carbon dioxide is converted into bicarbonate (Boutton 1991). St. Lucie Estuary dolphins were also found to be significantly more enriched than dolphins in the other segments based on nitrogen isotope values. Southern parts of the IRL and the St. Lucie Estuary receive large amounts of runoff from flood control drainage canals, including that from agricultural watersheds which introduce large amounts of pesticides, pollutants, and fertilizers (Mazzoil *et al.* 2008a). The input of these fertilizers most likely contributes to a higher nitrogen level in this segment which is then carried up the food chain.

The IRL contains seven species of seagrasses and over one hundred species of algae (Clementz and Tuross 2006). Surveys have found that primary producers in the northern segments of the IRL are largely seagrass based, whereas southern parts of the IRL are more so algae based (Woodward-Clyde Consultants 1994). Stable isotope studies done on seagrasses and macroalgae have shown that seagrasses tend to be more enriched in carbon and macroalgae is more enriched in nitrogen (e.g., Clementz and Tuross 2006, Alves 2007). This would support the trends seen in the current study with nitrogen values being highest in the southern segments of the IRL and becoming more depleted towards the northern segments as well as the carbon

values being the most enriched in the northern segments and decreasing in the southern segments.

Even though the majority of individual fatty acids found in blubber are derived directly from the diet, the overall fatty acid profile of a consumer is still not identical to that of the prey they have consumed because there is always some selective metabolism and biosynthesis by that consumer. Even in the absence of a full understanding of fatty acid metabolism, fatty acid signature analysis has been used to infer spatial and temporal differences in diet both within and between a number of different species (Iverson *et al.* 1997). A lot can be gleaned from a basic fatty acid analysis as has been shown repeatedly with other species (e.g., Samuel and Worthy 2004, Smith and Worthy 2006, Krahn *et al.* 2007, Walton *et al.* 2007, Budge *et al.* 2008). Differences in fatty acid signatures indicate differences in feeding habits. Iverson *et al.* (1997) showed that Alaskan harbor seals using different haul-out sites were distinguishable, based on FASA, with >95% accuracy and that this was consistent with tracking data showing that these seals were feeding in different geographic areas. Samuel and Worthy (2005) examined the feeding ecology and fatty acid signatures of spotted (*Stenella attenuata*) and spinner (*S. longirostris*) dolphins in the Eastern Tropical Pacific (ETP) Ocean. They found significant differences between these two *Stenella* species and also between sexes within each species. When combined with stable isotope analysis, fatty acid signature analysis can add a more detailed dimension to the picture. Stable isotope analysis can give us information about the trophic level an animal is feeding at and an indication of where that animal is feeding, whereas, fatty acid signature analysis can provide more specific information about the prey items being ingested due to the large number of fatty acids being compared (21 in the current study).

The current study is the first to predict bottlenose dolphin diet in the Indian River using stable isotopic signatures of skin in a mixing model and is unique as it was able to validate a Bayesian mixing model using isotope values from a controlled feeding study. Historically, dietary mixing models have had limitations such as the number of contributing sources a model could handle (number of isotopes analyzed plus one) or not being able to account for variability of input parameters. Recent models have remedied these constraints (Phillips 2012). Bayesian mixing models such as SIAR tackle some of the limitations of previous models by allowing an uncapped number of dietary sources to be inputted, as well as accounting for variation in both the isotope values and the diet-tissue fractionation factors, making it a more rigorous model (Parnell *et al.* 2008, Drago *et al.* 2010, Parnell *et al.* 2010). With the expanding use of stable isotopes in ecology and the desire to determine relative source contributions to the mixture of interest, validation of these mixing models through controlled studies is relevant. The current study utilized captive dolphins held at two exhibits at SeaWorld Orlando in Florida. These animals were being fed known diet mixtures and consistent proportions which allows for ground-truthing runs of the mixing model. For the “Key West” exhibit animals, the model was able to very closely predict the mean contribution of each prey group with the largest discrepancy being a difference of 4.5% for adult female dolphins. Dolphins held at the “Whales and Dolphins” exhibit were fed prey items of capelin and herring, both of which had very similar carbon and nitrogen signatures. Although it is recommended to combine similar sources into groups (Phillips *et al.* 2005), in the current study, these similar prey were kept as separate prey items to determine if SIAR was able to estimate relative proportions. Although the model predicted contributions to the diet were not as precise as seen with the “Key West” animals, SIAR was still able to make some predictions regarding proportions. The current study demonstrates that when

the prey signatures are very close to one another the model still has the ability to discriminate but that the precision increases as the prey signatures are more distinct from each other.

Modeling predictions made in the current study using SIAR supports the assumption that IRL bottlenose dolphins are generalist feeders, consuming a variety of species. Across years, there tended to be two or three species that made up the bulk of the dolphin diet with remaining species contributing in smaller amounts. Barros (1993) conducted a study inspecting stomach contents of dead, stranded IRL bottlenose dolphins and determined the prey species of most importance were spotted seatrout, silver perch, striped mullet, Atlantic croaker, and oyster toadfish. Of lesser importance were pinfish, pigfish, and spot. When compared to the predictions of diet compositions modeled by SIAR in the current study, spotted seatrout was considered to be one of the top diet contributors in three of the four sampling years. Silver perch was ranked in importance in the top three source groups for three of the four sampling years as well. Striped mullet was the largest contributor to dolphin diet in 2006 and was third in abundance in 2004. In the current study, pinfish and spot were not significantly different in their isotopic signatures and were therefore grouped together as “pinspot”. Contrary to stomach content analysis findings, “pinspot” was found to be an important contributor to dolphin diet and in three (2004-2006) of the four sampling years, this source group was the second highest contributor. Similar to the findings of Barros (1993), in the current study, pigfish was a dietary source group that contributed least to dolphin diet. The exception to this is in 2007 where pigfish were one of the top contributing species, along with spotted seatrout. However, comparisons to stomach content studies must be done with caution as dolphins in the Barros (1993) study were dead, stranded individuals and may have been unhealthy and not representative of the healthy portion of the population.

Stable isotope signatures were significantly different across years for dolphins, and specifically, collection year 2006 was significantly more depleted in nitrogen when compared to other collection years. SIAR model predictions show that spotted seatrout, which is the most nitrogen enriched species in the current study, was the number one contributing source in 2004 and 2005, making up 24% and 30% of the diet respectively. However, in 2006, spotted seatrout dropped in importance to the second last contributing source at 11%. This decrease in spotted seatrout in the diet of dolphins would explain the decrease in $\delta^{15}\text{N}$ in dolphin skin. Instead, striped mullet became the most important contributing prey group at 30% compared to previous years where it was of lesser importance at 18% (2004) and 16% (2005). Striped mullet are considered detritivores and a large portion of their diet consists of detritus and algae (De Silva and Wijeyarante 1977). An increase in algae within the Indian River may explain a population increase of striped mullet, and, the increased importance of this fish species to the diet of dolphins in 2006. Hypothetically, an increase in algae can negatively affect seagrass growth by limiting light attenuation and spotted seatrout are often affiliated with seagrass beds (Wenner *et al.* 1990).

Estimated contributions of prey sources to dolphin diet in 2007 were substantially different than previous years. All six source groups used as input into the model were fairly evenly responsible for contributing to diet, whereas, in other collection years, two or three source groups tended to dominate contribution amounts. This indicates that dolphins in 2007 were eating a more diverse diet than previous years. In addition, pigfish were responsible for approximately 19% of the make-up of dolphin diet which was an increase from 2006 (6%) and 2005 (8%).

Bottlenose dolphins in the Indian River tend to be found in small groups (e.g., 1-3 individuals) (Odell and Asper 1990). Their feeding strategies reflect this, typically targeting species that do not form large schools such as spotted seatrout, pinfish, pigfish and spot, despite the presence of large schooling fish such as menhaden (Barros 1993). It has been suggested that passive listening may be a foraging strategy employed by dolphins and the current study reveals that some of the contributing prey species are soniferous such as spotted seatrout and pigfish (Gannon *et al.* 2005). These findings suggest that some populations of dolphins may be more selective in prey items than previously thought.

In the model validation portion of the study, it was seen that the model's ability to discriminate among sources is dependent on prey signatures being distinct; therefore, some SIAR predictions may be confounded by the fact that some of the potential prey sources did not have significantly different stable isotope ratios. This was the case for pinfish and spot which were subsequently grouped together to form one source group, "pinspot". Spot are bottom feeders that eat algae and copepods (Kjelson and Johnson 1976), whereas pinfish are omnivorous, eating minnows, crustaceans, seagrasses and algae (Luczkovich *et al.* 1995). Adult pinfish tend to be more herbivorous, foraging more on plant material and algae which lends to why stable isotopic signatures between pinfish and spot were similar (Luczkovich *et al.* 1995). Because these two species were indistinguishable in regards to stable isotope signatures, it is impossible to state which, if any, contributed more towards the diet of IRL bottlenose dolphins, however, according to Barros (1993) both species were fairly similar in their importance to dolphin diet with pinfish having a slightly higher frequency of occurrence in stomachs.

Over the past 15 years, bottlenose dolphins in the IRL have exhibited increased numbers of strandings (Stolen *et al.* 2007), Unusual Mortality Events (Marine Mammal Commission

2002), and have shown increased accounts of pathological issues such as infections and inflammatory diseases (Bossart *et al.* 2003) necessitating more studies into their ecology and biology. They are apex predators within this important, biodiverse estuary and may serve as indicators for the health of the overall system. The stable isotope analysis done here clearly shows the differences in feeding ecology among age classes of bottlenose dolphins, collection years, and among IRL segments and demonstrates the usefulness of this methodology. Fatty acid analysis was able to tease apart some differences in the diets of males and females that the stable isotope data did not reveal. Collectively the isotope and fatty acid data presented here indicate that IRL dolphins in different sub-regions are either feeding on different prey, or that the relative proportions of those prey in their respective diets differ. The conclusions, based on the fatty acid data, are consistent with the independent conclusions of our isotope analysis and collectively demonstrate that IRL bottlenose dolphins are flexible in their dietary choices and that they quickly respond to changes in the availability, and possibly energetic/nutritional quality, of the resident ichthyofauna. These analyses suggest that there is great potential in using dolphin blubber/skin to monitor ecological changes that may be occurring in the IRL. Clearly the IRL is undergoing dramatic changes (e.g., Bossart *et al.* 2006) and the current study provides insight into bottlenose dolphin ecology which can be utilized in conservation efforts. Future research efforts should include comparisons of stable isotope and fatty acid signatures between healthy and diseased resident dolphins to evaluate the relationship between the recent surge of disease in the population and feeding habits and/or habitat usage. With the increasing deposition of fertilizers and pollutants into the southern portions of the IRL, continued monitoring of nitrogen isotope signatures in these apex predators would be a useful indicator of important ecological changes for managers. Inter-annual differences observed in the current study could not be

explained by changes in salinity or water temperature or seemed to correspond with any other notable event (algal blooms, unusual mortality events, etc.). Further research into changes of stable isotope and fatty acid signatures in the IRL over years may reveal new patterns and dynamics which illuminate some of the ecosystem processes and characteristics. Future work should concentrate on sample collection over continuous consecutive years, larger sample sizes and perhaps collection of additional environmental data such as light attenuation values. These works may lend insight to events such as mortality events. The current study presented a unique opportunity to validate a Bayesian mixing model utilizing isotope values measured from animals on controlled diets and incorporating diet-tissue discrimination factors calculated from the same study animals. Based on current findings, SIAR is an appropriate choice for modeling source contributions to a consumer's diet. The current study is also the first of its kind to attempt to model bottlenose dolphin diet in the Indian River using stable isotopes. Additional studies into the feeding ecology of Indian River bottlenose dolphins are needed to assist in our understanding of their resource usage (Durden *et al.* 2011). Food, being a primary driver for many species, can lend explanation of things like movement patterns, habitat usage, competition, reproductive success, survival, and the spread of diseases, which has been an issue in Indian River dolphins in recent years. Dietary information modeled in this study provided new data for the relative contribution of a suite of potential prey to an apex predator in the Indian River. Stable isotope analysis and the use of SIAR are relatively easy and reliable tools for investigating dolphin feeding ecology and can be used in any future cetacean feeding studies.

Table 4.1: Mean (\pm SE) values of stable carbon and nitrogen isotopes and sample sizes of potential IRL dolphin prey categories. Prey species were collected with a 183 m center-bag seine during May of 2004 through 2007 in the IRL. Pinfish and spot were condensed to “Pinspot” based on no statistical difference for either carbon or nitrogen stable isotopes.

Source Group	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Pigfish	9	10.19 ± 0.43	-14.29 ± 0.68
“Pinspot”	34	9.95 ± 1.17	-16.67 ± 1.55
Silver Perch	43	11.04 ± 0.93	-16.42 ± 1.62
Spotted seatrout	25	13.36 ± 0.99	-18.22 ± 0.75
Striped mullet	31	8.19 ± 1.26	-15.88 ± 1.98
White mullet	45	8.16 ± 0.97	-14.05 ± 1.51
	187	9.95 ± 2.05	-15.94 ± 2.03

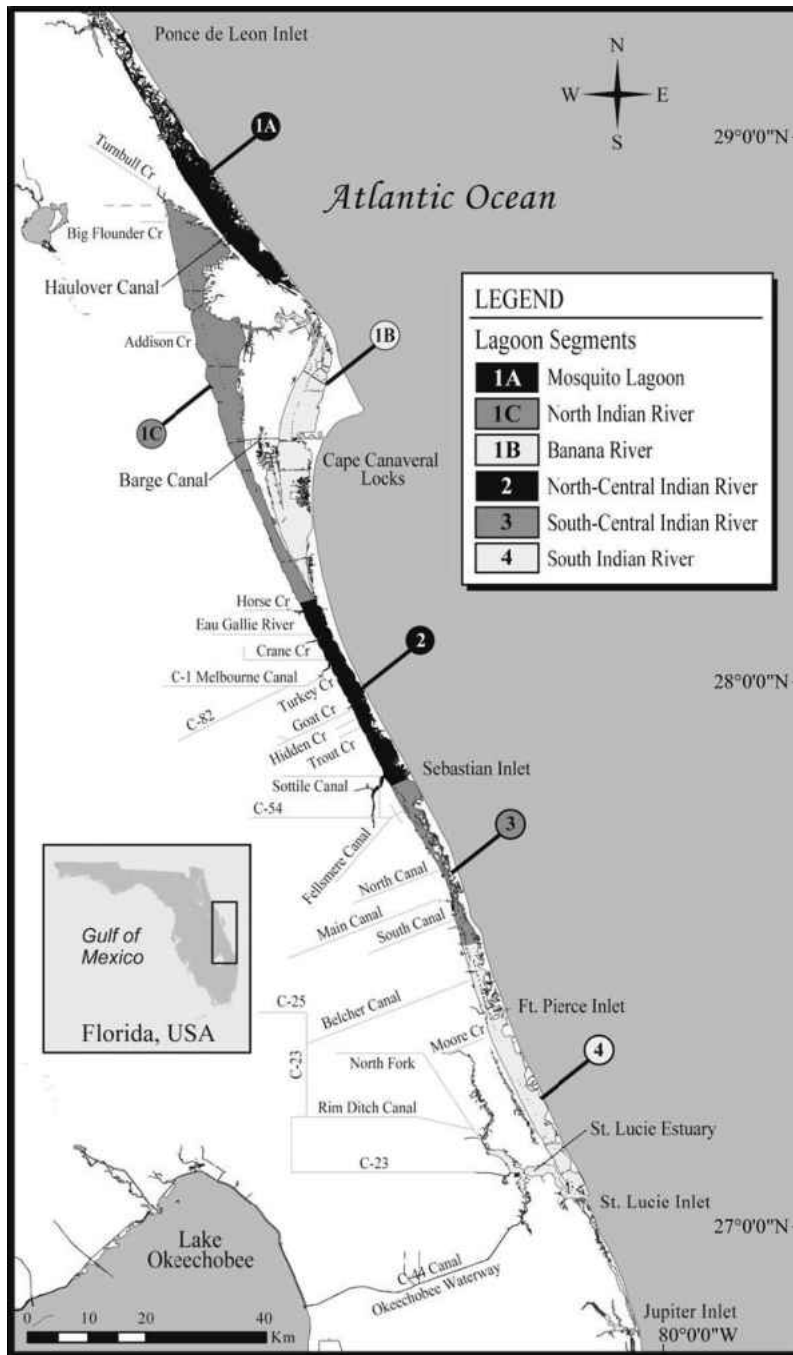


Figure 4.1: The Indian River Lagoon (IRL) located on the east coast of Florida was split into six segments to facilitate management projects (Mazzoil *et al.* 2008a).

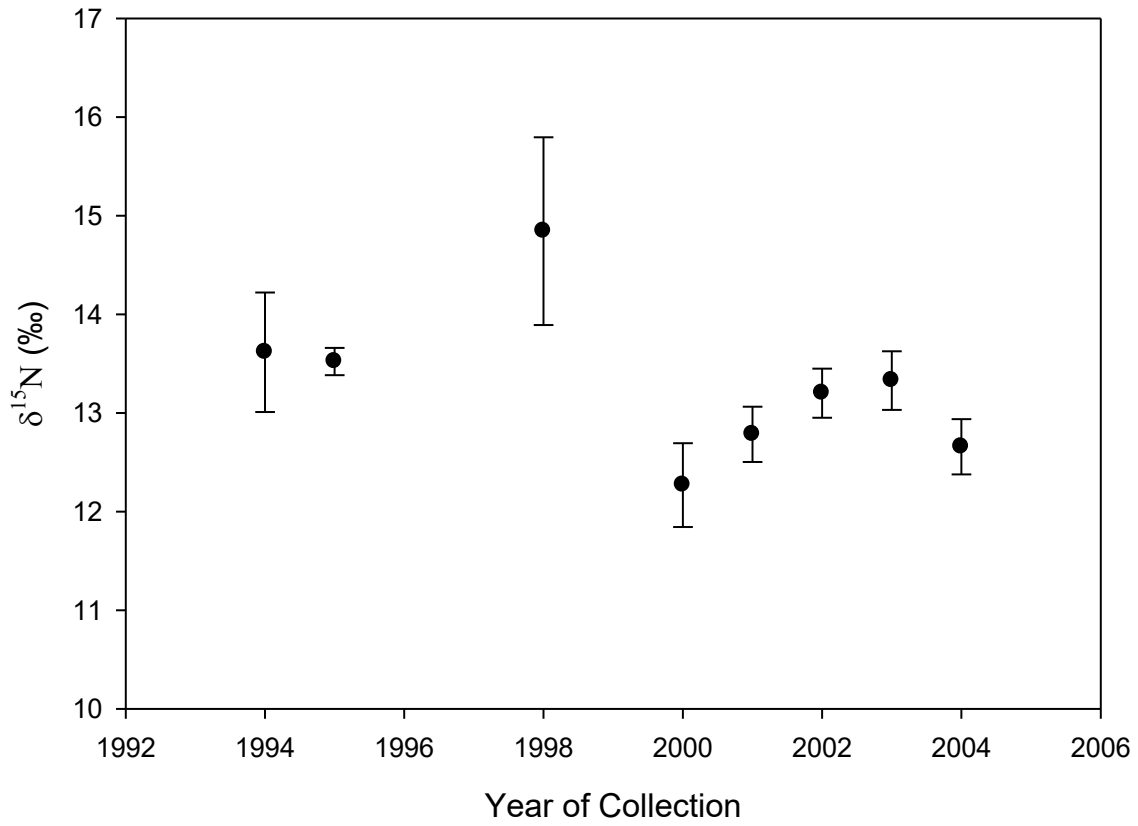


Figure 4.2: Mean nitrogen values (\pm S.E.) of IRL stranded bottlenose dolphins across collection years.

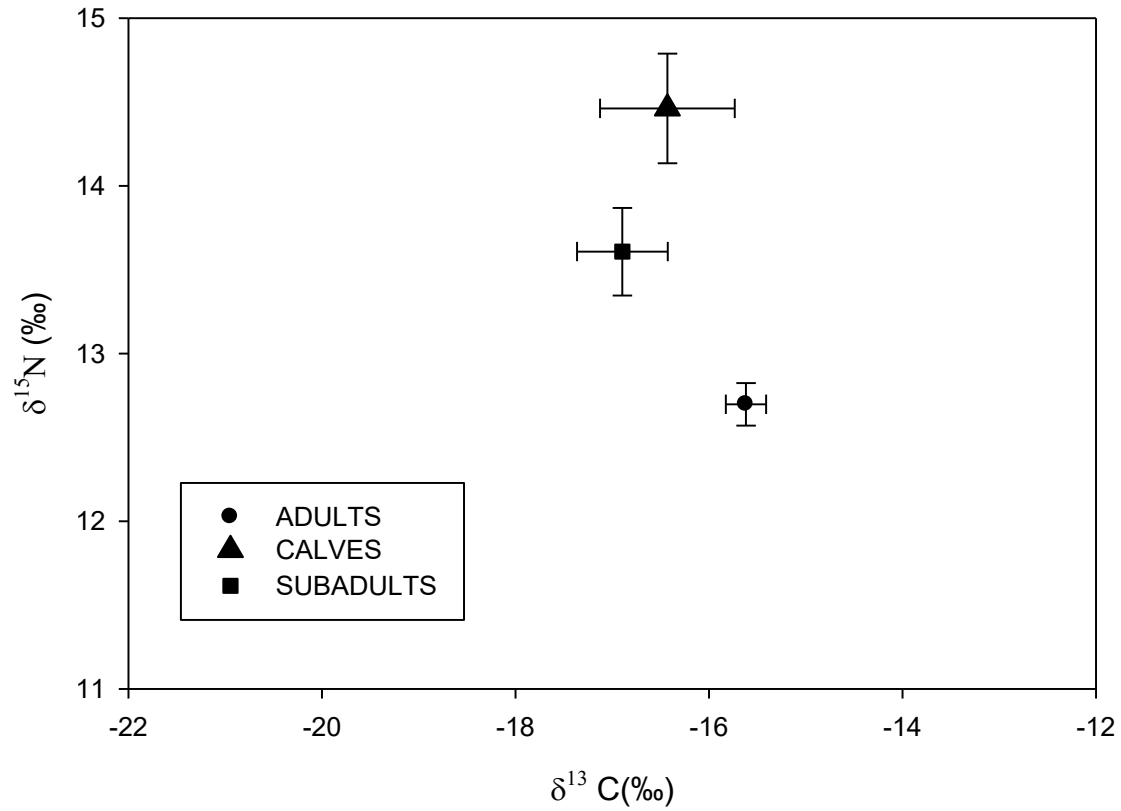


Figure 4.3: Carbon and nitrogen values (mean \pm S.E.) for different age classes of stranded IRL bottlenose dolphins.

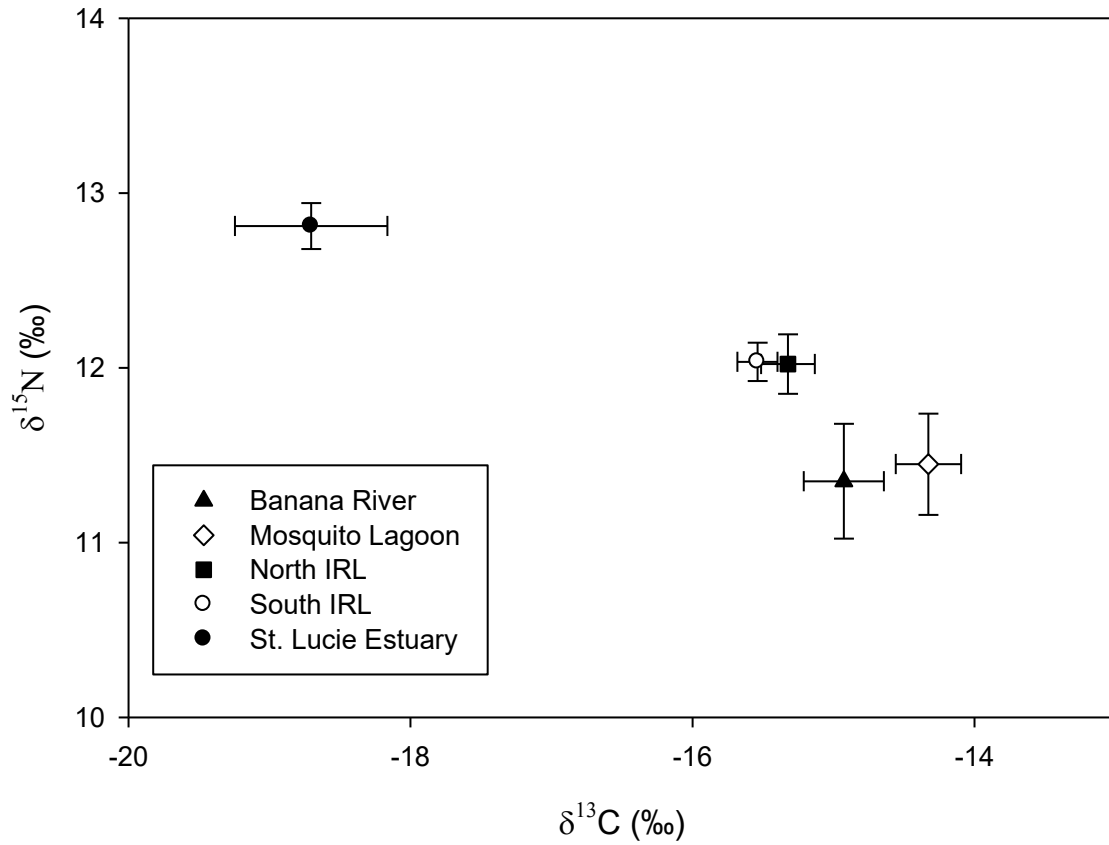


Figure 4.4: Carbon and nitrogen values (mean \pm S.E.) for different IRL segments of live biopsied IRL bottlenose dolphins.

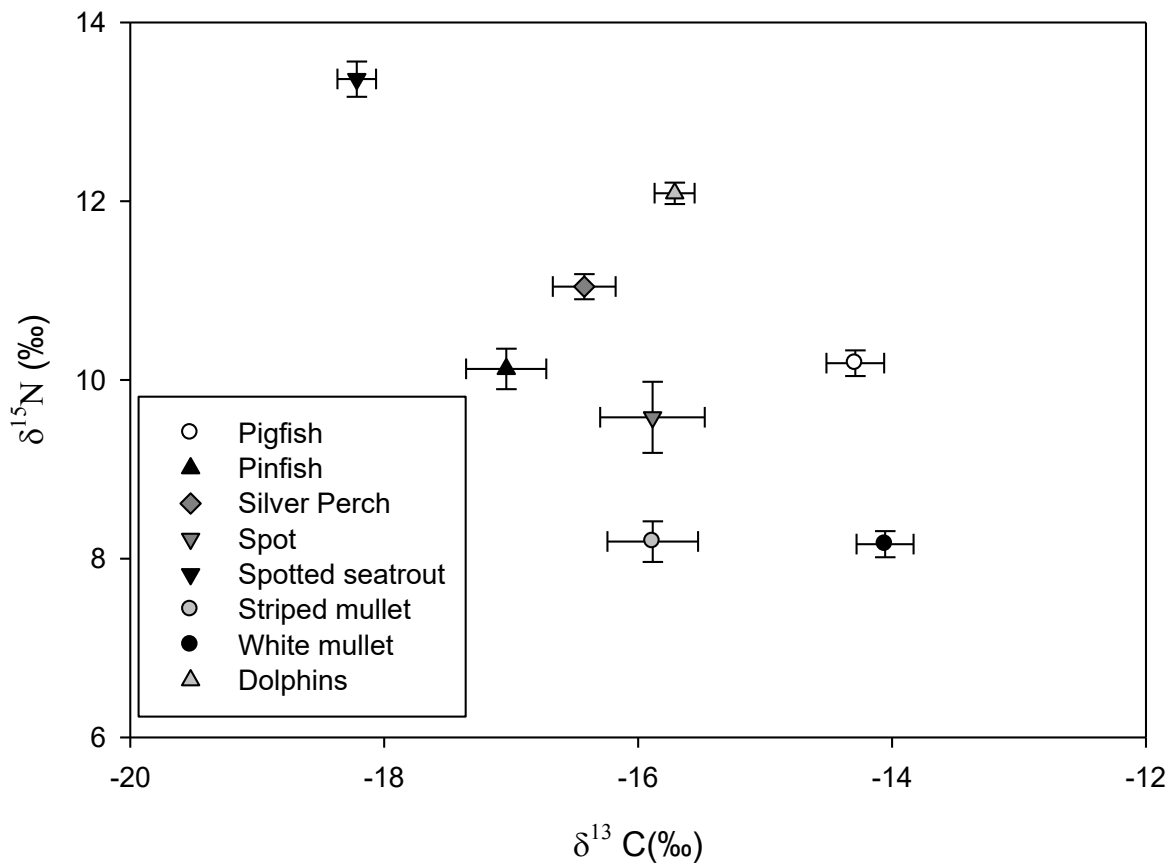


Figure 4.5: Carbon and nitrogen values (mean \pm SE) of Indian River bottlenose dolphins and their potential prey collected 2004-2007.

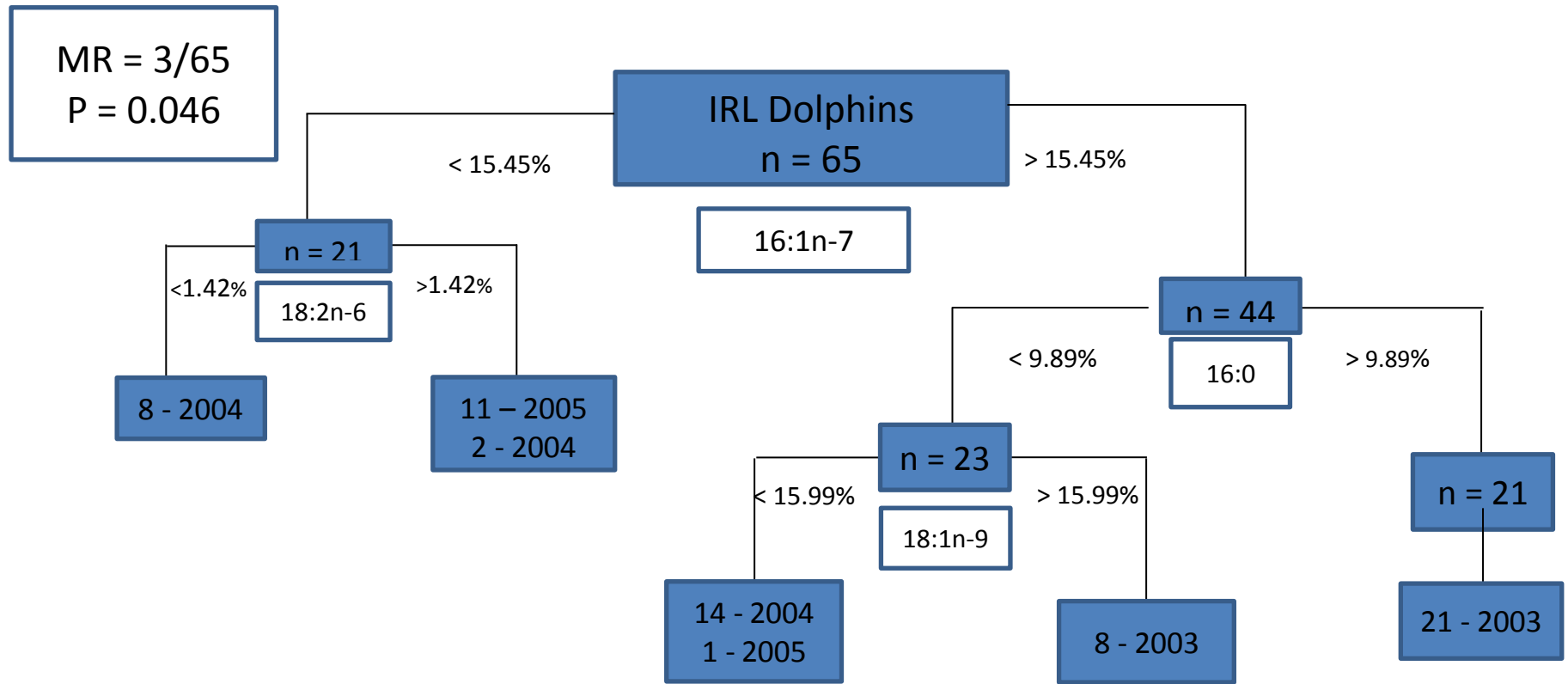


Figure 4.6: CART analysis of biopsies from live IRL bottlenose dolphins. Split points are identified with the specific fatty acid being used to split the dataset as well as the absolute concentrations of that fatty acid that determine the separation. Terminal nodes indicate the year of sample collection (and number) assigned to the node as well as the number of any misclassified samples.

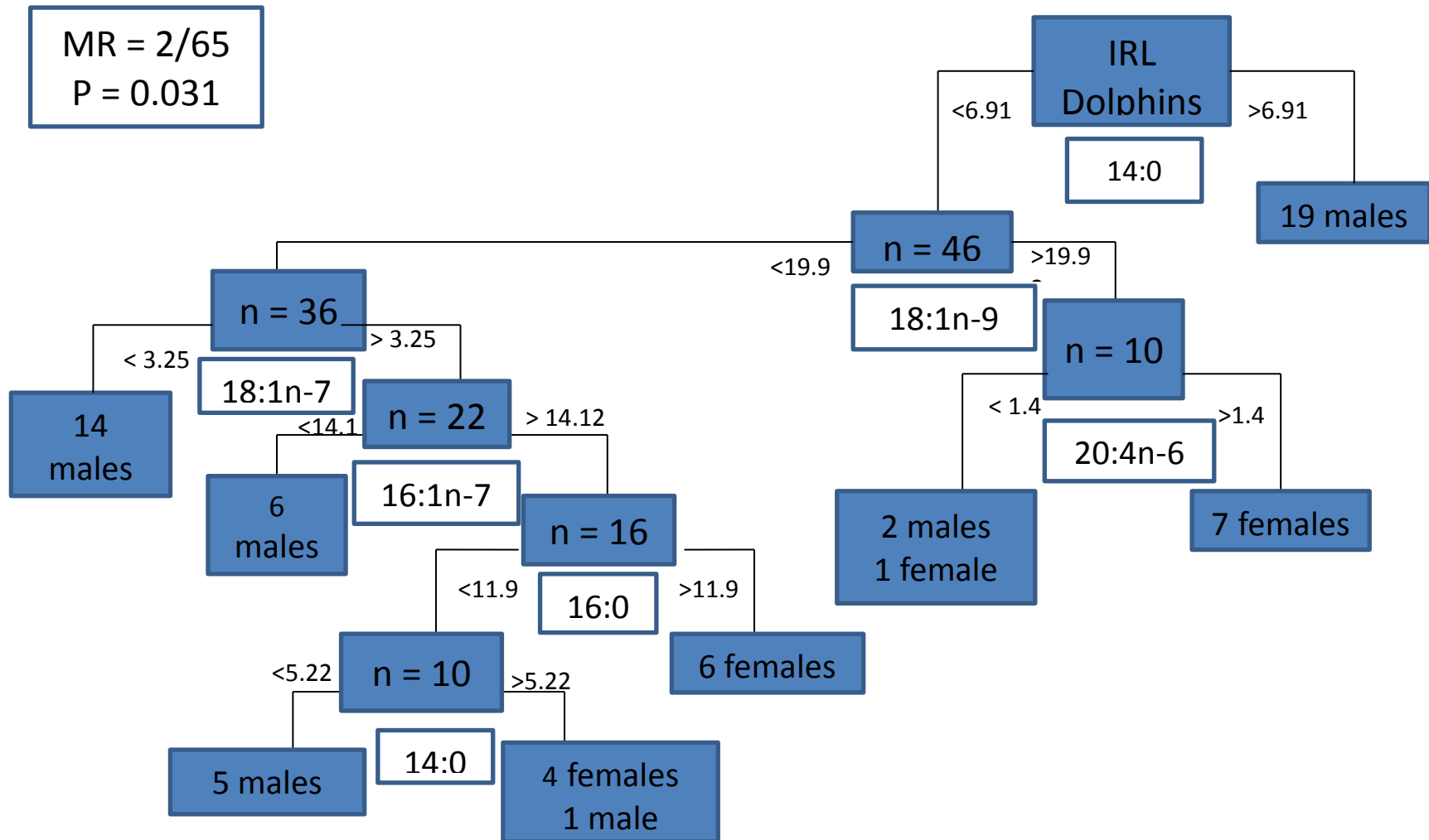


Figure 4.7: CART analysis of biopsies from live IRL bottlenose dolphins. Split points are identified with the specific fatty acid being used to split the dataset as well as the absolute concentrations of that fatty acid that determine the separation. Terminal nodes indicate the sex of the dolphin (and number) assigned to the node as well as the number of any misclassified samples.

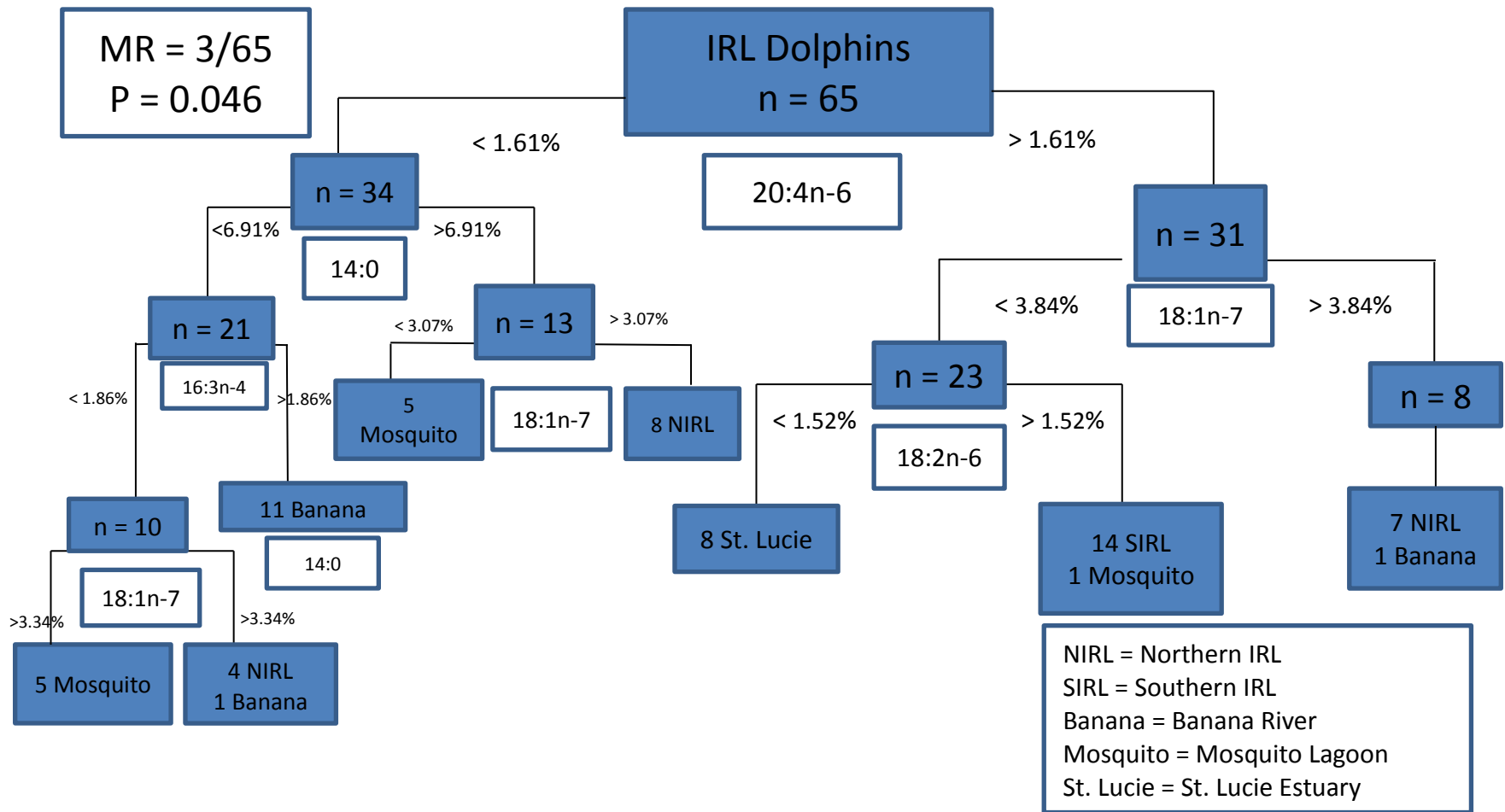


Figure 4.8: CART analysis of biopsies from live IRL bottlenose dolphins. Split points are identified with the specific fatty acid being used to split the dataset as well as the absolute concentrations of that fatty acid that determine the separation. Terminal nodes indicate the IRL segment (and number) assigned to the node as well as the number of any misclassified samples.

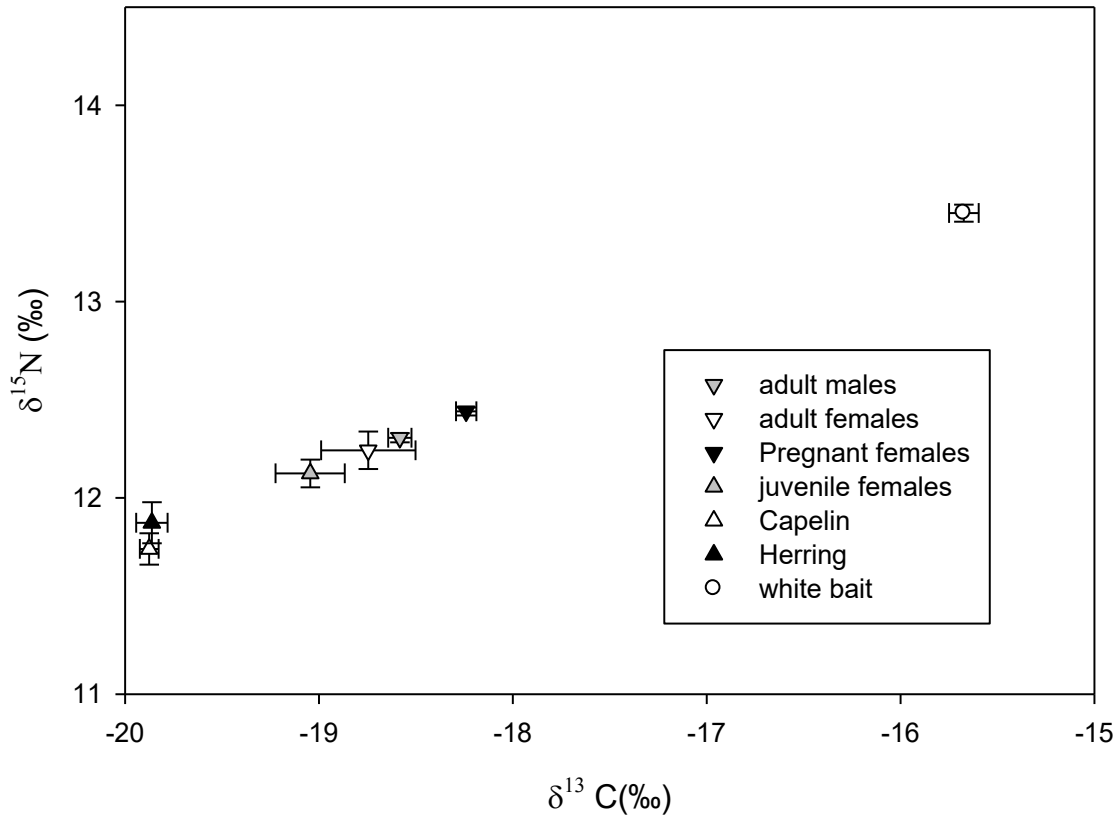


Figure 4.9: Scatterplot showing isotopic values (mean \pm SE) for dolphins held at SeaWorld Orlando in the “Key West” exhibit (consumers) and their food (sources) inputted into the Bayesian mixing model SIAR. Dolphins are grouped according to reproductive status.

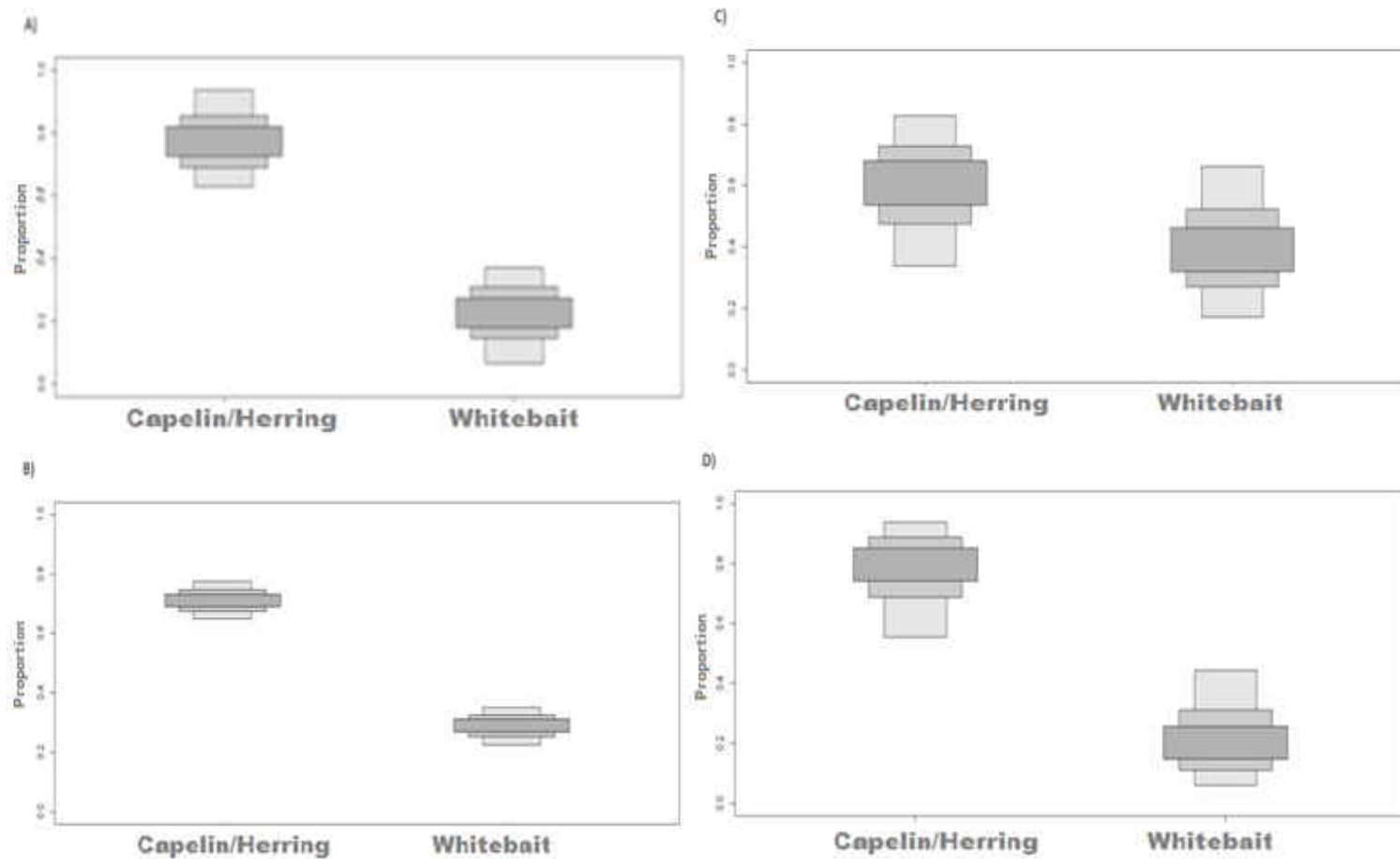


Figure 4.10: Boxplots of Bayesian SIAR mixing model results for SeaWorld dolphins in the “Key West” exhibit (model 1) showing estimates (50%, 75%, and 95% credibility intervals) of diet composition for bottlenose dolphins for each reproductive status category: adult females (A), adult males (B), pregnant females(C), and juveniles(D). Capelin and herring have been grouped into one source group due to similar isotopic signatures.

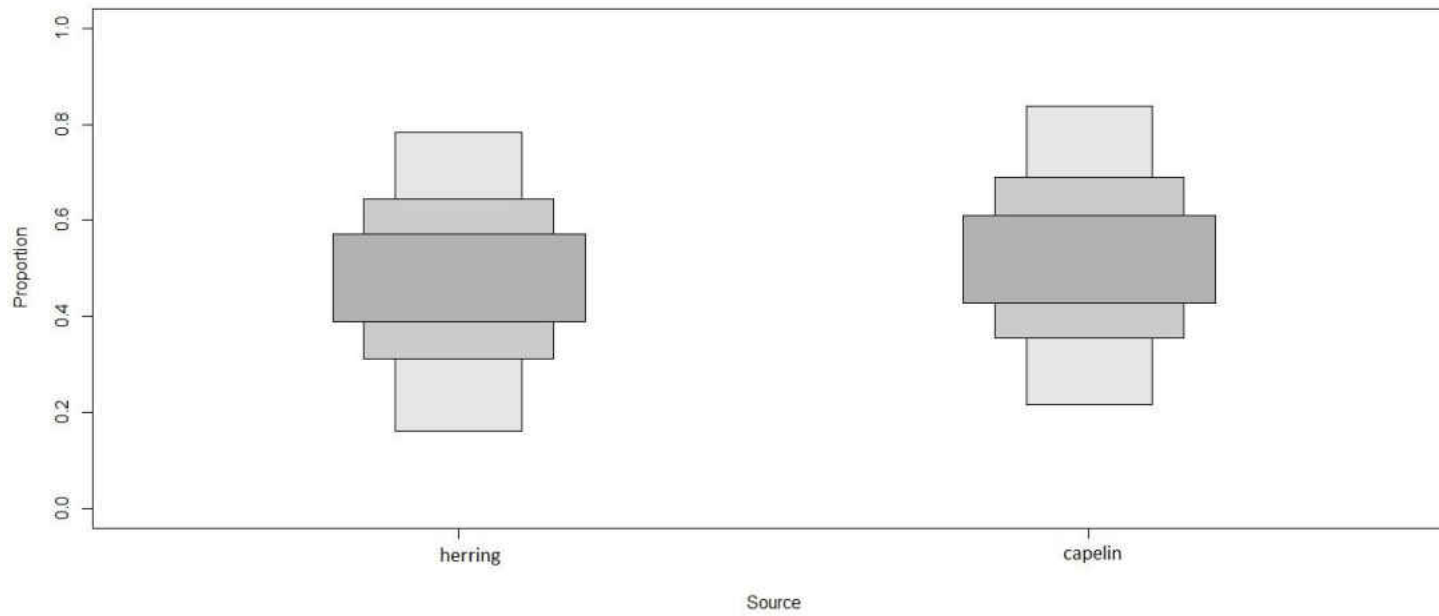


Figure 4.11: Results of Bayesian SIAR mixing model for SeaWorld “Whales and Dolphins” exhibit animals showing predictions (50%, 75%, and 95% credibility intervals) of diet composition for bottlenose dolphins.

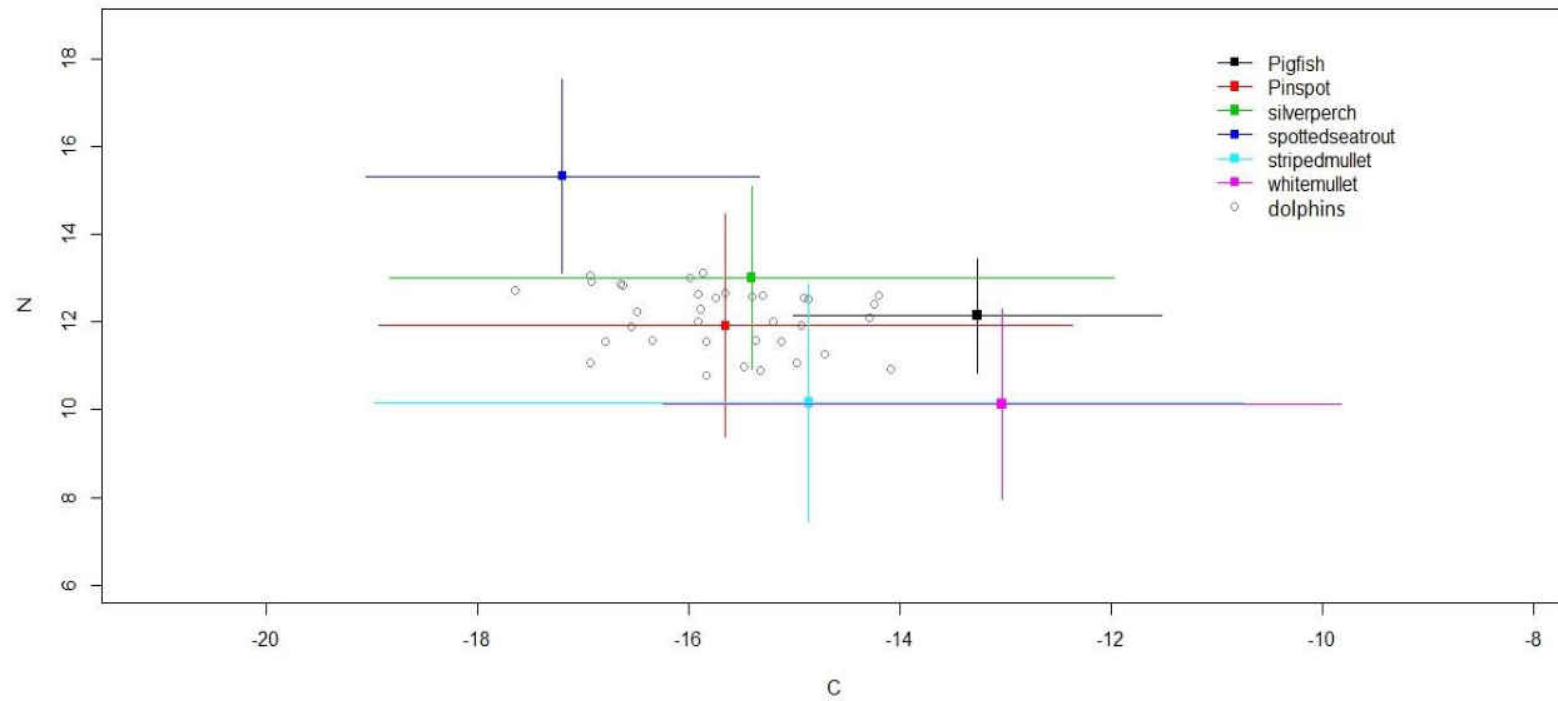


Figure 4.12: Scatterplot showing mean \pm SE for all IRL source groups (fish) and consumers (dolphins) inputted into the Bayesian mixing model SIAR. Dolphins are grouped across collection years (2004-2007).

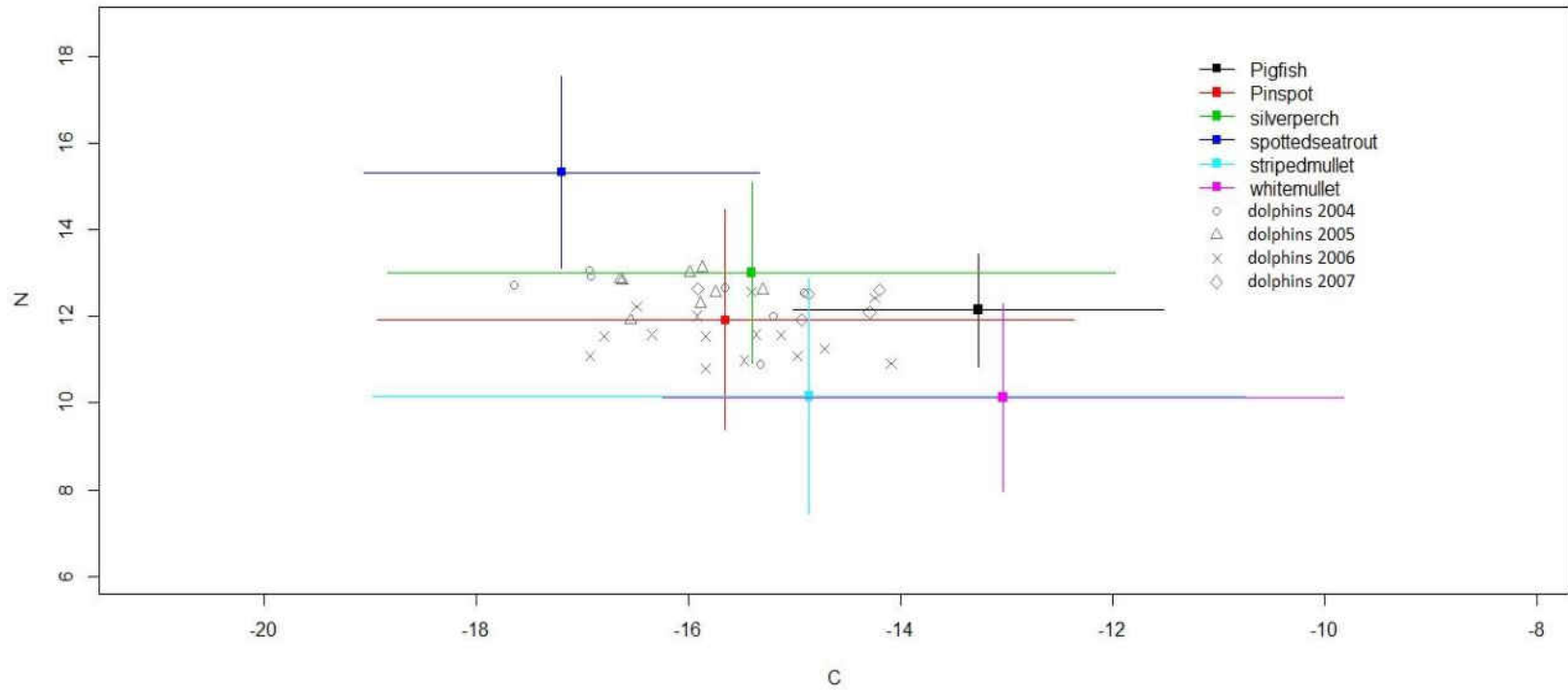


Figure 4.13: Scatterplot showing mean \pm SE for all IRL source groups (fish) and consumers (dolphins) inputted into the Bayesian mixing model SIAR. Dolphins are separated by collection years (2004-2007).

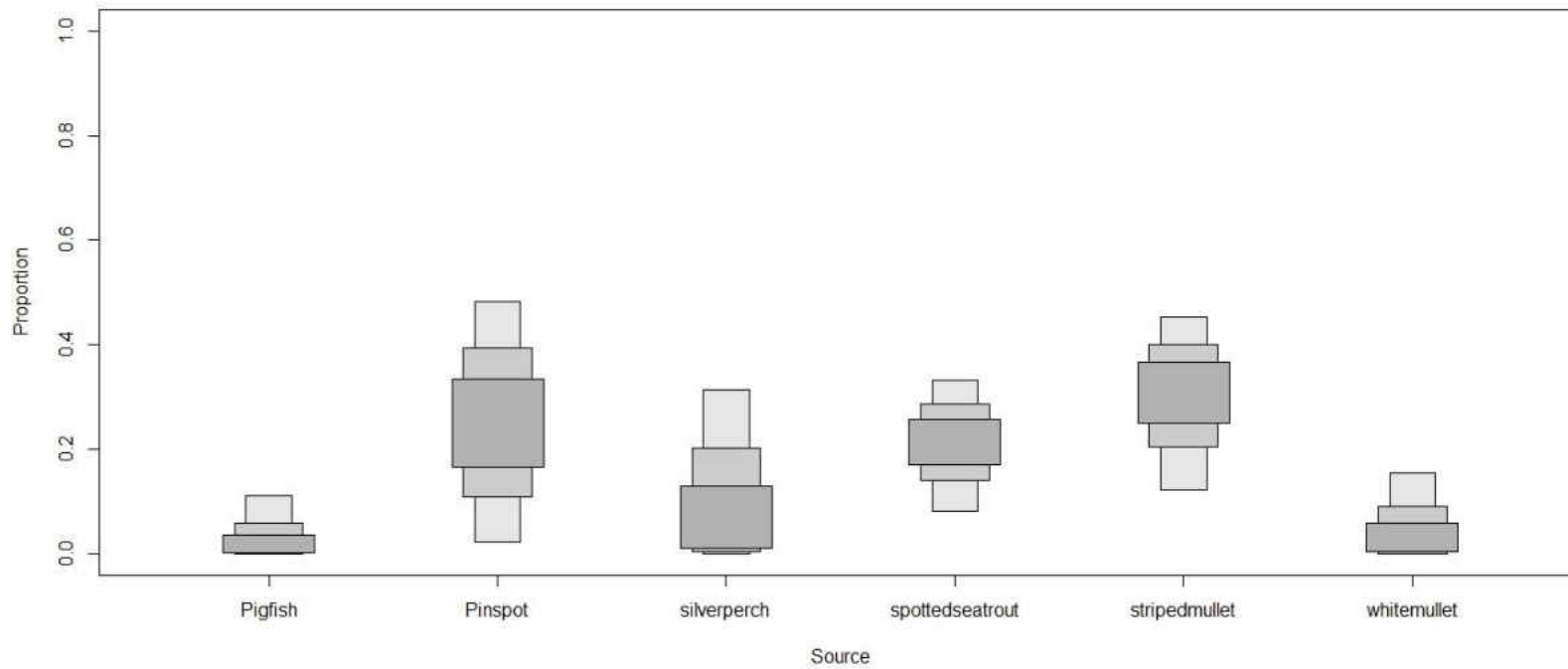


Figure 4.14: Results of Bayesian SIAR mixing model for IRL model 1 showing estimates (50%, 75%, and 95% credibility intervals) of diet composition for bottlenose dolphins with all sample years combined.

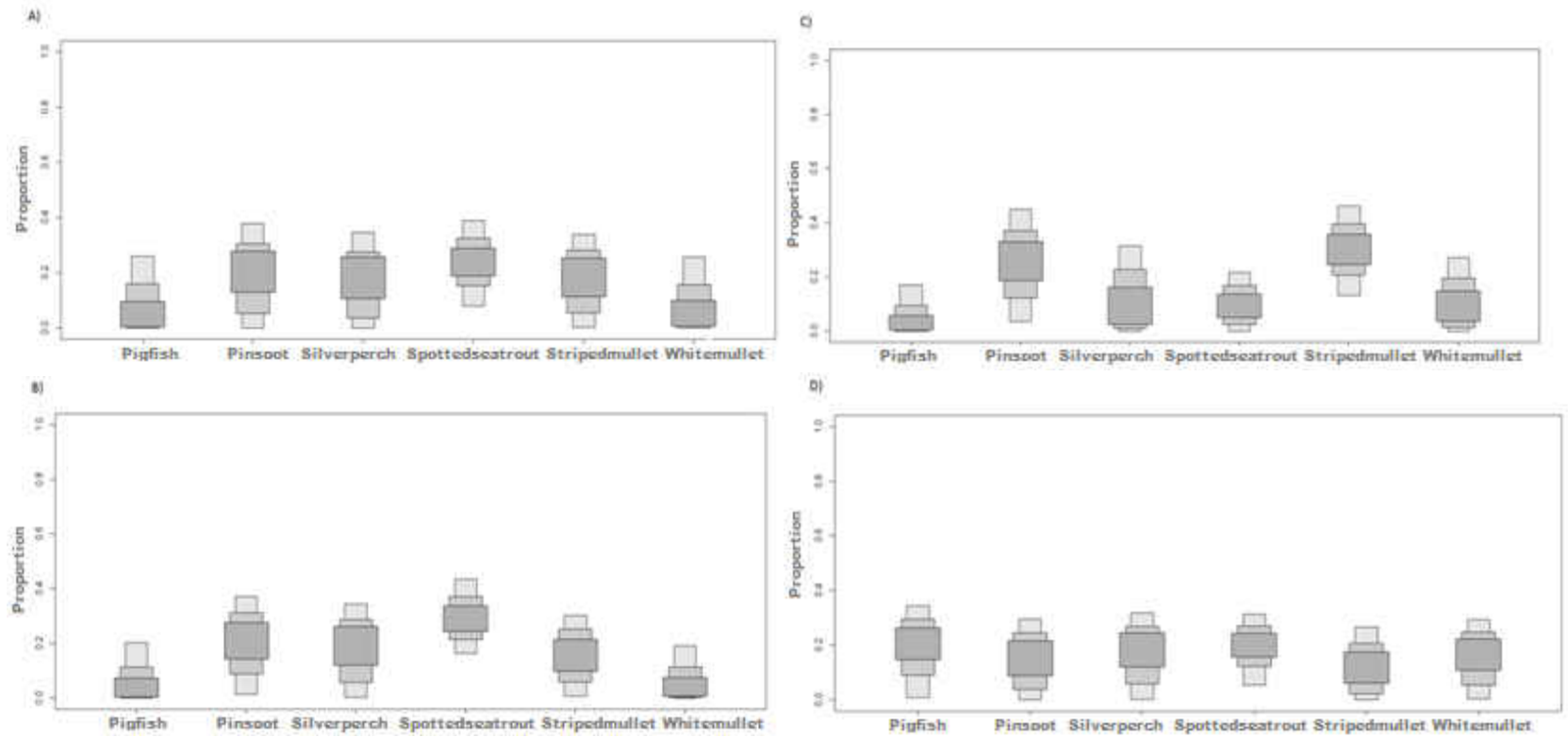


Figure 4.15: Results of Bayesian SIAR mixing model for IRL model 2 showing estimates (50%, 75%, and 95% credibility intervals) of diet composition for bottlenose dolphins for each sampling year: 2004 (A), 2005 (B), 2006 (C), and 2007 (D).

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CHAPTER 5: CONCLUSION

The results documented in this dissertation represent a novel examination of delphinid feeding ecology and habitat utilization primarily through the analysis of stable isotopes. Prey availability affects many aspects of a predator's life history and is considered a primary factor influencing habitat selection and movements whether they are small scale movements within a habitat or large scale movements like the migration patterns of many birds and whales (Hobson 1999, Kenney *et al.* 2001, Witteveen *et al.* 2009, Boyle *et al.* 2011, Wasko and Sasa 2012). This theory also holds true for delphinid species where it has been documented that the behaviors and movements of dolphins are strongly influenced by food availability (e.g., Shane 1977, Hanson and Defran 1993). The feeding ecology and habitat usage of many dolphin species is poorly understood and therefore, a large gap exists in the understanding of these animals' life histories. Many methodologies used to explore these facets of dolphin ecology have limitations and constraints or are logistically infeasible. Fortunately, stable isotope analysis provides a methodology that overcomes some of these limitations and provides information regarding relative trophic levels and feeding origins which lends insight into habitat usage.

Gannes *et al.* (1997) predicted an explosion in the usage of stable isotope analysis and argued that controlled studies are needed to establish species and tissue specific baseline values that can be applied in field studies. Many isotope studies have had to rely on diet-tissue discrimination values and turnover rates of tissues and species other than the ones being examined. This could lead to unreliable data and misleading conclusions being drawn. Chapter 2 presented calculated values for the diet-tissue discrimination factors and turnover rates for both carbon and nitrogen in bottlenose dolphin skin through the employment of controlled diet trials

with animals held at Farglory Ocean Park, Taiwan and SeaWorld Orlando, Florida. Up until now, cetacean researches have been forced to use values calculated through controlled studies of other species such as pinnipeds, birds and small mammals as surrogates (Hobson *et al.* 1996, Tieszan *et al.* 1983). This study represents the first of its kind and makes a significant contribution to stable isotope cetacean ecology. Traditionally, cetacean ecologists have utilized generalized published fractionation values of 1‰ for ^{13}C (Deniro and Epstein 1978) and 2-5‰ for ^{15}N (e.g., Rau *et al.* 1983, Minagawa and Wada 1984, Hobson *et al.* 1994). Average discrimination values determined in the present study were slightly lower, 0.82‰, for carbon and, on the lower end of the range, 2.20‰, for nitrogen. Diet-tissue discrimination factors are influenced by numerous including metabolic rate of the animal and/or tissue being studied, age of the animal, nutritional quality of the diet, tissue being sampled, and the taxon being investigated (e.g., Hobson and Clark 1992, Caut *et al.* 2009, Martinez del Rio *et al.* 2009, Newsome *et al.* 2010, Borrell *et al.* 2012). The present study examined this parameter and determined that nitrogen discrimination values showed evidence of significant differences based on the quality of the diet (lipid content) being consumed and maturity of the dolphin sampled. Juvenile dolphins had a significantly higher $\Delta^{15}\text{N}$ ($2.46 \pm 0.17\text{‰}$) than adults consuming the same diet ($1.92 \pm 0.14\text{‰}$) but did not differ significantly in $\Delta^{13}\text{C}$ ($0.53 \pm 0.17\text{‰}$ versus $0.84 \pm 0.18\text{‰}$). Dolphins consuming low fat diets, and hence high protein, had significantly lower $\Delta^{15}\text{N}$ ($1.68 \pm 0.11\text{‰}$) than those consuming a higher fat diet, and hence lower protein, ($2.96 \pm 0.12\text{‰}$) but both diets resulted in similar $\Delta^{13}\text{C}$ ($1.6 \pm 0.09\text{‰}$ versus $2.04 \pm 0.14\text{‰}$). It has been suggested that $\Delta^{15}\text{N}$ should decrease as dietary protein quality increases (quality hypothesis) (e.g., Roth and Hobson 2000, Robbins *et al.* 2010). Juvenile dolphins in the present study were continuing to nurse and hence, were receiving a higher fat diet compared to older individuals only consuming

fish. The quality hypothesis may help explain their higher $\Delta^{15}\text{N}$ when compared to adults. Collectively, results of the present study suggest that prey composition could be a significant factor to consider when applying discrimination factors to field studies. My results suggest that while diet-tissue discrimination factors for $\Delta^{13}\text{C}$ may be approximately 1‰ in odontocetes, $\Delta^{15}\text{N}$ may be closer to 2‰ and may show significant variability as dietary quality changes on a seasonal basis, but also as a function of the trophic level of the prey being consumed.

Turnover rates quantify the period of time it takes for the isotopic signature of consumer tissues to reflect a new diet. It has been estimated that it takes approximately 2-3 half-lives for the complete integration of a new isotopic signature into consumer tissues (e.g., Hobson and Clark 1992). In the present study, nitrogen had a half-life, depending on diet, of 17.2 ± 1.3 days and carbon had a comparable half-life of between 13.9 ± 4.8 (diet B) and 22.1 ± 0.5 days (diet A). Until now there were no other published studies of isotopic turnover rates in cetacean skin. Isotopic turnover rates vary among tissue types, species, body sizes, nutritional states, and protein turnover rates which in turn can be influenced by diet quality (Martinez del Rio *et al.* 2009, Newsome *et al.* 2010) making comparisons among studies difficult. The only previous study in the literature that has investigated turnover rates in marine mammal skin was done with manatees (Alves-Stanley and Worthy 2009). This latter study investigated half-lives of carbon and nitrogen in the skin of both coastal and riverine manatees. Coastal manatees had a mean carbon half-life of 53 days and nitrogen half-life of 27 days, while riverine manatees had a half-life for carbon of 59 days and nitrogen of 58 days. Turnover rates may be slower in manatees than in bottlenose dolphins in part, due to the difference in body size (1000 kg versus 260 kg respectively) (Kastelein *et al.* 2002), but also the lower metabolic rate of manatees (Irvine 1983).

Despite wide-spread application of stable isotope analysis, the literature is still lacking information on discrimination values for most cetacean tissue types and turnover rates are virtually non-existent. Over the past few years, there have been repeated calls for direct measurements of the basic assumptions and parameters involved in stable isotope ecology (e.g., Gannes *et al.* 1997; Caut *et al.* 2009, Martinez del Rio *et al.* 2009, Wolf *et al.* 2009, Wyatt *et al.* 2010). Tissue specific discrimination values, especially skin, allow ecologists to establish trophic levels for organisms and turnover rate data is necessary for interpreting temporal scales. Both of these values are necessary when trying to recreate dietary history and discrimination factors are critical for use in mixing models (see Chapter 4) (e.g., Phillips and Gregg 2001, Phillips and Gregg 2003, Parnell *et al.* 2010). This portion of the current study is the first of its kind and presents data that will allow cetacean biologists to more accurately and appropriately interpret isotope data.

Many species of delphinids co-exist in the same geographic region or have overlapping distributions. It is believed these sympatric species partition resources through variations in habitat use, temporal activity, and dietary preferences with the result that coexistence is possible and competition is reduced (Baird and Whitehead 2000, Parra 2006, Saayman and Taylor 1973, Spitz *et al.* 2011, Wang *et al.* 2012). Chapter 3 demonstrated this phenomenon by illustrating resource partitioning in four South African delphinid species. Of the two isotopes tested, $\delta^{13}\text{C}$ indicates source of feeding location while $\delta^{15}\text{N}$ reflects trophic position (Hobson and Welch 1992, Rau *et al.* 1992, Hobson and Wassenaar 1999, Kelly 2000). Significant differences in nitrogen signatures were observed among the four species that I examined, indicating differences in the prey types or proportions of prey taken in by these delphinids and illustrates some degree of resource partitioning. Humpback dolphins were most enriched in $\delta^{15}\text{N}$ and averaged 3‰

greater than the most nitrogen depleted of the four species, the striped dolphin. This difference suggests that humpback dolphins forage approximately one trophic level above striped dolphins in this ecosystem (Rau *et al.* 1983). The results of the current study are generally consistent with reported analyses of stomach contents. Cockcroft and Ross (1983) and Young and Cockcroft (1994) examined stomach contents of South African delphinids and found that common dolphins preyed primarily on pilchard (*Sardinops ocellatus*), which are offshore pelagic fish that are filter feeders, whereas bottlenose and humpback dolphins fed mostly on inshore species. The prey of importance to bottlenose dolphins were piggies (*Pomadasys olivaceum*), a benthic predator that feeds on prey such as small shrimp and flatworms, and cuttlefish (*Sepia spp.*), while the species that were most commonly found in the stomachs of humpback dolphins were glassnoses (*Thrissa vitirostris*), striped grunter (*Pomadasys striatum*) and cuttlefish (*Sepia spp.*).

In aquatic ecosystems, carbon isotope ratios reflect differences between freshwater and marine sources as well as offshore/pelagic habitats versus inshore/benthic sources. Carbon isotope values tend to become more enriched from offshore to inshore locations, serving as a potential indicator of habitat usage (Gomez-Campos 2011). In the present study, humpback dolphins had the most enriched $\delta^{13}\text{C}$ values while striped dolphins were most depleted. Common dolphins were not significantly different from humpback dolphins although on average, had slightly lower $\delta^{13}\text{C}$ values. Values for bottlenose dolphins fell between common and striped dolphins. These findings suggest some spatial separation when it comes to foraging, despite overlapping ranges. Based on carbon values in the current study, humpback and common dolphins are foraging closest inshore, followed by bottlenose dolphins and then finally striped dolphins which would be feeding in more offshore waters.

Males and females differed significantly in nitrogen values, indicating that dolphins of different sexes might be feeding at different trophic levels, hence utilizing resources differently. While data on dietary preferences between male and female dolphins differ in the literature (e.g., Young and Cockcroft 1994, Chou *et al.* 1995, Silva 1999, Amir *et al.* 2005), Cockcroft and Ross (1990) examined stomach contents from animals in this same region and concluded there were significant differences in prey choice between sexes.

Food is a primary driver for movement, habitat usage, and behavior and therefore, is an important factor of any organism's biology to understand. Several of these South African delphinids are considered to be vulnerable or data deficient with calls for more studies that will illuminate their ecology. The findings of chapter three of this work does just that by providing new insight into the feeding ecology, habitat usage and resource partitioning of these dolphins. This is the first study of its kind to evaluate resource partitioning of *in situ* South African dolphins through stable isotope signatures.

Chapter 4 incorporated both stable isotope and fatty acid analyses to examine habitat utilization and feeding ecology of a resident population of bottlenose dolphins in the Indian River Lagoon, Florida. The Indian River Lagoon (IRL) is a 250 km long estuary comprised of four distinct bodies of water running along the east coast of Florida including Mosquito Lagoon, Indian River, Banana River and the St. Lucie Estuary. In 1990 the United States Environmental Protection Agency designated the IRL as an Estuary of National Significance to help preserve one of the most biodiverse estuaries in North America. Pressures from human population growth, overfishing, habitat degradation and inflows of pollutants, nutrients and freshwater made it necessary to put conservation efforts into force. It is estimated that there are approximately 300 resident dolphins in the IRL that rarely, if ever, leave the lagoon (Mazzoil *et al.* 2008). In

addition to the conservation concerns stated above, dolphins in the IRL have been showing a substantial increase in disease over the last decade and hence, research efforts have increased in an attempt to learn more about their ecology. The conclusions presented in chapter 4 were based on samples derived from both dead stranded and live biopsied animals. The use of stranded animals allowed for measurement of all age classes whereas calves would not typically be targeted for sample collection. $\delta^{13}\text{C}$ was not significantly different among these animals indicating they were all feeding in the same location; however $\delta^{15}\text{N}$ was significantly different for all three age classes. It has been demonstrated that nursing mammals will have enriched nitrogen values (e.g., Hobson *et al.* 1997, Knoff *et al.* 2007), and the results of this chapter reflect that. In chapter 4 it was also demonstrated that locational differences could be determined through carbon signatures, reflecting that dolphins were feeding in different parts of the Indian River Lagoon. This system, made up of four major basins, is known to vary in abundance of seagrasses and algae which will be reflected in the carbon signature of the consumers (Woodward-Clyde Consultants 1994). The addition of fatty acid analysis allowed for finer resolution of feeding differences, detecting differences between male and female dolphins in the Indian River Lagoon which were not differentiated by stable isotope analysis on its own. This may indicate that males and females are eating in similar locations and at a similar trophic level, however, are varying in the types or proportions of prey at this level. The combination of these parallel types of analyses results in a powerful tool for assessing feeding ecology.

Fish species residing in the IRL have been well studied and stable isotope values of seven species of fish that were collected for a different study were available for inclusion in mixing models to estimate the proportions of each prey species that IRL dolphins might be consuming. Dietary mixing models have been used widely throughout the ecology literature and several

variations have been developed in recent years. Bayesian mixing models, in particular, allow for the input of diet-tissue discrimination factors and also account for variability in these factors as well as that in dietary sources (Phillips 2012). They also allow for inclusion of a larger number of dietary sources than some of the older models that were restricted to $n + 1$, where n represents the number of isotopes being measured (Phillips and Gregg 2003). Ground-truthing of these mixing models using data collected from controlled diet studies is needed to ascertain their degree of certainty when predicting diet composition. Chapter 4 utilizes *ex situ* animals to obtain the parameters needed for dietary mixing models and provides an opportunity to evaluate the accuracy of a Bayesian mixing model (SIAR). The model demonstrated the ability to discern between prey items with the largest discrepancy being a difference of 4.5% for adult female dolphins, and increased in accuracy as prey items differed in isotopic signatures.

Bottlenose dolphins are apex predators in the Indian River and as such, play an important role in influencing this important, ecologically diverse habitat (Bossart 2010). Species of dolphins have been found to consume fish, squid, and crustaceans, depending on their habitat, and are commonly considered to be generalists in prey selections. The diet of IRL bottlenose dolphins has not been studied closely and information in the published literature is scarce. The same dietary mixing model was used to predict proportions of each fish species the dolphins were consuming. Modeling predictions made in the current study using SIAR supports the assumption that IRL bottlenose dolphins are generalist feeders, consuming a variety of species. Across years, there tended to be two or three species that made up the bulk of the dolphin diet with remaining species contributing in smaller amounts.

The work of chapter 4 presented a unique opportunity to validate a Bayesian mixing model utilizing isotope values measured from animals on controlled diets and incorporating diet-

tissue discrimination factors calculated from the same study animals. It was demonstrated that SIAR performed with accuracy in predicting the relative contributions of differing sources and that it even had some abilities to make predictions regarding sources that were similar in isotopic signatures.

The studies described in the chapters of this dissertation demonstrate an application of stable isotope ecology on both a fine and broad scale. Results from each chapter present novel findings and contribute to the understanding of delphinid feeding ecology and habitat utilization, as well as being the first to present important and needed stable isotope values and validating frequently used mixing models. These works demonstrate the benefits and successful application of stable isotope analysis, as well as fatty acid signature analysis to the ecology of delphinids.

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