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Stabel Isotope Turnover Rates And Diet-tissue Discrimination In The Skin Of West Indian Manatees: Implcations For Evaluating Their Feeding Ecology And Habitat Use

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STABLE ISOTOPE TURNOVER RATES AND DIET-TISSUE DISCRIMINATION IN THE SKIN OF WEST INDIAN MANATEES: IMPLICATIONS FOR EVALUATING THEIR FEEDING ECOLOGY AND HABITAT USE

by

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ABSTRACT

The West Indian manatee (*Trichechus manatus*) is an herbivorous marine mammal that occupies freshwater, estuarine, and marine habitats. Despite being considered endangered, relatively little is known about the feeding ecology of either of the two recognized subspecies, the Florida manatee (*T.m. latirostris*) and Caribbean or Antillean manatee (*T.m. manatus*). A better understanding of their respective feeding preferences and habitat use is essential to establish criteria on which conservation plans can be based. The present study expands on previous work on manatee feeding ecology by both assessing the application of stable isotope analysis to manatee tissue and providing critical baseline parameters for accurate isotopic data interpretation.

The present study was the first to calculate stable isotope turnover rate in the skin of any marine mammal. Stable carbon and nitrogen isotope ratios were examined over a period of more than one year in the epidermis of rescued Florida manatees that were transitioning from a diet of aquatic forage to terrestrial forage (lettuce) in captivity. Mean half-life for ^{13}C turnover in manatee epidermis was 55 days and mean half-life for ^{15}N turnover was 42 days. Due to these slow turnover rates, carbon and nitrogen stable isotope analysis in manatee epidermis is useful in summarizing average dietary intake over a long period of time rather than assessing recent diet. In addition to turnover rate, a diet-tissue discrimination value of 2.8‰ for ^{13}C was calculated for long-term captive manatees on a lettuce diet.

Turnover and diet-tissue discrimination results were subsequently used to interpret carbon and nitrogen stable isotope data in epidermis samples collected from

free-ranging manatees in Florida, Belize, and Puerto Rico. This study was the first application of stable isotope analysis to Antillean manatees. Regional differences in stable isotope ratios in manatee skin were consistent with ratios in plant samples collected in those regions. Signatures in the skin of manatees sampled in Belize and Puerto Rico indicated a diet composed mainly of seagrasses, whereas those of Florida manatees exhibited greater variation. Mixing model results indicated manatees sampled from Crystal River and Homosassa Springs had an overall average intake of primarily freshwater vegetation whereas manatees sampled from Big Bend Power Plant, Ten Thousand Islands, and Warm Mineral Springs fed primarily on seagrasses. Possible diet-tissue discrimination values for ^{15}N ranged from 1.0 to 1.5‰. Stable isotope analysis can be successfully applied to interpret manatee feeding behavior over a long period of time, specifically the use of freshwater vegetation vs. seagrasses, and can aid in improving conservation efforts.

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CHAPTER 1: INTRODUCTION

The West Indian manatee (*Trichechus manatus*) is an herbivorous marine mammal comprised of two subspecies, the Florida manatee (*T.m. latirostris*) and the Antillean or Caribbean manatee (*T.m. manatus).* Of the four extant sirenian species, the West Indian manatee is one of only two (*T. manatus* and *T. senegalensis*) that occupy marine, estuarine, and freshwater habitats (Hartman 1979, Best 1981). Florida manatees are found in Floridian waters year-round but during warm seasons their range extends west along the Gulf coast to Texas, and as far north as the Carolinas and Virginia (Fertl et al. 2005). The Antillean manatee is distributed along the coastlines of the Caribbean, Central America, and the northern coast of South America.

Florida and Antillean manatees are endangered and both human-related and natural threats compromise their populations. Injury due to watercraft and poaching rank the highest among human-related causes of mortality in Florida and Antillean populations, respectively (UNEP Caribbean Environment Programme 1995, U.S. Fish and Wildlife Service 2001). Less common, yet still threatening, are entanglement in fishing gear, ingestion of debris, and crushing/drowning in flood gates or canal locks. Natural causes of mortality may include cold stress, red-tide poisoning, disease, or birth complications. Habitat threats that can and are affecting both populations include scarring of seagrass beds due to boat traffic, loss of seagrass beds, coastal development, and pollution (Smith 1993, Duarte 2002).

Relatively little is known about manatee feeding ecology and habitat use since they often occupy shallow, turbid water. The Florida Manatee Recovery Plan (U.S. Fish

and Wildlife Service 2001) and the Regional Management Plan for the West Indian Manatee (UNEP Caribbean Environment Programme 1995) include identifying and evaluating manatee habitats through studies of their feeding ecology as one of many actions needed for species recovery and/or protection. The present study focused on the feeding ecology of manatees in Florida, Belize and Puerto Rico.

The Florida manatee has been divided regionally into four subpopulations: Northwest, Southwest, Atlantic (including the lower St. Johns River), and the upper St. Johns River. Three of the four subpopulations occur in regions that contain marine, estuarine, and freshwater habitats, while the upper St. Johns River is exclusively a freshwater habitat. There is some movement of individual manatees between subpopulations, but movements between the east and west coasts of Florida have not been documented (Reid et al. 1991). Manatee habitat use in Florida may be characterized by access to freshwater, adequate and appropriate vegetation, bathymetry, currents, and/or shelter (reviewed by Lefebvre et al. 1989). It is presumed that the need for regular access to fresh drinking water is based upon osmoregulatory requirements (Ortiz et al. 1998, Ortiz et al. 1999). If water temperatures drop below 20C, manatees in Florida aggregate at natural (springs) or artificial (power plant outflows) warm water sources due to physiological constraints on thermal tolerances (Irvine 1983, Worthy et al. 2000, Bossart et al. 2003).

Habitat use by Antillean manatees in Mexico has been characterized most strongly by proximity to a freshwater source (Olivera-Gomez & Mellink 2005). The tropical habitat occupied by Antillean manatees does not experience the same fluctuations in water temperatures that necessitate manatee migration during cold seasons

in Florida. Antillean manatee distribution is patchy, with population counts in most countries totaling fewer than 100 individuals (summarized in O'Shea & Salisbury 1991). Within the range of the Antillean manatee, Belize has the largest population counts at approximately 100-250 individuals (O'Shea & Salisbury 1991, Morales-Vela et al. 2000). In Puerto Rico, the distribution is patchy, with most manatees being spotted along the southern and northeastern coasts. Aerial survey counts in this region suggest a total population of 60-100 individuals (UNEP Caribbean Environment Programme 1995).

Manatees are considered generalist herbivores and have been known to consume some 60 species of submerged, emergent, and floating vegetation in marine, estuarine, and freshwater habitats (Hartman 1979, Best 1981, Bengtson 1983). Manatees may also incidentally ingest a considerable amount of encrusting organisms and/or algae found in the roots and foliage of aquatic vegetation (Hartman 1979, Mignucci-Giannoni & Beck 1998, Courbis & Worthy 2003). Additionally, there is evidence of manatees feeding on dead fish in the wild and captivity (Powell 1978, C. D. Alves pers. obs.) as well as terrestrial vegetation (e.g., O'Shea 1986). Manatee feeding preference on different aquatic plant species may be influenced by species abundance and/or location, nutritional quality, sediment character, or water depth (e.g., Hartman 1979). Depending on the plant type, nutritional quality, and/or palatability, sometimes manatees feed only on leaves and stalks whereas other plants are consumed whole (Hartman 1979, Best 1981).

Seagrass species present in Florida and the Caribbean include *Thalassia testudinum* (turtle grass), *Syringodium filiforme* (manatee grass), *Halodule wrightii* (shoal grass), and *Halophila spp.*, the first three of which are the most common. Manatees have been observed both cropping the tops of seagrass blades and consuming the whole plant

including rhizomes (Hartman 1979, Packard 1984, Lefebvre & Powell 1990). While consuming seagrass blades, it is also possible for manatees to inadvertently ingest invertebrates found in the seagrass community, and/or epiphytic algae on the blades.

Evaluating feeding behavior and habitat use in marine mammals is challenging on multiple levels (reviewed by Pierce & Boyle 1991). Feeding habits are difficult to observe directly since many marine mammal species feed at depth, are fast-moving, and may have expansive migration patterns. Satellite transponders can be attached to a species during which movement patterns and time-depth analysis can provide information about feeding ecology (e.g., Laidre et al. 2003). However, this analysis is costly and provides information only on specific individuals for a limited time period. Stomach content analysis is another technique applied to marine mammal feeding research that provides evidence of recent diet (e.g., Spitz et al. 2006). Stomach contents are either obtained from dead animals, or removed from live animals (gastric lavage). Identification of the ingested material is often difficult and may be biased towards undigested hard parts such as otaliths or beaks. Ingested material can also be analyzed in the feces (e.g., Sinclair & Zeppelin 2002). However, in the marine environment collection of fecal material and identification of the source individual can be problematic. Recently, the use of fatty acid signature analysis (FASA) has expanded as a dietary analysis methodology used in marine mammal research (reviewed by Budge et al. 2006). Potentially, FASA could distinguish between prey species through investigation of the proportions of fatty acids in the blubber layer of the consumer as they relate to those in the diet. However, data interpretation is still in developmental stages and sampling is somewhat invasive, requiring full blubber depth. FASA is also difficult to apply to

species that undergo fermentation, such as manatees, since fatty acids are a product of the fermentation process and would complicate tracing of dietary fatty acids.

While the dietary preferences of manatees are understood within some habitats, it is unclear whether they are feeding in all habitats they occupy, and how their feeding habits vary. While some work has been done, my goal is to expand on previous work by assessing the application of stable isotope analysis to manatee tissue, and providing critical baseline parameters for accurate isotopic data interpretation.

Stable isotope analysis is a relatively new technique and its use in ecological research has expanded rapidly in recent years (reviewed by Kelly 2000). Isotopic analysis has some advantages over the previously discussed techniques in feeding ecology because it provides information about assimilated nutrients, not just those ingested. Also, sampling does not require sacrificing the animal because tissues such as skin, hair, or blood can be collected from live animals. For marine mammals, this sampling method is often logistically easier than collecting stomach content and/or fecal samples and less invasive than sampling the full blubber depth required for FASA. Finally, stable isotope composition can provide information about both previous and current diet since isotope turnover rates vary between different tissues (Hobson & Clark 1992a).

Ratios of heavy to light stable isotopes naturally occurring in the diet are reflected in the tissues of the consumer. These ratios can be used to make predictions about food web dynamics, diet composition, and even habitat use and migratory patterns (Deniro & Epstein 1978, 1981, Fry 1981, Peterson & Fry 1987, Hobson 1999, Jones & Waldron

2003, Cerling et al. 2006). Two common naturally occurring stable isotope ratios used in ecological studies are $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (expressed as $\delta^{13}C$ and $\delta^{15}N$, respectively).

Carbon and nitrogen stable isotope analyses of food source and consumer tissue can be used to make predictions about habitat use because freshwater and marine food webs have distinct isotopic compositions (e.g., Smith et al. 1996), as do terrestrial and aquatic food webs (e.g., Fry & Sherr 1984, Cree et al. 1999), as well as benthic and pelagic regions (e.g., Vizzini et al. 2002). Specifically, carbon and nitrogen stable isotope ratios differ between the sources of primary production due to the differing rates of heavy (^{13}C, ^{15}N) vs. light (^{12}C, ^{14}N) isotope incorporation during biogeochemical reactions.

The stable isotope ratios of consumer tissue differ slightly from those of the diet. This difference is referred to as diet-tissue discrimination (formerly fractionation) and occurs due to the biochemical processes that show affinity for either the heavier or lighter isotope (Ehleringer & Rundel 1989). For example, most enzymes have an affinity for the lighter isotope. It is important to calculate an accurate diet-discrimination value in order to properly analyze stable isotope results. Carbon diet-tissue discrimination may occur due to one or more of the following processes: preferential loss of $^{12}CO_2$ during respiration, preferential uptake of ^{13}C during digestion, and/or metabolic discrimination during the synthesis of new tissues (Deniro & Epstein 1978, Tieszen et al. 1983). Consumer tissue is enriched in ^{13}C compared to the diet by an average of approximately 1‰ (Deniro & Epstein 1978). Nitrogen is incorporated into the body through digestion and lost through excretion. During both processes, there is a preferential loss of ^{14}N (Minagawa & Wada 1984). This diet-tissue enrichment for nitrogen (3‰ on average)

progresses step-wise up trophic levels and is what allows for the interpretation of trophic position in food web studies (Deniro & Epstein 1981, Peterson & Fry 1987).

In terrestrial plants, carbon in the atmosphere in the form of CO_2 is incorporated into the plant through stomata. Discrimination against the heavier $^{13}CO_2$ takes place during diffusion into the cytoplasm and during carbon fixation, resulting in low $^{13}C/^{12}C$ ratios (Criss 1999). The two enzymes involved in carbon fixation are PEP carboxylase and RuBP carboxylase, the later of which discriminates more strongly against ^{13}C. Consequently, C_4 and C_3 plants, which use these enzymes respectively, have distinct $\delta^{13}C$ values that differ from each other by approximately 14‰ (Smith & Epstein 1971). CAM plants may use PEP or RuBP carboxylase depending on environmental and/or developmental factors. As a result, the range of carbon isotopic ratios for CAM plants overlaps with those of C_3 (-35 to -20‰) and C_4 plants (-14 to -9‰) (Deines 1980). Much less is known about the dynamics of $^{15}N/^{14}N$ ratios in terrestrial plants. Nitrogen isotopes may differ between plants that fix atmospheric nitrogen, and those that rely on soil nitrogen. There is also a degree of discrimination that occurs during nitrogen metabolism in the plant (Lajtha & Marshall 1994).

In aquatic plants, carbon and nitrogen are incorporated into the plant from the dissolved inorganic carbon (DIC) and dissolved inorganic nitrogen (DIN) available in the water, respectively. Aquatic plants have $\delta^{13}C$ values that range from -30 to -8‰ (Ehleringer & Rundel 1989). Ratios depend on the form and isotope ratios of the carbon source (CO_2 vs. bicarbonate) and the photosynthetic pathway. The $\delta^{15}N$ values in aquatic plants range from -2 to 17‰ (McClelland et al. 1997, Cloern et al. 2002) and depend on nitrogen source and metabolism. These factors enable both carbon and nitrogen stable

isotope ratios to be used to distinguish between aquatic plants in fresh, estuarine, and marine habitats (e.g., Reich & Worthy 2006).

Variables that can affect carbon signatures in aquatic plants include light, pH, and temperature. Light can affect metabolic rates of aquatic plants (which may be a result of depth, season, turbidity, or community species composition), which in turn impacts isotopic ratios (e.g., Cooper & Deniro 1989). The pH can affect the relative amounts of dissolved free CO_2 and bicarbonate in water (Bade & Cole 2006), consequently affecting plant isotopic ratios. Changes in temperature (lower temperatures allow for greater gas solubility) can also impact isotopic ratio (e.g., Rau et al. 1989). Many anthropogenic factors may affect ^{15}N signatures in aquatic habitats such as fertilizer runoff, sewage, or animal waste runoff from agricultural regions (e.g., Vizzini & Mazzola 2006).

As diet changes over time, the turnover rate in consumer tissues must be known in order to compare isotope composition of tissues to that of the current diet (Bosley et al. 2002). One can then infer changes in habitat, diet, or migratory pattern. An effective method to determine turnover rates is to switch an animal experimentally from one known diet to another isotopically distinct diet. Stable isotope turnover studies have shown that tissues with higher metabolic activity (e.g., blood, liver) have faster turnover rates than less active tissues (e.g., bone) (e.g., Hobson & Clark 1992a). Stable isotope ratios in tissues with faster turnover rates will represent a recent diet whereas isotopic composition of tissues with slower turnover rates will represent feeding habits from some time previous. Despite the large number of studies that have used stable isotope analysis across the marine mammal taxa (e.g., Ramsay & Hobson 1991, Hobson & Welch 1992, Ames et al. 1996, Walker et al. 1999, Clementz & Koch 2001, Kurle & Worthy 2002,

Yamamuro et al. 2004, Lee et al. 2005, Newsome et al. 2006, Reich & Worthy 2006), stable isotope turnover rates have only been calculated for seal vibrissae (Zhao & Schell 2004, Hall-Aspland et al. 2005). Because turnover rates often differ between tissues and species, it is crucial to broaden marine mammal isotope turnover research in order to accurately interpret stable isotope data.

The overall objective of this study was to use stable isotope analysis of the skin of Florida and Antillean manatees to assess feeding ecology. To accomplish this objective, I first determined stable isotope turnover rates and diet-tissue discrimination values in manatee skin in order to accurately interpret isotopic results. Chapter 2 focuses on determining ^{13}C and ^{15}N turnover rates in the epidermis of rehabilitated Florida manatees. This study was the first to measure stable isotope turnover in marine mammal skin. Manatees that were brought into captivity for medical treatment and in need of rehabilitation were immediately transitioned to a diet consisting of mainly romaine lettuce, which has an isotopic signature distinct from that of aquatic vegetation. Skin samples were collected over a period of up to 418 days following the diet switch. Carbon diet-tissue discrimination values were also calculated. Chapter 3 applies stable isotope analysis of skin tissue to free-ranging manatees in Florida, Belize, and Puerto Rico. This study was the first to apply stable isotope analysis to Antillean manatees. Regional differences in carbon and nitrogen isotopic signatures were compared between manatees. Additionally, factors including sex, season, and age class were explored. Aquatic plants within the presumed manatee diet were also analyzed for carbon and nitrogen stable isotopes. Using calculated and estimated diet-tissue discrimination values, a mixing

model was used to compare proportions of freshwater vegetation, estuarine vegetation, and seagrasses in the diet of Florida manatees that winter in different regions.

CHAPTER 2: CARBON AND NITROGEN STABLE ISOTOPE TURNOVER RATES IN THE SKIN OF THE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

Introduction

The use of stable isotope ratios in ecological research has expanded rapidly in recent years (reviewed by Kelly 2000). Isotopic composition of consumer tissues reflects those of local food webs and can be used to predict diet composition, the trophic level at which the consumer is feeding, and even habitat use and migratory patterns (Deniro & Epstein 1978, 1981, Fry 1981, Peterson & Fry 1987, reviewed by Hobson 1999). Two common stable isotope ratios analyzed in feeding ecology studies are those of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). Carbon stable isotope ratios indicate the source of primary production and have been used to differentiate between C_3 and C_4 plants, terrestrial and marine ecosystems, and benthic and pelagic aquatic systems (Cloern et al. 2002, Hall-Aspland et al. 2005). Nitrogen stable isotope ratios exhibit a predictable, step-wise enrichment between trophic levels and also have been shown to differ between terrestrial and marine ecosystems (Hobson & Welch 1992).

Stable isotope analysis is especially advantageous when investigating the feeding ecology and habitat use of marine mammals for which it is often difficult to directly observe feeding or migratory behavior. Tissue samples such as skin or blubber may be analyzed for stable isotope ratios without sacrificing the animal. In addition, isotope ratios reflect assimilated nutrients rather than only those ingested. Stable isotope analysis has been successfully applied to marine mammals including mysticetes (e.g., Lee et al. 2005), odontocetes (e.g., Walker et al. 1999), pinnipeds (e.g., Hobson & Welch 1992,

Kurle & Worthy 2002, Newsome et al. 2006), sirenians (Ames et al. 1996, MacFadden et al. 2004, Yamamuro et al. 2004, Reich & Worthy 2006), sea otters (Clementz & Koch 2001), and polar bears (e.g., Ramsay & Hobson 1991).

In order to accurately interpret isotopic results, it is imperative to determine both isotopic discrimination (the difference in isotopic ratios between consumer tissue and diet) and turnover rate (the time it takes for the isotope to be assimilated into the consumer's tissue) of the sampled tissue. Diet-tissue discrimination may be difficult to determine for animals feeding on multiple, isotopically distinct prey items for which the proportions of contribution to the diet are unknown. However, controlled captive studies on a variety of taxa have allowed for more precise measurements (Roth & Hobson 2000, Cherel et al. 2005, Logan et al. 2006, Seminoff et al. 2006). In addition to diet-tissue discrimination, turnover rates in tissues must be determined in order to assess whether the isotope signature of the tissue represents the most recent diet or the long-term diet. An effective method to determine turnover rate is to switch an animal experimentally from one known diet to another isotopically distinct diet. Turnover rates of stable isotopes have been calculated using this method for mammals (e.g., Tieszen et al. 1983), birds (e.g., Hobson & Clark 1992a), fish (e.g., Bosley et al. 2002), and invertebrates (e.g., Olive et al. 2003). These studies have shown that tissues with higher metabolic activity (e.g., blood, liver) have faster turnover rates than less active tissues (e.g., bone).

The endangered Florida manatee (*Trichechus manatus latirostris*) is known to feed on aquatic plants in fresh, estuarine, and marine habitats (Campbell & Irvine 1977, Hartman 1979, Best 1981), each of which has a distinct isotopic signature (Alves Chapter 3, Fry & Sherr 1984, Reich & Worthy 2006). Little is known about manatee feeding

ecology and habitat use since they often occupy shallow, turbid water. In addition, manatee population counts and trends remain unclear (Lefebvre et al. 1995, U.S. Fish and Wildlife Service 2001). It has become increasingly important to understand manatee feeding ecology and its relation to habitat use in order to improve conservation efforts.

The present study used tissue samples from Florida manatees transitioning between two isotopically distinct diets (terrestrial and aquatic) to determine turnover rates in epidermis tissue. Manatees were part of the rehabilitation program at SeaWorld Orlando, and were in need of captive care for reasons including physical trauma, nutritional stress, and/or cold stress. The objectives of this study were to (1) determine ^{13}C and ^{15}N turnover rates in epidermis tissue using skin samples from rescued Florida manatees, (2) calculate diet-tissue discrimination values for carbon and nitrogen stable isotopes in the skin of captive manatees held long-term, and (3) suggest application of these findings as they relate to the analysis of stable isotope data for free-ranging manatees.

Materials and Methods

Sample collection

Food items fed to manatees in captivity at SeaWorld Orlando (Orlando, FL) were collected during 2003 and 2004 (n = 35, Appendix A). Manatees held long-term at SeaWorld Orlando were fed a diet consisting primarily of lettuce with minimal amounts of other terrestrial vegetation including spinach, carrots, and cabbage. Occasionally, monkey chow biscuits (Mazuri) were given as a protein supplement. Rescued manatees held at SeaWorld for rehabilitation were fed primarily romaine lettuce with minimal amounts of spinach. Additionally, upon rescue, manatees were fed a gruel mixture through a stomach tube for the first few weeks consisting of romaine lettuce, spinach, water, and monkey chow (P. L. Ramos-Navarrete pers. comm.). Manatees are known to engage in coprophagy (Hartman 1979, C. D. Alves pers. obs.) and therefore fecal material from captive manatees was also collected and analyzed for stable isotope ratios (n = 4) for a comparison to the fed diet items.

Skin samples were collected from captive Florida manatees at SeaWorld Orlando. Some of these manatees were rescued animals being held temporarily in captivity for rehabilitation (n = 8, Appendix B), while others were animals held long-term for over one year (n = 9, Appendix C). Skin samples from long-term captive animals were used to calculate diet-tissue discrimination and to determine isotopic values in manatee skin that were fully representative of a captive diet. To determine turnover rates, samples were obtained opportunistically from rescued animals at various time intervals as they transitioned from wild forage to a captive diet (Appendix B). Frequency of sample

collection depended on logistic feasibility. Carbon and nitrogen stable isotope turnover rates in skin were calculated for Florida manatees rescued near Naples on the gulf coast and Cape Canaveral on the central east coast (Fig. 2.1). These manatees were referred to as "coastal." Turnover rates in skin were also calculated for Florida manatees rescued in a region of the lower St. Johns River near Jacksonville (Fig. 2.1). These manatees were referred to as "riverine."

After rehabilitation, some manatees were fitted with satellite tags and tracked when released. Tracking provided an opportunity to collect skin samples from two animals that were released and had transitioned from a diet of terrestrial forage in captivity to aquatic forage in the wild. Post-release sampling occurred during routine physical examinations (coordinated by the Manatee Rehabilitation Consortium) which were scheduled two, six, and twelve months post-release. Slight deviations from this schedule often occurred due to availability of staff, weather conditions, and health condition of the animal.

Biopsies of epidermal tissue were collected from the edge of the paddle using either a scalpel or ronguers. Sloughed epidermis was collected if biopsies were not available. Body mass and sex were determined, and length measurements were taken, where body length was measured as the straight distance from snout to paddle (Fig. 2.2). Manatees were categorized into three age classes based on body length measurements (adults: >275 cm, subadults/late juveniles: 176-275 cm, and calves: <176, O'Shea et al. 1985).

Manatee skin samples in previous studies were obtained by gathering sloughed-off tissue (Ames et al. 1996) or by using skin samples derived from dead manatees (Reich

& Worthy 2006). I compared stable isotope ratios between a sloughed skin and biopsy sample from the same recently rescued manatees (n = 9) to determine precision between collection methods and analyses.

Sample preparation and analysis

Stable isotope ratios were expressed in ppt (‰) using delta notation:

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

in which R_{sample} and $R_{standard}$ are the absolute isotope ratios of the sample and standard, respectively, X is ^{13}C or ^{15}N, and the standards are PeeDee belemnite (from the Cretaceous marine fossil, Belemnitella americana, from the PeeDee formation in South Carolina, Craig 1957) and atmospheric N_2, respectively.

Turnover rate was calculated using the exponential model of Hobson and Clark (1992a):

$$y = a + be^{ct} \quad (2)$$

in which y is δX, a is the value approached asymptotically, b is the total change in value after diet switch, c is turnover rate, and t is time (days) since diet switch. Turnover rate was expressed in terms of half-life, the time it takes for the isotopic composition of the tissue to reach a midpoint between the initial and final values:

$$\frac{\ln 0.5}{c} \quad (3)$$

In order to better fit turnover data to the exponential model, an "anchor point" based on the mean stable isotope ratio ($\delta^{13}C$ = -24.4 ± 0.2‰ SE, $\delta^{15}N$ = 2.7 ± 0.2‰ SE) of skin samples from nine long-term captive manatees at SeaWorld Orlando, was set at 600 days. These animals were fed a diet of mainly lettuce for multiple years. The

position of the anchor point at 600 days was chosen because it was well beyond the maximum sampling time for all rescued manatees (no manatee was sampled later than 418 days), plots for almost all skin samples reached an asymptote at or before this point, and positions greater than 600 days did not alter results. Goodness of fit was first expressed by calculating the coefficient of determination (R^2) using the anchor point as part of the data set. To further illustrate fit, data for skin from each rescued manatee were paired with each individual data point contributing to the mean anchor point and minimum and maximum R^2 values were computed.

All statistical analyses were judged to be significant at <0.05. Data were tested for normality using the Shapiro-Wilk test. Levene's F and Box's M were used to test homogeneity of variance between factors and homogeneity of covariance, respectively. Differences in $\delta^{13}C$ and $\delta^{15}N$ values were tested using parametric and non-parametric analyses as appropriate. Means are presented ± SE.

Results

Stable isotope ratios were significantly different between diet items (MANOVA: Wilks' Lambda, $F_{8,50} = 16.79$, $p < 0.001$). Specifically, $\delta^{13}C$ values differed (ANOVA: $F_{4,26} = 75.60$, $p < 0.001$), but $\delta^{15}N$ values did not ($F_{4,26} = 0.35$, $p = 0.84$). Gruel and monkey chow were both significantly enriched in ^{13}C compared to romaine lettuce, spinach, and fecal samples (all p values < 0.01, Tukey HSD, Table 2.1).

Skin from long-term captive manatees held at SeaWorld Orlando (mean $\delta^{13}C$ = -24.4 ± 0.2‰) was enriched in ^{13}C compared to the major diet components (romaine lettuce and spinach) by an average of 2.8‰. The $\delta^{15}N$ values did not differ between manatee skin (mean = 2.7 ± 0.2‰) and the diet (Table 2.1).

Turnover rates

Isotopic ratios in biopsy samples and sloughed skin from the same rehabilitated manatees were compared to determine the effect of differing collection methods. The $\delta^{13}C$ values did not differ significantly between biopsy and sloughed samples (paired t-test: $t = 0.15$, $df = 8$, $p = 0.89$). However, sloughed samples were significantly enriched in ^{15}N compared to biopsy samples (mean enrichment = 1.3 ± 0.3‰, $t = 4.32$, $df = 8$, $p = 0.003$). To account for this difference, all $\delta^{15}N$ values for sloughed samples were adjusted by the mean enrichment value.

Coastal manatees

Skin from manatees rescued from the Cape Canaveral region and Naples was greatly enriched in ^{13}C relative to that of long-term captive manatees (mean enrichment =

13.8‰). The $\delta^{13}C$ values from four coastal manatees were fit to the exponential decay model (Fig. 2.3). Carbon turnover half-lives in skin ranged from 42 to 63 days with a mean of 53 days (Table 2.2).

Skin from manatees rescued from the Cape Canaveral region and Naples was only slightly enriched in ^{15}N relative to that of captive manatees (mean enrichment = 3.5‰). The $\delta^{15}N$ values for four rescued manatees were fit to the exponential decay model (Fig. 2.4). Nitrogen half-lives in the skin of coastal manatees ranged from 14 to 36 days with a mean of 27 days (Table 2.2) and were significantly shorter than carbon half-lives (paired t-test: $t = 10.33$, $df = 3$, $p = 0.002$).

Riverine manatees

Skin from manatees rescued from the St. Johns River had only slightly enriched or very similar carbon signatures relative to that of captive manatees (mean enrichment = 4.3‰). The $\delta^{13}C$ values in the skin of four riverine manatees were fit to the exponential decay model (Fig. 2.5). However, half-life was not calculated for manatee 0341 because the equation fit to the data points showed little to no change in signature over time (Table 2.2, Fig. 2.5). Carbon turnover half-lives in the skin of riverine manatees ranged from 39 to 72 days with a mean of 59 days (Table 2.2).

Skin from manatees rescued from the St. Johns River was enriched in ^{15}N relative to that of captive manatees (mean enrichment = 6.7‰). The $\delta^{15}N$ values in the skin of four rescued manatees were fit to the exponential decay model (Fig. 2.6). Nitrogen half-lives in the skin of riverine manatees ranged from 21 to 115 days with a mean of 58 days (Table 2.2) and were not significantly different from carbon half-lives (paired t-test, t =

0.13, df = 2, p = 0.91). MANOVA results indicated there were no significant differences in stable carbon or nitrogen isotope half-lives between manatees rescued from riverine vs. coastal regions (F test: $F_{2,4} = 0.58$, p = 0.60).

After 434 days in captivity at SeaWorld Orlando, manatees 0340 and 0341 were successfully rehabilitated and released at Blue Spring on the St. Johns River (Figure 2.1). Skin samples were collected from both animals 77 days after release during a recapture coordinated by the Manatee Rehabilitation Consortium. The exponential decay equations calculated for nitrogen turnover in the skin of those two manatees were reversed and plotted to show a change in signature after a diet switch from lettuce in captivity to freshwater aquatic plants in the St. Johns River (Fig. 2.7). Skin from both manatees was enriched in ^{15}N after release compared to values while on a captive diet. The post release $\delta^{15}N$ value for manatee 0340 (3.7‰) fell short of the model prediction (7.8‰) by 4.1‰. The post release $\delta^{15}N$ value for manatee 0341 (3.1‰) fell short of the model prediction (5.7‰) by 2.6‰. This calculation was not made for $\delta^{13}C$ values due to the similarity of stable isotope ratios between skin from these riverine manatees and those of long-term captive manatees at SeaWorld Orlando (Fig. 2.5).

Discussion

Diet-tissue discrimination

The carbon enrichment value calculated in the skin of captive manatees relative to the diet (2.8‰) was similar to values previously reported. Ames et al. (1996) found sloughed skin from captive manatees to be enriched in ^{13}C by an average of 4.1‰ compared to lettuce. Reich and Worthy (2006) assumed a carbon enrichment value of 3.0‰ in manatee skin when applying the technique to diet interpretation of free-ranging manatees. The only other known study on diet-tissue discrimination in skin is that of Hobson et al. (1996). Seal skin was enriched in ^{13}C relative to diet by 2.8‰. In the present study, nitrogen enrichment could not be determined due to the variability of nitrogen signatures in the diet. Typically, diet-tissue discrimination values for nitrogen are in the range of 2-5‰ (Peterson & Fry 1987, Kelly 2000).

Turnover rates

Carbon turnover

Manatees rescued from coastal regions were ideal subjects for carbon turnover calculations because carbon signatures in their skin differed dramatically from those of captive manatees. Interpreting $\delta^{13}C$ values in the skin of riverine manatees was problematic due to variability in values at the time of rescue and similarity of $\delta^{13}C$ values between skin from rescued manatees and those of long-term captive manatees. The half-lives in skin from riverine manatees were not significantly different than those of skin from coastal manatees; however, the carbon turnover data for manatees rescued from

riverine regions did not fit the exponential decay models as closely as those for coastal manatees (Table 2.2).

The carbon half-life calculated for manatee epidermis was very slow compared to previous turnover studies on other species. Stable isotope turnover rates can differ based on the particular isotope, tissue and/or taxon analyzed, diet, physiological state, feeding rate, and/or growth rate of the animal (Fry & Arnold 1982, Bosley et al. 2002, Hobson & Bairlein 2003, Olive et al. 2003). Additionally, some studies removed lipids from samples while others did not. Therefore, direct comparisons between studies are difficult. Dalerum & Angerbjorn (2005) cautioned comparisons of turnover rates should be made between the same tissues to avoid these complications. Additionally, turnover rates in tissues should be compared between animals of similar body size since metabolic rates have an effect on isotope turnover (Sponheimer et al. 2006).

This study was the first to calculate stable isotope turnover rates in marine mammal skin. Isotope turnover rates that have been reported for large terrestrial mammals, including bears (Hilderbrand et al. 1996), alpacas (Sponheimer et al. 2006), and domestic cattle and horses (Schwertl et al. 2003, Ayliffe et al. 2004), were determined using blood, muscle and liver, and hair, respectively. Since no appropriate comparison between turnover rates in the skin of large mammals was possible, the results from this study will be cautiously compared to others. Studies on stable isotope ratios in hair were omitted from this comparison since hair is a metabolically inert tissue in which the isotopic composition represents the period of growth.

The only reported carbon half-lives in mammal tissue that were greater than that of manatee epidermis tissue were those of alpaca muscle (179 days, Sponheimer et al.

2006), and bat wing membrane (>100 days) and whole blood (>100 days, Voigt et al. 2003). In the alpaca study, muscle tissues were not lipid extracted so direct comparisons may not be applicable. Voigt et al. (2003) suggested the slow turnover rate in bat wing membrane was due to the tissue being composed primarily of collagen and elastin, which are known to have slow turnover rates in bone. Additionally, Voigt et al. (2003) attributed the slow turnover rate in bat blood to long-lived erythrocytes. Other reported carbon half-lives for muscle, liver, fat, blood, and brain tissue in mammals (5 to 37 days, Tieszen et al. 1983, Hilderbrand et al. 1996, Sponheimer et al. 2006) ranging in body size from gerbils (70 g) to American black bears (140 kg) were less than that calculated for manatee skin (average manatee body mass is approximately 1,000 kg, U.S. Fish and Wildlife Service 2001).

Metabolic rate in adult Florida manatees has been shown to be slower than predicted based on their body size (15-40% of predicted values, Irvine 1983, Worthy et al. 2000). Slow metabolism was likely a contributing factor to the slow carbon turnover rate in manatee skin. It is also possible that feeding rate had an impact on turnover rate (as discussed in Post 2002). Manatees use hindgut fermentation and the calculated passage rate in the digestive tract was 146 hours (Lomolino & Ewel 1984). The manatees sampled in the present study were rescued for reasons including cold stress, entanglement, and watercraft injuries (Appendix B). Due to their physical condition, their intake rates may have been slower than those of manatees not in need of rehabilitation.

Epidermal tissue is composed of keratin in the epithelial and collagen and elastin in the basal lamina. Manatee epidermis has been described as thick and possesses the

characteristic of hyperkeratosis (Sokolov 1982, Graham et al. 2003). It is possible that a slow replacement of keratin in manatee epidermis and the presence of collagen and elastin in the basal lamina also contributed to the slow isotope turnover rate in the skin.

The gruel mixture fed for the first few weeks of rehabilitation was enriched in ^{13}C compared to the main diet components, romaine lettuce and spinach. It is possible that the gruel in the diet affected the turnover rate slightly because essentially a change in the isotopic composition of the diet occurred a few weeks into the sampling period. Also, while lettuce is offered from day one, the rate and/or frequency at which individual manatees begin feeding on the lettuce can vary. It is presumed that the initial supplementation of gruel in the diet would only have had a small impact, if any on the carbon turnover rate since turnover was already very slow. Also, the carbon half-lives calculated were on the same order (approximately 1.5 to 2.5 months) among individual manatees.

There was no significant difference in $\delta^{13}C$ values between biopsy and sloughed skin samples, so the differing sample types had no effect on carbon turnover rate. Carbon isotope ratios of manatee fecal material did not differ from those of the main diet items, so even if manatees were engaging in coprophagy, it would not have had any effect on carbon turnover rate in the skin. This result is further indication that stable isotope analysis of fecal material is useful in assessing short-term, recent diet.

Nitrogen Turnover

Manatees rescued from coastal regions were less than ideal subjects for nitrogen turnover calculations due to variability in initial $\delta^{15}N$ values at time of rescue, similarity

of $\delta^{15}N$ values between skin from rescued animals and those of captive animals, and variability in $\delta^{15}N$ values in captive diet items. Consequently, the nitrogen half-lives calculated were inconsistent (Table 2.2). However, manatees rescued from the St. Johns River were better subjects because there was a greater difference in nitrogen signatures of skin between rescued and long-term captive animals and the data was a closer fit to the exponential model. Even so, nitrogen half-lives for skin from riverine manatees were still variable. Variability in half-lives was most likely due to the variability in nitrogen signatures of romaine lettuce and spinach fed in captivity (Table 2.1). The lettuce and spinach in the captive manatee diet often originated from different agricultural producers and it is possible that different fertilization techniques were used. Differences in fertilization techniques have been shown to contribute to variability in $\delta^{15}N$ values of plants (Georgi et al. 2005).

There are very few studies on nitrogen turnover in other species. Nitrogen half-lives in mammal tissues have been calculated for blood plasma and cells in black bears (3 and 22 days, respectively, Hilderbrand et al. 1996). Nitrogen half-lives have also been calculated in avian whole blood (10.0 to 14.4 days, Bearhop et al. 2002, Hobson & Bairlein 2003, Ogden et al. 2004) and plasma (0.5 to 1.7 days, Pearson et al. 2003). Nitrogen turnover in manatee skin was relatively slow compared to the results of these studies and is most likely due to the slow metabolic rate in manatees. It might additionally be a result of epidermal tissue composition and/or feeding rate as previously discussed in terms of carbon turnover.

Another factor that may affect nitrogen turnover is that nitrogen signatures in animal tissues may be dependant on nutritional state. Hobson et al. (1993) found that

nutritional stress in birds resulted in elevated $\delta^{15}N$ values, and suggested that this physiological effect be taken into consideration for other species. Due to the variability in nitrogen signatures, it was not possible to determine whether enriched nitrogen signatures in skin of the rescued manatees were an indication of nutritional stress, or if nutritional stress might have had an effect on turnover rate. At the time of rescue, each manatee's body length and mass were recorded (Appendix B). Those rescued for cold stress had the lowest mass to length ratio. However, these manatees were also some of the smallest in size and a low mass to length ratio is typical of younger animals. Of the eight rescued manatees, only two had a mass to length ratio less than 1:1. Average adult manatee mass (1,000 kg) and length (300 cm) (U.S. Fish and Wildlife Service 2001) give a ratio of approximately 3:1.

There was no compounding effect of the gruel supplement on nitrogen turnover rate since the signature of the gruel was not significantly different from that of romaine lettuce and spinach. Likewise, coprophagy would have had no effect on nitrogen turnover rate in manatee skin since the $\delta^{15}N$ values of manatee fecal material did not differ from those of the main diet items. However, sloughed skin samples were significantly enriched in ^{15}N compared to biopsy samples. Though $\delta^{15}N$ values were adjusted to account for this enrichment, variability in nitrogen turnover rates and lack of fit indicate that sample type may have contributed to the difficulty in calculating more precise half-lives. At the present time, it is unclear as to why sloughed samples differed in $\delta^{15}N$ values, but not $\delta^{13}C$ values, from biopsy samples.

Two rehabilitated manatees were sampled after release into the St Johns River. As expected, the post release nitrogen signatures in the skin began to approach the

original, free-ranging signature at time of capture. It would be interesting to extend sampling of released manatees over a period of one year to determine whether the nitrogen turnover rate remains similar to that during rehabilitation. It is not clear why the nitrogen ratios of the post-release skin samples fell short of the model. However, seasonal variability in aquatic plant $\delta^{15}N$ values in this region (Alves Chapter 3) may complicate post release turnover analysis. Additionally, recently released animals may not eat for a period of time after release while adjusting to their new environment. Finally, both samples were sloughed skin and $\delta^{15}N$ values were adjusted as previously mentioned. Regardless of the adjustment, both samples still fell short of the model.

Application to studies on free-ranging manatees

When proportions of food sources contributing to a mixed diet are unknown, mixing models are often used to aid in estimating these proportions (e.g., Newsome et al. 2004). If a change in diet occurs, the resulting signature may not be representative of the current diet, but in fact, may be some intermediate value between the two distinct diets. While this result is the case in all stable isotope analyses, turnover rates in tissues with high metabolic activity, or turnover rates in tissues of other species with faster metabolic rates, are often fast enough to limit this complication. Free-ranging manatees are known to switch diet sources (Best 1981, Lefebvre et al. 2000), and the very slow turnover rates for carbon and nitrogen stable isotopes in epidermis tissue complicate the interpretation of isotopic analyses. Unless the manatee has been feeding on the same diet for an extended period of time, the skin signature will always be in a transitional state. Slow turnover rates in manatee skin especially complicate the estimation of freshwater,

estuarine, and marine proportions of the diet because $\delta^{13}C$ values for estuarine vegetation are intermediate between those of freshwater vegetation and seagrasses (Alves Chapter 3). For example, it might be assumed that an intermediate carbon signature in manatee skin is indicative of diet of primarily estuarine vegetation. However, it is entirely possible that the manatee underwent a diet switch from freshwater vegetation to seagrass without ever consuming estuarine vegetation. The incorporation of nitrogen stable isotope analysis aids in further separation of these three diet sources since nitrogen signatures for freshwater and estuarine vegetation differ from those of seagrasses (Alves Chapter 3). The only known location in Florida where manatees may feed on the same type of vegetation for long periods of time is in freshwater habitat of the St. Johns River. Manatees that winter in the upper St. Johns tend to spend the remainder of the year in the lower St. Johns (Bengston 1981).

Computing a precise diet-tissue discrimination value is essential when interpreting isotopic results. Discrimination values for carbon have been calculated in the skin of manatees on a captive diet (present study, Ames et al. 1996) and discrimination values for carbon and nitrogen have been estimated in the skin of free-ranging manatees on possible diets of freshwater, estuarine, and/or marine vegetation (Alves Chapter 3, Reich & Worthy 2006). It is unknown whether diet-tissue discrimination in manatee skin differs between diet types as has been shown in other studies (e.g., Hobson & Clark 1992b).

Carbon and nitrogen stable isotope analysis of manatee epidermal tissue is difficult if not impossible to use when assessing short-term or recent changes in diet and habitat use because of slow turnover rates. This technique would potentially have more

direct application in summarizing average dietary intake over longer periods of time. In order to accurately interpret isotopic analyses, determining diet-tissue discrimination factors and turnover rates in the tissue are essential. The difficulty with most studies is that isotope discrimination and turnover are best calculated under controlled situations in captivity. Other marine mammals with faster metabolic rates such as dolphins and seals (Williams et al. 2001) should have a faster isotopic turnover rate in skin compared to that of manatees. Mixing model results for tissues with slow turnover rates should be interpreted with caution, especially in species that may be switching between diets in which an intermediate isotope ratio may be mistakenly described as indicating a single diet source instead of a mixture of others.

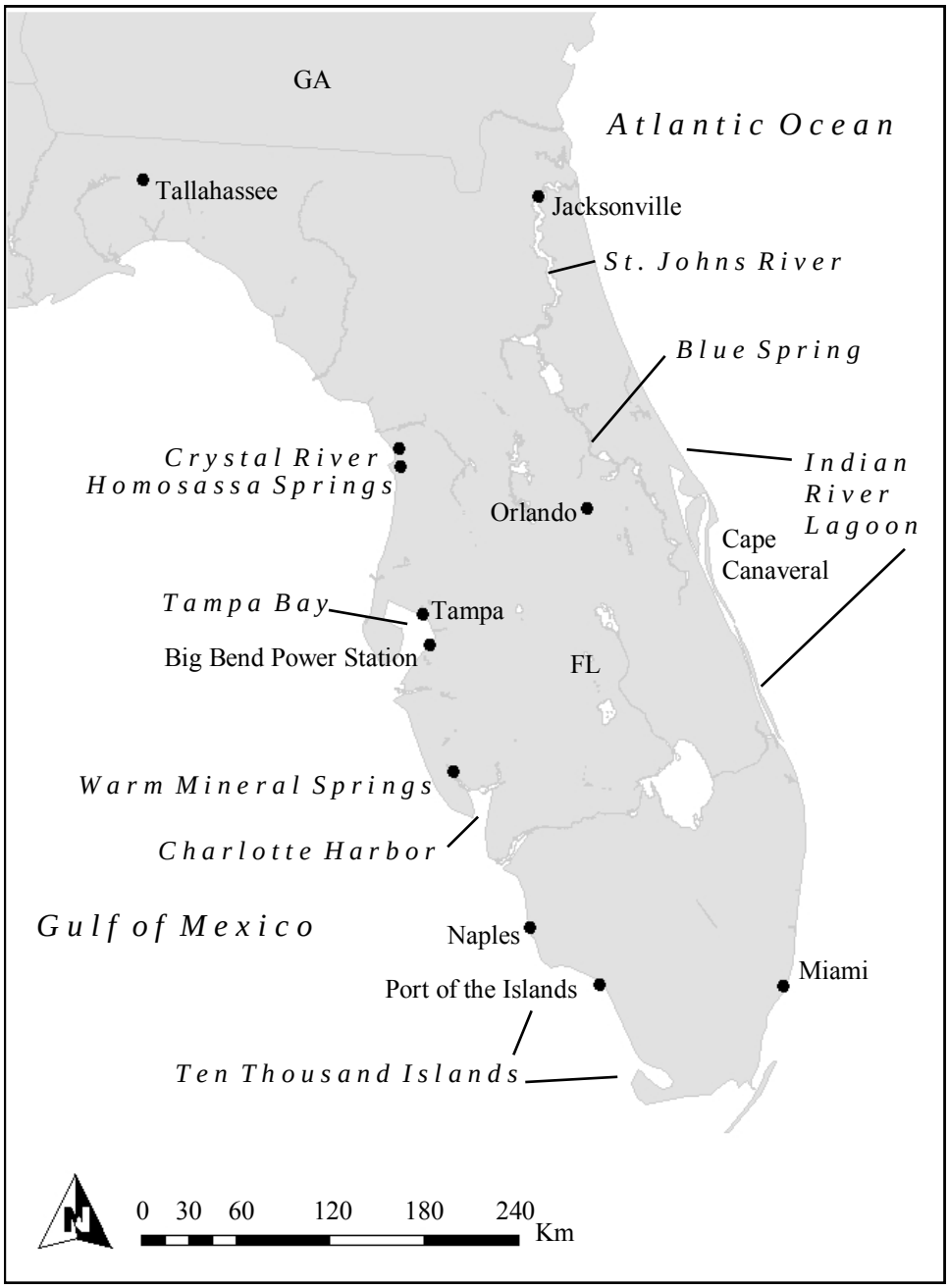

Figure 2.1. Map of Florida.

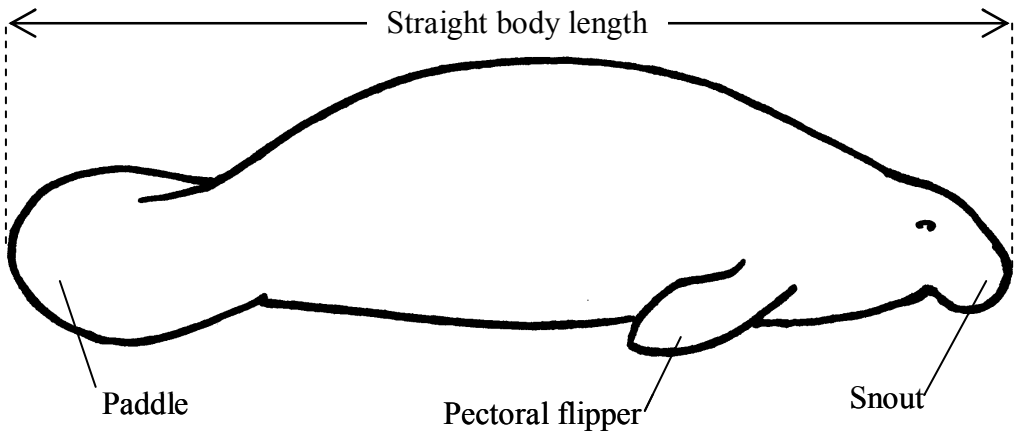

Figure 2.2. Straight line body length measurement in manatees.

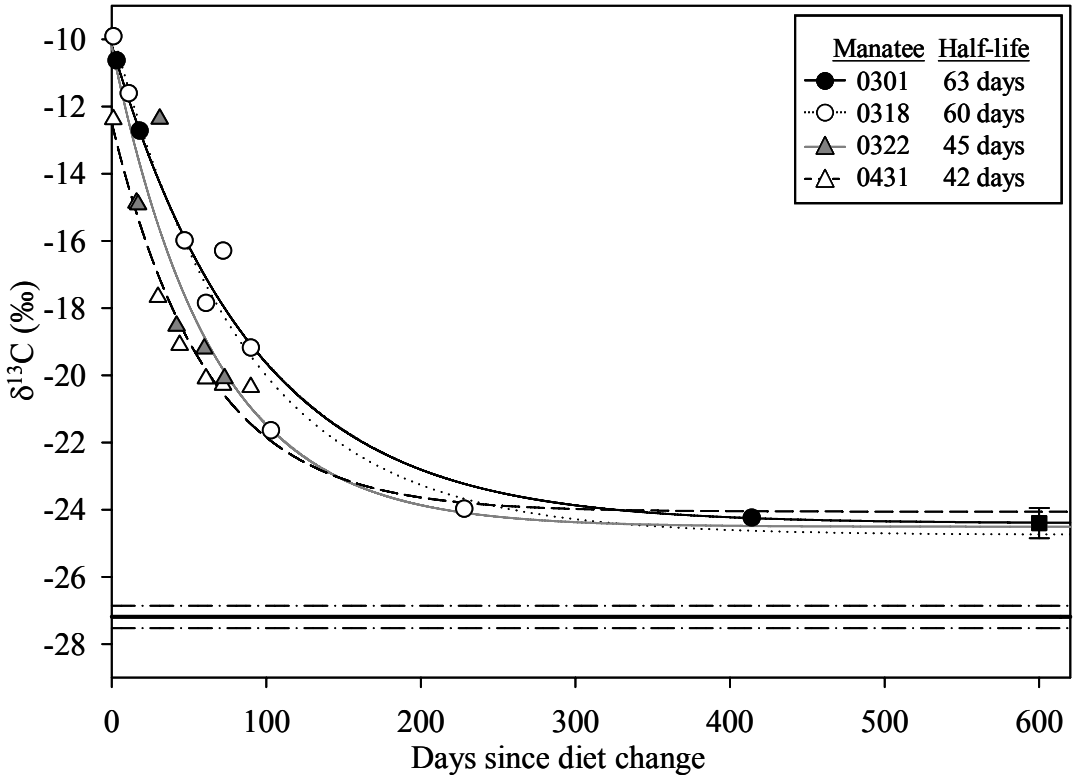

Figure 2.3. ^{13}C turnover in epidermis from manatees rescued from coastal regions in Florida.

Mean (±95% CI) stable isotope ratio for skin from long-term captive manatees (■) was used as an "anchor point" set at 600 day to better fit the model. Mean (±95% CI) stable isotope ratio for the main diet items fed in captivity (romaine lettuce and spinach) were plotted for comparison and are indicated by a horizontal solid black line and horizontal dash-dot lines.

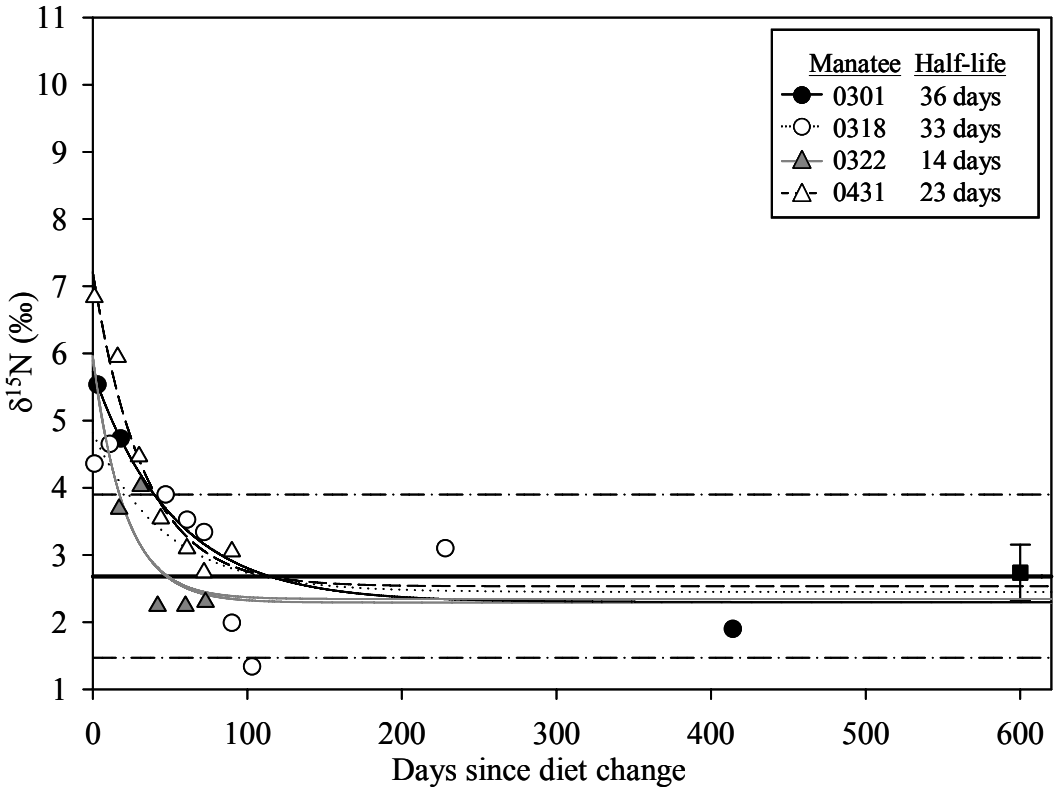

Figure 2.4. ^{15}N turnover in epidermis from manatees rescued from coastal regions in Florida.

Mean (±95% CI) stable isotope ratio for skin from long-term captive manatees (■) was used as an "anchor point" set at 600 days to better fit the model. Mean (±95% CI) stable isotope ratio for the main diet items fed in captivity (romaine lettuce and spinach) were plotted for comparison and are indicated by a horizontal solid black line and horizontal dash-dot lines.

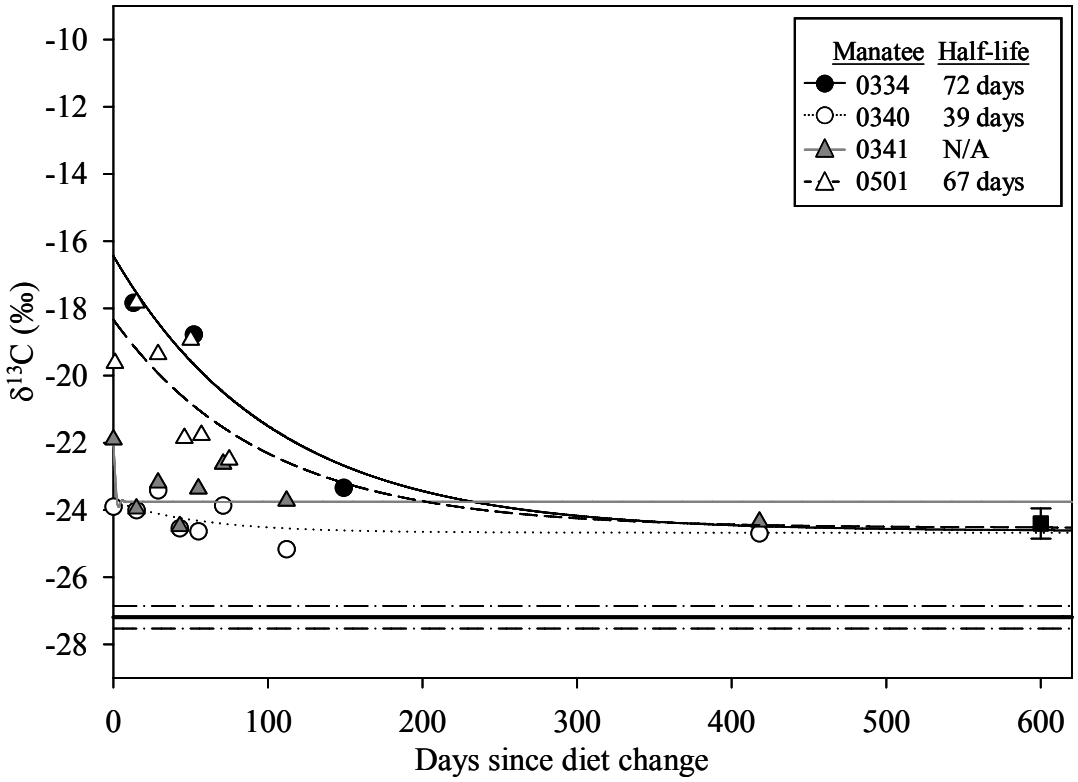

Figure 2.5. ^{13}C turnover in epidermis from manatees rescued from the St. Johns River in Florida.

Mean (±95% CI) stable isotope ratio for skin from long-term captive manatees (■) was used as an "anchor point" set at 600 days to better fit the model. Mean (±95% CI) stable isotope ratio for the main diet items fed in captivity (romaine lettuce and spinach) were plotted for comparison and are indicated by a horizontal solid black line and horizontal dash-dot lines.

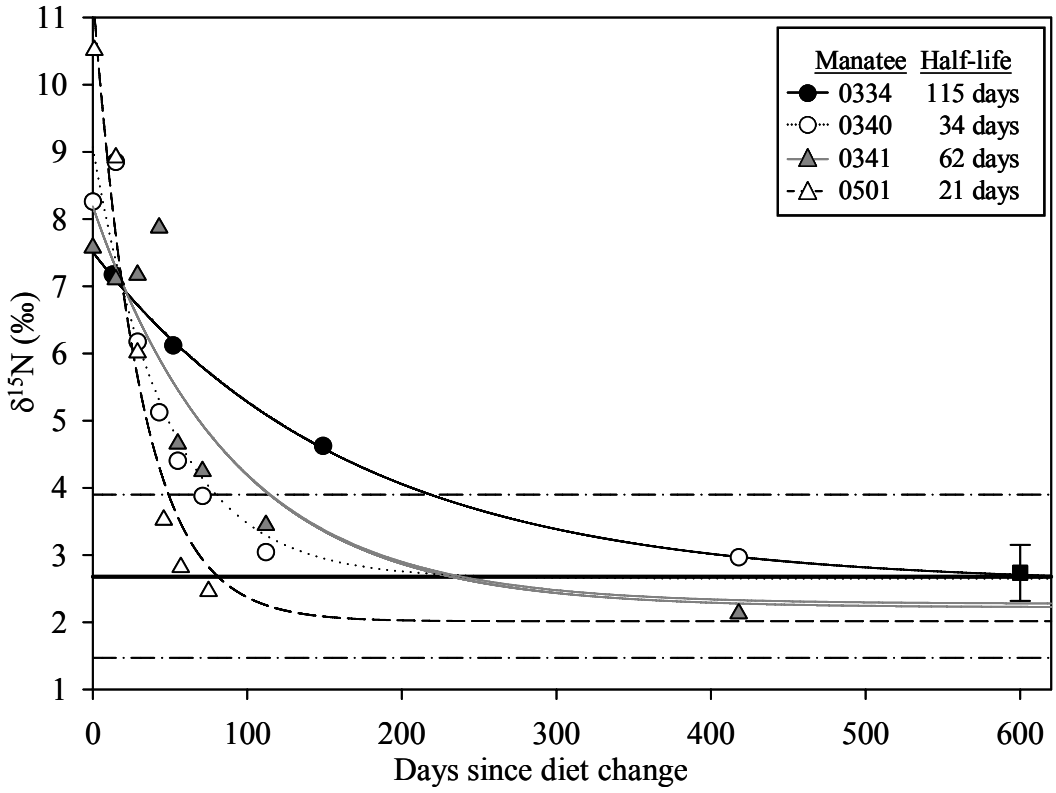

Figure 2.6. ^{15}N turnover in epidermis from manatees rescued from the St. Johns River in Florida.

Mean (±95% CI) stable isotope ratio for skin from long-term captive manatees (■) was used as an "anchor point" set at 600 days to better fit the model. Mean (±95% CI) stable isotope ratio for the main diet items fed in captivity (romaine lettuce and spinach) were plotted for comparison and are indicated by a horizontal solid black line and horizontal dash-dot lines.

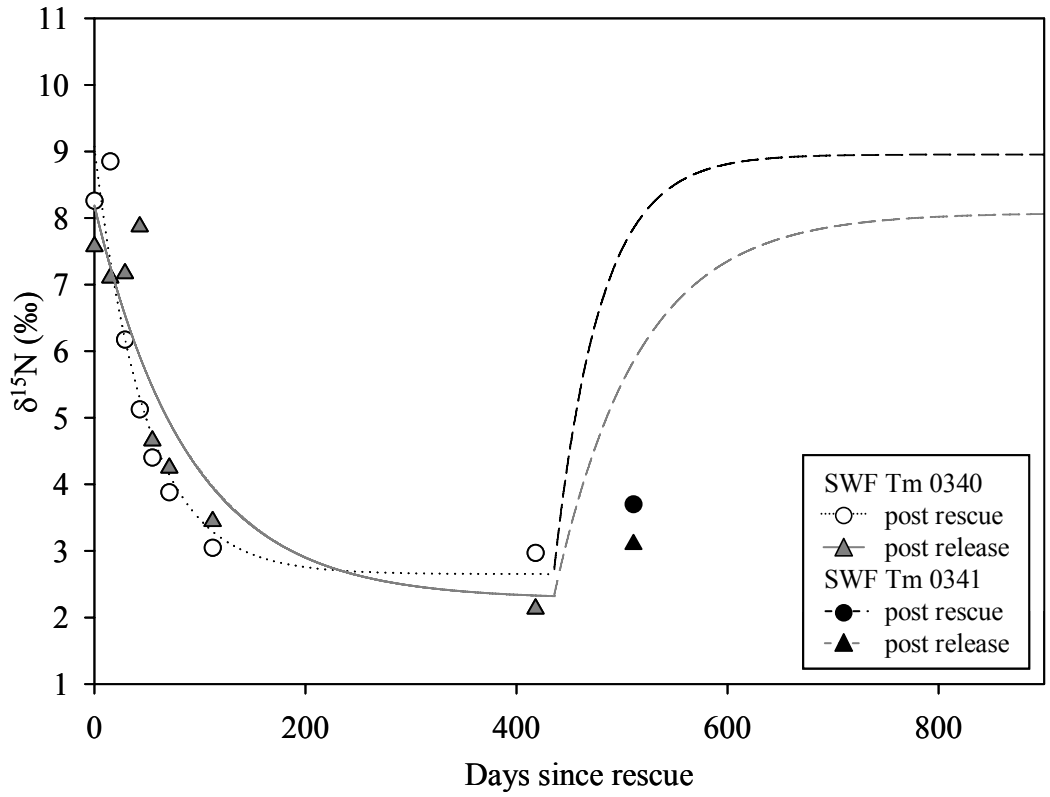

Figure 2.7. ^{15}N turnover in epidermis from two manatees rescued from and later released in the St. Johns River.

Both manatees remained at SeaWorld Orlando and were fed a diet of mainly romaine lettuce and spinach for 434 days before they were released. Skin samples were collected 77 days post release for analysis. The exponential decay model was reversed to show a change in stable isotope ratios post release.

Table 2.1. Stable isotope ratios of diet items fed to captive manatees at SeaWorld Orlando. The gruel mixture was composed of romaine lettuce, spinach, monkey chow, and water. Fecal material was analyzed because manatees are known to engage in coprophagy.

		$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Diet item	n	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum
Romaine lettuce	16	-27.2 ± 0.2	-28.8	-25.8	2.9 ± 0.7	-0.1	8.9
Spinach	3	-27.2 ± 0.3	-27.8	-26.7	1.8 ± 1.2	-0.7	3.0
Monkey chow	4	-21.2 ± 0.6	-23.0	-20.1	2.9 ± 0.4	2.1	4.1
Gruel	4	-20.3 ± 0.3	-21.0	-19.7	2.5 ± 0.3	1.9	3.2
Fecal material	4	-27.9 ± 0.8	-29.0	-25.6	3.7 ± 0.7	2.2	5.2

Table 2.2. Exponential decay equations and half-lives representing stable isotope turnover in epidermis sampled from rehabilitated Florida manatees.

Carbon turnover

	Animal ID	Equation	R^2	Half-life (days)	$\delta^{13}C$ at day 0 (‰)	R^2 range Min	Max
Coastal manatees	0301	$y = -24.4 + 14.2e^{-0.01094x}$	1.00	63	-10.2	1.00	1.00
	0318	$y = -24.8 + 15.1e^{-0.01158x}$	0.97	60	-9.7	0.96	0.97
	0322	$y = -24.5 + 14.3e^{-0.01550x}$	0.83	45	-10.2	0.79	0.84
	0431	$y = -24.1 + 11.6e^{-0.01657x}$	0.97	42	-12.5	0.97	0.97
			Mean	53	-10.7		
Riverine manatees	0334	$y = -24.6 + 8.2e^{-0.00963x}$	0.95	72	-16.4	0.90	0.96
	0340	$y = -24.7 + 0.9e^{-0.01787x}$	0.36	39	-23.8	0.05	0.51
	0341	$y = -23.8 + 1.9e^{-20920x}$	0.50	N/A	-21.9	0.43	0.51
	0501	$y = -24.5 + 6.2e^{-0.01028x}$	0.70	67	-18.3	0.59	0.75
			Mean	59	-20.1		

Nitrogen turnover

	Animal ID	Equation	R^2	Half-life (days)	$\delta^{15}N$ at day 0 (‰)	R^2 range Min	Max
Coastal manatees	0301	$y = 2.3 + 3.4e^{-0.01918x}$	0.96	36	5.7	0.83	1.00
	0318	$y = 2.4 + 2.4e^{-0.02125x}$	0.57	33	4.8	0.46	0.62
	0322	$y = 2.3 + 3.6e^{-0.04898x}$	0.56	14	5.9	0.29	0.68
	0431	$y = 2.5 + 4.7e^{-0.03038x}$	0.97	23	7.2	0.91	0.97
			Mean	27	5.9		
Riverine manatees	0334	$y = 2.6 + 4.9e^{-0.00601x}$	1.00	115	7.5	0.98	1.00
	0340	$y = 2.7 + 6.4e^{-0.02055x}$	0.92	34	9.1	0.90	0.92
	0341	$y = 2.3 + 5.9e^{-0.01125x}$	0.85	62	8.2	0.80	0.87
	0501	$y = 2.0 + 9.4e^{-0.03273x}$	0.95	21	11.4	0.92	0.97
			Mean	58	9.1		

CHAPTER 3: STABLE ISOTOPE ANALYSIS OF THE SKIN OF THE WEST INDIAN MANATEE *(TRICHECHUS MANATUS*) IN FLORIDA, BELIZE, AND PUERTO RICO

Introduction

The endangered West Indian manatee (*Trichechus manatus*) is one of only two extant sirenian species (*T. manatus* and *T. senegalensis*) that occupy marine, estuarine, and freshwater habitats (Hartman 1979, Best 1981). This herbivorous species has two recognized subspecies, the Florida manatee (*T.m. latirostris*) and the Antillean or Caribbean manatee (*T.m. manatus*). Relatively little is known about manatee feeding ecology and habitat use since they often occupy shallow, turbid water. As a result, most feeding studies have been conducted in captivity (e.g., Marshall et al. 2000) or in the clear waters near natural springs (e.g., Hartman 1979). Reliable population estimates for both subspecies remain elusive (Lefebvre et al. 1995, UNEP Caribbean Environment Programme 1995, U.S. Fish and Wildlife Service 2001) and a better understanding of manatee feeding ecology and its relation to habitat use is essential in order to improve conservation efforts.

Though there are few morphological differences between the two subspecies (Antilleans tend to be smaller in body size than Florida manatees and have differing skull features, Converse et al. 1994), there are many other differences in habitat, climate, human impact, and population size that have unique effects on the success of each subspecies. Both subspecies appear to require regular year-round access to a freshwater source (Lefebvre et al. 1989, Olivera-Gomez & Mellink 2005), a requirement that is presumed to be due to osmoregulatory constraints (Ortiz et al. 1998, Ortiz et al. 1999).

Winter habitat use by Florida manatees is primarily influenced by water temperature whereby physiological thermal constraints require that they aggregate at natural (springs) and/or artificial (power plant outflows) warm water sources when water temperatures drop below 20C (Irvine 1983, Worthy et al. 2000, Bossart et al. 2003). This type of migration is unnecessary for Antillean manatees since their range lies in the tropics and lacks large seasonal fluctuations in water temperature. In Florida, the primary human-related cause of manatee mortality is injury due to watercraft. Less common, yet still evident, are mortalities due to entanglement in fishery equipment, crushing/drowning in flood gates or canal locks, or ingestion of debris (U.S. Fish and Wildlife Service 2001). Human impact on the Antillean manatee population is dominated by injuries due to watercraft as well, but poaching is also a significant threat (UNEP Caribbean Environment Programme 1995). Both the Florida and Antillean manatee habitats have been affected by coastal development, but it is a more prominent issue in Florida. Seagrass beds may be affected by runoff in developed areas (e.g., Lewis et al. 2002) as well as direct damage from boat traffic including propellers "scarring" or tearing seagrasses from the substrate (e.g., Uhrin & Holmquist 2003). Population estimates for the Florida manatee suggest counts as high as 3,276 (U.S. Fish and Wildlife Service 2001), whereas Antillean manatees in most countries are thought to number fewer than 100 individuals (summarized in O'Shea & Salisbury 1991). The largest Antillean manatee population is located in Belize (approximately 100-250 individuals, O'Shea & Salisbury 1991, Morales-Vela et al. 2000). In Puerto Rico, manatee distribution is patchy and population counts suggest fewer than 100 individuals (summarized in O'Shea & Salisbury 1991).

Manatees are often considered generalist feeders and have been known to consume approximately 60 different species of vegetation in marine, estuarine, and freshwater habitats (Hartman 1979, Best 1981, Bengtson 1983). Florida manatees appear to exhibit regional (Reich & Worthy 2006) and possibly seasonal differences in diet composition. Because of the previously mentioned habitat and climate differences between the two West Indian manatee subspecies, seagrasses tend to make up a much larger portion of the manatee diet in Puerto Rico than do freshwater and/or estuarine vegetation (Mignucci-Giannoni & Beck 1998). Even less is known of the feeding habits of Belize manatees, but seasonal differences in lagoon water levels may lead to changes in habitat use (Morales-Vela et al. 2000) and consequently diet composition. There has been little research done on the Antillean subspecies and expanded effort is crucial to provide measures on which conservation plans can be based.

It can be challenging to evaluating feeding behavior and habitat use in marine mammals since they are difficult to observe directly, and may have expansive migration patterns. A variety of methods have been used, including satellite tracking (e.g., Laidre et al. 2003), stomach content analysis (e.g., Spitz et al. 2006), fecal analysis (e.g., Sinclair & Zeppelin 2002), and fatty acid signature analysis (e.g., Iverson et al. 1997). Some complications involved in these methods include logistics, cost, invasiveness, difficulty in identifying and estimating the proportion of diet components, and the inability to assess long-term feeding history. The application of stable isotope analysis to ecological research has expanded in recent years (reviewed by Kelly 2000) and has some advantages over these techniques. Isotopic ratios of local food webs are incorporated into the tissues of the consumer and can be used to predict diet composition, the trophic level at which

the consumer is feeding, and even habitat use and migratory patterns (Deniro & Epstein 1978, 1981, Fry 1981, Peterson & Fry 1987, Hobson 1999). Tissues such as hair and skin can be sampled from live animals, and both recent and long-term feeding history can be assessed through analyzing tissues that incorporate nutrients at different rates. In order to accurately interpret isotopic results it is important to know the diet-tissue discrimination value (the difference in isotope ratios between the diet and consumer tissue) as well as the turnover rate (the amount of time required to incorporate isotopes from the diet into the tissue). Previous studies have calculated or estimated diet-tissue discrimination values (Alves Chapter 2, Ames et al. 1996, Reich & Worthy 2006) and turnover rates (Alves Chapter 2) for carbon and nitrogen isotopes in manatee skin, allowing for more accurate interpretation of stable isotope data.

The present study is the first to apply stable isotope analysis to Antillean manatees and further expands the assessment of the feeding ecology of Florida manatees. The objectives of this study were to (1) analyze $\delta^{13}C$ and $\delta^{15}N$ values in epidermis samples collected from free-ranging manatees in Florida, Belize, and Puerto Rico, (2) compare signatures in the skin to those of fresh, estuarine, and marine vegetation within the presumed manatee diet, and (3) assess possible differences in feeding preferences by region, sex, age class, and season.

Materials and Methods

Sample collection

Aquatic plants

Samples of aquatic vegetation were collected from several regions within Florida during the summer of 2001, the fall, winter, and spring of 2004 and 2005 (Charlotte Harbor: n = 15, Crystal River: n = 5, Indian River Lagoon: n = 21, St. Johns River near Blue Spring: n = 29, Tampa Bay: n = 16, and Ten Thousand Islands: n = 15), and from Belize during the summer of 2002 (Drowned Cayes: n = 18) (Figs. 3.1 & 3.2, Appendix D). Each sample consisted of 2-3 whole plants or 20-30 seagrass blades of each available species at each site. Collection sites in St Johns River were sampled repeatedly during different months to determine seasonal variability.

Free-ranging manatees

Skin samples from free-ranging manatees in Florida (n = 118), Belize (n = 68), and Puerto Rico (n = 23) were collected during 2002-2005 (Appendix E) and provided by Bob Bonde, Sirenia Project, U.S. Geological Survey (permit number MA791721) as part of a larger study examining genetic relatedness. Sampling locations in Florida included Crystal River (city of Crystal River), Homosassa Springs (city of Homosassa Springs), Tampa Bay (Big Bend Power Station, Tampa), Ten Thousand Islands (Port of the Islands, Naples), and Charlotte Harbor (Warm Mineral Springs) (Fig. 2.1). Most Florida manatees (n = 113) were sampled during the fall and winter months when they often congregate in large numbers at natural or artificial warm water sources. Sampling

locations in Belize included the Northern Lagoon, Southern Lagoon, Western Lagoon (part of the Southern Lagoon), and Drowned Cayes (Fig. 3.2). Locations in Puerto Rico included Boqueron (Cabo Rojo), Ceiba, Guayanilla, and Salinas (Fig. 3.3). Samples from Antillean manatees in Belize and Puerto Rico were collected opportunistically over multiple years. For seasonal comparisons, winter was defined as December through February, spring as March through May, summer as June through August, and fall as September through November.

Epidermal tissue was collected from the edge of the paddle using a cattle ear notch tool. Sex was determined by observing the position of the urogenital slit and body length was measured as the straight distance from snout to paddle (Fig. 2.2). Florida manatees were categorized into three age classes based on body length measurements (adults: >275 cm, subadults/late juveniles: 176-275 cm, and calves: <176 cm, O'Shea et al. 1985). Antillean manatees were also categorized into age classes, although the classes were defined by different body length measurements since Antillean manatees are slightly smaller (adults: >225 cm, subadults/late juveniles: 176-225 cm, and calves: <176 cm, Mignucci-Giannoni et al. 2000).

Sample preparation and analysis

All manatee tissue and plant samples were frozen within two hours of collection and held at -20C until time of analysis. Samples were then rinsed with distilled water and oven dried at 60C for 24 hours to remove water. Lipids were removed using petroleum ether in a Soxhlet extractor for 24 hours to remove the effect of lipids on $\delta^{13}C$ values (Rau et al. 1992). Samples were then oven dried at 60C for 24 hours to remove any

remaining solvent. Samples were ground and homogenized using a SPEX 8000 Mixer/Mill (CertiPrep, Metuchen, NJ), Wig-L-Bug Amalgamator (Crescent Dental Manufacturing Co., Chicago, IL), or were chopped by hand using a scalpel. Approximately 1.0 mg of manatee tissue or 2.5 mg of plants were transferred to 5 mm x 9 mm tin capsules and analyzed by mass spectrometry (Thermo Finnigan DELTAplus and DELTA C, Bremen, Germany) for carbon and nitrogen stable isotope ratios at the Stable Isotope and Ecology Lab, University of Georgia, Athens, GA.

Seagrass samples were analyzed with and without epiphytes when possible. Seagrasses analyzed with epiphytes were prepared as discussed previously for other aquatic plants, with the exception that they were rinsed in saltwater instead of distilled water to prevent further removal of epiphytes.

Data analysis

Stable isotope ratios were expressed in ppt (‰) using delta notation:

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

in which R_{sample} and $R_{standard}$ are the absolute isotope ratios of the sample and standard, respectively, X is ^{13}C or ^{15}N, and the standards are PeeDee belemnite (from the Cretaceous marine fossil, Belemnitella americana, from the PeeDee formation in South Carolina, Craig 1957) and atmospheric N_2, respectively.

Statistical analysis

All statistical analyses were judged to be significant at <0.05. Data were tested for normality using the Shapiro-Wilk ($n < 50$) and Kolmogorov-Smirnov ($n > 50$) tests. Levene's F and Box's M were used to test homogeneity of variance between factors and

homogeneity of covariance, respectively. Differences in $\delta^{13}C$ and $\delta^{15}N$ values were tested using parametric and non-parametric analyses as appropriate. Diet-tissue discrimination values and proportions of freshwater vegetation, estuarine vegetation, and seagrasses contributing to the Florida manatee diet were estimated using the stable isotope mixing model, IsoError (Phillips & Gregg 2001). Means are presented ± SE.

Results

Quality assurance of mass spectrometer analysis

Quality assurance of mass spectrometer results was tested by running two standard samples before and after every twelve unknown samples. Standard samples were bovine tissue (n = 110) and poplar (n = 9). Standard errors of the mean for bovine samples were ±0.04‰ for $\delta^{13}C$ and ±0.01‰ for $\delta^{15}N$. Standard errors for poplar samples were ±0.04‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Precision of mass spectrometer analysis within a sample was tested by running a subset of manatee skin samples in duplicate. Neither $\delta^{13}C$ (paired t-test: $t = 0.17$, $df = 8$, $p = 0.87$) nor $\delta^{15}N$ values ($t = 1.36$, $df = 8$, $p = 0.21$) differed between duplicate samples, therefore single samples were run for further analyses.

Diet analysis

Florida aquatic plants

Because the seagrass *Thalassia testudinum* often has a large amount of epiphytic algae and other encrusting organisms attached to the blades, $\delta^{3}C$ and $\delta^{15}N$ values for clean and epiphytic blades were initially compared. Paired t-tests indicated no significant difference in $\delta^{13}C$ ($t = 2.15$, $df = 10$, $p = 0.057$) or $\delta^{15}N$ values ($t = 1.13$, $df = 10$, $p = 0.29$) between clean blades (mean $\delta^{13}C = -11.9 \pm 0.8$‰, mean $\delta^{15}N = 2.1 \pm 0.4$‰) and those with epiphytes present (mean $\delta^{13}C = -12.9 \pm 0.8$‰, mean $\delta^{15}N = 2.4 \pm 0.5$‰, so samples were grouped for further analyses.

Stable isotope signatures of aquatic vegetation were compared between collection locations in order to show distinctions between freshwater plants, estuarine plants, and seagrasses. Welch ANOVA results indicated that $\delta^{13}C$ values differed significantly ($F_{6,27} = 189.27$, $p < 0.001$). Freshwater plants were the most depleted in ^{13}C, seagrasses were the most enriched in ^{13}C, and plants collected from Crystal River as well as marine algae from the Indian River Lagoon, having intermediate $\delta^{13}C$ values, were categorized as estuarine vegetation (Table 3.1, Figs. 3.4 & 3.5). Regardless of collection location, $\delta^{13}C$ values differed between all three plant types (Tamhane's T2: all p values < 0.021). Welch ANOVA results also indicated that $\delta^{13}N$ values differed significantly between collection locations ($F_{6,34} = 38.19$, $p < 0.001$). Regardless of collection location, all seagrasses were significantly depleted in ^{15}N compared to freshwater and estuarine vegetation (Tamhane's T2: all p values < 0.002), but $\delta^{15}N$ values did not differ between freshwater and estuarine vegetation (all p values > 0.61, Table 3.1, Figs. 3.4 & 3.5).

Within all seagrass samples, $\delta^{13}C$ values differed significantly between collection locations (Welch ANOVA: $F_{3,27} = 5.51$, $p = 0.004$). Seagrasses from Tampa Bay were significantly depleted in ^{13}C by an average of 3.8‰ compared to those from Charlotte Harbor (Tamhane's T2: $p = 0.003$, Table 3.1, Fig. 3.5). No other significant differences were calculated for $\delta^{13}C$ values between any other collection locations (Tamhane's T2: all p values > 0.26). The $\delta^{13}C$ values did not differ between seagrass species regardless of how locations were grouped (ANOVA: $F < 1.1$, all p values > 0.37). Finally, $\delta^{15}N$ values for seagrasses did not differ between collection locations (Welch ANOVA: $F_{3,26} = 1.79$, $p = 0.17$, Table 3.1, Fig. 3.5) or species (ANOVA: $F_{2,53} = 2.34$, $p = 0.11$).

Halophila engelmannii was collected at one location (Ten Thousand Islands), preventing inclusion in this analysis.

Freshwater plants from St. Johns River were collected during the months of April, July, and December, allowing for a seasonal comparison. Main effects for month and plant species were tested using MANOVA. There was a significant effect for month (Wilks' Lambda: $F_{4,28} = 4.22$, $p = 0.009$), but there was no effect for species ($F_{16,28} = 1.63$, $p = 0.13$). The $\delta^{13}C$ values of freshwater plants did not differ between months (ANOVA: $F_{2,23} = 2.83$, $p = 0.080$), but $\delta^{15}N$ values were significantly different ($F_{2,23} = 12.73$, $p < 0.001$). Plants collected in July were depleted in ^{15}N by an average of 3.9‰ compared to those collected in December and April (both p values < 0.002, Tukey HSD, Fig. 3.6).

Belize aquatic plants

Samples of *T. testudinum* and *H. wrightii* were collected opportunistically from the Drowned Cayes in Belize (Fig. 3.2, Appendix D). The $\delta^{13}C$ values ranged from -11.9‰ to -2.9‰ and $\delta^{15}N$ values ranged from -5.1‰ to 2.6‰ (Table 3.1). Species comparisons were not calculated due to small sample size, but $\delta^{13}C$ and $\delta^{15}N$ values for *H. wrightii* fell within the range of those of *T. testudinum* (Table 3.1). There was no effect of collection month (July-September, MANOVA: Wilks' Lambda: $F_{4,28} = 0.97$, $p = 0.44$) between seagrasses in Belize.

Free-ranging manatees

Florida manatees

The $\delta^{13}C$ values for manatee skin increased from very depleted signatures for skin collected in freshwater regions to more enriched signatures for skin collected in coastal regions. The $\delta^{15}N$ values for manatee skin decreased from enriched signatures for skin collected in freshwater regions to depleted signatures for skin collected in coastal regions (Table 3.2, Fig. 3.7).

MANOVA was run for $\delta^{13}C$ and $\delta^{15}N$ values using the following main and interaction effects: sex, age class, location, sex and age class, and sex and location. No significant effects were noted for sex (F test: $F_{2,99} = 0.28$, $p = 0.76$), age class (Wilks' Lambda: $F_{4,198} = 0.76$, $p = 0.55$), or either of the interaction effects ($F < 0.66$, both p values > 0.73), but there was a significant effect for location (Wilks' Lambda: $F_{8,198} = 13.49$, $p < 0.001$).

Both $\delta^{13}C$ (ANOVA: $F_{4,113} = 43.39$, $p < 0.001$) and $\delta^{15}N$ values ($F_{4,113} = 20.15$, $p < 0.001$) for manatee skin differed significantly based on location. Skin from manatees sampled in Crystal River and Homosassa Springs was depleted on average in ^{13}C by 6.2‰ compared to skin from manatees at the Big Bend Power Plant, Port of the Islands, and Warm Mineral Springs (Tukey HSD: all p values < 0.010, Table 3.2, Fig. 3.8). The $\delta^{15}N$ values differed significantly between skin from manatees at the Big Bend Power Plant and Port of the Islands by an average of 1.8‰ (Tukey HSD: $p = 0.020$, Table 3.2, Fig. 3.8). The $\delta^{15}N$ values did not significantly differ between skin from manatees at any other locations (Tukey HSD: all p values > 0.10). Because there were no significant

differences in $\delta^{13}C$ or $\delta^{15}N$ values in skin from manatees sampled in Crystal River and Homosassa Springs, data from these two Florida locations were pooled ("riverine manatees") for further analyses (Table 3.2, Fig. 3.8). In addition, because there were no significant differences in $\delta^{13}C$ values in skin from manatees at the Big Bend Power Plant, Port of the Islands, and Warm Mineral Springs, data from these three Florida locations were pooled ("coastal manatees") for further analyses.

Age class comparisons were further investigated since sample distribution did not allow to test for an interaction effect between age class and location. Within riverine manatees, all skin samples were from calves or subadults. MANOVA showed no effect for age class (F test: $F_{2,85} = 0.09$, $p = 0.91$). For skin from coastal manatees, $\delta^{13}C$ values did not differ between any of the three age classes (ANOVA: $F_{2,27} = 3.27$, $p = 0.054$). Regardless of how locations were grouped for coastal manatees, $\delta^{15}N$ values of skin also did not differ between age classes (all test statistics < 1.9, all p values > 0.082).

Month of skin sample collection was further investigated as a possible effect on carbon and nitrogen stable isotope ratios. For skin from riverine manatees, MANOVA failed to show an effect for month (October – February, Wilks' Lambda: $F_{8,160} = 0.72$, $p = 0.67$). For skin from coastal manatees, $\delta^{13}C$ values did not differ between months (January, December, and April, ANOVA: $F_{2,27} = 1.57$, $p = 0.23$). The $\delta^{15}N$ values of skin also did not differ between months, regardless of how the locations were grouped for coastal manatees ($F < 0.2$, all p values > 0.82).

Antillean manatees

Belize

The $\delta^{13}C$ values for manatee skin ranged from depleted for skin collected from lagoon regions to more enriched for skin collected from the Drowned Cayes (Table 3.2, Fig. 3.9). Differences in $\delta^{13}C$ values were tested using ANOVA with the following main and interaction effects: sex, age class, season, location, sex and location, sex and season, and age class and season. There were significant effects for location ($F_{3,71} = 21.41$, $p < 0.001$) and season (F test: $F_{2,71} = 3.77$, $p = 0.028$) but no other effects were significant ($F < 2.00$, all p values > 0.13).

Skin from manatees sampled in the Drowned Cayes was significantly enriched on average in ^{13}C by 5.2‰ compared to samples from the three lagoons (Tukey HSD: all p values < 0.001, Table 3.2, Fig. 3.10), but there were no differences in $\delta^{13}C$ values between lagoon samples (all p values > 0.37). The $\delta^{15}N$ values also differed significantly between manatee skin from different locations (Welch ANOVA: $F_{3,22} = 17.86$, $p < 0.001$). There was a difference in $\delta^{15}N$ values between manatees sampled in the Drowned Cayes and Southern Lagoon (Tamhane's T2: $p < 0.001$). Additionally, for $\delta^{15}N$ values, skin samples from the Northern Lagoon differed significantly from those of the Southern ($p < 0.001$) and Western Lagoons ($p = 0.036$, Table 3.2, Fig. 3.10).

Samples in Belize were collected during fall and spring (rainy and dry seasons, respectively). Results of t-tests indicated manatee skin collected in the spring was significantly enriched in both ^{13}C (Drowned Cayes, $t = 5.18$, $df = 11$, $p < 0.001$; Southern Lagoon, $t = 2.95$, $df = 35$, $p = 0.006$) and ^{15}N (Drowned Cayes, $t = 2.32$, $df = 11$, $p =$

0.041; Southern Lagoon, t = 2.41, df = 33, p = 0.022) compared to skin collected in the fall (Table 3.3). There was no seasonal difference in $\delta^{13}C$ values for manatee skin collected in the Western Lagoon (t = 0.50, df = 8, p = 0.63), but skin collected in the spring was significantly depleted in ^{15}N compared to that collected in the fall (t = 6.22, df = 8, p < 0.001, Table 3.3). Manatees from the Northern Lagoon were sampled exclusively during the spring.

Puerto Rico

The $\delta^{13}C$ values for manatee skin ranged from -12.8‰ for an animal from Ceiba to a more enriched value of -7.5‰ for an animal from Guayanilla. $\delta^{15}N$ values ranged from 3.7‰ for an animal from Boqueron to 6.9‰ for an animal from Guayanilla (Table 3.2, Fig. 3.11).

Only one skin sample was analyzed from Salinas so it was not included in the following analyses. Differences in $\delta^{13}C$ and $\delta^{15}N$ were tested using MANOVA with the following main and interaction effects: sex, age, season, location, sex and location, sex and age class, and sex and season. There was a significant effect for location (F test: $F_{2,11} = 4.22$, p = 0.043), but no other effects were significant (F < 2.60, all p values > 0.11). There were no significant differences in $\delta^{13}C$ values (ANOVA: $F_{2,19} = 0.32$, p = 0.73) between the three locations (Guayanilla, Boqueron, and Ceiba), but there was a significant difference in $\delta^{15}N$ values ($F_{2,19} = 11.76$, p < 0.001). Samples from Guayanilla were enriched in ^{15}N by an average of 1.4‰ compared to those from both Boqueron and Ceiba (Tukey HSD: both p values < 0.01, Table 3.2, Fig. 3.12).

Mixing model

Carbon and nitrogen signatures for freshwater vegetation, estuarine vegetation, and seagrasses as possible manatee diet sources in Florida were plotted with a polygon connecting mean values (Fig. 3.13). The diet source means for $\delta^{13}C$ values were corrected for enrichment by 2.8‰ (Alves Chapter 2). In order to fit the isotopic signature of the consumer within the diet source polygon, IsoError calculations indicated possible nitrogen enrichment values ranging from 1.0 to 1.5‰.

As is a function of the model, IsoError results differed based upon the nitrogen enrichment value. Either freshwater or estuarine vegetation was the main diet component for Florida riverine manatees, while seagrasses were always the main diet component for Florida coastal manatees regardless of the nitrogen enrichment value (Table 3.4). It was possible that riverine manatees had no seagrass component in their diet for only one nitrogen enrichment value (1.0‰). However, results for coastal manatees indicated the possibility of no freshwater diet component regardless of the nitrogen enrichment value.

Discussion

Diet analysis

For aquatic plants collected in Florida and Belize, seagrasses were the most enriched in ^{13}C, while freshwater plants were the most depleted, and estuarine plants had intermediate values. This pattern is consistent with findings in previous studies (e.g., Reich & Worthy 2006). Aquatic plants incorporate carbon from the dissolved inorganic carbon (DIC) in the surrounding water into their tissues. DIC in freshwater is depleted in ^{13}C compared to that of seawater due to the isotope discrimination that takes place upon the conversion of CO_2 into bicarbonate and the contribution of decomposing terrestrial matter (Boutton 1991). Freshwater and estuarine plants collected in Florida did not differ in their nitrogen signatures, but both plant types were significantly enriched in ^{15}N compared to seagrasses. Differences in nitrogen ratios have been shown to be based on nitrogen source, specifically in freshwater vs. marine ecosystems (e.g., Bardonnet & Riera 2005); however, the understanding of nitrogen isotope patterns especially in aquatic plants is limited compared to those of carbon isotopes. The combined use of carbon and nitrogen stable isotope analysis further distinguishes the three aquatic plant types in this study.

No significant differences were found in $\delta^{13}C$ or $\delta^{15}N$ values between *Thalassia* blades with or without epiphytes. Previous studies have conflicting results, in which some did find differences between isotope ratios in seagrasses vs. epiphytes (e.g., Moncreiff & Sullivan 2001) while others did not (e.g., Fry et al. 1982). Typically, the epiphytes are depleted in ^{13}C and enriched in ^{15}N compared to the seagrass blades from

which they were removed. In terms of diet estimation, it is not likely that epiphyte signatures could cause seagrasses to be confused with signatures of freshwater plants. However, it is possible that the presence of epiphytes could cause carbon and nitrogen signatures of seagrasses to be more similar to those of estuarine vegetation.

Carbon and nitrogen stable isotope analyses were not precise enough to distinguish between aquatic plant species, but regional and seasonal differences in stable isotope ratios were found in Florida. Specifically, seagrasses collected from Charlotte Harbor were significantly enriched in ^{13}C compared to those from Tampa Bay. Variability in $\delta^{13}C$ values in seagrasses is mainly an effect of carbon source but can also vary with irradiance and temperature (Hemminga & Mateo 1996). Additionally, both carbon and nitrogen stable isotope ratios of seagrasses have been shown to exhibit seasonal and intra-annual variability (Anderson & Fourqurean 2003, Vizzini et al. 2003). Seagrasses from Charlotte Harbor were collected in late spring and those from Tampa Bay were collected during the summer. It is unknown whether differences in $\delta^{13}C$ between the two sites were due to location or a seasonal effect. In the St. Johns River, aquatic plants collected in July were significantly depleted in ^{15}N compared to those collected in December and April. Differences in nitrogen signatures in plants may be due to seasonal variation in the amount of fertilizer runoff, wastewater discharge, and/or animal waste runoff into the aquatic system (Vizzini & Mazzola 2006). Additionally, seasonal differences in water temperature, streamflow, and dissolved oxygen concentrations have been shown to affect nitrogen balance in the St. Johns River (Kroening 2004). Temporal and geographical variability in stable isotope ratios for

aquatic plants should be taken into consideration when estimating diet proportions for consumers.

Mixing model

Reliance on mixing model results to estimate manatee diet and/or habitat use through stable isotope analysis of epidermal tissue is problematic due to slow turnover rates in the skin (Alves Chapter 2). Free-ranging manatees are known to switch diet sources (Best 1981, Lefebvre et al. 2000) and an intermediate isotope signature often may be measured rather than one representing the more recent diet. Instead of attempting to predict manatee diet composition involving all three aquatic plant types, it may be more useful and more accurate to focus on the use of freshwater plants vs. seagrasses since they have very distinct $\delta^{13}C$ and $\delta^{15}N$ values, whereas the $\delta^{15}N$ values for estuarine plants do not differ from those of freshwater plants. Also, the slow turnover rate in epidermal tissue makes this analysis better fit as a representation of average overall dietary intake over a long period of time rather than an indication of recent intake (Alves Chapter 2). Finally, when interpreting mixing model results, upper and lower 95% CI should be considered in addition to the mean values as a complete representation of possible diet source proportions (Phillips & Gregg 2003).

Seagrasses were the most frequently "required" component present in the diets of Florida manatees. Due to slow turnover rates, seagrasses were therefore not necessarily a recent diet component, but all Florida manatees in this study likely fed on seagrasses sometime in the past months prior to sampling. Seagrasses were an even more critical component in the diet of manatees sampled at the Big Bend Power Plant, Port of the

Islands, and Warm Mineral Springs. For manatees from these locations, it was even possible that they consumed no freshwater vegetation in past months. Manatees from Crystal River and Homosassa Springs had a more varied reliance on plant types with freshwater vegetation most often being the main component.

Reich & Worthy (2006) used IsoError to estimate diet source proportions using skin from stranded manatee carcasses, but comparisons to the results of their study may not be appropriate. Diet-tissue discrimination values used for carbon were consistent (2.8‰, present study; 3.0‰, Reich & Worthy), but those for nitrogen were not (1.0 to 1.5‰, present study; 5.0‰, Reich & Worthy). Computing an accurate diet-tissue discrimination value is essential when using mixing models to estimate proportions of a multiple source diet (Phillips & Koch 2002). The $\delta^{15}N$ enrichment values for whole body samples average 3‰ (Deniro & Epstein 1981, Minagawa & Wada 1984, Peterson & Fry 1987). Most tissues are enriched in ^{15}N by 2-5‰ compared to the diet (Peterson & Fry 1987, Kelly 2000), yet values ranging from <1 to 6‰ have also been calculated for mammal tissue (Vanderklift & Ponsard 2003). Hobson et al. (1996) calculated the only known diet-tissue discrimination value for nitrogen (2.3‰) in the skin of marine mammals (phocid seals). Further captive study is needed to determine an accurate diet-tissue discrimination value for nitrogen in manatee skin and thus improve the accuracy of diet source estimation. Another difference is Reich and Worthy (2006) grouped manatees by much larger geographical regions. For example, the entire northwest Florida coast was grouped (including Tampa Bay). In the present study, manatees sampled in Crystal River and Homosassa Springs (northwest Florida) were grouped separately from those sampled at the Big Bend Power Plant (Tampa Bay) because stable carbon and

nitrogen isotope ratios were significantly different in the skin. Consequently, it is likely too much of a generalization to categorize manatee feeding across larger geographical regions. Finally, it should be noted that no study involving manatee stable isotope analysis has used a concentration-dependent model such as that of Phillips & Kock (2002) which may further refine results.

Free-ranging manatees

Though the unavailability of freshwater and estuarine plant samples prevented mixing model analysis for Antillean manatees, general comparisons and interpretations were made based on known patterns. Skin from manatees sampled in the Drowned Cayes in Belize was enriched in ^{13}C compared to that of lagoon manatees, which is consistent with a coastal diet of predominantly seagrasses, while the lagoon animals probably relied more heavily on estuarine and/or freshwater vegetation. There were no differences in carbon signatures among manatees from the three lagoons. However, skin from Northern lagoon manatees was enriched in ^{15}N compared to that of the Southern and Western Lagoon manatees. The Western Lagoon is part of the Southern Lagoon, while the Northern Lagoon is a more distinct body of water (Fig. 3.2). It is possible that regional differences in nitrogen input from surrounding terrestrial sources had an effect on the nitrogen signatures of lagoon vegetation.

Enriched carbon ratios in the skin of manatees sampled in Puerto Rico indicate that seagrasses are likely the main component of their diet. The lack of significant regional differences in carbon ratios suggests this reliance on seagrasses is consistent among manatees in Puerto Rico. There were, however, significant regional differences in

nitrogen ratios in the skin of Puerto Rico manatees. As in the Belize analysis, it is possible that differences in coastal development, pollution, and agriculture had an effect on nitrogen input in the surrounding waters. The stable isotope results for free-ranging manatees agree with the hypothesis of Lefebvre et al. (2000) that due to thermally driven seasonal migrations, Florida manatees are less specialized grazers than manatees in Puerto Rico, for which seagrasses are the main diet component.

There were no sex based differences in isotopic composition of Florida or Antillean manatee skin. In the manatee social system there are not separate breeding or feeding grounds as in other animals systems in which we may expect to see sex based differences in diet and thus isotopic composition of tissues. Additionally, no differences were found in stable carbon or nitrogen isotope signatures in Florida manatee skin based on the collection month. Skin samples were collected mainly during the months of November, December, and January. This sampling period coincided with the large number of manatees aggregated at warm water sources. In general, all manatee skin sampling was opportunistic, so it does not necessarily mean that a manatee sampled in November arrived at a warm water refuge earlier than a manatee sampled in January. A manatee sampled in January may have been frequenting the refuge since November, and was just not able to be sampled until later that season. It is also unknown how long each manatee spent in the refuge or whether it left, traveling a long distance (giving it access to alternative diet sources) and then returned again. For these reasons, it is understandable that we do not see a progression towards a greater number of manatee skin signatures representative of the vegetation available near the refuge over time.

Seasonal differences in $\delta^{13}C$ and/or $\delta^{15}N$ values were found within the skin of Belize manatees; however, results for manatees from the Drowned Cayes and Western Lagoon should be interpreted with caution due to small sample sizes. Reported differences may be due to seasonal changes in manatee feeding, habitat, and/or seasonal variability in the stable isotope ratios of aquatic plants, the later two of which have been previously documented in Belize manatees (Self-Sulivan et al. 2003) and Florida aquatic plants, respectively (present study, Anderson & Fourqurean 2003).

There was no difference in $\delta^{13}C$ or $\delta^{15}N$ values in manatee skin between any of the three age classes for Florida or Antillean manatees. Calves (<176 cm) are considered nutritionally dependent but manatees up to body lengths of 260cm may nurse in addition to feeding on aquatic vegetation (O'Shea et al. 1985). Most calves that were sampled were of body lengths ≥170 cm, near the calf/subadult division. It is likely that these manatees were already feeding on aquatic vegetation and possibly only nursing occasionally. In that case, we would not expect to see signatures different from those of subadults, especially if some manatees of subadult-length occasionally nurse. Had the manatees been very young calves, whose diet consisted of predominantly milk, we might expect $\delta^{15}N$ values of their skin to be enriched compared to adults. Nursing animals are consuming milk derived from the mother's tissues and are in essence, feeding at a higher trophic level (e.g., Fogel et al. 1989). Skin from the smallest manatees sampled (both body lengths = 130 cm, Florida) did not have enriched $\delta^{15}N$ values (5.8 and 4.3‰) compared to those of longer body lengths. Finally, the variability in nitrogen signatures found for aquatic plants in Florida (-2.9 to 10.3‰) makes it difficult to assess possible differences in trophic level between nursing and weaned manatees. The age class

division between subadults and adults is a gross estimate based on sexual maturity and is mainly used to assess manatee mortality rates and population structure (O'Shea et al. 1985). It has no relation to any specific feeding distinction.

Conservation implications

The interpretation of mixing model results warrants caution as discussed. However, if we are to make general estimates in relation to manatee conservation, seagrasses were a required component for Florida manatees from all regions sampled in this study (Gulf Coast) and even likely made up over half of the diet of manatees wintering at the Big Bend Power Plant in Tampa Bay, Warm Mineral Springs near Charlotte Harbor, and Port of the Islands in Ten Thousand Islands. In an effort to provide more suitable habitat for Florida manatees, focus should be on reducing the further loss of seagrass beds especially near the regions mentioned above. Finally, individual variation in $\delta^{13}C$ and $\delta^{15}N$ values in Florida manatee skin within a location suggests these warm water sources provide refuge for manatees previously feeding in differing habitats. Consequently, there may not be one single conservation solution regarding feeding habitat that caters to all manatees wintering at one of the specific locations in this study.

It is apparent that seagrasses are also an important component in the diet of Antillean manatees in Belize and even more so for those in Puerto Rico. Management plans here should also emphasize the protection of seagrass beds.

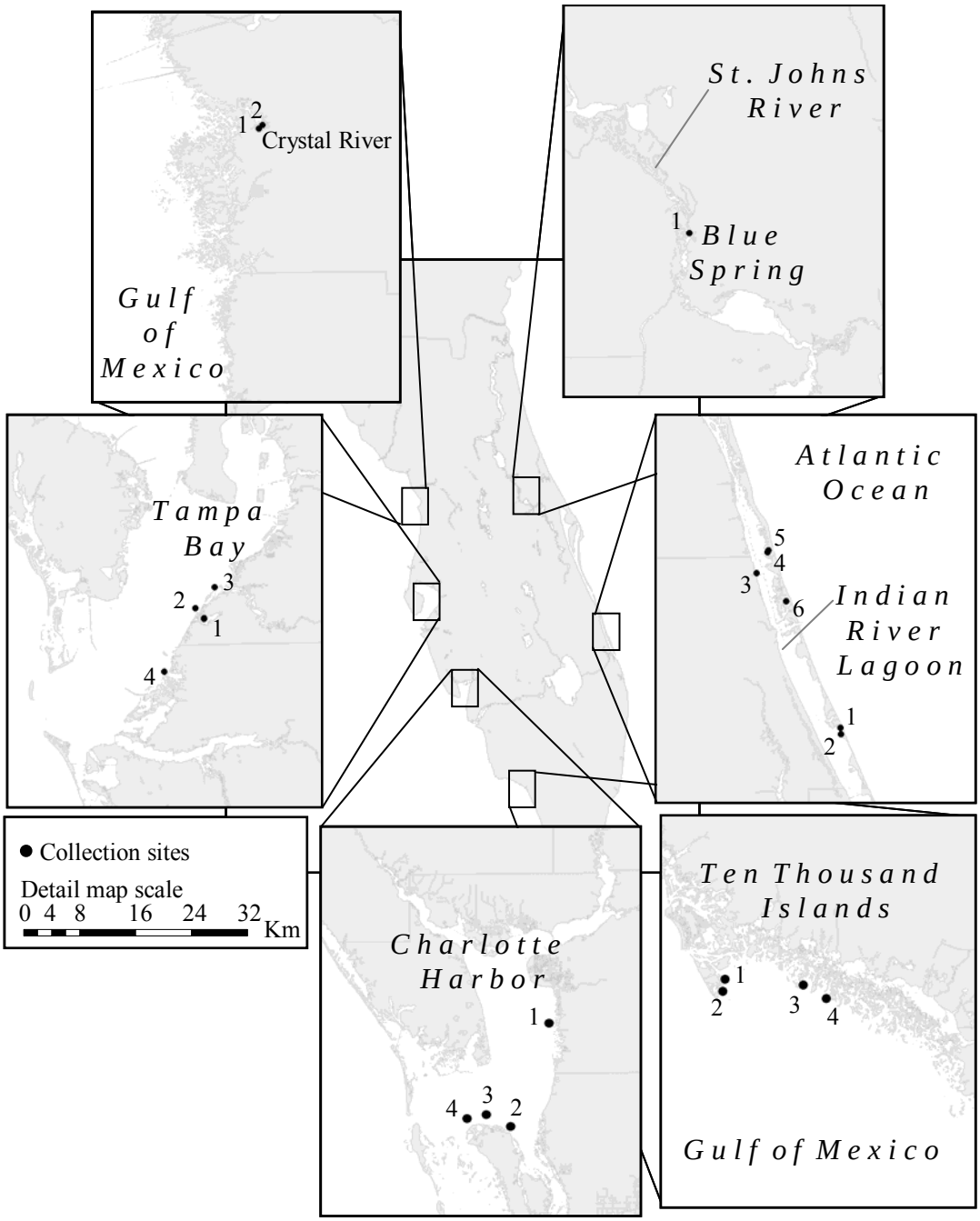

Figure 3.1. Aquatic plant collection sites in Florida.

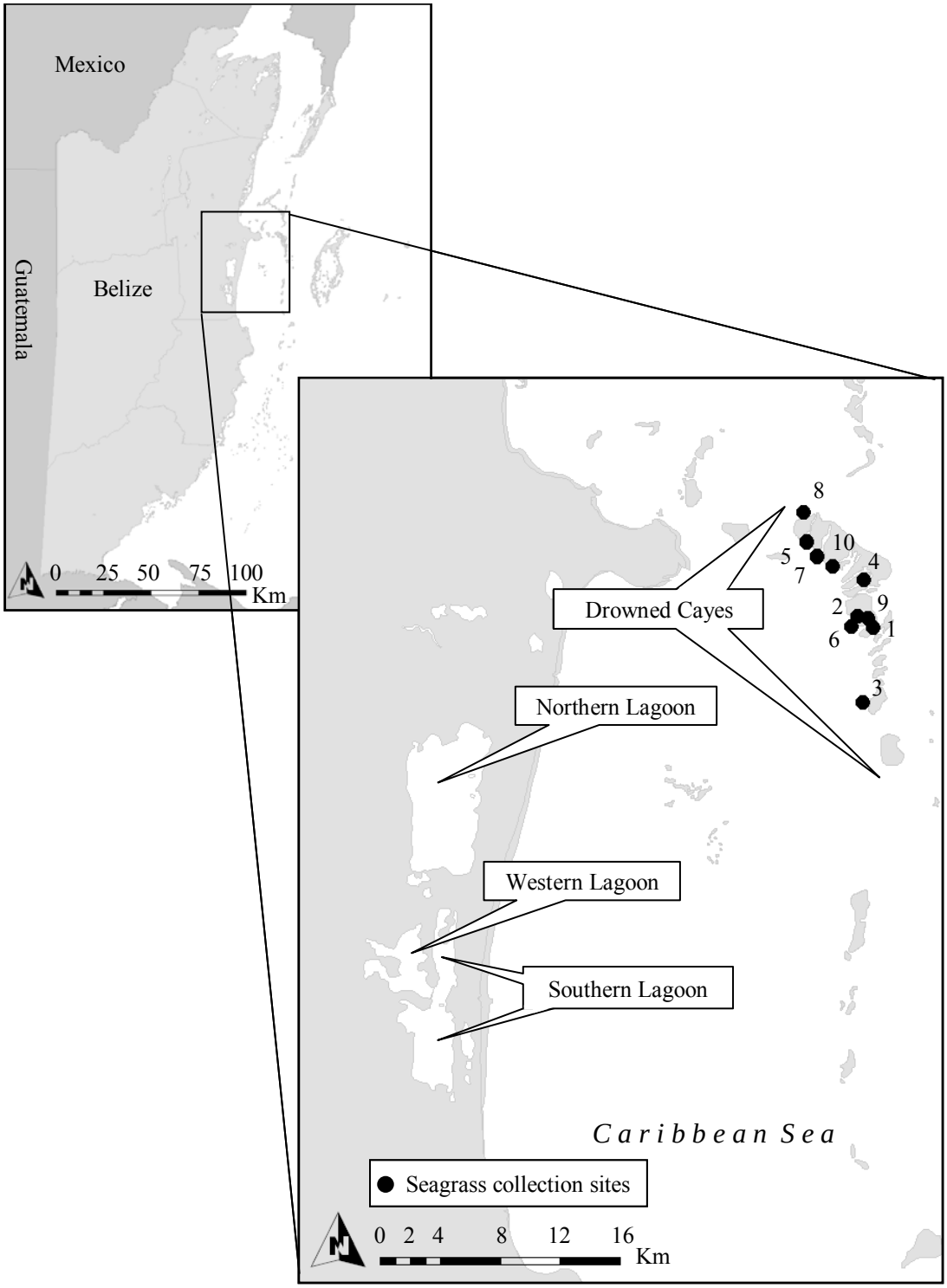

Figure 3.2. Manatee skin and seagrass collection locations in Belize.

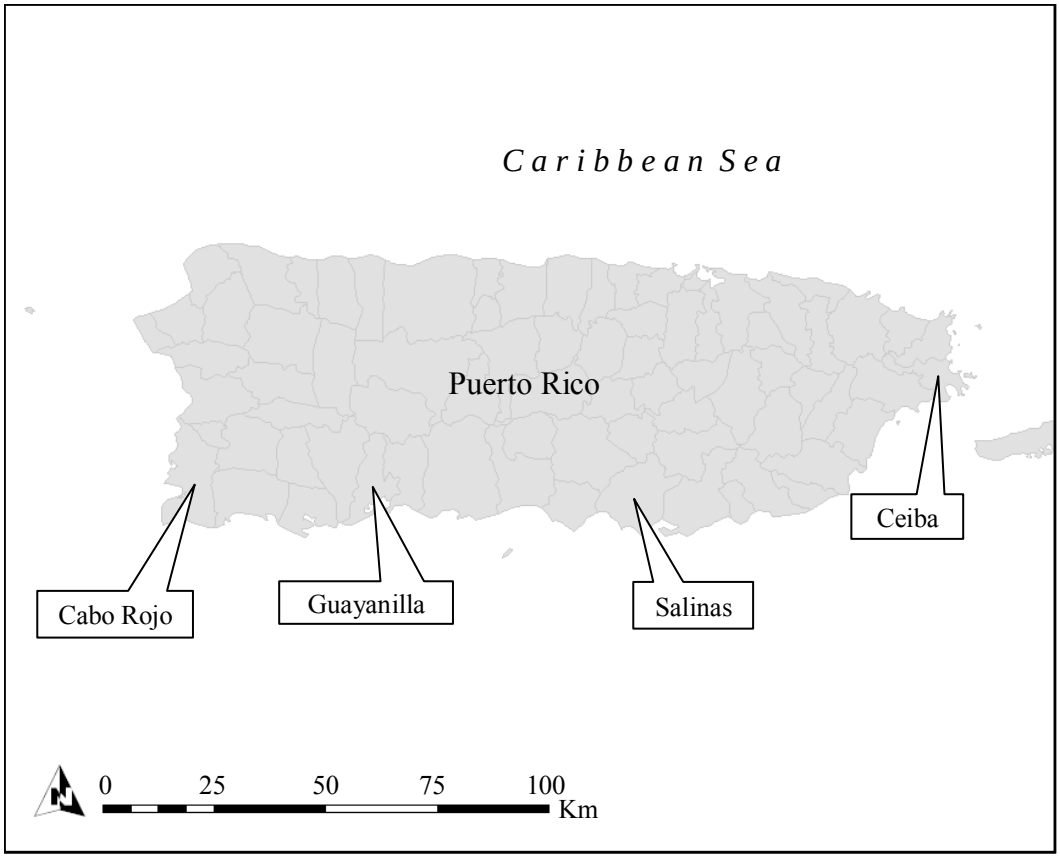

Figure 3.3. Manatee skin collection locations in Puerto Rico.

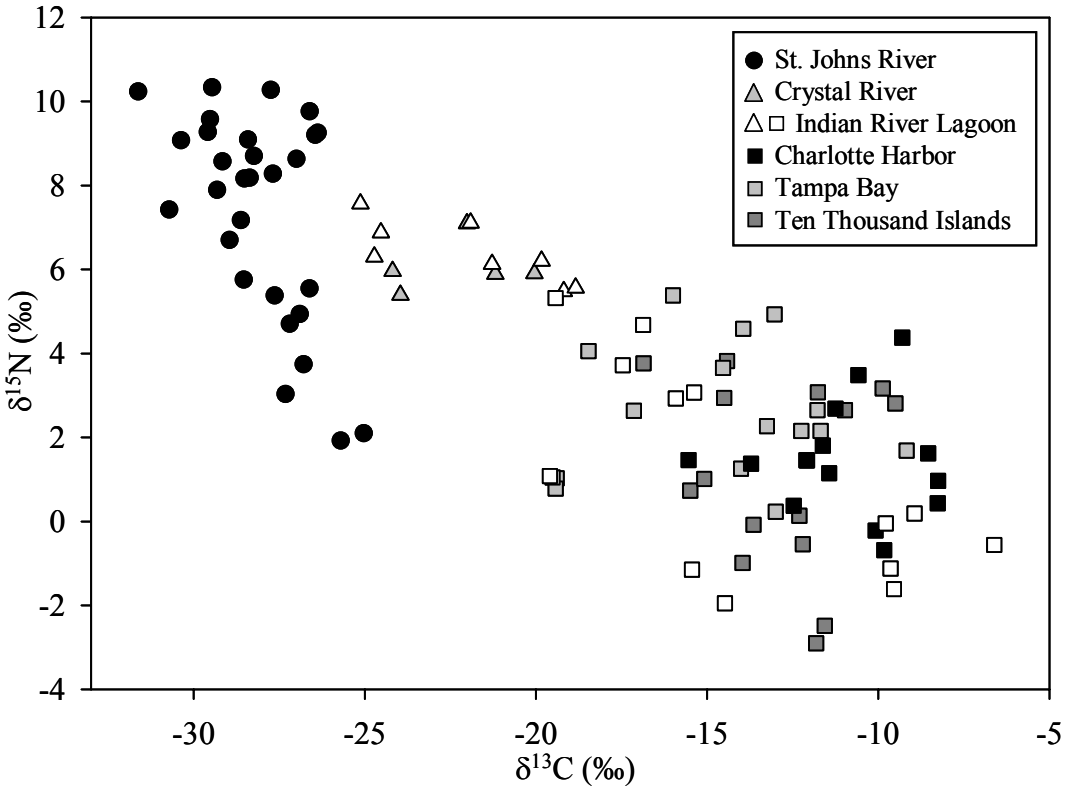

Figure 3.4. $\delta^{13}C$ and $\delta^{15}N$ values for aquatic plants in Florida.

Circles indicate freshwater vegetation, triangles indicate estuarine vegetation, and squares indicate seagrasses.

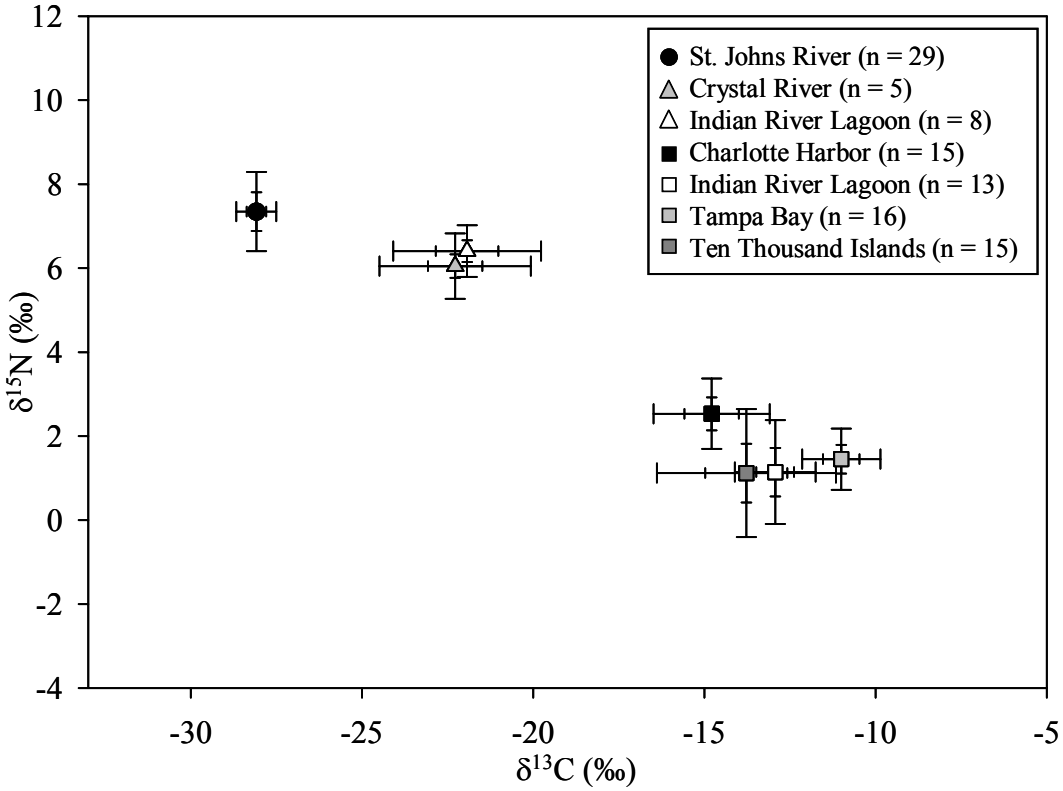

Figure 3.5. Regional differences in $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE and 95% CI) for aquatic plants in Florida.

Circles indicate freshwater vegetation, triangles indicate estuarine vegetation, and squares indicate seagrasses.

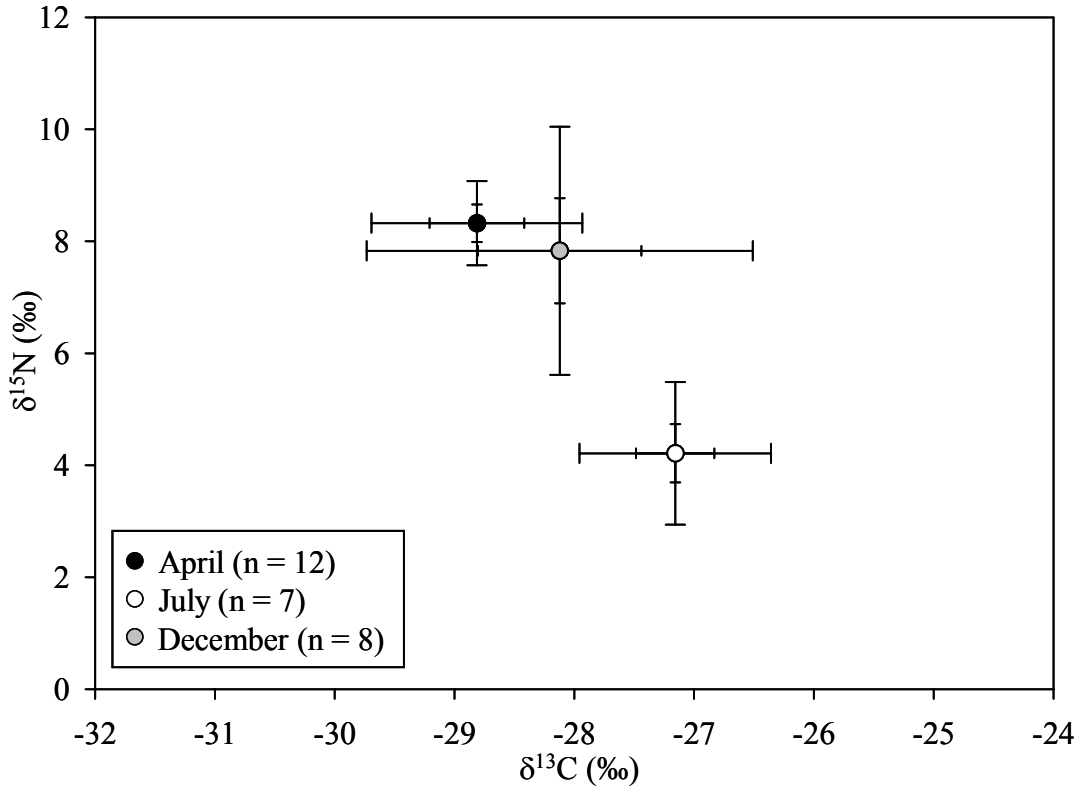

Figure 3.6. Seasonal differences in $\delta^{13}C$ and $\delta^{15}N$ values in freshwater aquatic plants from the St. Johns River (mean ± SE and 95% CI).

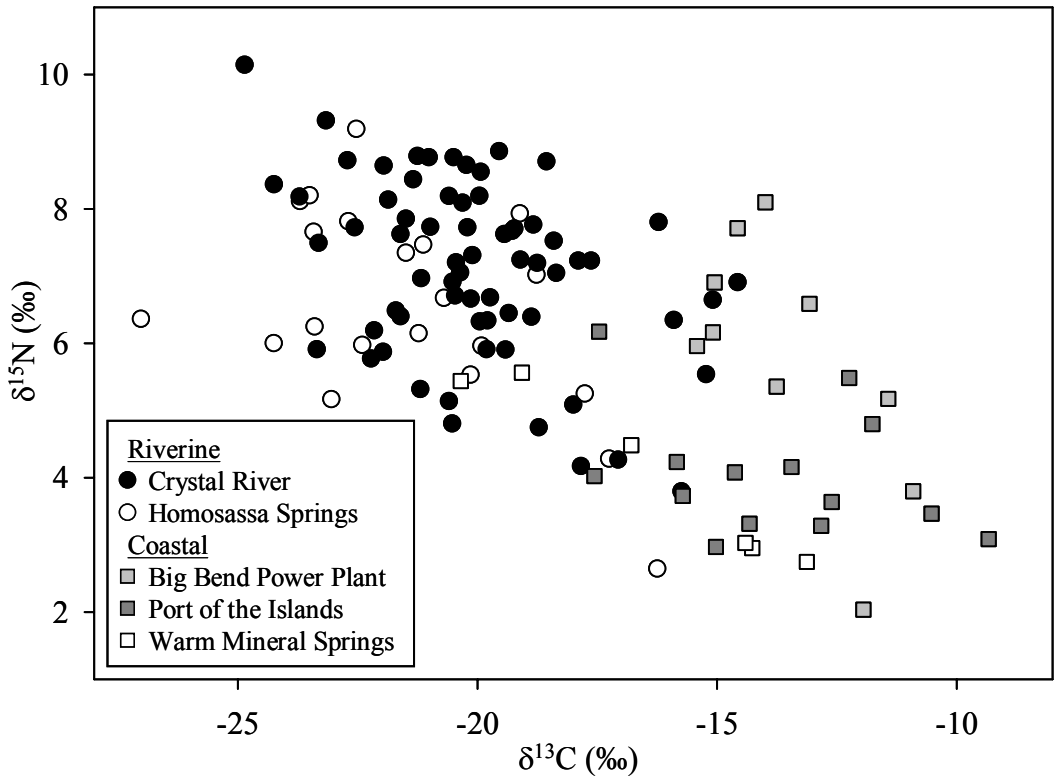

Figure 3.7. $\delta^{13}C$ and $\delta^{15}N$ values in manatee skin from free-ranging animals in Florida.

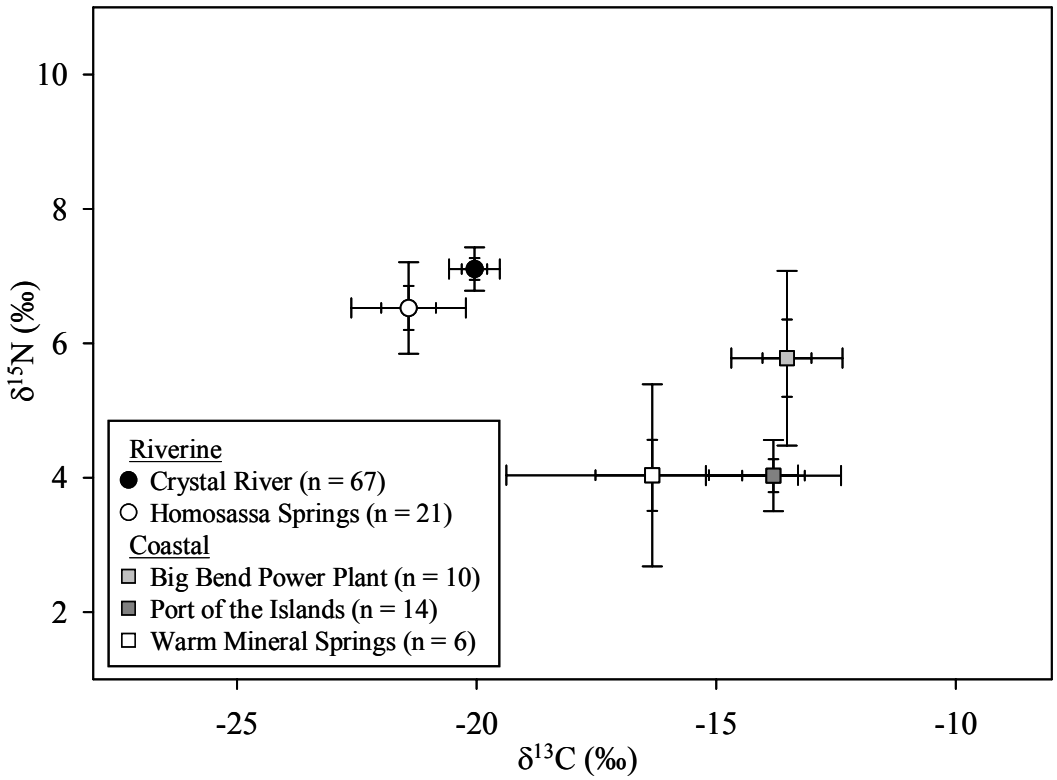

Figure 3.8. Regional differences in $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE and 95% CI) in manatee skin from free-ranging animals in Florida.

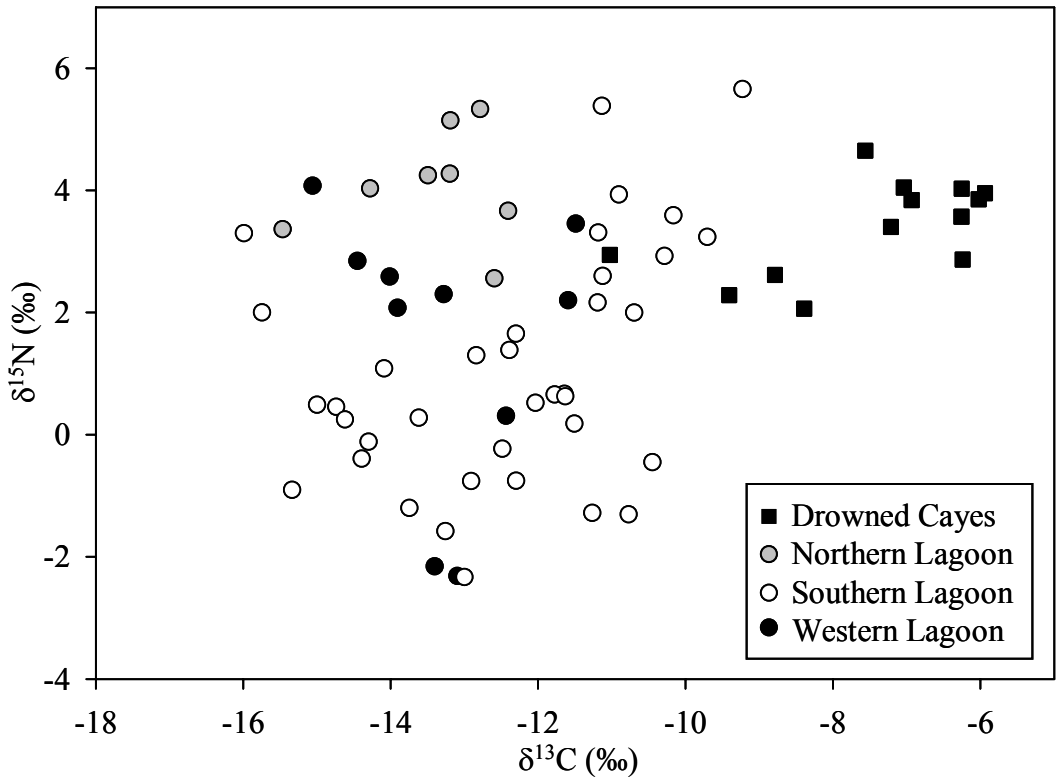

Figure 3.9. $\delta^{13}C$ and $\delta^{15}N$ values in manatee skin collected from free-ranging animals in Belize.

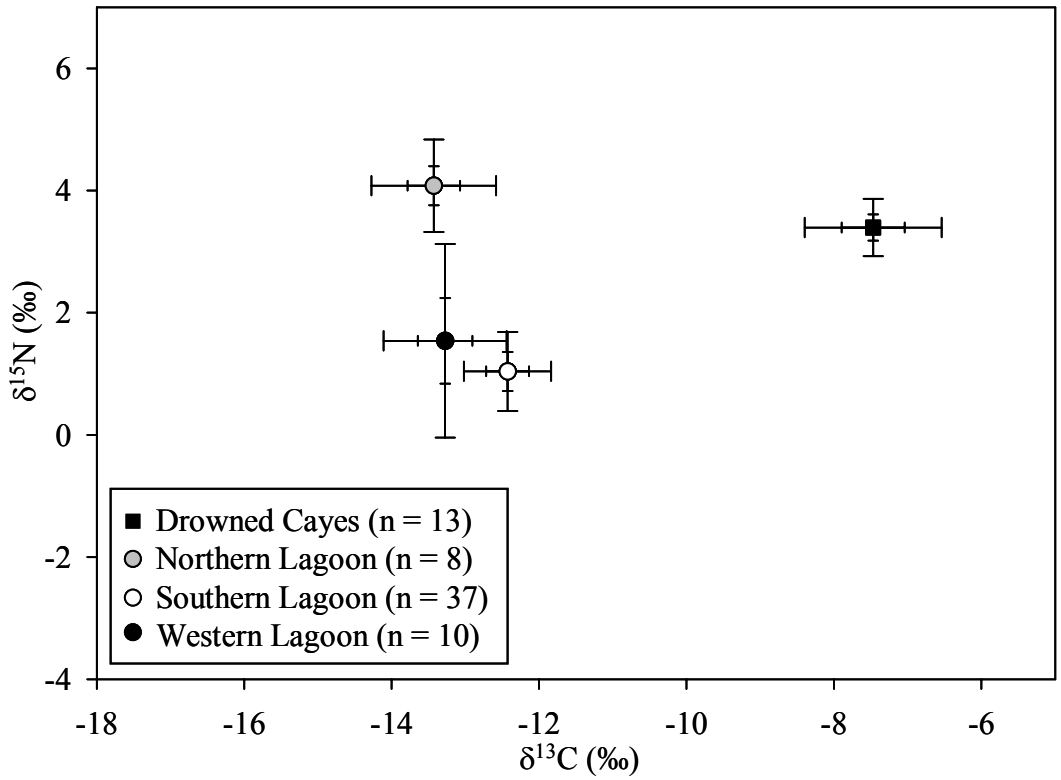

Figure 3.10. Regional differences in $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE and 95% CI) in manatee skin from Belize.

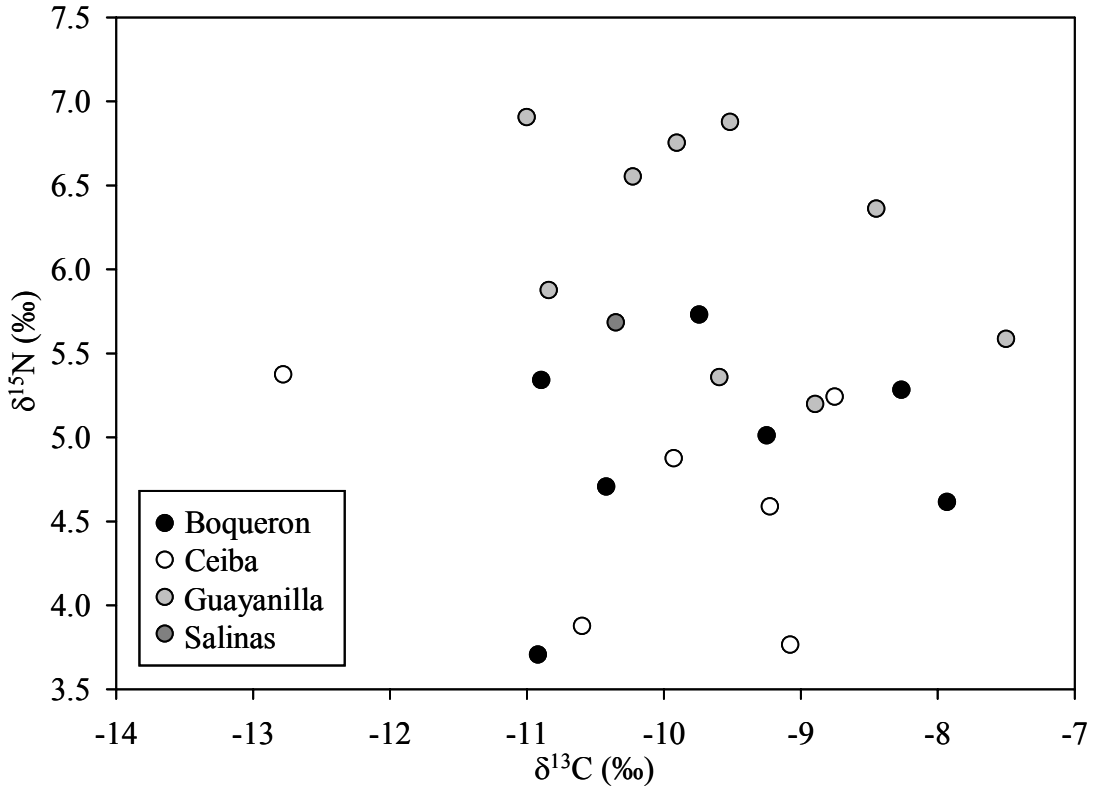

Figure 3.11. $\delta^{13}C$ and $\delta^{15}N$ values in manatee skin from free-ranging animals in Puerto Rico.

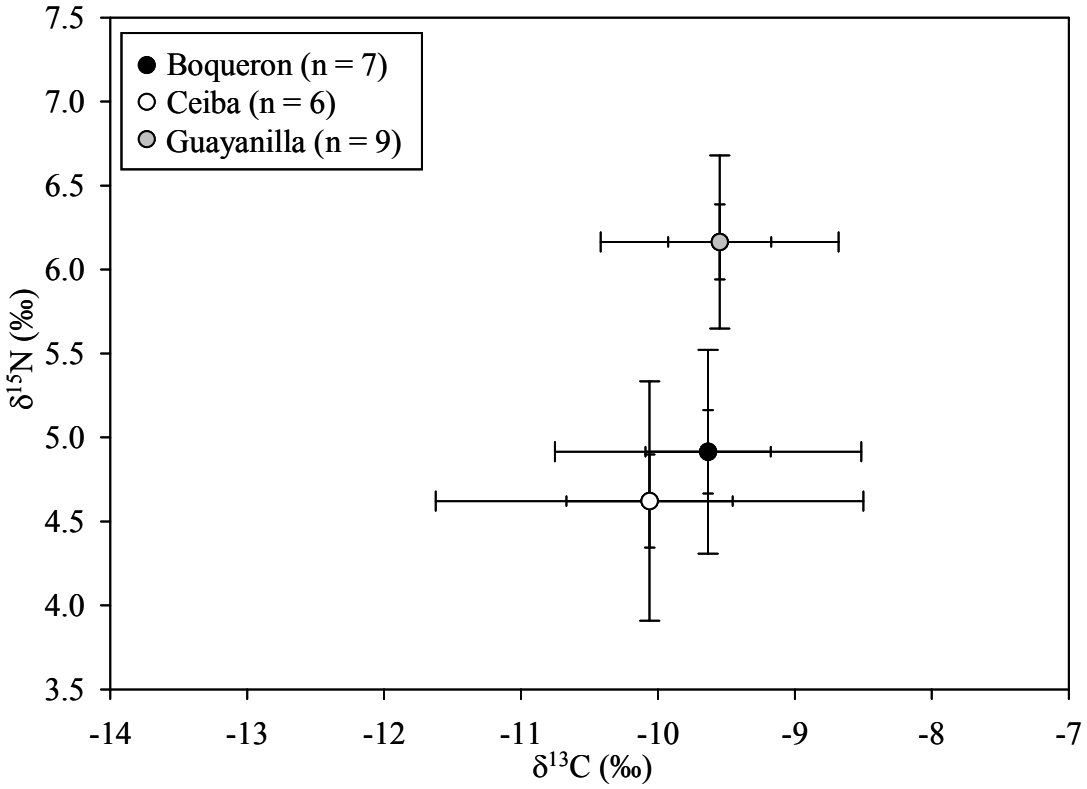

Figure 3.12. Regional differences in $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE and 95% CI) for manatee skin from free-ranging animals in Puerto Rico.

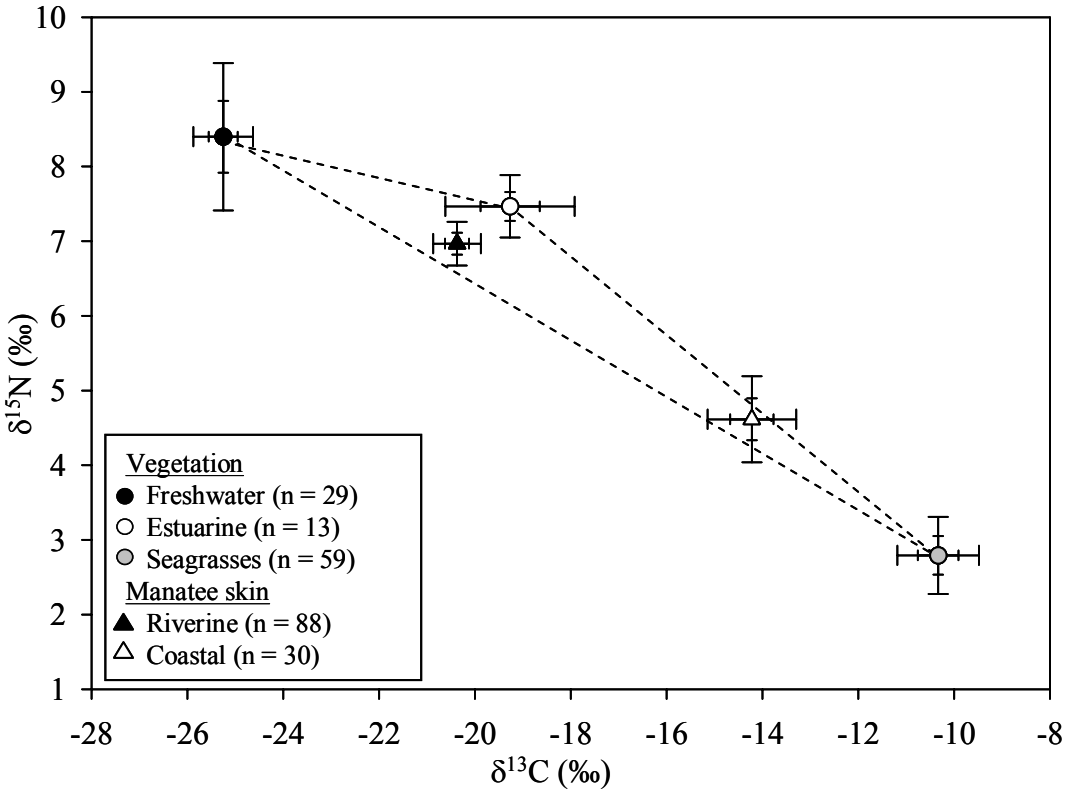

Figure 3.13. Estimation of diet-tissue discrimination in the skin of free-ranging Florida manatees.

The $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE and 95% CI) for aquatic vegetation and manatee skin from Florida were compared. The three possible diets sources (corrected for enrichment) were connected by a polygon. The accepted enrichment values for manatee skin necessary for the means to fall within the polygon were 2.8‰ for carbon and ranged from 1.0 to 1.5‰ for nitrogen (1.2‰ was plotted).

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Table 3.1. Stable isotope ratios of aquatic plants collected in Florida and Belize.

Plant type	n	$\delta^{13}C$ (‰) Mean ± SE	Minimum	Maximum	$\delta^{15}N$ (‰) Mean ± SE	Minimum	Maximum
Florida							
Freshwater vegetation							
St. Johns River	29	-28.1 ± 0.3	-31.6	-25.0	7.3 ± 0.5	1.9	10.3
Estuarine vegetation	13	-22.1 ± 0.6	-25.1	-18.8	6.3 ± 0.2	5.4	7.6
Crystal River	5	-22.3 ± 0.8	-24.2	-20.1	6.0 ± 0.3	5.4	7.1
Indian River Lagoon	8	-21.9 ± 0.9	-25.1	-18.8	6.4 ± 0.3	5.5	7.6
Seagrasses	59	-13.1 ± 0.4	-19.6	-6.6	1.6 ± 0.3	-2.9	5.4
Charlotte Harbor	15	-11.0 ± 0.5	-15.5	-8.3	1.4 ± 0.3	-0.7	4.4
Indian River Lagoon	13	-13.8 ± 1.2	-19.6	-6.6	1.1 ± 0.7	-2.0	5.3
Tampa Bay	16	-14.8 ± 0.8	-19.5	-9.2	2.5 ± 0.4	0.2	5.4
Ten Thousand Islands	15	-12.9 ± 0.5	-16.9	-9.5	1.1 ± 0.6	-2.9	3.8
Belize							
Seagrasses	18	-7.2 ± 0.5	-11.9	-2.9	-0.6 ± 0.5	-5.1	2.6

Table 3.2. Stable isotope ratios in manatee skin from free-ranging animals in Florida, Belize, and Puerto Rico.

		$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Location	n	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum
Florida							
Riverine manatees	88	-20.4 ± 0.3	-27.0	-14.6	7.0 ± 0.1	2.7	10.1
Crystal River	67	-20.0 ± 0.3	-24.9	-14.6	7.1 ± 0.2	3.8	10.1
Homosassa Springs	21	-21.4 ± 0.6	-27.0	-16.3	6.5 ± 0.3	2.7	9.2
Coastal manatees	30	-14.2 ± 0.5	-20.4	-9.3	4.6 ± 0.3	2.0	8.1
Big Bend Power Station	10	-13.5 ± 0.5	-15.4	-10.9	5.8 ± 0.6	2.0	8.1
Port of the Islands	14	-13.8 ± 0.7	-17.6	-9.3	4.0 ± 0.2	3.0	6.2
Warm Mineral Springs	6	-16.3 ± 1.2	-20.4	-13.1	4.0 ± 0.5	2.8	5.6
Belize							
Drowned Cayes	13	-7.5 ± 0.4	-11.0	-5.9	3.4 ± 0.2	2.1	4.7
All lagoons	55	-12.7 ± 0.2	-16.0	-9.2	1.6 ± 0.3	-2.3	5.7
Northern Lagoon	8	-13.4 ± 0.4	-15.5	-12.4	4.1 ± 0.3	2.6	5.3
Southern Lagoon	37	-12.4 ± 0.3	-16.0	-9.2	1.0 ± 0.3	-2.3	5.7
Western Lagoon	10	-13.3 ± 0.4	-15.1	-11.5	1.5 ± 0.7	-2.3	4.1
Puerto Rico							
Boqueron	7	-9.6 ± 0.5	-10.9	-7.9	4.9 ± 0.2	3.7	5.7
Ceiba	6	-10.1 ± 0.6	-12.8	-8.8	4.6 ± 0.3	3.8	5.4
Guayanilla	9	-9.6 ± 0.4	-11.0	-7.5	6.2 ± 0.2	5.2	6.9
Salinas	1	-10.4	-10.4	-10.4	5.7	5.7	5.7

Table 3.3. Seasonal differences in stable isotope ratios of manatee skin from Belize.

		$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Location	n	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum
Drowned Cayes							
fall	3	-9.7 ± 0.7	-11.0	-8.8	2.6 ± 0.2	2.3	2.9
spring	10	-6.8 ± 0.2	-8.4	-5.9	3.6 ± 0.2	2.1	4.7
Southern Lagoon							
fall	11	-13.6 ± 0.4	-15.8	-11.6	0.1 ± 0.3	-1.6	2.0
spring	26	-11.9 ± 0.3	-16.0	-9.2	1.4 ± 0.4	-2.3	5.7
Western Lagoon							
fall	7	-13.4 ± 0.5	-15.1	-11.5	2.8 ± 0.3	2.1	4.1
spring	3	-13.0 ± 0.3	-13.4	-12.4	-1.4 ± 0.8	-2.3	0.3

Table 3.4. IsoError results representing possible proportions of freshwater vegetation, estuarine vegetation, and seagrasses contributing to the manatee diet. Diet source means were corrected for enrichment by 2.8‰ for carbon. Model criteria allowed enrichment factors for nitrogen ranging from 1.0 to 1.5‰, so three possible solutions are shown below for riverine and coastal manatees.

	δ^{15}N enrichment = 1.0‰			**δ^{15}N enrichment = 1.2‰**			**δ^{15}N enrichment = 1.5‰**		
Diet source	Mean ± SE (%)	Lower 95% CI (%)	Upper 95% CI (%)	Mean ± SE (%)	Lower 95% CI (%)	Upper 95% CI (%)	Mean ± SE (%)	Lower 95% CI (%)	Upper 95% CI (%)
Riverine manatees									
Freshwater vegetation	41 ± 12	16	66	50 ± 13	24	76	64 ± 15	34	95
Estuarine vegetation	44 ± 19	5	82	28 ± 21	0	70	5 ± 25	0	55
Seagrasses	15 ± 8	0	30	21 ± 8	4	38	31 ± 10	10	51
Coastal manatees									
Freshwater vegetation	2 ± 17	0	36	11 ± 17	0	45	25 ± 18	0	61
Estuarine vegetation	40 ± 26	0	92	25 ± 26	0	77	1 ± 28	0	57
Seagrasses	58 ± 10	39	77	64 ± 10	44	84	73 ± 11	52	95

CHAPTER 4: CONCLUSIONS

The overall objective of this study was to assess feeding ecology of Florida and Antillean manatees through the use of carbon and nitrogen stable isotope analysis of manatee skin. This was accomplished by first calculating isotope turnover rates and diet-tissue discrimination values in manatee skin, parameters that must be known in order to accurately interpret isotopic data. Turnover rates and discrimination values, paired with isotopic analysis of aquatic vegetation, were then used to assess $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the skin of free-ranging manatees in Florida, Belize, and Puerto Rico.

The present study was the first to calculate stable isotope turnover rates in the skin of a marine mammal. Turnover rates were determined by collecting epidermis tissue over a period of more than one year from manatees transitioning between two isotopically distinct diets (terrestrial and aquatic vegetation). These manatees were in need of rehabilitation, were brought into captivity at SeaWorld Orlando, and were immediately transitioned to a diet of primarily lettuce. Mean stable carbon and nitrogen isotope half-lives in the skin (55 and 42 days, respectively) were slower than most other half-lives calculated in metabolically active tissues in mammals. Slow turnover rate was most likely a consequence of the manatee's slow metabolic rate. Manatees rescued from coastal regions were the best subjects for carbon turnover and those rescued from riverine regions were the best subjects for nitrogen turnover due to distinct isotope signatures compared to those of terrestrial vegetation. Because of slow turnover rates in manatee epidermis, analysis of carbon and nitrogen stable isotopes in this tissue is more useful in

summarizing average dietary intake over a long period of time rather than assessing short-term or recent changes in diet, and thus habitat use.

Diet-tissue discrimination values for ^{13}C were also calculated in the skin of manatees on a lettuce diet for more than one year. Manatee skin was enriched in ^{13}C relative to diet by an average of 2.8‰. Nitrogen stable isotope enrichment values were unable to be calculated during this portion of the study due to variability in the $\delta^{15}N$ values for lettuce fed in captivity.

These established values for turnover and diet-tissue discrimination, along with an analysis of aquatic plant isotopic data, were used to interpret $\delta^{13}C$ and $\delta^{15}N$ values in epidermis collected from free-ranging manatees in Florida, Belize, and Puerto Rico. This was the first application of stable isotope analysis to Antillean manatees.

Significant differences in $\delta^{13}C$ and $\delta^{15}N$ values between freshwater plants, estuarine plants, and seagrasses allowed for an assessment of manatee feeding in differing habitats. Specifically, freshwater plants were the most depleted in ^{13}C and enriched in ^{15}N whereas seagrasses were most the most enriched in ^{13}C and depleted in ^{15}N. Stable isotope analysis of aquatic vegetation was not powerful enough to distinguish between plant species. Regional differences in isotope ratios in manatee skin were consistent with expected dietary intake from that region. Variability in $\delta^{13}C$ and $\delta^{15}N$ values in the skin of individual manatees within a region suggested that individuals were previously feeding in different habitats.

No differences in stable isotope ratios of manatee skin were found with respect to sex or age class. Based upon results from manatees from Belize, there may be seasonal differences in the isotope ratios in manatee skin representing seasonal changes in diet

composition, or seasonal changes in stable isotope ratios of aquatic vegetation. This would also be expected in the skin of Florida manatees since they seasonally aggregate at warm water sites. However in this study, Florida samples were collected primarily during winter months so it was not possible to assess seasonality.

A mixing model (IsoError) and the previously calculated diet-tissue discrimination value for ^{13}C were used to estimate nitrogen discrimination in manatee skin. Enrichment values for ^{15}N ranged from 1.0 to 1.5‰. IsoError was then used to estimate proportions of freshwater plants, estuarine plants, and seagrasses contributing to the diet of Florida manatees. In general, manatees sampled in Crystal River and Homosassa Springs likely fed on a diet of predominantly freshwater vegetation whereas manatees sampled at the Big Bend Power Plant (Tampa Bay), Port of the Islands (Ten Thousand Islands), and Warm Mineral Springs (near Charlotte Harbor) likely fed on a diet of predominantly seagrasses. It was also likely that all Florida manatees sampled consumed seagrasses sometime in the previous months. Mixing model results should be considered only an approximation since diet source proportions varied with enrichment values and slow stable isotope turnover rates in manatee tissue complicates analyses.

This study contributes to the further refinement of stable isotope analysis as a technique used to investigate feeding ecology. The appropriate use of the analysis is especially important when it is applied to endangered or threatened species for which conservation and management decisions are crucial.

Future studies

Establishing a more precise nitrogen stable isotope turnover rate in manatee epidermis would further aid in stable isotope data interpretation and would be better achieved in future studies by assuring a consistent nitrogen signature in the new diet. For diet transitions between aquatic and terrestrial vegetation, manatees rescued from riverine regions would be more ideal subjects for nitrogen turnover studies than those from coastal regions since there was a greater difference in $\delta^{15}N$ values between the skin of riverine manatees and the lettuce diet. For isotope analysis of short-term changes in manatee diet, more metabolically active tissues could be used such as blood, or possibly the most recent hair or vibrissae growth. Taking deeper tissues from live manatees such as muscle is likely not feasible due to the invasive procedure and risk of infection.

Diet-tissue discrimination values for ^{15}N in manatee skin are inconsistent between studies, so caution should be taken in estimating diet proportions through the use of mixing models, as model results are highly dependent upon an accurately calculated diet-tissue discrimination value. These values have been estimated for manatee skin based on free-ranging data; however a controlled study on a known diet with little variability in nitrogen isotope signature is needed. Additionally, it would be useful to examine whether diet-tissue discrimination in manatee tissue differs based upon diet source.

As the first application of stable isotope analysis to Antillean manatees, regional differences in isotopic ratios in manatee skin were presented. A thorough investigation of isotope signatures in aquatic plants in Belize and Puerto Rico (including an analysis of freshwater, estuarine, and seagrass vegetation in different regions and during different seasons) would allow for a more detailed interpretation of results.

APPENDIX A: CAPTIVE MANATEE DIET ITEMS COLLECTED FROM SEAWORLD ORLANDO

Collection date	Item
13-Feb-03	Romaine lettuce
19-Feb-03	Romaine lettuce
21-Feb-03	Romaine lettuce
01-Apr-03	Romaine lettuce
12-Sep-03	Romaine lettuce
11-Oct-03	Romaine lettuce
24-Oct-03	Romaine lettuce
14-Nov-03	Romaine lettuce
02-Jan-04	Romaine lettuce
06-Jan-04	Romaine lettuce
26-Feb-04	Romaine lettuce
15-Apr-04	Romaine lettuce
17-Jun-04	Romaine lettuce
24-Jun-04	Romaine lettuce
unknown	Romaine lettuce
unknown	Romaine lettuce
19-Feb-03	Spinach
01-Apr-03	Spinach
06-May-03	Spinach
24-Feb-03	Gruel
12-Sep-03	Gruel
24-Jun-04	Gruel
unknown	Gruel
21-Feb-03	Monkey chow
14-Nov-03	Monkey chow
06-Jan-04	Monkey chow
18-May-04	Monkey chow
24-Oct-03	Fecal material
08-Apr-04	Fecal material
15-Apr-04	Fecal material
24-Jun-04	Fecal material

APPENDIX B: RESCUED FLORIDA MANATEES SAMPLED DURING REHABILITATION AND POST RELEASE

Animal ID	Sex	Length (cm)	Mass (kg)	Location of rescue	Reason for rescue	Sample collection dates	Days since diet switch	Sample type
SWF Tm 0301	M	174	105	Cape Canaveral	Cold stress	24-Feb-03	3	B
						09-Mar-03	18	B
						08-Apr-04	414	B
SWF Tm 0318	M	255	273	Cape Canaveral	Watercraft injury	14-Jul-03	1	B
						24-Jul-03	11	B
						29-Aug-03	47	B
						12-Sep-03	61	B, S
						23-Sep-03	72	S
						11-Oct-03	90	S
						24-Oct-03	103	S
						26-Feb-04	228	B
SWF Tm 0322	F	248	305	Cape Canaveral	Watercraft injury	29-Aug-03	17	B
						12-Sep-03	31	B
						23-Sep-03	42	S
						11-Oct-03	60	S
						24-Oct-03	73	S
SWF Tm 0334	F	298	593	Jacksonville	Entanglement	24-Nov-03	13	B
						02-Jan-04	52	S
						08-Apr-04	149	B
SWF Tm 0340	M	222	232	Jacksonville	Cold stress	18-Dec-03	0	B
						02-Jan-04	15	B, S
						16-Jan-04	29	B, S
						30-Jan-04	43	B, S
						11-Feb-04	55	B
						27-Feb-04	71	B
						08-Apr-04	112	B
						08-Feb-05	418	B
						12-May-05	77 *	S
SWF Tm 0341	F	208	209	Jacksonville	Cold stress	18-Dec-03	0	B
						02-Jan-04	15	B, S
						16-Jan-04	29	B, S
						30-Jan-04	43	B
						11-Feb-04	55	B
						27-Feb-04	71	B
						08-Apr-04	112	B
						08-Feb-05	418	B, S
						12-May-05	77 *	S

Animal ID	Sex	Length (cm)	Mass (kg)	Location of rescue	Reason for rescue	Sample collection dates	Days since diet switch	Sample type
SWF Tm 0431	F	90	125	Naples	Unknown	30-Dec-04	1	B
						14-Jan-05	16	B
						28-Jan-05	30	B
						11-Feb-05	44	B
						28-Feb-05	61	B
						11-Mar-05	72	B
						29-Mar-05	90	B
SWF Tm 0501	F	216	177	Jacksonville	Cold stress	14-Jan-05	1	B
						28-Jan-05	15	B
						11-Feb-05	29	B
						28-Feb-05	46	B
						04-Mar-05	50	B
						11-Mar-05	57	B
						29-Mar-05	75	B

(SWF Tm) = SeaWorld Florida *Trichechus manatus*
(B) = Biopsy, (S) = Sloughed skin
* Sample collected post release

APPENDIX C: LONG-TERM CAPTIVE FLORIDA MANATEES SAMPLED

Animal ID/name	Sex	Mass (kg)	Length (cm)	Date	Days on lettuce diet
Bo	M	unknown	>176	15-Jul-04	512
Charlotte	F	1136	330	17-Jun-04	>365
Primo	F	494	277	27-Jul-04	>365
Rita	F	>900	>275	17-Jun-04	>365
Sarah	F	1136	325	17-Jun-04	>365
Stubby	F	823	252*	27-Jul-04	>365
SWF Tm 0110	F	367	255	27-Jul-04	1231
SWF Tm 0302	F	unknown	>176	15-Jul-04	512
SWF Tm 0338	F	unknown	>176	08-Feb-05	429

(SWF Tm) = SeaWorld Florida *Trichechus manatus*
* Missing large portion of paddle

APPENDIX D: AQUATIC PLANT COLLECTIONS IN FLORIDA AND BELIZE

Date	Location	Site	Latitude	Longitude	Temperature (C)	Salinity (‰)	Depth (m)	Species
Florida								
6-Apr-04	St. Johns River	1	28.94507	-81.34730	U	<5	2.0	*A.p., E.c., H., L.v., M.a., N.l., P.s., P.c.,* U.g.
5-Jul-04	St. Johns River	1	28.94507	-81.34730	U	<5	1.5	*A.p., E.c., H., L.v., N.l., P.s.,* U.g.
9-Dec-04	St. Johns River	1	28.94507	-81.34730	<24.0	<5	2.2	*A.p., E.c., H., L.v., M.a., N.l., P.s.,* U.g.
12-Jan-05	Ten Thousand Islands	1	25.86982	-81.66802	21.7	40	<0.5	*H.e., H.w., T.t.*
12-Jan-05	Ten Thousand Islands	2	25.85423	-81.67165	21.7	41	<0.5	*H.w., T.t.*
12-Jan-05	Ten Thousand Islands	3	25.86032	-81.55838	21.1	40	1.0	*H.e., H.w., S.f., T.t.*
12-Jan-05	Ten Thousand Islands	4	25.84282	-81.52627	21.1	40	1.1	*H.e., H.w., T.t.*
18-Feb-05	Crystal River	1	28.87935	-82.60023	<24.0	8	<0.5	*H.v., C., M.s.*
18-Feb-05	Crystal River	2	28.88392	-82.59533	<24.0	6	<0.5	*H.v., M.s.*
23-May-05	Charlotte Harbor	1	26.83600	-82.06693	30.0	26	0.9	*H.w., T.t.*
23-May-05	Charlotte Harbor	2	26.70182	-82.12158	28.3	30	0.8	*H.w., S.f., T.t.*
23-May-05	Charlotte Harbor	3	26.71743	-82.15440	28.3	35	1.1	*H.w., S.f., T.t.*
23-May-05	Charlotte Harbor	4	26.71282	-82.18127	28.3	35	0.7	*H.w., S.f., T.t.*
27-Jul-05	Tampa Bay	1	27.67750	-82.51780	32.2	26	<0.5	*H.w., S.f., T.t.*
27-Jul-05	Tampa Bay	2	27.69050	-82.52932	31.7	28	0.3	*H.w., S.f., T.t.*
27-Jul-05	Tampa Bay	3	27.71640	-82.50247	31.7	22	0.6	*H.w., S.f., T.t.*
27-Jul-05	Tampa Bay	4	27.61210	-82.57463	32.8	28	0.8	*H.w., S.f., T.t.*
20-Jun-01	Indian River Lagoon	1	27.33944	-80.23717	32.0	35	<0.5	*H.w.*
9-Jul-01	Indian River Lagoon	1	27.33944	-80.23717	30.0	34	<0.5	*H.w.*
25-Jul-01	Indian River Lagoon	1	27.33944	-80.23717	29.0	30	0.5	*H.w.*
7-Aug-01	Indian River Lagoon	1	27.33944	-80.23717	29.5	24	0.5	*H.w.*
25-Jun-01	Indian River Lagoon	2	27.33158	-80.23681	28.5	33	1.4	*G.*
7-Aug-01	Indian River Lagoon	2	27.33158	-80.23681	29.5	26	1.3	*H.w., G.*
10-Jul-01	Indian River Lagoon	2	27.33158	-80.23681	29.0	35	1.2	*G.*
25-Jul-01	Indian River Lagoon	2	27.33158	-80.23681	28.0	31	1.3	*G.*
29-Jun-01	Indian River Lagoon	3	27.53603	-80.34847	29.5	35	0.7	*H.w., S.f., T.t.*
17-Jul-01	Indian River Lagoon	3	27.53603	-80.34847	31.0	25	0.9	*S.f.*
30-Jun-01	Indian River Lagoon	4	27.56422	-80.33078	33.0	36	0.6	*G.*
18-Jul-01	Indian River Lagoon	4	27.56422	-80.33078	30.5	25	0.9	*G.*
30-Jul-01	Indian River Lagoon	4	27.56422	-80.33078	31.0	25	0.9	*G.*
18-Jul-01	Indian River Lagoon	5	27.56225	-80.33239	30.5	25	0.9	*G., S.f.*

Date	Location	Site	Latitude	Longitude	Temperature (C)	Salinity (‰)	Depth (m)	Species
Florida								
30-Jul-01	Indian River Lagoon	5	27.56225	-80.33239	31.0	25	0.9	*G., H.w., S.f.*
30-Jun-01	Indian River Lagoon	5	27.56225	-80.33239	33.0	36	0.6	*H.w., S.f.*
13-Aug-01	Indian River Lagoon	6	27.49972	-80.30783	34.0	25	0.5	*H.w.*
Belize								
1-Sep-02	Drowned Cayes	1	17.45349	-88.06873	>24.0	37	U	*T.t.*
31-Jul-02	Drowned Cayes	2	17.46028	-88.07813	>24.0	35	U	*H.w.*
30-Aug-02	Drowned Cayes	3	17.40824	-88.07497	>24.0	35	U	*T.t.*
7-Jul-02	Drowned Cayes	4	17.48239	-88.07482	>24.0	37	U	*T.t.*
2-Jul-02	Drowned Cayes	5	17.50538	-88.10929	>24.0	39	U	*T.t.*
15-Aug-02	Drowned Cayes	5	17.50494	-88.10907	>24.0	37	U	*T.t.*
26-Jul-02	Drowned Cayes	6	17.45407	-88.08223	>24.0	37	U	*T.t.*
8-Jul-02	Drowned Cayes	7	17.49657	-88.10286	>24.0	37	U	*T.t.*
28-Jul-02	Drowned Cayes	7	17.49646	-88.10277	>24.0	38	U	*T.t.*
29-Aug-02	Drowned Cayes	7	17.49631	-88.10280	>24.0	37	U	*H.w.*
3-Jul-02	Drowned Cayes	8	17.52261	-88.11116	>24.0	37	U	*T.t.*
2-Sep-02	Drowned Cayes	9	17.45920	-88.07199	>24.0	U	U	*T.t.*
14-Aug-02	Drowned Cayes	10	17.49054	-88.09344	>24.0	36	U	*T.t.*

(U) = Unknown

Species abbreviations

A.p.	*Alternanthera philoxeroides*	*M.a.*	*Myriophyllum aquaticum*
C.	*Chara* sp.	*M.s.*	*Myriophyllum spicatum*
E.c.	*Eichhornia crassipes*	*N.l.*	*Nuphar luteum*
G.	*Gracilaria* sp.	*P.c.*	*Pontederia cordata*
H.	*Hydrocotyle* sp.	*P.s.*	*Pistia stratiotes*
H.e.	*Halophila engelmannii*	*S.f.*	*Syringodium filiforme*
H.v.	*Hydrilla verticillata*	*T.t.*	*Thalassia testudinum*
H.w.	*Halodule wrightii*	U.g.	Unknown grass
L.v.	*Lemna valdiviana*		

APPENDIX E: FREE-RANGING MANATEES SAMPLED IN FLORIDA, BELIZE, AND PUERTO RICO

Animal ID	Sex	Length (cm)	Age class	Date	Location
Florida					
02-232-1	M	180	subadult	20-Aug-02	Homosassa Springs
02-303-1	F	200	subadult	30-Oct-02	Crystal River
02-303-2	M	170	calf	30-Oct-02	Crystal River
02-318-1	F	170	calf	14-Nov-02	Crystal River
02-323-1	M	200	subadult	19-Nov-02	Crystal River
02-323-2	F	200	subadult	19-Nov-02	Crystal River
02-323-3	M	230	subadult	19-Nov-02	Homosassa Springs
02-324-1	M	170	calf	20-Nov-02	Crystal River
02-324-2	M	220	subadult	20-Nov-02	Crystal River
02-324-3	F	180	subadult	20-Nov-02	Homosassa Springs
02-324-4	F	200	subadult	20-Nov-02	Homosassa Springs
02-329-1	M	180	subadult	25-Nov-02	Crystal River
02-330-1	M	220	subadult	26-Nov-02	Crystal River
02-330-2	M	170	calf	26-Nov-02	Crystal River
02-330-3	M	220	subadult	26-Nov-02	Homosassa Springs
02-336-1	M	210	subadult	02-Dec-02	Crystal River
02-336-2	M	170	calf	02-Dec-02	Crystal River
02-336-3	M	180	subadult	02-Dec-02	Crystal River
02-336-4	M	190	subadult	02-Dec-02	Crystal River
02-336-5	F	180	subadult	02-Dec-02	Crystal River
02-336-6	F	220	subadult	02-Dec-02	Homosassa Springs
02-337-1	U	200	subadult	03-Dec-02	Crystal River
02-337-2	M	190	subadult	03-Dec-02	Crystal River
02-337-3	F	230	subadult	03-Dec-02	Crystal River
02-337-4	F	210	subadult	03-Dec-02	Crystal River
02-337-5	M	180	subadult	03-Dec-02	Crystal River
02-337-6	F	170	calf	03-Dec-02	Crystal River
02-337-7	F	235	subadult	03-Dec-02	Homosassa Springs
02-340-1	M	284	adult	06-Dec-02	Port of the Islands
02-340-2	M	256	subadult	06-Dec-02	Port of the Islands
02-340-3	F	266	subadult	06-Dec-02	Port of the Islands
02-341-1	M	272	subadult	07-Dec-02	Port of the Islands
02-341-2	F	323	adult	07-Dec-02	Port of the Islands
02-346-1	U	230	subadult	12-Dec-02	Crystal River
02-346-2	M	225	subadult	12-Dec-02	Crystal River
02-347-1	F	290	adult	13-Dec-02	Big Bend Power Station
02-347-2	F	259	subadult	13-Dec-02	Big Bend Power Station
02-347-3	M	290	adult	13-Dec-02	Big Bend Power Station
02-350-1	M	160	calf	16-Dec-02	Crystal River
02-350-2	U	180	subadult	16-Dec-02	Crystal River
02-350-3	M	190	subadult	16-Dec-02	Crystal River
02-350-4	F	190	subadult	16-Dec-02	Crystal River
02-350-5	F	170	calf	16-Dec-02	Crystal River
02-350-6	F	170	calf	16-Dec-02	Crystal River
02-350-7	M	170	calf	16-Dec-02	Homosassa Springs
02-350-8	M	170	calf	16-Dec-02	Homosassa Springs
02-352-1	F	180	subadult	18-Dec-02	Crystal River

Animal ID	Sex	Length (cm)	Age class	Date	Location
Florida					
02-352-2	M	180	subadult	18-Dec-02	Crystal River
02-352-3	F	200	subadult	18-Dec-02	Crystal River
02-364-1	M	170	calf	30-Dec-02	Crystal River
02-364-2	M	200	subadult	30-Dec-02	Crystal River
02-364-3	F	200	subadult	30-Dec-02	Homosassa Springs
03-008-1	M	180	subadult	08-Jan-03	Crystal River
03-008-2	M	230	subadult	08-Jan-03	Crystal River
03-008-3	M	190	subadult	08-Jan-03	Crystal River
03-010-1	M	180	subadult	10-Jan-03	Crystal River
03-010-2	M	190	subadult	10-Jan-03	Crystal River
03-010-3	F	200	subadult	10-Jan-03	Homosassa Springs
03-013-1	F	241	subadult	13-Jan-03	Warm Mineral Springs
03-013-2	F	259	subadult	13-Jan-03	Warm Mineral Springs
03-014-1	M	224	subadult	14-Jan-03	Warm Mineral Springs
03-015-1	M	236	subadult	15-Jan-03	Warm Mineral Springs
03-017-1	F	210	subadult	17-Jan-03	Crystal River
03-017-2	F	170	calf	17-Jan-03	Crystal River
03-017-3	F	210	subadult	17-Jan-03	Crystal River
03-017-4	F	180	subadult	17-Jan-03	Crystal River
03-022-1	F	180	subadult	22-Jan-03	Crystal River
03-024-1	F	180	subadult	24-Jan-03	Crystal River
03-024-2	F	210	subadult	24-Jan-03	Crystal River
03-028-1	M	190	subadult	28-Jan-03	Crystal River
03-028-2	F	180	subadult	28-Jan-03	Crystal River
03-028-3	F	277	adult	28-Jan-03	Warm Mineral Springs
03-028-4	F	277	adult	28-Jan-03	Warm Mineral Springs
03-029-1	U	190	subadult	29-Jan-03	Crystal River
03-036-1	M	200	subadult	05-Feb-03	Homosassa Springs
03-038-1	F	210	subadult	07-Feb-03	Crystal River
03-315-1	M	190	subadult	11-Nov-03	Crystal River
03-315-2	M	170	calf	11-Nov-03	Crystal River
03-317-1	M	130	calf	13-Nov-03	Crystal River
03-317-1B	M	130	calf	13-Nov-03	Crystal River
03-345-1	F	200	subadult	11-Dec-03	Crystal River
03-345-2	F	220	subadult	11-Dec-03	Crystal River
03-345-3	M	210	subadult	11-Dec-03	Crystal River
04-010-1	M	180	subadult	10-Jan-04	Crystal River
04-010-2	M	180	subadult	10-Jan-04	Crystal River
04-022-1	F	150	calf	22-Jan-04	Crystal River
04-068-1	M	220	subadult	08-Mar-04	Crystal River
04-357-1	M	170	calf	22-Dec-04	Homosassa Springs
05-019-1	F	220	subadult	19-Jan-05	Homosassa Springs
05-019-2	F	190	subadult	19-Jan-05	Homosassa Springs
05-019-3	M	160	calf	19-Jan-05	Homosassa Springs
05-022-1	F	180	subadult	22-Jan-05	Crystal River
05-022-2	M	190	subadult	22-Jan-05	Crystal River
05-022-3	M	230	subadult	22-Jan-05	Crystal River

Animal ID	Sex	Length (cm)	Age class	Date	Location
Florida					
05-022-4	F	190	subadult	22-Jan-05	Crystal River
05-022-5	M	210	subadult	22-Jan-05	Crystal River
05-022-6	M	220	subadult	22-Jan-05	Homosassa Springs
05-022-7	M	230	subadult	22-Jan-05	Homosassa Springs
05-022-8	F	170	calf	22-Jan-05	Homosassa Springs
05-025-1	F	180	subadult	25-Jan-05	Homosassa Springs
05-028-1	M	230	subadult	28-Jan-05	Homosassa Springs
05-043-1	F	190	subadult	12-Feb-05	Crystal River
CNP-04-01	M	245	subadult	17-Jan-04	Port of the Islands
CTB-042	M	177	subadult	14-Dec-04	Big Bend Power Station
CTB-043	F	174	calf	14-Dec-04	Big Bend Power Station
CTB-044	M	147	calf	14-Dec-04	Big Bend Power Station
CTB-045	F	173	calf	14-Dec-04	Big Bend Power Station
CTB-046	F	212	subadult	14-Dec-04	Big Bend Power Station
TNP-25	M	242	subadult	15-Jan-04	Port of the Islands
TNP-26	M	312	adult	15-Jan-04	Port of the Islands
TNP-27	M	267	subadult	16-Jan-04	Port of the Islands
TNP-28	M	234	subadult	16-Jan-04	Port of the Islands
TNP-29	F	289	adult	18-Apr-04	Port of the Islands
TNP-30	M	274	subadult	18-Apr-04	Port of the Islands
TNP-31	F	308	adult	19-Apr-04	Port of the Islands
TNP-32	M	306	adult	19-Apr-04	Port of the Islands
TTB-109	F	282	adult	30-Dec-03	Big Bend Power Station
TTB-110	F	295	adult	30-Dec-03	Big Bend Power Station
Belize					
BZ01F18	F	276	adult	08-Mar-02	Western Lagoon
BZ02F20	F	254	adult	07-Mar-02	Southern Lagoon
BZ02F21	F	188	subadult	07-Mar-02	Southern Lagoon
BZ02F22	F	244	adult	10-Mar-02	Southern Lagoon
BZ02F26	F	279	adult	07-Nov-02	Southern Lagoon
BZ02F27	F	242	adult	08-Nov-02	Western Lagoon
BZ02M23	M	248	adult	13-Mar-02	Northern Lagoon
BZ02M24	M	240	adult	13-Mar-02	Western Lagoon
BZ02M25	M	289	adult	07-Nov-02	Western Lagoon
BZ03F28	F	190	subadult	09-Apr-03	Southern Lagoon
BZ03F29	F	277	adult	07-May-04	Southern Lagoon
BZ03F31	F	302	adult	10-Apr-03	Southern Lagoon
BZ03F35	F	300	adult	12-Apr-03	Southern Lagoon
BZ03F39	F	234	adult	13-Apr-03	Northern Lagoon
BZ03F41	F	296	adult	14-Apr-03	Western Lagoon
BZ03F42	F	318	adult	15-Apr-03	Northern Lagoon
BZ03F44	F	>275	adult	15-Apr-03	Northern Lagoon
BZ03F47	M	205	subadult	20-Nov-03	Western Lagoon
BZ03F48	F	159	calf	20-Nov-03	Western Lagoon
BZ03F50	F	224	subadult	21-Nov-03	Western Lagoon
BZ03M30	M	293	adult	09-Apr-03	Southern Lagoon

Animal ID	Sex	Length (cm)	Age class	Date	Location
Belize					
BZ03M32	M	245	adult	11-Apr-03	Southern Lagoon
BZ03M33	M	296	adult	11-Apr-03	Northern Lagoon
BZ03M34	M	248	adult	12-Apr-03	Southern Lagoon
BZ03M36	M	249	adult	12-Apr-03	Southern Lagoon
BZ03M37	M	324	adult	13-Apr-03	Northern Lagoon
BZ03M38	M	257	adult	13-Apr-03	Northern Lagoon
BZ03M40	M	211	subadult	14-Apr-03	Southern Lagoon
BZ03M43	M	272	adult	15-Apr-03	Northern Lagoon
BZ03M45	M	250	adult	16-Apr-03	Southern Lagoon
BZ03M46	M	230	adult	16-Apr-03	Southern Lagoon
BZ03M49	M	269	adult	21-Nov-03	Western Lagoon
BZ04F52	F	205	subadult	05-May-04	Southern Lagoon
BZ04F53	F	285	adult	05-May-04	Southern Lagoon
BZ04F55	F	292	adult	06-May-04	Southern Lagoon
BZ04F56	F	170	calf	06-May-04	Southern Lagoon
BZ04F57	F	154	calf	07-May-04	Southern Lagoon
BZ04F60	F	273	adult	11-May-04	Drowned Cayes
BZ04F61	F	259	adult	12-May-04	Drowned Cayes
BZ04F62	F	298	adult	12-May-04	Drowned Cayes
BZ04F65	F	204	subadult	17-Nov-04	Drowned Cayes
BZ04F67	F	219	subadult	17-Nov-04	Drowned Cayes
BZ04F68	F	252	adult	19-Nov-04	Southern Lagoon
BZ04F69	F	285	adult	20-Nov-04	Southern Lagoon
BZ04F72	F	263	adult	21-Nov-04	Southern Lagoon
BZ04F73	F	283	adult	22-Nov-04	Southern Lagoon
BZ04F75	F	260	adult	22-Nov-04	Southern Lagoon
BZ04F76	F	172	calf	22-Nov-04	Southern Lagoon
BZ04M51	M	215	subadult	04-May-04	Southern Lagoon
BZ04M54	M	270	adult	06-May-04	Southern Lagoon
BZ04M58	M	293	adult	10-May-04	Drowned Cayes
BZ04M59	M	241	adult	11-May-04	Drowned Cayes
BZ04M63	M	238	adult	13-May-04	Drowned Cayes
BZ04M64	M	284	adult	13-May-04	Drowned Cayes
BZ04M66	M	277	adult	17-Nov-04	Drowned Cayes
BZ04M70	M	192	subadult	20-Nov-04	Southern Lagoon
BZ04M71	M	250	adult	21-Nov-04	Southern Lagoon
BZ04M74	M	286	adult	22-Nov-04	Southern Lagoon
BZ04M77	M	256	adult	22-Nov-04	Southern Lagoon
BZ05F80	F	234	adult	17-Apr-05	Southern Lagoon
BZ05F81	F	212	subadult	17-Apr-05	Southern Lagoon
BZ05F82	F	273	adult	22-Apr-05	Drowned Cayes
BZ05M79	M	225	subadult	17-Apr-05	Southern Lagoon
BZ05M83	M	286	adult	23-Apr-05	Drowned Cayes
BZ05M84	M	253	adult	23-Apr-05	Drowned Cayes
BZ97M01	M	306	adult	07-Nov-02	Western Lagoon
BZ98M06	M	285	adult	07-May-04	Southern Lagoon
BZ99M10	M	255	adult	09-Mar-02	Southern Lagoon

Animal ID	Sex	Length (cm)	Age class	Date	Location
Puerto Rico					
CPR-03-01	M	212	subadult	20-Jul-02	Guayanilla
CPR-03-02	F	251	adult	21-Jul-03	Guayanilla
CPR-03-03	F	236	adult	07-Nov-03	Boqueron
CPR-04-01	F	193	subadult	10-Jun-04	Boqueron
NEPST-872	U	U	adult	15-Nov-03	Salinas
TPR-07	M	265	adult	01-May-05	Ceiba
TPR-11	F	296	adult	03-Nov-03	Boqueron
TPR-13	F	299	adult	17-Jul-03	Boqueron
TPR-14	M	250	adult	18-Jul-03	Boqueron
TPR-15	F	296	adult	20-Jul-03	Guayanilla
TPR-16	M	287	adult	04-Nov-03	Boqueron
TPR-17	M	310	adult	05-Nov-03	Guayanilla
TPR-18	F	267	adult	05-Nov-03	Guayanilla
TPR-19	M	249	adult	06-Nov-03	Guayanilla
TPR-20	F	288	adult	07-Jun-04	Guayanilla
TPR-21	F	297	adult	07-Jun-04	Guayanilla
TPR-22	M	256	adult	08-Jun-04	Guayanilla
TPR-23	F	261	adult	10-Jun-04	Boqueron
TPR-28	M	273	adult	29-Apr-05	Ceiba
TPR-29	F	264	adult	29-Apr-05	Ceiba
TPR-31	F	250	adult	30-Apr-05	Ceiba
TPR-32	F	250	adult	01-May-05	Ceiba
TPR-33	M	252	adult	02-May-05	Ceiba

(U) = Unknown

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