SPATIO-TEMPORAL EVIDENCE OF ANTHROPOGENIC NITROGEN IN FLORIDA WATERS USING STABLE ISOTOPE ANALYSIS OF MANATEE BONES

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I would like to dedicate this thesis to my mother, Marilou Estes, for giving me the best opportunity to succeed and go after my dreams. Your sacrifice through the years and unwavering support in my academic career mean a lot. Even though you may not know my topic completely well, the fact that you are constantly there for me regardless is something I still cannot quite comprehend. Many hours of planning, researching, analyzing, and editing went into making this thesis possible. I can only hope that this slight but feeble attempt at repaying your generosity is a testament for the gratitude and appreciation that I feel for you. Thanks and I love you.

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ABSTRACT

Nutrient pollution from human activities leads to coastal eutrophication and degradation of critical habitat for threatened species, including the Florida manatees (*Trichechus manatus latirostris*). Recovery of these damaged habitats relies on water quality assessments over a long period of time. With the absence of long-term diet and water quality data, manatee bones will serve as proxies for environmental reconstruction. Isotopic composition of skeletal nitrogen (N) reflects plants that manatees consume and the predominant N source driving primary productivity within the ecosystem. Sewage, as a consequence of rapid coastal development, is isotopically distinct from natural sources of N. Thus, $\delta^{15}N$ values are predicted to increase over time and would be highest in densely-populated areas. Collagen in manatee bones were analyzed from 173 necropsied individuals since 1975. Mean $\delta^{15}N$ values have decreased from 8.8‰ in the 1970s to 6.3‰ in 2010 mainly because fertilizer is the source of depleted N. Coastal and regional mean $\delta^{15}N$ values were very high, suggesting a mixing of enriched N sources that include sewage and atmospheric deposition.

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CHAPTER 1

INTRODUCTION

Overview

The goal of this study is to identify sources of nitrogen (N) pollution in Florida waters. This is important because a decline in water quality often limits photosynthetic capabilities of plants below the water due to a reduction in water clarity or overabundance of algal mats on the surface precluding light from filtering through. This issue is affecting the critical habitats for manatees because seagrass habitats have also experienced a decline in coverage due to nutrient over-enrichment caused by increased synthetic fertilizer use and sewage inputs into the environment. While reductions of N into aquatic systems have taken place, recovery is still slow because of the severe damage caused to these environments. In order to properly address this issue and take corrective actions going forward, the difficult task of distinguishing one dominant source of N pollution from other potential sources must first be accomplished.

Determining water quality typically involves measuring, among other things, the turbidity, suspended solids, and nitrates in water samples for an overall health assessment of that body of water. While comprehensive water quality data are available for more recent assessments, long-term data (35+ years) are very limited or unavailable. This study is unique because it reconstructs past environmental conditions indirectly by using bones from Florida manatees as proxies for the type of N pollution occurring in state waters. This is possible because N incorporated in manatee bones is obtained from their diet of aquatic vegetation, which in turn takes them up from the environment during primary production. The type and proportion of N found in bones (in the form of isotopic ratio) will also indicate if its origin is natural or anthropogenic (manmade). Therefore, bones from manatees can indicate how N pollution has changed over time.

Stable Isotope Analysis

This study will use stable isotope analysis, a well-established method of determining the source of N found in biological materials (Kelly, 2000; Walker & Macko, 1999) including from sirenians. For instance, MacFadden, Higgins, Clementz, and Jones (2004) and Clementz, Goswami, Gingerich, and Koch (2006) analyzed teeth of fossilized sirenians to show historical ecological habitats using carbon (C) and oxygen (O) isotopes. They found that extinct sirenians lived along marine coastlines with a diet of predominantly seagrass with almost no freshwater vegetation component. Clementz, Koch, and Beck (2007) followed up by explaining how modern manatees incorporate much more freshwater vegetation into their diet and that their C isotope ratios overlap with those of terrestrial ungulates. Walker and Macko (1999) investigated trophic levels using teeth from a variety of animals to show that herbivorous manatees occupy the lowest trophic level of all marine mammals. Analysis of skin C and N isotopes by Alves-Stanley, Worthy, and Bonde (2010) estimated fractionation factors, or discrimination of N isotopes, between diet and tissue in wild Florida manatees. Skin of rescued Florida manatees indicated that their C overall turnover rate is lower (53 to 58 days) compared to N (27 to 58 days) (Alves-Stanley & Worthy, 2009). However, no study has attempted to correlate isotope ratios of manatees as a method for determining dominant sources of N in coastal environments.

Stable isotope analysis of N (δ^{15}N in units ‰) is a particularly effective technique for quantifying the amount of anthropogenically derived N is in an organism. δ^{15}N value represents the ratio of enriched N isotope (^{15}N) relative to the most common form (^{14}N) found in natural sources. It provides a general indication for the source of N in their diet because N is transferred up the food chain. Therefore, organisms whose diet is enriched in N will reflect a much higher δ^{15}N value relative to diet from a depleted N source. N in tissues and bones from diet is cumulative and represents an enrichment of approximately 3‰ for each increase in trophic level, as Table 1 shows a summary of $\delta^{15}N$ values from manatees compared to those of higher trophic level organisms. $\delta^{15}N$ value is obtained by using the equation: $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is the ratio of $^{15}N/^{14}N$ of the sample and standard (atmospheric N^2). This technique is particularly helpful because the N sources used as end members in this study (fertilizer and sewage) can be distinguished from each other because their $\delta^{15}N$ values are distinct. Agriculture-derived $\delta^{15}N$ ranges from –3 to +3‰ (Bateman & Kelly, 2007; McClelland, Valiela, & Michener, 1997) while $\delta^{15}N$ from sewage is at least +8‰ (Costanzo, Udy, Longstaff, & Jones, 2005; McClelland et al., 1997). An increase in $\delta^{15}N$ over time is strong evidence for sewage-derived N pollution because it correlates with a rapid coastal development, particularly in states such as Florida.

Obtaining C isotopic values ($\delta^{13}C$ in units ‰) from samples are determined in the same manner as described above with N analysis, the difference is that Pee Dee Belemnite (PDB) is used as a standard and $^{13}C/^{12}C$ ratios are relative to atmospheric CO_2 with a $\delta^{13}C$ of –8‰ (Gannes, del Rio, & Koch, 1998). Stable isotope analysis of C forms the basis for dietary reconstruction because it represents the type of vegetation consumed (terrestrial, marine, estuarine, freshwater). For example, terrestrial and freshwater plants utilizing C3 photosynthesis contain $\delta^{13}C$ values that are depleted in heavy carbon (–34‰ to –22‰) while terrestrial and seagrasses that perform the C4 photosynthetic pathway have $\delta^{13}C$ values enriched in heavy carbon (–20‰ to –9‰) (Gannes et al., 1998; Gannes, OBrien, & delRio, 1997). Plants with $\delta^{13}C$ values that fall within –13‰ to –23‰ are believed to come from estuarine environments (Reich & Worthy, 2006).

Table 1. Summary of Published $\delta^{15}N$ Values from Various Body Samples

Organism	**Skin**		**Teeth**		**Bone**	
	Low	High	Low	High	Low	High
Manatee	5.7 [e] 4.8 [f]	9.6 [e] 11.4 [f]	6.4 [c]	9.0 [c]	4.6 [g]	13.3 [g]
Stellar sea cow [†] [g]					10.4 9.9	12.3 17.8
Marine mammals						
Planktivores [a]					11.7	15.8
Right whale [g]					7.8	12.4
Humpback whale [g]					11.5	16.3
Carnivores [a]					12.1	23.0
Pilot whales [b]	11.3	14.2	12.8	15.1		
Sperm whale			11.3 [c]	14.3 [c]	14.0 [g]	15.7 [g]
Bottlenose dolphins			13.6 [c] 13.6 [d]	15.6 [c] 18.1 [d]		
Killer whale			16.3 [c]	19.9 [c]	15.1 [g]	15.8 [g]
Terrestrial mammals [a]						
Ungulates					3.4	7.3
Others					1.9	10.0
Fish [a]						
Freshwater					6.6	9.5
Marine					11.1	16.0

[a] (Schoeninger & Deniro, 1984), [b] (Abend & Smith, 1995), [c] (Walker & Macko, 1999), [d] (Walker, Potter, & Macko, 1999), [e] (Reich & Worthy, 2006), [f] (Alves-Stanley & Worthy, 2009), [g] (Clementz, Fox-Dobbs, Wheatley, Koch, & Doak, 2009). [†] signifies an extinct organism.

Basis for the Study

The main rationale is to understand the environment in which these manatees lived as it relates to N. Since water quality data are unavailable; therefore, we have to rely on proxies for reconstructing environmental conditions in Florida. Manatee bones are particularly useful in this study because $\delta^{15}N$ in bone collagen represents N assimilated over a long period of time. Skeletal N reflects plants that manatees consume (Keegan & Deniro, 1988) and thus the predominant N source driving primary productivity within the ecosystem. While freshwater plants and seagrasses provide a more direct record of N source, a limited archive of those samples exist. Therefore, stable isotope ratios of archived manatee bones will be analyzed instead to quantify changes in $\delta^{15}N$ values over time, which is reflective of change in $\delta^{15}N$ in their food sources. Though an indirect assessment, manatee bones will still serve as long-term indicators of environmental changes as part of this study's overall historical investigation.

Bones have been shown to retain their isotopic signatures for reconstructive purposes much better than other organic materials, including soft tissues. Previous studies by Clementz et al. (2007, 2009) used bones from modern and historic manatee samples to show both ecological and paleo-ecological dietary preferences. Even bones subjected to heat (Deniro, Schoeninger, & Hastorf, 1985) and preservation materials (Moore, Murray, & Schoeninger, 1989) maintain their isotopic signatures. In contrast, stomach contents with a residence time in the order of several hours to a few days (J. E. Reynolds & Rommel, 1996) and epidermal tissue with a mean half-life ranging from 30 days to 2 months (Alves-Stanley & Worthy, 2009) provide only short term isotopic signatures and are eventually shed from the body. Bones, on the other hand, retain older isotopic signatures as well as new ones, providing a long-term integration encompassing the lifespan of that individual.

Connection of Nutrient Pollution to the Study

The eutrophication issue in Florida is primarily driven by nutrient-rich sewage which is detrimental to aquatic ecosystems. In order to determine the severity of this nutrient pollution, this study will focus on N contained in sewage and will assess if improvements in sewage treatment has properly addressed this ongoing issue. On the other hand, this study will also highlight the contribution of agricultural fertilizer into the ecosystem as a source of much depleted source of nitrogen over many decades. The remaining details that follow will outline the impact that these sources of N may have played in the overall ecology of the organism of interest in this study, the Florida manatees.

Manatee Life History

The manatee is one of four extant species in the order Sirenia (J.E. Reynolds & Odell, 1991) and is likely a descendant of marine-dwelling proboscideans and terrestrial hyraxes (Clementz et al., 2006; Hoson, Kawada, & Oda, 2009; Liu, Seiffert, & Simons, 2008; MacFadden et al., 2004). The family Trichechidae, one of two in the order, is represented by three species—the Amazonian, West African, and the West Indian—which occur over a wide range of habitats along the Atlantic basin. The Amazonian manatee is strictly a freshwater species living exclusively in the Amazon River. The West African manatee inhabits the coastlines and inland rivers of the continent; however, very little is known about them. The two subspecies of the West Indian manatee are morphologically and genetically distinct from each other (Haubold, Deutsch, & Fonnesbeck, 2006; Hunter et al., 2010) and thus have distinct regional distributions encompassing tropical to subtropical climates. The Antillean manatee (*Trichechus manatus manatus*) is found along the Caribbean and the Central and South American coasts. The Florida manatee (*Trichechus manatus latirostris*) represents the most northern range of this species, living along the southeast coast of the United States, most particularly in the peninsula of Florida (Laist & Reynolds, 2005; J.E. Reynolds & Odell, 1991).

Much of what is known about the behavior and migratory patterns of the Florida manatee comes from a wealth of studies utilizing photo-identification, radio telemetry, aerial surveys, satellite tracking data, and necropsy data (Deutsch et al., 2003; "Manatee Mortality Statistics," 2012; Nabor & Patton, 1989). They reveal that during warmer months, manatees tend to travel north along the Atlantic coast and west along the Gulf of Mexico. In colder months (October to March), especially when water temperatures approach 20 °C (68 °F), manatees congregate along warm water refuges (i.e., natural springs and power plant discharges) because of their low metabolism and limited insulating blubber to sustain thermoregulatory controls during the cold period between November and March (Deutsch et al., 2003).

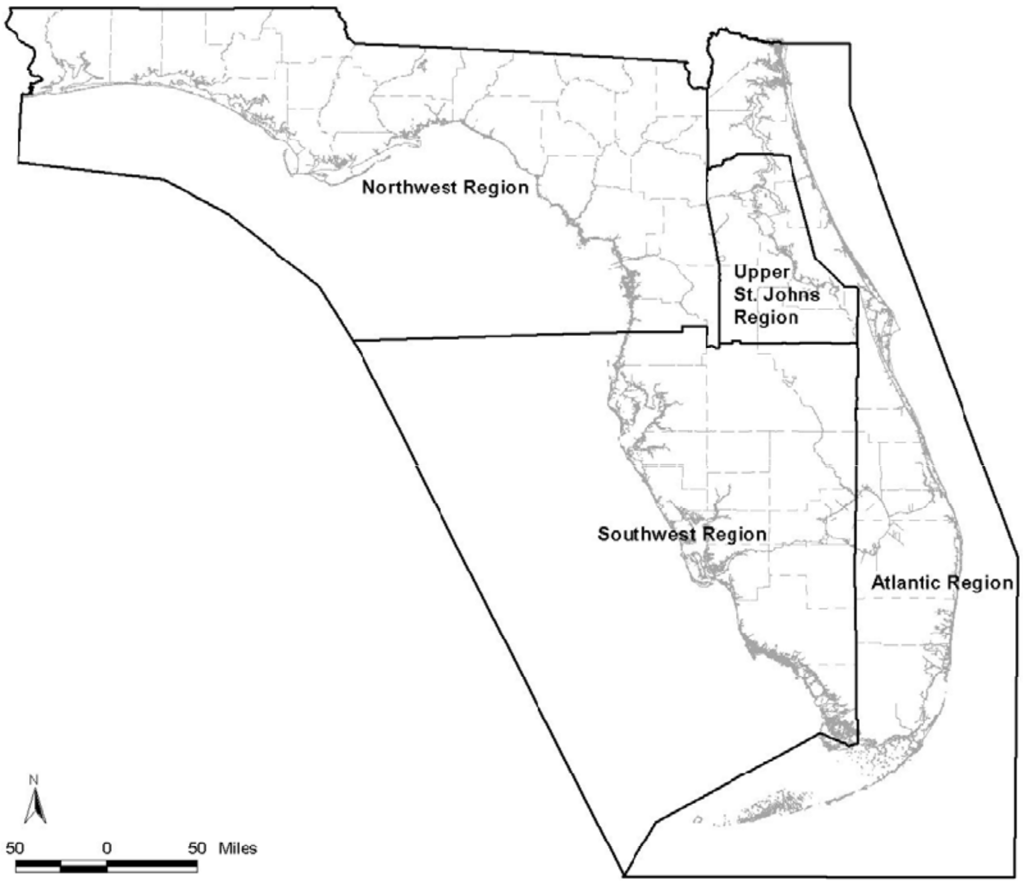

Figure 1. Florida Manatee Subpopulations According to US-FWS. Adapted from Haubold et al. (2006).

Studies that have tracked the movement of individuals (i.e., via photograph and radio telemetry) revealed that manatees tend to use the same warm water locations but utilize specific locales (rivers and protected habitats) for food and refuge. This evidence forms the basis for establishing various subpopulation designations within the state and dictates creation of subsequent management areas based on their geographic range (Deutsch et al., 2003; Haubold et al., 2006; Laist & Reynolds, 2005). The four recognized subpopulations of the Florida manatee are: Upper St. Johns River, Atlantic region, Southwest region, and Northwest region (Figure 1).

Although one of the earliest studies indicated that genetic variability for the Florida manatee is not an issue (McClenaghan & O'Shea, 1988), subsequent research since then has suggested that this conclusion may not be the case (R.K. Bonde, 2009; Haubold et al., 2006). Among the reasons for this lack of gene flow include little, if any, contact between coastal subpopulations due to physical barrier provided by the Everglades and the strong currents along the Gulf of Mexico and the Florida Bay (R.K. Bonde, 2009; Deutsch et al., 2003; Haubold et al., 2006). Scarcity of available food and access to freshwater may also be contributing factors, although these are all hypothetical explanations that have not been confirmed.

Representing one of four species of fully herbivorous aquatic mammals, Florida manatees are found in freshwater, estuarine, and marine environments. Their generalist feeding strategy is advantageous in such diverse habitats, allowing them to utilize as many as 60 plant species (Alves-Stanley et al., 2010; Clementz et al., 2007; Reich & Worthy, 2006). Tables 2 and 3 below provide a comprehensive summary of their dietary preferences, ranging from freshwater aquatic vegetation to seagrasses. They possess low basal metabolic rates that are 15–33% lower than terrestrial mammals of similar sizes (Clementz et al., 2007). Despite their slow metabolism, it has been suggested that Florida manatees can easily consume approximately 10–15% of their body weight's worth of vegetation on a daily basis (Robert K. Bonde, Aguirre, & Powell, 2004; "Manatee Facts," 2012). Although manatees have been observed feeding on other food sources (detritus, algae, acorns, mangrove propagules, invertebrates attached to vegetations), this behavior is likely accidental and not intentional as has been proposed (Courbis & Worthy, 2003).

Florida manatees can grow to an average length of 3 m and weigh approximately 454 kg (Van Meter & Wiegert, 2001), although their body length can be greater than 4 m and approach

Table 2. Published Mean $\delta^{13}C$ and $\delta^{15}N$ Values of Plants in East Coast of Florida

Location	Plant Type	Taxon	$\delta^{13}C$	$\delta^{15}N$
Banana River	Estuarine [a]	*Caulerpa sp.* (alga)	-14.8	6.1
		Halodule wrightii (shoal grass)	-16.5	2.7
		Ruppia maritima (widgeon grass)	-13.8	1.4
	Marine [a]	*Syringodium filiforme* (manatee grass)	-8.8	1.1
Blue Springs	Freshwater [a]	Unidentified grass	-28.3	6.3
		Eichornia crassipes (water hyacinth)	-29.0	5.9
		Pistia stratiotes (water lettuce)	-26.6	4.1
Indian River Lagoon	Estuarine [c]	*Gracilaria sp.* (red alga) *Halodule wrightii* *Syringodium filiforme* *Thalassia testudinum* (turtle grass)	-21.9	6.4
	Marine [b]	*Halophila decipiens* (paddle grass)	-10.1	
		Syringodium filiforme	-10.9	
		Thalassia testudinum	-13.6	
	Marine [c]	*Gracilaria sp.* *Halodule wrightii* *Syringodium filiforme* *Thalassia testudinum*	-13.8	1.1
St. Johns River	Freshwater [c]	*Alternanthera philoxeroides* (alligatorweed) *Eichornia crassipes* *Hydrocotyle sp.* *Lemna valdiviana* (duckweed) *Myriophyllum aquaticum* (parrotfeather) *Nuphar luteum* (spatterdock) *Pistia stratiotes* *Pontederia cordata* (pickerel weed)	-28.1	7.3
Florida Keys	Marine [b]	*Thalassia testudinum*	-8.4	1.8
Palm Beach County	Freshwater [a]	Mangrove	-26.9	1.5
		Spartina alterniflora	-13.4	3.5
	Marine [a]	*Halodule wrightii*	-14.1	2.0
		Thalassia testudinum	-10.8	1.2
		Syringodium filiforme	-8.7	1.1

[a] (Reich & Worthy, 2006), [b] (Clementz et al., 2007), [c] (Alves-Stanley et al., 2010)

1,361 kg in many cases ("Manatee Facts," 2012; J.E. Reynolds & Odell, 1991). They have long life spans, supported by earbone growth layer group (GLG) data revealing that they can live up to 60 years (Haubold et al., 2006; Marmontel, Humphrey, & OShea, 1997). Males tend to reach sexual maturity around three years old while the median age of first reproduction in females

Table 3. Published Mean $\delta^{13}C$ and $\delta^{15}N$ Values of Plants in West Coast of Florida

Location	Plant Type	Taxon	$\delta^{13}C$	$\delta^{15}N$
Apalachicola Bay	Terrestrial C3 [b]	*Cladium jamaicense* (sawgrass)	-25.4	
	Terrestrial C4 [b]	*Spartina spp.* (cordgrass)	-13.2	
	Freshwater [b]	*Typha sp.* (cattail)	-26.1	
		Eichhornia crassipes	-28.2	
		Vallisneria sp. (tapegrass)	-25.2	
		Ruppia maritima	-25.7	
	Estuarine [b]	*Gracilaria sp.*	-19.8	
		Ulva lactua (sea lettuce)	-17.8	
	Marine [b]	*Halodule wrightii*	-14.4	
Crystal River	Freshwater [a]	*Ceratophyllum demersum* (hornwort)	-24.3	-0.7
		Eichornia crassipes	-23.6	4.1
		Pistia stratiotes	-27.9	3.9
	Estuarine [a]	*Hydrilla verticillata* (hydrilla)	-21.7	2.4
		Myriophyllum sp. (watermilfoil)	-21.2	3.8
		Potomageten sp. (pondweed)	-19.4	-1.2
		Vallisneria americana	-18.1	4.7
	Estuarine [c]	*Chara sp.* (stonewort) *Hydrilla verticillata* (hydrilla) *Myriophyllum spicatum*	-22.3	6.0
Homossasa River	Freshwater [a]	*Najas guadalupensis* (waternymph)	-27.3	5.5
		Panicum sp.	-26.0	6.4
		Spicatum	-24.1	5.9
		Typha sp.	-27.5	4.0
		Unidentified algae	-26.6	4.6
Tampa Bay	Freshwater [a]	Mangrove	-26.9	2.0
	Estuarine [a]	*Spartina alterniflora*	-13.6	3.7
	Marine [a]	*Enteromorpha* (green alga)	-11.4	0.1
		Halodule wrightii	-10.8	-0.8
		Syringodium filiforme	-8.8	0.3
		Thalassia testudinum	-9.6	1.4
	Marine [c]	*Halodule wrightii* *Syringodium filiforme* *Thalassia testudinum*	-14.8	2.5
Charlotte Harbor	Marine [c]	*Halodule wrightii* *Syringodium filiforme* *Thalassia testudinum*	-11.0	1.4
Ten Thousand Islands	Marine [c]	*Halodule wrightii* *Halophila engelmannii* (star grass) *Syringodium filiforme* *Thalassia testudinum*	-12.9	1.1

[a] (Reich & Worthy, 2006), [b] (Clementz et al., 2007), [c] (Alves-Stanley et al., 2010)

occurs at five years old (Haubold et al., 2006; Marmontel et al., 1997). There is no recognized breeding season because manatees are observed mating throughout the year. They tend to give birth to one calf, though twin births are known and have been documented (Haubold et al., 2006; "Manatee Facts," 2012; Marmontel et al., 1997; Van Meter & Wiegert, 2001). After a gestation period of approximately one year, mom-calf pairs stay with each other for one to two years, even shortly after weaning, for the purpose of learning migratory patterns, foraging behavior, and locating warm water refuges (Deutsch et al., 2003; Marmontel et al., 1997; Van Meter & Wiegert, 2001).

Endangered Status

Since the 1700s, and even much earlier, manatees have been hunted for their hide, meat, bones, and oil (Nabor & Patton, 1989; Van Meter & Wiegert, 2001). European settlers, as did the Native Americans before them, used manatees not only for sustenance but also for shipping and trading purposes. These practices were halted in 1893 when Florida declared killing these animals as illegal and carried a fine along with incarceration (Clifton, Yan, Mecholsky, & Reep, 2005; Haubold et al., 2006; Nabor & Patton, 1989; J.E. Reynolds & Odell, 1991). Protection of these animals were further strengthened with the passage of both the Marine Mammal Protection Act (MMPA) in 1972 and the Endangered Species Act (ESA) of 1973, which declared them on the brink of extinction (Clifton et al., 2005; Nabor & Patton, 1989). The entire state was declared a protected area through the Florida Manatee Sanctuary Act in 1978, paving the way for implementing a recovery plan (Clifton et al., 2005; Haubold et al., 2006).

Critical to the success of the manatee recovery plan is determining an accurate population estimate to assess what constitutes maximum sustainable levels to warrant removal from the endangered species list (Clifton et al., 2005). Manatee counts have remained variable through the decades, with estimates ranging from 738–850 individuals in the mid-1970s, 1,200 individuals in 1985 (Nabor & Patton, 1989), 1,856 in 1992 (Marmontel et al., 1997), and 3,276 animals in 2001 (Haubold et al., 2006; Laist & Reynolds, 2005). As of January 2011, the minimum count stood at 4,480 manatees ("Manatee Facts," 2012) and is subject to adjustment due to observer error and difficulty deciphering individual manatees and from other objects, particularly in turbid waters (Deutsch et al., 2003). Since enacting the Sanctuary Act in 1978, studies utilizing a combination of radio telemetry, satellite tracking, aerial surveys, boat monitoring, and photo-ID have given way to establishing boat speed regulations, protective safe zones, and even management of seagrass habitats (Deutsch et al., 2003).

Florida manatees continue to carry this endangered status at the IUCN, federal, and state level. However, when Florida Fish and Wildlife Conservation Commission (FWC) adjusted its classification system in 1999, they did not align as closely to the same level of protection as the IUCN red list category (Haubold et al., 2006; "Manatee Facts," 2012). Specifically, a Threatened category under IUCN consisted of three levels of specific listing: Critically Endangered, Endangered, and Vulnerable that equated to Endangered, Threatened, and Species of Special Concern, respectively, at the state level. This misalignment became even more contentious when a biological review panel (BRP) tasked with evaluating the current listing status recommended in 2006 that Florida manatees be down-listed to Threatened (Haubold et al., 2006). Even though this change in status would have kept the Endangered status at the IUCN and federal levels, the down-listing process was postponed indefinitely in 2007 at the request of then governor Charlie Crist, multiple private agencies, and the public at large ("Manatee Facts," 2012).

Threats to Conservation

Threats to Florida manatee's survival can be grouped into two categories: natural and anthropogenic. Natural sources of mortality include weather-related cold stress and red tide outbreaks. Since mortality records were documented in 1974, there has been a dramatic increase in these two natural sources. In the last decade (2000–2010), six of those winters were above the yearly average of 27 cold-stress related mortalities, the most notable during the winter of 2009–2010 in which cold-stress mortality was blamed for at least 529 deaths ("Manatee Mortality Statistics," 2012). Before 2010, the record year for mortality was in 2009 with 429 deaths. Since then, the total mortality for 2010 was 766; for 2011, it was down to 453 individuals with at least 113 (25%) as confirmed cold stress. Moreover, in a 16-year period from 1996 to 2011, all but 1998 and 2010 saw confirmed red tide mortalities with the highest counts in 1996, 2003, and 2005 at 151, 100, and 93 individuals, respectively ("Manatee Mortality Statistics," 2012).

The anthropogenic threats can be divided further into direct and indirect effects. The best example of a direct effect is also one that is most visible: watercraft mortality, accounting for nearly 25% of all deaths from 1974–2007 (Halvorsen & Keith, 2008; Haubold et al., 2006). That proportion alone represents approximately 85% of all anthropogenic-related mortalities that continue to rise at a steady rate of 7% annually based on a study conducted between 1992 to 2004 (Clifton et al., 2005). Despite ongoing boat speed regulations, statistics indicate that as long as manatee and people co-exist, manatee mortalities will continue to be high (Deutsch et al., 2003). The same is the case for other human-related mortalities, including entrapment in flood gates and canal structures as well as ingestion and entanglement of fishing equipment (Haubold et al., 2006; Van Meter & Wiegert, 2001).

One example of an indirect effect is nutrient over-enrichment (eutrophication) of coastal waters from sources such as agriculture fertilizer, sewage, storm runoff, and industrial wastes.

Since the 1950s, nutrient pollution through N over-enrichment has led to widespread coastal ecosystem collapse (Brand, 2001; *Clean Coastal Waters*, 2000). This “cultural” eutrophication is a concern because N pollution can manifest itself immediately in the form of frequent, more intense harmful algal blooms (HABs) near shore. These events have been linked to large increases in manatee deaths since mortality records were first documented in the early 1970s (Haubold et al., 2006; "Manatee Mortality Statistics," 2012). Long-lasting effects of N pollution include massive seagrass dieoffs along Tampa Bay in the 1960s and 1970s (*Clean Coastal Waters*, 2000) and in the Florida Bay beginning in 1987 (Boyer, Fourqurean, & Jones, 1999; Brand, 2001). While these ecosystems are in recovery due in large part to reductions of N into the system, these examples underscore the kind of damage that N pollution can impart on critical manatee habitats and the environment in general.

Sewage inputs are of special concern because they contain pathogens along with a suite of toxins and heavy metals (*Clean Coastal Waters*, 2000; Laws, 2000). These contaminants are also problematic in that they might expose manatees to certain diseases or lead to long-term immune dysfunctions (Belanger & Wittnich, 2008; Bossart, 2007). Compounding the issue is that few historical records exist about the condition of the coastal environments in Florida prior to the 1950s pertaining to water pollution levels. It was not until an event in 1971 when massive fish kills associated with a noticeable decline in aesthetic quality of the surface water in Escambia Bay introduced the public to the idea of industrial development causing nutrient enrichment and oxygen dead zones (Laws, 2000). While few anecdotal reports can shed light on what was truly happening in Florida waters even as recently as the 1980s, such examples point to a growing body of evidence that point to an aquatic environment already in rapid decline.

N, which is often limiting in marine environments (Laws, 2000), drives primary productivity within the ecosystem (Kelly, 2000; MacFadden et al., 2004; Reich & Worthy, 2006) It enters coastal environments from multiple sources, both natural and anthropogenic. Natural sources of N are derived from terrestrial organic matter and N-fixation by marine diazotrophs (Laws, 2000). The most common anthropogenic sources are agricultural fertilizers (Bateman & Kelly, 2007) and sewage (Brand, 2001). The widespread use of synthetic fertilizers since the Green Revolution has dramatically increased crop yield, while also increasing coastal eutrophication (*Clean Coastal Waters*, 2000). Sewage, on the other hand, is synonymous with rapid development in increasingly urbanized locations of the globe (Cabana & Rasmussen, 1996). The increasing demand on treatment plants to process more wastewater than their infrastructure can handle increases the likelihood of raw sewage overflows (Laws, 2000). This does not include non-point sources from leaky septic tanks and cesspits, still widely used even in developed countries. Industrial wastes originate in ammonia and nitrate factories, meat, poultry and vegetable packing companies, and even from winery wastewater (Barile & Lapointe, 2005; *Clean Coastal Waters*, 2000). Another emerging anthropogenic source is atmospheric deposition of volatile N products from agriculture and fossil fuel combustion, which can enrich marine habitats far removed from the source (Baker, Webster, & Kim, 2010; Barile & Lapointe, 2005). The multitude of N sources, both natural and anthropogenic, makes it extremely difficult to identify and mitigate the damage caused by nutrient over-enrichment.

Human Population Growth in Florida

Florida has seen a dramatic increase in population since it became the 27th state in 1845. While the population was estimated to be at 87,445 in 1850 ("Florida Department of State," 2012), this number grew to approximately 140,000 by the 1860 and quickly rose to 528,542 after the 1900 census (Forstall, 1995). It was not until the 1930 census when the population hit over a million (1,468,211) at the start of the Great Depression. The population nearly doubled 20 years later at 2,771,305. The height of the Green Revolution may have contributed to another doubling of the state population in just a decade, to a staggering 4,951,560 in 1960. Ever since the 1980 estimate of 9,746,961, the state's population has added approximately 3 million people each decade (Forstall, 1995) and has now reached over 18 million (18,801,310) according to the recent 2010 census ("Florida Census," 2012).

Changes in Pollution Sources

The natural beauty and abundant land provided several opportunities for agriculture to flourish in the latter half of the 19th century. Canals were built to drain much of the swamp to make way for agriculture, resulting in a boom in cattle farming, citrus industry, and even phosphate mining (Brand, 2001; "Florida Department of State," 2012; "Florida Water Management History," 2012). Although much of this industry has remained unchanged for most of the 20th century, the proportion of lands devoted to strictly agricultural purposes may have been altered in large part to urbanization and a variety of technology-driven industries. A wide distribution of agricultural industries can still be seen in distinct regions ("Florida Agriculture Facts and Statistics," 2012). For example, the forest industry is situated in the northern half of the state while a combination of citrus, field crops, fruits, and vegetables dominate the interior along with livestock. The seafood industry is mostly situated on the west coast, in areas known for great fishing grounds such as the Apalachicola Bay, Tampa Bay, and along Charlotte Harbor.

The prominence of agricultural industry in the earlier history in Florida could be attributed to a greater proportion of agriculture-driven pollution. Still, this was not ignored because the first of many water pollution laws was enacted in 1868 specifically to conserve springs and sources of drinking water ("Florida Department of State," 2012). The effects of agriculture may have had more of an impact after the Green Revolution began shortly after 1940s ("Facts About Florida," 2012). This period was driven primarily by improvements in design and technology along with better farm practices to improve yield. The biggest contributor to success in this industry was the rise of synthetic fertilizer ("Florida Agriculture Facts and Statistics," 2012). Nevertheless, pollution derived from agricultural fertilizer use (Figure 2) is considered non-point source because of its association with surface runoff that is difficult to quantify (Laws, 2000).

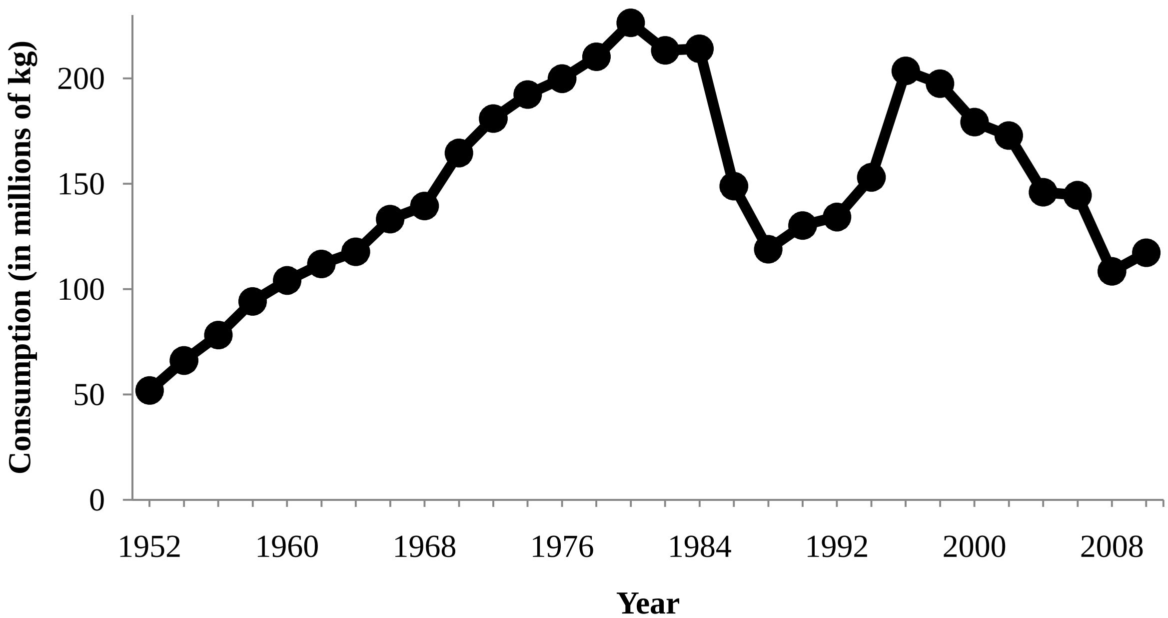

Figure 2. Nitrogen Fertilizer Consumption in Farms throughout Florida Since 1952.

A greater demand for agriculture products drove a dramatic growth in the population within Florida (Forstall, 1995) and likely contributed to the increased inputs of sewage into lakes and coastal waters. One of the earliest evidence of this sewage pollution occurred in 1947 when Lake Apopka experienced its first reported case of algal blooms, affecting both its aesthetic quality and sport fishing ("Florida Water Management History," 2012). As industry expanded post-WWII and beyond, it was clear that sewage pollution was synonymous with urban growth, providing an even greater necessity to properly treat wastewater from domestic and industrial sources. Although in existence even to this day, leaky septic tanks, cesspits, and direct discharge of sewage into the ground are problematic given the limited capability of treatment plants to keep up with an ever increasing demand to process all wastes (Brand, 2001; Laws, 2000).

Sewage Treatment

The capacity and design of a treatment plant will determine what kind of processing of sewage will take place. Primary treatment involves removing suspended solids (SS), including garbage, from the effluent using a variety of mechanical methods while the rest is pumped into an anaerobic digester (Laws, 2000). Secondary treatment uses biological processes to consume as much of the organic matter as possible, thus reducing the amount of biochemical oxygen demand (BOD) in the effluent that can be oxidized later on (Bloetscher & Gokgoz, 2001). Tertiary treatment in the U.S. primarily removes nutrients from sewage, although SS and BOD are further removed in the process. Regardless of treatment type, the resulting effluent does not leave the facility and into the environment until it is disinfected through chlorination to reduce the amount of pathogens (Laws, 2000).

The process of reducing SS, BOD, pathogens and nutrients to some extent are carefully regulated to minimize the harmful effects of treated wastewater in aquatic environments. To provide a framework for why this process is crucial, raw sewage typically contains 200 mg/L of SS, 200 mg/L of BOD, 40 mg/L of N, and 10 mg/L of phosphorus (P) in the form of phosphate (Laws, 2000). As of 1996, 75% of all wastewater in the U.S. flowed through treatment plants, commonly known as publicly owned treatment works (POTWs). This statistic roughly translates to about 50 billion m^3 of raw sewage per year.

The passage of the Clean Water Act in 1972 established federal mandates requiring sewage treatment plants to focus mainly on SS and BOD removal, so it is no surprise that many municipal treatment plants are not as efficient in removing nutrients from sewage due to space and financial limitations. N removal is typically on the order of 5–15% and no more than 30–40%, while removing P from sewage is more efficient at 30–50% and as high as 70–90% (Bloetscher & Gokgoz, 2001; "Domestic Wastewater," 2012).

Response to Federal Mandates

The 19,400 municipal POTWs in the 1996 data underscore the extent of waste produced in the country and the constant need to be on the cutting edge of keeping up with an ever increasing demand to treat waste. In Florida, each person generates approximately 378.5 L (100 gallons) of wastewater per day which is either stored in septic tanks or processed by one of the 2,300 domestic municipal treatment facilities ("Domestic Wastewater," 2012). The end products are disposed in landfills or are incinerated. Some are sold as fertilizer while the more processed effluent is used for irrigation. The more common method is surface discharge into waterways or deep well injections (Brand, 2001; "Domestic Wastewater," 2012). Though recent technology has allowed for better processing of raw sewage, the issue of improper disposal through direct discharge into cesspits and leaky tanks still pose an environmental issue because, as already mentioned, treated and untreated sewage will eventually make their way into aquatic systems, including the groundwater supply.

As a demonstration of federal standards being followed by sewage treatment facilities in the U.S., effluent samples collected from 12 secondary treatment plants in Pinellas county, FL, indicated that their treated wastewater values met or even exceeded the 30 mg/L threshold for discharge in terms of total N (12–25 mg/L), organic N (1.6–11 mg/L), and a range of N-species (ammonia: 5–23 mg/L; nitrite: 0–3.4 mg/L; nitrate: 0–3.2 mg/L) (Rosenshein & Hickey, 1976). Strict adherence to federal nutrient standards should become an even greater priority as the volume of wastewater continues to increase with population. This is not always the case because pollution of surface waters have been well documented in the Florida Bay throughout the years due to severe impact of eutrophication brought on by excessive nutrients from sewage, particularly N (Boyer et al., 1999; Brand, 2001).

Florida Nutrient Criteria

The overall goal of the Clean Water Act was to establish water quality standards in bodies of water, to be enforced by the EPA (Migliaccio, Li, & Obreza, 2007). It turns out that these guidelines have not been strictly followed in certain states, among them Florida. By enacting the Florida Watershed Restoration Act in 1999 as a means of addressing the EPA mandate to identify impaired and threatened waters, the state developed a set of guidelines to return these waters to acceptable levels that suit their intended use (Wymyslo, 2012). However, the state of Florida has resorted to developing a qualitative guideline called an "imbalance criterion" stating that nutrient concentrations must not be altered in such a way that it affects the flora and fauna of that ecosystem. At best, this was a blanket statement not based on any quantitative data.

The state declared in 2008 that nearly 1,600 km (1,000 miles) of waterways and thousands of hectares of lakes and estuaries were impaired by nutrient pollution. Environmental groups responded by filing a lawsuit urging the EPA to establish nutrient criteria because the state failed at accomplishing that goal. In 2009, the EPA settled the suit and agreed to set numeric criteria to be implemented by March of 2012, which was put on hold (Migliaccio et al., 2007). The delay has mainly stemmed from industry groups' opposition to the federal guidelines, arguing that they may be too stringent and expensive to implement. In response to this, the Florida DEP proposed its own set of quantitative nutrient criteria which was approved by the state legislature. However, the courts intervened and ruled in favor of the EPA's guidelines, instead. The state is currently awaiting approval from the EPA on its proposed limits while the EPA has until July 2012 before its own guidelines, the first portion devoted to inland waters, are given full effect (Wymyslo, 2012).

Overall Study Objectives

Given the complexity involved in undertaking a long-term environmental reconstruction over such a wide geographic area, the overarching theme for this study is: Has the source of nitrogen changed over time? If so, can we detect these changes at state, coastal, regional, or even county-level scales?

Provided that sewage and agriculture are the dominant sources of N in coastal areas and that the changes in population growth and agriculture practices are known, the following predictions will be tested:

Prediction 1: $\delta^{15}N$ values will increase over time, indicating greater inputs of sewage-derived N in the environment as a consequence of rapid coastal development as well as poor management and treatment of wastewater.

Prediction 2: Highest $\delta^{15}N$ values will be exhibited along the East coast as a reflection of more densely-populated areas and less contribution from agriculture.

CHAPTER 2

MATERIALS AND METHODS

Background

Prior to sampling, a research permit for collecting endangered marine mammals was granted by the US Fish and Wildlife Service (FWS) in October 2010 (MA14932A–0, Expiration: October 2012) allowing me to collect bone samples from up to 500 previously necropsied Florida manatee individuals. Subsequent authorization from Florida Fish and Wildlife Conservation Commission (FWC) and Florida Museum of Natural History (FMNH) provided access to a much larger collection.

Basis for Opportunistic Skeletal Sampling

Unlike previous research on manatees which only focused on the skull and rib bones, this study was the first to test an entire skeleton for a comprehensive assessment of $\delta^{15}N$ variations within an animal and to determine if variations among bone types would be significantly different. Establishing which bones to sample was crucial because institutions in possession of skeletons did not necessarily have the same bones, so it was ideal not to be restricted to a particular bone type when conducting large scale sampling.

Bone fragments (~1 g) taken from previously necropsied individuals (n=4) were sub-sampled based on 19 pre-determined locations within the entire skeleton (Figure 3). Specifically, the manatee skeleton was divided according to its appendicular and axial anatomical zones. Appendicular skeleton was associated with the appendages and included the scapula (SC), humerus (HM), ulna (UL), radius (RA), digits (DG), and the pelvic bones (PV). The axial skeleton consisted of the skull and the vertebral column. The skull included the cranium (UP), mandible (LW), earbones (pars petrosa=PP, periotic dome=PD) and teeth (lower jaw=LT, upper jaw=UT). The vertebral column was composed of the cervical (C), thoracic (T), lumbar (L), caudal (CA) vertebrae along with the sternum (ST), ribs (RB) and chevron bones (CV).

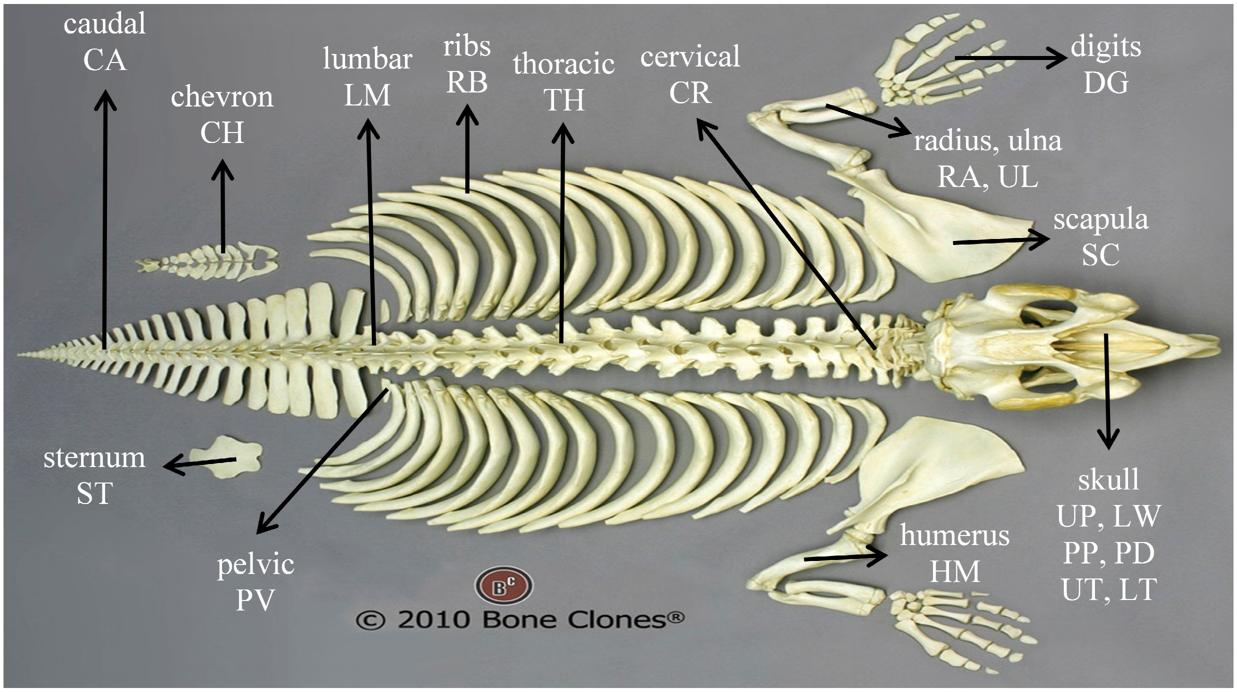

Figure 3. Bone Types for Sub-sampling. Bones associated with appendicular and axial skeletons described above.

Field Sampling Protocol

Sampling duration took place from December 9–23, 2010. Most of the trip was spent driving to various institutions throughout Florida when no access to replicate samples was determined in FWC or FMNH databases. The remaining sampling days were spent at the Marine Mammal Pathobiology Laboratory (MMPL) in St. Petersburg where bones from previously necropsied individuals were stored.

The locations from which the specimens originally stranded also reflect the scope of coverage of this study because manatees inhabit the coastal regions of Florida and beyond, even as far west as Texas and Mexico and as far north as the New England states (Deutsch et al., 2003). Because of this, the study will only focus on the Florida coastline. Therefore, bone samples from manatee individuals represent different regions of the state, based on the regional designations used by FWC marine patrol and MMPL: Northeast (NE), East Central (EC), Southeast (SE), Southwest (SW), and Northwest (NW). For this study, I simplified these five categories into four by combining NE with EC as a combined NE region (Figure 4, Table 4).

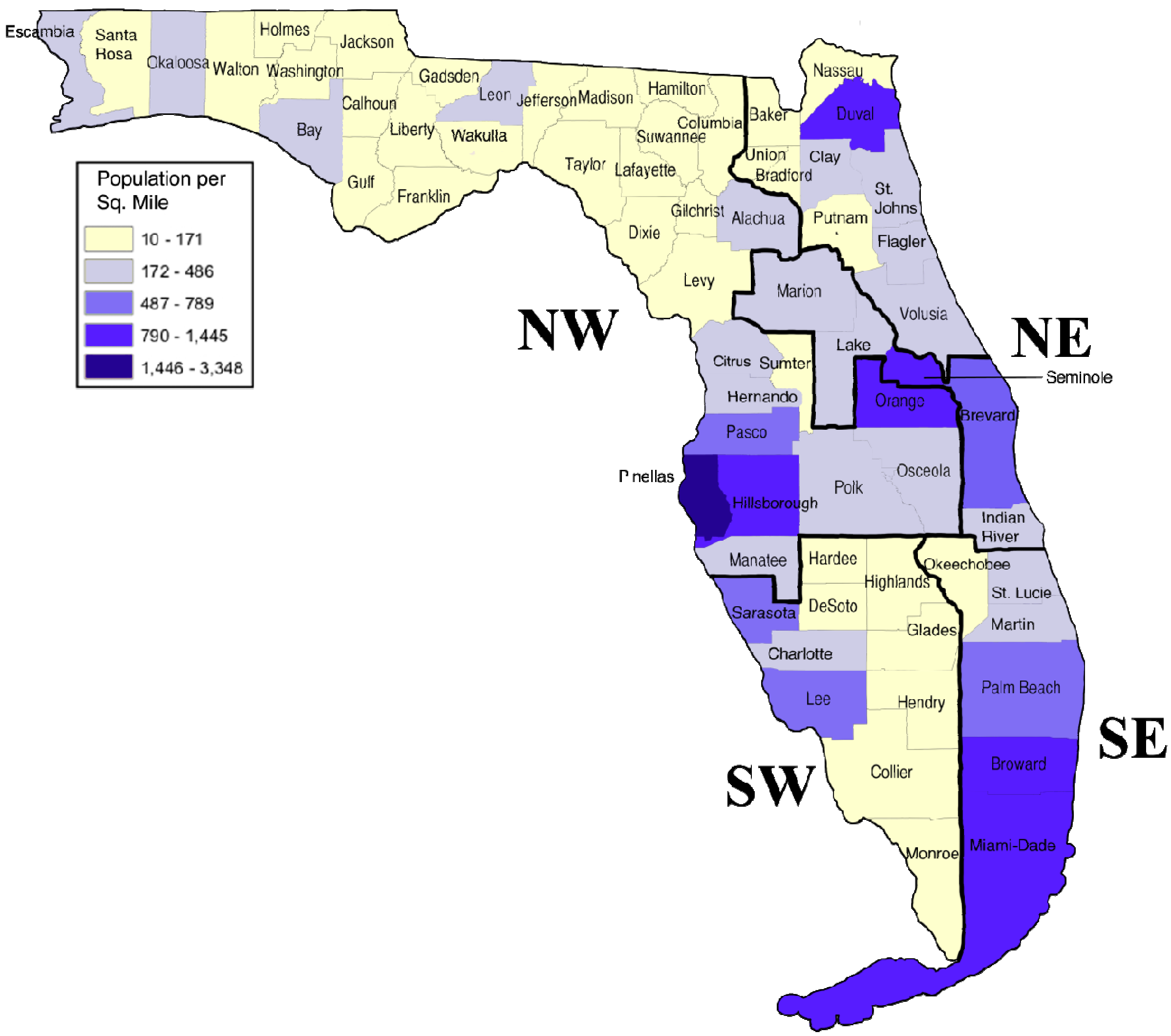

Figure 4. State of Florida Divided into Four Sampling Regions.

Table 4. Collected Bone Samples by Location

Coast	Region	County	Total
EAST n=101	Northeast (NE) n=74	Brevard	41
		Duval	12
		Flagler	1
		Indian River	6
		Nassau	2
		Putnam	2
		Seminole	1
		Volusia	9
	Southeast (SE) n=27	Broward	7
		Martin	8
		Miami-Dade	7
		Palm Beach	2
		St. Lucie	3
WEST n=72	Northwest (NW) n=19	Citrus	5
		Dixie	1
		Hernando	1
		Hillsborough	4
		Levy	1
		Manatee	3
		Pinellas	3
		Wakulla	1
	Southwest (SW) n=53	Charlotte	3
		Collier	9
		Glades	4
		Hendry	3
		Lee	31
		Monroe	3
Grand Total			173

Sampling of individuals was limited by a number of factors. First, I was limited by sample availability for a particular year. Second, age classes were constrained to mostly juveniles and adults since calves and newborns still rely on mother's milk during the first years of life, equating to a higher trophic level. In other words, their diet would not be reflective of plants but of biologically processed byproduct, instead. Third, these age classes correspond to total lengths of 200–275 cm for juveniles and at least 276 cm for adults, based on gross necropsy findings and not necessarily on live animals frequently observed in photo-identification (Deutsch et al., 2003; J. E. Reynolds & Rommel, 1996). Fourth, it was difficult to obtain a comparable number of bone samples per region because of the uneven distribution of mortalities through the years. Moreover, specimens kept in storage are often determined at random and vary in frequency due to limited space at the collection facility. Bones from certain manatees are given high priority if they come from known animals, those that suffered from trauma, or those carrying remodeled skeletons from previous injuries.

Individuals matching the criteria mentioned above were sampled regardless of which bone types were available in collection, as findings from preliminary analysis indicated no significant variations in $\delta^{15}N$ values among different bone types (see Figure 5A and B under Results). Although much of the emphasis was focused on collecting skull and rib bones as previous manatee research had done, when they were not available other bones were used, instead.

Sample Treatment

A method for obtaining collagen for N isotopic analysis in marine animals was first demonstrated by Schoeninger and Deniro (1984). Since then, the process has been modified to account for type of tissue (soft or hard), equipment (power drill, sonicator, lyophilizer), source of specimen (fresh, museum, fossil), and duration of chemical treatments (24–48 h). For this study, I adopted the methods followed by Schoeninger and Deniro (1984) and Clementz et al. (2009) with some modifications.

Bone samples from 173 individuals, spanning from 1975 to 2010, were obtained by using a hand file to scrape off approximately 50 mg of powder from the bone surface. The resulting powder was collected on a sheet of aluminum foil and funneled into a 1.5 mL microcentrifuge tube and subjected to a series of chemical treatments. Samples of bone powder were decalcified using 0.5 mol/L of HCL for 48 h in refrigeration (4 °C) followed by 5 rinses with de-ionized water. Removal of lipids involved 3 rinses with a 2:1 chloroform:methanol solution and sonicated for 20 minutes. Another series of 5 rinses using de-ionized water was performed prior to drying in an oven at ~40 °C for 24 h.

Processing

Approximately 1 mg of the final product was packaged in a tin capsule (3.5 x 5.0 mm) for analysis by isotope ratio mass spectrometry (Thermo Delta V coupled to a Carlo-Erba NC2500 via a Conflo III open-split interface) at the Carnegie Institution of Washington Geophysical Laboratory. While $\delta^{15}N$ is the focus in this study, $\delta^{13}C$ values are determined simultaneously, and C:N ratios will be used to confirm effective pre-treatment for collagen isolation (Clementz et al., 2009). The analytical precision of samples to standard was estimated at 0.3‰ for N and C.

Data Analysis

Data obtained were screened for normality and homogeneity of variance. ANCOVA was the preferred model when linear regression and ANOVA (one-way, two-way) by itself could not account for any temporal and spatial interaction. Where significant effects were detected, post-hoc Tukey-Kramer HSD t-tests were employed. Data analysis was conducted using the following software packages: JMP 4.0 (SAS, Inc.), R 2.15 (GNU Project), and SPSS 20.0 (IBM).

CHAPTER 3

RESULTS

Analysis of Bone Types

Mean $\delta^{15}N$ value for the appendicular skeleton was 7.2 ± 1.70‰ and 8.3 ± 1.17‰ for axial skeleton (Figure 5A). This indicated that no significant differences existed between skeletal anatomical zones and among bone types (p>0.05). The range of mean $\delta^{15}N$ values among bone types were 5.2 ± 0.39‰ and 11.0 ± 3.53‰ (Figure 5B).

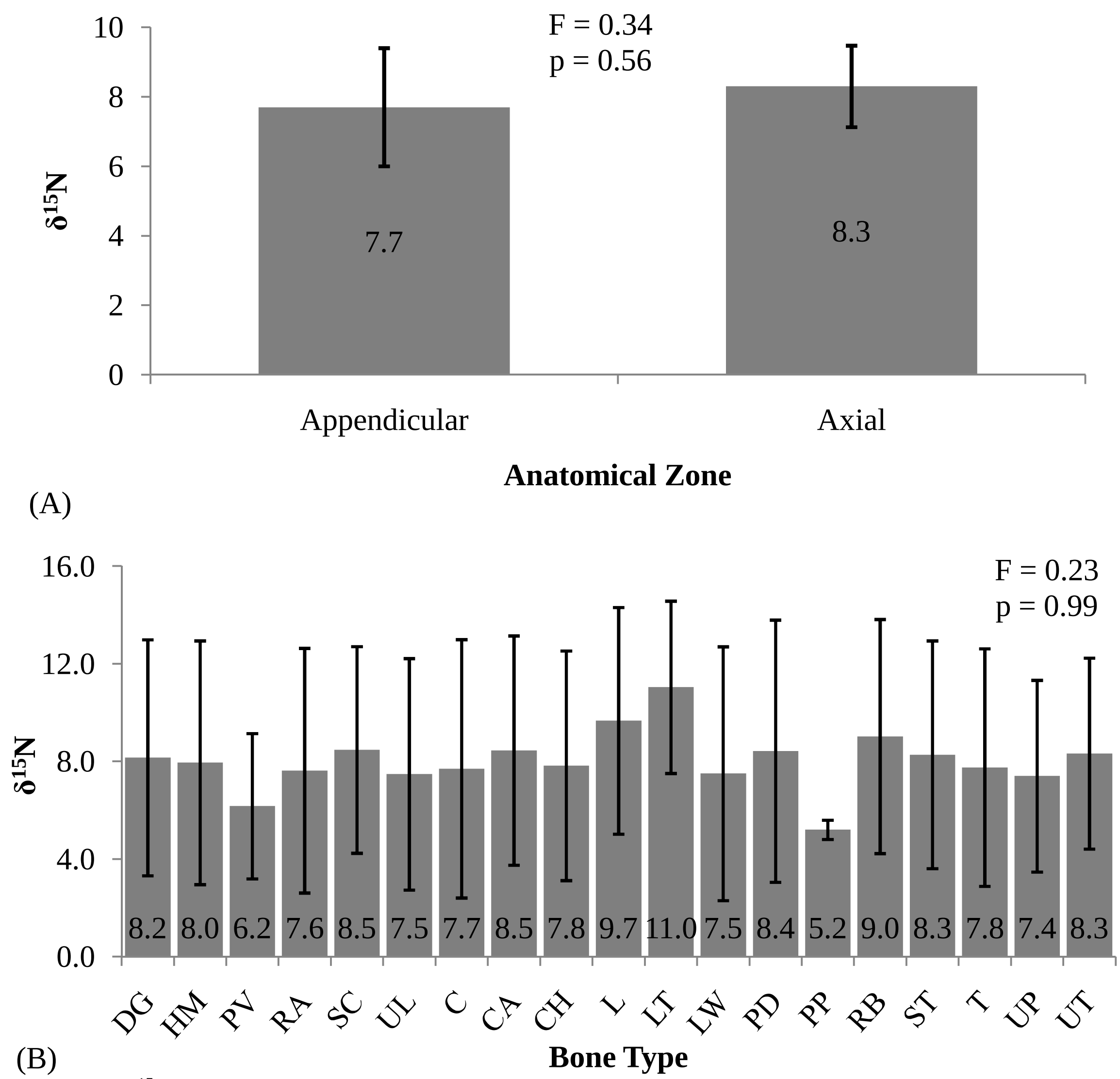

Figure 5. Mean $\delta^{15}N$ Values with a 95% Confidence Interval Between (A) Anatomical Zones of Appendicular (n=23) and Axial (n=51) Skeleton and (B) Bone Types. Sample sizes are greater than 3 for all others but PP=2.

A total of 173 individuals were included for this study, representing both coastlines and four regions in Florida (Figure 4, Table 4). Observed $\delta^{15}N$ values ranged from 2.8 to 15.3‰ with a mean $\delta^{15}N$ of 8.0‰ (Table 5).

Table 5. Summary Statistics of $\delta^{15}N$ in the Study

Total Number of Samples		173
Mean ± SE		8.0 ± 0.19‰
95% Confidence Interval	Upper	8.4‰
	Lower	7.7‰
Range	Highest	15.3‰
	Median	7.8‰
	Lowest	2.8‰

An ANCOVA model including temporal, spatial, and interactions of each variable revealed temporal and spatial effects but no interactions between time and space (Table 6). The spatial effects included coast ($p<0.001$) and latitude ($p<0.05$). An interaction only existed between coast and latitude ($p<0.01$), essentially equating to four regions.

Table 6. Whole Model ANCOVA Summary

Parameter	DF	Mean Square	F Ratio	P-value
Model	7	33.77	6.90	<0.0001
Decade	1	55.28	11.29	**0.0010**
Latitude	1	31.02	6.34	**0.0128**
Coast	1	59.41	12.13	**0.0006**
Latitude*Coast	1	33.87	6.92	**0.0094**
Decade*Latitude	1	2.25	0.46	0.4989
Decade*Coast	1	0.76	0.15	0.6948
Decade*Latitude*Coast	1	10.03	2.05	0.1544

Temporal $\delta^{15}N$ Pattern

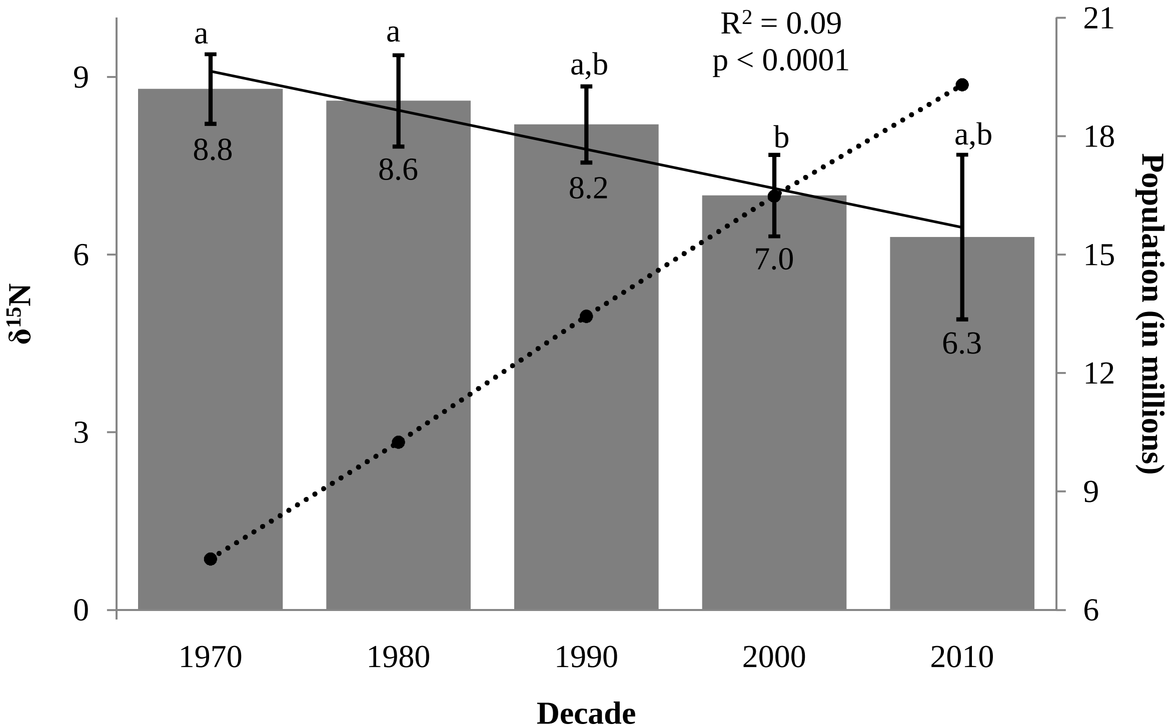

Figure 6. Linear Regression Shows a Negative Relationship Between $\delta^{15}N$ and Time. Mean $\delta^{15}N$ values by decade with a 95% confidence interval against population (dotted line). Different letters indicate significantly different means. Sample sizes for the following decades: 1970 (n=38), 1980 (n=35), 1990 (n=42), 2000 (n=54), and 2010 (n=4).

A decrease in $\delta^{15}N$ over time was statistically significant ($F_{1,171}$=16.96, p<0.0001; Figure 6). This pattern of decline from the 1970s to 2010 was apparent when analyzing mean differences using ANOVA ($F_{4,168}$=4.75, p<0.01). Post-hoc analysis showed that mean $\delta^{15}N$ values for 1970 (8.8 ± 0.71‰) and 1980 (8.6 ± 0.77‰) are significantly different from 2000 (7.0 ± 0.66‰), but not to each other. Mean $\delta^{15}N$ values for 1990 (8.2 ± 0.66‰) and 2010 (6.3 ± 1.72‰) overlap with all other decades.

<u>Temporal $\delta^{13}C$ Pattern</u>

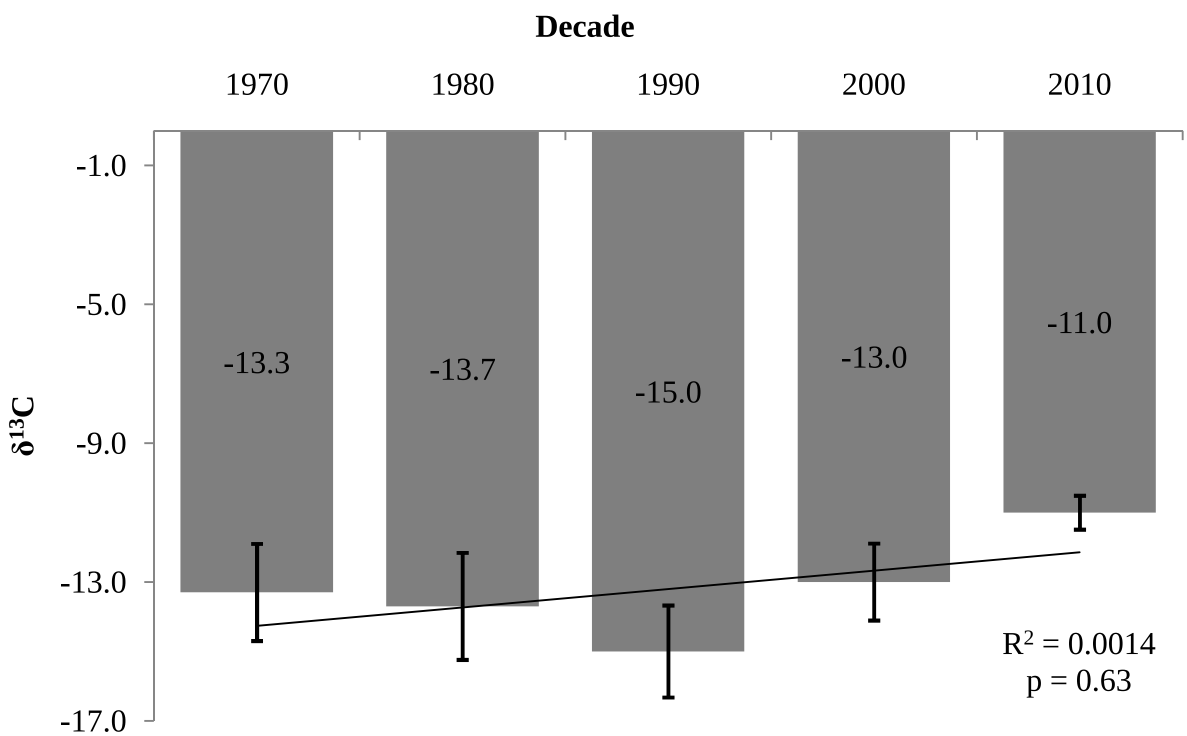

Figure 7. Linear Regression Shows No Relationship Between $\delta^{15}N$ and Time. Mean $\delta^{13}C$ values by decade with a 95% confidence interval. Sample sizes for the following decades: 1970 (n=38), 1980 (n=35), 1990 (n=42), 2000 (n=54), and 2010 (n=4).

No relationship was observed between $\delta^{13}C$ and time ($F_{1,171}=0.24$, $p>0.5$; Figure 7). Although the data suggest $\delta^{13}C$ enrichment of approximately –2.3‰ from 1970 to 2010, this pattern was not statistically significant.

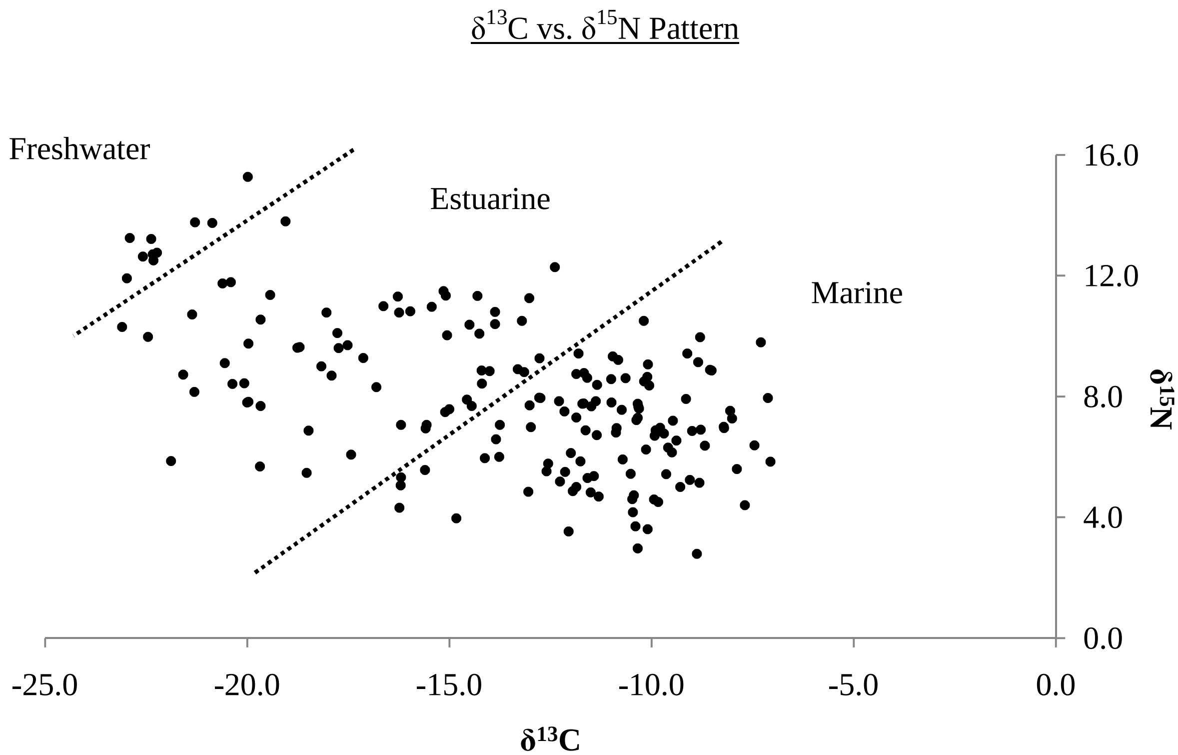

Figure 8. General Distribution of Manatee Individuals Based on Diet ($\delta^{13}C$) and Nitrogen Source ($\delta^{15}N$).

According to $\delta^{13}C$ and $\delta^{15}N$ data, coastal manatees feeding on seagrasses had $\delta^{13}C$ values of –16.2‰ to –7.1‰ and $\delta^{15}N$ values of 2.8‰ to 10.5‰. Individuals feeding on freshwater vegetations had $\delta^{13}C$ values of –23.0‰ to –20.0‰ and $\delta^{15}N$ values of 11.9‰ to 15.3‰. Individuals with intermediate values indicated estuarine-based diets (Figure 8). The demarcation lines through the data were adapted from estimates on feeding strategy work by Reich and Worthy (2006) and Alves-Stanley et al. (2010) utilizing different plant types taken from multiple geographic locations in Florida.

Overall Spatial Patterns

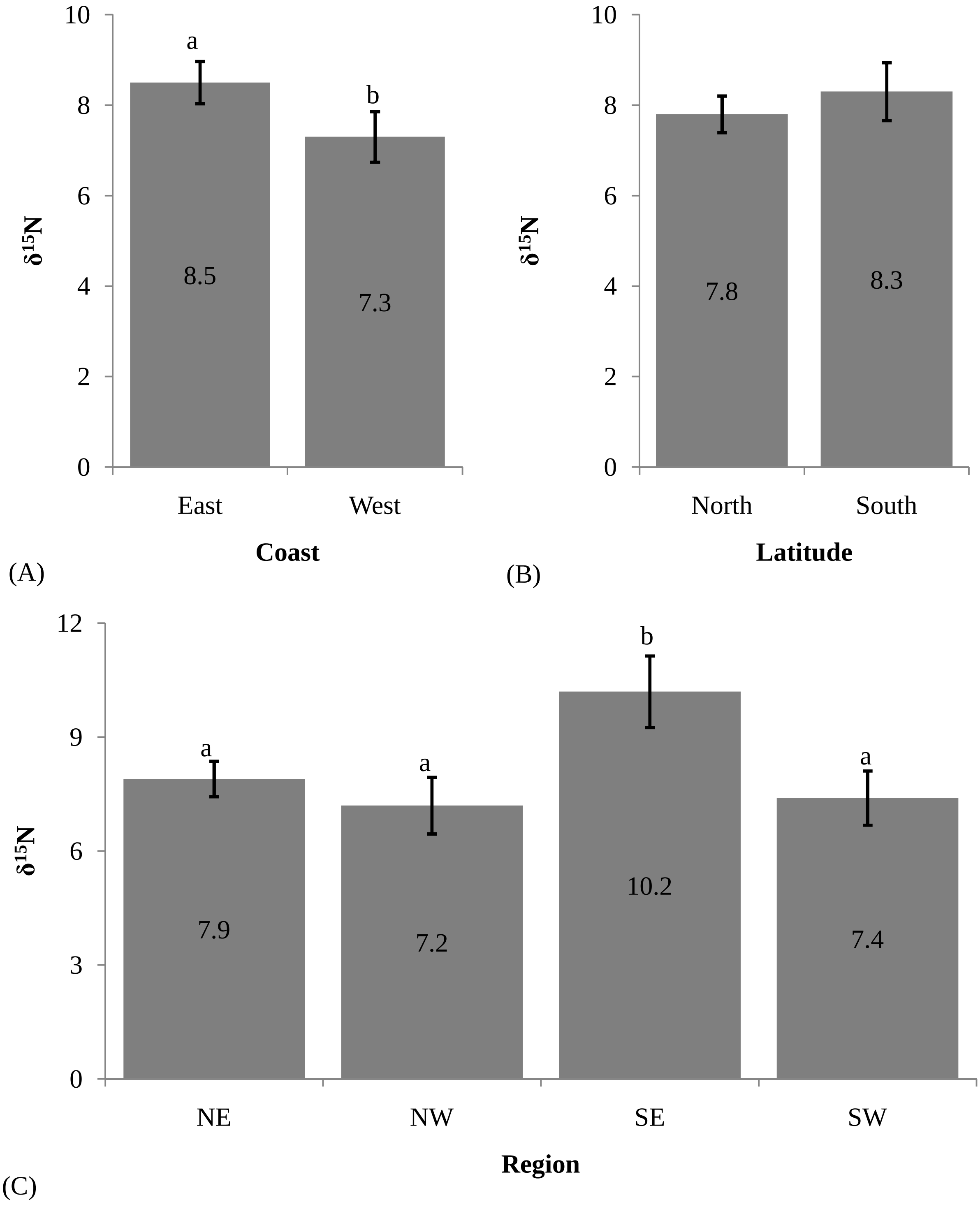

Figure 9. Mean $\delta^{15}N$ Values with a 95% Confidence Interval Between (A) East (n=101) and West (n=72) Coasts, (B) North (n=93) and South (n=80), and (C) Across Four Regions: NE (n=74), NW (n=19), SE (n=27), and SW (n=53). Different letters indicate significantly different means.

Spatial Pattern by Coast

East coast mean $\delta^{15}N$ value (8.5 ± 0.47‰) was much greater than in West coast (7.3 ± 0.56‰). Mean $\delta^{15}N$ values between coasts were significantly different ($F_{1,171}=10.07$, $p<0.01$; Figure 9A).

Spatial Pattern by Latitude

Mean $\delta^{15}N$ value in the North (7.8 ± 0.40‰) was higher than in the South (8.3 ± 0.64‰). However, mean $\delta^{15}N$ values for the northern and southern portions of the state were not significantly different ($F_{1,171}=2.20$, $p>0.10$; Figure 9B).

Spatial Pattern by Region

Table 7. Updated Whole Model ANCOVA Summary

Parameter	DF	Mean Square	F Ratio	P-value
Model	7	33.77	6.90	<0.0001
Decade	1	55.28	11.29	**0.0010**
Region	3	36.74	7.50	**<0.0001**
Decade*Region	3	4.81	0.98	0.4022

The latitudinal effect observed in Table 6 was better represented as an interaction with each coast. Subsequent ANCOVA determined a regional effect, showing a much stronger spatial relationship that divided the state into four regions (Table 7). Similarly, no temporal and spatial interactions were observed.

Post-hoc analysis of regional mean $\delta^{15}N$ values revealed that NE (7.9 ± 0.47‰), NW (7.2 ± 0.75‰), and SW (7.4 ± 0.71‰) regions were significantly different from SE (10.2 ± 0.94‰), but not to each other ($F_{3,169}=10.11$, $p<0.0001$; Figure 9C). Much of the discussion will focus along this SE region.

$\delta^{15}N$ Pattern in SE Region

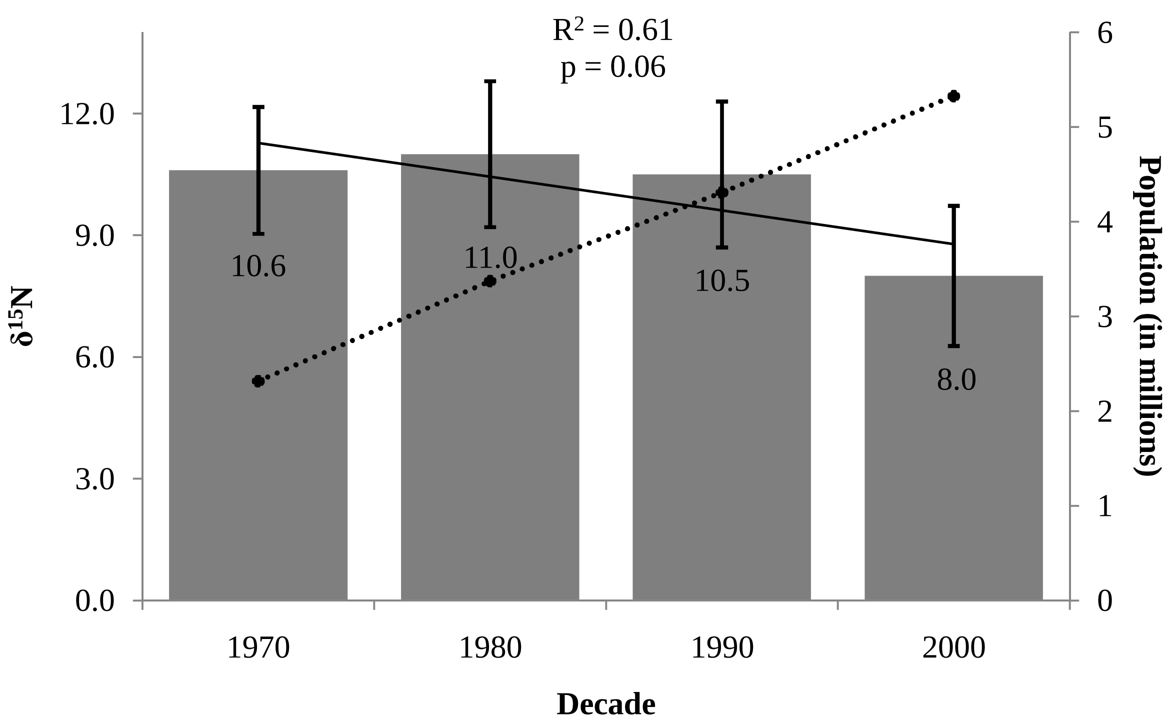

Figure 10. Correlation of $\delta^{15}N$ with a 95% Confidence Interval as a Function of Population by Decade (dotted line) in the SE Region. Sample sizes for the following decades: 1970 (n=7), 1980 (n=9), 1990 (n=5), and 2000 (n=6).

A decline in $\delta^{15}N$ was apparent as human population levels in the SE region increased by approximately 1 million individuals per decade since the 1970s. This pattern of $\delta^{15}N$ decline was not statistically significant (Figure 10).

Statewide County-level Patterns

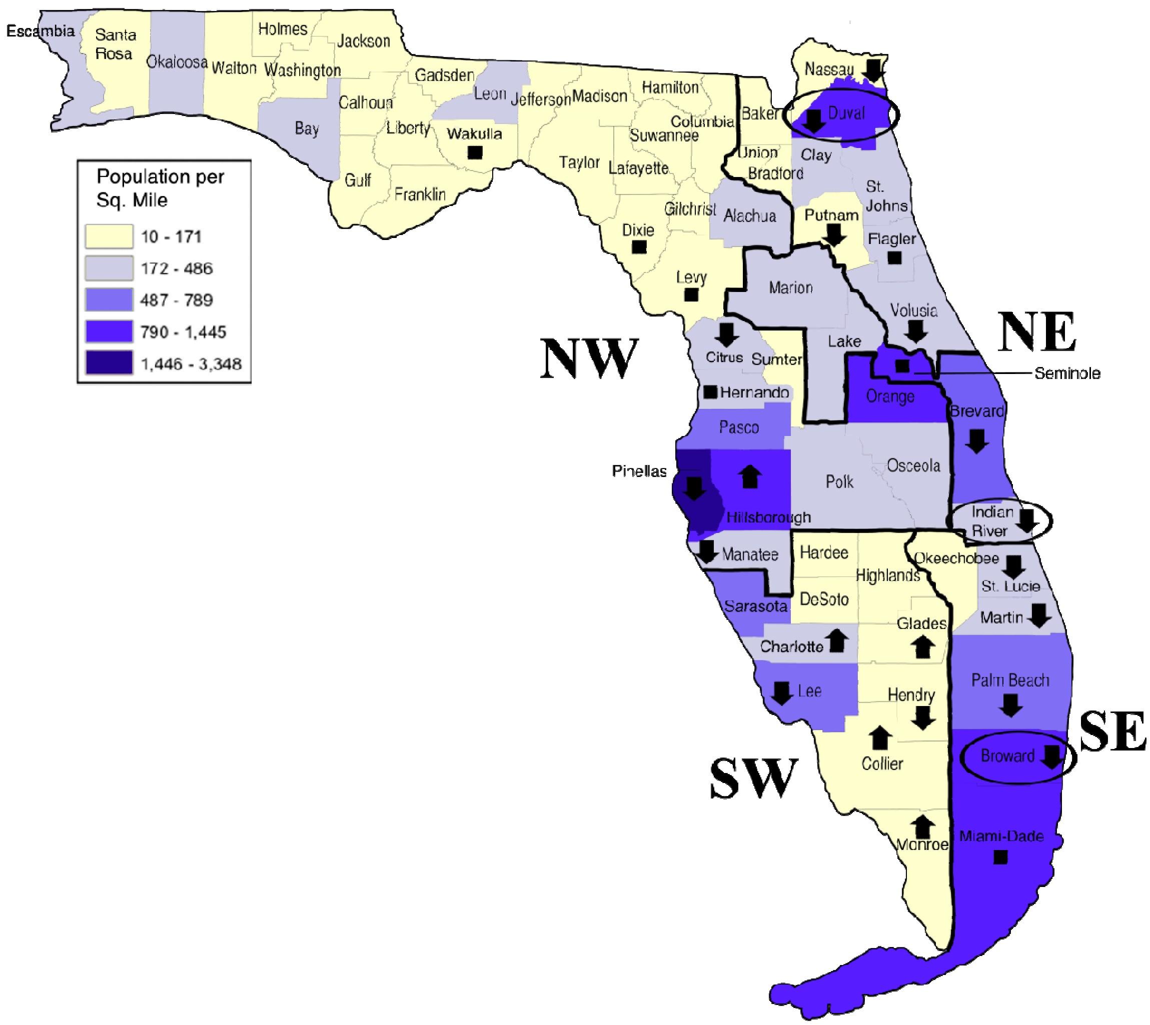

Figure 11. County-level $\delta^{15}N$ Patterns Since the 1970s, as Shown by their Corresponding Arrows. Squares denote a steady pattern or n=1. Circles indicate significant trends. Population density data based on 2010 census.

The declining $\delta^{15}N$ trend in this study since the 1970s has only been observed at the statewide level and not at the coastal, latitudinal, and regional scales (Table 6). This finding is largely substantiated by data from Figure 11 and Table 8 showing that $\delta^{15}N$ declines at the county level were not as consistent throughout the state. However, other patterns were evident. First, all counties along the East coast except for Flagler, Miami-Dade, and Seminole had declining $\delta^{15}N$. Mean values for three counties (Broward, Duval, and Indian River) were statistically significant ($p<0.05$). Second, counties along the West coast showed variable $\delta^{15}N$ patterns. Only five counties (Citrus, Hendry, Lee, Manatee, and Pinellas) indicated a decline while five counties (Charlotte, Collier, Glades, Hillsborough, and Monroe) showed an increase. Mean values for all West coast counties were not statistically significant.

Table 8. Statewide County-level $\delta^{15}N$ Patterns Over Time

Region	County	n	Pattern
Northeast (NE) n=74	Brevard	41	Decrease
	Duval	12	Decrease *
	Flagler	1	n/a
	Indian River	6	Decrease *
	Nassau	2	Decrease
	Putnam	2	Decrease
	Seminole	1	n/a
	Volusia	9	Decrease
Southeast (SE) n=27	Broward	7	Decrease *
	Martin	8	Decrease
	Miami-Dade	7	Steady
	Palm Beach	2	Decrease
	St. Lucie	3	Decrease
Northwest (NW) n=19	Citrus	5	Decrease
	Dixie	1	n/a
	Hernando	1	n/a
	Hillsborough	4	Increase
	Levy	1	n/a
	Manatee	3	Decrease
	Pinellas	3	Decrease
	Wakulla	1	n/a
Southwest (SW) n=53	Charlotte	3	Increase
	Collier	9	Increase
	Glades	4	Increase
	Hendry	3	Decrease
	Lee	31	Decrease
	Monroe	3	Increase

* indicates statistically significant pattern.

CHAPTER 4

DISCUSSION

The preliminary findings into this study (Figure 5) demonstrated that manatee bones are good substitutes for determining N sources to quantify the degree of nutrient pollution in Florida. Even with relatively limited temporal scale (35 years) and no direct water quality measurements, manatee bones were still able to show important $\delta^{15}N$ patterns temporally (Figure 6) and spatially (Figure 9). Therefore, manatee bones can function as environmental proxies for nutrient pollution in the absence of long-term diet and water quality data.

The use of stable isotope analysis to identify enriched N sources is not novel, but its application to assess the role of coastal eutrophication is comparatively recent (Costanzo et al., 2005; McClelland et al., 1997; Piñón-Gimate, Soto-Jiménez, Ochoa-Izaguirre, García-Pagés, & Páez-Osuna, 2009). Among those examples that use an ecological approach as a central theme in their work include the direct effects of terrestrial-driven sources in aquatic systems by investigating the overabundance of N from plants and algae (Costanzo et al., 2005; McClelland & Valiela, 1998; McClelland et al., 1997; Piñón-Gimate et al., 2009) to coral reef systems (Baker et al., 2010; Carrie Futch, Griffin, Banks, & Lipp, 2011; Sherwood, Lapointe, Risk, & Jamieson, 2010; Webster, 2007). My study, on the other hand, is the first to explicitly link N sources using manatee bones. However, this particular work is one of several studies that point to a growing evidence of depleted N sources influencing $\delta^{15}N$ patterns through time. Similar works include coral skeletons in Indonesia (Marion et al., 2005) and Caribbean octocorals (Baker et al., 2010). This study hinges on one important question: Has the source of N changed over time? I will address this question in two parts. First, by exploring alternative hypotheses to explain the observed trends in $\delta^{15}N$ at the state level; and second, at the coastal and regional levels.

Temporal Explanations

A change in N source from highly enriched to more depleted sources has indeed occurred during the 35 years covered by this study, from a high of 8.8‰ in the 1970s to 6.3‰ in 2010 (Figure 6). This finding does not support the prediction that $\delta^{15}N$ values would increase as a result of rapid coastal development and greater inputs of sewage in the environment. This significant decrease of approximately 2.5‰ is equivalent to a decline by one trophic level. Below, I explore several hypotheses to explain this overall trend in $\delta^{15}N$.

Hypothesis 1: Shift in Dietary Source Since the 1970s

The overall temporal hypothesis was that the source of N would change, with the prediction stating that $\delta^{15}N$ values would increase due to enriched N sources. Instead, a decline in $\delta^{15}N$ over time was observed due to the influence of depleted N sources. Figure 7 indicated a decline in $\delta^{13}C$ in more recent manatees (2000 and beyond), some clustering around marine food sources. However, the overall pattern over time showed that their diet composition has not changed much during these 35 years, as demonstrated by the high p-value. Data in Figure 8 also demonstrated that a large proportion of manatees from this study were marine-dwelling that fed mainly on seagrass. While this pattern suggests that they fed in locations dominated by marine and estuarine plants, the fact that their overall distribution since 1975 is continuous along three habitats support their generalist strategy. More importantly, there is no clear dividing line between each habitat which means that manatees were neither constrained by plant types as their food source nor their ability to travel from one diet source to the next. It has been suggested in literature, though, that individuals may preferentially feed on seagrasses because they are more nutritious (Deutsch et al., 2003). Another explanation could be that seagrass beds are abundant and readily available along their daily and seasonal migration routes particularly when using the coastline, not rivers, as a travel corridor. However, the only way that diet could lead to a sharp decline in $\delta^{15}N$ values in bones would be if manatees suddenly discriminated against one type (i.e., freshwater plants) over others (seagrass) that have distinct $\delta^{15}N$ signatures. This has not been the case, even from data in more recent manatees. Therefore, a shift in dietary preference over time is not a likely explanation for this decline in $\delta^{15}N$.

Hypothesis 2: Infrastructure for Treating Sewage Has Improved

Attributing the overall decline from improvements in sewage treatment process alone is a strong statement but also problematic because the Clean Water Act regulations only targeted suspended solids (SS) and biochemical oxygen demand (BOD) in raw sewage (*Clean Coastal Waters*, 2000; Laws, 2000). The 1972 amendment to the Clean Water Act was actually an extension of earlier mandate of the 1948 Federal Water Pollution Control Act addressing only point source pollution (Laws, 2000; Migliaccio et al., 2007). It was years later that regulations were directed specifically at non-point source pollution, particularly from agricultural fertilizer runoff, to put greater emphasis on addressing pollutants associated with nutrient enrichment.

It would be easy to acknowledge that a large part of this $\delta^{15}N$ decline was due to treatment plant upgrades. However, many of the largest publicly owned treatment plants even in the 1960s were not designed to provide tertiary treatment (York & Potts, 1995). A 1966 inventory revealed that of the 593 treatment facilities, the 14 largest facilities making up about 2 percent of the total number of facilities actually accounted for 40 percent of the state capacity. Interestingly, these treatment plants discharged their effluent along surface waters. Even in 1993, 6 out of 27 largest treatment plants provided tertiary treatment while the overall capacity to treat large volumes of sewage increased considerably, from a combined total of over 784 million liters daily, mld (or 207 million gallons daily, mgd) in 1966 to nearly 4.1 billion liters daily, bld (1,075 mgd) in 1993 (York & Potts, 1995). As of 2011, there were over 3,700 permitted wastewater facilities in Florida, of which 2,300 are dedicated domestic wastewater facilities with a combined capacity to treat over 9.5 bld (2.5 billion gallons per day, bgd) ("Domestic Wastewater," 2012). It is evident from these numbers that structural upgrades exist to keep up with the large demand of treating SS and BOD in sewage rather than nutrients, especially N. More importantly, any structural improvements could not account for the apparent decline in $\delta^{15}N$ many years prior, much less at a state level given that tertiary plants were few and localized. Any substantial effect from tertiary treatment is likely exhibited at a localized level (Figure 11 and Table 8) but not sufficient enough to drive down the overall statewide $\delta^{15}N$ value during this study period.

Hypothesis 3: Elevated Use of Synthetic Fertilizer as a Depleted Source of N

An increase in synthetic fertilizer use is a strong explanation for driving down the overall statewide $\delta^{15}N$ over time because agriculture within the state has been a dominant feature ever since the onset of the Green Revolution shortly after WWII (*Clean Coastal Waters*, 2000). While $\delta^{15}N$ values from the 1970s to the 1990s remained above 8‰ (Figure 6), only the presence of a depleted N source with widespread coverage throughout the state could have contributed to such a significant decrease in $\delta^{15}N$ by 2.5‰ over a 35-year period.

Agricultural census in Florida from 1968, for example, revealed that a proportion of the state's land, 40.5%, comprised of farms while this number decreased significantly to approximately 26.9% in 2007 ("Florida Agriculture Facts and Statistics," 2012). This decline in the proportion of farm land is due in large part to a massive influx of population moving into the state during this study period. Loss of farms due to greater urbanization has not necessarily affected the state's economic contribution from agricultural industry because it still remains a leader in the production of fruits and vegetables ("Florida Agriculture Facts and Statistics," 2012). Since the 1950s, the steady loss of farmlands as a result of urbanization actually increased synthetic N fertilizer use (Figure 2). Approximately 51.9 million kg (57,233 tons) was used in 1952 and 164.6 million kg (181,472 tons) in 1970, with peak farm consumption in 1980 at 226.4 million kg (249,569 tons) ("Consumption of Commercial Fertilizers"). Although total use declined sharply to 119 million kg (131,152 tons) in 1988 and 2008, it has been followed with a steady increase shortly after ("Archive Fertilizer Tonnage Data," 2011). Nevertheless, overall N fertilizer use in Florida has remained well above 1952 levels and has never dropped below 100 million kg since 1960 when N fertilizer use reached 104.1 million kg (114,770 tons). This number is expected to increase with even greater demand worldwide, with projections of nearly 50% increase by 2030 in North and Central America alone (Zhang & Zhang, 2007). If this scenario continues, then N fertilizer will continue to be the dominant source of depleted N in aquatic environments for many decades to come.

Hypothesis 4: Increased N-fixation by Diazotrophs

N-fixation remains one natural source of depleted N since organisms utilizing this method essentially convert inert N into its biologically active form (Knapp, DiFiore, Deutsch, & Sigman, 2008). The resulting N pool is less enriched where it gets fractionated by organisms and is propagated up the food chain. This process is believed to be utilized by organisms when its environment becomes N-limited, such as marine systems (Vitousek et al., 2002). However, data from this study indicate coastal environments that have remained eutrophic since the 1970s and are no longer limited by N. This assumption has led many to suggest that coastal environments are unlikely to be dominated by N-fixation by diazotrophs anymore because they have been largely outcompeted by diatoms and small phytoplankton for available iron and phosphorus, instead (Krishnamurthy, Moore, Zender, & Luo, 2006). Thus, the magnitude of bacterial and algal N-fixation is probably not great enough to counteract the effects of enriched N sources observed in this study.

Hypothesis 5: Atmospheric Deposition of N onto Surface Waters Has Increased

Anthropogenic introduction of N into the atmosphere has increased 9-fold from 1890 to 1990 relative to a 3.5-fold increase in global population for the same time period (Galloway & Cowling, 2002). Combustion of fossil fuels in cars and factories as well as volatilization of ammonia from fertilizer and animal wastes have contributed to large proportions of N being delivered back to the surface in the form of gaseous and wet deposition far removed from its source (Gao, 2002; Garten JR, 1996).

While N deposition has been documented as a major contributing source in temperate regions such as the northeastern portions of the U.S.(Elliot et al., 2009; Krishnamurthy et al., 2006), results from this study do not support the same conclusions in previous research nor account for the significant decline in $\delta^{15}N$ statewide. One primary reason for this is that isotopic values of nitrate (–8.86 to 1.35‰), ammonia (–5.38 to 5.19‰), and pollution-derived NO_x (–5 to 6‰) have overlapping ranges that make it difficult to decipher if the depositional composition is depleted or enriched, let alone provide a clue to its original source (Gao, 2002; Saurer, Cherubini, Ammann, De Cinti, & Siegwolf, 2004). Second, a study by Elliot et al. (2009) showed a seasonal pattern of low $\delta^{15}N$-nitrate in the summer and high $\delta^{15}N$-nitrate in winter that coincided with peak heating demand. No such seasonal relationship was observed in this Florida study, which meant that any proportion of N deposition from such sources were negligible.

Even if this N deposition contributed more to surface waters than expected, one study concluded that stationary sources (factories) tended to be associated more with dry deposition (gaseous and particulate) than wet deposition (precipitation) (Garten JR, 1996). This suggests that the effects of N deposition are more localized and not statewide. Available isotopic data indicate that NO_x from coal power plants are more enriched (6 to 13‰) than those emitted from vehicles (–13 to –2‰) (Garten JR, 1996), although others have reported vehicle combustion values between 3.5 to 5.7‰ (Elliot et al., 2009). Given these sources are more prevalent in certain locations in Florida than others, N deposition may help to explain increasing $\delta^{15}N$ patterns observed in Figure 11 and Table 8 as a result of relative proximities (downwind or eastward) to coal power plants. Localized N depositions from vehicles may also have some support from studies showing that vehicle NO_x emissions are not transported very far relative to stationary sources (Elliot et al., 2009; Saurer et al., 2004). In this scenario, large metropolitan centers with high vehicle traffic (automobile, boat) could be receiving a majority of depleted N from exhausts, not fertilizer. For example, the effect of automobile combustion in Duval, Brevard, Broward, and Pinellas counties may be magnified by recreational and fishing boaters as well as shipping traffic (Figure 11 and Table 8).

Spatial Explanations

The decreasing $\delta^{15}N$ trend statewide was attributed to synthetic fertilizer as a dominant source of depleted N, even though high $\delta^{15}N$ values indicated an environment that was still quite enriched (Figure 6). While no temporal and spatial relationship was observed (Tables 6 and 7), likely mechanisms acting in different geographic locations will be explored in the context of $\delta^{15}N$ to support the prediction that highest $\delta^{15}N$ values would be exhibited along the East coast as a reflection of more densely-populated areas and less contribution from agriculture.

Coastal: East vs. West

Figure 9a showed that both coastal areas are characterized as having enriched N sources, indicating $\delta^{15}N$ values greater than 5‰. The East coast had significantly higher mean $\delta^{15}N$ (8.5‰) than West coast (7.3‰). An explanation for this, the high $\delta^{15}N$ value along the East coast, is a combination of enriched sources, namely sewage and atmospheric deposition of power plant emissions.

The significantly high $\delta^{15}N$ value along the East coast was associated with a greater volume of sewage as reflected in vast improvements and increases in the number of sewage treatment plants. In the 1966 inventory of the 14 largest treatment facilities, seven (50%) was along the East coast, five (36%) was on the West coast mainly situated along the Tampa Bay area, while the rest was located in the interior parts of the state (York & Potts, 1995). In the 1993 inventory of 27 largest facilities, there was an equal distribution of treatment plants (11 each). The fact that larger facilities are spread out along the East coast underscores the enormity of sewage wastewater contributions from numerous, dense population centers. In contrast, large facilities along the West coast are located mainly around Tampa Bay and the Tallahassee region of the panhandle. As previously discussed, though, having greater capacity to treat larger sewage inputs as a response to increasing population pressure is not necessarily synonymous with better nutrient removal. As long as nutrients such as N are not removed from wastewater before discharge, coastal environments will remain eutrophic the way they are depicted in this study.

Atmospheric deposition is a likely contributor of enriched N because of a number of coal-driven power plants along the East coast (Figure 12). As already discussed, coal emissions with enriched $\delta^{15}N$ values (6 to 13‰) are deposited as wet and dry particles (Gao, 2002; Garten JR, 1996), leading to enrichment of surface waters nearby and in downstream locations. While this scenario may also be true on the West coast, this enrichment effect is magnified on the East coast because of even greater inputs of wastewater with limited to no nutrient removal.

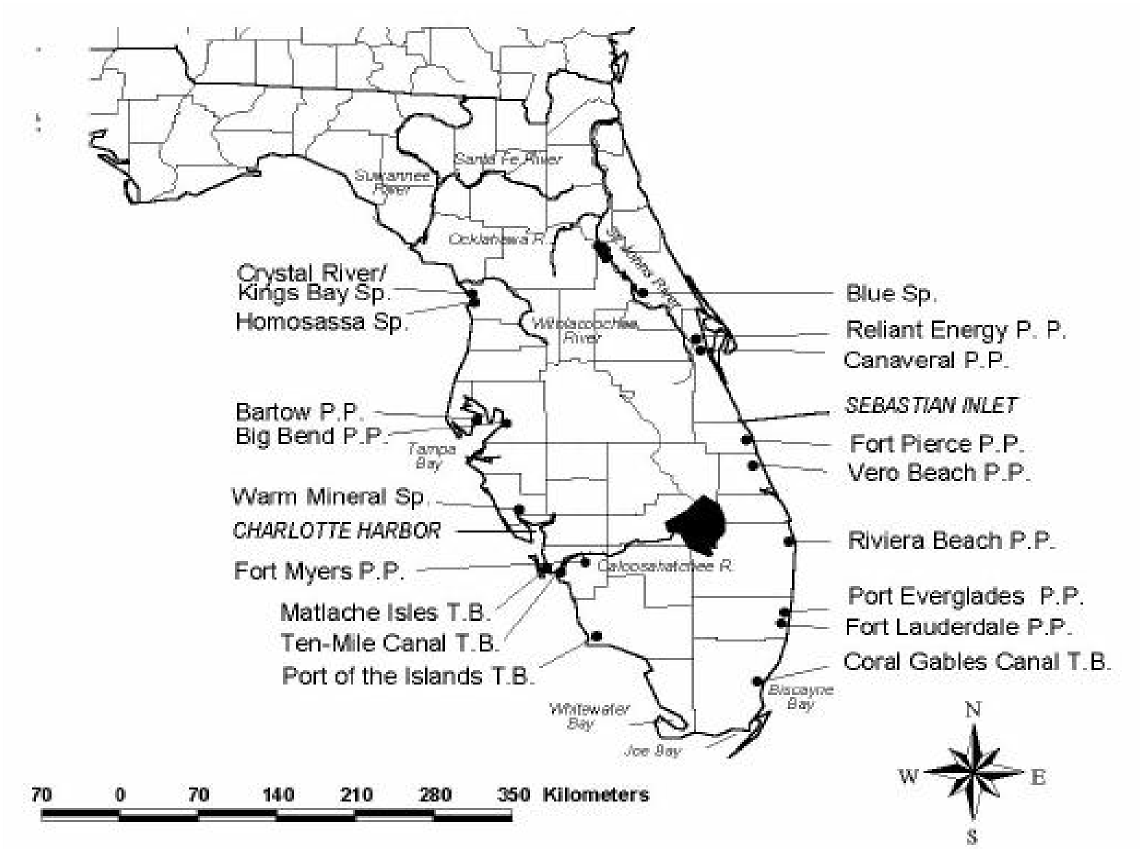

Figure 12. Location of Warm-water Refuges (Sp. = natural springs, P.P. = power plants, T.B. = thermal basin). Taken from Laist and Reynolds (2005).

On the West coast, however, one possibility why the mean $\delta^{15}N$ value is significantly lower there could be due to numerous rivers carrying agriculture water from inland locations that is less enriched (Figure 12). This notion is supported by a similar study by Mayer and colleagues in 2002 that showed lower mean $\delta^{15}N$ values by approximately 2‰ in forested and less developed areas compared to sewage-driven, urban locations.

Regional: SE vs. NE, NW, and SW

At the regional level, SE has the highest $\delta^{15}N$ values (Figure 9c), with an average of 10.2‰. This finding is consistent with the idea that densely-populated areas would correlate with the highest mean $\delta^{15}N$ value. Over the past decades, this region has consistently made up at least 33% of the state's total population ("Facts About Florida," 2012; Forstall, 1995). Many of the large cities and metropolitan areas are situated here, making it the eighth densely populated area in the U.S. (Carrie Futch et al., 2011). Thus, sewage pollution is most likely a major contributor of enriched N into aquatic systems in the same way that describes the entire East coast. While $\delta^{15}N$ did not change over time (Figure 10), the continued increase in human population in the SE region supports the idea that sewage is still a dominant source of enriched N. It is evident from the 1966 inventory that 6 of the 14 largest treatment plants were located in the SE while, in 1993, 10 of the 11 treatment plants along the East coast were situated along this area (York & Potts, 1995). In both inventories, none of those listed facilities provided beyond secondary sewage treatment (Bloetscher & Gokgoz, 2001). Most of the effluent was discharged on surface waters which could explain the very high $\delta^{15}N$ value.

Atmospheric deposition from coal burning implicated in the overall East coast enrichment cannot be excluded as a likely contributor in the SE region because several power plants are located along here (Figure 12). While the magnitude of this N source may not be as great relative to sewage, the combined effects of these enriched sources are more apparent here than elsewhere, including the NE region.

The fact that mean $\delta^{15}N$ values for the other regions are within 0.7‰ of each other (Figure 9c) suggests that mixing of enriched sources (sewage and atmospheric deposition from coal combustion) exist but is also influenced by other factors. For instance, the mean $\delta^{15}N$

difference between SE and NE is 2.3‰. Though they are located on the same coast, the primary difference between them lies in the greater pollution effect along the SE region that accounts for its very high mean $\delta^{15}N$ value. Both regions along the West coast, however, have mean $\delta^{15}N$ values that are significantly lower by at least 2.8‰ compared to SE. The explanation here is that the enrichment effect of sewage and coal burning is constrained by the presence of depleted N sources in the form of fertilizer and atmospheric deposition from vehicle combustion, resulting in overall mean $\delta^{15}N$ values that are much lower relative to the SE region.

Conclusions and Management Implications

The statewide decline in $\delta^{15}N$ over the last 35 years is most likely driven by agricultural fertilizer as a source of depleted N relative to other sources of N. High $\delta^{15}N$ values at coastal and regional scales point to sewage pollution and atmospheric deposition from power plants as potential sources of enriched N keeping $\delta^{15}N$ values at elevated levels. While $\delta^{15}N$ values along coastal waters in Florida appear to be on a downward trend, as observed through bone $\delta^{15}N$ values, much work is still needed to address the ongoing N pollution in light of this study's findings that mixing of various, anthropogenic N sources is likely occurring statewide. As water quality becomes an even bigger issue going forward, it is also important to emphasize that any management actions related to N pollution should be targeted to localized, specific sources unique to a particular area as well as the species and ecosystem that may be under threat.

APPENDIX A

RAW SUMMARY OF DATA

Reference ID	Field ID	Bone Type	$\delta^{15}N$	$\delta^{13}C$	Decade	Year	Month	Coast	Latitude	Region	County
AU059	UF13429 (M5)	LW	8.6	-10.6	1970	1975	February	East	North	NE	Brevard
AU035	M38	UP	7.3	-8.0	1970	1976	November	East	North	NE	Brevard
AU058	UF13888 (M39)	CA	9.3	-12.8	1970	1976	December	East	North	NE	Duval
AU057	UF13877 (M36)	LW	9.6	-18.8	1970	1976	November	East	North	NE	Duval
AU072	UF14039 (M82)	CA	7.3	-10.3	1970	1977	April	East	North	NE	Brevard
AU047	UF13918 (M67)	RB	6.1	-17.4	1970	1977	February	East	North	NE	Brevard
AU071	UF14033 (M80)	DG	6.9	-9.9	1970	1977	May	East	North	NE	Brevard
AU049	UF13991 (M78)	LW	11.3	-13.0	1970	1977	April	East	North	NE	Duval
AU048	UF13920 (M69)	LW	10.8	-16.0	1970	1977	February	East	North	NE	Duval
AU099	UF15162 (M7822)	LW	9.4	-9.1	1970	1978	March	East	South	SE	Martin
AU093	UF15161 (M7812)	LW	5.3	-16.2	1970	1978	February	West	South	SW	Collier
AU092	UF15160 (M787)	LW	11.0	-16.6	1970	1978	February	West	South	SW	Lee
AU094	UF15158 (M783)	LW	7.1	-13.7	1970	1978	January	West	South	SW	Lee
AU061	UF15117	RB	4.4	-7.7	1970	1979	June	East	North	NE	Brevard
AU079	UF15114 (M153)	LW	7.7	-10.3	1970	1979	May	East	North	NE	Brevard
AU073	UF15110 (M147)	LW	7.8	-11.0	1970	1979	May	East	North	NE	Brevard
AU138	NFWL (M152)	LW	9.4	-11.8	1970	1979	May	East	North	NE	Brevard
AU088	UF15127 (M174)	LW	6.4	-7.5	1970	1979	October	East	North	NE	Brevard
AU087	UF15125 (M170)	LW	7.3	-11.9	1970	1979	September	East	North	NE	Brevard
AU086	UF15123 (M168)	LW	10.8	-16.2	1970	1979	August	East	North	NE	Duval
AU075	UF15120 (M164)	RB	8.6	-11.6	1970	1979	July	East	North	NE	Duval
AU076	UF15121 (M165)	RB	9.7	-17.5	1970	1979	July	East	North	NE	Duval
AU078	UF15115 (M157)	LW	9.1	-10.1	1970	1979	June	East	North	NE	Duval
AU080	UF15113 (M151)	LW	9.6	-18.7	1970	1979	May	East	North	NE	Duval
AU074	UF15111 (M149)	LW	12.7	-22.3	1970	1979	May	East	North	NE	Putnam
AU085	UF15122 (M166)	UP	10.5	-19.7	1970	1979	August	East	North	NE	Volusia
AU110	UF15174 (M7921)	LW	13.8	-19.1	1970	1979	September	East	South	SE	Broward

AU101	UF15167 (M794)	LW	7.0	-8.2	1970	1979	February	East	South	SE	Martin
AU106	UF15173 (M7920)	LW	11.2	-2.3	1970	1979	September	East	South	SE	Miami-Dade
AU081	UF15112 (M150)	LW	11.7	-20.6	1970	1979	May	East	South	SE	Martin
AU104	UF15171 (M7917)	LW	10.8	-13.9	1970	1979	August	East	South	SE	Miami-Dade
AU105	UF15172 (M7918)	LW	10.4	-13.9	1970	1979	August	East	South	SE	Miami-Dade
AU077	UF15116 (M158)	LW	9.1	-8.8	1970	1979	June	West	North	NW	Manatee
AU103	UF15170 (M799)	LW	5.0	-9.3	1970	1979	February	West	South	SW	Charlotte
AU102	UF15169 (M798)	LW	7.6	-15.0	1970	1979	February	West	South	SW	Collier
AU109	UF15175 (M7922)	RB	10.1	-14.3	1970	1979	October	West	South	SW	Collier
AU108	UF15177 (M7925)	LW	10.1	-17.8	1970	1979	November	West	South	SW	Glades
AU100	UF15166 (M7903)	RB	4.8	-13.0	1970	1979	February	West	South	SW	Lee
AU090	UF15130 (M182)	LW	5.6	-7.9	1980	1980	January	East	North	NE	Brevard
AU067	UF15195 (M8017)	LW	13.7	-20.9	1980	1980	July	East	South	SE	Broward
AU069	UF15193 (M8016)	LW	13.8	-21.3	1980	1980	June	East	South	SE	Broward
AU063	UF15185 (M807)	LW	7.5	-8.1	1980	1980	February	East	South	SE	Martin
AU065	UF15187 (M8010)	LW	8.9	-14.2	1980	1980	March	East	South	SE	Martin
AU062	UF15184 (M806)	LW	10.5	-10.2	1980	1980	February	East	South	SE	Miami-Dade
AU068	UF15192 (M8015)	LW	11.8	-20.4	1980	1980	May	East	South	SE	St. Lucie
AU089	UF15129 (M180)	LW	8.7	-21.6	1980	1980	January	West	North	NW	Dixie
AU066	UF15190 (M8013)	LW	4.7	-11.3	1980	1980	April	West	South	SW	Lee
AU070	UF15191 (M8014)	LW	7.7	-14.4	1980	1980	April	West	South	SW	Lee
AU107	UF15183 (M805)	LW	11.3	-14.3	1980	1980	January	West	South	SW	Lee
AU064	UF15186 (M809)	LW	6.2	-10.1	1980	1980	February	West	South	SW	Monroe
AU008	M8132	LW	9.3	-11.0	1980	1981	March	East	South	SE	Miami-Dade
AU091	UF19134 (M8156)	LW	4.3	-16.2	1980	1981	July	West	South	SW	Collier
AU137	M8135 (MSW8135)	LW	5.3	-11.6	1980	1981	March	West	South	SW	Monroe
AU083	UF24977 (M396)	LW	8.5	-10.2	1980	1984	July	East	North	NE	Brevard
AU095	UF24980 (M401)	LW	7.8	-11.7	1980	1984	September	East	North	NE	Brevard
AU054	UF24969 (M376)	LW	8.4	-10.1	1980	1984	March	East	North	NE	Duval
AU082	UF24972 (M389)	UP	9.2	-10.8	1980	1984	June	East	North	NE	Indian River
AU084	UF24978 (M398)	LW	5.8	-7.1	1980	1984	August	East	North	NE	Volusia
AU045	UF24970	LW	7.7	-19.7	1980	1984	March	West	North	NW	Citrus
AU046	UF24971	LW	7.8	-20.0	1980	1984	April	West	North	NW	Levy

AU144	MSW37	RB	11.4	-19.4	1980	1984	November	West	South	SW	Hendry
AU098	UF24991 (M426)	HM	7.6	-10.3	1980	1985	February	East	North	NE	Brevard
AU096	UF24988 (M417)	LW	10.0	-15.1	1980	1985	January	East	North	NE	Brevard
AU116	UF25003 (M456)	LW	8.0	-7.1	1980	1985	June	East	North	NE	Brevard
AU060	UF25005 (M457)	LW	11.5	-15.1	1980	1985	June	East	North	NE	Brevard
AU114	UF25000	RB	6.3	-9.6	1980	1985	April	East	North	NE	Volusia
AU117	UF25004	LW	6.7	-9.9	1980	1985	June	n.d.	n.d.	n.d.	n.d.
AU097	UF24989 (M422)	LW	8.4	-20.4	1980	1985	February	West	North	NW	Citrus
AU111	UF24996 (M445)	LW	8.7	-10.1	1980	1985	April	West	North	NW	Hernando
AU113	UF24997 (M447)	LW	6.9	-8.8	1980	1985	April	West	North	NW	Hillsborough
AU168	M8613	LW	15.3	-20.0	1980	1986	March	East	South	SE	Broward
AU043	M8628	RB	8.4	-14.2	1980	1986	December	East	South	SE	Palm Beach
AU141	MSW75	RB	7.1	-15.6	1980	1986	March	West	South	SW	Collier
AU011	UCF9054	RB	11.3	-15.1	1990	1990	August	East	North	NE	Brevard
AU031	UCF9012	LW	8.8	-11.9	1990	1990	January	East	North	NE	Brevard
AU182	MJAV9037	LW	5.8	-12.6	1990	1990	August	East	North	NE	Duval
AU056	MSW270	LW	8.7	-17.9	1990	1990	April	West	South	SW	Collier
AU042	MSW267	UP	5.2	-12.3	1990	1990	April	West	South	SW	Lee
AU120	MSW288	RB	9.0	-18.2	1990	1990	December	West	South	SW	Lee
AU148	MSW240	RB	11.0	-15.4	1990	1990	January	West	South	SW	Lee
AU002	UCF9110	LW	11.3	-16.3	1990	1991	March	East	North	NE	Brevard
AU039	UCF9118	LW	6.9	-9.0	1990	1991	May	East	North	NE	Brevard
AU121	MNE9127	CA	10.8	-18.0	1990	1991	September	East	North	NE	Nassau
AU030	MSW9144	LW	5.5	-18.5	1990	1991	December	West	South	SW	Lee
AU036	UCF9232	HM	9.8	-7.3	1990	1992	December	East	North	NE	Brevard
AU015	UCF9231	PP	8.9	-13.3	1990	1992	December	East	North	NE	Brevard
AU130	MSE9234	UP	7.9	-9.1	1990	1992	December	East	South	SE	Martin
AU181	MEC9317	LW	5.7	-19.7	1990	1993	July	East	North	NE	Volusia
AU183	MNE9409	RB	7.0	-13.0	1990	1994	July	East	North	NE	Nassau
AU032	MSE9404	LW	10.4	-14.5	1990	1994	February	East	South	SE	Broward
AU129	MSE9406	UP	9.3	-17.1	1990	1994	February	East	South	SE	Miami-Dade
AU020	MEC9512	LW	5.5	-12.6	1990	1995	May	East	North	NE	Brevard
AU169	MSE9529	LW	12.5	-22.3	1990	1995	October	East	South	SE	Broward

AU027	MEC9664	UP	8.9	-8.6	1990	1996	October	East	North	NE	Indian River
AU021	MEC9660	RB	10.0	-22.5	1990	1996	September	East	North	NE	Indian River
AU146	MSE9618	LW	12.6	-22.6	1990	1996	May	East	South	SE	Miami-Dade
AU038	MNW9606	LW	5.9	-10.7	1990	1996	March	West	North	NW	Hillsborough
AU040	MSW96206	LW	8.8	-14.0	1990	1996	July	West	South	SW	Charlotte
AU005	MSW96141	RB	10.3	-23.1	1990	1996	April	West	South	SW	Collier
AU178	MSW96220	LW	5.8	-11.8	1990	1996	August	West	South	SW	Lee
AU180	MSW9627	LW	7.9	-14.6	1990	1996	February	West	South	SW	Lee
AU179	MSW9623	LW	5.6	-15.6	1990	1996	February	West	South	SW	Lee
AU125	MSW9619	CA	11.9	-23.0	1990	1996	January	West	South	SW	Lee
AU033	MSW9658	RB	7.0	-9.8	1990	1996	March	West	South	SW	Lee
AU142	MSW96125	RB	6.0	-14.1	1990	1996	March	West	South	SW	Lee
AU186	MEC9750	LW	7.8	-20.0	1990	1997	August	East	North	NE	Seminole
AU128	MNW9703	LW	8.6	-11.0	1990	1997	January	West	North	NW	Hillsborough
AU149	MSW9776	LW	9.6	-17.7	1990	1997	December	West	South	SW	Lee
AU143	MEC9844	LW	5.4	-10.5	1990	1998	August	East	North	NE	Brevard
AU166	MEC9843	LW	5.9	-21.9	1990	1998	August	East	North	NE	Brevard
AU026	MEC9858	LW	8.8	-11.7	1990	1998	December	East	North	NE	Brevard
AU022	MEC9807	LW	6.8	-10.9	1990	1998	February	East	North	NE	Brevard
AU172	MNW9823	LW	7.7	-13.0	1990	1998	August	West	North	NW	Pinellas
AU136	MSW9842	RB	5.1	-16.2	1990	1998	August	West	South	SW	Collier
AU171	MNW9937	LW	7.8	-12.3	1990	1999	December	West	North	NW	Hillsborough
AU025	MEC0001	LW	10.0	-8.8	2000	2000	January	East	North	NE	Brevard
AU123	MNW0029	RB	6.9	-15.6	2000	2000	October	West	North	NW	Citrus
AU135	MNW0023	RB	6.9	-18.5	2000	2000	June	West	North	NW	Wakulla
AU147	MSW00107	RB	13.2	-22.4	2000	2000	December	West	South	SW	Glades
AU009	MSW00103	LW	13.2	-22.9	2000	2000	November	West	South	SW	Glades
AU167	MSW0096	LW	6.8	-9.7	2000	2000	October	West	South	SW	Lee
AU017	MEC0113	LW	2.8	-8.9	2000	2001	February	East	North	NE	Brevard
AU024	MEC0112	SC	8.8	-13.2	2000	2001	February	East	North	NE	Brevard
AU134	MEC0121	RB	6.4	-8.7	2000	2001	March	East	North	NE	Brevard
AU160	SWFTm0119b	RB	8.9	-8.5	2000	2001	August	East	North	NE	Volusia
AU159	MSW01106	LW	4.8	-11.5	2000	2001	December	West	South	SW	Lee

AU170	MSW0166	LW	6.9	-11.6	2000	2001	May	West	South	SW	Lee
AU124	MSW0206	RB	5.1	-8.8	2000	2002	January	West	South	SW	Lee
AU044	MSE0337	HM	7.5	-12.2	2000	2003	December	East	South	SE	Palm Beach
AU007	MNW0339	LW	7.2	-10.4	2000	2003	September	West	North	NW	Pinellas
AU127	MSW0337	CA	8.2	-21.3	2000	2003	March	West	South	SW	Collier
AU164	MSW03167	LW	4.9	-11.9	2000	2003	November	West	South	SW	Glades
AU133	MSW0329	LW	3.5	-12.1	2000	2003	February	West	South	SW	Hendry
AU150	MSW03183	RB	4.7	-10.4	2000	2003	December	West	South	SW	Lee
AU153	MNE0419	RB	8.4	-11.3	2000	2004	June	East	North	NE	Duval
AU037	MSW0452	LW	6.6	-13.8	2000	2004	June	West	South	SW	Charlotte
AU185	MSW0462	LW	3.6	-10.1	2000	2004	July	West	South	SW	Lee
AU003	MEC0575	RB	7.8	-11.7	2000	2005	December	East	North	NE	Brevard
AU041	MEC0556	RB	6.0	-13.8	2000	2005	July	East	North	NE	Brevard
AU163	MSE0533	RB	6.2	-9.5	2000	2005	August	East	South	SE	Martin
AU028	MSW0547	C	8.4	-20.1	2000	2005	March	West	South	SW	Hendry
AU157	MSW05150	T	8.3	-16.8	2000	2005	December	West	South	SW	Lee
AU122	MSW0524	RB	5.4	-11.4	2000	2005	February	West	South	SW	Lee
AU012	MNE0631	LW	5.2	-9.1	2000	2006	September	East	North	NE	Flagler
AU140	MSE0650	RB	7.8	-10.3	2000	2006	November	East	South	SE	Broward
AU184	MNW0608	RB	4.0	-14.8	2000	2006	March	West	North	NW	Citrus
AU034	MNW0629	CA	5.5	-12.1	2000	2006	August	West	North	NW	Manatee
AU119	MSW0707	RB	10.7	-21.4	2000	2007	February	West	South	SW	Lee
AU176	MEC0820	PV	8.0	-12.7	2000	2008	April	East	North	NE	Brevard
AU013	MEC0881	HM	8.0	-12.8	2000	2008	November	East	North	NE	Brevard
AU156	MEC0826	RB	5.0	-11.9	2000	2008	April	East	North	NE	Indian River
AU006	MEC0843	LW	4.5	-9.8	2000	2008	June	East	North	NE	Indian River
AU016	MEC0870	RB	6.1	-12.0	2000	2008	October	East	North	NE	Indian River
AU132	MNE0813	RB	9.7	-20.0	2000	2008	May	East	North	NE	Putnam
AU155	MNE0806	LW	7.2	-9.5	2000	2008	April	East	North	NE	Volusia
AU177	MNE0811	RB	4.6	-9.9	2000	2008	May	East	North	NE	Volusia
AU131	MSE0812	LW	7.6	-10.7	2000	2008	March	East	South	SE	Martin
AU019	MSE0801	PV	12.3	-12.4	2000	2008	January	East	South	SE	St. Lucie
AU126	MSE0817	LW	7.0	-8.2	2000	2008	May	East	South	SE	St. Lucie

AU001	MNW0806	RB	9.1	-20.6	2000	2008	February	West	North	NW	Citrus
AU145	MNW0847	RB	3.0	-10.3	2000	2008	September	West	North	NW	Manatee
AU018	MNW0804	PV	7.1	-16.2	2000	2008	January	West	North	NW	Pinellas
AU139	MSW0829	RB	4.6	-10.5	2000	2008	April	West	South	SW	Lee
AU165	MSW0842	LW	12.8	-22.2	2000	2008	June	West	South	SW	Lee
AU154	MSW0821	RB	4.2	-10.5	2000	2008	March	West	South	SW	Lee
AU152	MSW0837	LW	5.4	-9.6	2000	2008	May	West	South	SW	Lee
AU175	MSW0836	LW	7.5	-15.1	2000	2008	May	West	South	SW	Lee
AU174	MSE0813	LW	6.5	-9.4	2000	2008	March	West	South	SW	Monroe
AU029	MEC0982	LW	7.8	-11.4	2000	2009	June	East	North	NE	Brevard
AU187	MEC1080	LW	3.7	-10.4	2010	2010	February	East	North	NE	Brevard
AU151	MEC10192	LW	7.7	-11.5	2010	2010	July	East	North	NE	Brevard
AU161	MEC10170	LW	7.0	-10.9	2010	2010	May	East	North	NE	Brevard
AU162	MNE1016	LW	6.7	-11.4	2010	2010	February	East	North	NE	Volusia

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