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Spatial distribution of submerged aquatic vegetation in the Shark River Estuary and implications for understanding movement and feeding patterns of manatees (Trichechus manatus latirostris)

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SPATIAL DISTRIBUTION OF SUBMERGED AQUATIC VEGETATION IN THE SHARK RIVER ESTUARY AND IMPLICATIONS FOR UNDERSTANDING MOVEMENT AND FEEDING PATTERNS OF MANATEES *(TRICHECHUS MANATUS LATIROSTRIS)*

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Virginia C. Cornett

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To: Interim Dean Mark D. Szuchman
College of Arts and Sciences

This thesis, written by Virginia C. Cornett, and entitled Spatial Distribution of Submerged Aquatic Vegetation in the Shark River Estuary and Implications for Understanding Movement and Feeding Patterns of Manatees (*Trichechus manatus latirostris*), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Date of Defense: July 14, 2006

The thesis of Virginia C. Cornett is approved.

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ABSTRACT OF THE THESIS

SPATIAL DISTRIBUTION OF SUBMERGED AQUATIC VEGETATION IN THE SHARK RIVER ESTUARY AND IMPLICATIONS FOR UNDERSTANDING MOVEMENT AND FEEDING PATTERNS OF MANATEES *(TRICHECHUS MANATUS LATIROSTRIS)*

by

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Florida International University, 2006

Miami, Florida

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The purposes of this study were to 1) characterize the distribution of submerged aquatic vegetation (SAV) in the Shark River Estuary, 2) determine water quality parameters driving distribution of SAV, 3) document the stable isotopic and elemental content of potential food sources for manatees, and 4) explore the utility of natural variability in isotope ratios of SAV in determining feeding patterns of manatees using isotopic compositions of manatee hairs as analytical tools. The marine/freshwater interface of the estuary was found to be the main factor driving SAV distribution. $\delta^{13}C$ of manatee hairs showed significant differences between captive (mean = -24‰) and wild (-15.7‰) manatees. These results show that manatee hairs reflect the isotopic compositions of their food sources. This provides researchers with the ability to explore manatee travel routes and food consumption habits across a broad spatial range using non-invasive techniques.

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Introduction

Tropical and subtropical estuaries are dynamic ecosystems known to be highly productive and ecologically-significant habitats (Zieman 1982; Twilley 1985; Zieman et al. 1989). The estuarine seagrass-mangrove communities of the Everglades ecosystem of southern Florida provide substantial primary and secondary productivity as well as nursery habitat and shelter, sediment stability, significant input into the detrital food chain, and serve as a food source for small invertebrate animals as well as macroherbivores such as sea turtles and manatees (Bjorndal 1980; Zieman et al. 1989; Fleming et al. 1990).

To date, there is little documentation of the submerged aquatic vegetation (SAV) communities of the ecologically-vital mangrove-dominated rivers and estuaries of southwest Florida. SAV beds are known to be primary habitat for many ecologically- and commercially-important animals in south Florida, including the endangered Florida manatee (*Trichechus manatus latirostris*), a large obligate herbivore that can consume up to 80 kg of vegetation per day (Bengtson 1983; Etheridge et al. 1985; Marshall et al. 2000). This animal, a subspecies of the West Indian manatee, is the largest aquatic herbivore in south Florida and is the only marine mammal in the U.S. known to feed directly on seagrasses (Bertram 1968; Thayer et al. 1984). Manatees' exceptional osmoregulatory capabilities allow them to move between fresh and salt water and to feed within all natural levels of salinity (Campbell and Irvine 1977; Ortiz et al. 1998); as such, they are known to consume considerable amounts of a wide range of vegetation from

both freshwater and marine environments (Bengtson 1983; Etheridge et al. 1985; Ledder 1986).

The Everglades, one of the largest wetland ecosystems in the world, is also one of the most threatened in the U.S. Historically, fresh water flowed into the rivers through the broad, shallow slough systems of the Everglades via sheet flow draining into Florida Bay in the south (through Taylor Slough) and the Florida Shelf to the southwest (through Shark River Slough [SRS]) (Light and Dineen 1994). Starting in the early 1900's, however, the natural flow of fresh water from the Kissimmee River into Lake Okeechobee and through the Everglades was gradually diverted via an extensive network of canals to accommodate agricultural and urban advancements. Construction of the Tamiami Trail highway in 1928 served as a further impediment to the southward water flow through Shark River Slough and the Everglades (Smith et al. 1989; Light and Dineen 1994). Since the 1960's, freshwater discharge to Shark River Slough has been almost completely controlled through water conservation areas and canals (Smith et al. 1989). These land-use and water management practices have had adverse effects on the ecosystems of southwest Florida. In an attempt to mitigate previous damage caused to the ecosystem, plans are underway to "... capture, store, and redistribute fresh water previously lost to tide and regulate the quality, quantity, timing and distribution of water flows" into south Florida through the Comprehensive Everglades Restoration Plan (CERP). This large-scale, interdisciplinary program is designed to restore more natural water flows into the Everglades while continuing to provide for the other water-related needs of the region. According to the Plan, an average of 26% more water will be delivered to the Shark River Slough than under current conditions, resulting in the

delivery of nearly a half million acre-feet of additional water to the slough (for a description of CERP, see Ogden et al. 2003).

Increasing the freshwater flow into the Everglades has the potential to significantly alter the habitat and change the distribution of vegetation throughout the ecosystem. The coastal areas of southwest Florida are considered "critical habitat" for the federally-protected and endangered Florida manatee (Fig. 1), which feeds on submerged aquatic vegetation in the area (Weigle et al. 2001). As such, a better understanding of the community structure of manatee's potential food sources and controls of food composition is essential in their protection and management.

There are two individual components to this thesis. Chapter 2 describes water quality parameters and biogeochemical characteristics and distribution of SAV, a potential food source for manatees, in the Shark River in Everglades National Park (ENP) on the southwest coast of Florida, providing information on the vegetation communities upon which manatees are known to feed. Chapter 3 discusses the utility of stable isotopic composition of hairs for investigating feeding ecology of manatees, as well as the factors controlling nutritive values of SAV and stable isotopic and elemental content. The wide variety of SAV present in the sheltered rivers of ENP is potentially a critical food source for these endangered mammals. A more thorough understanding of the parameters driving the distribution and abundance of SAV in the area will provide valuable information to managers and engineers for current and future modifications to the environment and their potential affects on manatees.

Fig. 1. Florida Manatee Critical Habitat (black areas) as defined by Florida Fish & Wildlife Service, September 1976, Federal Register 41. Study area is identified in red.

Chapter 2

Spatial Distribution of Submerged Aquatic Vegetation in the Shark River Estuary in Relation to Water Quality

Introduction

The Shark River estuary is located in western Everglades National Park (ENP) and is formed from the freshwater runoff of the Shark River Slough (SRS). It is one of the largest estuaries of the southwest Florida coast (Fig. 2). The emergent marsh plant *Cladium* sp. dominates the freshwater wetland areas, mangrove forests fringe the coastal area, and marine subtidal vegetation is dominated by seagrasses (*Thalassia testudinum* and *H. wrightii*) (Zieman et al. 1989; Fleming et al. 1990; Jaffe et al. 2001). The mangroves, which line the river up to 20 km inland, give the river waters a dark brown coloration as a result of the tannin complexes produced by the trees. Lying within subtropical latitudes, this area experiences extreme wet and dry seasons with between 60% to 75% of the rainfall occurring during the wet season of May through October (Schomer and Drew 1982; Chen and Twilley 1999) and has a typical tidal range of approximately 1.1 m (Provost 1973; Twilley 1985). The phosphorus-limited freshwater and nitrogen-limited intertidal areas (Jaffe et al. 2001), coupled with extreme wet/dry seasonal patterns and varying tidal inundations, narrow the breadth of vegetation suited to survive in such extreme environments (Boyer et al. 1997; Chen and Twilley 1999; Jaffe et al. 2001).

Seasonal patterns of rainfall, water level, and temperature strongly influence the water quality in the Shark River (McPherson 1970). Higher salinities and lower water

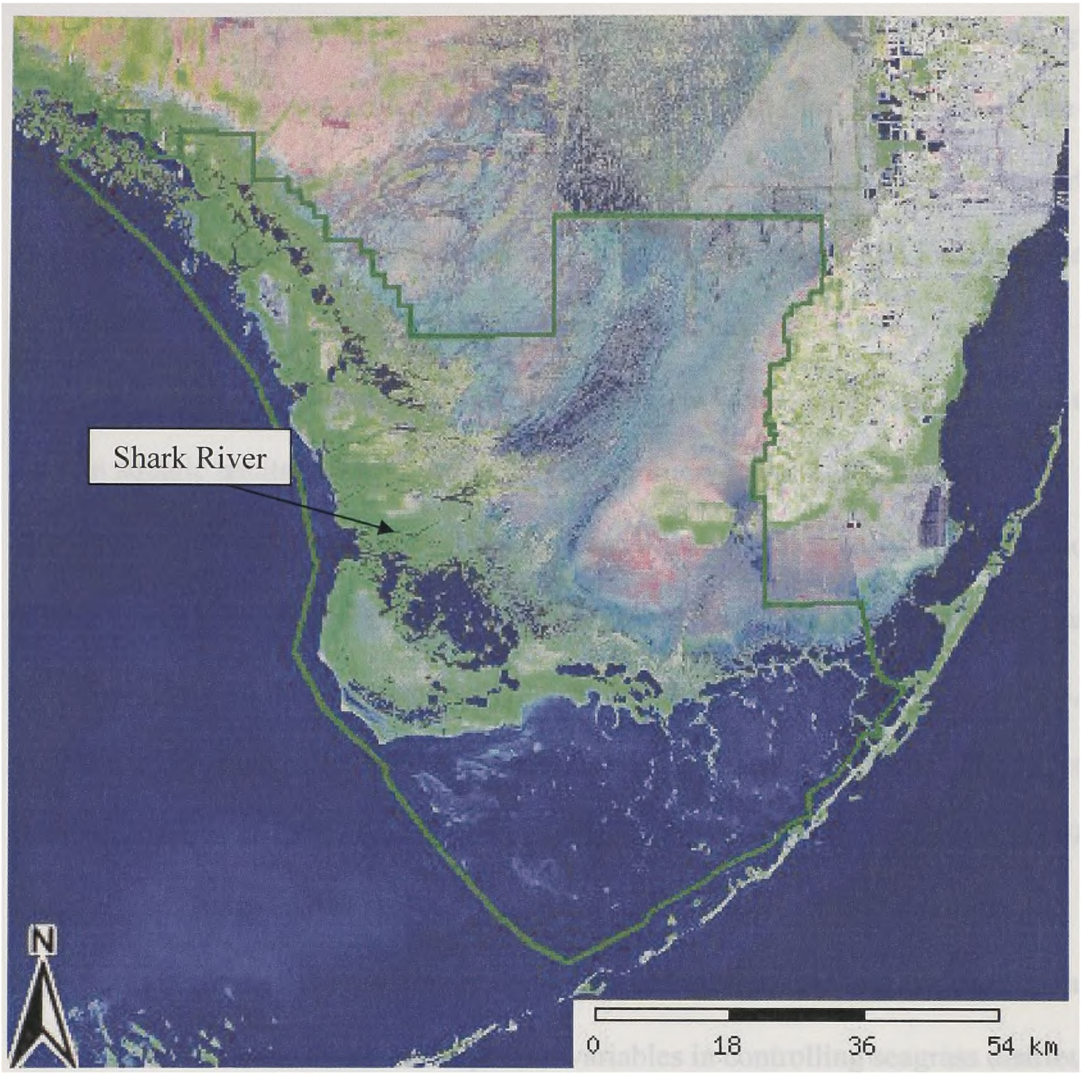


Fig. 2. Satellite representation of the study region. Green line delineates the boundaries of Everglades National Park. (www.fcelter.fiu.edu/maps/)

depths occur during dry seasons (November – April) and the reverse during wet seasons (May – October). Salinity also exhibits a spatial pattern with fresh waters at the headwaters, brackish south and west of Tarpon Bay and marine near the mouth. The bottoms of these rivers is mainly exposed limestone, but organic debris can be found in the more still waters upstream and along the peat/marl banks.

To date, there is little documentation of the SAV communities of the ecologically-vital mangrove-dominated rivers and estuaries of southwest Florida. SAV beds are known to be primary habitat for many of the ecologically- and commercially-important animals in south Florida, including shrimp, fishes, and the endangered Florida manatee (*T. manatus latirostris*) (Odell 1976; Hartman 1979; Thayer et al. 1984).

A variety of physical, chemical, and biological factors control distributions of SAV in aquatic habitats. Light availability is a primary factor determining the distribution of aquatic autotrophs (Dennison and Alberte 1985; Hall et al. 1999), though depth, turbidity, physical disturbance, current velocity, tidal inundation frequency and amplitude, grazing, nutrient availability, mean salinity, salinity variability, siltation, and sedimentary characteristics can also be important (Dennison and Alberte 1985; Snedaker 1989; Robblee et al. 1991; Gunderson 1994; McIntyre et al. 1996; Vermaat et al. 1996; Cahoon et al. 1999; Hall et al. 1999; Longstaff and Dennison 1999). For example, salinity, salinity variability, sediment depth, amount of light reaching the benthos, and mean nutrient concentrations are important variables in controlling seagrass distribution in Florida Bay (Fourqurean et al. 1993). There is a high degree of interrelatedness between many of these factors that make it difficult to determine the primary factor (e.g. depth and turbidity both affect light attenuation) affecting SAV distribution. Further confounding this is the fact that different species of submerged plants have different habitat requirements (Koch 2001).

Human activities often have negative effects on estuarine communities. As such, it is important to understand the relationships between water quality and submerged macrophytes, which are a dominant feature of the nearshore marine environment in south

Florida. Multiple water quality parameters can control the distribution of vegetation in aquatic habitats (Vannote et al. 1980; Dennison et al. 1993; Longstaff and Dennison 1999). The goal of this project was to describe the community composition of the SAV in the Shark River and explore the relationships between water quality and the composition of the SAV communities. A survey to document the distribution, density, and species composition of SAV in the Shark River was conducted during a 12-month period beginning July 2002. The SRS is the largest freshwater source in the Everglades and contains representatives of most habitat types in the region and was therefore selected as the location for this study.

Methods

Study Sites

Two different sampling designs were employed to investigate temporal and spatial distributions of SAV along the Shark River. Six primary survey sites were selected at about 5-km increments along the river beginning at Ponce de Leon Inlet in the Gulf of Mexico and ending approximately 20-km upstream at the river's headwaters (Fig. 3). These sites were selected to coordinate with existing water quality monitoring programs in the Shark River and were visited monthly from July 2002 through June were located at Florida Coastal Everglades Long-Term Ecological Research (FCE-LTER) water quality monitoring sites (SRS 3, 4, 5, and 6) (Table 1). SERC 39 and LTER 5 are located at the same coordinates. Site 20 was not surveyed for benthic vegetation due to inaccessibility by boat.

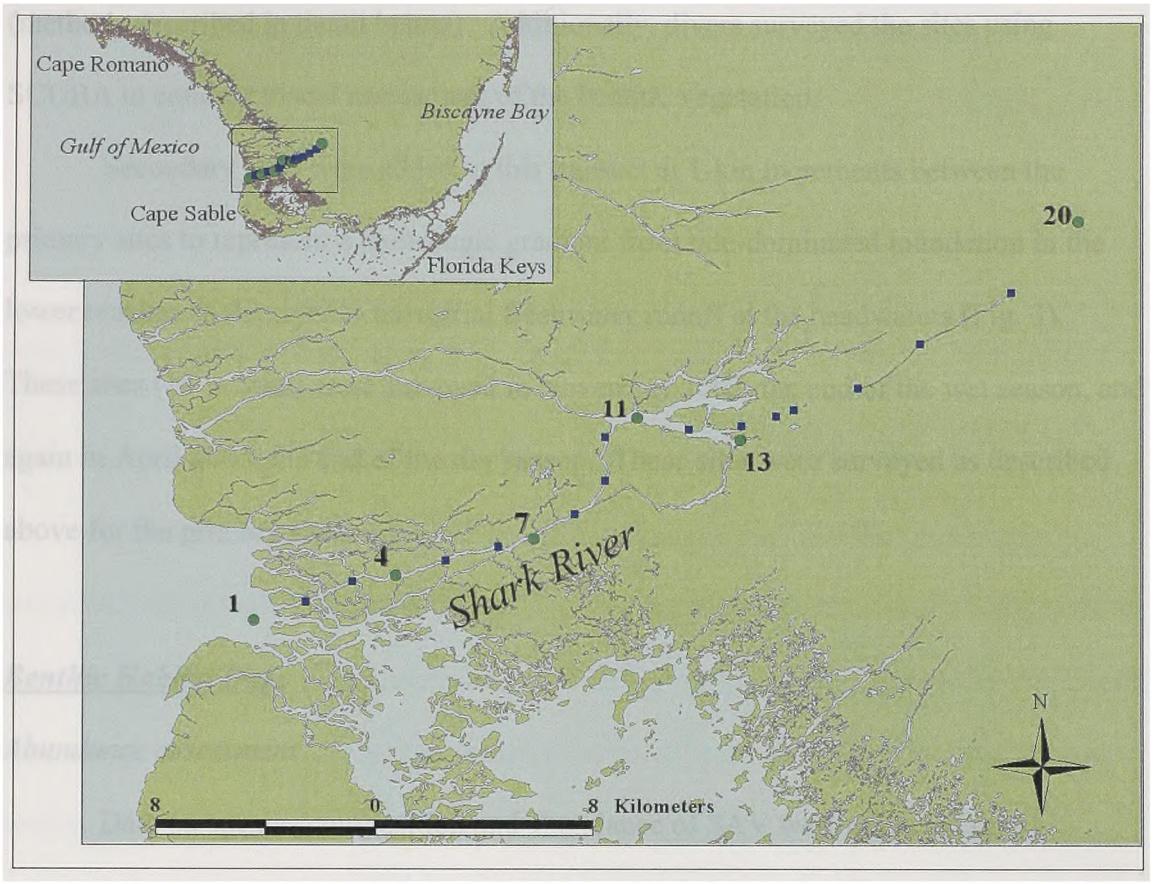


Fig. 3. Map of sites included in this project. Primary sites are represented by green symbols, secondary sites are represented by blue symbols.

	This Project	SERC	LTER	Latitude (°N)	Longitude (°W)
Ponce de Leon Inlet	1	40	---	25.3497	-81.1246
Shark River	4	---	6	25.3646	-81.0780
Gunboat Island	7	39	5	25.3770	-81.0323
Tarpon Bay	11	38	---	25.4173	-80.9984
Avocado Creek	13	---	4	25.4098	-80.9643
Sawgrass Marsh	20	---	3	25.4682	-80.8533

Table 1. Shark River Slough area primary site coordinates. Primary site locations were coordinated with Southeast Environmental Research Center (SERC) and Florida Coastal Everglades Long-Term Ecological Research (FCE-LTER) monitoring site locations.

Each month and at each of the primary sites, temperature, salinity, dissolved oxygen, turbidity, secchi depth, water depth, and light attenuation were recorded

(methods described in detail below). Additionally, divers surveyed the sites using SCUBA to conduct visual assessment of the benthic vegetation.

Secondary sites were added to this transect at 1-km increments between the primary sites to represent a hydrologic gradient from tide-dominated inundation in the lower reaches of the river to terrestrial freshwater runoff at the headwaters (Fig. 3). These sites (14 in total) were surveyed in November 2002, the end of the wet season, and again in April 2003, the end of the dry season. These sites were surveyed as described above for the primary sites.

Benthic Habitat Data

Abundance assessment

Data on species composition and abundance of SAV were collected on 81 occasions at a total of 19 sites along the Shark River between July 2002 and June 2003. At each site, Braun-Blanquet cover-abundance surveys were conducted (Braun-Blanquet 1972) to assess abundance of seagrass and macroalgae. Percent coverage of benthic taxa was recorded from 10 0.25m^2 quadrats haphazardly located within 10-m of the differential global positioning system (DGPS) coordinates of the station and examined using SCUBA. All macrophyte species occurring in each quadrat were listed and a score based on the cover of the species in that quadrat was assigned (Table 2). Cover is defined as the fraction of the total quadrat area that is obscured by a particular species when viewed from directly above. Braun Blanquet density for each taxon, i, (D_i) was calculated by summing the abundance scores for that taxon for all quadrats at a site, and then dividing by the number of observed quadrats.

Score	Cover
0	Taxa absent from quadrat
0.1	Taxa represented by a solitary shoot, <5% cover
0.5	Taxa represented by a few (<5) shoots, <5% cover
1	Taxa represented by many (>5) shoots, <5% cover
2	Taxa represented by many (>5) shoots, 5% to 25% cover
3	Taxa represented by many (>5) shoots, 25% to 50% cover
4	Taxa represented by many (>5) shoots, 50% to 75% cover
5	Taxa represented by many (>5) shoots, 75% to 100% cover

Table 2. Braun-Blanquet density scores.

Statistical Analyses of Benthic Vegetation

Each sampling occasion was treated as a separate case and Braun-Blanquet densities were used to define the groups. Site 20 was omitted from this analysis as there were no benthic vegetation data available. Number and percentage of sites in which each taxa occurred and number and percentage of surveys in which each taxa occurred were recorded. Braun-Blanquet density scores were calculated and shown as mean, median, minimum, and maximum score for each site. A hierarchical clustering algorithm using the presence/absence (presence = 1, absence = 0) of each vegetation type was used to group areas of similar benthic vegetation. Binary values were assigned based on whether a taxa had been present or absent at each site (19 total) during the entire sampling period (12 months). Similarities were measured using the binary Jaccard coefficient, which measures degree of overlap between two sets.

Water Quality Data

Data Collection

Water quality data were collected on 83 occasions at a total of 19 sites along the Shark River between July 2002 and June 2003. Water temperature, salinity, and

dissolved oxygen values were measured using an Orion (model 142) meter. Upon arrival at a site, the meter probe was placed approximately 0.5-m below the surface of the water to obtain a surface measurement. Time was allowed for values to stabilize before recording the data. The probe then was lowered to within 0.5-m of the bottom to a obtain bottom measurement. Temperature was recorded in degrees Celsius, salinity was recorded in parts per thousand, and dissolved oxygen was recorded in mg/L.

Upon arrival at a site, two standard plastic scintillation vials were filled with seawater for assessment of turbidity. These replicate water samples were analyzed using a DRT-15CE turbidimeter (HF Scientific, Inc.). Samples were shaken before being placed in the Turbidimeter and a value was read after a 10-second stabilization period. The replicate sample readings were averaged together and the numeric value obtained is reported to the nearest tenth in nephlometric turbidity units (NTU).

Secchi values are used to describe water clarity at a given point in time. Upon arrival at a site, and if water clarity was such that the bottom could not be seen, a standard limnology/oceanography secchi disk (alternating black and white pattern) was lowered into the water until the point that it was no longer visible; that depth then was measured in centimeters and recorded. If the bottom was visible, secchi values were recorded as the depth of that particular site in centimeters.

The underwater light environment was measured using a 4 π PAR sensor. Profiles of light intensity in the water column were obtained and attenuation coefficients were calculated by fitting the profiles to the Lambert-Beer Law, $I_z=I_0e^{kz}$, where $I_0 = 1$, and z = depth. The amount of light reaching the benthic vegetation was calculated as the fraction of the incoming solar radiation measured at the bottom of the water column.

Sediment depth was recorded at each quadrat at each site. Sediment depth was measured using a steel rod marked in 5-cm increments. The rod was pushed into the sediment until it contacted solid rock or reached a depth >50 cm. Sediment texture was qualitatively assessed and categorized as mud, muddy-sand, sandy mud, sand, and rock.

Water Column Nutrient Analyses

Water samples for SERC and LTER sites were collected from 10-cm below the surface of the water. Samples were analyzed for chlorophyll *a*, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), alkaline phosphatase activity, turbidity, soluble reactive phosphorus, nitrate, nitrite, and ammonium. Analytical methods are described in Boyer et al 1999.

Water quality samples for LTER sites also were collected using ISCO autosamplers. The autosamplers contain 24 1L bottles. Water is sampled by programming the autosamplers to take composite samples once every 3 days. These samples are a composite of four 250-mL subsamples drawn every 18 hours (a sampling scheme that captures a dawn, noon, dusk, and midnight sample in every 3-day composite). The samples are collected from the autosamplers every 3-4 weeks and analyzed for TP, TN, and salinity using the methods described in Boyer et al. 1999.

Statistical Analyses of Water Quality

Preliminary descriptions of the water quality data were calculated as mean, standard deviation (SD), median, and minimum and maximum values at each site and

time. Bivariate correlations were constructed to investigate covariances between the water quality parameters.

Based on results of the correlations, variables that did not covary with each other were chosen as proxy variables to represent the group of correlated variables. TN, TP, TOC, irradiance at bottom, and sediment depth each adequately described most of the variation in the data and were chosen so as to reduce the large quantity of data to a manageable size. Salinity did covary with all three nutrient components but was added as a proxy variable to help describe the conditions along a salinity gradient.

Since water quality and benthic habitat data were not necessarily collected at the same times, continuous surfaces for these water quality parameters were interpolated from the survey data points and were used to portray spatial and temporal variation in the proxy water quality parameters as a function of date and position in the river. These continuous surfaces were calculated using triangulation with linear interpolation using Golden Software, Inc.'s surface mapping system Surfer ® (v. 7). In order to direct emphasis to the spatial rather than temporal scale, the number of lines in the grid line geometry was set at 50 for the x axis and 100 for the y axis and anisotropy for triangulation was set at 0.1. This anisotropy setting maximized the influence of points collected during a sampling event on the interpolation to give more realistic representations of the marine-estuarine gradient. This method is an exact interpolator and uses an algorithm to draw lines between data points to create triangles resulting in a patchwork of triangular zones over the extent of the grid. This method honors the true data very precisely because the original data are used to define the triangles. These

continuous surfaces were then sampled at the time and location of the benthic data collections so that a complete data set could be generated.

For each benthic habitat cluster, a mean, SD, and coefficient of variation (CV) (CV = SD/mean) was calculated for each of the proxy variables.

Results

Benthic Habitat Data

Visual assessment surveys identified 21 taxa of submerged plants at the 19 surveyed sites (Table 3). In addition, eight taxa were noted as present at a site but not found within any observed quadrat. Submerged vegetation was very sparse in the study area and each site had a unique composition of the benthic macrophyte community. Only four vascular plant species, *H. wrightii, Halophila decipiens*, *Ruppia maritima*, and *Najas guadalupensis*, were identified at any site. *H. wrightii* was located near Site 1 but only after a search of Ponce de Leon Bay that extended well beyond a 50-m radius of the site coordinates; it was located north of the site and in shallow (<2 m) water in fine-mud sediment. *H. decipiens* was present only at Site 1, occurring at 26.3% of the surveys and only in July and March, April, May, and June (Table 4). The highest density for *H decipiens* was 1.5, which occurred in June. *R. maritima* was present only at Site 13 and only in October and November with densities of 0.20 and 0.30, respectively. *N. guadalupensis* was present only at Sites 14 (visual observation, species not present within any quadrat) and 16 (density of 1.4) and only during November.

Taxa	Site 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Seagrass																			
Halophila decipiens	*																		
Halodule wrightii	***																		
Algae																			
Acanthophora sp.	*			**															
Caloglossa sp.	*				*	*													
Caulerpa fastigiata	*	*	*	*	*	*	*	*	*	*	*	*							
Caulerpa mexicana	**																		
Caulerpa prolifera	**																		
Caulerpa sertularoides	*																		
Caulerpa verticillata	*	*	*	*	*														
Chara sp.													*	*					
Chondria sp.	**			**															
Coralline Red Algae	*	*																	
Dasya sp.	*	**																	
Gracilaria sp.	*			*	**	*	*	*			*								
Halymenia sp.	*																		
Laurencia sp.	*																		
Rhizoclonium sp.											*		*					*	*
Solieria sp.	*																		
Udotea sp.	*	**	*																
Ulva sp.	***																		
Green Other	*	*					*												
Red Other	*			*			*				*								
Drift Red	*																		
Turf		*	*	*	*	*	*	*	*	*	*								
Freshwater Vascular																			
Najas guadalupensis														**		*			
Ruppia maritima													*						
Animals																			
Hydroids	*	*	*	*	*														
Octocorals	*																		
Tunicates	*		*	*		*													
Sponges	*	*	*	*															
Total # of Taxa	**20**	**7**	**7**	**8**	**5**	**5**	**5**	**3**	**2**	**2**	**5**	**1**	**3**	**2**	**0**	**1**	**0**	**1**	**1**

Table 3. Presence/Absence occurrence matrix of benthic taxa along Shark River.

* taxa present in at least one quadrat; no symbol signifies taxa not observed in any quadrat at site
** taxa identified at site but not within quadrat
*** taxa identified near site but not within 50 meters of coordinates

Five species of the genus *Caulerpa* (Division Chlorophyta) were identified as occurring along the river – *Caulerpa fastigiata*, *C. mexicana*, *C. prolifera*, *C. sertularoides*, and *C. verticillata*. *C. fastigiata* was the most commonly occurring

Taxa	# of sites at which taxa occurred	% of sites at which taxa occurred	# of surveys in which taxa occurred	% of surveys in which taxa occurred	Mean Braun-Blanquet Density	Median Braun-Blanquet Density	Max Braun-Blanquet Density
Seagrass							
Halophila decipiens	1	5.3%	5	26.3%	0.65	0.55	1.5
Algae							
Acanthophora sp.	1	5.3%	2	10.5%	0.15	0.15	0.15
Caloglossa sp.	3	15.8%	1	5.3%	0.04	0.05	0.05
Caulerpa fastigiata	12	63.2%	8	42.1%	1.36	0.75	3.9
Caulerpa sertularoides	1	5.3%	2	10.5%	0.06	0.06	0.1
Caulerpa verticillata	5	26.3%	10	52.6%	0.97	0.90	2.3
Chara sp.	2	10.5%	6	31.6%	0.37	0.40	0.9
Coralline Red Algae	2	10.5%	8	42.1%	0.73	0.70	1.4
Dasya sp.	1	5.3%	2	10.5%	0.21	0.21	0.35
Gracilaria sp.	6	31.6%	9	47.4%	0.36	0.28	1.4
Halymenia sp.	1	5.3%	2	10.5%	0.06	0.06	0.06
Laurencia sp.	1	5.3%	1	5.3%	0.10	0.05	0.1
Rhizoclonium sp.	4	21.1%	3	15.8%	0.94	1.12	1.3
Solieria sp.	1	5.3%	4	21.1%	0.29	0.35	0.41
Udotea sp.	2	10.5%	3	15.8%	0.07	0.05	0.1
Green Other	3	15.8%	2	10.5%	0.05	0.05	0.05
Red Other	4	21.1%	6	31.6%	0.21	0.11	0.8
Drift Red	1	5.3%	2	10.5%	0.15	0.15	0.2
Turf	10	52.6%	4	21.1%	0.52	1.38	3
Freshwater Vascular							
Najas guadalupensis	1	5.3%	1	5.3%	1.40	0.00	1.4
Ruppia maritima	1	5.3%	2	10.5%	0.25	0.25	0.3
Animals							
Hydroids	5	26.3%	8	42.1%	0.29	0.26	0.7
Octocorals	1	5.3%	2	10.5%	0.06	0.06	0.1
Tunicates	4	21.1%	5	26.3%	0.56	0.40	1.8
Sponges	4	21.05%	11	57.9%	0.98	0.80	2.6

Table 4. Summary of taxa distribution, including the number and percentage of sites and surveys where each taxa was present and the maximum, median, and mean Braun-Blanquet density scores for each taxa.

Caulerpa species and was present at 63.2% of the sites and 10% of the time at Site 1, 36% of the time at Site 4, 80% of the time at Site 7, 55% of the time at Site 11, and never encountered at Site 13. *C. fastigiata* occurred most frequently during the dry season sampling where it was present at 80% of the sites. *C. fastigiata* occurred at only 30% of the sites during the wet season sampling. Dry season densities for *C. fastigiata* were

highest, reaching 3.90 at Site 9. *C. mexicana* and *C. prolifera* were observed at Site 1 but were not located within a quadrat. *C. verticillata* occurred only at Sites 1-5 and was present in at least one quadrat at Site 1 during every sampling. *C. verticillata* reached its highest densities at Sites 1, 2, and 3 with densities ranging from 0.10 to 2.30 with a median of 1.30 for those sites. *C. sertularoides* was observed only at Site 1 and only during July, October, and November, with a median density of 0.20.

The alga *Chara* sp. (Division Charophyta) occurred only at Sites 13 and 14. It was present 55% of the time at Site 13 and during the wet season sampling at Site 14. Densities at Site 13 ranged from 0.01 to 0.90 with a median of 0.40. It was always patchily distributed in very fine mud sediment of >50 cm. The green alga *Rhizoclonium* sp. (Division Chlorophyta) occurred at Sites 11, 13, 18, and 19. At Site 13, *Rhizoclonium* sp. was usually found intermingled with *Chara* sp.

Ulva sp. (Division Chlorophyta) was identified near Site 1 but not within 50 m of the site coordinates and was observed only once. It was found attached to *Rhizophora mangle* prop roots along the northern edge of Ponce de Leon Inlet; this area was investigated only during collection of terrestrial species present along the mangrove fringe of the river.

The genus *Udotea* was encountered only at Sites 1 and 3. This calcareous green alga was present 18% of the time at Site 1 and only in the dry season at Site 3. Density for Site 3 was 0.10 and median density for Site 1 was 0.05.

Red algae (Division Rhodophyta) were represented at 42% of the sites and were most abundant at Site 1, occurring 90% of the time with densities reaching 1.60 with a median density of 0.80. Seasonal patterns in red algal density at this site occurred in the

months of February through June with a median value of 0.80 for that time period and 0.30 for the remainder of the year. Red algal species identified include *Acanthophora* sp., *Laurencia* sp., *Gracilaria* sp., *Dasya* sp., *Halymenia* sp., *Solieria* sp., and *Caloglossa* sp. Coralline red algae accounted for the majority of red algae encountered at Site 1 throughout the survey, representing 100% of red algal density in the months from October through January. Other red algal species at this site occurred most frequently in the months of January through May, with *Solieria* sp. and an unidentified red (Red Other) occurring most frequently at 40% and 50% of the time, respectively with mean densities of 0.12 and 0.08, respectively. Red algal presence was highest in the months from February through June, appearing an average of 56% of the time during those months as opposed to an average of 11% of the time during the remaining months. Red algal species were present at Sites 1 through 11 with the exceptions of Sites 3, 9, and 10. *Gracilaria* sp. was present at 31.6% of these sites with coralline red algae, *Caloglossa* sp., and Red Other present at 10.5%, 15.8% and 21.1% of these sites, respectively. *Gracilaria* sp. densities were highest at Sites 6, 7, and 8, representing an average of 0.34 during the wet season sampling and 0.57 during the dry season sampling period.

The functional group called "Turf" was created to represent a group of algal species that was not possible to accurately identify in a field setting. This group was found attached to bedrock and other hard surfaces in the lower reaches of the river. Representatives of this group are *Gelidium* sp., *Bostrychia* sp., *Polysiphonia* sp. and similar taxa. The Turf group was represented at 52.6% of the sites but was seen only at Sites 2 through 9 and only present at the sites during the wet season sampling period; it

was seen at Site 7 during October as well. Turf densities reached 3.00 at Site 7 and represented a mean density of 1.66.

The only animals included in the data were hydroids, octocorals, sponges, and tunicates. Hydroids were present only at Sites 1 through 5. They were present 60% of the time at Site 1 and 30% of the time at Site 4; they did not occur at either of these sites between July and November. Highest densities were reached at Site 1 with a median density of 0.25. Octocorals were represented only at Site 1 and were present only 20% of the time and in densities of 0.1 and 0.01 for the months of April and June, respectively. Sponges occurred only at Sites 1 through 5 (26% of the sites). They occurred in at least one quadrat during each sampling at Site 1 and 26% of the time at Site 4. Sponge densities averaged 0.79 at Site 1 and 0.92 at Site 4. Tunicates occurred only at Sites 1, 3, 4, and 6, most frequently at Site 1 (30% of the time) but in highest densities at Sites 4 (0.88) and 5 (1.1).

Overall, Site 1 had the highest number of benthic taxa present at any time where Sites 15 and 17 had the lowest with no benthic taxa present; 20 taxa were identified at Site 1, 8 at Site 4, 5 at Site 7, 5 at Site 11, and 3 at Site 13. Only five taxa were found at sites other than Site 1. *R. maritima*, *N. guadalupensis*, *Chara* sp., *Rhizoclonium* sp., and the turf algal complex did not occur at Site 1.

Of the 81 sampling events (includes each site sampled for each time sampled), cluster analysis identified five distinct benthic macrophyte community types defined by species composition and density (Table 5, Fig. 4). Cluster 1 ($n = 11$) included only Site 1 (Ponce de Leon Inlet) and is identified as the "Gulf" cluster. Cluster 2 ($n = 17$) contained Sites 2 through 5, sites at the lower reaches of the river, and is thus identified as the

	Taxa	Community Type (Cluster Membership) Gulf (n = 11)	Lower (n = 17)	Middle (n = 31)	Upper Soft (n = 17)	Upper Hard (n = 6)
Braun-Blanquet density (Mean ± SE)	*Acanthophora* sp.	0.03 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Coralline Red algae	0.60 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Caulerpa sertularoides	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Caulerpa verticillata	0.99 ± 0.22	0.33 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Dasya sp.	0.04 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Drift Red Algae	0.03 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Halymenia sp.	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Halophila decipiens	0.30 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Hydroids	0.23 ± 0.07	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Laurencia sp.	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Octocorals	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Red Other	0.07 ± 0.03	0.01 ± 0.01	0.04 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
	Solieria sp.	0.11 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Sponges	0.72 ± 0.13	0.64 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Udotea sp.	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Tunicates	0.04 ± 0.02	0.21 ± 0.11	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
	Turf Algae	0.00 ± 0.00	0.16 ± 0.08	0.55 ± 0.19	0.00 ± 0.00	0.00 ± 0.00
	Caulerpa fastigiata	0.01 ± 0.01	0.07 ± 0.04	1.10 ± 0.24	0.00 ± 0.00	0.00 ± 0.00
	Gracilaria sp.	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
	Chara sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.06	0.00 ± 0.00
	Rhizoclonium sp.	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.21 ± 0.11	0.00 ± 0.00
	Ruppia maritima	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00
	Najas guadalupensis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.06

Table 5. Density, on a Braun-Blanquet scale, of benthic vegetation in each of the benthic habitat classes. The number of sites (n) supporting each habitat type is indicated. Values given are mean density ± 1 Standard Error (SE).

"Lower" cluster. Sites 6 through 12, which lie along the mid-reaches of the river, fell into a single cluster identified as the "Middle" cluster (n = 31). The sites in the upper river fell into two distinct clusters: Cluster 4 (n = 17) included Sites 13, 14, 18, and 19, and the remaining three sites, 15 through 17, fell into their own cluster, Cluster 5 (n = 6). Cluster 4 is identified as the "Upper Soft" cluster and Cluster 5 as the "Upper Hard" cluster due to their unique sediment depths.

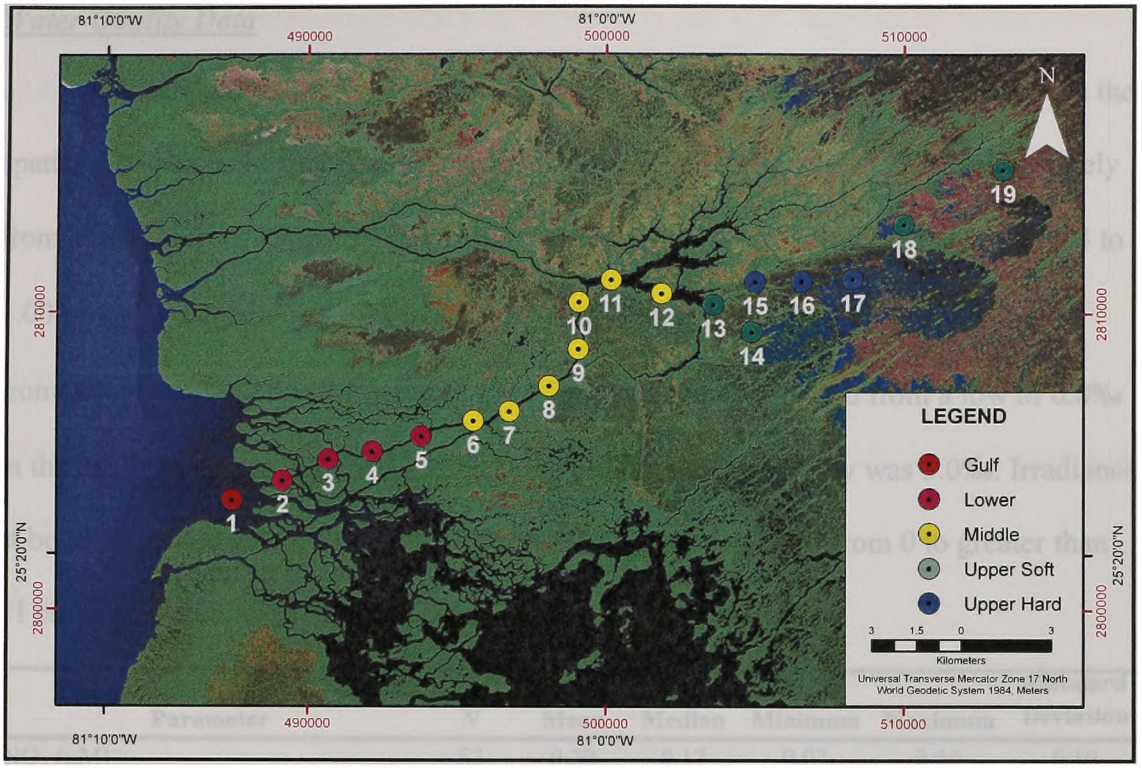


Fig. 4. Memberships of benthic vegetation as a result of cluster analysis. Gulf cluster is red, Lower is pink, Middle is yellow, Upper Soft is light blue, and Upper Hard is dark blue.

The Gulf cluster had the greatest taxa diversity (14 species) and had highest densities for *C. verticillata* (0.99 ± 0.22) and sponges (0.72 ± 0.13). The Lower cluster contained four taxa with sponges (0.64 ± 0.22) representing the highest density. The Middle cluster contained three total species: *C. fastigiata* (1.10 ± 0.24), *Gracilaria* sp. (0.18 ± 0.06), and Turf algae (0.55 ± 0.19). *Chara* sp., *Rhizoclonium* sp., and *R. maritima* were the only species present in the Upper Soft cluster, accounting for densities of 0.15 ± 0.06, 0.21 ± 0.11, and 0.03 ± 0.02, respectively. *N. guadalupensis* was the sole representative in the Upper Hard cluster, accounting for a density of 0.06 ± 0.06.

Water Quality Data

Water quality data displayed wide ranges (Table 6) which was expected given the spatial distribution of monitoring stations along this salinity gradient. TN varied widely from a minimum of 19.29 to 160.49 μM (median of 37.64 μM). TP ranged from 0.04 to 1.00 μM with a median of 0.24 μM. TOC concentrations were very high and ranged from 405.79 to 2267.50 μM (median 1114.79 μM). Salinity ranged from a low of 0.0‰ in the headwaters to a high of 32.22‰ at the outlet; median salinity was 5.0‰. Irradiance at bottom varied from 9.50% to 66.70%. Sediment depth ranged from 0 to greater than 51 cm with a median of 8.30 cm.

Parameter	*N*	Mean	Median	Minimum	Maximum	Standard Deviation
NO_2 (μM)	83	0.20	0.17	0.03	0.54	0.10
NO_3 (μM)	83	1.61	1.36	0.04	5.83	1.27
NH_4 (μM)	81	1.95	1.48	0.40	8.68	1.62
Total organic nitrogen (μM)	36	37.98	34.72	13.50	82.06	17.32
Total nitrogen (μM)	515	43.83	37.64	19.29	160.49	21.49
Total phosphorus (μM)	495	0.26	0.24	0.04	1.00	0.17
Soluble reactive phosphorus (μM)	71	0.07	0.06	0.00	0.21	0.05
Total organic carbon (μM)	79	1075.83	1114.79	405.79	2267.50	345.46
$SiOH^-$	12	102.48	59.07	22.48	224.04	76.88
Turbidity (NTU)	113	2.50	1.60	0.44	17.75	2.69
Chlorophyll *a* (mg l^{-1})	36	2.08	1.68	0.32	8.76	1.56
Salinity (‰)	605	9.30	5.00	0.00	32.22	9.98
Temperature (°C)	116	25.88	26.36	16.76	31.60	3.63
Dissolved oxygen (mg l^{-1})	116	5.36	5.05	1.84	11.57	1.52
k_d (PAR) (m^{-1})	83	1.04	0.99	0.41	2.35	0.38
Irradiance at bottom	83	36.84	37.00	9.50	66.70	11.49
pH	36	7.69	7.73	7.25	8.11	0.20
Depth (m)	83	2.45	2.59	0.00	4.10	0.92
Secchi (cm)	73	146.15	144.00	61.00	248.00	47.78
Sediment depth	81	14.75	8.30	0.00	51.00	17.68

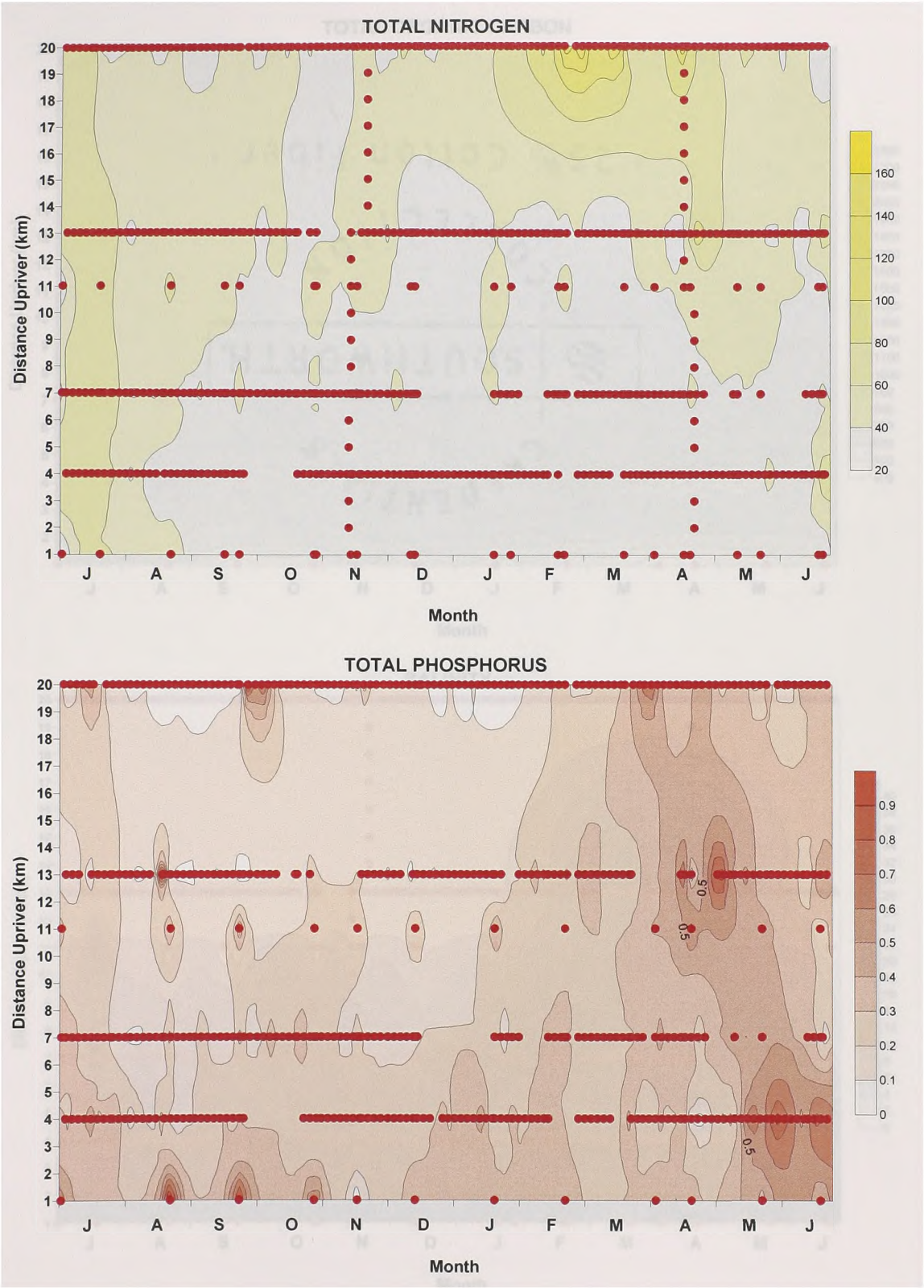
Table 6. Summary statistics for all observations in Shark River between July 1, 2002 and June 30, 2003.

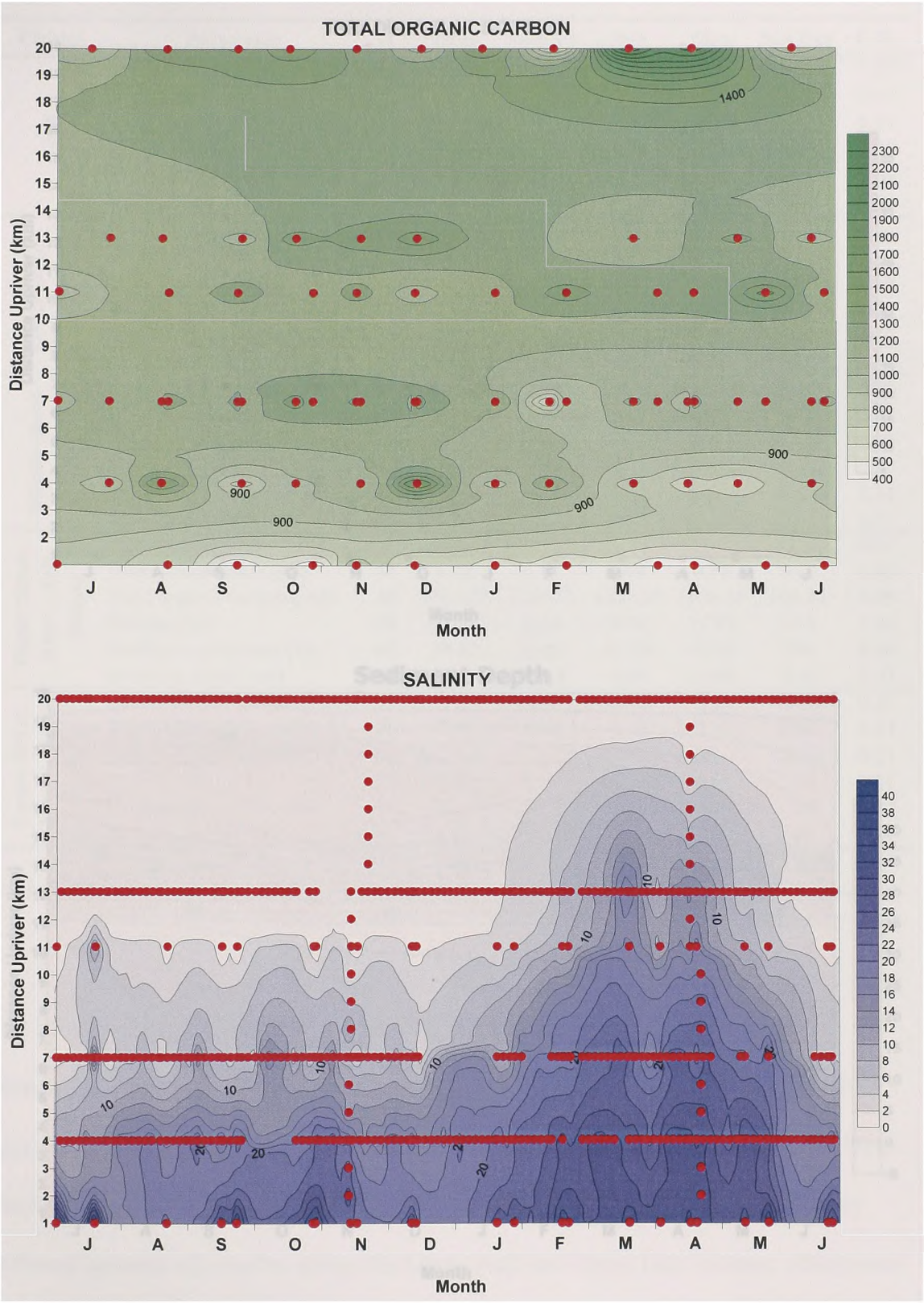
Interpolated continuous surfaces showed TN and TOC had highest concentrations upstream, but TP values were higher in the Gulf and Lower clusters of the river (Fig. 5). TP and TOC showed highest concentrations during the dry season months. Salinity showed a pattern consistent with a tidal river system with the lowest salinities in the upper ranges of the river and the highest at the mouth. Irradiance reaching the bottom showed higher intensity in the late dry season months but no distinct upstream/downstream trends. Sediment depth was consistently greater in the Upper Soft sites.

Relating Benthic Communities to Water Quality

TN means ranged from 32.44 μM in the Gulf to 52.55 μM at sites in the Upper Soft clusters (Table 7). Mean values for TP decreased with distance upstream and ranged from 0.37 to 0.22 μM. TOC values increased with distance upstream from 600.31 to 1266.1 μM, recorded in the Upper Soft cluster. Salinity showed lowest values (mean =1.94‰) in the clusters farthest upstream (Upper Hard and Upper Soft) with the widest range occurring in the Middle cluster (SD = 6.73). Lowest mean values for irradiance at bottom occurred in the Middle cluster (34.67%) but did not vary significantly from the other four clusters. Sediment depths ranged from 0.00 cm in the Gulf, Lower, Middle, and Upper Hard clusters to greater than 51.00 cm in the Upper Soft cluster; mean values for sediment depth ranged from 2.40 to 11.7 cm at all clusters except for the Upper Soft cluster which showed a mean of 19.00.

ANOVA showed all water quality parameters were significantly different between clusters ($p < 0.001$) except for amount of light reaching the bottom ($p = 0.377$) (Fig. 6). Post-hoc comparisons showed TN was not different between the Gulf, Lower


TOTAL NITROGEN
Distance Upriver (km)
Month
J A S O N D J F M A M J
160
140
120
100
80
60
40
20
TOTAL PHOSPHORUS
Distance Upriver (km)
Month
J A S O N D J F M A M J
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0


TOTAL ORGANIC CARBON
Distance Upriver (km)
Month
J A S O N D J F M A M J
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900
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
SALINITY
Distance Upriver (km)
Month
J A S O N D J F M A M J
10
20
40
38
36
34
32
30
28
26
24
22
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18
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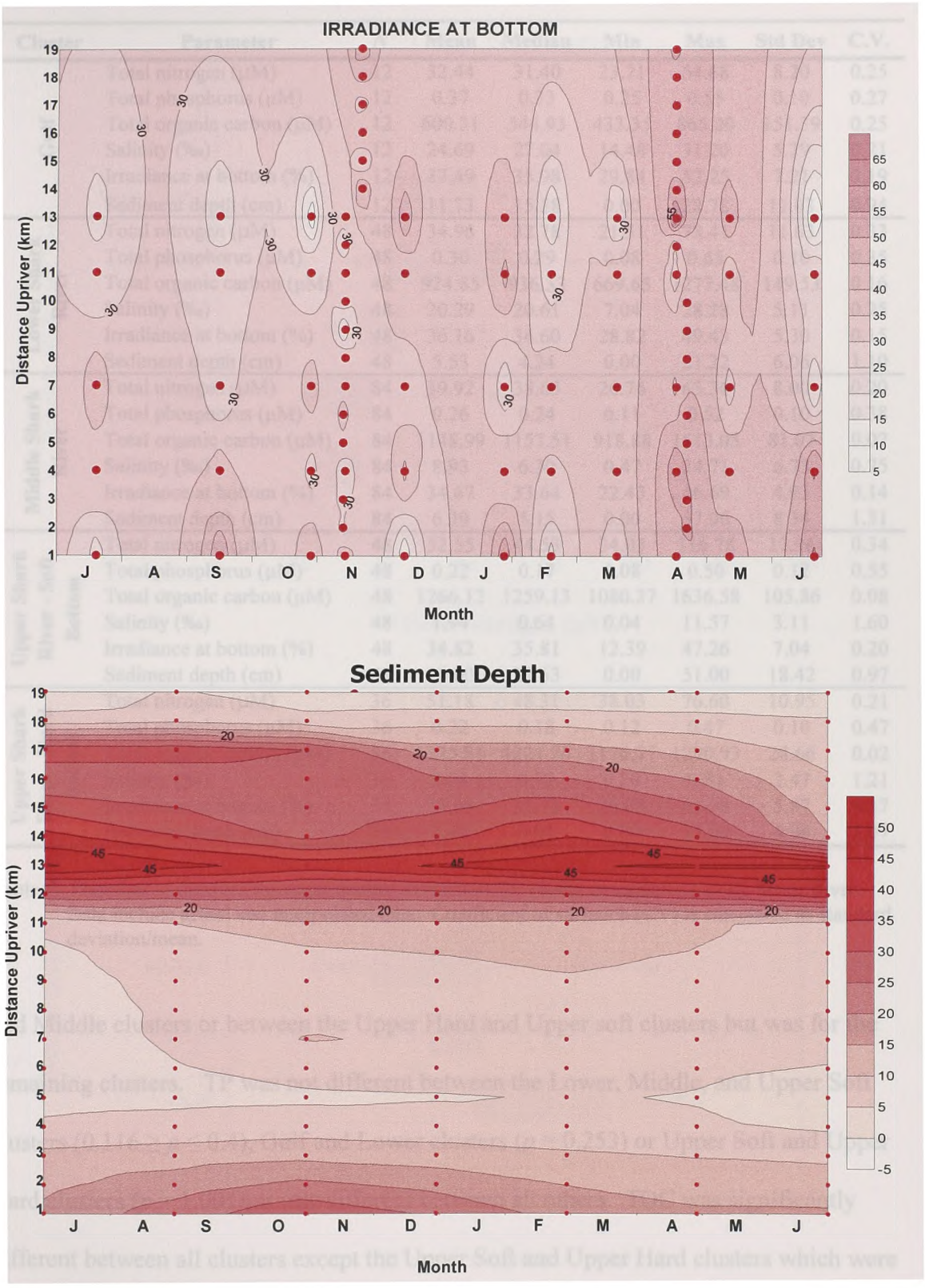


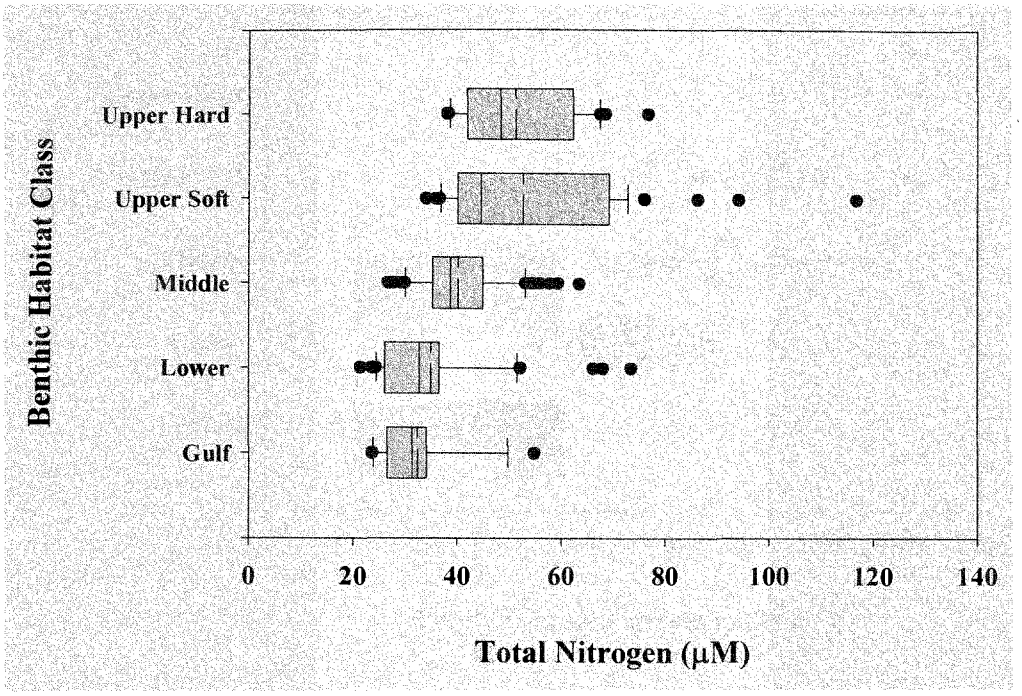
Fig. 5. Contour plots showing temporal and spatial distributions of six proxy variables.

Cluster	Parameter	N	Mean	Median	Min	Max	Std Dev	C.V.
Gulf	Total nitrogen (μM)	12	32.44	31.40	23.71	54.68	8.20	0.25
	Total phosphorus (μM)	12	0.37	0.33	0.25	0.55	0.10	0.27
	Total organic carbon (μM)	12	600.31	544.93	433.35	865.20	151.39	0.25
	Salinity (‰)	12	24.69	27.04	14.48	31.20	5.29	0.21
	Irradiance at bottom (%)	12	37.49	35.98	29.84	52.25	7.21	0.19
	Sediment depth (cm)	12	11.73	15.58	0.00	28.78	11.03	0.94
Lower Shark River	Total nitrogen (μM)	48	34.96	32.78	21.51	73.41	11.63	0.33
	Total phosphorus (μM)	48	0.30	0.29	0.08	0.65	0.10	0.35
	Total organic carbon (μM)	48	924.85	936.33	669.65	1277.48	149.53	0.16
	Salinity (‰)	48	20.29	20.61	7.04	28.28	5.11	0.25
	Irradiance at bottom (%)	48	36.16	34.60	28.82	49.43	5.30	0.15
	Sediment depth (cm)	48	5.53	4.24	0.00	21.22	6.06	1.10
Middle Shark River	Total nitrogen (μM)	84	39.92	38.65	26.76	63.36	8.00	0.20
	Total phosphorus (μM)	84	0.26	0.24	0.11	0.52	0.10	0.38
	Total organic carbon (μM)	84	1148.99	1157.51	918.88	1513.05	81.07	0.07
	Salinity (‰)	84	8.93	6.30	0.47	24.71	6.73	0.75
	Irradiance at bottom (%)	84	34.67	33.64	22.45	46.69	4.93	0.14
	Sediment depth (cm)	84	6.39	5.15	0.00	37.06	8.34	1.31
Upper Shark River - Soft Bottom	Total nitrogen (μM)	48	52.55	44.53	34.03	116.78	17.96	0.34
	Total phosphorus (μM)	48	0.22	0.17	0.08	0.50	0.12	0.55
	Total organic carbon (μM)	48	1266.12	1259.13	1080.37	1636.58	105.86	0.08
	Salinity (‰)	48	1.94	0.64	0.04	11.57	3.11	1.60
	Irradiance at bottom (%)	48	34.82	35.81	12.39	47.26	7.04	0.20
	Sediment depth (cm)	48	19.00	22.63	0.00	51.00	18.42	0.97
Upper Shark River - Hard Bottom	Total nitrogen (μM)	36	51.18	48.31	38.03	76.60	10.95	0.21
	Total phosphorus (μM)	36	0.22	0.18	0.12	0.47	0.10	0.47
	Total organic carbon (μM)	36	1225.81	1224.26	1176.77	1290.93	28.66	0.02
	Salinity (‰)	36	2.04	0.72	0.20	9.51	2.47	1.21
	Irradiance at bottom (%)	36	35.05	35.73	26.08	47.08	5.87	0.17
	Sediment depth (cm)	36	2.40	0.00	0.00	17.00	4.96	2.07

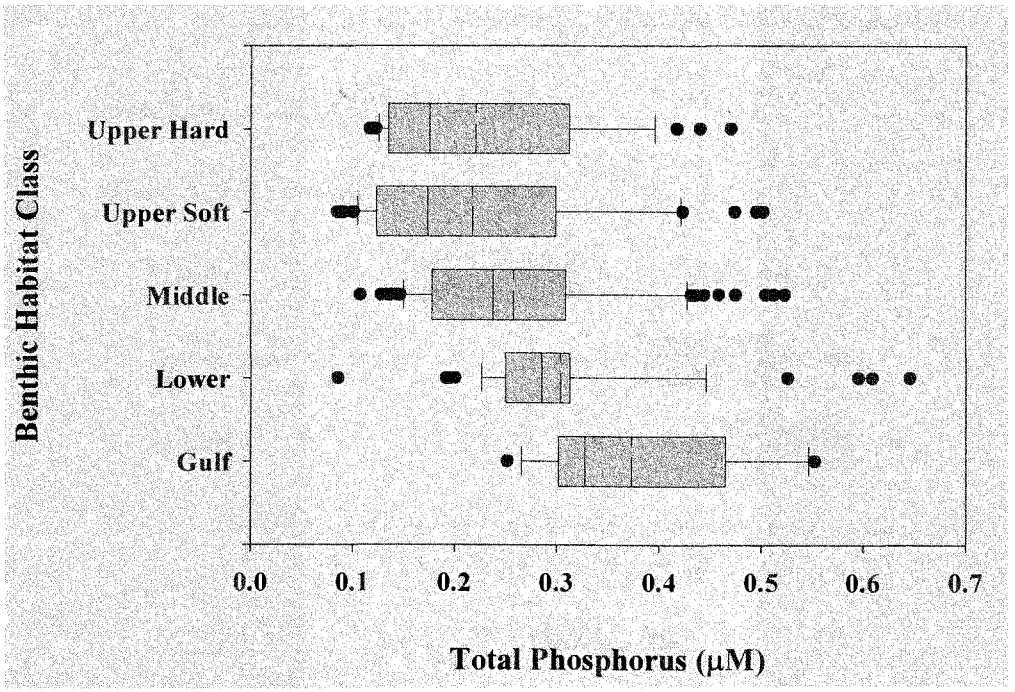
Table 7. Descriptive statistics for water quality parameters for cluster designations along Shark River. Data include actual and interpolated data. Coefficient of variation (CV) is calculated as standard deviation/mean.

and Middle clusters or between the Upper Hard and Upper soft clusters but was for the remaining clusters. TP was not different between the Lower, Middle, and Upper Soft clusters ($0.116 \geq p \leq 0.4$), Gulf and Lower clusters ($p = 0.253$) or Upper Soft and Upper Hard clusters ($p = 1.00$) but was different between all others. TOC was significantly different between all clusters except the Upper Soft and Upper Hard clusters which were

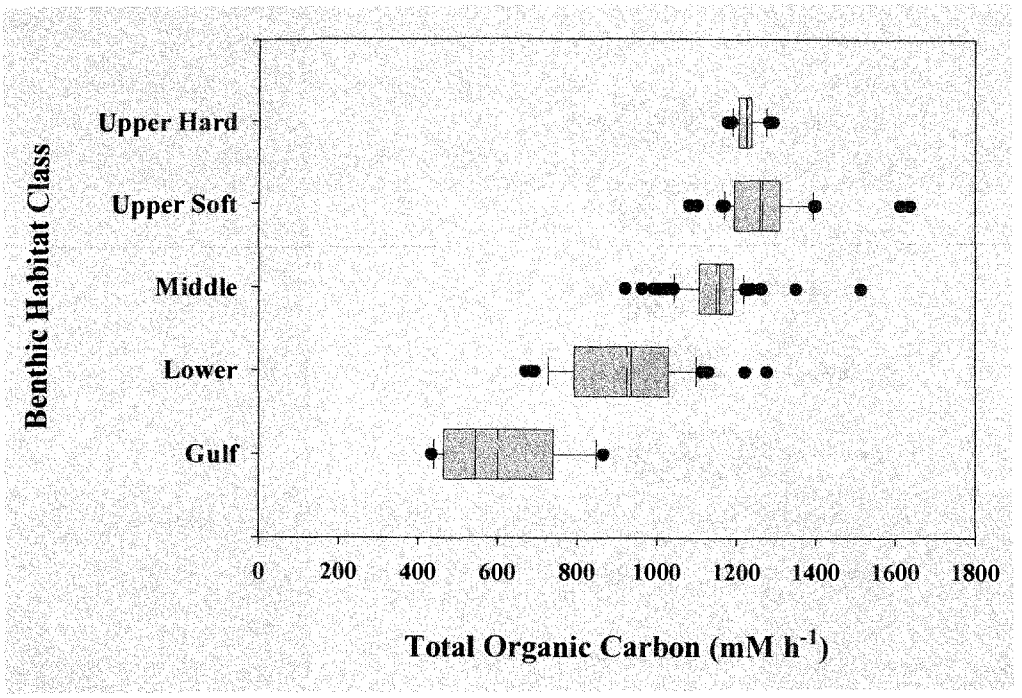
Total Nitrogen



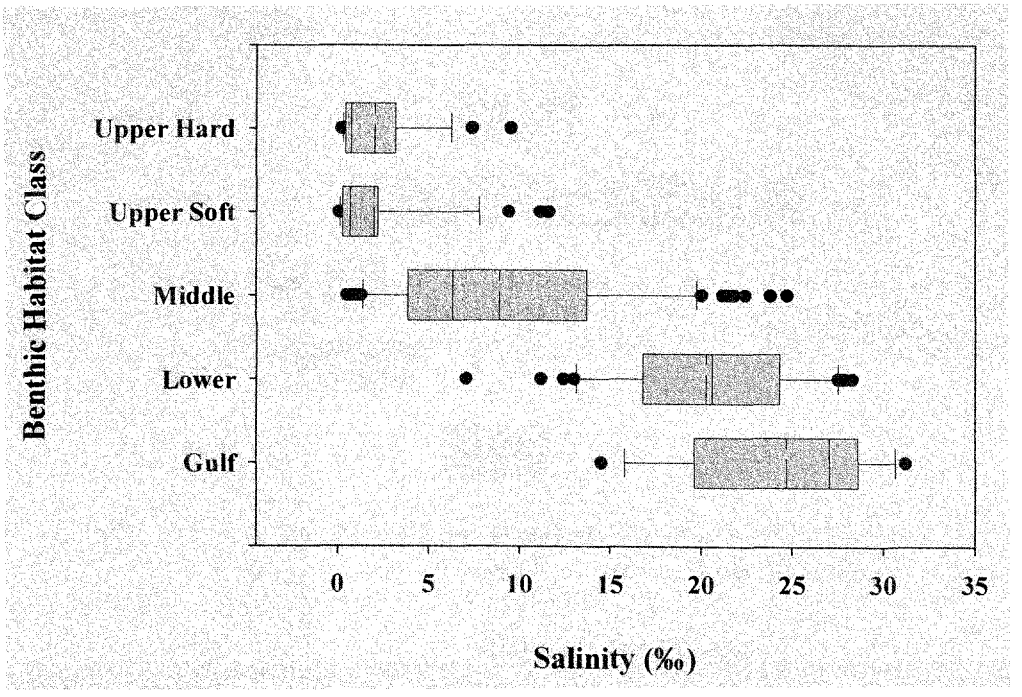
Total Phosphorus



Total Organic Carbon



Salinity



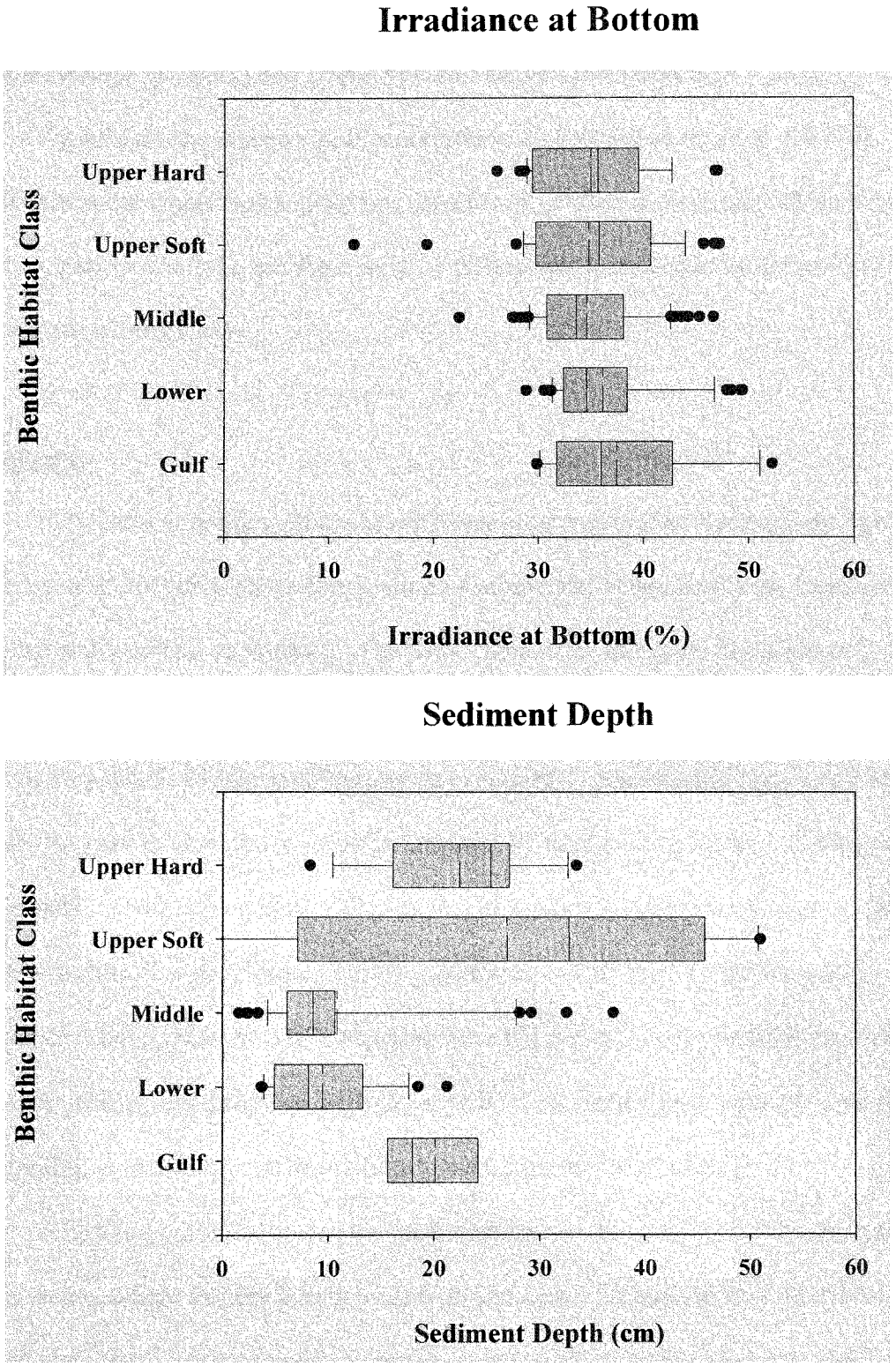


Fig. 6. Boxplots comparing water quality to cluster membership. End bars represent 10^{th} and 90^{th} percentiles. Solid bar is median, dotted line is mean.

not significantly different ($p = 0.399$). Salinity was only different between the Gulf and Lower clusters ($p = 0.67$) and Upper Soft and Upper Hard clusters ($p = 1.00$).

Irradiance showed no significant differences between clusters ($p = 0.377$). Sediment at the Upper Soft cluster was significantly different from each of the remaining four clusters ($p < 0.001$), but there were no differences in sediment depth between or among any other cluster.

Discussion

The salinity gradient of the Shark River estuary provides a prime opportunity to investigate factors driving the distribution of submerged vegetation in an economically and culturally critical ecosystem. The SAV community along the Shark River is considerably depauperate compared to those in nearby estuarine environments (i.e. Florida Bay, Gulf of Mexico) (Fourqurean et al. 2001). Salinity and nutrient distributions follow the pattern common in estuaries affected by tidal mixing from a carbonate marine environment (Fourqurean et al. 1993; Chen and Twilley 1999) with nitrogen, organic carbon, and fresh water flushing into the Gulf waters from the Everglades and phosphorus being brought upriver from richer offshore waters during tidal inundation. Nitrogen availability decreases with distance from the headwaters while phosphorus availability increases due to inputs from prevailing offshore currents.

The SAV community was the most diverse and abundant near the mouth of the Shark River, where salinity is relatively high and stable. It appears that there is also a strong correlation between current strength and SAV distribution along the Shark River. Although current speed was not directly measured, sediment depth data, along with

anecdotal evidence, substantiates this assertion. The areas of the river that experience the highest energy – Gulf, Lower, Middle, and Upper Hard clusters – are essentially barren of the appropriate substrate to support all but the hardiest species of vegetation. Vegetation with the ability to adapt to the lack of sediment and extreme flow exists in these areas but in relatively small amounts. Morphological characteristics of taxa found in these high current areas, such as *Gracilaria* sp., *C. fastigiata* and the turf algal complex, provide these taxa with the ability to "holdfast" to the bare substratum. Taxa requiring some depth of sediment, such as *R. maritima* and *H. decipiens*, were found only in areas where the current flow was low and therefore appropriate sediment existed.

The qualities of the turf complex at the Shark River sites parallel those of the "*Bostrychietum* complex" described in King (1990). These associations are the dominant phycoflora in mangrove areas of the Gulf of Mexico where soft sediment and typical estuarine conditions occur (Ortegon-Aznar, 1997). The "turf" complex was the dominant vegetation in the Shark River. It showed a median density of 1.38, the highest median density shown during the surveys, and a maximum density of 3, which is second only to *C. fastigiata* at the Shark River sites.

In a comprehensive listing of algal associations with Mexican mangroves, Collado-Vides (2000) identified 32 genera (including the *Bostrychietum* complex as a single genera) and 76 species. Of this list, 12% of the algal species and 43.7% of the algal genera (including the *Bostrychietum* complex and turf algal complex as single genera) recorded at Shark River were included.

The amount of light reaching the substrate was, *a priori*, expected to be a controlling parameter of the SAV. These results show, however, that sufficient light is

reaching the bottom in most of this system. Minimum light required for seagrass and submerged aquatic vascular plant survival is considered to be 10% to 30% of surface irradiance (Dennison, et al. 1993; Kemp, et al. 2004); macroalgae require even less light. Irradiance at sites along the Shark River ranged from 9.5% to 66.7% (mean of 36.8%), which appears to be sufficient for SAV and macroalgal survival. Increased absorption, however, may significantly alter the useable light reaching the chloroplasts of the vegetation. High amounts of dissolved organic matter, primarily tannins, in these blackwater rivers absorb the lower wavelengths of light entering the water column, leaving only higher wavelengths of photosynthetically active radiation (PAR) available for plant use. These results did not show significant correlations between irradiance levels and turbidity or chlorophyll *a*, which would have been suspected. articulate organic matter was not quantitatively measured so there is no way to directly correlate this parameter with any trends in light attenuation along the river. It is presumed, however, that the intensity of photosynthetically utilizable radiation (PUR) reaching the vegetation is significantly overestimated by using only a PAR sensor to estimate the attenuation coefficient and not correcting for the absorption of blue light by dissolved organic matter in the water and that the optical quality of the water would contribute to changes in the benthic community (Gallegos, 2005).

Freshwater flow, and therefore nutrient concentration, has been altered significantly in the past century as extensive channels divert the historical sheet flow into the Atlantic (Smith et al. 1989; Light and Dineen 1994; Childers et al. 2003). Projects are underway to restore historical hydrological characteristics of the Everglades. This alteration will increase freshwater flow into the Shark River Slough, which will not only

reduce salinities along the river but also will increase the inputs of carbon and nitrogen into the estuary and surrounding Gulf waters. As carbon, nitrogen, and salinity have been shown to be controlling factors in the distribution of SAV along the Shark River, the potential increases in these inputs could significantly affect the benthic communities in the river and surrounding areas.

CHAPTER 3

Elemental and Isotopic Composition of Submerged Plants from a Mangrove River and their Roles as Potential Food for the Endangered West Indian Manatee (*Trichechus manatus latirostris*)

Introduction

Sirenians (manatees and dugongs) worldwide are endangered and the prospect of survival of these species is uncertain. As such, proper management of the habitat for these animals will require collaborative inputs of information on all aspects of manatee population biology, movement patterns, dietary requirements, and life history.

The Florida manatee (*T. manatus latirostris*) is the largest aquatic herbivore in south Florida and the only herbivorous marine mammal in the U. S. (Bertram 1968; Thayer et al. 1984). Manatees' exceptional osmoregulatory capabilities allow them to move between fresh and salt water and feed within all natural salinity levels (Campbell and Irvine 1977; Ortiz et al. 1998). These large obligate herbivores are known to consume a wide range of vegetation from both freshwater and marine environments (Bengtson 1983; Etheridge et al. 1985; Ledder 1986).

Due to their long life spans and highly mobile nature, the population status of manatees in Florida is difficult to measure. The Florida Fish and Wildlife Conservation Commission (FWC) reports there are 1,433.3 km^2 of federally-defined critical habitat for manatees in South Florida (Fig. 1, Chapter 1), of which 867.6 km^2 are in ENP (Haddad 2003). Little is known about the manatee inhabitants in ENP though they comprise a large percentage of the manatee population in Florida and recent population models

identify a decrease in the growth rate for this subpopulation (Doyle, 2001; Reid et al. 2003).

In the past, the feeding ecology of manatees was assessed using visual observation or analysis of gut contents. However, the potential for observer error and the fact that ingested materials are not all assimilated, do not remain in the digestive tract for the same amount of time for different tissues, and represent only recent feeding bouts (Tieszen et al 1983) make these techniques unreliable and provide incomplete information.

The role of SAV beds from the southwest Florida region as food for the manatee is poorly known. There are observations of these animals traveling and feeding in the area (Snow 1991; Ackerman 1995; Lefebvre et al. 1995; Weigle et al. 2001), but the relative importance of SAV from different areas (marine, estuarine, freshwater) and of different species within SAV beds has yet to be explored. Tracking data and field observations from the U.S.G.S. Sirenia Project show that the spatial distribution of SAV influences manatee movements in the northern Everglades (Reid et al. 2003). Food quality as well as availability may vary significantly across the landscape, as nutrient contents of seagrasses are spatially and temporally variable (Fourqurean et al. 1997).

Questions about the importance of different foods to the diets of consumers have been addressed by analyzing carbon and nitrogen stable isotopic compositions of consumers and their food sources (Fry et al. 1978; Fry and Parker 1979; Rau et al. 1983; France 1995; Branstrator et al. 2000). As plants uptake carbon (C) and nitrogen (N) into their cells, heavier isotopes (^{13}C and ^{15}N) tend to be discriminated against during numerous biogeochemical stages in preference of the lighter and much more abundant

elemental isotopes (^{12}C and ^{14}N) (Park and Epstein 1961; Peterson and Fry 1987). This discrimination by both terrestrial and aquatic plants results in species-specific ratios that are different from those of the C or N source (Chisholm et al. 1982; Peterson and Fry 1987).

Distinct SAV communities form along a salinity gradient with plants tolerant of higher salinities persisting in marine (downstream) areas and those requiring lower salinities persisting in fresh (upstream) areas (see Chapter 2). In the mid- and/or tidally-influenced areas of a river, plants tolerant of both extremes may exist, though the stress of such a fluctuating salinity regime limits plant diversity. These distinct assemblages, and the unique isotopic ratios of plants from these assemblages, lend themselves nicely to the study of an animal's utilization of marine v. freshwater or terrestrial food webs and other dietary information using stable isotopes. For example, freshwater and terrestrial plants typically have $\delta^{13}C$ signatures that are 10‰ to15‰ lighter (more negative) than marine species such as seagrasses (Smith and Epstein 1971; Fry 1984); as a result, animals consuming primarily marine plants show distinctly different $\delta^{13}C$ signatures than those consuming freshwater or terrestrial vegetation (Chisholm et al. 1982; Cree et al. 1999). Marine plants, however, tend to have higher $^{14}N/^{15}N$ ratios than do terrestrial plants, thus, marine animals tend to have higher $^{14}N/^{15}N$ ratios than do animals feeding from terrestrial sources (Schoeninger et al. 1983; Schoeninger and DeNiro 1984).

The purposes of this study were to 1) document the stable isotopic and elemental content of potential food sources for manatees in the mangrove-lined rivers of southwest Florida, and 2) explore the utility of natural variability in isotope ratios retained in hairs to augment data gained from traditional techniques (visual observations and gut content

analyses) for examining the feeding ecology of manatees. In essence, do manatee hairs reflect isotopic compositions of their food sources? Since animal tissues reflect the isotopic composition of their food sources, the isotopic composition of animal tissue can indicate their dietary source (Chisolm et al 1982, Peterson and Fry 1987). Though manatee tissues (skin, muscle, kidney, blubber, and liver) have been analyzed for isotopic composition to investigate feeding history and dietary habits (Ames et al. 1996), manatee hairs have not yet been considered. Hairs may be well-suited for isotopic investigation of feeding ecology since they are retained on the body for extended periods and therefore have the potential to integrate isotopic information over longer time periods (Tieszen et al. 1983). Perhaps more importantly, Florida manatees are endangered and federally-protected making it imperative that samples are collected non-invasively; hairs can be sampled with no harm to the animal.

The use of stable isotopes, particularly of ^{13}C and ^{15}N, is a powerful tool that has enabled researchers to investigate aquatic food web structures and dietary patterns in estuaries based on the significant and consistent differences in isotopic compositions of different types of primary producers (Fry and Sherr 1984; Hobson and Clark 1992; Boon and Bunn 1994; Deegan and Garritt 1997).

The Use of $\delta^{13}C$ in Investigating Feeding Ecology

Because of differences in biochemical mechanisms of processing different elements and the resulting differences in observed fractionation of different elements by different processes, variability in isotope ratios of the light elements C and N have properties that make them useful for the study of organic matter in ecosystems. The

discrimination against ^{13}C by both terrestrial and aquatic plants results in unique, species-specific ratios that are lower than those of atmospheric and oceanic CO_2; these predictable ratio variations have been used to trace the source of original C and energy for consumers (Park and Epstein 1961; Peterson and Fry 1987; Farquhar et al. 1989; Deegan and Garritt 1997).

Significant differences in carbon isotope ratios between C_3 and C_4 plants occur due to different photosynthetic pathways used (i.e. Rubisco v. PEP carboxylase). Atmospheric CO_2 has an average $\delta^{13}C$ value of ~ -7‰ (Park and Epstein 1960) providing relatively consistent ranges of $\delta^{13}C$ values between taxa of -6‰ to -17‰ for C_4 and -18‰ to -33‰ for C_3 terrestrial plants (Park and Epstein 1961; Berry 1989; Farquhar et al. 1989).

Similarly, differences between terrestrial and marine plants can be further investigated with information on C isotopic compositions. In terrestrial plants, C is taken in directly from the atmosphere in the form of $CO_{2(g)}$ (Berry 1989; Farquhar et al. 1989) with fractionation occurring during uptake of the gas into the cytoplasm via diffusion and then again during carboxylation (Park and Epstein 1961; Christeller and Laing 1976; O'Leary 1984; Durako 1993).

In an aquatic medium, however, there are additional factors resulting in variations in $\delta^{13}C$ values (Degens et al. 1968; Keely et al. 1986; Thompson and Calvert 1994; Grice et al. 1996). In water, fractionation of C isotopes occurs not only during diffusion and assimilation, as in terrestrial plants, but also during the dissolution of atmospheric CO_2 into water (approximately ¯0.5‰ to ¯1.4‰) and again approximately 8‰ during the dehydration of dissolved CO_2 into bicarbonate ions (HCO_3^-) (Mook et al. 1974; Dring

1982; Thompson and Calvert 1994; Zhang et al. 1995). Additionally, the solubility of CO_2 in seawater is relatively low, concentrations in water being 10% to 15% lower than that of CO_2 in the atmosphere (Chen and Durbin 1994; Beer and Rehnberg 1997; Hemminga and Duarte 2000).

Environmental factors cause further differences in final isotopic compositions of aquatic plants (Durako and Hall 1992; MacLeod and Barton 1998; Burkhardt et al. 1999). For example, light intensity has been shown to affect the isotopic composition of aquatic plants by affecting their metabolic rate; a decrease in metabolic functioning would result in a reduced demand for photosynthetic carbon thus allowing for greater discrimination against $\delta^{13}C$ (Farquhar 1983). More simply, high light levels increase C demand which therefore reduces discrimination.

Increased light levels can trigger an increase in pH by promoting higher rates of photosynthetic activity (Thompson and Calvert 1994) which decreases isotopic discrimination in the diffusion of CO_2 due to the lower CO_2 concentration of the dissolved inorganic carbon (DIC) pool but also contributes to the uptake of $\delta^{13}C$-depleted HCO_3^- in some plants. Given the different isotopic signatures of dissolved CO_2 and HCO_3^- ions, this potential for shifting sources of inorganic carbon for aquatic plants leads to considerable differences in isotopic ratios between and among terrestrial and aquatic plants (Benedict et al. 1980; Bjork et al. 1997; Burkhardt et al. 1999).

These known parameters affecting the ratio of ^{13}C to ^{12}C isotopic ratios in primary producers allow investigators to trace the flow of C in food webs by establishing the fractional composition of dietary intakes of consumers (McConnaughey and McRoy 1979; Teeri and Schoeller 1979; Fry and Sherr 1984; Jackson and Harkness 1987). Cree

et al. (1999) used stable C isotope ratios as indicators of marine versus terrestrial inputs to the diet of *Sphenodon punctatus* (tuatara, a lizard-like reptile) to infer different life history stages between males, females, and juveniles. Deegan and Garritt (1997) used C isotopic compositions of primary producers to determine the relative importances of phytoplankton, benthic microalgae, fresh and salt marsh emergent plants, and terrestrial organic matter in an estuarine food web. A clear spatial pattern of C signatures in primary producers emerged along the gradient from the upper estuary with high freshwater inputs to the lower estuary connected directly to the open ocean. Terrestrial inputs and freshwater phytoplankton exhibited values of -28.4‰ and -27.9‰, respectively, while downstream fresh marsh components presented slightly heavier $\delta^{13}C$ values of -25.8‰ and -21.5‰, respectively. Salt marsh producers further downstream were considerably enriched with values of -13.1‰ and -15.8‰, respectively.

The Use of $\delta^{15}N$ in Investigating Feeding Ecology

Nitrogen isotope compositions of consumers are typically used to estimate trophic position (DeNiro and Epstein 1981). $\delta^{15}N$ ratios are often combined with those of $\delta^{13}C$ to discern particular reference material (Marguillier et al. 1997; Lawson and Hobson 2000) – $\delta^{15}N$ provides trophic information whereas $\delta^{13}C$ reveals the source of C incorporated into primary producers. An average of 3.2‰ enrichment of ^{15}N occurs for each trophic level (DeNiro and Epstein 1981; Peterson and Fry 1987). This results from isotopic discrimination against lighter isotopes during assimilatory and excretory functions within the consumer (Marguillier et al. 1997). As with C, initial source of N essentially dictates the $\delta^{15}N$ values along a trophic gradient. Nitrogen derived from plants that fix N (e.g.

legumes, cyanobacteria) is typically depleted in ^{15}N than those plants not capable of N fixation (DeNiro and Epstein 1981; Cabana and Rasmussen 1996). This signature difference allows the potential to discern source of feeding for consumers in an area of anthropogenic influence.

The potential to trace seagrass N using their relatively distinctive $\delta^{15}N$ signatures arises from the presumption that phytoplankton do not fix N and that seagrasses derive some of their N from N fixation associated with the sediment microflora (Fry and Sherr 1984). Low $\delta^{15}N$ values (0‰ to ¯2.3‰) are commonly found in plants that fix N (Macko et al. 1982). Plants that fix atmospheric (N_2) nitrogen tend to show less positive values than those that do not (Macko et al. 1982; Fry and Sherr 1984).

In investigating trophic relationships, spatial and temporal differences in dietary contribution of marine and terrestrial mammals have been successfully determined using $\delta^{15}N$ compositions: harp seal (*Pagophilus groenlandicus*), (Lawson and Hobson 2000), northern fur seal (*Callorhinus ursinus*),(Kurle and Worthy 2001), black bear (*Ursus americanus*), and grizzly bear (*Ursus arctos*) (Hobson et al. 2000).

The accumulation of isotopically-enriched terrigenously-derived N along the riverine systems of southwest Florida and its subsequent discharge along the Florida Shelf provides a "textbook opportunity" to explore estuarine trophic relationships (of the very short manatee herbivore food chain) as well as to illustrate the downstream gradient in ^{15}N content of SAV of the area.

As there is significant fractionation at each trophic level, N isotopes in food web interactions are primarily used to discern trophic position. Since the animal of concern in this study is an herbivore, $\delta^{15}N$ values will be of limited use as a trophic level predictor

but may serve as good tracers of source organic matter. There should, however, be a slight differentiation between flora collected that are capable of N fixation (e.g. seagrasses) and those unable to do so (e.g. macroalgae, mangroves).

The Use of Manatee Hairs to Infer Feeding Ecology

Hairs and other keratinous tissues have been used to explore feeding history and ecology of various types of mammals. Hairs were taken from steers fed diets of C_4, C_3, and C_4 plant species in sequence to provide evidence of a relationship between $\delta^{13}C$ of diet and hair of animals (Jones et al. 1981). The study showed that as hair grows from the base, it incorporates the isotope compositions of the diet at the time of the hair formation. By determining the time interval in equilibrating between feed switches, it is therefore possible to provide a timeline of movement between food sources.

Schell et al. (1989) analyzed the keratinous baleen plates of bowhead whales (*Balaena mysticetus*) for isotopic composition. They compared their data with information on atmospheric inputs of ^{14}C from nuclear weapons testing in the late 1950's and early 1960's and were able to corroborate the validity of their findings. Their study, which utilized the baleen plates and muscle tissue of specimens collected from 1952 to1972, provides evidence that the stable isotopic abundances of keratinous materials, like hair, can be useful in indicating changes in migratory movements and seasonal changes in diet.

More recently, Macko et al. (1999) applied stable isotope techniques to a paleoecological study of diet in ancient human populations. They were able to determine principle food components (C_3 v. C_4 and terrestrial v. marine) as well as the range of food

habits from omnivory to herbivory by analyzing the sulfur, N, and C isotopic compositions of preserved hairs.

Other studies have shown that hairs from bears (Hobson et al. 1997; Hobson et al. 2000), pinnipeds (Hobson et al. 1997; Kurle and Worthy 2001), gerbils (Tieszen et al. 1983), and primates (Schoeninger et al. 1999) are reliable indicators of trophic position. The use of stable isotopic information retained in hairs would make the determination of food source, trophic level, and regional feeding regimes of manatees much more accessible and dependable to the investigator. Manatees feed on a wide variety of vegetation from diverse environments (freshwater, marine and brackish). Before proceeding in applying these assumptions to manatees, baseline investigations are required to determine if the stable isotopic compositions of hair on manatees, like hairs on other mammals, are indicative of the isotope ratios of their food sources.

Methods

Sample Collection

Potential manatee food sources from both captive and wild environments were tested for C and N isotopic composition and C, N and P elemental content. Vascular submerged plants including *H. decipiens*, *H. wrightii* and *R. maritima* as well as algal taxa including *Caulerpa* spp., *Chara* sp. and *Gracilaria* sp. were collected from the Shark River during a 12-month survey (see Chapter 1). *T. testudinum* was also collected from various areas in southwest Florida but was not located in the direct vicinity of the Shark River study. High Protein Monkey Diet® (a commercially-available animal feed manufactured by Purina-Mills, Inc.) and romaine lettuce, the primary components of

manatee diet at the Miami Seaquarium, were provided by the aquarium staff along with various other fruits and vegetables consumed in small amounts by the manatees. Terrestrial species of plants, including *R. mangle*, *Laguncularia racemosa*, *Typha domingensis*, *Cladium jamaicense*, and *Conocarpus erectus*, also were collected as possible manatee food sources and as an elemental and isotopic catalog of vegetation along the Shark River.

Vegetation samples were cleaned of adhering epiphytes by gently scraping with a razor blade. Materials were dried at 80°C then ground to a fine powder using a mortar and pestle.

For elemental content, subsamples of each sample were analyzed in duplicate for C and N content using a combustion-GC (gas chromatography) analysis technique (Fisons NA1500). Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean et al. 1992). Elemental content was calculated on a dry weight basis; elemental ratios will be calculated on a mole:mole basis.

Hair samples were collected from captive and wild manatees and analyzed for isotopic composition. Hairs from captive manatees were taken from four animals at the Miami Seaquarium in April 2001. Three of these animals (hereinafter referred to as C1, C2, and C3) had been residents at the Seaquarium for over 1 year. During the year prior to sample collection, these animals had been on a diet consisting of 90% Romaine lettuce, 5% High Protein Monkey Diet®, and 5% assorted fruits and vegetables. The fourth manatee, a nursing female calf, arrived at the facility in April 2001. Her hair samples represent a transition from her status in the wild to that of captivity; her data are referred

to as T1 for the samples taken immediately upon arrival at the facility (when it is assumed that her hair reflected a "wild" state) and T2 for those taken a month after her arrival. Hairs from wild manatees (W1 and W2) were taken from animals in southwest Florida (near the Port of the Islands, *ca.* 25.9°N, -81.5°W).

Hairs were plucked using tweezers and placed into plastic containers until processed. Three hairs were collected from each of three areas: neck, side, and peduncle. Hair samples were cleaned with deionized water and wiped with tissues to remove superficial debris then dried at 80°C. The sheath surrounding the base of the hairs was gently removed with tweezers. Only the basal portion of the hair (2 to 3 mm in length) was used for measurement purposes as it provided the most recent isotopic information, given that hairs grow from the base.

Hairs and food sources were then analyzed for $\delta^{13}C$ and $\delta^{15}N$ using standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS) procedures at the SERC Stable Isotope Laboratory. Stable C and N isotope values are expressed in δ notation using the standard convention:

$$\delta X\,(‰) = [(R_{sample})/R_{standard}) - 1]) \times 10^3$$

where X is ^{13}C or ^{15}N and R is the abundance ratio of the heavy to light isotopes of the element (i.e. ^{13}C:^{12}C). Standards are Peedee Belemnite (PDB) for C and air for N. An organism (sample) showing a δ value of $^{+}10$ would have a $^{13}X/^{12}X$ ratio greater than the standard by 10‰; the larger value is therefore considered "enriched" in the heavier isotope (the reverse applies to δ values lower than standard being "depleted").

Statistical Analyses

ANOVA and post hoc comparisons were employed to explore differences in $\delta^{13}C$ and $\delta^{15}N$ signatures between placement on body and between captive, transitional, and wild animals as well as differences in these signatures between captive and wild manatees. Means were weighted to investigate the influence of food source on isotopic compositions in the hairs.

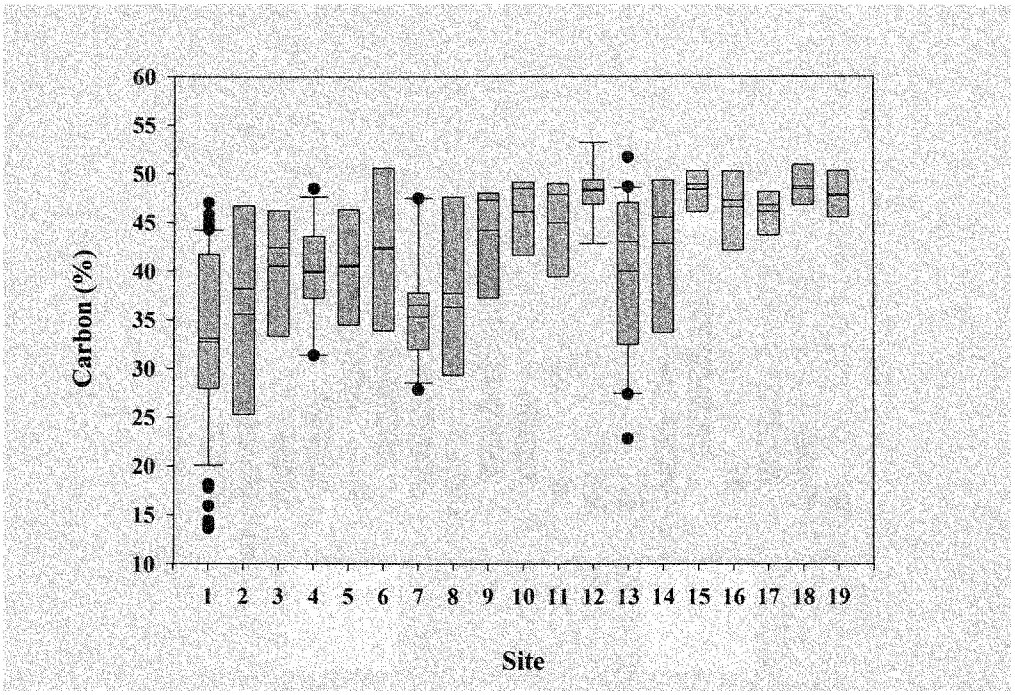
Summary descriptive statistics were created for elemental and isotopic compositions of vegetation. ANOVA and bivariate correlations were used to describe relationships between and within parameters (elemental and isotopic composition, vegetation category, and species).

Results

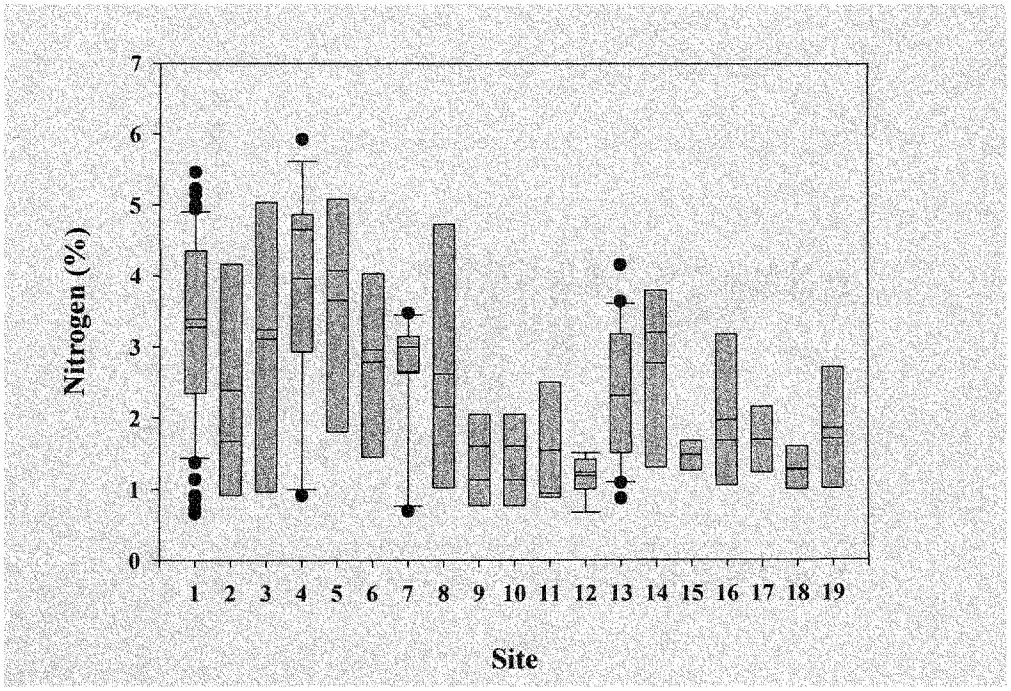
Isotopic and elemental content of potential food sources for manatees

ANOVA to test for differences in isotopic and elemental content of vegetation between site (n = 19) and type of vegetation (seagrass, algae, freshwater vascular, and terrestrial) (n = 4) showed a significant difference for site only with $\delta^{15}N$ ($p = 0.026$) (Fig. 7). All components, C, N, P, $\delta^{15}N$, were significantly different between type of vegetation ($p < 0.001$) (Fig. 8). There were no significant differences for site * type ($p > 0.351$). Bivariate correlations showed all elemental (C, N, P) and isotopic (^{13}C and ^{15}N) compositions were significantly correlated with each other ($p < 0.035$) except for P which was only correlated with nitrogen and ^{13}C (Table 8).

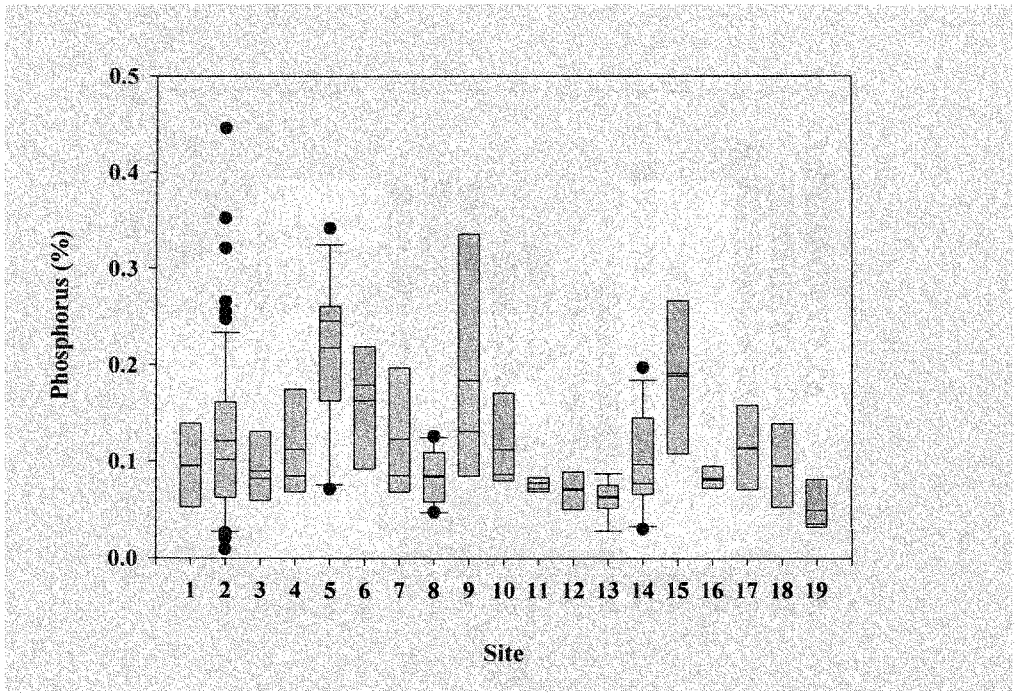
% Carbon in Vegetation along Shark River



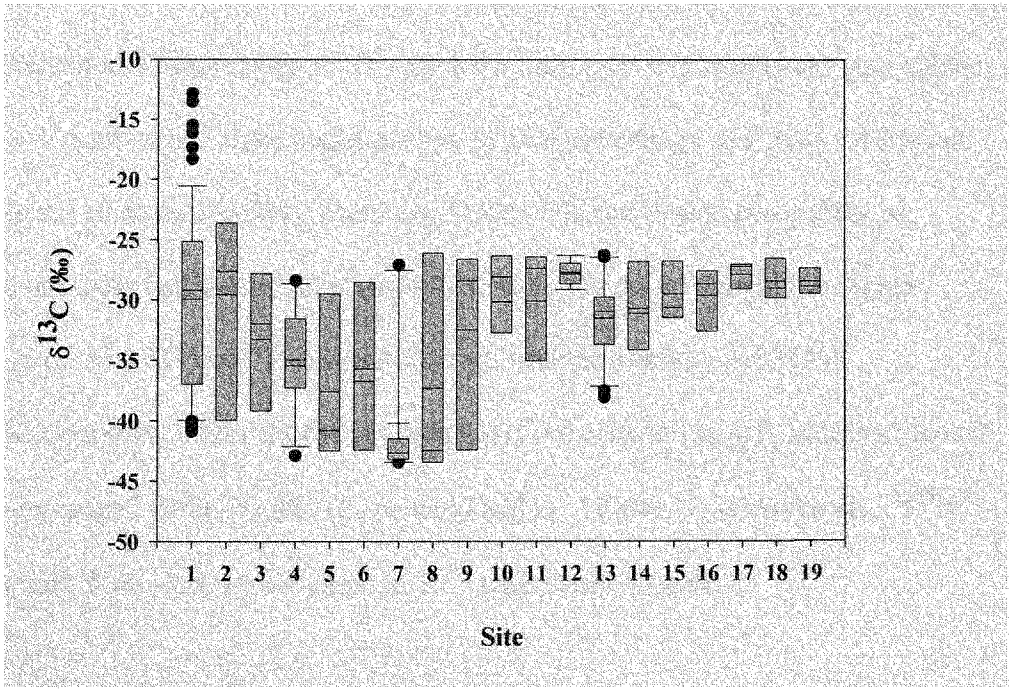
% Nitrogen in Vegetation along Shark River



% Phosphorus in Vegetation along Shark River



$\delta^{13}C$ in Vegetation along the Shark River



$\delta^{15}N$ of Vegetation along Shark River

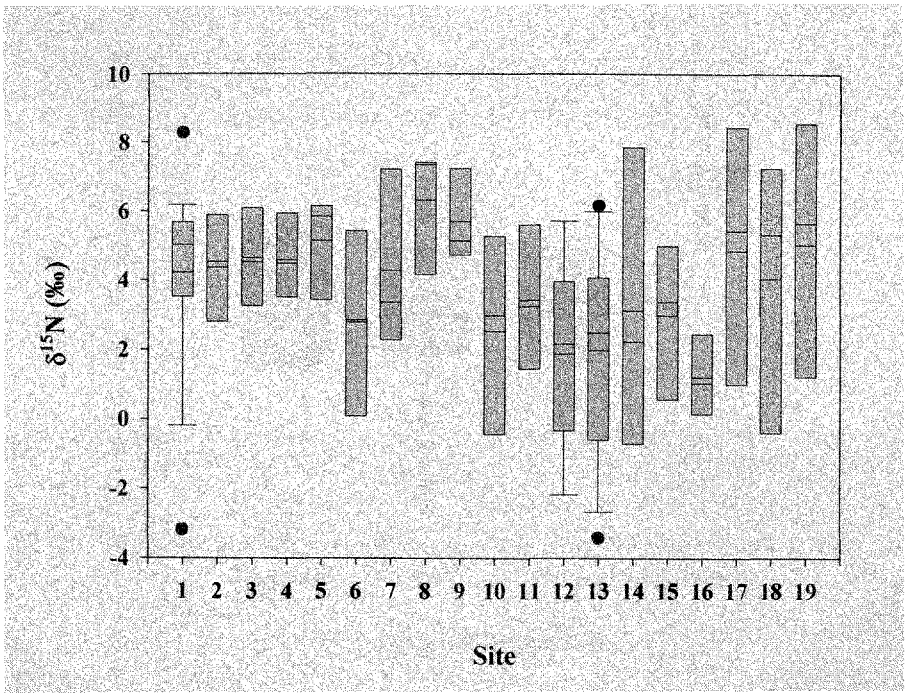
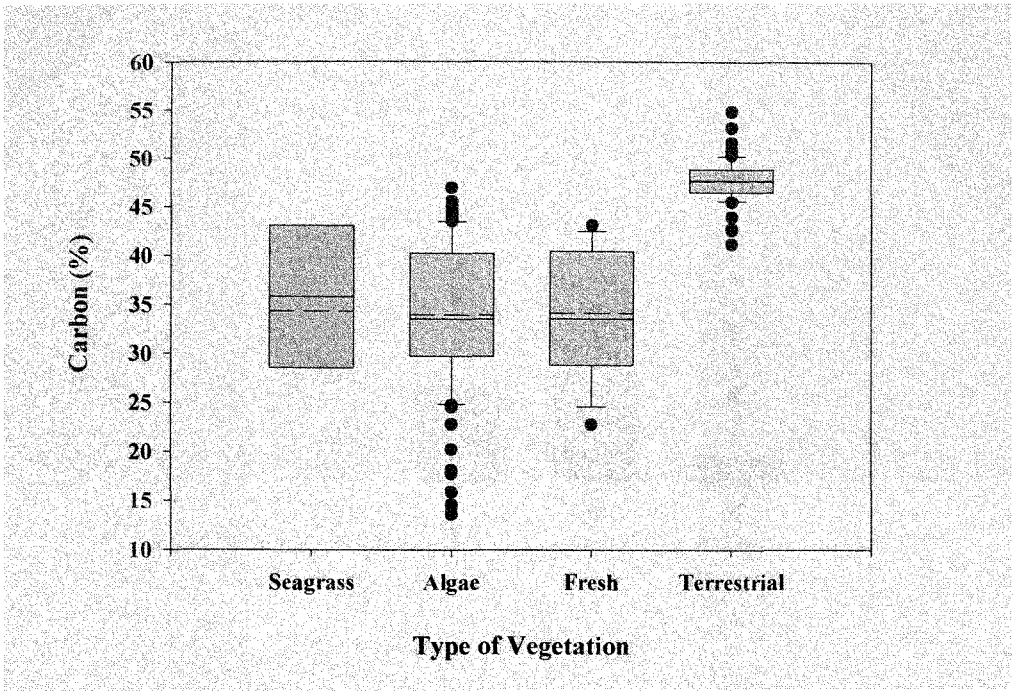


Fig. 7. Elemental and isotopic contents of vegetation along the Shark River. Values are composites of all vegetation (seagrass, algae, freshwater vascular, and terrestrial) found at each site. End bars are 10th & 90th percentiles. Solid line is median,

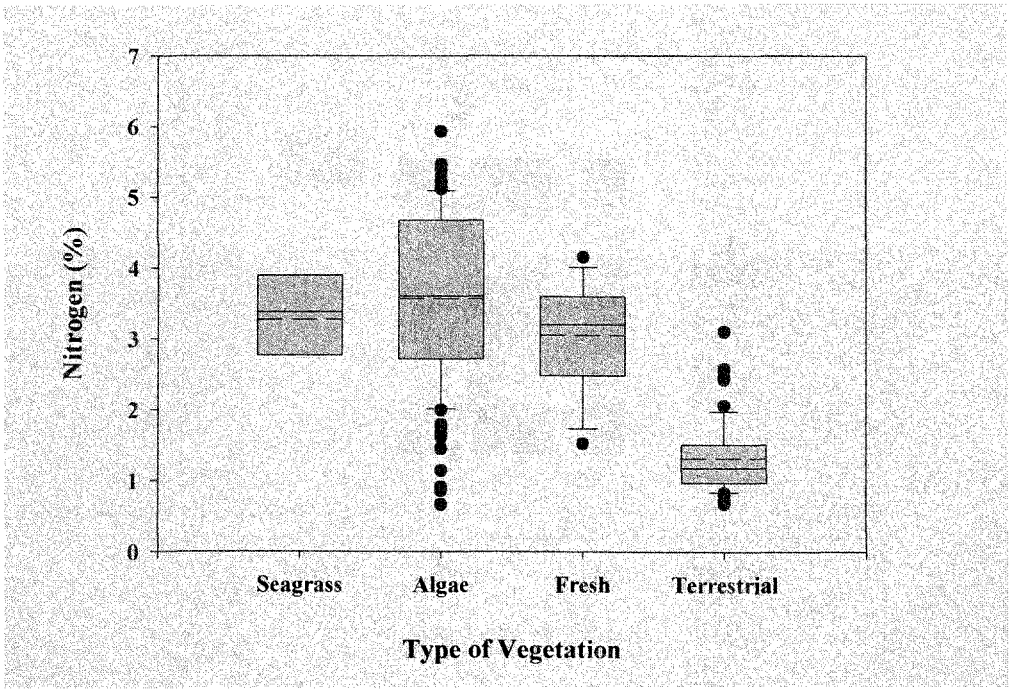
Elemental compositions of all vegetation analyzed had widely ranging values (Table 9). Coralline red algae contained the largest percentage of C with 14.2% and *R. mangle* the smallest (53.8%). Romaine lettuce had the largest percentage of N (5.6%) and pear had the smallest (0.3%). *Gracilaria* sp. contained the highest percentage of P (0.227) and *T. domingensis* sp. had the smallest (0.031%).

Isotopic compositions of vegetation analyzed also had widely ranging values. $\delta^{13}C$ values ranged from -40.9‰ (*C. verticillata*) to -12.4‰ (*T. testudinum*). $\delta^{15}N$ values ranged from -5.4 (*H. wrightii*) to 10.7 (*Salix caroliniana*).

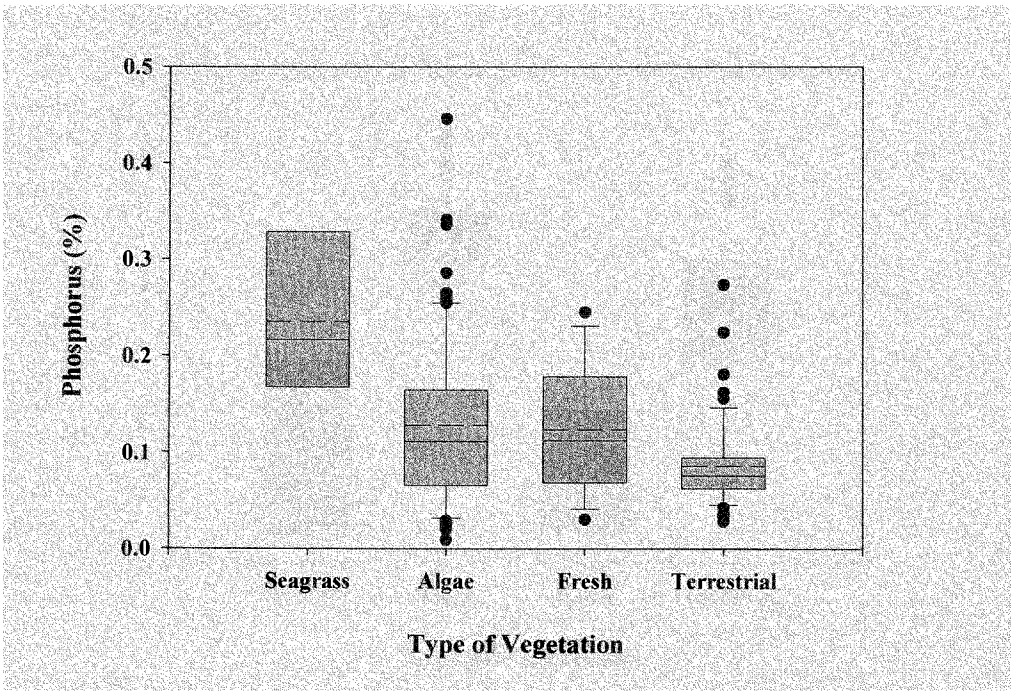
% Carbon in Vegetation along Shark River



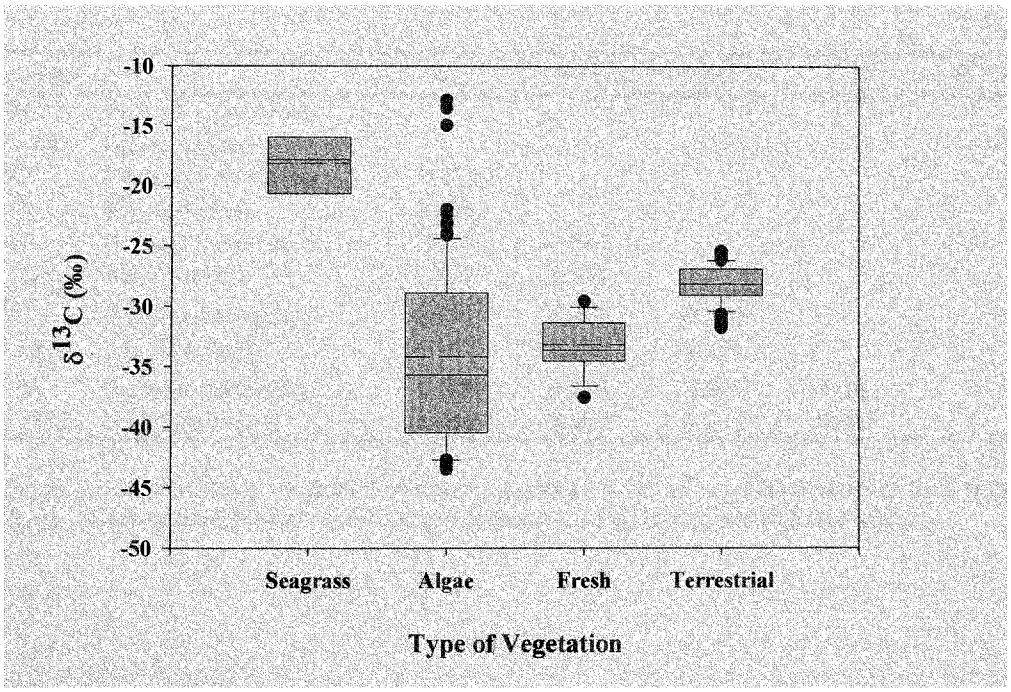
% Nitrogen in Vegetation along Shark River



% Phorphorus in Vegetation along the Shark River



$\delta^{13}C$ in Vegetation along the Shark River



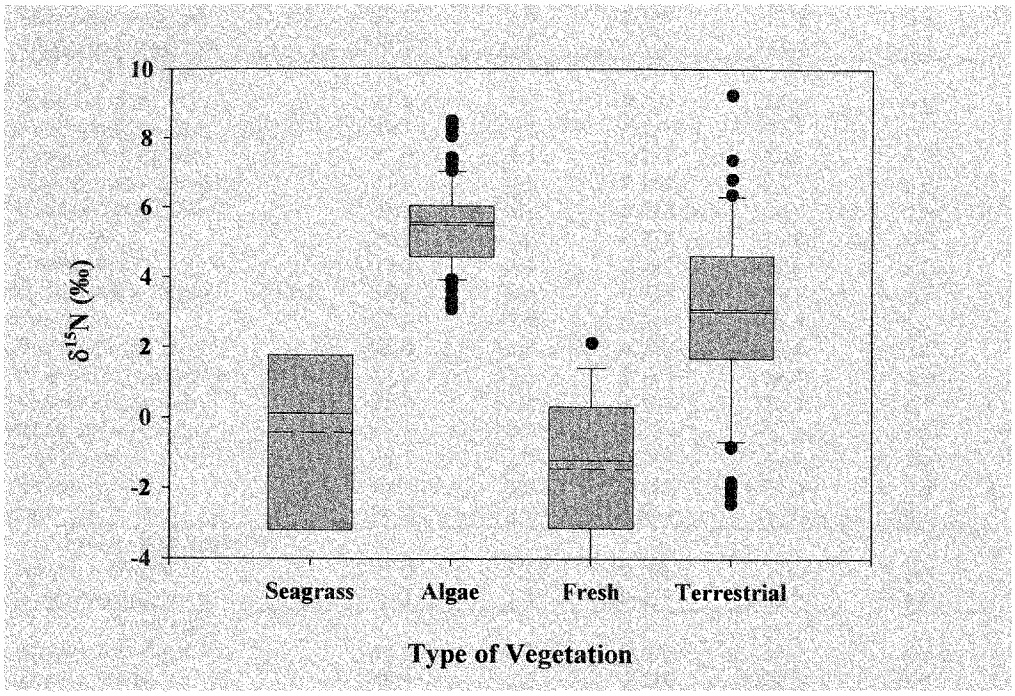


Fig. 8. Elemental and isotopic contents of vegetation along the Shark River. Values are for individual types of vegetation (seagrass, algae, freshwater vascular, and terrestrial) found along the river. End bars represent 10th & 90th percentiles. Solid bar is median, dotted bar is mean.

Component		%C	%N	%P	$\delta^{13}C$	$\delta^{15}N$
%C	Pearson Correlation					
(*n* = 188)	Sig. (2-tailed)					
%N	Pearson Correlation	-0.178				
(*n* = 187)	Sig. (2-tailed)	**0.015**				
%P	Pearson Correlation	0.017	0.610			
(*n* = 182)	Sig. (2-tailed)	0.820	**0.000**			
$\delta^{13}C$	Pearson Correlation	0.101	-0.498	-0.156		
(*n* = 187)	Sig. (2-tailed)	0.167	**0.000**	**0.035**		
$\delta^{15}N$	Pearson Correlation	-0.209	0.218	-0.010	-0.343	
(*n* = 187)	Sig. (2-tailed)	**0.004**	**0.003**	0.888	**0.000**	

Table 8. Bivariate correlations of elemental and isotopic compositions of vegetation samples from Shark River, Southwestern Florida, and Miami Seaquarium. Significant correlations bolded.

Taxa	Content				
	%C	%N	%P	$\delta^{13}C‰$	$\delta^{15}N‰$
Seagrass					
Halodule wrightii	41.8	2.8	0.200	-19.0	-5.4
Halophila decipiens	32.6	3.3	0.219	-17.7	0.1
Thalassia testudinum	36.2	2.2	0.156	-12.4	5.5
Algae					
Acanthophora sp.	31.6	3.1	0.035	-28.8	5.6
Caulerpa fastigiata	39.7	4.7	0.217	-37.1	5.7
Caulerpa prolifera	43.4	4.4	0.173	-23.3	4.0
Caulerpa sertularoides	42.5	4.4	0.153	-28.3	5.0
Caulerpa verticillata	41.7	5.1	0.159	-40.9	5.8
Chara sp.	30.0	2.6	0.088	-31.9	-0.6
Chondria sp.	29.7	2.9	0.052	-29.9	5.3
Coralline Red Algae	14.2	0.8	0.073	-13.8	5.5
Dasya sp.	27.3	2.6	0.112	-39.4	5.7
Gracilaria sp.	33.0	3.3	0.227	-38.8	6.1
Halymenia echinophysa	31.9	4.3	0.163	-38.6	5.0
Halymenia floresia	25.7	2.1	0.107	-38.7	5.7
Rhizoclonium sp.	41.3	3.7	0.197	-34.2	0.7
Solieria sp.	25.1	2.7	0.119	-31.1	5.9
Udotea sp.	29.7	3.0	0.089	-27.0	3.9
Ulva sp.	30.4	1.1	0.090	-29.8	8.3
Freshwater Vascular					
Ruppia maritima	41.0	3.3	0.191	-31.2	-3.4
Najas guadalupensis	43.3	2.3	0.037	-38.1	4.4
Terrestrial					
Acrostichum aureum	42.8	2.7	0.189	-27.0	4.3
Annona glabra	46.0	2.6	0.144	-28.0	7.3
Baccharis sp.	46.6	2.1	0.162	-28.7	3.0
Blechum pyramidatum	53.3	2.2	0.107	-30.9	-0.7
Chrysobalanus icaco	46.8	1.8	0.098	-27.1	-2.6
Cladium jamaicense	50.4	2.0	0.156	-29.5	6.2
Conocarpus erectus	44.8	1.6	0.074	-27.0	1.9
Crinum americanum	47.6	1.7	0.113	-30.0	5.9
Dalbergia ecastaphyllum	47.9	1.6	0.145	-26.2	2.0
Hippocratea volubilis	50.9	1.6	0.032	-29.2	-2.0
Ilex cassine	48.8	1.5	0.064	-26.3	-0.9
Laguncularia racemosa	50.6	1.5	0.079	-25.5	0.3
Metopium toxiferum	48.5	1.2	0.065	-30.3	0.8
Myrica cerifera	48.2	1.2	0.084	-27.2	3.2
Rhabdadenia biflora	46.1	1.1	0.077	-27.2	1.0
Rhizophora mangle	53.8	1.2	0.055	-28.7	-2.4
Sabal palmetto	46.6	1.0	0.040	-29.5	4.7
Salix caroliniana	50.3	1.0	0.046	-28.7	10.7
Typha domingensis	48.0	0.9	0.031	-28.6	6.0
Vitis aestivalis	47.5	0.8	0.069	-28.2	3.6
Captive					
Sweet Potato	41.7	0.6	0.174	-24.9	-1.7
Monkey Diet	44.1	3.6	0.198	-27.2	-1.1
Carrot	41.4	1.4	0.197	-25.7	-0.6
Romaine lettuce	42.9	5.6	0.191	-26.4	0.4
Apple	41.9	0.3	0.196	-21.2	3.4
Beet	39.2	2.7	0.197	-28.3	3.5
Pear	42.2	0.3	0.172	-25.9	10.4

Table 9. Means of carbon , nitrogen, phosphorus, d13C, and d15N compositions of vegetation collected for this project. Vegetation is grouped by category: seagrass, algae, freshwater vascular, terrestrial, and captive.

The $\delta^{13}C$ values of the freshwater vascular species, *N. guadalupensis* ($\delta^{13}C$ mean -38.1‰) and *R. maritima* ($\delta^{13}C$ mean = -31.2‰), were considerably lower than those of the marine seagrasses *H. wrightii* ($\delta^{13}C$ mean = -19.0‰), *T. testudinum* ($\delta^{13}C$ mean = -12.4‰) and *H. decipiens* ($\delta^{13}C$ mean = -17.7‰). The $\delta^{15}N$ values of *N. guadalupensis* ($\delta^{15}N$ mean = 4.4‰), *R. maritima* ($\delta^{15}N$ mean = -3.4‰), *H. wrightii* ($\delta^{15}N$ mean = -5.4‰), *T. testudinum* ($\delta^{15}N$ mean = 5.5‰) and *H. decipiens* ($\delta^{15}N$ mean = 0.1‰) were not significantly different from each other.

The $\delta^{13}C$ values for algal species ranged from -42.9‰ (*Gracilaria* sp.) to -13.8‰ (coralline red algae). The $\delta^{15}N$ values ranged from -0.6‰ (*Chara* sp.) to 8.3‰ (*Ulva* sp.).

The $\delta^{13}C$ values for terrestrial vegetation ranged from -30.9‰ (*Blechum pyramidatum*) to -25.5‰ (*L. racemosa*) (mean = -28.2‰ ± 1.5‰). $\delta^{15}N$ values were between -2.6‰ (*Chrysobalanus icaco*) and 10.7‰ (*S. caroliniana*) (mean = 3.1‰ ± 2.7‰).

Romaine lettuce, which comprises ~ 90% of manatee diet at Miami Seaquarium, had average $\delta^{13}C$ and $\delta^{15}N$ values of -26.4‰ and +0.4‰, respectively. Monkey Diet, a cereal-based diet amended with animal meal and vitamins, which accounts for about 5% of the manatee diet at the Seaquarium, had average $\delta^{13}C$ and $\delta^{15}N$ values of -27.2‰ and -1.1‰, respectively. Other minor components of captive manatee diet, including pears, yams, apples, carrots, beets, had $\delta^{13}C$ values ranging from -28.3‰ to -21.2‰ (mean = -26.0‰ ± 0.9‰) and $\delta^{15}N$ values ranging from -1.7‰ to +10.4‰ (mean = 1.46‰ ± 5.1‰).

Relationships of Elemental and Isotopic Contents of Vegetation to Water Quality

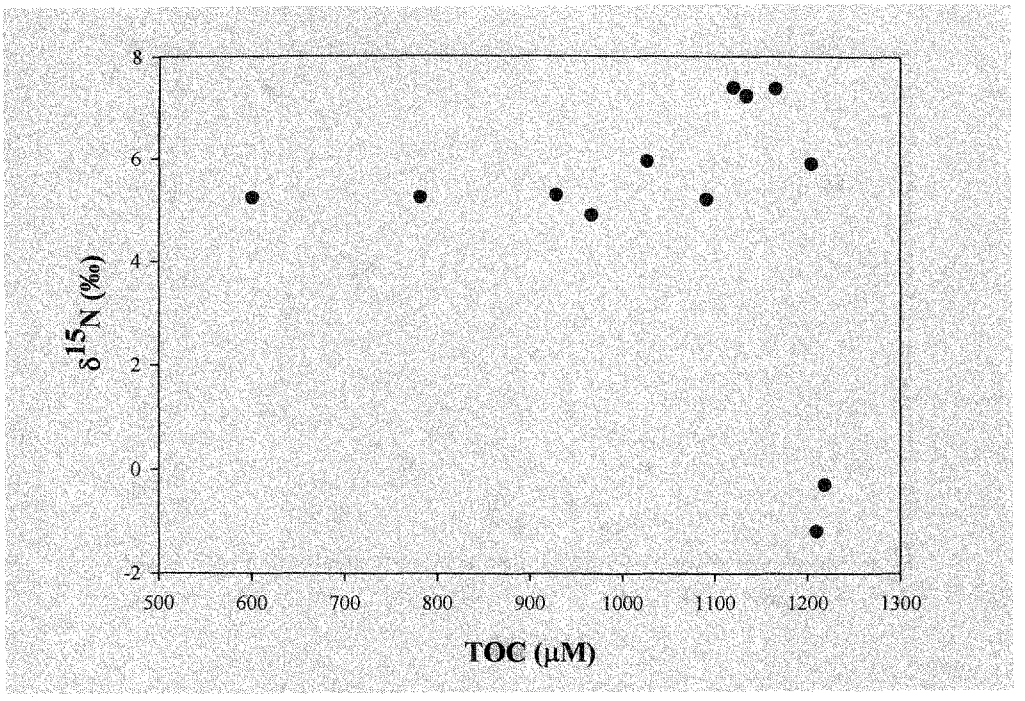
Bivariate correlations of water quality proxy variables and elemental and isotopic contents of SAV from Shark River showed 10 significant correlations (Table 10; Fig. 9). Due to lack of distribution for marine and freshwater vascular vegetations, only algae and terrestrial vegetation were included in the correlations. Sediment depth was correlated with %C, %N, $\delta^{15}N$, and $\delta^{13}C$ in algae ($p < 0.031$). TOC was correlated with P in

Content and Vegetation Type		TN	TP	TOC	Salinity	% Light at Bottom	Sediment Depth
% C Algae (n = 13)	Pearson Correlation	-0.298	0.035	0.047	0.237	0.133	-0.609
	Sig. (2-tailed)	0.323	0.909	0.879	0.435	0.664	**0.027**
% C Terrestrial (n = 19)	Pearson Correlation	0.229	-0.374	0.433	-0.365	-0.371	0.085
	Sig. (2-tailed)	0.345	0.115	0.064	0.125	0.118	0.729
% N Algae (n = 13)	Pearson Correlation	-0.179	0.099	0.054	0.141	0.123	-0.678
	Sig. (2-tailed)	0.559	0.748	0.860	0.646	0.690	**0.011**
% N Terrestrial (n = 19)	Pearson Correlation	0.820	-0.717	0.671	-0.700	-0.222	0.235
	Sig. (2-tailed)	**0.000**	**0.001**	**0.002**	**0.001**	0.361	0.332
% P Algae (n = 13)	Pearson Correlation	-0.172	-0.014	0.015	0.193	0.083	-0.496
	Sig. (2-tailed)	0.575	0.964	0.962	0.527	0.788	0.085
% P Terrestrial (n = 19)	Pearson Correlation	0.268	-0.289	0.181	-0.226	-0.175	0.217
	Sig. (2-tailed)	0.268	0.230	0.458	0.353	0.473	0.372
$\delta^{15}N$ Algae (n = 13)	Pearson Correlation	-0.100	0.419	-0.623	0.422	0.395	0.597
	Sig. (2-tailed)	0.745	0.154	**0.023**	0.151	0.182	**0.031**
$\delta^{15}N$ Terrestrial (n = 19)	Pearson Correlation	-0.398	0.342	-0.209	0.296	0.064	-0.281
	Sig. (2-tailed)	0.092	0.152	0.391	0.218	0.796	0.244
$\delta^{13}C$ Algae (n = 13)	Pearson Correlation	-0.625	0.403	-0.189	0.347	0.419	-0.876
	Sig. (2-tailed)	**0.022**	0.172	0.537	0.246	0.154	**0.000**
$\delta^{13}C$ Terrestrial (n = 19)	Pearson Correlation	0.237	-0.234	0.170	-0.254	-0.056	0.049
	Sig. (2-tailed)	0.328	0.335	0.486	0.293	0.821	0.841

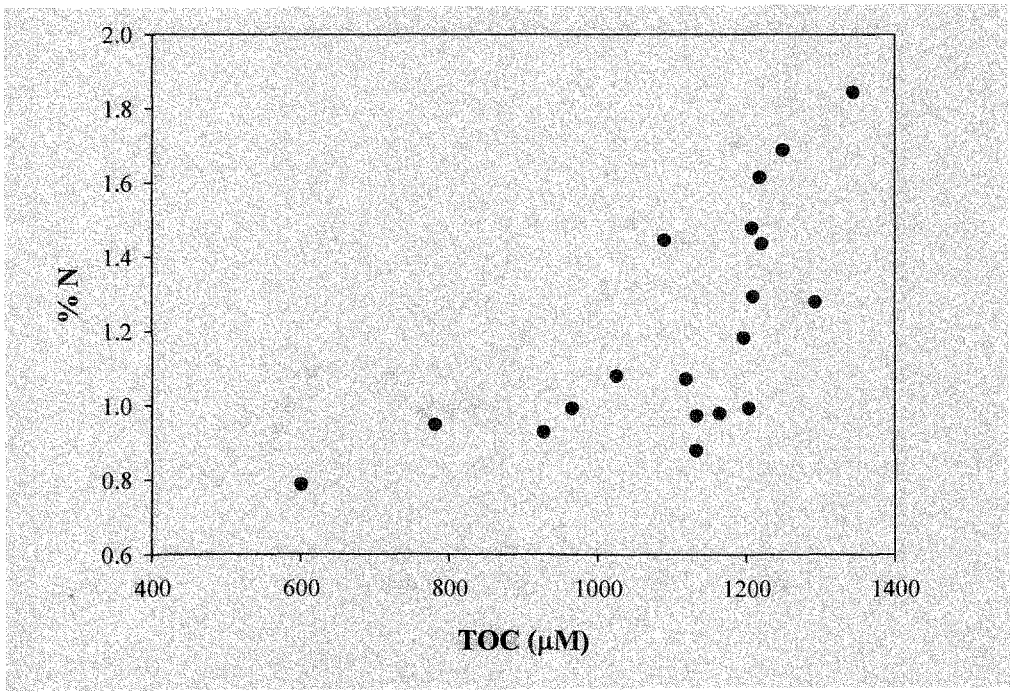
Table 10. Bivariate correlations for elemental and isotopic content of vegetation with water quality.

terrestrial vegetation ($p = 0.002$) and with $\delta^{15}N$ in algae ($p = 0.023$). Nitrogen in terrestrial vegetation was also correlated with TN, TP, and salinity. TN was correlated with $\delta^{13}C$ in algae ($p = 0.022$).

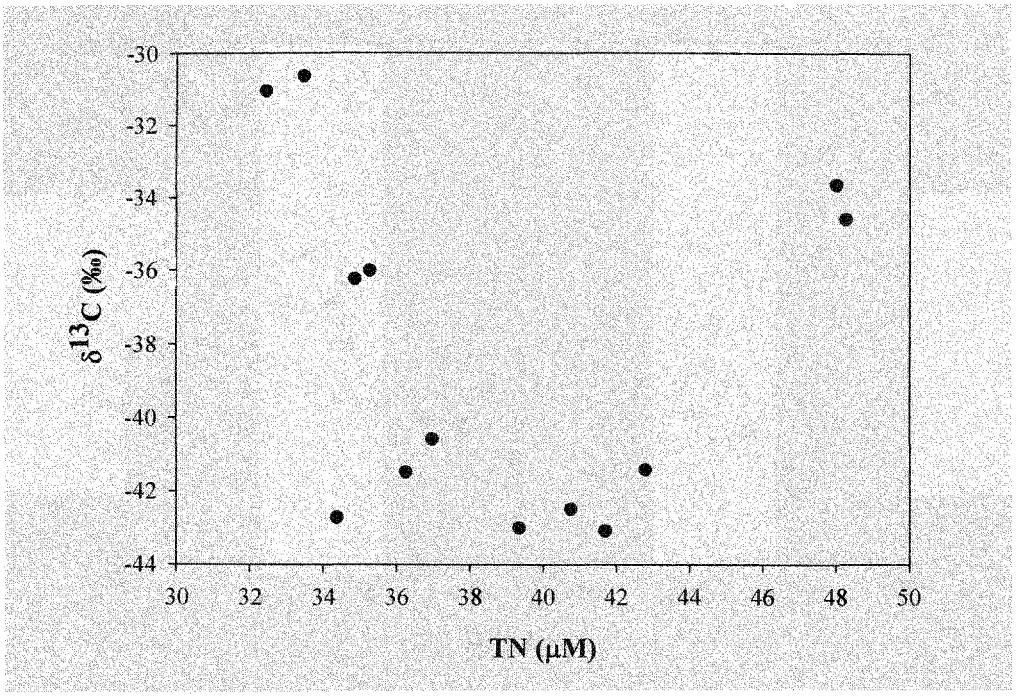
$\delta^{15}N$ in Algae v TOC in Water



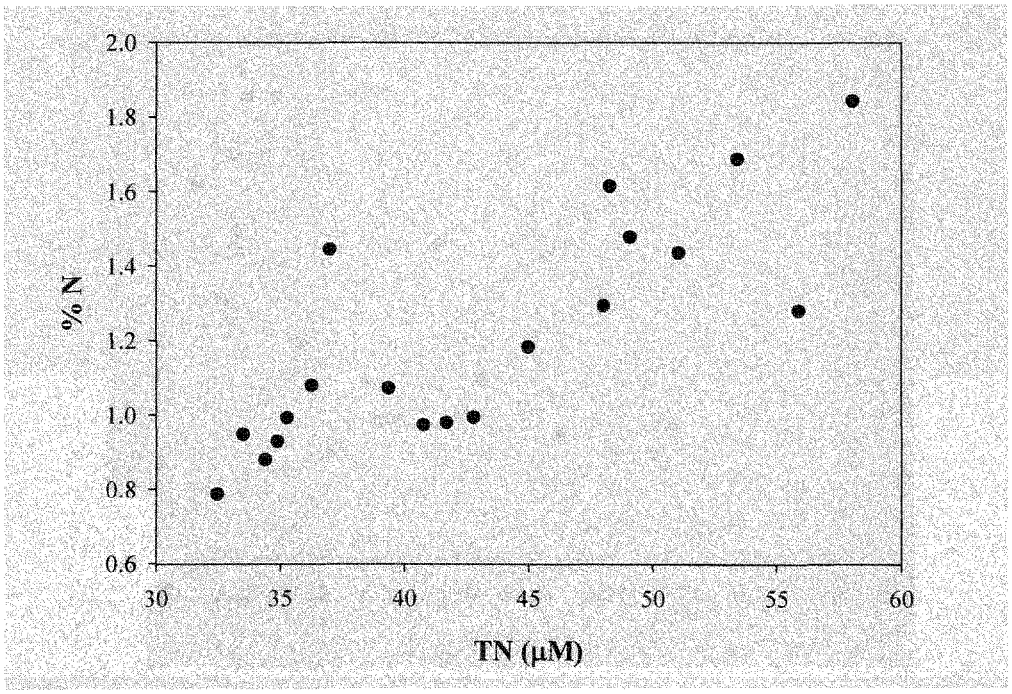
% N in Terrestrial Vegetation v TOC in Water



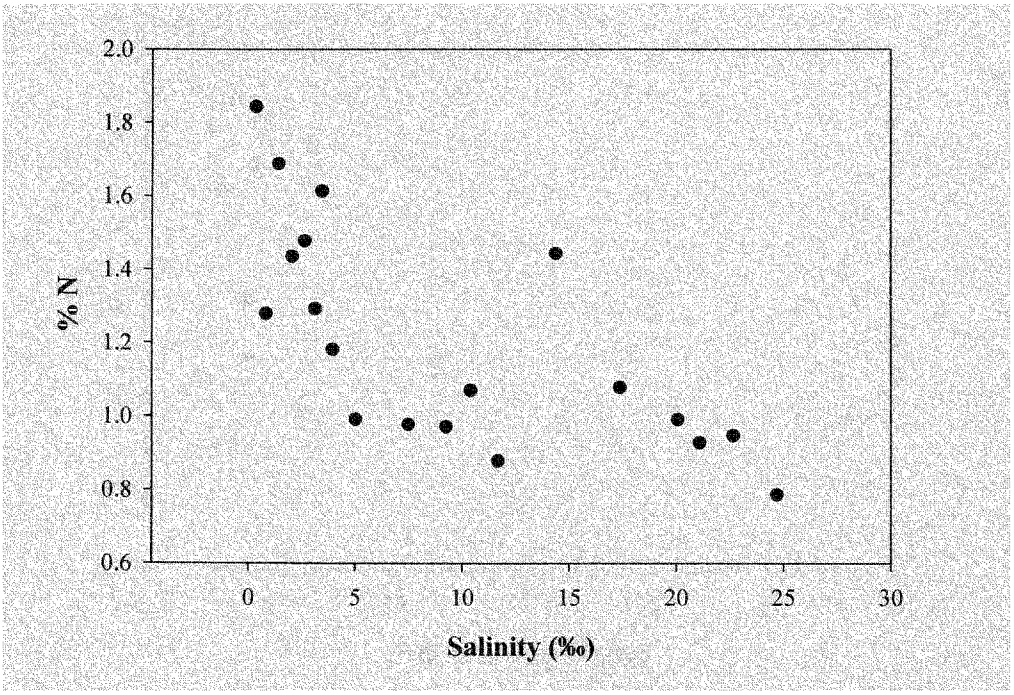
$\delta^{13}C$ in Algae v TN in Water



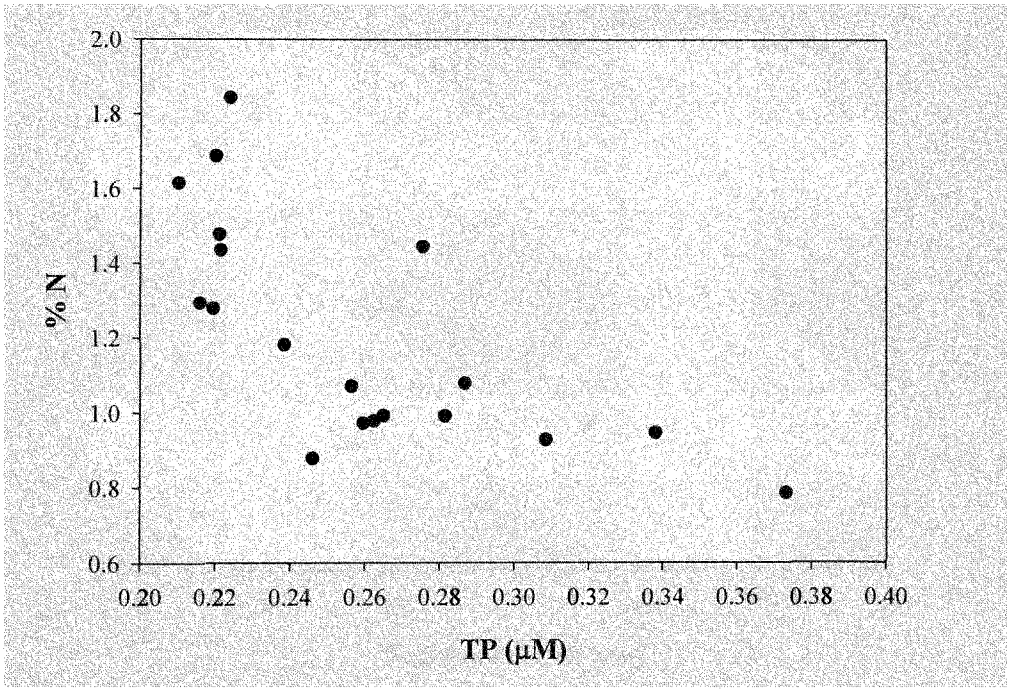
% N in Terrestrial Vegetation v TN in Water



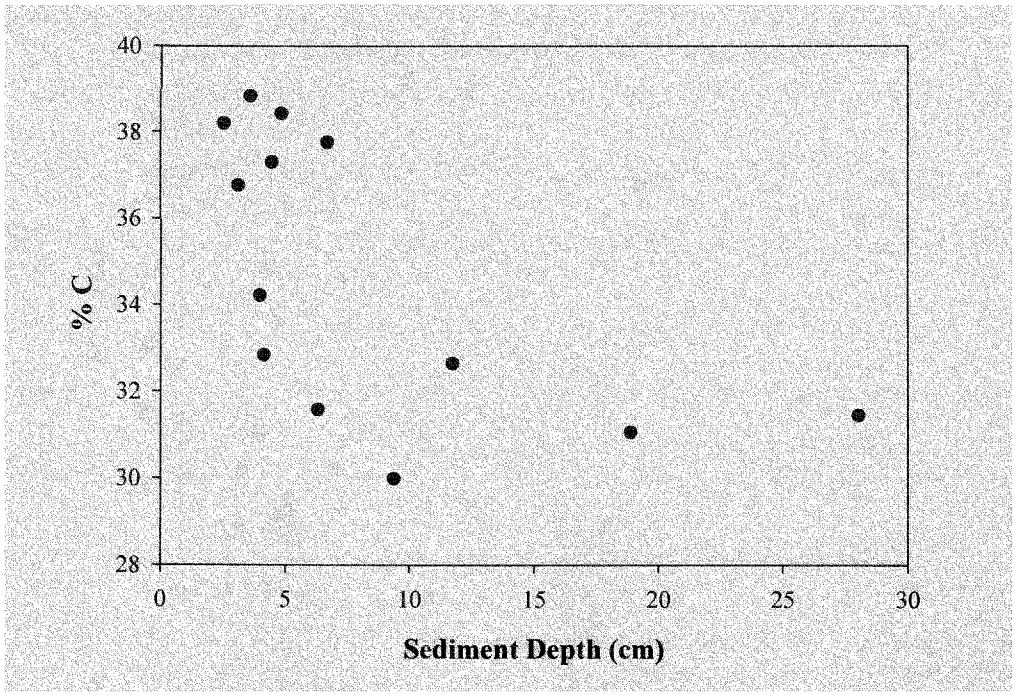
% N in Terrestrial Vegetation v. Salinity of Water



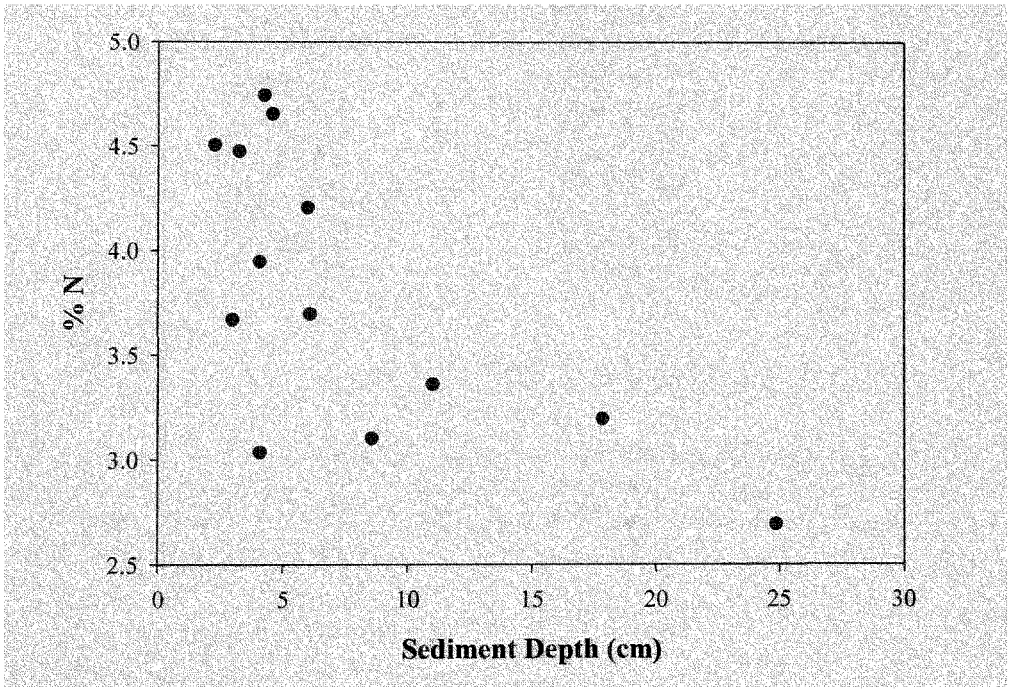
% N in Terrestrial Vegetation v TP in Water



% C in Algae v Sediment Depth



% N in Algae v Sediment Depth



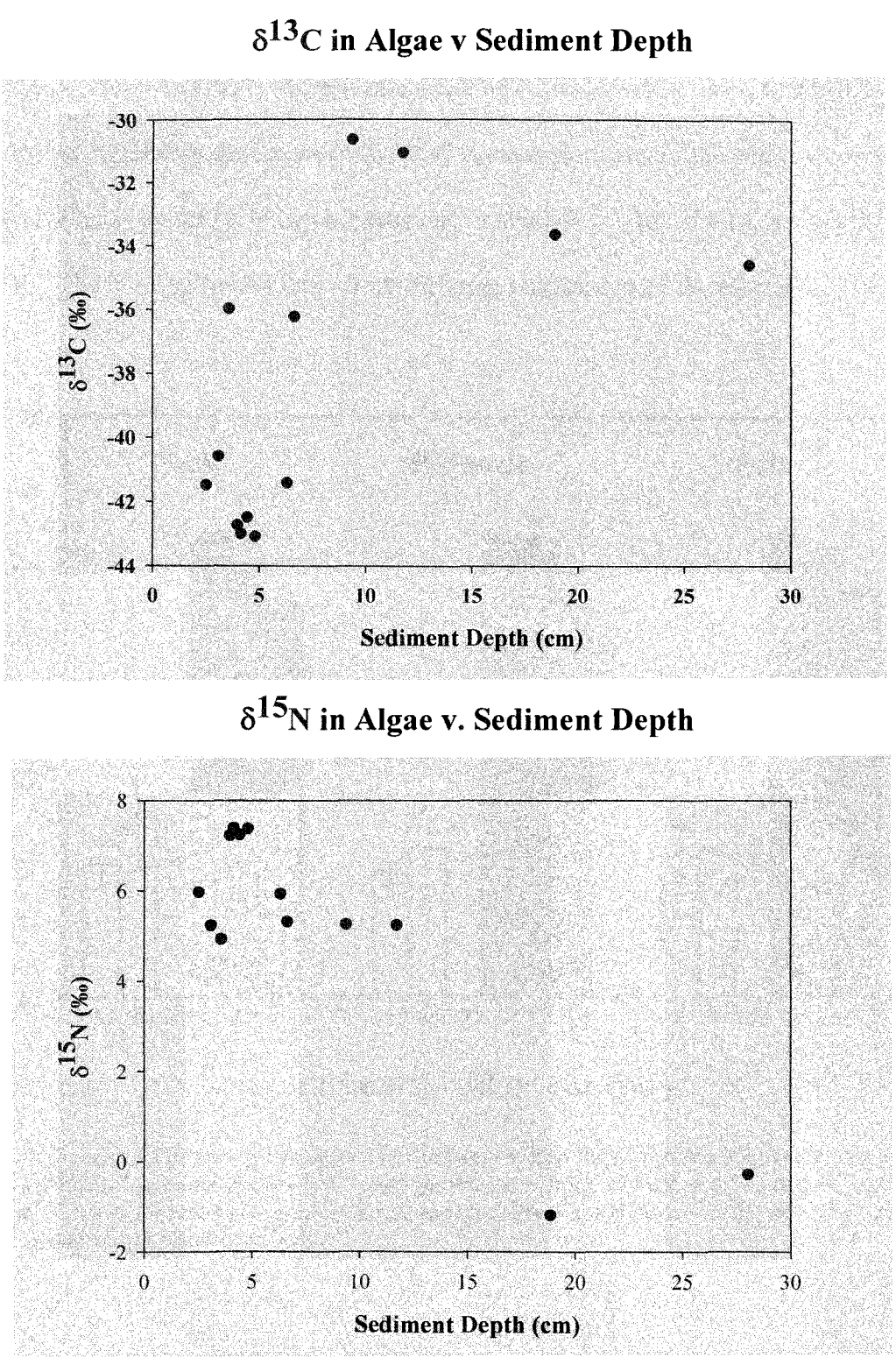


Fig. 9 Comparisons of elemental and isotopic compositions of vegetation from the Shark River with water quality parameters from the same area. Data points for water quality parameters are time-averaged means.

Isotopic content of wild and captive manatee hairs

A one-way ANOVA, which nested location on body within individual manatee, identified no significant difference in the $\delta^{13}C$ signatures on any of the animals between locations of hairs on the body (neck, peduncle, or side) ($F = 1.18$, $df = 14$, $p = 0.345$) (Fig. 10). The $\delta^{13}C$ values for hairs from different locations were therefore pooled for

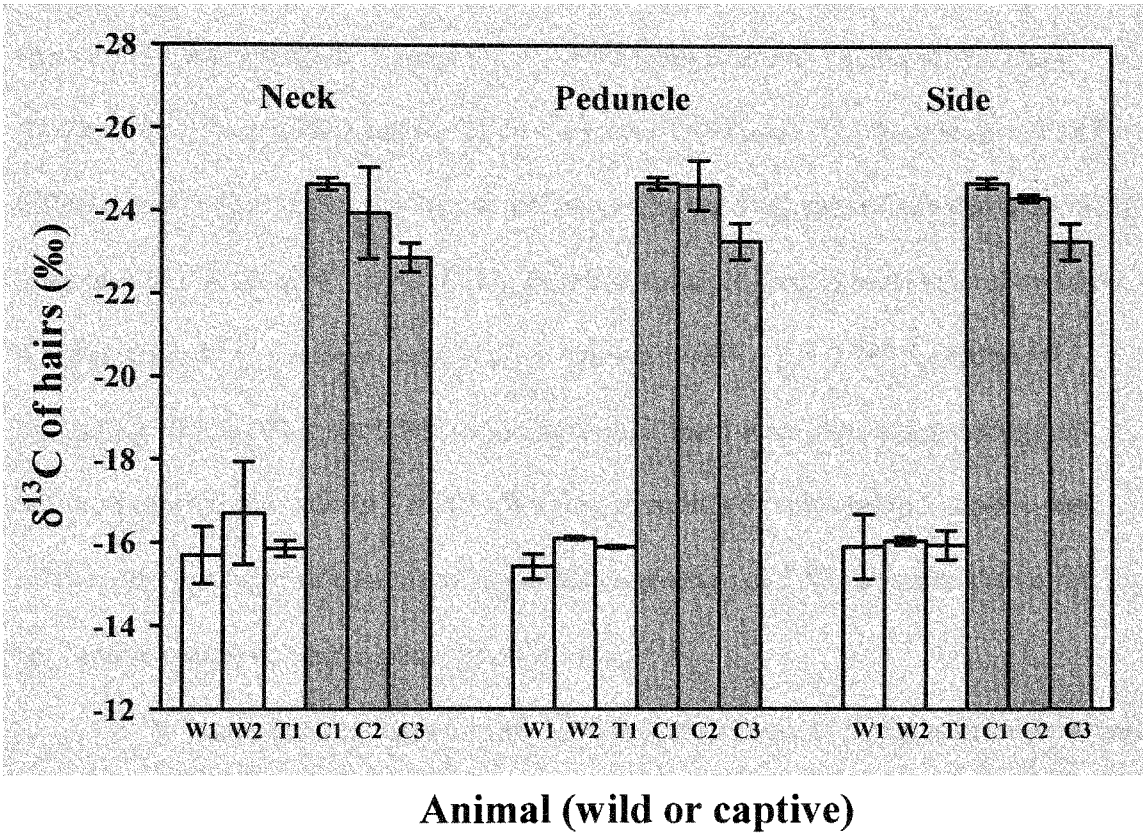


Fig. 10. $\delta^{13}C$ values for hairs of wild and captive manatees on different areas of the body (neck, peduncle, and side). Light bars indicate $\delta^{13}C$ values for wild manatees (W1, W2, and T1); dark bars indicate $\delta^{13}C$ values for captive manatees (C1, C2, and C3). Error bars indicate ± 1 SD. N = 3 for each animal at each location on body.

further analysis. A similar statistical comparison on the $\delta^{15}N$ could not be completed because some of the hair samples were not large enough to analyze for both and $\delta^{13}C$ and $\delta^{15}N$.

$\delta^{13}C$ values for manatees hairs ranged from -25.1% to -15.2% (Table 11). Captive manatee hairs ($n = 3$) showed $\delta^{13}C$ values from -25.1‰ to -22.1‰ (mean = -24‰ ± 0.8‰) while those from wild manatees ($n = 2$) ranged from -16.1‰ to -14.9‰ (mean = -15.7‰ ± 0.5‰). Values for T1 ($n = 1$) ranged from -16.2‰ to -15.7‰ (mean = -15.9 ‰ ± 0.2‰) and for T2 ($n = 1$) from -20.0‰ to -17.6‰ (mean = -19.4‰ ± 0.9‰). ANOVA revealed significant differences in the $\delta^{13}C$ values between individual manatees ($F = 8.10$, $df = 5$, $p < 0.001$). Post hoc multiple comparisons tests showed no differences in $\delta^{13}C$ values within the three captive ($0.054 < p < 0.982$) or three wild (including T1) ($0.194 < p < 0.965$) animals though there were significant differences between captive and wild ($p < 0.001$) Within one month of consuming a captive diet, $\delta^{13}C$ values for the transition manatee shifted an average of 3.5‰ towards the values for the captive manatees on the same diet (Fig. 11a).

$\delta^{15}N$ values in hairs ranged from 3.2% to 7.3% (Table 11). Nitrogen isotope data were only available for manatees C1, T1, T2, W1, and W2. $\delta^{15}N$ values ranged from 3.2% and 7.3%. These values for captive manatees ($n = 1$) were between 5.4 and 5.6 (mean = 5.5‰ ± 0.1‰) and for wild manatees ($n = 2$) were between 3.2 and 7.3 (6.9‰ ± 1.5‰).

$\delta^{13}C$	C1	C2	C3	C4	T1	T2	W1	W2
N	6	6	6	6	6	6	6	6
Mean	-23.2	-23.8	-24.3	-24.7	-15.9	-19.4	-15.7	-16.3
Median	-23.3	-24.1	-24.3	-24.7	-15.9	-19.7	-15.5	-16.1
Minimum	-23.7	-24.4	-25.1	-24.8	-16.2	-20.0	-16.5	-17.6
Maximum	-22.6	-22.1	-23.2	-24.5	-15.7	-17.6	-15.2	-15.8
Standard Deviation	0.4	0.8	0.6	0.1	0.2	0.9	0.5	0.6

$\delta^{15}N$	C1	C2	C3	C4	T1	T2	W1	W2
N	3	N/D	N/D	N/D	6	6	3	3
Mean	5.5	N/D	N/D	N/D	6.9	6.0	3.7	4.3
Median	5.5	N/D	N/D	N/D	7.1	5.9	3.4	3.9
Minimum	5.4	N/D	N/D	N/D	6.3	5.8	3.2	3.4
Maximum	5.6	N/D	N/D	N/D	7.3	6.2	4.6	5.6
Standard Deviation	0.1	N/D	N/D	N/D	0.5	0.2	0.8	1.1

Table 11. Summary statistics for $\delta^{13}C$ and $\delta^{15}N$ values for hairs of captive, transition, and wild manatees. Carbon isotopic ratios for all captive (n = 4) and all wild (n = 2) were pooled for captive and wild descriptive statistics.

difference between C1 and T2 ($p = 0.749$). Neither W1 nor W2 were different from each other ($p = 0.966$) but were different from T1 ($p < 0.001$) and T2 ($p = 0.002$ and 0.005, respectively) (Fig. 11b).

The data show an average 7.4‰ enrichment of the $\delta^{13}C$ signatures in hairs in wild manatees (mean = -15.9‰ ± 0.6‰) compared to captive animals (mean = -23.9‰ ± 0.8‰).

Relating Manatee Hairs to Potential Food Sources

The $\delta^{13}C$ signatures for hair samples of captive manatees were higher by an average of 4.7‰ over diet. The captive animal's diet had a weighted mean $\delta^{13}C$ of -27.9‰, with the weighted mean calculated as *weighted mean* = Σ from i = 1 to j $X_i \cdot \delta^{13}C_i$;

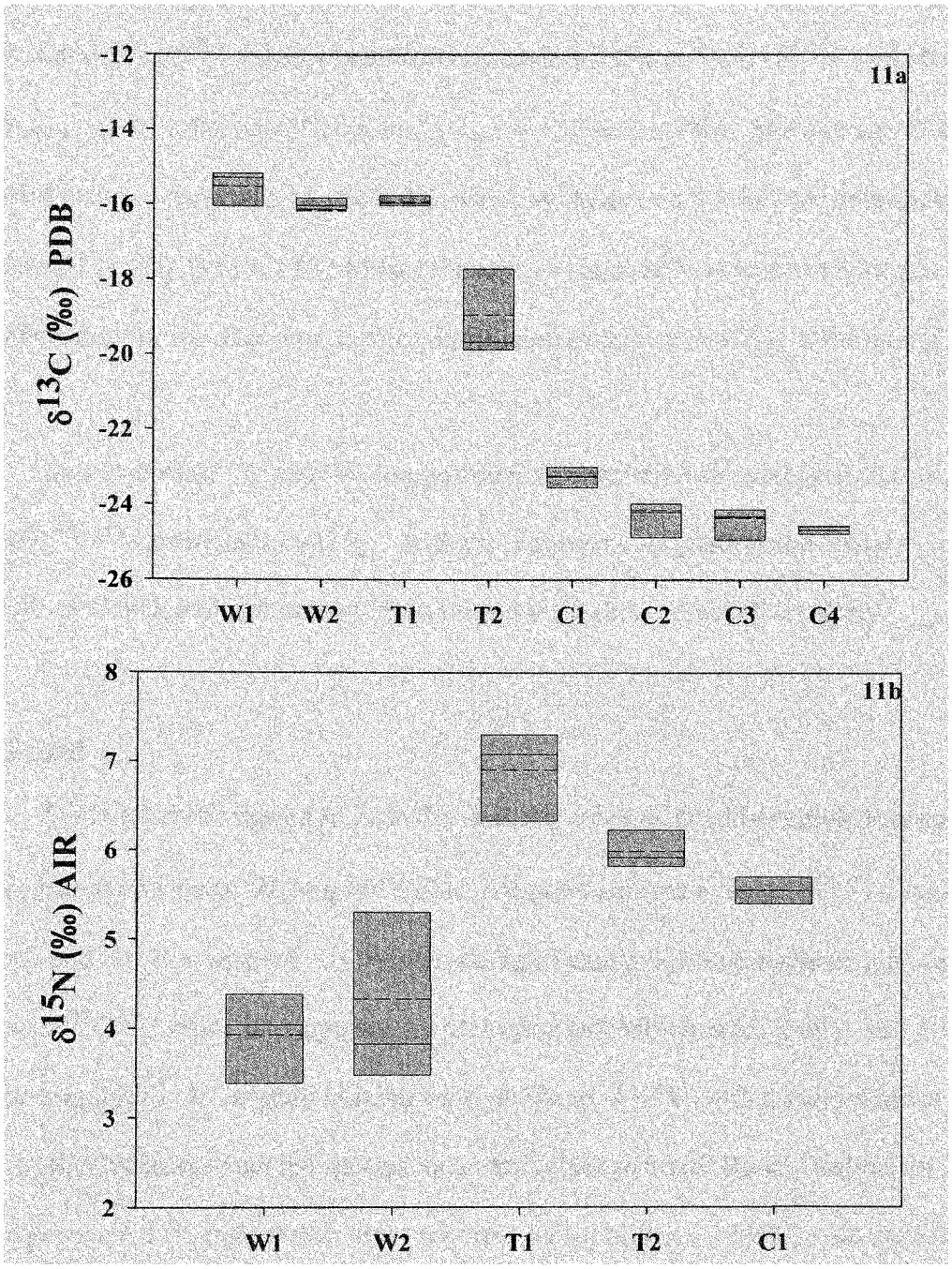


Fig. 11. δ13C (11a) and $\delta^{15}N$ (11b) values of manatee hairs of two wild (W1 and W2), two transitional (T1 and T2), and four captive (C1, C2, C3, and C4) manatees. Data for only one captive manatee (C1)were available for $\delta^{15}N$ comparisons. Arrows show the trend of isotopic depletion in the Transitional manatee towards values of captive manatees. Solid bar is median, dotted bar is mean.

where Σ from i = 1 to j $X_i = 1.0$, i represents an individual food source, and j is the last food source. The $\delta^{15}N$ values of hairs were enriched 2.1‰ over diet ($\delta^{15}N$ weighted $mean_{captive\ diet}$ = +3.4‰ and $\delta^{15}N$ $mean_{captive\ hair}$ = +5.5‰ ± 0.2‰). The average $\delta^{15}N$ of the wild manatee hairs was +4.0‰ ± 0.4, which is closer to our measured average marine seagrass $\delta^{15}N$ of 0.43‰ ± 2.83. Means for captive manatee food were weighted to accommodate for the disproportionate contribution of lettuce (~ 90%) to the animals' diet.

Scatterplots of ^{13}C and ^{15}N compositions of vegetation showed general category-specific $^{13}C/^{15}N$ compositions (Fig. 12a & b). Terrestrial vegetation and captive vegetation (which included terrestrial fruits and vegetables) grouped similarly.

Discussion

$\delta^{13}C$ values of vegetation from Shark River are considerably depleted compared to most reported values. *H. wrightii* and *H. decipiens* showed average $\delta^{13}C$ values of -19.0‰ and -17.7‰, respectively, which are significantly lighter than those reported in a review of $^{12}C/^{13}C$ ratios in seagrasses by McMillan (McMillan et al. 1980). The review reported values for *H. wrightii* ranging from -8.5‰ to -13.3‰ with a species mean of -10.8, 43% lower than the species mean for values in Shark River. Values for *H. decipiens* are 42% lighter than those reported by McMillan (-10.2‰) with an average mean of -17.7‰. $\delta^{13}C$ values for algae were between -12.9‰ and -43.5‰ with a mean of -34.2‰ ± 7.2‰. This is mainly due to the DIC pool from which the alga take their carbon; mean $\delta^{13}C$ values for *R. mangle*, *L. racemosa*, and *C. erectus* along the Shark

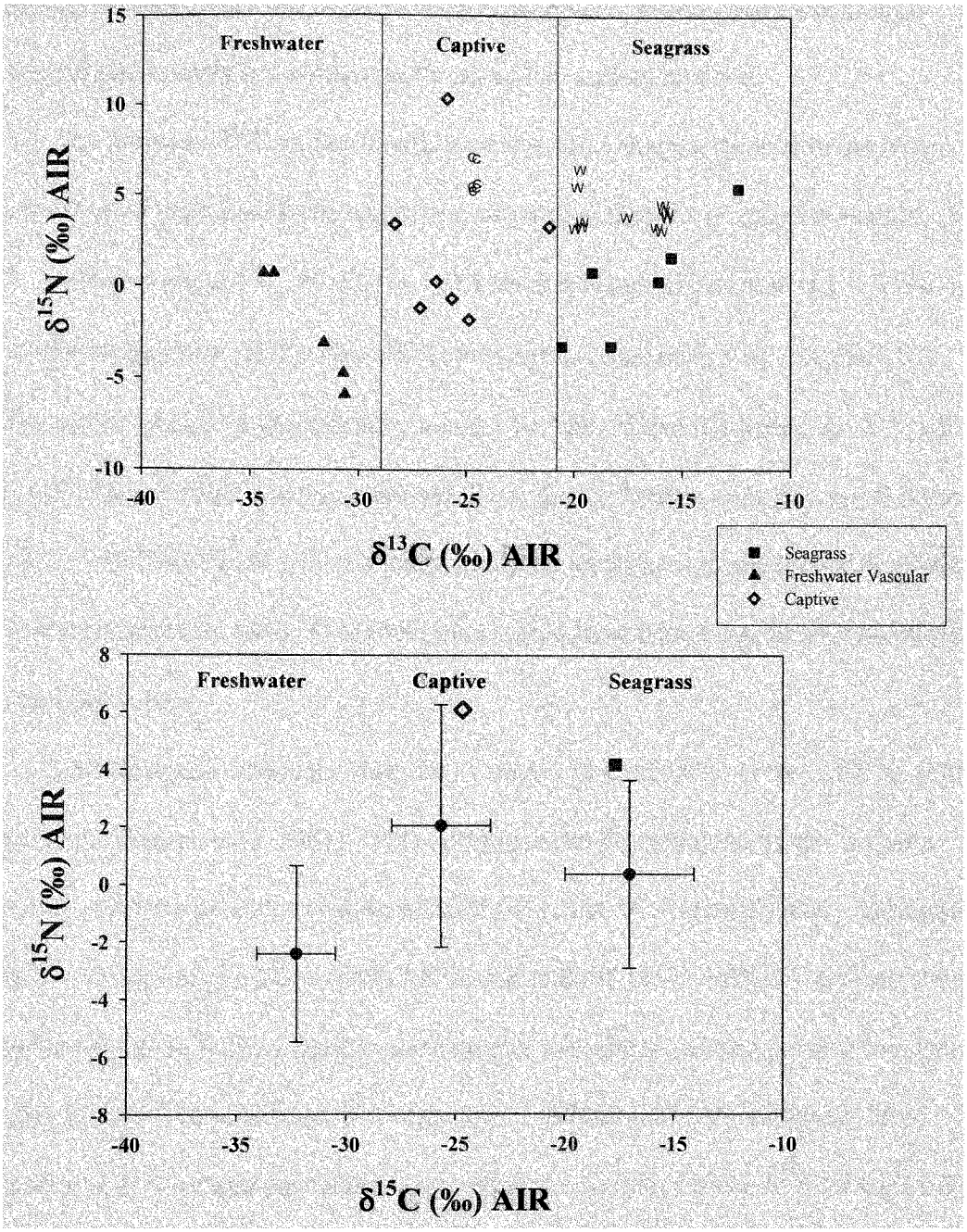


Fig. 12. Plots of $\delta^{13}C$ and $\delta^{15}N$ values for vegetation (freshwater vascular and seagrass from Shark River) and captive food items (from Miami Seaquarium) compared with $\delta^{13}C$ and $\delta^{15}N$ values of manatee hairs. 12a) Scatterplot of vegetation and manatee hairs (C = captive and W = wild). 12b) Bidirectional error bars for $\delta^{13}C$ and $\delta^{15}N$ compositions of vegetation. Error bars include 95% confidence interval. $\delta^{13}C$ and $\delta^{15}N$ values for hairs of captive manatees represented by open diamond symbol; those for wild manatees represented by closed square symbol.

River are -27.19 ± 1.06, -28.21 ± 0.92 and -27.23 ± 0.65, respectively. Low light levels at the sites further contribute to the isotopically-depleted numbers as there is less photosynthetic activity occurring and therefore less carbon demand.

The enriched $\delta^{13}C$ values in wild manatee hairs suggest these animals obtain a large portion of their food from seagrasses rather than from freshwater vegetation. This study showed average $\delta^{13}C$ of -33.1‰ ± 0.5 for freshwater vegetation and -15.5‰ ± 0.6 for marine seagrasses. The average $\delta^{13}C$ for wild manatee hairs was -16.0‰ ± 0.2, similar to that of their suggested food source. For the captive manatees, the $\delta^{13}C$ of their hairs (-23.2‰ ± 0.8‰) is isotopically-similar to that of lettuce, their primary food source (-25.2‰ ± 0.03‰). If the wild manatees had been feeding on the isotopically lighter freshwater vegetation, the $\delta^{13}C$ of their hairs would have been considerably heavier than the resulting values.

This difference is greater than the average 1‰ increase over diet of $\delta^{13}C$ values in gerbil hairs (Tieszen et al. 1983). This difference can be explained by the variable vegetable combinations provided to manatees in captivity. Tieszen's study had tightly fixed $\delta^{13}C$ values of -12.2‰ ± 0.4‰ for C_4 diet and -21.8‰ ± 0.3‰ for C_3 diet. Lettuce comprises the great bulk of captive manatee diet with the remaining portion consisting of Monkey Diet and various fruits and vegetables. Additionally, the relatively large difference in $\delta^{13}C$ of manatee hairs compared to the weighted mean $\delta^{13}C$ of their offered diet may indicate that the manatees were selectively feeding on the food source with isotopically heavier ratios, such as the Monkey Diet. The Monkey Diet is a cereal-based diet consisting mainly of soy, wheat, corn, animal fat preserved with BHA, beet pulp, fish

meal, alfalfa, plus additional vitamins and minerals. Not surprisingly, the isotope values of this mixture of foods comprise an isotopic composition appropriately reflecting this amalgamation. Corn and alfalfa are C_4 plants, which tend to have heavier $\delta^{13}C$ values than those of C_3 plants like soybean, beets, and wheat. Similarly, the fish meal and animal fat would have more enriched $\delta^{15}N$ values than that of primary producers, since heterotrophs become enriched by ~3‰ at every trophic level.

The $\delta^{15}N$ information obtained from T1/T2 was initially confusing as it was expected that T1 would have had a $\delta^{15}N$ composition similar to that of the wild animals as was true with her $\delta^{13}C$ values. Instead, her $\delta^{15}N$ values are an average of 2.0‰ greater than those of the two wild manatees (Fig. 11b). These differences are likely a result of her feeding history prior to capture. Based on the contents and consistency of her fecal matter, the Seaquarium staff determined that this animal had been primarily nursing prior to being brought to the facility. Nitrogen isotopic values become heavier by approximately 3‰ to 5‰ upon trophic transfer (DeNiro and Epstein 1981), so a calf feeding on mother's milk would be expected to be isotopically heavier than her mother. It is precisely such a difference that is seen between this animal and the two wild ones. Other investigators have encountered similar diet enrichments in nursing young (Fogel et al. 1989; Hobson et al. 1997; Hobson et al. 2000; Jenkins et al. 2001). Additionally, both the $\delta^{15}N$ and $\delta^{13}C$ in the hair samples taken from T1 in May, after a month of eating the diet of the captive manatees, show a shift towards that of the captives. The $\delta^{15}N$ of the wild manatee hairs serves to confirm the above conclusion that the wild manatees sampled were feeding primarily on seagrasses.

The results of this study show that manatee hairs reflect the isotopic compositions of their food source. They also show that nutrient and elemental content of SAV vary but tend to fall into common ranges of value. Total nitrogen content in the water is greatest in the upstream regions due to terrestrial inputs; high TOC values upstream occur due to the longer residence time of mangrove C in low-energy "nooks" of the river. Phosphorus levels decrease with distance upstream as is common in estuarine environments where P inputs come from offshore upwelling currents driven in by tidal flow. Higher nutrient content in plants means potentially higher food quality and animals may specifically seek higher nutrient vegetation for feeding (Preen 1995; Valentine and Heck 2001). The nutrient contents of SAV in the Shark River are generally correlated with the salinity gradient, thus changes in freshwater regime in the estuary could influence nutrient quality of foods sought out by manatees.

For these results to be more useful in field research, it will be important to understand turnover time for hairs, which is unknown at present. Currently, little is known about the growth of manatee hairs. Anecdotal evidence exists suggesting hairs grow longer on captive manatees than on wild since there are fewer sources of abrasion in captivity. If the growth rate of hairs was known, then it would be possible to develop feeding chronologies along single hairs if $\delta^{13}C$ and $\delta^{15}N$ values were determined for small hair segments. It is known that more metabolically-active tissues, such as liver and fat, have faster turnover rates than bone and connective tissues (Libby et al. 1964). As such, it is expected that turnover rate for body hairs would be relatively slow but the exact rate is unknown. Hairs grow by adding length at their base; keratin is formed in the basal portions under the skin (Chase 1954). If new hair isotopically matches the current

food source of the animal, the changes in isotopic composition of the hairs along their length would represent a record of the seasonal fluctuations in diet and migration patterns (Schwertl et al. 2003). Another consequence of this growth pattern will be relatively rapid change in the stable isotope composition of whole hairs immediately after a diet shift with an asymptotic approach to the new food isotopic value. In an experiment switching gerbils (*Meriones unguienlatus*) from a C_4-based diet to a C_3-based diet, hairs had still not reached the isotopic composition of the food source even after 155 days (Tieszen et al. 1983). Determination of growth rate for body hairs can be achieved using captive manatees and a prescribed marking technique, perhaps adding labeled nitrogen or carbon to a food source. Understanding this growth rate coupled with the information presented above will present a more complete picture of manatee dietary habits and movement patterns.

The information gleaned from this investigation provides researchers with the ability to explore manatee travel routes and food consumption habitats across a broad spatial range. This concept will be a particularly beneficial tool for managers and scientists working on the grand-scale restoration activities occurring in ENP. This ecosystem stands to be significantly affected by restoration management activities. The research provided in these two chapters shows that the benthic vegetation communities of the Shark River are particularly susceptible to changes in salinity regime. As the CERP calls for delivering an additional 26% of water flow through the Everglades (with an obvious increase in the Shark River, the main estuary), it is likely that the species composition and distribution of vegetation along the river will shift up or downstream to

accommodate lower salinities. These effects have significant potential to affect the feeding and movement patterns of manatees along the southwestern Florida coast.

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