

**Proximate Nutrient Analyses of Four Species of Submerged Aquatic Vegetation Consumed by Florida Manatee (*Trichechus manatus latirostris*) Compared to Romaine Lettuce (*Lactuca sativa* var. *longifolia*)**

Author(s): Jessica L. Siegal-Willott, D.V.M., Dipl. A.C.Z.M., Kendal Harr, D.V.M., M.S., Dipl. A.C.V.P., Lee-Ann C. Hayek, Ph.D., Karen C. Scott, Ph.D., Trevor Gerlach, B.S., Paul Sirois, M.S., Mike Reuter, B.S., David W. Crewz, M.S., and Richard C. Hill, M.A., Vet.M.B., Ph.D., M.R.C.V.S.

Source: Journal of Zoo and Wildlife Medicine, 41(4):594-602. 2010.

Published By: American Association of Zoo Veterinarians

DOI: 10.1638/2009-0118.1

URL: <http://www.bioone.org/doi/full/10.1638/2009-0118.1>

---

BioOne ([www.bioone.org](http://www.bioone.org)) is an electronic aggregator of bioscience research content, and the online home to over 160 journals and books published by not-for-profit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/page/terms\\_of\\_use](http://www.bioone.org/page/terms_of_use).

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

## PROXIMATE NUTRIENT ANALYSES OF FOUR SPECIES OF SUBMERGED AQUATIC VEGETATION CONSUMED BY FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*) COMPARED TO ROMAINE LETTUCE (*LACTUCA SATIVA* VAR. *LONGIFOLIA*)

Jessica L. Siegal-Willott, D.V.M., Dipl. A.C.Z.M., Kendal Harr, D.V.M., M.S., Dipl. A.C.V.P., Lee-Ann C. Hayek, Ph.D., Karen C. Scott, Ph.D., Trevor Gerlach, B.S., Paul Sirois, M.S., Mike Reuter, B.S., David W. Crewz, M.S., and Richard C. Hill, M.A., Vet.M.B., Ph.D., M.R.C.V.S.

**Abstract:** Free-ranging Florida manatees (*Trichechus manatus latirostris*) consume a variety of sea grasses and algae. This study compared the dry matter (DM) content, proximate nutrients (crude protein [CP], ether-extracted crude fat [EE], nonfiber carbohydrate [NFC], and ash), and the calculated digestible energy (DE) of sea grasses (*Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*) collected in spring, summer, and winter, and an alga (*Chara* sp.) with those of romaine lettuce (*Lactuca sativa* var. *longifolia*). Neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and lignin (L) measured after ash-extraction were also compared. Results of statistical tests ( $\alpha = 0.01$ ) revealed DM content was higher in aquatic vegetation than in lettuce ( $P = 0.0001$ ), but NDF and ADF were up to threefold greater, EE ( $P = 0.00001$ ) and CP ( $P = 0.00001$ ) were 2–9 times less, and NFC ( $P = 0.0001$ ) was 2–6 times lower in sea grass than in lettuce, on a DM basis. *Chara* was lower in NDF, ADF, L, EE, CP, and NFC relative to lettuce on a DM basis. Ash content (DM basis) was higher ( $P = 0.0001$ ), and DE was 2–6 times lower in aquatic vegetation than in lettuce. Sea grass rhizomes had lower L and higher ash contents (DM basis) than sea grass leaves. Based on the nutrient analyses, romaine lettuce and sea grasses are not equivalent forages, which suggests that the current diet of captive Florida manatees should be reassessed.

**Key words:** *Halodule wrightii*, manatee, nutrient analysis, *Syringodium filiforme*, *Thalassia testudinum*, *Trichechus manatus*.

### INTRODUCTION

Free-ranging manatees consume more than 60 species of shoreline plants and submerged aquatic vegetation (SAV), including sea grasses such as *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*, marine algae, and freshwater algae such as *Chara* spp.<sup>1,5,16,17,20,21,27,30,31,35</sup> In contrast, captive manatees are fed a diet composed principally of romaine lettuce (*Lactuca sativa* var.

*longifolia*) supplemented with commercial pelleted foods marketed for various other species, as well as other leafy foods (green-leaf lettuce, cabbage, endive, kale), fruits (apples), and vegetables (sweet potatoes, carrots).<sup>1,19</sup>

Previous research into manatee nutrition focused on manatee digestive morphology, physiology, and efficiency, and on the types of aquatic plants consumed.<sup>1,3–5,13,15–17,19,20,25,27,30</sup> Previous reports concerned with aquatic plants consumed by manatees have evaluated the nutrient content of plants consumed and the variability in plant nutrient content based on species, collection site, location within the community, plant turnover, and time of year, but have not evaluated *Chara* algae or directly compared such data with that of romaine lettuce in diets of captive animals.<sup>1,3–13,18,20,25,27,30</sup> Free-ranging manatees consume entire plants of sea grasses, including both rhizomes and leaves.<sup>20,27</sup> Previous investigations found SAV to be high in ash and insoluble carbohydrate, and low in percent protein, lipid, lignin, and soluble carbohydrates.<sup>6,7,9,10,27</sup> During periods of growth, blade protein levels have been shown to increase, rhizome soluble carbohydrates decrease, and lipid levels remain relatively stable in certain species of SAV.<sup>6,7,9,10</sup> The nutrient content of sea

---

From the College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, Florida 32610, USA (Department of Small Animal Hospital (Siegal-Willott, Scott, Gerlach, Hill) and Department of Large Animal Hospital (Harr); Mathematics and Statistics, the Smithsonian Institution, MRC-121, Washington, D.C. 20560, USA (Hayek); the Dairy One Forage Laboratory, 730 Warren Road, Ithaca, New York 14850, USA (Sirois, Reuter); and the Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Avenue, St. Petersburg, Florida 33701, USA (Crewz). Present address (Siegal-Willott): Department of Animal Health, Smithsonian National Zoological Park, 3001 Connecticut Avenue NW, Washington, D.C. 20008, USA; (Harr): Idexx Laboratories, Inc.–Canada, 5645-199 Street, Langley, British Columbia V3A 1H9, Canada. Correspondence should be directed to Dr. Siegal-Willott (jess\_sw24@hotmail.com).

grasses also varies among species and among portions of the plant. Thus, entire plants of each species consumed by free-ranging manatees must be analyzed on several occasions throughout the year to obtain a representative evaluation of the diet.

Nevertheless, the proximate-nutrient composition of the diet of free-ranging manatees has not been compared with that of captive manatees. Differences in nutrient composition among diets could affect captive-manatee health.<sup>19</sup> Thus, the aim of this study was to compare the nutrient analysis of romaine lettuce with those of four species of SAV known to be consumed regularly by free-ranging Florida manatees, with a view to improving captive-manatee nutrition.

## MATERIALS AND METHODS

### SAV and romaine lettuce collection and storage

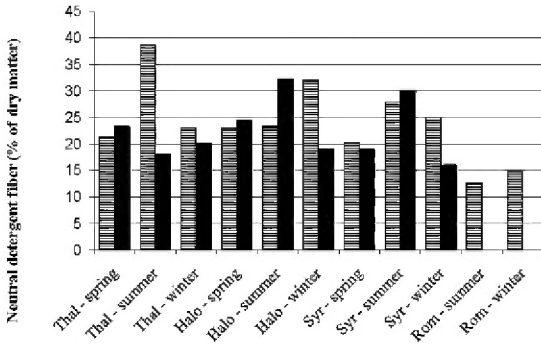
Sea grass samples were collected under a de minimus permit issued to the Florida Fish and Wildlife Research Institute (St. Petersburg, Florida 33071, USA). Sea grass samples were collected from two shallow-water locations in Tampa Bay, Florida, adjacent to Apollo Beach (APB), (27°45'46N, 82°26'33W), and Sunshine Skyway causeway (SKY), (27°39'24N, 82°40'35W). Collection sites were chosen where manatees are known to graze.<sup>17</sup> *Halodule wrightii* and *Thalassia testudinum* were collected both at APB and SKY. *Syringodium filiforme* occurred only at SKY. Sea grass samples were collected at three time points during 2006 to incorporate seasonal variations in nutrient composition: spring (March 2006), summer (June 2006), and winter (December 2006). *Chara* sp. was collected from the Everglades National Park (ENP) (25°18'28N, 81°1'31W) in June 2006 only; limited accessibility to ENP restricted *Chara* sp. collection to a single site and time. Romaine lettuce was purchased from a local supermarket in June and December. Romaine lettuce analysis was not included in the original study design and, therefore, was not collected at the March time period.

All parts of sea grass plants (including roots, horizontal and vertical rhizomes, and leaf blades) were collected every few meters along approximately 100 m or more of the sea grass community and combined to make one sample of about 1 kg wet weight for each species at each location. Two 1-kg samples of *Thalassia* (APB, SKY), and *Halodule* (APB, SKY), and a single 1-kg sample each of *Syringodium* (SKY) and *Chara* (ENP) were collected. The sea grasses were cleaned of

most shells, sand, and other biota; rinsed; and then patted dry with unbleached paper towels. Samples were weighed to obtain a field wet weight and placed into plastic bags in a cooler that contained wet ice to protect them from light and heat during transfer to the laboratory. In the laboratory, sea grass leaf blades were manually separated from rhizomes and some attached roots. Neither romaine lettuce nor *Chara* parts were separated for analysis. Whether the two supermarket purchases of romaine lettuce in June and December were the same cultivar is not known, nor is the agricultural source of the romaine lettuce known. Once separated, sea grass leaves and rhizomes were weighed (laboratory wet weight) and stored at -80°C. Samples were lyophilized in a freeze dryer (FTS Systems Inc., Stone Ridge, New York 12484, USA) to stable weight (laboratory dry weight). Romaine lettuce and *Chara* samples were treated similarly to those of the sea grasses. Percent dry matter (DM) of each sample was determined as laboratory dry weight  $\times$  100/laboratory wet weight. Percent DM of leaf portion relative to rhizome portion was calculated as laboratory dry weight leaf/(laboratory dry weight leaf portion + laboratory dry weight rhizome portion) and laboratory dry weight rhizome/(laboratory dry weight leaf portion + laboratory dry weight rhizome portion).

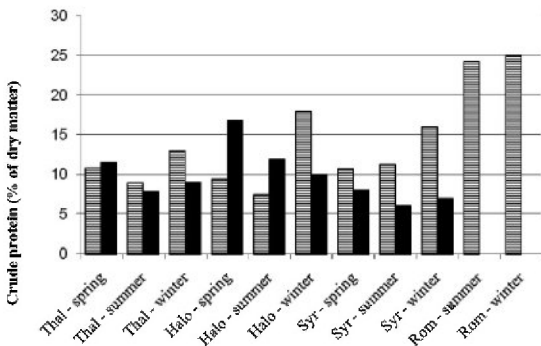
### Forage analysis

Proximate-nutrient analyses were performed in duplicate on subsamples of each ground, lyophilized sample after thorough mixing. Sample moisture was measured by using near infrared reflectance spectroscopy (Model 6500, Foss North America, Eden Prairie, Minnesota 55344, USA; AOAC method 991.01) to account for any moisture obtained during shipment; crude protein (CP) was determined with an autoanalyzer (Leco FP-528, LECO Corporation, St. Joseph, Michigan 49805, USA; AOAC method 990.03); ether-extracted crude fat (EE) and ash were determined by ashing by using AOAC methods 2003.05 and 942.05, respectively. Percent acid-detergent fiber (ADF), neutral-detergent fiber (NDF), and lignin (L) were determined by using a filter-bag technique (ANKOM A200, ANKOM Technology, Macedon, New York 14502, USA). The high percentage of ash in the sea grasses interfered with standard fiber-analysis methods. Therefore, sequential, ash-free fiber analyses were performed as follows (P. Sirois, pers. comm.). After analysis for NDF was completed, the neutral-detergent insoluble residue was subjected

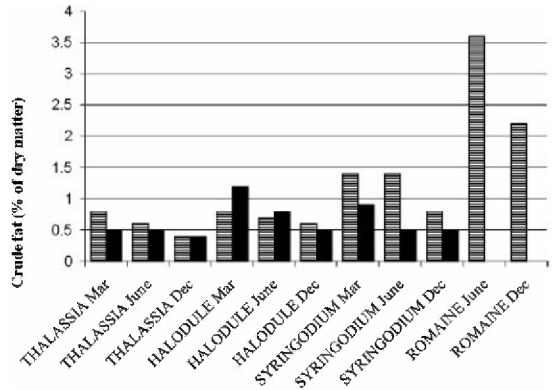


**Figure 1.** Mean neutral-detergent fiber content (dry matter basis) for three species of seagrass (Thal, *Thalassia testudinum*; Halo, *Halodule wrightii*; and Syr, *Syringodium filiforme*) collected in three seasons (spring, March; summer, June; and winter, December) in 2006 from Tampa Bay, Florida, USA, and romaine lettuce purchased at two time points from a local supermarket. Seagrass leaf (hatched box) and rhizome (solid black box) portions are presented separately.

to ADF analysis. The acid-detergent insoluble residue was then used for standard L analysis. The final step in the L procedure involved ashing of the L residue, which was then factored into the final calculation of NDF, ADF, and L. Percent non-fiber carbohydrate (NFC) was defined as  $100\% - (CP + (NDF - NDICP + EE + ash))$ . The NDICP refers to neutral-detergent insoluble CP and represents the estimated portion of the undegradable protein available to the animal (April 2009; Dairy One Forage Laboratory, [www.dairyone.com/Forage/FactSheet/ForageAnalysis.htm](http://www.dairyone.com/Forage/FactSheet/ForageAnalysis.htm)).

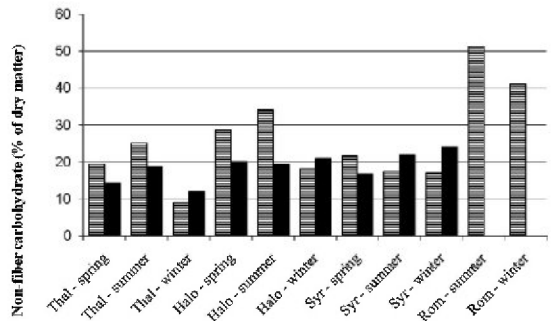


**Figure 2.** Mean crude protein content (dry matter basis) for three species of seagrass (Thal, *Thalassia testudinum*; Halo, *Halodule wrightii*; and Syr, *Syringodium filiforme*) collected in three seasons (spring, March; summer, June; and winter, December) in 2006 from Tampa Bay, Florida, USA, and romaine lettuce purchased at two time points from a local supermarket. Seagrass leaf (hatched box) and rhizome (solid black box) portions are presented separately.



**Figure 3.** Mean crude fat content (dry matter basis) for three species of seagrass (Thal, *Thalassia testudinum*; Halo, *Halodule wrightii*; and Syr, *Syringodium filiforme*) collected in three seasons (spring, March; summer, June; and winter, December) in 2006 from Tampa Bay, Florida, USA and romaine lettuce purchased at two time points from a local supermarket. Seagrass leaf (hatched box) and rhizome (solid black box) portions are presented separately.

Because the digestion efficiency of manatees is unknown and may lie between ruminants and equids, digestible energy (kcal/kg DM) was calculated by using equations for both horses (DEh) and cattle (DER) at maintenance.<sup>22,23</sup> In Figures 1–4 and Table 1, proximate-nutrient data were averaged for the two Tampa Bay collection sites for each species of sea grass (except for the *Syringodium*). All results are presented on a DM basis.



**Figure 4.** Mean nonfiber carbohydrate content (dry matter basis) for three species of seagrass (Thal, *Thalassia testudinum*; Halo, *Halodule wrightii*; and Syr, *Syringodium filiforme*) collected in three seasons (spring, March; summer, June; and winter, December) in 2006 from Tampa Bay, Florida, USA, and romaine lettuce purchased at two time points from a local supermarket. Seagrass leaf (hatched box) and rhizome (solid black box) portions are presented separately.

**Table 1.** Proximate nutrients (minimum–maximum ranges) and digestible energy contents of three seagrass species, a fresh-water alga (*Chara* sp.), and romaine lettuce (*Lactuca sativa* var. *longifolia*).<sup>a,b,c</sup>

Plant	Plant portion <sup>d</sup>	DM (%) <sup>e</sup>	NDF (%)	ADF (%)	Lignin (%)	CP (%)	EE (%)	NFC (%)	Ash (%)	DEr (kcal/kg)	DEh (kcal/kg)
Thal	Rhizome	19–20	18–23	11–18	0.9–1.4	8–12	0.4–0.5	12–19	61–67	900–1053	623–886
Halo	Rhizome	21–27	19–32	10–19	1.0–2.6	10–17	0.5–1.2	19–21	48–50	680–1390	557–1248
Syr	Rhizome	26–30	16–30	12–27	0.9–2.4	6–8	0.5–0.9	17–24	52–70	1310–1853	1220–1721
Thal	Leaf	16–28	21–39	14–26	1.7–2.0	9–13	0.4–0.8	9–25	53–59	1125–1690	787–1321
Halo	Leaf	23–25	23–32	17–19	1.4–2.3	8–18	0.6–0.8	18–34	34–41	1190–1955	956–1615
Syr	Leaf	17–19	20–28	14–18	1.5–2.6	11–16	0.8–1.4	17–22	40–51	1520–1855	1270–1460
<i>Chara</i>	Leaf	17	9	5	1.2	10	0.9	25	56	1340	1254
Rom <sup>e</sup>	Leaf	5, 8	13, 15	12, 13	1.9, 2.1	24, 25	2.2, 3.6	41, 51	11, 18	3120, 3460	2758, 3212

<sup>a</sup> Results are presented as ranges for all seasons of collection (seagrasses [March, June, December 2006]) or purchase dates (romaine lettuce [June, December]). *Chara* was collected only in June.

<sup>b</sup> All results expressed on a dry matter basis.

<sup>c</sup> DM, dry matter; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; CP, crude protein; EE, ether extract (measurement of crude fat); NFC, nonfiber carbohydrate; DEr, digestible energy for ruminants; DEh, digestible energy for horses; Thal, *Thalassia testudinum*; Halo, *Halodule wrightii*; Syr, *Syringodium filiforme*; Rom, romaine lettuce.

<sup>d</sup> Rhizome includes vertical and horizontal rhizome portions and some roots; leaf includes only seagrass blades. All parts of romaine lettuce and *Chara* were analyzed.

<sup>e</sup> Romaine lettuce values given for both collection times (June, December).

## Statistical methods

Transformation of percentage and other data for analysis was determined by Box-Cox procedures. General linear models were constructed that incorporated both variance and means univariate analysis of variance models for testing. For each test, an alpha level of 0.01 was used, and the observed *P* value was cited. When heterogeneity was detected, Satterthwaite procedures were assessed. Both descriptive and inferential statistical statements were obtained from SPSS version 17 (IBM SPSS Statistics version 17.0.2 March 2009, IBM, Inc. New York, New York 10504, USA) and MathCad (MathCad 2009 version 14.0, PTC Corporation, Needham, Massachusetts 02494, USA). The primary data set consisted of 19 observations of SAV and two of romaine for each nutrient assessment. An examination by statistical procedures assured this as a representative sample and adequate for analyses. However, as noted in the text on a case-by-case basis, when the size of the effect was large and the alternative hypothesis would have more effectively

been evaluated with a larger sample size replication of this study by using the observed effect size for sample size determination was recommended.<sup>33</sup>

## RESULTS

The proximate nutrient composition of the SAV differed substantially from that of the romaine lettuce (Figs. 1–4; Table 1). DM was two- to sixfold higher in the SAV (16–30%) than in romaine lettuce (5, 8%), with the DM arithmetic mean for SAV significantly greater than that for romaine lettuce ( $F = 4.46$ ;  $P = 0.005$ ). Percent leaf DM ranged from 21% to 59.1%, and percent rhizome DM ranged from 40.9% to 79%, depending on season and plant species (Table 2). On a DM basis, values for percent NDF were up to three times greater in both sea grass rhizomes (range: 16–32%) and leaves (range: 20–39%) than in romaine lettuce (13% summer, 15% summer) or *Chara* (9%). Mean NDF for the SAV leaves ( $26.03 \pm 5.95\%$ ) (excluding *Chara*) and mean NDF for SAV rhizomes ( $22.46 \pm 5.19\%$ ) were significantly greater than the value

**Table 2.** Percent of leaf and rhizome dry matter from three seagrass species.<sup>a,b</sup>

Seagrass	Spring		Summer		Winter	
	Leaf (%) <sup>a</sup>	Rhizome (%) <sup>b</sup>	Leaf (%)	Rhizome (%)	Leaf (%)	Rhizome (%)
<i>Thalassia testudinum</i>	29.1	70.9	36.4	63.6	27.2	72.8
<i>Halodule wrightii</i>	30.2	69.8	35.2	64.8	21	79
<i>Syringodium filiforme</i>	59.1	40.9	51.3	48.7	49.1	50.9

<sup>a</sup> All results on a dry matter basis.

<sup>b</sup> Rhizome includes vertical and horizontal rhizome portions and some roots; leaf includes only seagrass blades.

of NDF of *Chara* (8.50%) and mean NDF of romaine lettuce ( $13.75 \pm 1.77\%$ ). Percent ADF in sea grasses (10–27%) were similar ( $P = 0.220$ ) to romaine lettuce (12% summer, 13% winter). Small amounts of L were found in all plants (0.9–2.6%), and was similar ( $P = 0.099$ ) between SAV ( $1.71 \pm 0.56\%$ ) and romaine lettuce ( $2.00 \pm 0.14\%$ ).

Percent CP was up to threefold greater in romaine lettuce (24% summer, 25% winter DM) than in the SAV (6–18% DM). The mean CP for SAV ( $10.70 \pm 3.30\%$ ) was significantly lower ( $P = 0.0006$ ) than CP for romaine lettuce ( $24.60 \pm 0.57\%$ ). There was a trend for sea grass rhizome CP content to be greater in spring, whereas CP content in the leaves tended to be greater in winter (Fig. 2; Table 1). The power of the test for seasonal variation in CP was too low to provide reliable statistical results.

Percent EE in romaine lettuce (2.2% summer, 3.6% winter DM) was 2–9 times greater than those of the sea grasses (0.4–1.4% DM). Mean EE for romaine lettuce ( $2.9 \pm 0.99\%$ ) was significantly greater ( $P = 0.0006$ ) than EE for the SAV ( $0.745 \pm 0.31\%$ ). For each species of sea grass, EE content tended to be greatest in spring samples of sea grass rhizome and leaf portions (Fig. 3; Table 1). For *Thalassia* and *Syringodium*, leaves contained more EE than the rhizomes; the opposite was true for *Halodule*. Protein and fat contents were greatest in spring samples of sea grass rhizomes (Figs. 2, 3).

Percent NFC of romaine lettuce (41% summer, 51% winter DM) was approximately two- to sixfold greater than in the sea grasses (9–34% DM), with the mean NFC for the SAV ( $20.17 \pm 5.77\%$ ) significantly less than the mean NFC for romaine lettuce ( $46.05 \pm 7.14\%$ ;  $P = 0.009$ ) (Fig 4; Table 1). The mean value for NFC for SAV leaves ( $21.11 \pm 7.32\%$ ) was not significantly different from the mean NFC for SAV rhizomes ( $18.64 \pm 3.79\%$ ). However, NFC content was variable, both in the rhizome and leaf portions of sea grasses seasonally, but, in all cases was 2–6 times less ( $P = 0.009$ ) than that of romaine lettuce ( $46.05 \pm 7.14\%$ ). Total DEh of romaine lettuce (2758 kcal/kg summer, 3212 kcal/kg winter DM) was two- to sixfold greater than that of sea grass leaves (787–1615 kcal/kg DM), sea grass rhizomes (557–1721 kcal/kg DM), and *Chara* (1254 kcal/kg DM). The DEh of sea grasses varied both in rhizome and leaf portions of sea grasses with no apparent trend, but, in all cases, significantly less ( $P = 0.0001$ ) than that of romaine lettuce (Table 1). Similarly, total DER of romaine lettuce

(3120 kcal/kg summer, 3460 kcal/kg winter DM) was two- to fivefold greater ( $P = 0.005$ ) than that of sea grass leaves (1125–1955 kcal/kg DM), sea grass rhizomes (900–1852 kcal/kg DM), and *Chara* (1340 kcal/kg DM), with no trend in seasonal variability (Table 1).

Ash was 3–7 times higher (Table 1) in the SAV ( $52.74 \pm 10.50\%$ ) than in romaine lettuce ( $14.38 \pm 5.12\%$ ), with a significant mean difference of 38.36 percentage units ( $P = 0.0001$ ). *Chara* was lower in NDF (8.5%), ADF (5.20%), L (1.20%), EE (0.85%), CP (9.50%), NFC (25.40%), DER, and DEH relative to those variables in romaine lettuce (Table 1).

## DISCUSSION

Nutrient analyses of SAV consumed by manatees in the wild differed from those of romaine lettuce commonly provided to captive manatees. The SAV contained significantly more DM, and the DM contained significantly greater amounts of NDF and lower amounts of digestible nutrients (protein, fat, NFCs) than the lettuce. Difference in mean ADF was not statistically significant between SAV and romaine lettuce, possibly because of a lack of preassessment of a representative effect size.<sup>33</sup> As a consequence, SAV contained less digestible energy than romaine lettuce on a DM basis. Rhizomes constituted a greater percentage DM than leaves for *Thalassia* and *Halodule*, with equal contributions of rhizome versus leaves for *Syringodium* (Table 2).

Formulas for calculating DE in manatees have not been established to date. Nevertheless, a large, vat-like stomach and large intestine, lengthy intestinal tract, and slow gastrointestinal transit time (6–7 days) should allow extensive fermentation of high-fiber foods by intestinal microbes and suggests that manatees and dugongs are probably intermediate between equids and ruminants in fiber-digestion efficiency.<sup>1,3,4,15,18,25,26</sup> Therefore, DE by using the ruminant and equine-based equations was calculated, and it is expected that the true value lies between these two values.<sup>22,23</sup> Both DEh and DER are significantly greater in lettuce than in SAV on a DM basis (Table 1). The comparative values for DEh and DER for leaves versus rhizomes in the sea grasses were both nonsignificant. Mean DEh and DER for sea grass leaves were less than those reported previously for *Thalassia* (2.6 kcal/g DM), *Halodule* (2.8 kcal/g DM), and *Syringodium* (2.9 kcal/g DM).<sup>7</sup> Differences in DE values in this study compared with previous reports may be related to changes in nutrient

availability, sampling or analytical technique, sampling site, method of calculating energy content, failure to include ash content of sea grasses in calculation of energy content, or changes in environmental parameters (water quality, soil quality, anthropogenic effects, etc.).<sup>1,6,7</sup> In some herbivores, DM determines the amount of food consumed.<sup>22</sup> If this is also true in manatees, then captive manatees that consume mostly romaine lettuce are likely to consume more calories relative to DM and are more likely to become obese than when consuming SAV.

The amount of NDF, ADF, and L were also much greater in sea grasses than in romaine lettuce, but, only L had a small effect size, whereas, the others had greater values for effect size. The observed percentage unit difference between the mean values for the SAV and romaine of 3.54 for ADF and 9.67 for NDF coupled with homogeneity of variances appears to justify repetition of this study with preset effect sizes for determining effective sample sizes to be able to detect these representative differences as significant.<sup>33</sup> At present, there is no indication of critical values for these nutrients for manatees. The mean concentrations of NDF (Thal = 21.3–41% DM; Halo = 28.3–49.7% DM; Syr = 31.5–42% DM), ADF (Thal = 17.9–34% DM; Halo = 12.8–35.7% DM; Syr = 20.5–27.3% DM), and L (Thal = 0–2.3% DM; Halo = 0–3.9% DM; Syr = 0.6–2.5% DM) previously reported for sea grass leaves were comparable with those in this report, although less than values reported for terrestrial or shoreline grasses.<sup>6,7,9,10,32</sup> Romaine lettuce NDF (16.3% DM) and ADF (14.1% DM) values<sup>29</sup> were similar to those reported here. The amount, composition, and physical properties of fiber have marked effects on intestinal function and intestinal disease in other herbivores. Potential problems that have been associated with a low-fiber diet in herbivores include obesity, diabetes mellitus, gastric acidosis, inflammatory changes (such as laminitis), dysbiosis, poor digestion, thermoregulatory disorders, diarrhea, gastrointestinal tract bloat, colic, torsion, or displacement of the intestines. Excess gas formation could also potentially cause buoyancy disorders in manatees.<sup>14,22,24,27,29</sup>

Thus, manatees that consume lettuce that contains less fiber and that is higher in easily digestible carbohydrates may be at risk for health problems, especially in captive settings, where natural foraging and migratory behaviors are reduced by space limitations. Poor skin health and immune function in captive manatees has

been detected.<sup>1,19</sup> Obesity has been noted in captive manatees (Siegal-Willott, Harr, pers. obs.), and gastric ulceration has been observed (Menchaca, pers. comm.), but the overall prevalence of diet-related disorders may be underdetected or underreported. Therefore, further investigation of the effect of a low-fiber diet on manatee intestinal function and health is warranted.

*Chara* was also lower in fat, protein, energy, and NFC than romaine lettuce but did not share the higher fiber content found in sea grasses. Further investigations of seasonal and geographical differences in *Chara* spp. are warranted for a more complete understanding of the role of these algal species in manatee nutrition.

Analyses revealed large percentages of ash in the SAV (34–70%; mean  $52.74 \pm 10.50\%$ ). The high ash contents of these sea grasses have been previously noted (Thal = 24–44% DM; Halo = 14–48% DM; Syr = 16–41% DM).<sup>1,6,7,9,10,12,27</sup> Romaine lettuce ash content ( $14.38 \pm 5.12\%$ ) was also similar to that previously reported (9.0% DM).<sup>29</sup> Ash represents the mineral portion of the plant and is of no direct energetic value but contains minerals essential for normal metabolism. How such large differences in ash concentration in forage affect mineral absorption, microbial fermentation, and other aspects of intestinal function needs further evaluation.

Seasonal variation in lettuce composition was not assessed because of low sample number and lack of a spring collection sample. Romaine lettuce DM, CP, and EE at the two collection times were similar to previous lettuce composition reports (Table 1).<sup>29</sup> Sea grass leaf NDF generally increased linearly from spring to summer collections, followed by decreased NDF in winter samples, whereas sea grass rhizome NDF did not present any seasonal trends (Fig. 1). However, with the exception of *Thalassia* rhizomes and *Halodule* leaves, total carbohydrates (NDF + NFC) were generally higher in summer. Protein and fat content were greatest in spring samples of sea grass rhizomes, similar to other findings reported for sea grasses.<sup>1,6,27</sup> Leaf CP content of sea grasses increased from summer to winter, with CP content of SAV leaves collected in the spring intermediate (Fig. 2). These seasonal variations represent a normal nutrient-fluctuation pattern for sea grasses, which differs from those for terrestrial grasses.<sup>7,9,10,12</sup>

Seasonal variations in sea grass leaf and rhizome composition are related to normal life cycle and variable annual growth in a subtropical environment. Variation in sea grass proximate

constituents with season results from changes in water quality (light penetration, turbidity, salinity, temperature) that invoke changes in nutrient availability for adding biomass or to counter stresses.<sup>6,7</sup> Therefore, nutrient uptake rates and assimilation by SAV vary, depending on photosynthetic abilities.<sup>6</sup> Shallow-water, subtropical sea grasses undergo a period of winter regression (above-sediment biomass losses) but regain rapid continuous growth during late spring and summer, tapering off in autumn months.<sup>6–10,12</sup> This pattern promotes lower protein in the leaf portions during rapid summer growth, relative to increasing carbohydrate contents. Rhizomes function as storage organs and as anchoring structures are not usually directly affected by photosynthetic stresses as seen in sea grass leaves and, therefore, vary less in proximate nutrient content seasonally.<sup>9,10,12</sup> Generally, rhizome CP was higher over winter–spring and lowest in summer.

Although sea grass leaves in general may contain caloric values similar to those of terrestrial grasses (true grasses, family Poaceae), they generally have small amounts of lignin (0–3.9%) and lower amounts of structural carbohydrates, such as hemicelluloses and cellulose (NDF and ADF).<sup>7</sup> Terrestrial grasses may contain 6–10% lignin. Seasonally, sea grasses do not vary much in total caloric value, even though certain components may fluctuate. Low values of lignin relative to those of terrestrial grasses permit greater year-round digestibility. Drift and attached macroalgae also enhance manatee diets during times of lower seasonal sea grass abundance.<sup>10</sup> Therefore, the total caloric value of a sea grass bed for manatees may remain fairly constant throughout the year in the Tampa Bay region.

Other contributing factors to seasonal proximate-nutrient variations may include the sample collection method, collection site within a sea grass bed (outer fringe versus inner sea grass bed), sample number and quantity, epiphyte biomass and associated light attenuation, nutrient-limited growth, temperature and salinity at time of harvest, or laboratory error.<sup>2,6,8,11,12,28</sup> Typically, proximate-nutrient analysis is performed on a 0.5-kg subsample obtained from a 2–5-kg composite sample (Dairy One Forage Laboratory, [www.dairyone.com/Forage/services/Forage\\_Good\\_Sample.htm](http://www.dairyone.com/Forage/services/Forage_Good_Sample.htm)). Because sea grasses are protected by Florida state law, collection under a de minimus permit is minimized to protect local distribution and the health of these plants. Collection of *Syringodium* was also

limited geographically, given its absence from one of the study sites. The lack of *Syringodium* at the APB site may be related to overgrazing by manatees, failure of this species to colonize this location, eradication by anthropogenic stressors (pollution, etc.), or elimination by environmental conditions (sediment quality, water depth, etc.) (Bonde, Carlson, pers. comm.).<sup>5,17</sup> Although low sample number and volume collected may have contributed to seasonal-analysis variations, these variations were for the most part not significant, and values for sea grass proximate nutrients were comparable with those obtained previously and are thus likely representative samples.<sup>1,7,9</sup> Similarly, values for romaine proximate analyses were similar to previously reported values.<sup>1,29</sup>

Based on recommendations from the Manatee Rehabilitation Partnership Consortium, captive manatees have recently been released to their natural environment, after rehabilitation or maintenance in captivity. From the differences determined in this study, recommendations for a gradual transition to a more natural diet or one that closely mimics it before release of captive manatees into the wild is supported. This will ensure that the animal possesses optimal immune health and successful rehabilitation for transition to a free-ranging lifestyle.<sup>19</sup> Further evaluation of the gastrointestinal tract physiology and overall metabolic health of captive manatees is warranted, along with additional SAV proximate-nutrient analyses.

## CONCLUSIONS

Differences in proximate nutrients of four species of SAV commonly consumed by manatees in Florida compared with that of romaine lettuce, suggest that captive manatees should be provided a diet higher in fiber and lower in fat, protein, digestible carbohydrates, and digestible energy to more closely mimic the diet of free-ranging manatees. Romaine lettuce may not represent the best staple diet for long-term maintenance of captive manatees because the staple diet may have overall undesired effects on manatee health. Further investigations of alternative food sources for captive manatees are warranted.

*Acknowledgments:* The authors wish to extend thanks to the sea grass and manatee groups at the Florida Fish and Wildlife Research Institute for providing boats and personnel (Donna Berns, Jennifer Kunzelman) and for assisting with sea grass collections. Thanks also to the staff at



Everglades National Park for assisting with collection of *Chara*. This research was funded by Dr. Darryl Heard through the Zoological Medicine Research Fund, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA.

### LITERATURE CITED

1. Best, R. C. 1981. Foods and feeding habits of wild and captive Sirenia. *Mammal Rev.* 11: 3–29.
2. Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. *Mar. Biol.* 87: 211–218.
3. Burn, D. M. 1986. The digestive strategy and efficiency of the West Indian manatee, *Trichechus manatus*. *Comp. Biochem. Physiol.* 85: 139–142.
4. Burn, D. M., and D. K. Odell. 1987. Volatile fatty acid concentrations in the digestive tract of the West Indian manatee, *Trichechus manatus*. *Comp. Biochem. Physiol.* 88B: 47–49.
5. Campbell, H. W., and A. B. Irvine. 1977. Feeding ecology of the West Indian manatee *Trichechus manatus* Linnaeus. *Aquaculture.* 12: 249–251.
6. Dawes, C., M. Chan, R. Chinn, E. W. Koch, A. Lazar, and D. Tomasko. 1987. Proximate composition, photosynthetic and respiratory responses of the seagrass *Halophila engelmannii* from Florida. *Aquat. Bot.* 27: 195–201.
7. Dawes, C. J. 1986. Seasonal proximate constituents and caloric values in seagrasses and algae on the west coast of Florida. *J. Coastal Res.* 2: 25–32.
8. Dawes, C. J., M. O. Hall, and R. K. Riechert. 1985. Seasonal biomass and energy content in seagrass communities on the west coast of Florida. *J. Coastal Res.* 3: 255–262.
9. Dawes, C. J., and J. M. Lawrence. 1979. Effects of blade removal on the proximate composition of the rhizome of the seagrass *Thalassia testudinum* Banks ex König. *Aquat. Bot.* 7: 255–266.
10. Dawes, C. J., and L. M. Lawrence. 1980. Seasonal changes in the proximate constituents of the seagrasses *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*. *Aquat. Bot.* 8: 371–380.
11. Duarte, C. M. 1990. Seagrass nutrient content. *Mar. Ecol. Prog. Ser.* 67: 201–207.
12. Durako, M. J., and M. D. Moffler. 1985. Spatial influences on temporal variations in leaf growth and chemical composition of *Thalassia testudinum* Banks ex König in Tampa Bay, Florida. *Gulf Res. Rep.* 8: 43–49.
13. Etheridge, K., G. B. Rathbun, J. A. Powell, and H. I. Kochman. 1985. Consumption of aquatic plants by the West Indian manatee. *J. Aquat. Plant Manag.* 23: 21–25.
14. Kahn, C. M. 2005. The Merck Veterinary Manual, 9th ed. Merck & Co., Duluth, Georgia.
15. Lanyon, J. M., and H. Marsh. 1995. Digesta passage times in the dugong. *Aust. J. Zool.* 4: 119–127.
16. Lefebvre, L. W., J. P. Reid, W. J. Kenworthy, and J. A. Powell. 2000. Characterizing manatee habitat use and seagrass grazing in Florida and Puerto Rico: implications for conservation and management. *Pacific Conserv. Biol.* 5: 289–298.
17. Lewis, R. R., J. M. Carlton, and R. Lombardo. 1984. Algal consumption by the manatee (*Trichechus manatus*) in Tampa Bay, Florida. *Florida Sci.* 47: 189–191.
18. Lomolino, M. V., and K. C. Ewel. 1984. Digestive efficiencies of the West Indian manatee (*Trichechus manatus*). *Florida Sci.* 47: 176–179.
19. Manire, C. A., C. J. Walsh, H. L. Rhinehart, D. E. Colbert, D. R. Noyes, and C. A. Luer. 2003. Alterations in blood and urine parameters in two Florida manatees (*Trichechus manatus latirostris*) from simulated conditions of release following rehabilitation. *Zoo Biol.* 22: 103–120.
20. Mignucci-Giannoni, A. A., and C. Beck. 1998. The diet of the manatee (*Trichechus manatus*) in Puerto Rico. *Mar. Mammal Sci.* 14: 394–397.
21. Murphy, D. 2003. Sirenia. In: Fowler, M. E., and R. E. Miller (eds.), *Zoo and Wild Animal Medicine*, 5th ed. Saunders, St. Louis, Missouri. Pp. 476–482.
22. National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*, 7th ed. National Academies Press, Washington, D.C.
23. National Research Council. 2007a. *Nutrient Requirements of Horses*, 6th ed. National Academies Press, Washington, D.C.
24. National Research Council. 2007b. *Nutrient Requirements of Small Ruminants*. National Academies Press, Washington, D.C.
25. Reynolds, J. E., and S. A. Rommel. 1996. Structure and function of the gastrointestinal tract of the Florida manatee, *Trichechus manatus latirostris*. *Anat. Rec.* 245: 539–558.
26. Rommel, S. A., and L. J. Lowenstine. 2001. Gross and microscopic anatomy. In: Dierauf, L. A., and F. M. D. Gulland (eds.), *Marine Mammal Medicine*, 2nd ed. CRC Press, New York, New York. Pp. 146–147.
27. Rommel, S. A., J. E. Reynolds, and H. A. Lynch. 2003. Adaptations of the herbivorous marine mammals. *Proc. 2003 VI Int. Symp. Nutr. Herbivores*. Pp. 287–306.
28. Sand-Jensen, K. 1990. Epiphyte shading: its role in resulting depth distribution of submerged aquatic macrophytes. *Fol. Geobot. Phytotaxon.* 25: 315–320.
29. Schmidt, D. A., M. S. Kerley, J. H. Porter, and J. L. Dempsey. 2005. Structural and nonstructural carbohydrate, fat, and protein composition of commercially available, whole produce. *Zoo Biol.* 24: 359–373.
30. Snipes, R. L. 1984. Anatomy of the cecum of the West Indian manatee, *Trichechus manatus* (Mammalia, Sirenia). *Zoomorphology.* 104: 67–78.
31. Stith, B. M., D. H. Slone, and J. P. Reid. 2006. Review and Synthesis of Manatee Data in Everglades National Park. U.S. Geological Survey, Gainesville, Florida. P. 110.

32. Tharel, L. N. 1981. Determining Predictability Equations for Digestibility of a Tropical and Temperate Forage at Three Stages of Maturity. Ph.D. Dissertation, Univ. Arkansas, Fayetteville, Arkansas. P. 86.
33. Virnstein, R., L. C. Hayek, and L. M. Morris. 2009. Pulsating patches: a model for the spatial and temporal dynamics of the threatened seagrass *Halophila johnsonii*. Mar. Ecol. Prog. Ser. 385: 97–109.
34. Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Animal Feed Sci. and Technol. 39: 95–110.
35. Woods, C. A., and F. E. Sergile (eds.). 2001. Biogeography of the West Indies: Patterns and Perspectives, 2nd ed. CRC Press, Boca Raton, Florida.

*Received for publication 9 June 2009*