

# Digestive Efficiencies of Ex Situ and In Situ West Indian Manatees (*Trichechus manatus latirostris*)\*

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## ABSTRACT

Digestive efficiencies ( $D_m$ ) of ex situ and in situ manatees (*Trichechus manatus latirostris*) were, for the first time, assessed using manganese ( $Mn^{2+}$ ) as a naturally occurring marker. The  $D_m$  of ex situ manatees determined using  $[Mn^{2+}]$  did not differ significantly from the  $D_m$  assessed using lignin, supporting the efficacy of the manganese approach. Gastrointestinal tract samples, obtained from recently dead animals, showed  $[Mn^{2+}]$  concentrations were lowest in the stomach and remained low in the duodenum and small intestine but increased in the cecum, colon, and rectum, consistent with colonic digestion and absorption. In situ manatees consuming marine vegetation had significantly lower  $D_m$  (mean  $\pm$  SE, 46.9%  $\pm$  1.8%;  $n = 8$ ) than did in situ manatees consuming freshwater vegetation (77.8%  $\pm$  2.6%;  $n = 7$ ), which in turn had significantly lower values than did ex situ manatees consuming lettuce (84.0%  $\pm$  0.7%;  $n = 37$ ). In situ manatees eating seagrasses had significantly higher  $D_m$  than did long-term ex situ animals consuming seagrass for short periods of time (46.9%  $\pm$  1.8% vs. 36.2%  $\pm$  1.2%, respectively), suggesting potential modification of gut flora over time. One significant ramification of our results is that manatees consuming seagrasses would require a greater standing biomass to support their needs than would be required if they were eating freshwater vegetation. This reinforces the critical need to implement habitat conservation and protection before considering downlisting or delisting manatees as an endangered species.

## Introduction

Conservation physiology integrates whole-organism function, such as metabolism, thermal relationships, and nutrition, with conservation problems (Wikelski and Cooke 2006), and one key aspect of this relates to understanding the efficiency of energy acquisition and processing relative to energy demands. Resource acquisition is critical at all levels of ecological integration, and digestive efficiency determines how much time and effort will be spent collecting food to meet energy requirements and ultimately how much energy is available for basic maintenance, growth, and reproduction. Clemens and Maloiy (1982) concluded that assessing the digestive efficiency of an endangered species is a critical consideration when developing conservation management strategies.

The West Indian manatee (*Trichechus manatus latirostris*) is a member of a small group of mammals referred to as mega-herbivores (Owen-Smith 1988). This group consists of mammals that exceed 1,000 kg in adult mass and in addition to the fully aquatic manatee includes the elephants, hippopotamus, rhinoceroses, and giraffe (Owen-Smith 1988). As a result of their large size, these mammals have a unique ecology and physiology. This group shares another characteristic: because of their size, they have large habitat demands, a trait that frequently conflicts with human needs, and therefore, these species are likely to become extirpated or they are threatened/endangered throughout their range.

West Indian manatees are considered to be opportunistic herbivores, consuming up to 60 different species of vegetation in marine, estuarine, and/or freshwater habitats (e.g., Campbell and Irvine 1977; Hartman 1979; Best 1981; Bengtson 1983; Etheridge et al. 1985; Ledder 1986). They also appear to exhibit regional and possibly seasonal differences in diet composition (Reich and Worthy 2006; Alves-Stanley and Worthy 2009; Alves-Stanley et al. 2010). Even though manatees are known to feed on a wide variety of plant species, a few dominant species of submerged freshwater plants are preferred: hydrilla (*Hydrilla verticillata*), eel grass (*Vallisneria americana*), coontail (*Ceratophyllum demersum*), water milfoil (*Myriophyllum spicatum*), water hyacinth (*Eichornia crassipes*), parrot feather (*Myriophyllum aquaticum*), and alligator weed (*Alternanthera philoxeroides*; e.g., Campbell and Irvine 1977; Hartman 1979; Etheridge et al. 1985; Ledder 1986). Coastally, seagrasses are a dietary staple (e.g., Campbell and Irvine 1977; Hartman 1979; Ledder 1986; Provancha and Hall 1991; Koelsch 1997; Lefebvre et al. 2000), with manatees apparently preferring manatee grass (*Syringodium filiforme*) over turtle grass (*Thalassia testudinum*) in mixed-species stands (Hartman 1979; Provancha and Hall 1991). To a lesser extent manatees may also eat macroalgae

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(e.g., *Gracillaria tikvahiae*), invertebrates, terrestrial grasses growing close enough to the shore to reach, acorns that have fallen into the water, and fish caught in gill nets (e.g., Powell 1978; Hartman 1979; Reeves et al. 1992; Courbis and Worthy 2003). Ex situ manatees are fed a diet consisting predominately of romaine lettuce (*Lactuca sativa*), which differs significantly from their in situ diet in both nutritional value and composition (Siegall-Willott et al. 2010).

In spite of manatees having been held in captivity for more than 130 years (e.g., Garrod 1877), there are relatively few data regarding energy assimilation from the various types of forage they consume, and understanding their nutritional needs has been identified as critical to enhancing their long-term viability in the wild (USFWS 2001). Plant leaves, particularly those of grasses, contain a high proportion of cellulose that mammals cannot digest without the aid of gut microorganisms (e.g., Karasov and Martinez del Rio 2007). Species have evolved mechanisms to slow the passage rate of materials through the gut and facilitate the fermentation process. It has been suggested that mean retention time (MRT) increases as an animal's body mass increases (e.g., Ilius and Gordon 1992) and therefore megaherbivores would theoretically benefit from their large size. This interpretation is complicated by the fact that in reality there is a complex relationship between retention time and metabolic rate, forage quality, and fermentation strategy. More recently, it has been found that there is actually little influence of body mass on MRT and instead that there was a stronger relationship with digestion type (ruminant vs. hindgut fermenter) and metabolic rate (Steuer et al. 2011; Müller et al. 2013). A longer MRT is considered to be an advantage for species that consume food that is either hard to digest or low in nutritional value (Robbins 1993). Manatees utilize hindgut (colonic) fermentation (Reynolds 1980; Best 1981; Snipes 1984), and while there is a paucity of data on the MRT of manatees, the limited data that are available for sirenians suggest that they have an MRT that is significantly longer than in most other species of similar body mass (Steuer et al. 2011, Müller et al. 2013), ranging from 120 to 168 h (e.g., Lanyon and Marsh 1995; Larkin et al. 2007).

There are also suggestions of differing strategies in MRT and digestibility in mammals that consume forage with high fiber content (e.g., Owen-Smith 1988; Steuer et al. 2011). Species can be ranked on a continuum between those having a relatively long MRT and high digestibility (a typical ruminant strategy) and those with a relatively short MRT and low digestibility (many hindgut fermenters; e.g., Owen-Smith 1988; Steuer et al. 2011). MRTs for manatees are significantly longer than those of similar-sized hindgut fermenters (32 h) or ruminants (66 h; Steuer et al. 2011), suggesting that manatees may have higher digestibilities than would be predicted even when consuming poor-quality forage. Ultimately, traditional explanations for herbivore niche differentiation along a body mass gradient should not be based solely on either the allometry of digestive physiology or fermentation strategy (Müller et al. 2013). Müller et al. (2013) suggest instead that larger species can tolerate lower-quality diets because their intake has a higher allometric

scaling than their basal metabolism, allowing them to eat relatively more of a lower-quality food without having to increase digestive efficiency. This implies that manatees, with their low basal metabolic rate, large body size, and long MRT, could have a very efficient digestive system.

Digestive efficiency ( $D_m$ ) is defined as the percentage of ingested material that is absorbed across the gut wall and is ultimately available for maintenance and growth. This is of particular interest because a low efficiency would translate into more time spent foraging to meet energetic needs. The  $D_m$  has traditionally been measured using inassimilable markers that are added to the diet (e.g.,  $^{51}\text{Cr}$  or chromic oxide; Karasov and Martinez del Rio 2007) or by undertaking a total collection of feces produced over an extended period of time. These methods ultimately require controlled access to animals and the ability to manipulate what they are consuming. Applying these approaches to either in situ or ex situ manatees is problematic, and therefore the ability to use naturally occurring inert markers is advantageous (e.g., acid insoluble ash, acid detergent lignin). Some elements, such as manganese (Mn), are so poorly absorbed (Kaufman et al. 1976; Nagy 1977; Nagy and Milton 1979; Nagy and Montgomery 1980) that they have been determined to be potential markers for assessing  $D_m$  (e.g., Fadely et al. 1990; Fisher et al. 1992; Wikelski et al. 1993; Mårtensson et al. 1994; Lawson et al. 1997; Rosen and Trites 2000). When naturally occurring Mn was used as a marker for measuring  $D_m$  of ex situ northern fur seals (*Callorhinus ursinus*), results comparable to those derived using standard markers,  $^{51}\text{Cr}$  and chromic oxide, were obtained (Fadely et al. 1990). While this approach has not previously been utilized in any megaherbivore, the potential advantage of using Mn is that it can be used to determine the  $D_m$  of in situ animals.

Understanding the nutritional ecology of a species is a critical component of any conservation management plan, as well as being a necessity for effective ex situ husbandry; however, it has historically been difficult to quantify digestive efficiencies for manatees. Because of their protected status, there are limited numbers of ex situ animals with nominal availability for research, including restrictions on the experimental protocols that can be employed. Access to in situ manatees is even more problematic. The use of naturally occurring Mn as an inert marker allows us to measure digestive efficiencies of ex situ and in situ manatees and thereby assess impacts of varying environmental conditions on their nutritional status, as well as explore the relationship between MRT and digestibility in these hindgut fermenters. An understanding of how effectively manatees exploit their environment is critical in assessing how much time and effort this species must spend to meet energy requirements and ultimately how this relates to future habitat protection.

## Material and Methods

### Diet Sampling

Samples of foodstuffs consumed by ex situ manatees (see below) were collected and labeled with species name, date of

collection, and institution. Representative samples of marine and freshwater plant species were also collected from locations that in situ manatees frequently visited based on satellite telemetry and capture information (fig. 1). Study animals had been captured from these areas, released back into them, or frequently used them as feeding grounds. Wild plants were collected during both the wet season (May 1–October 31) and the dry season (November 1–April 30; Black 1993) in order to assess potential seasonal variability in manganese concentration. Freshwater plants were collected from DeLeon Springs, Crystal River, and St. John's River (fig. 1), and marine vegetation was sampled from Biscayne Bay, Tampa Bay, Charlotte Harbor, Ten Thousand Islands, and Indian River Lagoon (fig. 1). Multiple sites per locale were sampled whenever possible. All samples were stored in a cooler until they were returned to the laboratory, where they were stored at  $-20^{\circ}\text{C}$  until further processing.

#### *Ex Situ Feeding Trials*

Fecal samples were collected opportunistically from known individual manatees held at SeaWorld of Florida (SWF), Orlando, Florida; Lowry Park Zoo (LPZ), Tampa, Florida; Homosassa Springs State Wildlife Park (HSSWP), Homosassa, Florida; Epcot's Living Seas (ELS), Orlando; Miami Seaquarium (MSQ), Miami, Florida; and Columbus Zoo (CZ), Columbus, Ohio. Standard diets varied between institutions; manatees at SWF and ELS received a romaine lettuce-only diet; manatees at CZ and MSQ consumed a mixed romaine lettuce/vegetable diet; manatees at LPZ were fed a mixed romaine lettuce/vegetable diet supplemented with freshwater plant species; and manatees at HSSWP consumed a mixed romaine lettuce/vegetable diet but also ingested some natural forage. The proportions of different foodstuffs consumed by individual manatees were estimated using the facility feeding records documenting proportions offered, which were assumed to apply to all manatees being housed together at that facility. Fecal samples used in this study were collected during periods when manatees were consuming only lettuce and mixed vegetables and not being supplemented with either freshwater or marine vegetation. Several facilities supplemented their diets with monkey biscuits (Zupreem, Premium Nutritional Products, Shawnee, KS), but this accounted for <1% of total intake (wet mass [WM]).

Fecal samples were opportunistically collected from any identifiable ex situ animal whose recent diet was known with certainty. Samples were collected immediately after defecation using a dip net. Fecal samples were also collected from ex situ and recaptured in situ animals during health assessments and routine veterinary care. In these latter cases, animals were out of water while procedures were under way. If defecation occurred, the animal was rolled onto its side and the fecal sample collected. Sample sizes were generally 50–100 g, and samples were stored at  $-20^{\circ}\text{C}$  until further processing.

*Manatees on Consistent Diets.* Ex situ manatees were classified into the following dietary categories: standard ex situ diet (ro-

maine lettuce with mixed vegetables), standard ex situ diet supplemented with freshwater vegetation, seagrass diet (see below), or transitioning between two diet categories (see below). In situ manatees were assumed to be consuming their preferred plant species specific to the region/time of year in which the animal was sampled. Intake [Mn] used in  $D_m$  calculations of in situ manatees was based on mean values for preferred plant species associated with the area of capture (see above). Any manatee located in an area that was not represented in the plant collection data set was assigned an intake value based on the overall mean of preferred plant species in the appropriate habitat type.

*Animals Undergoing Diet Changes.* Animals in the transitional group included newly rescued manatees as well as animals that had recently been released back to the wild. For manatees that were newly arrived at a facility, fecal samples were collected upon capture and weekly thereafter, for 4 wk. For released animals, fecal samples were collected on the day of release and during routine scheduled health assessments at 3, 6, and 12 mo postrelease. Based on average gut transit times (Larkin et al. 2007), any fecal sample collected from a manatee  $\leq 7$  d after a diet change was considered to represent the original diet category, and any sample collected  $\geq 10$  d after a diet change was considered to represent the new diet.

*Ex Situ Manatees Transitioned to a Seagrass Diet.* A controlled feeding trial using a strictly seagrass diet was undertaken at MSQ. Prior to the commencement of this feeding trial, manatees ( $n = 5$ ) at MSQ were offered a diet consisting of 80% (WM) romaine lettuce; 19% various items, including bananas (*Musa* sp.,  $[\text{Mn}^{2+}] = 9.4$  ppm dry mass [DM]), apples (*Malus domestica*,  $[\text{Mn}^{2+}] = 2.6$  ppm DM), and sweet potatoes (*Ipomoea batatas*,  $[\text{Mn}^{2+}] = 35.5$  ppm DM), and <1% monkey chow biscuits ( $[\text{Mn}^{2+}] = 113.5 \pm 4.9$  ppm DM). Once the seagrass diet trials began, sufficient quantities of manatee grass (*Syringodium filiforme*; up to a total of 140 kg/d WM), with small amounts of turtle grass (*Thalassia testudinum*), were collected fresh daily and fed to manatees at their normal feeding times. Seagrass was fed to manatees for a total of 17 d, and samples of diet and feces were opportunistically collected on a daily basis and frozen at  $-20^{\circ}\text{C}$  for future analysis. Fecal samples collected >10 d after the diet change were considered to represent the seagrass diet.

#### *Manatee Gut Content Collections*

Digesta samples were collected from manatees ( $n = 6$ ) that had been rescued and treated for an extended period (weeks to months) but that eventually succumbed to the injuries that had necessitated their initial rescue. During treatment, these manatees had exhibited  $D_m$  comparable to other healthy ex situ manatees. Samples were collected during necropsy from several locations along the gastrointestinal tract: stomach; duodenum; proximal, mid-, and distal small intestine; cecum; proximal and distal colon; and rectum. Samples (50–100 g) were collected

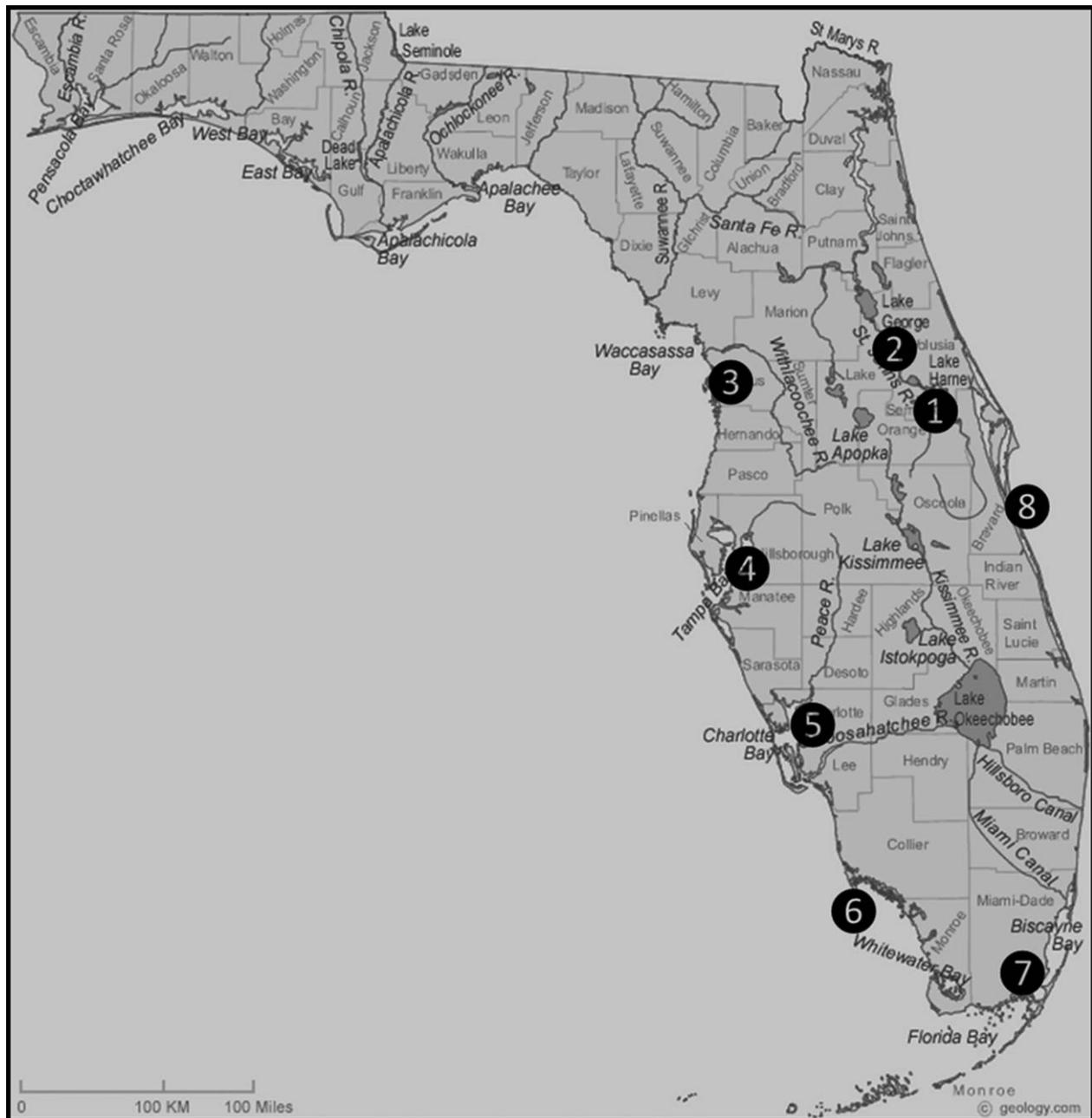


Figure 1. Representative samples of forage plants potentially consumed by manatees were collected at multiple sampling stations in each of three freshwater sites (1 = Blue Springs, St. John's River; 2 = DeLeon Springs, St. John's River; and 3 = Crystal River) and five salt/brackish water sites (4 = Tampa Bay; 5 = Charlotte Harbor; 6 = Ten Thousand Islands; 7 = Biscayne Bay; and 8 = Indian River Lagoon). A color version of this figure is available online.

into plastic snap cap vials and stored at  $-20^{\circ}\text{C}$  until further processing.

#### Sample Processing

**Manganese Analysis.** Plant and fecal samples were freeze-dried to constant mass using a lyophilizer (LabConco, Kansas City, MO), ground with a SPEX 8000 Mixer/Mill (Spex SamplePrep,

Metuchen, NJ), and stored in polycarbonate vials prior to analysis. Samples were subsequently digested using a modified version of Environmental Protection Agency (EPA) method 3050B (EPA 2007) to prepare for inductively coupled plasma atomic emission spectrometry (ICP-AES). Reagent-grade chemicals were used for all procedures. Samples ( $\sim 10$  mL) were analyzed in batches of 230 samples using ICP-AES analysis on an IRIS Intrepid Thermo Elemental analyzer (Waltham, MA). Samples

were run in their digestion batches with the appropriate blank and blank spike. If a batch was split into separate runs or sample reruns were required, the associated method blank and blank spikes were run multiple times. The instrument was calibrated with a set of manganese standards at 0.5, 2, and 5 ppm, and a check standard of 2 ppm was run periodically. Resulting manganese values are reported in parts per million (DM).

Reportable and accurate results were ensured by the use of stringent quality control measures as suggested by EPA (2007) in method 3050B. Method blanks demonstrated that no contamination resulted from the chemicals or equipment used or the technique of the analyst. Matrix spikes and spiked duplicate samples were run on 10% of samples in each matrix. Matrix spikes and duplicates demonstrated precision and repeatability in the methodology. Finally, a National Institute of Standards and Technology standard reference material for spinach was digested for each batch. This standard has a certified manganese value. Any sample value higher than 13.5 (90% of linear range) was considered out of range, diluted, and rerun.

**Lignin Analysis.** A subset of romaine lettuce ( $n = 10$ ) and fecal samples ( $n = 20$ ) from manatees consuming a lettuce-only diet was sent to Dairy One Forage Lab in Ithaca, New York, for acid detergent lignin analysis (Daisy II; ANKOM Technology 2011). Results were reported as percent dry matter. Digestive efficiencies calculated using lignin and manganese as the marker were compared.

**Digestive Efficiency Calculation.** Digestive efficiency ( $D_m$ ) was calculated based on the increased concentration of manganese or lignin in the feces compared to that ingested in the food:

$$D_m = \left(1 - \frac{C_i}{C_f}\right) \times 100, \quad (1)$$

where  $C$  is the concentration (expressed on a dry mass basis) of  $[\text{Mn}^{2+}]$  or lignin in the ingesta (i) or feces (f; e.g., Fadely et al. 1990; Robbins 1993).

**Estimation of Food Intake Rates of In Situ and Ex Situ Manatees.** An estimate of gross energy (GE) intake requirements can be obtained by working backward through the bioenergetic scheme (e.g., Worthy 2001). Using estimates of basal metabolism and the cost of activity and thermoregulation, an estimate of metabolizable energy (ME) requirements can be calculated, and by accounting for assumed metabolic losses in urine, methane production, and digestive efficiency, GE requirements can be calculated. Linking these to published energy densities of potential foodstuffs, the amount of food necessary to meet these requirements can be estimated. In the absence of measured values for either urinary energy losses or methane production and the apparent lack of a heat increment of feeding (Gallivan and Best 1986), ME was assumed to be 90% of digestible energy (Robbins 1993; Clauss et al. 2005).

Standard metabolic rate (SMR) was calculated using the Kleiber (1975) equation ( $\text{SMR} = 3.4 \text{ mass}^{0.75}$ ) and converting to

megajoules per day. It was assumed that adult manatees have a metabolic requirement of 35% of predicted by the Kleiber (1975) equation and that juveniles (animals weighing less than 300 kg) have metabolic rates that are 70% of predicted (Irvine 1983; Worthy et al. 2000; G. A. J. Worthy and T. A. M. Worthy, unpublished data). Field metabolic rate (FMR) was calculated as  $2 \times \text{SMR}$ , and temperatures below the lower critical temperature of the thermoneutral zone resulted in a further 50% increase in metabolic costs (Worthy et al. 2000).

GE intake rates were calculated for animals living under ex situ conditions (eating lettuce) or in situ conditions eating one of two species of seagrass. Lettuce intake estimates were based on SMR and  $D_m = 84\%$  (this study); consumption rates of *Syringodium* and *Thalassia* used FMR and  $D_m = 47\%$  (this study). Energy densities of lettuce, *Syringodium*, and *Thalassia* ( $0.7 \text{ MJ kg}^{-1} \text{ GE}$ ,  $1.1 \text{ MJ kg}^{-1} \text{ GE}$ , and  $2.5 \text{ MJ kg}^{-1} \text{ GE}$ , respectively; Lobel and Ogden 1981; Siegall-Willott et al. 2010) were applied to the estimated GE requirement to calculate required daily intake rates.

**Statistical Analyses.** Statistical analyses were carried out using SPSS, version 20.0 (SPSS, Chicago, IL). Sample distributions were analyzed for normality using a Shapiro-Wilk test, and homogeneity of variance was tested using Levene's  $F$ . Proportional data were arcsine transformed prior to analysis, and an average value was used to represent individual manatees with repeated measures of  $D_m$ . Data were analyzed using ANOVA with statistical significance set at  $P < 0.05$ . Means are reported  $\pm \text{SE}$ .

## Results

### Manganese Content of Diet

**Standard Ex Situ Diet.** There were significant differences between dietary items potentially available to be fed to ex situ manatees (ANOVA,  $F_{7,47} = 19.74$ ,  $P < 0.0001$ ; table 1). Romaine lettuce did not differ significantly between facilities or time of

Table 1: Manganese concentrations  $[\text{Mn}^{2+}]$  (ppm DM) measured in foodstuffs fed to ex situ manatees

Foodstuff	Location	$[\text{Mn}^{2+}] \pm \text{SE}$	$n$
Alfalfa	LPZ, HSSWP	$41.4 \pm 10.1$	6
Cabbage	HSSWP	$24.9 \pm 3.7$	6
Endive	LPZ	$62.0 \pm 7.3$	3
Escarole	LPZ	$94.2 \pm 16.4$	3
Kale	LPZ	$38.0 \pm 7.3$	5
Romaine lettuce	CZ, ELS, LPZ, SWE, MSQ, HSSWP	$47.9 \pm 4.9$	15
Monkey chow	CZ, ELS, LPZ, SWE, MSQ	$113.5 \pm 4.9$	6

Note. There were no significant differences between facilities. CZ = Columbus Zoo, Columbus, OH; ELS = Epcot's Living Seas, Orlando, FL; HSSWP = Homosassa Springs State Wildlife Park, Homosassa, FL; LPZ = Lowry Park Zoo, Tampa, FL; MSQ = Miami Seaquarium, Miami, FL; SWF = SeaWorld of Florida, Orlando.

year and had an overall  $[\text{Mn}^{2+}]$  of  $47.9 \pm 4.9$  ppm DM ( $n = 15$ ). Romaine lettuce did not differ significantly from cabbage, kale, alfalfa, and endives (table 1). Monkey chow and escarole had significantly higher  $[\text{Mn}^{2+}]$  than romaine lettuce, and carrots had significantly lower  $[\text{Mn}^{2+}]$  (table 1).

**Freshwater Vegetation.** Fifteen species of freshwater plants commonly consumed by manatees were sampled at three locations and across seasons (table 2). There were no significant geographic differences in  $[\text{Mn}^{2+}]$  within a species in a given season, but there were significant differences between species (ANOVA,  $F_{19,90} = 26.557$ ,  $P < 0.0001$ ). Hydrilla, duckweed, and water hyacinth also exhibited significant differences between wet and dry seasons (table 2). Of the species that manatees prefer to consume (hydrilla, tapegrass, coontail, parrot feather, and alligator weed; Hartman 1979; Ledder 1986), hydrilla (wet season), alligator weed, and coontail were not significantly different from each other (Tukey,  $P > 0.05$ ; table 2). Parrotfeather and tapegrass had significantly higher  $[\text{Mn}^{2+}]$  (table 2). Water milfoil, water hyacinth (dry season), tapegrass, and hydrilla (dry season) also had significantly greater  $[\text{Mn}^{2+}]$  than hydrilla (wet season; Tukey,  $P < 0.05$ ; table 2).

**Marine Vegetation.** Four species of marine plants representing known or suspected manatee dietary components were collected from six sampling sites and across both seasons (table 3). When marine species were examined individually, shoal grass did not differ significantly between sampling locations, with the exception of samples collected in Cockroach Bay (south shore of Tampa Bay, FL;  $F_{5,19} = 23.409$ ,  $P < 0.001$ ; Tukey,

$P < 0.05$ ). Similarly, red weed ( $F_{1,11} = 121.961$ ,  $P < 0.001$ ), manatee grass ( $F_{3,13} = 33.356$ ,  $P < 0.001$ ), and turtle grass ( $F_{5,23} = 27.145$ ,  $P < 0.001$ ) had significantly higher  $[\text{Mn}^{2+}]$  in Cockroach Bay or nearby sites (table 3). Interspecifically, there were no significant differences between any sampled marine species if Cockroach Bay was excluded (ANOVA,  $F_{3,54} = 2.092$ ,  $P = 0.113$ ), and no seasonal differences were apparent.

#### Assessment of Manganese as a Measure of Digestive Efficiency

**Comparison of Manganese and Lignin Methods of Calculating Digestive Efficiency.** Romaine lettuce had a lignin concentration of  $2.3\% \pm 0.4\%$  DM ( $n = 10$ ), similar to that measured by Siegall-Willott et al. (2010), and a  $[\text{Mn}^{2+}]$  of  $45.0 \pm 2.7$  ppm DM ( $n = 10$ ). Fecal lignin concentration was  $14.8\% \pm 0.6\%$  DM ( $n = 20$ ), while  $[\text{Mn}^{2+}]$  in the same fecal sample was  $313.6 \pm 19.7$  ppm DM ( $n = 20$ ). There was no significant difference between  $D_m$  calculated using lignin ( $83.8\% \pm 0.7\%$ ) and  $D_m$  calculated using manganese ( $84.7\% \pm 0.7\%$ ; paired  $t$ -test,  $t = -1.447$ ,  $df = 19$ ,  $P = 0.16$ ).

**Gut Content Data.** Six manatee carcasses were sampled for  $[\text{Mn}^{2+}]$  of their gut contents. Calculated  $D_m$  values for these animals (using  $[\text{Mn}^{2+}]$  of stomach contents to estimate intake values) ranged from 78.8% to 90.3% ( $86.8\% \pm 1.9\%$ ), comparable to other ex situ manatees consuming lettuce. Manganese concentrations were lowest in the stomach (SWF animals,  $51.2 \pm 3.8$  ppm DM,  $n = 4$ ; LPZ animals,  $15.3 \pm 1.8$  ppm DM,  $n = 2$ ; fig. 2) and were similar to romaine lettuce samples consumed prior to death at the respective holding facility (SWF

Table 2: Manganese concentrations  $[\text{Mn}^{2+}]$  (ppm DM) measured in freshwater vegetation commonly consumed by manatees

Scientific name	Common name	Location	Season	$[\text{Mn}^{2+}] \pm \text{SE}$	$n$
<i>Alternanthera philoxeroides</i>	Alligator weed	DLS, SJR	Both	$223.6 \pm 18.3$	9
<i>Ceratophyllum demersum</i>	Coontail	DLS	Wet	$432.8 \pm 10.1$	5
<i>Eichornia crassipes</i>	Water hyacinth	DLS	Wet	$152.4 \pm 25.8$	6
<i>E. crassipes</i>	Water hyacinth	DLS, SJR	Dry	$929.9 \pm 48.8$	6
<i>Hydrilla verticillata</i>	Hydrilla	CR, DLS	Wet	$177.1 \pm 22.8$	7
<i>H. verticillata</i>	Hydrilla	DLS	Dry	$650.1 \pm 43.6$	6
<i>Hydrocotyle</i> sp.	Pennywort	DLS	Both	$189.0 \pm 15.5$	10
<i>Lemna valdiana</i>	Duckweed	DLS	Wet	$276.4 \pm 57.1$	3
<i>L. valdiana</i>	Duckweed	DLS	Dry	$547.6 \pm 101.6$	3
<i>Limnophila sessiliflora</i>	Limnophila	DLS	Dry	$358.4 \pm 28.9$	2
<i>Myriophyllum aquaticum</i>	Parrotfeather	DLS, SJR	Dry	$506.3 \pm 69.9$	3
<i>Myriophyllum spicatum</i>	Water milfoil	CR	Dry	$120.4 \pm 7.0$	3
<i>M. spicatum</i>	Water milfoil	DLS	Dry	$912.4 \pm 69.7$	6
<i>Nuphar polysepala</i>	Spatterdock	SJR	Both	$132.3 \pm 2.5$	4
<i>Nymphaea odorata</i>	Water lily	DLS	Wet	$83.1 \pm 3.4$	3
<i>Panicum hemitomo</i>	Maidencane	DLS, SJR	Both	$154.1 \pm 13.3$	5
<i>Pistia stratiotes</i>	Water lettuce	DLS, SJR	Dry	$472.9 \pm 60.8$	10
<i>Pontederia cordata</i>	Pickerel weed	DLS	Both	$162.1 \pm 16.7$	7
<i>Vallisneria americana</i>	Eel grass	DLS	Dry	$657.9 \pm 71.4$	3

Note. CR = Crystal River; DLS = DeLeon Springs; SJR = St. John's River (see fig. 1 for locations). Wet season = May 1–October 31; dry season = November 1–April 30 (Black 1993).

Table 3: Manganese concentrations [ $\text{Mn}^{2+}$ ] (ppm DM) measured in marine vegetation commonly consumed by manatees

Scientific name	Common name	Location	Season	[ $\text{Mn}^{2+}$ ] $\pm$ SE	<i>n</i>
<i>Gracillaria tikvahiae</i>	Red weed	IRL	Wet	162.6 $\pm$ 19.0	6
<i>G. tikvahiae</i>	Red weed	CB	Wet	2,016.9 $\pm$ 184.5	5
<i>Halodule wrightii</i>	Shoal grass	BB, CH, IRL, TTI, SP	Wet	226.8 $\pm$ 23.9	18
<i>H. wrightii</i>	Shoal grass	CB, TECO	Wet	864.9 $\pm$ 106.4	3
<i>Syringodium filiforme</i>	Manatee grass	CH, IRL, BB	Wet	171.4 $\pm$ 20.6	12
<i>S. filiforme</i>	Manatee grass	CB	Wet	506.1 $\pm$ 20.0	3
<i>Thalassia testudinum</i>	Turtle grass	BB, CH, IRL, TTI	Wet/dry	161.5 $\pm$ 12.4	17
<i>T. testudinum</i>	Turtle grass	CB, TECO	Wet	578.6 $\pm$ 44.7	8

Note. BB = Biscayne Bay; CB = Cockroach Bay, Tampa Bay; CH = Charlotte Harbor; IRL = Indian River Lagoon; SP = Simmons Park, Tampa Bay; TECO = Tampa Bay Electric Company, Tampa Bay; TTI = Ten Thousand Islands (see fig. 1 for locations). Wet season = May 1–October 31; dry season = November 1–April 30 (Black 1993).

lettuce,  $54.0 \pm 3.2$  ppm DM,  $n = 6$ ; LPZ lettuce,  $15.2 \pm 0.5$  ppm DM,  $n = 3$ ). Manganese concentrations remained low in the duodenum and small intestine but increased in the cecum, colon, and rectum (fig. 2).

#### Manatee Digestive Efficiency

*Ex Situ Animals on Standard Diets (Romaine with Mixed Vegetables).* Fecal samples were collected from a total of 37 individual ex situ manatees (table 4). Assuming a dietary [ $\text{Mn}^{2+}$ ] calculated for the specific diet (based on weights of food offered by the facility) at the time of fecal sampling, there were no significant differences in digestive efficiencies between sexes (male,  $n = 16$ ; female,  $n = 21$ ; ANOVA,  $F_{1,37} = 3.531$ ,  $P = 0.07$ ) or for animals held at different facilities (ANOVA,  $F_{5,37} = 1.836$ ,  $P = 0.134$ ). Overall, ex situ manatees consuming a romaine lettuce/mixed vegetable diet had a  $D_m$  of  $84.0\% \pm 0.7\%$  ( $n = 37$ ; table 4).

*Animals Undergoing Dietary Changes.* Four individual manatees were followed from initial rescue through the rehabilitation process (fig. 3). Two of these animals were rescued from a freshwater area (St. John's River) and two from a marine environment (Indian River Lagoon) due to injuries sustained from either boat impacts or net entanglement and did not suffer from any condition that would be anticipated to affect digestive efficiency. Manatees rescued from the freshwater area had an initial  $D_m$  of  $73.5\% \pm 3.0\%$  ( $n = 2$ ), while those rescued from the marine environment had an initial  $D_m$  of  $45.0\% \pm 2.7\%$  ( $n = 2$ ). All rescued manatees exhibited  $D_m$  when consuming their lettuce diets, similar to other ex situ animals within a short time of rescue. Efficiencies ranged from 76.0% to 89.8% (fig. 3), averaging  $83.6\% \pm 4.5\%$  ( $n = 4$ ).

*Ex Situ Manatees Consuming a Seagrass Diet.* Manganese concentrations of manatee grass and turtle grass collected specifically for this experiment from Biscayne Bay were not significantly different from each other ( $174.9 \pm 47.1$  ppm DM

[ $n = 15$ ] and  $173.5 \pm 36.1$  ppm DM [ $n = 6$ ], respectively) or from previously collected values (table 3). [ $\text{Mn}^{2+}$ ] of the preexperiment ex situ maintenance diet was  $54.3 \pm 7.1$  ppm DM.

[ $\text{Mn}^{2+}$ ] in feces of manatees consuming their maintenance diet was  $290.5 \pm 16.69$  ppm DM, dropping to  $288.4 \pm 8.6$  ppm DM after  $\geq 10$  d of consuming the seagrass diet. This nonsignificant change in fecal [ $\text{Mn}^{2+}$ ] (paired  $t$ -test,  $t = 0.202$ ,  $df = 12$ ,  $P = 0.843$ ) was associated with a significant increase in dietary [ $\text{Mn}^{2+}$ ]. Preexperiment, manatees consuming their normal maintenance diet had a  $D_m$  of  $81.6\% \pm 1.1\%$  ( $n = 4$ ). After consuming strictly seagrass for  $\geq 10$  d,  $D_m$  was  $36.2\% \pm 1.2\%$  ( $n = 5$ ).

*In Situ Manatees Consuming Wild Diets.* Digestive efficiency was estimated for eight in situ manatees consuming a marine diet for longer than 90 d and for seven manatees consuming freshwater vegetation for extended periods (table 5). These animals had been released after rehabilitation but were recaptured for routine scheduled health assessments at 3-, 6-, and 12-mo intervals after release. In situ manatees consuming marine vegetation had significantly lower  $D_m$  ( $46.9\% \pm 1.8\%$ ;  $n = 8$ ) than in situ manatees consuming freshwater vegetation ( $77.8\% \pm 2.6\%$ ;  $n = 7$ ), which in turn were significantly lower than ex situ manatees consuming lettuce/mixed vegetables ( $84.0\% \pm 0.7\%$ ;  $n = 37$ ; ANOVA,  $F_{2,51} = 143.70$ ,  $P < 0.0001$ ; tables 4, 5). In situ manatees eating seagrasses had significantly higher  $D_m$  than long-term ex situ animals consuming seagrass for short periods of time (see above;  $46.9\% \pm 1.8\%$  vs.  $36.2\% \pm 1.2\%$ , respectively;  $t$ -test,  $t = 2.458$ ,  $df = 12$ ,  $P < 0.05$ ).

#### Estimation of Food Intake Rates of In Situ and Ex Situ Manatees

Estimates of food intake required to meet daily GE requirements of ex situ manatees consuming lettuce ranged from  $12.3 \text{ kg d}^{-1}$  (WM) for a 100-kg juvenile to  $34.6 \text{ kg d}^{-1}$  (WM) for a 1,000-kg adult (table 6). These estimates were based on a SMR of 35% of that predicted by Kleiber (1975), an energy density of  $0.7 \text{ MJ kg}^{-1}$  (WM), and a  $D_m$  of 84%. Estimates of GE requirements for in situ manatees were impacted by greater

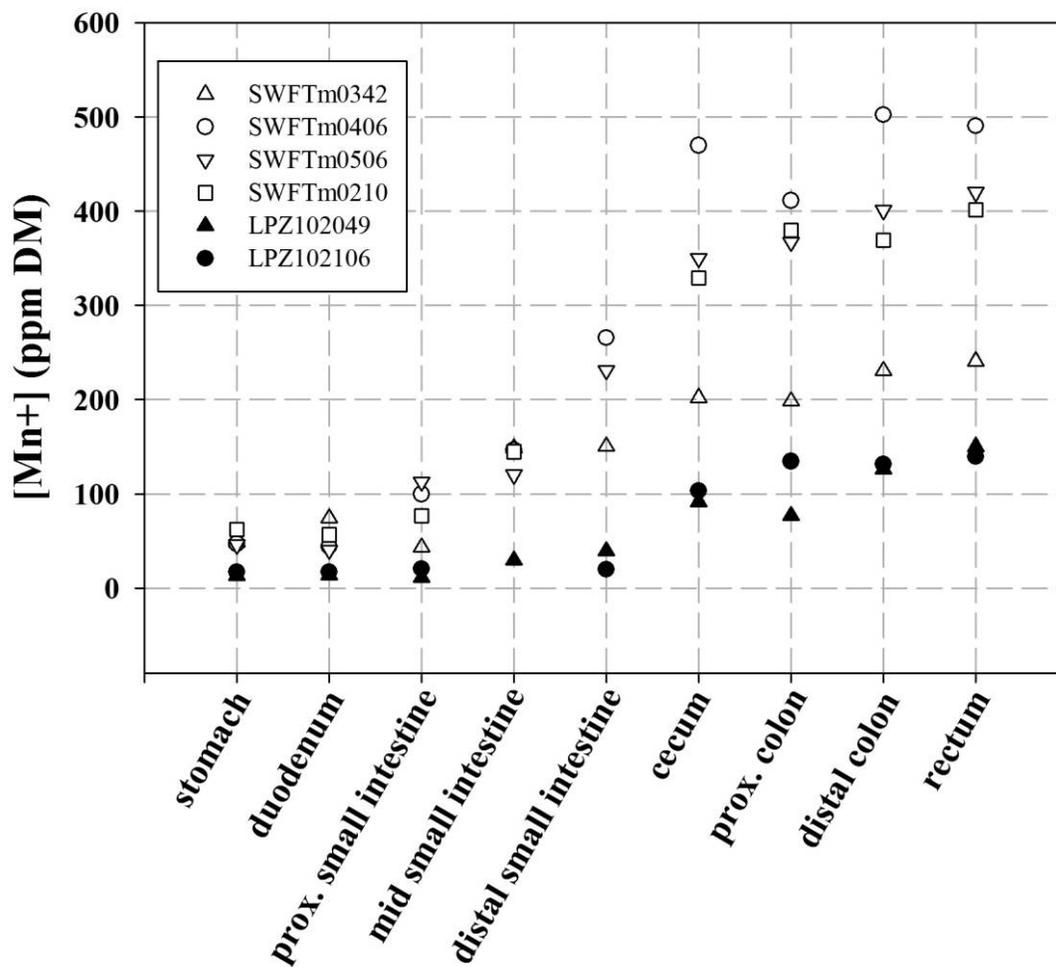


Figure 2. Manganese concentrations (ppm DM) of digesta recovered from various sections of the gut of recently dead manatees. Stomach content values mirror manganese concentrations of food consumed in the days preceding death.

activity levels (with FMR being equivalent to  $2 \times$  SMR) and a lower digestibility for seagrasses ( $D_m$  of 47%). Under thermoneutral conditions, GE requirements ranged from  $30.8 \text{ MJ d}^{-1}$  (GE) for a 100-kg juvenile to  $86.5 \text{ MJ d}^{-1}$  (GE) for a 1,000-kg adult (table 6). Using published energy densities (*Syringodium* and *Thalassia*; 1.1 and  $2.5 \text{ MJ kg}^{-1}$  [WM], respectively; Lobel and Ogden 1981; Siegall-Willott et al. 2010), a juvenile manatee would need to consume  $12.3 \text{ kg d}^{-1}$  (WM) of *Thalassia* or  $28.0 \text{ kg d}^{-1}$  (WM) of *Syringodium*, while an adult would require  $34.6 \text{ kg d}^{-1}$  (WM) of *Thalassia* or  $78.7 \text{ kg d}^{-1}$  (WM) of *Syringodium* (table 6). These consumption requirements would increase under cold conditions, when a juvenile manatee would need to consume  $18.5 \text{ kg d}^{-1}$  (WM) of *Thalassia* or  $42.0 \text{ kg d}^{-1}$  (WM) of *Syringodium* and an adult would require  $51.9 \text{ kg d}^{-1}$  (WM) of *Thalassia* or  $118.0 \text{ kg d}^{-1}$  (WM) of *Syringodium* (table 6).

## Discussion

The historical approach taken in manatee digestive studies has been to examine gastrointestinal function on deceased animals (e.g., Snipes 1984; Reynolds and Rommel 1996) or to estimate digestive efficiencies by undertaking a total collection trial (e.g., Lomolino and Ewell 1984). This study was the first to use an inert trace mineral as a dietary marker in manatees or in any megaherbivore. Comparison of the lignin and manganese methods of determining digestive efficiency demonstrates a lack of significant difference between traditional methods and this inassimilable marker methodology despite the suggestion that using acid detergent lignin as an internal marker generally works well only if the diet contains  $\geq 5\%$  lignin (DM; Van Soest 1994).

This study showed that manatees have a  $D_m$  of approximately 84% when consuming lettuce/mixed vegetables, decreasing to

Table 4: Digestive efficiencies (%) of long-term ex situ manatees eating standard oceanaria diets

ID	Facility	Sex	Days on diet	$D_m$ (% $\pm$ SE)	$n$
HS 8601	HSSWP	F	>365	89.3 $\pm$ 4.9	3
LPZ 102102	LPZ	M	63	76.5 $\pm$ 2.1	2
HS 9002	HSSWP	F	>365	77.4 $\pm$ 3.2	3
SWF Tm 0408	SWF	F	57	90.6 $\pm$ 3.6	2
SWF Tm 0116	ELS	M	>365	77.3	1
SWF Tm 0510	SWF	F	12	84.7 $\pm$ 4.1	7
SWF Tm 0521	SWF	M	15	83.0 $\pm$ 4.8	5
MSQ 9507	SWF	M	281	73.6 $\pm$ 2.1	2
LPZ 100379	LPZ	M	>365	87.1 $\pm$ 1.0	4
SWF Tm 0304	SWF	F	149	85.8	1
SWF Tm 7708	CZ	M	>365	84.6 $\pm$ .6	3
SWF Tm 9539	CZ	F	>365	87.7 $\pm$ 4.6	2
LPZ 100432	LPZ	M	>365	88.7 $\pm$ 2.3	3
SWF Tm 0522	LPZ	F	23	87.5	1
SWF Tm 0501	SWF	F	14	80.4	1
MSQ 5801	MSQ	F	>365	80.5 $\pm$ 5.1	4
LPZ 102150	LPZ	F	223	87.8 $\pm$ 4.4	3
MSQ 1	HSSWP	F	>365	86.2 $\pm$ .3	2
SWF Tm 0015	ELS	M	>365	77.4	1
SWF Tm 9417	ELS	M	>365	80.9 $\pm$ 2.6	2
MSQ 9304	MSQ	F	>365	86.7 $\pm$ 4.1	5
MSQ 9701	MSQ	F	>365	79.6	1
SWF Tm 0413	SWF	F	33	86.6	1
SWF Tm 0411	SWF	M	>365	87.5	1
MSQ 5701	MSQ	M	>365	82.0 $\pm$ 2.0	4
HS 252	HSSWP	F	>365	86.6	1
SWF Tm 0210	SWF	M	>365	89.3 $\pm$ 1.3	2
SWF Tm 0322	SWF	F	73	74.7	1
SWF Tm 9535	CZ	F	>365	86.3 $\pm$ .1	2
MSQ Tm 0701	MSQ	M	>365	81.2	1
SWF Tm 0512	SWF	F	27	84.8 $\pm$ .9	5
SWF Tm 0312	CZ	M	>365	87.1	1
SWF Tm 0511	SWF	M	16	82.9 $\pm$ 3.8	4
SWF Tm 0517	SWF	F	12	86.8 $\pm$ 1.0	4
SWF Tm 0338	SWF	F	130	87.8 $\pm$ 1.0	4
SWF Tm 0514	SWF	F	21	84.5 $\pm$ 2.6	6
SWF Tm 9519	CZ	M	>365	85.6 $\pm$ 3.6	3

Note. CZ = Columbus Zoo, Columbus, OH; ELS = Epcot's Living Seas, Orlando, FL; HSSWP = Homosassa Springs State Wildlife Park, Homosassa, FL; LPZ = Lowry Park Zoo, Tampa, FL; MSQ = Miami Seaquarium, Miami, FL; SWF = SeaWorld of Florida, Orlando.

approximately 78% when consuming freshwater vegetation and decreasing further to approximately 47% when feeding on seagrasses. A direct correlation between fiber content and digestibility has been shown to exist in dugongs (Lanyon and Sanson 2006). Some seagrasses (e.g., *Halophila* and *Zostera*) have high fiber (neutral detergent fiber [NDF]) content (42%–63% DM; Lanyon and Sanson 2006), while other species of seagrass (e.g., *Thalassia*, *Syringodium*, and *Halodule*) have mean NDF levels of approximately 26% DM (Dawes and Lawrence 1980; Carlson et al. 2003; Siegel-Willott et al. 2010). In contrast, freshwater

aquatic plants, such as hydrilla, duckweed, and water lettuce (*Pistia stratiotes*), contain relatively low NDF (5.4%–26.7% DM; Tan 1970) and would be expected to be more digestible. Romaine lettuce has very low NDF content (2% DM; Rubatzky and Yamaguchi 1997; Siegel-Willott et al. 2010) and is higher in crude fat, crude protein, carbohydrates, and digestible energy than seagrasses (Siegel-Willott et al. 2010). Consistent with these fiber values, our data show that romaine lettuce is more digestible than freshwater plants, which in turn are more digestible than seagrasses.

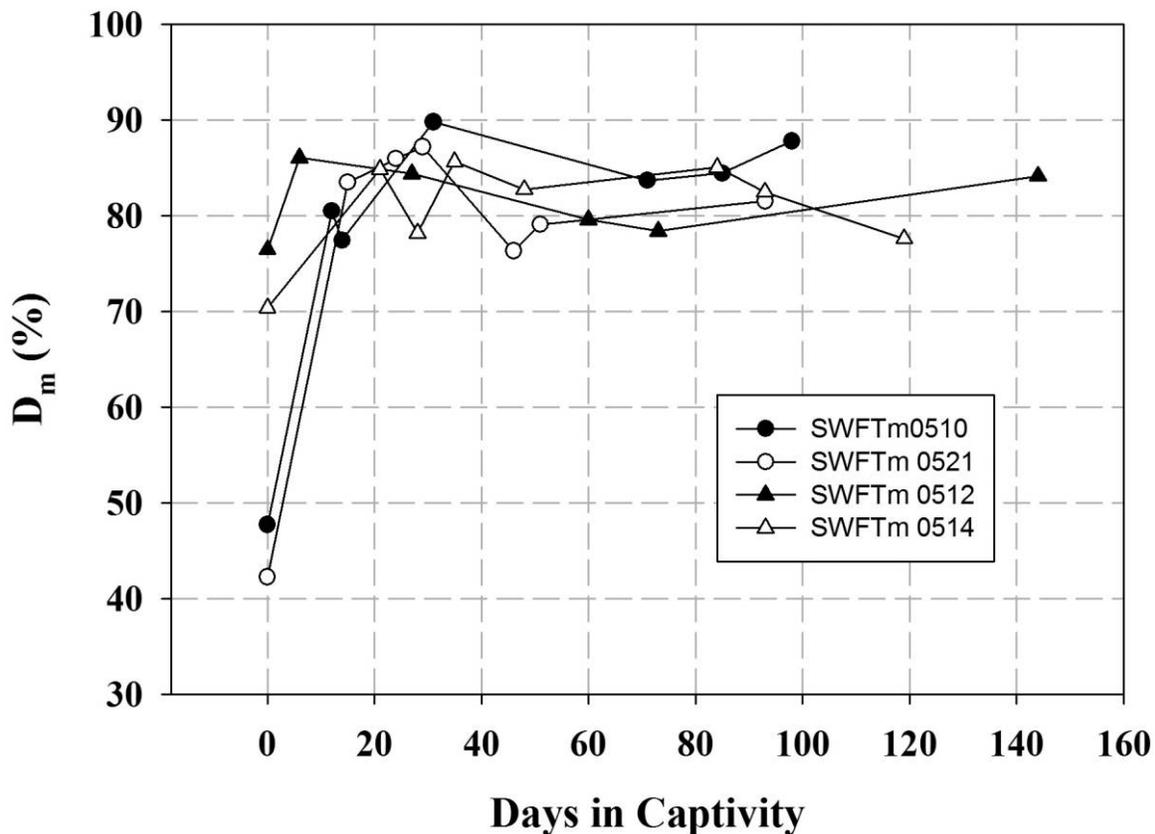


Figure 3. Manatees transitioning from an in situ diet to an ex situ lettuce diet. Two manatees were recovered from the Indian River Lagoon (circles), and two others were recovered from the St. John's River (triangles). Initial  $D_m$  values (%) were estimated based on plant characteristics of the region of capture.

The significant differences in  $D_m$  for manatees consuming freshwater and marine diets have major implications for their natural history and, ultimately, their conservation. Differences in  $D_m$  between ex situ and in situ manatees consuming seagrasses were not unexpected. Drastic dietary changes have been shown to result in modification of the gut flora in other mammalian species (e.g., Rowland et al. 1985; Juliand 2005), and the sudden change in diet that our ex situ manatees experienced when they were switched to a seagrass diet could account for the difference in  $D_m$  seen between them and in situ animals in the marine environment. Our data also indicate that manatees consuming seagrasses differ significantly in their  $D_m$  from dugongs on a seagrass diet (Murray et al. 1977; Aketa et al. 2001, 2003; Goto et al. 2004, 2008).

Different species of seagrasses can vary dramatically in their digestibility, with dugongs having a  $D_m$  of up to 85% when consuming seagrass (Aketa et al. 2001, 2003; Goto et al. 2008). Several authors have suggested that dugongs must consume plant species that are highly digestible (*Halophila*) and have high nutrient contents (*Halodule*) to maximize nutrient intake (Preen 1992; Lanyon and March 1995; Aragonés 1996; Aragonés and Marsh 2000; Lanyon and Sanson 2006). Preen et al. (1995)

further suggested that the highly specialized dietary requirements of dugongs may limit the number of seagrass meadows that are suitable as dugong habitat. Manatees have a broader distribution and, as a result of the wider diversity of forage species they consume, may be less specialized in their feeding and digestive strategies than dugongs (Lefebvre et al. 2000).

Our  $D_m$  results are consistent with the only previous study that estimated the  $D_m$  of a single ex situ West Indian manatee, which was consuming either lettuce or water hyacinth (Lomolino and Ewel 1984). Lomolino and Ewel (1984) used a total fecal collection method with a manatee that was individually housed for the duration of their study. They measured a  $D_m$  of 91.4% for lettuce and 82.6% for water hyacinth, similar to the results of this study. Best (1981) reported preliminary digestibility values between 44% and 68% for Amazonian manatees (*Trichechus inunguis*) consuming freshwater vegetation and recommended using more conservative estimates of digestibility than those suggested by Lomolino and Ewel (1984).

Documented feeding habits suggest that manatees are selecting vascular plants that are rich in cellulose and require some degree of microbial fermentation (Snipes 1984). Manatees are hindgut fermenters but are unusual in that they combine

Table 5: Estimated digestive efficiencies (%) of manatees consuming either marine or freshwater in situ diets

ID	Sex	Length (cm)	Mass (kg)	Time on natural forage (d)	Region	Assumed dietary [Mn <sup>2+</sup> ] (ppm DM)	Measured fecal [Mn <sup>2+</sup> ] (ppm DM)	D <sub>m</sub> (%)
Marine:								
SWF Tm 0301	M	NA	NA	373	BB	162	306.1	47.1
MS 9507	M	269	445.0	60	BB	162	349.3	53.6
SWF Tm 0510	F	289	447.7	SCR	IRL	162	303.7	46.7
SWF Tm 0521	M	270	313.2	SCR	IRL	162	280.4	42.2
LPZ 101735	M	NA	NA	231	TB	353	594.5	40.6
SWF Tm 9417	M	280	386.4	367	TB	353	689.9	48.8
LPZ 101754	M	249	318.2	399	TB	353	758.5	53.5
SWF Tm 0431	F	242	288.6	91	CH	162	281.2	42.4
Freshwater:								
SWF Tm 0523	F	NA	NA	SCR	CR	393	1,732.6	77.4
SWF Tm 0522	F	254	NA	SCR	SJR	393	3,372.2	88.3
SWF Tm 0341	F	240	178.6	77	SJR	393	2,490.6	84.2
SWF Tm 0340	M	257	190.9	77	SJR	393	1,834.3	78.6
SWF Tm 0512	F	290	209.5	SCR	SJR	393	1,669.9	76.5
SWF Tm 0312	M	278	422.7	262	SJR	393	1,245.8	68.9
SWF Tm 0514	F	257	338.6	SCR	SJR	393	1,327.3	70.4

Note. Overall dietary manganese concentration (ppm DM) was estimated for each geographic region based on the composition of the preferred forage species in the specific region utilized by the individual manatee. Length and/or mass data were not always available (NA). M = male; F = female. SCR = sample collected at time of rescue. BB = Biscayne Bay; CH = Charlotte Harbor; CR = Crystal Labor; IRL = Indian River Lagoon; SJR = St. John's River; TB = Tampa Bay.

this hindgut fermentation with a prolonged digesta transit time (Burn 1986; Reynolds and Rommel 1996). Numerous authors (e.g., Lomolino 1977; Best 1981; Burn 1986; Reynolds and Rommel 1996) have noted that this slow passage (up to 7 d; Larkin et al. 2007) should aid in their ability to digest the generally low-quality (i.e., relatively high crude fiber, low crude protein, and low caloric value) food they consume.

Manatees are colonic fermenters, having a cecum that is not particularly complex or differentiated (Reynolds 1980; Snipes 1984). The large intestine and its contents can account for 10% of body mass, and the colon of an adult manatee can be >20 m in length and 15 cm in diameter (Reynolds 1980). Larkin et al. (2007) suggested that gut contents pass through the tract as a bolus with minimal mixing, consistent with the absence of a "fermentation vat." Burn and Odell (1987) assessed volatile fatty acid concentrations and concluded that the cecum and colon are the primary sites of microbial fermentation. If this is the case, then most absorption would occur in the hindgut—consistent with our observed trend in manganese concentrations in the cecum and colon. Burn (1986) and Burn and Odell (1987) also determined that the primary site of water and nutrient absorption was the proximal large intestine.

Understanding nutritional ecology and digestion is important to both caregivers and ecologists. Digestive processes determine how efficiently animals extract energy and nutrients from their foods, and low digestive efficiencies translate into more time and effort spent collecting food to meet a fixed

required energy input determined by metabolic requirements. These concerns are particularly important when one is dealing with an endangered species. Key habitat types that have been recognized in the Manatee Recovery Plan (USFWS 2001) include those that are used for (1) thermoregulation at warm-water refuges; (2) feeding, reproduction, and shelter; and (3) travel and migration. During cold periods, manatees remain in thermal refugia and do not feed, but during periods of milder winter temperatures, manatees move to nearby seagrass beds to feed (Deutsch 2000). Adequate feeding habitat in close proximity to warm-water refuge sites is critically important to the overall recovery of the Florida manatee, but in order to properly assess habitat requirements, estimates of feeding needs are critical.

There are limited data describing consumption rates of in situ manatees, but Bengtson (1981, 1983) and Etheridge et al. (1985) reported that manatees feeding in the upper St. Johns River had mean consumption rates of 4%–9% (WM) of their body weight per day, depending on season, with lactating females consuming as much as 13% WM per day. Etheridge et al. (1985) estimated daily consumption rates by adults, juveniles, and calves eating hydrilla at 7.1%, 9.6%, and 15.7% (WM) of body mass per day, respectively. At the present time it is not technically feasible to directly measure feeding rates of in situ manatees, but we can use a simple bioenergetic approach to estimate their requirements. Daily food intake rates can be estimated by working backward through the bioenergetic

Table 6: Estimated gross energy (GE) requirements and calculated food intake ( $\text{kg d}^{-1}$  wet mass) required for each of three potential diets

Mass (kg)	Relative water temperature	SMR ( $\text{MJ d}^{-1}$ )	FMR ( $\text{MJ d}^{-1}$ )	GE ( $\text{MJ d}^{-1}$ )	Lettuce intake ( $\text{kg d}^{-1}$ )	<i>Syringodium</i> intake ( $\text{kg d}^{-1}$ )	<i>Thalassia</i> intake ( $\text{kg d}^{-1}$ )
100	TNZ	6.5	...	8.6	12.3	...	...
	TNZ	...	13.0	30.8	...	28.0	12.3
	Cold	...	19.5	46.2	...	42.0	18.5
250	TNZ	12.9	...	17.1	24.5	...	...
	TNZ	...	25.9	61.2	...	55.6	24.5
	Cold	...	38.8	91.8	...	83.4	36.7
500	TNZ	10.9	...	14.4	20.6	...	...
	TNZ	...	21.8	51.4	...	46.8	20.6
	Cold	...	32.6	77.2	...	70.2	30.9
750	TNZ	14.7	...	19.5	27.9	...	...
	TNZ	...	29.5	69.7	...	63.4	27.9
	Cold	...	44.2	104.6	...	95.1	41.8
1,000	TNZ	18.3	...	24.2	34.6	...	...
	TNZ	...	36.6	86.5	...	78.7	34.6
	Cold	...	54.9	129.8	...	118.0	51.9

Note. The three diets consisted of an ex situ lettuce diet, an in situ *Syringodium* diet, or an in situ *Thalassia* diet for manatees living under thermoneutral conditions (TNZ) or as a result of a 50% increase in field metabolic rate (FMR) due to exposure to cold-water conditions (below TNZ; Worthy et al. 2000; G. A. J. Worthy and T. A. M. Worthy, unpublished data). See "Material and Methods" for energy calculations.

scheme, by combining energy needs (metabolic rate; Irvine 1983; Worthy et al. 2000; G. A. J. Worthy and T. A. M. Worthy, unpublished data) with digestive efficiencies (this study) and published energy densities of potential foodstuffs (table 6). This simple approach predicts that an ex situ adult manatee weighing 1,000 kg would consume  $32.7 \text{ kg d}^{-1}$  (WM) of lettuce, while a similarly sized in situ manatee would eat  $36.6 \text{ kg d}^{-1}$  of turtle grass or  $83.2 \text{ kg d}^{-1}$  (WM) of manatee grass (table 6). This estimate of biomass intake for ex situ animals is consistent with what manatees were consuming during this study. Consumption requirements for manatee grass and turtle grass are opposite of what you might predict given that manatees prefer to eat manatee grass over turtle grass (Hartman 1979; Provan-cha and Hall 1991). These intake estimates are all less than the theoretical maximum daily intake rate for a manatee of this size (9% of body mass, or  $90 \text{ kg d}^{-1}$ ; Bengtson 1983) and well below the  $130 \text{ kg d}^{-1}$  (i.e., 13% of body mass) that has been suggested for lactating females (Bengtson 1983). Juvenile manatees would need to consume more than 9% of their body mass per day on a seagrass diet to meet their estimated energetic demands, although they could balance needs on a lettuce diet at a lower intake rate (table 6).

When manatees are exposed to water temperatures below the lower critical limit of the thermoneutral zone ( $20^\circ\text{C}$ ; Irvine 1983; Worthy et al. 2000), metabolic rate can increase by more than 50% (Worthy et al. 2000; G. A. J. Worthy and T. A. M. Worthy, unpublished data) and animals would need to increase consumption rates (table 6). A 1,000-kg manatee, under these conditions, could meet requirements by consuming  $124.7 \text{ kg d}^{-1}$  (WM) of *Syringodium* (12.5% of its body mass) or  $54.7 \text{ kg}$

$\text{d}^{-1}$  (WM) of *Thalassia*, and a 250-kg juvenile manatee would need to consume  $88 \text{ kg d}^{-1}$  (WM) of *Syringodium* (table 6), or 35% of body mass. This latter consumption rate is well above the theoretical maximum rate and is clearly not feasible.

Estimates of consumption rates are critical considerations when assessing the carrying capacity of a region. The implication of low digestive efficiency means that manatees eating seagrasses require a greater standing biomass to support their needs than would be calculated using the higher  $D_m$  that manatees have when eating either freshwater vegetation or lettuce (Lomolino and Ewel 1984; this study). It also suggests that if manatee metabolic requirements increase dramatically due to cold water temperatures, it may be impossible for them to meet daily energy needs by eating seagrass alone. The results of this study have significant ramifications for both government agencies and caregivers who need to take digestive efficiencies into consideration when planning for the future of manatee conservation. Given the large quantities of seagrass required to sustain manatees, especially during cold weather, it is critical to implement habitat conservation and protection before considering downlisting or delisting manatees as an endangered species (USFWS 2001).

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