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RESEARCH ARTICLE

Digesta Passage Rates in the Florida Manatee (*Trichechus manatus latirostris*)

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The Florida manatee, *Trichechus manatus latirostris* (Sirenia: Trichechidae), is an herbivorous marine mammal found within coastal areas throughout the state of Florida, which feeds on both fresh and salt water sea grasses. Manatees, like other Sirenians, are a tropical species with little tolerance for water temperatures below 20°C, rely on a relatively poor nutritional food source, and have a low metabolic rate. Although manatees are hindgut fermenting herbivores, they are very efficient at extracting nutrients from the plants on which they feed. Slow passage rates of digesta have been suggested to be a factor in this increased efficiency. Two studies monitored the digesta passage times and mixing of particulate digesta within the manatee digestive tract using MicroGrits colored corncob grit as a fecal marker. Fecal samples were collected subsequently from four manatees in Study 1 and 3 manatees in Study 2, grit pieces removed, counted, and measured. The digesta passage times ranged from 6 and 10 days in Study 1, and 4.3 and 8.3 days in Study 2, supporting data presented in previous studies. When two different colored markers were administered on sequential days, minimal to no mixing was seen in recovered feces, suggesting that the digesta from a given day traveled through the tract as a bolus. Less than 1% of the marker fed was recovered and we

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hypothesize that perpendicular folds of the large intestine may be the major contributing factor, with pieces being retained and eventually digested. Zoo Biol 26:503–515, 2007.

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INTRODUCTION

The Florida manatee (*Trichechus manatus latirostris*) is a subspecies of the West Indian manatee, within the order Sirenia. This endangered species, along with the remaining extant Sirenia, has evolved to inhabit a tropical distribution in both fresh and/or salt coastal waterways [Owen, 1855; Bertram and Bertram, 1973; Domning, 1982; Savage et al., 1994]. Unlike other marine mammals, manatees and dugongs (*Dugong dugon*) have a relatively low metabolic rate and are not adapted to cold water temperatures [Scholander and Irving, 1941; Irvine, 1983].

The Sirenia represent the only marine mammals that are obligate herbivores. Florida manatees are generalist hindgut fermenting herbivores, spending 6–8 hr a day feeding on numerous species of aquatic plants, in both fresh and salt water [Best, 1981]. As nonruminants, manatees have an enlarged hindgut, but with some rarely seen specialized adaptations such as "a discrete cardiac gland, submucosal mucous glands along the greater curvature of the stomach, and unkeratinized, stratified squamous epithelial cells overlying the glandular mucosae of the pyloric antrum, midgut cecum, colon, and rectum" [Reynolds and Rommel, 1996]. Other hindgut fermenting herbivores, such as the horse (*Equus caballus*), have evolved a strategy of feeding on large amounts of relatively low-quality feed and maintaining a faster but less efficient digestive rate. By comparison, many large ruminants have a slower, more efficient digestive strategy preventing them from thriving on low-quality feed in a similar manner [Janis, 1976; Reynolds and Rommel, 1996]. Manatees are unusually efficient in their extraction of nutrients, particularly cellulose, from the aquatic plants on which they feed, compared to another hindgut herbivore, the horse [Burn, 1986]. Longer passage rates have been correlated with greater efficiency of cellulose digestion [Parra, 1978]. Characteristics of the manatee large intestine anatomy, where most cellulolysis occurs, are consistent with slower rates of digesta passage. These include its immense size (complete tract and contents, up to 23% of total body weight, 40 m or more in length) and numerous ridges perpendicular to the flow of digesta [Reynolds, 1980; Burn, 1986; Reynolds and Rommel, 1996]. However, some evidence indicates shorter retention rates for digesta in the stomach and the relatively long small intestine. For example, both organs generally contain little plant material, digesta in the stomach is enveloped in a thick coat of mucus which would minimize nutrient absorption, and rather than having a predominance of absorptive cells, goblet cells are the dominant cell type in the manatee small intestine [Burn, 1986; Reynolds and Rommel, 1996].

Measurement of the passage rate for food from the time of ingestion until it is defecated can be informative with respect to gastrointestinal function as well as to indicate time delays in hormone concentrations when comparing values from serum and fecal samples. Utilization of fecal markers has been well established in digestive studies looking at the passage rates of digesta and fecal composition [Warner, 1981;

Van Soest, 1982]. Both liquid and solid phase markers are available. Previous studies reported the digesta passage rates of manatees by changing the diet of a single captive manatee from lettuce (*Lactuca lactuca*) to water hyacinth (*Eichhornia crassipes*) and noting the color change of the feces [Lomolino and Ewel, 1984]. Lomolino and Ewel [1984] indicated a retention time of 146 hr (~6 days). Another unpublished study cited within Best [1981] also found retention rates of 120–140 hr (~5–6 days), however, these times seemed excessive in comparison to most other large herbivorous species and have been brought into question [Burn, 1986; see Table 1]. Horses, which are similar to manatees in using hindgut fermentation, have a mean gut retention time ranging from 28 to 38 hr depending on the diet fed. Ruminants such as cattle (*Bos taurus*) or buffalo (*Bubalus bubalis*) have mean retention times that range 68.8 ± 28.2 hr SD and 94.8 ± 3.3 hr SD, respectively. In fact, even elephants (*Loxodonta africana* and *Elephas maximus*) have a mean retention time between 21 and 46 hr [Warner, 1981; Rees, 1982]. In contrast, a study of digestive passage rates in two captive dugongs (*Dugong dugon*), a related Sirenian, found mouth-to-anus retention times of 146–166 hr (~6–7 days) [Lanyon and Marsh, 1995], which would corroborate the previous Florida manatee data [Best, 1981; Lomolino and Ewel, 1984].

We conducted two studies to further clarify the passage rate of digesta in the Florida manatee gastrointestinal tract. The first study describes two parameters of digesta passage rates: transit time, measuring the time from first feeding of a fecal marker through the gut to its first identification in the fecal samples; and retention time, the mean time for the marker in the gut to clear the digestive tract once feeding of a particular color ended [Warner, 1981; Van Soest, 1994; Stevens and Hume, 1995]. The second study looked at transit and retention, but also focused on how the digesta travels through the gut. This study looked more closely at the fecal marker pieces that traversed the gut, the percentage of fecal marker recovery, and extent to which mixing of different colored markers fed at different times would occur. One possibility is that the digesta spreads out within the gut by retention in the colonic ridges lining the colon [Reynolds, 1980] to such an extent that food ingested one day would mix with food retained from the previous day. Another possibility is that digesta travels through the gut more or less as a bolus or linear flow, is slowed by the cecum and colonic ridges, but minimal mixing takes place between meals from different days. We hypothesized that digesta travels as a linear flow.

METHODS

Animals

The animals involved in both studies were housed at Miami Seaquarium, Florida. General information such as age, length, and weight during each study is included in Table 2. Study 1 included two males, Romeo and Newton, and two females, Phoenix and Juliet. Study 2 conducted 2 years later included three females, Juliet, Millie, and Phoenix. At the time of the studies, Romeo and Juliet were older animals, >50 years of age, Newton and Phoenix were both younger, 6–10 years of age, but still considered adults, and Millie, at 2 years old during Study 2, was considered a weaned, large calf/subadult. Males and females are housed in separate tanks and in both male and female tanks additional animals not included in the

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TABLE 1. A comparison of mean digestive marker retention times

Species	Body mass (kg)	Diet	Marker method	Mean retention time (hr)	Reference
Foregut fermenters					
Cow (*Bos Taurus*)	220 & 610; 509	Grass hay; alfalfa hay and maize silage	Chromium-mordanted timothy *Phleum pretense*; Cr-mordanted hay	55 & 66; 64.5	[Udén et al., 1982; Bartocci et al., 1997]
Sheep (*Ovis aries*)	49.5; 70	Pelleted alfalfa hay plus oats; alfalfa hay and maize silage	^{103}Ru-phen; Cr-mordanted hay	50; 58.4	[Faichney and White, 1988; Bartocci et al., 1997]
Sloth (*Bradypus variegates*); *Bradypus tridactylus*)	4–8.5; 1.2–3.9	Leaves, twigs, fruit; foliage (*Cecropia palmate*)	Glass beads; $YbCl_3$, LaCl3, SmCl3, Cr-EDTA, Co-EDTA, and Fe-EDTA	200–300; 147–152	[Montgomery and Sunquist, 1978; Foley et al., 1995]
Eastern grey kangaroo (*Macropus giganteus*); Red kangaroo (*Macropus rufus*)	20.8; 26	Chopped alfalfa hay; Lucerne hay and oaten hay	^{103}Ru-phen; Cr-mordanted hay	30; 28.6 and 30.9	[Dellow, 1982; Munn and Dawson, 2006]
Hindgut fermenters					
Manatee (*Trichechus manatus*)	182–1136	Lettuce, plus some fruits and vegetables	Dyed corn	147	Present study
Dugong (*Dugong dugon*)	147 & 114	Seagrass *Syringodium isoetifolium*	Inert plastic beads	145–166	[Lanyon and Marsh, 1995]
Horse (*Equus caballus*)	210	Hay, oats	Cr-mordanted hay, Yb-marked oats	26–27	[Orton et al., 1985; Rosenfeld et al., 2006]
Elephant (*Loxodonta africana*); (*Elephas maximus*)	888–4013	Hay, straw; Hay, pelleted feed, oats, red clover, and beets	Beetroot; chromium oxide	21.4–46; 21.4–32.1	[Rees, 1982; Clauss et al., 2003]
Hyrax (*Procavia capensis*)	3.5	Alfalfa pellets	Chromic oxide	106	[Paul-Murphy et al., 1982]
Pig (*Sus scrofa*)	176; 45–50	Hay/grain; rice bran, maize, fish meal and soy bean meal	2 mm marker; Cr-mordanted grass and Co-EDTA	48; 22.2–38.9	[Warner, 1981; Dung et al., 2002]
Koala (*Phascolarctos cinereus*)	6.55	Eucalyptus foliage	^{103}Ru-phen	130	[Cork and Warner, 1983]
Rabbit (*Oryctolagus cuniculus*)	1.76	44% alfalfa meal, 33% rolled barley	Cr-mordanted Italian ryegrass	17	[Sakaguchi et al., 1992]

EDTA, ethylenediaminetetraacetic acid.

TABLE 2. Captive Florida manatee (*Trichechus manatus latirostris*) characteristics for each study

Name	Gender	Age (yr)	Length (m)	Weight (kg)
Study 1				
Juliet	Female	50	3.8	1136
Phoenix	Female	8	3.0	909
Newton	Male	7	–	409
Romeo	Male	43	3.2	818
Study 2				
Juliet	Female	52	3.8	1136
Millie	Female	2	1.7	182
Phoenix	Female	10	3.3	890

research were housed with the study animals. Animals were housed in brackish water tanks with water temperature kept constant at 31–32°C. The two pool sizes measured 11 m in diameter, holding 272.3 kl of water and 6 m in diameter, holding 92.7 kl of water. Animals were considered healthy throughout the duration of the study periods.

The composition of the manatee diet remained relatively constant throughout both studies, approximately 80% iceberg and romaine lettuce, 19% sweet potatoes, carrots, apples and bananas, and 1% monkey biscuit (Zupreem, http://www.zupreem.com/ or Purina/Mazuri, http://www.mazuri.com/). Manatees were fed the daily diet as a group in each tank, free choice. Lettuce was divided into morning and evening portions and the rest fed throughout the day at the discretion of the animal care staff. The total diet was fed at a rate of 8–10% of body weight per animal based on as-is weight of food.

All fecal samples collected in this study were covered under Endangered Species Permit PRT-79172, issued to US Geological Survey's Sirenia Project. The University of Florida project IACUC approval number to use animal samples was A164.

Fecal Marker and Collection

There is special concern in feeding endangered species fecal markers, especially in such a closely managed species as the Florida manatee, which falls under both the Endangered Species Act (1973) and Marine Mammal Protection Act (1972), plus is supported by the Florida Manatee Sanctuary Act (1978), and Florida Manatee Recovery Plan [USF&W, 2001]. All animals in captivity are part of the rescue, rehabilitation, and release program, so all individuals must be considered potential candidates for release back to the wild. Accommodating these concerns to estimate the relative digesta passage time, MicroGrits colored corncob grit (1420 grade, GreenTru Product, Micro Tracers Inc., San Francisco, CA) was used as a marker. Micro Tracers dyed the markers red, blue, orange, or purple using a Food and Drug Administration approved dye. The corncob grit, on average 1.09 ± 0.54 mm diameter, with a specific gravity between 1.2 and 1.4, is naturally digestible if retained within the digestive tract for an extended period of time, unlike plastic beads as highlighted by $\leq 14\%$ recovery in dugongs [Lanyon and Marsh, 1995]. This corncob grit marker was prepared according to the following formula: 2 L of water were heated in a beaker until boiling. To the water, 180 g of gelatin (Sigma, http://www.sigma-aldrich.com) were added slowly and mixed for 5 min. The mixture was then removed from the heat and 300 g of monkey biscuit powder, after grinding in a blender, were then added for flavor and stirred with a spoon. Four hundred and eighty grams of colored grits were added and stirred again. The mixture was spooned into nine ice cube trays and refrigerated overnight. In both studies, the single dose for each animal was one tray, consisting of 14 cubes equaling ~54 g of the marker.

For Study 1, the animals were already receiving the fecal marker as part of another research project. Thus, changing the color of the corn marker allowed an opportunity to determine how long it took for the new color to be observed in the fecal samples. The color was then changed back to the original, providing a second opportunity to measure the time for the color change to be observed in the feces collected. For Study 2, manatees were only fed fecal markers for 6 days. The animals ate one tray of red fecal marker each morning at 9:00 am for days 1–3. Subsequently, the animals were fed one tray of blue fecal marker each morning for days 4–6. For both studies, all fecal samples were collected daily, during normal park hours (9:00 am–6:00 pm), until the study color was no longer found in samples collected. As herbivores, manatees defecate often and the process may take several minutes so samples could be identified from specific individuals and be removed from the water with a pool net. Transit time was measured in days or hours from first feeding of the marker until first observation of the marker in fecal samples. Retention time was similarly measured as the mean from last feeding of a marker until last observation of that marker in fecal samples.

Experimental Design

Study 1: gut transit time collections

Four manatees, two females and two males (Table 2), were already receiving the colored corn to mark their fecal samples for another research project before the start of Study 1. Two individuals were receiving red marker and the other two receiving blue marker. With the start of Study 1, the marker fed to the animals was changed from red to orange and from blue to purple for each of the respective individuals. All identified fecal samples were collected throughout the day during normal park hours. Once the new color, orange or purple, was identified in the fecal samples, the marker fed to the animal was changed back to the original, red or blue. With this design we were able to measure the transit and retention times twice for each animal, one time with the original marker and a second with the new study color (Table 3).

Study 2: digesta mixing characteristics

To identify to what extent any mixing of digesta occurred over time, two colors, red and blue, of the corncob grit fecal marker were used in three female manatees (Table 2). On days 1–3 one tray per manatee of red corn was fed at 9:00 am and on days 4–6 the blue corn was similarly fed. All fecal samples were collected daily during the park day time hours (9:00 am–6:00 pm), and all MicroGrits found in the fecal samples were removed, counted, and measured. Digesta passage times were then calculated for each manatee.

TABLE 3. Study 1—digestive marker transit and retention times for captive Florida manatees (*Trichechus manatus latirostris*)

	Marker transit time[a]		Marker retention time[b]	
	Time (days)		Time (days)	
Manatee	New color	Old color	Old color	New color
Newton	7	7	6	8
Phoenix	8	9	8	10
Romeo	6	6	8	8
Juliet	7	6	8	8
Average	7.0 days (±1.1 STD)		8.0 days (±1.1 STD)	

Time Line: → Original 'Old' color (fed prior to Study 1 to mark fecals) → New color (fed for Experiment One) → Old color (fed to resume original fecal marking colors).
[a]Time, in days, it takes the fecal marker to get from first ingestion, to first be seen in the feces.
[b]Time, in days, it takes the fecal marker from last ingestion until last visible traces in feces.

A schedule of one feeding per day over 3 days for each color was used after a preliminary experiment found that manatees fed a single 1-day bolus of red marker (of the same formula), and a single bolus of blue marker the next day, failed to produce significant marker to count in the feces.

Descriptive statistics, linear regressions, and Mann–Whitney Rank Sum tests were run using SigmaStat for Windows Version 3.5, Build 3.5.0.54, Copyright© 2006 Systat Software, Inc.

RESULTS

Study 1

From the four animals monitored at Miami Seaquarium, the mean number of days for corncob grit to first be seen in the fecal sample after first feeding, or gut transit time, was 7 days ±1.1 standard deviation (STD), with a range of 6–9 days (Fig. 1). The mean number of days for corn marker to leave the digestive tract once feeding of that marker ended, or gut retention time, was 8 days ±1.1 STD, with a range of 6–10 days. There was no significant correlation of transit or retention time with age or weight of manatee, as assessed by linear regression.

Study 2

The digesta transit times for the three adult manatees in this study ranged between 103 and 149 hr (4.3–6.2 days) and averaged 130 hr ±22 STD (5.4 days) (Table 4). The digesta retention times ranged from 135 to 191 hr (5.6–8.0 days) and averaged 160 hr ±21 STD (6.7 days). After 178 hr since its first administration, no blue marker was identified in samples collected from Phoenix. Times of defecation that occurred during the night were unknown. It is possible we missed the samples that may have contained blue marker from Phoenix at night. During the 140 hr, from the first collection of feces containing marker until no marker was present, there was

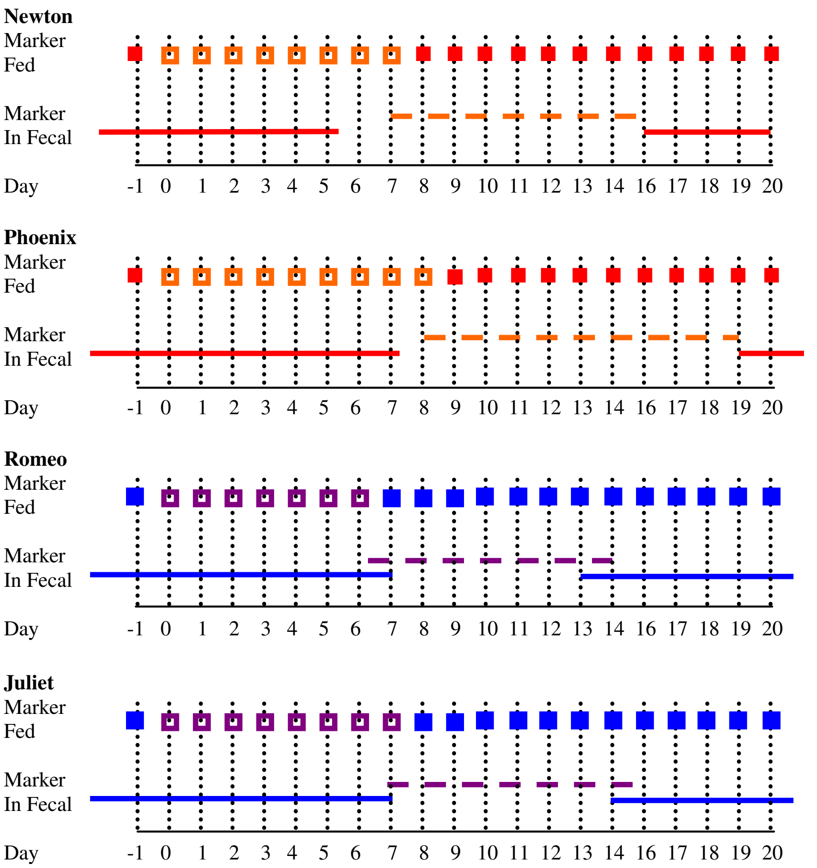

Fig. 1. Fecal marker feeding schedule and timeline of marker recovered in fecal samples for Study 1. Boxes represent the color marker fed to a manatee on a given morning. Lines represent the type of marker recovered in fecal samples on a given day. Solid squares and lines represent the original color given to the animal before and after the study. Open squares and dashed lines represent the new color used in this study to measure transit and retention times.

TABLE 4. Study 2—marker recovery and times for transit and retention in captive Florida manatees (*Trichechus manatus latirostris*)

Manatee	Marker color	Estimated no. of particles ingested	Number/percent of particles recovered	Transit time in hours/days	Retention time in hours/days
Juliet	Red	200,000	1183/0.59	103/4.3	172/7.2
	Blue	200,000	107/0.05	146/6.1[a]	152/6.3
Millie	Red	200,000	176/0.09	144/6.0	153/6.4
	Blue	200,000	915/0.46	109/4.5	135/5.6
Phoenix	Red	200,000	201/0.10	149/6.2	191/8.0
	Blue	200,000	0/0	–	–
		Mean values		130/5.4	160/6.7

[a]A single piece of blue corn was collected at 76 hr.

vast variation in the amount of times a manatee defecated during the daytime hours, between 6 and 15 times, and the animals had no regular pattern of defecation. Less than 1% of the corn grits fed to each of the animals was recovered. An estimated one-eighth of the mixture was lost to the water while feeding the manatees.

The number of grit pieces counted per fecal sample was plotted cumulatively against hours after administration for each manatee (Fig. 2). Mixing of the corn marker varied with each animal. Juliet showed some mixing over a 5-hr period,

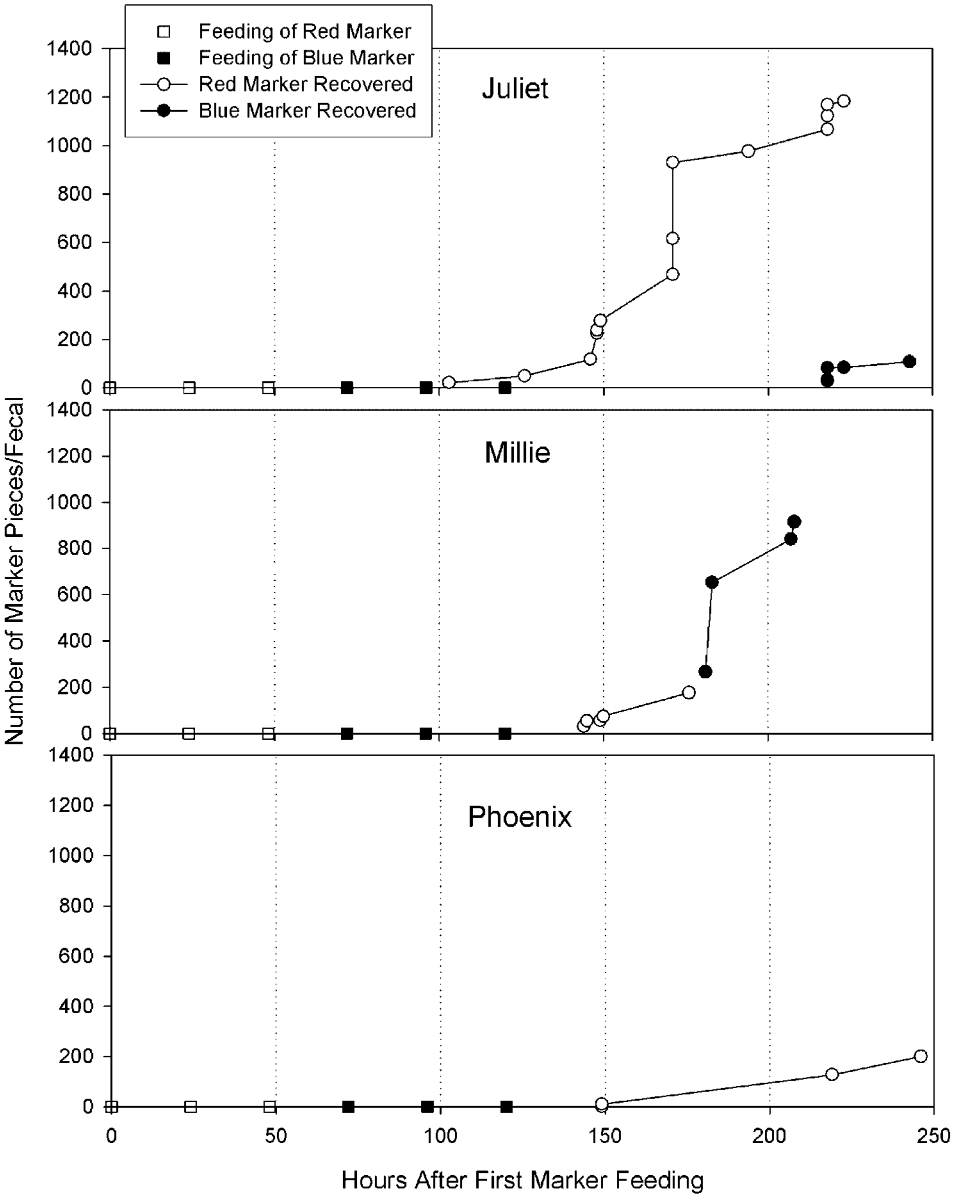

Fig. 2. Number of grit pieces counted per fecal sample plotted cumulatively against hours after administration for each manatee in Study 2.

between 218 and 223 hr. Millie showed no mixing, with the sample collected at 176 hr containing only red marker and the next sample at 181 hr (109 hr after administration of the blue marker) contained only blue marker. Phoenix passed no blue marker for the duration of the study.

The size of marker pieces was measured and plotted from each sample collected (Fig. 3). The range of marker pieces was similar across each of the animals and did

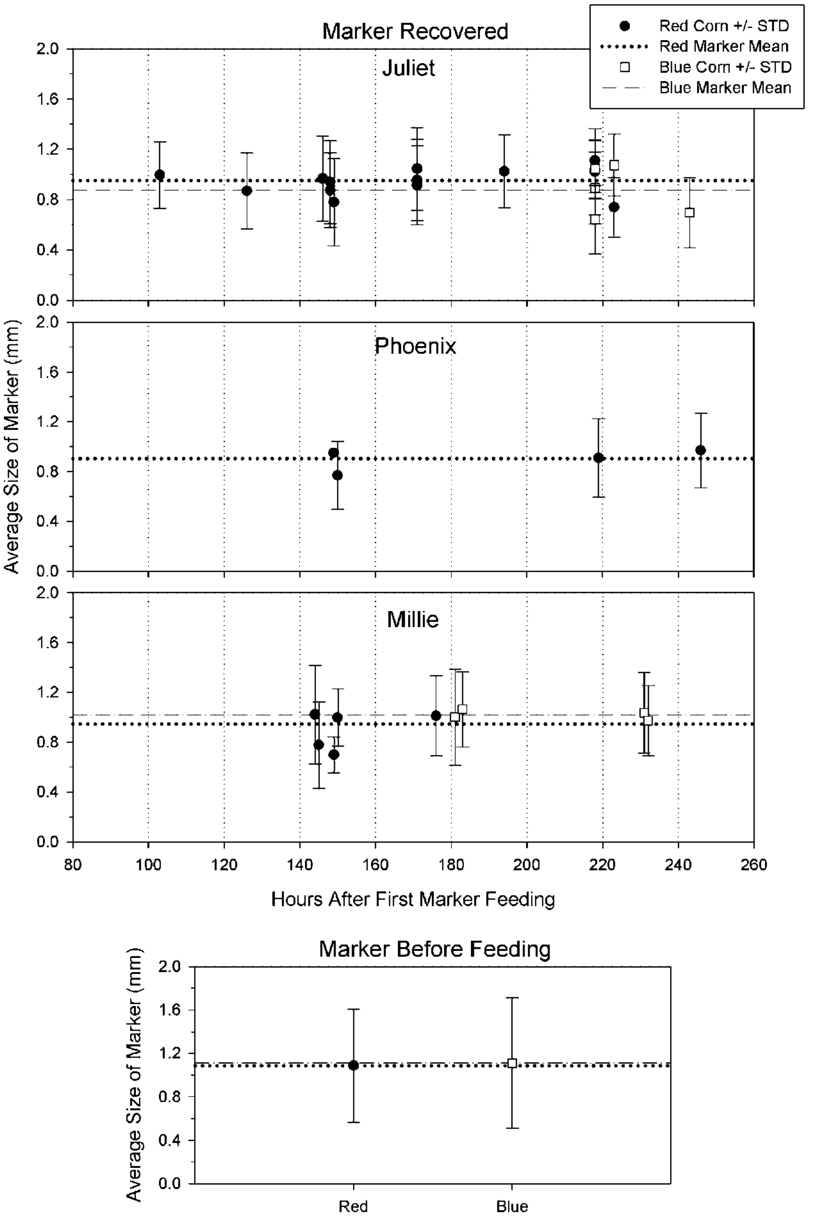

Fig. 3. Marker size measured before feeding and after recovered from fecal samples in Study 2.

not vary over time. The average of all marker pieces measured from all fecal samples collected (0.948 mm $\pm$0.316 STD) was significantly smaller ($P = 0.037$) than the average of marker pieces measured before feeding (1.099 mm $\pm$ 0.557) using a Mann–Whitney Rank Sum test.

DISCUSSION

The relatively slow retention rate averaging 5.4–7 days across both studies measured in Florida manatees confirms the data presented by earlier studies [Best, 1981; Lomolino and Ewel, 1984] and is congruous with the immense size of the manatee large intestine [Reynolds and Rommel, 1996], low metabolic rate (17–22% less than predicted across eutherian values) [Irvine, 1983], plus the efficient breakdown of fibrous plant material, comparable to ruminants [Burn, 1986]. On a primarily lettuce-based diet, the digesta mean retention times for particulate matter to pass through the digestive tract are very slow compared to other species such as foregut fermenting herbivores (ruminants), carnivores, and other much larger hindgut fermenting herbivores (nonruminants), such as the elephant (order *Proboscidea*). Table 1 provides a comparison across some species that are more commonly studied, a few that are specialists, and those that are most closely related to manatees. Species that are within the same range of mean retention times as manatees are highly specialized and include the koala (*Phascolarctos cinereus*) [Cork and Warner, 1983] and sloths (the three-toed sloth, *Bradypus tridactylus* and the two-toed sloth, *Choleopus didactylus*) [Foley et al., 1995; Montgomery and Sunquist, 1978; Warner, 1981]. The digesta transit times are similar to another Sirenian, the dugong, when fed the seagrass, *Syringodium isoetifolium* [Lanyon and Marsh, 1995]. The hyrax (*Procavia capensis*—Order Hyracoidea), which is evolutionarily related, within the Paenungulata clade, to manatees and elephants [Kellogg et al., 2007], also has a long mean retention time relative to its size, 106 hr [Paul-Murphy et al., 1982], especially when compared to a similarly sized rabbit, 17 hr [*Oryctolagus cuniculus*; Sakaguchi et al., 1992].

Although use of the MicroGrits avoided some managerial concerns of feeding nondigestible beads or liquid markers with heavy metals or other chemicals, this technique has limitations when used to measure digesta retention time. Grits retained in the gut for extended periods of time were digested and this was the majority of marker fed with $<1\%$ recovered in Study 2. The colonic ridges oriented perpendicular to the flow of digesta [Reynolds, 1980; Reynolds and Rommel, 1996] would be prime areas for small particles to be held up until broken down. However, a low recovery of marker ($\leq 14\%$) was also seen in dugongs given plastic beads to mark digesta transit [Lanyon and Marsh, 1995]. Coprophagy and loss of feces and marker to the filtration system were listed as possible mechanisms of marker loss and would certainly be applicable to our study as well. Subsequent studies would need to devise some methods to overcome these limitations and also should consider liquid markers. It should also be noted that the diet of manatees in captivity, fed primarily lettuce and supplemented with sweet potatoes, carrots, apples, bananas, and monkey biscuits, can differ significantly in fiber, nutrients, calories, protein, and water content compared to a wild diet of fresh and marine aquatic vegetation depending on season and location [Dawes and Lawrence, 1980; Best, 1981]. Manatees are generalist herbivores and are known to feed on over 60 species of aquatic plants, plus grasses and leaves when tides are high enough [Best, 1981; Hartman, 1979]. Manatees seem to be strategic in their feeding patterns, traveling long distances to reach certain sea grass beds when local, more abundant freshwater species may be available. These behaviors are thought to be influenced by nutrients and taste, however much of this aspect still needs further study [Reep and Bonde, 2006].

Despite the above mentioned limitations, the transit data should still reflect a realistic time line for the shortest mouth to anus transit for a particulate marker, and at a mean of 7 (Study 1) and 5.4 days (Study 2) this still highlights the incredible specialization with which manatees digest vegetation. The use of two different colored markers indicated that there was limited mixing of digesta in large volumes within the gut, thus suggesting that digesta travels through the tract as a linear flow in a similar manner as dugongs [Lanyon and Marsh, 1995].

These data are useful for estimating time-lines when measuring hormone concentrations noninvasively from fecal samples, as is commonly done now in many zoological facilities. Fecal assays can be used to monitor steroid hormones correlated with stress and reproductive cycles in species that are difficult to handle for various reasons. The analysis of study results indicates that steroid hormones measured from fecal samples should exhibit similar patterns of concentration as found in urine and blood samples, although shifted approximately 5–7 days, depending on individual variation.

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