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Carbon and nitrogen stable isotope turnover rates and diet–tissue discrimination in Florida manatees (*Trichechus manatus latirostris*)

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SUMMARY

The Florida manatee (*Trichechus manatus latirostris*) is a herbivorous marine mammal that occupies freshwater, estuarine and marine habitats. Despite being considered endangered, relatively little is known about its feeding ecology. The present study expands on previous work on manatee feeding ecology by providing critical baseline parameters for accurate isotopic data interpretation. Stable carbon and nitrogen isotope ratios were examined over a period of more than 1 year in the epidermis of rescued Florida manatees that were transitioning from a diet of aquatic forage to terrestrial forage (lettuce). The mean half-life for ^{13}C turnover was 53 and 59 days for skin from manatees rescued from coastal and riverine regions, respectively. The mean half-life for ^{15}N turnover was 27 and 58 days, respectively. Because of these slow turnover rates, carbon and nitrogen stable isotope analysis in manatee epidermis is useful in summarizing average dietary intake over a long period of time rather than assessing recent diet. In addition to turnover rate, a diet–tissue discrimination value of 2.8‰ for ^{13}C was calculated for long-term captive manatees on a lettuce diet. Determining both turnover rate and diet–tissue discrimination is essential in order to accurately interpret stable isotope data.

Key words: turnover, stable isotope, Florida manatee, diet–tissue discrimination, ^{13}C, ^{15}N, *Trichechus manatus*, feeding ecology.

INTRODUCTION

The isotopic composition of consumer tissues reflects that of local food webs and can be used to predict diet composition, the trophic level at which the consumer is feeding, and even habitat use and migratory patterns (e.g. Deniro and Epstein, 1978; Deniro and Epstein, 1981; Fry, 1981; Peterson and Fry, 1987) (reviewed by Hobson, 1999). Two stable isotope ratios commonly analyzed in feeding ecology studies are those of carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N). Carbon isotope ratios indicate the likely source of primary production and have been used to differentiate between C_3 and C_4 plants, terrestrial and marine ecosystems, deep forest and open habitat consumers, and benthic and pelagic aquatic systems (e.g. Cloern et al., 2002; Cerling et al., 2004; Hall-Aspland et al., 2005). Nitrogen isotope ratios exhibit a predictable, step-wise enrichment between trophic levels and also have been shown to differ between terrestrial and marine ecosystems (e.g. Hobson and Welch, 1992).

Stable isotope analysis is especially advantageous when investigating the feeding ecology and habitat use of marine mammals where it is often impossible to directly observe feeding or migratory behavior. Tissue samples, such as skin or blubber, may be analyzed for stable isotope ratios without sacrificing the animal and this approach has been successfully applied to a variety of marine mammal species including mysticetes (e.g. Lee et al., 2005), odontocetes (e.g. Walker et al., 1999), pinnipeds (e.g. Hobson and Welch, 1992; Kurle and Worthy, 2002; Newsome et al., 2006), sirenians (e.g. Ames et al., 1996; MacFadden et al., 2004; Yamamuro et al., 2004; Reich and Worthy, 2006), sea otters (Clementz and Koch, 2001) and polar bears (e.g. Ramsay and Hobson, 1991).

In order to accurately interpret isotopic results, it is imperative to determine both isotopic discrimination (the difference in isotopic ratios between consumer tissue and diet) and turnover rate (the time it takes for the isotope to be assimilated into the consumer's tissue) of the sampled tissue. Diet–tissue discrimination may be difficult to determine for animals feeding on multiple, isotopically distinct prey items for which the proportions contributing to the diet are unknown. However, controlled captive studies on a variety of taxa have allowed for more precise measurements (e.g. Roth and Hobson, 2000; Cherel et al., 2005; Logan et al., 2006; Seminoff et al., 2006). In addition to diet–tissue discrimination, turnover rates in tissues must be determined in order to assess whether the isotope signature of the tissue represents the most recent diet or the long-term diet. An effective method to determine turnover rate is to experimentally switch an animal from one known diet to another isotopically distinct diet. Turnover rates of stable isotopes have been calculated using this method for mammals (e.g. Tieszen et al., 1983), birds (e.g. Hobson and Clark, 1992a), fish (e.g. Bosley et al., 2002) and invertebrates (e.g. Olive et al., 2003). These studies have shown that tissues with higher metabolic activity (e.g. blood, liver) have faster turnover rates than less active tissue (e.g. bone).

The endangered Florida manatee (*Trichechus manatus latirostris* L.) is known to feed on a variety of aquatic plants in fresh, estuarine and marine habitats (e.g. Campbell and Irvine, 1977; Hartman, 1979; Best, 1981), each of which has a distinct isotopic signature (e.g. Fry and Sherr, 1984; Reich and Worthy, 2006) (C.D.A.-S. and G.A.J.W., in preparation). Little is known about fine-scale manatee feeding ecology and habitat use because manatees often occupy shallow, turbid water. In addition, manatee population counts and trends remain unclear (Lefebvre et al., 1995; US Fish and Wildlife Service, 2001). Consequently, it has become increasingly important to understand manatee feeding ecology and its relation to habitat use in order to improve conservation efforts.

Table 1. Rescued manatees sampled during rehabilitation

Animal ID	Sex	Mass (kg)	Length (cm)	Age class	Rescue site	Reason for rescue
SWF Tm 0301	M	105	174	Juvenile	Cape Canaveral	Cold stress
SWF Tm 0318	M	273	255	Subadult	Cape Canaveral	Watercraft injury
SWF Tm 0322	F	305	248	Subadult	Cape Canaveral	Watercraft injury
SWF Tm 0334	F	593	298	Adult	Jacksonville	Entanglement
SWF Tm 0340	M	232	222	Subadult	Jacksonville	Cold stress
SWF Tm 0341	F	209	208	Subadult	Jacksonville	Cold stress
SWF Tm 0431	F	125	90	Juvenile	Naples	Unknown
SWF Tm 0501	F	177	216	Subadult	Jacksonville	Cold stress

The present study used skin samples from Florida manatees transitioning between two isotopically distinct diets (aquatic to terrestrial) to determine turnover rates and diet–tissue discrimination in epidermis tissue. Manatees were part of the rehabilitation program at SeaWorld of Florida, and were in need of captive care for reasons including physical trauma, nutritional stress and/or cold stress. The overall objectives of the present study were to determine ^{13}C and ^{15}N turnover rates in epidermis tissue and to calculate diet–tissue discrimination values for carbon and nitrogen stable isotopes in manatee skin.

MATERIALS AND METHODS

Sample collection

Manatees held long-term at SeaWorld of Florida (Orlando, FL, USA) were fed a diet consisting primarily of romaine lettuce (>90% by mass) with minimal amounts of other terrestrial vegetation (<10%) including spinach, carrots and cabbage, with monkey chow biscuits (Mazuri; www.mazuri.com) given as an occasional protein supplement. Rescued manatees, temporarily held at SeaWorld for rehabilitation, were similarly fed romaine lettuce with minimal amounts of spinach. Initially, after rescue, this second group of manatees were fed a gruel mixture consisting of romaine lettuce, spinach, water and monkey chow (P. L. Ramos-Navarrete, personal communication). Samples of all food items fed to these manatees were collected during 2003 and 2004 (N=35). Manatees are known to engage in coprophagy (Hartman, 1979) and therefore fecal materials from captive manatees were also collected and analyzed for isotope ratios (N=4) for comparison with the fed diet items.

Skin samples were collected from eight rescued manatees, being held temporarily in captivity for rehabilitation (Table 1) and nine manatees held long-term for periods greater than 1 year (Table 2). Skin samples from long-term captive animals were used to calculate diet–tissue discrimination and to determine isotopic values in manatee skin that were representative of a captive diet. To determine turnover rates, samples were obtained opportunistically from rescued animals at various time intervals as they transitioned from wild forage to a captive diet. Carbon and nitrogen stable isotope turnover rates in skin were calculated for manatees rescued from two habitats in Florida: 'coastal' (Naples on the Gulf coast and Cape Canaveral on the central east coast) and 'riverine' or fresh water (St Johns River near Jacksonville; Fig. 1). The terms 'coastal' and 'riverine' represent rescue locations only, and are not intended to describe overall habitat use.

Table 2. Long-term captive manatees

Animal ID/name	Sex	Mass (kg)	Length (cm)	Age class	Days on lettuce diet
Bo	M	Unknown	>176	Subadult–adult	512
Charlotte	F	1136	330	Adult	>365
Primo	F	494	277	Adult	>365
Rita	F	>900	>275	Adult	>365
Sarah	F	1136	325	Adult	>365
Stubby	F	823	252*	Subadult–adult	>365
SWF Tm 0110	F	367	255	Subadult	1231
SWF Tm 0302	F	Unknown	>176	Subadult–adult	512
SWF Tm 0338	F	Unknown	>176	Subadult–adult	429

*Missing large portion of paddle.

Biopsies of epidermal tissue were collected from the trailing edge of the paddle using either a scalpel or ronguers (samples were approximately 10 mm×5 mm, full depth of the paddle). Partially sloughed epidermis was collected directly off individual manatees (approximately 2 cm×2 cm) if biopsies were not available. Body mass and sex were determined, and length measurements taken, where body length was measured as the straight distance from snout to paddle (O'Shea et al., 1985). Manatees were categorized into three age classes based on body length measurements (adults >275 cm, subadults/late juveniles 176–275 cm, and calves <176 cm; Tables 1 and 2).

Sample preparation and analysis

All manatee tissue and food items were frozen within 2 h of collection and held at –20°C until time of analysis. Samples were initially rinsed with distilled water and oven dried at 60°C for 24 h (fecal samples were freeze dried) to remove water. Lipids were removed using petroleum ether in a Soxhlet extractor for 24 h to eliminate the effect of lipids on carbon isotope ratios (Rau et al., 1992). Samples were then oven dried at 60°C for 24 h to remove any remaining solvent. Samples were ground and homogenized using either an 8000 Mixer/Mill (SPEX CertiPrep, Metuchen, NJ, USA) or Wig-L-Bug Amalgamator (Crescent Dental Manufacturing, Chicago, IL, USA), or were chopped by hand using a scalpel. Approximately 1.0 mg of manatee tissue or 2.5 mg of food items was transferred to 5 mm×9 mm tin capsules and analyzed by mass spectrometry (Thermo Finnigan DELTAplus and DELTA C, Bremen, Germany) for carbon and nitrogen isotopes at the Stable Isotope and Ecology Lab, University of Georgia, Athens, GA, USA. Analytical precision for both carbon and nitrogen was 0.10‰.

Stable isotope ratios were expressed in p.p.t. (‰) using delta notation:

$$\delta X = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000 , \quad (1)$$

in which R_{sample} and R_{standard} are the absolute isotope ratios of the sample and standard, respectively, X is ^{13}C or ^{15}N, and the standards are PeeDee belemnite (Craig, 1957) and atmospheric N_2, respectively.

Turnover rate was calculated using the exponential model of Hobson and Clark (Hobson and Clark, 1992a):

$$y = a + be^{ct} , \quad (2)$$

in which y is δX, a is the value approached asymptotically, b is the total change in value after diet switch, c is turnover rate, and t is

Fig. 1. Rescue locations of manatees in Florida.

time (days) since diet switch. Turnover rate was expressed in terms of half-life, the time it takes for the isotopic composition of the tissue to reach a midpoint between the initial and final values:

$$X = (\ln 0.5) / c\,, \quad (3)$$

In order to better fit turnover data to the exponential model, an 'anchor point' based on the mean stable isotope ratio ($\delta^{13}C$=–24.4±0.6‰, $\delta^{15}N$=2.7±0.5‰, means ± s.e.) of skin samples from nine long-term captive manatees at SeaWorld Of Florida was set at 600 days. These animals had been fed a diet of mainly lettuce for multiple years. The position of the anchor point at 600 days was chosen for several reasons: it was beyond the maximum sampling time for all rescued manatees (no manatee was sampled later than 418 days), plots generally reached an asymptote at or before this point, and positions greater than 600 days did not alter results. Goodness of fit was first expressed by calculating the coefficient of determination (R^2) using the anchor point as part of the data set. To further illustrate fit, data for skin from each rescued manatee were paired with each individual data point contributing to the mean anchor point and minimum and maximum R^2 values were computed.

All statistical analyses were judged to be significant at P<0.05. Data were tested for normality using the Shapiro–Wilk test. Levene's F and Box's M were used to test homogeneity of variance between factors and homogeneity of covariance, respectively. Differences in $\delta^{13}C$ and $\delta^{15}N$ values were tested using parametric and non-parametric analyses as appropriate. Data are presented as means with standard error.

RESULTS

Diet and enrichment

Stable isotope ratios were significantly different between diet items (MANOVA: Wilks' lambda, $F_{8,50}$=16.79, P<0.001). Specifically, $\delta^{13}C$ values differed (ANOVA: $F_{4,26}$=75.60, P<0.001), but $\delta^{15}N$ values did not ($F_{4,26}$=0.35, P=0.84). Gruel and monkey chow were both significantly enriched in ^{13}C compared with romaine lettuce, spinach and fecal samples (all P<0.01, Tukey HSD; Table 3).

Skin from long-term captive manatees held at SeaWorld of Florida (mean $\delta^{13}C$=–24.4±0.6‰) was significantly enriched in ^{13}C compared with the major diet components (romaine lettuce and spinach) by an average of 2.8±0.9‰ (t-test: t=10.41, d.f.=26, P<0.001). $\delta^{15}N$ values did not differ between manatee skin (mean=2.7±0.5‰) and the diet (t=0.09, d.f.=21, P=0.93; Table 3).

Turnover rates

Isotopic ratios in biopsy samples and sloughed skin from the same rehabilitated manatees were compared to determine the effect of differing collection methods. $\delta^{13}C$ values did not differ significantly between biopsy and sloughed samples (paired t-test: t=0.15, d.f.=8, P=0.89). However, sloughed samples were significantly enriched in ^{15}N compared with biopsy samples (mean enrichment=1.3±0.9‰, t=4.32, d.f.=8, P=0.003). To account for this difference, all $\delta^{15}N$ values for sloughed samples were adjusted by the mean enrichment value.

Coastal manatees

Skin samples from coastal manatees were greatly enriched in ^{13}C (calculated at day 0) relative to those of long-term captive manatees (mean enrichment=13.8±1.2‰). $\delta^{13}C$ values from four coastal manatees were fitted to the exponential decay model and carbon turnover half-lives in skin ranged from 42 to 63 days with a mean of 53±11 days (Fig. 2; Table 4).

Skin samples from these coastal manatees were only slightly enriched in ^{15}N relative to those of captive manatees (mean enrichment=3.2±1.0‰). Nitrogen half-lives in skin from coastal manatees ranged from 14 to 36 days with a mean of 27±10 days (Fig. 2; Table 4), significantly shorter than carbon half-lives (paired t-test: t=10.33, d.f.=3, P=0.002).

Riverine manatees

Skin from riverine manatees had carbon signatures (calculated at day 0) that were similar to those of captive manatees (mean enrichment=4.3±3.0‰). $\delta^{13}C$ values in the skin of four riverine

Table 3. Stable isotope ratios of diet items fed to captive manatees at SeaWorld of Florida

		$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Diet item	N	Mean ± s.e.	Minimum	Maximum	Mean ± s.e.	Minimum	Maximum
Romaine lettuce	16	–27.2±0.7	–28.8	–25.8	2.9±2.6	–0.1	8.9
Spinach	3	–27.2±0.5	–27.8	–26.7	1.8±2.1	–0.7	3.0
Monkey chow	4	–21.2±1.3	–23.0	–20.1	2.9±0.9	2.1	4.1
Gruel	4	–20.3±0.6	–21.0	–19.7	2.5±0.6	1.9	3.2
Fecal material	4	–27.9±1.6	–29.0	–25.6	3.7±1.5	2.2	5.2

Gruel was composed of romaine lettuce, spinach, monkey chow and water. Fecal material was analyzed because manatees are known to engage in coprophagy.

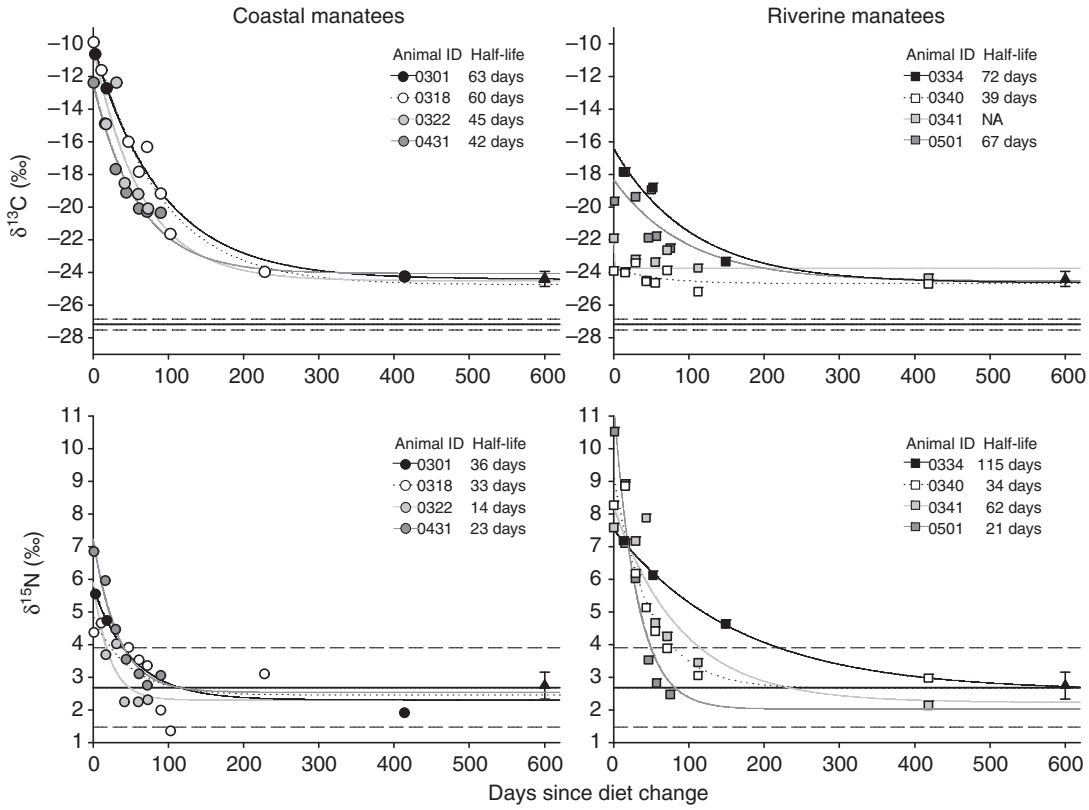

Fig. 2. ^{13}C and ^{15}N turnover in epidermis from manatees rescued near Naples and Cape Canaveral (coastal manatees) and from the St Johns River (riverine manatees) in Florida. Mean (±95% CI) stable isotope ratios for skin from long-term captive manatees (▲) were used as 'anchor points' set at 600 days to better fit the models. To illustrate diet–tissue discrimination, mean (±95% CI) stable isotope ratios for the main diet items fed in captivity (romaine lettuce and spinach) are indicated by a horizontal solid black line and horizontal dashed lines.

manatees were also fitted to the exponential decay model (Fig. 2). Carbon turnover half-lives in the skin of riverine manatees ranged from 39 to 72 days with a mean of 59±18 days; however, a half-life was not calculated for manatee 0341 because the equation showed little to no change in signature over time (Fig. 2; Table 4).

Skin samples from these riverine manatees were also enriched in ^{15}N relative to that of captive manatees (mean enrichment= 6.3±1.6‰) and nitrogen half-lives ranged from 21 to 115 days with a mean of 58±42 days (Fig. 2; Table 4). These turnover times were not significantly different from carbon half-lives (paired *t*-test, t=0.13, d.f.=2, P=0.91). MANOVA results indicated there were no significant differences in stable carbon or nitrogen isotope half-lives between manatees rescued from riverine *vs* coastal regions (*F*-test: $F_{2,4}$=0.58, P=0.60).

DISCUSSION

Carbon enrichment values calculated in the present study (2.8±0.9‰) were similar to values previously reported. Ames and colleagues (Ames et al., 1996) found sloughed skin from captive manatees to be enriched in ^{13}C by an average of 4.1‰ compared with lettuce. Reich and Worthy (Reich and Worthy, 2006) assumed a carbon enrichment value of 3.0‰ in manatee skin when applying an isotope mixing model (Phillips and Gregg, 2001) to diet interpretation of free-ranging manatees. The only other known study on diet–tissue discrimination in any mammalian skin is that of Hobson and colleagues (Hobson et al., 1996) in which seal skin was found to be enriched in ^{13}C relative to diet by 2.8‰. In the present study, nitrogen enrichment could not be determined because of the high degree of variability of nitrogen signatures in the lettuce diet. Typically, diet–tissue discrimination values for nitrogen are in the range 2–5‰ (Peterson and Fry, 1987; Kelly, 2000).

Manatees rescued from coastal regions were ideal subjects for carbon isotope turnover calculations because carbon signatures in their skin differed dramatically from those of captive manatees. Interpreting $\delta^{13}C$ values in the skin of riverine manatees was problematic because of variability in values at the time of rescue and the similarity of $\delta^{13}C$ values between the skin of rescued manatees and that of long-term captive manatees. The half-lives in skin from riverine manatees were not significantly different from those of skin from coastal manatees; however, the carbon turnover data for manatees rescued from riverine regions did not fit the exponential decay models as closely as those for coastal manatees.

The carbon half-life calculated for manatee epidermis was very slow compared with previous turnover studies on other species. Stable isotope turnover rates can differ based on the particular isotope, tissue and/or taxon analyzed, diet, physiological state, feeding rate and/or growth rate of the animal (e.g. Fry and Arnold, 1982; Bosley et al., 2002; Hobson and Bairlein, 2003; Olive et al., 2003). Additionally, some studies removed lipids from samples while others did not. Therefore, direct comparisons between studies are difficult. Dalerum and Angerbjorn (Dalerum and Angerbjorn, 2005) cautioned that comparisons of turnover rates should be made between the same tissues to avoid these complications. Additionally, turnover rates in tissues should be compared between animals of similar body size as metabolic rates have an effect on isotope turnover (Sponheimer et al., 2006).

Table 4. Exponential decay equations and half-lives representing stable isotope turnover in epidermis sampled from rehabilitated Florida manatees

Carbon turnover	Animal ID	Equation	R^2	Half-life (days)	$\delta^{13}C$ at day 0 (‰)	R^2 range Min.	R^2 range Max.
Coastal manatees	0301	$y=-24.4+14.2e^{-0.01094x}$	1.00	63	–10.2	1.00	1.00
	0318	$y=-24.8+15.1e^{-0.01158x}$	0.97	60	–9.7	0.96	0.97
	0322	$y=-24.5+14.3e^{-0.01550x}$	0.83	45	–10.2	0.79	0.84
	0431	$y=-24.1+11.6e^{-0.01657x}$	0.97	42	–12.5	0.97	0.97
			Mean	53	–10.7		
Riverine manatees	0334	$y=-24.6+8.2e^{-0.00963x}$	0.95	72	–16.4	0.90	0.96
	0340	$y=-24.7+0.9e^{-0.01787x}$	0.36	39	–23.8	0.05	0.51
	0341	$y=-23.8+1.9e^{-20920x}$	0.50	n.d.	–21.9	0.43	0.51
	0501	$y=-24.5+6.2e^{-0.01028x}$	0.70	67	–18.3	0.59	0.75
			Mean	59	–20.1		

Nitrogen turnover	Animal ID	Equation	R^2	Half-life (days)	$\delta^{15}N$ at day 0 (‰)	R^2 range Min.	R^2 range Max.
Coastal manatees	0301	$y=2.3+3.4e^{-0.01918x}$	0.96	36	5.7	0.83	1.00
	0318	$y=2.4+2.4e^{-0.02125x}$	0.57	33	4.8	0.46	0.62
	0322	$y=2.3+3.6e^{-0.04898x}$	0.56	14	5.9	0.29	0.68
	0431	$y=2.5+4.7e^{-0.03038x}$	0.97	23	7.2	0.91	0.97
			Mean	27	5.9		
Riverine manatees	0334	$y=2.6+4.9e^{-0.00601x}$	1.00	115	7.5	0.98	1.00
	0340	$y=2.7+6.4e^{-0.02055x}$	0.92	34	9.1	0.90	0.92
	0341	$y=2.3+5.9e^{-0.01125x}$	0.85	62	8.2	0.80	0.87
	0501	$y=2.0+9.4e^{-0.03273x}$	0.95	21	11.4	0.92	0.97
			Mean	58	9.1		

n.d., not determined (see text).

The present study is the first to calculate stable isotope turnover rates in the skin of any marine mammal species. Isotope turnover rates that have been reported for large terrestrial mammals including bears (Hilderbrand et al., 1996), alpacas (Sponheimer et al., 2006), and domestic cattle and horses (Schwertl et al., 2003; Ayliffe et al., 2004) were determined using blood, muscle and liver, and hair, respectively. As no appropriate comparison between turnover rates in the skin of large mammals was possible, the results from this study will be cautiously compared with others. Previous studies on stable isotope ratios in hair were omitted from this comparison as hair is a metabolically inert tissue in which the isotopic composition represents the period of growth.

The only reported carbon half-lives in animal tissue that are greater than those of manatee epidermis are those of alpaca muscle [179 days (Sponheimer et al., 2006)], bat wing membrane and whole blood [102–134 days (Voigt et al., 2003)], and quail bone collagen [173 days (Hobson and Clark, 1992a)]. In the alpaca study, muscle tissues were not lipid extracted so direct comparisons may be problematic. Voigt and colleagues (Voigt et al., 2003) suggested the slow turnover rate in bat wing membrane was largely due to the tissue being composed primarily of collagen and elastin, which are known to have slow turnover rates in bone. Additionally, Voigt and colleagues (Voigt et al., 2003) attributed the slow turnover rate in bat blood to their long-lived erythrocytes. Finally, the long half-life in quail bone aligns with collagen being a less metabolically active tissue. Epidermal tissue is composed of keratin in the epithelial lamina and collagen and elastin in the basal lamina. Manatee epidermis has been described as thick and possesses the characteristic of hyperkeratosis (Sokolov, 1982; Graham et al., 2003). It is possible that a slow replacement of keratin in manatee epidermis and the presence of collagen and elastin in the basal lamina also contributed to the slow isotope turnover rate in the skin.

Another potentially significant factor impacting on turnover rate is the manatees' overall slow metabolism. Metabolic rates in adult Florida manatees have been shown to be lower than those predicted based on body size [15–40% of predicted values (Irvine, 1983; Worthy et al., 2000)]. It is also possible that food passage time impacts on turnover rate [as discussed by Post (Post, 2002)]. Manatees use hindgut fermentation and have a passage rate through the digestive tract of 146–147 h (Lomolino and Ewel, 1984; Larkin et al., 2007). This rate is consistent with that of the dugong, another sirenian [145–166 h (Lanyon and Marsh, 1995)], but much slower than those of other large hindgut fermenters such as elephants [21–46 h (Rees, 1982)], horses [26–27 h (Rosenfeld et al., 2006)] and rhinoceros [61 h (Clauss et al., 2005)]. Manatees sampled in the present study were rescued for reasons including cold stress, entanglement and watercraft injuries. Because of their physical condition, their intake rates may have been slower than those of manatees not in need of rehabilitation.

The gruel mixture fed to the manatees for the first few weeks of rehabilitation was enriched in ^{13}C compared with lettuce and spinach. It is presumed that the initial supplementation of the diet with gruel would have had minimal to no impact on the carbon turnover rate as turnover was already very slow. There were no significant differences in $\delta^{13}C$ values between biopsy and sloughed skin samples, so the differing sample types had no effect on carbon turnover rate. Carbon isotope ratios of manatee fecal material did not differ from those of the main diet items, so even if manatees were engaging in coprophagy it would not have had any effect on carbon turnover rate in the skin. This result is another indication

that stable isotope analysis of fecal material has great potential in assessing short-term, recent dietary choices.

Phillips and Koch (Phillips and Koch, 2002) suggested incorporating carbon and nitrogen concentration analysis to aid in stable isotope dietary reconstruction, especially when there are great differences in C and N concentration between diet items. We were unable to include a concentration analysis in this study; however, it may be a useful addition to future analyses.

Coastal manatees were poor subjects for nitrogen turnover calculations because of the high variability in their initial $\delta^{15}N$ values at the time of rescue, the similarity of $\delta^{15}N$ values between the skin of rescued animals and that of captive animals, and the high variability in $\delta^{15}N$ values of captive diet items. Consequently, calculated nitrogen half-lives were inconsistent. Riverine manatees were better subjects because there was a greater difference in nitrogen signatures of skin between rescued and long-term captive animals. Even then, nitrogen half-lives for skin from riverine manatees were still variable, most likely because of the variability in nitrogen signatures of romaine lettuce and spinach fed in captivity. The lettuce and spinach in the captive manatee diet often originated from different agricultural producers and it is possible that different fertilization techniques were used resulting in high variability in $\delta^{15}N$ values (see Georgi et al., 2005).

There are very few studies on nitrogen turnover in other species. Nitrogen half-lives in mammalian tissues have been calculated for blood plasma and cells in black bears [3 and 22 days, respectively (Hilderbrand et al., 1996)] and avian whole blood [10.0–14.4 days (Bearhop et al., 2002; Hobson and Bairlein, 2003; Ogden et al., 2004)] and plasma [0.5–1.7 days (Pearson et al., 2003)]. Nitrogen turnover in manatee skin was relatively slow compared with the results of these studies, most likely owing to the low metabolic rate of manatees, epidermal tissue composition and/or food passage rate as previously discussed in terms of carbon turnover.

There was no compounding effect of the gruel supplement on nitrogen turnover rate as the signature of the gruel was not significantly different from that of romaine lettuce and spinach. Likewise, coprophagy would have had no effect on nitrogen turnover rate in manatee skin as the $\delta^{15}N$ values of manatee fecal material did not differ from those of the main diet items. However, sloughed skin samples were significantly enriched in ^{15}N compared with biopsy samples. Though $\delta^{15}N$ values were adjusted to account for this enrichment, variability in nitrogen turnover rates and lack of fit indicate that sample type may have contributed to the difficulty in calculating more precise half-lives. At the present time, it is unclear why sloughed samples differed in $\delta^{15}N$ values, but not $\delta^{13}C$ values, from biopsy samples. It is possible that materials were redistributed within the skin while sloughing, and sloughed samples also contained less epidermal depth than biopsies. Both of these factors could have contributed to differing $\delta^{15}N$ values between sample types.

Cerling and colleagues (Cerling et al., 2007) and Ayliffe and colleagues (Ayliffe et al., 2004) have suggested that some stable isotope turnover data may be better fitted to a multiple-pool model than to the traditional single-pool, exponential decay model used in the present study. Proper application requires knowledge of each pool *in vivo* (Sponheimer et al., 2006), which at this time is unknown for manatees. Consequently, we did not incorporate a multiple-pool analysis. Single-pool analyses are still useful for intraspecific and interspecific comparisons of stable isotope turnover (Sponheimer et al., 2006).

When proportions of food sources contributing to a mixed diet are unknown, mixing models are often used to aid in estimating these proportions (e.g. Phillips and Gregg, 2001; Newsome et al., 2004; Reich and Worthy, 2006). If a change in diet occurs, the resulting signature may not be representative of the current diet but, in fact, may be some intermediate value between two distinct diets. While this possibility is true for all stable isotope analyses, the impact is minimized in tissues with high turnover rates because the time frame is short. Free-ranging manatees are known to switch diet sources (Best, 1981; Lefebvre et al., 2000), and the very slow turnover rates for carbon and nitrogen stable isotopes in epidermis tissue mean that unless the manatee has been feeding on the same diet for an extended period of time, the skin signature will always be in some transitional state. Slow turnover rates in manatee skin especially complicate estimation of the proportions of freshwater, estuarine and marine sources in the diet because $\delta^{13}C$ values for estuarine vegetation are intermediate between those of freshwater vegetation and seagrasses (Reich and Worthy, 2006) (C.D.A.-S. and G.A.J.W., in preparation). The incorporation of nitrogen stable isotope analysis can aid in further separation of these three diet sources as nitrogen signatures for freshwater and estuarine vegetation differ from those of seagrasses (C.D.A.-S. and G.A.J.W., in preparation).

Computing a precise diet–tissue discrimination value is essential when interpreting isotopic results. Discrimination values for carbon have previously been calculated for manatee skin on a captive diet (Ames et al., 1996) and discrimination values for carbon and nitrogen have been estimated in the skin of free-ranging manatees on possible diets of freshwater, estuarine and/or marine vegetation (Reich and Worthy, 2006). It is unknown whether diet–tissue discrimination in manatee skin differs between diet types as has been shown in other studies (e.g. Hobson and Clark, 1992b). The results of the present study are the most extensive thus far.

Carbon and nitrogen stable isotope analysis of manatee epidermal tissue is difficult, if not impossible, to use when assessing short-term or recent changes in diet and habitat use because of slow turnover rates. This technique would potentially have more direct application in summarizing average dietary intake over longer periods of time. In order to accurately interpret isotopic analyses, determining diet–tissue discrimination factors and turnover rates in the tissue is essential. The difficulty with most studies is that isotope discrimination and turnover are best calculated under controlled situations in captivity. Mixing model results for tissues with slow turnover rates should be interpreted with caution, especially in species that may be switching between diets in which an intermediate isotope ratio may be mistakenly described as indicating a single diet source instead of a mixture of sources.

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References

Ames, A. L., VanVleet, E. S. and Sackett, W. M. (1996). The use of stable carbon isotope analysis for determining the dietary habits of the Florida manatee, *Trichechus manatus latirostris*. *Mar. Mamm. Sci.* **12**, 555-563.

Ayliffe, L. K., Cerling, T. E., Robinson, T., West, A. G., Sponheimer, M., Passey, B. H., Hammer, J., Roeder, B., Dearing, M. D. and Ehleringer, J. R. (2004). Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. *Oecologia* **139**, 11-22.

Bearhop, S., Waldron, S., Votier, S. C. and Furness, R. W. (2002). Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiol. Biochem. Zool.* **75**, 451-458.
Best, R. C. (1981). Foods and feeding habits of wild and captive Sirenia. *Mamm. Rev.* **11**, 3-29.
Bosley, K. L., Witting, D. A., Chambers, R. C. and Wainright, S. C. (2002). Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Mar. Ecol. Prog. Ser.* **236**, 233-240.
Campbell, H. W. and Irvine, A. B. (1977). Feeding ecology of West Indian manatee *Trichechus manatus* Linnaeus. *Aquaculture* **12**, 249-251.
Cerling, T. E., Hart, J. A. and Hart, T. B. (2004). Stable isotope ecology in the Ituri Forest. *Oecologia* **138**, 5-12.
Cerling, T. E., Ayliffe, L. K., Dearing, M. D., Ehleringer, J. R., Passey, B. H., Podlesak, D. W. A., T. and West, A. G. (2007). Determining biological tissue turnover using stable isotopes: the reaction progress variable. *Oecologia* **151**, 175-189.
Cherel, Y., Hobson, K. A. and Hassani, S. (2005). Isotopic discrimination between food and blood and feathers of captive penguins: implications for dietary studies in the wild. *Physiol. Biochem. Zool.* **78**, 106-115.
Clauss, M., Polster, C., Kienzle, E., Wiesner, H., Baumgartner, K., von Houwald, F., Ortmann, S., Streich, W. J. and Dierenfeld, E. S. (2005). Studies on digestive physiology and feed digestibilities in captive Indian rhinoceros (*Rhinoceros unicornis*). *J. Anim. Physiol. Anim. Nutr.* **89**, 229-237.
Clementz, M. T. and Koch, P. L. (2001). Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia* **129**, 461-472.
Cloern, J. E., Canuel, E. A. and Harris, D. (2002). Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnol. Oceanogr.* **47**, 713-729.
Craig, H. (1957). Isotopic standards for hydrogen and oxygen and correlation factors for mass-spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta* **42**, 495-506.
Dalerum, F. and Angerbjorn, A. (2005). Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* **144**, 647-658.
Deniro, M. J. and Epstein, S. (1978). Influence of diet on distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**, 495-506.
Deniro, M. J. and Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* **45**, 341-351.
Fry, B. (1981). Natural stable carbon isotope tag traces Texas shrimp migrations. *Fish. Bull.* **79**, 337-345.
Fry, B. and Arnold, C. (1982). Rapid C-13/C-12 turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* **54**, 200-204.
Fry, B. and Sherr, E. B. (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sci.* **27**, 13-47.
Georgi, M., Voerkelius, S., Rossmann, A., Grassmann, J. and Schnitzler, W. H. (2005). Multielement isotope ratios of vegetables from integrated and organic production. *Plant Soil* **275**, 93-100.
Graham, A. R., Samuelson, D. A., Isaza, R. and Lewis, P. A. (2003). Histological comparison of manatee and elephant integument. In *15th Biennial Conference on the Biology of Marine Mammals*, pp. 62-63. Greensboro, NC: Society for Marine Mammalogy.
Hall-Aspland, S. A., Rogers, T. L. and Canfield, R. B. (2005). Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. *Mar. Ecol. Prog. Ser.* **305**, 249-259.
Hartman, D. S. (1979). Ecology and behavior of the manatee (*Trichechus manatus*) in Florida: The American Society of Mammalogists Special Publication No. 5.
Hilderbrand, G. V., Farley, S. D., Robbins, C. T., Hanley, T. A., Titus, K. and Servheen, C. (1996). Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.* **74**, 2080-2088.
Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* **120**, 314-326.
Hobson, K. A. and Bairlein, F. (2003). Isotopic fractionation and turnover in captive garden warblers (*Sylvia borin*): implications for delineating dietary and migratory associations in wild passerines. *Can. J. Zool.* **81**, 1630-1635.
Hobson, K. A. and Clark, R. G. (1992a). Assessing avian diets using stable isotopes 1. Turnover of ^{13}C in tissues. *Condor* **94**, 181-188.
Hobson, K. A. and Clark, R. G. (1992b). Assessing avian diets using stable isotopes 2. Factors influencing diet-tissue fractionation. *Condor* **94**, 189-197.
Hobson, K. A. and Welch, H. E. (1992). Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Mar. Ecol. Prog. Ser.* **84**, 9-18.
Hobson, K. A., Schell, D. M., Renouf, D. and Noseworthy, E. (1996). Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can. J. Fish Aquat. Sci.* **53**, 528-533.
Irvine, A. B. (1983). Manatee metabolism and its influence on distribution in Florida. *Biol. Conserv.* **25**, 315-334.
Kelly, J. F. (2000). Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* **78**, 1-27.
Kurle, C. M. and Worthy, G. A. J. (2002). Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. *Mar. Ecol. Prog. Ser.* **236**, 289-300.
Lanyon, J. M. and Marsh, H. (1995). Digesta passage times in the dugong. *Aust. J. Zool.* **43**, 119-127.
Larkin, I. L. V., Fowler, V. F. and Reep, R. L. (2007). Digesta passage rates in the Florida manatee (*Trichechus manatus latirostris*). *Zoo Biol.* **26**, 503-515.
Lee, S. H., Schell, D. M., McDonald, T. L. and Richardson, W. J. (2005). Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. *Mar. Ecol. Prog. Ser.* **285**, 271-287.
Lefebvre, L. W., Ackerman, B. B., Portier, K. M. and Pollock, K. H. (1995). Aerial survey as a technique for estimating trends in manatee population size: problems and prospects. In *Population biology of the Florida Manatee* (ed. T. J. O'Shea, B. B. Ackerman and H. F. Percival), pp. 63-74. Washington, DC: Information and Technology Report 1, National Biological Service.
Lefebvre, L. W., Reid, J. P., Kenworthy, W. J. and Powell, J. A. (2000). Characterizing manatee habitat use and seagrass grazing in Florida and Puerto Rico: implications for conservation and management. *Pac. Conserv. Biol.* **5**, 289-298.
Logan, J., Haas, H., Deegan, L. and Gaines, E. (2006). Turnover rates of nitrogen stable isotopes in the salt marsh mummichog, *Fundulus heteroclitus*, following a laboratory diet switch. *Oecologia* **147**, 391-395.
Lomolino, M. W. and Ewel, K. C. (1984). Digestive efficiencies of the West Indian manatee (*Trichechus manatus*). *Florida Sci.* **47**, 176-179.
MacFadden, B. J., Higgins, P., Clementz, M. T. and Jones, D. S. (2004). Diets, habitat preferences, and niche differentiation of Cenozoic sirenians from Florida: evidence from stable isotopes. *Paleobiology* **30**, 297-324.
Newsome, S. D., Phillips, D. L., Culleton, B. J., Guilderson, T. P. and Koch, P. L. (2004). Dietary reconstruction of an early to middle Holocene human population from the central California coast: insights from advanced stable isotope mixing models. *J. Archaeol. Sci.* **31**, 1101-1115.
Newsome, S. D., Koch, P. L., Etnier, M. A. and Aurioles-Gambao, D. (2006). Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. *Mar. Mamm. Sci.* **22**, 556-572.
O'Shea, T. J., Beck, C. A., Bonde, R. K., Kochman, H. I. and Odell, D. K. (1985). An analysis of manatee mortality patterns in Florida, 1976-81. *J. Wildl. Manage.* **49**, 1-11.
Ogden, L. J. E., Hobson, K. A. and Lank, D. B. (2004). Blood isotopic ($d^{13}C$ and $d^{15}N$) turnover and diet-tissue fractionation factors in captive Dunlin (*Calidris alpina pacifica*). *Auk* **121**, 170-177.
Olive, P. J. W., Pinnegar, J. K., Polunin, N. V. C., Richards, G. and Welch, R. (2003). Isotope trophic-step fractionation: a dynamic equilibrium model. *J. Anim. Ecol.* **72**, 608-617.
Pearson, S. F., Levey, D. J., Greenberg, C. H. and del Rio, C. M. (2003). Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* **135**, 516-523.
Peterson, B. J. and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **18**, 293-320.
Phillips, D. L. and Gregg, J. W. (2001). Uncertainty in source partitioning using stable isotopes. *Oecologia* **127**, 171-179.
Phillips, D. L. and Koch, P. L. (2002). Incorporating concentration dependence in stable isotope mixing models. *Oecologia* **130**, 114-125.
Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703-718.
Ramsay, M. A. and Hobson, K. A. (1991). Polar bears make little use of terrestrial food webs: evidence from stable-carbon isotope analysis. *Oecologia* **86**, 598-600.
Rau, G. H., Ainley, D. G., Bengtson, J. L., Torres, J. J. and Hopkins, T. L. (1992). $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea birds, seals, and fish: implications for diet and trophic structure. *Mar. Ecol. Prog. Ser.* **84**, 1-8.
Rees, R. A. (1982). Gross assimilation efficiency and food passage time in the African elephant. *Afr. J. Ecol.* **20**, 193-198.
Reich, K. J. and Worthy, G. A. J. (2006). An isotopic assessment of the feeding habits of free-ranging manatees. *Mar. Ecol. Prog. Ser.* **322**, 303-309.
Rosenfeld, I., Austbo, D. and Volden, H. (2006). Models for estimating digesta passage kinetics in the gastrointestinal tract of the horse. *J. Anim. Sci.* **83**, 3321-3328.
Roth, J. D. and Hobson, K. A. (2000). Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Can. J. Zool.* **78**, 848-852.
Schwertl, M., Auerswald, K. and Schnyder, H. (2003). Reconstruction of the isotopic history of animal diets by hair segmental analysis. *Rapid Commun. Mass Spectrom.* **17**, 1312-1318.
Seminoff, J. A., Jones, T. T., Eguchi, T., Jones, D. R. and Dutton, P. H. (2006). Stable isotope discrimination ($\delta^{13}C$ and $\delta^{15}N$) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. *Mar. Ecol. Prog. Ser.* **308**, 271-278.
Sokolov, V. E. (1982). *Mammal Skin*. Berkeley, CA: University of California Press.
Sponheimer, M., Robinson, T. F., Cerling, T. E., Tegland, L., Roeder, B. L., Ayliffe, L., Dearing, M. D. and Ehleringer, J. R. (2006). Turnover of stable carbon isotopes in the muscle, liver, and breath CO_2 of alpacas (*Lama pacos*). *Rapid Commun. Mass Spectrom.* **20**, 1395-1399.
Tieszen, L. L., Boutton, T. W., Tesdahl, K. G. and Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* **57**, 32-37.
US Fish and Wildlife Service (2001). *Florida Manatee Recovery Plan* (*Trichechus manatus latirostris*), 3rd revision. Atlanta, GA: US Fish and Wildlife Service.
Voigt, C. C., Matt, F., Michener, R. and Kunz, T. H. (2003). Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. *J. Exp. Biol.* **206**, 1419-1427.
Walker, J. L., Potter, C. W. and Macko, S. A. (1999). The diets of modern and historic bottlenose dolphin populations reflected through stable isotopes. *Mar. Mamm. Sci.* **15**, 335-350.
Worthy, G. A. J., Miculka, T. A. and Wright, S. D. (2000). Manatee response to cold: how cold is too cold? In *Florida Manatees and Warm Water: Proceedings of the Warm Water Workshop* (ed. W. Perkins), pp. 1-6. Jacksonville, FL: US Fish and Wildlife Service.
Yamamuro, M., Aketa, K. and Uchida, S. (2004). Carbon and nitrogen stable isotope ratios of the tissues and gut contents of a dugong from the temperate coast of Japan. *Mammal Study* **29**, 179-183.