Stable Isotope Analysis of Manatee Vibrissae to Investigate Individual Patterns of Resource Use in the Northern Gulf of Mexico

Levette Tucker
University of South Alabama, levette.tucker@gmail.com

Mackenzie L. Russell
Dauphin Island Sea Lab, mrussell@disl.org

Elizabeth E. Hieb
Dauphin Island Sea Lab, ehieb@disl.org

Ruth H. Carmichael
Dauphin Island Sea Lab, rcarmichael@disl.org

See next page for additional authors

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GULF AND CARIBBEAN

R E S E A R C H

SHORT COMMUNICATION

STABLE ISOTOPE ANALYSIS OF MANATEE VIBRISSAE TO INVESTIGATE INDIVIDUAL PATTERNS OF RESOURCE USE IN THE NORTHERN GULF OF MEXICO[§]

Levette Tucker[1], Mackenzie L. Russell[2], Elizabeth E. Hieb[2], Ruth H. Carmichael[1,2], and Carl S. Cloyed[1,2*]

[1]*Stokes School of Marine and Environmental Sciences, University of South Alabama, 600 Clinic Drive, Mobile, Mobile, AL 36608;*
[2]*Dauphin Island Sea Lab, 101 Bienville Blvd, Dauphin Island, AL 36528; *Corresponding author, email: ccloyed@disl.org*

INTRODUCTION

Many species are undergoing range shifts and inhabiting new areas because global warming is changing environmental conditions (Hastings et al. 2020, Pinsky et al. 2020, Osland et al. 2021). Many of those species are moving into habitats at higher latitudes, shifting their geographic ranges poleward (Hastings et al. 2020, Pinsky et al. 2020, Osland et al. 2021). For example, West—Indian manatees, *Trichechus manatus*, are a tropical, herbivorous marine mammal that has become increasingly common in the northern Gulf of Mexico (nGOM), which are sub—tropical waters that historically were not part of the primary range for manatees in the southeastern United States (Hieb et al. 2017, Cloyed et al. 2021a, 2022). In many ways, manatees are a flagship species in the accelerating tropicalization of the nGOM, where the number of plant and animal species with tropical ranges are increasing (Fodrie et al. 2010, Heck et al. 2015, Osland et al. 2021). Because manatees are large, herbivorous, marine mammals, they can have large impacts on aquatic plant communities and local ecosystems (Hauxwell et al. 2004, Lefebvre et al. 2017, Littles et al. 2019). Understanding how manatees use habitats in the nGOM will provide important insights into how manatees affect or may be affected by both tropical and native vegetation species in local communities as their range shifts.

Manatees forage in different types of habitats throughout their range, and these habitats can be distinguished by differences in stable carbon and nitrogen isotope ratios (Moncreiff and Sullivan 2001, Wissel et al. 2005, Reich and Worthy 2006, Vaslet et al. 2012). Manatees typically forage on a variety of available dietary resources across habitat types in the nGOM primarily during warm seasons (Lefebvre et al. 1999, Reich and Worthy 2006, Cloyed et al. 2022) and spring and autumn migrations to and from the region (Hieb et al. 2017, Cloyed et al. 2019, 2022). These habitats include freshwater or riverine habitats, estuarine habitats that include a mixture of riverine and marine influences, and seagrass habitats that are generally found in more marine—influenced sections of estuaries and adjacent nearshore areas (Reich and Worthy 2006, Alves—Stanley et al. 2010). The $\delta^{13}C$ values from these habitats also form a gradient. Submerged aquatic vegetation (SAV) from estuarine habitats dominated by freshwater flow from inland sources have $\delta^{13}C$ values ranging from −28‰ to −34‰ (Wissel et al. 2005, Reich and Worthy 2006, Alves—Stanley et al. 2010, Cloyed et al. 2021b). The SAV from lower parts of estuaries typically have higher $\delta^{13}C$ values ranging from −18‰ to −24‰, with areas more heavily influenced by marine waters having higher $\delta^{13}C$ values. Seagrass habitats that are typically found in more marine influenced parts of estuaries have the highest $\delta^{13}C$ values ranging from −8‰ to −14‰ (Peterson and Fry 1987, Moncreiff and Sullivan 2001, Reich and Worthy 2006, Vaslet et al. 2012, Wilson et al. 2017). Although $\delta^{15}N$ values typically vary with trophic level, increases in $\delta^{15}N$ values can indicate freshwater association or potential nutritional stress (Wissel et al. 2005, Hatch 2012). Seagrass meadows are common in the more tropical waters of Florida but do occur in the nGOM, especially in the Mississippi Sound (Lefebvre et al. 1999, Handley and Lockwood 2021). We generally know the habitats that manatees use in the nGOM, but we know little about the individual variation in habitat use and how frequently individuals may move among freshwater or marine—dominated estuarine habitats and more marine seagrass habitats.

Here, for the first time we analyzed stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in facial whiskers (perioral facial vibrissae) which aid in feeding (Marshall et al. 1998), to define individual variation in habitat use by West Indian manatees stranded in the nGOM. Perioral facial vibrissae are keratin based, metabolically inert, and estimated to grow at a rate of about 0.51 mm/d (Garcés Cuartas et al. 2020), with a range between 0.47 and 0.55 mm/d among animals in controlled feeding studies. As they grow, the vibrissae may incorporate stable isotopes that correspond to manatee diets foraged from associated habitats at a similar rate, with the root

representing the most recent diet and the tip the most distant (Zhao and Schell 2004). To determine if vibrissae record stable isotope ratios on timescales comparable to other tissues, we compared the isotope values in different segments of the vibrissae to values in liver, skin, and muscle that have varying isotopic incorporation rates (Alves—Stanley and Worthy 2009, Kurle 2009, Martínez del Rio et al. 2009). We predicted that isotope values in the root segment of vibrissae would be similar to values in liver because liver typically has faster isotopic incorporation than other tissues (turnover of 1—3 weeks; Kurle 2009, Martínez del Rio et al. 2009); values in mid—vibrissae to the tip would correspond to values in skin (turnover rate of 3—6 weeks; Alves—Stanley and Worthy 2009); and values in the tips of longer vibrissae could correspond to the values in muscle, which typically has slower incorporation than skin (6—8 weeks; Kurle 2009, Martínez del Rio et al. 2009). Facial vibrissae are a promising supplement or alternative to the use of soft tissues for stable isotope analyses because they resist rapid decomposition, are relatively easy to collect and store compared to soft tissues, which often require a necropsy to obtain, and could reduce the need to collect multiple tissues to define temporal variation in resource use (Zhao and Schell 2004, Newsome et al. 2010, Cloyed et al. 2023).

Materials and Methods

Study Site and Stranding Information

Manatees used in this study stranded dead along the nGOM coast between Bay St. Louis, MS and the Pensacola Bay area of the Florida panhandle (Figure S1). Of the 14 stranded manatees, 7 stranded in Mississippi, 5 in Alabama, and 2 in Florida, and 12 individuals were males and 2 were female. Twelve strandings took place during the cold season (November—April), and individuals that stranded during these months all had evidence of cold stress, even if they had other cause of deaths (e.g., trauma from vessel collisions). Two strandings that took place during the warm season (May—October) were caused by trauma and had no evidence of cold stress. Strandings occurred in a combination of coastal areas, barrier islands, and embayments like Pensacola Bay, Mobile Bay, and Mississippi Sound. All strandings were responded to by the Alabama Marine Mammal Stranding Network (ALMMSN) at the Dauphin Island Sea Lab under US Fish and Wildlife Service Letters of Authorization #LOAFC770191—H and #MA66525C.

Manatee Tissue Samples

We sub—sampled facial vibrissae and ~5 g each of liver, abdominal muscle, and skin from frozen samples (—20°C) that were initially collected during post—mortem examinations (necropsies) of manatees at the time carcasses were recovered. The longest available facial vibrissae were selected from each manatee. Each vibrissa was thoroughly cleaned by soaking in soapy (Sparkleen™, Fisher Scientific, Pittsburg, PA, USA) water for 72 h to loosen residual skin and fat, scrubbed with a soft toothbrush and isopropyl alcohol for final gross tissue removal, and submerged for 5 min in 2:1 chloroform:methanol to remove contaminating lipids from the surface. After clean-ing, lengths of vibrissae were measured, and vibrissae were cut into 0.5 cm segments, estimated to represent 9—11 d periods of time, when the error rate of these growth rates were factored into the calculation (Garcés Cuartas et al. 2020). Each segment was labeled alphabetically from root to tip.

Facial vibrissae and all soft tissues were dried in an oven for 48—72 h, until thoroughly dry. Vibrissae were homogenized using a hand press (Parr 2811 Pellet Press, Parr Instrument Company, Moline, IL, USA), and soft tissues were homogenized using mortar and pestle. Samples were weighed to 1.0 mg (0.2 mg) and packed into 3x5 mm tin capsules. Soft tissue samples were not lipid—extracted because previous research found this step unnecessary (Cloyed et al. 2020). Samples were sent to the University of California, Davis Stable Isotope Facility for analysis on a PDZ Europa ANCA—GSL elemental analyzer with a PDZ Europa 20—20 isotope—ratio mass spectrometer (Sercon Ltd, Cherise, UK). Isotopic values were expressed using delta notation (δ) in parts per thousand (‰), where $\delta X = (R_{sample} / R_{standard} - 1) \times 1{,}000$, with R_{sample} and $R_{standard}$ representing the molar ratios of C^{13}/C^{12} and N^{15}/N^{14} of the sample and standard reference material, respectively. The reference material was Vienna—Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Measurement standard deviations of these references were 0.04‰ for $\delta^{13}C$ and 0.04‰ for $\delta^{15}N$, which were done in—house and based on chitin (n = 11), amaranth flour (5), caffeiene (6), enriched alanine (6), glutamic acid (6), keratin (5), and nylon powder (37).

Analyses

We examined the $\delta^{13}C$ and $\delta^{15}N$ values along the length of each vibrissa, from root to tip, with segment *a*, nearest the root, corresponding to the most recent 10 d (±1 day) period before stranding, and each subsequent segment corresponding to subsequent 10 d periods. We compared the $\delta^{13}C$ values of each segment to known values in freshwater, marine, and seagrass sources in the nGOM (Moncreiff and Sullivan 2001, Wissel et al. 2005, Vaslet et al. 2012, Marth et al. 2023) to determine if individual manatees foraged among these habitats in the weeks and months prior to stranding. Although manatees are herbivores and should exhibit little variation in $\delta^{15}N$ (reflecting a consistently low trophic level), we compared $\delta^{15}N$ values along the length of vibrissae to test for variation in basal $\delta^{15}N$ values among forage habitats and for signs of nutritional stress (Hatch 2012, Patterson and Carmichael 2018, Funck et al. 2020), which may be detectable in animals experiencing cold stress syndrome (Bossart et al. 2003, Martony et al. 2019).

We used t—tests to determine if $\delta^{13}C$ values in different segments of vibrissae were similar to the $\delta^{13}C$ values in the different soft tissues based on their isotopic incorporation rates. We calculated the difference of $\delta^{13}C$ values between vibrissae and each soft tissue and then used t—tests to compare whether those differences were different from 0. To account for multiple comparisons within each soft tissue, we used a Bonferroni approach to adjust p values. We assumed an alpha = 0.05, and each tissue had 4 comparisons (segments *ab, bc, cd, de*), so adjusted alphas = 0.013 (0.05/4) and p values below this thresh-

old were considered significant. Different tissues undergo differential fractionation during metabolic processes leading to different isotopic values relative to diet, and we applied trophic discrimination factors to each tissue type based on previous studies. For $\delta^{13}C$ values, we applied trophic discrimination factors of 2.50‰ for facial vibrissae, and 3.62‰, 2.78‰, and 2.80‰ for liver, muscle, and skin, respectively (Stegall et al. 2008, Alves–Stanley and Worthy 2009, Newsome et al. 2010, Tyrrell et al. 2013, Stricker et al. 2015). For $\delta^{15}N$ values, we applied factors of 3.3‰ for vibrissae, and 3.90‰, 2.7‰, and 0.35‰ for liver, muscle, and skin, respectively (Stegall et al. 2008, Alves–Stanley and Worthy 2009, Newsome et al. 2010, Tyrrell et al. 2013, Stricker et al. 2015). We then averaged $\delta^{13}C$ and $\delta^{15}N$ values of pairwise segments (*ab*, *bc*, *cd*, and *de*) to characterize isotope values along the length of the vibrissae. We did not analyze beyond segment *e* because the majority of individuals had vibrissae with segments *a – e*. We calculated the difference in $\delta^{13}C$ and $\delta^{15}N$ values between each of those averaged pairwise segments and the $\delta^{13}C$ and $\delta^{15}N$ values in the soft tissues after the trophic discrimination factors had been applied.

RESULTS

The longest available vibrissae from each manatee (n = 14) varied in length. One individual had 3 segments (*a–c*), one had 4 segments (*a–d*), 10 had 5 segments (*a–e*), one had 6 segments (*a–f*), and one had 8 segments (*a–h*). Liver and skin samples were viable for analysis from 13 individuals and muscle from 12.

The $\delta^{13}C$ values in manatee vibrissae ranged from about −30 to −14‰, with some individuals having little variation along the length of the vibrissae and others having dramatic shifts of 5−10‰ from the root to the tip (Table 1; Figure 1A). Many of the individuals with $\delta^{13}C$ values that remained consistent along their vibrissae had higher $\delta^{13}C$ values (> −20‰), falling between the typical range of values in estuarine and seagrass sources (Figure 1A). Most individuals in which $\delta^{13}C$ values changed showed a decrease in $\delta^{13}C$ from values within the range of marine sources at the vibrissae root to values approaching freshwater sources at the vibrissae tip (Figure 1A). The individual with the longest vibrissa (8 segments) had $\delta^{13}C$ values in the range of marine sources at the root that shifted toward freshwater sources mid–vibrissae before rising again toward the range of marine sources at the tip (Table 1; Figure 1A). The $\delta^{15}N$ values in manatee vibrissae also showed considerable variation, ranging from about 1–8 ‰ (Table 1), with values in most animals decreasing 1–2 ‰ from root to tip (Figure 1B). In contrast, one individual had an increase in $\delta^{15}N$ values of > 2‰ from the second segment (b) to the tip (Figure 1B). Many of the individuals that had lower $\delta^{15}N$ values had the highest $\delta^{13}C$ values and stranded in MS or AL (Figure 1A, B), and many of the individuals with high $\delta^{15}N$ values had low $\delta^{13}C$ values (Figure 1A, B). Both FL individuals had relatively high $\delta^{15}N$ values (Figure 1B).
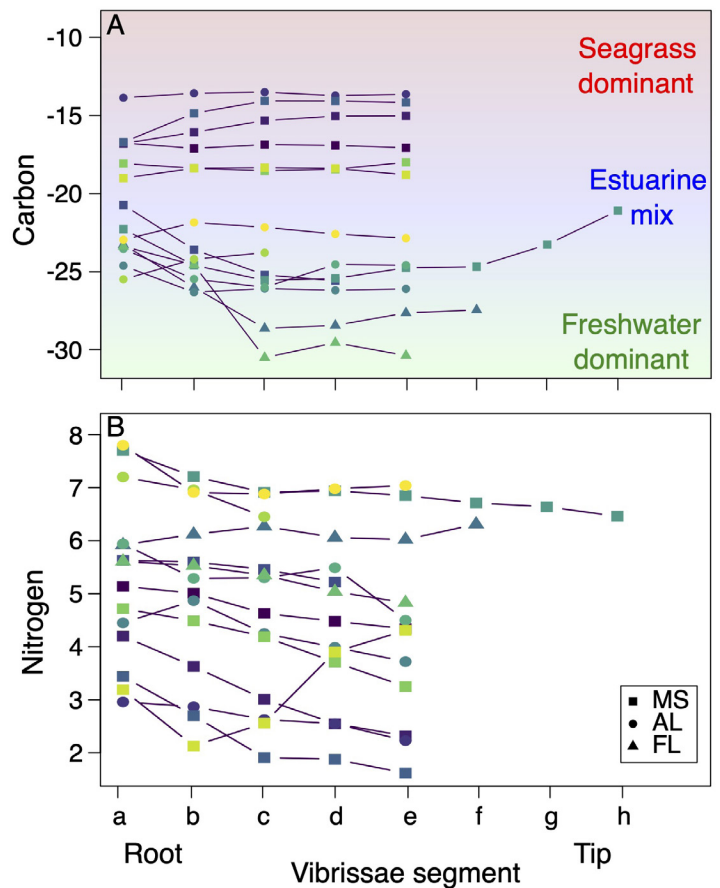
The results comparing the differences between segments of

FIGURE 1. *Isotope values along the length of individual manatee vibrissae from the root (a) to the tip (h). A. Carbon. B. Nitrogen.*

TABLE 1. *Mean stable carbon and nitrogen isotope values (± sd) for each vibrissae segment and soft tissue from manatees stranded in the northcentral Gulf of Mexico. Segment indicates 0.5 cm segments from root (a) to tip (h).*

Tissue	Segment	$\delta^{13}C$	$\delta^{15}N$	N
Vibrissae	a	−18.04 (3.62)	8.58 (1.58)	14
	b	−18.57 (4.49)	8.25 (1.63)	14
	c	−19.25 (5.58)	8.00 (1.67)	14
	d	−18.95 (7.38)	7.82 (2.61)	13
	e	−18.58 (8.53)	7.55 (3.18)	12
	f	−23.56 (NA)	9.81 (NA)	2
	g	−20.77 (NA)	9.94 (NA)	1
	h	−18.59 (NA)	9.76 (NA)	1
Liver		−19.15 (3.65)	9.20 (1.24)	13
Skin		−18.99 (4.35)	8.14 (1.67)	13
Muscle		−17.59 (3.67)	7.19 (1.88)	12

vibrissae to soft tissues were complex (Table S1). For liver and skin, $\delta^{13}C$ values were different between vibrissae segments and tissue (differences ≠ 0) at the root (*ab*) but became similar (differences = 0) moving towards the tip (*de*) (Table S1; Figure
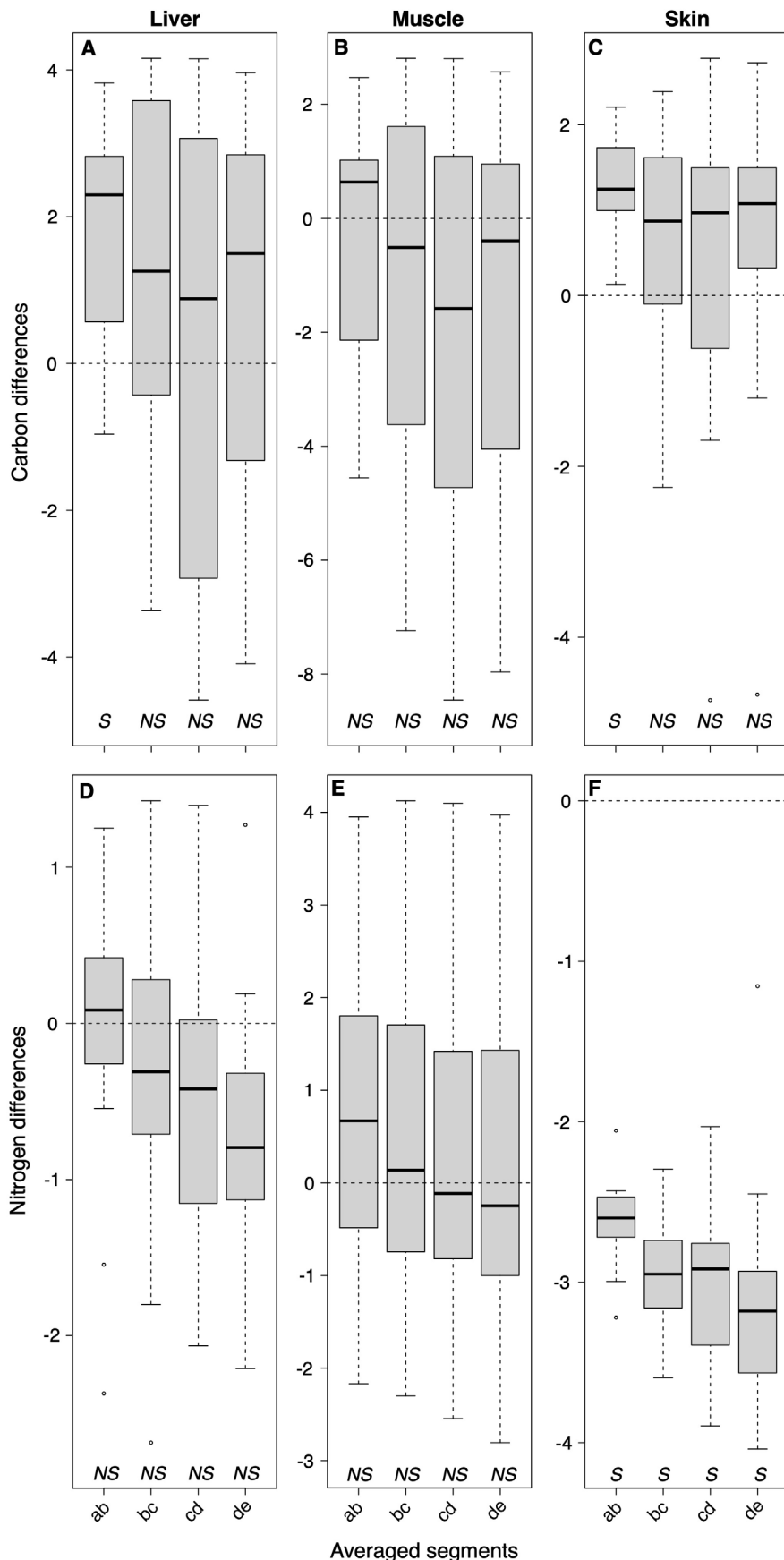
FIGURE 2. *Boxplots depict differences in stable isotope values between averaged pairwise segments of vibrissae and various manatee tissues. Vibrissae segments range from root (a) to tip (e). A. Carbon isotopes in vibrissae and liver. B. Carbon isotopes in vibrissae and muscle. C. Carbon isotopes in vibrissae and skin. D. Nitrogen isotopes in vibrissae and liver. E. Nitrogen isotopes in vibrissae and muscle. F. Nitrogen isotopes in vibrissae and skin. Solid bars indicate median, boxes represent upper and lower quartiles, error bars represent 1.5*interquartile range. S–Significant at p < 0.013 based on Bonferroni adjustment of Student t–test; NS–nonsignificant based on Bonferroni adjustment of Student t–test.*

2D). A similar pattern of differences in $\delta^{15}N$ values was observed between skin and along the length of vibrissae, but all values were different from zero (Figure 2F). The values in vibrissae segments and muscle were similar throughout the length of the vibrissae for both $\delta^{13}C$ and $\delta^{15}N$ (Table S1; Figure 2B, E).

DISCUSSION

The $\delta^{13}C$ and $\delta^{15}N$ values along the length of manatee facial vibrissae demonstrated variation in diet that reflected use of different forage habitats in the weeks and months prior to death. Differences in how $\delta^{13}C$ values changed along the length of the vibrissae revealed considerable individual variation in forage habitat use by manatees, particularly variation along a habitat gradient from freshwater–influenced habitats at one end to seagrass beds in marine–influenced habitats at the other end and estuarine habitats with a mixture of freshwater and marine resources in the middle (Wissel et al. 2005, Reich and Worthy 2006, Vaslet et al. 2012, Cloyed et al. 2022). Manatees are known to use a wide range of coastal and freshwater habitat types throughout their range, and in the nGOM are generally found in coastal or inshore habitats where submerged aquatic vegetation is readily available (Reich and Worthy 2006, Hieb et al. 2017, Cloyed et al. 2022). In general, manatees with high $\delta^{13}C$ values that are indictive of foraging in marine habitats containing seagrass showed little change among vibrissae segments in our study. Many of these individuals stranded in Mississippi, where patches of seagrass are present (Moncreiff 2007, Pham et al. 2014), and in addition to having high $\delta^{13}C$ values, they also had low $\delta^{15}N$ values. This combination of $\delta^{13}C$

2A, C). In contrast, $\delta^{15}N$ values were similar between vibrissae and liver at the root but were increasingly different towards the tip, but these differences were not significant (Table S1; Figure

and $\delta^{15}N$ values is highly indictive of a seagrass–based diet (Campbell and Fourqurean 2009, Barros et al. 2010). These manatees likely remained in marine and/or seagrass habitats

in the weeks and months prior to stranding. Manatees that had lower $\delta^{13}C$ values generally showed the greatest change in $\delta^{13}C$ values (typically increasing) along vibrissae, suggesting movement from freshwater–influenced towards marine–influenced estuarine habitats. Additionally, many of these individuals with low $\delta^{13}C$ values also had high values of $\delta^{15}N$, suggesting that these large variations in $\delta^{15}N$ values represent differences in habitat baselines. These results demonstrate the flexibility manatees can exhibit in their habitat use and associated foraging and their likelihood to move among a range of habitats within the scope of 1–3 weeks (Cloyed et al. 2021a). Although our sample size is relatively small given the spatial and temporal scales at which they were collected, our results represent a high degree of variation in habitat use and in movement among habitats among individuals, which is possible given the known behavior of manatees to use and move among these habitats (Lefebvre et al. 1999, Morales–Vela et al. 2000, Alves–Stanley et al. 2010). Furthermore, the relatively common change from lower to higher $\delta^{13}C$ values may reflect movements from upper estuarine, inshore habitats to more coastal habitats along which manatees would travel during their migration to thermal refugia located in peninsular Florida where they typically overwinter (Cloyed et al. 2019, 2021a). This idea is supported by the fact that most animals in this study (n = 12) died between November to late January, overlapping with the late fall migration period for manatees leaving nGOM waters (Cloyed et al. 2019, 2021a).

The increase in $\delta^{15}N$ values from tip to root of most vibrissae may be caused by nutritional deficiencies associated with cold stress syndrome in manatees (Hatch 2012, Martony et al. 2019). All but 2 of the manatees in our study either died from cold stress or had evidence of cold stress syndrome, even if their direct cause of death was something else (e.g., trauma from vessel collision). Cold stress occurs when manatees are exposed to water temperatures < 20°C for prolonged periods (Bossart et al. 2003), and causes reduced metabolism, nutritional deficiency, emaciation, and eventually starvation (Bossart et al. 2003, Martony et al. 2019). Organisms undergoing nutritional stress and starvation often pull energy from internal reserves such as fat and muscle (Hatch 2012). The result is an increase in $\delta^{15}N$ values due to catabolic processes that can mimic dietary consumption at higher trophic levels (Hatch 2012, Bowes et al. 2014, Funck et al. 2020). Of note, $\delta^{15}N$ values also increased slightly at the vibrissae root for the one animal that died during the warm season. This warm season animal increased 1.5‰ in $\delta^{15}N$, whereas many cold stressed animals had increased > 1.5‰. In these cases, other diagnostic criteria are needed to indicate cold stress because increased $\delta^{15}N$ values alone may indicate a shift into more freshwater–influenced parts of estuaries.

Comparing isotopic values along the length of manatee vibrissae to those in soft tissues revealed an interesting pattern in isotopic incorporation in manatees. If manatee vibrissae grow at a rate of 0.51 cm/day (± 0.04 sd; Garcés Cuartas et al. 2020), then the isotope values along the length of the vibrissae should correspond to soft tissues, depending on how quickly those tissues incorporate isotope values and given the tissue–specific discrimination factor. These patterns were different than expected in many cases. For example, $\delta^{13}C$ values between vibrissae and skin were similar to the patterns with liver, suggesting both tissues may turnover on the order of 10–30 days, which is faster than the 1–2 months reported by a controlled feeding experiment on captive manatees (Alves–Stanley and Worthy 2009, Kurle 2009). Some discrepancies between vibrissae segments and soft tissues maybe caused by the variation around vibrissae growth and isotopic incorporation rates. Growth rates of vibrissae maybe lower in wild manatees compared to those in captivity where growth experiments were performed. Additionally, because there is evidence that some wild manatees in our study died from cold stress, associated nutritional stress may affect vibrissae growth. Vibrissae growth rates may, therefore, also likely vary seasonally, with higher rates during the warm season when manatees are consuming large quantities of food and lower in the cold season when metabolic rates and food intake are reduced. Similarly, $\delta^{15}N$ values in skin were quite different from vibrissae, and this may be caused by incorrectly applied trophic discrimination factors. Of note, for liver and muscle, discrimination factors were determined from studies performed on taxonomically and ecologically similar species, but for skin, discrimination factors were obtained from the controlled feeding study (Alves–Stanley and Worthy 2009). Our data suggest that the values obtained from the controlled feeding study may not be accurate for wild manatees (Alves–Stanley and Worthy 2009). Additionally, the discrimination factors that were available for vibrissae came primarily from carnivores, generally from pinnipeds and sea otters, and variation in discrimination factors between carnivores and herbivores is unknown. It is also possible that discrimination factors may vary with other demographic factors such as sex or age, but most discrimination patterns do not differ between the sexes; and our animals were primarily males and none were calves, so it unlikely these factors had a meaningful effect on the analysis (Kurle et al. 2014). Despite potential caveats to comparing stable isotopes in vibrissae to soft tissues, the pattern of differences in $\delta^{15}N$ values along the length of the vibrissae is very similar to the pattern of differences in $\delta^{13}C$, suggesting that once the discrimination of $\delta^{15}N$ in skin has been correctly accounted for, turnover is likely to be on the order of 10–20 days (similar to the timescale defined using $\delta^{13}C$).

This study is the first to use vibrissae for stable isotope analysis in manatees. These tissues resist rapid decomposition that can complicate use of soft tissues from carcasses (the major source of manatee tissues broadly available for isotope analyses) and are relatively easy, safe, and inexpensive to collect. With additional study, it also may be possible to use individual vibrissae in place of multiple tissues to provide information on diet and habitat use from multiple points in time prior to stranding. These data may be particularly informative to define shifts in habitat use following migration to or seasonal residence in regions like the nGOM to help define critical food resources and habitats in these areas outside core parts of their range in peninsular Florida. The Gulf of Mexico, in particular, is experiencing warming at nearly twice the rate as oceans worldwide

(Wang et al. 2023). As such, the effects of climate change in the nGOM are coming into focus, and with the warming temperatures come more tropical–associated species at higher latitudes (Fodrie et al. 2010, Heck et al. 2015, Osland et al. 2021). Because manatees are large herbivores, they may have outsized effects on plant communities (Hauxwell et al. 2004, Lefebvre et al. 2017, Littles et al. 2019). Understanding where manatees forage and how they use habitats will help ecologists, conser-

vationists, and managers understand the food and habitat resources important to manatee conservation, as well as how manatees may affect the composition of future communities. Using a promising new tissue for monitoring, our study reveals that manatees use a range of forage habitats, from freshwater– to marine–influenced parts of estuaries to seagrass dominated habitats within the nGOM.

Literature Cited

Alves–Stanley, C.D. and G.A. Worthy. 2009. Carbon and nitrogen stable isotope turnover rates and diet–tissue discrimination in Florida manatees (*Trichechus manatus latirostris*). Journal of Experimental Biology 212:2349-2355. https://doi.org/10.1242/jeb.027565

Alves–Stanley, C.D., G.A. Worthy, and R.K. Bonde. 2010. Feeding preferences of West Indian manatees in Florida, Belize, and Puerto Rico as indicated by stable isotope analysis. Marine Ecology Progress Series 402:255-267. https://doi.org/10.3354/meps08450

Barros, N.B., P. Ostrom, C.A. Stricker, and R. Wells. 2010. Stable isotopes differentiate bottlenose dolphins off west–central Florida. Marine Mammal Science 26:324-336. https://doi.org/10.1111/j.1748–7692.2009.00315.x

Bossart, G.D., R.A. Meisner, S. Rommel, S. Ghim, and A.B. Jenson. 2003. Pathological features of the Florida manatee cold stress syndrome. Aquatic Mammals 29:9-17. https://doi.org/10.1578/016754203101024031

Bowes, R.E., M.H. Lafferty, and J.H. Thorp. 2014. Less means more: Nutrient stress leads to higher $\delta^{15}N$ ratios in fish. Freshwater Biology 59:1926-1931. https://doi.org/10.1111/fwb.12396

Campbell, J.E. and J.W. Fourqurean. 2009. Interspecific variation in the elemental and stable isotope content of seagrasses in South Florida. Marine Ecology Progress Series 387:109-123. https://doi.org/10.3354/meps08093

Cloyed, C.S., E.E. Hieb, M.K. Collins, K.P. DaCosta, and R.H. Carmichael. 2019. Linking use of ship channels by West Indian manatees (*Trichechus manatus*) to seasonal migration and habitat use. Frontiers in Marine Science 6:318. https://doi.org/10.3389/fmars.2019.00318

Cloyed, C.S., K.P. DaCosta, M R. Hodanbosi, and R.H. Carmichael. 2020. The effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values and use of lipid–correction models across tissues, taxa and trophic groups. Methods in Ecology and Evolution 11:751-762. https://doi.org/10.1111/2041–210X.13386

Cloyed, C.S., E.E. Hieb, K. DaCosta, M. Ross, and R.H. Car-

michael. 2021a. West Indian manatees use partial migration to expand their geographic range into the northern Gulf of Mexico. Frontiers in Marine Science 8:1354. https://doi.org/10.3389/fmars.2021.725837

Cloyed, C.S., R.M. Wilson, B.C. Balmer, A.A. Hohn, L.H. Schwacke, E.S. Zolman, M.C. Tumlin, R S. Wells, A.A. Barleycorn, J.B. Allen, and R.H. Carmichael. 2021b. Specialization of a mobile, apex predator affects trophic coupling among adjacent habitats. Scientific Reports 11:19611. https://doi.org/10.1038/s41598–021–99017–1

Cloyed, C.S., E.E. Hieb, K.P. DaCosta, M. Ross, and R.H. Carmichael. 2022. Habitat selection and abundance of West Indian manatees *Trichechus manatus* at the margins of their expanding range. Marine Ecology Progress Series 696:151-167. https://doi.org/10.3354/meps14116

Cloyed, C.S., C. Johnson, K.P. DaCosta, L.R. Clance, M.L. Russell, C. Díaz Clark, E.E. Hieb, and R.H. Carmichael. 2023. Effects of tissue decomposition on stable isotope ratios and implications for use of stranded animals in research. Ecosphere 14:e4385. https://doi.org/10.1002/ecs2.4385

Fodrie, F.J., K.L. Heck, Jr., S.P. Powers, W.M. Graham, and K.L. Robinson. 2010. Climate–related, decadal–scale assemblage changes of seagrass–associated fishes in the northern Gulf of Mexico. Global Change Biology 16:48-59. https://doi.org/10.1111/j.1365–2486.2009.01889.x

Funck, J., C. Kellam, C.T. Seaton, and M. J. Wooller. 2020. Stable isotopic signatures in modern wood bison (*Bison bison athabascae*) hairs as telltale biomarkers of nutritional stress. Canadian Journal of Zoology 98:505-514. https://doi.org/10.1139/cjz–2019–0185

Garcés–Cuartas, N., C.A. Niño–Torres, and D.N. Castelblanco–Martínez. 2020. Vibrissae growth rate of captive Antillean manatees (*Trichechus manatus manatus* Linnaeus, 1758). Marine Mammal Science 36:344-353. https://doi.org/10.1111/mms.12638

Handley, L. and C. Lockwood. 2021. Seagrass status and trends update for the northern Gulf of Mexico: 2002-2017. Final

Report, Gulf of Mexico Alliance, Ocean Springs, MS, USA. https://doi.org/10.3133/sir20065287

Hastings, R.A., L.A. Rutterford, J.J. Freer, R.A. Collins, S.D. Simpson, and M.J. Genner. 2020. Climate change drives poleward increases and equatorward declines in marine species. Current Biology 30:1572–1577. https://doi.org/10.1016/j.cub.2020.02.043

Hatch, K A. 2012. The use and application of stable isotope analysis to the study of starvation, fasting, and nutritional stress in animals. In: M. McCue, ed. Comparative Physiology of Fasting, Starvation, and Food Limitation. Springer, Berlin, p. 337–364. https://doi.org/10.1007/978–3–642–29056–5_20

Hauxwell, J., C.W. Osenberg, and T.K. Frazer. 2004. Conflicting management goals: Manatees and invasive competitors inhibit restoration of a native macrophyte. Ecological Applications 14:571–586. https://doi.org/10.1890/02–5250

Heck, K., Jr., F. Fodrie, S. Madsen, C. Baillie, and D. Byron. 2015. Seagrass consumption by native and a tropically associated fish species: Potential impacts of the tropicalization of the northern Gulf of Mexico. Marine Ecology Progress Series 520:165–173. https://doi.org/10.3354/meps11104

Hieb, E.E., R.H. Carmichael, A. Aven, C. Nelson–Seely, and N. Taylor. 2017. Sighting demographics of the West Indian manatee *Trichechus manatus* in the north–central Gulf of Mexico supported by citizen–sourced data. Endangered Species Research 32:321–332. https://doi.org/10.3354/esr00817

Kurle, C.M. 2009. Interpreting temporal variation in omnivore foraging ecology via stable isotope modelling. Functional Ecology 23:733–744. https://doi.org/10.1111/j.1365–2435.2009.01553.x

Kurle, C.M., P.L. Koch, B.R. Tershy, and D.A. Croll. 2014. The effects of sex, tissue type, and dietary components on stable isotope discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) in mammalian omnivores. Isotopes in Environmental and Health Studies 50:307–321. https://doi.org/10.1080/10256016.2014.908872

Lefebvre, L.W., J.P. Reid, W.J. Kenworthy, and J.A. Powell. 1999. Characterizing manatee habitat use and seagrass grazing in Florida and Puerto Rico: Implications for conservation and management. Pacific Conservation Biology 5:289–298. https://doi.org/10.1071/PC000289

Lefebvre, L.W., J.A. Provancha, D.H. Slone, and W.J. Kenworthy. 2017. Manatee grazing impacts on a mixed species seagrass bed. Marine Ecology Progress Series 564:29–45. https://doi.org/10.3354/meps11986

Littles, C.J., R.K. Bonde, S.M. Butler, C.A. Jacoby, S.K. Notestein, J.P. Reid, D.H. Slone, and T.K. Frazer. 2019. Coastal habitat change and marine megafauna behavior: Florida manatees encountering reduced food provisions in a prominent winter refuge. Endangered Species Research 38:29–43. https://doi.org/10.3354/esr00933

Marshall, C., G. Huth, V. Edmonds, D. Halin, and R. Reep. 1998. Prehensile use of perioral bristles during feeding and associated behaviors of the Florida manatee (*Trichechus manatus latirostris*). Marine Mammal Science 14:274–289. https://doi.org/10.1111/j.1748–7692.1998.tb00716.x

Marth, E.C., C.S. Cloyed, and R.H. Carmichael. 2023. Identifying stable isotope patterns among taxa, sites, and environmental variables in the eastern Mississippi Sound. Gulf and Caribbean Research 34:SC6–SC12. https://doi.org/10.18785/gcr.3401.02

Martínez del Rio, C., N. Wolf, S.A. Carleton, and L Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84:91–111. https://doi.org/10.1111/j.1469–185x.2008.00064.x

Martony, M., J.A. Hernandez, M. de Wit, J.S. Leger, C. Erlacher–Reid, J. Vandenberg, and N.I. Stacy. 2019. Clinicopathological prognostic indicators of survival and pathological findings in cold–stressed Florida manatees *Trichechus manatus latirostris*. Diseases of aquatic organisms 132:85–97. https://doi.org/10.3354/dao03306

Moncreiff, C.A. 2007. Statewide summary for Mississippi. In: D. Altsman and R. DeMay, eds. Seagrass Status and Trends in the Northern Gulf of Mexico: 1940–2002. United States of Geological Survey, Florida–Caribbean Water Science Center, Tampa, FL, USA, p. 72–74. https://doi.org/10.3133/sir20065287

Moncreiff, C.A. and M.J. Sullivan. 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: Evidence from multiple stable isotope analyses. Marine Ecology Progress Series 215:93–106. https://doi.org/10.3354/meps215093

Morales–Vela, B., D. Olivera–Gómez, J.E. Reynolds III, and G.B. Rathbun. 2000. Distribution and habitat use by manatees (*Trichechus manatus manatus*) in Belize and Chetumal Bay, Mexico. Biological Conservation 95:67–75. https://doi.org/10.1016/S0006–3207(00)00009–4

Newsome, S.D., G.B. Bentall, M.T. Tinker, O.T. Oftedal, K. Ralls, J.A. Estes, and M.L. Fogel. 2010. Variation in $\delta^{13}C$ and $\delta^{15}N$ diet-vibrissae trophic discrimination factors in a wild population of California sea otters. Ecological Applications 20:1744–1752. https://doi.org/10.1890/09–1502.1

Osland, M.J., P.W. Stevens, M.M. Lamont, R.C. Brusca, K.M. Hart, J.H. Waddle, C.A. Langtimm, C.M. Williams, B.D. Keim, and A.J. Terando. 2021. Tropicalization of temperate ecosystems in North America: The northward range expansion of tropical organisms in response to warming winter temperatures. Global Change Biology 27:3009–3034. https://doi.org/10.1111/gcb.15563

Patterson, H.K. and R.H. Carmichael. 2018. Dissolved oxygen concentration affects $\delta^{15}N$ values in oyster tissues: Implications for stable isotope ecology. Ecosphere 9:e02154. https://doi.org/10.1002/ecs2.2154

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review in Ecology and Systematics 18:293–320. https://doi.org/10.1146/annurev.es.18.110187.001453

Pham, L.T., P.D. Biber, and G.A. Carter. 2014. Seagrasses in the Mississippi and Chandeleur Sounds and problems associated with decadal–scale change detection. Gulf of Mexico Science 32:3. https://doi.org/10.18785/goms.3201.03

Pinsky, M.L., R.L. Selden, and Z.J. Kitchel. 2020. Climate–driven shifts in marine species ranges: Scaling from organisms to

communities. Annual Review of Marine Science 12:153–179. https://doi.org/10.1146/annurev—marine—010419—010916

Reich, K.J. and G.A. Worthy. 2006. An isotopic assessment of the feeding habits of free—ranging manatees. Marine Ecology Progress Series 322:303–309. http://dx.doi.org/10.3354/meps322303

Stegall, V.K., S.D. Farley, L.D. Rea, K.W. Pitcher, R O. Rye, C.L. Kester, C.A. Stricker, and C.R. Bern. 2008. Discrimination of carbon and nitrogen isotopes from milk to serum and vibrissae in Alaska Steller sea lions (*Eumetopias jubatus*). Canadian Journal of Zoology 86:17–23. https://doi.org/10.1139/Z07—115

Stricker, C.A., A.M. Christ, M.B. Wunder, A.C. Doll, S.D. Farley, L.D. Rea, D.A. Rosen, R. Scherer, and D.J. Tollit. 2015. Stable carbon and nitrogen isotope trophic enrichment factors for Steller sea lion vibrissae relative to milk and fish/invertebrate diets. Marine Ecology Progress Series 523:255–266. https://doi.org/10.3354/meps11205

Tyrrell, L.P., S.D. Newsome, M.L. Fogel, M. Viens, R. Bowden, and M.J. Murray. 2013. Vibrissae growth rates and trophic discrimination factors in captive southern sea otters (*Enhydra lutris nereis*). Journal of Mammalogy 94:331–338. https://doi.org/10.1644/12—MAMM—A—035.1

Vaslet, A., D. Phillips, C. France, I.C. Feller, and C.C. Baldwin. 2012. The relative importance of mangroves and seagrass beds as feeding areas for resident and transient fishes among different mangrove habitats in Florida and Belize: Evidence from dietary and stable—isotope analyses. Journal of Experimental Marine Biology and Ecology 434:81–93. https://doi.org/10.1016/j.jembe.2012.07.024

Wang, Z., T. Boyer, J. Reagan, and P. Hogan. 2023. Upper—oceanic warming in the Gulf of Mexico between 1950 and 2020. Journal of Climate 36:2721–2734. https://doi.org/10.1175/JCLI—D—22—0409.1

Wilson, R.M., R.B. Tyson, J.A. Nelson, B.C. Balmer, J.P. Chanton, and D.P. Nowacek. 2017. Niche differentiation and prey selectivity among common bottlenose dolphins (*Tursiops truncatus*) sighted in St. George Sound, Gulf of Mexico. Frontiers in Marine Science 4:235. https://doi.org/10.3389/fmars.2017.00235

Wissel, B., A. Gaçe, and B. Fry. 2005. Tracing river influences on phytoplankton dynamics in two Louisiana estuaries. Ecology 86:2751–2762. https://doi.org/10.1890/04—1714

Zhao, L. and D.M. Schell. 2004. Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: Effects of growth patterns on ecological records. Marine Ecology Progress Series 281:267–273. https://doi.org/10.3354/meps281267