Latin American Journal of Aquatic Mammals

ISSN: 2236-1057 | Vol. 21 No. 2, October 2026

Inferring gestational diet in free-ranging Greater Caribbean manatee (*Trichechus manatus manatus*) using newborn hair isotopic signatures

Helena Gurjão Pinheiro do Val[1,*], Lucas Inácio dos Santos Melo[2], Vitor Luz Carvalho[3], Aline Ramos Souza[3], Larissa Rosa de Oliveira[3], Fernanda Loffler Niemeyer Attademo[2,4], Fábia de Oliveira Luna[2], and Marcelo Zacharias Moreira[5]

[1]Programa de Pós-graduação em Ecologia, Universidade de São Paulo, São Paulo, Brasil
[2]Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos, Instituto Chico Mendes para Conservação da Biodiversidade, Ilha de Itamaracá, Brasil
[3]Associação de Pesquisa e Preservação de Ecossistemas Aquáticos, Caucaia, Brasil
[4]Universidade Federal Rural do Semi-Árido, Mossoró, Brasil
[5]Centro de Energia Nuclear na Agricultura, Universidade de São Paulo/ Campus Luiz de Queiroz, Piracicaba, Brasil

*Corresponding author: helena.gurjao@yahoo.com.br

Abstract

Species feeding habits can be accessed in a variety of ways, such as fecal samples and stomach contents. However, these methods reflect only the individual's recent diet, disregarding a broader perspective of feeding habits along a temporal scale. Such differences can be evaluated by analyzing inert biological samples, like hair and skin tissues, which absorb and preserve isotopic signatures over time. In this study we analyzed hair samples from nine stranded manatee calves, in order to trace their mothers' diet during gestation, an innovative attempt for this species which is currently classified as Critically Endangered of extinction in Brazil. Our sample, however small, contained data distributed along four Brazilian states, covering half of the current occurrence area of the species in the Brazilian coast. $\delta^{13}C$ values showed variability among calves, particularly in individuals aged 0.9 - 1.5 months. In contrast $\delta^{15}N$ values, carbon and nitrogen concentrations presented very similar values, regardless of calf age. $\delta^{13}C$ values had a strong positive correlation with size at stranding even when controlled by age, suggesting that enriched $\delta^{13}C$ signatures correspond to the production of larger calves. Despite our small sample size, here we present a substantial contribution to the knowledge of the diet of free-ranging females, use of habitat during pregnancy and how it varies according to resource availability. Furthermore, our findings support the hypothesis that nutritional intake during gestation periods might have a long-term impact on calf growth and development.

Keywords:
calf, habitat use, maternal, $\delta^{13}C$, $\delta^{15}N$, Sirenian

ARTICLE INFO

Manuscript type: Article

Article History
Received: 24 July 2026
Received in revised form: 31 August 2026
Accepted: 31 August 2026
Available online: 07 October 2026
Handling Editor: Nataly Castelblanco Martinez

Citation:
do Val, H. G. P., Melo, L. I. S., Carvalho, V. L., Souza, A. R., Oliveira, L. R., Attademo, F. L. N., Luna, F. O., & Moreira, M. Z. (2026). Inferring gestational diet in free-ranging Greater Caribbean manatee (*Trichechus manatus manatus*) using newborn hair isotopic signatures. *Latin American Journal of Aquatic Mammals, 21*(2), 91-97. https://doi.org/10.5597/lajam00374

Introduction

The Greater Caribbean manatee (*Trichechus manatus manatus* Linnaeus, 1758) is an aquatic mammal from the order Sirenia, which is currently classified as Critically Endangered of extinction in Brazil (Attademo et al., 2024). Its occurrence area in the country ranges from the coastal and estuarine areas from Amapá to Alagoas states, where the species inhabits shallow waters and feeds on seagrasses and freshwater vegetation (Borges et al., 2008; Alves et al., 2013; Normande et al., 2016). Studies in the last decade have indicated that female manatees use estuaries and mangrove ecosystems to give birth and care for their calves during their first stages of life, as those function as shelter habitats from the tidal waves and marine currents (Balensiefer et al., 2017).

Feeding habits of wild manatees can be analyzed in a variety of ways, from direct to indirect observations, such as faecal samples and stomach contents (*e.g.* Allen et al., 2022; Arévalo-González et al., 2026). However, these methods capture only a 'snapshot' of an individual's recent dietary intake, generally covering a period of approximately three days, disregarding a broader perspective of feeding habits and how it can change over months (Boecklen

et al., 2011). Contrasting to other traditional methodologies, the isotopic approach uses stable isotope signatures of non-radioactive elements (*e.g.* carbon, nitrogen, and other elements) that are fixed in biological tissues, such as blood (Jenkins et al., 2001), hair (Stegall et al., 2008; Magioli et al., 2019), skin (Gelippi et al., 2020), and bones (York et al., 2008, Bacalan et al., 2018) to yield information on diet, use of habitat, and even movement of individuals (Smith et al., 2021). The most used elements, carbon and nitrogen, are related to the type of vegetation source (*e.g.* seagrass, algae, freshwater plants) (Seminoff et al., 2006) and trophic level and enrichment (Duarte et al., 2018), respectively allowing researchers to observe how feeding habits can vary within populations of the same species (Navarro et al., 2021).

Researchers have used stable isotopes analysis to evaluate diet of individuals both directly and indirectly. The first occurs when the samples are derived from the studied individual (Ben-David & Flaherty 2012; Bacalan et al., 2018; Magioli et al., 2023), while the indirect happens when attempting to track one's diet using another individual's sample, such as milk and via placental relationship between mother and the offspring (Gelippi et al., 2020; Cherney et al., 2021; Ruiz-Sagalés et al., 2025). Although stable isotopic analysis has become popular among the scientific community in the last decades and spreading through different taxa (Newsome et al., 2010; Niella et al., 2021; Jaroměřská et al., 2023), such an approach is still poorly explored to study manatees. Studies based on stable isotope analysis have been conducted for sirenian species such as dugong (*Dugong dugong*) (Thibault et al., 2024), Amazonian manatee (*T. inunguis*) (Carvalho et al., 2022), and the Greater Caribbean manatee (*T. manatus manatus*) (Garcés-Cuartas et al., 2025). In Brazil, studies with the Greater Caribbean manatee are restricted to bone and tooth samples (Ciotti, 2012; Carvalho et al., 2026) to analyze isotopic signature from deceased free ranging adults. To the best of our knowledge, there has been no attempt so far to investigate gestational diet by rescued calves' hair isotope signature. However, fetal $\delta^{15}N$ and $\delta^{13}C$ values in keratin structures closely align with maternal signatures, suggesting that the isotopic decomposition might occur simultaneously between individuals in baleen whales (Ruiz-Sagalés et al., 2025), and maximum maternal-fetus isotopic difference is estimated at ~1.4‰ for $\delta^{13}C$ and between 1 and 1.5‰ for $\delta^{15}N$ values for epidermal tissues in whales (Gelippi et al., 2020).

The present study aims to 1) evaluate pregnant female manatees' diet composition and use of habitat by analyzing the isotope values of newborn manatee calves as a proxy for their mothers' feeding habits throughout pregnancy and; 2) verify potential relationships between mother feeding choice and calf body size.

Materials and Methods

Data collection

The hair samples were collected from 2023 to 2026 by two Brazilian institutions responsible for rescuing and rehabilitating stranded Greater Caribbean manatees on the Brazilian coast: Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (CMA/ICMBio) and Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (Aquasis). The samples were collected during calves' aid and care after stranding, cutting the bulk of calves' hair along their body. Samples were cleaned with alcohol and preserved on labeled eppendorf vials. Sample size had a mean of 0.464 mg (range 0.228 - 0.698 mg).

Data analysis

Manatees are born with hair. Considering their early age, the contamination with consumed vegetables in the hair tissue is quite low, suggesting that isotopic values reflect their mothers' feeding. The bulk analysis was performed following the procedures of Magioli et al. (2023), which consisted of storing the samples in tin capsules and combusting each capsule individually. The gases originated by the combustion were separated in a chromatographic column and loaded on a continuous flow isotope ratio mass spectrometer (Delta Plus, Thermo Scientific, Bremen, Germany), which provides the isotopic composition of the samples for both carbon and nitrogen. The isotopic values of those elements were indicated in delta notation ($\delta^{13}C$, $\delta^{15}N$) in parts per thousand (‰) following the V-PDB (Vienna-Pee Dee Belemnite) and atmospheric N^2 standards, respectively. Delta values were calculated based on the standards using the adopted

Table 1. Data on sampled calves. Calf ID: identification code; Stranding site: specific site/Brazilian state abbreviation); Size strand.: size at stranding, in cm; Est. age: estimated age at stranding, in months; sex: M:male/F:female; and stable isotopic values of $\delta^{13}C$ and $\delta^{15}N$ (‰), %C: percentage of carbon in the hair sample; C/N: carbon and nitrogen ratio in the sample. CE: Ceará, MA: Maranhão, PB: Paraíba; PI: Piauí

Calf ID	Stranding site	Size strand.	Est. age	Sex	$\delta^{1}5N$	%N	$\delta^{13}C$	%C	C/N
01S0111/380	Praia de Santo Amaro/ MA	124.5	1.5	M	7.13	12.61	-14.89	41.45	3.28
01S0111/381	Praia da Pedra de Sal/ PI	126	1.0	M	6.66	13.84	-9.63	43.29	3.12
01S0112/382	Praia de Atalaia/ PI	117	0.9	F	6.24	13.49	-13.38	42.53	3.15
01S0111/385	Praia do Oiteiro/ PB	93	0.1	M	8.10	9.15	-20.63	36.52	3.99
02S0111/114	Praia de Canoa Quebrada/ CE	139	0.1	M	5.63	13.98	-10.47	47.38	3.38
02S0112/120	Praia de Melancias/ CE	124	0.1	F	6.19	13.61	-10.60	45.07	3.31
02S0111/123	Praia de Peroba/ CE	140	0.1	M	5.43	13.54	-10.00	44.34	3.27
02S0112/124	Praia de Carnaubinhas/ PI	123	0.1	F	5.10	13.98	-10.48	44.59	3.18
02S0111/125	Praia do Uruaú/ CE	144	0.1	M	6.71	13.69	-10.09	41.15	3.00

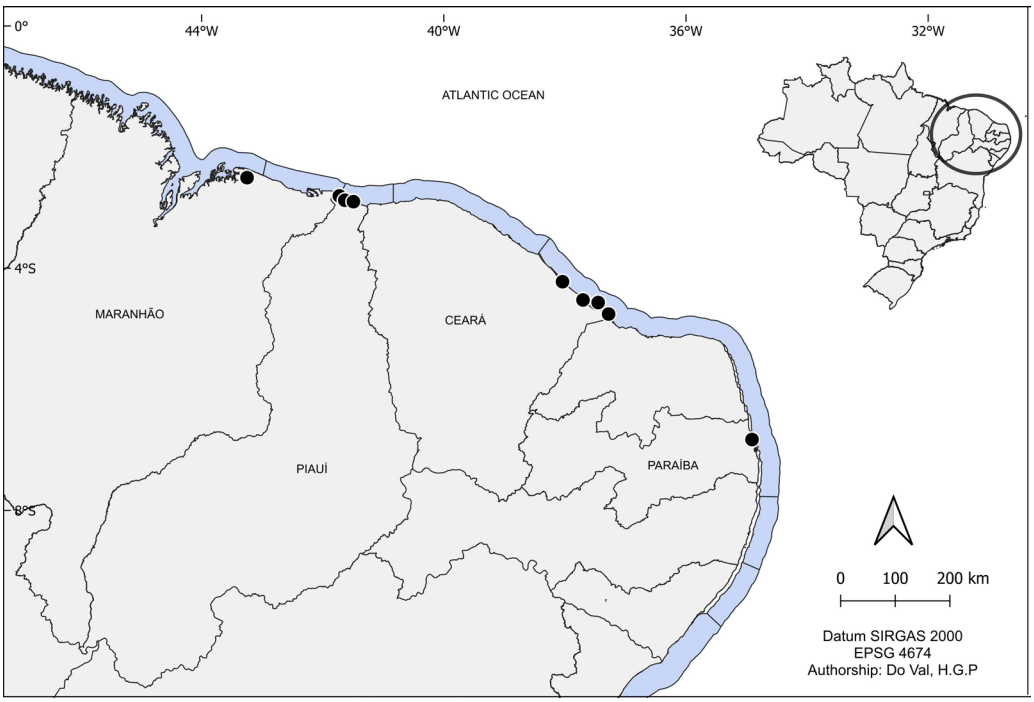


Figure 1. Map of the northeast Brazilian coast, indicating the Greater Caribbean manatee (*Trichechus manatus manatus*) stranding sites in black dots and the species distribution in light blue (IUCN, 2023).

equation δX = [(Rsample/Rstandard) − 1] multiplied by 1000, where X represents the stable carbon or nitrogen isotopes (^{13}C or ^{15}N), and R the isotopes ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

Age was estimated according to the evaluation of umbilical cord fragments and how fresh tail wrinkles were, given that manatee calves' tails stay bent in the uterus until birth. Size at stranding correspond to the total length from nostrils to end of the tail.

After exploratory analysis, we performed two multiple regression models using the *'lm( )'* function from R software version 4.5.3 (R Core Team, 2015), to verify how total body length (CT) is influenced by $\delta^{13}C$, $\delta^{15}N$ and estimated age, as follows: i) CT ~ $\delta^{13}C$ + Age and ii) CT ~ $\delta^{15}N$ + Age. We also employed a Pearson's correlation test to ensure association between body size and delta values, removing the effect of a potential confounding variable.

Results

Hair samples from nine individuals distributed along four Brazilian states were analysed in this study: one in Maranhão (MA), one in Paraíba (PB), three in Piauí (PI) and four in Ceará (CE) (Figure 1).

$\delta^{13}C$ values showed variability among calves, particularly in individuals aged 0.9 - 1.5 month. In contrast, $\delta^{15}N$ values, carbon and nitrogen concentration presented very similar values regardless of calf age. An outlier was observed and the interpretation of those values compared to the whole sample are thoroughly discussed. Table 1 presents calf's data as identification, origin, biometry, and hair isotopic signature for the nine individuals.

Regarding body size relationship with food isotopic signature, the multiple regression analysis showed that although $\delta^{15}N$ has no significant effect on stranding size (p = 0.10), a strong positive correlation was observed between $\delta^{13}C$ and size at stranding (r = 0.85, p = 0.0036). This relationship remained significant after controlling for age in multiple regression (β = 3.61 ± 0.92 SE, p = 0.0078), while age had no detectable effect (p = 0.995). Those results suggest that body size is somehow related to enriched (less negative) $\delta^{13}C$ signatures.

Discussion

Our results present a substantial contribution on the gestational health profile of free ranging manatees, by showing data on biochemical (%N and %C) and source level ($\delta^{15}N$ and $\delta^{13}C$), of a quite elusive and difficult species to study in the wild. Six of the nine neonates were nearly three days old when they stranded, and so the obtained values mirrored the maternal late-term pregnancy diet.

The most important result concerns an outlier (calf 01S0111/385), which in comparison with the other five individuals of the same age, needed to be regarded carefully. While carbon and nitrogen ratios provide information on food quality, the delta values account for the source and its trophic level, as a chemical fingerprint (Duarte et al., 2018). Considering the age of the six individuals within 0.1 month (Table 1), it would be expected that if all calves had the same nursing period before stranding and if the environment was homogeneous, $\delta^{15}N$ would not have such variation. The higher $\delta^{15}N$ (8.10‰) compared to other calves might be related to specific features of the aquatic environment for this calf, such as anthropogenic waste (McClelland & Valiela 1997, 1998; Cole et al., 2004; Bertelli et al., 2020); inhibition of nitrifying bacteria (Tino et al., 2025) nutritional stress (Kempster et al., 2007; Loudon et al., 2007), and length of starvation (Doi et al., 2017). In the latter context, $\delta^{15}N$ values can be applied to

monitor not only dietary intake but also the nitrogen balance within the individuals (Fuller et al., 2004). As for $\delta^{13}C$, it yields information on the type of plants that are being consumed by the primary consumer (in this case, the mother) (Seminoff et al., 2006). Seagrasses and marine algae generally have $\delta^{13}C$ values ranging from -10.6‰ to -16.6‰, respectively, contrasting to values from mangroves, which are more carbon depleted, around -28.6‰ (Garcés-Cuartas et al., 2021). Thus, the outlier calf (-20.63) suggests a variability in the female's diet, including resources from mangroves, probably while inhabiting canals, instead of marine open waters (Alves-Stanley et al., 2010). In the present study, due to the limited size of the hair samples (< 1 cm), we did not discriminate isotopic composition along hair length, which could have resulted in a higher resolution of change in female diet (Tucker et al., 2024).

Our data on both $\delta^{13}C$ and $\delta^{15}N$ are also spatially corroborated by Carvalho et al. (2026), who found similar delta values for manatees living on the coast of Ceará, Piauí, and Paraíba states. However, the $\delta^{13}C$ value found for the Paraíba outlier calf was still extreme compared to the others. Data on distribution of seagrass meadows from Deeks et al. (2024) show a large concentration of seagrass resources in the State of Ceará (118 km²), compared to Paraíba (13 km²), which in turn had a higher proportion of coral reefs. This data, along with our results on $\delta^{13}C$, indicates that female manatees might indeed rely on different substrates given their occurrence and distribution on the Brazilian coast, reflecting spatial heterogeneity in foraging habitats (Fry, 2006; Newsome et al., 2010).

Furthermore, variation in $\delta^{13}C$ and the relationships with body mass can be attributed to variability in carbon sourcing (Wolf et al., 2024). Mangrove and freshwater vegetation has consistently less crude protein than marine seagrass and algae (Rodrigues et al., 2021; Arévalo-González et al., 2024). Recent studies on livestock animals have demonstrated that protein-restricted environments can have several impacts on offspring health, such as on metabolism, immunity, reproduction, and gut health (Xiao et al., 2025); fetal growth retardation and compromised glucose regulation in early life (Maresca et al., 2018); and offspring birth weight, weaning weight, and daily weight gain (Barcelos et al., 2022). Also, this nutritional effect might have an extended impact on calves' body condition not only at birth, but probably in the long-term. An empirical observation regarding the small size of a manatee calf born in semi-captivity and its progenitor's diet (mangrove plants) and calf 01S0111/385 are examples that support such a hypothesis. The outlier calf (01S0111/385) was the smallest one at stranding time (93 cm), far from the mean size for neonates (100 - 120 cm) (Attademo et al., 2022); and, in spite of diet and health assistance, its growth is slower compared to other offspring of the same age (L.I.S. Melo, pers. obs.).

In conclusion, our study contributes to the knowledge of the pregnancy diet, habitat use, and how resource availability influences these behaviors in free-ranging Greater Caribbean manatee females. However, we also acknowledge the remaining important gaps such as the temporal scale of diet shifts and the triggers of such change (*e.g.*, water temperature and salinity, pregnancy stages). As this was a pilot study, we present only preliminary data and invite researchers to delve deeper into this ecological approach, to improve our knowledge of this elusive and threatened species. Furthermore, our findings open the hypothesis that nutritional intake during gestation periods might impact long-term calf growth and development, thus being a good source of information for rescuing and rehabilitating programs.

Acknowledgments

We would like to acknowledge the teams from both institutions, especially Cássia Farias (CMA/ICMBio) for the outstanding help throughout this project. This work was approved by Instituto Chico Mendes para Conservação da Biodiversidade (ICMBio). Research Permit SISBIO 93999-1 relates to the permit for collection and use of manatee biological samples. The collection of biological samples conducted by Aquasis was supported by the Potiguar Basin Beach Monitoring Programme (PMP-BP), conducted by Petrobras and licensed by IBAMA of the Brazilian Ministry of the Environment.

References

Allen, A. C., Beck, C. A., Sattelberger, D. C., & Kiszka, J. J. (2022). Evidence of a dietary shift by the Florida manatee (*Trichechus manatus latirostris*) in the Indian River Lagoon inferred from stomach content analyses. *Estuarine, Coastal and Shelf Science, 268*, 107788. https://doi.org/10.1016/j.ecss.2022.107788

Alves, R. R. N., Sassi, R., & Santana, G. G. (2013). The mangrove forest at the Bucatu Lagoon, Northeast Brazil: structural characterization and anthropic impacts. *Tropical Conservation Science, 6*(2), 254-267. https://doi.org/10.1177/194008291300600207

Alves-Stanley, C. D., Worthy, G. A. J., & Bonde, R. K. (2010). Feeding ecology of Florida manatees (*Trichechus manatus latirostris*) as replicated by stable isotope analysis. *Marine Ecology Progress Series, 402*, 255-267. https://doi.org/10.3354/meps08450

Arévalo-González, K., Castelblanco-Martínez, N., Serrano, A., & Pereira-Corona, A. (2024). Nutritional composition of manatee's diet in the Western Caribbean: from the river to the reef. *Ethology Ecology & Evolution, 37*(3), 247–264. https://doi.org/10.1080/03949370.2024.2411506

Arévalo-González, G. K., Jiménez-Pino, N., Alviz, A., Lancheros-Redondo, O. H., & Castelblanco-Martínez, D. N. (2026). First look at the Greater Caribbean manatee's diet in the Magdalena River Basin, Colombia, through fecal analysis. *Mammalian Biology, 106*, 9-14. https://doi.org/10.1007/s42991-025-00544-5

Attademo, F. L. N., Miranda, A. V., Torres-Florez, J. P., Sousa, G. P., Fruet, P. F., & Luna, F. O. (2022). *Atendimento a Peixes-Bois Encalhados e Transporte de Filhotes* (Nº 978-65-5693-043-5). ICMBio. https://www.gov.br/icmbio/pt-br/centrais-de-conteudo/publicacoes/publicacoes-diversas/outros/Protocolo_Atendimento_a_Peixes_Bois_Encalhados_e_Transporte_de_Filhotes1_compressed.pdf

Attademo, F. L. N., Borges, J. C. G., Marmontel, M., Lucchini, K. M. S., Meirelles, A. C. O., Farro, A. P. C., Barreto, A. S., Andriolo, A., Kolesnikovas, C. K. M., Luna, F. O., Perez, F. S., Silva, F. J. L., Cunha, H. A., Lucena, J. J. C., Brito Jr, J. L., Di Tullio, J.

C., Groch, K. R., Pavanelli, L., Oliveira, L. R., Wedekin, L. L., Rosa, L. D., Frias, M. P., Cremer, M. J., Marcondes, M. C. C., Fruet, P. F., Botta, S., Silva, V. M. F., Gravena, W., Kantek, D. L. Z., Nogueira, I. X. B., Albuquerque, L., Ciotti, L. L., & Domit, C. (2024). *Trichechus manatus*. Sistema de Avaliação do Risco de Extinção da Biodiversidade. Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio. https://doi.org/10.37002/salve.ficha.14523.3

Bacalan, V., Poinsatte, T., Baker, D. M., Fogel, M. L., & Kim, K. (2018). Stable isotope analyses of manatee bones measure historical nitrogen pollution in Florida waters, 1975–2010. *Marine Biology, 165*, 85. https://doi.org/10.1007/s00227-018-3349-4

Balensiefer, D. C., Attademo, F. L. N., Sousa, G. P., Freire, A. C. B., Cunha, F. A. G. C., Alencar, A. E. B., Silva, F. J. S., & Luna, F. O. (2017). Three decades of Antillean manatee (*Trichechus manatus manatus*) stranding along the Brazilian coast. *Tropical Conservation Science, 10*, 1-9. https://doi.org/10.1177/1940082917728375

Barcelos, S. S., Nascimento, K. B., Silva, T. E. D., Mezzomo, R., Alves, K. S., de Souza Duarte, M., & Gionbelli, M. P. (2022). The effects of prenatal diet on calf performance and perspectives for fetal programming studies: a meta-analytical investigation. *Animals, 12*(16), 2145. https://doi.org/10.3390/ani12162145

Ben-David, M., & Flaherty, E. A. (2012). Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy, 93*(2), 312-328. https://doi.org/10.1644/11-MAMM-S-166.1

Bertelli, C. M., Creed, J. C., Nuuttila, H. K., & Unsworth, R. K. F. (2020). The response of the seagrass *Halodule wrightii* Ascherson to environmental stressors. *Estuarine, Coastal and Shelf Science, 238*, 106693. https://doi.org/10.1016/j.ecss.2020.106693

Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution and Systematics, 42*, 411-440. https://doi.org/10.1146/annurev-ecolsys-102209-144726

Borges, J. C. G., Araújo, P. G., Anzolin, D. G., & De Miranda, G. E. C. (2008). Identificação de itens alimentares constituintes da dieta dos peixes-boi marinhos (*Trichechus manatus*) na região Nordeste do Brasil. *Biotemas, 21*(2), 77-81. https://doi.org/10.5007/2175-7925.2008v21n2p77

Carvalho, C. C., Marmontel, M., Botta, S., Emin-Lima, R., Costa, A. F., Crema, L. C., Silva, V. M. F., Piedade, M. T. F., & Secchi, E. R. (2022). Spatio-temporal, ontogenetic and sex-related patterns in resource use of Amazonian manatees across floodplains and estuaries as inferred by $\delta^{13}C$ and $\delta^{15}N$ isotopic values. *Aquatic Conservation: Marine and Freshwater Ecosystems, 32*(6), 967-980. https://doi.org/10.1002/aqc.3833

Carvalho, C. C., Deeks, E., Ciotti, L. L., Botta, S., Marmontel, M., Copertino, M., Luna F. O., Attademo, F. N. L., Carvalho, V. L., Borges, J. C. G., Costa, A. F., Emin-Lima, R., Kratina, P., & Secchi, E. R. (2026). American manatees adjust their diet composition and trophic niche breadth across different coastal regions. *Limnology and Oceanography Letters, 11*(3), e70127. https://doi.org/10.1002/lol2.70127

Cherney, M. D., Fisher, D. C., Hren, M. T., & Shirley, E. A. (2021). Stable isotope records of nursing and weaning: A case study in elephants with implications for paleobiological investigations. *Palaeogeography, Palaeoclimatology, Palaeoecology, 567*, 110223. https://doi.org/10.1016/j.palaeo.2021.110223

Ciotti, L. L. (2012). *Isótopos estáveis de carbono e nitrogênio aplicados ao estudo da ecologia trófica do peixe-boi marinho* (Trichechus manatus) *no Brasil.* [Doctoral dissertation, Universidade Federal do Rio Grande].

Cole, M. L., Kroeger, K. D., McClelland, J. W., & Valiela, I. (2004). Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. *Journal of Environmental Quality, 33*(1), 124–132. https://doi.org/10.2134/jeq2004.1240

Deeks, E., Magalhães, K., Traganos, D., Ward, R., Normande, I., Dawson, T. P, & Kratina, P. (2024). Seagrass mapping of north-eastern Brazil using Google Earth Engine and Sentinel-2 imagery. *Environmental and Sustainability Indicators, 24*, 100489. https://doi.org/10.1016/j.indic.2024.100489

Doi, H., Akamatsu, F., & González, A. L. (2017). Starvation effects on nitrogen and carbon stable isotopes of animals: an insight from meta-analysis of fasting experiments. *Royal Society of Open Science, 4*(8),170633. https://doi.org/10.1098/rsos.170633

Duarte, C. M., Delgado-Huertas, A., Anton, A., Carrillo-de-Albornoz, P., López-Sandoval, D. C., Agustí, S., Almahasheer, H., Marbá, N., Hendriks, I. E., Krause-Jensen, D., & Garcias-Bonet, N. (2018). Stable isotope $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, δD composition and nutrient concentration of Red Sea primary producers. *Frontiers in Marine Science, 5*, 298. https://doi.org/10.3389/fmars.2018.00298

Fry, B. (2006). *Stable Isotope Ecology*. Springer.

Fuller, B. T., Fuller, J. L., Sage, N. E., Harris, D. A., O'Connell, T. C., & Hedges, R. E. M. (2004). Nitrogen balance and $\delta^{15}N$: why you're not what you eat during pregnancy. *Rapid Communications in Mass Spectrometry, 18*(23), 2889-2896. https://doi.org/10.1002/rcm.1708

Garcés-Cuartas, N., Niño-Torres, C. A., Castelblanco-Martínez, D. N., Delgado-Huertas, A., Cetz-Navarro, N. P., Ortiz-Pulido, R., & Cuevas, J. (2021). Isotopic composition of aquatic and semiaquatic plants from the Mexican Caribbean: A baseline for regional ecological studies. *Estuarine, Coastal and Shelf Science, 260*, 107489. https://doi.org/10.1016/j.ecss.2021.107489

Garcés-Cuartas, N., Niño-Torres, C. A., Castelblanco-Martínez, D. N., Delgado-Huertas, & Cuevas, J. (2025). Stable isotope analysis in bone collagen reveals new insights into the feeding ecology of free-ranging manatees in the Mexican Caribbean. *Marine Ecology Progress Series, 758*, 179-194. https://doi.org/10.3354/meps14826

Gelippi, M., Popp, B., Gauger, M. F. W., & Caraveo-Patiño, J. (2020). Tracing gestation and lactation in free ranging gray whales using the stable isotopic composition of epidermis layers. *PLoS ONE, 15*(10), e0240171. https://doi.org/10.1371/journal.pone.0240171

International Union for Conservation of Nature - IUCN. (2023). *Trichechus manatus*. The IUCN Red List of Threatened Species. https://www.iucnredlist.org.

Jaroměřská, T. N., Ambrosini, R., Mazurkiewicz, M., Franzetti, A., Klimaszyk, P., Rozwalak, P., Poniecka, E., Vondrovicová, L., & Zawierucha, K. (2023). Spatial distribution and stable isotopic composition of invertebrates uncover differences between habitats on the glacier surface in the Alps. *Limnology,*

24, 83–93. https://doi.org/10.1007/s10201-023-00713-w

Jenkins, S. G., Partridge, S. T., Stephenson, T. R., Farley, S. D., & Robbins, C. T. (2001). Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia, 129*, 336–341. https://doi.org/10.1007/s004420100755

Kempster, B., Zanette, L., Longstaffe, F. J., MacDougall-Shackleton, S. A., Wingfield, J. C., & Clinchy, M. (2007). Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. *Oecologia, 151*, 365-371. https://doi.org/10.1007/s00442-006-0597-7

Loudon, J. E., Sponheimer, M., Sauther, M. L., & Cuozzo, F. P. (2007). Intraspecific variation in hair $\delta^{13}C$ and $\delta^{15}N$ values of ring-tailed lemurs (*Lemur catta*) with known individual histories, behavior, and feeding ecology. *American Journal of Physical Anthropology, 133*(3), 978-985. https://doi.org/10.1002/ajpa.20605

Magioli, M., Moreira, M. Z., Fonseca, R. C. B., Ribeiro, M. C., Rodrigues, M. G., & Ferraz, K. M. P. M. B. (2019). Human-modified landscapes alter mammal resource and habitat use and trophic structure. *Proceedings of the National Academy of Sciences, 116*(37), 18466-18472. https://doi.org/10.1073/pnas.1904384116

Magioli, M., Attias, N., Massocato, G., Kluyber, D., Moreira, M. Z., Ferraz, K. M. P. M. B., Chiarello, A. G., & Desbiez, A. L. J. (2023). What a few hairs can tell us about the resource use of giant armadillos. *Integrative Zoology, 18*(1), 129–142. https://doi.org/10.1111/1749-4877.12644

Maresca, S., Lopez Valiente, S., Rodriguez, A. M., Lang, N. M., Pavan, E., & Quintans, G. (2018). Effect of protein restriction of bovine dams during late gestation on offspring postnatal growth, glucose-insulin metabolism and IGF-1 concentration. *Livestock Science, 212*, 120-126. https://doi.org/10.1016/j.livsci.2018.04.009

McClelland, J. W., & Valiela, I. (1997). Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnology and Oceanography, 42*(5), 930-937. https://doi.org/10.4319/lo.1997.42.5.0930

McClelland, J. W., & Valiela, I. (1998). Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography, 43*(4), 577–585. https://doi.org/10.4319/lo.1998.43.4.0577

Navarro, A. B., Magioli, M., Bogoni, J. A., Moreira, M. Z., Silveira, L. F., Alexandrino, E. R., Luz, D. T. A., Pizo, M. A., Silva, W. R., Oliveira, V. C., Donatelli, R. J., Christianini, A. V., Piratelli, A. J., & Ferraz, K. M. P. M. B. (2021). Human-modified landscapes narrow the isotopic niche of Neotropical birds. *Oecologia, 196*, 171-184. https://doi.org/10.1007/s00442-021-04908-9

Newsome, S. D., Clementz, M. T., & Koch, P. L. (2010). Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science, 26*(3), 509-572. https://doi.org/10.1111/j.1748-7692.2009.00354.x

Niella, Y., Raoult, V., Gaston, T., Peddemors, V. M., Harcourt, R., & Smoothey, A. F. (2021). Overcoming multi-year impacts of maternal isotope signatures using multi-tracers and fast turnover tissues in juvenile sharks. *Chemosphere, 269*, 129393. https://doi.org/10.1016/j.chemosphere.2020.129393

Normande, I. C., Malhado, A. C. M., Reid, J., Viana Júnior, P. C., Savaget, P. V. S., Correia, R. A., Luna, F. O., & Ladle, R. J. (2016). Post-release monitoring of Antillean manatees: an assessment of the Brazilian rehabilitation and release programme. *Animal Conservation, 19*(3), 235-246. https://doi.org/10.1111/acv.12236

R Core Team (2015). *R: A language and environment for statistical computing.* [Computer Software]. R Foundation for Statistical Computing. https://www.R-project.org/

Rodrigues, F. M., Marin, A. K. V., Rebelo, V. A., Marmontel, M., Borges, J. C., Vergara-Parente, J. E., & Miyagi, E. S. (2021). Nutritional composition of food items consumed by Antillean manatees (*Trichechus manatus manatus*) along the coast of Paraíba, northeastern Brazil. *Aquatic Botany, 168*, 103324. https://doi.org/10.1016/j.aquabot.2020.103324

Ruiz-Sagalés, M., Borrell, A., Halldórsson, S. D., Chosson, V., Sigurðsson, G. M.,& Aguilar, A. (2025). Multielement stable isotope ratios in the baleen of maternal and fetal fin whales provide evidence for the most recently formed segment of Balaenopteridae baleen. *Marine Mammal Science, 42*(1), e70105. https://doi.org/10.1111/mms.70105

Seminoff, J. A., Jones, T. T., Eguchi, T., Jones, D. R., & 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. *Marine Ecology Progress Series, 308*, 271-278. https://doi.org/10.3354/meps308271

Smith, K. J., Trueman, C. N., France, C. A. M., Sparks, J. P., Brownlow, A. C., Dähne, M., Davison, N. J., Guðmundsson, G., Khidas, K., Kitchener, A. C., Langeveld, B. W., Lesage, V., Meijer, H. J. M., Ososky, J. J., Sabin, R. C., Timmons, Z. L., Víkingsson, G. A., Wenzel, F. W., & Peterson, M. J. (2021) Stable isotope analysis of specimens of opportunity reveals ocean-scale site fidelity in an elusive whale species. *Frontiers in Conservation Science, 2*, 653766. https://doi.org/10.3389/fcosc.2021.653766

Stegall, V. K., Farley, S. D., Rea, L. D., Pitcher, K. W., Rye, R. O., Kester, C. L., Stricker, C. A., & Bern, C. R. (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*(1), 17-23. https://doi.org/10.1139/Z07-115

Thibault, M., Letourneur, Y., Cleguer, C., Bonneville, C., Briand, M. J., Derville, S., Bustamante, P. C., & Garrigue, C. (2024). C and N stable isotopes enlighten the trophic behaviour of the dugong (*Dugong dugon*). *Scientific Reports, 14*(1), 896. https://doi.org/10.1038/s41598-023-50578-3

Tino, C. J., Stüeken, E. E., Gregory, D. D., & Lyons, T. W. (2025). Elevated $\delta^{15}N$ linked to inhibited nitrification coupled to ammonia volatilization in sediments of shallow alkaline-hypersaline lakes. *Geobiology, 23*(2), e70018. https://doi.org/10.1111/gbi.70018

Tucker, L., Russell, M. L., Hieb, E. E., Carmichael, R. H., & Cloyed, C. S. (2024). Stable isotope analysis of manatee vibrissae to investigate individual patterns of resource use in the northern Gulf of Mexico. *Gulf and Caribbean Research, 35*(1), SC12-SC19. https://doi.org/10.18785/gcr.3501.08

Wolf, N., Garcia, S., Harris, B. P., & Howard, K. G. (2024). Stable isotopes, morphology, and body condition metrics suggest similarity in the trophic level and diversity in the carbon sources of freshwater and early marine diets of Chinook

salmon. *Marine Biology, 171*, 75. https://doi.org/10.1007/s00227-024-04392-8

Xiao, S., Liu, W., Zhang, S., & Schroyen, M. (2025). The role of maternal dietary protein on livestock development, production and health. *Animal Reproduction Science, 276*, 107835. https://doi.org/10.1016/j.anireprosci.2025.107835

York, A. E., Thomason, J. R., Sinclair, E. H., & Hobson, K. A. (2008). Stable carbon and nitrogen isotope values in teeth of Steller sea lions: Age of weaning and the impact of the 1975–1976 regime shift in the North Pacific Ocean. *Canadian Journal of Zoology, 86*(1), 33-44. https://doi.org/10.1139/Z07-108