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ISÓTOPOS ESTÁVEIS DE CARBONO E NITROGÊNIO APLICADOS AO ESTUDO DA ECOLOGIA TRÓFICA DO PEIXE-BOI MARINHO (*Trichechus manatus*) NO BRASIL

LEANDRO LAZZARI CIOTTI

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Orientador: Eduardo Resende Secchi

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Mas tenho promessas a cumprir e milhas
a percorrer antes de dormir
(Robert Frost)

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Índice

Resumo

Na costa brasileira, o peixe-boi marinho, *Trichechus manatus*, apresenta distribuição fragmentada e restrita a algumas regiões com características ecológicas distintas. É considerado um herbívoro generalista e oportunista, que consome uma ampla variedade de vegetação de rios, mares e estuários, porém, existem poucas informações sobre a ecologia da espécie no país. O objetivo do presente estudo foi o de estudar a ecologia trófica de *T. manatus* no Brasil por meio de análise de isótopos estáveis. Amostras de dentes (n=22) e ossos (n=21) de peixes-bois encalhados e amostras de vegetação foram coletadas na região nordeste do país para a análise de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$). Os animais foram agrupados em cinco regiões: Alagoas, Ceará, Maranhão, Paraíba (que também inclui animais do Rio Grande do Norte e Pernambuco) e Piauí. As plantas, obtidas destas mesmas regiões, foram categorizadas em quatro grupos: fanerógamas marinhas, macroalgas, mangues e de marismas. O modelo de mistura bayesiano para isótopos estáveis SIAR (Stable Isotopes Analysis in R) foi utilizado para estimar as proporções das fontes nas dietas dos grupos de peixes-bois. Não foram verificadas diferenças nos valores de $\delta^{13}C$ e $\delta^{15}N$ entre dentes e ossos, classes de idade ou sexos. Foram verificadas, entretanto, diferenças nas composições isotópicas dos peixes-bois entre as regiões, principalmente com relação ao carbono: os peixes-bois do Ceará (-7,0 ±0,5‰) apresentaram as composições mais enriquecidas em ^{13}C, enquanto os valores mais empobrecidos foram encontrados nos animais do Maranhão (-15,7 ±1,6‰). Valores intermediários foram observados nos indivíduos de Alagoas (-9,3 ±0,4‰), Piauí (-9,3 ±0,6‰) e Paraíba (-11,4 ±2,8‰). Com relação aos isótopos de nitrogênio, os animais da Paraíba (8,8 ±1,2‰) apresentaram composições mais enriquecidas em ^{15}N do que os indivíduos das outras regiões. O modelo de mistura também mostrou diferenças espaciais na ecologia trófica dos peixes-bois. Os animais do Ceará consumiram predominantemente fanerógamas (97,3%), enquanto as fanerógamas (69,4%) e as macroalgas (64,4%) foram mais importantes para os indivíduos de Alagoas e Paraíba, respectivamente. Apesar dos manguezais predominarem no Maranhão, a vegetação de marisma foi a que apresentou a maior contribuição (68,7%) na dieta dos peixes-bois do região. Diferentemente das demais regiões, os indivíduos do Piauí apresentaram uma dieta mais diversificada, na qual os quatro grupos de macrófitas contribuíram em proporções similares (fanerógamas: 28,5%; marismas: 27,1%; macroalgas: 24,0%; mangues: 20,4%). Os resultados demonstram diferenças espaciais nas estratégias alimentares, bem como a importância dos diferentes grupos de macrófitas na dieta dos peixes-bois. O presente estudo é o primeiro a aplicar a análise de isótopos estáveis em peixes-bois marinhos na América do Sul, amplia o conhecimento sobre a ecologia trófica da espécie no país e fornece informações importantes para o estabelecimento de estratégias para a conservação de *T. manatus* e seu habitat no Brasil.

Palavras-chave: Sirênios, peixe-boi, ecologia trófica, dieta, uso do habitat, macrófitas marinhas, composição isotópica.

Abstract

On the Brazilian coast, the manatee *Trichechus manatus* has a fragmented distribution, restricted to a few regions with different ecological characteristics. It is considered a generalist and opportunistic herbivore, consuming a wide range of vegetation from rivers, seas and estuaries, however, information about the ecology of the species in the country is scarce. The aim of this study was to study the trophic ecology of *T. manatus* in Brazil through stable isotopes. Samples of teeth (n=22) and bones (n=21) of stranded manatees and plants were collected in the northeastern region of the country for the analysis of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). The animals were grouped in five regions: Alagoas, Ceara, Maranhão, Paraíba (that includes individuals from Pernambuco and Rio Grande do Norte) and Piauí. The plants, obtained from these same areas, were categorized in four main groups: macroalgae, mangroves, seagrasses and saltmarsh plants. The bayesian mixture model for stable isotopes SIAR (Stable Isotopes Analysis in R) was used to estimate the proportions of the sources in the diets of the groups of manatees. There were no differences in the values of $\delta^{13}C$ and $\delta^{15}N$ among teeth and bones, age classes or between sexes. There were, however, regional differences in isotopic compositions of manatees, especially for $\delta^{13}C$. Carbon stable isotope compositions ranged from depleted in ^{13}C in Maranhão (-15,7±1.6‰), to more enriched in Ceará (-7.0±0.5‰), whereas Alagoas (-9.3±0.4‰), Piauí (-9.3±0.6‰) and Paraíba (-11.4±2.8‰) presented intermediate values. Manatees from Paraíba had higher ^{15}N values (8.8±1.2‰) than those from other regions. The mixture model also indicated regional variation on the manatees diet. Animals from Ceará feed almost exclusively on seagrasses (97.3%), while manatees from Alagoas and

Paraíba graze mainly upon seagrasses (69.4%) and macroalgae (64.4%), respectively. Individuals from Maranhão were feeding mainly on saltmarsh plants (68.7%), despite mangrove dominate the area. Individuals from Piauí, on the other hand, had a more diversified diet, where seagrasses (28.5%), saltmarsh plants (27.1%), macroalgae (24.0%) and mangroves (20.4%) were assimilated in similar proportions. This study is the first to use stable isotopes do investigate the feeding ecology of manatees in South America. The spatial variation in the manatee’s diet demonstrates that proper conservation strategies for the species should include the maintenance of different macrophyte habitats along the northeastern Brazil.

Key Words: Sirenians, manatees, trophic ecology, diet, habitat use, marine macrophytes, isotopic composition.

1. Introdução

1.1 O Peixe-boi Marinho *Trichechus manatus*

Os peixes-bois são mamíferos aquáticos herbívoros de grande porte que vivem nas águas tropicais e subtropicais dos continentes americano e africano (Whitehead 1977). Pertencem à ordem Sirenia, que possui duas famílias: Trichechidae, da qual os peixes-bois fazem parte, e Dugongidae, representada atualmente apenas pelo dugongo, *Dugong dugon,* espécie que habita os oceanos Índico e Pacífico. A família Trichechidae é representada por três espécies: o peixe-boi marinho, *Trichechus manatus*, o peixe-boi da Amazônia, *Trichechus inunguis,* e o peixe-boi africano, *Trichechus senegalensis* (Bertram & Bertram 1973). As duas primeiras espécies ocorrem em águas brasileiras (Domning 1981).

O peixe-boi marinho, *T. manatus,* é um dos mamíferos aquáticos mais ameaçados de extinção do mundo, e vem sendo listado desde 1982 como vulnerável pela IUCN - União Internacional para Conservação da Natureza (IUCN 2012), principalmente em virtude de fatores antrópicos, como perda e degradação de habitat, colisão com embarcações motorizadas, caça ilegal, captura incidental em redes de pesca, poluição, entre outros (Deutsch *et al.* 2008).

A espécie se distribui pela região costeira Atlântica oeste, desde o sudeste dos EUA até o nordeste do Brasil, podendo ingressar em estuários, lagoas e rios costeiros (Bertram & Bertram 1973). O peixe-boi é considerado generalista e oportunista, e consome uma ampla variedade de plantas aquáticas e semi-aquáticas (Best 1981), geralmente em águas rasas e, preferencialmente, sobre vegetação submersa (Hartmam

1979). Duas subespécies têm sido propostas: o peixe-boi das Antilhas, *T. m. manatus,* para as populações das Américas do Sul e Central, e o peixe-boi da Flórida, *T. m. latirostris,* para os animais da América do Norte (Domning & Hayek 1986).

A temperatura da água é um dos fatores ambientais que controlam a distribuição e o comportamento dos indivíduos. Irvine (1983) observou que os peixes-bois têm taxas metabólicas excepcionalmente baixas para um animal de seu porte e não apresentam adaptação apropriada ao frio, sugerindo que a temperatura mínima da água adequada para a espécie seria de 20ºC. Nos EUA, a distribuição do peixe-boi da Flórida está relacionada às variações da temperatura da água ao longo das estações do ano: no inverno, quando a temperatura cai abaixo de 20ºC, a distribuição se restringe à península da Flórida, onde os animais se agregam em fontes de água quente naturais ou artificiais (efluentes de termelétricas) (Irvine 1983, Alves-Stanley *et al.* 2010); durante as estações quentes, quando a temperatura da água aumenta, os peixes-bois se dispersam, principalmente, ao longo da Flórida, Geórgia, Carolina do Sul, Carolina do Norte (Rathbun *et al.* 1982, Powell & Rathbun 1984, Deutsch *et al.* 2003). Os peixes-bois das Antilhas, por outro lado, não apresentam este tipo de padrão de movimentação, visto que se distribuem ao longo da região tropical, onde a temperatura da água é mais constante (Alves-Stanley *et al.* 2010).

Em razão destas diferenças, as duas subespécies utilizam o habitat de distintas formas, bem como apresentam diferentes estratégias alimentares. Lefebvre *et al.* (2000) sugerem que os peixes-bois da Flórida, por realizarem migrações sazonais e utilizarem uma maior variedade de recursos, seriam herbívoros menos especializados do que peixes-bois de Porto Rico, que se alimentam quase que exclusivamente de fanerógamas. Nos EUA, os animais se alimentam do que estiver disponível próximo aos refúgios térmicos ou ao longo das rotas de deslocamento, enquanto que os peixes-bois de Porto Rico não estão sujeitos aos regimes termais, assim, desenvolveram estratégias alimentares mais especializadas e que maximizam o ganho energético.

Uma característica da espécie é a substituição aparentemente ilimitada de seus dentes, onde novos molares se desenvolvem na parte distal da arcada dentária e empurram os demais dentes para a parte frontal (Domning & Hayek 1984). Este fenômeno, conhecido como substituição contínua dos dentes (SCD), é uma adaptação para compensar o desgaste decorrente da maceração de plantas resistentes e abrasivas ingeridas pelos animais, algumas vezes com sedimento. Três características principais possibilitam a SCD: (1) ocorrência de molares supernumerários; (2) movimento mesial dos dentes, que corresponde ao deslocamento horizontal dos molares para o lado frontal do arco dentário, como resultado de uma pressão em seu lado distal; e (3) erupção tardia dos dentes, onde a dentição definitiva ocorre apenas na idade adulta (Rodrigues *et al.* 2011). A SCD ocorre por meio da reabsorção do osso no lado posterior de cada septo intra-alveolar e deposição no lado anterior, sendo provavelmente controlada pelo estresse mecânico da mastigação, relacionado à qualidade e quantidade da planta ingerida. A taxa de movimentação de dentes de peixes-bois é de aproximadamente 1mm por mês, e se inicia com o desmame do filhote, quando o animal passa a ingerir alimento sólido; assim, o indivíduo produziria aproximadamente 27 dentes em cada arcada dentária em 20 anos (Domning & Hayek 1984).

No Brasil, a espécie apresenta distribuição descontínua, ocorrendo desde o estado do Amapá (4ºN) até o estado de Alagoas (10ºS) (Lima 1997; Luna *et al.* 2008), porém, registros históricos (manuscritos e relatórios escritos) sugerem que a distribuição se estendia mais ao sul, até o estado do Espírito Santo (20ºS) (Whitehead 1977, 1978). Na região nordeste, existem três grandes áreas de descontinuidade na ocorrência da espécie: (1) entre Barra do Camaragibe (AL) e Recife (PE), (2) entre Iguape (CE) e Jericoacoara (CE) e (3) entre o Delta do Paranaíba e os Lençóis Maranhenses (MA) (Luna *et al.* 2008). Apesar da ampla distribuição, o tamanho da população no país é extremamente baixo, com estimativa de apenas 450 indivíduos (Luna *et al.* 2008). A intensa caça que os animais sofreram historicamente parece ser a responsável pelo drástico declínio da população, bem como pelo desaparecimento da espécie em algumas áreas do litoral brasileiro (Lima 1997). Atualmente, as principais ameaças aos animais no país são a caça ilegal, a captura incidental em artes de pesca e a degradação do habitat (Parente *et al.* 2004; Luna *et al.* 2008; Meirelles 2008).

Estudos mostram que os peixes-bois forrageiam sobre uma ampla variedade de vegetação aquática no Brasil, incluindo fanerógamas marinhas, macroalgas e folhas de mangues. Lima (1997) relata que os principais componentes da dieta dos animais seriam fanerógamas marinhas (*e.g. Halodule* sp. e *Halophila* sp.); Borges *et al.* (2008) afirmam que macroalgas, principalmente vermelhas (*e.g. Gracilaria* sp. e *Hypnea musciformis*), também seriam importantes recursos para a espécie; Best (1981) atesta que, em ambientes estuarinos, os animais se alimentam de folhas e brotos de mangues (*e.g. Rhizophora mangle* e *Laguncularia racemosa*) e de plantas de marisma (*e.g. Spartina alterniflora*). No entanto, pouco se sabe sobre a importância relativa dos diferentes tipos de vegetação, bem como sobre a influência dos distintos habitats na dieta e ecologia alimentar dos grupos de peixes-bois no Brasil.

1.2. A Análise da Dieta Por Meio de Isótopos Estáveis (AIE)

Isótopos estáveis são átomos de um mesmo elemento químico que possuem o mesmo número de prótons, porém, diferentes números de nêutrons, ou seja, possuem massas diferentes, mas que não decaem ao longo do tempo. A ideia central da AIE se baseia no fato de que as composições de isótopos de consumidores refletem as composições isotópicas dos produtores primários, os quais, por sua vez, refletem as características do ambiente em que ocorrem (Reich & Worthy 2006). Em estudos de ecologia, dois elementos corriqueiramente utilizados são os isótopos de carbono (^{13}C e ^{12}C) e os de nitrogênio (^{15}N e ^{14}N), sendo que a composição isotópica de uma amostra é obtida usando-se a notação δ, em partes por mil (‰):

$$\delta X = [(R_{amostra}/R_{padrão}) -1] * 1000,$$

onde X refere-se a ^{13}C ou ^{15}N, $R_{amostra}$ é a relação $^{13}C/^{12}C$ ou $^{15}N/^{14}N$ e $R_{padrão}$ é o elemento de referência (belemnita da formação PeeDee (PDB) para $\delta^{13}C$ e N_2 atmosférico para $\delta^{15}N$) (Peterson & Fry 1987). Quando a razão entre os isótopos pesado e leve de uma amostra é maior do que a razão do padrão, o valor δ é positivo (composição enriquecida), e quando a razão entre os isótopos pesado e leve da amostra é menor do que a razão do padrão, o valor δ é negativo (composição empobrecida).

A AIE apresenta algumas vantagens sobre os métodos tradicionais de estudo de dieta, como, por exemplo, o registro da alimentação assimilada e não simplesmente ingerida pelo animal, a possibilidade de obtenção de amostras a partir de coleções e museus e a inferência sobre a dieta em diferentes escalas de tempo (Walker & Macko 1999). A técnica vem sendo utilizada em estudos de ecologia alimentar, uso de habitat, migrações, fisiologia e paleoecologia de mamíferos marinhos (Newsome *et al.* 2010) e, mais recentemente, para a identificação de estoques ecológicos (Barros *et al.* 2010, Botta 2011).

Fracionamento isotópico

A diferença no número de nêutrons não tem influência na reatividade química dos isótopos, porém, eles se comportam de formas distintas em reações cinéticas: o isótopo mais leve usualmente reage de maneira mais rápida do que o pesado, ou seja, a razão entre os isótopos pesado e leve ao final de uma reação (produto) é diferente da razão inicial entre eles (substrato). Este comportamento isotópico distinto, que leva à variação na proporção entre isótopos ao passar por um processo físico-químico, é conhecido como fracionamento isotópico. Em razão do fracionamento, consumidores apresentam composições isotópicas enriquecidas em relação a suas fontes alimentares. O enriquecimento de nitrogênio entre níveis tróficos varia, tipicamente, entre 2 e 5‰ (Peterson & Fry 1987), enquanto que, para carbono, varia entre 1 e 2‰ (DeNiro & Espstein 1978). Assim, em virtude da diferença no enriquecimento trófico apresentado pelos elementos, usualmente isótopos de nitrogênio são usados para estimar a posição trófica de um indivíduo em uma cadeia alimentar, enquanto que isótopos de carbono são usados para caracterizar a dieta dos consumidores (Peterson & Fry 1987).

Taxa de renovação isotópica

A taxa de renovação (conversão ou *turnover*) consiste na síntese de tecido novo em um organismo, assim, um aspecto importante que deve ser considerado em AIE é o tecido analisado. A escala de tempo representada por uma amostra dependerá, em parte, da taxa de renovação do tecido (Hobson & Clark 1992). Em geral, tecidos metabolicamente mais ativos, como a gordura, têm taxas de conversão mais altas, assim, refletiriam uma dieta consumida mais recentemente. Tecidos metabolicamente menos ativos, como o osso, apresentam taxas de renovação mais lentas, assim, refletem uma dieta ingerida em um período de tempo maior (Tieszen *et al.* 1983; Hobson & Clark 1992). Por outro lado, tecidos inertes, como a dentina, uma vez sintetizados, não são posteriormente remodelados (Mendes *et al.* 2007; Newsome *et al.* 2010). Portanto, a análise de dentes que apresentam crescimento contínuo por acresção de dentina, como observado em odontocetos e pinípedes, tem o potencial de registrar mudanças na alimentação de um indivíduo relacionadas a condições ambientais, assim como possibilita obter um registro cronológico da dieta (Hobson & Sease 1998). A utilização simultânea de ossos e dentes podem fornecer informações sobre a dieta em diferentes escalas de tempo e possíveis variações na alimentação, além de possuírem grande potencial de preservação *postmortem* (Clementz *et al.* 2007; Koch 2007), assim, vêm sendo utilizados com frequência em estudos de ecologia de mamíferos marinhos que envolvem AIE (ver Newsome *et al.* 2010 para uma revisão).

Composições isotópicas dos produtores primários

As diferenças nos valores de $\delta^{13}C$ entre produtores ocorrem, principalmente, em razão do sistema fotossintético das plantas, onde espécies com ciclo C4 geralmente apresentam composições mais enriquecidas em ^{13}C do que plantas C3 (Peterson & Fry 1987). Os valores médios de $\delta^{13}C$ entre plantas de água doce (-27‰), macroalgas (-18.5‰) e fanerógamas marinhas (-11‰), por exemplo, são estatisticamente distintos, o que possibilita determinar a proporção de cada tipo na dieta dos consumidores

(Clementz *et al.* 2007). Além disso, os ecossistemas aquáticos apresentam diferenças espaciais nos valores de $\delta^{13}C$: de uma maneira geral, ambientes marinhos costeiros apresentam composições mais enriquecidas do que ambientes estuarinos e de água doce (Clementz & Koch 2001). Por sua vez, os valores de $\delta^{15}N$ dos produtores podem ser afetados, por exemplo, por processos naturais (*e.g.* florações de fitoplâncton) ou antrópicos (*e.g.* descarga de efluentes domésticos e industriais em corpos d'água), que lançam compostos nitrogenados no ambiente (Peterson 1999).

Isótopos estáveis em estudos com peixes-bois

Estudos sobre ecologia alimentar de peixes-bois são realizados, tradicionalmente, pela observação direta de animais na natureza e pela análise do conteúdo estomacal e fecal (*e.g.* Mignucci-Giannoni & Beck 1998, Borges *et al.* 2008, Castelblanco-Martínez *et al.* 2009). Porém, devido à dificuldade na realização de estudos observacionais no ambiente natural e às limitações das análises estomacais e fecais, que proporcionam informações sobre os itens alimentares recentemente ingeridos e cujas coletas na natureza são trabalhosas, a AIE vem se tornando uma ferramenta útil em estudos de ecologia de *T. manatus*, e o número de estudos com sirênios, que utilizam tal ferramenta, tem crescido nos últimos anos (Ames *et al.* 1996, McFadden *et al.* 2004, Reich & Worthy 2006, Clementz *et al.* 2007; Alves-Stanley & Worthy 2009; Alves-Stanley *et al.* 2010).

Assim, a AIE em tecidos de peixes-bois pode representar uma abordagem alternativa para investigar as fontes de alimentação dos animais, relacionando-os com o tipo de habitat e região geográfica. Além disso, pode proporcionar um melhor entendimento sobre a importância relativa dos diferentes grupos vegetais na dieta do peixe-boi, sobre sua ecologia trófica e sobre a relevância dos distintos ambientes para a espécie. Tais informações são cruciais para o desenvolvimento e implementação de estratégias para a conservação da espécie e seu habitat no Brasil. O presente estudo é o primeiro a utilizar a técnica de AIE em peixes-bois marinhos na América do Sul.

2. Objetivos

A dissertação tem como objetivo geral estudar a ecologia trófica de *T. manatus* no nordeste do Brasil por meio de isótopos estáveis. Os objetivos específicos do estudo são: (1) comparar as razões de isótopos de carbono e nitrogênio entre ossos e dentes em diferentes posições na arcada dentária de peixes-bois, (2) determinar as composições de isótopos de carbono e nitrogênio em peixes-bois de distintas regiões do litoral nordeste do Brasil, avaliando as diferenças entre regiões e (3) analisar a contribuição relativa dos diferentes grupos vegetais, bem como a influência dos distintos habitats, na dieta dos grupos de *T. manatus*.

3. Material & Métodos

3.1. Área de estudo

A costa nordeste do Brasil oferece habitats favoráveis aos peixes-bois, como a ocorrência de águas quentes e rasas e disponibilidade de alimento e de áreas protegidas (Lima *et al.* 1992). Estuários, recifes de coral, manguezais e pradarias de fanerógamas são importantes habitats costeiros na região, que pode ser dividida em três grandes unidades, cujas condições climáticas e fisiográficas são relativamente uniformes: (1) da Ponta Curuçá (PA) até a Ponta Mangues Secos (PI); (2) da Ponta Mangues Secos até o Cabo Calcanhar (RN); e (3) do Cabo Calcanhar até o Recôncavo Baiano (BA) (Schaeffer-Novelli et al. 1990). A unidade (1) é caracterizada por manguezais bem desenvolvidos, por uma menor diversidade de macroalgas e pela ausência de fanerógamas marinhas. A unidade (2) caracteriza-se por manguezais pouco desenvolvidos e por uma maior diversidade de macroalgas e fanerógamas, limitadas, porém, a algumas regiões. A unidade (3) é caracterizada por manguezais desenvolvidos e por uma maior diversidade de macroalgas e fanerógamas, distribuídas por toda a região (Schaeffer-Novelli et al. 1990, Figueiredo et al. 2008, Marques & Creed 2008).

O litoral do Maranhão é caracterizado pela presença de muito rios, deltas e estuários, permitindo a formação de densos manguezais e marismas (Souza–Filho 2005, Ab'Saber 2008). A fronteira dos estados do Piauí e Ceará se caracteriza por um mosaico de habitats, incluindo estuários, manguezais e pradarias de fanerógamas marinhas e campos de macroalgas (Mai and Loebmann 2010), onde quatro rios (Camurupim, Cardoso, Timonha e Ubatuba) formam uma extensa área estuarina (Meirelles 2008). O litoral leste do Ceará é caracterizado por uma linha de costa retilínea, presença de praias arenosas e de pradarias de fanerógamas marinhas e campos de macroalgas (Pinheiro-Joventino *et al.* 1998, Barros 2008), porém, com exceção de alguns poucos rios e estuários, não existem lagunas, baías ou outras áreas protegidas (Meirelles 2008). No norte da Paraíba, o estuário do rio Mamanguape, uma das principais áreas de ocorrência de peixes-bois no nordeste do Brasil, se caracteriza por uma rica floresta de manguezal, por uma escassa pradaria de fanerógamas marinhas e por um recife de franja que corre paralelo à costa, coberto por macroalgas (Xavier *et al.* 2012). A costa de Alagoas é também caracterizada por uma diversidade de ecossistemas, incluindo praias arenosas, estuários, lagunas e recifes, que suportam uma grande biodiversidade, como manguezais, pradarias de fanerógamas marinhas e macroalgas (Marques 1991).

3.2. Amostras

As amostras foram obtidas com o Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos do Instituto Chico Mendes de Conservação da Biodiversidade (CMA/ICMBio) e com a Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (Aquasis), instituições parceiras na execução do estudo.

Composições de isótopos em dentes e ossos dos peixes-bois

Dentes e ossos de cinco peixes-bois marinhos encalhados na costa nordeste do Brasil entre os anos de 2003 e 2009 foram incluídos no estudo (Tabela 1, Anexo I). Seis molares funcionais foram coletados da mandíbula direita de cada indivíduo e registrados como M1 (posição frontal da mandíbula) a M6 (posição distal da mandíbula) (Figura 1, Anexo I). Como as variações nos valores de $\delta^{13}C$ e $\delta^{15}N$ no colágeno em diferentes ossos de um mesmo indivíduo são pequenas (DeNiro & Schoeninger 1983), a amostra de osso foi obtida a partir do côndilo mandibular direito dos animais. Com o objetivo de minimizar uma possível influência materna nos valores de isótopos, apenas adultos (comprimento total: > 225 cm, > 7 anos de idade; Mignucci-Giannoni *et al.* 2000) foram usados nas análises.

Composições isotópicas e dieta dos peixes-bois

Dentes (n=22) e ossos (n=21) de *T. manatus* encalhados entre os anos de 1989 e 2010 no litoral nordeste do Brasil foram incluídos no estudo. Os animais foram agrupados em cinco regiões de acordo com os locais onde encalharam: Maranhão (MA, n=5), Piauí (PI, n=2), Ceará (CE, n=10), Paraíba (PB, n=23) e Alagoas (AL, n=3). A divisão nestes cinco grupos foi feita de modo que fossem abrangidas tanto as áreas de ocorrência de *T. manatus* quanto as regiões de descontinuidades na sua distribuição ao longo do nordeste do país (Figura 1, Anexo II).

Amostras triplicadas das principais espécies de plantas consumidas pelos animais foram coletadas manualmente, entre setembro de 2011 e fevereiro de 2012, nas mesmas cinco regiões, considerando os locais de maior ocorrência de animais em cada região: Porto de Pedras (AL), Icapuí (CE), Humberto de Campos e São José de Ribamar (MA), Barra de Mamanguape (PB) e Cajueiro da Praia (PI). As plantas foram categorizadas em quatro grupos vegetais: fanerógamas marinhas, macroalgas, mangues e plantas de marismas. Foram coletadas folhas de mangues e de plantas de marismas, parte aérea e subterrânea de fanerógamas marinhas e talos de macroalgas.

3.3. Preparação das Amostras

As amostras de dentes e ossos foram preparadas para análise de isótopos estáveis seguindo protocolo descrito em Walker & Macko (1999): passaram por um processo de limpeza e remoção de resíduos, músculo e outros tecidos aderidos, com uso de uma escova; em seguida, foram enxaguadas com água destilada e secadas em estufa a 60ºC por 48 horas. Os dentes foram cortados longitudinalmente com uma serra circular diamantada de rotação lenta para expor a dentina e, assim como os ossos, transformados em pó com auxílio de uma broca. Como dentes e ossos são compostos por uma parte orgânica (colágeno) e outra mineral (bioapatita) (Koch 2007), as amostras foram acidificadas com HCl a 30% para eliminar o carbono inorgânico e, em seguida, secadas em estufa a 60ºC por 2 horas para remoção de qualquer resíduo de ácido clorídrico. Para avaliar o efeito da acidificação nos valores de δ^{13}C e δ^{15}N, foram obtidas amostras duplicadas em cindo dentes de peixes-bois, das quais uma foi acidificação enquanto a outra foi mantida como controle, isto é, não sofreu tratamento. Não foram observadas diferenças significativas tanto para δ^{13}C (Mann-Whitney: U=11,0; p=0,835), como para δ^{15}N (Mann-Whitney: U=11,5; p=0,917).

As amostras de plantas foram limpas com água doce e destilada, para retirada de impurezas e epífitas aderidas, e secadas em estufa a 60ºC por 48 horas. Em seguida, maceradas com gral e pistilo até tornarem-se pó. Aproximadamente 1.0mg das amostras de dentes e ossos e entre 2.5 e 3.0 mg das amostras de plantas, foram armazenadas em cápsulas de estanho de 5x9 mm.

3.4. Análise Isotópica

As análises isotópicas para determinação das razões δ^{13}C e δ^{15}N foram realizadas no Laboratório Núcleo de Isótopos Estáveis da Universidade do Estado de Washington (Stable Isotope Core Laboratory - Washington State University), utilizando um analisador elementar (ECS 4010, Costech Analytical, Valencia, CA) acoplado a um espectrômetro de massas de razão isotópica de fluxo contínuo (Delta PlusXP, Thermofinnigan, Bremen).

3.5. Análise dos Dados

Os dados foram testados para normalidade utilizando-se teste de Shapiro-Wilk e, para homogeneidade de variâncias, teste de Levene. As diferenças estatísticas foram consideradas significativas para $p < 0,05$.

Composições de isótopos em dentes e ossos dos peixes-bois

Análise de Variância (ANOVA) foi usada para comparar os valores de $\delta^{13}C$ e $\delta^{15}N$ entre ossos e dentes em cada posição nas arcadas dentárias dos cinco animais. Para verificar se houve tendência nos valores de $\delta^{13}C$ e $\delta^{15}N$ com as posições dos dentes na arcada dentária e osso de cada indivíduo, utilizou-se coeficiente de correlação de Pearson. Gráfico de dispersão ($\delta^{13}C$ versus $\delta^{15}N$) foi usado para visualizar os valores de isótopos dos dentes e osso em cada indivíduo. Os resultados médios estão apresentados como média ± desvio-padrão.

Composições isotópicas e dieta dos peixes-bois

Os dados foram testados para normalidade utilizando-se teste de Shapiro-Wilk e, para homogeneidade de variâncias, teste de Levene. Análise de Variância (ANOVA) e teste de Kruskall-Wallis foram usados para comparar os valores de $\delta^{13}C$ e $\delta^{15}N$ entre classes de idade (filhotes: < 175cm comprimento total, < 2 anos de idade; subadultos: 176 – 225cm, 3 – 7 anos de idade; adultos: > 225cm, > 7 anos de idade; Mignucci-Giannoni *et al.* 2000) e entre grupos de peixes-bois, seguidos de teste *a posteriori* de Bonferroni. Testes t-Student e Mann-Whitney foram utilizados para comparar os valores de $\delta^{13}C$ e $\delta^{15}N$ entre machos e fêmeas. Gráficos de dispersão ($\delta^{15}N$ versus $\delta^{13}C$) foram usados para visualizar os valores de isótopos dos grupos de peixes-bois. Os resultados médios estão apresentados como média ± erro-padrão.

O modelo de mistura bayesiano para isótopos estáveis, SIAR (Stable Isotopes Analysis in R), foi utilizado para estimar a proporção dos diferentes tipos de vegetação na dieta dos peixes-bois. O SIAR oferece algumas vantagens em relação a outros modelos de mistura, como, por exemplo, a incorporação de variabilidade nas fontes e nos fatores de enriquecimento trófico (TEF) e, além disso, gera como resultados distribuições de probabilidades (Parnell *et al.* 2010).

A AIE em estudos sobre dieta animal requerem uma estimativa do TEF entre o indivíduo e sua dieta ($\Delta^{13}C$ e $\Delta^{15}N$), que variam, por exemplo, com o tipo de tecido analisado e a qualidade da dieta (Newsome *et al.* 2010). Clementz *et al.* (2007) propuseram valores de $\Delta^{13}C$ no colágeno em *T. manatus* com diferentes dietas: 1,8‰ para animais cuja dieta é baseada em fanerógamas e plantas de água doce, e 6,2‰ para indivíduos que se alimentam de vegetação de água doce. Assim, no presente estudo, como os animais se alimentam de vegetação de água doce (principalmente mangues), estuarinas e marinhas, o valor de $\Delta^{13}C$ considerado foi 1,8‰. Por outro lado, com relação ao nitrogênio, não existem estudos com sirênios, no entanto, Schoeninger & DeNiro (1984), em um trabalho envolvendo pinípedes e cetáceos, sugerem 3‰ como valor de $\Delta^{15}N$. Como, provavelmente, o TEF de nitrogênio para sirênios seria menor do que para mamíferos marinhos carnívoros (Newsome *et al.* 2010), o valor adotado para $\Delta^{15}N$ no presente estudo foi 2‰, o qual é coerente com as composições isotópicas encontradas para os animais e as fontes.

4. Resultados

4.1. Composições de Isótopos em Dentes e Ossos dos Peixes-bois

As composições de isótopos estáveis de carbono e nitrogênio dos dentes e ossos dos peixes-bois apresentaram baixa variabilidade intraindividual (Figura 2, Anexo I). Porém, alguns indivíduos apresentaram diferenças maiores do que 2‰ entre dentes, tanto para $\delta^{13}C$ (indivíduo PB-132) como para $\delta^{15}N$ (indivíduo PB-145). Apesar de valores mais empobrecidos em isótopos de nitrogênio serem mais frequentemente observados nos ossos, a diferença não foi significativa (ANOVA; F=0,222; p=0,966). Também não houve diferença significativa nos valores isotópicos de carbono entre dentes e ossos (ANOVA; F=0,042; p=0,999), bem como não foi observada correlação entre $\delta^{13}C$ e $\delta^{15}N$ e as posições dos dentes e osso em nenhum dos animais ($p>0,05$ para todos os indivíduos; Figura 3, Anexo I).

4.2. Composições Isotópicas dos Peixes-bois

Os peixes-bois da costa nordeste do Brasil apresentaram composições isotópicas médias de -10,6‰ ± 0,5‰ para $\delta^{13}C$ (máximo: -5,9‰; mínimo: -17,4‰) e 8,1‰ ± 0,3‰ para $\delta^{15}N$ (máximo: 11,0‰; mínimo: 5,3‰) (Tabela 1, Anexo II), Não foram verificadas diferenças significativas nos valores de isótopos entre sexos, tanto para $\delta^{13}C$ (fêmeas: -10,2‰, n=18; machos: -10,3‰, n=17; Mann-Whitney: U=144; p=0,779) como para $\delta^{15}N$ (fêmeas: 8,1‰, n=18; machos: 7,8‰, n=17; t-Student: t=0,023; p=0,951), Da mesma forma, não houve diferenças nas composições isotópicas entre classes de idade, tanto para $\delta^{13}C$ (filhotes: -9,6‰, n=15; subadultos: -8,5‰, n=4; adultos: -11,0‰, n=18; Kruskall-Wallis: H=2,905; p=0,234) como para $\delta^{15}N$ (filhotes: 8,0‰, n=15; subadultos: 7,1‰, n=4; adultos: 8,5‰, n=18; Kruskall-Wallis: H=2,761; p=0,252), No entanto, os grupos de peixes-bois apresentaram diferenças significativas, tanto para carbono (Kruskall-Wallis: H=26,7; p<0,001) como para nitrogênio (Kruskall-Wallis: H=14,8; p=0,005),

As maiores diferenças entre grupos foram observadas para isótopos de carbono, que variou de composições empobrecidas nos animais do MA (-15,7 ± 0,7‰), até composições mais enriquecidas nos indivíduos do CE (-7,0 ± 0,2‰), Os peixes-bois de AL (-9,3 ± 0,2‰), PI (-9,3 ± 0,4‰) e PB (-11,4 ± 0,6‰) apresentaram valores intermediários, cujas diferenças não foram significativas (Figura 2, Anexo II),

Por sua vez, as diferenças nos valores de isótopos de nitrogênio entre os grupos de peixes-bois foram menores. Os valores médios de $\delta^{15}N$ variaram de composições empobrecidas nos animais do PI (6,6 ± 1,2‰) e MA (6,6 ± 0,6‰), até valores um pouco mais enriquecidos, como apresentados pelos indivíduos da PB (8,8 ± 0,3‰). Já os animais de AL (6,9 ± 0,7‰) e CE (7,7 ± 0,4‰) apresentaram valores intermediários. Os peixes-bois da PB foram os únicos que apresentaram diferenças significativas em relação aos demais.

4.3. Dieta dos Peixes-bois

De um modo geral, mangues exibiram as composições de isótopos de carbono mais empobrecidas e fanerógamas e marismas, as composições mais enriquecidas, já macroalgas exibiram composições de isótopos de nitrogênio maiores do que os demais grupos vegetais (Tabela 4, Anexo II).

O SIAR mostrou diferenças nas composições das dietas dos grupos de peixes-bois (Figura 3, Anexo II). Observou-se que os indivíduos do Ceará alimentaram-se fundamentalmente de fanerógamas marinhas, que foram responsáveis por aproximadamente 97,3% da dieta dos peixes-bois na região, enquanto os indivíduos do PI apresentaram a dieta mais diversificada (fanerógamas: 28,5%; marisma: 27,1%; algas: 24,0%; mangue: 20,4%). Para os animais de AL, a maior contribuição foi de fanerógamas (69,4%) e, em menor proporção, de macroalgas (25,1%), ao passo que os animais da PB se alimentaram principalmente de macroalgas (64,4%) e, em menor proporção, de fanerógamas (33,5%). Na dieta dos animais do MA, a vegetação de marisma foi responsável pela maior contribuição (68,7%), superando a vegetação de mangue (31,3%), que predomina na região.

5. Conclusões

- A análise de isótopos estáveis em dentes e ossos de peixes-bois marinhos é uma ferramenta útil em estudos sobre ecologia trófica da espécie no Brasil;

- Não há diferenças significativas nas composições de isótopos de carbono e nitrogênio de dentes e ossos de peixes-bois. Porém, as diferenças intra-individuais podem ser maiores que as inter-individuais ou regionais;
- Os isótopos estáveis de carbono permitem verificar mudanças temporais e se obter um registro cronológico da dieta dos últimos anos de vida de um indivíduo;
- Existem variações espaciais na composição isotópica de nitrogênio e, especialmente, de carbono nos peixes-bois marinhos ao longo de sua distribuição no Brasil. Com relação a isótopos de carbono, os peixes-bois do Ceará apresentaram as composições mais enriquecidas, enquanto os animais do Maranhão mostraram os valores mais empobrecidos. Os indivíduos de Alagoas, Paraíba e Piauí apresentaram valores intermediários. Com relação a isótopos de nitrogênio, os animais da Paraíba apresentaram composições mais enriquecidas do que os demais;
- Foram verificadas diferenças nas dietas dos grupos de peixes-bois nas distintas regiões: os animais do Ceará alimentam-se predominantemente de fanerógamas marinhas; os indivíduos do Maranhão, principalmente de vegetação de marisma; os peixes-bois de Alagoas, de fanerógamas marinhas; os animais da Paraíba, de macroalgas; e os indivíduos do Piauí apresentam uma dieta mais diversificada, composta por proporções semelhantes dos quatro grupos vegetais.

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Anexo I

Intra-individual variation in $\delta^{13}C$ and $\delta^{15}N$ composition in the Antillean manatee *Trichechus manatus* from northeastern Brazil

Leandro L. Ciotti, Fábia O. Luna, Eduardo R. Secchi

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LEANDRO L. CIOTTI

Programa de Pós-graduação em Oceanografia Biológica
Laboratório de Tartarugas e Mamíferos Marinhos
Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG
Av. Itália km 8, Caixa Postal 474
Rio Grande, RS, Brazil, 96.201-900
E-mail: llciotti@yahoo.com.br

FÁBIA O. LUNA

Centro Mamíferos Aquáticos – CMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Estrada do Forte Orange, s/ nº
Orange, Itamaracá, PE, Brazil, 53.900-000

EDUARDO R. SECCHI

Laboratório de Tartarugas e Mamíferos Marinhos
Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG
Av. Itália km 8, Caixa Postal 474
Rio Grande, RS, Brazil, 96.201-900

ABSTRACT

Manatees continuously replace their teeth throughout their life, thus the objectives of this study were to verify if there are intra-individual differences in carbon and nitrogen stable isotopes in teeth and bones of Antillean manatees, and to assess the potential use of stable isotopes analysis as a temporal record of changes in the individual's diet. We determined δ^{13}C and δ^{15}N values in six teeth and a piece of bone from the lower jaw of five manatees stranded in the northeastern Brazilian coast. There was no significant difference in carbon values among teeth and bones, though the later were slightly more depleted in ^{15}N. Although some animals presented differences greater than 2‰ among teeth for both carbon and nitrogen, in general, manatees showed low intra-individual variability in the isotopic composition. The results suggest that bones and teeth, regardless of their position in the dental arcade, can be used in comparative studies on the feeding ecology of manatees and teeth can potentially be used to detect temporal changes in their diet. Nevertheless, when samples size is reduced, the use of at least two teeth from each individual is recommended.

Key Words: *Trichechus manatus*, sirenians, continuous teeth replacement, stable isotopes, Brazil

The manatee *Trichechus manatus* (Linnaeus, 1758) inhabits the tropical Atlantic coast of America and Caribbean (Bertram and Bertram 1973). Two subspecies have been proposed: the Antillean manatee *T. m. manatus* for the populations of Central and South Americas, and the Florida manatee *T. m. latirostris* for animals from North America (Domning and Hayek 1986). The species is considered vulnerable by the International Union for Conservation of Nature (IUCN 2012).

In Brazil, the manatee presents a discontinuous distribution, occurring from Amapá (4ºN) to Alagoas (10ºS) states (Lima 1997, Luna *et al.* 2008a), but historical records suggest that the species extended further south to the Espírito Santo state (20°S) (Whitehead 1977). The intensive hunting that the animals suffered historically seems to be the reason for the decline in population size, as well as the disappearance of the species in some areas of the Brazilian coast (Lima 1997, Luna *et al.* 2008b). Today, the main threats to the animals in the country are illegal hunting, incidental catch in fishing nets and habitat degradation (Parente *et al.* 2004, Luna *et al.* 2008a, Meirelles 2008).

Manatees are opportunistic herbivores that consume macrophytes from freshwater, estuarine and marine environments (Best 1981), usually in shallow water and preferably on submerged vegetation (Hartman 1979). In Brazil, the species forages on a wide range of vegetation, including seagrasses, macroalgae, and mangrove leaves (Best 1981, Paludo 1997, Borges *et al.* 2008). Studies on the feeding ecology of manatees are performed traditionally by direct observation of wild animals and by analysis of stomach contents and scats (*e.g.* Mignucci-Giannoni and Beck 1998, Borges *et al.* 2008, Castelblanco-Martínez *et al.* 2009). However, because of the difficulty in conducting observational studies in nature and the limitations of stomach and fecal analyses, which provide information only about recently ingested food and whose sampling and storing are time consuming and logistic-dependent, the stable isotopes analysis (SIA) has become a useful tool in studies of feeding ecology of *T. manatus* (Reich and Worthy 2006). SIA presents some advantages over the traditional methods: it provides information on the food assimilated and not simply ingested by the animal, allows to make inference about diet on different time scales, and samples can be easily obtained from research collections and museums (Walker and Macko 1999).

The SIA assumes that the isotopic composition of a consumer reflects the isotopic composition of its diet. Therefore, from the determination of the ratios between heavy and light isotopes in plants and animals, for example, isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), it is possible to make inferences about diet and trophic relationships between individuals in a same food chain (Peterson and Fry 1987).

An important aspect that must be considered in SIA is the tissue used for the analysis. The time scale represented by a sample will depend, in part, on the turnover rate of the tissue (Hobson and Clark 1992). In general, metabolically active tissues, such as fat, have higher turnover rates and reflect a diet consumed more recently, while less metabolically active tissues, such as bone, reflect a diet ingested along a longer period of time (Tieszen *et al.* 1983, Hobson and Clark 1992). Furthermore, inert tissues, such as dentin, once synthesized are no further remodeled (Mendes *et al.* 2007, Newsome *et al.* 2010), therefore the analysis of teeth that grow by continuous accretion of dentin, as in most aquatic mammals, can provide a chronological record of the diet (Hobson and Sease 1998) and may potentially record temporal changes in the diet which could be related to environmental conditions or individual's behavior such as movements between different foraging sites or resources selection. Thus, the great *postmortem* preservation of bones and teeth (Koch 2007) as well as their potential in providing information about the diet at different time scales turned them ideal tissues commonly used in ecological studies of marine mammals (see Newsome *et al.* 2010 for a review).

Unlike most aquatic mammals, whose teeth grow by accretion of dentin layers, the manatees apparently replace their teeth continuously, where new molars develop in the distal part of the dental arcade and push the other teeth towards the front (Domning and Hayek 1984). This phenomenon, known as continuous teeth replacement (CTR), is an adaptation to compensate the wear of the teeth caused by maceration of resistant and abrasive plants eaten by the animals, sometimes with sediment. Three key features enable the CTR: (1) occurrence of supernumerary molars; (2) mesial movement of teeth, that corresponds to horizontal displacement of molars to the frontal end of the dental arcade, as a result of pressure on its distal end; and (3) delayed eruption of teeth, where permanent teeth appear only during adulthood (Rodrigues *et al.* 2011). The CTR occurs via bone resorption on the posterior side of each inter-alveolar septum and deposition in the anterior side, and is probably controlled by the mechanical stress of chewing, related to the quality and quantity of ingested plants (Domning and Hayek 1984). As result of this CTR, the development of teeth in manatees occurs in a shorter time than would happen for most mammals, thus the isotopic compositions of their teeth reflect the food assimilated during a short period (Walker and Macko 1999).

The rate of manatee's teeth movement is approximately 1mm per month, and it begins when the animal starts to ingest solid food. A manatee would produce about 27 teeth in each dental row in 20 years (Domning and Hayek 1984), which represents approximately the formation of a new tooth every nine months. As each tooth is formed in a distinct period and does not undergo subsequent reworking, such tissues could show different isotopic compositions if the animal's diet is subject to temporal variation. Considering that manatees have five to seven functional teeth in each dental arcade (Husar 1978), and each tooth reflects distinct and successive feeding periods, it would be possible to assess changes in the diet over the last five to six years of the individual's life. Therefore, the present study aims (1) to compare intra-individual $\delta^{13}C$ and $\delta^{15}N$ values in teeth and bones of manatees, (2) to test differences in carbon and nitrogen isotopes in the teeth according to their position in the dental arcade and (3) to assess the potential of using intra-individual differences in stable isotopes compositions as a temporal record of diet changes.

METHODS

Samples

Teeth and bones of five manatees stranded between the years 2003 and 2009 in northeastern Brazilian coast were obtained from the Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos – Instituto Chico Mendes de Conservação da Biodiversidade (CMA – ICMBio). To minimize any possible maternal influence on the values of isotopes, only adults (total length: >225cm, >7 years old; Mignucci-Giannoni *et al.* 2000) were used in the study (Table 1). Six functional molars were collected from the right mandible of each individual and recorded as M1 (frontal position of the jaw, oldest molar) to M6 (distal position of the jaw, newest molar) (Figure 1). As the differences in $\delta^{13}C$ and $\delta^{15}N$ values in the collagen of different bones in a same individual are small (DeNiro and Schoeninger 1983), the bone sample was obtained from the right mandibular condyle and recorded as B. Among the five adult animals analyzed, whose had the full set of teeth in one hemimandible, three out of these came from Paraiba state, one of the regions with highest occurrence of manatees in northeastern Brazil (Silva *et al.* 1992).

Sample preparation

Samples were prepared for stable isotope analysis following the protocol described in Walker and Macko (1999). Samples passed through a cleaning process for removing residues, muscle and other adhered tissues, rinsed in distilled water and dried in an oven at 60°C for 48 hours. The teeth were longitudinally cut with a slow rotation diamond circular saw to expose the dentine. Both teeth and bones were powdered with a drill bit. As these tissues consist of an organic (collagen) and a mineral (bioapatite) portion (Koch 2007), samples were acidified with 30% hydrochloric acid (HCl) to remove the inorganic carbon, and then dried again in oven at 60°C for 2 hours to remove any residual HCl. Approximately 1.0 mg of the powdered samples was stored in a 5x9 mm tin capsule for the analyses.

Isotope Analysis

The isotopic analysis was conducted at the Stable Isotope Core Laboratory - Washington State University, using an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA) coupled to a continuous flow isotope ratio mass spectrometer (Delta PlusXP, ThermoFinnigan, Bremen). The isotopic ratio δ (in ‰) is expressed by $\delta X = [(R_{sample}/R_{standard}) - 1] * 1000$, where X refers to ^{13}C or ^{15}N, R_{sample} is the ratio of isotopes of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and $R_{standard}$ is the ratio of isotopes of the reference (VPDB - Vienna PeeDee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). Standard deviations were estimated at 0.14‰ and 0.10‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Data Analysis

The data were tested for normality using Shapiro-Wilk’s test, and Levene's test for homogeneity of variances. Analysis of Variance (ANOVA) was used to compare the values of $\delta^{13}C$ and $\delta^{15}N$ of bones and teeth in each position in the dental hemimandible among the five animals. Pearson’s coefficient was used to verify if there was an intra-individual correlation between $\delta^{13}C$ or $\delta^{15}N$ values and the positions of the teeth in the dental arcade. The results are presented as mean ± standard deviation, and statistical analyses were considered significant for $p<0.05$.

RESULTS

The carbon (Table 2) and nitrogen (Table 3) stable isotope compositions of teeth and bones of the manatees showed a low intra-individual and a high inter-individual variability, particularly for carbon (Figure 2). However, some animals presented differences greater than 2‰ among teeth for $\delta^{13}C$ (individual PB-132) and $\delta^{15}N$ (individual PB-145). Such differences were even greater than some mean differences found between individuals and locations (Tables 2 and 3). There was no significant difference in carbon values among teeth and bones (ANOVA, $F=0.042$, $p>0.9$), and, although bones were generally slightly more depleted in ^{15}N than teeth (exception for individual PB-214), the difference was non-significant (ANOVA; $F=0.222$; $p>0.9$). There was no correlation between $\delta^{13}C$ or $\delta^{15}N$ values and the position of teeth in the dental arcade of the animals, except for the individual RN-152, that showed a weak negative correlation in $\delta^{15}N$ ($p<0.04$) ($p>0.05$ for the other individuals; Figure 3).

DISCUSSION

The overall intra-individual difference in carbon and nitrogen stable isotope compositions between teeth and bones of *T. manatus* were non-significant, and no correlations among $\delta^{13}C$ or $\delta^{15}N$ values and the positions occupied by teeth in the dental arcade were found. Studies with Florida manatees showed similar results: Walker and Macko (1999) compared the values of carbon and nitrogen isotopes of the front and back teeth of three two-years old *T. m. latirostris*, *i.e.* still subject to the influence of milk, and found no trends between the two molars; MacFadden *et al.* 2004 analyzed carbon and oxygen isotopic compositions of six teeth from three Florida manatees born in captivity, exposed mainly to lettuce, and found few differences in both isotopes, as expected from a uniform diet. Thus, although methodologically different, the results of these two works seem to agree with the results of the present study, which reinforces the interpretation that there are no significant intra-individual differences or trends in the isotopic compositions of teeth in manatees.

Nevertheless, a slight depletion in $\delta^{15}N$ in bones compared to teeth was observed. Because of the milk-based diet at the early stages of an animal's life, enrichment in ^{15}N is expected in calves and young mammals. Teeth represent food assimilated from birth to adulthood in animals which teeth grow by continuous accretion of dentin layers. Bones, on the other hand, are constantly remodeled and record assimilation spanning a shorter period. As a result, $\delta^{15}N$ values are generally higher in teeth than in bones in a same individual (Bocherens *et al.* 1996). Although manatees continuously replace their teeth, it is possible that, in relatively young individuals, the oldest teeth on the dental arcade were formed during lactation, which may last up to two years in manatees (Hartmann 1979, Reid *et al.* 1995) and, therefore, would result in higher $\delta^{15}N$ than in bone.

Furthermore, two individuals showed differences between teeth and bones greater than 2‰ for $\delta^{13}C$ or $\delta^{15}N$. It has been observed in homophiodontic marine mammals with continuous dental growth that changes in feeding related to environmental circumstances can be detected by the isotopic analysis of different growth layers of the teeth (Hobson and Sease 1998). In the study, the manatee PB-132, for example, showed the greatest difference between teeth for carbon isotopes (2.4‰, Table 1). It is noticed that the average $\delta^{13}C$ for teeth was -9.7‰, and only the tooth M2 (-11.5‰) showed discrepant isotopic composition. Assuming a tooth replacement rate of about nine months (*i.e.* 27 teeth in 20 years) as observed by Domning and Hayek (1984), the formation of tooth M2 had probably occurred between four and five years before the death of the animal. This might suggest that a few years prior to its death, the animal experienced a change in its diet that led to a marked drop in $\delta^{13}C$ value, corresponding to the time of the genesis of M2 tooth. This change in the diet, as indicated by carbon isotopes ratio, could have results either from a variation in environmental conditions, such as changes in community structure, or a change in the animal's behavior, such as movement to a different foraging site.

The input of continental freshwater in coasts and estuaries is one of the factors that influence the community structure of these environments (Lirman *et al.* 2008). In northeastern Brazil, changes in community structure could affect the manatees: higher rainfall during winter, for example, can contribute to remove sand and expose the rhizomes of *Halodule wrightii,* making possible to increase biomass of *Gracilaria* spp. (Silva *et al.* 1987). Alternatively, this lower $\delta^{13}C$ value for tooth M2 could have been caused by a change in the behavior of the animal: the manatee could have moved to areas dominated by vegetation depleted in $\delta^{13}C$. The difference in $\delta^{13}C$ between seagrasses (~ -11‰) and macroalgae (~ -18.5‰) (Clementz *et al.* 2007) – region where the manatee PB-132 was found dead, and are typically found at Paraíba state (Figueiredo *et al.* 2008)– might indicate that the importance of macroalgae decreased at the moment of the genesis of M2 tooth. Moreover, this individual showed a much less depleted $\delta^{13}C$ values than the other two individuals stranded in Paraíba state, which might suggest that it generally feed on seagrasses, but during the formation of M2 it fed more on macroalgae as the other two animals from this region. The same rationale could be applied to the other animals, such as PB-145, which showed differences in nitrogen isotopes among teeth.

The overall results of this study suggest that bones and teeth of manatees, regardless of their position in the dental arcade, can be used in comparative studies of diet and trophic ecology involving analysis of stable isotopes. However, attention should be given to the fact there is a tendency of bones to be slightly poorer in ^{15}N compared to teeth, and that within individual differences in both δ^{13}C (*e.g.* individual PB-132) and δ^{15}N (*e.g.* individual PB-145) might eventually be higher than mean differences between locations. Moreover, as some animals showed a relative high intra-individual variability in the isotopic composition of teeth, we recommend the use of bones or at least two teeth in ecological studies with manatees. These intra-individual differences seem to confirm that the analysis of stable isotopes in manatee's teeth provides a chronological record of diet during the last years of the animal's life and can potentially be used to detect temporal changes in the diet of individual manatees.

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Table 1. Gender, total length, stranding date and location of the five manatees *T. manatus* analysed in this study.

Manatee	Stranding date	Stranding location	Sex	Length (cm)
PB-132	14/03/2003	Praia de Acaú, PB	Male	228
PB-145	17/03/2004	Praia de Acaú, PB	Female	287
PB-214	03/05/2009	Tramataia, PB	Male	299
PE-215	02/05/2009	Praia de Catuama, PE	Female	340
RN-152	14/05/2004	Touros, RN	Male	297

Table 2. δ^{13}C values for *T. manatus* teeth (M1 to M6) and bone (B, right mandibular condyle) from northeastern Brazil. Mean ± standard deviation and difference between maximum and minimum values.

Manatee	M_1	M_2	M_3	M_4	M_5	M_6	Mean	Difference	B
PB-132	-9.9	-11.5	-9.2	-9.3	-9.1	-9.2	-9.7 ± 0.9	2.4	-9.4
PB-145	-15.6	-15.5	-16.0	-16.0	-15.1	-15.6	-15.6 ± 0.3	0.9	-15.6
PB-214	-14.5	-14.9	-14.7	-14.8	-14.3	-14.9	-14.7 ± 0.2	0.6	-16.6
PE-215	-12.4	-12.2	-12.7	-12.3	-11.7	-11.9	-12.2 ± 0.3	1.0	-11.4
RN-152	-9.5	-9.8	-10.0	-9.8	-10.7	-9.9	-9.9 ± 0.4	1.2	-11.5
Mean	-12.4 ± 2.5	-12.8 ± 1.9	-12.5 ± 3.0	-12.4 ± 3.0	-12.2 ± 2.7	-12.3 ± 2.9			-12.9 ± 3.4

Table3. $\delta^{15}N$ values for *T. manatus* teeth (M1 to M6) and bones (B, right mandibular condyle) from northeastern Brazil. Mean ± standard deviation and difference between maximum and minimum values.

Manatee	M_1	M_2	M_3	M_4	M_5	M_6	Mean	Difference	B
PB-132	8.8	9.1	9.2	9.4	8.7	8.0	8.9 ± 0.5	1.4	7.9
PB-145	9.1	9.3	9.9	8.8	8.9	7.8	9.0 ± 0.7	2.1	8.4
PB-214	6.9	7.2	7.1	7.0	6.5	7.5	7.0 ± 0.3	1.0	7.6
PE-215	11.0	10.2	10.7	10.8	10.8	10.7	10.7 ± 0.3	0.7	10.1
RN-152	9.8	9.5	9.1	9.1	8.8	8.7	9.2 ± 0.4	1.1	8.7
Mean	9.1 ± 1.5	9.1 ± 1.2	9.2 ± 1.3	9.0 ± 1.4	8.7 ± 1.5	8.5 ± 1.3			8.5 ± 1.0

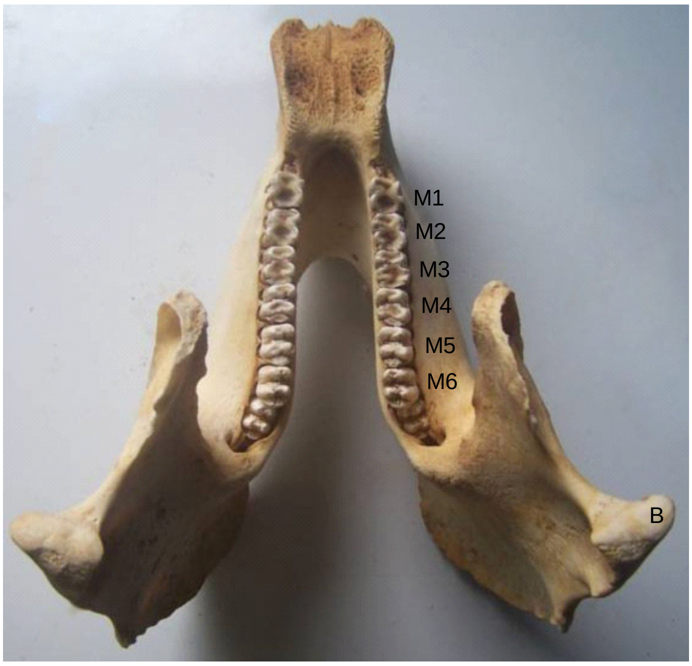

Figure 1. Mandible of *T. manatus* showing the six functional teeth (M1: frontal, oldest molar; to M6: distal, newest molar) and mandibular condyle (B). Picture taken by Leandro Lazzari Ciotti.

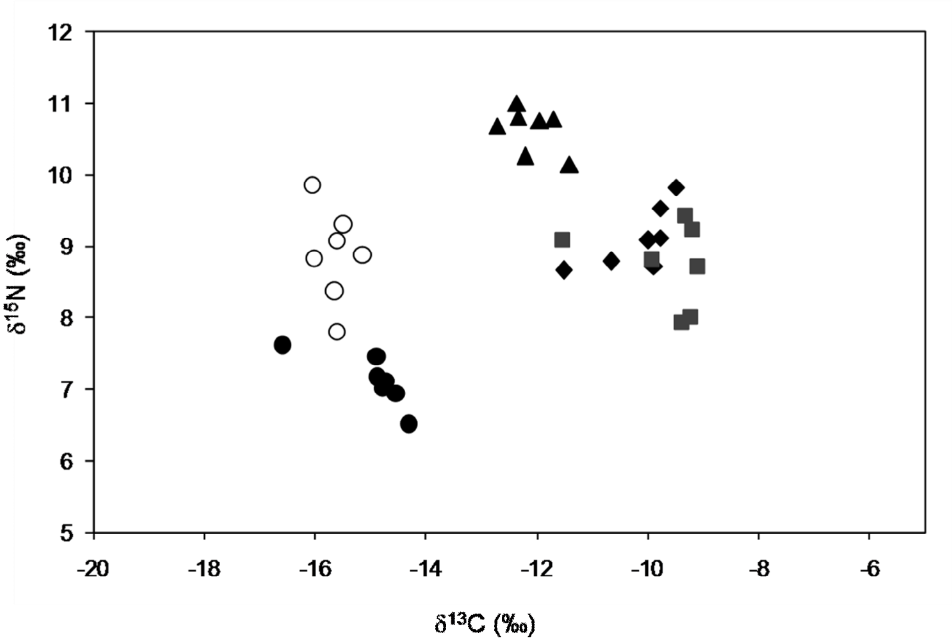

Figure 2. δ^{13}C and δ^{15}N values in teeth and bones of the five manatees *T. manatus* from northeastern Brazil. The animals showed low intraindividual variability between tissues. PB-132 (■), PB-145 (○), PB-214 (●), PE-215 (▲) e RN-152 (♦).

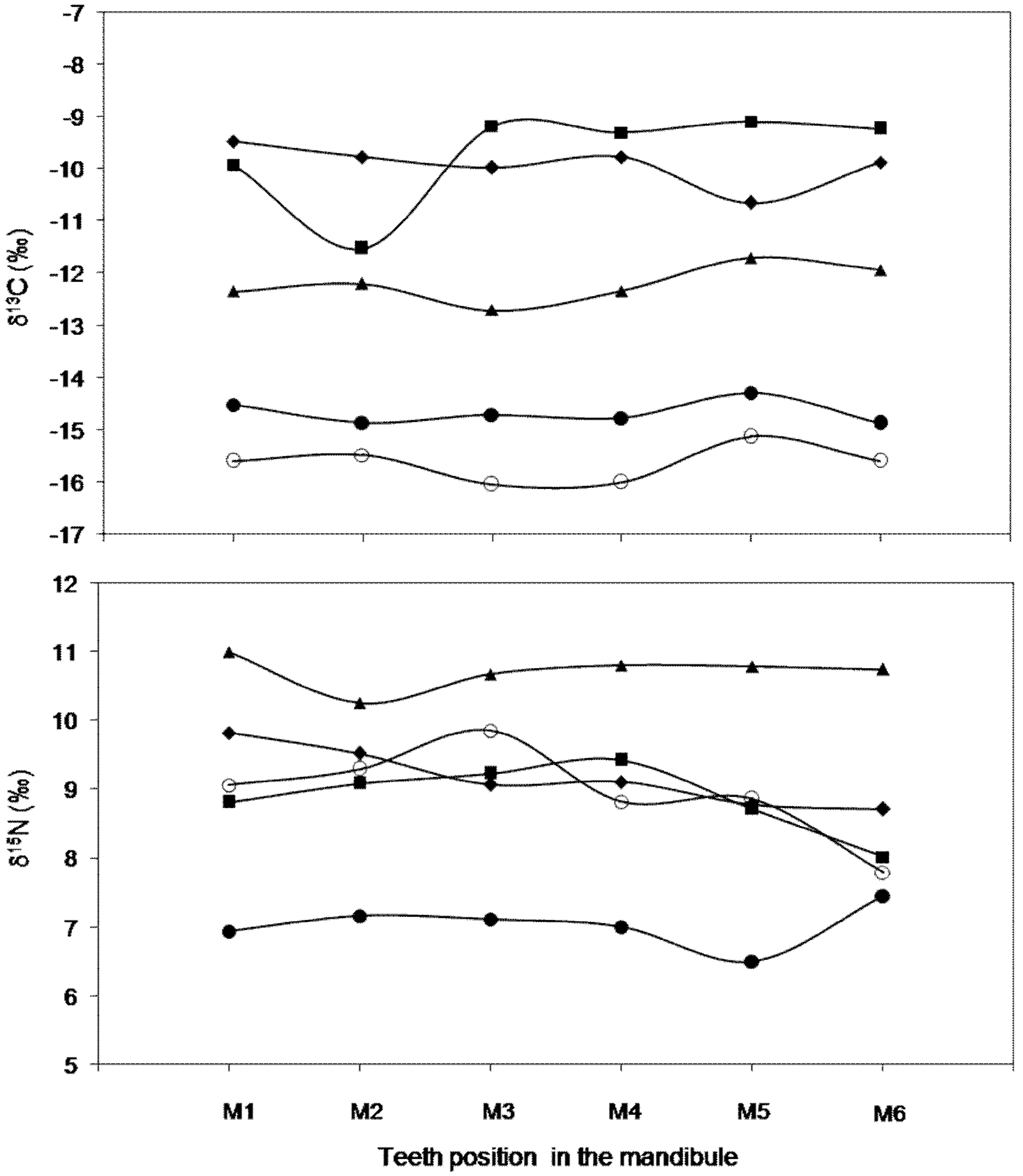

Figure 3. δ^{13}C and δ^{15}N values in teeth according to their position in the mandibule (M1: frontal, oldest tooth; to M6: distal, newest tooth) of the five manatees *T. manatus* from northeastern Brazil. PB-132 (■), PB-145 (○), PB-214 (●), PE-215 (▲) and RN-152 (♦).

Anexo II

Spatial variation in the feeding ecology of the Antillean manatee *Trichechus manatus* in Brazil as inferred by C and N stable isotopes

Leandro L. Ciotti, Inês L. Serrano, Ana Carolina O. Meirelles, Eduardo R. Sechhi

(A ser submetido à Marine Mammal Science)

Spatial variation in the feeding ecology of the Antillean manatee *Trichechus manatus* in Brazil as inferred by C and N stable isotopes

LEANDRO L. CIOTTI

Programa de Pós-graduação em Oceanografia Biológica

Laboratório de Tartarugas e Mamíferos Marinhos

Instituto de Oceanografia – Universidade Federal do Rio Grande – FURG

Av. Itália km 8, Caixa Postal 474

Rio Grande, RS, Brazil, 96.201-900

E-mail: llciotti@yahoo.com.br

INÊS L. SERRANO

Centro Mamíferos Aquáticos – CMA

Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio

Estrada do Forte Orange, s/ nº

Orange, Itamaracá, PE, Brazil, 53.900-000

ANA CAROLINA O. MEIRELLES

Associação de Pesquisa e Preservação de Ecossistemas Aquáticos – AQUASIS

SESC Iparana, Praia de Iparana, s/ nº

Caucaia, CE, Brazil, 61.627-010

EDUARDO R. SECCHI

Laboratório de Tartarugas e Mamíferos Marinhos

Instituto de Oceanografia – Universidade Federal do Rio Grande – FURG
Av. Itália km 8, Caixa Postal 474
Rio Grande, RS, Brazil, 96.201-900

ABSTRACT

In northeastern Brazil, the Antillean manatee *Trichechus manatus* has a fragmented distribution, apparently forming discreet ecological groups exploring a variety of habitats. We investigated the feeding ecology of the species by means of carbon and nitrogen stable isotope compositions. Teeth and bones from 43 stranded manatees were used for C and N stable isotopes analysis. The individuals were clumped into five groups according to areas of occurrence: Alagoas, Ceará, Maranhão, Paraíba and Piauí states. Dominant species of macroalgae, seagrasses, leaves of mangroves and saltmarsh plants were collected from areas of high occurrence of manatees within those regions. The contribution of each feeding source to the manatees's diet was determined through the bayesian mixture model SIAR. The major differences were observed for carbon isotopes: animals from Ceará (-7.0±0.5‰) showed the most enriched values, whereas the lower $\delta^{13}C$ values were found in individuals from Maranhão (-15.7±1.6‰); manatees from Alagoas (-9.3±0.4‰), Piauí (-9.3±0.6‰) and Paraíba (-11.4±2.8‰) showed intermediate values. In turn, manatees did not show large differences in nitrogen isotopes, however, animals from Paraíba (8.8±1.2‰) presented higher values than the other groups. The SIAR indicated regional variation on the manatees diet. Animals from Ceará feed almost exclusively on seagrasses, while individuals from Piauí, on the other hand, had a more diversified diet, where macroalgae, seagrasses and saltmarsh plants were assimilated in similar proportions. Seagrasses, macroalgae and salt marsh plants showed a higher dietary contribution to animals from Alagoas, Paraiba and Maranhão, respectively. The spatial variation in the manatee’s diet demonstrates that proper conservation strategies for the species should include the maintenance of different macrophyte habitats along the northeastern Brazil.

Key Words: Manatees, trophic ecology, diet, habitat use, macrophytes, isotopic composition, mixing model

The manatee *Trichechus manatus* is one of the most endangered aquatic mammals of the world, and has been listed since 1982 as vulnerable by the IUCN – International Union for Conservation of Nature (IUCN 2012). It is classified in this threat category because of anthropogenic factors, such as habitat loss and degradation, collision with watercrafts, illegal hunting, incidental catch in fishing nets and pollution (Deutsch *et al.* 2008). The species inhabits tropical and subtropical western Atlantic coast, from southeastern U.S.A. to northeastern Brazil, where it roves between sea, estuaries, lagoons and coastal rivers (Bertram and Bertram 1973). This sirenian is the only herbivorous aquatic mammal and it is considered generalist and opportunistic, consuming a wide range of aquatic and semi–aquatic plants (Best 1981), usually in shallow water and preferably on submerged vegetation (Hartmam 1979). Two subspecies have been proposed: the Antillean manatee *T. m. manatus* for the populations of Central and South Americas, and the Florida manatee *T. m. latirostris* for the individuals of North America (Domning and Hayek 1986).

One of the environmental factors that controls the distribution and behavior of the individuals is water temperature. Irvine (1983) noted that manatees have exceptionally low metabolic rates and do not have appropriate adaptation to cold, suggesting that the minimum water temperature tolerated by the species would be 20ºC. The distribution of manatees in Florida (U.S.A.) is related to variations in water temperature throughout the seasons: in winter, when the temperature drops below 20ºC, the distribution is restricted to the Florida peninsula, where the animals aggregate in natural and artificial hot springs (Irvine 1983, Alves–Stanley *et al.* 2010); during the warm seasons, when the water temperature rises, manatees disperse throughout Florida, Georgia, South Carolina, North Carolina, and other surrounding states (Deutsch *et al.* 2003). On the other hand, the Antillean manatees do not present this movement pattern because the subspecies is distributed along the tropics, where the water temperature is more constant (Alves–Stanley *et al.* 2010).

Because of these differences, the two subspecies use the habitat in different ways and present different feeding strategies. Lefebvre *et al.* (2000) suggest that the Florida manatees, for doing seasonal migrations and using a wider range of resources, would be less specialized herbivorous than manatees in Puerto Rico. In the U.S.A., animals feed on resources that are available near the thermal refuges or along the travel routes, while manatees in Puerto Rico, not subjected to thermal variations, developed more specialized feeding strategies to maximize the energy intake (Lefebvre *et al.* 2000). Studies in Belize, Mexico and Puerto Rico showed that Antillean manatees feed mainly on seagrasses (Mignucci-Giannoni and Beck 1998, Castelblanco-Martinez *et al.* 2009, Alves-Stanley *et al.* 2010). In Florida, despite some regional differences (Reich & Worthy 2006, Alves-Stanley *et al.* 2010), seagrasses are also the most frequent component in the manatee’s diet (Ledder 1986, Alves-Stanley *et al.* 2010).

In Brazil, the Antillean manatee has a discontinuous distribution from Amapá (4ºN) to Alagoas (10ºS) states (Lima 1997, Luna *et al.* 2008), but historical records suggest that the species distribution extended further south up to Espírito Santo state (20°S) (Whitehead 1977). Despite the widespread distribution, the size of the population in the country is extremely small, with rough estimates of only 450 individuals (Luna *et al.* 2008). The intensive hunting that the animals suffered historically seems to be responsible for the the disappearance of the species in some areas along the Brazilian coast (Lima 1997). Nowadays, the main threats to the animals in the country are illegal hunting, incidental capture in fishing gear and habitat degradation (Parente *et al.* 2004, Luna *et al.* 2008, Meirelles 2008).

Studies show that manatees forage on a wide range of aquatic vegetation in Brazil, including seagrasses, macroalgae and mangrove leaves. Lima (1997) reports that the main component of the animal's diet would be seagrasses (*e.g. Halodule wrightii*, and *Halophila decipiens*). Borges *et al.* (2008) state that macroalgae (*e.g. Gracilaria* spp. and *Hypnea musciformis*), would also be an important resource for the species. Best (1981) reports that animals feed on young shoots and leaves of mangrove trees (*e.g. Rhizophora mangle* and *Laguncularia racemosa*) and saltmarsh plants (*Spartina alterniflora*) in estuarine environments.

Studies on the feeding ecology of manatees are performed traditionally by direct observation of wild animals and by analysis of stomach contents and scats (*e.g.* Mignucci–Giannoni and Beck 1998, Borges *et al.* 2008, Castelblanco–Martínez *et al.* 2009). However, because of the complexity in conducting observational studies in nature and the limitations of stomach and fecal analyzes, which provide information only about the recently ingested food and whose collections are difficult, the stable isotopes analysis (SIA) has become a useful tool in studies of feeding ecology of *T. manatus* (Ames *et al.* 1996, Reich and Worthy 2006, Clementz *et al.* 2007, Alves–Stanley *et al.* 2010). SIA presents some advantages over the traditional methods: it provides information on assimilated food and not simply ingested by the animal, it allows making inference about diet on different time scales and samples can be easily obtained from research collections and museums (Walker and Macko 1999). The technique has been used to identify ecological stocks (Barros *et al.* 2010, Botta 2011) and in studies of feeding ecology, habitat use, migration, physiology and paleoecology of marine mammals (see Newsome *et al.* 2010 for a review).

The SIA assumes that the isotopic composition of a consumer reflects the isotopic composition of its diet. Therefore, from the determination of the ratios between heavy and light isotopes in plants and animals, for example, isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), it is possible to make inferences about diet and trophic relationships between individuals in a same food chain, considering that the isotopic composition in a consumer is typically heavier than composition of its diet (Peterson and Fry 1987).

Differences in stable isotope composition of consumers reflect differences in isotopic ratios of primary producers, which have distinct isotopic values, particularly for carbon. The average $\delta^{13}C$ of freshwater plants (–27‰), macroalgae (–18.5‰) and seagrasses (–11‰) are different, making possible to determine the proportion of each type in the diet of a consumer (Clementz *et al.* 2007). In addition, aquatic ecosystems have spatial gradients in $\delta^{13}C$ values, with samples derived from coastal marine environments typically being more enriched than those from estuaries and freshwater systems (Clementz and Koch 2001).

However, very little is known about the relative importance of the different types of vegetation in the diet, as well as about the influence of the different habitats in the feeding ecology and distribution of manatees in Brazil. Thus, a better understanding on the feeding ecology of *T. manatus* is critical for the development and implementation of strategies for the conservation of the species and its habitat in the country. The present study aims (1) to analyze carbon and nitrogen stable isotope compositions of manatees from northeastern Brazil; (2) to assess spatial variation in the diet; and (3) to determine the relative contribution of different macrophytes and the influence of different habitats on the manatees diet.

METHODS

Samples and study area

Teeth (n = 22) and bones (n = 21) of *T. manatus* stranded between the years 1989 and 2010 on the northeastern Brazilian coast were obtained from the Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos – Instituto Chico Mendes de Conservação da Biodiversidade (CMA – ICMBio) and the Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS). The animals were grouped in five regions according to the places where they stranded: Maranhão (MA, n=5), Piauí (PI, n=2), Ceará (CE, n=10), Paraíba (PB, n=23) and Alagoas (AL, n=3). As teeth and bones do not show differences in carbon and nitrogen isotopes, both were included in the analysis to increase the number of samples (Ciotti *et al.*, Anexo I). The division took the main areas of occurrence of manatees, as well as the discontinuities in distribution into account (Figure 1).

Samples of the major plant species consumed by manatees were collected between September 2011 and February 2012, in places of high occurrence of animals within those five regions: Humberto de Campos and São José de Ribamar (MA), Cajueiro da Praia (PI), Icapuí (CE), Barra de Mamanguape (PB) and Porto de Pedras (AL) (Figure 1). Vegetation was categorized into four main types: seagrasses, macroalgae, mangroves and salt marshes plants. We collected by hand samples of thallus of macroalgae , whole body of seagrasses and fresh live leaves of mangroves and salt marsh plants.

The Maranhão state is characterized by the presence of several rivers, wetlands areas, deltas and estuaries, allowing for the formation of dense mangroves and salt marshes (Souza–Filho 2005, Ab'Saber 2008). The border of Piauí and Ceará states is characterized by a mosaic of habitats, including estuaries, mangroves, seagrass meadows and macroalgae beds (Mai and Loebmann 2010). The occurrence of manatees is frequent in the area, that is formed by the mouth of four rivers (Camurupim, Cardoso, Timonha and Ubatuba). The region remains little impacted by human activities and presents protected areas that are appropriate for manatees' feeding and calving (Meirelles 2008). The eastern Ceará state is characterized by a straight coastline, sandy beaches, seagrasses meadows and macroalgae beds (Pinheiro-Joventino *et al.* 1998), however, except for a few rivers and estuaries, there are no bays, lagoons or other protected environments (Meirelles 2008). The Barra do Mamanguape is a Marine Protected Area in northern Paraíba state, and is characterized by a dense mangrove forest, scarce seagrass meadows and a fringe reef that runs parallel to the coast, covered by macroalgae beds (Xavier *et al.* 2012). The coast of Alagoas is also characterized by a diversity of ecosystems, including sandy beaches, estuaries, lagoons and reefs, that support a high biodiversity, such as mangroves, seagrass meadows and macroalgae beds (Marques 1991).

Sample preparation

Samples of teeth and bones were prepared for stable isotope analysis following the protocol described in Walker and Macko (1999). Samples passed through a cleaning process for removing residues, muscle and other adhered tissues; then, they were rinsed in distilled water and dried in oven at 60°C for 48 hours. The teeth were longitudinally cut with a slow rotation diamond circular saw to expose the dentine. Both teeth and bones were powdered with a drill bit. As these tissues consist of an organic (collagen) and a mineral (bioapatita) portion (Koch 2007), samples was acidified with 30% hydrochloric acid (HCl) to remove the inorganic carbon, and then dried again in oven at 60°C for 2 hours to remove any residual HCl. Plants were rinsed first in freshwater and then in distilled water to remove impurities and attached epiphytes, and also dried in oven at 60ºC for 48 hours. Once dried, plants were ground with pestle and mortar until become a fine powder. Plants were not acidified because this treatment can modify $\delta^{15}N$ values (Bunn *et al.* 1995). Approximately 1.0 mg of bones and teeth samples and between 2.5 and 3.0 mg of plant samples were stored in 5x9 mm tin capsules for the analyses.

Isotope Analysis

The isotopic analyses were performed at the Stable Isotope Core Laboratory – Washington State University, using an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA) coupled to a continuous flow isotope ratio mass spectrometer

(Delta PlusXP, Thermofinnigan, Bremen). The isotopic ratio δ (in ‰) is expressed by: $\delta X = [(R_{sample}/R_{standard}) - 1] * 1000$, where X refers to ^{13}C or ^{15}N, R_{sample} is the ratio of isotopes of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and $R_{standard}$ is the ratio of isotopes of the reference (VPDB – Vienna PeeDee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). Standard deviations were estimated at 0.14‰ and 0.10‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Data Analysis

Data were tested for normality and homogeneity of variances through Shapiro–Wilk and Levene's tests, respectively. Analysis of variance (ANOVA) and Kruskal–Wallis tests, followed by a *post hoc* pairwise comparisons (Bonferroni test), were used to compare the values of $\delta^{13}C$ and $\delta^{15}N$ for age classes as inferred from total body length (calves: <175cm, < 2 years old; subadults: 176 – 225cm, 3-7 years old; and adults: >225cm, > 7 years old; Mignucci–Giannoni *et al.* 2000), and for regional groups of manatees. Student–t and Mann–Whitney tests were used to compare the mean values of $\delta^{13}C$ and $\delta^{15}N$ between males and females. Results are presented as mean ± standard–error and the statistical differences were considered significant at $p < 0.05$.

The relative contribution of the different vegetation types in the diet of manatees was determined by the SIAR (Stable Isotopes Analysis in R), a Bayesian isotopic mixing model. The SIAR offers some advantages compared to other mixing models, like the incorporation of variability in sources and generates results as probability distributions (Parnell *et al.* 2010).

The SIA in ecological studies requires an estimate for the TEF between the individual and its diet ($\Delta^{13}C$ and $\Delta^{15}N$), which vary, for example, with the type of tissue and quality of the diet (Newsome *et al.* 2010). Clementz *et al.* (2007) proposed $\Delta^{13}C$ values in collagen of *T. manatus* ranging from 1.8‰ for animals whose diet is based on seagrass and freshwater plants, to 6.2‰ for individuals that feed only on freshwater vegetation. Thus, in this study, as the animals feed on freshwater, estuarine and marine plants, the value for $\Delta^{13}C$ was considered 1.8‰. Relative to nitrogen, there are no studies with sirenians, however, Schoeninger and DeNiro (1984) suggested a $\Delta^{15}N$ of 3‰ for pinnipeds and cetaceans. As some studies indicate that nitrogen TEF for sirenians would probably be lower than for carnivore marine mammals (Newsome *et al.* 2010), the value adopted for $\Delta^{15}N$ in this study was 2‰, that is smaller than for other groups of marine mammals and is consistent to the isotopic compositions found for the animals and plants.

RESULTS

Isotopic composition

The Antillean manatees of the northeastern Brazil showed mean isotopic composition of –10.6‰ ± 0.5‰ for $\delta^{13}C$ (minimum: –17.4‰; maximum: –5.9‰) and 8.1‰ ± 0.3‰ for $\delta^{15}N$ (minimum: 5.3‰; maximum: 11.0‰) (Table 1). There were no significant differences in isotopic values between sexes for both $\delta^{13}C$ (females: –10.2‰, n=18; males: –10.3‰, n=17; Mann–Whitney: U=144, p>0.7) and $\delta^{15}N$ (females: 8.1‰, n=18; males: 7.8‰, n=17; t–Student: t=0.023, p>0.9). Likewise, there were no differences in mean isotopic compositions among age classes for both $\delta^{13}C$ (calves: –9.6‰, n=15; sub adults: –8.5‰, n=4; adults: –11.0‰, n=18; Kruskall–Wallis: H=2.905, p>0.2) and $\delta^{15}N$ (calves: 8.0‰, n=15; sub adults: 7.1‰, n=4; adults: 8.5‰, n=18; Kruskall–Wallis: H=2.761, p>0.2). However, significant differences were observed in the mean isotopic compositions among the regional groups of manatees for both carbon (Kruskall–Wallis: H=26.7; $p<0.001$) and nitrogen (Kruskall–Wallis: H=14.8; $p<0.005$).

The greatest differences among groups were observed for carbon isotopes, ranging from depleted compositions in the animals from MA (–15.7 ± 0.7‰) to more enriched compositions in individuals from CE (–7.0 ± 0.2‰). The manatees from AL (–9.3 ± 0.2‰), PI (–9.3 ± 0.4‰) and PB (–11.4 ± 0.6‰) showed intermediate values and no significant differences, as well as MA and PI, that showed no significant differences either (Figure 2, Table 2). In turn, the differences in nitrogen values among groups were lower. Mean δ^{15}N values ranged from depleted compositions in the individuals from PI (6.6 ± 1.2‰), MA (6.6 ± 0.6‰) and AL (6.9 ± 0.7‰) to values slightly more enriched in δ^{15}N in manatees from PB (8.8 ± 0.3‰). The animals from CE (7.7 ± 0.4‰) presented intermediate values. The manatees from PB were the only to show significantly different mean δ^{15}N with respect to AL, CE and MA (Table 3).

Diet of the manatees

The plant groups presented distinct isotopic compositions, particularly for carbon. In general, mangrove leaves exhibited low δ^{13}C values, seagrasses and leaves of salt marsh plants presented high δ^{13}C, whereas macroalgae showed intermediate values. Regarding δ^{15}N, differences between groups were lower than those for carbon, but in general macroalgae presented more enriched compositions in ^{15}N than the other macrophytes within each region (Table 4).

The SIAR (Figure 3) suggested spatial differences in the diet composition of the manatees: individuals from CE feed mainly on seagrasses (mean: 97.4%; 0.95 CI: 92% to 100%), while manatees from PI presented a more diversified diet, with the four sources contributing in relatively similar proportions (seagrasses – mean: 27.7%; 0.95 CI: 0% to 54%; saltmarsh plants – mean: 26.9%; 0.95 CI: 0% to 52%; macroalgae – mean: 24.2%; 0.95 CI: 0% to 48%; and mangroves – mean: 21.3%; 0.95 CI: 0% to 44%). Manatees from AL also graze mostly upon seagrasses (mean: 69.7%; 0.95 CI: 28% to 96%) and, to a lesser extent, on macroalgae (mean: 24.9%; 0.95 CI: 0% to 61%); whereas animals from PB feed mainly on macroalgae (mean: 64.3%; 0.95 CI: 51% to 76%) and, to a lesser extent, on seagrasses (mean: 33.6%; 0.95 CI: 21% to 47%). In MA, manatees feed on salt marsh plants (mean: 68.8%; 0.95 CI: 54% to 83%), surpassing mangrove leaves (mean: 31.2%; 0.95 CI: 17% to 46%) (Figure 3).

Discussion

Estuaries, reefs, mangroves and seagrasses meadows are important coastal habitats in northeastern Brazilian coast (Schaeffer–Novelli *et al.* 1990, Figueiredo *et al.* 2008, Marques and Creed 2008), and this diversity of habitats is reflected in the variability of isotopic compositions of the manatees, which showed distinct ratios among regions and demonstrate that the groups use different resources along their distribution range.

Carbon stable isotope composition

The high $\delta^{13}C$ composition presented by the manatees from CE is probably related to the foraging in coastal marine areas, that present higher $\delta^{13}C$ than estuarine and freshwater environments, which seems to confirm that the animals do not use the rivers and estuaries in the region. Although today there are no records of manatees in rivers and estuaries in eastern CE, they occurred in the Jaguaribe river in the past. Increased boating activity and shrimp farming in the area have caused destruction of mangroves and river siltation, limiting the access to the estuary and driving the animals to more coastal or marine areas (Meirelles 2008). In contrast, manatees from MA showed lower $\delta^{13}C$ values, which reflect the differences between the two environments. The coast of MA is characterized by dense mangroves and salt marshes, and the depleted $\delta^{13}C$ suggest that manatees use these estuarine and freshwater habitats.

The manatees from Alagoas (–9.3‰), Piauí (–9.3‰) and Paraíba (–11.4‰) presented intermediate carbon compositions. All three regions present a mosaic of different habitats, such as mangrove forests, estuaries and coastal beaches, that could determine the intermediate isotopic composition of the manatees, and suggest that the individuals feed on resources from both marine and estuarine environments. The wide variation in $\delta^{13}C$ values presented by the manatees from PB (–17.4‰ to –6.9 ‰) could also indicate the use of different environments by the individuals. Manatees concentrate at two neighbors areas of northeastern Brasil in summer, Barra do Mamanguape and Sagi (Rio Grande do Norte state), for breeding and calving, and probably disperse to other areas of the coast during the other seasons (Silva et al. 1992, Paludo and Langguth 2002).

Nitrogen stable isotope composition

The manatees did not presented large differences in $\delta^{15}N$ values among regions, however, animals from PB (8.8‰) showed compositions higher than those from other areas. The coastal area of Paraíba, Pernambuco and Rio Grande do Norte states is extensively altered by plantation of sugar cane and the capitals of these states together sum approximately 5,755,926 inhabitants (IBGE 2010). Sewage and industrial effluents from these large cities and the runoff from agriculture are sources of nitrogen to aquatic environments, which may lead to habitat degradation (Carpenter *et al.* 1998), as well as to an increase in $\delta^{15}N$ values in organisms' tissues (Rau *et al.* 1981, Wayland and Hobson 2001), $\delta^{15}N$ values in primary producers reflect the nitrogen inputs from anthropogenic wastes to aquatic systems (McClelland *et al.* 1997; Cole *et al.* 2004), and $\delta^{15}N$ signals in primary consumers are strongly linked to human population density (Cabana and Rasmussen 1996). Thus, the high $\delta^{15}N$ values presented by manatees in the region could be a result of the nitrogen released into the environment due to anthropogenic activities. Indeed, this high $\delta^{15}N$ presented by individuals from Paraíba could be a result from a diet based on macroalgae, that have higher $\delta^{15}N$ values than other macrophytes (Dillon and Chanton 2008) and appear to be better indicators of anthropogenic inputs in water bodies (Cole *et al.* 2004; Dillon and Chanton 2008). This is corroborated by higher values of some trace metals (*e.g.* aluminum, lead, cadmium) in the blood of the individuals from Barra do Mamanguape than those from another regions, like Alagoas (Anzolin *et al.* 2012). Alves–Stanley *et al.* (2010) also found differences in nitrogen compositions in manatees from Belize and Puerto Rico, and suggested that higher values are related to coastal development, pollution and farming.

Diet of the manatees

The results have demonstrated that manatees explore different resources along the northeastern Brazil, suggesting different feeding strategies and levels of specialization among groups. This variety of feeding habits is probably a result of the diversity of environments and different types of vegetation available throughout the region.

The SIAR (Figure 3) suggest that animals from MA feed mostly on saltmarsh plants and, to a lesser extent, on mangroves. Thus, the diet is mainly linked to the animals’ access to freshwater and estuaries and the plant resources present in these environments. The macrotidal regime (*ca.* 4.2m) and mean annual precipitation (*ca.* 2,000 mm) in area are suitable for the development of mangroves and, in low energy depositional environments, it is common the colonization by *Spartina alterniflora* (Schaeffer–Novelli *et al.* 1999).

The manatees from CE feed primarily on seagrasses, and this appears to be related to the limited access of the animals to freshwater on the eastern coast of the state, which is characterized by occurrence of a few rivers and human–impacted estuaries, that restricts the distribution of animals to marine areas (Meirelles 2008). Despite the great diversity of macroalgae species, the eastern CE has a relatively low biomass of seaweeds (I. B. de Castro, personal communication).

As opposed to the monotonous diet presented by animals from Ceará, manatees from Piauí showed a generalist diet, with a relatively even contribution from the four plant groups. The coast of Piauí is biologically diverse, with a high primary productivity, which provide food and habitat for many animal species, including the manatee (Mai and Loebmann 2010). However, contributions of the vegetable groups to diet of individuals from Piauí should be interpreted with cautious, as sample size is small and the estimates present high credibility intervals (Figure 3).

Individuals from PB and AL also have different diets: in AL, manatees graze mainly upon seagrasses, while macroalgae has a higher contribution in the diet of animals from PB. This pattern is probably related to the floristic composition of the regions: while Paraíba, Pernambuco and Rio Grande do Norte states have high diversity of macroalgae associated to the coastal reefs, the coast of Alagoas has a low diversity of benthic algae (Figueiredo *et al.* 2008). Moreover, J. C. Borges (personal communication) noted that macroalgae are important food items for the animals in Barra de Mamanguape and Paludo and Langguth (2002) suggest that the occurrence of manatees in the region of Sagi is apparently related to the presence of macroalgae beds associated with the reefs of the region.

Although manatees probably select plants for palatability, digestibility and nutritional value (Heinshon and Birch 1972, Hartmann 1979), diet and feeding strategy seem to be related, in large part, to the availability of the dominant resources in the environments. Hartmann (1979) states that some populations of *T. m. latirostris* feed on the dominant submerged plant in the regions in which they occur, and Lefebvre *et al.* (2000) found that manatees in Florida and Puerto Rico feed on the most abundant vegetation in the area. In the present study, the manatees in Brazil apparently feed on the most common and abundant food items available, such as macroalgae in PB and seagrasses in AL. At the same time, the animals appear to have preferences for certain items, such as in MA, where animals fed mainly on salt marsh plants, despite the availability of macroalgae.

Conclusion

The northeastern Brazilian coast provides favorable habitats for manatees, such as warm, shallow and sheltered waters, as well as food supply. Estuaries, reefs, mangroves and seagrass meadows are important coastal habitats in the region, and also important for the manatees, that have different diets and strategies among regions. Salt marsh plants in Maranhão, seagrasses in Ceará and Alagoas, macroalgae in Paraíba and a variety of aquatic plants in Piauí are important item foods for the manatees. This study is the first to use stable isotopes to investigate the feeding ecology of Antillean manatees in South America, and the information presented here demonstrates the importance of the diversity of habitats to the diet and feeding ecology of *T. m. manatus* in northeastern Brazil, and is crucial to formulate regional strategies for the conservation of the species and its habitats.

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Table 1. Mean, minimum and maximum δ^{13}C and δ^{15}N for teeth and bones of Antillean manatee *Trichechus manatus* in Alagoas (AL), Ceará (CE), Maranhão (MA), Paraíba (PB) and Piauí (PI) states, northeastern Brazil. Number of samples in parentheses.

	δ^{13}C (‰)			δ^{15}N (‰)		
Region	Mean	Minimum	Maximum	Mean	Minimum	Maximum
MA (5)	–15.7 ± 0.7	–17.4	–13.2	6.6 ± 0.6	5.9	9.2
PI (2)	–9.3 ± 0.4	–9.7	–8.8	6.6 ± 1.2	5.5	7.8
CE (10)	–7.0 ± 0.2	–7.9	–5.9	7.7 ± 0.4	6.0	9.8
PB (23)	–11.4 ± 0.6	–17.4	–6.9	8.8 ± 0.3	5.3	11.0
AL (3)	–9.3 ± 0.2	–9.7	–9.0	6.9 ± 0.7	6.0	8.2
Overall (43)	–10.6 ± 0.5	–17.4	–5.9	8.1 ± 0.2	5.3	11.0

Table 2. Bonferroni’s test values of comparisons of δ^{13}C values for teeth and bones of Antillean manatee *T.manatus* in Alagoas (AL), Ceará (CE), Maranhão (MA), Paraíba (PB) and Piauí (PI) states, northeastern Brazil.

Region	AL	CE	MA	PB	PI
AL	–	0.014*	0.037*	0.149	0.773
CE	–	–	0.003*	0.000*	0.041*
MA	–	–	–	0.008*	0.081
PB	–	–	–	–	0.249
PI	–	–	–	–	–

* Significant difference

Table 3. Bonferroni's test values of comparisons of $\delta^{15}N$ values for teeth and bones of Antillean manatee *T. manatus* in Alagoas (AL), Ceará (CE), Maranhão (MA), Paraíba (PB) and Piauí (PI) states, northeastern Brazil.

Region	AL	CE	MA	PB	PI
AL	–	0.272	0.371	0.033*	0.773
CE	–	–	0.076	0.016*	0.334
MA	–	–	–	0.018*	0.847
PB	–	–	–	–	0.080
PI	–	–	–	–	–

* Significant difference

Table 4. Mean values of $\delta^{13}C$ and $\delta^{15}N$ of plant species collected at Alagoas (AL), Ceará (CE), Maranhão (MA), Paraíba (PB) and Piauí (PI) states, northeastern Brazil. Number of samples in parentheses.

Region	Species	Vegetable type	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Alagoas	*Gracilaria caudata*	Macroalgae(11)	–16.5 ± 0.6	4.4 ± 0.3
	Caulerpa sp.		–12.4 ± 0.1	5.6 ± 0.3
	Gracilaria sp.		–17.4 ± 0.2	5.0 ± 0.1
	Sargassum vulgare		–18.1 ± 1.0	4.5 ± 0.1
	Halodule wrightii	Seagrass (3)	–9.6 ± 0.1	2.4 ± 0.1
	Rizophora mangle	Mangrove (6)	–32.0 ± 0.8	0.9 ± 1.1
	Laguncularia racemosa		–29.7 ± 1.2	1.5 ± 1.9
Ceará	*Hypnea musciformes*	Macroalgae(11)	–17.4 ± 0.5	8.7 ± 1.1
	Caulerpa prolifera		–14.1 ± 0.9	9.0 ± 0.1
	Spataglossum schoederi		–20.4 ± 0.3	6.9 ± 0.1
	Gracilaria sp.		–19.6 ± 0.2	8.5 ± 0.2
	Halodule wrightii	Seagrass (3)	–10.4 ± 0.2	5.9 ± 0.2
Maranhão	*Rizophora mangle*	Mangrove (9)	–28.6 ± 1.0	4.3 ± 0.4
	Avicennia germinans		–26.6 ± 0.5	5.3 ± 0.8
	Laguncularia racemosa		–27.3 ± 0.3	2.1 ± 2.8
	Spartina alterniflora	Saltmarsh (3)	–13.3 ± 0.1	5.0 ± 1.0
Paraíba	*Hypnea musciformes*	Macroalgae (6)	–20.0 ± 0.7	9.0 ± 0.3
	Gracilaria birdiae		–17.0 ± 1.0	7.6 ± 0.1
	Halodule wrightii	Seagrass (3)	–14.2 ± 0.4	3.2 ± 1.7
	Laguncularia racemosa	Mangrove (6)	–28.0 ± 1.5	5.2 ± 1.2
	Rizophora mangle		–28.4 ± 0.2	3.1 ± 2.1
Piauí	*Caulerpa sp.*	Macroalgae(12)	–16.9 ± 0.2	3.9 ± 0.4
	Hypnea musciformes		–21.2 ± 0.3	6.0 ± 0.2
	Gracilaria sp.		–19.1 ± 0.1	6.6 ± 0.2
	Gelidiella sp.		–23.4 ± 0.3	5.3 ± 0.1
	Halodule wrightii	Seagrass (3)	–11.5 ± 0.3	3.8 ± 0.1
	Laguncularia racemosa	Mangrove (6)	–28.2 ± 0.5	5.3 ± 0.1
	Rizophora mangle		–28.2 ± 0.2	6.1 ± 0.2
	Spartina alterniflora	Saltmarsh (3)	–13.4 ± 0.1	3.7 ± 0.5

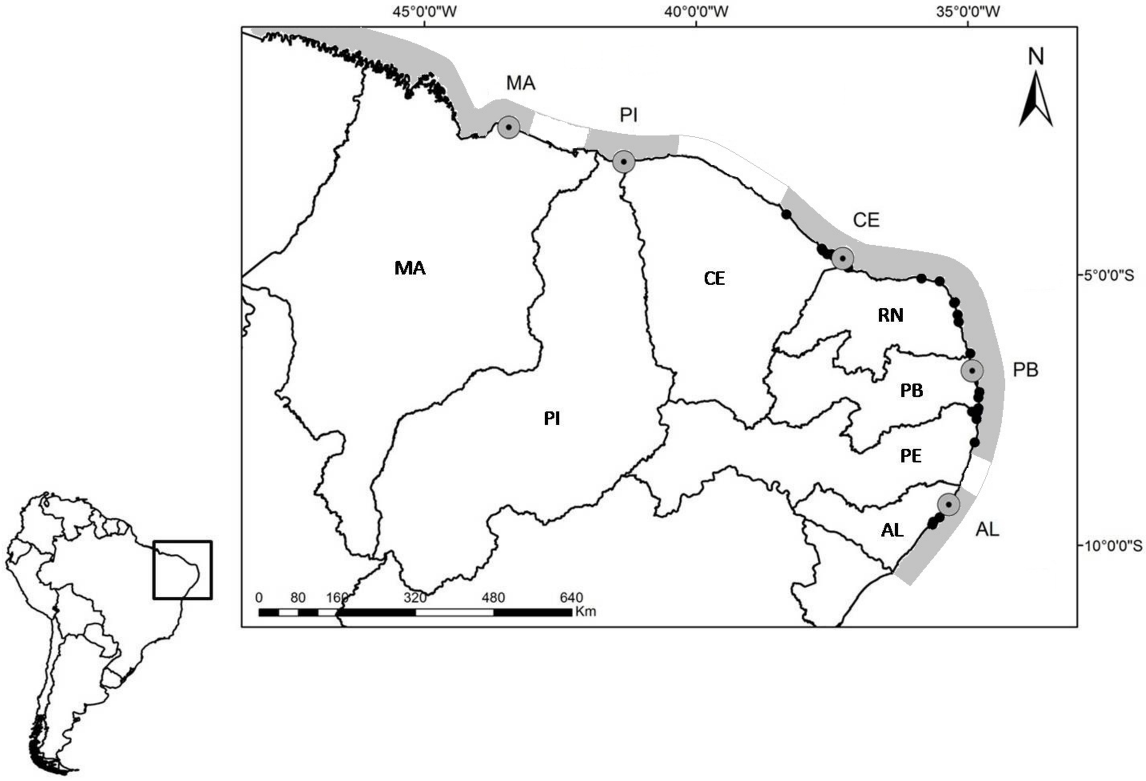

Figure 1. Areas of occurrence (in gray) and discontinuities of Antillean manatee *T. manatus* along the northeastern Brazilian coast, and location where individuals (•) and macrophytes (ʘ) were sampled. MA = Maranhão, PI = Piauí, CE = Ceará, PB = Paraíba and AL = Alagoas.

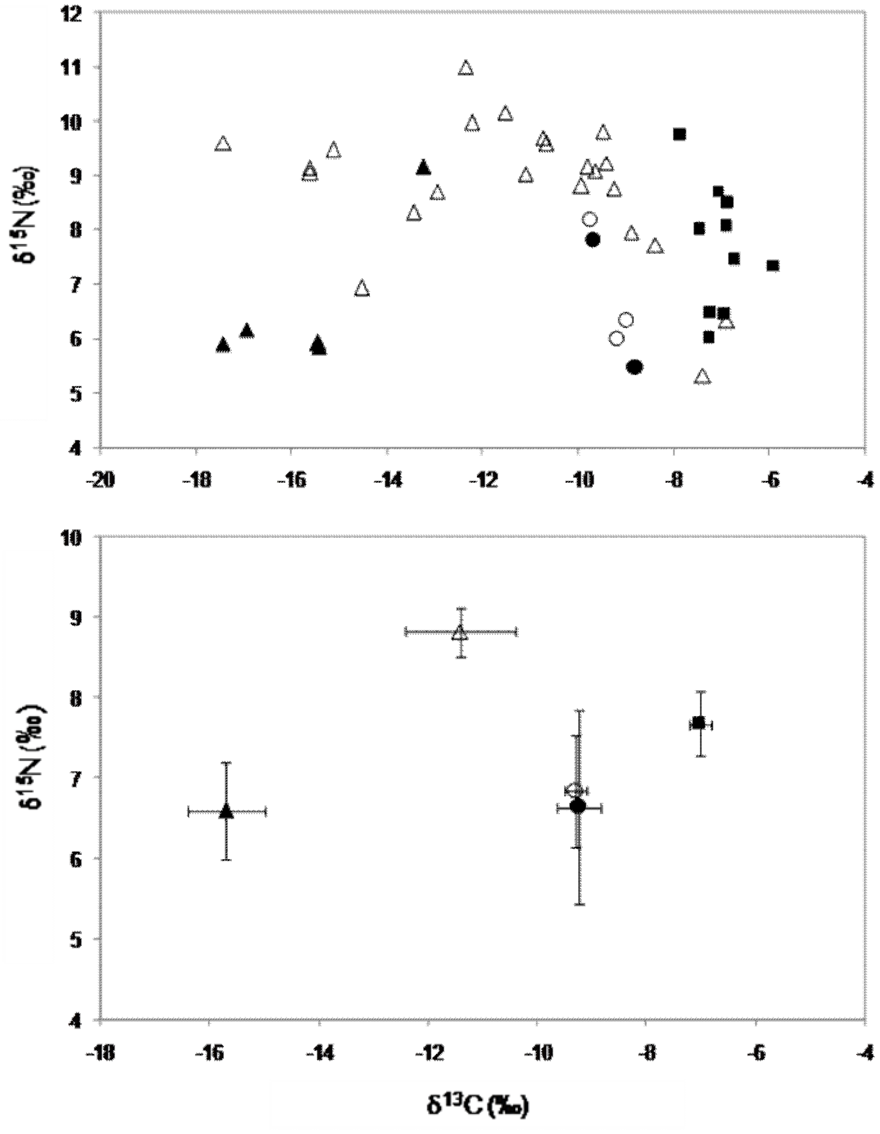

Figure 2. Stable isotope values (scatterplot and mean±standard–error) for teeth and bones of Antillean manatee *T. manatus* in northeastern Brazil, from Maranhão (▲), Piauí (●), Ceará (■), Paraíba (Δ) and Alagoas (○).

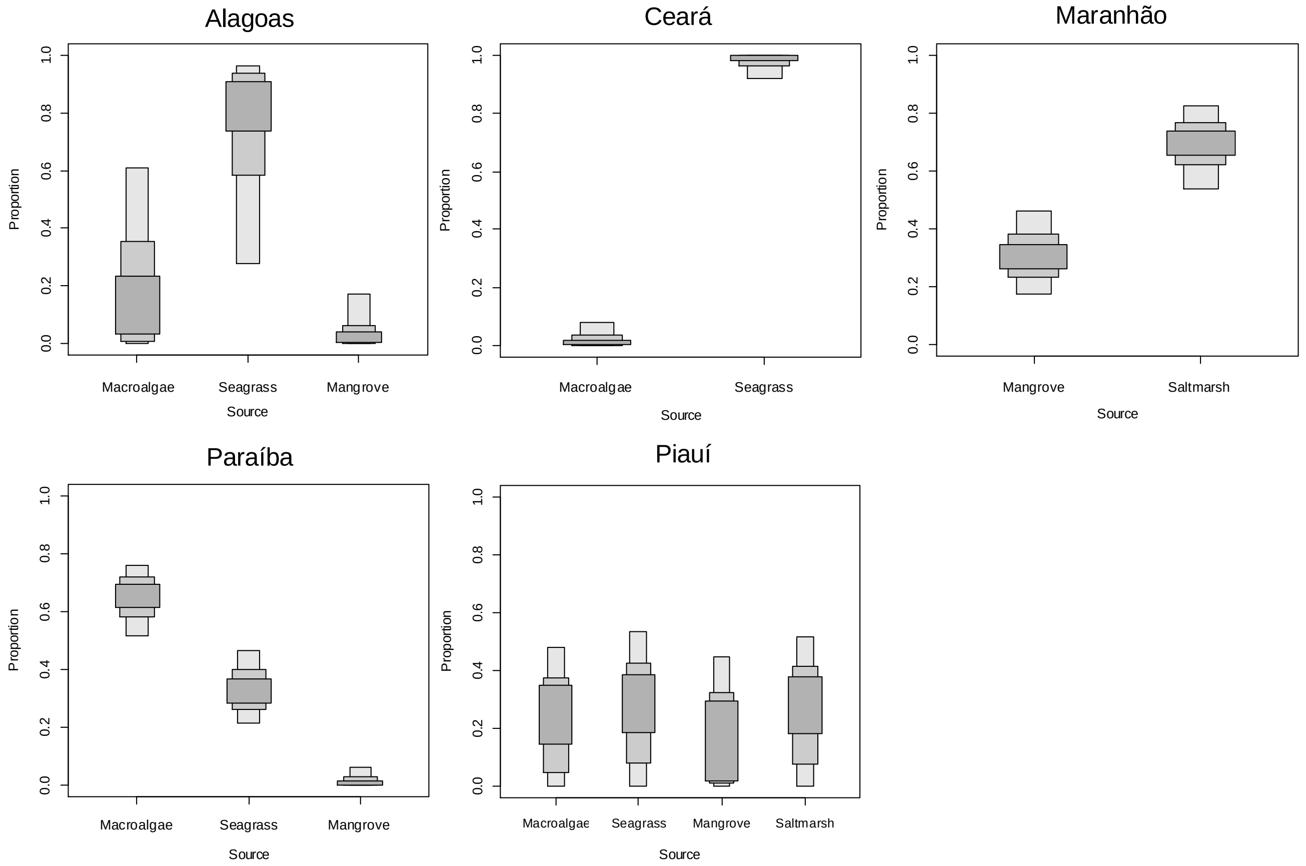

Figure 3. Contribution (%) of vegetable groups (seagrass, macroalgae, mangrove and salt marsh plants) in the diet of Antillean manatee *T. manatus* in different regions of northeastern Brazil, from mixing model SIAR (decreasing bar widths represent 95%, 75% and 25% credibility intervals).