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ECOLOGIA ALIMENTAR DE PEIXES-BOI NO BRASIL COM BASE EM ANÁLISES DE ISÓTOPOS ESTÁVEIS DE CARBONO E NITROGÊNIO

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RESUMO

Estudar a ecologia alimentar é essencial para entender questões comportamentais, incluindo aspectos de uso de habitat importantes para subsidiar estratégias de conservação. Existem poucos estudos com enfoque na dieta para as duas espécies de sirênios que ocorrem no Brasil, o peixe-boi-amazônico (*Trichechus inunguis*) e o peixe-boi-marinho (*T. manatus*). Além de haver poucas informações disponíveis, essas espécies apresentam ampla distribuição, podendo assim, apresentar variações espaciais na dieta. Com o objetivo de compreender a ecologia alimentar dessas espécies, as variações na dieta e o uso do habitat das duas espécies foram avaliados por meio da análise de isótopos estáveis. Para *T. inunguis*, as amostras de dentes depositadas em coleções científicas foram utilizadas. Modelos de mistura isotópica foram utilizados para estimar a composição da dieta dos indivíduos provenientes do médio Rio Solimões, Estuário do Rio Amazonas, rios Negro e Tapajós. Os peixes-boi apresentaram diferenças significativas nas médias de $\delta^{13}C$ e $\delta^{15}N$ entre os diferentes habitats. Os animais do Rio Negro apresentaram menores valores de $\delta^{13}C$ (-28,7 ± 1,8 ‰) e de $\delta^{15}N$ (7,5 ± 1,7 ‰) e os animais do médio Solimões maiores valores de $\delta^{13}C$ (-17,0 ± 2,7 ‰) e de $\delta^{15}N$ (9,3 ± 1,1 ‰). Para os animais do médio Rio Solimões, houve um efeito significativo da classe ontogenética nos valores de $\delta^{13}C$ (p= 0.002), enquanto não foi observado efeito significativo da classe ontogenética, sexo e da interação entre os fatores nos valores de $\delta^{15}N$. Os modelos de mistura mostraram que as plantas C_4 e plantas aquáticas C_3 contribuem em similar proporção para a dieta do peixe-boi no médio Solimões e no Estuário do Rio Amazonas (a diferença de contribuição de 0,04 a 0,06). Modelos de mistura para peixes-boi do Estuário do Rio Amazonas apresentaram alta contribuição de macroalgas. No caso de *T. manatus*, amostras das regiões norte e nordeste do Brasil foram obtidas de coleções científicas. A área estudada foi dividida em Litoral Amazônico (LA), Litoral Setentrional do Nordeste (LS-NE) e Litoral Oriental do Nordeste (LO-NE). Os nichos isotópicos dos indivíduos de cada área foram calculados com base nas composições isotópicas dos indivíduos coletados em cada região e mapas isotópicos foram gerados. Os valores de $\delta^{13}C$ foram mais altos para os peixes-boi-marinhos do LS-NE (-8.1 ± 1.2 ‰) e mais baixos para os animais do LA (18.7 ± 5.5 ‰). O nicho isotópico mostrou-se mais amplo para os peixes-boi-marinhos do LO-NE (SEAc = 18,2 $‰^2$), seguido dos animais do LA e (SEAc = 6,4 $‰^2$) e LS-NE (SEAc = 5,0 $‰^2$). Diferenças

nas disponibilidades de tipos de plantas entre habitats (água doce, estuarino, marinho) provavelmente são responsáveis pela maior parte da variação espacial encontrada. Diferentes requerimentos energéticos associados ao crescimento e reprodução também podem estar relacionados às variações na dieta. Aparentemente, tanto *T. inunguis* quanto *T. manatus* são espécies oportunistas e generalistas, alimentando-se de uma ampla gama de recursos alimentares de acordo com a sua disponibilidade no ambiente.

Palavras-chave: *Trichechus inunguis*, *Trichechus manatus*, Amazônia, Costa brasileira, nicho isotópico, *isoscapes*

ABSTRACT

Feeding-ecology studies are essential to understand behavioural questions, including habitat-use patterns, and to subsidize conservation strategies In Brazil, few studies have focused on the diet of the Amazon manatee (*Trichechus inunguis*) and the West Indian manatee (*Trichechus manatus*), both species have present a wide distribution, and diet possibly varies spatially. To understand the feeding ecology of manatee species in Brazil, diet and habitat-use variation were assessed through stable-isotope analysis. For *T. inunguis*, teeth samples were obtained from scientific collections. Mixing models were used to estimate the diet composition of manatees from the Middle-Amazon, Amazon River Estuary, and from the Negro and Tapajós Rivers. The animals from Rio Negro presented lower values of $\delta^{13}C$ (-28.7 ± 1.8 ‰) and $\delta^{15}N$ (7.5 ± 1.7 ‰) and animals from the middle Amazon had higher values of $\delta^{13}C$ (-17.0 ± 2.7 ‰) and $\delta^{15}N$ (9.3 ± 1.1 ‰). For animals from the middle Amazon, there was a significant effect of the ontogenetic class on the $\delta^{13}C$ values ($p = 0.002$), while no significant effect of the ontogenetic class, sex and the interaction between the factors on the $\delta^{15}N$ values was observed. Mixing models showed that C_4 plants and C_3 aquatic plants contribute in a similar proportion to the manatee diet in the middle Amazon and the Amazon River Estuary (difference range: 0.04 - 0.06). Manatee mixing models from the Amazon River Estuary showed a high contribution of macroalgae. Teeth samples from *T. manatus* were obtained from scientific collections located in the North and Northeastern regions. The study area was divided into North Coast (NC), Dry-Northeastern Coast (Dry-NEC) and Humid-Northeastern Coast (Humid-NEC). The isotopic niche of all individuals was calculated as area of the isoscapes generated from isotopic values. The $\delta^{13}C$ values for animals from Dry-NEC were higher (-8.1 ± 1.2 ‰) than for animals from the North Coast (18.7 ± 5.5 ‰). The isotopic-niche width of manatees was greater in Humid-NEC (SEAc = 18.2 ‰^{2}), followed by North Coast (SEAc = 6.4 ‰^{2}) and Dry-NEC (SEAc = 5.0 ‰^{2}). Differences in the availability of food sources and habitats (freshwater, estuarine and marine) are probable explanations for most of the spatial variation. Also, distinct energetic requirements related to growing and reproduction may be related to diet variation. Both, *T inunguis* and *T. manatus* are opportunist and generalist species, feeding from a large diversity of food plant types in different habitats presumably due mainly to differences in availability.

Keywords: *Trichechus inunguis*, *Trichechus manatus*, Amazon, Brazilian coast, isotopic niche, *isoscapes*

1. INTRODUÇÃO GERAL

Estudos aprofundados sobre a ecologia alimentar são essenciais para a compreensão de processos evolucionários, fisiológicos e comportamentais (Cherel & Hobson 2007). A aquisição de recursos alimentares afeta a sobrevivência individual, o crescimento e a fecundidade dos animais, acarretando efeitos diretos na dinâmica das populações (Elser et al. 1996).

Dentre os mamíferos aquáticos, muitas espécies são consideradas generalistas oportunistas, entretanto selecionam os recursos alimentares quando há opções disponíveis (Berta et al. 2006). É esperado que grandes herbívoros aquáticos, como os sirênios, tentem maximizar a ingestão de recursos de maior qualidade (maiores concentrações de energia) e minimizar o consumo de recursos de menor qualidade (alto teor de fibras e componentes secundários) (Marsh et al. 2012).

Ao longo de sua vida, a estratégia que maximiza o ganho de energia irá envolver um compromisso entre aumentar o ganho de energia a curto prazo e minimizar os riscos à sobrevivência (Heithaus & Dill 2006). Sendo assim, a dieta e o comportamento de forrageamento são afetados por fatores demográficos como a idade, sexo, estado reprodutivo, características fisiológicas e anatômicas, risco de predação, competição, e distribuição e abundância dos potenciais recursos alimentares (Berta et al. 2006).

Frequentemente são observadas diferenças na dieta de indivíduos de diferentes sexos e idade/classes ontogenéticas. Essas diferenças podem estar associadas a demandas da reprodução (p. ex. lactação), mudanças fisiológicas, importância relativa da ingestão de energia (Kim et al. 2012), além do grau de experiência e período de aprendizado requerido para o uso apropriado de determinadas táticas (Lundström et al. 2010, Henning et al. 2018). Sendo assim, estudos são necessários para compreender as relações da dieta e comportamento de forrageamento com as características dos indivíduos (sexo, tamanho, classe ontogenética) e do habitat (Berta et al. 2006).

1.1.Herbivoria em mamíferos aquáticos

O único grupo de mamíferos aquáticos exclusivamente herbívoros são os sirênios (Ordem Sirenia). Mesmo com a alta disponibilidade de plantas aquáticas, nenhum outro grupo de mamíferos marinhos (cetáceos e pinípedes) evoluiu para explorar esse recurso

alimentar. A história evolutiva dos sirênios remonta a 50 milhões de anos (Eoceno), atingindo uma maior diversidade no Oligoceno e Mioceno, quando se deu início o declínio das espécies do grupo em função do resfriamento global, mudanças oceanográficas e impactos antrópicos (Domning 2006). Somente dois gêneros e quatro espécies são encontrados atualmente (Figura 1): três espécies da família Trichechidae - os peixes-boi (*Trichechus*), que se distribuem pela região costeira do Oceano Atlântico e rios no continente americano (*Trichechus manatus*) e costa oeste da África (*Trichechus senengalensis*), sendo o peixe-boi-amazônico (*Trichechus inunguis*) encontrado somente nas águas doces da Bacia Amazônica; e o dugongo (*Dugon dugong*), único membro vivente da família Dugongidae que se distribui no Oceano Índico e sudoeste do Pacífico (Marsh et al. 2012).

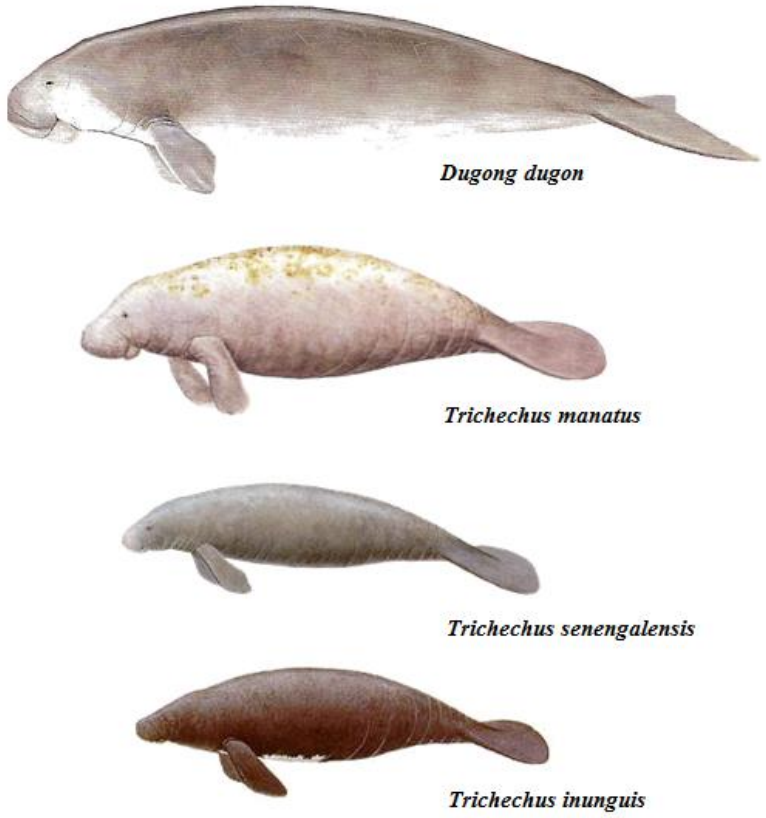


Figura 1. Representantes viventes da Ordem Sirenia. Desenhos de Peter Folkens (Adaptado de: Reeves et al. 1992).

Os primeiros sirênios (família Prorastomidae e Protosirenidae – Eoceno) eram do tamanho de um porco, possuíam quatro patas e apresentavam características anfíbias. Mesmo apresentando deslocamento terrestre, já apresentavam dieta herbívora e

dependiam de gramas marinhas e outras angiospermas aquáticas. As formas posteriores desenvolveram uma variedade de estratégias de forrageamento, em adaptações a mudanças climáticas, geológicas, oceanográficas e condições biológicas dentro dos limites da megaherbivoria aquática (Berta et al. 2006).

1.2.Sirênios no Brasil

No Brasil ocorrem duas espécies de sirênios pertencentes à família Trichechidae: o peixe-boi-amazônico e o peixe-boi-marinho.

O peixe-boi-amazônico se distribui por toda a drenagem do rio Amazonas até a ilha do Marajó, incluindo regiões de lagos, tributários maiores e as áreas alagadas (várzeas e igapós) associados (Marmontel et al. 2016). As áreas de descontinuidade em sua distribuição são locais a montante de corredeiras como em Santo Antônio no Rio Madeira, Cachoeira do Espelho no Rio Xingú, São Luiz no Rio Tapajós, Cachoeira do Bem Querer no Rio Branco, São Gabriel da Cachoeira no Rio Negro, Cachoeira Porteira no Rio Trombetas, Cachoeira Comprida no Rio Nhamundá, Cachoeira Panamá no Rio Parú e Cachoeira Aurora no Rio Jarí (da Silva et al. 2018).

A espécie ocorre em todos os tipos de rios amazônicos (de águas claras, brancas e pretas) e apresenta comportamento adaptado às grandes flutuações dos níveis da água (>15 m), concentrando-se em locais de maiores profundidades no período da seca (Arraut et al. 2010; 2017). Não há estimativas robustas de tamanho populacional até o presente momento, mas há registros históricos de uma drástica redução populacional devido à caça comercial, onde cerca de 80 a 140 mil peixes-boi foram abatidos entre os anos de 1935 e 1954 (Domning, 1981). Estudos genéticos sugerem um tamanho efetivo populacional de fêmeas de aproximadamente 455.000 indivíduos (Cantanhede et al. 2005).

O peixe-boi-amazônico é o menor representante dos sirênios, podendo atingir 3 m de comprimento e 450 kg. A maioria dos indivíduos apresenta uma mancha mais clara (branca ou rosada) na região ventral e não apresenta unhas nas nadadeiras peitorais (Reeves et al. 1992). A espécie não apresenta dimorfismo sexual pronunciado, e os sexos são diferenciados pela localização da genitália: na fêmea a abertura localiza-se próxima ao ânus e nos machos próxima à cicatriz umbilical (Figura 2).

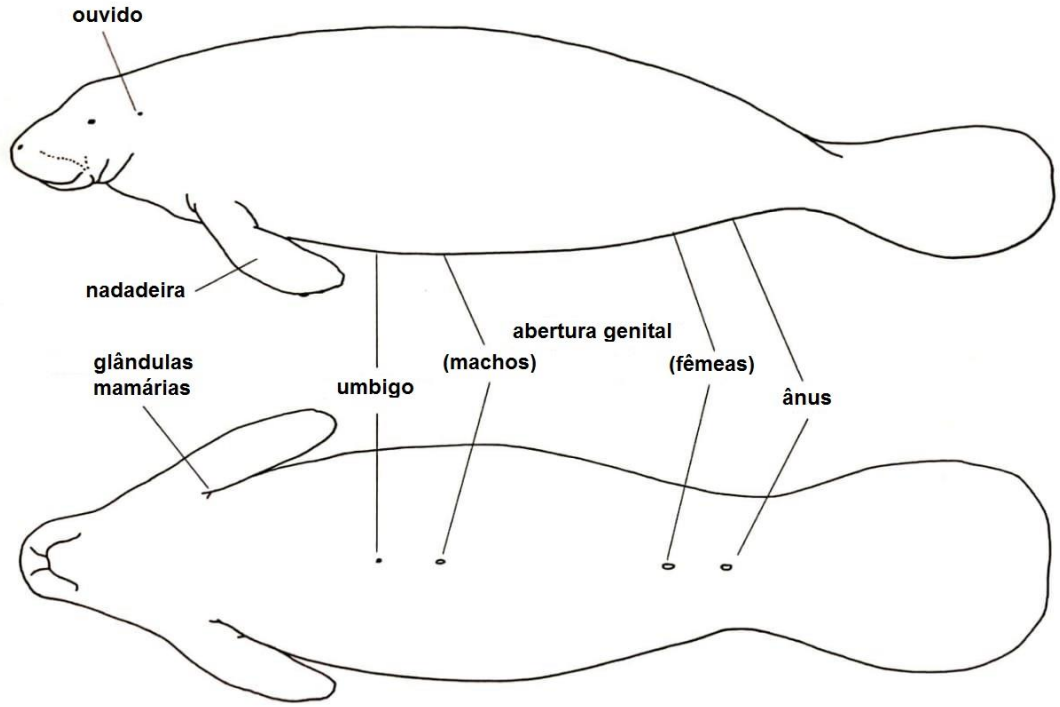


Figura 2. Morfologia externa de peixe-boi (Adaptado de: Geraci & Lounsbury, 2005).

A maturidade sexual ocorre aproximadamente aos sete anos para machos e fêmeas, sendo razoável considerar um indivíduo adulto quando possui mais de nove anos e 200 cm de comprimento total (Amaral et al. 2017). O sistema de acasalamento é do tipo promíscuo, onde observa-se mais de um macho tentando copular com uma mesma fêmea (Carvalho et al. 2017), formando grandes agregações chamadas "*mating herds*" (Reynolds et al. 2004). Acredita-se que a época reprodutiva ocorra no período da cheia, quando há maior disponibilidade de alimentos para os animais que nascem e fêmeas lactantes (Best 1982), dado que o tempo de gestação é de aproximadamente 12 meses. A espécie apresenta baixa taxa reprodutiva, com intervalos de nascimento de aproximadamente três anos (filhotes mamam por aproximadamente dois anos; Rosas 1994).

Embora tenham sofrido a drástica redução populacional no passado, as populações atuais não apresentam fragmentação e nem efeitos de gargalo populacional; o peixe-boi-amazônico apresenta elevado fluxo gênico e uma alta diversidade genética em relação a *T. manatus* (Cantanhede et al. 2005). Atualmente, as principais ameaças são a caça de subsistência e para comercialização em pequena escala nos centros urbanos de cidades da região Norte (Calvimontes 2009, da Silva et al. 2018); o aumento do uso de redes de emalhe (30% dos animais resgatados pelo INPA são provenientes de emalhamento) e

eventos de secas extremas (que podem ser agravados com as mudanças climáticas, Fearnside 2013). Além disso, são afetados diretamente por poluentes organoclorados, hidrocarbonetos e metais pesados provenientes de atividades antrópicas, desmatamento (relacionado com o assoreamento dos rios) (da Silva et al. 2018) e com a construção de hidrelétricas (Arraut & Marmontel 2016).

No Brasil, o peixe-boi-marinho ocorre desde o estado do Amapá até o estado de Alagoas (Lima 1997; Luna et al. 2008). A espécie pode utilizar diferentes tipos de ambiente, tanto os de água doce e estuarino quanto locais exclusivamente marinhos, e restringem-se a águas calmas e rasas onde há predominância de vegetação aquática (gramas marinhas e macroalgas). Os estuários, além de serem utilizados para a ingestão de água doce, funcionam como uma espécie de berçário no período reprodutivo, onde as fêmeas parem os filhotes e prestam os primeiros cuidados (Luna et al. 2018).

A espécie marinha também sofreu com a intensa caça comercial associada aos episódios de caça de subsistência, acarretando em um declínio populacional (Domning 1981) e o desaparecimento da espécie em algumas áreas (p. ex. do litoral do Espírito Santo, Bahia e Sergipe, onde há registros históricos da ocorrência da espécie; Whitehead 1978). Apesar da distribuição da espécie por uma ampla faixa do litoral brasileiro, apenas 278 animais haviam sido estimados para a região NE (Alagoas ao Piauí) e 207 para a região NO (Maranhão até Amapá) através de entrevistas com pescadores locais (Luna et al 2008). Estimativas realizadas a partir de censo aéreo estimaram uma média de 1104 indivíduos para a região Nordeste do Brasil, porém com um amplo intervalo de credibilidade (Alves et al. 2015).

O peixe-boi-marinho pode medir até 4,5 m e pesar mais de 600 kg e assim como a espécie de água doce o peixe-boi marinho não apresenta dimorfismo sexual pronunciado, diferenciando-se macho e fêmea pela posição das aberturas genitais. As nadadeiras peitorais, diferem das do peixe-boi-amazônico em possuem três ou quatro unhas (Reeves et al. 1992).

A espécie é descrita como promíscua formando grupos compostos por fêmeas e vários machos adultos, denominados "*mating herds*" que podem estender-se por períodos de uma semana a mais de um mês e percorrer centenas de quilômetros (Bengtson 1981). Durante esse período, estimado nos meses de setembro a abril devido ao aumento das ocorrências de encalhe de filhotes, a fêmea acasala com vários machos. A maturidade sexual ocorre aos 3 e 4 anos (Rathbun et al. 1995) alcançando sucesso reprodutivo máximo entre 5 e 8 anos (Marmontel et al. 1992).

Ao contrário do peixe-boi-amazônico, a variabilidade genética na população de peixe-boi-marinho no Brasil é baixa com grande variação geográfica na estrutura genética (Luna et al. 2012). Até o momento não se tem informações do grau de isolamento dos indivíduos e das subpopulações. As principais ameaças descritas atualmente são a perda e a degradação do habitat, especialmente os habitats de gramas marinhas (Copertino et al., 2016). Atividades antrópicas como a carcinocultura, portos, pesca, dragagem e intensificação da ocupação humana na região costeira também podem afetar negativamente a espécie através da ingestão de lixo, captura em redes de pesca, atropelamento por embarcações e assoreamento dos estuários. Além disso, a caça de subsistência ainda ocorre em alguns locais do litoral Norte (Luna et al. 2018).

Na região do Estuário Amazônico e proximidades, há registro de híbridos entre as duas espécies que ocorrem no Brasil (*T. inunguis e T. manatus*) (Cantanhede et al. 2005; Vianna et al. 2006). Eventos de hibridização podem comprometer o *pool* genético reduzindo o *fitness* dos indivíduos, ou aumentar a diversidade genética de forma de aumentar a adaptabilidade da espécie, sendo de extrema importância ampliar os estudos de ambas as espécies nessa região.

1.3.Adaptações para a herbivoria

Vários aspectos da morfologia dos sirênios influenciam sua ecologia trófica e a capacidade de adquirir diferentes tipos de recursos alimentares. Dentre elas pode-se destacar (i) deflexão do rostro; (ii) sistema de troca contínua dos dentes; (iii) adaptação da musculatura facial e labial; e (iv) conjunto de adaptações no sistema digestório.

O grau de deflexão no rostro em relação ao plano palatal é diferente entre as espécies de sirênios viventes. Acredita-se que o grau de deflexão do crânio está relacionado com o local preferencial de alimentação na coluna da água (p. ex. superfície ou fundo) e com a capacidade de captura de plantas submersas, flutuantes e emergentes (Domning 1982; Domning & Hayek 1986). O peixe-boi-amazônico tem os menores graus de deflexão (25-41º) e a musculatura do pescoço diferenciada, provavelmente uma adaptação para alimentar-se próximo à superfície. A deflexão do peixe-boi-marinho tem valores intermediários (29-52º), refletindo um nicho mais amplo de forrageamento (Domning & Hayek 1986).

Para a mastigação de plantas abrasivas, com alto conteúdo de sílica, os peixes-boi desenvolveram um sistema de troca contínua dos dentes (Figura 3). Os dentes funcionais

(de 5 a 8) em cada quadrante da mandíbula e maxila são substituídos horizontalmente em uma série de molares supranumerários (Domning & Hayek 1984). Ao longo da vida do animal, um número de 20 a 30 dentes em cada mandíbula é possível de serem formados (média de formação de um novo dente a cada nove meses; Domning & Hayek 1984). Após o desmame, o aumento da ingestão de comida sólida e, consequentemente, da mastigação, age como um mecanismo de estímulo para o movimento dos dentes. Em comparação a outras espécies de sirênios, o peixe-boi-amazônico possui uma dentição melhor adaptada a uma dieta mais abrasiva (provavelmente devido ao alto consumo de grama verdadeira – família Poaceae) (Domning 1982; Domning & Hayek 1986; Guterres-Pazin et al. 2014).

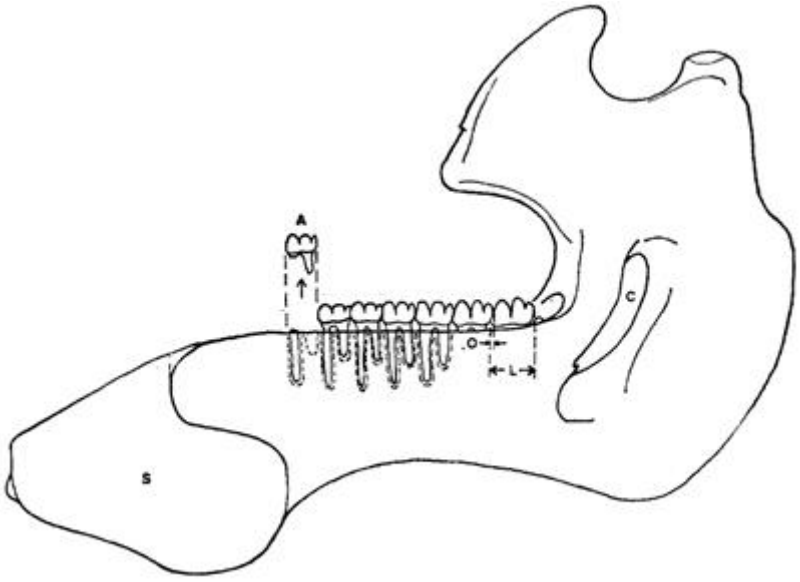


Figura 3. Visão diagramática da mandíbula direita de *Trichechus inunguis*; C = cápsula dental; L= comprimento da coroa; O = sobreposição de coroas adjacentes; S = sínfise mandibular. Nota-se a progressiva reabsorção das raízes posteriores conforme o dente se move para frente, e a retenção da raiz anterior quando o dente (A) é perdido (Adaptado de: Domning & Hayek 1984).

1.4.Ecologia alimentar

Enquanto se tem bastante informação sobre a ecologia de peixes-boi em habitats estuarinos, marinhos e cristalinos de água doce, ainda há poucos estudos sobre a disponibilidade, sazonalidade e dieta em ambientes de águas turvas (Marsh et al. 2012).

Os desafios de desenvolver estudos nesses ambientes estão relacionados à dificuldade de observar a espécie (visto que expõem somente uma pequena parte do rostro para respirar) e à alta riqueza de herbáceas aquáticas. Por exemplo, em torno de 388 espécies são encontradas nas várzeas amazônicas (Junk & Piedade 1993).

Os sirênios podem consumir em torno de 8% do seu peso total diariamente (Figura 4) e estima-se que passem em torno de 4 a 7 horas por dia se alimentando (Bengtson 1981; Etheridge 1985). Dessa forma, as informações de dieta também contribuem bastante para o entendimento do uso do habitat.



Figura 4. Peixes-boi-amazônicos em cativeiro alimentando-se das plantas flutuantes *Azolla* sp. Foto: Sônia Vill

Até o momento, estudos mais detalhados da ecologia alimentar de peixe-boi-amazônico foram realizados na região do médio Rio Solimões: áreas próximas a Manaus e nas Reservas de Desenvolvimento Sustentável Amanã (RDSA) e Mamirauá (RDSM). Esses estudos utilizaram a análise de conteúdos estomacais, fezes, conhecimento tradicional e identificaram entre 22 e 49 fontes alimentares, sendo em sua maioria plantas emergentes pertencentes à família Poaceae, principalmente as espécies *Paspalum repens* e *Echynochloa polystachya (*Colares & Colares 2002, Guterres-Pazin et al. 2014), ambas espécies de via fotossintética C_4. Um estudo nos rios Negro e Tapajós concluiu que os peixes-boi podem variar sua dieta de acordo com a disponibilidade de recursos em seu

ambiente local (Crema et al. 2019). Os autores também encontraram uma grande variedade de itens alimentares, incluindo macroalgas e plantas vasculares de diferentes vias fotossintéticas, com proporções de contribuição que variaram de acordo com o local e classe ontogenética.

Para o peixe-boi-marinho, análises de conteúdo estomacal realizadas no Nordeste brasileiro com animais provenientes dos estados do Rio Grande do Norte, Paraíba e Alagoas, encontraram 21 taxa componentes da dieta, entre macroalgas verdes, pardas, vermelhas e gramas marinhas (Borges et al. 2008). Na região estuarina, área de simpatria entre as duas espécies, Domning (1981) identificou alguns itens alimentares baseado em entrevistas e no conhecimento ecológico local, e Sousa et al. (2013) identificaram 33 espécies de plantas na zona entre marés da costa norte da Ilha do Marajó, treze das quais já haviam sido registradas como componentes da dieta de peixes-boi. Lins et al. (2014) também reportaram espécies consumidas por *T. manatus*, incluindo as plantas *Blutaparon portulacoides*, *Eleocharis geniculata*, *Crenea maritima* e *Echinochloa polystachchya*.

1.5.Estudo da dieta através de isótopos estáveis

A análise de isótopos estáveis (AIE) tem sido amplamente utilizada para o estudo da dieta de diversas espécies de mamíferos aquáticos, incluindo os sirênios (Ames et al. 1996; Reich & Worthy 2006; Alves-Stanley & Worthy 2009; Alves-Stanley et al. 2010; Ciotti 2012; Crema et al. 2019). Os isótopos são formas de um mesmo elemento que possuem diferentes massas atômicas pelo fato de haver variação no número de nêutrons (Fry 2006).

A AIE se baseia na premissa de que a composição de isótopos dos consumidores reflete as razões isotópicas das fontes alimentares (DeNiro & Epstein 1978). Os isótopos mais comumente utilizados nos estudos ecológicos são os isótopos de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$). Os valores de $\delta^{13}C$ variam entre os produtores de acordo com as vias fotossintéticas, e permanecem quase inalterados (~ 1 ‰) ao longo da cadeia alimentar, sendo um *proxy* da origem das fontes alimentares (DeNiro & Epstein 1978). Por outro lado, os valores de $\delta^{15}N$ apresentam um enriquecimento trófico acentuado e previsível entre os tecidos dos consumidores e os de sua dieta (~ 3-5 ‰) sendo usados principalmente para estimar a posição trófica (Post 2002). No entanto, os valores de ^{15}N também podem fornecer informações sobre locais de alimentação e condições ambientais, pois existem diferenças isotópicas entre diferentes locais, como regiões áridas e úmidas

(Schoeninger & DeNiro 1984; Ambrose e De Niro 1986; Vogel et al. 1990), e ambientes intocados e impactados (Abreu et al. 2006).

A composição isotópica consiste na razão entre o isótopo mais pesado em relação ao mais leve ($^{14}C/^{13}C$, $^{15}N/^{14}N$) e é representada pela notação delta (δ) em partes por mil (‰), ou seja, por seu desvio em relação à razão conhecida do padrão internacional estabelecido. No caso do carbono, o padrão é o *Pee dee Belamite* (PDB) e do nitrogênio, o ar atmosférico (Peterson & Fry 1987).

A AIE ainda possui a vantagem de inferir sobre a dieta assimilada em vez de apenas itens ingeridos e, por permitir a amostragem de tecidos com diferentes taxas de renovação, pode fornecer informações em escalas de tempo variáveis. A utilização de tecidos inertes como a dentina (Newsome et al. 2010) possibilita a inferência da dieta em uma ampla escala de tempo, o uso de amostras de coleções e o estudo de animais de difícil observação (Walker & Macko 1999).

1.5.1 Nicho isotópico

A AIE também pode ser utilizada para a investigação dos componentes intra- e interindividuais na amplitude do nicho isotópico (Newsome et al. 2007). Os dados apresentados nessa abordagem ecológica-isotópica consistem de pontos em espaços cartesianos onde os eixos representam os valores de $\delta^{13}C$ e $\delta^{15}N$. Esse δ-espaço relaciona-se com o espaço n-dimensional que abrange o nicho ecológico (Bearhop et al. 2004). Embora essa abordagem apresente inúmeras limitações, tem sido amplamente usada em estudos para comparar amplitudes de nicho entre localidades (Carrasco et al. 2019) e verificar variações sexual nos hábitos alimentares (Kernaléguen et al. 2015, Lima et al. 2019), mudanças ontogenéticas no uso do habitat e recursos (Albernaz et al. 2017) e sobreposição de nicho entre espécies simpátricas (Drago et al. 2017; Botta et al. 2018). Até o presente momento nenhum trabalho utilizou essa abordagem para sirênios.

1.5.2 Isoscapes

A variabilidade geográfica na dieta pode ser avaliada através de AIE e exibida graficamente como paisagens isotópicas (*isoscapes*) (e.g. Ceriani et al. 2012; 2014). Esses gradientes espaciais isotópicos foram observados para carbono e nitrogênio em

plataformas continentais marinhas através dos valores de produtores primários, consumidores e matéria orgânica em sedimentos superficiais (Radabaugh et al. 2013). Para os isótopos de carbono, a fixação do dióxido de carbono pelas plantas é o principal processo de diferenciação das fontes, visto que a magnitude do fracionamento varia em função das vias fotossintéticas (C_3, C_4 e CAM, DeNiro e Epstein 1978). Além disso, os animais que se alimentam em habitats marinhos e estuarinos geralmente têm valores de $\delta^{13}C$ mais elevados do que aqueles que se alimentam em ambientes de água doce e terrestres (Schoeninger & DeNiro 1984). Essas diferenças criam padrões de larga escala nos valores de $\delta^{13}C$ de acordo com a distribuição geográfica dos tipos de plantas (produtores primários) e condições ambientais (Bowen 2010).

Dados isotópicos de carbono podem ser combinados com isótopos de nitrogênio para identificar os ambientes de alimentação utilizados por mamíferos marinhos (Hobson & Schell 1998; Newsome et al. 2007; Graham et al. 2010). Desta forma, fontes de nutrientes, transformações biológicas, fracionamento isotópico e efeitos do tamanho do *pool* de nutrientes devem ser levados em conta (McMahon et al. 2013). Os valores de $\delta^{15}N$ também podem ser usados como marcadores do *input* de nitrogênio, especialmente de fontes antropogênicas (por exemplo, descarga de efluentes e fertilizantes; Bowen 2010).

1.6.Objetivos

1.6.1. Geral

Descrever a ecologia alimentar do peixe-boi-amazônico e do peixe-boi-marinho ao longo dos diferentes habitats das suas distribuições no Brasil, através da análise de isótopos estáveis de carbono e nitrogênio.

1.6.2. Objetivos específicos

- Identificar os principais grupos de fontes alimentares que contribuem na dieta do peixe-boi-amazônico no médio Rio Solimões e Estuário do Rio Amazonas;
- Investigar a variação ontogenética e sexual na dieta de peixe-boi-amazônico no médio Rio Solimões;

- Verificar a existência de variação espacial na dieta de peixe-boi-amazônico para os animais encontrados nos rios Tapajós e Negro, médio Rio Solimões e Estuário do Rio Amazonas;
- Determinar a variação na amplitude de nicho isotópico dos peixes-boi-marinhos ao longo do litoral norte e nordeste do Brasil;
- Descrever a variação espacial e padrões na dieta de peixe-boi-marinho através da construção de *isoscapes* com os valores isotópicos de carbono e nitrogênio.

2. MATERIAL E MÉTODOS

2.1 Área de estudo

2.1.1 Peixe-boi-amazônico

A Bacia Amazônica, maior bacia hidrográfica do mundo, abrange uma área de 6.879.761 km^2 (Duncan & Fernandes 2010), com uma média de descarga de 200.900 m^3s^{-1} (Molinier et al. 1992). Ao longo de sua extensão apresenta uma variedade de habitats terrestres e aquáticos que sustentam uma alta diversidade biológica.

A classificação dos rios amazônicos baseia-se nas características físico-químicas, que refletem as propriedades do solo dos locais de drenagem (Sioli 1984; 1985). Os rios são geralmente classificados em três categorias: (i) rios de água branca - têm coloração barrenta, altas concentrações de solutos dissolvidos, variação do pH de alcalino para neutro e uma alta carga de sedimentos originados da região andina; (ii) rios de água preta - possuem coloração escura devido à alta concentração de material orgânico dissolvido, apresentam pouco sedimento em suspensão, transparências médias, são muito diluídos em íons dissolvidos e geralmente são ácidos; e (iii) rios de águas claras - transparentes, tipicamente tem baixa carga de sedimentos dissolvidos devido à sua origem nos Escudos Pré-Cambrianos (Guianas e do Brasil Central), exibem valores baixos de condutividade elétrica e o pH varia do ácido para o alcalino.

Uma complexa rede de lagos e áreas alagadas constituem as áreas úmidas (*wetlands*) da Amazônia abrangendo uma área de aproximadamente 300.000 km^2 (6%) da Bacia Amazônica, (Junk 1989). As áreas alagadas também são classificadas de acordo com as propriedades físico-químicas das águas em (i) várzeas, regiões altamente

produtivas alagadas por águas brancas que compreendem uma área de 200.000 km^2; e (ii) igapós, alagados por rios de águas pretas com alta concentração de material orgânico dissolvido (ácidos húmicos) ou claras, ambas apresentando pH ácido, totalizando uma área de aproximadamente 100.000 km^2 (Junk e Howard-Williams 1984; Ferreira et al. 2010).

A extensão da área alagada depende da precipitação, descarga, topografia, e mudanças ao longo do curso dos rios (Junk et al. 1989). As oscilações do nível da água são de 10 m em média na foz do Rio Negro e a jusante, em Santarém, de 6 a 7 m (Sioli 1985). No estuário, as variações do nível anuais da descarga fluvial sobrepujadas pela oscilação diurna das marés. A influência da maré ocorre até o município de Óbidos (PA), que se encontra a uma distância de 1.000 km da foz do rio Amazonas.

Os ciclos de inundações promovem a conectividade entre os rios e outros corpos da água, como os lagos de várzea, favorecendo a expansão da cobertura de vegetação flutuante herbácea, transportada durante os períodos de enchente e cheia. No período da cheia, um grande número de macrófitas aquáticas (de diferentes formas de vida) podem ser encontradas colonizando os lagos temporários e permanentes (Ferreira et al. 2010) e podem cobrir mais de 70% das áreas abertas nos lagos de várzea (Bayley 1989).

Os locais de amostragem do presente estudo incluirem a região do médio Rio Solimões, englobando os corpos d'água presentes nas Reservas de Desenvolvimento Sustentável Mamirauá (RDSM – 02º15'36" S, 65º40'48" W) e a Reserva de Desenvolvimento Sustentável Amanã (RDSA – 02º13'07" S, 64º22'47" W), os rios Negro e Tapajós e a área estuarina da foz do Rio Amazonas (Figura 5).

As RDSM e RDSA estão localizadas na confluência dos rios Solimões e Japurá, e representam uma área extensa de floresta de aproximadamente 3.474.000 hectares. Ambas as reservas apresentam habitats de várzea, que permanecem inundados durante seis meses do ano, e possuem uma das maiores flutuações no nível da água na Amazônia (maior do que 11 metros da estação cheia para a seca), limitando o número de espécies que sobrevivem a essa dinâmica (IDSM 2019).

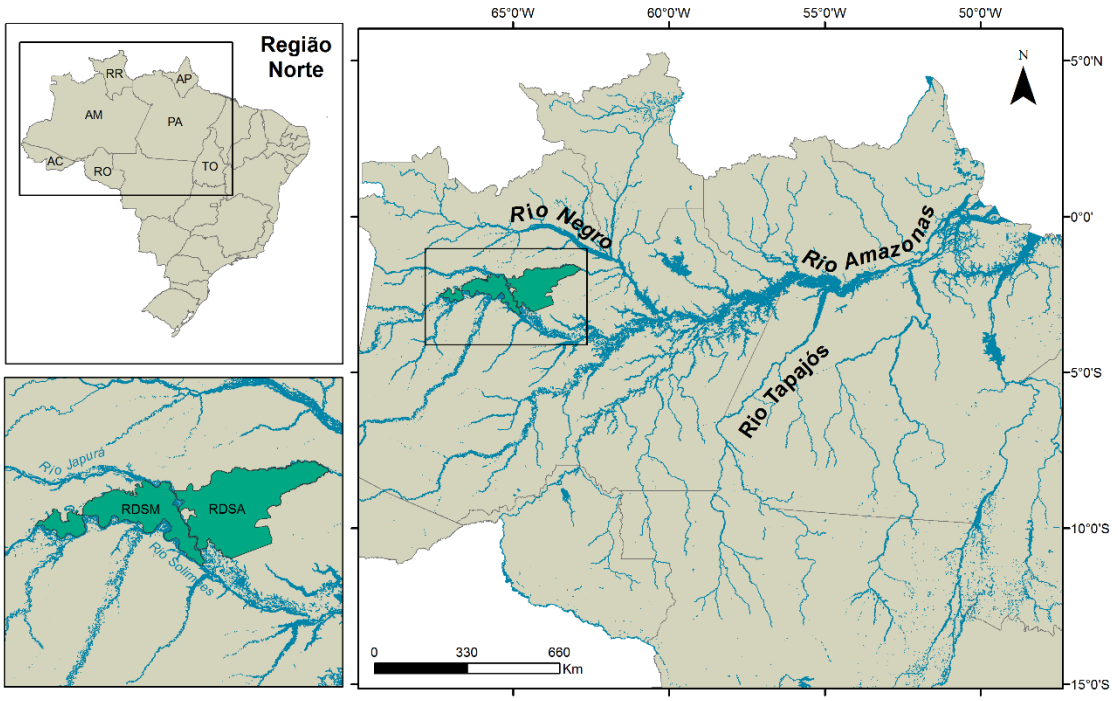


Figura 5. Área de distribuição das coletas de amostras de dentes de peixe-boi amazônico *T. inunguis* utilizadas para a análise de isótopos estáveis. O mapa inferior à esquerda representa a região do médio Rio Solimões com enfoque nas Reservas de Desenvolvimento Sustentável Amanã (RDSA) e Mamirauá (RDSM).

O Rio Amazonas [pH = 6,6 ± 0,2; condutividade = 44,8 ± 24,8 µS.cm^{-1}; total de sólidos dissolvidos = 23,9 ± 17,8 mg/l] (Duncan & Fernandes 2010) é um rio de água branca que apresenta 7.000 km de extensão, nasce nos Andes peruanos e apresenta diversos nomes ao longo de seu curso; no Brasil começa a ser chamado de Solimões e, a partir da confluência do com o Rio Negro, recebe o nome de Amazonas. Todo o sistema do Rio Amazonas engloba uma extensa área alagada com uma rede de canais de drenagem e lagos permanentes de tamanho diferentes que se comunicam com o rio principal durante as inundações periódicas e permanecem isolados durante a água baixa (Junk et al. 1989).

O Rio Tapajós, por sua vez, classificado como rio de água clara, é um dos maiores tributários do Rio Amazonas, possui aproximadamente 2.000 km de comprimento e uma média de descarga de aproximadamente 12.440 m^{3}s^{-1} [pH = 6,5 ± 0,4; condutividade= 14,4 ± 13,1 µS.cm^{-1}, total de sólidos dissolvidos = 7,7 ± 5,6 mg/l] (Duncan & Fernandes 2010). O Rio Negro flui através de aproximadamente 1.700 km com descarga média de 28.000 m^{3}s^{-1}, e é o principal representante dos rios de água preta [pH = 4,5 ± 0,9;

condutividade= 17,0 ± 15,2 µS.cm^{-1}, total de sólidos dissolvidos = 7,1 ± 6,7 mg/l] (Duncan & Fernandes 2010).

No Estuário do Rio Amazonas, as oscilações nos níveis da água resultam de dois principais fatores: a descarga dos rios e das mesomarés (Gallo & Vinzon 2005; Prestes et al. 2017). A bacia dos rios Araguaia e Tocantins pode ser considerada a principal fonte de água doce para essa região (Rosário et al. 2016), resultando em valores de condutividade = 104.3 ± 0.3 µS/cm e total de sólidos dissolvidos = 67.0± 0.2 mg/l (Duncan & Fernandes 2010). No período de cheia, a salinidade é zero e no período de seca, quando há uma baixa descarga de água doce, a salinidade pode atingir o valor máximo de 4,6 (Rosário et al., 2016). A vegetação na área consiste principalmente de de manguezais bem desenvolvidos e áreas com predominância de plantas das famílias Cyperaceae, Poaceae e Lythraceae, que também foram registrados como parte da dieta de peixe-boi (Lins et al., 2014).

2.1.2 Peixe-boi-marinho

As amostras obtidas para *T. manatus* são provenientes de diversas localidades do litoral brasileiro, sendo o limite superior o estado do Amapá (04º18’12” N, 51º18’42” W) e inferior o estado do Alagoas (10º31’57” S, 36º19’10” W). Esta região abrange áreas com características litorâneas distintas que podem ser agrupadas em (1) Litoral Amazônico; (2) Litoral Setentrional do Nordeste e (3) Litoral Oriental do Nordeste (Ab’Saber 2005) (Figura 6).

O Litoral Amazônico (Amapá/AP, Pará/PA e Maranhão/MA) apresenta 1.850 km de extensão (Ab’Saber 2005), é caracterizado por manguezais bem desenvolvidos, baixa diversidade de macroalgas (Giarizzo et al. 2011) e praticamente ausência de gramas marinhas (presença de *Ruppia marítima* somente em lagoas internas no MA; Copertino et al. 2016). Nessa região a costa é aluvial e as águas rasas estendem-se por grandes distâncias na plataforma continental (Schaeffer-Novelli et al. 1990).

No AP as florestas de manguezais são dominadas pelo mangue-preto (*Avicennia* sp.) que colonizam as margens de rios, estendendo-se por longas distâncias no continente (Souza-Filho, 2005). Nesses locais próximos de rios, também estão presentes macrófitas do gênero *Montrichardia* (Schaeffer-Novelli et al. 1990), descrita como item alimentar de peixe-boi (Guterres-Pazin et al. 2014).

Na região do delta do Rio Amazonas (AP/PA), onde há presença de diversas ilhas, o manguezal é pouco desenvolvido devido a alta descarga de água doce. No MA, a planície costeira também é permeada por estuários que penetram em direção ao interior por vários quilômetros. Nos manguezais há predomínio de *Rhizophora mangle*, e ambientes deposicionais de baixa energia podem apresentar colonização por *Spartina* (Schaeffer-Novelli et al. 1990).

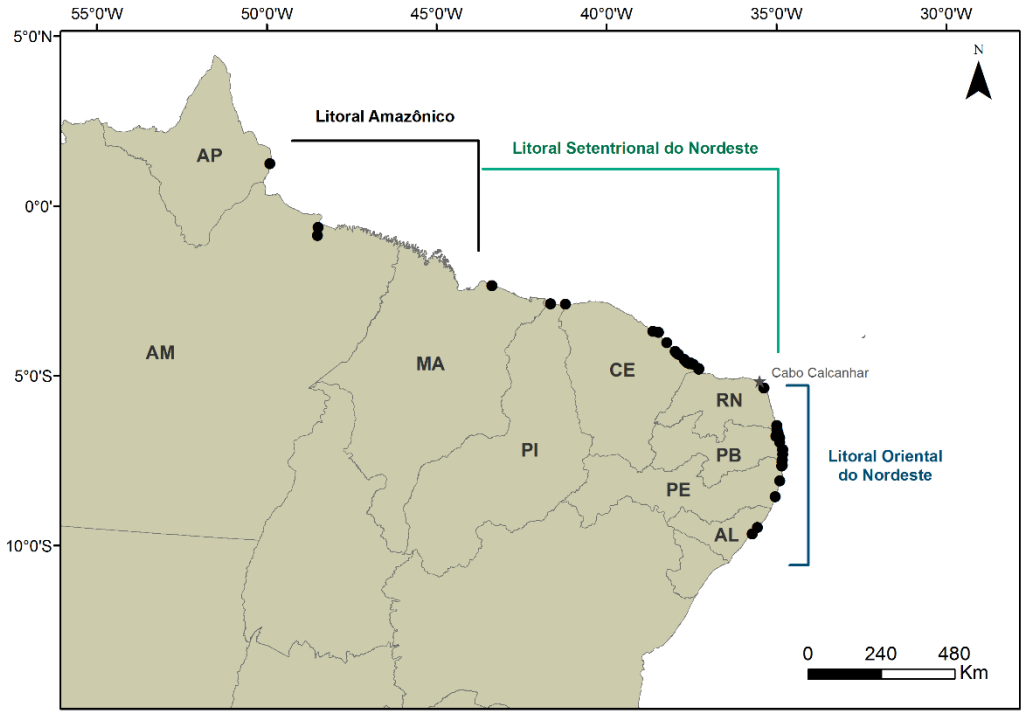


Figura 6. Área de distribuição das amostras de dentes de peixe-boi marinho *Trichechus manatus* na costa brasileira utilizadas para a análise de isótopos estáveis.

O Litoral Setentrional do Nordeste estende-se por aproximadamente 1.250 km, desde metade do MA até o estado do Rio Grande do Norte (RN) no Cabo Calcanhar (Ab'Saber 2005), englobando os estados do Piauí (PI) e Ceará (CE). Essa região apresenta o litoral exposto a alta energia das ondas, resultando em praias arenosas, dunas de areia e penhascos de arenito. O clima é seco, com uma pronunciada estação seca e com a presença de poucos rios (Schaeffer-Novelli et al. 1990). No CE não há presença de baías, lagoas e áreas costeiras protegidas, somente poucos rios e estuários (Meirelles 2008) e encontra-se uma grande diversidade de bancos de macroalgas com elevada produção (Pinheiro-Joventino et al. 1998). As condições ambientais citadas acima resultam em manguezais pouco desenvolvidos e maior diversidade de fanerógamas (desde o PI:

Halodule wrightii, *Halophila decipens* e *Halophila bailonis* e desde o CE: *Halodule emerginata;* Copertino et al. 2016)

O Litoral Oriental do Nordeste, a partir do Cabo Calcanhar no RN até o estado de Alagoas, é caracterizado por praias retilíneas, estreitas e arenosas expostas a alta energia das ondas. Nessa região predominam águas tropicais com baixa disponibilidade de nutrientes e podem ser encontradas formações recifais paralelas ao litoral (Schaeffer-Novelli et al. 1990), com bancos de macroalgas associados. Devido à alta energia das ondas, os manguezais se desenvolvem em áreas protegidas, associados a estuários e lagoas costeiras, como ocorre na região da Baía de Mamanguape – Paraíba/PB (Xavier et al. 2012). Na Ilha de Itamaracá em Pernambuco (PE), a espécie de macroalga *Gracilaria* spp., associada a macroalgas vermelhas e à macroalga verde *Caulerpa* spp., alcança elevadas biomassas no verão (Figueiredo et al. 2008).

2.2 Amostras

2.2.1 Peixe-boi-amazônico

As amostras de dentes de *T. inunguis* foram obtidas a partir de material de coleção do acervo do Instituto de Desenvolvimento Sustentável Mamirauá (IDSM) (Figura 7) – região do médio Rio Solimões e do Museu Paraense Emílio Goeldi (MPEG) – região estuarina. Os dados da composição isotópica dos dentes de animais provenientes dos rios Negro e Tapajós foram obtidos de Crema et al. (2019).

Os animais foram agrupados em quatro sub-regiões: médio Rio Solimões (inclui o Rio Solimões, Rio Japurá e outros corpos da água associados como o Lago Amanã, que embora apresente água preta, recebe influência das águas brancas); Rio Negro; Rio Tapajós e Estuário do Rio Amazonas.



Figura 7. Coleta das amostras de dentes no acervo mastozoológico do Instituto de Desenvolvimento Sustentável Mamirauá. Foto: Daniel Gonzalez-Socoloske.

2.2.2 Peixe-boi-marinho

As amostras de dentes de peixe-boi-marinho foram obtidas nos acervos das instituições parceiras: Fundação Mamíferos Aquáticos (FMA); Associação de Pesquisa e Conservação de Ecossistemas Costeiros (Aquasis); Museu Paraense Emilio Goeldi (MPEG); e Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (CMA)/Centro de Pesquisa e Conservação da Biodiversidade Marinha do Nordeste (CEPENE) do Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). Como citado acima, os animais foram agrupados em três regiões de acordo com as características do local: (1) Litoral Amazônico; (2) Litoral Setentrional do Nordeste e (3) Litoral Oriental do Nordeste.

De cada crânio foram coletados os molares mais recentes (M6 ou M7) do lado direito da mandíbula. Quando estes não estavam presentes, optou-se pelo 1) molar mais recente do lado direito, mesmo não sendo a última posição, 2) molar mais recente do lado

esquerdo da mandíbula e 3) molar superior mais recente da maxila, pois Ciotti et al. (2014) e Crema et al. (2019) mostraram que não há diferença significativa na composição isotópica entre as posições dos dentes.

2.3 Coleta de itens alimentares

Foram coletadas 49 potenciais fontes alimentares (macroalgas e plantas vasculares) do peixe-boi-amazônico na região do médio Rio Solimões, com base nos trabalhos de descrição da dieta (Colares & Colares 2002, Guterres et al. 2008, Guterres-Pazin et al. 2014). Os itens alimentares foram coletados em quatro áreas distintas localizadas nas RDSA e RDSM, em lagos de várzea e igarapés com influência de água branca. As coletas foram realizadas no período da cheia (Jul) e início da vazante (Dez) no ano de 2017. Os dados de fontes alimentares para os rios Negro e Tapajós foram obtidos do trabalho de Crema et al. (2019). Amostras de plantas e macroalgas da região do estuário foram coletadas em igarapés e na região litorânea do Pará nos períodos de seca e cheia no ano de 2012 (Costa 2015).

2.4 Processamento dos dentes

Inicialmente, os dentes foram escovados com água destilada para remoção de tecidos e resíduos. Em seguida, os dentes foram cortados longitudinalmente ao meio utilizando uma serra circular diamantada, escovados, enxaguados com água destilada e secos em temperatura ambiente por 24 h. A dentina foi extraída com uma broca, sendo o pó resultante representativo da dieta de aproximadamente 9 meses, visto que uma característica única desses animais é a troca contínua dos dentes. O pó resultante foi acidificado em uma cuba contendo ácido clorídrico (HCl) a 30% para remoção de carbono inorgânico. Para remoção dos resíduos do HCl, a dentina extraída foi seca em estufa por um período de duas horas. Como não foi observada mudança nos valores de carbono e nitrogênio nos dentes de peixe-boi decorrentes do processo de acidificação (Ciotti 2012), a mesma amostra acidificada foi utilizada para a análise dos isótopos de carbono e nitrogênio. Aproximadamente 1 mg do pó foi armazenado em cápsulas de estanho e enviado para análise.

2.5 Processamento das fontes alimentares

As plantas coletadas foram lavadas com água destilada e secas em estufa a 60ºC por 48 h. As plantas secas foram maceradas utilizando o gral e pistilo e aproximadamente 3 mg do material foi armazenado em cápsulas de estanho e enviado para análise.

2.6 Análise de isótopos estáveis

As análises isotópicas para determinar as razões de $\delta^{13}C$ e $\delta^{15}N$ foram realizadas no *Center for Stable Isotopes* na *University of New Mexico* (Albuquerque, Novo México) utilizando um analisador elementar Costech 4010 (Costech, Valencia, CA) acoplado a um espectrômetro de massas de razão isotópica Thermo Scientific Delta V (Thermo Scientific, Bremen, Germany). As razões isotópicas ($^{13}C/^{12}C$ e $^{15}N/^{14}N$) das amostras foram expressas através da notação delta (δ) em partes por mil (‰) dos padrões internacionais Vienna Pee Dee Belemnite (VPDB) para carbono e ar atmosférico para nitrogênio. A razão isotópica de cada elemento analisado na amostra foi comparada com os padrões internacionais para determinar a composição isotópica da dentina segundo a equação:

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

$R_{amostra\ e}\ R_{padrão}$ são as razões de $^{13}C/^{12}C$ e $^{15}N/^{14}N$ da amostra e do padrão, respectivamente. Os valores delta foram calculados utilizando a normalização de múltiplos pontos. Os padrões do laboratório utilizadas foram proteína de soja, caseína, tuna, proteína whey, IAEA-N1, IAEA-N2, USGS-4 e USGS-43. A precisão analítica dos padrões do laboratório utilizados foi: $\delta^{13}C$ <0,04 ‰ e $\delta^{15}N$ < 0,2 ‰.

Para os dados de peixe-boi marinho, como foram utilizadas algumas amostras que haviam sido analisadas em outro laboratório, um procedimento de calibração foi aplicado. Os valores isotópicos dos dentes revelaram diferença significativa apenas para os valores de nitrogênio, sendo calculado o valor corrigido através da equação:

$$\textbf{δ}^{\textbf{15}}\textbf{N corrigido} = 0{,}6504\ (\delta^{15}N\text{-Laboratório WSU}) + 3{,}9792$$

onde Laboratório WSU representa os valores de $\delta^{13}C$ e $\delta^{15}N$ obtidos no Stable Isotope Core Laboratory, Washington State University.

2.7 Análise dos dados

2.7.1 Dentes

Os dados foram testados para homogeneidade utilizando o teste de Levene. Diferenças na composição isotópica entre os diferentes habitats foram testados com uma análise de variância (ANOVA) de uma via. Para identificar a diferença entre os pares foi aplicado o teste *post hoc* de Tukey. As diferenças na composição isotópica entre os sexos e classes ontogenéticas (filhotes, juvenis e adultos) para os animais do médio-Solimões foram testadas através de uma ANOVA de duas vias. As classes foram definidas de acordo com Amaral et al. (2017): as faixas de idade e comprimento total para filhotes, juvenis e adultos são < 3 anos e < 161 cm; 3 – 9 anos e 161 – 200 cm; e > 9 anos e > 200 cm, respectivamente. As idades para a maioria dos indivíduos haviam sido estimadas previamente por Vergara-Parente (2009) através da contagem de camadas de crescimento no osso do ouvido de acordo com Marmontel et al. (1996).

2.7.2 Plantas

Os dados de $\delta^{13}C$ e $\delta^{15}N$ também foram testados para normalidade e homogeneidade utilizando os testes de Shapiro-Wilk e Levene, respectivamente. Os dados isotópicos que apresentaram distribuição normal e homocedasticidade foram comparados através do teste *t* de Student e os dados que não atenderam a esses critérios foram comparados utilizando os testes Wilcoxon e Kruskal-Wallis. Os valores médios de $\delta^{13}C$ e $\delta^{15}N$ para as fontes alimentares do médio Rio Solimões foram comparadas entre as estações (cheia e seca) e entre as partes das plantas (flor, folha, raiz e talo) para os grupos C_3 e C_4 separadamente. Além disso, para os itens coletados no médio Rio Solimões e no Estuário do Rio Amazonas, os valores isotópicos foram comparados entre as classes de grupo utilizadas nos modelos de mistura para validação.

2.7.3 Polígonos e modelos de mistura

O modelo bayesiano *Stable Isotope Mixing Models* (pacote 'simmr', Parnell & Inger 2016) foi utilizado para estimar a proporção das diferentes fontes alimentares na dieta dos peixes-boi-amazônicos nos diferentes ambientes. Foram gerados modelos separados para os animais do médio Rio Solimões e Estuário do Rio Amazonas. Além disso, baseando-se nos resultados dos modelos lineares generalizados, foram gerados modelos separados para adultos e juvenis e machos e fêmeas adultas do médio Rio Solimões.

Os grupos de fontes alimentares foram definidos com base em estudos anteriores com dieta de peixe-boi-amazônico (Colares & Colares 2002; Guterres-Pazin et al. 2014; Crema et al. 2019) e de acordo com as semelhanças dos valores de $\delta^{13}C$ e $\delta^{15}N$, levando em consideração a via fotossintética e hábito das plantas. Os modelos para o médio Rio Solimões incluíram plantas aquáticas C_3, terrestres C_3 e plantas C_4. As plantas terrestres podem ser definidas como espécies que permanecem a maior parte do ano em um ambiente seco, mas ocupam áreas alagadas na estação das águas altas (Junk & Piedade 1993), tornando-se disponíveis para peixes-boi. Macroalgas foram incluídas no grupo de plantas aquáticas C_3 por apresentarem valores isotópicos similares. As fontes alimentares incluídas nos modelos do Estuário do Rio Amazonas foram agrupadas em macroalgas, plantas C_3 e C_4.

Fatores de discriminação trófica (FDT - a diferença entre os valores isotópicos do consumidor e sua dieta) são necessários para a execução dos modelos de mistura isotópica. Como não havia fatores de discriminação de colágeno determinados experimentalmente para peixes-boi ou animais relacionados, foram testados diferentes valores encontrados na literatura através de simulação dos polígonos de mistura usando os pacotes 'sp' e 'splancs' (Smith et al. 2013). Os polígonos de mistura foram comparados visualmente e o TDF escolhido foi $\Delta^{13}C = 6.2 \pm 0.5$ ‰ proposto para sirênios (Clementz et al., 2007) e $\Delta^{15}N = 4.2 \pm 0.5$ ‰ estimado para *Bos taurus* (Steele & Daniel 1978). Para os testes e modelos estatísticos foi considerado nível de significância de 0,05. Todas as análises foram realizadas utilizando o software R v.3.4.2 (R Development Core Team 2019).

2.7.4 Nicho isotópico

Os nichos isotópicos para *T. manatus* das três sub-regiões definidas foram calculadas utilizando o pacote *Stable Isotope Bayesian Ellipses* (SIBER; Jackson et al. 2011) no software R (R Development Core Team 2019). As elipses padrão foram corrigidas para pequeno número de amostras (SEAc expressado como ‰²), contendo aproximadamente 40% dos dados (Syväranta et al. 2013), e utilizadas como uma medida da amplitude do nicho isotópico.

2.7.5 Isoscapes

Para construir as *isoscapes*, coordenadas geográficas foram atribuídas a cada amostra de *T. manatus*. Um limite de 100 km da costa foi criado anteriormente à geração do mapa, dado que a espécie apresenta hábito costeiro (Normande et al. 2016). As interpolações foram realizadas através do método de krigagem com modelos esféricos, utilizando a ferramenta *Spatial Analyst* no ArcMAP 10.1 software (ESRI 2011).

3. SÍNTESE DOS RESULTADOS

3.1 *Spatial, sexual and ontogenetic variation in the diet of Amazonian manatees* (Trichechus inunguis) *along riverine and estuarine habitats in Brazil*

- Os peixes-boi apresentaram diferenças significativas nas médias de $\delta^{13}C$ e $\delta^{15}N$ entre os diferentes habitats. Os animais do Rio Negro apresentaram menores valores de $\delta^{13}C$ (-28,7 ± 1,8 ‰) e de $\delta^{15}N$ (7,5 ± 1,7 ‰) e os animais do médio Solimões maiores valores de $\delta^{13}C$ (-17,0 ± 2,7 ‰) e de $\delta^{15}N$ (9,3 ± 1,1 ‰).
- Para os animais do médio Rio Solimões, houve um efeito significativo da classe ontogenética nos valores de $\delta^{13}C$ ($p= 0.002$). A diferença entre os pares mostrou que os filhotes apresentaram menores valores de $\delta^{13}C$ (-20.0 ± 2.6‰) do que os adultos (-16.1 ± 2.8‰) (teste post hoc de Tukey, $p = 0.003$).
- Não foi observado efeito significativo da classe ontogenética, sexo e da interação entre os fatores nos valores de $\delta^{15}N$.
- Os modelos de mistura mostraram que as plantas C_4 e plantas aquáticas C_3 contribuem em similar proporção para a dieta do peixe-boi no médio Solimões e

no Estuário do Rio Amazonas (a diferença de contribuição entre os grupos foram pequenas, variando de 0,04 a 0,06).

- As plantas C_3 terrestres foram o grupo que menos contribuiu para a dieta, considerando todas as classes. Apresentaram maior contribuição para as dietas de fêmeas adultas e juvenis.
- Modelos de mistura para peixes-boi do Estuário do Rio Amazonas apresentaram alta contribuição de macroalgas seguidas de plantas C_4 e C_3 em proporção similar.

3.2 Carbon and nitrogen isoscapes of Antillean manatee (Trichechus manatus manatus) *along the Brazilian coast*

- Os valores médios de $\delta^{13}C$ foram mais altos para os peixes-boi-marinhos do Litoral Setentrional do Nordeste (-8,1 ± 1,2 ‰), enquanto os menores valores foram encontrados para os animais do Litoral Amazônico (-18,7 ± 5,5 ‰). Os valores médios de $\delta^{15}N$ mais altos foram encontrados no Litoral Oriental do Nordeste (9,6 ± 1,6 ‰) e os menores valores também no Litoral Amazônico (7,7 ± 0,3 ‰).
- O nicho isotópico mostrou-se mais amplo para os peixes-boi-marinhos do Litoral Oriental do Nordeste (SEAc = 18,17 $‰^2$), seguido dos animais do Litoral Amazônico e (SEAc = 6,41 $‰^2$) e Litoral Setentrional do Nordeste (SEAc = 4,99 $‰^2$)
- Os valores de $\delta^{13}C$ e $\delta^{15}N$ dos dentes de peixe-boi-marinho variaram consideravelmente ao longo da área amostrada no litoral brasileiro como observado nas *isoscapes*, sendo que áreas contíguas apresentaram composições isotópicas distintas, evidenciando a relação dos valores isotópicos com a disponibilidade de habitats e itens alimentares.

4. CONCLUSÕES PRINCIPAIS

A dieta de peixe-boi-amazônico e peixe-boi-marinho varia nos diferentes locais de sua distribuição no Brasil. A variação na dieta está relacionada com a disponibilidade das fontes alimentares e de habitats. Os resultados encontrados corroboram o comportamento generalista e oportunista previamente descrito para as espécies. Além disso, a identificação de variações espaciais e ontogenética na dieta deve ser levada em consideração no processo de tomada de decisões, melhorando os critérios de zoneamento e delimitação de áreas críticas para a conservação.

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APÊNDICE 1

SPATIAL AND ONTOGENETIC VARIATION IN THE DIET OF AMAZONIAN MANATEES (*Trichechus inunguis*) ALONG RIVERINE AND ESTUARINE HABITATS IN BRAZIL

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(Manuscrito submetido ao periódico *Freshwater biology*)

Spatial and ontogenetic variation in the diet of Amazonian manatees (*Trichechus inunguis*) along riverine and estuarine habitats in Brazil

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Summary

1. The feeding ecology pattern of a species is directly related to effects on population dynamics. To investigate the main food sources and feeding habitats is crucial to determine priority areas for species conservation. Feeding ecology information is

usually scarce for animals that are difficult to observe and with large geographic ranges within highly remote areas, such as the Amazonian manatee (*Trichechus inunguis*).

2. Stable $\delta^{13}C$ and $\delta^{15}N$ isotopic data of Amazonian manatees and their potential food sources were used to assess the diet of manatees from the middle reaches and the estuary of the Amazon River. These data were compared to previous published data of the Negro (blackwater) and Tapajós River (clearwater). The large sample size for middle Amazon manatees also allowed the comparison of the isotopic data among ontogenetic classes and sex.
3. Mean $\delta^{13}C$ and $\delta^{15}N$ isotopic values varied among manatees from different sites: $\delta^{13}C$ values were lower in Negro (-28.7 ± 1.8‰) and Tapajós (-26.8 ± 2.8‰) rivers, and higher in the Amazon Estuary (-17.6 ± 3.6‰) and in the middle reaches of the Amazon (-17.0 ± 2.7‰). $\delta^{13}C$ values were significantly lower in nursing calves than juveniles and adults, while $\delta^{15}N$ values were higher in the former, although the difference was non-significant. These patters were probably related to the lactation period. Isotopic values did not differ between sexes. Isotopic mixing models showed a similar contribution of C_3 and C_4 plants to the manatee diet in middle Amazon and a highest contribution of macroalgae to the diet of manatees from the estuary.
4. A high proportion of C_4 plants were consumed by manatees from the middle reaches of the Amazon River, due to the predominance of whitewater floodplain areas with high biomass of these species, differing from the black and clearwater floodplains. Although macroalgae were highlighted as important food resource for manatees from estuary, it is important to improve the model with additional samples and corroborate the result with stomach content analysis. Amazonian manatee is an opportunist and generalist species, feeding upon a large diversity of food items according mostly to their availability.

Introduction

The intake of food resources by animals affects individual survival, growth and fecundity with direct effects on population dynamics (Elser et al., 1996). Understanding the complexity of interactions involved in the feeding ecology such as habitat use and

preference (*e.g*. Heithaus & Dill, 2002; Sheppard et al., 2007; Benoit-Bird et al., 2013), selection of food resources (*e.g*. Preen, 1992; Sheppard et al., 2010), competition (*e.g*. André et al., 2005), resource partitioning (*e.g*. Franco-Trecu et al., 2017; Botta et al., 2018) and temporal variation (e.g. Secchi et al., 2016) is crucial for the conservation of species and their habitat.

Aquatic mammals exhibit a diversity of feeding strategies and tactics to locate and capture food (Heithaus & Dill, 2009). Sirenians have a strictly herbivore diet, are hindgut fermenters, have large body sizes and ingest approximately 7-8% of their body mass daily (Best, 1984; Etheridge et al., 1985; Goto et al., 2004). All manatee species present several morphological and physiological adaptations related to feeding activities: (i) the use of facial musculature, lips and perioral bristles to pinch and grasp food (Marshall et al., 1998, 2000, 2003); (ii) an accessory digestive gland associated with the stomach; (iii) a digestive tract with a long and relatively narrow colon (Marsh et al., 2012); and (iv) the continuous horizontal tooth replacement (Domning & Hayek, 1984).

The Amazonian manatee (*Trichechus inunguis*), endemic of the Amazon Basin, is the only representative of the order that is exclusive of freshwater environments. Its distribution extends from the Colombia and Peru borders down to the Amazon River Estuary in Brazil, encompassing lakes, floodplains and major tributaries of clear, black and whitewaters (Reeves et al., 1992; Luna et al., 2010; Marmontel et al., 2016). In comparison with the other species, the Amazonian manatee has smaller teeth with more complex loph patterns, and a dentition that seems better adapted to an abrasive diet (Domning, 1982; Domning & Hayek, 1986).

The first records of the Amazonian manatee feeding in the wild were provided by naturalists, local fishermen and hunters (Pereira, 1944; Best, 1981). These direct observations, while useful for first insights on feeding habits, are often biased towards emergent and floating plants (Marsh et al., 2012). Despite this, a more recent study on ethnobiology allied to the ecosystem dynamics, lists also submerged plants and vine species in Amazonian manatee diet (Crema et al., 2019a). Analysis of gastrointestinal tract and feces contents have complemented dietary information and increased the number of food items reported (*e.g.* Colares & Colares, 2002; Guterres-Pazin et al., 2014). All these authors identified a variety of C_4 and C_3 (aquatic and semi aquatic) plants as food resource and reported a seasonal variation in the food items consumed, with apparently more opportunistic and distinct consumption during the low water period (Colares & Colares, 2002). Since annual changes in the structure and composition of

herbaceous vegetation are caused by the flood pulse and related water level fluctuations in the Amazon Basin (Junk, 1989; Junk & Piedade, 1997; Piedade & Junk, 2000), the manatees' diet is also likely to vary spatially, as a consequence of site-related differences in plant diversity and availability (Crema et al., 2019a).

Amazonian floodplains differ in plant composition. Floodplains surrounded by nutrients-enriched whitewater environments (*várzeas*) have high productivity and proportion of C_4 photosynthetic plants (Sioli, 1984; Piedade et al., 1991). Blackwater and clearwater floodplain forests (*igapós*) present low to intermediate levels of nutrients and a smaller diversity of herbaceous vegetation (Crema, 2017). The Amazon River Estuary shows a complex mosaic of vegetation types, including well-developed mangroves with associated macroalgae species (Fernandes & Alves, 2011) and estuarine *várzea* areas, which differ from upriver *várzea*. The flooding in the estuarine *várzea* is caused by tidal oscillations, rather than the annual flood pulse, resulting in low plant diversity and predominance of palm trees (Pires & Prance, 1985).

Until now, Crema et al. (2019a) was the only study that reported spatial variation and assessed ontogenetic differences in Amazonian manatee diet through stable isotope analysis (SIA). SIA rests on the premise that the isotopic composition in consumer tissues reflects the isotopic values of their diet (Newsome et al., 2010) and has been widely used to investigate the feeding ecology of aquatic mammals. Carbon and nitrogen are the most common elements used in ecological studies and can provide complementary information. $\delta^{13}C$ values vary among producers according to photosynthetic pathways, and remain almost unchanged up the food web (~ 1‰), thus being a proxy on the origin of feeding sources (DeNiro & Epstein, 1978). On the other hand, $\delta^{15}N$ values presents an accentuated and varied trophic enrichment between the consumer tissues and those of its diet (~3-5‰; Post, 2002). The variation observed in trophic discrimination factors (TDF- the difference between the isotopic values of the consumer and their diet) values can be a function of a range of environmental and physiological factors as habitat type (terrestrial, freshwater, marine), trophic level, taxon, tissue, metabolic rate, nitrogenous excretion, and sample treatment procedures (Vanderklift and Ponsard 2003; Dalerum and Angerbjörn 2005). Nevertheless, $\delta^{15}N$ values can also provide information about feeding locations and environmental conditions since there are isotopic differences between arid and wet regions (Schoeninger & DeNiro, 1984; Ambrose & De Niro, 1986; Vogel et al., 1990), pristine and impacted environments (Abreu et al., 2006), among others. In this sense, SIA allows the inference of the assimilated diet instead of the ingested items and

by sampling tissues with different turnover rates, SIA can give information over variable time scales. In the case of hard tissues, such as teeth and bones, long-term diet data can be identified (Walker & Macko, 1999; Newsome et al., 2010).

Crema et al. (2019a) showed that nursing females prioritize the consumption of C_4 plant species compared to juveniles and adults. However, further investigation is needed to verify if this pattern occurs in other areas of the Amazonian manatee's distribution. It is also possible that male manatees feed in distinct areas, upon more energetic resources, due to distances traveled for reproduction. Previous studies showed that radio-tracked male Florida manatees (*Trichechus manatus latirostris*) travel longer distances and more often (Bengtson, 1981; Deutsch et al., 2003) to search for receptive females. This behavior may also occur in areas where Amazonian manatee males migrate in Central Amazonia (Arraut et al., 2010).

Spatial, sexual and ontogenetic variations in C and N stable isotopes are important to determine habitat and feeding resource requirements for the Amazonian manatee. The species is classified as Vulnerable in the Red List of the International Union for Conservation of Nature (Marmontel et al., 2016) and in the Brazilian National List (MMA, 2014). The main threats are hunting (Calvimontes, 2009; Crema et al., 2019b), entanglement in fishing nets (ICMBio, 2018), habitat loss due to building hydroelectric dams (Arraut & Marmontel, 2016; Arraut et al., 2017), deforestation causing silting of rivers and water contamination (ICMBio, 2018), as well as climate-change effects (*e.g*. impacts in the timing and height of flooding; Fearnside, 2013). Understanding the relevant aspects of the Amazonian manatee ecology, such as patterns of habitat use and preferred diet may foster the development and implementation of science-oriented conservation strategies. Therefore, in order to provide information on the feeding and spatial ecology of manatee in the middle reaches and estuary of the Amazon River, this study had the following aims: (i) use stable isotopes to identify the relative contributions of the food sources to the diet of manatees from riverine and estuarine environments; and (ii) assess spatial, sexual and ontogenetic variation in manatee diet, among individuals from estuary and from whitewater, clearwater and blackwater floodplains.

Methods

Study area

Mid reaches of the Amazon River

Samples were obtained from Amazonian manatee carcasses within and around Mamirauá and Amanã Sustainable Development Reserves (MSDR and ASDR, respectively). These are located approximately 550 km from Manaus, in the central Brazilian Amazon, at the confluence of the Amazon (Solimões) and Japurá rivers (Figure 1). The Amazon and Japurá are white-water rivers characterized by neutral pH, high conductivity and an elevated total dissolved solids. The annual water-level fluctuations averaged 10 – 11 m (Ramalho et al., 2009). These river systems encompass an extensive wetland area formed by a network of drainage channels, temporary and permanent lakes, which have physical and chemical characteristics different from the main water bodies. The vegetation on the lower banks of the river channels is predominantly of aquatic and semi-aquatic plants (mainly herbaceous grasses of the Poaceae family, such as *Echinochloa polystachya* and *Paspalum* spp.) and pioneer-tree species (Junk & Piedade, 1997; Wittmann et al., 2002).

Tapajós and Negro Rivers

Isotopic data of Amazonian manatees from the Tapajós and Negro Rivers published in a previous study (Crema et al., 2019a) were used for comparisons. The Tapajós River is one of the largest tributaries of the Amazon River (approximately 2,000 km long) and is classified as a clearwater river (acid to alkaline pH, low conductivity and total solid dissolved), flowing from the state of Mato Grosso to Pará (Duncan & Fernandes, 2010). The Negro River is the largest blackwater river (acid waters and high concentration of dissolved organic matter) and flows approximately 1,700 km to its confluence with the Amazon River near Manaus. The annual water-level fluctuations average 9.47 m in the Negro River and 4.79 m in the Tapajós River (Junk et al., 2013). Trees and shrubs with lower growth rates than *várzea* plants are present in these areas (Schöngart et al. 2010). Floating and emergent species predominate in black-water environments, while in clear water, besides the occurrence of emergents, such as *E. polystachya* and *P. repens* that also occur in the *várzeas* habitats, the transparency of the water allows the development of submerged plants (Junk, 1983; Crema, 2017).

Amazon River Estuary

The estuarine samples were from the east coast of the Marajó Island (PA). This estuary receives a discharge of 10^4 m^3s^{-1} over 300 km of longitudinal extension (Prestes et al., 2014) resulting in higher conductivity and total dissolved solids than upriver Duncan & Fernandes, 2010). Water-level oscillation results from the combination of river flow and the tidal fluctuations (mesotides) (Gallo & Vinzon, 2005; Prestes et al., 2017). In the high-discharge period, salinity is low; and in the low-discharge period, it can reach a maximum of 4.6 ppm (Rosario et al., 2016). The vegetation is dominated by the families Cyperaceae, Poaceae and Lythraceae, which include species consumed by manatees (Lins et al., 2014). Halophytic herbs or subshrubs, vines and marshes of salt-tolerant grasses are associated with mangrove forests on less-frequently inundated sandy soils (Mehlig et al., 2010).

Sample collection and processing

Teeth

A total of 109 Amazonian manatee teeth deposited in research institutions were used for SIA (S1). When the most recently erupted right-side molar of the mandible (pattern chosen) was not present, the following sampling criteria were adopted: 1) the most recently erupted molar of the right mandible available, even if not at the last position; 2) the most recent molar on the left mandible; 3) the most recent right maxillary molar; and 4) the most recent left maxillary molar. This choice was based in the assumption that the isotopic composition of Amazonian manatee teeth did not differ among teeth from different positions in the dental arcade (Crema et al., 2019a).

Teeth were initially brushed with distilled water to remove soft tissues and debris. Each molar was cut in half longitudinally with a diamond circular saw to access the dentin. Teeth halves were brushed again with distilled water and oven-dried at room temperature for 24 hours. Dentin was extracted with a drill and the resulting powder was acidified with a 30% hydrochloric acid (HCl) solution for 24 hours to remove inorganic carbon. The extracted dentin was then oven-dried (60°C) for two hours to remove HCl residues. Approximately 1 mg of the dentin powder was stored in tin capsules for isotopic analysis.

Manatee tooth samples (dentin) represent the diet of approximately a nine-month period, which reflects the replacement rate of new molars (Domning & Hayek, 1984).

Food sources

Samples of potential food sources were collected in the Middle Amazon River region based on previous diet studies (Colares & Colares, 2002; Guterres et al., 2008; Guterres-Pazin et al., 2014). Two boat surveys were undertaken in four floodplain lakes and streams with whitewater influence located in the MSDR and ASDR. Fieldwork was carried out in 2017, during the high-water period (July) and as water levels started to rise (early December). Food sources collected in the Amazon River Estuary included three macroalgae (one Chlorophyta and two Rhodophyta) and nine species of vascular plants (belonging to eight families). Surveys were undertaken in small channels in the coastal region of Pará State during low and high-water seasons in 2012 (Costa, 2015). In each survey, when a macrophyte bank was identified, all the plants that have been registered as manatee food sources and macroalgae were collected. At least three samples of the same species were collected for comparisons among locations. Plant samples were separated in parts (leaf, root, flower, stem) for isotopic analysis. The macroalgae species and vascular plant parts were washed with distilled water and oven-dried (60ºC) for 48 hours. The dried material was macerated using mortar and pestle and approximately 3 mg of the material was stored in tin capsules for isotope analysis. Data on food sources from the Negro and Tapajós Rivers were obtained from Crema et al. (2019a).

Stable-isotopic analysis

Manatee teeth and food sources from the Middle Amazon and Amazon River Estuary were analyzed at the Center for Stable Isotopes at the University of New Mexico (Albuquerque, New Mexico) using a Costech 4010 elemental analyzer (Costech, Valencia, CA) coupled to a Thermo Scientific Delta V (Thermo Scientific, Bremen, Germany) isotope ratio mass spectrometer. The isotopic ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of the samples were expressed as delta notation (δ) in parts per thousand (‰) of the international Vienna Pee Dee Belemnite (VPDB) standards for carbon and atmospheric air for nitrogen. The isotopic ratio of each element analyzed in the sample was compared

with that of the international standards to determine the isotopic composition of the dentin in the equation:

$$\delta X\ (‰) = [(R_{sample} / R_{standard}) - 1]$$

where R_{sample} and $R_{standard}$ are the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of the sample and standard, respectively. Delta values were calculated using multipoint normalization. The laboratory standards used were soy protein, casein, tuna, whey protein, IAEA-N1, IAEA-N2, USGS-4 and USGS-43. The analytical precision of the laboratory standards used was <0.04‰ for $\delta^{13}C$ and <0.2‰ for $\delta^{15}N$.

Data analysis

Teeth

Differences in isotopic composition among sites were assessed using a one-way analysis of variance (ANOVA). The data were previously tested for homogeneity of variance using Levene's test and met the homocedasticity assumption. Pairwise differences among sites were assessed using a Tukey's HSD post hoc test. For this comparison, only data from mature individuals were used (see next paragraph).

Differences in isotopic composition between sexes and ontogenetic classes (nursing calves, juveniles and adults) for manatees from middle reaches of the Amazon were assessed using a two-way ANOVA. Classes were defined following Amaral et al. (2017): the ranges of age and total length for calf, juvenile, and adult are < 3yr old and <161 cm; 3–9 yr old and 161–200 cm; and >9 yr old and >200 cm, respectively. These analyses were only performed for this site because of the larger sample size and the age data. Ages for the most individuals were previously determined by Vergara-Parente (2009) through Growth Layer Group (GLG) counts in periotic bone according to Marmontel et al. (1996). Samples with unknown age or when age and length did not correspond to the same ontogenetic class were excluded from the analysis.

Food sources

$\delta^{13}C$ and $\delta^{15}N$ data were tested for homogeneity of variance using the Levene's test. Homocedastic isotopic data were compared by Student t-tests and data that did not attend

to these requirements were compared using Wilcoxon and Krukal-Wallis tests. Mean $\delta^{13}C$ and $\delta^{15}N$ values were compared among seasons (high and low water) and plant parts (flower, leaf, stems and roots) for C_3 and C_4 groups separately.

Isotope mixing models

Bayesian Stable Isotope Mixing Models (package 'simmr' Parnell & Inger, 2016) were used to estimate the proportion of different food sources to the diet of the manatees from different habitats. Each model was run using 10,000 iterations thinned by 20, with an initial discard of 1,000 iterations. Separated mixing models were generated by sex and ontogenetic class (excluding nursing calves) for manatees from the middle reaches of the Amazon River.

Food-source groups were defined based on previous studies of Amazonian manatee diet (Colares & Colares, 2002, Guterres-Pazin et al., 2014; Crema et al., 2019a, b) and according to similarities of the plant $\delta^{13}C$ and $\delta^{15}N$ values. Kruskal-Wallis test was used to compare the isotopic values among food sources groups. Photosynthetic pathway, plant habit and functional group were also considered. Models for the Middle Amazon include aquatic C_3, terrestrial C_3 and C_4 plants. Terrestrial plants are species that remain most time of the year in a dry environment but occupy flooded areas in the high-water season (Junk & Piedade, 1993) and become available for manatees. Macroalgae were included within the C_3 plants group because of the similar isotopic values. Food sources included in the models of the Amazon River Estuary were grouped as macroalgae, C_3 or C_4 plants. TDF are required for running the isotopic mixing models. Since there were no experimentally determined diet-collagen discrimination factors for manatees, a range of values was tested through simulated mixing polygons - using the packages ‘sp’ and ‘splancs’ in R (Smith et al., 2013; Fig. S2 Supplementary material). The mixing polygons were visually compared (Figs. S3 and S4 Supplementary material) and the TDF chosen was $\Delta^{13}C = 6.2 \pm 0.5$ ‰ proposed for sirenians (Clementz et al., 2007) and $\Delta^{15}N = 4.2 \pm 0.5$ ‰ estimated for *Bos taurus* (Steele & Daniel 1978). Statistical tests and models were performed at a significance level of 0.05, using the software R v. 3.4.2 (R Development Core Team, 2018).

Results

Spatial differences in manatee-tooth isotopic composition

Mean $\delta^{13}C$ values of mature manatees from the Negro River (-28.7 ± 1.8‰) and Tapajós River (-26.8 ± 2.8‰) were lower than in animals from the Amazon River estuary (-17.6 ± 3.6‰) and middle reaches (-17.0 ± 2.7‰; Figure 2) (ANOVA: $F_{3,74} = 29.4$; $p < 0.001$). Pairwise differences were significant for almost all combinations (Tukey's HSD post hoc test, $p < 0.003$), except for middle Amazon – Amazon Estuary ($p=0.68$) and Negro – Tapajós River comparisons ($p=0.85$). Isotopic nitrogen values were also statistically different (ANOVA: $F_{3,74} = 7.1$; $p < 0.03$) presenting lower values in Negro River (7.5 ± 1.7 ‰), intermediate values in Amazon Estuary (8.8 ± 1.4 ‰) and Tapajós River (8.8 ± 0.5 ‰) and higher values in middle Amazon (9.3 ± 1.1 ‰). However, pairwise differences showed that the manatees Middle Amazon had higher $\delta^{15}N$ than those from Negro River (Tukey's HSD post hoc test, $p = 0.02$).

Ontogenetic and sex-related differences in manatee-tooth isotopic composition in Middle Amazon

Two-way ANOVA results showed a significant effect of ontogenetic class ($F_{2,38} = 6.9$; $p = 0.002$) but not of sex ($F_{1,38} = 2.8$; $p = 0.10$) and the interaction between the factors ($F_{2,38} = 1.0$; $p = 0.38$) on $\delta^{13}C$ values of manatees in the middle reaches of the Amazon River. Pairwise differences showed that nursing calves had lower $\delta^{13}C$ values (-20.0 ± 2.6‰) than adults (-16.1 ± 2.8‰) (Tukey's HSD post hoc test, $p = 0.003$). For $\delta^{15}N$ values, no significant effects of sex ($F_{1,38} = 4.0$; $p = 0.05$), ontogenetic class ($F_{2,38} = 1.3$; $p = 0.28$) or the interaction factor ($F_{2,37} = 0.2$; $p = 0.83$) were found. (Figure 3).

$\delta^{13}C$ and $\delta^{15}N$ composition of potential manatee food sources

<u>Middle Amazon</u>

A total of 49 species of plants (44 plants from the C_3 group and 5 from C_4), belonging to 27 families, and two samples of unidentified macroalgae species were analyzed from the Middle Amazon region. Plants with different photosynthetic pathways showed distinct isotopic values as expected; C_4 plants showed much higher $\delta^{13}C$ values (-12.6 ± 0.5‰) than C_3 plants (-30.5 ± 1.9‰) (Wilcoxon-test, $p < 0.0001$) for $\delta^{13}C$ and no difference was observed in $\delta^{15}N$ values (t-test, $t = 0.34$, $df = 40.941$, $p = 0.73$) (S5, Figure 4).

$\delta^{13}C$ and $\delta^{15}N$ values showed no difference among different parts (*e.g.* leaf, flower, stem and root) of both C_3 (Kruskal-Wallis, $p > 0.05$) and C_4 plants (Kruskal-Wallis, $p > 0.05$). In addition, no difference was found between high- and low-water periods for either isotope. The $\delta^{13}C$ and $\delta^{15}N$ values of food groups selected for the mixing models (aquatic C_3, terrestrial C_3 and C_4 plants) were significantly different (Kruskal-Wallis test, $p < 0.003$).

Amazon River Estuary

Macroalgae had $\delta^{13}C$ isotopic composition (-24.9 ± 2.8‰) intermediate between C_3 and C_4 plants and a wide range of values (-30.4 to -20.3‰ – Figure 4). Mangrove trees (e.g. *Laguncularia racemosa*) showed the lowest $\delta^{13}C$ and $\delta^{15}N$ values (-28.4 ± 0.6‰ and 3.4 ± 1.9‰, respectively). The $\delta^{13}C$ and $\delta^{15}N$ values of food groups chosen for mixing models (macroalgae, C_3 and C_4 plants) were significantly different (Kruskal-Wallis test, $p < 0.001$).

Mixing models

Bayesian mixing models showed that C_4 and aquatic C_3 plants contribute almost equally for manatee diet in the Middle Amazon and Amazon River Estuary (i.e. differences in contribution between these plant groups were small, ranging from 0.04 to 0.06 - Figure 5). Terrestrial C_3 plants were the group that least contributed to the diet considering all classes, although they had higher contribution for adult-female and juvenile diets. Mixing models for manatees from the Amazon River Estuary showed high contribution of macroalgae followed by C_4 and C_3 plants in similar proportion (Figure 6).

Discussion

Spatial variation in Amazonian manatee's diet

C_4 and aquatic C_3 plants contributed most to the diet of Amazonian manatees in Middle Amazon, while macroalgae constituted the food source that most contributed in the

Amazon River Estuary. These results indicate high variability in Amazonian manatee diet that seems related to the availability of food items.

Approximately 390 species of herbaceous plants have been identified in v*árzea* floodplains, the environment found in Middle Amazon (Junk & Piedade, 1993). Most of them are C_3 species, and a group of C_4 plants of Poaceae family (e.g. *Paspalum repens*, *P. fasciculatum* and *Echinochloa polystachya*) that are fast growing, form monospecific stands and produce high values of biomass (Junk & Piedade, 1993; 1997; Piedade et al., 2010) are found in high abundance. In the mixing models, 46 aquatic C_3 plants and five C_4 species were included. Therefore, the high contribution of C_4 plants can be attributed to the abundance of grasses in *várzeas*, whereas the high consumption of C_3 aquatic plants can be related to the high number of species of this group.

These results corroborate previous diet studies with stomach content and faeces of Amazonian manatees carried out in Central Amazon (ASDR, MSDR and proximities). Colares & Colares (2002) identified that the two more frequent species in samples were the C_4 plants of the Poaceae family: *Paspalum repens* (17.5 %) and *Echinochloa polystachya* (13.8 %), while Guterres-Pazin et al. (2014) recorded a high occurrence of the C_3 species of this family: *Hymenachne amplexicaulis* (58.5 %) and *Oryza grandiglumis (*44.7 %). Both studies included data from the dry and high-water seasons and showed the importance of these two main groups with distinct photosynthetic pathways for Amazonian manatees' diet. Although terrestrial C_3 plants were the group that least contributed to the diet of Amazonian manatees in the present study, they may be important during the high water season, when parts of the terrestrial plants become submerged and accessible for grazing (Guterres-Pazin et al., 2014; Crema et al., 2009ab) and floating plants may scatter and become less available.

The Amazon River Estuary, differing from the other flooded areas, presents a higher diversity of macroalgae, the main group consumed according to mixing models. Macroalgae species included in the model were *Bostrychia* sp., *Ulva* sp. and *Catenella* sp., usually found in association with mangrove trees.

Macroalgae have been repeatedly reported as a food item for dugongs (*Dugong dugon*; Heinsohn & Birch, 1972; Spain & Heinsohn, 1973; Preen, 1995; Whiting, 2002) and West Indian manatees (*Trichechus manatus*; Hartman, 1979; Borges et al., 2008; Ciotti, 2012), with a total of 34 genera of non-epiphytic macroalgae listed as diet components for the latter (Marsh et al., 2012). However, the contribution of macroalgae in the diet of these species remains a controversial issue. Some studies showed low to moderate consumption

rates associated with the seagrasses unavailability (Heinsohn & Birch, 1972; Spain & Heinsohn, 1973; Preen & Marsh, 1995; Hartman, 1979; Marsh et al., 2012). On the other hand, where the diversity of seagrasses is low in Brazil (Copertino et al., 2016), 17 macroalgae species were registered as food items for Antillean manatees (*T. manatus manatus*) in Rio Grande do Norte, Paraíba and Alagoas states (Borges et al., 2008).

Few studies focused on diet studies in Amazonian manatee inhabiting the Amazon River Estuary and described the consumption of plants belonging to Poaceae and Cyperaceae family, as well as the species *Crenea maritima* and *Blutaparon portulacoides,* however, did not mention algae as a food item for the species (Domning, 1981, Lins et al., 2014).

Therefore, since there was an apparent relationship between macroalgae consumption and unavailability of other food sources and considering that manatees are generalist herbivores, it is plausible that Amazonian manatees effectively rely on the consumption of macroalgae in the Amazon River Estuary. Nevertheless, this result needs to be interpreted with caution due to the small sample size (n = 4), which resulted in wide credibility intervals for each resource group. Additionally, the wide range of macroalgae isotopic values may also introduce uncertainties in the model, probably overestimating the importance of this item to the diet of the manatees.

Although this study showed a high contribution of C_4 plants for adults in middle Amazon, SIA diet study on Amazonian manatees in the Negro and Tapajós rivers showed that C_3 plants from the *igapós* (blackwater/clearwater-flooded area) were the item that most contributed to adult diets in general. This result reflects the diversity and plant composition in the distinct areas. In blackwater-river floodplains, the diversity of herbaceous vegetation is low, free-floating plants are absent and the most abundant plants are climbers, terrestrial herbs and shrubs (Junk et al., 2011; Crema, 2017), mostly C_3 plants. There are only few species in common in white-water and clearwater floodplains (Crema, 2017), with blackwater *igapós* being characterized by high levels of endemism and low abundance of C_4 species (Junk et al., 2011).

In the Tapajós River, on the other hand, C_4 species are present throughout the year, though some seasonal variation occurs due to the increase of current strength (Junk & Piedade, 1997; Piedade et al., 2010; Ferreira et al., 2010). The most adapted plants remained in these areas, including the C_4 plants *E. polystachya*, *P. repens* and species of Cyperaceae family (Junk & Howard-Williams, 1984; Crema, 2017). Although C_4 species were present in this area, no data are available about the biomass in clearwater rivers. Considering the physical and chemical characteristics of the water types, a lower biomass than in *várzea*

locations is expected, which might explain the small contributions of C_4 plants to the manatee's diet in Tapajós River.

Ontogenetic and sex-related differences in manatee-tooth isotopic composition in the Middle Amazon

Lower $\delta^{13}C$ and higher $\delta^{15}N$ values than those in juveniles and adults were previously reported for aquatic mammal calves (Polischuk et al., 2001; Knoff et al., 2008; Orr et al., 2012; Drago et al., 2015). Low $\delta^{13}C$ values could be explained by the typical ^{13}C-depletion found in milk lipids (DeNiro & Epstein, 1977; Hobson et al., 2004) and $\delta^{15}N$ values were expected to be higher because the synthesis of milk results from the catabolism of mother's tissues (Fogel et al., 1989; Hobson & Sease, 1998; Newsome et al., 2006; Cherel et al., 2015). In the current study, this pattern was also observed although there was high variation in calf $\delta^{13}C$ and $\delta^{15}N$ values. Crema et al. (2019a) found an opposite pattern, higher $\delta^{13}C$ values for calves than juveniles and adults in manatees from Tapajós and Negro rivers. These results were possibly related to the high consumption of C_4 plants by the mothers in nearest whitewater floodplain areas, while adult females in middle Amazon were consuming C_3 and C_4 plants in almost equally proportions.

Besides variation in mothers´ diets among locations, there is evidence that sirenians start eating plants a few weeks after birth (Hartman, 1979; Marsh et al., 1982; Odell, 1982). Amazonian manatee calves stay with the mother for at least two years, and during this period the calves can consume aquatic plants together with milk (Rosas, 1994). In Middle Amazon floodplains there are many small floating C_3 species that are probably easy for a manatee calf to manipulate and ingest. Some of these species, such as *Pistia stratiotes*, *Salvinia minima* and *Azolla* sp., have high protein and mineral content, which might complement the diet (Di Santo, 2013). Therefore, the low $\delta^{13}C$ values of manatee-calf teeth from the Middle Amazon might be also related to ingestion of these C_3 plants.

Calves and juveniles in Middle Solimões showed high variability in $\delta^{15}N$ values, with the young animals showing both the lowest and the second highest values. This variation could be related to different phases of transition to adult diet, as proposed by Crema et al. (2019a) for juveniles in Tapajós River. Due to the wide range of $\delta^{15}N$ values in food sources and manatee samples, assessing differences in trophic level between ontogenetic classes is difficult (*e.g*. Reich & Worthy, 2006). It is possible that $\delta^{15}N$ values of suckling calves were also masked by the incorporation of solid food during the weaning period.

However, the small sample size for calves (n= 7) and juveniles (n=4) in the Middle Amazon restricts the interpretation of the results. It is also important to point out that this study and the study of Crema et al. (2019a) used different ontogenetic classification systems, both with different associated errors, which could be responsible for the disagreements in the results. A standardization of ontogenetic classification criteria is required for further studies and a different approach (*e.g.* stomach and feces content analysis) is still needed to verify the proportion of solid food that is ingested by calves during the nursing period.

Despite the use of different approaches to investigate feeding ecology of several sirenian species, no sex-related differences have been observed for dugongs (Johnstone & Hudson, 1981), West Indian manatees (Ledder, 1986; Castelblanco-Martinez et al., 2009; Alves-Stanley et al., 2010) or Amazonian manatee (Guterres-Pazin et al., 2014). In this study, no difference was also found in $\delta^{13}C$ and $\delta^{15}N$ values between sexes.

Differences in resource use could be related with sexual dimorphism and reproduction requirements (Ruckstuhl & Neuhaus, 2002; Kernaléguen et al., 2015). Although female sirenians are often slightly larger than males (Hartman, 1979; Marsh, 1980; Marmontel et al., 1992), difference is minimal to justify body size-related variation in energetic requirements (Ono & Boness, 1996). There is also no evidence to support different diet between sexes due to reproduction requirements. The Amazonian manatee reproductive system, like those of other sirenians, is based on male and female promiscuity, with a single estrous female pursued by males that coalesce into larger groups termed "mating herds" (Hartman, 1979; Reynolds, 1981; Carvalho et al., 2017). It was reported that male Florida manatees travel more widely and more often than females (Bengtson, 1981; Deutsch et al., 2003) to search for receptive females, but no data is available for the Amazonian species. The only telemetry study with manatees in Central Amazon reported that males travel more than 150 km in a short period during the seasonal migration, and related these movements to the search of food and habitat (Arraut et al., 2010; 2017). To better understand ontogenetic and sex-related variation in diet, telemetry studies with representative groups and investigation of the nutritional requirements by age classes and nutritional values of food sources are needed.

Conclusion

Amazonian manatee diet varies among physically and chemically distinct floodplains and estuarine habitats. Apparently, the variation is related to food availability and abundance in each environment. This result reinforces the consensus in the literature that manatees have generalist and opportunist feeding behavior.

The isotopic values for Amazonian manatee calves in middle reaches of the Amazon showed the expected lower $\delta^{13}C$ and higher $\delta^{15}N$ values, previously seen in other mammal species. However, they differed from those values recorded from Amazonian manatees in the Tapajós and Negro Rivers probably due to variation in mothers' diet Juveniles exhibit a wide range of isotopic values, highlighting a gradual transition to adult diet.

Macroalgae appear as important food source for Amazonian manatees in the Amazon River Estuary, however, it needs further investigation with a larger sample size and complementary stomach and gut content analysis to confirm the intentional ingestion.

Since Amazonian manatees are distributed in different habitats and rivers throughout the Amazon basin, these results are important to show that there is variation in consumption among the estuary and floodplains (*igapós* and *várzeas*) and that this should be taken into account in decision-making. This research can subsidize conservation strategies to protect areas by improving the zoning criteria, considering the critical areas used by the species as feeding grounds.

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Figure captions

Figure 1. Areas from which *T. inunguis* teeth samples used for the analysis of stable isotopes. The lower left map represents the Middle-Solimões region with a focus on the Amanã (ASDR) and Mamirauá (MSDR) Sustainable Development Reserves.

Figure 2. Graphic representation of the isotopic composition of manatees from different habitat by ontogenetic class (whitewater= middle Amazon, clearwater= Tapajós River, blackwater = Negro River and Amazon River estuary).

Figure 3. Mean (points) and sd (bars) $\delta^{13}C$ and $\delta^{15}N$ values for middle Amazon manatees grouped by sex and ontogenetic class.

Figure 4. Carbon and nitrogen (min and max) stable isotope biplots for Middle Solimões (MS – black squares) and Amazon River Estuary (ARE – blue squares) (top) and Tapajós (green) and Negro River (orange) food sources obtained from Crema et al., 2019a (bottom). Black circles and bars represent Amazonian manatee isotopic values (mean ± sd).

Figure 5. Results of simmr Bayesian mixing models showing proportional estimates (mean, 25% and 75% credibility interval) of diet composition for different ontogenetic classes and sexes for manatees in Middle Amazon.

Figure 6. Results of simmr Bayesian mixing models showing proportional estimates (mean, 25% and 75% credibility interval) of diet composition for Amazonian manatees in the Amazon River Estuary.

FIGURE 1

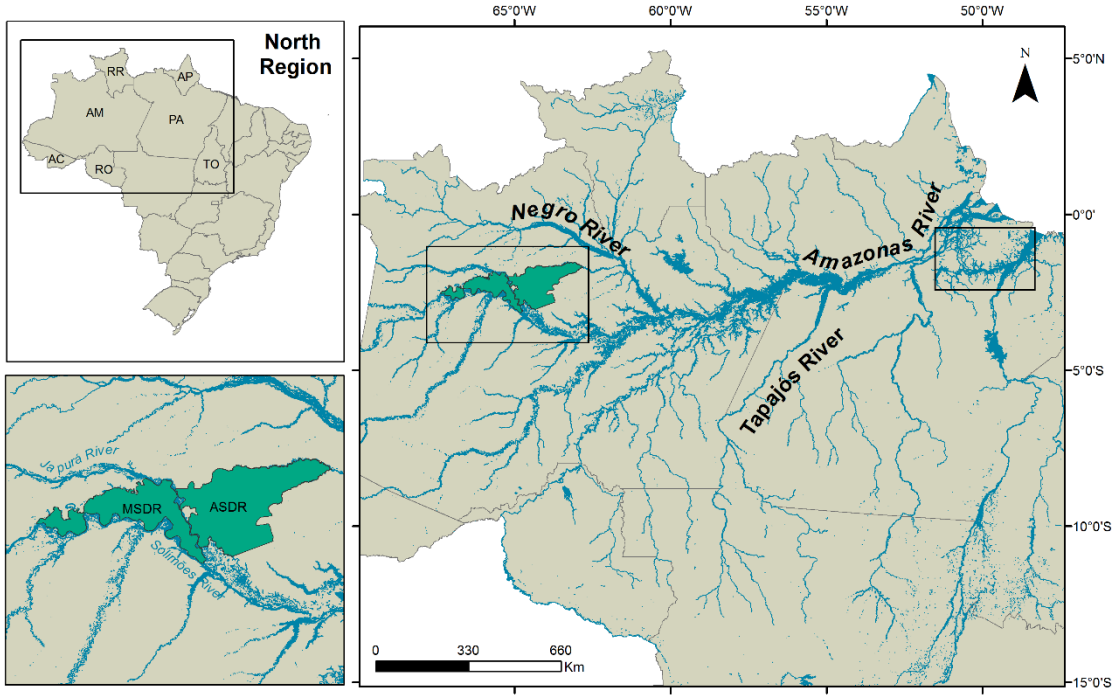


Figure 1. Areas from which *T. inunguis* teeth samples used for the analysis of stable isotopes. The lower left map represents the Middle-Solimões region with a focus on the Amanã (ASDR) and Mamirauá (MSDR) Sustainable Development Reserves.

FIGURE 2

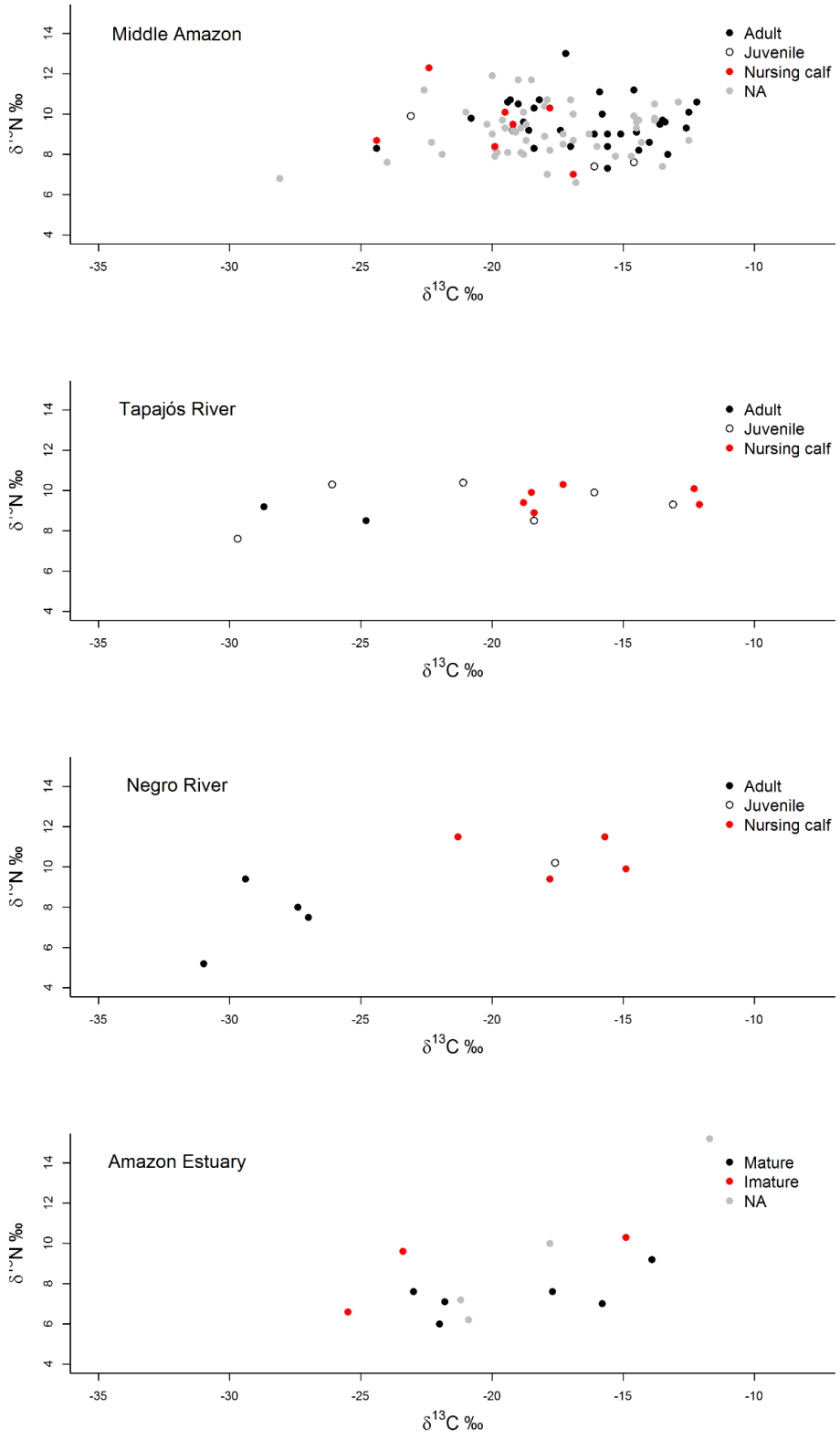


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FIGURE 3

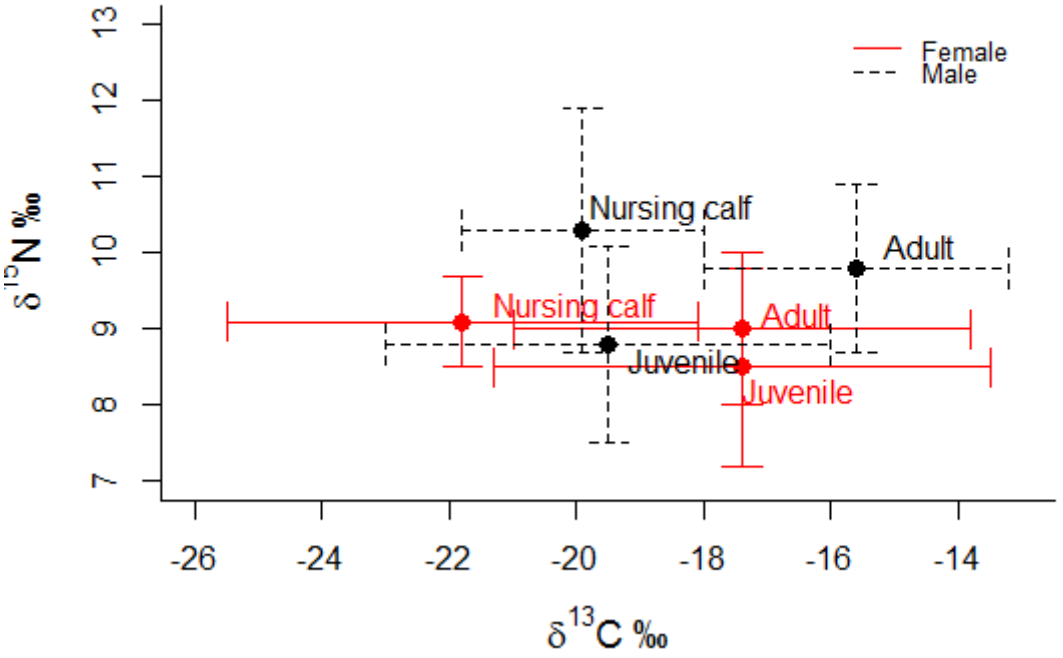


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FIGURE 4

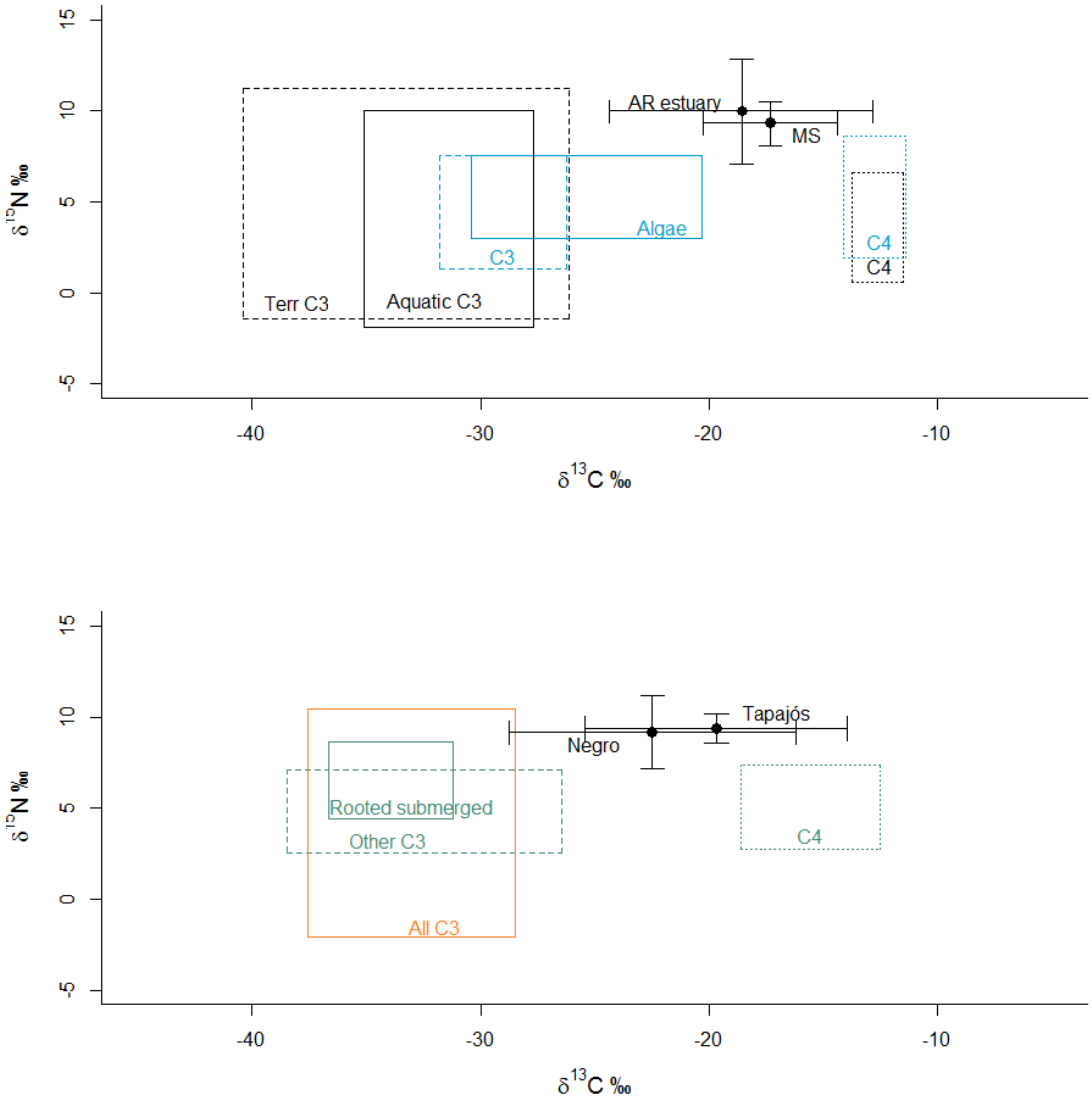


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FIGURE 5

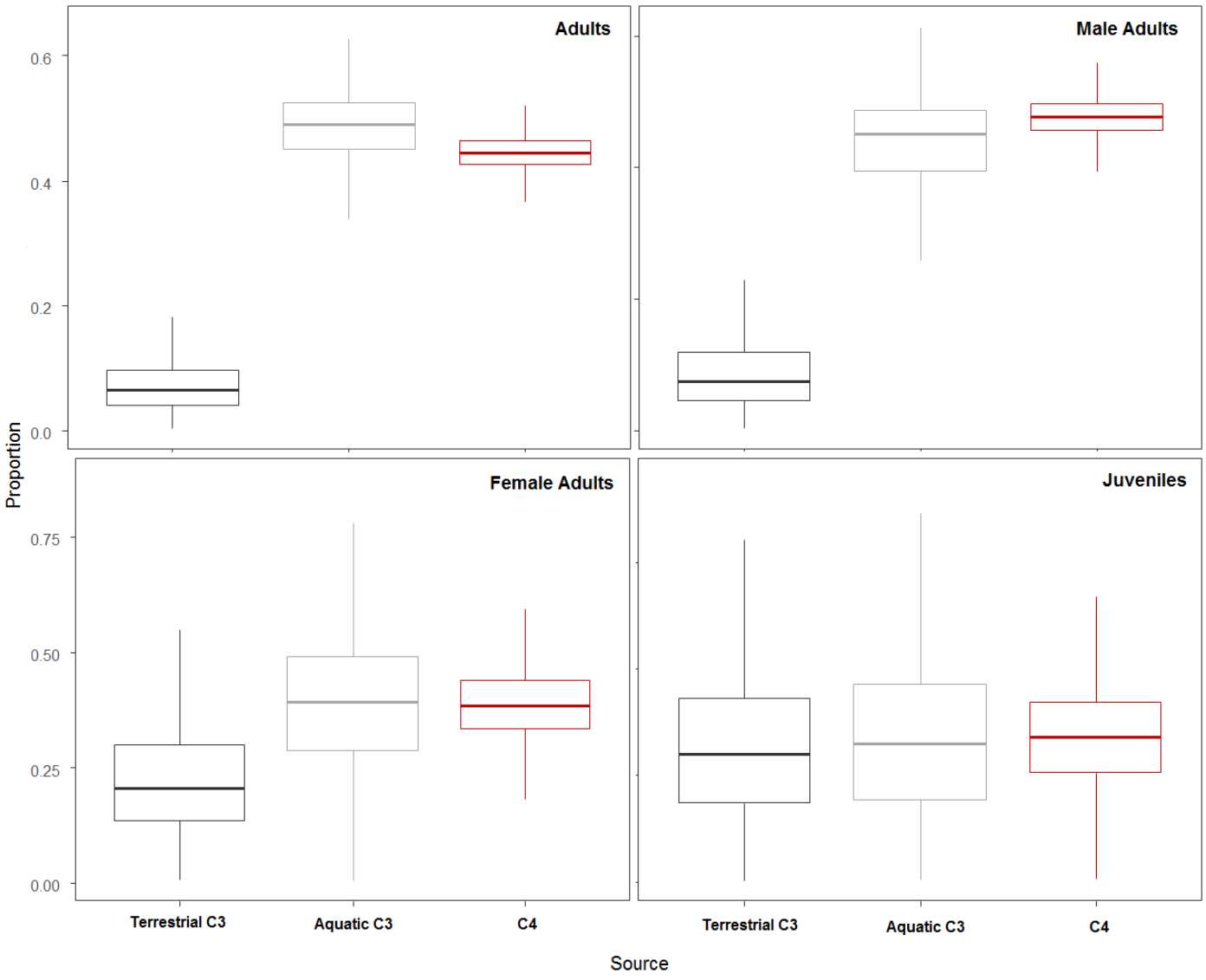


Figure 5. Results of simmr Bayesian mixing models showing proportional estimates (mean, 25% and 75% credibility interval) of diet composition for different ontogenetic classes and sexes for manatees in Middle Amazon.

FIGURE 6

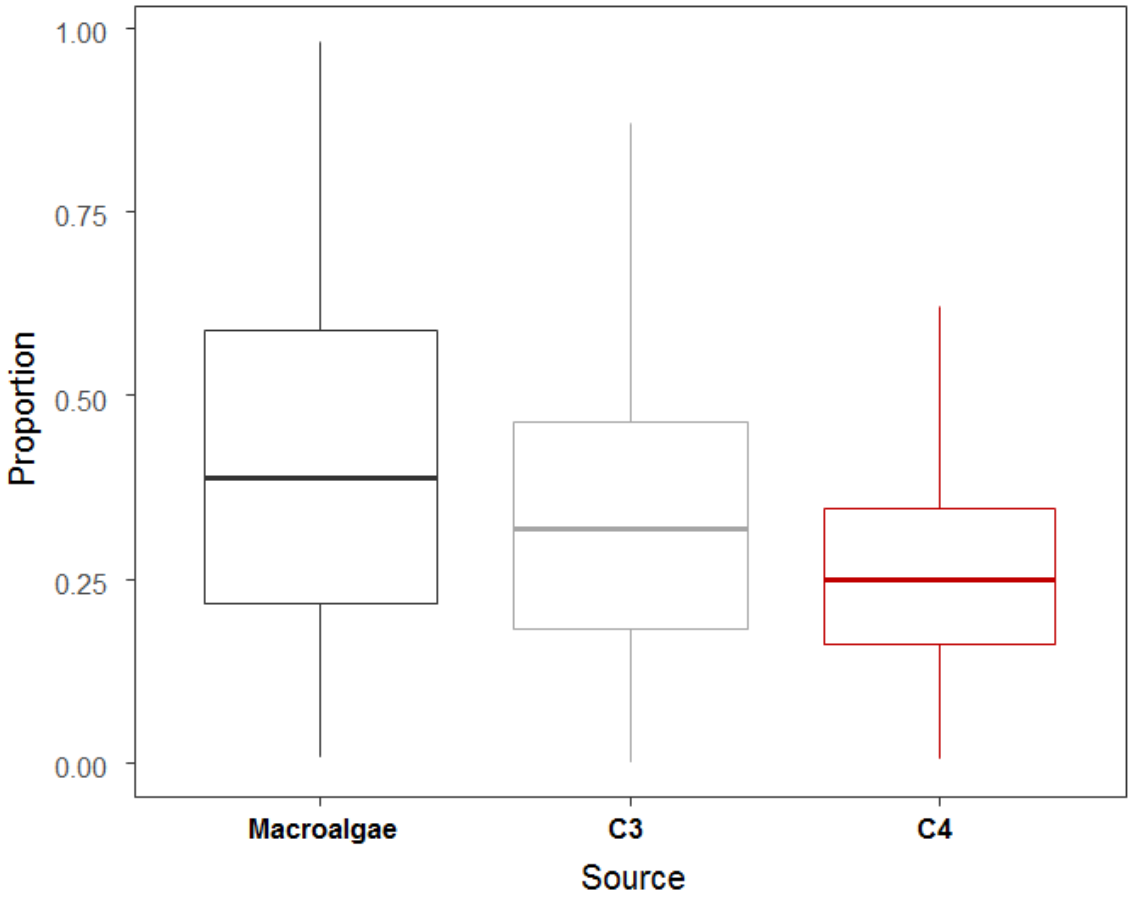


Figure 6. Results of simmr Bayesian mixing models showing proportional estimates (mean, 25% and 75% credibility interval) of diet composition for Amazonian manatees in the Amazon River Estuary.

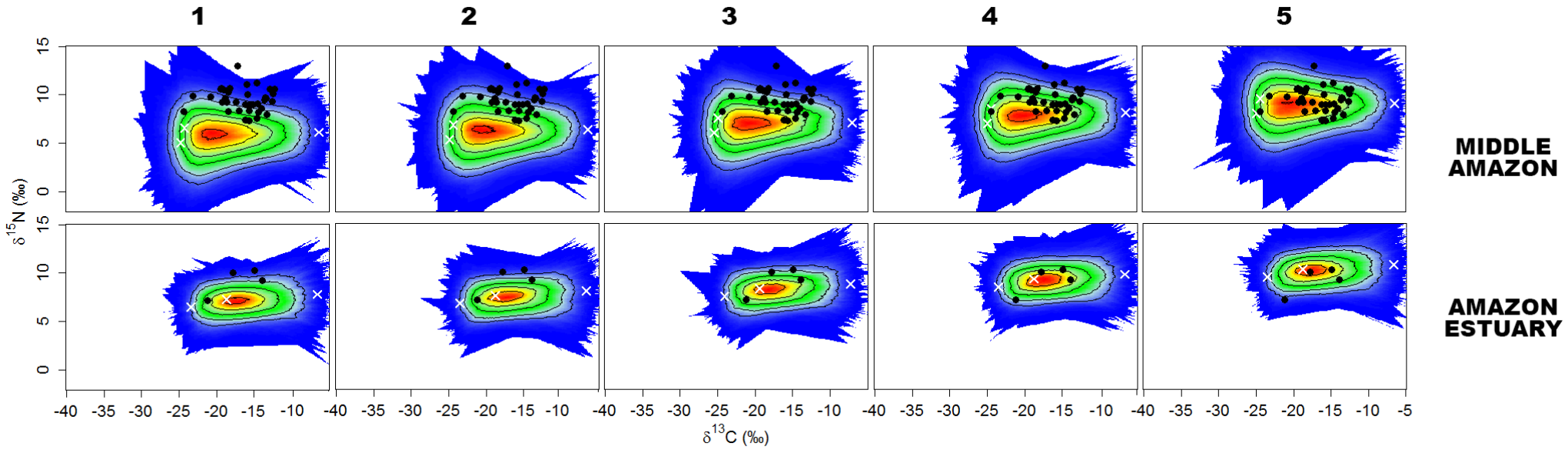
SUPPLEMENTARY MATERIAL

S1. Complete data from Amazonian manatee samples. Id = Collection number.

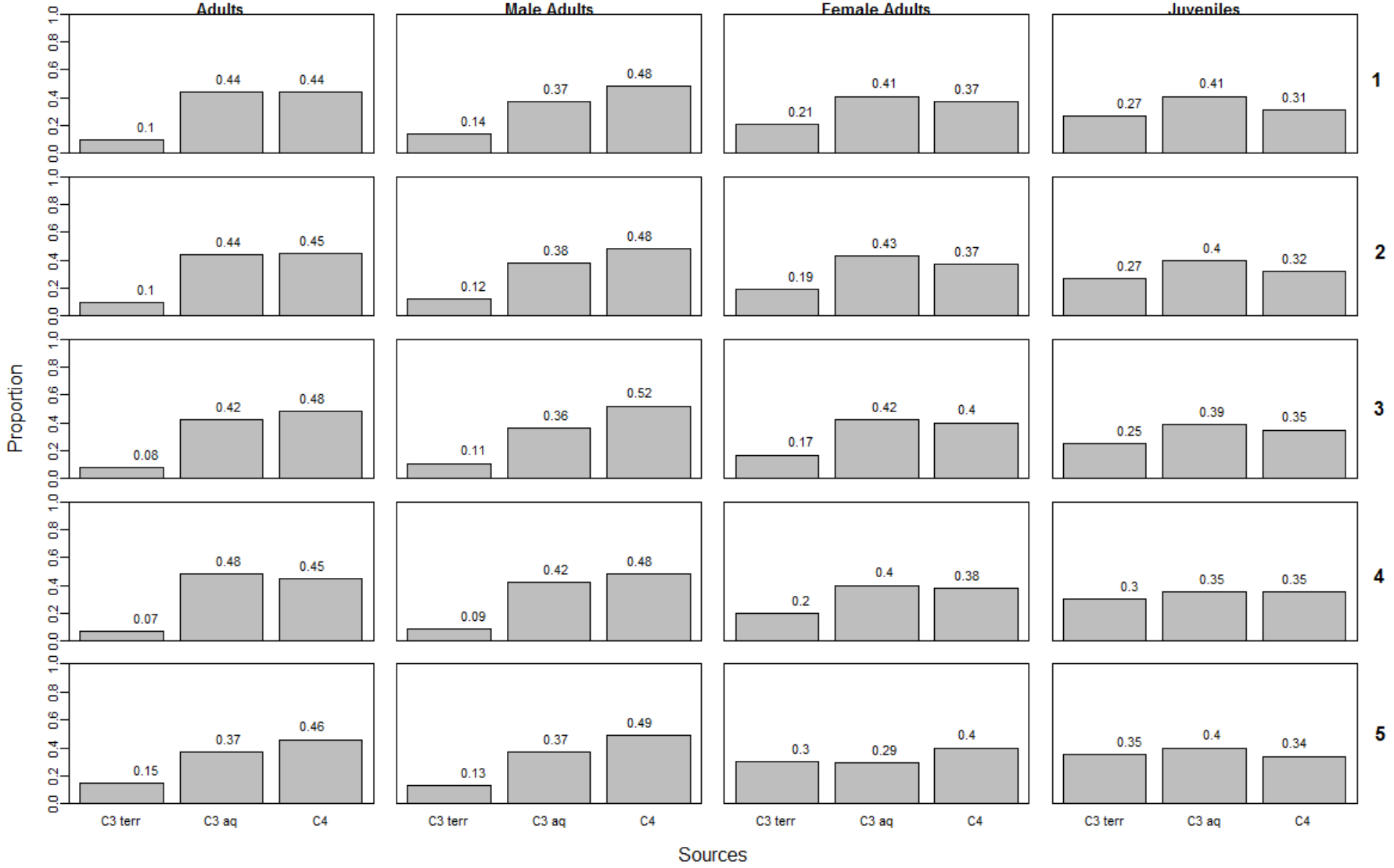
Middle Amazon – samples from Instituto de Desenvolvimento Sustentável Mamirauá (IDSM) – TeféBR								
Id	**Sample year**	**Sex**	**Length (cm)**	**Age**	**C**	**N**	**Ontogenetic class (age and length)**	**Mature**
1994 - 03	1994	M	160	-	-18.9	8.1	-	No
1994 - 04	1994	M	180	-	-21	10.1	-	Yes
1994-01	1994	M	220	9	-14.6	11.2	A	Yes
1994-05	1994	F	240	5	-20.2	9.5	J	Yes
1994-06	1994	M	140	0	-19.9	8.4	NC	No
1994-09	1994	-	120	2	-16.9	7	NC	No
1995-03	1994	M	220	0	-19	11.7	-	Yes
1995 - 02	1995	M	260	17	-15.1	9	A	Yes
1995 - 08	1995	M	153	1	-19.5	10.1	NC	No
1995 - 10	1995	M	200	8	-14.4	8.2	A	Yes
1995 - 13	1995	F	200	-	-22.6	11.2	-	Yes
1995-11	1995	M	200	-	-16	8.4	-	Yes
1995-14	1995	M	180	-	-19.8	8.1	-	Yes
1995-14 (17)	1995	F	260	5	-19.6	9.7	-	Yes
1995-23	1995	M	240	9	-12.2	10.6	A	Yes
1996 - 02	1996	F	260	3	-20	11.9	-	Yes
1996-08	1996	F	220	8	-19.3	10.7	A	Yes
1996-24	1996	M	200	10	-12.9	10.6	A	Yes
9702	1997	F	240	-	-14.7	7.9	-	Yes
1997 - 11	1997	F	243	14	-12.5	10.1	A	Yes
1997 - 15	1997	M	164	2	-22.4	12.3	NC	No
1997-02	1997	M	200	5	-18.8	9.6	A	Yes
1997-03	1997	M	140	-	-22.3	8.6	-	No
1997-04	1997	M	220	-	-14.6	9.9	-	Yes
1997-05	1997	F	140	22	-18.8	10.1	-	No
1997-06 (14)	1997	M	200	-	-17	10.7	-	Yes
1997-07	1997	M	211	13	-13.4	9.6	A	Yes
1997-09	1997	F	114	0	-24.4	8.7	NC	No
1997-11	1997	M	152	1	-17.8	10.3	NC	No
1997-13	1997	M	227	9	-19.4	10.6	A	Yes
1998-03	1998	M	240	12	-15.9	11.1	A	Yes
1999-04	1998	F	230	13	-15.6	7.3	A	Yes
1996-26	1999	F	260	-	-19.9	8.3	-	Yes
1999 - 16	1999	M	240	14	-14.5	9.1	A	Yes
1999 - 17	1999	F	140	4	-14.6	7.6	J	No
1999 - 23	1999	M	160	3	-23.1	9.9	J	No
1999-11	1999	M	160	3	-16.1	7.4	J	No
1999-21	1999	M	240	12	-13.3	8	A	Yes

2000 - 14	2000	M	230	13	-15.8	10	A	Yes
2000-06	2000	F	140	2	-19.2	9.5	NC	No
2000-06	2000	M	240	25	-13.5	9.7	A	Yes
2000-08	2000	F	140	-	-15.3	7.9	-	No
2000-10	2000	F	220	4	-18.8	8	-	Yes
2001-08	2000	F	-	-	-12.9	10.6	-	-
2001-09	2000	M	-	-	-14.5	9.6	-	-
2001 - 62	2001	F	240	-	-19.2	9.2	-	Yes
2001-13	2001	M	200	7	-18.4	8.3	A	Yes
2001-14 (13)	2001	M	180	3	-19.2	9.2	J	Yes
2001-17	2001	M	200	29	-15.6	8.4	A	Yes
2001-18	2001	M	220	13	-12.6	9.3	A	Yes
2001-21	2001	M	-	-	-13.8	9.8	-	-
2001-35	2001	-	220	-	-13.8	10.5	-	Yes
2001-36	2001	-	160	-	-18.5	11.7	-	No
2001-36 (33)	2001	F	202	2	-17.9	10.7	-	Yes
2001-42 (61)	2001	F	200	7	-20.8	9.8	A	Yes
2001-43	2001	M	226	15	-18.2	10.7	A	Yes
2001-65	2001	M	254.5	25	-17.2	13	A	Yes
2002 - 16	2002	F	220	-	-16.3	9	-	Yes
2002 - 17	2002	M	260	23	-16.1	9	A	Yes
2002-19	2002	F	240	25	-14	8.6	A	Yes
2003 - 04	2003	M	240	10	-18.4	10.3	A	Yes
2003 - 06	2003	M	250	29	-12.6	9.3	A	Yes
2003 - 07	2003	M	200	9	-18.6	9.2	A	Yes
2003-11	2003	M	190	-	-18	8.9	-	Yes
2005-02 (69)	2005	M	160	7	-24	7.6	-	No
2005-27	2005	F	210	9	-24.4	8.3	A	Yes
2005-55	2005	F	225	12	-15.6	9	A	Yes
2005-66	2005	M	247	27	-19	10.5	A	Yes
2005-67	2005	M	194	13	-13.6	9.5	A	Yes
2005-68	2005	F	240	12	-17.4	9.2	A	Yes
2006-25	2006	F	225	9	-17	8.4	A	Yes
2006-52	2006	-	160	-	-19.5	9.3	-	No
2006-53	2006	F	220	-	-19.4	8.1	-	Yes
2007-02	2006	F	220	-	-16	8.4	-	Yes
2007-	2007	M	240	-	-18.7	9.5	-	Yes
2007-03	2007	F	200	-	-19.9	7.9	-	Yes
2007-04	2007	F	280	-	-17.3	9	-	Yes
2007-06	2007	M	160	-	-14.4	9.7	-	No
2007-08	2007	M	220	-	-16.9	8.7	-	Yes
2007-09	2007	F	260	-	-28.1	6.8	-	Yes
2007-10	2007	F	280	-	-18	10.4	-	Yes

2007-12	2007	M	220	-	-17.8	8.2	-	Yes
2009-	2009	M	200	-	-18.7	8.7	-	Yes
11/04/2010	2010	M	-	-	-14.5	9.3	-	-
20/06/2011	2011	F	216	-	-21.9	8	-	Yes
2011-	2011	F	-	-	-17.3	8.5	-	-
07/12/2012	2012	M	290	-	-19.1	9.1	-	Yes
2012-	2012	M	-	-	-16.9	10	-	-
18/07/2013	2013	F	180	-	-16.8	6.6	-	Yes
07/08/2013	2013	F	220	-	-20	9	-	Yes
21/11/2013	2013	F	320	-	-13.8	9.7	-	Yes
2013-	2013	F	220	-	-13.5	7.4	-	Yes
30/07/2014	2014	F	180	-	-12.5	8.7	-	Yes
19/08/2014	2014	-	220	-	-18.9	9.3	-	Yes
99/08/2014	2014	M	170	-	-17.9	7	-	No
Sid	-	M	220	-	-14.3	8.6	-	Yes
Amazon River Estuary – samples from Museu Paraense Emílio Goeldi (MPEG) – Pará - BR								
4636	1916	-	-	-	-22	6	-	Yes
4638	1916	F	-	-	-15.8	7	-	Yes
4639	1917	F	-	-	-17.7	7.6	-	Yes
22428	1931	M	-	-	-20.9	6.2	-	-
4637	1934	F	-	-	-23	7.6	-	Yes
1518	1958	M	-	-	-21.8	7.1	-	Yes
7959	1973	F	-	-	-25.5	6.6	-	No
11198	1983	F	85	-	-23.4	9.6	-	No
GEMAM 429	2008	F	170	-	-14.9	10.3	-	No
42181	2012	F	219	-	-13.9	9.2	-	Yes
GEMAM 419	2012	-	-	-	-21.2	7.2	-	Yes
749	2018	M	-	-	-17.8	10	-	Yes
9138	-	-	-	-	-11.7	15.2	-	-

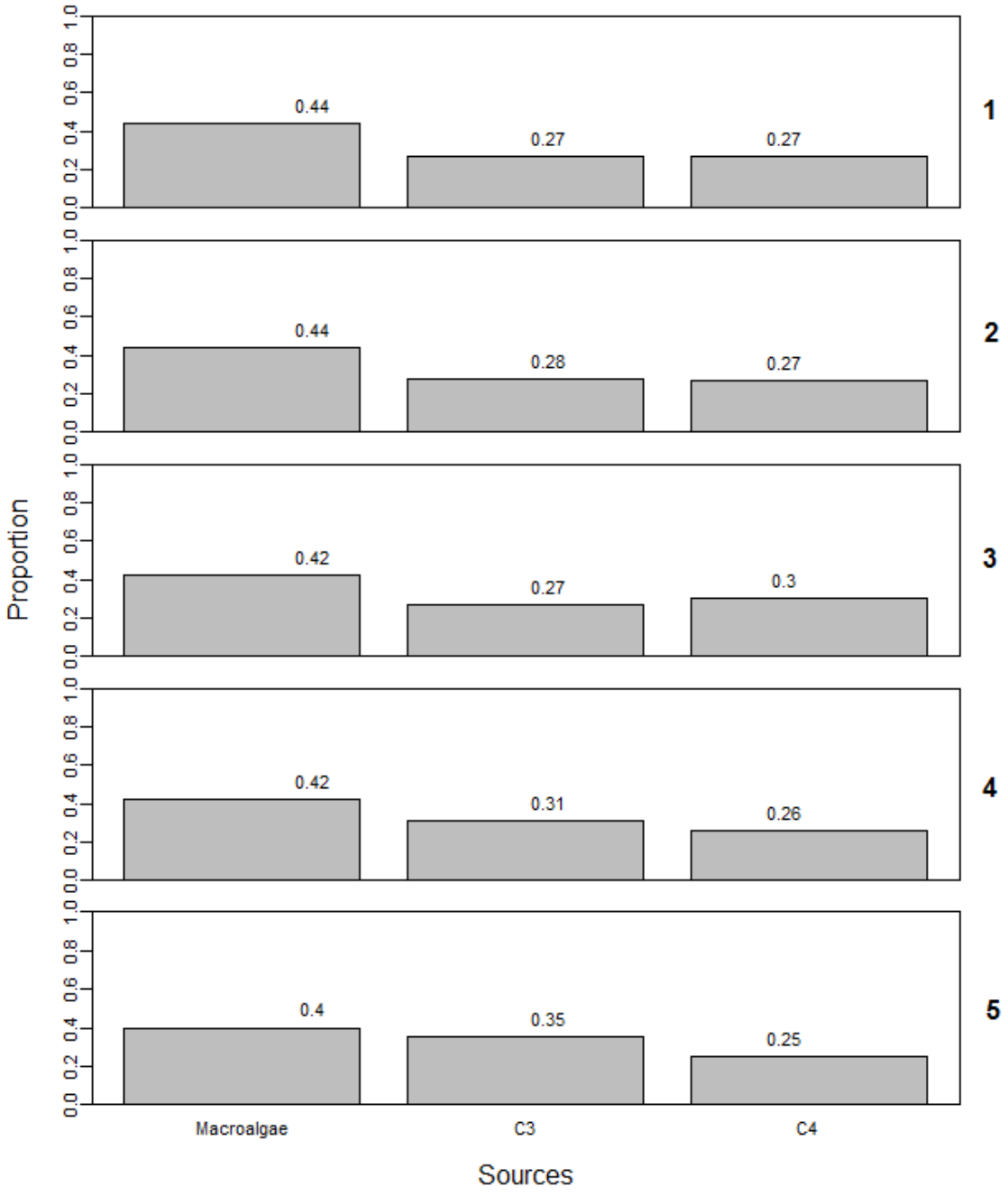


S2. Simulated mixing polygon for the Amazonian ma-tee dentin $\delta^{13}C$ and $\delta^{15}N$ values (black circles) and mean isotopic ratios of food sources (white crosses) from Middle Amazon and Amazon River Estuary. Polygons were generated with different trophic discrimi-tion factor (TDF) for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), in that order are: 6.2‰ and 2.0‰ (Ciotti, 2012); 6.1‰ and 2.3‰ (Han et al., 2016; Crema et al., 2019a); 5.5‰ and 3‰ (Vogel et al., 1990); 6.2‰ and 4‰ (Steele & Daniel, 1978; Clementz et al., 2007); 6.1‰ and 5‰ (best adjustment).



S3. Stable isotope Mixing Models (SIMMR) estimates of the mean proportio-l contribution (%) of each source group to the diet of adults, male adult, female adult and juvenlies from middle Amazon River. SIMMR models (1–5) with different trophic discrimi-tion factor (TDF) for carbon

($\delta^{13}C$) and nitrogen ($\delta^{15}N$), in that order are: 6.2‰ and 2.0‰ (Ciotti 2012); 6.1‰ and 2.3‰ (Han et al. 2016; Crema et al 2019a); 5.5‰ and 3‰ (Vogel et al. 1990); 6.1‰ and 4‰ (Steele & Daniel, 1978; Han et al. 2016); 6.1‰ and 5‰ (best adjustment based on the simulated mixing polygons). Food sources are terrestrial C_3 plants, aquatic C_3 plants and C_4 plants.



S4. Stable isotope Mixing Models (SIMMR) estimates of the mean proportio-l contribution (%) of each source group to the diet of adults from Amazon River Estuary. SIMMR models (1–5) with different trophic discrimi-tion factor (TDF) for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), in that order are: 6.2‰ and 2.0‰ (Ciotti 2012); 6.1‰ and 2.3‰ (Han et al. 2016; Crema et al 2019a); 5.5‰ and 3‰ (Vogel et al. 1990); 6.1‰ and 4‰ (Steele & Daniel, 1978; Han et al. 2016); 6.1‰ and 5‰ (best adjustment based on the simulated mixing polygons). Food sources are macroalgae, aquatic C_3 vascular plants and C_4 vascular plants.

S5. Mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ (‰) of feeding sources potentially consumed by *Trichechus inunguis* (Guterres et al., 2008) collected in creeks, lakes and channels of Amanã and Mamirauá Sustai-ble Development Reserve (Middle Amazon) and in the Amazon River Estuary. Plants were classified in the following life forms: emergent (emer), amphibious (amp), free floating (ff), rooted submerged with floating leaves (rsff), free floating submerged (ffs), algae (alg), vine (vin), shrubs and trees (tree).

Family	Species	Life form	Grouping class	Mean $\delta^{15}N$	Mean $\delta^{13}C$	n
Middle Amazon						
	algae	alg	aquatic C_3	6.0 ± 1.6	-36.7 ± 1.4	2
Acanthaceae	*Justicia laevilinguis* (Nees) Lindau	amp	aquatic C_3	3.6 ± 2.8	-29.8 ± 1.4	2
Alismataceae	*Sagittaria sprucei* Micheli	emer	aquatic C_3	2.8	-30.7	1
Anno-ceae	*Oxandra riedelia*- R.E.Fr.	tree	terrestrial C_3	3.1	-33.1	1
Apocy-ceae	*Rhabdadenia madida* (Vell.) Miers	vin	aquatic C_3	5.9	-31.6	1
	Tassadia trailia- (Benth.) Fontella	vin	aquatic C_3	3.3 ± 1.2	-31.4 ± 0.7	2
Araceae	*Montrichardia* sp.	emer	aquatic C_3	5.5 ± 1.3	-27.7 ± 0.1	2
	Pistia stratiotes L.	ff	aquatic C_3	5.3 ± 1.4	-30.2 ± 1.0	8
Araliaceae	*Hydrocotyle ranunculoides* L.f.	rsff	aquatic C_3	10.5	-29.9	1
Azollaceae	*Azolla* sp.	ff	aquatic C_3	-0.8 ± 0.3	-29.8 ± 0.8	8
Convolvulaceae	*Ipomea* sp.	vin	aquatic C_3	6.1 ± 3.2	-30.1 ± 2.2	5
Cyperaceae	*Cyperus esculentus* L.	amp	aquatic C_3	4.7	-31.5	1
	Cyperus sphacelatus Rottb.	emer, amp	C_4	2.0 ± 1.1	-12.7 ± 0.3	4
	Eleocharis subarticulata (Nees) Boeckeler	emer. amp	aquatic C_3	4.3	-27.6	1
	Eleocharis ochrostachys Steud.	emer. amp	aquatic C_3	2.1	-30.2	1
	Cyperus odoratus L.	emer. amp	C_4	4.9 ± 0.1	-12.4 ± 0.1	2
Euphorbiaceae	*Caperonia castaneifolia* (L.) A.St.-Hil.	amp	aquatic C_3	5.2 ± 2.1	-30.5 ± 0.8	6
	Mabea nitida Spruce ex Benth.	tree	terrestrial C_3	-0.1	-35.1	1
Fabaceae	*Aeschynomene sensitiva* Sw.	amp	aquatic C_3	2.3 ± 2.8	-31.1 ± 1.8	4
	Campsiandra angustifolia Spruce ex Benth.	tree	terrestrial C_3	3.1	-31.1	1
	Macrolobium acaciifolium (Benth.) Benth.	tree	terrestrial C_3	2.4	-31.1	1
	Neptunia oleracea Lour.	emer. amp	aquatic C_3	0.4 ± 1.1	-32.1 ± 0.8	2
	Vig- lasiocarpa (Mart.ex Benth.) Verdc.	vin	aquatic C_3	0.3 ± 2.6	-31.3 ± 1.8	5
Hydrocharitaceae	*Limnobium laevigatum* (Humb. & Bonpl. ex Willd.) Heine	ff	aquatic C_3	4.6 ± 1.5	-28.6 ± 1.7	4
Lentibulariaceae	*Utricularia* sp.	ffs	aquatic C_3	6.6 ± 2.5	-34.5 ± 4.3	6
Malpighiaceae	*Byrsonima japurensis* A.Juss.	tree	terrestrial C_3	0.6	-32.5	1

Myrtaceae	*Eugenia inundata* DC.	tree	terrestrial C_3	1.9 ± 1.1	-32.5 ± 1.3	2
Nymphaeaceae	*Nymphaea glandulífera* Rodschied	rsff	aquatic C_3	5.2	-33.6	1
O-graceae	*Ludwigia helminthorrhiza* (Mart.) H.Hara	ff	aquatic C_3	6.5 ± 2.4	-29.7 ± 1.6	6
	Ludwigia leptocarpa (Nutt.) H.Hara	emer. amp	aquatic C_3	3.2	-30.8	1
Phyllanthaceae	*Phyllanthus fluitans* Benth. ex Müll.Arg.	ff	aquatic C_3	3.7 ± 2.7	-31.1 ± 0.4	4
Poaceae	*Echinochloa polystachya* (Kunth) Hitchc.	emer	C_4	5.3 ± 1.5	-12.0 ± 0.5	6
	Hyme-chne amplexicaulis (Rudge) Nees	amp	aquatic C_3	5.5 ± 1.9	-29.8 ± 1.7	6
	Leersia hexandra Sw.	amp	aquatic C_3	5.1 ± 2.2	-28.1 ± 1.2	3
	Luziola sprucea- Benth. ex Döll	rsff	aquatic C_3	2.3 ± 2.4	-29.3 ± 1.1	3
	Oryza grandiglumis (Döll) Prod.	emer	aquatic C_3	6.8 ± 1.1	-29.7 ± 1.7	7
	Paspalum fasciculatum Willd. ex Flüggé	amp	C_4	2.8 ± 1.5	-12.7 ± 0.3	4
	Paspalum repens P.J.Bergius	emer	C_4	4.8 ± 1.4	-12.9 ± 0.4	8
Polygo-ceae	*Polygonum ferrugineum* Wedd.	emer	aquatic C_3	6.0 ± 1.1	-30.1 ± 0.3	4
	Symmeria paniculata Benth.	tree	terrestrial C_3	4.8 ± 2.2	-30.0 ± 2.0	6
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	ff	aquatic C_3	5.6 ± 1.7	-30.9 ± 0.5	6
	Pontederia rotundifolia L.f.	ff	aquatic C_3	5.0 ± 2.4	-31.1 ± 0.9	6
Pteridaceae	*Ceratopteris pteridoides* (Hook.) Hieron.	ff	aquatic C_3	4.8 ± 1.4	-29.7 ± 0.7	5
Ricciaceae	*Ricciocarpus -tans* (L.) Corda	ff	aquatic C_3	4.4 ± 1.0	-28.8 ± 0.1	3
Rubiaceae	*Genipa america*- L.	tree	terrestrial C_3	2.0 ± 0.3	-30.2 ± 0.1	2
	Oldenlandia herbacea (L.) Roxb.	emer	aquatic C_3	6.6	-30.3	1
Salvi-ceae	*Salvinia* sp.	ff	aquatic C_3	4.4 ± 1.3	-30.6 ± 1.0	15
Sapotaceae	*Elaeoluma glabrescens* (Mart. & Eichler) Aubrév.	tree	terrestrial C_3	2.4	-32	1
Urticaceae	*Cecropia latiloba* Miq.	tree	terrestrial C_3	1.6 ± 0.3	-29.4 ± 1.91	2
Amazon Estuary						
Acanthaceae	*Avicennia germi-ns* (L.) L.	tree	C_3	4.6 ± 0.5	-29.1 ± 1.4	7
Amaranthaceae	*Blutaparon* sp.	emer	C_4	7.4 ± 1.5	-13.4 ± 0	3
Araceae	*Montrichardia* sp.	amp	C_3	4.4 ± 1.0	-29.5 ± 0.5	3
Caulacanthaceae	*Catenella* sp.	alg	alga	5.5 ± 1.3	-24.6 ± 1.0	9
Combretaceae	*Laguncularia racemosa* (L.) C.F.Gaertn.	tree	C_3	3.4 ± 1.9	-29.4 ± 0.6	5
Cyperaceae	*Eleocharis geniculata* (L.) Roem. & Schult.	amp	C_3	5.6 ± 0.8	-29.1 ± 0.4	5

Lythraceae	*Crenea marítima* Aubl.	emer				
Poaceae	*Sparti-* sp.	amp	C_4	4.4 ± 1.5	-12.8 ± 1.0	6
	Sporobolus sp.	emer	C_4	6.9 ± 0.5	-11.9 ± 0.6	3
.Rhizophoraceae	*Rhizophora mangle* L.	tree	C_3	3.6 ± 1.2	-29.6 ± 1.1	7
Rhodomelaceae	*Bostrychia* sp.	alg	alga	5.5 ± 1.3	-24.6 ± 1.0	9
Ulvaceae	*Ulva* sp.	alg	alga	6.3 ± 0.8	-23.4 ± 2.0	5

APÊNDICE II

CARBON AND NITROGEN ISOSCAPES OF ANTILLEAN MANATEE (*Trichechus manatus manatus*) ALONG THE BRAZILIAN COAST

Carvalho CC, Marmontel M, Botta S, Luna FO, Attademo FNL, Meirelles AC, Gomes JCB, Secchi ER

Carbon and nitrogen isoscapes of Antillean manatee (*Trichechus manatus manatus*) along the Brazilian coast

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Oceanografia Biológica – FURG, under the supervision of E. R. Secchi and M. Marmontel.

Introduction

Marine mammals show high variability and flexibility in their foraging strategies. Feeding tactics are related with the local environmental characteristics and may vary among populations, ontogenetic classes, sexes and individuals (Heithaus & Dill, 2009). The chosen feeding strategy for each group will be a result of the trade-off between maximizing energy intake (food quality) and minimizing predation risks (Lima & Dill, 1990), and feeding strategies that maximize fitness presumably will be favored. For large herbivores, food quality is determined by nutritional characteristics and energy (McArthur et al., 2014). Although their high abundance and availability, plants are generally high in fiber and low in protein (low energetic food; Marsh et al., 2012). Therefore, food selection is also a result of a trade-off, since each food source differs in the energy required for search and handling and the amount of energy resulting for the consumers (Heithaus & Dill, 2009).

These trade-offs could determine habitat use patterns; the relative importance of habitats varying among areas. West Indian manatees (*Trichechus manatus*), a strictly herbivorous marine mammal described as a habitat generalist, uses resources across marine, estuarine and freshwater habitats (Ledder, 1986). It has been reported that in some areas manatees only feed on freshwater vegetation (Bengtson 1981; Reich & Worthy, 2006), while in other areas they use habitats that are exclusively estuarine or marine (Deutsch et al., 2003; Reich & Worthy, 2006).

In Brazil, Antillean manatees (*Trichechus manatus manatus*) have a discontinuous distribution from Amapá (4ºN) to Alagoas state (10°S; Lima, 1997; Luna et al., 2008). The discontinuous distribution is a result of hunting activities in the past (Domning, 1981), and is likely to jeopardize genetic exchange between populations, since Antillean manatees do not undertake large-scale movements (mean of maximum linear distance traveled was 30 km), with the exception of a few individuals (Normande et al., 2016). Despite the widespread distribution, the number of individuals of this subspecies was estimated recently from aerial survey as only 1,104 (Credibility Interval from 485 to

2221) along the northeastern coast (from the Parnaíba River mouth in Piauí state to th mouth of the São Francisco River in Alagoas State; Alves et al., 2016).

This tropical Brazilian coast area were manatees are distributed has a variety of habitats, including well-developed mangroves and saltmarshes (Schaeffer-Novelli et al., 2016), sandy beaches, dunes (Amaral et al., 2016), seagrass beds (Copertino et al., 2016) and rocky shores with associated macroalgal communities (Coutinho et al., 2016). It is not well known how Antillean manatees uses this variety of habitats and the relation with feeding ecology. First attempts to understand this complex scenario suggested spatial variation in diet: manatees from Northeastern Coast showing predominance of seagrasses and macroalgae in diet and manatees from North Coast presenting high contribution of salt marshes and mangroves (Ciotti, 2012). Nevertheless, it is plausible that the diet will reflect the local environmental conditions, due to the resident habit of the species.

Few studies have investigated the diet of the species and the differences in contribution of marine, estuarine and freshwater resources among distinct areas. Lima (1997) reported that seagrasses were the main component of manatee diets, especially *Halodule* sp., while Borges et al. (2008) showed the importance of macroalgae species (red, brown and green), and also identified seagrasses (*Halodule wrightii*, *Halophila* sp.) and cnidarians, as food items. Best (1981) reported the consumption of young shoots and leaves of mangrove trees (*Rhizophora mangle*, *Laguncularia racemosa*) and saltmarsh plants (*Spartina alterniflora*).

Geographical variability in diet can be assessed through stable-isotope analyses, and can be visually accessed through the resulting isotopic maps (isoscapes). These isotopic spatial gradients have been found on marine continental shelves in the carbon and nitrogen values of primary producers, consumers and organic matter in surface sediments (Radabaugh et al., 2013). For carbon isotopes, the fixation of carbon dioxide by plants is the key process, whereas the magnitude of the fractionation varies across the photosynthetic pathways (C_3, C_4 and CAM; DeNiro & Epstein, 1978). Additionally, animals feeding in marine and estuarine habitats usually have higher $\delta^{13}C$ values than those feeding in riverine and terrestrial environments, due to factors such as nutrient sources and variations in primary producers (Schoeninger & DeNiro, 1984). These differences create large-scale patterns in plant $\delta^{13}C$ values according to the geographical distribution of plant types and environmental conditions (Bowen, 2010). In the marine

environment, it is possible to infer the geographic origin of the consumers based on the $\delta^{13}C$ values of their tissues that result from the assimilation of primary producers through diet (Graham et al., 2010).

Carbon isotopic data can be combined with nitrogen isotopes to identify the feeding environments used by marine mammals (Hobson & Schell, 1998; Newsome et al., 2007; Graham et al., 2010). The pattern of $\delta^{15}N$ is not as clear because there is no dominant process influencing geographic $\delta^{15}N$ distributions (Bowen, 2010). Nutrient source, biological transformations, isotopic fractionation and nutrient pool size are among the factors likely to influence these isotopic values (McMahon et al., 2013). In addition, $\delta^{15}N$ values can also be used as a tracer of nitrogen inputs from anthropogenic sources, such as discharge of effluents and fertilizers (Bowen, 2010).

The identification of feeding sources and habitat requirements of a species is essential for its conservation. The Antillean manatee is currently classified as Endangered in the Brazilian (MMA, 2014) and in the IUCN (Self-Sullivan et al., 2008) Red Lists of Threatened Species. The main threats are habitat degradation, mangrove and riparian forest deforestation, coastal occupation, boat traffic and incidental capture in nets (Luna et al. 2018). In the face of ongoing habitat loss or degradation, information of Antillean manatee feeding ecology is essential to delineate conservation and coastal management strategies. The aim of this study was to investigate the spatial variation in the Antillean manatee diet and habitat use along the species´ distribution in coastal Brazilian waters through the development of isoscapes. We hypothesized that isotopic composition will vary between the distribution area as a function of habitat and plants availability, showing higher $\delta^{13}C$ values in Dry-NEC and lower values in the North Coast.

Methods

Samples and study area

Tooth samples of Antillean manatees were collected throughout their distribution area in the Brazilian northern and northeastern coasts, from the state of Amapá (AP; 04º18’12”N, 51º18’42”W) to the state of Alagoas (AL; 10º31’57”S, 36º19’10”W, Table 1, Figure 1).

All samples were taken from dead specimens found washed ashore or entangled in fishing nets.

Sampling collection sites were divided in three areas according to their contrasting environmental characteristics: Northern Coast (NC); Dry Northeastern Coast (Dry-NEC) and Humid Northeastern Coast (Humid-NEC) (Ab'Saber, 2008; Muehe, 2010).

Northern Coast

The Northern Coast comprises the states of Amapá, Pará and Maranhão (04º26'N 51º32'W to 02º11'S 44º27'W) extending for approximately 1200 km. This area is highly influenced by the water discharge and mud deposition of the Amazon River. Water-level oscillation results from the combination of two main factors: river flow and the tidal pulses – macrotides associated with strong tidal currents (Muehe, 2010) Well-developed mangroves colonize the riverbanks, extending for long distances (Souza-Filho, 2005), with the predominance of *Rhizophora* and low energy depositional environments that may be colonized by *Spartina* spp. (Schaeffer-Novelli et al., 1990; Schaeffer-Novelli et al., 2016). These mangroves forests do not present connection to other nearshore habitats as seagrasses and macroalgae beds (Giarrizzo, 2011). Seagrasses (*Ruppia maritima*) were reported only in coastal lagoons in Maranhão state (Copertino et al., 2016), a region with no manatee presence.

The Northeastern coast is characterized by the dominance of sedimentary cliffs and can be classified according the degree of water deficit: the semi-arid Dry-Northeastern Coast and the Humid-Northeastern Coast (Muhue, 2010).

Dry Northeastern Coast

The Dry Northeastern Coast extends for approximately 1,250 km, from the middle of Maranhão State (02º15'S 43º42'W) to Cape Calcanhar in Rio Grande do Norte State (05º09'S 35º29'W) (Ab'Saber, 2008; Muehe, 2010). This section has a coastline exposed to high-energy waves, resulting in sandy beaches, dunes and sandstone cliffs. The climate is dry with a pronounced dry season (Schaeffer-Novelli et al., 1990). In the eastern region

is possible to observe the meeting of the caatinga (desert vegetation) with the sea (Amaral et al., 2016). Mangroves are less developed and there is a greater diversity of phanerogam species (*Halodule wrightii*, *Halophila decipens*, *Halophila bailonis* and *Halodule emerginata*, Copertino et al., 2016) than in the other two regions.

Humid Northeastern Coast

The Humid Northeastern Coast, extends from Cape Calcanhar in Rio Grande do Norte State (05º09'S 35º29'W) to the state of Alagoas (10º29'S 36º25'W). The Humid-NEC is characterized by narrow and sandy beaches partly located between sandstone reefs in parallel to the coast, which can reach several kilometers long and are often associated with calcareous algae and corals. This formation acts protecting the coast from high-energy coastal dynamics, and consequently, is responsible for a great diversity of coastal landscapes as: exposed and protected beaches, rocky shores, seagrasses beds and tidal flats with mangrove vegetation associated (Amaral et al., 2016). Some seaweeds, such as *Gracilaria* spp., are found around Itamaracá Island (07º44'S 34º49'W) associated with red algae and the green algae *Caulerpa* spp. (Figueiredo et al., 2008). Mangrove formations are well developed within protected areas associated with estuaries and coastal lagoons, mainly in Mamanguape Bay (06º46'S 34º55'W; Xavier et al., 2012).

Sample collection

We selected the most recently erupted tooth from the right side of the mandible of each manatee skull. When that tooth was absent, we used the following criteria: 1) the most recent molar of the right side even if it was not in the last position (Ciotti et al., 2014; Crema et al., 2019); 2) the most recent molar on the left side of the mandible; and 3) the most recent right maxillary molar. Data from tooth and bone samples from a previous study (Ciotti et al. 2014) were also included in the analysis.

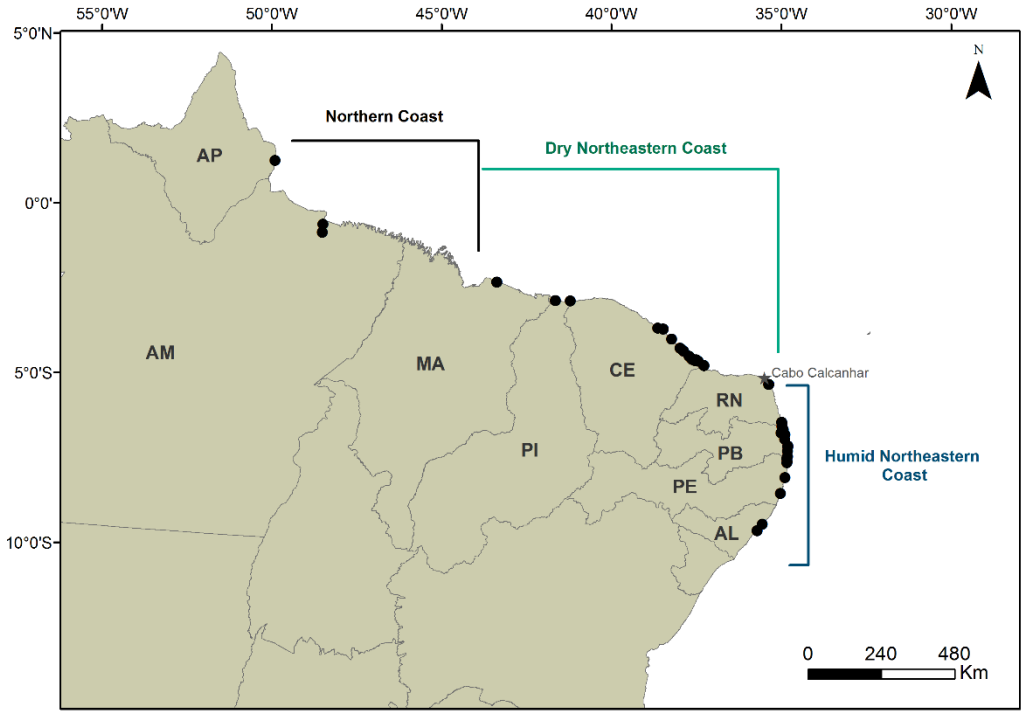


Figure 1. Location of manatee-tooth samples (black points) used in the study.

Stable-isotope analysis

All manatee teeth were brushed with distilled water to remove tissue and debris. One tooth of each individual was longitudinally cut in half with a circular low-speed diamond saw in order to expose the dentin. Tooth halves were brushed again with distilled water and dried at room temperature for 24 hours. Dentin was extracted with a drill and the resulting powder was acidified with 30% hydrochloric acid (HCl) to remove inorganic carbon. The powdered dentin was oven-dried at 60°C for a period of two hours to remove HCl residues. Approximately 1 mg of the dentin powder was stored in tin capsules.

Isotopic analyses were performed at the Center for Stable Isotopes at the University of New Mexico (Albuquerque, New Mexico) using a Costech 4010 elemental analyzer (Costech, Valencia, CA) coupled to a Thermo Scientific Delta V (Thermo Scientific, Bremen, Germany) isotope ratio mass spectrometer. The isotopic ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of the samples were expressed as delta notation ($\delta^{13}C$ and $\delta^{15}N$) in parts per thousand (‰) of the international Vienna Pee Dee Belemnite (VPDB) standards for carbon and atmospheric air for nitrogen, following the equation:

$$\delta X\ (‰) = [(R_{sample} / R_{standard}) -1]$$

where R_{sample} and $R_{standard}$ are the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of the sample and standard, respectively. Delta values were calculated using multipoint normalization. The laboratory standards used were soy protein, casein, tuna, whey protein, IAEA-N1, IAEA-N2, USGS-4 and USGS-43. The analytical precision of the laboratory standards used was: $\delta^{13}C$ <0.04 ‰ and $\delta^{15}N$ <0.2 ‰.

Previous data used were analyzed in a different laboratory, then a calibration procedure was applied. Isotopic values of teeth and bone of the Antillean manatee revealed significant differences in $\delta^{15}N$ values between paired-samples analyzed by UNM–CSI and SICL–WSU laboratories. Therefore, $\delta^{15}N$ values of our samples analyzed in SICL–WSU were corrected using following equation:

$$\delta^{15}N \text{ corrected} = 0.6504\ (\delta WSU) + 3.9792$$

where δWSU represents the $\delta^{15}N$ values obtained at the Stable Isotope Core Laboratory, Washington State University.

Statistical analysis

The isotopic niches of the Antillean manatees of the three areas were calculated using Stable Isotope Bayesian Ellipses (SIBER; Jackson et al., 2011) in the R statistical environment (R Development Core Team, 2018). This method can incorporate uncertainties such as sampling biases and small sample sizes (Jackson et al., 2011). The standard ellipse area corrected for small sample sizes (SEAc, expressed as ‰²), containing approximately 40% of data (Syväranta et al., 2013), was used as the measure of isotopic niche width. Ellipses were obtained for all animals (1 adult and 4 with absence of length data) from NC, due to the small sample size, and for the adults from the Dry-NEC and Humid-NEC.

Isoscapes

$\delta^{13}C$ and $\delta^{15}N$ isoscapes were generated using geographical coordinates attributed to each Antillean manatee sample location. A buffer of 100 km from the coast was created before the generation of isoscapes, corresponding to the coastal habitat limit of the Antillean manatee (Normande et al., 2016). The interpolations were conducted by ordinary kriging methods with spherical models, utilizing the Spatial Analyst tool in the ArcMAP 10.1 software (ESRI, 2019).

Results

Mean tooth $\delta^{13}C$ values were higher for Antillean manatees from Dry-NEC and lower values were found for animals from the NC. Higher and lower mean $\delta^{15}N$ values were found respectively in manatees from the Humid-NEC and the NC (Table 1).

Table 1. Minimum (Min), maximum (Max), sample size (n), mean and standard deviation (S) $\delta^{13}C$ and $\delta^{15}N$ values in teeth and bones of Antillean manatees, *Trichechus manatus manatus*, from each Brazilian State within the study area and grouped by the three areas: Northern Coast (Amapá, Pará, Maranhão), Dry Northeastern Coast (Piauí, Ceará, Dry-Rio Grande do Norte) and Humid Northeastern Coast (Rio Grande do Norte, Paraíba, Pernambuco and Alagoas).

			$\delta^{13}C$ (‰)			**$\delta^{15}N$ (‰)**		
Site	**Coordinates**	**n**	**Min**	**Mean ± sd**	**Max**	**Min**	**Mean ± sd**	**Max**
Northern Coast	4º26'N 51º30' to 2º13'S 43º38'W	**5**	**-28.5**	**-20.5 ± 7.0**	**-15.4**	**7.2**	**7.7 ± 0.5**	**8.1**
Amapá	4º26"N 51º30'W	1	-16.8	-16.8	-16.8	7.2	7.2	7.2
Pará	0º45'N 50º06'W	1	-28.5	-28.5	-28.5	8.1	8.1	8.1
Maranhão	1º05'S 46º03'W	3	-17.4	-16.1 ± 1.1	-15.4	7.8	7.8 ± 0.0	7.8
Dry Northeastern Coast	2º13'S 43º38'W to 5º09'S35º29'W	**34**	**-12.8**	**-8.6 ± 0.5**	**-5.9**	**5.3**	**8.8 ± 0.4**	**12.3**
Piauí	2º43'S 41º48'W	2	-9.7	-9.2 ± 0.6	-8.8	7.5	8.3 ± 1.1	9.1
Ceará	2º55'S 41º19'W	31	-12.8	-8.2 ± 1.4	-5.9	5.3	9.0 ± 1.5	12.3
Rio Grande do Norte - D	4º50'S 37º15'W	1	-8.4	-8.4	-8.4	9.0	9.0	9.0
Humid Northeastern Coast	5º09'S35º29'W to 10ª29'S 36º23'W	**42**	**-20.7**	**-11.4 ± 1.5**	**-6.9**	**3.4**	**9.2 ± 0.6**	**12.2**
Rio Grande do Norte - H	5º09'S 35º29'W	9	-20.7	-12.1 ± 4.8	-6.9	7.4	9.6 ± 1.1	10.9
Paraíba	6º29'S 34º58'W	26	-19.9	-12.9 ± 3.7	-8.1	3.4	9.7 ± 1.6	12.2
Pernambuco	7º33'S 34º49'W	4	-16.1	-11.3 ± 3.8	-8.1	7.4	9.3 ± 1.8	11.3
Alagoas	8º54'S 35º09'W	3	-9.7	-9.3 ± 0.3	-9.1	7.8	8.3 ± 0.8	9.3

Isotopic niche area was wider in Antillean manatees from Humid-NEC (SEAc = 11.46 ‰^{2}), followed by Northern Coast (SEAc = 6.41 ‰^{2}), and Dry-NEC (SEAc = 4.84 ‰^{2}) (Figure 2).

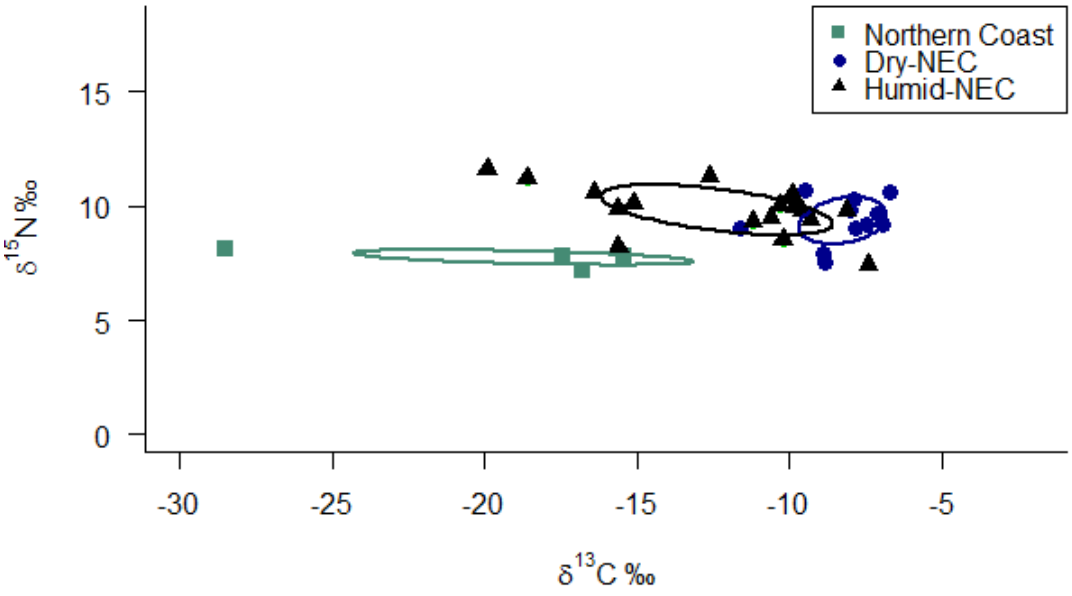


Figure 2. Isotopic niche area, represented as standard ellipse area corrected for small sample sizes (SEAc), based on $\delta^{13}C$ and $\delta^{15}N$ in teeth and bones of Antillean manatees (*Trichechus manatus manatus*) from different regions along the Brazilian coast.

Isoscapes

Manatee's $\delta^{13}C$ and $\delta^{15}N$ values varied considerably throughout the sampling area (Figures 3 and 4). $\delta^{13}C$ values were higher in the Dry-NEC coast and again in the southern portion of the Humid-NEC, while the NC coast showed the lowest values. In the case of the $\delta^{15}N$ values, an increasing trend was observed from NC towards Humid-NEC.

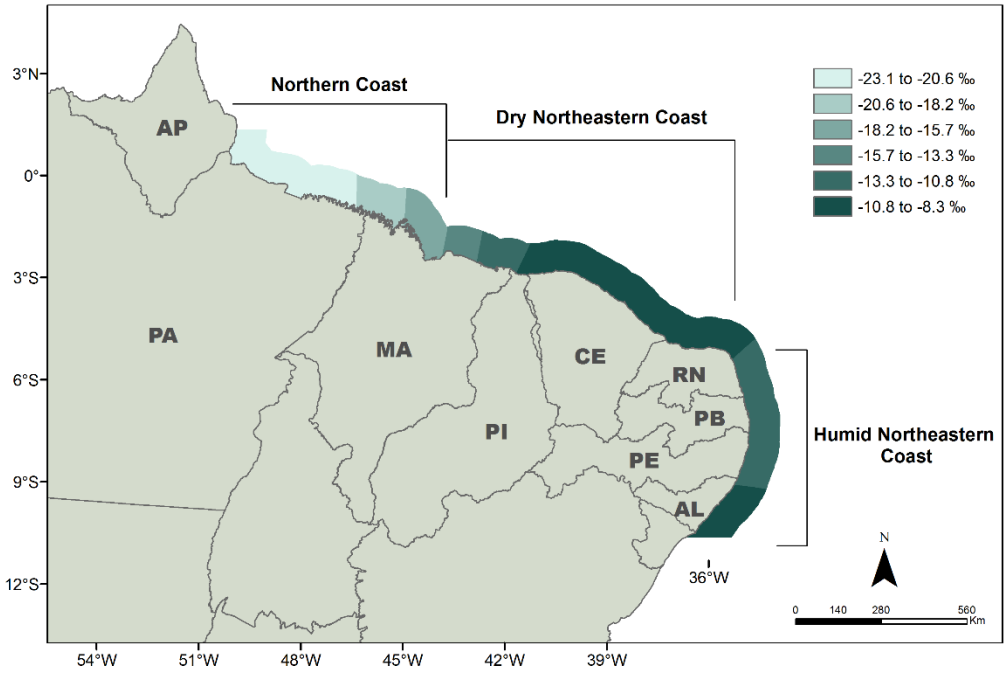


Figure 3. $\delta^{13}C$ isoscape produced from the interpolation of isotopic values of Antillean manatee (*Trichechus manatus manatus*) samples.

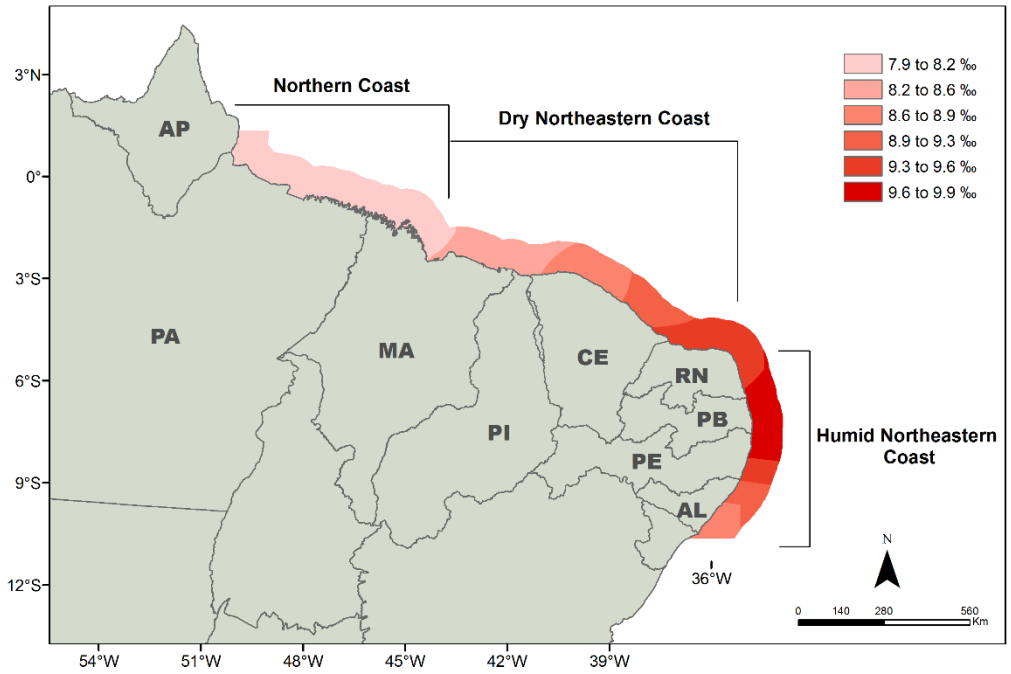


Figure 4. $\delta^{15}N$ isoscape produced from the interpolation of isotopic values of Antillean manatee (*Trichechus manatus manatus*) samples.

Discussion

The geographic variation in carbon and nitrogen isotopic values of manatee teeth reflects the environmental characteristics of coastal habitats; $\delta^{13}C$ values were lower in manatees from areas with predominantly mangrove, riverine and estuarine habitats and $\delta^{15}N$ values increased in the most dense and urbanized areas along the Humid-NEC.

$\delta^{13}C$ isotopic values

Manatees from the Northern Coast had the lower $\delta^{13}C$ values than the Dry and Humid-NEC. This result might be explained by the physical characteristics and vegetation type of this area. The Northern Coast encompasses a complex estuarine region and receives large amounts of freshwater discharge due to the input of the Amazon, Tocantins and Parnaíba rivers (Dominguez, 2009). This region is controlled by macrotidal processes (higher than 4 meters) with wide estuary mouths that can extend more than 100 km into the continent. In the rainy period, the estuarine area may have salinities near zero (Rosario et al., 2016).

The tooth-isotope values are probably mainly driven by the composition of producers that are found within these regions. In the NC there is a predominance of C_3 plants due to the presence of one of the largest mangrove systems (approximately 40 km in its maximum extension) in the world, with more than 7,000 km² (Souza Filho, 2005). The local characteristics also favor the development of saltmarsh plants, mainly *Spartina alterniflora* (Schaeffer-Novelli et al., 2016). These saltmarsh plants have C_4 photosynthetic pathways, and are present in low abundance.

Mangrove shoots and leaves have been described as manatee food items (Borges et al., 2008; Castelblanco-Martinez et al., 2009). In NC, one individual had a $\delta^{13}C$ value (around -28 ‰) like mangroves. The other individuals low $\delta^{13}C$ values probably had more mixed diets (including saltmarsh plants and macroalgae species) in riverine and estuarine feeding areas.-

The highest $\delta^{13}C$ values were found for manatees from Dry-NEC, followed by Humid-NEC. In the Dry-NEC, manatees are probably feeding in exclusive marine areas. This interpretation is supported by the fact that there are no records of manatees using rivers and estuaries in Ceará state, except for some past occurrences in the Jaguaribe river. It is

possible that the estuarine and riverine areas are no longer used by manatees because of human activities, such as boat traffic and shrimp farming that lead to mangrove destruction and river siltation, limiting the access to estuaries and restricting the use of the marine environment (Meirelles, 2008). In the other states of this region there are fewer riverine and estuarine environments than in other regions.

Another factor that favors marine-habitat use in Dry-NEC is the abundance of seagrasses, with the dominance of *Halodule wrightii* meadows. The seagrass beds are found between a barrier reef and beaches, within the reef lagoon (Copertino et al., 2016). *H. wrightii* has been previously described as an important item in the diet of manatees along the Brazilian coast (Lima, 1997; Borges et al., 2008). Therefore, the highest $\delta^{13}C$ values probably imply the use of marine environments, high consumption of seagrasses and low ingestion of macroalgae and mangroves.

In the Humid-NEC, a mosaic of habitats are present, including mangroves, estuaries and coastal beaches. This variety of habitats may be responsible for the intermediate $\delta^{13}C$ values for manatees from this area. The geomorphological characteristics of the coast determines the type of food sources found by manatees: the Humid-NEC comprises a barrier reef coast that provides ideal conditions for the development of seagrasses in dense beds (Copertino et al., 2016), and coral algal reefs on hard substrates (beach rocks) (Figueiredo et al., 2008). Paludo and Langguth (2002) suggested that the occurrence of macroalgal beds, especially those with red algae, was the main factor explaining the presence of manatees in Sagi (Rio Grande do Norte) in the summer, which was corroborated by the results of Borges et al. (2008). Since macroalgae shows intermediate $\delta^{13}C$ values (Clementz et al., 2007), the consumption of seaweed, in addition to the estuarine habitat use, can explain the intermediate values found for manatees in the Humid-NEC.

$\delta^{15}N$ isotopic values

While the carbon isotopes reflect the habitats used and the vegetation type consumed, the $\delta^{15}N$ seems more related to anthropogenic characteristics of the coast. The NC was also the region comprising manatee samples with the lowest $\delta^{15}N$ values. This result possibly reflects the lack of urbanized and impacted areas where the carcasses were collected. It has been previously demonstrated that human-impacted areas have higher $\delta^{15}N$ than pristine habitats, this being a useful tool to assess pollution in estuarine (Cole et al., 2004;

Abreu et al., 2006; Dillon & Chanton, 2008) and riverine environments (Wayland & Hobson, 2001). In the NC, there is buffalo breeding in Amapá and Pará States, but this activity contributes less to nitrogen inputs to the environment than wastewater and agriculture, as shown in studies developed in northeastern states (Lacerda, 2006).

In the Northeastern Brazilian coast, many estuaries are impacted by shrimp farming, sewage input, solid waste and wastewaters, urban runoff, cattle husbandry and fertilizers used in agriculture (Lacerda, 2006). According to Brazilian governmental reports, only one third of Brazilian cities treat effluents before releasing them into the environment (IBGE, 2008). The Dry-NEC showed $\delta^{15}N$ values similar to the Humid-NEC, with the latter having the highest values. Although the Dry-NEC has low population densities (with the exception of Fortaleza city, capital of Ceará State), there are considerable emissions of N coming from agriculture, cattle husbandry and shrimp farming (Lacerda, 2006). Shrimp aquiculture is on the rise in the NEC region, representing 99% of the national production. Of the total shrimp production in this region, 71.5% is from Ceará (452 shrimp farms) and Rio Grande do Norte (384 shrimp farms) States, adding up to 49,724 ton in 13,120 ha (Rodrigues & Borba, 2013). This activity has been the target of criticism due to the environmental impacts associated with the emission of large amounts of N in estuaries (Burford et al., 2003), as reported for the Jaguaribe River estuary (Meirelles, 2008). This expanding aquiculture scenario could explain the high $\delta^{15}N$ values found in this region.

In the Humid-NEC, which had the highest $\delta^{15}N$ values, aquiculture is not as extensive as in the Dry-NEC. A study in Pernambuco State showed that wastewater was the main source of nutrients to coastal areas, accounting for 51% of N from anthropogenic and natural sources (Noriega & Araújo, 2009). In approximately 400 km of coast, there are the capitals of the four States, totaling more than three million inhabitants (IBGE, 2007). The second highest source of N was sugar cane cultivation, which has a high demand for N fertilization, totalizing 35% (Noriega & Araújo, 2009). The States of Pernambuco and Alagoas have large areas of sugar cane cultivation, 321,994 and 258,361 ha respectively; Rio Grande do Norte and Paraíba also show representative areas (97,269 and 62,729 ha; UNICADATA, 2017). The relative contribution of these sources varies with the degree of urbanization, population and area of agricultural land (Lacerda, 2006; Noriega & Araújo, 2009). This a worrying scenario, since the population in these States continues to grow (IBGE, 2019), which will result in the increase of nutrient input. The Humid-NEC

appears to be the area with the highest contribution of anthropogenic N from different sources, probably explaining the high $\delta^{15}N$ values.

Some studies have also shown that there are differences among N values in macroalgae and seagrasses (two main food sources for manatees in marine areas), essentially because the seagrasses acquire N from the sediment and the macroalgae through the fronds (Duarte, 1995). Since $\delta^{15}N$ in sediment differs from the water column, these producers have different $\delta^{15}N$ values (Cole et al., 2004), with the highest values found in macroalgae. Therefore, the highest $\delta^{15}N$ values from manatees from the Humid-NEC could be a result of the anthropogenic sources associated with a higher macroalgae consumption in this area than in the other two regions.

It is important to highlight that all these impacts related to the high input of N can lead to the loss of seagrasses beds (Cole et al., 2004). In addition to water quality, degradation resulting from human-altered watersheds and effects of aquaculture, indirect effects of invasive species, overfishing, coastal engineering and global climate change also negatively affect seagrass beds (Waycott et al., 2009). Unfortunately, it is not well understood the interaction between megaherbivores and seagrasses in Brazil, but since these plants are found as a mainly diet component of manatees in different areas, it will probably affects negatively the manatee population. Efforts are, therefore, required to decrease inputs from agricultural, aquiculture and urban sources to maintain adequate underwater light conditions for seagrass growth (Waycott et al., 2009).

Conclusion

Isoscapes proved to be an effective tool for the study of feeding ecology and habitat use by manatees. Since the species is resident, opportunist and a primary consumer, the results also reflect the isotopic trophic baseline in this part of the Brazilian coast. Isoscapes patterns showed that manatees choose feeding habitats (estuarine and marine) according to availability. In the case of nitrogen, the high $\delta^{15}N$ values also provide an indication of contamination by anthropogenic activities.

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