

**INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA
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**CARACTERIZAÇÃO DE IGAPÓS DE ÁGUAS CLARAS E PRETAS E SUAS
DISPONIBILIDADES ALIMENTARES PARA O PEIXE-BOI-DA-AMAZÔNIA
(*Trichechus inunguis*)**

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Manaus, Amazonas

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(*Trichechus inunguis*)**

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Tese apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Doutora em Ciências Biológicas, área de concentração Biologia de Água Doce e Pesca Interior

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SINOPSE:

Foram estudados a dinâmica de disponibilidade alimentar para o peixe-boi-da-Amazônia, sua biologia e dieta nas diferentes fases ontogenéticas. Essas informações foram avaliadas por meio de estudos de campo em lagos de igapós de águas pretas (rio Negro) e claras (rio Tapajós), entrevistas com populações tradicionais em Unidades de Conservação Federais e uso de análises de isótopos estáveis de C e N em dentes e ossos dessa espécie ameaçada de extinção

Palavras-chave: plantas aquáticas, parâmetros físicos e químicos da água, entrevistas, etnobiologia, ecologia alimentar, modelos basianos de mistura, isótopos estáveis.

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Entre você e Deus

Muitas vezes, as pessoas são egocêntricas, ilógicas e insensatas.
Perdoe-as, assim mesmo.

Se você é gentil, as pessoas podem acusá-lo de egoísta, interesseiro.
Seja gentil, assim mesmo.

Se você é um vencedor, terá alguns falsos amigos e inimigos verdadeiros.
Vença, assim mesmo.

Se você é honesto e franco, as pessoas podem enganá-lo.
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Veja você que, no final das contas é
Entre Você e Deus e não entre você e os homens.

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RESUMO

O peixe-boi-da-Amazônia (*Trichechus inunguis*) é herbívoro, endêmico da bacia amazônica, e sua distribuição está ligada ao pulso de inundação e à disponibilidade de plantas aquáticas. Sabe-se que ele habita ambientes bem diferenciados entre si, como igapós de águas pretas e claras. Atualmente se encontra na categoria de ameaça “vulnerável” devido aos eventos de caça (histórica e atual), sua baixa taxa reprodutiva e a destruição de seus habitats. Embora sejam de extrema importância, grande parte dos estudos sobre alimentação de peixe-boi-da-Amazônia foi desenvolvida utilizando conteúdos estomacais ou observações de campo, que trazem informações de curto prazo. Por meio de estudos de campo em lagos de igapós de águas pretas (rio Negro) e claras (rio Tapajós), entrevistas com populações tradicionais em Unidades de Conservação (UCs) Federais e uso de análises de isótopos estáveis de C e N em dentes e ossos de peixe-boi-da-Amazônia, obtive informações sobre a dinâmica de disponibilidade alimentar para a espécie, dados sobre sua biologia e dieta nas diferentes fases ontogenéticas. Houve uma grande variação nos parâmetros físicos e químicos nas águas dos lagos estudados nas diferentes fases do pulso de inundação. Das 57 espécies de plantas registradas, apenas 13 ocorreram nos dois ecossistemas. No rio Negro a disponibilidade de herbáceas é escassa e limitada a trepadeiras durante a fase de águas altas, sendo elas as de maior saliência cognitiva na dieta do peixe-boi, segundo os entrevistados. No Tapajós há grande riqueza de herbáceas ao longo de todo o pulso de inundação. As plantas com maior saliência cognitiva foram as submersas com folhas flutuantes e submersas enraizadas. Apesar de não haver variação nos sinais de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ entre dentes, as diferenças entre indivíduos indicam que a dieta do peixe-boi-da-Amazônia está relacionada com a disponibilidade de recursos, preferência alimentar ou necessidades nutricionais. Além disso, a espécie se alimenta de uma variedade de plantas e algas, cujas proporções variam de acordo com a classe ontogenética e o ecossistema. A disponibilidade alimentar pode representar um fator determinante na migração trófica do peixe-boi-da-Amazônia entre igapós e várzea, especialmente no caso das fêmeas lactantes do baixo rio Negro. Por isso, a adoção de iniciativas interinstitucionais para garantir a proteção regional da espécie, considerando as possibilidades de movimentação entre habitats, requer atenção especial. Além disso, com a finalidade de reduzir ameaças à espécie e aumentar a efetividade das UCs é necessário maior envolvimento da população local e de áreas adjacentes em atividades que ampliem sua percepção sobre a relevância da conservação de ambientes naturais e de espécies ameaçadas como o peixe-boi-da-Amazônia.

ABSTRACT

The Amazonian manatee (*Trichechus inunguis*) is a herbivore, endemic to the Amazon basin, and its distribution is related to the flood pulse and the availability of aquatic plants. It is known that it inhabits well differentiated environments, such as igapós of clear and blackwaters. It is currently in the threat category "vulnerable" due to hunting events (historical and current), its low reproductive rate and the destruction of its habitats. Although they are extremely important, most of the studies on the Amazonian manatee's feeding were developed using stomach contents or field observations, which provide information on short-term. Through field surveys in lakes of igapós of black (Negro River) and clearwaters (Tapajós River), interviews with traditional communities in Federal Protected Areas (PAs) and analyses of stable isotopes of C and N in teeth and bones of the Amazonian manatee, I obtained information on the dynamics of food availability, biology and diet in different ontogenetic classes. Physical and chemical parameters of the water of the studied lakes varied greatly during different phases of the flood pulse. Of all the 57 species of plants registered, only 13 occurred in the two ecosystems. In the Negro River, availability of herbaceous plants is scarce and limited to vines during the period of high waters, when they had the great cognitive salience in manatees' diet, according to interviewees. In Tapajós River there is a great richness of herbaceous plants throughout the flood pulse. The plants with highest cognitive salience were submerged with floating leaves and rooted submerged. Although there is no variation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signs between teeth, the differences among individuals indicate that the Amazonian manatee diet is related to the availability of resources, and their food preferences or nutritional needs. In addition, the species feeds on a variety of plants and algae, whose proportions vary according to the ontogenetic class and ecosystem. Food availability may be a determining factor in the trophic migration of the Amazonian manatee between igapós and várzeas, especially in the case of the lactating females of the lower Negro River. For this reason, the establishment of interinstitutional initiatives to guarantee the regional protection of the species, considering the possibility of movement between habitats, requires special attention. In order to reduce species threats and increase the effectiveness of PAs, greater involvement of local people and adjacent areas is needed in activities that increase their awareness of the conservation relevance of natural environments and endangered species like the Amazonia manatee.

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INTRODUÇÃO GERAL

A Bacia Amazônica é dominada e caracterizada por uma densa trama de rios de diversas ordens e tipologias ligadas à química, física e geomorfologia das áreas de captação (Sioli, 1968; Stallard e Edmond, 1983). Independentemente do tipo das águas, a principal força motriz que determina a dinâmica ecológica das planícies alagáveis amazônicas é o pulso de inundação (Junk *et al.*, 1989). Esse conceito tem como base as características hidrológicas do corpo de água, bacia de drenagem e planície inundável (Junk, 1997). Ele exerce uma forte influência sobre a vegetação e composição florística dos ecossistemas (Worbes *et al.*, 1992, Wittmann *et al.*, 2002) que, tomando como base a tipologia das águas (Sioli, 1984) e as florestas associadas (Prance, 1980), são divididos em várzeas e igapós.

As várzeas são periodicamente inundadas por rios de águas brancas, dominam a planície amazônica e ocorrem em formações do Quaternário recente, constituídas por sedimentos ricos em nutrientes e com elevada dinâmica hidrogeomorfológica (Prance, 1979). Possuem fertilidade relativamente elevada, que se reflete no crescimento rápido de árvores (Parolin e Ferreira, 1998), da vegetação herbácea (Piedade *et al.*, 1991; Junk e Piedade, 1993; 1997), na presença de elevados teores de minerais nos tecidos de espécies lenhosas (Klinge *et al.*, 1983) e na rápida reciclagem de nutrientes (Junk e Furch, 1991; Furch e Junk, 1992; 1997). Os ambientes de igapó, por sua vez, são áreas alagáveis por rios de águas pretas ou claras, que drenam formações mais antigas do período Terciário, apresentam níveis de nutrientes baixos a intermediários e pH ácido (Fittkau *et al.*, 1975; Sioli, 1984). Estes ambientes são colonizados por comunidades de plantas lenhosas com taxas de crescimento menores e densidades da madeira maiores que as da várzea (Schöngart *et al.*, 2010). Com relação às herbáceas aquáticas, nos igapós de águas pretas há predominância de espécies vegetais flutuantes ou emersas, sendo comuns as espécies *Nymphaea gardineriana* (Nymphaeaceae) e *Montrichardia arborescens* (Araceae) (Ferreira, 2000). Já nos igapós de águas claras, apesar da presença de espécies emergentes, é evidente a presença de plantas submersas, favorecidas pela elevada transparência da água, sendo frequente a colonização por *Eleocharis* spp. (Cyperaceae) e *Utricularia foliosa* [Lentibulariaceae; (Crema, 2012, observ. pess.)].

O peixe-boi-da-Amazônia, *Trichechus inunguis*, é considerado “vulnerável” na Lista Nacional de Espécies da Fauna Brasileira Ameaçada de Extinção (MMA, 2014) e na Lista Vermelha da União Internacional para Conservação da Natureza (IUCN, 2016). É o menor dos peixes-bois e essencialmente fluvial, pode atingir até três metros de comprimento e pesar em torno de 420 kg (Amaral *et al.*, 2010). Ocorre

desde a nascente de rios da Bacia Amazônica na Colômbia, Peru e Equador (Best, 1984) e, no Brasil, ocorre praticamente em todas as bacias dos principais rios da Amazônia, estando limitado por grandes corredeiras e cachoeiras (da Silva *et al.*, 2008). Sua distribuição está relacionada ao pulso de inundação (Junk e da Silva, 1997) e à disponibilidade de macrófitas aquáticas e outras plantas importantes em sua dieta (Best, 1984; Colares e Colares, 2002; Guterres-Pazin *et al.*, 2014). O peixe-boi-da-Amazônia não é territorialista e efetua migrações anuais das áreas de várzea ou igapós, onde permanece se alimentando no período de enchente e cheia, para lagos perenes e canais mais profundos de rios, ficando protegido durante a estação seca, quando se torna mais vulnerável à predação (Best, 1982; 1984; Arraut *et al.*, 2010).

O conhecimento tradicional é um corpo cumulativo de conhecimento e crenças, transmitido culturalmente através de gerações, sobre a relação dos seres vivos entre si e com o meio ambiente (Berkes, 1993; Berkes *et al.*, 2000). Ele permite entender processos ecossistêmicos em escalas temporais ou espaciais dificilmente observados na pesquisa científica convencional (Huntington, 1999), já que é originado por interações diárias com o meio ao longo de anos (Berkes *et al.*, 2000, Fabricius e Koch, 2004). Dessa forma, pode auxiliar na orientação de esforços para proteção de habitats (Huntington, 2000; Johannes e Yeeting, 2001; Rajamani, 2013;), no planejamento de ações de conservação em nível de espécie (Huntington 1999; Fraser *et al.*, 2006; Brook e McLachlan, 2008), como também na obtenção de informações sobre ecologia alimentar, distribuição e reprodução de espécies elusivas (Franzini *et al.*, 2013).

Além do conhecimento tradicional, outra ferramenta importante na identificação das fontes alimentares de um animal é a utilização de análise de isótopos estáveis (AIE). A premissa suportando o uso dessa técnica é de que as composições isotópicas dos consumidores refletem as dos produtores primários que, por sua vez, refletem as composições isotópicas do ecossistema onde habitam (Reich e Worthy 2006; Newsome *et al.*, 2010). Os dois elementos corriqueiramente utilizados em estudos de ecologia trófica são os isótopos de carbono (^{13}C e ^{12}C) e os de nitrogênio (^{15}N e ^{14}N) (Martinelli *et al.*, 2009). O uso de AIE apresenta vantagens sobre os métodos tradicionais de observação direta e de análise do conteúdo estomacal, pois possibilita o registro da alimentação assimilada e não somente a ingerida durante as observações e coletas, além de permitir a utilização de amostras a partir de coleções zoológicas [p. ex. ossos, dentes, pelo, (Walker e Macko, 1999)].

Estudos ecológicos na região Amazônica têm utilizado frequentemente AIE (Araujo-Lima *et al.*, 1986; Forsberg *et al.*, 1993; Villamarín *et al.*, 2017), já que com esta técnica é possível conhecer a composição isotópica do animal e de sua dieta. Funciona como ferramenta para traçar fluxos energéticos em teias alimentares, assim

como no estabelecimento de vias de ciclagem de nutrientes em ecossistemas terrestres e aquáticos (Vander Zanden e Rasmussen, 2001; Manetta e Benedito-Cecílio, 2003; Thomé-Souza, 2005; Caraballo, 2010; Marshall, 2010). Além disso, a utilização de AIE a partir de tecidos de peixes-bois pode também fornecer dados complementares sobre suas fontes alimentares e auxiliar na identificação de relações entre recursos e habitats (Ciotti *et al.*, 2014).

Os registros da espécie em vida livre são muito raros devido ao comportamento tímido e arisco do animal (Best, 1982), que pouco interage com outros indivíduos na mesma área (Pereira, 1944). Além disto, sua visualização é difícil devido à turbidez das águas onde ocorre (Junk e da Silva, 1997) o que, entretanto, não impede a intensa caça predatória (da Silva *et al.*, 2008). Desta forma, o estudo dos ecossistemas de ocorrência e a alimentação do peixe-boi amazônico podem ampliar o conhecimento sobre a ecologia trófica e uso sazonal de habitats desta importante e vulnerável espécie.

Diante do exposto, o presente estudo visa caracterizar áreas de igapós de águas pretas e claras da bacia amazônica, suas variações sazonais ao longo do pulso de inundação anual, disponibilidades de recursos vegetais em cada uma delas, bem como as dietas preponderantes para as populações de *T. inunguis* nesses dois ecossistemas contrastantes. Para uma melhor compreensão dos resultados encontrados, a tese foi dividida em três capítulos. Em síntese, o primeiro capítulo objetiva analisar igapós de águas pretas e claras relacionando as variáveis limnológicas e a estrutura e composição de plantas herbáceas aquáticas, bem como outros tipos de plantas disponíveis aos organismos aquáticos na interface entre ecossistemas aquáticos e terrestres. O segundo investiga o conhecimento tradicional sobre *T. inunguis*, de forma a caracterizar a biologia e ecologia alimentar da espécie e contribuir para a gestão das áreas protegidas onde ela habita. O terceiro capítulo avalia as mudanças na dieta do peixe-boi-da-Amazônia de igapós de águas pretas e claras, analisando como os isótopos de carbono e nitrogênio variam nos indivíduos, entre indivíduos, classes ontogenéticas e ecossistemas.

OBJETIVOS

Geral: Caracterizar sazonalmente igapós de águas pretas e claras e avaliar as dietas preponderantes do peixe-boi-da-Amazônia (*Trichechus inunguis*) presente nesses ecossistemas.

Específicos:

- Avaliar a variação da estrutura e composição de comunidade de plantas em igapós de águas pretas e claras em função do ciclo hidrológico e dos parâmetros físico químicos da água;
- Verificar se a estrutura e a composição da comunidade de plantas disponíveis para alimentação de herbívoros aquáticos diferem entre igapós de águas pretas e claras;
- Aumentar a compreensão sobre a biologia e ecologia alimentar do peixe-boi-da-Amazônia (*Trichechus inunguis*) por meio do conhecimento tradicional de comunidades localizadas em Unidades de Conservação Federais e em suas redondezas;
- Avaliar variações intra e interindividuais nos valores de $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ em dentes e ossos do peixe-boi-da-Amazônia de igapós de águas pretas e claras;
- Avaliar a composição da dieta do peixe-boi-da-Amazônia de igapós de águas pretas e claras e sua variação em relação com a idade.
- Contribuir com informações para a gestão do peixe-boi-da-Amazônia nas Unidades de Conservação Federais envolvidas no estudo, por meio do conhecimento tradicional e científico.

Capítulo 1

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Flood pulse and water quality drive aquatic and palustric plant
communities in Amazonian lakes.

Flood pulse and water quality drive aquatic and palustric plant communities in Amazonian lakes

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Abstract

Igapó forests are seasonally flooded by blackwater or clearwater rivers that drain ancient geological formations in the Amazon. Blackwater rivers contain lower levels of nutrients and higher acidity than their clearwater counterparts. Aquatic plants contribute to nutrient cycling and provide fauna with both habitat and food. We analysed physicochemical parameters of water as well as species richness, abundance and biomass of plants available for aquatic herbivores in 21 igapó forest lakes in Brazilian protected areas: Resex Tapajós Arapiuns and Flona do Tapajós at lower Tapajós River (clearwater), and Parna de Anavilhanas at lower Negro River (blackwater). We sampled emerged and submerged plots (0.25m²) in four phases of the flood pulse: rising water, flood, receding water and drought. We found a pronounced variation in physicochemical parameters of water between hydrological phases. In the Tapajós River, we found 48 species of herbaceous aquatic plants in 36 genera and 19 families, whilst in the Negro River we found 44 species of herbaceous plants, climbers and shrubs in 38 genera and 24 families. Of all 78 species, 13 occurred in both ecosystems. However, submerged plants were available in all phases in the Tapajós River. In the Negro River, the availability of herbaceous plants was scarce and restricted to rising water and flood, when climbers are abundant on flooded trees and shrubs. We concluded that igapó forests in the Tapajós River are richer in herbaceous plants than in Negro River, providing food for aquatic herbivores all year long.

Keywords: Tapajós River, Negro River, protected areas, aquatic plants, physicochemical water parameters.

Introduction

The existence of floodplains in the Amazon basin is related to periodic flood pulses (Junk et al. 1989) associated with water-level fluctuations in rivers. The amplitude of the water level can reach 14 m between peaks of flood (aquatic phase) and drought (terrestrial phase), with inundation periods of 50 to 279 days per year, depending on the location along the flooding gradient (Junk 1980; Junk et al., 2011). Plants need to alternate their life cycles in response to this dynamic, emphasizing the strong influence that flood pulses have upon the structure and floristic composition of these ecosystems (Worbes et al. 1992; Wittmann et al. 2002).

Within the typological classification for Amazonian waters described by Sioli (1950), and the associated forest formations (Prance 1980), igapó and várzea are highlighted. Igapó forests are seasonally flooded by blackwater or clearwater rivers that drain ancient geological formations, dating from older formations of the tertiary period, and exhibit from low to intermediate levels of nutrients and acidity (Fittkau et al. 1975; Sioli 1984; Ayres 1986). They are colonized by trees and shrubs with lower growth rate and higher wood density than those in várzeas (Schöngart et al. 2010). Herbaceous aquatic plants in blackwater igapós are predominantly floating or emergent species, as in the Nymphaeaceae, Marantaceae, Araceae and Pontederiaceae families (Lopes et al. 2014, Ferreira 2000; Junk 1983). In contrast, clearwater igapós favour not only emergent species - such as *Paspalum repens* and *Echinochloa polystachya* - but also submerged ones, due to greater water transparency (Junk 1983).

Flood pulses affect the distribution and diversity of wetland plants by influencing important factors such as: (1) color and nutritional condition of waters flooding the forest (Junk 1993; Ferreira et al. 2010; Piedade and Junk 2000; Piedade et al. 2010a), (2) duration of aquatic and terrestrial phases (Junk 1989; Junk and Piedade 1997; Neiff 2001; Piedade et al. 2010b; Wang et al. 2016), (3) physical stability of the substrate, related to sedimentation, erosion and water current action (Junk and Piedade 1997; Piedade and Junk 2000), (4) underwater irradiance (Rooney and Kalff 2000; Pezzato and Camargo 2003; Tavechio and Thomaz 2003; Caffrey et al. 2007), (5) processes of plant succession in each habitat (Junk and Piedade 1997, Piedade et al. 2010b), and (6) anthropogenic action (Junk and Piedade 1997; Piedade et al. 2010a). Interactions among these factors determine germination, establishment and reproduction of plant species in each level of the flood gradient (Junk and Piedade 1997, Piedade et al. 2010b; Ferreira et al. 2010).

Herbaceous aquatic plants constitute a key source of habitat for aquatic and terrestrial fauna, as they produce large amounts of biomass and occupy extensive areas (Piedade and Junk 2000; Petry et al. 2003). They support invertebrate and

vertebrate communities within their root systems; provide suitable substrate for egg-laying and refuge for zooplankton (Murphy 1988; Esteves 1998); juvenile fish can forage and shelter from predators within stems, roots and foliage (Piedade et al. 2005; Gopal 2016). The Amazonian manatee (*Trichechus inunguis*) and capybara (*Hydrochoerus hydrochaeris*) are the main species of mammal that consume aquatic herbaceous plants in the Amazon (Best 1984; Junk 1986; Junk and da Silva 1997; Piedade et al. 2010a).

Aquatic herbaceous plants suffer substantial herbivory, which often affects their relative abundance and productivity (Lodge 1991; Newman 1991), and potentially changes the structure and functioning of aquatic systems (Bakker 2016). However, the foraging behaviour of generalist herbivores depends on various characteristics of plants (Boiché 2011). Nutritious value and defensive compounds are recognized as particularly important in determining herbivore performance, which may disguise the effective relationship between herbivore and productivity (Bakker 2016).

Whichever type of water, igapó lakes provide essential habitats in floodplains as many lakes remain flooded during low water periods (Fernandes et al. 2009), and offer abundant sources of food (Junk 1980). But despite food availability, these ecosystems suffer dramatic ecological changes caused by the alternation between aquatic and terrestrial phases that requires many organisms to develop morphologic, physiologic or ethologic adaptations in order to survive (Junk 1980).

We explore the relationships between limnological variables and the structure and composition of communities of aquatic herbaceous plants (and other types of plants available to aquatic organisms in the interface between aquatic and terrestrial ecosystems) in blackwater and clearwater igapó lakes throughout phases of the flood pulse. Our questions are: (1) How the structure and composition of aquatic and palustric plants communities vary with the flood pulse in black and clearwater igapós? (2) Plants available as food source for herbivores differ between clearwater and blackwater igapós?

Methods

Study site

Our study site comprised igapó lakes inside federal Conservation Units (*Unidades de Conservação*, henceforth UCs); protected areas that integrate the Brazilian National System of Conservation Units (*Sistema Nacional de Unidades de Conservação*) (BRASIL, 2000). We included 12 blackwater igapó lakes located at Parque Nacional de Anavilhanas (PARNA de Anavilhanas) in the lower Negro River, and nine clearwater igapó lakes at Floresta Nacional do Tapajós (FLONA do Tapajós)

and Reserva Extrativista Tapajós Arapiuns (RESEX Tapajós Arapiuns) in the lower Tapajós River (Fig. 1).

PARNA de Anavilhanas is located in the state of Amazonas (02° 03' - 03°02'S and 60° 22' - 61°12'W), Novo Airão municipality, approximately 60 km North of the city of Manaus, and covers an area of 647,610 ha (ICMBio 2017). In Novo Airão, lower Negro River, the climate is similar to the one in Manaus: tropical rainforest (Af of the Köppen classification), with annual average temperature between 26 and 27 °C, average relative air humidity around 90% and rainfall varying between 1,750 and 2,500 mm (Irion et al. 1997; IBAMA 1999). FLONA do Tapajós is located in the state of Pará (02° 45' and 4°10'S - 54°45' and 55°30'W), and includes the municipalities of Aveiro, Belterra, Rurópolis and Placas, with an estimated size of 600,000 ha (IBAMA 2004). RESEX Tapajós-Arapiuns, also in the state of Pará (02°20' and 03°40'S - 55°00' and 56°00'W), includes the municipalities of Santarém and Aveiro, comprising 647.610 ha (ICMBio 2014). These two UCs are separated by the Tapajós River, with FLONA do Tapajós on the right bank and RESEX Tapajós Arapiuns on the left bank. In the region of Santarém, lower Tapajós River, the climate is Am (in the Köppen classification), with average annual temperature between 26 and 27 °C, relative air humidity around 86%, annual precipitation varying from 1,287 to 2,538 mm, and markedly seasonal (IBAMA 2004).

The Negro River flows through approximately 1,700 km from its source at the Eastern plains of Colombia until its mouth near Manaus, with an average discharge of 28,000 m³.s⁻¹, an average speed of 1m.s⁻¹, and connecting with the riverine system in the North, West, East and Northeast of the Amazon Basin. It is the principal representative of Amazonian blackwater rivers (conductivity from 9.3 to 9.9 µS.cm⁻¹), poor in nutrients and electrolytes (Furch and Junk 1997) as it drains through highly weathered soils (Goulding *et al.*, 1988). The water colour is reddish brown and pH (4.8 – 5.1) is highly acidic, both due to high levels of dissolved humic and fulvic substances (Furch and Junk 1997). The Tapajós River is one of the greatest tributaries of the Amazon River, starting in the state of Mato Grosso and flowing through the state of Pará, in the lower Amazonas River. It has an approximate length of 2,000 km, average discharge of around 12,440 m³.s⁻¹, and average speed of 0.40 m.s⁻¹. It is a typical representative of clearwater rivers, with characteristics lying in between those of the Amazon and Negro rivers (pH 6.5 ± 0.4; conductivity= 14.4 ± 13.1 µS.cm⁻¹; total dissolved solids= 7.7 ± 5.6 mg.L⁻¹) (Duncan and Fernandes 2010).

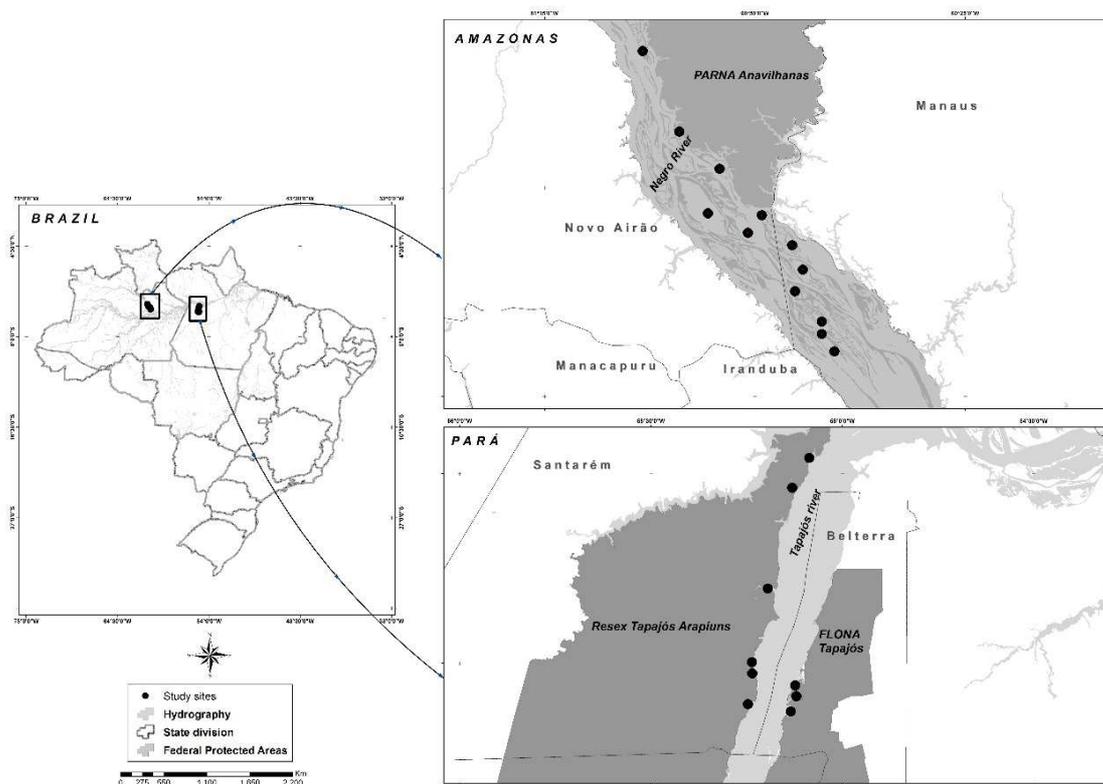


Fig. 1 Sample points in the RESEX Tapajós-Arapiuns and FLONA do Tapajós, in the lower Tapajós River (clearwater), and in the PARNA de Anavilhanas, lower Negro River (blackwater).

Field survey

We carried out sampling throughout 2012 in the lower Negro River, and 2013 as well as 2014 in the lower Tapajós River in order to include all phases of the flood pulse: rising water (February- March), flood (May-July), receding water (August-October) and drought (November-January). This temporal difference occurred due to the logistic impossibility of carrying out simultaneous samplings because the two ecosystems are about 700 km far from each other. However, this was of minor influence in the flood regime of the study period since 2012, 2013 and 2014 were influenced by a weak “La Niña”, resulting in little interannual variability (NOOA, 2017). We measured the following limnological variables in three to four sampling points for each lake (including source, middle course and mouth): temperature, dissolved oxygen, electrical conductivity, pH, turbidity, dissolved solids, water transparency and depth. We collected samples of superficial water at the same sampling points in order to quantify Total Nitrogen ($\text{mg N}\cdot\text{L}^{-1}$), Nitrite ($\text{mg NO}_2\cdot\text{L}^{-1}$), and Nitrate ($\text{mg NO}_3\cdot\text{L}^{-1}$) based on Mackereth et al. (1978), Ammonium ($\text{mg NH}_4^+\cdot\text{L}^{-1}$) based on Koroleff (1976), Total Phosphorus ($\text{mg P}\cdot\text{L}^{-1}$) and Phosphate ($\text{mg PO}_4^{3-}\cdot\text{L}^{-1}$) based on Golterman et al. (1978).

Abundance (% cover) of plants available to aquatic herbivores, we measured in line transects with quadrats (0.50 x 0.50 m) distributed on the vegetated banks at the margins of lakes (always in the interface between aquatic and terrestrial ecosystems). We performed 15 m transects and a total of 30 quadrats for species in the interface between water and air, whilst for subaquatic species we used 5 m transects and a total of 10 quadrats. We collected subaquatic species by scuba diving. In addition to quadrats, we visually inspected and collected herbaceous plants located on shores as well as climbers and shrubs for identification purposes. The sampling of these life forms at a metric unit was not possible owing to methodological limitations. In order to calculate biomass of species in the Tapajós River, we used the months of August and November 2013 (receding water and drought, respectively) and April 2014 (flood) as reference. In the laboratory, botanical material collected from quadrats on lakes was washed in order to remove sediments, periphytic algae and deposited particles. We then separated samples per site, and oven-dried them at 60 °C for 72 hours until reaching a constant weight on digital scale, and determined biomass per area unit for each lake and for each phase of the flood pulse. The collected and identified samples of each species were deposited in the herbaria at the Federal University of Amazonas and the National Institute of Amazonian Research (Manaus, Brazil). We grouped species based on life-form (adapted from Junk 1986), that refers to different plant growth habits, and classified them as: terrestrial, emergent, amphibious, free floating, free floating submerged, rooted submerged, rooted submerged with floating leaves, climbers, and shrubs. We followed the binomial nomenclature for species based on the standard classification of Forzza et al (2016).

Statistical analysis

We performed analyses of variance to examine differences in limnological data across the four phases of the flood pulse (rising water, flood, receding water and drought), in each ecosystem. We used ANOVA when its requirements were met, and Kruskal-Wallis for non-parametric data, as well as post-hoc Tukey test. We used a principal component analysis (PCA) to explore differences in limnological data among phases of the flood pulse, followed by an ANOVA on the PCA scores as well as Tukey test.

In order to evaluate differences in general species composition between the two ecosystems, in species composition in each ecosystem separately, and in species richness of different life-forms through phases of the flood, we performed non-metric multidimensional scaling (NMDS). We used Bray-Curtis index for generalized dissimilarity matrices, followed by multivariate permutation test (999 permutations)

(Kruskal 1964). In addition, we explored on the same dataset the multivariate homogeneity of group dispersions (Anderson et al. 2006), obtained using the function `betadisper` on the `Vegan` package (Oksanen et al. 2011). We did this by calculating the similarity of species composition and richness of different life-forms between phases of the flood pulse, also based on Bray-Curtis index followed by permutation test (999 permutations). Differences between groups corresponded to differences between distances of individual points (phases) to the centroid of its own group, on the tridimensional space calculated on a Principal Coordinate Analysis (PCoA) (Anderson et al. 2001). We used two-way ANOVA followed by Tukey test (Zar 1999) to investigate potential differences between phases of the flood pulse for abundance and biomass of different life-forms. We performed all analyses using the `Vegan` package in R environment (R Development Core Team, 2016).

Results

Physicochemical parameters of water in sampled lakes

Lakes in the lower Tapajós River showed 1.6 times higher electrical conductivity and 3.8 times greater water transparency than in the lower Negro River in all phases of the flood pulse (see Table S1 in Electronic Supplement). In addition, lakes in both river systems were strongly acidic, except during rising water period in the Tapajós River, when pH increased to slightly acidic ($H=18.56$, $P<0.001$). During rising water and flood, water depth in lakes in the Negro River was nearly three times greater than it was in lakes in the Tapajós River. Some nutrient concentrations in lakes of the Tapajós River were the lowest regardless of phase; sometimes lower than the minimum detectable by our equipment (see Table S1 in Electronic Supplement). On the other hand, nutrient concentrations in lakes of the Negro River varied between phases, especially total nitrogen (for which the average remained greater than 0.65 mg.L^{-1} during all phases; see Table S1 in Electronic Supplement).

Axes 1 and 2 in the PCA analysis explained 33.14 and 21.06% of variation in physicochemical parameters of water in the Tapajós River, respectively, and in the Negro River 44.4 and 12.0%. In addition, significant changes in these parameters indicated differences between phases of the flood pulse in axes 1 and 2 ($F=31.78$, $P<0.001$ and $F=3.88$, $P<0.05$, respectively) of the PCA in the Tapajós River (Fig. 2a) and axes 1 and 2 ($F=127.87$, $P<0.001$ and $F=6.24$, $P<0.001$) of the PCA in the Negro River (Fig. 2b). When looking at differences between phases of the flood pulse, drought in the Tapajós River was different from all others in axis 1 of the PCA, whilst receding water was different from rising water in axis 2. In the Negro River, drought and

receding water were different from the remaining phases in axis 1 of the PCA, whilst in axis 2 only receding water was different. In both ecosystems (i.e. clearwater and blackwater), physicochemical parameters remained more constant during receding water and drought - when there is a lower volume of water - than they did during the other phases.

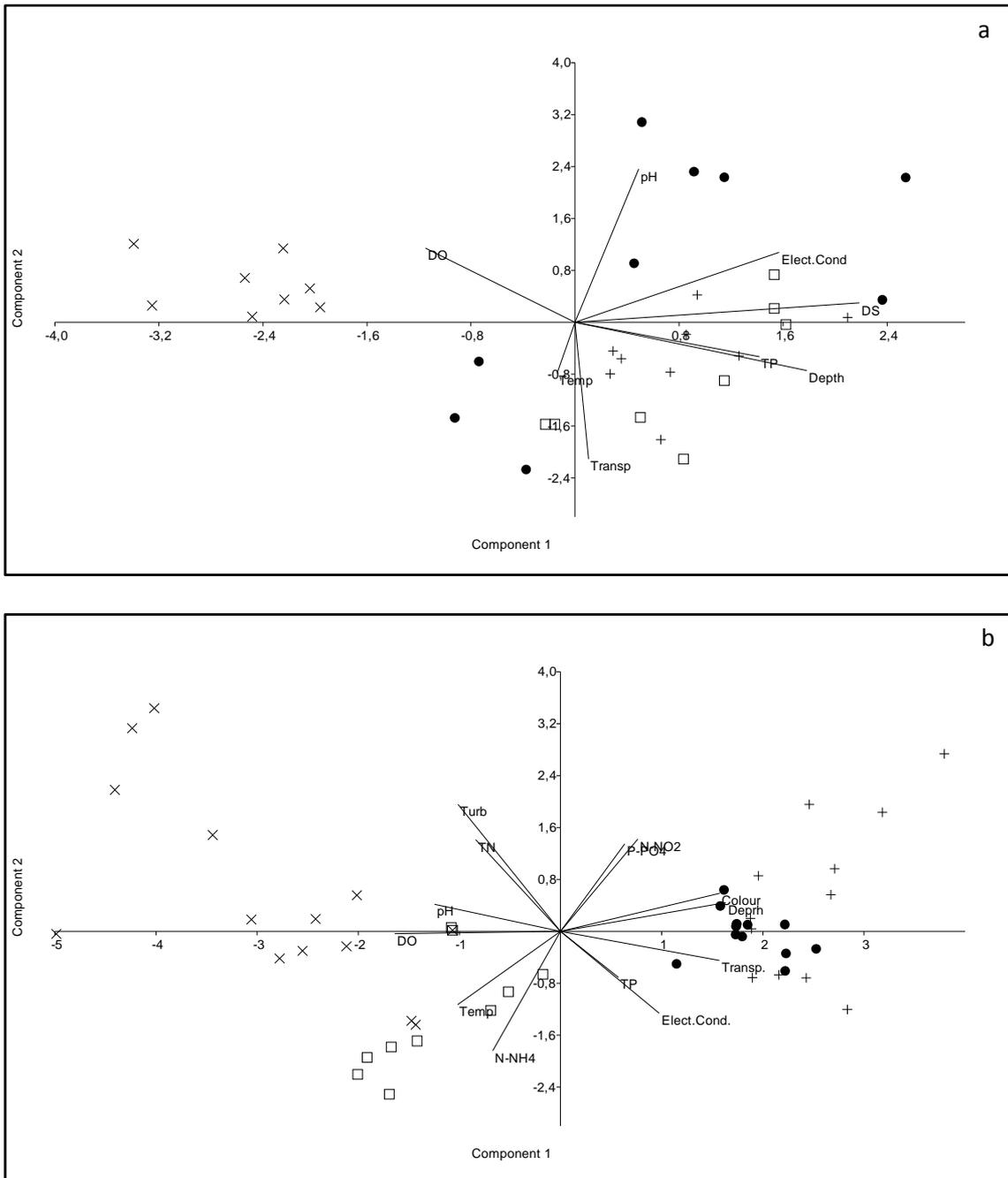


Fig. 2 Principal component analysis of physicochemical parameters in clearwater lakes of the lower Tapajós River basin(a) and in blackwater lakes of the lower Negro River (b). Rising water (●), flood (+), receding water (□) and drought (x).

Richness, composition and abundance of plants

Richness in clearwater and blackwater ecosystems

In lakes in the lower Tapajós River, we found 48 species of herbaceous aquatic plants in 36 genera and in 20 families (Table 1). The most numerous forms of life were amphibious (29.2%) and rooted submerged (16.7%). The most represented genus was *Eleocharis* with six species. Families with most species were Poaceae (18.8%) and Cyperaceae (22.9%), corresponding to 41.7% of all plants. In lakes in the lower Negro River, we found 43 species of herbaceous plants, climbers, and shrubs in 38 genera and 23 families (Table 1). The main life-forms were climbers (25.6%), herbaceous terrestrial (18.6%), and shrubs (18.6%). Similarly to what we found in the Tapajós River, the most represented families were Poaceae (14.0%) and Cyperaceae (11.6%), which corresponded to 25.6% of all plants.

Of the 78 species, only 13 occurred in both ecosystems: emergent – *Oryza glumaepatula* and *Echinochloa polystachya*; amphibious – *Caperonia castaneifolia*, *Hymenacne amplexicaulis* and *Paspalum repens*; free floating – *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia auriculata*; rooted submerged and terrestrial – *Eleocharis minima*; free floating submerged – *Utricularia foliosa*; and climbers – *Cissus erosa*, *Tassadia berteriana* and *Vigna lasiocarpa* (Table 1). Although some species were shared, the non-metric multidimensional scaling (NMDS) separated species composition for both ecosystems (Permanova, $F=24.00$ $P=0.001$) (Fig. 3a). In addition, permutation tests for homogeneity and multivariate dispersion showed differences, albeit small (Permdisp2, $F=3.42$, $P=0.067$), that indicated variation in species composition between the two ecosystems (Fig. 3b).

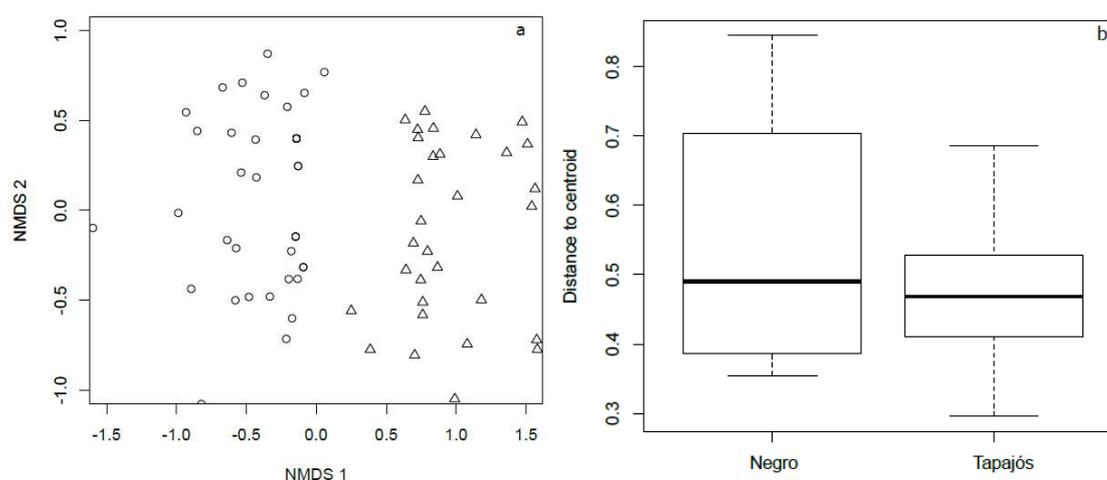


Fig. 3 (a) Non-metric multidimensional scaling of floristic composition in the lower Tapajós River (Δ) (clearwater) and lower Negro River (\circ) (blackwater) (stress=0.15). (b) Principal Coordinate Analysis average distances to centroid of species in the lower Tapajós River and lower Negro River.

Table 1 Floristic composition and life-forms in lakes in the lower Tapajós River (T) and lower Negro River (N), where: eme (emergent), amp (amphibious), fle (free floating), rs (rooted submerged), rsff (rooted submerged with floating leaves), ffs (free floating submerged), terr (terrestrial), vin (vine), tre (shrubs and trees).

Family	Specie	life-form	River
Apocynaceae	<i>Mesechites trifida</i> (Jacq.) Müll.Arg.	vin	N
	<i>Tassadia berteriana</i> (Spreng.) W.D.Stevens	vin	N,T
Araceae	<i>Montrichardia linifera</i> (Arruda) Schott	emer	T
	<i>Lemna minuta</i> Kunth	ff	N
	<i>Montrichardia arborescens</i> (L.) Schott	emer	T
	<i>Pistia stratiotes</i> L.	ff	N,T
Asteraceae	<i>Mikania micrantha</i> Kunth	vin	N
	<i>Cabomba aquatica</i> Aubl.	rs	T
	<i>Cabomba furcata</i> Schult. & Schult.f.	rs	T
Convolvulaceae	<i>Ipomoea</i> sp. L.	vin	N
Costaceae	<i>Costus arabicus</i> L.	emer	N
Curcubitaceae	<i>Cayaponia cruegeri</i> (Naudin) Cogn.	vin	N
	<i>Pteropepon deltoideus</i> Cogn.	vin	N
Cyperaceae	Cyperaceae sp.1	terr	N
	Cyperaceae sp.2	terr	N
	Cyperaceae sp.3	terr	T
	<i>Cyperus haspan</i> L.	amp	T
	<i>Cyperus laetus</i> J.Presl & C.Presl	terr	N
	<i>Cyperus odoratus</i> L.	terr	N
	<i>Eleocharis confervoides</i> (Poir.) Steud.	rs	T
	<i>Eleocharis fluctuans</i> (L.T. Eiten) E.H. Roalson & C.E. Hinchliff	rs	T
	<i>Eleocharis interstincta</i> (Vahl) Roem. & Schult.	emer	T
	<i>Eleocharis minima</i> Kunth	terr, sub	N,T
	<i>Eleocharis mutata</i> (L.) Roem. & Schult.	amp	T
	<i>Eleocharis plicarhachis</i> (Griseb.) Svenson	amp	T
	<i>Oxycaryum cubense</i> (Poepp. & Kunth) Lye	amp	T
	<i>Rhynchospora holoschoenoides</i> (Rich.) Herter	amp	T
	<i>Scleria gaertneri</i> Raddi	emer	T
Eriocaulaceae	<i>Eriocaulon guyanense</i> Körn.	terr	N
Euphorbiaceae	<i>Alchornea discolor</i> Poepp.	tre	N
	<i>Caperonia castaneifolia</i> (L.) A.St.-Hil.	amp	N,T
Fabaceae	<i>Aeschynomene</i> sp. L.	amp	N
	<i>Clitoria glycinoides</i> DC.	vin	N
	<i>Cymbosema roseum</i> Benth.	vin	T
	<i>Sesbania exasperata</i> Kunth	tre	T
	<i>Vigna lasiocarpa</i> (Mart.ex Benth.) Verdc	vin	N,T
Hydrocaritaceae	<i>Limnobium laevigatum</i> (Humb. & Bonpl. ex Willd.) Heine	ff	N
	<i>Apalanthe granatensis</i> (Bonpl.) Planch	rs	T
Lentibulariaceae	<i>Utricularia breviscapa</i> C.Wright ex Griseb.	ffs	T
	<i>Utricularia foliosa</i> L.	ffs	N,T
Malpighiaceae	<i>Heteropterys orinocensis</i> (Kunth) A.Juss.	tre	N
Malvaceae	<i>Pavonia fruticosa</i> (Mill.) Fawc. & Rendle	tre	N
Mayacaceae	<i>Mayaca fluviatilis</i> Aubl.	rs	T
	<i>Mayaca longipes</i> Gand.	rs	T
Menyanthaceae	<i>Nymphoides indica</i> (L.) Kuntze	rsff	T
Nymphaeaceae	<i>Nymphaea potamophila</i> Wiersema	rsff	T
	<i>Nymphaea rudgeana</i> G.Mey.	rsff	T
Ochinaceae	<i>Ouratea discophora</i> Ducke	tre	N
Onagraceae	<i>Ludwigia octovalvis</i> (Jacq.) P.H.Raven	amp	N
	<i>Ludwigia sedoides</i> (Humb. & Bonpl.) H.Hara	rsff	T
Passifloraceae	<i>Passiflora misera</i> Kunth	vin	N
	<i>Passiflora pohlii</i> Mast.	vin	N
Plantaginaceae	<i>Bacopa reflexa</i> (Benth.) Edwall	rs	T
Poaceae	<i>Echinochloa</i> sp. P.Beauv.	terr	N
	<i>Echinochloa polystachya</i> (Kunth) Hitchc.	emer	N,T
	<i>Fimbristylis dichotoma</i> (L.) Vah	terr	N
	<i>Hymenachne amplexicaulis</i> (Rudge) Nees	amp	N,T
	<i>Leersia hexandra</i> Sw.	amp	T
	<i>Louisiella elephantipes</i> (Nees ex Trin.) Zuloaga	amp	T
	<i>Luziola subintegra</i> Swallen	amp	T

	<i>Oryza glumaepatula</i> Steud	emer	N,T
	<i>Panicum dichotomiflorum</i> Michx.	amp	T
	<i>Paspalum repens</i> P.J.Bergius	emer	N,T
	<i>Trachypogon macroglossus</i> Trin	amp	T
Polygonaceae	<i>Polygonum acuminatum</i> Kunth	amp	T
	<i>Ruprechtia cf. tangarana</i> Standl.	tre	N
	<i>Symmeria paniculata</i> Benth.	tre	N
Pontederiaceae	<i>Eichhornia azurea</i> (Sw.) Kunth	rsff	T
	<i>Eichhornia crassipes</i> (Mart.) Solms	ff	N,T
	<i>Pontederia rotundifolia</i> L.f.	ff	T
Pteridaceae	<i>Ceratopteris pteridoides</i> (Hook.) Hieron.	ff	N
Salvinaceae	<i>Azolla filiculoides</i> Lam.	ff	T
	<i>Salvinia auriculata</i> Aubl.	ff	N,T
	<i>Salvinia</i> sp.	ff	N
	<i>Schwenckia grandiflora</i> Benth.	tre	N
	<i>Solanum subinerme</i> Jacq.	tre	N
Vitaceae	<i>Cissus erosa</i> Rich.	vin	N,T
Xyridaceae	<i>Xyris Gronov. ex L.</i>	amp	T

Differences between phases of the flood pulse

On the analysis of community structure associated with the flood pulse in each ecosystem, we found no differences between phases in the Tapajós River based on the ordinated composition in the axes of the NMDS, or on the multivariate dispersion of species (Permanova $F=1.25$, $P=0.243$ and Permdispe2 $F= 0.038$, $P=0.989$, respectively), which indicates homogeneity in species composition throughout the flood pulse (Figs. 4a and 4b). In contrast, species composition ordinated in the axes of the NMDS was grouped per phase of the flood pulse in the Negro River (Permanova $F=9.14$, $P=0.001$), and showed heterogeneity in the multivariate dispersion (Permdispe2 $F=4.42$, $P=0.005$), which indicates that species composition during drought is more homogenous than it is during flood (Tukey $P=0.004$) (Figs. 4c and 4d).

In both ecosystems, species richness of different life-forms changed between phases of the flood pulse (Permanova $F=2.15$, $P=0.038$ in the Tapajós River and $F=16.11$, $P=0.001$ in the Negro River), as indicated by the NMDS groups (Figs. 5a and 5c). In the Tapajós River, rising water showed greatest richness of life-forms with predominantly emergent and rooted submerged with floating leaves ($n=13$ and $n=06$, respectively), whilst rooted submerged species were predominant during drought ($n=08$). In the Negro River, flood showed greatest richness of all life-forms, predominantly climbers ($n=11$), shrubs ($n=6$) and amphibious ($n=4$). In contrast, during drought we found less species richness within life-forms, with herbaceous terrestrial the richest ($n=4$). However, we found no variation based on multivariate dispersion analysis of life-forms in the Tapajós River (Permanova $F=0.004$, $P=0.99$) or in the Negro River (Permdispe2 $F=1.87$, $P=0.148$). Therefore, although richness of life-forms varied between phases of the flood pulse, this variation was similar between phases (Figs. 5b and 5d).

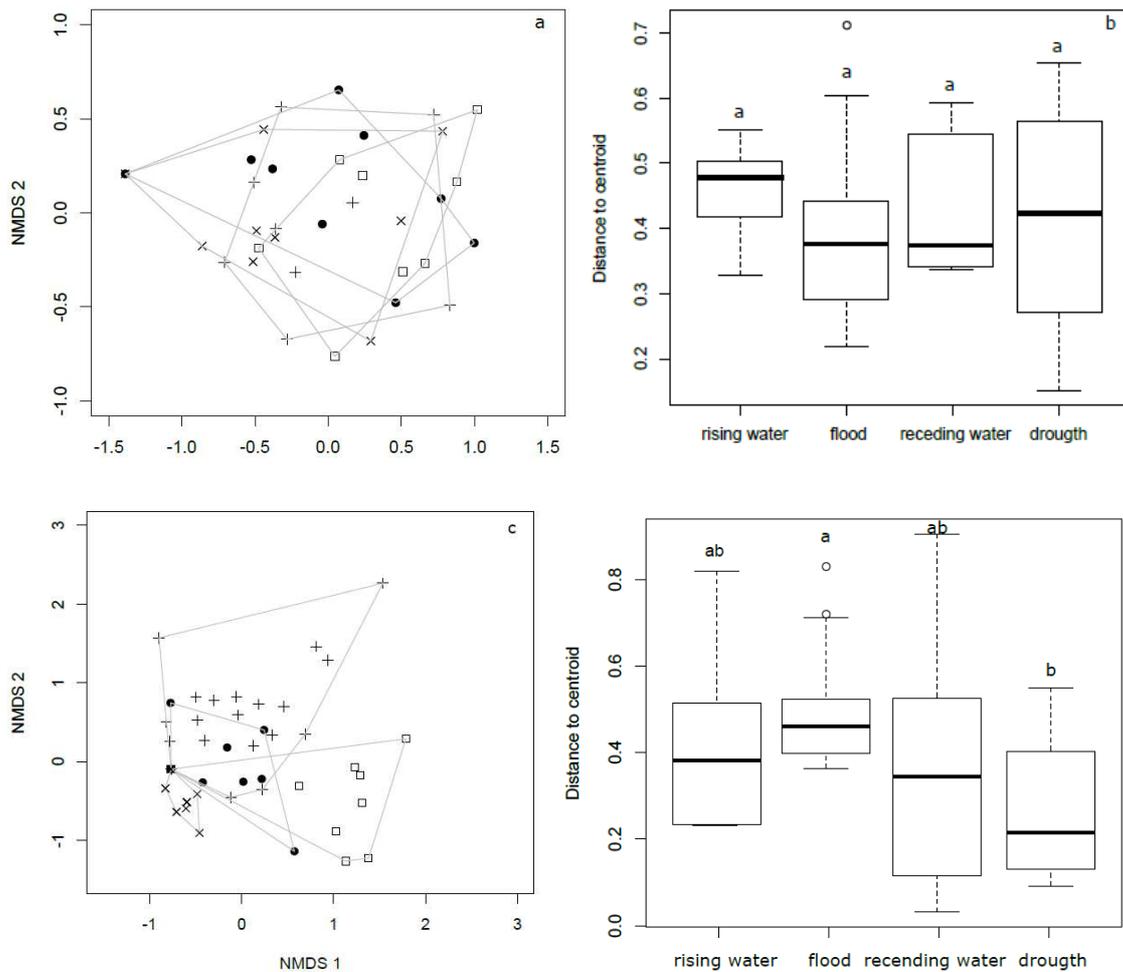


Fig. 4 Non-metric multidimensional scaling of floristic composition throughout the flood pulse in the lower Tapajós River (a) (clearwater) (stress=0.16) and lower Negro River (c) (blackwater). (stress=0.17). Principal Coordinate Analysis average distances to centroid of species in the lower Tapajós River (b) and lower Negro River (d) throughout the flood pulse. Distances that do not share the same letter are statistically different from one another ($P < 0.05$). Rising water (●), flood (+), receding water (□) and drought (x).

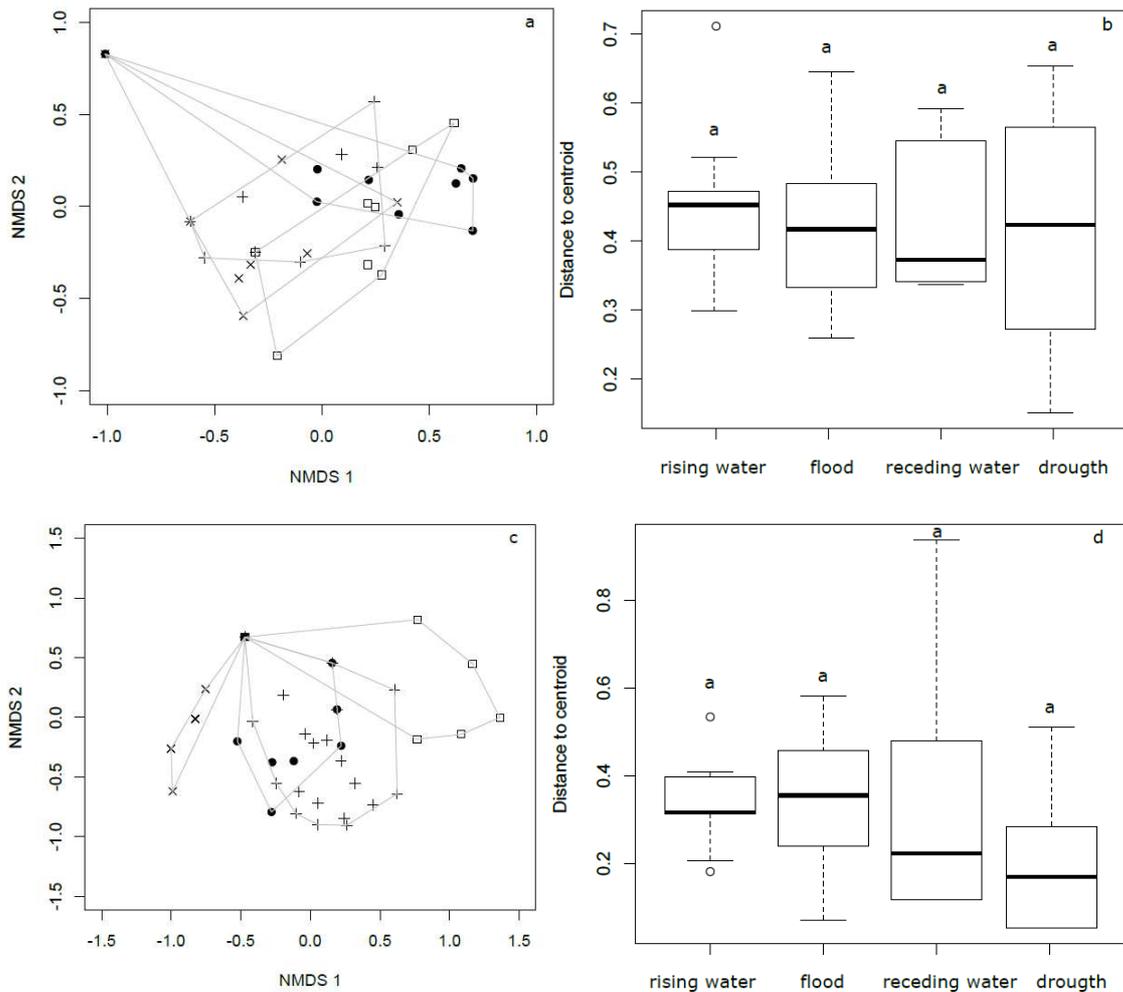


Fig. 5 Non-metric multidimensional scaling of richness of life-forms throughout the flood pulse in the lower Tapajós River (a) (clearwater) (stress = 0.11) and lower Negro River (c) (blackwater). (stress = 0.11). Principal Coordinate Analysis average distances to centroid of richness of life-forms in the lower Tapajós River (b) and lower Negro River (d) throughout the floodpulse. Distances that do not share the same letter are statistically different from one another ($P < 0.05$). Rising water (●), flood (+), receding water (□) and drought (x).

Abundance of life-forms

Despite differences in species composition within life-forms in the Tapajós River (Anova $F=4.91$, $P<0.001$), abundance of each life-form did not vary between phases of the flood pulse. Rooted submerged species remained the most abundant in all phases (Fig. 6a). In the Negro River, the abundance of each life-form differed significantly between phases (Anova $F=98.18$, $P<0.001$) (Fig. 6b). Although we did not measure abundance of climbers, which were highly represented during flood, we observed that free floating emerged species were abundant near climbers during this phase. Emergent species were the most abundant life-form during both drought (when environmental conditions favour seedling establishment) and rising water (when

seedlings grow) (Fig. 6b). During receding water, abundance of all life-forms was null, as there was no vegetation in any of our sampling points.

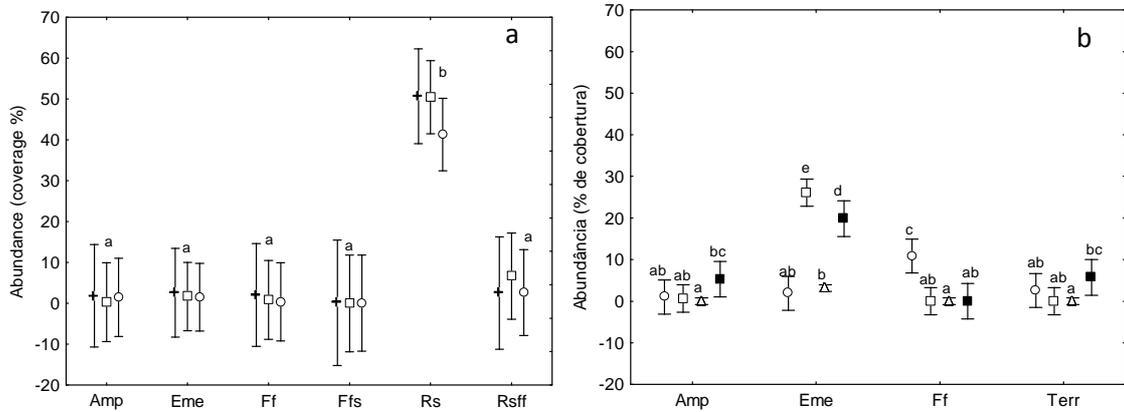


Fig. 6 Abundance of different life-forms throughout the flood pulse in the lower Tapajós River (a) (clearwater) and lower Negro River (b) (blackwater). Amp: amphibious, Eme: emergent, Ff: free floating, Ffs: free floating submerged, Rs: rooted submerged, Rsff: rooted submerged with floating leaves, Terr: terrestrial. Means that do not share the same letter are statistically different from one another ($P < 0.05$). Receding water (+), drought (\square), rising water (Δ), flood (\circ) and drought 2 (\bullet)

Biomass of plants in the Tapajós River

We only observed significant changes in biomass between phases of the flood pulse (Anova $F=36.64$, $P<0.001$) for emergent species, with greater biomass during rising water and flood. However, rooted submerged species showed greatest biomass in all phases (Fig. 7).

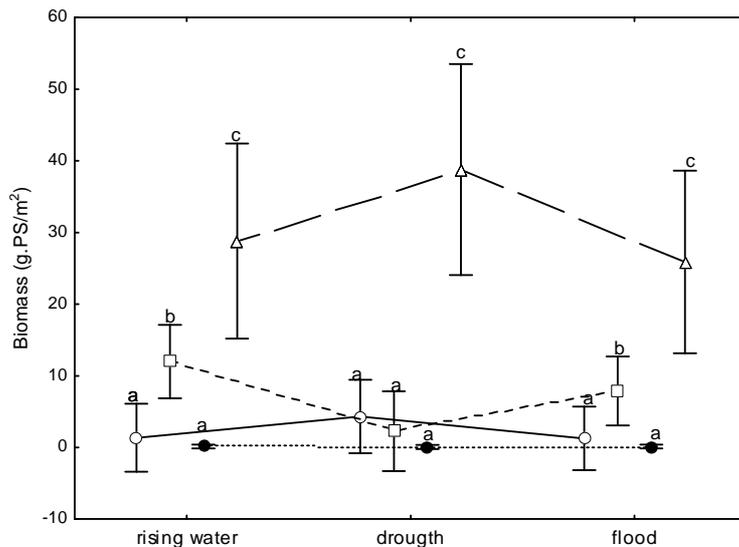


Fig. 7 Biomass (g.PS.m^{-2}) of main life-forms of aquatic plants during receding water, drought and flood in the lower Tapajós River (clearwater). Means that do not share the same letter are statistically different from one another ($P < 0.05$). Emergent (\square), free floating (\bullet), rooted submerged with floating leaves (\circ) and rooted submerged (Δ).

Discussion

Influence of physicochemical parameters of water on community structure

Despite physicochemical differences in the water, igapós in the lower Tapajós River (clearwater) and lower Negro River (blackwater) showed similar number of species, genera and families. However, our total species richness of 78 is far below the 388 reported for várzea (Junk and Piedade 1993) in a survey near Manaus. This contrast may be related to environmental differences, as igapós have lower pH and concentration of nutrients in water and soil (Sioli 1950; Junk et al. 1989, Junk and Piedade 1993), which limits species adaptability (Piedade et al. 2010b). However, in this study and others the most common families in igapó and várzea are the same: Cyperaceae and Poaceae (Mormul et al. 2013, Lopes et al. 2014, Ferreira et al. 2015, Junk and Piedade 1993). Many aquatic herbaceous species in these families (França et al. 2003; Pivari et al. 2008), colonize a variety of aquatic environments and ecotones (Cook 1996; Leite et al. 2009). Their success of colonization relates to the presence of some structures such as berhizomes, tubers, and stolons that facilitate vegetative propagation (Pott et al. 1989; Bove et al. 2003; França et al. 2003; Matias et al. 2003).

On the other hand, clearwater and blackwater igapós in our study shared only thirteen species (16%), revealing a great floristic distinction between the two ecosystems. Water parameters that may influence distribution and diversity of vegetation in floodplains include transparency and quality of waters (Sioli 1950, 1957, 1985; Furch 1984; Junk and Furch 1985). The most noticeable difference between them was the greater transparency in the Tapajós River (from 1.00 to 3.75m in our study), which enables colonization of rooted submerged herbaceous species (Junk and Howard-Williams 1984). Indeed, we found eight species of this life-form in the Tapajós River (17% of species in this ecosystem), and none in the Negro River. Dissolved minerals in clearwater range from low to intermediate, whilst in blackwater it is low alongside pH. Also, blackwaters have higher concentration of dissolved humic substances, which darken the water (Sioli 1957; Santos et al. 1985; Santos and Ribeiro 1988; Konhauser et al. 1994; Dupré et al. 1996; Gaillardet et al. 1997) and limits underwater irradiance (Junk and Furch 1985). These conditions favour few species (Ferreira et al. 2010).

Environmental gradients structure herbaceous plant assemblages and their respective trophic chains (Mormul et al. 2013). As expected, we found different temporal changes in the two igapós. In the selected lakes of the Tapajós River, limnological variables differ between phases of the flood pulse, with most contrast during receding water and drought; floodplains are the most distinctive when water levels are low (Thomaz et al. 2007). However, these changes did not affect species

composition throughout the flood pulse, as composition is commonly not related to all physicochemical parameters of water in lakes (Jones et al. 2003; Reid and Quinn 2004; Pätzig et al. 2012), and vegetation responses to these parameters are not necessarily linear (Rolon and Maltchik 2006). In these phases, changes in limnological variables such as lowering of electrical conductivity, dissolved solids and water transparency, as well as increase in oxygen concentration may not reach restricting levels such that could affect community stability. In the Tapajós River, water transparency - which determines colonization of herbaceous species - varies throughout the flood pulse but the depth of underwater irradiance (Crema, unpublished data) is great and constant enough for submerged species to grow. In the Negro River, physicochemical parameters of water in lakes changed considerably during drought and - contrarily to what we observed in the Tapajós River – there were fewer species and composition was more homogenous in this phase. The establishment of plant species in the gradient may follow their needs and adaptations to specific habitat conditions (Piedade et al. 2010b). During drought, lakes are very shallow and increased turbidity hinders light penetration, thus few species which complete the annual life cycle on exposed soil are able to colonize them (Junk 1983; Piedade et al. 2005, Ferreira et al. 2010). Therefore, local processes in the habitat scale are more important in determining the structure of biological communities in this phase (Rodriguez and Lewis Jr. 1997). In this way, the colonization of herbaceous plants in the Tapajós has proved to be more homogeneous than in the Negro River, where changes among phases of the flood pulse were drastic.

Richness of life-forms, however, showed a different response to phases of the flood pulse, as changes in the availability of habitats and nutrients influence the spatial distribution and percentage cover of plant populations (Furck and Junk 1997; Thomaz et al. 2007, Piedade et al. 2010b; Mormul et al. 2013). According to Junk (1983), there is no significant growth of herbaceous plants in the Negro River and the majority of aquatic or semi-aquatic plants are absent due to low concentration of electrolytes and pH (Junk and Piedade 1997). However, the region of PARNA de Anavilhanas is influenced by the Branco River - an affluent of the Negro River that supplies sediments, which increase fertility, and also floristic elements (Junk et al. 2015). This results in increased nutrient concentration and pH, favouring colonization for some species of aquatic plants, such as the amphibious *Paspalum repens* (Junk 1983; Junk and Howard-Williams 1984; Junk and Piedade 1997; Junk et al. 2015). Nevertheless, the most frequent life-forms of aquatic herbaceous plants during flood are climbers over flooded trees and shrubs, and emergent and floating species to a lesser extent. During drought, the decrease in water column favours only emergent species (Mormul et al.

2013). During this phase, humid areas with lower elevation are mostly covered by *O. glumaepatula*, resistant to acid pH (Piedade et al. 2010a), and annual amphibious or terrestrial species. Exposed seeds during drought germinate as soon as rain wets the soil. Establishment ensues rapidly, and within two to three months, terrestrial plants seed and die, whilst water levels continue to rise and inundate the soil (Howard-Williams and Lenton 1975; Ferreira et al. 2010). Emergent and amphibious species persist and develop to maximum growth. During rising and receding water, few species of aquatic herbaceous plants were recorded, frequently *O. glumaepatula* at low average abundance.

Lakes in the lower Tapajós show a different dynamic. During rising water, there is great richness of all life-forms of herbaceous plants, predominantly emergent, amphibious and rooted submerged with floating leaves. Richness decreases during flood, as species require adaptations - such as observed in amphibious, floating and some submerged species - for growing together with increasing water levels (Junk and Howard-Williams 1984). This fact suggests an inverse correlation between water level and macrophyte cover (Silva et al. 2010). During receding water and drought, lower water levels become detrimental to emergent and floating species; margins become less humid and most plants grown during more aquatic phases dry out and are carried away by the currents (Junk and Howard-Williams 1984; Junk and Piedade 1997; Piedade et al. 2010b; Ferreira et al. 2010). In contrast, rooted submerged species become more numerous during these phases, often covering the whole water column and altering the ecosystem structure. Irradiance coupled with temperature and nutrient supply are limiting factors for the growth of submerged species (Spencer and Bowes 1990; Kirk 1994; Madsen and Sand-Jensen 1994; Camargo *et al.* 2003; Pezatto and Camargo 2003), and their presence is greatly influenced by light availability (Sand-Jensen 1989; Kirk 1994; Bini and Thomaz 2005). Water transparency allowing for 1-4% of light incidence determines the depth at which submerged species grow (Sculthorpe 1967). In the lower Tapajós River, average underwater irradiance that can occur all year in the margins of lakes ($9.27 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ in 3 m depth; Crema, unpublished data) corresponds to 1.4% incidence and is suitable for growth of submerged species in the whole range of water levels in the flood pulse. Although they represent only 17% of all recorded species, depending on the phase of the flood pulse, the abundance and biomass of submerged species can be twice that of other life-forms. According to Wang et al. (2016), the range of water levels has little effect on performance of submerged species communities, as they are adapted to such variations through morphological plasticity and relocation of biomass (Zhang et al. 2013; Cao et al. 2012; Riis 2002). Despite changes in richness of life-forms, in the lower Tapajós River there was no

variation in abundance or biomass over time - submerged species were always dominant.

Food availability

Many attributes (e.g. duration, frequency, and timing) of fluctuations in water level caused by inundation in floodplains can have various effects on the biota (Junk et al. 1989; Maltchik et al. 2005). They influence physical and chemical characteristics of ecosystems, thus affecting floristic composition, abundance or biomass. Therefore, it is expected that organisms - herbivores in particular - alternate food sources according to changes in the relative abundance of resources (Lowe-McConnell 1987; Winemiller 1989; Wootton 1999).

It is clear that differences between clearwater and blackwater igapós lie not only on the water physicochemical characteristics or composition of plant species, but also on food availability for aquatic herbivores. Igapó lakes from Negro River appear to provide low food variety and biomass to herbivores in all phases of the flood pulse. This is because herbaceous plants are scarce for most of the year, and during drought there is difficult access for aquatic herbivores in the few humid areas of the lakes where moderate abundance of emergent and terrestrial plants occurs. During flood, climbers associated with flooded forests become predominant alongside - to a lesser extent - the emergent species *O. glumaepatula*. Terrestrial plants and those above the water are generally less consumed, due to carbon-rich structural compounds that strengthen cell walls, increase resistance to herbivory and reduce digestibility (Lodge 1991; Bakker et al. 2016). Therefore, climbers are the most important food source in this ecosystem, albeit available only where their leaves submerge during flood. Foraging marks left by Amazonian manatees are noticeable in these areas throughout the whole study site. Variation in composition of herbaceous plants in lakes in the lower Negro River indicates plasticity of feeding habits for various organisms - as recorded for fish (Andrian et al. 1994; Abelha et al. 2001; Freitas et al. 2011).

On the other hand, plant abundance and biomass in the lower Tapajós River do not vary; food sources are plenty and regularly available, with predominant rooted submerged species in all phases of the flood pulse. Differences in herbivory are often attributed to variation in plant quality based on herbivore perception (Lodge 1991; Cronin et al. 2002). Generally, there is a positive relationship between herbivory and nitrogen content in plant tissue (Cebrian and Lartigue 2004). Subaquatic species require fewer structural components as they are supported by the aerenchyma, resulting in greater nitrogen content and herbivory than the observed in other aquatic plants (Moss 1980; Bakker et al. 2016). Moreover, concentration of phenolic defensive

compounds - which is lower in plants above the water - suggests an enhanced palatability due to nutritional value as well as scarcity of resistance tissues and chemical compounds (Hay and Fenical 1988; Su et al. 1973; Planas et al. 1981; Newman et al. 1990; Lodge 1991; Bakker et al. 2016). In addition to such abundant source of food, the dense maze of herbaceous plants in lakes in the lower Tapajós River also provide a physical structure that improves complexity and heterogeneity of habitats (Søndergaard et al. 1998). These features benefit fish diversity - particularly for small juveniles such as cichlids (e.g.: *Apistogramma* sp.) and characids (e.g.: *Bryconops* sp.) (Montag et al. 2014), and large predators like pirarucu (*Arapaima gigas*) and tucunaré (*Cichla* spp.) - as well as aquatic mammals such as the Amazonian manatee.

We conclude that igapó lakes in the lower Tapajós River are richer in species and life-forms of herbaceous aquatic plants than that in the lower Negro River. Despite variation in limnological parameters, the plants composition in Tapajós varies very little over the year and this ecosystem has abundant and regular biomass of submerged species in all phases of the flood pulse. In contrast, the changes in limnological parameters in igapó lakes in the lower Negro River influence more strongly the composition of herbaceous plants. This ecosystem exhibits great differences in the colonization of the different forms of life and the greater abundance of herbaceous occurs during flood, when climbers are on the top of trees and shrubs but maintaining their leaves underwater.

Compliance with Ethical Standards

Informed consent

Permission for collecting samples in the field was obtained via Sistema de Autorização e Informação da Biodiversidade (SISBio) of Instituto Chico Mendes de Conservação da Biodiversidade, under permit number 25347-1. None of the species sampled for this study was protected or under risk of extinction at the time of writing this report.

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Flood pulse and water quality drive aquatic and palustric plant communities in Amazonian lakes

Electronic Supplement 1: Additional Table

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Table S1 Mean and standard deviation of physicochemical parameters of water in lakes in the Tapajós River (clearwater) and Negro River (blackwater) throughout the flood pulse. Different letters indicate statistically significant differences ($P < 0.05$). Temp: temperature, DO: dissolved oxygen, Elect. Cond: electric conductivity, DS: dissolved solids, Transp: transparency, NO₂: nitrite, NO₃: nitrate, NH₄⁺: ammonium, TN: total nitrogen, PO₄³⁻: orthophosphate and TP: total phosphorus, <L.D.: Lower than the minimum detectable.

Rivers		Temp (°C)	pH	D.O (mg.L ⁻¹)	Elect. Cond. (uS.cm ⁻¹)	Depth (m)	D.S. (ppm)	Turb. (NTU)	Transp (m)	NO ₂ (mg.L ⁻¹)	NO ₃ (mg.L ⁻¹)	NH ₄ ⁺ (mg.L ⁻¹)	TN (mg.L ⁻¹)	PO ₄ ³⁻ (mg.L ⁻¹)	TP (mg.L ⁻¹)
Tapajós	Rising water	29.15±1.21(a)	6.39±1.88(a)	4.44±1.51(a)	14.96±4.92(a)	2.77±0.70(a)	7.76±2.7(a)	-	2.11±0.91(a)	0.005±0.002(a)	<L.D.	0.005±0.006(a)	0.37±0.08(a)	0.019±0.004	0.038±0.0(a)
	Flood	29.23±1.26(a)	4.58±0.41(b)	4.69±1.01(a)	13.57±2.00(a)	4.46±0.60(b)	6.79±1.0(a)	1.86±0.74(a)	2.08±0.42(a)	0.003±0.001(a)	<L.D.	0.014±0.036(a)	0.40±0.24(a)	<L.D.	0.051±0.0(ab)
	Receding water	30.64±1.50(a)	4.54±0.68(b)	4.38±1.48(a)	15.53±3.67(a)	2.95±0.50(a)	7.47±1.5(a)	1.39±0.41(a)	2.54±0.49(a)	0.005±0.002(a)	<L.D.	<L.D.	0.38±0.19(a)	<L.D.	0.067±0.0(ab)
	Droughth	29.61±1.56(a)	4.84±0.27(b)	6.75±2.09(b)	10.76±4.77(a)	0.87±0.30(c)	1.63±1.3(b)	6.68±1.57(b)	1.57±0.75(a)	0.011±0.006(b)	0.150±0.08	<L.D.	<L.D.	<L.D.	0.01±0.0(bc)
Negro	Rising water	29.74±0.78(a)	4.80±0.19(a)	2.52±0.38(a)	9.73±0.80 (a)	7.89±0.67(a)	-	1.63±0.23(a)	1.11±0.10(a)	<0.01	0.940±0.123(a)	0.001±0.004(a)	2.22±0.58(a)	0.006±0.020(a)	0.03±0.05(a)
	Flood	29.65±0.71(a)	5.17±0.35(a)	2.14±0.61(a)	9.82±1.26(a)	9.29±0.87(b)	-	1.44±0.26(a)	0.99±0.07(a)	0.037±0.030(a)	<L.D.	0.026±0.000(b)	0.65±0.15(b)	0.026±0.045(a)	0.05±0.09(a)
	Receding water	30.79±1.26(b)	5.60±0.30(b)	4.31±0.59(b)	9.76±1.76(a)	2.04±0.71(c)	-	3.03±0.66(a)	0.65±0.09(b)	0.006±0.001(b)	<L.D.	0.055±0.021(c)	0.71±0.39(b)	0.018±0.006(a)	0.03±0.008(a)
	Droughth	30.99±1.48(b)	5.80±0.44(b)	5.75±0.81(c)	7.80±1.12(b)	0.86±0.63(d)	-	27.34±35.44(b)	0.50±0.21(b)	0.014±0.010(b)	0.530±0.440(a)	0.023±0.029(b)	3.28±2.49(a)	0.013±0.010(a)	0.01±0.01(a)

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Capítulo 2

Crema, L.C; da Silva, V.M.F.; Piedade, M.T.F. Contributions of traditional knowledge of Amazonian manatee (*Trichechus inunguis*) to the management of Brazilian Federal Protected Areas. *Biodiversity and Conservation* (formatado)

Contributions of traditional knowledge of Amazonian manatee (*Trichechus inunguis*) to the management of Brazilian Federal Protected Areas

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Abstract

During 2012-2014, 90 interviews were conducted with riverine people in the Anavilhanas National Park region, lower Rio Negro (blackwater), Tapajós-Arapiuns Extractive Reserve, and Tapajós National Forest, lower Rio Tapajós (clearwater). The goal was to evaluate Amazonian manatee (*Trichechus inunguis*) conservation in Federal Protected Areas (PAs), increase understanding of their biology and so contribute to effective PAs management. In Anavilhanas National Park, 31 consumed plants by the Amazonian manatee were identified. Vines as *Ipomea alba* and *Mikania micranta* had the highest Cognitive Saliency Index ($S=0.34$), even though they were only available to manatees during high water seasons. In the Tapajós PAs, 37 plant species were identified; the submerged species with floating leaves, *Nymphaea* spp. ($S=0.38$), and rooted submerged, *Eleocharis minima* ($S=0.24$) had greatest prominence. While previous studies suggested that, in clear water, *T. inunguis* preferred emergent or floating plants, we found submerged plants drive the Amazonian manatee's diet, due to great abundance and biomass. Interviewees considered that although hunting has declined, it still occurs in Anavilhanas, an area very susceptible to environmental crimes due to the proximity of large urban centers. In contrast, in the Tapajós PAs, manatee hunting seems to be infrequent and have little impact in manatee population. To reduce threats and increase effectiveness of manatee conservation within PAs, greater local population involvement is necessary in activities that broaden understanding of the relevance of natural environments and threatened species conservation.

Keywords: feeding, Rio Negro, Rio Tapajós, interview, ethnobiology

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Introduction

The Amazonian manatee (*Trichechus inunguis*) is still the most hunted aquatic mammal in Brazil (da Silva et al. 2008). The intense and indiscriminate hunting of this animal since pre-colonial Brazil culminated in the drastic reduction of its populations and its classification as “vulnerable” on Brazil's official list of endangered species (MMA 2014) and the Red List of the International Union for Conservation of Nature (IUCN 2016). In addition, the Amazonian manatee is listed in Appendix I of CITES [Convention on International Trade in Endangered Species of Wild Fauna and Flora Species (CITES 2011)]. While the intensity of hunting has decreased (Calvimontes 2009; Marmontel et al. 2012; Souza 2014), the consumption of manatee meat is still considered traditional in the Amazon (ICMBio 2011; Rosas 1994), representing an important source of protein for the riverine people (da Silva et al. 2008).

Trichechus inunguis is the smallest of the four extant members of the Order Sirenia (manatees and dugongs) manatee taxa. Essentially fluvial, it can reach up to three meters in length and weigh some 420 kg (Amaral et al. 2010). The species is herbivorous, feeding predominantly on aquatic and semi-aquatic macrophytes, roots and vegetation from flooded areas within the Amazon Basin (Best 1984; Colares and Colares 2002; Guterres-Pazin et al. 2014). It inhabits floodplains with very different physical and chemical properties, such as black and clear water flooded forests (igapós), and those on white water rivers (várzeas) (Best 1983; Rosas 1994; Junk and da Silva 1997). The differences between these environments are paralleled in the variations in richness and diversity of plants this animal feeds upon (Chapter 1), indicating a great dietary breadth and adaptability, which facilitates its survival in these contrasting ecosystems.

The management of natural areas for wildlife protection is one of the most important governmental instruments for the conservation of biodiversity, not only in the Brazilian Amazon, but around the world (Azevedo-Ramos et al. 2006; Nolte and Agrawal 2012; Mintzer et al. 2015). However, for numerous species of mammals a lack of knowledge hinders conservation efforts and the appropriate management of their habitats within such areas (Tardio and da Silveira 2015). The study of the Amazonian manatee in the wild is challenging due to its cryptic behaviour (it is generally only visible when exposing a small part of its body when it breathes), the turbidity of its favorite habitats (Best 1984; Timm et al. 1986; Rosas 1994) and because it is able to spend long periods beneath extensive banks of aquatic macrophytes (Junk and da Silva 1997). Despite the difficulties in obtaining information about distribution, abundance and behaviour of the Amazonian manatee, human populations living traditional river-dwelling lifestyles have been able to share detailed knowledge about

this species, as historically they have shared the same environment, and used the manatee for a variety of purposes (Calvimontes and Marmontel 2010).

Traditional knowledge is a cumulative body of expertise, awareness and beliefs, culturally transmitted across generations, concerning the relationships of living beings among themselves and with their environment (Berkes 1993; Berkes et al. 2000). Through greater proximity between man and nature, ethnoscience treats groups of humans as social subjects who, in their intellectual actions (knowledge, perception, beliefs), decide and execute practical operations in order to exercise a measure of control over the ecosystem (Toledo 1992). As traditional knowledge originates from daily interactions with the environment (Berkes et al. 2000, Fabricius and Koch 2004), it allows us to understand ecosystemic processes in temporal and spacial scales that would otherwise be difficult to record in conventional scientific research (Huntington 1999). As such, it can guide efforts in the protection of habitats (Johannes and Yeeting 2001; Rajamani, 2013; Huntington 2000), support the planning of conservation actions at the species level (Huntington 1999; Fraser et al, 2006; Brook and McLachlan 2008), and assist in the attaining of information about feeding, distribution and reproduction of elusive species (Franzini et al. 2013).

The current research aimed to access traditional knowledge about *T. inuguis* in Federal protected areas (PAs) situated in black and clear water igapós in the Brazilian Amazon in order to: 1) Increase our knowledge of the biology and feeding ecology of the species; 2) Contribute to the management of the PAs involved and others in the region. Interviews were undertaken with community members of the region surrounding the Anavilhanas National Park (NP), the residents of the Tapajós-Arapiuns Extractive Reserve (RESEX) and the Tapajós National Forest (FLONA), to obtain the following information regarding the Amazonian manatee: places of occurrence, reproductive season, plants eaten, hunting, and the cultural usage and importance of the species.

Materials and methods

Study region

Research on traditional knowledge of the Amazonian manatee was undertaken in two regions, the first in the surrounding areas of Anavilhanas NP, including residents of the municipality of Novo Airão and 14 communities (Fig. 1) of the three protected areas in the Amazonas State: Areas of Environmental Protection (APA) of the Right Margin of the Rio Negro Paduari-Solimões Sector, Rio Negro State Park (PAREST) – North Sector, and the APA of the Left Margin of the Rio Negro Aturiá-Apuauzinho Sector. In the lower Tapajós region, residents from six communities of the Tapajós-Arapiuns RESEX and from three of the Tapajós FLONA were interviewed (Fig. 1).

Anavilhanas National Park is located in the Amazonas State ($02^{\circ} 03' - 03^{\circ}02' S$ and $60^{\circ} 22' - 61^{\circ}12' W$), in the municipality of Novo Airão, approximately 60 km north of the city of Manaus, with an area of 647,610 ha (ICMBio 2017). It was created in 1981, as an Ecological Station, and was recategorized as a national park in 2008. The majority of its area comprises seasonally flooded, fluvial islands, and the remainder is the never flooded terra firme forest. The Tapajós FLONA, created in 1974, is located in Pará State ($02^{\circ} 45'$ and $4^{\circ}10' S - 54^{\circ}45'$ e $55^{\circ}30' W$), within the municipalities of Aveiro, Belterra, Rurópolis and Placas, and has an estimated area of 600.000 ha (IBAMA 2004). The Tapajós-Arapiuns RESEX, created in 1998, is located in Pará State ($02^{\circ}20'$ and $03^{\circ}40' S - 55^{\circ}00'$ and $56^{\circ}00' W$), within the municipalities of Santarém and Aveiro, with an area of 647,610 ha (ICMBio 2014). These two PAs are separated by the Rio Tapajós, with the FLONA located on the right and the RESEX on the left margin. In both margins the habitat is primarily terra firme forest, with ria lakes along the banks, and thin strips of igapó forest.

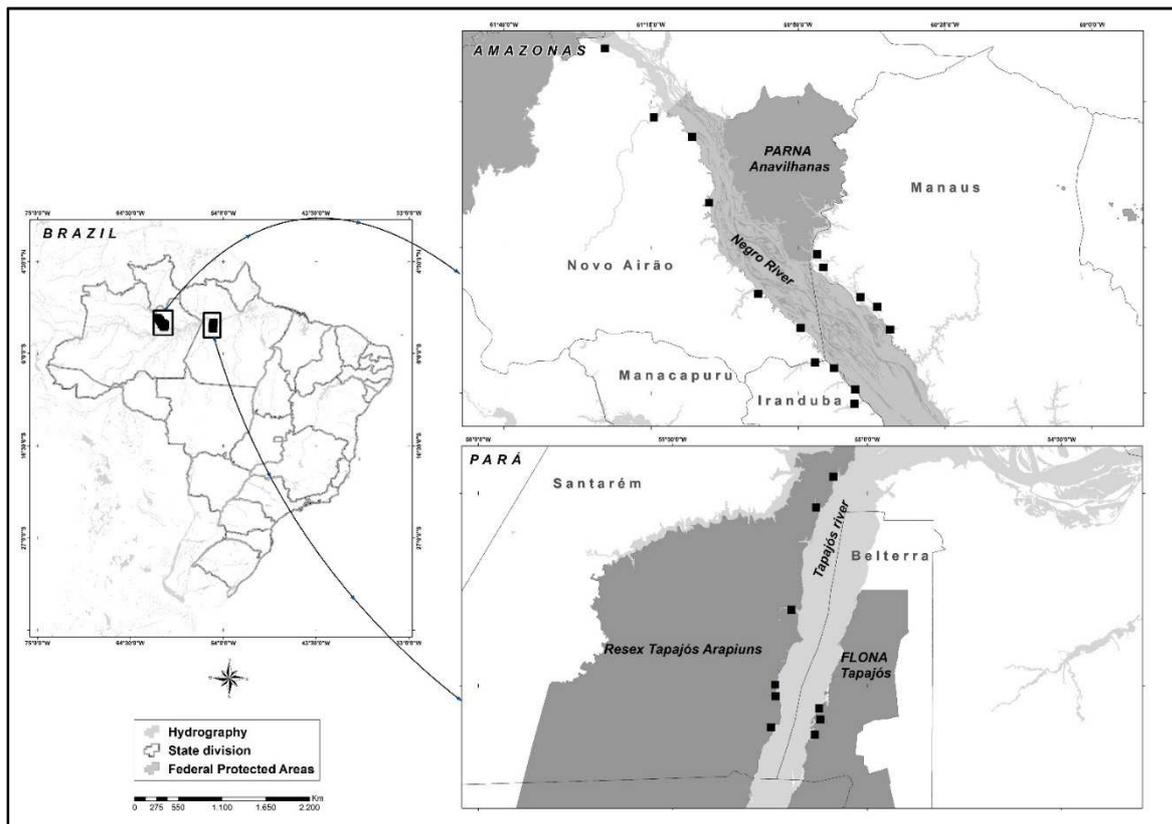


Fig. 1 Map of the studied area showing the origin of the interviewees.

Interview protocol and questionnaire

The information was obtained via individual interviews with the members of the communities. This approach is used as a data collection strategy in qualitative research assumes that if the questions are correctly formulated, then the expressions of the interviewees' experiences will reflect their reality (Borgatti 1998; Albuquerque et al 2014; Miller and Glassner 2016). The interviews allowed the collection of detailed accounts by the participants, thoughts, attitudes, beliefs and knowledge about the uses and interactions between plants and animals, such as the manatee. Requirements for the selection of the interviewees were: (i) experience in hunting manatees or any direct contact with the species; (ii) number of years living in that particular area; (iii) community leaders' recommendation. Interviews took place from March 2012 to October 2014 with 29 men and 1 woman in the NP region, 34 men and 5 women in the RESEX, and 19 men and 3 women in FLONA. According to the PA managers, the greatest sources of income for the communities derives from subsistence agriculture, manioc flour production and fishing; the families are made up of "riverine peoples" or "caboclos", whose ancestry represents a mix of indigenous, Afro-Brazilians and Europeans (Lima-Ayres 1992).

Initially, meetings were held with the presidents and/or other elected community representatives, and permission to undertake the study requested. Once authorization was granted, community homes and socialization centers were visited in order to recruit manatee experts. Each one of them named others that could contribute to the study so as to reach the greatest diversity possible. Each interview was held either in the interviewee's house, in the expedition boat or in communal structures, such as schools or community centers. In Novo Airão, the interviews were held with members of the Boat Pilots Association, during the Anavilhanas NP Consultative Board meetings or in extraordinary meetings. Summing all three sites, 90 interviews were undertaken in total.

INPA's Human Research Ethics Committee approved this study. We conducted semi-structured interviews (see the form in Supplementary Material) where questions about the occurrence and biology of the Amazonian manatee, with a focus on its feeding ecology were included. One part of the interview consisted of a free-listing activity (Borgatti 1998) in which people were asked to name all plants eaten by the Amazonian manatee. A board with photos of 39 study region plant species was shown to the interviewees, who were asked if they could recognize and name them. In the open questions category, answers were grouped according to their similarities. To assay local knowledge, all interviews were transcribed and used in this study with the subject's consent and maintaining anonymity. Maps were made based on the

observation and occurrence of manatees, and showing the locations where interviewees said they had seen the species, strategic visualization spots. In addition, locations of feeding marks in the vegetation (“comidias”) were obtained from monitoring the lakes in the PAs (see methods in Chapter 1). The collected and identified samples of each cited species were deposited in the herbaria at the Federal University of Amazonas (Manaus, Brazil). We grouped species based on life-form (adapted from Junk 1986). We followed the binomial nomenclature for species based on the standard classification of Forzza et al (2016).

The answers were analyzed in a descriptive form, and the data from the free listing exercise of plant species was used to calculate the cognitive salience index (Sutrop 2001) for each morphospecies said to be a manatee food resource. The cognitive salience index (S) represents the summed importance of each plant considering all interviews with local observers, and combines the frequency of citation of a specific item (F) with its mean position on all lists (mP): $S = F/(N \cdot mP)$, where N is the total number of free-list interviews. S varies between 0 and 1, with higher values suggesting a greater cognitive salience for a specific item during the processes of recalling and listing. Names of plants mentioned only once were excluded from the analysis. The cognitive salience index for plants was compared with the floristic inventory made during the same period (Chapter 1).

Results

General knowledge

Interviewees declared they were engaged in subsistence agriculture and fishing; average age was 52 years (range 32 to 83 years old) in Anavilhanas NP region, lower Rio Negro, and 55 years (range 19 to 82 years old) in the PAs of the lower Rio Tapajós. None of the respondents claimed to be currently engaged in the manatee hunting. However, all said that the animal occurs in the region where they live, and that they have occasionally seen or heard of animals in the wild or slaughtered and were able to identify feeding areas and feces.

Among the interviewees, 20% reported having seen a free-range manatee in the last two years in the Anavilhanas PN region, and 56% in Tapajós PAs. Records show that the number of animals killed in the last 10 years was much higher in Anavilhanas (30% of the interviewees) than in the Tapajós PAs (5%). Most of the interviewees considered the high water level season, the period with the highest food availability, to be the best time of year to see manatees (59% in Anavilhanas and 80% in the Tapajós river PAs). The great majority of interviewees (94%) showed high levels of knowledge about Amazonian manatee ecology, describing in detail aspects of its behavior and

biology. Reports confirming the presence of the animal assisted in mapping the most likely sighting sites. In the field manatee presence was detected by several means: feces presence, feeding traces, mortality or direct visualizations. In the Anavilhanas NP region, 43 sites were identified, five of them outside the PA (Fig. 2a); 30 sites were recorded in the Tapajós FLONA and in the Tapajós-Arapiuns RESEX (Fig. 2b).

Most interviewees in the Anavilhanas region (72%) and 32% of the community members of the Tapajós PAs stated that the manatee moves in pairs, often a female with a calf. In the Tapajós, 40% have seen groups of up to four animals and, in Anavilhanas, 33% of the interviewees said they had seen groupings of more than 10 individuals, especially during the low water season. In Anavilhanas, 68% of the interviewees, and 95% in the Tapajós PAs, stated that the species migrates during the low water season to deeper water areas known as “poços”, and to the bottom of the Rio Negro (41% of respondents in Anavilhanas) and Rio Tapajós (72% of respondents in Tapajós PAs). In Anavilhanas, 81% of interviewees say they did not know the species' breeding season, while in the Tapajós PAs, 60% said that this occurred during the flooding season. All interviewees stated that the manatee has good hearing, a good sense of smell, but reduced vision.

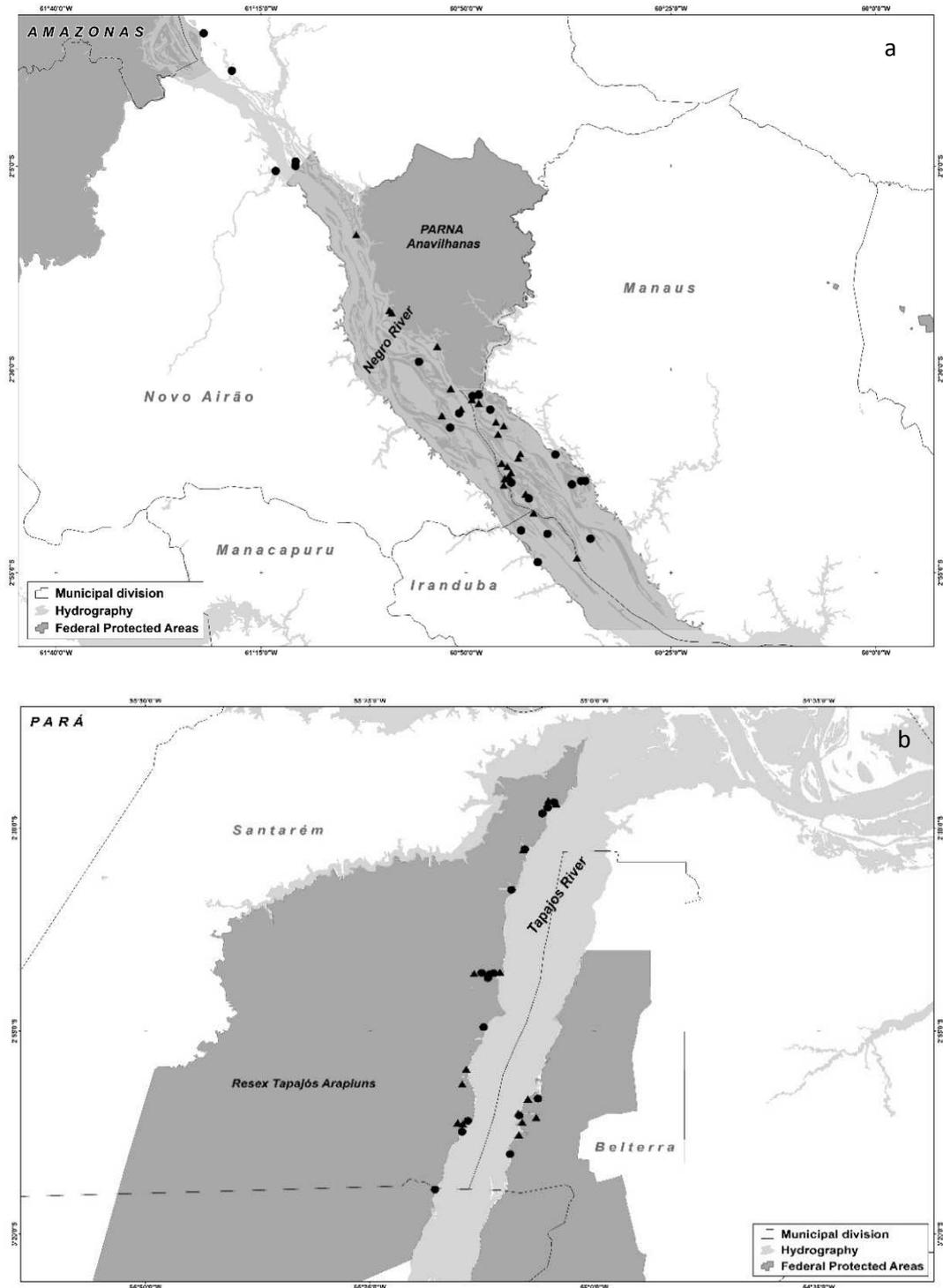


Fig. 2 Locations of direct and indirect records of manatees made during this study: sightings (●) feeding evidence in vegetation (▲), a – Anavilhanas National Park and b- Tapajós-Arapiuns Extractive Reserve and Tapajós National Forest.

Feeding habits of Amazonian manatee

Interviewees indicated 56 plant species consumed by the Amazonian manatee (Table 1) based on direct observations or evidence of vegetation consumption on lake

banks, macrophyte banks, floating remains of submerged macrophytes or clusters of vines on flooded trees and shrubs. 31 species of plants on the Rio Negro, and 37 species on the Rio Tapajós were identified as potential food sources, with 12 species common to both sites (Table 1). Regardless of the area, the most frequently cited plant families were Poaceae, Cyperaceae and Fabaceae (with 9, 6 and 5 spp., respectively). The most cited in Anavilhanas was Poaceae (5 spp.), while in the Tapajós PAs it was Cyperaceae (6 spp.). In terms of growth forms, in Anavilhanas, the highest number of citations were for vines (12 spp.), followed by free floating plants (7 spp.), amphibious (5 spp.) and shrubs (4 spp.); whereas in the Tapajós PAs, the rooted submerged (7 spp.), free floating (5 spp.) and the rooted submerged with floating leaves (4 spp.) were observed (Table 1).

In Anavilhanas, the plant species with the highest cognitive salience index were vines, such as batataranas - *Ipomea alba* (Convolvulaceae) and *Mikania micranta* (Asteraceae) ($S = 0.34$), cipó-icicas - *Tassadia berteriana* and *Mesechites trifida* (Apocynaceae) ($S = 0.23$), followed by shrubs such as maracarana - *Coccoloba pichuna* (Polygonaceae) ($S = 0.19$) and terrestrial vegetation ($S = 0.30$) (Table 2). For the Tapajós PAs, the plant species with the highest cognitive salience index were herbaceous plants with floating leaves and submerged roots, such as the apé - *Nymphaea spp.* (Nymphaeaceae) ($S = 0.38$) and rooted submerged plants, such as capins do fundo - *Eleocharis minima* and *E. fluctuans* (Cyperaceae) ($S = 0.24$) (Table 3).

Plants parts mostly consumed by manatees, according to interviewees were: leaves (59.2% and 44.6% of species cited), whole plant (22.5% and 28.6% of species), respectively in Anavilhanas and the Tapajós PAs. In addition, 70% of the respondents from the Tapajós PAs said that manatees also feed on submerged plant roots in the sediment, mainly of *Nymphaea* species.

Table 1 List of plant species in the diet of *Trichechus inunguis* obtained by interviews in Anavilhanas NP, lower Rio Negro (N), and FLONA do Tapajós and RESEX Tapajós-Arapiuns, lower Rio Tapajós (T), complemented with reports from the literature. Growth form: amp (amphibious), eme (emergent), ff (free floating), rs (rooted submerged), rsff (rooted submerged with floating leaves), ffs (free floating submerged), terr (terrestrial), vin (vine), tre (trees and shrubs).

Family	Species	Growth Form	Vernacular name	Locality
Apocynaceae	<i>Mesechites trifida</i> (Jacq.) Müll.Arg.	vin	Cipó	N
	<i>Tassadia berteriana</i> (Spreng.) W.D.Stevens	vin	Cipó-icica	N,T
Araceae	<i>Lemna minuta</i> Kunth	ff	Murerizinho	N
	<i>Montrichardia arborescens</i> ^{2,4}	eme	Aninga	T
	<i>Montrichardia linifera</i> (Arruda) Schott	eme	Aninga	T
	<i>Pistia stratiotes</i> L. ^{1,2,5}	ff	Aguapé flor d'água	N,T
Cabombaceae	<i>Cabomba aquatica</i> Aubl. ^{1,4}	rs	Mururé-redondinho	T
	<i>Cabomba furcata</i> Schult. & Schult.f.	rs	Samambaia	T
Asteraceae	<i>Mikania micrantha</i> Kunth	vin	Batatarana	N
Convolvulaceae	<i>Ipomoea alba</i> L.	vin	Batatarana	N
Curcubitaceae	<i>Cayaponia cruegeri</i> (Naudin) Cogn. ⁵	vin	Jamarurana	N
	<i>Pteropepon deltoideus</i> Cogn.	vin	-	N
Cyperaceae	<i>Eleocharis confervoides</i> (Poir.) Steud.	rs	Amã	T
	<i>Eleocharis fluctuans</i> (L.T. Eiten) E.H. Roalson & C.E. Hinchliff	rs	Capim de fundo, capinzinho	T
	<i>Eleocharis interstincta</i> (Vahl) Roem. & Schult ³	amp	Junco	T
	<i>Eleocharis minima</i> Kunth	terr, sub	Capim de fundo, capinzinho	N,T
	<i>Eleocharis mutata</i> (L.) Roem. & Schult.	amp	Junquinho	T
	<i>Eleocharis plicarhachis</i> (Griseb.) Svenson	amp	Junco	T
Euphorbiaceae	<i>Alchornea discolor</i> Poepp.	tre	Supiarana	N
Fabaceae	<i>Aeschynomene</i> L.	amp	Dormideira	N
	<i>Clitoria glycinoides</i> DC.	vin	Feijão de praia	N
	<i>Sesbania exasperata</i> Kunth	tre	Paricá	T
	<i>Symbosema roseum</i> Benth.	vin	Feijão graúdo	N
	<i>Vigna lasiocarpa</i> (Mart.ex Benth.) Verdc	vin	Feijãoarana	N,T
Hydrocaritaceae	<i>Apalanthe granatensis</i> (Bonpl.) Planch	rs	Samamanbaia	T
	<i>Limnobium laevigatum</i> (Humb. & Bonpl. ex Willd.) Heine	ffs	Mureru	N
Lentibulariaceae	<i>Utricularia breviscapa</i> C.Wright ex Griseb.	ffs	Samambaia, lodo	T
	<i>Utricularia foliosa</i> L.	ffs	Lodo, samambaia	N,T
Mayacaceae	<i>Mayaca fluviatilis</i> Aubl.	rs	mureré do campo	T
Menyanthaceae	<i>Nymphoides indica</i> (L.) Kuntze	rsff	Apé	T
Nymphaeaceae	<i>Nymphaea potamophila</i> Wiersema	rsff	Apézinho	T
	<i>Nymphaea rudgeana</i> G.Mey.	rsff	Apé	T
Passifloraceae	<i>Passiflora misera</i> Kunth	vin	Maracujázinho	N
	<i>Passiflora pohlii</i> Mast.	vin	Maracujázinho	N
Plantaginaceae	<i>Bacopa reflexa</i> (Benth.) Edwall	rs	Samambaia d'água	T
Poaceae	<i>Echinochloa</i> sp. P.Beauv	amp	Canarana	N
	<i>Echinochloa polystachya</i> (Kunth) Hitchc. ^{1,2,4,5}	amp	Canarana	N,T
	<i>Hymenachne amplexicaulis</i> (Rudge) Nees ^{1,2,5}	eme	Bucho de pato	N,T
	<i>Leersia hexandra</i> Sw ^{2,4,5}	amp	Capim navalha	T
	<i>Louisiella elephantipes</i> (Nees ex Trin.) Zuloaga	amp	Capim	T
	<i>Luziola subintegra</i> Swallen	amp	Capim buchudo	T
	<i>Oryza glumaepatula</i> Steud	eme	Arroz, capim de arroz	N,T
	<i>Panicum dichotomiflorum</i> Michx. ⁵	amp	Capim	T
	<i>Paspalum repens</i> P.J.Bergius ^{1,2,4,5}	amp	Membeca, premembeca	N,T
Polygonaceae	<i>Polygonum acuminatum</i> Kunth	amp	Tabacarana, boieira	T
	<i>Coccoloba pichuna</i> Huber	tre	Maracarana	N
Pontederiaceae	<i>Eichhornia azurea</i> (Sw.) Kunth ^{2,4}	rsff	Mureru	T
	<i>Eichhornia crassipes</i> (Mart.) Solms ^{1,2,3,4,5}	ff	Mureru	N,T
	<i>Pontederia rotundifolia</i> L.f. ⁵	ff	Mureru	T
Pteridaceae	<i>Ceratopteris pteridoides</i> (Hook.) Hieron. ⁵	ff	Mureru véu	N
Salvinaceae	<i>Azolla filiculoides</i> Lam.	ff	Mureruzinho	T
	<i>Salvinia auriculata</i> Aubl. ^{1,2,4}	ff	Lentilha d'água	N,T
	<i>Salvinia</i> sp.	ff	Mureru	N
Solanaceae	<i>Schwenckia grandiflora</i> Benth.	tre	Cipó	N
	<i>Solanum subinerme</i> Jacq.	tre	Jurubeba	N
Vitaceae	<i>Cissus erosa</i> Rich.	vin	Cipó	N,T
-	-	-	Lodo/lama	N,T

Table 2 Citation frequency, mean citation position and cognitive salience index of each plant reported by inhabitants of Anavilhanas NP, lower Rio Negro, as being in the diet of *Trichechus inunguis*. Growth form: amp (amphibious), eme (emergent), ff (free floating), ffs (free floating submerged), terr (terrestrial), vin (vine), tre (trees and shrubs).

Plants	Vernacular name	Growth Form	Citation Frequency	Mean citation position	Cognitive salience index
<i>Ipomoea alba</i> L.	Batatarana	vin	12	1.75	0.44
<i>Mikania micrantha</i> Kunth					
<i>Tassadia berteriana</i> (Spreng.) W.D.Stevens	Cipó-íca	vin	12	2.58	0.23
<i>Mesechites trifida</i> (Jacq.) Müll.Arg.					
<i>Coccoloba pichuna</i> Huber	Maracarana	tre	09	2.33	0.19
Cyperaceae	Capim	terr, amp	08	2.25	0.17
<i>Oryza glumaepatula</i> Steud	Arroz	eme	13	4.00	0.16
<i>Vigna lasiocarpa</i> (Mart.ex Benth.) Verdc	Feijãoarana	vin	07	3.57	0.10
<i>Salvinia auriculata</i> Aubl	Mureru	ff	05	2.60	0.10
<i>Echinochloa polystachya</i> (Kunth) Hitchc.	Canarana	amp	05	4.00	0.06
<i>Utricularia foliosa</i> L.	Camarão quissá	ffs	04	4.50	0.04
<i>Paspalum repens</i> P.J.Bergius	Membeca	eme	03	5.00	0.03
<i>Solanum subinerme</i> Jacq.	Jurubeba	tre	02	5.00	0.03
Perifiton	Lodo	alga	02	5.00	0.02

Table 3 Citation frequency, mean citation position and cognitive salience index of each plant reported by inhabitants of the Tapajós FLONA and Tapajós-Arapiuns RESEX, lower Rio Tapajós, as being in the diet of *Trichechus inunguis*. Growth form: amp (amphibious), eme (emergent), ff (free floating), ffs (free floating submerged), terr (terrestrial), vin (vine).

Plants	Vernacular name	Growth Form	Citation Frequency	Mean citation position	Cognitive salience index
<i>Nymphaea potamophila</i> Wiersema	Apé	rsff	43	1.88	0.38
<i>Nymphaea rudgeana</i> G.Mey					
<i>Eleocharis minima</i> Kunth	Capim de fundo	terr, rs	31	2.01	0.24
<i>Eleocharis fluctuans</i> (L.T. Eiten) E.H. Roalson & C.E. Hinchliff	Junco	rs	11	1.81	0.10
<i>Oryza glumaepatula</i> Steud	Arroz	eme	17	3.05	0.09
<i>Montrichardia linifera</i> (Arruda) Schott	Aninga	eme	15	3.06	0.08
<i>Montrichardia arborescens</i> (L.) Schott		eme			
<i>Echinochloa polystachya</i> (Kunth) Hitchc.	Canarana	eme	18	3.83	0.08
<i>Utricularia foliosa</i> L.	Samambaia	ffs	18	3.83	0.08
<i>Hymenachne amplexicaulis</i> (Rudge) Nees	Premembeca	eme	14	3.14	0.07
<i>Ipomoea</i> L.	Batatarana	vin	10	9.90	0.04
<i>Eichhornia azurea</i> (Sw.) Kunth	Mureru	rsff	10	4.00	0.04
<i>Eichhornia crassipes</i> (Mart.) Solms		ff			
<i>Eleocharis confervoides</i> (Poir.) Steud.	Amã	rs	07	3.00	0.04
Cyperaceae Juss.	Capim amolado	amp	05	3.40	0.02
<i>Louisiella elephantipes</i> (Nees ex Trin.) Zuloaga	Taboca	amp	05	3.80	0.02
<i>Vigna lasiocarpa</i> (Mart.ex Benth.) Verdc	Feijãoarana	vin	05	4.20	0.02
<i>Scleria gaertneri</i> Raddi	Tiririca	terr	03	3.33	0.01
<i>Cecropia</i> Loefl	Embaúba	amp	02	3.50	0.01

Amazonian manatee hunting

Hunting is still the main cause of death of the species (as stated by 62% and 52% of the respondents in Anavilhanas and the Tapajós PAs, respectively), but on the Tapajós there were records of stranding and entanglements in fishing nets (both 16%).

Most reported hunting records predate 2003 (as cited by 42% of the interviewees in Anavilhanas and 75% in the Tapajós PAs). During the research period, 26% of the Anavilhanas interviewees were aware of hunting events, whereas only 2% in the Tapajós. In both locations, the most cited hunting instrument was the harpoon, and high water season as the hydrological phase with the highest stated hunting intensity (68% in Anavilhanas and 90% in the Tapajós PAs), although 23% of respondents in Anavilhanas described that hunting occurs throughout the year. The most cited motivation was subsistence; however, trade was also mentioned (22% of the interviewees). The majority (65% of the interviewees) admitted to have consumed manatee meat, at least in their youth, and some interviewees from the region of Anavilhanas said they knew of current commercialization sites. Manatee meat was confirmed by all interviewees as the most used part of the animal. Most of the interviewees (77.3% in Anavilhanas and 91.5% in the Tapajós PAs) claimed that manatee fat can be used in cooking, deep frying and roasting of cassava flour, while medicinal use was more frequently cited in the Tapajós (32%). The hide was given as an item of lesser interest, although some interviewees described it being used in some dishes, and, in decades past, that it had been used as a raw material in tanneries. Some respondents (27%) said they knew of the use of bones in the manufacturing of manual tools. According to interviewees, while manatee hunting has decreased, it still occurs in the Anavilhanas NP region. Hunting appears much less frequent in the Tapajós PAs.

Discussion

General Knowledge of the Amazonian manatee

The manatee is well known by the community members of the studied regions, whose proximity to the environment provide them with frequent opportunities to observe the resources that surround them (Berkes et al. 2000). However, interviewees emphasized that not all of them understand the biology of this animal, since the species has cryptic behavior and acute hearing, making it difficult to observe. This fact is supported by such interviewee statements as: "It is a sensitive, subtle animal; you have to be silent to see it", and "the manatee hears well, it's very clever, not easy to see." These characteristics, also mentioned in other studies, underscore the importance and utility of obtaining information about the species from that sector of human society who encounter it most frequently, "ribeirinhos", the rural river-dwellers of Amazonia (Calvimontes and Marmontel 2010; de Freitas et al. 2015; da Silva et al. 2017).

The distribution of Amazonian manatees is closely related to seasonal ecosystem changes (Best et al. 1984, Junk and da Silva, 1997, da Silva et al. 2008, Arraut et al. 2010, Marmontel et al. 2012). During the high water phase, water bodies interconnect, so increasing the availability of habitats, and the diversity and supply of plant species and their nutritional value (Junk et al. 1989). In contrast, during the low water phase, aquatic environments become more restricted, movement is consequently limited and the production of herbaceous vegetation is limited to some biotypes and is available to herbivores, such as manatees, only in deeper water locations (Chapter 1). Interviewees also commented on this: "During high water period, they eat in the shallow places, at night", while another says that "In the summer [low water season] we cannot find it, there is no food, nothing. They only surface in lakes with deep areas", describing that even though the animals concentrate in deeper sites, these are limited in size and it is difficult to reach them. Migration behaviour, previously documented for *T. inunguis* and other sirenians (Sheppard et al. 2006), was described by Arraut et al. (2010; 2017) for Central Amazonian manatee populations in Mamirauá and Amanã Sustainable Development Reserves (SDR). These authors noted that these animals move to those localities where the chances of surviving the prevailing hydrological conditions are greatest, both in terms of water depth and food availability. In our study, according to the interviewees, the Amazonian manatees move every year, leaving the igapós lakes near their communities to deeper places, such as the main channels of the Rio Negro and Rio Tapajós.

Although the interviewees' level of knowledge of the timing of manatee reproduction differed between the areas, the mating attempt is, in both locations, known as the "vadia" or "vadiação" (Zaniolo 2006; Calvimontes 2009; Calvimontes and Marmontel 2010). In Anavilhanas, most respondents said they did not know when manatees bred, while in the Tapajós PAs, many individuals described in detail: "During the vadia, there are more than 20 manatees together, several following a female. This happens as the rivers rise in the flooding season. The male pushes the female to shallow water". This agrees with what Pereira (1974) has described for reproductive behaviour of a female with several males. Most interviewees had little information on the birth of calves, but Best (1982) reports that it occurs as waters rise, when there is greater availability of plants and their nutritional value is high. This synchrony facilitates the energetic recovery of females at the end of gestation and onset of lactation, as well as the beginning of weaning of calves, using small quantities of plants, in one or two months after birth (Barbour 1973; Best 1982; Hartman 1971).

With the exception of the relationship between the female and her calf, individuals are normally solitary (Rosas 1994). Interviewees confirmed this, but also

described having seen large groups of animals, especially in low water season, in places of refuge. One interviewee commented: "Sometimes manatees stay in groups of up to 20, they urinate like cattle, when they breathe they make sounds like cattle". It should also be noted that the Portuguese name for manatees, "peixe boi", means "fish bull". In fact, other types of aggregations are cited, both at feeding sites (Best 1982; Calvimontes 2009; Denkinger 2010) as well as during the reproductive period (Pereira 1944; Souza 2015).

Feeding habits of Amazonian manatee

To date, 128 species have been listed as potential *T. inunguis* food plants (Best 1981; da Silveira 1988; Colares 1991; Colares and Colares 2002; Zaniolo 2006; Franzini et al. 2013; Guterres-Pazin et al. 2014). In this study, from the interviews and the collection of plants in lakes of the lower Rio Negro and lower Rio Tapajós basins (Chapter 1), 57 species have been catalogued, of which 34 (60%) had not been mentioned previously, highlighting a range of information still to be studied on the feeding ecology of wild Amazonian manatees.

Trichechus inunguis feeds on a variety of aquatic and semi-aquatic plants, mostly from the families Cyperace and Poaceae (Best 1981; da Silveira 1988; Colares and Colares 2002; Franzini et al. 2013; Guterres-Pazin et al. 2014). On the Rio Negro, however, the cognitive salience index indicated that a number of other species were also important, including vine species: batatarana (*Mikania micrantha* and *Ipomoea alba* L), cipó-icica (*Tassadia berteriana*) and feijãoarana (*Vigna lasiocarpa*), as well as shrubs, such as the maracarana (*Coccoloba pichuna*) (Table 1). Unlike many of the emergent and floating plants, these species only become accessible to the manatees during the high water season, when the shrubs are flooded and the lower leaves of the vines became available for consumption (Chapter 1).

A study in central Amazonian igapó at Lake Amanã identified a variety of plant species in feces and stomach contents of manatees during the low water phase (Guerres-Pazin et al. 2014). However, in the igapó lakes of Anavilhanas there is no evidence of consumption in this period. According to the Chapter 1, on the lower Rio Negro, the igapó lakes appear not to have significant availability or variety of food for aquatic herbivores, since, as for most of the year, these lakes have low aquatic plant availability. In the low water phase, emergent species and those of terrestrial cycles predominate only in perpetually wet places such as the lowest parts of seasonal lakes. Such areas are generally, inaccessible to an aquatic herbivore with the size and habits of the manatee. In the high water phase, the manatee shows selective feeding behavior, while during the low water period, such behavior is opportunistic (Colares

1991; Colares and Colares 2002). Consequently, selectivity is linked to the availability of plants, as determined by the flood pulse (Guterres-Pazin et al. 2014). During high waters, the supply and variety of food is great, and animals accumulate the fat that will allow them to survive for several months during the dry season, when forage is poor or non-existent (Best 1983). This period is called by Zaniolo (2006) as "months of famine", when animals eventually resort to consuming dead plants at the bottom of lakes (Best 1983) or exploit vegetation in deeper waters (Arraut et al. 2010).

On the Rio Tapajós, from the community dwellers' point of view, the species preferred rooted plants with floating leaves, as one interviewee describes: "Manatee eat apé [*Nymphaea* spp.] yes, but what they really like are its bulbs [botanically corms] that are on the bottom", or the fixed submerged plants as the capins de fundo *Eleocharis fluctuans* and *E. minima* (Table 2). Although manatees feed preferentially on leaves, riverine interviewed on the UCs of Rio Tapajós, said that manatees would dig up the lake or river-bottom around *Nymphaea* spp. to consume their corms and roots, in addition to eating large quantities of submerged plants throughout the year. Changes in plant tissue quality can be perceived by herbivores, because there is generally a positive relationship between the nitrogen content of the plant and the rate of ingestion (Cebrian and Lartigue 2004). This may lead to variations in the rates of consumption of certain items (Lodge 1991, Cronin et al. 2002), Underwater species differ because they require fewer structural components as they are supported by the water and by the aerenchyma, resulting in proportionately high nitrogen concentration and, consequently, high rates of herbivory (Moss 1980; Bakker et al. 2016). In addition, *T. inunguis* feeds preferentially on plants with low fiber content and high nutritional value, and seems to avoid plants with natural toxins (Best 1981). As the amount of these toxins decreases from emergent plants to floating and from these to submerged, the palatability of submerged plants may be greater, not only due to the nutritional quality, but also due to the presence of lower concentrations of defense chemicals (Hay and Fenical 1988; Su et al. 1973; Planas et al. 1981; Newman et al. 1990; Lodge 1991; Bakker et al. 2016).

On the lower Tapajós, many lakes retain sufficient water during low water period, to permit the persistence of both submerged macrophytes and manatees (Chapter 1). Previous studies have associated degrees of rostral deflection in the sirenian skulls to the adaptation of feeding at different depths (Domning 1978; Domning and Hayek 1986) and have postulated that the Amazonian manatee prefers plant species with emergent or floating habits (Rosas 1994; Guterres- Pazin et al. 2014). In clear waters, such as those of the Rio Tapajós, there is no evidence that this propensity occurs. There are numerous reports of the manatee's preference for the roots and corms of

plants fixed in the sediment and for submerged species, which is compatible with their high abundance and biomass due to the high transparency of the water (Chapter 1).

Amazonian manatee hunting

As reported by several authors (Pereira 1944; Franzini et al. 2013, Marmontel et al. 2012; Souza 2015), it is consensus among the interviewees that a manatee hunter must have specific skills and mastery of the habitat and behavior of the species. One of the most used indicators to locate manatees in both study sites are the "comidias" (Fig. 3), open places in the dense aquatic vegetation resulting from the removal of parts of the floating or emergent plants by feeding manatee, or marks left behind by bites on leaves and stems while feeding (Franzini et al. 2013; Calvimontes and Marmontel 2010). Harpoon hunting is still the primary cause of death for Amazonian manatees (Marmontel et al. 2012), a fact confirmed by interviewees in the current study. However, incidental catches in fishing nets were considered by interviewees to be the second most common cause of death. This factor has also been described by Aguilar (2003) for the lower Tapajós PAs, and by Zaniolo (2006) for Anavilhanas NP. Such impacts can substantially reduce populations across the year (Rosas 1994). In the Amazon, young animals are the most vulnerable to fishing nets; if they survive, hunters can sell them or donate them for consumption or as pets (Marmontel et al. 2012). However, in this study, respondents did not relate manatee size to incidental catches.

Residents of the PAs related the seasonality of Amazonian manatee hunting to their variations of habitat use. This matched previous data from Calvimontes (2009) and Souza (2015) for *T. inunguis* and for *T. manatus manatus* in the Colombian part of the Orinoco (Castelblanco-Martinez 2004). The interviewees emphasized the greater ease with which manatees can be detected at "comidias" during the high waters, a fact also reported by Arraut et al. (2010). Some authors (Best 1982; Calvimontes and Marmontel 2010; Denkinger 2010) and the interviewees of this study reported that during the low waters, hunting is restricted to pools where several individuals congregate and many community members' fish, increasing the likelihood of encounters with manatees (Mayaka et al. 2013).

The international demand for *T. inunguis* hide was intense from the beginning of the 18th century, with a peak in 1938, when about 16 thousand manatees were hunted (Domning 1982; Antunes et al. 2016). Since the creation of the Fauna Protection Act in 1967, there has been no record of this type of activity. As such, the main motivation for their hunting, according to the interviewees, is small-scale commercialization and an appreciation of the flavour of the meat, and - to some extent - tradition; both because the meat is much appreciated by ribeirinhos, as pointed out in other studies (Lima 1997;

Calvimontes and Marmontel 2010; Mayaka et al. 2013). This fact was emphasized by one of the interviewees: "Dying, make the most of everything (laughing), just put it in the tucupi. You can grill it, make sausage and mixira" (dish made from fried manatee meat and stored in manatee fat).

Interviewees described how each manatee provided two or three types of meat: red meat, similar to cow flesh, located in the ventral part of the body; white meat, which is compared to fish, lighter in color and present in the caudal fin; some interviewees likened its taste to that of pork. Such differences have also been recorded by other studies, both of *T. manatus* (Lima et al. 1997; Castelblanco-Martínez et al. 2004), and *T. inunguis* (Aguilar 2007; da Silva et al. 2017). A study by Marmontel et al. (2012) reported that in some parts of Amazonia, there are communities that differentiate up to seven flavors for the manatee meat. Interviewees in the current study confirmed the use of manatee lard in the preparation of the mixira, but also in other local culinary dishes such as beiju, tapioca, roasting farinha (manioc flour), as well for deep-frying. Interviewees in both this study and that of Zaniolo (2006) said that community members in the Tapajós PAs region also use manatee skin as a source of protein, adding it to bean casseroles.

In addition, interviewees in this and other studies state that manatee oil (the lightest elements only, derived by warming and filtering the fat) has a medicinal effect (Lima et al. 1997; O'Shea et al. 1998; Hines et al. 2005; Rajamani et al. 2006; Souto et al. 2011; Rajamani 2013; Ferreira et al. 2013), and can be used to treat rheumatism, muscular pain, thrombosis, allergy and inflammation (Alves and Rosa 2007). Other authors have reported its use for healing of asthma, as a general healing ointment for hernias (Zaniolo 2006). A mixture of manatee oil, Andiroba oil (*Carapa guianensis* Aubl., Meliaceae), and manatee skin is reportedly used for bone disease and to massage bruises (Lima 1997; Costa-Neto 1999; Andrade and Costa-Neto 2005). A tea can be made from the roasted and ground skin and used to treat hernia, respiratory diseases and sexual impotence. Because it is an animal with acute hearing, either drinking a tea made from the powder of the stirrup (one of the bones of the middle ear), or wearing the bone hung around the neck, will cure deafness (Lima et al. 2007).



Fig. 3 “Comidias”, bite marks on the leaves and branches of floating vegetation, and vegetation bent down by Amazonian manatee. Photographs by Luciana Crema.

From the point of view of the Protected Areas

Even though *T. inunguis* is known to be distributed throughout the Amazon basin (Best 1984), refining this information, especially in protected areas, can be useful for conservation activity management at the local or regional levels. The locations shown on the maps were generated from interviews and records of food traces, and correspond to places of rest, refuge or foraging of the Amazonian manatee in the different phases of the flood pulse. Most of these areas lie within in PAs already protected by law, and zoned into areas of differing use categories (including restriction and prohibition of hunting and extractive activities). This is fundamental to define, for example, locations for species reintroduction and management. Reintroductions were carried out in 2007, at Lake Anumã in the Tapajós-Arapiuns RESEX, with two manatees rehabilitated in captivity (Luna et al. 2008). In Amanã SDR, reintroductions of five Amazonian manatees were carried out in 2012 (Rodrigues 2012). While on the Rio Cuieiras, in the Rio Negro State Park South Sector, Amazonas, four animals were reintroduced between 2008 and 2009 (Souza et al. 2010), and four manatees have been reintroduced more recently into the lakes of the Piagaçu-Purus SDR, and have been monitored with the participation of community members and former hunters (Diogo Souza pers. comm.).

Although the study areas are PAs, threats to the manatee cannot be ruled out. Even with the reduction in the frequency of hunting events, in the Anavilhanas NP almost 30% of the respondents reported at least one episode of manatee hunting during the last two years. The situation seems to be disturbing many of them, as one of the interviewees describes: "There are still manatees, but not many. Last year [2011] there was a lot of hunting. It's happening. Moto-taxis offer manatee meat. It is a frequent occurrence. There are people here who live on that alone. If they have a harpoon, they are hunting the manatee. Sad reality." The Anavilhanas PN is the most

visited park in the Central Amazon, either by authorized tourists or by local people exploiting its resources, even though by law it has total protection, its proximity to urban centers such as Manaus city (2 million inhabitants) and the town of Novo Airão (18,000 inhabitants) makes it vulnerable to anthropogenic disturbances (Tardio and da Silveira 2015). Despite attempts by Chico Mendes Institute for Biodiversity Conservation, the State Government, non-governmental organizations and local entities for an integrated management approach in the environment surrounding the NP, a number of illegal activities, including hunting, logging and commercial fishing are still recorded (ICMBio 2017).

The number of hunting events recorded in the last years in the lower Tapajós PAs, is smaller than at Anavilhanas. This can be interpreted in two ways: (i) a fear of reporting these events (since the interviewees know that hunting the Amazonian manatee is illegal), or (ii) that hunting practiced in this region is truly smaller in extent. Considering the first interpretation, Ortega-Argueta et al. (2012) warn of this bias, when the interviewee avoids reporting illegal activities, for fear of potential retribution, either from perpetrators or from legal authorities. Both the Tapajós-Arapiuns RESEX and the Tapajós FLONA are categorized as sustainable use PAs, where the rational exploitation of natural resources is permitted, and both have approved and implemented sustainable management plans. In these PAs, there are large numbers of families that are considered traditional inhabitants, since they have a history of multi-generational residence and subsistence use of natural resources, and use low impact technologies derived from patrimonial knowledge, which normally, are sustainable (Berkes 1993; Arruda 1999). A study in the Mamirauá SDR of the Amazon river dolphin (*Inia geoffrensis*) found a positive relationship between the involvement of resident ribeirinhos in research projects and their opinions of conservation projects (Mintzer et al. 2015). On the Tapajós, both RESEX and FLONA, true to the purpose for which they were created, develop not only activities that involve management and conservation, but also those aimed at encouraging the autonomy of member communities. Despite this, there are reports of a few hunters who remain active, but without the establishment of formal trade, suggesting activities that are isolated, culturally embedded and low-impact. As one interviewee reported: "Every year, Mr. X kills a manatee to eat, but it's only one. This year (2013) he has already killed."

Trichechus inunguis is protected by Brazilian legislation, and the 1967 hunting ban was an important step towards its conservation, but the implementation of laws conserving natural resources in the Amazon is often hampered by the logistics size, difficulties of access to areas, a lack of monitoring and inspection, and scarce financial and human resources. Even so, the implementation of protected areas continues to be

an important instrument for the maintenance of biodiversity, even if the creation of the area does not prevent illicit activities from occurring within it. Protected areas have the additional responsibility of maintaining vulnerable vertebrate species in populations large enough that they maintain their ecosystem functions. However, this role is neither always compatible with the scarce financial and human resources, nor with the growing human population presence (Terborgh 1999). Although Anavilhanas NP has no legal permanent residents, 70% of Amazon parks do have resident populations (Terborgh and Peres 2002). In addition, the majority of the protected areas currently being created explicitly include people, and FLONAs and RESEXs are legally occupied, often by non-traditional populations, subsisting wholly or partially on hunting, with no sustainable basis.

In Anavilhanas NP, according to the interviewees, vines were the most cited species (40% of all citations, and the highest cognitive salience index) in the manatee diet, even though they are only available during the high water period. In this region of igapó, for most of the year, there is little or no food available to aquatic herbivores (Chapter 1). As such, it is to be expected that the manatee will move to areas with greater food supply. The Solimões river floodplain, some 130 km from the PARNA in a straight line and with a greater diversity and abundance of more nutritious plants (Piedade et al. 2010; Ferreira et al. 2010) may be one of them. Arraut et al. (2010; 2017) showed that the Amazonian manatee makes annual migrations of tens of kilometres between the igapó and the floodplain in Amanã and Mamirauá SDRs, and that this movement is determined by food availability and predator avoidance. Thus, the occurrence of *T. inunguis* in Anavilhanas NP, and the initiatives for its conservation carried out only in the municipality of Novo Airão, in which the Park is located, may be insufficient to ensure its protection, since ideally the extension of activities should cover the entire life area of the species where human influence may exist.

Worldwide, attitudes related to environmental conservation are influenced by the educational level (Fiallo and Jacobson 1995; Sah and Heinen 2002). This may be due to greater access to information, different means of communication or greater contact with informed people, all of which would aid assimilation of the importance and the need to preserve endangered resources. For example, the continued work of Tamar, a conservation project of sea turtles in many parts of the Brazilian coast, have helped fishers to undertake a more conservationist stance that is not necessarily (but can be) a reflection of realities in their day-to-day (Braga and Schiavetti 2013). The Mamirauá Institute of Sustainable Development and the Projeto Boto, through fishers' involvement with ecotourism and scientific research, have had a significant effect in

promoting positive attitudes for conservation of *Inia geoffrensis* in protected area (Mintzer et al. 2015).

Thus, a suitable strategy for effective manatee protection must not only limit hunting, but also increase the involvement of residents and surrounding populations of PAs in activities related to sustainable management and research to enhance the effectiveness of these areas. In this context it is key to provide training and support to community management, involving local personnel in practices of faunal and floral management, in on-going education and environmental awareness initiatives, as well as training of community agents (Ortega-Argueta et al. 2012; Rajamani 2013; Mintzer et al. 2015). With regards to species conservation, in-depth studies should be conducted to determine their home range, including those beyond the boundaries of the PAs, so that joint actions between government, non-governmental institutions and, especially, local human communities and key stakeholders can be planned. Therefore, it is considered that the management of protected areas combined with the active participation of its inhabitants and with their surroundings represents one of the greatest challenges and opportunities for conservation (Harmon 1998; Fragoso et al. 2000; CBD 2008; Shepard et al. 2010), including that of the Amazonia manatee.

Conclusions

Ribeirinhos in the vicinity of Anavilhanas NP and those of the Tapajós-Arapicums RESEX and the Tapajós FLONA have a detailed knowledge of Amazonian manatee biology. This contributed greatly to several areas of knowledge concerning manatee biology, including distribution, movement, feeding and hunting, and information on plant species that the literature had not previously reported as part of their diet. On the Rio Negro, the cognitive salience index indicated that vines were the commonest diet species, although their availability is restricted to the high water season. On the Rio Tapajós, submerged plants were the species with higher cognitive salience index, due to their greater abundance and biomass, a feature related to the high transparency of the clear waters of the lakes. According to the interviewees, even though manatee hunting has diminished, it still occurs in Anavilhanas NP, an area very susceptible to environmental crimes due to the proximity to large urban centers. In the Tapajós PAs, hunting appears to be less frequent and of lower impact. As a way to reduce threats to the manatee, and increase the effectiveness of these protected areas, we suggest the constant inclusion of local populations in activities directly related to the PAs, as practices of faunal and floral management, in addition to their participation in the Management Councils, as initiatives to disseminate the importance of the protected areas, support research projects and management actions, and training of community

agents, so as to bring the users closer of the management unit, increasing the perception of the value of their participation in conservation actions.

Compliance with Ethical Standards

This study was authorized by the Secretaria de Estado de Meio Ambiente do Amazonas, responsible for the management of the PAs: APA da Margem Direita do Rio Negro Setor Puduari-Solimões, PAREST Rio Negro - Setor Norte and APA do Margin Esquerda do Rio Negro, Aturiá-Apuauzinho (permissão 70/2014) and by the Instituto Chico Mendes de Conservação da Biodiversidade, responsible for the management of the Federal PAs: Anavilhanas NP, the Tapajós-Arapiuns RESEX and the Tapajós FLONA (permission SISBIO 25347), according to the Brazilian legislation for scientific research in Protected Areas.

The Human Research Ethics Committee (HRE) of the National Institute of Amazonian Research approved our protocol (501,473/2013), according to Brazilian legislation that involves access to traditional knowledge on biodiversity. All the interviewees signed the Free and Informed Consent Form.

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Biodiversity and Conservation

Electronic Supplementary Material

Contributions of traditional knowledge of Amazonian manatee (*Trichechus inunguis*) to the management of Brazilian Federal Protected Areas

Luciana Carvalho Crema • Vera Maria Ferreira da Silva • Maria Teresa Fernandez Piedade

Questionnaire

1. Are you aware of the occurrence of the manatee in the region? (yes) (not)
2. Have you seen the Amazonian manatee? (yes) (not)
3. In case you have already seen manatees, in which situation it was? (dead), (alive) (dead/alive). If dead, do you know the cause of death? (stranding and entanglements in fishing nets), (hit by boats), (stranding), (hunting), (others, which ones?) (Does not know).
4. Where did you see the manatee (which lake)? How many times? When (month/year)?
5. What time of year are manatees the most sighted? (receding-water), (drought), (rising-water), (flood), (do not know).
6. Do manatees move in groups? (yes) (not) (do not know). In the case of a positive response, how many manatees are sighted normally at the same time? (2), (3), (4), more ().
7. When was the last time you heard of a manatee that was hunted? (this year) (last year), (more than two years), (more than two years), (more than ten years), (do not know), Exact date (_____).
8. In the case of hunting, which form is predominant? (subsistence) (trade-sale or exchange).
9. What was harnessed from the animal? (meat), (skin), (oil), other (.....)
10. What was done with the oil that was extracted?
11. What was done with the skin?
12. Which hunting instrument is most used? (harpoon), (nets), (other.....), (do not know).
13. Which time of year are the manatees most hunted? (receding-water), (drought), (rising-water), (flood), (do not know).
14. What animal is most hunted? (male), (female), (both). If it is a single answer (male or female), what is the reason?
15. Does the manatee carry out movements (migration)? (yes), (not), (do not know). Explain this.
16. If so, what is the time of year that these movements occur? (receding-water), (drought), (rising-water), (flood), (do not know).
17. According to the time of year, could you indicate to where the manatees go? (várzea), (terra firme lakes), (others/which ones?) _____
18. When are the pups of manatee born? (receding-water), (drought), (rising-water), (flood), (do not know).
19. Generally, with how many calves, adult manatees are seen?
20. Have you already seen manatees' "comedia"? (yes) (not) (do not know). If so, where? Which plant?
21. Do you know which plants the manatee feeds? (yes) (not)
22. If so, can you name the plants?
23. Could you identify the plants you mentioned on the photo board?.....
24. Is there any other plant from which the manatee feeds that is not present on the photo board? (yes) (not). If so, which ones?
25. From which parts of each plant does it feed? (all), (leaf), (stem), (root), (mud), (flower), (fruit), (seed).
26. Have you ever seen or heard of cases where some manatees have eaten a toxic plant and caused death of the animal? (yes) (not). Which plant?
27. Have you ever seen manatee feces? (yes) (not). If so, where?
28. Can you indicate a location that you think is ideal for viewing the manatee? (yes) (not). If so, where?
29. Do you know anyone who may have manatee bones? (yes) (not). If so, who is this person? Where can we find it?

Capítulo 3

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Ontogenetic variation in the trophic ecology of Amazonian manatee, *Trichechus inunguis*, using stable isotopes

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Abstract

We assessed the feeding habits of the Amazonian manatee inhabiting blackwater (Negro River) and clearwater (Tapajós River) igapós (floodplain) using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of dentin and bone collagen from different ontogenetic classes. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of teeth did not vary with tooth position. Bones were more depleted in ^{13}C and ^{15}N compared to teeth, and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bone differed between classes. Food sources had $\delta^{13}\text{C}$ signatures typical of plants using the C_3 and C_4 photosynthetic pathways. Mixing models showed that nursing females (inferred from isotopic values from nursing calves) had a preference for the consumption of C_4 plants. Juveniles had a mixed diet, while adults consumed primarily C_3 -plants in both the Tapajós and Negro igapó. Because of the low abundance of C_4 -plants in the Negro, we hypothesize that the C_4 signal of calves results from females' movement to várzea, abundant in C_4 -species. Results demonstrate that manatee diets vary with ontogenetic classes and water typology. Individuals from Tapajós may be resident, as C_3 and C_4 plants are available during the flood pulse. Preservation of both habitats (igapó and várzea) is therefore required for the survival of manatees.

Key-words: diet, aquatic mammal, feeding ecology, Sirenia, Bayesian mixing models, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Introduction

The Amazonian manatee (*Trichechus inunguis*) is an endemic and highly vulnerable species that is currently on Brazil's official list of endangered species (MMA, 2014) and in the Red List of the International Union for Conservation of Nature (IUCN, 2016). The species is essentially fluvial and herbivorous, occurring in the headwaters of the Amazon basin, in Colombia, Ecuador and Peru (Best, 1984), and in all main rivers of the Brazilian Amazon, with their extent limited by large rapids and waterfalls (Domning, 1981; da Silva et al., 2008). The distribution of the species in the Amazon basin is related to the seasonal flood pulse (Junk & da Silva, 1997) and the availability in the regions' distinct types of floodplains (várzea and igapó, Prance, 1980). Previous studies of manatee diet in Brazil indicated that they predominately eat emergent aquatic plants (Best, 1981; Colares & Colares, 2002; Guterres-Pazin et al., 2014), while in Peru, manatees are found to consume more free-floating aquatic species (Marmol, 1976). Although the feeding ecology of the species has been assessed, studies to date were mainly based on fecal analysis and stomach contents, precluding the evaluation of how feeding may vary among sexes or ontogenetic classes. The Amazonian manatee is also known to migrate long distances in response to changes in water level, which modifies the availability of food and suitable habitats for protection and survival, particularly in low water periods (Best 1981; 1983 Arraut et al., 2010). However, Guterres-Pazin et al. (2014) based on the analysis of the feeding habits of the manatee postulated that the trophic pressure alone may not be the main trigger for manatee migrations, but can be associated to the physical constraints of the environment and nutritional value of plants.

Igapó floodplains are seasonally inundated by black or clearwater rivers that drain ancient geological formations and exhibit low to intermediate levels of nutrients and acidity (Fittkau, et al. 1975; Sioli, 1984; Ayres, 1986). Trees and shrubs colonizing igapós have lower growth rates and higher wood densities than those in várzeas (Schöngart et al., 2010). In blackwater igapós, floating or emergent species, as in the Nymphaeaceae, Marantaceae, Araceae and Pontederiaceae families (Junk, 1983, Ferreira, 2000, Lopes et al., 2014) are common. In contrast, clearwater igapós favour not only emergent species, but also submerged ones, due to greater water transparency (Junk, 1983).

The identification of food sources through traditional methodologies, such as direct observation or analysis of stomach and feces contents have several disadvantages. They reflect only short-term diets, and overestimate the importance of more slowly digested materials (Alves-Stanley & Worth, 2009; Bowen & Iverson, 2013). The observation of feeding behavior is difficult in Amazonian manatee due to its cryptic

behaviour, and the collection of stomach contents and feces is challenged by logistical and cost issues (Alves-Stanley et al., 2010; Ciotti et al., 2014). In this context, the use of alternative and complementary methods such as stable isotopes, provide a powerful tool for studying the foraging ecology of manatees. Indeed, stable isotopes of carbon and nitrogen are commonly used in ecological studies examining diet and trophic level of a variety of organisms including groups of aquatic mammals (Abend & Smith, 1995; Pablo-Rodrigues et al., 2016; Secchi et al., 2016).

The analysis of stable isotopes of consumers provides information on the assimilated food, and may not exactly represent the volume of ingested materials. Isotopic signatures of subaerial plants mainly reflect their photosynthetic pathways, C₃ (from -24‰ to -38‰) or C₄ (from -11‰ to -15‰) (Smith & Epstein, 1970). Aquatic plants can incorporate HCO₃⁻ (dissolved inorganic carbon) in addition to CO₂ during photosynthesis, (Smith & Walker, 1980; Osmond et al., 1981). Bicarbonate (HCO₃⁻) is typically enriched in ¹³C (by 7–11‰) compared to dissolved CO₂ (Mook et al., 1974; Maberly & Madsen, 1998; 2002; Lamb et al., 2006). Variations in ¹³C isotopic signature can also reflect environmental conditions (Boon & Bunn, 1994) as well as differences in the chemical makeup (i.e. lignin versus cellulose) in plant tissues (Clementz et al., 2006).

Mammals have tissues with different compositions, growth rates and turnover rates (Newsome et al., 2010) that can further add variability in bulk stable isotope content. Active metabolic tissues such as fat, skin, muscle, hair, red blood cells, and plasma turn over rapidly and reflect recently consumed materials, while less active tissues, such as bone, can integrate diet ingested over a long period of time (Tieszen et al., 1983, Hobson & Clark, 1992; Clementz et al., 2007). Consequently, analysis of stable isotopes allows both short-term and long-term estimation of trophic ecology by sampling tissues with different turnover rates. For tissues like bones and teeth, obtaining information integrates several years of feeding and offers the possibility of using samples from zoological collections (Walker & Macko, 1999). These are valuable advantages for a species such as the Amazonian manatee that is difficult to sample and study.

All Sirenians including Amazonian manatees have teeth that continuously grow and are replaced, in order to compensate for the wear caused by the chewing of plants with high silica content (Domning & Magor, 1978; Domning, 1983; Domning & Hayek, 1986). The development of teeth occurs quickly and the isotopic composition reflects food assimilated during their formation (Walker & Macko, 1999). Because Amazonian manatees have from five to seven functional teeth in each arcade (Husar, 1978) and they are formed in different periods of time, it is possible to access a history of diet over

five to six years from the complete set of teeth in a single jaw. This fact may allow the tracking ontogenetic changes, which may be related to environmental or behavioural aspects of the animal, such as migration, resource selection by energy demand or food preference (Clementz et al., 2007; Ciotti et al., 2014).

Although stable isotopes have already been used in several studies of the Florida manatee - *T. manatus latirostris* (Ames et al., 1996; Walker & Macko, 1999; MacFadden et al., 2004; Reich & Worthy 2006, Alves-Stanley & Worthy 2009), and the Antillean manatee - *T. m. manatus* (Ciotti et al., 2014; Pablo-Rodrigues et al., 2016), they were not to date applied to study wild Amazonian manatees. Our study aimed to use stable isotopes to investigate if feeding ecology and diet of Amazonian manatees, reflect the type of water habitat (black or clearwater igapó), and assess the potential to use stable isotopes to track animal movements. More specifically, we analyzed stable isotopes in teeth and bone collagen to: (1) evaluate the intra and interindividual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in teeth and bones and (2) evaluate the diet composition of Amazonian manatees from black and clearwater igapós and its variation with broadly defined ontogenetic classes (nursing females, juveniles, adults).

Material and methods

Studied areas and sample collection

The Tapajos River (Tapajós; Fig. 1) is one of the greatest Amazon river tributaries, flowing from the state of Mato Grosso to the state of Pará, southeast to northeast. It has an approximate length of 2,000 km, an average discharge of around 12,440 m³ s and it is classified as a clearwater river, with characteristics [pH 6.5 ± 0.4; conductivity= 14.4 ± 13.1 μS cm, total dissolved solids= 7.7 ± 5.6 mg l] that lie between those of the Amazonas and the Negro rivers (Duncan & Fernandes, 2010)]. The Negro River (Negro) flows through approximately 1,700 km from its source at the Eastern plains of Colombia until its mouth, with an average discharge of 28,000 m³ s. It is the principal representative of Amazonian blackwater rivers (conductivity from 9.3 to 9.9 μS.cm⁻¹), with an acidic pH (4.8 – 5.1), resulting from high levels of humic and fulvic substances, being poor in nutrients and electrolytes (Goulding *et al.*, 1988; Furch and Junk 1997) (Fig. 1).

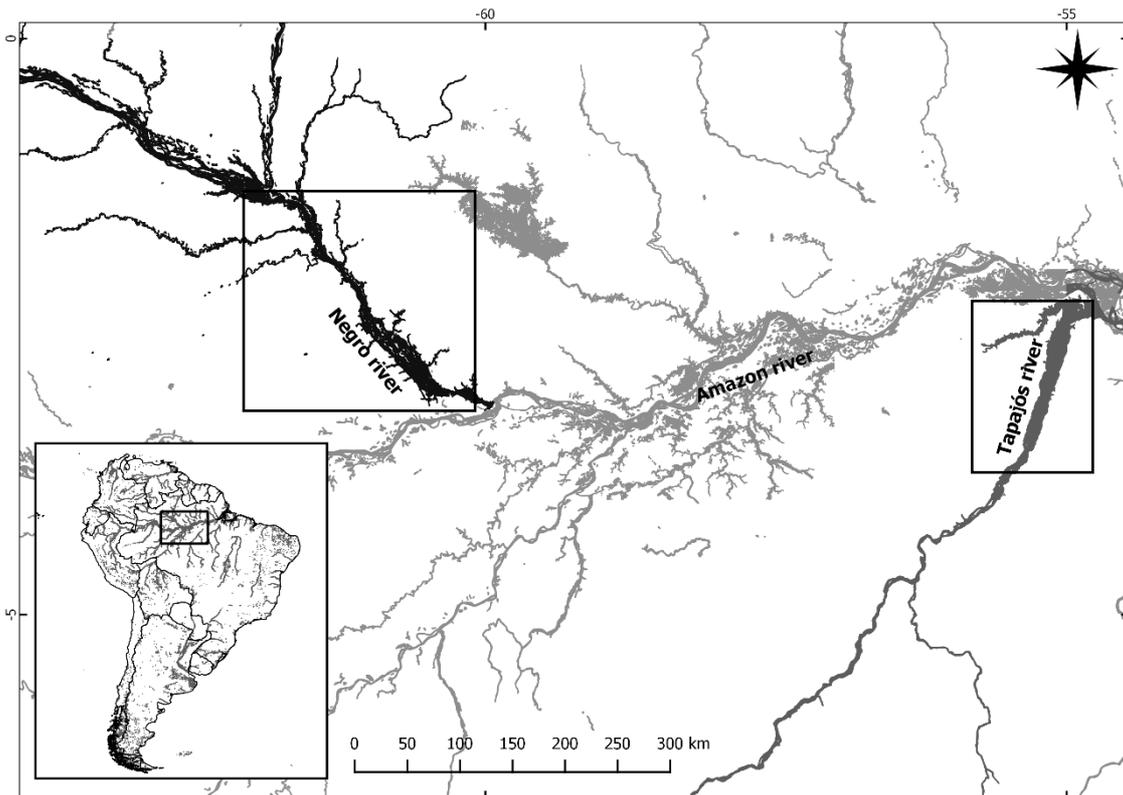


Fig.1 Map showing the origin of the samples in Negro and Tapajós Rivers, in relation to the Amazon River, Amazonia, Brazil.

Teeth and bone samples of Amazonian manatees used for this study were collected from the igapó floodplain forests of the lower Tapajós River (Tapajós, n=17) and the lower Negro River (Negro, n=19) and archived in zoological collections (Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos - Instituto Chico Mendes de Conservação da Biodiversidade, Zoológico das Faculdades Integradas do Tapajós and Coleção de Mamíferos Aquáticos - Instituto Nacional de Pesquisas da Amazônia). Most of the animals in these collections are derived from illegal and accidental hunting, so the information about them, i.e. exact location of collection, is often scarce.

A maximum of six and a minimum of two functional molars recorded as M1 (oldest molar, in the most anterior position of the jaw) to M6 (newest molar, in the most distal position), according to the position in the hemimandible (Ciotti et al., 2014), were removed from 14 animals from Tapajós and 9 individuals from Negro. Additionally, approximately 3.0 cm³ of bone samples (B) were taken from the right mandibular condyle. When this bone was not available, samples from ribs, skull or scapula were collected (Table S1 in Online Resource). Differences in collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of different bones from the same individual are considered small (DeNiro & Schoeninger, 1983) and therefore samples obtained from different bones in the same

individual were assumed to be equivalent. Specimens were classified into three ontogenetic classes according to the ossification pattern of the basicranial sutures in the genus *Trichechus* (Hoson et al., 2012; Valdevino, 2016) and the wear of the teeth: lactating calves (suture open with no ossification and teeth with no wear); juveniles (suture partially closed to closed, but still clearly observed and a small wear on the teeth); and adults (suture partially obscured by ossification or closed and completely obliterated and a strong wear on teeth). Information on sex, collection site and age class of samples are presented in Table S1 in Online Resources.

Samples of plants reported as food sources for the manatee (Best, 1981; da Silveira, 1988; Colares, 1991; Colares & Colares, 2002; Zaniolo, 2006; Franzini et al., 2013; Guterres-Pazin et al., 2014, Chapter 2.) were collected in igapós from the Tapajós (18 samples) and Negro (27 samples) rivers throughout all phases of the seasonal flood pulse (rising water, flood, receding water and drought) between 2012 and 2014. Plants and phytoplankton (seston) were collected directly from the vegetated banks at the margins of lakes (always in the interface between aquatic and terrestrial ecosystems) and subaquatic species were collected by scuba diving. The type of structure collected (e.g. leaves, roots, etc.) varied according to the species (Table S2 in Online Resource). Isotopic data from plants from várzea and periphyton (attached filamentous algae) from igapó were obtained from the literature (Marshall et al., 2008; Oliveira et al., 2006).

Stable Isotope Analysis

Tooth and bone samples were prepared for stable isotope analysis following the protocol described by Walker & Macko (1999). Samples were cleaned to remove residues and external soft tissues, rinsed in distilled water, and oven dried at 60°C for 48 h. Teeth were cut longitudinally with a diamond wafer blade mounted on a low speed saw to expose the dentine and then powdered with a drill bit. Bones were cleaned, cut with a manual saw and then powdered with a drill bit. As these tissues consist of organic (collagen) and mineral (bioapatite) components (Koch, 2007), samples of bones and teeth were acidified with 30% hydrochloric acid (HCl) to remove inorganic carbon, and then oven-dried again at 60°C for 2 h to remove residual acid. Plant and phytoplankton (seston) samples were cleaned with distilled water, oven dried at 60°C for 48 h and ground with mortar and pestle.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses of all samples were performed at the Max-Planck-Institute for Biogeochemistry (Germany). The analyses were conducted on a Delta+ isotope ratio mass spectrometer (MAT Finnigan, now Thermo Finnigan) coupled to an

EA1100 CN elemental analyzer (CE Instruments, Rodano, Italy) via a Conflow III open split interface. Approximately 1.0 mg (for $\delta^{13}\text{C}$) and 4.0 mg (for $\delta^{15}\text{N}$) of tooth/bone collagen or plant powdered samples were packed in tin capsules from IVA (Viersen, Germany) and combusted at 1,020°C. The reduction furnace was kept at 650°C, and the reflow rate was 85 ml min. Samples were referenced using in house standards, that have themselves been calibrated against NBS19 and LSVEC (for $\delta^{13}\text{C}$) and against IAEA-N-1 and IAEA-N-2 (for $\delta^{15}\text{N}$). Details on data handling can be found in Werner and Brand (2001).

The isotopic ratios were expressed in *per mil* (‰) using delta notation: $\delta x = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000$, where X refers to ^{13}C or ^{15}N , R_{sample} is the ratio of isotopes of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$), and R_{standard} is the ratio of isotopes of the standard (VPDB–Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$). The daily measurement precision was better than 0.67‰ SD for $\delta^{13}\text{C}$ and 0.33‰ SD for $\delta^{15}\text{N}$ analyses.

Statistical analyses

Isotopic data were tested for normality and homogeneity of variances using Shapiro-Wilk's and Levene's tests, respectively. Spearman's coefficient was used to test whether there was an intraindividual correlation between $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values and the positions of the teeth in the dental arcade (M1 to M6) for lactating calves, juveniles and adults of each area. Kruskal-Wallis' test was used to compare $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values among teeth of different positions (M1 to M6) within the hemimandible in manatees of the same ontogenetic class.

Additionally, Kruskal-Wallis' test was used to compare values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bones (B) and teeth (only the M3 position) between lactating calves, juveniles and adults from Tapajós and Negro rivers. For teeth from the Negro river, we used the Mann-Whitney *U* test because we had only very few data from the juvenile class. Wilcoxon matched-pairs test was used to compare $\delta^{13}\text{C}$ and in $\delta^{15}\text{N}$ values from teeth (median among M1 to M6) and bones. The Mann-Whitney *U* test was used to compare $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values in teeth and bones from each ontogenetic class (lactating calves, juveniles and adults) between Tapajós and Negro River basins.

The Kruskal-Wallis test was used to compare mean stable isotope values of feeding sources for manatees from Tapajós and the Student's t-test for independent samples by group was used to compare our data from Negro (C_3 plants) with periphyton (attached filamentous algae) data published by Marshall et al. (2008).

Mixing Models and Diet Composition

Bayesian mixing models for stable isotope data (simmr package; Parnell, 2017, a package developed for the R statistical environment (R Development Core Team, 2016), were used to estimate the relative contribution of plants with differing isotopic signatures to the manatee diet. These models address natural variation and uncertainty of stable isotopic compositions to generate probability distributions of the sources' proportional contribution to the diet of the consumer (Parnell et al., 2010). Each model was run using 10,000 iterations thinned by 20, with an initial discard of 1,000 iterations.

Separate models were run for manatees from the Negro and Tapajós rivers. Bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were used for modelling manatee's diet due to the larger sample size for this tissue (see Table S1 in Online Resource). Stable isotope values for each area were classified in three groups: nursing females, juveniles and adult manatees. Isotopic values for nursing females were inferred from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of calves, to which we added a trophic enrichment factor correction of -2‰ for $\delta^{15}\text{N}$ values in order to account for the discrimination against the light isotope during the assimilation of milk by the offspring (Newsome et al., 2006; 2009; Drago et al., 2015). No trophic correction was applied in the case of $\delta^{13}\text{C}$ values.

Source inputs were grouped *a priori* according to similarities of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Four groups were included as sources for Tapajós manatees: C_3 rooted submerged, other C_3 (terrestrial, emergent, amphibious, free floating, free floating submerged, rooted submerged with floating leaves; see Table S2 in Online Resource), C_4 plants and periphyton (Table 1). For modelling the diet of Negro river manatees, five groups were considered: C_3 and C_4 plants (see Table S2 in Online Resource) and algae (isotopic data reported in Marshal et al., 2008) from igapós, and C_3 and C_4 plants from várzea (mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values reported in Oliveira et al. 2006). The latter were included due to the potential movement and feeding of Negro animals into this region during the flood pulse (Arraut et al., 2010).

Table 1 Mean \pm standard deviation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of feeding sources used in the Bayesian mixing models to estimate the diet of the Amazonian manatee in lakes of the lower Tapajós and Negro River basins.

Ecosystem	Sources	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	Reference
Igapó of Tapajós river	rooted submerged	-33.9 ± 2.8	5.1 ± 3.9	5	This study
	Phytoplankton (seston)	-38.5 ± 2.3	5.0 ± 0.2	2	This study
	other C_3	-29.6 ± 2.5	4.5 ± 1.7	9	This study
	C_4	-13.0 ± 0.5	5.0 ± 2.7	4	This study
Igapó of Negro river	C_3	-30.7 ± 1.2	5.2 ± 2.5	2	This study
	Periphyton (attached filamentous algae)	-37.6 ± 4.1	4.0 ± 2.0	2	Marshall et al. 2008
Várzea (Amazon river)	C_3	-29.1 ± 1.2	4.5 ± 2.0	1	Oliveira et al. 2006
				6	
	C_4	-12.9 ± 0.7	6.7 ± 0.1	2	Oliveira et al. 2006

After running the models, pairwise correlations between estimated source contributions (matrix output plot) were explored and we detected highly negative correlations between sources. These negative correlations are usually present whenever sources overlap or are opposed in the isotopic space and indicate that the model may be having difficulty in distinguishing between them (Parnell et al., 2010). When identified, the proportions for negatively correlated sources were combined *a posteriori* (Phillips et al., 2014). There are no experimentally determined diet-collagen discrimination factors for manatees, thus based on diet similarities and taxonomic proximity, Trophic Discrimination Factor (TDF) values calculated for the giant panda (*Ailuropoda melanoleuca*) were used ($6.1 \pm 0.5\%$ for $\delta^{13}\text{C}$ and $2.3 \pm 0.5\%$ for $\delta^{15}\text{N}$ [Han et al., 2016]). Clementz et al. (2007) calculated similar TDF values for Antillean manatees (*T. manatus*) identified as freshwater foragers as the difference between mean isotopic values of bone collagen and $\delta^{13}\text{C}$ values of dietary resources. Their TDF values ($5.9 \pm 1.8\%$) were similar to those we used, thus allowed us to be confident in applying these values in mixing models for both Tapajós and Negro manatees.

Results are presented as mean \pm standard deviation, and statistical analyses were considered significant for $p < 0.05$. All statistics were run within R 3.2.3 (R Development Core Team, 2016).

Results

Isotopic composition in bones and teeth

Differences among teeth in the dental arcade

Manatees continuously replace their teeth, with a tooth movement rate, for captive animals, of approximately 1mm/mo after the lost of the first tooth. It begins with the weaning of the calve, when the animal starts to ingest solid food; thus, an individual would produce approximately 27 teeth in each dental arcade in 20 years, which

represents approximately a new tooth formation every nine months (Domning & Kayek, 1986). In general, carbon (Table 2) and nitrogen (Table 4) stable isotope compositions of manatee's teeth showed only small variations among teeth in a single individual but large differences between individuals. However, two manatees from Tapajós (# 2 and 8) and three individuals from Negro (# 33, 34 and 35) - all lactating calves – had variations in $\delta^{13}\text{C}$ between teeth in the same individual that were greater than 2‰. For $\delta^{15}\text{N}$ values just one manatee from Tapajós (individual #9) had differences greater than 2‰ between teeth within the same individual (Table 3).

There were no significant correlations between $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values and the position of teeth in the dental arcade of lactating calves (Spearman, $r_s=-0.05$, $p=0.74$ for $\delta^{13}\text{C}$ and $r_s=0.26$, $p=0.06$ for $\delta^{15}\text{N}$), juveniles (Spearman, $r_s=-0.07$, $p=0.71$ for $\delta^{13}\text{C}$ and $r_s=-0.15$, $p=0.43$ for $\delta^{15}\text{N}$) and adults (Spearman, $r_s=0.008$, $p=0.97$ for $\delta^{13}\text{C}$ and $r_s=0.21$, $p=0.30$ for $\delta^{15}\text{N}$). Also, there were no differences in $\delta^{13}\text{C}$ or in $\delta^{15}\text{N}$ values among teeth of different positions of nursing calves (Kruskall-Wallis, $H_{5,52}=1.97$ $p=0.99$ for $\delta^{13}\text{C}$ and $H_{5,52}=7.12$ $p=0.21$ for $\delta^{15}\text{N}$), juveniles (Kruskall-Wallis, $H_{5,28}=0.33$ $p=0.99$ for $\delta^{13}\text{C}$ and $H_{5,28}=2.97$ $p=0.70$ for $\delta^{15}\text{N}$) and adult animals (Kruskall-Wallis, $H_{4,26}=1.00$ $p=0.90$ for $\delta^{13}\text{C}$ and $H_{4,26}=1.72$ $p=0.78$ for $\delta^{15}\text{N}$).

Table 2 $\delta^{13}\text{C}$ values (‰) in the Amazonian manatee teeth (M1 - oldest to M6 - youngest) and bones (B) from Tapajós and Negro rivers. Mean \pm standard deviation and difference between maximum and minimum values are indicated. Ages are represented for lactating calves (LC), juveniles (J) and adults (A).

River	Animal	Age	M1	M2	M3	M4	M5	M6	Mean \pm SD	Difference	B
Tapajós	2	LC		-15.6	-14.6	-12.2	-12.2	-12.3	-13.4 \pm 1.6	2.4	-12.6
	8	LC	-14.9	-16.0	-15.5	-14.0	-16.7	-17.3	-15.7 \pm 1.2	3.3	-18.1
	10	LC		-12.1	-12.1				-12.1 \pm 0.0	0.0	-15.2
	12	LC		-20.1	-19.8	-19.7	-18.6	-18.4	-19.3 \pm 0.7	1.7	-21.2
	13	LC	-19.6	-19.1	-19.0	-19.0	-18.8		-19.1 \pm 0.3	0.8	-18.4
	15	LC			-19.0	-18.9	-18.5	-18.5	-18.7 \pm 0.2	0.5	-19.7
	1	J		-28.6	-29.0	-29.5	-29.8	-29.7	-29.3 \pm 0.5	1.2	-32.2
	6	J		-20.1	-20.8	-20.4	-21.1		-20.6 \pm 0.5	1.1	-21.6
	7	J		-13.7	-13.9	-13.4	-13.5	-13.1	-13.5 \pm 0.3	0.8	-15.7
	9	J	-16.5	-15.7	-15.7	-16.1	-16.1		-16.0 \pm 0.3	0.5	-15.8
	11	J		-17.7	-19.8	-17.6	-18.4		-18.1 \pm 0.6	1.3	-19.3
	14	J	-25.4	-25.2	-25.3	-25.5	-26.1		-25.5 \pm 0.3	0.9	-27.9
	3	A		-25.9	-26.1	-25.6	-24.8		-25.6 \pm 0.6	1.3	-29.3
	16	A			-29.4	-29.1	-28.6	-28.7	-29.0 \pm 0.4	0.8	-30.5
	Negro	32	LC		-15.7	-15.7	-15.7	-15.6	-14.9	-15.5 \pm 0.4	0.8
33		LC		-14.3	-13.4	-15.0	-17.8		-15.1 \pm 1.9	4.4	-15.4
34		LC	-17.4	-16.9	-18.2	-17.1	-16.9	-21.3	-18.0 \pm 1.7	4.4	-17.8
35		LC	-13.6	-13.1	-15.1	-16.2	-14.1	-15.7	-14.6 \pm 1.2	3.1	-13.9
21		J			-16.5	-16.2	-16.3	-17.6	-16.7 \pm 0.7	1.4	-20.2
19		A			-27.9	-27.7	-27.3	-27.4	-27.6 \pm 0.3	0.6	-27.1
25		A		-31.3	-30.4	-29.9	-30.2	-31.0	-30.5 \pm 0.6	1.1	-31.3
26		A		-26.9	-27.5	-27.4	-27.4	-27.0	-27.3 \pm 0.3	0.6	-29.0
31		A			-30.4	-30.1	-29.7	-29.4	-29.9 \pm 0.4	1.0	-32.3

Table 3 $\delta^{15}\text{N}$ values (‰) in the Amazonian manatee teeth (M1 - oldest to M6 - youngest) and bones (B) from Tapajós and Negro rivers. Mean \pm standard deviation and difference between maximum and minimum values are indicated. Ages are represented for lactating calves (LC), juveniles (J) and adults (A).

River	Animal	Age	M1	M2	M3	M4	M5	M6	Mean \pm SD	Difference	B	
Tapajós	2	LC		8.3	8.6	8.8	9.5	10.1	9.1 \pm 0.7	1.8	8.3	
	8	LC	9.5	9.3	9.3	8.8	9.5	10.3	9.5 \pm 0.5	1.5	8.3	
	10	LC		9.4	9.3				9.3 \pm 0.1	0.1	8.2	
	12	LC		10.0	9.7	9.5	9.3	8.9	9.5 \pm 0.4	0.7	7.9	
	13	LC	8.7	9.0	9.0	9.2	9.4		9.1 \pm 0.2	0.4	8.7	
	15	LC			9.0	9.4	9.6	9.9	9.5 \pm 0.4	1.0	9.3	
	1	J		9.0	8.3	8.4	8.2	7.6	8.3 \pm 0.5	0.8	8.3	
	6	J		10.3	10.1	10.3	10.4		10.3 \pm 0.2	0.3	9.0	
	7	J		9.7	9.4	9.7	9.7	9.3	9.5 \pm 0.2	0.4	7.6	
	9	J	7.7	10.3	9.4	9.3	9.9		9.3 \pm 1.0	2.6	8.2	
	11	J	8.5	8.3	8.4	8.5			8.4 \pm 0.1	0.2	6.8	
	14	J	10.4	10.5	10.0	10.3	10.3		10.3 \pm 0.2	0.5	8.6	
	3	A			8.3	8.0	8.4	8.5		8.3 \pm 0.2	0.2	7.1
	16	A				8.6	9.0	9.0	9.2	9.0 \pm 0.3	0.6	7.3
	Negro	32	LC		9.7	9.3	9.9	10.1	9.9	9.8 \pm 0.3	0.8	10.2
		33	LC		9.3	9.2	9.5	9.4		9.3 \pm 0.1	0.3	8.1
34		LC	10.8	11.0	11.1	11.2	11.2	11.5	11.2 \pm 0.2	0.7	10.9	
35		LC	11.1	11.2	10.8	11.1	11.7	11.5	11.2 \pm 0.3	0.9	10.7	
21		J			9.7	9.18	9.82	10.2	9.9 \pm 0.3	0.5	9.8	
19		A			7.4	7.7	7.8	8.0	7.7 \pm 0.2	0.6	6.6	
25		A		5.7	5.7	5.9	5.8	5.2	5.7 \pm	0.6	3.8	
26		A		6.6	7.4	7.4	7.3	7.5	7.20 \pm 0.4	0.9	6.3	
31		A		8.9	9.6	9.1	9.3	9.4	9.3 \pm 0.2	0.6	7.3	

Isotopic differences among ontogenetic classes

Carbon and nitrogen isotopic compositions in teeth of animals from Tapajós were similar among ontogenetic classes ($\delta^{13}\text{C}$ - Kruskal-Wallis $H=5.34$, $p=0.07$; $\delta^{15}\text{N}$ - Kruskal-Wallis, $H=2.34$, $p=0.30$) (Table 4). For bones, lactating calves were more enriched in $\delta^{13}\text{C}$ than adults (Kruskal-Wallis $H_7=6.06$, $p=0.05$), but there were no significant differences in the isotopic composition of nitrogen between ontogenetic classes (Kruskal-Wallis, $H=3.63$, $p=0.16$) (Table 4). For animals from the Negro river, lactating calves had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that were more enriched in the heavier isotopes than adults in both teeth (Mann-Whitney U , $Z=2.31$ $p=0.02$) and bones (Kruskal-Wallis $H_{2,19}=8.85$, $p=0.01$ for $\delta^{13}\text{C}$ and $H_{2,19}=8.71$, $p=0.01$ for $\delta^{15}\text{N}$) (Table 4).

Differences between teeth and bones

There were significant differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between paired teeth (median between M1 to M6 of each individual) and bones of manatees (Figs. 2a and 2b). Bones from the same individual were more depleted in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than teeth (see Tables 3 and 4).

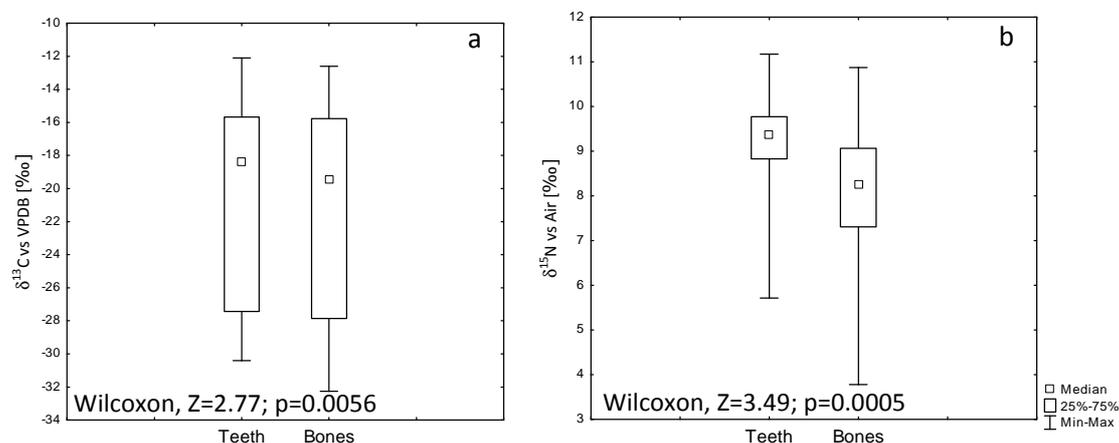


Fig. 2 Box plots (Median, 25-75%, min-max): a- of $\delta^{13}\text{C}$ and b- $\delta^{15}\text{N}$ of teeth and bones from Amazonian manatees from Tapajós River and Negro River.

Table 4 Means, standard deviations and sample numbers of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (in ‰) in teeth (M3) and bones of Amazonian manatees from Tapajós and Negro rivers. Age classes were divided in lactating calves (LC), juveniles (J) and adults (A).

River	Age	M3 Teeth				n	Bones				n
		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$			$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		
		Mean±SD	Min/Max	Mean±SD	Min/Max		Mean±SD	Min/Max	Mean±SD	Min/Max	
Tapajós	LC	-16.3±2.7	-19.0/-12.1	8.9±0.4	8.4/9.3	06	-17.5±3.1	-21.2/-12.6	8.3±0.6	7.6/9.3	06
	J	-20.4±5.8	-28.9/-13.9	9.3±0.8	8.2/10.4	06	-20.9±6.9	-32.2/-13.7	8.5±0.8	6.8/9.1	07
	A	-24.6±6.8	-29.4/-26.1	8.2±0.5	7.9/8.6	02	-26.3±4.2	-30.5/-22.6	7.5±0.8	6.8/7.3	04
	All	-19.8±5.6	-29.4/-12.1	9.1±0.6	8.2/10.4	14	-21.0±5.9	-32.2/-12.6	8.2±0.8	6.8/9.3	17
Negro	LC	-15.2±2.0	-18.1/-13.4	10.1±1.0	9.2/11.1	04	-15.9±1.6	-17.8/-13.9	10.0±1.2	8.1/10.9	04
	J	-16.4		9.7		01	-23.6±4.2	-28.4/-20.2	8.5±2.7	5.4/10.4	03
	A	-29.1±1.6	-30.4/-27.5	7.4±1.3	5.7/8.9	04	-25.2±4.2	-31.3/-17.5	6.5±1.2	3.8/8.1	12
	All	-21.7±7.2	-30.4/-13.4	8.9±1.7	5.7/11.1	09	-23.0±5.3	-31.2/-13.9	7.6±2.0	3.8/10.9	19

Isotopic differences between the two sampling areas

No significant differences were found among isotopic compositions of carbon and nitrogen in any age class for bones or teeth of manatees from the Tapajós and Negro rivers (Figs. 3a, 3b and 3c).

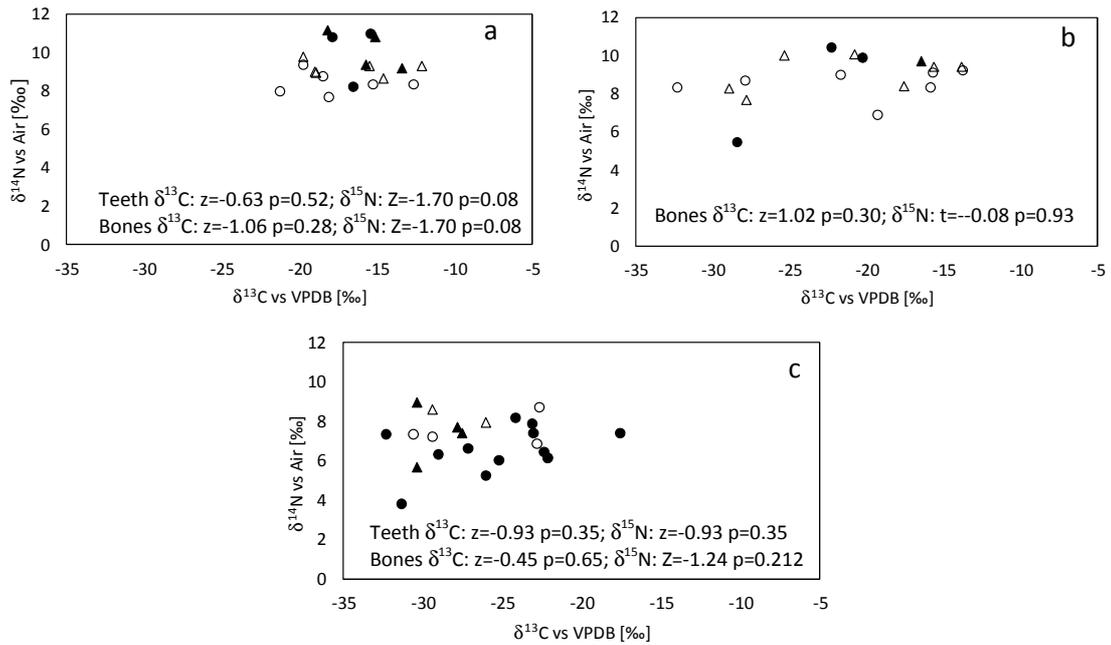


Fig. 3 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bones (\circ) and M3 teeth (Δ) of lactating calves (a), juveniles(b) and adults (c) of Amazonian manatees from the Tapajós river (open symbols) and Negro river (black).

Food Sources

Significant differences were found in Tapajós for $\delta^{13}\text{C}$ values (Kruskal-Wallis, $H=18.51$, $p=0.003$), but not for $\delta^{15}\text{N}$ (Kruskal-Wallis, $H=0.81$, $p=0.84$). We observed the expected differences in $\delta^{13}\text{C}$ values among plants with different photosynthetic pathways: phytoplankton (seston) and rooted submerged plants had the most depleted $\delta^{13}\text{C}$ values, followed by other C_3 plants. The most ^{13}C -enriched were the C_4 plants (Table 1, Fig. 4). In the Negro river, periphyton (Marshall et al., 2008) was more depleted in ^{13}C than C_3 plants ($t=-7.49$, $p=0.00$), but there was no significant difference in $\delta^{15}\text{N}$ ($t=1.58$, $p=0.12$) (Table 1, Fig. 4)

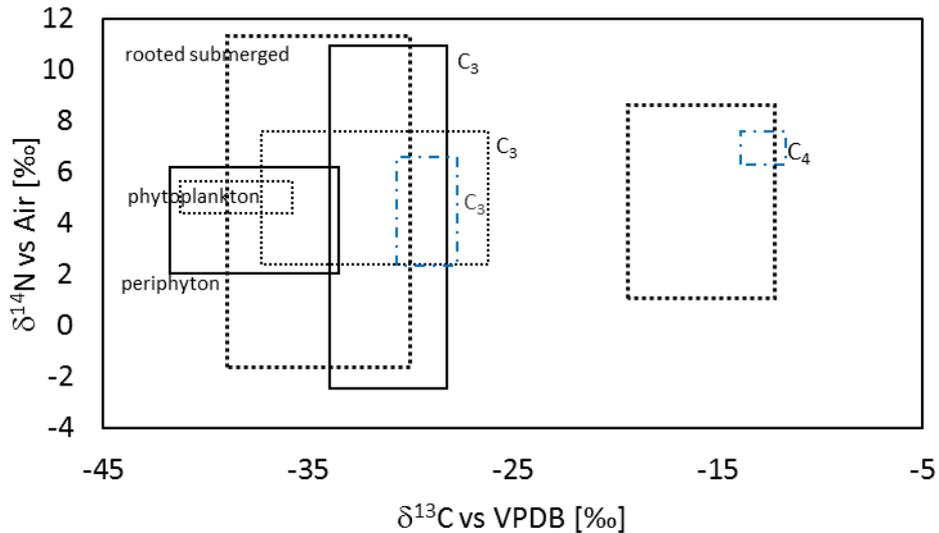


Fig. 4 Stable isotope values for food sources of the Amazonian manatee from lakes of the Tapajós (dotted line) and Negro (solid line) river basins, and the várzea (blue line). Data for periphyton was taken from Marshall et al. (2008) and those of várzea data from Oliveira et al. (2006).

Diet Composition based on stable isotopes

Negro River samples

Bayesian stable isotope mixing models showed that nursing females preferentially consumed C₄-plants with a mean proportion of 47% (95% credibility interval = 22-62%), followed by C₃-plants from várzea (19%, 2-47%), and igapó (18%, 2-45%) and periphyton (15%, 2-36%). The probability that the contribution of C₄-plants was higher than the periphyton in the nursing females' diet was high (0.97). For juveniles, the differences in the mean contribution of sources were small: C₃-plants from igapó (29%, 3-77%) and várzea (26%, 2-72%), periphyton (26%, 3-65%) and C₄ (19%, 2-53%). For adults, the highest source contributor was periphyton (0.41, 0.12-0.68), followed by C₃-plants from várzea (27%, 4-58%) and igapó (22%, 3-57%) and a small contribution of C₄-plants (10%, 2-21%). The probability that the contribution of periphyton was higher than C₄-plants in the adults' diet was high (99%).

Pairwise comparisons indicated strong negative correlations between C₃-plants from igapó and periphyton for nursing females (-0.40), juveniles (-0.46) and adults (-0.57). Because of this correlation, we summed these two sources into an "all-C₃ igapó" category (Fig. 6). The combined group contributed 33% (11-53%) of the diet of nursing females, 55% (13-89%) for juveniles and 63% (33-86%) for adults (Figs. 5a, 5b and 5c).

Overall, C₄-plants contributed the highest proportion (roughly two-thirds) to the diets of nursing females, but less than 19% to the diets of juveniles and adults. The

probability of “all-C3 igapó” having a larger contribution to adult’s diets than for nursing females and juveniles’ was relatively high (45%).

Tapajós River samples

Overall differences in the diet of manatees living in the Tapajos River were not as pronounced as those from the Negro river. Nursing females consumed C₄-plants with a mean proportion of 41% (95% credibility interval = 17–59), followed by other C₃-plants (28%, 4–62%), periphyton (16%, 2-40%) and submerged plants (14%, 2-37%). However, unlike in the Negro river, the probability that the contribution of C₄-plants was higher than the other sources in the nursing females’ diet was low (0.16%). For juveniles, the differences in the mean contribution of sources were small: C₄-plants (29%, 5-54%), other C₃-plants (25%, 3-68%), phytoplankton (seston) (24%, 4–62%) and submerged plants (22%, 3-52%). Adults consumed preferentially phytoplankton (seston) with a mean proportion of 41% (95% credibility interval = 6-75%), followed by other C₃-plants (26%, 3–69%), rooted submerged (19%, 2-55%) and C₄ plants (15%, 2-35%). The probability that the contribution of phytoplankton (seston) plants was higher than other sources in adults’ diet was low (13%).

Pairwise comparisons showed strong negative correlations between C₃-plants and phytoplankton (seston) for nursing females (-0.53), juveniles (-0,51) and adults (-0.64). Therefore, we summed these two sources *a posteriori* (Phillips et al. 2014) into the “C₃ all” category (Fig. 7). After combining these sources, the “C3 all” group contributed with a mean proportion of 44% (17–73%) for nursing females (Fig. 6a), 49% (20–82%) for juveniles (Fig. 6b) and 67% (29-91%) for adults (Fig. 6c).

Our results showed that the relative contributions of different plant sources to the diets of manatees from the Tapajós river was different between groups. The probability of C₄-plants being proportionately greater in the diet of nursing females was 60%. On the other hand, C₃ all plants had a probability of 40% of showing a greater contribution to adults’ diets than to nursing females. Rooted submerged plants did not present a great proportion in any ontogenetic class.

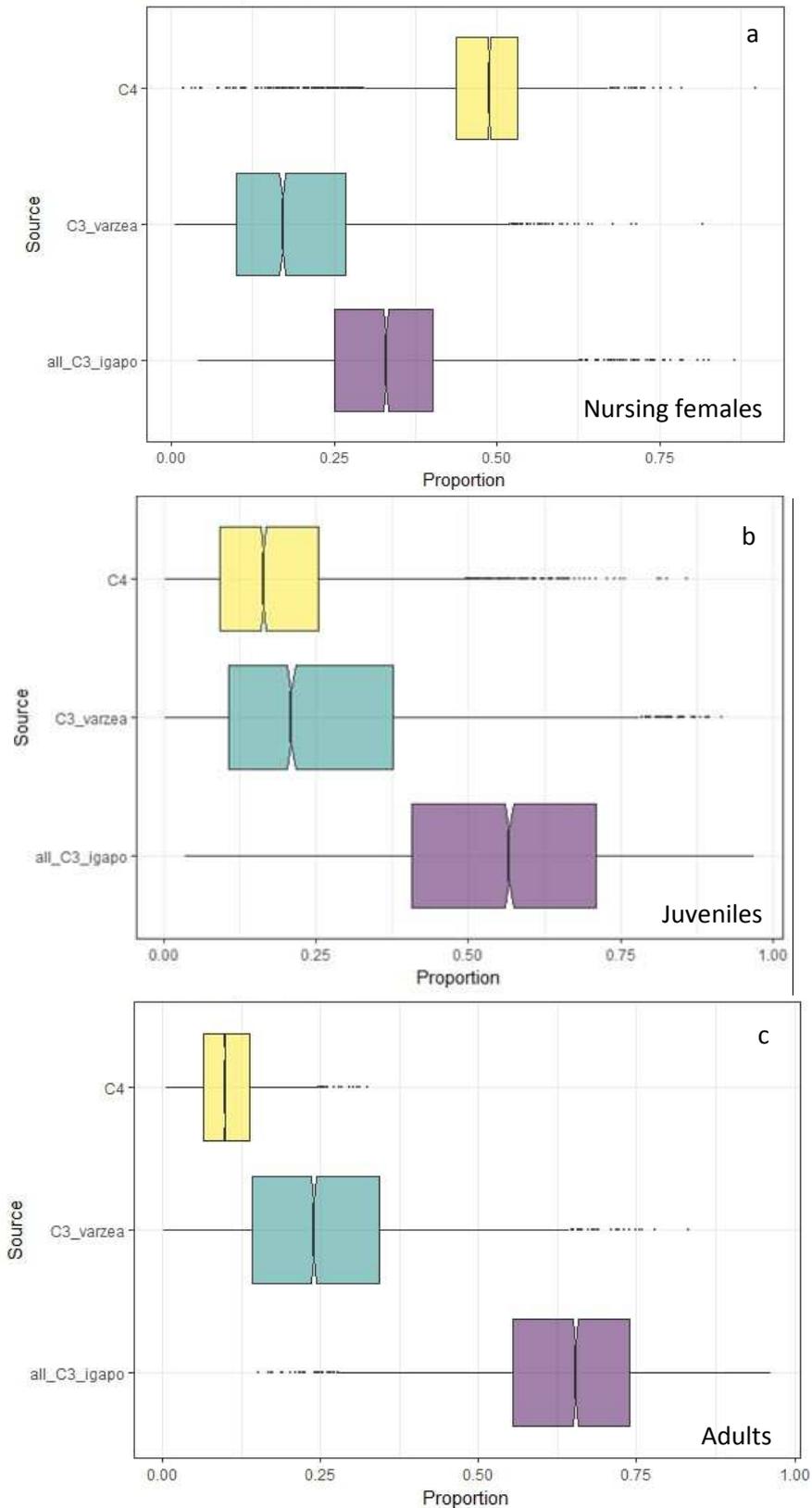


Fig. 5 Results of simmr Bayesian mixing models showing proportional estimates (mean, 25% and 75% percentiles) of diet composition of nursing females (a) juveniles (b) and adults (c) Amazonian manatees from Negro River following *a posteriori* combining of Periphyton and C3 igapó plants into “all C3 igapó” group. C4 (C_4 -plants from várzea), and C3_várzea (C_3 -plants from várzea).

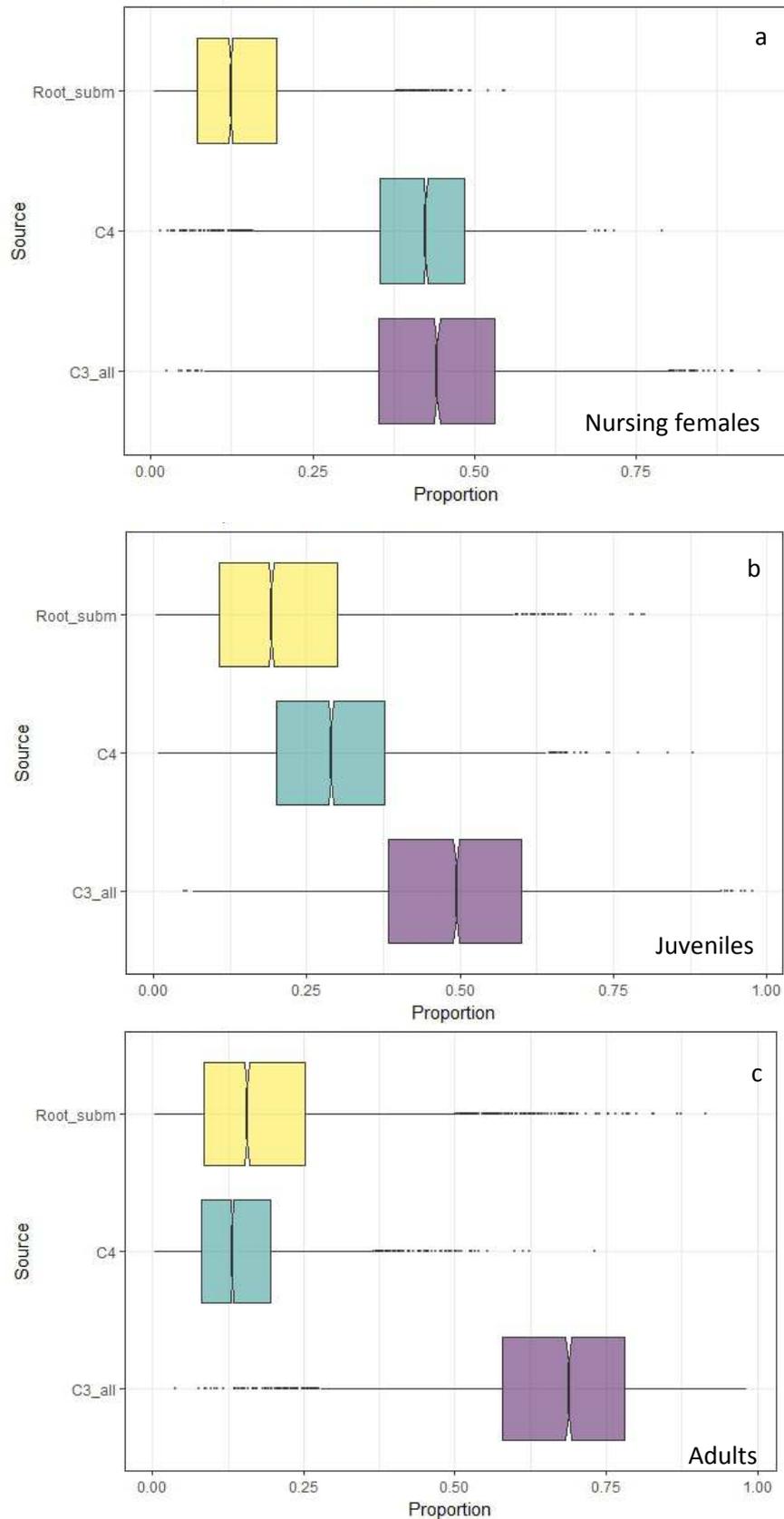


Fig. 6 Results of simmr Bayesian mixing models showing proportional estimates (mean, 25% and 75% percentiles) of diet composition of nursing females (a) juveniles (b) and adults (c) Amazonian manatees from Tapajós River following *a posteriori* combining of phytoplankton seston and C₃-plants into the “C₃ all” group: Root_subm (rooted submerged plants), C4 (C₄-plants).

Discussion

Isotopic composition in bones and teeth

Differences among teeth in the dental arcade

The only individuals that showed differences in $\delta^{13}\text{C}$ among different teeth were all nursing calves and the isotopic values found might be related to their mother's diet that was further transfer to the pups during lactation (Jenkins et al., 2001; Sare et al., 2005). A significant portion of carbon in milk is used in the synthesis of dentin, particularly during periods of fast growth in the first year of life (Newsome et al., 2006), and the $\delta^{13}\text{C}$ difference between calves and adults is often minimal and not expressive when animals live in the same habitat (Hobson & Quirk, 2014; Hanson et al., 2009, Albernaz et al., 2016). The change in diet (difference $<2\text{‰}$), as indicated by the carbon isotope composition, could have resulted either from a movement of the nursing female to a different foraging site (Arraut et al., 2010; Ciotti et al., 2014) or a change in their preference of diet (Best, 1981; Lefebvre et al., 2000; Clementez et al., 2007; see section about diet composition).

For young mammals an enrichment in ^{15}N in tissues after birth is expected because of the exclusively milk diet (Michener & Schell, 1994; Polischuk et al., 2001; Cherel et al., 2015). However, in our study, just one animal, classified as juvenile (#9), showed intraindividual differences in $\delta^{15}\text{N}$ values greater than 2‰ among teeth, with the lowest value in the oldest teeth (M6), rejecting the possibility of the influence of breastfeeding. Some studies comparing carbon and nitrogen signals of isotope values from frontal and distal teeth of *T. manatus*, under the influence of breast milk or in a solid diet, also found no pattern (Walker & Macko, 1999; MacFadden et al., 2004; Ciotti et al., 2014). This lack of difference may be related to the continuous replacement of teeth that occurs in the Amazon manatee, with a movement of approximately 1mm/mo (Domning, 1983; Domning & Kayek, 1986). As juveniles can have ages of more than two years, one reasonable explanation for the lack of $\delta^{15}\text{N}$ variation within individuals is that some teeth formed during breastfeeding have been replaced by new ones and therefore the difference between phases of life could not be observed.

Differences among ontogenetic classes

Carbon stable isotope compositions of manatees' teeth and bones from both Tapajós and Negro rivers varied more between individuals than teeth of a single individual (Tables 2 and 3). This variation was higher among juveniles from Tapajós and lower among calves or adults from both rivers. Some studies with marine mammals have reported differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between adults and pups. In

such cases, pups fed on a diet based on milk rich in lipids, indicated that pups were feeding at a higher trophic level than older individuals (Lowther & Goldsworthy, 2011; Orr et al., 2012; Vales et al., 2015). The milk produced and source of carbon transferred to the young is isotopically depleted in ^{13}C with respect to the mother's diet (Witt & Ayliffe, 2001), which is caused by stable isotope discrimination from the female to the pup during nursing (Newsome et al., 2006). However, the differences between the youngest (neonates and fetus) and adults approached 0~4‰ (Nelson et al., 1998; Witt & Ayliffe, 2001; Newsome et al., 2006), much smaller than the mean differences we observed for teeth (-10.1‰ and -13.0‰, Tapajós and Negro respectively) and bones (9.3‰ and 9.1‰, Tapajós and Negro respectively).

In our study, teeth and bones of calves were more ^{13}C -enriched than adults and some juveniles, which is the opposite of what would be expected given the high lipid content of milk in aquatic mammals [i.e. low $\delta^{13}\text{C}$ values, (Pervaiz & Brew, 1986; Arnould & Boyd, 1995)]. Therefore, we can only explain the highly ^{13}C -enriched carbon isotopic values found in calves as being related to a diet for the nursing mother that is rich in C_4 plants with high $\delta^{13}\text{C}$ values. Adults, in contrast, had $\delta^{13}\text{C}$ values more depleted in ^{13}C , indicating a feeding preference for C_3 -plants. The juveniles exhibited a large variation in $\delta^{13}\text{C}$ (e.g. animal bones from Tapajós ranged between -15.7‰ and -32.2‰). This variation may be related to differences in feeding behavior during this ontogenetic class, like a change in diet from pup to adult. Parental care by Amazonian manatee lasts about more than two years (Rosas, 1994; Junk & da Silva, 1997), so, it is expected that some of these animals would still be following their mothers, with a diet close to that observed in nursing females while others would have started a more independent diet, such as that observed in adults.

High interindividual variation in $\delta^{15}\text{N}$ values, mainly due to significant differences between lactating calves and adults, was seen only in animals from Negro. This may be related to the fractionation between calves and breastmilk (Newsome et al., 2006; Troina et al., 2016). Theoretically, the nursing offspring is consuming its mother by catabolism and, therefore, it would be a trophic level higher than its mother until weaning begins (Hobson et al., 1993; Newsome et al., 2006; Cherel et al. 2015), but in practice these differences are less than one trophic level [(~1-4‰) Schoeninger & DeNiro, 1984; Hobson et al., 2000; Sare et al., 2005]. The fact that there was low variation within a single individual and no differences in $\delta^{15}\text{N}$ between ontogenetic classes in the Tapajós may be related to the limited sample size of adults or to the generalist feeding behavior of this manatee population. Juveniles, on the other hand, showed $\delta^{15}\text{N}$ values similar to those of the lactating calves and had both teeth and bone with $\delta^{15}\text{N}$ values slightly more enriched in ^{15}N (8.3 to 9.0‰) than adults. This

difference, however, was not significant which might be reflecting that the breastfeeding $\delta^{15}\text{N}$ signal may persist sometime after weaning in bone tissue due to a low turnover rate (Newsome et al., 2006, Drago et al., 2009). Moreover, bone resorption only occurs in larger manatees with ages greater than 15 years and body lengths greater than 300 cm. (Marmontel et al. 1996; Maciel & da Silva, 2009).

Differences between teeth and bones

Collagen contains approximately 35% carbon and 11–16% nitrogen by weight (van Klinken, 1999). The organic constitution of bones is basically of fibrous protein, collagen and, to a lesser extent, non-collagenous protein. In the teeth, despite a smaller amount in relation to the bones, collagen is the main organic component of the dentin and is absent in the enamel (Boskey, 2007). Therefore, the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between bones and teeth may be related to the different amounts of lipids and proteins (i.e. different aminoacid compositions) of these tissues, which may contribute to the differences in isotopic values (Newsome et al., 2010).

Within the same individual, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in bones were more depleted than those in teeth. Collagen takes a considerable time to turn over (~7 years), thus the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen show the average isotopic values of the dietary protein over a lifetime (Schwarcz & Schoeninger, 1991; Jim, 2000; Clementz et al., 2007), while the tooth dentin reflects the isotopic value of the diet at the moment of its formation (Jim, 2000; Tykot, 2004). Although the C in tooth dentin is not exchanged once deposited, in the case of species which have constant replacement of teeth, such as manatees or horses (Domning & Magor, 1978; Stevens & Hedges, 2004), it is expected, in general, a similar $\delta^{15}\text{N}$ collagen composition in bones and teeth (Bocherens et al., 1996).

Food sources

All plant samples we analyzed had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within the range reported for plants using the C_3 and C_4 photosynthetic pathways for inorganic carbon fixation (Smith & Epstein, 1971; Martinelli & Devol, 1991; Forsberg et al., 1993; Ometto et al., 2002). In Tapajós, phytoplankton and submerged rotted plants and, in Negro, periphyton, showed the lowest $\delta^{13}\text{C}$ values, followed by other C_3 -plants and the C_4 -plants, the most ^{13}C -enriched in $\delta^{13}\text{C}$ source. Phytoplankton and periphyton are usually lighter than vascular plants carbon (Hamilton & Lewis, 1992, Victoria et al., 1992) and their $\delta^{13}\text{C}$ values were similar to others registered for the Amazon basin (Araújo-Lima et al., 1986; Forsberg et al., 1993; Benedito-Celílio et al., 2000). The causes for the depletion of $\delta^{13}\text{C}$ in phytoplankton, periphyton and submerged rooted would be the ^{13}C

depleted source of carbon provided by respiration inside the water (Rau, 1979; Osmond et al., 1981). The other groups (C_3 and C_4 -plants) showed the classic distinction created by fractionation of carbon during photosynthesis (Fry, 2007) as seen in different studies in Amazon (Martinelli et al., 1994; Benedito-Celílio et al., 2000; Leite et al., 2002).

Diet Composition

Amazonian manatees from black and clearwater igapós feed on C_3 and C_4 -plants of different growth forms to different degrees, with differences in diet between nursing females and adults.

Negro River samples

According to our mixing model, nursing females had a strong preference for the consumption of C_4 compared to juveniles and adults. Juveniles had a more mixed diet, while adults had a strong preference for the consumption of C_3 -plants and periphyton (C_3 igapó all class). Differences in food consumption between classes are evident and related to seasonal changes in the ecosystem (Junk et al., 1989), individual preferences (Lefebvre et al., 2000) or even nutritional needs (Best, 1981).

Both the scientific (Best, 1982), and the traditional knowledge (Chapter 2), describes the birth of Amazonian manatee pups during high water phases, which coincides with abundance of herbaceous, mainly C_4 -plants in várzeas, but not in the igapós. This synchrony is assumed to facilitate the energy recovery of females at the end of gestation and early lactation (Barbour, 1973; Hartman, 1971; Best, 1982). Carbon isotope compositions enriched in ^{13}C found in lactating calves are related to a greater contribution of C_4 -plants in their mother diets and the consequent transfer through milk, and C_4 -plants were reported as very common in Amazonian manatee diets (Colares & Colares, 2002; Guterres-Pazin et al., 2014). However, C_4 -plants in the Negro river are not abundant, and are found mainly during the phases of low water (Junk, 1983; Chapter 1). Therefore, it is possible that the nursing females, to supply the increase in energy demand, shift to várzea during the high and receding waters, where C_4 species such as *Paspalum repens*, *Echinochola polystachya* and *Hymenachne amplexicaulis* are abundant (Junk & Piedade, 1993; 1997) highly productive (Piedade et al., 1991; Piedade et al., 1993) and provide food of higher nutritional content (Junk & Howard-Williams, 1984; Best, 1982). It is known that serenians do migrations to the phase of the flood pulse (Sheppard et al., 2006; Self-Sullivan et al., 2003; Deutsch et al., 2003; Castelblanco-Martínez et al., 2009). This fact was detailed for the Amazonian manatee by Arraut et al. (2010) in the Sustainable Development Reserves (RDS)

Mamirauá and Amanã, Central Amazonia. During high waters (May to June), manatees stay in várzea eating a high variety of selected macrophytes. When the waters recede (October – November), food availability is drastically reduced, and there is a limitation of space. As a result, animals migrate to deep water lakes to find better conditions for survival.

Juveniles eat a more mixed diet, including both C₃ (predominant) and C₄-plants. In this group, some animals could be influenced by both breastmilk (from mother that was feeding on várzea) and solid foods (both C₃ and C₄ plants), since the beginning of weaning process, within the first one or two months after birth, they can eat small quantities of plants to supplement their diet (Barbour, 1973; Best, 1982; Hartman, 1971). Unlike nursing females, that may move to várzea to feed on C₄-plants, and juveniles, adults appear to remain in the lakes of igapós and could feed on the abundant vines, trees and shrubs covered by periphyton available underwater during the flood (Chapter 2). According to Putz et al. (1997), green algae are common attached to leaves of trees and aquatic macrophytes, with a production of 1.2 gC m² day in their roots (Doyle, 1991). These algae can be ingested also through submerged tree trunks (pers. comm., Valdecir do Nascimento) or in symbiosis with plant leaves (Putz et al. 1997; Reich & Worth, 2006).

Tapajós River samples

Although the differences between classes are not as evident as in the Negro, nursing females expressed a higher consumption of C₄ plants and adults expressed strong signals of all classes of C₃ (phytoplankton and C₃-plants together).

These different consumption rates are related to the dynamics of plants in the Tapajós lakes during the flood pulse. These ecosystems are colonized by herbaceous aquatic species (C₃ and C₄ plants) throughout the year, in spite of differences in water level caused by the flood pulse (Chapter 1). During the flood, the richness and abundance of plants increase (range of 5.8 to 114.6 gPS m²), but as the water level decreases, there is a decline remaining only the most adapted, including also several C₄ plants: *E. polystachya*, *H. amplexicaulis*, *Paspalum repens* and Cyperaceae spp. (Junk & Howard-Williams, 1984; Chapter 1). During low water, the lakes become shallow and most of the plants dry out or are carried away by the currents (Junk & Piedade, 1997; Piedade et al., 2010; Ferreira et al., 2010). At the same time, increases the occurrence of phytoplankton, periphyton and submerged rotted species (all C₃ plants, with a range of 121.0 to 161.9 gPS m²), the latter often occupying the entire water column (Chapter 1) favored by the great light penetration in clearwater (Sand-Jensen, 1989; Kirk, 1994; Bini & Thomaz, 2005).

Unlike the Negro river, the Tapajós river offers a diversity of plant species with different growth forms and distinct photosynthetic pathways that can supply nutritional needs for all ontogenetic classes. Therefore, we suggest that in the Tapajós the necessity of movement by the nursing females to the várzea to access C₄-plants is not mandatory. However, it is not impossible, given to the connectivity between the Tapajós and the Amazon Rivers and the proximity of about 80 km between the study site and the Amazon.

As in the Negro, in the Tapajós river the juveniles showed a more mixed diet. For calves, this variation in diet are related to the shift from a milk-diet to a solid-diet, a normally a gradual process of microbial-gut adaptation. Some juveniles showed a diet similar to nursing females; perhaps they followed their mothers and feed on the same sources. Furthermore, better food quality during early life stages speeds growth and reduces the possibility of death before reproduction (Struck et al., 2004).

In case of adults, the diet was based on C₃-plants and phytoplankton, corroborating the fact that the Tapajós offers a great diversity and biomass of these kinds of plants during all flood pulse. Moreover, according to Fittkau et al. (2012), the production of phytoplankton in the Tapajós can be greater than in várzea (>3 tonsC/ha), despite de poor nutrient supply and because of the favorable light conditions. Since phytoplankton attaches on macrophytes and may form large agglomerates, relevant quantities of phytoplankton can be ingested by Amazonian manatees. However, according to the mixing model, sources with greater abundance along the flood pulse and wich serve as habitat for phytoplankton, as submerged rotted plants (Chapter 1), were not the most consumed. This may be related to muscle and joint neck peculiarities of manatees, reinforcing the hypothesis that the species is more specialized to feed on the surface of the water (Domning, 1978; Guterres-Pazin et al., 2014), although it is able to obtain food (C₃ or C₄ plants) efficiently in any depth of water column (Domning, 1980).

We can conclude that, although there is no change in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among teeth the large variation in these signals between individuals indicates the Amazonian manatee may switch its diet according to the availability of resources in its local environment, and also its food preference or nutritional needs. Moreover, the species feed on a great variety of plants and algae (phytoplankton and periphyton) from different photosynthetic pathways, with proportions that vary according to the ontogenetic class and the ecosystem. Nursing females from the Negro river may be induced to perform trophic movement to várzea to supply their nutritional needs consuming nutrient rich plants. On the other hand, nursing females and other individuals from the Tapajós River may have not need to migrate, as the igapós of this

area can offer appropriate habitats and abundant plant nutrition during all the phases of the flood pulse.

Implications for conservation and future works

C₄-plants made up an important fraction of the diet for species from both igapó regions sampled in this study, with likely up to 50% of the diet of nursing females derived from C₄ vegetation. Some of the sampled individuals in this study were collected in igapós located within Federal Protected Areas - PAs (Tapajós-Arapiuns Extractive Reserve and Tapajós Nacional Forest and the region of Anavilhanas National Park) and the ¹³C stable isotope suggests that gestating or nursing females move out of this area (largely without C₄ plants) to feed on C₄ plants in the nearby várzeas. This is a matter of concern, as some of these várzea areas are not protected, exposing the animals to a greater probability of hunting, pollution or other risks as they are very productive and populous regions. Therefore, regional management plans should emphasize the protection of this species in várzea areas outside existing reserves. In addition, it is important to stress that the creation of any protected area should incorporate information on habitat use of representative, and especially of threatened species, so that they include the core areas of habitat use.

This study demonstrated the use of stable isotopes in Amazonian manatees and suggested changes in diet for lactating females. However, the number of individuals analyzed was limited. Future work should emphasize testing of the suggestion from ¹³C that some individuals move to várzeas from nearby igapós to obtain nutrition when lactating. As most individuals had a mixed diet, further information on the trophic ecology of the species must also be augmented by means of radio tracking, especially to test the hypothesis of the regional movements.

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Ontogenetic variation in the trophic ecology of Amazonian manatee, *Trichechus inunguis*, using stable isotopes

Luciana Carvalho Crema¹ • Vera Maria Ferreira da Silva • Silvina Botta • Susan Trumbore • Maria Teresa Fernandez Piedade

Table S1 Age, sex and sample type of manatees, *T. inunguis*, from Tapajós and Negro rivers analyzed in this study. Lactating calves (LC), juveniles/adults (J/A), females (F), males (M), unidentified sex (UN), teeth (T), mandibular condyle (C), ribs (R).

River	Zoo Collection/year	Animal	Age	Sex	Sample Type
Tapajós	CMA - 2003	2	LC	M	T, C
	ZooFit-2013	8	LC	F	T, C
	ZooFit-2013	10	LC	M	T, C
	LMA - 2013	12	LC	UN	T, C
	LMA - 2012	13	LC	UN	T, C
	LMA - 2013	15	LC	UN	T, C
	CMA - 2003	1	J	UN	T, C
	ZooFit-2012	6	J	F	T, C
	ZooFit-2014	7	J	M	T, C
	ZooFit - 2013	9	J	F	T, C
	ZooFit-2013	11	J	M	T, C
	LMA - 2014	14	J	UN	T, C
	LMA - 2013	19	J	F	C
	CMA	3	A	UN	T, C
	LMA – 2013	16	A	UN	T, C
	LMA - 2014	17	A	UN	R
	LMA - 2014	18	A	UN	C
	Negro	LMA/1979	32	LC	M
LMA		33	LC	F	T, C
LMA/1981		34	LC	F	T, C
LMA/1980		35	LC	F	T, C
LMA/1976		21	J	F	T, C
LMA		27	J	UN	R
LMA/1978		30	J	UN	R
LMA/1976		19	A	F	T, C
LMA/1975		20	A	F	S
LMA/1976		22	A	UN	R
LMA/1976		23A	A	F	T, C
LMA/1976		23B	A	F	T, C
LMA/1977		24A	A	F	K,
LMA/1977		24B	A	F	R
LMA/1977		25	A	UN	T,C, S
LMA/1978		26	A	UN	T, C
LMA		28	A	UN	T, C
LMA	29	A	UN	R	
LMA	31	A	M	T, C	

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Table S2 Means of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) and life-forms of plants used in *Trichechus inunguis* feeding, collected in igapós of Tapajós River (T) and Negro River (N) where: eme (emergent), amph (amphibious), ffl (free floating), rsub (rooted submerged), rsubl (rooted submerged with floating leaves), ffls (free floating submerged), alg (algae), terr (terrestrial), clim (climber), tre (shrubs and trees).

River	Family	Specie names	Life-form	Grouping class	Mean $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$
Tapajós	Cabombaceae	<i>Cabomba aquatica</i> Aubl.	rsub	rooted submerged	-35.2	4.4
		<i>Cabomba furcata</i> Schult. & Schult.f.	rsub	rooted submerged	-31.2	5.0
	Cyperaceae	Cyperaceae sp.1	amph	others C ₃	-29.1	3.2
		<i>Echinochloa polystachya</i> (Kunth) Hitchc.	emer	C ₄	-12.5	7.4
		<i>Eleocharis fluctuans</i> (L.T. Eiten) E.H. Roalson & C.E. Hinchliff	rsub	rooted submerged	-33.2	1.0
		<i>Eleocharis minima</i> Kunth	terr, rsub	rooted submerged	-34.4	6.9
	Cyperaceae	Cyperaceae sp.1	terr	C ₄	-12.9	4.6
		Onagraceae	<i>Ludwigia octovalvis</i> (Jacq.) P.H.Raven	amph	others C ₃	-28.4
	Lentibulariaceae	<i>Utricularia breviscapa</i> C.Wright ex Griseb.	ffls	others C ₃	-31.9	7.0
			ffls	others C ₃	-33.7	5.2
	Mayacaceae	<i>Mayaca fluviatilis</i> Aubl.	rsub	rooted submerged	-36.6	8.7
	Araceae	<i>Montrichardia linifera</i> (Arruda) Schott	emer	others C ₃	-26.4	4.7
	Nymphaeaceae	<i>Nymphaea potamophila</i> Wiersema	rsubl	others C ₃	-29.1	4.5
			rsubl	others C ₃	-34.3	2.5
	Poaceae	<i>Hymenachne amplexicaulis</i> (Rudge) Nees	amph	C ₄	-13.4	2.7
			emer	others C ₃	-28.1	5.2
			emer	C ₄	-18.6	3.4
Pontederiaceae	<i>Eichhornia azurea</i> (Sw.) Kunth	rsubl	others C ₃	-32.2	6.8	
Salvinaceae	<i>Salvinia auriculata</i> Aubl.	ffl	others C ₃	-29.9	3.3	
*	Phytoplankton (seston)	alg	phytoplankton	-38.5	5.0	
Negro	Apocynaceae	<i>Tassadia berteriana</i> (Spreng.) W.D.Stevens	clim	C ₃	-31.9	5.3
		<i>Mesechites trifida</i> (Jacq.) Müll.Arg.	clim	C ₃	-30.8	4.9
	Asteraceae	<i>Mikania micrantha</i> Kunth	clim	C ₃	-30.9	4.2
	Convolvulaceae	<i>Ipomea</i> sp	clim	C ₃	-31.3	6.3
	Fabaceae	<i>Vigna</i> sp.1	clim	C ₃	-29.9	4.4
		<i>Vigna</i> sp.2	clim	C ₃	-30.4	6.5
		<i>Clitoria glycyinoides</i> DC.	clim	C ₃	-30.6	5.6
		<i>Vigna lasiocarpa</i> (Mart.ex Benth.) Verdc	clim	C ₃	-33.5	4.6
	Passifloraceae	<i>Passiflora pohlii</i> Mast.	clim	C ₃	-30.0	6.9
		<i>Passiflora misera</i> Kunth	clim	C ₃	-29.7	6.4
	Poaceae	Poaceae sp.1	amph	C ₃	-29.0	10.5
		Poaceae sp.2	amph	C ₃	-30.1	8.9
		<i>Oryza glumaepatula</i> Steud	emer	C ₃	-30.9	7.7
	Polygonaceae	<i>Coccoloba pichuna</i> Huber	tre	C ₃	-29.5	3.1
		<i>Symmeria paniculata</i> Benth.	tre	C ₃	-31.7	5.3
	Solanaceae	<i>Schwenckia grandiflora</i> Benth.	tre	C ₃	-28.5	6.8
		<i>Solanum subinerme</i> Jacq.	tre	C ₃	-30.6	6.6
	Urticaceae	<i>Cecropia</i> sp	tre	C ₃	-28.7	-2.1
	Vitaceae	<i>Cissus erosa</i> Rich.	clim	C ₃	-31.1	5.3
	*	Shrubs leaves mix	tre	C ₃	-30.6	2.9
	*	Trees leaves mix	tre	C ₃	-32.6	3.0

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SÍNTESE

Embora o peixe-boi-da-Amazônia se alimente de plantas aquáticas e semiaquáticas, e de raízes da vegetação de áreas inundáveis (Best, 1984; Colares e Colares, 2002; Franzini *et al.*, 2013; Guterres-Pazin *et al.*, 2014), sua dieta varia entre ecossistemas e fases ontogenéticas. Essa diferença está relacionada à disponibilidade de plantas, que é fortemente influenciada pelas mudanças nas variáveis físicas e químicas da água provocadas pelo pulso de inundação ou mesmo por preferências individuais ou necessidades energéticas (Best, 1981; Junk *et al.*, 1989; Lefebvre *et al.*, 2000).

Os resultados deste estudo mostram que o compartilhamento de espécies vegetais herbáceas entre igapós de águas claras e pretas é pequeno, havendo também diferenças nos parâmetros físicos e químicos da água dos dois ecossistemas ao longo do pulso de inundação. Os lagos de igapó do baixo rio Negro possuem sazonalidade marcada na composição da comunidade de herbáceas e oferecem para herbívoros aquáticos, somente durante a cheia, grande abundância de espécies trepadeiras. Estas, por sua vez, se apresentaram com maior saliência cognitiva na alimentação do peixe-boi-da-Amazônia, no ponto de vista dos ribeirinhos entrevistados, conhecedores de seu comportamento. No entanto, fêmeas lactantes podem ser induzidas a realizar miração para se proteger e suplementar suas necessidades nutricionais, se deslocando para regiões com condições mais favoráveis à sua sobrevivência, como a várzea. Isso porque, segundo o modelo de mistura aplicado no estudo, essas fêmeas apresentaram dietas com predominância de plantas C_4 . É justamente durante a época de águas altas que ocorre o pico de nascimento de filhotes de peixe-boi (Best, 1983) concomitante ao aumento de biomassa de espécies de plantas de ciclo C_4 nas várzeas, muitas delas ricas em nitrogênio, proteínas e potássio (Junk e Harward-Williams, 1984). Por outro lado, os adultos machos e as fêmeas não lactantes têm uma dieta mais baseada em plantas típicas do igapó de águas pretas como plantas do ciclo C_3 e algas, abundantemente encontradas aderidas a essas plantas. Durante a cheia, troncos, trepadeiras, folhas de árvores e de arbustos ficam submersos, e disponíveis como substrato a algas (Putz, 1997) e também como fonte alimentar para animais aquáticos. Já os juvenis de peixe-boi-da-Amazônia têm dieta mais variada, podendo acompanhar e refletir a dieta de sua mãe em regiões de várzea até aproximadamente dois anos de idade, período aproximado de cuidado parental (Rosas, 1994; Junk e da Silva, 1997), ou mesmo apresentar uma dieta mais independente, semelhante à de adultos em igapós.

Apesar de os moradores entrevistados da região do baixo Tapajós indicarem plantas submersas enraizadas com folhas flutuantes (p. ex. *Nymphaea* spp.) e submersas enraizadas (p. ex. *Eleocharis* spp.) como as preferidas pelo peixe-boi-da-Amazônia, seus lagos são mais ricos em espécies e formas de vida de herbáceas aquáticas que os do baixo rio Negro. Além disso, embora haja variação em sua composição de herbáceas ao longo do ano, esses lagos são capazes de assegurar, durante todo o pulso de inundação, fontes alimentares para herbívoros aquáticos, com abundância e biomassa constantes. Este fato pode propiciar a permanência de indivíduos de peixes-bois da Amazônia nesses ecossistemas, reduzindo a pressão para realizarem migrações nas épocas de águas baixas, desde que a profundidade do corpo de água assim os permita. Segundo os resultados das análises de isótopos estáveis, os indivíduos de peixes-bois que vivem no Tapajós se alimentam de uma variedade de plantas e algas, tanto de ciclo C_3 como C_4 . Embora os sinais de fêmeas lactantes do Tapajós também se relacionem a plantas C_4 , a disponibilidade desse grupo vegetal e as maiores profundidades de seus lagos associados indicam que, para elas, a migração para fora desse ecossistema pode não ser tão determinante quanto no rio Negro.

Por meio das entrevistas com populações tradicionais residentes ou do entorno de Unidades de Conservação (UCs) Federais, foram apontadas localidades que correspondem a lugares de descanso, refúgio ou forrageio do peixe-boi-da-Amazônia nas diferentes fases do pulso de inundação. Estes locais podem ser usados como região para manejo, reintrodução da espécie ou alvo de zoneamento nos Planos de Manejo das UCs. Além disso, apesar da percepção geral da redução da caça nos ambientes estudados, no Parque Nacional de Anavilhanas, por ser mais próximo a grandes centros populacionais como Manaus e Novo Airão, a demanda pela carne do peixe-boi ainda existe e pode ser um risco à espécie nessa região. Além disso, a possibilidade de migração das fêmeas prenhas ou com filhotes para locais fora dos limites de UCs deve ser levada em consideração. Apesar de o peixe-boi ser um animal protegido por lei, assim como as UCs estudadas, a execução de políticas públicas acerca de recursos naturais na Amazônia é muitas vezes dificultada pelas dimensões, problemas de acesso às áreas e, pela escassez de aplicação em recursos financeiros e humanos. Dessa forma, uma estratégia adequada não só para limitar a caça, mas também para aprimorar a efetividade das UCs e da conservação do peixe-boi, é aumentar o envolvimento dos residentes, bem como de populações adjacentes a estas áreas em atividades relacionadas à gestão, manejo e pesquisa. Assim, esses moradores seriam motivados a reconhecer o valor da biodiversidade e até mesmo contribuir para a defesa do meio ambiente. Além disso, criar iniciativas

interinstitucionais em todos os níveis para garantir a proteção regional da espécie, considerando a possibilidade de movimentação entre áreas protegidas ou não (p. exe. diferentes igapós e várzeas), até mesmo dentro de bacias transfronteiriças, deve ser prioridade em ações de conservação para o peixe-boi-da-Amazônia.

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APÊNCIE A

Ata da Qualificação de Doutorado



PARECER AULA DE QUALIFICAÇÃO

Estudante: LUCIANA CARVALHO CREMA
Programa: Biologia de Água Doce e Pesca Interior Curso: Doutorado
Orientadora: Dra. Maria Teresa Fernandez Piedade

TÍTULO

"Vida das plantas na planície de inundação com ênfase em herbáceas aquáticas de igapós."

BANCA JULGADORA

TITULARES

Maria Anete Leite Rubim – FCA/UFAM
Jefferson da Cruz – ICB/UFAM
Maria de Lourdes da Costa Soares Moraes - INPA

SUPLENTES

Aline Lopes – Max Planck/INPA
Florian Karl Wittmann - Max-Planck/INPA

EXAMINADORES

PARECER

ASSINATURA

Maria Anete Leite Rubim	<input checked="" type="checkbox"/> Aprovado () Reprovado	
Jefferson da Cruz	<input checked="" type="checkbox"/> Aprovado () Reprovado	
Maria de Lourdes C. Soares Moraes	<input checked="" type="checkbox"/> Aprovado () Reprovado	
Aline Lopes	() Aprovado () Reprovado	_____
Florian Karl Wittmann	() Aprovado () Reprovado	_____

COMENTÁRIOS:



Manaus (AM), 11 de Maio de 2015.

APÊNCIE B

Ata da Defesa de doutorado



MINISTÉRIO DA
CIÊNCIA, TECNOLOGIA,
INOVAÇÕES E COMUNICAÇÕES



ATA DA DEFESA PÚBLICA PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA DE ÁGUA DOCE E PESCA INTERIOR

No dia 18 de Agosto 2017, às 09:00 horas, no Auditório do PPG BADPI, Campus Aleixo II – INPA, reuniu-se a Banca Julgadora da DEFESA PÚBLICA de DOUTORADO, composta pelos seguintes Doutores(as), membros titulares: Cristiano Queiroz de Albuquerque, Joana D'Arc de Paula, Charles Roland Clement, Rodrigo de Souza Amaral e Eduardo Antonio Rios-Villamizar; tendo como membro suplente: Jefferson Cruz, a fim de proceder a arguição pública da TESE da discente LUCIANA CARVALHO CREMA, intitulada: CARACTERIZAÇÃO DE IGAPÓS DE ÁGUAS CLARAS E PRETAS E SUAS DISPONIBILIDADES ALIMENTARES PARA O PEIXE-BOI-DA-AMAZÔNIA (*Trichechus inunguis*). O estudo foi conduzido sob a orientação da Dra. Maria Teresa Fernandez Piedade e coorientação da Dra. Vera Maria Ferreira da Silva.

Após a exposição da aula, dentro do tempo regulamentar, (o)a discente foi arguido(a) oralmente pelos membros da Banca Julgadora, tendo recebido o conceito final:

- Aprovada (o) por unanimidade MENÇÃO: - Com "Distinção"
- Aprovada (o) por maioria - Com "Distinção e Louvor"
- Reprovada

Foi lavrada a ata e assinada pelos membros presentes da Banca Julgadora.

Cristiano Queiroz de Albuquerque – UFERSA

Joana D'Arc de Paula - UniniltonLins

Charles Roland Clement – INPA

Rodrigo de Souza Amaral – IFAM

Eduardo Antonio Rios- Villamizar – INPA

Jefferson Cruz– UFAM

Claudia Pereira de Deus
Dra. Claudia Pereira de Deus
Coordenadora do PPG BADPI
PO. 244/2016 – INPA/MCT-PR



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