INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

O USO DE ISÓTOPOS ESTÁVEIS DE NITROGÊNIO ($\delta^{15}N$) EVIDENCIA A POSIÇÃO TRÓFICA DO PIRARUCU (*Arapaima* sp.)?

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Manaus, Amazonas

Março, 2020

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Dissertação apresentada ao
Instituto Nacional de Pesquisas da
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Sinopse:

Estudou-se o uso da razão de isótopos estáveis de nitrogênio ($\delta^{15}N$) para estimar a posição trófica do pirarucu (*Arapaima* sp.) e análises de conteúdo estomacal aliadas ao conhecimento de moradores locais para investigar a dieta do pirarucu do médio rio Juruá, Amazonas.

Palavras-chave: Ecologia trófica, conteúdo estomacal, isótopos estáveis, água doce, Juruá

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Meu muito obrigada!

Este título de mestrado é de todos vocês!

RESUMO

Estudos tróficos são essenciais para se entender a regulação e transferência de energia entre indivíduos e ecossistemas. Nós exploramos estimativas de posição trófica realizando análises de conteúdo estomacal e análises da razão natural dos isótopos estáveis de nitrogênio ($\delta^{15}N$) do fígado e do músculo de pirarucus de uma ampla gama de tamanhos corporais de lagos do médio rio Juruá, Amazonas. Também aliamos análises de conteúdos estomacais com o conhecimento empírico de moradores locais para expandir o entendimento sobre a alimentação do pirarucu na área do estudo. O tamanho total do pirarucu explicou a maior parte da variação da posição trófica estimada com $\delta^{15}N$ do fígado e do músculo, mostrando que o tamanho corporal tem um efeito mais forte do que a posição trófica das presas nos valores do $\delta^{15}N$. Isso reforça a necessidade de um melhor entendimento dos fatores que afetam os valores de $\delta^{15}N$ que não são relacionados à posição trófica da dieta. Análises de conteúdo estomacal aliadas ao conhecimento empírico apresentaram informações complementares indicando que pirarucus jovens se alimentam de peixes e invertebrados e adultos se alimentam exclusivamente de peixes, mas de uma ampla gama de espécies e principalmente de baixas posições tróficas. Os moradores entrevistados apresentam um conhecimento ecológico consistente da dieta do pirarucu, que poderia contribuir na implementação de futuros projetos de manejo na região. Isótopos estáveis podem adicionar informações complementares em estudos tróficos, mas análises de conteúdo estomacal continuam sendo necessárias para desvendar a ecologia trófica de peixes predadores em cada área de interesse.

ABSTRACT

Trophic studies are essential to understand the regulation and transfer of energy among individuals and ecosystems. We explored estimates of trophic position using stomach-content analysis and $\delta^{15}N$ in liver and muscle in a broad size range of arapaima from lakes in the middle Juruá River, Amazonas. We also combined stomach-content analysis with the empirical knowledge of local dwellers to expand the understanding of arapaima feeding in the study area. Arapaima total length explained most of the variation in trophic-position values estimated from liver and muscle $\delta^{15}N$, showing that body size has more effect than prey trophic position on $\delta^{15}N$ values. This highlights the need for a better understanding of the factors that affect values of $\delta^{15}N$ that are unrelated to diet trophic position. Stomach content analysis combined with empirical knowledge provided complementary information indicating that young arapaima eat fish and invertebrates and adults feed exclusively on fish, but from a wide range of species and mainly from low trophic positions. The interviewees had consistent ecological knowledge of the arapaima feeding and could contribute to the implementation of future management projects in the region. Stable isotopes may add supplementary information in trophic studies, but stomach-content analysis is still needed to unravel the trophic ecology of predatory fishes in each area of interest.

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INTRODUÇÃO GERAL

O entendimento da regulação e transferência de energia nos ecossistemas fornece informações essenciais para o desenvolvimento de estratégias voltadas para o manejo sustentável e conservação da biodiversidade. O tamanho corporal dos organismos é uma das variáveis que podem determinar interações entre espécies e influenciar a estrutura de cadeias alimentares em diferentes níveis ecológicos (Woodward et al., 2005). O pirarucu (*Arapaima* spp. - Osteoglossidae) é o maior peixe de escamas de água doce do mundo, podendo pesar mais de 200 kg e atingir três metros de comprimento total (Nelson, 1994; Queiroz, 2000). É um peixe amplamente distribuído na bacia do Amazonas (Queiroz, 2000; Castello, 2008; Araripe et al., 2013) e estudos indicam a possibilidade deste peixe pertencer a diferentes linhagens e provavelmente diferentes espécies dentro do gênero (Castello & Stewart, 2009), mas ainda não há consenso em relação a este tema (Farias et al 2019) e todos os indivíduos são popularmente chamados de pirarucu. Sua carne é de alta qualidade e grande importância comercial e tradicional na região amazônica, e o seu consumo desenfreado o caracteriza como uma espécie superexplorada na maior parte da sua distribuição geográfica (Castello et al., 2015). Entretanto, apesar de sua importância cultural, ecológica e econômica, dados sobre a dieta do pirarucu ainda são escassos. Espera-se que a espécie quando abundante influencie a estrutura de teias alimentares através de mudanças na quantidade, comportamento e uso de habitat de suas presas. Pirarucus da Reserva de Desenvolvimento Sustentável Mamirauá (RDS Mamiráua) com menos de 50 cm de comprimento apresentam uma dieta mais variada, composta por crustáceos, peixes, insetos e moluscos, passando a se alimentar majoritariamente de peixes com o aumento do tamanho corporal (Queiroz, 2000), evidenciando a importância do tamanho nas relações tróficas da cadeia alimentar.

A análise de conteúdo estomacal é uma técnica frequentemente usada nos estudos sobre a ecologia de peixes, visando informações sobre a estrutura das redes alimentares e plasticidade trófica. A técnica consiste numa análise qualitativa, identificando os itens ingeridos pelo organismo de estudo e, uma análise quantitativa, como a frequência de ocorrência e/ou proporção de cada item (Teixeira & Gurgel, 2002). É uma técnica trabalhosa e onerosa, uma vez que são necessárias grandes quantidades de coletas durante um longo período para abranger diferenças temporais e espaciais na ingestão de recursos. Além disso, certos itens alimentares podem ser de difícil identificação devido ao estado de decomposição ou devido ao seu pequeno tamanho.

Experimentos de laboratório realizados no início da década de 1980 (DeNiro e Epstein 1981) analisaram uma variedade de animais tratados com dietas que diferiam isotopicamente e encontraram que a razão isotópica de nitrogênio do consumidor aumentava em relação à sua dieta. Posteriormente, investigações em campo indicaram uma média de enriquecimento da razão isotópica de nitrogênio ($\delta^{15}N$) de 3.4‰ (Minagawa & Wada 1984), um número que atualmente é usado como o padrão para uma ampla gama de organismos (Post, 2002). A média de enriquecimento é conhecida como fator de discriminação trófica (FDT) e é este valor que diferencia os organismos em relação às posições tróficas na cadeia alimentar usando isótopos estáveis. Dessa forma, um consumidor deveria ter em média uma diferença isotópica de 3.4% a mais em relação as suas presas e este FDT entre o tecido do consumidor e da sua dieta deveria ser constante. Em geral, esse padrão ($\delta^{15}N$ do consumidor > $\delta^{15}N$ da dieta) é amplamente usado para inferir a posição trófica em estudos ecológicos. Ao se estimar a posição trófica de um organismo com a $\delta^{15}N$, devem ser consideradas ainda mais duas variáveis: a linha de base e a taxa de substituição. A linha de base é a caracterização isotópica do ambiente onde o organismo de interesse vive e precisa ser conhecida para estimar a posição trófica. Consumidores primários costumam ser coletados como indicadores da linha de base porque seu tamanho corporal maior e maior longevidade em relação aos produtores primários resultam em menor sazonalidade nas assinaturas de $\delta^{15}N$ (Cabana & Rasmussen, 1996). A taxa de substituição reflete o período de tempo necessário para a composição isotópica do tecido do consumidor refletir a composição isotópica da dieta e esse período de tempo pode variar entre tecidos. Os tecidos que têm alto nível de atividade metabólica, como sangue e fígado, apresentam rápida taxa de substituição em relação aos tecidos menos ativos, como o músculo (Manetta & Benedito-Cecilio, 2003).

O $\delta^{15}N$ claramente aumenta ao longo da cadeia alimentar, entretanto, estudos mais recentes vêm sugerindo que a magnitude da mudança para cada nível trófico é mais complexa do que o assumido na maioria dos trabalhos. Trueman et al. (2005) realizou experimentos com alimentação controlada e demonstrou que o FDT entre o tecido do salmão do Atlântico (*Salmo salar*) e a sua dieta não era constante durante o crescimento do peixe, variando inversamente com a sua taxa de crescimento. Os autores sugeriram que os requerimentos metabólicos ou as consequências do crescimento devem afetar a diferença entre os valores do $\delta^{15}N$ da dieta e tecido. Gorokhova (2018) também sugeriu que a taxa de crescimento é um fator determinante na discriminação trófica de crustáceos do gênero Neomysis. Villamarín e colaboradores (2018) estudaram crocodilianos amazônicos, organismos ectotérmicos que variam bastante em tamanho, e concluíram que as mudanças ontogenéticas no nível trófico de crocodilianos baseadas na dieta eram mínimas e diferiam das estimativas de nível trófico usando isótopos estáveis. Os autores hipotetizaram que isso poderia ser resultado de processos metabólicos relacionados com o tamanho corporal dos indivíduos, o que poderia influenciar a discriminação trófica. Logo, se o FDT é influenciado por algo que não apenas a dieta, usar a $\delta^{15}N$ para diferenciar posições tróficas assumindo que toda variação isotópica é em função da dieta pode gerar resultados não confiáveis.

Usando isótopos estáveis, Carvalho et al. (2018) encontraram que os valores do $\delta^{15}N$ do tecido muscular do pirarucu amazônico (*Arapaima* sp.) cresciam com o aumento do tamanho corporal do peixe e sugeriram que esse aumento refletiria mudanças na dieta do pirarucu. Isso indicaria que pirarucus maiores consumiriam presas de níveis tróficos mais altos que pirarucus menores, mas análises de conteúdo estomacal não apoiaram esta hipótese. Estômagos de adultos continham principalmente peixes de baixas posições tróficas, como detritívoros e omnívoros (Queiroz, 2000). Além disso, características morfológicas, como o intestino tendo em média 1,45 vezes o tamanho do comprimento total do corpo, dentes relativamente pequenos e numerosos suportam a ideia de que o pirarucu é um consumidor secundário (Watson et al., 2013).

Considerando estas incongruências notadas entre os estudos, nós investigamos se é possível estimar corretamente a posição trófica do pirarucu usando valores do $\delta^{15}N$ do fígado e do músculo de uma ampla gama de tamanhos de pirarucus de lagos do médio rio Juruá, comparando estimativas de posição trófica baseadas em isótopos estáveis com a análise da composição da sua dieta. Assim, no Capítulo I, avaliamos até que ponto a dieta explica mudanças nas estimativas de posição trófica com base nos valores de $\delta^{15}N$, como assumido na literatura (Vander Zanden et al., 1997). Havendo pouca relação entre os dados alimentares e isotópicos, fica evidente que os valores de $\delta^{15}N$ do pirarucu resultam de mecanismos adicionais à assimilação da dieta e queríamos testar se tais mecanismos podem estar relacionados ao tamanho corporal do indivíduo. Por apresentar uma grande variação em tamanho ao longo da sua vida, o pirarucu pode ser um bom modelo para entender a importância de se levar em consideração o tamanho do organismo nas estimativas tróficas utilizando o $\delta^{15}N$. Também avaliamos se existe diferença de assimilação do nitrogênio da dieta entre o fígado e o músculo, já que o fígado apresenta uma taxa metabólica mais alta.

Adicionalmente, como não há dados sobre a dieta do pirarucu na região deste estudo, o médio rio Juruá, também buscamos expandir o conhecimento sobre a alimentação da espécie na região (Capítulo II). O médio Juruá é uma das regiões do Amazonas onde é permitida a pesca do pirarucu uma vez ao ano durante atividades de manejo da espécie. Este manejo vem apresentando grande sucesso (Campos-Silva et al., 2019) uma vez que a densidade de pirarucus vem aumentando consideravelmente, de forma a contribuir com a conservação da espécie e com a fonte de renda e alimentação dos moradores locais (Campos-Silva and Peres, 2016). Realizamos análises de conteúdos estomacais de pirarucus desta região e aliamos esta fonte de informação ao conhecimento empírico de moradores envolvidos nas atividades de manejo do médio Juruá, pretendendo contribuir com informações importantes para a conservação da espécie e melhoria das atividades de manejo na região.

OBJETIVOS GERAIS

O objetivo geral deste trabalho foi descrever a dieta e nível trófico do pirarucu usando isótopos estáveis de nitrogênio ($\delta^{15}N$) do fígado e do músculo e a descrição taxonômica dos itens no conteúdo estomacal.

OBJETIVOS ESPECÍFICOS

- Estimar as posições tróficas de pirarucus de uma ampla faixa de tamanhos corporais do Médio Rio Juruá (AM) com base na identificação dos seus conteúdos estomacais;

- Investigar se a variação isotópica dos conteúdos estomacais dos pirarucus está relacionada às estimativas de posição trófica das presas destes conteúdos;

- Explorar estimativas de posição trófica baseadas na razão natural de isótopos estáveis de nitrogênio ($\delta^{15}N$) do tecido muscular e do fígado de pirarucus de uma ampla faixa de tamanhos corporais e investigar se estas estimativas de posição trófica estão relacionadas à dieta presente no conteúdo estomacal;

- Avaliar se as estimativas de posição trófica baseadas em $\delta^{15}N$ do fígado e do músculo apresentam diferenças na relação com a dieta, considerando que a taxa de turnover do fígado é mais rápida que o músculo;

- Investigar se o aumento do tamanho corporal do pirarucu pode explicar parte da variação das estimativas de posição trófica baseadas na $\delta^{15}N$;

- Melhorar a compreensão da alimentação do pirarucu do sistema de manejo dos lagos de várzea do médio rio Juruá, unindo informações da identificação de conteúdos estomacais com o conhecimento empírico de moradores da região.

Capítulo I.

Jacobi, C.M.; Villamarín, F.; Jardine, T.; Magnusson, W.E.

Uncertainties associated with trophic discrimination factor and body size complicate calculation of δ^{15}N-derived trophic positions in *Arapaima* sp.

Manuscrito em revisão: Ecology of Freshwater Fish

RESEARCH ARTICLE

Uncertainties associated with trophic discrimination factor and body size complicate calculation of δ^{15}N-derived trophic positions in *Arapaima* sp.

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Short running title: **Uncertainties to calculate δ^{15}N-derived trophic position**

ABSTRACT

Stable-isotope ratios of nitrogen ($\delta^{15}N$) have been used to estimate trophic position (TP) of organisms due to the predictable enrichment of nitrogen-15 in consumer tissues relative to their diet. We explored estimates of trophic position using liver and muscle $\delta^{15}N$ and stomach-content analysis in a broad size range of *Arapaima* sp. from Amazonian floodplain lakes. Estimates of TP based on liver $\delta^{15}N$ were more closely related to the stomach-content data than estimates based on muscle $\delta^{15}N$, possibly because of the higher turnover of nitrogen in liver. Total length and season explained most of the variation in TP values estimated from $\delta^{15}N$, showing that they have more effect than prey trophic position on $\delta^{15}N$ values. The TP estimated by identification of stomach content was 3.6 and was unrelated to the size of the arapaima. This highlights the need for a better understanding of the factors that affect values of $\delta^{15}N$ and stomach-content analysis is still needed to unravel the trophic ecology of predatory fishes.

key words: trophic ecology, fish, stomach-content, stable isotopes, freshwater, Amazon

1. INTRODUCTION

Knowledge of the trophic ecology of species is essential to understand the regulation and transfer of energy in ecosystems (Lindeman, 1942; Kerr & Martin, 1970; Morales-Zárate et al., 2004) and this can be used to develop strategies for the sustainable management of species. Arapaima (*Arapaima* spp. - Osteoglossidae) are the largest scaled freshwater fish in the world, reaching up to 200 kg and over 3 meters in total length (Nelson, 1994; Queiroz, 2000). This species can influence the structure of food webs through changes in the abundance, behavior and habitat use of its prey. Arapaima are widely distributed in the Amazon basin (Queiroz, 2000; Castello, 2008; Araripe et al., 2013) and while recent studies indicate different lineages and probably distinct species within the range of the genus (Castello & Stewart, 2009), little is known of species boundaries and all are called pirarucu by local fishers. They are important food sources for riverine people and have high economic value (Castello et al., 2014). Stomach-content analysis indicates that the diet of arapaima varies throughout the year because of changes in food and habitat availability, and also changes with age and increases in size (Queiroz, 2000); however, this does not necessarily mean that large-bodied arapaima feed on higher-trophic-level prey because many large-bodied tropical species occupy low trophic positions (Layman et al., 2005). Arapaima smaller than 50 cm in total length have a more varied diet, composed of crustaceans, fish, insects, and mollusks, but larger individuals feed mainly on fish (Queiroz, 2000). Dietary data can provide an accurate measure of trophic position for individuals within a population, but it is necessary to have detailed gut-content data from large numbers of fish, sampled throughout the year, a situation that is rare in dietary studies.

In recent years, analysis of stable-isotope ratios of nitrogen ($\delta^{15}N$) has become an increasingly important tool to understand and complement trophic studies based on stomach-content analysis, being used to estimate trophic position of organisms as a continuous measure (Post, 2002). The principle behind this technique is that $\delta^{15}N$ values generally increase at each trophic-level transition due to enrichment in the heavier isotope (^{15}N) during the fractionation of nitrogen. The increase in $\delta^{15}N$ varies from 2 to 5‰ with an average of approximately 3.4 ‰ (DeNiro & Epstein, 1981, Post 2002, McCutchan et al. 2003) and is known as the trophic discrimination factor (TDF). This provides the metric for estimating differences between trophic positions in the food chain. Therefore, it is expected that the difference between the isotopic values of the consumer's tissue and that of its diet is relatively constant and that the $\delta^{15}N$ present in the consumer's tissue reflects that of its diet with a correction for the TDF (Ponsard & Averbuch, 1999; Olive et al., 2003). This general pattern ($\delta^{15}N$ of consumer > $\delta^{15}N$ of diet) is widely used to infer trophic position in ecological studies.

Using stable nitrogen isotopes in the Amazon basin, Carvalho et al. (2018) found that $\delta^{15}N$ muscle values of arapaima increased with body size and suggested that this increase reflects changes in arapaima diet. This would indicate that larger arapaima feed on prey from higher trophic levels than do small arapaima, but stomach-content analysis did not support this hypothesis. Stomachs of adults mostly contained fish from low trophic positions, such as detritivores and omnivores (Queiroz, 2000). Morphological characteristics, such as an intestine averaging 1.45 times total body length, relatively small teeth, and numerous, closely-spaced gill rakers support the idea that arapaima are secondary consumers and may be better characterized as omnivores and not top predators (Watson et al., 2013). In a study of Amazonian crocodilians, which are also ectothermic predators that vary greatly in size, changes in trophic level based on stomach-content data were minimal and differed from trophic levels estimated using stable isotopes (Villamarín et al., 2018), perhaps due to slow isotopic turnover. The period of time required for the isotopic composition of the consumer tissue to reflect the diet isotopic composition (turnover) may vary among tissues. Those with a high level of metabolic activity, such as blood and liver, have a rapid turnover compared to less active tissues, such as muscle (Manetta & Benedito-Cecilio, 2003). However, Villamarín et al. (2018) argued that differences between isotopic ratio and diet may be due to the influence of factors other than dietary assimilation. Such factors may be linked to metabolic processes related to growth that vary with the size of individuals, which could influence $\delta^{15}N$ trophic discrimination. If this discrimination is influenced by something other than diet, using $\delta^{15}N$ to differentiate trophic positions may not be reliable. Based on the discrepancies noted in previous studies, we investigated to what extent it is possible to correctly estimate the trophic position of arapaima using $\delta^{15}N$.

Our goals were to 1) estimate arapaima trophic positions of a broad size range by identifying stomach contents; 2) explore estimates of arapaima trophic positions using $\delta^{15}N$ of liver and muscle, as the liver has a faster isotopic turnover, and; 3) relate these values to the proportional contributions of prey from different trophic levels in stomach contents. By doing so, we evaluate the extent to which diet is coupled with shifts in trophic-position estimates based on $\delta^{15}N$ values, as is assumed in the literature (Vander Zanden et al., 1997). Uncoupled trends between dietary and isotopic data would suggest that the values of $\delta^{15}N$ of arapaima result from mechanisms additional to dietary assimilation and we wanted to know whether such mechanisms might be related to body size.

2. METHODS

2.1. Study area

The study was conducted in eight floodplain lakes (Fig. 1) located inside two protected areas (Reserva de Desenvolvimento Sustentável Uacari and Reserva Extrativista do Médio Juruá), along the Juruá River, a major tributary of the Amazon River, in Amazonas State, Brazil. This region is influenced by pronounced and predictable hydrology, with the flood period characterized as the high water levels from January to June and the dry period corresponding to the low water levels from August to November (Hawes and Peres, 2016).

2.2. Sample collection

Arapaima of different body sizes were captured in the period of low water (September 2018) and high water levels (June 2019) with the use of gill nets and the help of local fishers. Most samples came from the low-water period because we opportunistically collected samples associated with managed harvesting at that time (Campos-Silva & Peres 2016). All individuals were measured (total length to tip of tail) and dissected to collect muscle tissue, liver and stomach contents. We also collected invertebrate primary consumers (Chironomidae, Ephemeroptera, snails, zooplankton) in the shallows and in the middle of each lake to be used as a baseline for the $\delta^{15}N$ isotopic composition. These were collected with D-frame kick nets and with vertical tows of a plankton net. Samples were frozen at -20°C in the field for later analysis.

Data collection was authorized by the Sistema de Autorização e Informação em Biodiversidade (SISBIO), Departamento de Mudanças Climáticas e Gestão de Unidades de Conservação (DEMUC) of the Secretaria Estadual de Meio Ambiente do Amazonas (SEMA) and by the Ethics Committee of the Instituto Nacional de Pesquisas da Amazônia (INPA), with permits 62427-1, 41/2018 and 040/2018, respectively.

2.3. Estimates of trophic position with stomach-content analysis

Food items in each stomach were separated, weighed and identified to the lowest possible taxonomic level. We then estimated trophic position of individuals based on the composition of all food items in each stomach ($TP_{stomach}$) through the following equation modified from Cortés (1999):

$$TP_{stomach} = [\sum_{j=1}^{n} P_j * TL_j]$$

where the trophic position is the sum of the proportion of each food-item category (j) in the predator diet (P_j) multiplied by the trophic level of each food-item category (TL_j) and (n) is the total number of different food types in the stomach. Values for trophic levels of prey were classified according to their diets with the help of the literature and local expert knowledge, and assigned as follows: plants = 1, herbivores = 2, detritivores that consume mostly organic matter from primary producers = 2, detritivores that consume mostly from trophic levels higher than primary producers = 2.5, omnivores = 2.5, carnivores = 3 and carnivores that sometimes can eat other predators = 3.5. For prey that we were unable to identify at the species level, we estimated values according to the species most probable for the region. Since plants may have been ingested incidentally, we performed a secondary calculation that removed plants from the analysis. This metric is referred to as TP_{prey} and includes only animal prey.

2.4. Stable-isotope ratios

The baseline organisms, liver, muscle and stomach-content samples were dried at 60 °C for 48 hours and sent to the University of Saskatchewan in Canada, where analyses of stable carbon and nitrogen isotopes were undertaken using mass spectrometry. These techniques measure the ratio of heavy and light isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$) in the samples in relation to Pee Dee Belemnite (PDB) and atmospheric nitrogen, respectively. Isotopic ratios (δ) are expressed in parts per thousand (‰), defined as δ (‰) = (R_{sample} / $R_{standard}$ - 1) ^{x} 1000, where R_{sample} and $R_{standard}$ are the isotopic ratios of the sample and the standard, respectively. A protein standard analyzed repeatedly (n = 29) alongside samples had standard deviations of 0.14‰ and 0.11‰ for C and N, respectively.

2.5. Estimates of trophic position with $\delta^{15}N$ values

Estimates of trophic position based on stable-isotope analysis (SIA) of muscle ($TP_{sia\text{-}M}$) and liver ($TP_{sia\text{-}L}$) $\delta^{15}N$ values were calculated with the following equation (modified from Post, 2002):

$$TP_{sia\text{-}M} \text{ or } TP_{sia\text{-}L} = \lambda + (\delta^{15}N_{muscle\ or\ liver} - \delta^{15}N_{baseline}) / TDF$$

where the estimated trophic position (TP) with stable isotopes is equal to the trophic level of the organisms used as baseline (λ) plus the difference between the muscle or liver $\delta^{15}N$ and the $\delta^{15}N$ value of the baseline divided by the trophic discrimination factor (TDF). We opted to use a TDF of 3.4‰ (Post 2002) for calculations but also report arapaima-specific TDFs by subtracting the $\delta^{15}N$ of the stomach contents of each fish from the $\delta^{15}N$ of the muscle and the liver ($\delta^{15}N_{muscle\ or\ liver} - \delta^{15}N_{stomach\ or\ prey}$). The baseline was calculated as the $\delta^{15}N$ mean of primary consumers from each lake and ranged from 4.7‰ to 8.1‰ (Table 2). The baseline was composed of Chironomidae, Ephemeroptera, snails and zooplankton (Vander Zanden & Rasmussen 1999).

The nitrogen isotope values of $TP_{stomach}$ and TP_{prey}, hereafter referred to as $\delta^{15}N_{stomach}$ and $\delta^{15}N_{prey}$, were calculated according the relative biomass of each item in the stomach and we also subtracted the baseline from this values ($\delta^{15}N_{stomach\text{-}baseline}$ and $\delta^{15}N_{prey\text{-}baseline}$).

2.6. Data analysis

A linear regression was made between the $\delta^{15}N$ value of all stomach contents ($\delta^{15}N_{stomach}$) and $TP_{stomach}$ to determine if the relationship between estimates of trophic position of food based on stable isotopes and those from direct observations were related. The same was made with $\delta^{15}N$ value of animal prey ($\delta^{15}N_{prey}$) and $TP_{prey.}$ These four trophic estimates from stomach contents, as well as body size (total length, TL), were then regressed against TP_{sia-M} of arapaima to determine if trophic shifts occurred as arapaima grew in size. The same analysis was repeated for TP_{sia-L}, and these values were also regressed on TP_{sia-M} values to estimate whether TP_{sia-M} can predict TP_{sia-L} and vice-versa. To test if there was a change in the trophic level of prey ingested by arapaima ($TP_{stomach}$ and TP_{prey}) between seasons (high and low water levels) we performed a Wilcoxon-Mann-Whitney test. We also used a generalized linear model (GLM) to determine if arapaima total length was related to the presence or absence of prey in stomach contents.

Analysis of covariance was used to determine which of the variables used (diet, size and season) best explain the TP_{sia-M} and TP_{sia-L} variation. For diet we considered $TP_{stomach}$, TP_{prey}, $\delta^{15}N_{stomach}$ and $\delta^{15}N_{prey}$. Season was added as a categorical variable (high and low water levels) to take into account possible differences in isotopic values among seasons. All statistical analyses and graphics were run using R software (R Core Team, 2017).

3. RESULTS

3.1. Stomach contents

During the dry season we collected liver, muscle and stomach-content samples from 76 arapaima with total lengths (TL) between 60 and 235 centimeters. However, only 28 individuals had animal prey in the stomachs and we used only samples from individuals with identifiable contents in the stomach. Prey were identifiable in twenty-two stomachs (29%) and consisted mostly of omnivorous (44%), carnivorous (33%) and detritivorous (19%) fish. Nineteen prey types were identified, including fish of the orders Characiformes (33%), Siluriformes (33%), Osteoglossiformes (11%) and Perciformes (11%), as well as shrimps

(*Macrobrachium amazonicum*, 11%) (Table 1). Eighteen stomachs also contained plant material.

In the falling-water period we obtained samples of only five individuals due to the difficulty in catching fish at high water levels. These stomachs contained omnivorous (83%) and detritivorous prey (17%). Six prey types were identified, including fish of the orders Siluriformes (33%), Characiformes (17%) and Gymnotiformes (17%), and invertebrates, such as Decapoda (*Macrobrachium amazonicum* 17%) and Ephemeroptera (17%) (Table 1). All stomachs contained plant material.

Empty stomachs occurred across the size range and there was no relationship between body size and the presence/absence of prey in stomachs ($p = 0.366$, average size of individuals with empty stomachs = 183 cm, those with prey in stomachs = 171 cm). Trophic positions of arapaima based on estimates of all stomach contents ($TP_{stomach}$ + 1 trophic level) were 3.6 (standard deviation = 0.5) and based only on the animal prey (TP_{prey} + 1 trophic level) were 3.7 (standard deviation = 0.4) (Table 2).

3.2. Stable isotopes

Arapaima $\delta^{15}N_{muscle}$ averaged 9.6‰ and ranged from 7.9‰ to 11.4‰ (Fig. 2A). The mean TDF between all-stomach-content or only-animal-prey $\delta^{15}N$ and muscle $\delta^{15}N$ was 1.0‰, ranging from -2.3‰ to 3.4‰ for $\delta^{15}N_{stomach}$ (Fig. 2B) and from -1.7‰ to 3.4‰ for $\delta^{15}N_{prey}$ (Fig. 2B). Using a literature-based value of 3.4‰ for the TDF yielded a mean $TP_{sia\text{-}M}$ of 3.2 (2.3 to 3.7).

When all stomach contents, including plant matter, were included, $TP_{stomach}$ had a positive influence on $\delta^{15}N_{stomach}$ ($\delta^{15}N_{stomach} = -2.506 + 2.203*TP_{stomach}$, $F_{1,25} = 17.58$, $r^2 = 0.41$, $p < 0.01$; Fig. 3A), suggesting that the stable isotopes reflected, at least in part, our trophic-level categories. However, $TP_{stomach}$ had no relationship with $TP_{sia\text{-}M}$ values ($F_{1,25} = 1.66$, $r^2 = 0.06$, $p = 0.21$; Fig. 4A) but $\delta^{15}N_{stomach}$ had a positive relationship with $TP_{sia\text{-}M}$ ($TP_{sia\text{-}M} = 2.81 +$

0.12* $\delta^{15}N_{stomach,}$ $F_{1,25}$ = 11.3, r^2 = 0.31, p < 0.01; Fig. 4B). When $\delta^{15}N$ values of stomach contents were based only on animal prey, without considering plant material, TP_{prey} had a positive relationship with $\delta^{15}N_{prey}$ ($\delta^{15}N_{prey}$ = -2.96 + 2.31*TP_{prey}, $F_{1,25}$ = 15.37, r^2 = 0.38, p < 0.01; Fig. 3B). However just $\delta^{15}N_{prey}$ was related to $TP_{sia\text{-}M}$ ($TP_{sia\text{-}M}$ = 2.77 + 0.13* $\delta^{15}N_{prey,}$ $F_{1,25}$ = 14.01, r^2 = 0.36, p < 0.01, Fig. 4D) while TP_{prey} was not ($F_{1,25}$ = 2.71, r^2 = 0.10, p = 0.11, Fig. 4C).

The $\delta^{15}N_{liver}$ values averaged 9.4‰ and ranged from 8.1‰ to 11.9‰ (Fig. 2C). The mean difference between liver $\delta^{15}N$ and stomach-content or prey $\delta^{15}N$ was 0.7‰, ranging from -1.5‰ to 2.9‰ for $\delta^{15}N_{stomach}$ (Fig. 3D, ▽) and from -1.3‰ to 2.9‰ for $\delta^{15}N_{prey}$ (Fig. 2D, ×). Trophic positions estimated with liver $\delta^{15}N$ ($TP_{sia\text{-}L}$) and muscle $\delta^{15}N$ ($TP_{sia\text{-}M}$) were positively related ($TP_{sia\text{-}M}$ = -0.06 + 1.03*$TP_{sia\text{-}L}$, $F_{1,25}$ = 90.55, r^2 = 0.78, p < 0.01, Supplementary Fig. S1). $TP_{sia\text{-}L}$ also averaged 3.2 when using a TDF of 3.4‰. Unlike $TP_{sia\text{-}M}$, $TP_{sia\text{-}L}$ had a significant, positive relationship with $TP_{stomach}$ ($TP_{sia\text{-}L}$ = 2.52 + 0.24*$TP_{stomach,}$ $F_{1,25}$ = 4.2, r^2 = 0.14, p = 0.05; Fig. 5A) and TP_{prey} ($TP_{sia\text{-}L}$ = 2.26 + 0.34*TP_{prey}, $F_{1,25}$ = 7.0, r^2 = 0.22, p = 0.01; Fig. 5C). $\delta^{15}N_{stomach}$ and $\delta^{15}N_{prey}$ had also a positive relationship with $TP_{sia\text{-}L}$ ($TP_{sia\text{-}L}$ = 2.79 + 0.12* $\delta^{15}N_{stomach,}$; $F_{1,25}$ = 15.12, r^2 = 0.38, p < 0.01; Fig. 5C; $TP_{sia\text{-}L}$ = 2.76 + 0.12* $\delta^{15}N_{prey,}$; $F_{1,25}$ = 17.44, r^2 = 0.41, p < 0.01; Fig. 5D).

3.3. Effects of body size and season

Estimates of trophic position of all stomach contents and only animal prey ($TP_{stomach}$ and TP_{prey}) were not related to the total length of arapaima ($F_{1,25}$ = 1.64, r^2 = 0.06, p = 0.21 and $F_{1,25}$ = 2.67, r^2 = 0.10, p = 0.11, respectively; Supplementary Fig. S2). There was no significant difference in the mean trophic level of $TP_{stomach}$ and TP_{prey} between the high- and low-water seasons (w = 44.5, p = 0.53; w = 40, p = 0.32; Supplementary Fig. S3). We regressed $TP_{sia\text{-}M}$ and $TP_{sia\text{-}L}$ on total length to evaluate the magnitude of the effect of increase in length on these trophic estimates without the diet effect (TP_{sia-M} = 2.20 + 0.006*TL, $F_{1,25}$ = 20.68, r^2 = 0.45, p < 0.01; TP_{sia-L} = 2.27 + 0.005*TL, $F_{1,25}$ = 23.07, r^2 = 0.48, p < 0.01). One value was an outlier with large leverage. Removal of the outlier resulted in an even stronger relationships (TP_{sia-M} = 1.94 + 0.007*TL, $F_{1,24}$ = 24.9, r^2 = 0.51, p < 0.01, Fig. 6A; TP_{sia-L} = 2.04 + 0.006*TL, $F_{1,24}$ = 28.22, r^2 = 0.54, p < 0.01; Fig. 6B) which indicates that TP_{sia-M} and TP_{sia-L} both increase about 0.01‰ for each centimeter increase in total length despite no increase in the estimated TP of ingested prey.

Analysis of covariance indicated that TP_{sia-M} and TP_{sia-L} were related to the combination of diet ($TP_{stomach}$ or TP_{prey}, $\delta^{15}N_{stomach-baseline}$ or $\delta^{15}N_{prey-baseline}$), total length, season, and interactions of season with diet and total length. However, only total length and the interactions between season and $\delta^{15}N_{stomach-baseline}$ and $\delta^{15}N_{prey–baseline}$ contributed positively to the relationships while the interaction between season and TL, diet and season alone did not (Table 3).

Although there were significant effects of season and its interaction on TP_{sia-M}, removing season and its interaction from the analysis reduced the variance explained by the model between 20% and 24%. (Table 3). Without season it was related to the combination of diet and TL with a significant contribution from TL but not diet (Table 3). For TP_{sia-L}, the combination of $\delta^{15}N_{stomach-baseline}$, $\delta^{15}N_{prey-baseline}$ and TL contributed significantly to the relationship and the model explained between 15% and 19% less removing season and its interactions (Table 3).

Removing total length, the models remained significant for TP_{sia-M} with significant contributions of $\delta^{15}N_{prey-baseline}$ and season (Table 3). It also remained significant for TP_{sia-L} with $\delta^{15}N_{stomach-baseline}$, $\delta^{15}N_{prey-baseline}$ and TP_{prey} contributing positively to the relationship

(Table 3). When present, TL always contributed significantly in the analysis, while the same was not so for diet and season.

DISCUSSION

The TDF calculated for arapaima using $\delta^{15}N$ values was much smaller than the values frequently used in the literature (Post, 2000; McCutchan et al., 2003) and with large variation among individuals. Possibly due to faster turnover, $TP_{sia\text{-}L}$ showed a better match than $TP_{sia\text{-}M}$ with the stomach-content data. However, body size and season explained most of the variation in stable-isotope values in muscle and liver, and hence estimates of arapaima trophic position based on these isotope data ($TP_{sia\text{-}M}$, $TP_{sia\text{-}L}$). Both $TP_{sia\text{-}L}$ and $TP_{sia\text{-}M}$ increased with arapaima body size and diet had little effect on these relationships. There is a need for a better comprehension of the factors besides diet trophic position that can influence $\delta^{15}N$ values before estimates of trophic position can be based only on stable isotopes.

Low TDFs have also been suggested for crocodilians (Marques et al., 2014) and could be common in large-bodied ectothermic predators. However, it is unlikely that most of the prey taken by arapaima also have such low TDFs. As such, we chose to use a common literature-derived value for our TP calculations. Since these calculations are sensitive to TDF (Post, 2002) as it is the denominator used in the equation, we may be underestimating TPs for arapaima. Yet our estimated TDF values from ingested prey (1.0‰ for muscle, 0.7‰ for liver) would have led to extremely high and unrealistic values. The uncertainties in TDF shown for arapaima here and also suggested for caimans (Villamarín et al., 2018), are likely to apply to different species and are a complicating factor when using $\delta^{15}N$ to estimate TP.

It is assumed that $\delta^{15}N$ reflects the assimilation of dietary intake over a long time span including the differential assimilation of different types of food (Peterson & Fry, 1987). Therefore, muscle $\delta^{15}N$ may reflect what was eaten some time before the animal was captured, especially in larger individuals (Thomas & Crowther, 2014), and seasonal differences in prey availability might mask the relationship between stomach-content $\delta^{15}N$ and muscle $\delta^{15}N$. However, liver $\delta^{15}N$ often turns over faster than muscle $\delta^{15}N$ (Manetta & Benedito, 2003; Perga & Gerdeaux, 2005; Logan et al., 2006) and in spite of $TP_{sia\text{-}M}$ and $TP_{sia\text{-}L}$ being related ($r^2 = 0.78$), $TP_{sia\text{-}L}$ was positively and statistically related to the diet in more analysis probably because of the faster turnover rates. There was no seasonal difference in trophic level of prey based on stomach contents. Even taking season into account, the influence of total length was stronger than diet in $TP_{sia\text{-}L}$ values, which also increased with body size, suggesting that temporal lags in prey assimilation are unlikely to be responsible for the lack of relationship between trophic position of prey and stable-isotope estimates of TP of arapaima.

Although it is often assumed that the $\delta^{15}N$ of a predator increases predictably with increase in trophic level of its prey (DeNiro & Epstein, 1981**;** Minagawa & Wada, 1984; Manetta & Benedito, 2003), several studies have questioned whether TDFs remain constant throughout an animal's life span (Davis et al., 2012; Villamarín et al., 2018), especially when there are expected ontogenetic changes in trophic level (Overmann & Parrish, 2001). A previous study based on $\delta^{15}N$ found that the presumed trophic position of arapaima individuals increased with size by approximately 3‰, or one trophic level, over a size range from 60 to >200 cm length (Carvalho et al., 2018), similar to our estimates from stable isotopes. However, the data based on the classification of stomach contents of the same individuals of that study showed no clear increase in the trophic level of ingested prey as arapaima size increased, with a low occurrence of piscivorous prey in arapaima stomachs. Villamarín et al. (2018) studied species of caimans, which are ectothermic predators similar in size and habitat to arapaima, and concluded that changes in muscle $\delta^{15}N$ were more closely related to size than to the trophic levels of their prey, and TDFs were related to growth rates of the caiman. Our overall results indicate that, as with caiman, TP based on muscle and liver $\delta^{15}N$ values are more related to size than to the diet trophic level or diet $\delta^{15}N$. There was no consistent increase with size in the trophic level of the prey in stomach contents, and this result held whether we considered all stomach contents, including plants, or only animal prey. Due the short-term nature of stomach contents, additional stomach content data can help to confirm if indeed there is no shift towards higher trophic position prey with increased body size. The generalization of trophic level for large prey groups like Curimatidae, can be a source of error; however, we minimized this by opting for the most probable trophic level for species of the family in the region of study. Also, the majority of prey items from this family that we identified at least to the genus level are from low and intermediate trophic levels.

Many arapaima stomachs were empty or with only traces of plant material, such as branches and leaves. Carvalho et al. (2018) also found a great proportion (80%) of empty stomachs in arapaima from floodplain lakes, possibly because in the dry season the lakes are isolated from the main river and prey becomes limiting. Piscivorous fishes often have empty stomachs (Arrington et al., 2002). In our study, arapaima commonly regurgitated when captured. This phenomenon has been found in predatory fishes captured with gill nets, especially when water temperatures exceed 21ºC (Treasurer, 1988). Although this may have affected the total number of prey detected, there is no reason to believe that arapaima selectively regurgitate prey from different trophic levels.

As the diet, represented by all-stomach-contents or only-animal-prey $\delta^{15}N$ and its estimated trophic positions, are not related to total length, arapaima TP should not increase with body size. Physiological processes associated with size that are unrelated to trophic level could lead to higher or lower $\delta^{15}N$ than expected. We do not know why estimates of TP based on stable isotopes increase with size in arapaima independent of the trophic level of prey.

Possibilities include ^{15}N discrimination that can be affected by rates of nitrogen excretion relative to assimilation (McCutchan et al., 2003), or that discrimination is dependent on the protein content of the diet (Florin et al., 2011). Poor protein quality leads to greater ^{15}N enrichment in tissues, which could explain the higher $\delta^{15}N$ values in larger individuals if their prey were of inferior quality. Nevertheless, larger arapaima ate mainly fish, which presumably are more easily digested than the invertebrates that are more common in the diets of smaller individuals.

Similar to the results for crocodilians (Villamarín et al., 2018), arapaima body size explained shifts in estimates of $\delta^{15}N$-based TP better than dietary observations. Season also seems to influence arapaima $\delta^{15}N$ values despite the fact that there was no difference in trophic levels of prey between seasons. As we obtained only a few samples from the flood season we are not able to offer an explanation as to why this occurs. According to Queiroz (2000) arapaima diet composition varies along the seasonal cycle, but there is no evidence that this effects the mean trophic level of prey. During periods of rising water and floods, fish-prey densities decrease because of the increasing area and volume of water, and crustaceans were more present in these periods while mollusks also appear during rising water (Quieroz 2000). One possibility is that our small number of samples in the flood period are non-representative. We also did not find differences in the prey trophic level ingested between seasons but the samples from the flood period cover only a small size range, being all samples from individuals smaller than 180cm while in the dry season we have samples of individuals as high as 235cm in total length. Perhaps this is why the interaction between season and $\delta^{15}N_{stomach}$ or $\delta^{15}N_{prey}$ are significant in the models while the interactions between season and $TP_{stomach}$ or TP_{prey} are not.

This study show that arapaima $\delta^{15}N$ is not a simple reflection of diet alone but other factors associated with size and season are also having an influence. These influences must be better understood in order to be able to use $\delta^{15}N$ to estimate trophic position. Until then, $\delta^{15}N$ must be used with caution when estimating trophic positions of ectotherms with large variation in size and seasonal variations. Despite the stomach-content method being more laborious, providing only information about the most recent feeding prior to animal capture, and other uncertainties caused by the large proportion of empty stomachs, it remains the most reliable technique to estimate trophic position when sample sizes are sufficient. In the meantime, it is important to recognize potential biases in both approaches (stable isotopes and stomach-content analysis) for estimating food web relationships; this will lead to more rigorous determinations of animal diet and energy acquisition to unravel the trophic ecology of predatory fishes.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Research Program on Biodiversity (PPBio/Data ONE) repository, https://ppbio.inpa.gov.br/repositorio/dados.

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TABLES

Table 1. Prey identified in arapaima stomach-contents and their respective trophic group and trophic level based on diet. Trophic groups were defined as follows: (C) carnivore; (D) detritivore; (H) herbivore; (O) omnivore and (P) producer.

Prey type	**Trophic group**	**Trophic level**
Charachiformes		
Anostomidae	D/O	2.5
Characinae	C	3
Curimatidae	D	2
Hydrolycus scomberoides	C	3.5
Metynnis sp.	H	2
Prochilodus nigricans	D	2
Rhaphiodon vulpinus	C	3
Serrasalmidae	O	2.5
Triportheus sp.	O	2.5
Decapoda		
Macrobrachium amazonicum	O	2.5
Ephemeroptera	D	2
Gymnotiformes		
Adontosternarchus sp.	O	2.5
Osteoglossiformes		
Osteoglossum bicirrhosum	C	3.5
Perciformes		
Cichlidae	O	2.5
Siluriformes		
Doradidae	O	2.5
Hypophthalmus sp.	O	2.5
Hypostomus sp.	D	2.5
Loricariichthys sp.	D	2.5
Loricariinae	D	2.5
Pimelodina flavipinnis	C	3
Pimelodus blochii	O	2.5
Pimelodus sp.	O	2.5
Trachelyopterus sp.	O	2.5
Seeds, branches or leaves	P	1

Table 2. Lake, baseline, season, total length (TL = total length in centimeters), stable-isotope ratios and trophic-position data for each arapaima analyzed. $\delta^{15}N_{muscle}$ = stable-isotope ratio of nitrogen in arapaima muscle; $\delta^{15}N_{liver}$ = stable-isotope ratio of nitrogen in arapaima liver; $\delta^{15}N_{stomach}$ = stable-isotope ratio of nitrogen of arapaima stomach-contents; $\delta^{15}N_{prey}$ = stable-isotope ratio of animal prey present in the arapaima stomach-contents; $TP_{stomach}$ = trophic position estimated based on trophic level of prey in stomach-contents; TP_{prey} = trophic position estimated based only on trophic level of animal prey present in the stomach contents; $TP_{sia\text{-}M}$ trophic position estimated with $\delta^{15}N$ from arapaima muscle; $TP_{sia\text{-}L}$ trophic position estimated with $\delta^{15}N$ from arapaima liver.

Lake	**δ^{15}NBaseline**	**Season**	**TL (cm)**	**$\delta^{15}N_{muscle}$**	**$\delta^{15}N_{liver}$**	**$\delta^{15}N_{stomach}$**	**$\delta^{15}N_{prey}$**	**$TP_{stomach}$**	**TP_{prey}**	**TP_{sia}-M**	**TP_{sia}-L**
Santa Clara	6.7	Dry	61.0	9.94	9.86	6.95	6.95	2.50	2.50	2.95	2.93
Samauma	6.9	Falling	98.6	9.86	9.75	9.43	9.43	2.50	2.50	2.88	2.84
Mandioca	4.7	Dry	118.0	8.42	8.58	9.64	9.64	2.50	2.50	3.09	3.13
Mandioca	4.7	Dry	123.0	8.16	8.21	5.48	5.67	2.17	2.50	3.01	3.02
Marari Grande	7.9	Falling	131.0	9.62	9.78	9.47	9.76	2.42	2.50	2.49	2.54
Mandioca	8.1	Falling	134.0	9.15	9.36	8.13	8.19	2.42	2.50	2.31	2.37
Mandioca	4.7	Dry	138.0	7.95	8.22	8.00	7.37	2.90	2.94	2.95	3.03
Janiceto	5.9	Dry	148.0	10.17	9.48	9.46	9.09	2.79	2.82	3.24	3.04
Samauma	5.9	Dry	164.0	10.31	10.42	9.25	9.11	2.35	2.50	3.29	3.32
Santo Antonio	5.9	Dry	166.0	9.13	9.03	10.14	10.15	2.48	2.50	2.94	2.91
Mandioca	4.7	Dry	169.9	9.32	8.15	9.67	9.25	2.38	2.50	3.35	3.00
Sacado do Juburi	7.1	Falling	170.0	9.87	10.28	9.15	9.45	2.34	2.50	2.81	2.94
Mandioca	4.7	Dry	174.2	8.74	8.05	7.33	7.37	2.04	2.04	3.18	2.98
Mandioca	4.7	Dry	176.0	8.90	8.50	7.32	7.17	1.93	2.00	3.23	3.11

Lake	**δ^{15}NBaseline**	**Season**	**TL (cm)**	**$\delta^{15}N_{muscle}$**	**$\delta^{15}N_{liver}$**	**$\delta^{15}N_{stomach}$**	**$\delta^{15}N_{prey}$**	**$TP_{stomach}$**	**TP_{prey}**	**TP_{sia}-M**	**TP_{sia}-L**
Santa Clara	6.9	Falling	180.0	10.59	10.69	9.59	9.63	2.47	2.50	3.09	3.11
Mandioca	4.7	Dry	184.0	10.36	9.32	10.54	10.66	3.50	3.50	3.66	3.35
Mandioca	4.7	Dry	187.2	8.98	9.07	6.74	6.68	2.66	2.67	3.25	3.28
Mandioca	4.7	Dry	192.4	7.86	9.44	10.19	9.53	3.47	3.50	2.92	3.39
Mandioca	4.7	Dry	199.0	10.67	10.14	9.75	10.09	2.97	3.00	3.75	3.59
Mandioca	4.7	Dry	202.0	9.83	9.40	7.98	8.72	1.99	2.50	3.50	3.37
Mandioca	4.7	Dry	206.3	9.70	9.05	7.82	8.00	2.93	2.93	3.46	3.27
Mandioca	4.7	Dry	208.0	10.24	10.25	7.84	8.12	2.10	2.50	3.62	3.62
Veado	6.3	Dry	209.0	10.29	10.06	8.84	9.39	1.93	2.00	3.17	3.11
Mandioca	4.7	Dry	210.2	9.93	9.35	7.20	7.05	2.35	2.50	3.53	3.36
Mandioca	4.7	Dry	211.0	10.25	9.31	6.84	6.84	2.50	2.50	3.62	3.35
Samauma	5.9	Dry	230.0	11.44	11.90	11.80	11.90	3.50	3.50	3.62	3.75
Mandioca	4.7	Dry	234.9	10.01	9.46	10.50	10.50	3.50	3.50	3.55	3.39
							Mean TP	2.6	2.7	3.2	3.2

Table 3. Models used in the analysis of covariance to determine which variables (diet, size and season) best explain the arapaima trophic positions based on muscle and liver nitrogen. In **bold** are the variables that contributed positively in the models.

	Model	**Multiple R-squared**	**F-statistic**	**p-value**
TP_{sia-M}	$TP_{stomach}$ + **TL** + season + Tpstomach * season + TL * season	0.7041	9.995 on 5 and 21 DF	5.28E-05
	$\delta^{15}N_{stomach}$ + **TL** + season + **$\delta^{15}N_{stomach}$ * season** + TL * season	0.7558	13 on 5 and 21 DF	7.74E-06
	Tpprey + **TL** + season + TP_{prey} * season + TL * season	0.6685	11.09 on 4 and 22 DF	4.44E-05
	$\delta^{15}N_{prey}$ + **TL** + season + **$\delta^{15}N_{prey}$ * season** + TL * season	0.7513	12.69 on 5 and 21 DF	9.30E-06
	$TP_{stomach}$ + **TL**	0.46	10.22 on 2 and 24 DF	0.0006149
	$\delta^{15}N_{stomach}$ + **TL**	0.5171	12.85 on 2 and 24 DF	0.0001609
	TP_{prey}+ **TL**	0.4647	10.42 on 2 and 24 DF	0.0005541
	$\delta^{15}N_{prey}$ + **TL**	0.5275	13.39 on 2 and 24 DF	0.0001239
	$TP_{stomach}$ + season + Tpstomach * season	0.4665	6.703 on 3 and 23 DF	0.002052
	$\delta^{15}N_{stomach}$ + **season** + $\delta^{15}N_{stomach}$ * season	0.5887	10.97 on 3 and 23 DF	0.0001139
	TP_{prey} + **season** + TP_{prey} * season	0.4706	10.67 on 2 and 24 DF	0.0004849
	$\delta^{15}N_{prey}$ + **season** + $\delta^{15}N_{prey}$ * season	0.6155	12.27 on 3 and 23 DF	5.35E-05

	Model	Multiple R-squared	F-statistic	p-value
TP_{sia-L}	$TP_{stomach}$ + **TL** + season + Tpstomach * season + TL * season	0.7111	10.34 on 5 and 21 DF	4.17E-05
	$\delta^{15}N_{stomach}$ + **TL** + season + **$\delta^{15}N_{stomach}$ * season** + TL * season	0.7606	13.34 on 5 and 21 DF	6.34E-06
	Tpprey + **TL** + season + TP_{prey} * season + TL * season	0.705	13.14 on 4 and 22 DF	1.29E-05
	$\delta^{15}N_{prey}$ + **TL** + season + **$\delta^{15}N_{prey}$ * season** + TL * season	0.7586	13.2 on 5 and 21 DF	6.87E-06
	$TP_{stomach}$ + **TL**	0.5257	13.3 on 2 and 24 DF	0.0001296
	$\delta^{15}N_{stomach}$ + **TL**	0.5739	16.16 on 2 and 24 DF	3.58E-05
	Tpprey + **TL**	0.5505	14.7 on 2 and 24 DF	6.80E-05
	$\delta^{15}N_{prey}$ + **TL**	0.5761	16.31 on 2 and 24 DF	3.37E-05
	$TP_{stomach}$ + season + Tpstomach * season	0.4698	6.793 on 3 and 23 DF	0.001915
	$\delta^{15}N_{stomach}$ + **season** + $\delta^{15}N_{stomach}$ * season	0.5981	11.41 on 3 and 23 DF	8.79E-05
	TP_{prey} + **season** + TP_{prey} * season	0.5136	12.67 on 2 and 24 DF	0.0001752
	$\delta^{15}N_{prey}$ + **season** + $\delta^{15}N_{prey - baseline}$ * season	0.6237	12.71 on 3 and 23 DF	4.20E-05

FIGURES

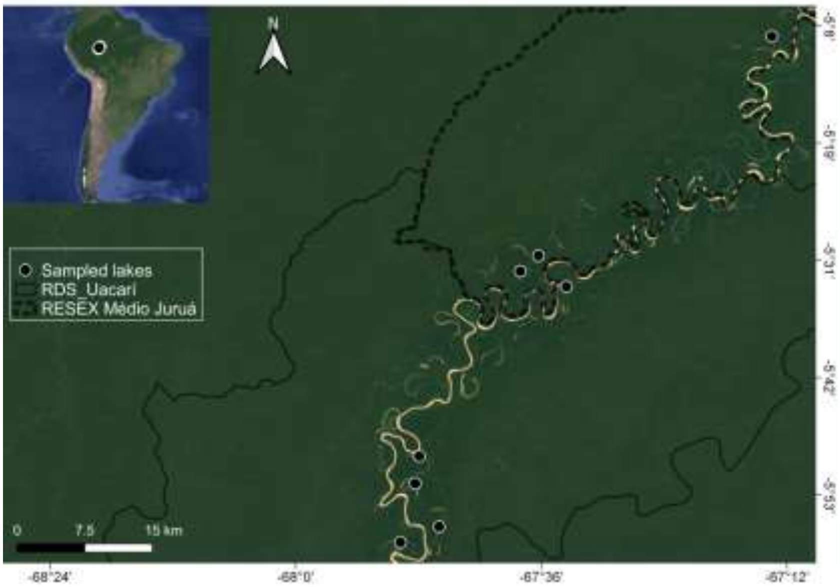

Fig. 1. Locations of the lakes along the Juruá River sampled in the study area, which included two protected areas.

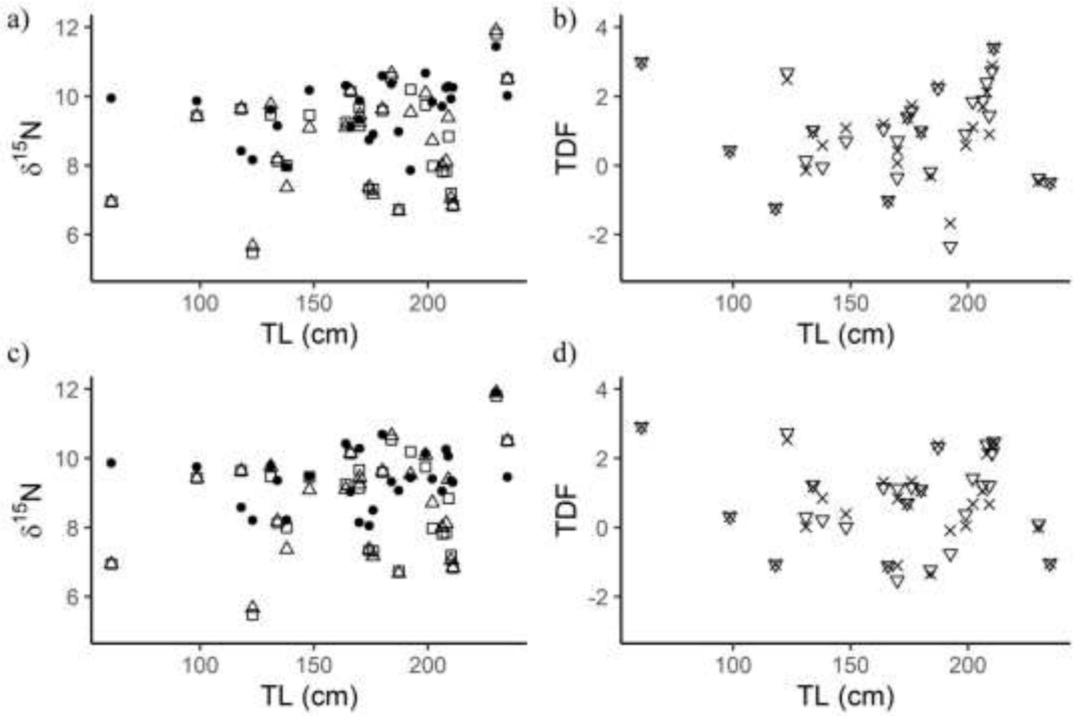

Fig. 2. (A) Variation in $\delta^{15}N$ values of muscle, all stomach contents and only-animal prey (● = muscle, □ = all stomach contents, △ = prey animals) relative to arapaima body size (total

length, TL). (B) Trophic discrimination factor (TDF) between muscle and all-stomach-contents $\delta^{15}N$ (▽) and between muscle and only-animal-prey $\delta^{15}N$ (×) relative to arapaima body size (total length, TL). (C) Variation in $\delta^{15}N$ values of liver, all stomach contents and animal prey in the stomach contents (● = liver, □ = stomach-content, △ = prey) relative to arapaima body size (total length, TL). (D) Trophic discrimination between liver and all stomach contents $\delta^{15}N$ (▽) and between liver and animal prey $\delta^{15}N$ (×) relative to arapaima body size (total length, TL).

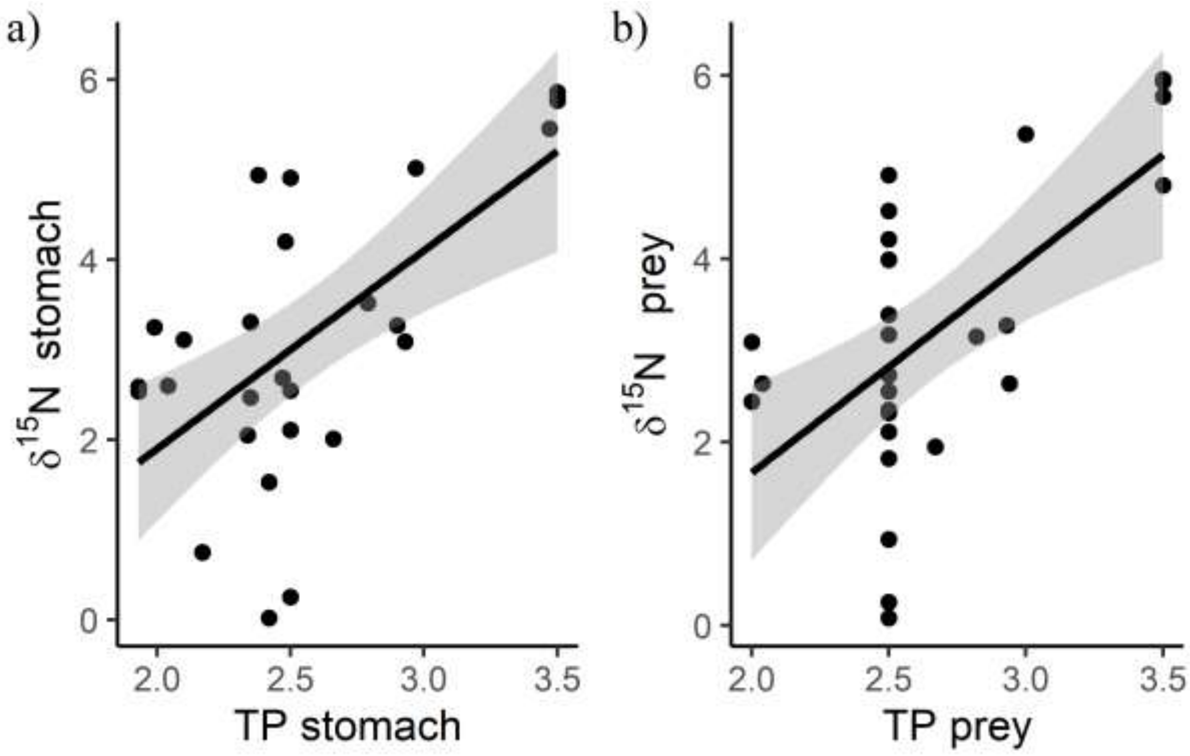

Fig. 3. (A) Relationships between visual trophic-position estimates of arapaima stomach-content ($TP_{stomach}$) and all-stomach-content $\delta^{15}N$ values. (B) Relationships between visual trophic position estimates of the arapaima animal prey present in stomach-content and animal prey $\delta^{15}N$ values.

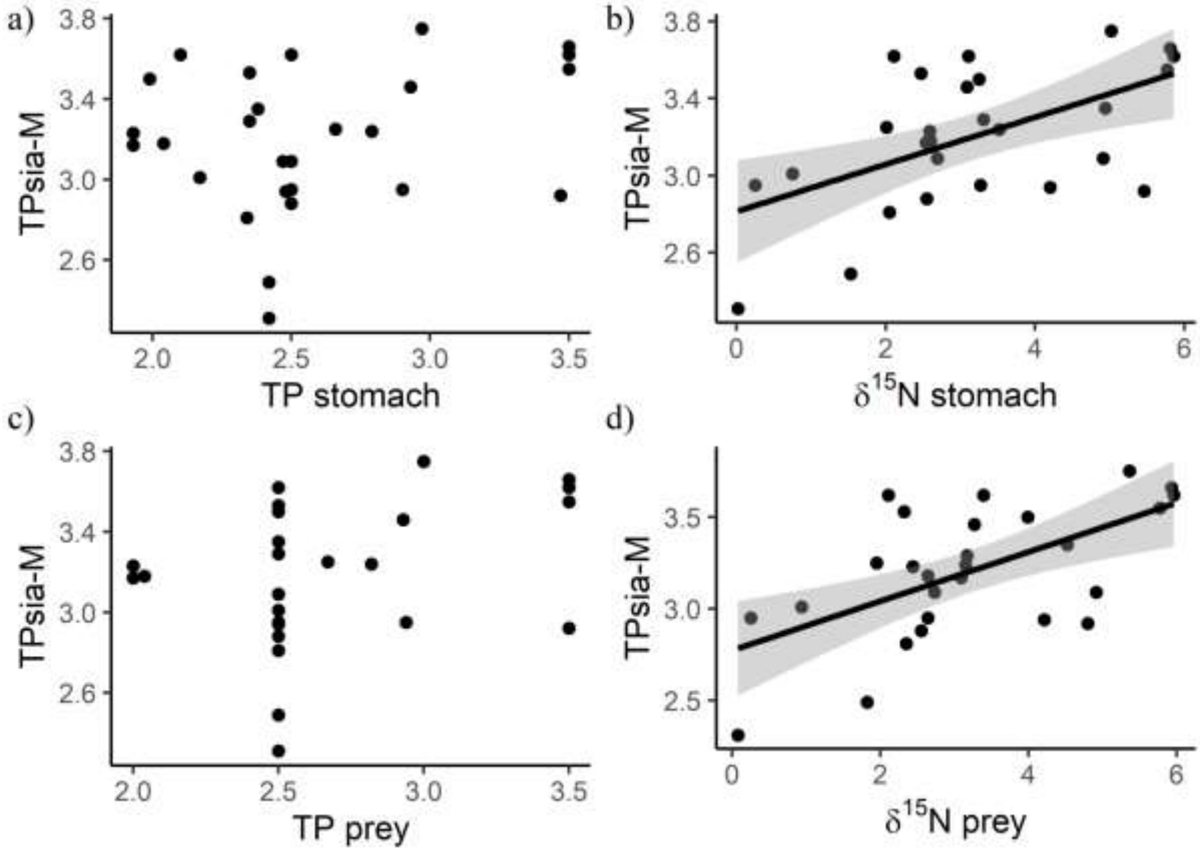

Fig. 4. Relationships between (A) trophic position derived from muscle $\delta^{15}N$ ($TP_{sia\text{-}M}$) and trophic position estimated visually for the stomach-content ($TP_{stomach}$); (B) $TP_{sia\text{-}M}$ and the $\delta^{15}N$ of stomach-content; (C) $TP_{sia\text{-}M}$ and trophic position estimated visually for the animal prey present in the stomach-content (TP_{prey}); (D) $TP_{sia\text{-}M}$ and animal prey $\delta^{15}N$.

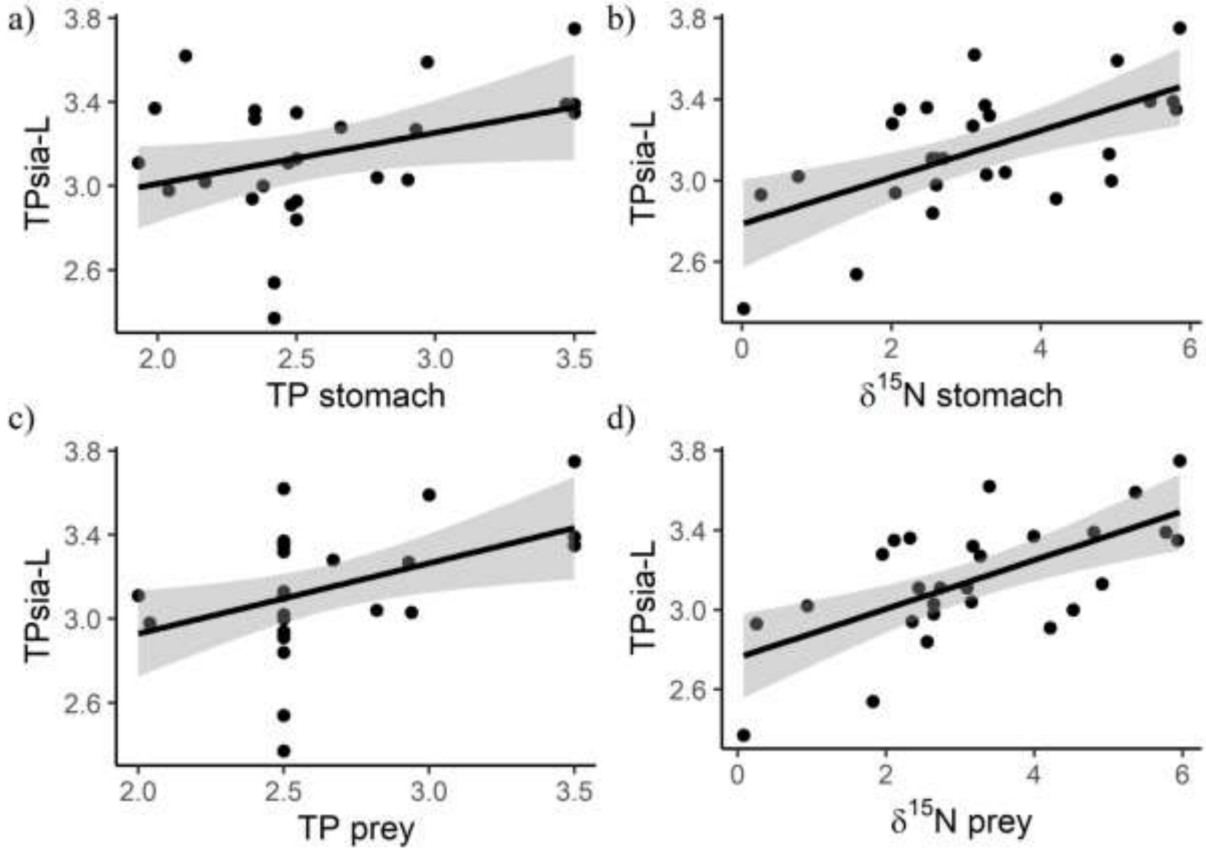

Fig. 5. Relationships between (A) trophic position derived from liver $\delta^{15}N$ ($TP_{sia\text{-}L}$) and trophic position estimated visually for the stomach-contents ($TP_{stomach}$); (B) Relationship between $TP_{sia\text{-}L}$ and $\delta^{15}N$ of all stomach contents ($\delta^{15}N_{stomach}$); (C) Relationship between $TP_{sia\text{-}L}$ and estimated trophic position of animal prey present in stomach content (TP_{prey}); (D) Relationship between $TP_{sia\text{-}L}$ and $\delta^{15}N$ of animal prey ($\delta^{15}N_{prey}$).

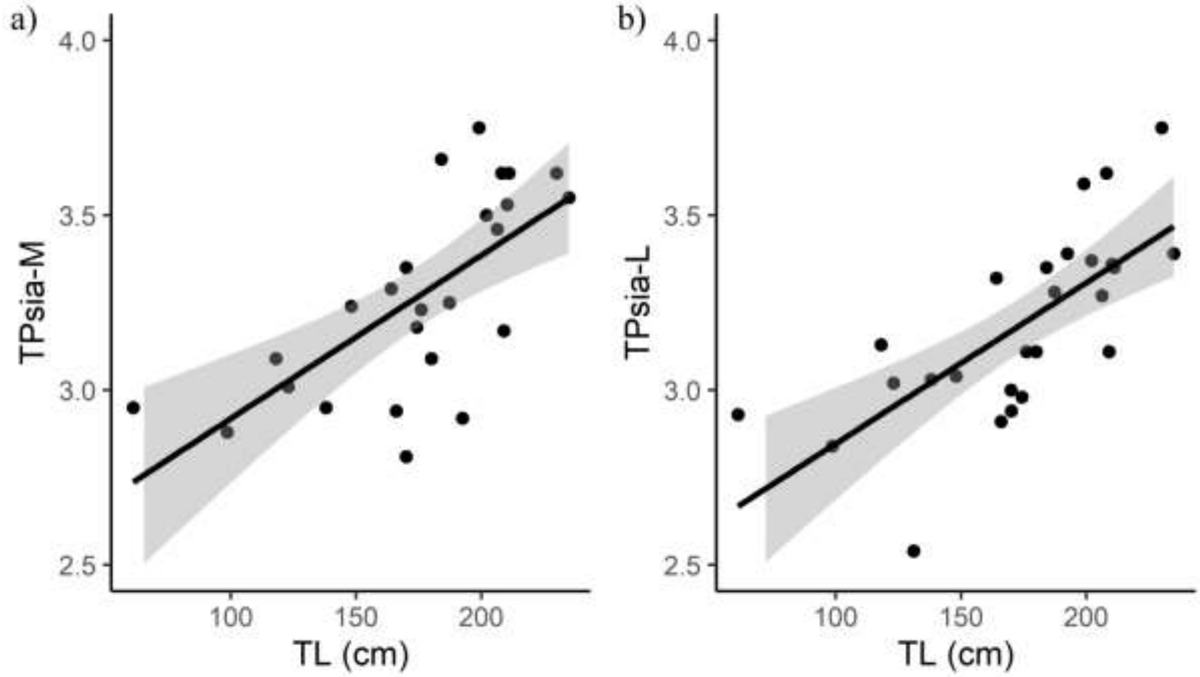

Fig. 6. (A) Relationship between estimated trophic positions based on $\delta^{15}N$ muscle values (TP_{sia-M}) and total length in centimeters (TL (cm)). (B) Relationship between estimated trophic positions based on $\delta^{15}N$ liver values (TP_{sia-L}) and TL (cm).

SUPPORTING INFORMATION

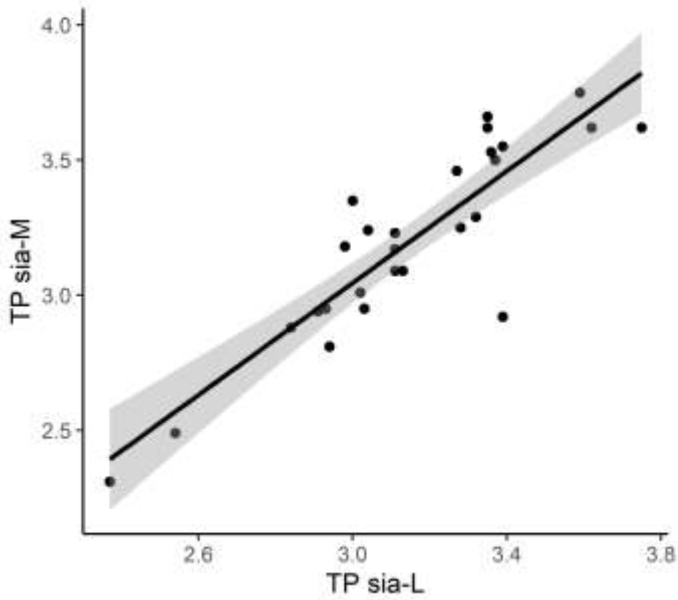

Fig. S1. Relationship between the trophic position estimated with $\delta^{15}N$ from arapaima muscle ($TP_{muscle\text{-}sia}$) and trophic position estimated from $\delta^{15}N$ in arapaima liver ($TP_{liver\text{-}sia}$).

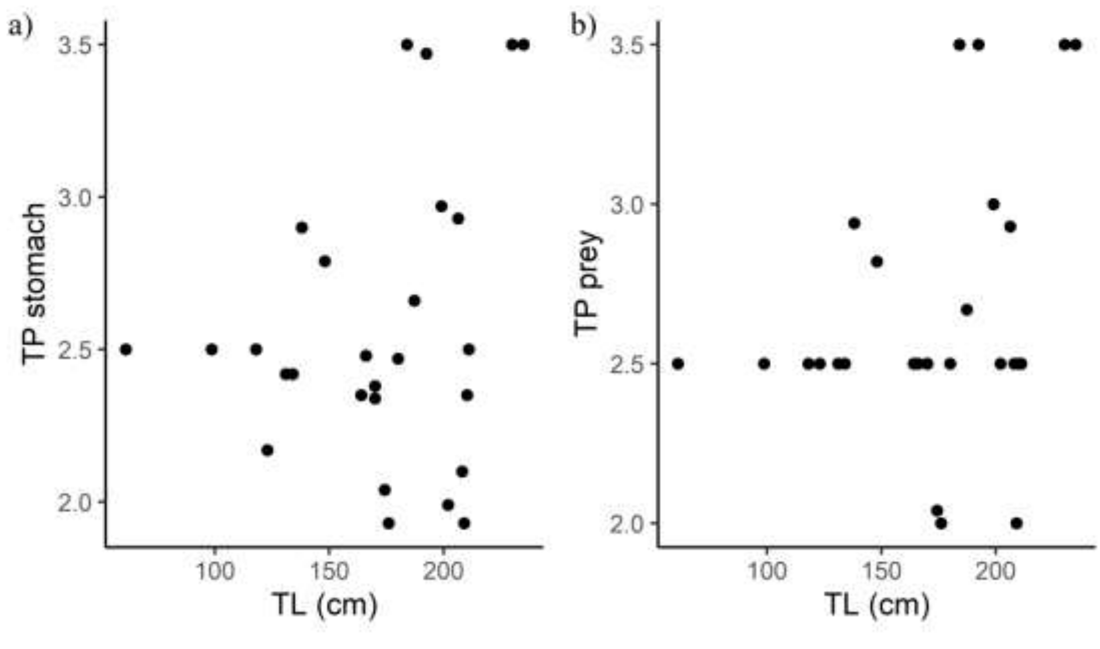

Fig. S2. Relationship between total length (cm) and trophic position estimated based on trophic level of all items in the arapaima stomach content (A) and only from animal prey in stomach contents (B).

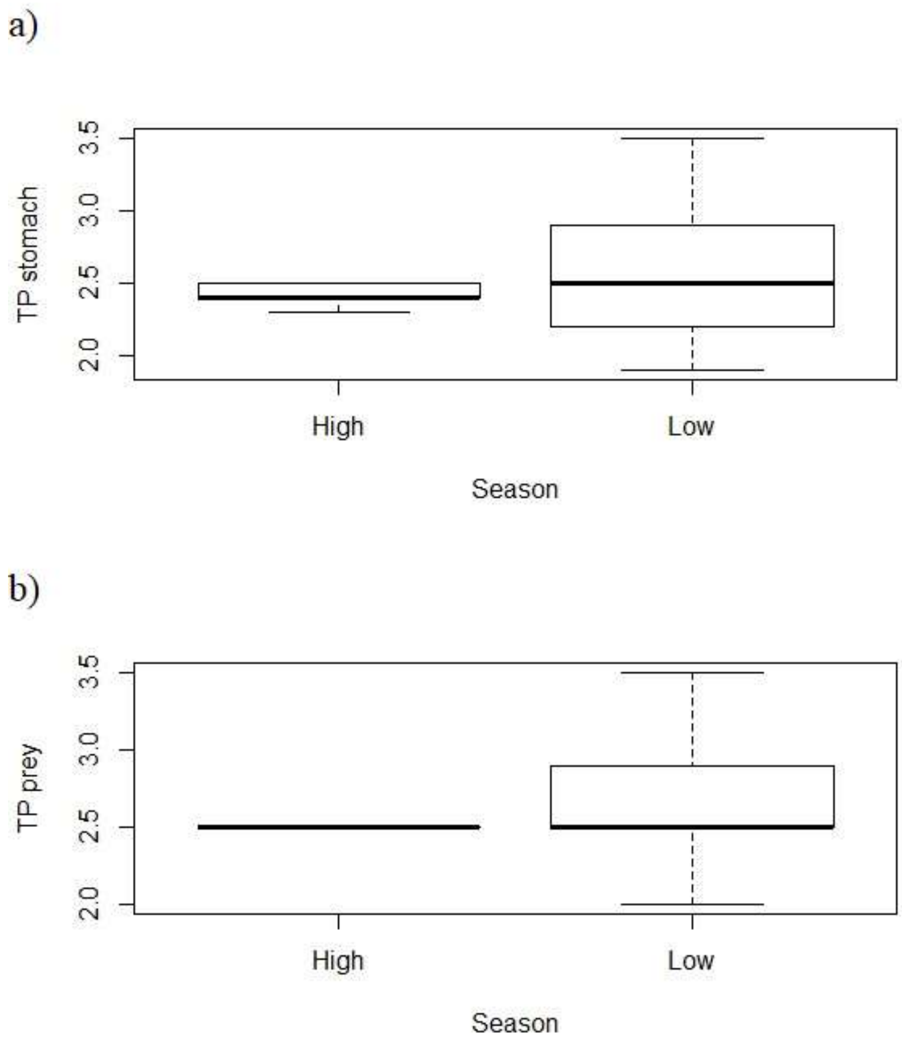

Fig. S3. Arapaima stomach and prey trophic level ($TP_{stomach}$ and TP_{prey}) between the high and low-water seasons.

Capítulo II.

Jacobi, C.M.; Villamarín, F.; Campos-Silva, J. V.; Jardine, T.; Magnusson, W.E.

Feeding of *Arapaima* sp.: integrating stomach contents and local ecological knowledge

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RESEARCH ARTICLE

Feeding of *Arapaima* sp.: integrating stomach contents and local ecological knowledge

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Abstract: The giant arapaima (*Arapaima* sp.*)* has been described as a fish of change in Amazonia. However, despite the cultural, ecological and economic importance of arapaima, data on diet are scarce. Aiming to expand knowledge about arapaima diet in western Amazonia, we integrate scientific knowledge with the knowledge of local dwellers. During the low-water period (September 2018) and the falling-water period (June 2019) we collected arapaima stomachs from 11 floodplain lakes in the middle Juruá River. All fishes were measured (TL – total length) and sexed. Food items from each stomach were categorized as fishes, invertebrates, plants and bone remains, and weighed. Also, in the latter period we interviewed experienced local fishers about arapaima feeding. Our integrated approach revealed that young arapaima eat fish and invertebrates, but adult arapaima eat fish of a wide range of species, which were mainly of low and intermediate trophic positions. We report the first case of cannibalism for arapaima, and we also show that during the low-water period, many individuals had empty stomachs or with only some small fish bone remains and/or plant material. Arapaima sex and total length had no influence on the absence of prey in stomach contents. Overall, we conclude that local people had consistent ethnobiological knowledge of arapaima feeding ecology that could be useful within management projects in the region.

Key words: Amazon, diet, ethnobiology, ichthyology, predation

INTRODUCTION

Arapaima sp., also known as pirarucu or paiche, is the largest freshwater scaled fish in the world. It can weigh up to 200 kg and reach up to about 3 meters in total length (TL; Nelson, 1994). It is endemic to the Amazon basin, inhabiting mainly floodplain lakes and flooded-forests. During rising and high-water levels, arapaima move from lakes to flooded-forest habitats, exploiting spatially and temporally heterogeneous resources of the floodplain, which presumably improves growth and reproduction (Castello, 2008; Campos-Silva *et al*., 2019). Five species of the genus have been proposed (Castello & Stewart, 2010; Stewart, 2013a, 2013b), but there is still no consensus on its taxonomy (Farias *et al*., 2019). Arapaima is also traditionally and commercially fished in the Amazon basin due to the quality of its meat, being highly overexploited over most of its geographical range and currently facing local extinctions in many localities (Castello *et al*., 2015). Despite its importance, arapaima is listed as data deficient by the IUCN (2020) and included in CITES Appendix II (the Convention on International Trade in Endangered Species of Wild Fauna and Flora).

Due to population declines of the species in some natural environments, arapaima fishing is prohibited in some regions of the Amazon, such as most of the state of Amazonas, Brazil. However, community-based management (CBM) programs have been established to recover and maintain arapaima stocks and improve local people's income (Castello *et al*., 2009). These initiatives have been successfully recovering wild populations of arapaima, combining biodiversity conservation with social development (Castello *et al*., 2009; Campos-Silva & Peres, 2016; Petersen *et al*., 2016; Campos-Silva *et al*., 2017; Campos-Silva *et al*., 2019; Freitas *et al*., 2020). The CBM of arapaima is largely based on harvest zoning systems, in which lakes are classified in three management categories: open-access lakes, where commercial fishing activities are allowed with no restrictions, (2) subsistence-use lakes, where fishing is allowed to supply local subsistence needs and (3) protected lakes, where fishing is banned except for a brief arapaima harvest period each year. In the protected lakes, population monitoring is undertaken through annual population counts carried out by local inhabitants. Based on this information, environmental authorities allow the removal of 30% of the adult population during the yearly harvesting period. In the Jurua River, a major tributary of the Amazon River, the CBM of arapaima has induced recovery of wild populations, with increases of more than 420% within 11 years (Campos-Silva *et al*., 2019). This is an example of a win-win program, allowing stock recovery and providing food and income for riverine people (Campos-Silva & Peres, 2016).

Despite the cultural, ecological and economic importance of arapaima, data on diet, which may provide information to improve conservation and management plans for the species, are still scarce. Some authors consider adult arapaima as apex predators that occupy relatively high positions on the food chain (Carvalho *et al*., 2018). Others have concluded that arapaima are omnivorous, typically secondary consumers, feeding relatively low or in the middle of the food web (Watson *et al*., 2013). Queiroz (2000) concluded that arapaima diet is composed mainly of fishes, and characterized the species as mainly piscivorous though the smallest individuals also complement their diet with invertebrates.

Many studies have demonstrated the value and usefulness of fisher's ecological knowledge to research and management (Braga & Rebêlo, 2017; da Silva *et al*., 2019; Nunes *et al*., 2019), showing agreement between information derived from interviews and what was found with scientific methods. In a study from Africa's Lake Tanganyika, Bulengela et al. (2019) concluded that fishers' local knowledge of ecological conditions, fish availability and fishing pressures could benefit fisheries management. Braga & Rebêlo (2017) also found that fishers from the lower Juruá River presented an extensive and detailed knowledge of the reproductive behavior of the region's fish species. Sometimes, due to difficulties in finding and accessing academic literature, local knowledge can be the only available data and can indicate directions to decisions makers. Researchers can also gain considerable insight from interviews with experienced fishers (Silvano & Valbo-Jørgensen, 2008).

Taking this into account, to increase knowledge on the trophic ecology of arapaima in the Juruá River, we conducted stomach-content analyses and interviews with experienced fishers involved in the arapaima CBM program. The knowledge of local dwellers is a promising strategy to assess arapaima feeding ecology, due to their generations of empirical observations. In other regions, combining distinct knowledge sources has proven useful in developing a fuller understanding of ecological phenomena (e.g. Jackson *et al*., 2014; Mantyka-Pringle *et al*., 2017; Abu *et al*., 2019). A multi-pronged approach to knowledge generation could therefore improve understanding of arapaima feeding in the floodplain lake management system in western Amazonia and offer an example for such knowledge synthesis elsewhere.

METHODS

Data collection

We collected data in 11 lakes along the middle section of the Juruá River (Figure 1), a tributary of the Amazon River, including two protected areas (Reserva de Desenvolvimento Sustentável Uacari and Reserva Extrativista do Médio Juruá). The middle Juruá River region is influenced by pronounced and predictable hydrology, with the period of high water levels from January to June and the period of low water levels from August to November (Hawes & Peres, 2016).

During the low-water period in September 2018, we collected arapaima stomachs from individuals caught by fishers using gill nets as part of the CBM program. We also collected stomachs in June 2019 to include samples from the season when water levels are falling. These latter individuals were captured by fishers using a traditional harpoon method. All fishes were measured from tip of snout to tail (TL – total length in cm) and sexed. The stomachs were stored on ice in the field. At a field station, food items from each stomach were separated into the following categories: fish, invertebrates, plants and bone remains, and weighed. Later, animal prey were identified to the lowest possible taxonomic level with the aid of a fish taxonomist. We then calculated the proportion of each item in each stomach according to the item's weight.

During the falling-water period, in June 2019, we also conducted interviews with experienced fishers who were over 21 years old and involved in the CBM program. Interviews were conducted through informal conversations using simple and commonly used vocabulary, where we always included the same specific questions present in a semi structured questionnaire (Supporting information Table S1). Broadly, we were interested in knowing the experience and perceptions about arapaima feeding as a function of body size, season and lake management status. Before the interview, we obtained the consent of each participant to be interviewed.

Data collection was authorized by the Sistema de Autorização e Informação em Biodiversidade (SISBIO - 62427-1), Departamento de Mudanças Climáticas e Gestão de Unidades de Conservação (DEMUC – 41/2018) of the Secretaria Estadual de Meio Ambiente do Amazonas (SEMA), and by the Ethics Committee of the Instituto Nacional de Pesquisas da Amazônia (INPA) permits 040/2018 and 3.474.092.

Data analysis

Data obtained from stomach contents and interviews were analyzed separately and then compared, as they are complementary sources of information. We tried to identify and classify the fish common names cited in the interviews according to scientific names from specific literature for the region's fauna (Santos *et al*., 2006; Silvano *et al*., 2001). As some stomachs were empty we performed a generalized linear model (GLM) to determine if arapaima size or sex influenced the presence or absence of prey in stomach contents. The model formula was as follows: stomach (with or without prey) ~ total length + sex, family = "binomial". Given that presence or absence of stomach contents is a categorical variable we used a binomial distribution in the model. Since we expected differences in prey items associated with ontogeny (Oliveira et al., 2004; Queiroz, 2000; Wu and Culver, 1992), we regressed arapaima TL (predictor variable) against prey trophic level and prey maximum length (response variables). Trophic level and maximum length of fish prey were recorded at the species level and obtained from Fishbase (www.fishbase.org). For shrimp, we obtained only maximum length (Moraes-Riodades & Valenti, 2002). All analyses were run using RStudio software (RStudio Team, 2016).

RESULTS

Stomach contents

We collected 113 stomachs during the CBM harvesting activities in September 2018 (low-water period) and five stomachs in the falling-water period (June, 2019). Total length (TL) of sampled arapaima varied from 60 to 245 centimeters (Supporting information Table S2). Thirty one stomachs (26%) were empty and all of these were from the low-water period. The mean TLs of arapaima with and without prey in stomachs were respectively 171 cm and 175 cm and neither TL ($p = 0.69$) nor sex ($p = 0.40$ male and 0.76 female) influenced the presence or absence of prey in the stomachs. Overall, 41 stomachs (35%) had animal prey, but only in 35 could the prey be identified (Supporting information Table S2). The remaining 46 stomachs (39%) contained only plant material (pieces of leaves, branches and seeds) and/or fish-bone remains (Supporting information Table S2). Plant material was found in 79 stomachs (Supporting information Table S2).

Vertebrates were the most common prey (Supporting information Table S3) and were represented only by fish from the orders Characiformes (47%), Siluriformes (30%), Osteoglossiformes (7%), Perciformes (3%) and Gymnotiformes (3%). Invertebrates were represented by the orders Decapoda (3%), Ephemeroptera (3%) and Hemiptera (3%) (Supporting information Table S3). The smaller arapaima had higher proportions of invertebrates in their stomachs than adults, whose diets were composed almost entirely of fish (Figure 2). Invertebrates were found only in arapaima less than 160 cm TL (Supporting information Table S2).

The most common prey type in our samples consisted of fish from the genus *Pimelodus* (six stomachs) (Supporting information Table S2). In one case, we found a young arapaima individual, weighing 650 grams and measuring approximately 60 centimeters TL in the stomach of a large male (208 centimeters TL), captured in Santo Antônio Lake (5º33'9.06"S; 67º33'33.43"W). The size of the arapaima eaten indicates that it was at least 10 months old (Lima et al., 2017). We observed a positive relationship between maximum length of ingested prey and arapaima TL (Prey maximum length = -20.66 + 0.40*arapaima TL, $F_{1,16} = 6.85$, $r^2 = 0.30$, $p = 0.02$; Figure 3) but we excluded the cannibalistic event because the maximum length for arapaima is far larger than the individual that consumed it. Some taxa, such as *Macrobrachium amazonicum*, were found in multiple small individuals and others such as *Osteoglossum bicirrhosum* only occurred in stomachs of large individuals. No relationship was found between prey trophic level and arapaima total length ($F_{1,12} < 0.01$, $r^2 < 0.01$, $p = 0.96$; Figure 4).

Interviews

Sixteen fishers aged 21 to 64 from eight communities and actively involved in arapaima-fishing activities were interviewed (Supporting information Table S4). These interviews produced a list of prey commonly found in arapaima stomachs, represented by 21 types of fish (Figure 5), shrimps and crabs. Interviewed participants also mentioned the presence of mud and plant material, such as fruits and grass. For approximately 40% of those interviewed, "cascuda" (*Psectrogaster rutiloides, P. amazonica*) is the preferred prey of arapaima, followed by "acará" (*Apistogramma* spp., *Heros appendiculatus, Mesonauta insignis* - 12.5%) and "mocinha" (*Potamorhina altamazonica* - 12.5%). Cascuda was also mentioned as being the species most commonly found in arapaima stomach contents (37.5%) (Supporting information Table S4).

According to all interviewees, there were no feeding differences between managed (protected or subsistence-use lakes) and unmanaged lakes (open-access lakes) (Supporting information Table S4). When asked about differences in arapaima feeding between low- and high-water periods, 56% of those interviewed said that arapaima eat the same types of prey throughout the year. Some fishers said that arapaima eat more during the high-water period (12.5%) and that "traíra" (*Hoplias malabaricus*) is the prey most eaten in high-water season (25%). "Cascuda" and "mocinha" were cited more often (25%) as the most consumed prey during low water (Supporting information Table S4). We also asked if young arapaima ate the same type of prey as adult arapaima. Most interviewed (81%) said that young and adult arapaima eat the same prey types. However, some interviewees commented that younger individuals eat more shrimps and crickets, and adults eat fish (Supporting information Table S4). One of the interviewees said that the only difference among age classes is that adult arapaima can eat prey of larger size than young individuals. When asked if adult arapaima eat smaller arapaima (cannibalism), 31% of those interviewed said yes and 25% said that they had seen it firsthand (Supporting information Table S4).

The relative contribution from each source of information (stomach-content analyses and local knowledge) is illustrated in Figure 6, considering the fish popular names cited by local stakeholders and the fish identified to genus or species level in arapaima stomach contents, totaling 29 types of fish ingested by arapaima.

DISCUSSION

The different knowledge sources used in this study, understanding of local fishers and stomach-content analysis, provided complementary information about arapaima feeding. In general, there was agreement that young arapaima have a generalist feeding habit, eating fish and invertebrates, but adult arapaima had eaten fish almost exclusively in our samples, including the possibility of cannibalism. With increases in body length, arapaima are able to feed on prey of larger sizes but also continue to eat small prey, but this increase in prey size is not associated with an increase in prey trophic level. During the low-water season, many arapaima had empty stomachs or stomachs with just some small bone remains and plant material, suggesting that the species may undergo periods of fasting like other large predatory fishes (Arrington *et al*., 2002).

Comparisons between fish species found in stomach contents and those mentioned by interviewees is difficult in this species-rich ecosystem, especially because many popular names may represent more than one species. For example, “bodó” or “cascudo” is a popular name for different species of siluriformes. Despite these challenges, we conclude that most fish species identified in stomach contents were also cited by interviewees, including “aruanã” (*Osteoglossum bicirrhosum*), “bodó” (*Hypostomus* sp., *Loricariichthys* sp.), “cascuda”

(*Psectrogaster amazonica),* “curimatã” (*Prochilodus nigricans*), “mandí” (*Pimelodina flavipinnis, Pimelodus blochii)*, “mapará” (*Hypophthalmus* sp.), “mocinha/branquinha” (*Potamorhina altamazonica*, *Potamorhina pristigaster*), “pacú” (*Metynnis* sp.), “sarapó” (*Adontosternarchus* sp.) and “sardinha” (*Triportheus* sp.). This list of 14 taxa in common was greater than the sum of species observed in only one information source, which suggests a general agreement between the two methods. On the other hand, species such as *Rhaphiodon vulpinus* and *Hydrolycus scomberoides,* both popularly known as “cachorra”, were not mentioned by the interviewees, but were identified in arapaima stomach contents. Other species not found in stomach contents were cited by interviewees, greatly contributing to knowledge of arapaima feeding. These included “acará” (can be many species of cichlids: *Astronotus crassipinnis*, *Chaetobranchus semifasciatus*, *Heros efasciatus*, *Satanoperca jurupari*), “agulhão” (*Potamorrhaphis* sp.), “arari” (*Chalceus erythrurus*), “cangati” (*Auchenipterus nuchalis*), “charuto” (*Hemiodus* sp.), “jeju” (*Hoplerythrinus unitaeniatus*), “piaba”(can be many species belonging to the Characidae), “piau” (can be many species belonging to the Anostomidae: *Leporinus* spp., *Schizodon fasciatus*, *Abramites hypselonotus*), “tambaqui” (*Colossoma macropomum*), “traíra” (*Hoplias malabaricus*), and “tucunaré” (*Cichla* sp.).

Our study contrasts to some degree with that of Queiroz (2000) conducted in Mamirauá Reserve (Brazil), in which “tamoatá” (*Hoplosternum thoracatum*) was the most important fish in the arapaima diet in all seasons, except during the high-water period when “branquinha” (*Potamorhina* sp.) was most consumed. We did not register tamoatá in either stomach contents or interviews, even though it is known to occur in the Jurua River. Instead, Curimatidae and Pimelodidae were the most common fish families in arapaima stomachs in the Juruá. These families are mainly known in the region by common names, such as cascuda, branquinha or mocinha and mandí and are very abundant in shoals in lakes and lentic waters

(Santos *et al*., 2006). These most common species are of low and intermediate trophic levels (e. g. *Potamorhina altamazonica, P. pristigaster, Psectrogaster amazonica, Pimelodus blochi, Pimelodina flavipinnis* – respective trophic levels according to Fishbase: 2, 2.5, 2, 3.1 and 3.2), as are many of the individuals we identified only to family or genera, such as Loricariidae that is composed mainly of detritivorous and herbivorous species.

The size of the arapaima appeared to influence the prey type ingested. Prey size eaten often increases with predator size (Mittelbach & Persson, 1998; Scharf *et al*., 2000), which can implies higher trophic positions but this is not expected in diverse tropical food webs (Layman *et al*., 2005) such as the Juruá. The capacity to ingest larger prey increases as arapaima grow, but large individuals may still ingest small prey as well (Queiroz, 2000). However, invertebrate prey were only found in smaller arapaima and in general, the largest prey species, such as *Hydrolycus scomberoides* (max. length 117cm - Fishbase), *Hypophthalmus* sp. (max. length 57.5 cm - Fishbase), and *Osteoglossum bicirrhosum* (max. length 90 cm - Fishbase) were ingested only by larger arapaima. These species, while large-bodied, show a large variation in trophic level based on information in Fishbase (4.5, 2.9 and 3.4 respectively). Despite the fact that mean prey size increased with arapaima ontogeny, there was no evidence of an increase in prey trophic level.

Lima and Batista (2012) conducted interviews with local arapaima fishers in the Mamirauá Reserve and, similar to our interviews, fishers said that arapaima had a diversified diet with fish and shrimp as the principle prey. They did not mention the presence of other types of invertebrate prey, such as Ephemeroptera and Belostomatidae. This may be because these organisms are generally very small and difficult to identify in stomachs, or because these invertebrates were found only in arapaima smaller than 160 cm and arapaima fishing during the CBM is only allowed for individuals larger than 150 cm. This is also the reason for the small number of samples of younger arapaima in our data set.

We did not make diet comparisons between low and falling water because of the small number of arapaima sampled during the latter period. However, according to the interviewees, there are few changes in prey types ingested during the high and low water seasons but the food supply decreases during the dry season. During low water, many fish species show marked decreases in feeding activity (Junk, 1985) and we also observed a large number of stomachs without prey in the low-water period that was not related to arapaima sex or total length. In contrast, all five fish sampled at falling water had fish in their stomachs.

During the low-water season, empty stomachs or stomachs with only plant material (seeds, branches or leaves) were also common in floodplain lakes in the Purús River (Carvalho *et al*., 2018). Little or no connectivity between floodplains and the main river during the dry season can cause a reduction in prey availability. Also, we observed that some arapaima regurgitated prey when they were captured (Jacobi et al. in review); however, it was not possible to quantify the frequency of this occurrence. It is still not clear why arapaima ingest plant material. Queiroz (2000) inferred that plant materials found in arapaima stomachs were ingested accidentally during suction feeding on animal prey. Also, plant material has high cellulose content that can be slow to digest, so it can accumulate in the stomach.

In our 118 arapaima with stomach contents, we observed only one incidence of cannibalism. Although some interviewees said that they had seen one arapaima eating another, this is the only cannibalistic event scientifically documented for this species, highlighting the importance of local knowledge in affirming scientific observations and suggesting that cannibalism might be more common than previously thought. The overall incidence of these events in protected lakes where arapaima densities have been steadily increasing (Campos-Silva & Peres 2016) remains a subject worth studying.

In summary, an adult arapaima can best be classified as an opportunistic piscivore, which feeds on a wide range of fish species, being able to consume larger prey with the increase in body size. This generalist feeding behavior suggests that management of particular prey species is not necessary in these lakes, but the maintenance of a diversity of prey including large-bodied species would be beneficial in sustaining a range of arapaima life stages. This information is important in light of community-based management of arapaima because there are some initiatives to manage other high-value fish species during the harvesting season, including tambaqui (*Colossoma macrpomum*) and pirapitinga (*Piractus brachypomus*). Drawing on local knowledge, diet during the dry season may not be greatly different from what would be seen at other times of the year. Arapaima also has different predominant prey species in different areas, such as the lower Japura River (Queiroz, 2000) and the middle Jurua River (this study). Therefore, diet studies need to be undertaken in each area of interest. The local fishers involved in the CBM program of the middle Juruá River had consistent ethnobiological knowledge of arapaima feeding ecology. This experience could be used in future fisheries-management projects in the region and also, by expanding the inclusion of fishermen’s local knowledge, these knowledge holders will realize that their information is valued, motivating them to contribute to sustainable management practices.

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FIGURES

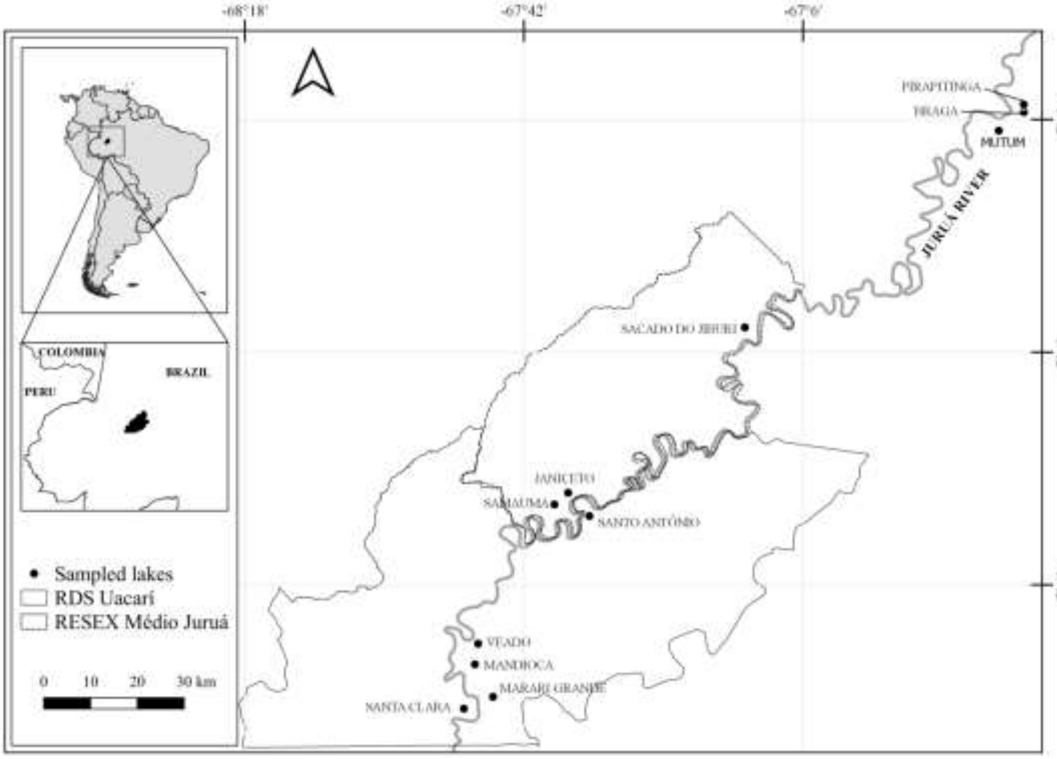

Figure 1. Location of the 11 lakes where stomach contents were collected along the middle Juruá River (Amazonas, BR) including two protected areas (Reserva de Desenvolvimento Sustentável Uacari and Reserva Extrativista do Médio Juruá).

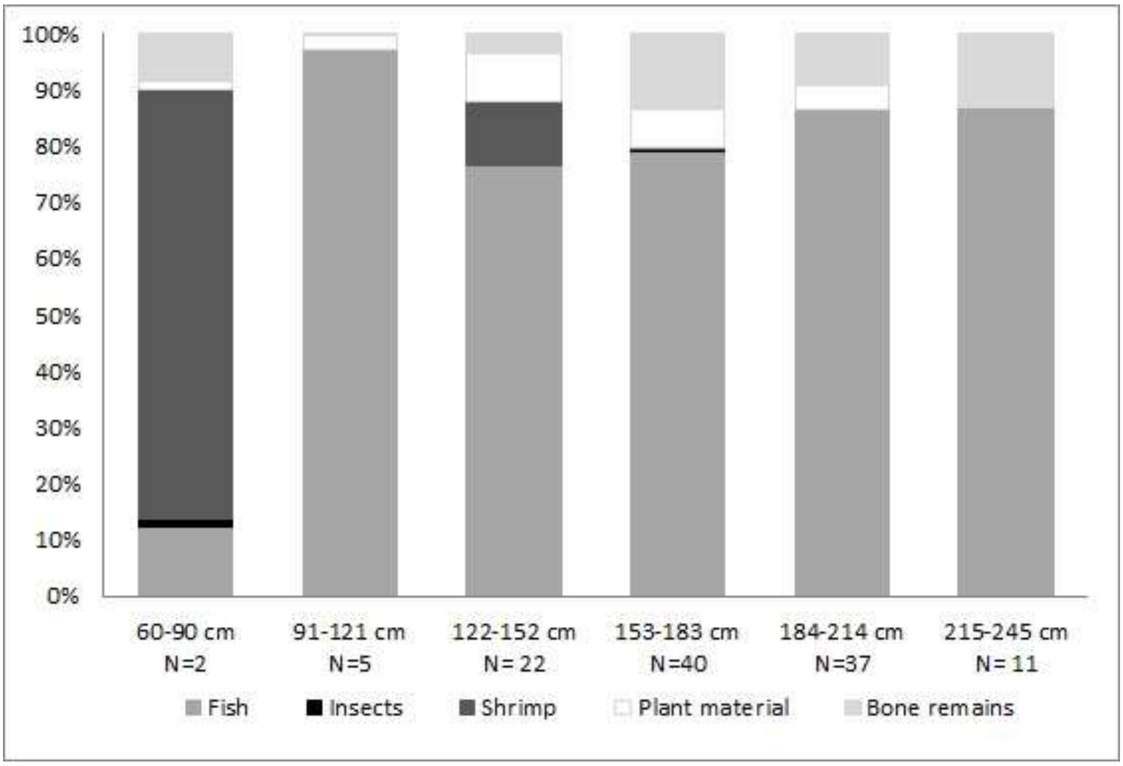

Figure 2. Proportional contribution by mass (g) of prey types present in arapaima stomach contents by arapaima size category (30cm difference in each size category). Prey types include bone remains, fish, insects, plant materials and shrimp.

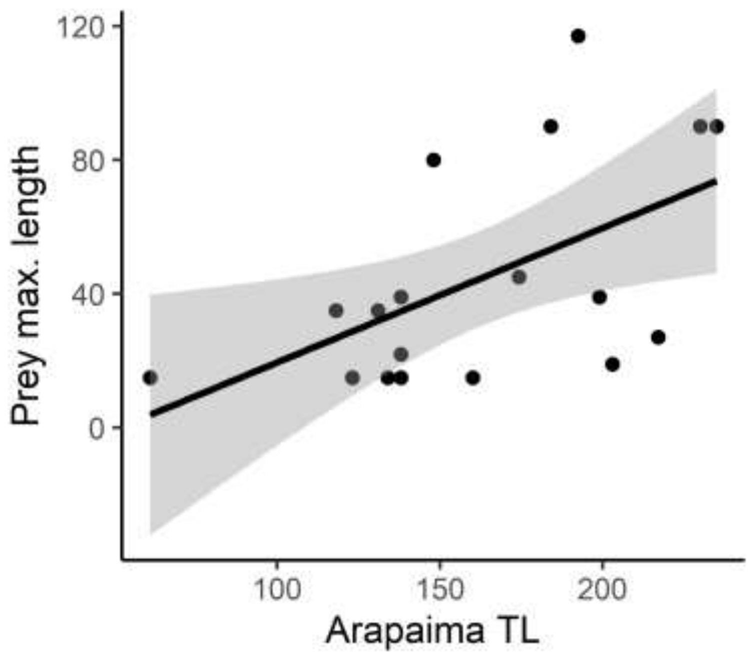

Figure 3. Relationship between arapaima total length (TL) and maximum length of prey ingested and identified to species level.

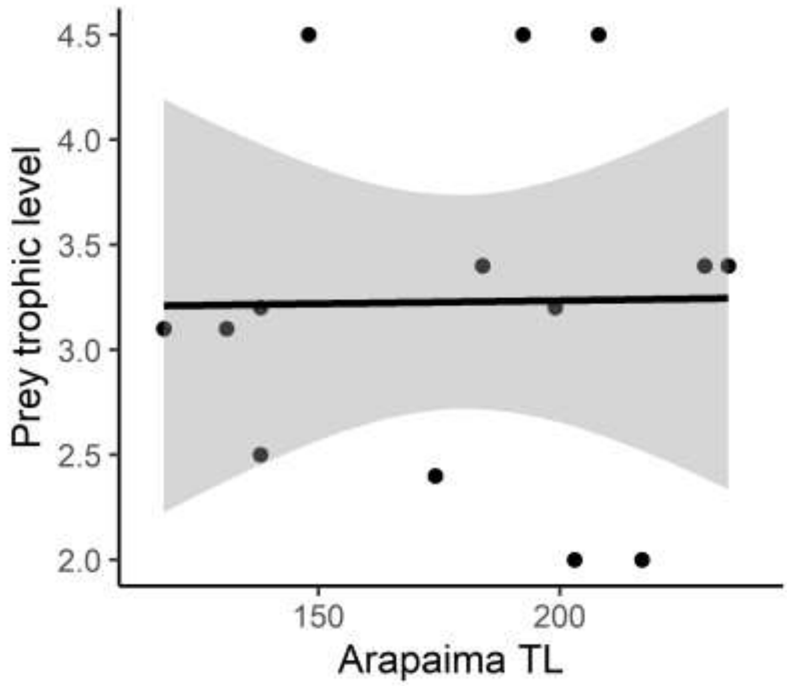

Figure 4. Relationships between arapaima total length (TL) and fishprey trophic level identified to species level.

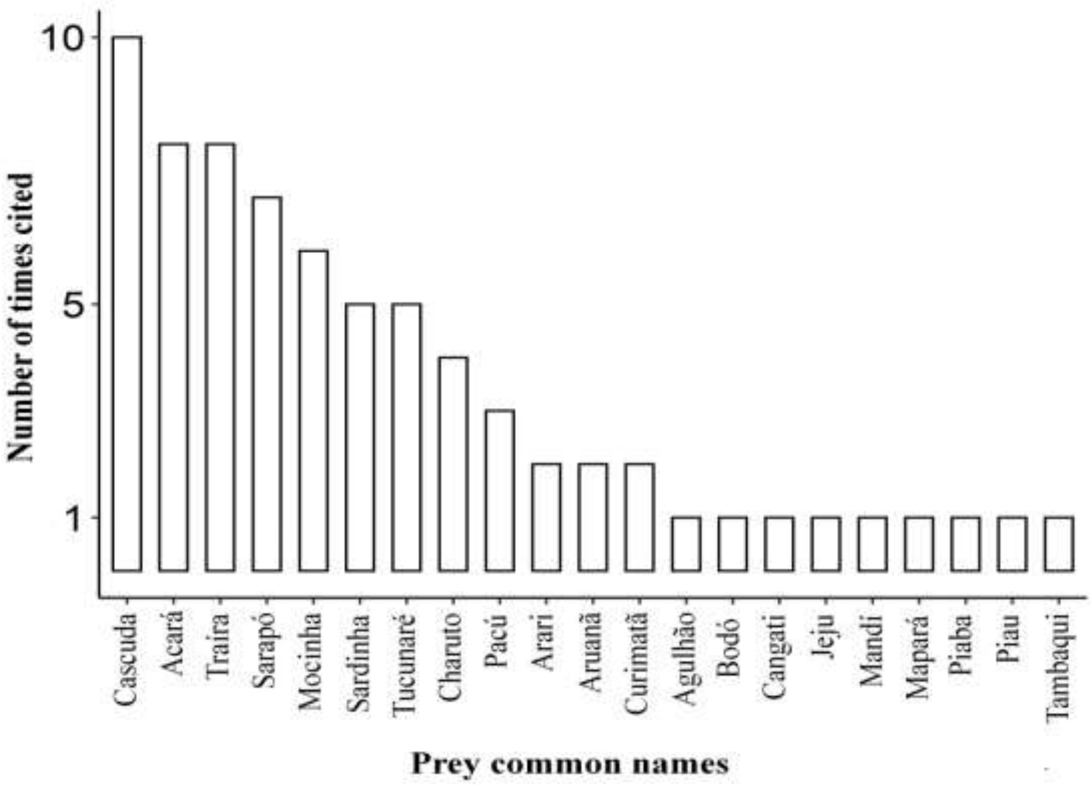

Figure 5. Popular names of fishes and number of times cited by interviewed fishers.

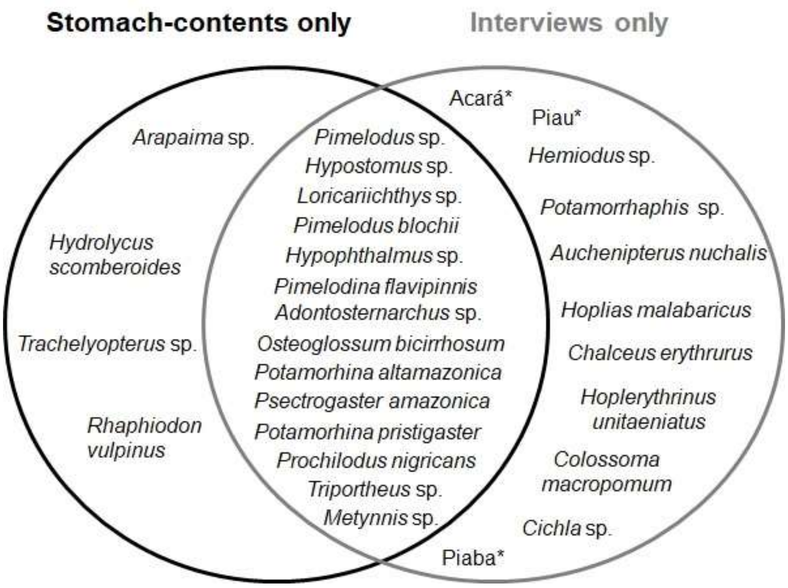

Figure 6. Venn diagram showing the number of genera or species in arapaima stomach-contents but not in interviews (left), the number of species cited in the interviews but not observed in stomach contents (right) and the number of genera or species observed in both (center). * Popular name that can be many species.

SUPPORTING INFORMATION

Table S1. Questionnaire used in the interviews.

	Questions
1	What is your age?
2	In which community do you live?
3	What types of animals have you found in arapaima stomachs?
4	What types of fish have you found in arapaima stomachs?
5	Do arapaima have a preference for some species?
6	What is the most common species to find in arapaima stomachs?
7	Do arapaima from managed lakes eat the same prey as arapaima from unmanaged lakes?
8	Is arapaima feeding different between high and low water levels?
9	Is there a difference in feeding between young and adult arapaima?
10	Do adult arapaima eat smaller arapaima? If yes, how many times have you seen it?

Table S2. Data of each individual: arapaima identification, lake sampled, arapaima total length (TL), sex, stomach content, presence of plant material, presence of fish bones and sampled period.

Arapaima	**Lake**	**TL (cm)**	**Sex**	**Stomach-content**	**Plant material**	**Bones**	**Water level**
FVJ_1655	Mandioca	138.0	F	*Pimelodina flavipinnis*, *Macrobrachium amazonicum*	x	x	Low
FVJ_1656	Mandioca	123.0	M	*Macrobrachium amazonicum*	x	x	Low
FVJ_1657	Mandioca	118.0	F	*Pimelodus Blochi*			Low
FVJ_1658	Mandioca	138.0	M	Empty			Low
FVJ_1725	Santa Clara	61.0		*Macrobrachium amazonicum*			Low
FVJ_1750	Mandioca	184.0	F	*Osteoglossum bicirrhosum*		x	Low
FVJ_1751	Mandioca	198.0	M	-	x	x	Low
FVJ_1752	Mandioca	228.0	F	Empty			Low
FVJ_1753	Mandioca	190.0	M	-	x	x	Low
FVJ_1754	Mandioca	208.0	M	*Trachelyopterus* sp.	x	x	Low
FVJ_1755	Mandioca	223.0	M	Empty			Low
FVJ_1756	Mandioca	199.0	M	*Pimelodina flavipinnis*	x	x	Low
FVJ_1757	Mandioca	134.0		-	x		Low
FVJ_1758	Mandioca	176.0	F	*Metynnis* sp.	x	x	Low
FVJ_1759	Mandioca	168.0	F	-	x	x	Low
FVJ_1760	Mandioca	200.0	F	-	x	x	Low
FVJ_1761	Mandioca	174.2	M	*Prochilodus nigricans*, Cichlidae	x	x	Low
FVJ_1762	Mandioca	181.2	M	-	x	x	Low
FVJ_1763	Mandioca	187.2	F	*Hypostomus* sp., Characinae	x	x	Low
FVJ_1764	Mandioca	205.1	M	-	x	x	Low
FVJ_1823	Mandioca	211.0	F	*Triportheus* sp.	x	x	Low
FVJ_1824	Mandioca	206.3	M	Loricariinae, Characinae			Low

Arapaima	**Lake**	**TL (cm)**	**Sex**	**Stomach-content**	**Plant material**	**Bones**	**Water level**
FVJ_1825	Mandioca	206.0	F	-	x	x	Low
FVJ_1826	Mandioca	192.2	F	-	x	x	Low
FVJ_1827	Mandioca	194.0	M	-	x	x	Low
FVJ_1828	Mandioca	210.2	M	*Hypophtalmus* sp.	x	x	Low
FVJ_1829	Mandioca	228.9	M	-	x	x	Low
FVJ_1831	Mandioca	203.0	M	*Psectrogaster amazonica*, Characiforme	x	x	Low
FVJ_1832	Mandioca	204.9	F	-	x	x	Low
FVJ_1833	Mandioca	217.0	M	*Potamorhina altamazonica*	x		Low
FVJ_1835	Veado	159.0	M	Curimatidae	x	x	Low
FVJ_1836	Veado	183.5	M	-	x		Low
FVJ_1837	Veado	206.0	F	Empty			Low
FVJ_1838	Mandioca	192.4	F	*Hydrolycus scomberoides*	x	x	Low
FVJ_1839	Veado	161.0	M	Unidentified fish	x	x	Low
FVJ_1895	Mandioca	202.0	M	Serrasalmidae	x	x	Low
FVJ_1897	Mandioca	169.9	M	*Pimelodus* sp.	x	x	Low
FVJ_1900	Mandioca	234.9	F	*Osteoglossum bicirrhosum*	x		Low
FVJ_1901	Veado	196.0	M	-		x	Low
FVJ_1902	Veado	177.0	F	-	x		Low
FVJ_1903	Veado	190.5	F	Unidentified fish	x	x	Low
FVJ_1904	Veado	164.0	M	-	x	x	Low
FVJ_1905	Veado	209.0	F	Curimatidae	x	x	Low
FVJ_1906	Veado	202.0	M	Empty			Low
FVJ_1907	Veado	165.0	M	Empty			Low
FVJ_1908	Veado	221.0	F	Empty			Low
FVJ_1909	Veado	242.0	M	Characidae		x	Low

Arapaima	Lake	TL (cm)	Sex	Stomach-content	Plant material	Bones	Water level
FVJ_1910	Veado	192.0	F	-	x	x	Low
FVJ_1911	Veado	235.0	M	-		x	Low
FVJ_1937	Janiceto	124.5		Empty			Low
FVJ_1938	Janiceto	121.5	M	-	x	x	Low
FVJ_1939	Janiceto	138.5	M	Empty			Low
FVJ_1940	Janiceto	114.5	F	Empty			Low
FVJ_1941	Janiceto	143.0		Unidentified fish	x	x	Low
FVJ_1942	Janiceto	148.0	M	*Rhaphiodon vulpinus*, Cichlidae	x	x	Low
FVJ_1943	Santo Antônio	167.0	M	-	x		Low
FVJ_1944	Santo Antônio	166.0	F	Cichlidae	x	x	Low
FVJ_1945	Santo Antônio	171.0	M	-	x	x	Low
FVJ_1946	Santo Antônio	175.0	M	-	x	x	Low
FVJ_1947	Janiceto	160.0	M	Empty			Low
FVJ_1948	Janiceto	165.0	F	Empty			Low
FVJ_1949	Janiceto	160.0	F	-	x	x	Low
FVJ_1950	Samauma	164.0	M	-	x	x	Low
FVJ_1951	Samauma	230.0	M	*Osteoglossum bicirrhosum*		x	Low
FVJ_1952	Samauma	166.0	M	-	x		Low
FVJ_1954	Samauma	150.0		-	x	x	Low
FVJ_1955	Samauma	159.0	F	-	x	x	Low
FVJ_1956	Samauma	179.0	F	-	x		Low
FVJ_1957	Samauma	199.0	M	Empty			Low
FVJ_1958	Samauma	198.0	F	-	x	x	Low
FVJ_1959	Samauma	172.0	M	-	x	x	Low
FVJ_2013	Samauma	189.0	M	Empty			Low

Arapaima	Lake	TL (cm)	Sex	Stomach-content	Plant material	Bones	Water level
FVJ_2014	Samauma	164.0	F	*Loricariichthys* sp.	x	x	Low
FVJ_2015	Samauma	212.0	F	Empty			Low
FVJ_2016	Samauma	178.0		Empty			Low
FVJ_2017	Samauma	184.0	F	Empty			Low
FVJ_2018	Samauma		M	-	x	x	Low
FVJ_2037	Marari Grande	186.0	M	Empty			Low
FVJ_2038	Marari Grande	183.0	M	Empty			Low
FVJ_2039	Marari Grande	190.0	M	Empty			Low
FVJ_2040	Marari Grande	163.0	M	Empty			Low
FVJ_2041	Marari Grande	175.0	F	Empty			Low
FVJ_2042	Marari Grande	170.0	M	-	x		Low
FVJ_2043	Marari Grande	155.0		Empty			Low
FVJ_2044	Marari Grande	183.0	F	Empty			Low
FVJ_2045	Marari Grande	176.0	M	-	x		Low
FVJ_2046	Marari Grande	203.0	M	-	x	x	Low
FVJ_2047	Marari Grande	140.0	M	Empty			Low
FVJ_2048	Marari Grande	190.0	F	Empty			Low
FVJ_2049	Marari Grande	176.0	F	Empty			Low
FVJ_2050	Marari Grande	160.0	M	*Macrobrachium amazonicum*	x	x	Low
FVJ_2051	Marari Grande	189.0	F	-	x	x	Low
FVJ_2052	Marari Grande	173.0	M	-	x		Low
FVJ_2053	Marari Grande	221.0	F	-	x	x	Low
FVJ_2054	Marari Grande	186.0	F	-	x	x	Low
FVJ_2055	Marari Grande	160.0	F	Empty			Low
FVJ_2056	Marari Grande	217.0	F	-	x		Low

Arapaima	Lake	TL (cm)	Sex	Stomach-content	Plant material	Bones	Water level
FVJ_2057	Marari Grande	185.0	F	Empty			Low
FVJ_2069	Santo Antônio	208.0	M	*Arapaima* sp.	x	x	Low
FVJ_2070	Samauma	170.0	F	Empty			Low
FVJ_2081	Lago do Mutum	141.0	F	-	x		Low
FVJ_2082	Lago do Mutum	110.0	M	-	x		Low
FVJ_2083	Lago do Mutum	138.0	F	*Potamorhina pristigaster*	x		Low
FVJ_2084	Lago do Mutum	112.0	M	Unidentified fish	x		Low
FVJ_2085	Lago do Mutum	129.0	F	-	x		Low
FVJ_2086	Lago do Mutum	154.0	M	Belostomatidae	x		Low
FVJ_2087	Lago de Pirapitinga	144.5	M	Unidentified fish	x		Low
FVJ_2088	Lago de Pirapitinga	144.5	M	-	x	x	Low
FVJ_2089	Lago do Braga	147.0	F	-	x	x	Low
FVJ_2090	Lago do Mutum	124.0	F	Empty			Low
FVJ_2091	Lago de Pirapitinga	144.0	M	-	x		Low
FVJ_2092	Lago de Pirapitinga	141.0	M	-	x	x	Low
FVJ_2093	Lago do Braga	147.0	F	Unidentified fish	x		Low
FVJ_2173	Santa Clara	180.0	F	Anostomidae	x	x	Falling
FVJ_2237	Marari Grande	131.0	M	*Adontosternarchus* sp., *Pimelodus* cf *blochii*	x	x	Falling
FVJ_2274	Mandioca	134.0		*Macrobrachium amazonicum*, Doradidae, Pimelodidae, *Adontosternarchus* sp.	x	x	Falling
FVJ_2433	Samauma	98.6		*Adontosternarchus* sp., *Pimelodus* sp., Ephemeroptera	x	x	Falling
FVJ_2498	Sacado do Juburi	170.0	F	*Pimelodus* sp., Doradidae	x	x	Falling

Table S3. Systematic classification (class, order, family, specie and popular name) of prey identified in arapaima stomach contents.

Class	Order	Family	Species	Popular name
Actinopterygii	Characiformes	Anostomidae	-	-
Actinopterygii	Characiformes	-	-	-
Actinopterygii	Characiformes	Characidae	-	-
Actinopterygii	Characiformes	Characidae	*Triportheus* sp.	Sardinha
Actinopterygii	Characiformes	Characidae	Characinae	-
Actinopterygii	Characiformes	Curimatidae	*Potamorhina altamazonica*	Mocinha/Branquinha
Actinopterygii	Characiformes	Curimatidae	*Potamorhina pristigaster*	Mocinha/Branquinha
Actinopterygii	Characiformes	Curimatidae	*Psectrogaster amazonica*	Cascuda
Actinopterygii	Characiformes	Curimatidae	-	-
Actinopterygii	Characiformes	Cynodontidae	*Hydrolycus scomberoides*	Cachorra; Pirandirá
Actinopterygii	Characiformes	Cynodontidae	*Rhaphiodon vulpinus*	Cachorra; Ripa
Actinopterygii	Characiformes	Prochilodontidae	*Prochilodus nigricans*	Curimatã
Actinopterygii	Characiformes	Serrasalmidae	*Metynnis* sp.	Pacú
Actinopterygii	Characiformes	Serrasalmidae	-	-
Actinopterygii	Gymnotiformes	Gymnotidae	*Adontosternarchus* sp.	Sarapó
Actinopterygii	Osteoglossiformes	Osteoglossidae	*Arapaima* sp.	Pirarucu
Actinopterygii	Osteoglossiformes	Osteoglossidae	*Osteoglossum bicirrhosum*	Aruana
Actinopterygii	Perciformes	Cichlidae	-	-
Actinopterygii	Siluriformes	Auchenipteridae	*Trachelyopterus* sp.	-
Actinopterygii	Siluriformes	Doradidae	-	Bodó/Cascudo/Bagre
Actinopterygii	Siluriformes	Loricariidae	*Hypostomus* sp.	Bodó/Cascudo/Bagre
Actinopterygii	Siluriformes	Loricariidae	*Loricariichthys sp.*	Bodó/Cascudo/Bagre
Actinopterygii	Siluriformes	Loricariidae	Loricariinae	Bodó/Cascudo/Bagre
Actinopterygii	Siluriformes	Pimelodidae	*Hypophthalmus* sp.	Mapará
Class	**Order**	**Family**	**Species**	**Popular name**

Actinopterygii	Siluriformes	Pimelodidae	*Pimelodus blochii*	Mandí
Actinopterygii	Siluriformes	Pimelodidae	*Pimelodus* sp.	Mandí
Insecta	Hemiptera	Belostomatidae	-	Barata-d'água
Insecta	Ephemeroptera	-	-	-
Malacostraca	Decapoda	Palaemonidae	*Macrobrachium amazonicum*	Camarão da Amazônia

Table S4. Summary of questionnaire answers. (Q1 = question 1; Q2 = question 2…).

Q1	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**
21	Morro Alto	fish, fruits	aguião, piaba, tucunaré	aguião	aguião
22	Vila Medeiros	fish,shrimp, crab	cascuda, charuto, mocinha, sardinha, traíra	cascuda	cascuda
23	Morro Alto	fish	cascuda, mocinha, traíra	-	all similar
24	Xibauazinho	fish	acará, cascuda, charuto, mocinha, pacú, traíra	cascuda and mocinha	cascuda, mocinha
24	São Francisco	fish, grass	all kinds	smaller fish because it's easier to catch	piranha, sardinha
26	Toari	fish	acará, acará-açú, cascuda	cascuda	cascuda
29	São Sebastião	fish	acara-açú, cascuda, mapará, sarapó, sardinha, tucunaré	-	-
30	Fortuna	fish, shrimp, crab	acará, cangati, mandí, sarapó, traíra	-	acará, traíra
31	Bom Fim	fish	acará, cascuda, traíra	cascuda	cascuda
36	Xibauazinho	fish	acará, cascuda, curimatã, sarapó, sardinha	cascuda	cascuda
44	São Francisco	fish	arari, jiju, sarapó, sardinha, traíra	sarapó	sarapó, traíra
50	Toari	fish, crab	aruanã, cascuda, tambaqui, traíra, tucunaré	Jeju	jeju
52	Bom Fim	fish, shrimp	acará, curimatã, piau, sarapó, tucunaré	acará	acará, jeju, traíra
Q1	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**

53	São Sebastião	fish	acará, aruanã, mocinha, pacú, sarapó, tucunaré	No, eat everything	-
64	Vila Medeiros	fish,shrimp, crab	arari, cascuda, charuto, mocinha, pacú, sarapó, traíra	acará	acará, sarapó, traíra
64	Morro Alto	fish, shrimp, crab	bodó, cascuda, charuto, mocinha, sardinha	cascuda and mocinha	cascuda, mocinha

Q7	**Q8 (Low water level)**	**Q8 (high water level)**	**Q9**	**Q10**
No difference	Is the same	Is the same	No difference	No
No difference	Eat more cascuda	Eat more traíra	No difference	Yes; once
No difference	Is the same	Is the same	No difference	No
No difference	Eat more mocinha, pacú	Eat more traíra	No difference	No
No difference	Is the same but in the high water level also eat fruits		No difference	
No difference	Is the same	Is the same	No difference	No
No difference	-	eat more, same species	No difference	No
No difference	Is the same	Is the same	Young eat more piaba, cricket and adult more fish	Yes; never
Eat more in protected lakes but there is no difference in what they eat	Is the same	Is the same	No difference	No
No difference	Eat more cascuda, sardinha, sargo, mocinha	Eat more cará, piranha, traíra, arari	No difference	No
No difference	-	Eat more, same species	No difference	No
No difference	Is the same	Is the same	No difference	No
No difference	Eat more acará, jeju	Eat more	Young eat more shrimps	Yes; once
No difference	-	Eat more species, because move more	No difference	Yes; once
Q7	Q8 (Low water level)	Q8 (high water level)	Q9	Q10

No difference	Is the same	-	No difference	Yes; once
No difference	Eat more cascuda, mocinha, charuto, shrimp	Eat more traíra	Difference in the prey size	No

SÍNTESE

A posição trófica do pirarucu estimada com base na análise de conteúdos estomacais foi de 3.6 (erro padrão = 0.1). E as estimativas de posição trófica baseadas na análise do conteúdo estomacal estão relacionadas à variação isotópica deste mesmo conteúdo.

O fator de discriminação trófico (FDT) do pirarucu variou bastante entre indivíduos e o valor médio foi menor do que a média usada na literatura. A posição trófica estimada com valores de $\delta^{15}N$ do fígado do pirarucu apresentou uma relação maior com a dieta do que a posição trófica estimada com valores de $\delta^{15}N$ do músculo, entretanto, a variação da posição trófica estimada com a $\delta^{15}N$ foi mais explicada pelo tamanho corporal do que pela dieta. É necessária uma melhor compreensão dos fatores que podem influenciar o $\delta^{15}N$ e análises de conteúdo estomacal continuam sendo necessárias para estimar a posição trófica de animais ectotérmicos com grande variação em tamanho.

O pirarucu adulto pode ser classificado como um piscívoro oportunista, que se alimenta de uma ampla gama de peixes de baixo e médio níveis tróficos. O tamanho do pirarucu parece influenciar o tipo de presa ingerido. Em geral, presas maiores foram ingeridas por pirarucus maiores e invertebrados apenas por pirarucus menores. Entretanto, os pirarucus grandes continuam ingerindo presas pequenas. Durante o período de águas baixas, muitos pirarucus apresentaram os estômagos vazios ou apenas com restos de ossos de peixes e pedaços de plantas. Encontramos um caso de canibalismo, sendo o primeiro já registrado para o pirarucu. Os moradores da região do médio rio Juruá possuem conhecimentos ecológicos consistentes sobre a alimentação do pirarucu, podendo contribuir na implementação de futuros projetos de manejo da espécie na região.

Este estudo permitiu um melhor entendimento da ecologia trófica do pirarucu da Amazônia Ocidental, aliando dados de conteúdos estomacais, conhecimentos empíricos e isótopos estáveis de nitrogênio.

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