



Diet and seasonal distribution of piranha populations in the São Miguel River, Rondônia, Brazil

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ABSTRACT

This study analyzed the spatial and seasonal distribution of piranha species and their respective diets. Samples were collected monthly from August 2020 to July 2021 using various types of fishing gear in different sections of the river. Limnological data were also analyzed to assess correlations with the distribution of piranha species. A total of 326 fish were identified, encompassing nine species and three genera. The most abundant species were *Serrasalmus rhombeus* (N = 155), *Pygocentrus nattereri* (N = 89), and *Serrasalmus spilopleura* (N = 23), which were found in the headwaters, intermediate section and river mouth, respectively. The stomach contents of 56 piranhas were analyzed and revealed that 55.35% of the stomachs were full and 44.65% were empty. The results indicated that fish remains were the most relevant food items at all the collection points. The results demonstrated spatial effects among the fish communities in the headwaters, intermediate section and river mouth, along with variations in water levels, with differences between high- and low-water periods. The redundancy analysis indicated significant effects for temperature, but not for other water parameters. It can be concluded that the piranha populations of the São Miguel River are distributed according to the water levels and the different sections of the river, though they utilize the same type of food resource.

Keywords: Piranhas; Diet; Population distribution; Guaporé River.

Dieta e distribuição sazonal das populações de piranhas no Rio São Miguel, Rondônia, Brasil

RESUMO

Este estudo analisou a distribuição espacial e sazonal das espécies de piranhas e suas respectivas dietas. As amostras foram coletadas mensalmente de agosto de 2020 a julho de 2021, utilizando diversos tipos de apetrecho de pesca em diferentes trechos do rio. Dados limnológicos também foram analisados para avaliar correlações com a distribuição das espécies de piranhas. Foram identificados 326 peixes, distribuídos em nove espécies e três gêneros. As espécies mais abundantes foram *Serrasalmus rhombeus* (N = 155), *Pygocentrus nattereri* (N = 89) e *Serrasalmus spilopleura* (N = 23), as quais foram encontradas nas cabeceiras, seção intermediária e foz, respectivamente. O conteúdo estomacal de 56 piranhas foi analisado e revelou que 55,35% dos estômagos estavam cheios e 44,65% vazios. Os resultados indicaram que restos de peixe foram os itens alimentares mais relevantes em todos os pontos de coleta. Foram observados efeitos espaciais entre as comunidades de peixes nas cabeceiras, seção intermediária e foz, relacionados com as variações nos níveis de água, com diferenças entre os períodos de águas altas e baixas. A análise de redundância indicou efeitos significativos para a temperatura, mas não para outros parâmetros da água. Pôde-se concluir que as populações de piranhas do Rio São Miguel estão distribuídas de acordo com os níveis de água nos diferentes trechos do rio, porém utilizam o mesmo tipo de recurso alimentar.

Palavras-chave: Piranhas; Dieta; Distribuição populacional; Rio Guaporé.

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INTRODUCTION

The population imbalance of piranhas can cause various problems for aquatic fauna, as they can threaten resident species and also compete for resources, negatively impact fishing, and even transmit diseases (Agostinho, 2003; Agostinho & Júlio Jr., 2002; Melo et al., 2017; Sales-Ribeiro et al., 2023).

The Amazon basin is home to the greatest diversity of flora and fauna on the planet, as well as the largest freshwater network in the world (Neill et al., 2006). Among the rivers in this region, the São Miguel River stands out. It is a tributary of the Guaporé River and is located in the state of Rondônia, Brazil (Teixeira, 2008), and is known for its exuberant beauty and great abundance of fish species, attracting commercial, subsistence, and sport fishers alike. This abundance of fish generates income for riverine populations and other communities that depend on fishing in the region (Gérard, 2007).

Among the fish species found in the São Miguel River, piranhas are particularly abundant. Although they have low commercial value, they are an important food source for riverine communities (Ruffino, 2004). Piranhas belong to the order Characiformes and are distributed in five genera: *Serrasalmus*, *Pygocentrus*, *Pristobrycon*, *Catoprion*, and *Pygopristis* (Braga, 1975) and 99 species that make up the Serrasalmidae family (Fricke et al., 2020).

Although piranhas are often regarded as voracious predators due to their sharp and robust dentition (Silva et al., 1999), their diet is varied and includes arthropods, fish (including fins and scales), other vertebrates, and plant matter, such as seeds, leaves, fruits, and decaying material (Goulding, 1980).

Depending on the species, piranhas can reach up to 45 cm in length and are distinguished by their rhomboid and laterally compressed body, with a front jaw that is equipped with sharp, sculpted teeth, which are used to tear prey (Diemer et al., 2017). They live in groups of three to 20 individuals, which facilitates hunting and provides protection against predators (Siqueira et al., 2006). Spawning occurs in batches, with a population increase during the high-water period, when they lay their eggs on submerged tree roots (Ferreira et al., 1996).

Although their populations grow rapidly, competition for space and food with other species, including forage fish, can negatively affect other fish species (Teles & Godinho, 1997), since predators directly impact commercial fish (Oesterwind & Piatkowski, 2023).

Nevertheless, piranhas play an important role in the aquatic ecosystem by consuming dead animals, helping to clean rivers and maintain the natural balance (Silva & Silva, 1995). Even though

they are carnivorous, their ontogenetic changes result in a broad diet that adapts to the availability of resources in the environment (Sazima & Machado, 1990), which is also commonly observed in several predatory species (Gül et al., 2023).

Dietary variations are associated with survival strategies, adjustments to different environments and the frequency of available food during high- and low-water periods (Godoy, 1959; Junk et al., 1989), for which several models have been applied to evaluate fish diets (Hernvann et al., 2022). Therefore, piranhas should not be classified as strictly carnivorous, as their diet is varied (Machado-Allison & Garcia, 1986).

Analyzing the stomach contents of fish is a way of investigating the role of food in the bioecological community (Hyslop, 1980). For an accurate description of food resources, the researcher must be able to recognize the items in the animal's diet (Braga, 1999).

Stomach content analysis techniques can be quantitative, involving the identification of organisms and the analysis of the degree of digestion, or qualitative, which include methods such as numerical frequency (Hynes, 1950; Hyslop, 1980), direct or indirect volumetric estimates (Uieda, 1994), gravimetry (Hyslop, 1980) and repletion indices (Pillay, 1952).

In this sense, the study of fish provides important information for a better understanding of the relationships between members of the ichthyofauna and other organisms in the aquatic community, offering an efficient approach to understanding the trophic structures of the community (Bachiller et al., 2021; Polis & Winemiller, 1996). In view of the above, this study aimed to understand the spatial distribution and diet of piranhas along the São Miguel River, evaluating their population composition, diet, and spatial distribution during periods of high and low water and in different environments of the river.

MATERIALS AND METHODS

Study area

Samplings of the fish were carried out in three sectors (headwaters, intermediate section, and river mouth) along the main channel of the São Miguel River, which is a tributary of the Guaporé River. This river covers an area of 10,293.61 km² (Simões et al., 2019) and passes through the municipalities of São Miguel do Guaporé, Seringueiras, and São Francisco do Guaporé, in the state of Rondônia. The collection sites were selected due to their distinct environmental characteristics: the headwaters are characterized by contemplating an area that has greater forest coverage and the river is narrower (Fig. 1a),

the intermediate section is more open and the riparian forest is further away from the shore (Fig. 1b), and the river mouth (Fig. 1c), where the São Miguel River meets the Guaporé River, presents a wider and more open area, and contains stretches of beaches on the banks.

Sample collection

Experimental fisheries were conducted monthly from August 2020 to July 2021, totaling 12 samplings. Four excursions per river section (headwaters, intermediate, and river mouth) were carried out to monitor water levels (low water: June to November; high water: December to May) across different sections of the São Miguel River.

For the sampling of the piranhas, gill nets were used with meshes measuring 40, 60, 80, 100, 120, and 180 mm between opposite knots (with a height of 2.5 m and length of 20 m), These were submerged for a period of 48 hours, with inspections every six hours for the removal of fish (6 a.m., 12 p.m., 6 p.m., and 0 a.m.). Fishing lines were also used in the fisheries and were cast for 60 minutes shortly after the inspections of the gill nets.

After collecting the samples, while still in the field, the piranhas were identified with the help of specific taxonomic keys (Efrem et al., 1998; Ohara et al., 2017). During the removal of the fish from the nets, the limnological parameters of the water were also measured, these being: hydrogenionic potential (pH), electrical conductivity (μ S•cm⁻¹), dissolved oxygen, and temperature (°C).

The experimental fisheries were authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), under license number 75092-1, and was approved by the Ethics Commission on the Use of Animals at the Universidade Federal de Rondônia, under approval No. 006/2020.

Analysis of stomach contents

The stomachs of the piranhas were removed and stored individually in glass jars with lids in a 10% formaldehyde solution and later in 70% alcohol for conservation purposes, according to Gandini et al. (2012). They were then transported to the laboratory, where the weight of the total stomach contents was obtained, and evaluated individually for the contents of animal matter, plant material, scales, fins and worms/larvae.

Food items were measured using the feeding index (IAi) proposed by Kawakami and Vazzoler (1980), which was modified by replacing the volume percentage by the weight percentage, according to Eq. 1:

$$IAi = \frac{Fi \, x \, Wi}{\sum_{i=1}^{n} (Fi \, x \, Wi)} \, x \, 100 \tag{1}$$

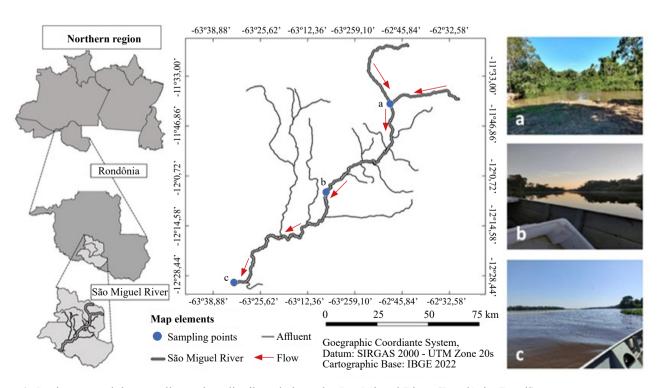


Figure 1. Study area and the sampling points distributed along the São Miguel River (Rondônia, Brazil).

Where: IAi= feeding index; i= 1, 2, ...;n= certain food item; Fi= frequency of occurrence of food (%); Wi= weight of occurrence of the given item (%).

Diversity indicators

The diversity indexes were calculated based on the numerical distribution of the individuals of the different species as a function of the total number of individuals in the analyzed sample. The biodiversity was evaluated using the Margalef (1956) index (Eq. 2):

$$D_{M\sigma} = (S-1)/\ln(N) \tag{2}$$

Where: D_{mg} = Margalef biodiversity index; S= number of species sampled; ln= natural log; N= total number of individuals in all species.

Dominance was estimated according to the Simpson index (Eq. 3):

$$D = 1 - \Sigma(pi^2) \tag{3}$$

Where: D= dominance index; pi= proportion of individuals (number of fish of a given species divided by N) (Simpson, 1949).

The Shannon diversity index was calculated using the Eq. 4:

$$H' = -\Sigma pi x lnpi$$
 (4)

Where: pi= the proportion of individuals; lnpi= the natural logarithm of pi.

The Pielou equability was calculated using Eq. 5:

$$J = H'/H_{max}$$
(5)

Where: J= equitability; H'= Shannon diversity index (Shannon & Weaver, 1949); $H_{max} = \ln(S)$; S= richness (number of species sampled).

Statistical analysis

A two-way permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was used (Anderson et al., 2011) to evaluate whether the environmental parameters (pH, electrical conductivity, dissolved oxygen, and temperature) differed along the São Miguel River (headwaters, intermediate section, and river mouth) during periods of high and low water. This analysis considered the following factors: sampling site (headwaters, intermediate section, and river mouth) and water level (high and low). The response variable was a Bray-Curtis distance matrix, which was estimated from the species abundance data by sampling and was previously standardized using the Hellinger transformation (Borcard et al., 2018; Legendre & Gallagher, 2001).

Furthermore, PERMANOVA was conducted alongside a distance-based redundancy analysis (db-RDA) to further investigate the relationship between fish assemblage variability and environmental variables. While PERMANOVA tests whether the differences in fish assemblages are significant based on the factors (site and water level), db-RDA allows the exploration of which environmental variables (pH, dissolved oxygen, temperature, and electrical conductivity) are responsible for the observed differences. The db-RDA, like the PERMANOVA, was performed using a species abundance matrix that was previously standardized by the Hellinger transformation. This combined approach provides a comprehensive view of the environmental influence on fish assemblages and diversity patterns.

All the analyses were performed in the statistical program R, using the vegan package (Oksanen et al., 2019).

RESULTS

The observations of the physical-chemical values of the water of the São Miguel River showed a lower temperature value (T) for the headwater region in the low-water period (21.6 ± 3.9) , and the pH (6.4 ± 1.2) was more acidic. The lowest dissolved oxygen level (2.1 ± 1.5) occurred in the high-water period for the intermediate section (Table 1).

The experimental fishing reached a total of 326 specimens of piranhas along the São Miguel River, which included nine species distributed in three genera. The most abundant species was *Serrasalmus rhombeus* (Linnaeus, 1766), with 155 individuals, followed by *Pygocentrus nattereri* (Kner, 1858), with 89, and *Serrasalmus spilopleura* (Kner, 1858), with 23 specimens; however, this last species did not show any values for the river mouth (Table 2).

Diversity indexes

In the intermediate section (N = 116) and at the river mouth (N = 72) of the São Miguel River, in the low-water period, the highest abundance of piranhas was evidenced; however, in the headwaters, the high-water period was when the highest abundance was observed (N = 6). The highest species richness occurred in the intermediate section (S = 9), with a lower value in the headwaters and river mouth in the low-water period (S = 3), Table 3.

The intermediate section of the São Miguel River presented the highest diversity indexes in both periods (Dmg = 2.42 and 1.47), while the lowest value for the headwaters (Dmg = 0.91) and

Parameter	Headwaters		Intermedi	ate section	River mouth		
	High water	Low water	High water	Low water	High water	Low water	
pН	6.7 ± 0.3	7.1 ± 0.5	6.4 ± 1.2	6.6 ± 0.3	6.4 ± 1.2	6.8 ± 0.3	
EC µS.cm ⁻¹	80.1 ± 52.0	74.8 ± 60.5	88.2 ± 30.7	64.9 ± 54.3	60.4 ± 28.6	102.0 ± 36.4	
DO mg.L ⁻¹	5.4 ± 3.4	6.5 ± 1.7	2.1 ± 1.5	4.6 ± 3.4	3.79 ± 3.1	4.53 ± 1.3	
T°C	28.1 ± 1.8	21.6 ± 3.9	27.5 ± 1.19	26.6 ± 3.2	28.0 ± 1.8	25.0 ± 1.7	

Table 1. Mean values of seasonal limnological parameters found in different stretches of the São Miguel River, Rondônia, Brazil.

EC= electrical conductivity; DO= dissolved oxygen.

Table 2. Distribution and taxonomy of piranhas captured in different stretches of the São Miguel River, Rondônia, Brazil, in the high- and low-water periods.

Tarana	Cada	Headwaters		Intermediate section		River mouth		N
Taxonomy	Code -	High water	Low water	High water	Low water	High water	Low water	Ν
Characiformes								
Serrasalmidae Catoprion mento (Cuvier, 1819)		1	0	1	17	2	0	21
Pygocentrus nattereri (Kner, 1858)	Pna	1	1	1	47	17	22	89
Serrasalmus compressus (Jégu et al., 1991)	Sco	6	0	4	2	6	0	18
Serrasalmus elongatus (Kner, 1858)	Selon	0	0	1	4	0	1	6
Serrasalmus hollandi (Eigenmann, 1915)	Sho	3	2	1	5	0	0	11
Serrasalmus manueli (Fernández-Yépez, 1967)	Sman	0	0	2	0	0	0	2
Serrasalmus marginatus (Valenciennes, 1837)	Sma	0	0	0	1	0	0	1
Serrasalmus rhombeus (Linnaeus, 1766)	Srh	25	6	7	36	32	49	155
Serrasalmus spilopleura (Kner, 1858)	Ssp	4	0	1	4	14	0	23
Total		40	9	18	116	71	72	326

Code= abbreviation of the name of the species.

Table 3. Diversity indexes for piranha populations, estimated according to water levels and the section of the São Miguel River.

Index	Headwaters		Intern sect	nediate tion	River mouth		
	High water	Low water	High water	Low water	High water	Low water	
N	40	9	18	116	71	72	
S	6	3	8	8	5	3	
Dmg	1.36	0.91	2.42	1.47	0.94	0.47	
D	0.43	0.51	0.23	0.29	0.31	0.56	
Н'	1.19	0.85	1.75	1.49	1.33	0.68	
J	0.66	0.77	0.84	0.72	0.83	0.62	

N= abundance; S= richness; Dmg= Margalef index; D= Simpson dominance; H'= Shannon index; J= Pielou equitability. river mouth (Dmg = 0.47) occurred in the low-water period. The highest values of dominance (D = 0.56 and 0.51) occurred in the river mouth and headwaters in the low-water period, and the lowest values were for the intermediate section (0.29 and 0.23). The Shannon diversity index was higher in the intermediate section for the high-water period (1.75) and lower in the river mouth for the low-water period (0.68). The Pielou equitability was higher in the intermediate section in the high-water period (J = 0.84) and lower in the river mouth in the low-water period (J = 0.62). These values are shown in Table 3.

Stomach contents

The stomach contents of 56 piranha specimens were analyzed; 55.35% of the stomachs were full and 44.65% were empty. In the headwaters, there was the highest incidence of empty stomachs

(54.55%), while the intermediate section had the lowest amount (40%) of stomachs without contents.

The food items with the highest frequency of occurrence for the headwaters were exclusively animal matter (100%), while in the intermediate section there was a greater variety of items, *e.g.*, animal matter (77.78%), vegetable matter and scales (33.33%), and finally worms/larvae, which were represented only in this stretch, with a frequency of occurrence of 11.11%.

At the mouth of the São Miguel River, there was also a greater variety of stomach contents, *e.g.*, animal matter (95.24%), vegetable matter and scales (9.52%), and fins (4.76%). Despite this diversity of foods, the weight of occurrence of animal matter corresponded to 98.04% of the weight of the total content. The feeding index (IAi) was higher for animal matter at all the sampling points, especially at the mouth of the river (Iai = 0.7843) (Table 4).

Distance-based redundancy analysis

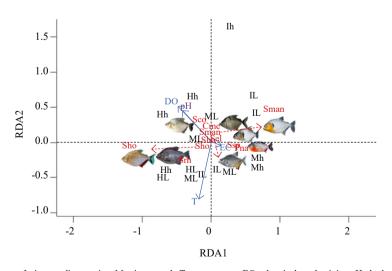
In the evaluation of the interaction of piranha species with the sections of the river, water levels and environmental parameters, the PERMANOVA indicated the existence of spatial effects, with differences between the fish communities of the headwaters, intermediate section, and the river mouth (F = 2.590, degrees of freedom – df = 2, 19, p = 0.044), the water levels, with differences between the high- and low-water period (F = 3.140, df = 1, 19, p = 0.045), and no interaction effect between the spatial and water level factors (F = 1.465, df = 2, 19, p = 0.20).

The redundancy analysis indicated significant effects for temperature (F = 3.236, p = 0.010), but no effects involving the other water parameters (Fig. 2). It also explained the distribution of the data in the coordinate matrix, whereby on the X-axis (RDA1) they were grouped exclusively on the left side, representing the headwaters of the São Miguel River with

Table 4. Feeding index of the stomach contents of p	piranhas captured along	g the São Miguel Riv	ver, Rondônia, Brazil.
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Food item	Headwaters		Inte	Intermediate section			River mouth		
	Fi%	Pi%	Iai	Fi%	Pi%	Iai	Fi?	% Pi%	Iai
Animal material	100	100	1	77.78	66.21	0.3310	95.2	98.04	0.7843
Plant material				33.33	2.04	0.0044	9.5	2 1.40	0.0011
Scales				33.33	31.46	0.0674	9.5	2 0.55	0.0004
Fins							4.7	6 0.01	0.0000
Worms/larvae				11.11	0.29	0.0002			
Total	100	100		*	100		*	100	

IAi= feeding index; Fi%= frequency (%) of the food item; Pi%= weight (%) of the food item; *items that were found in most of the animals analyzed.



L= low water; h= high water; H= headwaters; I= intermediate section; M= river mouth; T= temperature; EC= electrical conductivity; pH= hydrogenionic potential; DO= dissolved oxygen. **Figure 2.** Seasonal and spatial distribution of piranha species in the São Miguel River, Rondônia, Brazil. The species codes for the piranhas are given in Table 2.

the population of *S. compressus* piranhas allocated in the upper corner of the Y-axis (RDA2), with the influence of dissolved oxygen and pH in the high-water period. In the headwater region, the species *S. rhombeus* was allocated in the lower corner (Y-axis) for the low-water period, where temperature influenced with this distribution.

On the other hand, the populations of *C. mento* and *S. manueli* were allocated on the right side of the X axis and upper part of the Y axis, representing the region of the intermediate section in the low-water period. Also on the right side of the X axis, but in the lower part of the Y axis, the species *S. spilopleura* and *P. nattereri* were grouped for the headwater region. These species clustered in the high-water period, in the intermediate section and river mouth, where electrical conductivity was the environmental parameter with the highest intensity in the distribution of these scenarios (Fig. 2).

DISCUSSION

The limnological parameters of the waters of the São Miguel River, in Rondônia, did not show significant statistical differences regarding the interaction effect, nor between spatial and seasonal factors. However, differences were identified between sampling points and water levels, indicating distinct environmental conditions to which piranhas are adapted due to the seasonal variations, Amazon region's characteristic (Junk et al., 1989; Melack & Fisher, 1990). The pH showed little variation, which is a positive aspect, as it affects the physiology of several fish species (Esteves, 1998).

The lower level of dissolved oxygen (2.1 ± 1.5) observed in the intermediate section during the high-water period is concerning, as levels between 2-4 ppm allow only a few species of fish and insects to survive (Embrapa, 2011). This may result from an atypical condition at the sampling site, related to the decomposition of organic matter from the flooded forest by microorganisms (ANA, 2017), which may also have affected the pH levels and contributed to the reduced number of piranha specimens captured in this region.

The indices of abundance and richness were lower in the headwater region, especially during the low-water period, which may indicate a lack of prey, as many individuals had empty stomachs in the stomach content analysis. In contrast, the intermediate section and the river mouth showed higher diversity indices, correlated with the species *S. spilopleura* and *P. nattereri*, which also presented the largest number of individuals with full stomachs. This suggests that diversity is directly related to food

availability and more favorable environmental conditions in these regions.

According to Macedo et al. (2015), *P. nattereri* and *S. rhombeus* were also the most abundant species in floodplain lakes located in the lower Solimões River, which demonstrates these species' great adaptability to environmental variations. However, differences in specific abundance may indicate competition in different stretches of the river, as observed by Behr et al. (2008) in the Ibicuí River, Rio Grande do Sul.

The distribution of piranhas in the São Miguel River was heterogeneous along different stretches of the river, with variations in the number of individuals. The river mouth and the intermediate section presented the greatest number of piranhas, while the headwater region showed a lower number, especially during the low-water period. This variation may be related to environmental factors, such as food availability and water quality, which influence the distribution and abundance of forage species (Boyd et al., 2017).

The species *P. nattereri* and *S. rhombeus* were the most frequent in all the fisheries conducted, indicating their wide geographical distribution in the São Miguel River. The presence of empty stomachs in some areas of the river suggests the possibility of seasonal variations in food availability and lower predator activity, which may impact the ecology and behavior of these species (Carpentieri et al., 2006). Additionally, the reduced concentration of dissolved oxygen in the intermediate section, where the lowest number of captured piranhas was observed, may have contributed to this feeding pattern (Embrapa, 2011).

These results indicated that the piranhas of the São Miguel River follow a piscivorous feeding pattern, which is consistent with the findings for these species in other basins (Leão et al., 1991; Piorski et al., 2005). They also consume other items, such as plants and insects, suggesting an omnivorous diet with a tendency towards piscivory (Almeida et al., 1998; Leão et al., 1991).

Therefore, diet may vary depending on prey availability and the ratio of predator to prey size (Piorski et al., 2005), and their occupation of different environments along the river channel may indicate spatial (Vannote et al., 1980) and seasonal (Junk et al., 1989) adaptation.

CONCLUSION

The piranha species demonstrated similar feeding preferences in the intermediate section and river mouth, but different in the headwaters, where the contents analyzed were 100% of animal origin. This difference in their diet can be explained by the fact that they occupy different stretches of the São Miguel River at different times of the year, indicating a possible division of ecological niches. The information obtained in this study can provide environmental agency managers with knowledge about part of the feeding biology of piranhas for the development of appropriate management strategies for the study region and assist in the maintenance of local fishery resources and, consequently, in the sustainability of regional fisheries.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data sets were generated or analyzed in the current study.

AUTHORS' CONTRIBUTIONS

Conceptualization:Bezerra-Neto,E.B.,Alves,J.A.;Writingoriginal draft: Bezerra-Neto, E.B.; Data curation: Bezerra-Neto, E.B.; Writing – review & edition: Bezerra-Neto, E.B.; Supervision: Sousa, R.G.C.; Validation: Sousa, R.G.C.; Writing – review & edition: Sousa, R.G.C.; Final approval: Sousa, R.G.C.; Resources: Alves, J.A.; Investigation: Alves, J.A.; Software: Faria Junior, C.H.; Data curation: Faria Junior, C.H.; Final approval: Sousa, R.G.C.

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