

Bromatological characteristics of fillet fish of *Leporinus agassizi* and *Leporinus fasciatus* (Pisces: Characidae) in its natural habitat

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ABSTRACT

Bromatological parameters in 39 Warakú verdadero (*Leporinus agassizi*) and 21 Warakú pinima (*Leporinus fasciatus*) fillets were analyzed at three different sites of the Vaupés River, Colombia sampled during a hydrobiological cycle. The bromatological parameters did not shown significant differences in relation to the sites and season of capture. At point 3 and during the descending water season *Leporinus agassizi* had the highest values for dry matter (25.24%), protein (19.82%) and ash (2.16%). The highest lipid value for this species was found in low waters at point 1 (2.93%). *Leporinus fasciatus* showed the highest levels of dry matter (25.19%) and ash (2.16%) at point 3 during ascendant and high waters, respectively. The bromatological parameters tends to vary regarding the time of year. It was possible to determined that these fish species have great potential for continental aquaculture thus constituting a healthy food model.

Keywords: hydrological cycle, nutritional composition, protein, Warakú.

Características bromatológicas de filetes de *Leporinus agassizi* y *Leporinus fasciatus* (Pisces: Characidae) en su hábitat natural

RESUMEN

Se analizaron los parámetros bromatológicos de 39 filetes de Warakú verdadero (*Leporinus agassizi*) y 21 filetes de Warakú pinima (*Leporinus fasciatus*) muestreados durante un ciclo hidrológico en tres puntos diferentes del Río Vaupés, Colombia. Los parámetros evaluados no presentaron diferencias significativas en relación al sitio y época de captura. En el punto 3 y durante la temporada de aguas descendentes *Leporinus agassizi* tuvo los valores más altos para materia seca (25,24%), proteína (19,82%) y cenizas (2,16%). Los mayores niveles de lípidos para esta especie fueron encontrados en aguas bajas del punto 1 (2,93%). *Leporinus fasciatus* presentó los niveles más altos de materia seca (25,19%) y cenizas (2,16%) en el punto 3 durante la temporada de aguas ascendentes

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y altas, respectivamente. Las variables bromatológicas tendieron a variar de acuerdo con la época del año. Fue posible determinar que estas especies de peces poseen un gran potencial para la acuicultura de aguas continentales constituyendo así un modelo de alimentación saludable.

Palabras clave: ciclo hidrológico, composición nutricional, proteína, Warakú.

INTRODUCTION

Proximate analysis is one of the important techniques used to determine the chemical-structural characteristics and the nutritional composition of a food. The method, based on well-established physicochemical techniques, consists in the analytical determination of water (moisture), proteins, fats, carbohydrates, minerals and some elements such as vitamins, additives, preservatives, dyes and antioxidants (Greenfield and Southgate 2006; Del Angel 2013). Although these studies have become indispensable to know the nutritional structure of some food sources, it is becoming increasingly necessary to determine other specific components such as amino acids and the type of fatty acids, in order to obtain a total knowledge of the nutritional value of the food.

According to the FAO (2014), determining the proximal composition of a certain species of fish is a very important factor when it is wanted to make a characterization of it. The physical, chemical and bacteriological characteristics of fish can vary both between and within the same species due to feeding habits, seasonal variability, spawning cycles, methods of catching, size, age and geographical and regional differences (Ali *et al.* 2013; Gököglü and Yerlikaya 2015).

Fish of the genus *Leporinus* are considered a potential species for continental fish farming in South America (Saint 1986)

and one of the main sources of food in the Amazon region. Within this genus are frequently found the species *Leporinus friderici*, *Leporinus agassizi* and *Leporinus fasciatus*, which have become the most important in this area. Cubillos *et al.* (2019) identified that three species of *Leporinus* fish (including *L. fasciatus*), are consumed during gestation and menstruation by women of the Sikuni indigenous tribe, located in eastern Colombia. This is due to specific dietary behaviors of the indigenous populations of this region of the country and related to cultural, health and/or magical-religious celebrations (Madden and Chamberlain 2010).

In other regions, for example in the Atrato River basin, they occupy the second place in fishing (Rivas *et al.* 2003), which shows the importance of these freshwater fish. One of the main difficulties that are presented for that study is that there is still not enough information on the production and nutritional quality of these species to establish them in the production systems, and their nutritional contribution for human consumption is still unknown. However, pioneering studies such as that of Santos (1982) on biological and ecological aspects of *Leporinus* species and Velasco-Santamaría *et al.* (2017) on the feeding habits of *Leporinus friderici* during a hydrobiological cycle in the Vaupés River favor the increase of the biological and ecological knowledge of these species.

Regarding the hydrological aspect, the Vaupés region has two large rivers, Vaupés and Apaporis, as well as small tributaries that irrigate these bodies of water from North to Southeast (N-SW) towards the border between Colombia and Brazil, interrupted by rock formations, jumps, rapids and screes. Fishing is in turn an everyday activity and its product is the regular complement of the indigenous diet; this is affected by the migrations of some schools of fish that are governed by the hydrographic cycle during rising and falling water (Correa 2010). In this way, in the present study the bromatological and

nutritional characteristics of the Warakú verdadero (*Leporinus agassizi*) and Warakú pinima (*Leporinus fasciatus*) are shown, and how they are influenced under the effects of hydrological inflows.

MATERIALS AND METHODS

Study area

The sampling of the fillets was done on fish caught in the Vaupés River (extreme east of Colombia) in key points determined by the indigenous communities according to the biodiversity of the species. The point

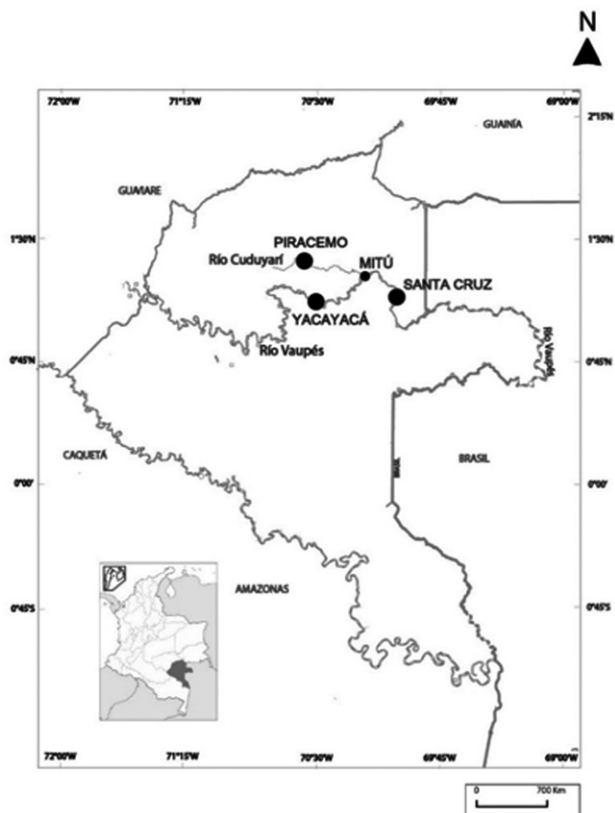


FIGURE 1 Location of sampling sites, Point 1 (Yacayacá) (01° 05'00.5"N–070° 28'19,4"W), Point 2 (Piracemo) (01° 19'55,8"N–70° 23' 00.8"W) and Point 3 (Santa Cruz) (01° 09'27"N–70° 00'24.3"W) in the department of Vaupés, Colombia. **Source:** Taken from Velasco-Santamaría *et al.* (2017).

1 [Yacayacá; South-West (N 01° 05' 00.5 "W 070° 28' 19.4") at 12.49 km from the municipal seat of Mitú]. The point 2 [Piracemo; North-West (N 01° 19' 55.8 "W 70° 23' 00.8") 6.1 km from the city of Mitú] and point 3 [Santa Cruz; to the South-East at 28.31 km from the city of Mitú (N 01° 09' 27 "W 70° 00' 24.3")] (Figure 1). Subsequently, the samples were transported to the Universidad de los Llanos located in the department of Meta, Colombia.

Biological material

The fish was caught by native anglers of the indigenous communities surrounding each sampling point, considering their customs and fishing techniques, as well as a fishing work schedule established between 5:00 a.m. and 10:00 a.m. The fish sampling were done during a complete hydrobiological cycle (March 2014 to May 2015), corresponding to months between March and April (ascendant waters), May to July (high waters), August to October (descending waters) and November to February (low waters).

Fish were anaesthetized by immersion in 2-phenoxyethanol (400 ppm, Sigma Chemical Co., St. Louis, Missouri, USA). All procedures were performed in accordance with the standards established by the Resolution No. 008430 of 1993 of the Ministry of Health, Colombia and also approved by the Bioethical Committee of the Universidad de los Llanos.

Sixty fillets of fish of the genus *Leporinus* were analyzed, which 39 were of the species *Leporinus agassizi* (Warakú verdadero) and 21 to *Leporinus fasciatus* (Warakú pinima). The fillets were vacuum packed in Ziploc® ice bags, as established in Colombian Technical Norm (NTC) 5443 of 2006, and then stored at -20°C until processing.

Proximate analysis

The proximate composition was analyzed in each sample under the parameters of the Association of Official Analytical Chemists (AOAC 2007), according to the protocols for moisture (%) (Protocol N°. 952.08 with modifications), crude protein (%) (N°. 955.04 with modifications), crude lipids (%) (N°. 948.15 with modifications) and ashes (%) (N°. 938.08 with modifications). These modifications were made based on the methodologies proposed by the Nutrition Laboratory of the Universidad de los Llanos. The samples were processed in the Laboratory of Animal Nutrition of the Faculty of Agricultural Sciences and Natural Resources (FCARN) and the Nutrition Laboratory of the Institute of Aquaculture of the Universidad de los Llanos, using the methodologies previously mentioned, as follows:

Dry matter and moisture

After drying dried the sample (in an oven at 60°C for 72 hours), 2 to 3 grams were placed in a porcelain dish in an oven at 60°C for 48 hours. The weight of the sample, the weight of the capsule and the weight of the capsule plus dry matter were recorded.

Ashes

In a pre-weighed porcelain crucible, 1 gram of sample was added, later samples were cremated in the Muffle at 600°C for 3 hours and after 16 hours, the crucible was weighed.

Ethereal extract or fats by Soxhlet method

1 gram of the sample wrapped in absorbent paper was weighed. This was then placed in the Soxhlet's collection bottle. Once the balloon was weighed, 210 to

270 ml of petroleum ether were added to wash the fat for 1 hour, then each balloon was distilled to obtain the solvent again. Finally, the balloon was subjected to 60°C for 24 hours.

Crude protein by Kjeldahl method

0.1 gram of the sample were weighed and wrapped in absorbent paper, placed in Kjeldahl balloons and 100 ml tubes, adding 0.9 grams of catalyst (MgO: magnesium oxide). Then 5 ml of 98% sulfuric acid were added. The digestion was done in a thermo-digester equipment at 360°C for 1 hour, obtaining a green crystalline coloration as a product. The samples were allowed to cool and 5 ml of distilled water were added. In a 50 ml Erlenmeyer flask, 3 ml of boric acid 4% and 2 drops of mixed indicator were added and then placed in a Kjeldahl distiller. After the sample was added, 10 ml of 40% sodium hydroxide (NaOH) was added to begin the distillation. When the Erlenmeyer reached a volume of 30 ml, it was removed from the equipment. Said solution was titrated with 0.1 N hydrochloric acid until obtaining red-orange color.

Statistical analysis

A descriptive analysis was made showing the data as mean \pm standard deviation (SD). Before making any statistical analysis of the results, homogeneity of variance assumptions were evaluated by the Bartlett test and the normality of the data with the Kolmogorov-Smirnov test, which allowed guiding the type of statistical analysis to be performed. Subsequently, a two-way analysis of variance (ANOVA) was carried out, where factor 1 corresponded to the collection site and factor 2 to the time of year (low, ascendant, high and descending waters). Finally, a Pearson correlation was

made. In all cases, $p < 0.05$ was used as a statistical criterion to reveal significant differences. The data was analyzed with SAS® software (version 9.2, 2009) and GraphPad® (version 5.03, 2009).

RESULTS AND DISCUSSION

Table 1 shows the bromatological analysis of specimens of *Leporinus agassizi* (N = 39) and *Leporinus fasciatus* (N = 21) without considering the fishing sites or the time of capture.

Leporinus agassizi

Table 2 corresponds to the analysis made according to the different fishing sites and the hydrobiological cycles in which the species could be collected with greater success. In fish caught at Point 1 (Yacayacá), *L. agassizi* had the highest amount of dry matter and fat at low waters (23.52% and 2.93%, respectively); protein at descending waters (17.30%) and ashes at high waters (2.08%) In fish caught at Point 2 (Piracemo), only values were obtained in low waters: dry matter (19.76%), protein (15.07%), ash (1.44%) and fat (0.46%). In fish caught at Point 3 (Santa Cruz), the highest values were obtained for descending waters, as follows: dry matter (25.24%), protein (19.82%), ashes (2.16%), and fat (1.58%). The results showed no statistically significant differences when relating the capture site with the provenance ($p > 0.05$).

Leporinus fasciatus

The analysis made according to the different fishing sites and hydrobiological cycles in which the species could be collected with greater success is shown in Table 3. In fish caught at Point 1, the higher value of dry matter was obtained at high

TABLE 1. Bromatological analysis of *Leporinus agassizi* and *Leporinus fasciatus* caught in the Vaupés River, Colombia. Data expressed as mean ± SD. SD: standard deviation

Variable	<i>Leporinus agassizi</i>	<i>Leporinus fasciatus</i>
Dry matter	22.34% ± 2.22	22.7% ± 3.19
Moisture	77.65% ± 2.23	77.3% ± 0.80
Protein	16.59% ± 2.35	17.6% ± 3.1
Fat	1.39% ± 0.74	1% ± 0.53
Ash	1.60% ± 0.52	1.7% ± 0.56

TABLE 2. Bromatological analysis of *Leporinus agassizi* caught in the three sampling points of the Vaupés River, Colombia, over four hydrobiological cycles. Data expressed as mean ± SD (standard deviation).

Sampling site	Season	Dry matter (%)	Moisture (%)	Protein (%)	Fat (%)	Ashes (%)
Point 1 Yacayacá	Ascendant	21.69 ± 1.46	78.31 ± 1.46	16.56 ± 1.30	1.26 ± 0.82	1.29 ± 0.31
	High	20.93 ± 2.50	79.07 ± 2.50	16.37 ± 1.53	0.71 ± 0.40	2.08 ± 1.07
	Descending	23.27 ± 1.76	76.73 ± 1.76	17.30 ± 2.83	1.85 ± 1.33	1.72 ± 0.33
	Low	23.52 ± 1.38	76.48 ± 1.38	15.80 ± 2.41	2.93 ± 0.75	1.16 ± 0.41
Point 2 Piracemo	Ascendant	NA	NA	NA	NA	NA
	High	NA	NA	NA	NA	NA
	Descending	NA	NA	NA	NA	NA
	Low	19.76 ± 1.64	80.24 ± 1.64	15.07 ± 1.15	0.46 ± 0.25	1.44 ± 0.40
Point 3 Santa Cruz	Ascendant	NA	NA	NA	NA	NA
	High	23.32 ± 2.24	76.68 ± 2.24	15.90 ± 3.17	1.54 ± 1.18	1.38 ± 0.47
	Descending	25.24 ± 1.07	74.76 ± 1.07	19.82 ± 1.54	1.58 ± 0.67	2.16 ± 0.33
	Low	21.00 ± 1.55	79.00 ± 1.55	15.87 ± 2.29	0.82 ± 0.44	1.58 ± 0.25

NA: Not analyzed.

waters (22.95%); the protein was higher at ascendant waters (18.08%), and the fat and ashes were higher at low waters (0.64% and 1.51%, respectively). In fish caught at Point 2, the highest amounts were obtained in low waters: dry matter (22.35%), protein (17.42%), ashes (1.78%), and fat (1.44%) In fish caught at Point 3, high values were obtained for ascendant waters, dry matter (25.19%), protein and fat at low waters (17.93% and 1.20%, respectively) and ashes at high waters (2.16%) Similarly, to *Leporinus agassizi* the results showed no statistically significant differences when relating

the capture site with the provenance ($p > 0.05$).

To identify the degree of relationship between the bromatological variables of *L. agassizi* and *L. fasciatus* a Pearson correlation analysis was done (tables 4 and 5). A significant correlation for dry matter/moisture, dry matter/protein, dry matter/ash and ash/protein parameters were observed in *L. agassizi* and for dry matter/moisture, dry matter/protein, dry matter/ash and dry matter/fat parameters was observed in *L. fasciatus*.

In the present study was possible to establish that the bromatological characte-

TABLE 3. Bromatological analysis of *Leporinus fasciatus* caught in the three sampling points of the Vaupés River, Colombia, over four hydrobiological cycles. Data expressed as mean \pm SD (standard deviation).

Sampling site	Season	Dry matter (%)	Moisture (%)	Protein (%)	Fat (%)	Ashes (%)
Point 1 Yacayacá	Ascendant	20.44 \pm 0.36	79.56 \pm 0.11	18.08 \pm 2.13	0.33 \pm 1.07	1.36 \pm 0.69
	High	22.95 \pm 0.54	77.05 \pm 0.54	NA	NA	NA
	Descending	NA	NA	NA	NA	NA
	Low	21.17 \pm 1.65	78.83 \pm 1.65	17.08 \pm 1.00	0.64 \pm 0.93	1.51 \pm 0.56
Point 2 Piracemo	Ascendant	NA	NA	NA	NA	NA
	High	NA	NA	NA	NA	NA
	Descending	23.33 \pm 2.23	76.67 \pm 1.64	NA	NA	NA
	Low	23.35 \pm 3.19	77.65 \pm 3.19	17.42 \pm 3.09	1.44 \pm 1.24	1.78 \pm 0.45
Point 3 Santa Cruz	Ascendant	25.19 \pm 2.04	74.81 \pm 1.56	NA	NA	NA
	High	22.83 \pm 0.80	77.17 \pm 0.80	17.65 \pm 1.31	0.70 \pm 1.22	2.16 \pm 0.12
	Descending	NA	NA	NA	NA	NA
	Low	23.06 \pm 1.60	76.94 \pm 1.60	17.93 \pm 1.87	1.20 \pm 0.53	1.64 \pm 0.29

NA: Not analyzed.

TABLE 4. Pearson correlation for the bromatological analysis of *Leporinus agassizi* caught in the Vaupés River. Correlations with a value of $p < 0.05$ are indicated in bold italics. Correlations with a value of $p < 0.001$ appear in bold type.

Variable	Dry matter	Moisture	Ashes	Protein	Fat
Dry matter		-100%	20%	67%	48%
Moisture	-100%		-20%	-67%	-48%
Ashes	20%	-20%		46%	-11%
Protein	67%	-67%	46%		24%
Fat	48%	-48%	-11%	24%	

TABLE 5. Pearson correlation for the bromatological analysis of *Leporinus fasciatus* caught in the Vaupés River. Correlations with a value of $p < 0.05$ are indicated in bold italics. Correlations with a value of $p < 0.001$ appear in bold type.

Variable	Dry matter	Moisture	Ashes	Protein	Fat
Dry matter		-100%	52%	81%	-73%
Moisture	-100%		-52%	-81%	73%
Ashes	52%	-52%		47%	36%
Protein	81%	-81%	47%		47%
Fat	73%	-73%	-36%	47%	

istics of the species *Leporinus agassizi* and *Leporinus fasciatus* are very similar to those of other well-known fish species, and even species that live in similar environmental conditions. Both *Leporinus agassizi* and *Leporinus fasciatus* present values within the ranges of macronutrients found in other species consumed in Colombia, such as rainbow trout (*Oncorhynchus mykiss*), red tilapia (*Oreochromis sp.*), Cachama (*Colosoma macropomum*), Bocachico (*Prochilodus reticulatus magdalenae*) and Bagre or catfish (*Pseudoplatystoma fasciatum*), which makes them a source of similar nutritional contribution. Contrasting the protein percentage of *P. reticulatus* (16.36-20.44%) and *C. macropomum* (16.7-19.34%); humidity of *P. reticulatus* (75.16–78.07%), and *C.*

macropomum (74.81–79.28%); fat percentage of Bagre or catfish (0.35–1.94%) and *P. reticulatus* (1.34–5.15%) (Perea *et al.* 2008). Regarding the values of ash, it was established that the species of *Leporinus* studied have a higher percentage compared to the previous fish species.

The bromatological analysis of other species present in the Orinoco and Amazonas basins, such as the Arawana (*Osteoglossum bicirrhosum*) and the Mapará (*Hypophthalmus edentatus*), showed higher moisture values for the Arawana (83.91%) and lower for the Mapará (65.18%) (Costa 2006), compared to the obtained in the present study. However, the ash values of the two Warakú species are higher than those values obtained by that author.

In the fillets of the species of *L. agassizi* and *L. fasciatus* were found similar levels of protein to Pacú (*Piaractus mesopotamicus*) (~19%) (Pavón *et al.* 2018). Likewise, the protein obtained in the present study for *Leporinus fasciatus* (17.6%) and *Leporinus agassizi* (16.59%), can be contrasted with the average protein of the species *Leporinus obtusidens*, which was 16.56% on average (Lazzari *et al.* 2015). Similarly, the values found for protein, ash and fat in the present study were higher in comparison with the results obtained in fresh and eviscerated Tilapia (*Oreochromis* sp.) (Sena *et al.* 2014).

When several specimens of *Leporinus obtusidens* were subjected to dietary feeding with and without cooking, the results referring to protein are similar and lower for the percentage of ashes. Considering that the fish feed of the present study was different from the one used by the mentioned author, the evaluated parameters show a quite similar behavior (Lazzari *et al.* 2007). Although the ash values of Warakú *verdadero* and Warakú *pinima* resemble different species, they are superior to the values found for fillets of Pacú (*Piaractus mesopotamicus*) (~1.20%) (Pavón *et al.* 2018)

Although no statistically significant differences were found in the present study ($p > 0.05$) related to the hydrobiological cycle in the different capture sites, it should be considered that the factors that affect the chemical/nutritional composition of the fish are numerous, being either an intrinsic nature bearing upon genetics, morphology, physiology or environmental factors, relating to the living conditions and food (Jacquot 1961; Borgstrom 2012). In turn, it is reported that the lipid content of severe species of fish, including common carp, can vary widely depending on the feeding habits of animal and the feed used (Ćirković *et al.* 2012).

In the present study, both *L. agassizi* and *L. fasciatus* showed the lowest values of dry matter and protein (percentage) during the low water period, which suggests that the consumption of aquatic plants and macrophytes generates a lower protein conversion in the fish fillet. The diet of the fish community may change depending on the hydrological period, due to the increase in biodiversity offered for feeding the fish, especially during times when the water level is high (Pérez y Prada 2011). In periods of low water, the availability of food is restricted, which implies that the fish have an herbivorous behavior since the availability of insects is limited (Medeiros *et al.* 2014). This was observed in *L. friderici*, where the animals had greater herbivore preference in low waters in the Point 1 and Point 3 (Velasco-Santamaría *et al.* 2017). On the other hand, during the increase of the flow (ascendant-high waters) sediments and biological materials are dragged, allowing the greater food availability of the fish (Petry *et al.* 2011). Nearby species such as *L. friderici*, present an important consumption of insects of the genus *Blattodea*, *Coleoptera* and *Diptera*, and of invertebrates such as nematodes and annelids, leaves and bark, in greater proportions during high and descending waters (Velasco-Santamaría *et al.* 2017).

Unlike the reports in the literature, in the present study the bromatological parameters were generally higher in levels of descending waters, although during the moments of high waters the percentage amount had similarity. This suggests that the fish has a varied diet that although is not as diversified and rich as in high waters, is not as limited as in the low water levels.

There is a classification of fish according to the percentages of fat and protein present in their meat, known as the Stansby

classification (Stansby 1962). This classification has five categories, category A being: fish with a percentage <5% of fat and a high protein content of 15-20%, such as Bacalao of Pacific or Pacific cod (*Gadus macrocephalus*). Category B: 5-15% fat and 15-20% protein, the case of Caballa or mackerel (*Scomber scombrus*). Category C: >15% fat and <15% protein, Salvelinus (*Salvelinus namaycush siscowet*). Category D: <5% fat and >20% protein, Tuna (*Katsuwunos pelamis*). Category E: <5% fat and <15% protein. According to the results obtained for the percentage amounts of fat and protein, the species of *L. agassizi* and *L. fasciatus* are include in the category A Stansby.

CONCLUSION

Although the study did not show significant statistically differences, and it is assumed that in the different sites of the river the nutritional contribution of the fish is going to be the same, during periods like high and descending waters, the best levels of each bromatological variable were found. These results support the hypothesis that during this type of water level the diversity of food is greater; however, additional studies in these species should be carried out, considering another variety of natural and anthropological factors, as well as, eating habits. The results obtained in this study, contribute with the scientific knowledge and also a contribution to food security and its possible introduction to productive systems through continental aquaculture.

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Conflict of Interest: none.

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