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ZOOLOGY

Daily variation feeding of *Astyanax lacustris* (Lütken 1875) in a subtropical river

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ABSTRACT. This study aimed to know the daily variation of *Astyanax lacustris* (Lütken 1875) feeding in a lotic environment. Fish were caught with a net for three days every three hours in the summer to capture ten individuals per hour in a stretch of the Ijuí River, Middle Uruguay River, Brazil. The captured specimens had their stomachs removed and the content analyzed and separated into seven food categories with the aid of stereomicroscope. For the analysis of food items were used the frequency of occurrence methods, volumetric method and applied the Alimentary Index (IA*i*). Two hundred stomachs were analyzed, of which 95% had food content. The most abundant items were algae and autochthonous insects. *Astyanax lacustris* feeds throughout the day especially early in the morning (9 hours) and reduces its feeding at night (24 hours and 3 hours). It was also verified variation of AI of different food items throughout the day. It is concluded that *A. lacustris* feeds throughout the day, especially in the daytime and that throughout the 24 hours it varies its diet due to photoperiod and food availability.

Keyword: Temporal variation; trophic ecology; neotropical fish; photoperiod; diet.

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Introduction

Neotropical ichthyofauna is characterized by high feeding flexibility, using a wide variety of alimentary resources present in the aquatic environment (microorganisms, primary producers, invertebrates, vertebrates) and even terrestrial environment (primary producers and insects) (Petry, Thomaz, & Esteves, 2011; Meurer & Zaniboni-Filho, 2012). Feeding studies provide a better understanding of the behavior related to alimentary availability, seasonal and daily variation (Corrêa & Silva, 2010).

Throughout the day the fish present differences in feeding consumption, often related to the ecosystem variations. These variations may be determined by biotic factors such as alimentary availability, predator presence and inter-specific competition (Falcón, Migaud, Muñoz-Cueto, & Carrillo, 2010; Evangelista, Boiche, Lecerf, & Cucherousset, 2014) and also by abiotic factors such as hydrodynamics of the environment, water temperature and photoperiod (Veras et al., 2013).

The fish of the genus *Astyanax* forage all trophic levels, presenting abilities to adapt rapidly to a new feeding and to continue feeding even in unfavorable environmental conditions (Gomiero & Braga, 2003; Corrêa & Silva, 2010). These individuals inhabit the middle and surface strata, either in lentic or lotic waters, to collect items dragged by the water, characterized by frequent omnivorous feeding (Gomiero & Braga, 2003; Falcón et al., 2010).

The species *Astyanax lacustris* (Lütken, 1875) is considered omnivorous for feeding on animal and plant items (Silva, Pessoa, Costa, Chellappa, & Chellappa, 2012). Authors such as Andrian, Silva, & Peretti (2001) and Dias, Castelo Branco, & Lopes (2005) emphasize the predominance of plant remains and insects in their diet, characterizing them as omnivores with a herbivore-insectivorous propensity. However, in the literature, it is still not clear if this omnivorous species may feed at whatever day hour or present a variation in the quantity and quality of the items ingested during the day.

In this way, the present study aimed to evaluate the *A. lacustris* diet at different times of the day, supposing that *A. lacustris* in Middle Uruguay presents a daily variation in the quantity and quality of its feeding, based on studies with other species (Hahn, Loureiro, & Delariva, 1999; Figueiredo & Vieira, 2005).

Material and methods

Study area

The study occurred in Salto Pirapó, located on the Ijuí River, the watershed of the Uruguay River and the border of the municipalities of Dezesseis de Novembro and Roque Gonzales, RS, Brazil (Figure 1). The section where the samples were collected is about 325 meters wide and 500 meters long and it is situated 2.2 kilometers downstream from the spillway of the Passo São João Hydroelectric Plant (HPP). It is a lotic environment, followed by water waterfalls with many rapids and small backwaters where is possible to observe several species fish jumping up the river in times of spawning.

The water flow of this site is regulated by the Passo São João HPP, according to the intensity of the rainfall upstream of the dam. During periods of drought, as recorded in the study, the water is retained by the floodgates, where only the ecological flow is released, and in periods of high rainfall, there is greater water release through the gates, raising the water level of the Salto Pirapó.

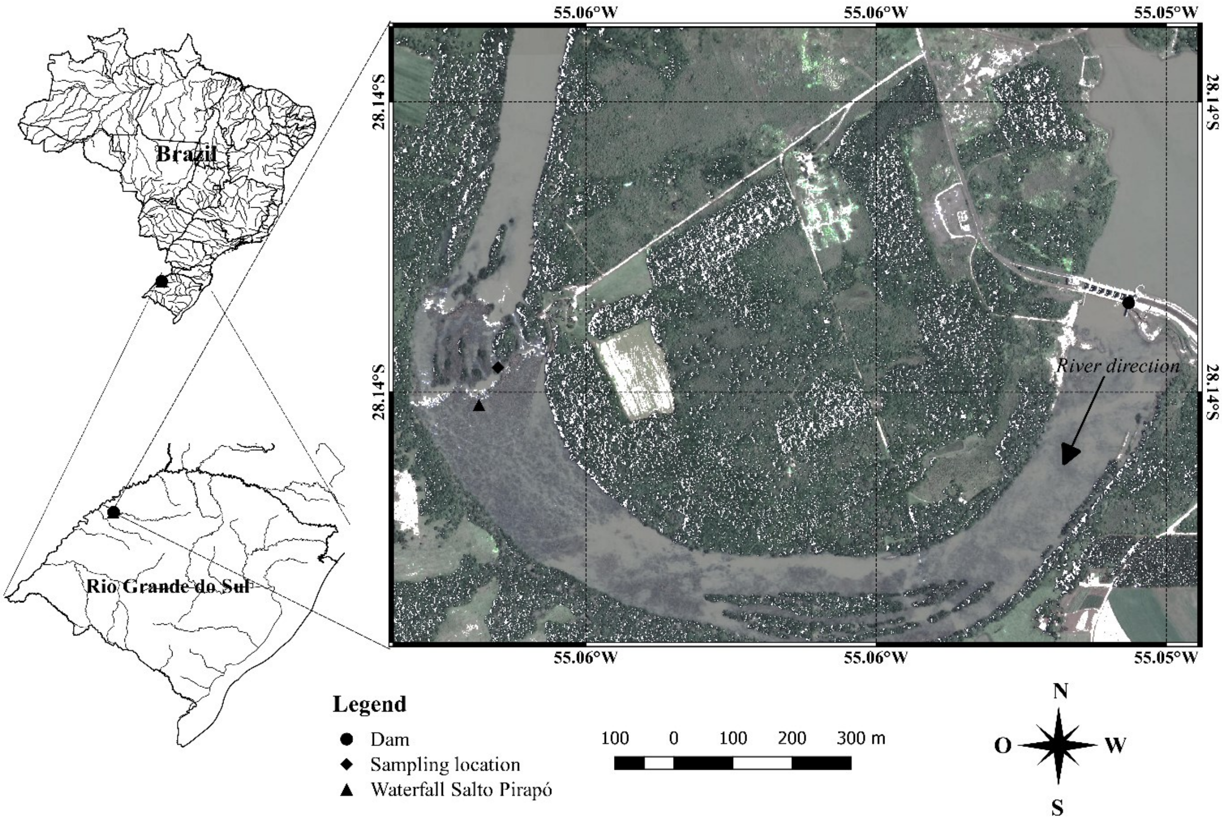

Figure 1. Location of sampling site in the Ijuí river, Middle Uruguay, Brazil.

Sampling

Fishes were sampled eight times, at a 3 hours interval over a 24 hours period: 6, 9, 12, 15, 18, 21, 24, and 3 hours and represented by treatments H6, H9, H12, H15, H18, H21, H24, and H3, respectively. This sample capture occurred for three days in the summer (December 2017), totaling 24 samplings. The individuals were captured using a casting net with 12 mm mesh between the nodes and a 15 meters radius. For each sampling, the period of one hour was established with successive sets using a casting net in an extension of approximately 100 meters. In each sampling captured, ten individuals of *Astyanax lacustris* were randomly selected for stomach removal and immediately frozen (-10 °C) until analysis. At some day-hours such as H24, H3, and H6 due to low fish movement, the number of captured individuals were less than ten per hour, but never less than five individuals per day. During the sampling capture, some individuals of the species were put in 10% formalin and deposited in the Museum of the State University of Maringá (NUP20566). This study is part of Project 534 entitled "Fish Ecology of the Middle Uruguay", carried out under permits 55011-2 (ICMBio). The ethics committee of the Federal University of Fronteira Sul, Brazil, approved this Project (number 23.205.004977/2015-90).

Laboratory processing

In the laboratory, fishes were measured (ST, cm to the nearest mm), weighet (Wt, gr) and eviscerated. Stomach weight (Wg) was registered to the nearest 0.01 g. Stomach content was removed, separated from mucus and measured (wet mass of stomach content; Wc) to the nearest 0.01 g.

Furthermore it was performed a visual evaluation of the degree of repletion, being: empty = no item; partially empty = until a quarter filled; partially filled = partial filling greater than a quarter; filled = fully filled (Hahn et al., 1999). In the end, the stomachs were conditioned in sealed pots containing 4% of formaldehyde.

The alimentary items present in the stomach contents of each fish were identified by a stereomicroscope to the lowest possible taxonomic level and grouped according to their origin: fish (F), autochthonous insects (AUI), allochthonous insects (ALI), vegetable matter (VM), algae (AL), sediment (S) and others (Hahn and Fugi 2007). Each identified item was quantified in volume (mm^3) using millimeter paper with a fixed height of one millimeter. After that, the data were recorded and reconditioned items in pots with 70% alcohol.

Statistics Analysis

The frequency of occurrence (F%) method was used to analyze the data, which represents the percentage frequency of the number of stomachs with a specific alimentary item in relation to the total number of evaluated stomachs that presented some type of food (Hyslop, 1980). It was also used the volumetric method (V%), which consists of expressing the percentage volume considering the total volume occupied by a specific alimentary item in relation to the total volume of all items present in the stomach (Hynes, 1950). These two methods were integrated for feeding analysis using the Alimentary Index expressed as percentage IA_i (%), as proposed by (Kawakami & Vazzoler, 1980). The index is expressed by the equation $IA_i = (F_i * V_i) / \Sigma\, F_i * V_i) * 100$, where i = 1, 2, ... n alimentary items; F_i = frequency of occurrence of a specific alimentary item; and V_i = volume of a specific alimentary item.

The Shapiro-Wilks test to test normality and the Levene test to evaluate homoscedasticity were used to determine the use of the parametric method ANOVA, this analysis was used to verify the differences between the volume of food consumed and the Ia_i between the different times of the day (Zar, 1999). In the presence of statistical differences, the Tukey's test *a posteriori* was used. All analyses were performed in the statistical program Statistic 7.1.

Non-metric multidimensional scaling analysis (nMDS) was used to examine temporal variation in diet using the alimentary index of each item. The dissimilarity matrix used in the ordination was constructed using the Bray-Curtis index, with this analysis carried out using the PCORD software (version 5.1) (Hammer, Harper, & Ryan, 2001). In nMDS analysis a low-stress value (0.1), corresponding to a good ordering, ensuring good reliability in the interpretation of the results. The significance level of 0.05 was used for all statistical analyses.

Results

A total of 200 individuals, ranging from 67 to 156 mm with a mean of 134 ± 27 mm, were collected. Related to the degree of repletion, 87% of the stomachs were filled, 5% partially filled, 3% partially unfilled, and only 5% had no stomach contents. It was verified that the species fed on animal and plant material, although there was greater participation of plant origin. Among the most prominent items, were filamentous algae, followed by autochthonous insects, mainly from the orders Trichoptera and Ephemeroptera (Table 1). The category others was composed of the digested material that composed most of the rare items of small value, such as feathers, insect eggs, free-living nematodes, annelids, arachnids, and microplastic (< 2.0 mm). This last item was found in the stomach of three samples.

The ANOVA showed that there was a significant difference in the total volume of the items consumed by *A. lacrustris* throughout the day ($P < 0.05$). The highest consumption was verified in H9 ($V = 3.280 mm^3$, $P < 0.05$), and lower consumptions in H24 ($V = 478 mm^3$) and H3 ($V = 361 mm^3$) (Tukey, $P < 0.05$, Figure 2).

ANOVA also showed that there is significant variance in all alimentary items ($P < 0.05$). For the autochthonous insects, the highest values of IA_i were at 9 and 21 hour (Tukey, $P < 0.05$, Figure 3A). For allochthonous insects, the highest values were at 21 and 24 hour (Tukey, $P < 0.05$, Figure 3B). For algae, the highest values were 6 hour and in the period between 12 and 18 hour (Tukey, $P < 0.05$, Figure 3C). For the item vegetal matter and sediments, the highest IA_i values were at 9 hour, (Tukey, $P < 0.05$, Figures 3E and 3F).

Table 1. Alimentary index (IAi) of the items consumed by *Astyanax lacustris* (Lütken 1875) during different times of the day in the Ijuí river, Uruguay River basin.

Alimentary items	Time							
	H6	H9	H12	H15	H18	H21	H24	H3
Autochthonous insects								
Coleoptera	0.21	0.32	0.17	0.39	0.08	0.16		
Diptera	1.73	4.03	2.94	8.54	2.94	3.88	0.22	2.25
Ephemeroptera	5.04	0.36	0.69	4.37	1.49	18.92	4.13	1.53
Lepidoptera	0.20	0.64	0.09	1.37	0.03	0.70	1.69	5.59
Trichoptera	19.30	21.37	3.26	5.45	1.80	24.18	2.22	0.44
Insects fragments	3.01	1.89	1.03	3.50	1.04	4.95	1.59	10.47
Allochthonous insects								
Coleoptera		0.14	0.01	0.05	0.13	3.08	2.63	0.44
Diptera	0.04	0.05		0.01		0.10		
Hymenoptera	0.02	0.01		0.15	0.04	0.03		
Lepidoptera		0.61				0.06		0.18
Trichoptera	0.10			0.04		0.11	1.15	
Insects fragments	0.05			0.04		0.24	2.58	
Algae	66.16	41.05	75.43	63.62	90.88	19.86	44.64	41.53
Vegetable matter	1.99	21.85	13.53	2.25	0.28	13.42	6.13	1.24
Fish								
Scales	0.06	2.53	1.82	2.60	0.82		1.17	4.91
Fish fragments						6.23	9.41	28.42
Sediments		0.31	0.17	0.04	0.01		0.21	
Others	2.09	4.84	0.86	7.58	0.42	4.08	22.23	3.00

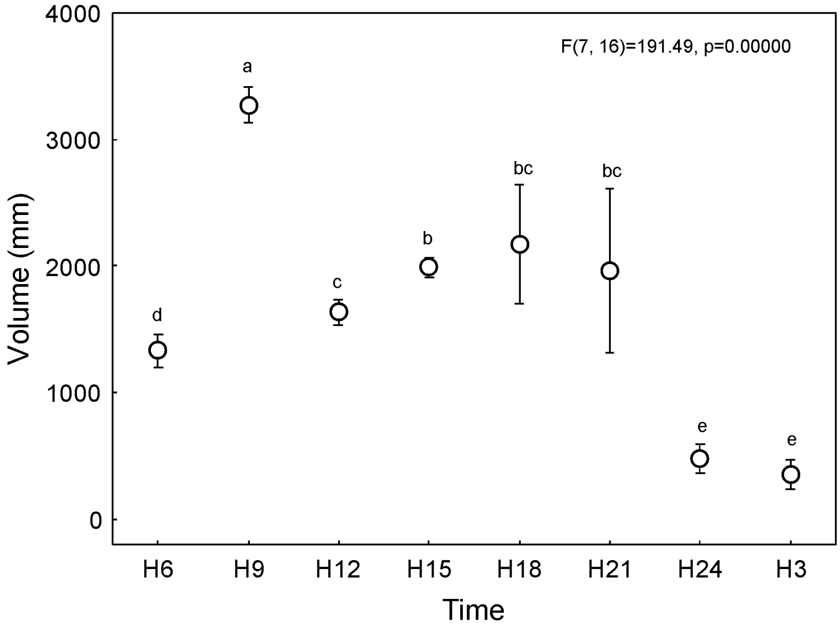

Figure 2. ANOVA result, mean values and standard deviation of stomach content volume found in *Astyanax lacustris* (Lütken 1875) in the Ijuí river, Middle Uruguay, Brazil.

The nMDS summarized the composition of the different alimentary items present in the stomach of *A. lacustris* and separated the different times of the day, (Figure 4). The stability criterion was met with a final stress of 0.09 for a two-dimensional solution. The proportion of variance represented by each axis, based on the R^2 between distance in the ordination space and distance in the original space, was 0.44 for the nMDS 1 and 0.25 for the nMDS 2. The result revealed three main groups: times H6+H12+H15+H18, times H24+H3 and times H9+H21, nMDS 1 was affected primarily by allochthonous insects (r=0.52), fish (r=0.85) and algae (r=-0.91), nMDS 2 was affected primarily by algae (r=0.75), allochthonous insects (r=-0.73), fish (r=-0.60) and vegetable matter (r=-0.56).

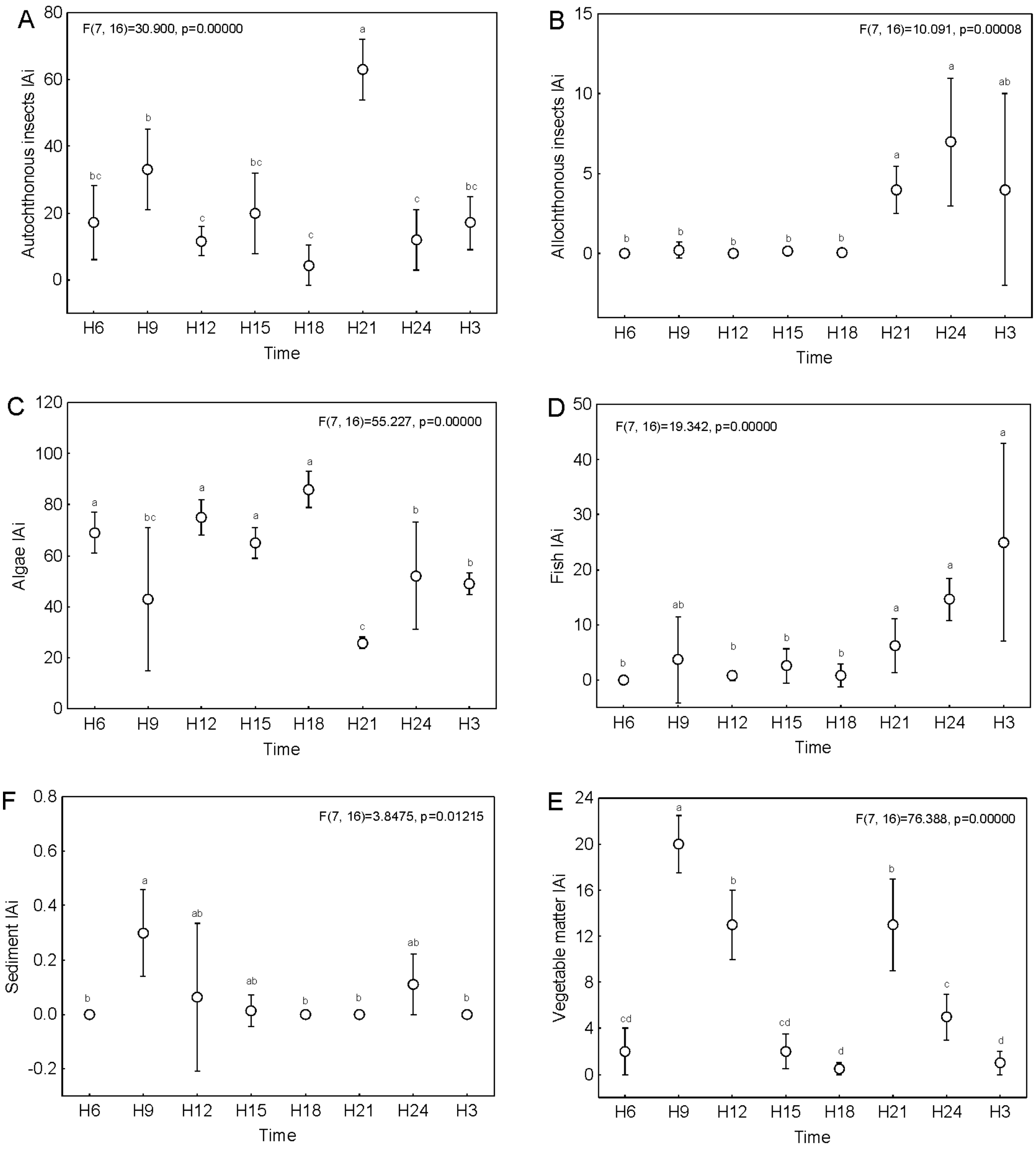

Figure 3. ANOVA result, mean values and standard deviation of Alimentary index (IA*i*) for: (A) autochthonous insects, (B) allochthonous insects, (C) algae, (D) material vegetable, (E) fish and (F) Sediments ingested by *Astyanax lacustris* (Lütken 1875) in the Ijuí river, Middle Uruguay, Brazil.

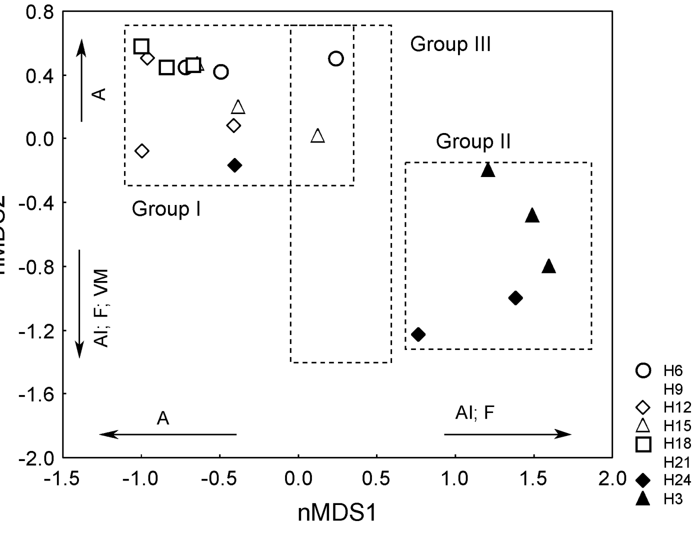

Figure 4. nMDS result applied to the alimentarey index of each food item found in the *Astyanax lacustris* (Lütken 1875) stomachs captured in the Ijui River, Middle Uruguay, Brazil.

Discussion

The diversity of alimentary items in the stomach of *A. lacustris* shows that this species is omnivorous, which confirms what was verified in other studies (Rocha, Cassati, & Pereira, 2009; Silva et al., 2012). The high incidence of filled stomachs at all times indicates that this lambari feeds at all times of the day. Nevertheless, it does not consume all items homogeneously during the day, there are variations in the volume, frequency, and order of importance of the items consumed.

The most abundant item in the diet of *A. lacustris* was algae. The filamentous algae had a noticeable constancy and volume in the diet of the species during all the periods, especially in the hours of greater luminosity, being a significant source of autochthonous food for the species, which guaranteed high IA*i* values at practically all times. The fact of this item is one of the most frequent in the lambaris stomach also indicates that these organisms are abundant downstream of the dam.

The Ijuí River is characterized by being a turbid river due to the erosive processes presents in its upstream stretches (Didoné, Minella, & Evrard, 2017). Thus, during the year water transparency is usually less than 30 cm, which certainly interferes with the light entering the water. However, this high turbidity is not observed downstream of the Passo São João reservoir due to sedimentation processes occurring in the lakes of the São José and Passo São João reservoirs located upstream of the study area, hence in the Salto Pirapó transparencies are greater than 80 cm are common, which allows the entrance of light promoting the proliferation of filamentous algae.

Although the algae were the main item in the stomachs of *A. lacustris*, they were not abundant in the nocturnal periods. Several studies have shown that the lambaris of the genus *Astyanax* use the vision primarily to capture their prey (Orsi, Carvalho, & Foresti, 2004). Thus, we hypothesized that the reduction of algae in the consumption at night is related to the difficulty of *A. lacustris* in visualizing and predating these organisms.

Another resource widely used by the species at all times was the autochthonous insects, which showed peaks consumption in the early morning and at the end of the day. We believe that the peaks of consumption are related to the increase of macroinvertebrate movement during these periods. Movements made by these organisms are known as drift. Studies on insect drift point to photoperiod as an important regulatory factor, so that the highest rates of macroinvertebrate drift are observed at dawn and dusk when light intensity is lower (Benson & Pearson, 1987; Hansen & Closs, 2007; Lancaster, 1992; Castro, Hughes, & Callisto, 2013).

Also, the presence of allochthonous insects in the stomach contents was verified. This item appeared mainly at night time. The highest consumption of these organisms at night may be related to the nocturnal flock, a period of greater activity of the insects when compared to the hot hours of the day. A study realized with Siluriform fish in New Zealand rivers shows that the feeding of these fish is related to the high activity time of the allochthonous invertebrates, which usually occurs at night (Scrimgeour & Winterbourn, 1987; Silva, Fugi, Carniatto, & Ganassin, 2014). A similar explanation was offered for the nocturnal feeding of some fishes from neotropical environments (Hahn, Andrian, Fugi, & Almeida, 1997; Silva, Gubiani, & Delariva, 2014). We propose that *A. lacustris* may have exhibited opportunistic behavior to capture allochthonous insects that fell into the river at night.

The consumption of vegetable matter was also significant and occurred mainly in the early morning when the highest total volume of food was found in the stomach of this lambari. The dominance of vegetable matter and autochthonous insects at peak feeding time (9 hours) seems to indicate that these two items are important in the diet to meet the metabolic needs of *A. lacustris* after the nocturnal period in which consumption is reduced. Other studies have demonstrated the importance of insects and vegetable matter in this lambari diet (Cassemiro, Hahn, & Fugi, 2002; Silva et al., 2012).

The sediment consumption was reduced and its consumption peaks coincided with the higher consumption of autochthonous insects. We theorize that this coincidence is related to the capture of the autochthonous insects that are usually found in the substrate. As a result, when consuming the insects, the sediment would be ingested simultaneously.

Significant variations during 24 hours of the day were also recorded for the ingestion of the fish item (Table 1) by the *A. lacustris*. The highest IA*i* values for this item were verified at night, where they were found in the stomachs, fish muscles, spine, and relatively whole fish, which explains the higher volume and IA*i* of this item in this period. At daytime, the IA*i* of the fish item was low and related mainly to the recording of scales in the stomach. The presence of the scales in the stomach at daytime may be related to intraspecific agonistic encounters (Vilella, Becker, & Hartz, 2002) or the longer digestion time of this structure of the fish that were consumed at night. We infer that for this study the second hypothesis is the most probable.

The nMDS synthesizes the explained by the ANOVA result and explained previously and joins the alimentary items in three alimentary groups preferably consumed throughout the day. The group I containing filamentous algae are mainly consumed during the daytime hours, Group II which contains the fish and the allochthonous insects that are consumed mainly at night and the group III that includes the first daytime and nocturnal schedule in which is verified the greater voracity of lambaris and where all the alimentary items of group I and II are consumed plus indigenous insects. A summary of the daily variation of *A. lacustris* feeding in the Ijuí river is presented in Figure 5.

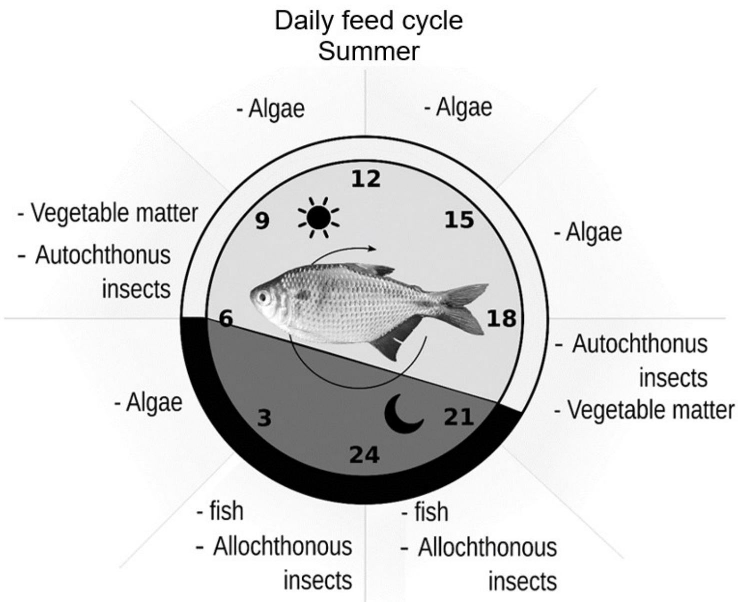

Figure 5. Representative model of *Astyanax lacustris* (Lütken 1875) daily feeding in the Ijuí River, Brazil.

In general, we suggest that for *A. lacustris*, the dominance of the alimentary items found in the stomachs does not necessarily mean the choice of the food, but it may reflect the abundance and/or availability of the food on time due to the omnivorous and opportunistic habit of this species (Abelha, Goulart, Kashiwaqui, & Silva, 2006; Silva et al., 2012).

In addition to alimentary items, other items appeared to a lesser amount mainly at night time, such as insect eggs, feathers, annelids, and microplastics. Reports of microplastic ingestion by fish are frequent (Jabeen et al., 2017; Jovanović, 2017). Studies occurred in the last decade in marine and freshwater environments in Brazil have been showing the pollution of these ecosystems by plastic components (Barboza, Vethaak, Lavorante, Lundebye, & Guilhermino, 2018), while for Brazilian freshwater rivers belonging to the Atlantic Forest biome are still scarce. We propose that the species at the time of ingesting some other item, such as filamentous algae, for example, ingested this material in the channel region. The fact of exists this registration in three samples is worrisome and shows the high availability of these materials in the Ijuí River.

Through this study, we confirm our initial hypothesis, showing that there is a daily variation in *A. lacustris* feeding. Variations in feeding during the day were also observed for other species. For *Micropogonias furnieri* (Desmarest, 1823), the highest volumes of stomach contents were recorded during the day, and the species presented a reduction in feeding during the night period (Figueiredo & Vieira, 2005). For the species *Plagioscion squamosissimus* (Heckel, 1840), the feeding occurs 24 hours a day, but it was discovered that feeding was more intense during the day, especially in the early morning (Hahn et al., 1999). In general, for fish, it is supposed that food availability (Barthem, 1987; Abelha, Agostinho, & Goulart, 2001) and photoperiod (Reebs, 2002; Zhdanova & Reebs, 2006) are the main factors driving feed throughout the day. These factors also appear to be the ones that control the feeding of *A. lacustris*.

In addition to the daily changes, changes in the fish diet may still be governed by seasonal changes, considering the distinct periods of the year that presents different abiotic conditions and food availability. In subtropical latitudes, most of the seasonal variations are caused mainly by variations in water temperature and photoperiod that govern the development of many aquatic and terrestrial organisms (Payne, 1986;

Agostinho & Julio Junior, 1999) and also by hydrometric oscillations, which lead to the flooding of adjacent regions to the channel (Goulding, 1980; Vazzoler, Agostinho, & Hahn, 1997). These three factors certainly vary the abundance of all alimentary items observed in this study.

As the present work occurred during the summer with the river slightly low and with the temperature of the water and the photoperiod increasing, other studies should evaluate if these results are confirmed in other seasons of the year and with other water conditions. Moreover, whether other species of *Astyanax* exhibit similar feeding behavior.

Conclusion

Finally, it was concluded that *A. lacustris* is an omnivorous species which feeds throughout the day and presents significant quantitative variations of alimentary items consumed in a daily cycle due mainly to variations in the photoperiod and availability of alimentary items.

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