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Forest fragmentation influences the diet of cichlids *Apistogramma agassizii* (Steindachner, 1875) and *Aequidens tetramerus* (Heckel, 1840) (Actinopterygii: Cichliformes) in streams of the Western Amazon

A fragmentação florestal influencia a dieta dos ciclídeos, *Apistogramma agassizii* (Steindachner, 1875) e *Aequidens tetramerus* (Heckel, 1840) (Actinopterygii: Cichliformes) em riachos da Amazônia Ocidental

Lucena Rocha Virgilio[1]*, Cleyton Holanda de Brito[2], Monik da Silveira Suçuarana[2],
Lisandro Juno Soares Vieira[2]

[1]Universidade Federal do Acre, Campus Floresta, Estrada do Canela Fina, Km 12, CEP 69980-000, Cruzeiro do Sul, AC, Brasil.

[2]Universidade Federal do Acre, Programa de Pós-graduação em Ecologia e Manejo de Recursos Naturais, BR-364, Km 4, CEP 69915-900, Distrito Industrial, Rio Branco, AC, Brasil
*e-mail: lucena_gymnor@hotmail.com

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Abstract: Aims: To evaluate the effects of replacing riparian vegetation with pasture in the diet of *Apistogramma agassizii* and *Aequidens tetramerus*. **Methods:** The study was carried out in the municipality of Senador Guiomar, Acre. We sampled three streams divided into three stretches each (100 m), bordered by forest, pasture, and both forest and pasture (edge). Quarterly collections using fishnets were performed between January and October 2008. **Results:** The species *A. agassizii* had a diet based on the consumption of adult insects in stretches of forest, different from the pasture environment, in which the consumption of insect larvae was higher. In the diet of *A. tetramerus*, the most important item was insect larvae in all environments, mainly in the pasture. We found low amplitude of trophic niche for *A. agassizii* in the forest environment, due to the consumption of adult insects, and for *A. tetramerus* in the pasture, due to the consumption of insect larvae. In the pasture areas, we found no difference between the items consumed by the species, which showed a high trophic niche overlap, influenced by the consumption of Diptera larvae. **Conclusion:** Thus, we show that allochthonous items, such as adult insects, are food resources indicative of riparian vegetation conservation and are important for both species, mainly *A. agassizii*. However, this item has been replaced by autochthonous items, such as Diptera larvae, in the *A. agassizii* diet and become predominant in the *A. tetramerus* diet as the riparian vegetation deteriorates.

Keywords: food overlap; land-use change, trophic niche, riparian vegetation.

Resumo: Objetivos: Avaliar os efeitos da substituição da vegetação ripária por pastagem na dieta de *Apistogramma agassizii* e *Aequidens tetramerus*. **Métodos:** O estudo foi realizado no município de Senador Guiomar, Acre. Foram amostrados três riachos separados em dois trechos (100 metros de extensão cada), sendo um trecho margeado por floresta, borda e outro por pastagem. Foram realizadas coletas trimestrais entre janeiro e outubro de 2008, com auxílio de puçás. **Resultados:** A espécie *A. agassizii* em trechos de floresta teve uma dieta baseada no consumo de insetos adultos, diferente do ambiente de pastagem, no qual o consumo de larvas de insetos foi maior. Já na dieta de *A. tetramerus* larvas de insetos foi o item mais importante em todos os ambientes, principalmente no de pastagem. Houve baixa amplitude de nicho trófico para *A. agassizii* em ambiente de floresta, devido ao consumo de insetos adultos, e para *A. tetramerus* na pastagem, devido ao consumo de larvas de insetos. Nas áreas de pastagem, não houve diferença nos itens consumidos pelas espécies, no qual apresentaram elevada sobreposição de nicho trófico, influenciado pelo consumo de larvas de Diptera. **Conclusão:** Assim, evidenciamos que os itens alóctones, como insetos adultos são recursos alimentares indicadores de conservação da vegetação ripária e importantes para ambas as espécies, principalmente *A. agassizii*. No entanto observou-se a substituição na dieta de *A. agassizii* por itens autóctones, como larvas de Diptera, e o predomínio deste item na dieta de *A. tetramerus* conforme ocorre a degradação da vegetação ripária.

Palavras-chave: sobreposição alimentar; mudança do uso do solo; nicho trófico; mata ripária.

1. Introduction

The Amazon basin has the greatest fish diversity in the world (Reis et al., 2016). These freshwater ecosystems cover between 14% and 29% of all Amazon basin area (Castello et al., 2013). The basin's vast dimension explains the existence of highly complex ecosystems, which influence its rich diversity (Kandziora et al., 2013).

Small streams networks, regionally called igarapés (Junk, 1983), part of the extensive Amazonian basin, are formed in elevated portions of the basin and harbor a uniquely rich ichthyofauna (Sabino & Zuanon, 1998). These streams are relatively fragile systems with low autochthonous production, thus dependent on riparian vegetation to maintain their biotic integrity (Barrella et al., 2000; Henry, 2003).

In the last decades, the land-use change that leads to the conversion of native vegetation, called deforestation, has changed at least 697,770 km^2 (10%) of the Amazon basin, largely due to the expansion of agriculture, mining and livestock (Eva et al., 2004). In these regions, areas of extensive tropical forest can be found concurrently with high deforestation rates (Velasco Gomez et al., 2015). Thus, the habitat loss and degradation in these areas of small water bodies lead to the decline of several species (Keinath et al., 2017).

Although studies dealing with the effects of forest fragmentation on individual species are complex (Purvis et al., 2005), the ecological specialization, such as in studies regarding the diet, population density (Newmark, 1991), and body size (Cardillo et al., 2005), can predict species sensibility to physical and chemical variation in certain areas.

Thus, because the fish diet is often associated with its environment, which is probably influenced by the presence and type of riparian vegetation (Ferreira et al., 2012), the following study indicated the diet analysis as a tool to explain the effect of land/soil change on fish populations. Lorion & Kennedy (2009) and Ceneviva-Bastos & Casatti (2007) have used trophic ecology in their studies to show that as the riparian zone changes, the trophic structure fish is altered so that the species remain generalist or opportunistic.

Moreover, studies regarding fish diet have been of great importance because they describe the processes of obtaining energy and help to understand the activities involved in the processes of development, growth, reproduction, and maintenance of the organism (Ribeiro et al., 2014).

The Cichlidae family is an interesting group to study potentially adaptive processes in the evolution of neotropical fish. They are geographically widespread and diversified enough to represent a good part of the neotropical diversity (López-Fernández et al., 2010). They belong to the order Cichliformes, being the third most diversified family of neotropical freshwater fish (Reis et al., 2003). South American cichlids, especially small ones, have a wide variety of reproductive and food adaptive strategies (Sampaio & Goulart, 2011) and, therefore, can respond to changes in the environment (Costa & Soares, 2015).

The cichlid species *Apistogramma agassizii* (Steindachner, 1875) and *Aequidens tetramerus* (Heckel, 1840) can be found foraging in the same microhabitat (Costa & Soares, 2015). They are widely distributed in a variety of habitats, such as lowland lakes, aquatic vegetation, and small streams, where aquatic insects are the dietary basis of these two species (Cardoso & Couceiro, 2017). Because they are considered opportunistic species, they can be used as indicators of preserved or degraded areas (Ferreira et al., 2018; Virgilio et al., 2018), as they show high tolerance and generality due to their adaptation strategies to different environmental conditions (Burress et al., 2013). In this regard, studies about the diet of these two species in preserved and degraded areas can provide decisive information on how the removal of riparian vegetation can influence the environment.

Therefore, the present study aims to evaluate the effects of replacing riparian vegetation with pasture in the diet of *Apistogramma agassizii* and *Aequidens tetramerus*. Specifically, the present study (I) evaluated the influence of riparian vegetation removal on the food resources most consumed by *A. agassizii* and *A. tetramerus*; (II) analyzed the influence of pasture areas on the amplitude and overlap of the trophic niche in the use of food resources by species; and (III) evaluated if there is a difference in the consumption of food items by the two species between the forest, edge, and pasture environments.

2. Material and methods

2.1. Study area

This study was conducted in the southwestern Brazilian Amazon in the Catuaba Experimental Reserve (REC) (10°04'S, 67°37'W, 214 m a.s.l), municipality of Senador Guiomard, State of Acre, Brazil (Figure 1). This area has 2111 ha of tropical rainforest cover, composed mainly of open rainforest with bamboo and palms (Morato et al., 2005). In this region, the forest removal is due to cattle farming, which has transformed most of the once vast areas of rainforest into a mosaic of grasslands with sparse forest fragments (Fonseca, 2006). The three seasonal periods can be defined as rainy (from December to March), dry (July to August), and two transition seasons (September to October and April to May).

We chose three low-order streams to use as model ecosystems for this study: *Bambú* stream (10º04'39,6" S; 067º36'48,4" W) and *Poço* stream (10º05'19,5" S; 067º36'51,3" W), both first-order, and *Floresta* stream (10º04'15,4" S; 067º36'52,6" W), a second-order stream. We selected three stretches of 100 m in each stream, distributed as follows: a stretch inside the forest (Forest), inserted 200 m from the edge; a stretch bordered by forest and grassland (Edge), inserted in the forest fragment edge; and a stretch bordered by grassland (Pasture), inserted 200 m outside the forest fragment. In total, we sampled fish in nine stretches.

In general, stretches of igarapés bordered by forest had a high level of preservation. In these environments, we observed the presence of a great abundance of arboreal, shrub, and herbaceous layers, as well as epiphytes and closed canopy, conferring low light to streams beds. The stretches inserted in pasture environment had a low degree of preservation, mainly due to the reduced abundance of arboreal vegetation, direct exposure of the aquatic environment, and the initial stage of siltation.

2.2. Sampling

Data collection occurred between January and October 2008, at quarterly intervals, contemplating wet, ebb, and dry periods (Permit ICMBio No.

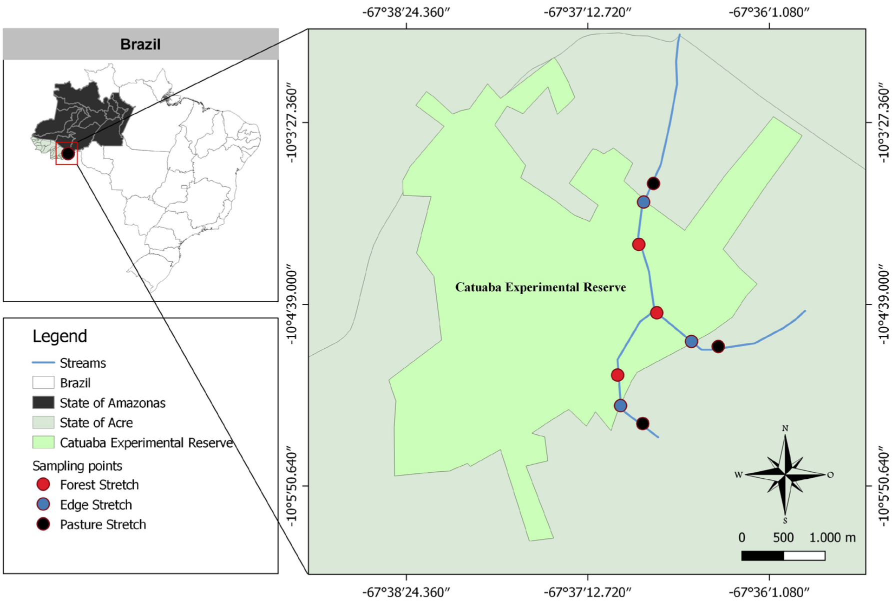

Figure 1. Geographical location of edge, forest, and pasture stretches sampled in three low-order streams of the Catuaba Experimental Reserve, municipality of Senador Guiomard, Acre, Brazil.

11185-1 de 27/10/2007). In seasonal period, each section was sampled in the morning (8:00 a.m.) and afternoon (5:00 p.m.). In each stretch, a course of 100 meters was delimited, where the fish collection was performed using two fishnets (80 cm x 60 cm mesh size), operated by two people simultaneously, during two consecutive hours. Sampling was performed upstream and included different biotopes, such as backwaters, riverbeds, and marginal ponds. A 1.5 mm mesh nylon net was used to isolate the sampled area to minimize the fish escape effect.

The fish collected were immediately fixed in a 10% formaldehyde solution, preserved in 70% alcohol, and then deposited in the Ichthyological Collection of the Federal University of Acre, catalog numbers MUFAC-IC 778 to MUFAC-IC 935. The fish were submitted to biometry and ventral incision for stomach removal and subsequent analysis in the Laboratory of Ichthyology and Aquatic Ecology of the Federal University of Acre.

2.3. Stomach content analysis

Each stomach was dissected in a petri dish under a stereo microscope, with its contents removed and visually separated. We used bibliographic references and taxonomic keys to identify the items found to the lowest possible taxonomic level. After sorting, each food item was deposited into a petri dish with a ruled graph paper attached to the bottom and positioned between two 1 mm high glass coverslips. Another slide was superimposed on the coverslips, allowing us to calculate the volumetric measurement in mm^3 ($1mm^3 = 0.001mL$). In this way, the food item volume was obtained by counting the millimeters occupied by the content. Some items found were kept in large clades due to the high level of material degradation and/or their low representativeness, such as items of plant origin, other algae, adult insects, arachnids, and crustaceans.

2.4. Data analysis

The Alimentary Index (IAi; Kawakami & Vazzoler 1980) was used for the diet analysis, which is calculated through the frequency of occurrence (FO%), i.e., the ratio of the number of times that a given item is present to the total stomachs; and relative volume (V%), i.e., the ratio of the volume of each item to the total volume (Hynes, 1950). This index is used as a final analysis and was proposed because it allows assessing the relative importance of each item (Liedke et al., 2016).

The trophic niche breadth (diet breadth) was calculated for the pasture, edge, and forest areas for each species, using the Levin's standardized index. This index ranges from 0 when species consumed only one type of food, and 1, when species consumed several types of food. The index was calculated using the following formula: $Ba = [(\Sigma jP^2ij)^{-1} - 1] (n - 1)^{-1}$, where: Ba = standard trophic niche breadth; Pij = food item proportion j in the diet of species i; n = total number of food items.

The food overlap was estimated for the forest, edge, and pasture areas for each species, using the Pianka (1973) index. This index was calculated using the following formula: $\alpha = \Sigma U_{1j} \times \Sigma U_{2j} / \Sigma (U_{1j})^2 \times (U_{2j})^2$, where α = niche overlap index; $\mathbf{U_{1j}}$ = use of food resource or foraging environment **j** by species 1; and $\mathbf{U_{2j}}$ = use of food resource or foraging environment **j** by species 2. This index allows the analysis of spatial and dietary overlap between two species, on a scale ranging from 0 to 1. The value 0 indicates that two species are completely dissimilar, while the value 1 refers to total overlap.

The original data matrix was randomized to assess whether the observed overlap pattern differs from a random pattern. Using a null model, the volume ratios of observed food items for each species were randomized 10,000 times, and for each randomization, a Pianka index was calculated. Thus, the statistical significance was determined through the comparison of observed overlap with the distribution of null values, considering a significance level of $\alpha < 0.05$. For this analysis, we used the EcoSim software (Gotelli & Entsminger, 2006).

The matrix of relative volume data obtained from food items found in the stomach of fish species in the pasture, edge, and forest areas, was transformed into a distance matrix using the Bray-Curtis similarity coefficient. PERMANOVA (Permutational Multivariate Analysis of Variance) was applied to the Bray-Curtis similarity matrix to assess differences in the volume of food items consumed between species, in different environments (pasture, edge, and forest) and to assess the difference in the volume of items consumed between environments (pasture, edge, and forest), for each fish species. Bray-Curtis distance matrices together with PERMANOVA were performed using the PRIMER 6 & PERMANOVA software version 6.0 (Anderson et al., 2008).

3. Results

We analyzed the stomachs of 17 *A. tetramerus* individuals from the forest fragment, 41 from the fragment border (edge), and 45 from the pasture fragment. For *A. agassizii*, we analyzed 41 stomachs from individuals from the forest fragment,43 from the fragment border (edge), and 43 from the pasture fragment.

The diet of *A. agassizii* consisted of insect fragments of allochthonous origin, in the forest and edge areas, with an index of alimentary importance above 60%. However, larvae were the most consumed item by this species in pasture environments (58%), followed by insect fragments (37%) (Table 1). Among the larvae consumed by this species, Diptera was the most important group in the three types of environments (pasture, edge, and forest) (Table 1). Despite the presence of plant fragments in some examined stomachs, the species was considered carnivorous in the three environments with a tendency to insectivory.

The diet of *A. tetramerus* mainly consisted of Diptera larvae, but although this item was the most important in the three environments studied, we found an increase in its importance towards the forest → border → pasture, representing 54.1% of the diet in the forest, 55.93% in the border, and 85% in the pasture areas (Table 2). On the other hand, items of allochthonous origin, such as insect fragments, had their importance increased in the opposite direction, corresponding to 5.3% in pasture areas and reaching 42.16% of the diet in forest environments (Table 2).

The high consumption of insect fragments by *A. agassizii* suggests a diet with low niche amplitude in forest environments (Ba = 0.35) and moderate in edge (Ba = 0.50) and pasture environments (Ba = 0.55). *Aequidens tetramerus*, on the other hand, showed moderate niche amplitude in the forest (Ba = 0.57) and edge environments (Ba = 0.55) and lower in pasture environments (Ba = 0.30).

Interspecific food overlap values were considered low in the forest (Ojk = 0.38) (simulated mean = 0.18; p = 0.001) and edge environments (Ojk = 0.41) (simulated mean = 0.40; p = 0.01) and moderate in pasture areas (Ojk = 0.65) (simulated mean = 0.37; p = 0.002). These values were considered significantly higher than expected by chance. Consequently. the observed values could not be generated by chance and, therefore, reflect biological processes.

We found a difference between the volumes of items consumed by *A. agassizii* and *A. tetramerus* between the environments (Pseudo-F = 4.23; p = 0.001). The difference occurred in the forest (p = 0.01) and edge (p = 0.001) environments. The items consumed by *A. agassizii* and *A. tetramerus* individuals varied between the areas of pasture and forest (p = 0.001; p = 0.02, respectively).

4. Discussion

The results of the present study showed the influence of replacing riparian vegetation with pasture in the diet of *Apistogramma agassizii* and *Aequidens tetramerus*. *Apistogramma agassizii*

Table 1. Composition of stomach contents of *Apistogramma agassizii* individuals from the forest, edge, and pasture areas.

Food item	Class	Forest			Edge			Pasture		
		%V	%F	%IAi	%V	%F	%IAi	%V	%F	%IAi
Insecta fragments	Allo	33.12	67.62	**73.78**	38.48	49.34	**65.81**	28.19	33.14	**37.09**
Plant fragments	Allo	3.01	3.05	0.30	1.09	1.54	0.06	5.20	2.29	0.47
Arachnida	Allo	1.55	1.63	0.08	1.88	1.54	0.10	1.60	3.43	0.22
Hymenoptera (adult)	Allo	0.00	0.00	0.00	0.68	1.54	0.04	2.35	1.43	0.13
Bacillariophyceae	Allo	0.02	0.20	0.00	0.35	0.88	0.01	0.35	3.71	0.05
Thin particle matter	Allo	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.29	0.00
Diptera larvae	Aut	41.1	17.52	**23.74**	37.60	24.56	**32.01**	43.05	34.00	**58.11**
Coleoptera larvae	Aut	4.59	2.85	0.43	5.85	2.41	0.49	8.38	6.86	2.28
Nematoda	Aut	0.31	0.61	0.01	3.66	4.17	0.53	4.76	4.29	0.81
Crustacea	Aut	10.30	4.68	1.59	2.36	5.26	0.43	3.23	5.71	0.73
Trichoptera larvae	Aut	0.90	0.61	0.002	2.30	1.10	0.09	0.67	1.14	0.03
Ephemeroptera larvae	Aut	0.00	0.00	0.00	0.33	0.88	0.01	0.52	1.43	0.03
Odonata larvae	Aut	0.70	0.41	0.00	2.46	3.51	0.30	1.03	0.29	0.01
Chlorophyceae	Aut	0.00	0.00	0.00	0.53	0.66	0.01	0.32	1.14	0.01
Rotifera	Aut	4.00	0.20	0.03	1.42	2.19	0.11	0.00	0.00	0.00
Algae	Aut	0.83	0.61	0.02	1.01	0.44	0.02	0.11	0.86	0.00

%F = frequency of occurrence, %V = volume, and % IAi = alimentary index. Allo=Allochthonous, Aut=Autochthonous.

Table 2. Composition of stomach contents of *Aequidens tetramerus* individuals from the forest, edge, and pasture areas.

Food item	Class	Forest			Edge			Pasture		
		%V	%F	%IAi	%V	%F	%IAi	%V	%F	%IAi
Insecta fragments	Allo	42.52	27.78	**42.16**	30.31	24.34	**35.45**	11.86	11.80	**5.03**
Hymenoptera (adult)	Allo	0.00	0.00	0.00	2.17	2.05	0.23	6.38	4.55	1.05
Arachnida	Allo	10.82	3.70	1.43	4.30	2.05	0.45	1.45	1.66	0.09
Plant fragments	Allo	0.87	1.85	0.06	6.84	2.93	1.02	6.95	3.73	0.93
Coleoptera larvae	Aut	6.97	3.70	0.92	12.33	7.62	4.77	9.58	11.80	4.07
Diptera larvae	Aut	32.73	46.30	**54.10**	26.64	39.88	**55.93**	44.04	55.28	**87.59**
Nematoda	Aut	0.00	0.00	0.00	0.89	2.64	0.12	5.21	2.48	0.47
Odonata larvae	Aut	0.00	0.00	0.00	1.77	1.17	0.11	3.72	2.28	0.31
Thin particle matter	Aut	0.00	0.00	0.00	2.23	1.76	0.20	5.42	1.24	0.24
Crustacea	Aut	0.00	0.00	0.00	3.57	4.99	0.90	1.46	1.66	0.09
Algae	Aut	0.00	0.00	0.00	0.70	1.17	0.04	1.52	1.24	0.07
Chlorophyceae	Aut	0.00	0.00	0.00	3.71	0.59	0.11	1.77	0.83	0.05
Trichoptera larvae	Aut	4.11	1.85	0.27	2.07	1.76	0.19	0.42	0.83	0.01
Ephemeroptera larvae	Aut	0.00	0.00	0.00	0.82	0.59	0.02	0.23	0.62	0.01
Bacillariophyceae	Aut	1.99	14.81	1.05	1.56	5.87	0.46	0.00	0.00	0.00
Rotifera	Aut	0.00	0.00	0.00	0.09	0.59	0.00	0.00	0.00	0.00

%F = frequency of occurrence, %V = volume, and %IAi = alimentary importance index. Allo=Allochthonous, Aut=Autochthonous.

maintained the preference for consumption of insects of allochthonous origin in forested areas, where the importance was decreasing according to the change in riparian vegetation in the forest, edge, and pasture direction. Thus, in pasture areas, the feeding preference of this species was for items of autochthonous origin such as Diptera larvae. However, *A. tetramerus* maintained a preference for insect larvae in the three environments. The consumption of allochthonous insects by this species varied, where the highest consumption occurred within the fragment and the lowest in pasture areas, showing a relationship between the use of allochthonous food and the type of marginal vegetation. These results corroborate some studies that found a direct relationship between riparian vegetation and the presence of allochthonous material (e.g., adult insects) in the fish diet, as well as the increase in consumption of items of autochthonous origin in fish from deforested areas (Chan et al., 2008; Schneider et al., 2011; Ferreira et al., 2012).

In areas with preserved vegetation such as forest environments, the present study showed a difference in prey consumption between *A. agassizii* and *A. tetramerus*, which decreased in pasture areas. We observed a similarity in the consumption of insect larvae by the species, mainly Diptera, with the change of the surrounding vegetation. According to Zeni & Casatti (2014), in streams with homogeneous vegetation, such as pasture areas, there was an increase in the consumption of Diptera larvae by the fish assemblage. This order of aquatic insects is among the aquatic invertebrates most consumed by fish, which is associated with the high abundance of this group in many environments including those impacted (Delariva et al., 2011; Souza & Lima-Junior, 2013). They have adaptations that allow them to become the first colonists in degraded areas (Rossaro, 1991), which may justify the high volume of these larvae in the stomachs of species.

Despite the variety of food items consumed in stream environments, the trophic niche amplitude values were, in general, low or moderate, due to the dominance of few items in the diet of species. The more restricted range of *A. agassizii* is associated with the high consumption of adult insects in forest environments. However, this fact does not suggest a specialization for these items of allochthonous origin for this species, because as the riparian vegetation has changed, there has been a moderate expansion of the trophic niche towards the consumption of other items, mainly insect larvae. In this sense, we suggest that *A. agassizii* prefers to consume more energetically favorable items in forest environments, which only preserved environments can offer due to the variety of allochthonous items provided by the riparian vegetation (Ceneviva-Bastos & Casatti, 2007). However, in pasture areas, they end up consuming items of greater predominance, such as Diptera larvae. According to Callisto et al. (2001), degraded sites generally have low species diversity and high density of organisms, and are restricted to more tolerant groups, such as Diptera larvae. That is, in preserved areas, this species may be consuming higher-quality items, and with the degradation of the environment, they end up opting for higher-quantity items instead.

In contrast, *A. tetramerus* showed a moderate niche range in the edge and forest environments, and narrower in the pasture environments, due to the predominance of insect larvae in their diet. This may be associated with the feeding behavior of this species, which forages on the substrate (Bührnheim 2002). This behavior facilitates the ingestion of autochthonous items, unlike other fish species that swim and eat in the water column, ingesting a large number of allochthonous items (Mazzoni & Iglesias-Rios, 2002). In this regard, the feeding behavior and the abundance of larvae influenced the trophic niche reduction of this species in the environments.

We found a high trophic niche overlap between *A. agassizii* and *A. tetramerus* in pasture areas. However, we found a difference in the consumption of food items and a low niche overlap between species in forest environments. Thus, the results suggest that the change of riparian vegetation homogenized the species' diet. According to Casatti et al. (2006) and Casatti et al. (2012), anthropized areas have a small supply of food items since the diet reflects what is available in the environment (Wootton, 1990). Diptera larvae were abundant in the stomachs of the species then we can suggest that these food items were available in great abundance in pasture environments. Thus, justifying this food resource being shared by species in pasture areas.

In conclusion, we show that the allochthonous items indicate the conservation of riparian vegetation and are relevant for both species, mainly *A. agassizii*. However, these items are being replaced by autochthonous items in the diet of *A. agassizii*, such as insect larvae, which are also and becoming predominant in the diet of *A. tetramerus* as the riparian vegetation changes. In this sense, the present study reinforces the importance of conserving the riparian vegetation of small streams in the Amazon and demonstrates the importance of evaluating the diet as a tool to verify the influence of anthropic actions on aquatic organisms. Accordingly, our results reinforce the importance of maintaining and expanding forested areas in small water bodies, and assists in the implementation of public policies aimed at recovering this basin, so that the water does not become even scarcer and the aquatic environments lose environmental quality.

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