The seasonal diet of *Aequidens tetramerus* (Cichlidae) in a small forest stream in the Machado River basin, Rondônia, Brazil

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ABSTRACT

Trophic relationships in fish communities are affected by the availability of resources, which in turn is affected by spatial and temporal variations throughout the year. The aims of this study were to characterize the diet of *A. tetramerus* in a streamlet in the north of Brazil and compare its composition in different hydrological seasons (wet and dry seasons). Collections were performed every two months from October 2011 to September 2012 with the aid of seine nets, hand net and fishing traps in the streamlet located in the Machado River drainage basin in the Rondônia state. Most of the specimens collected were quite small (< 40 mm) and had empty stomachs. Our results showed that *A. tetramerus* feeds on a wide variety of items of plant origin, such as algae, seeds and leaves, as well as items of animal origin, including bryozoans, crustaceans, fish scales, terrestrial insects and detritus. The data also indicated higher consumption of aquatic insects than other food items, suggesting a primarily insect-based diet. Items of plant and allochthonous origin were consumed more in the wet season than in the dry season, but there were no seasonal differences in the consumption of animal and autochthonous items.

KEYWORDS: Fish, Perciformes, Amazon, feeding habits, ornamental fish.

* Dieta sazonal de *Aequidens tetramerus* (Cichlidae) em um igarapé da bacia do rio Machado, Rondônia, Brasil

RESUMO

Relações tróficas das comunidades de peixes são afetadas por variações espaciais e temporais, que alteraram a disponibilidade de recursos ao longo do ano. Os objetivos deste estudo foram caracterizar a dieta de *A. tetramerus* no igarapé estudado, bem como comparar se a composição dos itens da dieta desta espécie dependem da estação hidrológica (período chuvoso e seco). As coletas foram realizadas bimestralmente, de Outubro de 2011 a Setembro de 2012, com auxílio de picaré, puçá e covos em um igarapé localizado na bacia do rio Machado no estado de Rondônia. A maioria dos espécimes eram pequenos (< 40 mm) e tinham estômago vazio. Observou-se que *A. tetramerus* se alimenta de uma grande variedade de itens de origem vegetal, como algas, sementes e folhas; e de itens de origem animal como: briozoários, crustáceos, escamas, insetos terrestres e detritos. Constatou-se que há maior consumo de insetos aquáticos, o que sugere uma dieta insetívora. Itens de origem vegetal e alóctones foram mais consumidos na estação chuvosa do que na estação seca. Não houve diferença na composição da dieta dos peixes entre as estações de seca e chuva.

PALAVRAS-CHAVE: Peixe, Perciformes, Amazônia, hábitos alimentares, peixe ornamental.
INTRODUCTION

One approach for evaluating interactive processes in aquatic communities that yields consistent results is to investigate the diet of fishes (Reis and Santos 2014), whose feeding habits can be influenced by environmental conditions, the biological characteristics of the species (e.g., development of individuals) (Abelha et al. 2001) and spatial and temporal variations (Ximenes et al. 2011).

Small forest streams (regionally known as igarapé) are watercourses that are poor in nutrients and have low primary production (Mendonça et al. 2008). Food chains in these watercourses therefore depend on allochthonous matter from nearby forests (Abilhoa 2009). A further characteristic of these streams is that the water level is subject to frequent, rapid flood pulses (Carvalho et al. 2013). After the onset of heavy rains, the water level rises, often overflowing and reaching the surrounding valley (Espírito-Santo et al. 2009).

Trophic relationships in fish communities are affected by spatial and temporal variations (Ferreira et al. 2014), as these affect the qualitative and quantitative availability of resources throughout the year (Ximenes et al. 2011). Several studies have shown that fish select food resources based on their availability in the environment (e.g., Gonçalves et al. 2013; Nogueira and Costa 2014).

Trophic ecology studies have demonstrated that most teleost fishes exhibit considerable feeding versatility (Abelha et al. 2001). However, South American cichlids appear not to have a great variety of feeding tactics; some of these tactics (e.g., plowing sandy and rocky substrate and preying on invertebrates in macrophytes and the water column) may be related to certain types of microhabitat occupancy (Sampaio and Goulart 2011), similarly to the tactics of Aequidens tetramerus, the species studied here.

Aequidens tetramerus (Heckel, 1840), also known as “saddle cichlid”, is a native neotropical fish (Moreira and Zuanon 2002) of ornamental importance (Regulatory Directive 001/2012-Mapa/Mma) that has a small body and short snout (Montaña and Winemiller 2013) and is coded as follows: 0 (empty), 1 (volume < 25%), 2 (25% - 75%) and 3 (75% - 100%). The frequency (F%i) and volume (V%i) methods were used to analyze stomach contents (Hyslop 1980). The volume of each item was obtained using a petri dish with a millimeter scale and was then converted into milliliters (Hellawell and Hamada et al. 2014) using a stereoscopic microscope. Repletion degree (RD) was determined according to the scale proposed by Hahn et al. (1999) and was coded as follows: 0 (empty), 1 (volume < 25%), 2 (25% - 75%) and 3 (75% - 100%). The frequency of occurrence (F%i) and volume (V%i) methods were used to analyze stomach contents (Hyslop 1980). The volume of each item was obtained using a petri dish with a millimeter scale and was then converted into milliliters (Hellawell and Abel 1971). These data were combined in an alimentary index (IAi) proposed by Kawakami and Vazzoler (1980). The index is given by the equation IAi = (F%i * V%i / Σ F%i * V%i) * 100, where

MATERIALS AND METHODS

Study Area

The streamlet studied here is known as the do Noive streamlet (61°51'24"S; 11°15'31"W) and is located in the Machado River drainage basin in the east of the state of Rondônia (Figure 1). The temperature varies between 19 and 33 °C, and annual precipitation is about 2,500 mm (Krusche et al. 2005). The dry season extends from April to September, and the wet season from October to March (SedaM 2010). The streamlet is a lower (first) order one, with a sandy substratum, pastures in the adjacent matrix and a small amount of riparian vegetation consisting of grass and small aquatic plants. In the dry season the mean depth was 100 ± 0.1 cm, mean width 60 ± 0.1cm, mean water speed 0.1 ± 0.0 m s⁻¹ and mean flow 1.2 ± 0.1 m³ s⁻¹, while in the wet season the corresponding figures were 150 ± 0.1 cm, 120 ± 0.5 cm, 0.2 ± 0.0 m s⁻¹ and 1.3 ± 0.2 m³ s⁻¹.

Sampling and data analysis

Samples were collected every two months from October 2011 to September 2012 (totaling three samples for each season) along a 50 m stretch of the streamlet. Seine nets (picaré) measuring 2.0 x 1.20 m with a mesh size of 2 mm and a hand net (puçã) with a 0.46 x 0.33 m opening and a mesh size of 2 mm were used simultaneously along the whole stretch for one hour. In addition, ten fishing traps (covos) made with 2 L PET bottles containing ground corn bait were placed 20 m upstream from the area where active collections were made. The bottles were placed 2 m apart along the whole stretch, with the opening facing downstream. They were left for 24 h and checked every 4 h. Before sampling, each end of the 50 m sampling site was blocked with small-mesh nets (5 mm between opposite knots) to prevent fishes from escaping. Each specimen collected was fixed in 10% formalin and preserved in 70% ethanol.

The standard length (SL) of each specimen was measured to identify length classes (LCs). Stomach contents were then stored in 70% alcohol, and food items were identified down to the lowest taxonomic level with the aid of Hamada and Ferreira-Keppler (2012) and Hamada et al. (2014) using a stereoscopic microscope. Repletion degree (RD) was determined according to the scale proposed by Hahn et al. (1999) and was coded as follows: 0 (empty), 1 (volume < 25%), 2 (25% - 75%) and 3 (75% - 100%). The frequency of occurrence (F%i) and volume (V%i) methods were used to analyze stomach contents (Hyslop 1980). The volume of each item was obtained using a petri dish with a millimeter scale and was then converted into milliliters (Hellawell and Abel 1971). These data were combined in an alimentary index (IAi) proposed by Kawakami and Vazzoler (1980). The index is given by the equation IAi = (F%i * V%i / Σ F%i * V%i) * 100, where
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\[ i = 1, 2, \ldots, n \] food items; \( F_i \) = frequency of occurrence of a given food item; and \( V_i \) = volume of a given food item. Food items were grouped according to type (animal or plant) and origin (autochthonous or allochthonous).

The Kolmogorov-Smirnov test of normality and Levene’s test of homoscedasticity were used to determine whether parametric ANOVA or the non-parametric Kruskal-Wallis test should be used to test for differences between LC (length classes) and RD (repletion degree) (Zar 1999). Chi squared tests \( (\chi^2) \) were applied to data that had a normal distribution and exhibited homoscedasticity to detect seasonal differences (dry versus wet season) in absolute frequency of LC, RD and type and origin of food items (Zar 1999).

Non-metric multidimensional scaling analysis (nMDS) was used to examine temporal variation in diet using the total volume values of each item. The analyses of variance (ANOVA and Kruskal-Wallis) and \( \chi^2 \) tests were performed with Statistica 7.1 (StatSoft 2005). NMDS was carried out using the PAST package (version 2.1.7) (Hammer et al. 2001). Results were considered significant when \( p \leq 0.05 \).

**Figure 1.** Map of the Machado River basin with the study sites indicated (black circle).
RESULTS

A total of 59 specimens were analyzed; 25 were collected in the wet season and 34 in the dry season. Most of the specimens were longer than 10 mm, and the length class with the greatest absolute frequency was LC 10-20 mm (n = 22), followed by LC 21-30 mm (n = 19), LC 31-40 mm (n = 9) and LC 41-50 mm (n = 5) (Figure 2). No significant differences were found between the length classes analyzed (ANOVA, F = 4.54; p = 0.02; df = 1). Only the absolute frequency of smaller individuals (10-20 mm) differed significantly in the dry season (n = 19) and wet season (n = 3) (χ² = 11.6; p < 0.05).

No significant differences were found between the values of repletion degree analyzed (ANOVA, F = 2.36; p = 0.20; df = 1) or between these values for the two seasons (Table 1).

*Aequidens tetramerus* ingested a wide variety of food items, including material of plant origin, such as algae, seeds and leaves; material of animal origin, such as bryozoans, crustaceans, scales and terrestrial and aquatic insects at different stages of the life cycle; and undefined matter (detritus/mud). Fragments of aquatic insects were the most consumed item in both seasons (dry, IAᵢ = 52.76%; wet, IAᵢ = 38.47%), followed by trichopterans (IAᵢ = 18.70%), seeds (IAᵢ = 12.68%) and plant matter (IAᵢ = 12.16%) in the wet season and detritus (IAᵢ = 26.16%) in the dry season (Table 2).

Items of plant origin were consumed more in the wet season than in the dry season (χ² = 19.0, p < 0.05). There were no significant differences between seasons for food items of animal origin (χ² = 3.0, p > 0.05). While allochthonous items were consumed more in the wet season than in the dry season (χ² = 16.0, p < 0.05), there were no significant differences between seasons for autochthonous items (χ² = 3.4, p > 0.05). Food items of animal origin were more abundant than items of plant origin in the wet season (χ² = 25.0, p < 0.05) and dry season (χ² = 92.0, p < 0.05). Autochthonous items were consumed more than allochthonous items in both seasons (wet: χ² = 17.6, p < 0.05; dry: χ² = 81.0, p < 0.05).

![Figure 2. Absolute frequency (n) of *A. tetramerus* specimens from the do Nove streamlet in each standard length (SL) class.](image-url)

nMDS analysis based on the total volume of each item of animal (stress = 0.47), plant (stress = 0.47), autochthonous (stress = 0.55) and allochthonous origin (stress = 0.46) in each season failed to show any separation on axis 1 and 2 retained for interpretation (Figure 3).

Table 1. Absolute frequency of *A. tetramerus* specimens from the do Nove streamlet for each stomach repletion degree class. Repletion degree 6 (RD 6 = empty), repletion degree 1 (RD 1 = < 25%), repletion degree 2 (RD 2 = 25% - 75%) and repletion degree 3 (RD 3 = 75% - 100%); χ² = values of the chi-square test; p = significance.

<table>
<thead>
<tr>
<th>Repletion degree</th>
<th>Absolute frequency</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet season</td>
<td>Dry season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RD 0</td>
<td>9</td>
<td>11</td>
<td>0.20</td>
</tr>
<tr>
<td>RD 1</td>
<td>8</td>
<td>9</td>
<td>0.52</td>
</tr>
<tr>
<td>RD 2</td>
<td>4</td>
<td>8</td>
<td>1.33</td>
</tr>
<tr>
<td>RD 3</td>
<td>4</td>
<td>6</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Table 2. Percentage composition by volume (V%), occurrence (Fi) and alimentary index (IAᵢ) of each food item identified in the diet of *A. tetramerus* during the wet and dry seasons in the do Nove streamlet in the Machado River basin, Rondônia. Allochthonous items*: autochthonous items**.

<table>
<thead>
<tr>
<th>Food item</th>
<th>Wet season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V%</td>
<td>F%</td>
</tr>
<tr>
<td>Plant origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filamentous algae**</td>
<td>3.49</td>
<td>1.54</td>
</tr>
<tr>
<td>Algae (Bacillariophyceae)**</td>
<td>0.04</td>
<td>2.38</td>
</tr>
<tr>
<td>Seeds*</td>
<td>19.50</td>
<td>7.14</td>
</tr>
<tr>
<td>Plant matter (leaves and twigs)*</td>
<td>11.21</td>
<td>11.90</td>
</tr>
<tr>
<td>Animal origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bryozoa**</td>
<td>3.71</td>
<td>11.90</td>
</tr>
<tr>
<td>Diptera*</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ceratopogonidae**</td>
<td>2.32</td>
<td>7.14</td>
</tr>
<tr>
<td>Chironomidae**</td>
<td>6.86</td>
<td>7.14</td>
</tr>
<tr>
<td>Coleoptera*</td>
<td>8.43</td>
<td>4.76</td>
</tr>
<tr>
<td>Hymenoptera*</td>
<td>3.33</td>
<td>3.08</td>
</tr>
<tr>
<td>Lepidoptera*</td>
<td>0.42</td>
<td>1.54</td>
</tr>
<tr>
<td>Plecoptera**</td>
<td>1.07</td>
<td>2.38</td>
</tr>
<tr>
<td>Trichoptera**</td>
<td>17.25</td>
<td>11.90</td>
</tr>
<tr>
<td>Cladocera**</td>
<td>0.11</td>
<td>2.38</td>
</tr>
<tr>
<td>Copepod**</td>
<td>0.93</td>
<td>1.54</td>
</tr>
<tr>
<td>Fragments of aquatic insects**</td>
<td>22.18</td>
<td>19.05</td>
</tr>
<tr>
<td>Scales**</td>
<td>3.82</td>
<td>9.52</td>
</tr>
</tbody>
</table>

Unidentified Material

| Detritus/mud**              | 3.50| 2.38| 0.76 |

| Detritus/mud**              | 26.95| 10.77| 26.16|
Streamlets in mainland forests are important components of the Amazon fluvial system as they shelter many fish species (Casatti et al. 2013), particularly small species with a total length of less than 100 mm (Bührnheim 2002). The family Cichlidae includes approximately 105 genera, with 1,300 species of freshwater and brackish water fish described, and is considered one of the largest families of fish although Kullander (2003) believes the number of species has probably been underestimated. It is also considered one of the most specious and common families in streamlets in the Amazon Forest (Mendonça et al. 2008; Casatti et al. 2013).

In contrast to our results, Soares (1979) found greater abundance of *A. tetramerus* in LC > 40 mm. This difference is probably related to the fact that the streams studied by Soares were larger (higher order, with a mean width of 260 ± 29 cm) than the streams in the present study. According to Bührnheim (2002), most of the fish species in mainland streams are small and use shallow habitats with large marginal areas as shelter against predators. Juveniles of *Aequidens* sp. measuring less than 30 mm in length tend to remain in groups in the middle of the vegetation in shallow waters under parental care (Goldstein 1973).

According to Benitez and Suárez (2009), hydrological differences, such as flow variability and the physical and chemical characteristics of the water, affect the population characteristics of the species. We infer that the lower abundance of small individuals in the rainy season can be explained by the greater dispersion of individuals to marginal environments, where there are rooted emergent macrophytes, and to the increase in channel depth, which provides a greater...
number of habitats and resources to be exploited, making the fish less susceptible to capture by our active fishing equipment.

A study by Soares (1979) carried out in a streamlet in the Brazilian state of Mato Grosso describes a high frequency of empty stomachs (50%) for *A. tetramerus*. Although we found no differences between the seasons for repletion degree in our study, we observed a lower frequency of individuals with full stomachs than Soares. According to Moreira and Zuanon (2002), this indicates that the feeding strategy used by cichlids is based on constant foraging during the day, making it unusual to find a completely full stomach.

Changes in fish diet may be determined by seasonal conditions, as different sites and periods have different abiotic conditions and also offer other feeding opportunities (Gandini *et al.* 2012), creating periods of higher or lower feeding intensity or efficiency. No seasonal differences were observed in stomach fullness, which was correlated with the high occurrence and prevalence of fragments of aquatic insects in the stomach of *A. tetramerus*. This can be explained by the fact that this resource is widely available all year round (Sonoda 2010).

Some studies report that the genus *Aequidens* may have either omnivorous or malacophagous habits (Ximenes *et al.* 2011; Montaña and Winemiller 2013). The existence of different trophic guilds probably reflects differences in physical environment and resource availability, as well as morphological variations (Gonçalves *et al.* 2013; Nogueira and Costa 2014).

According to Agostinho *et al.* (2007), the omnivorous habits reported for *Aequidens* sp. are an important strategy in altered environments for increasing energy acquisition according to the availability and quality of food. It has repeatedly been mentioned in the literature that animal items such as aquatic larvae and aquatic and terrestrial adult insects of autochthonous or allochthonous origin are important elements in food chains in streams and an important food source for stream-dwelling fishes (Carvalho *et al.* 2007; Nogueira and Costa 2014). The food chain in streams seems to depend essentially on items of allochthonous origin, as aquatic insects depend on organic matter from riparian forest (Horn 1998), such as aquatic insects that have a chitin exoskeleton (Sampaio and Goulart 2011).

Digestive process, and their function is to crush and grind hard elements in food chains in streams and an important food source for stream-dwelling fishes (Carvalho *et al.* 2007; Nogueira and Costa 2014). The food chain in streams seems to depend essentially on items of allochthonous origin, as aquatic insects depend on organic matter from riparian forest (Horn 1998), such as aquatic insects that have a chitin exoskeleton (Sampaio and Goulart 2011).

Pharyngeal teeth, which are located behind the oral cavity and are functionally dissociated from the jaws, exhibit major diversification of form and function (Meyer 1993). They are important in the initial stage of the digestive process, and their function is to crush and grind hard prey (Horn 1998), such as aquatic insects that have a chitin exoskeleton (Sampaio and Goulart 2011).

**CONCLUSION**

The species *A. tetramerus* has insectivorous feeding habits, and autochthonous insects constitute a large part of its diet, 2007). They also consume aquatic invertebrates and detritus. The low occurrence of allochthonous items registered in stomachs during the dry season supports the hypothesis that allochthonous input in tropical ecosystems varies seasonally and is scarce during the dry season (Lowe-McConnell 1999).

Our results confirm those of Rezende and Mazzoni (2005), who found a predominance of autochthonous prey in stretches with a more open canopy predominantly during the dry season. The fact that many species are restricted to areas with vegetation cover has been attributed to the greater amount of allochthonous matter, particularly terrestrial insects, in these areas than in areas without vegetation cover (Mazzoni and Iglesias-Ríos 2002). Thus, the high consumption of autochthonous items by *A. tetramerus* may also be related to the reduced vegetation cover in the streamlet analyzed.

*Aequidens tetramerus* consumes predominantly food of animal origin (Soares 1979). The predominance of autochthonous items of animal origin in the stomachs of individuals from this species observed here is in accordance with the fact that autochthonous prey predominates in environments without vegetation cover (Silva *et al.* 2014) and the behavioral, morphological and feeding characteristics of genus *Aequidens*, such as greater activity during the day (Lowe-McConnell 1999), an improved visual system (Soares 1979) and foraging based on exploration of the substrate (Bührnheim 2002). The feeding behavior of *A. tetramerus* facilitates ingestion of autochthonous items, unlike that of other stream-dwelling fish species that swim and feed in midwater, ingesting large amounts of allochthonous items (Mazzoni and Iglesias-Ríos 2002).

The high diversity of Cichlidae has been explained by the versatile design of the pharyngeal dentigerous plates used for grinding food (Kullander 2003), as well as the shape and orientation of the mouth, the development of the lips and the capacity for jaw protrusion, reflecting where these fishes capture their food (at the surface, bottom or middle of the water column) (Sampaio and Goulart 2011). This is the case with *Crenicichla britskii*, which has its mouth in the terminal position and is classified as a generalist insectivore, consuming mainly immature insects in autochthonous material (Casatti 2002). In cichlids, the pharyngeal teeth, which are located behind the oral cavity and are functionally dissociated from the jaws, exhibit major diversification of form and function (Meyer 1993). They are important in the initial stage of the digestive process, and their function is to crush and grind hard prey (Horn 1998), such as aquatic insects that have a chitin exoskeleton (Sampaio and Goulart 2011).
possibly because autochthonous animal items are of greater importance in fishes’ diets. There was no evidence of seasonal changes in the diet of *A. tetramerus* except for items consumed less frequently, probably because of the constant availability of food and the broad spectrum of food consumed by this species.

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