Gut content analysis confirms the feeding plasticity of a generalist fish species in a tropical river

Análise do conteúdo estomacal confirma a plasticidade alimentar de uma espécie de peixe generalista em um rio tropical

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**Abstract:** Aim: In this paper, we compared the diet composition of the South American silver croaker, *Plagioscion squamosissimus* in preserved and impacted areas (agrarian land use) of an Amazonian river. Our objective was to quantify the plasticity in diet across different habitats and evaluate the importance of a carnivorous generalist species as an environmental indicator based on its feeding variation. Methods: We analysed the stomach contents of 135 individuals and compared the trophic level of *P*. *squamosissimus* and the source of ingested food items in the impacted and non-impacted habitats. Results: The trophic level values in both areas were similar. In both areas, *P*. *squamosissimus* used a wide variety of food items, consuming mainly fish and invertebrates of autochthonous origin. However, in terms of composition of food items, small pelagic fish and autochthonous items were more frequently consumed in the preserved area, while in the impacted area fish and benthic invertebrates were predominant in the diet. Conclusions: Our gut analysis suggests plasticity in *P squamosissimus* diet across varying areas, which point to the ability of *P*. *squamosissimus* to modify their diet in the impacted situation, utilizing more benthic material on impacted area in order to maintain a similar trophic position.

**Keywords:** carnivorous; diet plasticity; trophic ecology; diet composition; Machado River (Brazil).

**Resumo:** Objetivo: Neste artigo, comparamos a composição da dieta da pescada branca *Plagioscion squamosissimus* em áreas preservadas e impactadas (área agrária) de um rio amazônico, a fim de quantificar a plasticidade trófica em diferentes habitats e avaliar a importância de uma espécie carnívora generalista como um indicador ambiental baseado em sua variação alimentar. Métodos: Analisamos o conteúdo estomacal de 135 indivíduos e compararamos o nível trófico de *P. squamosissimus* e a origem dos itens alimentares nos habitats impactados e preservados. Resultados: Os valores do nível trófico em ambas as áreas foram semelhantes. Nas duas áreas, *P. squamosissimus* utilizou uma grande variedade de itens alimentares, consumindo principalmente peixes e invertebrados de origem autóctone. No entanto, em relação à composição dos itens alimentares, pequenos peixes pelágicos e itens autóctones foram consumidos com maior frequência na área preservada, enquanto na área impactada predominaram os peixes e invertebrados bentônicos na dieta. Conclusões: Nossa análise...
intestinal sugere a plasticidade na dieta de *P. squamosissimus* em diferentes áreas, sendo útil apontar a capacidade de *P. squamosissimus* de modificar sua dieta na situação impactada, utilizando mais material bentônico na área impactada, a fim de manter uma posição trófica semelhante.

**Palavras-chave:** carnívoro; plasticidade alimentar; ecologia trófica; composição da dieta; rio Machado (Brasil).

### 1. Introduction

Deforestation is one of the major impacts in the tropical forest, affecting terrestrial and aquatic environments, changing the relations among organisms (Lorion & Kennedy, 2009; Frederico et al., 2016) and the structure and composition of aquatic communities (Schneider & Winemiller, 2008; Carvalho & Tejerina-Garro, 2015). In this modified scenario, species’ ability to change the use of preferred food items is key for determining which species will remain in the ecosystems (Zeni et al., 2019).

Many species characteristics could influence their ability to change diet in new habitat conditions, as body size (Van der Lee & Koops, 2015), dispersal mechanisms (Gubiani et al., 2007), and feeding behavior (Bornatowski et al., 2014). Accordingly, generalist species have a large dietary breadth and can restructure food webs affected by different environmental impacts (Bartley et al., 2019).

Modifications in fish diets can be caused by spatial, seasonal and human changes in habitats, which influence abiotic conditions (e.g. oxygen concentration, temperature, pH) and food availability (Whitehouse et al., 2016). Generalist carnivorous species have large feeding spectrum, consuming different food resources that are appropriate for their morphology, feeding behavior and digestive capacity (Bennemann et al., 2000; Neves et al., 2015).

Fish diets may also be influenced by the interface between land and river (riparian zone) in a watershed and are important for the exchange of organic matter between terrestrial and aquatic ecosystems (Pusey & Arthington, 2003; Manna et al., 2012; Casatti et al., 2012; Zeni & Casatti, 2014). Allochthonous organic matter, such as leaves and branches, are consumed directly by benthic macroinvertebrates (Rosi-Marshall et al., 2016) and serves as substrate for the development of microorganisms that are consumed by aquatic invertebrates and fish (Ferreira et al., 2012).

Most teleost fishes in tropical rivers have notable versatility in their feeding habits (Moyle & Cech, 2004), which is a remarkable aspect of tropical rivers (Lowe-McConnell, 1999; Correa & Winemiller, 2014). The South American silver croaker, *Plagioscion squamosissimus* (Heckel, 1840), is a sedentary fish native to the Amazonian region (Santos et al., 2006) and is considered a valuable resource for human consumption and recreational fishing (Barros et al., 2012). In several environments and biomes (Hahn et al., 1999; Bennemann et al., 2000, 2006; Santos et al., 2016), *P. squamosissimus* is considered piscivorous (e.g. large rivers) (Hahn et al., 1997) or generalist carnivore (e.g. reservoirs) (Rocha et al., 2015). Due to its wide geographical distribution, abundance and tolerance to environmental stress in dammed river systems, *P. squamosissimus* could help as aquatic bioindicators in tropical and subtropical aquatic environments (Wunderlich et al., 2015).

To estimate the trophic position of this species, gut content analysis remains a useful method in order to comprehend the qualitative and quantitative diet of fish species, providing indicators of feeding habits (Baker et al., 2014). In this article, we hypothesized that there are differences in food resources consumed by *Plagioscion squamosissimus* (a predator) between a protected area (Biological Reserve of Jaru) and an unprotected area of the Machado River (Amazon Forest), reflecting trophic interactions in both food chains.

### 2. Material and Methods

#### 2.1. Study area

The study was carried out in the Machado River (commonly referred to as Ji-Paraná River) basin, which covers 75,400 km² in the State of Rondônia, Brazil (Figure 1 and 2).

This seventh-order river annually drains about 700 m³.s⁻¹ into the middle course of the Madeira River (Krusche et al., 2005). The Machado River has large individual rocks, rocky portions, as well as trunks and branches observed during the low water period with low sediment loads, typifying it as a clear-water Amazonian river (Goulding et al., 2003). The Machado River runs through the Jaru Biological Reserve (ReBio Jaru, Figure 1), which has a total area of 47,733 km² (Brasil, 2010), with preserved riparian zone covered by ombrophalous
Gut content analysis confirms...

Figure 1. Machado River and sampling sites (See details in Figure 2), Rondônia State = black square, Brazil.
forest that is mainly open and has low floristic variations (IBGE, 1992).

The preserved area has a riverine zone constituted of shrubby and woody angiosperms that establish a primary forest (2c), which could be considered reproduction hotspot and feeding for fish species, where fishing is prohibited (ICMBIO, 2010). The impacted area (poção site = ~ 50 km and São Sebastião site = ~ 5 km upstream of the ReBio Jaru) is composed of pasture, with a riverine zone on the right bank composed of grasses, and a narrow strip with a few woody angiosperms and stretches of bare bushes on the left bank (Figure 2f). In this area there is constant sand dredging (Figure 2e) and fishing activities (artisanal and recreational; Figure 2d).

2.2. Fish sampling

Samplings were performed bimonthly from June 2013 to March 2015 at five locations, three within the preserved area in ReBio Jaru (Carmita, Farofo and Suretama), and two (São Sebastião and Poção) in the impacted area (Figure 1).

Eight sets of gillnets (2 × 20m with mesh sizes varying from 30 to 100 mm) were used. Sampling effort was standardized, and scientific fishing was carried out for 24 hours continuously at each sampling site. A specimen was fixed in 10% formalin and preserved in 70% ethanol. Subsequently, this specimen was deposited in the Ichthyology collection at the Universidade Federal de Rondônia (voucher specimens: UFRO-ICT 023107).

Figure 2. Sampling sites in the Machado River Basin, Rondônia, Brazil: (A) Carmita; (B) Farofo; (C) Suretama (preserved area); (D) São Sebastião, fishing activities; (E) sand dredging activity; and (F) Poção, pasture, few woody angiosperms and stretches of bare bushes, degraded local banks and (impacted area).
2.3. Diet analysis

The abdominal cavities of 135 specimens were opened, and their stomachs were removed. Then, gut contents were stored in 70% alcohol, and food items were analyzed and identified to the lowest taxonomic level (Hamada & Ferreira-Keppler, 2012; Hamada et al., 2014). The occurrence frequency (Fi%) and the method of volumetric frequency (Vi%) were used to quantify the gut contents (Hyslop, 1980). The occurrence frequency method, is the number of stomachs a diet item is found in divided by total stomachs containing food. The stomachs with no food were used for the Fullness index (FI) calculations and later discarded. For the volumetric frequency, the volume of each item was obtained using the percentage in relation to the total value of all gut contents. The volume was obtained using a gridded dish, and cubic millimeters were converted to milliliter (Hellawell & Abel, 1971). All values were combined in a feeding index (IAi), given by Equation 1:

\[ IA_i = \frac{(F_i \times V_i)}{\sum (F_i \times V_i)} \times 100 \]  

(1)

where \( i = 1, 2, \ldots n \) food items; \( F_i \) = Frequency of occurrence of food item \( i \); and \( V_i \) = Volume of food item \( i \), proposed by Kawakami & Vazzoler (1980).

Food items were grouped according to type (animal or plant) and origin (autochthonous or allochthonous). The FI was determined according to Hahn et al. (1999) and gut contents were coded as follows: 0 (empty), 1 (volume < 25%), 2 (25% < volume < 75%) and 3 (75% < volume < 100%).

The trophic level (TL) of \( P. \) squamosissimus was calculated using the formula \( TL = 1 + \) (weighed average of TLs of each prey) (Pauly & Christensen, 1999). Trophic level and maximum length of \( P. \) squamosissimus fish prey were determined using FishBase platform (Froese & Pauly, 2019) and SeaLifeBase (2018).

2.4. Data analysis

In all analyzed individuals, the standard length \( (L_5 \) in cm) was measured. The Shapiro-Wilk test of normality and Levene’s test of homoscedasticity were used to determine whether parametric \( t \)-test or non-parametric Mann-Whitney \( U \) test should be used to test for differences in the \( L_5 \) between preserved habitat and area impacted.

Chi-squared \( (\chi^2) \) tests were applied to a contingency table (Zar, 1999) in order to verify the association between the absolute frequency of FI class in each study area. To compare areas, \( \chi^2 \) tests were applied between food origin (allochthonous or autochthonous), food type (plant or animal), and trophic level (TL) values. A non-metric multidimensional scaling analysis (nMDS) was used to examine multidimensional spatial variation in diet using the total volume of each item. The dissimilarity matrix used in the ordination was built using the Bray-Curtis index, which was performed in the software PAST (version 2.1.7 Hammer et al., 2001). Statistical tests were performed using R software, version 3.5.2 (R Development Core Team, 2018) and the packages vegan for parametric test \( t \) or non-parametric Mann-Whitney \( U \) test and \( \chi^2 \).

3. Results

A total of 135 specimens were analyzed: 75 were collected in the preserved area and 60 in the impacted area. The mean \( L_5 \) in the preserved area \( (L_5 = 49.1 \pm 5.3 \, \text{cm}) \) was greater than that of the impacted area \( (L_5 = 44.2 \pm 8.3 \, \text{cm}) \) (U Mann-Whitney, \( U = 83.5; p = 0.01; \, df = 1 \)). The Fullness Index (FI) did not differ significantly between preserved and impacted sites (Table 1).

\[ Plasioecion squamosissimus \] ingested a wide variety of food items as fish, shrimps, terrestrial and aquatic insects, as well as plant (Table 2). \( Astyanax bimaculatus \) (Linnaeus, 1758) was the most important food item in the preserved area, as well as unidentified fish fragments and terrestrial insects (Table 2, Figure 3a).

Compared to the preserved area, \( Pimelodus blochii \) Valenciennes, 1840 was the most important food item in the impacted area, as well as the shrimp, \( Tenellus trimaculatus \) (Boulenger, 1898) and \( Hypostomus plecostomus \) (Linnaeus, 1758) (Table 2, Figure 3b).

In both areas, \( P. \) squamosissimus showed similar trophic level values (mean of \( TL_{\text{preserved}} = 3.42; \, TL_{\text{impacted}} = 3.62; \, \chi^2 = 0.0; \, p = 0.95; \, df = 1 \)). However, there was higher consumption of autochthonous material (\( \chi^2 = 5 \).

<table>
<thead>
<tr>
<th>FI</th>
<th>Absolute frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preserved area</td>
</tr>
<tr>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
</tr>
</tbody>
</table>

\( FI = 0 \): empty; \( FI = 1 \): < 25%; \( FI = 2 \): between 25% and 75% and \( FI = 3 \): between 75% and 100%. Samplings made at the Machado River, Brazil (June 2013 to March 2015). Contingency table \( (\chi^2 = 1.40, p = 0.06, df = 3) \).
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Table 2. Trophic level (TL), occurrence frequency (F%), volumetric frequency (V%), and feeding index (IAi) for diet items from *P. squamosissimus* at preserved area and impacted area of Machado River, Brazil (June 2013 to March 2015). Item relevance ranking, based on IAi, was tested using χ2.

<table>
<thead>
<tr>
<th>Food Items</th>
<th>TL</th>
<th>Preserved Area</th>
<th>Impacted Area</th>
<th>Item relevance by area (p&lt;0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F%</td>
<td>V%</td>
<td>IAi</td>
</tr>
<tr>
<td><strong>Animal origin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pimelodus blochii</td>
<td>3.2</td>
<td>1.88</td>
<td>1.43</td>
<td>0.01</td>
</tr>
<tr>
<td>Astyanax bimaculatus</td>
<td>2.5</td>
<td>18.75</td>
<td>15.01</td>
<td>1.81</td>
</tr>
<tr>
<td>Pterygoplichthys pardalii</td>
<td>2</td>
<td>9.43</td>
<td>7.19</td>
<td>0.04</td>
</tr>
<tr>
<td>Serrasalmus rhomaeus</td>
<td>4</td>
<td>1.88</td>
<td>1.43</td>
<td>0.01</td>
</tr>
<tr>
<td>Moenkhausia lepidura</td>
<td>3.2</td>
<td>1.88</td>
<td>4.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Hyphostomus plecostumus</td>
<td>2</td>
<td></td>
<td>18.75</td>
<td>9</td>
</tr>
<tr>
<td>Tenellus trimaculatus</td>
<td>2.8</td>
<td>6.96</td>
<td>5</td>
<td>0.25</td>
</tr>
<tr>
<td>Sternoptygus macrurus</td>
<td>3.2</td>
<td></td>
<td>12.5</td>
<td>3</td>
</tr>
<tr>
<td>Fish no identified</td>
<td>2.9</td>
<td>24.5</td>
<td>29.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Scales</td>
<td>2.9</td>
<td>7.54</td>
<td>5.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Aquatic insects</td>
<td>2</td>
<td>15</td>
<td>15.8</td>
<td>0.16</td>
</tr>
<tr>
<td>Terrestrial insects</td>
<td>2</td>
<td>7.54</td>
<td>10</td>
<td>0.34</td>
</tr>
<tr>
<td>Nematodes</td>
<td>2</td>
<td>5.6</td>
<td>4.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Shrimp</td>
<td>2</td>
<td></td>
<td>6.25</td>
<td>2.56</td>
</tr>
<tr>
<td><strong>Vegetal origin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant no identified</td>
<td>1</td>
<td>5.66</td>
<td>4.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Leaves</td>
<td>1</td>
<td>3.77</td>
<td>2.87</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Figure 3. Simplified food chain for *Plagioscion squamosissimus* (*Ps*) in preserved area (A) and impacted area (B) in Machado River (Brazil). Arrow width illustrates importance in the diet Y-axis = trophic level (TL), X-axis: maximum length (cm). (TL and maximum length are from Froese & Pauly, 2019 and or literature). Ps = Plagioscion squamosissimus; Sr = Serrasalmus rhombeus; Ml = Moenkhausia lepidura; Pb = Pimelodus blochii; Ab = Astyanax bimaculatus; Pp = Pterygoplichthys pardalii; Sm = Sternoptygus macrurus; Tt = Tenellus trimaculatus; Hp = Hypostomus plecostumus; Sh = Shrimp; Ai = Aquatic insect; and Ti = Terrestrial insect.

Figure 4. Non-metric multidimensional scaling analysis (nMDS) of volumetric data of the gut contents of *P. squamosissimus* in preserved area (circle) and impacted area (square), in Machado River (Brazilian Amazon), from June 2013 to March 2015. Carmita (dark gray circle), Farofa, (light gray circle), Suretama (white circle); São Sebastião (light gray square) and Poção (white square).

12.1; p < 0.05; df = 1) and animal items (χ2 = 12.6, p < 0.05, df = 1) in the preserved area.

In the nMDS analysis, clustering was observed based on the distinct use of food resources with a “stress” of 0.11 (Figure 4).

The food items that showed higher volumetric frequency in the gut contents of *P. squamosissimus* in the preserved area were non-identifiable fish
Gut content analysis confirms... (29.3%), followed by aquatic insects (15.8%) and *A. bimaculatus* (15.0%). For the impacted area, *P. blochii* (30.2%), followed by non-identifiable fish (10.1%) and *H. plecostomus* (9.0%) were the most representative (Figure 5).

There was no considerable overlap of items from animal (stress = 0.11; Figure 6a), plant (stress = 0.08; Figure 6b), autochthonous (stress = 0.20; Figure 6c), and allochthonous origin (stress = 0.23; Figure 6d) consumed by *P. squamosissimus* in each area.

**Figure 5.** Volumetric data (%) of the gut contents of *P. squamosissimus* in preserved area (black bar) and impacted area (gray bar), in Machado River (Brazilian Amazon), from June 2013 to March 2015. Fni = Fish no identified; Ai = Aquatic insects; Ab = *A. bimaculatus*; Ti = Terrestrial insects; Pp = *P. pardalis*; Sc = Scales; Ml = *M. lepidura*; Ne = Nematodes; Pni = Plant no identified; Le = Leaves; Pb = *P. blochii*; Sr = *S. rhomabeus*; Hp = *H. plecostomus*; Tt = *T. trimaculatus*; Sm = *S. macrurus*; Sh = Shrimp.

**Figure 6.** Non-metric multidimensional scaling analysis of diet items origin of *P. squamosissimus* in preserved area (circle) and impacted area (square). Item sources: animal (A), plant (B), autochthonous (C) and allochthonous (D) in Machado River (Brazilian Amazon), from June 2013 to March 2015.
4. Discussion

In our study, it was clear that *P. squamosissimus* consumed different food items in preserved and impacted areas. Small pelagic fish (*A. bimaculatus*) were most commonly found in its diet in the preserved area, while more benthic organisms (*P. blochii*, *H. plecostomus* and shrimps) were consumed in the impacted area. Nevertheless, trophic level values for *P. squamosissimus* were similar in both areas, which could indicate that its feeding plasticity refers to habitat use, varying from pelagic to benthic food chains. This is an opportunistic behavior already identified for this species, in other environments (Bennemann & Shibatta, 2002). We highlight that the trophic level calculated for *P. squamosissimus* for both areas analyzed was based on values in the literature (FishBase platform and SeaLifeBase), however it is evident the segregation in the use of food resources by the studied species, based mainly on the composition and volume of the fishes. food items consumed (see Figures 4 and 5).

Large Generalist Predators (LGP) can modify prey-predator interactions, because their bigger body size confer them the ability to feed on a wide variety of prey of different sizes (Woodson et al., 2018). We speculate that behavior responses of *P. squamosissimus* feeding lower on food chains in both areas evolved because, in higher temperatures, animals have higher relative demand for carbon and digestion of plant tissue is easier, inducing higher rates of herbivory and omnivory (Woodson et al., 2018; Dantas et al., 2019). Generalist and opportunistic species as *P. squamosissimus* are fundamental to restructure impacted food webs, since they could change interactions and trigger trophic cascades on the new environmental conditions (Bartley et al., 2019) despite to remain in the original trophic position.

Since we studied the diet composition of the same generalist species in two contiguous areas, but with different land use, and in the same river, we contend that our gut content analysis was helpful to show the availability of food resources in both areas and the ability of *P. squamosissimus* in changing their diet in the impacted situation, utilizing more benthic material on impacted area in order to maintain a similar trophic position. Therefore, the high abundance of omnivorous fish (*P. blochii*) and shrimp in gut content of *P. squamosissimus* in impacted areas could be an indicator of degraded aquatic systems, since the omnivorous and detritivorous species use a broad spectrum of food items (Pinto & Araújo, 2007). Accordingly, and following these above conditions, differences in gut content analysis and its metrics (Fi%, Vi% and IAI) could be used as an indicator to evaluate the capacity of modification of the species as well as the environmental conditions.

As already described, the TL values for both areas analyzed were somewhat dissimilar, and this index was calculated using literature values of the trophic level for each diet item. We emphasize that this is a limitation of the present study, considering that the trophic level of each food item consumed by *P. squamosissimus* was not analyzed in the laboratory, we speculate that such analysis may have resulted in a high variation in the measurement of the trophic level of the *P. squamosissimus*. However, we observed that this is a common practice in the literature and that the analysis of stomach contents, mainly from carnivorous fish, is quite hampered by the high speed of digestion and deterioration of food items.

The fishermen report a decrease in the abundance and size of the *P. squamosissimus* specimens, mainly related to the decreasing of the size in the mesh of fishing equipment and the increase in environmental degradation in the Machado River basin. Drenner & Hambright (2002) describe that predators generally have generalist habits (or omnivorous) and feed on prey in more than one trophic level, potentially spreading the effects of predation throughout the food chain, not focusing on particular trophic levels. As fishing pressure is eased through management efforts to restore overexploited stocks, making it necessary to enter information about interactions between species, especially trophic interactions between prey and predators, in models that promote the prediction and management of recovered fish stocks effectively (Jennings & Kaiser, 1998). For this, it is necessary to understand the trophic relationships between species and the factors that influence prey-predator interactions.

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