Understanding the factors that control production of commercially important fishes in the Amazon are necessary to effectively manage fisheries resources in the region. The first step of this understanding demands the identification of plants that contribute to the energy flow in fish's food web. The objective of this study was to investigate which group of plants supports the production of Characiformes during their juvenile life phase, in which these fishes inhabit an environment formed by C₃ and C₄ plants. A carbon isotope analysis was conducted for juvenile Characiformes collected from herbaceous aquatic stands of the Solimões River floodplain. 

The diet of these fishes was also analyzed to identify possible trophic links. The results indicate that although C₃ plants are the main energy sources of juveniles, the juvenile stage is that the sampled species have higher contribution from group of C₄ plants. A diet analysis revealed that juveniles of Brycon amazonicus, Mylossoma duriventris and Tripotherus angulatus consume energy sources through invertebrate herbivores, while Semaprochilodus insignis fed the sources directly on detritus. The dependence of juvenile fish diets on both C₃ and C₄ plants suggests that the maintenance of herbaceous aquatic environment is extremely important for fish communities in Amazonian floodplains.

Keywords: Fish; stable isotopes; food web; floodplain; diet.

Contribution of autotrophic C₃ and C₄ sources for juvenile Characiformes in the aquatic herbaceous plants in the Solimões River, Central Amazon, Brazil

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Contrução de fontes autotróficas C₃ e C₄ para Characiformes juvenis em plantas herbáceas aquáticas no rio Solimões, Amazônia Central, Brasil

A compreensão dos fatores que controlam a produção de peixes comercialmente importantes na Amazônia é necessária para gerenciar efetivamente os recursos pesqueiros da região. O primeiro passo deste entendimento exige a identificação de plantas que contribuam para o fluxo de energia nas redes alimentares dos peixes. O objetivo deste estudo foi investigar qual grupo de plantas suporta a produção de Characiformes durante a fase juvenil, em que esses peixes habitam um ambiente formado por plantas C₃ e C₄. Uma análise isotópica de carbono foi conduzida para Characiformes juvenis coletados em plantas herbáceas aquáticas na planície de inundação do rio Solimões. A dieta desses peixes também foi analisada para identificar possíveis elos tróficos. Os resultados indicam que embora as plantas C₃ sejam as principais fontes de energia dos juvenis, a fase juvenil é a fase em que as espécies amostradas têm maior contribuição do grupo de plantas C₄. A análise da dieta revelou que juvenis de Brycon amazonicus, Mylossoma duriventris e Tripotherus angulatus consumem fontes de energia através de invertebrados herbívoros, enquanto Semaprochilodus insignis alimenta-se diretamente através de detritos. A dependência das dietas dos peixes juvenis das plantas C₃ e C₄ sugere que a manutenção do ambiente das herbáceas aquáticas é extremamente importante para as comunidades de peixes nas várzeas da Amazônia.

Palavras-chave: peixe, isotopos estáveis, teia alimentar, várzea, dieta.
Material and Methods

Study area

Fish samples were collected in herbaceous aquatics stands, at a total of forty sites located in two areas of the Solimões River: ten sites upstream (●) and ten sites downstream (●) of the confluence of Solimões River with the Lake Coari (3°54’42.9”S, 63°17’29.6”W - 3°59’07.3”S, 62°52’23.8”W, respectively); and ten sites upstream (●) and ten sites of the downstream (●) of the confluence of the Solimões River with the Negro River (3°16’72.7”S, 60°03’43.3”W - 3°02’68.1”S, 59°46’88.9”W) (Figure 1), the distance between each site was one kilometer. The objective of this sampling methodology was to examine the spatial variation in carbon stable isotope ratios of the species sampled (Figure 1).

Sampling was conducted monthly between December 2007 and April 2008 during the rising water period, which coincides with the spawning period of the migratory fishes’ species (BAYLEY; PETRERE, 1989). The initial development of these species occurs in the main river channel where they feed off their yolk reserves, but after the total absorption of these reserves, Characidiform larvae colonize places where they find food and protection against predators (GOULDING, 1980; BAYLEY; PETRERE, 1989). The selection of species was based on their commercial importance (BARTHEM; GOULDING, 2007). *B. amazonicus, M. duriventris, S. insignis* e *T. angulatus* were frequent in the group Characidiform which represents more than 50% of the total juveniles captured in the herbaceous aquatics stands of the Solimões River.

Sampling methodology

Fishes were collected using dip-nets (1.1 x 0.8 m diameter and 5 mm mesh size) and seine nets (10 x 3 m diameter and 5 mm mesh size). Upon capture, specimens were placed on ice before being preserved in 10% formalin and transported to Ichthyoplankton laboratory of the National Institute of Amazonian Research (INPA) in Manaus, AM, Brazil.

In the laboratory, fishes were identified to the level of species based on ontogenetic sequence and counting of vertebrae and patterns of pigmentation (ARAÚJO-LIMA et al., 1993; ARAÚJO-LIMA; DONALD, 1988; ARAÚJO-LIMA; HARDY, 1987). Juveniles were also identified with the help of experts and compared with specimens previously identified in the Ichthyoplankton Laboratory. Later with the objective of verifying the progressive ontogenetic variation in feeding due to the increase in size during the juvenile development, the specimens were divided into two classes of development according to their standard length (SL) constituting class I (juveniles between 15 to 30 mm) and class II (juveniles between 31 and 60 mm).

Stomach content analysis

Stomach content analysis was performed for each species and analyzed in relation to size class and collection area. Diet composition was determined according to the following recognized categories: fish (including fins, scales and muscle tissue); zooplankton (including cladocerans, copepods, ostracods and rotifers); detritus (particulate organic matter); insects (adults, larvae, pupae and dismembered pieces); spiders; and vegetable matter (including pieces of leaves, fruits, roots and plant stock). The importance of each food item was calculated according to the feeding index proposed by Kawakami and Vazzoler (1980).

Stable isotope analysis of carbon ($\delta^{13}C$)

Individuals from each species and size class were sampled for stable isotope analysis of carbon. A small sample of muscle tissue was removed from the dorsal area, rinsed with distilled water and oven dried at 60°C for 48 hours. After drying, the samples were grounded into a fine powder using a mortar and pestle, placed in tin capsules and shipped to the Environmental Stable Isotope Center in the Bioscience Institute at the State University of São Paulo, Botucatu Campus, SP, Brazil for analysis of carbon isotope ratios using an elemental analyzer (EA 1108 CHN) coupled with a isotope ratio mass spectrometer (DELTA-S Finnigan MAT). The carbon ($\delta^{13}C$) isotope ratios were expressed by the following formula:

\[
\text{Sample} \% = \frac{R_{\text{sample}} - R_{\text{standards}}}{R_{\text{standards}}} \times 1000
\]

Later, the carbon isotope values were corrected because the samples were preserved in formalin solution and this substance caused a 1.65‰ reduction in carbon isotopic signatures (SARAKINOS et al., 2002). To determine the relative contribution of $C_{\text{plants}}$ plants for each fish species was used the following two end-member mixing model (FORSBERG et al., 1993):

\[
\%C_{\text{plants}} = \left[ 1 - \frac{\delta^{13}C_{\text{fish}} - \delta^{13}C_{\text{plants}}}{\delta^{13}C_{\text{fish}} - \delta^{13}C_{\text{C}} \text{C}_{\text{C}} \text{C}_{\text{C}}} \right] \times 100
\]

Where: $\%C_{\text{plants}}$ is the percentage of contribution of $C_{\text{plants}}$, $\delta^{13}C_{\text{fish}}$ is the mean value of $\delta^{13}C$ for fish, $\delta^{13}C_{\text{plants}}$ is the mean value of $\delta^{13}C$ for $C_{\text{plants}}$ plants, and $\delta^{13}C_{\text{C}}$ is the mean value of $\delta^{13}C$ for $C_{\text{C}}$ plants.

In the present study, was used the carbon isotope data of local autotrophic sources registered at Benedito-Cedillo et al. (2000) because they are representative of the region studied: $C_{\text{C}}$ = -13.4‰, Periphytic algae = -28.3‰ and Phytoplankton = -37.2‰. These values were obtained from spatial and seasonal variations in 495 samples of plants along an 1800 km west-to-east (i.e. upstream-downstream) transect of Amazon River between the cities of Vargem Grande (3° 16’ S, 67° 55’ W) and Óbidos (1° 55’ S, 55° 30’ W), Brazil during the low and high water seasons between October 1993 and July 1998.

In order to verify if there was a significant influence of juvenile development stage on the isotopic ratios of $\delta^{13}C$ and if there were significant variations among the species, the Student’s t-test was used with the Shapiro-Wilk and Levene tests a priori and Tukey’s test (p=0.05) a posteriori. The analyzes were conducted with the aid of the Statistical Program Paleontological STatistics - PAST, version 3.15 (HAMMER, 2017).

Results

Average $\delta^{13}C$ values and standard deviation of the juvenile fish ranged from a maximum of -24.6‰ ± 1.0 for class II of *B. amazonicus*, in the confluence of the Solimões River with the Negro River (Manaus) to a minimum of -31.2‰ ± 1.2 for class II of *T. angulatus*, in the confluence of Solimões River (Coari) with the Lake Coari (Table 1).
Table 1. Averages and standard deviations of δ C values and estimates of minimum and maximum contributions of C plants for Brycon amazonicus, Mylossoma duriventre, Semaprochilodus insignis and Triportheus elongatus. (n = number of samples; Min = minimum; Max = maximum; SD = standard deviation; Size classes: 1-15 to 30; 31-51 to 60 mm SL).

<table>
<thead>
<tr>
<th>Species</th>
<th>Size classes</th>
<th>COARI</th>
<th>MANAUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Avg.</td>
<td>SD</td>
</tr>
<tr>
<td>Brycon amazonicus</td>
<td>I</td>
<td>20</td>
<td>-28.6</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>28</td>
<td>-27.6</td>
</tr>
<tr>
<td>Mylossoma duriventre</td>
<td>I</td>
<td>20</td>
<td>-27.0</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>24</td>
<td>-28.1</td>
</tr>
<tr>
<td>Semaprochilodus insignis</td>
<td>I</td>
<td>20</td>
<td>-25.9</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>39</td>
<td>-27.5</td>
</tr>
<tr>
<td>Triportheus elongatus</td>
<td>I</td>
<td>20</td>
<td>-30.3</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>40</td>
<td>-31.2</td>
</tr>
<tr>
<td>AVG</td>
<td></td>
<td>11.5</td>
<td></td>
</tr>
</tbody>
</table>

The normality assumptions and homocedasticity were met and later it was verified with the T test that there was no significant difference between development classes of each species (p>0.05 = 0.3254; t = 0.5966) therefore, sampling site were pooled across developmental class of the species for both sampling regions: Coari and Manaus.

Only the species T. angulatus and B. amazonicus significantly differ between the sampling sites because values of δ C were lower in Coari and higher in Manaus (Tuley p<0.05 = 0.0132). There were also significant differences between development classes of both species (Tuley p<0.05 = 0.0001). T. angulatus was enriched in δ C in Manaus and impoverished in Coari, while B. amazonicus enriched in δ C in Coari and Manaus with increasing (Figure 2d; 2a). There was no significant difference between the size of M. duriventre and S. insignis (p>0.05 = 0.5853) (Figure 2b; 2c).

The analysis performed with the two end-member mixing model revealed variations in relative contributions of C plants between the two collection sites, because the species B. amazonicus and T. angulatus in Manaus presented in their two classes of development a contribution of plants C superior that in Coari, while the relative contribution of plants C to M. duriventre and S. insignis did not present changes (Table 1). The estimates of average relative contributions of C, grasses to the autotrophic energy sources of the juvenile fish varied from a minimum of 11.5% to a maximum of 38.1% in Coari and a minimum of 12.5% to a maximum of 43.8% in Manaus (Table 1).

For stomach content analyzes were used 1250 stomachs, of which 1181 (94.5%) contained food items. Stomach content analysis did not present significant differences in the diet of the species with respect to the collection areas (Coari and Manaus, downstream and upstream locations) (p>0.05 = 0.6318). Therefore, the analysis was only applied to the development classes of each species, eliminating the need to analysis by geographic location. The diet of juveniles of B. amazonicus, M. duriventre and T. angulatus were high in animal content, principally insects and zooplankton, presenting a higher percentage in class I (88.3%) than class II (575%) (Figure 3a; 3b; 3d). For the same species, there were also large differences in plant matter content, which varied from 1.3% in class I to 39.9% in class II. In comparison, S. insignis presented a diet low in animal content (21% in class I and 19% in class II), composed only of zooplankton, while 60% constituted a mixture of detritus and algae (Figure 3c).

Discussion

The δ C values of B. amazonicus, M. duriventre, S. insignis and T. angulatus indicate that although such juveniles inhabit an environment where plants of the group C predominates over the group C3, it is likely they selectively consume and/or assimilate C plants, following patterns observed in adult fish.

Figure 2. Averages and standard deviations of δ C (‰) of a) Brycon amazonicus, b) Mylossoma duriventre, c) Semaprochilodus insignis and d) Triportheus elongatus collected in aquatic herbaceous plants. δ Cof plants from the Amazon floodplain reported by Benefício-Cassio et al. (2000) for the same region. For species that there was no significant difference between the classes of development and/or sampling sites (M. duriventre and S. insignis) is shown only mean δ C of species. The rectangle indicates the range of autotrophic sources in which the values of δ C of species are inserted: COARI = Coari; MAO = Manaus; Size classes (mm SL): 1-15 to 30; 31-51 to 60 mm SL.

Figure 3. Frequency of occurrence (%) of the main food items identified in the stomachs of a) Brycon amazonicus (n= CL I: 114; CL II: 96), b) Mylossoma duriventre (n= CL I: 16; CL II: 38), c) Semaprochilodus insignis (n= CL I: 12; CL II: 109) and d) Triportheus elongatus (n= CL I: 21; CL II: 22) collected in aquatic herbaceous plants.
These results reiterate the studies that indicate that the fish present food selectivity being this tendentious to the consumption of plants of the group \(C\) (GINDERDEUREN et al., 2014; NANDI; SAIKIA, 2015; DENG et al., 2018).

In this sense, previous studies have shown that herbivorous and detritivorous fish generally avoid \(C\) plant foods because these plant sources, because they are rich in fiber, have low levels of nutrients and phenolic compounds that reduce their digestibility and palatability (FORSBERG et al., 1993; MORTILLARO et al., 2015). On the other hand, \(C\) plants have nutritional characteristics that attract herbivores and detritivores, since herbaceous \(C\) leaves, all algae, seeds and fruits of the trees have the highest average levels of minerals and proteins, are poorer in fiber and are, therefore, a source of energy assimilated more quickly by the body and also more nutritious (MORTILLARO et al., 2016; DENG et al., 2018).

However, the results of this study suggest that the juvenile stage is the only stage of development in which the fish show greater consumption of \(C\) plants. This observation derives from the fact that while the maximum contribution of the \(C\) plants to juveniles of the analyzed species was 38.1% in Coari and 43.8% in Manaus while the largest contribution of \(C\) plants to the larvae of Brycon cephalus, who was later identified the Brycon amazonicus (LEITE, 2004), M. duriventre, S. insignis and T. angulatus, collected in amazonian floodplain lakes, was only 22% (LEITE et al., 2002). Likewise, for adult specimens of T. angulatus, M. duriventre and S. insignis, the maximum contributions of \(C\) plants calculated by Forsberg et al. (1993) were 0%, 20% and 23.4%, respectively. These results probably reflect the large dilution of \(C\) plant energy by the assimilation of \(C\) plant energy during the adult phase of development, which occurs mainly in the flooded forest where \(C\) plant sources dominate. It is likely that movements among habitats during different growth phases explain the varying contributions of plant sources, corroborating the studies of Hobson and Clark (1992), who suggested that the isotopic signals encountered in animals' tissues depend on a mixture of diet and habitat displacements.

On the other hand, Oliveira et al. (2010) found that juvenile Characiformes, of the species Colossoma macropomum, fed in an artificial environment, synthesized muscle tissue more rapidly with diet of \(C\) plants. In addition, the quality of the dietary amino acids \(C\) plants is inadequate to promote the development of the species. As this study is based fish captured in the wild, the most plausible explanation for the great contribution from \(C\) plants at this stage of development of Characiformes is effect of the higher abundance of \(C\) group. Although stands of herbaceous is an association of \(C\) and \(C\) plants, the \(C\) herbaceous are the most abundant plant within the group herbaceous aquatic community in the Solimões river floodplain, resulting in a greater volume of organic matter from that group of plants and available aquatic environment (SOARES et al., 2014).

The analysis of the diet of the species revealed that the juveniles assimilate the energy of the \(C\) plants in an indirect way, that is, through trophic links, among which are the herbivorous invertebrates of autochthonous and allochthonous origin that inhabit the herbaceous stands. These results confirm the importance of trophic links in the transfer of autotrophic energy to the sustenance and maintenance of the aquatic food chain (POST, 2002; CAMPOS et al., 2015). In addition, it is also possible that subtle variations in the isotopic composition of species such as B. amazonicus and T. angulatus are due to the consumption of aquatic invertebrates which is susceptible to variations depending on the fish's food preference and the availability of the resource in the environment (UIEDA; PINTO, 2011; DIAS et al., 2017).

Although it is not possible to state with precision which plant group \(C\) was the most important source for S. insignis, apparently these juveniles do not use phytoplankton as their main source of autotrophic energy because its isotopic signature remained in the range of sources as periphyton \(C\), \(C\) herbaceous and flooded parts of the forest, plants that are common on the banks of herbaceous, probably because S. insignis forages exclusively on herbaceous bank because Araújo-Lima and Hardy (1987) reported these juveniles sucking submerged stems of herbaceous aquatic structures in which they adhere periphytic algae, detritus and other organic materials. Since for adults of S. insignis in the Amazon floodplain, the phytoplankton is indicated as the main source of carbon because this phase of life this species forage in other environments where the phytoplankton is abundant as per example, in the open waters of the floodplain lakes and the main river (ARAÚJO-LIMA et al., 1986; FORSBERG et al., 1993; BENEDITO-CECÍLIO; ARAÚJO-LIMA, 2002).

Conclusion

In conclusion, the analysis of carbon isotope showed that the contribution of \(C\) plants to the development of juveniles is higher than for larvae and adults because the juvenile stage is the only period in which the analyzed species live and forage exclusively in herbaceous banks. In combination with diet analysis, was observed that the consumption of energy sources by autotrophic juvenile of the species B. amazonicus, M. duriventre and T. angulatus occurs by the invertebrate herbivores in these fish's food chains and in S. insignis occurs directly through detritus. Although large contribution of \(C\) plants to juveniles' diets, \(C\) plants are the most important energy source for the four species throughout the juvenile phase. Therefore, the results of this study indicate that \(C\) and \(C\) plant groups together can ensure future fish stocks, therefore the maintenance of herbaceous aquatic environments is extremely important for the fish communities in the floodplains of the Amazon.

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