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# LIPID PROFILES OF THREE SPECIES OF TROGLOBITIC FISH FROM BRAZIL

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## ABSTRACT

In this work, three species of troglobitic fish (*Ituglanis passensis*, *Trichomycterus itacarambiensis* and *Stygichthys typhlops*), each found in a different region of Brazil were tested. Brazil has a high abundance of caves, but most of them have not been discovered or biologically explored. Consequently, the fauna associated with cave environments are threatened by ignorance towards their potential extinction of the fauna. With the intention of preserving the species and environments associated with them, the lipid composition of each fish was studied to determine the influence of the subterranean environment on the metabolism of these animals, especially those that were altered due to the medium they inhabited. Gas chromatography with flame ionization was used to analyze and identify the fatty acids in each species; experiments showed 25 fatty acids in *Ituglanis passensis*, 28 in *Trichomycterus itacarambiensis* and 25 in *Stygichthys typhlops*. Some of the acids were present in higher concentrations in relation to others for all species; these were palmitic (C16:0), stearic (C18:0), oleic (C18:1 ω9c), and linoleic (C18:2 ω6c) acids. Results demonstrated that the lipidic compositions of each analyzed species were influenced by conditions of their habitat; mainly feeding.

**Keywords:** cavefish, troglobitic, lipid profile, fatty acid, *Trichomycterus itacarambiensis*, *Ituglanis passensis*, *Stygichthys typhlops*, Brazil.

  

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## INTRODUCTION

The introduction of protective measures to conserve the underground environments of freshwater caves and streams is extremely important for the fauna in these ecosystems. These protective measures avert imbalances caused by pollution and disorderly tourism.

Organic resources that maintain cave communities almost always originate from external environments, being brought to the caves by physical (e.g. water, wind) or biological (e.g. trogloxenes, roots, microbes) means (Gnaspini-Neto, 1989; Ferreira and Pompeu, 1997). Accordingly, cave animals have adapted metabolisms to cope with the food scarcity conditions, prevalent in most caves. During reproductive periods, all fish species are influenced by external factors and require a suitable energy supply for survival and appropriate reproductive development (Navarro, 2010). Because the endocrine system is influenced by the external environment, it is considered to be one of the factors that regulate reproduction (Colares de Melo, 1989).

In non-cavefish species, the nutritional requirements for reproductive phases are different from other stages of development, such as post-larval, juvenile and fattening. Moreover, many of the deficiencies and nutritional problems observed in each of these phases are directly related to feeding methods and generally include the type and amount of nutrients supplied by breeders (Izquierdo et al., 2001). The amount and bioavailability of nutrients are examples of parameters that interfere with animal development.

Lipids are composed of different types of fatty acids and can be divided into storage and membrane lipids (Lehninger et al., 2006). In fish and other animals, polar lipids such as phospholipids, act as structural components responsible for maintaining the fluidity of membranes. Meanwhile, neutral lipids act as energy stores and are used as fuel for basic physiological functions such as growth, locomotion and gametogenesis (Brown and Murphy, 1995).

The composition, distribution and relationship between the fatty acids in fish are influenced primarily by three factors: metabolism (for example the species and stage of development), environment (temperature and salinity) and nutrition (Visentainer et al., 2003). The lipid composition of each fish species is a reflection of the aliment consumed by the animal. As such,

the lipid profile of cavefish may be utilized as a strong tool for preservation, as well as a way to provide information on the metabolic functions and the influence of food resources present in underground systems.

  

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Consequently, the study of lipid composition and fatty acid patterns aimed to provide a better understanding of the dynamics of these compounds in subterranean fish tissue during different stages of development, as well as the effect of environmental and seasonal changes in lipid metabolism of cavefish. The main objective of this work was therefore to determine the lipid metabolism of some cavefish species from Brazil, with the hope that this information can help to conserve the species and their environments.

## MATERIALS AND METHODS

### Samples

Experiments were conducted using two individuals from each species: Ituglanis passensis (Fernández and Bichuette, 2002), Trichomycterus itacarambiensis (Trajano and Pinna, 1996), and Stygichthys typhlops (Brittan and Böhlke, 1965). The small number of individuals analyzed is due to the small population sizes of each species, allied to their endangered status. The specimens were collected under the license n.13295-2 of the Brazilian Institute of the Environment and Renewable Resources (IBAMA).

Ituglanis passensis (Figure 1A) is endemic to the Passa Três cave, located in the São Domingos region, in the state of Goiás, Brazil. Trichomycterus itacarambiensis (Figure 1C), is endemic to the Olhos D'Água cave, located in the city of Itacarambi, in the northern section of Minas Gerais (Trajano and Pinna, 1996). The last species analyzed, Stygichthys typhlops (Figure 1B), occurs in groundwater in Jaíba municipality, north of Minas Gerais (Brittan and Böhlke, 1965).

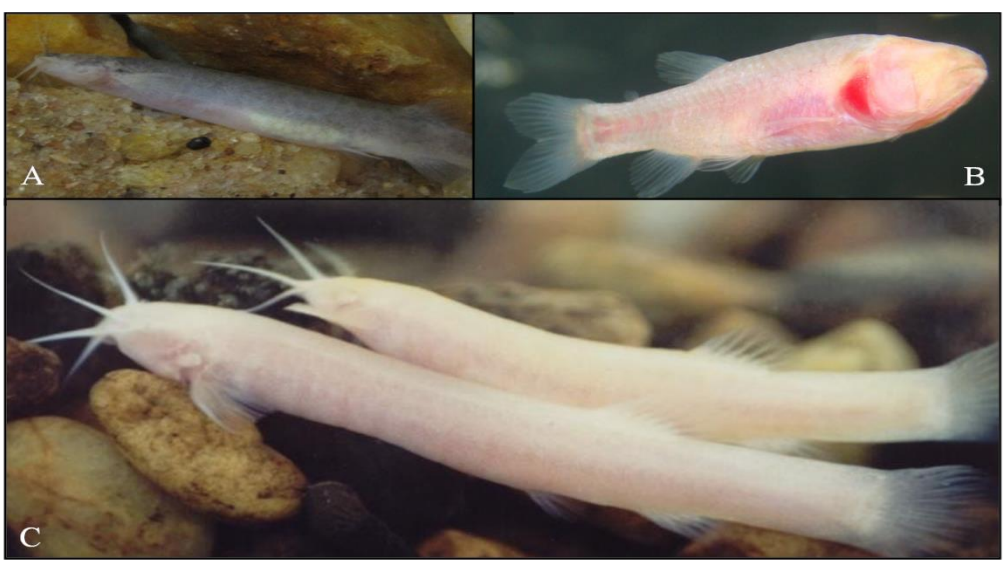


**Figure 1. The troglobitic fishes studied: *Ituglanis passensis* (A); *Stygichthys typhlops* (B) and *Trichomycterus itacarambiensis* (C).**

  

The fishes were collected with hand nets, transported in plastic containers with aeration and temperature monitoring. In the laboratory, the fish were sacrificed through their placement in an anesthetic containing container.

The samples were properly wrapped and maintained in a freezer (-20 ºC) until the beginning of the analyses, when then they were thawed to room temperature (≈ 25 ºC).

**Characterization of Fatty Acids**

Lipid extraction and esterification was performed using methodology published by Folch and collaborators (1957).

The fatty acid composition was determined by gas chromatography using a GC-2010 (Shimadzu) chromatograph, equipped with a flame ionization detector and a fused silica capillary column of 100 m length and 0.25 mm inner diameter, containing polyethylene glycol as liquid stationary phase. The standard used was a mixture of 37 methyl esters considered the most important for fish metabolism (SupelcoTM 37 Component FAME Mix). The following operational parameters were used: split injection mode, split ratio 1:100; 1 µL injection volume; 260 ºC detector temperature; 260 ºC injector temperature; oven temperature program: hold at 60 °C for 1 minute, ramp at 4 ºC/min. to 140 ºC, hold for 5 minutes; ramp at 4 ºC/min. to 240 ºC, hold for 30 minutes. Samples were dissolved in 0.30 mL of n-hexane. Peak identification (Table 1) was resolved by comparing the retention times of the fatty acid methyl ester standards with the retention times of the observed peaks.

**Principal Component Analysis**

The variation of the fatty acids and lipidic fractional components were determined by principal component analysis (PCA) grouping. PCA was performed beginning from a data matrix made up of the fatty acid levels for each sample. Before the analysis, the data were auto scaled in the Matlab program (2007).

**RESULTS AND DISCUSSIONS**

The fatty acids identified in the fish samples show an assorted composition of food material and results demonstrate which fatty acids are responsible for the prevalent differences among the three species studied.

Table 1 displays the influence of each fatty acid in the genetic separation between the species. When all three species were compared, Ituglanis passensis presented higher percentages for the fatty acids C12:0 (1), C17:1 (8), C18:2 ω6 (10), C20:4 ω6 (13). Meanwhile, Trichomycterus itacarambiensis demonstrated higher percentages of the fatty acids C13:0 (2), C14:0 (3), C14:1

  

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(4), C16:0 (6) and C17:0 (7); these were observed as the main acids responsible for the distinction of the species. Finally, Stygichthys typhlops was characterized by presenting higher levels of the fatty acids C18:0 (9), C20:0 (11), C21:0 (12) and C23:0 (14) when compared to the previous two species. An important strategy for the fish's adaptation to environmental temperature increases is to increase the proportion of unsaturated fatty acids in the membrane phospholipids. The level of unsaturated fatty acids in the body depends not only on the composition of the diet but also on the type of desaturation of the fatty acids (Ribeiro, 2007).

**Table 1. Fatty acid composition of the total lipid fraction in each species.**

| | Fatty acid | *Ituglanis passensis* (%) | *Trichomycterus itacarambiensis* (%) | *Stygichthys typhlops* (%) |
|---|---|---|---|---|
| 1 | **C12:0** | 0.63 | 0.18 | 0.02 |
| 2 | **C13:0** | 0.02 | 0.45 | 0.03 |
| 3 | **C14:0** | 1.52 | 2.68 | 0.95 |
| 4 | **C14:1** | 0.03 | 0.11 | 0.00 |
| 5 | **C15:0** | 1.09 | 1.23 | 0.92 |
| 6 | **C16:0** | 23.11 | 26.79 | 26.28 |
| 7 | **C17:0** | 1.68 | 1.87 | 1.58 |
| 8 | **C17:1** | 0.60 | 0.00 | 0.00 |
| 9 | **C18:0** | 8.42 | 8.38 | 12.60 |
| 10 | **C18:2 ω6c** | 11.15 | 6.72 | 6.32 |
| 11 | **C20:0** | 0.38 | 0.20 | 0.49 |
| 12 | **C21:0** | 0.07 | 0.25 | 0.32 |
| 13 | **C20:4 ω6** | 7.15 | 2.37 | 2.40 |
| 14 | **C23:0** | 0.00 | 0.06 | 0.26 |
| 15 | **C22:2** | 0.23 | 0.17 | 0.06 |

  

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The lipid composition of the studied species varied according to the characteristics of the specific environment to which they were live, including the abundance or lack of food; however, it cannot be determined which of the environmental factors contributed to the observed results, since the available food in each species' habitat was not quantified in this study.

The influence of environmental factors such as temperature and feeding frequency have been shown to influence the metabolism of different species of fish, which affects their demands for fatty acids and, therefore, can be used as an indication of the nutritional profile of the species (Perez, 2007).

An important strategy for the adaptation to temperature of many freshwater, is the increase in the proportion of unsaturated fatty acids in membrane phospholipids. The level of unsaturated fatty acids in the body not depends only on the composition of the diet as well as the degree of desaturation of fatty acids.

Desaturation of the fatty acid membrane is considered an important mechanism for the adaptation to thermal stress in fish. Changes in fatty acid composition of the phospholipid cell membranes show adaptation to cold in order to maintain fluidity, and the most significant responses to this stress is the increased levels of unsaturated fatty acids, which were observed in the results based on the Ituglanis passensis species (Navarro, 2010).

PCA allowed for better verification of the fatty acid composition of each studied species. The PCA results are presented in Figures 2A and 2B. The numbers on the graph correspond to the fatty acids described in Table 1.

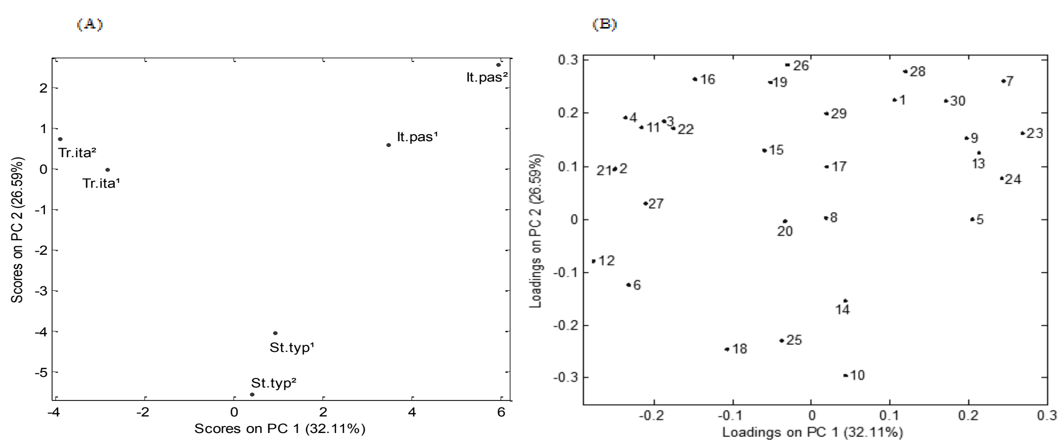


**Figure 2. Scores derived from principal component analysis for the studied species. Relationship involving the analyzed specimens (A) and the fatty acid levels (B).**

The graph score (Figure 2A) indicated that there was a distinction among the species Ituglanis passensis, Trichomycterus itacarambiensis and Stygichthys typhlops, as a function of the fatty acid composition of each.

The PCA derived scores, considering the analyzed specimens, are presented in Figure 2B, whose numbering represents the fatty acid described in the Table 1. The observed differences can be explained by the fact of their being of different species, besides being a troglophile and a troglobite, respectively. Both were collected from different places, however, with considerable food availability.

Finally, we can suggest some observed effects resulting from the distinct food availability related to the different lipid profiles in the studied species. In the case of Ituglanis passensis, the differences can be related to the fact that the individuals inhabit a cave with an entrance that encompasses a drain, elevating the food availability (organic matter) introduced from the relatively well preserved forest present in the external environment adjacent to the cave. This environmental condition could explain the presence of more different types of fatty acids (especially unsaturated fatty acids) for this species in comparison with the other species. For Trichomycterus itacarambiensis, the food availability was much lower (the cave entrance experiences resurgence) and the levels of some fatty acids were higher in relation to the other species. This result may eventually indicate the great capacity that such species have to store fat for later use according to the needs of the animal.

## CONCLUSIONS

The results from the evaluation of lipid profiles studied for the three species demonstrate the influence that adverse conditions may have on underground environments compared to epigean environments; primarily the quantity and quality of food provide metabolic adaptations in the body of the cavefish to allow for the deposition of lipid reserves necessary to sustain food shortages.

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