

Changes in macroinvertebrate biogeography: Symbiotic analysis in ichthyofauna trophic chains.

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Abstract. Aquatic biogeography is formed by a panorama of biodiversity that adds a pattern subordinate to the tree of life. Considering this concept, dietary plasticity in freshwater fish is a factor of extreme scientific relevance, mainly for analyzing the ecosystem changes that occur in the aquatic environment. Given the concerns resulting from climate change, which range from ecological phenomena, biological behaviors, to impacts caused on the biodiversity of rivers and marine surfaces, it is necessary to investigate trophic chains from a phylogenetic perspective through analyzes of fish stomach contents, on a panorama of studies that make it possible to understand the autoecology of species and their role in the ecosystem. The qualitative nature used in the database follows the standards of the volumetric and gravimetric method, both with a directional plane of volume and mass, which proposes observing the diet of 298 fish from 17 species, captured at the peak of 24 hours, but precisely night, through the use of nets with 12mm mesh between us, within a radius of 15m. It is concluded from the above, an abundance in the biodiversity of macroinvertebrates of the orders Ephemeroptera, Plecoptera and Trichoptera, overlapping them with analyzes of alternative molecular-based hypotheses; When examining the stomach contents of fish, under the observation of an optical microscope, spores of the Endomelanconiosis fungus were found in macroinvertebrates from the Caenidae family, hydropsychidae, Leptohiphidae e Perlidae, showing a strong concern in the behavioral change of aquatic microorganisms in parasitic symbiotic actions; the analyzes also elucidated the observational bias of changes in aquatic biogeography, indicating preliminary effects of climate change.

Key words. Ichthyofauna, Food chain, Comparative biogeography, Phylogeny, macroinvertebrates.

1. Introduction

Ecosystem studies on the trophic chain through the analysis of fish stomach contents have gained important visibility in recent decades, whose observations support a comparative taxonomic system in aquatic biogeography, reflecting on the

understanding of a feeding organization in watercourses of rivers or environments marine (Teixeira *et al.* 2005? Blaber, 2000; Rozas & Zimmerman, 2000). The biota associated with the substrates constructs a variable understood as benthos, which certainly connects a broad but at the same time complex diversity, from fish and algae to

macroinvertebrates, other arthropods, sediments and other vegetation. We also consider that food has an essential role in the flow of energy, considering the trophic modifications of food chains (WOOTTON, 1990).

1.2 Morphological sampling plan of ichthyofauna

Fish are aquatic vertebrates that have gills, a body supported by an internal cartilaginous or bony skeleton, whose appendages, when present, are shaped like fins. These vertebrates are extremely adapted to the aquatic environment where they live, given that their great ecological diversity is reflected in the immense variety of shapes, colors and different types of locomotion (BEMVENUTI; FISCHER, 2010).

Studies on the feeding of freshwater fish in the southern part of Brazil have contributed to the verification of bioindicators (Teixeira and Bennemann, 2007; Chaves e Vendel, 2008; Oliveira and Bennemann, 2005). This means that fish can be used in assessments of environmental impacts on aquatic ecosystems, being considered potential bioindicators, due to their sensitivity to contaminants in the environment (MORON, 2006).

1.3 Fish feeding behavior

By thoroughly reviewing such literature, we consider that the population dynamics of aquatic animals, when compared to their feeding between species, it becomes complex to predict their behavior, to the point of having variable dietary levels (Kawakami & Amaral, 1983; Hahn & Delariva, 2003; Hobson & Chess, 1986; Sazima, 1986; Edgar & Shaw, 1995). Based on this curiosity, Bastin (p.13-22, 2018) in his monograph paraphrases that, "studies on fish feeding allow us to recognize distinct trophic categories and infer about the structure and degree of importance of different trophic levels", which we believe are embedded in the interrelationships between its components.

1.4 Trophic plasticity in fish

Once defined the dietary plasticity in freshwater fish, it becomes feasible to observe certain variants in the digestive tract, both of cartilaginous and bony fish, allowing remarkable observations, which incorporate molecular and morphological data, trying to be as exhaustive as possible, from an evolutionary point of view. We argue in this context, the (arthropods) which are a group of triploblastic animals belonging to the clade that groups other animals with cuticular moulting (Aguinaldo et al., 1997; Giribet y Ribera, 1998; Giribet, 1999), which bring into their phylogeny problems that are most debated in taxonomy zoological which range from ecological phenomena to biological behaviors, which are also included in the diet of freshwater fish.

On another, somewhat significant note, Abelha and Goulart (p.431, 2001) point out that "the occurrence of a flexible diet is a striking characteristic of tropical riverine ichthyofauna", this indicates that most species can switch from one food to another. another, as soon as oscillations occur in the relative abundance of the food resource in use, motivated by spatio-temporal environmental changes (PAGLIARINI, p.119, 2018; ABELHA and GOULART, 2001; RAMOS et al., 2022).

The objective of our work intensifies in the search for new discoveries, both at the taxonomic margins and in the predominance of the investigative content of the evolution of species in aquatic biogeography, highlighting comparative studies on the mechanisms of the diet of freshwater fish in southern Brazil. The observations propose definitions of recurring phenomena in ecological interactions between living beings, in the face of global climate disorders. In this same context, the abundance and scarcity of aquatic biodiversity is also observed, based on macro or micro fauna. When starting from a preliminary observation of this sampling plan, there is a synchronism between taxonomy and evolutionary biology on the functioning of the ecosystem, as well as concerns about morphological changes in the clades of benthic macroinvertebrates and other invertebrates that inhabit these environments (GIRIBET et al., 1999)

2. Methodology

The qualitative nature used in the database follows the standards of the volumetric and gravimetric method, both with a directional plane of volume and mass. According to this scheme, publications by Hynes (1950), Hyslop (1980) and Bowen (1992) are among the most cited reviews on methods used in the study of fish feeding.

The study was carried out in June 2023 in the city of Cerro Largo, precisely on the Ijuí river, located in the Uruguay river basin, northwestern region of Rio Grande do Sul in Brazil, during a technical field trip in one of the monitoring activities in biodiversity and phylogeny of the main groups of hexapods, together with the pedagogical residency project at the Federal University of Fronteira Sul (UFFS), *Campus* Cerro-Largo, financed by the Coordination for the Improvement of Higher Education Personnel Foundation (CAPES).

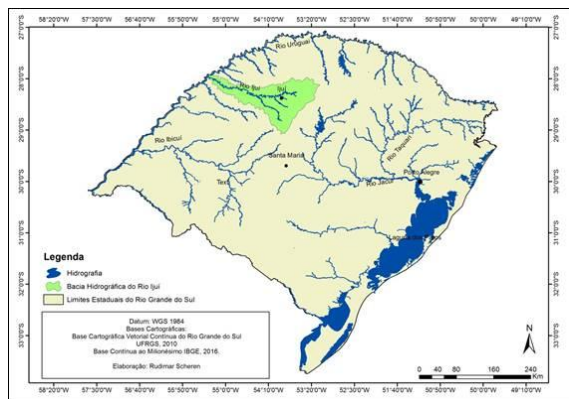


Figure 1 – Location map of the Ijuí River watershed (highlighted in green), by (Scheren et al., 2019).

For this work, 298 fish of 17 species were collected over a period of 24 hours, captured using cast nets with a 12 mm mesh, between nodes and a radius of 15 m, of 17 species, which were identified by categories. Among the conditioned groups are: (PV) *Parapimelodus valenciennis*(121); (THE) *Iheringichthys labrosus* (42); (LS) *Loricariichthys spp.* (37); (SB) *Steindachneridion brevipinna*(32); (AP) *Acestrorhynchus pantaneiro*(20); (CK) *Cynopotamus kincaidii*(17); (SM) *Serrasalmus spotted*(8); (HC) *We hypothesize a merchant*(5); (HA) *Hoplias aff. malabaricus* (4); (AL) *Astynax lacustris*(2); (HI) *Hypostomus isbrueckeri* (2); (SN) *Schizodon nasutus* (2); (AA) *Apareiodon is related*(2); (GH) *Galeocharax humeralis* (1); (PF) *Psalidodon fasciatus*(1); (CM) *Missionary Crenicichla*(1); is (this) *Eigenmannia virescens*(1).

After collection, the total length (in mm) and total weight (in grams) were verified using an ichthyometer and precision scale. Then, the stomachs were removed through an abdominal incision that starts at the anal opening and ends near the pectoral fin region. Finally, measurements of the weight of the stomachs (in grams) were taken and visually assessed regarding the degree of fullness, as follows: Empty (V.) when there is no food item; partially empty (P.V.) when it is filled up to ¼; partially full (P.C.) when partial filling is greater than ¼; and full (C.) when the stomach is completely filled. In order to organize the food categories, the abbreviation for each food item was identified in the table: Pisces (PX1); Algae(AG1); macroinvertebrates (MI1); other arthropods (AT2); sediments(ST1) and other vegetation(VT2).

Following this observation, the stomach contents were exposed in petri dishes under a stereoscopic microscope for analysis and identification of the food items consumed. However, with the help of a control card, whose usefulness was to mark each food item found, the analyzes continued, where the experiments continued with the construction of slides, with the purpose of examining microorganisms, which were subsequently subjected to observations under an optical

microscope. The items found were identified at the lowest taxonomic level for algae, insects and fungi and taxonomic category at family level for macroinvertebrates. Such food nutrition were analyzed according to the length (mm), the (M.E.), the stage of Replexion (E.R.) and Stomach Weight (g) as shown in Table 1 below, representing eight specimens of the species analyzed:

Aba.1- Variation of food resources in freshwater fish.

species	mm	E.M	E.R	(g)
(AP)	175	AG1	P.V	0,217
(PV)	150	AG1; ST1 MI1	C.	0,254
(SB)	143	MI1	P.C	0,071
(SM)	120	PX1	C.	0,531
(PF)	120	AG1; MI1	P.V	0,394
(THE)	193	MI1;AG1 ;ST1	C.	0,466
(CK)	190	MI1	P.C	2,065
(LS)	180	MI1	P.C	0,483

By understanding ecological relationships in microfauna, as being the most intriguing among organisms and perhaps the most complex, we chose to observe these behaviors closely, following an analysis protocol that lasted 28 days on time. Based on the keys already worked on by Nilsson (1964); Ingold (1975); Marvanová (1997), where we identified the samples as being (AMT-F1); then we sterilize the inoculation thread, starting from the colli handle to the nickel chrome needle; lactic acid was then added to the center of the slide; after this, the mycelium was removed from the edge of the colony from each food item (as long as they were macroinvertebrates) and these mycelia were subsequently spread in lactic acid using the laminula to open them; a drop of lugol was added, bearing in mind that its excess was removed with paper towel and, finally,, the coverslip was placed at a 45-degree angle on top of the mycelium. We reiterate again that this process was repeated for each sample individually between 10 to 15 samples for each fungal colony.

3. Development

The most representative species found were *Parapimelodus valenciennis* with 121

individuals, representative of the Pimelodidae family, its main characteristics are; body covered in leather, three pairs of barbels, well-developed adipose fin, strong and serrated spines on the pectoral fins, popularly known as catfish. Another representative of the same family also appeared with a significant number of individuals is the *theringiichthys labrosus* with 42 individuals captured, popularly known as Mandi bicudo, which is a species that has an elongated and slightly compressed body, morphologically high, at the beginning of the dorsal fin, tapering towards the head and the caudal fin, its head is conical with the eyes located laterally and has a forked caudal fin and numerous barbels around the mouth. Another individual in the family *Loricariinae* with 37 representatives captured is *Loricariichthys spp.* Its animal anatomy exposes a body covered with four or more longitudinal series of bony plates, suction cup-shaped lips, with a rudimentary barbel on each side of the mouth. According to Baumgartner's literary descriptions *et al.*, (2012), both species mentioned above refer to the same order: (Siluriformes) represented by Bagres and Cascudos.

Following taxonomic sequencing, the *Steindachneridion brevipinna* appear with 32 individuals, and are representatives of the order Characiforme, and the family *Curimatidae*, then The characteristic features are large scales, with 33 to 37 scales with pores on the lateral line, scales with pores, slightly pigmented in black, forming a horizontal line on the side of the body, a diffuse black spot in the central region and close to the base of the dorsal fin. They are also known as branquinhas, curimatã, or curimbatã. When following this clade, we observe the family *Acestrorhynchidae*, scientifically described, as *Acestrorhynchus pantaneiro* with 20 representatives, highlighting the characteristics of the laterally compressed body, it has an adipose fin, an anal fin with a long base, larger than the dorsal fin, a falcate anal fin, with a very long anterior lobe, furred caudal fin, very small scales, large mouth, with well-developed canine teeth, popularly known as dog fish (LATINI...*et al.*, 2004).

The fact of the enormous quantity of freshwater fish is due to the range of different habitats (rivers, lakes, ponds, streams), being considered by Hahn, n.s. & Delariva, r.l. (2003) the vertebrates with the greatest number of habitats. Considering (Figure 1) regarding the location of the study, we emphasize the constant climate changes that were not common at the time that biomonitoring emerged, considering the hot climate reaching high degrees between 38°C and 40°C, instead of the cold that it usually happens in winter, suitable for this time of year, which results in major changes in the ecological attributes of river systems, directly interfering in the composition and abundance of the original ecosystem (FERREIRA; FERRAZA; HARTAMNN, 2011).

Among the species analyzed, according to (Table 1), it is observed that the majority of Neotropical fish have sufficient capacity (dietary plasticity) to adjust their diet (HAHN & FUGI,*et al.* 2007) and, when a food becomes available, many species are able to take advantage of this opportunity (GERKING,*et al.* 1994) exploring the food categories present in greater quantities. However, another aspect that draws attention due to its quantity present in the food items examined, refers to benthic macroinvertebrates (MI1), being present in 194 fish stomachs from 298 analyzes carried out, highlighting the Mayflies (72 individuals), which played a leading role in the families *Caenidae* and *Leptohyphidae*; *Plecoptera* (58 individuals), highlighting the *Perlidae*; *Trichopteros* (64 individuals) where the family was present in large numbers *Hydropsychidae*.

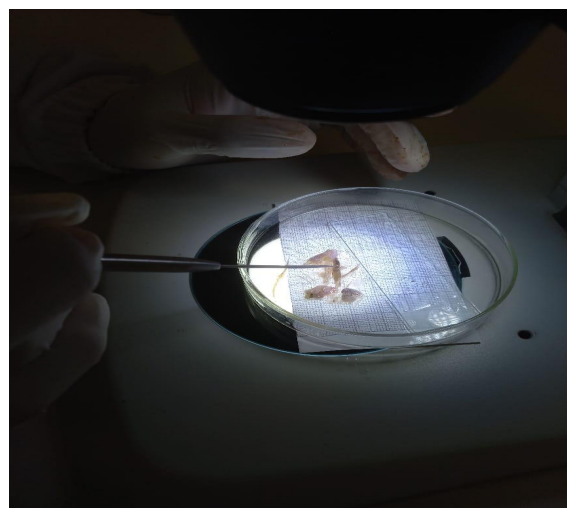


Figure 2 – Fish stomach sample (CK) consisting of a colony of ascomycete fungi from gender *Endomelanconiopsis* in Ephemeroptera. By RODRIGUES (2023).

Trophic plasticity, also called adaptability, versatility or dietary flexibility, was described by Gerking (1994) as the ability of certain species to take advantage of the most available food resources in the environment for a certain period of time (Corrêa, Cláudia & Smith, Welber. p.6. 2019). However, when we look at the scientific literature on symbiosis in microfauna, referring to macroinvertebrates and fungi, there is a lack of analysis, showing that this can be an extraordinary event on the sporadic branch of the tree of life. When analyzing all (AMT-F1) we observed ascomycete spores, present in macroinvertebrates from the families *Caenidae*, *Hydropsychidae* and *Leptohyphidae*. According to the taxonomic contributions of Rojas El,*et al.*, (2008) the genus found, it is the *Endomelanconiopsis*, which is characterized by eustromatic conidiomas and unicellular, brown, non-apiculate, holoblastically produced conidia, each with a longitudinal germ

cleft. In this way, new samples are being collected in the spring period which lasts from September to December 2023, as well as in the period from March to June, being the autumn season of 2024, with the purpose of deepening these symbiotic relationships in the microfauna present in the aquatic biogeography.

4. Conclusions

When examining the stomach contents of freshwater fish, a diversity of benthic macroinvertebrates was noted as the predominant food item, with emphasis on the orders Ephemeroptera, Plecoptera and Trichoptera. However, colonies of fungi of the genus *Endomelanconiopsis* in macroinvertebrates from the Caenidae families, Hydropsychidae, Leptohiphidae and Perlidae, revealing a strong concern in the behavioral change of aquatic microorganisms in parasitic symbiotic actions. When making a comparison between fish, macroinvertebrates and the relationship between fungi and these organisms, it was noticeable to observe changes in aquatic biogeography, indicating preliminary effects of climate change, also considering, hypothetically, the harm caused by urban centers indicating traces of pollution. In a genomic centrality, effects on the behavioral changes of aquatic microfauna organisms, mainly benthic macroinvertebrates, are considered. Therefore, there was a need for new investigations, supported by biological samples (only from macroinvertebrates) to determine whether changes are occurring in animal morphology and anatomy, which will rely on molecular observations. The investigation of such assumptions, in the genomic contexts in the phylogeny of macroinvertebrates, is posed by a problem seen only in the symmetry of Ephemeroptera, Plecoptera and Trichoptera, the main ones being harmed in the symbiotic relationships of aquatic fungi, without affecting the ichthyofauna.

5. Recognition

To Professor Dr. Gonzalo Giribet do Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, **Harvard University**, 26 Oxford Street, Cambridge, MA 02138, USA, for all the support and guidance in the development of the entire project.

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