



PATRÍCIA SANTOS FRÁGUAS

**TROPHIC ASPECTS OF THE ICHTHYOFaUNA:
IMPOUNDMENT EFFECTS AND SAMPLING SUFFICIENCY**

LAVRAS – MG

2020

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração Ecologia e Conservação de Recursos em Paisagens Fragmentadas e Agrossistemas para obtenção do título de Mestre.

Prof. Dr. Paulo dos Santos Pompeu
Orientador

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TROPHIC ASPECTS OF THE ICHTHYOFaUNA: IMPOUNDMENT EFFECTS AND SAMPLING SUFFICIENCY

ASPECTOS TRÓFICOS DA ICTIOFAUNA: EFEITOS DE BARRAMENTOS E SUFICIÊNCIA AMOSTRAL

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração Ecologia e Conservação de Recursos em Paisagens Fragmentadas e Agrossistemas para obtenção do título de Mestre.

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RESUMO

As alterações de fluxo sazonais têm grande influência sobre o funcionamento e integridade dos ecossistemas aquáticos. Os períodos de cheia aumentam a disponibilidade de abrigo, aporte de recursos alimentares e são importantes para a manutenção da biodiversidade nestes ecossistemas. A construção de usinas hidrelétricas acarreta diversas alterações nos ambientes aquáticos que consequentemente irão alterar dinâmicas da fauna local. Os barramentos interrompem rotas de migração, retêm sedimentos e nutrientes na região a montante, e interferem na qualidade e disponibilidade de recursos alimentares para os peixes a jusante do barramento. O estudo do conteúdo estomacal de peixes possibilita a distinção de grupos tróficos, auxilia na compreensão da estrutura trófica e das relações entre os componentes de uma comunidade. A alimentação, por ser um processo dinâmico, pode gerar alterações na estrutura trófica de comunidades em decorrência de alterações de condições ecológicas. Para que inferências robustas possam ser feitas através das informações fornecidas pelos estudos de análise de conteúdo estomacal, é necessário que o número de indivíduos coletados seja suficiente para descrever a dieta das espécies apropriadamente. Neste trabalho será discutido como os impactos gerados pela instalação de usinas hidrelétricas afetam a estrutura trófica da comunidade de peixes nas regiões de influência de uma barragem (Capítulo 1), e também utilizamos três espécies de peixe como modelo a fim de avaliar a suficiência amostral para a descrição da dieta (Capítulo 2).

PALAVRAS-CHAVE: Barragem. Conteúdo estomacal. Metodologia. Rio Jequitinhonha.

ABSTRACT

Seasonal flow changes have major influence on the functioning and integrity of aquatic ecosystems. Flooding periods are important for maintaining biodiversity in these ecosystems and also increase the availability of shelter and food resources. Hydroelectric plants constructions causes several changes in the aquatic environments that will consequently change the dynamics of the local fauna. Dams interrupt migration routes, retain sediment and nutrients in the upstream region, and interfere with the quality and availability of food resources for fish downstream the dam. Stomachal content studies of fishes allows the distinction of trophic groups, helps to understand the trophic structure and the relationships between the components of a community. As feeding is a dynamic process, it can generate changes in the trophic structure of communities due to changes in ecological conditions. In order for robust inferences to be made through the information provided by stomach content analysis studies, it is necessary that the number of individuals collected is sufficient to describe the species' diet appropriately. In this work, we discuss how the impacts generated by the installation of hydroelectric plants affect the trophic structure of the fish community in the regions of influence of a dam (Chapter 1), and we used three species of fish as a model to evaluate the sampling sufficiency for the description of the diet (Chapter 2).

KEYWORDS: Dam. Jequitinhonha River. Methodology. Stomachal content.

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A construção de usinas hidrelétricas acarreta diversas alterações nos ambientes aquáticos (AGOSTINHO; GOMES; PELICICE, 2007), como as alterações hidrológicas nos cursos dos rios. As alterações de fluxo nos sistemas de água corrente são de grande importância, pois sua magnitude, frequência, duração e periodicidade têm grande influência sobre o funcionamento e integridade dos ecossistemas aquáticos (POFF et al., 1997).

Os organismos inseridos neste ambiente, dentre eles a ictiofauna, estão intimamente ligados à estas dinâmicas. As inundações sazonais aumentam a área física alagada, conectando diferentes habitats e aumentando a disponibilidade de abrigo (FERNANDES et al., 2009). Durante estes eventos há maior disponibilidade de recursos alimentares, por meio da entrada de material alóctone que é carreado para dentro do leito, enriquecendo o ambiente em nutrientes (LUZ-AGOSTINHO et al., 2009). Como os eventos de cheias e secas que ocorrem naturalmente são importantes para manter a biodiversidade nestes ecossistemas (MALMQVIST; RUNDLE, 2002), espera-se que alterações no regime de fluxo natural irão refletir em alterações nas dinâmicas da fauna local.

O represamento de rios leva a alterações profundas no ambiente aquático (BAXTER, 1977). Uma das modificações é a regulação do regime de fluxo, que interrompe rotas de migração, e retém sedimentos e nutrientes na região a montante (AGOSTINHO; PELICICE; GOMES, 2008). Devido ao controle do regime hidrológico, a qualidade e disponibilidade de recursos alimentares para os peixes a jusante também fica comprometida (MONTEIRO et al., 2008).

O conhecimento da dieta da assembleia de peixes possibilita a distinção de grupos tróficos, auxilia na compreensão da estrutura trófica e das relações entre os componentes de uma comunidade (AGOSTINHO et al., 1997). A alimentação, por ser um processo dinâmico, pode gerar alterações na estrutura trófica de comunidades em decorrência de alterações de condições ecológicas (BRODEUR et al., 2017). Para que inferências robustas possam ser feitas através das informações fornecidas pelos estudos de análise de conteúdo estomacal, é necessário que o número de indivíduos coletados seja suficiente para descrever a dieta das espécies apropriadamente (FERRY; CAILLIET, 1996).

Portanto, este trabalho busca entender como os impactos gerados pela instalação de usinas hidrelétricas afetam a estrutura trófica da assembleia de peixes nas regiões de influência

de uma barragem (Capítulo 1), e também utilizou três espécies de peixe como modelo a fim de avaliar a suficiência amostral para a descrição da dieta destas espécies (Capítulo 2).

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SEGUNDA PARTE

MANUSCRITO 1**Hydropower affects fish trophic structure both downstream of the dam and upstream of the reservoir**

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Abstract

Dams cause drastic changes in freshwater environments and can compromise the quality and availability of food resources on rivers. This study aims to analyse the influence of the construction of a dam on the trophic structure of the fish assemblage on lotic areas both upstream and downstream of Irapé power plant. Fish sampling occurred before (2003 to 2005) and after the impoundment (2011 to 2017). The trophic structure and species composition before the dam construction were similar upstream and downstream. After the dam closure, both aspects of the assemblages changed on the lotic stretches. After the dam implementation, while upstream there was an increase of detritivores biomass and decrease of piscivores and omnivores, downstream went exactly the opposite, causing the differentiation between the two assemblages. Because lotic areas upstream of reservoirs are also impacted by river damming, efforts for mitigation of impacts should also focus on these areas.

Keywords: Dam, feeding, fish assemblage, trophic guild, Irapé, Jequitinhonha River.

Introduction

Dams fragment river systems around the world (Nilsson 2005), causing drastic changes in freshwater environments (Agostinho et al. 2008). Impoundments affect the natural flow regime, which is a key driver of river ecology (Bunn and Arthington 2002) by determining channel morphology, habitat diversity and substrate stability (Power et al. 1995; Nilsson and Svedmark 2002). Alterations on natural flow regime change the availability of basal resources (detritus and phytoplankton) that provide energy to support food webs (Angradi 1994) compromising the quality of food resources downstream (Baxter 1977; Abujanra et al. 2009). These modifications contribute to influence the distribution, abundance and diversity of organisms of these ecosystems (Poff and Zimmerman 2010).

Feeding is a dynamic process and the diet can change based on the shift of ecologic conditions. These changes will affect not only the transfer of energy through the system but also the growth, survival, and demographics of assemblages (Brodeur et al. 2017). Information regarding the feeding habits of the fish assemblage can help with the separation of trophic groups, the understanding of the trophic structure and interspecific relations in a community (Agostinho et al. 1997). Comprehending the structure of fish assemblages after human-induced alterations can help to develop conservation and management strategies for freshwater environments (Pompeu and Godinho 2003).

Trophic studies on rivers affected by dams have mostly focused on diet and trophic structure on changes related to the reservoir creation (Albrecht et al. 2003; Mériona et al. 2005; Luz-Agostinho et al. 2006; Delariva et al. 2013). Although reservoirs undergo the most pronounced changes, creating a new habitat (Baxter 1977), the river regions upstream of the reservoir and downstream of the dam can also be affected. The influence

of impoundments on the trophic ecology of downstream fish assemblages have also been frequently addressed (Power et al. 1996), including studies for the tropical region (Mérona et al. 2001; Gandini et al. 2012; Peressin et al. 2016). However, evaluations comprising both upstream and downstream trophic responses of fish assemblages to hydroelectric dams are scarce (Sá-Oliveira et al. 2015).

Therefore, the aim of our study is to analyse the influence of the construction of a hydropower plant on the trophic structure of the fish assemblage before and after the construction of the dam on the river stretches both upstream of the reservoir and downstream of the dam. We tested (i) if trophic structure changed upstream and downstream after the construction of the dam, and (ii) if eventual changes occurred because of species substitution or changes in the relative abundance of species.

Material and Methods

Study area The Jequitinhonha River basin drains an area of approximately 70,315 km² on the states of Minas Gerais and Bahia, Brazil. Mean annual rainfall averages around 600 and 1.600 mm irregularly distributed throughout the year. Rainfall season occurs between October and March (IBGE 1997). The Jequitinhonha River rises in the Espinhaço complex mountain and runs 1,086 km into the Atlantic Ocean (Guerrero 2009).

Sampling points were placed on lotic stretches upstream and downstream of Irapé Hydroelectric Power Plant (16°44'S, 42°34'W), in the Jequitinhonha River (Figure 1). Sampling point 3 is placed immediately downstream of Irapé dam, and its flow is completely determined by the hydropower plant operation. Irapé hydroelectric plant started its operation in 2006. The reservoir has 142.95 km² of area, an installed power capacity of 360MW, and accumulates 5,954.88x10⁶ m³ of water (Cachapuz 2006). This is the highest dam in Brazil, with 205 meters (Cachapuz 2006).

Upstream of the reservoir the hydrological patterns have remained; while downstream of the dam, the regulation by the power plant was evident (Figure 2).

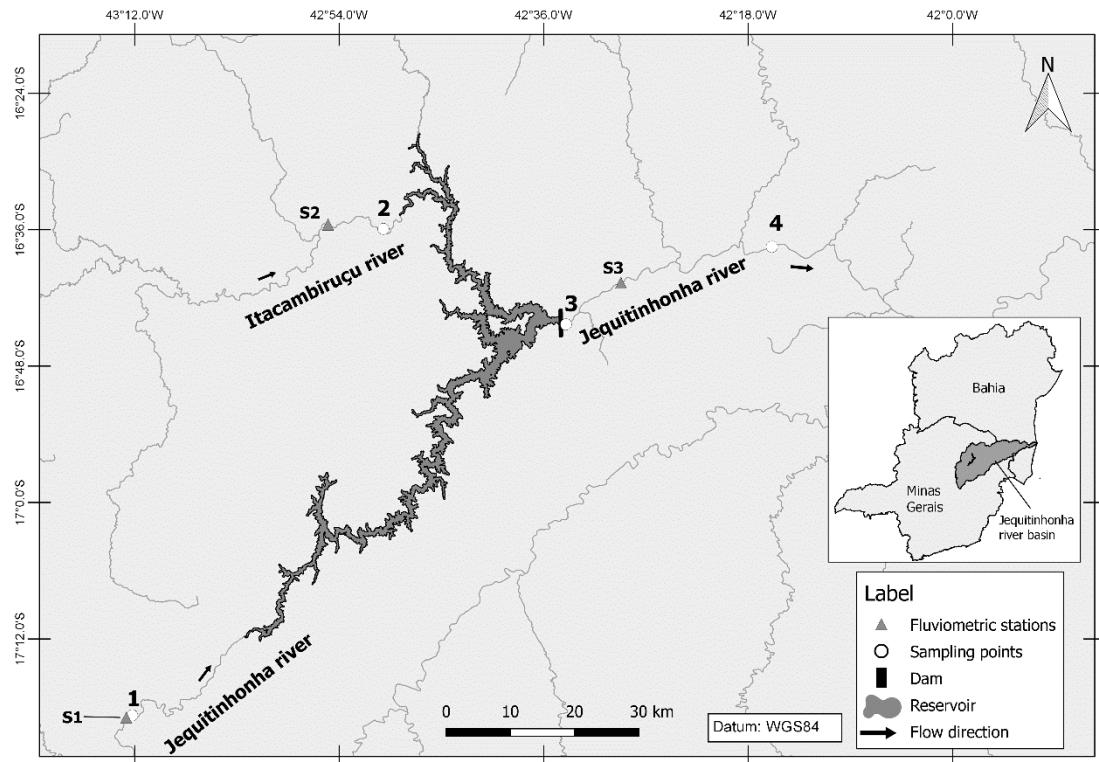


Figure 1 Map of Irapé hydroelectric power plant showing the fish sampling points (1 to 4) and fluviometric stations (S1, S2, S3)

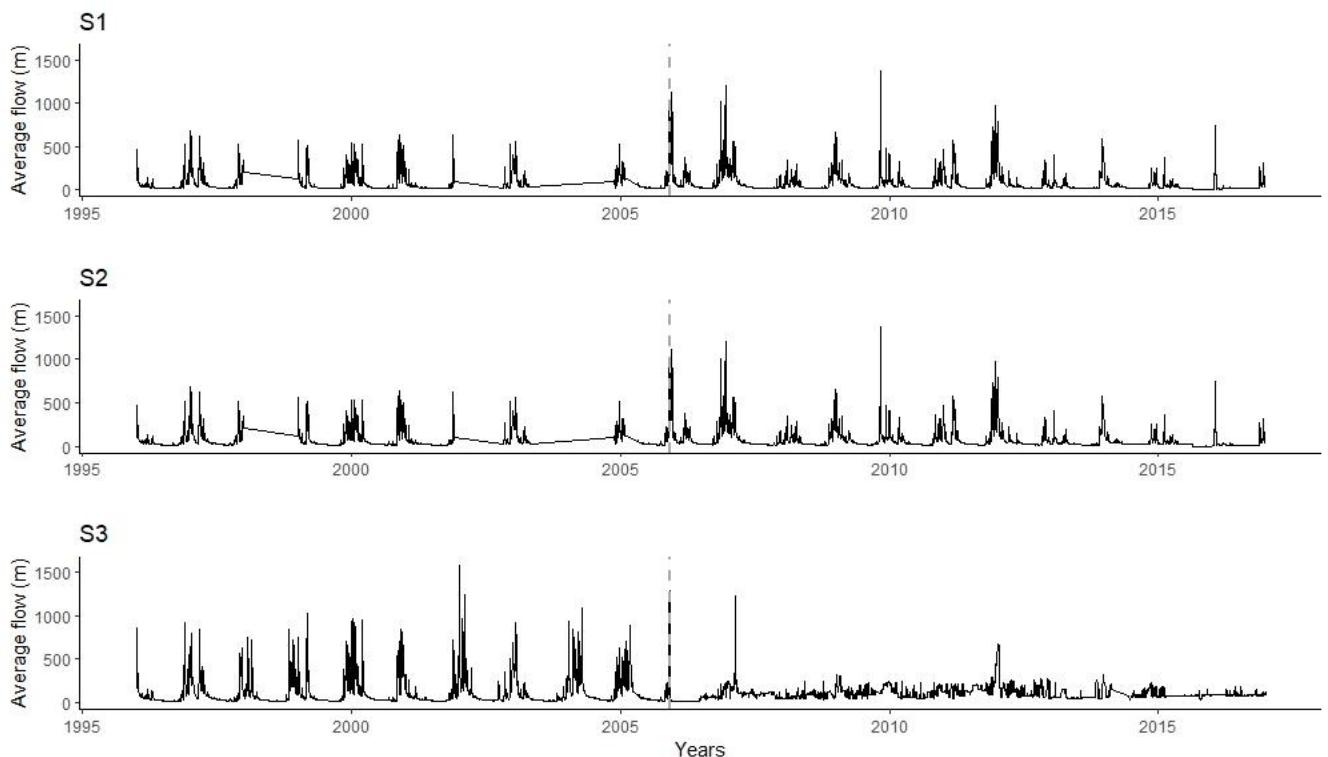


Figure 2 Daily average flow for three fluviometric stations. S1 and S2 are located upstream of the reservoir, and S3 is located downstream of the dam. The gray dashed line

represents the date in which the dam was closed. The Energy Company of Minas Gerais (CEMIG) provided historical flow data (1996 – 2016) of the fluvimetric stations nº 1743023 (S1), 1642029 (S2), and 1642044 (S3)

Data sampling Fish sampling prior to the construction of the dam occurred from 2003 to 2005, and after from 2011 to 2017 (Table 1) on both rainy and dry seasons. For fish collection, we used gillnets in sizes 2.4, 3, 4, 5, 6, 7, 8, 10, 12, 14 and 16 cm between opposite nodes. The effort of each mesh was 30 m, except for the 2.4 mesh net that used 20 m. The nets were exposed for 12 hours. We fixed the individuals in formaldehyde and preserved them in alcohol in the laboratory. Fish had their stomachs removed for subsequent analysis. Under a stereomicroscope, stomach contents were analysed to the lowest possible taxonomic level. Identification of food items was made with the aid of the bibliography (Ward and Whipple 1918; Costa et al. 2006; Mugnai et al. 2010). The wet weight of the items was determined with a precision scale (Ohaus Adventurer, AR 2140 - 0,0001g).

Table 1 Number of sampling for each fish sampling point

Period	Sampling point	Number of samplings
Before		
	1	6
	2	5
	4	6
After		
	1	7
	2	14
	3	27
	4	11

Data Analysis We classified the species into trophic guilds through a bibliographic review and stomach content analysis. For species without feeding descriptions on the literature, we analysed up to 10 stomachs. To determine the importance of each food item, the food index (IAi) (Kawakami and Vazzoler 1980) was

calculated, but the volume of the items was replaced by their weight (Bennemann et al. 2006) as shown in the following formula: $IAi = \frac{Fi \times Pi}{\sum_{i=1}^n (Fi \times Pi)} \times 100$

Where: IAi: Alimentary Index; Fi: Frequency of occurrence (%) of food item i; Pi: Weight (%) of food item i.

We grouped the guilds in broad categories: detritivore, herbivore, omnivore, piscivore and insectivore. To determine the trophic structure, we summed the biomass of the individuals of the species belonging to each guild.

In order to evaluate trophic structure changes upstream and downstream, the fish assemblages were compared considering four groups according to the location (upstream / downstream) and time (before or after) relative to the dam construction: before/upstream (BU); before/downstream (BD); after/upstream (AU) and after/downstream (AD). Data was standardized by the total of samples. These groups were sorted by non-metric multidimensional scaling analysis (NMDS) using the Bray-Curtis similarity index and compared by the similarity analysis test (ANOSIM). The similarity percentage analysis (SIMPER) was used to evaluate which guild/species contributed most to the differentiation of these groups. NMDS, ANOSIM and SIMPER tests were performed in the Primer 6 & Permanova + software (Clarke and Gorley 2006).

To test for species substitution or changes in the relative abundance of species we compared the ANOSIM results for two NMDS analysis: one based on the biomass of each species using the Bray-Curtis similarity index and the other based on the presence/absence of species using Jaccard similarity index to test for species substitution.

Results

Trophic structure A number of 4055 individuals belonging to 31 species were sampled. Regarding the feeding habits, 10 species were classified as detritivores, two as herbivores, three as insectivores, 10 as omnivores and six as piscivores (Table 2) (S1).

Table 2 List of species, their classification in trophic guilds, occurrence and abundance on sampling points

Species	Trophic guild	Before Upstream	Before Downstream	After Upstream	After Downstream
<i>Astyanax bimaculatus</i> (Linnaeus, 1758)	Herbivore	10	12	37	327
<i>Astyanax fasciatus</i> (Cuvier, 1819)	Omnivore	2	2	372	298
<i>Brycon devillei</i> (Castelnau, 1855)	Omnivore			10	2
<i>Brycon howesi</i> Lima, 2017	Omnivore	10			
<i>Delturus brevis</i> Reis & Pereira, 2006	Detritivore			89	
<i>Geophagus brasiliensis</i> (Quoy & Gaimard, 1824)	Insectivore				1
<i>Gymnotus carapo</i> Linnaeus, 1758	Insectivore		5	4	6
<i>Harttia garavelloii</i> Oyakawa, 1993	Detritivore			3	
<i>Hoplias brasiliensis</i> (Spix & Agassiz, 1829)	Piscivore	13	2	10	37
<i>Hoplias malabaricus</i> (Bloch, 1794)	Piscivore	6	4	14	63
<i>Hypostomus</i> sp. Lacepède, 1803	Detritivore	39	13	268	81
<i>Leporinus steindachneri</i> Eigenmann, 1907	Omnivore	6	1	3	43
<i>Megaleporinus elongatus</i> (Valenciennes, 1850)	Insectivore	5		12	41
<i>Megaleporinus garmani</i> (Borodin, 1929)	Herbivore	38	1	238	171
<i>Moenkhausia intermedia</i> Eigenmann, 1908	Omnivore		1		
<i>Moenkhausia costae</i> (Steindachner, 1907)	Omnivore				542
<i>Oligosarcus macrolepis</i> (Steindachner, 1877)	Piscivore	2	1	20	37
<i>Pareiorhaphis</i> sp. Miranda Ribeiro, 1918	Detritivore			39	
<i>Pareiorhaphis stephanus</i> (Oliveira & Oyakawa, 1999)	Detritivore			36	
<i>Pimelodella</i> sp. Eigenmann & Eigenmann, 1888	Omnivore			1	
<i>Prochilodus argenteus</i> Spix & Agassiz, 1829	Detritivore				8
<i>Prochilodus costatus</i> Valenciennes, 1850	Detritivore				8
<i>Prochilodus hartii</i> Steindachner, 1875	Detritivore	16	6	88	69
<i>Prochilodus</i> sp. Agassiz, 1829	Detritivore				2
<i>Pseudoplatystoma</i> sp. Bleeker, 1862	Piscivore	1			1
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	Omnivore	1		8	3
<i>Serrasalmus brandtii</i> Lütken, 1875	Piscivore			2	92
<i>Steindachneridion amblyurum</i> (Eigenmann & Eigenmann, 1888)	Piscivore	2			
<i>Steindachnerina elegans</i> (Steindachner, 1875)	Detritivore	1	46	4	14
<i>Trachelyopterus galeatus</i> (Linnaeus, 1766)	Omnivore	22	15	18	191
<i>Wertheimeria maculata</i> Steindachner, 1877	Omnivore	3	2	1	453

Before the construction of the dam, there was no difference in the trophic structure between the river segments upstream and downstream. After the construction of the dam, not only the two regions differed from the previous situation, but also became different from each other (Table 3, Figure 3).

After the dam implementation, while upstream there was an increase of detritivores biomass and decrease of piscivores and omnivores, downstream went exactly the opposite, causing the differentiation between the two assemblages (Table 4).

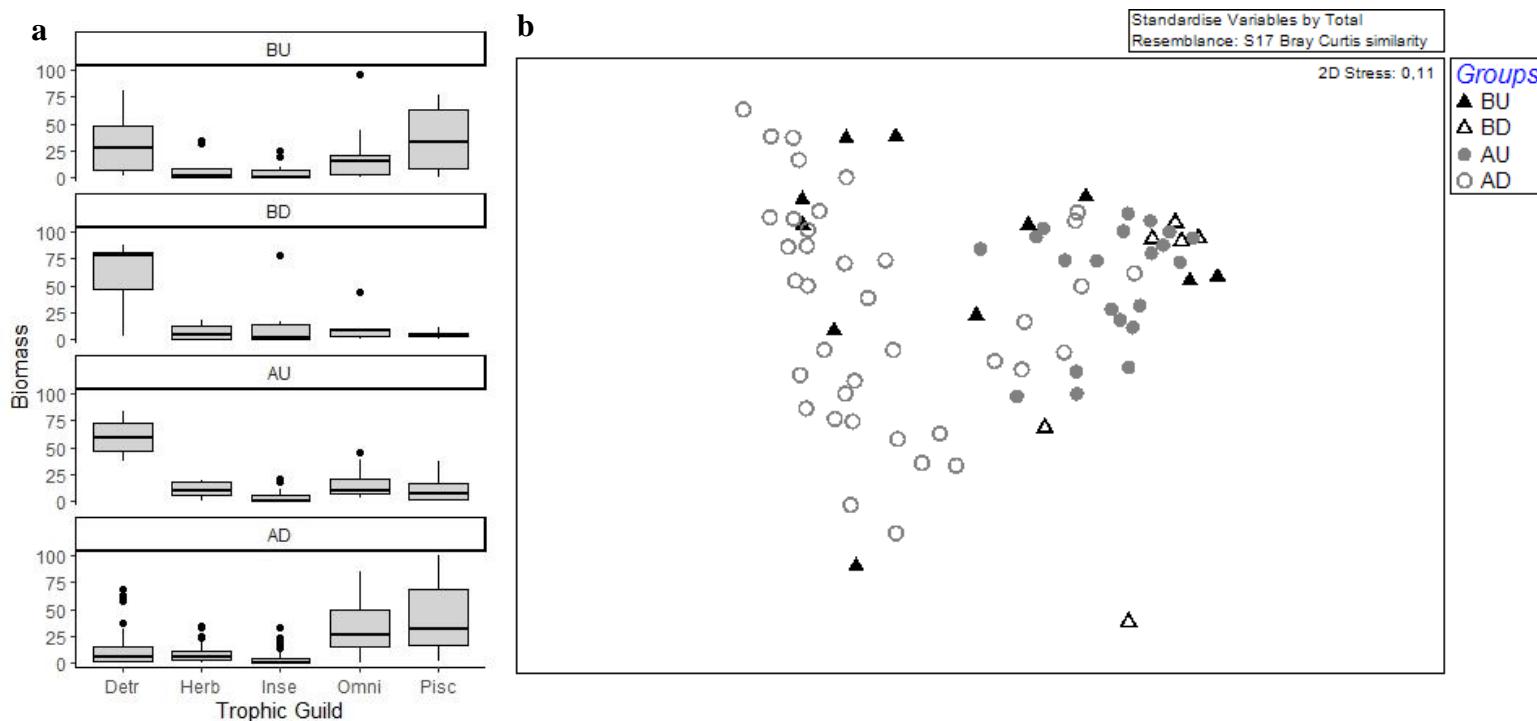


Figure 3 a) Trophic structure of the fish assemblage represented by relative biomass of the trophic guilds. Line inside the box = median; box = 25th and 75th percentiles; whiskers = 1.5 x IQR; dots = outliers b) Non-metrical multidimensional scaling of the relative biomass for each trophic guild. (Detr= Detritivores, Herb= Herbivores, Inse= Insectivores, Omni= Omnivore, Pisc= Piscivore, BU= Before/upstream, BD= Before/downstream, AU= After /upstream, AD=After/downstream)

Table 3 ANOSIM tests for trophic structure and species composition (BU= Before/upstream, BD= Before/downstream, AU= After /upstream, AD=After/downstream) Level of significance p<0.05

Groups	Trophic Structure		Species composition			
			Species substitution		Relative abundance	
	R statistical	p value	R statistical	p value	R statistical	p value
BU, AU	0.483	0.001	0.450	0.001	0.378	0.002
BD, AD	0.615	0.001	0.482	0.004	0.547	0.001
AU, AD	0.477	0.001	0.424	0.001	0.39	0.001
BU, BD	0.139	0.102	0.013	0.402	0.077	0.214

Table 4 Similarity percentage analysis of the trophic structure between the groups (BU= Before/upstream, BD= Before/downstream, AU= After /upstream, AD=After/downstream)

Trophic Guilds	Groups			
	Before Upstream	Before Downstream	After Upstream	After Downstream
BU x AU Average dissimilarity = 53.39		Average Abundance		Contribution% Cumulative %
Detritivore	30.67	59.97		34.56 34.56
Piscivore	35.9	10.37		29.82 64.38
Omnivore	20.53	14.77		18.14 82.52
Herbivore	7.8	10.7		11.02 93.54
BD x AD Average dissimilarity = 71.33				
Detritivore	60.93		13.9	36.38 36.38
Piscivore	4.21		40.86	25.83 62.21
Omnivore	11.8		32.62	19.3 81.51
Insectivore	16.43		4.42	12.47 93.98
AU x AD Average dissimilarity = 60.50				
Detritivore		59.97	13.9	39.38 39.38
Piscivore		10.37	40.86	27.57 66.95
Omnivore		14.77	32.62	20.33 87.29
Herbivore		10.7	8.2	7.36 94.65

Species composition The observed changes on trophic structure occurred due to both species composition and relative abundance changes (Table 3, Figure 4). Considering the relative abundance of species, the detritivore species *Prochilodus hartii* accounted for almost 20% of the changes among the three groups comparisons (Table 5).

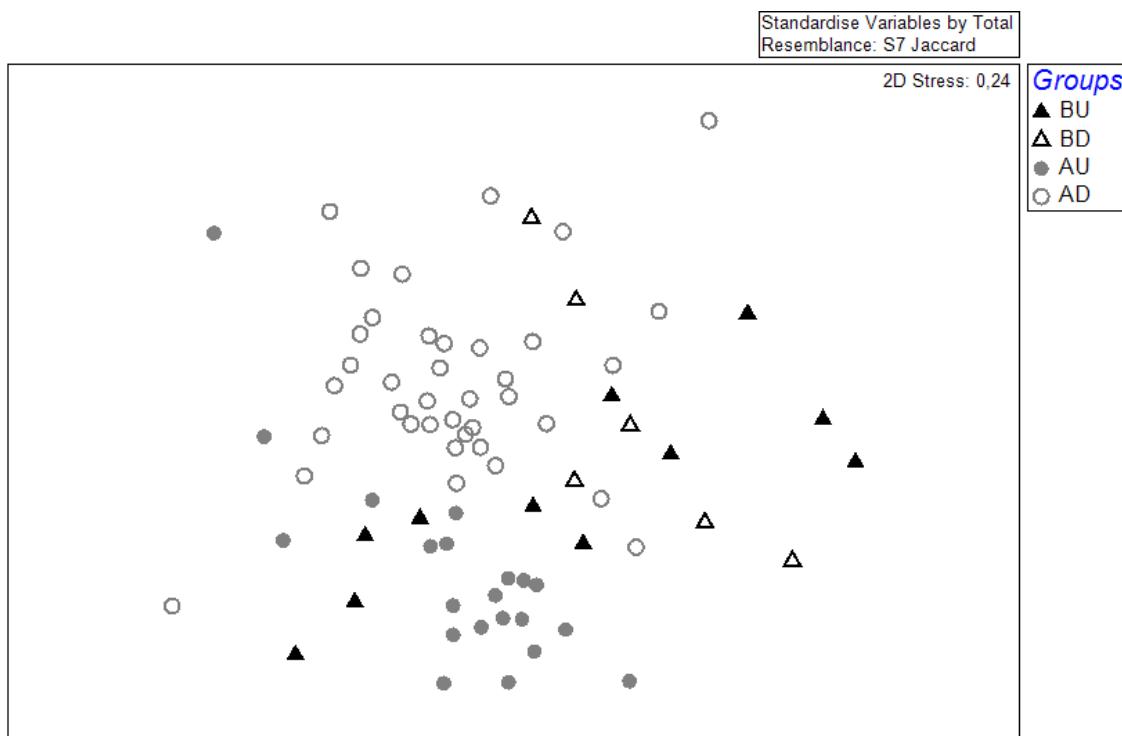


Figure 4 Non-metrical multidimensional scaling of species composition. (BU= Before/upstream, BD= Before/downstream, AU= After /upstream, AD=After/downstream)

Table 5 Similarity percentage analysis of species composition between groups (BU= Before/upstream, BD= Before/downstream, AU= After /upstream, AD=After/downstream)

Species	Groups				
	Before Upstream	Before Downstream	After Upstream	After Downstream	
BU x AU Average Dissimilarity= 73.09		Average Abundance		Contribution%	Cumulative %
<i>Prochilodus hartii</i>	17.92	35.14		19.19	19.19
<i>Hoplias malabaricus</i>	13.54	4.43		10.62	29.8
<i>Hoplias brasiliensis</i>	12.39	4.48		9.42	39.22
<i>Hypostomus</i> sp.	12.68	12.85		8.73	47.94
<i>Megaleporinus garmani</i>	7.22	9.86		7.76	55.7
<i>Leporinus steindachneri</i>	10.68	1.18		7.67	63.37
<i>Delturus brevis</i>	0	8.14		5.57	68.94
<i>Astyanax fasciatus</i>	0.19	7.45		4.99	73.93
<i>Pseudoplatystoma</i> sp.	6.99	0		4.78	78.71
<i>Megaleporinus elongatus</i>	5.11	3.56		4.53	83.25
<i>Wertheimeria maculata</i>	4.31	0.49		3.21	86.45
<i>Trachelyopterus galeatus</i>	3.3	2.04		2.93	89.38
<i>Pareiorhaphis</i> sp.	0	3.24		2.21	91.59
BD x AD Average dissimilarity = 87.12					
<i>Prochilodus hartii</i>	29.36	7.73	16.87	16.87	
<i>Wertheimeria maculata</i>	0.93	16.68	9.43	26.3	
<i>Gymnotus carapo</i>	16.43	0.06	9.43	35.73	
<i>Steindachnerina elegans</i>	15.84	0.17	9.06	44.79	
<i>Hypostomus</i> sp.	15.73	1.7	8.53	53.32	
<i>Hoplias brasiliensis</i>	1.13	14.22	8.1	61.42	
<i>Serrasalmus brandtii</i>	0	12.76	7.32	68.74	
<i>Hoplias malabaricus</i>	2.91	11.82	6.51	75.26	
<i>Trachelyopterus galeatus</i>	8.92	4.8	5.62	80.87	
<i>Astyanax bimaculatus</i>	5.67	4.14	3.78	84.66	
<i>Leporinus steindachneri</i>	1.34	4.42	2.8	87.46	
<i>Megaleporinus elongatus</i>	0	4.35	2.5	89.96	
<i>Megaleporinus garmani</i>	0.96	4.06	2.38	92.34	
AU x AD Average dissimilarity = 80.47					
<i>Prochilodus hartii</i>	35.14	7.73	18.8	18.8	
<i>Wertheimeria maculata</i>	0.49	16.68	10.35	29.15	
<i>Hoplias brasiliensis</i>	4.48	14.22	9.26	38.41	
<i>Serrasalmus brandtii</i>	0.78	12.76	7.99	46.41	
<i>Hoplias malabaricus</i>	4.43	11.82	7.83	54.24	
<i>Hypostomus</i> sp.	12.85	1.7	7.53	61.77	

Table 5 (continued)

Species	Groups					
	Before Upstream	Before Downstream	After Upstream	After Downstream		
<i>Megaleporinus garmani</i>			9.86	4.06	5.12	66.89
<i>Delturus brevis</i>			8.14	0	5.06	71.94
<i>Astyanax fasciatus</i>			7.45	3.83	4.43	76.38
<i>Megaleporinus elongatus</i>			3.56	4.35	3.79	80.17
<i>Trachelyopterus galeatus</i>			2.04	4.8	3.27	83.43
<i>Leporinus steindachneri</i>			1.18	4.42	3.04	86.47
<i>Astyanax bimaculatus</i>			0.84	4.14	2.52	89
<i>Pareiorhaphis</i> sp.			3.24	0	2.01	91.01

Discussion

The trophic structure and species composition before the dam construction were similar upstream and downstream. After the dam closure, both aspects of the assemblages changed on the lotic stretches upstream and downstream. Changes in the river flow could have altered the trophic structure downstream of the dam. In this area, three trophic groups were affected: piscivores, omnivores increasing their abundance and detritivores decreasing their abundance. However, the fish assemblage on the upstream river stretch might have been impacted by other factors, such as the blockage of migration by the dam. The detritivores were the most affected group upstream of the reservoir increasing their abundance.

The blockage of migration routes can lead to agglomeration of fish downstream of dams (Mérona et al. 2001), increasing the availability of fish as food resource. After the dam closure, we observed an increase of the relative biomass of piscivores downstream, the same pattern was observed in other dams (Mérona et al. 2001; Delariva et al. 2013; Peressin et al. 2016). Alterations in the water level and transparency can also make fish more vulnerable to predation (Granzotti et al. 2018) favouring visual predators.

We also observed an increase of omnivores after the installation of the dam in the downstream area. Some studies comparing fish diet on a spatio-temporal scale (river/reservoir and pre and post impoundment) show that fish tend to restrict their feeding spectrum post impoundment, changing their trophic guild, leading to a decrease in omnivores abundance (Mérona et al. 2001, 2005; Delariva et al. 2013). However, it is important to highlight that this study focused on the lotic areas upstream and downstream of dams, and that we did not evaluate the changes in trophic guilds by comparisons of stomach content analysis before and after the impoundment, like other studies. Even though changes in trophic guilds were not assessed, the increase in the abundance of the omnivores can reflect their generalist feeding habits, preying on a broad spectrum of food resources (Gerking 1994), and then using resources according to their availability on the river. In that case, the shift on the river's available resources would not affect the resource availability for these species, as observed for a fish assemblage under peak flow due to reservoir operation (Gandini et al. 2014).

The decrease of detritivores relative abundance downstream of the dam observed in our study, was also found downstream of the Tucurui Dam (Mérona et al. 2001), and detritus were the less consumed food item on the river downstream of 12 hydroelectric power plants (Peressin et al. 2016). Impoundments affect the reproductive aspects of migratory species (Agostinho et al. 2004), and flow alterations can affect negatively detritivore species (Abujanra et al. 2009). Dams trap sediments and nutrients in the reservoir (Baxter 1977; Roberto et al. 2009), decreasing turbidity and nutrient load modifying water quality downstream (Agostinho et al. 2008). These fish have morphological adaptations to detritivory (Bowen 1983), and therefore they were unfavoured on food resources downstream. In our study, *Prochilodus hartii*, a detritivore migratory species, had lower abundances after the construction of the dam. On upper

Paraná River, populations of *Prochilodus lineatus* were unable to colonize marginal lagoons due to flow regulation (Gubiani et al. 2007). On Jequitinhonha river, the blockage of migration has a negative effect on reproductive aspects of *P. hartii* (Abdo et al. 2018), and the low quality of foraging resources and inadequate spawning areas may have led to the decrease of this species biomass downstream of the dam.

Upstream of the reservoir, changes on the trophic structure are not expected to occur due to changes on resources availability and could be explained by interferences of the hydropower on the migratory fish movements. If the breeding sites of *P. hartii* are located upstream, the barrier represented by the reservoir (Pelicice et al. 2015) could be confining most of the population in such a region. Moreover, the diminishing in the relative abundance of other long migratory species, such as *Megaleporinus garmani* and *Pseudoplatystoma* sp. could also be related to the impact of the dam and reservoir on their migrations.

The construction of the Irapé hydropower plant has led to changes on the trophic structure on lotic river stretches both upstream of the reservoir and downstream of the dam. Therefore, impoundments will cause drastic changes not only in the reservoir area but also to the adjacent areas that were not affected by the transition from lotic to lentic regimes. The trophic structure changed by changes in the species composition, that consequently altered species abundance in both upstream and downstream river stretches. The comprehension of the most affected trophic groups on the lotic stretches adjacent to dams is useful to determine conservation strategies for the fish fauna. Hydropower plants could implement artificial seasonal flows, to emulate natural variation of flow and reduce the effects of flow regulation downstream. Our results also hinders the use of upstream areas as a control for changes in the downstream ones.

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S1 Bibliographic review and Alimentary Index (IAi) for classification on trophic guilds

Species	Trophic guild	Reference	n	Obs	Most consumed items	n (IAi)	IAi (%)
<i>Astyanax bimaculatus</i> (Linnaeus, 1758)	Herbivore	Luiz EA, Agostinho AA, Gomes LC, Hahn NS (1998) Ecologia trófica de peixes em dois riachos da bacia do rio Paraná. Rev. Bras. Biol. 58:273–285	67	Plant remains	10	Filamentous Algae - 59.93	
<i>Astyanax fasciatus</i> (Cuvier, 1819)	Omnivore	Vilella FS, Becker FG, Hartz SM (2002) Diet of <i>Astyanax</i> species (Teleostei, Characidae) in an Atlantic Forest River in Southern Brazil. Brazilian Arch Biol Technol 45:223–232. https://doi.org/10.1590/S1516-89132002000200015	34	Algae, insect remains, plant remains	-	-	
<i>Brycon devillei</i> (Castelnau, 1855)	Omnivore	Azevedo PG, Melo RMC, Young RJ (2011) Feeding and social behavior of the piabanha, <i>Brycon devillei</i> (Castelnau, 1855) (Characidae: Bryconinae) in the wild, with a note on following behavior. Neotrop Ichthyol 9:807–814. https://doi.org/10.1590/S1679-62252011005000046		Observations of feeding in loci	-	-	
<i>Brycon howesi</i> Lima, 2017	Omnivore	Gomiero LM, Manzatto AG, Braga FM de S (2008) The role of riverine forests for food supply for the omnivorous fish <i>Brycon opalinus</i> Cuvier, 1819 (Characidae) in the Serra do Mar, Southeast Brazil. Braz J Biol 68:321–328	256	Study with congeneric species (<i>B. opalinus</i>)	Insects, fruit and seeds	-	-
<i>Delturus brevis</i> Reis & Pereira, 2006	Detritivore	-	-	-	-	10	Detritus - 99.93
<i>Geophagus brasiliensis</i> (Quoy & Gaimard, 1824)	Insectivore	de Moraes MFPG, de Freitas Barbola I, Duboc LF (2004) Feeding habits and morphometry of digestive tracts of <i>Geophagus brasiliensis</i> (Osteichthyes, Cichlidae), in a lagoon of high Tibagi River, Paraná State, Brazil. Publ UEPG Ciências biológicas e da saúde 10:	83	Ephemeroptera, Odonata, Trichoptera and Diptera larvae	Diptera - 28.40 Insect Fragments - 17.79 Trichoptera - 6.75	10	Diptera - 28.40 Insect Fragments - 17.79 Trichoptera - 6.75
<i>Gymnotus carapo</i> Linnaeus, 1758	Insectivore	da Rocha FC, Casatti L, Pereira DC (2009) Structure and feeding of a stream fish assemblage in Southeastern Brazil: evidence of low seasonal influences. Acta Limnol Bras 123–134	15	Aquatic insect debris	Odonata - 94.36 Hemiptera - 5.64	4	Odonata - 94.36 Hemiptera - 5.64
<i>Harttia garavelloii</i> Oyakawa, 1993	Detritivore	Sa-Oliveira JC, Angelini R, Isaac-Nahum VJ (2014) Diet and niche breadth and overlap in fish communities within the area affected by an Amazonian reservoir (Amapá, Brazil). An Acad Bras Cienc 86:383–406	12	Study with congeneric species (<i>H. duriventris</i>)	Detritus	6	Detritus - 100
<i>Hoplias brasiliensis</i> (Spix & Agassiz, 1829)	Piscivore	Carvalho LN, Fernandes CHV, Moreira VSS (2002) Alimentação de <i>Hoplias malabaricus</i> (Bloch, 1794)(Osteichthyes, Erythrinidae) no rio Vermelho, Pantanal Sul Mato-Grossense. Rev Bras Zoociências 4:	40	Study with congeneric species (<i>H. malabaricus</i>)	Fish Remains	4	Fish remains - 99.41
<i>Hoplias malabaricus</i> (Bloch, 1794)	Piscivore	Carvalho LN, Fernandes CHV, Moreira VSS (2002) Alimentação de <i>Hoplias malabaricus</i> (Bloch, 1794)(Osteichthyes, Erythrinidae) no rio Vermelho, Pantanal Sul Mato-Grossense. Rev Bras Zoociências 4:	40	Fish Remains	3	Fish remains - 99.57	

S1 (continued)

Species	Trophic guild	Reference	n	Obs	Most consumed items	n (IAi)	IAi (%)
<i>Hypostomus</i> sp. Lacepède, 1803	Detritivore	Villares-Junior GA, Cardone IB, Goitein R (2016) Comparative feeding ecology of four syntopic Hypostomus species in a Brazilian southeastern river. <i>Braz J Biol</i> 76:692–699	582		Particulate Organic material	8	Detritus - 98.46
<i>Leporinus steindachneri</i> Eigenmann, 1907	Omnivore	Hahn NS, Agostinho AA, Gomes LC, Bini LM (2018) Estrutura trófica da ictiofauna do reservatório de Itaipu (Paraná-Brasil) nos primeiros anos de sua formação			Vegetal items	-	-
<i>Megaleporinus elongatus</i> (Valenciennes, 1850)	Insectivore	Balassa GC, Fugi R, Hahn NS, Galina AB (2004) Dieta de espécies de Anostomidae (Teleostei, Characiformes) na área de influência do reservatório de Manso, Mato Grosso, Brasil. <i>Iheringia Ser Zool</i> 94:77–82	24		Chironomidae larvae	-	-
<i>Megaleporinus garmani</i> (Borodin, 1929)	Herbivore	Araújo RTN de (2015) ALIMENTAÇÃO DA ICTIOFAUNA DA REGIÃO DE INFLUÊNCIA DE UM RESERVATÓRIO DO ALTO JEQUITINHONHA, MG. PUC Minas	89		Vegetal remains	10	Algae - 51.93 Macrophyte - 37.12
<i>Moenkhausia intermedia</i> Eigenmann, 1908	Omnivore	Esteves KE, Galetti Jr PM (1994) Feeding ecology of Moenkhausia intermedia (Pisces, Characidae) in a small oxbow lake of Mogi-Guaçu river, São Paulo, Brazil. <i>Int Vereinigung für Theor und Angew Limnol Verhandlungen</i> 25:2198–2204	179		Organic matter, insects and zooplankton	-	-
<i>Moenkhausia costae</i> (Steindachner, 1907)	Omnivore	Esteves KE, Galetti Jr PM (1994) Feeding ecology of Moenkhausia intermedia (Pisces, Characidae) in a small oxbow lake of Mogi-Guaçu river, São Paulo, Brazil. <i>Int Vereinigung für Theor und Angew Limnol Verhandlungen</i> 25:2198–2204	179	Study with congeneric species (<i>M. intermedia</i>)	Organic matter, insects and zooplankton	6	Fish scale - 61.51 Insect remains - 35.15
<i>Oligosarcus macrolepis</i> (Steindachner, 1877)	Piscivore	Nunes DM, Hartz SM (2006) Feeding dynamics and ecomorphology of Oligosarcus jenynsii (Gunther, 1864) and Oligosarcus robustus (Menezes, 1969) in the Lagoa Fortaleza, southern Brazil. <i>Braz J Biol</i> 66:121–132	405	Study with congeneric species (<i>O. robustus</i>)	Fishes	-	-
<i>Pareiorhaphis</i> sp. Miranda Ribeiro, 1918	Detritivore	Dias TS, Fialho CB (2011) Comparative dietary analysis of Eurycheilichthys pantherinus and Pareiorhaphis hystrix: two Loricariidae species (Ostariophysi, Siluriformes) from Campos Sulinos biome, southern Brazil. <i>Iheringia Ser Zool</i> 101:49–55	60	Study with congeneric species (<i>P. hystrix</i>)	Detritus	-	-
<i>Pareiorhaphis stephanus</i> (Oliveira & Oyakawa, 1999)	Detritivore	Dias TS, Fialho CB (2011) Comparative dietary analysis of Eurycheilichthys pantherinus and Pareiorhaphis hystrix: two Loricariidae species (Ostariophysi, Siluriformes) from Campos Sulinos biome, southern Brazil. <i>Iheringia Ser Zool</i> 101:49–55	60	Study with (<i>P. hystrix</i>)	Detritus	9	Detritus 89.64
<i>Pimelodella</i> sp. Eigenmann & Eigenmann, 1888	Omnivore	Viana LF, dos Santos SL, Lima-Junior SE (2006) Variação sazonal na alimentação de Pimelodella cf. gracilis (Osteichthyes, Siluriformes, Pimelodidae) no rio Amambai, Estado de Mato Grosso do Sul. <i>Acta Sci Biol Sci</i> 28:123–128	232	Study with congeneric species (<i>P. gracilis</i>)	Terrestrial insects, plants	-	-

S1 (continued)

Species	Trophic guild	Reference	n	Obs	Most consumed items	n (IAi)	IAi (%)
<i>Prochilodus argenteus</i> Spix & Agassiz, 1829	Detritivore	Bowen SH (1983) Detritivory in neotropical fish communities. Environ Biol Fishes 9:137–144. https://doi.org/10.1007/BF00690858			Detritus	-	-
<i>Prochilodus costatus</i> Valenciennes, 1850	Detritivore	Bowen SH (1983) Detritivory in neotropical fish communities. Environ Biol Fishes 9:137–144. https://doi.org/10.1007/BF00690858			Detritus	-	-
<i>Prochilodus hartii</i> Steindachner, 1875	Detritivore	Bowen SH (1983) Detritivory in neotropical fish communities. Environ Biol Fishes 9:137–144. https://doi.org/10.1007/BF00690858			Detritus	10	Detritus 99.78
<i>Prochilodus</i> sp. Agassiz, 1829	Detritivore	Bowen SH (1983) Detritivory in neotropical fish communities. Environ Biol Fishes 9:137–144. https://doi.org/10.1007/BF00690858			Detritus	-	-
<i>Pseudoplatystoma</i> sp. Bleeker, 1862	Piscivore	Barbarino Duque A, Winemiller KO (2003) Dietary segregation among large catfishes of the Apure and Arauca Rivers, Venezuela. J Fish Biol 63:410–427	575		Fish	-	-
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	Omnivore	Pagotto JPA, Goulart E, Oliveira EF, Yamamura CB (2011) Trophic ecomorphology of Siluriformes (Pisces, Osteichthyes) from a tropical stream. Braz J Biol 71:469–479	14		Plants and terrestrial insects	-	-
<i>Serrasalmus brandtii</i> Lütken, 1875	Piscivore	Pompeu PDS (1999) Dieta da pirambeba <i>Serrasalmus brandtii</i> Reinhardt (Teleostei, Characidae) em quatro lagoas marginais do rio São Francisco, Brasil. Rev Bras Zool 16:19–26. https://doi.org/10.1590/S0101-81751999000600003	212		Fish, fin, and fish scales	10	Fish remains - 86.96
<i>Steindachneridion amblyurum</i> (Eigenmann & Eigemann, 1888)	Piscivore	Agostinho AA, Gomes LC, Suzuki HI, Júlio-JR HF (2003) Migratory fishes of the Upper Paraná River Basin. In: Migratory Fishes of South America. pp 23–78			Fish	-	-
<i>Steindachnerina elegans</i> (Steindachner, 1875)	Detritivore	de Amorim Teixeira JL, Gurgel H de CB (2004) Dinâmica da nutrição e alimentação natural de <i>Steindachnerina notonota</i> (Miranda-Ribeiro, 1937) (Pisces, Curimatidae), Açude de Riacho da Cruz, Rio Grande do Norte, Brasil. Rev Bras Zoociências 6:	33	Study with congeneric species (<i>S. notonota</i>)	Detritus, sediment	-	-
<i>Trachelyopterus galeatus</i> (Linnaeus, 1766)	Omnivore	Santin M, Lopes TM, Baggio MM, et al (2015) Mudanças ontogenéticas no trato digestório e na dieta de <i>Trachelyopterus galeatus</i> . Bol do Inst Pesca 41:57–68	61		Aquatic insects, microcrustaceans, plant remains, fish larvae	10	Plant remains - 45.11 Fish remains - 36.68
<i>Wertheimeria maculata</i> Steindachner, 1877	Omnivore	Vono V, Birindelli JLO (2007) Natural history of <i>Wertheimeria maculata</i> , a basal doradid catfish endemic to eastern Brazil (Siluriformes: Doradidae). Ichthyol. Explor. Freshwaters 18:183–191	106		Plant material, terrestrial insects, aquatic macroinvertebrates, fish scales and macrozooplankton	10	Plant Remains - 33.85 Odonata - 25.47 Fish remains - 15.40 Fish scale - 12.49

MANUSCRITO 2**Sampling sufficiency in stomach content surveys**

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Abstract

This work aims to estimate the number of stomachs required to describe a fish species diet through cumulative prey curves and using the Alimentary Index to define trophic groups. The stomach content of three species were analysed, *Megaleporinus garmani* (174), *Trachelyopterus galeatus* (99) and *Wertheimeria maculata* (68). The cumulative curve for prey items did not reach an asymptote for any of the species. However, comparing with the richness estimators used, the observed prey richness achieved ranged between 73 to 91% for all the species. Considering the Alimentary Index, 30 to 60 stomachs indicate adequately the predominant items on the diet. To determine a feeding guild a lower number of stomachs (25 to 35) were required.

Keywords Alimentary Index, Feeding, Fish diet, Methodology, Richness estimators, Sample size.

Introduction

The analysis of gut contents provides information on fish feeding patterns, that comprise species interactions, population dynamics between predator and preys, energy intake and transfer (Brodeur *et al.*, 2017; Manko, 2016). Feeding studies on fish enlightens aspects of fish ecology on individual, population and ecosystem levels. The broad applications of such studies led to several approaches and techniques to analyse this type of data (Manko, 2016). A series of reviews focusing on methodology and statistical analysis (Cortés, 1997; Ferry and Cailliet, 1996; Hahn and Delariva, 2003; Hyslop, 1980) have compiled the various stomach content analysis methods highlighting their strengths and weakness.

One type of approach to stomach content analysis is based on the use of compound indices, consisting in the combination of two or more measures such as number, mass, volume and occurrence of the items, in order to express more information (Sagar *et al.*, 2018). The Alimentary Index (Kawakami and Vazzoler, 1980) is based on the frequency of occurrence and the volume of food items, and presents the relative importance of each item of the diet. Besides analysing the predominant items present on the diet, the diet breadth can also be studied, assessing all the prey items consumed by a species.

The food items can be accessed by estimating the richness of those items in the species stomach. Species richness may be defined as the number of species present in a particular community; this is one of the simplest measures of diversity used to characterize a community (Chao, 2006). Although the simplicity of species richness metric, data sampling to measure this variable is usually difficult. Richness observed in

the samples tends to underestimate the real number of species present in the community (Gotelli and Colwell, 2011). The sampling effort required to collect all species of a community is hard to obtain in practice (Chao and Chiu, 2014). Due to these difficulties, some approaches allow the estimation of species richness in a community, enabling to compare species richness among different communities. The non-asymptotic methods, rarefaction and extrapolation, allow comparison between samples of standardized size. In the asymptotic method, we estimate the value at which the curve of accumulation of species reaches its asymptote, and this value is used to compare different communities (Chao and Chiu, 2014).

Given the impacts of anthropogenic pressures on natural communities, species richness is a metric often used in efforts to conserve biodiversity (Gotelli and Chao, 2013). Despite the great importance and diverse applications about richness estimation, few studies in the literature address richness estimation for fish diet (see Côté *et al.*, 2013). In addition, after reviewing 200 papers about fish feeding, Ferry and Cailliet (1996) stated that none of the papers used any technique to determine if a sufficient size sample was used to make comparisons between diets. Therefore, this work aims to use three fish species (*Megaleporinus garmani* (Borodin, 1929), *Trachelyopterus galeatus* (Linnaeus, 1766) and *Wertheimeria maculata* (Steindachner, 1877)) as models to (i) apply richness estimation methods for food items using species accumulation curve and (ii) estimate the necessary number of individuals for properly calculate the Alimentary Index and define trophic groups.

Material and Methods

Study area The Jequitinhonha River basin drains an area of approximately 70,315 km² taking place on the states of Minas Gerais and Bahia, Brazil. Mean annual rainfall

averages around 600 and 1.600 mm irregularly distributed throughout the year. Rainfall season occurs between October and March (IBGE, 1997). The Jequitinhonha River rises in the Espinhaço complex mountain range in Serro - MG and runs 1,086 km to the municipality of Belmonte - BA where it flows into the Atlantic Ocean (Guerrero, 2009). Two of the the main tributaries on the left bank are the Itacambiruçu and Vacaria rivers (IBGE,1997). Three species that occur in the Jequitinhonha River basin were used in this study: *Megaleporinus garmani*, *Trachelyopterus galeatus* and *Wertheimeria maculata*. Such species have been classified as an obligate digger omnivorous (Azevedo *et al.*, 2011), omnivorous with a tendency to herbivory (Santin *et al.*, 2015) and omnivorous with a tendency to herbivory (Vono and Birindelli, 2007) respectively.

Data sampling. Fish sampling took place on Jequitinhonha, Itacambiruçu and Vacaria rivers between 2011 to 2013, 2015 and 2017 to 2018 (Table 1).

Table 1. Sampling points

Sampling Point	River	Location
IR1	Jequitinhonha	S17°18'44.8" 43°12'14.9"
IR2	Jequitinhonha	S16°46'25.8" W42°38'47.8"
IR3	Itacambiruçu	S16°43'44.3" W42°40'49.1"
IR4	Jequitinhonha	S16°44'20.6" W42°34'02.3"
IR5	Jequitinhonha	S16°62'54.9" W42°26'82.8"
IR6	Vacaria	S16°38'37.0" W42°47'59.0"
IR7	Itacambiruçu	S16°35'56.4" W42°50'07.9"
IR8	Jequitinhonha	S16°44'06.9" W42°34'34.1"
IR9	Itacambiruçu	S16°32'08.2" W42°47'51.9"

For fish collection, we used gillnets in sizes 2.4, 3, 4, 5, 6, 7, 8, 10, 12, 14 and 16 cm between opposite nodes. The effort of each mesh was 30 m, except for the 2.4 mesh net that used 20 m. The nets were exposed for 12 hours. We fixed the individuals in formaldehyde and preserved them in alcohol in the laboratory. Specimens were measured (total and standard length), weighed, and then dissected to remove stomachs, which were

stored in 70% alcohol solution. Under a stereomicroscope, stomach contents were analysed to the lowest possible taxonomic level. Identification was made with the aid of the bibliography (Costa *et al.*, 2006; Mugnai *et al.*, 2010; Ward and Whipple, 1918). The wet weight of the items was determined with a precision scale (Ohaus Adventurer, AR 2140 - 0,0001g).

Data analysis. To determine the importance of each food item, the food index (IAi) (Kawakami and Vazzoler, 1980) was calculated, but the volume of the items was replaced by weight (Bennemann *et al.*, 2006) as shown in the following formula:

$$\text{IAi} = \frac{\text{Fi} \times \text{Pi}}{\sum_{i=1}^n (\text{Fi} \times \text{Pi})} \times 100 \text{ Where,}$$

IAi: Alimentary Index

Fi: Frequency of occurrence (%) of food item i

Pi: Weight (%) of food item i

To create the graph of the alimentary index (IAi), we calculated cumulatively the IAi for the stomachs analysed in a random order, considering the items that accounted for 90% of the alimentary index for the species at the end of the analysis. The items were grouped on the following categories: Algae, Cladocera (macrozooplankton), filamentous algae, fish remains, fish scale, Hymenoptera, insect remains, macrophytes, Odonata, plant remains and sediment. We also created graphics cumulatively integrating the IAi into five feeding guilds: detritivore, invertivore, herbivore, piscivore and zooplantctivore. The graphics were made with R software (R Development Core Team, 2015) with the ggplot2 package (Wickham, 2016).

Species accumulation curves were constructed by adding cumulatively the items found in the stomachs, in order to verify the sample sufficiency for the three species. The addition order of stomachs to the analysis can interfere with the shape of the curve

(Colwell and Coddington, 1994), so sampling was randomized 100 times. Richness values and richness estimators were calculated with Estimate S v 9.1.0 software (Colwell, 2013) and the graphics were made with R software (R Development Core Team, 2015) with ggplot2 package (Wickham, 2016). Three non-parametric estimators were used: Chao2, Jackknife 1 and bootstrap. These estimators focus on rare items in the sample to calculate the frequency of items that were not sampled (Gotelli and Chao, 2013). We did not count the number of individuals ingested in each stomach, so the estimators were based on the incidence of the different items in each stomach. The Chao2 estimator takes into account the uniques (items present in only one stomach) and the duplicates, (items present in two stomachs); the estimator of Jackknife 1 considers only the presence of uniques. Bootstrap uses frequency data of all items to calculate richness. Such non-parametric estimators have presented robust results in the analyses, especially Jackknife and Chao (Chao and Chiu, 2014; Walther and Moore, 2005).

Results

We analysed 192 stomachs of *Megaleporinus garmani*, 157 of *Trachelyopterus galeatus*, and 86 of *Wertheimeria maculata*. Empty stomachs were removed from the analysis, remaining 174, 99 and 68 respectively. *M. garmani* ingested 22 different food items, *T. galeatus* 19 and *W. maculata* 17 (Table 2).

We used the most detailed categories of food items to construct the species accumulation curve, and none of them reached an asymptote (Figure 1). For *M. garmani* the number of prey found in one (uniques) or two (duplicates) stomachs were stable at maximum sample size, and the curves for the richness asymptotic estimators continued to increase. For *T. galeatus* and *W. maculata*, the number of uniques started to decrease and the number of duplicates was stable. At maximum sample size, Chao 2 and Jackknife

estimators still present higher values than observed richness and tend to plateau at approximately 60 sampled stomachs of *T. galeatus* and at 35 sampled stomachs for *W. maculata*. Considering the three asymptotic richness estimators, observed prey richness ranged between 73 – 87% for *M. garmani*, 82 – 90% for *T. galeatus* and 85 – 91% for *W. maculata*. Bootstrap was the only richness estimator within the confidence interval for the three analysed species.

Table 2. Alimentary index for the food items each species consumed

Item	Alimentary index (%)		
	<i>Megaleporinus garmani</i>	<i>Trachelyopterus galeatus</i>	<i>Wertheimeria maculata</i>
Acari	<0.01%	<0.01%	
Algae	77.64%	0.05%	
Algae (Filamentous)			5.07%
Amphipoda	<0.01%		
Bivalvia	<0.01%		
Cladocera	<0.01%		4.09%
Coleoptera	<0.01%	3.77%	
detritus	<0.01%		
Diptera	1.30%	<0.01%	1.53%
Ephemeroptera	0.29%	0.04%	0.42%
Fish remains	<0.01%	21.35%	23.00%
Fish scale	<0.01%	4.28%	19.58%
Hemiptera	<0.01%	0.03%	
Hymenoptera	<0.01%	42.31%	2.35%
Insect remains		23.19%	19.48%
Lepidoptera	<0.01%		
Macrophyte	8.65%		1.16%
Nematoda		0.03%	
Odonata	<0.01%	0.66%	6.76%
Orthoptera		0.36%	
Ostracoda	<0.01%		<0.01%
Plant remains	0.22%	3.83%	12.85%
Plecoptera		<0.01%	
Sediment	9.82%	0.05%	1.62%
Trichoptera	0.04%	0.03%	<0.01%
Unknown	1.98%	0.02%	2.05%
Zooplankton	<0.01%		0.03%

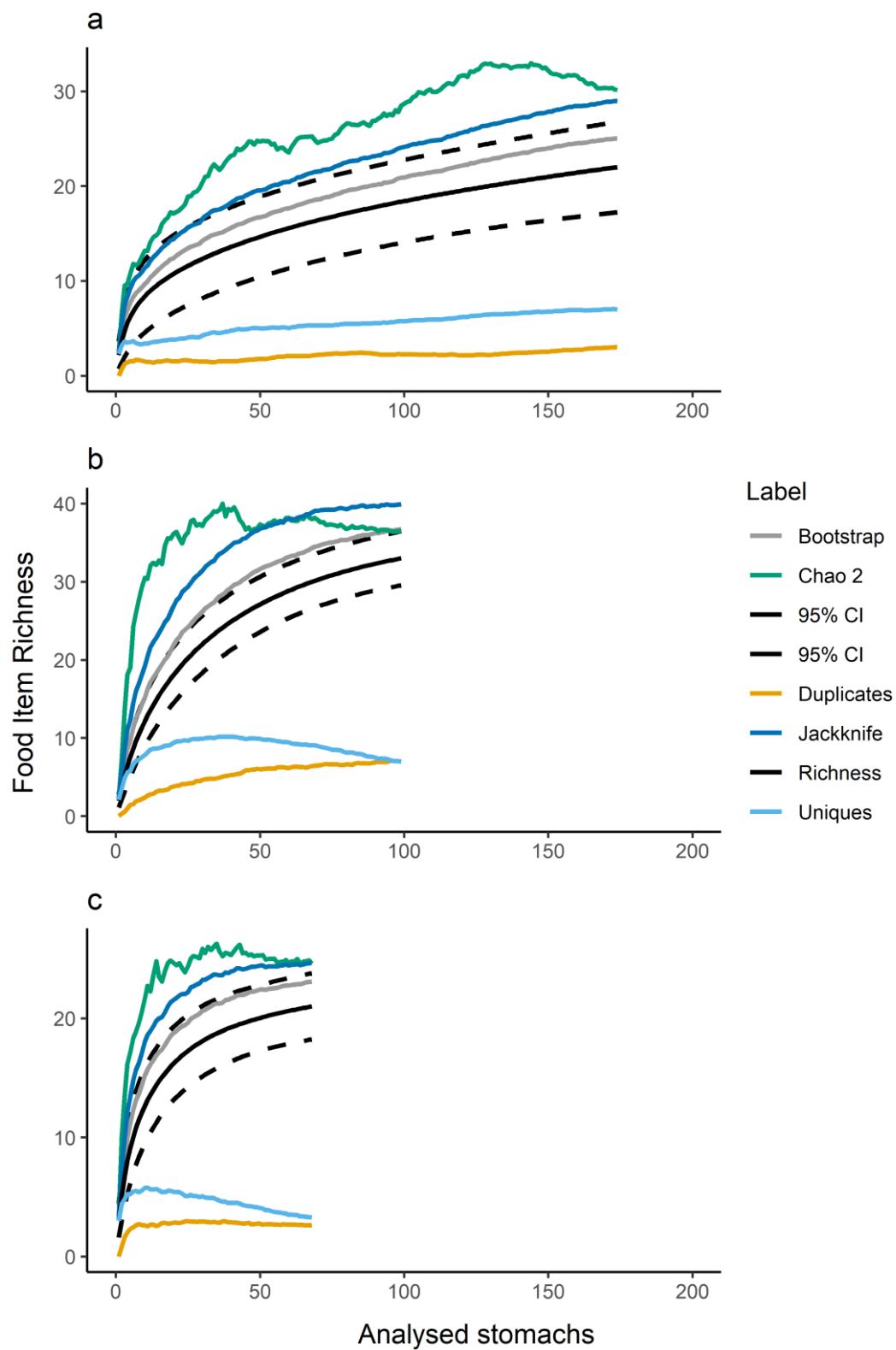


Figure 1. Richness estimation of food items to (a) *Megaleporinus garmani*, (b) *Trachelyopterus galeatus*, and (c) *Wertheimeria maculata*. CI – 95% Confidence interval

The alimentary index graphics show the variation of the items that were more important for each species. The alimentary index stabilized after the analysis of 30 stomachs for *M. garmani*, 60 stomachs for *T. galeatus* and 35 for *W. maculata*. Still, the trophic guilds of the species stabilized after the analysis of 25 stomachs for *M. garmani*, 25 for *T. galeatus* and 35 for *W. maculata* (Figure 2).

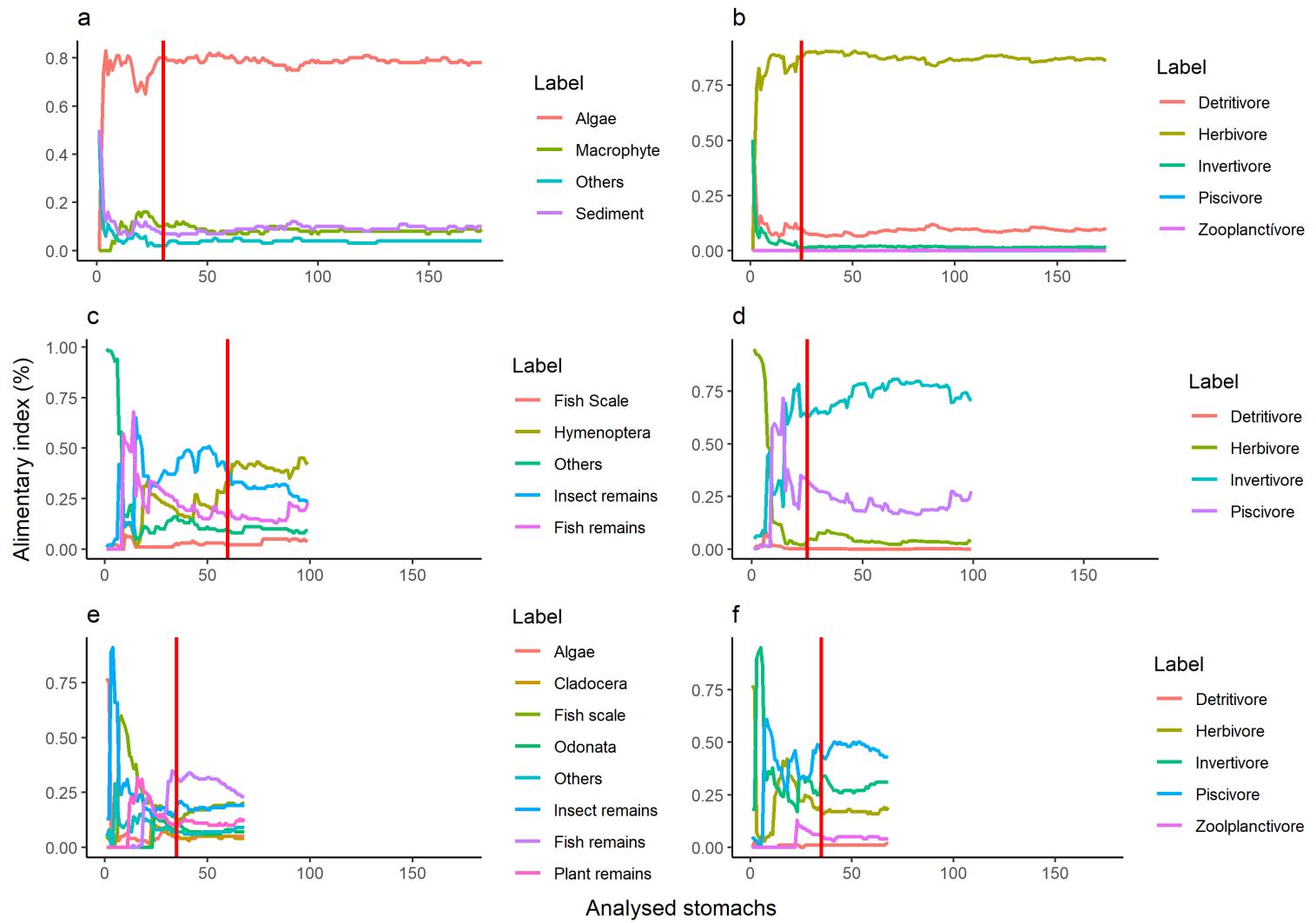


Figure 2. Alimentary Index for food items (left column) and for trophic guilds (right column) for *Megaleporinus garmani* (a, b), *Trachelyopterus galeatus* (c, d), and *Wertheimeria maculata* (e, f). The red lines indicate the number of stomachs needed to stabilize the variations.

Discussion

The accumulation curves for food item richness did not reach an asymptote for any of the three species considered. However, when a broader category is considered, from 30 to 60 stomachs would produce a satisfactory alimentary index, and a lower number (25 to 35) would suffice to determine a trophic guild. These estimations were also species-dependent, with a lower number of necessary stomachs for the more specialised species (*M. garmani*).

Several authors use cumulative prey curves to determine sample size sufficiency (Eddy *et al.*, 2016; Karpov and Cailliet, 1979; Logan *et al.*, 2013; Pielou, 1966). The asymptote of the prey curve would indicate the required number of stomachs to describe the diet, and a quantitative method to evaluate the slope of the curve was developed (Bizzarro *et al.*, 2007). Complete sampling of prey items is especially useful when stomach contents may offer a useful sampling tool in studies of benthic macroinvertebrates, especially in large, deep, fast rivers that are difficult to sample with traditional sediment sampling gear (Tupinambás *et al.*, 2015). Also, when stomach content analysis is used to assess the impact of invasive species on preys of the invaded habitat (Côté *et al.*, 2013). However, it is harder to reach sample sufficiency since fish feed on various food items. Chao *et al.* (2009) demonstrated through simulations that the sampling effort needed to collect all the rare species in a community is substantially large. An alternative to this issue can be aiming to achieve a lower percentage of species richness, such as 95%, that would yield more realistic sampling effort since a complete sampling is hardly achieved with real data samplings (Chang and Colwell, 2005; Walther and Martin, 2001).

Based on the asymptotic richness estimators, the estimated richness was 12 to 26%

higher than observed richness for *M. garmani*, 9 to 17% higher for *T. galeatus* and 8 to 14% higher to *W. maculata*. The species that had the observed richness values closer to estimated richness (*M. garmani* and *W. maculata*) also presented decreasing number of uniques towards maximum sampling size. The number of uniques decreases with increased sampling, and when all uniques disappear, it can be assumed that total richness has been achieved (Walther and Moore, 2005).

The level of taxonomic detail of the prey species influenced on the number of stomachs needed to stabilize variations of their determination, and as expected, the detailed categories on the Alimentary Index analysis required more stomachs than the trophic guilds categories. Fewer samples are required to describe prey diversity than to describe prey richness (Dunn, 2009). It is important to point out that since the broad classification of food items synthesize information, the number of stomachs required to stop variation can be small and may not represent true diet.

Depending on the aim of the work, such as assessing prey assemblage, the most detailed categories are useful for presenting the diet breadth. Studies that aim at describing the predominant items of the diet can use the cumulative plot of alimentary indices of the sampled stomachs in order to find the sample size needed to describe feeding guilds. Even though it is not always possible to reach optimal sample size, it is important to take in consideration techniques to evaluate sample size sufficiency in order to achieve more reliable, comparative and robust results.

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