



## EFFECTS OF ENVIRONMENTAL FEATURES ON FISH ASSEMBLAGE STRUCTURES IN THE MACHADO RIVER, AMAZON BASIN, BRAZIL

### EFEITOS DAS VARIÁVEIS AMBIENTAIS NAS ESTRUTURAS DA ASSEMBLÉIA DE PEIXES NO RIO MACHADO, BACIA AMAZÔNIA, BRASIL

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**RESUMO** Neste estudo, examinamos a influência das características ambientais de três microbacias do rio Machado na distribuição de comunidades de peixes, verificando se elas são influenciadas por fatores abióticos. A amostragem foi realizada em riachos de primeira ordem nas microbacias do rio Machado, Estado de Rondônia, Brasil. Em cada fluxo, selecionamos dois trechos (150 cada) no canal principal. Os peixes foram capturados com diferentes tamanhos de redes. Após a coleta, os peixes foram armazenados em sacos de plástico, para posterior identificação taxonômica. Em cada local, as variáveis físico-químicas da água (temperatura, oxigênio dissolvido, pH e condutividade elétrica) foram medidos. Assim, abundância, riqueza e diversidade de Hill foram estimados. A análise multivariada Permutacional da variância (Permanova) foi aplicada para determinar efeitos na abundância, riqueza e diversidade, considerando como variáveis explicativas as microbacias, parâmetros físico-químicos da água e a sazonalidade. Uma análise de redundância baseada na distância (dBRDA) foi realizada para determinar a influência das propriedades físico-químicas da água nas três microbacias e seu efeito na composição dos peixes. Foram amostrados 6.432 indivíduos pertencentes a 4 ordens, 19 famílias e 56 espécies. *Aequidens tetramerus*, *Serrapinnus* cff. *microdon* e *Serrapinnus* aff. *notomelas* foram as espécies mais abundantes. A condutividade foi a única variável que apresentou diferença significativas com as três variáveis respostas analisadas. A dBRDA indicou que 44,57% da variância, foi explicada pelas variáveis utilizadas e estas apresentaram correlação significativa com a abundância e composição da ictiofauna ( $F = 1.88$ ,  $p = 0.035$ ), sendo a condutividade e o pH os fatores mais relevantes. Uma associação significativa entre as comunidades piscícolas e os fatores abióticos sustenta a hipótese de que os padrões em larga escala nas comunidades piscícolas em pequenos córregos Amazônicos são, pelo menos em parte, estruturados por condições abióticas.

**Palavras-chave:** Rio Madeira, microbacias, córregos.

**ABSTRACT** In this study, we examined the influence of the environmental characteristics of three microbasins of the Machado River on the distribution of fish assemblages in order to predict if its fish communities are influenced by abiotic factors. Sampling was conducted in first order streams in the microbasins of the Machado River, state of Rondônia, Brazil. In each stream, we selected two 150 m-long stretches on the main channel. The fish were sampled with different sizes of nets, stored in plastic bags, and taxonomically identified. At each site, the physiochemical parameters of the water (temperature, dissolved oxygen, pH, and electrical conductivity) were measured. Thus, abundance, richness and Hill's diversity were estimated. The Permutational Multivariate Analysis of Variance (Permanova) was applied to determine the effect in fish abundance, richness and diversity, considering microbasins, physio-chemical water and seasons as explanatory variables. A distance-based redundancy analysis (dBRDA) was performed to determine the influence of physio-chemical properties of the water across the three microbasins and their effect on fish composition. We sampled a total of 6,432 individuals belonging to 4 orders, 19 families, and 56 species. *Aequidens tetramerus*, *Serrapinnus* cff. *microdon* and *Serrapinnus* aff. *notomelas* were the most abundant species. Conductivity was the only explanatory variable in which a significant difference was detected for the three response variables. The dBRDA indicated that 44.57% of the total variance was explained by the variables used and were significantly correlated with the abundance and composition of the ichthyofauna ( $F = 1.88$ ,  $p = 0.035$ ), conductivity and pH were the most relevant factors. A significant association between fish communities and abiotic factors supports the hypothesis that broad-scale patterns in fish communities in Amazonian small streams are, at least in part, structured by abiotic conditions.

**Key words:** Madeira River, microbasin, streams.

## Introdução

Despite the environmental heterogeneity and dynamism of streams, some studies appear to have explored the variability of the fish assemblages found in this type of ecosystem in the Amazon. Our understanding of this dynamism is based on mesoscale (e.g. river basin location, rainfall regime) and microscale characteristics (e.g., water depth, current velocity, and substrate) (Gosselin, Maddock & Petts, 2012), which influence the abundance and composition of fish species (Florentino et al., 2016; Wegscheider, Linnansaari & Curry, 2020). In this way, the wet season, which occurs on the macroscale (affected locally by the rainfall regime), is a determining factor of hydrologic variation in Amazonian streams (as flood pulse), which as a consequence promotes abrupt changes in the local basin (Hurd et al., 2016). These changes cause mixing in the substrate of local microhabitats, and alter the physicochemical characteristics that are an important environmental source for fish breeding. At the same time, these habitat modifications can eliminate sensitive species and thereby alter the organization of fish communities (Felipe & Suárez, 2010). Besides the changes in geomorphology, natural disturbance regimes and vegetation dynamics also have an impact on the interaction between biological, physical and chemical factors within a watershed (Ortega, Dias, Petry, Oliveira & Agostinho, 2014). Such factors reflect the lotic system's characteristics, i.e., mainly physical habitat and water quality variables that influence richness and abundance in fish assemblages (Vieira & Tejerina-Garro, 2020).

The natural effects, intensities, and variation of the hydrologic seasons have been studied on small and large scales in the streams of the Amazon Basin (Garcez & Freitas, 2008; Hurd et al., 2016). Regionally, environmental factors often play a big role in determining assemblage patterns of local species in aquatic systems and should not be neglected (Qu, Guse & Fohrer, 2018; Massaro, Pachla, Bastian, Pelicice & Reynalte-Tataje, 2019; Roa-Fuentes et al., 2020). Although a number of abiotic and biotic variables can influence lotic community composition and structure, the relative importance of certain variables can vary for different groups of taxa (i.e., community concordance), and can provide a better understanding of the effects of broad-scale environmental features on ichthyofauna assemblages (Neff & Jackson, 2013). Previous studies suggest that community interactions between different groups of aquatic taxa and environmental factors do exist, although results vary depending on the spatial and temporal scale, as well as the abiotic factors in question (Jackson & Harvey, 1993; Kilgour & Barton, 1999; Bowman et al., 2008; Felipe & Suárez, 2010). Ultimately, this type of analysis allows us to broaden the scope of interpretation and the generality of our ecological understanding (Neff & Jackson, 2013).

When considering the Amazonian river systems, the most studied theory about aquatic inland environments is the flood-pulse concept (Junk, Bayley & Sparks, 1989; Hurd et al., 2016), but this assumption does not consider streams in areas of *terra firme* as part of this environmental model. Moreover, the seasonal flooding of *terra firme* streams is mainly regulated by local rain incidence (Mendonça, Magnusson & Zuanon, 2005; Tomasella et al., 2008). Thus, a vast study was done on this subject for land use and pasture areas, but there is still a lack of consistent information regarding ichthyofaunal spatial distribution and fish community composition from these small streams in the Amazon Basin (Mendonça, Magnusson & Zuanon, 2005; Mojica Castellanos & Lobón-Cerviá, 2009; Brejão, Hoehinghaus, Pérez-Mayorga, Ferraz & Casatti, 2018; Leitão et al., 2018).

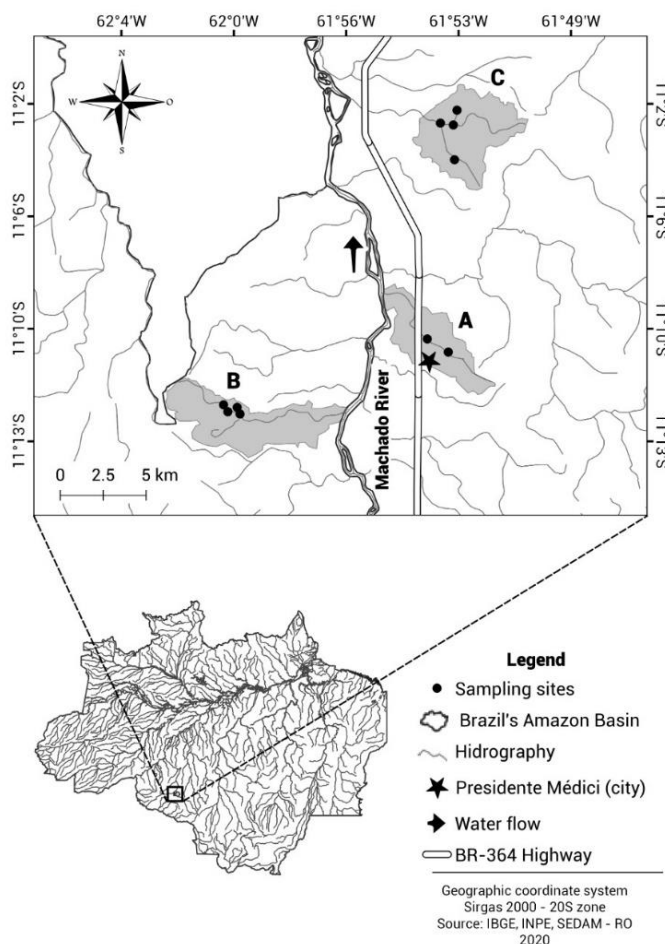
In this study, we investigated whether fish communities are influenced by abiotic factors within streams and if there is a strong correlation with the environment in the different study sites. A significant association between the fish community and abiotic factors supports the hypothesis that broad-scale patterns in fish communities in Amazon small streams are, at least in part, structured by abiotic conditions (Taylor, Winston & Matthews, 1993). Therefore, the present study aims to evaluate whether the evenness, richness, and species composition of the fish assemblage are influenced by environmental characteristics, and which variables are most important in determining the distribution of fish species in the streams of the Machado River basin, situated in Rondônia State, western Brazilian Amazon. Thus, we tested the hypothesis that there is no degree of interaction between fish assemblage structure with the annual seasons (dry and wet), and environmental physicochemical parameters existent along the streams in the selected study area.

## Material e Métodos

### STUDY AREA

Field sampling was conducted in three microbasins, in first order streams (A, B, and C) in the Machado River basin (Figure 1). This basin covers approximately 1,243 km of river course; its headwater is the

Pimenta Bueno River, which flows into the Madeira River, in the northern part of Rondônia (Fernandes & Guimarães, 2002). The regional climate has temperatures ranging between 19 °C to 33 °C (66.2-91.4 °F), and the average annual rainfall is approximately 2,500 mm (Krusche et al., 2005). In this region, seasons are well-defined; the dry season (late May to September) and the wet season (October to April) (Fernandes & Guimarães, 2002). Microbasins A and B, are located in regions with fish farming activity and agricultural areas, predominantly pasture for livestock, and, consequently, a reduction in native vegetation cover, Table 1. Microbasin C possesses a larger proportion of forest fragments with its more conservative ciliary vegetation.



**Figure 1.** Sampling site locations in headstreams along the Machado River basin (microbasins A, B and C). Arrow indicate the river water flow.

**Table 1.** Environmental characteristics and status of the tree microbasins along the Machado River basin.

Environmental characteristics	Microbasins		
	A	B	C
Cross river length (m)	2.75 ± 0.65	1.20 ± 0.81	1.09 ± 0.14
Downstream deepness (m)	0.85 ± 0.49	0.20 ± 0.07	0.18 ± 0.05
<b>Environmental status</b>	Grass land, rock and sand ground, lentic waters.	Grass land, riparian forest, microphite presence, sand ground, and lentic waters.	Primary and secondary forest, microphite presence, sand ground, and lentic water

## FISH SAMPLING

Data on the fish were obtained from three microbasin tributaries of the Machado River basin, from June 2014 to March 2015 (Figure 1). Ten small-stream locations were sampled. Sampling events (two separate days in each location per season) were performed in both seasons: dry (June and September 2014) and wet (December 2014 and March 2015), according to rainfall patterns in the region.

In each stream, we selected two 150-meter-long stretches on the main channel; the sections were approximately 1,000 m apart from each other. We used three sampling methods for fish collection, in order to maximize the probability of covering all fish species from the sampling locations. Equipment included two gillnets (mesh size 20 mm, length 5 m); a ring net trap (2 m diameter, 10 mm between opposite knots); and a specialized fishing net with 2 mm knotted nylon mesh, coupled to handles and hoops (1 m diameter). Traps were set over 4 h; 2 h during the night (18:00-20:00 h) and 2 h during the day (06:00- 08:00 h), respectively. Gillnets were submerged for four hours and inspected every 30 min. The ring net was employed for two hours along the stretches, followed by the specialized net at the streams edge. After collection, all fishes were tagged and stored in plastic bags, for subsequent taxonomical identification at the Aquaculture and Fisheries Laboratory of the Universidade Federal de Rondônia (UNIR). At the lab, all fishes were fixed in 10% formalin, counted, identified, and subsequently transferred to 70% alcohol. Taxonomic identification of species was performed using specialized literature (Queiros et al., 2013), with the assistance of experts from several institutions.

## ENVIRONMENTAL VARIABLES

In each sampling event, in order to characterize each stream's physicochemical parameters, we measured temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), pH, and electrical conductivity (µS cm<sup>-1</sup>) with a portable multiparameter probe.

## DATA ANALYSIS

The number of individuals captured was separated by season and microbasins. The relative importance of species was considered according to the Garcia and Vieira (2001) classification when %N was greater than 100/S, where S is the number of species recorded in the area. A species was defined as frequent when its % FO (frequent of occurrence) value for a given area was greater than 50%. The combination of these parameters allowed the species to be classified into four categories: abundant and frequent (%N>100/S and %FO≥50%); abundant but infrequent (%N>100/S and %FO<50%); less abundant but frequent (%N<100/S and %FO≥50%) and less abundant and infrequent (%N<100/S and %FO<50%).

Fish assemblages were described by their abundance, richness and diversity. The total number of individuals (abundance) and total number of species (richness) were calculated based on observations from each sampling event at each location. The Hill's numbers were used to estimate the diversity (Jost, 2006), derived from these numbers, which can be used to compute diversity indices instead of popular formulae for Shannon entropy and because it is more likely to have a linear relationship with environmental variables (Borcard, Gillet & Legendre, 2018).

Permutational multivariate analysis of variance (Permanova - Bray-Curtis index obtained with 9999 random permutations), a nonparametric method to test homogeneity of multivariate dispersions (Anderson, Ellingsen & Mcardle, 2006). We evaluate the effect on fish abundance, richness, and diversity were the response variables, and microbasins, electrical conductivity, pH, dissolved oxygen and seasons were included as explanatory variables.

In addition, in order to determine the influence of physicochemical parameters of the water (electrical conductivity, pH and dissolved oxygen) across the three microbasins, a distance-based redundancy analysis, dbRDA, was performed (Legendre & Anderson, 1999), based on Bray-Curtis similarity matrices (Legendre & Legendre, 2012) on square root transformed data to enhance the contribution of the less abundant taxa (Clarke & Warwick, 2001). The global significance was tested by permutation analysis. All processing and analyses were conducted in R version 4.0.3 (R Core Team, 2020), using the vegan package (Oksanen et al., 2019). The level of 5% significance was adopted in the analysis.

## Results

A total of 6,432 individuals belonging to 4 orders, 19 families, and 56 species were captured in the three microbasins of the Machado River (Table 2). The five most abundant species (*Aequidens tetramerus*, n = 1,017; *Serrapinnus* cff. *microdon*, n = 908; *Serrapinnus* aff. *notomelas*, n = 892; *Steindachnerina fasciata*, n = 796 and *Satanoperca jurupari*, n = 739) accounted for approximately 69% of all fish recorded in this study. Characiformes was also the richest order in seasons and all three microbasins (Microbasin A: 18 species; Microbasin B: 16 species; Microbasin C: 29 species).

In the dry and wet seasons, 45 and 50 species were identified, respectively, 39 of which were found in both periods. The five most abundant species remained the same in dry and wet, corresponding to 69 and 67%, respectively.

The relative importance of species in the three microbasins, in general, there were more fish classified as less abundant but frequent (Microbasin A: 74%; Microbasin B: 61%; Microbasin C: 51%). The number of abundant and frequent species in microbasins A, B and C were six, eight and eight, respectively. The species *Serrapinnus* cff. *microdon*, *Serrapinnus* aff. *notomelas*, *Aequidens tetramerus* and *Satanoperca jurupari* were abundant and frequent in the three microbasins. Ten specimens of *Oreochromis niloticus*, an exotic fish in the region, were also encountered and classified in microbasins B and C as less abundant but frequent and less abundant and infrequent, respectively.

**Table 2.** Abundance of fish species collected in three microbasins of the Machado River, Rondônia State. Relative importance: 1, abundant and frequent; 2, abundant and infrequent; 3, less abundant but frequent; 4, less abundant and infrequent.

Order/Family/Species	Code	Season		Microbasins			Relative importance Microbasins		
		Dry	Wet	A	B	C	A	B	C
<b>CHARACIFORMES</b>									
<b>Anostomidae</b>									
<i>Leporinus friderici</i> (Bloch, 1794)	Lf	31	19	41	5	4	3	3	3
<b>Acestrorhynchidae</b>									
<i>Acestrorhynchus falcatus</i> (Bloch, 1794)	Af	2	4	0	0	6			3
<b>Characidae</b>									
<i>Astyanax</i> aff. <i>bimaculatus</i> (Linnaeus, 1758)	Ab	168	239	72	294	41	3	1	1
<i>Astyanax</i> cf. <i>maximus</i> (Steindachner, 1876)	Am	31	12	1	27	15	3	3	3
<i>Brachyhalcinus copei</i> (Steindachner, 1882)	Bc	4	19	0	19	4		3	3
<i>Bryconops</i> cf. <i>giacopinii</i> (Fernández-Yépez, 1950)	Bg	95	70	12	51	102	3	3	1
<i>Hypheobrycon agulha</i> (Fowler, 1913)	Ha	1	0	0	0	1			4
<i>Jupiaba anteroides</i> (Géry, 1965)	Ja	1	4	0	0	5			3
<i>Jupiaba</i> cf. <i>apenima</i> (Zanata, 1997)	Jc	3	2	0	0	5			3
<i>Knodus</i> cf. <i>heteresthes</i> (Eigenmann, 1908)	Kh	69	27	1	95	0	3	1	
<i>Moenkhausia</i> cf. <i>pankiloptyx</i> (Bertaco and Lucinda, 2006)	Mp	0	24	0	20	4		4	4
<i>Moenkhausia cotinho</i> (Eigenmann, 1908)	Mc	5	0	0	0	5			4
<i>Moenkhausia oligolepis</i> (Günther, 1864)	Mo	25	39	1	41	22	3	3	3
<i>Phenacogaster</i> cf. <i>beni</i> (Eigenmann, 1911)	Pb	0	1	0	1	0		4	
<i>Poptella compressa</i> (Günther, 1864)	Pc	6	3	0	0	9			3
<i>Roeboides affinis</i> (Günther, 1868)	Ra	0	2	0	0	2			4
<i>Serrapinnus</i> aff. <i>notomelas</i> (Eigenmann, 1915)	Sn	362	530	271	333	288	1	1	1
<i>Serrapinnus</i> cff. <i>microdon</i> (Eigenmann, 1915)	Sm	337	571	477	372	59	1	1	1

**Table 2** (cont.)

<b>Curimatidae</b>									
<i>Curimata inornata</i> (Vari, 1989)	Cl	0	1	0	0	1			4
<i>Curimata ocellata</i> (Eigenmann and Eigenmann, 1889)	Co	2	0	0	0	2			4
<i>Curimatella dorsalis</i> (Eigenman Eigenmann, 1889)	Cd	107	120	129	0	98	1		1
<i>Steindachnerina fasciata</i> (Vari and Géry, 1985)	Cf	423	373	532	249	15	1	1	3
<i>Cyphocharax notatus</i> (Steindachner, 1908)	Cn	0	1	0	0	1			4
<b>Crenuchidae</b>									
<i>Characidium aff. zebra</i> (Eigenmann, 1909)	Cz	4	4	8	0	0	3		
<b>Erythrinidae</b>									
<i>Hoplias malabaricus</i> (Bloch, 1794)	Hm	70	23	31	42	20	3	3	3
<b>Hemiodontidae</b>									
<i>Hemiodus unimaculatus</i> (Block, 1794)	Hu	0	1	0	0	1			4
<b>Prochilodontidae</b>									
<i>Prochilodus nigricans</i> (Spix and Agassiz, 1829)	Pn	22	6	1	10	17	3	3	3
<b>Parodontidae</b>									
<i>Paradon bucleyi</i> (Boulenger, 1887)	Pi	3	0	0	3	0		3	
<b>Serrasalminidae</b>									
<i>Serrasalmus altispinis</i> (Merckx, Jégu and Mendes dos Santos, 2000)	As	29	84	105	0	8	2		3
<i>Myleus asterias</i> (Müller and Troschel 1844)	Ma	8	0	0	7	1		4	4
<b>GYMNOTIFORMES</b>									
<b>Apteronotidae</b>									
<i>Apteronotus albifrons</i> (Linnaeus, 1766)	Aa	1	3	0	1	3		4	3
<b>Gymnotidae</b>									
<i>Gymnotus carapo</i> Linnaeus, 1758	Gc	62	53	21	75	19	3	1	3
<b>Sternopygidae</b>									
<i>Eigenmannia</i> sp.	E	36	27	17	29	17	3	3	3
<i>Sternopygus macrurus</i> (Bloch and Schneider, 1801)	S	17	21	8	25	5	3	3	3
<b>PERCIFORMES</b>									
<b>Cichlidae</b>									
<i>Aequidens tetramerus</i> (Heckel, 1840)	At	581	436	185	504	328	1	1	1
<i>Cichla monoculus</i> Agassiz, 1831	Cm	3	12	3	3	9	3	4	3
<i>Crenicichla lepidota</i> Heckel, 1840	Cl	34	23	29	16	12	3	3	3
<i>Crenicichla</i> sp.	C	9	57	0	46	20		3	3
<i>Oreochromis niloticus</i> (Linnaeus, 1758)	On	4	6	0	9	1		3	4
<i>Satanoperca jurupari</i> (Heckel, 1840)	Sj	403	336	510	84	145	1	1	1
<b>SILURIFORMES</b>									
<b>Auchenipteridae</b>									
<i>Parauchenipterus galeatus</i> (Linnaeus, 1766)	Pg	0	1	0	0	1			4
<b>Callichthyidae</b>									
<i>Callichthys callichthys</i> (Linnaeus, 1758)	Cc	17	10	0	11	16		3	3
<i>Corydoras aeneus</i> (Gill, 1858)	Ca	35	5	0	40	0		3	
<i>Corydoras</i> cf. <i>trilenatus</i> Cope, 1872	Ct	0	1	0	1	0		4	
<i>Hoplosternum littorale</i> (Hancock, 1828)	Hl	21	7	3	25	0	3	3	

**Table 2** (cont.)

<b>Heptapteridae</b>									
<i>Rhamdia quelen</i> (Quoy and Gaimard, 1824)	Rq	8	4	3	9	0	3	3	
<i>Phenacorhamdia</i> sp.	P	0	1	0	0	1			4
<i>Pimelodella serrata</i> Eigenmann, 1917	Os	3	13	0	16	0		3	
<b>Loricariidae</b>									
<i>Ancistrus</i> cf. <i>dubius</i> Eigenmann and Eigenmann, 1889	Ad	18	16	4	26	4	3	3	3
<i>Farlowella oxyrhyncha</i> (Kner, 1853)	Fo	5	5	0	7	3		3	3
<i>Hypostomus pyrineusi</i> (Miranda Ribeiro, 1920)	Hp	47	26	6	36	31	3	3	1
<i>Hypostomus</i> sp.	H	5	0	0	2	3		4	4
<i>Pterygoplichthys lituratus</i> (Kner, 1854)	Pl	0	7	2	0	5	3		3
<i>Rineloricaria lanceolata</i> (Günther, 1868)	Rl	18	4	0	21	1		3	4
<i>Rineloricaria</i> sp.	R	25	18	20	17	6	3	3	3
<b>Trichomycteridae</b>									
<i>Ituglanis</i> cf. <i>amazonicus</i> (Steindachner, 1882)	la	0	1	0	0	1			4

The concentration and descriptive statistics of physicochemical parameters in dry and wet season of three microbasins A, B and C are shown in Table 3. During the study monitoring, hydrogen-ion potential, dissolved oxygen, electrical conductivity and temperature in three microbasins showed some seasonal variation and ranged from 4.2 to 7.3; 1.2 to 4.4 mg L<sup>-1</sup>; 11.0 to 147.1 µS cm<sup>-1</sup>; 20.7 to 30.4 °C respectively.

Mean pH values in all the microbasins were superficially acidic. Microbasin A showed the lowest pH value (4.2) during the wet season. The mean DO was higher in the wet season than in the dry season in all the microbasins, and the lowest value was found in microbasin B (1.2 mg L<sup>-1</sup>). The highest EC value were registered in microbasin B (118.5 and 147.1 µS cm<sup>-1</sup>) for both the dry and the wet seasons, and the lowest EC value was in microbasin C (11.0 µS cm<sup>-1</sup>). The temperature was the highest during the dry season and the lowest during the wet season, and the highest water temperature values were recorded in microbasin C during the dry season (30.4 °C) and the wet season (29.6 °C).

**Table 3.** Descriptive statistics of the physicochemical parameters measured during the dry season and the wet season in the three microbasins of the Madeira River.

	Microbasin A				Microbasin B				Microbasin C			
	pH	DO	EC	TC	pH	DO	EC	TC	pH	DO	EC	TC
Dry season												
Maximum	6.8	4.9	73.0	29.5	6.8	4.8	118.5	30.3	5.7	6.0	83.0	30.4
Minimum	5.2	1.8	45.4	27.5	4.3	1.2	80.8	24.3	4.6	2.7	11.0	25.0
Mean	6.0	3.6	55.6	28.3	6.1	2.7	100.0	25.9	6.6	4.7	36.83	27.8
Std Error	0.4	0.6	6.4	0.4	0.3	0.4	5.4	0.6	0.2	0.3	9.5	0.6
Std Dev	0.8	1.3	12.9	0.9	0.9	1.2	15.2	1.8	0.6	1.0	26.9	1.9
Variance	0.7	1.7	168.2	0.8	0.8	1.4	233.8	3.4	0.4	1.0	726.6	3.6
Wet season												
Maximum	6.9	9.0	72.7	29.2	7.3	5.5	147.1	27.6	6.8	9.0	97.6	29.6
Minimum	4.2	2.3	50.4	25.6	5.0	3.9	98.4	25.0	4.5	4.4	11.1	20.7
Mean	6.1	4.6	59.5	27.5	6.4	4.2	116.0	26.6	5.8	5.5	32.37	25.6
Std Error	0.6	1.4	4.8	0.7	0.3	0.5	6.7	0.3	0.3	0.5	10.3	1.1
Std Dev	1.3	2.9	9.7	1.5	0.9	1.5	19.1	1.0	0.8	1.5	29.3	3.3
Variance	1.7	8.9	94.7	2.5	0.7	2.4	365.0	1.0	0.7	2.3	862.3	11.2

TC= temperature, EC = electrical conductivity, DO = dissolved oxygen.

In fish abundance, fish richness and fish diversity, conductivity (COND) was the only response variable in which a significant difference was detected with Permanova for the three response variables, but there was also an emphasis on location (microbasins) which was significant for fish abundance and fish richness, and presented a marginal difference for fish diversity (Table 2). In the abundance of fish, the strongest correlation occurred with the microbasin variable ( $R^2 = 0.60$ ), and in addition to the variables mentioned above, there were significant differences for pH and marginal distinctions for temperature (TC) and seasons. In fish richness, the strongest correlation was also with the microbasin variable ( $R^2 = 0.37$ ), and other variables with significant differences were temperature (TC), pH and dissolved oxygen (DO). Fish diversity was the only response variable in which conductivity had the strongest correlation ( $R^2 = 0.44$ ), the explanatory variables temperature and dissolved oxygen showed a significant difference (Table 4).

**Table 4.** Permanova based on Bray-Curtis similarity for total abundance (transformed into square root) of fish caught in Machado River basin in Rondônia State. Factors: Microbasins, Electrical conductivity - COND, Hydrogen-ion potential - pH, Dissolved oxygen - DO and Seasons. df - degrees of freedom; SS - square sum;  $R^2$  - correlation coefficient; F - pseudo-F; P - p-value.

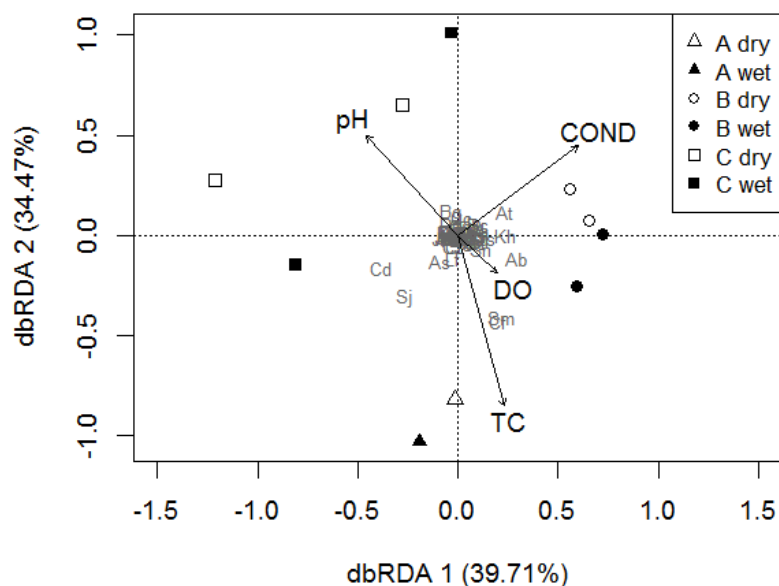
Variables	df	Fish abundance				Fish richness				Fish diversity			
		SS	$R^2$	F	P	SS	$R^2$	F	P	SS	$R^2$	F	P
Microbasins	2	0.816	0.60	13.81 6	0.001	0.018	0.37	70.872	0.012	0.009	0.11	8.338	0.064
TC	1	0.080	0.06	2.709	0.067	0.007	0.13	50.800	0.013	0.011	0.14	20.051	0.038
COND	1	0.152	0.11	5.161	0.006	0.010	0.20	78.508	0.001	0.035	0.44	64.530	0.007
pH	1	0.138	0.10	4.688	0.009	0.007	0.14	52.707	0.014	0.005	0.06	8.663	0.111
DO	1	0.039	0.03	1.325	0.324	0.007	0.14	52.009	0.014	0.019	0.24	34.681	0.016
Seasons	1	0.075	0.06	2.553	0.086	0.001	0.02	6.494	0.112	$5 \times 10^{-5}$	0.00	0.097	0.866
Residual	2	0.059	0.04			0.000	0.01			0.001	0.01		
Total	9	1.360	1.00			0.050	1.00			0.080	1.00		

df = degree of freedom applied to all analyses.

The variations in conductivity, temperature, pH and dissolved oxygen were significantly correlated with the abundance and composition of the ichthyofauna ( $F = 1.88$ ,  $p = 0.035$ ) in the analysis with dbRDA (Figure 2). In the dbRDA, 44.57% of the total variance (the fitted variance) was explained by the variables used, with 74.18% of this proportion being represented by the first two canonical axes. Thus, separating the fish species among the water's physicochemical parameters and microbasins at axis 1, likewise between hydrological season at axis 2. In axis 1, conductivity and pH were the most relevant factors, also related to dry season, and exhibited at the superior part of axis 2. In addition, in axis 2, temperature and dissolved oxygen were correlated to the wet season at the bottom of the plotted area. Axis 1 also organized all fish collections from microbasins B on the right side, and, for microbasins A and C, on the left side of the plotted matrix (Figure 2).

The species *Curimatella dorsalis* (Cd), *Satanoperca jurupari* (Sj), and *Serrasalmus altispinis* (As) were present in the basin C and linked to wet and dry seasons, also associated to the pH parameter on the left side of axis 1. The fish species *Aequidens tetramerus* (At) and *Knodus cf. hetesthes* (Kh) were correlated with electrical conductivity (COND) during the dry season at the microbasin B. Also, *Astyanax aff. bimaculatus* (Ab), *Serrapinus cff. microdon* (Sm) and *Steindachnerina fasciata* (Cf) showed clear relation with dissolved oxygen (OD) and temperature (TC) for the wet season, associated with microbasin B (right side of the axis 1 and in the bottom of axis 2). For the microbasin A, the corrected correlation between fish species and temperature parameter (TC) were difficult to be finished for both seasons (Figure 2).





**Figure 2.** Distance-based redundancy analysis (dbRDA) between fish assemblage structure and the explanatory variables (Temperature, TC; Electrical conductivity, COND; Hydrogen-ion potential, pH; and Dissolved oxygen, DO) among the seasons and microbasins of the Machado River basin in the state of Rondônia. Triangle, circle and square symbols are microbasins A, B and C, respectively (dry - empty symbols; wet - filled symbols).

## Discussion

In this study, we hypothesized that fish species richness is positively correlated to the annual seasons (dry and wet), and to environmental physicochemical factors (e.g. temperature, dissolved oxygen and Ph) in the microbasins. We therefore expected to see a strong degree of interaction between fish assemblage structure and these factors, since they differ across locations (Súarez & Petrere Júnior, 2007). Significant relationships between fish communities and abiotic factors supported the hypothesis that broad-scale patterns in Amazon small stream fish communities are, at least in part, structured by abiotic and seasonal conditions.

The present study resulted in a confirmation that evenness, richness, and species composition values from these fish assemblages are influenced by environmental characteristics, such as the streams' physicochemical parameters, the seasons, and locations of the microbasins, which determine the distribution of fish species in the Machado River basin. This pattern was observed for floodplain fish assemblages in the Amazon basin, where the seasonality of the river level was associated with other abiotic variables (Brejão et al., 2018). These abiotic (environmental) factors impact the richness and species composition of fish assemblages in these areas, where such species are more resistant to environmental pressures (Junk, 1999; Freitas & Garcez, 2004; Suárez & Petrere Júnior, 2007; Garcez & Freitas, 2008; Hurd et al., 2016).

The most abundant order was the Characiformes, as reported in most studies of South American aquatic ecosystems (Freitas & Garcez, 2004; Garcez & Freitas, 2008). This order's wide occurrence could be related to the ability of these species to adjust to diverse types of habitats (Reis et al., 2016). However, *Aequidens tetramerus*, a Perciformes species, presented a greater distribution in the three microbasins sampled, which indicates that it is able to explore opportunities in different microhabitats. This may be related to the drought, which interrupts hydrological connectivity and causes direct impact on abiotic factors, in this case water. As such, there are adaptations of species never previously reported and which are intrinsically related to physicochemical contexts of water (Pires, Pires, Collares-Pereira & Magalhães, 2010).

Droughts are seasonally predictable events since they occur each year, but notably vary in intensity between years. The understanding of the mechanisms and seasonal patterns studied in the microbasins of the Madeira River was particularly important in order for us to identify the predominance or adaptations of fish species, since these demonstrate increasing recognition of the complexity and high dynamism of aquatic systems (Brejão et al., 2018).

Another important factor was the location of the microbasin in the river, which allowed great distribution of the species *Aequidens tetramerus*. This happens due to the high forest quality of the location, which contributes by providing food for the diet of fish, especially in their greater consumption of allochthonous organic matter (organic material formed outside the ecosystem). Autochthonous prey may predominate in environments with low vegetation cover, thus favoring its wide distribution (Silva, Fugii, Carniatio & Ganassin, 2014; Costa & Soares, 2015). It is noted that the fish species found at that location became tolerant to adverse conditions and were resistant to seasonal variations.

Our results showed that the *A. tetramerus* were more abundant in the microbasin B, also relating this species with the electrical conductivity parameter. This may be due to this microbasin and abiotic variable being linked to environmental degradation (deforestation, siltation and stream bank impoverishment) and, this fact may have contributed to fish assemblage preference (Brejão et al., 2018).

The difference in species richness distributed between the three microbasins may be related to variations in flow (related to cross river length and deepness), landscape structure, chemical characteristics of water and environmental integrity, since these changes all tend to affect fish species distribution and structure (Brejão et al., 2018; Ilha, Schiesari, Yanagawa, Jankowski & Navas2018). The microbasin study sites presented different environmental conditions (e.g., land use and habitat degradation, and different water physicochemical parameters between the seasons) and suffered different impacts proportions, which may have influenced the large number of rare fish species, as changes in the environment became more intense, thus the fish assemblages also respond to the habitats changes and thus the best adapted resident fish individuals turn out to be predominate (Benitez & Suárez, 2009; Gonçalves, Souza, Braga & Casatti, 2018; Ilha et al., 2018; Teresa & Casatti, 2017).

In this context, we found that microbasin C presented greater species richness in relation to microbasins A and B, and this finding may be related to the high quality of the surrounding environmental conditions, since this location is situated inside a larger forest fragment. Thus, the maintenance of forest areas protects channel stability by providing habitat heterogeneity, which in turn also ensures the presence of important sources of food for fish, ideal temperatures and, consequently favors a greater number of fish species in this environment (Lorion & Kennedy, 2009; Gonçalves et al., 2018; Ilha et al., 2018).

The environment's physicochemical parameters were important descriptors for the determination of fish assemblage distribution among the streams of the microbasins, and configure the composition of the communities according to their adaptive affinities (e.g., migratory reproductive processes), though this may also be related to the environmental condition of each site species (Arantes, Santos, Rizzo, Sato & Bazzoli, 2011; Murgas et al., 2012; Suárez, Silva & Viana, 2017). In microbasin A, the values of temperature and pH were those that most influenced fish distribution. By contrast, in microbasin B, the electrical conductivity of the water was the biggest influence, and it's may be related to the land use conditions of the region, and indicative of low vegetation cover (mainly caused by these anthropic interference), which causes changes in sediment transported through the channel (Brejão et al., 2018) that can affect water conductivity. In this sense, the variation in environmental conditions in microbasins A and B indicate limitations for the occurrence of certain species, leading to a decrease in species richness. In microbasin B, a greater occurrence of the non-native species *Oreochromis niloticus*, introduced in the region for breeding in fish farms, was also verified. However, it is a fish tolerant to adverse environmental conditions (Melo, Ribeiro, Luz, Bazzoli & Rizzo, 2016), which may have influenced the imbalance of native fish assemblages in microbasin B, once this specie is reported as omnivorous, highly invasive and, negative impact on the local aquatic environments (Froese & Pauly, 2020).

The wet season and dissolved oxygen concentration values were the main factors that contributed to the greater species richness in microbasin C, but also, this could be attributed to the higher proportions of forest fragments present in this region, which may result in the better quality of the limnological components and functional structure of fish assemblages. The strong rainfall during this period also contributes to a greater supply of food resources of allochthonous input, derived from plant and animal material directly from the forest, which can contribute to food maintenance for the fish species (Freitas, Siqueira-Souza, Humston & Hurd, 2013).

However, for microbasin C, the pH was relevant for the fish species distribution that occurred during the dry season. This behavior could be related to the increase in temperatures that affect them in dry periods and the decrease in water volume, which change the electrical conductivity drastically and consequently retain less dissolved oxygen, since the salts present in the water tend to concentrate, and cause changes in pH. As expected, the water temperature was higher in the dry period than in the wet periods.

Due to this fact, the fish assemblages are vulnerable to alterations related to abiotic factors and thus explain the variability of the results found. The pH affects the ability of fish and other aquatic organisms to regulate the maintenance of basic life processes. When the pH exceeds the levels that the organism can tolerate, it causes negative effects on the fish, reducing the growth rate and even causing mortality (Kodom, Onoyinka, Mkude, Out & Yeboah, 2018). This confirms the hypothesis that the rainy and dry period have some influence on the variables studied.

The minimum values of dissolved oxygen observed for the three microbasins, which ranged between (1.2 to 2.7 mg L<sup>-1</sup>) in the drought period, also enabled considerable microbial activity, since this process consumes a lot of oxygen, which is necessary for the degradation of organic matter (Eliku & Leta, 2018). Dissolved oxygen is one of the most important parameters in surface water systems in order to determine the quality of life of aquatic ecosystems (Santos, Simões & Sonoda, 2018).

However, the low fish species richness occurring in microbasins A and B could be related to the local seasonal hydrology pattern, which could influence the abiotic (pH and conductivity) and biotic (fish assemblages) interactions in the fish trophic chain (Súarez & Petrere Júnior, 2007). This may amplify the competition for space and food among fish species, and between prey and predatory fish during the dry season, which, later on, probably reduces competitiveness during the wet season (Hurd et al., 2016). In addition, also this pattern could be amplified by the local climate changes (e.g., temperature) related to environmental degradation (Teresa & Casatti, 2017; Scarabotti, Demonte & Pouilly, 2017).

The ichthyofauna of small streams is affected by environmental factors (Sousa & Freitas, 2008; Brejão et al., 2018). Nevertheless, abiotic factors, especially those related to wet and dry seasons, directly affect the existing biota in these microbasins. In both cases, the influence of rainfall on the level of the Machado River tributaries contributes to the transport of allochthonous foods to these streambeds, which in turn interferes with the behaviors of existing fish assemblages and their trophic relationships (Teresa & Casatti, 2017; Brejão et al., 2018). This variation in the seasonal dynamics of watersheds in streams, mainly the flow that can increase during the wet (rainy) season and decrease during the dry season, interferes in the composition of fish species found therein, and in modifications of the environment's physicochemical parameters, such as temperature, dissolved oxygen, and pH (Felipe & Suárez, 2010; Hurd et al., 2016; Gonçalves et al., 2018; Ilha et al., 2018).

Given the above, measures to mitigate environmental impacts should be applied in the Machado River basin region. These should include: i) actions related to the recovery of riparian forests, ii) mandatory fish farming rules, especially in regards to farms utilizing native fishes in their production, and iii) extension activities involving farmers with the aim of creating medium and long-term awareness in relation to the ecological importance of small fish assemblages to the local food chain.

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