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ORIGINAL ARTICLE

Effect of environmental and spatial factors on small-sized fish assemblages in a tropical river

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ABSTRACT

The river continuum concept predicts that gradual changes in river geomorphology and hydrology can drive longitudinal changes in aquatic community structure. Accordingly, we evaluated how environmental variables (electrical conductivity, pH, water transparency and water velocity), spatial factors (distance from headwaters and Moran's eigen vector maps – MEMs), and the presence of dams affect small-sized fish assemblages along a 105-km stretch of the upper Branco River, a tributary in the Madeira River, Amazonas Basin, Brazil. Seine-net based collections were carried out at 15 sites up- and downriver from dams during the 2019 dry season. We captured a total of 4,330 individual fish belonging to three orders, nine families and 26 species (and a hybrid individual). Electrical conductivity and pH were affected by the presence of dams and the distance from headwaters. Species richness and abundance did not vary in response to environmental variables. While species richness showed no significant variation along the sampled river section, abundance showed a negative relationship with distance from headwaters. Species composition varied significantly in response to pH, linear spatial factors and the presence of dams. Our results suggest that change in species composition of small-sized fish assemblages in the upper Branco River occurs due to variation in water characteristics, inherent dispersal limitation, and in response to the presence of dams.

KEYWORDS: distance from headwaters, pH, longitudinal gradient, Amazonas basin

Efeito de fatores ambientais e espaciais sobre assembléias de peixes de pequeno porte em um rio tropical

RESUMO

O conceito de rio continuo prediz que mudanças graduais na geomorfologia e hidrologia de um rio podem causar mudanças longitudinais na estrutura de comunidades aquáticas. Nesse contexto, avaliamos como variáveis ambientais (condutividade elétrica, pH, transparência e velocidade da água), fatores espaciais (distância da cabeceira e Moran's *eigen vector maps* – MEMs) e presença de barragens afetam as assembleias de peixes de pequeno porte ao longo de um trecho de 105 km do alto Rio Branco, um tributário do Rio Madeira, bacia Amazônica, Brasil. Coletas com rede de arrasto foram realizadas em 15 pontos a jusante e montante de barragens durante a estação seca de 2019. Capturamos um total de 4.330 indivíduos, distribuídos em três ordens, nove famílias e 26 espécies (e um indivíduo híbrido). Condutividade elétrica e pH foram afetados significativamente pela presença de barragens e pela distância da cabeceira. A riqueza de espécies e a abundância não variaram em função das variáveis ambientais. Ao contrário da riqueza de espécies, que não variou ao longo do trecho amostrado, a abundância apresentou uma relação negativa com a distância da cabeceira. A composição de espécies variou significativamente em resposta ao pH, a fatores espaciais lineares e à presença de barragens. Nossos resultados sugerem que a mudanças na composição de espécies de peixes de pequeno porte no alto Rio Branco ocorre devido à variação em características da água, em função da limitação na dispersão inerente das espécies e em resposta à presença de barragens.

PALAVRAS-CHAVE: distância da cabeceira, pH, gradiente longitudinal, bacia do Rio Amazonas

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INTRODUCTION

The river continuum concept (Vannote *et al.* 1980) predicts changes in community structure and ecosystem processes accompanying the gradual changes in geomorphology and hydrology that occur from a river's headwaters to its mouth (Foubert *et al.* 2018). This vision of a longitudinal gradient has dominated riverine ecological studies in recent years (Benda *et al.* 2004). However, this linear perspective does not take into account the presence of natural (waterfalls), anthropogenic (dams) barriers (Ward and Stanford 1995; Torrente-Vilara *et al.* 2011) and the spatial and temporal dynamics that regulate riverine ecosystem diversity (Altermatt 2013; Tonkin *et al.* 2017).

Drainage basins are dendritic structures, where rivers function as corridors through which energy, matter and living organisms are transported (Rodriguez-Iturbe *et al.* 2009). In this structure, headwater sites, being less productive and more isolated, tend to have high endemism, lower species richness and species assemblages that are more structured by environmental variables than assamblages located downstream, which have higher connectivity and are structured by dispersal (Henriques-Silva *et al.* 2019). In riverine systems, longitudinal connectivity is considered an important aspect as it allows fish and other organisms to disperse through a water course and migrate between upstream and downstream habitats (Fullerton *et al.* 2010; Branco *et al.* 2012), especially during the reproductive period (Lucas and Batley 1996).

Among the main factors responsible for the modification of longitudinal connectivity within the dendritic structure of a riverine network, is the construction of dams for reservoir formation and power generation (Fukushima *et al.* 2007; Fullerton *et al.* 2010; Winemiller *et al.* 2016). River damming causes changes in the natural flow of rivers and their associated physical and chemical characteristics (Shao *et al.* 2019). This is likely to negatively affect organisms not capable of adapting to the new environmental conditions, such as large and migratory fish, and positively impact those species that can take advantage of the new environments, such as small-sized or sedentary fish species with parental care (Agostinho *et al.* 2016).

In spite of the small-sized fish being dominant in tropical rivers, large-sized migratory fish have received more attention (Fullerton *et al.* 2010; Torrente-Vilara *et al.* 2011), as they are more important to commercial and recreational fisheries (Agostinho *et al.* 2007a; Santos *et al.* 2018). Small-sized fish are found mainly in marginal regions associated with aquatic vegetation, where a complex habitat structure composed by leaves, stems and roots provides suitable shelter and food (Dias *et al.* 2011). Besides this, small-sized fish need a small area to complete their life cycle (Agostinho *et al.* 2007b), and respond to fine-scale environmental variations rather than to spatial factors (Fernandes *et al.* 2015).

Within this context, this study aimed to evaluate how the structure of small-sized fish assemblages in the Branco River, a tributary in the Madeira River basin in the southwestern Brazilian Amazon, are influenced by: i) environmental factors (electrical conductivity, pH, water transparency and water velocity); ii) linear spatial factors (distance from headwaters and Moran's eigen vector map predictors); and iii) the presence of barriers (dams).

MATERIAL AND METHODS

Study area

The Branco River is a tributary of the Madeira River (Figure 1), one of the main tributaries in the Amazonas River basin. The Branco River has an extension of 391.88 km and a drainage basin of 9,340,615 km^2. The regional climate is of the Am type (tropical monsoon), according to the Köppen classification, with an annual precipitation that varies from 1800 to 2300 mm, and average annual temperature between 22 and 26 °C (Alvares *et al.* 2014). A dry period lasts from May to October, and the rainy season from November to April (Butt *et al.* 2011). Due to an altimetric variation of 368 m between the headwaters and the mouth of the river, rapids and waterfalls are formed, which confer a great potential

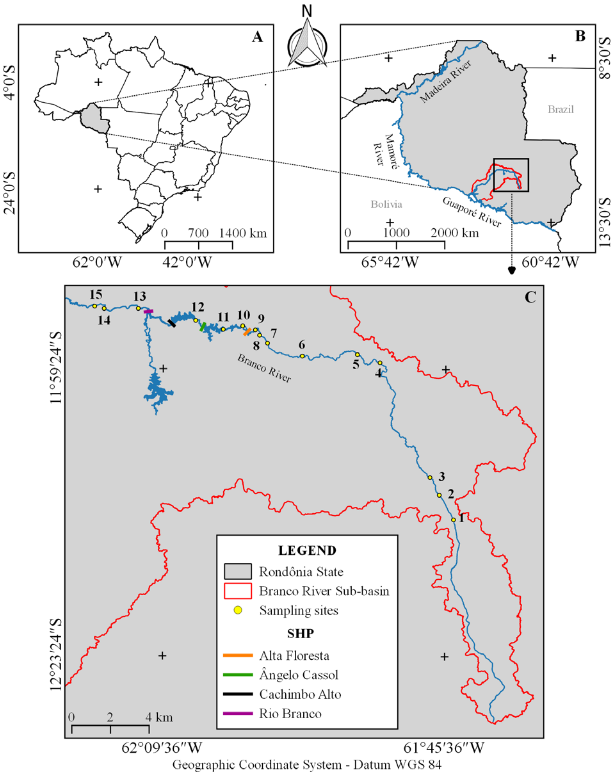

Figure 1. A - Location of the study area in Rondônia state, Brazil; B - Branco River sub-basin, showing the location of sampling sites in the upper Branco River; C - Upper Branco River, showing the location of the 15 sampling sites and four hydroelectric dams. This figure is in color in the electronic version.

for energy generation (Reis *et al.* 2020). So far, four small hydroelectric power plants (SHPs) are active along the Branco River (Figure 1), with generation capacity of 5 MW (SHP Alta Floresta, in operation since 2000), 6.9 MW (SHP Rio Branco, in operation since 2005), 3.6 MW (SHP Angelo Cassol, in operation since 2011) and 8.9 MW (SHP Cachimbo Alto, in operation since 2017).

Fish sampling

Small fish (adults up to 15 cm in length; Castro and Polaz 2020) samples were collected in June, 2019 (dry season) at 15 sampling sites (P1 – P15) distributed along approximately 105 km of the upper Branco River (eight upstream from the four dams, four in dam reservoires (one at SPH Alta Floresta, two at SHP Angelo Cassol, and one at SPH Cachimbo Alto) and three downstream from the dams) (Figure 1; Table 1). The dry season was chosen for sampling due to the low flow and reduced water volume of the river, increasing the concentration of fish, and hence sampling efficiency. To collect fish, we used a seine net of 6 m in length, 2 m in height, and a mesh size of 2.5 mm between opposing knots. Each site was sampled once during daylight hours using a standardized effort of three throws. We used only active fishing gear because small fish are mostly sedentary and do not conduct reproductive migrations, so that they are found in the same environment throughout the year and are not efficiently sampled by passive fishing gear. At all sites, we sampled close to the river margin, in the presence of aquatic vegetation and on sandy substrates (Lapointe *et al.* 2011).

Captured fish were anesthetized using eugenol (0.20 mL, equivalent to 200 mg, of clove oil per 500 mL of water; Fernandes *et al.* 2017), fixed in 10% formalin solution, preserved in 70% alcohol, identified and later deposited in the ichthyological collection of Universidade Federal de Mato Grosso (UFMT), Cuiabá, Mato Grosso state, Brazil. Fish identification was carried out with the help of regional keys (Queiroz *et al.* 2013), and articles describing specific species. Collection was authorized by SISBIO license # 57920–2 issued by the Brazilian national environmental agency (Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio) and by the Ethics Committee on Animal Use of Universidade Federal de Rondonônia (protocol # 001/2021 - CEUA/UNIR).

Environmental factors

Geographical coordinates of each sampling location were taken using GPS devices (Datum WGS84). pH, and electrical conductivity (µS cm^{-1}) were measured *in situ* with an AK88 multiparameter probe inserted to an approximate depth of 20 cm. Water transparency was measured with a Secchi disk and graduated cord. Water velocity (m s^{-1}) was measured by calculating the time a small styrofoam ball took to float from one end to the other of a 50-cm wooden rod (average of three measures). Water temperature and oxygen concentration were measured, but were subsequently discarded due to a technical problem with the AK88 multiparameter probe sensor.

Spatial factors

The distance from headwaters was estimated as the distance from each sampling site along the river to the most distant point in the headwater region considered as the source of the Branco River. We also used Moran's eigen vector maps

Table 1. Sampling sites, environmental variables and spatial factors measured for each of 15 sampling sites of small-sized fish assemblages in the upper Branco River, Madeira River basin, Brazilian Amazon.

				Environmental variables				Spatial factors	
Sampling site	Latitude	Longitude	Regions	Electrical conductivity (µS cm^{-1})	pH	Transparency (cm)	Water velocity (m s^{-1})	Distance from headwaters (km)	MEM2
P1	12°11'57.24"S	61°44'53.78"W	Upstream	14.3	7.2	79	0	40.400	-0.2782
P2	12° 9'54.27"S	61°46'6.11"W	Upstream	8.8	6.48	160	0.13	45.239	0.2396
P3	12° 8'25.14"S	61°46'53.83"W	Upstream	11.1	6.94	115	0.08	50.493	0.0350
P4	11°58'51.53"S	61°51'11.95"W	Upstream	35.7	7.02	82	0.07	79.928	-0.0017
P5	11°58'9.71"S	61°53'6.85"W	Upstream	34.3	7.19	58	0.12	86.377	0.0084
P6	11°58'18.41"S	61°57'47.80"W	Upstream	41.11	7.3	67	0.17	100.804	-0.0074
P7	11°57'13.68"S	62°0'44.98"W	Upstream	43.9	7.3	50	0.5	108.731	-0.0384
P8	11°56'35.35"S	62°1'25.88"W	Upstream	44.5	7.54	62	0.15	110.684	0.2051
P9	11°56'0.20"S	62°1'38.14"W	Reservoir	50.5	7.61	66	0.12	112.062	-0.1690
P10	11°55'49.23"S	62° 2'52.30"W	Reservoir	46.4	7.75	71	0	115.457	-0.0018
P11	11°56'9.86"S	62° 4'28.26"W	Reservoir	48.1	7.66	88	0	118.929	0.6238
P12	11°55'22.47"S	62° 6'50.80"W	Reservoir	50.4	7.71	101	0.13	124.611	-0.6343
P13	11°54'22.31"S	62°11'44.33"W	Downstream	50.2	7.53	96	0.1	136.526	0.0048
P14	11°54'34.70"S	62°14'12.32"W	Downstream	50.2	7.72	84	0.3	142.209	0.0023
P15	11°54'12.90"S	62°15'29.40"W	Downstream	51.2	7.6	102	0.12	145.602	0.0116

– MEMs (Borcard *et al*., 2004), which are spatial predictors generated using a topological distance matrix (distances among all the sampling sites along the river), to assess the magnitude of fish dispersal along the water course (Altermatt 2013). To build the spatial predictors, the "pcnm" function of the *vegan* package (Oksanen *et al*. 2019) from the R Program was used. This procedure resulted in eight spatial predictors ranging from broad (MEM1) to fine scale (MEM8), representing both the effect of dispersal limitation and variables that were not measured but are spatially structured (Peres-Neto and Legendre 2010; Fernandes *et al*. 2014).

Data analysis

To assess the effect of dam presence, the data were grouped in three regions: sites dowstream from the dams, sites within reservoires and sites upstream from the dams. Water electrical conductivity, pH, transparency and velocity were compared between regions with a Kruskal-Wallis analysis, as the data were not normally distributed (according to a Shapiro-Wilk test) and did not show homogeneous variance (according to a Bartlett test) (Sokal and Rohlf 1995). When test results were significant ($p < 0.05$), the post hoc test proposed by Siegel and Castellan (1988) was used to determine in which region the environmental variables differed significantly. This analysis was made using the "kruskalmc" function of the *pgirmess* package (Giraudoux *et al.* 2018). The effect of distance from headwaters on environmental variables was assessed using linear regression (Zar 1999). Extent of collinearity among the variables was assessed using variance inflation factors (VIF). When the variable showed a VIF value larger than 3, it was dropped from the multiple regression (Zuur *et al.* 2010). Electrical conductivity showed a high positive correlation with pH ($r = 0.87$), and a negative correlation with water transparency ($r = -0.51$), and pH showed a negativie correlation with water transparency ($r = -0.51$). Thus, both electrical conductivity and pH showed VIF > 3 and were removed from the analysis.

The effect of dam presence on species richness and abundance was evaluated using Kruskal-Wallis tests, while the effect of the continuous variables transparency, water velocity, and distance from headwaters was assessed with a multiple regression.

To evaluate the effect of environmental factors [E] (electrical conductivity, pH, transparency and water velocity), and spatial factors [S] (distance from headwaters and Moran's eigen vector maps - MEMs) on species composition, an analysis of variance partition was used (Peres-Neto *et al.* 2006). Variance partition is based on partial redundancy analysis (pRDA), and allows reduction of the explained variance percentage (r^2) for each predictor variable (Borcard *et al*. 1992; Legendre 1993; Legendre *et al*. 2005; Peres-Neto and Legendre 2010). The environmental and spatial factors were selected using the "forward.sel" function of the *adespatial* package (Dray *et al*. 2020).

To evaluate the effect of dam presence on species composition, a non-parametric multivariate analysis of variance (PERMANOVA) was used (Anderson 2001). The distance matrix between sites was calculated using Bray-Curtis distances (Legendre and Legendre 2012; Clarke and Warwick 2014), after species composition data was transformed using the Hellinger method (Legendre and Gallagher 2001). The statistical test used for PERMANOVA is a pseudo-F, and is calculated directly from any symmetric distance or dissimilarity matrix, with P values calculated for 999 permutations. The analysis was made using the "pairwise.perm.manova" function from the RVAideMemoire package (Hervé 2020). All analyses were performed in the R Program environment (R Core Team 2020).

RESULTS

Ichthyofauna

A total of 4,330 individuals were captured, distributed among three orders, nine families and 26 species (Table 2). The only species that fell outside the small-fish size criterion was *Hoplias malabaricus*, that has adults that can reach up to 65 cm. The dominant order was Characiformes, with 22 species, followed by Siluriformes and Cichliformes, with two species each. *Serrapinnus* cf. *microdon*, with 2,459 individuals (56.8%), *Serrapinnus micropterus*, with 804 (18.6%), *Moenkhausia* sp. 2, with 265 (6.1%) and *Bryconops* cf. *caudomaculatus*, with 211 (4.9%) were the most abundant species (Table 2). A sole hybrid individual (locally known as *jundiara*) resulting from a cross between *Leiarius marmoratus* and *Pseudoplatystoma punctifer* was captured in the SHP Ângelo Cassol reservoir (P10 in Table 2 and Figure 1).

Fish abundance varied from 12 individuals at P11 to 762 at P2 (Table 1), with an mean of 288 individuals per site. On average, 4.4 species were captured per sampling site. The highest species richness ($S = 7$) was found at P10, and the lowest at P4 ($S = 2$).

Environmental characteristics

The water in the studied stretch of the Branco River was relatively alkaline with low electrical conductivity (Table 1). Significantly higher values for these variables were found in the reservoirs and in the downstream region (Table 3). In the three regions, the water was transparent and with low velocity (Table 1), and the two variables did not differ significantly among the regions (Table 3). Conductivity ($r^2 = 0.90$, $p < 0.001$; Figure 2) and pH ($r^2 = 0.68$, $p < 0.001$; Figure 3) increased significantly with the distance from the headwaters. Transparency ($r^2 = 0.03$, $p = 0.24$) and water velocity ($r^2 = 0.01$, $p = 0.34$) did not show any relationship with distance from the headwaters.

Table 2. List of small-sized fish species captured at 15 sampling sites along the upper Branco River, Madeira River basin, in the southwestern Brazilian Amazon. Values are the number of individuals captured at each site and overall (TTL).

Order/Family/Species	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	TTL	Voucher
CHARACIFORMES																	
Curimatidae																	
Steindachnerina guentheri (Eigenmann & Eigenmann, 1889)	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	4	CPUFMT6900
Crenuchidae																	
Characidium zebra Eigenmann, 1909	1	10	13	0	59	2	0	0	1	0	0	0	1	0	0	87	CPUFMT6894
Iguanodectidae																	
Bryconops cf. *caudomaculatus* (Günther, 1864)	60	0	142	0	0	7	2	0	0	0	0	0	0	0	0	211	CPUFMT6892
Bryconops cf. *giacopinii* (Fernández-Yépez, 1950)	0	116	0	0	0	0	0	29	0	13	0	0	0	0	0	158	CPUFMT6903
Characidae																	
Aphyocharax sp.	0	0	0	0	0	0	0	0	0	0	0	0	18	63	20	101	CPUFMT6887
Astyanax sp.	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	8	CPUFMT6897
Hemigrammus lunatus Durbin, 1918	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	CPUFMT6907
Hemigrammus stictus (Durbin, 1909)																1	CPUFMT6889
Knodus cf. *heteresthes* (Eigenmann, 1908)	0	0	19	29	0	0	0	0	0	10	0	0	0	0	0	58	CPUFMT6893
Knodus cf. *orteguasae* (Fowler, 1943)	0	0	0	0	0	3	52	0	0	0	0	0	0	3	0	58	CPUFMT6899
Knodus cf. *smithi* (Fowler, 1913)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	CPUFMT6905
Moenkhausia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	33	CPUFMT6888
Moenkhausia sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5	CPUFMT6890
Moenkhausia sp. 2	0	0	0	0	0	0	0	0	0	36	0	228	1	0	0	256	CPUFMT6901
Moenkhausia oligolepis (Günther, 1864)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	CPUFMT6906
Odontostilbe nareuda Bührnheim & Malabarba, 2006	0	0	0	0	0	0	0	0	0	1	0	0	12	11	5	29	CPUFMT6891
Roeboides affinis (Günther, 1868)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	CPUFMT6908
Serrapinnus cf. *microdon* (Eigenmann, 1915)	0	631	371	393	491	531	41	1	0	0	0	0	0	0	0	2459	CPUFMT6895
Serrapinnus cf. *notomelas* (Eigenmann, 1915)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	CPUFMT6898
Serrapinnus micropterus (Eigenmann, 1907)	695	0	0	0	0	0	0	0	105	0	0	1	3	0	0	804	CPUFMT6885
Acestrorhynchidae																	
Acestrorhynchus falcatus (Bloch, 1794)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	CPUFMT6883
Erythrinidae																	
Hoplias malabaricus (Bloch, 1794)	0	0	0	0	0	0	0	0	1	1	2	0	0	0	1	5	CPUFMT6896
CICHLIFORMES																	
Cichlidae																	
Crenicichla lepidota Heckel, 1840	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3	CPUFMT6884
Satanoperca curupira Ota, Kullander, Deprá, da Graça & Pavanelli, 2018	0	3	0	0	0	0	0	6	0	0	1	5	0	0	0	15	CPUFMT6902
SILURIFORMES																	
Heptapteridae																	
Pimelodella boliviana Eigenmann, 1917	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	18	CPUFMT6904
Loricariidae																	
Rineloricaria sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	CPUFMT6886
Total abundance	758	760	545	422	552	561	95	38	108	64	12	234	36	116	29	4330	

Table 3. Variation of limnological variables by sampling region along the upper Branco River, Madeira River basin, in the southwestern Brazilian Amazon. Values are the median followed by the range.

Limnological variable	Upstream	Reservoir	Downstream	X^2	p-value
Electrical conductivity (µS cm^{-1})	35.0 (8.8 - 44.5)^a	49.2 (46.4 - 50.5)^b	50.2 (50.2 - 51.2)^b	10.6	0.004
pH	7.1 (6.4 - 7.5)^a	7.6^b (7.6 - 7.7)^b	7.6 (7.5 - 7.7)^{ab}	10.1	0.006
Transparency (cm)	73.0 (50 - 160)	79.5 (66 - 101)	96.0 (84 - 102)	1.8	0.395
Water velocity (m s^{-1})	0.12 (0.0 - 0.50)	0.06 (0.0 -0.13)	0.12 (0.1 - 0.3)	1.6	0.447

Different superscript letters in a line indicate significant differences according to a Kruskal-Wallis test and a Tukey post-hoc test.

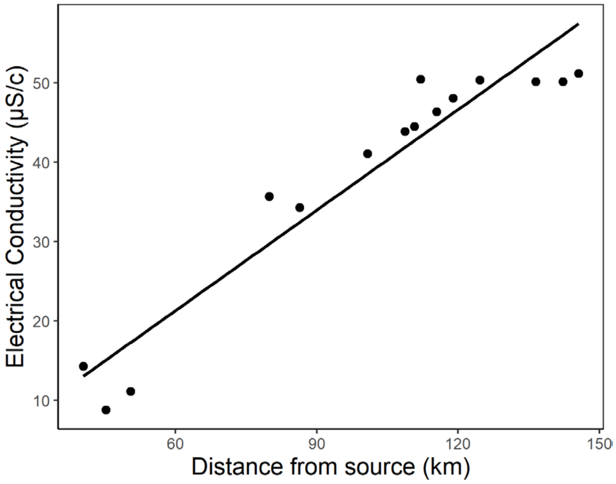

Figure 2. Relationship between electrical conductivity and distance from headwaters along the upper Branco River, Rondônia state, Brazil.

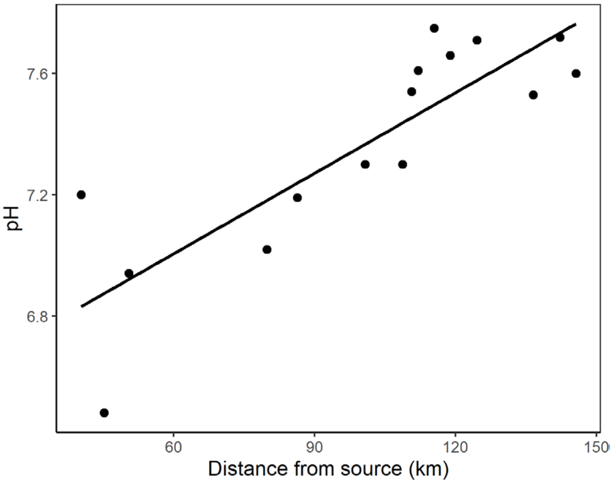

Figure 3. Relationship between pH and distance from headwaters along the upper Branco River, Rondônia state, Brazil.

Effect of environmental and spatial factors on fish assemblages

Fish abundance (Kruskal-Wallis $X^2 = 6.02$, $p = 0.05$) and species richness (Kruskal-Wallis $X^2 = 6.04$, $p = 0.05$) did not vary significantly between the three regions, and were not significantly related to water transparency (abundance: $r^2 = 0.52$, $p = 0.98$; species richness: $r^2 = -0.004$, $p = 0.66$), or water velocity (abundance: $r^2 = 0.52$, $p = 0.32$; species richness: $r^2 = -0.004$, $p = 0.38$). Fish abundance showed a significantly negative relationship with distance from headwaters ($r^2 = 0.52$, $p = 0.001$; Figure 4), while species richness showed no significant variation along the longitudinal gradient ($r^2 = -0.004$, $p = 0.41$).

Of the environmental factors, only pH was selected using the "forward.sel" function, while, of the spatial factors, only MEM2 was selected. Accordingly, both MEM2 and distance from headwaters were used as spatial predictors. Variance partitioning (pRDA) indicated that environmental factors [E] contributed 12% of the variance in species composition (r^2 adjusted = 0.12, $p = 0.003$), spatial factors [S] contributed 15% of the variance (r^2 adjusted = 0.15, $p = 0.001$) while 3% of the variance in species composition was shared between environmental and spatial factors [E|S], and 70% of the variance was not explained by any of the variables considered in this study (Residual = 0.70). Species composition varied significantly between regions (PERMANOVA: $r^2 = 0.44$, $F = 4.72$, $p = 0.001$; $p < 0.01$ for all pairwise comparisons).

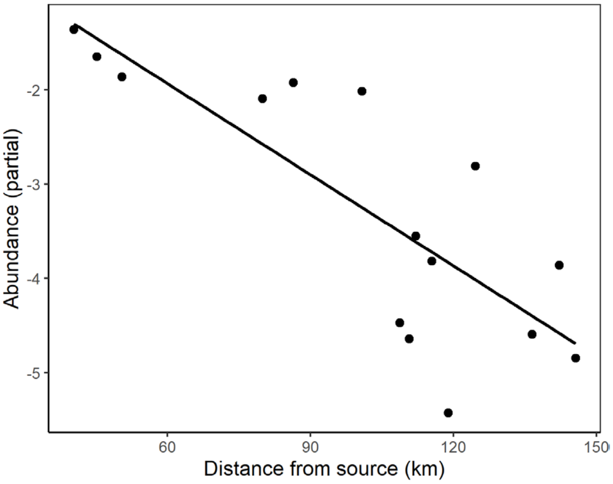

Figure 4. Relationship between fish abundance and distance from headwaters along the upper Branco River, Rondônia state, Brazil.

DISCUSSION

Ichthyofauna

The dominance of members of the order Characiformes in our samples is in accordance with other studies carried out in the Amazon (Lowe-McConnel 1999; Torrente-Vilara *et al.* 2011), and can be explained by the dominance of this order in the Amazon region (there are approximately 1,063 known valid species of Characiformes in this region; Dagosta and De Pinna 2019). The presence of one hybrid individual of *jundiara* in the Branco River is probably the results of the rupture of fish ponds, which are built close to the banks of rivers or through the damming of small watercourses. The impact of *jundiaras* on aquatic ecosystms is still uncertain, however, their high growth rates (Barros *et al.* 2020) and morphological, ecological and reproductive similarity to native species (Nobile *et al.* 2020) can facilitate their settlement and may pose a threat to native species. This the first record of the hybrid *jundiara* captured in nature in the Amazon basin.

Environmental characteristics

The highest electrical conductivity and pH values were found in the regions furthest from the headwaters, in the reservoirs and in the downstream region. Electrical conductivity and pH quantify the volume of total ions dissolved in the water and vary depending on the rock type forming a basin's soil, the amount of organic matter, and the rate of oxygen input from photosynthesis (Niemistö *et al.* 2011; Hayashi *et al.* 2012). Additionally, the concentration of nutrients, sediment, pollutants and organic matter increases towards the river mouth, so that sampling sites located in downstream regions show higher nutrient concentrations (Swanson et al. 2006; Altermatt 2013; Forsberg *et al.* 2017) and, consequently, higher electrical conductivity.

The uppermost sampling locations (P1 to P3) lie on the Casa Branca Formation, composed mainly of micaceous sandstones, while sites P4 and P5 lie on the São Felipe Formation, formed by gneiss and granite subtypes (Quadros and Rizzotto 2007). These rocks are acidic, and, when weathered, tend to lower soil pH (Scandolara *et al.* 1999). Sites P6 to P15 were located on the Rio Branco Formation, which has a predominance of metagabro and amphibolites, basic rocks, which, when weathered, tend to raise soil pH (Scandolara *et al.* 1999). Thus, the downstream increase in pH and electrical contuctivity is likely linked to the input of nutrients from agricultural effluents and primary productivity mediated by the geological formations underlying the study area.

Effect of environmental and spatial factors on fish assemblages

Although some studies have shown that within-assemblage abundance increases in downstream direction (Henriques-Silva *et al.* 2019; Borthagaray et al. 2020), we found a negative relationship between abundance and distance from headwaters. This was due to that three of the most abundant species (*Serrapinnus* cf. *microdon*, *Serrapinnus micropterus* and *Bryconops* cf. *caudomaculatus*), which accounted for 80.3% of catches, dominanted in the headwater sites, probably owing to higher abundance of subaquatic vegetation at these sites, favouring higher fish density. Due to their small body size, these species have low dispersal capacity and often live in association with aquatic plants, where they find food resources and protection from predators (Petry *et al.* 2003; Agostinho *et al.* 2007b).

The spatial distribution of small-sized fish assemblages in the Branco River was influenced by environmental filters, dispersal limitation, distance from headwaters and presence of dams. Effects of pH on fish-assemblage composition has been documented in both tropical and temperate regions (Rahel 1984; Lourenço *et al.* 2012). Water with high pH (basic water) or low pH (acidic water) can affect gill function (Wurts and Durborow 1992) and, consequently, gas exchange and osmoregulation, impairing biological process (Rahel 1984). Despite having explained only 12% of the variance in species composition, pH was the most relevant of the evaluated environmental filters to influence small-fish species composition in the upper Branco River.

The effect of the spatial factor (MEM2) may be related to the limited dispersal capacity of small-sized fish species at larger scales (Griffiths 2006; Bie *et al.* 2012; Henriques-Silva *et al.* 2013; Fernandes *et al.* 2014). Small fish are mostly short-distance migrants, and respond mainly to fine-scale environmental variations like water depth and habitat structure (Lamouroux *et al.* 1999; Fernandes *et al.* 2015). Likewise, the effect of the distance from headwaters also points to limited dispersal capacity, as predicted by the network position hypothesis (Henriques-Silva *et al.* 2019; Borthagaray *et al.* 2020). Environmental and spatial factors explained only 30% of the variation in our data, which indicates that important variables, such as functional connectivity, and ecological interactions, such as competition, known to be important in regulating composition and structure of local assemblages (Cadotte and Tucker 2017) were not considered.

Some studies have shown that hydroelectric dams alter the characteristics of the aquatic environment (Forsberg *et al.* 2017) and reduce up- and downstream connectivity, further limiting the large-scale dispersal of fish (Falke and Gido 2006; Barbosa *et al.* 2015), resulting in changes in fish assemblage structure (Torrente-Vilara *et al.* 2011; Vitorino Júnior *et al.* 2016; Cella-Ribeiro *et al.* 2017), and reductions in fishery stocks (Santos *et al.* 2018). Previous studies have generally focused on species with larger body size, which are more likely to be migratory and rheophilic (Couto *et al.* 2021), unlike the small-sized fish considered in our study, which are mostly short-distance migrants (Castro and Polaz 2020). Our results indicate that the presence of dams also affects small-sized margin-dwelling fish assemblages. However, the unbalanced sampling design between the three regions, with a higher number of sites and range of distances among sites in the upstream region, may have influenced species composition, richness and abundance estimates.

CONCLUSIONS

The small-sized fish assemblage from the upper Branco River was dominated by order Characiformes and the variance in species composition was explained mainly by environmental (pH) and spatial factors (MEM2 and distance from headwaters), as well as the presence of dams. Environmental and spatial factors explained 30% of the variation in our data, which indicates that important variables were not mensured. Future studies that assess the longitudinal gradient in the riverine landscape in the Amazon basin should take into account the effect of other environmental variables and further evaluate the effect of the alterations in the natural river dynamics on the connectivity in small-sized fish species.

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