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Fish Ecology of the Alto Madre de Dios River Basin (Peru): Notes on Electrofishing Surveys, Elevation, Palm Swamp and Headwater Fishes

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Abstract: Our study analyzes the distribution of fish communities related to the environmental variables of the Alto Madre de Dios River, an Andean-Amazon watershed of southern Peru, between 300 and 2811 m a.s.l. within the Manu Biosphere Reserve. We provide new ecological and diversity data on fishes for these poorly studied rivers and new data for palm swamp habitats. With electric fishing techniques, we collected a total of 1934 fish specimens belonging to 78 species, 42 genera and 15 families. To assess main patterns of diversity we combined SIMPER and ANOSIM with canonical correspondence analysis to obtain an overview of the community structure of fish and their distribution related to aquatic habitats. Our results show an important shift on fish diversity at 700 m a.s.l. separating headwater and middle-lowland communities. Electrofishing was a hindrance due to the depth, flow and low conductivity of the rivers, but also allowed us to capture fish not observed with other techniques. We also compared the use of elevation with slope as an alternative variable for statistical analysis. Our results show that slope offers a solid and equivalent explanation for fish distribution variability, avoids redundancy, and instead of giving geographical data offers ecologically solid information.

Keywords: Tropical Andes; Manu Biosphere Reserve; *Astroblepus*; *Trichomycterus*; *Mauritia flexuosa*

1. Introduction

Freshwater ecosystems are often referenced among those that are most altered and threatened by anthropogenic impacts [1] and their fauna is at greater risk than any other animal and plant groups [2]. In this respect, precise knowledge of ecological aspects like species distribution and requirements are a key point for conservation strategies, especially when the focal species are threatened or endangered [3]. Unfortunately, our limited taxonomic knowledge and incomplete information on species distributions for broad territories is very high and represents an insurmountable obstacle for documentation of imperilment and extinction of freshwater biodiversity [4]. In particular, fish assemblage variations in mountain streams of the Andes are poorly understood [5,6].

The Tropical Andes is regarded as the richest of the 25 recognized global biodiversity “hotspots”. It includes Andean ecosystems above 500 m a.s.l., extending from Chile and Argentina, through Bolivia, Peru, Ecuador, to Colombia and Venezuela. This hotspot supports an estimated 45,000 plant and 3400 vertebrate species (excluding fishes), which represents about 15% and 12% of all globally known species, respectively, being nearly half of them endemic to the area [7].

Peru is home to 1064 fish species [8], more than the 7% of all the globally known freshwater species. Most of them (more than 800) are found in the Amazonian Basin. Only during the first decade of the XXI century, 155 new species were named in Peru and the experts estimate the total number of Peruvian freshwater fishes at approximately 1200 species [8]. However, as seen before, there have been few ecological surveys focusing on fish and the majority point out the uneven level of knowledge for this group of vertebrates [9,10], with some taxa itemized at the species level, whereas others routinely are catalogued at family level, order or even phylum [4].

Reviewing research on the Department of Madre de Dios where our study was carried out (Figure 1), Pitman et al. [11] reported that all the scientific studies generated for the department found that only 2.8% of the analyzed manuscripts focus on fish or hydrobiology. Only lichens received less attention than fish. Other research works regarding plants or mammals comprise 21.3% and 16.6% of the total scientific literature, respectively.

Streams of the Tropical Andes are also ecologically important as the headwaters of the megadiverse lowland river systems in South America [12]. They are responsible of delivering major loads of inorganic sediment and organic carbon to lowlands [13] and play a key role in the ecological processes along the Andes-to-Amazon fluvial continuum [14]. Furthermore, the Andean flank of the Amazon hosts the highest biodiversity rates and has been least affected by historical climate variability and land use [15].

However, the Andean-Amazonian piedmont is a rapidly changing landscape, part of the “arc of deforestation” [16], caused foremost by the expansion of cattle and soybean production [17]. The recent growth of human populations, the exploitation of natural resources and the proliferation of hydroelectric dams are leading to extensive reductions in habitats and subsequent impacts on rivers [18–20]. Fortunately, many opportunities for protecting these habitats yet exist, particularly in Peru, where entire river systems are still relatively intact and where there are few large dams and other major structural changes to river channels [21].

Our study area, the Alto Madre de Dios River Basin, in the south west of Peru, is an almost pristine basin. It is located in the Andean-Amazonian piedmont, in the transition zone of the Manu Biosphere Reserve, bordered on the northwest by the Manu National Park, and on the southeast by the Amaraeri Communal Reserve, two important protected areas of the Peruvian Amazon. This region has long been known to tropical biologists as a region high in species diversity, as well as some of the world’s largest expanses of pristine tropical forest [22].

Given the pessimistic predictions for the sustainability and conservation of the Andean biodiversity due to environmental alterations [23,24], studies on the relationships among biotic and abiotic factors and the abundance and distribution of Andean fish are critical to document the primary characteristics of the original communities and thereby contribute to the delimitation of appropriate conservation areas and/or to recovery strategies.

One of the most frequent variables used for studying ecology and biodiversity distribution patterns is elevation. Ecologist all around the globe have demonstrated the strong correlation between altitude and changes in community composition, for plants [25], insects [26], amphibians [27], birds [28], and other organisms. Elevation is also commonly used for studying freshwater biodiversity distribution patterns along the watersheds [29], and together with the distance to the mouth or the distance to the source, they are among the most repeated variables for analyzing fish distribution patterns [10,30]. Nevertheless, the use of those geographical variables can be problematic. We aspire to discuss it and evaluate the use of slope as an alternative environmental variable.

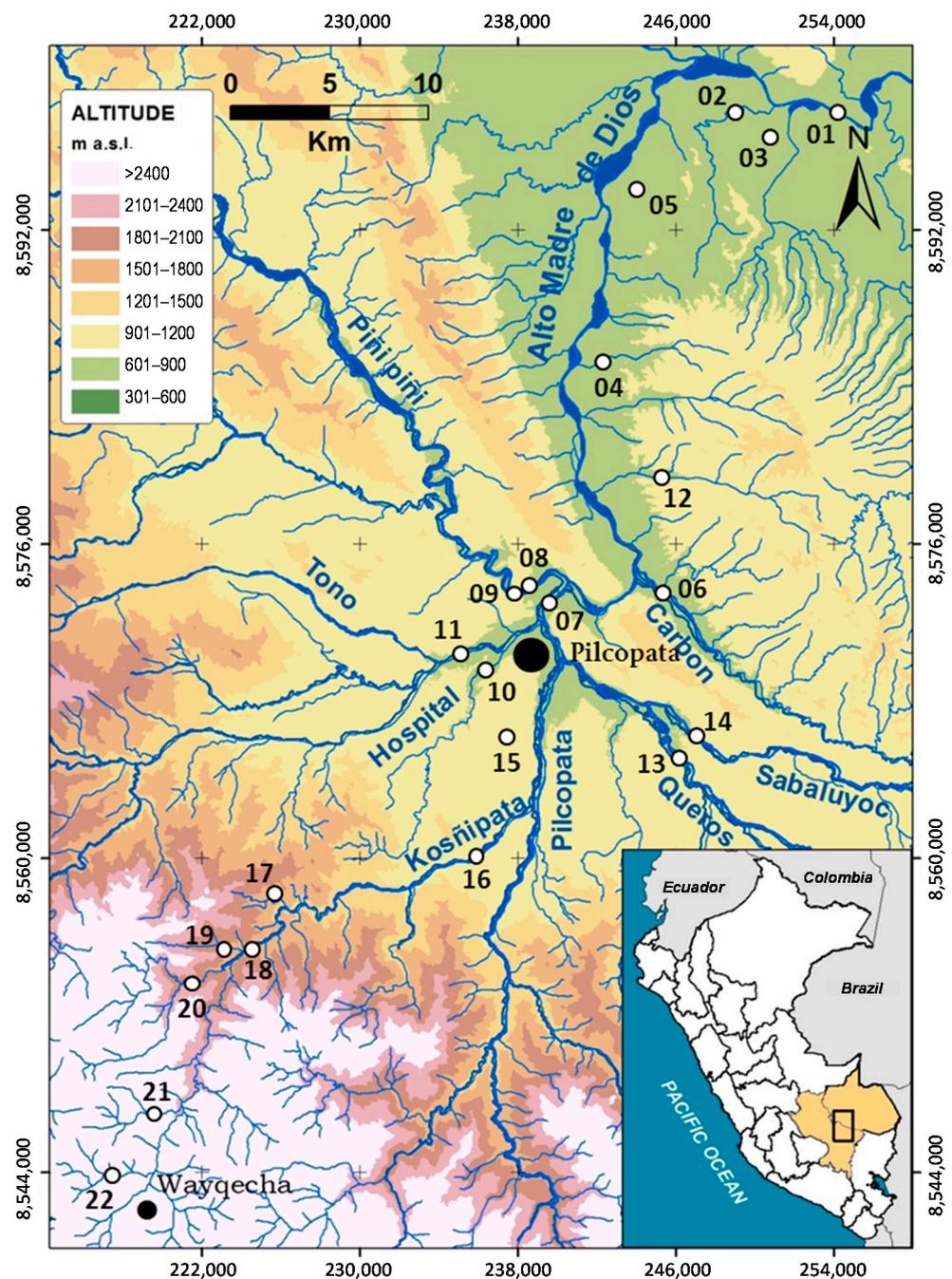


Figure 1. Sampling sites in the Alto Madre de Dios River, Peru (white circles).

In the present study we examine the freshwater ecology for the Alto Madre de Dios River focusing on its ichthyofauna and evaluate the environmental integrity of the studied ecosystems. Our specific objectives are to (1) describe spatial variation of fish assemblage of an unstudied Andean-Amazonian stream, (2) to identify patterns of association between fish assemblages and habitat variables, and to (3) discuss the use of elevation for ecological and fish distribution analysis.

2. Materials and Methods

2.1. Study Area

The Manu National Park has been considered one of the world's most important tropical protected areas since its creation in 1973 (Shepard et al., 2010). Located in the southern Peruvian Amazon rainforest constitutes the core of the Manu Biosphere Reserve

and an IUCN World Heritage Site [31]. The southern buffer zone of Manu Biosphere Reserve includes the Alto Madre de Dios basin ($11^{\circ}00' - 13^{\circ}30' \text{ S}$, $73^{\circ}30' - 68^{\circ}30' \text{ W}$) (Figure 1). The river flows, south to north, for 275 km through the rainforest of Cusco and Madre de Dios Departments, draining an area of approximately 1600 km². The altitude in the basin varies from up to 3500 to 300 m a.s.l. in the Manu River junction, spanning five Andean vegetation zones: puna (4500–3500 m), upper cloud forest (3500–2500 m), lower cloud forest (2500–1000 m), piedmont (1000–400 m) and lowlands (400–50 m) [32]. Our highest sampling site was located at 2411 m a.s.l. while to lowest was at 398 m a.s.l., 80 kms downstream. Rivers flow through deep and narrow valleys and steep slopes from the source to around 700 m a.s.l., where the valleys open to wide and flat lowland floodplains. Flow regime is highly responsive to rainfall and presents the greatest discharge period from November to April, decreasing progressively in the dry season (from May to October). The rivers remain turbid through the year, with highest sediment loads during the rainy season.

Human population density in the Alto Madre de Dios valley is low, with scattered settlements that are under the administration of the village of Pilcopata in the District of Kosñipata (pop. 4790 in 2007, according to the Statistical National Institute of the Peruvian Government), Department of Cusco.

Nowadays, most of the land is still covered by primary Amazonian forest, although some areas have been deforested and are used for cattle raising. Tourism facilities—lodges and a network of forest trails—are used by a relatively low number of tourists in comparison with other lodges in Madre de Dios area [22].

2.2. Field Sampling and Analysis

Our survey was conducted on twenty-two sampling sites in June 2012, during the dry season (Figure 1). The following environmental variables were recorded at each site using a multiparametric probe (Hanna Instrument, HI 98129 Combo Waterproof, Woonsocket, RI, USA): conductivity ($\mu\text{S}/\text{cm}$), water temperature ($^{\circ}\text{C}$), and pH. Characterization of habitat structure was done using transects according to Armantrout (1998), and included depth (m), width (m), water velocity ($\text{m}\cdot\text{s}^{-1}$), percent tree canopy shading and dominant substrate categorized as fines (<2 mm), gravels (~2–64 mm), pebbles (~64–256 mm), boulders (>256 mm), or bedrock and concrete. Additionally, two habitat quality indices were measured: Qualitative Habitat Evaluation Index—QHEI (Rankin, 1989), and Andean adapted version of the riparian forest quality index QBR (Acosta et al., 2009) (Table 1). QHEI jointly considers different habitat parameters, such as bottom substrate and embeddedness, instream structure, velocity and depth regime, canopy cover, channel alteration and pool/riffle or run/bend ratios. QBR index includes aspects of the riparian forest such as total riparian vegetation cover, cover structure, cover quality and channel alterations.

Fish sampling was carried out in wadeable stream stretches (<1 m of height) by electrofishing surveys, using a backpack electrofishing gear (Hans Grassl model IG200/2D, 300–600 V, 0.2–2 A). Surveys were conducted following a single-run depletion methodology and estimating fish abundance based on catch per unit effort (CPUE) (Meador, McIntyre & Pollock, 2003). Collected fish were anesthetized and subsequently counted, measured to the nearest 0.1 cm total length (TL), weighed with a digital scale to an accuracy of 0.05 g and released after the survey, except for some voucher specimens kept to confirm identification.

Voucher specimens were deposited in the fish collection of the Natural History Museum of National University of San Marcos (Lima, Peru). In the laboratory, fish were preserved in alcohol (75%) and identified by Ana María Cortijo, Jessica Espino and Hernán Ortega, members of the Department of Ichthyology of Museum. Scientific names were validated according to W. N. Eschmeyer's Catalog of Fishes [33].

Table 1. Sampling sites and environmental variables in Alto Madre de Dios River, Peru.

Code	River Place	Date	Alt	pH	Ta	Tw	Cd	Wh	Dh	Vc	QBR	QHEI
01R	Aguas Calientes	13 June 2012	398	7.95	24.3	21.7	105	18.2	25.1	0.51	100	72
02R	Shintuya	12 June 2012	424	8.07	24.4	24.4	99	11.2	13.7	0.23	90	72
03A	Anaconda	13 June 2012	428	7.37	26.4	21.4	119	3.45	16.8	0.05	95	61
04Q	Mascuitania	14 June 2012	459	6.82	29.0	25.4	44	7.86	25.6	0.25	85	67
05Q	Puente a km 2,5	14 June 2012	462	8.03	26.1	22.4	169	5.54	18.7	0.15	100	74
06R	Carbón	11 June 2012	499	8.78	26.3	25.3	102	14.4	34.5	0.41	100	85
07R	Pilcopata	10 June 2012	511	6.94	24.8	25.0	119	15.7	15.6	0.04	100	74
08R	Piñi-Piñi	7 June 2012	518	6.86	22.3	22.0	131	6.86	44.1	0.19	100	88
09Q	Villa Carmen	7 June 2012	518	7.20	22.3	20.6	126	4.51	5.11	0.13	100	62
10R	Hospital	8 June 2012	547	7.63	23.1	19.9	26	28.3	36.6	0.74	100	84
11R	Tono	8 June 2012	551	7.70	19.1	18.5	50	6.94	22.1	0.30	100	68
12R	Salvación	11 June 2012	558	7.26	24.0	23.2	33	4.42	30.1	0.39	100	66
13R	Queros	9 June 2012	587	8.00	20.0	18.3	61	10.4	39.5	0.31	80	82
14R	Sabaloyacu	9 June 2012	593	7.70	19.7	21.2	44	22.9	31.0	0.43	100	87
15A	Aguajal	17 June 2012	622	5.77	23.9	22.0	26	2.49	57.4	0.04	50	63
16R	Asunción	10 June 2012	720	7.40	26.4	19.3	65	5.16	19.9	0.32	100	78
17Q	Quitacalzon	16 June 2012	1064	7.83	20.9	18.7	52	4.07	31.4	0.45	100	88
18R	Kosñipata	16 June 2012	1347	7.53	22.2	16.0	89	6.42	24.4	0.15	95	70
19R	San Pedro	16 June 2012	1394	7.81	20.1	18.5	43	7.74	49.4	0.48	95	88
20R	Unión	17 June 2012	1673	7.72	20.2	15.3	64	8.41	32.0	0.72	75	66
21Q	Wayquecha	18 June 2012	2218	7.40	17.3	11.4	51	3.99	30.2	0.83	100	84
22Q	Pacchayoc	18 June 2012	2411	4.41	16.1	11.7	188	6.11	23.9	0.89	100	82

Alt: Altitude (m), Ta: Air temperature (°C), Tw: Water temperature (°C), Cd: Water conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), Wh: Mean width (m), Dh: Mean depth (cm), Vc: mean water velocity ($\text{m}\cdot\text{s}^{-1}$), QBR: riparian forest quality index and QHEI: qualitative habitat evaluation index.

2.3. Data Analysis

From fish community compositional data, richness (Margalef's index $R = (S - 1)/\ln N$), and diversity (Shannon–Wiener index $H' = -\sum p_i \log^2 p_i$, and Simpson's index $D = \sum p_i^2$) measures were calculated, where p_i is the proportion of species "i" at a given site, N is the total number of collected specimens, and S is the number of species [34].

First, polynomial regressions were set among the mentioned indices and elevation, distance to the mouth or slope. We wanted to describe and compare patterns of diversity considering these three variables related to location along the basin.

Second, several multiparametric statistical approaches were used to establish the main spatial patterns in the fish community composition [35]. To detect patterns among fish communities, we used a permutation-based test with a nonparametric one-way analysis of similarity (ANOSIM) using a Bray–Curtis similarity index based on abundance data [36,37]. The ANOSIM statistic compares the mean of ranked dissimilarities among groups to the mean of ranked dissimilarities within groups. An R-value close to 1 suggests dissimilarity among groups while an R value close to 0 suggests an even distribution of high and low ranks within and among groups. Post-hoc tests with Bonferroni correction in p -values (which were multiplied by the number of comparisons) are done. This correction is very conservative (produces large p -values). The sequential Bonferroni option does not output corrected p -values, but significance is decided based on step-down sequential Bonferroni, which is slightly more powerful than simple Bonferroni. If ANOSIM revealed a statistically significant result, the relative contribution of each species to group dissimilarities was quantified using a similarity percentage analysis (SIMPER), with a cut-off criterion of 90% (Bray–Curtis similarity index), to identify subsets of the most important species [36]. SIMPER calculates the average Bray–Curtis dissimilarity among all pairs of inter-group samples, expressed in terms of the average contribution from each species. Previously, we created three categorical variables (type of mass of water (river vs. stream), elevation (upstream–downstream 700 m), and stream order). Fish assemblages were compared considering these three categories.

For the choice of linear or unimodal analysis, a preliminary DCA (Detrended Correspondence Analysis) was performed [38]. This analysis evaluates the species turnover (the length of gradients) through the first DCA axis. The criterion for choosing linear vs. unimodal ordinations models is to obtain a $SD < 3$ [39]. Our results showed a $SD > 3$

because of the large number of species and their frequency of occurrence. To examine relationships between community composition and several environmental variables a Canonical Correspondence Analysis (CCA, Unimodal response; length of gradient $SD > 3$) was used through the program CANOCO 4.5 [39,40] based on DCA results. To reduce the number of graphics and considering the similar patterns shown, altitude was selected over distance to mouth ($r = 0.964$, p -value < 0.01) as representant of the geographical variables, to compare it with slope. Two parallel CCA were carried out with altitude or slope as key variables related to location along the headwater-downstream gradient. This direct gradient analysis technique provides ordination axes linearly related to the explanatory variables. The main result is a scatterplot ordination diagram displaying the pattern of fish assemblage variation along the environmental variables shown as vectors. Vector direction and length indicate the relative magnitude and influence of a particular variable on fish assemblages. The significance of the analyses was assessed by a permutation test with 1000 random permutations. Habitat structure and water quality variables were also included in the analysis. To improve linearity, environmental variables were log transformed. Proportional data and abundance fish data were transformed (arcsine and root transformed, respectively). Peebles was removed to avoid a high variance inflation factor (VIF) [41].

All multiparametric analysis were done for species that were captured in more than two sites (i.e., 10% occurrence) and were performed using the R project software version 3.2.3 [42] with the package "vegan" version 2.3–3 [43] except in the case of CCA which had been developed in CANOCO 4.56 [40].

Finally, fish species have been classified according to their trophic specialization [6,44,45], with the aim to analyze the distribution of these trophic groups in the elevation gradient.

3. Results

A total of 1934 specimens were collected, and 78 fish taxa were identified belonging to 43 genera and 14 families (Dataset S1). Twenty-seven species were undetermined, ten were identified as species *affinis*, and 41 species were correctly identified. Characids were the most diverse family with 43% of the species, followed by loricarids (14%), trichomycterids (8%), astroblepids, cichlids and heptapterids (7% each one) and crenuchids (3%). The remaining fish belonged to ten families with only one represented species.

Regarding diversity indices, values presented a marked altitudinal pattern. Shannon–Wiener and Margalef's indices showed negative correlations with altitude, distance to the mouth and slope, whereas Simpson's index showed positive correlation (Figure 2). Site 13 presented the highest diversity and hosted alone 28% of the total fish species (22 species). On the other hand, highest sites, 21 and 20, only had two species (*Astroblepus* sp. and *Trichomycterus* sp.). Finally, site 15 significantly different from the rest of the sampling points. It was a swamp ecosystem isolated from the main river and due to its unique environmental characteristics, it was discarded for subsequent analysis.

Results of one-way ANOSIM showed significant differences in community composition as indicated the high R values (global R: 0.786, $p < 0.001$) with the cut-off point at 700 m a.s.l. This makes fish composition upstream and downstream 700 m a.s.l. well distinguishable. Mass of water and stream order showed not significant differences (global R: 0.04 and R: 0.22, respectively; $p > 0.05$). The SIMPER procedure indicated that using three species was possible to explain more than 38% of variation among elevation categories. *Astroblepus*, *Trichomycterus* and *Knodus* were the most important species explaining the variation in community composition (Table 2).

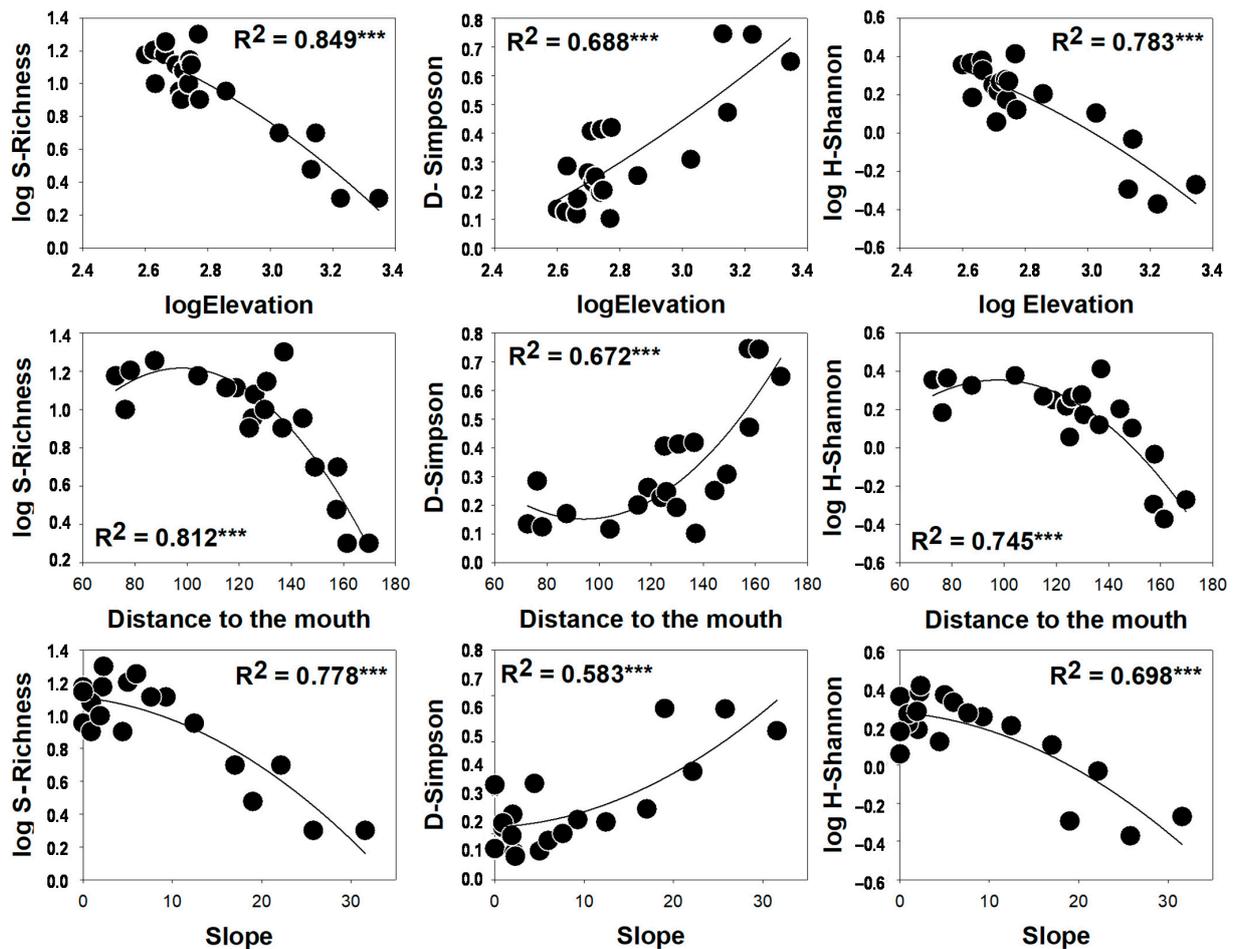


Figure 2. Estimates of the fish community diversity indices along the Alto Madre de Dios River, regarding to elevation, distance to the mouth and slope of sampling points. *** significant coefficient of determination (R^2).

Table 2. Relative abundance of taxa presents under (U) and above (A) 700 m of elevation. Taxa are ordered by their overall weight in the SIMPER analysis (taxa showed with a cut-point of 90% of contribution). Percent of contribution of each taxon accord to SIMPER is show for each group (%contri). In the last row richness of each category of groups are shows.

Taxa	% Contri.	Elevation-700 m	
		U	A
<i>Astroblepus</i>	18.36	1.03	9.00
<i>Trichomycterus</i>	10.76	0.78	5.78
<i>Knodus</i>	8.91	3.92	-
<i>Chaetostoma</i>	7.67	3.54	1.93
<i>Creagrutus</i>	6.35	3.09	-
<i>Bryconamericus</i>	6.01	1.84	2.64
<i>Ceratobranchia</i>	5.88	3.22	-
<i>Astyanax</i>	5.26	2.45	-
<i>Ancistrus</i>	4.57	2.48	0.28
<i>Bujurquina</i>	3.42	1.73	-
<i>Hemibrycon</i>	3.36	1.63	-
<i>Characidium</i>	3.01	1.33	0.28
<i>Aphyocharax</i>	2.67	1.52	-
<i>Rineloricaria</i>	2.36	1.05	-
<i>Hoplias</i>	1.96	0.87	-
Richness		24	6

The relationship between fish and environmental variables are shown in Figure 3 and Tables 3 and 4. The importance of environmental variables is indicated by the marginal effect values (λ_A): for both analysis conductivity (0.22), boulders (0.25), water velocity (0.25) and water temperature (0.38) were most significant. The variables with the highest marginal effect were slope (0.44) for the first CCA (Table 3a) and elevation (0.59) for the second CCA (Table 3b).

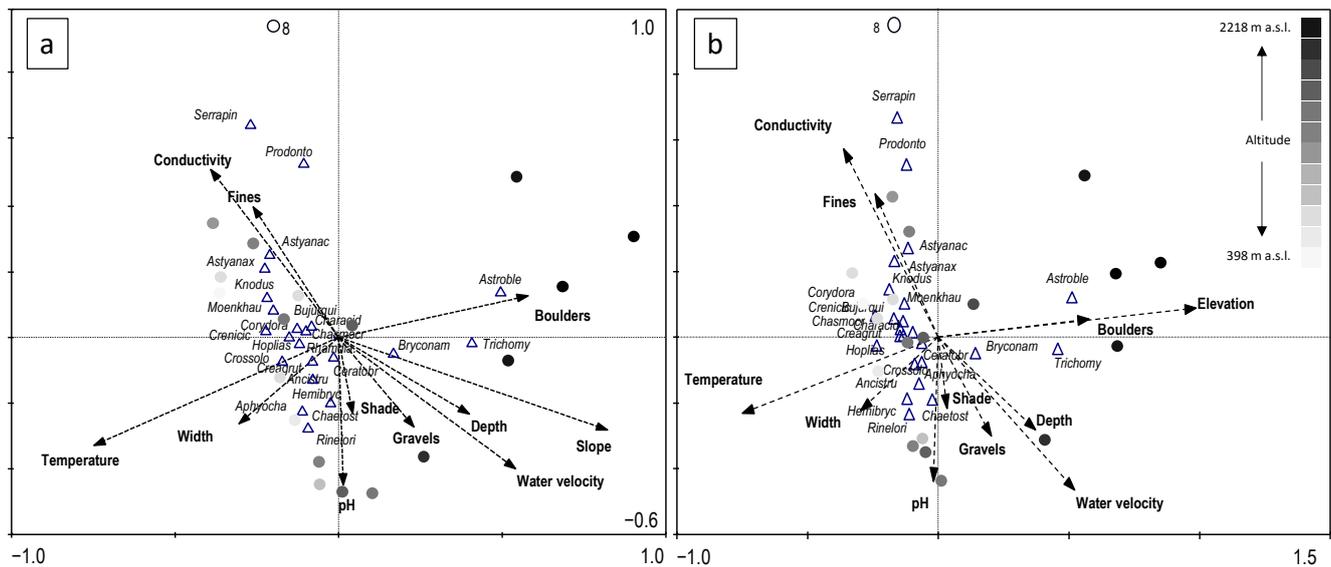


Figure 3. Triplot of results of canonical correspondence analysis carried out with slope (a) or elevation (b) as key variables related to location, showing site scores (circles), the environmental variables (vectors) and fish abundances (triangles) on the first two canonical axes. The code for site scores and environmental variables refers to sampling sites according to Table 1.

Table 3. Results of canonical correspondence analyses (CCA) carried out with slope (a) or elevation (b) as key variables related to location along the headwater-downstream gradient, showing canonical coefficients and weighted intraset correlation coefficients of explanatory variables with the first two axes of the CCA. Importance of environmental variables using marginal effects are shown λ_A .

(a) Slope	Marginal Effects	Intraset Correlations		Regression/Canonical Coefficients	
Groups	λ_A	CCA1	CCA2	CCA1	CCA2
Slope	0.44	0.786	−0.261	0.755	−0.114
Water temperature	0.38	−0.715	−0.304	−0.418	−0.433
Water velocity	0.25	0.517	−0.370	0.047	0.002
Boulders	0.25	0.554	0.118	0.006	0.111
Conductivity	0.22	−0.374	0.472	0.007	0.987
Depth	0.19	0.382	−0.216	−0.076	0.377
Fines	0.15	−0.250	0.368	−0.149	0.282
Width	0.14	−0.291	−0.243	−0.132	0.064
pH	0.13	0.014	−0.415	−0.090	−0.662
Gravels	0.07	0.220	−0.252	0.018	0.236
Shade	0.07	0.041	−0.214	−0.266	−0.289
(b) Elevation	Marginal Effects	Intraset Correlations		Regression/Canonical Coefficients	
Groups	λ_A	CCA1	CCA2	CCA1	CCA2
Elevation	0.59	0.963	0.081	1.069	0.077
Water temperature	0.38	−0.730	−0.213	0.189	−0.330
Water velocity	0.25	0.510	−0.427	0.074	−0.032
Boulders	0.25	0.563	0.051	0.091	−0.010
Conductivity	0.22	−0.354	0.526	−0.068	1.064
Depth	0.19	0.364	−0.259	−0.080	0.402
Fines	0.15	−0.236	0.400	0.045	0.287
Width	0.14	−0.289	−0.205	−0.084	0.184
pH	0.13	−0.018	−0.403	0.164	−0.787
Gravels	0.07	0.198	−0.276	−0.035	0.261
Shade	0.07	0.034	−0.199	−0.083	−0.250

Table 4. Summary statistics table for CCA ordination presented, with slope (a) or elevation (b) as key variables.

(a) Slope	CCA Axis	1	2	3	4
	Eigenvalues	0.579	0.264	0.167	0.126
	Species-environmental correlations	0.956	0.924	0.884	0.950
	Cumulative percentage variance of species data	27.9	40.6	48.7	54.7
	of species-environmental relation	38.4	55.9	67.0	75.4
(b) Elevation	CCA Axis	1	2	3	4
	Eigenvalues	0.597	0.263	0.170	0.132
	Species-environmental correlations	0.976	0.918	0.903	0.931
	Cumulative percentage variance of species data	28.8	41.5	49.7	56.0
	of species-environmental relation	38.8	55.9	67.0	75.5

For (a) Significance of the axis by the Monte Carlo permutation test is given by $F = 3.10$ ($p < 0.05$). All canonical axes were significant. Values in bold indicate significant difference at $p < 0.05$. For (b) Significance of the axis by the Monte Carlo permutation test is given by $F = 3.23$ ($p < 0.05$). All canonical axes were significant. Values in bold indicate significant difference at $p < 0.05$.

Outcomes were equivalent using slope and elevation as main variables, therefore, only results for CCA with slope are described next. The inertia in the assemblage composition in the studied sites was 0.579 for axis 1, 0.264 for axis 2, 0.167 for axis 3 and 0.126 for axis 4. The CCA ordination revealed strong relationships between species abundances and measured environmental variables explaining 72.6% of species distribution. The first canonical axis (axis 1) accounted for 38.4% of the variation in the data set, the second axis (axis 2) accounted for 17.5% of the variation in the data set. An unrestricted Monte Carlo permutation test indicated that all canonical axes were significant ($p < 0.05$).

First Canonical Correspondence Analysis CCA1 eigenvalue accounts 0.579 and it is composed by slope, boulders and water velocity on the positive side and water temperature on the opposite side (Figure 3a). First Canonical Correspondence Analysis was interpreted as a longitudinal gradient from high to lowlands with sites above 700 m a.s.l. being distributed on the right area of the graph (except for site 12, slightly right from the axis) and sites below that altitude spread in the left area. Second Canonical Correspondence Analysis CCA2 (eigenvalue = 0.264) is dominated by conductivity on the positive side and pH and water temperature on the negative side. Second Canonical Correspondence Analysis was interpreted as hydrochemical parameters (Table 3a; Figure 3a).

On the right area of the graph (Figure 3a) a first species group composed by *Trichomycterus* (Figure 4) and *Astroblepus* genera (Figure 5) was separated from the remaining and it was positively related to headwaters showing distinctive features: high velocities of water, big boulders and lower temperatures. *Bryconamericus* genera species were also related to these headwater streams, although they do not appear so high in the altitudinal gradient. A second group of fish represented by *Serrapinnus*, *Prodonotocharax*, *Astyanax*, *Astyanacinus* and *Knodus* genera, located on the top-left area of the graph, was positively related to still waters with low velocities, higher conductivities and fine substrates (mainly sands). A third cluster, located on the top-left area of the graph, included *Rineloricaria*, *Chaetostoma*, *Aphyocharax*, *Hemibrycon*, *Ancistrus*, *Creagrutus* and *Crossoloricaria*. They were related to open places with highest water temperatures and wide riverbeds. The rest of the genera were distributed between these two groups along the first axis on the left of the graph, including *Hoplias*, *Rhamdia*, *Chasmocranus* and *Characidium*.

Attending to fish distribution and trophic specialization [6] along the altitudinal gradient (Figure 6), no fish were found above 2200 m a.s.l. *Astroblepus* and *Trichomycterus* were the only genus found on highest reaches (above 1400 m a.s.l.), feeding exclusively on invertebrates. Even though they appear tightly related to headwaters, they were found all along the basin with occasional and scarce occurrence also in lowland streams. At 1400 m a.s.l. three new genera appeared: one invertivorous *Bryconamericus*, and the

first two herbivore-detritivores *Chaetostoma* and *Ancistrus* genera. Next invertivorous *Characidium* genus was found below 1100 m. Above the 700 m other three invertivorous genera were found: *Rhamdia*, *Creagrutus* and *Chasmocranus*. The rest of the fish taxa were found below 622 m, representing most of the diversity. The first piscivorous species, *Hoplias malabaricus* and *Crenicichla semicineta* were found at this altitude.

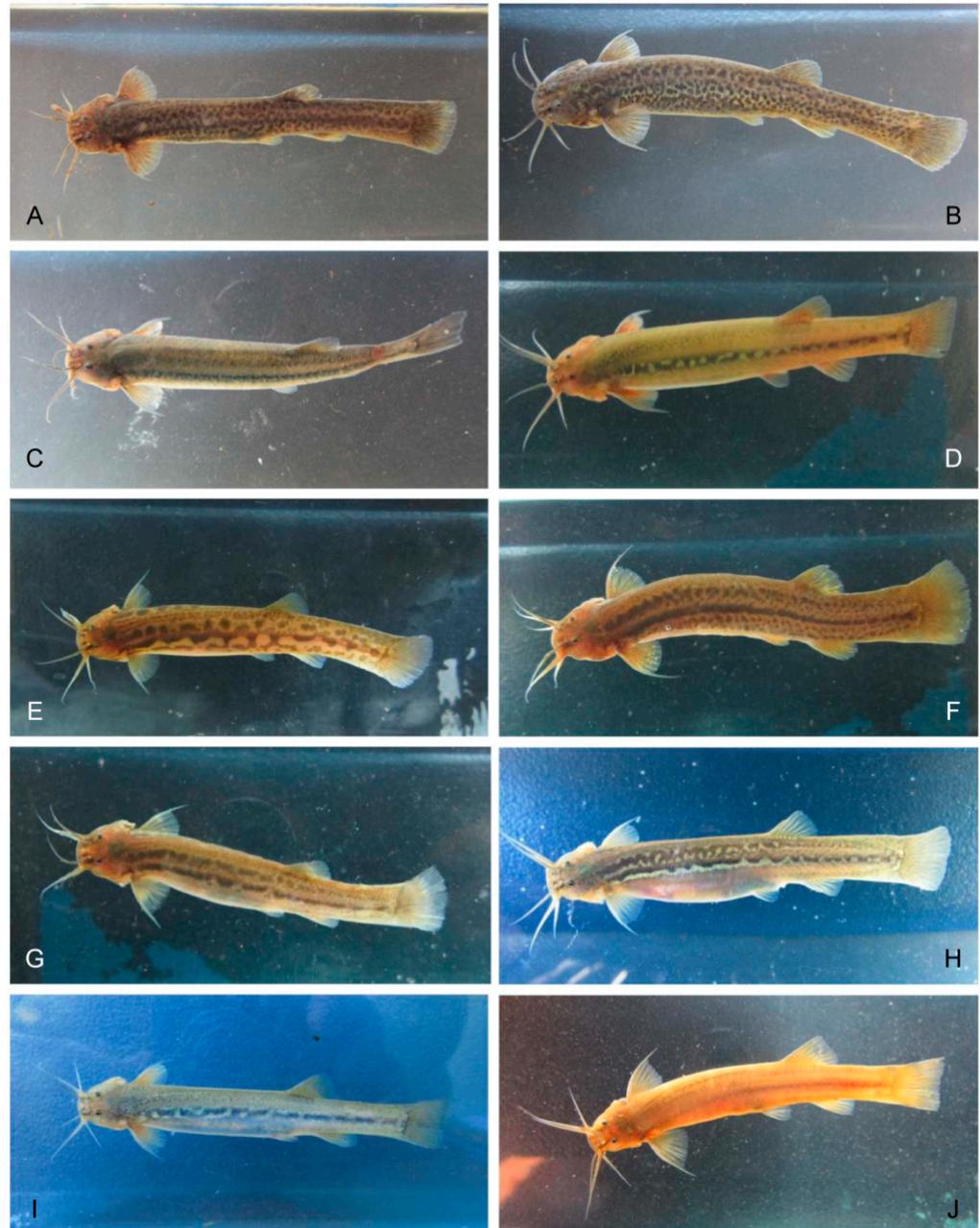


Figure 4. Species and morphotypes of genus *Trichomycterus* collected in the upper Madre de Dios River. (A,B) *Trichomycterus* sp.3 collected on Américo stream (21Q), (C) *Trichomycterus* sp.2 collected on Unión River (20R), (D) *Trichomycterus* sp.2 collected on San Pedro River (19R), (E) *Trichomycterus* sp.3 collected on Quitacalzon stream (17Q), (F,G) *Trichomycterus* sp.5 collected on Quitacalzon stream (17Q), (H) *Trichomycterus* sp.4 collected on Salvación River (12R), (I) *Trichomycterus* sp.6 collected on Kosñipata River (16R), and (J) *Trichomycterus* sp.1 collected on Queros River (13R).

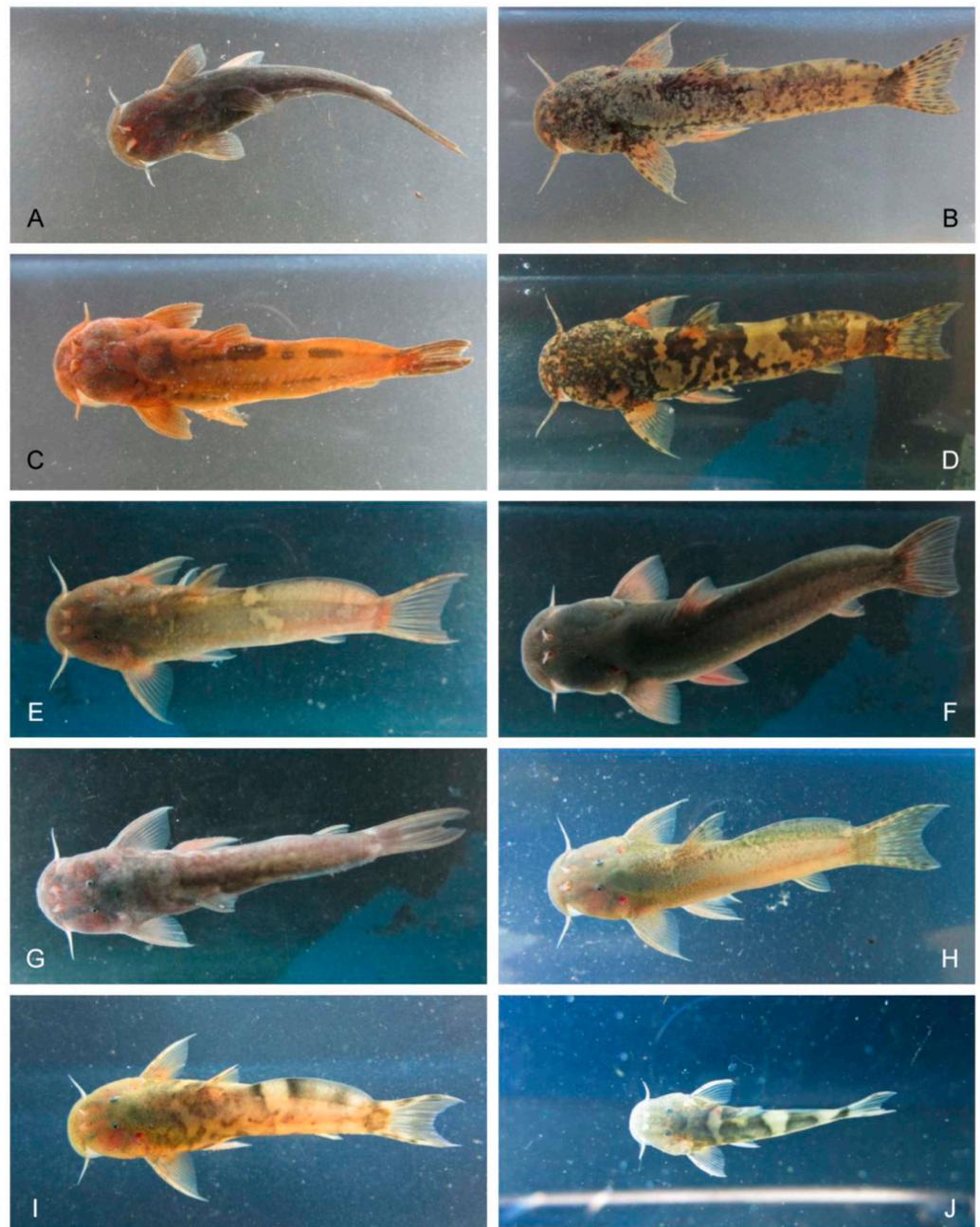


Figure 5. Species and morphotypes of genus *Astroblepus* collected in the upper Madre de Dios River. (A) *Astroblepus* sp.2 collected on Américo stream (21Q), (B) *Astroblepus* sp.1 collected on Unión River (20R), (C) Reddish specimen of *Astroblepus* sp.1 collected on Unión River (20R), (D) *Astroblepus* sp.1 collected on San Pedro River (19R), (E) *Astroblepus mancoi* collected on San Pedro River (19R), (F) *Astroblepus* sp.2 collected on San Pedro River (19R), (G) *Astroblepus* sp.1 collected on Quitacalzon stream (17Q), (H) *Astroblepus* aff. *longifilis* collected on Kosñipata River (16R), (I) *Astroblepus mancoi* collected on Queros River (13R), and (J) *Astroblepus* aff. *trifasciatus* collected on Salvación River (12R).

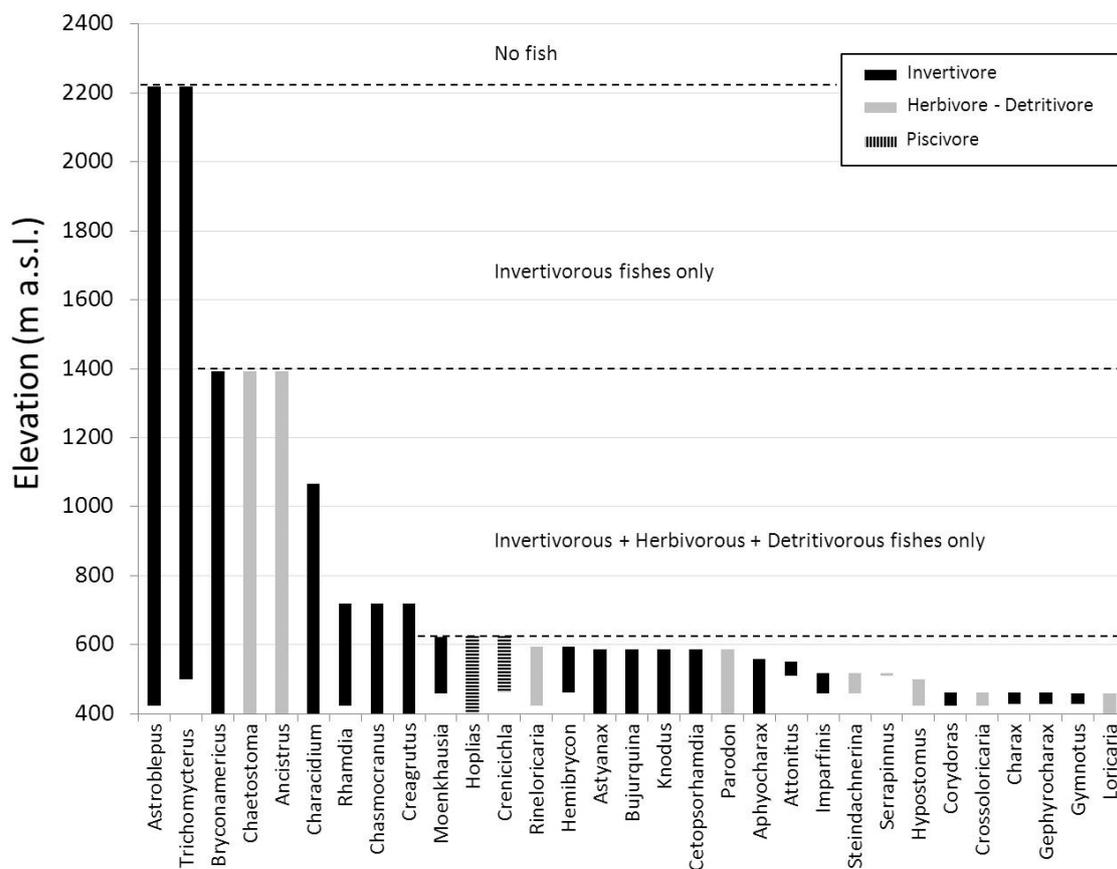


Figure 6. Elevational ranges and trophic specializations of fish species occurring at elevations >400 m a.s.l. in tributary and main-channel sites within the Alto Madre de Dios watershed, south-eastern Peru.

4. Discussion

A gradual increase in species richness and diversity is expected along longitudinal gradients in lotic systems, associated with changes in resource availability, channel morphology, flow regime and substrate type [46,47]. Addition of species is usually related to an increase of habitats motivated for the structural diversification of the environment downstream [48]. Our study confirms this pattern (Figure 2), with the only exception of one sampling point 15, that does not fit the trend due to its low diversity values, explained by its peculiar features as discussed below.

All the studied environmental variables were correspondent with the values reported by other works for natural and unaltered streams of this territory [6,30,49]. Quality indices of riparian and fluvial habitat (QBR and QHEI) got very high scores in all the sampling points, highlighting the good environmental quality, with some remarkable exceptions (Table 1). Riparian forest (and adjacent jungle) on the right bank of the sampling point 13, in the Queros River, was lacking due to deforestation for extensive cattle raising.

Besides, sampling point 15, located at 622 m a.s.l., showed significant differences both in environmental and diversity features, as already mentioned. This site belongs to a special type of aquatic ecosystem of Amazon forest known as *aguajal*. They are back swamp forests, defined as forest on low-lying areas outside of streams courses, only connected to them during high flood season. They are usually dominated by palms (*Mauritia flexuosa*), although other tree species like figs (*Ficus* species) can be abundant as well [50]. They also tend to be clear and acidic, in contrast to the higher turbidity and circumneutral pH of the streams around [51,52]. This *aguajal* was confined by extensive cattle pastures surrounding it and was used as drinking reservoir for cattle, showing a remarkable eutrophication. Sampling point 15 presented the described characteristics

and showed low habitat quality indices (Table 1) and low diversity of fishes (Figure 2), with only three extant species: *Moenkhausia oligolepis* (70 specimens), *Crenicichla semicincta* (three specimens) and *Hoplias malabaricus* (one specimen). However, diversity might increase in the rainy season, when this habitat is connected to the river and other species present in the river could occupy this place. Species of genera *Astroblepus*, *Trichomycterus*, *Bryconamericus*, *Ceratobranchia*, *Creagutus* and *Rhamdia* are present in the nearest sampling points (Sites 14 and 16).

Because of its special and no-comparable characteristics, this peculiar sampling point has been excluded for the general comparative analysis. Although the importance of the *aguajales* has been widely recognized because of their ecological and social relevance [53,54], no fish species records are available on the literature. Our fish and ecological data throw some light on these poorly studied freshwater habitats.

When analyzing richness, diversity and dominance in relation with position from headwaters-downstream indicated by elevation, distance to mouth and slope, results were solid and similar for the three variables (Figure 2). The Canonical Correspondence Analyses through the marginal effect values (λ_A) highlighted principal role of elevation (0.59) or slope (0.44) for explaining fish distribution along the basin (Table 3). The resulting graphics were also analogous when using both variables (Figure 3). Elevation and distance to mouth are widely used for multivariate analysis [55,56]. Nevertheless, they do not give environmental information, they are geographical variables and indicate spatial position. Although they show strong correlation with ecologic changes, their use for ecological analysis together with environmental variables may be problematic. Instead, slope is a purely environmental variable that measures a geophysical characteristic of the site. Furthermore, it is strongly related to position along the basin but is independent from other variables, something that avoids “noise” on multivariate analyses. After validating its robustness for explaining fish distribution as well as elevation, we recommend the use of slope as alternative variable to elevation or distances to mouth/source.

Fish community composition along the basin presented two distinct groups with a clear cut-off point occurring at 700 m a.s.l as indicated by the ANOSIM, separating mountainous fish from piedmont communities. The boundary between both zones is marked by geomorphological changes on the basin, when it shifts from the steep mountainous streams flowing through narrow valleys, to flat and wide floodplains where river channels widen. As pointed out by SIMPER procedure *Astroblepus* and *Trichomycterus* are the most distinctive species from the mountainous streams, while *Knodus* was the most important species from lowlands explaining the variation in community composition (Table 2).

Fish abundance and distribution in response to environmental variables was represented using the CCA analysis and showed similar results using slope or elevation (Figure 3). The observed distribution of species along the longitudinal gradient of the basin was comparable to other fish assemblages found in rivers with similar characteristics in adjacent areas [10,30,49]. This biotic zonation corresponds to discontinuities in river geomorphology or abiotic conditions and are usually related to smooth transitions of abiotic factors contributing to nested patterns of assemblage composition along the altitudinal gradient [57]. The graph highlights this zonation, distributing headwater sites and their fish assemblages on the right of the diagram, related to fast running waters, steep slope, boulders and low water temperatures, opposed to middle and lowland streams on the left of the graph related to higher water temperatures, wider streams, low velocities, higher conductivity and fine substrates.

Genera *Astroblepus* and *Trichomycterus* co-occur in high reaches, being the unique species present on the sites above 1400 m a.s.l. These genera are adapted to headwaters, related to rocky substrates and cold, clean and well oxygenated water (Figure 3). They are benthonic species without scales and a powerful sucker mouth or opercular odontodes [58]. Nevertheless, they occasionally occur in lower areas, although their distribution is usually limited to elevations greater than 400 m.a.s.l. (Lujan et al., 2013). *Bryconamericus*, a rheophile genus, is the next genus joining the headwater assemblage at 1400 m a.s.l. (Figure 6).

They prefer areas of moderate to strong current and present even in the torrential flows, occupying intermediate places between mountain upstream and jungle downstream [30,59]. We also found some *Chaetostoma* and *Ancistrus* fishes at these reaches, although they appeared closely related with lower middle section streams (Figure 3).

We found most of the species related to these middle-low reaches, distributed along the left area of the CCA (Figure 3): habitats with fine substrata, very slow water velocities, mild slope and more conductivity, related with *Serrapinus*, *Prodontocharax*, *Astyanancistrus*, *Astyanas*, *Knodus* and *Moenkhausia*, on the top-left area of the diagram (Figure 3b) [60]; other lotic sites presenting the widest habitats with highest temperature and higher pH, were distributed on the bottom left of the diagram, related with *Aphyocharax* and *Hemibrycon* genera characids and loricarids like *Rineloricaria*, *Chaetostoma* and *Ancistrus* [47,59,61].

We also observed a remarkable zonation related to trophic niches (Figure 6). Headwaters were dominated exclusively by invertivorous *Astroblepus* (Figure 5) and *Trichomycterus* (Figure 4) fishes due to the scarcity of other food resources in these clean waters [9,58]. Although the first herbivore-detritivore *Chaetostoma* and *Ancistrus* individuals were found starting at 1400 m a.s.l., they were more abundant below 1000 m a.s.l., once the river carries enough organic matter coming from the surrounding forest offering a new trophic niche [9,49]. The first piscivorous species, *Hoplias malabaricus* and *Crenicichla semicincta*, were distributed around 600 m a.s.l. and below, first found at site 15, the *aguajal*, where the *Moenkhausia oligolepis* community was very abundant providing enough biomass to feed the predators. This distribution pattern is according with the observations of Lujan et al. [6].

According to some ecological studies and inventories carried out in this area [8,62], more than 130 species of freshwater fishes have been reported for the Alto Madre de Dios Basin. In the present study, only 78 species were collected, due to two main reasons: (1) the highest biodiversity is found on the lowest reaches of the basin and our lowest sampling was at 398 m a.s.l.; (2) electrofishing technique has some limitations on these ecosystems.

Regarding electrofishing, only wadeable stretches were sampled, whereas larger courses or deep stretches were avoided. This limitation directed our sampling efforts to smaller rivers and tributaries where the electrofisher gained efficiency [63]. Although this represents a significant impediment for carrying out a complete biological survey, also brings up new opportunities. Most of the ichthyofaunal studies undertaken in Amazonia have focused on the large rivers and commercially valuable species, therefore small and noncommercial fishes and secondary streams have been usually overlooked [64]. Guided by our limitations, part of our surveying efforts was concentrated on this poorly studied ecosystems and species.

Besides the restrictions for choosing samplings sites, fish resistance to electrofishing was significant. This resistance was favored by low water conductivity and temperature, high velocities, shelter's abundance or low visibility [65]. Furthermore, capture efficiency depended on the mobility of species: little benthic fish were collected easily, in contrast to larger, strong-swimming species that escaped the electrofishers range. However, according to other authors [65,66] electrofishing is the best sampling method when your objectives are to estimate and quantify freshwater fish populations in streams and wadable rivers and to correlate these abundances with environmental features, habitat characteristics, hydrochemical parameters or other ecological measures. Besides, although electrofishing surveys have been widely achieved on the world, they have been scarce in remote places like the Alto Madre de Dios Basin due to the more complex logistics.

If we match our results with the survey carried out with seine nets by Araújo-Flores in 2013 [62] we find remarkable differences. Comparing the total 44 species reported by Araújo-Flores [62] with our 78 species only 17 of them were captured by both studies. Nevertheless, survey of Araújo-Flores focused on two rivers and its tributaries (Pilcopata and Piñi-Piñi Rivers), distributing ten sampling sites in a smaller area with a limited altitudinal range (500–600 m a.s.l.). If we compare our data for the three sites coinciding with the survey of Araújo-Flores (sites 7, 8 and 9), although our sampling effort was lower (3 vs. 10 sites) and our captured diversity smaller (26 vs. 44 species), we collected 15 species

missing in the survey of Araújo-Flores [62]. Therefore, although electrofishing is not as effective in tropical streams as it is for temperate streams, it may be more effective for capturing some species that seem to be underestimated by other techniques. Consequently, as we focused on understudied small rivers and streams, catching poorly known small fish, using an uncommon technique for tropical freshwaters, our study presents new and relevant taxonomic and ecological information, providing data for some fish never captured before by previous surveys in the area.

Although our results highlight the good conservation status of the Alto Madre de Dios Basin, the territory endures severe threats: logging [22,31], gold mining [67], climate change [15,23], fossil fuel extraction [68], and hydropower projects [20,23]. The development and implementation of management plans is crucial for anticipating and mitigating future impacts. Nevertheless, the principal threat for this peculiar area, with high endemic biodiversity and very good conservation status, is the lack of knowledge regarding its fauna and flora. Studies of the fish fauna are critical to document the primary characteristics of the original communities and thereby contribute to the delimitation of appropriate conservation areas and/or to recovery strategies for degraded streams [69]. Therefore, Peruvian freshwater fish faunal inventory (including the Alto Madre de Dios River Basin) is a priority [8,69]. There are many remote areas, with poor accessibility, where knowledge of freshwater fish fauna is negligible and for most fish taxa basic taxonomic work is still required, not only for this area, but for all the Amazon and adjacent basins [8]. Besides, there is even less information, in some cases null, on the ecology of many species. This knowledge gap highlights the necessity of basic taxonomic works and the creation of field guides providing new research projects an essential tool [70]. Fortunately, there are some remarkable guides for the Madre de Dios Basin [52,61] and adjacent areas [71,72] that offer inestimable material about freshwater fishes in this region, although, the information in these guides is incomplete for many species. In this sense, the lack of basic knowledge may hamper the development of community-level analyses [3,73]. Although fish diversity for the hotspot is unclear yet, some papers estimate fish richness for the Andean region more than 600 species [69], with more species found at low elevations compared to higher reaches [5]. Around 2700 species have been recognized for the hotspot inside the Amazon Basin territory [74]. In contrast to richness, endemism tends to increase at higher elevations and particularly concentrates in isolated patches of habitat such as valleys and mountain tops [7]. An IUCN report evaluated fish fauna conservation status for the Tropical Andes [75] considering 666 endemic species: 13 spp. critically endangered; 33 spp. vulnerable; 36 spp. near threatened; 341 spp. least concern; 215 spp. data deficient. One of the main conclusions points out the significant information gap: 32% of the evaluated fish were poorly known.

Concerning this taxonomic constraint, freshwater fishes of high headwaters demand special attention. All of them are Andean species and live on high altitudes, areas where human settlements and their subsequent impact has a longer and more intense presence, compared with Amazonian streams [23]. We found five different species of *Astroblepus* (Figure 5), two of them undetermined, five species of *Bryconamericus*, and six species of *Trichomycterus* all undetermined (Figure 4). Astroblepids and trichomycterids are typically restricted in their geographical distribution and endemic to single or adjacent river systems of the Andes, and their taxonomy is poorly known and in constant revision [76–78]. Advances on the taxonomic and ecological knowledge of these high mountain species, related to tropical montane cloud forests, are mandatory for the design of conservation and management plans in the area.

According to Ortega et al. [8], there is not an official national Peruvian red list of freshwater fishes because of this lack of knowledge (despite several attempts and proposals). Although conservation lists have their limitations and critics, they represent an essential tool required for protecting biodiversity [79]. Therefore, it is imperative to continue with the study of freshwater fish species in Peru with the aim of improving management actions and conservation plans.

In the light of this scenario where no Andean-Amazon Basin will remain untouched, the Alto Madre de Dios River Basin still preserves healthy ecosystems, with mild human impacts affecting some stretches, but showing a good environmental quality overall. Therefore, this makes the basin a perfect candidate for being preserved and considered as a reference basin for these seriously endangered ecosystems.

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