

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

**Metacommunity analysis of fishes of the Cube River Basin,
Esmeraldas: an intermittent system**

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Biología

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**HOJA DE CALIFICACIÓN
DE TRABAJO DE FIN DE CARRERA**

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intermittent system**

Karla Sofía Barragán Figueroa

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RESUMEN

Los ríos intermitentes son los cuerpos de agua corriente más comunes del planeta. Sin embargo, existe información limitada sobre estos ecosistemas en la región neotropical. La interrupción del flujo en estos ríos causa que las comunidades acuáticas residentes se reorganicen constantemente. Las metacomunidades de peces están determinadas por variables locales y regionales y la magnitud del efecto de cada una puede estar relacionado a la intermitencia. En el presente estudio hipotetizamos que la intermitencia afectará el impacto de las variables locales y regionales en la configuración de la composición de la comunidad de peces (diversidad beta). El estudio se realizó en la cuenca del río Cube, noroeste del Ecuador. El muestreo se realizó durante la temporada lluviosa (abril-mayo) de 2021, bajo el marco del proyecto DRYvER. Nuestros resultados indican que la composición de la comunidad local de peces (diversidad alfa) y las variables ambientales locales difieren significativamente entre sitios perennes e intermitentes. La temperatura y el pH tienen una fuerte correlación negativa con la diversidad alfa. Se capturó un total de 1033 individuos de 20 especies de peces, donde *Pseudopoecilia fria* y *Eretmobrycon ecuadoriensis* representan las especies más abundantes del muestreo. Los sitios perennes son más diversos y tienen un mayor número de especies que los sitios intermitentes. *Roadsia minor* y *Andinoacara blombergi* son especies indicadoras de sitios perennes, mientras que *Trichomycterus spilossoma* y *Astroblepus fissidens* son indicadoras de sitios intermitentes. Finalmente, los análisis de metacomunidades muestran que, tanto en los sitios intermitentes como en los perennes, la intensidad en la que las variables locales y regionales determinan la diversidad beta es similar. Por lo tanto, la intermitencia no modula la fuerza con la que ambos conjuntos de variables determinan la composición de la comunidad de peces. Este estudio contribuye

al entendimiento de cómo las comunidades de peces se agrupan en sistemas intermitentes tropicales, altamente diversos y amenazados.

Palabras clave: Metacomunidad, peces de agua dulce, intermitencia, Ecuador.

ABSTRACT

Intermittent rivers and ephemeral streams are the most common flowing waters on Earth. However, they are still poorly understood in the neotropical region. Flow interruption in these ecosystems leads to complex processes that pushes resident communities of aquatic taxa to be constantly rearranged. Fish metacommunities are shaped by both local and regional variables and the magnitude of the effect of each may be affected by flow interruption. In the present study, we hypothesize that the effect of local and regional variables in shaping fish community composition (beta diversity), is affected by intermittency. The study was conducted in the Cube River Basin, Northwestern Ecuador. Sampling was performed during the wet season (Apr-May) of 2021 under the scope of the DRYVER project. Local fish community composition (alpha diversity) and local environmental variables differ significantly between perennial and intermittent sites. Temperature and pH have strong negative correlations with alpha diversity. A total of 1033 individuals of 20 fish species were found, where *Pseudopoecilia fria* and *Eretmobrycon ecuadoriensis* represent were the most abundant species of our sampling. Perennial sites appeared to be more diverse and had more species than intermittent sites. *Roadsia minor* and *Andinoacara blombergi* were indicative species of perennial sites, while *Trichomycterus spilossoma* and *Astroblepus fissidens* were indicative of intermittent sites. Finally, metacommunity analysis show that in both, intermittent and perennial sites, the intensity in which local and regional variables affect beta diversity, was similar. Thus, intermittency is not modulating the strength in which both sets of variables shape community composition. This study broadens the understanding of how fish communities assemble in tropical intermittent systems, on a threatened, and highly diverse basin.

Key words: Metacommunity, freshwater fish, intermittent, Ecuador

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INTRODUCTION

Intermittent rivers and ephemeral streams (IRES) are found on every continent and they are said to be the most common flowing waters on Earth (Datry, Bonada, & Boulton, 2017).

However, they are often overlooked for regional estimations of flow regimes, management and legislative protection (Datry, Singer, et al., 2017). IRES use “Intermittent” and “ephemeral” as descriptions of flow patterns, which suggest interrupted or shorter flow duration (Datry, Bonada, et al., 2017). Thus, the acronym IRES has been proposed to account for “all flowing waters that cease flow and/or dry completely at some point along their course” as per Datry, Bonada, & Boulton et al. (2017).

Several natural and anthropogenic conditions generate and/or control intermittence on flowing waters. This include climate, water source (rainfall, phreatic waters, soil type; Gutiérrez-Jurado et al., 2019), channel features (topography, geology) and land use (riparian vegetation; Datry et al., 2017a; Fovet et al., 2021). It is predicted that in the next century, the flow permanence of some IRES will be further reduced and that some perennial flowing waters will become IRES due to climate change, increased water use pressure and land-cover change (Acuña et al., 2014; Sauquet et al., 2021). Recently, IRES have gained more scientific attention because of their ecosystems services (Datry, Larned, & Tockner, 2014) (Fovet et al., 2021), however, IRES ecosystems are still poorly understood, especially in the context of climate change.

Water flow cessation makes IRES very complex habitats. These comprise mixtures of lotic, lentic and terrestrial landscapes that can vary through time and space, according to the

changes in surface discharge (rainfall) and ground level (freatic waters; Datry et al., 2017a). Water flow variation causes resident communities to be constantly rearranged and this dynamic has prompted aquatic organisms to develop morphological and/or behavioral adaptations to enhance or accommodate their dispersal abilities and migration patterns, reproductive cycles (Rangel-Serpa & Torres, 2015), life history traits (Lanés, Keppeler, & Maltchik, 2014) and to develop resistance to adverse conditions (de Freitas Barros Neto, Frigo, Gavilan, de Moura, & Lima, 2020).

Fish dynamics in IRES have been studied in Africa, Europe, Australia and North America (Kerezszy, Gido, Magalhães, & Skelton, 2017), but scientific research on the Neotropics is still scarce. In Ecuador, as in many other tropical ecosystems, headwaters of several rivers are intermittent or ephemeral, driven primarily by rainfall (Datry, Bonada, et al., 2017; Molinero et al., 2019). Little scientific literature is available on fish dynamics on Ecuadorian IRES.

Continental Ecuador is divided by the Andean ridge, where the western (occidental) drainages (trans-Andean region) flow into the Pacific Ocean. Rivers from this region tend to be smaller in longitude and drier than their eastern counterparts (Amazon Basin), including several intermittent tributaries, mostly on their headwaters. There is a precipitation gradient along the western-territory, where North occidental territories exhibit higher precipitation levels that periodically diminish towards the south (Jimenez & Valdiviezo, 2021). The lowland tropical forests in northwestern Ecuador correspond to the Chocó bioregion, one of the 25 biodiversity hotspots in the world (Fagua & Ramsey, 2019). Despite

of being an area with priority for conservation due to its high biodiversity and endemism, it exhibits one of the highest rates of deforestation in South America (Fagua & Ramsey, 2019). The Chocó presents a bimodal hydrological seasonality (Arias et al., 2021) where the wet season occurs from January to May and the dry season from June to December (Molinero et al., 2019).

Freshwater fish in Ecuador are within the most diverse but less studied vertebrates of the country (Jimenez & Valdiviezo, 2021). In Ecuador, 124 species have been reported for the western slopes (Jiménez Prado et al., 2015). Western species stand out for having high levels of endemism (38.4%), unusual morphological adaptations, and possibly much cryptic diversity undescribed yet (Jiménez Prado et al., 2015). Three fish biogeographic regions have been described for the Ecuadorian Chocó: (1) Esmeraldas, (2) Santiago-Cayapas, and (3) Mira-Mataje (Barriga, 2012; Hualpa Vivanco & Rivadeneira Romero, 2020; Intriago & Sánchez, 2013).

The Cube River, Esmeraldas River basin, is part of the Esmeraldas fish biogeographic region, and is a seasonally intermittent river. The Cube River sub-basin presents different levels of alteration, ranging from highly deforested areas by intensive agriculture and livestock, to pristine or well-preserved riparian zones. The miss-management of the land makes this basin vulnerable to a decrease in the quality and quantity of water available for human consumption and the maintenance of its ecological properties (Molinero et al., 2019). The location of the Cube basin within the Chocó bioregion, coupled with its characteristic

intermittency and with the different levels of anthropogenic disturbances, makes an ideal study area to research the determinants of diversity patterns among aquatic communities.

At the Cube basin, 22 species of freshwater fish have been reported to occur (W. Aguirre et al., 2019; Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015; Leberg et al., 2021). Even though ichthyological studies in the Cube have documented the diversity of fish (Hualpa Vivanco & Rivadeneira Romero, 2020) and their richness and abundance in fragmented landscapes (Leberg et al., 2021), analyses at the level of fish metacommunities are still needed.

Classic community ecology studies local patterns of abundance, distribution and interaction of species, assuming that communities are closed and isolated (Leibold et al., 2004). The novel metacommunity approach proposes that the composition of local communities, and their spatial variation, is influenced not only by local factors (e.g., abiotic factors of a locality) but also by regional factors (e.g., altitudinal gradients, spatial distances, fragmentation; Presley, 2020). However, much of the available knowledge on fish community ecology in the tropics is focused at the local scale as metacommunities studies are relatively recent in the zone (Heino, 2013; Leibold et al., 2004). Studies that take into account various scales provide a finer understanding on the determinants of diversity patterns for Neotropical IRES; information that could be applicable on similar, less-studied contexts for management purposes and biological conservation.

Leibold et al. (2004) proposed four models to explain how metacommunities are arranged in different ecosystems. For freshwater ecosystems, species sorting which is a model that suggests that communities are shaped by processes like biotic interactions and environmental filtering, seem to be the one that better describes metacommunities (Heino, 2011) (Datry, Melo, et al., 2016; Leibold et al., 2004). Fish metacommunity studies have revealed that in ecosystems with benign conditions (e.g. some perennial rivers), species sorting is more important than dispersal in shaping aquatic communities (López-Delgado, Winemiller, & Villa-Navarro, 2020). Reversly, under moderate environmental harshness, such as conditions IRES present, dispersal, spatial distance and other regional factors better determine community structure (Datry, Melo, et al., 2016) as the dominant pathway in supporting community recovery is recolonization processes from perennial refugees after rewetting (Datry, Moya, Zubieta, & Oberdorff, 2016).

In the present study, we aim to understand what are the determinants of fish communities within the intermittent Cube River Basin, in Esmeraldas-Ecuador. Specifically, we aim to (1) explore how perennial and intermittent sites vary in environmental variables and local diversity and (2) analyze whether intermittency affects the influence of local and regional environmental factors in community composition. For this, we use metacommunity analysis with field-collected data from the wet season (Apr-May) of 2021. We hypothesize that when rivers and streams exhibit intermittency, regional spatial factors will be better predictors of fish community assemblage than local environmental factors, whilst in perennial rivers and streams, local factors (species sorting) will be the determinants of fish assemblage composition.

This study is encompassed under the Securing biodiversity, functional integrity and ecosystem services in DRYing riVER networks project (DRYvER), which is an international and multidisciplinary effort that aims to investigate nine drying river networks under the scope of climate change. The project is funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 869226.

METHODS

Study area

The Cube River (165.15 km² drainage area) is a seasonally intermittent system, part of the drainage of the Esmeraldas River Basin, northwestern Ecuador. At the northwest it limits with the Viche River, at the east with the Mache-Chindul ridge and at the south with the Bilsa Biological Reserve (Figure 1). Most of its area is located in the Mache-Chindul Ecological Reserve (REMACH), which extends through the Esmeraldas province and is shared between farmers and conservation entities. These are the last remnants of the Chocó bioregion in Ecuador.

To study the effect of local and regional variables on fish assemblages within the Cube basin, we sampled 20 different locations during the wet season (Apr-May 2021), 7 of which are intermittent streams or rivers. Intermittent (INT) sites were classified as so whenever they presented temporal pools as the dominant ecosystem (~70% of the area) for at least half of the year, whilst perennial (PER) sites present other water ecosystems, such as rapids, and waterfalls. Intermittency of the sites was confirmed by observation during the dry season (Oct-Dic) and with knowledge of the local people. Sampling site CuB_14, although it dried

completely in the dry season, failed to fit on the proposed classification of intermittency, therefore, it was excluded of the analysis. Nineteen localities were accounted for the analysis: 7 INT and 12 PER. Localities were distributed along an altitudinal gradient between 52 to 490 m.a.s.l (Figure 1,

Table 1. Coordinates and environmental variables of sampling sites. Latitude (Lat), longitude (Lon), altitude (Alt), temperature (Temp), conductivity (Cond), dissolved oxygen (DO), discharge (Dis)) and presented different levels of riparian vegetation cover and land use.

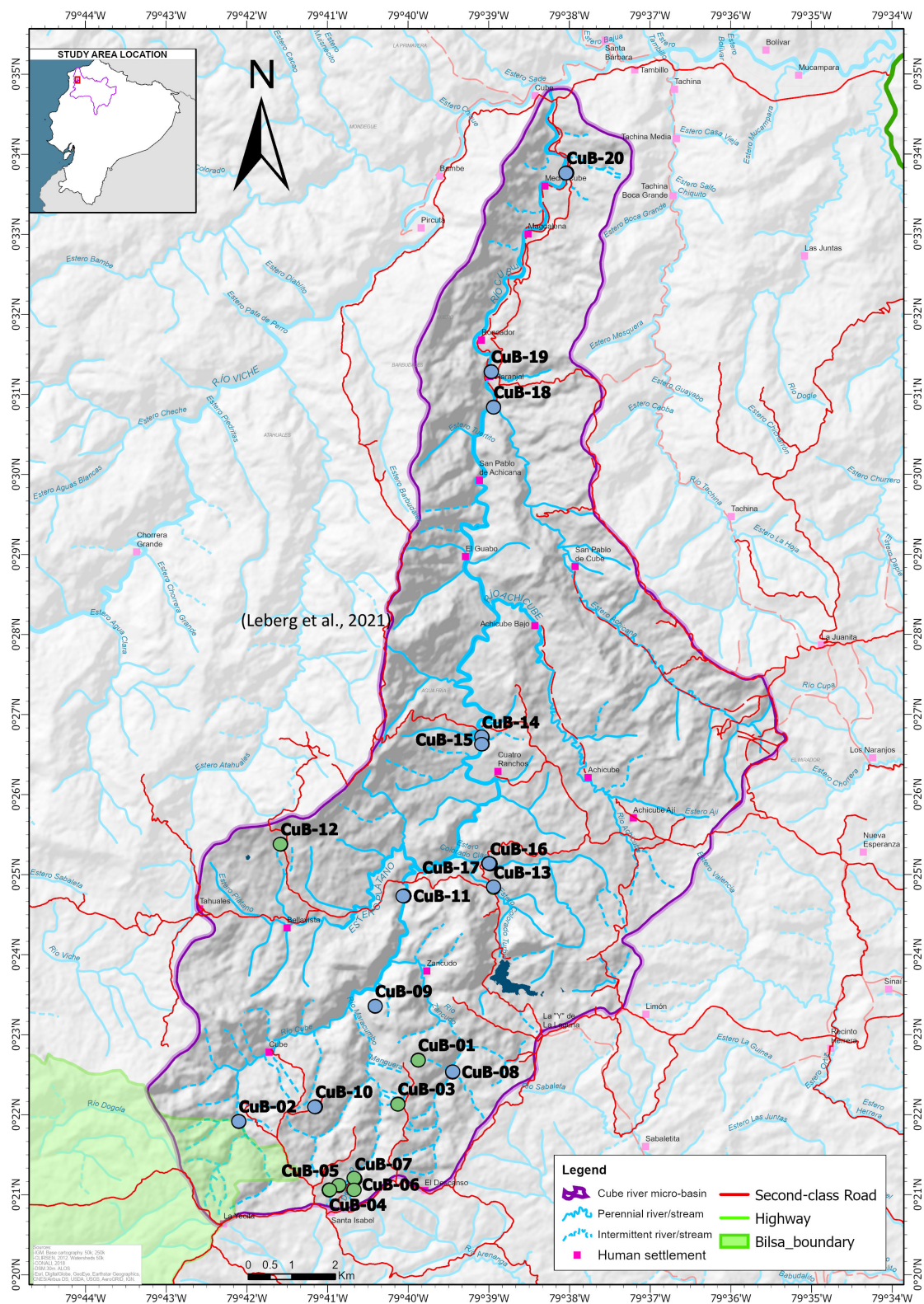


Figure 1. Map of the Cube River basin, Esmeraldas province, northwestern Ecuador. Sampling points are shown as circles where red denotes intermittent (INT) sites and green denotes perennial (PER) sites. Map credits: José Daza

Table 1. Coordinates and environmental variables of sampling sites. Latitude (Lat), longitude (Lon), altitude (Alt), temperature (Temp), conductivity (Cond), dissolved oxygen (DO), discharge (Dis)

Site code	Site name	Drainage	Lat	Lon	Flow	Alt	Temp (°C)	pH	Cond (uS/cm)	DO (mg/l)	Width (m)	Depth (cm)	Dis (l/s)
CuB_01	Estero Mata Caballo	Zancudo	0.3780	-79.6646	INT	490	22.3	7.2	55.5	8.56	2.37	13.65	12.10
CuB_02	Cuchilla bajo Bilsa	Zancudo	0.3652	-79.7016	PER	342	22.3	7.8	92.4	8.58	6.24	42.77	85.50
CuB_03	Entrada olor-zorrillos	Maracumbo	0.3688	-79.6688	INT	472	22.2	6.57	37.6	7.65	1.19	5.92	1.63
CuB_04	Estero Puas	Maracumbo	0.3510	-79.6830	INT	518	22.7	6.25	38.33	8.33	3.07	14.36	61.45
CuB_05	Don Adrian-Pozas	Maracumbo	0.3519	-79.6810	INT	521	22.7	7.53	52.9	8.5	3,00	10.45	16.63
CuB_06	Don Adrian-pequeño	Maracumbo	0.3512	-79.6780	INT	464	23.3	7.36	61.2	8.51	1.78	15.13	24.45
CuB_07	Don Adrian-Sensor	Maracumbo	0.3532	-79.6776	INT	447	22.2	7.22	60.2	8.45	4.72	16.11	67.70
CuB_08	Zancudo	Zancudo	0.3755	-79.6575	PER	325	23.2	7.63	87,00	8.73	4.6	23.87	144.23
CuB_09	Maracumbo	Maracumbo	0.3892	-79.6735	PER	351	23,00	7.44	66.4	8.71	15.91	35,00	260.77
CuB_10	Chanchera	Cube	0.3683	-79.6858	PER	526	23.2	7.21	83,00	8.57	13.3	71.93	2781.85
CuB_11	Don Cortez	Cube	0.4122	-79.6675	PER	198	23.4	7.97	131.3	8.76	17.89	88.56	884.84
CuB_12	Platano/Caca	Cube	0.4230	-79.6930	INT	376	24.1	7.1	208.6	7.48	1.42	9.86	137.76
CuB_13	Colorado turbio	Cube	0.4140	-79.6490	PER	215	26.2	7.4	107.7	7.94	4.7	35.5	396.32
CuB_15	Cube medio-Cristopher	Cube	0.4438	-79.6513	PER	131	25.8	7.52	241.6	8.13	18.2	45.43	4075.72
CuB_16	Cabecera Achicube	Cube	0.4188	-79.6497	PER	207	25.9	8.12	208.6	8.65	6.95	21.24	293.72
CuB_17	Colorado Claro	Cube	0.4190	-79.6500	PER	208	24.6	7.69	325.1	8.07	3.48	15.82	173.97
CuB_18	Achicana	Cube	0.5140	-79.6490	PER	86	26.7	7.57	308.5	8.22	12.02	33.31	436.62
CuB_19	Naranjal	Cube	0.5213	-79.6495	PER	70	26.9	7.59	303.5	8.11	28.43	70.68	13920.81
CuB_20	Cube-bajo-Viche	Cube	0.5627	-79.6338	PER	52	26.4	7.79	350.6	8.12	29.51	48,00	8089.06

Environmental variables

Six environmental variables (water temperature, pH, conductivity, dissolved oxygen, stream width and depth) were taken into account for alpha diversity analysis, as per local community assemblage scale. At each locality a transect varying from 50 to 150 m, depending on river or stream width, was sampled. Every variable, except for depth, was measured five times at different sections of the river or stream and then averaged. Physico-chemical variables were measured with a YSI pro DSS sensor. River width was measured with a Bosch laser rangefinder (GLM 40). Three depth measurements were taken (one on each riverside and one on the center, when possible) every 10 m for big and medium-sized rivers, and 5 m for small streams, within the determined sampling transect, following the DRYvER protocols ("DRYvER - Drying rivers and climate change," n.d.). Sites coordinates and altitudes were recorded *in situ* with a Garmin GPS.

Fish sampling

The transect length at each site depended on river width, water depth and habitat variation, with a minimum of 20 m length for <5 m of width, 50 m for 5-15 m width, and >50 m on one or both sides for >15 m width ("DRYvER - Drying rivers and climate change," n.d.). For medium-sized rivers, fishing was carried out using hand nets, stop nets and seine nets (5mm mesh size, 6m or 10 m long, and 2m high). For smaller streams sampling was aided by an electrofisher (EFKO- Elektrofischfanggeräte, Typ FEG 1500), with a voltage between 200-400 V, and amperage between 0-1 A adjusted to the local water conductivity. Fishing with electrofisher was performed in zig-zag, while walking at a constant speed. Both ends of the

transect were blocked with seine nets. All caught fish were anesthetized with eugenol oil, identified to species level (Froese & Pauly, 2020; Jiménez Prado et al., 2015), measured standard and total length with a caliper, and weighted on a pocket balance (Kern CM 60-2N, $\pm 0,01$ g). After recovery, fish were released back at the same sampling point. Some specimens were photographed live and a voucher for each species was collected to aid with further identification.

Data analysis

Diversity patterns and local environmental variables.

Potential differences in composition of fish communities between INT and PER sites were explored by running a non-metric multidimensional scaling (NMDS) analysis. Data is automatically square-root-transformed and standardized by Winsconsin double standardization by the metaMDS() function of the vegan package (Oksanen et al., 2020), not to meet normality, but to reduce the influence of extreme values, and to make comparable, variables measured in different scales (Oksanen et al., 2020). Then a multivariate homogeneity of groups dispersions analysis (betadisper) was performed to test the assumption for a permutational multivariate analysis of variance (ADONIS), which is homogeneity of dispersion (variances) among groups. ADONIS was ran to test differences between INT and PER sites. An indicator value analysis (INDVAL) was performed to determine indicator species for INT and PER sites. Subsequently, NMDS, betadisper and ADONIS were also performed to analyze sites agrupations related to environmental variables, plus PCA correlation circles to show the relationship between the variables and the quality of representation of each variable, for each group of sites (INT and PER). All statistical analyses

were performed on R Studio software (R Core Team, 2021), using the *vegan* (Oksanen et al., 2020), *indicspecies* (Cáceres, 2013), *FactoMineR* (Lê, Josse, & Husson, 2008), *factoextra* (Kassambara & Mundt, 2020), *ggplot2* (Gómez-Rubio, 2017), *permute* (Simpson, 2014), *lattice* (Sarkar, 2017), *viridis* (Garnier, 2017), *cowplot* (Wilke, 2016), *magrittr* (Bache & Wickham, 2020) and *reshape2* (Wickham, 2017) packages.

To analyze alpha diversity, we calculated Shannon's H' , richness and Pielou's evenness for each site. We then ran an ANOVA to test for differences within INT and PER sites related with these three parameters and graphed boxplots to visualize the results. Other independent ANOVAS for Shannon's H' and each environmental variable were posteriorly ran. Subsequently, simple linear models were performed to observe relationships between environmental variables and Shannon diversity H' .

Metacommunity structure.

To analyse how our local and regional variables relate to the beta diversity in our basin, metacommunity analysis were performed by means of Mantel tests. Mantel tests are correlation statisticals that compare two (similarity or distance) matrices computed about the same elements (Diniz-Filho et al., 2013). We built three matrices for each INT and PER sites, respectively. First, a matrix for the explanatory variables at a local scale comes from environmental distances (ENVI) calculated from six environmental variables, with the *vegdist()* function, using Euclidean distances. Second, a matrix for the explanatory variable at a regional scale comes from watercourse distances (WACO) between sampling points, which were calculated using Network Analyst extension in ArcGIS Pro which constructs an Origin-Destination Cost Matrix. Third, a final matrix for the response variable was

constructed from community composition dissimilarity (beta diversity) analysis based on Bray-Curtis dissimilarity. In addition, scatter plots were graphed to visualize the relations between Bray-Curtis dissimilarity with ENVI and WACO, respectively, for PER and INT sites. We expected to observe a strong correlation between ENVI and beta diversity, plus a weak correlation between WACO and beta diversity for PER sites, and a reversed pattern for INT sites.

RESULTS

Local environmental variables.

Regards sites environmental variation within the Cube basin, channels varied deeply in wetted width, from small to medium-sized rivers (1-29m width), channel discharge (2-13920 l/s), depth (6-89 cm), altitude (52-490 m.a.s.l.), and physico-chemical characteristics (Table 1). NMDS ordinations show that PER and INT sites, in relation to local environmental variables, group separately (Figure 2). However, betadisper ($P=0.001$) indicates that groups don't present homogenous variance, which means that the significant differences seen in the ADONIS ($P=0.002$) could be either explained by the heterogenous distribution of variances within groups or by the INT or PER quality. To further examine this patterns we performed PCA correlation circle plots for each group of sites (Figure 3), which additionally indicate the quality of representation of each variable.

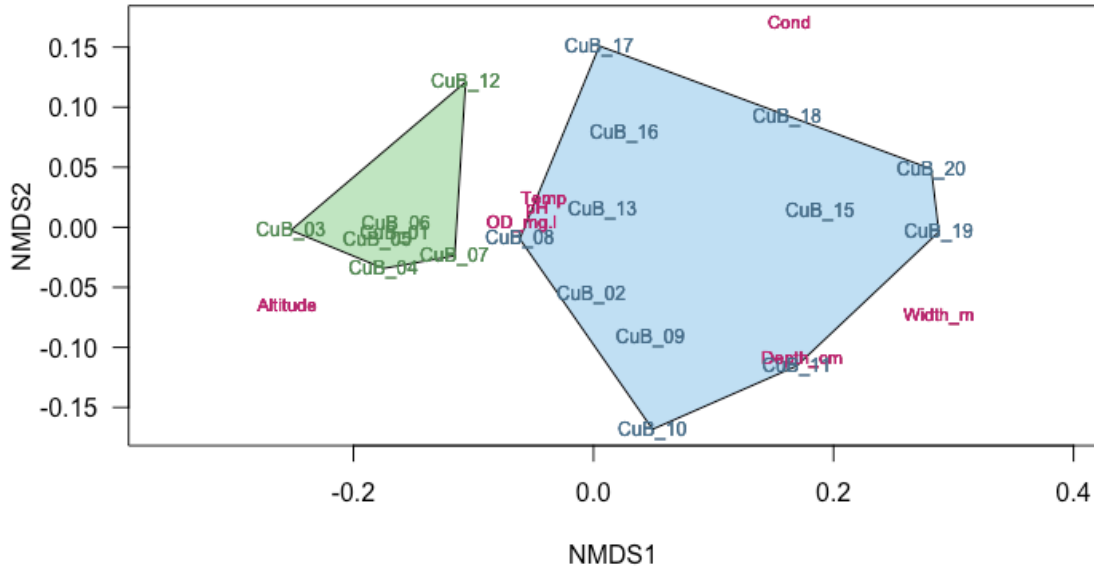


Figure 2. NMDS ordinations of sites associated with local environmental variables using square-root-transformed data. Green represents INT sites (n=7) and sky blue PER sites (n=12). Stress= 0.031

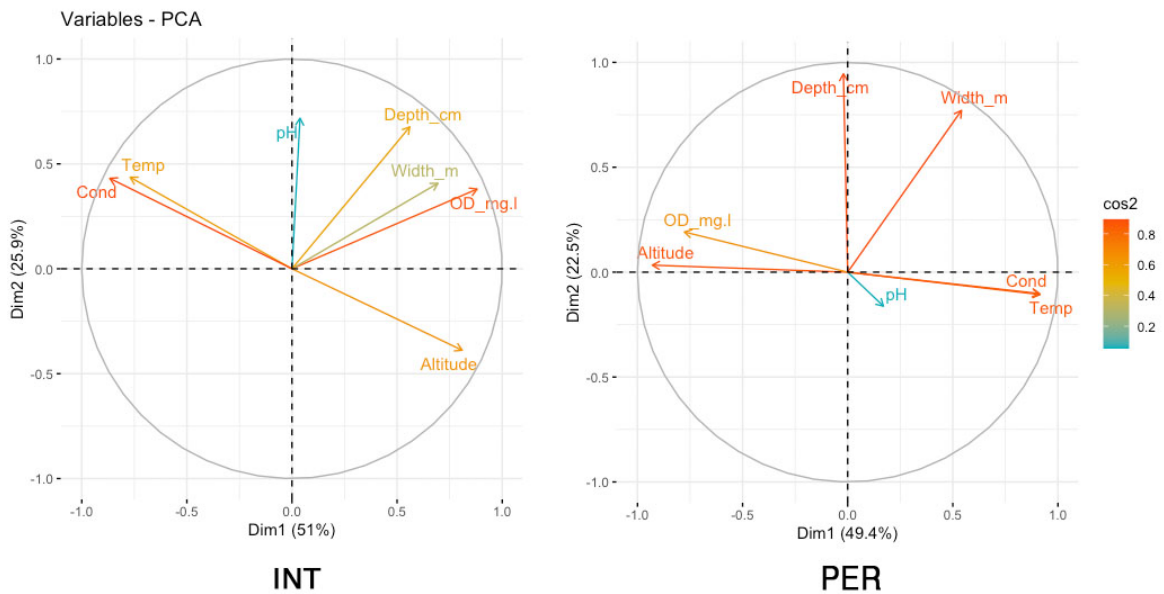


Figure 3. PCA correlation circle plot for environmental variables for INT (left) and PER (right) sites. \cos^2 indicates the quality of representation of the variable on the factor map.

The first two axes of the PCA explain 76.9% of the total variance in the environmental variables for INT sites (Figure 3, left). Conductivity and temperature are positively correlated

with each other and negatively correlated to altitude. Depth, width and dissolved oxygen are also positively correlated; pH has a low quality of representation (\cos^2). For PER sites, the first two axes explain 71.9% of the total variance (Figure 3, right). Conductivity and temperature have also a positive correlation with each other and a negative correlation with altitude, plus dissolved oxygen is also positively correlated with altitude. Depth and width are less grouped together in this PER scenario, but pH still presents a low quality of representation.

Alpha diversity patterns

1033 fish individuals were captured, belonging to 20 species (2 introduced) from 19 genera, 13 families and 6 orders (Table 2; APPENDIX A). 14 species were previously reported in the literature for the Mache-Chindul Ecological Reserve (APPENDIX C). The most abundant species were *Pseudopoecilia fria* (n=283) and *Eretmobrycon ecuadoriensis* (n=278), and the least common *Synbranchus marmoratus* (n=1, Table 2). The average number of individuals per site was 52 with a maximum of 171 and minimum of eight.

Table 2. Order, family, species and total abundance of the species reported in the study

Order	Family	Species	Total abundance
Cyprinodontiformes	Poeciliidae	<i>Pseudopoecilia fria</i>	283
Characiformes	Characidae	<i>Eretmobrycon ecuadoriensis</i>	278
Characiformes	Characidae	<i>Rhoadsia minor</i>	149
Characiformes	Characidae	<i>Pseudochalceus lineatus*</i>	96
Siluriformes	Trichomycteridae	<i>Trichomycterus spilossoma</i>	87
Siluriformes	Astroblepidae	<i>Astroblepus fissidens</i>	33
Cichliformes	Cichlidae	<i>Andinoacara blombergi</i>	32
Characiformes	Bryconidae	<i>Brycon dentex</i>	21
Characiformes	Characidae	<i>Eretmobrycon brevirostris</i>	12
Gobiiformes	Gobiidae	<i>Awaous transandeanus</i>	7
Characiformes	Curimatidae	<i>Pseudocurimata boehlkei</i>	6

Characiformes	Lebiasinidae	<i>Lebiasina bimaculata</i>	5
Cyprinodontiformes	Poeciliidae	<i>Xiphophorus maculatus</i> *	5
Siluriformes	Heptateridae	<i>Pimelodella grisea</i>	5
Cichliformes	Cichlidae	<i>Mesoheros festae</i>	4
Cyprinodontiformes	Poeciliidae	<i>Poecilia reticulata</i> *	3
Characiformes	Erythrinidae	<i>Hoplias malabaricus</i>	2
Gobiiformes	Eleotridae	<i>Gobiomorus maculatus</i>	2
Siluriformes	Loricariidae	<i>Chaetostoma aequinoctiale</i>	2
Synbranchiformes	Synbranchidae	<i>Synbranchus marmoratus</i>	1

* indicates introduced species

Analysis of alpha diversity at all sites indicate that Shannon's H' index has a significant positive relationship with two environmental variables: temperature and pH (ANOVA, $P < 0.05$; Figure 4). Altitude has a negative but non-significant relation and conductivity, width and depth have positive but non-significant relationships (ANOVA, $P > 0.05$). It is also noteworthy that stream depth exhibited near-significant values (ANOVA, $P = 0.066$).

Species accumulation curve indicated that the number of species is near-to-be stabilized, probably only rare species being left to be sampled (

APPENDIX B).

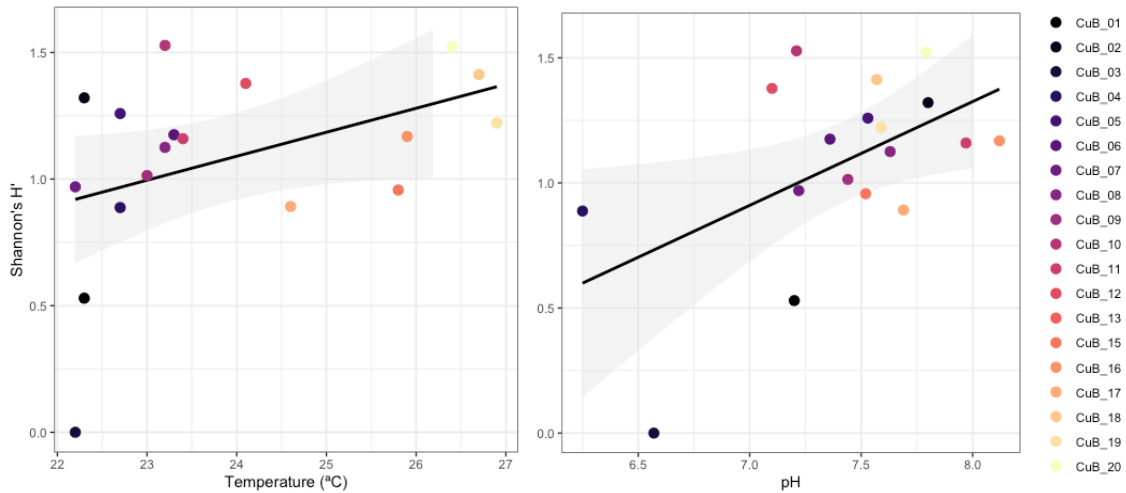


Figure 4. Simple linear regressions for Shannon's H' with temperature (right) and pH (left).

Shannon's diversity index (H') ranged from $H'=0$ to $H'=1.74$ within all locations, CuB_03 (INT) being the least and CuB_13 (PER) the most diverse sites (Figure 5A). Regarding richness, the average number of species per site was 5; where CuB_19 (PER) presented the most species ($n=10$) and CuB_03 (PER) the least ($n=1$; Figure 5B). CuB_20 (PER) and CuB_02 (INT) have both high Pielou's evenness values ($J'=0.953$; $J'=0.946$ respectively), while CuB_15 (PER) has the lowest evenness value ($J'=0.460$; Figure 5C). CuB_03 is not represented as it contains only one specie.

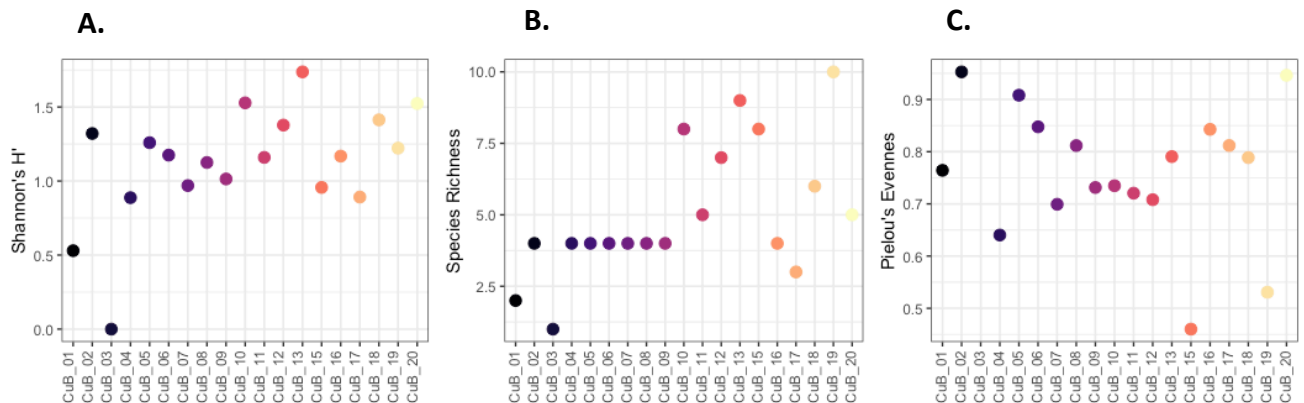


Figure 5. (A) Shannon diversity index (H'), (B) species richness and (C) Pielou's evenness at each sampling site.

Local fish community composition was different between PER and INT sites (betadisper, $P=0.057$; ADONIS, $P=0.001$; Figure 6) according to NMDS ordinations. ANOVA between type of site and Shannon's H' index (alpha diversity) also show that there is a significant difference between PER and INT sites (ANOVA, $P=0.041$), PER sites having higher H' values (Figure 7). Species richness and Pielou's evenness don't show significant differences (ANOVA, $P=0.057$; $P=0.987$), although richness' P value was almost significant. PER sites have a mean of 6 species and INT sites of 4 species.

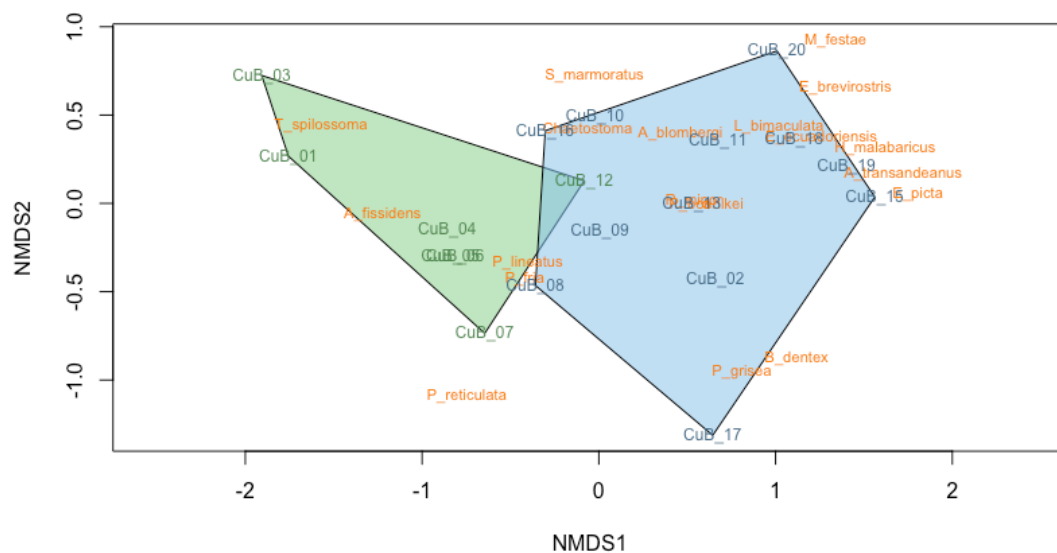


Figure 6. NMDS ordinations of sites associated with local fish communities using square-root-transformed abundances. Green represents INT sites ($n=7$) and sky blue PER sites ($n=12$). Stress= 0.086

A.

B.

C.

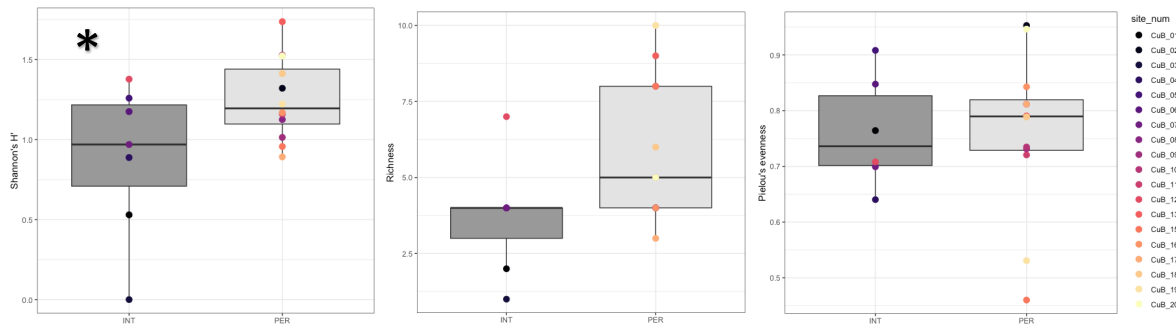


Figure 7. Box plots for (A) Shannon's H' , (B) richness and (C) Pielou's evenness for INT and PER sites. Horizontal lines indicate (from top to bottom) the first, second (median) and third quartiles. * indicates significant differences in the ANOVA.

The INDVAL analysis demonstrate that *Roadsia minor* ($P=0.007$) and *Andinoacara blombergi* ($P=0.009$) are indicator species of PER sites, while *Trichomycterus spilossoma* ($P=0.001$) and *Astroblepus fissidens* (INDVAL, $P=0.004$) are indicative of INT sites (APPENDIX A).

1

Metacommunity structure

Metacommunity analysis were performed for the basin as a whole and for INT and PER sites independently. Table 3 indicates the relationships for the whole basin and shows that, Bray-Curtis dissimilarity (beta diversity) is significant and positively correlated to both ENVI and WACO (Table 3, Figure 8). WACO and ENVI are also significantly and positively correlated, meaning that when distance between sites increases, environmental distance also increases (Table 3).

Table 3. Mantel tests correlations for spatial (WACO=watercourse) and environmental distances (ENVI), and correlations between dissimilarity matrices for abundance data based on Bray-Curtis dissimilarity and environmental distances or spatial distances for all sites.

	ENVI	WACO
WACO	0.572 (**)	-

Bray-Curtis dissimilarity	0.481 (**)	0.254 (*)
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Values correspond to Mantel R and associated P value: NS - $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

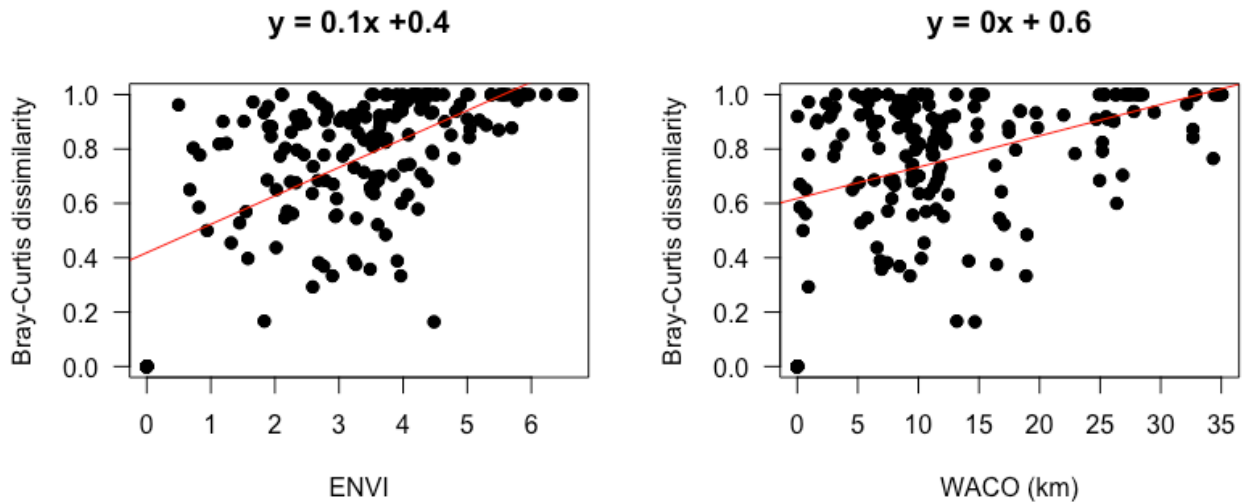


Figure 8. Relationship between Bray-Curtis dissimilarity and environmental (ENVI) or watercourse (WACO) distances for all sites. Equation numbers are rounded to a single digit so values inferior to 0.05 may appear as 0.

For both, INT and PER sites, separately, fish community dissimilarity was not explained by either ENVI nor WACO distance matrices (Table 4). Although, ENVI distances correlation with dissimilarity matrix was almost significant for INT sites (Mantel $R=0.06$). When ENVI and WACO distances increase, community dissimilarity also increases (Figure 9).

Table 4. Mantel tests correlations for dissimilarity matrices for abundance data based on Bray-Curtis dissimilarity and environmental distances (ENVI) or spatial distances (WACO=watercourse)

Site type	ENVI	WACO
PER	0.201 (NS)	0.066 (NS)
INT	-0.083 (NS)	0.006 (NS)

Values correspond to Mantel R and associated P value: NS - $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

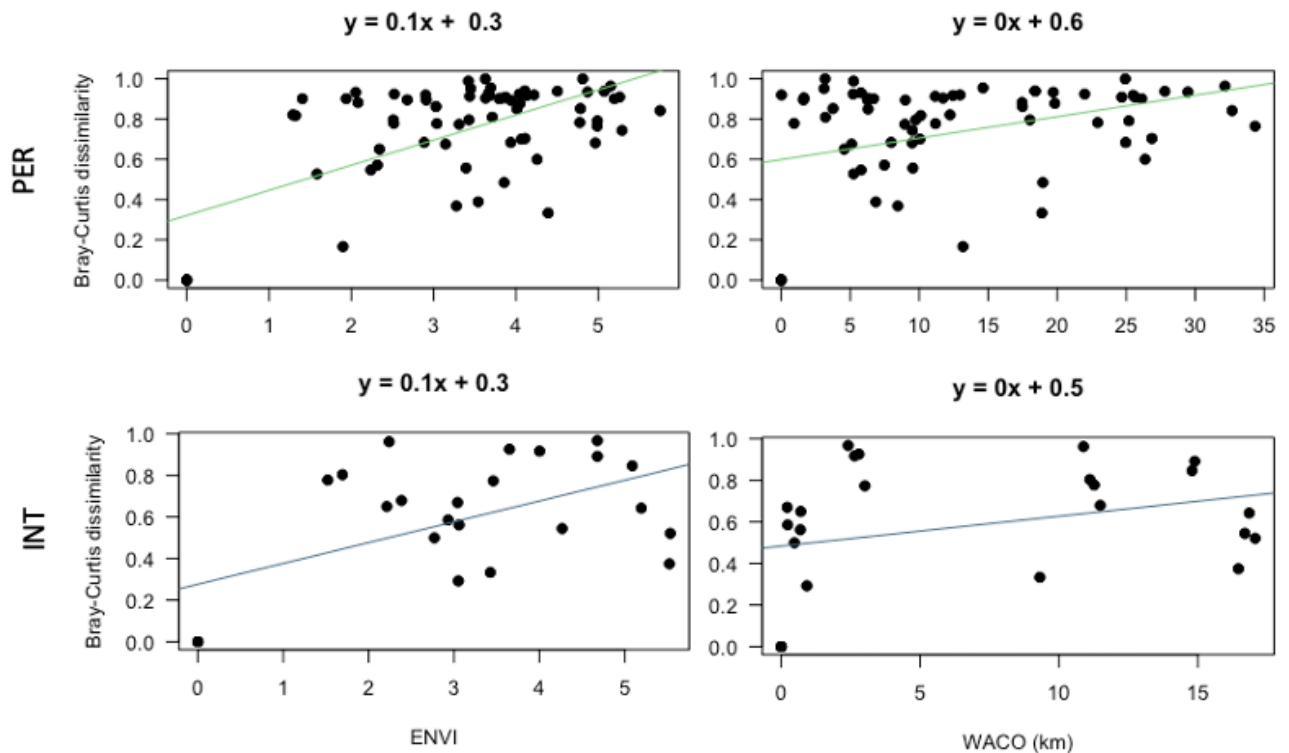


Figure 9. Relationship between Bray-Curtis dissimilarity and environmental (ENVI) or watercourse (WACO) distances for perennial (PER) and intermittent (INT) sites. Equation numbers are rounded to a single digit so values inferior to 0.05 may appear as 0.

DISCUSSION

Our results indicate that INT and PER environments are significantly different from each other in their abiotic factors (environmental variables) and local fish diversity. Although locally distinct, the change of species between sites (beta diversity) seems to be influenced by local and regional factors in the same intensity for INT and PER sites. Thus, species sorting seems to be the mechanism defining fish communities locally, but the change of diversity from one site to the other is influenced by a combination between environmental and spatial distances, independently from flow regime.

Intermittent and Perennial sites according to local environmental variables and alpha diversity.

INT and PER sites differ in their environmental variables. However, in both sites most variables correlate similarly. Physicochemical parameters like water temperature and conductivity are negatively correlated with altitude, where higher altitude localities present lower temperatures and conductivity, patterns commonly reported in the literature (Caissie, 2006). For PER sites temperature is negatively correlated with dissolved oxygen because it is more soluble in cold water (Khani & Rajaei, 2017); although some studies for tropical Andean rivers have presented the opposite trend, suggesting that in high altitude places with lower water temperatures, low levels of dissolved oxygen may be explained by low levels of partial pressures of atmospheric oxygen (Miranda et al., 2021). pH doesn't appear to be correlated with any of the analyzed variables for neither INT nor PER sites because it is usually more dependent of CO₂ concentrations bound to photosynthesis and respiration processes (Hamid, Sami, Bhat, & Jehangir, 2019), as well as decomposition of organic matter in the streams (Ghisi et al., 2017). Substrate composition, specifically related to carbonate forms on the surrounding bedrock is another factor that alters water pH (Hamid et al., 2019). Other anthropogenic causes of variation include wastewater and mining (Ghisi et al., 2017). Hydromorphological parameters such as depth and width are positively correlated with each other in both sites and also correlated with dissolved oxygen for INT sites. Overall, environmental variables behave (associate) similarly in both groups of sites, but locally, the parameters vary significantly between INT and PER sites (Figure 3).

Twenty species were sampled during this study, most already previously reported for the Mache-Chindul Ecological Reserve. Two introduced species were found (*Xiphophorus maculatus* and *Poecilia reticulata*). Both poecilids are commonly commercialized as

ornamental species, thus, the introduction to the zone may be product of liberation of captive individuals. Additionally, a third introduced species not reported on our study, *Oreochromis mossambicus*, locally known as tilapia has been reported in the literature for the reserve (Hualpa Vivanco & Rivadeneira Romero, 2020 ; Appendix C). This is a highly commercial species (Froese & Pauly, 2020), commonly cultured and consumed locally.

Temperature and pH were positively related with Shannon's H diversity index. Water temperature has been found to be a primary determinant of the assemblage structure of fish species in tropical Andean rivers, as it directly affects metabolism, fecundity and growth (Miranda et al., 2021). Water temperature has been found to be negatively correlated with fish abundances of *Astroblepus* species, and positively correlated with abundances of *Bryconamericus* (sister taxa of *Eretmobrycon*), *Brycon* and *Pimelodella* (Miranda et al., 2021). pH, in contrast, has been reported to be a good predictor of species richness, along with water velocity (Kwik & Yeo, 2015). Characin genera like *Bryconamericus*, *Brycon* and *Pseudochalceus* are commonly related to high pH values (Miranda et al., 2021). Contrary to what was expected, altitude wasn't significantly correlated with alpha diversity, although studies suggest that fish populations tend to be markedly distributed through an altitudinal gradient (Miranda et al., 2021; Suárez & Petrere, 2007). Additionally, hydromorphological variables such as steepness of stretches, substrate composition and water velocity, are also relevant to fish community composition (Miranda et al., 2021). However, these variables were not analysed in this study. Analyzing more environmental variables could provide a broader picture of the ecology of local fish communities.

In running waters, which are lotic unidirectional continuous ecosystems, species are continuously replaced rather than occurring in discrete stages, as proposed by de River Continuum Concept (RCC; Junk & Wantzen, 2004). Nevertheless, in intermittent systems the channel continuum is spatially or temporally interrupted, creating different community composition patterns when compared to perennial ecosystems. Our results showed that, similar to what was seen with environmental variables, INT and PER sites are different from each other in species richness and abundance, but similar within the groups. PER sites are more diverse (Shannon's H') than INT sites, and have higher numbers of species. Evenness is very similar for both (Figure 5). Several studies have shown that INT systems are less diverse than their PER counterparts, possibly due to low recolonization after periods of drought (Datry, Moya, et al., 2016). Furthermore, diversity of aquatic organisms is predicted to be lower in the headwaters and in the lower parts of the rivers, and highest at the middle reaches where the variability of physico-chemical variables such as temperature is also higher, offering a variety of thermal optima for numerous taxa to develop (Junk & Wantzen, 2004). Catadromous species such as *Awaous transandeanus* and *Gobiomorus maculatus* are, thus, able to subsist only on the middle and lower reaches of the basin. Most PER sites are located on the middle reaches of the basin, which could partially explain the higher diversity of fish species.

Two silurid species were indicative of INT sites, *Trichomycterus spilossoma* and *Astroblepus fissidens*. The species of the genus *Astroblepus* are known as climbing catfishes and prefer cold shallow waters and narrow stretches, conditions present at our INT sites (Miranda et al., 2021). *Trichomycterus spilossoma* are found in sympatry with *A. fissidens* and must also

be associated with the same ecological conditions. For PER sites, *Roadsia minor* and *Andinoacara blombergi* were indicative species. *Roadsia* tend to be more common at lower elevations and prefer warmer temperatures and wider and deeper water habitats (W. E. Aguirre et al., 2016) much like the conditions present at PER sites (Table 1). The ecology of *A. blombergi* is still unknown, but sister species like *A. rivulatus* are usually present on low-current ecosystems like big pools but may also be found in more rushing waters, mostly associated with vegetation or submerged logs (Jiménez Prado et al., 2015).

Metacommunity analysis

It was traditionally assumed that abiotic environmental variables are the primary, if not the only, responsables of the variation in the community structure for aquatic environments (Heino, 2011). Spatial distance and local environmental conditions have been recognized to determine which species are able to colonize and later settle in local communities (Heino, Soininen, Alahuhta, Lappalainen, & Virtanen, 2015). Furthermore, it has been proven that environmental harshness, which is a result of a combination of environmental factors such as climate type, altitudinal range, and flow regime, can affect the relative contribution of local environmental factors (species sorting) and regional spatial distances in shapping metacommunities (Datry, Melo, et al., 2016), during dry seasons.

Our results analyzed only flow regime as a proxy of environmental harshness and show that intermittency, in this scenario, is not modulating the strength in which local and regional variables correlate with community dissimilarity of fishes between sites (beta diversity). For the Cube basin in the wet season, fish community dissimilarity is positively correlated with both local environmental variables and regional spatial variables (Figure 9). Contrary to our

predictions, community dissimilarity increases with environmental distance and watercourse distance for both INT and PER sites. In PER sites, previous studies have reported that, in the dry season, community dissimilarities are exclusively correlated with environmental distances, suggesting that environmental filtering is the principal mechanism assembling communities, regardless of inter-site distance (Datry, Moya, et al., 2016). Other studies suggest that although environmental variables are the main factors structuring fish communities during dry season, in the wet season spatial distances are also important as connectivity in the streams increases and habitat isolation is broken (Benone, Ligeiro, Juen, & Montag, 2018). On the wet season, where our sampling was performed, connectivity within all the Cube basin was higher, making regional spatial variables and dispersion important not only for INT sites but also for PER sites. In INT sites, where we expected only regional variables to explain community dissimilarity, local environmental variables may be also related because environmental distances and spatial distances are significantly correlated with each other (Table 3). In addition, assemblage of beta diversity in INT sites is more resemblant to the patterns we would have expected for PER sites during wet season, as an increase in flooding and decrease in isolation allows for species to move from PER reservoirs to INT sites (dispersal) but also be affected by local environmental factors (species sorting). Sampling during the dry season could probably expose patterns more similar to what we hypothesized.

CONCLUSIONS

Intermittent rivers are complex and dynamic ecosystems that differ greatly from their perennial counterparts, both in diversity and in their environmental characteristics. Our

study shows that fish communities are shaped by a combination of local and regional variables, although in different patterns than what has been seen for other intermittent basins. The variability in results highlights the need for more research in tropical IRES. Plus, the dynamics of fish community assemblages in IRES are hardly known for the wet season, thus our research remarks how important it is to acknowledge both seasons, to further understand the temporal dynamics of these ecosystems. In Ecuador, minimal information is available for IRES and their associated organisms. The Cube River basin presents a great opportunity to understand fish diversity patterns in an intermittent system, and may serve as model to understand other tropical IRES.

On future scenarios of climate change and increase in land use, it is predicted that flow patterns of IRES may change drastically by presenting harder and longer drought periods or, on the contrary, more rain-water input, and that some perennial systems will become intermittent (Acuña et al., 2014; Sauquet et al., 2021). Therefore, understanding natural-intermittent systems, such as the Cube River basin, is key for management and biological conservation, but also to predict how will diversity respond to future global climatic and land changes.

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APPENDIX A: LIVE PHOTOGRAPHS OF THE CUBE RIVER'S FISH SPECIES

A.



B.



C.



D.



E.



F.



G.



H.



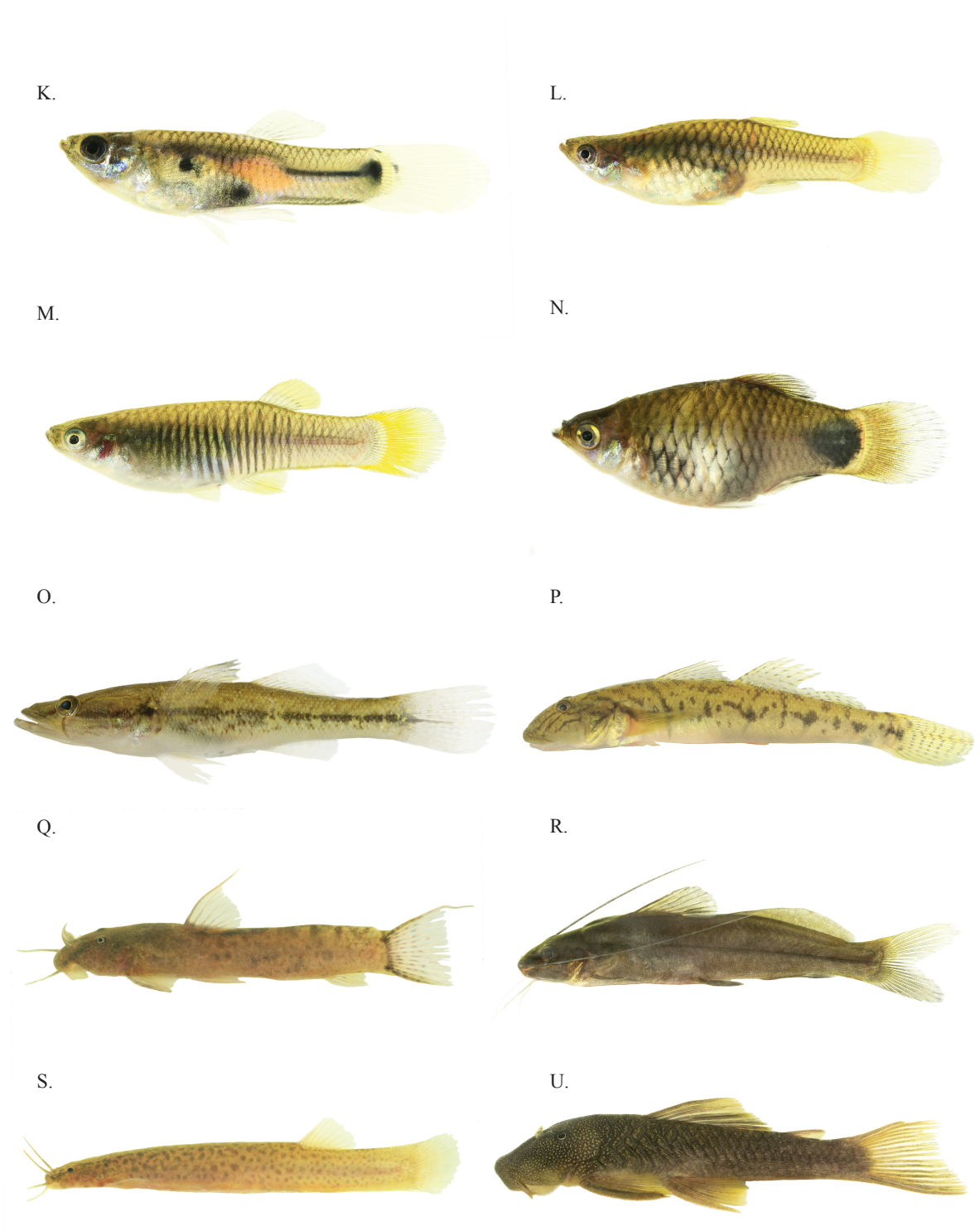
I.



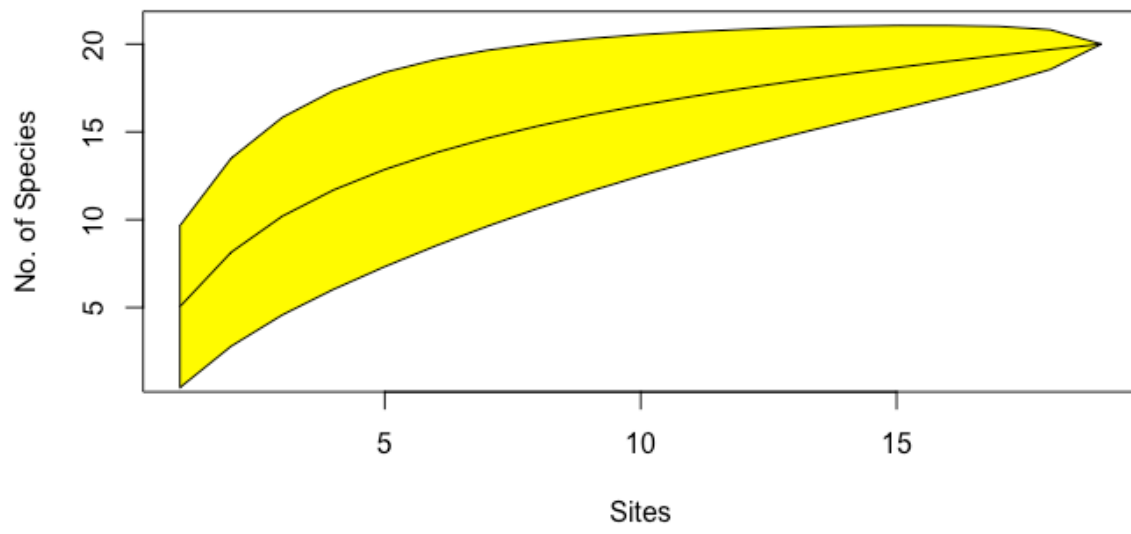
J.



A. *Brycon dentex*, B. *Eretmobrycon dahli*, C. *Eretmobrycon ecuadoriensis*, D. *Pseudochalceus lineatus*, E. *Roodsia minor*, F. *Pseudocurimata bohlkei*, G. *Hoplias malabaricus*, H. *Lebiasina bimaculata*, I. *Andinoacara blombergi*, J. *Mesoheros festae*.



K. *Poecilia reticulata* (macho), L. *Poecilia reticulata* (hembra), M. *Pseudopoecilia fria*, N. *Xiphophorus maculatus*, O. *Gobiomorus maculatus*, P. *Awaous transandeanus*, Q. *Astroblepus fissidens*, R. *Pimelodella grisea*, S. *Trichomycterus spilossoma* , U. *Chaetostoma aequinoctiale*

APPENDIX B: SPECIES ACCUMULATION CURVE FOR ALL LOCALITIES

APPENDIX C. SPECIES OF THE CUBE RIVER BASIN REPORTED IN THE LITERATURE

Order	Family	Species	Type	References
Characiformes	Characidae	<i>Eretmobrycon dahli</i>	Endemic	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015)
Characiformes	Characidae	<i>Eretmobrycon ecuadoriensis</i>	Endemic	(Leberg et al., 2021)
Characiformes	Characidae	<i>Rhoadsia altipinna</i>	Native	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015)
Characiformes	Characidae	<i>Rhoadsia minor</i>	Endemic	(Jiménez Prado et al., 2015; Leberg et al., 2021)
Characiformes	Characidae	<i>Pseudochalceus boehlkei</i>	Endemic	(Jiménez Prado et al., 2015; Leberg et al., 2021)
Characiformes	Bryconidae	<i>Brycon dentex</i>	Native	(Jiménez Prado et al., 2015; Leberg et al., 2021)
Characiformes	Curimatidae	<i>Pseudocurimata boehlkei</i>	Endemic	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015)
Characiformes	Lebiasinidae	<i>Lebiasina bimaculata</i>	Native	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015; Leberg et al., 2021)
Characiformes	Erythrinidae	<i>Hoplias malabaricus</i>	Native	(Jiménez Prado et al., 2015; Leberg et al., 2021)
Cichliformes	Cichlidae	<i>Andinoacara blombergi</i>	Endemic	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015; Leberg et al., 2021)
Cichliformes	Cichlidae	<i>Mesoheros festae</i>	Native	(Jiménez Prado et al., 2015; Leberg et al., 2021)

Cichliformes	Cichlidae	<i>Oreochromis mossambicus</i>	Introduced	(Hualpa Vivanco & Rivadeneira Romero, 2020)
Cyprinodontiformes	Poeciliidae	<i>Pseudopoecilia fría</i>	Endemic	(Jiménez Prado et al., 2015; Leberg et al., 2021)
Cyprinodontiformes	Poeciliidae	<i>Poecilia reticulata</i>	Introduced	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015)
Siluriformes	Loricariidae	<i>Chaetostoma aequinoctiale</i>	Endemic	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015; Leberg et al., 2021)
Siluriformes	Loricariidae	<i>Rineloricaria jubata</i>	Native	(Jiménez Prado et al., 2015; Leberg et al., 2021)
Siluriformes	Heptateridae	<i>Rhamdia quelen</i>	Native	(Hualpa Vivanco & Rivadeneira Romero, 2020; Jiménez Prado et al., 2015; Leberg et al., 2021)
Siluriformes	Pimelodidae	<i>Pimelodella grisea</i>	Native	(Jiménez Prado et al., 2015; Leberg et al., 2021)
Siluriformes	Astroblepidae	<i>Astroblepus cf. fissidens</i>	Endemic	(Leberg et al., 2021)
Siluriformes	Trychomycteridae	<i>Trichomycterus aff. spilossoma</i>	Native	(Leberg et al., 2021)
Siluriformes	Pseudopimelodidae	<i>Batrochoglanis transmontanus</i>	Native	(Jiménez Prado et al., 2015; Leberg et al., 2021)

Table source: Escobar et al. unpublished