COMMUNITY ASSEMBLY RULES OF FISH ASSEMBLAGES ALONG A LARGE TROPICAL RIVER CATCHMENT: A VIEW FROM DIFFERENT PERSPECTIVES

A Dissertation

by

EDWIN ORLANDO LÓPEZ DELGADO

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Chair of Committee,	Kirk O. Winemiller
Committee Members,	Lee Fitzgerald
	Kevin Conway
	Michell Lawing
Head of Department,	David Caldwell

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ABSTRACT

Community ecologist have studied biodiversity worldwide principally focusing on species richness. However, little is known about how species composition varies in space, time and even less about the species role in the ecosystem functioning, which is critical for natural resource management and conservation. In this study, I investigated mechanisms underlying community structure and how environmental and spatial factors influence fish taxonomic and functional (α,β) diversity along a longitudinal gradient in a nearly pristine Neotropical river. Standardized surveys were conducted during the low-water period at 34 sites within the Bita River Basin in the Colombian Llanos. Physical, chemical and landscape parameters were recorded at each site. Asymmetric eigenvector maps were used as spatial variables. To examine the relative influence of spatial and environmental variables on taxonomic and functional diversity, a distance-based redundancy analysis (db-RDA) and variation partitioning analysis were conducted. Results of these two facets of biodiversity showed that environmental filtering and stochastic dynamics might be structuring fish communities in this river system. Variation partitioning analyses for both biodiversity facets revealed that environmental features related to in-stream cover might act as filters structuring fish communities, therefore, in order to preserve fish biodiversity in this diverse river, it is crucial that habitat variability (in-stream cover) and connectivity be preserved.

DEDICATION

To my family and friends who gave courage to continue this adventure far from home.

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Contributors

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The Neotropical region harbors the greatest diversity of living organisms on Earth. For fish, it is estimated that roughly 6,000 species inhabit the Neotropics, representing almost 50% of all freshwater fish species, and many more are yet to be discover (Reis, 2013; Winemiller, Agostinho & Caramaschi, 2008). With about 1,000 fish species registered to date, the Orinoco River Basin is an important area for biodiversity conservation (Lasso, Machado-Allison & Taphorn, 2016). This region contains diverse aquatic habitats, including rivers, creeks, oxbows, floodplain lakes, flooded savanna and forest, and some special biotopes, such as 'Morichales' (small, low-gradient streams lined with palms) (Lasso *et al.*, 2011). Among the main tributaries of the Orinoco Basin, the Bita River has, until recently, escaped major anthropogenic impacts and maintains relatively natural conditions, providing an excellent opportunity to study processes behind diversity patterns of fish assemblages under natural environmental conditions.

Given the current crisis of biodiversity loss due to human impacts (e.g., dams, climate change, mining, land use), a key goal of ecology is to understand patterns structuring biological communities in order to identify to what extent these disturbances may affect species diversity and ecosystem functions (Cardinale *et al.*, 2012). However, a major challenge for community ecology is the measurement of biodiversity, and this is because of its multifaceted nature. Biodiversity has been studied using different perspectives, including analysis of taxonomic (Diamond, 1975; Clements, 1916; Gleason, 1926; Hubbell, 2001; Simberloff, 1983; Whittaker, 1960), functional (Mouillot *et al.*, 2011; Villéger, Grenouillet & Brosse, 2013), phylogenetic

(Faith, 1992; Webb et al. 2002), and multifaceted approaches (Kluge & Kessler, 2011; Pavoine et al., 2011; Weinstein et al., 2014). A multifaceted approach has proven to be an effective means to elucidate mechanisms that create and maintain biodiversity (Swenson, 2011b; Swenson, 2011a).

Several mechanisms have been proposed to explain patterns of biodiversity in aquatic ecosystems (Baselga, 2012; Cucherousset & Villéger, 2015; Podani, Ricotta & Schmera, 2013; Pool, Grenouillet & Villéger, 2014; Swenson, 2011a). Much scientific literature has discussed three processes structuring local communities – environmental filtering, limiting similarity and neutral processes – that may act sequentially, simultaneously or interact at any given scale (Chase, 2010; Carvalho & Tejerina-Garro, 2015; Pease *et al.*, 2012; Mason *et al.*, 2007; Troia & Gido, 2014). In the present study, I used a multifaceted approach to elucidated the processes having strongest effects on fish assemblages along the longitudinal fluvial gradient of the Bita River Basin and within four sections distributed along this gradient.

In chapter II, I adopted a metacommunity approach to investigate spatial variation in fish assemblage structure along the longitudinal gradient and within the four river sections. Results from this chapter revealed that species in this system respond as groups to the environmental gradient (Clementsian distribution), implying a potential effect of environmental filtering and species sorting. In an effort to explore more deeply the taxonomic facet of biodiversity, in chapter III, I evaluated β diversity patterns along the longitudinal gradient and whether or not position within the river network had an effect on assemblage taxonomic dissimilarity. Results showed that high β diversity was consistent among species assemblages no matter the spatial scale of analysis or position within the river network. Species replacement (turnover) dominated beta diversity, suggesting again the importance of environmental filtering and species sorting in this system.

It is well known, that a taxonomic based approach provides an incomplete view of biodiversity, and it is insufficient to explain processes including multispecies interactions, species distributions, and how diversity affects ecosystem functions (Cadotte, Carscadden & Mirotchnick, 2011; Pavoine et al., 2011; Pool, Grenouillet & Villéger, 2014). Therefore, in chapter IV, I used a functional approach to examine changes in functional diversity patterns along the longitudinal gradient and within river sections. Findings from this chapter revealed low values of functional richness and high values of functional β diversity across the entire basin and within each river section. These findings, combined with the results of the other chapters, support the idea that environmental filtering plays an important role in structuring fish assemblages in this species-rich tropical river. By addressing assembly processes in different ways, I achieved a more robust assessment of mechanisms that influence biodiversity and assemblage structure in an undisturbed system.

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2. DO METACOMMUNITY THEORIES EXPLAIN SPATIAL VARIATION IN FISH ASSEMBLAGE STRUCTURE IN A PRISTINE TROPICAL RIVER?*

2.1. Introduction

The spatial distributions of species and resultant diversity of local assemblages are affected by multiple factors operating at different temporal and spatial scales (Pease et al., 2012; Presley, Higgins & Willig, 2010), and understanding how these factors influence patterns has been the main objective in community ecology (Landeiro et al., 2011). Stream ecologists have been particularly active in research addressing relationships of assemblage patterns with abiotic (Mouillot, Dumay & Tomasini, 2007), biotic (MacArthur & Levins, 1967), spatial (Peres-Neto & Legendre, 2010) and stochastic influences (Chase et al., 2011). These factors can act independently, sequentially or simultaneously, and often interact (Carvalho & Tejerina-Garro, 2015).

Until recently, most field studies have focused on associations between local species assemblages and local environmental factors. During the past decade there has been a rapid increase in research showing how local communities are influenced by dispersal of organisms across various spatial scales and involving ecological processes such as habitat selection and responses to disturbances or competition (Erős et al., 2017; Erős, 2017; Leibold & Mikkelson, 2002; Leibold et al., 2004; Presley, Higgins & Willig, 2010).

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*Reprinted with permission from “Do metacommunity theories explain spatial variation in fish assemblage structure in a pristine tropical river?” by Authors Edwin O. López-Delgado, Kirk O. Winemiller, Francisco A. Villa-Navarro. 2019. *Freshwater Biology,* 64 2, 367-379, Copyright (2018) by John Wiley and Sons.

Metacommunity theory explores how local interactions and regional processes (dispersal, sub-population extirpation) shape community structure. The word metacommunity is used to describe a network of local communities of interacting populations linked via dispersal. The metacommunity paradigm has stimulated investigation on how populations interact at regional as well as local scales (Logue et al., 2011). At least four models of metacommunity dynamics have been proposed, each defined by the relative influences of dispersal, environmental filtering, habitat selection, habitat disturbance, biotic interactions, and stochastic factors (Leibold et al., 2004).

Three statistical approaches have been developed to study metacommunities. These are Elements of Metacommunity Structure (EMS), variation partitioning, and zero-sum multinomial distribution (Logue et al., 2011; Monteiro, Paiva & Peres-Neto, 2017). The current study adopts the first two approaches to test metacommunity theories by analyzing spatial variation in fish assemblages in a pristine Neotropical river.

The EMS method (Leibold & Mikkelson, 2002) analyzes species distribution patterns along environmental gradients to reveal multiple elements of metacommunity structure. EMS uses an ordered sites-by-species incidence matrix by reciprocal averaging to evaluate assemblage coherence, turnover, and boundary clumping. The method can distinguish among six possible patterns of species distribution: checkerboard (nonrandom patterns of species co-occurrence), Clementsian (clumps of co-occurring species distributed along spatial gradients), Gleasonian (independent species distributions along spatial gradients), evenly spaced (hyperdispersed species distributions), nested subsets, and random (Leibold & Mikkelson, 2002; Presley, Higgins & Willig, 2010). Because the focal units of analysis are species distributions rather than assemblage structure among sites, this approach allows testing of hypotheses about competitive exclusion (negative coherence-checkerboard distribution), random assemblies (non-significant coherence), and environmental filtering (positive coherence-Clementsian and Gleasonian distributions) (Meynard et al., 2013).

Variation partitioning, another commonly used method in community ecology, was designed to reveal potential mechanisms responsible for spatial variation in assemblage composition (Peres-Neto et al., 2006). This approach integrates environmental variation and spatial processes and facilitates inferences about the likelihood that one or more of the four metacommunity processes (species sorting, mass effect, patch dynamics, null model) account for observed assemblage patterns (Cottenie, 2005).

Spatial configurations, such as dendritic structures of river networks, also affect fish assemblage structure (Vitorino-Júnior et al., 2016). Brown & Swan (2010) found that processes structuring aquatic communities differed depending on the network location (headwaters streams vs main stem). Headwaters streams are relatively isolated within river networks, possess high habitat heterogeneity, and tend to have strong disturbance regimes. Patch dynamics may dominate metacommunity dynamics under these conditions (Winemiller, Flecker & Hoeinghaus, 2010). Conversely, habitats in lower reaches are much less isolated with greater potential for dispersal, conditions that should facilitate species sorting and the mass effect. Community structure along river longitudinal gradients also could be affected by the unidirectional flow of water. Datry et al. (2016) inferred that species diversity increases from upstream to downstream due to the combined effect of headwater isolation and water flow limiting upstream dispersal.

Relatively few investigations have examined metacommunity patterns along longitudinal fluvial gradients (Almeida & Cetra, 2016; Carvajal-Quintero et al., 2015; Datry et al., 2016; Vitorino-Júnior et al., 2016), and even less research has been conducted in relatively pristine rivers with watersheds lacking significant human impacts. Knowledge gained from unperturbed systems enables predictions about future impacts to biodiversity, and can help to guide conservation and restoration efforts for rivers that have already been degraded.

I investigated spatial variation in fish assemblage structure along the longitudinal fluvial gradient of the Bita River, a nearly pristine tributary of the Orinoco River in the Llanos region of Colombia. I analyzed fish species distributions across the longitudinal gradient in an effort to reveal relative influences of abiotic environmental and spatial factors, and to infer metacommunity models that may account for patterns. I predicted that, at the basin-scale, dispersal rates are sufficiently low to allow local assemblages to track spatial environmental variation (e.g., species sorting), with a major fraction of variation in structure explained by a pure environmental effect (Heino et al., 2015). Given that environmental conditions should have greater variation over broader spatial scales, metacommunity patterns should reveal high turnover and fairly discrete local assemblages with a Clementsian distribution. At a more regional scale (localities within a river section), assemblages in upstream and downstream regions may reflect different metacommunity processes. Because headwater streams are relatively isolated within the river network, they should receive fewer migrants and may have local assemblages strongly influenced by local environmental conditions and species sorting. Metacommunity patterns of headwaters should reveal, high turnover, with three possible distributional patterns: Clementsian, Gleasonian or evenly spaced. Conversely, sites located downstream and closer to the river mouth should be influenced by high dispersal, resulting in a greater importance of spatial factors and the mass effect. In the downstream section, in-channel environmental variation will be lower, and metacommunity patterns may be nested along the longitudinal gradient.

2.2. Methods

2.2.1. Study area

The Bita River Basin is located in the eastern plains of the Colombian Llanos in the Vichada department, draining an area of 812,312 ha flowing east roughly 700 km to the Orinoco River. As in most natural drainage systems, stream environmental characteristics in the Bita Basin vary along the longitudinal gradient. Most headwater streams begin at an elevation of 300 m asl. The middle and lower sections are characterized by a broad, sinuous channel and floodplains with numerous lagoons and seasonally flooded forests and savannas. The average elevational gradient is low (0.357 m/km), with the river entering the Orinoco River at an elevation of 50 m asl near the municipality of Puerto Carreño. Riparian vegetation is comprised of dense gallery forests dominated by Caraipa llanorum (Calophyllaceae), Astrocaryum jauari (Arecaceae) and Parahancornia oblonga (Apocynaceae) and savannas dominated by Axonopus anceps, Panicum cayennense (Poaceae), Bulbostylis capillaris, Cyperus haspan, Rhynchospora cephalotes, (Cyperaceae), Caladium macrotites (Araceae), and Caraipa llanorum (Calophyllaceae).(Trujillo & Lasso, 2017). The main river and streams contain multiple habitats that vary mainly in substrate composition, riparian vegetation, and depth. During May throughout November, the river floods adjacent areas creating complex and much diverse habitats. Average air temperature in the region is 27 °C, and average annual precipitation is 2,300 mm with most rainfall from May–August (Trujillo & Lasso, 2017).

Two field expeditions, each of which lasted 30 days, were performed during January and March 2016 when low-water conditions facilitate efficient capture of fishes and relationships between fish assemblage structure and habitat should be strongest (Pease et al., 2012). I selected 34 survey sites distributed along the entire basin (E1 uppermost to E34 lowest) following the river’s longitudinal gradient. The basin was divided into four sections (high, mid-high, mid-low, low) using the linear distance of the basin (265 km) divided it by the number of sections (four). This value (66.5 km) was used to define the distance between each section along the longitudinal gradient (Figure 2.1).

2.2.2. Fish surveys

At each survey site, I selected a 200-m reach encompassing all apparent macrohabitats to collect fishes and data for local environmental variables. Fishes were collected using a seine (10 x 1.5 m, 3-mm mesh) and two gill nets (10 x 2 m, 100-mm mesh). Within each study reach, six seine hauls of 20 m were performed, and the gill net was deployed for 2 h. After fishes were removed from nets, they were anesthetized according to an approved Texas A&M university animal use protocol (IACUC 2015-0360) by immersion in tricaine methane sulfonate (MS-222) and euthanized in an overdose of MS-222. Specimens were fixed in 10% formalin, transported to the laboratory, and transferred to 70% ethanol for preservation. All specimens were identified, cataloged and deposited in the voucher collections of the Universidad del Tolima and Instituto von Humboldt in Colombia.

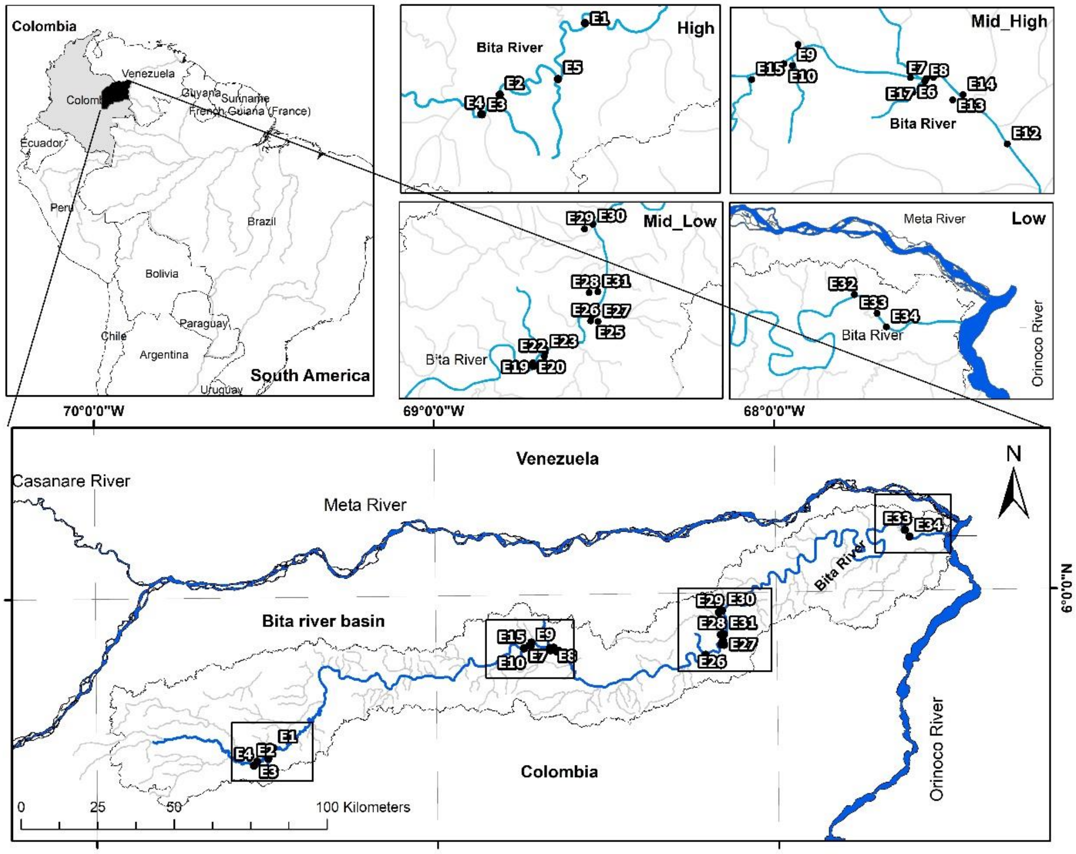

Figure 2.1 Map of the Bita River Basin showing the four sections and the 34 survey sites.

2.2.3. Environmental variables

Environmental variables were divided into six categories; water parameters, substrate, instream cover, channel morphology, local riparian buffer, and landscape variables following Pease *et al.*, (2012). Prior to fish sampling, water quality parameters, such as pH, conductivity (µS), water temperature (°C), dissolved oxygen (mg/L) and total solids, were measured at each survey site using a multiparameter water quality meter (YSI model 85). To characterize substrate and instream cover, the percentage of cobble (diameter 6-25 cm), sand (0.06–2 mm), mud (<0.06 mm), filamentous algae, large woody debris (> 50 cm), small woody debris (< 50 cm), submerged roots, overhanging terrestrial material and leaf litter were visually estimated along the

200-m reach. Variables such as; width of riparian buffer (m), area of the riparian forest (ha) and landscape (Presence of roads, crops, distance to the Orinoco River and altitude) were measured by georeferenced satellite images using ArcMap (Version 10.3.1) in a circular buffer of 1 km. Stream order was calculated using the function stream order in ArcMap (Version 10.3.1) method Strahler's classification.

2.2.4. Data analysis

Analyses were performed at basin (no. sites= 34) and regional scales. Regional-scale datasets were grouped according to four sections along the longitudinal fluvial gradient (high, mid-high, mid-low, low). This approach aimed to explore the possibility that environmental or spatial variation within river sections influences assemblage patterns according to metacommunity processes that may differ among sections. Using the method of Dufrene and Legendre (1997), I calculated indicator values for species most common within a given river section. This method is based on species abundance (specificity) and frequency (fidelity) at survey sites. Values from this method range from 0 to 1, with 1 being a perfect indicator of a river section. Significance of indicator values was evaluated using the difference between the observed value and the mean of values obtained from 1,000 random permutations. Species indicator analysis was performed using the function *Indval* of the package labdsv (Roberts, 2007) in the R statistical language (Version 3.4.1) (R Core Team, 2017).

Spatial variables

Because fish dispersal in rivers is difficult to estimate, I used spatial predictors as proxies for dispersal potential. Spatial eigenvectors were used as predictors to control for spatial autocorrelation and estimate the influence of spatial distance on species distributions and patterns of assemblage structure (Peres-Neto & Legendre, 2010). Spatial patterns were modeled through Asymmetric Eigenvector Maps (AEM), an approach proposed by Blanchet, Legendre and Borcard (2008b). This method takes into account the directional aspect of inter-site distances of systems such as river networks (e.g., water flow, relative position), a limitation of methods such as Principal Coordinates of Neighbor Matrices (PCNM) and Moran's Eigenvector Maps (MEM) (Blanchet *et al.*, 2011; Sharma *et al.*, 2011).

To perform the AEM, I used the distribution of sites across the basin to construct a connection diagram that links sites to one another according to pathways within the river network (Figure 2.1) following the flow direction from upstream to downstream. Using the connection diagram, a sites-by-edges matrix was constructed, and this matrix was used to calculate asymmetrical spatial eigenfunctions through singular value decomposition, which were used later as spatial descriptors. AEM eigenfunctions were calculated using the function *aem* from the package adespatial in R (Version 3.4.1) (Dray *et al.*, 2017). To obtain an additional spatial descriptor, I measured the watercourse distance as the distance from each point to the river mouth along the watercourse using georeferenced satellite images in ArcMap (Version 10.3.1). This variable is more relevant to fish dispersal than straight-line distance that includes overland segments (albeit such dispersal routes are possible during the annual flood pulse).

Elements of the metacommunity structure (EMS)

Using the sites-by-species incidence matrix (excluding rare species that occurred at only one site) as input for reciprocal averaging (RA), elements of metacommunity structure were analyzed based on species scores on the first two axes. To evaluate patterns with respect to six idealized distributional patterns: 1) Checkerboard (Diamond, 1975), 2) nested subsets (Patterson & Atmar, 1986), 3) Clementsian (Clements, 1916), 4) Gleasonian (Gleason, 1926), 5) evenly spaced (Tilman, 1982) and 6) random (Simberloff, 1983), I followed the framework proposed by Leibold and Mikkelson (2002) and Presley, Higgins and Willig (2010).

Coherence was assessed by calculating the number of gaps in species range (observed absences) from the ordered matrix from RA. Statistical significance of coherence was evaluated using the z-score test comparing observed absences with those expected based on 1,000 randomized simulations. Whenever observed absences are greater than expected (negative coherence), there is a checkerboard pattern. Conversely, if observed absences are less than expected under the null model (positive coherence), the analysis tests for evidence of species turnover and boundary clumping. No significant difference between observed and expected absences indicates random distributions (no coherence) (Leibold & Mikkelson, 2002).

Turnover was evaluated by calculating the number of species replacements between sites. I used a z-score test to compare observed species replacements with the mean for species replacements obtained from 1,000 null simulations. Significantly fewer observed replacements are indicative of low turnover and distributions that are nested subsets, whereas more observed replacements indicate high turnover. Finally, boundary clumping was evaluated using Morisita's index, for which a value of one indicates that boundaries are not clumped, values greater than one indicate clumping, and less than one indicates hyperdispersion. Statistical significance of Morisita's index was determined using the chi-squared test. More information about inference of idealized patterns can be found in figure 1 of Presley, Higgins and Willig (2010).

I applied a fixed proportional null model (i.e. “r1” null model in *Metacommunity* function in R) to test significance of coherence and turnover. This null model maintains species richness of each sampling site, but fills species ranges based on their marginal probabilities. Random simulated matrices were calculated using 1,000 simulations (Dallas, 2014). EMS were assessed using the *Metacommunity* function of the Metacom package (Dallas, 2014) in R.

Variation partitioning

I used variation partitioning analysis (Peres-Neto *et al.*, 2006) as an additional means to identify metacommunity types and to assess the relative contribution of environmental and spatial predictors on fish distributions at basin and regional scales. This method allowed us to determine the contribution of environmental conditions independent of space, and vice versa, controlling for type I error as a product of spatial autocorrelation in the environmental component (Peres-Neto & Legendre, 2010). Type I error may lead to spurious conclusions, generating significant species-environment relationships that are artifacts. Partitioning analysis was conducted using the adjusted R-squared in Redundancy Analysis (RDA) on the Hellinger-transformed species-by-sites abundance matrix and two sets of predictors; environment [E] and space [S].

To create each set of predictors, first, I reduced the number of variables in each matrix, because they reduce the statistical power of the test to identify unique and significant environmental and spatial contributions (Peres-Neto & Legendre, 2010). I performed a variable selection for each set of predictors to identify significant variables ($P < 0.05$) associated with the species-by-sites abundance matrix. For the [E] matrix, I transformed the environmental variables due to nonhomogeneous units and verified that the distribution was normal. Those variables expressed as proportions were transformed to the arcsine of their square root. The remaining variables were log(x+1) transformed, with the exception of ordinal and categorical data for which no transformation was done. Data for all variables were standardized and centered by calculating *z*-scores (mean = 0; standard deviation=1) (Borcard, Gillet & Legendre, 2011; Falke & Fausch, 2010). The function *forward.sel* from the R library adespatial (Dray *et al.*, 2017) was used to perform variable selection using 999 permutations with a significance level of P= 0.05. Selected variables comprised the environmental matrix [E] used for the partition analysis.

Spatial predictors from the AEM analysis and inter-site distance data were tested for inclusion in the spatial matrix [S] using the *forward.sel* function of the R library adespatial, using the same approach as described for the [E] matrix. Selected variables were included in the [S] matrix for the partitioning analysis. Finally, with our three datasets (Hellinger-transformed sites-by-species matrix, [E] and [S]), I applied the *varpart* function of the vegan library in R to perform variation partitioning analysis as proposed by Blanchet, Legendre and Borcard (2008a) and Borcard, Gillet and Legendre (2011).

The significance levels of the components produced by the variation partitioning were used to classify the fish metacommunity according to one of the four metacommunity paradigms; species sorting, mass effect, neutral model and patch dynamics (Leibold *et al.*, 2004). To choose the proper paradigm I applied the decision tree proposed by Cottenie (2005).

I used two approaches to identify the underlying environmental and spatial factors that correlate with observed patterns in the metacommunity organization at the basin and regional level. First, I calculated Spearman-rank correlations between the two dominant axes extracted from the RA used in the EMS framework and each variable from the set of environmental and spatial predictors. Second, a variable selection procedure was performed for each group (environmental and spatial) using the *forward.sel* function of the R library adespatial. Correlations were calculated using the function *cor.test* of the stats library in R.

2.3. Results

High and mid-high sections were characterized by higher values of dissolved oxygen, elevation, distance from the main source, savanna area, flow and percentages of grasses and filamentous algae (Table 2.1). Channel width, water temperature, and total solids tended to be lower at upper-basin sites. Substratum and instream cover were heterogeneous within all four sections. Sites located in the lower sections tended to have more extensive riparian forest, more lagoons in adjacent floodplains, wider channels, and lower flow velocity (Table 2.1).

A total of 25,928 fish specimens, representing 201 species, 39 families and 10 orders, were collected during the study. Sixty species were collected in the high section, 148 in the mid-high, 142 in the mid-low, and 67 in the low section. Average number of specimens per site was762, and species richness varied from 4 to 55. The most abundant species were *Amazonprattus scintilla* (Engraulidae) and two species of the genus *Hemigrammus* (Characidae), *H. elegans* and *H. geisleri*. These three-species represented nearly 30% of the collected fish specimens (Appendix A). Fifty-one rare species were collected at single sites. Several species were identified as indicators for the low (12 spp.) and high (6 spp.) sections, and few or none were identified for the mid-high (1 sp.) and low (0 sp.) sections (Appendix B).

Table 2.1 Environmental variables measured in the study, values are untransformed mean ± standard error.

Category	Variable	High	Mid-high	Mid-low	Low
Water parameters	pH	8.9 ± 0.33	6.89 ± 0.18	7.88 ± 0.26	8.55 ± 0.67
	Conductivity	3.76 ± 0.16	4.48 ± 0.09	31.4 ± 11.36	7.16 ± 1.22
	Water temperature	28.1 ± 0.39	28.89 ± 0.25	29.3 ± 0.44	30.76 ± 0.89
	Dissolved oxygen	10.92 ± 0.49	9.42 ± 0.53	8.83 ± 0.69	8.20 ± 1.25
	Total solids	2.68 ± 0.09	3.2 ± 0.06	19.15 ± 7.62	5.06 ± 0.81
Substratum	(%) Cobble (6–25 cm)	14 ± 14	7.5 ± 7.5	0 ± 0	0 ± 0
	(%) Sand (0.06–2 mm)	63 ± 16.85	42.5 ± 12.13	37.14 ± 12.06	46.66 ± 29.05
	(%) Mud or silt (<0.06 mm)	16 ± 16	46.66 ± 12.92	50.71 ± 11.06	46.66 ± 24.03
Instream cover	(%) Filamentous algae	10 ± 5.47	1.66 ± 1.12	1.42 ± 0.97	0 ± 0
	(%) Large woody debris	8 ± 3.74	8.33 ± 3.21	8.57 ± 2.53	6.66 ± 3.33
	(%) Submerged leaf packs	29 ± 11	37.08 ± 8.22	28.57 ± 4.90	33.33 ± 12.01
	(%) Grass	2 ± 2	0 ± 0	0 ± 0	0 ± 0
	(%) Small woody debris	14 ± 2.45	17.08 ± 3.91	13.21 ± 3.21	20 ± 10
	(%) Overhanging terrestrial vegetation	2 ± 2	3.75 ± 2.22	8.21 ± 2.65	6.66 ± 3.33
	(%) Submerged roots	8 ± 5.83	2.5 ± 1.79	10 ± 3.18	3.33 ± 3.33

Table 2.1 Continued

Category	**Variable**	**High**	**Mid-high**	**Mid-low**	**Low**
Channel morphology	Depth (1: <1m; 2:>1-2m; 3:>2m)	2.80 ± 0.20	2.50 ± 0.15	2 ± 0.18	2.33 ± 0.33
	Width (m)	26.22 ± 10.04	45.18 ± 11.05	51.57 ± 10.25	55.39 ± 36.72
	Flow (1: High; 2: Medium; 3: Low)	2 ± 0.31	1.25 ± 0.17	1.28 ± 0.12	1 ± 0
Local riparian buffer	Width of riparian buffer (m)	2.8 ± 0.2	2.5 ± 0.15	2 ± 0.18	2.33 ± 0.33
	(%) Stream shaded by tree canopy	0.34 ± 0.17	0.23 ± 0.07	0.22 ± 0.06	0.2 ± 0.1
	Area Riparian Forest (ha)	18.14 ± 3.26	40.04 ± 3.44	52.39 ± 4.16	47.90 ± 8.23
	Area Savanna (ha)	51.81 ± 2.61	27.50 ± 4.29	11.32± 4.71	10.12 ± 7.89
Landscape variables	Altitude (m)	86.8 ± 0.58	70.92 ± 0.63	64.29 ± 1.31	48.67 ± 1.20
	Stream order (1 to 4)	3.20 ± 0.58	3.33 ± 0.63	3.57 ± 1.31	2.67 ± 1.20
Landscape variables	Near to crop areas (1: Presence; 0: Absence)	0.40 ± 0.24	0 ± 0	0.14 ± 0.1	1 ± 0
	Near to roads (1: Presence; 0: Absence)	0.80 ± 0.20	0.33 ± 0.14	0.29 ± 0.13	1 ± 0
	Distance to the Río Orinoco (km)	627 ± 2.975	380 ± 2.29	256 ± 3.12	19 2.45

2.3.1. Elements of metacommunity structure

At the basin scale (34 sites), the EMS revealed positive coherence with a Clementsian distribution along both of the dominant axes modeling gradients of fish assemblage structure (RA1 & RA2) (Table 2.2, Figure 2.2). Thus, at a broad spatial scale, species seem to respond as groups to environmental factors, leading to relatively discrete assemblages along the longitudinal gradient. The Clementsian pattern identified for RA1 was positively correlated with channel width, stream order and spatial predictors (AEM21, AEM25), and negatively correlated with variables associated to substrate, instream cover and local riparian vegetation (Table 2.3). For RA2, only the spatial predictor AEM1 (positive) and flow (negative) were correlated with site scores (Table 3).

Metacommunity structure was analyzed for sites within each of the four sections along the longitudinal fluvial gradient (high, mid-high, mid-low and low). Assemblages in the high and low sections had negative coherence and a random pattern for both RA axes in the high section and RA1 in the low section; RA2 in the low section revealed a nested distribution. No significant correlations between assemblage structure and environmental or spatial factors were found for the high and low sections (Table 2.2 & 2.3, Figure 2.3).

Mid-high and mid-low sections displayed Clementsian patterns of assemblage structure for both the first and second RA axes. For the mid-high section, no significant correlations were obtained between RA1 and environmental or spatial variables, however, RA2 was positively correlated with the presence of roads and the spatial predictor AEM1 (Table 2.2 & 2.3, Figure 2.3). For the mid-low section, RA1 was positively correlated with substrate and instream cover and RA2 with the spatial predictor AEM1 (Table 2.2 & 2.3, Figure 2.3).

Table 2.2 Results of EMS analysis of fish metacommunity structure at the level of the entire Bita Basin and at the regional level for high, mid-high, mid-low and low sections of the river. Statistically significant values (p< 0.05) are shown in bold type.

Axis of variation	Basin	High	Mid-high	Mid-low	Low
Species	145	37	84	85	55
Sites	34	5	12	14	3
Primary axis					
Coherence					
Observed absences	2506	55	318	453	14
Expected absences	3032	41	445	554	14
P value	**<0.001**	0.127	**<0.001**	**0.001**	0.9
Turnover					
Observed replacements	347752	543	20498	27534	630
Expected replacements	186586	624	14397	20631	621
P value	**<0.001**	0.15	**<0.001**	**<0.001**	0.89
Clumping					
Morisita's index	1.58	1.02	1.37	1.14	1
P value	**<0.001**	0.17	**<0.001**	**<0.001**	1
Best-fit patterns	Clementsian	Random	Clementsian	Clementsian	Random
Secondary axis					
Coherence					
Observed absences	2706	40	383	541	22
Expected absences	3030	40.32	447	557	13
P value	**0.001**	0.96	**0.03**	0.64	**0.007**
Turnover					
Observed replacements	366210	597	19630	14070	377
Expected replacements	186480	622	14397	20697	541
P value	**<0.001**	0.65	**<0.001**	**<0.001**	**0.004**
Clumping					
Morisita's index	1.55	1.36	1.37	1.17	1
P value	**<0.001**	**0.004**	**<0.001**	**<0.001**	1
Best-fit patterns	Clementsian	Random	Clementsian	Random	Nested distribution

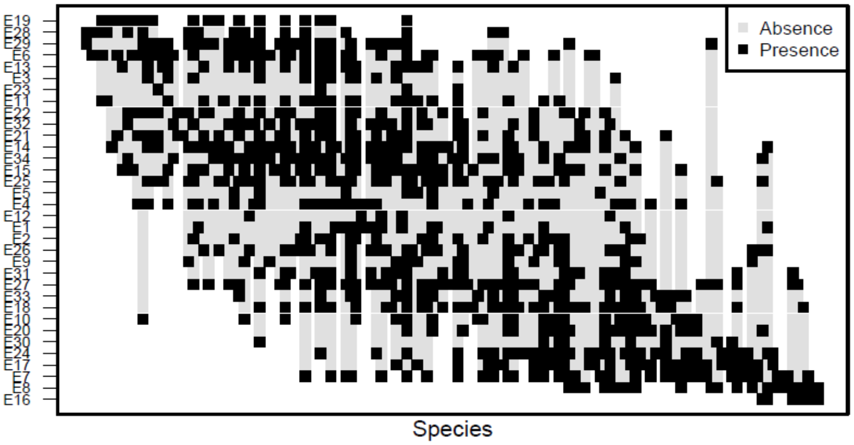

Figure 2.2 Species distributions among 34 sites in the Bita Basin ordered according to scores on the first axis of the reciprocal averaging, revealing a Clementsian gradient. Sites are in rows; species are in columns.

Table 2.3 Spearman-rank correlations between predictors and scores obtained from the first two axes from the reciprocal averaging ordination of the sites-by-species incidence matrix in the EMS framework. Only statistically significant values ($p< 0.05$) are shown. No significant correlations were found for high and low sections.

Variables	Basin		Mid-High		Mid-Low	
	r	p	r	p	r	p
Primary axis						
(%) Mud	-0.55	< 0.01			0.61	0.02
(%) Large woody debris	-0.48	< 0.01				
(%) Small woody debris	-0.48	< 0.01			0.55	0.04
(%) Submerged roots	-0.48	< 0.01			0.61	0.02
(%) Stream shaded by tree canopy	-0.47	< 0.01			0.61	0.02
Width	0.37	0.03				
Stream order	0.52	< 0.01				
AEM21	0.39	0.02				
AEM25	0.39	0.02				
Secondary axis						
(%) Mud			-0.71	0.01		
(%) Large woody debris			-0.66	0.02		
(%) Small woody debris			-0.75	< 0.01		
(%) Stream shaded by tree canopy			-0.75	< 0.01		
Flow	-0.47	< 0.01				
Near to roads			0.66	0.02		
AEM1	0.51	< 0.01	0.6	0.04	0.69	0.01

2.3.2. Variation partitioning analysis

At the basin scale, all the components from variation partitioning (E, S, E+S, E|S, S|E) explained significant ($P < 0.05$) variation in fish assemblage structure, supporting the idea that metacommunity dynamics influence patterns at that scale (Table 2.4). Around, 19% of total explained variation (E+S) was modeled by the combined influence of environment and spatial predictors, 6.9% by a pure environmental component (E|S), and 4.6% by a pure spatial component (S|E). Eighty-one percent of the variation was unexplained. Environmental variation at the basin scale was significantly influenced by percentage of mud substrate, small woody debris, and water conductivity (Table 2.4). Only four of 33 spatial predictors were significant (AEM1, AEM, 16, AEM18, AEM21) (Table 2.4).

At the regional scale, the number of sites within the high and low sections were too small for statistical analysis, and only the mid-high and mid-low sections were analyzed. Only the environmental component (E) explained significant variation in fish assemblage structure within each of these sections (Table 2.4), indicating that it was not possible to infer any metacommunity model. In the mid-high section, a significant environmental component influenced most strongly by percentage of mud substrate and small woody debris explained 16% of assemblage variation. In the mid-low section, 14% of assemblage variation was explained by an environmental component, with conductivity and percentage of small woody debris most influential.

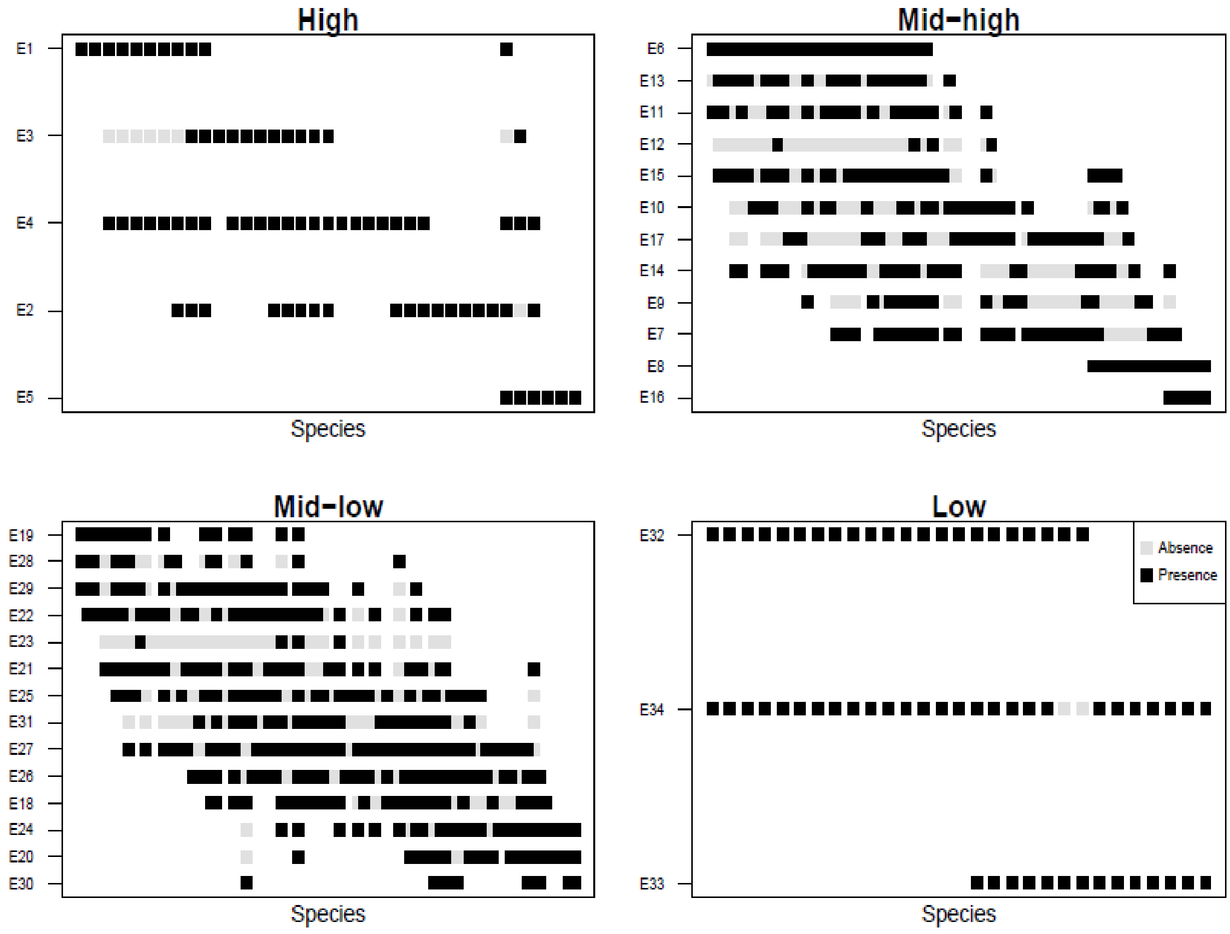

Figure 2.3 Species distributions within four river sections ordered according to scores on the first the reciprocal (RA) axis.

Drivers of fish community structure

The set of variables selected by variation partitioning included most of the ones that had highest Spearman rank correlations. At the basin level, environmental variables related to substrate and instream cover were identified by both methods (Table 2.3 & 2.4). Environmental predictors, such as percentages of mud substrate and small woody debris and the spatial predictor AEM1, were significant at both basin and regional scales (Table 2.3 & 2.4).

Table 2.4 Variation partitioning analysis for fish assemblages and selected groups of environmental and spatial variables at basin and regional scales. AdjR^2= adjusted R^2 for the percentage of variation. p= p-value, significant values (P <0.05) are shown in bold. E= environmental variation, S= spatial variation, E+S= Total explained variation, E|S= Pure environmental variation, S|E= Pure spatial variation, b= Variation shared by environmental and spatial factors, R= Unexplained variation (Residual).

Component	Bita basin		Mid-high		Mid-low	
	AdjR^2	p	AdjR^2	p	AdjR^2	p
E	0.14	**0.001**	0.16	**0.007**	0.14	**0.002**
S	0.12	**0.001**	0.00	0.5	0.00	0.56
E+S	0.19	**0.001**	0.18	0.2	0.14	0.13
E|S	0.069	**0.001**	0.20	0.3	0.16	0.16
S|E	0.046	**0.025**	0.02	0.6	0.00	0.51
b	0.075					
R	0.81		0.82		0.86	
Variables selected	%Mud [E] %Small woody debris [E] Conductivity [E] AEM1 [S] AEM16 [S] AEM18 [S] AEM21 [S]		%Mud [E] %Small woody debris [E]		Conductivity [E] %Small woody debris [E]	

2.4. Discussion

In this study, I applied complementary statistical analyses to investigate fish species distributions and variation in assemblage structure across a longitudinal fluvial gradient to reveal the relative influence of factors associated with environmental conditions (e.g., habitat features, environmental filtering) and spatial relationships influencing dispersal. Results from these analyses provided a basis to infer the relative influence of biotic interactions and stochastic processes in community assembly at two spatial scales. Our results suggest that the fish metacommunity in the Bita River exhibits a Clementsian distribution, implying that species respond to the environmental and fluvial gradient as groups with fairly consistent compositions (Clements, 1916). This pattern also implies a potential effect of environmental filtering at the scale of the entire basin. Similarly, the variation partitioning analysis showed that the variation explained by the pure environmental component was higher than the pure spatial component, which also is consistent with species sorting. Species sorting occurs when organisms select habitats that convey relatively high fitness, and/or avoid or are otherwise eliminated from habitats that reduce their fitness (Soininen, 2014). These findings support our prediction that at the basin scale, dispersal rates are low, and assemblage structure is strongly influenced by species sorting.

Regional-scale results also revealed a Clementsian distribution of local assemblage composition. This pattern could have been caused by species responses to spatial variation of abiotic and biotic environmental factors. Spearman correlation between environmental variables and site scores on gradients of assemblage variation indicated that stream order and channel size had strong associations with local assemblage structure. Variables associated with substrate composition and instream cover also had significant associations. Fishes in the Bita River apparently respond to these environmental variables as relatively clumped groups. Clementsian assemblage structure has been found in plants (Meynard *et al.*, 2013; Willig *et al.*, 2011), mammals (López-González *et al.*, 2012; Presley & Willig, 2010) and various freshwater organisms, including plankton, macroinvertebrates and fish (Dallas & Drake, 2014; Erős *et al.*, 2017; Fernandes *et al.*, 2014; Tonkin *et al.*, 2016; Torres & Higgins, 2016).

I proposed that some metacommunity processes and resultant patterns would not be the same at two different spatial scales of analysis. I found positive coherence and turnover in upstream regions, and positive coherence and negative turnover with nested distributions in downstream regions. These patterns were not predicted a priori. At the regional scale, a Clementsian distribution was found only in the mid-high and mid-low sections of the basin, suggesting that species responded as groups to environmental filters more strongly in these middle sections of longitudinal gradient. Assemblage coherence was non-significant (i.e., not statistically different from a random distribution) for the high and low sections of the river. According to Presley, Higgins and Willig (2010), non-significant coherence is not always an indicator of random structures. Dallas and Drake (2014) found that in most studies in which random patterns were found, samples contained few species, few sites or both, and therefore there was not enough statistical power to test coherence using randomization procedures. In my study, there were fewer survey sites for the highest and lowest sections of the river, which may account for non-significant coherence in those sections. Nonetheless, Figure 3 reveals a pattern of species distribution with high turnover and boundary clumping in the high section; such a pattern is less discernable for the low section.

In the low section of the river, distribution patterns were different for the two assemblage ordination axes. Random distributions were inferred based on the first ordination axis, and nested distributions were found for the second axis. Results of the second axis support my hypothesis that environmental gradients over smaller spatial scales in the low section may promote nested distribution patterns. Sites in this section were characterized by substrates comprised almost entirely of sand with patches of leaf litter and low variation in water physicochemistry. Distribution patterns may be more likely to be nested when sample size is small and environmental gradients are short (Tonkin *et al.*, 2016). Conversely, large sample sizes and long environmental gradients may increase the likelihood of finding Clementsian and Gleasonian patterns.

At the basin scale, the total variation in assemblage structure captured by the model (19%) was decomposed into 6.9% purely environmental, 7.5% shared by environmental and spatial, and 4.6% purely spatial components (each fraction with $P< 0.001$). The pure environmental component was strongly associated with the percentage of mud substrate, small woody debris, and conductivity. Shared environmental and spatial factors also were associated with the percentage of mud and conductivity. The pure spatial component was represented by eigenvectors from asymmetric eigenvector mapping (AEM), predictors that are not easily interpretable because they integrate multiple spatial scales (Peres-Neto & Legendre, 2010), with small-scale spatial variables tending to be associated with mass effect dynamics and large-scale spatial variables tending to be associated with dispersal limitation (Heino *et al.*, 2015). At the scale of the entire basin, both processes probably occur simultaneously.

The percentage of variation explained by the partitioning analysis was low (19%), a common finding in community ecology studies (Castillo-Escrivà *et al.*, 2016; Devercelli *et al.*, 2016; Erős *et al.*, 2017; Legendre & Legendre, 2012; Ter Braak & Šmilauer, 2012). The large amount of unexplained variation could be due to several factors, such as failure to include other relevant environmental variables and spatial predictors or variables related to other processes such as biotic interactions. Of course, there also could be a large influence of stochastic dynamics. Soininen (2016) reviewed 322 datasets and found that spatial predictors obtained from spatial eigenvector analysis generally explained a small fraction of total assemblage variation, which suggests that spatial predictors from asymmetric eigenvector mapping (AEM) may perform poorly in capturing patterns reflecting dispersal dynamics. Monteiro, Paiva and Peres-Neto (2017) proposed the use of patch connectivity metrics as predictors of dispersal dynamics rather than spatial eigenvectors. They found that the patch connectivity framework increased the amount of explained variation by as much as 50%.

Because both pure environmental and pure spatial components were statistically significant, both species sorting and mass effect dynamics could have influenced fish assemblage structure at the basin scale (Cottenie, 2005). However, because the proportion explained by the pure environmental fraction was larger than the spatial fraction (6.9% vs. 4.6%), species sorting likely was the more influential component structuring fish assemblages in this system. These results are in accordance with Heino, Melo, & Bini, 2015. They found that an intermediate amount of dispersal was needed in order to species sorting process. Since species need to disperse from one place to another searching for sites with the appropriate environmental conditions to thrive.

Soininen (2014) reviewed 326 investigations that used variation partitioning analysis, finding that environmental variables usually explained the most variation in assemblage composition and species sorting was most often inferred. Species sorting may be the prevalent metacommunity model for river and stream fish assemblages (Cottenie, 2005; Falke & Fausch, 2010; Heino *et al.*, 2015).

At a smaller spatial scale, I predicted that species sorting strongly influences assemblages in headwaters where habitats are relatively isolated within the river network, whereas assemblages in downstream reaches would have greater dispersal and be more influenced by mass effect dynamics. These predictions were not confirmed. Based on variation partitioning analysis and the decision tree of Cottenie (2005), no evidence was obtained to support any of the metacommunity paradigms proposed by Leibold *et al.* (2004). The variation partitioning procedure could only be performed for the mid-high and mid-low sections that had sufficiently large samples. Only the environmental fraction was significant, and this result can be prone to type I error.

In summary, variation partitioning and metacommunity structure analyses provided complementary findings to infer processes structuring fish assemblages in the Bita River. Both approaches identified environmental filtering and species sorting as the principal structuring processes in this system. Variation partitioning analysis implied that dispersal plays a significant role at the basin scale, however, the environmental fraction explained more variation. Both analyses revealed assemblage patterns correlated with components of habitat structure. Similar conclusions have been reported from studies conducted at smaller spatial scales in other tropical rivers (Arrington, Winemiller & Layman, 2005; Willis, Winemiller & Lopez-Fernandez, 2005), with some also inferring that biotic interactions play a significant role in structuring fish assemblages within local habitats (Montaña et al., 2014). In a study of the Cinaruco River, an Orinoco tributary in the Venezuelan Llanos with characteristics similar to the Bita River, Willis et al., (2005) found that habitat structural complexity was strongly correlated with the functional diversity of fish assemblages. These findings combined with mine, suggest that fish community structure of Neotropical rivers and streams is influenced by local environmental conditions, especially aspects associated with substrate and habitat structural complexity, that in turn affect species sorting that results in Clementsian patterns of species distribution. Improved understanding of the influence of dispersal, environmental filtering and biotic interactions on species assemblages in pristine systems will be essential for efforts to conserve and restore biodiversity. For example, if the spatial distribution of fishes in the Bita River derives largely from dispersal and environmental filtering, then strategies to conserve fish diversity must emphasize maintenance of habitat heterogeneity and connectivity at appropriate spatial and temporal scales.

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3. EXPLORING EFFECTS OF ENVIRONMENTAL AND SPATIAL FACTORS ON FISH BETA DIVERSITY IN A SPECIES-RICH NEOTROPICAL RIVER

3.1. Introduction

Biodiversity is a broad concept that extends beyond the number of species found in a certain place to include various types of organisms and levels of organization from genes to ecosystems as well as processes (ecological and evolutionary) that maintain them (Mouchet et al., 2010). The measurement of biodiversity has been a major challenge in community ecology (Villéger, Grenouillet & Brosse, 2013), and multiple concepts and approaches have been adopted (Clements, 1916; Diamond, 1975; Gleason, 1926; Hubbell, 2001; Simberloff, 1983; Whittaker, 1960). Traditionally, ecologists have focused on factors that affect communities locally. However, during the past 25 years there has been a rapid increase in studies showing how local communities exchange organisms to form metacommunities, and how the processes of speciation, extinction and dispersal interact at variable spatial and temporal scales to structure regional communities (Dray et al., 2012; Leibold et al., 2004; Ricklefs & Schluter, 1993).

Theoretical and empirical work on metacommunities has given rise to four conceptual models: patch dynamics, species sorting, mass effects and neutral paradigm (Leibold et al., 2004; Leibold & Mikkelson, 2002). In riverine systems, the concept of patch dynamics has been recognized as being generally applicable to benthic algae and aquatic macroinvertebrate metacommunities, the mass effect paradigm to intertidal invertebrates and coral reef fishes, and the species sorting paradigm to numerous taxa, including algae, meiofauna, macroinvertebrates and fishes (Winemiller, Flecker & Hoeinghaus, 2010). In an African river floodplain Jackson et al. (2013) found that mass effect, patch dynamics and species sorting concepts all were consistent with fish assemblage structure, and López-Delgado, Winemiller & Villa-Navarro (2019) inferred that species sorting had a strong influence on spatial variation of fish assemblages in a Neotropical river system. Recently, Brown et al. (2017) criticized research aimed at confirming alternative metacommunity paradigms and proposed the existence of structures beyond the four proposed by Leibold et al. (2004).

This expanded view of community ecology has been accompanied by new conceptual and statistical methods that facilitate better understanding of how community composition changes in space and time (Baselga, 2012; Legendre, 2014; Podani & Schmera, 2011; Podani, Ricotta & Schmera, 2013). Variation in species composition was defined as beta diversity by Whittaker (1960). Because beta diversity evaluates more than species richness, and biotic composition contains more information about ecological and evolutionary processes at different scales (Baselga, Gómez-Rodríguez & Lobo, 2012; Legendre & De Cáceres, 2013), this biodiversity measure has attracted great attention from community ecologists during the past 25 years. Beta diversity analysis allows ecologists to test hypotheses about the processes that generate and maintain biodiversity (Legendre & De Cáceres, 2013), which is crucial for understanding ecosystem functioning as well as conservation of biodiversity. Variation in species composition can be decomposed into species replacement and richness difference, metrics that are antithetical (Legendre, 2014).

Species replacement (i.e. turnover) and richness difference decompose dissimilarity coefficients that can be used to measure species variation (β diversity) (Legendre, 2014). Species replacement describes the simultaneous gain and loss of species along spatial, temporal gradients that potentially are caused by environmental filtering, biotic interactions or historical factors (Baselga & Orme, 2012; Legendre & De Cáceres, 2013). On the other hand, a species richness difference occurs when one community includes a larger set of species than other, or when one site has a nested subset of species that are present at another. Richness difference may reflect niche differences across the spatial or temporal scales and could be due to species loss, barriers to dispersal, or various mechanisms of colonization and extinction (Baselga, 2012; Legendre, 2014; Ricklefs & He, 2016; Ricklefs, 2006; Schmera & Podani, 2011).

The dendritic structure of fluvial networks has been shown to affect beta diversity patterns of fish assemblages in Neotropical rivers. Vitorino Júnior et al. (2016) found that species replacement explained beta diversity patterns in branches and upper parts of river networks via environmental filtering and dispersal constraints. In contrast, species turnover was lower in the river mainstem, likely due to the mass effect and less dispersal limitation. Datry et al. (2016) proposed that changes in beta diversity along a longitudinal gradient are related to the unidirectional flow of water and passive dispersal of organisms "downstream" in river networks, generating an increase in species diversity from upstream to downstream. Consequently, spatial variation in community structure is strongly influenced by dispersal (mass effect) and habitat selection (species sorting). Upstream drainages are relatively isolated and the unidirectional flow of water constrains dispersal, and communities should more strongly reflect species sorting. Conversely, high connectivity in the river mainstem results in local communities that more strongly reflect the mass effect.

Local community structure also should be influenced by location within the fluvial network. The network position hypothesis (NPH) predicts that environmental factors structure communities at headwaters sites of river systems, and downstream sites communities are structure mainly by dispersal and mass effect (Brown & Swan, 2010). According to Schmera et al. (2018), predictions of the NPH have been observed only in macroinvertebrate and fish communities. Heino, Melo & Bini (2015) proposed that when dispersal rates are intermediate, there is a strong relation between environmental heterogeneity and variation in species composition (beta diversity). Gianuca et al. (2017) examined the potential role of dispersal and environmental heterogeneity in determining beta diversity and its components in zooplankton communities. They found that dispersal limitation increased beta diversity via species replacement within environmentally homogeneous landscapes. In environmentally heterogeneous landscapes, the importance of beta diversity components changed depending on dispersal rates.

The goal of my study was to elucidate patterns and potential drivers of beta diversity and species replacement in fish metacommunities along the longitudinal gradient of the Bita River, a lowland river with an undisturbed watershed in the Llanos region of Colombia. In particular, I investigated whether or not position within the river network had an effect on patterns of beta diversity. At the basin-level, given that environmental heterogeneity is high and dispersal rates are intermediate, I proposed that species track suitable environmental conditions, therefore beta diversity should be high and mainly due to species replacement (i.e. turnover) through species sorting. At a finer scale (localities within a river section) beta diversity should show patterns that vary depending on position within the river network. Because headwaters sites are typically environmentally heterogeneous and isolated within the river network and therefore receiving fewer migrants, beta diversity should be high and mainly driven by species turnover due to species sorting. Conversely, at downstream sites close to the river mouth, where sites are more connected, and high dispersal rates should give rise to a strong mass effect that tends to reduce beta diversity. As a result, species turnover should be relatively low, with spatial differences in species richness reflecting a nested pattern.

3.2. Methods

3.2.1. Study area

In 2018 The Bita River Basin was declared as a wetland of international importance under the Ramsar convention, being one of the few free-flowing rivers protected in the world (World Wide Fund For Nature, 2018). This watershed is located between the municipalities of La Primavera and Puerto Carreño in Vichada Department within the eastern Colombian Llanos. The Bita River Basin drains an area of 812,312 ha and has a channel length of roughly 700 km flowing east to the Orinoco River. During its course the Bita River watershed is fed by approximately 5,070 streams of different orders, with orders one and two (Strahler's classification) most abundant. The climate is tropical and hot with an average temperature of 27-28 °C, and average annual precipitation of 2,300 mm. The region has two well-defined periods a harsh dry period with very low precipitations between December and March and a wet season with high precipitations between April and November (Trujillo & Lasso, 2017).

Despite the fact that the basin has a low elevational gradient (0.357 m/km), with most headwater streams located at 300 m asl and the river's mouth at 50 m asl, environmental features vary along its longitudinal gradient. The study area has a great diversity of aquatic habitats, including river channels, creeks, oxbows, floodplain lakes, flooded savanna and forest, and some special biotopes, such as 'morichales' (small low-gradient streams lined with palms) (Lasso et al., 2011). The Bita Basin contains a mosaic of gallery forests and savannas. From April through November, the river floods adjacent areas to create a complex of diverse aquatic habitats, including flooded forest and savannas crucial for maintenance of fish diversity (Trujillo & Lasso, 2017).

3.2.2. Surveys

Two field expeditions, each of which lasted 30 days, were performed during January and March 2016 when low-water conditions facilitated efficient capture of fishes and relationships between fish assemblage structure and local habitat features should be strong due to high per-unit-area fish densities within contracted aquatic habitat and reduced opportunities for dispersal (Pease et al., 2012). I selected 34 survey sites distributed along the entire longitudinal gradient of the river basin (Figure 3.1). The basin was divided into four sections (high, mid-high, mid-low, low) of equal length (66 km) along the basin's longitudinal gradient (Figure 3.1).

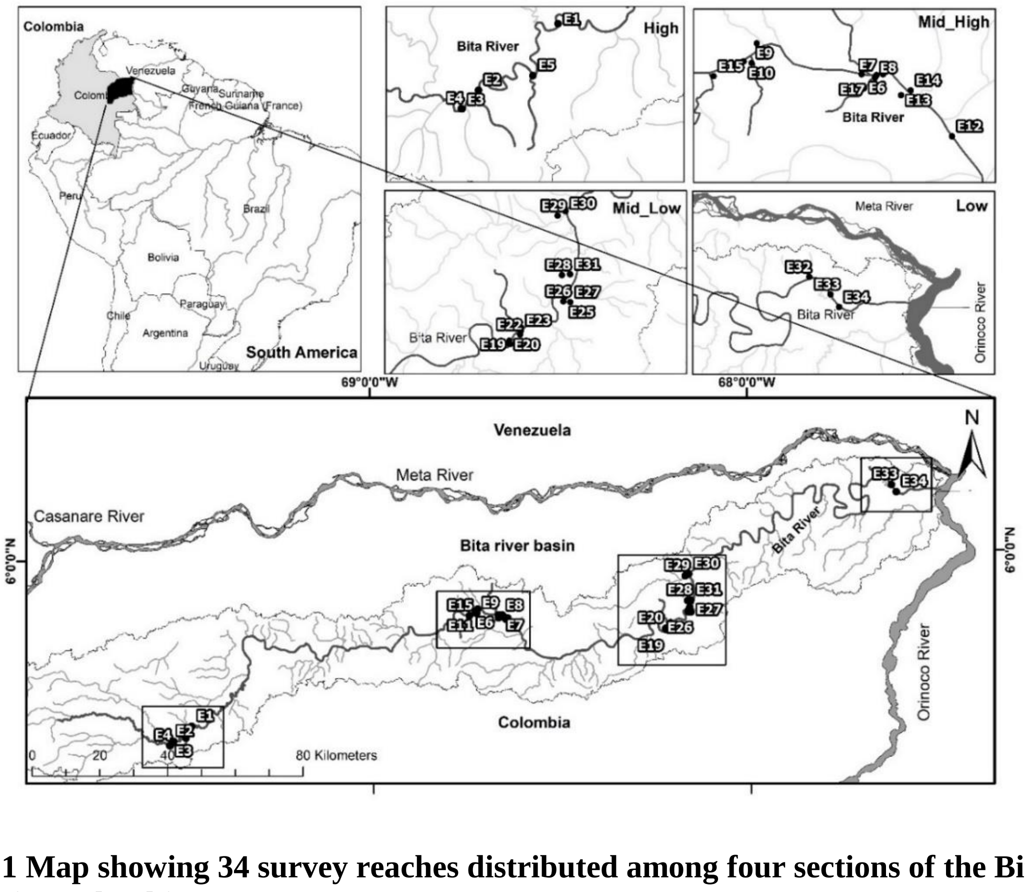

Figure 3.1 Map showing 34 survey reaches distributed among four sections of the Bita River Basin, Colombia.

At each survey site, I selected a 200-m reach encompassing all available macrohabitats to collect fishes and data for local environmental variables. Fishes were collected using a seine (10 x 1.5 m, 3-mm mesh) and two gill nets (10 x 2 m, 100-mm mesh). Within each study reach, six seine hauls of 20 m were performed, and gill nets were deployed for 2 h. After fishes were removed from nets, they were anesthetized according to an approved Texas A&M university animal use protocol (IACUC 2015-0360) by immersion in tricaine methane sulfonate (MS-222) and then euthanized in an overdose of MS-222. Specimens were fixed in 10% formalin, transported to the laboratory, and transferred to 70% ethanol for preservation. All specimens were identified, cataloged and deposited in the voucher collections of the Universidad del Tolima (CZUT-IC) and Instituto von Humboldt (IAvH-P) in Colombia.

3.2.3. Environmental variables

Environmental variables were divided into six categories; water parameters, substrate, instream cover, channel morphology, local riparian buffer, and landscape variables following Pease et al. (2012) (Table 2.1). Prior to fish sampling, water quality parameters, such as pH, conductivity (µS), water temperature (°C), dissolved oxygen (mg/L) and total solids, were measured at each survey site using a multiparameter water quality meter (YSI model 85). To characterize substrate and instream cover, the percentage of cobble (diameter 6-25 cm), sand (0.06–2 mm), mud (<0.06 mm), filamentous algae, large woody debris (> 50 cm), small woody debris (< 50 cm), submerged roots, overhanging terrestrial material and leaf litter were visually estimated along the 200-m reach. Variables such as; width of riparian buffer (m), area of the riparian forest (ha) and landscape (Presence of roads, crops, distance to the Orinoco River and altitude) were measured by georeferenced satellite images using ArcMap (Version 10.3.1) in a circular buffer of 1 km. Stream order was calculated using the function stream order in ArcMap (Version 10.3.1) method Strahler's classification.

3.2.4. Spatial variables

To compose the spatial predictors, I used Asymmetric Eigenvector Maps (AEM) (Blanchet, Legendre & Borcard, 2008) which are known to be an efficient way to model simple and complex spatial patterns at different scales. To performed AEM, a connection diagram was built using the sites distribution across the entire watershed linking sites to one another according to their position within the river network. With the connection diagram, a sites-by-edges matrix was constructed and this matrix was used to performed an AEM eigenfunction analysis using the aem function from the package adespatial (Dray et al., 2017). More information on the calculation of the AEM can be found in López-Delgado, Winemiller & Villa-Navarro (2019).

3.2.5. Data analysis

Analyses were conducted at two regional unit levels. At the basin level, I used the overall dataset (34 sites) and ecoregion level I used the four sections distributed along the longitudinal fluvial gradient (high, mid-high, mid-low, low). Each section represents a regional unit that encompasses a regional pool of species of a number of aggregated sampling sites. This approach allowed me to test whether β-diversity patterns differ depending on position within the river network. In addition, I performed analyses using both species presence-absence and abundance data, because both provide useful and complementary information about mechanisms structuring communities at different spatial scales (Gianuca et al., 2017; Legendre, 2014).

Decomposing beta diversity into richness difference and replacement components

Using sites-by- species presence-absence and abundance matrices, β diversity was assessed. For presence absence data, Jaccard dissimilarity coefficients were used; for species abundance data, I used Ružička dissimilarity. Species abundance was transformed using the square-root transformation to minimize differences due to the high abundance of some species. Next, β diversity was partitioned using the Podani family decompositions into richness/abundance difference and species/abundance replacement components as described by Legendre (2014), using the function *beta.div.comp* from the library adespatial (Dray *et al.,* 2017) in R (Version 3.4.1) (R Core Team, 2017).

Once β diversity and its components (richness difference and replacement) were estimated, the next step was to identify if these values differ as function of the position within the river network. To do that, I used PERMDISP (Anderson, Ellingsen & McArdle, 2006), an analysis that tests if β diversity and its components (response variables) differ among the four river sections (factors). Tukey's HSD was then used to test for significant pair-wise differences between river sections. Both analyses were performed using the *betadisper* function from the vegan library (Oksanen, McGlinn & Wagner, 2018) in R.

Simplex analysis

Given that species richness difference (Richdiff) and replacement (Repl) combine to equal β diversity (D) and similarity (S) equal to 1-D, Podani & Schmera (2011) and Podani, Ricotta & Schmera (2013) proposed a way to represent these three values (S + Repl + RichDiff = 1) in a triangular graph that they called an SDR-simplex plot. Each side of the triangle represents one of the three components. The scatter of points among all possible pairs of sites reflects the relative importance of the richness difference and replacement. SDR-simplex plots were created to vizualize the relative contribution of richness difference and replacement components to β diversity at the basin scale and for each of four sections within the river network. SDR-simplex plots were created using the function *ggtern* form the ggtern package in R. In additional to the triangular plot, SDR-Simplex analysis provides information for the percent contribution of each component. More information about how to perform and interpret the SDR simplex analysis can be found in Podani, Ricotta & Schmera (2013).

Local contributions to beta diversity (LCBD)

To measure the contribution of each site to β diversity, I calculated the LCBD indices, these are comparative values of the ecological uniqueness of the sampling sites across the entire basin. LCBD indices were calculated for the β diversity and its components; richness difference and replacement, using the function LCBD.comp from the adespatial package in R. Large LCBD values indicate sites that may have high conservation status for their particular species combinations or sites with a very low number of species that may be candidates for ecological restoration (Legendre & De Cáceres, 2013). To identify if LCBD indices were related to local richness and abundance, Spearman correlation tests were performed using the function cor.test from the library stats. This method was selected because some variables did not have normal distributions.

Explaining variation in beta diversity and its components

To test if variation in assemblage dissimilarity matrices (β diversity, richness difference and replacement) were related to environmental and spatial factors along the longitudinal fluvial gradient, the distance-based redundancy analysis (db-RDA) method was applied as proposed by Legendre & Anderson (1999). This method works well when the dissimilarity matrix is Euclidean. For that reason, Podani decomposition was used. According to Legendre (2014), matrices produced by this family are Euclidean and can be fully represented in the Euclidean space without any transformation. db-RDA was calculated using the function dbRDA.D from appendix S4 in Legendre (2014), this function produces a F-test of significance between the response data matrix (β diversity and its components) and a set of explanatory variables (environmental and spatial).

To select the environmental and spatial variables that were significant related to β diversity and its components, a forward selection procedure was conducted using the complete set of principal coordinates produced by the db-RDA computed from each of the dissimilarity matrices as response data (one by one) and the environmental and spatial variables (separated) as explanatory data following Legendre (2014). Prior to the db-RDA and forward procedure, environmental variables were transformed due to their nonhomogeneous nature. Variables expressed as proportions were transformed to the arcsine of their square root. The remaining variables were log(x+1) transformed, with the exception of ordinal and categorical data for which no transformation was performed. Data for all variables were standardized and centered by calculating z-scores (mean = 0; standard deviation=1). The function forward.sel from the adespatial package in R was used with 999 permutations and a significance level = 0.05.

Variation partitioning

To determine the relative influence of environmental and spatial variables on each of the dissimilarity matrices, the variance partitioning procedure was applied (Peres-Neto, Legendre, Dray & Borcard, 2006). According to Clappe, Dray & Peres-Neto (2018) this method is routinely used in ecology to assess the importance of environmental and spatial variables on metacommunities. Because, dissimilarity matrices are being used as response data, this method uses db-RDA to partition the variation into the pure components of environment, space and their shared contribution to the explanation of the β diversity and its components. To test the significance of each testable component, an ANOVA-like permutation test for the db-RDA was performed with 999 permutations and alpha level of 0.05. These analyses were carried out using the functions varpart and anova from the vegan package in R.

3.3. Results

I recorded 201 species in the study area among a total of 25,928 fish specimens, with 60 species occurring in the high section, 148 in the mid-high, 142 in the mid-low and 67 in the low section (Appendix A). Local richness ranged from 4 to 55 species, with an average number of 28 species per site. At the basin level, percentage of β diversity was high for both data types, although slightly higher for abundance data (Table 3.1). When presence-absence (P/A) was considered, the fish community was dominated by species replacement, which accounted for 55.7% of β diversity reflecting the continuous turnover of fish species along the longitudinal gradient of Bita River. The simplex diagram confirmed these results, and most of the site pairs were concentrated close to the beta diversity edge, with the centroid of the point cloud near to the species replacement corner (Figure 3.2). Results obtained using abundance data were nearly identical (Table 3.1, Figure 3.2).

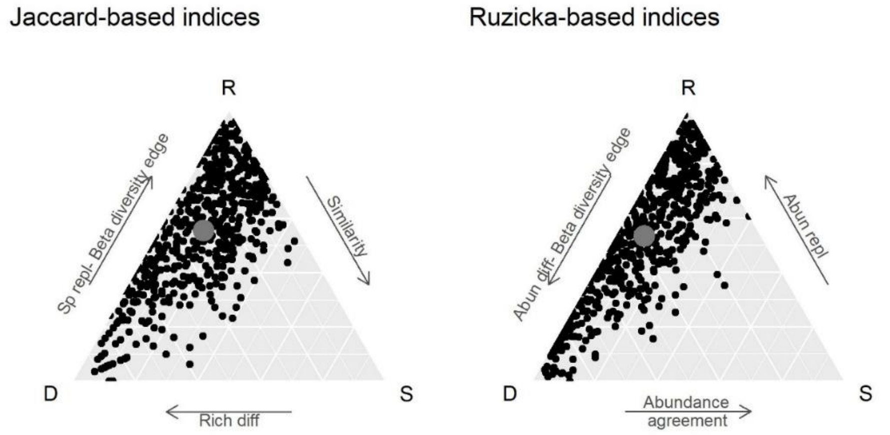

Figure 3.2 SDR-simplex plots for survey sites in the Bita River along its longitudinal fluvial gradient. Each black dot represents a pair of sites. The large gray dot represents the centroid of the point cloud. S (similarity), D(difference), R(replacement), Sp repl and Abun repl (species and abundance replacement), Abun diff and Rich diff (abundance and richness difference). Jaccard dissimilarity coefficients were used for P/ A data, and Ružička dissimilarity was used for species abundance data.

Table 3.1 Beta diversity and percentage contributions of its components for abundance and presence/absence (P/A) data based on the entire basin and four sections of the Bita River. Nestedness was calculated according to Podani et al. (2013); S denotes similarity, D is difference, R is replacement, β is beta diversity, and Nest is nestedness.

Scale	Data	S	R	D	β	Nest
Basin	P/A	13.7	55.8	30.5	86.3	41.2
	Abundance	9.4	53.2	37.5	90.7	42.9
High	P/A	15.2	55.7	29.1	84.8	43.9
	Abundance	13.0	53.3	33.8	87.0	46.7
Mid-high	P/A	12.2	55.8	32.0	87.8	36.1
	Abundance	8.1	52.0	39.9	91.9	37.0
Mid-low	P/A	16.1	51.7	32.3	83.9	47.6
	Abundance	10.9	54.3	34.8	89.1	45.0
Low	P/A	23.9	59.1	17.0	76.1	40.9
	Abundance	16.4	62.3	21.3	83.6	37.6

Beta diversity patterns were analyzed for sites within each of the four river sections. For P/A, percentages of β diversity ranged from 76.1% to 87.7% with species replacement dominating β diversity along the river network. Results from the simplex diagrams showed that in all four sections, sites pairs are concentrated near to the beta diversity edge and the centroids of the point clouds were close to the replacement corner, reflecting that species replacement is constant no matter the position within the river network (Figure 3.3). According to the results of PERMDISP analysis, only β diversity was significantly different among the river sections (p= 0.023). Tukey HSD pair-wise comparisons revealed that the low section was different from the mid-high and mid-low sections (Table 3.2).

When species abundance was considered, percentages of β diversity were higher than values computed from P/A data (Table 3.1). These ranged from 83.6% to 91.9%. Results from the simplex diagrams were similar to those for P/A data, with centroids of the point clouds close to the replacement corner. PERMDISP revealed a significant difference only in β diversity (P= 0.001), and pair-wise comparisons showed that the low section was significantly different from the mid-high and mid-low sections (Table 3.2).

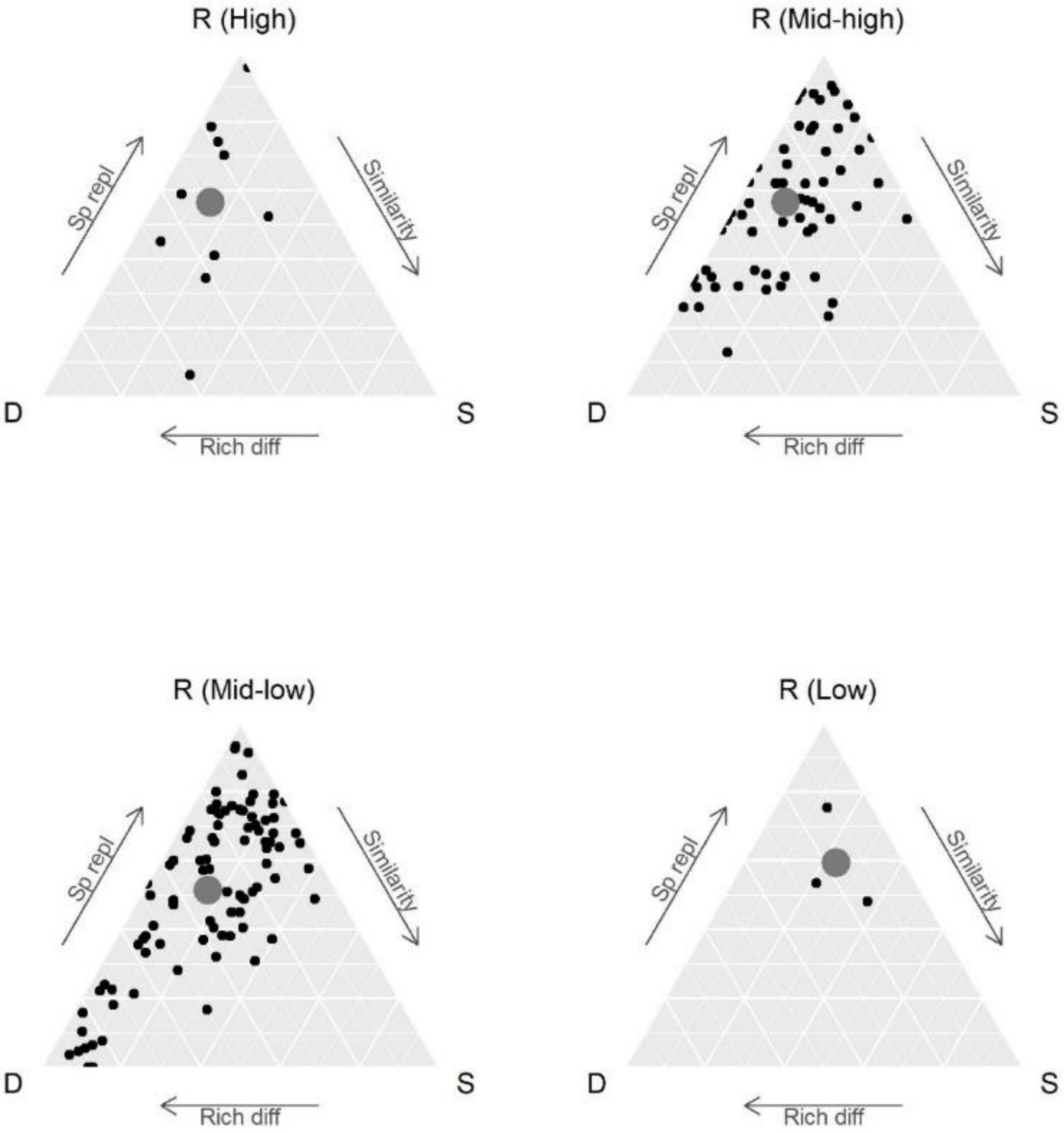

Figure 3.3 Results of the SDR simplex method within each section of the Bita river basin using presence absence data. Each black dot represents a pair of sites. The large gray dot represents the centroid of the point cloud. S (similarity), D(difference), R(replacement), Sp repl (species replacement), Rich diff (richness difference).

Table 3.2 Variation in beta diversity and its components richness/abundance difference and species/abundance replacement for presence-absence (Jaccard dissimilarity) and abundance data (Ružička dissimilarity), within each section of the Bita river basin, calculated using PERMDISP and "TukeyHSD" pot-hoc test. *diff* stands for difference between river sections. Statistically significant values (p< 0.05) are shown in bold type.

Beta diversity Jaccard	***diff***	***p*-value**	**Beta diversity Ružička**	***diff***	***p*-value**
Low-High	-0.096	0.309	Low-High	-0.067	0.402
Mid-high-High	0.060	0.444	Mid-high-High	0.073	0.101
Mid-low-High	0.037	0.775	Mid-low-High	0.058	0.240
Mid-high-Low	0.155	**0.015**	Mid-high-Low	0.140	**0.004**
Mid-low-Low	0.132	**0.041**	Mid-low-Low	0.125	**0.010**
Mid-low-Mid-high	-0.023	0.864	Mid-low-Mid-high	-0.015	0.903
Richness difference			**Abundance difference**		
Low-High	-0.096	0.781	Low-High	-0.074	0.961
Mid-high-High	0.044	0.933	Mid-high-High	0.034	0.990
Mid-low-High	0.037	0.956	Mid-low-High	-0.003	1.000
Mid-high-Low	0.140	0.415	Mid-high-Low	0.108	0.851
Mid-low-Low	0.133	0.447	Mid-low-Low	0.072	0.948
Mid-low-Mid-high	-0.007	0.999	Mid-low-Mid-high	-0.037	0.969
Species replacement			**Abundance replacement**		
Low-High	-0.030	0.984	Low-High	-0.079	0.835
Mid-high-High	0.022	0.985	Mid-high-High	-0.102	0.457
Mid-low-High	0.022	0.983	Mid-low-High	-0.122	0.284
Mid-high-Low	0.052	0.897	Mid-high-Low	-0.023	0.992
Mid-low-Low	0.052	0.891	Mid-low-Low	-0.043	0.952
Mid-low-Mid-high	0.000	1.000	Mid-low-Mid-high	-0.020	0.978

LCBD indices were computed for β diversity and its two components, richness difference and replacement, using P/A and abundance data (Figure 3.4). LCBD values for the β diversity were similar for both data types; sites located in the mid-high section (E5, E8, E12, E16) had the highest values. Spearman rank correlation results showed that LCBD values were negatively related with local richness and abundance, and, therefore, these sites were exceptional due to their particular species combinations, low richness and low abundance (Table 3.3).

Table 3.3 Spearman rank correlations of the local contribution to beta diversity (LCBD) indices for presence-absence (P/A) and abundance data and local richness and local abundance. Statistically significant values (p< 0.05) are shown in bold type. rho= coefficient of correlation, p= p-value.

	Local richness		Local abundance	
LCBD	rho	p	rho	p
P/A data				
Jaccard β-diversity	-0.57	**<0.001**	-0.35	**0.03**
Richness difference	-0.29	0.09	-0.17	0.33
Species replacement	0.19	0.26	0.2	0.25
Abundance data				
Ružička β-diversity	-0.52	**0.01**	-0.39	**0.02**
Abundance difference	-0.18	0.29	-0.34	**0.04**
Abundance replacement	0.16	0.35	0.25	0.15

High LCBD values for richness difference values were obtained for sites E3, E12, E27 and E30 (Figure 3.4). These sites were located in high, mid-high and mid-low sections. Local richness and abundance were not significantly correlated with LCBD richness difference values

(Table 3.3), however, most of the sites had low richness, with the exception of site E27 which was the richest site with 55 species.

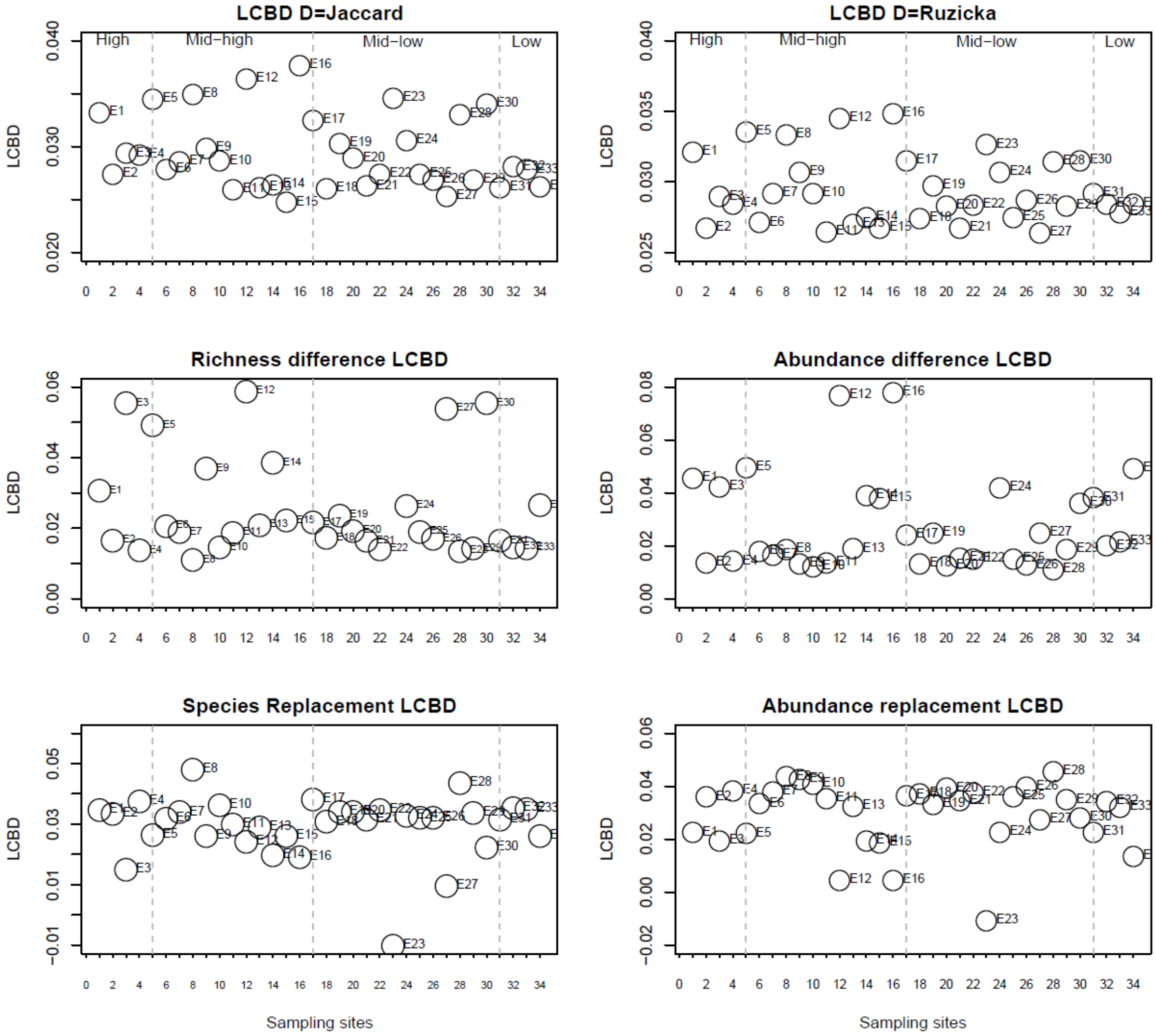

Figure 3.4 Results of the LCBD values of β diversity and its components (species-abundance) difference and (species-abundance) replacement for presence-absence (P/A-Jaccard dissimilarity) and abundance data (Ružička dissimilarity) for the Bita River.

High LCBD values for abundance difference were found at sites E12 and E16 located in the two middle sections (Figure 3.4). These were negatively correlated with local abundance (Table 3.3) and had low values for species richness and abundance. LCBD values for species and abundance replacement were relatively stable for most sampling sites across the longitudinal gradient (Figure 3.4), and were not significantly related to local richness or abundance (Table 3.3).

The forward selection procedure for the P/A data selected 14 environmental variables and six AEM spatial predictors for β diversity. For richness difference, 15 environmental variables and one spatial variable were selected, and for species replacement, 14 environmental and three spatial variables were selected. Most of the environmental variables were associated with habitat instream cover (Table 3.4), reflecting the importance of habitat for β-diversity patterns in the Bita River. Regarding abundance data, selection procedure identified four environmental and 12 spatial variables related to β diversity. For abundance difference, 14 environmental and one spatial variable were selected, and for abundance replacement, 14 environmental and eight spatial variables were selected (Table 3.5). Environmental variables selected for both data types were almost the same; consequently, these variables were subsequently used in a variation partitioning analysis.

All components from the variation partitioning analysis (E, S, E+S, E|S, S|E) for P/A data explained significant ($p< 0.05$) variation in β diversity (Table 3.6). Around 8.4% of the variation was modeled by the pure environmental fraction, 6.4% by the combined influence of environment and space and 2.4% by the pure spatial predictors (Figure 3.5). Variation in richness difference was only significantly explained by the pure spatial component, unlike species replacement where pure environmental and spatial fractions were significant, with the environmental factor explaining more variation (Figure 3.5).

Table 3.4 Environmental and spatial variables selected by the *forward selection* procedure for the beta diversity and its components richness difference and species replacement for presence-absence data (Jaccard dissimilarity). Only statistically significant values (p< 0.05) are shown

Variables	Beta diversity		Richness difference		Species replacement	
	R^2	p	R^2	p	R^2	p
Environmental						
%Mud	0.445	0.001	0.102	0.001	0.105	0.001
Width	0.150	0.001	0.103	0.010		
%Swoode	0.095	0.001	0.032	0.006	0.477	0.001
Cond	0.067	0.002			0.037	0.005
%SubRoots	0.058	0.003	0.024	0.015	0.035	0.001
pH	0.026	0.014	0.120	0.009	0.040	0.009
%Leafpacks	0.027	0.011	0.299	0.001	0.046	0.002
%Lwoode	0.021	0.019	0.069	0.002		
%FilAlgae	0.024	0.007	0.026	0.009		
%Cobble	0.037	0.001	0.023	0.003	0.043	0.016
%CanopyShade	0.019	0.001	0.073	0.006	0.016	0.002
O2	0.008	0.008	0.010	0.001	0.009	0.001
C	0.007	0.002	0.038	0.008	0.116	0.001
%Sand	0.005	0.002	0.026	0.046	0.031	0.01
S.T			0.025	0.046	0.020	0.003
%Grass			0.022	0.001	0.008	0.01
%OverhaTerrVeg					0.006	0.008
Spatial (AEM)						
V1	0.133	0.011			0.151	0.011
V16	0.117	0.011				
V25	0.101	0.013			0.097	0.021
V29	0.066	0.043			0.080	0.033
V21	0.064	0.033				
V12	0.063	0.031				
V10			0.108	0.034		

Table 3.5 Environmental and spatial variables selected by the *forward selection* procedure for the beta diversity and its components abundance difference and abundance replacement for abundance data (Ružička dissimilarity). Only statistically significant values (p< 0.05) are shown

	Beta diversity		Abundance Difference		Abundance Replacement	
	R^2	p	R^2	p	R^2	p
Environmental						
%.Mud	0.497	0.001	0.087	0.001	0.138	0.001
%.Swoode	0.224	0.001	0.016	0.003	0.472	0.001
Cond	0.064	0.003	0.017	0.008	0.135	0.002
%.SubRoots	0.032	0.014	0.067	0.004	0.033	0.013
%Leafpacks			0.289	0.004	0.026	0.016
%.Cobble			0.129	0.013	0.012	0.001
Width			0.106	0.007	0.038	0.013
pH			0.092	0.010	0.009	0.005
%Lwoode			0.048	0.030	0.006	0.019
O2			0.043	0.035		
%CanopyShade			0.036	0.041		
S.T			0.035	0.002	0.013	0.005
%Sand			0.014	0.001	0.027	0.020
%OverhaTerrVeg			0.011	0.001	0.034	0.001
%.Grass					0.016	0.047
%.FilAlgae					0.026	0.005
Spatial (AEM)						
V1	0.160	0.003			0.158	0.009
V16	0.134	0.006	0.120	0.021		
V21	0.081	0.031			0.077	0.031
V25	0.075	0.028			0.077	0.028
V12	0.072	0.021			0.075	0.018
V3	0.052	0.049			0.053	0.014
V29	0.049	0.045				
V23	0.046	0.031			0.100	0.009
V32	0.044	0.031			0.057	0.028
V11	0.036	0.035				
V22	0.033	0.049				
V30	0.031	0.030				
V18					0.105	0.021

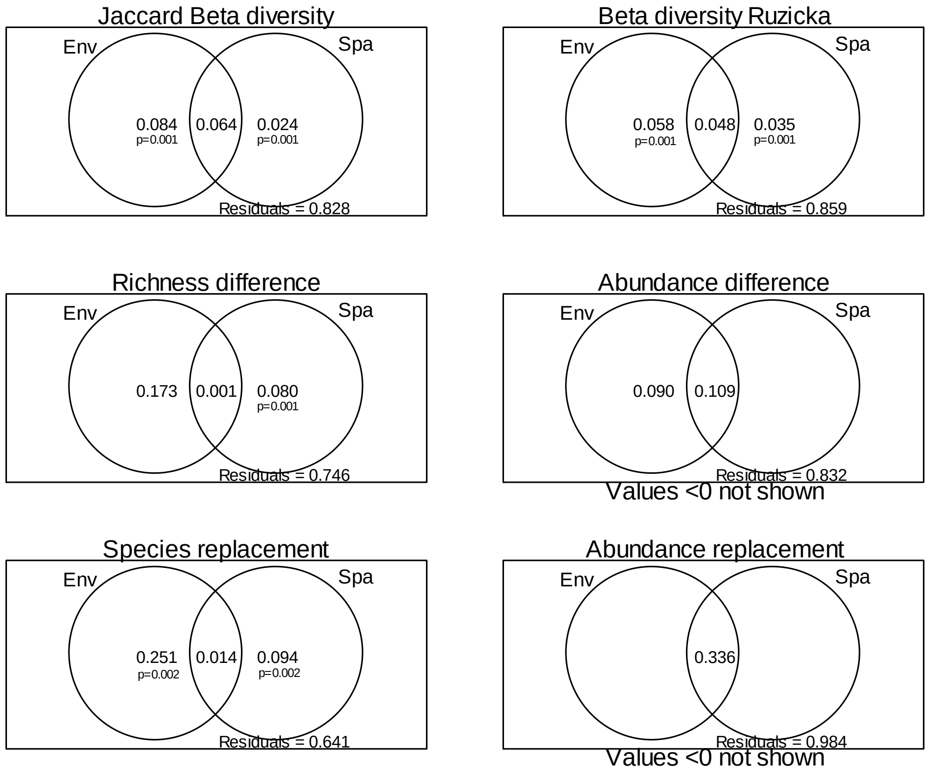

Figure 3.5 Variation partitioning analysis for β diversity and its components richness difference and species replacement for presence-absence (Jaccard dissimilarity) and abundance data (Ružička dissimilarity) and selected groups of environmental and spatial variables. Circles are not drawn to proportions, values represent the adjusted R^2, negative fractions values are not presented.

For the analysis based on abundance data, all components from the variation partitioning analysis (E, S, E+S, E|S, S|E) explained significant variation in β diversity ($P< 0.05$). Environmental factors explained 5.8% of the variation, the combined influence of environment and spatial factors explained 4.8%, and 3.5% was explained by spatial factors. No environmental or spatial variables were found to significantly influence abundance difference and replacement components of β diversity (Figure 3.5, Table 3.6).

Table 3.6 Variation partitioning analysis for beta diversity and its components (Species-abundance) difference and (Species-abundance) replacement for presence-absence (P/A-Jaccard dissimilarity) and abundance data (Ružička dissimilarity) and selected groups of environmental and spatial variables. AdjR^2= adjusted R^2 for the percentage of variation. p= p-value, significant values (P <0.05) are shown in bold. E= environmental variation, S= spatial variation, E+S= Total explained variation, E|S= Pure environmental variation, S|E= Pure spatial variation, b= Variation shared by environmental and spatial factors, R= Unexplained variation (Residual). Statistically significant values (p< 0.05) are shown in bold type.

Component	**Beta diversity**		**Difference**		**Replacement**	
	AdjR^2	p	AdjR^2	p	AdjR^2	p
Jaccar dissimilarity P/A						
E	0.148	**0.001**	0.174	0.182	0.265	**0.006**
S	0.088	**0.001**	0.081	**0.044**	0.107	**0.002**
E+S	0.172	**0.001**	0.254	0.12	0.359	**0.003**
E|S	0.084	**0.001**	0.173	0.122	0.251	**0.002**
S|E	0.024	**0.001**	0.080	**0.001**	0.094	**0.002**
b	0.064		0.001		0.014	
R	0.828		0.746		0.641	
Ružička dissimilarity Abundance						
E	0.106	**0.001**	0.199	0.160	0.166	**0.001**
S	0.084	**0.001**	0.078	**0.046**	0.185	**0.006**
E+S	0.141	**0.001**	0.168	0.269	0.016	0.204
E|S	0.058	**0.001**	0.090	0.282	-0.169	0.220
S|E	0.035	**0.001**	-0.031	0.271	-0.150	0.188
b	0.048		0.109		0.336	
R	0.859		0.83206		0.984	

3.4. Discussion

This study examined patterns of β diversity and its components, in the fish metacommunity of the Bita River at two different spatial levels. At the basin level, results from both presence/absence and abundance data showed that high β diversity was a consistent feature of fish metacommunities and was dominated by species and abundance replacement rather than richness difference, reflecting the importance of species turnover over along the longitudinal gradient. Similar results have been obtained for phytoplankton (Maloufi *et al.*, 2016), aquatic macroinvertebrate (Hill *et al.*, 2017), beetle (Tonkin *et al.*, 2016b), fish (Leprieur *et al.*, 2012), amphibian and reptile (Laurencio & Fitzgerald, 2010) bird, and mammal communities (Melo, Rangel & Diniz-Filho, 2009; Si *et al.*, 2016).

Variation in β diversity and its components that was explained by the pure environmental fraction was higher than the spatial and shared fractions, implying that environmental filters constrain species distributions and abundances, which is consistent with the metacommunity concept of species sorting (Córdova-Tapia, Hernández-Marroquín & Zambrano, 2018; Soininen, 2014). These findings support my first hypothesis that, at the basin level, environmental heterogeneity is high and species are able to select suitable environmental conditions, resulting in high β diversity driven by species replacement.

Heino, Melo & Bini (2015) found that, at the basin level, environmental factors control β diversity patterns when dispersal rates are intermediate, producing variability in species composition among sites (Chase & Leibold, 2003; Soininen, 2014). They also proposed that there is a positive β diversity-environmental heterogeneity relationship. Similar results were found in the present study, with most of the environmental variables selected being associated with habitat instream cover, reflecting the importance of habitat heterogeneity for fish metacommunity structure.

In the Bita River, habitat features apparently act as a filter that selects for particular combination of traits associated with locomotion, feeding, defense and reproduction. Some examples are *Dicrossus filamentosus, Elachocharax pulcher, Crenicichla wallacii* and

Nannostomus unifasciatus, species that principally occurred in habitats containing leafpacks and woody debris. Other species, such as *Eigenmannia macrops, Gymnorhamphichthys rondoni, Mastiglanis asopos, Amazonsprattus scintilla, Bivibranchia fowleri, Knodus cinarucoense,* and *Acestrocephalus sardina*, were found mainly on submerged sandbanks, habitats with homogeneous instream cover.

Variation partitioning analysis revealed different relationships between β diversity components and variables depending on the type of data analyzed. For presence/absence data, species replacement was significantly associated with both pure environmental and spatial factors. However, the richness difference was only significantly related with spatial factors, which could be related to the limited dispersal capabilities of certain species. This supports the idea that moderate dispersal is required for species to track suitable environmental conditions (Heino *et al.*, 2015). With regards to species abundance data, none of the β diversity components were significantly associated with environment or space. This, together with large unexplained variation found in all partitioning analyses, could be due to several reasons, such as the failure to include other relevant environmental and spatial predictors, or variables related to other processes such as biotic interactions and/or stochastic events (Chase, 2010; Devercelli *et al.*, 2016).

At the ecoregion level, I expected to find distinct patterns of β diversity derived from species abundance replacement and richness abundance difference depending on position within the river network. The headwaters metacommunity should have high β diversity driven mainly by species turnover. Within the downstream reach close to the river mouth, high dispersal rates (strong mass effect) should reduce β diversity as well as the turnover component (Ferreira *et al.*, 2018; Tonkin *et al.*, 2016a). However, these predictions were not supported by results obtained for fish assemblages in the four sections of the Bita River. β diversity was high in all river sections, with strong species and abundance replacement (turnover) rather than richness differences.

My results from analyses performed at the ecoregion level are consistent with the environmental control model proposed by Heino, Melo & Bini (2015), which assumes that species sorting prevails across multiple scales when dispersal rates are insufficient to overwhelm the environmental filtering process. These authors also stated that when communities are dominated by organisms with limited dispersal rates and short dispersal capabilities, a strong positive β diversity-environmental heterogeneity relationship should be detected at different spatial scales, which is in accordance with the results obtained for Bita River fishes. De Bie *et al*., (2012) also reported strong species sorting in small freshwater organisms and concluded that this was associated with their demography (short generation times, rapid population growth potential) and dispersal limitation.

Fish assemblages in near-shore habitats of the Bita River were dominated by small species, such as *Amazonprattus scintilla* (Engraulidae), *Hemigrammus elegans, H. geisleri, H. analis* and *Hyphessobrycon diancistrus* (Characidae). Species that tend to occupy areas with deeper water, for instance large catfishes (Pimelodidae., Doradidae) and croakers (Sciaenidae) as well as those with body shapes that have low susceptibility to capture in gillnets (e.g., stingrays (Potamotrygonidae), could have been underestimated in my samples. However, my surveys captured nearly 80% of all fish species reported from the Bita River Basin (Trujillo & Lasso, 2017) and 30% of all fish species reported for the Colombian Orinoco hydrographic region (DoNascimiento et al., 2017). Similar species richness has been reported for other rivers in the region, including the Casiquiare (174 spp.), Arauca (191 spp.), Tomo (282 spp.) and Cinaruco

(238 spp.) (Lasso et al., 2004; Lasso, Machado-Allison & Taphorn, 2016; Maldonado-Ocampo et al., 2006).

My surveys were conducted during the annual low-water period when fish dispersal opportunities are lowest, conditions that should promote β diversity via environmental control *sensu* Heino, Melo & Bini (2015). Different patterns might be obtained during the wet season when the river floods vast areas to create a complex of aquatic habitats with high lateral and longitudinal connectivity. In Venezuela's Cinaruco River, a Llanos river very similar to the Bita River, Arrington & Winemiller (2006) found that composition of fish assemblages in structurally complex habitats was nonrandom during the low-water period, but during the period of rising water, fish assemblages reflected a strong influence of stochastic colonization dynamics. The annual flood pulse promotes fish dispersal, frequent restructuring of fish assemblages over variable spatial scales, a tendency toward homogenization of assemblage composition, and a decline in β diversity. Gutiérrez, Ortega & Agostinho (2018) evaluated the effects of environmental heterogeneity and floods on fish β diversity in a floodplain reach of the Upper Paraná River and found that for species with high dispersal capabilities, there was no clear relation between the flood pulse, environmental heterogeneity and β diversity. Only those fishes with limited dispersal rates, especially substrate-nesting species with brood care, revealed patterns associated with hydrological and environmental variation.

Large LCBD values indicate local assemblages (sites) with strongly divergent species composition compared with the regional average. Relatively undisturbed habitats supporting these local assemblages are particularly important for biodiversity conservation because of their unique features that support species with limited distributions in the riverscape. Conversely, some sites with large LCBD values may represent divergent assemblage structures that reflect impacted habitats that may be candidates for ecological restoration (Legendre, 2014). In this system, large values were observed in the two middle sections of the Bita River, and this was the case for both data types. Most of these sites had very high species richness, but a few sites had relatively low species richness and fish abundance compared with sites in the upper and lower sections. Sites with high species richness might explained by the transitional nature of the fluvial landscape within the middle sections where the elevational gradient and flow velocity decline and channel meandering increases, creating extensive aquatic habitat in floodplains with seasonally fluctuating connectivity (Schlosser, 1982). Sites that yielded low species richness and fish abundance might have been influenced by sampling bias or human impacts, such as fishing or watershed impacts, although I found little evidence of overfishing or habitat alteration in any of the sampling sites.

Regardless of the regional unit level (basin or within sections) or type of assemblage data (presence/absence vs. abundance), species sorting appeared to have a strong influence on β diversity and species turnover in the Bita River during the dry season, a finding consistent with other studies of riverine fish metacommunities (Bini *et al.*, 2014; Cottenie *et al.*, 2003; Heino *et al.*, 2012; Hill *et al.*, 2017; Soininen, 2014; Roa-Fuentes *et al.*, 2019). Spatial variables were only weakly associated with β diversity and its components. The strong pattern of species turnover in the Bita River, found at both regional unit levels, suggests that conservation strategies for this system should focus on protecting regions that encompass divergent species assemblages and habitat features.

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4. FISH FUNCTIONAL DIVERSITY CHANGES ALONG THE LONGITUDINAL GRADIENT OF A SPECIES-RICH TROPICAL RIVER

4.1. Introduction

Biodiversity is a multifaceted concept that encompasses variation across a broad hierarchical spectrum from genes to species and regional biotas, as well as the ecological and evolutionary processes that maintains this variation (Mouchet et al., 2010). A long standing challenge for ecologists is to identify and understand mechanisms that create and maintain biodiversity at various scales. In the past, most biodiversity studies have focused on classical diversity measures, such as species richness (e.g., Shannon index) and evenness (e.g., Pielou index). However, it is well known that this species-based approach provides an incomplete view of biodiversity and is insufficient to understand how biodiversity affects ecosystem functions (Mouillot et al., 2011; Villéger, Grenouillet & Brosse, 2013).

One way to address the larger question of how does biodiversity influence community function is to apply a trait-based approach to study the functional organization of communities. According to Villéger et al. (2017) scientific publications based on a functional approach in community ecology have increased in recent years, especially for plants, fishes, and birds. Functional ecology examines patterns and processes involving features of organisms that affect performance and fitness (Villéger et al., 2010). Functional ecology also provides insights into the roles of different species within a particular ecosystem (Teresa & Casatti, 2012) as well as an effective means to make spatial and temporal comparisons and to examine how organisms respond to environmental gradients, disturbances, and biotic interactions.

Ecological communities can be assembled through stochastic or deterministic processes such as niche filtering or limiting similarity (Mouillot, 2007). Under the niche filtering mechanism, environmental factors (i.e., abiotic environmental parameters, habitat features) only allow establishment and persistence of species with appropriate trait combinations, resulting in low functional diversity and high functional redundancy (i.e., trait under-dispersion within the community). Conversely, under limiting similarity, biotic interactions, such as competition and predation, constrain the trait combinations that are able to coexist, resulting in patterns of high functional diversity (i.e., trait over-dispersion within the community) (Cadotte & Tucker, 2017; Mouillot, 2007; Mouillot, Dumay & Tomasini, 2007; Mouchet et al., 2010; Mouillot et al., 2013). Environmental filtering and limiting similarity have been studied along environmental gradients at various spatial scales and in ecosystems involving various taxa, including terrestrial snails (Astor et al., 2014), subtropical forests (Liu et al., 2013), Neotropical birds (Arruda Almeida et al., 2018), amphibians (Dayton & Fitzgerald, 2001; Riemann et al., 2017), macroinvertebrates (Brown & Swan, 2010) and tropical and temperate fishes (Casatti et al., 2015; Teresa & Casatti, 2012; Vitorino Júnior et al., 2016; Villéger, Grenouillet & Brosse, 2013).

Functional diversity measures how traits (morphological, physiological and phenological) vary among species in a community (Maire et al., 2015), but it also can be used to estimate patterns of trait variation that may influence community assembly and ecosystem processes. Since the early 2000s, several functional diversity metrics have been proposed, some of which are based on multiple traits and some able to incorporate species abundance (Villéger, Mason & Mouillot, 2008). Some indices are influenced by species richness, and this has stimulated considerable discussion about how to account for this interaction (Cardoso et al., 2014; Villéger, Mason & Mouillot, 2008; Villéger et al., 2010). Villéger, Mason & Mouillot, (2008) and Laliberté & Legendre (2010) devised four metrics to estimate different aspects of functional diversity; functional richness, functional evenness, functional divergence and functional dispersion. These distinctions can help to disentangle community assembly mechanisms along spatial and temporal environmental gradients. Teresa & Casatti (2017) evaluated the potential use of several functional diversity indices as bioindicators, and found that these four metrics were good predictors of environmental degradation.

Studies of dissimilarities in species composition (taxonomic β diversity) have been widely used in ecology and biogeography to investigate biodiversity patterns, community assembly rules, and longer term evolutionary and ecological processes (Baselga, 2012; Legendre, 2014; Podani, Ricotta & Schmera, 2013). New metrics of functional diversity have recently been developed to evaluate the level of trait variation among communities (Cardoso et al., 2014; Swenson, 2011; Villéger et al., 2012). There is much research on taxonomic β diversity, and now there is growing interest in how functional β diversity changes along spatial and temporal gradients (Cilleros et al., 2016). Research on functional β diversity has the potential to reveal community assembly mechanisms and provide insights into how species influence ecosystem structures and functions (Villéger et al., 2012).

Two assemblages can differ in species composition (high species turnover) but be very similar in terms of functional traits (low functional turnover). Functional β diversity provides an effective means to reveal such patterns. Similar to taxonomic β diversity, functional β diversity can be partitioned into functional turnover and functional nestedness (Baselga, 2012; Villéger, Grenouillet & Brosse, 2013). Functional turnover measures the overlap within the functional space between two communities based on the convex hull volume, whereas functional nestedness represents the case when one community fills only a small subset of the functional spaced filled by the other community (Villéger, Grenouillet & Brosse, 2013). To date, two frameworks have been proposed to decompose overall functional β diversity. One is based on the functional richness (FRic) index, a measure of the convex hull volume of two communities (Villéger, Mason & Mouillot, 2008; Villéger, Grenouillet & Brosse, 2013), and the other is based on functional clustering trees, in which functional β diversity between two communities is calculated using the distances of the branch length (Cardoso et al., 2014; Loiseau et al., 2017; Swenson, 2014).

High functional β diversity can be due to a high functional turnover, when species from two communities have different sets of traits combinations or when there is a low functional turnover and high functional nestedness, because the species in one community represent a small subset of the functional space occupied by other community. On the other hand, low functional β diversity would be observed when species from two communities, despite being taxonomically different, have similar functional trait distributions (Villéger, Grenouillet & Brosse, 2013). Functional diversity is predicted to be associated with mechanisms for species coexistence. For instance, if assemblages are strongly influenced by environmental features (environmental niche filtering), species should be functionally similar with low functional diversity and high functional turnover. Conversely, if competition is strong (limiting similarity), only species with divergent trait combinations would coexist and functional diversity would be high, with functional beta diversity depending on the functional composition of local assemblages (Mouchet et al., 2010). Dispersal limitation is another mechanism proposed to explain functional beta diversity patterns (Cilleros et al., 2016). When dispersal rates are high and environmental filtering is null (neutral dynamics), both taxonomic and functional β diversity will be low

(Weinstein et al., 2014). In contrast, when dispersal limitation is high and environmental variation is low, local communities should assemble independently, with high species turnover and low functional β diversity (Cilleros et al., 2016).

Research on functional β diversity is relatively sparse compared to studies of taxonomic β diversity (Villéger, Grenouillet & Brosse, 2013), and studies decomposing functional β diversity are even less common. Some studies have considered three facets of biodiversity; for instance, Weinstein et al. (2014) and Pool, Grenouillet & Villéger (2014) evaluated taxonomic, phylogenetic and functional β diversity patterns. Variation in taxonomic and functional β diversity has been examined in tropical freshwater (Cilleros et al., 2016) and estuarine fishes (Villéger et al., 2012), temperate freshwater fishes (Villéger, Grenouillet & Brosse, 2013), and montane ant communities (Bishop et al., 2015). Recently, Vitorino Júnior et al. (2016) evaluated the influence of the river network on patterns of taxonomic and functional β diversity of Neotropical fishes.

In this study, I used a functional diversity approach to examine the potential influence of environmental filtering, limiting similarity and dispersal processes in shaping fish community structure along the longitudinal fluvial gradient of the Bita River Basin, a nearly pristine river in the Llanos region of Colombia. My goal was to test if spatial scale and position within the river channel would have an effect on functional diversity patterns. I hypothesized that, at the basin scale, functional α diversity should be low and functional β diversity high and driven by functional turnover due to environmental niche filtering along the longitudinal gradient (Cardoso et al., 2014; Kluge & Kessler, 2011). At a finer scale, functional α and β diversity patterns should reveal different patterns depending on the position within the river network. In headwaters, I proposed that, due to dispersal limitation and the influence of abiotic factors, communities should be structured by environmental filtering. Consequently, functional α diversity should be low and functional β diversity should be high, because patches that are close to each other might exhibit very different environmental features. In contrast, downstream areas where connectivity is higher (close to river mouth) and environmental conditions are more predictable with more gradual changes, biotic interactions (limiting similarity) should have a stronger influence on assemblage structure, resulting in high functional α diversity and low functional β diversity because patches are environmentally similar and highly connected.

4.2. Methods

4.2.1. Data collection

Fish surveys were performed across the longitudinal gradient (700 km) of the Bita River Basin, a free-flowing river recently declared as a wetland of international importance under the Ramsar convention (World Wide Fund For Nature, 2018). The Bita River Basin drains an area of 812.312 ha before flowing into the Orinoco River. The regional climate is tropical with a mean annual precipitation of 2,300 mm and mean temperature of 28°C (Trujillo & Lasso, 2017). The river's hydrology is strongly seasonal with two well-defined seasons, a dry period with low flows during December to March, and a wet period with high water levels during April to November (Arrington, Winemiller & Layman, 2005; Trujillo & Lasso, 2017). During the dry season, aquatic habitats are restricted to the river mainstem and lateral channels, creeks, and lakes in the floodplains. Substrates in the main channel are dominated by sand, and in floodplain habitats substrates are composed of sand, leafpacks and woody debris. In the wet season the river floods adjacent areas of forest and savannas increasing aquatic habitat volume and connectivity.

Fishes were collected during the dry season when low-water conditions improve the efficiency of the sampling methods (López-Delgado, Winemiller & Villa-Navarro, 2019; Pease et al., 2012). To study changes within the river network, I divided the watershed into four sections of equal length (high, mid-high, mid-low, low). A total of 34 sites were surveyed (Figure 4.1) using a seine (10 x 1.5 m, 3-mm mesh) and two gill nets (10 x 2 m, 100-mm mesh). In each survey site, a 200-m reach was assessed performing six hauls of 20 m each and two hours/site per gill net. Surveys yielded a total of 25.928 specimens representing 201 species (López-Delgado, Winemiller & Villa-Navarro, 2019) that were deposited in the voucher collections of the Instituto von Humboldt and the Universidad del Tolima in Colombia.

4.2.2. Functional traits data

Functional traits were measured on 3-10 individuals of 132 species that had more than three specimens (Figure 4.2, Appendix C) yielding a total of 2,219 specimens measured. Juvenile fish were not used in order to avoid ontogenetic variation. Traits were classified into functional categories, reflecting three niche dimensions (habitat, defense and feeding) sensu Winemiller et al. (2015) (Appendix D). Twenty morphological traits were related to locomotion and habitat use, four to defense, and 13 to feeding (Appendix D). Traits were measured following the methodology proposed by Gatz (1979) and Winemiller (1991) using calipers to the nearest 0.1 mm. As proposed by Su, Villéger & Brosse (2018), values were set to 0 for traits that were inapplicable or difficult to locate in particular species (e.g., dorsal fin in knifefishes and eels).

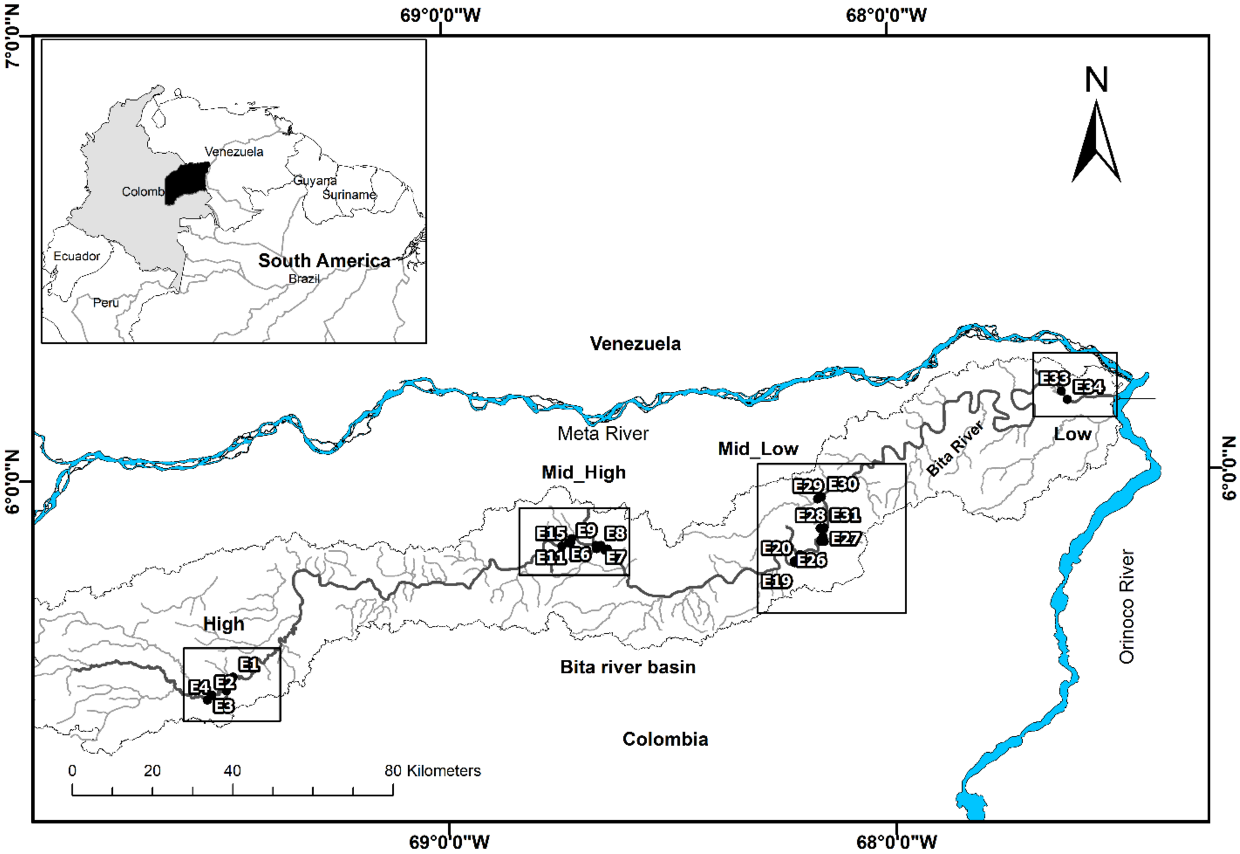

Figure 4.1 Location of the 34 sites sampled within the Bita river Basin. Rectangles represent each section sampled following the river longitudinal gradient.

4.2.3. Abiotic environmental factors

Environmental and spatial variables were recorded at each survey site. Environmental variables were measured following Pease et al. (2012) and were divided into six categories that describe physical and chemical features of aquatic habitats (Table 2.1). Water quality parameters were measured using a multiparameter probe (YSI model 85), substrate and instream cover were estimated visually along a 200-m reach, and variables associated with the riparian area and landcover were estimated using georeferenced satellite images and ArcMap (Version 10.3.1). Spatial patterns were modelled using Asymmetric Eigenvector Maps, connection diagrams that link survey sites across the entire river network, and variables obtained from this analysis were used as proxies for dispersal (see López-Delgado, Winemiller & Villa-Navarro (2019) for a complete description of the methods to measure environmental parameters and to model spatial variables).

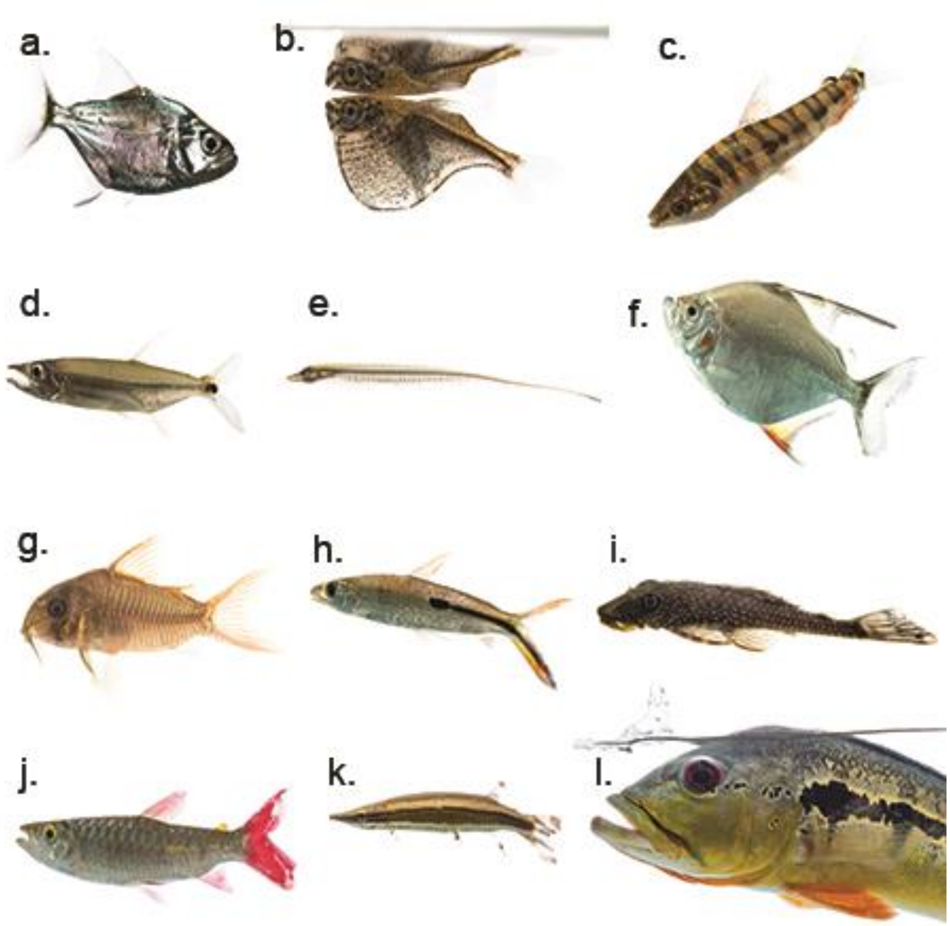

Figure 4.2 Representative examples of fishes included in this study. A. *Serrasalmus sp.* b. *Carnegiella marthae,* c. *Leporinus fasciatus,* d. *Acestrorhynchus minimus,* e. *Gymnorhamphichthys rondoni,* f. *Catoprion mento,* g. *Corydoras sp,* h. *Hemiodus semitaeniatus,* i. *Dekeyseria scaphirhyncha,* j. *Chalceus macrolepidotus* , k. *Boulengerella lateristriga,* l. *Cichla intermedia.*

4.2.4. Data Analysis

To determine if functional diversity patterns differed depending on the spatial scale and position within the river network, analyses were performed at both the basin scale (34 sites) and regional scale (four sections).

Functional Diversity

In total, 26 continuous and 10 categorical traits (Appendix D) were measured for each species. Continuous traits were converted to unitless ratios to serve as shape components of the body and head, as recommended by Winemiller (1991). This standardization reduces the chance that species ordination would be dominated by a few traits (i.e. body length). To calculate functional diversity indices, I used the mean of the standardized trait values for each species. Functional distance matrix between species was calculated using Gower's distance because this allows for use of different type of traits (categorical, ordinal, numeric) (Villéger, Grenouillet & Brosse, 2013). Principal coordinate analysis (PCoA) was performed on the functional distance matrix to obtain species coordinates in multidimensional trait space. Because PCoA produces as many axes as the number of species minus one, it is important to select those axes that truly reflect the original functional variation between species (quality of functional space). Therefore, I applied Maire et al.'s (2015) method to calculate the quality of the functional space that best represent the functional information. Following a tradeoff between quality and computational time, I used the species coordinates from the first three PCoA axes to calculate functional α and β diversity indices. These analyses were performed using the function quality_funct_space in r (R Core Team, 2017) available in the appendix S1 from Maire et al. (2015).

To compare changes in community assembly within the river network, I compared functional α diversity indices among the river sections. I used six functional diversity metrics that according to Mouchet et al. (2010) are good predictors of assembly processes. Functional richness (FRic) was measured as the proportion of the functional space occupied by the species in a community. Functional divergence (FDiv) is the deviation of species abundance to the center of the functional space occupied by the species pool. Functional evenness (FEve) is the evenness of the species abundance in the functional space. Functional dispersion (FDis) is the mean distance to the centroid of the species present in the community divided by half the maximum distance among all the species in the set of assemblages. Functional specialization (FSep) is the mean distance to the centroid of the species pool. Functional originality (FOri) as the mean distance to the closest neighbor from the species pool divided by the maximum distance to the nearest neighbor (Córdova-Tapia, Hernández-Marroquín & Zambrano, 2018; Mouillot et al., 2013). These indices were calculated using the function multidimFD in r available from Villeger (2017). In addition, functional redundancy was calculated according to the method proposed by Pillar et al. (2013) using the Gini-Simpson index minus the Rao's quadratic entropy in r. The Kruskal-Wallis test was used to compare differences in functional α diversity metrics among river sections.

To test which of the environmental filtering and limiting similarity hypotheses best explain community assembly, I used a null model to identify whether the observed values for functional □ diversity metrics differed from random expectations. This was calculated by generating 999 random community matrices using the independent swap algorithm and recalculating functional □ diversity metrics. Standardized effect sizes (SES) were calculated as a measure of departure from random expectation; values greater than 0 indicate that the observed value is greater than expected by chance (trait over dispersion–limiting similarity), and values smaller than 0 indicate observed is less than expected (trait underdispersion indicating environmental filtering) (Mouillot, Dumay & Tomasini, 2007). Statistical significance of dispersion patterns was assessed using a bilateral test; p-values greater or equal to 0.975 and less than or equal to 0.025 were considered significant (Swenson, 2014). Randomizations were conducted using the function randomizeMatrix in the package picante in r (Kembel et al., 2010), SES and p-values were calculated according to Swenson (2014).

To calculate pairwise functional β diversity, I used the convex hull volume of species and measured the overlap in the functional space between two assemblages; a value equal to one indicates that there is no overlap between two assemblages, and a value equal to 0 that there is complete overlap. This method was proposed by Villéger, Grenouillet & Brosse (2013) as an analogy of taxonomic β diversity proposed by Baselga (2012). Functional β diversity was then calculated based on the Jaccard dissimilarity index and partitioned into functional turnover and functional nestedness-resultant dissimilarity components using the function functional.beta.pair in the package betapart in r. To identify possible differences in mean functional β diversity and its components among river sections, I used a test of homogeneity of dispersion PERMDIST (Anderson, Ellingsen & McArdle, 2006), and then Tukey's HSD was applied to identify significant pair-wise differences among river sections. Analyses were conducted using the function betadisper from the vegan library in r.

I tested if functional β diversity values differ from random expectations following the method proposed by Swenson (2014). The community data matrix was randomized 999 times using the independent swap algorithm, pairwise functional dissimilarity between each pair of communities was calculated for each iteration, and then a two-tailed test was performed, with p-values greater or equal to 0.975 indicating that the observed value was were greater than expected by chance, and an observed value less than or equal to 0.025 indicated a value lower than expected by chance. Analysis were performed in r using the function randomizeMatrix from the library picante.

Explaining variation in functional β diversity along environmental and spatial gradients

To test whether variation in functional dissimilarity matrices (functional β diversity, functional turnover, functional nestedness) between pairs of local assemblages was best explained by environmental or spatial factors, I used distance-based redundancy analysis (db-RDA) and a variation partitioning method (Legendre, 2014; Peres-Neto et al., 2006). To select the environmental and spatial variables to include in the variation partitioning analysis, first a db-RDA was performed using each of the dissimilarity matrices and the environmental and spatial variables, and then a forward selection procedure was applied on the set of principal coordinates produced by the db-RDA. Environmental variables were transformed and standardized (mean=0; standard deviation=1) prior to analysis; all variables expressed as proportions were transformed to the arcsine of their square root, and the remaining variables (except categorical) were log(x+1) transformed. Selected variables (environmental and spatial) by the forward selection procedure were used in the variation partitioning analysis. Statistical significance of each component from the variation partitioning was assessed based on an ANOVA-like permutation (999 permutations; $\alpha=0.05$). Analyses were performed in r using the functions forward.sel from the adespatial package (999 permutations; $\alpha = 0.05$), dbRDA.D from appendix S4 in Legendre (2014) and varpart and anova from the vegan package.

4.3. Results

In general, values of functional diversity metrics were low (min 0.14; max:0.46) (Table 4.1, Appendix E), with the exception of functional divergence (mean ± SD; 0.74±0.1) and functional redundancy (mean ± SD; 0.75±0.14). Functional richness per section ranged from 19 to 32 % of the complete functional space filled by the species pool (132 species), with a mean of 27 ± 0.22% (Table 4.1). Highest values of diversity metrics were observed in the two middle sections; however, these were not significantly different among river sections (Figure 4.3, 4.4).

Standardized effect size values from the null model analysis revealed that trait underdispersion was the dominant pattern throughout the entire basin. However, for most of the diversity metrics, observed values were not different from random expectations, with the exception of functional divergence (FDiv), in which 91% of the values were significantly different from random (Appendix E).

Table 4.1 Functional α diversity metrics (mean±SD) for fish assemblages based on functional trait analyses that included samples for the entire Bita River Basin and analysis for the four sections separately.

Scale	FRic	FDiv	FEve	FDis	FSpe	FOri
Entire Basin	0.27±0.22	0.74±0.12	0.43±0.15	0.33±0.15	0.36±0.09	0.16±0.07
High	0.19±0.2	0.73±0.12	0.43±0.22	0.28±0.14	0.36±0.05	0.17±0.1
Mid-high	0.24±0.22	0.78±0.13	0.46±0.13	0.32±0.17	0.38±0.1	0.16±0.09
Mid-low	0.32±0.21	0.71±0.08	0.41±0.13	0.35±0.13	0.36±0.06	0.16±0.03
Low	0.27±0.17	0.74±0.06	0.39±0.06	0.3±0.1	0.33±0.04	0.14±0.02

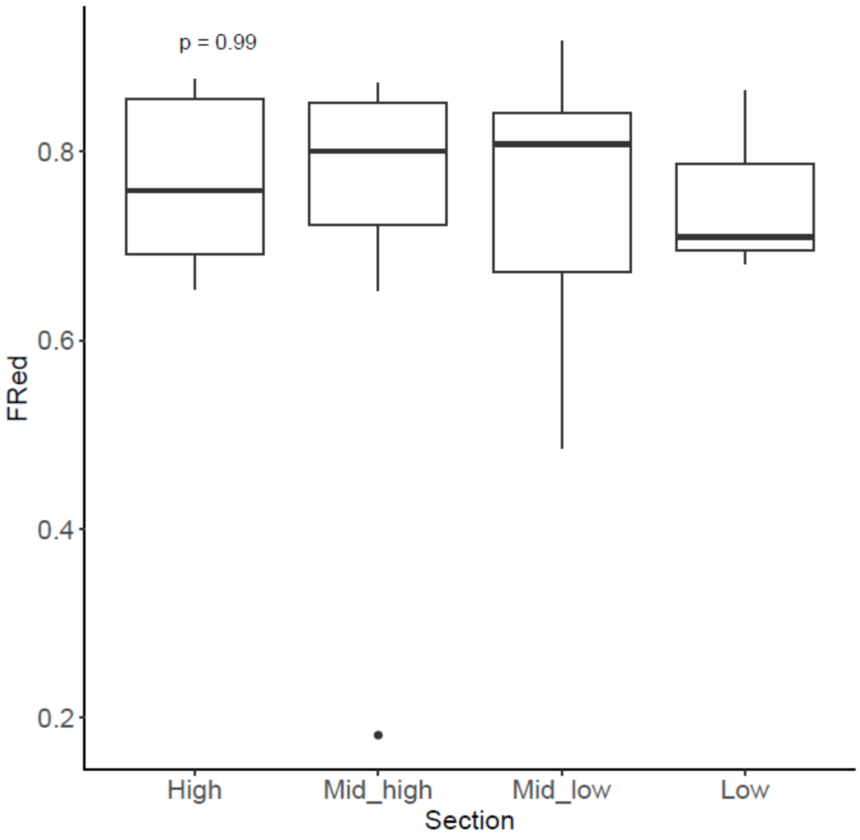

Figure 4.3 Comparison of the functional redundancy of the local assemblages in four sections of the Bita River Basin.

Functional β diversity and its components

At the basin scale, functional β diversity ranged from 0.25 to 1, with a mean value of 0.74±0.19 (Table 4.2). Functional turnover contributed 43%, whereas the functional nestedness-resultant component was 57% (Table 4.2). At the finer scale, functional β diversity generally was high, with values greater than 0.64 and the nestedness-resultant component dominating functional β diversity in all four river sections (Table 4.2). According to results from PERMDISP analysis, only functional turnover was significantly different among the river sections (p=0.001). The Tukey HSD test revealed that the low section was significantly different from the mid-high section, and the mid-low and mid-high sections also were significantly different (Appendix F). Null model analyses revealed that 149 of the 561 observed pairwise values of functional β diversity were significantly greater than expected by chance, 47 were significantly lower, and 365 pairs (65%) were not significantly different from random

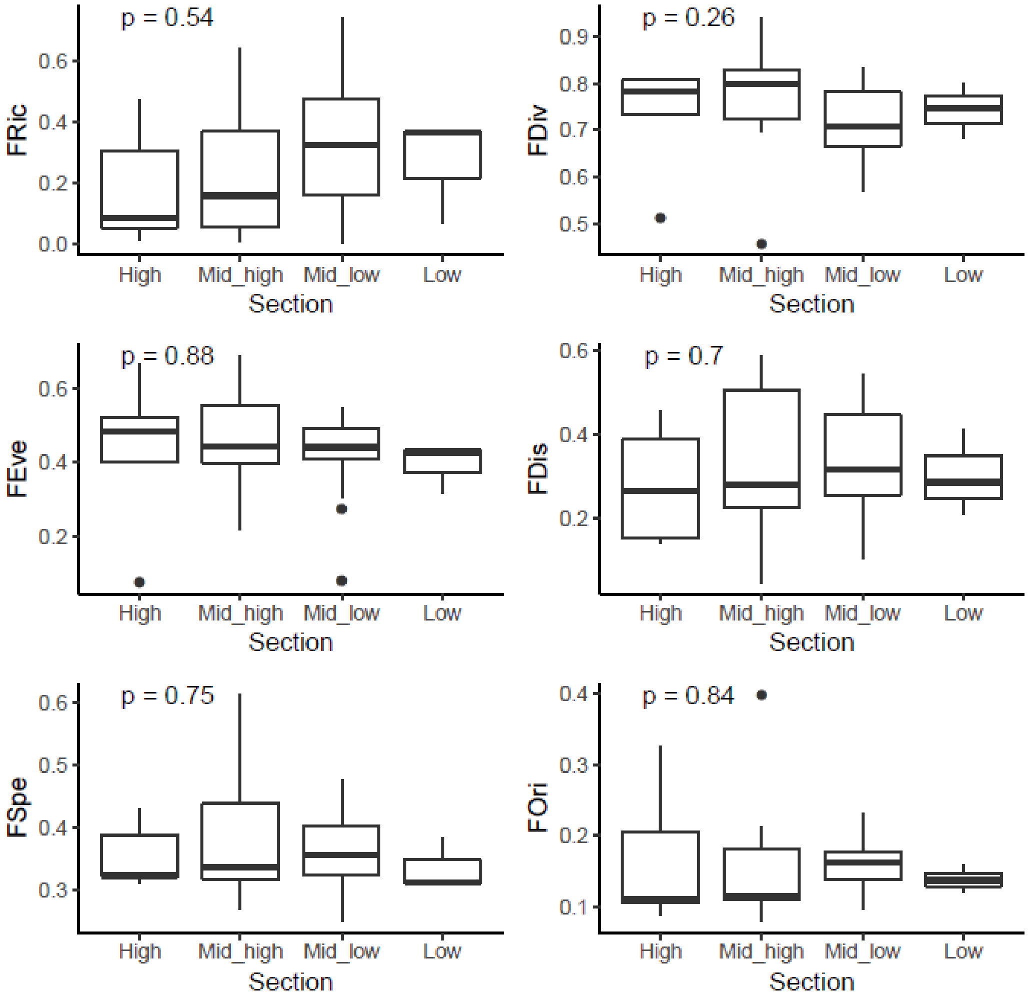

Figure 4.4 Comparison of functional diversity indices of local fish assemblages in four sections of the Bita River Basin. Functional richness (FRic), functional divergence (FDiv), functional eveness (FEve), functional dispersion (FDis), Functional specialization (FSpe) and functional originality (FOri). P-values based on the Kruskal-Wallis test.

Table 4.2 Mean values ± standard deviations of functional beta diversity (FBeta), functional turnover (FTurn) and functional nestedness-resultant (FNes) calculated at the entire basin and each section. Fpturn refers to the contribution of turnover to functional beta diversity (Fpturn=FTurn/FBeta).

Scale	FBeta	FTurn	FNes	Fpturn
Entire basin	0.74±0.19	0.30±0.27	0.43±0.31	0.43±0.34
High	0.83±0.17	0.37±0.29	0.45±0.29	0.45±0.31
Mid-high	0.80±0.17	0.40±0.32	0.40±0.30	0.49±0.35
Mid-low	0.68±0.20	0.23±0.18	0.45±0.30	0.38±0.32
Low	0.65±0.30	0.23±0.06	0.42±0.29	0.48±0.43

Functional β diversity and abiotic variables

The forward selection procedure selected four environmental and four spatial variables that modeled functional β diversity (Appendix G). For functional turnover, six environmental and one spatial variable were selected; and for functional nestedness-resultant, five environmental and four spatial variables were selected (Appendix G). Most of the selected environmental variables were associated with habitat features. Results from the variation partitioning analysis showed that all fractions (E, S, E+S, E|S, S|E) explained significant variation in functional β diversity and its components (Appendix H). The percentage of variation explained by the pure spatial fraction was higher than the pure environmental fraction for functional β diversity and functional nestedness (10.6% and 19.9%, respectively) (Figure 4.5). Conversely, 43% of the variation in functional turnover was explained by the pure environmental fraction, and 11.6% was explained by the spatial fraction (Figure 4.5).

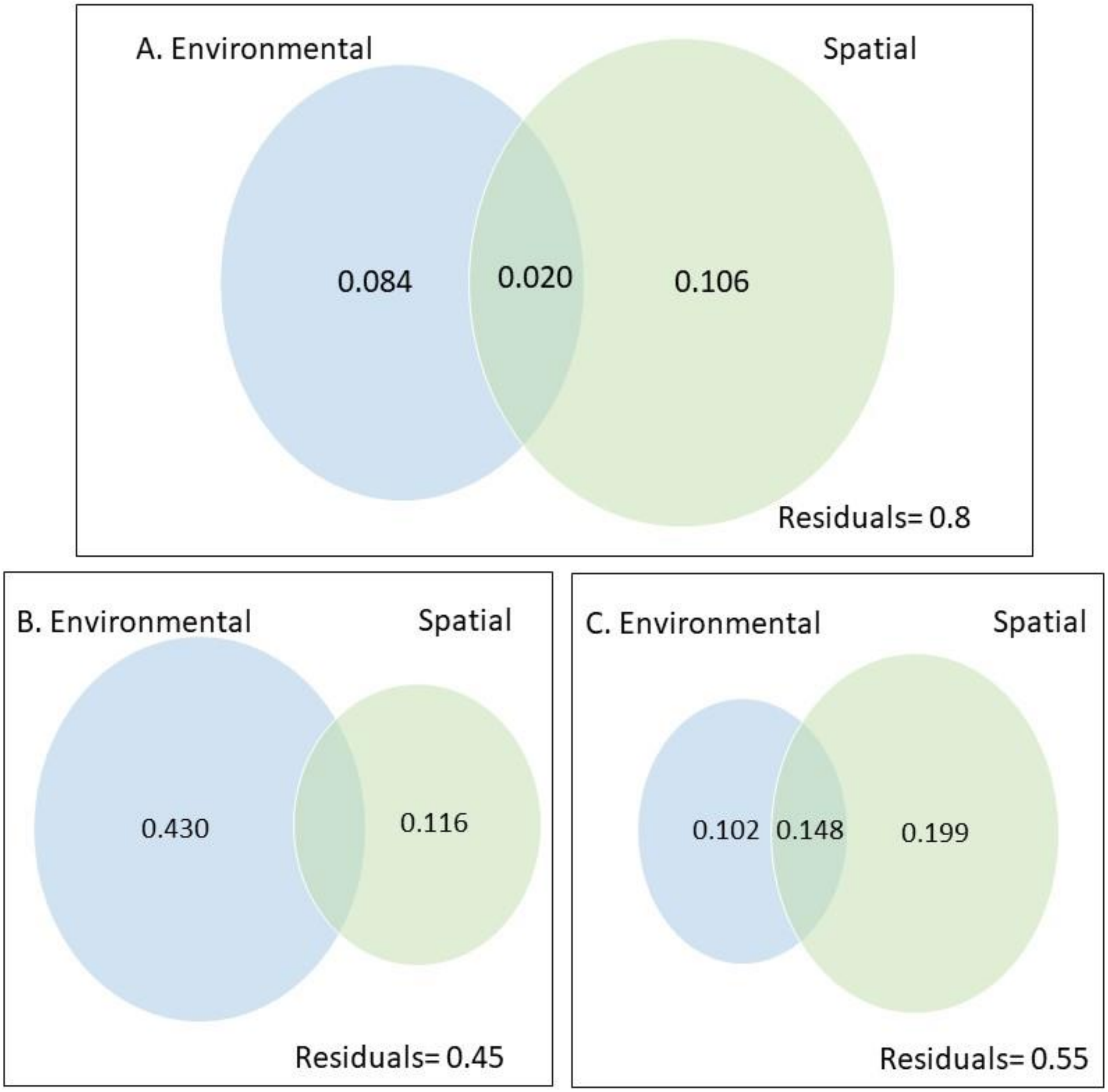

Figure 4.5 Variation partitioning analysis for functional β diversity and its components functional turnover and functional nestedness for selected groups of environmental and spatial variables. Circles are not drawn to scale, values represent the adjusted R^2, negative fractions values are not shown.

4.4. Discussion

In this study, my objective was to identify the processes structuring fish communities at two spatial scales using a functional traits approach. Results from both scales of analysis showed functional richness was low and functional β diversity was high and driven mostly by functional nestedness, reflecting the importance of environmental filtering at both scales. These results partially confirm my initial prediction. At the basin scale, I expected low functional α diversity and high functional β diversity driven by functional turnover. However, results showed that functional nestedness was the dominant pattern. Low values of functional α diversity metrics along the longitudinal gradient reflect trait underdispersion, suggesting that local assemblages are being filtered by the environment and only species with similar trait combinations are able to colonize and persist in a given habitat (Mouillot, 2007), indicating high functional redundancy within assemblages. These findings are consistent with that of Casatti et al. (2015) who found high functional redundancy in species-high tropical rivers.

At a finer scale, I expected to find distinct patterns of functional diversity depending on position within the river network. Headwater assemblages should be more strongly influenced by environmental filtering and have low functional α diversity and high functional β diversity and functional turnover (Vitorino Júnior et al., 2016). Conversely, downstream assemblages should be influenced by biotic interactions to a greater degree owing to high connectivity (facilitating high dispersal and species sorting) resulting in higher functional α diversity. Overall, my results only support the hypothesis regarding headwater assemblages; environmental filtering was the dominant pattern no matter the position within the river network. Evidence was lacking to support a strong influence of biotic interactions on downstream assemblages. Several studies have shown that factors influencing community structure vary according to spatial scale (Brown, Fox & Kelt, 2000; Mason *et al.*, 2008; Vitorino Júnior *et al.*, 2016); however, my results did not reveal significant differences in functional diversity for analyses performed at basin-wide versus reach scales.

Results from my study are consistent with the environmental control model proposed by Heino, Melo & Bini (2015), which assumes that environmental filtering prevails at multiple spatial scales when communities are dominated by species with limited dispersal rates. Several studies have found that environmental filtering constrained both species occurrence and abundance. As a result, the most abundant species were expected to share similar trait combinations that allowed them to tolerate conditions imposed by the environment (Cadotte & Tucker, 2017). This idea was supported by my results for the Bita River; for example, small characids were by far the most abundant and diverse fishes (López-Delgado, Winemiller & Villa-Navarro, 2019), and they shared similar traits including limited dispersal ability, omnivorous feeding habits, and life history strategies.

Evidence for environmental filtering is extensive in the literature (Cadotte & Tucker, 2017; Kedy, 1992; Kluge & Kessler, 2011; Lamanna et al., 2014; Thakur & Wright, 2017). This community assembly mechanism can result in trait under-dispersion and low functional richness (Mouillot, Dumay & Tomasini, 2007). In the Bita River, low functional richness and high functional redundancy were consistent patterns throughout the longitudinal fluvial gradient, suggesting that despite being a species-rich system, locally co-occurring species have relatively consistent sets of functional traits (Clementsian distribution). Which are defined in this study as any traits related directly with species performance (Mouillot et al., 2013). Within particular types of habitats, fishes with similar traits should perform similar functions, even if the taxonomic composition of these groups varies (see discussion chapter 3 for the species associated with habitat features). In fact, high functional redundancy has been found in several Neotropical systems (Casatti et al., 2015) and this often is inferred to promote community and ecosystem stability and resilience (Cadotte, Carscadden & Mirotchnick, 2011). High levels of functional redundancy reduce the probability that local extinctions affect ecosystem functions (Fonseca & Ganade, 2001). In a global biodiversity assessment performed by Toussaint et al. (2016), it was found that the loss of threated species in the Neotropics might result in a limited loss of functional diversity (but see comments by Vitule et al., 2017).

High functional β diversity along the entire basin and within river sections probably reflects the fact that this fluvial system is highly heterogeneous with a mosaic of in-channel and floodplain habitats. This habitat heterogeneity undoubtedly influences fish assemblage structure, resulting in high functional turnover associated with the environmental component. Such patterns may reflect adaptation to different selection pressures within local habitats (Weinstein et al., 2014; Teresa & Casatti, 2012). It is likely that local environmental conditions are associated with functional assemblage structure, and functional groups turnover according local environmental conditions, and there is some degree of nestedness in this pattern. But low functional diversity within local assemblages is remarkably consistent. Therefore, it seems that certain local habitat types are present throughout the basin, and similar sets of functional traits appear over and over again within a given habitat type (like leafpacks vs. sandbanks). So functional richness is low but species turnover high in space, which causes taxonomic β diversity to be higher in this system (see discussion chapter 3 for high β diversity and species turnover). Contrary to my expectations, contribution of functional nestedness to functional β diversity was slightly higher than functional turnover, which could indicate that changes in functional β diversity in Neotropical systems are product of a complex mixture of turnover and nestedness patterns operating along the longitudinal gradient. Similar mixed results were found by Bishop et al. (2015) for ant communities of southern Africa.

Null model analysis showed that approximately 65% of the observed values of functional α and β diversity were not different from random expectations based on the regional species pool. Therefore, in addition to environmental filtering, stochastic processes associated with dispersal and survival also structure local assemblages (Chase, 2007; Devercelli et al., 2016; Spasojevic & Suding, 2012; Thorp, Thoms & Delong, 2006; Villéger, Grenouillet & Brosse, 2013). In addition, there are potential sources of error that could influence the ability for community metrics to detect community assembly rules (Mouchet et al., 2010; Aiba et al., 2013; Botta-Dukát & Czúcz, 2016), such as the null model used (Swenson, 2014) and the set of traits used (Fitzgerald et al., 2017). For instance, Swenson (2014) argues that the independent swap algorithm is not appropriate for functional beta diversity analysis, since this method does not maintain species spatial structure in the system. In a study of fish assemblages in the Xingu River, a major Amazon tributary, Fitzgerald et al. (2017) obtained different results when using different null models. They found that dividing the full trait set according to different functional subsets (feeding vs. locomotion) produced different patterns.

In an attempt to identify abiotic variables associated with variation in functional β diversity and its components, I performed a variation partitioning analysis using environmental variables and spatial variables. Results of this analyses varied depending on the dissimilarity matrix used. For functional β diversity the percentage of unexplained variation was high (84%), which is fairly common in ecological studies (Legendre & Legendre, 2012; Erős et al., 2017; Castillo-Escrivà et al., 2016). Low percentage of variation explained by the variation partitioning analysis might suggest that stochastic processes not linked to network connectivity play an important role in structuring fish communities in this system. This also could result from failure to include key abiotic variables or functional traits. Perhaps, if an analysis were conducted using different functional traits, including traits that influence additional niche dimensions (e.g., Winemiller et al., 2015) or trait subsets, the percentage of explained variation would be greater.

Environmental and spatial variables explained more variation in functional turnover and nestedness (albeit in different percentages) than in functional β diversity per se. Functional turnover seems to be driven more by environmental variables and functional nestedness influenced more by spatial factors. Functional turnover seemed to be associated with structural habitat features, especially the percentages of cobble, leafpacks, small woody debris and large woody debris, whereas nestedness was most strongly associated with percentages of mud, grass and filamentous algae. Thus, particular habitat features seem to influence patterns of functional β diversity at the basin scale. These results were consistent with those of Santos et al. (2019) who found significant relation between environmental heterogeneity and functional structure of fish assemblages in Amazonian rivers.

Some community ecologists have cast doubt about the ability to infer environmental filtering and whether or not this idea should be abandoned (Cadotte & Tucker, 2017; Thakur & Wright, 2017), because competition can yield similar patterns and most of the studies have been conducted on observational data. However, in this study, I have found that some of the observed values are lower than expected by chance, which suggests trait underdispersion via environmental filtering across the longitudinal gradient. In addition, I found significant association between functional dissimilarity metrics and environmental variables associated with habitat features, which suggests that at least some influential environmental features had been identified.

As stated by Mouillot (2007) the question in ecology is no longer what mechanisms may play a role in structuring communities, but which mechanism has the strongest effect. In my study, it seems there is a large influence of stochastic dynamics and environmental filtering along the longitudinal fluvial gradient. Evidence of deterministic and stochastic processes acting simultaneously have been found in studies of assemblage structuring in other Neotropical rivers (Arrington, Winemiller & Layman, 2005; Córdova-Tapia, Hernández-Marroquín & Zambrano, 2018; Fitzgerald et al., 2017) and other freshwater organisms (Devercelli et al., 2016; Heino et al., 2015; Soininen, Korhonen & Luoto, 2013; Soininen, McDonald & Hillebrand, 2007). High stochasticity has been found to be responsible of high β diversity even in localities that are not environmental heterogeneous and spatially distant, specially when the regional species pool is diverse and there is a high functional redundancy (Chase, 2003; Fukami, 2015; Leibold & Chase, 2017), which is consistent with my findings.

Results from this research reflect functional diversity patterns only during the annual low-water period, when fish functional groups are expected to be structured mainly by environmental filters due to reduced availability of habitat, high fish densities, and, in some cases, harsh abiotic environmental conditions (Winemiller, 1990; Córdova-Tapia & Zambrano, 2016). However, several authors have shown that tropical freshwater fish assemblages vary in systems with strong seasonal hydrology (Winemiller, 1990; Arrington & Winemiller, 2006; Winemiller et al., 2018). Fitzgerald et al. (2017) inferred that stochastic factors strongly influenced dry-season fish assemblages in the Xingu River, and a combination of assembly mechanisms were influential during the wet season. In contrast, Córdova-Tapia & Zambrano (2016) found that environmental conditions act as filters during the dry season, and during the wet season niche partitioning might allow species to coexist.

My study appears to be the first to evaluate fish community assembly using functional α and β diversity as well as their respective turnover and nestedness-resultant components along the entire longitudinal fluvial gradient of a major Neotropical river. I inferred that environmental filtering is an important process structuring fish communities of the Bita River at both basin and reach scales, which suggests a strong dependence of fish diversity on environmental features related to substrate composition and structural complexity. One lesson from these findings is that to preserve fish biodiversity in the Bita River, it is probably crucial that sufficient levels of habitat heterogeneity and connectivity be maintained. Without this heterogeneity, the patterns of functional diversity which explain fish biodiversity may not persist in this tropical river.

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5. CONCLUSIONS

Identifying processes behind fish community assembly in tropical rivers is critical to gain understanding of ecosystem processes and to preserve biodiversity in these hyperdiverse systems (Córdova-Tapia, Hernández-Marroquín & Zambrano, 2018). Species assemblages may be structured by either stochastic or deterministic processes (Chase *et al.*, 2011), such as environmental filtering and biotic interactions (Mouillot, Dumay & Tomasini, 2007) at different scales. Stochastic and deterministic processes are not mutually exclusive and both exert variable effects at different scales of space and time. In this dissertation, I attempted to disentangle the relative roles these processes play at two spatial scales. Specifically, I sought to understand how different assemblages of fish species were influenced by environmental factors by using multiple analytical approaches.

Results from the taxonomic facet of biodiversity showed that fish metacommunities in this river network followed a pattern consistent with a Clementsian distribution, which implies that species responded as groups to the environmental and fluvial gradients via species sorting (López-Delgado, Winemiller & Villa-Navarro, 2019). Analyses of taxonomic diversity showed that species richness was high (201 species) and taxonomic β diversity were both high throughout the longitudinal fluvial gradient. The β diversity partitioning analysis revealed a continuous turnover of fish species along the longitudinal gradient and within four river sections. Species turnover was associated with environmental variables, especially structural features of habitats. These findings suggest that environmental filtering probably played an important role in shaping fish assemblage structure.

Research into the functional facets of fish communities in the Bita River produced interesting findings compared with those based strictly on taxonomic analysis. Functional richness was low along the longitudinal fluvial gradient and within river sections, reflecting a high redundancy in species attributes that are presumed to influence performance and fitness. In contrast, values of functional β diversity were high at both spatial scales, with functional nestedness being slightly higher than functional turnover. In addition, results from the null models revealed that approximately 65% of the observed values were not different from random expectations, suggesting that in this species-rich tropical system, both stochastic dynamics and environmental filtering are structuring fish assemblages. Additionally, I did not find significant differences in functional diversity within river sections, and as for taxonomic diversity, environmental variables that described structural habitat features were associated with variation in functional dissimilarity.

One important facet of biodiversity not addressed in this research is the phylogenetic diversity. By using this approach in futures analysis, it is likely that the proportion of unexplained variation (stochasticity) found in the taxonomic and functional facets reduce. Phylogenetic diversity takes into account the evolutionary relationship between species, reflecting the importance of evolutionary history in shaping community structure, which might provide a deeper understanding of metacommunity assembly processes by connecting local processes with trait evolution, speciation and dispersal (Elias et al. 2009; Leprieur et al., 2012; Swenson, 2014; Webb et al. 2002; Webb, 2000). Processes such as environmental filtering and biotic interaction can also be studied with this facet. For instance, if fish communities in the Bita River are shaped by environmental filtering as suggested by the taxonomic and functional approaches, closely related species will tend to share similar niches resulting in phylogenetic clustering. On the contrary, if metacommunities are structured by biotic interactions species will tend to exclude each other leading to a phylogenetic overdispersion within local species assemblages (Webb et al. 2002). I hope that future research in this basin aims to combine taxonomic, functional and phylogenetic information to gain a more robust assessment of mechanisms that influence biodiversity and assemblage structure in this nearly pristine system.

The Bita River, a free-flowing system with minimum watershed or channel disturbances, was recently declared a wetland of international importance under the RAMSAR convention (World Wide Fund For Nature, 2018). My findings for two facets of biodiversity, taxonomic and functional, were consistent in that environmental features related to substrate composition and structural complexity had strong associations with fish assemblage structure in this river network. The fish survey data and community analyses performed for my dissertation research serve to enhance ecologists' understanding of how fish communities are assembled and maintained under natural conditions. I consider that such an understanding is fundamental for ecological restoration of tropical river systems. This work also provides insights useful for biological monitoring that goes beyond documenting lists of species present at sites.

One lesson from this research is that to preserve fish biodiversity in the Bita River, it is probably crucial that sufficient levels of habitat heterogeneity and connectivity be maintained. Without this heterogeneity, the patterns of taxonomic and functional diversity which explain overall patterns of diversity may not persist. An important issue not addressed directly by my study is the influence of temporal environmental variation on processes affecting population dynamics and community assembly. Seasonal hydrology has a strong influence on ecological dynamics in river-floodplain systems (Arrington & Winemiller, 2006; Córdova-Tapia, Hernández-Marroquín & Zambrano, 2018), and future research is needed to reveal how temporal environmental variation affects α and β diversity. Conservationists and ecosystem managers often concentrate their efforts in areas identified as having high species richness or threatened and endangered species; however, given the scope of the current biodiversity crisis (Maire *et al.*, 2015), effective conservation of multiple dimensions of biodiversity will require better understanding of how natural communities are structured and how they respond to disturbances.

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APPENDIX A

LIST OF SPECIES CAPTURED IN THE BITA RIVER BASIN AND ABUNDANCE ACROSS THE FOUR SECTIONS

Species	High	Mid-high	Mid-low	Low	Total
Beloniformes					
Belonidae					
Belonion dibranchodon		7	229	5	241
Characiformes					
Acestrorhynchidae					
Acestrorhynchus falcatus			1		1
Acestrorhynchus falcirostris		5	12		17
Acestrorhynchus microlepis		9	8	1	18
Acestrorhynchus minimus	1	1		5	7
Acestrorhyncus sp			1		1
Gnathocharax steindachneri		1	2		3
Heterocharax leptogrammus		120	52		172
Hoplocharax goethei		2	19		21
Loncogenys ilisha			8		8
Anostomidae					
Anostomus ternetzi		10			10
Leporinus fasciatus		1	21		22
Leporinus friderici		2	3		5
Pseudanos winterbottomi		3			3
Schizodon scotorhabdotus		28	9		37
Bryconidae					
Brycon melanopterus		2	11	1	14
Brycon pesu				3	3
Chalceidae					
Chalceus macrolepidotus	1	8	28	7	44
Characidae					
Acestrocephalus sardina		19	21		40
Aphyocharax alburnus	5	292	119	91	507

Appendix A. Continued

Species	High	Mid-high	Mid-low	Low	Total
Characiformes					
Characidae					
Brittanichthys sp		83	19	290	392
Bryconamericus sp		126	22		148
Bryconamericus sp "Blanco"		8			8
Characidae sp			10		10
Charax condei			7		7
Creagrutus phasma		43	17		60
Creagrutus sp		9			9
Hemigrammus elegans	120	532	597	1619	2868
Hemigrammus geisleri		299	1576	772	2647
Hemigrammus micropterus			59		59
Hemigrammus microstomus		15	275		290
Hemigrammus newboldi	102	769	303	169	1343
Hemigrammus rhodostomus		37	8	78	123
Hemigrammus schmardae	36		1		37
Hemigrammus sp		1			1
Hemigrammus sp "pseudomicropterus"	26	549			575
Hemigrammus stictus			10		10
Hyphessobrycon acaciae	29	2			31
Hyphessobrycon diancistrus	1	1621	49	9	1680
Hyphessobrycon mavro	1				1
Hyphessobrycon sp			1		1
Hyphessobrycon sweglesi		27	17		44
Knodus cinarucoense	95	412	283		790
Knodus sp		132		14	146
Microschemobrycon callops		76			76
Microschemobrycon casiquiare	11	481	821	101	1414
Moenkhausia ceros	1	25	47	7	80
Moenkhausia collettii	1	328	416	29	774
Moenkhausia copei	14	34	126		174
Moenkhausia lepidura	34	278	316	10	638
Moenkhausia oligolepis		2	8		10
Moenkhausia sp				1	1
Moenkhausia sp "blanco"		9			9
Paracheirodon innesi			14		14
Parapristella georgiae	134	94	31	36	295
Phenacogaster megalostictus		23	2		25
Phenacogaster prolatus		1			1

Appendix A. Continued

Species	High	Mid-high	Mid-low	Low	Total
Characiformes					
Characidae					
Priocharax ariel		16	3		19
Roeboides affinis			2		2
Serrabrycon magoi	1			1	2
Tetragonopterus argenteus		2	4		6
Tyttobrycon sp	4	269	26	409	708
Chilodontidae					
Caenotropus labyrinthicus		25	5		30
Crenuchidae					
Ammocryptocharax elegans	38		1		39
Elachocharax pulcher	4	13	14		31
Melanocharacidium dispilomma	5		3		8
Melanocharacidium sp			4		4
Microcharacidium weitzmani			1		1
Poecilocharax weitzmani			29		29
Ctenoluciidae					
Boulengerella cuvieri		26	56		82
Boulengerella lateristriga	1				1
Boulengerella maculata		4	5		9
Curimatidae					
Curimata vittata			1		1
Curimatopsis evelynae			10	21	31
Cyphocharax spilurus	1	168	22	26	217
Psectrogaster ciliata		1			1
Steindachnerina argentea		53	3		56
Steindachnerina sp		4	6		10
Cynodontidae					
Cynodon septenarius		10			10
Hydrolycus armatus		5	2		7
Erythrinidae					
Hoplerythrinus unitaeniatus			1		1
Hoplias malabaricus	1	5	6	1	13

Appendix A. Continued

Species	High	Mid-high	Mid-low	Low	Total
Gasteropelecidae					
Carnegiella marthae		30	2		32
Hemiodontidae					
Argonectes longiceps		4	13		17
Bivibranchia fowleri		33	81	21	135
Bivibranchia sp			6		6
Bivibranchia velox		5			5
Hemiodus gracilis		7	2	8	17
Hemiodus immaculatus		4	14		18
Hemiodus semitaeniatus	6	13	63	8	90
Hemiodus unimaculatus		18	44		62
Iguanodectidae					
Iguanodectes spilurus		3			3
Lebiasinidae					
Copella arnoldi		16	2		18
Copella eigenmanni	1				1
Copella meinkeni				6	6
Copella nattereri		6	316		322
Copella sp		1	15		16
Nannostomus eques		84	21		105
Nannostomus marilynae	40	3	160	65	268
Nannostomus trifasciatus			14		14
Nannostomus unifasciatus		7	104	188	299
Serrasalmidae					
Catoprion mento		4			4
Metynnis argenteus		1			1
Myleus schomburgkii			3		3
Pristobrycon striolatus		1	3		4
Pygopristis denticulatus		1			1
Serrasalmus altuvei		3	8		11
Serrasalmus irritans		1			1
Serrasalmus manueli			1		1
Serrasalmus rhombeus		4			4
Serrasalmus sp	1	1	6		8

Appendix A. Continued

Species	High	Mid-high	Mid-low	Low	Total
Triportheidae					
Agoniates halecinus		6	3	1	10
Triportheus brachipomus		13	1		14
Cichliformes					
Cichlidae					
Acaronia vultuosa		1		2	3
Aequidens sp	1				1
Aequidens tetramerus		2	3		5
Apistogramma hongsloi	8	153	142		303
Apistogramma minima		4		193	197
Apistogramma sp			1		1
Biotodoma wavrini		16	114	4	134
Biotoecus dicentrarchus		3		35	38
Cichla intermedia		1	1		2
Cichla orinocensis			4		4
Cichla temensis	1				1
Cichlidae sp				2	2
Crenicichla saxatilis	1				1
Crenicichla wallacii		31	31	11	73
Dicrossus filamentosus	6	154	165	137	462
Geophagus abalios		1			1
Geophagus dicrozoster	6	40	30	11	87
Heros severus		2	5		7
Hoplarchus psittacus		1	2		3
Laetacara fulvipinnis			2		2
Mesonauta egregius		4	1		5
Satanoperca daemon	5	2	4		11
Satanoperca jurupari		1			1
Satanoperca sp		1			1
Clupeiformes					
Engraulidae					
Amazonsprattus scintilla	213	517	769	698	2197
Anchoviella guianensis	12	92	10	1	115
Pristigasteridae		1			1
Pellona castelnaeana		1			1

Appendix A. Continued

Species	High	Mid-high	Mid-low	Low	Total
Cyprinodontiformes					
Poeciliidae					
Fluviphylax pygmaeus				81	81
Gymnotiformes					
Hypopomidae					
Hypopygus lepturus		15	14		29
Microsternarchus bilineatus	1	3	1		5
Rhamphichthydae	5	5	5		15
Gymnorhamphichthys rondoni	5	5	5		15
Sternopygidae					
Eigenmannia macrops		20	6		26
Eigenmannia virescens			1		1
Perciformes					
Eleotridae					
Microphilypnus ternetzi		12	16	34	62
Scianidae					
Pachyurus schomburgki		4			4
Plagioscion squamosissimus		3			3
Pleuronectiformes		2	1		3
Achiridae					
Achirus novoae		2	1		3
Siluriformes					
Aspredinidae					
Bunocephalus aloikae	1				1
Auchenipteridae					
Ageneiosus inermis		13			13
Auchenipterichthys longimanus	1	70	4		75
Tatia galaxias	1	3			4
Tatia marthae	1	84	194	2	281
Tatia nigra		28	61	10	99
Trachycorystes trachycorystes				2	2

Appendix A. Continued

Species	High	Mid-high	Mid-low	Low	Total
Siluriformes					
Callichthyidae					
Corydoras sp		2	2		4
Cetopsidae					
Cetopsidium morenoi	4	2	10		16
Helogenes castaneus			1		1
Doradidae					
Acanthodoras cataphractus		3	7	2	12
Amblydoras bolivarensis		5	7		12
Leptodoras linnelli		2	1		3
Physopyxis lyra			14		14
Platydoras hancockii	1	3	4	1	9
Scorpiodoras heckelii	3	3	2	10	18
Heptapteridae					
Gladioglanis sp	4	16	2		22
Goeldiella eques		1			1
Mastiglanis asopos	8		2		10
Microglanis poecilus		8	10	2	20
Phenacorhamdia anisura	3	1			4
Pimelodella sp		34	14		48
Loricariidae					
Acestridium colombiensis		2	7		9
Ancistrus macropthalmus		5			5
Ancistrus sp	4		6		10
Dekeyseria scaphirhyncha	1	5	7	2	15
Farlowella vittata	18	1	1		20
Limatulichthys griseus		5		1	6
Limatulichthys sp			1		1
Loricariichthys brunneus				6	6
Panaqolus maccus		2			2
Parotocinclus eppleyi	22				22
Peckoltia sp		2			2
Pterygoplichthys gibbiceps			8		8
Rineloricaria formosa		1	1	2	4
Rineloricaria sp			1		1

Appendix A. Continued

Species	High	Mid-high	Mid-low	Low	Total
Siluriformes					
Pimelodidae					
Pimelodus albofasciatus		11			11
Pseudoplatystoma metaense		2			2
Sorubim elongatus		28			28
Pseudopimelodidae		6	6	3	15
Batrochoglanis villosus		6	6	3	15
Trichomycteridae					
Haemomaster venezuelae			2	1	3
Ochmacanthus alternus	1	1		6	8
Paravandellia sp	12	36	34	1	83
Synbranchiformes					
Synbranchidae					
Synbranchus marmoratus		10	9	3	22
Total	1330	9646	8948	6004	25928

APPENDIX B

SUMMARY OF THE INDICATOR SPECIES ANALYSIS FOR EACH SECTION IN THE BITA BASIN

Specie	Section	Indicator value
Hemigrammus schmardae	High	0.59
Parotocinclus eppleyi	High	0.40
Ammocryptocharax elegans	High	0.40
Farlowella vittata	High	0.38
Mastiglanis asopos	High	0.37
Phenacorhamdia anisura	High	0.35
Steindachnerina argentea	Mid-High	0.48
Nannostomus unifasciatus	Low	0.89
Microphilypnus ternetzi	Low	0.84
Hemigrammus elegans	Low	0.83
Apistogramma minima	Low	0.66
Brittanichthys sp	Low	0.61
Curimatopsis evelynae	Low	0.60
Hemigrammus analis	Low	0.60
Acaronia vultuosa	Low	0.59
Hemigrammus rhodostomus	Low	0.58
Ochmacanthus alternus	Low	0.58
Acestrorhynchus minimus	Low	0.57
Hemigrammus barrigonae	Low	0.33

APPENDIX C

LIST OF SPECIES MEASURED IN THE BITA RIVER BASIN

Species
Clupeiformes
Engraulidae
Amazonsprattus scintilla
Anchoviella guianensis
Characiformes
Crenuchidae
Ammocryptocharax elegans
Elachocharax pulcher
Melanocharacidium dispilomma
Poecilocharax weitzmani
Erythrinidae
Hoplias malabaricus
Cynodontidae
Hydrolycus armatus
Serrasalmidae
Catoprion mento
Pristobrycon striolatus
Serrasalmus altuvei
Hemiodontidae
Argonectes longiceps
Bivibranchia fowleri
Bivibranchia velox
Hemiodus gracilis
Hemiodus immaculatus
Hemiodus semitaeniatus
Hemiodus unimaculatus
Anostomidae
Anostomus ternetzi

Species
Characiformes
Anostomidae
Leporinus fasciatus
Leporinus friderici
Pseudanos winterbottomi
Schizodon scotorhabdotus
Curimatidae
Curimatopsis evelynae
Cyphocharax spilurus
Steindachnerina argentea
Steindachnerina sp
Lebiasinidae
Copella arnoldi
Copella nattereri
Copella sp
Nannostomus eques
Nannostomus marilynae
Nannostomus trifasciatus
Nannostomus unifasciatus
Chalceidae
Chalceus macrolepidotus
Ctenoluciidae
Boulengerella cuvieri
Boulengerella lateristriga
Gasteropelecidae
Carnegiella marthae

APPENDIX C Continued.

Species
Characiformes
Bryconidae
Brycon pesu
Brycon melanopterus
Acestrorhynchidae
Acestrorhynchus falcirostris
Acestrorhynchus microlepis
Acestrorhynchus minimus
Heterocharax leptogrammus
Loncogenys ilisha
Characidae
Acestrocephalus sardina
Aphyocharax alburnus
Astyanax bimaculatus
Brittanichthys sp
Bryconamericus sp
Charax condei
Creagrutus phasma
Hemigrammus elegans
Hemigrammus geisleri
Hemigrammus micropterus
Hemigrammus microstomus
Hemigrammus newboldi
Hemigrammus rhodostomus
Hemigrammus schmardae
Hemigrammus sp
Hemigrammus sp "pseudomicropterus"
Hemigrammus stictus
Hyphessobrycon acaciae
Hyphessobrycon diancistrus
Hyphessobrycon sweglesi
Knodus cinarucoense
Microschemobrycon callops
Microschemobrycon casiquiare
Characiformes
Characidae
Moenkhausia ceros
Moenkhausia collettii
Moenkhausia copei
Moenkhausia lepidura
Moenkhausia oligolepis
Moenkhausia sp "blanco"
Paracheirodon innesi
Parapristella georgiae
Phenacogaster megalostictus
Priocharax ariel
Tetragonopterus argenteus
Tyttobrycon sp
Gymnotiformes
Hypopomidae
Hypopygus lepturus
Microsternarchus bilineatus
Rhamphichthydae
Gymnorhamphichthys rondoni
Sternopygidae
Eigenmannia macrops
Siluriformes
Auchenipteridae
Auchenipterichthys longimanus
Tatia galaxias
Tatia marthae
Tatia nigra
Callichthyidae
Corydoras sp
Cetopsidae
Cetopsidium morenoi

APPENDIX C Continued.

Species
Doradidae
Acanthodoras cataphractus
Amblydoras bolivarensis
Leptodoras linnelli
Physopyxis lyra
Platydoras hancockii
Scorpiodoras heckelii
Heptapteridae
Gladioglanis sp
Mastiglanis asopos
Microglanis poecilus
Heptapteridae
Phenacorhamdia anisura
Pimelodella sp
Loricariidae
Acestridium colombiensis
Ancistrus macropthalmus
Ancistrus sp
Dekeyseria scaphirhyncha
Farlowella vittata
Parotocinclus eppleyi
Pterygoplichthys gibbiceps
Pimelodidae
Pimelodus albofasciatus
Pseudopimelodidae
Batrochoglanis villosus
Trichomycteridae
Haemomaster venezuelae
Ochmacanthus alternus
Trichomycteridae
Paravandellia sp
Synbranchiformes
Synbranchidae
Synbranchus marmoratus
Cichliformes
Cichlidae
Aequidens tetramerus
Apistogramma hongsloi
Apistogramma minima
Biotodoma wavrini
Biotoecus dicentrarchus
Cichla orinocensis
Crenicichla wallacii
Dicrossus filamentosus
Geophagus dicrozoster
Cyprinodontiformes
Poeciliidae
Fluviphylax pygmaeus
Beloniformes
Belonidae
Belonion dibranchodon
Perciformes
Eleotridae
Microphilypnus ternetzi

APPENDIX D

FUNCTIONAL TRAITS USED FOR THE BITA RIVER BASIN. TRAIT DEFINITION AND ABBREVIATIONS FOLLOW GATZ (1979) AND WINEMILLER (1991).

Trait	Abbreviation	Functional category	Observations
Standard length	SL	Locomotion	
Maximum body width	BODW		
Caudal peduncle length	PEDL		
Caudal peduncle depth	PEDD		
Caudal peduncle width	PEDW		
Dorsal fin height	DORH		
Dorsal fin length	DORL		
Caudal fin length	CAUL		
Caudal fin height	CAUH		
Anal Fin height	ANAH		
Anal fin Length	ANAL		
Shape of caudal fin	SHCF		0) absent, 1) rounded, 2) trunked, 3) forked
Swim bladder length	SWBL		
Lateral line	LLIE		1) complete, 2) incomplete, 3) interrupted, 0) absent
Maximum Body depth	BODD	Habitat use, Locomotion	
Body depth below midline	BDBM		
Pectoral fin height	PECH		
Pectoral fin length	PECL		
Pelvic fin length	PELVL		
Pelvic fin height	PELVH		
Spines	SPN	Defense	0) none, 1) few short & weakly serrated, 2) few long or few short & strongly serrated, 3) many long (dorsal, anal), 4) long & massive
Venom	VEN		0) none, 1) pectoral & dorsal spines with venom
Armor	ARM		0) none, 1) massive skull, 2) thin bony plates covering body, 3) thick

APPENDIX D Continued.

Trait	Abbreviation	Functional category	Observations
Crypsis	CRY	Defense	0) none, 1) mottling for blending with vegetation, 2) mimicry of vegetation of woody debris
Head length	HEAL	Feeding	
Head depth	HEAD		
Head width	HEAW		
Eye position	EYEP		
Eye diameter	EYED		
Mouth position	MOUPO		1) superior, 2) terminal, 3) subterminal, 4) inferior and 5) bottom
Mouth width	MOUW		
Mouth height	MOUH		
Number of barbels	NBAR		
Protrusion	PRO		0) absent, 1) present
Tooth shape	TSHA		0) absent, 1) unicuspid (rasping), 2) multicuspid (crushing), 3) short conical (grasping) 4) long conical (piercing) 5) triangular serrated (shearing)
Gill raker	GRAK		0) absent, 1) short, blunt, or toothlike, 2) intermediate or long and sparse, and 4) long and comb-like
GUT length	GUTL		

APPENDIX E

RESULTS OF THE FUNCTIONAL DIVERSITY INDICES AND THE STANDARDIZED EFFECT SIZES (SES), BASED ON THE NULL MODELING APPROACH

Site	**Section**	**FRic**		**FDiv**		**FEve**		**FDis**		**FSpe**		**FOri**	
		FD	**SES**	**FD**	**SES**	**FD**	**SES**	**FD**	**SES**	**FD**	**SES**	**FD**	**SES**
E1	High	0.31	0.62	0.73	-0.34	0.67	-0.34	0.39	-1.43	0.43	-1.43	0.33	1.55
E2	High	0.08	-1.45	0.81	1.61	0.40	**-4.85**	0.26	**-2.94**	0.32	-2.94	0.11	**-3.05**
E3	High	0.05	-1.75	0.78	0.93	0.48	**-3.46**	0.15	**-4.32**	0.32	-4.32	0.09	**-3.49**
E4	High	0.47	**2.18**	0.81	1.52	0.52	**-2.83**	0.46	-0.59	0.39	-0.59	0.20	-1.03
E5	High	0.01	**-2.15**	0.51	**-5.81**	0.07	**-10.38**	0.14	**-4.45**	0.31	-4.45	0.11	**-3.12**
E6	Mid-high	0.65	**3.80**	0.91	**4.21**	0.41	**-4.62**	0.27	**-2.84**	0.39	-2.84	0.11	**-2.98**
E7	Mid-high	0.31	0.69	0.72	-0.75	0.32	**-6.24**	0.24	**-3.22**	0.32	-3.22	0.11	**-3.02**
E8	Mid-high	0.06	-1.70	0.81	1.49	0.69	-0.01	0.53	0.36	0.43	0.36	0.18	-1.46
E9	Mid-high	0.04	**-1.84**	0.46	**-7.22**	0.40	**-4.91**	0.05	**-5.61**	0.27	**-5.61**	0.08	**-3.69**
E10	Mid-high	0.08	-1.46	0.80	1.22	0.57	-1.92	0.31	**-2.43**	0.32	-2.43	0.18	-1.54
E11	Mid-high	0.12	-1.09	0.72	-0.54	0.22	**-7.92**	0.23	**-3.33**	0.32	-3.33	0.11	**-3.05**
E12	Mid-high	0.04	**-1.83**	0.80	1.37	0.55	**-2.30**	0.59	1.01	0.61	**1.01**	0.40	**3.09**
E13	Mid-high	0.19	-0.44	0.80	1.39	0.40	**-4.92**	0.52	0.25	0.45	0.25	0.18	-1.57
E14	Mid-high	0.59	**3.25**	0.94	**4.87**	0.47	**-3.71**	0.29	**-2.65**	0.35	-2.65	0.12	**-2.91**
E15	Mid-high	0.36	1.17	0.77	0.71	0.47	**-3.73**	0.20	**-3.75**	0.33	-3.75	0.11	**-3.01**
E16	Mid-high	0.01	**-2.16**	0.89	**3.65**	0.56	**-2.13**	0.50	-0.06	0.46	-0.06	0.21	-0.85
E17	Mid-high	0.38	1.32	0.70	-1.26	0.41	**-4.61**	0.17	**-4.07**	0.29	**-4.07**	0.11	**-3.02**
E18	Mid-low	0.36	1.14	0.80	1.40	0.41	**-4.64**	0.41	-1.09	0.40	-1.09	0.16	**-2.03**
E19	Mid-low	0.28	0.35	0.68	-1.66	0.52	**-2.91**	0.28	**-2.80**	0.36	-2.80	0.10	**-3.14**
E20	Mid-low	0.13	-1.03	0.70	-1.21	0.27	**-7.06**	0.30	-2.48	0.32	-2.48	0.17	-1.75
E21	Mid-low	0.50	**2.43**	0.74	-0.27	0.41	**-4.75**	0.39	-1.39	0.38	-1.39	0.13	**-2.53**
E22	Mid-low	0.32	0.79	0.66	**-2.13**	0.47	**-3.67**	0.31	-2.34	0.33	-2.34	0.16	**-1.86**
E23	Mid-low	0.00	**-2.24**	0.61	**-3.51**	0.08	**-10.29**	0.25	**-3.13**	0.35	-3.13	0.13	**-2.51**
E24	Mid-low	0.33	0.83	0.69	-1.31	0.42	**-4.52**	0.10	**-4.91**	0.28	**-4.91**	0.10	**-3.33**
E25	Mid-low	0.75	**4.74**	0.83	**2.12**	0.49	**-3.33**	0.54	0.41	0.48	0.41	0.16	**-1.97**
E26	Mid-low	0.51	**2.52**	0.72	-0.68	0.49	**-3.43**	0.54	0.48	0.43	0.48	0.23	-0.44

APPENDIX E Continued.

Site	Section	FRic		FDiv		FEve		FDis		FSpe		FOri	
		FD	**SES**	**FD**	**SES**	**FD**	**SES**	**FD**	**SES**	**FD**	**SES**	**FD**	**SES**
E27	Mid-low	0.52	**2.62**	0.77	0.66	0.53	**-2.72**	0.45	-0.60	0.39	-0.60	0.19	-1.42
E28	Mid-low	0.06	-1.70	0.63	**-2.89**	0.30	**-6.53**	0.23	**-3.29**	0.25	**-3.29**	0.15	**-2.25**
E29	Mid-low	0.41	1.63	0.57	**-4.45**	0.46	**-3.87**	0.32	**-2.26**	0.30	**-2.26**	0.18	-1.49
E30	Mid-low	0.06	-1.67	0.78	0.94	0.42	**-4.55**	0.24	**-3.29**	0.32	-3.29	0.17	**-1.85**
E31	Mid-low	0.24	0.05	0.79	1.00	0.55	**-2.38**	0.49	-0.17	0.41	-0.17	0.18	-1.55
E32	Low	0.37	1.18	0.68	-1.60	0.32	**-6.27**	0.41	-1.15	0.38	-1.15	0.16	**-2.01**
E33	Low	0.07	-1.58	0.75	0.02	0.43	**-4.40**	0.28	**-2.68**	0.31	-2.68	0.14	**-2.46**
E34	Low	0.37	1.20	0.80	1.33	0.43	**-4.37**	0.21	**-3.61**	0.31	-3.61	0.12	**-2.82**
% Significant values		35.29		29.41		91.18		55.88		17.65		67.65	

APPENDIX F

VARIATION IN FUNCTIONAL B DIVERSITY (FBETA) AND ITS COMPONENTS; FUNCTIONAL TURNOVER (FTURN) AND FUNCTIONAL NESTEDNESS-RESULTANT (FNES), WITHIN EACH SECTION OF THE BITA RIVER BASIN, CALCULATED USING PERMDISP AND "TUKEYHSD" POT-HOC TEST

FBeta	diff	p-value
Low-High	0.143	0.415
Mid_high-High	0.017	0.994
Mid_low-High	-0.069	0.722
Mid_high-Low	0.160	0.221
Mid_low-Low	0.075	0.787
Mid_low-Mid_high	-0.085	0.328
FTurn		
Low-High	-0.197	0.079
Mid_high-High	0.031	0.946
Mid_low-High	-0.130	0.118
Mid_high-Low	0.228	**0.013**
Mid_low-Low	0.067	0.759
Mid_low-Mid_high	-0.161	**0.003**
FNes		
Low-High	-0.088	0.868
Mid_high-High	-0.061	0.887
Mid_low-High	-0.041	0.958
Mid_high-Low	0.028	0.993
Mid_low-Low	0.047	0.965
Mid_low-Mid_high	0.019	0.989

APPENDIX G

ENVIRONMENTAL AND SPATIAL VARIABLES SELECTED BY THE FORWARD SELECTION PROCEDURE FOR THE FUNCTIONAL BETA DIVERSITY AND ITS COMPONENTS FUNCTIONAL TURNOVER AND FUNCTIONAL NESTEDNESS (JACCARD DISSIMILARITY)

Variables	FBeta		FTurn		FNes	
	R^2	p	R^2	p	R^2	p
Environmental						
X.SubRoots	0.18	0.01				
X.Cobble	0.10	0.02	0.36	0.003		
Elevation	0.09	0.03	0.10	0.002		
X.Leafpacks	0.08	0.02	0.03	0.041		
X.Lwoode			0.18	0.001		
X.Swoode			0.09	0.002		
pH			0.04	0.018		
Cond					0.18	0.005
X.Mud					0.11	0.029
X.Grass					0.08	0.043
X.FilAlgae					0.10	0.011
C					0.07	0.024
Spatial						
V3	0.13	0.01			0.09	0.033
V6	0.10	0.02			0.12	0.028
V7	0.10	0.02			0.10	0.045
V16	0.08	0.02	0.19	0.004		
V10					0.08	0.038

APPENDIX H

VARIATION PARTITIONING ANALYSIS FOR THE FUNCTIONAL BETA DIVERSITY AND ITS COMPONENTS FUNCTIONAL TURNOVER AND FUNCTIONAL NESTEDNESS (JACCARD DISSIMILARITY) AND SELECTED GROUPS OF ENVIRONMENTAL AND SPATIAL VARIABLES.

Component	FBeta		FTurn		FNes	
	AdjR^2	p	AdjR^2	p	AdjR^2	p
Jaccar dissimilarity						
E	0.10	**0.001**	0.43	**0.001**	0.25	**0.007**
S	0.12	**0.001**	0.11	**0.025**	0.35	**0.001**
E+S	0.21	**0.001**	0.54	**0.001**	0.45	**0.002**
E|S	0.08	**0.001**	0.43	**0.001**	0.10	**0.002**
S|E	0.11	**0.001**	0.12	**0.001**	0.20	**0.001**
b	0.01		0.00		0.15	
R	0.79		0.46		0.55	