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Composition and distribution of hyporheic and macrobenthic fauna in a Neotropical mountain river, Colombia

Yojana Valentina Ballesteros Navia[1,*], Jaime Ricardo Cantera Kintz[1], Enrique Javier Peña Salamanca[1] and Gabriel Pinilla Agudelo[2]

[1] Departmento de Biología, Universidad del Valle, Cali, Colombia.
[2] Departamento de Biología, Universidad Nacional de Colombia, Bogotá, Colombia.

[*] Corresponding author: yojana.ballesteros@correounivalle.edu.co

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ABSTRACT

Composition and distribution of hyporheic and macrobenthic fauna in a Neotropical mountain river, Colombia

This study examined the composition and diversity of the invertebrate communities that inhabit the hyporheic zone (HZ) in the upper and lower basins of the Dagua River, a tropical mountain stream, and their relationship with the macrobenthic fauna. In the HZ, different taxa were collected, many of which have been reported in several regions worldwide, suggesting that the biodiversity of the hyporheic community in Colombian rivers may be high. Although the hyporheic and macrobenthic fauna shared a moderate percentage (> 50 %) of taxa, their ecological structures were different. The analysis of hyporheic filtrate water showed the presence of microplastic (MP) fibers as well as fauna, a condition that, based on the literature review, could represent a risk for the normal functioning of this river ecosystem.

Key words: benthic fauna, Dagua River, environmental risk, hyporheic zone, hyporheos, microplastic fibers

RESUMEN

Composición y distribución de la fauna hiporreica y macrobentónica de un río neotropical de montaña, Colombia

En este estudio se examinó la composición y diversidad de la comunidad de invertebrados que habita en la zona hiporréica (HZ) en la cuenca alta y baja del río Dagua, un curso de agua tropical, y su relación con la fauna macrobentónica. En la HZ se recolectaron diferentes taxones, de los cuales muchos han sido reportados en varias regiones del mundo; esto sugiere que la biodiversidad de las comunidades hiporreicas de ríos colombianos podría ser alta. Aunque las faunas hiporreica y macrobentónica compartieron un moderado porcentaje (> 50 %) de taxa, su estructura ecológica fue diferente. El análisis del filtrado de aguas hiporreicas mostró la presencia de fibras de microplástico junto con la fauna, una condición que, con base en la revisión de literatura, podría representar un riesgo para el funcionamiento normal de este ecosistema de río.

Palabras clave: *fauna bentónica, río Dagua, riesgo ambiental, zona hiporreica, hiporreos, fibras de microplástico*

INTRODUCTION

Invertebrate fauna associated with the bottom of lotic ecosystems has two main components: one is the macrobenthic fauna associated with the substrates on the riverbed (rocks, gravel, boulders, sand, silt, and clay), and the other is hyporheic organisms that live under the bed of the river, an area called the hyporheic zone (HZ) (Fraser & Williams, 1998). This area constitutes an interstitial flow path along which surface water descends toward the subsurface sediment, and after passing through, mixes with groundwater and returns to the surface current (Hakenkamp & Palmer, 2000). The HZ has been designated as an interstitial biotope, hyporheic biotope, interstitial environment (Williams & Hynes, 1974), and ecotone (Boulton et al., 1998; Sabater & Vila, 1991; Williams, 2003). It harbors hyporheic fauna, considered meiofauna due to its size (Palmer et al., 2007), and is composed of typical taxa of the interstitial environment, as well as other taxonomic groups present in groundwater (phreatic or hypogeal organisms) and the benthic region (epigeal organisms) (Ruffo, 1961; Kirchengast, 1984; Gibert, 1991).

Hyporheos play a role in the maintenance of porosity, modification of the redox gradient, and stimulation of biofilm activity (Gibert & Deharveng, 2002; Humphreys, 2002). Some of these activities cause the elimination of dissolved substances from water and their conversion into particulate forms (Mickleburgh et al., 1984; Kaplan & Bott, 1985). These processes can also provide nourishment for hyporheic invertebrates (Williams, 1981), which in turn serve as food sources for organisms at higher trophic levels; that is, the hyporheic fauna transfers carbon from microbial biofilms to larger consumers of invertebrates (Hakenkamp & Palmer, 2000).

The idea of sampling in excavations at the margins of streams was introduced by Karaman (1935) and Chappuis (1942), who collected samples from rivers in Yugoslavia and Romania. This technique probably played a catalyst role in the discovery of the hyporheic fauna. According to Orghidan (1959, cited by Käser, 2010), this methodology led to the discovery of many genera and species that inhabit subsurface aquatic systems, such as turbellarians, nematodes, amphipods, isopods, and aquatic mites. Based on these pioneering investigations, interest in the ecological aspects of the HZ, such as its biodiversity and biogeographic importance, increased (Marmonier et al., 1993). For several years, studies on lotic ecosystems considered only two dimensions, lateral and longitudinal, but at present, the importance of the vertical dimension and its role in the assemblages of macrobenthic and hyporheic invertebrates is recognized (Dole-Olivier, 1998; Davy-Bowker et al., 2006; Mathers & Wood, 2016). Likewise, the processes resulting from vertical flow in the interstitial zone effect sediment composition and porosity (Gomez-Velez et al., 2014), bed topography (Wildhaber et al., 2014), and hydrological conditions (Dudley-Southern & Binley, 2015).

The HZ comprises complex and dense networks of interstitial spaces suitable for invertebrate colonization along a vertical spatial gradient, allowing habitation by fauna at different depths (Stanford & Gaufin, 1974; Dole-Olivier & Marmonier, 1992). The connectivity between the subsurface environment and the surface current allows the upward movement of organisms, which is one of the colonization patterns of macrobenthic fauna, rendering the subsurface (hyporheic) substrate a factor in the processes of biological exchange with the fluvial benthos (Williams & Hynes, 1976; Townsend & Hildrew, 1994). One of the explanations for the existence and composition of hyporheic fauna is the hypothesis of the hyporheic refuge (HHR) (Angelier, 1953; Orghidan, 1959), which states that the refuge is a place where the effects of disturbances in the surrounding area are minimized (Lancaster & Belyea, 1997). Williams & Hynes (1974) supported this hypothesis based on the favorable physicochemical conditions for HZ fauna and the morphology of invertebrates associated with displacement capacity in the substrate. Other arguments in favor of this hypothesis are the decrease in flow in the HZ (Humphries & Baldwin, 2003; Murray et al., 2003), reduction in abrasion that organisms may experience due to erosive processes (Statzner et al., 1988), protection against biological interactions (Franken et al., 2006), and lower variations in temperature (Benkebil et al., 2021). The HZ contributes to the diversity of the aquatic invertebrate community via patches created by hydrological exchange, the variability in the time of residence of the water, and the biological activity of the distinct taxa (Boulton, 2000).

Very little is known about HZs, including both their physical characteristics and ecological conditions, in Neotropical regions. Mugnai et al. (2015a) reviewed investigations carried out in Mexico, Honduras, Venezuela, Chile, and Argentina; more recent studies have also been conducted in Brazil (Veras et al., 2018; Mugnai et al., 2019). Research on HZs in Colombian rivers, which are mostly unstudied, is important because of the richness of fluvial systems in the country; additionally, many rivers have had drastic decreases in their flow rates and their flood plains. It is necessary to understand and conserve this zone, given that it is involved in stream dynamics and functionality at the local to global levels. The study of HZs will allow better guidance to address threats to water resources, such as climate change (Stubbington et al., 2009a). Understanding the functions of the HZ is a challenge that requires a holistic and interdisciplinary approach (Robertson & Wood, 2010). Therefore, efforts to compile information about hyporheic fauna will help deepen the understanding of its response to disturbances (Leigh et al., 2013).

In recent decades, studies on contaminants in the aquatic environment have drawn attention to the environmental occurrence of a variety of newly identified compounds of anthropogenic origin, known as emerging pollutants. Currently, there are many anthropogenic impacts on HZs (Hancock, 2002), including contaminants such as microplastics (MPs) (Drummond et al., 2019; Frei et al., 2019) and chemical substances associated with the pharmaceutical industry and personal care products, and endocrine disruptors (Gogoi et al., 2018). They are not toxic *per se* but can have a significant impact on aquatic habitats because they become substrates for toxic substances and bacterial growth (Kovač Viršek et al., 2017; Godoy et al., 2019). Aquatic organisms can easily ingest MPs that are similar in size to their food source (Cole et al., 2013; Kaposi et al., 2014; Tanaka & Takada, 2016), thereby transferring the chemicals on the MPs to the organisms (Tanaka et al., 2015; Besseling et al., 2017); MPs can then reach humans through the food chain (Wright & Kelly, 2017; Carbery et al., 2018).

The data reported in this research represent the first assessment of the invertebrate fauna of the Dagua River's HZ from the high basin to its mouth in the Bay of Buenaventura in the Eastern Pacific Ocean. The spatial and temporal distributions of the hyporheic and macrobenthic fauna are described, and comparisons of their compositions and diversity are analyzed. Given the sensitivity of aquatic invertebrates, pollutants present in the HZ can represent a risk to their survival and their function (Rode et al., 2015), which is why some preliminary data on the presence of MPs in the HZ of this river are also reported.

MATERIALS AND METHODS

Study region

The Dagua River starts approximately 2200 meters above sea level in a humid montane forest zone. It has a length of 101 km, a drainage basin of 142 500 hectares, and an average slope of 2.6 %; a large part of its route passes through urban sectors (Aguirre et al., 2017). In its upper basin, the length is approximately 74 km. The river descends gently from the western flank of the Western Cordillera; at the middle of the mountain, it enters a canyon and then gradually descends into the Pacific coastal plain in the department of Valle del Cauca, Colombia, South America. Five monitoring locations were selected considering their access and anthropogenic impacts: four in the upper basin and one in the lower basin (Fig. 1). General descriptions, limnological characteristics (dissolved oxygen, temperature, and pH), and abbreviations for each sampling station are shown in Table 1. It should be noted that plastic waste, represented mainly by bottles, food containers, and mesh waste used for agricultural and domestic activities, was present in the riverbank areas; the latter has become a great contributor to plastic pollution since on many occasions during sampling, waste was observed trapped between stones and bedding materials.

Sampling periods and collection of invertebrates and MPs

Five field samplings were carried out: 1) April 2017, 2) July 2017, 3) October 2017, 4) January

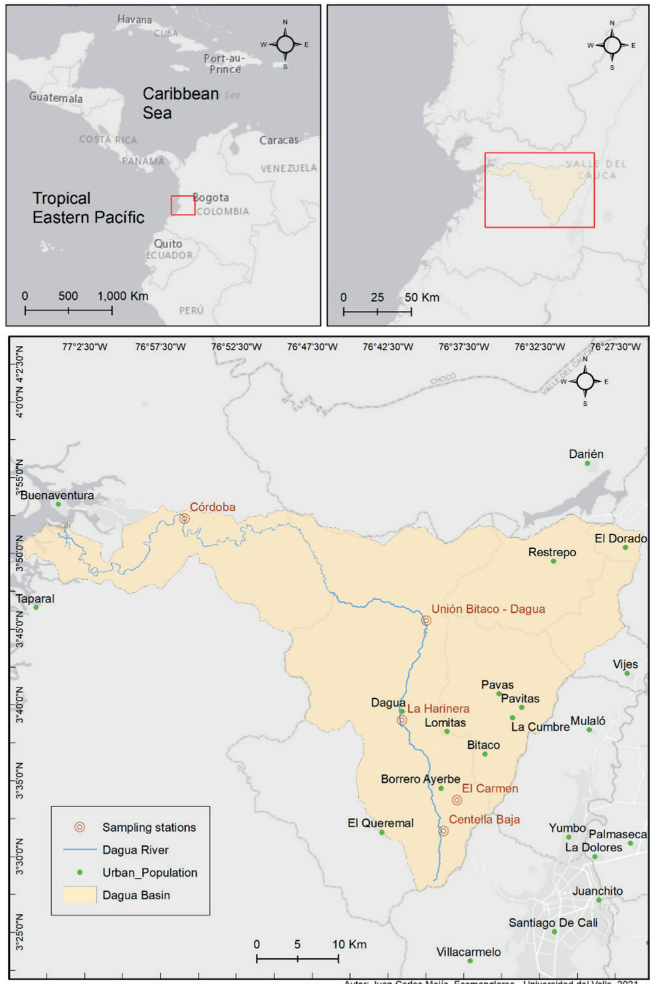

Figure 1. Locations of the Dagua River basin and sampling stations. *Localización de la cuenca del Río Dagua y distribución de las estaciones de muestreo.*

2018, and 5) December 2018. April and October are the months with the highest precipitation; therefore, the river flow is increased during these months. The Bitaco-Dagua sampling station (the lowest in the mid-upper basin) and the location located at a lower altitude (Córdoba station, Table 1) had a higher river flow than the other stations (Fig. 2). The Bou Rouch pumping method for the extraction of hyporheic fauna cannot be applied in hard beds, such as those found at the Dagua River's headwaters. Therefore, to standardize sampling among all the stations, the Karaman-Chappuis method was chosen. The volume of water extracted from the sediment was the same for each sample, but the sampling area of the HZ was dependent on the porosity and permeability of the sediment. For this method, the substrate must contain a certain amount of gravel (Pryce et al., 2010) and sand to allow water circulation. At each site, three wells, separated by 10 m to maintain differentiation, were excavated; each well was 40 cm in diameter and 60 cm deep according to the river water level. Each well was initially georeferenced, but there was notable variability in the shore physiography due to hydrological seasonality and bedding material ex-

Table 1. Features of the hyporheic sampling stations established in the Dagua River. *Características de las estaciones de muestreo de hiporreos establecidas en el Río Dagua.*

Basin	Sampling station	Abbreviation	Elevation (masl)	Average monthly rainfall (mm)	Surface water parameters			Human interventions
					Temperature (°C)	Dissolved oxygen (ppm)	pH (Units)	
High	El Carmen	**EC**	1502	2.87	19.07	5.58	7.39	Tourism, agricultural activities, alteration of the riverbank forest; presence of a dispersed population.
	Centella Baja	**CB**	1408	2.1	20.9	5.87	7.44	Inadequate management of agricultural (agrochemical) systems, degradation of soils and water; moderate disturbance of the riverside forest; presence of a dispersed population.
	La Harinera	**LH**	878	4.47	21.75	6.47	7.9	Extraction of stone and sand, detergent inputs, modifications of the channel for access roads; alteration of the riverbank forest; presence of a dispersed population.
	Bitaco-Dagua	**BD**	649	13.8	21.51	5.78	7	Organic spills; gravel and sand extraction; alteration of the riverbank forest; increase in human settlements.
Low	Córdoba	**CO**	52	15.5	24.24	5.91	6.92	Gold, gravel and sand mining; alteration of the riverbank forest; increase in human settlements.

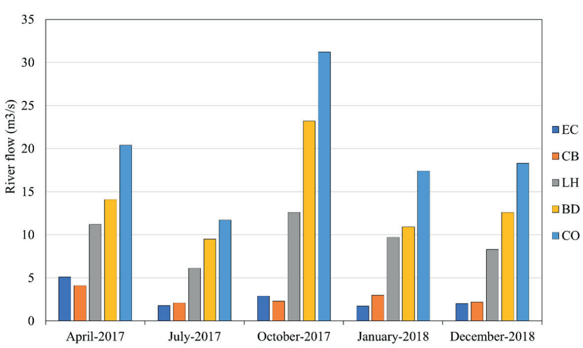

Figure 2. River flow (m^3/s) registered during the sampling periods. See Table 1 for the site abbreviations. *Flujo del río (m^3/s) registrado durante los períodos de muestreo. Ver tabla 1 para las abreviaturas de los sitios.*

traction activities, so wells dug on each date were located as close as possible to the initial coordinates. Well drilling was performed manually; 5 L of water was collected and filtered through a 63 µ pore sieve; and the filtrate was preserved in 80 % ethanol for subsequent transport. Macrobenthic fauna were collected with a Surber net with a side of 0.3 m and a pore size of 300 µ. According to the recommendations of Jiménez-Valverde and Hortal (2003), it was estimated that three net samplings for one minute each at each site would capture 95 % of the species present. The Surber net was placed in front of each well, and a composite sample was obtained from each station.

When analyzing the faunal samples from the HZ, many MP fibers were observed; therefore, a preliminary analysis of their abundance and frequency was carried out. MP fiber estimations ranged from 500 µ to 5000 µ (Löder & Gerdts, 2015). Given the possible influence of air currents on the number of plastic fibers in the samples handled in the laboratory, it was decided to follow the protocol proposed by Jiang et al. (2019). Due to their high abundance, a subsample was obtained by homogenizing the sediment mixture in alcohol, filtering it, and removing one gram of sediment from the filtrate from which all the fibers were extracted; the value obtained was extrapolated to the total grams of filtered material.

Data were recorded as the number of fibers/L of filtered hyporheic water. Visual observation of the samples was conducted following methods described by Löder & Gerdts (2015), who demonstrated that for particles over 500 μ in size, ocular examinations are suitable for identification.

A Nikon SMZ 645 microscope (Capovani Brothers Inc. Scotia, NY, United States) was used for extraction of the invertebrates and MP fibers. Identification was performed to the lowest possible taxonomic level. Organisms in the phylum Arthropoda, class Entognatha were identified to order, while those in the class Insecta were mostly identified to genus; aquatic mites were grouped as Hydrachnidiae, crustaceans were identified to order, and the myriapods Pauropoda and Symphyla were identified to class. The phyla Platyhelminthes and Annelida were identified at the family level. Organisms in the Mollusca phylum were mostly identified at the genus level, and nematodes were identified at the phylum level. The taxonomic keys of Silva et al. (2007); Galassi et al. (2009); Sendra Mocholí (2015); Baquero & Jordana (2015); Linares et al. (2018); Poinar (2015); Rodríguez Domínguez (2015), and Baltanás & Mesquita-Joanes (2015) were used. We also had the support of specialists for certain taxonomic groups: Pilar Rodríguez (University of Basque Country) for Oligochaeta; Marcela Peralta (Fundación Miguel Lillo) for Copepoda and Harpacticoida; Gabriela Cuezzo (Conicet) for Gasteropoda; and María del Carmen Zuñiga, Ranulfo González, and Marcela Gonzáles (Universidad del Valle) for Insecta.

Statistical analysis

Nonmetric multidimensional scaling (NMDS) was used to explore the structures of the hyporheic and macrobenthic invertebrate communities. To determine whether there were significant differences in the community structures between the macrobenthic and hyporheic fauna, nonparametric analysis of similarity (ANOSIM) based on the Bray–Curtis index and 9999 permutations was performed. ANOSIM tests whether the distances between groups of macrobenthic fauna and hyporheic fauna are greater than those within groups. The comparison of the Shannon diversity (H') and Margalef richness indices of the two groups of fauna was performed by ANOVA after confirming the normality of the data using the Shapiro–Wilk test; since the Pielou equity index did not meet this requirement, the comparison for this index was performed with the nonparametric Kruskal–Wallis test.

To assess whether the amount of MP in the samples was influenced by the monitoring stations, the sampling periods, or spatial and temporal interactions (considering the repeated sampling of wells over time), analysis of variance (ANOVA) with a linear model was adjusted to ANOVA with a mixed linear model, taking into account the Akaike and Bayesian information criteria. Due to the lack of independent data, the assumption of normality of the residuals was validated using a Q-Q (quantile-quantile) plot. Significant differences between the factor levels were assessed using the Tukey multiple comparison test. Statistical analyses were carried out with the freely available program R, version 3.5.1 (R Development Core Team, 2019), and the significance level was set at 5 %.

RESULTS

Hyporheic and macrobenthic fauna

Table 2 lists the hyporheic invertebrates collected in the Dagua River and their abundance, the diversity index (H'), and richness of taxa (S) along the river course and during different sampling seasons. A total of 1843 hyporheic fauna specimens were collected, of which 41 taxa belonging to the phyla Arthropoda, Annelida, Nematoda, Mollusca, and Platyhelminthes were identified. Arthropoda was the dominant group, with 1253 individuals, and within this group, the Hexanauplia (subclass Copepoda with 558 individuals), Insecta (373 individuals), and Arachnida (Hidrachnidiae, 272 individuals) classes predominated. Within the Insecta class, the most representative taxa were, in decreasing order, Diptera, Trichoptera, Coleoptera, and Ephemeroptera. Other Arthropoda from the Entognatha (Collembola of the order

Table 2. Checklist of invertebrate taxa in each phylum (Ph.). Sampling stations (codes as in Table 1) and spatiotemporal estimations of abundance, richness and diversity (H'). *Lista de verificación de taxones de invertebrados incluidos en cada filo (Ph.). Estaciones de muestreo (códigos como en el cuadro 1) y estimación espaciotemporal de abundancia, riqueza y diversidad (H').*

Taxon	EL CARMEN					CENTELLA BAJA					LA HARINERA					BITACO DAGUA					CORDOBA				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
Ph. Arthropoda																									
Collembola	0	1	1	4	3	0	0	1	6	1	0	0	1	0	0	1	1	0	2	0	0	0	0	0	0
Japygidae	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Corydalus spp.	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Dolichopodidae	4	0	2	0	1	2	0	5	0	1	0	0	1	0	0	0	0	3	3	0	0	1	1	1	0
Bezzia spp.	0	0	1	1	0	0	0	3	0	0	3	1	3	1	1	1	4	0	2	5	5	0	0	0	2
Syrphidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0
Chironomidae	1	1	3	3	1	0	0	0	0	0	2	0	0	0	7	9	3	4	5	1	11	0	15	5	0
Chaoboridae	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellulidae	0	2	0	0	0	0	0	0	0	1	2	0	1	0	1	1	0	0	0	2	0	0	0	0	1
Coenagrionidae	2	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrelmis spp.	3	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Heterelmis spp.	0	0	0	1	0	0	0	2	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2
Huleechius spp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Staphylinidae	0	0	2	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Psephenus spp.	3	0	1	1	1	0	1	0	3	1	1	0	1	3	4	0	0	1	0	0	1	0	0	0	0
Hidrophilidae	0	0	0	5	0	0	0	0	4	0	0	0	0	3	0	0	0	0	1	0	0	2	0	0	0
Anacroneuria spp.	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Cryphocricos spp.	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicopsyche spp.	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Atopsyche spp.	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Hydropsychidae	3	6	4	3	7	8	1	1	1	2	1	1	4	2	3	4	3	4	2	3	3	7	0	9	2
*Trycorythode*s spp.	1	1	6	2	2	1	4	0	3	0	0	0	6	0	2	0	1	0	0	0	0	0	0	1	1
Leptohyphes spp.	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Thraulodes spp.	0	1	1	1	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Camelobaetidius spp.	1	1	0	0	0	0	0	0	0	3	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	1	0	1	0	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0
Hydrachnidia	3	3	6	4	8	9	3	3	8	14	12	9	4	20	13	9	11	10	17	12	27	3	28	12	24
Pauropoda	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Podocopida	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	9
Cyclopoida	13	4	7	13	8	8	7	8	15	7	14	3	7	6	6	15	8	15	6	10	21	13	16	5	7
Parastenocarididae	26	32	46	6	13	6	4	21	7	7	5	15	2	23	11	8	8	22	11	13	5	10	4	9	5
Symphyla	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ph. Platyhelminthes																									
Rhabditophora	0	0	3	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	2	0
Ph. Annelida																									
Naididae	8	3	7	12	15	4	9	2	17	3	8	9	11	8	20	4	4	7	11	8	14	9	12	3	13
Annelida	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	2	0	0	8	5	8	9
Ph.Mollusca																									
Cochliopidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Doryssa spp.	0	0	1	1	0	3	0	0	0	0	2	0	0	3	0	0	0	0	2	0	0	2	0	0	0
Physidae	0	0	0	0	1	0	3	0	0	0	0	0	1	0	0	2	0	3	0	0	0	0	0	0	2
Gundlachia spp.	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Melanoides spp.	5	0	0	0	0	0	1	0	0	2	0	0	0	0	7	0	2	0	0	1	4	7	6	8	1
Ph. Nematoda																									
Nematoda	1	5	10	4	11	11	7	3	9	4	6	6	13	25	4	9	16	9	23	2	10	21	17	13	25
Relative Abundance	77	65	105	64	77	54	44	56	73	52	57	45	62	97	81	65	68	86	92	59	101	85	105	76	104
Taxa Richness (S)	18	16	20	18	18		14	15	10	17	12	8	19	12	14	13	17	15	18	12	10	12	10	12	15
Shannon Diversity (H´)	2,3	2,0	2,2	2,5	2,4	2,1	2,3	2,2	2,1	2,4	2,1	1,7	2,5	2,0	2,3	2,2	2,4	2,3	2,3	2,1	2,0	2,2	2,0	2,3	2,2

M= Sampling periods

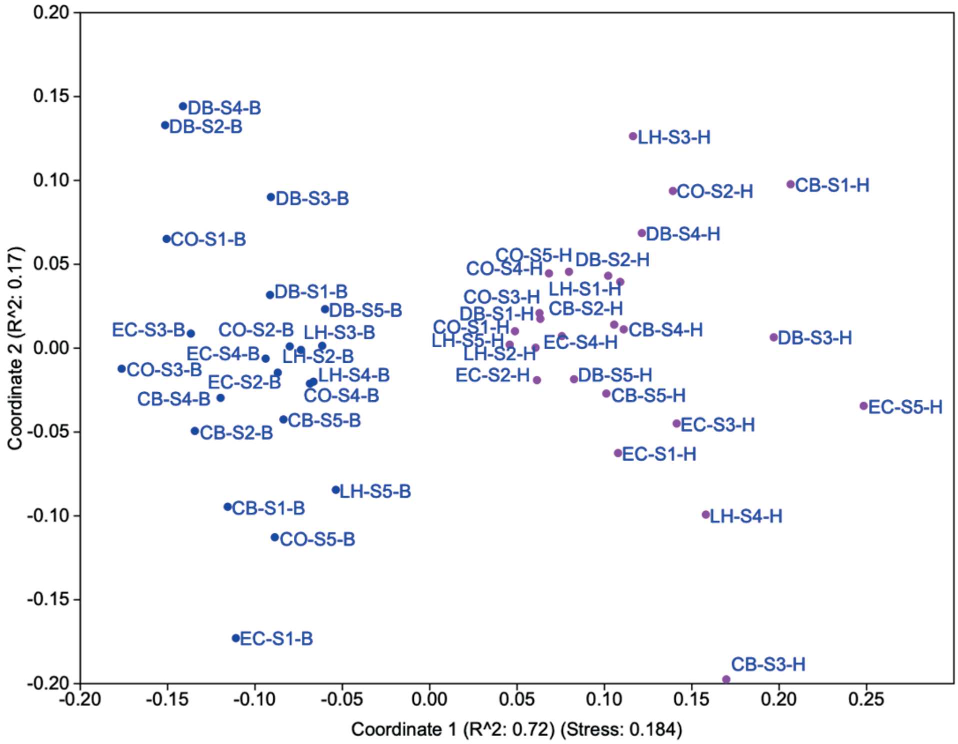

Figure 3. NMDS diagram showing the composition and spatiotemporal distribution of hyporheic fauna (H-points) and macrobenthic fauna (-B points). *Diagrama NMDS que muestra la composición y distribución espaciotemporal de la fauna hiporreica (puntos H) y macrobentónica (puntos B).*

Ellipura and Japygidae of the order Diplura), Pauropoda, Ostracoda, and Symphyla classes were not abundant. The phyla Annelida and Nematoda presented similar abundances, with 262 and 254 individuals, respectively. Mollusca and Platyhelminthes were poorly represented. The average Shannon diversity index (H') of the hyporheic invertebrate community was relatively high (H' = 2.18; σ = 0.18). The average richness of taxa was moderate (S = 14.2; σ = 3.3).

From the macrobenthic community, 450 organisms were collected, including three phyla: Arthropoda (92.4 %), Platyhelminthes (6.2), and Mollusca (5.8 %). Within Arthropoda, 92 % of organisms belonged to the Insecta class, while only 0.4 % belonged to the Arachnida class (Hydracnidiae). The comparison of hyporheic and macrobenthic invertebrates showed that of the 41 taxa collected in the HZ, 23 were common to the benthic zone (53 %), but the hyporheic organ-

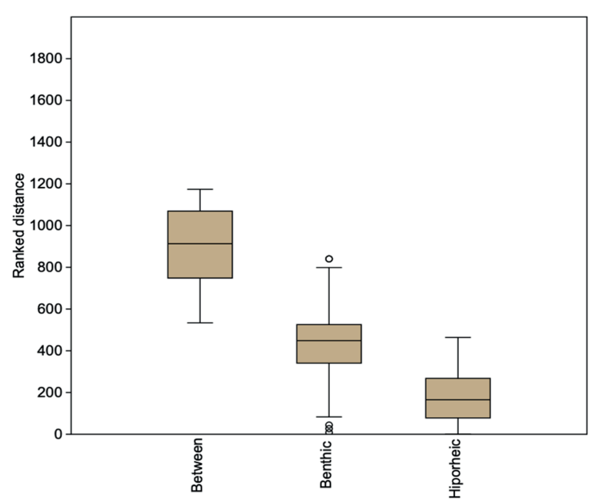

Figure 4. Boxplot showing similarity distances between the macrobenthic fauna and the hyporheic fauna from the ANOSIM results. *Diagrama de cajas que muestra las distancias en la similitud entre la fauna macrobentónica e hiporreica a partir de los resultados del ANOSIM.*

isms presented premature development compared to those in the benthic zone. The common taxa were *Corydalus* spp., *Bezzia* spp., Chironomidae, Libellulidae, Cenagrionidae, *Macrelmis* spp., *Heterelmis* spp., *Psephenus* spp., Hydrophilidae, *Anacroneuria* spp., *Cryphocricos* spp., *Helicopsyche* spp., *Atopsyche* spp., Hydropsychidae, *Tricorythodes* spp., *Leptohyphes* spp., *Thraulodes* spp., *Camelobaetidius* spp., Baetidae, Hydrachnidia, Planariidae, Physidae, and *Melanoides* spp.

The Shannon diversity index of the macrobenthic fauna tended to decrease from the headwaters (2.05) to the lower basin site (1.83). The richness presented a similar pattern, with ten taxa collected in the headwaters and seven taxa collected in the lower basin. The hyporheic fauna had a similar Shannon diversity index throughout the basin (approximately 2.15), but the richness gradually decreased, from 18 taxa in the headwaters to 11 taxa in the lower basin. When comparing the composition and abundance of the hyporheic and macrobenthic communities, the NMDS analysis (Fig. 3) revealed that the macrobenthic fauna samples (-B points, located to the left of the ordination graph) separated from the hyporheic fauna samples (points -H, located to the right of the arrangement). The ANOSIM had an R of 0.98 (p = 0.0001), indicating that there were significant differences between the compositions and

Table 3. Abundance of MPs per liter collected from filtered hyporheic water of the Dagua River. See Table 1 for site abbreviations. *Abundancia de MP por litro recolectadas de aguas hiporreicas filtradas del Río Dagua. Ver tabla 1 para las abreviaturas de los sitios.*

Monitoring period			Monitoring station	
1	April_17	465 ± 276	EC	333 ± 214
2	July_17	468 ± 373	CB	315 ± 186
3	Oct_17	717 ± 522	LH	436 ± 363
4	Jan_18	354 ± 179	BD	558 ± 368
5	Dec_18	518 ± 481	CO	880 ± 506

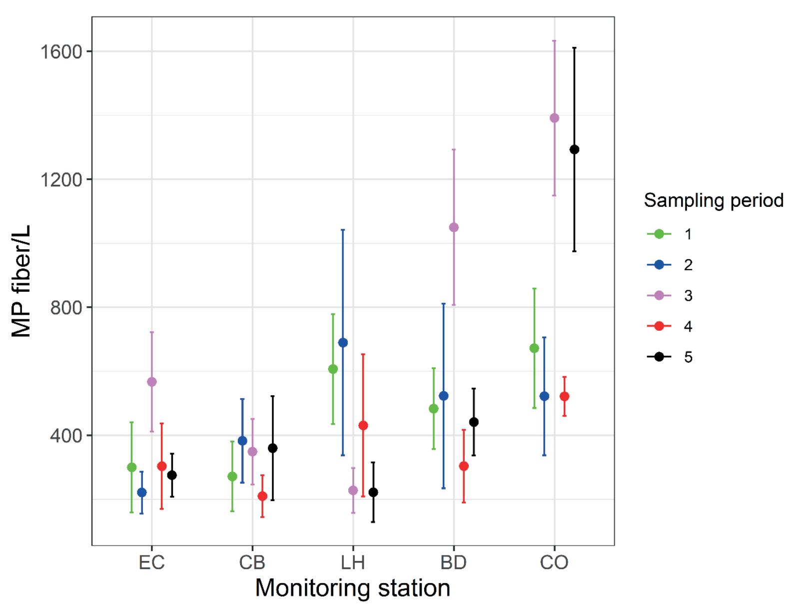

Figure 5. Spatiotemporal behavior of the average abundance of MP fibers at each sampling station (El Carmen EC, Centella Baja CB, La Harinera LH, Bitaco Dagua BD, and Córdoba CO) and in each sampling period (1 green: April 2017, 2 blue: July 2017, 3 pink: October 2017, 4 red: January 2018, and 5 black: December 2018). The vertical bars correspond to the standard deviation. *Comportamiento espaciotemporal de la abundancia promedio de las fibras de MP en cada estación de muestreo (El Carmen EC, Centella Baja CB, La Harinera LH, Bitaco Dagua BD y Córdoba CO) y en cada período de muestreo (1 verde: abril 2017, 2 azul: julio 2017, 3 rosado: octubre 2017, 4 rojo: enero 2018 y 5 negro: diciembre 2018). Las barras verticales corresponden a la desviación estándar.*

abundance of the hyporheic and macrobenthic fauna (Fig. 4). The statistical comparison of the diversity indices of the two groups of invertebrates showed that in all cases, there were significant differences in both the Shannon diversity (F = 11.75, p = 0.0012) and the richness of Margalef (F = 43.64, p = 3.2E-08) averages as well as the median Pielou equitability (tie corrected = 21.35, p = 3.83E-06).

Presence of MPs

Despite the methodological difficulties associated with the wells' locations and the types of samples obtained (semiquantitative), as mentioned in the methodology, all the samples collected during the monitoring periods yielded MPs, either at high or low concentrations. The plastic waste observed in the riverbed and its banks is probably the principal source of the MPs observed in the hyporheic interstitial water of the Dagua River. The average abundance of MPs had a high standard deviation, both spatially and temporally, with a greater range of variation in the middle section of the watershed; this range of variation decreased toward the lower basin. For the sampling periods, the variations were also wide and were especially high in October 2017 and December 2018 (Table 3). The abundance of MPs was higher at the lowest point of the basin, with great variability (Fig. 5). The ANOVA (Table 4) evaluating the interaction between the monitoring stations and samplings indicated that the concentrations of MPs depended on the interactions between these factors (*p value* = 0.003).

Comparisons of MP abundance between sampling periods for each site, performed using Tukey's test (df = 40; *p value* < 0.05), showed that October 2017 was different, with a greater abundance of MPs; in this month, a higher flow was reported (Fig. 2), and the abundance of MPs increased, especially at the intermediate stations and in the lower basin (LH, BD, and CO). Tukey's tests between monitoring sites for each sampling period (df = 10; *p value* < 0.05) showed that as the river descended from the headwater sites (EC and CB) to the intermediate sites (LH and BD) and the lower basin site (CO), the abundance of MPs increased (Fig. 5).

DISCUSSION

Hyporheic and macrobenthic fauna

In this work, the composition and diversity of hyporheic invertebrate communities in a Colombian Neotropical river are described for the first time; this data supplements studies conducted in the American tropics on this group of aquatic organisms, which are notably scarce (Mugnai et al., 2015a). The initial descriptive objective was complemented with the discovery of plastic fibers in the hyporheic environment, which drew our attention due to potential environmental problems associated with this type of emerging pollutant. New information about not only on the biological aspects of aquatic invertebrates in the Dagua River but also pollution by plastic fibers is reported, and these data are important for limnologists and the scientific community in general who are interested in the conservation of the biological diversity of the tropics.

Table 4. ANOVA of the MP abundance at the monitoring stations and in the sampling periods and their interactions, adjusted to a mixed linear model. DF: degrees of freedom; num: numerator; den: denominator; F: value of the F test. *ANOVA de la abundancia de MP en las estaciones de monitoreo, período de muestreo y su interacción, ajustada a un modelo lineal mixto. DF: grados de libertad; num: numerador; den: denominador; F: valores de la prueba F.*

Factors	numDF	denDF	F value	*p value*
(Intercept)	1	40	9 072 078	< 0.001
Monitoring station	4	10	381 037	0.0392
Sampling period	4	40	421 402	0.0061
Monitoring station/Sampling period	16	40	292 028	0.0030

Based on the richness and diversity of the taxa collected from the Dagua River and considering that Colombia has a large number of lotic systems, it is possible to suppose that the country has a high potential for hyporheos biodiversity. Most of the taxonomic groups collected from the Dagua River (Table 2) have been reported by various authors in different locations worldwide (e.g., Barrera González et al., 2014; Boon et al., 2016; Di Lorenzo et al., 2013; Moldovan

Table 5. Comparison of the number of hyporheic taxa recorded in the Dagua River with those of other studies. *Comparación del número de taxones hiporreicos registrados en el Río Dagua con otros estudios realizados en otras regiones. H: diversidad de Shannon; S: riqueza de Margalef; D: dominancia de Simpson.*

Ecosystems	Dominant groups of hyporheos and diversity data	Reference
Dagua River (Colombia)	Copepoda and Insecta (Diptera, Trichoptera, Coleoptera and Ephemeroptera Orders). Number of taxa: 41 H´ = 2.18, σ = 0.18; S = 14.2, σ = 3.3	This study
Speed River (Canada)	Chironomidae, Ephemeroptera, Copepoda, Oligochaeta, Hydracarina. Number of taxa: approx. 25	(Coleman & Hynes, 1970)
South Platte River (Colorado, USA)	Copepods: Parastenocaris. Number of taxa: 142	(Ward & Voelz, 1994)
16 streams in Oklahoma (USA)	Cyclopoida, Harpacticoida, Nematoda, Class Insecta (Chironomidae), Isopoda. S = 11.5 Number of taxa: 43	(Hunt & Stanley, 2003)
Kye Burn Stream (New Zealand)	Coleoptera, Ephemeroptera. S= (12,9-19 - 16,6); H'= 0.66 – 0.88). Number of taxa: 47	(Olsen & Townsend, 2003)
Tributary of Bigelow Brook (Massachusetts, USA)	Chironomidae, Elmidae, Hydrophilidae, Hydropsychidae, Leuctridae, Nemouridae, Tipulidae. H' (0,623-1,98); S = 31. Number of taxa: 31	(Collins et al., 2007)
Selwyn River (New Zealand)	Copepods, mites, oligochaetes, nematodes, and ostracods. Number of taxa: 56	(Datry et al., 2007)
Delour River (Ireland)	Number of taxa: 74	(Kibichii et al., 2009)
Turia and Palancia rivers (Spain)	Diptera (Chironomidae), Collembola, Copepoda (Cyclopoida), Annelida (Naididae). Number of taxa: 35	(Barrera González et al., 2014)
Tijuca River (Brazil)	Diptera and Copepoda; Number of taxa: 31; H’ = 2.5; D = 0.9	(Mugnai et al., 2015a)
Ashop, Black Brook and Lathkill rivers (United Kingdom)	Oligochaeta, Chironomidae. Number of taxa: 48	(Stubbington et al., 2016)
Ain, Bienne and Albarine rivers (France)	Orthocladiinae, Oligochaeta. Number of taxa: 63	(Stubbington et al., 2016)
Piddle and Frome rivers (United Kingdom)	Chironomidae, Nematoda, Oligochata. Number of taxa: 17	(Pacioglu & Robertson, 2017)
Lee and Rib reivers (United Kingdom)	Chironomidae, Nematoda, Harpacticoida, Oligochaeta. Number of taxa: 15	(Pacioglu & Robertson, 2017)

& Levei, 2015; Mugnai et al., 2015a; Pacioglu & Robertson, 2017; Peralta-Maraver et al., 2018; Pérez Fernández & Pérez Ruiz, 2015; Pryce et al., 2010; Stubbington et al., 2016; Tione et al., 2014). In the Neotropical region, Fernández (2002) and Fernández and Fossati-Gaschignard (2011) registered the Hydrachnidia and Oligochaete (Rodríguez, 2002) groups. In the Tijuca River in Brazil, Mugnai et al. (2015b) collected 31 taxa, of which almost 50 % (Copepoda, Diptera) were common with those found in the Dagua River. Table 5 shows a comparison of the biodiversity estimators obtained for the hyporheic fauna of the Dagua River with those from other regions of the world. Hyporheic fauna seem to have a widespread and cosmopolitan distribution, at least at gross taxonomic levels. However, it is necessary to further study the taxonomy since there could be Neotropical endemisms not yet described.

The HZ provides protection against predation and adverse conditions (e.g., floods, droughts, and pollution) and thus serves as a preservation habitat for organisms in the early stages of development, allowing future benthic colonization once surface flow conditions are restored (Stead et al., 2005). The common taxa between the hyporheic and macrobenthic fauna in the Dagua River, especially taxa in the incipient stages of development in the HZ, seem to confirm that this underground habitat acts as a refuge zone. The hypothesis of the HZ as a refuge area (Stubbington et al., 2009b; Dole-Olivier, 2011) has been controversial because direct comparisons between benthic and hyporheic environments are not easy due to sampling discrepancies, since there are no unified methods for sampling the two communities (Pugsley & Hynes, 1983; Williams & Hynes, 1974). Nevertheless, it is interesting to note that, unlike other investigations that have shown high similarity between the macrobenthic and hyporheic fauna (Bretschko, 1981; Bretschko & Leichtfried, 1988; Bretschko, 1991), in the Dagua River, this similarity was not so marked. The similarity in taxonomic composition was approximately 50 %, and there were significant differences in the abundances, Margalef richness, Shannon diversity, and Pielou equitability between the two groups of invertebrates.

Differences in geomorphological conditions, sampling depths, types of disturbances, and stream orders have led to the HHR being reconsidered as the “hyporheic refuge concept” (CRH) (Dole-Olivier, 2011), which takes into account infiltration, survival, and emigration. These aspects have not been studied simultaneously in tropical rivers. Some hyporheic taxa in the de Dagua River are classified as stygobites (obligate, or strictly subterranean, aquatic animals; Lopes et al., 2001), which includes copepods, ostracods, and diplurans in the Japygidae family (Gibert, 1991; Hahn & Matzke, 2005; Halse, 2018; Kayo et al., 2012; Ruffo, 1961). The presence of such stygofauna reaffirms the consideration of the HZ as an ecotone that contains a combination of epigeal aquatic fauna and other animals from the surrounding systems (Sabater & Vila, 1991). Benthic fauna migrate toward the HZ (Dole-Olivier, 2011; Stubbington et al., 2011; Williams & Hynes, 1974, 1976), but few studies have analyzed this process in conjunction with hydrological aspects (Datry, 2011; Stubbington et al., 2015).

The decrease in the hyporheic and macrobenthic taxa richness from the upper to the lower basin could be associated with habitat conditions. The stations in the headwaters (EC, CB) had narrow channels with rocky and stony beds that were hard to drill, steep slopes, high water velocities but low flows, and better coverage of riparian vegetation (Allan & Castillo, 2007; Buffington & Tonina, 2009). In contrast, stations in the middle and low basins (LH, BD, and CO) had wider channels, minor slopes, low current speeds, smaller numbers of stones and gravel but finer sediments, and higher solar radiation due to riparian vegetation deforestation. Seemingly, gravel riverbeds can support rich and diversified assemblages of invertebrates with the capacity to actively move within the substrate (Bo et al., 2006), while fine sediment prevents the vertical migration of organisms (Brunke & Gonser, 1997; Vadher et al., 2015; Vervier et al., 1992). The extraction of gravel and sand at stations LH and BD may also drastically affect the conservation of the heterogeneity of the river habitat. Geomorphologic diversity is a determinant factor in the colonization capacity and distribution of aquatic communities (Erman & Erman, 1984; Townsend &

Hildrew, 1994; J. V. Ward & Stanford, 1979); furthermore, food availability (Dobson & Hildrew, 1992) and hydraulic conditions (Quinn & Hickey, 1994) are affected. Physical heterogeneity influences hydrological exchange, which affects the migration capacity of invertebrates between benthic, hyporheic, and subterranean zones, influencing the community composition (Boulton & Foster, 1998; Dole-Olivier & Marmonier, 1992; Fowler & Scarsbrook, 2010; Olsen & Townsend, 2003; Varricchione et al., 2005). In summary, in the Dagua River, the macrobenthic and hyporheic faunal abundances decreased toward the lower basin, probably because the riverbed in this lower sector was obstructed by fine sediments, reducing the quality of habitats for invertebrates (Descloux et al., 2014).

Presence of plastic microfibers

The technique used to obtain MPs could be affected by river flow fluctuations, which could generate variations in their quantification. However, it is feasible to suppose that MP particles in hyporheic interstitial water are probably generated by the "partitioning" or fragmentation of plastic transported by the river (Chaukura et al., 2021). From the headwaters to the mouth of the Dagua River, plastic debris was observed on the banks. It was common to observe nurseries and other constructions for poultry breeding for which plastic fibers are used and exposed to the weather, which could degrade these materials to fibers similar to those observed trapped among the river stones. These elements, plus other types of plastics that are disposed directly into the river, are transported downstream.

In the Dagua River, MP particles can vertically penetrate the sediment, reaching the HZ due to hydrodynamic processes occurring in the benthos (Cardenas, 2015; Harvey & Bencala, 1993). In this way and considering the abundance of fibers per volume of hyporheic water in the Dagua River, the HZ could already be or become a potential reservoir for MP pollution, the effects of which are still to be elucidated. Comparing the MP abundances obtained in this study with those recorded in other rivers is challenging since microfibers have been evaluated in streams and sediments but not in interstitial water (Jiang et al., 2019), as is the case for our samples, which represent filtrates obtained from the hyporheic sediment. Another aspect that makes comparison difficult is that a standard MP abundance unit has not been agreed upon. Authors have expressed the abundance of MP as m^3 per water volume, kg per sediment weight, or m^2 per water or sediment area, applying other sampling methods, such as the Bou Rouch or freezing core method. The presence of MP fibers in the hyporheic water of the Dagua River is worrisome in the context of invertebrate community health and the general functioning of the river ecosystem; this should be studied more deeply.

CONCLUSIONS

The hyporheic fauna in the Dagua River showed a notable diversity and richness, which may be higher than those reported in other studies if complementary sampling techniques are used, is deepened and knowledge about the taxonomy of these organisms. The data from the Dagua River suggest that the biodiversity of these communities in Colombian rivers may be high, as has been observed for macrobenthic invertebrates. Although the composition of the hyporheic fauna was notably similar to that of the macrobenthic invertebrate community, its structure, measured by ecological indices, was different.

The variety of taxa collected from the HZ of the Dagua River expands the knowledge about the hyporheos in tropical rivers. Given the general lack of information, it is important to improve our understanding about the hyporheic fauna in tropical zones, recognize its role in the different processes associated with rivers, and generate alerts about the risks to these ecosystems by plastic pollutants. The presence of MPs in the HZ throughout the Dagua River basin could affect the meiofauna that inhabit the hyporheic habitat. Considering the abundance of fibers per volume of hyporheic water, we suggest that the HZ could be or become a future reservoir for MP pollution. It is essential to carry out interdisciplinary studies throughout the basin that consider other aspects, such as geology, sediment composition, land use, hydrodynamics, and water quality.

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