



Effects of human disturbance gradient on aquatic macroinvertebrate diversity: A study in a river of the Sierra Nevada de Santa Marta

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ABSTRACT

Understanding the environmental factors that shape macroinvertebrate diversity is particularly important because rivers in the neotropics are continuously affected by changes in land use. The main objective of this work was to analyze how environmental variables associated with different levels of human activity shape the macroinvertebrate community in the Ranchería River. Various environmental variables were measured at each of the nine sampling stations distributed across three gradients of human activity in the Ranchería River Basin. The sampling design included three stations in the high elevation zone (P9, P8, P7; 595 to 308 masl), three in the medium elevation zone (P6, P5, P4; 153 to 112 masl), and three in the low elevation zone (P3, P2, P1; 90 to 22 masl). For aquatic macroinvertebrates, five monthly samples were taken from January to May 2010 from these nine stations. A total of 4,615 individuals were collected, distributed across 16 orders, 54 families, and 83 taxa. The taxa with the highest relative abundance were *Simulium* (Diptera), *Melanoides* sp. (Thiaridae), and Orthocladiinae (Diptera: Chironomidae), with 20, 11, and 9% respectively. It was found that anthropogenic impacts shape the environmental variables and the structure of macroinvertebrate communities in the Ranchería River Basin.

Keywords: anthropic affectations, canonical correspondence analysis, Hill numbers, Rancheria River, Sierra Nevada de Santa Marta.



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Efeitos do gradiente de perturbação humana na diversidade de macroinvertebrados aquáticos: Um estudo em um rio da Sierra Nevada de Santa Marta

RESUMO

Compreender os fatores ambientais que moldam a diversidade de macroinvertebrados é particularmente importante porque os rios nos neotrópicos são continuamente afetados por mudanças no uso da terra. O principal objetivo deste trabalho foi analisar como as variáveis ambientais associadas a diferentes níveis de atividade humana moldam a comunidade de macroinvertebrados no rio Ranchería. Várias variáveis ambientais foram medidas em cada uma das nove estações de amostragem distribuídas ao longo de três gradientes de atividade humana na bacia do rio Ranchería. O desenho de amostragem incluiu três estações na zona de elevação alta (P9, P8, P7; 595 a 308 metros acima do nível do mar), três na zona de elevação média (P6, P5, P4; 153 a 112 metros acima do nível do mar) e três na zona de baixa elevação (P3, P2, P1; 90 a 22 metros acima do nível do mar). Para os macroinvertebrados aquáticos, foram coletadas cinco amostras mensais de janeiro a maio de 2010 nestas nove estações. Um total de 4.615 indivíduos foram coletados, distribuídos em 16 ordens, 54 famílias e 83 táxons. Os táxons com maior abundância relativa foram *Simulium* (Diptera), *Melanoides* sp. (Thiaridae) e *Orthocladiinae* (Diptera: Chironomidae), com 20, 11 e 9% respectivamente. Constatou-se que os impactos antropogênicos moldam as variáveis ambientais e a estrutura das comunidades de macroinvertebrados na bacia do rio Ranchería.

Palavras-chave: afetações antrópicas, análise de correspondência canônica, números de Hill, Rio Ranchería, Sierra Nevada de Santa Marta.

1. INTRODUCTION

Aquatic macroinvertebrates are organisms with a body size greater than 250 µm and a direct relationship to the aquatic environment during at least one stage of their life cycle (Hauer and Resh, 2007). This group includes mollusca, annelida, nematoda, platyhelminthes, and arthropoda, with arthropods typically being the most numerous and diverse, especially insects (Hauer and Resh, 2007). The most ecologically significant insect orders in their larval stage in rivers tend to be Ephemeroptera, Trichoptera, Plecoptera, Diptera, Coleoptera, Megaloptera, and Odonata (Giller and Malmqvist, 1998).

Macroinvertebrates are found in practically all continental aquatic ecosystems, with the vast majority developing in lotic systems (Hynes, 1970). Most macroinvertebrates have specific habitat requirements (Lencioni *et al.*, 2018), such that some species more sensitive to minimal changes in water quality can serve as indicators of lotic system health in freshwater biomonitoring assessments (Paggi, 2003). Similarly, macroinvertebrates require specific hydrological regimes to complete reproductive and other life-cycle stages (Merritt and Cummins, 1996). Thus, the overall success of diverse macroinvertebrate communities depends largely on intact physical environments (Karr *et al.*, 1986). For example, multiple adverse effects of stream sedimentation on macroinvertebrate community composition and structure have been reported (Krynak and Yates, 2018; Ryan, 1991; Bilotto and Brazier, 2008; Moore *et al.*, 2014), including decreased diversity and density (Quinn *et al.*, 1992), and reduced habitat availability (López-López *et al.*, 2015). Mining, agriculture and urban expansion have altered the physicochemical and biotic environment of aquatic systems in the neotropics (Villamarín *et al.*, 2021) through changes in nutrient inputs, sediment transport, flow, temperature, and invasive species introductions (Ríos-Touma and Ramírez, 2019) which have impacted macroinvertebrate communities and functional diversity (Prat *et al.*, 2009). Given the

importance and diversity of macroinvertebrates in aquatic environments, advancing ecological knowledge to understand how environmental stressors modify aquatic macroinvertebrate community structure and composition is fundamental.

The Sierra Nevada de Santa Marta (SNSM), an isolated mountain range located in northern Colombia along the Caribbean coast, is renowned for its exceptional levels of endemism across diverse taxonomic groups. Despite its biological significance, studies characterizing the aquatic macroinvertebrate fauna, including any potential endemic species, are limited. Most research has focused on the Gaira River Basin (Granados-Martínez *et al.*, 2016; Tamaris-Turizo *et al.*, 2013; 2020; Oliveros-Villanueva *et al.*, 2020). Studies of different areas of the SNSM include those reported by Jaimes-Contreras and Granados-Martínez (2016), Barros-Núñez and Granados-Martínez (2016) and Barragán *et al.*, (2016). Currently, only one study has been conducted in the upper Ranchería River Basin (Barragán *et al.*, 2016). Given that neotropical rivers are continually affected by land use changes (Ríos-Touma and Ramírez, 2019), understanding the environmental factors shaping macroinvertebrate diversity is critical. The main objective of this study was to analyze how environmental variables associated with different levels of human activity influence the macroinvertebrate community in the Ranchería River.

2. MATERIAL AND METHODS

2.1. Study Area

The Ranchería River Basin is located in the Department of La Guajira, in northernmost Colombia. The basin originates in the eastern flank of the SNSM mountain range, specifically in the Chirigua Páramo at an elevation of 3,875 meters above sea level (masl). After a course of approximately 248 km, the river drains a watershed area of 4,070 km² and discharges into the Caribbean Sea near the city of Riohacha (Corpoguajira, 2012; Pérez *et al.*, 2018). The basin is in the northern region of La Guajira, which is the most arid zone of Colombia, with annual rainfall ranging from 501 to 1,000 mm (Corpoguajira, 2012). Precipitation is highest in the headwaters of the basin and gradually decreases northward. Sampling was conducted in three zones of slight elevation differences: the high part from 595 to 308 masl, the middle part from 153 to 112 masl, and the low part from 90 to 22 masl (Figure 1). The high elevation areas were considered as reference points due to minimal human influence in the zone. The middle elevation areas were near urban zones and agricultural activities, while the low elevation areas were close to an open-pit coal mine. This gradient of human activity allows for the assessment of anthropogenic impacts on the river ecosystem.

Various environmental variables were measured at each of the nine sampling stations distributed across three gradients of human activity in the Ranchería River Basin. The sampling design included three stations in the high elevation zone (P9, P8, P7; 595 to 308 masl), three in the medium elevation zone (P6, P5, P4; 153 to 112 masl), and three in the low elevation zone (P3, P2, P1; 90 to 22 masl) (Figure 1). Discharge was estimated at each station by float method. Water quality parameters including temperature (°C), dissolved oxygen (DO, mg/L), conductivity (µS/cm), and pH were measured using a WTW 350 multi-parameter probe. The probe measures conductivity and calculates an approximation of total dissolved solids (TDS) based on an assumed relationship, using the following equation: TDS (ppm) = EC (µS/cm) × conversion factor, where the conversion factor typically ranges between 0.55 and 0.8 depending on the chemical composition of the water (Walton, 1989). Additionally, water samples were collected in sterilized polyethylene bottles at each station for subsequent laboratory analyses of nutrients and microbiological components. These samples were processed using Standard Methods protocols at the University of La Guajira water quality laboratory to quantify nitrite (NO₂⁻), nitrate (NO₃⁻), ammonium NH₄⁺ (mg/L), phosphate (PO₄³⁻), total coliforms (TC) and fecal coliforms (FC).

2.2. Biological sample collection

Macroinvertebrate sampling was conducted at each of the nine stations distributed across three gradients of human disturbance in the Ranchería River Basin. These stations were located in zones of slight elevation differences, which nonetheless corresponded to varying levels of human impact. At each station, a 100-m reach was selected, and five ($n=5$) samples were collected with a Surber net (0.09-m² area, 250-µm mesh) that integrated all substrate types present within each stretch, following the methodology of Chará (2004). Sampling was performed monthly from January to May 2010. The collected material was cleaned, sorted, and stored in labeled plastic vials containing 96% ethanol for preservation. Samples were not deposited in an entomological collection since they were intended for subsequent diet analysis. Macroinvertebrates were identified to genus and/or morphotype using a stereo microscope and taxonomic keys including Domínguez and Fernández (2009), Posada-García (2003), Roldán (1988), and Springer (2010).

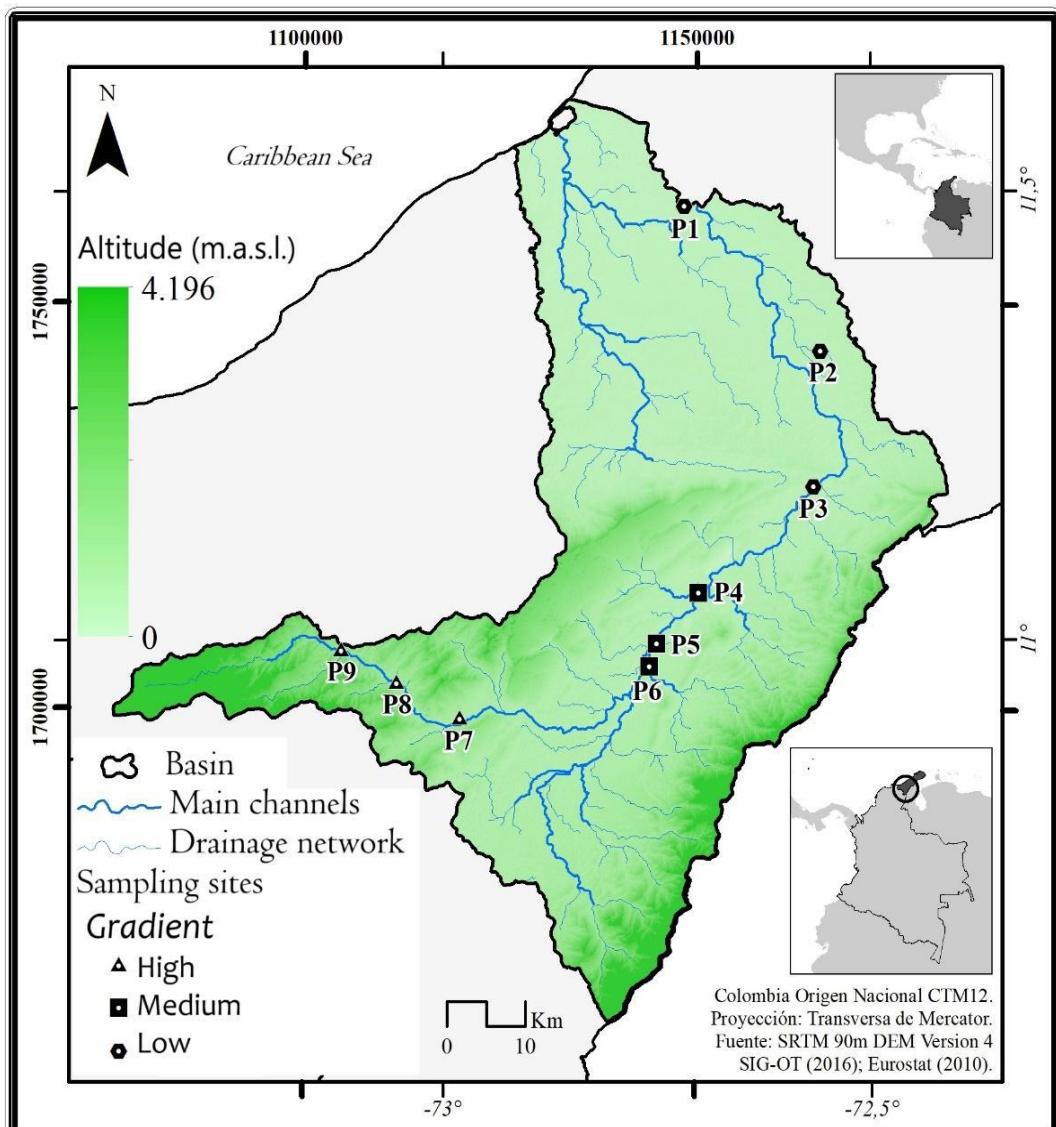


Figure 1. Sampling stations along a gradient of human influence in the Ranchería River. The high elevation areas (P7, P8, P9) were considered as reference points due to minimal human influence in the zone. The middle elevation areas (P4, P5, P6) were near urban zones and agricultural activities, while the low elevation areas (P1, P2, P3) were close to an open-pit coal mine.

2.3. Data analysis

Data analysis was conducted to determine differences in environmental and microbiological variables between sampling stations. Due to the failure to meet the assumptions required for parametric tests, the non-parametric Kruskal-Wallis test was applied to all variables. This test was used to assess differences across sampling stations without relying on the assumptions of normality and homoscedasticity. Results were considered statistically significant at a p-value threshold of 0.05. These analyses were carried out using PAST Version 2.0. To demonstrate the variability pattern between human disturbance gradient and environmental and microbiological variables, a canonical discriminant analysis was performed using R Version 2.15.2 (R Core Team, 2015) and RWizar (Guisande, 2015). Results from this analysis were considered significant when the p-value was less than 0.05.

To determine the relationship between human disturbance gradient and abundance of the most representative taxa, canonical discriminant analysis was applied, followed by ANOSIM with Bonferroni correction to detect which taxa contributed most to dissimilarity among gradients. Similarity percentage analysis (SIMPER) was also used. All analyses were performed using PAST Version 2.0.

The effective number of species was determined as a measure of alpha diversity through Hill numbers (Jost, 2006). Rarefaction-extrapolation curves were generated for the first three Hill numbers ($q=0$, $q=1$, and $q=2$). Order zero diversity ($q=0$) represents richness, order one diversity ($q=1$) weights typical species by calculating the exponential of the Shannon entropy index, and order two diversity ($q=2$) gives more weight to dominant species by calculating the inverse Simpson index (Jost, 2006; Chao *et al.*, 2014). Rarefaction-extrapolation curves expressed the accumulation of observed and estimated species based on individuals captured, with a 95% confidence interval obtained through bootstrap. Analyses were performed using R Version 3.0.3 (R Core Team, 2015) with the "iNEXT" package 2.0.5 (Hsieh *et al.*, 2016). In order to infer relationships between environmental variables, microbiological variables, the taxa found and gradient, a canonical correspondence analysis (CCA) was performed. The data were transformed using $\log_{10}(X+1)$ to meet the assumptions of the analysis. CCA is a multivariate technique suitable for exploring associations among sets of variables. Transforming the data is often necessary to fulfill the analysis requirements, and $\log_{10}(X+1)$ is common for count data that may contain zeros. This approach provides an effective way to understand the relationships between the variables of interest along a gradient. The CCA was conducted using PAST Version 2.0 software.

3. RESULTS

Discharge, conductivity, pH, and temperature increased along the gradient of human activity. We recorded significant differences along the human disturbance gradient for conductivity, pH, and temperature. In contrast, dissolved oxygen (DO) decreased as human activity increased. For TDS, the highest concentration occurred in areas with moderate human activity, with significant differences along the gradient. Overall, nutrient concentrations were very low. Phosphate and ammonium concentrations increased with increasing human activity, while nitrate and nitrite concentrations peaked in areas with moderate human influence. However, only nitrate exhibited statistically significant differences along the human disturbance gradient, while differences in phosphate, ammonium, and nitrite concentrations were not statistically significant. Microbiological parameters also increased with increasing human activity, with the lowest fecal and total coliform concentrations in areas with minimal human influence (Table 1). Figure 2 illustrates differences along the human disturbance gradient, with 93% cumulative variation explained by the first canonical axis. Dissolved oxygen (DO) and coliform concentrations followed similar patterns along the gradient, with higher DO concentrations and lower coliform concentrations observed in the upper river stations compared

to the lower river stations.

Table 1. Mean values, standard deviation (SD) of physicochemical variables, nutrients, and microbiological parameters at different stations along the gradient of human disturbance in the Ranchería River. Asterix indicate significant differences. (High: minimal human influence, Medium: near urban and agricultural areas, Low: close to open-pit coal mine).

Variables	High	SD	Medium	SD	Low	SD
Q (m^3/s)	11.9	25.0	15.5	105.6	17.5	177.4
$\mu\text{S}/\text{cm}$	42.8	0.6	226.3	0.7	278.3*	1.9
DO (mg L^{-1})	7.9	0.4	7.5	0.5	6.0*	2.0
pH	7.3	1.9	7.9	1.4	7.2	7.4
T ($^{\circ}\text{C}$)	22.7	0.1	27.5*	0.2	26.4	0.3
PO_4 (mg L^{-1})	0.2	0.3	0.3	0.4	0.4	0.6
NH_4 (mg L^{-1})	0.4	0.0	0.6	0.2	0.7	0.2
NO_3 (mg L^{-1})	0.1	0.0	0.4*	0.1	0.3*	0.0
NO_2 (mg L^{-1})	0.01	7.0	0.04	65.9	0.02	81.8
Solids (ppm)	61.5	14.5	487.3*	168.3	441.6*	104.1
FC (MPN/100 mL)	2912.0	12353.8	4163.3	14563.0	8046.0	31446.1
TC (MPN/100 mL)	5771.3	6.4	11516.0	8.6	18874.7	8.4

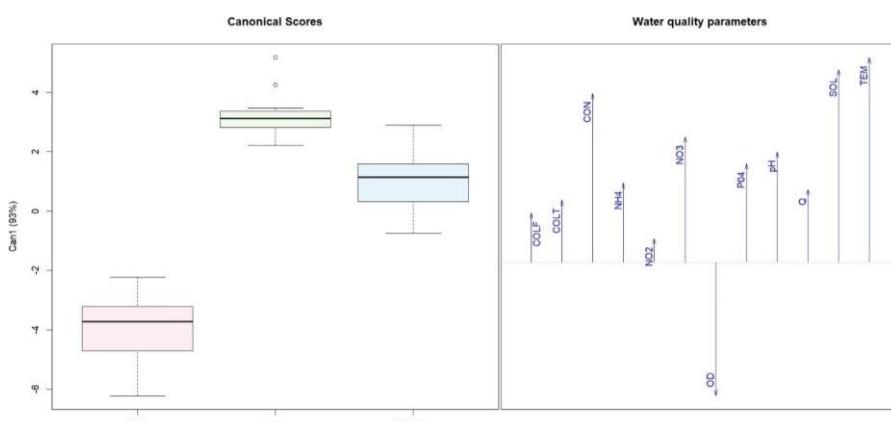


Figure 2. Canonical Discriminant Analysis applied to physicochemical and microbiological variables along the gradient of human influence in the Ranchería River. (High: minimal human influence, Medium: near urban and agricultural areas, Low: close to open-pit coal mine).

A total of 4,615 individual macroinvertebrates were collected, distributed across five phyla, eight classes, 16 orders, 54 families, and 83 taxa (Table 2). The most abundant orders across all sampling were Diptera, Ephemeroptera, and Mesogastropoda, representing 31%, 26%, and 12% of relative abundance, respectively. Megaloptera and Veneroida were the least represented orders. The families with the highest representation were Simuliidae, Leptohyphidae, and Thiaridae, with 21%, 12%, and 11%, respectively. The most abundant taxa were *Simulium*, *Melanoides*, and Orthocladiinae, comprising 20%, 11%, and 9% of individuals, respectively. In the areas with minimal human influence (reference points), 16 orders distributed among 47 families and 66 taxa were found. The areas near urban zones and agricultural activities had 17 orders distributed in 45 families and 60 taxa, while the areas close to the open-pit coal mine contained 14 orders distributed among 38 families and 46 taxa. Completeness values were satisfactory for each zone of human activity with values above 0.95 at all stations and the highest value of 0.99 in the areas with minimal human influence (Table 3).

Table 2. Taxonomic list of aquatic macroinvertebrates associated with a gradient of human disturbance in the Ranchería River Basin. (High: minimal human influence, Medium: near urban and agricultural areas, Low: close to open-pit coal mine).

Order	Family	TAXON	HIGH	LOW	MEDIUM	TOTAL
Coleoptera	Planorbidae	<i>Gyralus</i>	6	1	3	10
		<i>Austrolimnius</i>	80	2	6	88
		<i>Microcylloepus</i>	59	0	8	67
		<i>Cylloepus</i>	36	1	27	64
		<i>Heterelmis</i>	23	0	15	38
	Elmidae	<i>Pseudodisersus</i>	18	1	4	23
		<i>Phanocerus</i>	6	0	11	17
		<i>Macrelmis</i>	1	0	7	8
		<i>Onychelmis</i>	6	1	0	7
		<i>Hexanchorus</i>	0	0	1	1
Decapoda	Gyrinidae	<i>Gyrinus</i>	2	0	0	2
	Hydraenidae	<i>Ochtebius</i>	0	0	3	3
		<i>Hydraena</i>	1	0	0	1
	Hydrophilidae	<i>Hydrophilus</i>	2	0	0	2
	Psephenidae	<i>Psephenops</i>	10	1	0	11
	Atyidae	<i>Atya</i>	0	57	0	57
	Palaemonidae	<i>Macrobrachium</i>	2	55	17	74
	Trichodactylidae	<i>Trichodactylus</i>	0	1	0	1
	Blepharoceridae	<i>Limonicola</i>	2	0	0	2
	Ceratopogonidae	<i>Alluaudomyia</i>	0	8	23	31
		<i>Probezzia</i>	0	1	0	1
Diptera	Chironomidae	<i>Orthocladiinae</i>	214	39	112	365
		<i>Tanypodinae</i>	22	5	2	29
		<i>Chironomus</i>	2	0	0	2
	Empididae	<i>Hemerodromia</i>	1	0	1	2
	Psychodidae	<i>Maurina</i>	5	0	1	6
	Simuliidae	<i>Simulium</i>	732	3	225	960
	Stratiomyidae	<i>Stratiomys</i>	0	1	0	1
	Tabanidae	<i>Tabanus</i>	1	0	1	2
	Tipulidae	<i>Hexatoma</i>	0	0	24	24
	Baetidae	<i>Baetodes</i>	200	9	31	240
		<i>Americabaetis</i>	48	37	28	113
		<i>Camelobaetidius</i>	53	11	10	74
		<i>Guajirolus</i>	3	1	2	6
Ephemeroptera	Leptohyphidae	<i>Tricorythodes</i>	187	42	120	349
		<i>Leptohypes</i>	140	7	87	234
	Leptophlebiidae	<i>Farrodes</i>	2	29	61	92
		<i>Traulodes</i>	2	0	0	2
	Oligoneuriidae	<i>Lachlania</i>	72	9	6	87

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	Gerridae	<i>Eurygerris</i>	1	1	1	3
Hemiptera	Naucoridae	<i>Ambrysus</i>	1	7	5	13
		<i>Limnocoris</i>	10	2	1	13
		<i>Pelicoris</i>	1	0	0	1
	Mesoveliidae	<i>Mesovelia</i>	2	5	4	11
	Veliidae	<i>Rhagovelia</i>	7	58	65	130
Lepidoptera	Crambidae	<i>Piralidae</i>	83	1	5	83
		<i>Petrophila</i>	3	0	0	3
Megaloptera	Corydalidae	<i>Corydalus</i>	2	0	2	4
Mesogastropoda	Ancylidae	<i>Ferrissia</i>	0	0	1	1
	Hydrobiidae	<i>Amnicola</i>	0	0	2	2
	Pleuroceridae	<i>Gonobiasis</i>	0	0	58	58
	Thiaridae	<i>Melanoides</i>	0	393	125	518
Basommatophora	Lymnaeidae	<i>Lymnaea</i>	0	2	4	6
Veneroida	Sphaeridae	<i>Pisidium</i>	0	0	1	1
Odonata	Aeshnidae	<i>Anax</i>	24	9	0	33
	Calopterigidae	<i>Hetaerina</i>	3	1	2	6
	Coenagrionidae	<i>Argia</i>	3	20	3	26
	Gomphidae	<i>Progomphus</i>	3	0	1	4
	Libellulidae	<i>Brechmoroga</i>	0	0	4	4
Plecoptera	Perlidae	<i>Anacroneuria</i>	63	21	9	93
	Calamoceratidae	<i>Phylloicus</i>	7	1	1	9
	Glossosomatidae	<i>Protoptila</i>	18	0	0	18
		<i>Culoptila</i>	5	0	0	5
	Helicopsychidae	<i>Helicopsyche</i>	5	0	0	5
	Hydrobiosidae	<i>Atopsyche</i>	5	0	0	5
	Hydropsychidae	<i>Smicridea</i>	146	16	79	241
Trichoptera	Hydroptilidae	<i>Metrichia</i>	0	0	1	1
		<i>Hydroptila</i>	1	0	0	1
		<i>Ochrotrichia</i>	21	0	0	21
		<i>Metrichia</i>	8	0	0	8
		<i>Hydroptila</i>	1	0	2	3
	Leptoceridae	<i>Atanatolica</i>	40	0	0	40
		<i>Grumichella</i>	32	0	0	32
		<i>Oecetis</i>	14	0	1	15
		<i>Nectopsyche</i>	0	5	2	7
	Odontoceridae	<i>Marilia</i>	2	0	1	3
	Philopotamidae	<i>Chimarra</i>	15	0	4	19
	Polycentropodidae	<i>Polycentropus</i>	0	2	0	2
	Xiphocentronidae	<i>Xiphocentronidae</i>	0	1	0	1
Tricladida	Planariidae	<i>Dugesia</i>	1	0	3	4
Trombidiformes	Arrenuridae	<i>Arrenurus</i>	5	24	8	37
Tubifida	Tubificidae	<i>Limnodrilus</i>	0	17	8	25
		<i>Tubifex</i>	2	0	2	4
		Total				4615

Table 3. Macroinvertebrate sampling completeness in the Ranchería River, where total abundance represents the number of recorded individuals, Sobs is the observed richness, and inventory completeness is calculated through Cm. (High: minimal human influence, Medium: near urban and agricultural areas, Low: close to open-pit coal mine).

Gradient	Total abundance	Sobs	Completeness (Cm)
High	2475	66	0.99
Medium	1242	60	0.98
Low	911	46	0.98

Figure 3 shows differences along the gradient of human activity. These differences were driven by the taxa *Americabaetis*, *Anacroneuria*, *Austrolimnius*, *Baedodes*, *Camelobaetidius*, *Lachlania*, *Orthocladiinae*, *Simulium*, *Smicridea*, and *Tricorythodes*, which displayed higher abundance values in areas with minimal human influence (reference points). In contrast, the taxa *Melanoides* and *Rhagovelia* were more abundant in areas with moderate to high human activity (near urban zones, agricultural activities, and the open-pit coal mine). ANOSIM analysis confirmed significant differences ($R = 0.17$, $P = 0.0002$) in macroinvertebrate communities associated with the level of human disturbance, with distinct assemblages in minimally disturbed areas compared to moderately and highly disturbed stations. SIMPER analysis indicated the taxa contributing most to differences between gradients were *Simulium* (21%), *Melanoides* (17%), *Orthocladiinae/morphospecies 1* (9%), *Tricorythodes* (7%), and *Rhagovelia* (7%), which is a similar pattern as the CDA results.

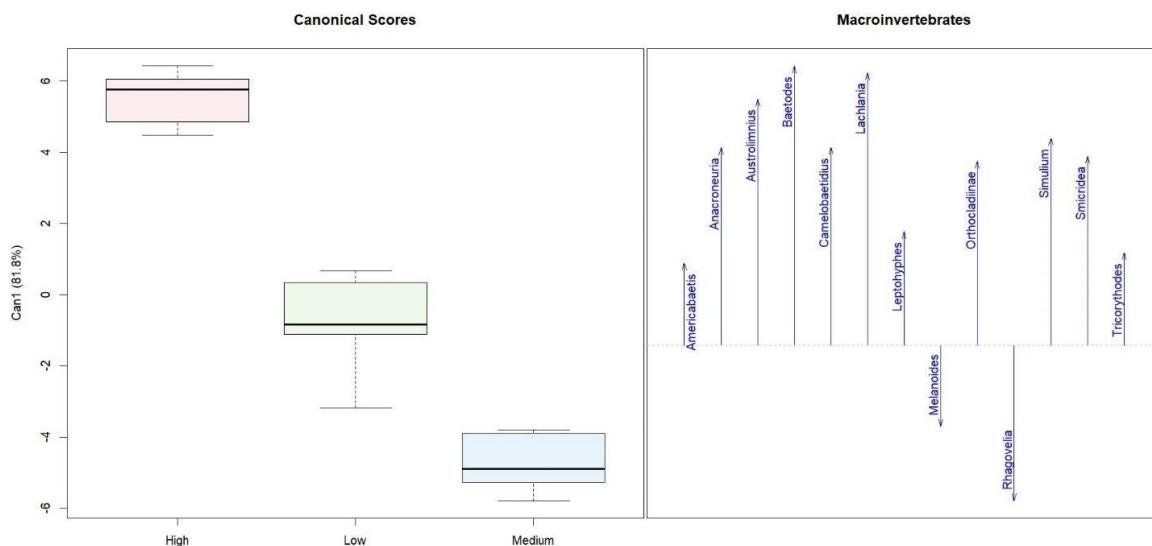


Figure 3. Canonical discriminant analysis of the altitudinal gradients based on the most abundant macroinvertebrates in the Ranchería River. (High: minimal human influence, Medium: near urban and agricultural areas, Low: close to open-pit coal mine).

Total abundance was lower in the areas with high human activity (near the open-pit coal mine) ($n=911$) compared to the areas with moderate human activity (near urban zones and agricultural activities) ($n=1242$) and areas with minimal human influence (reference points) ($n=2475$). When comparing effective species richness between macroinvertebrates collected at different levels of human disturbance, relative to the lowest total abundance (high human activity area) (vertical dashed line, Figure 4), the effective number of species ($q=0$) was 53.8 in minimally disturbed areas, 57.1 in moderately disturbed areas, and 45.9 in highly disturbed areas, with no significant differences. The diversity of moderately abundant species,

corresponding to the exponent of Shannon diversity ($q=1$), differed significantly along the gradient, with values of 17.4 in minimally disturbed areas, 19.9 in moderately disturbed areas, and 11.3 in highly disturbed areas (Figure 4). Furthermore, $q=2$ (the inverse Simpson index) also showed significant differences across the three levels of human disturbance, with the lowest number of dominant species in the highly disturbed area ($q=2 = 4.8$), the highest value in the moderately disturbed area ($q=2 = 12.6$), and a value of 8.9 in the minimally disturbed area (Figure 4).

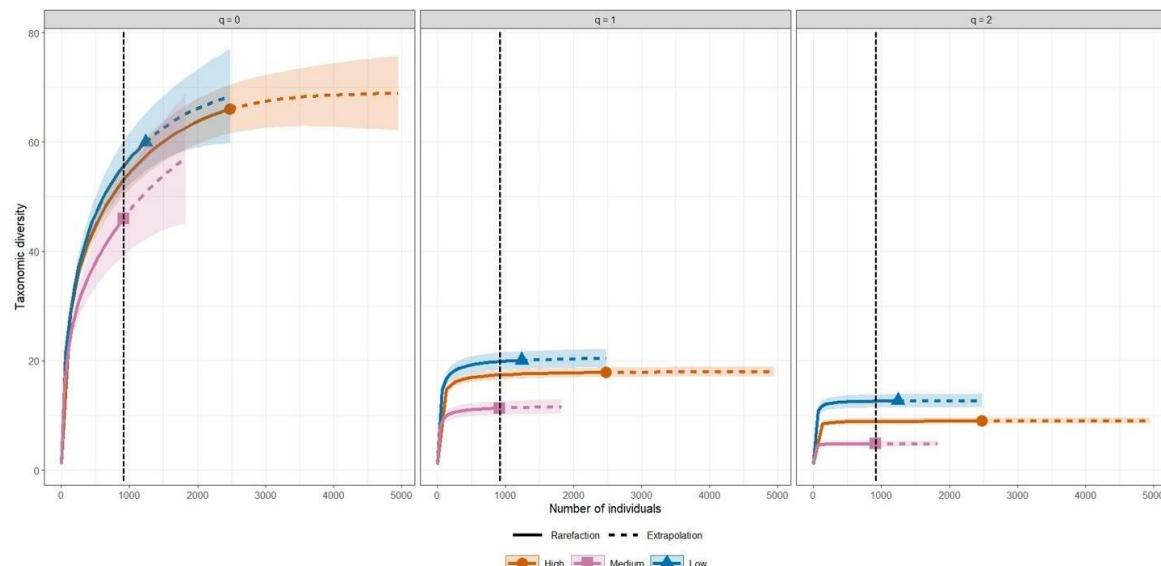


Figure 4. Interpolation (rarefaction)/extrapolation curves of macroinvertebrate diversity based on Hill numbers collected at different level of human disturbance (High: minimal human influence, Medium: near urban and agricultural areas, Low: close to open-pit coal mine). The bands correspond to the 95% confidence interval.

According to first-order Hill numbers (without weighting species abundance), areas with moderate human activity were more diverse than areas with minimal human influence by only three (3) species, while areas with high human activity were less diverse by 37 species, representing a significant difference in diversity compared to minimally and moderately disturbed sites. This pattern held with extrapolation of the curves by increasing sampling effort (Figure 4a). Variations in total abundance across the three levels of human disturbance (minimal, moderate, high) showed statistical differences ($H = 31.8$; $P < 0.05$), where mean abundance in highly disturbed areas was completely distinct from the other two gradients..

The canonical correspondence analysis (CCA) explained 99% of the total variance on Axes 1 and 2, as indicated by the inertia values of the matrix. The first axis positively associated the upper section with DO, along with the *Anacroneuria*, *Smicridea*, *Cylloepus*, *Microcylloepus*, *Simulium*, *Leptohyphes*, *Baetodes*, *Piralidae*, *Lachlania*, *Smicridea*, and *Atanatolica* taxa. In contrast, the negative end of Axis 1 was associated with the middle and lower zones the temperature, TDS, conductivity, discharge and pH variables, and the *Camelobaetidius*, *Americabaetis*, *Orthocladinae*, *Tricorythodes*, *Rhagovelia*, *Melanoides* and *Atya* taxa (Figure 5).

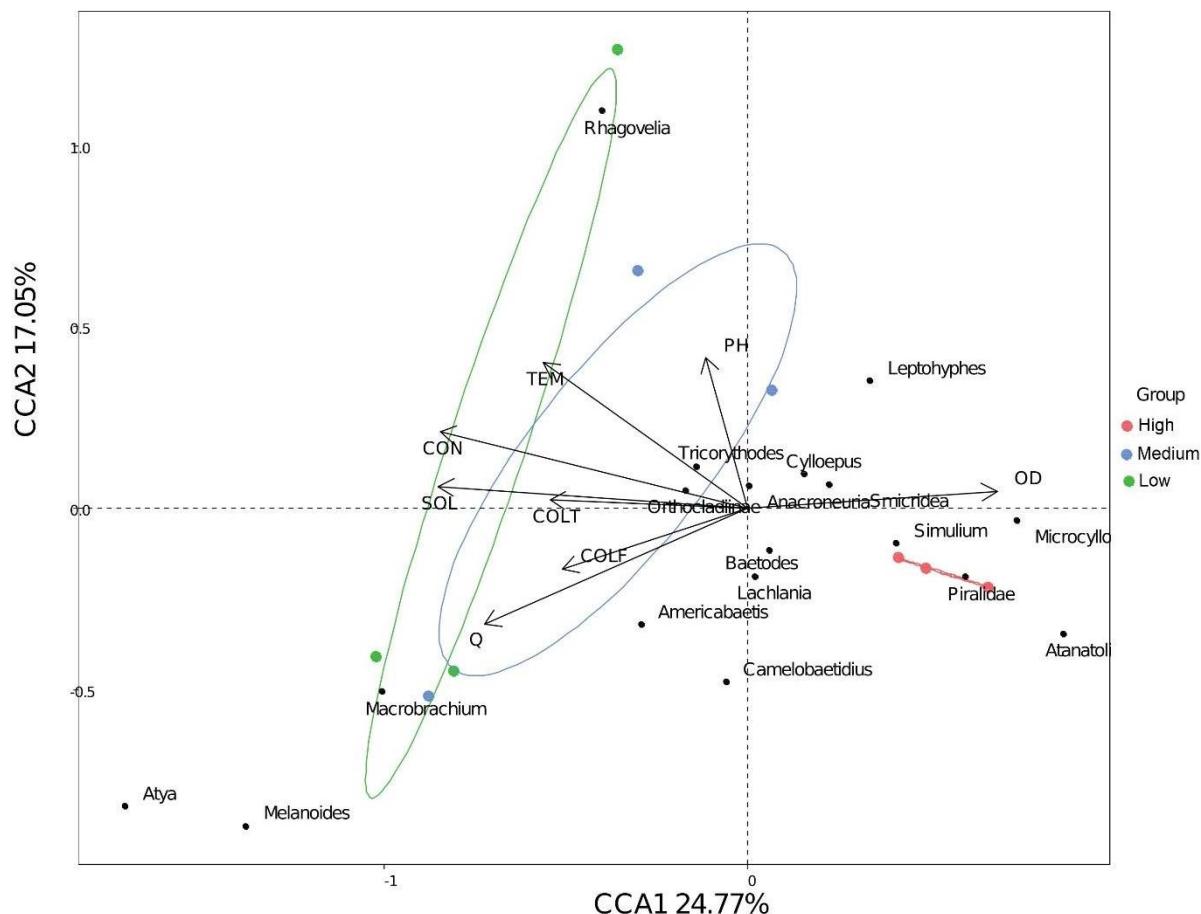


Figure 5. Canonical correspondence analysis diagram of the relationships between gradients, environmental variables, and the most abundant taxa found in the Ranchería River. (High: minimal human influence, Medium: near urban and agricultural areas, Low: close to open-pit coal mine).

4. DISCUSSION

Overall, pH values were slightly basic, consistent with several other studies that found rivers in the SNSM (Jaimes-Contreras and Granados-Martínez, 2016; Barros-Núñez and Granados-Martínez, 2016) are characterized by igneous and metamorphic rocks with alkaline composition such as granite, which enhance the buffering capacity of rivers originating in this area (Idárraga-García *et al.*, 2011). Temperature increased in areas with higher human activity, likely due to the impact of urban and agricultural land uses and the scarcity of riparian vegetation. Conductivity and TDS were also higher in areas with moderate to high human activity compared to the minimally disturbed areas. The increase in conductivity and TDS could be explained by agricultural and urban activities in the surrounding areas, which also corresponded to higher microbiological levels. Dissolved oxygen concentrations were similar to those reported by other studies in SNSM rivers (Serna *et al.*, 2015; Jaimes-Contreras and Granados-Martínez, 2016, Barros-Núñez and Granados-Martínez, 2016). The dissolved oxygen levels found in the Ranchería River favor the survival of present biota (Roldán and Ramírez, 2008). Flow increased downstream, likely explained by additional tributary inputs. Nutrient levels were very low, in some cases undetectable, and everywhere below the limits established by Colombian regulations for water use. According to the Colombian Ministry of Environment and Sustainable Development, the maximum permissible limits for nutrients in water bodies intended for human consumption and domestic use are as follows: 10 mg/L for nitrates, 1 mg/L for nitrites, and 0.5 mg/L for total phosphorus (MADS, 2015). Microbiological parameters increased downstream, as at lower elevations microbial activity tends to be higher according to

Acosta and Prat (2010). The observed exceedances of coliform concentrations in the Ranchería River are a cause for concern, as they surpass the limits established by Colombian regulations for water use. These high coliform levels indicate that the river water may not be suitable for human consumption or domestic use without prior treatment, as they could pose a risk to public health and promote the spread of waterborne diseases. These exceedances have been previously reported by Pérez *et al.* (2018). Overall, nutrients and microbiological parameters were highest in the areas with moderate to high human activity. This pattern could be explained by the agricultural and urban land use characteristic of these areas. Other studies have found a strong influence of environmental variables associated with human disturbance, where substrate composition, vegetation, and allochthonous inputs from ecosystems and urban areas determine environmental gradients (Villamarín *et al.*, 2021; Scheibler *et al.*, 2008; Acosta and Prat 2010; Oviedo-Machado and Reinoso-Flórez, 2018). The areas with minimal human influence (reference points) showed lower levels of nutrients and microbiological parameters compared to the areas near urban zones, agricultural activities, and the open-pit coal mine. These variations along the gradient of human activity could influence biotic and abiotic components and define the structure and composition of macroinvertebrates in the Ranchería River. This pattern was also evidenced in the structure and composition of macroinvertebrates across the different levels of human disturbance, from minimal to high impact areas.

According to Moreno *et al.* (2011), determining sample coverage is important because the more complete the sample, the lower the likelihood of collecting an individual of an unrecorded species. In this study, C_m values above 0.96 were obtained, indicating a complete sample given the effort for the objectives and low probability of finding undetected species with this sampling method. Estimating diversity through effective numbers is widely recommended when comparing the diversity of different communities, as it acknowledges samples are incomplete representations (Moreno *et al.*, 2011). The results here showed a similar trend using diversity of order q = 0 and q = 1, confirming distinct assemblages in areas with high human activity compared to those with moderate and minimal human influence. This decrease in species richness in highly impacted areas could be due to biotic and abiotic factors typical of the basin and increased anthropogenic impacts, such as changes in food availability and environmental conditions.

Diptera and Ephemeroptera were the most abundant orders, consistent with Padilla-Garcia *et al.* (2022) in the SNSM, and Lasso and Granados-Martínez (2015) and Pérez-Rodríguez *et al.* (2021) in the Serranía de la Macuira in the department of La Guajira. These groups are known for their wide distribution and abundance in Colombia (González-Córdoba *et al.*, 2020). Comparing richness, abundance, and diversity along the gradient of human activity showed higher values in areas with minimal and moderate human influence. This pattern was observed in the CDA and rarefaction-extrapolation curves, where macroinvertebrate community structure and composition in areas with high human activity (near the open-pit coal mine) diverged from those in areas with moderate human activity (near urban zones and agricultural activities) and minimal human influence (reference points).

Human activities significantly impact the composition of aquatic organisms (Serna *et al.*, 2022). Baumgartner and Robinson (2017) state that due to natural gradients (stream order, substrate, elevation, etc.) and the dendritic structure of river networks, cumulative environmental factors are expected to affect biotic assemblages at human-dominated sites. Serna *et al.* (2022) found changes in the distribution of aquatic macroinvertebrates across functional feeding groups (FFG). According to Dallas (2007), variations in aquatic organism composition and structure are due to factors like colonization potential of biotopes, increased water turbidity, and elevated water temperature, which were similar to conditions found in this study. Several authors, including Whittaker *et al.* (2001) and Willig *et al.* (2003), note that biodiversity tends to decline with increasing elevation gradients in response to more restrictive

environmental factors (Vannote *et al.*, 1980; Rahbek, 1995). Previous studies have reported varying trends in taxonomic richness along gradients of human disturbance in different regions. For instance, Hepp *et al.* (2010) found that macroinvertebrate richness and diversity decreased with increasing urban land use in southern Brazilian streams. Similarly, Miserendino *et al.* (2011) observed that taxonomic richness was negatively affected by human activities such as urbanization and agriculture in Patagonian streams. However, some studies have shown more complex patterns. For example, Sponseller *et al.* (2001) found that macroinvertebrate diversity peaked at intermediate levels of catchment urbanization in streams in Virginia, USA. The present study aligns with these findings, showing the highest diversity values in areas with moderate human influence, while areas with high human activity (near the open-pit coal mine) exhibited the lowest diversity. This pattern suggests that moderate levels of disturbance might promote diversity, possibly due to increased habitat heterogeneity, while high levels of disturbance lead to biodiversity loss, likely due to degradation of water quality and habitat conditions (Allan, 2004; Vörösmarty *et al.*, 2010).

Although the study area did not encompass a steep elevation gradient, a clear effect of human activity was observed along the river. This gradient of anthropogenic impact, ranging from minimal disturbance in the reference points to high disturbance near the open-pit coal mine, significantly shaped the environmental variables and macroinvertebrate community structure in the Ranchería River. The interplay between natural environmental variations and increasing human activities along the river course resulted in distinct patterns of macroinvertebrate diversity and composition across the study sites.

The CCA showed that Coleoptera such as *Cylloepus*, *Microcylloepus*, Ephemeroptera like *Baetodes*, *Leptohyphae*, *Lachlania*, *Camelobaetidius*, Diptera including *Simulium*, *Orthocladiinae*, *Trichoptera* such as *Smicridea*, *Atanatolica*, and the Lepidoptera family *Piralidae* were associated with areas of minimal human influence (reference points) and, in turn, high dissolved oxygen levels. Elmidae beetles, especially, have been documented as characteristic of less disturbed areas with higher oxygen content (Manzo, 2013; González-Córdoba *et al.*, 2020). This same pattern has been reported for larvae of the genus *Simulium* (Coscarón-Arias, 2009), consistent with the current findings. *Baetodes*, *Leptohyphes*, *Camelobaetidius* and *Lachlania* have been found in conditions similar to this study's reference points and in good environmental conditions (Gutiérrez and Dias, 2015; Barros-Núñez and Granados-Martínez, 2016; Arana-Maestre *et al.*, 2021). The Trichoptera *Smicridea* and *Atanatolica* have been documented in SNSM rivers in comparable conditions, with the former exhibiting a broader distribution range (Jaimes-Contreras and Granados-Martínez, 2016). For areas with moderate human influence, the Ephemeroptera *Farrodes*, the Hemiptera *Rhagovelia*, and the snail *Melanoides* were associated with higher temperatures, greater flow, increased solids, and higher conductivity values. *Rhagovelia* individuals are characterized by inhabiting the surface films of freshwater ecosystems and have been reported at altitudes from 160 to 590 masl (Padilla-Gil, 2016). On the other hand, the Thiaridae snail family originates from southern China, Taiwan, the Philippines and East Indies (Malek, 1962) and eastern Africa (Facon *et al.*, 2005). Their introduction appears to be due to commercial exchange between countries, establishing populations in the Neotropics (Pointier *et al.*, 1994). This species has high ecological importance as an invader due to its impact on native snail diversity (Cruz-Ascencio *et al.*, 2003; Facon *et al.*, 2005), displacing and threatening disappearance or at least decline of native mollusk populations. Due to its high biotic potential, prolific reproduction, and high reproductive rate, this snail is considered an invasive exotic species. Finally, the Ephemeroptera *Americabaetis*, the Plecoptera *Anacroneuria*, and the shrimps *Macrobrachium* and *Atya* were associated with areas of high human influence and higher fecal and total coliform values. *Americabaetis* exhibits a broad distribution across different levels of human disturbance, recorded in both SNSM rivers and other Andean mountain rivers under various environmental

conditions (Gutiérrez and Dias, 2015; Barros-Núñez and Granados-Martínez, 2016). *Anacroneuria* has been documented across varying elevations in the SNSM, exhibiting a broad environmental tolerance (Guzmán-Soto and Tamarís-Turizo, 2014). The shrimps *Macrobrachium* and *Atya* have been previously reported from areas of the SNSM rivers that are now experiencing higher levels of human influence (Guzmán-Soto and Tamarís-Turizo, 2014), suggesting their ability to persist in more disturbed conditions.

This study found variations in environmental variables and macroinvertebrate assemblages along a gradient of human disturbance in the Ranchería River. Dissolved oxygen was highest in areas with minimal human influence (reference points), while temperature, conductivity, solids, and microbiological levels increased in areas with higher human activity. Macroinvertebrate richness, abundance, and diversity were highest in areas with moderate human influence compared to areas with minimal and high human impact. This pattern suggests that moderate levels of disturbance might promote diversity, possibly due to increased habitat heterogeneity, while high levels of disturbance lead to biodiversity loss, likely due to degradation of water quality and habitat conditions.

5. CONCLUSIONS

The canonical correspondence analysis showed associations between macroinvertebrate taxa and environmental variables along the Ranchería River's gradient of human disturbance. The level of human activity shaped the structure and composition of assemblages. Higher dissolved oxygen in areas with minimal human influence was related to Ephemeroptera, Coleoptera, Trichoptera, and Lepidoptera taxa adapted to these conditions. Higher temperatures and TDS concentrations in areas with moderate human impact were associated with the presence of *Rhagovelia* and *Melanoides*. Lastly, high coliform levels in areas with high human activity were linked to *Americabaetis*, *Anacroneuria*, and shrimp taxa.

Overall, environmental factors related to the gradient of human disturbance determined macroinvertebrate assemblage patterns along the Ranchería River. This study provides a baseline for future biomonitoring and conservation efforts in the region, highlighting the importance of managing human activities to maintain aquatic biodiversity.

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