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Coffee processing affect the strcucture of mayflies assemblages in streams of the Sierra Nevada de Santa Marta, Colombia

Carmen Villalobos-Borre [1], Grace Maria Núñez-Sarmiento [2], Cesar Enrique Tamaris-Turizo [3]

Abstract

The order Ephemeroptera is widely used as a bioindicator of water quality due to its ecological characteristics, due to this group is ideal for studying the impact of activities like as coffeee hulling on stream ecosystems. Objective: To evaluate the effect of coffee crop on the abundance and diversity of nymphs of Ephemeroptera. Methodology: 2 sites affected by coffee hulling (Palmichal and Guáimaro) and two localities without stressors (reference sites: Libertad and Nuevo mundo) were studied during the months of November and December of 2021. In each site, Ephemeroptera were collected with surber and type D nets in the microhabitats present. Additionally, physicochemical data were taken. To establish the effect of coffee hulling on the order Ephemeroptera, the abundance and richness of genera in disturbed and conserved places were compared. Results: Cluster analysis based on incidence showed the formation of two groups: one constituted by Libertad, Nuevo mundo, Palmichal sites and another by Guáimaro. Likewise, there were significant differences among the abundance of the study sites (ANOSIM R=0.48 p= 0.0007). In the environmentally affected sites *Thraulodes* and *Camelobaetidius* dominated in Guáimaro and *Leptohyphes* and *Baetodes* in Palmichal, with respect to the reference sites: *Baetodes*, *Leptohyphes*, *Mayobaetis* and *Nanomis* dominated. Conclusions: The results of the present study showed that the effect of water produced by coffee hulling affects the structure of mayflies, which can be observed in the decreasing of abundance and richness, in the differentiation of dominant taxa, in comparison with reference sites.

Keywords: mayfly, diversity, microhabitats, coffee hulling, enviromental impact.

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[1] Bióloga, Universidad del Magdalena, Santa Marta, Colombia, carmenvillaloboseb@unimagdalena.edu.co
[2] Bióloga, Universidad del Magdalena, Santa Marta, Colombia, gracenunezms@unimagdalena.edu.co
Cesar Enrique Tamaris-Turizo
[3] Ph.D. Universidad del Magdalena, Santa Marta, Colombia,
orcid.org/0000-0002-9600-4641 **Google Scholar**

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El procesamiento del café afecta la estructura de los ensamblajes de efemerópteros en ríos de la Sierra Nevada de Santa Marta, Colombia

Resumen

El orden Ephemeroptera es ampliamente utilizado como bioindicador de la calidad del agua por sus características ecológicas, por lo cual, es ideal para estudiar el impacto que producen actividades como el despulpe del café sobre ecosistemas fluviales. Objetivo. Evaluar el efecto de la caficultura sobre la abundancia y diversidad de ninfas de Ephemeroptera. Metodología. Se muestrearon 2 sitios afectados por la caficultura (Palmichal y Guaímaro) y 2 sitios sin afectación antrópica (sitios de referencia: Libertad y Nuevo mundo) durante los meses de noviembre y diciembre del 2021. En cada sitio se realizaron recolectas de efemerópteros con redes Surber y tipo D en los microhábitats presentes. Adicionalmente, se tomaron datos fisicoquímicos. Para establecer el efecto de la caficultura sobre el orden Ephemeroptera, se comparó la abundancia y riqueza de géneros en los lugares perturbados y conservados. Resultados: El análisis clúster evidenció la conformación de dos grupos: uno constituido por sitios Libertad, Nuevo mundo, Palmichal y otro por Guáimaro. Así mismo, se presentaron diferencias significativas entre la abundancia de los sitios (ANOSIM R=0,48 p= 0,0007). Con relación a la composición, se observó que en los sitios intervenidos dominaron *Thraulodes* y *Camelobaetidius* en Guáimaro y *Leptohyphes* y *Baetodes* en Palmichal; mientras que en los sitios de referencia dominaron *Baetodes*, *Leptohyphes*, *Mayobaetis* y *Nanomis*. Conclusiones: Los resultados del presente estudio evidenciaron que el efecto de las aguas producto del procesamiento del café afecta la estructura de los efemerópteros, lo cual se refleja en la disminución de la abundancia y riqueza y en la diferenciación de taxones dominantes, en comparación con los sitios de referencia.

Palabras claves: efemerópteros, diversidad, despulpe del café, impacto ambiental.

Introduction

Mayflies are a group of aquatic insects with approximately 3,500 described species, of these, 820 are found in the Neotropical region (SALLES *et al.*, 2018). In Colombia, this order is represented by 9 families, 53 genera, and 118 species (DOMÍNGUEZ *et al.*, 2019). The nymphs live from several months to about a year and only in this stage they can feed (FLOWERS & DE LA ROSA, 2010; GUTIÉRREZ, 2018). They are typically associated with leaf litter and stone substrates, where sediment accumulates, and are part of a variety of functional feeding groups such as collectors, filterers, scrapers, shredders, herbivores, and some predators (BRITTAIN & SARTORI, 2003). This makes them crucial for the functioning of lotic ecosystems, connecting primary and secondary producers by being preyed upon by fish and aquatic invertebrates (GONZÁLES *et al.*, 2009). On the other hand, mayflies have been widely used in bioindication studies due to their wide distribution, long life cycles in their nymph stage, and sensitivity to environmental pollution, for this reason this group is commonly used by assessing water quality (ROLDÁN, 2003).

Some studies indicate that aquatic insect communities are affected by agricultural activities, leading to a reduction in the richness and dominance of non-resistant taxa to chemical and organic pollution (DELONG & BRUSVEN, 1998; ALLAN, 2004; SONG *et al.*, 2008). This is a consequence of the indiscriminate use of fertilizers and pesticides from agricultural activities, causing eutrophication of water bodies (RAP-AL, 2010; DIOSEFAT, 2018). In the Magdalena department (northen Colombia), coffee crop has a significant impact due to its large-scale production, with an estimated 20,000 hectares of cultivated area. Ciénaga municipality stands out as the quintessential coffee-producing, with over 50% of the cultivated area, and this situation is expected to increase (VILORIA, 2019).

The study area is located in the municipality of Ciénaga, in the San Pedro and San Javier districts, surrounded by the Frío and Sevilla rivers, providing water to an estimated 72,000 people (CORPAMAG, 2016). Although coffee farmers have addressed sustainable resource use issues; extensive coffee plantations affect the ecosystem directly due to the extensive use of fertilizers, pesticides, and pulping (ZULUAGA & ZAMBRANO, 1993). Bodies of water are particularly affected by coffee processing, as the coffee hulling process demands high water consumption and receives water from coffee washing (SOTTO *et al.*, 2020).

Theses physicochemical changes impact directly on sensitive organisms, for example to the Ephemeroptera order (FORERO-CÉSPEDES, 2013; TÜRKME & KAZANCI, 2015; CASTILLO-FIGUEROA *et al.*, 2018). Due to the sensitivity of these organisms to environmental changes, they are important in environmental impact and biodiversity studies. The presence or absence of certain genera can be indicative of the ecological state of freshwater ecosystems (GONZÁLEZ *et al.*, 2008). Therefore, the aim of this study was to evaluate the effect of water discharge resulting from coffee hulling on the assemblage composition of the Ephemeroptera order in the municipalities of San Pedro and San Javier, Sierra Nevada de Santa Marta. We hypothesize that mayflies will have lower abundance and richness in sites with coffee hulling affectation, in comparation with undisturbed sites. Moreover, the dominant taxa are expected to differ among contransting sites.

Methods

Study area

Sampling sites are distributed along the middle part of the Río Frío and Sevilla (between 465 to 1224 altitude) basins, which is characterized by high slopes (up 10%), and crops of coffee and cocoa and low development of cattle raising activities. We established three located in the San Pedro township and, one in San Javier township: Nuevo mundo (tributary of the Río Frío River) and Libertad (tributary of the Sevilla River) streams were selected as reference sites, the surrounding vegetation of these sites exhibit a dense canopy, no-introduced species. Moreover Palmichal (tributary of the Río Frío River) and Guáimaro (tributary of the Sevilla River) were streams altered by water washed product of the coffee hulling (Table 1, Figure 1).

Tabla 1. Details regarding the study sites, name of the principally Rivers, including altitude and geographic coordinates.

Site	River	Category	Altitude (m)	Geographic coordinates	
				Latitude	Longitude
Palmichal	Frío	Caficulture	1150	10° 55´ 23” N	74° 01´ 17” W
Guáimaro	Sevilla	Caficulture	465	10° 53´ 06” N	74° 05´ 50” W
Libertad	Sevilla	Reference	1174	10° 51´ 11” N	74° 01´ 35” W
Nuevo mundo	Frío	Reference	1224	10° 55´ 05” N	74° 00´ 45” W

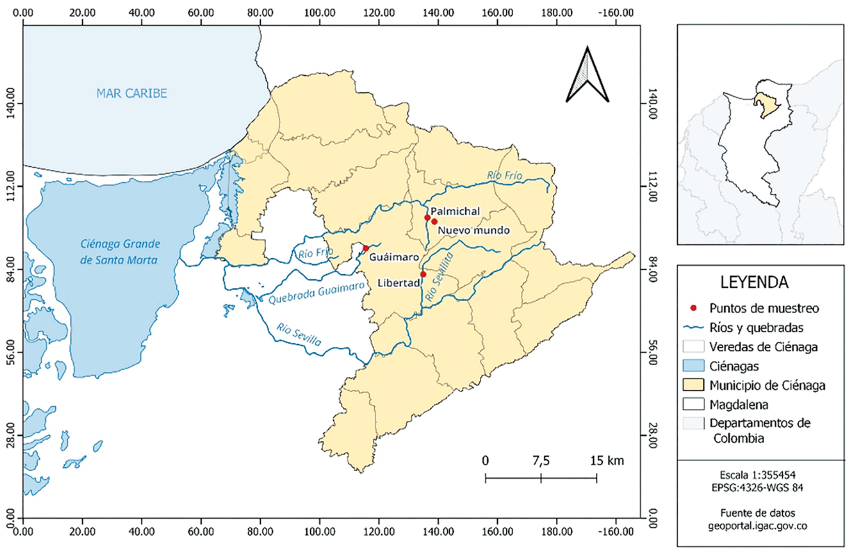


Figura 1. Details regarding the study sites, name of the principally Rivers, including altitude and geographic coordinates.

Sampling and processing of biological samples

Two samplings were conducted during the period of coffee processing: November and December 2021. To collect biological samples, a 300 m reach was established at each site. The collected samples of organims followed the methodology of ZÚÑIGA & CARDONA (2009), which involves gathering organisms found in microhabitats such as leaf litter, rocks, and macrophytes using a Surber net (mesh size: 250 µm, area: 0.09 m^2). For the collection of individuals in the rock microhabitat, the Surber net was positioned downstream, ensuring that upon the removal of substrates, the individuals remained within the mesh. This procedure was carried out in the leaf litter and macrophyte microhabitats, but employing a D-type net (mesh pore size: 250 µm, area: 0.09 m^2). Finally, the collected samples were preserved in 96% etanol. The sampling period at each site was 30 minutes. To describe physicochemical variables, a multi-parameter OHAUS was utilized, from which data on pH, temperature (°C), conductivity (µS/cm), and total dissolved solids (mg/L) were recorded.

The samples were sorted manually using entomological forceps. The mayflies were identified until genera level with a ZEISS Stemi 305 stereoscope, following the taxonomic keys (DOMÍNGUEZ *et al.*, 2006; SALLES *et al.*, 2018).

Data analysis

To assess taxonomic diversity (D^q) in each zone, effective species numbers (q = 0 and q = 2) were employed, as proposed by Jost (2010). Here, q represents the diversity of order D, where q = 0 indicate absolute richness (S), q=1 indicate the number of common species, which is calculated with the exponential of Shannon entropy index, and q = 2 representing the number of the species number, caltulated with using the inverse of the Simpson concentration index. Effective numbers allow the inclusion of incidence data (presence-absence) while adhering to mathematical properties that align with the intuitive interpretation of diversity concepts, such as the duplication property (MORENO *et al.*, 2011). For this, the iNext platform (https://chao.shinyapps.io/iNEXTOnline/) (Chao, 2016) was utilized. To assess species turnover between study sites, the Whittaker index (β=S/α-1) was employed. This index results from the ratio between species richness recorded in all samples by site (γ=S) and the average number of species in the simples by site. Additionally, a Cluster Analysis based on a Jaccard similarity matrix was conducted using presence-absence data. Furthermore, to understand the abundance of genera in relation to the sites, a Simple Correspondence Analysis (SCA) was performed with Past software version 4.03 (HAMMER & HARPER, 2006). To evaluate differences in abundances among the 4 sampling sites, an ANOSIM (Analysis of Similarity) test with 999 permutations and a significance level of 5% was conducted. Subsequently, a SIMPER (Similarity Percentage) test was performed to identify the contribution of genera to the differentiation found in the previous procedure.

Results

Environmental characterization

The water bodies exhibited pH values close to neutrality, exception of Palmichal (pH = 5.8), indicating a degree of acidity. Temperature, conductivity, and total dissolved solids showed higher values in the sites altered by coffee hilling (Palmichal and Guáimaro) in contrast to the reference sites (Libertad y Nuevo mundo) (Table 2).

Tabla 2. Average values of the measured physicochemical variables from the four sampling sites.

Variable	Palmichal	Guáimaro	Libertad	Nuevo mundo
PH	5,8 ± 1,1	6,7 ± 0,5	7,0 ± 0,0	7,0 ± 0,1
Temperature (ºC)	21,5 ± 0,7	23,3 ± 0,4	16,5 ± 0,7	18,2 ± 0,2
Conductivity (µS/cm)	111,2 ± 1,2	104,5 ± 0,8	98,7 ± 5,2	99,1 ± 1,6
Total dissolved solids (mg/L)	64,9 ± 1,4	60,7 ± 3,5	57,7 ± 3,5	59,1 ± 2,8

Assemblages structure

A total of 1138 individuals were collected, grouped in 4 families and 9 genera. The reference sites (Libertad and Nuevo_Mundo) showed the highest incidence (frecuency based on presence) and richness of genera (Table 3, Figure 2), with Libertad being the site with the highest incidence (63 individuals) and richness (9 genera). In contrast, the disturbed sites (Palmichal and Guáimaro) exhibited lower values, with Palmichal recording 26 individuals distributed among 6 genera, and Guáimaro with 15 individuals and 5 genera.

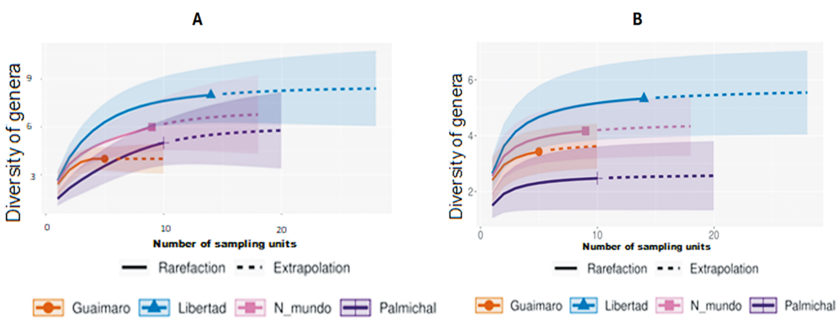


Figura 2. Rarefaction and extrapolation curves of mayfly incidence in the sampling sites. A) Comparison of effective genus (q = 0) between the four sampling sites. B) Comparation of dominant genera (q = 2) in the four sites.

Tabla 3. Data of diversity of rarefaction and extrapolation. q= order of the diversity (q=0: number of species, q=1: number of common species, q=2: number of dominant species), F= frecuency, Riq= richness, ^qD= diversity stimated, Lm Low= lower limit, Lm Up= upper limit, Cob= coverage.

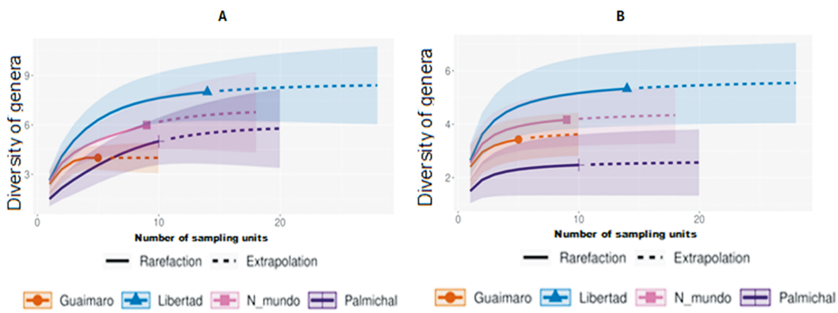
Sitio	q	F	Riq	^qD	Lm Low Riq	Lm Up Riq	Cob	Lm Up Cob	Lm Low Cob
Palmichal	0	26	6	6	4,7	7,3	1,0	1,0	1
	1	26	6	3	2,5	3,6	1,0	1,0	1
	2	26	6	2	2,1	2,9	1,0	1,0	1
Guáimaro	0	15	5	5	4,7	5,3	1,0	1,0	1
	1	15	5	3	2,6	3,7	1,0	1,0	1
	2	15	5	3	2,0	3,0	1,0	1,0	1
Libertad	0	63	9	9	8,5	9,5	1,0	1,0	1
	1	63	9	3	3,4	3,7	1,0	1,0	1
	2	63	9	2	2,3	2,7	1,0	1,0	1
Nuevo_mundo	0	41	7	7	6,0	8,0	1,0	1,0	1
	1	41	7	4	3,5	4,1	1,0	1,0	1
	2	41	7	3	3,0	3,5	1,0	1,0	1

Rocks and leaf litter were the microhabitats were present in all localities, while macrophytes were found in Palmichal and Libertad. Mayflies showed higher abundance (690 individuals) and richness (9 genera) in rock microhabitats at the reference sites (Libertad and Nuevo mundo) (Table 4). In Libertad, 409 individuals were found, grouped into 9 genera, while in Nuevo mundo, 212 individuals were grouped in 6 genera. Similar results were observed in the leaf litter microhabitat: Nuevo mundo recorded 134 individuals, followed by Libertad with 124, while Palmichal and Guáimaro registered 71 and 57 individuals, respectively.

All sites had significant sampling coverage (close to 100%), indicating that the sampling effort was representative. Coverage in leaf litter and rock microhabitats was 100% in most sites, except for Palmichal (94% in the rock microhabitat). Meanwhile, macrophytes showed a coverage of 97% in Libertad and 73% in Palmichal (Table 4).

Tabla 4. Rarefaction and extrapolation in the microhábitats analyzed. H= leaf litter, M= macrophytes, R= rock, Cob=coverage, ^{q}D= diversity stimated fo the oreder q, Ab=abundance, F= frecuency, Riq=richness.

	Guáimaro			Libertad			Nuevo_mundo			Palmichal		
	H	**M**	**R**	**H**	**M**	**R**	**H**	**M**	**R**	**H**	**M**	**R**
Cob0	1		1	1	0,97	1	1		1	1	0,73	0,94
Cob1	1		1	1	0,97	1	1		1	1	0,73	0,94
Cob2	1		1	1	0,97	1	1		1	1	0,73	0,94
^{0}D	4		4	7	5	9	4		6	3	3	5
^{1}D	3		3	4	2	2	2		3	2	3	3
^{2}D	3		3	3	2	2	1		2	2	2	2
F	57		38	124	56	409	134		212	71	5	31
Ri^{q}	4		4	7	5	9	4		6	3	3	5



Turnover analysis

Beta diversity analysis revealed a maximum dissimilarity of approximately 45% between the Guáimaro and Palmichal sites. The similarity dendrogram subsequently delineated two distinct clusters: the first comprised Libertad, Nuevo Mundo, and Palmichal, while the second consisted exclusively of Guáimaro (Figure 3; Table 5). Within the first cluster, the composition and abundance of the Nuevo Mundo reference site were found to be statistically comparable to those documented for the perturbed locations.

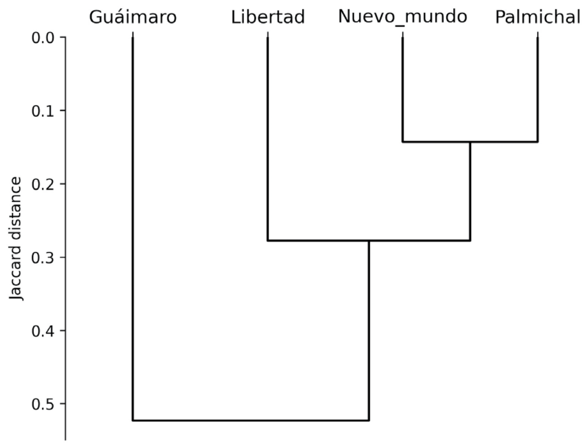


Figura 3. Cluster dissimilarity analysis using Jaccard distance, based on incidence of mayflies in the localities.

Tabla 5. Replacement of genera in the localities, using Whittaker index.

	Guaimaro	Libertad	Nuevo_mundo	Palmichal
Guaimaro	0	0,29	0,33	0,45
Libertad		0,00	0,13	0,20
Nuevo_mundo			0,00	0,08
Palmichal				0

The ANOSIM test confirmed a clear differentiation in genus abundance across the four sites (R=0.48, p=0.007). On the other hand, the Simple Correspondence Analysis (SCA) displayed a cumulative variance of 92.2% across the first two axes, revealing an association between the disturbed sites and dominant taxa. Especially, Guáimaro was dominated by *Thraulodes* and *Camelobaetidius*, while Palmichal by *Leptohyphes* and *Baetodes*. Libertad showed the highest abundance of *Baetodes*, followed by *Lachlania*, standing out as the only site where the *Tricorythodes* genus was found. Finally, Nuevo mundo presented the highest abundance of *Mayobaetis* and *Nanomis* (Figure 4).

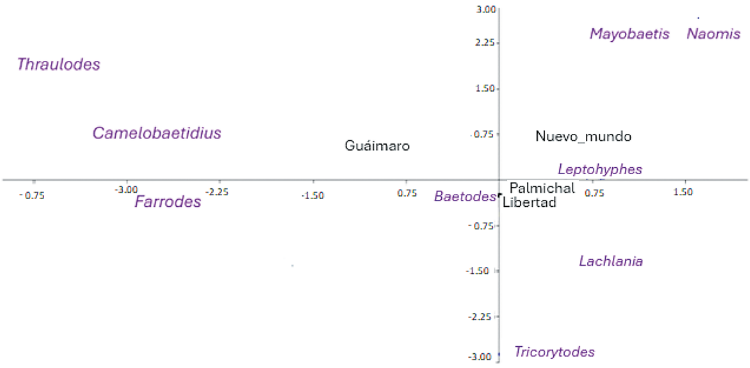


Figura 4. Simple Correspondence analysis of genus abundance in the sampling sites.

The SIMPER analysis revealed that *Mayobaetis* provided the highest contribution in the analyzed group pairs, with 87% similarity between Nuevo mundo and Palmichal. *Thraulodes* and *Leptohyphes* contributed over 80% in Guáimaro and Palmichal, as well as Libertad and Palmichal. In the reference sites, *Baetodes* and *Leptohyphes* contributed the most (*Leptohyphes*: 74%, *Baetodes*: 49%). However, no significant differences were observed. On the other hand, disturbed sites were represented by *Baetodes*, *Leptohyphes*, and *Thraulodes*, with the latter contributing 83% (Table 6).

Tabla 6. Percentage of similarity in SIMPER indicating the contribution of different genera in pairs of sites., ** p < 0,05, ***p < 0,01. Taxa with significant sifferences are showed.

Comparison between sites	Contribution (%)	P-value
	Guáimaro-Nuevo mundo	
Mayobaetis	84	0.001 ***
	Guáimaro-Libertad	
Baetodes	54	0.003 **
	Guáimaro-Palmichal	
Thraulodes	83	0.001 ***
	Libertad-Palmichal	
Baetodes	61	0.003 **
	Nuevo mundo-Palmichal	
Mayobaetis	87	0.001 ***

Discussion

We found that the diversity of Ephemeroptera in the sites were similar to that recorded in previous studies conducted in the SNSM (GRANADOS-MARTÍNEZ, 2013; BARROS-NÚÑEZ & GRANADOS-MARTÍNEZ, 2016; RÚA *et al.*, 2015; SCHMITT *et al.*, 2016). The diversity observed in this study could be related to presence of rainfall during the samplings, highlighting that hydrological variations (CAMBINDO *et al.*, 2020) could have caused changes in the structure of the aquatic insect community (GARCÍA-ALZATE *et al.*, 2010; GUZMÁN-SOTO & TAMARIS-TURIZO, 2014). Additionally, GONZÁLEZ *et al.*, (2008) found that discharge of contaminated water from coffee processing alters the waters quality, resulting in a decrease in the abundance of aquatic insects and dominance in taxa like Chironomidae family. In Guáimaro and Palmichal, the discharge of coffee hulling was observed in field and evidencie of that was the observation of coffe ground remains, abundant biofilm and filamentous alge, this fact was related to low values of abundance and richness, especially in Palmichal, where low pH values were also recorded, because the hulling coffee acidifies the water (Torres-Valenzuela, *et al.,* 2019). These results are consistent with previous studies, as Ephemeroptera is a group sensitive to enviromental pollution, which can be reflected in a decrease in abundance and taxonomic composition of the assemblage (ROLDÁN, 2003; FERNÁNDEZ *et al.*, 2007; BRAGA & GUTJAHR, 2018).

Commonly, the order Ephemeroptera is more abundant and diverse in fluvial systems with presence of rocky substrates (ZÚÑIGA & ROJAS, 1995; FLOWERS & DE LA ROSA, 2010). This coincides with the results obtained by different researchers (PÉREZ & SEGNINI, 2005; VÁSQUEZ-RAMOS & REINOSO, 2012) who affirm that this is related to a higher dissolved oxygen and low temperature, therefore, creating suitable conditions for their colonization. However, it was evident in the Libertad and Nuevo mundo sites where 936 individuals were found, of which 621 were in the rock microhabitat (Table 4). Additionally, high richness was found in macrophytes, with genera such as *Nanomis*, *Mayobaetis*, *Baetodes*, and *Lachlania*, which may be related to this microhabitat offering food resources and hábitat to a variety of herbivorous, scrapers, and pircering aquatic organisms (FONTANARROSA *et al.*, 2013; GLIME, 2017). The productivity of these aquatic plants can be associated with the physicochemical variables of the rivers, such as substrate type, pH, temperature, and conductivity, characteristics evident in Libertad, which presented low temperature and high conductivity (Table 2). It is noteworthy that macrophytes were only found in the second sampling, in the preserved Libertad site and in the disturbed Palmichal site, where this type of vegetation was observed, althrought in smaller proportions. Rivers affected by coffee hulling areas tend to have less diversity due changes in environmental variables (ROMERO, 2015; RODRÍGUEZ *et al.*, 2017).

Baetodes and *Leptohyphes* were present in all sites with high abundance, these results are similar like to those recorded by GUTIÉRREZ AND REINOSO (2010), GRANADOS-MARTINEZ (2013), GUTIÉRREZ AND DÍAS (2015), and PÉREZ-RODRÍGUEZ *et al.* (2021). These genera are considered tolerant to various levels of contamination (ROLDÁN, 1996). Similar results were reported by SALINAS *et al.* (2019), who evaluated the diversity of the Baetidae family in tributaries affected by extensive agriculture and livestock farming in the La Vieja River, Valle del Cauca. On the other hand, the findings regarding *Mayobaetis* are interesting, as it showed higher abundance in reference sites, primarily in Nuevo_ mundo, followed by Libertad, with low abundance in Palmichal and absence in Guáimaro, due to restriction in the distribution of this taxon, wich usually found in high elevations. This aligns with descriptions by (FLOWERS & DE LA ROSA, 2010; GARCÍA, 2014; RÚA *et al.*, 2015), who indicate that this genus is commonly found in well-oxygenated and undisturbed, being physiologically less tolerant to changes in environmental factors. Furthermore, it was evident that, although there was similarity in the taxonomic composition in the Nuevo mundo and Palmichal Rivers, it mau be explained by their proximity. Palmichal showed less intervention than Guaimaro, highlighting that few individuals manage to survive the disturbance caused by agriculture, which was markedly acidic pH recorded at this site.

Conclusions

In this study, a dissimilarity in the distribution of Ephemeroptera was observed, and was associated with the effects of coffee hulling in the studied water bodies. The results determined that these insects showed a considerable level of pollution sensitivity, as the highest abundances in the reference sites. This suggests that these insects tend to high dominant and high rechness in clean and less disturbed waters. Finally, ours results will help comprehend the effect of agricultural expansion on river systems of the Sierra Nevada de Santa Marta.

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