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ARTICLE

Effects of physical and chemical factors on Ephemeroptera (Insecta) assemblages in an urban river of the eastern Colombian Llanos

Jose Ismael Rojas-Peña[1,4]; Jesús Manuel Vásquez-Ramos[2]; Luis Gonzalo Salinas-Jiménez[3]; Diana Paola Osorio-Ramirez[1,5] & Clara Inés Caro-Caro[1,6]

[1] Universidad de los Llanos (UNILLANOS), Instituto de Ciencias Ambientales de la Orinoquia Colombiana (ICAOC), Grupo de Investigación en Gestión Ambiental Sostenible (GIGAS). Villavicencio, Meta, Colombia.
[2] Universidad de los Llanos (UNILLANOS), Facultad de Ciencias Básicas e Ingeniería (FCBI), Departamento de Biología y Química, Grupo de Investigación Evaluación, Manejo y Conservación de Recursos Hidrobiológicos y Pesqueros (GIREHPES). Villavicencio, Meta, Colombia. ORCID: http://orcid.org/0000-0003-1465-9407. E-mail: jvasquez@unillanos.edu.co
[3] Universidade Federal de Viçosa (UFV), Departamento de Entomologia (DDE). Viçosa, MG, Brasil. ORCID: http://orcid.org/0000-0002-8973-4996. E-mail: biobaetodes@gmail.com
[4] ORCID: http://orcid.org/0000-0002-4005-0825. E-mail: jose.rojas.pena@unillanos.edu.co (correspondence author)
[5] ORCID: http://orcid.org/0000-0002-7643-3369. E-mail: dosorio@unillanos.edu.co
[6] ORCID: http://orcid.org/0000-0003-1589-6535. E-mail: clarainescaro@unillanos.edu.co

Abstract. The mayflies are insects widely known as indicators of aquatic quality in freshwater systems, they are abundant and diverse in tropical streams. In this study, it was determined the influence of physical, chemical and bacteriological characteristics on the Ephemeroptera assembly in a period of low precipitation in the Ocoa river of Meta department. There were 5,332 nymphs belonging to 4 families, 10 genera and 3 species. Three new genera records and one species are presented for Meta: *Cloeodes, Zelusia, Lumahyphes* and *Americabaetis alphus*. The genera *Camelobaetidius* and *Varipes* were found associated with conditions of contamination by organic matter and water mineralization. Likewise, *Americabaetis, Thraulodes, Lumahyphes* and *Tricorythodes* were associated with contamination conditions, especially with nitrites and nitrates. *Nanomis, Cloeodes* and *Zelusia* showed greater sensitivity to the contamination condition and were related to high percentages of dissolved oxygen in the water and they were in the sampling stations of the upper part in the river. It is important to mention that there were not Ephemeroptera in most of the stations associated with the urban area. That reflects their high sensitivity to polluting conditions at low precipitation period.

Keywords. Water quality; Mayflies; Pollution; Distribution; Orinoquia.

INTRODUCTION

The effect of pollution on aquatic ecosystems by anthropogenic impacts (*e.g.,* irrigation of crops, urbanization, domestic and industrial discharges, and channelization) are the main cause of loss of their natural characteristics (Mallin & Cahoon, 2003; Blann *et al.,* 2009; Tanaka *et al.,* 2016). Those impacts generate a homogeneous environment and significantly affect the stability of aquatic communities (Bauernfeind & Moog, 2000), and deteriorate the water resource for society and the environment (WWAP, 2017). Since these activities can change the water quality, the managements have been used the physicochemical aspects of water to monitor and evaluate the ecological condition of the streams (Ramírez & Viña, 1998). Last years, they have integrated the organisms as bioindicators and physicochemical variables (Prat *et al.,* 2009).

Initially, the physical and chemical indices were used to evaluate the water quality, however they have several limitations to respond and discriminate against different human activities (Sharifinia *et al.,* 2016). Then it was implemented the use of aquatic macroinvertebrates. They have been recognized as good bioindicators of water quality due to the ability to respond to different types of environmental disturbances (Hodkinson & Jackson, 2005; Miranda, 1987; Prat *et al.,* 2009; Sharifinia *et al.,* 2016; Springer, 2010). Nevertheless, the taxonomic and ecological complexity of aquatic macroinvertebrates are high. Therefore, it has been suggested the analysis of a single representative biological group like Ephemeroptera, which presents different levels of tolerance to en-

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vironmental disturbances and contamination (Arimoro & Muller, 2010; Buss & Salles, 2007; Hubbard & Peters, 1978; Savić *et al.,* 2011; Shimano *et al.,* 2013; Zedková *et al.,* 2014). Mayflies have been widely cited as bioindicators in monitoring programs owing to: (1) their high abundance and diversity; (2) have a broad geographical distribution; (3) their well-known taxonomy and based mainly on immature stages; (4) their capacity for colonize variety of substrates; (5) and their good taxonomic resolution in the neotropical region (Barber-James *et al.,* 2008; Buss & Salles, 2007; Edsall, 2001; Forero-Céspedes & Reinoso-Flórez, 2013; Menetrey *et al.,* 2008; Moog *et al.,* 1997; Prat *et al.,* 2009; Souto *et al.,* 2011).

Colombia is recognized worldwide for its water wealth, specifically the Eastern region with Orinoco and Amazon basins, shared with several countries (Roldán & Ramírez, 2008). However, they are suffering the impact of population growth with the urbanization and demand for water resources for the cities (Correa, 2014). Among these is the Ocoa river, which starts and ends within the municipality of Villavicencio and collects water from 42 tributaries; it is a source of water for domestic consumption, agricultural, forestry and mining activities, as well as being 80% recipient of the wastewater discharges (Osorio-Ramírez *et al.,* 2015). Based on the above, the objective of this research was: 1. Determine the diversity of Ephemeroptera assembly and 2. Determine the influence of physical, chemical and bacteriological characteristics on the Ephemeroptera assembly in a period of low rainfall in the Ocoa river, department of Meta, Colombia.

MATERIAL AND METHODS

Study area: The Ocoa river basin rises in the southwestern municipality of Villavicencio (Meta department) in the rural settlement Samaria (1,155 m.a.s.l.). It flows east to Guatiquía river in the Murujuy sector, between the villages El Guamo and Indostan (225 m.a.s.l.). It covers longitudinally 54.89 km with an area of 28,290 ha (Fig. 1). The climate regime of the basin presents a precipitation among 2,700 mm/year in low zone to 5,000 mm/year in the high zone. Likewise, the atmospheric humidity and temperature varies from the low zone (79%, 19.5-33.5°C) to the upper zone (84%, 15.5-30°C) (Osorio-Ramírez *et al.,* 2015).

Collection of specimens: The Ephemeroptera collections were made in the low rainfall period, at 15 sampling stations (a representative reach of the Ocoa river and some tributaries: 100 m) that have variations in land use, vegetation cover and discharges (Table 1). The collection of organisms was made with surber sampler (300 µm, 900 cm^2 area) (Domínguez *et al.,* 2009). 15 subsamples were randomly taken in each sampling station on pebble substrate. In the laboratory, the organisms were preserved in 90% alcohol and were identified at the lowest taxonomic level using the keys and descriptions of Domínguez *et al.* (2009) and Domínguez *et al.* (2006).

Physical, chemical and bacteriological variables of water: At each sampling station, 5 variables were measured in situ: pH (pH units), dissolved oxygen (mg/L O_2), temperature (°C), electrical conductivity (µS/cm) and flow (m^3/s). Fourteen variables were measured *ex situ:* ammonium (mg/L NH_3), bicarbonates (mg/L HCO_3), COD (mg/L O_2), BOD_5 (mg/L O_2), iron (mg/L Fe), nitrates (mg/L) NO_3^-), nitrites (mg/L NO_2), orthophosphates (mg/L PO_4), total dissolved solids (ppm STD), sulfates (mg/L SO_4), turbidity (UNT), surfactants (mg/L SAAM), zinc (mg/L Zn) and total coliforms (NMP/100 ml), analyzed by the ANALQUIM laboratory, certified by the Instituto de Hidrología, Meteorología y Estudios Ambientales (IDEAM) in Colombia. The Organic Matter Contamination

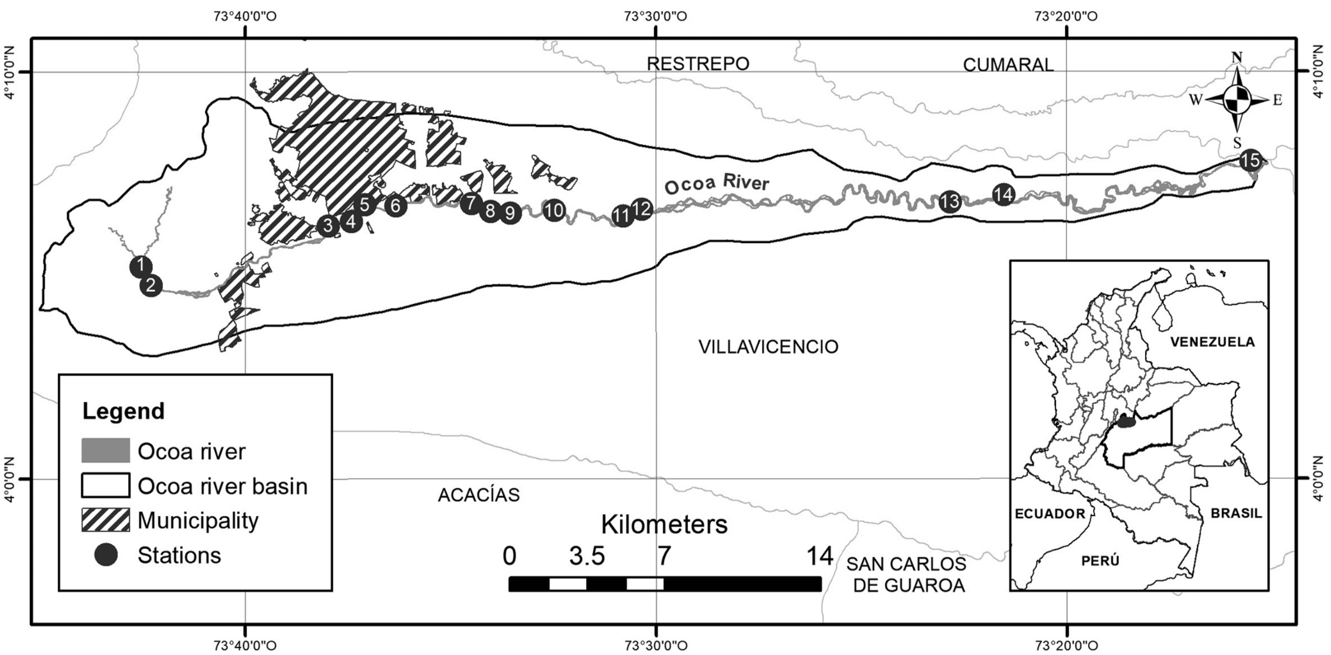

Figure 1. Geographical location of the Ocoa river basin and sampling stations.

Table 1. Description of the Ocoa river sampling stations.

Sampling Station	Name	Coordinates	Altitude (m.a.s.l.)	Station's characteristics (vegetation cover, land use, dumping)
1	Union Q/da Blanca y río Ocoa	04°05′13.3″N, 73°42′32.6″W	532	Dense forest. Scattered residential and livestock. Domestic dumping of farms and neighboring houses.
2	San Luis de Ocoa	04°04′45.8″N, 73°42′18.2″W	504	Wooded pastures. Residential. Domestic dumping of farmhouses, poultry farmers and use of water for recreation.
3	Puente caído	04°06′13.5″N, 73°37′58.9″W	419	Urban areas and clean pastures. Residential. Domestic and industrial discharges such as: oil company, thermo-electric power plant, fish farms, car washes and pig beneficiary plant.
4	Before caño Buque	04°06′19.6″N, 73°37′25.3″W	420	Urban areas and clean pastures. Residential. Industrial and domestic dumping and car washes.
5	Caño Buque	04°06′44.8″N, 73°37′05.2″W	415	Urban areas and clean pastures. Residential. Domestic dumping and waste collected by caño Buque in Villavicencio urban area.
6	Bridge Chorillano	04°06′42.9″N, 73°36′20.5″W	399	Urban areas and clean pastures. Residential. Domestic dumping, car washes, restaurants and grills.
7	Before caño La Cuerera	04°06′42.3″N, 73°34′19.4″W	381	Urban areas and clean pastures. Residential. Industrial and domestic dumping.
8	Caño La Cuerera	04°06′50.0″N, 73°34′20.0″W	381	Urban areas and clean pastures. Residential. Industrial and domestic dumping, close to an old sanitary landfill.
9	After caño La Cuerera	04°06′41.0″N, 73°34′14.4″W	377	Clean / managed pastures. Residential. Industrial and domestic dumping, close to an old sanitary landfill.
10	Before caño Maizaro	04°06′30.5″N, 73°33′43.8″W	374	Clean / managed pastures. Residential and agricultural Domestic discharges associated with farms near the river and livestock activities.
11	Caño Maizaro	04°06′30.0″N, 73°30′40.0″W	345	Clean / managed pastures. Residential, agricultural and livestock. Domestic dumping and waste of tires, plastics, mattresses and paper from the city.
12	After caño Maizaro	04°06′32.9″N, 73°30′39.2″W	345	Clean / managed pastures. Agricultural and livestock. Domestic and industrial dumping and livestock activities near the river.
13	Discharge Ecopetrol-Apiay	04°06′48.4″N, 73°22′50.0″W	259	Clean / managed pastures. Agricultural and livestock. Domestic and industrial dumping associated with the oil sector.
14	Alcaraván	04°06′59.0″N, 73°21′31.8″W	276	Weeds / stubble. Agricultural. Domestic and industrial dumping and changes in the channel due to the transit of heavy-duty cars.
15	Mouth of Ocoa river	04°07′48.8″N, 73°15′30.1″W	241	Heterogeneous crops. Agricultural. Domestic dumping of farms and water collection for rice and oil palm crops.

Index (ICOMO) was applied (Ramírez *et al.,* 1997), considering that the main sources of pollution are generated by wastewater from urbanization, which flow directly and indirectly into the Ocoa river.

Data Analysis: The taxonomic composition and abundance of the Ephemeroptera were determined for sampling station. Richness (0D), Shannon-Wiener diversity (1D) and Simpson dominance (2D) metrics were calculated in order to know the degree of equity and dominance in the sampling stations by means of effective numbers of genera or Hill numbers (Hill, 1973; Jost, 2006). For this, we used the PAST program (Hammer *et al.,* 2009) and the Real Statistics Resource Pack software (Release 6.8) (Zaiontz, 2020). In order to identify the physical and chemical variables that significantly affect the richness (0D) of Ephemeroptera, Generalized Linear Model (GLM) were tested and fitted to data (Model = quasi-Poisson, link = Log function, test = Chi square) (Dunn & Smyth, 2018). Previously, the collinearity of predictor variables was analyzed with Pearson's linear correlation (variables with $|r| > 0.7$ were omitted to compensate for collinearity) (Dormann *et al.,* 2013). In this way, the chosen variables for analysis were flow, temperature, dissolved oxygen, conductivity, nitrites, nitrates, phosphorous, total coliforms and ICOMO. Models were constructed with all possible combinations of parameters and they were considered adequate and retained when differing less than 2 AIC from the model with the lowest (best) AIC value (Dos Reis Oliveira *et al.,* 2020). The R2 values were used to measuring the goodness of fit of GLM's (Crawley, 2007; García, 2020). These analyses were performed in RStudio (RStudio Team, 2015), using the packages *vegan* (Oksanen *et al.,* 2019) for GLM analysis and *MuMIn* version 1.43.17 (Bartoń, 2020) for model selection.

In addition, direct ordination methods were explored to analyze the relationships between biotic variation and the environmental variables. Redundancy analysis (RDA) was adopted after detrended correspondence analysis (DCA) performed showing that lengths of former four axes were less than 2. Environmental variables data was standardized to z-cores. Forward selection procedure was realized in order to select the most important variables (Blanchet *et al.,* 2008). Biotic data were submitted to Hellinger transformation (Peres-Neto *et al.,* 2006). The statistical analyses were performed with *vegan* (Oksanen *et al.,* 2019) and *adespatial* (Dray *et al.,* 2019) packages in RStudio (RStudio Team, 2015).

RESULTS

Taxonomic composition of the Ephemeroptera assembly: We quantified 5,332 nymphs belonging to 4 families, 10 genera and 3 species (*Americabaetis alphus* Lugo-Ortiz & McCafferty, 1996, *Camelobaetidius edmundsi* Dominique *et al.,* 2001, *Nanomis galera* Lugo-Ortiz & McCafferty, 1999). The most abundant families were Baetidae (61.96%) and Leptohyphidae (31.12%), and the least abundant were Leptophlebiidae (6.90%) and Caenidae (0.02%). The most abundant genera were *Americabaetis* (56.54%) and *Lumahyphes* (29.06%), and the least abundant were *Caenis* (0.02%), *Nanomis* (0.02%) and *Cloeodes* (0.04%) (Table 2). The sampling stations with the highest number of nymphs were 14 and 15, with *Americabaetis* and *Lumahyphes* being the most important genera; while the sampling stations with lower abundance were 1, 2 and 13 (with 6, 19 and 26 organisms, respectively). On the other hand, any nymph was recorded at sampling stations 6, 7, 8, 9, 11 and 12 (Table 3).

Diversity of Ephemeroptera: Stations 14 and 15 located in the lower zone, with higher flow and inside an agricultural area, present the greatest richness (0D) with 5 and 6 taxa, respectively. Shannon diversity (1D) displayed that stations 1, 3, 4, 5 and 15 were the most diverse, with values between 2.74 to 1.9 effective number of genera; station 1 is located in the confluence area of two streams in the upper part of the river, while stations 3, 4 and 5 are in the upper urban area and station 15 is in the area with greatest richness. While sampling stations 2, 10, 13 and 14 recorded the lowest values of 1D (1.0 to 1.31 effective number of genera). It is interesting to note that sampling stations 1, 3, 4 and 15 presented twice as much diversity (1D) as the others, except the 5. Simpson's dominance (2D) showed a tendency similar to 1D, with few dominant taxa in the different sampling stations; however, in the 15 the dominance of few genera in the assembly was observed (2.02 effective number of genera) (Fig. 2).

Physical, chemical and bacteriological variables: In general, the physical, chemical and bacteriological variables in Ocoa river presented differences in the coefficient of variation. The pH and temperature showed little variation along the river (0.07 and 0.15, respectively). Others variables exhibited very high standard deviations and coefficients of variation (nitrates, nitrites, ammonium, orthophosphates, total coliforms, BOD_5, COD, surfactants and zinc) (Table 4).

Table 2. Composition of the Ephemeroptera fauna in the Ocoa river, during a period of low precipitation.

Family	Genera / Species	Relative abundance (%)
Baetidae (61.94%)	*Americabaetis alphus*	56.56
	Camelobaetidius edmundsi	4.74
	Cloeodes	0.04
	Nanomis galera	0.02
	Zelusia	0.41
	Varipes	0.17
Caenidae (0.02%)	*Caenis*	0.02
Leptohyphidae (31.13%)	*Lumahyphes*	29.07
	Tricorythodes	2.06
Leptophlebiidae (6.90%)	*Thraulodes*	6.9

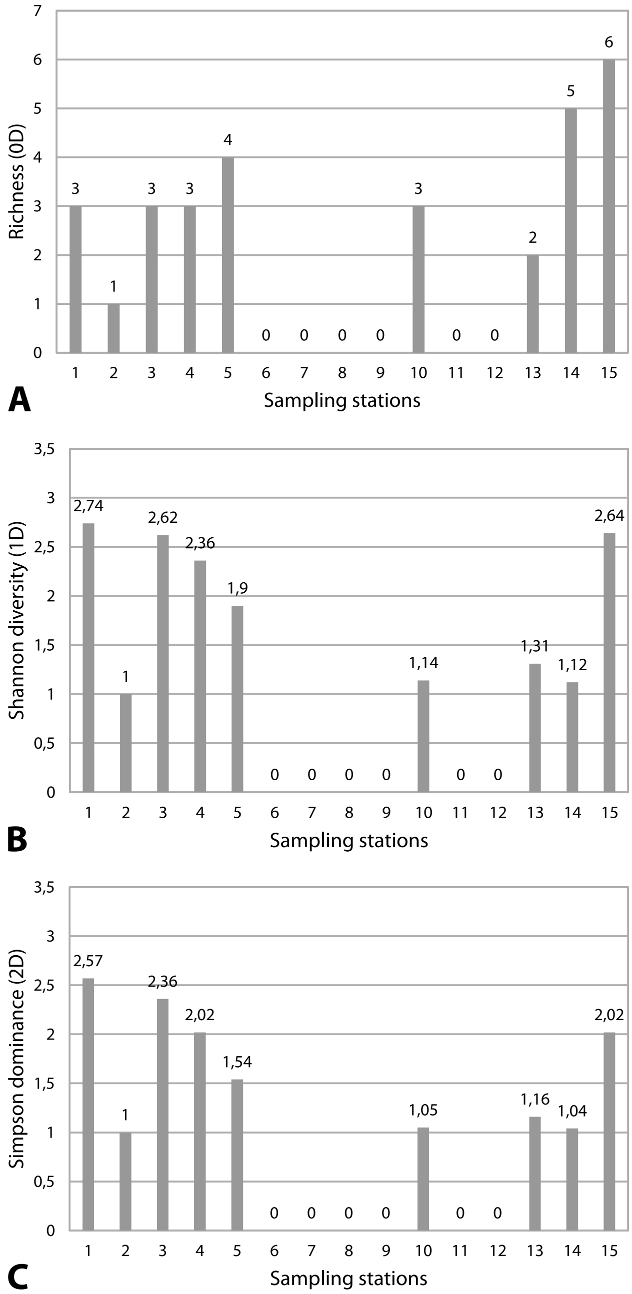

Figure 2. Diversity of the Ephemeroptera assemblage at sampling stations in the Ocoa river. (A) Richness (0D, number of taxa); (B) Shannon diversity (1D, Hill numbers unit); C. Simpson dominance (2D, Hill numbers unit).

Table 3. Distribution of Ephemeroptera abundance in the sampling stations of the Ocoa river.

Species	Sampling stations														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A. alphus	0	0	37	43	1	0	0	0	0	148	0	0	24	2370	393
C. edmundsi	0	0	70	16	162	0	0	0	0	0	0	0	0	0	5
Cloeodes	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. galera	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Varipes	0	0	0	0	8	0	0	0	0	0	0	0	0	1	0
Zelusia	3	19	0	0	0	0	0	0	0	0	0	0	0	0	0
Lumahyphes	0	0	0	0	0	0	0	0	0	1	0	0	0	2	1547
Tricorythodes	0	0	0	0	0	0	0	0	0	0	0	0	0	2	108
Thraulodes	0	0	18	7	35	0	0	0	0	3	0	0	2	52	251
Caenis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total	**6**	**19**	**125**	**66**	**206**	**0**	**0**	**0**	**0**	**152**	**0**	**0**	**26**	**2427**	**2305**

Table 4. Measures of central tendency of the physical, chemical and bacteriological variables.

Variable	Arithmetic average	Coefficient of variation
Temperature (°C)	26.33	0.07
Dissolved oxygen (mg/L)	3.84	3.14
pH (unidades de pH)	6.84	0.15
Electric conductivity (uS/cm)	252.22	0.61
Total dissolved solids (ppm STD)	111.40	0.53
Turbidity (UNT)	17.78	0.70
Bicarbonates (mg/L $CaCO_3$)	61.07	0.77
Nitrates (mg/L NO_3^-)	0.68	1.70
Nitrites (mg/L NO_2^-)	0.08	1.61
Ammonium (mg/L NH_4^+)	4.48	1.67
Orthophosphates (mg/L PO_4^{3-})	8.09	2.92
Sulfates (mg/L SO_4^{2-})	20.94	0.56
Total coliforms (NMP/100 ml)	11124502.67	2.09
$DBO_{5-20°C}$ (mg/L O_2)	25.47	1.67
DQO (mg/L O_2)	63.53	1.08
Anionic surfactants (mg/L)	1.20	1.64
Zinc (mg/L Zn)	0.17	1.75
Iron (mg/L Fe)	1.50	0.42
Flow (m^3/s)	2.68	0.47

The ICOMO's lowest values were presented in sampling stations 1 and 2 (< 0.2), which reflected an "optimal" water quality. Sampling station 3 was located in the urban area and presented a value close to 0.5, which indicates that the quality of the water is in a "doubtful" category. From sampling station 4 to 11, with the exception of 10, the values were higher than 0.8, which represents a high degree of contamination. However, that trend changes in the last sampling stations (12, 13, 14 and 15), where the values of the index gradually decrease from an "awful" to a "dubious" water quality (ICOMO from 0.9 to 0.6) (Fig. 3). It is important to note that the highest values were recorded in sampling stations 5, 8 and 11, which correspond to the three tributaries evaluated (Caño Buque, Caño La Cuerera and Caño Maizaro, respectively). A different behavior was registered in sampling station 10 which presented a low index value (ICOMO = 0.54, "doubtful" category) in comparison to the neighboring sampling stations.

Besides, in contrast to the diversity measures, the sampling stations (6, 7, 8, 9, 11 and 12) with pollution high (ICOMO = 0.9 to 1.0) the richness and diversity (0D, 1D and 2D) was zero (except the sampling stations 4 and 5). Sampling stations 1, 2, 3, 13, 14 and 15 registered high richness and diversity while the ICOMO was decreasing.

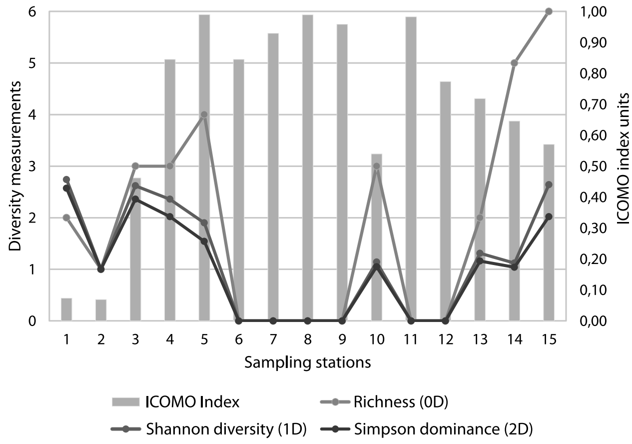

Figure 3. Trends in the spatial variation of the diversity measures: Richness (0D), Shannon diversity (1D) and Simpson dominance (2D) in the primary axis and the ICOMO index in the axis secondary. Aquatic quality values: Optimum (0-0.2); Good (0.2-0.4); Doubtful (0.4-0.6); Inadequate (0.6-0.8); Awful (0.8-1).

The GLM for Ephemeroptera's richness resulted in six models from lowest (best) AIC value. Coliform totals, ICOMO, nitrates, nitrites and temperature were significative variables in the models. The model six (6) had the best goodness of fit (R2 = 0,91); ICOMO and nitrates were the signiticative predictor variables with possitive effect in the model for the richness (0D) (Table 5).

Relationship between the physical chemical and bacteriological variables with the Ephemeroptera: The RDA model explained 47.19% of the variation in the stream in relation to pH, nitrites and flow, in a significant relationship between response and explanatory variables ($P < 0.012$, R^2 Adjusted = 49.5%). The first two axes

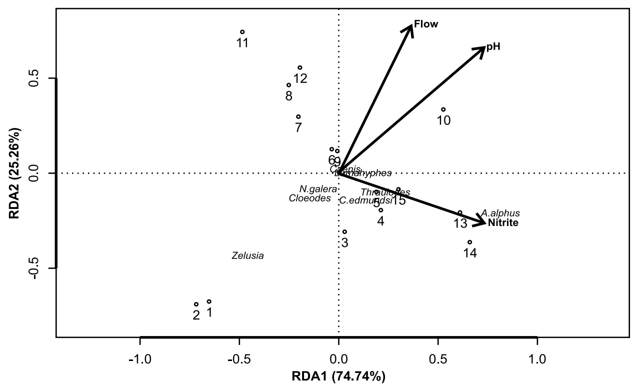

Figure 4. RDA classification diagram of the Ephemeroptera assemblage with respect to physical, chemical and bacteriological variables and sampling stations.

Table 5. Generalized linear model (GLM) for Ephemeroptera's richness (0D). Effects of each predictor on richness (0D). Variables not selected for the model are shown as (—). N = 15 for the models. Significant values (p < 0.05, test = Chi square) are in bold face.

Model	(Intercept)	Caud	Col.Tot	ICOMO	Nitra	Nitri	OD	Temp	AIC	R^2
1	-16.999	-2.172	—	—	—	**11.052**	—	**0.811**	50.254	0.6743
2	0.979	—	**0.000000032**	**-2.185**	**0.590**	—	—	—	51.649	0.6353
3	-1.101	—	**0.000000031**	—	**0.546**	—	0.015	—	51.672	0.6347
4	-1.983	—	**0.000000042**	—	**0.417**	4.132	0.021	—	51.762	0.7626
5	-4.841	-2.877	—	**10.643**	**0.390**	17.384	0.062	—	52.218	0.9130
6	-18.448	-2.304	0.000	—	—	**12.352**	—	**0.864**	52.885	0.7312

of the RDA explained 51.5% of this variation and the first axis explained 38.3% of this variation (eigenvalue = 0.18). It was possible to visualize that *Americabaetis, Thraulodes* and *Camelobaetidius* were positively related with Nitrite and the sampling stations 5, 13, 14 and 15, and negatively with the sampling stations 7, 8, 11 and 12. The second axis explained 13.19% of variation (eigenvalue = 0.062) and *Zelusia, Cloeodes* and *Nanomis,* in the sampling stations 1 and 2, were associated negatively to flow and pH, and the sampling station 10. The remaining genera represented by the cloud of points near the origin (point 0,0) apparently do not indicate any relationship with any sampling station or variable (Fig. 4).

DISCUSSION

Taxonomic composition of the Ephemeroptera assembly: There are nine Ephemeroptera families reported for Colombia (Salles *et al.,* 2020), four were recorded in this study: Baetidae, Leptohyphidae, Leptophlebiidae and Caenidae. These correspond to 44.4% of registered families in the country and they have the largest number of genera and species recorded (Dias *et al.,* 2009; Forero-Céspedes *et al.,* 2014; García *et al.,* 2013; Gutiérrez & Reinoso-Flórez, 2010; Gutiérrez *et al.,* 2013; Gutiérrez & Llano, 2015; Hoyos *et al.,* 2014; Molineri, 2014; Molineri *et al.,* 2011; Motta-Díaz *et al.,* 2012; Rozo & Salinas, 2016; Salinas *et al.,* 2011, 2012, 2013; Salinas-Jiménez *et al.,* 2017, 2018; Vinasco-Mondragón & Zúñiga, 2016; Zúñiga *et al.,* 2014, 2015; Zúñiga & Torres-Zambrano, 2015). Baetidae and Leptohyphidae were the most abundant and frequent in the different sampling stations, similar to what was found by Zamora (2015) in Orinoquia piedmont streams, where he further reported Leptophlebiidae and Oligoneuriidae. Likewise, studies conducted at east of the Meta (Franco *et al.,* 2012) and Casanare department (Camacho-Reyes & Camacho-Rozo, 2010) have been registered these families along with Caenidae, in savanna rivers and esteros. However, it can exist difference in the taxonomic composition (genera and species) due to the local biological, physical, chemical, hydrological and geomorphological attributes of these ecosystems compared to a piedmont river as the Ocoa (Donato & Galvis, 2008).

In this work three new registers of genera are reported for the Meta department: *Cloeodes, Lumahyphes* and *Zelusia.* Until now, 18 genera and 11 species were reported for this department (Dias *et al.,* 2009; Salinas-Jiménez *et al.,* 2017, 2018, 2019; Zamora, 2015; Zúñiga & Torres-Zambrano, 2015). With this research the genera registered increases to 21, which correspond to 38% of the approximately 55 genera recorded in Colombia (Dias *et al.,* 2009; Forero-Céspedes *et al.,* 2014; García *et al.,* 2013; Gutiérrez & Reinoso-Flórez, 2010; Gutiérrez *et al.,* 2013; Gutiérrez & Llano, 2015; Hoyos *et al.,* 2014; Molineri, 2014; Molineri *et al.,* 2011; Motta-Díaz *et al.,* 2012; Rozo & Salinas, 2016; Salinas *et al.,* 2011, 2012, 2013; Salinas-Jiménez *et al.,* 2017, 2018, 2019; Zúñiga *et al.,* 2014, 2015; Zúñiga & Torres-Zambrano, 2015). In addition, *A. alphus* is reported for the first time for this region, increasing the number of species to 12.

The variation in composition of mayflies at sampling stations was characterized by the abundance of *A. alphus* and *Lumahyphes.* The first was found in seven sampling stations (3, 4, 5, 10, 13, 14, 15); and second was reported in three sampling stations (10, 14, 15). This situation could be explained by particularity in their lifecycles; for example, the coincidence of sampling date with a pre-emergence period of nymphs (Domínguez *et al.,* 2009; Brittain, 1982), particularly at the sampling stations 14 *(A. alphus)* and 15 *(Lumahyphes).* Other reason may be that the supply nutrients decreased in these stations and this could favor them (Roldán & Ramírez, 2008); the above can be contrasted with ICOMO, in effect it was low to sampling station 3, 14 and 15, while it was increasing at sampling station 4 to 13.

Nanomis, Zelusia and *Cloeodes* registered the lowest abundance and frequency in the sampling stations before the urban center (1 and 2). They are not very tolerant organisms of pollution and environmental disturbance (Buss & Salles, 2007; Chacón & Segnini, 2007), and they have particular characteristics of distribution (Domínguez *et al.,* 2009). The register of *Caenis* at sampling station 15 is associated with the preference of these nymphs to live in slow to moderate currents with sediment areas, as well as lakes and floodplains (Domínguez *et al.,* 2009; Flowers & De La Rosa, 2010; Menetrey *et al.,* 2008; Zúñiga *et al.,* 1997). However, it has also been reported that Ephemeroptera nymphs are part of the drift of the rivers, as a defensive behavior, dispersion, feeding activities, contamination or unfavorable changes in the water quality and their habitat (Brittain & Eikeland, 1988; Elliott, 1967; Flowers & De La Rosa, 2010).

Diversity of the Ephemeroptera assembly: The lotic ecosystems provide a multiplicity of suitable microhabitats for the establishment of macroinvertebrates (Beisel *et al.,* 1998). They are susceptible to loss the structure and function of their natural characteristics due to the effects of pollution, especially in urban rivers (Bauernfeind & Moog, 2000; Prat *et al.,* 2009; Ramírez & Viña, 1998). The richness (0D), diversity of Shannon (1D) and dominance (2D) of Ephemeroptera recorded in this study were affected by urbanization (predominant land use matrix along the river). In urbanized zones the diversity was low and in six stations there were none nymph (sampling stations 6, 7, 8, 9, 11 and 12), including tributaries (Caño La Cuerera and Caño Maizaro). This is because the Ocoa river is the main sink for discharges of Villavicencio city; along with its tributaries Caño Grande, Buque, Maizaro, La Cuerera, Pendejo, Tigre, Arenoso and Siete vueltas, with more than 100 discharges identified, in approximately 17 km of distance, which greatly affects the functioning of the ecosystem (Osorio-Ramírez *et al.,* 2015). It has been reported in rivers of other regions, where the main factor influencing the richness and diversity of aquatic insects is the urbanization, along with domestic and industrial wastewater, deforestation, erosion processes, sediment drag, groundwater contamination, among others, that cause negative effects by partially or completely transforming the natural characteristics of aquatic ecosystems (Bauernfeind & Moog, 2000; Bispo & Oliveira, 2007; Moore & Palmer, 2005; Roldán & Ramírez, 2008).

On the other hand, the highest values of diversity were recorded in the highest stations (1, 2, 3 and 4) and in the lowest (14 and 15). The first ones still do not present a high degree of contamination and the last ones are favored by the recovery and dilution by healthy tributaries, which allows the establishment of mayflies' nymphs. Additionally, the matrix of land use in such stations corresponds to agriculture and livestock, which could favor the diversity and richness of these insects, as occurs in sampling stations 1, 3 and 15. In other studies it has been found that the diversity of aquatic insects in sites with agricultural influence is greater with respect to urbanized sites (Moore & Palmer, 2005; Cortezzi *et al.,* 2009).

Physical, chemical and bacteriological variables: In general, most variables showed high variation (CV > 1.0), possibly due to the heterogeneity of anthropogenic activities that develop along the river; for example, water catchments for domestic use and impacts by tourism are characteristic of the upper zone; in the middle prevail the domestic discharges on direct tributaries, in addition to discharges by oil industries, animal benefit plants, among others; and finally, in the lower area, the contribution of domestic discharges, the influence of oil activity and catchments for agricultural use (Osorio-Ramírez *et al.,* 2015).

The highest values of dissolved oxygen were registered in the first three sampling stations and decreased significantly on the last (located after the urban area: 14 and 15). This could be related to the impact generated by the contribution of organic matter, which increase the activity of chemical and microbiological processes and allow the establishment of tolerant organisms such as Chironomidae, Oligochaeta and Gastropoda; these organisms can tolerate low oxygen conditions in the water (Jiménez & Vélez, 2006). This is validated by the high values of total coliforms (from 170,000 to 79, 900,000 NMP/100 ml) at the stations located in the urban area. Those values infringe the specifications of article 38 of Decree 1594 of 1984 and indirectly influence the behavior of BOD_5, COD and dissolved oxygen; that situation represents an evident risk factor for the health of people who are related to the use of water resources in these sectors.

Nitrites and nitrates showed low values from sampling station 1 to 10, but gradually increased towards station 15. This is possibly related to livestock and agricultural crops (such as rice and palm) characteristic of this basin (Osorio-Ramírez *et al.,* 2015). The presence of ammonium is an indicator of contamination associated with chemical processes of degradation of organic matter (Pacheco *et al.,* 2002) and according to Decree 1594 of 1984, the admissible limit for the destination of the resource for the preservation of flora and fauna in freshwater should not exceed 0.1 mg/L. The sampling stations that were found below that value were 1 and 2, while the 4, 6, 11, 12 and 13 had values higher than 4.5 mg/L; the highest value of ammonium was registered in the tributary Caño Buque, with 30 mg/L, which indicates a potential danger to public health, as well as for the establishment of aquatic communities. Additionally, the flow values showed a gradual increase downstream, however they decreased in the last stations (14 and 15). That situation could be related to water abstraction destined for extensive livestock and agricultural crops such as palms or flow deviations (Osorio-Ramírez *et al.,* 2015), which puts in serious problems the preservation of aquatic life.

Respect to ICOMO, the sampling stations with category of "optimal" water aquatic were 1 and 2; which could be associated with the fact that the anthropic impact associated with them is lower magnitude (Table 1) compared to the urban area's sampling sites. Sampling station 3, the first in the urban area presented the category of "doubtful" water quality because at this point there are accumulated wastewater from the urbanization and industry, oil companies, fish farms, animal benefit plants for chickens, and so on, which could affect the structure and function of the ecosystem (Bauernfeind & Moog, 2000; Prat *et al.,* 2009). From sampling station 4 to 11, except 10, the index values rise drastically. This represents a high degree of contamination, but in the last sampling stations (12, 13, 14 and 15) it is observed how the values of the index decrease, when changing from "awful" to "doubtful" water quality. The flow of water and the biotic activities facilitate the processing of the organic matter and therefore the recovery of river (Roldán & Ramírez, 2008). The low pollution value of sampling station 10, compared to the surrounding ones, may be due to the dilution effect between 9 and 10 for the Caños Chavicure and Campoalegre (Osorio-Ramírez *et al.,* 2015). Then, better environmental conditions contribute to the dilution of the pollutant load of the main channel (Roldán & Ramírez, 2008).

The GLM resulted in that coliform totals, ICOMO, nitrates, nitrites and temperature were significative variables predictor in the six models. This is congruent with the behavior observed and discuted about of variation physical, chemical and bacteriological variables with the diversity. The highest stations (1, 2, 3, 4) and lowest (14, 15) presented a low contamination's pressure and the best Ephemeroptera's richness. The best model (6) (R^2 = 0.91, Table 5) had the ICOMO and nitrates like signiticative predictor variables for richness (0D); for that reason it's possible that the decision makers could employ this predictor variables like indicators about the health situation of the Ocoa river.

Relationship between physical, chemical and bacteriological variables with Ephemeroptera: *Americabaetis alphus* was the most abundant and frequent at Ocoa river, associated mainly with rapids and rocky substrates with high contents of detritus or particulate organic matter. It was presented in stations from "doubtful" to "awful" water quality, according to ICOMO (Fig. 4) and it had greater degree of association with nitrites, principally in the stations 13 and 14 (Fig. 3). Unlike found by Buss & Salles (2007), who mention that this species was found predominantly in litter substrate and in less proportion in rocky substrate. Additionally, nymphs *Americabaetis* has been reported associated with alkalinity, electrical conductivity, SDT, pH, hardness, flow, temperature, chlorides and phosphates in a urban stream in the Tolima department (Forero-Céspedes & Reinoso-Flórez, 2013), variables that denote a gradient of contamination by water mineralization (Ramírez *et al.,* 1997; Ramírez & Viña, 1998).

Camelobaetidius was recorded in fast currents and sampling stations with a degree of aquatic contamination from "doubtful" to "awful" (Fig. 4). *Camelobaetidius* is a detritivore genus, scraper of plant material from rocky surfaces and aquatic plants, with morphological adaptations such as setae in the cerci that help it to swim and spatulate claws to scrape and grapple to the substrate. They are considered to be not very sensitive to being found in clean and contaminated waters (Buss & Salles, 2007; Rojas *et al.,* 1993). Likewise, Forero-Céspedes & Reinoso-Flórez (2013) found this genus associated with variables that indicate contamination by water mineralization, such as alkalinity, hardness, electrical conductivity, SDT, pH, flow rate, temperature, chlorides and phosphates.

Varipes was found in rapids and rocky substrates, in waters from "inadequate" to "awful" quality (Fig. 4). Therefore, it could be inferred that this genus is tolerant to the contamination condition. Castillo & Pérez (2011) found it associated to gravel and sand substrates, and some degree of tolerance to habitat disturbance due to human activities, given the characteristics of the places where it was recorded. Additionally, Reynaga & Dos Santos (2012) found that this genus had characteristics such as moderate body hardness, oval-shaped gills, elongated body, claws to face the flow and high tolerance to oxygen deficit.

Nanomis, Zelusia, and *Cloeodes* were found in rapids and rocky substrates, "optimum" water quality (Fig. 4) and associated with high oxygen conditions, on the sampling stations of the high zone (1 and 2, with dissolved oxygen values of 7.96 and 8.19 mg O_2/L respectively); this would allow to suppose that these taxa are sensitive to conditions of water contamination. As reported by Buss & Salles (2007), *Zelusia* is a genus with very sensitive organisms, good swimmers of fast and slow currents. Chacón & Segnini (2007) reported that *Nanomis* is little tolerant to contamination, and associated with very low levels of water mineralization (expressed by the variables conductivity, hardness and alkalinity). *Cloeodes* organisms have a hydrodynamic body, which helps them resist the current and inhabit the stones (Buss & Salles, 2007). Forero-Céspedes & Reinoso-Flórez (2013) found it associated with variables related to mineralization processes: alkalinity, hardness, electrical conductivity, SDT, pH, flow, temperature, chlorides and phosphates. In general, *Cloeodes* is a sensitive genus to contamination condition; however, this qualifier may change with respect to the species (Buss & Salles, 2007).

Lumahyphes was found in slow currents and rocky substrates with sand; it was the second most abundant genus (with greater importance in station 15), related to stations with "doubtful" and "inadequate" water quality (Fig. 4), with some degree of tolerance to contamination conditions. Any information was found associated with ecological aspects of *Lumahyphes,* most of the information corresponds to taxonomic reports and species distribution (Boldrini *et al.,* 2015; Molineri, 2010; Molineri & Zúñiga, 2004); so this study is one of the first to provide information on ecological aspects of the genus.

The genus *Tricorythodes* was found in slow and rapid streams of rocky substrates with sandy bottoms. It showed association with stations of "uncertain" or "inadequate" water quality (Fig. 4). These results coincide with that reported by Rojas *et al.* (1993) who point out that it lives from warm to cold currents, their body have setae abundant, with filtering habits and they inhabit sandbanks at water bottom. In addition, they can be found from clean to slightly contaminated waters (Zúñiga *et al.,* 1997), and exhibit a greater degree of tolerance to high values of conductivity and hardness (Chacón & Segnini, 2007).

Thraulodes was one of the most frequent genus in the sampling stations, in fast currents with rocky substrates, with conditions from "doubtful" to "awful" water quality (Fig. 4) and associated with nitrates (Fig. 3). Zúñiga *et al.* (1997) indicated that these nymphs can be found in clean to slightly polluted waters, as reported by Chacón & Segnini (2007) that consider *Thraulodes* as a slightly tolerant, associated with low mineralization of water and sensitive to contamination by organic matter and oxygen deficiency.

Otherwise, the genus *Caenis* presented a single individual in station 15, in a microhabitat of slow current and rocky substrate, and associated with nitrates in "doubtful" water quality (Fig. 4). Zúñiga *et al.* (1997) signalized that the organisms of this genus are typical of waters with little or any current, associated with areas of mud, vegetation or muddy banks at water bottom and that rarely inhabit the middle or superficial part of the water column. It is known that *Caenis* can tolerate high polluted and eutrophic waters with high temperatures and low oxygen levels (Flowers & De La Rosa, 2010).

CONCLUSIONS

This study highlights the effect of water pollution on the Ephemeroptera diversity in a low precipitation period. The direct and diffuse domestic and industrial waste discharges that the Ocoa river collects along, as well as tributaries with the same nature, showed that most stations located in the urban area of Villavicencio did not register mayflies. The stations at the ends of the city increased abundance and their diversity. We found that the residual load discharged into this river represents a public health problem, also an ecological degradation that limits the availability and diversity of habitats for the establishment of Ephemeroptera nymph. Most water pollution problems are generated as a result of the development of human activities, so it is important that they begin to generate strategies from the field of environmental management to minimize the impact on water resources.

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AUTHORS' CONTRIBUTIONS

Formulation research question: J.I.R.P., D.P.O.R., J.M.V.R.; Taxonomic determination: J.I.R.P., L.G.S.J.; Data analysis: J.I.R.P., J.M.V.R.; Supervision: D.P.O.R., J.M.V.R.; Writing-original draft: J.I.R.P.; Funding acquisition, experimental design and project administration: D.P.O.R., C.I.C.C. All authors read, writing-review, editing and approved the final document.

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