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Black Fly (Diptera: Simuliidae) Assemblages of High Andean Rivers Respond to Environmental and Pollution Gradients

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Abstract

Simuliid larvae are common inhabitants of mountain rivers throughout the world, where they can serve as ecological indicators. Black flies were sampled during three seasons in four rivers in the upper basin of the Bogotá River in the Colombian Andes, and physical, chemical, and hydrological data were recorded. Multivariate methods were used to determine the relationships between the presence and abundance of simuliid species and environmental characteristics. Fourteen species were found: eight in the genus *Gigantodax* (Enderlein, Diptera, Simuliidae) and six in the genus *Simulium* (Latreille, Diptera, Simuliidae). Dissolved oxygen, dissolved solids, redox potential, chemical oxygen demand, and nutrients contributed to an explanation of species distributions. Species in clean waters and in more polluted waters had narrow niches; those in low to moderately polluted waters had broader niches. Species in the lower reaches of the watercourses had greater turnover, perhaps because the most sensitive species had disappeared and been replaced by more tolerant species. Thus, simuliids can be used as predictors of environmental characteristics of Andean rivers and can be useful in the evaluation and management of these watersheds.

Key words: bioindicator, high-mountain river, macroinvertebrate, Neotropical Region

Black flies (Simuliidae) are dipteran insects whose immature stages are exclusively aquatic (Dobson 2013). Adults are of medical importance in vector-borne diseases such as onchocerciasis and mansonellosis. In addition, they have been considered good indicators of water quality (Docile et al. 2015). The larvae filter small particles from the water column and egest large numbers of fecal pellets (Hershey et al. 1996, Malmqvist et al. 2001, Wotton and Malmqvist 2001). They have a high rate of intestinal yield, and despite their low assimilation efficiency (Wotton 1978), their trophic function is remarkable, given that the biomass they process can be consumed by organisms of higher trophic levels, such as macroinvertebrates and fish (Wallace and Webster 1996, Allan and Castillo 2007). However, ecological studies of Simuliidae in the Neotropics are scarce (Hamada et al. 2002; McCreadie et al. 2004, 2017; Figueiró et al. 2006). Ecological information about black flies in Colombian rivers is particularly scarce (Mantilla et al. 2018), but the taxonomy of the family is well known in some regions of the country (Moncada et al. 2017).

Many studies in different areas of the world suggest that environmental factors play a significant role in determining the distribution of black flies (Gallardo-Mayenco and Toja 2002, Hamada et al. 2002, McCreadie et al. 2006, Landeiro et al. 2009, Pachón and Walton 2011, McCreadie and Adler 2012, Srisuka et al. 2015). In South America, studies have indicated the importance of dissolved oxygen, current velocity, elevation, flow, temperature, and light intensity (Grillet and Barrera 1997; Hamada et al. 2002; Figueiró et al. 2006, 2012; Landeiro et al. 2009), but in Colombia, few studies have associated simuliids with physical characteristics of streams (Rojas 2013, Buitrago-Guacaneme et al. 2018, Mantilla et al. 2018). The high Colombian tropical mountains are subject to seasonal variation in precipitation and day–night thermal differences, which could influence the ecological responses of black flies, compared with other areas in the Neotropical Region. Furthermore, the taxa composition is different in the high Andean mountains, as happens with *Gigantodax*, a genus that has a distribution above 2,200 masl and is not found in Brazil (Moncada et al. 2017).

The upper basin of the Bogotá River is in the paramo and Andean forest and is included in a conservation program, but its middle basin receives effluent from wastewater and solid waste when it passes through the most populated areas. Thus, this middle sector takes in effluent from industrial and domestic uses of several municipalities. The catchment is currently coupled with a decontamination plan in accordance with the Colombian Laws requirements (Judgment of the Council of State of Colombia, Güiza et al. 2015). Simuliids are principally found in clean to moderately contaminated watercourses (Roldán 2003), but because their aquatic larvae require fine particulate organic matter, and urban areas provide significant organic input, some taxa are expected in waters with higher levels of contamination. Streams of the Bogotá Basin present a huge physicochemical gradient and, therefore, can be used to understand, and possibly model, the distribution of simuliids. Thus, these dipterans offer potential as bioindicators of river pollution.

The aim of this study was to correlate physical, chemical, and hydrological characteristics with the presence and abundance of simuliid species in watercourses of the Bogotá River Basin. The following hypotheses were tested: 1) environmental degradation of water quality leads to decreased richness of black flies, 2) organic pollution leads to increased abundance of tolerant species, and 3) given wide environmental variations in these rivers, turnover of simuliid species is expected along gradients.

Materials and Methods

Sampling Sites

Four watercourses of different conservation conditions were included (Table 1). One was the Bogotá River in its upper basin (second-order according to the classification of Harrel 1966), where the highest sampling point was in a protected and well-conserved area, and the other two sampling points were in agricultural and cattle-raising zones. The other three watercourses were small to intermediate streams of the Eastern Hills of Bogotá city: Fucha river (second order), where two sampling points were in a protected forest area, and the third (lowest) was strongly influenced by domestic waste; Arzobispo stream (first order), which was influenced by hiking and urban activities even at the headwaters; and La Vieja stream (first order), which had moderate influence from tourism due to hiking routes, and with the lowest sampling point receiving anthropogenic influences (e.g., effluent) from human dwellings. These three streams are tributaries of the Bogotá River in its middle basin. Each watercourse was sampled in three different seasons: rainy (September), transition (November), and dry (March), from 2015 to 2016. During this period, Colombia was influenced by a strong El Niño Southern Oscillation (ENSO) phenomenon (L'Heureux et al. 2017). The three different sampling stations in each watercourse were referred to as 'high', middle', and 'low' reaches, and they were characterized by a pollution gradient from clean water in the headwaters to polluted in the lowest sectors, where fecal coliform increased greatly (see Supp Table 1 [online only]).

Biological, Hydrological, Qualitative, and Physicochemical Sampling

Larval and pupal black flies were collected by hand from all available substrates: rocks, trailing riparian herbs and leaves, submerged stems, leaf litter, wood (trunks), and artificial substrates such as discarded plastic. Each point was sampled for 1 h by two people (McCreadie et al. 2005). Specimens were fixed in 80% ethanol for identification by morphological and molecular methods, and those with white or gray histoblasts were fixed in Carnoy's solution (one part acetic acid: three parts 95% ethanol; Adler et al. 2004). The Carnoy's solution was changed twice within the first hour, and once more after 24 h, and stored at 4°C. Some pupae were kept in humid cages to permit emergence of adults to aid identification (McCreadie and Colbo 1992, Hamada and McCreadie 1999, Adler et al. 2004). Larvae with gray or black histoblasts and pupae were identified to species level with taxonomic keys of Wygodzinsky and Coscarón (1989) and Coscarón and Coscarón-Arias (2007), and descriptions of species by Bueno et al. (1979), Moncada et al. (1981), Muñoz de Hoyos et al. (1982, 1984), Muñoz de Hoyos (1990, 1995), Muñoz de Hoyos and Miranda-Esquivel (1997), and Muñoz de Hoyos and Coscarón (1999). Molecular methods based on the mitochondrial cytochrome oxidase subunit 1 (COI) gene (Folmer et al. 1994) and cytogenetic techniques (Adler et al. 2016) were used to confirm identifications of selected species.

Quantitative measurements were made for terrain (elevation and slope), hydrological (current velocity, channel depth, and flow), and physical, chemical, and bacteriological variables, including water temperature, dissolved oxygen, oxygen saturation, redox potential, electric conductivity, total dissolved solids, pH, nitrates, nitrites, ammonia nitrogen, orthophosphates, sulphates, Kjeldahl total nitrogen, total phosphorous, biological oxygen demand (BOD_5), chemical oxygen demand (COD), and fecal coliform density. Methodologies established by UNESCO-WHO (1978), APHA-AWWA-WPCF (1992), OMM (1994), and IDEAM (2007) were used. Categorical variables were type of riparian vegetation (native forest, intervened forest, agriculture, and cleared area), particle size of the riverbed (mud, sand, small stones, rubble, boulders, and bedrock), and canopy cover. Substrates were categorized as percentage of plastic, litter, mosses, wood (trunks), stones > 40 cm, and stones < 40 cm. Canopy was categorized as open, partial, and complete.

Data Analysis

The frequency of species in the streams was calculated in two ways: dividing the number of times the species were recorded by the total number of samples (36) and dividing the number of sites where the species were collected by the total number of sampled sites (12; Hamada et al. 2002). To obtain the richness and diversity for each stream (only for simuliid species), the following ecological descriptors were calculated: species richness (*S*), Shannon–Wiener diversity (*H*), Simpson dominance (*D*), and Pielou evenness (*J*; Magurran 2004). Only larvae with gray or black histoblasts and pupae were considered for the ecological indices (McCreadie and Adler 2014), which were calculated with the free software Past (Paleontological Statistics version 2.17; Hammer et al. 2001).

The analysis of environmental variables was performed using R version 3.3.3. (the R Foundation for Statistical Computing). When necessary, the variables were transformed with $\log_{10}$, or $\log_{10} x + 1$ when the variable contained a zero. After checking for a lack of extreme outliers, data were subjected to principal component analysis (PCA) to explore the organization of the sampling points. Of the 17 physical, chemical, and bacteriological variables measured, those that presented collinearity (correlation coefficient > 0.95) were discarded, and eight with the highest coefficient of variation were selected for the PCA. The highest PCA eigenvalues were used to detect the most important environmental variables in the set of watercourses. We used all variables for a redundancy analysis (RDA; Rao 1964, Peres-Neto et al. 2006) to find abiotic predictors of the presence and abundance of simuliids.

Table 1. General characteristics of sampling points in watercourses of the Upper Bogotá River Basin, Colombia

Stream	Reach	Coordinates	Elevation (masl)	Substrate	Vegetation and environmental conditions
Fucha	High	4° 32′ 51.8″ N 74° 3′ 19.4″ W	2,970	Rocks of different sizes	Dominance of *Pinus patula* (introduced exotic species), minimal human activities. CPI = 3.75
	Middle	4° 33′ 45″ N 74° 3′ 45″ W	2,850	Rocks of different sizes	*Eucalyptus globulus* and *Pinus patula* (both introduced exotic species); *Chusquea* sp., scarce human activities. CPI = 6.03
	Low	4° 34′ 5.2″ N 74° 4′ 3.6″ W	2,780	Large rocks	Shrublands of native and exotic species, meadows, urban effect, wastewater inlet. CPI = 146.27
La Vieja	High	4° 38′ 34.7″ N 74° 02′ 23.8″ W	2,924	Mostly leaf litter and rocks of middle sizes	Andean forest vegetation (*Smallanthus pyramidalis*, *Clusia murtiflora*, *Pteridium aquilinum*), hiking activities. CPI = 3.90
	Middle	4° 38′ 42.3″ N 74° 02′ 37.0″ W	2,825	Large rocks and litter	Andean forest and *Eucalyptus globulus*, hiking activities. CPI = 3.27
	Low	4° 39′ 0.4″ N 74° 02′ 57.7″ W	2,703	Rocks of different sizes	Mixture of native (*Symphytum officinale*, *Sambucus nigra*, *Fuchsia boliviana*) and exotic species (*Acacia melanoxylon*), urban influence. CPI = 3.59
Arzobispo	High	4° 37′ 18,3″ N 74° 03′ 1,9″ W	2,670	Large rocks and some wooden logs	Andean forest vegetation and *Eucalyptus globulus*, hiking activities. CPI = 3.00
	Middle	4° 37′3.56″ N 74° 3′13.8″ W	2,650	Rocks of middle sizes, wood, and litter	Andean forest vegetation and *Eucalyptus globulus*, hiking activities. CPI = 6.94
	Low	4° 37′ 25″ N 74° 02′ 23.9″ W	2,628	Rocks of middle and large sizes, floating leaves, and various inorganic substrates (mostly plastic)	*Eucalyptus globulus*, *Rubus ulmifolius,* meadows, influence of passive recreational activities. CPI = 11.83
Bogotá	High	5° 12′ 39.6″ N 73° 32′ 57.2″ W	3,157	Large rocks and some floating branches	Andean forest and paramo vegetation, minimal human activities. CPI = 2.01
	Middle	5° 13′ 20.3″ N 73° 34′ 7.0″ W	2,885	Medium-sized rocks, logs, and litter	Andean forest vegetation and *Eucalyptus globulus*. Influence of potato and corn crops. CPI = 3.02
	Low	5° 13′ 25.6″ N 73° 35′ 32.6″ W	2,780	Rocks of small, middle, and large sizes	*Eucalyptus globulus*, meadows for livestock, influence of agricultural crops. CPI = 4.91

CPI, chemical pollution index (according to Jiang and Shen 2005).

The chemical pollution index (CPI) for each site and species tolerance of environmental variables were explored by calculating species pollution values (SPVs), following the methodology of Jiang and Shen (2005); permissible values for human consumption of 10 water quality variables, according to Colombian legislation, were considered: conductivity, total dissolved solids, dissolved oxygen, pH, sulphates, nitrites, nitrates, orthophosphates, BOD_5, and fecal coliform. The following equation was used to calculate the CPI:

$$\mathrm{CPI} = \sum_{i=1}^{n} \frac{C}{\mathrm{LC}}$$

where C is the concentration of the variable, LC is the variable concentration limit, and n is the number of parameters. The pollution value of a specific taxon (SPV) was calculated as follows:

$$\mathrm{SPV} = \frac{\sum_{i=1}^{n} \left(\frac{\mathrm{Ln10CPI}}{n}\right) i}{N}$$

where n is the number of chemical variables, N is the number of stations, and i represents the stations where the taxon was collected. Spearman's correlation between CPI and species richness was calculated.

Niche breadths for the species were calculated with the Shannon–Wiener index (H): $H = (-\Sigma \mathrm{pi}\ \mathrm{Ln}\ \mathrm{pi})$, where pi is the number of individuals of the species at each site divided by the total sites occupied by that species (Colwell and Futuyma 1971). Turnover of simuliid species between sites of each river was assessed with Whittaker and Cody indices (Koleff et al. 2003).

Results

Overall Simuliid Prevalence

In the four watercourses, 14 species of black flies (8 in genus *Gigantodax* and 6 in genus *Simulium*) were collected (Table 2 contains the authors of the species names). The Bogotá River shared 10 (71.4%) of the 14 total species with the streams in the Eastern Hills of Bogotá city. The species found only in the Bogotá River were *S. arcabucense*, *S. sumapazense*, *S. tunja*, and *G. siberianus*, whereas *G. multituberculatus* was found only in La Vieja and *G. basinflatus* was found only at the highest points in all streams, with the exception of Arzobispo stream.

Species occurrence for the total samples (36), including the different seasons, was greatest for *S. muiscorum* (83.3%), *G. ortizi* (75.0%), and *S. ignescens* (69.4%). Middle frequencies corresponded to *S. furcillatum* (30.5%); *G. cervicornis* and *G. osornorum* (27.8%); *G. wygodzinskyi* (13.9%); *S. tunja* (11.1%); and *G. basinflatus*, *G. destitutus*, and *G multituberculatus* (8.3%). Taxa with lowest representation were *G. siberianus*, *S. arcabucense*, and *S. sumapazense*, each with a frequency of 2.8%. If the occurrence was considered only by sampling places (12), the frequency was *S. muiscorum*: 100%; *S. ignescens*: 91.7%; *G. ortizi*: 83.3%; *S. furcillatum*: 50.0%; *G cervicornis*: 41.7%; *G. wygodzinskyi*: 33.3%; *G. basinflatus*, *G. multituberculatus*, *G. destitutus*, and *G. osornorum*: 25.0%; and *G. siberianus*, *S. arcabucense*, *S. sumapazense*, and *S. tunja*: 8.3%. The abundance of species varied between sections of each river (Fig. 1). In the Arzobispo stream, total abundance was similar along the elevational gradient, but *S. muiscorum* and

G. ortizi were dominant in the high and middle sectors, whereas *G. cervicornis* was dominant in the low section (Fig. 1a). For the Bogotá River, the greatest abundance occurred in the middle sector, represented mainly by *S. ignescens*, whereas in the lower sector *S. tunja* had greater abundance (Fig. 1b). In Fucha river, the abundance decreased from the upper to the lower section, whereas the opposite occurred in La Vieja stream. The most representative taxa in Fucha were *S. muiscorum* and *S. ignescens* (Fig. 1c), and in La Vieja, they were *G. ortizi*, *S. muiscorum*, and *S. ignescens* (Fig. 1d).

The Bogotá River, considering all the sectors evaluated, was the richest in species (11), with relatively high diversity (noticing that it was calculated with the species of a single invertebrate family) and evenness and low dominance (H = 1.23, J = 0.51, D = 0.37). Among the watercourses, Fucha and La Vieja had nine and eight species, respectively, with low values of diversity (H = 1.0 and 0.95), major dominance (D = 0.53 and 0.49), and moderate evenness (J = 0.45 in both streams). Despite having fewer species (7), the Arzobispo stream had the greatest diversity, the least dominance, and the highest evenness (H = 1.29, D = 0.3, and J = 0.66). The diversity by sectors (Table 2) in Fucha and Arzobispo rivers increased in the lower reaches, whereas in La Vieja stream and the Bogotá River, diversity was higher in the upper reaches.

Environmental Description

Ordination of physical, chemical, and hydrological characteristics (Table 3, Fig. 2) showed that the four systems had different conditions and formed at least two groups. Fucha and Arzobispo streams were associated with high values of nitrites, ammonia nitrogen, orthophosphates, total phosphorus, sulphates, BOD_5, COD, and fecal coliform (i.e., variables indicating organic contamination). The second group was composed of the Bogotá River and La Vieja stream, which had better water quality, based on low values of the aforementioned variables. Eigenvalues of variables associated with PCA-1 (Fig. 2a) were as follows: nitrite (−1.14), orthophosphates (−0.80), fecal coliform (−1.02), and total phosphorous (−1.17). The associated variables for PCA-2 were ammonia nitrogen (0.76), BOD_5 (−0.95), and COD (−0.96).

The sampling points were separated mainly according to elevation and slope. In the PCA of hydrological and terrain variables (Fig. 2b), the slope (−1.09), depth (1.26), mean velocity (1.24), and discharge (1.51) had high eigenvalues with PCA-1. With PCA-2, elevation (−1.46) and slope (0.75) were the more important variables. This PCA identified three groups. One group had Fucha and Bogotá rivers, in which elevation, water velocity, stream depth, and discharge were higher. A second group corresponded to Arzobispo stream, with high slope, and the third group was represented by La Vieja stream, in an intermediate position between the previous two.

The sampling stations were separated mainly by vegetation cover and type of substrate (Fig. 2c). The highest eigenvalues associated with PCA-1 were rocks greater than 40 cm (ROC2, 1.39) and grade of cover (−1.29); with PCA-2, the associated variables were small rocks (ROC1, 0.76), leaf litter (−1.30), small trunks (0.92), and mosses (0.99). This PCA showed that each river had its own qualitative features. The Bogotá River was characterized by mosses and high Andean vegetation (cloud forest and paramo), especially at the highest elevation site. For the Arzobispo stream, plastic waste was a determinant substrate at the lower point. In La Vieja stream, the principal variables were greater cover of vegetation (Andean forest) and smaller rocks (ROC1).

The PCA performed with all variables separately for watercourses (Fig. 2d), seasons (Fig. 2e), and elevational reaches (Fig. 2f) confirmed many of the results shown by the previous PCA for each group of variables. Fucha was associated principally with larger rocks (ROC2), discharge, COD, and BOD_5; Arzobispo was related to slope, nitrite, and total phosphorus; La Vieja was related to canopy; and the Bogotá River was influenced by elevation, mosses, and depth

Table 2. Presence, richness, and diversity of simuliid species of *Gigantodax* and *Simulium* at each collection point in watercourses of the Upper Bogotá River Basin, Colombia

Taxa	Fucha River			Arzobispo stream			La Vieja stream			Bogotá River		
	High	Middle	Low	High	Middle	Low	High	Middle	Low	High	Middle	Low
G. basinflatus Wygodzinsky & Coscarón	R						R			R		
G. cervicornis Wygodzinsky	D	T, R, D	R, D			T, R, D			D			
G. destitutus Wygodzinsky & Coscarón	R						D	R				
G. multituberculatus Wygodzinsky & Coscarón				T	T	T	R	T	R			
G. osornorum Muñoz de Hoyos, Martínez, Mejía & Bueno	D	T		R		D	D	T		R, D	T, R, D	
G. ortizi Wygodzinsky	T, R, D	T, R, D	R, D	T, R, D	T, R, D	T, R, D	T, R	T, R, D	T, R, D		R, D	
G. siberianus Wygodzinsky & Coscarón										R		
G. wygodzinskyi Moncada, Muñoz de Hoyos & Bueno			D			R, D				D		T
S. arcabucense Coscarón										T		
S. sumapazense Coscarón & Py-Daniel										T		
S. furcillatum Wygodzinsky & Coscarón	R	T, R, D	T		T, R					T	T, R, D	
S. ignescens Roubaud	R	T, R, D	T, R, D	T, D	D	T, R, D		R	R, D	T, R, D	T, R, D	T, R, D
S. muiscorum Bueno, Moncada & Muñoz de Hoyos	T, R, D	T, R, D	T, R, D	T, R, D	T, R, D	T, D	T	T, R, D	T, R, D	R, T	T, R, D	T
S. tunja Coscarón											D	T, R, D
Richness	8	6	6	5	5	7	6	6	5	9	6	4
Shannon diversity	0.58	1.23	1.24	0.77	0.86	1.19	1.54	0.87	0.84	1.24	0.81	0.33

Authors of species names are included. R, rainy, T, transition, D, dry.

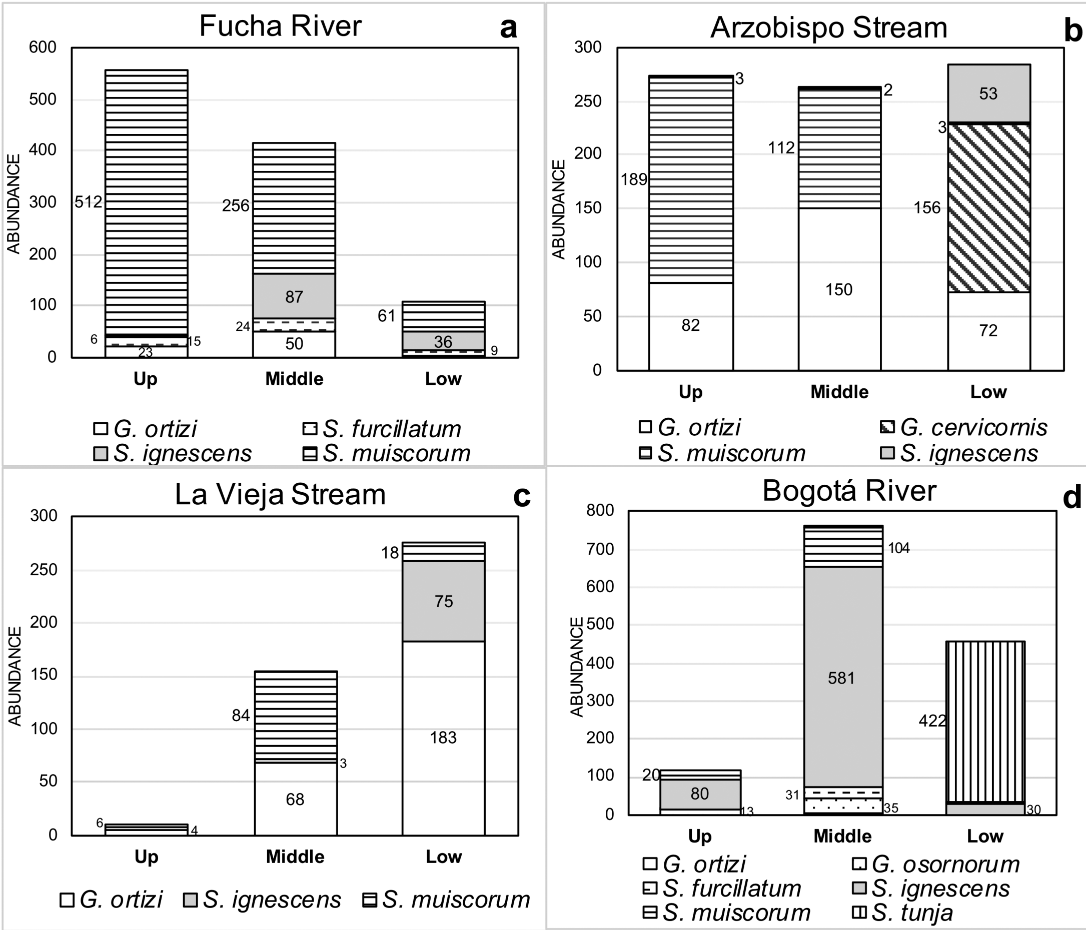

Fig. 1. Abundance of main simuliid species in the high, middle, and low reaches of the (a) Fucha, (b) Arzobispo, (c) La Vieja, and (d) Bogotá watercourses, 2015–2016.

(Fig. 2d). The seasons overlapped, with a tendency for major nutrients in the dry season (Fig. 2e). With regard to sampling sites along the elevational gradient, the upper points were grouped separately and associated with elevation, moss, plant cover, and slope. Middle and lower points shared variables of pollution, depth, and discharge (Fig. 2f).

Predicting Simuliid Distributions

The RDA showed dissolved oxygen, total dissolved solids, and redox potential as physical and chemical conditions predicting the presence of simuliid species ($P < 0.01$, Table 4). Indicator taxa are shown in Fig. 3a. *Simulium muiscorum* was associated with redox, dissolved oxygen, COD, sulphates, and total nitrogen. *Gigantodax cervicornis* was strongly related to a high concentration of fecal coliform; *G. ortizi* showed a slight association with orthophosphates, BOD_5, and total phosphorous, whereas *S. furcillatum*, *S. ignescens*, *S. tunja*, and *G. osornorum* were not related to any parameter. For terrain and hydrological variables, elevation, mean water velocity, and discharge were significant for presence of black flies ($P < 0.001$, Table 4). *Simulium ignescens* and *S. tunja* were most related to mean velocity; other taxa were not clearly related to any parameter (Fig. 3b). For qualitative variables, ROC2 was significant ($P < 0.01$, Table 4). In the RDA plot, *S. ignescens* and *S. tunja* were significantly associated with ROC2, *G. ortizi* with vegetation cover, and *G. cervicornis* with plastic waste (Fig. 3c). For all type of variables, the stream and the sampling point were highly significant ($P < 0.001$, Table 4).

In general, the lower stations had greater organic contamination, as shown by the chemical pollution index (CPI in Table 1), in contrast to the high sectors, where, except for La Vieja, contamination was less. Species richness and the CPI were not correlated (Spearman's $r = -0.109$, $P = 0.73$). Some black fly species (*G. cervicornis* and *G. wygodzinskyi*) showed higher tolerance to pollution (Table 5) and were found mainly in low sections of the watercourses, where there was greater anthropogenic influence. Other species (*G. siberianus*, *S. arcabucense*, and *S. sumapazense*) had SPVs indicative of greater sensitivity to water contamination and were recorded only in cleaner reaches of the Bogotá River (a narrow niche, Table 5), corresponding to the paramo area, where this lotic system originates. The remaining nine taxa had intermediate SPVs, but SPVs of the most common species, i.e., species with wider niches (*G. ortizi*, *S. muiscorum*, *S. ignescens*, and *S. furcillatum*), tended to be relatively high.

Turnover of species generally increased in the middle and lower reaches of each watercourse (Table 6), especially at the lower sites, which suffered greater deterioration and contamination and had higher values of the Whittaker and Cody indexes, meaning greater turnover of taxa. The Bogotá River showed the greatest turnover in its middle and lower sections, with 2.0 and 3.5 species that changed between sections, in comparison with the other stream systems.

Table 3. Physical, chemical, bacteriological, and hydrological characteristics of watercourses in the Upper Bogotá River Basin, Colombia

Variable	Fucha River		Arzobispo stream		La Vieja stream		Bogotá River	
	Average	SD	Average	SD	Average	SD	Average	SD
Temperature (°C)	13.0	1.7	12.7	0.9	12.1	0.5	12.5	1.9
Dissolved oxygen (mg/l)	7.64	0.3	7.37	0.4	7.73	0.2	7.64	0.4
Redox potential (mV)	184.2	91.93	183.3	77.03	225.5	98.3	222.2	87.97
Saturation of oxygen (%)	101.5	1.2	93.3	7.9	100.1	1.6	101.2	2.4
Conductivity (µS/cm)	33.4	15.4	18.3	6.55	8.8	3.04	10.8	7.68
pH (units)	6.81	0.41	6.37	0.5	6.01	0.63	5.65	0.53
Total dissolved solids (mg/l)	20.3	9.12	11	4.13	5.5	1.8	6.5	4.5
NO_3 (mg/l)	0.467	0.22	0.667	0.52	0.478	0.18	0.267	0.1
NO_2 (mg/l)	0.009	0.008	0.006	0.004	0.006	0.007	0.003	0.004
NH_4 (mg/l)	0.218	0.19	0.794	1.12	0.038	0.03	0.036	0.04
PO_4 (mg/l)	0.466	0.31	0.558	0.41	0.300	0.1	0.257	0.2
SO_4 (mg/l)	3.55	5.3	0.57	0.53	0.11	0.33	0.0	0.00
Fecal coliform (most probable number)	47,733	132,194.4	2,433	5,073.5	280	543.2	447	812.6
Biological oxygen demand (mg/l oxygen)	2.3	1.8	3.3	3.5	2.5	1.4	1.4	1.0
Chemical oxygen demand (mg/l oxygen)	16.7	12.1	3.3	3.5	12.7	6.1	1.4	1.0
Total phosphorous (mg/l)	0.166	0.155	0.151	0.090	0.080	0.047	0.050	0.000
Total nitrogen Kjeldahl (mg/l)	0.533	0.347	0.300	0.090	0.300	0.090	0.270	0.000
Meters above sea level	2,866	83.2	2,649	18.2	2,817	95.9	2,926	184.5
Slope (%)	8	1.5	18.7	9.5	14.6	4.0	4.0	2.3
Depth (m)	0.31	0.12	0.19	0.04	0.13	0.06	0.25	0.05
Speed (m/s)	0.26	0.09	0.10	0.05	0.27	0.28	0.27	0.28
Flow (m^3/s)	1.13	0.7	0.24	0.2	0.33	0.3	0.98	1.2

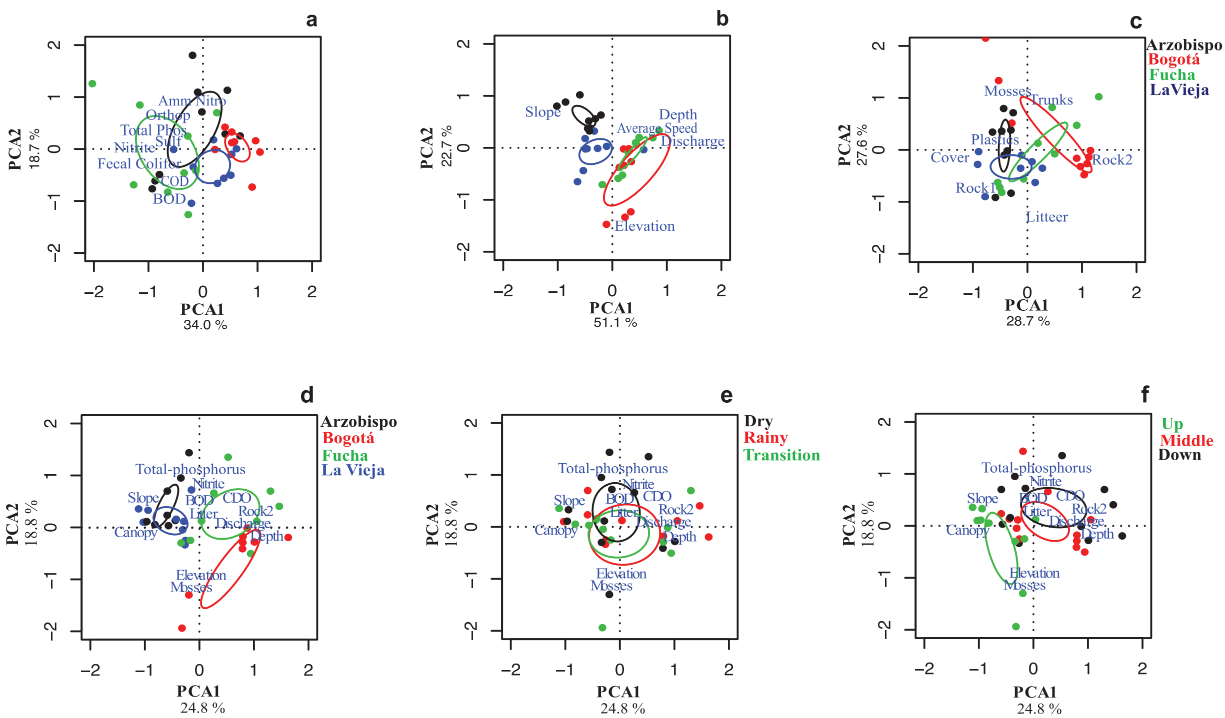

Fig. 2. Principal component analysis plots of abiotic variables in the Arzobispo, Bogotá, Fucha, and La Vieja watercourses, 2015–2016. (a) Physical, chemical, and bacteriological variables; (b) hydrological and terrain variables; (c) qualitative variables; (d) river and stream ordination based on all variables; (e) climatic ordination; (f) elevational ordination. ROC1: stones > 40 cm; ROC2: stones < 40 cm.

Discussion

Simuliid Abundance and Predictors of Richness

Some authors have suggested that species richness of the Simuliidae is higher in the Neotropical Region (Hamada et al. 2002); however, comparisons can be influenced by ecoregions and methodologies (Malmqvist et al. 2004, Srisuka et al. 2015, Ya'Cob et al. 2016, Sankarappan et al. 2018). Buitrago-Guacaneme et al. (2018) reported only five species in three of the watercourses that we studied, but they subsumed several species of the genus *Gigantodax* within a single taxonomic entity, because at that time the species could not be

Table 4. Redundancy analysis results of abiotic variables as predictors of Simuliidae abundance in the Arzobispo, Bogotá, Fucha, and La Vieja watercourses of Colombia, 2015–2016

	R^2 adjusted	df	AIC	F	P (>F)
Physical and chemical variables					
Stream	0.318	3	−34.24	6.44	0.002**
Point	0.416	2	−38.20	3.71	0.002**
Dissolved oxygen	0.454	1	−39.82	3.07	0.012*
Total dissolved solids	0.499	1	−42.20	3.61	0.014*
Redox	0.524	1	−43.36	2.48	0.042*
Hydrological variables					
Stream	0.318	3	−34.24	6.44	0.002**
Point	0.416	2	−38.20	3.71	0.002**
Elevation	0.475	1	−41.21	4.33	0.004**
Mean velocity	0.520	1	−43.73	3.74	0.010**
Discharge	0.561	1	−46.21	3.58	0.008**
Qualitative variables					
Stream	0.318	3	−34.24	6.44	0.002**
Point	0.416	2	−38.20	3.71	0.002**
ROC2	0.449	1	−39.51	2.79	0.034*

df, degrees of freedom; AIC, Akaike's information criterion; F, Fisher–Snedecor statistic; P, probability.
**$P < 0.001$, *$P < 0.01$.

reliably differentiated. Simuliid richness in some Neotropical areas can reach 35 species, e.g., in Central Amazonia, Orinoco Basin, and Brazilian Cerrado (Hamada et al. 2002, McCreadie et al. 2006, Figueiró et al. 2012), but more streams and larger elevational gradients were sampled than in the present work. In Colombia, 74 species of black flies have been reported (Moncada et al. 2017); 32 of these are found in high mountains, from Andean forest to paramo, of which 18 belong to *Gigantodax* and 14 to *Simulium*, so that about half of the richness of high Andean black flies exists in our study area.

Although Colombian species are considered multivoltine, they experience rainy to dry seasonality. Thus, some taxa are associated not only with water quality, but also with a specific climatic season or elevation. *Gigantodax basinflatus*, e.g., occurred only at the highest sampling points in three streams during the rainy season, a result that coincides with that reported for this species by Mantilla et al. (2018), who found that the species is associated with colder sites, that is, higher elevations. Other species, such as *S. tunja*, had higher occurrence in all seasons, but only in the low sector of the Bogotá River, possibly responding more to the elevational gradient. The elevational distribution in Colombia for *G. basinflatus* is 3,235–3,700 masl (Moncada et al. 2017), but the species probably can occur at lower elevations, including the studied range (2,924–3,157 masl), with drift from upper reaches. The most frequent and abundant species in our study (*S. muiscorum* and *G. ortizi*) were also the most prevalent in a previous study in the same watercourses (Buitrago-Gucaneme et al. 2018). Sampling in that study was done in 2012, indicating that the dominant species in the simuliid assemblages of these ecosystems did not change significantly between that year and 2015–2016.

Several researchers have found that seston, width, depth, velocity, flow, conductivity, pH, dissolved oxygen, and temperature explain simuliid presence and abundance (Corkum and Ciborowski 1988, Corkum 1989, Wade et al. 1989, Quinn and Hickey 1990, McCreadie and Colbo 1992, McCreadie and Adler 1998). Other authors have included elevation, type of streambed, land use, riparian vegetation, and channel morphology (McCreadie and Adler 1998, Nessimian et al. 2008, Docile et al. 2015). Given the lack of consensus as to which variables are associated with distributions of black flies, and because our study is one of the first for simuliids in a high-elevation region of the Colombian Andes, we explored the association by using a wide range of 22 variables. Previously, Buitrago-Guacaneme et al. (2018) determined that the variables influencing the species of black flies in streams of the Eastern Hills of Bogotá were oxygen, temperature, pH, and current velocity. In our study, species abundance and presence were influenced by the watercourse, sampling point, dissolved oxygen, dissolved total solids, redox, elevation, water velocity, and presence of larger rocks. The influence of the stream and the sampling point indicates that the environmental features explaining simuliid distribution may be affected by the particularities of each river. Further, the occurrence of some taxa only in certain seasons suggests that there may be unstudied ecological aspects that influence the distribution of simuliids, such as habitat modifications, predators, life cycle, and multiyear climatic variations (e.g., ENSO).

Niche Breadth, Organic Pollution, and Tolerant Species

Black flies are conventionally considered to develop in unpolluted to moderately polluted waters. The niche breadths suggested some degree of common response of the species to pollution. For example, the species with the narrowest niches ($H < 1.55$) were either the most tolerant to organic pollution or the most sensitive. The tendency of the more widely distributed taxa to exhibit high SPVs suggests that many species of simuliids can develop in moderately polluted water. Accordingly, species such as *G. ortizi*, *S. muiscorum*, and *S. furcillatum* could be considered indicators of good to moderately polluted water quality. Buitrago-Guacaneme et al. (2018) also found that *S. muiscorum* could be more tolerant to lower oxygen levels in relatively contaminated rivers.

Fucha, the most contaminated river, is impacted in the high, middle, and low sampling areas by trout farming, some domestic uses, and sewage, respectively, which did not exclude moderately sensitive species such as *G. ortizi*, *S. muiscorum*, *S. ignescens*, and *S. furcillatum*. Similar findings were described for streams of the Caí River watershed in Brazil by Strieder et al. (2006), who found that *S.* (*Chirostilbia*) *pertinax* Kollar occurred in polluted rural areas, where coliform bacteria levels were higher than in our watercourses. In the

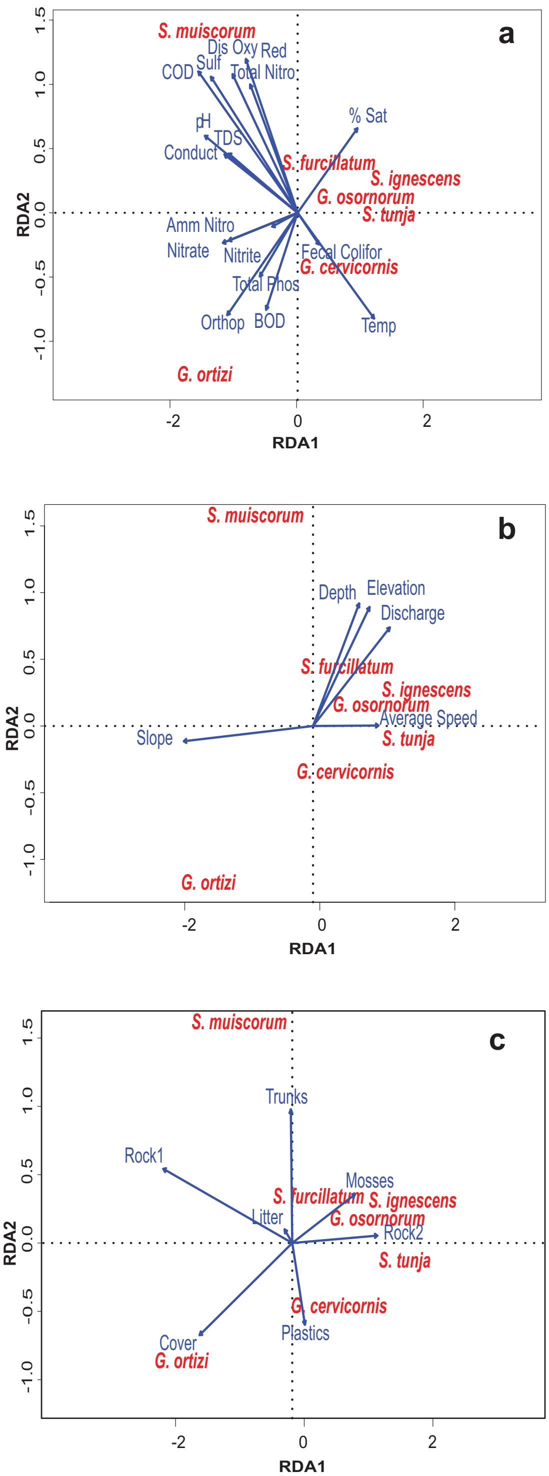

Fig. 3. Redundancy analysis diagrams of the effects of the physical and chemical variables on the species of Simuliidae for the Bogotá and Fucha rivers, and Arzobispo and La Vieja streams, 2015–2016. (a) Physical, chemical, and bacteriological variables; (b) hydrological and terrain variables; (c) qualitative variables.

Caí River Basin, *S. dinellii* (Joan) was present exclusively in the most impacted areas, such as areas with poultry and pig farming, associated with high concentrations of nitrates and nitrites (Strieder et al. 2006). The tolerance of simuliid taxa might be related to the ability of their larvae to survive in low oxygen conditions; Docile et al. (2015) mention that larvae of black flies can survive several hours in anoxic waters, but do not specify at what time of day. Further investigation is needed regarding the possible adaptation of the black flies to low oxygen potential, which might be more influential at night. Likewise, it will be necessary to measure the diurnal oscillations in pH and to relate them to the conditions of water redox.

Although in most cases, species richness decreased in the most contaminated sectors of the watercourses, these decreases were not as strong as expected (Spearman's $r = -0.109$). Apparently, the number of simuliid species does not indicate distinct reduction in water quality in the studied watercourses. Neither the index of diversity nor abundance revealed a clear answer regarding pollution. However, the degree of deterioration in water quality was indicated by the presence and abundance of species more tolerant to contamination, such as *G. cervicornis* and *G. wygodzinskyi*. This result was confirmed by the RDA, which associated *G. cervicornis* with a high quantity of coliform bacteria. The wide occurrence of other common and abundant taxa, such as *G. ortizi*, *S. muiscorum*, *S. ignescens*, and *S. furcillatum*, makes it difficult to establish their true indicator role, but the data suggest that these species are adapted to waters of low to moderate pollution, circumstances under which they have higher abundances compared with the most deteriorated sites. Previous studies also report *S. ignescens* as a species associated with waters of moderately high oxygen content (Buitrago-Guacaneme et al. 2018, Mantilla et al. 2018). Detailed autoecological studies are needed for each of these taxa to determine their indicator power. On the other hand, *G. siberianus*, *S. arcabucense*, and *S. sumapazense* were found only in clean waters.

Table 5. Niche breadth (*H*), estimated with the Shannon–Wiener index, and species pollution values (SPVs) of simuliid species of the Upper Bogotá River Basin, calculated according to Jiang and Shen (2005)

Taxa	*H*	SPV
G. ortizi	2.84	1.854
S. muiscorum	2.77	1.736
S. ignescens	2.25	1.770
S. furcillatum	2.09	1.975
G. osornorum	1.93	1.381
G. wygodzinskyi	1.52	2.437
G. multituberculatus	1.46	1.275
G. cervicornis	1.37	2.644
G. basinflatus	1.01	1.129
G. destitutus	0.95	1.290
S. tunja	0.82	1.349
G. siberianus	0	0.702
S. arcabucense	0	0.702
S. sumapazense	0	0.702

High SPV values correspond to the most tolerant species and low values to the most sensitive to pollution.

Turnover of Simuliid Species along Pollution Gradients

In some studies of macroinvertebrates, beta diversity increases as environmental stress becomes greater (Dimitriadis and Koutsoubas 2011, Hawkins et al. 2015); i.e., the turnover of species is greater at the most deteriorated sites. This pattern of beta diversity can be governed by the rate of loss of rare species along the environmental gradient (Dimitriadis and Koutsoubas 2011). In our study, this might be the reason for the higher rate of turnover in the lower sections of the watercourses, where species with reduced niche breadth disappear, such as *G. basinflatus*,

Table 6. Variation of species turnover between high (High), middle (Middle), and low (Low) reaches of watercourses in the Upper Bogotá River Basin, Colombia

	Arz-High	Arz-Middle	Arz-Low
Arz-High		0.20	0.16
Arz-Middle	1.0		0.33
Arz-Low	1.0	2.0	
	Bog-High	Bog-Middle	Bog-Low
Bog-High		0.46	0.53
Bog-Middle	3.5		0.40
Bog-Low	3.5	2.0	
	Fuc-High	Fuc-Middle	Fuc-Low
Fuc-High		0.14	0.28
Fuc-Middle	1.0		0.16
Fuc-Low	2.0	1.0	
	Vie-High	Vie-Middle	Vie-Low
Vie-High		0.16	0.45
Vie-Middle	1.0		0.27
Vie-Low	2.5	1.5	

Each upper triangle corresponds to the values of the Whittaker index and the lower portion to those of the Cody index. Arz, Arzobispo stream; Bog, Bogotá River; Fuc, Fucha River; Vie, La Vieja stream.

G. destitutus, *G. siberianus*, *S. arcabucense*, and *S. sumapazense*. Hawkins et al. (2015) argue that the increase in beta diversity may be due to an increase in the presence of rare but tolerant taxa and the decrease in the presence of some relatively common but sensitive species. Although it is difficult to infer why beta diversity increases when organic contamination is greater, we tentatively suggest that reserves of rare and sensitive simuliid species from these streams are affected by the increase in the amount of organic matter and the concentration of coliform bacteria. However, other variables, such as competition, predation, life cycle, and climatic fluctuations (e.g., ENSO), might also affect variation of beta diversity among local communities within a stream.

The presumption of a greater abundance of tolerant taxa in the most contaminated places was not conclusive, but the abundance of *G. ortizi* and *S. ignescens* in the most impacted sectors of the Arzobispo and La Vieja streams was conspicuous. *Gigantodax cervicornis* and *S. tunja*, also indicative of greater contamination, were more restricted to the Arzobispo and Bogotá systems, respectively. The elevational gradients of the studied watercourses coincide with the pollution gradients, i.e., at higher elevations water quality is better and deteriorates gradually with decreasing elevation. Greater contamination of the low sites could potentially reduce the longitudinal connectivity of the flow, which could create a degree of isolation between high and low sections, leading to a lower probability of dispersal between sites and reducing the turnover of species in the headwaters.

The Bogotá River and the streams of the Eastern Hills of Bogotá city are important for the conservation of biological diversity and provision of water for the city. Our results show that environmental degradation of these systems is reflected in the characteristics of the black fly assemblages. Thus, these organisms can be used to assess environmental deterioration. Based on this information, conservation and recovery measures can be proposed for these important fluvial ecosystems.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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