Chapter 12

Agrorural Ecosystem Effects on the Macroinvertebrate Assemblages of a Tropical River

Bert Kohlmann, Alejandra Arroyo,
Monika Springer and Danny Vásquez

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59073

1. Introduction

Costa Rica is an ideal reference point for global tropical ecology. It has an abundance of tropical forests, wetlands, rivers, estuaries, and active volcanoes. It supports one of the highest known species density (number of species per unit area) [1, 2] on the planet and possesses about 4 % of the world´s total species diversity [3]. Because of its tropical setting, it also serves as an important location for agricultural production, including cultivars such as coffee, bananas, palm hearts, and pineapples. The country has also attracted more ecotourists and adventure travelers per square kilometer than any other country in the world [4].

The agrorural frontier on the Caribbean side of Costa Rica started to spread during the 1970s, especially in its northeastern area. Migrations of land-poor people from the Pacific and mountain areas of the country started to colonize the land that the government had made available [5, 6]. These waves of immigrants tended to establish themselves along river systems. In this way, towns, small to medium-scale family farming, ranching, and plantation agriculture began to base themselves along the main river systems. It was during this time that the human settlements originated along the Dos Novillos River [7].

Residual waters produced by all of the aforementioned human activities are at present discharged into the river systems in the Costa Rican Caribbean area. Households not situated in the neighborhood of rivers will use septic tanks; homesteads situated along riverbanks will discharge their effluents directly into the rivers. Other activities like the production of residual waters from dairy farms, pigsties, banana packing plants, plantations´ excess fertilization, etc. will drain eventually into a river. The Dos Novillos River is no exception. Water sewage systems in this part of Costa Rica are almost non-existent.

Aquatic biomonitoring in Latin America started at the end of the last century, commencing in Colombia [8-10] and then spreading to other Latin American countries. Revisions of the use of aquatic biomonitoring indices in Latin America are given in de la Lanza Espino *et al*. [8], Prat *et al*. [9], and Springer [10]. In the case of Costa Rica, several studies based on the importance of macroinvertebrates for biomonitoring water quality and community structure, and function in banana and conventional and organic rice systems [14-22], as well as macroinvertebrate field-guides [23, 24] have been published. Some studies of macroinvertebrate assemblage structures also have been reported for rivers on the southern Caribbean coast of Costa Rica [25-34].

Although ecosystem studies have been used in Costa Rica for evaluating possible impacts caused by crop activities on river water quality at specific points, almost nothing is known about how a mix of other human activities can impact macroinvertebrate biodiversity and ecosystem structure along the length of a river. The aim of this study was to use macroinvertebrate biodiversity under the influence of different human activities along the length of a river in order to describe their impact on community structure and function in tropical agrorural environments.

2. Materials and methods

2.1. Study area

This study was conducted at the Dos Novillos River (Figure 1, Table 1) in the province of Limón, Costa Rica. Samples were collected two times a month from January 2005 to March 2005 and monthly from April 2005 to January 2006. The Dos Novillos River drains from the Central Cordillera at an elevation of approximately 2380 masl towards the Caribbean lowlands of the province of Limón. This river is part of the 2950 km^2 Parismina River watershed in a premontane wet forest and tropical moist forest region [35]. The underlying geology is represented by quaternary sedimentary and volcanic rocks under the influence of nearby volcanoes, with a flat to undulating topography and poorly drained alluvial soils susceptible to flooding [36]. Banana plantations have been developed on the lower reaches of this watershed. The study area is characterized by a humid tropical climate with a mean temperature of 25.8 °C, an average annual relative humidity of 87 %, and an annual precipitation average of 3460 mm ± 750 mm without a pronounced dry season. The sampling area runs in a straight line through the towns and localities of La Argentina, Pocora, and EARTH University in the Province of Limón. Each fore mentioned landmark is separated by approximately 4 km. The total sampling area runs along a length of 13.6 km, across an area with a mixture of a premontane wet forest, pastureland, small town, riparian tropical moist forest, and banana agricultural areas.

Six sampling sites were located along the Dos Novillos River (Figs. 1-7; Table 1) where macroinvertebrates were sampled. The first sampling site (Figure 2) (site 1, "Don Eladio") served as a reference site, being part of the rhithral region, located upstream of the first anthropogenic disturbance (pastureland). This site is surrounded by tropical rain forest;

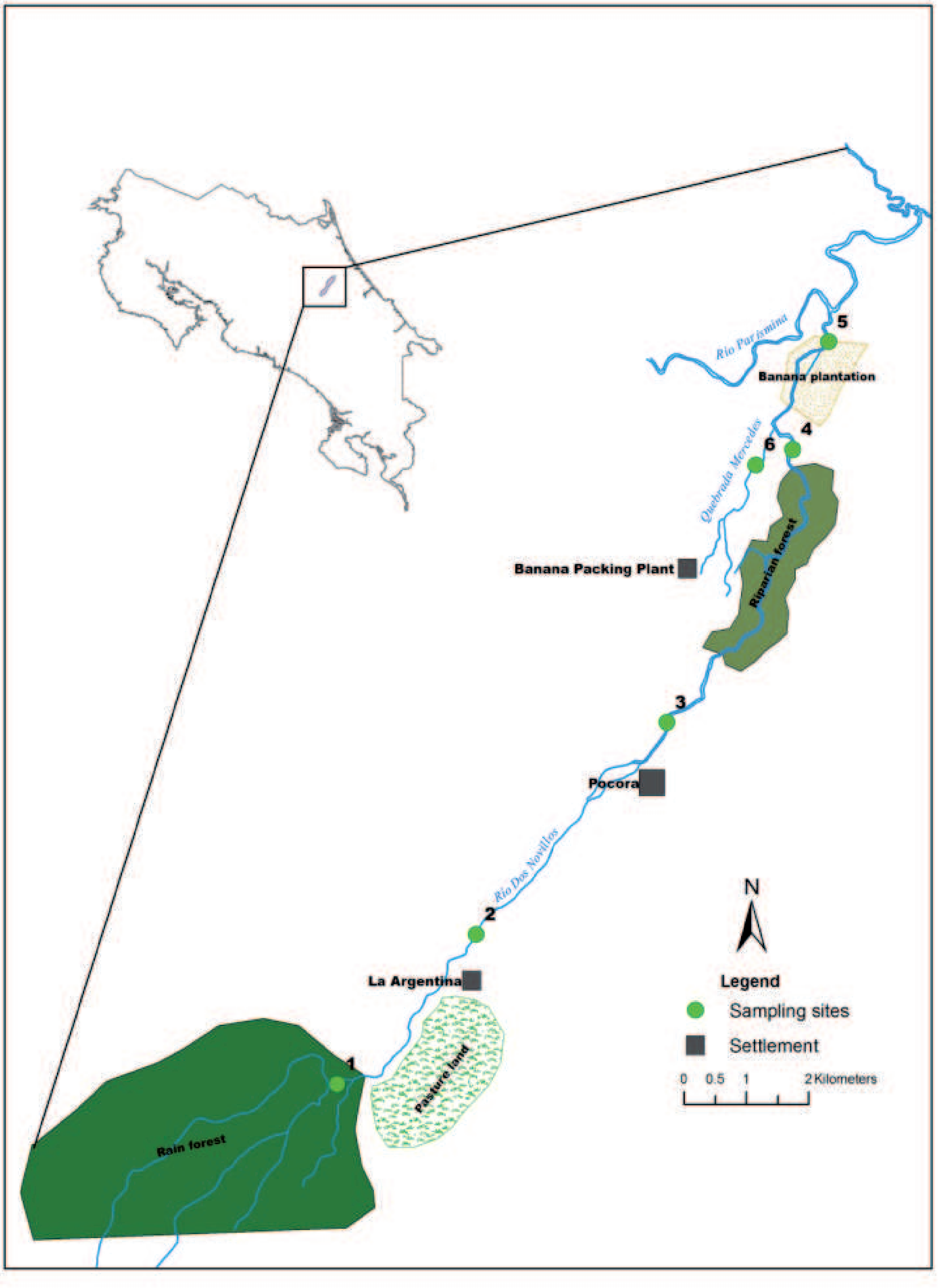

Figure 1. Sampling site location and areas of major potential anthropogenic disturbance at the Dos Novillos River, Guácimo, Limón, Costa Rica [modified from [37].

therefore, natural good water conditions were expected, as well as high taxa richness and an assemblage composition dominated by pollution-sensitive organisms. Site 2, "La Argentina" (Figure 3), was located approximately 5.5 km downstream from site 1. This site was selected

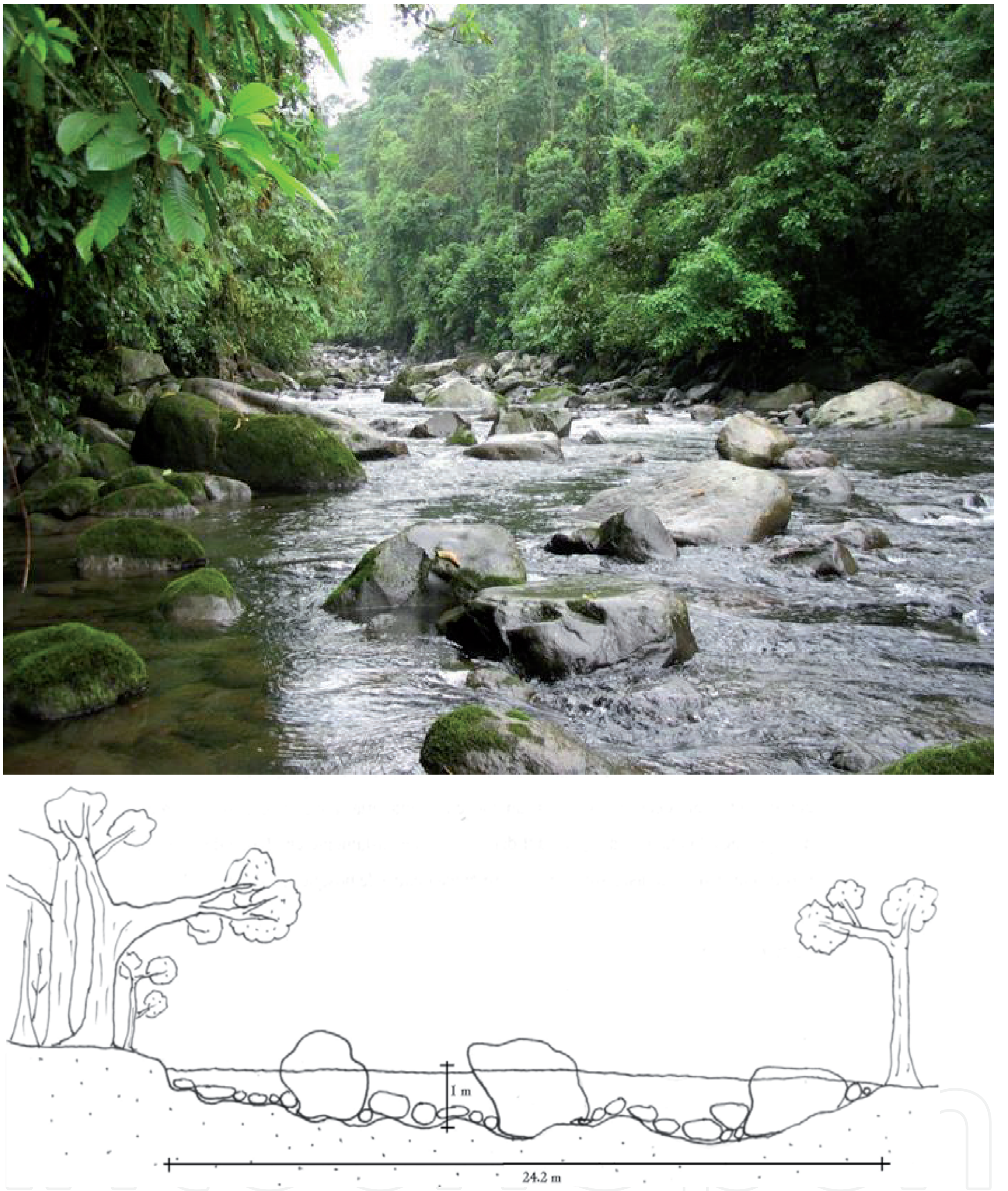

Figure 2. The first site (Don Eladio) was established as a control (reference) site and is located several miles upstream of La Argentina, Pocora, inside natural tropical forest. The site is characterized by a variety of current conditions, with fast flowing riffles, medium laminar flow and pools. The substrate is composed mostly of medium-sized rocks, many of them covered with moss, though large boulders also stand, where large numbers of insects live in the splash area.

to examine the possible extent that small livestock farming might have on river water quality. Site 3, "Chiquitín" (Figure 4), was located approximately 8 km downstream from site 1. High anthropogenic influence was expected at this site because the houses situated at the riverfront discharge their grey and black waters directly into the river. Site 4, "Puente La Hamaca" (Figure 5), was located within the property of EARTH University, approximately 2 km downstream

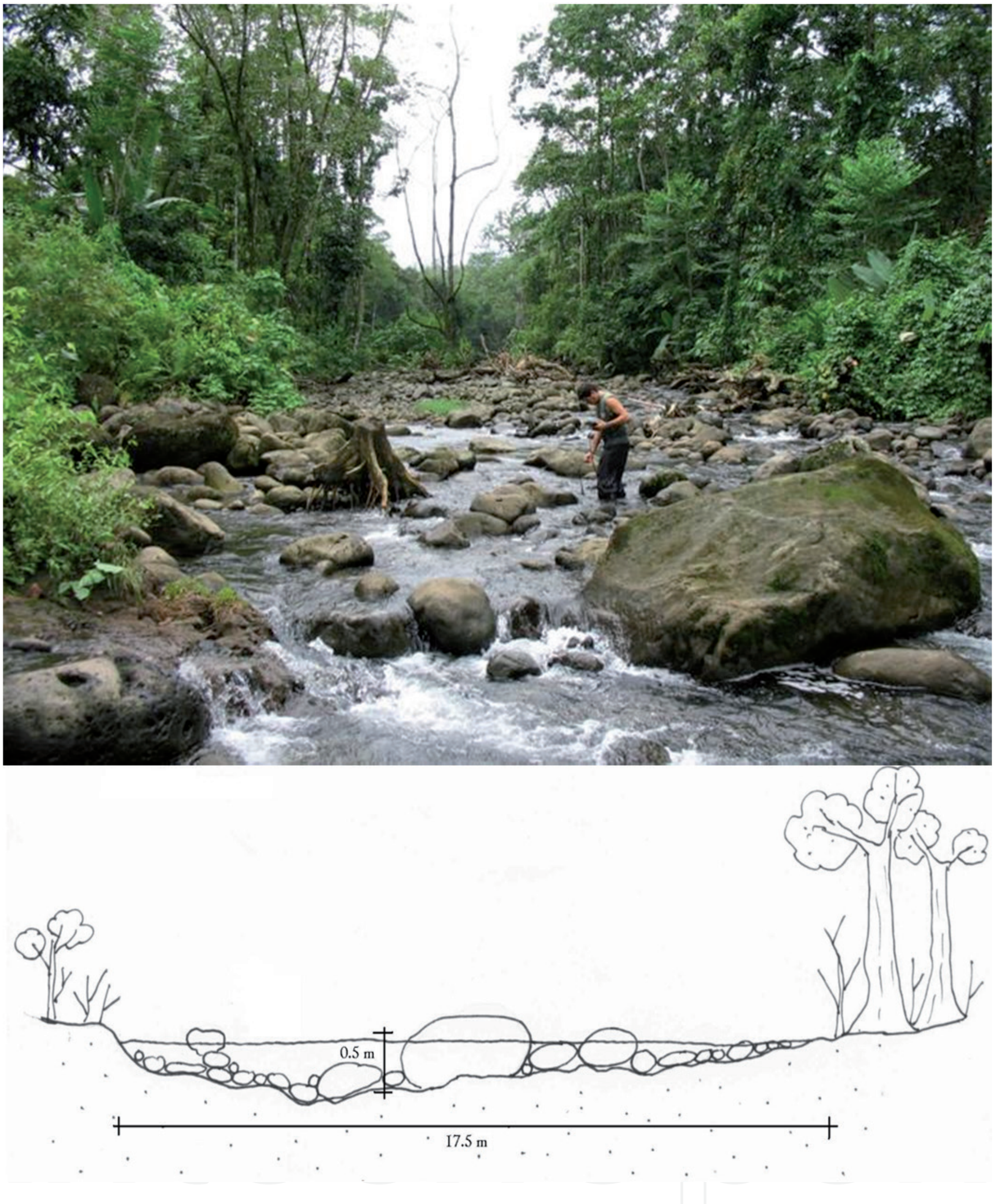

Figure 3. Site 2 (La Argentina) is located downstream of La Argentina. It is a place dominated by rocks of all sizes, although big boulders are less numerous than site 1. Current conditions are similar as in Site 1.

from site 3. As the intervening river length between sites 3 and 4 runs through forest areas, site 4 was selected in order to examine if water quality was improved by a forest filtering processes. Site 5, "Desembocadura" (Figure 6), is within the EARTH University campus and is located approximately 500 m upstream from the confluence of the Dos Novillos and Parismina Rivers. Site 5 was selected to analyze the impact of banana plantations on river water quality. Site 6, "Quebrada Mercedes" (Figure 7), is one tributary of the Dos Novillos River flowing through a forested area within the EARTH University campus, approximately 2 km downstream from site 3. Site 6 was chosen to examine if the intermittent discharge of a small drain of water used to wash bananas in a packing plant had any effect on the stream. Table 1 indicates the exact location, depth, width, and current conditions for each site.

Figure 4. This site (Chiquitín) is located in the center of the town of Pocora. It is at this point where the channel becomes wider and current is more laminar; the substrate is also rocky, but there is no presence of large boulders.

http://dx.doi.org/ 10.5772/59073

Figure 5. Site 4 (La Hamaca) is located within the EARTH University campus, specifically at the suspension bridge. The diversity of current conditions is similar to the other sites, although the rock size is much smaller as at the other three upstream sites. The gallery forest borders the channel at this point.

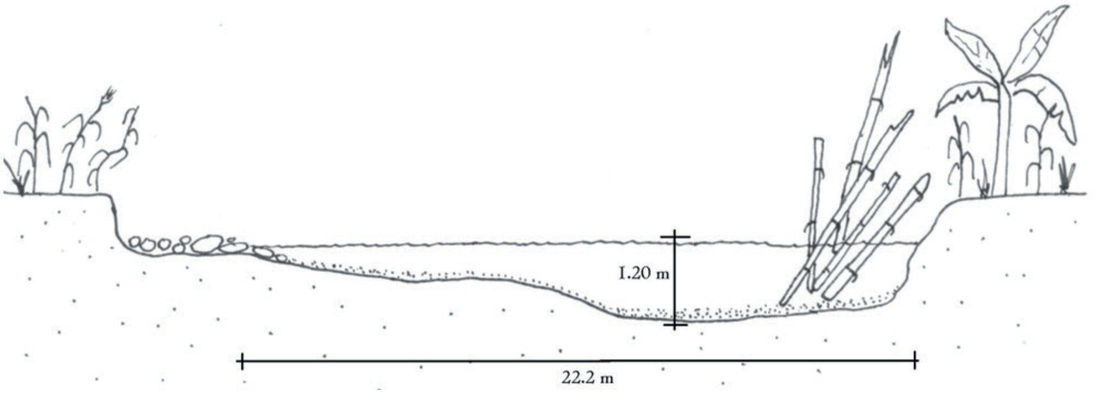

Figure 6. Site 5 (Desembocadura) corresponds to the mouth of the River Dos Novillos with the Parismina. The substrate consists almost entirely of sand. Big boulders are absent, small rocks are scarce and current flow is weak; tall grasses, banana plants, bamboo, and a few trees dominate vegetation along the channel. At this site, small airplanes were observed flying over the river while spraying pesticides on the surrounding banana plantations.

http://dx.doi.org/ 10.5772/59073

Figure 7. Shallow waters and a moderate current characterize the tributary Quebrada Mercedes (site 6). Small rocks are the predominant substrate and current is moderate and laminar with some faster flowing riffle areas.

Site number	1	2	3	4	5	6
and name	Don Eladio	La Argentina	Chiquitín	La Hamaca	Desembocadura	Quebrada Mercedes
Longitude (N)	10° 07′ 09.7′′	10° 09′ 14.3′′	10° 10′ 40.8′′	10° 13′ 00.9′′	10° 14′	10° 12′
Latitude (W)	83° 39′ 15.2′′	83° 37′ 24.7′′	83° 36′ 10.6′′	83° 35′ 18.4′′	83° 34′	83° 35′
Altitude (m)	441	187	90	51	40	44
Width (m)	24.2	17.5	30.5	24	22.2	9.5
Depth (m)	0.3-1.3	0.25-0.8	0.25-0.8	0.2-0.86	0.2 – 1.2	0.15-0.3
Current (m/s)	1.67	1.94	0.9	1.05	3.2	1.9
River bottom	Medium-sized rocks	Medium-sized rocks	Small rocks	Small rocks	Sand	Small rocks

Table 1. Geographical and physical characteristics of each sampling site at the Dos Novillos River (1-5) and the Mercedes Stream (6), Guácimo, Province of Limón, Costa Rica.

2.2. Sampling

A plastic strainer with a diameter of 20 cm and 0.5 mm mesh size, and tweezers were used for directly collecting macroinvertebrates. The main criterion for this semi-quantitative collecting method is time; there were no defined sampling areas. All types of microhabitats present at a particular site were examined equally for the macroinvertebrates. Collected organisms were fixed immediately in 70 % ethanol at the time of sampling. Exact details of the sampling methodology can be found in Stein *et al.* [37].

A presampling was carried out in order to determine sampling time [37]. Out of the achieved results, an accumulated taxa curve was elaborated, and 120 min were determined to be a representative sampling time per site. Sampling took place during early morning and under normal current situations in order to avoid the negative effects of flooding and high water conditions.

The government of Costa Rica has officially suggested this method under the water quality monitoring regulation [38]. This method is coupled with the use of a modified Biological Monitoring Working Party index for Costa Rica (BMWP′-CR), a biotic index utilized to define different levels of water quality. Each family of macroinvertebrates has a sensitivity value ranging from 1 to 10, reflecting tolerance to pollution based on the knowledge of distribution and abundance. The values for each family are then summed up independently from abundance and generic or species diversity. Sensitivity scores higher than 120 points indicate undisturbed aquatic ecosystems, while low values indicate serious contamination (mostly organic) of the environment [13, 23, 38,].

2.3. Data analysis

The analyzed data comprised the values of the physical-chemical water quality variables (Table 2) and macroinvertebrate abundances (Table 3) during a collecting period of 13 months (January 2005 to January 2006).

http://dx.doi.org/ 10.5772/59073

The following physical-chemical variables were measured: pH, temperature, O_2, O_2 saturation, suspended solids, turbidity, conductivity, NO_3^-, NH_4^+, PO_4^+, BOD, and COD. The water samples also were analyzed for the following agrochemicals associated with banana production using gas chromatography-MS and liquid chromatography-PDA: Chlorpyrifos, Diazinon, Dimethoate, Edifenfos, Etoprofos, Fenamifos, Malathion, Parathion-methyl, Parathion-ehtyl, Terbufos, Difenoconazol, Propiconazol, Imazalil, Ametrine, Atrazine, Hexazinone, Terbutylazine, Bromacil, Bitertanol, Chlorothalonil, and Thiabendazole. However, no traces of them could be detected in the river water samples. This does not come as a surprise because in order to monitor pesticides very frequent sampling would be required to detect peak concentrations during pesticide application periods [39], whereas low concentrations are very difficult to detect.

Following the suggestion of Ramírez and Gutiérrez-Fonseca [40], this study is also undertaking an ecosystem process analysis of the functional feeding groups (FGG) of the aquatic macroinvertebrates. This sort of analysis is based on two key aspects of macroinvertebrates: morphological characteristics related to the obtainment of food resources (*e.g.*, mouthparts and related structures) and behavioural mechanisms (*e.g.*, feeding behaviour). FGG is a very useful tool that provides valuable information on ecosystem functioning, facilitating stream ecosystem comparisons, and avoiding the traps of gut content analysis, which is more appropriate for assigning trophic guilds [40].

In this ecosystem study, the use of different parameters of the structure and composition of macroinvertebrate assemblages are presented: total and relative abundances, taxa richness, and functional feeding groups. Also, correlations of different genera and functional feeding groups with environmental variables were analyzed.

2.4. Statistical analysis

The model comparisons between physical-chemical variables, macroinvertebrate abundances, and the BMWP´-CR index at different collecting sites was done by performing an analysis of variance (ANOVA; α=0.05). For all three cases, the proposed hypothesis is to test the existence of significant variable differences between sites. Abundances were square root transformed in order to comply with error normality. Evaluation of the best model (homocedastic or heterocedastic) for each variable was performed using the Akaike information criterion (AIC), which is one of the benchmarks of mixed models based on penalized likelihood [41, 42]. When the model detected significant differences, a DGC (Di Rienzo – González – Casanoves) statistical test was performed for the comparison of means [43].

On the other hand, taxonomic groupings and FFG relative frequencies analyses were done using a Chi-square test in order to assay for statistically significant differences between sites. The Chi-square analysis is testing for independence between the sites and the studied variables. Any p value below 0.05 shows the existence of an association between the site and the studied variable.

PLS regression is a technique that combines Principal Component Analysis and Linear Regression [44]. It is applied when it is desired to predict a set of dependent variables (y), in this case the abundance of macroinvertebrate genera and FFG abundances and the BMWP index values, from a set of predictor variables (x), in this case physical-chemical variables. To represent the results obtained from the PLS analysis, a Triplot graph was superimposed on a Biplot graph [45], thus correlating all variables. Then, the observations appear ordered in a Triplot graph (sites), depending on the values of the dependent variables (macroinvertebrate and FGG abundances and BMWP′-CR index) and their correlation with the predictor variables (physical-chemical water-quality variables). For the macroinvertebrate genera PLS analysis, out of the 127 collected taxa (of which, 123 could be identified to the genera level and their different developmental stages: larva, pupa, adult), the multimetric analysis included only 58 taxa, which were chosen using a PCA (Principal Component Analysis). The rest were characterized for repeating the same information. These 58 taxa, composed of 15 688 individuals, showed high projection values on the first two principal components. All statistical analyses were done using the InfoStat program [46].

In order to correlate the abundance of macroinvertebrates, FFG, and the BMWP′-CR index values of different sites, these variables were correlated with physical-chemical variables using the Spearman rank correlation coefficient. In the present case, the hypothesis tries to establish if one variable can be effectively substituted by another one, due to the existence of a significant correlation. The Spearman correlation coefficient was selected, versus Pearson, because its use is recommended in the case of having a small sample.

Finally, in order to evince the consistency of the sites′ congruences arranged in one plane unto physicochemical variables and FFG and macroinvertebrate genera abundances, a Generalized Procrustes Analysis was performed. This analysis is used for harmonizing multivariate configurations obtained on the same set of observations with different types of variables or time points [47]. Alignment is performed through a series of steps including normalization, rotation, reflection, and scaling of data to obtain a consensus array between groups of variables. This series of steps should maintain the distances between individuals from the individual configurations and minimize the distance between similar points [44]. The result of this multivariate method is to present a graph that displays the configurations arrived at by each variable type and the consensus configuration. A percentage consensus analysis was also undertaken. A high consensus indicates that any group of variables characterizes the different sites in the same way; therefore, using any group of variables is indistinct for site characterization.

3. Results

3.1. Physical-chemical parameters

Table 2 shows the results of the physical-chemical analysis (ANOVA, DGC-test, p>0, 05). All sites presented neutral pH and high dissolved oxygen levels and saturation. Temperatures varied in a statistically significant way, site 1 being the coolest place and site 5 the warmest. Conductivity was quite low at all sites, but statistically significantly lower in sites 5 and 6;

http://dx.doi.org/ 10.5772/59073

whereas, turbidity was statistically significantly higher in site 6. NO_3 was statistically significantly higher in sites 5 and 6, and lowest in sites 1, 2, and 3.

Variables		Site 1 Don Eladio	Site 2 Argentina	Site 3 Chiquitín	Site 4 Hamaca	Site 5 Desembocadura	Site 6 Q. Mercedes
pH		7.0±0.5	7.1±0.6	7.1±0.7	7.1±0.7	7.0±0.7	7.2±0.7
Dissolved O_2	mg/L	7.2±1.8	7.4±1.8	7.5±1.2	7.7±1.6	7.00±2.1	7.0±1.8
O_2 Saturation	%	80.3±21.3	88.5±22.4	86.8±20.8	90.5±21.8	86.7±27.5	82.3±24.8
Temperature	°C	21.1±0.6c	23.6±1.3b	24.7±1.4b	24.3±0.8b	25.9±1.5a	24.0±1.0b
Conductivity	µS/cm	46.5±4.3b	49.3±4.4b	46.3±7.5b	43.3±17.5b	58.4±7.2a	56.0±14.5a
Turbidity	NTU	1.3±2.3b	0.8±0.5b	1.0±0.6b	1.5±1.2b	1.4±1.2b	3.4±2.6a
BOD	ppm	16.0±8.1	14.7±10.9	16.3±8.8	11.6±7.7	14.3±9.2	12.2±9.6
COD	ppm	14.9±10.7	10.2±8.0	12.8±7.2	10.4±6.3	15.4±14.9	14.9±9.7
NO_3	ppm	0.05±0.04c	0.03±0.02c	0.06±0.04c	0.10±0.05b	0.13±0.04a	0.15±0.07a
NH_4	ppm	0.06±0.14	0.04±0.06	0.03±0.05	0.06±0.10	0.07±0.17	0.05±0.10
PO_4	ppm	0.08±0.05	0.07±0.06	0.08±0.05	0.09±0.05	0.09±0.05	0.09±0.06
Susp. Solids	mg/l	46.2±27.5	38.3±22.3	28.5±27.2	39.7±29.2	42.1±22.8	42.2±31.8

Table 2. Mean values of physical-chemical variables with their standard deviations used for the PLS analysis at the six collecting sites (January 2005-January 2006). Means in the same row with the same lettering are not significantly different (ANOVA, DGC-test, homocedastic model for the physical-chemical variables, p>0, 05).

3.2. Diversity and composition of macroinvertebrate assemblages

The study collected a total of 17 163 specimens, distributed in the following 6 classes (number of total amount of specimens in parentheses): Clitellata (2), Turbellaria (10), Gastropoda (403), Arachnida (21), Malacostraca (40), and Insecta (16 706) with the following orders: Ephemeroptera (6299), Coleoptera (3150), Trichoptera (3010), Diptera (2868), Plecoptera (683), Odonata (435), Hemiptera (101), Megaloptera (91), Lepidoptera (64), Blattodea (2) (Table 3). The total abundance analysis (Figure 8) shows that Ephemeroptera, Coleoptera, Trichoptera, and Diptera were the most abundant groups, comprising 89.2 % of the collected macroinvertebrates.

Class/Order	Family	Genus	FFG	Site 1 Don Eladio	Site 2 La Argent.	Site 3 Chiquitín	Site 4 Hamaca	Site 5 Desembocadura	Site 6 Q.Merced	Total
Blattodea	Blaberidae	Epilampra		0	1	0	0	0	1	2
Coleoptera	Elmidae	Austrolimnius	CG	3	1	1	0	1	0	6
Coleoptera	Elmidae	Austrolimnius-a†	CG	6	15	5	0	1	0	27
Coleoptera	Elmidae	Cylloepus†	GC	0	0	0	3	4	3	10

Class/Order	Family	Genus	FFG	Site 1 Don Eladio	Site 2 La Argent.	Site 3 Chiquitín	Site 4 Hamaca	Site 5 Desembocadura	Site 6 Q.Merced	Total
Coleoptera	Elmidae	Cylloepus-a†	CG	13	16	10	2	5	0	46
Coleoptera	Elmidae	Heterelmis†	CG	46	50	26	42	21	3	188
Coleoptera	Elmidae	Heterelmis-a†	CG	206	87	6	3	0	17	319
Coleoptera	Elmidae	Disersus	CG	2	0	0	0	0	0	2
Coleoptera	Elmidae	Hexanchorus†	CG	105	142	10	40	2	2	301
Coleoptera	Elmidae	Hexanchorus-a†	CG	567	205	41	44	1	5	863
Coleoptera	Elmidae	Macrelmis†	CG	26	199	53	108	19	15	420
Coleoptera	Elmidae	Macrelmis-a†	CG	55	95	19	53	10	8	240
Coleoptera	Elmidae	Microcylloepus	CG	4	5	5	3	4	1	22
Coleoptera	Elmidae	Microcylloepus-a†	CG	17	53	1	5	8	6	90
Coleoptera	Elmidae	Neocylloepus	CG	0	0	0	0	0	2	2
Coleoptera	Elmidae	Neoelmis	CG	1	1	3	2	5	0	12
Coleoptera	Elmidae	Neoelmis-a†	CG	1	5	5	2	1	0	14
Coleoptera	Elmidae	Onychelmis	CG	0	0	0	0	1	0	1
Coleoptera	Elmidae	Phanocerus†	CG	29	27	4	20	10	6	96
Coleoptera	Elmidae	Phanocerus-a†	CG	32	9	0	13	10	6	70
Coleoptera	Elmidae	Pseudodisersus†	CG	1	1	0	0	0	0	2
Coleoptera	Elmidae	Stenhelmoides	CG	0	0	0	1	0	0	1
Coleoptera	Elmidae	Stenhelmoides-a	CG	0	0	1	0	0	0	1
Coleoptera	Gyrinidae	Gyretes-a	Pr	0	0	0	0	1	0	1
Coleoptera	Lutrochidae	Lutrochus	Sh	0	1	0	0	0	0	1
Coleoptera	Lutrochidae	Lutrochus-a	Sh	0	2	0	6	0	0	8
Coleoptera	Psephenidae	Psephenops-a	Sc	0	4	0	0	0	0	4
Coleoptera	Psephenidae	Psephenus†	Sc	52	128	87	67	13	12	359
Coleoptera	Ptilodactylidae	Anchytarsus†	Sh	4	15	10	6	2	7	44
Diptera	Blephariceridae	Paltostoma†	Sc	107	1	0	1	0	0	109
Diptera	Blephariceridae	Paltostoma-p†	Sc	2	3	0	0	0	0	5
Diptera	Chironomidae	Chironomus	CG	0	0	0	0	0	2	2
Diptera	Empididae	Hemerodromia†	Pr	1	8	4	7	2	1	23
Diptera	Psychodidae	Maruina†	Sc	263	274	142	24	0	0	703
Diptera	Psychodidae	Maruina-p†	Sc	0	6	0	0	0	0	6
Diptera	Simuliidae	Simulium†	Ft	36	588	442	244	412	130	1852
Diptera	Simuliidae	Simulium-p	Ft	0	5	6	0	0	0	11
Diptera	Tabanidae	Chrysops	Pr	0	0	0	0	0	1	
Diptera	Tipulidae	Hexatoma†	Pr	5	2	3	23	26	82	141
Diptera	Tipulidae	Hexatoma-p	Pr	0	0	3	0	0	2	5

Class/Order	Family	Genus	FFG	Site 1 Don Eladio	Site 2 La Argent.	Site 3 Chiquitín	Site 4 Hamaca	Site 5 Desembocadura	Site 6 Q.Merced	Total
Ephemeroptera	Baetidae	Americabaetis†	CG	3	4	13	197	512	65	794
Ephemeroptera	Baetidae	Baetodes†	Sc	116	246	127	62	17	28	596
Ephemeroptera	Baetidae	Camelobaetidius†	CG	91	179	198	84	21	8	581
Ephemeroptera	Baetidae	Cloeodes	CG	20	6	50	2	9	1	88
Ephemeroptera	Baetidae	Mayobaetis†	CG	119	96	0	0	0	0	215
Ephemeroptera	Caenidae	Caenis†	CG	0	0	0	0	2	4	6
Ephemeroptera	Heptageniidae	Stenonema	Sc	1	1	0	3	1	22	28
Ephemeroptera	Leptohyphidae	Asioplax†	CG	4	2	2	2	3	1	14
Ephemeroptera	Leptohyphidae	Epiphrades†	CG	0	1	0	11	54	17	83
Ephemeroptera	Leptohyphidae	Haplohyphes	CG	0	1	0	0	0	0	1
Ephemeroptera	Leptohyphidae	Leptohyphes†	CG	166	422	242	634	322	87	1873
Ephemeroptera	Leptohyphidae	Tricorythodes†	CG	143	44	74	74	80	64	479
Ephemeroptera	Leptohyphidae	Vacuperinus	CG	0	2	54	48	171	5	280
Ephemeroptera	Leptophlebiidae	Farrodes†	CG	51	40	51	123	223	135	623
Ephemeroptera	Leptophlebiidae	Hydrosmilodon	CG	0	1	0	0	0	0	1
Ephemeroptera	Leptophlebiidae	Terpides	CG	2	1	1	3	96	5	108
Ephemeroptera	Leptophlebiidae	Thraulodes†	CG	106	206	92	90	9	26	529
Hemiptera	Hebridae	Hebrus†	Pr	1	6	1	1	1	0	10
Hemiptera	Mesoveliidae	Mesovelia	Pr	1	6	4	0	1	2	14
Hemiptera	Naucoridae	Cryphocricos	Pr	0	1	0	0	0	4	5
Hemiptera	Naucoridae	Limnocoris	Pr	0	0	1	0	2	0	3
Hemiptera	Naucoridae	Limnocoris-a	Pr	0	0	0	1	1	0	2
Hemiptera	Ochteridae	Ochterus†	Pr	5	8	0	0	0	0	13
Hemiptera	Veliidae	Rhagovelia	Pr	0	17	2	5	24	6	54
Lepidoptera	Crambidae	Petrophila†	Sc	11	25	15	10	2	1	64
Megaloptera	Corydalidae	Chloronia†	Pr	2	0	0	0	2	0	4
Megaloptera	Corydalidae	Corydalus	Pr	13	27	5	36	4	2	87
Odonata	Calopterygidae	Hetaerina	Pr	9	9	6	18	35	11	88
Odonata	Coenagrionidae	Argia	Pr	21	19	39	22	44	22	167
Odonata	Coenagrionidae	Nehalenia†	Pr	0	0	0	0	2	1	3
Odonata	Corduliidae	Neocordulia	Pr	0	0	0	1	0	0	1
Odonata	Gomphidae	Agriogomphus	Pr	0	0	0	0	2	7	9
Odonata	Gomphidae	Desmogomphus†	Pr	4	2	0	0	0	0	6
Odonata	Gomphidae	Epigomphus	Pr	1	1	0	0	1	0	3
Odonata	Gomphidae	Erpetogomphus	Pr	1	1	2	1	4	24	33
Odonata	Gomphidae	Perigomphus	Pr	0	1	0	0	0	0	1

Class/Order	Family	Genus	FFG	Site 1 Don Eladio	Site 2 La Argent.	Site 3 Chiquitín	Site 4 Hamaca	Site 5 Desembocadura	Site 6 Q.Merced	Total
Odonata	Gomphidae	Phyllogomphoides	Pr	0	0	0	0	2	0	2
Odonata	Libellulidae	Brechmorhoga†	Pr	9	3	1	5	1	1	20
Odonata	Libellulidae	Dythemis†	Pr	0	0	0	2	15	4	21
Odonata	Libellulidae	Macrothemis†	Pr	0	7	0	0	3	0	10
Odonata	Libellulidae	Miathyria	Pr	0	0	0	0	1	0	1
Odonata	Megapodagrionidae	Heteragrion†	Pr	10	3	3	0	3	14	33
Odonata	Perilestidae	Perrisolestes†	Pr	0	0	0	0	1	1	2
Odonata	Platystictidae	Palaemnema	Pr	0	0	1	1	0	28	30
Odonata	Polythoridae	Cora†	Pr	1	3	1	0	0	0	5
Plecoptera	Perlidae	Anacroneuria†	Pr	226	258	24	22	5	148	683
Trichoptera	Anomalopsychidae	Contulma	Sc	1	0	0	0	0	0	1
Trichoptera	Calamoceratidae	Phylloicus	Sh	5	0	0	0	0	0	5
Trichoptera	Helicopsychidae	Cochliopsyche†	Sc	1	0	1	0	0	0	2
Trichoptera	Helicopsychidae	Helicopsyche	Sc	9	0	0	0	0	0	9
Trichoptera	Hydrobiosidae	Atopsyche	Pr	2	25	3	0	0	0	30
Trichoptera	Hydrobiosidae	Atopsyche-p†	Pr	0	1	0	1	0	0	2
Trichoptera	Hydropsychidae	Leptonema	Ft	11	43	13	124	37	153	381
Trichoptera	Hydropsychidae	Macronema	Ft	1	0	0	1	2	83	87
Trichoptera	Hydropsychidae	Smicridea	Ft	163	229	120	239	205	50	1006
Trichoptera	Hydropsychidae	Smicridea-p	Ft	0	1	0	0	0	0	1
Trichoptera	Hydroptilidae	Alisotrichia	Pc	0	0	0	4	0	0	4
Trichoptera	Hydroptilidae	Anchitrichia†	Pc	0	0	0	4	0	0	4
Trichoptera	Hydroptilidae	Anchitrichia-p	Pc	1	0	0	0	0	0	1
Trichoptera	Hydroptilidae	Brysopterix†	Pc	265	13	0	0	0	0	278
Trichoptera	Hydroptilidae	Brysopterix-p†	Pc	18	19	0	0	0	0	31
Trichoptera	Hydroptilidae	Leucotrichia	Pc	0	9	32	0	0	0	72
Trichoptera	Hydroptilidae	Leucotrichia-p	Pc	0	0	6	0	0	0	6
Trichoptera	Hydroptilidae	Ochrotrichia	Pc	411	2	0	0	0	0	413
Trichoptera	Hydroptilidae	Ochrotrichia-p	Pc	39	0	0	0	0	0	39
Trichoptera	Hydroptilidae	Oxyethira†	Pc	4	0	2	0	0	0	6
Trichoptera	Hydroptilidae	Oxyethira-p	Pc	2	0	0	0	0	0	2
Trichoptera	Hydroptilidae	Rhyacopsyche-p	Pc	0	73	12	1	0	0	86
Trichoptera	Leptoceridae	Atanatolica†	CG	20	5	0	0	0	0	25
Trichoptera	Leptoceridae	Atanatolica-p†	CG	1	0	0	0	0	0	1
Trichoptera	Leptoceridae	Nectopsyche	Sh	8	3	8	5	14	4	42
Trichoptera	Leptoceridae	Nectopsyche-p	Sh	9	5	5	0	0	5	24

Class/Order	Family	Genus	FFG	Site 1 Don Eladio	Site 2 La Argent.	Site 3 Chiquitín	Site 4 Hamaca	Site 5 Desembocadura	Site 6 Q.Merced	Total
Trichoptera	Leptoceridae	Oecetis	Pr	2	3	0	0	2	0	7
Trichoptera	Leptoceridae	Triplectides†	CG	2	0	0	0	0	2	4
Trichoptera	Philopotamidae	Chimarra†	Ft	22	116	79	130	15	89	451
Trichoptera	Polycentropodidae	Polycentropus†	Pr	5	2	2	0	0	0	9
Trombidiformes	Various families	Various genera	Pr	12	2	2	3	0	2	21
Decapoda	Atyidae	Atya	CG	3	2	1	1	1	1	9
Decapoda	Palaemonidae	Macrobrachium	Pr	7	6	3	4	3	2	25
Decapoda	Pseudothelphusidae	Pseudothelphusa	CG	2	1	1	1	1	0	6
Gastropoda	Ampullaridae	Pomacea	Sc	0	0	0	0	0	1	1
Gastropoda	Ancylidae	Gundlachia	Ft	0	0	2	0	0	0	2
Gastropoda	Hydrobiidae	Aroapyrgus	Sc	10	8	20	24	4	311	377
Gastropoda	Physidae	Gen. indet.	Sc	0	0	12	0	0	0	12
Gastropoda	Thiaridae	Melanoides	Sc	0	0	1	1	9	0	11
Lumbriculida	Lumbriculidae	Gen. indet.	CG	0	0	2	0	0	0	2
Tricladida	Planariidae	Gen. indet.	Pr	0	4	4	2	0	0	10
Total				3757	4170	2227	2722	2528	1759	17163

Table 3. Total number of individuals collected (abundance) per genera and its different life-forms along the studied sites (a=adult, p=pupa, no sign=larva, † taxa considered for the genera PLS analysis). Functional feeding group (FFG) categories: CG=Collector-Gatherers, Ft=Filterers, Pc=Piercers, Pr=Predators, Sc=Scrapers, and Sh=Shredders.

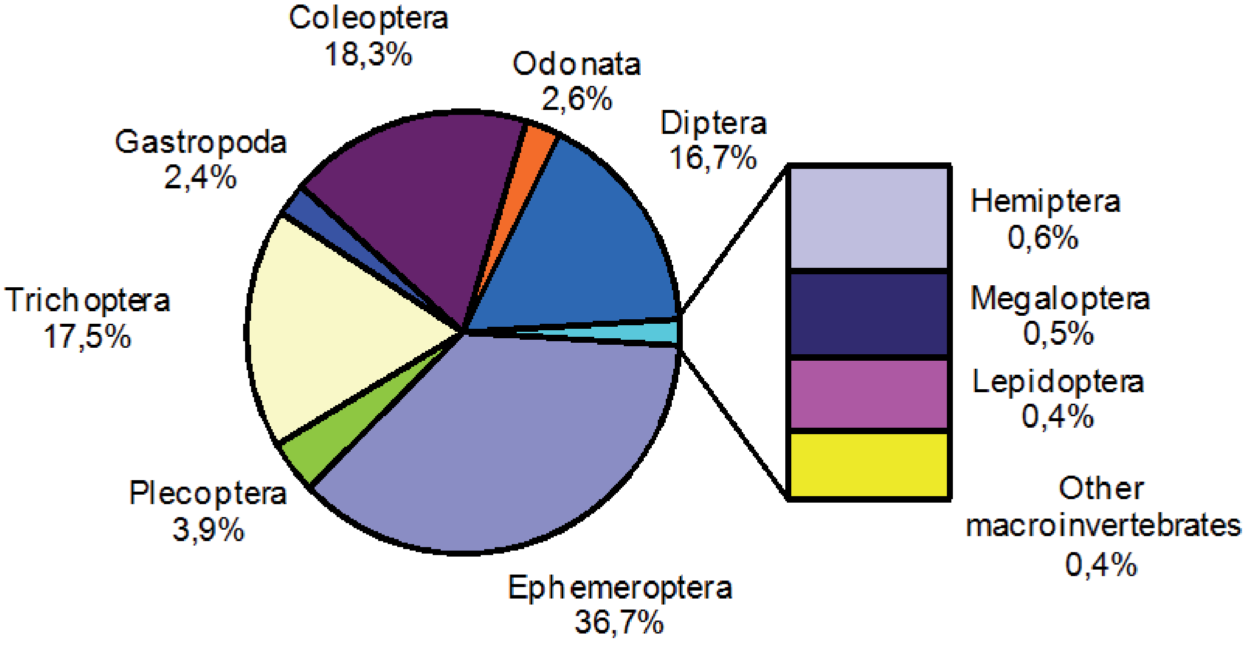

Figure 8. Graph indicating the percentage taxonomic composition of the total study sample of all six collecting sites.

3.3. Comparison between sampling sites with different agrorural influence

If we consider the mean total number of collected individuals per site, clear abundance differences are evinced (Figure 9). The greatest mean total numbers, with almost 300 individuals collected per sampling date, are found in the two least impacted sites (ANOVA, DGC-test; p>0.05),the reference (site 1) and the livestock-pasture site (site 2), whereas the sites under more intense human influence present statistically significantly reduced mean total values (around 150 individuals collected per month).

Table 4 presents a family, genera, and EPT richness analysis of the different collecting sites, as well as the mean BMWP-CR values and resulting water quality. The total family and genera richness does not show significant frequency differences between sampling sites, although the highest number of genera were found at the first two sites, with over 60 genera. The site with the lowest taxonomic richness, both on family and genus level, was site 5, close to the river mouth. The EPT taxa richness was highest at site 1 (14 EPT taxa), decreased downstream towards only nine EPT taxa at sites 5 and 6, although no statistically significant frequency differences were found using the Chi-square test. The BMWP'-CR index shows statistically significant differences (ANOVA, DGC-test; p>0.05), where the index values diminish according to the following site groupings: (sites 1-2)-(site 6)-(sites 3-4)-(site 5). The water quality, indicated by the mean BMWP'-CR index falls into the categories "good quality" (sites 1-2) and "regular quality" (sites 3-6).

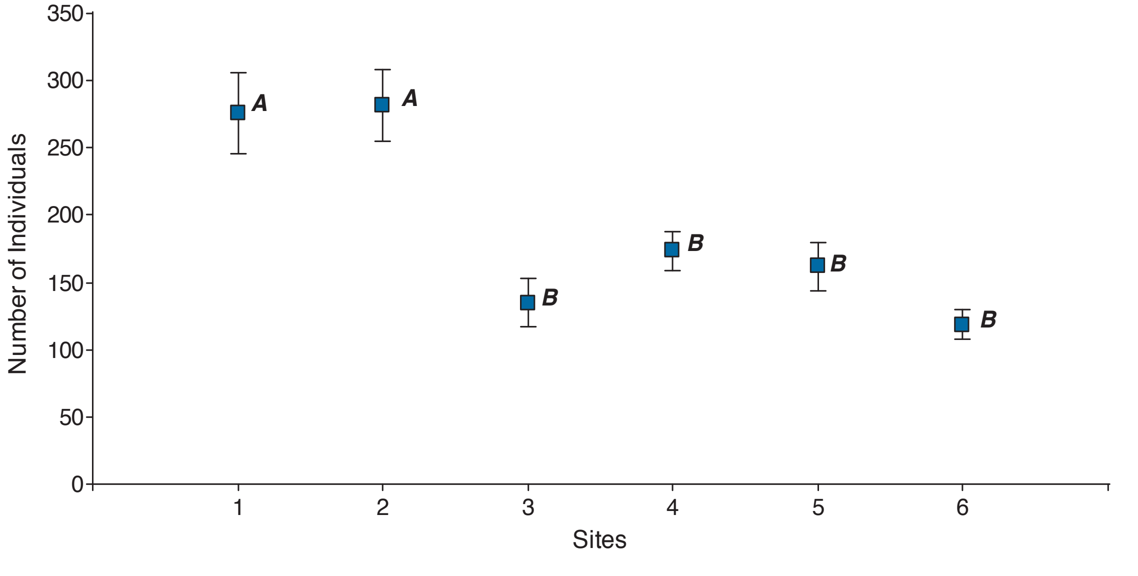

Figure 9. Comparison of the mean of the total number of collected individuals in the six sampling sites. Means with the same letters are not significantly different (ANOVA, DGC-test; p>0.05).

On the other hand, a very clear change in the structure of macroinvertebrate assemblages can be observed along the different sampling sites of the Dos Novillos River (Figure 10). At the first site, the undisturbed sampling point, beetles are significantly more abundant than at the other sites (Chi-square, p<0.0001), which are all under the influence of human impact. The last

Sites	Site 1 Don Eladio	Site 2 La Argentina	Site 3 Chiquitín	Site 4 La Hamaca	Site 5 Desembocadura	Site 6 Q. Mercedes
Families	37	39	40	36	33	35
Genera	63	67	53	52	45	53
EPT	14	11	11	10	9	9
BMWP'-CR	113.8±20.6[a]	119.8±16.3[a]	77.1±12.0[c]	83.9±8.4[c]	68.8±15.2[d]	93.6±13.2[b]
Water quality	good	good	regular	regular	regular	regular

Table 4. Total number of families, genera richness, EPT richness (Ephemeroptera-Plecoptera-Trichoptera), and mean values for the BMWP'-CR index with resulting biological water quality in the different collecting sites. No statistically significant frequency differences were found using the Chi – square test for families, genera and EPT richness (ANOVA, DGC-test, heterocedastic model for the BMWP analysis, p>0, 05).

site (site 6) shows a significantly greater abundance of Gastropoda, Odonata, and Plecoptera (Chi-square, p<0.0001). Here the banana packing plant discharges effluents, carrying small banana pieces and other suspended organic material, into the water of the stream. Also, a tendency of an increase in mayfly (Ephemeroptera) abundance can be observed towards sites 4 and 5, while caddisfly abundance is relatively steady throughout all sampling sites.

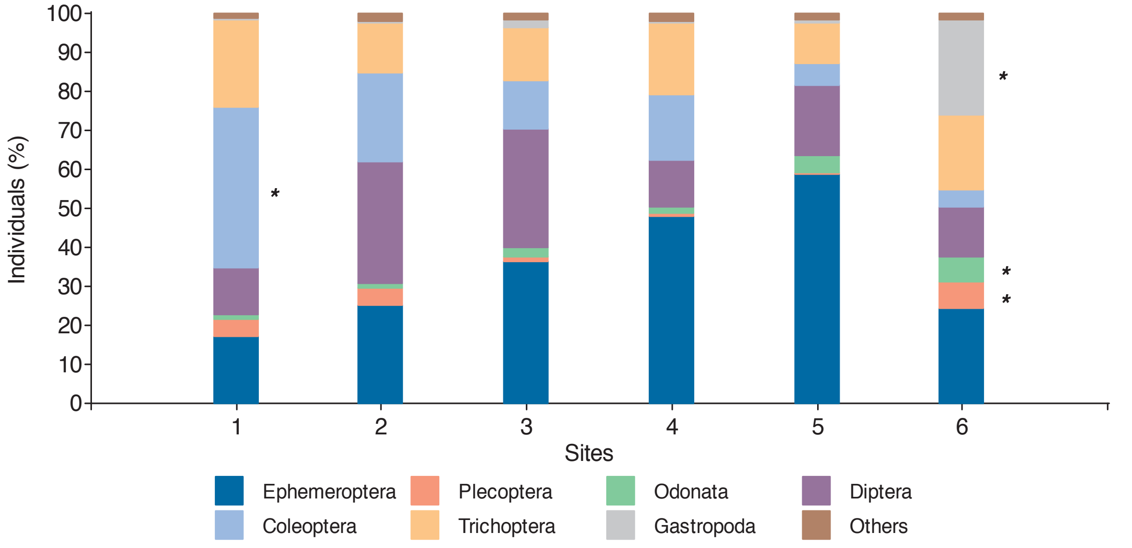

Figure 10. Percentage abundances of the seven most common taxonomic groups at the six different sampling sites. Groups marked with an asterisk (*) have statistically significant frequency differences (Chi-square; p<0.0001).

3.4. Functional feeding group analysis

The FFG analysis is presented in Figure 11 (Chi-square; p<0.0001). Collector-gatherers are the dominant group at all sites along the main river, with around 50% of all individuals collected; at Quebrada Mercedes (site 6), they are the second largest group after the filter-feeders. Filter-feeders show also high percentages at each site, with exception of site 1 (reference site), where they have statistically significant lower relative frequencies. One the other hand, piercers have a statistically significant greater relative frequency at this site, compared to the rest of the sampling sites. Finally, site 6 has a statistically significant lower relative frequency of collector-gatherers and greater relative frequencies of predators, scrapers, and shredders.

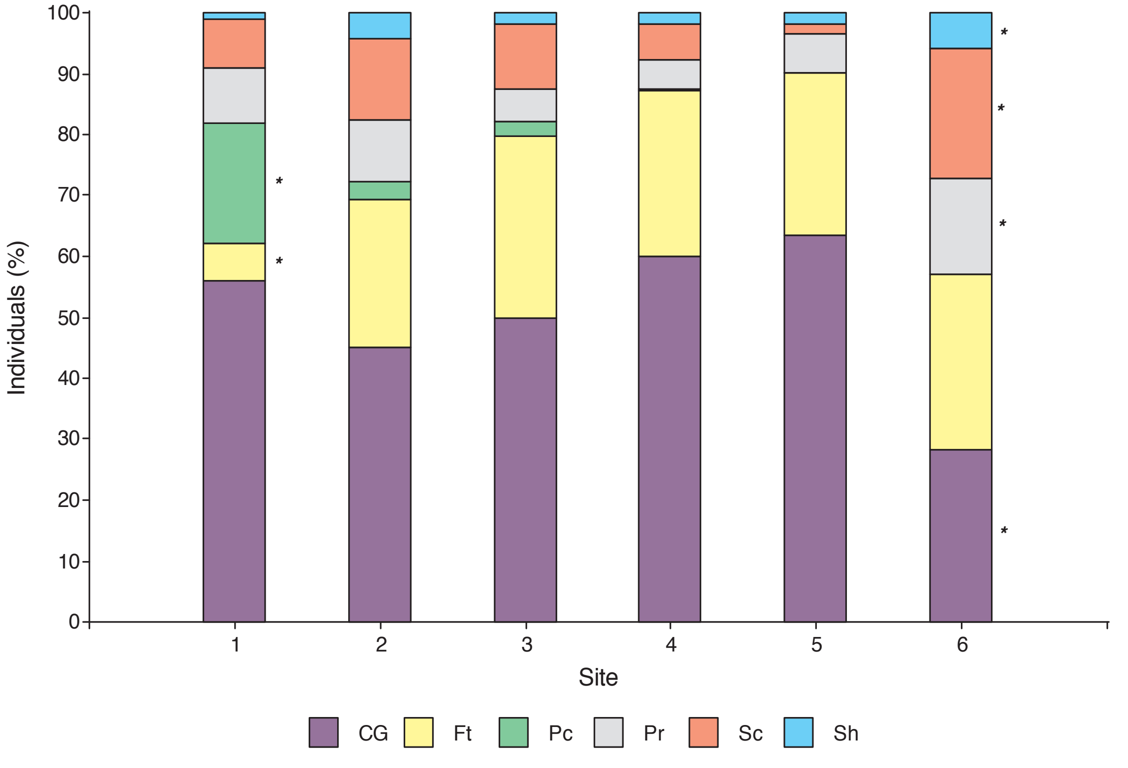

Figure 11. Percentage abundances of the functional feeding groups (FFG) at the six different sampling sites. Groups marked with an asterisk (*) have statistically significant frequency differences (Chi-square; $p<0.0001$). CG=Collector-Gatherers, Ft=Filterers, Pc=Piercers, Pr=Predators, Sc=Scrapers, and Sh=Shredders.

The FFG abundance PLS (Figure 12) presents high variation explanatory values, with factor 1 explaining 39.1 % of the total variance and factor 2 explaining 25.4 %. The variables´ temperature, as well as the piercer and predator abundances, mainly separates the different sites on the horizontal projection, as does the shredder abundance on the vertical projection. The FFG most closely allied to the reference site, is piercer abundance. On the other hand, the most closely allied variables to the agriculturally impacted areas, sites 5 and 6, are the variables turbidity, conductivity, and NO_3.

3.5. Relationship between macroinvertebrate assemblages, physical-chemical parameters and sampling sites

The macroinvertebrate abundance partial least square analysis (Figure 13) presents high variation explanatory values, with factor 1 explaining 42.0 % of the total variance and factor 2 explaining 29.9%. The variable $NO_{3,}$ and to a lesser degree conductivity and turbidity, separate mainly the different sites on the horizontal projection, as does suspended solids on the vertical

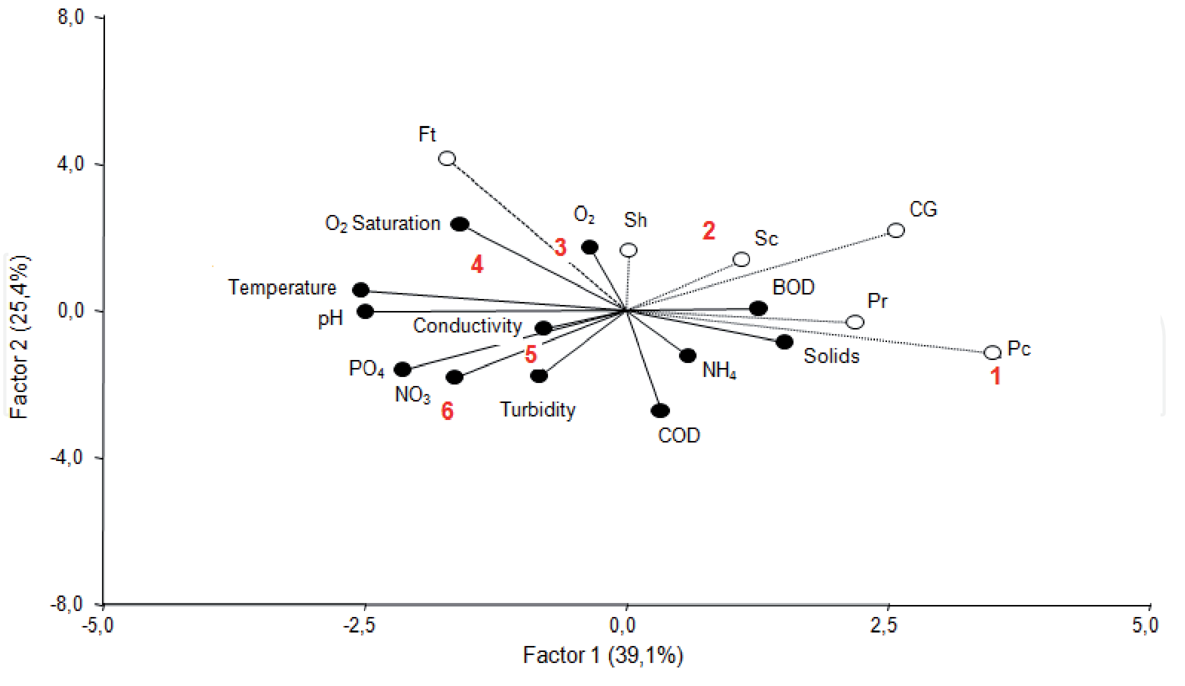

Figure 12. Site ordination (1-6) with a triplot PLS analysis using physical-chemical values as independent variables and functional feeding group abundances as dependent variables. CG=Collector-Gatherers, Ft=Filterers, Pc=Piercers, Pr=Predators, Sc=Scrapers, and Sh=Shredders.

one. The genera most closely allied to the reference site are: *Brysopterix*, *Heterelmis*, and *Paltostoma*. On the other hand, the most closely allied genera to the agriculturally impacted areas, sites 5 and 6, are larvae of the genera *Caenis*, *Dythemis, and Hexatoma*. Interestingly, the Spearman rank analysis has resulted in a plethora of correlations. The strongest physical-chemical/biological correlations are presented in Table 5.

Variable (1)	**Variable (2)**	**Spearman rank**	**p-value**
pH	Heterelmis	-0.99	0.0003
NO_3	Maruina	-0.99	0.0003
Temperature	Phanocerus	-0.97	0.0012
Turbidity	Atopsyche	-0.94	0.0051
Turbidity	*Austrolimnius*-a	-0.93	0.0077
NO_3	Farrodes	0.93	0.0077
O_2 Saturation	Hemerodromia	0.93	0.0077
NO_3	Piercers	-0.93	0.0077
PO_4	Collector-Gatherers	-0.93	0.0080
NO_3	Cora	-0.93	0 0080

Variable (1)	Variable (2)	Spearman rank	p-value
Turbidity	Cora	-0.93	0.0080
PO_4	*Cylloepus*-a	-0.93	0.0080
PO_4	*Macrelmis*-a	-0.93	0.0080
PO_4	pH	0.93	0.0080
NO_3	PO_4	0.93	0.0080

Table 5. Statistically significant associations of the Spearman rank correlation coefficient between physical-chemical, genera, and functional feeding groups (a=adults, p=pupae, genera with no lettering=larvae).

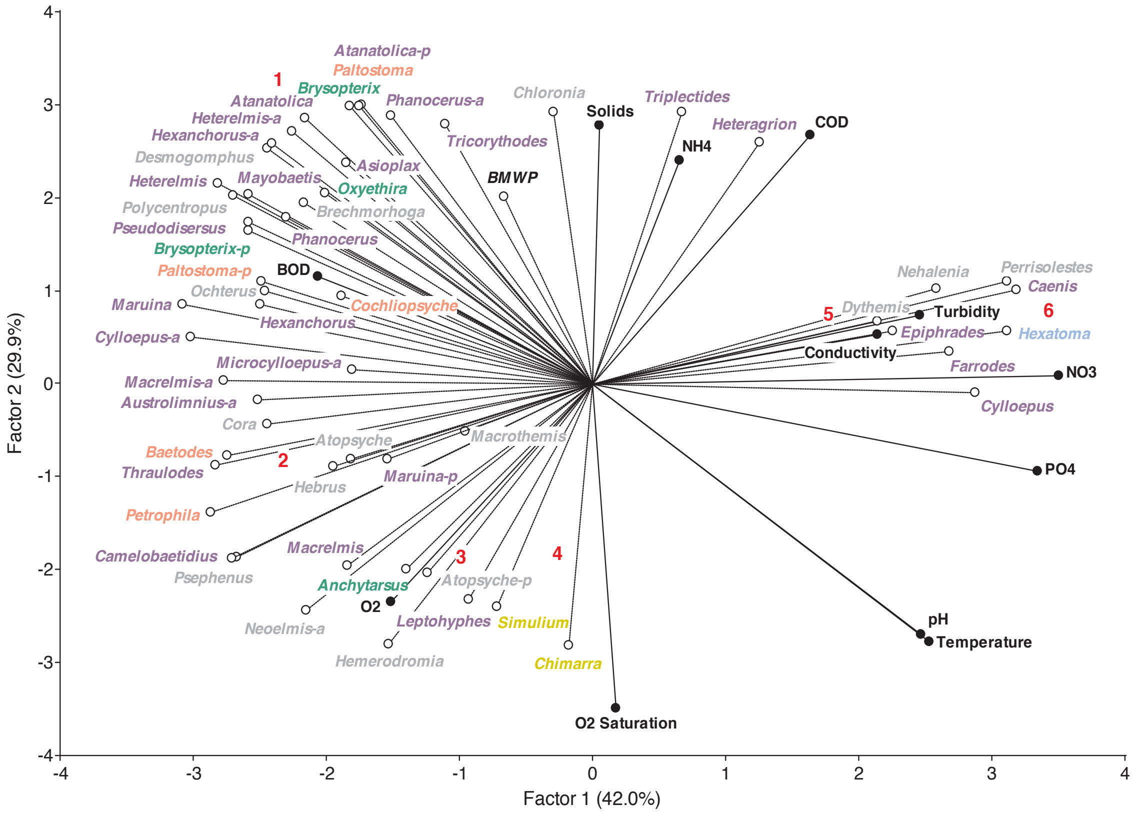

Figure 13. Site ordination (1-6) with a triplot PLS analysis, using physical-chemical values as independent variables, and macroinvertebrate generic abundance and the BMWP index as dependent variables (FFG categories: purple=collector-gatherers, yellow=filterers, green=piercers, grey=predators, red=scrapers, blue=shredders).

The Procrustes analysis produced a good site ordination consensus based on macroinvertebrate abundances, FFG, and the physical-chemical variables. The first axis explains 45.8 % of the variance and the second axis explains 33.8 % (Figure 14). The proportional consensus percentages are also good, ranging from 76 % to 92.4 %, with a mean value of 82.9% (Table 6). One can conclude that the site ordination has a good congruence with the biological and physical-chemical data sets.

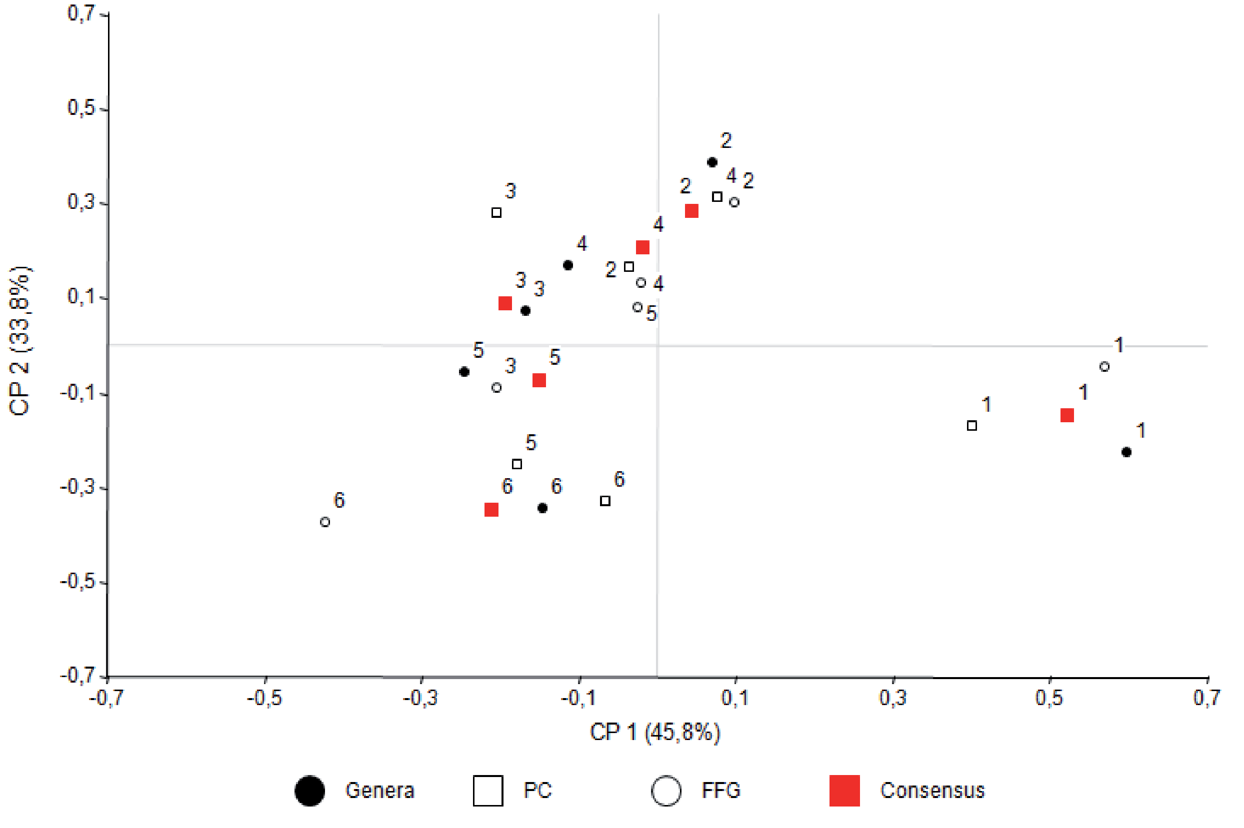

Figure 14. Site ordination configuration congruence according to physical-chemical values (PC), macroinvertebrate and functional feeding group (FFG) abundances (Genera) using a Procrustes analysis.

Variable	Proportional consensus (%)
Genera	92, 4
FFG	78, 3
PC	76, 0
Mean	82, 9

Table 6. Proportional consensus percentages as displayed by the configurations generated between macroinvertebrate generic abundances, physical-chemical values (PC), and functional feeding groups (FFG) with the generalized Procrustes ordination.

4. Discussion

An important consideration for the present study is the ability to distinguish between natural variability and human impacts [49]. In the present case, all sites are located along the same river, spanning only a small distance of 10 km (Figure 1). Moreover, elevation, stream size, and surface geology are relatively similar (Table 1) in order to properly assess human impacts and reduce natural gradients.

4.1. Diversity and composition of macroinvertebrate assemblages

The present study recorded the existence of 16 macroinvertebrate orders. In an analysis undertaken by Castillo *et al*. [17] very near to the present collecting localities, the authors reported the existence of 15-16 macroinvertebrate orders in their reference sites and 12-16 orders in the banana plantation sites. The results of the present study fall within the order range for similar studies in nearby regions. The present analysis resulted in 53 collected families (Table 3). This number compares well with the number of families collected by Lorion and Kennedy [27] and O'Callaghan and Kelly-Quinn [58] in Costa Rican and Honduran neotropical rivers, who reported 56 and 60 families, respectively. The present study also identified a total of 98 genera (Table 3). Montoya Moreno *et al*. [59] reported 69 genera for the Negro River in Colombia; whereas, Sánchez Argüello *et al*. [59] reported 96 genera in their study in Panama. Considering that some groups, such as water mites, were not identified to genus level in the present study, the total number of genera present in the Dos Novillos river and its tributaries is likely to be over 100 genera, reflecting a very high taxa richness.

It is interesting to compare the dominance results obtained in this study with analyses made in neighbouring areas under similar ecological conditions. Ramírez *et al*. [33] sampled the Carbón and Gandoca Rivers and found the following dominance gradient for total abundances: Ephemeroptera-Diptera-Trichoptera-Odonata. Castillo *et al*. [17], sampling on sites very near to the present ones, found the following order dominance in their reference sites: Ephemeroptera-Trichoptera-Coleoptera and the following one in banana plantation sites: Ephemeroptera-Diptera-Coleoptera-Gastropoda-Trichoptera. Lorion and Kennedy [27] studied several streams in the Sixaola River Valley. Considering the total number of individuals, they found a diminishing abundance gradient as follows: Ephemeroptera-Diptera-Coleoptera-Odonata. Gutiérrez-Fonseca and Ramírez [50] have reported at La Selva Biological Station, in a 15-year study, the following dominance sequence in unpolluted streams: Diptera-Trichoptera-Odonata. The present study (Figure 8) has found the following abundance dominance order: Ephemeroptera-Coleoptera-Trichoptera-Diptera. It would seem from these results that dominance sequences might vary depending on several factors dependent on the collecting site, such as substrate, current, and water quality, but also can be a result of different sampling device and mesh size [37]. However, Ephemeroptera would appear to be the most constant dominant group in most lowland rivers and streams in Costa Rica.

4.2. Comparison between sampling sites with different agrorural influence

The analysis of abundance differences demonstrates that less impacted sites clearly show statistically significantly higher abundances than sites under stronger human influence (Figure 9). Similarly, Paaby *et al*. [28] and Lorion and Kennedy [27] detected greater abundances in forested areas versus pastures under neotropical conditions; whereas, the study by Ramírez *et al*. [33] detects greater abundances in Costa Rican tropical riffle habitats than in other habitats.

Taxonomic composition also varies in a clear way along the sampling sites under the influence of different ecological impacts (Figure 10). The reference site, arguably not under the influence of human impact, presented a significantly greater relative abundance of Coleoptera. This is due to the high amount of individuals collected from the riffle beetle family (Elmidae), which are especially diverse and abundant in well oxygenized rivers and streams in forested areas (Springer, *unpubl*). A study of rivers in the Guanacaste area in Costa Rica (Kohlmann, *unpubl.*) has also found very high numbers of Coleoptera in unpolluted rivers. At the other end of the scale, the banana packing plant discharge, carrying banana debris and showing statistically significantly higher values of conductivity, turbidity, and NO_3, presents statistically significantly high numbers of Gastropoda, Odonata, and Plecoptera. This is very interesting because stoneflies (Plecoptera) have always been considered as good indicators of oxygenated, clean, and cool running waters [51-54]. Additionally, the triplot (Figure 12) and Spearman rank correlation analyses show a strong negative correlation between stonefly (*Anacroneuria*) abundance and temperature values (Spearman rank correlation value=-0.89, p=0.0476). Gutiérrez and Springer [55] reported the widespread species *A. holzenthali* from coffee plantations in Costa Rica. Tomanova and Tedesco [56], as well as Thorp and Covich [57] also indicated that stonefly presence is not necessarily a sure sign of water cleanliness. The results from these analyses seem to support this claim.

The EPT taxa richness (Table 4) showed a steady decline along the collecting sites, following an increasing anthropogenic impact trend, similar to what Lorion and Kennedy [27] reported following a forest-forest buffer-pasture gradient. The number of families per site (Table 4) did not vary much (33-40) along the collecting transect in this study (Table 4). An analysis by Castillo *et al*. [17] reported a family number variation going from 39 to 47 families. Similarly, their family numbers did not vary much between their reference (46-47) and their banana plantation collecting sites (39-46). Some families (Table 3) were restricted to only a single site (site number in parentheses), like (1): Anomalopsychidae, Calamoceratidae; (3): Ancylidae, Lumbriculidae, Physidae; (4): Corduliidae; (5): Gyrinidae; (6): Ampullaridae, Chironomidae, and Tabanidae. Certain genera (Table 3) appeared only once in a specific site: (1): *Chloronia, Contulma, Dythemis, Hemerodromia, Ochterus, Palaemnema,* and *Polycentropus*; (2): *Hebrus, Leptohyphes, Mayobaetis,* and *Psephenops*; (3) *Stenonema*; (4): *Anchitrichia* and *Neocylloepus*; (5): *Haplohyphes*; and (6): *Chironomus, Chrysops*. These taxa would seem to be closely associated with the ecological conditions of each site, *e.g.* Ampullaridae, Chironomidae, and Tabanidae in a banana packing-plant effluent with a high organic waste discharge versus Anomalopsychidae and Calamoceratidae in a forested undisturbed condition. Interestingly, the number of families and genera do not show the same trend among sampling sites. The reference and slightly disturbed sites (site 1 and 2, respectively) show a higher number of genera (over 60), in comparison to the more influenced sites (with around 40 to 50 genera), even though the number of families were similar at the latter, and even higher in one case (site 3). These results suggest that family richness is not necessarily an adequate indicator for biomonitoring, and generic identification is necessary to achieve results that are more reliable.

The BMWP index in its different variations has been popularly employed in Latin America. These studies usually have found this index to be satisfactory for reflecting water quality [10, 12, 22, 60-63], especially the Costa Rican adaptation [58]. Sánchez Argüello *et al*. [61] undertook in their Panamanian study a comparison between the Colombian and Costa Rican adaptations of the BMWP index and found the latter to be more unforgiving in its water quality evaluation. Rizo-Patrón *et al*. [22] undertook an analysis using the BMWP index modified for Costa Rica, studying the environmental impact caused by conventional and organic-irrigated rice fields on the macroinvertebrate communities. Their BMWP´-CR results show that the index values were greater in the organically irrigated rice fields. On the other hand, Fenoglio *et al*. [64] recommend, from their experience in Nicaragua, the use of the Indice Biotico Esteso [65] because of its ease of use and low cost. However, these studies have mostly assessed the comparative performance of the various indices; no attempts were made to correlate the BMWP index to specific physical-chemical variables.

The results of the BMWP'-CR index for the present analysis (Table 2) do indeed show a discriminating capacity of the index following a diminishing environmental quality site trend, especially under agricultural impact conditions (site 5), but it also shows a tendency of reporting a higher value when in river waters with high organic pollution (site 6). Interestingly, there is a statistically significant negative correlation of the BMWP'-CR index with temperature, not with pollution variables, as one could expect from the general assumption that the BMWP index reflects organic water pollution quality. These results generate some doubts about the reliability of the BMWP'-CR index as an environmentally representative tool, as the following studies indicate. Sermeño Chicas *et al*. [66] tried to implement the BMWP-CR index in El Salvador where rivers showed consistently high organic pollution conditions that were not reflected by the BMWP index [67]. In their analysis of selected macroinvertebrate-based biotic indices in Honduras, O´Callaghan and Kelly-Quinn [58] found that a BMWP-CR-based version of the ASPT index performed much better than the aforementioned index. Without doubt, more studies will be necessary in order to adjust the biotic indices used for aquatic biomonitoring in Costa Rica and Central America according to the different ecoregions.

4.3. Functional feeding group analysis

FFG relative abundances also change significantly depending on the human impact conditions on the quality of river water. It would seem that under undisturbed conditions filterers´ relative abundances tend to be minimal, their increase at disturbed sites might be a result of higher dissolved organic matter. In this study, under conditions of high organic pollution, shredders, scrapers, and predators tend to have maximal relative abundances while collector-gatherers tend to have minimal values. Finally, filter feeders seem to react positively to high concentrations of dissolved O_2 (Table 5), which is positively correlated with fast flowing waters, a condition that also favors the feeding mechanism of filterers.

The taxonomic grouping triplot analysis (Figure 13) suggests a correlation between the reference site and the piercers, and it would appear that the first axis is characterized by a piercers' abundance gradient, diminishing from the reference community (site 1) to the high organic waste discharge sites (site 6). In the present study, piercers are mainly represented by the caddisfly family Hydroptilidae, which is especially abundant in the splash zone of big rocks in riffle areas, which were characteristic for the reference sampling site. The second axis appears to be characterized by filterers, arranging the different agrorural ecosystems along a diminishing abundance gradient.

The FFG triplot analysis (Figure 12) supports a strong agrorural ecosystem ordination process mediated by the abundance of piercers along the main axis as suggested in the previous triplot analysis; however, as this analysis benefits from having more information (127 taxa versus 58 taxa), it also evinces the importance of predators and shredders as relevant ecosystem ordinating biological variables. The strong negative correlation between the piercer's abundance and NO_3 and PO_4 values (Table 5) stresses again the importance of the piercers as an ecosystem characterizing variable, although the presence of suitable microhabitats might be another important factor to consider.

4.4. Relationship between macroinvertebrate assemblages, physical-chemical parameters and sampling sites

The taxonomic grouping triplot analysis (Figure 13) and the Spearman rank correlation analysis (Table 5) show the existence of several genera and FFG that are highly correlated with physical-chemical variables and that possibly could be used as surrogates for these variables. The larvae of *Maruina* showed one of the strongest correlations with NO_3, although other genera like *Farrodes* and *Cora,* and the piercers´ functional feeding group were also significantly correlated with this chemical variable. Species of the genus *Caenis* have been found regularly in organically enriched streams [48]. Of the statistically significant genus list (Table 5), only *Heterelmis* and *Farrodes* already had been cited before as good quality bioindicators for toxicity and pollution-sensitivity testing by Castillo *et al.* [17] and Rizo-Patrón *et al.* [22], respectively, in a similar type of analysis.

Finally, the Procrustes analysis allows an assessment of the goodness of fit of the taxonomic, FFG, and physical-chemical analyses (Figure 14, Table 6). The analysis resulted in a good match of the collecting sites (landmarks) with the values derived from the three blocks of variables, indicating that any one of the three is describing in a similar way the ecology of each study site. The consensus values indicated in Table 6 show a very good concordance in this study between the different variable blocs and the consensus values, where generic abundances seem to generate a better ecological ordination. An environmental impact gradient also becomes very apparent on the first ordination axis, ranging from the undisturbed reference site inside the forest on the right of the graph to the banana packing-plant effluent on the left.

5. Conclusions

The present study clearly shows that tropical river macroinvertebrate diversity changes and at the same time characterizes and defines different river ecosystem conditions under various agrorural impacts. Changes in taxonomic composition and functional feeding group structure are very indicative of ecosystem function.

Ephemeroptera seem to be, in general, a rather constant and numerous group, present in the great majority of collections in neotropical rivers. However, high relative abundances of Coleoptera, especially from the family Elmidae, seem to be indicative of unpolluted conditions in tropical rivers; whereas, high relative abundances of Gastropoda, Odonata, and Plecoptera show up in sites with relatively high (plant-derived) organic pollution, although with well-oxygenized waters and forested stream margins. Addtionally, high numbers of individuals were found in unpolluted or slightly polluted sites; whereas, lower abundances were found in sites under human impact (town and fruit packing plant discharges, agricultural plantations).

Especially illustrative is the change in structure of functional feeding groups. Piercers showed the highest relative abundance in unpolluted sites and seem to be especially sensitive to human impacts because they quickly disappear under altered conditions, most probably reacting to the decrease of their microhabitat and food items. Filterers have the lowest relative abundance under unpolluted conditions and quickly become relatively more abundant under human impact conditions, most probably reflecting an increase in particles in the water column. At the other end of the spectrum, under high (plant-derived) organic pollution, shredders and scrapers show their highest relative abundance concomitantly with an increase in particulate organic matter, and probably as a response to it. Predators here show their highest relative abundance as well and at the same time, collector-gatherers here show their lowest relative abundance. It would seem then that predation, as well as scraping and shredding, increases significantly in river areas with high (plant-derived) organic pollution.

The biomonitoring analysis presented in this chapter used an adaptation of the BMWP index to Costa Rica. In all cases, the method revealed or indicated the existence of an anthropogenic/agricultural impact gradient going from the unpolluted site to the most perturbed locality. ANOVA tests evidenced the fact that the BMWP index has enough sensitivity and discriminating power to detect changes in macroinvertebrate biodiversity, which can be translated into statistically significant differences between sampling sites. The method determined the existence of changes in macroinvertebrate communities associated with agricultural areas, even when analytical methods could not detect the presence of pesticides in river water. A previous analysis of the BMWP score by Pinder and Farr [68, 69] found that it was significantly negatively correlated only with dissolved organic carbon. The present study detects a strong negative correlation between the BMWP score and temperature (Figure 12, Table 5). Due to its simplicity, speed of use, efficiency, and cheapness, this method shall undoubtedly continue to be a very popular one in the future. The BMWP is considered an extremely successful index according to Spellerberg [70]. However, there are some doubts about its suitability as a tool for detecting organic pollution in some regions of Latin America, especially if one considers that in this case the index was more sensitive to the impact of a banana plantation than the one caused by relatively high (plant-derived) organic pollution. The PLS/Procrustes analysis seemed, on this occasion, to be a more suitable method for describing and evaluating anthropogenic/agricultural environmental impacts.

The use of multivariate ordination for environmental studies is becoming more and more common. In particular, the PLS/Procrustes analyses represent a very powerful combination tool as they not only perform a site ordination but different taxa and environmental variables can be correlated at the same time. This makes this method extremely useful for taxa, physical-

http://dx.doi.org/ 10.5772/59073

chemical, and FFG variables' correlations as specific environmental variable surrogates. Castillo *et al*. [17] and Rizo-Patrón *et al*. [22] found similar promising results for the study of agricultural ecosystem analyses. Castillo *et al*. [17] indicated, based on their results, that multivariate analyses are more sensitive in distinguishing pesticide effects than toxicity tests. Therefore, multivariate analyses should be incorporated as an approach for future ecosystem/ biodiversity analyses.

Acknowledgements

We would like to thank Mildred Linkimer for her help in the design of some figures; as well as Roxana Araya for her help in the composition of tables and lists. Finally yet importantly, we are also indebted to Jane Yeomans and Kent McLeod for critically revising the text and to Pablo Gutiérrez for useful suggestions in organizing the results. This material is based upon work supported by the Department of Energy [National Nuclear Security Administration] under Award Number DE FG02-04ER 63856.

Author details

Bert Kohlmann[1*], Alejandra Arroyo[1], Monika Springer[2] and Danny Vásquez[3]

*Address all correspondence to: bkohlman@earth.ac.cr

1 EARTH University, San José, Costa Rica

2 School of Biology, University of Costa Rica, San José, Costa Rica

3 Private Consultant, San José, Costa Rica

References

[1] Valerio CE. Costa Rica. Ambiente y Sociedad. Santo Domingo de Heredia: Instituto Nacional de Biodiversidad; 1999.

[2] Obando V. Biodiversidad de Costa Rica. Estado del Conocimiento y Gestión. Santo Domingo de Heredia: Instituto Nacional de Biodiversidad; 2002.

[3] Jiménez J. Training parataxonomists and curators to help conservation: the biodiversity inventory. In: Bisonette, JA. and Krausman, PR. (eds.). Integrating people and wildlife for a sustainable future: proceeding of the First International Wildlife Congress. Bethesda: The Wildlife Society; 1995. p165-167.

[4] Kohlmann B, Mitsch WJ, Hansen DO. Ecological Management and Sustainable Development in the Humid Tropics of Costa Rica. Ecological Engineering 2008; 34(4): 254-266.

[5] Jones JR. Colonization and Environment: Land Settlement Projects in Central America. Tokio: The United Nations University; 1990.

[6] Seligson M. Peasants of Costa Rica and the Development of Agrarian Capitalism. Madison: University of Wisconsin Press; 1980.

[7] de Oñoro MT. El Asentamiento Neguev. Atlantic Zone Programme. Inf. Técnico No. 162, Programme Paper No. 7. Turrialba: Centro Agronómico Tropical de Investigación y Enseñanza; Universidad Agrícola de Wageningen; Ministerio de Agricultura y Ganadería; 1990.

[8] Roldán G., Builes J., Trujillo CM., Suárez A. Efectos de la Contaminación Industrial y Doméstica sobre la Fauna Béntica del Río Medellín. Actualidades Biológicas 1973; 2(4): 54-60.

[9] Roldán G. Los Macroinvertebrados y su Valor como Indicadores de la Calidad del Agua. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 1999; 23(88): 375-387.

[10] Roldán G. Bioindicación de la Calidad del Agua en Colombia. Antioquia: Universidad de Antioquia; 2003.

[11] de la Lanza Espino G. Criterios Generales para la Elección de Bioindicadores. In: de la Lanza Espino G., Hernández Pulido H., Carbajal Pérez JL. (eds.). Organismos Indicadores de la Calidad del Agua y de la Contaminación (Bioindicadores). México, D.F.: Plaza y Valdés, S.A de C.V.; 2000. p17-41.

[12] Prat N., Ríos B., Acosta R., Rieradevall M. Los Macroinvertebrados como Indicadores de la Calidad de las Aguas. In: Domínguez E., Fernández HR. (eds.). Macroinvertebrados Bentónicos Sudamericanos: Sistemática y Biología. Tucumán: Fundación Miguel Lillo; 2009. P631-654.

[13] Springer M. Biomonitoreo Acuático. In: Springer M., Ramírez A., Hanson P. (eds.). Macroinvertebrados de Agua Dulce de Costa Rica I. Revista de Biología Tropical 2012; 58(4): 53-59.

[14] Castillo LE. Pesticide impact of intensive banana production on aquatic ecosystems in Costa Rica. PhD thesis. Stockholm University: Stockholm; 2000.

[15] Castillo LE., De la Cruz E., Ruepert C. Ecotoxicology and Pesticides in Tropical Aquatic Ecosystems of Central America. Environ. Toxicol. Chem. 1997; 16: 41-51.

[16] Castillo LE., Ruepert C., Pinnock M. Acute toxicity and pesticide residues in surface waters in a pineapple growing area of Costa Rica. 10th International Symposium on Toxicity Assessment, Quebec City. 2001.

http://dx.doi.org/ 10.5772/59073

[17] Castillo E., Martínez E., Ruepert C., Savage C., Gilek M., Pinnock M., Solís E. Water Quality and Macroinvertebrate Community Response Following Pesticide Applications in a Banana Plantation, Limón, Costa Rica. Science of the Total Environment 2006; 367: 418-432.

[18] Martínez E. Utilización de organismos acuáticos macro-bentónicos en la determinación de la calidad de las aguas naturales en los arrozales de Bagatzí, Guanacaste. MSc thesis. Universidad Nacional: Heredia; 1998.

[19] Monge P., Partanen T., Wesseling C., Bravo V., Ruepert C.B.I. Assessment of Pesticide Exposure in the Agricultural Population of Costa Rica. Ann. Occup. Hyg. 2005; 49(5): 375-384.

[20] Fernández L., Springer M. El Efecto del Beneficiado del Café sobre los Insectos Acuáticos en Tres Ríos del Valle Central (Alajuela) de Costa Rica. Revista de Biología Tropical 2008; 56(4): 237-256.

[21] Rizo-Patrón, F. Estudio de los arrozales del proyecto Tamarindo: agroquímicos y macroinvertebrados bentónicos en relación al Parque Nacional Palo Verde, Guanacaste, Costa Rica. MSc thesis. Universidad Nacional: Heredia; 2003.

[22] Rizo-Patrón F., Kumar A., McCoy Colton MB., Springer M., Trama FA. Macroinvertebrate Communities as Bioindicators of Water Quality in Conventional and Organic Irrigated Rice Fields in Guanacaste, Costa Rica. Ecological Indicators 2013; 29: 68-78.

[23] Springer M., Vásquez D., Castro A., Kohlmann B. Field-guide for water quality bioindicators. San José: EARTH-UCR; 2007.

[24] Vásquez D., Springer M., Castro A., Kohlmann B. Bioindicadores de la Calidad del Agua: Cuenca del Río Tempisque. San José: Universidad EARTH; 2010.

[25] Boyero L., Bosch J. Spatial and Temporal Variation of Macroinvertebrate Drift in Two Neotropical Streams. Biotropica 2002; 34(4): 567-574.

[26] Boyero L., Bosch J. Multiscale Spatial Variation of Stone Recolonization by Macroinvertebrates in a Costa Rican Stream. Journal of Tropical Ecology 2004; 20(1): 85-95.

[27] Lorion CM., Kennedy BP. Relationship between Deforestation, Riparian Forest Buffers and Benthic Macroinvertebrates in Neotropical Headwater Streams. Freshwater Biology 2009; 54(1): 165-180.

[28] Paaby P., Ramírez A., Pringle CM. The Benthic Macroinvertebrate Community in Caribbean Costa Rican Streams and the Effect of Two Sampling Methods. Rev. Biol. Trop. 1998; 46(Supl. 6): 185-199.

[29] Pringle C.M., Ramírez A. Use of Both Benthic and Drift Sampling Techniques to Assess Tropical Stream Invertebrate Communities along an Altitudinal Gradient, Costa Rica. Freshwater Biology 1998; 39(2): 359-373.

[30] Ramírez A., Pringle CM. Invertebrate Drift and Benthic Community Dynamics in a Lowland Neotropical Stream, Costa Rica. Hydrobiologia 1998a; 11(1-3): 19-26.

[31] Ramírez A., Pringle CM. Structure and Production of a Benthic Insect Assemblage in a Neotropical Stream. J. N. Am. Benthol. Soc. 1998b; 17(4): 443-463.

[32] Ramírez A., Pringle CM. Spatial and Temporal Patterns of Invertebrate Drift in Streams Draining in a Neotropical Landscape. Freshwater Biology 2001; 46(1): 47-62.

[33] Ramírez A., Paaby P., Pringle M., Agüero G. Effect of Habitat Type on Benthic Macroinvertebrates in Two Lowland Tropical Streams, Costa Rica. Rev. Biol. Trop. 1998; 46(Supl. 6): 201-213.

[34] Rosemond AD., Pringle CM., Ramírez A. Macroconsumer Effects on Insect Detritivores and Detritus Processing in a Tropical Stream. Freshwater Biology 1998; 39(3): 515-523.

[35] Mitsch WJ., Tejada J., Nahlik A., Kohlmann B., Bernal B. Hernández C. Tropical Wetlands for Climate Change Research, Water Quality Management and Conservation Education on a University Campus in Costa Rica. Ecological Engineering 2008; 34(4): 276-288.

[36] Vásquez Morera A. Soils. In: Janzen DH. (ed.). Costa Rican Natural History. Chicago: The University of Chicago Press; 1983.

[37] Stein H., Springer M., Kohlmann B. Comparison of Two Sampling Methods for Biomonitoring Using Aquatic Macroinvertebrates in the Dos Novillos River, Costa Rica. In: Kohlmann B., and Mitsch MJ. (eds.). Ecological Engineering 2008; 34(4): 267-275.

[38] MINAE (Ministerio de Ambiente y Energía, CR). Reglamento para la Evaluación y Clasificación de la Calidad de Cuerpos de Agua Superficiales. Decreto Ejecutivo Nº 33903-MINAE-S. Alcance No. 8 a La Gaceta No. 78, lunes 17 de septiembre del 2007. San José, CR. Available from: http://historico.gaceta.go.cr/pub/2007/09/17/COMP_17_09_2007.html

[39] Liess M., Brown C., Dohmen P., Duquesne S., Hart A., Heimbach F., Krueger J., Lagadic L., Maund S., Reinert W., Streloke M., Tarazona JV. (eds.). Effects of pesticides in the field. Le Croisic: EU and SETAC Europe Workshop; 2003.

[40] Ramírez A., Gutiérrez-Fonseca PE. Functional Feeding Groups of Aquatic Insect Families in Latin America: A Critical Analysis and Review of Existing Literature. Rev. Biol. Trop. 2014; 62(Suppl. 2): 155-167.

[41] Akaike H. Information theory and an extension of the maximum likelihood principle. Tsahkadsor, Armenian SSR. 2nd International Symposium on Information Theory; 1973.

[42] Arroyo AT. Modelos mixtos en el análisis de *loci* de caracteres cuantitativos y su aplicación en mejoramiento vegetal. PhD thesis. Universidad Nacional de Córdoba: Córdoba; 2008.

[43] Di Rienzo JA., Guzmán AW., Casanoves F. A Multiple Comparisons Method Based on the Distribution of the Root Node Distance of a Binary Tree. Journal of Agricultural, Biological and Environment Statistics 2002; 7(2): 1-14.

[44] Balzarini MG., González L., Tablada M., Casanoves F., Di Rienzo JA., Robledo CW. Infostat. Manual del Usuario, Córdoba: Editorial Brujas; 2008.

[45] Gabriel KR. Biplot Display of Multivariate Matrices with Application to Principal Components Analysis. Biometrika, 1971; 58: 453-467

[46] Di Rienzo JA., Casanoves F., Balzarini MG., González L., Tablada M., Robledo C.W. InfoStat versión 2012. Universidad Nacional de Córdoba: Grupo InfoStat, FCA; 2012. Available from: http://www.infostat.com.ar

[47] Gower JC. Generalized Procrustes Analysis. Psychometrika 1975; 40:33-51.

[48] Hilsenhoff WL. An Improved Biotic Index of Organic Stream Pollution. Great Lakes Entomologist 1987; 20: 31-39.

[49] Moya, N, Hughes, R.M., Domínguez, E., Gibon, F.M., Goitia, E., Oberdorff, T. 2011. Macroinvertebrate-Based Multimetric Predictive Models for Evaluating the Human Impact on Biotic Conditions of Bolivian Streams. Ecol. Indic. 11: 840-847.

[50] Gutiérrez-Fonseca PE., Ramírez A. Long-term patterns of aquatic macroinvertbrate assemblages in lowland neotropical streams. San José. Poster presented at the 50th Anniversary Meeting of the Association of Tropical Biology and Conservation and Organization of Tropical Studies, 2013. Available at: https://atbc.confex.com/atbc/2013/webprogram/Paper1287.html

[51] Baur WH. Gewässergüte Bestimmen und Beurteilen. Berlin: Parey Buchverlag; 1998.

[52] Stewart KW., Stark B. Plecoptera. In: Merritt RW., Cummins KR., Berg MB.(eds.). An Introduction to the Aquatic Insects of North America. Dubuque: Kendall/Hunt; 2008. p311-384.

[53] Gutiérrez-Fonseca PE. Ecología, reproducción, taxonomía y distribución de *Anacroneuria* spp. Klapálek, 1909 (Insecta: Plecoptera: Perlidae) en Costa Rica. BSc thesis.Universidad de Costa Rica, San José; 2009.

[54] Salcedo S., Trama FA. Manual de Identificación de Macroinvertebrados Acuáticos de la Microcuenca San Alberto, Provincia de Oxapampa, Perú. Lima: CONCYTEC/FONDECYT; 2014.

[55] Gutiérrez-Fonseca PE., Springer M. Description of the final instar nymphs of seven species from *Anacroneuria* Klapalék (Plecoptera: Perlidae) in Costa Rica, and first record for an additional genus in Central America. Zootaxa 2011; 2965: 16-38.

[56] Tomanova S., Tedesco PA. Tamaño Corporal, Tolerancia Biológica y Potencial de Bioindicación de la Calidad del Agua de *Anacroneuria* spp. (Plecoptera: Perlidae) en América del Sur. Revista de Biología Tropical 2007; 55(1): 67-81.

[57] Thorp JH., Covich AP. Ecology and Classification of North American Freshwater Invertebrates. Academic Press; 2010.

[58] O´Callaghan P., Kelly-Quinn M. Performance of Selected Macroinvertebrate-Based Biotic Indices for Rivers Draining the Merendón Mountains Region of Honduras. Cuadernos de Investigación UNED 2013; 5(1): 45-54.

[59] Montoya Moreno Y., Acosta García Y., Zuluaga Zuluaga E., García A. Evaluación de la Biodiversidad de Insectos Acuáticos y de Calidad Fisicoquímica y Biológica del Río Negro (Antioquia-Colombia). Revista Universidad Católica de Oriente 2007; 23: 71-84.

[60] Sánchez Argüello R., Cornejo A., Boyero L., Santos Murgas A. Evaluación de la Calidad del Agua en la Cuenca del Río Capira, Panamá. Tecnociencia 2010; 12(2): 57-70.

[61] Miserendino ML., Pizzolón LA. Rapid Assessment of River Water Quality Using Macroinvertebrates: A Family Level Biotic Index for the Patagonic Andean Zone. Acta Limnologica Brasiliensia 1999; 11: 137-148.

[62] Mafla MH. Guía para Evaluaciones Ecológicas Rápidas con Indicadores Biológicos en Ríos de Tamaño Medio Talamanca – Costa Rica. Macroinvertebrados (BMWP – CR – Biological Monitoring Working Party) y Hábitat (SVAP – Stream Visual Assessment Protocol). Turrialba: Centro Agronómico Tropical de Investigación y Enseñanza (CATIE); 2005.

[63] Macchi PA. Calidad del agua en ecosistemas fluviales utilizando macroinvertebrados bentónicos. Cuenca del arroyo Pocahullo, San Martín de los Andes. BSc thesis. Universidad Nacional del Comuahue, Neuquén; 2007.

[64] Fenoglio S., Badino G., Bona F. Benthic Macroinvertebrate Communities as Indicators of River Environment Quality: An Experience in Nicaragua. Rev. Biol. Trop. 2002; 50(3-4): 1125-1131.

[65] Ghetti RE. Manuale di Applicazione dell’Indice Biotico Esteso (I.B.E.). Trento: Provincia Autonoma di Trento Press; 1997

[66] Sermeño Chicas JM., Serrano Cervantes L., Springer M., Paniagua Cienfuegos MR., Pérez D., Rivas Flores AW., Menjívar Rosa, RA., Bonilla de Torres BL., Carranza Estrada FA., Flores Tensos JM., Gonzáles C. de los A., Gutiérrez Fonseca PE., Hernández Martínez MA., Monterrosa Urías AJ., Arias de Linares AY. Determinación de la Calidad Ambiental de las Aguas de los Ríos de El Salvador, Utilizando Invertebrados Acuáticos: Índice Biológico a Nivel de Familias de Invertebrados Acuáticos en El Salvador (IBF-SC-2010). San Salvador: Editorial Universitaria (UES); 2010.

[67] Hilsenhoff WL. Rapid Field Assessment of Organic Pollution with a Family-Level Biotic Index. Journal of the North American Benthological Society 1988; 7: 65-68.

[68] Pinder LCV., Farr IS. Biological Surveillance of Water Quality 2. Temporal and Spatial Variation in the Macroinvertbrate Fauna of the River Frome, a Dorset Chalk Stream. Archives of Hydrobiology 1987a; 109: 321-331.

[69] Pinder LCV., Farr IS. Biological Surveillance of Water Quality 3. The Influence of Organic Enrichment on the Macroinvertebrate Fauna of a Small Chalk Stream. Archives of Hydrobiology 1987b; 109: 619-637.

[70] Spellerberg IF. Monitoring Ecological Change. Cambridge: Cambridge University Press; 2005.

IntechOpen