Riparian and microhabitat factors determine the structure of the EPT community in Andean headwater rivers of Ecuador

Vimos-Lojano D.J.[1,2], Martínez-Capel F.[2], Hampel H.[3,1]

[1]Laboratorio de Ecología Acuática, Departamento de Recursos Hídricos y Ciencias Ambientales, Universidad de Cuenca, Ecuador.

[2] Research Institute for Integrated Management of Coastal Areas (IGIC), Universitat Politècnica de València, Grao de Gandia, Spain

[3]Facultad de Ciencias Químicas, Universidad de Cuenca, Ecuador

Correspondence to: Diego Vimos-Lojano, Universidad de Cuenca, Departamento de Recursos Hídricos y Ciencias Ambientales, Universidad de Cuenca, Av. 12 de Abril, Cuenca, Ecuador. Tel./fax: + 593 74051000.

Email: diego.vimos.l@gmail.com

Running Head: Environmental factors driving EPT taxa in Ecuador

ABSTRACT

This research was conducted in the high-Andean basin of the Zhurucay River in southern Ecuador. In four river reaches 19 sampling campaigns were conducted per reach spread over a period of 35 months. The biotic samples were selected in the periods with greatest flow stability. Parallel to each sampling, 37 environmental variables grouped into three factors (riparian corridor, hydromorphology and water quality) were recorded. The study aimed to analyse during periods of stable flow the influence of these environmental factors on the structure and density of the EPT community (Ephemeroptera, Plecoptera, Trichoptera) in a quasi-pristine aquatic ecosystem. Multivariate statistical analysis revealed that the Froude number (Fr), gravel type, and width/depth ratio are the most relevant hydromorphological variables explaining variations in EPT density. Xiphocentronidae, *Contulma* and *Helicopsyche* were observed to have a relationship with the order of the river, while *Ochrotrichia*, *Nectopsyche* and *Phylloicus* varied with the type of riparian vegetation. *Phylloicus*, *Ochrotrichia* and *Nectopsyche* were common in lentic sites, while the proportion of gravel and the width/depth ratio restricted the genus *Helicopsyche*. The only relevant water quality factor was the total phosphorus which was related with two taxa. In conclusion, although macroinvertebrates are currently employed in water quality studies, riparian vegetation and hydromorphological factors are determinant for their communities in pristine Andean rivers. Such factors are therefore crucial in the study of environmental flows and the assessment of the ecological integrity.

Keywords: Andean streams, headwaters, riparian corridor, hydromorphology, macroinvertebrate, EPT.

INTRODUCTION

One of the concerns in aquatic ecology is the identification of environmental factors (i.e., physical habitat or water quality) that restrict the spatial distributions of aquatic communities (Allan *et al.*, 1997; Parsons *et al.*, 2003; Poff, 1997). Although the variations in macroinvertebrate communities across a wide range of environmental conditions are studied in the temperate regions of the northern hemisphere, very few studies have been performed in Andean rivers (Jacobsen and Encalada, 1998; Mesa, 2010; Ríos-Touma *et al.*, 2011). For example, several studies documented that changing land use, besides affecting flow (Buytaert *et al.*, 2006) and sediment production (Restrepo and Restrepo, 2005), can reduce the physical, chemical (Trimble, 1997) and biological quality of water (Miserendino *et al.*, 2011). Land use adjacent to a river is a major determinant of surface water quality and the state of aquatic communities (Miserendino *et al.*, 2011; Mesa, 2010), which are generally associated with the trophic resources of the river banks (Mesa, 2010).

Similarly, other important components influence aquatic communities such as the geomorphology of the riverbed (Wilcox *et al.*, 2008; Smits *et al.*, 2015), the hydraulic characteristics (Statzner *et al.*, 1988) and the biological interactions (Holomuzki *et al.*, 2010). The influence of physical conditions on aquatic biota has been studied for several decades (Allen and Vaughn, 2010; McIntosh *et al.*, 2002; Gibbins *et al.*, 2001; Danehy *et al.*, 1999; Statzner, 1981); specifically, recent advances have been made in understanding how the hydraulic conditions interact with the substrate to affect aquatic macroinvertebrate communities (Allen and Vaughn, 2010; Gibbins *et al.*, 2001). These studies include an analysis of the effects of flow rate on flow velocity and the composition of the substrate, home of different habitats colonized by different benthic organisms (Ward, 1992). For example, the Froude number (Fr) and the substrate are important interrelated characteristics of the physical habitats of rivers. The first factor encompasses water velocity and turbulence (Beschta and Jackson, 1979), and the second is defined by the size and the diversity of the bottom material (Boyero, 2003). As stated by Beisel *et al.* (2000) are the habitat conditions associated with a thick or heterogeneous substrate more stable, even under conditions of high flow velocity. Consequently, these habitats can shelter a higher number of taxa compared to those with fine substrates, which are prone to being eroded (Beisel *et al.*, 2000; Erman and Erman, 1984). In mountain areas rivers are prune to a high degree of sediment transport, impacting the benthic communities (Statzner *et al.*, 1988).

While studies such as the above have been relatively abundant in temperate geographical zones, in the high-altitude areas of the Andean region research on hydraulics and aquatic macroinvertebrates has been scarce (Jacobsen *et al.*, 2013; Cauvy-Fraunié *et al.*, 2014a; Cauvy-Fraunié *et al.*, 2014b;; Jacobsen *et al.*, 2014). These studies generally examine the influence of thaw (flood waves) on aquatic communities in association with the distance from a glacier located over 4,000 m above sea level (m a.s.l.) without accounting for specific variables, such as the Fr, velocity, water depth, among others. One limitation of these studies is that the altitudinal range of the target rivers restricts the presence of some important benthic groups, such as EPT (Ephemeroptera, Plecoptera and Trichoptera), due to the extreme conditions of these environments and the low concentration of dissolved oxygen in the water (Jacobsen *et al.*, 2003; Jacobsen *et al.*, 1997). However, a study by Ríos-Touma *et al.* (2011) included waters at lower altitudes and found that seasonality and flow rate critically affected the composition of aquatic communities.

An approach to understand the environmental processes that control the structure and behaviour of aquatic communities is through the analysis of quasi-pristine ecosystems. At present, the increase in water abstraction areas, plantations of exotic species, the burning of grasslands and the expansion of agricultural land use strongly reduces the existence of totally pristine ecosystems in many mountainous countries, especially Ecuador (Spehn *et al.*, 2006). Headwater basins become more and more exposed to extreme anthropogenic pressures (Jacobsen and Marín, 2008) and are susceptible to any type of disturbance (Meyer *et al.*, 2001). Indeed, these headwater basins are used as references or controls in studies of biological water quality to assess the effect of changes in land use or anthropogenic activities (Acosta *et al.*, 2009; Villamarín *et al.*, 2013). Given the vulnerability of mountain headwaters we focused our study on a headwater area, which under natural conditions provide the habitats for organisms' subject to extreme temperature, flow, predation, and exotic species invasion (Meyer *et al.*, 2007). Specifically, aquatic macroinvertebrates of the EPT orders, given their abundance and diversity, have been used in eco-hydraulic (Gibbins *et al.*, 2010; McIntosh *et al.*, 2002; Mérigoux *et al.*, 2009) and water quality studies (Bonada *et al.*, 2002; López-López and Sedeño-Díaz, 2015; Miserendino *et al.*, 2011). We concentrated our study on EPT for the following reasons: i) the adequate taxonomic knowledge of these invertebrates and the ease of sampling; ii) their sedentary nature (compared to fish), which provides a reliable spatial signal of their status in each sampled habitat (Johnson *et al.*, 2003); iii) the rapid changes in the trophic structure, composition and abundance of the benthic community in response to various types of natural and anthropogenic disturbances (Rice *et al.*, 2001); and iv) the lack of other native aquatic taxa that can serve as bio-indicator (e.g., *Astroblepus*; Vimos-Lojano unpublished) at these altitudes. Other taxa, such as those of the order *Diptera,* that are usually dominant in Andean rivers (Scheibler *et al.*, 2014) were not used as they are opportunists that quickly adapt to fluctuating conditions (Ladle *et al.*, 1985) and are therefore inappropriate for the purposes of this study.

The main objective of this study was to analyse the influence of three environmental factors (riparian corridor, hydromorphology, water quality) on the structure and density of aquatic macroinvertebrate communities, specifically EPT taxa, in a high-Andean, quasi-pristine aquatic ecosystem during periods of stable flow discarding short-term effects of high-flow events, corresponding basically to base flow conditions. In summary, the study aimed to answer the following questions: i) which environmental variables primarily determine the structure of the EPT community during base-flow in a quasi-pristine mountain river system? and, ii) how do the EPT communities respond to those environmental variables?

MATERIALS AND METHODS

Study area

This study was performed in the Andean micro-watershed of the Zhurucay River (with an area of 7.53 km^2), located in southern Ecuador (between coordinates 9662500 N, 9658750 S, 694630 W and 698010 E, PSAD56 - UTM Zone 17S), releasing its water into the Rircay River which drains a basin area of 826.16 km^2 and discharges on its turn into the Pacific Ocean via the Jubones River (Fig. 1). The study area is characterized by an unaltered geological condition and quasi-zero anthropogenic intervention (Hampel *et al.*, 2010), covered by herbaceous vegetation (scrubland) encompassing a few patches of quinoa trees (e.g., *Polylepis incana*

Kunth and *Polylepis reticulata* Kunth) and native shrubs. The only human activity that in the study area could affect the ecosystem is the sporadic burning of the tussock vegetation in preparation of the extension of grassland, a typical action in the high Andean regions (Matson and Bart, 2013). Four river reaches (segments of 50 m in length) were selected throughout the basin (approximately 3,600 m a.s.l.). The vegetation coverage of the riversides in the reaches at the sampling points encompassed tussock grass (TG1 and TG2) and quinoa forests (QF1 and QF2) (Fig. 1). Annual average rainfall fluctuates around 1,289 ± 142.3 mm and average daily temperature between 4.8 and 5.9ºC (Padrón, 2013).

Data collection

In each of the four reaches, five cross-sections for sampling were established in the most representative and abundant aquatic mesohabitats (pool, riffle and run). Biotic and abiotic samples were collected during eight19 field campaigns stretched between December 2011 and October 2013.

Sampling of biotic variables

Macroinvertebrate samples were collected using a Surber net (area of 625 cm^2, 250 μm net mesh, 30 seconds of sampling effort) at the centre of each cross-section. The substrate within the sampling area was removed to a depth of 6 cm and washed by hand so that all the organisms were dragged into the net. The material collected in the field was preserved in individual bottles containing water and a proportion of 4% formalin. In the laboratory, the samples were washed with tap water over a 250 μm mesh to remove excess formaldehyde, silt and sand. Individuals were identified, using specialized taxonomic keys and a stereo microscope (Olympus SZ-6145TR, Japan), to the most detailed taxonomic level (usually genus) except for those from the Xiphocentronidae family as the larvae are indistinguishable at the genus level (Domínguez *et al.*, 2009). In addition, the ash free dry mass (AFDM) content in the riverbed sediment was determined to estimate the organic matter content in accordance with the protocol established by Steinman *et al.* (2007).

Sampling of abiotic variables

A total of 37 environmental variables were measured in this study (Table 1) including the type of vegetative cover along the riparian corridor. The riverside of the QF1 reach is characterized by quinoa forest and shrubs with a coverage area of 300 m in length with lateral strips of 30 m on average. Similarly, the QF2 section also features quinoa forest and shrubs over a length of 250 m along the riverside with lateral strips of 27 m on average on both sides of the tributary. In the TG1 and TG2 reaches, the vegetation type along the channels was tussock grass with some small shrubs. The order of the river was identified using Strahler's (1975) classification and ArcGis 10.1 software (version 10.1; ESRI Inc., Redlands, CA, USA).

The hydromorphological characteristics of the river cross-section in each of the four river reaches were monitored at the sites where the biological samples were collected. The depth (m) and width of the water surface (m) were expressed in cm, and the average velocity (m s^{-1}) measured at 60% of the water depth (Wyżga *et al.*, 2012) using a propeller flow meter (Hydromate CMC3, Sydney, Australia). The composition of the substrate in each habitat was visually estimated (over the coverage area of the Surber net, i.e., 25 cm x 25 cm) based on the proportion of each type of substrate applying the simplified classification method of Elosegi (2009): silt (<0.006 mm), sand (between 0.006 and 0.2 mm), gravel (between 0.2 and 20 mm), pebble (between 20 and 60 mm), cobble (between 60 and 250 mm) and boulder (>250 mm).

The physicochemical variables in each river were measured using a portable multi-sensor (Horiba U-52, USA, 2010) and included water temperature (°C), pH, oxidation-reduction potential (ORP; mV), electrical conductivity (μS cm^{-1}), turbidity (NTU), dissolved oxygen (mg l^{-1}), and total dissolved solids (TDS; g l^{-1}) (Table 1). Additionally, water samples were collected in amber glass (100 cm^3) and plastic (500 cm^3) containers for laboratory analysis with the water quality sets using a colorimeter (HACH, DR / 890, USA, 2011). The following parameters were measured: nitrites (mg l^{-1}), total organic carbon (TOC, mg l^{-1}), ammonium (mg l^{-1}), total phosphorus (mg l^{-1}), total chlorine (mg l^{-1}), total hardness (mg $CaCO_3$ l^{-1}), alkalinity (mg $CaCO_3$ l^{-1}) and iron (mg l^{-1}). The extensive array of physicochemical water quality variables measured in the study area, is quite an exception with respect to the water quality variables that are usually collected in classical multi-metric studies in the Andes rivers above 3,000 m a.s.l. (Acosta *et al.*, 2009; Villamarín *et al.*, 2013).

Data pre-processing

To determine the effects of various environmental factors on EPT taxa, it is essential to eliminate the noise (and interactions) produced by other factors not under study (hydrological variations; Poff, 1997; Rolls *et al.*, 2012). For the prevention of the effect of noise caused by hydrological events that influence the presence/absence of certain flood-sensitive taxa, biological samples were only collected in periods with stable flow rate for a period at least of 30 days prior to sampling. The environmental conditions during the months of September, October, and November 2012 and April, July, August, September, and October 2013 had the highest stability in this study (Fig. 2) with an average monthly rainfall of 67.1±28.9 mm. In total, 8 sampling campaigns were carried out in the mentioned periods. The condition of stable flow rates was identified using gauging stations, equipped with DI1501 Mini-Diver and Baro-Diver DI500 measuring sensors (Schlumberger Water Services), next to each sampling site. The measurement interval was 5 min, and the daily average of the collected data were analysed. For much of the year, the Andes mountain area above 3,000 m is characterized by constant low-flow levels interrupted by high hydrological pulses of varying magnitude in response to heterogeneous rainfall events (Mosquera *et al.*, 2015). We believed that the absence of hydrological disturbances (pulses) over a range of four weeks is needed to allow the communities to recover (Flecker and Feifarek, 1994; Suren and Jowett, 2006). Unstable flows over longer periods (> four weeks) can cause significant decreases in the number of individual macroinvertebrates due to the drag force of the flow (Flecker and Feifarek, 1994; Ríos-Touma *et al.*, 2011).

With respect to the habitat information, three mesohabitat types were classified using the Froude number (Jowett, 1993); respectively $Fr < 0.18$ for pools, $0.18 < Fr < 0.41$ for runs and $Fr > 0.41$ for riffle. The number of mesohabitats was used to calculate the proportion of mesohabitats at a river reach.

To guarantee the robustness of the analysis, some samples were discarded based on the following criteria: a) those with richness in only a single taxon, b) those with an abundance of less than four individuals, and c) those whose abundance was outside the 95% confidence interval. In addition, data corresponding to rare taxa (<1% of the total abundance of EPT) were eliminated as determined by a Grubb test ($p < 0.05$) performed with Statgraphics Centurion XVI software (version 16.1.17; StatPoint Technologies Inc., Warrenton, Virginia) via the revision of the mean and the standard deviation.

Finally, direct measurements were used to calculate the hydraulic variables whose importance to aquatic communities was demonstrated in previous studies: Froude number (Fr) (Jowett, 1993), Reynolds number (Re) (Rempel *et al.*, 2000), velocity times water depth ($v \cdot d$), width/depth ratio (WDR) (Weigel *et al.*, 2003), relative roughness (k_v) (Lamouroux *et al.*, 2004; Statzner *et al.*, 1988), and shear stress (SS) (Almeida *et al.*, 2013; Cauvy-Fraunié *et al.*, 2014a). The Shannon-Wiener substrate diversity index (SuD) was calculated according Demars *et al.* (2012) with the proportions found in the field, which were subsequently transformed (arcsine). The transformation log(x+1) was used for the hydraulic and physicochemical variables, except for ordinal values and pH.

Data analyses

The 37 environmental variables were grouped into three environmental factors: riparian corridor, hydromorphology and water quality (Table 1). Taxa densities were log(x+1) transformed and standardized by dividing them by their average. For each environmental factor prior to analysis, we independently discarded variables that correlated to each other to avoid redundancy (Spearman's $r > 0.8$); this was also applied for the community metrics. Time was considered a control variable for the temporal variation in the results (ordinal number of the sampling campaign), as specifically indicated for each statistical test.

In a first step, we assessed which of the environmental variables are the main drivers of the EPT community structure, using the partial Canonical Correspondence Analysis (pCCA, Borcard *et al.*, 1992), available in the CANOCO software (version 5.02; Biometric, Plant Research International, The Netherlands, and P. Smilauer, Czech Republic) (Ter Braak, 1986). This type of analysis quantifies the relative contribution of each environmental factor group to the total variation in the structure of the macroinvertebrate community (Šmilauer and Lepš, 2014). For the pCCA, the variables whose influence was of direct interest were established as covariates (concomitant variables). For example, if the variables grouped under the riparian corridor factor were of interest, they were set as covariates of the hydromorphological factors and water quality variables; this was also performed for each group of variables under the remaining two factors. Furthermore, to prevent an artificial increase in the explained variation, significant environmental variables ($p < 0.05$) were selected with an automatic forward selection and a Bonferroni correction to avoid false positives in each group of variables. Finally, based on the global variation obtained from the pCCA tests (sum of all canonical eigenvalues), a partition variation analysis of each group or factor was conducted. This procedure allowed distinctions to be made between singular effects, i.e., the variance explained by a single set of variables, and joint effects, i.e., the variance jointly explained by three factors (Borcard *et al.*, 1992).

In the second phase of the analysis, several EPT community metrics were calculated for each cross-section and sampling date; richness (S), total density of individuals (ind. m^{-2}), Pielou's evenness (J), Shannon-Wiener diversity index (H'), and density of individuals in each of the Ephemeroptera, Plecoptera and Trichoptera orders. To ensure the temporal independence of the data, each community metric was analysed using the autocorrelation function (ACF) in Statistica software (version 8.0, StatSoft Inc., USA). The responses of the metrics to the environmental variables were explored by canonical correlation analysis (CCorA) using all the samples. The sampling sequence in time was considered as a variable (ordinal number of the sampling campaign; Table 1). This type of analysis allows two sets of composite variables to be analysed (canonical random variable) and maximizes the correlations between all possible pairs of canonical random variables (Quinn and Keough, 2002). The analysis included the environmental variables that maximize the explained variance to the greatest extent in the Principal Component Analysis (PCA) (Chester *et al.*, 1983), for a proper performance in the CCorA analysis. All analyses were made with PRIMER statistical software (version 6; PRIMER-E, Ivybridge, UK) and XLSTAT (version 03313; Addinsoft, NY, USA).

RESULTS

The abiotic characteristics of the 133 analysed samples taken in the subsequent river cross sections are summarized in Table 1. The average flow rate is of the same order in the TG1 and QF1 river reaches, with

QF1 possessing on average a 12.7% larger discharge. The average flow rate in the TG2 and QF2 study sections is 4 to 5 times smaller. There is a certain similarity in the flow of pairs of sections, for example, the sections with greater size and flow, TG1 (order 3) and QF1 (order 4), and the other pair, TG2 and QF2. However, the highest values of flow per unit area were recorded in the QF1 section, which also exhibited the maximum average water velocity value. The depth was very shallow in all sections, 18 cm for both TG1 and QF1 and ~10 cm for TG2 and QF2, and the average width of the water surface of the cross-sections of the stretches varied between 0.60 m and 1.2 m. The amount of AFDM in the river substrate was small and varied between 22 and 42 g m^{-2}. Regarding the composition of the substrate, similar proportions of thick (~50%) and small (~50%) substrates were found in the quinoa forest areas (QF1 and QF2), while the proportions differed in the sections with a bank of tussock grass. In terms of the hydraulic variables, the highest Fr and SS values were obtained in the TG2 stretch. Regarding water quality, the four micro-watersheds had similar average values of water temperature (8.9°C), pH (6.0), turbidity (2.5 NTU), dissolved oxygen (9.0 mg l^{-1}), total dissolved solids (0.035 g l^{-1}), total organic carbon (TOC, 3.5 mg l^{-1}), ammonium (0.019 mg l^{-1}), total chlorine (0.022 mg l^{-1}), hardness (15.9 mg $CaCO_3$ l^{-1}) and iron (0.274 mg l^{-1}). Finally, with respect to temporal variation, all statistical tests indicated that time was not a factor in the results; the results of the autocorrelation function of the community metrics indicated temporal independence.

A total of 3,820 individual EPT aquatic macroinvertebrates belonging to 14 genera and 12 families were collected, and the average density was 483 individuals per square meter (ind. m^{-2}). The dominance genera were *Metrichia* and *Contulma* in the mesohabitat pool, followed by *Helicopsyche* and *Ecuaphlebia* in the mesohabitat run (Table 2). In contrast, a poor number of individuals were obtained in *Andesiops* and *Mortionella*, generally located in areas with arboreal vegetation (QF, Table 2). In the first phase of the analysis, the riparian corridor factor explained 53% of the variance on the first axis and 36% on the second (Fig. 3a, b), which agreed with the results of the pCCA test. Due to the low number of significant variables (Strahler and TG), additional variables (Time and AFDM) were manually included in the pCCA to improve the interpretability of the results. The variable of river order (Strahler) was chosen by forward selection, although its explanatory power was low (6%, F: 5.5, p: 0.01), and a certain relationship was found between both the Xiphocentronidae family and the *Contulma* genus and the higher-order study sites, while the *Helicopsyche* genus (*Trichoptera*) was associated with lower-order sites. The tussock grass (TG) vegetation explained 6.8% of the variation (F: 7.5, *p*: 0.01) and was associated with the *Ochrotrichia* genus, whereas the abundances of the *Nectopsyche* and *Phylloicus* genera were negatively related with this type of vegetation. These genera were usually found in the presence of QF vegetation, a variable that was previously discarded due to its collinearity (Fig. 3a).

The hydromorphological variables explained 55% of the variance on the first axis and 15% on the second axis (Fig. 3c, d). Among the relevant variables, the Fr was found to contribute little to the variation in the community (4.8%, F: 5.7, *p*: 0.03), but it was related to several taxa. The *Phylloicus*, *Ochrotrichia* and *Nectopsyche* genera were associated with the pool mesohabitat (Fr <0.18), and the Xiphocentronidae family was associated with the riffle mesohabitat (Fr >0.41). The gravel variables (Gra) and the width/depth ratio (WDR) contributed 2.3% (F: 2.7, *p*: 0.03) and 2.2% (F: 2.7, *p*: 0.05) of the variation in the community, respectively, and the only genus that was positively associated with these two variables was *Helicopsyche*. The pCCA included additional variables (Depth, K_v and Silt) to improve visualization.

For water quality, despite finding an explanation of 54% of the variance on the first axis and 26% on the second axis, the only important variable in the pCCA was the total phosphorus (TotalP). We observed a clear negative relationship between the *Phylloicus* and Xiphocentronidae taxa and this variable. Additional variables (ORP Turb and TOC) were included to improve the quality and the interpretability of the figure (Fig. 3e, f). The partition analysis of the variation in the aquatic macroinvertebrate communities (Fig. 4) yielded low explanatory values for each environmental factor, which were expressed as the sum of the canonical eigenvalues: 18.9% for the riparian corridor, 17.0% for hydromorphology and 10.5% for water quality. The percentage of the variation that was not explained was 20.1%.

Finally, in the second phase of the analysis, a positive canonical correlation was observed between the average velocity of the current and the density of Plecoptera individuals, but velocity was negatively related to Pielou's evenness (Fig. 5). A positive relationship was also found between the density of individuals and the cobble substrate. The Shannon-Wiener diversity and richness metrics were negatively related to water velocity and temperature, and the density of Ephemeroptera individuals was similarly negative related to water temperature. At the temporal scale (Annex 1a, b) no significant influence of the environmental variables on the community metrics was found, although the results generally followed the same response pattern as those obtained at the global scale.

DISCUSSION

Going beyond previous studies, in high-Andean rivers, this piece of research analysed the ecology of aquatic macroinvertebrates and their relationship with the riparian corridor, hydromorphology and water quality. Based on the first exploratory analysis, the matrix of the data had a high variability of macroinvertebrate abundance corresponding to high and low flow periods. This study covers the analyses of stable conditions with moderate hydrological variability (average flow, SD: 8.5±7.3 mm, average peak flow: 36.5 mm), characterized by smaller and regular precipitations; the intense rain periods were discarded. Hence, the community is representative of all the taxa found in these rivers (Vimos-Lojano, unpublished). For this reason, the sample design represents the entire community without the ecological filtering on some taxa exerted by high flows. In addition, the pre-selection of non-autocorrelated data and the statistical analysis enabled the detection of the influence of abiotic factors on the composition and structure of communities of the orders Ephemeroptera, Plecoptera and Trichoptera. The analysis confirmed that the data were not autocorrelated and that time was not a relevant factor in the community, which supports the robustness of the results. The lack of a temporal effect is related to disturbance by floods, which reconfigures the communities (Flecker and Feifarek, 1994; Suren and Jowett, 2006).

Relevance of the riparian corridor

Headwater ecosystems usually feature riparian vegetation that shades much of the channel, which is characterized by low allochthones primary productivity (Vannote *et al.*, 1980). The leaf holding capacity of the riverbed depends on the hydraulic and geomorphological characteristics of the river and, to a lesser extent to the intrinsic characteristics of the leaves, such as their size, texture and shape (Canhoto and Graça, 1998). All allochthones material in the river is important for several aquatic organisms (Bastian *et al.*, 2008), explaining the positive response of the density of shredders to the arboreal riparian vegetation; an important source of energy for these trophic groups (Li and Dudgeon, 2008).

In this study, *Ochrotrichia* was the only taxon linked to TG vegetation. This may be related to the low percentage of shading of the channel by these herbaceous plants, causing the TG sections to have high primary productivity. The effect is detected mainly when the vegetation has been removed from the riparian corridors by human activity as it happens in the areas of median altitude (Scarsbrook and Halliday, 1999, Miserendino *et al.*, 2010). Specifically, this level of productivity positively affects the periphyton, the main food source of *Ochrotrichia* (Tomanová *et al.*, 2006). In the stretches of the riparian corridor with quinoa forest, which is the only native tree found at these altitudes (above 3,000 m a.s.l.) (Cázares-Martínez *et al.*, 2010), the results indicated that the allochthones contributions that reaches the river are relevant. It should be emphasized that the arboreal vegetation cover in the high-Andes region differs from the mountains at other latitudes, which are dominated by pine and spruce forests (Scarsbrook and Halliday, 1999). Therefore, the QF vegetation was related to the presence of the genera *Nectopsyche* and *Phylloicus*, organisms that are almost exclusively characteristic of sites with forest vegetation cover (QF) because they use the accumulated material from the riverbed (Bispo *et al.*, 2006). Although the TG2 site contained the double of the AFDM of the other study locations, this variable did not prove to be relevant, suggesting that the provision of a canopy over the river by the vegetation favoured the presence of groups associated with this resource, as opposed to the amount of organic matter (Encalada *et al.*, 2010; Graça, 2001; Albariño and Balseiro, 2002).

Additionally, taxonomic changes were observed in response to the size of the river; a variation in community expected under the river continuum concept (Vannote *et al.*, 1980). This result contrasted with the study by Haggerty *et al*. (2002) in Appalachian and Cascade mountain headwaters, where abundance and richness of the community did not differ with river order, likely due to the low number of individuals in that area (<134 ind. m^{-2}) in comparison with our research. Additionally, in our study the small spatial scale allowed us to observe taxa substitution as the order of the river increased. Specifically, the *Helicopsyche* genus occurred at lower order sites, its convex hemispherical shape results in a low resistance to high water velocity (Vaughn, 1985). This species was replaced by *Contulma* in larger channels, the latter being a common taxon in high velocity headwater streams (Holzenthal and Ríos-Touma, 2012). Therefore, we hypothesize that the relatively large size of *Contulma* in relation to the other taxa directly influenced their resilience and presence in larger as well as higher-order rivers. It is doubtless that the complexity of these environments, i.e., the diverse trophic resources and physical conditions of the habitats of headwater rivers, explains this result (Allan, 2004).

Relevance of Hydromorphology

Another important environmental factor in the spatial distribution of aquatic communities is the hydromorphological conditions of the river, which vary according the flow rate (hydrology) and the geomorphology of the channel (Wilcox *et al.*, 2008). During baseflow periods, the flow of high-Andean rivers varies moderately, which contributes to the maintenance of habitat quality through the constant washing of accumulated silt and periphyton resulting in a dominant coarse sediment as occurs at other latitudes (e.g., Biggs *et al*., 2008). In this research, we found the Fr and the gravel substrate are the variables that determine the composition of the aquatic EPT communities.

The Fr is a standardized numerical index (Shoffner and Royall, 2008). Individuals with hydrodynamic (flattened) bodies or structures attach themselves to the riverbed (anal or tarsal claws and suckers) persist in places with high Fr value (Statzner and Beche, 2010; Tomanová and Usseglio-Polatera, 2007). For example, the *Nectopsyche* and *Phylloicus* genera were most represented in slow flow habitats (pools), which have a low Fr, as these organisms use tubular structures composed of stones and leaves and are not well resistant to current (Tomanova *et al.*, 2008). These organisms create these structures with the material accumulated on the riverbed in these habitats (Houghton *et al.*, 2011). In contrast, the Xiphocentronidae family occurred at greater density in habitats with a high Fr, and the most likely reason is that the structures they employ to adhere to the riverbed (cases, anal and tarsal claws) provide greater flow resistance (Thirion, 2016) compared to other taxa.

One of the negative associations was found between the *Metrichia* genus and both the gravel substrate and high width/depth ratio. In addition, a positive relationship was found between this taxon and large substrates (Spearman's *r*: 0.247, $p < 0.01$), i.e., those that provide habitat stability (Erman and Erman, 1984). The opposite result was found for the *Helicopsyche* genus, because this taxon is associated with the presence of gravel substrate in high-mountain lentic areas. It should be noted that individuals of this genus could be the *Helicopsyche cotopaxi* (subgenera *Feropsyche*), the only species recorded over 3,000 m a.s.l. in the north of Ecuador (Rios-Touma et al., 2017, Johanson, 2002), without presenting information on its biology or ecology. In previous studies, the presence of *Helicopsyche cotopaxi* was associated with the presence of fine substrate, which provides the base material for the construction of the helical structures they manufacture (Vaughn, 1985). However, it is possible that the steep slope and irregular flow of the high-Andean rivers influence the low proportions of fine material (sand) by constantly washing it from lentic areas. Therefore, in some degree our results for *Helicopsyche* refute the suggestion by Schwendel *et al.* (2011), who considered this genus to be a good indicator of stable substrates in New Zealand streams.

Our results confirm that the Fr is a valid and good predictor of aquatic assemblages (Gibbins *et al.*, 2016), since the response of the macroinvertebrate community can be considered like that in temperate rivers (Wyżga *et al.*, 2012; Lamouroux *et al.*, 2004; Almeida *et al.*, 2013). The positive effect of the gravel substrate on the composition of the aquatic community is analogous to other rivers, despite the instability of this type of substrate against hydrological disturbances (Rice *et al.*, 2007).

Relevance of water quality

One of the factors determining the physicochemical quality of the water and the aquatic communities of a river is the dominant type of land use or land cover of the riparian corridor (Burt *et al.*, 2010; Miserendino *et al.*, 2011). Our study is one of the few studies in high-Andean Rivers above 3,000 m a.s.l. linking water quality variables with aquatic macroinvertebrate communities, apart from a summarized multi-metric analysis (Acosta *et al.*, 2009; Villamarín *et al.*, 2013). In this research, the only decisive physicochemical variable explaining the spatial distribution of some taxa (Xiphocentronidae and *Phylloicus*) was the total phosphorus concentration, which ranged between 0 and 0.880 mg l^{-1}. The total phosphorus seems to be a good indicator of possible anthropogenic influences that can alter biodiversity (Niyogi *et al.*, 2003; Villamarín *et al.*, 2013), although in some cases this variable did not differ significantly among land uses (e.g., pasture, pine and native forest, urban, etc.; Miserendino and Masi, 2010). Among the few studies at these altitudes, Villamarín *et al.* (2013) related phosphorus levels with contaminated areas but observed no taxonomic response. In our study are the natural concentrations of phosphorus determined by the high organic matter content and, in turn, the nutrients in these soils (Andisols) (Quichimbo *et al.*, 2012). Therefore, this study provides new information on the influence of water quality variables in quasi-pristine rivers above 3,000 m a.s.l.

Analysing the three environmental factors together explained a high percentage (~80%) of the spatial variation in the aquatic communities. However, other variables (hydrological) that were not considered in this study can have an important influence on the temporality of some organisms (Flecker and Feifarek, 1994; Gibbins *et al.*, 2001; Hannah, *et al.*, 2007). The influence of those variables on high-Andean communities deserves to be studied.

Finally, as for the physical and hydrological characteristics of these environments, these low-order or headwater rivers are characterized by very high spatial complexity (Allan, 2004; Meyer *et al.*, 2007), which includes variations in the depth, the width of the water surface, the Fr, SS and AFDM between sections and sites. At the same time, the channels have homogeneous substrates and variable flow, which in turn are linked with high abundance of aquatic macroinvertebrate communities in the Andean regions (Principe *et al.*, 2007); however, the diversity of these communities is limited at higher altitudes (Jacobsen and Marín, 2008). Under the conditions of spatial and temporal variability in these rivers (Mosquera *et al.*, 2015), the aquatic communities mostly seek stable environments that are characterized by a thick, heterogeneous substrate (Beisel *et al.*, 2000; Duan *et al.*, 2008). Given its stability, the cobble substrate (60-250 mm) was positively related with the density of individuals in the EPT orders, a result that has been found in studies conducted in Asian mountain rivers where the Ephemeroptera order is dominant (Duan *et al.*, 2008). On the other hand, the diversity and richness of EPT taxa diminished by increasing flow velocity, and this decrease was characterized by a decline in the most representative groups, i.e., Trichoptera order, whose biological features confer low resistance to high-velocity flows (Tomanova *et al.*, 2008). Additionally, a low number of individuals and taxa from other groups (Ephemeroptera and Plecoptera) were observed in general. By contrast, Plecoptera was the only order associated with high velocity conditions, and their presence was favoured by their large size and strength, which enabled them to adhere to the riverbed under more turbulent conditions (Peters, 1986; Tomanova and Tedesco, 2007).

In this context, the low number of Ephemeroptera taxa in high-Andean rivers is due to altitude (Jacobsen, 2003; Jacobsen and Marín, 2008; Jacobsen *et al.*, 1997). The preference of some species of mayflies for low temperature conditions has been shown in studies in the Patagonian Andes (Miserendino and Pizzolán, 2001). To explore this potential relationship, the density of individuals of the order Ephemeroptera, represented by the *Ecuaphlebia* genus (98% relative abundance), was graphed against temperature. This graph, concerning *Meridialaris chiloeensis* and *Metamonius* sp., indicated maximum abundances in a temperature range between 7.5 and 9.5°C, but no clear correlation was observed (Spearman r: -0.143, p >0.05; Miserendino and Pizzolán, 2001). Notwithstanding, the analysis suggested that temperatures higher than 9.5°C may cause a drastic decrease in the densities of these groups (Vimos-Lojano, unpublished), but for this to be confirmed, a detailed database of temperature and flow would be necessary to understand the phenological, behavioural and environmental effects, among others (Dallas and Ross-Gillespie, 2015). Although Jacobsen (2008) found a negative influence of temperature on diversity, in a short-term monitoring study (two days with time interval of 15 min) in an Altiplano river, that study did not show which taxa were affected or which is the influence during the base-flow periods.

CONCLUSIONS

Our results indicate that the habitats of the studied high-Andean aquatic headwater ecosystems show a very high natural heterogeneity, which plays an important role in shelter availability and the maintenance of macroinvertebrates biodiversity. By analysing various factors operating at different scales (riparian corridor, hydromorphology, water quality), this study demonstrated that macroinvertebrates belonging to the EPT orders showed strong associations with the type of natural vegetation surrounding the aquatic ecosystem. Furthermore, a few variables (average velocity, cobble substrate and water temperature) played a critical role in the patterns of aquatic EPT communities, specifically concerning the density, richness, diversity, evenness, and density of Ephemeroptera. The lentic zones were characterized by substrates of sand, gravel and boulders of varying size, which make the habitats more heterogeneous than the lentic areas of lower altitude. Consequently, such heterogeneity facilitates the presence and availability of shelters, which favour reduced mortality from disturbances (Lancaster and Belyea, 1997) related with the highly fluctuating flow regimes in this region. In terms of community structure, the orders that prevailed were those that have been directly correlated with the availability of a given trophic resource, or groups with biological traits that are adapted to certain physical habitat conditions. As expected, the still largely unknown and complex interactions (intra- and interspecific competition, the effect of introduced species, the variety of habitat preferences of different larval stages, hydrological variability, etc.) of Andean aquatic ecosystems hinders the understanding of their ecological processes, which should be further analysed in order to be able to assess the relative tolerance of aquatic organisms to hydrological disturbances and physical variables.

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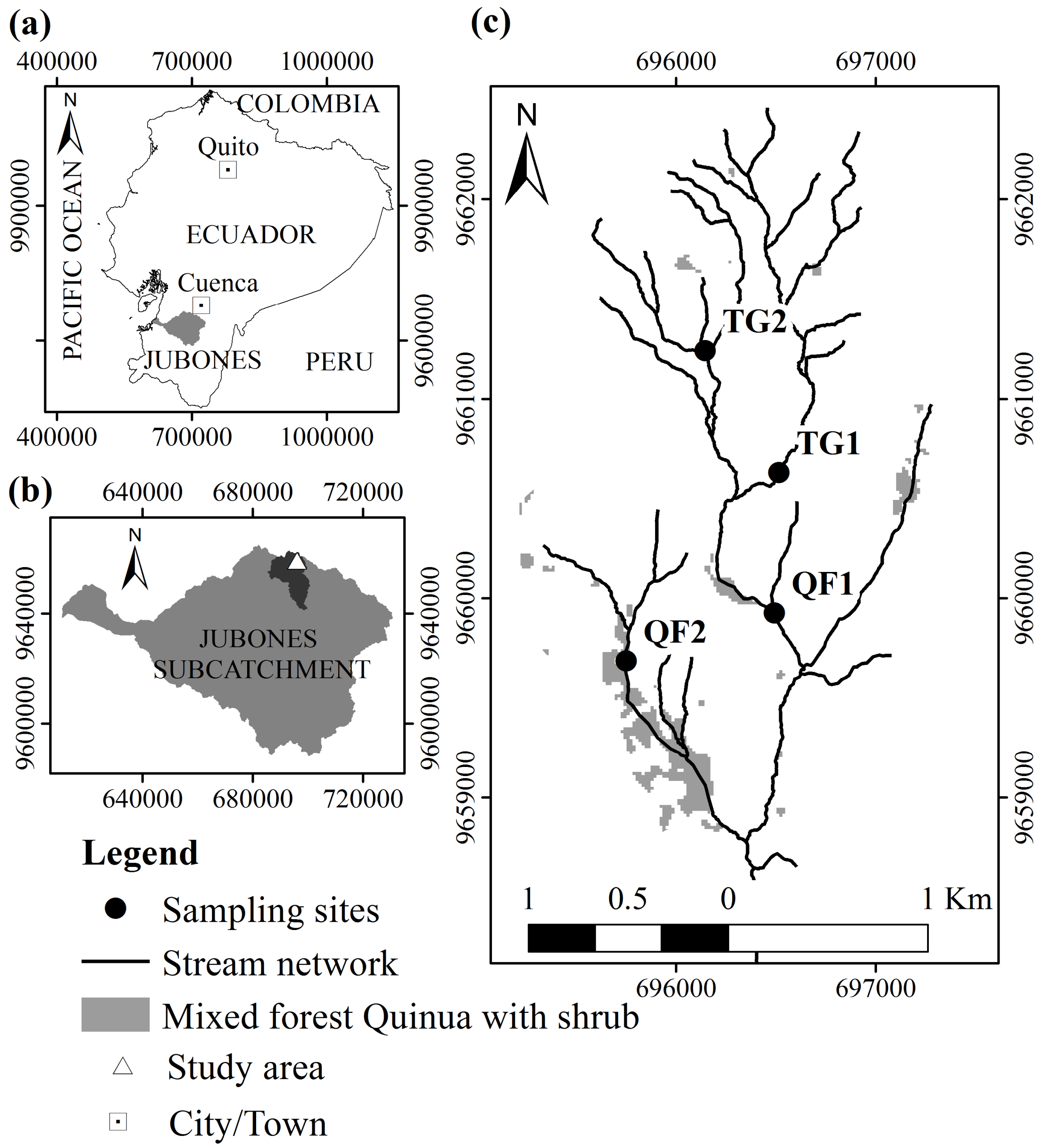

Figure 1. Location of the (a) Jubones River basin in Ecuador, (b) the micro-watershed of the Zhurucay River in the Jubones basin and (c) the sampling reaches in the upper micro-watershed of the Zhurucay River. The vegetation is indicated as tussock grass (TG) and quinoa forest (QF). Major cities are marked with rectangles; the study area is shown in the triangle.

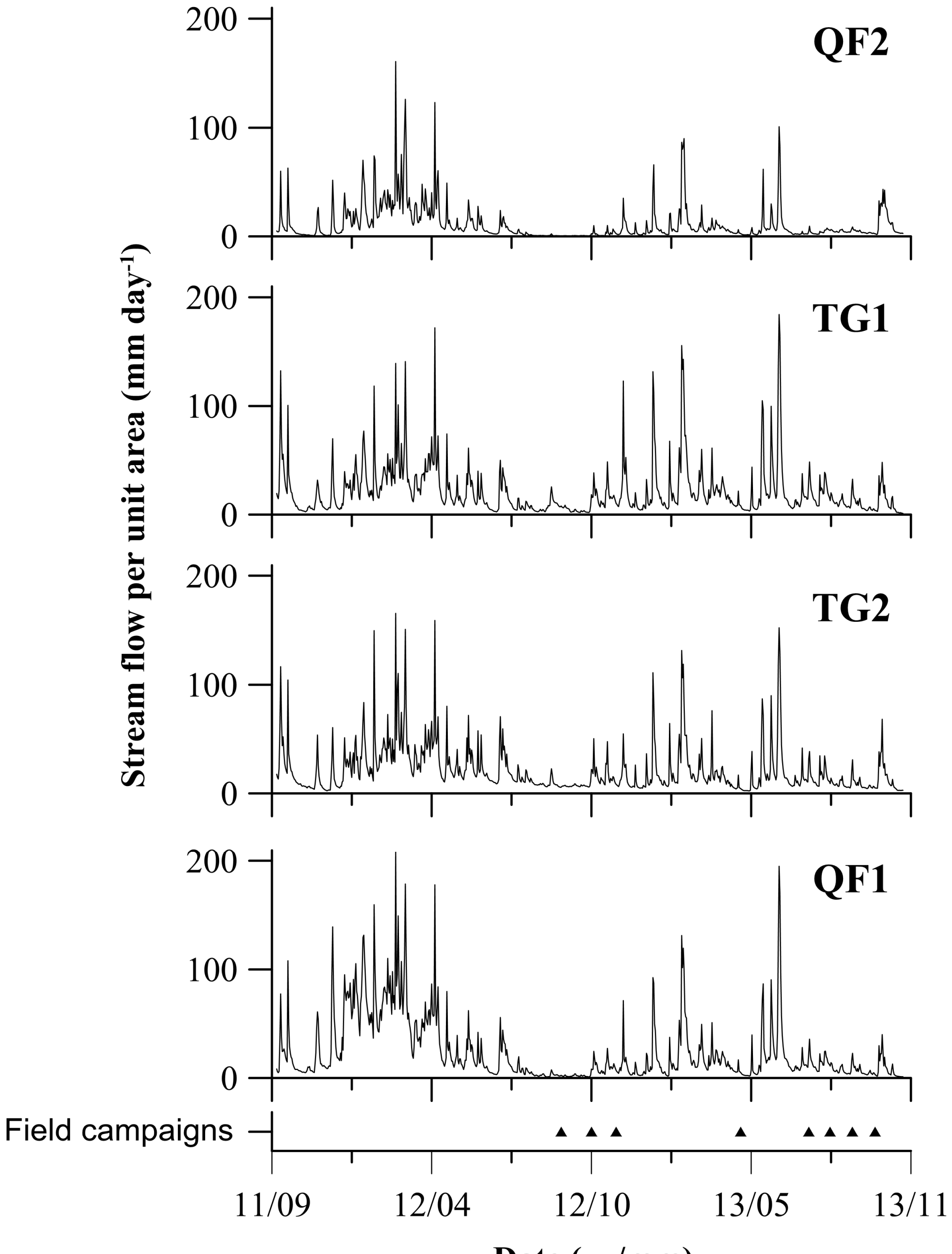

Figure 2. Hydrographs per area unit of the four study sites in the micro-watershed of the Zhurucay River. Tussock grass (TG) and quinoa forest (QF). Triangles indicate the sampling campaigns chosen for analysis.

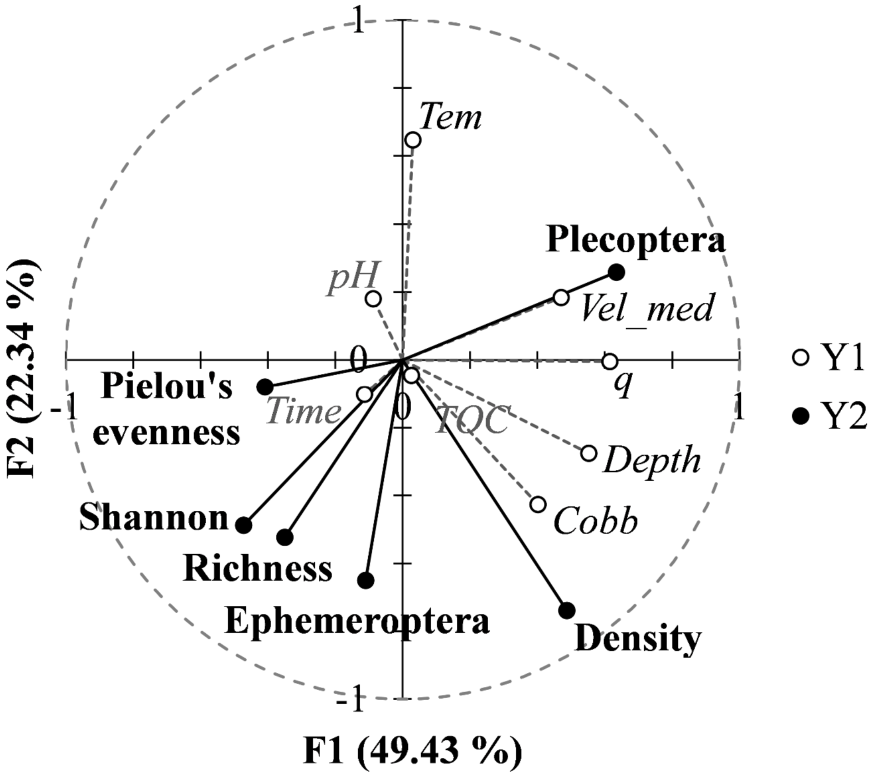

Figure 3. Partial canonical analysis (pCCA) of the eight most represented taxa, (a) (c) (e), using the (a) (b) riparian corridor (group 1), (c) (d) hydromorphology (group 2) and water quality (group 3) as explanatory variables. The significant variables are indicated in bold; other variables were automatically plotted to improve the visualization of the results. The spatial distribution of the sampling locations (b) (d) (f). TG: tussock grass, QF: quinoa forest, Time: sampling campaign, and AFDM: ash free dry mass of the fine riverbed sediments. Froude (Fr), relative roughness (K_V), width/depth ratio (WDR), gravel (Gra), turbidity (Turb), total organic carbon (TOC), and total phosphorus (TotalP).

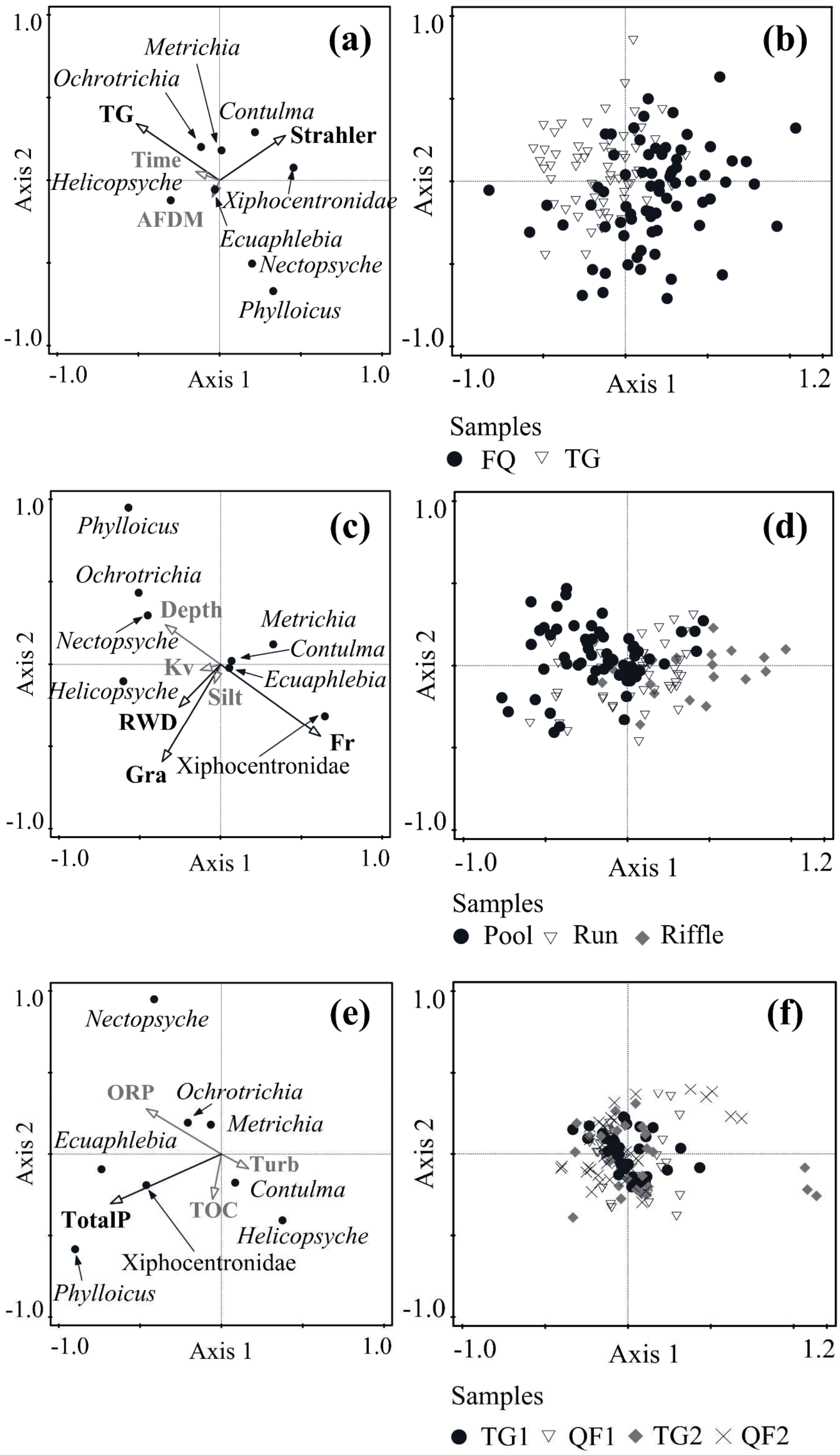

Figure 4. Partitioned variation in the aquatic macroinvertebrate community in the upper micro-watershed of the Zhurucay River.

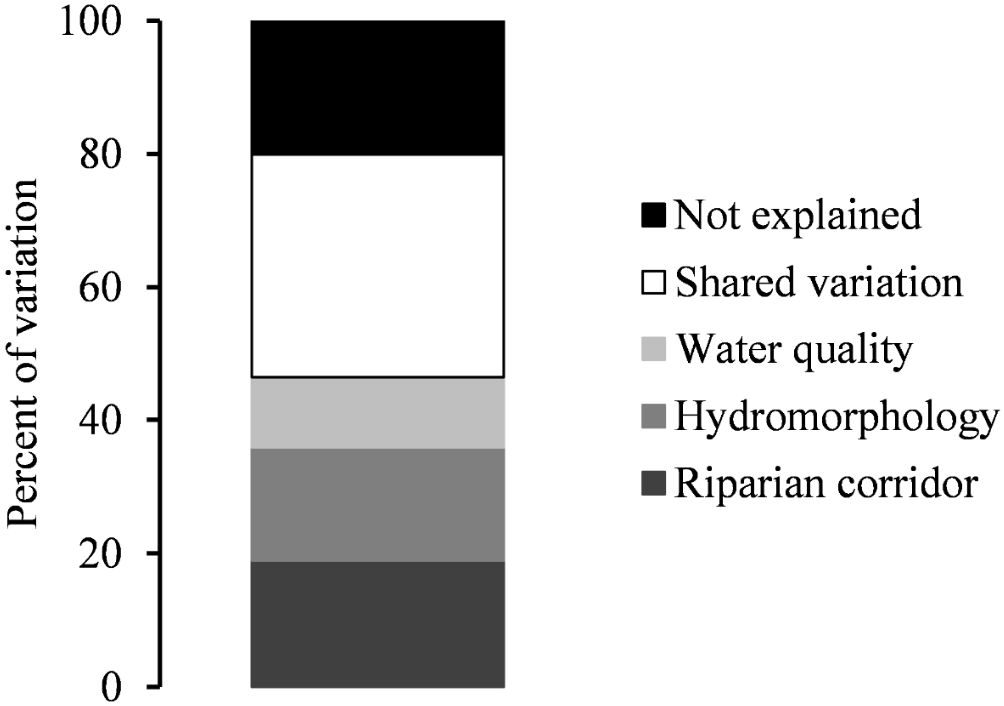

Figure 5. The results of the canonical correlation analysis of community metrics and environmental variables of the upper micro-watershed of the Zhurucay River. Alkal: alkalinity, Vel_med: mean velocity, TOC: total organic carbon, Cobb: cobble, v·d: velocity times water depth, and Shannon: Shannon-Wiener diversity.

Table 1. Average values ($\bar{x}$) and standard deviations (± SD) of the abiotic variables of the four study sections (TG1, TG2, QF1, and QF2, where TG represents tussock grass and QF represents quinoa forest) in the upper micro-watershed of the Zhurucay River. The acronyms for the variables are indicated by brackets [ ]. [a] Variable not included in the analysis. [b] The six analysed substrate categories summarized into three groups. The environmental factors (Env-Factors) indicate the 3 groups of factors considered: (1) riparian corridor and large-scale variables, (2) hydromorphological variables, and (3) physicochemical variables.

Variable (Units)	**TG1**	**QF1**	**TG2**	**QF2**	**Env-Factors**
Number of samples [N]	36	34	25	38	
Flow rate ($l\ s^{-1}$)[a] [q]	32.8 (±41.1)	36.96 (±31.1)	8.36 (±9.7)	7.31 (±6.3)	-
Riparian vegetation	TG	QF	TG	QF	1
Watershed area (km^2)	1.40	3.28	0.38	1.65	1
Strahler order	3	4	2	2	1
Ordinal number of the sampling campaign (range) (equivalent to Time) [Time]	1 - 8	1 - 8	1 - 8	1 - 8	1
Water temperature [T] (^0C)	8.4 (± 1.5)	8.7 (± 1.5)	8.9 (± 1.4)	9.5 (± 1.5)	1
Ash Free Dry Mass [AFDM] ($g\ m^{-2}$)	25.6 (± 63.2)	28.8 (± 38.3)	42 (± 46.1)	22.4 (± 30.1)	1
Velocity times water depth [v·d] ($m^2\ s^{-1}$)	0.049 (± 0.05)	0.075 (± 0.09)	0.034 (± 0.05)	0.013 (± 0.01)	2
Mean velocity ($m\ s^{-1}$)	0.186 (± 0.19)	0.337 (± 0.27)	0.128 (± 0.31)	0.019 (± 0.05)	2
Water depth (m)	0.18 (±0.08)	0.18 (±0.07)	0.10 (±0.05)	0.11 (±0.06)	2
Water width (m)	1.11 (±0.28)	0.95 (±0.33)	0.58 (±0.29)	1.18 (±0.22)	2
Coarse substrate (%)[b]	71.8 (±37.3)	47.2 (±44.4)	19.0 (±32.2)	50.1 (±37.2)	-
Medium substrate (%)[b]	27.4 (±37.5)	52.1 (±45.0)	79.4 (±31.6)	49.3 (±37.0)	-
Fine substrate (%)[b]	0.83 (±2.54)	0.74 (±2.5)	1.6 (±4.7)	0.53 (±2.3)	-
Substrate Shannon - Wiener diversity [SuD]	0.37 (± 0.356)	0.385 (± 0.326)	0.555 (± 0.263)	0.481 (± 0.335)	2
Froude number [Fr]	0.20 (± 0.11)	0.27 (± 0.14)	0.38 (± 0.31)	0.15 (± 0.15)	2
Relative roughness [k_v] (cm)	4.58 (± 0.88)	4.67 (± 0.86)	4.89 (± 0.42)	4.69 (± 0.59)	2
Ratio Width / Depth [RWD]	7.8 (± 5.4)	5.8 (± 2.2)	6.9 (± 4.1)	12.8 (± 5.6)	2
Shear stress [SS] ($m\ s^{-1}$)	45.1 (± 66.2)	98.9 (± 138.6)	174.8 (± 293.2)	33.7 (± 66.9)	2
Reynolds number [Re]	24450.0 (± 24321.8)	38101.8 (± 41076.2)	24169.5 (± 28935.6)	9551.4 (± 9406.4)	2
pH	5.8 (± 0.8)	6.1 (± 0.9)	6.1 (± 0.7)	6 (± 0.5)	3

Oxidation-Reduction Potential [ORP] (mV)	305.2 (± 64.2)	264.9 (± 76.6)	243.3 (± 95.0)	302.3 (± 48.5)	3
Electrical conductivity (μS cm^{-1})	52.3 (± 31.9)	53.0 (± 30.6)	78.8 (± 53.4)	50.0 (± 26.9)	3
Turbidity (NTU)	4.5 (± 10.4)	1.0 (± 1.2)	2.7 (± 3.0)	1.8 (± 2.4)	3
Dissolved oxygen (mg l^{-1})	9.4 (± 1.4)	8.9 (± 1.6)	9.8 (± 1.5)	7.9 (± 0.6)	3
Total dissolved solids [TDS] (g l^{-1})	0.03 (± 0.02)	0.03 (± 0.02)	0.05 (± 0.03)	0.03 (± 0.02)	3
Nitrites (mg l^{-1})	0.003 (± 0.003)	0.001 (± 0.001)	0.005 (± 0.005)	0.003 (± 0.002)	3
Total Organic Carbon [TOC] (mg l^{-1})	4.1 (± 2.6)	3.5 (± 2.2)	3.2 (± 2.6)	3.1 (± 1.8)	3
Ammonium (mg l^{-1})	0.034 (± 0.068)	0.013 (± 0.014)	0.01 (± 0.008)	0.017 (± 0.018)	3
Total Phosphorus (mg l^{-1})	0.265 (± 0.258)	0.221 (± 0.145)	0.236 (± 0.267)	0.105 (± 0.085)	3
Total Chlorine (mg l^{-1})	0.025 (± 0.03)	0.016 (± 0.01)	0.028 (± 0.021)	0.018 (± 0.009)	3
Ca Hardness (mg $CaCO^3$ l^{-1})	15.5 (± 7.3)	17.3 (± 7.0)	18.7 (± 3.6)	12.1 (± 6.5)	3
Alkalinity (mg $CaCO^3$ l^{-1})	13.2 (± 7.1)	16.6 (± 9.5)	21.9 (± 7.5)	15.7 (± 11.4)	3
Iron [Fe] (mg l^{-1})	0.214 (± 0.093)	0.289 (± 0.091)	0.272 (± 0.08)	0.319 (± 0.112)	3

Table 2. Average values (x̄) and standard error (± SD) of the absolutes abundances taxa in four study sections (TG1, TG2, QF1, and QF2, where TG represents tussock grass and QF represents quinoa forest) in the upper micro-watershed of the Zhurucay River. N = sample number include in this study.

		Pool				Run				Riffle			
Taxa		TG1	TG2	QF1	QF2	TG1	TG2	QF1	QF2	TG1	TG2	QF1	QF2
	N	**17**	**8**	**10**	**22**	**16**	**10**	**17**	**10**	**1**	**11**	**8**	**3**
Andesiops	x̄	0	0	0	4.4	0	0	2.8	10.5	0	2.9	0	0
	± SE	0	0	0	2.2	0	0	2.8	6.2	0	2	0	0
Ecuaphlebia	x̄	140.2	46	27.2	85.1	158.3	301.2	32.9	193.7	16	107.8	9.1	128
	± SE	35.2	28.6	12.6	25	38.7	226.5	10.3	62.1	0	53.3	7.1	60.6
Claudioperla	x̄	14.1	0	3.2	0	7	0	27.3	3.2	16	1.5	11.1	5.3
	± SE	4.5	0	2.1	0	3.6	0	27.3	2.1	0	1.5	7.7	5.3
Anacroneuria	x̄	0	28	0	5.1	0	40	0	12.1	0	27	0	0
	± SE	0	14.1	0	1.9	0	15.1	0	6.9	0	9.9	0	0
Contulma	x̄	86.6	2	40.9	2.9	258	14.5	79.1	3.2	832	32.8	231	5.3
	± SE	21.3	2	9.7	1.3	71.9	11.1	22.7	3.2	0	15.5	131	5.3
Phylloicus	x̄	1.9	2	16	21.8	0	0	0.9	57.2	0	0	0	16
	± SE	1.3	2	14.3	6.3	0	0	0.9	38.5	0	0	0	16
Mortoniella	x̄	0	0	0	9.5	0	0	0.9	14.4	0	0	0	32
	± SE	0	0	0	4.8	0	0	0.9	5.6	0	0	0	16
Helicopsyche	x̄	235.9	128	8	92.4	227.6	222.4	4.7	205	32	68.5	2	101.3
	± SE	53.3	42.9	2.7	16.4	51.6	73.5	2.3	76.3	0	24.2	2	45.6
Atopsyche	x̄	0.9	0	3.2	3.6	1	22.6	18.8	10.5	16	1.5	22	16
	± SE	0.9	0	2.1	1.8	1	22.6	5.5	7	0	1.5	10	9.2
Smicridea	x̄	0	0	0	0	0	140.6	0	0	0	135.5	0	0
	± SE	0	0	0	0	0	135.3	0	0	0	54	0	0
Metrichia	x̄	137	12	25.6	13.8	453.6	55.5	62.6	11.2	2240	370.8	202.4	26.7
	± SE	29	5.9	9	3.4	145.6	28.4	18.5	5.4	0	244.5	90.3	5.3
Ochrotrichia	x̄	47.1	0	6.4	9.5	54.1	0	4.7	1.6	32	0	16.1	0
	± SE	10.8	0	2.6	5.1	13.6	0	2.3	1.6	0	0	14	0
Atanatolica	x̄	0	0	0	0	25	100.1	6.6	0	96	183.6	0	0
	± SE	0	0	0	0	19.8	89.8	6.6	0	0	102.1	0	0

Nectopsyche	x̄	0	0	33.6	90.9	1	1.6	24.1	89.2	0	1.5	13.1	80
	± SE	0	0	26.7	19.6	1	1.6	6	44	0	1.5	7.5	27.7
Xiphocentronidae	x̄	8.5	0	9.6	0	18	0	30.2	1.6	0	0	66.5	0
	± SE	3.4	0	4.3	0	4.6	0	9.6	1.6	0	0	32.4	0

Annexes

Annex 1a. Temporal variation results of the canonical correlation analysis of community metrics against environmental variables. Letters a), b), c) and d) indicate four different sampling campaigns. Vel_med: mean velocity, Boul: boulder, Cobb: cobble, Pebb: pebble, Gra: gravel, K_V: relative roughness, WDR: width/depth ratio, Alkal: alkalinity, TOC: total organic carbon, TDS: total dissolved solutes, and Shannon: Shannon-Wiener diversity.

Annex 1b. Temporal variation results of the canonical correlation analysis of community metrics against environmental variables. Letters f), g), h) and i) indicate four different sampling campaigns. Vel_med: mean velocity, Boul: boulder, Cobb: cobble, Pebb: pebble, Gra: gravel, K_v: relative roughness, WDR: width/depth ratio, $v \cdot d$: velocity times water depth, Alkal: alkalinity, TOC: total organic carbon, TDS: total dissolved solutes, O2: dissolved oxygen, and Shannon: Shannon-Wiener diversity.