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POTENTIAL USE OF MACROINVERTEBRATES AS INDICATORS OF WATER QUALITY IN LOW-IMPACTED HIGHLAND WATERSHEDS: CASE OF SAN FRANCISCO DRAINAGE BASIN, URUGUAY

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ABSTRACT

Stream macroinvertebrate communities are modulated by different ecological processes that occur from the watershed to reaches and local sites, which can be directly or indirectly affected by human activities and changes in land use. This study evaluated the effects of water quality on macroinvertebrate structure in a low-impacted highland watershed, the San Francisco stream drainage basin, Lavalleja, Uruguay. We measured *in situ* physico-chemical water variables and collected a total of 50 macroinvertebrate samples using a Surber sampler in 10 reaches in different stream's order and basin size. Macroinvertebrate community structure was analyzed using density, richness, and two common bioindicator indexes: percentage of number of Ephemeroptera, Plecoptera and Trichoptera (%EPT) and percentage of Oligochaeta (%Oligochaeta). Although we did not find a significant relationship between physico-chemical water variables and stream order, the 1storder streams had comparatively higher water nutrient concentration and higher conductivity. The %EPT and %Oligochaeta indexes could largely be explained (positive and negatively, respectively) by stream order, but in general not by water quality per se. However, in 1st stream order reaches a connection with local water quality arose. Moreover, no patterns were found for density and taxonomic richness with stream order or water quality. Altogether, our evidence suggests the potential for using macroinvertebrate indexes as water quality indicators, even in well preserved drainage basins.

Key words: Benthos, %EPT, %Oligochaeta

RESUMEN

Potencial uso de los macroinvertebrados como indicadores de la calidad del agua en cuencas altas de bajo impacto: caso de la Cuenca del Arroyo San Francisco, Uruguay. Las comunidades de macroinvertebrados de arroyos están moduladas por diferentes procesos ecológicos que ocurren, desde la cuenca hasta los tramos locales, que pueden verse afectados directa o indirectamente por las actividades humanas y los cambios en el uso del suelo. Este estudio evaluó el efecto de la calidad del agua en la estructura de macroinvertebrados en una cuencaalta de bajo impacto, el arroyo San Francisco, Lavalleja, Uruguay. Medimos *in situ* variables fisicoquímicas del agua y recolectamos un total de 50 muestras de macroinvertebrados utilizando un muestreador Surber en 10 tramos de arroyos con diferente orden y tamaño de cuenca. Las estructuras de macroinvertebrados se analizaron utilizando densidad, riqueza y dos índices bioindicadores comunes; porcentaje de número de Ephemeroptera, Plecoptera y Trichoptera (% EPT) y porcentaje de Oligochaeta (% Oligochaeta). Aunque no se encontró una relación significativa entre las variables fisicoquímicas del agua y el orden de los arroyos, los arroyos de 1er orden tenían una concentración de nutrientes y conductividad de agua comparativamente más alta. Los índices %EPT y %Oligochaeta podrían explicarse en gran medida (positiva y negativamente, respectivamente) por el orden de los arroyos, pero en general no por la calidad del agua. Sin embargo, en los arroyos de 1er orden surgió una conexión con la calidad del agua local. Además, no se encontraron patrones de densidad y riqueza taxonómica con el orden de los arroyos o la

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calidad del agua. En conjunto, nuestra evidencia sugiere un promisorio potencial del uso de macroinvertebrados como indicadores de calidad ambiental, incluso en cuencas de drenaje bien conservadas.

Palabras claves: Bentos, %EPT, %Oligochaeta

INTRODUCTION

The structure and function of stream biota often reflects a broad set of geological, topographical, climatic, and biotic factors and processes; which can be in turn modulated by anthropogenic activities (Hynes, 1975; Allan y Castillo, 2007; Allan, Castillo y Capps, 2020). Streams connected in drainage basin are complex systems with great environmental heterogeneity at different spatial and temporal scales (Winemiller, Flecker y Hoeinghaus, 2010).

One of the most intensively studied riverine communities is the macroinvertebrate community, due to their important role in the transfer of energy from basal resources to apex consumers in food webs and due to the linkage within stream habitats and even between the aquatic and terrestrial ecosystems. Macroinvertebrate community structure is thus largely shaped by different processes occurring at different spatial scales, from the watershed to stream reaches and to the local scale (Stenedra et al., 2012; Collins, Matter, Buffam y Flotemersch, 2018). A potentially important driving factor is the longitudinal environmental gradient that occurs with the increase in stream order and the increase in basin size along the river network (Strahler, 1957; Vannote et al., 1980; Brown y Swan, 2010; Altermatt, 2013). The community can also be influenced by reach and local factors, from the characteristics of the riparian zones to microhabitats such as pools and riffles, to the occurrence and characteristics of aquatic plant beds, predators, etc. (Lamouroux, Dolédec y Gayraud, 2004; Thorp et al., 2006; Chung et al., 2012). At all scales, changes in land uses promoted by human activities can directly modify the physical habitats and water conditions affecting the structure of the macroinvertebrate communities (Stenedra et al., 2012). Macroinvertebrates are therefore often used as bioindicators in monitoring programs, since they are collectively sensitive to disturbances and changes in water quality (Resh y Rosenberg, 1993; Allan, 2004; Bonada, Prat, Resh y Statzner, 2006; Maasri et al., 2021).

Changes in land use and the modification of riparian zones could directly affect key environmental factors, for example by promoting changes in incoming light and temperature, the contribution of allochthonous organic matter, the susceptibility of bank erosion and in nutrient concentrations, often leading to lower biodiversity, lower functionality, and excessive algal production, among other signs of environmental deterioration (Carpenter, 2008; Giri y Qiu, 2016). As a consequence, changes in macroinvertebrate structure composition often occur since many invertebrate taxa are either particularly tolerant or sensitive to certain water conditions (Resh y Rosenberg, 1993; Jiang et al., 2010; Schmitt, Lemes da Silva, Macedo Soares, Petrucio y Siegloch, 2020; Wang, Zhang, Tan, Zheng y Zhang, 2021). The presence/absence of taxa or changes in different structural metrics are therefore widely used in the biological monitoring of freshwater ecosystems worldwide (Resh y Rosenberg, 1993; Chang, Lawrence, Rios-Touma y Resh, 2014). Structural variables, such as taxonomic density and richness, and the proportion of different indicator taxa, such as the percentage of Ephemeroptera, Plecoptera, Trichoptera (EPT), or the percentage of Oligochaeta individuals, are usually strong predictors of water quality (Miserendino, 2009; Hussain, 2012). If no major land use alterations occur, the taxonomic richness of macroinvertebrates is expected to be high in mid-order streams and in intermediate positions along a river network (Miserendino, 2009; Doretto, Piano y Larson, 2020), often being influenced by stream width (Chaves, Chainho, Costa, Prat y Costa, 2005), and the width of the riparian forest (Hentges et al., 2021). However, a decrease of macroinvertebrate richness and density in streams is generally a consequence of intensification in land use or spatially localized impacts (Chavez et al., 2005; Miserendino, 2009; Hentges et al., 2021; Sargac et al., 2021). Species richness of the EPT group sometimes decreases from upstream to mouth of the riverine system, responding to changes in land use in the basins (Miserendino and Pizzolon, 2003).

Uruguay has experienced a rapid intensification process since the early 2000's. An intensification of 65% of the agricultural-livestock land use has been estimated (2000-2017) (Gazzano, Achkar and Díaz, 2019) and the principal watersheds present some signs of eutrophication (Goyenola et al., 2021). The highlands of San Francisco stream drainage basin remain as a relatively low to medium- impact area of the country (Lavalleja county) (Meerhoff et al., 2017). This section of this drainage basin is particularly relevant since it provides the water source for the city of Minas.

The goal of this study was to evaluate the macroinvertebrate community structure in a low-impact highland basin (San Francisco stream), in relation to different potential drivers. We expected to find biological metrics that reflect local water physico-chemical conditions as appropriate indicators of water quality.

MATERIALS AND METHODS

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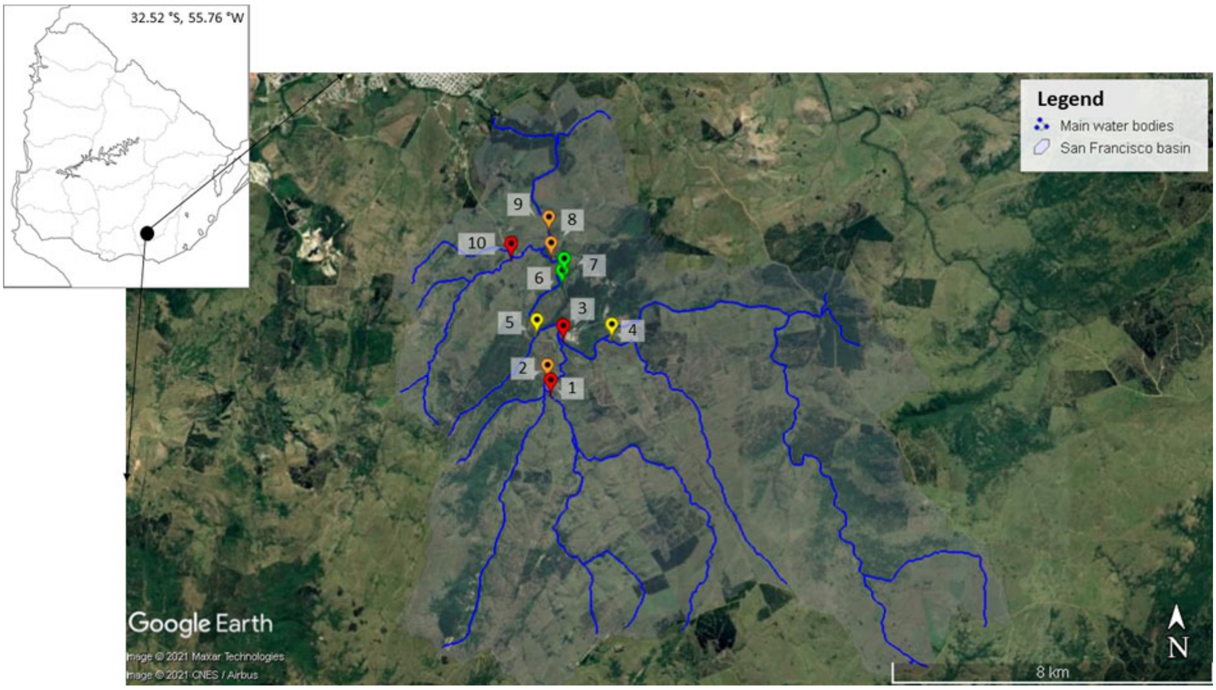

Fig. 1. Localization of sampling sites along San Francisco stream drainage basin, Lavalleja, Uruguay. 1- Stream Coronilla, 2- Stream Minas viejas, 3- Stream S. F. in Valencia mine, 4- Stream Minas y Olivos, 5- Stream Espinillo, 6- Stream UPA, 7- Stream Huerta, 8- Stream S. F. in Resort, 9- Stream S. F. in bridge, 10- Stream Hilos de la Vida. Colour codes: Green: Order 1; Yellow: Order 2; Red: Order 3; Orange: Order 4.

Study area

The study was carried out in the San Francisco stream drainage basin, upstream of the Maggiolo reservoir (which corresponds to the middle and upper basin of the whole stream, with an area of 139 km^2). The San Francisco stream drains to the headwaters of of Santa Lucía river, a key basin as it provides drinking water for most of the country's population. This basin is considered highland with respect to the relief of Uruguay (maximum elevation 513 m.a.s.l, Cerro Catedral), since its maximum point within the basin is 380 meters above sea level.

This study was part of a program to diagnose the environmental state of Maggiolo reservoir and its watershed (Meerhoff et al., 2017). This reservoir is used as the main drinking water supply of the city of Minas (-34,39° S; -55,21°W). The main economic activities in the basin include extensive cattle production and natural grasslands in a large proportion (area 113 km^2, 81% of the total area), afforestation (20 km^2, 14%), tourism and recreation parks (4.22 km^2, 3%), olive tree plantations and non-irrigated agriculture (3.19 km^2, 2%), and currently to a much lesser extent than in former periods, mining activity (0.53 km^2, 0.4%, which gave the name to the country´s capital city Minas) (Meerhoff et al., 2017). The general conservation status of water quality at the watershed level is considered good in terms of physico-chemical parameters and the structure of main biological communities (Meerhoff et al., 2017).

For the present study, 10 reaches were selected in the river network, with different stream orders and sub-basin sizes within the San Francisco (S.F.) drainage basin (Fig. 1). The selected sites included two sites of order 1, two sites of order 2, three sites of order 3 and three sites of order 4 (Fig. 1). Site selection was some how affected by accessibility to private properties.

Sampling

Physico-chemical water parameters were measured at each sampling site in late Spring 2015 (December 14, 2015). We measured temperature (Temp, °C), dissolved oxygen (DO, mg/L), pH, conductivity (Cond, mS/cm^2) and total dissolved solids (TDS, ppm) *in situ* using a YSI 6600 model multiparameter probe. In addition, water samples were collected (120 mL), refrigerated, and transported to the laboratory to analyze total nitrogen (TN, µg/L) and total phosphorus (TP, µg/L) concentrations (Valderrama, 1981). Stream order and sub-basin size (km^2) were determined by the Strahler method (Strahler, 1957) in a 1 cm:100 m scale and the size calculated by polygons

Table 1. Mean water physico-chemical variables along in the San Francisco stream drainage basin. The sites were ordered with increasing stream order and basin size. Temp= Temperature; Cond= Conductivity; DO= Dissolved Oxygen; TDS= Total Dissolved Solids; TN= Total Nitrogen; TP= Total Phosphorus.

Stream	Order	Size (km^2)	Temp (°C)	Cond (µS/cm)	DO (mg/L)	pH	TDS (ppm)	TN (µg/L)	TP (µg/L)
UPA	1	0,4	19,5	426	6,0	9,6	0,3	473	107
Huerta	1	0,4	22,0	517	6,7	9,5	0,3	950	195
Espinillo	2	2,8	25,5	286	11,3	9,6	0,2	251	27
Minas y Olivos	2	4,2	24,5	304	7,1	9,3	0,2	437	2
Hilos de la Vida	3	6,9	27,5	380	8,8	9,4	0,3	412	100
Coronilla	3	26,6	22,6	367	6,3	9,3	0,2	486	71
S.F. in Valencia mine	3	99,8	22,8	279	7,8	9,5	0,2	376	75
Minas Viejas	4	45,0	22,5	261	9,0	9,5	0,2	412	75
S.F. in Resort	4	110,0	23,1	340	8,4	9,6	0,2	327	73
S.F. in bridge	4	119,7	24,0	346	6,6	9,4	0,2	461	94

Table 2. Spearman correlation (rs) of the water physicochemical variables, stream order and size basin. Above the diagonal *p*–valor and below the rank-order correlation coefficients. In bold significant correlations.

	Size	Order	TN	TP	DO	pH	Cond	Temp	TDS
Size		**<0,01**	0,31	0,54	0,75	0,50	0,16	0,51	0,16
Order	0,93		0,28	0,58	0,39	0,43	0,15	0,50	0,15
TN	-0,36	-0,38		0,17	**<0,01**	0,25	**0,02**	0,08	**0,02**
TP	-0,22	-0,20	0,47		0,35	0,48	**0,05**	0,17	**0,05**
DO	0,12	0,31	-0,81	-0,33		0,61	**0,05**	0,11	**0,05**
pH	-0,24	-0,28	-0,40	0,26	0,18		0,93	0,45	0,93
Cond	-0,48	-0,49	0,71	0,63	-0,62	-0,03		0,47	**<0,01**
Temp	0,24	0,24	-0,57	-0,47	0,54	-0,27	-0,26		0,47
TDS	-0,48	-0,49	0,71	0,63	-0,62	-0,03	1.00	-0,26	

in Google Earth pro.

Macroinvertebrate community sampling was carried out at the same time using a Surber sampler of 14*14 cm, with a mesh size of 250 µm. At each site, a 50-m long section was selected where 5 replicated Surber samples were taken (total n= 50 samples). The samples were transported to the laboratory, washed on a 500 µm mesh sieve and fixed with 70% alcohol for subsequent counting and taxonomic identification of macroinvertebrates. The collected individuals were classified at the family level or the highest possible taxonomic resolution using regional taxonomic keys (Roldán, 1988; Domínguez y Fernández, 2009). The density of individuals (ind/m^2) and taxonomic richness (amount of taxa. M^2) were calculated for each sample. Also, the biotic indexes as percentage of Oligochaeta (% Oligochaeta) and percentage of Ephemeroptera, Trichoptera and Plecoptera (%EPT) individuals within the total density were calculated using the number of individuals of each taxa.

Data analysis

A principal component analysis (PCA) was

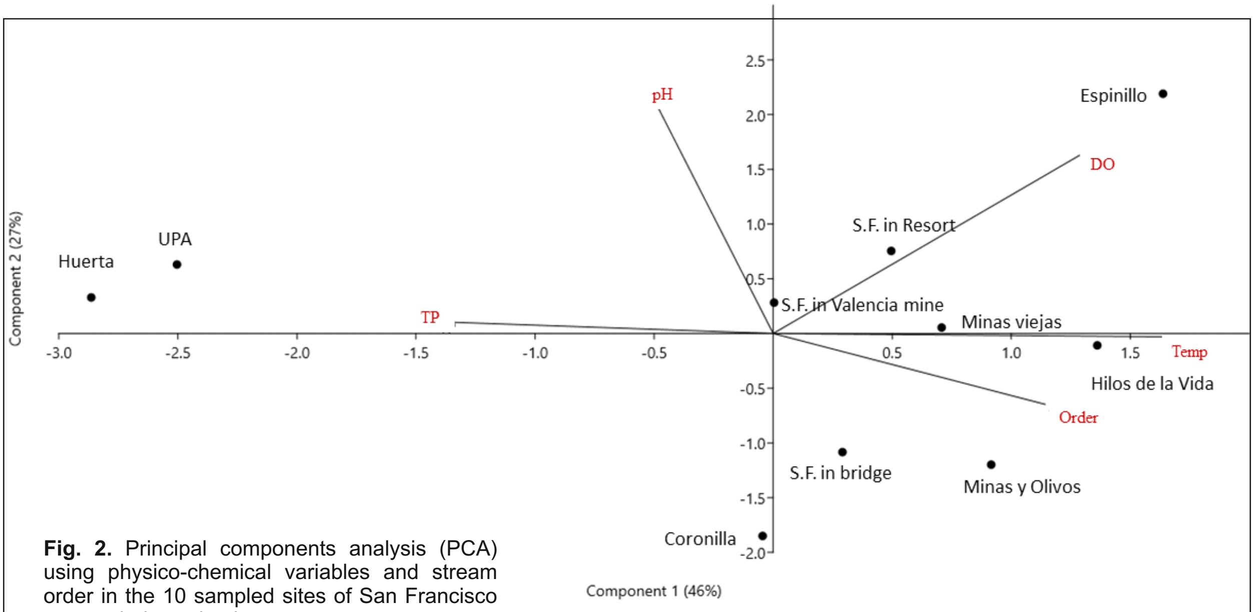

Fig. 2. Principal components analysis (PCA) using physico-chemical variables and stream order in the 10 sampled sites of San Francisco stream drainage basin.

performed using a correlation matrix with physico-chemical water variables. For this purpose, the correlation between the variables was previously analyzed using the Spearman's correlation coefficient and the variables selected for PCA (rs< 0.7) were Temp, DO, TP, pH and order. Given the high association of PCA first axis with Temp (correlation: 0.9), TP concentration (correlation: -0.8), DO (correlation: 0.7) and with order (correlation: 0.6), we thereafter used this axis as a proxy of the water quality in the community linear models. We analyzed the relationship between community variables (i.e., density, richness, %EPT and %Oligochaeta) and water variables such as stream order and physico-chemical parameters with simple linear models (e.g., the models followed the form "Variable ~ Stream order + PCA 1"). To obtain the model that best fit the data, the distribution of each response variable was analyzed, being normal for all community variables. Model validation was performed using visual QQplot diagrams, plots of observed and predicted residuals, and Shapiro tests. Since the stream order and watershed size were correlated (R^2=0.7, p<0.05), only the stream order variable was used in the models (Dormann et al., 2013). The relation between the above mentioned environmental factors and macroinvertebrate taxonomic composition in all sites was analyzed using a canonical correspondence analysis (CCA) (Legendre y Legendre, 1998).

Analyses were run using R Studio (R version 3.5.3; R Core Team, 2013) and PAST 4 (Hammer et al., 2001).

RESULTS

Environmental factors

The streams presented a temperature range between 19.5 and 27.5°C, a conductivity range of 261 to 517 µS/cm, high oxygenation (DO: 6.0 - 11.3 mg/L), alkaline pH (9.3 - 9.6) and a broad range of nutrient concentrations (251 - 950 µgTN /L; 2 - 195 µgTP /L) (Table 1).

The correlation between physico-chemical water variables showed that TDS, conductivity, and TN concentration tended to vary negatively with increasing stream order (Spearman correlation rs: -0.38 - -0.49; *p* = 0.15-0.28). Conductivity, TP, TN, and TDS were positively correlated between them (Spearman correlation rs: 0.63–1.0; *p*<0.05) (Table 2).

Regarding the PCA analysis, components 1 and 2, PC1 and PC2 respectively, explained 73% of the data variance. The PC1 explained 46% of the variance and was positively correlated with temperature, stream order, and dissolved oxygen concentration, and negatively with total phosphorus concentration. The PC2 explained 27% of the variance and was positively correlated mainly with pH (Fig. 2). Sampling sites were separated mainly by nutrient concentrations on one side (UPA and Huerta streams), and dissolved oxygen concentration on the other side of the axis gradient (Espinillo stream) (Fig. 2). The other sites were dispersed over an intermediate gradient of stream order, temperature, and dissolved oxygen concentration (Fig. 2).

Macroinvertebrate communities

The macroinvertebrate communities were represented by a total of 5880 individuals covering 28 different taxa. The sites with the highest mean density

Table 3. Mean and standard deviation of community parameters of benthic macroinvertebrates in 10 streams belonging to the San Francisco stream drainage basin.

Stream	Order	Density (n°. Ind/m^2)	Richness (n°. taxa)	EPT %	Oligochaeta %
UPA	1	4346,9 ± 295,5	7,8 ± 0,49	1,73 ± 1,04	15,20 ± 5,95
Huerta	1	6265,3 ± 1189,0	8,0 ± 1,13	9,98 ± 6,56	8,51 ± 2,89
Espinillo	2	4673,5 ± 1098,6	8,2 ± 0,86	9,17 ± 3,85	21,47 ± 5,02
Minas y Olivos	2	7571,4 ± 1681,3	10,2 ± 0,33	28,33 ± 6,11	6,10 ± 1,68
Hilos de la Vida	3	7661,2 ± 2196,2	8,4 ± 1,00	49,10 ± 8,52	1,87 ± 1,21
Coronilla	3	4244,9 ± 979,0	7,6 ± 0,68	52,28 ± 8,75	4,60 ± 1,99
S.F. in Valencia mine	3	5857,1 ± 959,5	8,0 ± 1,10	7,62 ± 1,53	3,30 ± 1,79
Minas Viejas	4	3704,1 ± 1328,2	8,8 ± 1.00	41,63 ± 8,57	0,44 ± 0,31
S.F. in Resort	4	8846,9 ± 1041,4	10,4 ± 1,12	34,64 ± 4,93	0,83 ± 0,34
S.F. in bridge	4	5112,2 ± 1332,9	8,0 ± 0,75	22,14 ± 2,51	5,80 ± 1,28

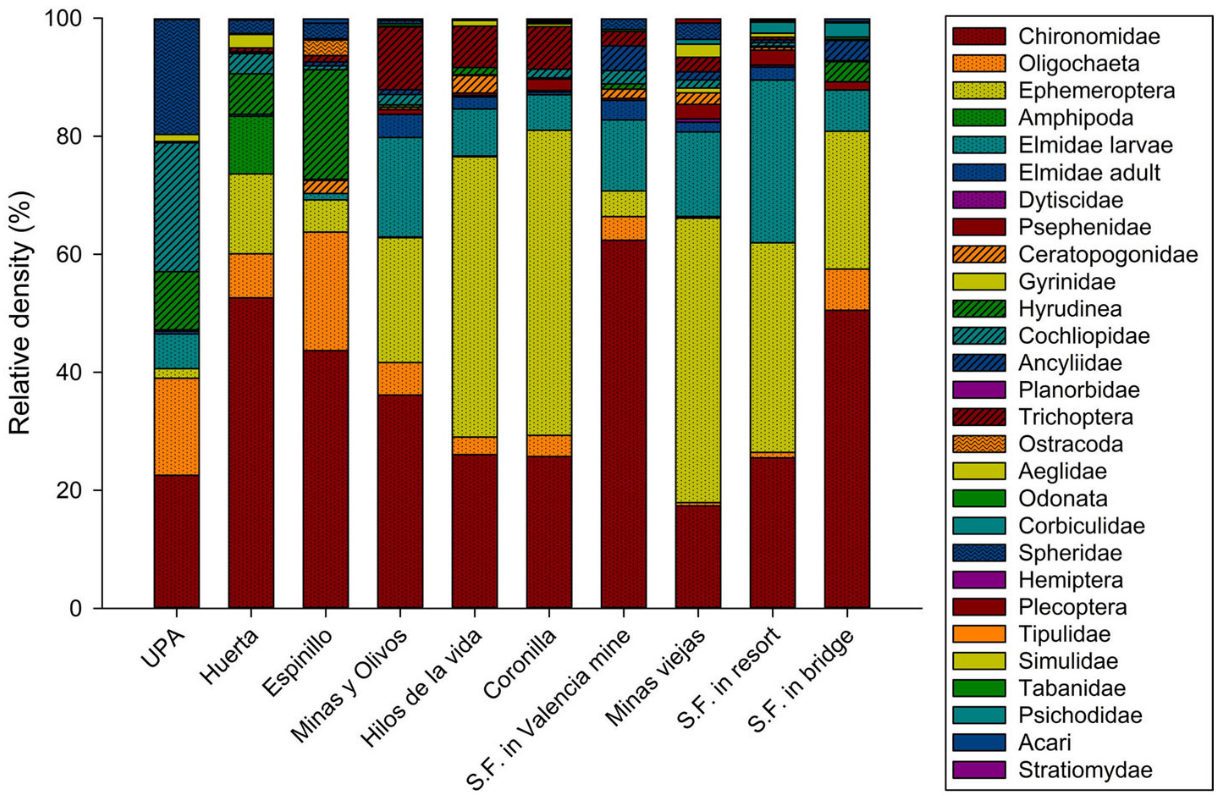

Fig. 3. Relative density (%) of different macroinvertebrate taxa in 10 streams belonging to the San Francisco stream drainage basin.

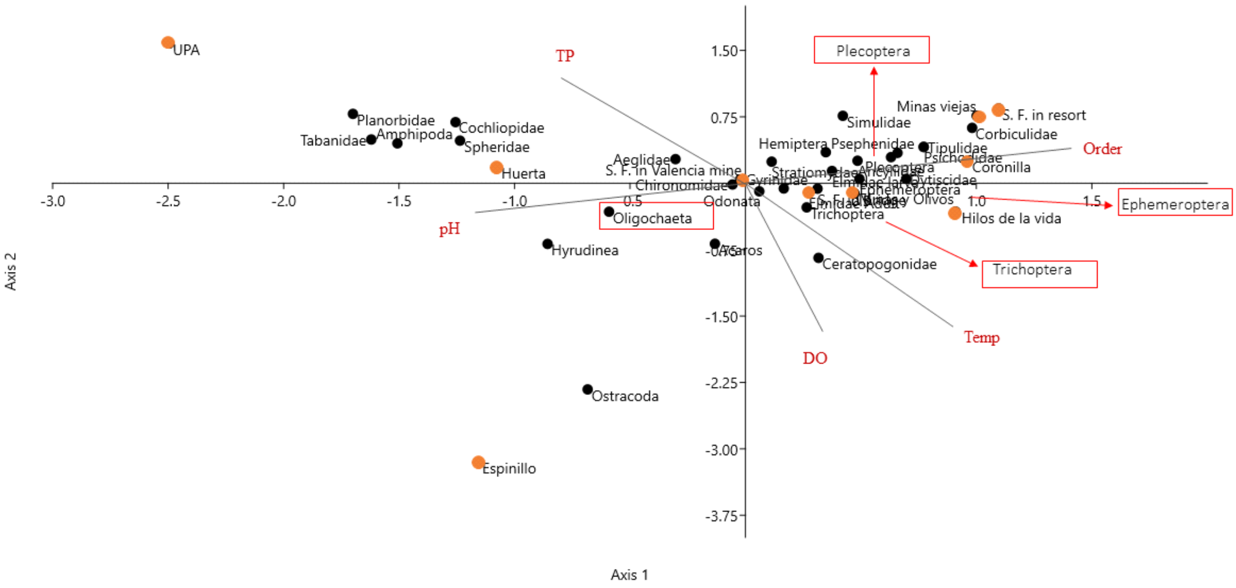

Fig. 4. Canonical correspondence analysis (CCA) using physico-chemical water variables and relative density of all taxa in the 10 streams belonging to the San Francisco stream basin. Sites are shown in orange colour. The taxa used for the biotic indexes are shown in red box.

and richness were San Francisco stream sampled in the location Resort, and Hilos de la Vida and Minas y Olivos streams (Table 3) in comparison with the others sites. The order 1 streams (i.e., UPA and Huerta) and order 2 (Espinillo) presented the largest mean percentage of Oligochaeta, whereas Coronilla and Hilos de la Vida (order 3) and Minas Viejas (order 4) presented the largest Ephemeroptera, Trichoptera and Plecoptera percentage compared with the others (Table 3).

The relative density of the Chironomidae, Oligochaeta, Ephemeroptera and Elmidae taxa represented in all streams > 40% of total macroinvertebrate densities (Fig. 3). The relationship between macroinvertebrate relative density and environmental variables (physico-chemical variables and order) in the CCA analysis showed that the %EPT and %Oligochaeta (indicated by red boxes in Fig. 4), responded to water quality.

Density and richness of benthic macroinvertebrates were not significantly related to stream order and PC1 (lineal model, $p > 0.05$). The indexes %EPT showed a positive (marginally significant) linear relationship with stream order (lineal model, Adjusted R= 0.28, p=0.07), while the %Oligochaeta showed a negative relationship with the same variable (Adjusted R= 0.45, p=0.02) (Fig. 5). For both indices, no significant relationship with PC1 was found (lm, p > 0.05).

DISCUSSION

We found evidences that some metrics of the structure of the benthic macroinvertebrate community can inform anthropically driven variations in stream water quality, despite the fact that the studied drainage basin is overall considered as well-preserved in comparison to other basins in Uruguay (Meerhoff et al., 2017; Goyenola et al., 2021).

The 10 sites differed in water physico-chemical characteristics. Three sites belong to the main channel of the San Francisco stream, while the rest are tributaries. The site ordination in the PCA showed no longitudinal gradient, as sites grouped according to local conditions. In the study carried out by Meerhoff et al., (2017) present that the UPA and Huerta streams are small, typically present low discharge, and receive the runoff of a recreational park with locally intense production activities, such as agricultural farming, and cattle and pork production, together with frequent high densities of people (Meerhoff et al., 2017). These conditions led to a local increase in water nutrient concentrations and conductivity, causing low water quality in the lower order sites selected, which is not the usual expectation shall the watersheds have a more random or uniform land uses. Despite these particular conditions, the water quality along the basin is comparatively good (Meerhoff et al., 2017).

In Uruguay, there are few studies focusing on stream macroinvertebrates. Most of these studies describe the community in relation to water quality (Chalar, 1994; Arocena, 1998, 1996; Chalar, Arocena,

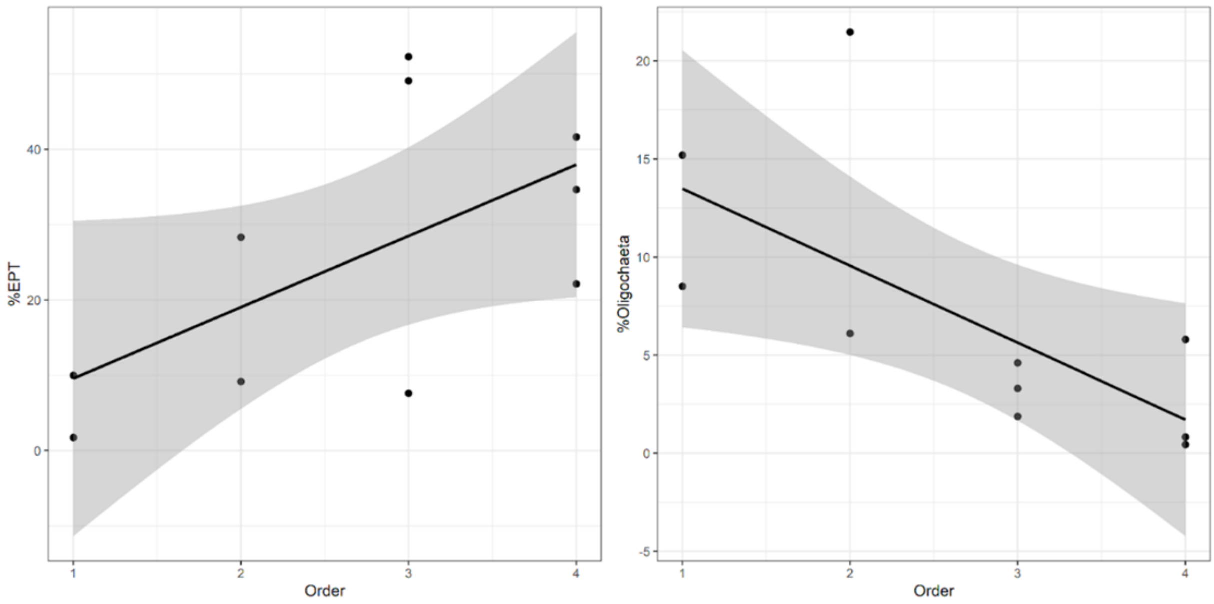

Fig. 5. Relationship between benthic macroinvertebrate community structure and stream order. The line indicates the linear relationship with a confidence interval in the gray zone (lineal model, EPT% Adjusted R= 0.28, *p*=0.07; %Oligochaeta Adjusted R= 0.45, *p*=0.02).

Pacheco y Fabián, 2011), include some ecosystem processes (Burwood et al., 2021), or are local studies of diversity in low order streams (Morelli y Verdi, 2014). The composition of the benthic macroinvertebrate communities in our study in general was similar to other studies in Uruguayan streams, i.e, with a large proportion of insects (Morelli y Verdi, 2014). The most abundant taxa in all sampled sites were Chironomidae, Oligochaeta, Ephemeroptera, and Elmidae taxa. These taxa, except Oligochaeta, are the most common in terms of abundance or density also in other streams in the region (Miserendino y Pizzolon, 2003; Miserendino, 2009; Morelli y Verdi, 2014; Hentges et al., 2021). The % Oligochaeta in our set of streams decreased when stream order increased, while %EPT showed an inverse pattern. Oligochaeta are frequently associated with pollution tolerance taxa, although there may be a certain variation in the sensitive-tolerant range depending on the particular subfamily (Mandaville, 2002; Marchese, 2009). On the other hand, the orders Ephemeroptera, Plecoptera and Trichoptera are often intolerant to pollution, so their numbers often decrease as pollution (or environmental degradation in general) increases (Hussain, 2012).

The causes underlying our biotic patterns seem to be the same as in other studies and highlight the importance of the impacts of changes in land uses on aquatic macroinvertebrate communities along a drainage basin (Miserendino y Pizzolon, 2003). Other parameters of the macroinvertebrate community analyzed in our study, such as richness and density, were in contrast not explained by our potentially explanatory variables. Most of the recent theories about aquatic ecology report the complexity of richness and abundance patterns and discuss a combination of discrete and longitudinal processes and mechanisms that may explain the structure of macroinvertebrates (Collins et al., 2018; Massri et al., 2021).

In our study the patterns observed in %Oligochaeta and %EPT reflected the responsiveness of this community to point and local stressors but also their capacity to recover downstream. Also, our results highlight the potential to use these relatively easy biological indexes in biomonitoring programs for this and other key drainage basins, given their importance for local biodiversity as for securing safe drinking water for society (such as Minas city).

CONCLUSIONS

This study contributes to understanding the structure of macroinvertebrate communities in highland streams of the subtropical regions. We found evidence of the potential for using easy macroinvertebrate indexes as water quality indicators, even in well preserved drainage basins, and advocate for including them in environmental monitoring programs particularly in reference basins.

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