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Benthic macroinvertebrates community structure and physical-chemical characteristics at Lauca River Basin high altitude wetlands, Altiplano, Chile

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ABSTRACT

Benthic macroinvertebrates community structure and physical-chemical characteristics at Lauca River Basin high-altitude wetlands, Altiplano, Chile

High Andean freshwater ecosystems are highly threatened by scarce water availability, species invasion, and global climate change, so generating knowledge about their ecological characteristics is extremely important for conservation decision-making. In this work, the seasonal variations and relationships between physico-chemical variables and the structure and composition of the macroinvertebrate community were analyzed considering "season", "type of ecosystem" and "site" factors. Five sites of representative ecosystems at Lauca River Basin of the Chilean Altiplano were sampled, two of them lentic and three lotic. Two field-sampling periods within a year according to the pre- and post-rain seasons characteristic of the Altiplano climate were considered. Thirty-five taxa were identified, and it was observed that the taxa Orthocladiinae, *Austrelmis* sp., *Hyallela cf kochi*, Podonominae and Helicopsychidae were indicator taxa that contributed the most to differences among sites or ecosystems (≥ 10 %.). No significant differences were found in the alpha diversity indicators used, except for some abundance values and Pielou's Evenness index (J´), which varied significantly between lotic and lentic systems. The results of the ordination analysis showed a significant differentiation considering physical and chemical variables and macroinvertebrates assemblages that responded to "sites" (ANOSIM *R* Global = 0.64, $p = 0.001$) and "type of ecosystem" factors (ANOSIM *R* Global = 0.31, $p = 0.02$). The "season" factor was not statistically significant to explain the variability of biological data (ANOSIM *R* Global = -0.003, $p = 0.47$) and was slight and marginally significant with the physical and chemical data (ANOSIM *R* Global = 0.1, $p = 0.04$). In addition, the linear redundancy analysis (RDA) showed that physico-chemical variables related to hardness, temperature, phosphorous, and nitrogen explained most of the variance in the biological data (the first two canonical axes RDA1 and RDA2 explained 45.23 % of the total variation, $p = 0.004$). These results support the relevance of local conditions for high altitude wetlands and how those environmental characteristics can be reflected in the macroinvertebrate assemblages that inhabit them.

Key words: biodiversity, endorheic basin, macroinvertebrate community, highland freshwater ecosystems

RESUMEN

Estructura de la comunidad de macroinvertebrados bentónicos y características físico-químicas en humedales de altura en la cuenca del río Lauca, Altiplano, Chile

Los ecosistemas de agua dulce altoandinos se encuentran altamente amenazados por la escasa disponibilidad de agua, la invasión de especies y el cambio climático global, por lo que generar conocimiento sobre sus características ecológicas es de suma relevancia para la toma de decisiones de conservación. Se analizó la variación estacional y relación de las variables fisicoquímicas, y la estructura y composición de la comunidad de macroinvertebrados en ecosistemas acuáticos considerando los factores de "temporada", "tipo de ecosistema" y "sitio". Se muestrearon cinco sitios de ecosistemas representativos de la cuenca del río Lauca en el altiplano chileno, dos lénticos y tres lóticos. Se hicieron campañas de muestreo en dos periodos del año, considerando las temporadas características del clima Altiplano pre- y post-lluvia. Se identificaron 35 taxones, de los cuales Orthocladiinae, Austrelmis *sp.,* Hyallela cf kochi*, Podonominae and Helicopsychidae fueron los taxones que coinciden y más contribuyen a las diferencias cuando se compara entre pares de sitios o ecosistemas (≥ 10 %.). No se encontraron diferencias significativas en los indicadores de diversidad alfa utilizados excepto algunos valores de abundancia y del índice de equidad de Pielou (J'), el cual varió significativamente entre sistema lótico versus léntico. Los resultados de los análisis de ordenación mostraron una marcada diferencia entre variables fisicoquímicas y entre el ensamble de macroinvertebrados que respondieron a factores de "sitio" (ANOSIM* R *Global = 0.64,* $p = 0.001$*) y "tipo de ecosistema" (ANOSIM* R *Global = 0.31,* $p = 0.02$*). El factor "temporada" no presentó diferencias significativas para explicar variabilidad de los datos biológicos (ANOSIM* R *Global = 0.003,* $p = 0.47$*) y fue leve y marginalmente significativo con los datos fisicoquímicos (ANOSIM* R *Global = 0.1,* $p = 0.04$*). Además, los resultados del análisis de redundancia lineal (RDA) muestran que las variables fisicoquímicas relacionadas con la dureza, la temperatura, el fosfato y el nitrógeno explican la varianza de los datos biológicos (RDA 1 y RDA 2 explican el 45.23 % de la variación total,* $p = 0.004$*). Además, se observó una baja similitud entre los sitios y tipo de ecosistema según las características fisicoquímicas de cada cuerpo de agua. Estos resultados respaldan la relevancia de las condiciones locales para los humedales de altura y cómo esas características ambientales pueden reflejarse en los conjuntos de macroinvertebrados que los habitan.*

Palabras clave: *biodiversidad, cuencas endorreicas, comunidad de macroinvertebrados, ecosistemas de agua dulce en altura*

INTRODUCTION

High-altitude wetlands are often extreme environments. In general, these ecosystems present altitudinal variation related to factors of temperature, atmospheric pressure, and partial pressure of gases such as CO_2, H_2O, and O_2, which decrease in the altitudinal gradient, while solar radiation (UV-b) increases (Körner, 2007). Other influencing factors in waterbodies are precipitation, cloudiness, and humidity, which can indeed change with altitude (Jacobsen & Dangles, 2017). Particularly, freshwater ecosystems in the Altiplano have been exposed to a long process of fragmentation and reconnection of the habitat due to climatic and geological events such as the uplift of the territory with the elevation of the Andean mountain range and volcanic activity, which have shaped the hydrographic basins since the late Eocene (Demergasso et al., 2010, Kött et al., 1995, Lambrinos et al., 2006, Rundel & Palma, 2000, Scott, 2010, Scott et al., 2015, Vila et al., 2013).

In the southern Andean Altiplano, particularly in the Lauca River Basin, landscape modulation has given rise to the availability of a singular habitat that has supported different substrates and biodiversity through several evolutionary processes (Cárcamo-Tejer et al., 2021, Guerrero et al., 2015, Guerrero et al., 2017, Oyadenel, 2013). Vegetation is herbaceous and shrubby with compact growth, which generates patches of azonal vegetation with species such as *Oxychloe andina* and *Distichia muscoide*, and sedges like *Zameoscirpus atacamensis* and *Philloscirpus acaulis*. There is also a unique ichthyofauna with a high degree of endemism. There are mainly two genera of endemic fishes: the catfishes *Trichomycterus* and the killifishes *Orestias* (Vila et al., 2007). For the genus *Orestias*, some researchers have described a high degree of speciation and adaptative radiation that reflects ecosystem heterogeneity and the aforementioned intermittent connection between waterbodies (Guerrero et al., 2017, Vila et al., 2013, Wirrman & Mourguiart, 1995). In addition, Márquez-García et al. (2009) showed that there is a difference in the composition of the aquatic biodiversity of some aquatic ecosystems in the Altiplano, depending on their chemical composition, including those of the Lauca River Basin. Macroinvertebrates play important functional roles within these high-altitude systems (Jacobsen & Dangles, 2017, Vila et al., 2020), and constitute the nexus of the transference of matter and energy from producers to consumers (Qazi & Ashok, 2012). It is known that the macroinvertebrate community varies according to the conditions of its habitat, including the types of substrates present and the physical and chemical characteristics of water (type of habitat, e.g., lotic or lentic systems). Therefore, they are a good indicator of environmental variability (Buffagni et al., 2020) and as such they facilitate the understanding of the functioning of aquatic ecosystems (Calabrese et al., 2020) and are useful for making conservational decisions.

In the context of climate change where impacts on aquatic ecosystems are expected to be due mainly to the increase in water temperature, hydrological cycle alterations (e.g., rainfall patterns, extreme frost, or drought events) and water quality, which may threaten macroinvertebrate assemblages (Jacobsen & Dangles, 2017). Although there are studies that have registered the biodiversity of benthic macroinvertebrate assemblages and how it relates to the environmental characteristics of their habitats in arid and semi-arid zones, the fewer investigations in the Altiplano report a low biodiversity and a high degree of endemism (Larsen et al, 2011).

Since aquatic communities have different responses to their environments, in this study we aim to describe the assemblage diversity of macroinvertebrates in the high-altitude wetlands of the Lauca River Basin and identify the main environmental drivers of these communities. It is expected that the results of this study contribute with relevant information to the further understanding of benthic communities in high-altitude water systems and promote their preservation.

METHODS

Study area

The study area focused on the Altiplano, an extensive flatland between 9° S and 27° S, situated at more than 3000 m a.s.l., framed by two cordilleras of the Andean mountain range. The hydrology of this plateau has been shaped largely by volcanism, tectonics, and climatic events since the late Eocene (≈ 40 Myr BP), giving rise to aquatic ecosystems such as lagoons, lakes, salt pants, springs, and rivers, forming numerous endorheic basins.

The endorheic basin of the Lauca River is located between the latitudes 18° 6’ 4’’ S - 18° 49’ 4’’ S and the longitudes 69° 12’ 41’’ W - 69° 9’ 42’’ W, occupying a surface area of 2350 km^2 in the Chilean territory. It is a protected area by the Lauca National Park and Las Vicuñas National Reserve. It is one of the western-most fluvial-lacustrine basins of Miocene-Pliocene origin in Chile (Feitl et al., 2019, Rundel & Palma, 2000), and is filled by clastic and volcaniclastic sediments of lacustrine and alluvial origin (Kött et al., 1995). The aquatic ecosystems of this basin have been the subject of various studies anent ecology, morphology, morphometry, karyotypes, phylogenetics and phylogeography, finding high endemism of the species that inhabit them (Cárcamo-Tejer et al., 2021, Collado et al., 2011, Collado et al., 2014, Guerrero et al., 2015, Guerrero et al., 2017, Scott, 2010, Vila et al., 2010, Vila et al., 2013).

The climate in this area is best described as semi-arid with annual rainfall influenced by the South American summer monsoons (Sáez et al., 2007). These climatic characteristics originate mainly two seasons of the year: a wet season where water flows and runoff increases due to intense rainfall concentrated from December to March, and a dry season between April and November where the absence of precipitation causes a decrease in water flows triggering an intermittent disconnection of some water bodies (Aceituno et al., 2021, Rondanelli et al., 2015, Sáez et al., 2007). In addition, the area is exposed to extreme climatic conditions such as a wide thermal oscillation between day and night (17 °C Day to -1 °C Night) and high solar radiation (> 1000W m^{-2}) (Aceituno, 1997, Aceituno et al., 2021).

Sampling

Two field samplings were carried out during May and October 2018, corresponding to the pre- and post-rainfall season within a year. Five sites distributed in different localities throughout the Lauca River Basin were sampled during each season with two replicates per site to obtain information from the main course (the Lauca River) and from the major aquatic ecosystems found in the basin. The sites were named according to the name of the locality in which they were sampled: Misitune (MST), Ancuta (ANC), Pisarata (PIS), Ancayuconi (ANY), and Paquisa (PAQ). The aquatic ecosystems sampled were classified according to their water flow characteristics to facilitate the analysis of lentic or lotic ecosystem (Fig.1, Table S1 (Supplementary information available at http://www.limnetica.net/es/limnetica)).

The lentic ecosystems (MST, PIS) are immersed in a matrix with a high cover (80-90 %) of herbaceous and cushion plants dominated by species of the *Oxychloe*, *Distichia* and *Deyeuxia* genera. The main macrophytes correspond to species of the genera *Myriophyllum*, *Lemna*, and *Stuckenia*. The substrates are mainly composed

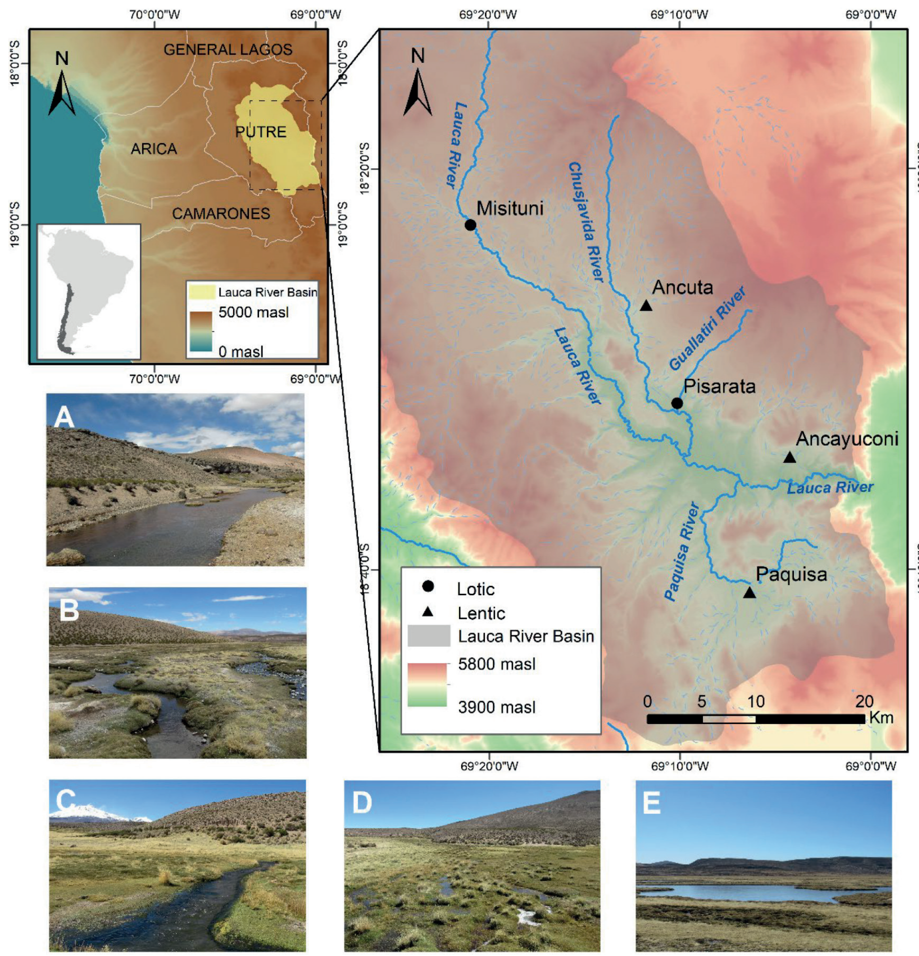

Figure 1. Location of the study area and delimitation of the basin. A: Misitune_Lotic ecosystem; B: Ancuta_Lentic ecosystem; C: Pisarata_Lotic ecosystem; D: Ancayuconi_Lentic ecosystem; E: Paquisa_Lentic ecosystem. *Localización del área de estudio y la delimitación de la cuenca. A: Misitune_ecosistema lótico; B: Ancuta_ecosistema léntico; C: Pisarata_ecosistema lótico; D: Ancayuconi_ecosistema léntico; E: Paquisa_ecosistema léntico.*

of silt, sand, and organic matter. Depths varied from 10 - 40 cm. The lotic ecosystems (ANC, ANY, and PAQ) are immersed in a more heterogeneous matrix than the lotic ones, where the cushion plants *Oxychloe* sp. and *Deyeuxia* sp. surround the channel and are followed by patchy distributions of *Distichia* sp. and *Festuca* sp. As for macrophytes, species of the genus *Myriophyllum* predominate. The substrates are mainly composed of gravel and sand with the presence of unstable rocks and silt in a smaller proportion. Depths varied from 40-70 cm, except in Paquisa where it reaches ≈ 1 m.

Water quality samples

During the sampling periods, we measured the physical and chemical water variables from the five sites with two replicates per site: conductivity, pH, salinity, and oxygen levels were determined *in situ* with a multiparameter probe WTW 3430 model. Two water samples of 500 ml were collected in each site and preserved for subsequent analysis according to standard methodology (APHA, American Public Health Association) (Baird & Bridgewater, 2017). The parameters analyzed were: Hardness (mg/l) with volumetry method with EDTA, alkalinity (mg/l), with Standard Method-230B; ammonium concentration (µg/l), colorimetry with blue indophenol; P/phosphates (µg/l), Murphy & Riley (1962). The other components chloride (mg/l), nitrite (µg/l), nitrate (µg/l), sulfate (mg/l), silica mg/l), N-Kjeldahl(µg/l), P-Kjeldahl (µg/l), sodium (mg/l), potassium(mg/l), magnesium (mg/l), and calcium(mg/l) were analyzed with Standard Method-4110B. (APHA, American Public Health Association) (Baird & Bridgewater, 2017).

Biological components samples

In order to characterize the macroinvertebrate communities, two replicates were taken randomly, distributed among the substrates covered by macrophytes and sediment from each site, each sample taken using a 250 µm Surber net (area of 0.09 m^2). The collected material was deposited in 350 cm^3 labelled flasks and fixed with 96 % alcohol. Subsequently, the samples were transferred to the Limnology Laboratory of the University of Chile, where they were identified to the lowest possible taxonomic level using a stereomicroscope (Leica, EZ4HD 40×) and identification keys (Domínguez & Fernández, 2009, Merritt et al., 2019, Tachet et al., 2010). Results were detailed in terms of density (individuals/m^2).

Statistical Analysis

In this study, three factors potentially influencing the macroinvertebrate communities were considered: season (pre-/post-rainfall), type of ecosystem (lentic/lotic) and site (MIS/PIS/ANC/ANY/PAQ). Alpha diversity was analyzed for community assemblage by determination of specific richness, relative abundance, Shannon and Pielou´s indexes, and was statistically determined by analysis of variance (ANOVA) considering the three factors. For beta diversity, we used a non-metric multidimensional scaling analysis (nMDS) to visualize a pattern for environmental and biological data, with a resemblance matrix using Bray-Curtis similarity index for biological data, and Euclidean distance for environmental data, elucidating how they are distributed in a two-dimensional space through season, type of ecosystem, and site factors. Then one-way analysis of similarities (ANOSIM) was carried out, to identify any differences/similarities in the communities. We made a similarity percentages analysis (SIMPER) based on the biological data to determine which taxa contributed in a higher proportion to the differences found. The analyses were performed in PRIMER 7 and PERMANOVA + software (Anderson et al., 2008, Clarke & Gorley, 2006). Finally, the association of biological and environmental data was estimated with a direct redundancy gradient analysis (RDA) as suggested by Šmilauer & Lepš (2014), previously log (x + 1) transformed using CANOCO 5 software (Šmilauer & Lepš, 2014, ter Braak & Šmilauer, 2012). This analysis measures the turnover of species directly related to environmental variables, with the restriction that the axes are combinations of linear variables. The stepwise "*forward selection*" method was applied to reduce the environmental variables input to avoid the risk of overfitting the RDA model (Sun et al., 2019). The statistical significance of the relationships found was determined using Monte Carlo simulations with 999 permutations (Manly, 1991).

RESULTS

Water quality samples

The physical and chemical variables measured *in situ* showed pH ranges between 7.8 (MST) and 9.4 (PAQ) -dissolved oxygen (mg/L) 7.4 (PIS)-8.6 (PAQ), electrical conductivity (µS/cm) ranges of 141 (ANC)-652 (PIS), and temperature 6.6 °C (PAQ)-17.8 °C (ANC). For the season and ecosystem factor, the main difference was in the electrical conductivity: in post-rainfall it was higher than pre-rainfall; and in lotic ecosystems it was higher than lentic. For more details see Table S2 (Supplementary information available at http://www.limnetica.net/es/limnetica).

Biological components samples

A total of 4638 macroinvertebrates were collected in the overall samples. We identified 35 taxa, 14 of them were only found in lentic systems including Nematoda, Mollusca, Entognatha, Hemiptera, and Odonata. While four taxa were only found in lotic systems including *Hidrachnidia* sp., Diamesinae, *Claudioperla* sp., and *Cailloma* sp. There were 17 taxa common to both systems (Table S3, Supplementary information available at http://www.limnetica.net/es/limnetica). Overall, macroinvertebrate assemblages were dominated by Orthocladiinae, *Austrelmis* sp., *Haementeria* sp., and *Hyallela cf kochi* with a frequency greater than 90 % in all sites and seasons of the year. The highest richness was obtained during the pre-rainfall season in the locality of Ancuta (ANC) and the lowest richness was measured in Paquisa (PAQ) in the post-rainfall season.

The relative abundance (%) with respect to the orders varied according to the site and the season (Figs. 2 A and B), showing in all the sampling sites the presence of individuals belonging to the order Malacostraca, represented by *Hyallela cf kochi* uniquely, which represents 69 % of the total of individuals in Paquisa (PAQ), and Diptera shows

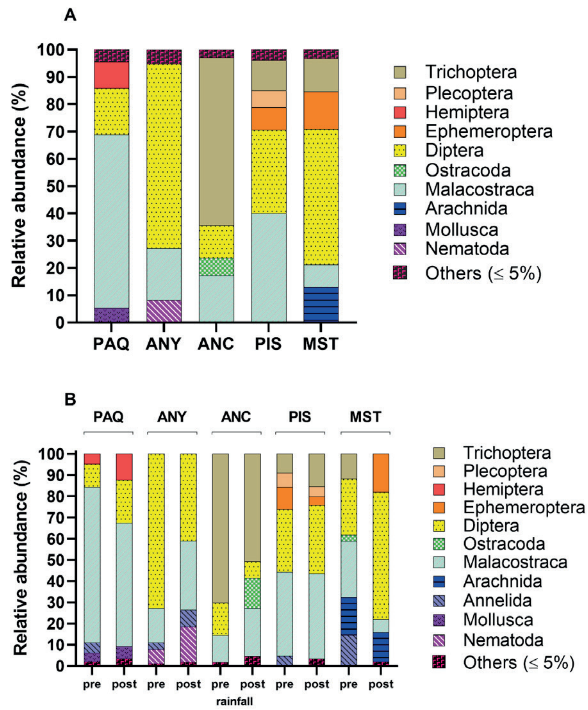

Figure 2. (A) Relative abundance (%) of macroinvertebrates main taxonomic groups at each sampling site. (B) Seasonal variation in the relative abundance (%) of macroinvertebrates in pre- and post-rainfall seasons. Orders with percentages less than 5 % were added and represented on the graph as "other". *(A) Abundancia relativa (%) de los principales grupos taxonómicos de macroinvertebrados en cada sitio de muestreo. (B) Variación estacional de la abundancia relativa (%) de macroinvertebrados en la temporada pre- y post-lluvia. Los órdenes con menos del 5 % fueron adicionados y representados en el gráfico como "otros".*

68 % in Ancayuconi (ANY). Figure 2B shows the relative abundance in terms of the season for each sampling site, and it is possible to observe that in Paquisa the percentage of individuals belonging to the order Malacostraca decreased in the post-rainfall season, while in the same season the percentage of individuals of the order Diptera and Hemiptera increased. In Ancayuconi (ANY) and Ancuta (ANC) the order Diptera - mostly represented by the Chironomidae - decreased by 50 % compared to the pre-rainfall season and the orders Malacostraca and Nematoda both increased. In Pisarata (PIS), individuals of the order Trichoptera increased in the post-rainfall season, while the order Ephemeroptera decreased by 60

%. Regarding Misitune (MST), individuals of the order Annelida and Trichoptera decreased by 100 % during the transition to the post-rainfall season, while the orders Diptera and Ephemeroptera both increased their representation by 56 % and 100 %, respectively.

The specific richness (S) values did not change among the five sampling sites (Fig. 3A). A higher specific richness was observed in lentic ecosystems in pre-rainfall season, while a higher richness index was observed in post-rainfall season in lotic ecosystems. The largest number of species was observed at the Ancayuconi (ANY) sampling point in the pre-rainfall season, and the abundance of individuals (N) is significantly different between seasons (Fig. 3B), with the highest number of individuals in the pre-rainfall season (843 ± 221). In the post-rainfall season, the highest number of individuals was observed in Paquisa (PAQ) and Ancuta (ANC), however it was not possible to establish statistical differences among sites. The Pielou's Evenness index (J′) presented similar values between seasons for all sampling sites (Fig. 3C). Nevertheless,

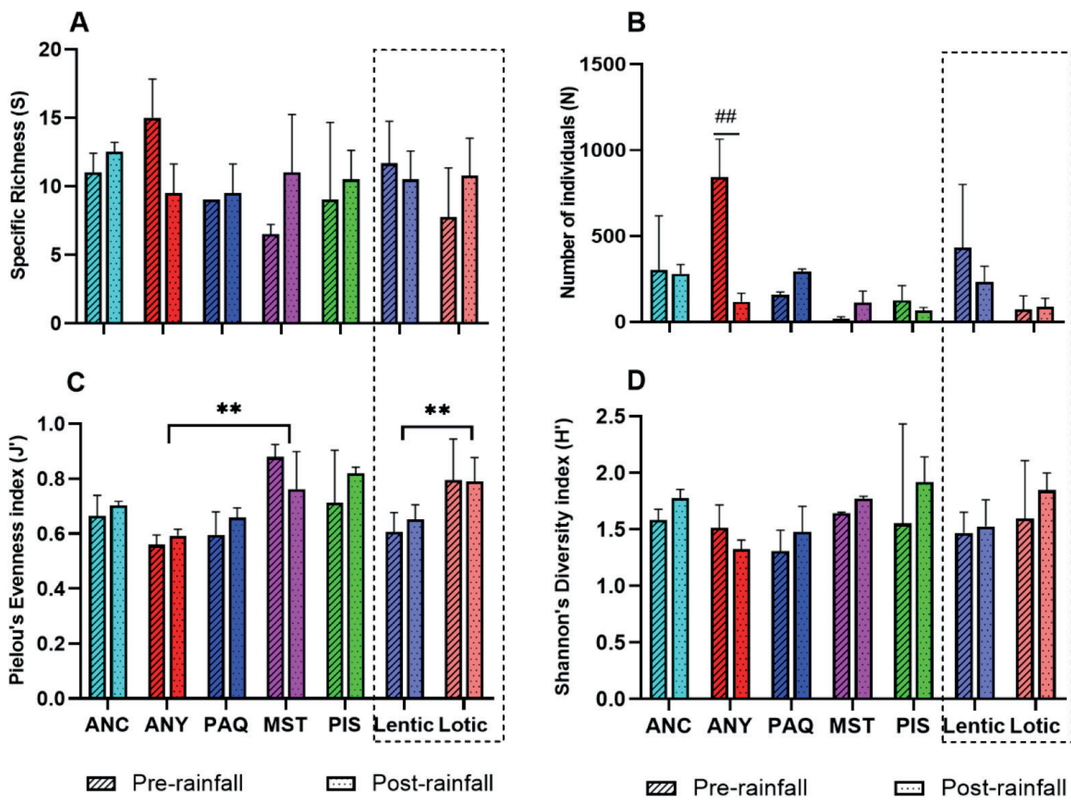

Figure 3. Diversity values and indices calculated at each sampling site in the pre- and post-rainfall seasons. A) Specific Richness, B) Abundance, C) Pielou's indexes, and D) Shannon diversity index. Diagonal line bars represent values calculated in the pre-rainfall season, while the empty bars represent values calculated in the post-rainfall season. * Represents statistically significant differences between sampling sites. # Represents statistical differences between seasons. In all the graphs the bars represent mean ± SD. For symbols that represent statistical significance: one symbol $p < 0.05$, double symbol $p < 0.01$. *Valores de diversidad e índices calculados en cada sitio de muestre en las temporadas pre- y post-lluvia. (A) Riqueza específica, B) Abundancia, C) Índice de Pielou y D) Índice de diversidad de Shannon. Las barras diagonales representan los valores calculados en la temporada de pre-lluvia, mientras que las barras vacías representan los valores calculados en la temporada de post-lluvia * Representa las diferencias estadísticamente significativas entre los sitios de muestreo. # Representa las diferencias estadísticamente significativas de las temporadas. En todos los gráficos las barras representan la media ± DS. Para los símbolos que representan significancia estadística: un símbolo* p *<0.05, doble símbolo* p *<0.01.*

samples from Misitune (MST) presented the maximum value (0.82 ± 0.08) compared to Ancayuconi (ANY) (0.57 ± 0.02), establishing a statistical significance (p = 0.009) between these sites. Furthermore, with this index, there was a statistical difference between types of ecosystems (p = 0.006), observing the highest value (0.79 ± 0.004) in the lotic ecosystem, mainly influenced by the Misitune site (MST), where it was possible to determine values closer to 1. The Shannon diversity index presented the lowest value in the Paquisa site (PAQ) (1.39 ± 0.12), while the highest value was observed in Pisarata (PIS) (1.73 ± 0.26). Although there were observable trends, it was not possible to determine a statistical significance between them (Fig. 3D) (Table S4, Supplementary information available at http://www.limnetica.net/es/limnetica).

The nMDS analysis of the macroinvertebrate community showed the clear formation of five groups according to the sampling sites (Fig. 4). According to the ANOSIM analysis, this grouping pattern presented highly significant differences (ANOSIM R Global = 0.64, p = 0.001). The result of the pairwise test showed that all pairs of sites presented significant differences except between sites Pisarata (PIS) and Misitune (MST) (ANOSIM pairwise test R = 0.271, p = 0.057) (Table S5, Supplementary information available at http://www.limnetica.net/es/limnetica). In relation to the type of ecosystem (lentic/lotic), the formation of two groups with significant differences between them was observed. However, the grouping had less clarity and significance than the site factor (ANOSIM R Global = 0.31, p = 0.02). Regarding the seasons, the invertebrate assemblage did not show a significant grouping pattern (Fig. 4). The nMDS analysis of the environmental data matrix showed a clear grouping with highly significant differences of the site factor (ANOSIM R Global = 0.80, p = 0.001). The result of the pairwise test showed that all pairs of sites presented significant differences except between sites Ancuta (ANC) and Misitune (MST) (ANOSIM pairwise test R = 0.309, p = 0.6) (Table S5). A grouping pattern was also observed that agreed with the two types of ecosystems (lentic and lotic), which presented significant differences between them. However, the grouping had less clarity and significance than for the site factor (ANOSIM R Global = 0.12, p = 0.03). The season factor also presented significant differences, but these were

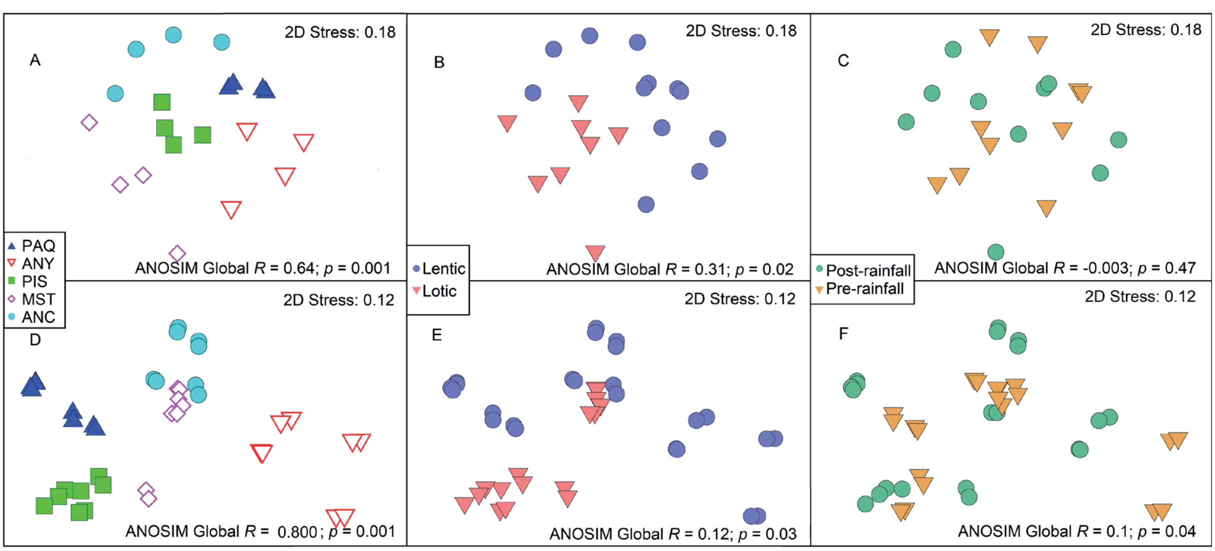

Figure 4. nMDS analysis of the biological (A, B and C) and environmental (D, E, F) matrix data according to the following factors: A-D = sites, B-E = type of ecosystem, C-F = season. *Análisis nMDS de las variables biológicas (A, B y C) y ambientales (D, E, F) de acuerdo con los siguientes factores A-D = sitios, B-E = tipo de ecosistema, C-F = temporada.*

Table 1. Similarity Percentage Analysis (SIMPER) identifying the contribution (%) of each taxon to the variance of the groups for the site and type of ecosystem factors. *Análisis de similitud porcentual (SIMPER) identificando la distribución de cada taxon en la varianza de los grupos, por sitio y tipo de factores de los ecosistemas.*

Taxa	Paq - Anc	Paq - Pis	Anc - Pis	Paq - Mst	Anc - Mst	Pis - Mst	Paq - Any	Anc - Any	Pis - Any	Mis - Any	Lentic - Lotic
Austrelmis sp	11.31	12		13.32			9.38		6.16	5.59	7.06
Ch. Orthocladiinae	11.16		13.75		14.7			11.09			7.69
Hyalella cf kochi	10.88	13.05	8.69	22.09	11.92	15.4	8.66	6.26		10.44	11.93
Plectidae *sp*	10.62		11.03		11.52			7.96			3.88
Ch. Podonominae	9.69	6.14	9.07		10.7	8.36		7.22			5.87
Ectemnostegella sp		10		9.2			6.47				
Simuliidae		7.65	6.15			9.77			6.16		4.08
Biomphalaria sp		7.11		6.56							
Ch. Chironominae			6.09		6.08						
Baetidae						7.24					3.79
Hydrachnidia						7.22					
Hydroptilidae						6.83					
Helicopsychidae							15.45	11.84	17.55	15.87	5.86
Leptoceridae							8.82	6.74	10.2	9.96	4.19
Sericostomatidae							6.24		7.39	6.89	
Ostracoda									6.21	6.07	

even less marked than the previous factors (ANOSIM *R* Global = 0.1, $p = 0.04$) (Fig. 4).

SIMPER's analysis indicates that most of the taxa contributed with a low percentage (ca. 10 %) to the differences. For the site factor, it was observed that the taxa *Austrelmis* sp., Orthocladiinae, *Hyalella cf kochi*, Plectidae sp., Podonominae, *Ectemnostegella* sp., Helicopsychidae, and Leptoceridae were indicators taxa that contribute the most differences when comparing between pairs of sites ($\geq$ 10 %.) For the type of system factor, the combination of taxa that most influenced these differences were the *Hyalella cf kochi*, Orthocladiinae, *Austrelmis* sp., Podonominae, and Helicopsychidae which together contributed more than 30 % of the differences. (Table 1). The SIMPER analysis was not carried out for the season factor since no significant differences were found.

The RDA triplot (Fig. 5) shows how the taxa are associated in a multivariate space with relation to the environmental variables according to the study sites. Ten of the 15 available environmental variables were considered in the model through the forward selection method, of which five were significant: P-Kjeldahl (p = 0.02), N-Kjeldahl ($p = 0.004$), Nitrite ($p = 0.004$), Nitrate ($p = 0.022$), hardness (p = 0.034) and temperature (p = 0.03). The adjusted explained variation of the model corresponded to 48.1 %. The first two canonical axes (RDA1 and RDA2) explained 45.23 % of the total variation (p = 0.004). Pisarata (PIS), Misitune (MST) and Paquisa (PAQ) did not present a degree of segregation between seasons,

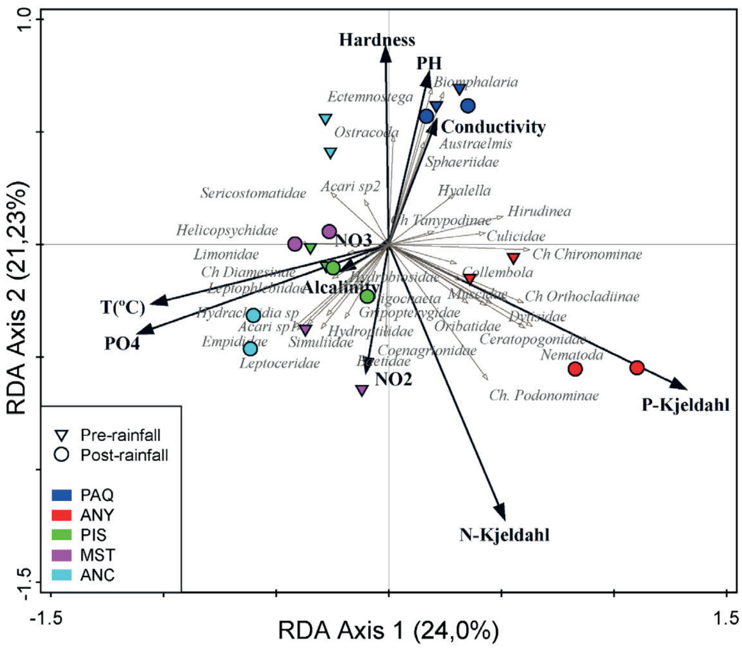

Figure 5. RDA for benthic macroinvertebrates. The first axis explains 45.23 % of the variance. Each of the sites is represented by a color and geometric figure indicates the season (triangle: pre-rainfall; circle: post-rainfall). *Análisis RDA para los macroinvertebrados bentónicos. El primer eje explica 45.23 % de la varianza. Cada uno de los sitios es representado por el color y la figura geométrica indica la temporada (triangulo: pre-lluvia; circulo: post-lluvia)..*

while in the sites of Ancuta (ANC) and Ancayuconi (ANY), segregation between seasons was observed in the second axis produced by environmental conditions.

DISCUSSION

Biodiversity components including abundance and composition can be shaped by the interaction of biotic and abiotic factors such as biological interactions, dispersal capacity, or by environmental conditions like climatic factors or habitat availability (Jacobsen & Dangles, 2017, Morán-Ordóñez et al., 2015). Previous studies have investigated how environmental factors are related to the benthic macroinvertebrate communities of high-altitude wetlands (Bertin et al., 2014, Llanquín-Rosas, 2019, Márquez-García et al., 2009, Villamarín et al., 2020, Vila et al., 2020). In this research found that, the variables P-Kjeldahl, N-Kjeldahl, Nitrite, Nitrate, hardness, and temperature are the major driving forces that affected the abundance and distribution of the macroinvertebrate assemblages in freshwater ecosystems of Lauca river basin.

Our *in situ* measurements´ values of physical and chemical variables were within the range of values registered in other highland aquatic ecosystems, such as those found in the Bolivian Altiplano (Molina et al., 2022) or the high wetlands of Argentina (Nieto et al., 2017) and Perú (Acosta et al., 2009). The conductivity values found in all studied sites are close to the lower limit but within the expected range (from 100 to 5000 uS/cm) of alkaline waters. Temperature had a wide range (6.6-17.8 °C), while the range of dissolved oxygen (7.4 - 8.6 °C) was more limited, similar to that indicated by Jacobsen & Marín (2008), that measured diel fluctuations of these variables on the Bolivian Altiplano.

As was expected for high Andean ecosystems, richness values were low (only 35 taxa) (Jacobsen & Dangles, 2017, Villamarín et al., 2020), yet the richness of taxa is linked to the level of identification, which is why this indicator could be underestimated as we used only the genus, family, or order level in most cases (Jones, 2008). Those taxa with the widest distribution among sites correspond to the classes Insecta, Malacostraca, and Clitellata, showing a high dominance of the phyla Arthropoda and Annelida, taxa with known adaptations that enable them to survive in regions with challenging environmental conditions (Glasbi et al., 2021, Yoshida & Tanaka, 2022). At the local scale, biotic factors such as the dispersion of species influence the adaptability of the benthic community to a particular type of environment and in consequence modulate the community in terms of composition and diversity (Bertin, 2014, Cottenie, 2005). This is in agreement with our results, since marked differences were found for the site and ecosystem type factors. It is relevant to mention that microhabitats within the sites have unique conditions (Pardo & Armitage, 1997), such as the source of origin of the water and the type of substrate or patches of macrophytes used as refuge by organisms. Therefore, multihabitat sampling within the sites could be required to fully characterize the diversity at a local and regional scale (González et al., 2020). Thus, taxa *Austrelmis* sp., Orthocladiinae, *Hyalella cf kochi*, Plectidae sp., and Helicopsychidae are the ones that most contributed to the differences found between the sites and were more consistent (see SIMPER results). For the ecosystem type factor, only *Hyalella cf kochi* contribute over 10 %, and taxa such as Orthochadiinae, *Austrelmis* sp., Podonominae, and Helicopsychidae also contribute to the dissimilarities between lentic and lotic ecosystems. The *Austrelmis* sp., and *Hyalella cf kochi* taxa are completely aquatic in habit, so their distribution is restricted to the ecosystem, preventing a greater degree of dispersion. in addition, *Hyalella cf kochi* has been described as a taxa that tends to occur in lentic environments (González, 2013). This is one of the few records of the macrobenthic community in the Lauca river basin. The results mainly indicate that the presence of rare abundant taxa is related to the characteristics of the ecosystem in which they inhabit, such as the presence of patches of macrophytes, substrate composition (e.g., sand, gravel, stones), lotic or lentic environments, which would reflect the adaptation of these specimens to particular environments. It is probable that in similar habitats that present similar environmental characteristics in the Altiplanic region of the Lauca river basin, community assemblages occur.

The physical and chemical variables that explained the variance in invertebrate communities among sites were the P-Kjeldahl, N-Kjeldahl, Nitrite, Nitrate, hardness, and temperature. Lentic ecosystems Misitune and Pisarata, where the substrate has a higher amount of organic matter, macrophytes and azonal vegetation were related to high values of nitrogen compounds and temperature. On the other hand, lotic ecosystems Paquisa and Ancayuconi, where a substrate with little to no organic matter, low coverage of macrophytes and azonal vegetation was observed, had higher values of hardness and P-Kjeldahl concentration. Ancuta was the site where the seasonality was more marked, where in the pre-rainfall season it was more related to high values of hardness while in post-rainfall it was more related to high values of temperature and nitrogen compounds. Nitrogen compounds have been reported as limiting primary production among organisms such as macrophytes and phytoplankton and could generate a bottom-up effect to the benthonic zoological community (Dorador et al., 2003, Llanquín-Rosas, 2019, Thiébaut, 2008, Wurtbaugh et al., 1991). Villamarin et al. (2020) carried out a study in tropical highland Andean, where the importance of environmental variability at the local level it was found determinant for the composition of the community, contrasting in two types of environments: montane forest and páramo-puna. In this sense, the composition of the substrate, such as the percentage of macrophyte patches or the flow of water, are abiotic factors that determine community assemblages.

The Lauca River Basin is host to unique flora and fauna with remarkable adaptations to the environmental conditions of the basin. However, despite being in a protected area, it confronts management challenges such as targeting conservation actions for rare or endangered species or human pressures such as tourism and mining activities in the region (Rundel & Palma, 2000). Furthermore, it has been reported that the effects of climate change will be especially severe in high altitude ecosystems where a faster temperature increase is expected than for lower lands. And regarding precipitation, it is expected that a change in rainfall patterns will occur, such as a wetter wet season and a drier dry season. These changes could also modify water flows, which are particularly relevant for arid or semi-arid ecosystems (Blin et al., 2022). Nevertheless, the lack of accurate climatic information about highland ecosystems limits the precision of future projections on climate change effects (Jacobsen & Dangles, 2017). Knowing both the components of biodiversity, as well as their relationship with biotic and abiotic factors, can help us to understand the functioning of biological communities and reinforce the basis for taking actions for its protection and conservation facing future environmental scenarios.

High altitude ecosystems are often hard environments as indicated by Jacobsen & Dangles (2017). These heterogeneous systems exhibit a wide variation in physical and chemical conditions, allowing an understanding of how environmental variation shapes biological patterns (Scheibler et al., 2020). This is one of the first studies providing basic information on different freshwater ecosystems present in the Lauca river basin, in terms of environmental characteristics, physical and chemical variables, and community records of the macroinvertebrate assemblages that inhabit them. This study is the first step to continue conducting research in this line to learn about these remote environments and thus, in the near future, identify conservation units in order to promote their preservation and good management of these habitats.

CONCLUSION

Here we described the diversity of the macroinvertebrate assemblage in the Lauca River Basin. 35 taxa were found, with dominance of Insecta, Malacostraca and Clytellata classes from the phyla Arthropoda and Annelida. A high heterogeneity was found between the studied sites and between the lentic and lotic ecosystem types, both in their macroinvertebrate assemblage and in the physical and chemical variables measured. In addition, seasonality (pre-rainfall versus post-rainfall) did not turn out to be a factor that significantly influenced the differences found. Finally, the physical and chemical variables that better explained the variance in invertebrate communities were P-Kjeldahl, N-Kjeldahl, Nitrite, Nitrate, hardness, and temperature. The results show the relevance of local conditions to shape the macroinvertebrates assemblages of these high-altitude systems, even in study sites belonging to the same basin. This information is relevant for decision-making in conservation.

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