

Taxonomic and functional structure of aquatic insect assemblages in headwaters from upland grasslands (Córdoba, Argentina)

Javier A. Márquez^{1,2} , María P. Rodríguez^{1,3} , Luciana Cibils-Martina^{1,2} , Ricardo J. Albariño⁴  and Romina E. Principe^{1,2,*} 

¹ Instituto de Ciencias de la Tierra, Biodiversidad y Ambiente (ICBIA), Universidad Nacional de Río Cuarto, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ruta 36 Km 601, CP X5804BYA, Río Cuarto, Argentina.

² Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina.

³ Departamento de Geología, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina.

⁴ Laboratorio de Fotobiología, INIBIOMA, CONICET, Universidad Nacional del Comahue, Quintral 1250, CP 8400, Bariloche, Argentina.

* Corresponding author: principe.romina@gmail.com

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ABSTRACT

Taxonomic and functional structure of aquatic insect assemblages in headwaters from upland grasslands (Córdoba, Argentina)

Although headwater streams play a key role in drainage catchments, they are among the most threatened habitats on earth. Natural open-canopy headwaters, in particular, have received little research attention in contrast to the most worldwide studied forested catchments. Then, the aim of this study was to assess the taxonomic and functional structure of insect assemblages in grassland headwaters contrasting three different habitats in two hydrological periods. Functional composition was quantified considering trophic and habit traits. We assessed the degree of assemblage overlapping among three habitat units (riffles, pools and macrophyte patches) and the differences in taxonomic and functional metrics among these stream habitats. Assemblages in macrophytes and riffles were rather overlapped and separated from the assemblage in pools. Macrophyte patches held the greatest insect abundance but richness and diversity were higher in riffles. Functional richness and diversity followed the same pattern found for the taxonomic analysis: higher in riffles, intermediate in macrophytes and lower in pools. The multivariate approach performed with functional groups concurred with the taxonomic analysis, being assemblages functionally different among habitats. Assemblages were dominated by gathering collectors, but the combined analysis of trophic and habit traits allowed disentangling the use of different habitats since collectors were mainly clingers in riffles and sprawlers in pools and macrophyte patches. Habitat characteristics affected the dominant habit within gathering collectors, with potential consequences for ecosystem processes (e.g. processing rates of fine organic material). This study provides valuable information on the taxonomic and functional structure of the insect community in grassland headwaters that can be useful for stream management and conservation.

Key words: macroinvertebrates, stream habitats, macrophyte, pool, riffle, functional diversity

RESUMEN

Estructura taxonómica y funcional del ensamble de insectos acuáticos en arroyos de cabecera en pastizales de altura (Córdoba, Argentina)

Aunque las cabeceras desempeñan un rol clave en las cuencas, se encuentran entre los hábitats más amenazados del planeta. Las cabeceras naturales no forestadas han sido poco investigadas en contraste con las forestadas. En consecuencia, el objetivo de este estudio fue analizar la estructura taxonómica y funcional del ensamble de insectos acuáticos en arroyos de cabecera en pastizales contrastando tres hábitats en dos periodos hidrológicos. La estructura funcional fue analizada considerando rasgos

alimentarios y hábitos. Evaluamos el grado de solapamiento entre rabiones, pozas y parches de macrófitas; y las diferencias en métricas taxonómicas y funcionales entre estos hábitats fluviales. Los ensambles en macrófitas y en rabiones se solaparon parcialmente y se separaron del ensamble de las pozas. Los parches de macrófitas alojaron la mayor abundancia, pero la riqueza y la diversidad fueron mayores en los rabiones. La diversidad y la riqueza funcional presentaron el mismo patrón que el análisis taxonómico: fueron mayores en los rabiones, intermedias en las macrófitas y menores en las pozas. El análisis multivariado de grupos funcionales coincidió con el análisis taxonómico siendo los ensambles funcionalmente diferentes entre los hábitats. Los recolectores fueron predominantes, pero el análisis combinado de los rasgos alimentarios y los hábitos permitió distinguir el uso de los diferentes hábitats ya que los recolectores fueron principalmente agarradores en rabiones y reptadores en pozas y macrófitas. Las características del hábitat afectaron en consecuencia el hábito dominante dentro de los recolectores, con consecuencias potenciales sobre los procesos del ecosistema (e. g. tasas de procesamiento de la materia orgánica fina). Este estudio provee valiosa información sobre la estructura taxonómica y funcional de la comunidad de insectos acuáticos en cabezeras en pastizales que puede ser útil para el manejo y la conservación del recurso.

Palabras clave: macroinvertebrados, hábitats fluviales, macrófitas, pozas, rabiones, diversidad funcional

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INTRODUCTION

Headwater streams play a key role in drainage catchments since they constitute areas of stream flow generation, they are source of organic matter and invertebrates, they represent critical sites for nutrient processing and they support unique biodiversity (Richardson, 2019). These freshwater ecosystems are particularly sensitive to stressors (dams, chemical pollution, exotic trees afforestation, etc.) and they are among the most threatened habitats on Earth (Wohl, 2017). Among the macroinvertebrates that inhabit headwater streams, aquatic insects are the most diverse and abundant taxonomic group and they are key contributors to ecosystem functions (Wallace & Webster, 1996). They are involved in organic matter processing, nutrient cycling and they are an important component of aquatic and terrestrial food webs (Covich *et al.*, 1999; Graça, 2001; Paetzold *et al.*, 2008). In addition, aquatic insects are widely used as bioindicators since they have proven to be an efficient diagnostic tool for monitoring water quality and ecological integrity of river systems (Bonada *et al.*, 2006a).

Spatial distribution pattern of aquatic insects among different habitats reflects the optimal adjustment between habit and physical environmental conditions (Merritt & Wallace, 2009). In headwater streams the spatiotemporal heterogeneity is represented by riffle-pool sequences (Brooks *et al.*, 2005). These habitats differ in current velocity,

depth and substrate type as well as in their capacity to transport, retain and produce basal resources (Hoover *et al.*, 2010). Although many studies have reported differences in the taxonomic and functional structure of invertebrate assemblages between riffle and pool habitats (e.g. Velásquez & Miserendino, 2003; Halwas *et al.*, 2005; Bonada *et al.*, 2006b; Tomanova & Usseglio-Polatera, 2007; Principe, 2008; Milesi *et al.*, 2016) there is still a lack of this information for open headwater streams, in which functional organization may be different to those of forested headwaters since sunlight constitute the basal resource.

Erosional habitats in streams with unshaded bottoms are usually covered by patches of macrophytes which increase habitat complexity (Thomaz & da Cunha, 2010). These heterogeneous areas on the streambed are colonized by great abundance and diversity of aquatic insects as they offer food and refuge (Hershey *et al.*, 2010). Although phytophilous invertebrate fauna have been widely studied in lowland streams and wetlands (i.e. Feldman, 2001; Ferreira *et al.*, 2011; Damborsky *et al.*, 2012; Tarjányi & Berczik, 2014) there are few studies addressing assemblages associated to macrophytes in mountain streams (Rodríguez *et al.*, 2017).

In central Argentina, in contrast to the most worldwide studied forested catchments, headwater streams drain upland grasslands and are located in a particular biogeographic province named Comechingones (Arana *et al.*, 2017). These

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mountain streams are autotrophic due to high light availability and their riparian zones are endangered mainly by pine afforestation and cattle grazing. The structure and composition of invertebrate assemblages in riffles have been already reported for these streams (Márquez et al., 2015) but information about the distribution of aquatic insects in other habitats such as pools and macrophyte patches is still missing. The knowledge of habitat heterogeneity and their associated biodiversity (taxonomic and functional) is important to properly establish the biological reference condition for water quality assessments (Oliveira & Cortes, 2005; Friberg et al., 2011; Curry et al., 2012), in particular, in open-canopy headwater streams from which research is scarce.

The aim of our study was to assess the taxonomic and functional structure of the insect assemblages in grassland headwater streams contrasting three different habitats in two hydrological periods. We assessed the degree of assemblage overlapping among habitat units (riffles, pools and macrophyte patches), we identified indicator taxa and evaluated the differences in taxonomic and functional metrics among stream habitats. We hypothesized that hydraulic characteristics and structural complexity of each habitat determine the structure and function of insect assemblages. Since more heterogeneous environments favor the establishment of more diverse communities (Warfe et al., 2008), we expect greater richness and abundance in macrophyte patches than in pools, being riffles intermediate sites. In addition, functional diversity is expected to be also higher in macrophyte patches due to the structural complexity of plants that offers a wide range of microhabitats.

MATERIALS AND METHODS

Study area

The study was carried out in streams of the Ctlamochita River upper basin, Córdoba, Argentina. This river is one of the main tributaries of the Carcaraña River and belongs to La Plata River basin. Headwaters of the Ctlamochita River are located in grasslands of the Comechingones Mountains between 900 and 1600 m a.s.l. The li-

thology is dominated by granitic rocks and localized patches of metamorphic rocks (gneiss, schist, migmatite) are also present. Annual precipitation in the region reaches 1000 mm occurring mostly between spring and the end of summer (austral region: October-March) (Cabido et al., 2003) and determining a relatively dry mountainous landscape. Maximum air temperature reaches 34 °C in summer (December-March) and decreases up to -5 °C in winter (June-September). Grasslands are dominated by *Festuca hieronymi* Hack., *Nassella filiculmis* (Delile) Barkworth, *Schizachyrium condensatum* (Kunth) Nees and *Eragrostis airoides* Nees (Oggero & Arana, 2012). They are primarily used for extensive livestock grazing and other human activities like extraction of medicinal and aromatic herbs that contribute to landscape modification (Cabido et al., 2003).

For our study we selected three first-order streams (Stream 1: 31° 58' 56" S, 64° 43' 25" W; Stream 2: 31° 58' 52" S, 64° 46' 29" W; Stream 3: 31° 58' 47" S, 64° 48' 41" W) in sites with similar exposure to sunlight, altitude (Stream 1: 1130 m a.s.l., Stream 2: 1157 m a.s.l., Stream 3: 1175 m a.s.l.) and drainage area (Stream 1: 31.2 ha, Stream 2: 84.4 ha, Stream 3: 86.7 ha). The study streams belong to the Comechingones biogeographic province according to the new regionalization of high altitude grasslands (Arana et al., 2017).

Field and laboratory methods

Aquatic insect samples were obtained in each stream in three fluvial habitats: riffles, pools and macrophyte patches during different seasons: summer (February 2012) and winter (August 2012). We consider these two opposite seasons that varied not only in discharge (winter: low flow, summer: high flow) but also in water temperature as contrasting conditions for stream fauna. Two replicate samples were collected with a Surber sampler (0.09 m²; 300 µm mesh size) in riffle habitats and three replicate samples were obtained with a Hess sampler (0.07 m²; 300 µm mesh size) in pools. For sampling of phytophilous insects, the most abundant macrophyte was determined by estimating the surface covered by each species in a 50 m reach. Since the macrophyte patches were mainly located in riffles, we con-

sider three riffles with macrophytes for sampling phytophilous insects and three other riffles without macrophytes for sampling insects in riffles *per se*. Then, three samples were taken in three different patches of the dominant macrophyte with an entomological water net (32 cm diameter; 300 μm mesh size).

Depth was measured in each sample site and current velocity was measured in riffles with a digital water velocity meter (Global Water flow meter FP101, Sacramento, California). Temperature, pH and conductivity measurements were taken in each stream and period with a portable sensor (Multiparameter PCS TESTR 35 Eutech - Oakton, Vernon Hills, Illinois). Substrate type was visually assessed (Gordon *et al.*, 2004) and channel width was measured with a measuring tape in pool and riffle sections. Finally, one water sample was obtained in each stream and hydrological period and brought back to the laboratory for chemical analysis according to standard methods (APHA, 1998).

Insect samples were preserved in 80 % ethanol and processed in the laboratory. Insects were identified to the lowest possible taxonomic level with specific keys (Domínguez & Fernández, 2009) and counted. Abundance was calculated as the number of individuals per m^2 . In the case of macrophyte patches, the surface covered by the plant was measured to estimate the area. Insects were also categorized into five trophic traits (i.e. functional feeding groups) and six habit trait groups according to Merritt & Cummins (1996), Tomanova *et al.* (2006), Principe *et al.* (2010) and Reynaga & Dos Santos (2012) resulting in 30 possible trait combinations. Functional feeding groups included shredders, gathering collectors, filtering collectors (hereafter gatherers and filterers), scrapers and predators. Habit trait groups included burrowers, climbers, clingers, divers, sprawlers and swimmers. Functional feeding groups thus refer to the feeding mode and food type, whereas habit trait groups provide information on the relative mobility and where food is obtained. Both of these characteristics may be important with regard to the functional roles of insects in stream ecosystems (Heino, 2005). Combinations of functional feeding groups and habit trait groups were used, and the 15 observed com-

binations were named subsequently as functional groups (Table S1, supplementary material available at <http://www.limnetica.net/en/limnetica>).

Data analyses

Two-way ANOVAs (habitat and season as fixed factors) were performed to compare depth and channel width between habitats and seasons. Current velocity in riffles was compared between seasons with one-way ANOVA (factor: season). We included streams and replicates as random factors (replicates nested within streams) in the models. In addition, proportional abundance of the different substrates was compared between riffles and pools with one-way ANOVAs (factor: habitat). Validations of simple assumptions of the models were performed by reviewing standardized residuals vs predicted plot and the Shapiro-Wilks test of normality. Depth values were $\text{Log}_{10} Y$ transformed and *a posteriori* comparisons were made using the DGC exclusionary group formation test (Di Rienzo-Guzman-Casanoves), a hierarchical method that controls type I error while maintaining acceptable power (Di Rienzo *et al.*, 2002). All analyses of variance were performed with INFOSTAT statistical software version 2012 (Di Rienzo *et al.*, 2012).

The degree of assemblage overlap (taxonomic and functional) were assessed among riffles, pools and macrophyte patches by non-metric multidimensional scaling (NMDS) and the analysis of similarities (ANOSIM) based on the Bray-Curtis dissimilarity index. ANOSIM is based on comparing distances between groups with distances within groups which are converted to ranks. A large positive R (up to 1) indicates dissimilarity between groups (Clarke, 1993). The significance was computed by permutation of group membership, with 10 000 replicates. We used two-way ANOSIM (factors: habitat and season) and abundance data were $\text{Log}_{10} Y+1$ transformed. Both analyses were performed with the software PAST version 3.13 (Hammer *et al.*, 2001).

The Indicator Value method (IndVal) (Dufrière & Legendre, 1997) was used to statistically determine the most representative insect taxa for each habitat. IndVal identifies characteristic taxa from a predefined group (i.e. habitats) based on

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the relative frequency of occurrence of a taxon in samples of one group and the mean relative abundance of that taxon in samples of one group compared with all groups. The indicator value ranges from 0 to 100 and the later corresponds to a perfect indication. Significance of each taxon was tested using Monte Carlo test with 1000 permutations. Taxa with significant indicator values ($p < 0.05$) were considered indicators of a specific habitat type. The IndVal method was carried out using PC-ORD version 5.0 (McCune & Mefford, 1999). In addition, taxonomic and functional rank abundance curves were built for each stream habitat and for both hydrological periods ordering taxa and functional groups by their relative abundance (only entities with relative abundance $>1\%$). Entities were ordered from the dominant to the less abundant in each habitat and hydrological period. Hence, these curves visually depict replacement in dominant species and functional groups, and evenness (i.e. reflected by plot slope) either among fluvial habitats or hydrological periods.

Rarefaction curves were performed using PAST version 3.13 in order to compare richness among the different habitats and seasons. This method allows comparing the number of expected species per site (i.e. habitat and season), based on the lowest number of individuals found in the sites to be compared. Additionally, three metrics were calculated to study the taxonomic structure of the insect assemblages: total abundance, diversity ($\exp^{\text{Shannon } H'}$, Jost 2006) and evenness (Shannon J'). In addition, three metrics were calculated to assess the assemblage functional structure: functional richness (number of functional trait groups), functional diversity ($\exp^{\text{Shannon } H'}$ of functional trait groups) and functional evenness (Shannon J' of functional trait groups). Ln was used for all Shannon H' and J' calculations. To compare these six metrics among habitats and seasons we used two-way ANOVAs. We included streams and replicates as nested random factors. Validations of simple assumptions of the models were performed by reviewing standardized residuals vs predicted plot and the Shapiro-Wilks test of normality. Total abundance values were $\text{Log}_{10} Y$ transformed and the DGC test was used for *a posteriori* comparisons. Two-way ANOVAs was carried out using Infostat version 2012 (Di Rienzo et al., 2012).

RESULTS

Physicochemical measurements and habitat characterization

Physicochemical variables were mostly similar among streams (Table S2, supplementary material, available at <http://www.limnetica.net/en/limnetica>). Water temperature was in average 15.2 ± 1.6 °C (mean \pm SE) during winter and 18.8 ± 1.1 °C during summer. Overall, pH was basic ranging between 7.6 and 8.6. Water conductivity was relatively low and similar among streams and also between seasons (winter: 80.67 ± 50.19 $\mu\text{S/cm}$, summer: 84.33 ± 9.35 $\mu\text{S/cm}$).

Current velocity in riffles was significantly faster in summer (rainy season) (ANOVA, $F = 7.75$, $p = 0.0387$; summer: 0.47 ± 0.04 m/s, winter: 0.27 ± 0.06 m/s). As current flow was barely perceptible in pools and macrophyte patches, current velocity could not be measured in these habitats since these low values were not detected by the current velocity meter. Differences in channel depth among fluvial habitats depended on seasons, but pool habitats were consistently deeper in both seasons (ANOVA, $F_{(\text{habitat} \times \text{season})} = 6.10$, $p = 0.0054$; pools in winter: 0.24 ± 0.02 m, pools in summer: 0.28 ± 0.03 m). Riffle and macrophytes only differed in summer, when macrophytes presented higher depth (ANOVA, $F_{(\text{habitat} \times \text{season})} = 6.10$, $p = 0.0054$; macrophytes in summer: 0.17 ± 0.03 , riffles in summer: 0.11 ± 0.03 m). In addition, channel width did not show differences between riffle and pool sections (ANOVA_(habitat): $F_{(1,12)} = 0.24$, $p = 0.630$; riffles: 1.24 ± 0.23 m; pools: 1.45 ± 0.25 m).

Substrate composition did not reveal major differences between riffle and pool habitats (Table S3, supplementary material, available at <http://www.limnetica.net/en/limnetica>). They were both predominantly composed by bedrock and boulders. Only sand proportion was higher in pool habitats (ANOVA: $F = 6.25$, $p = 0.025$; pools: $10.71 \pm 3.52\%$; riffles: $2.75 \pm 1.08\%$). Macrophytes were mostly located in riffle sections with fine substratum like sand, pebbles and gravels.

A total of 16 macrophyte species were identified in the studied streams (Table S4, supplementary material, available at <http://www.limnetica.net/en/limnetica>).

limnetica.net/en/limnetica) but in general macrophytes cover surface was less than 10 % (stream 2: 1 % cover, stream 3: 10 % cover), except in stream 1 in which the cover reached 70 %. *Hydrocotile* spp., *Rorippa nasturtium-aquaticum* (L.) Hayek and *Ranunculus flagelliformis* Sm. were the most frequent and abundant species from which insect samples were taken.

Table 1. Indicator values (IndVal) of insect taxa for riffles, pools and macrophyte patches. Monte Carlo test was used to assess the significance of each taxon as an indicator for a respective stream habitat. Only taxa with significant indicator values ($p < 0.05$) are listed. *Valores indicadores (IndVal) de insectos para rápidos, pozas y parches de macrófitas. La significancia de cada taxón como indicador de un hábitat respectivo se comprobó mediante el test de Monte Carlo. Se muestran solamente los taxa con valores indicadores significativos ($p < 0.05$).*

	IndVal	<i>p</i> -test
RIFFLES		
Psychodidae	54.0	0.0006
<i>Baetodes</i> spp.	47.4	0.0010
Empididae	47.0	0.0014
<i>Camelobaetidius</i> spp.	46.7	0.0002
Lutrochidae	41.0	0.0038
<i>Nanomis</i> spp.	40.8	0.0060
<i>Petrophila</i> spp.	40.0	0.0008
<i>Leptohyphes</i> spp.	39.3	0.0468
<i>Cyloepus</i> spp.	33.0	0.0038
Stratiomyidae	25.0	0.0140
POOLS		
<i>Caenis</i> spp.	67.1	0.0112
<i>Tanytarsus</i> spp.	60.8	0.0022
<i>Callibaetis</i> spp.	38.6	0.0088
Macropelopini	32.3	0.0124
MACROPHYTE PATCHES		
<i>Corynoneura</i> spp.	81.1	0.0002
<i>Rheotanytarsus</i> spp.	76.8	0.0006
<i>Americabaetis</i> spp.	68.6	0.0030
<i>Parametriocnemus</i> spp.	55.5	0.0308
<i>Heterelmis</i> spp.	54.4	0.0316
<i>Banyallarga</i> spp.	46.0	0.0114
Ceratopogonidae sp. 2	42.9	0.0198
<i>Oxyethira</i> spp.	35.3	0.0206
Aeshnidae	38.0	0.0434

Taxonomic composition and structure

A total of 79 taxa were found in the study streams (Table S1, supplementary material), 49.4 % of the taxa were shared by the three habitats, 25.3 % by two and 24.3 % were not shared among habitats and were evenly apportioned (7 taxa were exclusive of riffles, 6 only in pools and 7 in macrophytes). In relation to temporal composition, 57 taxa were present in both seasons, 12 taxa were only sampled during winter and 10 taxa during summer. The riffle beetle *Austrelmis* spp., the mayfly *Caenis* spp. and the non-biting midges *Cricotopus* spp. and *Pseudochironomus* spp. were the most frequent taxa since they were found in all analyzed samples. *Corynoneura* spp. and *Rheotanytarsus* spp. were the most abundant taxa followed by the mayfly *Americabaetis* spp.

The NMDS revealed significant differences in the assemblages among the three fluvial habitats, with riffles grouping close to macrophyte patches and pool assemblages isolated along axis 1 (Fig. 1; two-way ANOSIM, factor habitat: $R = 0.743$, $p = 0.0001$). Assemblages were also dissimilar between seasons (two-way ANOSIM, Factor season: $R = 0.741$, $p = 0.0001$). Indicator taxa (IndVal method) showed differences among fluvial habitats as well, since they varied in number and identity (Table 1). Riffle habitats were characterized by 10 indicator taxa being Psychodidae, *Baetodes* spp., Empididae and *Camelobaetidius* spp. those with the greatest indicator values. Macrophyte patches were characterized by nine indicator taxa, having *Corynoneura* spp. the highest score. In contrast, pool habitats were depicted only by four indicator taxa.

In winter, riffles were dominated by *Austrelmis* spp. and followed by *Corynoneura* spp. and the black fly *Simulium* spp. (Fig. 2). Although pools exhibit a great variety of chironomid fauna they were strongly dominated by *Caenis* spp. whereas macrophytes were dominated by *Parametriocnemus* spp. During summer, macrophytes changed their dominant taxa to *Corynoneura* spp. In riffles, the mayfly *Camelobaetidius* spp. strongly increased their abundance and the same happened with *Austrelmis* spp. in pools.

Rarefaction analysis differentiated two main groups of habitats according to their taxonom-

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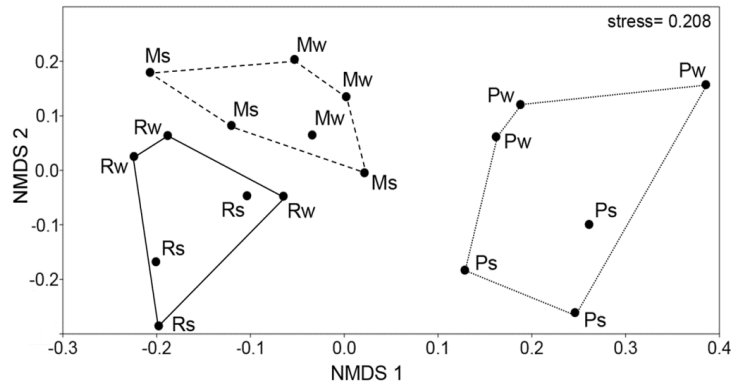


Figure 1. NMDS of the insect assemblages in three stream habitats (riffles: R, pools: P, macrophyte patches: M) of mountain grassland streams (Córdoba, Argentina) during two contrasting seasons (winter: w and summer: s). *NMDS de los ensambles de insectos en tres hábitats fluviales (rápidos: R, pozas: P, parches de macrófitas: M) de arroyos de montaña en pastizales (Córdoba, Argentina) en dos estaciones contrastantes (invierno: w y verano: s).*

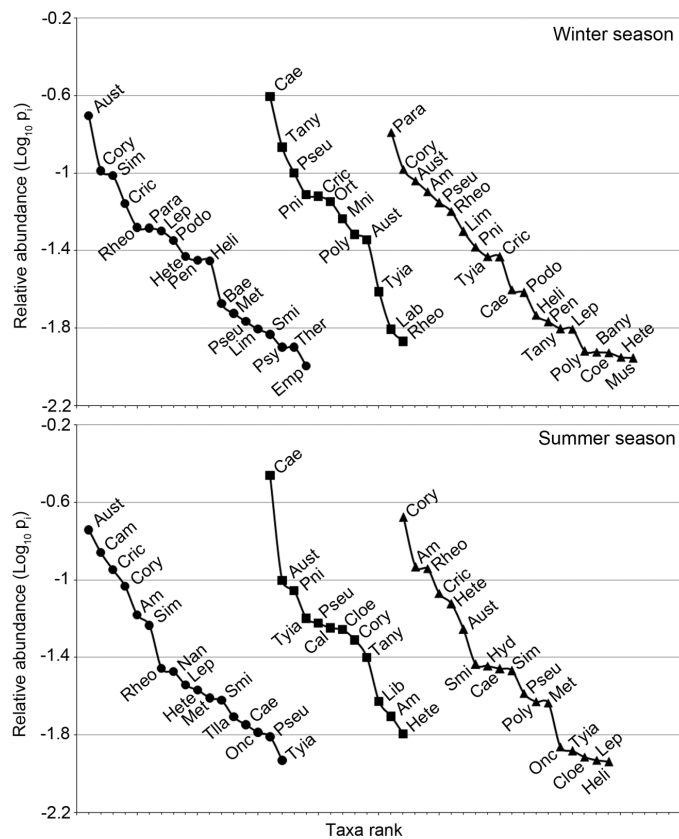


Figure 2. Rank abundance curves of insect assemblages in three stream habitats of mountain grasslands streams (Córdoba, Argentina) during two seasons (winter and summer): riffles (circles), pools (squares) and macrophyte patches (triangles). Taxa codes are included in Table S1, supplementary material. *Curvas de rango abundancia de los ensambles de insectos en tres hábitats fluviales de arroyos de montaña en pastizales (Córdoba, Argentina) en dos estaciones (invierno y verano): rápidos (círculos), pozas (cuadrados) y parches de macrófitas (triángulos). Los códigos de los taxa se incluyen en la Tabla S1 del material suplementario.*

ic richness (Fig. 3). In general, assemblages in pools (winter: 45, summer: 45.2 ± 2.9) and in macrophyte patches during summer (41.9 ± 2.9) were characterised by lower richness compared to those in riffles (winter: 50.1 ± 2.7 , summer: 48.5 ± 2.2) and macrophyte patches during winter (49.6 ± 2.0). Assemblages in macrophyte patches in summer reached the lowest estimated richness, whereas those in riffles during winter exhibited the highest one.

Metrics of community structure differed among fluvial habitats as shown by ANOVA (Table 2). Similarly to richness, diversity was highest in riffles, whereas pools exhibited the lowest values (mean values \pm SE: pools = 6.46 ± 0.55 , macrophytes = 10.27 ± 0.55 , riffles = 12.05 ± 0.67). Evenness was also lower in pools but did not differ between riffles and macrophytes (mean values \pm SE: pools = 0.67 ± 0.01 , riffles = 0.74 ± 0.02 , macrophytes = 0.76 ± 0.01). The difference in total abundance of insects among habitats was influenced by seasons. The highest abundance was registered in macrophyte patches in summer and the lowest values were found in pools in both seasons and in riffles during summer (Table 2).

Functional structure

The NMDS showed significant functional differences among the three fluvial habitats, with pool assemblages separated by the two ordination axes (Fig. 4; two way ANOSIM: factor habitat: $R = 0.469$, $p = 0.002$). Interestingly, different seasons did not influence community functional composition (two way ANOSIM: factor season: $R = 0.148$, $p = 0.172$). Gatherer clingers strongly dominated riffles in both seasons (Fig. 5). Scraper swimmers became abundant during summer whereas in winter gatherer sprawlers and filterer clingers were co-dominant. Pools were dominated by gatherer sprawlers and clingers as well as predator sprawlers during the winter, whereas gatherer sprawlers strongly dominated in summer. Gatherer sprawlers and clingers were also the most abundant functional groups in macrophyte patches in both seasons.

Functional attributes also distinguished assemblages among fluvial habitats, as shown by two-way ANOVAs (Table 2), but contrary to structural attributes, these differences depended on seasons. Functional richness was influenced by season only in riffle habitats in which the

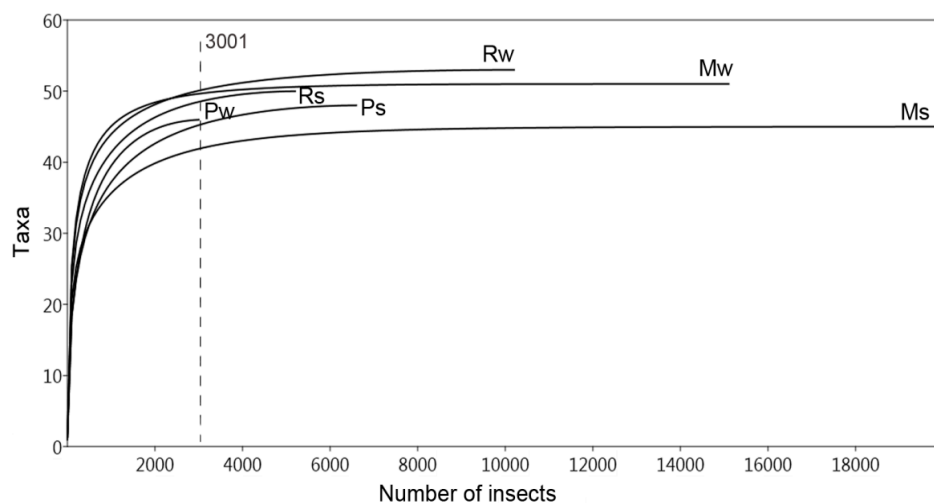


Figure 3. Rarefaction curves based on the number of individuals of the insect assemblages in three stream habitats (riffles: R, pools: P, macrophyte patches: M) of mountain grassland streams (Córdoba, Argentina) during two contrasting seasons (winter: w and summer: s). The minimum number of individuals is indicated with a dashed line. *Curvas de rarefacción basadas en el número de individuos de los ensambles de insectos en tres hábitats fluviales (rápidos: R, pozas: P, parches de macrófitas: M) de arroyos de montaña en pastizales (Córdoba, Argentina) en dos estaciones contrastantes (invierno: w y verano: s). El número mínimo de individuos está indicado con una línea punteada.*

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highest values were found in winter. The lowest functional richness was found in pools and macrophytes exhibited intermediate values (Table 2, mean values \pm SE: pools winter = 8.78 ± 0.19 , pools summer = 8.89 ± 0.19 , macrophytes win-

ter = 9.67 ± 0.19 , macrophytes summer = 9.89 ± 0.19 , riffles summer = 11.17 ± 0.23 , riffles winter = 12.50 ± 0.23). Functional diversity was influenced by seasons only in macrophyte patches. The highest values were found in riffles (both sea-

Table 2. Taxonomic and functional attributes of the insect assemblages compared by two-way ANOVAs using habitat (riffles, pools and macrophyte patches) and season (winter and summer) as fixed factors. Degree freedom(factor;error): habitat(2;34), season(1;34) and habitat x season(2;34). *Atributos taxonómicos y funcionales de los ensambles de insectos comparados mediante ANOVA de dos vías. Hábitat (rápidos, pozas y parches de macrófitas) y estación (invierno y verano) se consideraron como factores fijos. Grados de libertad (factor; error): hábitat(2;34), estación(1;34) and hábitat x estación(2;34).*

Variable	Factor	F	p	A posteriori
Total abundance	Habitat	52.04	<0.001	
	Season	<0.01	0.977	
Diversity	Habitat*Season	14.51	0.005	P _s = R _s = P _w < R _w = M _w < M _s
	Habitat	23.55	0.001	P < M < R
	Season	0.94	0.368	
Evenness	Habitat*Season	0.22	0.805	
	Habitat	15.68	0.004	P < R = M
	Season	1.79	0.229	
Functional richness	Habitat*Season	1.81	0.242	
	Habitat	82.70	<0.001	
	Season	11.27	0.015	
Functional Diversity	Habitat*Season	20.92	0.002	P _w = P _s < M _w = M _s = R _s < R _w
	Habitat	5.22	0.048	
	Season	3.71	0.102	
Functional evenness	Habitat*Season	6.24	0.041	P _w = M _s = P _s < R _w = R _s = M _w
	Habitat	2.09	0.204	
	Season	1.20	0.315	
	Habitat*Season	10.93	0.010	M _s = P _w = R _w = R _s = P _s < M _w

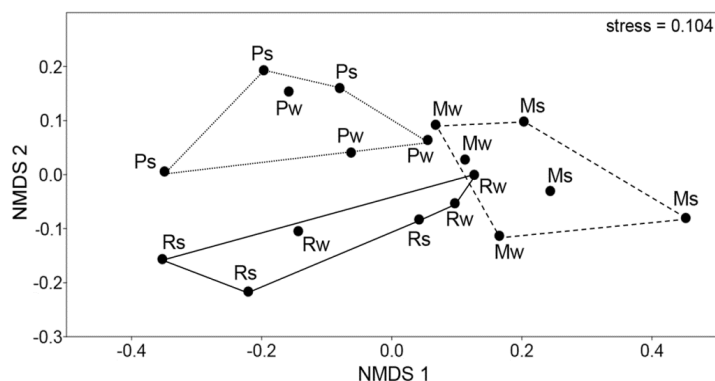


Figure 4. NMDS of the insect functional groups present in three stream habitats (riffles: R, pools: P, macrophyte patches: M) of mountain grassland stream (Córdoba, Argentina) during two contrasting seasons (winter: w and summer: s). *NMDS de los grupos funcionales de insectos presentes en tres hábitats fluviales (rápidos: R, pozas: P, parches de macrófitas: M) de arroyos de montaña en pastizales (Córdoba, Argentina) en dos estaciones contrastantes (invierno: w y verano: s).*

sons) and in macrophytes during winter whereas the lowest functional diversity was found in pools (both seasons) and macrophytes in summer (Table 2, mean values \pm SE: pools winter = 4.06 ± 0.26 , macrophytes summer = 4.28 ± 0.26 , pools summer = 4.41 ± 0.26 , riffles winter = 5.1 ± 0.32 , riffles summer = 5.12 ± 0.32 , macrophytes winter = 5.7 ± 0.26). Evenness showed the highest value in macrophytes during winter (Table 2, mean values \pm SE: macrophytes summer = 0.63 ± 0.02 , pools winter = 0.63 ± 0.02 , riffles winter = 0.64 ± 0.03 , riffles summer = 0.67 ± 0.03 , pools summer = 0.68 ± 0.02 , macrophytes winter = 0.76 ± 0.02).

DISCUSSION

Taxonomic composition and structure

Our results showed a clear distinction between assemblages at the habitat level. Riffles and macro-

phyte patches featured much more indicator taxa than pools. However, dipterans and ephemeropterans found in macrophytes and pools displayed the highest levels of habitat fidelity and specificity. Other studies had reported variable results in relation to habitat differentiation (McCulloch, 1986; Bonada *et al.*, 2006b). According to our results, pool-riffle sequences presented differences in depth, current velocity and substrate size distribution. Such differences shape the relative importance of basal resources, e.g., periphyton abundance (Rosenfeld & Hudson, 1997) and terrestrially derived organic matter (Hoover *et al.*, 2010). In concert, these differences in abiotic and trophic variables determine contrasting habitats which are either suitable for more lentic or rheophilic biota, delimiting then different insect assemblages in each stream habitat.

Riffles in naturally forested low order streams have been reported as stream habitats holding

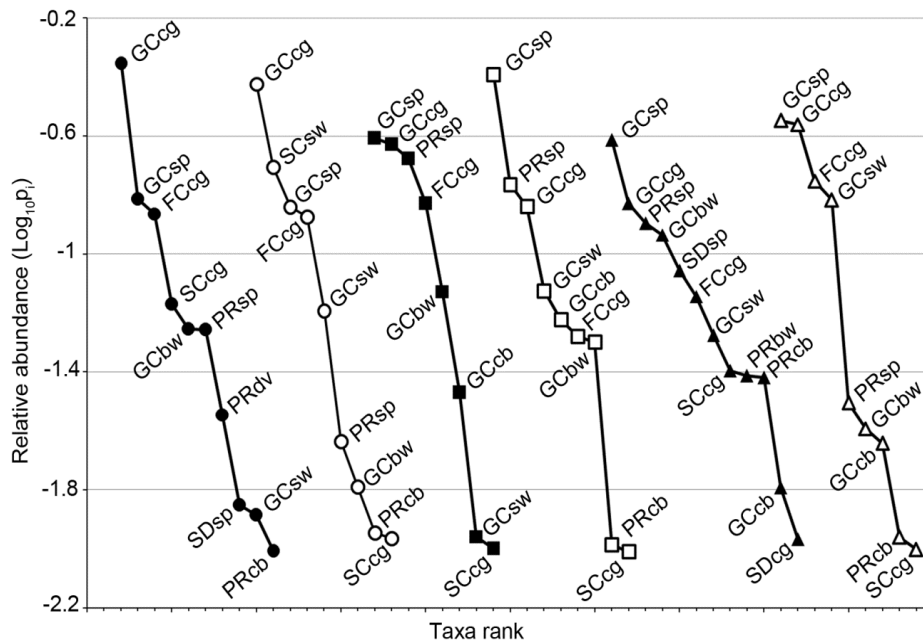


Figure 5. Rank abundance curves of insect functional groups in three stream habitats: riffles (circles), pools (squares) and macrophyte patches (triangles); during two seasons (winter and summer, filled and empty symbols, respectively). Functional group codes: GC (Gathering collectors), FC (filtering collectors), SC (scrapers), SD (shredders), PR (predators), cg (clingers), sp (sprawlers), sw (swimmers), dv (divers), cb (climbers), bw (burrowers). *Curvas de rango abundancia de los grupos funcionales de insectos en tres hábitats fluviales: rápidos (círculos), pozas (cuadrados) y parches de macrófitas (triángulos); en dos estaciones (invierno y verano, símbolos llenos y vacíos, respectivamente). Códigos de los grupos funcionales: GC (recolectores), FC (filtradores), SC (raspadores), SD (desmenuzadores), PR (depredadores), cg (agarradores), sp (reptadores), sw (nadadores), dv (buceadores), cb (trepadores), bw (excavadores).*

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higher invertebrate density and diversity than pools (Velásquez & Miserendino, 2003; Halwas et al., 2005; Bonada et al., 2006b; Principe, 2008), which is in agreement with our findings. However, other authors reported the opposite pattern (McCulloch, 1986; Boulton & Lake, 1992), or found no differences between habitats (Costa & Melo, 2008). In our study streams, macrophyte patches held the greatest insect abundance which agrees with other studies that also reported higher invertebrate densities in sites colonised by macrophytes (Hershey et al., 2010; Habib & Yousuf, 2015). Contrary to our expectations, taxonomic richness and diversity were higher in riffles or were similar to those found in macrophytes suggesting that riffles in unshaded grassland streams are the most heterogeneous environments. In addition, assemblage composition reflected the patterns of univariate metrics being macrophytes and riffles slightly overlapped and separated from pools. Although macrophyte cover was rather scarce in our study streams, our results suggest that patches of aquatic plants became a distinct habitat sustaining a particular insect assemblage.

When considering seasonal variation, insect density and richness were split into three main groups. Macrophytes supported the largest abundance but low richness and diversity during the summer. At the other end, pools had assemblages with low abundance, richness and diversity regardless seasons. Assemblages in macrophytes during winter and in riffles, in both seasons, had intermediate densities but held the highest richness and diversity. Then, our results showed that insect assemblages in macrophyte patches displayed the greatest seasonal variation probably because aquatic plants are more susceptible to disturbance engendered by floods (Janauer et al., 2010) that occur during summer (rainy season). On the other hand, pool habitats are less susceptible to flow variation since they are characterized by higher depth.

Functional structure

Functional structure of the insect assemblages was also different among the studied habitats. Overall, assemblages were dominated by gatherers. This finding agreed with the results found for third-or-

der streams in the area (Principe et al., 2010) and with the functional description of stream insect communities in other regions with natural riparian forest in headwaters (Velásquez & Miserendino, 2003; Fierro et al., 2015; Silva-Araújo et al., 2020). However, contrary to what we found in our open-canopy streams, shredders are abundant in forested headwaters and may be also represented by several insect taxa (Velásquez & Miserendino, 2003; Masese et al., 2014; Fierro et al., 2015). In our study, shredders were only represented by 3 taxa which showed low frequency and abundance (less than 5 % of proportional abundance) but scrapers were abundant in riffles, which is expected for streams in which benthic algae represent the primary production supporting food webs (Roberts et al., 2004; Cibils-Martina et al., 2017).

Contrary to our prediction, riffles exhibited greater functional diversity than macrophytes. However, the combined analysis of trophic and habit traits allowed disentangling the use of different habitats. For instance, gatherers, which dominated assemblages throughout the study, were represented by two basic habit traits: on the one hand by clingers, better adapted to riffles, and on the other hand by sprawlers with affinity to pools and macrophyte patches. Therefore, considering both the diversity of food resources and the place in which they can be obtained reveals more variability in functional data in order to reflect environmental conditions (Heino, 2005).

Functional richness and diversity followed the same pattern found for taxonomic analyses: higher in riffles, intermediate in macrophytes and lower in pools. In agreement, this similar response of taxonomical and functional indexes has also been reported by other studies (Schmera & Eros, 2004; Reynaga & Dos Santos, 2013; Tapolczai et al., 2017). In addition, the multivariate approach showed that the functional structure had the same pattern of taxonomic structure: assemblages were functionally different among habitats. These findings ratify the importance of environmental filters at the habitat scale in shaping functional structure of benthic insect assemblages in headwater grassland streams.

Although riffles were the most diverse habitat, not only for the taxonomic aspect but also for the functional one, it is important to take into

account that the other habitats sustained exclusive taxa characterized by particular functional traits. This finding may have important implications for food web structure considering that predators, for example, were mostly found in pools and macrophyte patches. As a consequence, food web may become unbalanced if any of these habitats are altered. In relation to this, Lecerf & Richardson (2011) found that the absence of large predatory invertebrates alters food webs increasing grazers abundance and consequently reducing biomass of benthic algae, which constitute the basal resource in food webs of the studied streams.

CONCLUSION

This study provides valuable information on the taxonomic and functional structure of stream insect communities in grassland headwaters of Central Argentina, a remote area that provides drinkable water to several cities and towns. Stream biodiversity and functioning in this area are being jeopardised by the ongoing invasion of exotic trees (mainly *Pinus elliottii* Engelm) that escape from extensive afforestations. Moreover, taking into account that some authors have pointed out that this region is still underrepresented in studies about functional diversity and macroinvertebrate functional traits (Schmera *et al.*, 2017; Reynaga & Dos Santos, 2013; Tomanova & Usseglio Polatera, 2007), this study contributes to this knowledge gap.

This research showed that taxonomic and functional composition differed among habitats. In this sense, habitat characteristic do affect, for example, whether clingers or sprawlers are dominant within the gathering collectors in these headwater ecosystems, with potential consequences for ecosystem processes (e.g. processing rates of fine organic material).

Finally, the present findings bear implications for the management and conservation of stream ecosystems in the region and other similar environments worldwide. The understanding of species distribution at the habitat level becomes essential to the development of proper management strategies (Thomson *et al.*, 2001) and future assessments will be more accurate when habitat preferences and indicator species are known (McGeoch & Chown, 1998; Curry *et al.*, 2012).

Based upon our results, riffles habitats were the most diverse, but pools and macrophytes sustained particular taxa which also differ in functional traits. Predators for example, which may have an important role in food webs (top-down control), were mainly associated to macrophyte and pools. As a consequence, water managers should attempt to preserve stream habitat heterogeneity, warranting the maintenance of, not only the most diverse habitat (i.e. riffles), but also pools and macrophyte patches. This management strategy will assure the presence of a range of habitats which is largely responsible for the resilience and resistance of the system (Hershkovitz & Gasith, 2013) and therefore of great importance to cope with both natural and human-made disturbances.

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