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# Occurrence of Hirudinea species in a post urban reach of a Patagonian mountain stream

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**ABSTRACT.** Temporal (May 2005 to February 2006) and habitat distribution (pools and riffles) of Hirudinea species was analyzed at a post urban reach from Esquel stream (Chubut province, Patagonia, Argentina). Site was located 5.7 km downstream a Waste Treatment Plant. Mean values of nutrients: ammonia, nitrates and soluble reactive phosphate, as well water conductivity, turbidity and total suspended solids indicated physical and organic pollution. Leeches assemblage was composed by the glossiphonids: *Helobdella scutifera* Blanchard, 1900, *H. michaelseni* (Blanchard, 1900), *H. simplex* (Moore, 1911), *Helobdella* sp., *H. hyalina* Ringuelet, 1942, *H. obscura* Ringuelet, 1942 and the semiscolecid *Patagoniobdella variabilis* (Blanchard, 1900). From these *H. hyalina* and *H. obscura* are new records for Chubut province. *Helobdella hyalina* (810 ind.m$^{-2}$) and *H. simplex* (465 ind. m$^{-2}$) clearly dominated the assemblage at the reach. Only *H. simplex* displayed a spatial preference being significantly more abundant in pools than in riffle habitats ($p<0.001$). Species recruitment occurred mostly at September, December and March when juveniles were very abundant. Although several species of *Helobdella* were able to live in the disturbed section of the stream, only *H. simplex* and *H. hyalina* sustained large populations at the site and can be considered as tolerant to organic enrichment. This information is valuable to future studies on stream condition assessment in mountainous areas in Patagonia, and in other areas in which these species are present.

**KEYWORDS.** Leeches, assemblages, streams, urbanization, organic pollution.

**RESUMEN. Ocurrencia de especies de Hirudinea en un tramo posturbano de un arroyo patagónico de montaña**. Se analizó la distribución temporal (mayo 2005 a febrero 2006) y espacial (pozones y rápidos) de las especies de hirudíneos en un segmento post urbano del arroyo Esquel (provincia de Chubut, Patagonia, Argentina). El sitio está localizado a 5,7 km aguas abajo de la planta de tratamiento de efluentes cloacales. Los valores promedio de nutrientes: amonio, nitratos y fósforo reactivo soluble, así como la conductividad del agua, turbidez y el total de sólidos suspendidos indican contaminación física y orgánica. Las especies de hirudíneos halladas fueron Glossiphoniidae: *Helobdella scutifera* Blanchard, 1900, *H. michaelseni* (Blanchard, 1900), *H. simplex* (Moore, 1911), *Helobdella* sp., *H. hyalina* Ringuelet, 1942 and *H. obscura* Ringuelet, 1942 y Semiscolecidae: *Patagoniobdella variabilis* (Blanchard, 1900). *Helobdella hyalina* and *H. obscura* representan nuevos registros para la provincia de Chubut. *Helobdella hyalina* (810 ind. m$^{-2}$) y *H. simplex* (465 ind. m$^{-2}$) fueron las especies claramente dominantes. Sólo *H. simplex* presentó una preferencia espacial siendo significativamente más abundante en los pozones que en los rápidos ($p<0.001$). El reclutamiento de las especies se observó principalmente en septiembre, diciembre y mayo donde los juveniles fueron muy abundantes. Aunque varias especies de *Helobdella* se registraron en este sector contaminado del arroyo, sólo *H. simplex* and *H. hyalina* presentaron poblaciones importantes y pueden ser consideradas como tolerantes al enriquecimiento orgánico. Esta información es de valor para futuros estudios sobre evaluación de calidad de los arroyos en áreas montañosas de la Patagonia y para otros donde estas especies están presentes.

**PALABRAS-CLAVE.** Sanguijuelas, ensambles, arroyos, urbanizaciones, contaminación orgánica.

In the Neotropical Region, leeches are important benthic members in rivers and streams usually displaying a high level of endemism (Ringuelet, 1944, 1978, 1985; Christoffersen, 2009). As part of the food chains play an important role as predators or as preys. They are intermediate host in life cycles of Digenea, Cestoda and parasitic Protozoa. Their medicinal use is also well known as well as their suitability as bioindicators in aquatic environments (Sawyer, 1986).

In the last time many contributions to the knowledge of this group were developed, particularly regarding to the discovery of new species (Siddall *et al*., 2001a,b; Siddal & Borda, 2004), redescription of others on the basis of molecular analysis (Mosser *et al.*, 2012a,b, 2013); phylogenetic studies (Siddall *et al.*, 2005); and inventories that increased the global diversity of Hirudinea (Sket & Trontelj, 2008).

In benthic environments, leeches are frequently associated to submersed vegetation where can be very abundant, they are absent at deeper areas in which aquatic plants, suitable substrates and food resources are scarce (Sawyer, 1986). Despite its importance as benthic members, it is not common to include them in studies dealing with benthic macroinvertebrates, because of their hard going identification.

A few studies on biodiversity and ecology of hirudineans have been conducted in Argentina (Gullo & Darrigran, 1991; Darrigran *et al.*, 1998; Gullo, 1998, 2007; César *et al.*, 2009). In particular, in the Patagonia region, leeches appeared usually in macroinvertebrates ecological research of streams and rivers (Miserendino & Pizzolón, 2003; Miserendino *et al*., 2011; Tagliaferro *et al*., 2013), listed in biotic index adapted to the area (Miserendino & Pizzolón, 1999; Figueroa *et al*., 2003) and reported as part of the diet of fish including native ones (Casaux & Di Prinzio, 2007), nevertheless the level of taxonomical resolution is not always fine.

At most studies of biological surveillance regarding water quality that have been conducted in Patagonia, hirudineans are proposed as moderately tolerant or tolerant to organic enrichment and pollution (Pizzolón & Miserendino, 2001). Nevertheless the specific response to disturbances at the species level remains unknown.

The aim of this study was to analyze the composition, the spatial distribution at the habitat scale (pools and riffles), and the seasonal pattern in density of Hirudinea species at a post urban stream affected by treated effluents in the Patagonian mountains.

## MATERIAL AND METHODS

Selected site (42°58'32"S, 71°23'47"W) was located on Esquel Stream (Futaleufú-Yelcho basin), 5.7 km downstream to the Waste Treatment Plant that processes domestic effluent of the homonymous town (30,977 inhabitants). At this point the stream is a $4^{th}$ order watercourse. Dominant riparian vegetation consists mostly of *Salix fragilis* and grasses. As other mountain waterways, Esquel stream have a pluvionival regime, with two seasonal peaks in flow. The peak in June-July is due to heavy winter precipitation and the other in September-October to melting ice and snow (Coronato & del Valle, 1988). An important coverage of de submersed *Myriophyllum quitense* Kunth, and of filamentous algae *Cladophora* sp. characterizes the site. Other species present are the submersed: *Callitriche lechleri* (Hegelm.) Fassett and *Ranunculus* sp., and the emergents *Mimulus gabratus* Kunth, *Veronica anagallis-aquatica* L., *Eleocharis* sp. and *Juncus arcticus* Wild. (Miserendino *et al*., 2011).

Sampling site was visited in May (autumn), September (winter), and December 2005 (spring) and February 2006 (summer), under stable environmental conditions. Percentages of boulder, cobble, gravel, pebble, and sand in the reach were estimated using a 1-$m^2$ grid. At each occasion current speed was measured in mid channel (average of three trials) by timing a float as it moved over a distance of 10 m (Gordon *et al*., 1994). Average depth was estimated from five measurements with a calibrated stick along one transverse profile across the channel. Wet and dry widths (from bank to bank) of the channel were also determined. Discharge was obtained by combining depth, wet width and current velocity as in Gordon *et al*. (1994). Air and water temperature were measured with a mercury thermometer.

In the mid channel section of the reach, specific conductance, pH, turbidity and dissolved oxygen were measured with a sensION 156 multiparameter probe. For nutrient analyses water samples were collected below the water surface, kept at 4°C and transported to the laboratory for analysis. Total suspended solids (TSS) were measured gravimetrically from separate water samples (plastic bottles 2000 ml). In the laboratory differences between final and initial weight of dried filters was obtained. Nitrate plus nitrite nitrogen ($NO_{3+}NO_2$), ammonia ($NH_4$), and soluble reactive phosphate (SRP) were analyzed using standard methods (APHA, 1999).

Quantitative samples of Hirudinea from riffles and pools ($n$=3) were obtained with a Surber sampler (0.09 $m^2$; 250 µm pore size). Samples were fixed in situ with 4% formaldehyde, and sorted in the laboratory under at least 5x magnification. Examination of external morphology and posterior dissection of specimens was accomplished with a Leica Wild M3Z stereomicroscope. Species were identified using regional keys (Ringuelet, 1985; Sawyer, 1986) and counted. The collected material was deposited in the Museo de La Plata - Other invertebrate section (MLP-OI) under the numbers 3830 to 3835.

Non parametric comparisons (Mann Withney U test) were performed to test for differences on species density between riffles and pools habitat (Sokal & Rohlf, 1995).

Principal Component Analysis (PCA) were carried out on log (x+1) transformed data to 1) identify the physical and chemical parameters that defined the environmental gradients along seasons, and to 2) examine the temporal variation on species density at pools and rifles (Ludwig & Reynolds, 1988).

## RESULTS

Water temperature ranged between 9 (September) and 13.1°C (December). As expected the base flow period occurred during February, whereas the highest records of water velocity, depth and discharge were documented on September. Conductivity values ranged from 224 to 390 $\mu S.cm^{-1}$, whereas pH values were circumneutral. The minimum value of dissolved oxygen occurred on December, where a peak in the level of total dissolved solids was detected (Tab. I). From May to September a declination in nutrient values was observed, followed by and increase of all soluble fractions from September to February. A marked peak in ammonia values was displayed on February in coincidence with the low discharge period (Tab. I).

The Hirudinea assemblage was composed by the glossiphonids: *Helobdella scutifera* Blanchard, 1900, *H. obscura* Ringuelet, 1942, *H. michaelseni* (Blanchard, 1900), *H. simplex* (Moore, 1911), *H. hyalina* Ringuelet, 1942 and *Helobdella* sp., and the semiscolecid *Patagoniobdella variabilis* (Blanchard, 1900). The species that contributed the most at the site were *Helobdella hyalina* and *H. simplex* with 55.6 and 32.1% respectively, followed by *Helobdella* sp. (4%), *P. variablilis* (3.3%) and *H. michaelseni* (3.2%), *H. obscura* (1.6%) and *H. scutifera* (0.12%) (Fig. 1).

The trend among season was also different for each species (Fig. 2). *Helobdella simplex* was the dominant leech species during May, whereas *H. hyalina* displayed maximum density on September and December. All species were present at September. *Patagoniobdella variabilis* displayed the highest density in February. Some peaks in species density were related with the recruitment period which occurred at September and December (*H. hyalina*), February and May (*H. simplex*), and March (*P. variabilis*) when juveniles were very abundant. Only *H. simplex* exhibited a spatial preference, being significantly more abundant at pools than at riffle habitats ($p<0.001$) (Tab. II).

Tab. I. Seasonal values of environmental quality variables at Esquel post urban reach (Chubut Province, Argentina) during the study period.

| | Dates | | | |
|---|---|---|---|---|
| Physicochemical variables | May 2005 | September 2005 | December 2005 | February 2006 |
| Water temperature (°C) | 11.0 | 9 | 13.1 | 11.9 |
| Wet width (m) | 9.3 | 10 | 10.3 | 9.8 |
| Depth (cm) | 23 | 32.74 | 27.5 | 23.10 |
| Current velocity ($m.s^{-1}$) | 0.75 | 1.25 | 1.07 | 0.53 |
| Discharge ($m^3.s^{-1}$) | 1.6 | 4.09 | 3.03 | 1.20 |
| pH | 6.74 | 7.50 | 7.20 | 7.15 |
| Conductivity 20º C ($\mu S. cm^{-1}$) | 390 | 273 | 224 | 366 |
| Dis. oxygen ($mg.l^{-1}$) | 11.88 | 10.02 | 7.86 | 8.31 |
| Turbidity (NTU) | 6 | 33 | 287 | 4 |
| Total suspended solids ($mg.l^{-1}$) | 6.9 | 16.1 | 31.4 | 4.1 |
| Ammonia ($\mu g.l^{-1}$) | 59.62 | 33.12 | 50.06 | 271.54 |
| Nitrate plus nitrite nitrogen ($\mu g.l^{-1}$) | 52.10 | 19.22 | 30.76 | 68.99 |
| Soluble reactive phosphate ($\mu g.l^{-1}$) | 20.25 | 6.25 | 7.07 | 20.96 |

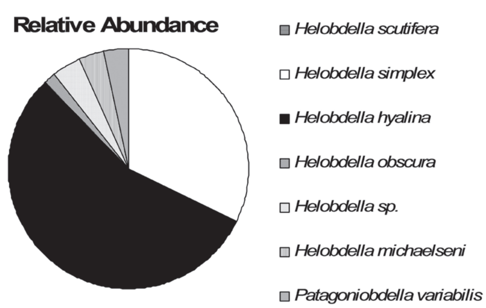



Fig. 1. Relative contribution on density (%) of Hirudinea species at the post urban reach of Esquel Stream (Chubut Province, Argentina).

Tab. II. Results of pair comparisons (*n*=12) after Mann Whitney U test (non parametric) between mean density (ind. 0.09 $m^{-2}$) values of Hirudinea species at riffles and pools during the study period (May 2005-February 2006). Data are from the post urban reach at the Esquel stream (Chubut Province, Patagonia, Argentina).

| | Riffle | Pool | p-level |
|---|---|---|---|
| Glosiphoniidae | | | |
| *Helobdella scutifera* | 144 | 156 | 0.317 |
| *Helobdella simplex* | 95.5 | 204.5 | 0.002 |
| *Helobdella hyalina* | 145 | 155 | 0.772 |
| *Helobdella obscura* | 148 | 152 | 0.859 |
| *Helobdella sp.* | 137.5 | 162.5 | 0.452 |
| *Helobdella michaelseni* | 132 | 168 | 0.071 |
| Semiscolecidae | | | |
| *Patagoniobdella variabilis* | 165.5 | 134.5 | 0.340 |

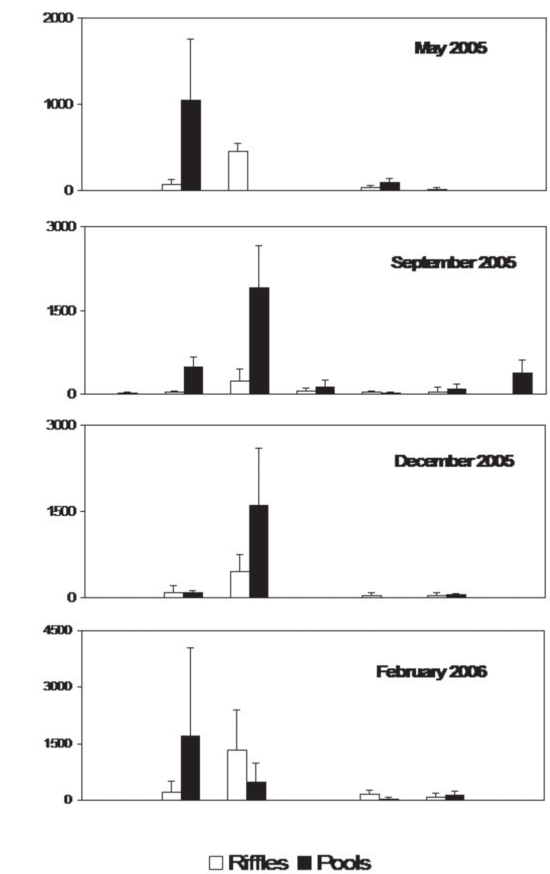
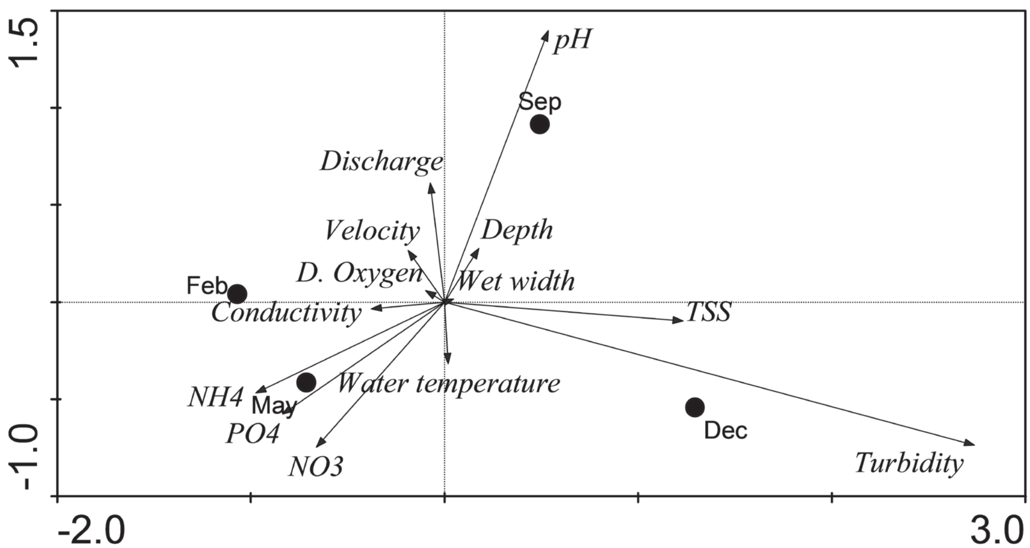



Fig. 2. Temporal patterns of Hirudinea species at riffles and pools (n=3) at the post urban reach of Esquel Stream (Chubut, Province, Argentina). Data are density values ($ind.m^{-2}$)(± 1SD).

According to the ordination procedure of the environmental PCA the variables with the highest loadings along axis 1 (variance explained: 79.7 %) were turbidity, total suspended solids and nutrients. The strong positive load of variables turbidity and total suspended solids separated clearly December from other months, whereas the negative loadings of ammonia, nitrates and soluble reactive phosphate located February and May on the left of PCA1 axis. Therefore, this axis highlighted the physical and chemical disturbance gradient at the study site along the year. The variable most important associated to PCA2 (variance explained: 10%) was pH, that displayed a maximum value during September. Other secondary variables associated to this axis where discharge and water temperature (Fig. 3). The PCA ordination based on abundance data showed that most taxa resulted associated to first axis, the exception was *H. simplex*, clearly linked with PCA2. The ordination reflected the variation in density of species across habitats during the year, with pools at September sustaining high densities of *H. michaelseni*, *H. scutifera*, *H. obscura* and *Helobdella* sp. (right upper quadrant) whereas pools on December displayed high density of *H. hyalina*. *Helobdella simplex* that peaked during May and February was positioned on the positive end of PCA2 (Fig. 4).

## DISCUSSION

As a result of this work and according the most recent revision of the genus *Helobdella* (Christoffersen, 2009), *H. hyalina* and *H. obscura* resulted new records for the Chubut province, being the rest of Hirudinea species new findings for the Esquel stream. This is not surprising because faunistic studies in Patagonia Argentina are not accomplished since approximately thirty years ago, being

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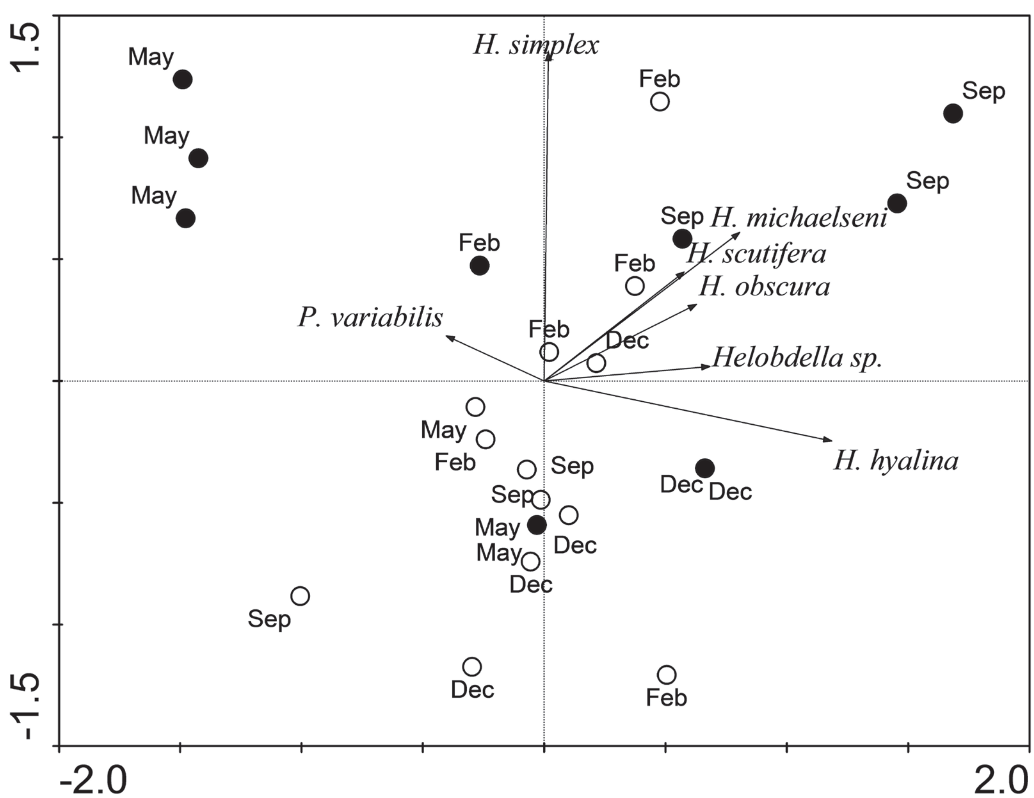


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Figs 3, 4. Ordination biplots according to the Principal Component Analysis of (3) environmental variables per season and (4) Hirudinea species per riffles (open circles) and pools (dark circles) during the study period in the Esquel Stream (Patagonia, Argentina).

the most significant works of taxonomical, biogeographical, and ecological aspects the vast contributions of RINGUELET (1981, 1985).

Nutrient values were high comparing with documented for undisturbed streams in the region (MISERENDINO, 2009) and similar to reported for grazed wetlands in the area (EPELE & ARCHANGESLKY, 2012). For this reason, the recorded species at the Esquel stream reach would be tolerant to physical and chemical disturbance whose origin is related with domestic treated effluents from the facilities. Given the urbanization size and the magnitude of the stream (4$^{th}$ order) a marked impact on water quality can be expectable. Previous works conducted at the same study site concluded that this part of the systems displays a moderate organic pollution evidenced by the biochemical oxygen demand (2-5 mg.l$^{-1}$), although extreme values of ammonia (500 to 3000 ug.l$^{-1}$) and nitrates (220-800 ug.l$^{-}$) were also recognized (MISERENDINO, 2001; MISERENDINO & BRAND, 2009). The toxic and lethal effect of some substances as for example ammonia on invertebrates has been widely reported (BEKETOV, 2004; LANGFORD *et al*., 2009). Our results suggest that species of Hirudinea recorded at this site were capable to support moderate to strong pollution which is in line with found for other species of Hirudinea including Glossiphoniidae (NELSON *et al*., 2000; LANGFORD *et al*., 2009).

It is known that food offer can regulate density patterns of invertebrate's species (ALLAN, 1995). According to our field observations and previous research (MISERENDINO *et al*., 2008), a rich and abundant assemblage of chironomids (*Pseudosmittia* sp. 1, *Parapsectrocladius* sp., *Paratrichocladius* sp.), oligochaetes (*Lumbriculus variegatus* and *Limnodrilus* spp.) and amphipods (*Hyalella araucana* and *Hyalella curvispina*) characterized the macrobenthic communities at the site, and leeches species probably fed on them. This is in agreement with reported by SAWYER (1986), who studied the food preferences for *Helobdella stagnalis* at different lakes, the diet consisted mainly of oligochaetes (28-32%), chironomids (23-25%) and mollusks (17-26%) being isopods an amphipods secondary preys. On the other hand, the presence of active predators as the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) that was very abundant as this section of the Esquel stream (DI PRINZIO *et al*., 2009) could explain the variation on some species abundance.

It was noticed that although *Patagoniobdella variabilis* was not abundant during the study, the species occurred at all dates. Possibly *P. variabilis* was present at other habitat types not sampled in the studied reach (e.g. shorelines, connected marginal ponds). For example the semicolescid *Semiscolex similis* (Weyenbergh, 1879) can be found in buddy bottoms of streams as well shorelines either vegetated or non-vegetated pools, being commonly present at polluted streams including those with sewage effluents (RINGUELET, 1944, 1985).

Although all species were able to live in the disturbed section of the stream, only *Helobdella hyalina* and *Helobdella simplex* dominated and sustained large populations at the site. For this reasons these species can be considered tolerant to organic enrichment as well other kinds of disturbances derived from urbanization phenomena (physical: turbidity, total suspended solids, etc.) (MISERENDINO & BRAND, 2009).

Given that these species display distributional ranges that exceeds the Patagonia ecoregion, this information is crucial to future studies dealing with stream condition assessment not only at mountainous South American areas, but also at systems in which these species are present.

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