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Does Type of Substratum Affects Chironomid Larvae Assemblage Composition? A Study in a River Catchment in Northern Patagonia, Argentina

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Abstract

Chironomid larvae assemblages were investigated at seven sampling stations in relation to stream habitat type in the Challhuaco-Ñireco river system located in the Nahuel Huapi National Park, in the North-Western part of Rio Negro Province, Argentina. A total of 2229 individuals were sampled and 43 Chironomidae taxa were recorded with Orthocladiinae (29) being the best represented subfamily, followed by Diamesinae (5), Tanypodinae (3), Podonominae (3) and Chironominae (3). *Stictocladius* spF, *Cricotopus* (*Paratrichocladius*) sp6, *Cricotopus* sp2, *Cricotopus* sp3 and *Parapsectrocladius* sp2 were the most abundant taxa. Sites near the source of the river system were dominated by *Podonomus* sp., *Limnophyes* sp., *Parapsectrocladius* sp. and *Stictocladius* spF, whereas sites close to the river mouth were dominated by Diamesinae sp5 and *Cricotopus* sp. Rank/abundance plots show that all the analysed sites displayed dominance of some species. *Stictocladius* spF, *Cricotopus* sp2, *Cricotopus* sp3, *Cricotopus* (*Paratrichocladius*) sp6, *Parapsectrocladius* sp. and *Limnophyes* sp. resulted as dominant species, or being part of a group of dominant species, at least in one sample. Eleven taxa were associated with habitats related with riffle areas and stable substrates with filamentous algae. Species–environment relationships were examined using ordination analysis. Elevation was the most significant environmental variable that explained 22% of the total variability of the chironomid assemblages, showing stronger relationships among sites within an altitudinal gradient than among habitat type. Abundance of chironomids increased from headwaters to the outflow in Nahuel Huapi Lake responding to an altitude gradient as well as some environmental factors such as coarse matter and nutrient concentrations.

Introduction

The family Chironomidae is the most ubiquitous and usually the most abundant insect group in all types of freshwater environments (Pinder 1983). Their immature stages are an important part of the diet of other invertebrates, fishes, amphibians and birds. The chironomid larvae carry out major trophic role in aquatic ecosystems such as predators, herbivores, detritivores, filter feeders and parasites (Berg 1995). They also have an extraordinary range of life habits with different species requirements of temperature, pH, oxygen concentrations, salinity, depth, current velocity and productivity. These features place Chironomidae as good environmental and climatic change indicators (Sæther 1979, Brooks 1995, Marziali *et al* 2006), and particularly when genus and species level identifications are achieved (Cranston 1995).

Like other members of aquatic invertebrate fauna, Chironomidae shows a high level of endemism in the Patagonian region (Cranston 1995, Donato *et al* 2008). In consequence, the knowledge of the Chironomidae community and its environmental relationships at the habitat scale are important for conservation purposes and for the management of low order streams in Patagonia.

Several studies attempted to explain the major underlying process and mechanism that shape Chironomidae community patterns in lotic environments. Thienemann (1954) stated that water temperature, among other variables such as dissolved oxygen and water current speed, is the major factor in determining the community structure. This statement was also suggested by Rossaro (1991a) in his review on the Chironomidae community in Italian streams and Lindegaard & Brodersen (1995) working on Holarctic, African and Australasian rivers.

Chironomid patterns in lotic systems are influenced by ecological and evolutionary factors at multiple scales (Allan & Castillo 2007, Heino *et al* 2015). The aquatic biota is supported by a combination of different physical variables of streams. Variables such as dissolved oxygen, pH and other physicochemical characteristics define the diversity distribution on a broad scale, whereas the kind of substrate and food availability tend to influence diversity distribution on a fine scale defining the structure of local community (Fesl 2002, Peeters *et al* 2004). Thus, the different niche dimensions allow larval species to differentiate and therefore reduce the overlap in resource utilization and perhaps avoiding interspecific competition (Tokeshi & Townsend 1987).

As a reflection of the adaptations of the major subfamilies to prevailing environmental conditions, the Andean chironomid fauna shows an increase in diversity and numbers of specimens decreasing from high altitude to lowlands of the Orthocladiinae, Podonominae and Diamesinae with a corresponding increase of the Chironominae and Tanypodinae. Orthocladiinae, Podonominae and Diamesinae can be regarded as an essentially cold-stenothermic predominantly rheophilic groups requiring high oxygen concentrations (Ashe *et al* 1987).

The composition and distribution of the Patagonian Chironomidae species in freshwater and their environmental relationships have been documented in different lotic environments, and the works of Paggi & Rodriguez Capítulo (2002) and Epele *et al* (2012) can be mentioned.

This study was undertaken on the Challhuaco-Ñireco river system in the Natural Reserve of Nahuel Huapi National Park (NHNP) in Río Negro Province, Patagonia, Argentina. Ñireco river has Challhuaco (2nd order) tributary stream close to its source surrounded by forest vegetation (Mauad *et al* 2015). As it moves towards the ecotonal forest-steppe area, the Ñireco river (3rd order) flows through the urban area of Bariloche where it is subjected to various anthropogenic impacts. The water quality of the upstream sampling area has remained relatively undisturbed (Mauad *et al* 2015). In contrast, the water quality of the downstream sampling area has been affected by an increase of human activities in the catchment itself. In addition, the urbanization of Bariloche City and other disturbances in the natural flow regime related to the extraction of water for residential and irrigation purposes probably resulted in an increase in the rate of decline in water quality in the last 20 years. This river system was studied recently by García & Añón Suarez (2007) whose objectives were to determine the composition, abundance, distribution and phenology of emergence of chironomid assemblages by the analysis of the pupal exuviae. These authors concluded that the subfamily Orthocladiinae was the most abundant taxa, most of the dominant and frequent species found were univoltine and the emergence peaks were coincident with higher temperature records.

The objectives of this study are to: (1) associate the most relevant environmental variables with the Chironomidae larvae assemblages along the habitat conditions and (2) describe and analyse the structure and composition of the Chironomidae larvae assemblages along an altitudinal gradient and their temporal variation during the summer (early and late summer). Based on the abovementioned objectives, we expected to find a probable habitat preference by chironomid larvae linked with the stability of the substratum and that Chironomidae larvae assemblages would respond to the environmental changes along the altitudinal gradient. Particularly, we assumed to find cold stenothermal taxa in high altitude sites and eurythermal taxa in low altitude sites.

Study Area

The Nahuel Huapi National Park (NHNP) is a biodiversity hotspot in northern Patagonia (Orme *et al* 2005, Donato *et al* 2008). It includes the ecotone between temperate montane rainforest and arid steppe which further increases the biodiversity of the region. Consequently, NHNP contains a wide diversity of wetland habitats. This study was carried out in the Ñireco catchment, an important lotic environment of the NHNP, located in the south western part of Río Negro Province (41°13′S, 71°19′W) and has different environmental features along its course. Ñireco river intercepts its tributary stream Challhuaco before arriving at the city of Bariloche and drains in the Nahuel Huapi lake at 700 m.a.s.l., fulfilling a distance of 40 km. The river source is located at 2.140 m.a.s.l. enclosed in a humid forest dominated by *Nothofagus pumilio* ("lenga") and its associated flora (*Berberis serrato-dentata, Maytenus chubutensis, Ribes magellanicus*, *Schinus patagonicus* and *Myoschilos oblongum*), whereas the lower area of the river runs across an open valley characterized by shrubs and bushes of Compositae and *Salix* sp. until the mouth in Nahuel Huapi lake. The hydrological regime of the area of Ñireco-Challhuaco system indicates high water discharge periods during autumn and spring due to precipitation and snow melting events, respectively. The substratum is bedrock with different sizes of stones (boulder, cobble, pebble or gravel) (Table 1). The

Table 1 Location and several characteristics of the seven sampling sites. SO, stream order.

Site	SO	Location	Substrate type	Elevation (m)	Riparian vegetation	Depth (cm)
I–II	1	UPPER	Cobble-pebble-sand	1321–1380	Lenga and Coihue closed forest	0.45–0.51
III–V	2–3	MIDDLE	Boulder-cobble-pebble	966–1029	Coihue and pino forest, Ñire and Rosa Mosqueta shrub	0.3–0.54
VI	3	LOWER	Boulder-cobble-pebble	892	Modified area	0.2
VII	3	OUTLET	Boulder-cobble	767	Post-urban area	0.75

seven sampled sites are located between the coordinates 41°15′35″S, 71°17′19″W to 41°07′49″S, 71°17′20″W and at an altitude from 1380 to 767 m (Fig 1). Throughout sites I to IV, the stream is narrow and shallow (1–3 m width) with a predominantly rocky bottom and large numbers of fallen leaves and branches due to the surrounding forest vegetation. As the stream crosses the ecotonal area, it becomes wider (6–11 m width) with a streambed that includes stones of different sizes, many of them partly covered by filamentous algae particularly at site VII.

Materials and Methods

Field methods: chironomid sampling and identification

To carry out this study, seven sites were sampled along Challhuaco-Ñireco river system during two sampling periods, corresponding to early summer (December of 2009) and late summer (February of 2010). The river system was divided into four sections: 1—upper section with two sample sites, immersed in a closed forest of Coihue (*Nothofagus dombeyi*) and Lenga (*N. pumilio*) and a bottom river characterized by cobble, pebble and sand; 2—middle section with three sample sites, surrounding an open forest of Coihue and shrubs of Ñire (*Nothofagus antarctica*) and the introduced Rosa mosqueta (*Rosa rubiginosa*); 3—lower section located within disturbed areas; and 4—outlet section that is directly affected by the urban settlement of Bariloche City (Table 1). For each site, the following variables were measured: water temperature, conductivity, total dissolved solids, pH and percentages of dissolved oxygen with a multi-parameter probe HANNA I-9828. For chemical analysis, water samples were collected below the water surface using 500-ml water bottles and kept refrigerated and transported to the laboratory. Nitrate and nitrite nitrogen ($\mathrm{N{-}NO_3}$, $\mathrm{N{-}NO_2}$), ammonia ($\mathrm{N{-}NH_4}$) and phosphates ($\mathrm{P{-}PO_4}$) were analysed using standard methods (American Publish Health Association APHA 1994). Several environmental characteristics such as typology of the river bank vegetation and substrate type were also estimated. Most abundant species composing the riparian areas in the sampled reach (50 m) were recorded and ranked as percentages of coverage in three categories: forest, shrubs and

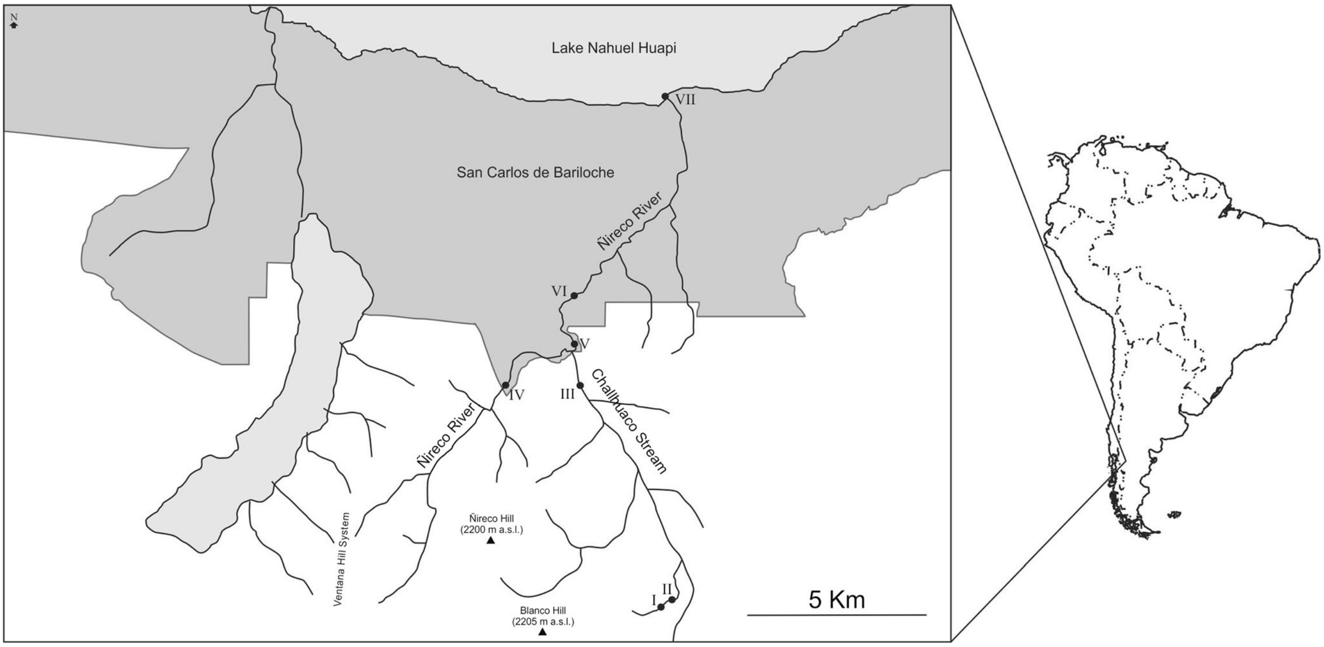

Fig 1 Map showing the seven sampling points I–VII along Challhuaco-Ñireco system in Northern Patagonia, Argentina. Urban area (San Carlos de Bariloche) is marked in *dark grey*.

herbaceous stratum. The percentages of boulder, cobble, gravel, pebble and sand in the reach were estimated using a 1 m^2 grid (Gordon *et al* 1994). Quantitative sampling was performed using a Surber net of 0.09 m^2 surface area and 250 µm mesh size with three replicates per site. Detritus was divided into fine (250–1000 µm) and coarse (>1000 µm) particulate fraction (FPOM and CPOM, respectively). In order to help in the determination, at specific level, of the chironomid larvae collected, we performed qualitative samples using manual and drift net for immatures and pupal exuviae, respectively. Chironomid larvae were identified by preparing permanent slides, clearing specimens with 10% KOH, neutralization with glacial acetic acid, dehydration in 80, 96 and 100% ethanol and mounting in Canada Balsam. Identification of larvae was performed using the following keys and papers: Brundin 1966, Cranston & Edwards 1999, Cranston 2010, Cranston & Krosch 2011, Epler 2001, Sæther & Cranston 2012 and Trivinho-Strixino 2011. Density of chironomids was calculated from counts of all the individuals of each taxon from the Surber samples and expressing the results as the number of individuals per surface area (m^2). Relative abundance was obtained as the number of each taxon divided by the total number of individuals in each sample. To compare the diversity between the two sampling periods, a rank/abundance plot was made where species were plotted in sequence from most to least abundant along the horizontal axis. To analyse the community structure and the species turnover among sites, diversity index of Shannon Weaver (H), equitability of Jaccard (J) and beta diversity (β_w) were calculated.

After the direct observation of the sampling sites, a total of five different habitat types were identified and three replicates per habitat were sampled. In depositional areas (pools), three habitat types were sampled: leaf-pack (LP), pebble with filamentous algae (PF) and gravel-sand (GS). In riffles areas, two different habitats were sampled: cobble-pebble (CP) and boulder with filamentous algae (BF). Habitat GS was present only at site II, whereas PF was present at sites IV and VI. The chironomid assemblages characteristic of each habitat was determined by the relative preference index (RPI) (Tickner *et al* 2000), which is calculated dividing the abundance of each taxon in a certain habitat by all the habitats. The RPI can vary between 0 and 1, meaning no preference and strong affinity for a particular habitat, respectively.

Multivariate methods

Ordinations were performed using CANOCO 4.5 (ter Braak & Smilauer 2002). Principal environmental variables and species density data were transformed ($\log_{10}X+1$) to achieve approximately normal distribution of the data. Redundancy analysis (RDA) was carried out in order to evaluate species–environment relationships in habitats at the altitudinal gradient. The environmental considered in the RDA were elevation, riparian coverage as % forest and herbaceous stratum and also percentages of cobble and pebble, temperature, stream order, ammonia, nitrites, nitrates, phosphate concentrations, CPOM and FPOM. Variables that covaried with other measured variables showed a high inflation factor ($VIF > 20$) and were removed from the analysis. Finally, we performed a subsequently RDA on the remaining variables with Monte Carlo permutation test with 999 permutations with manual forward selection to determine the statistical significance of environmental variables and canonical axes that contribute in explaining the variance in the chironomid assemblages.

Results

Environmental features

From the source to the mouth, where the river reaches the Nahuel Huapi lake, the elevation falls from 1380 to 767 m.a.s.l. (Table 1). The pH showed neutral to slightly alkaline values in all measurements. Mean water temperature increased gradually from the headwaters (5.8°C) to the mouth (11.08°C) (Table 2). Mean water conductivity was significantly different between the stations (Kruskal-Wallis test; $p < 0.05$), increasing from the headwaters (38 $\mu S_{20}\ cm^{-1}$) to the mouth (102.5 $\mu S_{20}\ cm^{-1}$). Dissolved solids content was low in the system; nevertheless, a slight increase could be observed at site VII (mean value 51 ± 14.1 ppm). Percentages of dissolved oxygen were >100% at all the sites during this study. Despite the low phosphate concentrations, there was an increase towards the mouth, with the highest mean value (50 μl^{-1}) recorded at site VII (Table 2). Ammonium increased towards the Ñireco mouth, with the minimum concentration at site II (6.25 μl^{-1}) and the maximum at site V (27.5 μl^{-1}). Nitrite mean values did not show significant variations; however, nitrate mean concentrations were high at site VII (220 μl^{-1}) (Table 2).

Distribution of chironomids assemblages

A total of 2229 individuals were counted (1099 in early summer and 1130 in late summer), 43 taxa were identified belonging to five subfamilies: Orthocladiinae (29), Diamesinae (5), Podonominae (3), Tanypodinae (3) and Chironominae (3) (a photographic on-line guide is available at http://gebica.blogspot.com.ar/2016/04/blog-post_70.html). The lowest richness was recorded at site I (15) with an abrupt increase in richness at site V (32). The Orthocladiinae were dominant at all sites (Fig 2). The Podonominae and the Tanypodinae were present at all sites except for site VII. Tanypodinae in early summer was present at site I and in late summer at site VI. However, Podonominae were most abundant in late summer, particularly at sites II and III. On the other hand, the Chironominae were recorded exclusively in early summer and were present only in the lower section of the basin

Table 2 Physico-chemical parameters at seven sampling sites in the Challhuaco-Ñireco System, during the study period (December 2009 and February 2010). Data are mean values ± SD ($n = 2$).

Variables	Sites						
	I	II	III	IV	V	VI	VII
pH	7.2 ± 0.3	7.7 ± 1	7.2 ± 1.7	7.2 ± 0.3	6.7 ± 1	7.4 ± 0.1	7.5 ± 0.7
t (°C)	5.8 ± 2	5.8 ± 1.8	8.2 ± 1.4	7.7 ± 3	7.8 ± 1.7	9.7 ± 2.2	11.08 ± 2.7
Conductivity (μs cm^{-1})	38	38.5 ± 0.7	63.5 ± 3.5	54.5 ± 10.6	53.5 ± 7.8	62 ± 7	102.5 ± 27.6
TDS (ppm)	19	19	31.5 ± 2.1	26.5 ± 5	26.5 ± 3.5	31 ± 2.8	51 ± 14.1
DO%	168.5 ± 108.3	205.5 ± 167.6	254.5 ± 222.7	231.9 ± 317.3	267 ± 238.4	224.1 ± 199.4	217.05 ± 210.8
P–PO$_4$ (μl^{-1})	5.1 ± 3.1	5.7 ± 2.06	7.7 ± 2.06	10 ± 1.41	22.5 ± 2.06	37 ± 1.04	50 ± 1
N–NH$_4$ (μl^{-1})	6.75 ± 8.13	6.25 ± 3.18	10 ± 6.36	21 ± 12.73	27.5 ± 26.16	9.5 ± 12.02	22 ± 12.73
N–NO$_2$ (μl^{-1})	1.5 ± 0.71	1.5 ± 0.71	1 ± 0	1.34 ± 0.23	1.36 ± 0.91	1 ± 0	2.7 ± 0.42
N–NO$_3$ (μl^{-1})	12.5 ± 5.6	13.95 ± 5.73	7.8 ± 5.94	15.4 ± 0.85	19.55 ± 3.46	11.65 ± 7.57	220 ± 2.83

and the outlet (sites IV and VII). The Diamesinae were dominant at late summer and present in the middle and lower section, with the highest percentage occurring at site III and VII (24 and 16%, respectively) (Fig 2). Concerning diversity assessment, Shannon index (H′) was highest at the lower section, with the highest value (2.6 bits) at site VI, whereas the lowest value (1.7 bits) was observed at site III (Table 3), showing that the sites belonging to the lower section of the basin were the most diverse. The mean values of equitability (J) ranged from 0.56 at site III to 0.8 at site VI (Table 3). Density at station V was significantly higher (978 ind m^{-2}; Kruskal-Wallis ($p < 0.05$) at both sampling periods, whereas station I had the lowest density (274 ind m^{-2}) (Table 3). Regarding richness (TR), sites V and VI showed the highest values with 32 and 25, respectively (Table 3). In contrast, site I evidenced the lowest value (15). Regarding temporal patterns of taxa distribution and concerning beta diversity, site I showed a total replacement of species from early to late summer, having a high beta diversity ($\beta_w = 0,9$). On the other hand, sites belonging to the outlet (V, VI and VII) had low values of beta diversity ($\beta_w = 0.4$–0.5) denoting a great number of shared species between the two sampled periods. Rank/abundance plots show that all the analysed sites displayed dominance of some species (Fig 3). *Stictocladius* spF, *Cricotopus* sp2, *Cricotopus* sp3, *Cricotopus* (*Paratrichocladius*) sp6, *Parapsectrocladius* sp. and *Limnophyes* resulted as dominant species, or being part of a group of dominant species, at least in one sample. In early summer, *Stictocladius* spF (Sæther & Cranston 2012) was the dominant taxon, mainly at the upper and middle section of the system (I, II, III, IV and V), being replaced at the outlet (VI and VII) by *Cricotopus* (*Paratrichocladius*) sp6 (Fig 3). *Cricotopus* sp2 and *C.* sp3 were abundant in sites III to VII, increasing their abundance from site III to the outlet. In late summer, *Parapsectrocladius* sp1 shared dominance with *Limnophyes* sp. at site I and more pronounced at site II. At site III, *Cricotopus* (*Paratrichocladius*) sp6 became the dominant species, though at site IV its dominance is shared with *Cricotopus* sp3 and *C.* sp2. From site III to the outlet, *Cricotopus* (*Paratrichocladius*) sp6 increased their abundance and at site VII shared dominance with *Cricotopus* sp2.

In relation to the RPI index (>0.5), at least 11 taxa were associated with CP and BF, 7 taxa with LP, 4 taxa with PF and none with GS (Table 4). At the upper section, *Thienemaniella* sp1 was linked to leaf-pack, while nr. *Psectrocladius* sp. and Orthocladiinae sp1 were associated with BF. At the middle section, *Botryocladius* sp1, *Barbadocladius* sp. and *Stictocladius* spF were associated with LP; Diamesinae sp4, Podonominae sp3, *Cricotopus* sp3, *C.* sp5, nr. *Bryophaenocladius* sp. and *Apedilum* were linked to BF; *Pentaneura* sp., Orthocladiinae sp4 and *Rheotanytarsus* sp. with CP; and *Botryocladius* sp3 was associated with PF. At the lower and outlet section of the basin, *Larsia* sp. and nr. *Riethia* sp. were linked with BF; Diamesinae sp2, Podonominae sp2, *Cricotopus* (*Paratrichocladius*) sp6, *Cricotopus* (*Paratrichocladius*) sp7 and *Botryocladius* sp2 were associated with CP; and Diamesinae sp3, Podonominae sp1 and *Parapsectrocladius* sp2 were linked to PF.

Chironomids species and environment relationships

The RDA with the subset of 15 variables (water temperature, elevation, stream order, % of boulder, % of cobble, % of pebble, % of forest, % of shrubs, % of herbaceous, CPOM, FPOM, phosphates, ammonium, nitrates and nitrites concentrations) displayed the eigenvalues of axes 1 and 2, 0.25 and 0.14 respectively, explaining 52% of the cumulative percentage variance (p value = 0.04). Species–environment factor correlation was RDA1 = 0.83 and RDA2 = 0.84, indicating high correlations of the environmental variables selected.

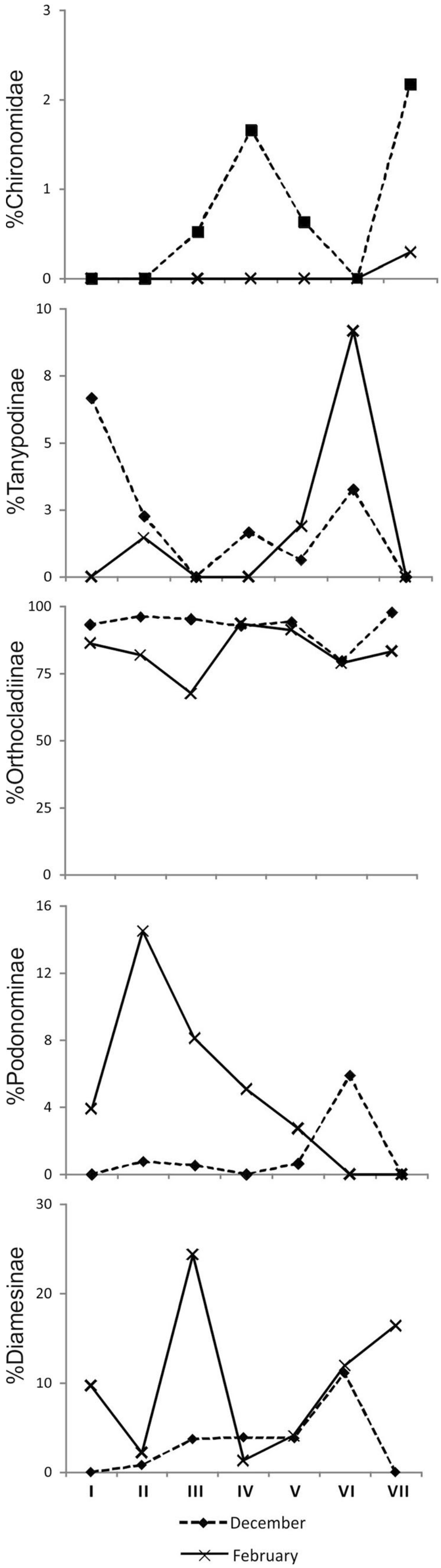

Fig 2 Relative abundance (%) of the five subfamilies at the seven sampling sites. D, December 2009; F, February 2010.

We performed an additional RDA on these five variables with Monte Carlo test with manual forward selection to determine the significance of these variables to explain the variance in the species data. Water temperature, stream order, % of cobble, % of herbaceous stratum, CPOM, phosphate and nitrate concentrations showed a high VIF, so they were removed from the analysis. Two of them were significant ($p \geq 0.05$) where the elevation explains 22% and CPOM 11% of the total variability. The eigenvalues of axes 1 and 2 were similar (0.249 and 0.139, respectively) than those obtained in the RDA considering the 15 environmental variables. The high cumulative percentage variance in the taxa environmental relationship suggests that these two selected variables are sufficient to explain the major distribution gradients in the chironomids species data.

The ordination diagram RDA strongly reflects the distribution of sites and species along axes 1 and 2. Axis 1 clearly shows a topographic gradient governed by elevation indicating stronger relationships among sites within an altitudinal gradient than among habitat type. Sites I and II and species belonging to headwaters (*Thienemanniella* sp1, Orthocladiinae sp1, nr. *Psectrocladius* sp. and Macropelopiini sp.) were located at the positive end of RDA axis 1 related to high altitude, low temperature and increased of CPOM (Fig 4). The increase in the level of nitrates in the lower section of the Ñireco system (sites VI and VII) was associated with the positive end of RDA axis 2. Therefore, the sites with higher levels of nitrate concentration were located on the left upper quadrant, and species that increased in density at those sites were grouped together (*Cricotopus* sp4, *Cricotopus* (*Paratrichocladius*) sp6, *Cricotopus* (*Paratrichocladius*) sp7, Orthocladiinae sp3, *Parapsectrocladius* sp1, *P.* sp2, *Botryocladius* sp2, *Onconeura* sp., *Limnophyes* sp., nr. *Riethia* sp., Diamesinae sp1, Diamesinae sp2, Diamesinaesp3, *Podonomus* sp1, *P.* sp2 and *Larsia* sp.). Finally, the middle section of the basin was separate into the lower quadrants, site III was associated with the positive end of axes 1 related with high altitude, while sites IV and V were associated with the negative values of RDA axis 1 and 2 and displayed high levels of phosphate concentrations and high percentages of herbaceous stratum and cobble in the reach. In terms of abundance and diversity, these sites were characterized mainly by Orthocladiinae and Chironominae species.

Discussion

According to our observations, in Challhuaco-Ñireco river system, the abundance of chironomids increased from headwaters to the outflow in Nahuel Huapi Lake, due to the altitude gradient as well as some environmental factors such as CPOM and nutrient concentrations. Nitrate concentrations

Table 3 Mean density values of taxa and morphotypes of Chironomidae, mean values of density (ind m^{-2}), Taxa Richness (TR), Shannon (H) and Pielou (J) diversity measures from de seven sites of Challhuaco-Ñireco system, Río Negro, Argentina during the study period ($n = 2$). Species codes are indicated in brackets.

Subfamily	Morphotype	Sites						
		I	II	III	IV	V	VI	VII
Diamesinae	Diamesinae sp1 (Dia1)			2	4	6	7	19
	Diamesinae sp2 (Dia2)			13		13	17	30
	Diamesinae sp3 (Dia3)			17		11	26	4
	Diamesinae sp4 (Dia4)	19	6		6	6	2	
	Diamesinae sp5 (Dia5)		2	11	6	4	4	54
Tanypodinae	*Pentaneura* sp. (Pen)		4		4	9	9	
	Larsia sp. (Lar)					6	13	
	Macropelopiini sp. (Macro)	6	6		2		6	
Podonominae	*Podonomus* sp1 (Podo1)	7	37	6	7	19	4	
	Podonomus sp2 (Podo2)		2	2		2	13	
	Podonomus sp3 (Podo3)			2				
Orthocladiinae	*Cricotopus* sp. 1 (Crico1)		2	11	4	28	7	4
	Cricotopus sp2 (Crico2)	4		67	115	246	50	185
	Cricotopus sp. 3 (Crico3)	4		96	80	107	50	128
	Cricotopus sp4 (Crico4)					4		
	Cricotopus sp5 (Crico5)							6
	Cricotopus (*Paratrichocladius*) sp6 (Crico6)	2	9	63	69	244	148	230
	Cricotopus (*Paratrichocladius*) sp7 (Crico7)		2	2	19	48	7	35
	Limnophyes sp. (Limno)	35	85	20	13	94	28	4
	Parapsectrocladius sp1 (Parap1)	69	85	2		2	6	7
	Parapsectrocladius sp2 (Parap2)	19	13		2	6	15	6
	nr. *Psectrochladius* sp. (nr.Pse)							2
	Thienemanniella sp1 (Thiene1)	30	17					
	Thienemaniella sp2 (Thiene2)	6	2				2	
	Onconeura sp. (Onco)		11			2		
	Orthocladiinae sp1 (O1)			2	9	7	19	
	Otrhocladiinae sp2 (O2)	2	7	2	2	4	2	2
	Orthocladiinae sp3 (O3)	2	4	28		7	7	11
	Orthocladiinae sp4 (O4)			4		2	20	
	Orthocladiinae sp5 (O5)				4	4		2
	Barbadocladius sp. (Barba)				2	4		
	nr. *Bryophaenocladius* sp. (nr.Bryo)				2			
	Corynoneura sp. (Cory)		2					
	Botryocladius sp1 (Botry1)					2		
	Botryocladius sp2 (Botry2)					2		
	Botryocladius sp3 (Botry3)				2			
	Stictocladius spF (StiF)	70	202	428	120	81	11	
	Stictocladius sp1 (Sti1)				4	4	13	
	Parakiefferiella sp. (Parak)	2	4		4	2		
	Metriocnemus sp. (Metrio)					2		
Chironominae	nr. *Riethia* sp. (nr.Rie)							2
	Rheotanytarsus sp. (Rheo)				6	2		
	Apedilum sp. (Ape)			4				2
Density (ind m^{-2})		274	500	780	481	978	485	730
TR		15	20	20	23	32	25	19
H		2.07	1.92	1.67	2.13	2.27	2.58	1.92
J		0.76	0.64	0.56	0.68	0.66	0.8	0.65

were noticeably high at site VII (maximum value: 240 µg l^{-1}), which is widely associated with organic pollution in urbanized areas that can result in severe impairment in river conditions, affecting the composition and density of chironomids. Previous studies carried out at Ñireco River by Albariño (1997) and García & Añón Suarez (2007), also found increases in conductivity at sites near to the mouth and documented significant increases in phosphate and nitrite concentrations towards the mouth of the Ñireco stream, concluding that those increases were linked to the anthropogenic impact of Bariloche City.

Our results suggest that chironomids are an important component of the benthic fauna, 43 taxa were recorded in the present study; this richness is comparable to that reported by other studies in the Patagonian region, such as Epele *et al* (2012) for two rivers in the Northwest of Chubut province, but is higher than that documented by Paggi & Rodriguez Capítulo (2002) in Limay River in Río Negro

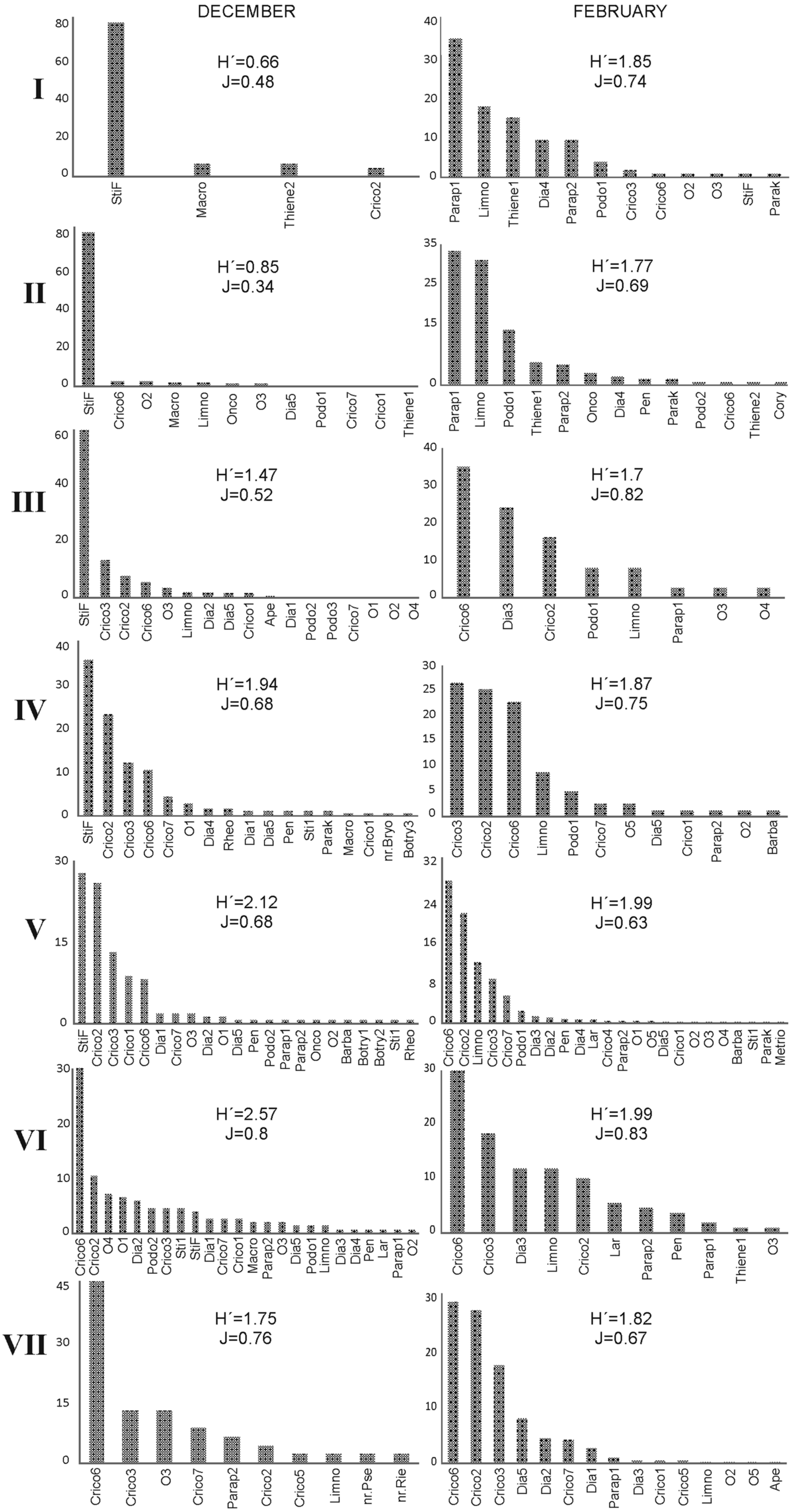

Fig 3 Rank/abundance plots diagram showing the diversity between the two sampling periods in the seven sampling sites in Challhuaco-Ñireco system, Río Negro, Argentina. Morphotypes codes are in Table 3.

Table 4 Chironomids associated with each functional habitat and their relative preference index (RPI ≥ 0.5) for the seven sampling sites.

Habitat	Taxon	RPI
BF	Diamesinae sp4	0.50
	Larsia sp.	1
	Podonomus sp3	1
	Cricotopus sp. 3	0.52
	Cricotopus sp5	1
	nr. *Psectrochladius* sp.	1
	Orthocladiinae sp1	0.50
	Otrhocladiinae sp2	0.75
	nr. *Bryophaenocladius* sp.	1
	nr. *Riethia* sp.	1
	Apedilum sp.	1
CP	Diamesinae sp2	0.67
	Pentaneura sp.	0.50
	Podonomus sp2	0.76
	Cricotopus (*Parapsectrocladius*) sp6	0.50
	Cricotopus (*Paratrichocladius*) sp7	0.53
	Thienemaniella sp2	1
	Orthocladinae sp4	0.96
	Botryocladius sp2	1
	Parakiefferiella sp.	1
	Rheotanytarsus sp.	0.50
LP	*Limnophyes* sp.	0.55
	Thienemanniella sp1	1
	Onconeura sp.	0.67
	Orthocladinae sp3	0.79
	Barbadocladius sp.	1
	Botryocladius sp1	1
	Stictocladius spF	0.63
PF	Diamesinae sp3	1
	Podonomus sp1	0.67
	Parapsectrocladius sp2	0.67
	Botryocladius sp3	1

Habitat codes: *BF* boulder with filamentous algae, *CP* cobble-pebble, *LP* leaf-pack, *PF* pebble with filamentous algae.

Province. However, chironomid richness is lower than that reported by a previous study carried out at Ñireco River by García & Añón Suarez (2007) which reported 55 taxa, but it is important to mention that pupal exuviae were used in that study, which certainly increased the level of taxonomic resolution. In Challhuaco-Ñireco system, the chironomid fauna found is characterized by the presence of the subfamily Orthocladiinae and Diamesinae, which together represented approximately the 80% of the total chironomid fauna, distributed along the basin, although Diamesinae richness increased at lower sites, whereas Podonominae and Tanypodinae represented the 14% and were recorded at all sites except for the outlet, and Chironominae represented 6% and were found in the middle and lower section of the basin and the outlet. Concerning the Podonominae and Diamesinae, our results show these subfamilies as most abundant in late summer. On the other hand, García & Añón Suarez (2007) mentioned the Podonominae and Diamesinae as spring emergers. Combining both results, they suggest that these subfamilies could overwinter as last instar larvae. Regarding taxa distributions, *Stictocladius* spF, *Parapsectrocladius* sp1 and *Limnophyes* shared dominance mainly at the upper and middle section of the system. The genus *Cricotopus* increased their abundance towards the outlet. García & Añón Suarez (2007) also found this pattern of distribution, but they suggested *Stictocladius* sp. at the middle, but this could be due to the water flow of the river that transports the exuviae to sites below. They also described *Cricotopus* sp. as the most abundant at the basin, but richness was underestimated because all morphotypes were grouped in the single genus (see in section *Cricotopus-Paratrichocladius* conflict in Online Resource 1).

According to Miserendino (2001) and Lencioni & Rossaro (2005), the number of organisms has been found to be directly related to the composition of the stream substrate, with most benthic insects concentrated where the food is most abundant; the substrate particles, especially those of small/medium size, act as collectors of detritus, whereas it has been stated that macrophytes sustained an important part of the chironomid community (Epele *et al* 2012). However, our results showed the two different habitats that described the riffle area (CP and BF) supporting the higher values of richness than the depositional area; at least 11 chironomid taxa were associated with CP and BF. These results suggest a probable habitat preference for all these taxa that could be explained by their adaptation to lotic environments with high water current speed. According to the continuum model (Vannote *et al* 1980), biodiversity in the middle reaches exhibit maximum values. Canopy open and riparian clearing reduced shading causing increases in streams temperatures, light penetration and enrichment of nutrient also increases autotrophic biomass and production resulting in proliferation of filamentous algae (Vannote *et al* 1980, Allan and Castillo 2007). This scenario could explain the increase of abundance and diversity in chironomids at the middle section of the basin. Our results showed sites V and VI at the most diverse sites and shared habitat related with stable substratum such as boulder and cobble with the addition of filamentous algae which could be interpreted as higher food availability. Therefore, we suggest further researches on the subject in future studies to clarify this issue.

Chironomid communities responded primarily to elevation and are sufficient to explain the major distribution gradients in the chironomids species data. Altitude seems to be important for chironomids distribution, and studies in altitudinal zonation show that species richness through an altitudinal gradient could

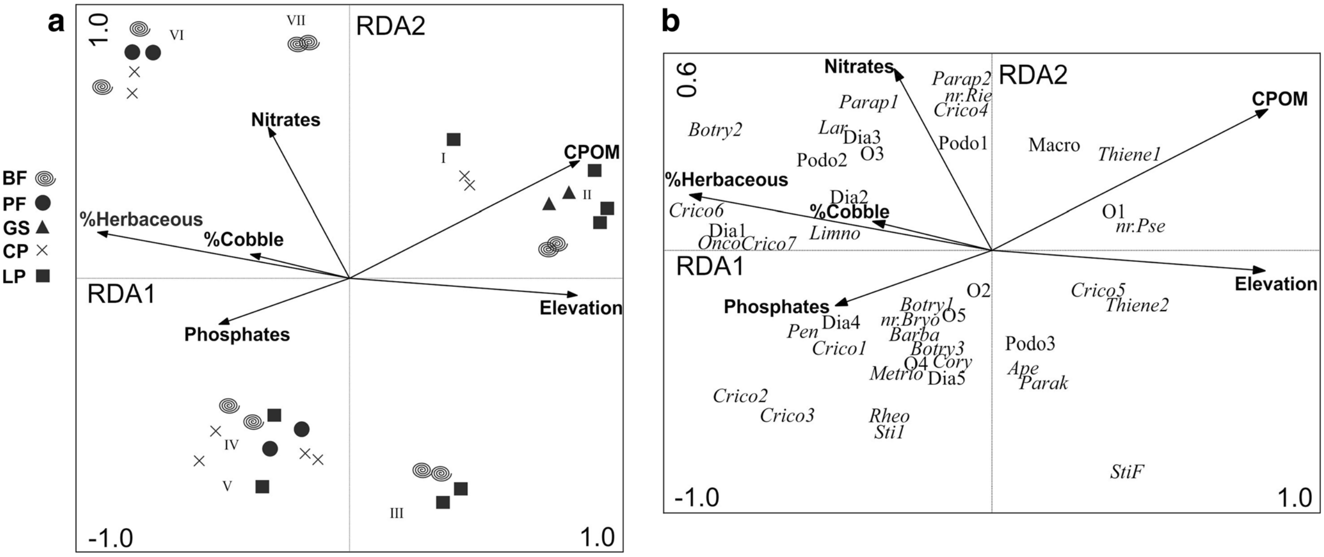

Fig 4 Redundancy Analysis (RDA) biplots for: (**A**) sites and environmental variables, (**B**) chironomid morphotypes/species and environmental variables in Challhuaco-Ñireco system, Río Negro, Argentina. *Plain arrows* represent significant ($p \leq 0.005$) environmental variables; codes of morphotypes in Table 3. Percentages of variance explanation of axes 1 and 2 after manual forward selection are also shown in the graph.

be related to temperature variation presented in different sections of the river (Vannote *et al* 1980, Ward 1994). Our results reflected the typical Andean pattern distribution of the subfamilies according to Ashe *et al* (1987). The ordination produced in the RDA in this work showed that upper sites (I and II) sustained populations of cold-stenothermic species. The opposite was observed for the low part of the Challhuaco-Ñireco system (sites VI and VII), with higher levels of conductivity as a consequence of an impoverishment in water quality, due to the elevated nutrient concentrations attributable to the anthropogenic disturbance cause by Bariloche City. Rossaro (1991b) and Rossaro *et al* (2006) showed that, in Alpine running waters, organic pollution does not appear to be a serious problem for the chironomid fauna; many species resulted favoured by moderate organic enrichment. These authors also revealed the importance of water temperature in chironomid fauna distribution in freshwater and observed that species that tolerate higher temperatures are likely to be tolerant to reduction in dissolved oxygen concentration as a consequence of an increase in organic pollution.

The results of this paper are in agreement with a previous study carried out in the area, in which the authors concluded that there is a longitudinal gradient of eutrophication as revealed by physicochemical features and also by plecopteran and chironomid compositions (Albariño 1997, García & Añón Suarez 2007.

The comparison of these studies with the analysis performed here reveals that many factors play a role in determining species richness of lotic assemblages of Chironomidae. Abiotic factors such as seasonality, temperature, current velocity, etc. determine the species assemblage of any one stream, but as pointed out by Cranston (1995), it is also important to consider the historical processes in providing the pool of species from which alpha-diversity is derived. This is evident viewing the presence in the Challhuaco-Ñireco river system of several Patagonian endemic genera and transantarctic genera, not present in the papers compared herein.

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