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Assessing the performance of macroinvertebrate metrics in the Challhuaco-Ñireco System (Northern Patagonia, Argentina)

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ABSTRACT. Seven sites were examined in the Challhuaco-Ñireco system, located in the reserve of the Nahuel Huapi National Park, however part of the catchment is urbanized, being San Carlos de Bariloche (150,000 inhabitants) placed in the lower part of the basin. Physico-chemical variables were measured and benthic macroinvertebrates were collected during three consecutive years at seven sites from the headwater to the river outlet. Sites near the source of the river were characterised by Plecoptera, Ephemeroptera, Trichoptera and Diptera, whereas sites close to the river mouth were dominated by Diptera, Oligochaeta and Mollusca. Regarding functional feeding groups, collector-gatherers were dominant at all sites and this pattern was consistent among years. Ordination Analysis (RDA) revealed that species assemblages distribution responded to the climatic and topographic gradient (temperature and elevation), but also were associated with variables related to human impact (conductivity, nitrate and phosphate contents). Species assemblages at headwaters were mostly represented by sensitive insects, whereas tolerant taxa such as Tubificidae, Lumbriculidae, Chironomidae and crustacean *Aegla* sp. were dominant at urbanised sites. Regarding macroinvertebrate metrics employed, total richness, EPT taxa, Shannon diversity index and Biotic Monitoring Patagonian Stream index resulted fairly consistent and evidenced different levels of disturbances at the stream, meaning that this measures are suitable for evaluation of the status of Patagonian mountain streams.

KEYWORDS. Patagonian, rivers, macroinvertebrates, biotic indexes, water quality.

RESUMEN. Evaluación del rendimiento de distintas métricas utilizando los macroinvertebrados en el sistema lótico de Challhuaco-Ñireco (Patagonia, Argentina). Siete sitios fueron examinados en el sistema lótico Challhuaco-Ñireco, ubicado dentro del Parque Nacional Nahuel Huapi (PNNH), sin embargo parte de esta cuenca está urbanizada ya que atraviesa la ciudad de San Carlos de Bariloche (150,000 habitantes). Se midieron variables físico-químicas y se colectaron los macroinvertebrados bentónicos durante tres años consecutivos en siete sitios desde la cabecera hasta la desembocadura. Plecoptera, Ephemeroptera, Trichoptera y Diptera predominaron en los sitios cercanos a la cabecera, mientras que Diptera, Oligochaeta y Mollusca dominaron los sitios más bajos cercanos a la desembocadura. En relación con los grupos funcionales, los colectores-recolectores fueron los dominantes en todos los sitios y este patrón fue consistente durante los tres años de muestreo. El análisis de ordenamiento (RDA) reveló que las distribuciones de los ensambles de especies respondieron al gradiente climático y topográfico (temperatura y elevación), pero también fueron asociados con variables relacionadas al impacto antrópico (conductividad, contenido de nitratos y fosfatos). Los ensambles en cabecera estuvieron representados por insectos sensibles, mientras que los taxones tolerantes como Tubificidae, Lumbriculidae, Chironomidae y el crustáceo *Aegla* sp. fueron dominantes en los sitios urbanizados. En relación con las métricas analizadas, la Riqueza total, el EPT taxa, la diversidad de Shannon y el índice biótico BMPS evidenciaron diferentes niveles de disturbio en el sistema, demostrando que estas medidas son una excelente herramienta para determinar el estado de los ríos de montaña de la Patagonia.

PALABRAS-CLAVE. Patagonia, ríos, macroinvertebrados, índices bióticos, calidad de agua

Among in streams communities benthic macroinvertebrates are one of the most conspicuous groups, inhabiting a variety of lotic environments. However, not all the existing species in those systems are ubiquitous nor their distributions are continuous (Cummins *et al.*, 1989). The organisms living in a particular ecosystem are adapted to the local environmental conditions reflecting the degree of preservation of the system. In general, the greater the intensity of natural or anthropogenic disturbances, the more intense are the ecological responses of the communities, revealed by changes in the relative abundance of some groups, increasing populations of opportunistic organisms or even exclusion of vulnerable species (Alba-Tercedor, 1996; Miserendino & Pizzolon, 2000; Ravera, 2001).

The riparian vegetation plays an important role in governing the productivity and the trophic relationships in lotic systems (Cummins *et al*., 1989; Melody & Richardson, 2007; Hoover *et al*., 2011). Therefore, the allochthonous and autochthonous inputs of organic matter are expected to determine the distribution patterns and abundance of macroinvertebrate functional feeding groups (FFG) (Vannote *et al*., 1980).

Biological assessment has been widely used for monitoring and management of quality and integrity of aquatic ecosystems, complementing traditional physical and chemical methods. Particularly, biomonitoring using benthic macroinvertebrates has been increasingly applied in the northern Hemisphere and is at present used and accepted as the most important tool for assessing water quality (Barbour *et al*., 1996, 2006; Walters *et al*., 2009; Bellucci *et al*., 2013). In the Argentinian Patagonia, the macroinvertebrate bioassessment is currently undertaking using the Biotic Monitoring Patagonian Streams (BMPS), developed by Miserendino & Pizzolon (1999) which is a modification of the UK designed Biological Monitoring Working Party (BMWP). At present several scientists had successfully employed the BMPS mostly in lotic systems in the Northwest area.

The Nahuel Huapi National Park (NHNP) is largely known as a biodiversity hotspot in northern Patagonia, is located in the ecotone between the temperate montane rainforest and the arid steppe which further increases the biodiversity of the region. This pristine area is appropriate for biodiversity and conservation studies and specially to establish reference sites, fact that usually is difficult in highly developed areas (Schmidt *et al*., 2009). Nevertheless, urbanization is one of the most increasing phenomena in some mountainous areas in Patagonia, as an example the Ñireco basin (NHNP) in which San Carlos de Bariloche city is located, suffered modifications due to an increase of anthropogenic activities in the last twenty years. One of the main disturbances detected in this basin is the disruption of flow regime due to water abstraction for residential and irrigation purposes. In addition, the inputs of wastewater due to the inadequate treatment of the urban sewage, is perceived by inhabitants and authorities as a problem. Since an increasing record of visitors for recreation was documented in the recent years, activities linked with eco-adventure tourism appear as another possible threat having negative consequences on the biodiversity of environments (Monjeau *et al*., 2005).

The knowledge of freshwater Patagonian benthic fauna is relatively recent and in some areas is poor and fragmentary. Although the functional and ecological aspects of Patagonian lotic ecosystems has received some attention (Miserendino, 2001; Velásquez & Miserendino, 2003; Miserendino & Pizzolon, 2004) most of the studies regarding water quality assessment using macroinvertebrates were carried out in the Northwest of Chubut province (Miserendino & Pizzolon, 2003, 2004; Miserendino, 2007). At urban streams most recent works were completed in nearby areas of small towns as Cholila, Esquel and Corcovado cities (<35,000 inhabitants) (Miserendino *et al*., 2008).

Previous studies conducted in the Ñireco basin refers to morphological and ecological approaches of the different macroinvertebrate functional feeding groups (FFG) (Albariño & Balseiro, 1998, 2001, 2002; Díaz Villanueva & Albariño, 1999; Balseiro & Albariño, 2006), and about species distribution along the altitudinal gradient (Albariño, 1997; García & Añón Suarez, 2007). Albeit all this work, there is still a lack effort in evaluating and testing the suitability and applicability of macroinvertebrate attributes and metrics combined with changes over the time to evaluate water quality at other Patagonian basins as the Ñireco.

The aim of this study was to examine the composition and functional structure of benthic macroinvertebrate communities along a vegetational and climatic gradient in the Ñireco valley and to establish the degree of the anthropogenic impact on this river. Our purpose was also to assess the usefulness of different macroinvertebrate metrics in a spatial dimension and in an inter-annual sequence, to detect water deterioration in this urbanized Patagonian basin. The results here obtained will be useful for biological recovery, conservation and restoration management of Patagonian watercourses. Given that the Nahuel Huapi National Park (NHNP) has been identified as one of the most important conservation areas in Argentinean Patagonia, the present study will accomplish the interest of the NHNP administration, regarding the impacts of increased tourism pressure on the aquatic ecosystems.

Study area. This study was carried out in the Challhuaco-Ñireco valley located in the south western of the Natural Reserve of NHNP in the Río Negro Province (41°13'S, 71°19'W), Patagonia Argentina. The river source is located at 2140 m.a.s.l. in Confluencia Hill, one of the highest elevations of the Ventana mountain range (Biedma, 1978). The headwaters of Ñireco river system is surrounded by the humid forest dominated by *Nothofagus pumilio* Poepp. & Endl. and its associated flora [*Berberis serratodentata* Lechl., *Maytenus chubutensis* (Speg.) Louteig, O'Donell & Sleumer, *Ribes magellanicum* Poir., S*chinus patagonicus* (Phil.) I.M. Johnst. and *Myoschilos oblongum* Ruiz & Pav.] whereas, the lower areas of the river goes through an open valley with ecotonal and steppe vegetation elements around the river banks, such as shrubs and brushes mostly dominated by Compositae and *Salix* sp. (Correa, 1998). The hydrological regime of the Ñireco system shows two high water discharge periods one due precipitation (autumn), and other to snowmelting events (spring). The substratum is bedrock (particle size: boulder-cobble-pebble-gravel) and the lotic order varies from the source to the mouth between 1 and 3 (Strahler, 1957).

Ñireco River intercepts its tributary, Challhuaco River, before arriving to the city of Bariloche, and drains into the Nahuel Huapi Lake at 700 m.a.s.l., fulfilling a distance of 40 Km (Fig. 1).

In the last few years, Bariloche city has suffered a growth of population improved by the touristic market, exceeding 150,000 permanent residents (Monjeau *et al*., 2005; Pereyra, 2007). Pluvial and waste waters from the urban area end in the Nahuel Huapi Lake. Even though there is a wastewater treatment plant (W.T.P.) located in the coast of the lake, it only processes one third of the entering sewage effluents. Furthermore, heavy metals from photography industry and gas stations are not treated in the W.T.P. (Pereyra, 2007). The Ñireco River receives a huge part of this waste produced in the city, mainly solid fuels and pluvial runoff, to finally drain into the lake.

MATERIALS AND METHODS

Seven sites located at the pre-urban (forest-ecotonal) and urban (ecotono-steppe) section of Bariloche city (41°09'S, 71°18'W) were sampled for benthic macroinvertebrates, on summer periods during three consecutive years (December 2009, February 2010, December 2010 and February 2011) (Fig. 1).

The environmental characterization included

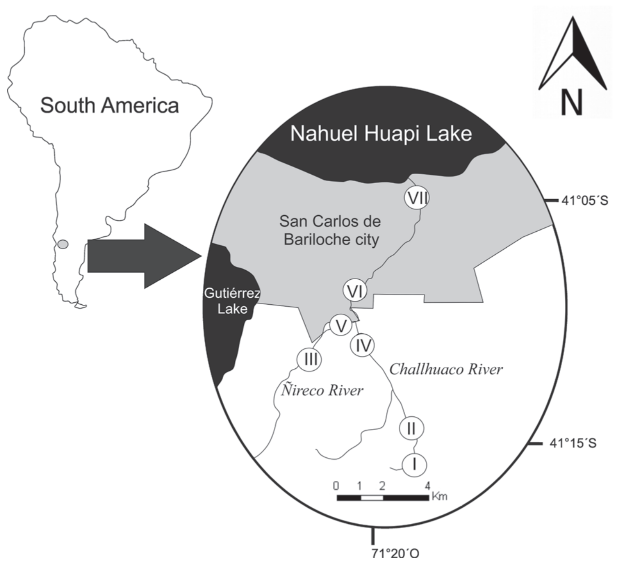

Fig. 1. Map with the seven sampling points along Chalhuaco-Ñireco system in Northern Patagonia, Argentina. (Grey: urban area of San Carlos de Bariloche).

physical and chemical variables such as pH, water temperature, conductivity, dissolved oxygen, NH_4, $N\text{-}NO_2$, $N\text{-}NO_3$ and $P\text{-}PO_4$ (Tab. II). Current speed was measured in mid-channel on three occasions by timing a float (average of three trials) as it moved over a distance of 10 m (GORDON *et al.*, 1994). Average depth was estimated from three measurements on a transect across the channel with a calibrated stick. Environmental features such as typology of the river bank, substrate type and vegetation were also estimated. Main 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 herbaceous stratum. The percentages of boulder, cobble, gravel, pebble, and sand in the reach were estimated using a $1m^2$ grid (GORDON *et al.*, 1994).

On each sampling occasion water temperature, pH, conductivity ($\mu S_{20}\ cm^{-1}$) and dissolved oxygen ($mg\ O_2\ l^{-1}$) were measured with a multi-parameter probe HANNA I-9828. For chemical analysis (NH_4, $N\text{-}NO_2$, $N\text{-}NO_3$ and $P\text{-}PO_4$) water samples were collected below the water surface using 500 ml water bottles, kept at 4°C prior to analysis at the laboratory, and analyzed using standard methods (APHA, 1999).

For macroinvertebrates, qualitative samples were obtained using a "kick-sampler" in order to get the maximum diversity for each site. To obtain comparable results, sampling was carried out along a 10 to 20 m section for a period of three minutes, as suggested for low order streams (DE PAUW & VANHOOREN, 1983). Simultaneously, quantitative samplings were performed using a Surber net of 0.09 m² surface area and 250 µm mesh size with three replicates per site (n=3).

In the laboratory, macroinvertebrate samples were washed and sieved through a set of filters (from 250 to 1000 µm). Detritus was analysed only in two samples sites (December 2010 and February 2011) and was divided into fine particulate organic matter (FPOM) (250-1000 µm) and coarse particulate organic matter (CPOM) (>1000 µm). After that, coarse detritus was separated into wood and leaves (mainly entire leaves) (VOELZ & WARD, 1990). All the fractions were dried (60°C for 24 h) and weighted. Macroinvertebrates were sorted in white trays and stored in alcohol 70% for subsequently taxonomical identification under stereo-microscope.

Invertebrates were identified to the lowest possible taxonomic level, using regional keys (LOPRETTO & TELL, 1995; DOMÍNGUEZ & FERNÁNDEZ, 2009) and classified into the different Functional Feeding Groups (FFG) that were recognised using available bibliography (MERRIT & CUMMINS, 1996; DOMINGUEZ, 1998; MISERENDINO & PIZZOLON, 2000): collector-gatherers (CG), collector-filterers (CF), scrapers/grazers (SC), shredders (S) and predators (P).

Absolute abundance was obtained counting individuals of each taxon and expressing the results as density (number of individuals per surface area m²). Relative abundance of FFG was obtained as the number of each group divided by the total number of macroinvertebrates at each sample.

To infer possible environmental impacts on the river, a set of macroinvertebrate community descriptors were estimated: the BMPS index (MISERENDINO & PIZZOLON, 1999) at family level, average of taxa richness (TR) which measures the overall variety of the macroinvertebrate assemblage, relative contribution of Chironomidae (% Chiro) and Ephemeroptera, Plecoptera and Trichoptera (% EPT) and abundance of EPT taxa (ROSENBERG & RESH, 1993).

To analyze the community structure, diversity index of Shannon Weaver (H), Simpson dominance (D) and Pielou eveness (J) were calculated, and differences were tested using Bayesian method (MCCARTHY, 2007; BOLSTAD, 2009). All macroinvertebrates numerical data were analysed in a Bayesian perspective using a Dirichlet non-informative distribution that belongs to the family of multivariate continuous distribution. To develop such indexes, a WinBUGS model (<http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml>) was run using R platform (R2WinBUGS) (<http://cran.r-project.org/web/packages/R2WinBUGS/>) (STURZ *et al.*, 2005; NTZOUFRAS, 2009). Analyses were carried out for the seven sites and at the four sampling periods on the biological data. In the procedure 100000 interactions were used (excluding the first 1000). The posterior distribution was expressed as average, standard deviation, Bayesian credibility intervals (95% probability that the true value is in the interval) and the posterior probability for each comparison.

To explore main relationships of metrics and water quality variables a Spearman rank correlations was performed, using Bonferroni adjustments (for the number of pair-wise comparisons) (RICE, 1989).

Detrended correspondence analysis (DCA), with down-weighting of rare taxa, was performed on log (x+1) transformed data to determine whether unimodal or linear ordination techniques would be most appropriate for modeling the relationships between macroinvertebrate assemblages and environmental variables. Based on the resulting axis 1gradient length of less than three, linear ordination techniques were performed (CANOCO statistical package, version 3.0). RDA was carried out in order to compare species-environment relationships during the three sampling years. For the RDA, fifteen variables (pH, conductivity, TDS, stream order, water temperature, dissolved oxygen (DO), elevation, P-PO_4, N-NO_2, N-NO_3, NH_4, riparian coverage as % forest and herbaceous stratum, and also percentages of cobble and pebble), twenty-eight samples and seventy-three taxa were used. Variance "inflation factor" (VIF) was examined to ascertain the environmental variables that covaried or/and were redundant with other variables. Then, variables with VIF >20 were removed prior the analysis. Finally, we performed a subsequently RDA on the remaining variables with Monte Carlo permutation test with manual forward selection, to determine the significant variables that contribute in explaining the variance in the macroinvertebrate taxa. Ordinations were performed using CANOCO 4.5 package (ter Braak & Smilauer, 2002).

RESULTS

Environmental features. The seven sampled sites studied display an altitudinal range from 767 to 1380 m.a.s.l. (Tab. I). Throughout sites I to IV, the stream is narrow and shallow (average depth at December 145.5 cm and at February 31 cm) with a predominantly rocky bottom and an important contribution of woody debris (fallen leaves and tree branches) from the surrounding forest. As the stream cross the ecotonal area it becomes wider with a streambed that includes stones of different sizes, many of them partly covered by Chlorophyta algae at site VII. Sites IV, V and VI are located within disturbed areas related to touristic activities (i.e. trekking, mountain bike, especially at site V that is a student recreational area), whereas site VII is directly affected by an urban settlement; San Carlos de Bariloche.

Table II summarises the main physico-chemical parameters measured at the seven sites in the Challhuaco-Ñireco System. We combined Decembers (2009 and 2010) and Februaries (2010 and 2011) to see if different environmental situations could be observed during early (December) and late (February) summer. In Northern Andes, early summer months are usually characterised by low precipitations and light runoff due to snowmelt (Paruelo *et al.*, 1998). A different situation can be recorded during late summer (February), with still low precipitation but low runoff as well. During the study, pH did not display important changes remaining from neutral to slightly alkaline values in all measures. Water temperature was higher at late summer and increased gradually from the headwaters to the mouth, while water conductivity did not show a regular longitudinal improve in values between site II to VI, but with the lowest value for site I and the highest for site VII. Dissolved solids content was low in the system; nevertheless, a slightly increase could be observed at site VII, particularly at late summer. Dissolved oxygen variation among sites was low at the whole study and the percentages of dissolved oxygen were superior to 80% in all the sites during this study. The maximum water speed was recorded at site VII and the minimum value at site V (Tab. II).

Phosphates concentrations were in general low; nevertheless, the variable displayed more concentrated at late summer and a brief increase towards the mouth, with the highest value recorded at site VII (Tab. II). The values of ammonium (NH_4), nitrite (NO_2) and nitrate (NO_3) also displayed more concentrated at late summer and a visible increase towards the Ñireco mouth.

Mean dry weight values of the detritus recorded ranged from 1.10 g.m^{-2} (FPOM at site IV) to 59.8 g.m^{-2} (CPOM at site I). As expected, from site I to VII it was observed a decreasing pattern of CPOM and an increase of FPOM (Fig. 2). From the CPOM fraction, the biomass of wood and leaves were higher at the sites located near to the headwaters (Fig. 2), showing the highest values at site I (142.8 g.m^{-2}) and the lowest at site IV (8.6 g.m^{-2}).

Taxa composition and functional organization. A total of 73 taxa (Tab. III) were identified in this study, being Insecta the most abundant and diverse group. The insect community was composed by five orders, with Plecoptera

Tab. I. Environmental features of the seven sampling sites in the Challhuaco-Ñireco system, Río Negro, Argentina during the study period. Mean values of depth (n=3).

Site	Stream Order	Elevation (m.a.s.l)	% Canopy			Substratetype (mm)			Depth (m)
			Forest	Shrub	Herbaceum	Boulder >256	Cobble 64-256	Pebble 16-64	
I	1	1380	100	0	0	0	40	50	0.51
II	1	1321	100	0	0	0	50	50	0.45
III	2	959	70	30	0	80	20	0	0.47
IV	2	1029	20	60	20	20	80	0	0.3
V	3	966	0	30	70	0	90	10	0.54
VI	3	892	0	30	70	40	50	10	0.2
VII	3	767	0	0	100	10	90	0	0.75

and Trichoptera displaying the highest richness (15 taxa each one).

Among Plecoptera, the Family Gripopterygidae (represented by 10 species) was dominant both in density and frequency (Tab. III). *Klapopteryx kuscheli*, *Notoperla fasciata* and *Austronemoura* sp. were mostly dominant at headwaters, *Notoperla archiplatae* and *Rhitroperla rossi* at the middle and mouth.

Leptophlebiidae was the family best represented among Ephemeroptera and from the three species of *Meridialaris* identified; *M. chiloeensis* was very frequent at all sites except VII. Concerning Baetidae, *Andesiops peruvianus* was most abundant at headwaters and *A. torrens* at the mouth.

Among Trichoptera, Hydropsychidae and Hydrobiosidae were the most abundant. *Smicridea dithyra* was the taxa most abundant, especially at sites I and II (Tab. III). The other Trichoptera taxa were represented by few individuals at all sites.

Among Diptera, Athericidae, Empididae, Simulidae and Tipulidae decreased in density towards the mouth, except for Chironomidae that increased from site I to VII (Tab. III). On the other hand, Blephariceridae, Dixidae and Tabanidae were found only at headwaters.

The Nannochoristidae family of Mecoptera and the turbellarian *Girardia* sp. were also found only at headwaters. Regarding Oligochaeta, Lumbriculidae was the most frequent and was found in all sites, but most Naididae at site I and Tubificidae at site VII.

From the 73 taxa recorded in the study (Tab. III), 5 of them were collector-filterers (CF), 12 shredders (S), 11 collector-gatherers (CG), 21 scraper/grazers (Sc) and 24 predators (P). Along the river CG and Sc were dominant, both together contributing with more than 60% of the total density at all sites (Fig. 3). Shredders reached 22% of relative density at site VI (35.5% at February 2010 and 44% at February 2011) but their density was negligible at headwaters and at sites located in the middle section of the river. Site VII was dominated by CG (almost 100%) with several species of Chironomidae and Oligochaeta in all the samples analyzed. Collector-filterers were absent at site VII in December 2009 and February of 2011, and shredders in December 2010. Sites II, III, IV and VI displayed high abundances of Scrappers (45%) during the whole sampling periods.

Macroinvertebrate metrics and biotic indexes. Sites I and II showed the highest taxonomical richness (TR), with the maximum values of 29 and 30 taxa respectively (Tab. IV). In contrast, site VII displayed the lowest value (5 taxa). The highest densities corresponded to sites II and VII (Tab. IV).

The % EPT was high at most sites (Tab. IV) during all the time (values between 30% and 70%) except at site VII where EPT contribution was the lowest (0.3% on February 2011 and 3.6% on December 2009). Sites III and VI showed the highest % EPT reflecting an important contribution of these three orders (>60%). The relative

Tab. II. Physico-chemical parameters measured at seven sampling sites in the Challhuaco-Ñireco System, Argentina. Data are mean values of December 2009 and December 2010 (D) and February 2010 and February 2011 (F), except for pH are mean values ± SD of the four sampling data, and for current velocity (CV) and depth measured only in December 2010 and February 2011.

Site	pH	T (°C)		Conductivity ($\mu S_{20}.cm^{-1}$)		TDS (ppm)		DO (mg $O_2 l^{-1}$)		CV ($m.s^{-1}$)		Depth (cm)		P-PO_4 (μl^{-1})		N-NH_4 (μl^{-1})		N-NO_2 (μl^{-1})		N-NO_3 (μl^{-1})	
		D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F
I	8.02 ±0.47	3.92	8.30	31.5	43	16	21.5	11.27	9.13	0.79	0.36	83.3	18.3	3.1	13	6.75	33.75	1.5	1	12.5	16.6
II	8.36 ±0.02	4.56	8.92	33.5	44	16.5	21.5	10.82	8.3	0.55	0.55	68.3	21.7	5.7	36	6.25	37	1.5	1.5	7.4	13.9
III	8.24 ±0.14	6.91	10.17	52.5	73	26	36.5	11.97	9.45	1.03	0.7	46.3	48.3	7.7	50	10	61	1	1	3.81	7.8
IV	7.6 ±1.35	7.10	9.35	37	67	18.5	32.5	12.58	7.78	0.97	0.29	24.3	36.3	10	45	21	51	1.34	1.5	9.2	15.4
V	7.88 ±0.88	7.13	10.26	38.5	68	19.5	33.5	12.6	9.63	0.76	0.22	32.3	36.7	22.5	42	7	27	1.36	1.5	3.5	19.5
VI	7.84 ±0.92	9.56	11.36	51.5	75	26	37	12.52	8	0.67	0.36	15.7	21.7	37	42	9.5	15	1	1.5	6.3	11.6
VII	8.43 ±0.45	11.68	13.51	73.5	128	36.5	64	11.31	6.6	1.61	0.93	63.3	86.7	50	56	22	70	2.7	14	127	220

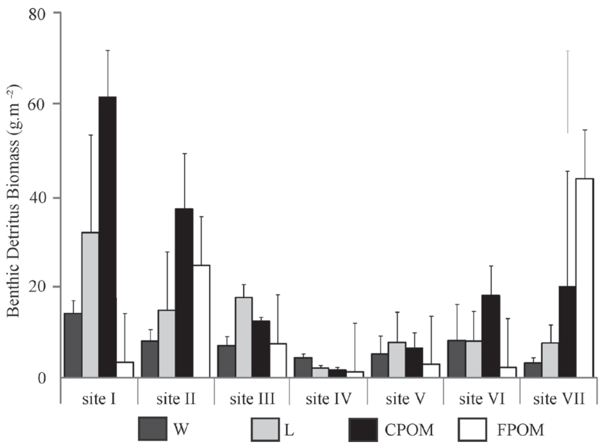

Fig. 2. Mean values of the benthic detritus (DM g.m^{-2} +1SD) at the seven sampling sites of the Chalhuaco-Ñireco system (Río Negro, Patagonia, Argentina) of December 2010 and February 2011 (FPOM, fine particulate organic matter; CPOM, coarse particulate organic matter; W, wood; L, leaves).

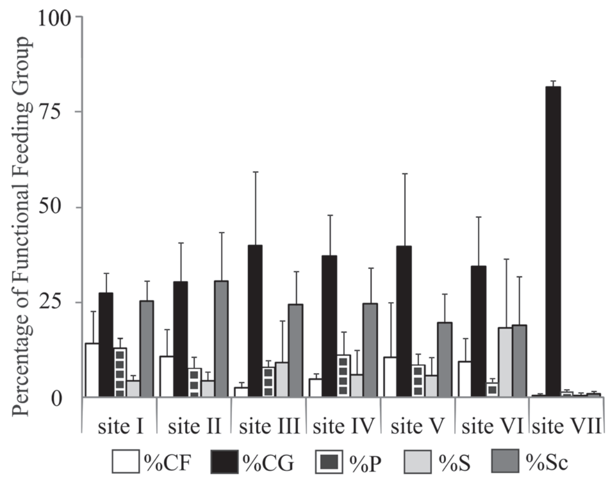

Fig. 3. Relative density (±1SD) of macroinvertebrates of each Functional Feeding Group, sampled at seven sites of the Chalhuaco-Ñireco system (Río Negro, Patagonia, Argentina) during the study period (CF, collector-filterers; CG, collector-gatherers; P, predators; S, shredders; Sc, Scraper/Grazers).

abundance of chironomids was substantially high at sites III and VII (70%) reaching the maximum values (100%) at site VII on February 2011.

Sites I and II showed the highest BMPS values (I: 142 February 2011, II: 141 December 2010) and corresponded to class I of unpolluted waters. Sites III, IV, V and VI (mean values between 68 and 86) displaying class II: "probably incipient pollution or other kind of perturbation". Finally, site VII accounted for the lowest mean value, corresponding to class III: "probably polluted waters"), being December 2009 the month that showed the minimum value (23) considered as class IV of "polluted waters".

According to Bayesian analysis and concerning the biotic indexes (H, D, J), the values observed at sites VII and I were significantly different from the rest ($p \leq 0.05$) (Tab. IV). Shannon-Weaver diversity values (H) decreased consistently from the headwater to the mouth. As expected, Simpson dominance (D) showed the opposite situation, increasing as Shannon diversity decreases, which means that the probability of an intra-specific encounter is higher when the community is homogeneous. Pielou equitability index (J) reflected a noticeable separation of site VII. For this reason, sites I and II that showed a highest H indicates an heterogeneous community composition whereas, sites close to the mouth that were less diverse and more uniform, showed a high D, at these particular sites D values were related with a community dominated by Chironomidae taxa.

Macroinvertebrate metrics and biotic indexes were correlated significantly with a range of water quality variables, as temperature, conductivity, TDS and nutrients concentration (Tab. V). As expected, the metrics TR, % EPT, EPT taxa, BMPS, H, D and J displayed several negative and significant responses to variables as conductivity, TDS and concentration of nitrates and nitrites. On the other hand, % Chiro reflected significant positive correlations with physico-chemical variables that indicated an environmental impoverishment, as conductivity, TDS and ammonia.

Species-environment relationships. In the RDA, the Monte Carlo permutation test with forward selection determined four significant variables: water temperature, elevation, P-PO_4 and N-NO_3 that accounted for the greatest amount of variance in the macroinvertebrate distribution along the river (47%). The eigenvalues of axes 1 and 2, were 0.309 and 0.109 respectively, and explained 73.5% of the cumulative percentage variance ($p = 0.002$) in the taxa environmental relationship data (Tab. VI).

Distribution of sites and species along axes 1 and 2 is displayed in Fig. 4. Axis 1 clearly shows an environmental/climatic gradient governed by temperature and elevation whereas axis 2 shows a nutrient gradient determined by nitrates and phosphates contents. Sites I and II and species that belongs to the group of sensitive insects (EPT), such as *Klapopteryx kuscheli*, *Austronemoura* sp., *Pelurgoperla personata*, *Notoperla* sp., *Senzilloides panguipulli*, *Nousia* sp., *Metamonius anceps*, *Andesiops peruvianus*, *Monocosmoecus* sp., *Austrocosmoecus*, *Smicridea dithyra*, *Sortosa* sp., *Rheochorema* sp. and *Neoatopsyche* sp. were located to the positive side of RDA axis 1 (Fig. 4). The increase in the level of phosphates in the low part of the Ñireco system was highlighted at the negative end of RDA axis 1, thus dates and sites having higher levels of phosphate contents were located on the left upper quadrant, and species that increased in density at those sites were grouped (*Andesiops torrens*, *Rhitroperla rossi*, *Notoperla archiplatae*, *Meridialaris laminata*, *Myotrichia murina*, *Smicridea annulicornis*, *Cailloma* sp.). On the other hand, sites V (D 09, D 10) and VI (D 10, D 09), and particularly site VII were associated to the negative values of RDA axis 2, with high levels of NO_3 and with the invertebrate assemblage composed by "tolerant" taxa such as Annelida (Lumbriculidae, Tubificidae and Naididae)

Tab. III. Mean density values of macroinvertebrates sampled at seven sites of Challhuaco-Ñireco system, Río Negro, Argentina, during the study period (December 2009 and 2010, February 2010 and 2011). Species codes (SC) used in the figures (Functional feeding groups - FFG: SC, scrapers; P, predators; S, shredders; CF, collector-filterers; CG- collector-gatherers).

Taxa	SC	FFG	I	II	III	IV	V	VI	VII
PLECOPTERA									
Austroperlidae									
Klapopteryx kuschelli Illies, 1960	kk	S	66	108	9	20	9	1	0
Gripopterygidae									
Alfonsoperla sp.	alfon	CG	2	0	0	0	0	0	0
Andiperlodes sp.	andi	CG	8	0	0	0	0	0	0
Antarctoperla sp.	antacto	S	0	0	5	1	0	7	1
Aubertoperla illiesi Froehlich, 1960	a i	SC	65	173	94	59	106	35	0
Limnoperla jaffueli Navás, 1928	limno	SC	0	0	3	0	2	50	1
Notoperla sp.	not	SC	1	0	0	0	0	0	0
Notoperla archiplatae Illies, 1958	not a	SC	3	2	33	33	32	4	1
Notoperla fasciata McLellan, 2006	not f	SC	39	52	0	2	6	2	0
Notoperla magnaspina McLellan, 2006	not m	SC	0	1	0	0	0	0	0
Pelurgoperla personata Illies, 1963	p p	CG	1	3	4	0	0	0	0
Rhitroperla rossi Froehlich, 1960	r r	S	1	5	104	69	81	373	4
Notonemouridae									
Austronemoura sp.	ausnem	SC	54	85	13	9	7	1	1
Udamocercia sp.	uda	SC	0	0	4	0	13	3	0
EPHEMEROPTERA									
Baetidae									
Andesiops peruvianus Ulmer, 1920	a p	SC	46	65	7	7	2	2	0
Andesiops torrens Lugo-Ortiz & McAfferty, 1999	a t	SC	6	6	135	72	70	91	3
Leptophlebiidae									
Meridialaris diguillina Demoulin, 1955	m di	SC	0	0	7	1	0	0	0
Meridialaris laminata Ulmer, 1920	m la	SC	0	0	10	0	3	81	3
Meridialaris chiloeensis Demoulin, 1955	m chi	SC	187	339	194	169	142	149	6
Nousia sp.	nou	SC	19	39	10	6	3	10	0
Nesameletidae									
Metamonius anceps Eaton, 1883	m a	SC	29	131	0	2	0	2	0
TRICHOPTERA									
Hydrobiosidae									
Cailloma sp.	c	P	0	0	9	0	2	0	2
Cailloma pumida Ross, 1956	c p	P	0	0	0	0	4	2	0
Cailloma rotunda Flint, 1967	c r	P	0	0	0	0	0	1	0
Neoatopsyche sp.	neoat	P	1	1	0	0	0	0	0
Neopsylochorema sp.	neop	P	0	1	0	0	0	0	0
Neopsylochorema tricarinatum Schmid, 1955	neop t	P	1	5	0	1	1	0	0
Rheochorema sp.	rheo	P	3	1	2	1	1	0	0
Rheochorema tenuispinum Schmid, 1955	rheo t	P	2	2	2	1	2	2	0
Hydropsychidae									
Smicridea sp.	sm	CF	0	2	0	0	0	0	0
Smicridea annulicornis Blanchard, 1851	sm a	CF	0	0	0	0	0	3	0
Smicridea dithyra Flint, 1974	sm di	CF	52	29	0	6	0	0	0
Leptoceridae									
Brachysetodes sp.	brachi	S	1	6	6	2	7	5	1
Limnephilidae									
Austrocosmoecus sp.	austro	S	1	0	0	1	0	0	0
Austrocosmoecus hirsutus Schmid, 1955	austro h	S	5	3	0	0	0	0	0
Monocosmoecus sp.	mono	S	2	6	0	0	0	0	0
Philopotamidae									
Sortosa sp.	sor	S	2	0	0	2	0	0	0
Sericostomatidae									
Myotrichia murina Schmid, 1955	m m	S	0	0	1	0	1	0	0
Parasericostoma sp.	para	S	0	0	0	1	2	2	0
DIPTERA									
Athericidae	athe	P	34	84	95	80	97	47	6
Blephariceridae	blepha	P	35	18	0	3	1	0	0
Ceratopogonidae	cerato	P	23	24	4	10	14	2	0
Chironomodae	chiro	CG	324	760	472	451	599	523	2231
Dixidae	dix	CF	0	2	0	1	0	0	0
Empididae	empi	P	15	31	7	13	22	3	2
Simulidae	sim	CF	230	368	45	60	165	162	8
Tabanidae	tab	P	2	0	0	0	0	0	0
Tipulidae	tip	P	86	45	12	61	2	2	1

Tab. III. Cont.

Taxa	SC	FFG	I	II	III	IV	V	VI	VII
MECOPTERA									
Nannochoristidae	nanno	P	5	13	0	0	0	0	0
CRUSTACEA									
Aeglidae									
Aegla sp.	aegla	P	0	0	0	0	0	0	1
TURBELLARIA									
Dugessidae									
Girardia sp.	gir	P	17	26	0	0	0	0	0
HIRUDINEA									
Glossiphonidae	hiru	P	1	0	0	0	0	0	0
OLIGOCHAETA									
Lumbriculidae	lumb	CG	7	16	15	0	2	17	16
Naididae	nai	CG	20	0	1	4	0	0	0
Tubificidae	tubi	CG	0	0	1	1	0	0	10

Tab. IV. Minimum and maximum values of Taxa Richness (TR) and mean values and standard deviation (± SD) of total density, relative abundance of EPT and Chironomidae, BMPS index, Shannon-Weaver (H), Simpson (D) and Pielou (J) diversity measures from the seven sites of the Challhuaco-Ñireco system, Río Negro, Argentina, sampled in four dates (December 2009 and 2010, February 2010 and 2011). Letters in round brackets show significant differences among sites ($P<0.05$).

Site	TR	Density	%EPT	%Chiro	BMPS	H	D	J
I	24-29	1550 ±533	40.62 ±10.7	20.74 ±6.5	124 ±16.8	4.7 ±0.009(f)	0.06 ±0.0002(e)	0.87 ±0.001(f)
II	22-30	2683 ±1122	43.18 ±18.9	29.87 ±14.2	124 ±17.6	4.5 ±0.007(e)	0.02 ±0.0003(a)	0.82 ±0.001(c)
III	14-21	1325 ±518	48.2 ±20.1	72 ±61.5	74 ±20	4.1 ±0.009(d)	0.02 ±0.0003(b)	0.84 ±0.002(e)
IV	11-28	1191 ±782	41.36 ±18	58 ±63	86 ±32.5	4 ±0.009(d)	0.03 ±0.0004(c)	0.84 ±0.002(e)
V	16-34	1410 ±217	34.5 ±13.5	32 ±26	86.5 ±4	4 ±0.01(c)	0.03 ±0.0005(d)	0.81 ±0.002(b)
VI	9-21	1576 ±394	48.8 ±19	36 ±18.7	68.25 ±26	3.9 ±0.008(b)	0.03 ±0.0003(c)	0.83 ±0.002(d)
VII	5-10	2298 ±2332	1.9 ±1.4	94.5 ±3.4	37.5 ±12.4	2.3 ±0.007(a)	0.1 ±0.001(f)	0.56 ±0.002(a)

Tab. V. Spearman rank correlations between environmental and biological variables determined at seven sites on the Challhuaco-Ñireco system, Río Negro, Argentina during the study period (December2009 and 2010; February2010 and 2011). Significant correlations after Bonferroni corrections are shown in bold (n=28; $P<0.05$) (TR, Taxa Richness; H, Shannon-Weaver; D, Simpson; J, Pielou).

Variables	TR	Density	% EPT	% Chiro	EPT taxa	BMPS	H	D	J
pH	-0.01	**0.77**	-0.52	0.25	-0.33	-0.25	-0.32	0.46	-0.54
Temperature	**-0.90**	0.09	-0.68	0.73	-0.61	-0.65	**-0.94**	**0.84**	**-0.82**
Conductivity	**-0.92**	0.08	**-0.76**	**0.84**	-0.72	-0.75	**-0.97**	**0.89**	**-0.87**
TDS	**-0.92**	0.07	**-0.76**	**0.83**	-0.73	**-0.76**	**-0.96**	**0.89**	**-0.87**
DO	0.34	0.13	0.16	-0.33	0.16	0.01	0.08	-0.07	-0.03
$P\text{-}PO_4$	-0.72	0.07	-0.30	0.74	-0.05	-0.01	-0.63	0.47	-0.50
$N\text{-}NH_4$	**-0.85**	-0.47	-0.32	**0.81**	-0.35	-0.31	-0.55	0.41	-0.33
$N\text{-}NO_2$	-0.68	0.54	**-0.99**	0.58	**-0.87**	-0.74	**-0.87**	**0.96**	**-0.94**
$N\text{-}NO_3$	-0.62	0.50	**-0.98**	0.49	**-0.91**	**-0.77**	**-0.80**	**0.91**	**-0.89**

Tab. VI. Eigenvalues and correlation of standardized environmental variables with the first two RDA axes. Significant correlations are presented in bold. Test of significance of first canonical axis: F=4.840, p-value=0.004. Test of significance of all canonical axes: F=1.909, p-value=0.002.

Axis	RDA1	RDA2
Eigenvalue	0.306	0.109
Species-environmental correlations	0.971	0.938
Cumulative percentages variance		
of species data	30.6	41.4
of species-environmental relation	41.6	56.3
Correlations		
Water temperature	-0.71	0.34
PO_4	-0.43	0.61
NO_3	-0.10	-0.56
Elevation	0.91	0.22

Chironomidae (Chironominae and Orthocladiinae) but also the Gripopterygidae *Limnoperla* sp.

DISCUSSION

Physic and chemical analysis, macroinvertebrate metrics and biotic indexes broadly indicated a good water quality in the Challhuaco-Ñireco river system. The exception was site VII, which suffered the increasing urban impact of San Carlos de Bariloche city. Generally speaking, this process was evidenced in high values of conductivity and nutrient concentration which were at some dates until five times (phosphates) and eight times (nitrates) higher at post urban sites than pre-urban ones. Despite these

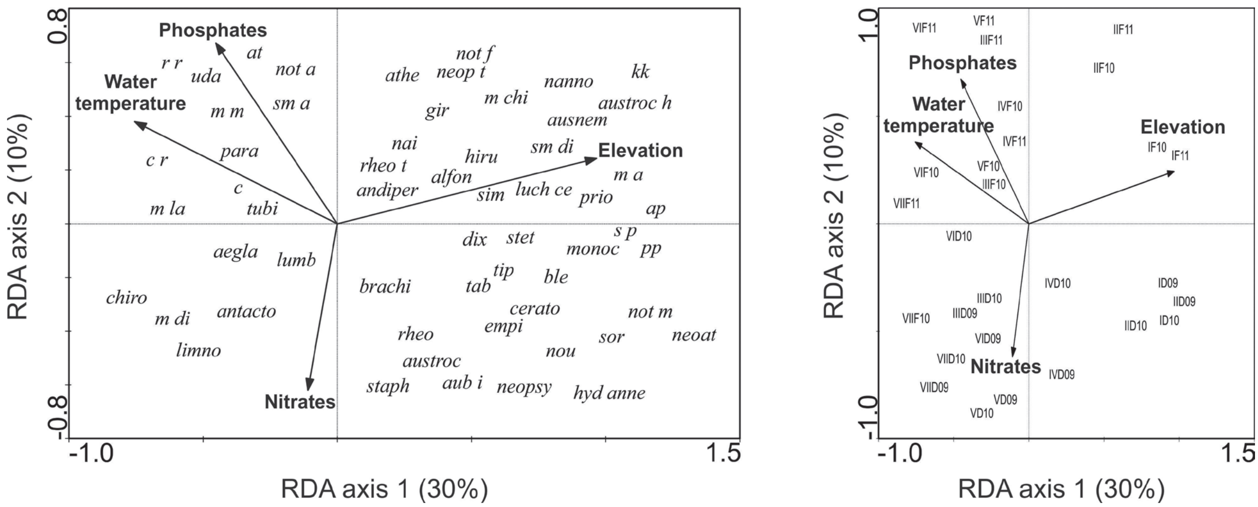

Fig. 4. Redundancy analysis (RDA) diagram in Ñireco catchment, Río Negro, Argentina. Left, species and environmental variables relationships; species codes are in Table III. Right, sites and environmental relationships, dates as follow: D09, December 2009; D10, December 2010; F10, February 2010; F11, February 2011. Plain arrows represent significant ($p<0.005$) environmental variables. Percentages of variance explanation of axes 1 and 2 after manual forward selection are also shown in the graph.

observed patterns can be partially explained by natural causes (e.g. elevation gradient, water flow, substrate composition), our study suggests that the discharged of residual water from Bariloche produced those modifications especially at site VII. Other previous studies carried out at Ñireco River (Albariño, 1997) also found increases in conductivity at sites near to the mouth (67.6 μS.cm^{-1}). Nitrate concentrations were noticeably high at site VII, fact that has been widely associated with organic pollution in urbanized areas (Alvarez Cabría *et al*., 2010). García & Añón Suarez (2007) also documented significant increases in phosphate and nitrites concentrations towards the mouth of the Ñireco stream, concluding that those increments were linked to anthropogenic impact of Bariloche city. Moreover, Temporetti (2006) reported an impoverishment of the water quality at the same basin and related that to a fire occurred in 1996 affecting 1,278 ha, the high nitrate levels in the water been a consequence of the high solubility of the compound and the poor retention of soils.

Rivers are highly interactive with the surrounding landscape, being affected by the disturbances produced by deforestation, pasture conversion and particularly urbanization (Ward, 1989; Paul & Meyer, 2001). This can results in a severe damage in the conditions of the river, affecting the composition and density of benthic communities. According to our observations, macroinvertebrate communities responded primarily to water temperature, elevation and nutrient concentrations ($P-PO_4$ and $N-NO_3$), and were useful to evidence incipient pollution or disturbance in the studied system.

As expected and in agreement with the RCC prediction (Vannote *et al.*, 1980), a decreasing pattern of CPOM and an increasing trend of FPOM were observed from headwaters to the mouth, strongly related with a downstream decrease in the percentage of the riparian cover vegetation. Previous works carried out in other Patagonian basins in the mountain and having similitude in forest cover concluded that functional organization at streams moderately fit to the RCC predictions (Velásquez & Miserendino, 2003; Miserendino, 2007). Collector-gatherers increased from headwaters to the river mouth; whereas collector-filterers, predators, scrapers and shredders decreased in the same way. Nevertheless the functional structure at site VII was strongly modified; collector-gatherers occupied more than the 95 % of total abundance. Castela *et al.* (2008) observed that nutrient enrichment, decrease of riparian coverage and impoverishment of the fluvial quality associated at urbanized sites resulted in important changes on the structure and function of macroinvertebrate communities, with dominance of collector-gatherers and filterers. These two groups are early colonisers and very tolerant groups to anthropogenic stress. Other studies conducted in urban Patagonian Rivers (Esquel stream), including those in plain areas (Chubut river), also showed high contribution of collectors (>90 %) (Miserendino *et al*., 2008; Miserendino & Brand, 2009).

Regarding the utilization of organic benthic resources, larvae of some plecopterans (*Klapoteryx kuscheli*, *Rhitroperla rossi* y *Senzilloides panguipulli)* and tricopterans (*Brachysetodes* sp., *Austrocosmoecus* sp., *Monocosmoecus* sp., *Sortosa* sp., *Parasericostoma* sp. and *Myotrichia* sp.) constituted the main shredders in the system, carrying an important role in the breakdown of *Nothofagus pumilio* leaves (Albariño & Valverde, 1998; Albariño & Balseiro, 1998, 2002; Díaz Villanueva & Albariño, 1999; Albariño & Díaz Villanueva, 2006) and in the cycling of detritus (Buria *et al*., 2007, 2009). However, the scrapers group, mostly represented by *Andesiops peruvianus*, *A. torrens*, *Meridialaris diguilina*, *M. laminata*, *M chiloeensis*, *Nousia* sp. and *Metamonius* *anceps*, is numerically the most abundant, both in closed and open areas such as sites IV, V, VI and VII. Sunlight irradiance and temperature of these reaches was higher, which favored primary production from periphyton (Miserendino, 2007; Modenutti *et al.*, 2010).

Concerning the BMPS index, the values obtained reflected a deterioration of the water quality from the source to the river outlet, with the first two sites classified as "non-polluted", the middle ones as "slightly polluted" and site VII as "contaminated". Similar results have been found by Pizzolon & Miserendino (2001), Miserendino *et al*. (2008), Miserendino & Masi (2010) and Macchi (2008) in studies developed in streams affected by urbanization in the mountainous area of Patagonia, showing. These studies also documented low values of BMPS, EPT richness and H at urban sites such as Esquel and Cholila (Chubut Province) and Duran stream (Río Negro Province). They also found negative relationship of the mentioned metrics with variables associated to pollution (turbidity, conductivity, nutrients and total suspended solids).

According to our results, site VII was clearly affected by the urbanization as revealed by low values of BMPS, % EPT and TR and high contribution of % chironomids, % CG and D, meaning that the settlement is producing a pervasive effect on macroinvertebrate community. Similarly, García & Añón Suárez (2007) anticipated a dominance of chironomids at sites close to the mouth in the Ñireco River. The ordination produced in RDA also evidenced good quality conditions at sites I and II which sustained populations of "sensitive" species and displayed low nutrient levels. An opposite situation was observed for sites V, VI and VII with high nutrient levels and high percentage of "tolerant" species. In addition to that, RDA also indicated high correlations between phosphate, water temperature and conductivity to samples taken on February, with low flow of water leading to high temperatures and more concentration of nutrients. There was also an increase of scrappers/grazers during this season that can be related with reduced discharges, higher temperatures and an increase in illumination that favoured primary production (Miserendino, 2007).

The intention of this study was to assess the water quality status of river Ñireco and to estimate the degree of human impact in this river. Our results reveals that sites I to VII showed decreasing water quality conditions being the worse condition recorded at VII, with low values of BMPS displaying class III "probably polluted waters"; indicating a high human influence. We conclude that human actions at urban areas produce modifications in Ñireco River. BMPS, % EPT, and % chironomids were the metrics that best reflected the environmental quality of the stream. Multivariate analysis supported these findings.

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