

Assessing Urban Impacts on Water Quality, Benthic Communities and Fish in Streams of the Andes Mountains, Patagonia (Argentina)

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Abstract Communities of aquatic macroinvertebrates, fish density and biomass, and environmental variables were investigated in three Patagonian mountain rivers affected by urbanization. The rivers Las Minas, Esquel and Carbón that flow through the towns of Cholila, Esquel and Corcovado, respectively (northwest Chubut, Argentina) were selected to assess the degree of impairment. A reference site and an urban site were established on each river. Water quality variables including conductivity, major nutrients, total suspended solids (TSS) and dissolved oxygen, habitat conditions and quality of riparian ecosystems were investigated in autumn, winter, spring and summer 2005–2006. Macroinvertebrates were sampled concurrently in three riffles and three pools at each site. Invertebrate species richness, EPT richness, the Shannon–Weaver diversity index, % EPT density, and the BMPS index were lower at urban sites, whereas % collectors increased. The most impaired site was below Esquel, the largest town. *Senzilloides panguipulli* (Plecoptera), *Polypedilum* and *Rheotanytarsus* species (Diptera: Chironomidae), *Nais communis* (Oligochaeta) and *Meridialaris chiloeensis* (Ephemeroptera) dominated assemblages at

reference and moderately impaired sites in summer, whereas the strongly polluted reach below Esquel had low flow in summer and a community dominated by *Limnodrilus* spp. (Oligochaeta), *Helobdella* spp. (Hirudinea), and two *Hyallela* species (Amphipoda). Canonical correspondence analysis indicated that ammonia, conductivity and TSS were important variables structuring invertebrate assemblages. In contrast, fish density and biomass varied in a non-systematic manner among sites. Overall, urbanization resulted in varying degrees of habitat degradation, sedimentation and nutrient enrichment that were reflected by the macroinvertebrate assemblages, which can be used effectively to monitor the effects of urban communities on Patagonian mountain streams.

Keywords Environmental relationships · Invertebrates · Nutrients · Pollution · Rivers

1 Introduction

Stream ecosystems are subject to extensive modification through human development worldwide (Mason 1991; Rosenberg and Resh 1993; Allan 1995). Urbanization results in frequent disturbances to streams and its pervasive effects reduce water quality and threaten aquatic biota (Prat 1997; Paul and Meyer 2001). The functional characteristics of urbanized catchments are altered as consequence of high

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nutrient concentrations (Meyer et al. 2005). Inputs of pollutants and sediments are common in urban streams and their effects on the biota have been documented extensively (Mason 1991; Finkenbine et al. 2000). Channelization and the realignment of rivers are also common practices in urban areas and result in substrate alteration and disruption of the riffle/pool sequences that provide a diversity of habitats for aquatic species (Nolan and Guthrie 1998). The clearing of riparian areas alongside urban rivers, including the modification and/or extirpation of streamside vegetation, can also alter the functioning of river ecosystems and disrupt fluxes of organic matter and energy (Vannote et al. 1980; Cummins et al. 1984). Urbanization can also result in modified flow regimes that can have major effects on stream communities (Rosenberg and Resh 1993; Death 1995) as increases in the area of impervious surfaces reduce groundwater recharge and increase run-off during storms (Suren 2000; Blakely and Harding 2005).

Benthic communities have been used extensively to assess urban river health. One of the most common effects in mountain rivers affected by urban discharges is EPT taxa reduction (Shieh et al. 2003; Ortiz and Puig 2007). In numerous places water pollution is brought about by inputs of organic effluents, which result in an increase in biochemical oxygen and nutrient enrichment, especially by ammonia and nitrates (Winter and Duthie 1998; Prat et al. 1999). Nutrient augmentation usually decreases macroinvertebrate richness (Paul and Meyer 2001) and the combination of high nutrient concentration and increased light in some urban reaches can result in dense stands of macrophytes and filamentous algae which result in further invertebrate habitat impoverishment. Benthic communities in organically impaired rivers typically have few taxa, with tolerant and opportunistic groups such as oligochaetes and chironomids prominent (Wilcock et al. 1999; Suren 2000).

In South America, the use of benthic indicator organisms in urban streams has received recent attention (Arocena 1998; Monaghan et al. 2000; Figueroa et al. 2003; Silveira et al. 2005) and in Argentina, benthic communities have been surveyed to assess anthropogenic effects on rivers (Gualdoni et al. 1994; Domínguez and Fernández 1998; Giorgi and Malacalza 2002; Jergentz et al. 2004; Pavé and Marchesse 2005; Vallania and Corigliano 2007).

The environmental characteristics of Patagonian streams and the nature of their macroinvertebrate communities have been investigated most intensively in areas adjacent to the Cordillera. These studies have resulted in the taxonomic composition of Patagonian stream faunas being fairly well known (Miserendino 2001; Miserendino and Pizzolón 2003, 2004) and have enabled indices to be developed for bioassessment purposes (Miserendino and Pizzolón 1992, 1999).

Two earlier studies carried out in the urban rivers considered here form a basis for comparison with the present research. First, Miserendino and Pizzolón (2000) investigated the ecology of benthic macroinvertebrates in the organically polluted Esquel–Percy river system when the town of Esquel did not have a local wastewater treatment plant, and second, the effects of water pollution on benthic communities during high and low flows was studied in Las Minas Stream near Cholila (Pizzolón et al. 1997).

To assess the degree of river ecosystem impairment of Patagonian mountain rivers by urbanization we carried out a seasonal research program from May 2005 to February 2006. In this paper we present the results of that investigation in which we contrasted water quality, macroinvertebrate communities, and fish density and biomass at sites above and below three different sized towns on three river systems.

2 Materials and Methods

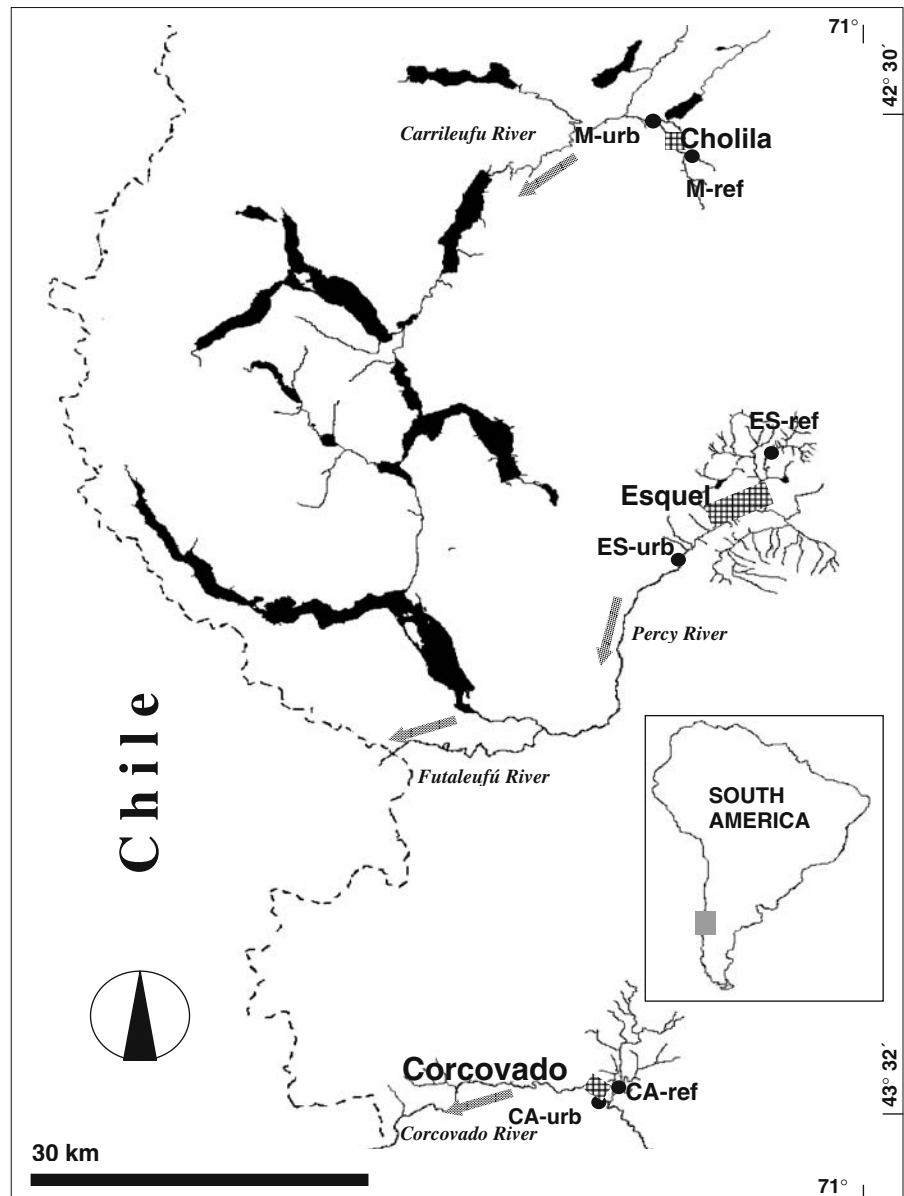
2.1 Study Area

The study area is located in the ecotone between the Patagonian Andes Cordillera and the Patagonian steppe and has a temperate climate with rainfall decreasing from the mountains to the east. The river catchments support sub-Antarctic forest characterized by perennial (*Austrocedrus chilensis*, *Nothofagus dombeyi* and *Maytenus boaria*) and deciduous species (*N. pumilio*, *N. antarctica*). Low-order streams were bordered by lengas (*N. pumilio*), whereas closer to the towns rivers were mainly flanked by *Salix fragilis* L. and *S. nigra* Marsh. The mountain rivers 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 and del Valle 1988).

The rivers selected for study flowed through the towns of Esquel (30,977 inhabitants), Cholila (2,190 inhabitants) and Corcovado (1,848 inhabitants) in Chubut province, Argentina (Fig. 1). Esquel Stream in the Futaleufú-Yelcho basin is third order above the town but fourth order below it as the Valle Chico Stream (third order) enters it within Esquel. Las Minas Stream is a second order stream in the Carrileufú basin, whereas Carbón Stream is a third order stream in the Corcovado basin. Reference sites (minimally disturbed) were located upstream of each

town and “urban” sites were located below them (Fig. 1). Sites were as similar as possible in terms of geology, substrate, water velocity, and background physico-chemistry. Because one of our objectives was to compare present data with previous studies, three of the sites located on Esquel and Las Minas streams were in the same locations used by Pizzolón et al. (1997) and Miserendino and Pizzolón (2000). However, in the year of our present surveys a concrete channel was being constructed at the entry to the town on Esquel Stream so the reference site had to be

Fig. 1 Location of sampling sites on Las Minas, Esquel and Carbon streams, Chubut Province, Patagonia, Argentina



positioned further upstream. Dredging and clearing of riparian areas below Cholila during September, resulted in increased sedimentation and turbidity of Las Minas Stream.

2.2 Field Methods

Sampling sites were visited in May (autumn), September (winter), December 2005 (spring) and February 2006 (summer), under stable environmental conditions; thus samples were not taken after rainstorms or extremely high discharge events. Substratum composition at each sampling site was estimated visually and recorded as percentages of boulders, cobbles, gravel, pebbles and sand (as defined by Cummins in Ward 1992). 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 five measurements on a transect across the channel with a calibrated stick. Wet and dry widths of the channel were also determined. Discharge was obtained by combining depth, wet width and current velocity as in Gordon et al. (1994). At each site, air and water temperature were measured with a mercury thermometer.

On each sampling occasion, specific conductance ($\mu\text{ S}_{20}\text{ cm}^{-1}$), pH, turbidity (NTU) and dissolved oxygen ($\text{mg O}_2\text{ l}^{-1}$) were measured with a Horiba U2-probe. For nutrient analyses water samples were collected below the water surface and kept at 4°C prior to analysis. At the laboratory nitrate plus nitrite nitrogen ($\text{NO}_3\text{-NO}_2$), ammonia (NH_4), soluble reactive phosphate (SRP) ($\pm 0.01\text{ }\mu\text{g l}^{-1}$), and total suspended solids (TSS; $\pm 0.1\text{ mg l}^{-1}$) were analyzed following APHA (1994). Statistical comparisons of values obtained for physical and chemical variables at reference and urban sites over were made with paired Kruskal–Wallis tests (Sokal and Rohlf 1995).

2.3 Habitat and Riparian Conditions

Attributes of the riparian vegetation were examined in each study reach using an adaptation of the QBR index for Patagonian streams (Munné et al. 1998; Kutschker et al. 2006). This index combines information on total cover, structure, complexity and naturalness of vegetation, and the degree of channel alteration (e.g. bank modifications, dredging, etc.).

The total QBR score can range from less than 25 points (extreme degradation) to more than 90 points (good quality, natural riparian forest).

We evaluated habitat quality using the assessment procedure for high gradient streams of Barbour et al. (1999). This method ranks 10 river channel features (scored 0 to 20), including epifaunal substrate availability, embeddedness, velocity and depth, sediment deposition, channel flow, channel modifications, frequency of riffles, condition of banks, bank vegetative protection, and riparian vegetative zone width. A score of 200 points indicates the river is natural and pristine and in its best possible condition. Both the QBR and habitat assessment indices are very useful as they provide an integrated picture of factors influencing the biological condition of a stream system.

2.4 Macroinvertebrate Analysis

Quantitative macroinvertebrate samples were taken with a Surber sampler (0.09 m^2 ; $250\text{ }\mu\text{m}$ pore size). On each sampling date, three samples were taken from riffles and three from pools. Samples were fixed in situ with 4% formaldehyde, and sorted in the laboratory under at least $5\times$ magnification. Macroinvertebrate species were identified using available keys (Domínguez et al. 1994; Angrisano 1995; Morrone and Coscarón 1998; Fernández and Domínguez 2001). Functional feeding groups (FFGs) were assigned using available references, knowledge of feeding modes (mouthpart morphology and behaviour), and analysis of gut contents (Merritt and Cummins 1996; Domínguez et al. 1994; Albariño and Balseiro 2002).

2.5 Fish Sampling

On each date fish communities were assessed at each site. Sampling was carried out by electro-fishing with a battery powered backpack machine (output 300 W) set at 300 V and a hand net. Reaches assessed were 100 m long; three passes were made at each site in all months by the same operator. Because our goal was to contrast fish abundance among reference sites and impaired sites, the data presented in this paper are measures of fish density and biomass. The Mann–Whitney U test was used to test for significance of fish biomass and fish density differences between urban and reference sites.

2.6 Invertebrate Data Analysis

We calculated 20 macroinvertebrate community descriptors for each site and sampling date, including richness measures: taxa richness (SR), which measures the overall variety of the macroinvertebrate assemblage; Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, which counts the number of mayfly, stonefly and caddisfly taxa; and total Chironomidae richness (C), which counts the number of recognized midge taxa.

We also calculated the Shannon–Weaver diversity index (H'), which incorporates measures of both richness and evenness, and Pielou evenness (J'). Both H' and J' were calculated from averaged macroinvertebrate densities at each site on each sampling date. We calculated % EPT density, % Plecoptera, % Ephemeroptera, % Trichoptera, % Chironomidae, relative abundance of EPT to Chironomidae (EPT/C), and % Orthoclaadiinae to Chironomidae (O/C; Rosenberg and Resh 1993).

Tolerance measures included density (ind m^{-2}) and % dominant taxon, which measures the dominance of the single most abundant taxon (Ludwig and Reynolds 1988; Barbour et al. 1999). Trophic measures were % scrapers and grazers, % predators, % collectors, and % shredders.

Two biotic indices previously adapted for use in the Patagonian region, the IAP (Indice Andino Patagónico) and BMPS (Biotic Monitoring Patagonian Streams), were calculated (Miserendino and Pizzolón 1999; Miserendino 2007). The IAP combines the level of sensitivity of some intolerant and tolerant taxa and is based on presence or absence of taxa (systematic units) in a sample. It ranges from 0 to 10, higher scores indicating very clean waters and lower scores indicating polluted waters. The BMPS is an adaptation of the BMWP (Biological Monitoring Working Party index; Armitage et al. 1983) and is calculated from a table of 95 families with different degrees of pollution sensitivity (scores 1–10) present in Patagonia. The total BMPS score is obtained by adding the scores for all families presents in a sample, and ranged from 0 to >150.

The sensitivity of each metric used to compare urban and reference sites employed was judged by the degree of interquartile overlap in box-and-whisker plots (Barbour et al. 1996). Metrics were judged to have one of 4 sensitivity values: a sensitivity of 3

(strong) if no overlap existed in the interquartile range; a sensitivity of 2 (strong) if there was some overlap that did not extend to the medians; a sensitivity of 1 (weak) if there was a moderate overlap of interquartile ranges but a t least 1 median was outside the range; and a sensitivity of 0 if the interquartile overlap was considerable; with no discrimination between reference and impaired sites (Barbour et al. 1996).

2.7 Data Analysis

Pearson's product-moment correlation coefficient (r) was used to analyze relationships between metrics and water quality values (Sokal and Rohlf 1995). Variables in these analyses, except pH values, were $\log(x+1)$ transformed to stabilize variances and normalize the data sets.

Canonical correspondence analysis (CCA) was run using CANOCO (ter Braak 1986; ter Braak and Smilauer 1999) to assess relationships between macroinvertebrate assemblages and environmental variables. Average seasonal values (means of six Surber samples per site) were used in the analysis. All environmental variables included in Tables 1 and 2, were used initially to evaluate the response of species and sites to environmental gradients. Variables (except pH) and species density were transformed ($\log x+1$), prior to analysis. Variables that were strongly inter-correlated with others (those with an inflation factor >10) in the initial analysis, were removed (dry channel width, stream order, phosphate, nitrite, depth, QBR) and a further analysis was carried out with the 12 remaining environmental variables. The forward selection option provided by CANOCO was applied and those variables with $p < 0.1$ (Monte Carlo permutation test) were kept for the analysis (pH, turbidity and water temperature were omitted). The final CCA was run using a set of independent and significant environmental variables (ter Braak and Smilauer 1998).

3 Results

3.1 Physicochemical Conditions

Substrate size was smaller at urban sites than reference sites at Esquel and Las Minas (Table 1).

Table 1 Locations and environmental features of the pre-urban (Upstream) and urban (Downstream) sites in Las Minas, Esquel and Carbón stream (Patagonia, Argentina) during the study period ($n=4$)

Variable	Las Minas		Esquel		Carbón	
	M-ref	M-urb	ES-ref	M-urb	CA-ref	CA-urb
Elevation (m)	573	555	721	491	384	403
Stream order	2	2	3	5	3	3
Dry width (m)	5	9	10.8	10.5	10.5	23.5
Wet width (m)	3.9±1.9	5.8±2.2	3.2±1.3	9.9±0.4**	8.7±0.7	16.3±7.6**
Depth (cm)	22.4±8.6	14±7.0	15±5.6	26.6±4.6**	26±5.3	28.4±6.9
Current velocity ($m s^{-1}$)	1±0.3	1.5±1.1	1.5±1	1.4±0.4	1.3±0.3	1.2±0.3
Water temperature (°C)	9.2±0.8	10.6±2.1	8.5±3.4	11.3±1.7	8.3±2.5	8.1±2.1
Discharge ($m^3 s^{-1}$)	0.9±0.5	1±0.5	0.7±0.5	3.7±1.2**	2.8±0.2	6.4±2**
Substratum size	Boul/cob	Cob/peb	Boul/cob	Peb/grav	Boul/cob	Boul/cob
% Macrophyte coverage	<5	<5	<5	>50	<5	<5

Variables are mean values (\pm SD).

Boul boulder, *Cob* cobble, *peb* pebble, *Grav* gravel.

Significance from paired Kruskal–Wallis test comparing the considered variable between reference site and urban site * $p<0.05$, ** $p<0.005$

Wet width and mean discharge were significantly higher at urban sites relative to reference sites at Carbon and Esquel streams ($p<0.004$), but mean depth was higher at ES-urb than ES-ref. Although mean dissolved oxygen concentration was higher at all reference sites than downstream sites, observed differences were not statistically significant. Conversely, mean turbidity values were lower at reference sites than downstream sites but not significant (Table 2).

Nitrate and ammonia had lower mean values at reference sites than urban sites, but observed

differences were significant only at ES-urb site ($p<0.004$). In this stream the highest ammonia values were found (range 33.1–271.5 $\mu g l^{-1}$) with maximum values in summer. SRP was also significantly higher at ES-urb than ES-ref. All urban sites had higher mean values of TSS than reference sites, but differences were not significant (Table 2). Dredging and vegetation removal at the urban site on Las Minas Stream during September resulted in a strong increase in TSS (102.7 $mg l^{-1}$) and turbidity values (215 NTU).

Table 2 Chemical variables of the pre-urban (Upstream) and urban (Downstream) sites in Las Minas, Esquel and Carbón stream (Patagonia, Argentina) during the study period ($n=4$)

Variable	Las Minas		Esquel		Carbón	
	M-ref	M-urb	ES-ref	ES-urb	CA-ref	CA-urb
pH	7.5±0.1	7.3±0.3	7.4±0.2	7.1±0.3	7.1±0.3	7.2±0.2
Dissolved oxygen ($mg l^{-1}$)	10.8±2.2	10.1±2.2	11.7±3.5	9.5±1.8	12.0±4.1	11.9±3.6
Turbidity (NTU)	41.8±77.5	55±106.7	29.3±39.4	82.5±137	4.3±5.9	7±7.8
Conductivity ($\mu S cm^{-1}$)	120.0±64.8	98.8±59.8	100.3±33.6	313.3±78.0**	72.5±22.6	70.3±18.4
Nitrate plus nitrite–nitrogen ($\mu g l^{-1}$)	6.0±8.2	8.4±14.3	0.50±0.89	42.76±22.16**	0.51±0.81	0.30±0.17
Ammonia ($\mu g l^{-1}$)	1.4±1.4	1.9±2.4	1.3±1.2	103.6±112.5**	1±0.6	1.1±0.7
Soluble reactive phosphate ($\mu g l^{-1}$)	0.65±0.36	0.49±0.22	0.72±0.35	13.63±8.06**	0.51±0.14	0.51±0.14
Total suspended solids ($mg l^{-1}$)	15.5±24.8	27±50.5	5.1±8.3	14.6±12.3	2.1±1.6	2.6±1.3

Variables are mean values (\pm SD).

Significance from paired Kruskal–Wallis test comparing the considered variable between reference site and urban site * $p<0.05$, ** $p<0.005$

3.2 Riparian Ecosystem and Habitat Conditions

Most of the reference sites had river channels and banks in good condition. At ES-ref and M-ref the riparian forest was composed mainly of native species, whereas at CA-ref the riparian corridor was dominated by *Salix* sp., *Populus nigra* and *P. alba* and the shrubs *Cytisus scoparia*, *Conium maculatum* and *Rosa rubiginosa*. At ES-urb and CA-urb the riparian forest was replaced by *Salix* sp. At M-urb banks were frequently disturbed by dredging. ES-urb had the highest coverage of aquatic plants, the dominant species being *Myriophyllum quitense* (Table 1).

The index of riparian ecosystem condition (QBR) indicated that the most degraded urban sites were M-urb (25.5) and CA-urb (35); the best reference site was ES-ref (75). Similarly, the habitat condition index had lower values at all urban sites than reference sites (Fig. 2). The lowest value for habitat condition was 69 at M-urb.

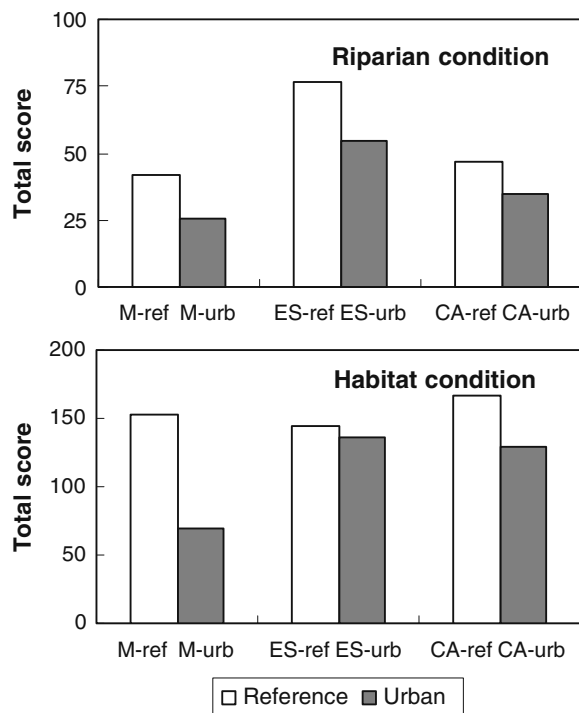


Fig. 2 Riparian ecosystem condition (*upper*) and habitat condition (*lower*) at reference and urban sites at Las Minas, Esquel and Carbón streams during the study period

3.3 Macroinvertebrate Community Analyses

A total of 104 taxa and 106,525 individuals were identified and counted in the study (Appendix). Mean total density was lower at most reference sites than urban sites, and was particularly marked in Esquel stream where macroinvertebrate density was seven times higher at ES-urb than ES-ref (Table 3). At ES-urb, benthic communities were dominated by Chironomidae (*Paratrichocladius* sp., *Parapsetrocladius* sp., *Parametrioctenus* sp.), Tubificidae (*Limnodrilus* sp.) and Hyalellidae (*H. araucana*, *H. curvispina*; Table 3, Appendix). Plecoptera were most abundant at reference sites as the gripopterygids *Aubertoperla illiesi* and *Notoperlopsis femina*, whereas Diptera and Annelida were most abundant at urban sites. In Las Minas and Esquel streams Trichoptera and Coleoptera density decreased from reference to urban sites (Table 3). The Trichoptera *Hudsonema flaminii*, *Brachysetodes major* and *Smicridea annulicornis*, and Elmidae spp. (Coleoptera) were best represented at M-ref and ES-ref, than at M-urb and ES-urb sites respectively (Appendix).

3.4 Metrics

Selected metrics were able to discriminate different degrees of biotic integrity. Species richness, EPT richness and H' were lower at all impaired sites than reference sites, however differences were only significant at Las Minas and Esquel streams (Fig. 3). The most dramatic changes were recorded at ES-urb.

% EPT density did not differ between M-urb and M-ref, but at ES-urb EPT taxa were practically absent. At Carbón stream differences between CA-ref site and CA-urb site were moderate (Fig. 3). Percentage of scrapers/grazers were similar at M-ref and M-urb, but were higher at ES-ref and CA-ref than ES-urb and CA-urb respectively. Percent of Orthoclaadiinae/Chironomidae increased from reference to urban sites in Esquel and Carbón streams, and was most pronounced in Esquel stream. Similarly, the percentage of collectors was greater at urban than reference sites, especially in Esquel Stream.

SR, EPT richness and H' values were higher in May and February than in September and December at all sites. In December the percentage of contribution of Chironomidae and dominant taxon were over 50% at M-urb and CA-urb. The lowest % EPT

Table 3 Mean total density and mean abundance of macroinvertebrate groups (\pm SD), biotic indexes scores, and relative abundance (percentage) of functional feeding groups in the studied rivers northwest Chubut, Patagonia, Argentina ($n=4$)

	Las Minas		Esquel		Carbón	
	M-ref	M-urb	ES-ref	ES-urb	CA-ref	CA-urb
Total density	371.4 (135.5)	843.1 (903)	271 (186.3)	2,011 (1,055)	351.1 (130)	591.5 (483)
Plecoptera	52.5 (74.6)	39.0 (54)	70.0 (62.3)	0.6 (1)	34.7 (3.9)	24.2 (13.4)
Ephemeroptera	55.6 (29.3)	111.3 (140)	78.2 (51.8)	0.7 (0.9)	72.0 (48.6)	95.5 (119.5)
Trichoptera	38.5 (30.1)	4.8 (4.3)	9.0 (16)	0	71.5 (47.6)	185.9 (198.4)
Diptera	193.1 (146.7)	555.1 (618.3)	57.2 (19.1)	935.5 (817.7)	119.8 (29.6)	141.7 (94)
Coleoptera	4.2 (2.6)	1.2 (0.6)	4.3 (3.2)	0.3	18.9 (17.1)	28.8 (43.4)
Hyalellidae	0.1 (0.3)	0.8 (1.6)	0	495.2 (270.3)	19.5 (38.9)	0
Annelida	27.3 (18.1)	129.9 (221.1)	51.2 (63.6)	563.2 (185)	55.7 (36.3)	110.4 (101)
Mollusca	0.1 (0.1)	0	0.1 (0.2)	15.2 (20.3)	0.2 (0.2)	0
IAP	10 (0)	10 (0)	10 (0)	6.1(2)	10 (0)	10 (0)
BMPS	115 (12)	101.5 (17)	93.0 (7.3)	37.3 (21)	126.5 (16)	108.3 (13)
FFG rel. abundance						
Shredders	12.5	0.7	6.1	0.0	7.6	9.1
Scrapers/grazers	28.1	34.5	42.1	0.1	30.8	23.0
Collectors	54.3	60.8	45.3	93.3	57.2	65.3
Predators	5.1	4.0	6.5	6.5	4.7	2.6

density was recorded in December at all sites in Las Minas and Esquel, whereas the % Orthocladiinae/Chironomidae was higher at urban sites than at reference sites in Esquel and Carbón streams, in all dates (Fig. 4a).

The relative abundance of different functional feeding groups varied among dates. The lowest contribution of shredders was found in September, month in which the relative abundance of scrapers/grazers was highest. The percentage of collectors was greatest in December at Las Minas and Esquel sites and in February at Carbón sites. In most sites, and when present, the contribution of Plecoptera was greatest in September. Total density was greatest in February at all urban sites. All reference sites showed BMPS values over 90 points in all dates (Fig. 4b).

Except for J and % Chironomidae, all metrics showed at least one significant correlation with water quality variables (Table 4). SR, EPT richness, % EPT richness, % Ephemeroptera, IAP and BMPS showed negative and significant relationships with turbidity, conductivity, nutrients and TSS. Macroinvertebrate density was positively correlated with conductivity, $\text{NO}_3\text{-NO}_2$, NH_4 and SRP; whereas % Scrapers/grazers, C, and EPT/C showed the opposite trend with those same variables. Collector's percentage was positively correlated with NH_4 and SRP (Table 4).

3.5 Biotic Indexes

Of the two biotic indexes only BMPS showed considerable variation between reference and urban sites (Table 3, Fig. 3). All urban sites had lower BMPS values than their respective reference sites, whereas the IAP index showed no difference between reference and urban sites on Las Minas and Carbón streams and had a score of 10 at all four sites. The IAP was able to detect impairment at ES-urb (mean score 6.1), however (Table 3).

3.6 Fish Analysis

The exotic salmonids *Oncorhynchus mykiss* and *Salmo trutta*, and the native *Hatcheria macraei* (Trichomycteridae) were present at ES-ref, but only *O. mykiss* was recorded at ES-urb. *O. mykiss* was the only fish species recorded at M-ref and M-urb, whereas both *O. mykiss* and *S. trutta* were found at CA-ref and CA-urb. Fish density patterns varied considerably among reference and urban sites. Thus, mean fish density was higher at reference sites on Las Minas and Esquel streams but this pattern was not statistically significant. Mean fish biomass was higher at the urban sites on Carbón and Esquel streams with the difference between reference and urban sites being

Fig. 3 Distribution of values of selected metrics for macroinvertebrates at reference and urban sites on the Las Minas, Esquel and Carbón rivers. *Range bars* show maxima and minima, *boxes* are interquartile ranges (25–75%), *small squares* are medians

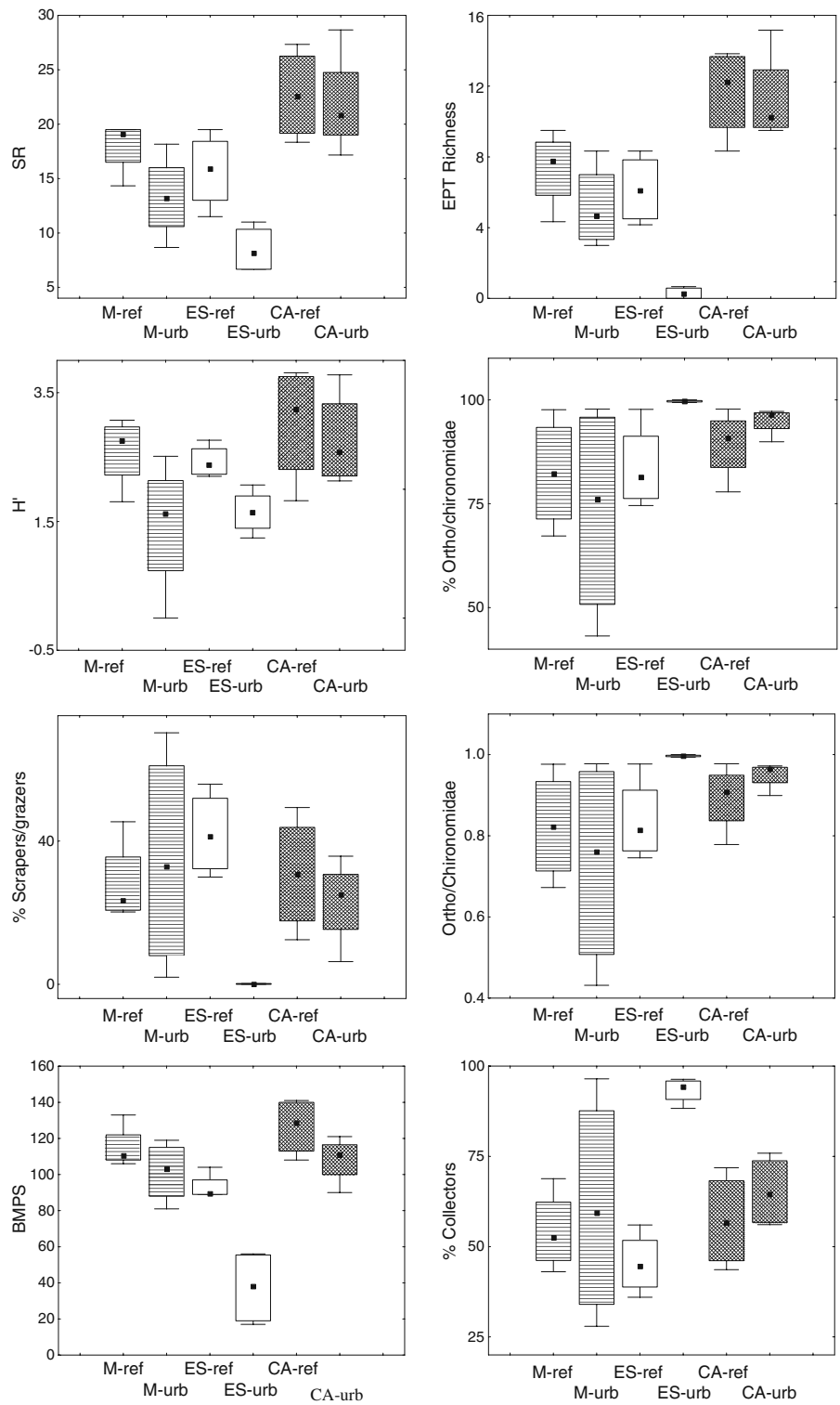


Fig. 4 a, b Seasonal values of metrics for macroinvertebrates at reference and urban sites on the Las Minas, Esquel and Carbón rivers during the study period. Density values are $\text{ind. m}^{-2} \times 1,000$

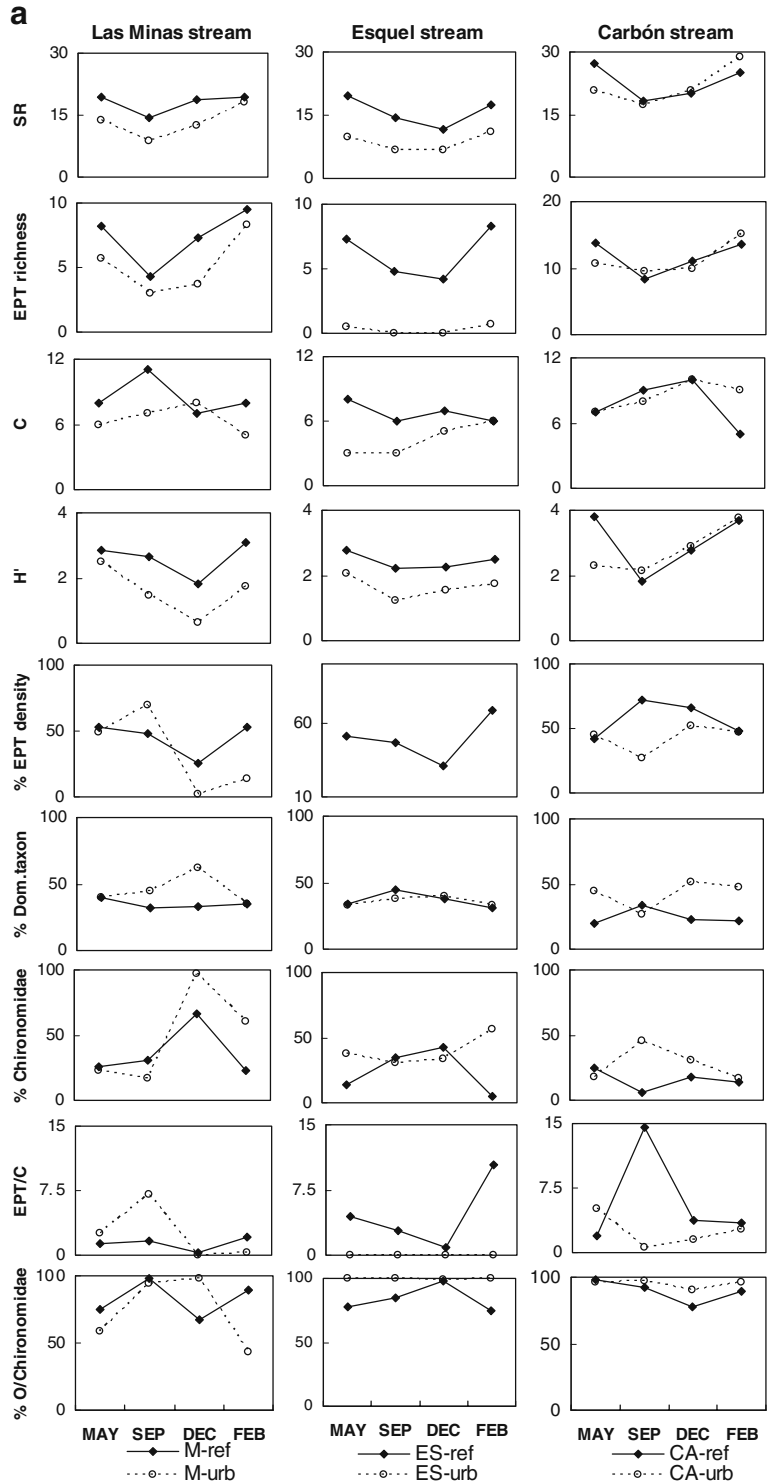


Fig. 4 (continued)

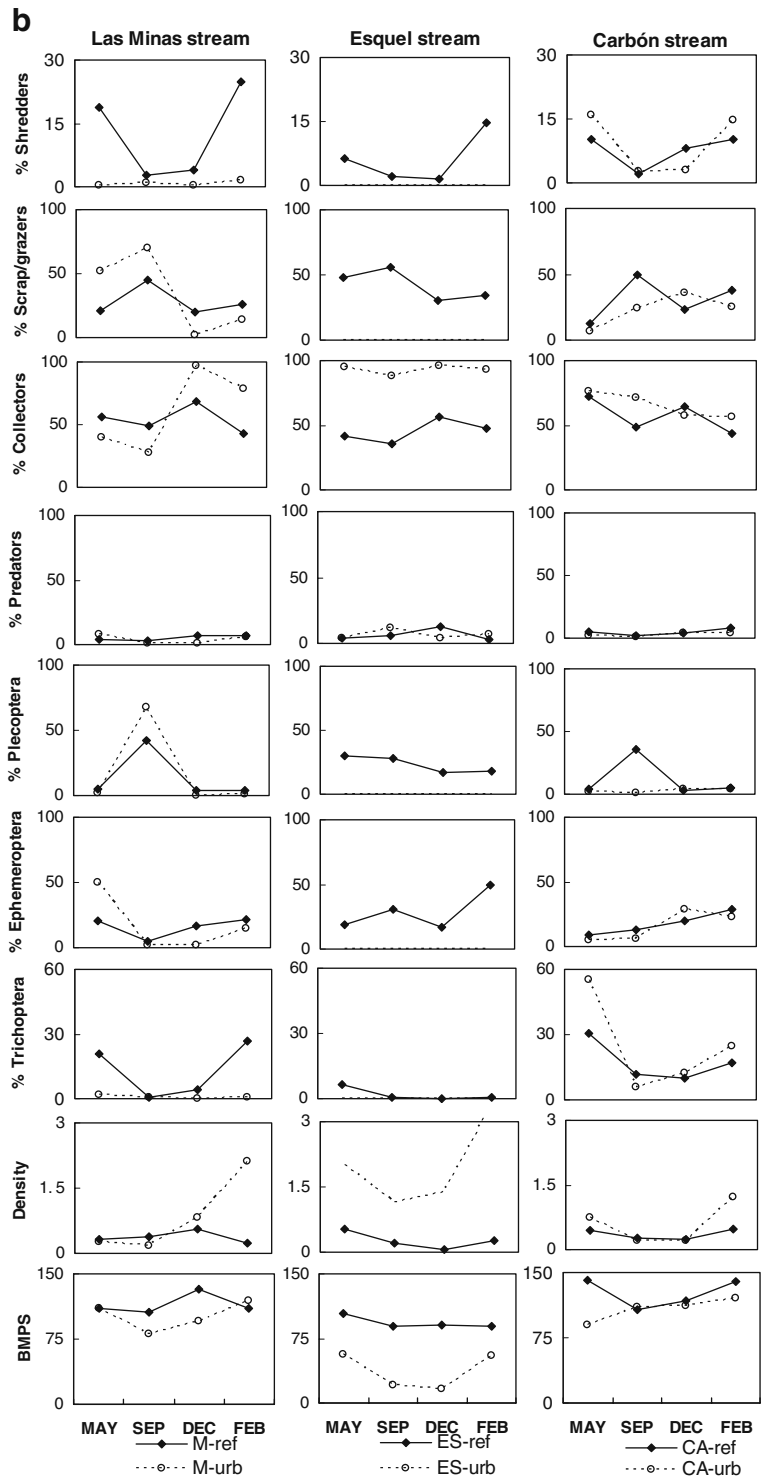


Table 4 Pearson correlation matrix between metrics and water quality variables in the studied rivers northwest Chubut, Patagonia, Argentina ($n=24$)

	Depth	Discharge	Water velocity	pH	Dissolved oxygen	Turbidity	Conductivity	NO ₃ –NO ₂	NH ₄	SRP	TSS
SR	ns	ns	ns	ns	ns	–0.68**	–0.44*	–0.71**	–0.75**	–0.66**	–0.68**
EPT richness	ns	ns	ns	ns	ns	–0.53*	–0.64**	–0.82**	–0.88**	–0.85**	–0.57**
C	ns	ns	–0.43*	ns	ns	ns	–0.67**	–0.46*	–0.53*	–0.62**	ns
H'	ns	ns	ns	ns	ns	–0.40*	ns	–0.40*	–0.45*	ns	ns
J'	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
% EPT density	ns	ns	ns	ns	ns	–0.43*	–0.63**	–0.70**	–0.86**	–0.84**	–0.44*
% Plecoptera	ns	ns	ns	ns	ns	ns	–0.66**	ns	–0.44*	–0.52*	ns
% Ephemeroptera	–0.45*	–0.36*	ns	ns	ns	–0.44*	–0.45*	–0.82**	–0.84**	–0.79**	–0.58**
% Trichoptera	ns	ns	ns	ns	0.46*	–0.53*	ns	–0.57**	–0.61**	–0.52*	–0.49*
% Chironomidae	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
EPT/C	ns	ns	ns	ns	ns	ns	–0.46*	–0.46*	–0.54*	–0.55*	ns
% O/C	0.69**	0.43*	–0.41*	–0.43*	ns	0.43*	ns	0.41*	0.44*	0.41*	ns
Density	ns	ns	0.48*	ns	ns	ns	0.59*	0.50*	0.56*	0.62**	ns
% Dominant taxon	ns	–0.40*	ns	ns	ns	ns	ns	ns	ns	ns	ns
% Shredders	ns	ns	ns	ns	ns	–0.43*	ns	–0.61**	–0.68**	–0.59*	–0.53*
% Scrapers/grazers	ns	–0.41*	ns	ns	ns	ns	–0.68**	–0.61**	–0.77**	–0.80**	ns
% Collectors	ns	0.52*	ns	ns	ns	ns	ns	ns	0.51*	0.54*	ns
% Predators	ns	ns	ns	ns	ns	ns	0.42*	ns	ns	ns	ns
IAP	ns	ns	ns	ns	ns	–0.43*	–0.61**	–0.63**	–0.71**	–0.72**	–0.41*
BMPS	ns	ns	ns	ns	ns	–0.55*	–0.59*	–0.66**	–0.77**	–0.73**	–0.47*

Significance: * $p < 0.05$, ** $p < 0.01$

significant in Carbón Stream (Mann–Whitney U test $p=0.04$; Fig. 5).

3.7 Multivariate Analysis

Results of the CCA (first three axes) are summarized in Table 4 and shown in Fig. 5. The environmental variables selected in the analysis are represented in the biplot by arrows, which point in the direction of maximum change in the value of the associated variable (Fig. 6). The species–environmental correlations were: 0.95, 0.94 and 0.94 for the first, second, and third axes, respectively (Table 5), indicating strong relationships with the environmental variables selected. Monte Carlo tests were significant for all axes (Table 5).

The strongest explanatory factors were physical and chemical variables, but only 36.1% of variation in the species data was accounted for by the environmental variables measured (Table 5). CCA axis 1 strongly reflected the distribution of sites along the pollution gradient. Ammonia concentration and conductivity had the strongest correlations with axis 1,

and TSS was also correlated with this axis. These three environmental variables are associated with stream impairment. Variables most strongly related to axis 2 were TSS, elevation, discharge and water velocity. Dissolved oxygen was also correlated with axis 2 but the correlation was low. Samples taken at ES-urb were clearly located at the positive end of axis 1, whereas sites showing lower ammonia and conductivity values were placed at the negative end of that axis (Fig. 6a). The positions of sites on axis 2 varied seasonally, and were associated with changes in temperature, rainfall, snow melt, discharge and dredging. For example, ammonia values at ES-urb were highest ($271.5 \mu\text{g l}^{-1}$) in the warmest month (February), and lowest in the high discharge period (September, $33.12 \mu\text{g l}^{-1}$; Fig. 6a).

Figure 6b shows the invertebrate assemblages along the same environmental gradients. A set of taxa exclusively recorded at Es-urb (Corixidae sp., *Biomphalaria peregrina*, *Diplodon chilensis* sp., Sciomyzidae sp.), or very abundant at that site (*Helobdella* spp., *Hyalella curvispina*, *Hyalella araucana* and *Limnodrilus* spp.) are positioned towards the positive end of

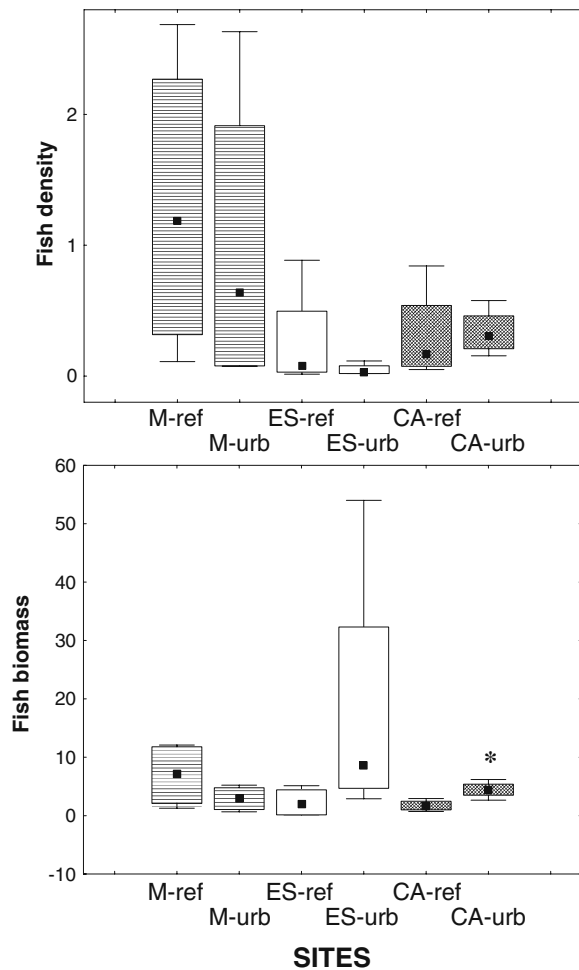


Fig. 5 Distribution of values of density and biomass of fish at reference and urban sites on the Las Minas, Esquel and Carbón rivers. Range bars show maxima and minima, boxes are interquartile ranges (25–75%), small squares are medians (*Mann–Whitney U test, $p=0.04$)

axis 1 (Appendix). *Girardia* sp., Telmatogetonidae sp.2 and a species of Orthocladiinae that peaked at ES-ref in December, *Aubertoperla illiesi* and *Cnesia dissimilis*, and a Psychodidae species that peaked at M-ref and M-urb in September, are found near the top of axis 2. Species that peaked in late summer during the low water period (February), such as *Senzilloides pangui-pulli*, *Polypedilum* sp., *Rheotanytarsus* sp., *Nais communis*, and *Meridialaris chiloeensis* are positioned in the upper left quadrant, whereas, species that were more abundant during the high water period (*K. genualis*, Orthocladiinae sp. 1, Podonominae sp., Leptoceridae sp., Philorheithridae and *Phreodrilus* sp.), are towards the bottom of the lower left quadrant.

4 Discussion

Our results indicate that metrics based on macro-invertebrate communities were useful to identify different degrees of pollution and disturbance in the studied urban streams. The site below Esquel was shown to be the most disturbed reach by the majority of metrics. Differences in stream order, discharge and depth between sites (ES-ref ES-urb) could have some influence determining a major coverage of macrophytes and changing substratum size, which in turn altered benthic assemblages. It is likely that input of nutrients at ES-urb enhanced primary productivity, which was reflected by an increase in macroinvertebrate density. However, at Es-urb, pollution-intolerant taxa of Plecoptera, Ephemeroptera and Trichoptera were almost totally absent, total species richness was very low and the community was dominated by taxa tolerant to moderate organic pollution (*Hyalella* spp., *Helobdella* spp. and some Orthocladiinae), or to sedimentation (*Limnodrilus* spp.; Marchese 1995; Paggi 1999; Miserendino 2001). Overall, the community was similar to that found before construction of the waste water treatment plant in 2001 when similar values for nutrients and a BOD of 5.6 mg l^{-1} indicated moderate pollution (Miserendino 1995; Miserendino and Pizzolón 2000). The predominance of Orthocladiinae rather than Chironominae as the most abundant midges is in agreement with the findings of Hicham and Lotfi (2007) for rivers having moderate levels of ammonia and high oxygen concentrations, and those of Shieh et al. (2003) for urban rivers downstream of wastewater treatment facilities.

We expected an improvement in water quality after construction of the wastewater treatment plant at Esquel, but none of the physico-chemical and biological parameters suggested stream water recovery. However, in the last few years Esquel has experienced a strong growth in population (1992, 17,000 to 2006, 31,000 inhabitants) and the ability of the plant to cope with the volume of waste produced now appears to be inadequate. Also, the impervious surfaces of the town have increased as more streets and neighborhood areas have been paved. In some areas the domestic sewage system is frequently flooded by storm-water, thus sewage and pluvial drainage systems are not always working as separate units. High discharge events associated with strong rains were frequent during our study and can be

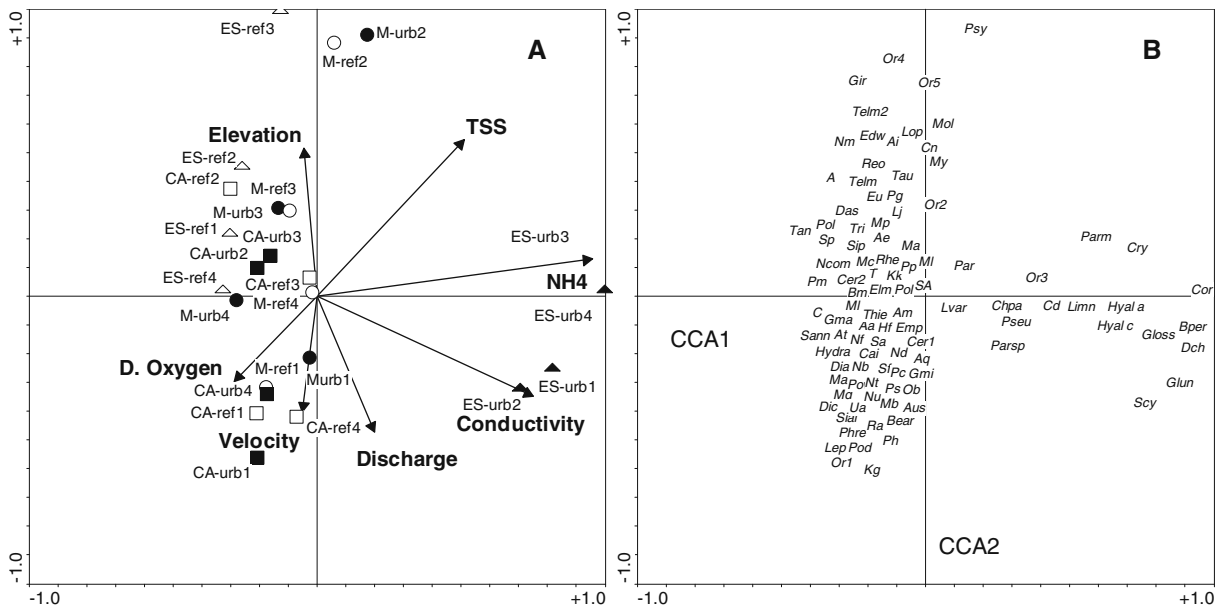


Fig. 6 **a** CCA ordination diagram for sites and environmental relationships based on abundance data for 107 macroinvertebrate taxa at reference and urban sites on the Las Minas (circles), Esquel (triangles) and Carbón (squares) rivers. Codes of taxa in Appendix. Code for months: 1=May, 2=September,

3=December and 4=February. Code for sites: open symbols are reference sites, dark symbols urban sites. **b** Ordination of macroinvertebrate species on the first two environmental axes of the CCA

expected to have resulted in inputs of nutrients and sediments to the river (Suren 2000; Blakely and Harding 2005). An expansion of the treatment plant facilities is planned for 2008 and monitoring of Esquel Stream should be maintained in order to evaluate its effect on the river.

Table 5 Axis eigenvalues and weighted intraset correlation between axes and environmental variables following canonical correspondence analysis of macroinvertebrate species abundance data from urban rivers in Chubut, Patagonia, Argentina

	CCA 1	CCA 2	CCA 3
Eigenvalues	0.49	0.26	0.19
Species–environment correlation	0.95	0.94	0.94
% variance of species data explained	19	29	36.1
Correlation with axes			
Ammonia (NH ₄)	0.91	0.12	0.07
Total suspended solids (TSS)	0.49	0.51	0.34
Conductivity	0.71	-0.32	-0.39
Dissolved oxygen	-0.27	-0.28	0.35
Water velocity	-0.05	-0.38	-0.36
Discharge	0.19	-0.45	0.64
Elevation	-0.04	0.48	-0.63

Significance of the axes by Montecarlo test is given. *P* values for Monte Carlo permutation test. Axis1: $F=3.75, p<0.001$. All canonical axes: $F=2.44, p<0.001$

Most values for physical and chemical variables at the urban site on Las Minas Stream were not significantly different from those found at the reference site. Nevertheless, there were some indications of habitat degradation as revealed by values of the indices of riparian quality and habitat condition. This habitat degradation probably affected the macroinvertebrate community, which had lower species richness, BMPS, *H'* and EPT richness than the community at the reference site. One of the strongest differences between M-ref and M-urb was the quality of the riparian corridor, which was very impoverished at the urban site. Riparian conditions affect nutrient run-off capture, sediments input, water temperature, energy sources utilized by stream biota and flow regime (Nolan and Guthrie 1998; Hall et al. 2001), and it is possible that some species were unable to complete their life cycles, especially after dredging and clearing of the riparian areas at M-urb. Additionally, losses of places for oviposition, and habitat for aerial stages of insects with aquatic larvae, can be critical if macroinvertebrate diversity is to be maintained (Ward 1992). Thus, where strips or patches of native riparian shrubs have been maintained alongside some Patagonian streams the effects of basin desertification appear to have been reduced and significant increases in EPT

richness have been found (Miserendino 2004). A reduction in taxon and EPT richness and an absence of some taxa with low tolerance to sedimentation was reported in an urban reach of the Quemquemtrey River (Río Negro Province; Miserendino and Pizzolón 2003), while in some rivers in New Zealand, the occurrence of EPT taxa seems to be affected by factors associated with riparian cover and catchment land use (Collier et al. 2000; Hall et al. 2001).

In contrast to the Esquel River, the present study provides a more optimistic picture about the ecological health of Las Minas stream than the earlier study (Pizzolón et al. 1997), which found significantly higher conductivity and nutrient levels. For example, summer values of nitrates and phosphates were over $100 \mu\text{g l}^{-1}$ in the earlier study, and the macroinvertebrate community was highly simplified and dominated by *Hyaella curvispina*, Glossiphonidae and *Chironomus* sp. By comparison, (c.f. summer nutrient values $<1 \mu\text{g l}^{-1}$ and community well represented by EPT taxa), results of the present study therefore suggest that the new domestic effluent system of Cholila is having a positive ecological effect on the Las Minas River ecosystem.

Carbón Stream was the less impaired watercourse and several metrics (SR, EPT richness, H' , % dominant taxon) did not differ significantly from those for the reference site. The index of habitat condition and the riparian quality index also displayed little difference between sites. Corcovado was the least populated town in the study and Carbón the largest river, two factors that likely ensured that the river maintained its macroinvertebrate diversity and ecological integrity below the urban area. CA-urb had important connectivity with the riparian corridor and high habitat availability unaffected by dredging or sedimentation. It was characterized by a high frequency of riffles and various combinations of depth and flow were represented. Macroinvertebrate community structure in the urban reach could therefore be linked with higher environmental heterogeneity as well as low nutrient inputs (Chessman 1995; Quinn et al. 1997; Collier et al. 2000).

With respect to functional feeding group representation, a high percentage of collectors appeared to be a good indicator of impairment. In particular, Tubificidae, Chironominae, Orthocladiinae, and Hyalellidae were increasingly abundant at impaired sites. The relative proportion of deposit feeding collectors was also greater at urban sites than rural sites in a

Southern Ontario stream (Winter and Duthie 1998), and Ortiz and Puig (2007) reported significant increases in the densities of collectors in an urban Mediterranean stream. It is possible that moderate increases in nutrients in the low conductivity Patagonian streams, favors collectors whose relative abundance was positively correlated with ammonia and soluble reactive phosphate. Comparable associations were reported by Shieh et al. (2003) in a study of macroinvertebrate production in a stream affected by urban and agricultural activities.

Except for a weak increase in fish biomass at ES-urb, our fish data showed no consistent effects of urbanization on fish density or biomass. Scott et al. (1986) found that salmonid production was higher at urban sites than forested reference sites in western North America, possibly because invertebrate biomass was greater in the urban stream. In contrast, other North American studies (Onorato et al. 2000; Morgan and Cushman 2005) suggest that fish diversity and abundance declines with increasing urbanization, and that relative abundance of tolerant fish taxa increases. Our results suggest that the fish community is unlikely to be a useful indicator of impairment in Patagonian mountain streams where the natural biodiversity of the fish fauna is low and there are many introduced species that make understanding and interpretation of field survey data difficult.

Patagonian urban streams frequently experiment alterations to their hydrological regimes, extensive channel and riparian modification (including regular dredging and clearing), and inputs of organic pollutants and sediments. An approach that combines direct biological assessment with physical, chemical and environmental analysis to diagnose stream degradation is needed for monitoring purposes and as shown by the present study can be effective and straightforward to interpret. In particular, our results show that macroinvertebrate community analyses are valuable for the detection of stream impairment in Patagonia and are recommended to monitor biological recovery, conservation and restoration efforts in the future.

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Appendix

Table 6 Mean annual abundance (ind. 0.09 m⁻²) of macroinvertebrate taxa collected at reference and urban sites on Las Minas, Esquel and Carbón streams in Northwest Chubut, Patagonia, Argentina

	Species code	M-ref	M-urb	ES-ref	ES-urb	CA-ref	CA-urb
Platyhelminthes							
<i>Girardia</i> sp.	Gir	0	0	0.3	0	0	0
Annelida							
<i>Lumbriculus variegatus</i>	Lvar	15.4	6.2	13.6	57.6	35.7	36.0
<i>Nais communis</i>	Ncom	11.3	116.4	36.3	0	0	0
<i>Phreodrilus</i> sp.	Phre	0	0	0	0	5.9	73.8
<i>Limnodrilus</i> spp.	Limn	0.7	7.1	1.3	381.5	12.3	0.7
<i>Helobdella</i> spp.	Gloss	0	0.3	0	124.1	1.8	0
Mollusca							
<i>Gundlachia concentrica</i>	Glun	0	0	0	0.5	0	0
<i>Chilina patagonica</i>	Chpa	0.1	0	0.1	0.3	0.1	0.1
<i>Biomphalaria peregrina</i>	Bper	0	0	0	0.9	0	0
<i>Diplodon chilensis</i>	Dch	0	0	0	13.5	0	0
Arthropoda							
Arachnida							
<i>Hydrachnidia</i> spp.	Hydra	0	1.1	0.5	0	0	4.7
Crustacea							
<i>Hyaella araucana</i>	Hyal a	0.1	0.8	0	389.4	15.4	0
<i>Hyaella curvispina</i>	Hyal c	0	0	0	105.8	4.1	0
<i>Aegla</i> sp.	Ae	0	0	0	0	0	0.1
Insecta							
Plecoptera							
<i>Senzilloides panguipulli</i>	Sp	0	0	7.3	0	0	0
<i>Potamoperla myrmidon</i>	Pm	0	0	0.1	0	0	0
<i>Limnoperla jaffueli</i>	Lj	0.1	0.2	2.0	0.3	3.8	6.0
<i>Notoperlopsis femina</i>	Nf	1.4	1.0	37.5	0	5.8	3.8
<i>Notoperla magnaspina</i>	Nm	0	0	1.6	0	0	0
<i>Aubertoperla illiesi</i>	Ai	43.6	29.9	14.5	0	19.8	8.6
<i>Antarctoperla michaelsoni</i>	Am	4.8	7.1	6.4	0.3	3.5	4.3
<i>Pelurgoperla personata</i>	Pp	0.1	0	0.1	0	0.4	0.1
<i>Udamocercia arumifera</i>	Ua	0	0	0	0	0	0.1
<i>Klapopteryx kuscheli</i>	Kk	2.4	0.8	0.5	0	1.3	1.3
<i>Pictetoperla gayi</i>	Pg	0	0	0.1	0	0.1	0
<i>Kempnyella genualis</i>	Kg	0	0	0	0	0	0.1
<i>Austronemoura quadrangularis</i>	Aq	0.1	0.1	0	0	0.1	0.1
Ephemeroptera							
<i>Siphonella</i> sp.	Sip	0	0	0	0	0	0.1
<i>Nousia delicata</i>	Nd	4.5	66.4	0.5	0.3	11.4	18.3
<i>Meridialaris chiloeensis</i>	Mc	40.3	17.2	57.6	0.1	25.9	19.4
<i>Meridialaris laminata</i>	Ml	8.9	22.5	0	0.2	15.8	10.1
<i>Meridialaris diguillina</i>	Md	0	0	3.3	0	10.3	17.2
<i>Penaphlebia chilensis</i>	Pc	0.1	1.8	0	0	1.3	1.7
<i>Rhigotopus andinensis</i>	Ra	0	0	0	0	0.1	0.4
<i>Andesiops ardua</i>	Aa	1.0	0.9	4.3	0.1	1.8	10.9
<i>Andesiops torrens</i>	At	0.7	1.9	12.5	0	5.5	17.5
<i>Metamoniuss anceps</i>	Ma	0.2	0	0	0	0	0
<i>Caenis</i> sp.	C	0	0.6	0	0	0	0

Table 6 (continued)

	Species code	M-ref	M-urb	ES-ref	ES-urb	CA-ref	CA-urb
Trichoptera							
<i>Atopsyche</i> sp.	A	0	0	0	0	0.1	0
<i>Neoatopsyche brevispina</i>	Nb	0.9	0	1.5	0	3.9	2.5
<i>Neoatopsyche unispina</i>	Nu	0	1.2	0	0	0	1.0
<i>Cailloma</i> sp.	Cai	0.7	0.5	0	0	0.7	1.3
<i>Neopsilochorema tricarinatum</i>	Nt	0	0.1	0.1	0	1.3	1.5
<i>Rheochorema</i> sp.	Reo	0	0.1	0.1	0	0	0
<i>Polycentropus</i> sp.	Pol	0	0	0.1	0	0	0
<i>Mastigoptila longicornuta</i>	MI	0	0	0	0	0.1	0.2
<i>Oxyethira bidentata</i>	Ob	0	0.1	0	0	0.1	0
<i>Metrichia patagonica</i>	Mp	0.2	0.1	0	0	0	0
<i>Austrotinodes</i> sp.	Aus	0	0	0	0	0.1	0
<i>Smicridea annulicornis</i>	Sa	7.4	1.5	1.9	0	37.1	93.3
<i>Smicridea frequens</i>	Sf	0	0.4	0	0	0	7.5
<i>Smicridea dithyra</i>	Sd	0	0	0.2	0	0	7.7
<i>Smicridea</i> sp. A	SA	0.1	0	0	0	0	0
Philorheitridae sp.	Ph	0	0	0	0	0.5	0
<i>Hudsonema flamini</i>	Hf	19.6	0.3	1.5	0	1.7	0.2
Leptoceridae sp. A	Lep	0	0	0	0	1.7	0.5
<i>Brachysetodes major</i>	Bm	9.6	0.5	3.8	0	12.4	2.6
<i>Parasericostoma ovale</i>	Pov	0	0.1	0	0	1.4	5.3
<i>Myotrichia murina</i>	My	0.1	0	0	0	0	0
Sericostomatidae sp. A	Ma	0	0	0	0	5.2	36.8
Sericostomatidae sp. B	Mb	0	0	0	0	5.3	25.8
Megaloptera							
<i>Sialis</i> sp.	Sial	0	0	0	0	0.1	0
Coleoptera							
Elmidae spp.	Elm	4.2	1.2	4.3	0.3	18.9	28.8
Hemiptera							
Corixidae sp.	Cor	0	0	0	0.1	0	0
Diptera							
<i>Edwardsina</i> sp.	Edw	0	0.2	0	0	0.3	0
<i>Diamessinae</i> sp.	Dia	0	0	0.2	0	0.2	0.1
<i>Tribelos</i> sp.	Tri	0	0.1	0	0	0	0.1
<i>Tanytarsus</i> sp.	Tan	0	0	1.7	0	0	0
<i>Rheotanytarsus</i> sp.	Rhe	27.5	88.5	0.8	0	2.0	2.0
<i>Chironomus decorus</i>	Cd	0	1.2	0	1.2	0.1	0
<i>Pseudochironomus</i> sp.	Ps	19.3	7.7	0	0	0	0
<i>Dicrotendipes</i> sp.	Dic	0	0	0	0	0	0.4
<i>Polypedilum</i> sp.	Pol	0	105.9	0	0.8	0	0
<i>Beardius</i> sp.	Bear	0	0	0	0	1.3	0.7
<i>Cryptochironomus</i> sp.	Cry	0	0	0	0.1	0	0
<i>Lopescladius</i> sp.	Lop	0.2	0	0	0	0	0.1
<i>Thienemanniella</i> sp.	Thie	14.0	0.4	0.7	0	7.7	9.1
<i>Pseudosmittia</i> sp.	Pseu	0	15.5	0	58.3	0.2	3.0
<i>Eukiefferiella</i> sp.	Eu	3.7	4.6	3.5	0	0.3	1.5
<i>Paratrachocladius</i> sp.	Par	64.2	282.7	22.5	260.5	31.2	70.8
<i>Parapsectrocladius</i> sp.	Parsp	5.8	0	3.8	429.9	7.5	18.9
<i>Orthoclaadiinae</i> sp. 1	Or1	0	0	0	0	7.1	6.3
<i>Orthoclaadiinae</i> sp. 2	Or2	9.2	0.6	0.3	0.3	0	0.4
<i>Orthoclaadiinae</i> sp. 3	Or3	0.8	0	1.6	77.8	4.2	0.1
<i>Orthoclaadiinae</i> sp. 4	Or4	0.5	0	0.3	0	0	0.1

Table 6 (continued)

	Species code	M-ref	M-urb	ES-ref	ES-urb	CA-ref	CA-urb
<i>Orthocladinae</i> sp. 5	Or5	0.3	0	0	0	0.1	0
<i>Parametrioctenus</i> sp.	Parm	10	1.7	0	105.8	4.6	0
<i>Telmatogetoninae</i> sp. 1	Telm	0.3	0.2	1.0	0	0.1	0.1
<i>Telmatogetoninae</i> sp. 2	Telm2	0.1	0.2	1.0	0	0	0
<i>Tanypodinae</i> spp.	T	8.2	26.0	2.0	0	1.9	4.7
<i>Podonominae</i> sp.	Pod	0	0	0	0	0.2	1.0
Empididae spp.	Emp	0.8	4.5	1.4	0.7	4.2	4.7
Ceratopogonidae sp. 1	Cer1	0	0	0.1	0	0.1	0
Ceratopogonidae sp. 2	Cer2	3.2	4.6	0.2	0	1.5	0.4
<i>Gigantodax minor</i>	Gmi	0.8	0.2	0.2	0	1.7	0
<i>Gigantodax marginalis</i>	Gma	2.3	1.8	1.1	0	11.1	5.0
<i>Simulium annulatum</i>	Sann	0	0	7.2	0	10.4	8.8
<i>Cnesia dissimilis</i>	Cn	16.2	7.2	0.2	0	17.6	2.7
<i>Tipulidae</i> sp.	Tau	0.5	0	0	0	0.1	0.1
<i>Hexatoma</i> spp.	Hex	2.5	1.1	0.3	0	2.9	0.2
<i>Molophilus</i> sp.	Mol	0.2	0	0	0	0.1	0
Psychodidae	Psy	0.1	0.1	0	0	0	0
Sciomyzidae	Scy	0	0	0	0.1	0	0
<i>Dasyoma</i> sp.	Das	2.7	0.3	7.0	0	1.5	0.5

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