# The performance of two regional biotic indices for running water quality in Northern Patagonian Andes

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**ABSTRACT: The performance of two regional biotic indices for running water quality in Northern Patagonian Andes.** Macroinvertebrate based biotic indices only have regional validity because of frequent endemisms in this group. The performance of two biotic indices for running water quality first proposed for Northern Patagonian Andes, the IAP (Andean Patagonian Index) and the BMPS (Biological Monitoring Patagonian Streams), was assessed against physical and chemical variables. Also family and species richness performance, and specific richness of some macroinvertebrate orders taken alone or in combination, were tested and compared, looking mainly for the best organic pollution estimators. The analysed data were collected along an important altitudinal gradient (> 1000 m) in a representative basin of the ecotone steppe-Sub-Antarctic forest, but having a great environmental heterogeneity and a strong wastewater pollution at the middle reach (Percey river). Fifteen sampling stations were monthly monitored for chemical variables, and monthly-bimonthly for macrozoobenthos community. Some variation ranges (absolute values) were 0-19 °C temperature, 9–654 $\mu$S cm$^{-1}$ conductivity, 0.25–3.26 meq L$^{-1}$ total alkalinity and 0.20–89.5 mg L$^{-1}$ BOD$_5$, 32–169 % oxygen saturation. Aerobic mesofilic bacteriae span over six orders of magnitude. Redundancy (RDA) and multiple regression analysis showed that BMPS and EPT (Ephemeroptera-Plecoptera –Trichoptera) group were the best indicators of organic matter enrichment; but at low polluted or non polluted sites (below the BOD threshold of 2.8-3.0 mg L$^{-1}$), all the proposed indices lost any significative relations with BOD; variables related with watershed natural features reached there more explanatory power. The IAP and BMPS are a useful tool for rapid assessment protocols in streams and wadeable rivers of the Andean Patagonian region, but they do not seem the best choice as an early warning system.
**Key words**: organic pollution, BOD, macrozoobenthos, biotic indices, calibration

**RESUMO: Performance de dois índices bióticos regionais para águas correntes no Norte da Patagônia Andina.** A avaliação da eficiência de dois índices bióticos para águas correntes, originalmente propostos para a região Norte da Patagônia Andina, o IAP (Índice da Patagônia Andina) e o BMPS (Monitoramento Biológico dos Riachos da Patagônia), foi determinada através de suas relações com variáveis físicas e químicas. A eficiência da riqueza no nível de família e espécie, e da riqueza específica de algumas ordens de macroinvertebrados utilizadas isoladamente ou combinadas, foi testada e comparada para determinação dos melhores indicadores, principalmente da poluição orgânica. Os dados analisados foram obtidos ao longo de um gradiente significativo de altitude (>1000m), numa bacia representativa do ecótono estepe-floresta Sub Antártica, que apresenta uma grande heterogeneidade ambiental e intensa poluição por despejos domésticos na sua extensão média (rio Percey). O monitoramento de quinze estações de amostragem foi realizado com periodicidade mensal para as variáveis químicas e mensal ou bimestral para a comunidade de macroinvertebrados. A amplitude de variação (valores absolutos) de alguns parâmetros é mostrada a seguir: 0-19°C temperature; 9-654 $\mu$S.cm$^{-1}$ conductivity, 0,25-3,26 meq.L$^{-1}$ alcalinidade total; 0,20-89,5 mg.L$^{-1}$ DBO; 32-169 % de saturação de oxigênio dissolvido. Bactérias aeróbicas mesofílicas variaram em 6

ordens de magnitude. As análises de redundância (RDA) e regressão múltipla mostraram que o BMPS e o grupo EPT (Ephemeroptera-Plecoptera-Trichoptera) foram os melhores indicadores do enriquecimento por matéria orgânica. Entretanto, nos locais não poluídos (DBO inferior à faixa entre 2,8 e 3,0 mg.L$^{-1}$) os índices propostos não apresentaram relação significativa com a DBO. O IAP e o BMPS são ferramentas úteis para avaliações rápidas de ambientes lóticos da Patagônia Andina, mas não parecem a melhor opção como sistema de alarme precoce.
**Palavras-chave:** poluição orgânica, DBO, macrozoobentos, índices bióticos, calibração.

# Introduction

Pollution of lotic environments modifies its physical properties, chemical composition and biocenosis structure (Welch & Lindell 1992). But, other kind of antropogenic disturbs modifies also its hydrological and thermal regime and its geomorphic features, which in turn affects the river physical and chemical template and its biodiversity (Wrigth et al. 1994; Resh et al. 1995; Ward 1998). The biocenosis is as important as physical and chemical factors in water quality assessment (Miettinen & Heinonen 1991; Welch & Lindell 1992; Wright et al. 1994). Qualitative indices based on macroinvertebrates incidence (presence/absence) are especially adapted to be used in extensive surveys (Wright et al. 1994; Resh et al. 1995) and in early warning systems (De Pauws & Roels 1987); family richness and species richness or species richness of Ephemeroptera (E), Diptera (D), Plecoptera (P), taken alone or in combination (EPT, EPD, PD), have also been used with similar purpose (Hawkes 1978; Paine & Gauffin 1956). Biological complexity reduction to a metric scale such as the above mentioned is useful to transfer information to natural resource or water managers (Armitage et al. 1983), but care has to be taken since by oversimplifying erroneous information can be transferred (Growns et al. 1995). The aim using biotic indices in stream surveys is to quantify its biotic integrity and indirectly the magnitude of anthropic disturbances. But, patterns of species or family distribution and related indices are affected also by natural factors in ways poorly known (Lake et al. 1994; Growns et al. 1995). In consequence, it is very important to assess it under the most diverse environmental conditions. Great efforts were carried out in some countries to predict the macroinvetebrate community at unpolluted sites (Wright 1995). Because of frequent endemisms and very few cosmopolitan species, macroinvertebrate based biotic indices only have a regional applicability (Resh et al. 1995; Wallace et al. 1996; Barbour et al. 1999). Patagonia is one of the most pristine areas of the world but many watercourses are showing the effects of growing human pressure, organic enrichment and others. Miserendino & Pizzolon (1992; 1999b) proposed two biotic indices of water quality, the IAP (Andean Patagonian Index) and the BMPS (Biotic Monitoring Patagonian Streams), respectively; species and some family richness were also used with similar purpose (Pizzolon et al. 1993). IAP and BMPS advantages and limitations were discussed in Miserendino & Pizzolon (1999b), but they still were not fully assessed against environmental variables. Only a short preliminary IAP evaluation was published in Pizzolon et al. (1992). In this study, the IAP and BMPS performance was assessed against physical and chemical variables. Also total specific richness, family richness, and specific richness of some macroinvertebrate orders taken alone or in several combinations were tested and compared, answering the following questions: (1) which of them responds best to different levels of organic matter?; (2) which is the $BOD_5$ threshold where they reach discriminant power? and (3) is it possible to use them in a predictive way? BOD was choosen as the main control variable, because it is a good estimator of organic matter concentration (Golterman et al. 1978) and it is also the most widely used chemical variable as organic pollution estimator (Sladececk 1988). Dissolved oxygen, oxygen saturation and bacteriological variables (total coliforms, faecal coliforms and aerobic mesofilic bacteriae) were also used as organic pollution estimators. The analysed data belong to the Percey River watershed, heavily polluted in the middle reach by the sewage discharge of Esquel.

## Study area

River Percey watershed is located at eastwards of the Andes (42° 54′ S; 71° 20′ W), discharging into the Pacific Ocean, through the binational Futaleufú-Yelcho basin. The Percey River watershed is limited by maxim heights near 2,200 m asl. Its has a surface area of 1,093 $km^2$, of which 349 $km^2$ belong to Esquel Stream watershed (Fig. 1). Lithology is dominated by mesosilicic vulcanites of different ages, with sedimentary

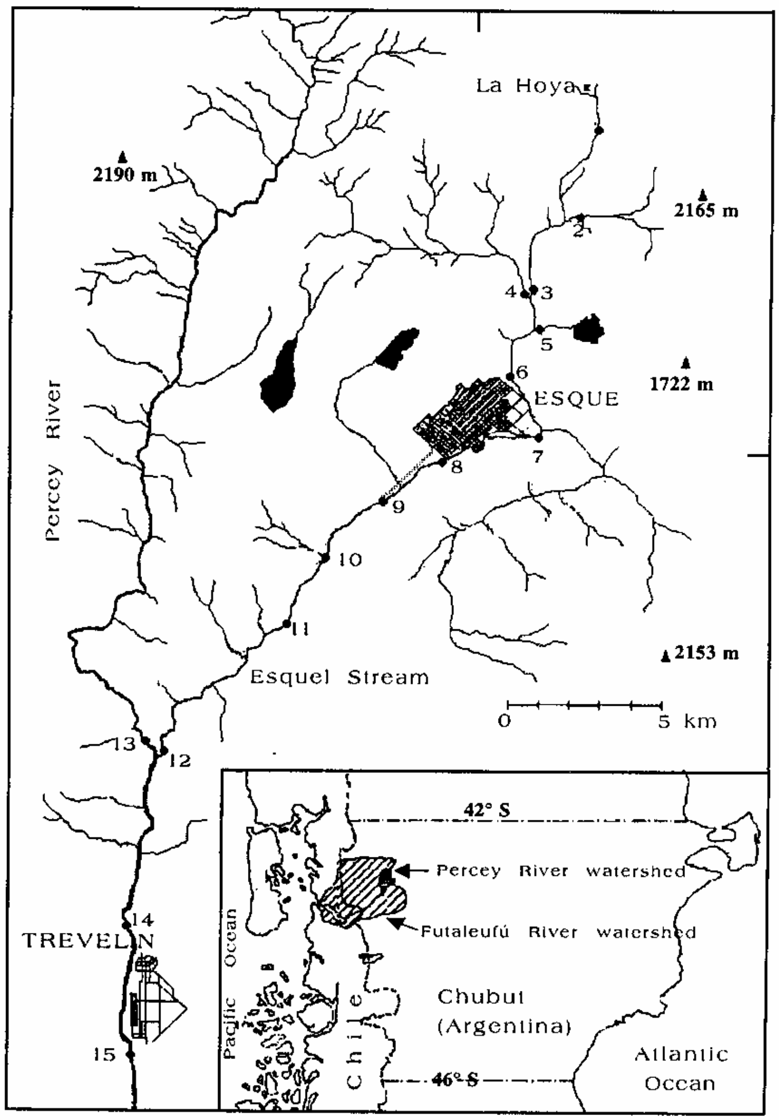


Figure 1: The Esquel-Percey lotic system and sampling sites location

continental and marine rocks, most of them covered by quaternary volcanic ashes. The climate is continental with marked daily and seasonal thermal oscillations, being the mean air temperature 8.9 °C. The mean annual rainfall is 500 - 600 mm at Esquel City, increasing to the West. Mean annual discharge of the Esquel Stream and Percey River are 1-2 $m^3.s^{-1}$ and 14 $m^3.s^{-1}$, respectively. The hydrological regime is bimodal, with two maxima due to rain and snowmelt, in winter and spring, respectively. The watershed is partially covered by the marginal andean-patagonian forest, which is composed mainly by southern beeches *Nothofagus pumilio*, a deciduous species growing from 800 m asl up to 1,700-1,800 m asl; perennial species dominant below 800 m asl, are *Maitenus boaria*, *Austrocedrus chilensis* and *Nothofagus dombeyii*. The herbaceous shrub-like steppe has a major coverage in the Esquel sub-basin. Below 700 m (close to S5 sampling station), the watercourses are flanked by *Salix fragilis* and *S. nigra*. The system is heavily polluted in the middle reach by the sewage discharge of Esquel (23,500 inhabitants). Overgrazing due to the extensive cattle ranching is responsible for notorious erosion processes. The general watershed features described for the River Percey are shared by many basins of the extended ecotonal strip Sub-Antarctic forest – Patagonian steppe.

# Methods

## Environmental variables

Water temperature, chemical variables, $BOD_5$, total and faecal coliforms and aerobic mesofilic bacteria were monthly sampled from November 1991 to October 1992 (n=11) in fifteen sampling stations (Fig. 1, Tab. I), following the method listed in Tab. II. Benthic macroinvertebrates were sampled during that period (n=8) at fourteen sampling stations. Nutrients were determined only in October 92. Sampling stations were distributed along 51 kilometres between the winter centre sports of La Hoya and Trevelin. Sampling task were carried out in the morning during three consecutive days. Analytical methods (A.P.H.A. 1978; Golterman et al. 1978) are specified in Tab. II. Altitude, lotic order and substrate size were also included as environmental variables. $BOD_5$ at S9 was estimated in samples diluted (1:4) with stream water taken before the sewage discharge. Total coliformes includes *Escherichia*, *Aerobacter*, *Citriobacter*, *Enterobacter* and *Klebsiella*. Aerobic mesofilic bacteriae (AMB) includes *Intermediae-aerogenes* and *Cloacae* (AWWA 1975).

Table I: Sampling stations names and soil use characteristics

| | Sampling sites | Lotic order | Land/river uses |
|---|---|---|---|
| 1 | La Hoya winter sport centre | 3 | winter sports/forest |
| 2 | R21 Stream | 3 | marginal forest |
| 3 | Los Bandidos Stream | 4 | marginal forest |
| 4 | La Calera Stream | 3 | livestock/shrubs |
| 5 | Willimanco Stream | 3 | livestock/shrubs |
| 6 | Esquel S. – upper bridge R. 259 | 4 | peri-urban |
| 7 | Esquel S. - Valle Chico S. | 5 | livestock/urban |
| 8 | Esquel S. - down city | 5 | point & non point urban disch. |
| 9 | Esquel S. - sewage discharge | 5 | urban sewage disch./livestok |
| 10 | Esquel S. - El Principio Farm | 5 | livestock |
| 11 | Esquel S. – lower bridge R. 259 | 5 | livestock/agriculture |
| 12 | Esquel S. – at the mouth | 5 | livestock/forest |
| 13 | upper Percey River | 6 | livestock/forest |
| 14 | Percy R. – bridge R. 71 | 6 | agriculture/livestock |
| 15 | Percy R. - Industrial Park | 6 | urban (Trevelin)/industrial |

a: tributary to the main course; *: the Esquel stream is formed at the junction of III and IV.

Table II: Parameters estimated and variables measured at the Esquel-Percey system

| | | | |
|---|---|---|---|
| altitude a.s.l | ALT | m | from topographic maps |
| stream order | SOR | | a/Strahler (1957) |
| substrate size | SS | | a/Ward (1992) |
| water temperature | Temp | °C | mercury thermometer |
| conductivity, | K20 | $\mu S\ cm^{-1}$ | Horiba U-27 water-checker |
| pH | pH | log units | potentiometric/Orion 720 SA |
| total alkalinity | TA | meq $l^{-1}$ | colorimetric end point/$CO_2$ stripping |
| carbonate alkalinity | CA | meq $l^{-1}$ | colorimetric end point/$CO_2$ stripping |
| dissolved oxygen | DO | mg $l^{-}$ | Winkler, sodium azide modification in duplicated samples |
| biochemical oxygen demand | $BOD_5$ | mg $l^{-1}$ | as DO; 5 days, 20 °C, dilution at S9 |
| Total coliforms | TC | mpn/100 ml, | AWWA 1975 |
| Faecal coliforms | FC | mpn/100 ml, | AWWA 1975 |
| Aerobic mesofilic bacteria | AMB | colony forming units/100 ml | AWWA 1975 |

## Biotic variables

Macroinvertebrates were sampled at the same sites, except S10, with a modified Surber net (0.90 $m^2$) and 250 mm mesh, pooling eight subsamples of each site, taken on different substrates. Only incidence data were used in this study, because we were looking for rapid water quality assessment protocols. The IAP, a species level qualitative biotic index, was estimated as in Miserendino & Pizzolon (1992) and the BMPS, a family level qualitative biotic index, as in Miserendino & Pizzolon (1999b). Total specific richness (SR) and family richness (FR) and specific richness of the main macroinvertebrate orders, taken alone (Diptera -D, Plecoptera -P, Ephemeroptera -E, Trichoptera –T) or in combination (PD, PE, EPT, EPD), were tested and compared.

## Statistical analysis

Environmental variables were analysed separately. Exploratory data analysis was carried out by the rank correlation coefficient of Spearman. HFA (Hierarchical Factor Analysis) was used to find the main sources of environmental variability, using the STATISTICA package (StatSoft Inc. 1993). Redundancy analysis (RDA) was conducted to determine the linear combinations of environmental variables that best explained variability patterns of biotic indices using CANOCO version 4.02 (ter Braak & Smilauer, 1999). RDA was chosen because preliminary analysis showed that indices variation was better described by linear response modeling than by unimodal models (ter Braak & Smilauer, 1998). Biotic variables were log (X+1) transformed, prior to statistical analysis to normalize and stabilize its variance. Among the environmental variables only conductivity and BOD were log transformed. To extract a reduced variable set, covariable environmental factors were excluded when the VIF (variable inflation factor) was >10 (ter Braak & Smilauer, 1998). A set of 10 environmental variables was used to perform the definitive RDA analysis.

If sufficient data are available, regression may result in more detailed descriptions and more accurate predictions than ordination (ter Braak 1995). Regression analysis express the response of a species as a function of one or more environmental variables. At the reverse, a biotic index predict values of an environmental variable as a function of species data and its construction is termed calibration (ter Braak 1995). Calibration problems differ from regression problems because the causal and statistical relations between species and environment are asymmetric (ter Braak 1995). The repetitive samples taken at the same site during the year prevent us from using multiple regression analysis on our complete data base (n=110), because this violates the basic assumption regarding the statistically independence of the observations (Kleimbaun & Kupper 1978). However, in order to give some preliminary model for predictive purposes, multiple regression analysis were performed on the annual mean (n=8) obtained at each sampling station (n=15). To avoid data transformations multiple regression were performed on a non-parametric correlation matrix (r Spearman), using the STATISTICA package, ver. 4.5. Inverse regression were used for calibration (ter Braak 1995) and some preliminary predictive models were proposed to be tested and improved with future data.

# Results

## Physical and chemical environmental factors

High absolute values of variability (maximum - minimum ranges) for abiotic variables were found at the Esquel-Percey system (Tab. IIIa). AMB span over six order of magnitude because of some extreme results in S9 and S10; the median gives a more reliable information than the average. The watershed shows a high environmental heterogeneity. Its strong altitudinal gradient (Fig. 2) is the main responsible for the temperatures found at S1 and S2 (Fig. 3), significantly lower than at other sites (T-test, $p<0.002$). The high value found at the end of the urban reach (S8), is attributable to small discharges, and probably to the higher mean air temperature in the urban area. A strong natural source of variation is introduced in the system at S5, by the Willimanco stream; due to geochemical factors its conductivity (K20) is more than seven times higher than that of the main course (Fig. 3); it also showed the lowest coefficient of variation in their physical and chemical characteristics, because it is fed mainly from a lentic water body. K20 and TA showed

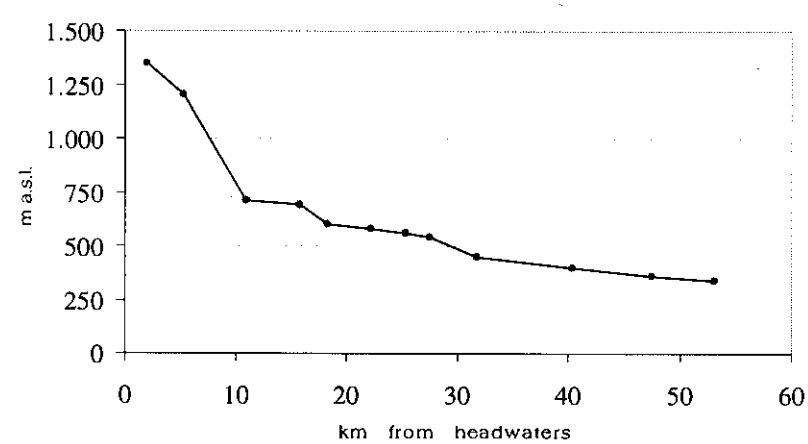


Figure 2: Longitudinal profile of the studied system

similar fluctuations. The K20 increase at mean reaches of Esquel stream was due to urban point and non-point sources of pollution. At S9, the sewage discharge introduces a strong third source of variability, well showed by $BOD_5$ (Fig. 3). Self-depuration seems to have taken place completely in the lower Esquel stream (S9 to S12).

Principal components analysis were carried out on a matrix of Spearman correlation coefficients instead of raw data, because of the highly asymmetric distribution of variables related with pollution (Kolmogorow-Smirnow test for normality). The first eigenvalue explained 37% of total variance, being 78% the accumulative variance explained by the first four eigenvalues. Hierarchical factor analysis (HFA) on principal components rotated by the varimax method gave four orthogonal and one oblique factor loadings (Tab. IV). The first independent factor loading comprised K20 and TA. Pollution variables (bacteriology and BOD) were a secondary factor, whose variance was shared with the first primary factor loading. This means that pollution variables were partially linked with geochemical variables. As example, K20 and TA were very high at S5 due to geochemical features of Willimanco basin, but it increased also from S7 to S11 (Fig. 3) due to urban discharges, notoriously at S9. LOR and ALT were the second independent factor loading. TEMP

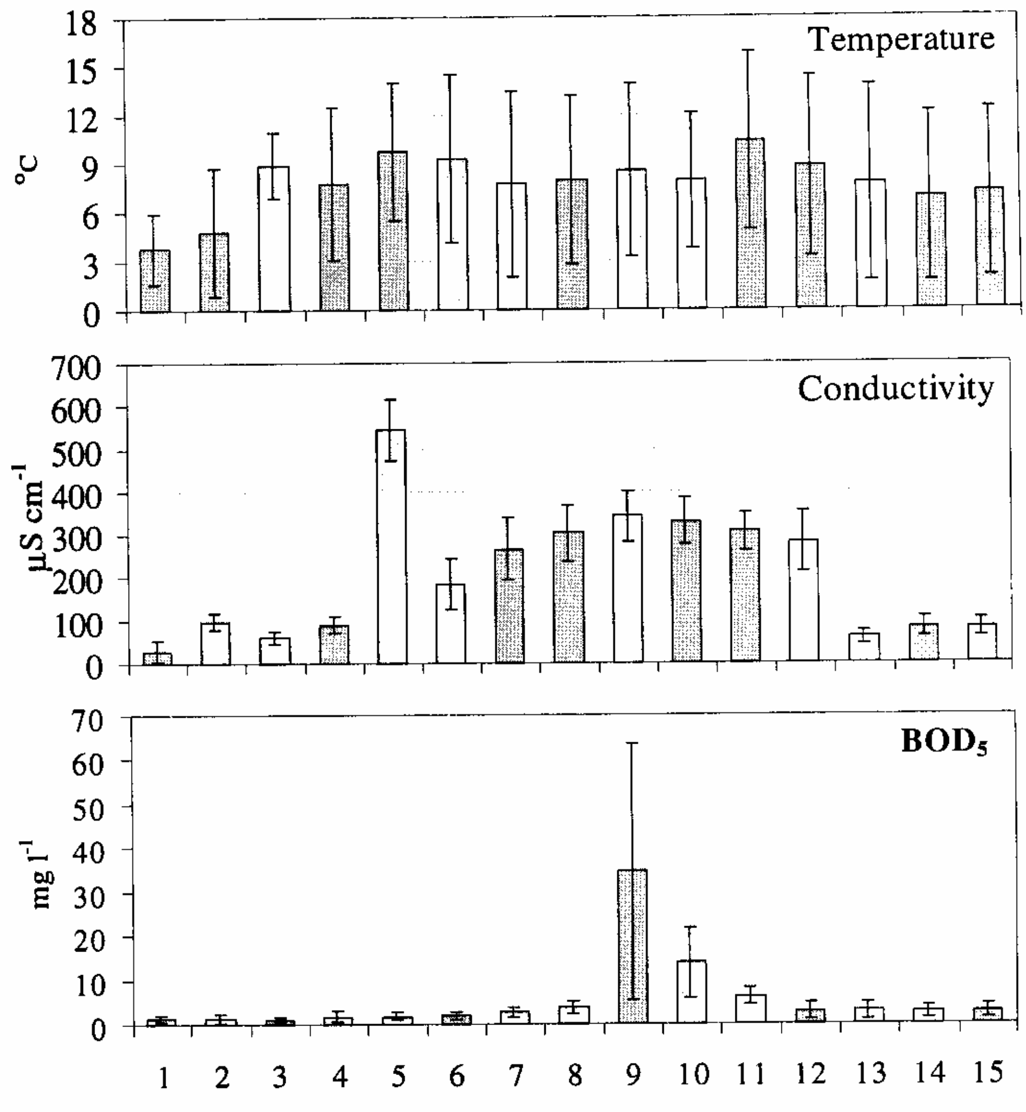


Figure 3: Annual mean values and standard deviation of representative variables monitored at 15 sampling stations in the Esquel-Percey system during 1990-91 (n=11; E1-E5 n=9 and E12, n=8).

and DO the third and pH the fourth. %DO was close to K20 and TA; CA was close to pH. CA was only found at highly polluted sites and sometimes at S5, being zero in the others. DO seem mainly controlled by the temperature of the site at the moment of sampling. In summary, the most consistent source of variability in the studied system could be grouped as K20/TA, ALT/LOR and TEMP/DO; bacteriological variables and BOD were less consistent as independent group, sharing its variance with the first. Bacteriological variables were screening together the environmental variables because they fluctuated following a similar pattern of BOD.

## Relationships between environmental factors and biotic variables

84 species and 42 families of macroinvertebrates were identified, most of them Insecta, with a range of 2-24 species per site (Tab. IIIb). FR and SR were highly correlated ($r^2$= 0.86; P< .000000; linear model) (Fig. 4). Further information on the benthic community could be found in Miserendino (1995) and Miserendino & Pizzolon (2000).

The 1st and 2nd axes in RDA biplots (Fig. 5) represent the two most important environmental gradients along which biological data are distributed. The first two axes accounted for the 46.9 % of the total variance in the biotic data (Tab. V). The first axis was significant at <0.005 probability level and it represented an environmental gradient mainly defined by organic matter (BOD) and secondarily by LOR and DO.

Table IIIa: Descriptive statistic of abiotic and bacteriological variables. 11 monthly samples taken at 15 sampling sites of the Percy watershed (N=152).

| | Temp °C | pH | K20 µS cm⁻¹ | TA meq l⁻¹ | CA meq l⁻¹ | DO mg l⁻¹ | Sat % | BOD mg l⁻¹ | AMB cfu | TC mpn | FC 100 ml |
|---|---|---|---|---|---|---|---|---|---|---|---|
| average | 7.9 | 7.8 | 205 | 1.57 | 0.02 | 12.26 | 110 | 5.30 | 114035 | 1417 | 827 |
| median | 7.0 | 7.7 | 168 | 1.45 | 0.00 | 12.74 | 114 | 2.56 | 320 | 2 | 1 |
| max | 19.0 | 9.3 | 654 | 3.26 | 0.50 | 19.17 | 182 | 89.50 | 5000000 | 69000 | 51200 |
| min | 0.0 | 6.7 | 9 | 0.25 | 0.00 | 1.53 | 15 | 0.24 | 0 | 0 | 0 |
| Std. dev. | 4.5 | 0.5 | 148 | 0.73 | 0.07 | 2.91 | 24 | 10.81 | 519359 | 6536 | 4611 |
| coef var. | 57 | 6 | 72 | 46 | | 24 | 22 | 204 | 455 | 461 | 558 |

Table IIIb: Descriptive statistic of biotic variables. 8 monthly samples taken at 14 sampling sites of the Percey watershed (N=108). P (plecopterans); E (ephemeropterans); D (dipterans); T(trichopterans); H (hirudineans)

| | P | E | D | T | H | PE | PD | EPT | EPD | SR | FR | IAP | BMPS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| average | 1.7 | 1.8 | 3.5 | 2.4 | 0.6 | 3.5 | 5.2 | 5.9 | 7.0 | 13.2 | 11.7 | 8.1 | 69.2 |
| median | 1.0 | 2.0 | 3.0 | 2.0 | 0.0 | 4.0 | 5.0 | 7.0 | 7.0 | 14.0 | 12.0 | 9.0 | 75.5 |
| max. | 6 | 4 | 8 | 8 | 3 | 8 | 12 | 12 | 15 | 24 | 20 | 10 | 140 |
| mín. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 3 |
| Std. dev. | 1.41 | 1.04 | 1.95 | 1.89 | 0.93 | 2.21 | 3.03 | 3.51 | 3.83 | 5.35 | 4.37 | 2.25 | 31.8 |
| Coef. var. | 83 | 58 | 56 | 79 | 154 | 63 | 58 | 60 | 55 | 40 | 37 | 28 | 46 |

Table IV: Hierarchical factor analysis (HFA): primary and secondary factor loadings (principal components varimax rotated) for physical, chemical and bacteriological data of Percey watershed.

| | Second. | Primary factor loading | | | |
|---|---|---|---|---|---|
| | 1 | 1 | 2 | 3 | 4 |
| ALT | -.47 | .07 | **-.81** | .03 | -.00 |
| LOR | .42 | -.13 | **.84** | -.08 | -.06 |
| TEMP | .13 | -.05 | .07 | **.95** | .09 |
| pH | .22 | -.05 | .01 | -.02 | **.83** |
| K20 | .45 | **.70** | -.22 | .07 | .10 |
| TA | .45 | **.71** | -.21 | .10 | .00 |
| CA | .30 | .17 | -.06 | -.08 | .66 |
| DO | -.13 | -.33 | .20 | **-.76** | .29 |
| % DO | -.26 | -.61 | .15 | .08 | .40 |
| BOD | .53 | .48 | .27 | -.51 | .07 |
| AMB | .66 | .48 | .38 | .25 | .00 |
| TC | .65 | .55 | .33 | .07 | -.04 |
| FC | .59 | .49 | .31 | .05 | -.04 |

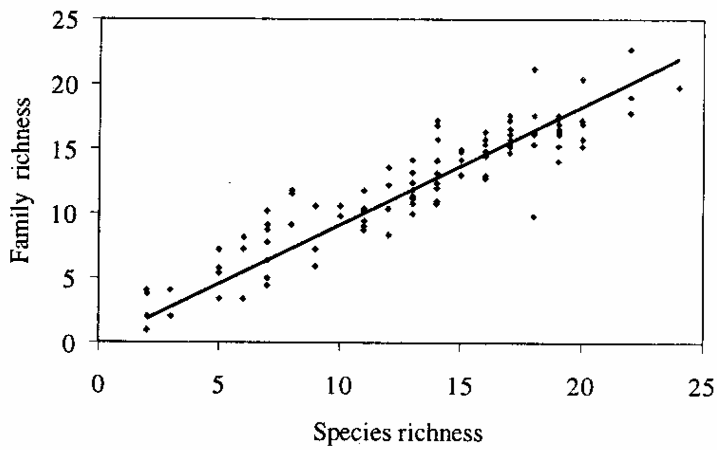


Figure 4: Species richness vs. family richness in 14 sampling stations along the Esquel-Percey system; 8 samplings during the period Nov. 90 - Oct. 91.

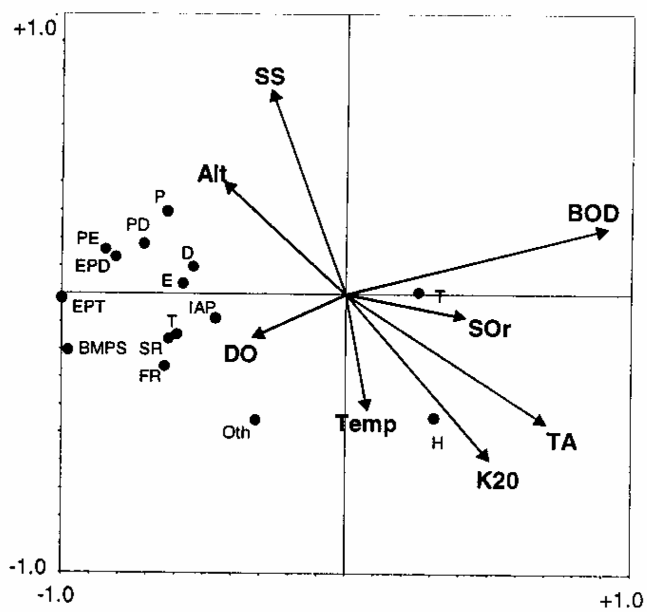


Figure 5: RDA biplot for the species-environment relationships at the Esquel-Percey system based on RDA analysis (P. Plecoptera; E. Ephemeroptera; D. Diptera; T. Trichoptera, Tu. Tubificidae; H. Hirudinea; Oth. others).

Table V: Results of redundancy analysis (RDA). The species-environmental correlations scale the strength of the relationship between species and environment for the axes. Montercarlo tests are listed for the first axis and for all the axes combined.

| **Axes** | **1** | **2** | **3** | **4** | **Total variance** |
|---|---|---|---|---|---|
| Eigenvalues | 0.421 | 0.049 | 0.020 | 0.011 | |
| Species-environment correlation | 0.782 | 0.729 | 0.633 | 0.525 | 1.000 |
| Cumulative percentage variance | | | | | |
| of species data | 42.1 | 46.9 | 48.9 | 50.0 | |
| of species-environment correlation | 81.8 | 91.2 | 95.1 | 97.2 | |

Test of significance:
Axis1: $F=71.191$ $p<0.005$
All canonical axes: $F=10.383$, $p<0.005$

The second axis represent a gradient of temperature and substrate type. Conductivity, total alkalinity and altitude were shared by both axes. Canonical coefficients, their approximate t-values and intraset correlations of the environmental variables are shown in Tab. VI. The species-environment correlations were 0.782 and 0.729 for the first two axes, and unrestricted Monte Carlo permutation test indicated that all the axes were significant (F=10.383; p<0.005, Tab. V). Macroinvertebrate groups present at headwaters, with very low BOD and high levels of DO, were positioned to the left, while Tubificidae (Tu), occurring in sites with organic enrichment were positioned in the right side (Fig. 5). Hirudinea (H), present in high conductivity and alkalinity waters were positioned at the lower right quadrat. The BMPS showed a strongest opposite position than IAP to the BOD in the first axis, meaning that this index could be a better organic pollution indicator than IAP. FR and SR were also positioned nearest to the first axis. Between the macroinvertebrate groups the EPT showed the strongest position with the first axis, with the lowest influence of the second axis. The best performance as organic matter indicator was showed by the EPT group (Fig. 5).

Multiple regression models (Tab. VII) were consistent with the RDA analysis in showing the strong influence of organic matter (BOD or one bacteriological variable) in determining the biotic indices, except for Ephemeroptera richness which was mainly determined by the total alkalinity. Lotic order, altitude, substrate size, were also significative factors in determining the BOD. Inverse regression models using mean annual values for predictive purposes were:

$BOD = 245\ BMPS^{-1.1}$ ($R^2 = 0.90$; $n=14$)

and $BOD = 181\ IAP^{-2.05}$ ($R^2 = 0.88$; $n=14$)

Table VI: Canonical coefficients, their approximate t-values and intraset correlations of the environmental variables. Significance level at p<0.05 in boldface.

| Environm. variables | Canonical coefficients RDA 1 | RDA 2 | t-values of regression coefficients RDA 1 | RDA 2 | Intraset correlation RDA1 | RDA2 |
|---|---|---|---|---|---|---|
| Altitude | **0.1949** | -0.0212 | **1.0356** | -0.0954 | **-0.4284** | 0.3965 |
| Lotic Order | **0.3550** | -0.0769 | **2.1211** | -0.3891 | **0.4139** | -0.0804 |
| Sustr. size | 0.0362 | **0.3621** | 0.3008 | **2.5475** | -0.2671 | **0.7223** |
| Temp | 0.0093 | **-0.4106** | 0.0713 | **-2.6748** | 0.0747 | **-0.4122** |
| Cond | 0.1944 | **-0.5037** | 0.8002 | **-1.7551** | 0 .5031 | **-0.5893** |
| pH | **-0.2916** | **0.2294** | **-2.3833** | **1.5873** | -0.1059 | -0.0964 |
| TA | **0.3197** | -0.2282 | **1.4481** | -0.8752 | **0.6985** | -0.4631 |
| CA | 0.0280 | -0.0504 | 0.2954 | -0.4501 | 0.1913 | 0.0484 |
| DO | **-0.1419** | **-0.5525** | **-1.2665** | **-4.1755** | **-0.3241** | -0.1547 |
| BOD | 0.5958 | **0.4592** | **4.2980** | **2.8044** | **0 .9100** | **0.2352** |

Table VII: Multiple regression models on mean annual data (n=8) at each station (n=14) of the Esquel-Percey system, estimated on a r-Spearman (not-parametric) correlation matrix.

| Eq. | Depend. var. | Intercept | Coef. 1 | Coef. 2 | Coef. 3 | Coef. 4 | R2 adj. | p< | F |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Diptera | 1.32 (ns) | -.52 BAM | .405 Sus | -.349 Temp | | .93 | .00003 | 36 |
| 2 | Plecoptera | 0.53 (ns) | -.81 BOD | .444 Sus | .313 LOR | -.17 DO | .97 | .000001 | 90 |
| 3 | Ephemeropt. | 1.76 (ns) | -.35 TA | -.41 BOD | -.287 Sus | | .67 | .002 | 10 |
| 4 | EPT | 1.94 (ns) | -1.1 BOD | .486 LOR | | | .72 | .00038 | 18 |
| 5 | SR | -0.67 (ns) | -1.1 BOD | .520 ALT | .390 Temp | | .79 | .00091 | 13 |
| 6 | FR | 10.05 (***) | -1.0 BOD | .510 ALT | | | .56 | .004 | 9 |
| 7 | IAP | -.35 (ns) | -1.1 BOD | .556 LOR | .360 SUS | | .83 | .0004 | 17 |
| 8 | BMPS | 78.8 (***) | -.87 BAM | | | | .73 | .00006 | 37 |
| 9 | BMPS s/bact | 85 (***) | -.86 BOD | | | | .72 | .00008 | 34 |

## BOD threshold

At high BOD values, biotic indices and macroinvertebrate groups were significantly correlated with BOD, but at unpolluted sites, variables related with the natural features of the watershed showed higher coefficients than pollution variables. The threshold values of BOD at which the biotic index reach significant ($p<0.05$) R-Spearman coefficients is 2.8-3.0 mg/OD $L^{-1}$. Bellow this value none of the proposed indices and macroinvertebrate groups reached significative coefficients (Fig. 6), except dipterans which showed significantly correlations up to BOD values near 2 $mg.L^{-1}$. This means that below 2.8 mg/OD.$L^{-1}$ biotic indices or macroinvertebrate groups variability was independent from BOD variability. Likewise it is necessary to take into account that at low level organic pollution (3-4 $mg.L^{-1}$), BOD explained significantly ($p<0.05$) only a very low proportion of indices variance; the R-Spearman coefficients were always comprised between -0.22 and -0.29. The highest coefficients were those of dipterans and BMPS. The significance level for trichopterans, plecopterans, EP, EPT and IAP decreased abruptly below the BOD threshold of 3 $mg.L^{-1}$.

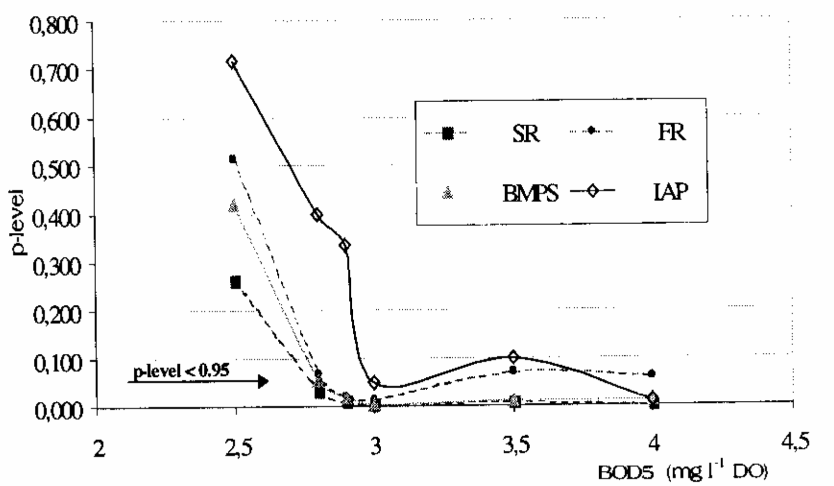


Figure 6: Significance level for the R-Spearman coefficient between BOD and SR, FR, IAP and BMPS taking data sub-sets having increased BOD values. Data from the Esquel-Percey system during 1990-91.

## Seasonal fluctuations

An exploratory analysis by the Spearman rank correlation coefficient between IAP, BMPS and chemical and bacteriological variables were seasonally estimated for autumn (May and June, n=26), spring (September, October and November, n=40) and summer (January, February and March, n=42) months. This analysis showed that IAP and BMPS were better correlated with TA and K20 in autumn and with TC and AMB in spring. In summer, IAP showed the highest coefficient with TA, and BMPS with BOD and TA.

## Self-depuration

The self-depuration of Esquel stream took place mostly along the last 15 km downstream the municipal sewage discharge, but the BOD recovery fitted to an inverse logarithmic model while the SR recovery fitted to a linear model (Fig. 7), as follows:

$BOD = 1 / (-3.24 + 0.9176 \ln km)$;  $r = 0.9997$; s.e.= 0.365

$SR = -22.14 + 0.71\ km$;  $r = 0.9998$; s.e.= 0.164

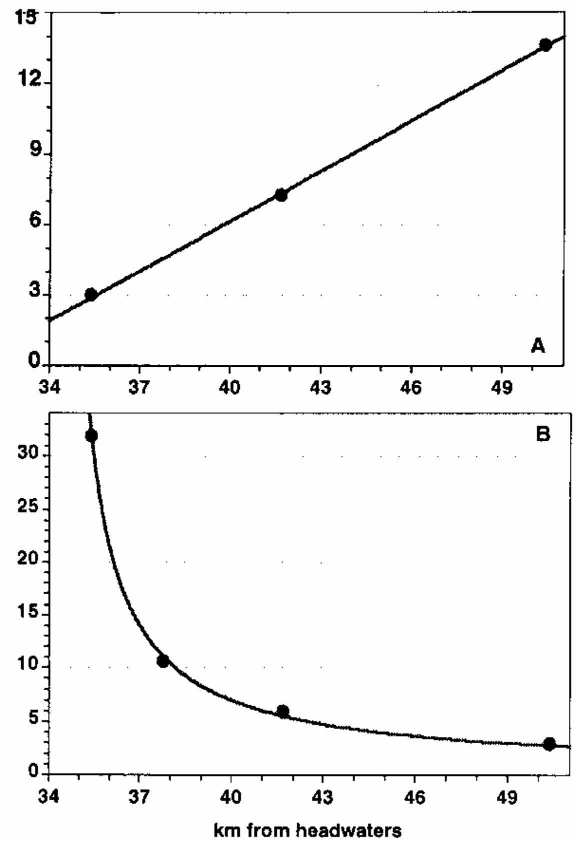


Figura 7: Specific richness (A) and Recovery of BOD (B) annual mean values along the recovery gradient of the Esquel stream, downstream the municipal sewage discharge

## Discussion

The performance of the biotic indices and specific richness of several macroinvertebrate orders were analysed in the Percey watershed, a system showing a high environmental complexity. The altitudinal gradient, one of the main factors influencing macroinvertebrate community (Ward 1992), is disrupted in this system by lateral inflows of naturally high conductivity and alkalinity waters and by the sewage input; moreover, the ionic spectrum at S5 is sulphate-calcium type, an uncommon feature in Patagonia (Pizzolon et al., 1989). This results in a cross gradient of environmental factors. The HFA analysis reflected this situation, showing that pollution variables were not independent from natural factors because they covariated with TA and K2O.

Biodiversity lost is expected at high or low levels of environmental disturbation (Ward & Stanford 1983). Intolerant taxa, such as plecopterans, ephemeropterans, trichopterans were the first to disappear (Wallace et al. 1996). A biotic index should be able to reflect this biodiversity lost (Wright et al. 1994) and the simplest biotic index is always the taxonomic richness.

Some biotic indices, i.e. SIGNAL, EPT, have a high degree of sensitivity to sewage pollution and a little response to gradients in natural factors (Chessman et al., 1997; Wallace et al., 1996). Other indices, i.e. the BMWP, seem to be responsive to many environmental factors (Wright et al. 1994). This also happened in our study. The two first axis of the RDA analysis (organic enrichment-DO, the first, and substrate size and temperature the second) explained 91% of species-environment correlation.

It is not surprising the high correlation between the IAP and BMPS with organic enrichment in the RDA bipolar, because both indices were constructed taking into account the sensitivity to the organic matter enrichment (Miserendino & Pizzolon. 1992; 1999). The river continuum concept (Vannote et al. 1980) remarks the importance of the longitudinal dimension in the riverine ecosystems structure. In fact, variables such as stream order. kilometers from headwater, substrate size, water temperature are highly dependent on altitude a.s.l., especially in mountain streams, and they are the major determinants of community structure at non polluted sites (Moss et al. 1987; Wright 1995). Rossaro & Pietrangelo (1993) showed that the community richness and biotic indices were explained by the longitudinal gradient and other physical factors. As several biotic indices resulted well correlated with these factors, these authors think that biotic indices are not a valid measure of water quality. Our results do no support these findings, but also stressed the importance of physical factors, such as substrate size. The multiple regression analysis also showed the importance of pollution and natural factor as determinant of the community richness and indices values.

RDA is only an ordination model and because both indices still lack a proper calibration process, we recommend its use with care. As stated by Moss et al. (1987), the relations between biotic and environmental variables established by this analysis were associative rather than causative. A low IAP or BMPS value does not always mean a low score in water quality. Hydrological factors, such as sites of high torrenciality and unstable substrates could cause the loss of biodiversity (Wright et al. 1994), diminishing also the indices value as found by Miserendino (1997). Macroinvertebrate community could need up to three years for a completely recovery after a great flood (Wright et al. 1994).

Inverse regression models were used for predictive purposes at Las Minas stream (Pizzolon et al. 1997), polluted by sewage discharges. The average difference between the expected and observed IAP and BMPS values were 9%, indicating a good performance of both indices, and lower differences for the IAP (6%). Despite this good agreement it is necessary to take into account that BOD underestimations up to 50% could be expected at lower stations (Percey River) and overestimations up to 30% at headstreams, as shown by residual analysis. However, they are a valuable first approximation to the use of IAP and BMPS as a predictive tool in Patagonian streams. The BMPS, whose scale extended from 0 to ›200 scores, seems to reach better discriminant power than IAP whose scale extended from 1 to 10 (Miserendino & Pizzolon 1999a,b).

## EPT index

Combining and testing taxa richness of several macroinvertebrate orders, EPT showed the higher correlations with the organic pollution axis in RDA (Fig. 5). Similar results were obtained by Wallace et al. (1996), who stressed that EPT index was especially sensitive to chemical quality of water while it remains relatively unaffected by natural disturbances such as extreme discharges. Wallace et al. (1996) showed that EPT had a faster and sharper response to experimental pollution and to water quality recovery processes than other indices. However it is necessary to take into account that EPT calculation requires considerable taxonomic task and it is more time consuming than BMPS or FR. Another drawback of EPT index could be that it shows different responses according to different disturbation factors. Wallace et al. (1996) report as example that EPT increased immediately after forest clear-cutting and the inverse when forest regrew. The authors suggest that EPT index may be not useful in regions with low richness of Ephemeroptera, Plecoptera and Trichoptera and its application seems to be restricted to streams of similar size and elevation within regional setting. Plecopterans were the single group better correlated with BOD.

## The IAP and BMPS performance in low or unpolluted sites

IAP and BMPS lost every significative relation with BOD below the BOD threshold of 2.8-3 mg DO $l^{-1}$. This means that at non polluted sites, factors others than BOD reached higher explanatory power for indices variance. Methodological errors in BOD determinations could be an important cause of this. The percentual error in BOD estimations increases exponentially as BOD values decreases, because BOD is the result of a substraction between two dissolved oxygen measures, each of which has its own error (Pizzolon 1991). Our Winkler method (precision about 0.04-0.05mg DO $l^{-1}$ in clean waters) gave significative BOD relationships with the indices only at urban and sub-urban sites and downstream wastewater discharge. However, despite the intrinsic error of BOD estimations, other authors have also found that a few physical variables are enough to explain and predict macrozoobenthos assemblages and biotic indices such as BMWP and ASPT at unpolluted sites (Armitage et al. 1983; Moss et al. 1987). Malmqvist & Mäki (1994) showed the great incidence of drainage area in species richness. In our study, at unpolluted sites IAP showed always very good water quality, but BMPS showed fluctuations related to unknown or not measured factors. Despite our first affirmations (Pizzolon et al. 1992; Miserendino & Pizzolon 1999b) this more accurate analysis demonstrates that these indices are not a good choice to assess the first stages of pollution. Similar results were found by Armitage et al. (1983), especially for family level biotic indices. In consequence they cannot be used, as an earlier warning system, as claimed by De Pauws & Roels (1987).

The assessing of indices performance at unpolluted sites remains an important task to be carried out in our region, as well as its proper calibration in a wider geographical area.

Regarding the routine use of the proposed indices the following remarks could be stressed:

1. BMPS and EPT were the groups better related with the organic enrichment in this system, but EPT, which implies considerable taxonomic knowledge at species level, requires a greater effort than the BMPS. Some times also orders could be used to assess inter-sites differences, but comparing the effect of different taxonomic levels on site classification, Furse et al. (1982) suggested that the BMWP, a family-level index (the parental index of the BMPS) provides adequate information to be used in routine monitoring.
2. The proper use of biotic indices requires a good selection of the sampling conditions, for example, never after a strong storm.
3. The seasonal samplings gave different results. Wallace et al. (1996) indicates that macrozoobenthic surveys have to integrate, at least, the result of sampling at spring, summer and autumn.
4. Armitage et al. (1983) examining several reviews for Great Britain concluded that no single index will satisfy all requirements and Wallace et al. (1996) stressed the importance to use always several indices in water quality assessment.

## Riverine landscapes and habitat assessment

We began to study water quality in Patagonian streams and rivers focused in a restricted water quality criteria. But the interactions between geomorphic features and fluvial dynamics as the major determinants of biodiversity patterns in riverine ecosystems (Ward 1998), require the interpretation of a water quality study within a broader conceptual framework. We were not able to explain the BMPS variability observed at unpolluted sites. From a broader perspective, the first step is to evaluate the habitat integrity, whose features are very important for the macrozoobenthos community (Hannaford et al. 1997; Barbour et al. 1999). The visual-based habitat assessment has become one of the most important tasks in assessing water resource conditions (Barbour et al. 1999; Resh et al. 1995). An holistic perspective considering the complete riverine landscape is necessary for a better interpretation of the taxonomic richness and biotic indices variability (Wright et al. 1994; Ward 1998; Bis et al. 2000).

Trophic relationships were not considered in this study, but they should not be ignored. Some studies have demonstrated that predators could remove some macroinvertebrates species (Reice 1991). Introduced Salmonidae are the most conspicuous predators in Patagonian streams; its effects could be important at the middle and lower course of streams, but not at headwaters, because of frequent waterfalls; this happens between S3 and S2 in the studied system.

Biotic indices do not allow the identification of disturbation factors, which is possible only by means of chemical or physical analysis (Wright et al. 1995), but the information of a single sampling of benthic macroinvertebrates is many times stronger than the meaning of a single chemical analysis, because the community of aquatic indigenous macroinvertebrates reveal structural modifications in the ecosystem due to chronical as well as episodic or rare disturbations. Despite the need to improve the performance of biotic indices, their use has become irreplaceable for a proper categorisation of water quality (Resh et al. 1995). We suggest that IAP and BMPS are applicable in wadeable watercourses of the oriental part of Valdivian forest, but a more extensive and representative data base is necessary for its proper calibration and validation. It will be very important to test its performance under different kinds of disturbations. Biotic indices are not still included in the environmental regulations of Argentina. We suggest, in agreement with Fore et al. (1996), that simple graphs and statistical models will be easier to adopt by decision makers in environmental protection than multidimensional plots derived from multidimensional analysis.

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