

# Acid stress evaluation using multimetric indices in the Carolina stream (San Luis –Argentina)

TRIPOLE<sup>1</sup>, E.S. & CORIGLIANO<sup>2</sup>, M. Del C.

<sup>1</sup>Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia, Area de Zoología, Chacabuco y Pedernera (5700) San Luis, Argentina. stripole@unsl.edu.ar

<sup>2</sup>Universidad Nacional de Río Cuarto, Departamento de Ciencias Naturales, AP 3, 5800. Río Cuarto, Argentina. mcorigliano@exa.unrc.edu.ar.

**ABSTRACT: Acid stress evaluation using multimetric indices in the Carolina stream (San Luis – Argentina).** The effect of water acidity on benthic macroinvertebrates using multimetric indices was evaluated. Biological integrity indices were adjusted for a stream reach affected by acid drainage from an abandoned mine. This permitted to demonstrate changes in the community of benthic macroinvertebrates subject to acid stress. Five sampling sites were established, in Grande river and in Carolina stream; four of them represented the reference condition. The latter were contrasted with the affected site to establish its degree of impairment. The sampling program was carried out between May 1997 and December 1998. The metric indices that discriminated between the reference situation and the problem site were selected while those that provided redundant information were eliminated. The range of metric values was divided into four categories and then translated in water quality judgments. After the selection, 13 metrics were considered reliable for the analysis and were used for the aggregation in the index and later evaluation of the biological quality of the water. The final evaluation of the multimetric index for the problem site was the sum of the scores of the selected metric indices. The biological condition of problem site was determined as moderately deteriorated environment. Biological monitoring by means of multimetric methods is sensitive to biological degradation in mining areas and effective when it is developed from the database of a regional reference condition.

**Key-words:** multimetric indices, acidity, macroinvertebrates.

**RESUMO: Avaliação do estresse ácido usando índices multimétricos no córrego Carolina (San Luis-Argentina).** O efeito da acidez da água sobre os macroinvertebrados bentônicos foi avaliada utilizando índices multimétricos. Índices de integridade biológica foram ajustados para um trecho do córrego que se encontra afetado pela drenagem ácida de uma mina abandonada. Assim foi possível demonstrar mudanças na comunidade de macroinvertebrados bentônicos submetidos ao estresse ácido. Cinco sítios de coletas foram estabelecidos no rio Grande e no córrego Carolina, dos quais quatro representaram a condição de referência, que foi contrastada com o sítio afetado para assim estabelecer seu grau de deterioração. O programa de amostragem foi realizado entre maio de 1997 e dezembro de 1998. Os índices métricos que discriminaram entre a situação de referência e do sítio problema foram selecionados, enquanto os outros, que forneceram informações redundantes foram eliminados. A série dos valores métricos foi dividida em quatro categorias e logo traduzida nos critérios de qualidade da água. Após a seleção, treze índices métricos foram considerados confiáveis para as análises e utilizados na avaliação posterior da qualidade biológica da água. A avaliação final dos índices métricos para o sítio afetado foi a resultante da soma dos valores dos índices métricos que foram selecionados. A condição biológica do trecho estudado foi classificada como um ambiente moderadamente deteriorado. O monitoramento biológico por meio dos métodos multimétricos é sensível à degradação biológica e eficaz quando desenvolvido a partir da base de dados de uma condição de referência.

**Palavras chave:** índices multimétricos, acidez, macroinvertebrados bentônicos.

---

## Introduction

The release of acid mine drainage (AMD) in natural waters of rivers and streams causes a significant impact on chemistry and biology. Acidity and increased dissolved ions are toxic to aquatic life and generate local problems in water quality (Monterroso & Macías, 1998; Aslibekian et al., 1999; Varner, 2001). The multiple effects caused by acid stress due to the AMD are often detrimental for the aquatic ecosystems, producing alterations in the benthic macroinvertebrate communities (Wiederholm & Eriksson, 1977; Allard & Moreau, 1987; Raddum et al., 1988; Mulholland et al., 1992; Herrmann et al., 1993; Appelberg et al., 1993; Varner, 2001).

To apply the present biomonitoring methods for water quality assessment it is necessary to know the reference condition, defined as the representative ecological state of a group of sites with minimal stress. These sites are organized by a set of selected biological, physical and chemical variables (Reynoldson et al., 1997), and are used to compare to an impacted site to be assessed. It is necessary to make comparisons with similar non-disturbed sites within the same ecoregion, distinguishing those that change due to natural variability (Barbour et al., 1999). The evaluation process begins with the assessment of habitat quality and the analysis of aquatic community attributes.

In order to evaluate different environmental stresses, Barbour et al. (1996) proposed Rapid Bioassessment Protocols (RBP) using multimetric indices. These are a combination of both diversity and ecological indices providing information about faunistic composition and ecological attributes. Multimetric indices permit to evaluate a biological community affected by non-natural disturbance and they can be applied in extensive areas with few modifications. Nevertheless, a regional calibration according to the type of impact is necessary (Corigliano et al., 1998).

In San Luis province, there are different environmental factors that affect the water quality of mountain streams: regulation by dams, urban and industrial effluents and diffuse contamination according to regional land use (Vallania et al., 1996). As this province is located in an arid region, water is a limited resource that must be conserved; hence, evaluations of affected sites are necessary for diagnosis and remediation. Acid mine drainage impacts are case study situations, which have not yet been evaluated in spite of the importance of potential mining development in the region. An examination of AMD impact in stream waters quality would allow comparisons with other study sites affected by acidity (Hämäläinen & Huttunen, 1990; Herrmann et al., 1993; Hämäläinen & Huttunen, 1996) making it possible to anticipate the effects of future mining activities. The purpose of this study is to evaluate the effect of acid mine drainage on benthic macroinvertebrates by means of multimetric indices applied to the problem sites and to reference conditions of San Luis mountain streams.

---

## Materials and methods

### Area of study

The study was carried out in the upper basin Grande River in the north center of the province of San Luis, Argentina. This basin drains an area of 291.3 Km<sup>2</sup>. One of its affluents, Carolina stream, flows through La Carolina town at 1620 masl, when it receives a drainage from a gold mine which has been out of work since 1930 (Fig. 1). The study river reach flows over siliceous materials with tertiary volcanic activity. We selected a site affected by acid drainage, site C<sub>2</sub>, and four study sites as reference conditions, RG<sub>1</sub>, RG<sub>2</sub> and RG<sub>3</sub> in the Grande river and C<sub>1</sub> in the Carolina stream, to evaluate the degree of deterioration produced by AMD.

### Field and laboratory methods

The sampling program was conducted from May 1997 to December 1998. Samples were obtained in study site C<sub>2</sub> monthly from April 1997 to January 1998 and simultaneously

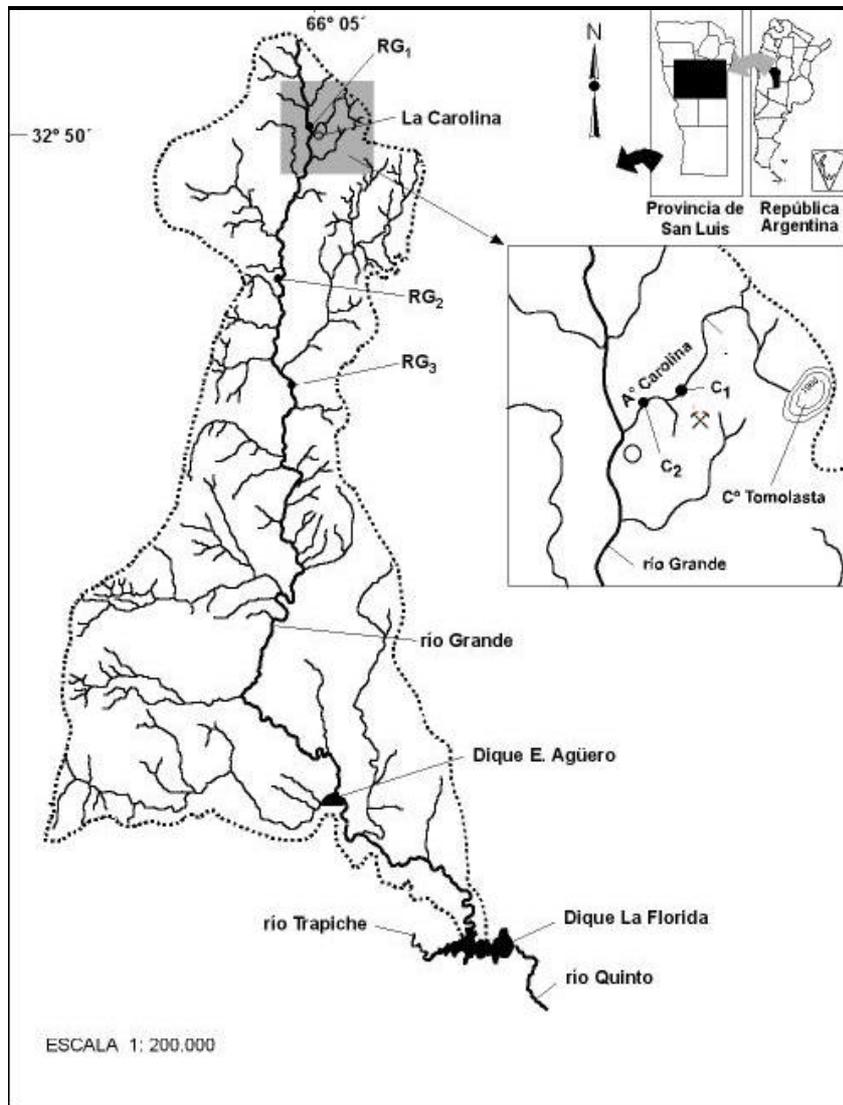


Figure 1: Sampling sites in the Grande river basin. Reference condition: RG<sub>1</sub>, RG<sub>2</sub>, RG<sub>3</sub> y C<sub>1</sub> and affected site: C<sub>2</sub>.

in the study sites C<sub>1</sub> and C<sub>2</sub> in February, March, August, October and December 1998; RG<sub>1</sub>, RG<sub>2</sub> and RG<sub>3</sub> were sampled in May, July, September, November 1997 and January, February, March and October 1998. Water temperature (°C), pH and conductivity (mS.cm<sup>-1</sup>) were measured directly in the field with portable sensors at each sample taking. Physical and biological variables (stream width, stream depth, bottom substrate type, aquatic vegetation) of the study sites were observed in the field, while geographic information of the locations (basin areas, distance from the spring source, stream order and altitude) was derived from 1: 20.000 topographical maps on the basis of aerial photographs of the sector. Stream velocity was registered by means of floaters and average depth was calculated from a cross-sectional profile measured every 30 cm. Discharge Q was calculated from speed and channel area. The dominant substrate was classified by granulometric composition criteria (Ward, 1992b). Precipitation records were obtained from zonal registers

during the sampling period. Water samples were collected and analyzed following standard procedures (APHA, 1992) for 12 variables: alkalinity, oxidability, turbidity, chlorides, sulfates, ortho-phosphates, nitrates, sodium, potassium, total hardness, carbonate hardness, Mg hardness, and total iron.

Macroinvertebrates were collected with a Surber sampler (0.30 x 0.30 m, 300 mm mesh size) following a random stratified sampling design across stream channel and preserved in 80% ethanol. The organisms were separated under stereoscopic microscope and counts were made in the entire sample. The identification of the organisms was made up to the family levels, and in some cases up to species and genera level. The identifications were made using specialized taxonomic keys: Physidae (Fernandez, 1981); Lymnaeidae (Castellanos & Landini, 1981); Planorbidae (Rumi, 1981); Oligochaeta (Brinkhurst & Marchese, 1991); Ephemeroptera (Domínguez et al., 1992); Odonata (Rodrigues, 1992); Heteroptera (Bachmann & Mazzucconi, 1995); Trichoptera (Angrisano, 1995); Coleoptera (Trémouilles et al., 1995); Simuliidae (Coscarón & Coscarón, 1995). Chironomidae were classified to subfamily and/or tribe level (Trivinho et al., 1995). When it was necessary, taxonomic experts were consulted for identifications of dubious or difficult taxa.

## Data analysis

IBSSL (San Luis Sierras Biotic Index) and multimetric indices were calculated, for high water period (March 1998 HW) and low water period (October 1998, LW) samples, to establish the deterioration of the problem site C<sub>2</sub>, during the two hydrological cycles. Variable standardizations were done by subtracting the mean and dividing by the standard deviation in order to put measurements on a common standard scale. A cluster analysis was performed with the hydrological, geographical and physical-chemical standardized variables to group study sites. Bray Curtis Similarity Index and weighted pair group average (WPGMA) method were used. Also, Jaccard Index and WPGMA method were used to develop a cluster analysis from a presence/absence macroinvertebrate matrix. Multimetric indices application was made according to the sequence developed by Barbour et al. (1999) who proposed a total of 65 metric, from which 31 were selected because they were the best indicators of stream acid stress. The discriminatory adequacy was evaluated by comparing reference condition and problem condition metric distributions on Box & Whisker plots. Metrics that were considered more effective were selected, while redundant metrics were excluded, after conducting Pearson correlations that evidenced which metrics provides the same information. The four metrics categories proposed by Resh et al. (1995) and Barbour et al. (1999) were selected: 1) Measures of diversity richness or variety of the association, 2) Measures of composition for identity and dominance, 3) Measures of tolerance that represent sensitivity to disturbance and 4) Feeding measures that provide information about the nutritional strategies.

As each metric is estimated at a different scale, the results were standardized transforming them into dimensionless numbers, before developing a total score. The criterion was based on metric value dispersions in all the evaluated sites. The values of each metric were ordered and the rank was divided into quartiles, assigning a score between 1 and 4 to each category. For those metrics whose value decreased under disturbance, value 1 was used for the lowest quartile of data distribution and for those that increased under disturbance, value 4 was used for the highest quartile. This standardization assumes that all the metrics have the same importance or weight to perform a summatory integration. The rank of the results of the metrics was divided in four categories that were translated in a water quality judgment, derived from biomonitoring protocols: not deteriorated (4), little deteriorated (3), moderately deteriorated (2) and severely deteriorated (1) (Resh et al., 1995). The concluding evaluation by multimetric indices for the study site C<sub>2</sub>, was calculated adding the score of each individual metric. The characterization of community condition was obtained considering the percentage of change from the reference condition.

## Results

Physical-chemical variables at study site C<sub>2</sub> evidenced an environmental shift in relation with regional water conditions (Tab. I). A greater similitude was observed among reference condition sites whereas C<sub>2</sub> was separated from the main group (Fig. 2). Benthic macroinvertebrates presence/ absence data cluster (Fig. 3), was similarly distributed; taxonomic richness and mean density decreased in C<sub>2</sub> (Tab. II).

Table I: Geographical, hydrological and physical-chemical parameters of the sampling sites, at Grande River Basin, San Luis province. Values shown are means ± standard deviation with ranges in parentheses.

	Problem Site		Reference Condition Sites		
	C <sub>2</sub>	C <sub>1</sub>	RG <sub>1</sub>	RG <sub>2</sub>	RG <sub>3</sub>
Elevation (m a. s. l.)	1690	1670	1660	1600	1560
Length (Km)	2.50	1.50	2.35	6.72	9.77
Stream Order	2°	2°	2°	3°	4°
Wide (m)	3.21± 0.29 (2.8 - 3.7)	3.21 ± 1.08 (1.8 - 4.8)	5.63 ± 1.07 (4.45 - 8.5)	10.14 ± 0.69 (9 - 11.2)	11.75 ± 6.14 (5.25 - 28.9)
Depth (m)	0.17 ± 0.04 (0.11 - 0.25)	0.08 ± 0.02 (0.06 - 0.11)	0.22 ± 0.06 (0.13 - 0.34)	0.07 ± 0.03 (0.03 - 0.14)	0.16 ± 0.5 (0.11 - 0.3)
Discharge (m <sup>3</sup> . seg <sup>-1</sup> )	0.03 ± 0.02 (0.009-0.088)	0.03 ± 0.03 (0.006 - 0.09)	0.11 ± 0.10 (0.02 - 0.39)	0.29 ± 0.25 (0.04 - 0.08)	0.63 ± 0.82 (0.04 - 2.84)
Water Temperature (° C)	16.8 ± 4.6 (8.5 - 22)	19.5 ± 4.3 (14 - 24)	15.7 ± 3.7 (11.0 - 22.0)	16 ± 3.5 (8.5 - 20)	15 ± 3.9 (7 - 19)
Turbidity (FTU)	4.87 ± 9.2 (0 - 36)	1.4 ± 1.3 (0.0 - 3.0)	1.2 ± 2.1 (0.0 - 8.0)	5.3 ± 14.7 (0 - 58)	0.9 ± 1.26 (0 - 4)
Alkalinity (mg. l <sup>-1</sup> CO <sub>3</sub> Ca)	2.73 ± 10.5 (0 - 41)	43 ± 2.0 (40 - 45)	127.3 ± 13.2 (94 - 144)	82.5 ± 20.1 (26 - 102)	84.6 ± 10.27 (68-99.6)
Conductivity (mS. cm <sup>-1</sup> )	420.7 ± 182.8 (163 - 700)	157.8 ± 23.2 (124 - 180)	215.9 ± 26.2 (175 - 260)	175.4 ± 27.3 (125 - 213)	165.5 ± 21.3 (124.8 - 191.6)
Total Hardness (mg. l <sup>-1</sup> CO <sub>3</sub> Ca)	140.4 ± 69.3 (38 - 263)	57.6 ± 25 (29 - 87)	100.3 ± 22.9 (43 - 130)	80.5 ± 25.2 (29 - 128)	75.15 ± 13.8 (33 - 90)
Ca Hardness (mg. l <sup>-1</sup> CO <sub>3</sub> Ca)	71.3 ± 32.8 (24 - 140)	36.6 ± 17.1 (19 - 58)	68.9 ± 20.9 (8.0 - 95 .0)	55.5 ± 14.4 (24 - 77)	52.4 ± 11.5 (24 - 69)
Mg Hardness (mg. l <sup>-1</sup> CO <sub>3</sub> Ca)	64.1 ± 33.5 (14 - 123)	21± 8.4 (10 - 29)	38.1± 17.7 (16 - 94)	25.1± 14.4 (5 - 56)	22.7 ± 5.5 (9 - 33)
Na <sup>+</sup> (mg. l <sup>-1</sup> )	8.1 ± 2.75 (4.3 - 12.8)	5.5 ± 1.9 (4.05 - 9)	7.7 ± 2.2 (5.0 - 14 .0)	7.3 ± 2.4 (4.2 - 14)	7.4 ± 1.9 (4.25 - 12)
K <sup>+</sup> (mg. l <sup>-1</sup> )	3.6 ± 2.25 (0.5 - 8.7)	1.7 ± 0.73 (0.5 - 2.4)	2.9 ± 1.52 (0.5 - 6.2)	2.5 ± 1.2 (0.5 - 5.0)	2.4 ± 1.19 (0.5 - 5.0)
SO <sub>4</sub> <sup>-</sup> (mg. l <sup>-1</sup> )	169.7 ± 83.5 (55 - 338)	34.8 ± 8.9 (25 - 48)	12.5 ± 6.12 (3.0 - 31.0)	15.6 ± 4.3 (8 - 22)	15.5 ± 4.05 (10 - 22)
Cl <sup>-</sup> (mg. l <sup>-1</sup> )	32.5 ± 8,25 (55 - 338)	16.8 ± 8.8 (7 - 28)	26.4 ± 8.5 (9.0 - 34.0)	26.5 ± 7.6 (10 - 35)	25.5 ± 6.5 (11 - 32)
NO <sub>3</sub> <sup>-</sup> (mg. l <sup>-1</sup> )	0,16 ± 0,11 (0,1 - 0,5)	0,9 ± 2,94 (0 - 11,2)	0,32 ± 0,41 (0,1 - 1,3)	? 0,1 ? 0,1	0,51 ± 0,62 (0,1 - 1,9)
PO <sub>4</sub> <sup>3-</sup> (2 g. l <sup>-1</sup> )	9,59 ± 7,85 (1,7 - 21)	5,9 ± 5,7 (0,2 - 21,3)	9,6 ± 6,24 (0,94 - 25)	10,8 ± 5,9 (5,3 - 19,4)	16,0 ± 18,5 (0,73 - 72,3)

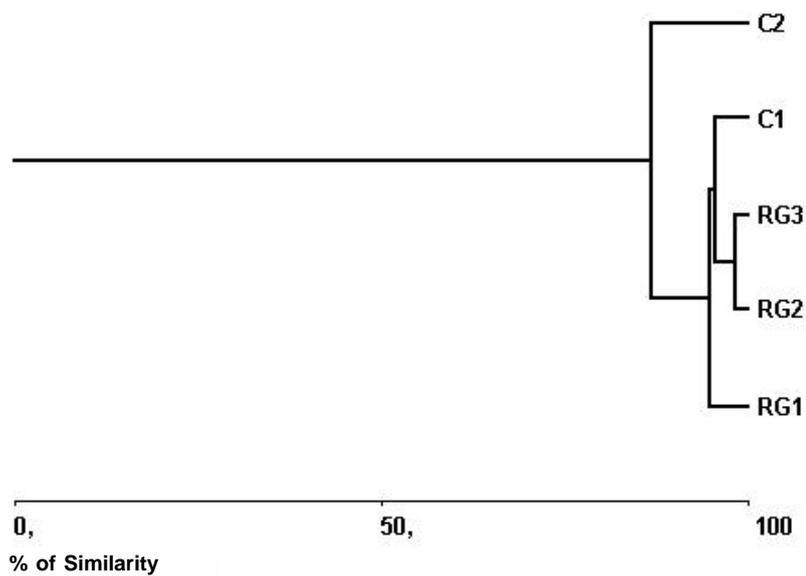


Figure 2: Similarity between reference condition (RG<sub>1</sub>, RG<sub>2</sub>, RG<sub>3</sub> and C<sub>1</sub>) and problem site C<sub>2</sub>, developed from hydrological, geographical, physical and chemical characteristics

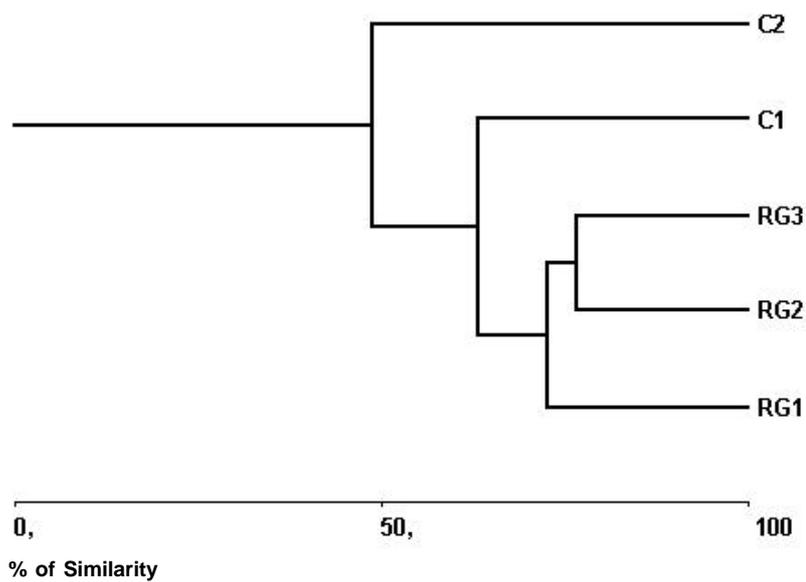


Figure 3: Similarity between the reference condition (RG<sub>1</sub>, RG<sub>2</sub>, RG<sub>3</sub> and C<sub>1</sub>) and the problem site C<sub>2</sub>, developed from benthic macroinvertebrates.

Table II: Mean density and relative abundance of benthic macroinvertebrate assemblages at the study sites in the Grande river basin. Reference condition: RG<sub>1</sub>, RG<sub>2</sub>, RG<sub>3</sub> y C<sub>1</sub> and affected site: C<sub>2</sub>.

Study Sites	RG <sub>1</sub>		RG <sub>2</sub>		RG <sub>3</sub>		C <sub>1</sub>		C <sub>2</sub>	
	Density	Abundance								
Taxa	Ind.m <sup>2</sup>	%								
Hydra sp.	279.57	0.81	15.52	0.08	0.00	0.00	295.91	1.25	4.40	0.25
Girardia sp.	46.38	0.13	8.14	0.04	0.00	0.00	137.41	0.52	0.00	0.00
Nematoda	147.47	0.43	187.57	0.91	11.33	0.16	484.21	1.84	2.96	0.16
Gordiodea	2.10	0.01	0.00	0.00	0.52	0.01	0.00	0.00	0.00	0.00
<b>Mollusca</b>										
Pelecypoda	1.33	0.01	0.00	0.00	0.00	0.00	185.19	0.70	0.00	0.00
Biomphalaria sp.	19.71	0.06	35.29	0.17	0.53	0.01	552.26	2.10	0.73	0.04
Stenophysa sp.	18.48	0.05	1419.86	6.87	5.24	0.07	0.00	0.00	0.00	0.00
Lymnaea sp.	0.00	0.00	0.00	0.00	0.00	0.00	20.18	0.08	0.00	0.00
Littoridina sp	0.00	0.00	0.00	0.00	0.00	0.00	44.13	0.17	0.00	0.00
<b>Anellida</b>										
Nais communis	2898.95	8.36	7105.57	34.40	442.90	6.27	10311.70	39.21	26.64	1.48
Nais variabilis	9.52	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nais elinguis	7.38	0.02	205.81	0.99	6.95	0.10	0.00	0.00	0.00	0.00
Chaetogaster sp.	466.90	1.35	989.76	4.79	132.67	1.88	1442.72	5.48	5.13	0.29
Pristinella sp.	68.28	0.20	101.19	0.49	4.00	0.06	758.01	2.88	0.00	0.00
Pristina sp.	32.24	0.09	21.90	0.11	2.62	0.04	815.18	3.10	56.22	3.13
Tubificidae	81.67	0.23	34.67	0.17	109.33	1.55	712.79	2.71	41.87	2.33
Enchytraeidae	21.19	0.06	210.67	1.02	1.05	0.01	231.10	0.88	27.58	1.54
Megadrili	0.00	0.00	53.62	0.26	2.67	0.04	10.33	0.04	6.64	0.37
Aeolosoma sp.	38.72	0.11	60.14	0.29	0.00	0.00	29.80	0.11	0.00	0.00
Arthropoda no Insecta										
Hyalella curvispina	286.81	0.83	0.00	0.00	6.81	0.09	17.53	0.07	0.00	0.00
Acari	590.00	1.70	3060.48	14.82	85.33	1.21	1535.30	5.84	193.44	10.77
<b>Heteroptera</b>										
Sigara sp.	40.00	0.11	0.00	0.00	0.52	0.07	18.60	0.07	10.09	0.56
Ectemnostega sp.	22.62	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lethocerus sp.	5.29	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ambrysus sp.	7.38	0.02	41.43	0.20	0.52	0.01	15.20	0.06	1.22	0.07
Notonectidae	169.29	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Odonota</b>										
Progonphus sp.	0.00	0.00	5.67	0.03	3.33	0.05	0.00	0.00	12.07	0.67
Aeshna sp.	14.57	0.04	11.57	0.06	0.00	0.00	7.73	0.03	0.00	0.00
Limnetron sp.	13.62	0.04	21.72	0.10	0.00	0.00	19.67	0.08	0.00	0.00
Coenagrionidae.	106.29	0.31	39.19	0.19	1.05	0.01	400.34	1.52	12.76	0.71
<b>Ephemeroptera</b>										
Tricorythodes popayanicus	59.24	0.17	279.67	1.35	1232.57	17.44	0.00	0.00	0.00	0.00
Leptohyphes sp.	12.67	0.04	110.57	0.53	648.52	9.18	0.00	0.00	0.00	0.00
Caenis sp.	9326.33	26.88	478.10	2.31	207.57	2.94	1021.71	3.88	11.80	0.66
Baetodes sp.	0.00	0.00	6.86	0.03	8.09	0.11	0.00	0.00	0.00	0.00
Camelobaetidius penai	2.90	0.01	42.29	0.20	107.86	1.53	0.00	0.00	0.00	0.00
Baetidae	874.19	2.52	331.95	1.61	1460.95	20.67	126.81	0.48	1.22	0.07

Table II. Cont..

Study Sites	RG <sub>1</sub>		RG <sub>2</sub>		RG <sub>3</sub>		C <sub>1</sub>		C <sub>2</sub>	
	Density	Abundance								
Taxa	Ind.m <sup>2</sup>	%								
<b>Trichoptera</b>										
Metrichia(M) neotropicalis	166.29	0.48	157.38	0.76	7.09	1.10	0.00	0.00	2.20	0.12
Hydroptila sp.	59.57	0.17	137.81	0.67	1.86	0.03	206.80	0.79	0.73	0.04
Oxyethira sp.	24.09	0.07	92.86	0.45	0.00	0.00	35.15	0.13	0.00	0.00
Marilia sp.	173.24	0.50	0.00	0.00	44.05	0.62	0.00	0.00	0.00	0.00
Helicopsyche sp.	847.62	2.44	69.76	0.34	6.29	0.09	650.53	2.47	0.24	0.01
Smicridia spp.	2.62	0.01	18.48	0.09	0.00	0.00	0.00	0.00	0.00	0.00
Polycentropus jeorgenseni	103.57	0.30	1.57	0.01	1.33	0.02	0.00	0.00	0.00	0.00
Protoptyla dubitans	2.09	0.01	0.00	0.00	0.00	0.00	41.52	0.16	0.00	0.00
Chimarra sp.	7.38	0.02	6.19	0.03	0.00	0.00	0.00	0.00	0.00	0.00
<b>Coleoptera</b>										
Dytiscidae (larvas)	129.90	0.37	0.00	0.00	0.52	0.07	42.97	0.16	0.00	0.00
Liodesuss sp.	366.52	1.06	6.71	0.03	0.52	0.07	61.50	0.23	62.87	3.50
Hydroporinae	37.00	0.11	0.76	0.01	12.38	0.17	4.13	0.02	34.20	1.90
Lancetes sp.	176.76	0.51	1.81	0.01	8.38	0.12	619.17	2.35	11.80	0.657
Elmidae (larvas)	991.10	2.86	2389.71	11.57	934.91	13.23	2.37	0.01	12.24	0.68
Austrelmis sp.	5.81	0.02	146.52	0.71	63.24	0.89	0.00	0.00	1.22	0.07
Austrolimnius sp.	2.09	0.01	10.91	0.05	8.29	0.12	0.00	0.00	0.00	0.00
Hydrophilidae	7.43	0.02	0.00	0.00	0.00	0.00	1.47	0.01	1.47	0.08
Berosus spp.	67.09	0.19	0.00	0.00	2.81	0.04	5.20	0.02	3.91	0.22
Hemiosus spp	0.52	0.01	1.57	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Gyrinus (O.) argentinus	17.62	0.05	0.00	0.00	0.00	0.00	0.00	0.00	2.47	0.14
Helichus cordubensis	0.00	0.00	10.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00
<b>Lepidoptera</b>										
Pyrilidae	56.86	0.16	73.43	0.35	0.52	0.01	0.00	0.00	0.00	0.00
<b>Diptera</b>										
Tipulidae	19.38	0.06	8.81	0.04	120.28	1.70	17.00	0.06	7.88	0.44
Psychodidae	1.05	0.03	0.00	0.00	0.00	0.00	8.67	0.03	11.80	0.66
Culicidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.56	0.59
Simulium lahilei	0.00	0.00	12.90	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Simulium wwolfueguelli	3.81	0.01	51.81	0.25	45.43	0.64	7.67	0.03	0.24	0.01
Ceratopogonidae	46.62	0.13	36.05	0.17	8.57	0.12	50.33	0.19	9.31	0.52
Muscidae	1.95	0.01	3.62	0.02	6.76	0.10	10.47	0.04	21.11	1.17
Dolichopodidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.93	0.16
Ephydriidae	4.24	0.01	2.67	0.01	16.61	0.23	9.67	0.04	3.18	0.18
Empididae	15.86	0.05	2.20	0.01	0.00	0.00	4.47	0.02	13.31	0.74
Stratiomidae	133.71	0.38	0.52	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Chironominae	9520.00	27.44	1458.62	7.06	534.95	7.57	2827.95	10.75	793.89	44.20
Orthoclaadiinae	1066.19	3.07	841.57	4.07	311.67	4.41	1633.04	6.21	321.04	17.87
Tanypodinae	4644.76	13.38	193.67	0.94	432.43	6.12	623.55	2.37	42.58	2.37
<b>Mean Density</b>	34693.47		20656.61		7067.09		26293.47		1791.11	
<b>Taxonomic Richness</b>	63		53		46		45		39	

The mean value obtaining during each sample taking was used for the multimetric analysis. Thirty-one metrics were selected in order to verify if they discriminated between reference condition and the problem site (Tab. III). From them, 19 metrics resulted reliable for C<sub>2</sub> stress evaluation because they represented the four metrics categories that best expressed ecological associations. For functional feeding groups only percentage (%) of scrapers could be considered. Reference conditions and study site C<sub>2</sub> metric values were compared to verify the discriminatory power of these indices to show the changes produced in the biota due to acid stress (Fig. 4). The metric % Diptera (r= 0.89) and % Chironomidae (r= 0.87) were eliminated because they provided the same information as

Table III. Definitions of best candidate among benthic metrics and predicted direction of metric response to increasing perturbation (compiled and adapted from Barbour et al., 1999).

<b>Metric</b>	<b>Definition</b>	<b>Expected response</b>
<b>Richness measures</b>		
No. EPT taxa	Number of taxa of Ephemeroptera, Plecoptera and Trichoptera	Decrease
Total No. taxa	Measures the overall variety of the macroinvertebrate assemblage	Decrease
No. Ephemeroptera taxa	Number of mayfly taxa (usually genus or species level)	Decrease
No. Trichoptera taxa	Number of caddisfly taxa (usually genus or species level)	Decrease
No. Coleoptera taxa	Number of beetle taxa (usually genus or species level)	Decrease
No. Diptera taxa	Number of "true" fly taxa (family level)	Decrease
No. Crustacea +Mollusca taxa	Sum of the number of calcium-depend taxa	Decrease
<b>Composition measures</b>		
EPT/EPT + Chironomidae	Relation between EPT and EPT plus Chironomidae	Decrease
% EPT	Percent of the composite of mayfly, stonefly, and caddisfly larvae	Decrease
% Ephemeroptera	Percent of mayfly nymphs	Decrease
% Trichoptera	Percent of caddisfly larvae	Decrease
% Odonata	Percent of dragonfly and damselfly nymphs	Decrease
% Coleoptera	Percent of coleopteran larvae	Decrease
% Diptera	Percent of all "true" fly larvae	Increase
% Chironomidae	Percent of midge larvae	Increase
% Orthoclaadiinae/Chironomidae	Relative abundance of Orthoclaadiinae larva	Increase
% Chironominae/Chironomidae	Relative abundance of Chironominae larva	Increase
% Oligochaeta	Percent of aquatic worms	Variable
% Crustacea + Mollusca	Percent of individuals classed as crustaceans plus molluscs	Decrease
% Gasteropoda	Percent of snails	Decrease
Shannon Wiener Index	Incorporates both richness and evenness in a measure of general diversity and composition	Decrease
<b>Tolerance/Intolerance measures</b>		
Abundance	Density in ind.m <sup>2</sup>	Decrease
% Baetidae/Ephemeroptera	Relative abundance of pollution tolerant mayflies (metric could also be regarded as a composition measure)	Increase
% Dominant taxon	Measures the dominance of the single most abundant taxon	Increase
San Luis' Sierras Biotic Index (SLSBI)	Use tolerance values of taxonomic Units to evaluates water biological quality	Decrease
<b>Feeding measures</b>		
No. of predator taxa	Number of taxa that feed upon other organisms or themselves in some instances	Variable
% Predators	Percent of the predator functional feeding group. Can be made restrictive to exclude omnivores	Variable
% Collectors Gatherers	Percent of the macrobenthos that "gather"	Variable
% Filterers	Percent of the macrobenthos that filter FPOM from either the water column or sediment	Variable
% Grazers and Scrapers	Percent of the macrobenthos that scrape or graze upon periphyton	Decrease
% Shredders	Percent of the macrobenthos that "shreds" leaf litter	Decrease

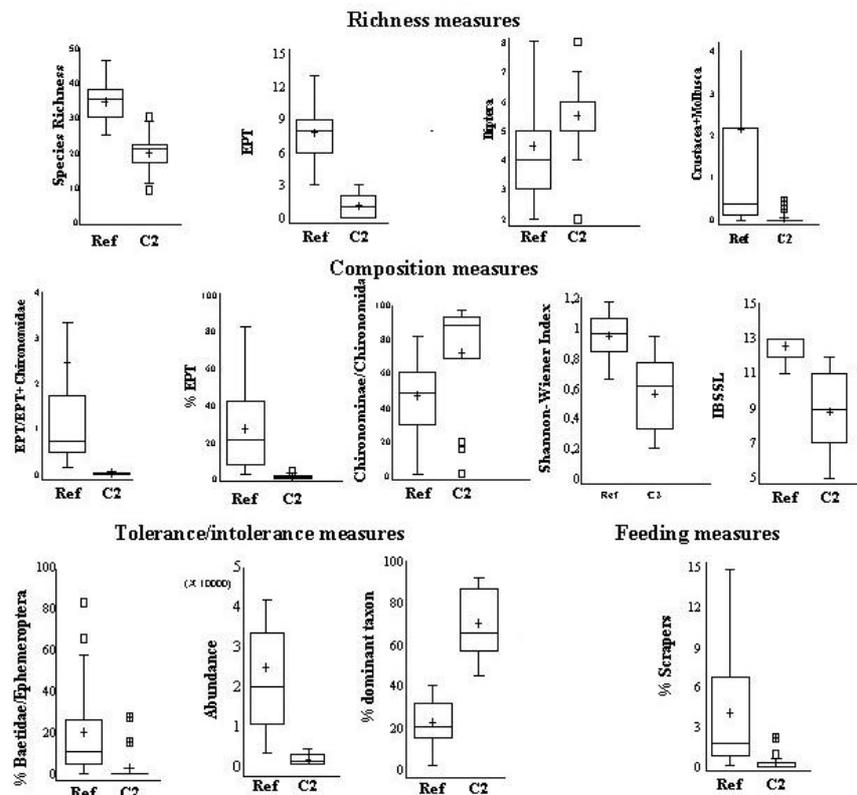


Figure 4: Discriminatory power analysis of selected multimetric indices. Ref: reference condition, C<sub>2</sub>: affected site.

% Dominant Taxa. Thus, the latter was selected because it provided an understanding of community structure and discriminated between reference condition and C<sub>2</sub>. The metrics No. Ephemeroptera taxa ( $r = 0.94$ ) and No. Trichoptera taxa ( $r = 0.94$ ) were also excluded because they correlated with No. EPT. So, % Ephemeroptera ( $r = 0.99$ ) and % Trichoptera ( $r = 0.81$ ) which correlated with % EPT ( $r =$  Pearson correlation,  $p < 0.01$ ,  $n = 20$ ) were also eliminated.

The selection provided 13 reliable indices. They were used for the multimetric score and the evaluation of the biological quality of the water (Tab. IV). They behaved according to the predicted response, except No. Diptera taxa. Chironominae/Chironomidae and % Dominant Taxa increased in stressed site; thus obtaining the minimal score (1) for the higher quartile of the data distribution

The evaluation of the study site C<sub>2</sub> resulted from the addition of the scores of the 13 selected metric indices, according to the criterion previously established (Tab. IV). A score of 21 for HW conditions represented a 40.4 % of change and a score of 18 for LW conditions represented a 34.4 % change in relation to the total value of the maximal score. This determined a quality judgment of moderately deteriorated environment, Class 2, in both periods. In LW the higher abundance was due to Orthoclaadiinae that elevated the total score of the index, although it was not enough to show an improvement in water quality. A community with low taxonomic richness and dominated by few species indicated a stressed environment. In the study site C<sub>2</sub> organisms considered particularly sensible to the acidification, such as those belonging to Ephemeroptera, Trichoptera and Mollusca were absent. The organisms of greater abundance were Chironomidae, generally reported as dominant in water bodies with low pH due to AMD and indicators of water pollution.

Table IV: Score of the selected metrics, and its application on study site C<sub>2</sub> during high water (HW) and low water (LW) hydrological conditions.

Metrics	Score criteria				C <sub>2</sub> metric value		C <sub>2</sub> score	
	1	2	3	4	HW	LW	HW	LW
Total No. Taxa	<21	21-29	30-36	>36	29	18	2	1
No. EPT Taxa	£1	2-5	6-8	>8	1	0	1	1
No. Diptera Taxa	>6	5-6	4-5	>4	5	6	2	2
No. Crustacea + Mollusca Taxa	0	1	1-2	3-4	1	0	2	1
EPT/EPT+ Chironomidae.	£0.02	0.03-0.3	0.4-0.7	>0.7	0.02	0	1	1
% de EPT	<1.2	1.2-6	7-27	>28	1.24	0	2	1
% Chironominae/Chironomidae	>80	80-58.2	58-32	<29	89.5	1.13	1	4
Shannon-Wiener Index	£0.69	0.7-0.82	0.84-0.9	>1	0.66	0.20	1	1
SLSBI	<10	10-11	12-13	>13	10	7	2	1
% Baetidae/ Ephemeroptera	0	1-4.8	5-16	³17	0	0	1	1
% Dominant Taxa	>57	32-56	19-32	<19	55	92	2	1
Abundance ind.m <sup>-2</sup> (x1000)	0.5- 1.9	2.7-8.7	9-22	23-87	2.7	3.6	2	2
% Scrapers	<0.1	0.1-0.8	0.9-2.4	>2.8	0.56	0	2	1
<b>Total Score</b>							<b>21</b>	<b>18</b>
<b>Percentage</b>							<b>40.4</b>	<b>34.6</b>

## Discussion

Many ecological studies that evaluated the biological integrity in acid environment (Hämäläinen & Huttunen 1990, 1996; Winterbourn & Mc Diffett, 1996) have been focused on a limited number of community attributes like presence/absence data, abundance, species richness and species distribution. Each attribute had been analyzed separately in order to give an interpretation of river health. Since these attributes change in natural conditions, it was necessary to develop a more integral model that reflected the specific and predictable responses of organisms to stressed conditions (Karr & Chu, 1997). The multimetric methods provide information of biological attributes that differ in their sensibility to deterioration (Resh et al., 1995), and after combination they operated like a comprehensive indicator of stream conditions.

The application of the multimetric indices has two advantages: their relative simplicity of calculation, and the fact that they are constructed on the central concepts of biomonitoring methods like tolerance, species richness and ecological relationships. Each metric evaluated a community attribute that is sensitive to deterioration, is firmly established on both empirical knowledge and tested experiences, and it responds to different stress gradients. Also, when they are properly constructed they avoid ambiguities, combining the biological understanding with the statistical power (Karr & Chu, 1997).

Aquatic organisms in our study site C<sub>2</sub> had to challenge low pH values that produced change in benthic community composition. Species richness and abundance decrease in the acid environments as has been reported on lakes (Wiederholm & Eriksson, 1977; Appelberg et al., 1993), on rivers (Raddum et al., 1988; Mulholland et al., 1992; Herrmann et al., 1993) and in experimental studies (Allard & Moreau, 1987). In streams contaminated by AMD at pH 3.75- 3.88, Varner (2001) found that densities oscillated between 56 to 70 individuals. m<sup>-2</sup> and observed very low proportions of EPT with respect to reference sites. But Winterbourn & Mc Diffett (1996), in New Zealand, observed a maximum of 16 taxa, 62 % EPT and 37 % Diptera taxa, including Chironomidae, at pH between 2.9- 3.9, and they concluded that the organisms which tolerate low pH also resist elevated concentrations of metals in the environment. The metric shifts in the study site behaved as predicted, with decreasing richness and abundance of organism belonging to

Ephemeroptera, Trichoptera and Mollusca that are sensible to acidification (Hämäläinen & Huttunen, 1990; Mulholland et al., 1992; Varner, 2001). Those more abundant were Chironomidae that are dominant under low pH condition (Zullo & Stahl, 1987; Appelberg et al., 1993; Tank & Winerbourn, 1995) especially under AMD effects (Harbrow, 2001) and are also good water quality bioindicators (Ragavan & Sada, 2000). These results confirm the concept that benthic macroinvertebrates are good indicators for water quality monitoring, not only under organic contamination, but also under acidification process after RBP regional adaptation.

---

## Acknowledgements

This study was financed by Secretaría de Ciencia y Técnica (UNSL). Professor Adriana Vallania (UNSL) is acknowledged for suggestions and practical help during the development of this survey. We are grateful to Mercedes Marchese (INALI, CONICET) and Elisa Angrisano (UBA) for their help during taxonomic determination of some Oligochaeta and Trichoptera, respectively.

---

## References

- Allard, M. & Moreau, G. 1987. Effects of experimental acidification on a lotic macroinvertebrate community. *Hydrobiologia*, 144:37-49.
- American Public Health Association - APHA. 1992. Standard Methods for the Water and Wastewater. 18<sup>th</sup> ed. American Public Health Association, Washington. 1268p.
- Angrisano, E.B. 1995. Insecta Trichoptera. In: Lopretto, E. & Tell, G. (eds.) *Ecosistemas de aguas continentales: metodologías para su estudio*. Ediciones Sur, La Plata. p.1199-1237.
- Appelberg, M., Henrikson, B.I., Henrikson, L. & Svedäng, M. 1993. Biotic interactions within the littoral community of Swedish forest lakes during acidification. *Ambio*, 22:290-296.
- Aslibekian, O., Gray, N. & Moles, R. 1999. Metal Contamination of surface water related to past mining activity in Ireland. In: IMWA - Mine, Water & Environment Congress. Sevilla. p.535-541.
- Bachmann, A.O. & Mazzucconi, S.A. 1995. Insecta Heteroptera (= Hemiptera s. str.). In: . Lopretto, E. & Tell, G. (eds.) *Ecosistemas de aguas continentales: metodologías para su estudio*. Ediciones Sur, La Plata. p.1291-1325.
- Barbour, M.T., Gerritsen, J., Griffith, G.E., Frydenborg, R., McCarron, E., White J.S & Bastian M.L. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. *J. North Am. Benthol. Soc.*, 15:185-211.
- Barbour, M.T., Gerritsen, J., Snyder, B.D. & Stribling, J.B. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. 2<sup>nd</sup> ed. U.S. Environmental Protection Agency, Office of Water, Washington. Chap.7, p.1-35.
- Brinkhurst, R.O. & Marchese, M.R. 1991. Guía para la identificación de oligoquetos acuáticos continentales de Sud y Centroamérica. *Asociación de Ciencias Naturales del Litoral, Santa Fé*. 207p. (Colección climax, 6).
- Corigliano, M.C., Gualdoni, C.M., Oberto, A.M. & Raffaini, G.B. 1998. Macroinvertebrados bentónicos en el examen de calidad ambiental de ecosistemas acuáticos en la subcuenca Carcarañá. In: *Anais do XVII Congreso Nacional del Agua*. Ed. Facultad de Ingeniería, Santa Fé, v.5, p.24-32.
- Castellanos, Z.A. & Landini, N.A. 1981. Mollusca Gasteropoda Lymnaeidae. In: Ringuelet, R.A. (ed.) *Fauna de agua dulce de la República Argentina*. FECIC, Buenos Aires. v.15, p.55-76.
- Coscarón, S. & Coscarón Arias, C. 1995. Insecta diptera simuliidae. In: Lopretto, E. & Tell, G. (eds.) *Ecosistemas de aguas continentales: metodologías para su estudio*. Ediciones Sur, La Plata. p.1269-1289.

- Domínguez, E., Hubbard, M.D. & Peters, W.L. 1992. Clave para ninfas y adultos de las familias y géneros de Ephemeroptera (Insecta) Sudamericanos. *Biol. Acuát.*, 16:1-41.
- Fernández, D. 1981. Mollusca gasteropoda physidae. In: Ringuelet, R.A. (ed.) Fauna de agua dulce de la República Argentina. FECIC, Buenos Aires. v.15, p.85-92.
- Hämäläinen, H. & Huttunen, P. 1990. Estimation of acidity in streams by means of benthic invertebrates: evaluation of two methods. In: Kauppi, P., Anttila, P., & Kenttämies, K. (eds.) Acidification in Finland. Springer Verlag, Berlin. p.1051-1070.
- Hämäläinen, H. & Huttunen, P. 1996. Inferring the minimum pH of streams from macro invertebrates using weighted averaging regression and calibration. *Freshwater Biol.*, 36:697-709.
- Harbrow, M. 2001 Ecology of streams effected by acid mine drainage near Westport South Island, New Zealand. <http://www.zool.canterbury.ac.nz/FERG/Streams.htm>
- Herrmann, J., Degerman, E., Gerhardt, A., Johansson, C., Lingdell, P. & Muniz, I. 1993. Acid-stress effects on stream biology. *Ambio*, 22:298-307.
- Karr, J & Chu, E.W. 1997. Biological monitoring: essential foundation for ecological risk assessment. *Hum. Ecol. Risk Assess.*, 3:993-1004.
- Monterroso, C. & Macías, F. 1998. Aguas de drenaje de mina afectadas por la oxidación de sulfuros. Variaciones estacionales de su composición. *Edafología*, 5:71-82.
- Mulholland, P., Driscoll, C.H., Elwood, J., Osgood, M., Palumbo, A., Rosemond, A., Smith M. & Schofield C. 1992. Relationships between stream acidity and bacteria, macroinvertebrates, and fish: a comparison of north temperate and south temperate mountain streams, USA. *Hydrobiología*, 239:7-24.
- Raddum, G., Fjellheim, A. & Hesthagen, T. 1988. Monitoring of acidification by the use of aquatic organisms. *Verh. Internat. Verein. Limnol.*, 23:2291-2297.
- Ragavan, A. & Sada, D. 2000. Biomonitoring of spring waters. <http://www.wmrs.edu/interns-2000/Ragavan/IndexRagavan.htm>
- Resh, V., Norris, R. & Barbour, M.R. 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Aust. J. Ecol.*, 20:108-121.
- Reynoldson, T., Norris, R., Resh, V., Day, K. & Rosenberg, D. 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. *J. North Am. Benthol. Soc.*, 16:833-852.
- Rodríguez Capítulo, A. 1992. Los Odonata de la República Argentina (Insecta). In: Castellanos, Z.A. (ed.). Fauna de agua dulce de la República Argentina. FECIC, Buenos Aires. v.34, p.5-91.
- Rumi, A. 1981. La Familia Planorbidae Rafinisque, 1815 en la República Argentina. In: Castellanos, Z.A. (ed.). Fauna de agua dulce de la República Argentina. FECIC, Buenos Aires. v.15, p.5-51.
- Tank, J. & Winterbourn, M. 1995. Biofilm development and invertebrate colonization of wood in four New Zealand streams of contrasting pH. *Freshwater Biol.*, 34:303-315.
- Trémouilles, E., Oliva, A. & Bachmann, A. 1995. Insecta Coleoptera. In: Lopretto, E & Tell, G. (eds.) Ecosistemas de aguas continentales: metodologías para su estudio. Ediciones Sur, La Plata. p.1133-1197.
- Trivinho-Strixino, S. & Strixino, G. 1995. Las larvas de Chironomidae (Diptera) do Estado de São Paulo. In: Santos, J.E. (ed.) Guía de identificação e diagnose dos gêneros. PRN/UFSCAR, São Carlos. 229p.
- Vallania, E.A., Garelis, P.A., Tripole, E.S. & Gil, M.A. 1996. Un índice biótico para las sierras de San Luis (Argentina). *Rev. UNRC*, 16:129-136.
- Varner, M. 2001. Variances in macroinvertebrates community structure along an acid mine drainage affected stream gradient in the central Appalachians of West Virginia. <http://www.nrac.wvu.edu/rm493-591/fall2001/students/varner/Index.htm>
- Ward, J.V. 1992. Aquatic insect ecology: biology and habitat. John Wiley & Sons, New York. 438p.
- Wiederholm, T. & Eriksson, L. 1977. Benthos of an acid lake. *Oikos*, 29:261-267.

- Winterbourn, M.J. & Mc Diffett, W.F. 1996. Benthic faunas of streams of low pH but contrasting water chemistry in New Zealand. *Hydrobiologia*, 341:101-111.
- Zullo, S. & Stahl, J.B. 1987. Abundance, distribution and life cycles of midges (chironomidae: diptera) in an acid strip-mine lake in Southern Illinois. *Am. Midl. Nat.*, 119:353-365.

**Received:** 14 June 2004

**Accepted:** 12 January 2005