Revista de Biología Marina y Oceanografía
Vol. 47, N°1: 23-33, abril 2012
Article

Benthic macroinvertebrate assemblages as indicators of water quality applying a modified biotic index in a spatio-seasonal context in a coastal basin of Southern Chile

Ensamble de macroinvertebrados bentónicos como indicadores de calidad de agua a través de la aplicación de un índice biótico modificado en un contexto espacio-estacional en una cuenca costera del sur de Chile

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Resumen.- Se caracterizó la calidad del agua en un río del sur de Chile a través de un Índice Biótico, en un contexto espacio-temporal y además se analizó la composición y distribución de macroinvertebrados bentónicos y su correlación con 14 parámetros físico-químicos. La toma de muestras ocurrió durante un año en 5 estaciones de muestreo en la cuenca del río Boroa. Se registró un total de 77 taxa, los órdenes más representativos fueron Ephemeroptera (43,30%), Diptera (24,16%) y Plecoptera (18,03%). Las especies más abundantes fueron *Meridialaris diguillina, Nousia maculata* (Ephemeroptera) y *Limnoperla jaffueli* (Plecoptera). Acordando al IBF modificado (Índice Biótico de Familias) los resultados indicaron excelente, muy buena y buena calidad de agua, la última calidad fue registrada principalmente en invierno. Los parámetros fisicoquímicos así mismo indicaron calidad de agua excepcional. Estos resultados indican un bajo nivel de impacto antropogénico en la cuenca. Al aplicar el Índice Biótico de Hilsenhoff modificado basado en macroinvertebrados, la influencia de la estacionalidad sobre la calidad del agua fue evidente.

Palabras clave: Estacionalidad, ríos chilenos

Abstract.- The water quality in a Southern Chile river was characterized using the Biotic Index in a spatio-seasonal context, to analyze the composition and distribution of benthic macroinvertebrates and their correlation with 14 physico-chemical parameters. Sampling was done over a year at 5 sampling stations in the basin of the Boroa River. A total of 77 taxa were recorded, with the most represented orders being the Ephemeroptera (43.30%), Diptera (24.16%) and Plecoptera (18.03%). The most abundant species were *Meridialaris diguillina*, *Nousia maculata* (Ephemeroptera) and *Limnoperla jaffueli* (Plecoptera). According to the modified FBI (Family Biotic Index), the findings indicated excellent, very good and good water quality, the latter quality being registered principally in winter. Likewise, the physico-chemical parameters indicated exceptional water quality. These results suggested a low level of anthropogenic impact in the basin. Applying the modified Hilsenhoff Biotic Index based on macroinvertebrates, the influence of seasonality on water quality was evident.

Key words: Seasonality, Chilean rivers

INTRODUCTION

Benthic macroinvertebrates constitute the most suitable group of organisms for evaluating river water quality for organic contamination (Figueroa *et al*. 2003, Giacometti & Bersosa 2006, Oscoz *et al*. 2007).This is due to the fact that: (1) They are relatively easy to sample qualitatively or quantitatively; (2) good identification keys exist for the majority of the orders (Zamora-Muñoz *et al*. 1995); (3) they interpret the state of the environment from the events of the preceding months (Alba-Tercedor 1996); (4) for most orders there are a number of studies on tolerance levels at the family level (Hilsenhoff 1988, Roldán 1999); and (5) they are very sensitive organisms with low mobility, therefore any alteration in the environment (natural or anthropogenic) may cause them to disappear or present reduced abundance. Species which are more tolerant to the contamination may be present in increased densities while others disappear (Alonso & Camargo 2005). The Family Biotic Index (FBI), initially developed for South African rivers by Chutter (1972), was modified by Hilsenhoff (1988) for application in the rivers of the Northern Hemisphere. This index uses the abundance and diversity of macroinvertebrates in a stretch of river, assigning each taxon a tolerance value. Figueroa *et al.* (2003, 2007), modified this index, adapting it to families present in Chilean waters. Importantly, even though this index only works for a certain taxonomic level, it is widely used, since it is a practical method which has the advantage of reducing the time needed to identify the fauna (Furse *et al.* 1984, Warwick 1988, Figueroa *et al.* 2005), however, the index assumes the same tolerance value for all the species or genera of a given family. For example, Mandaville (2002) established a certain tolerance value for a family Trichoptera, but assigned a different value to one genus belonging to this family; this may produce an underestimation or overestimation of the water quality according to the index. In this study we propose, in agreement with Guerold (2000) and Waite *et al.* (2004), that in order to obtain more precise results and a better ecological interpretation, the analysis should include lower taxonomic levels.

In the south-central zone of Chile (36°S to 47°S), studies of macroinvertebrates have been carried out in several regions: Biobío (Habit *et al.* 1998, Valdovinos 2001, Moya *et al.* 2009a,), Araucanía (Leiva 2004), Los Ríos (Guevara-Cardona *et al.* 2006), and Carlos Ibañez del Campo (Mercado & Elliott 2006, Oyanedel *et al.* 2008, Moya *et al.* 2009b); the majority of which only analyzed the structure of the ensemble of macroinvertebrates.

Coastal river basins are among the most dynamic ecosystems on earth, with enormous spatial and temporal complexity (Peña-Cortés *et al.* 2006). They are often influenced by continental run-off waters which are rich in nutrients derived from urban, agricultural and industrial activity (Martínez & Esteve 2007). Their rivers run down from coastal mountain ranges and are characterized by a great variability in space and time and in their physico-chemical characteristics. This is reflected in the structure and organization of the macroinvertebrate community (Hawkins & Sedell 1980, Vannote *et al.* 1980).

The objectives of this study were 1) to describe the composition and distribution of freshwater benthic macroinvertebrate communities and their correlation with the physico-chemical parameters of the water and, 2) to characterize the water quality in the Boroa River, Southern Chile, using a spatial and seasonal analysis, applying modified Hilsenhoff Family Biotic Index (IBF).

MATERIALS AND METHODS

Invertebrates were sampled in the Boroa River basin (12,910.73 ha), located in the Coast Range of the Araucanía Region (39°13'-39°21'S and 73°01'-73°10´W), whose waters drain into the Queule River (Fig. 1). The area has

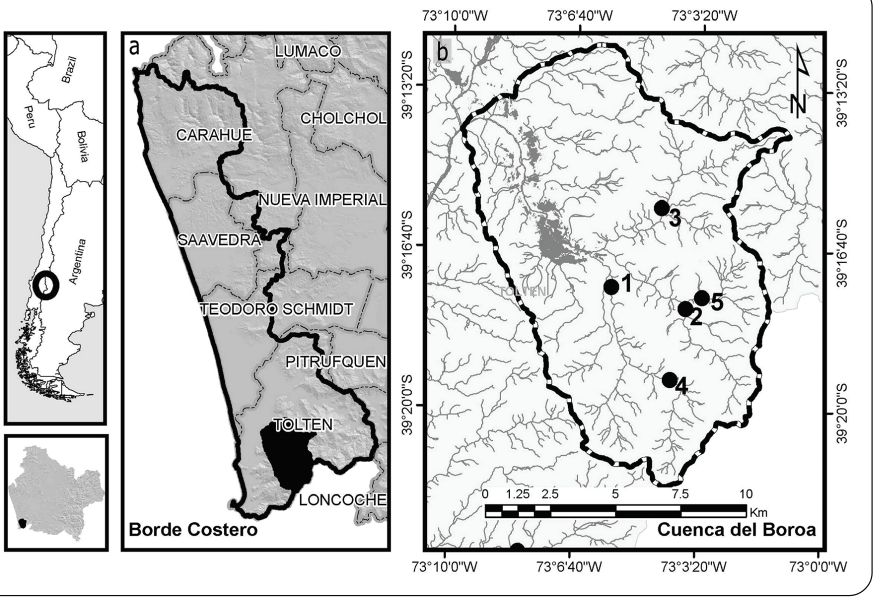

Figure 1. a) Location of the Boroa River basin in the coastal zone of the Araucanía Region. b) The sampling sites in the basin are indicated with numbers / a) Localización de la Cuenca del Río Boroa en la zona costera de la Región de la Araucanía. b) Las localizaciones de los sitios de muestreo en la cuenca están indicados con números

a temperate rainy climate with Mediterranean influence. The soil use is mixed, including native forest (*Nothofagus* spp.), forest plantations (*Eucalyptus* spp.), agriculture, cattle farming and swamp forests (Peña-Cortés *et al.* 2011). The principal river discharge period occurs in the months from May to July and is related to the winter rains. Sampling was carried out seasonally over a year: winter (2008), spring (2008), summer (2009) and autumn (2009). Five sampling stations were chosen, taking into account different levels of human impact (Fig. 1). Three replicates were taken in each site with a Surber net (0.3m x 0.3m; 0.5 mm mesh). Station 5 was not sampled in winter due to the heavy flow of the river. The samples were fixed in ethanol 70%, sorted and identified mostly by the use of taxonomic keys (Caamaño 1985, Fernández & Domínguez 2001, Domínguez & Fernández 2009), using a stereoscopic magnifier and a stereomicroscope.

Temperature, pH and conductivity were measured at each station. The water samples were collected in plastic bottles and taken to the laboratory for the determination of the following parameters: biochemical oxygen demand (BOD5), suspended solids, apparent colour, turbidity, dissolved oxygen, chlorides, sulphates, total dissolved solids, true colour, nitrates and phosphates. All the methods of analysis used followed those presented in APHA (1992) (Standard Methods for the Examination of Water and Wastewater). All these variables were used to obtain water qualities according to the secondary environmental quality standard for the protection of Chilean continental waters (NSCA) (Table 1).

Table 2. Water quality classification system based on the Family Biotic Index (FBI) values (Hilsenhoff 1988) / Sistema de clasificación de calidad de agua basados en los valores del Índice Biótico de Familias (IBF) (Hilsenhoff 1988)

Biotic index	Water quality	Degree of organic pollution
0.00 - 3.50	Excellent	Organic pollution unlikely
3.51 - 4.50	Very good	Possible slight organic pollution
4.51 - 5.50	Good	Some organic pollution probable
5.51 - 6.50	Fair	Fairly substantial pollution likely
6.51 - 7.50	Fairly poor	Substantial pollution likely
7.51 - 8.50	Poor	Very substantial pollution likely
8.51 - 10.00	Very poor	Severe organic pollution likely

For each station, the abundance of each taxonomic group and the total abundance were calculated, expressed as individuals m^{-2}. To estimate water quality the Hilsenhoff's Index (1988) was used, modified (FBI) for use at the species level (Appendix 1). Each taxon was assigned a tolerance value between 0 and 10, with 0 being the least tolerant to organic contamination and 10 the most tolerant. The tolerance values were established according to Barbour *et al.* (1999) and Mandaville (2002), and then classified into 7 quality classes (Table 2).

To determine the macroinvertebrate community structure we used the non-parametric similarity index of Bray-Curtis with Plymouth Routines in Multivariate Ecological Research software (PRIMER 6, Clarke & Warwick 1994). The physico-chemical variables were found by MDS analysis. To compare the physico-chemical

Table 1. Parameters determining the classes of water quality, according to the Secondary Environmental Quality Standard for the Protection of Chilean Continental Surface Waters (according CONAMA 2004) / Parámetros que determinan las clases de calidad de agua, de acuerdo a la Norma Secundaria de Calidad para la protección de aguas continentales superficiales (según CONAMA 2004)

Group of compounds or elements	Unit	Exception Class	Class 1	Class 2	Class 3
Electrical conductivity	µS cm^{-1}	< 600	750	1,500	2,250
BOD5	mg L^{-1}	< 2	5	10	20
Dissolved oxygen	mg L^{-1}	> 7.5	7.5	5.5	5
pH	-	6.5 - 8.5	6.5 - 8.5	6.5 - 8.5	6.5 - 8.5
Suspended solids	mg L^{-1}	< 24	30	50	80
Temperature	ΔT °C	< 0.5	1.5	1.5	3
Chlorides	mg L^{-1}	< 80	100	150	200
Apparent colour	Unit Pt Co^{-1}	< 16	20	100	> 100
Sulphate	mg L^{-1}	< 120	150	500	1,000
Total dissolved solids	mg L^{-1}	< 400	500	1,000	1,500

parameters among the sampling stations and seasons, a Principal Component Analysis (PCA) with physico-chemical data transformed to log (*x*+1) was carried out using the same statistical program. A Pearson correlation analysis was performed to search for relations between fauna and the physico-chemical variables.

RESULTS

MACROINVERTEBRATE COMMUNITY

The macroinvertebrate fauna consisted of 77 taxa corresponding to 19 orders, specifically immature states of insects; Ephemeroptera (43.30%), Diptera (24.16%), Plecoptera (18.03%), Coleoptera (5.41%), Trichoptera (1.54%) and others (7.56%). A total of 6,889 individuals were captured; the most abundant species were *Meridialaris diguillina*, *Nousia maculata* and *Limnoperla jaffueli*.

In winter 1,992 ind. m^{-2} were collected; the most abundant species was *Limnoperla jaffueli* with 570 ind. m^{-2}. In spring, 2,166 ind. m^{-2} were collected; the most abundant species was *Meridialaris diguillina* with 441 ind. m^{-2}. In summer we collected 11,055 ind. m^{-2}, of which *Nousia maculata* contributed 4,221 ind. m^{-2}. In autumn we recorded 5,451 ind. m^{-2}, the most abundant species again was *Limnoperla jaffueli* with 1,383 ind. m^{-2} (Table 3).

The cluster analysis showed that for all the sampling sites, existed was a 60% maximum similarity of taxa, specifically between sites 2 and 3 sampled during autumn. Two main groups were found through this analysis, according to the abundances of the taxa recorded, with 20% similarity (Fig. 2).

The Pearson correlation analysis between the community and physico-chemical parameters showed a negative correlation between abundance and phosphate in spring and summer (-0.97 and -0.11, respectively). In winter and autumn, a negative correlation was observed between abundance and nitrate (-0.97 and -0.85, respectively).

PHYSICO-CHEMICAL ANALYSIS OF THE WATER

Water temperatures ranged between 8.7°C (Station 1, autumn) and 21.6°C (Station 4, summer). The conductivity varied between 14.4 µS cm^{-1} (Station 2, winter) and 47.7 µS cm^{-1} (Station 1, summer). The pH varied between 6.07 (Station 3, autumn) and 6.90 (Station 1, summer), indicating slightly acidic waters (Table 4).

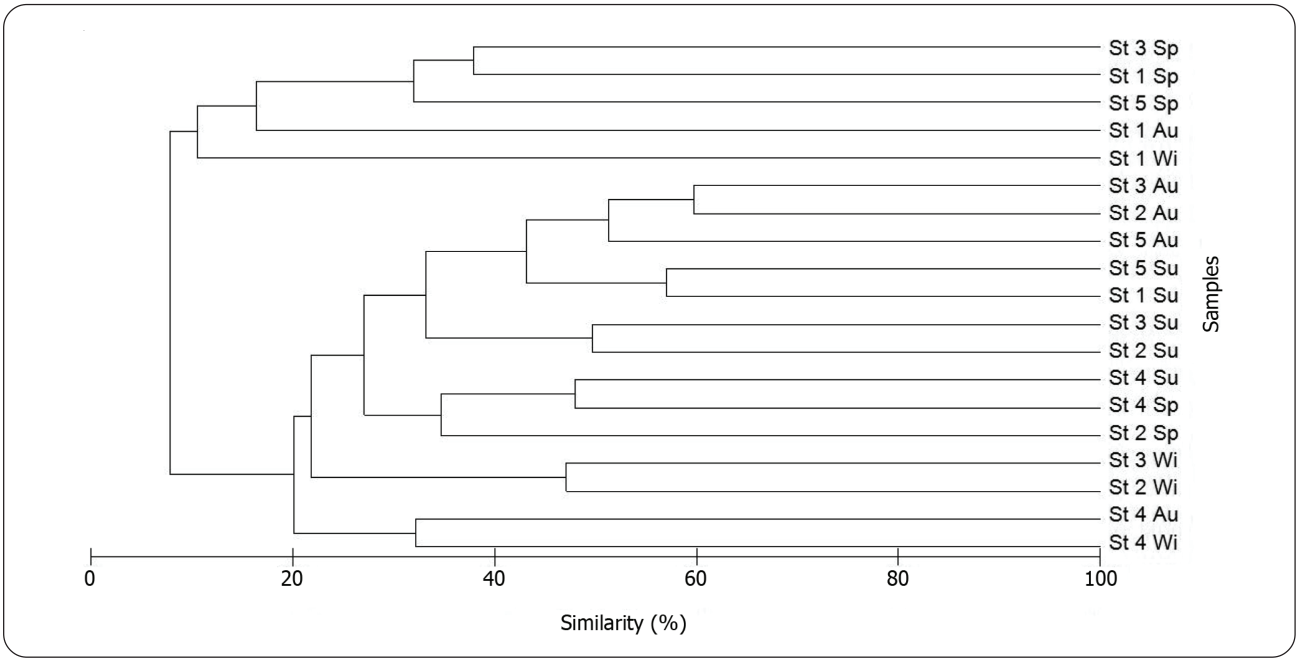

Figure 2. Cluster analysis grouping the sampling sites by the population abundance of macroinvertebrates from the Boroa River basin (Wi: winter, Sp: spring, Su: summer, Au: autumn) / Análisis de dendograma que agrupa los sitios de muestreo basado en la abundancia de la población de macroinvertebrados en la cuenca del Río Boroa (Wi: invierno, Sp: primavera, Su: verano, Au: otoño)

Table 3. Taxonomic list of the benthic macroinvertebrates and their abundances (ind. m^{-2}) recorded in the Boroa River basin. *: Indicates maximum abundance for each season of the year / Lista taxonómica de los macroinvertebrados bentónicos y sus abundancias (ind. m^{-2}) registradas en la Cuenca del Río Boroa. *: Indican máximas abundancias en cada estación del año

	Winter	Spring	Summer	Autumn
Plecoptera				
Klapopteryx armillata	30	48	51	9
Notoperlopsis femina	9	39	207	219
Antarctoperla michaelseni	0	3	0	15
Limnoperla jaffueli	570*	21	972	1383*
Perlugoperla personata	9	3	45	0
Senzilloides panguipullii	12	0	0	0
Aubertoperla illiesi	12	0	0	0
Ceratoperla schwabei	0	0	0	9
Kempnyella genualis	0	3	24	3
Diamphipnopsis samali	0	0	12	6
Diamphipnoa helgae	0	0	0	9
Udamocercia sp.	0	0	0	3
Ephemeroptera				
Chiloporter eatoni	3	51	6	27
Andesiops peruvianus	36	63	975	366
Andesiops torrens	72	24	3	147
Hapsiphlebia anastomosis	3	78	174	6
Meridialaris diguillina	93	441*	993	960
Nousia maculata	0	51	4221*	33
Nousia delicata	9	0	0	0
Penaphlebia chilensis	0	0	102	0
Rhigotopus andinensis	0	0	3	0
Trichoptera				
Brachysetodes sp.	12	36	0	0
Hydrobiosidae n/i	12	21	42	33
Neochorema sinuatum	0	3	0	0
Parasericostoma ovale	3	3	21	6
Smicridea annulicornis	0	18	30	18
Austrotinodes sp.	0	0	9	0
Ecnomidae n/i	0	0	0	15
Hydroptilidae n/i	0	0	6	0
Oxyethira sp.	0	0	12	0
Dolophilodes sp.	0	0	3	0
Polycentropus sp.	0	0	12	3
Coleoptera				
Tychepsephenus felix	0	15	9	9
Austrolimnius sp.	0	231	498	312
Luchoelmis sp.	0	6	9	0
Austrelmis sp.	24	0	0	0
Curculionidae n/i	0	3	0	0
Haliplidae n/i	0	0	3	0
Diptera				
Alluaudomyia sp.	12	42	12	3
Stilobezzia sp.	6	0	0	78
Atherix sp.	3	96	9	0
Hemerodromia sp.	0	21	6	3
Hexatoma sp.	129	216	87	696
Limonia sp.	0	84	12	78
Blephariceridae	0	0	0	3
Gigantodax sp.	21	0	3	24
Muscidae n/i	0	0	3	0
Symbiocladius wygodzinskyi	0	12	18	0
Corynoneura sp.	6	66	327	0
Eukiefferiella sp.	9	81	552	135
Lopescladius sp.	30	39	0	6
Orthocladius sp.	0	51	72	42
Thienemanniella sp.	27	12	54	0
Acricotopus sp.	9	0	0	0
Pentaneura sp.	3	123	453	9
Coelotanypus mendax	0	0	12	0
Rheotanytarsus sp.	36	0	180	273
Tanytarsus sp.	0	3	510	66
Dicrotendipes sp.	27	0	0	96
Podonomus albinervis	6	0	0	0
Collembola				
Collembola n/i	0	3	0	0
Odonata				
Neogomphus sp.	0	3	39	9
Hemiptera				
Corixa sp.	0	3	0	0
Decapoda				
Aegla araucaniensis	24	108	141	18
Samastacus spinifrons	0	0	9	0
Acariformes				
Hydracarina n/i	0	0	3	0
Torrenticola sp.	9	0	0	3
Basommatophora				
Chilina dombeyana	24	6	27	9
Mesogastropoda				
Littoridina cumingi	27	9	12	51
Pulmonata				
Lymnaea viator	0	0	3	6
Veneroida				
Pisidium chilense	0	0	3	0
Tubificida				
Tubifex sp.	345	0	54	234
Annelida				
Naididae	0	0	0	12
Stratiodrilus aeglaphilus	3	0	0	0
Arhynchobdellida				
Mesobdella sp.	3	0	0	0
Temnocephalida				
Temnocephala chilensis	324	27	3	0
Tricladida				
Dugesia anceps	0	0	9	6

n/i : no identificado

Table 4. Physico-chemical characteristics of the water at the 5 sampling stations during the 4 seasons of the year in the Boroa River basin / Características físico-químicas del agua en las 5 estaciones de muestreo durante las 4 estaciones del año en la Cuenca del Río Boroa

		Temperature (°C)	Conduc. (μS cm^{-})	TDS (mg L^{-1})	pH	Susp. solids (mg L^{-1})	DO (mg L^{-1})	BOD5 (mg L^{-1})	PO_4^{-3} (μg L^{-1})	NO^{3-} (mg L^{-1})	True colour (Pt Co^{-1})	Apparent colour (Pt Co^{-1})	Turbidity (NTU)	Chlorides (mg L^{-1})	Sulphates (mg L^{-1})
St 1	Winter	10.5	26.6	27	6.39 ± 0.05	5.74 ± 0.99	11.0 ± 2.0	1.0 ± 0.3	11.0 ± 0.3	0.7 ± 0.3	11.6	17	3	11.9	1.34
	Spring	14.3	43.9	45	6.81 ± 0.04	2.13 ± 0.54	10.0 ± 2.1	0.9 ± 0.0	40.3 ± 0.9	0.4 ± 0.1	22	48	2.2	14	1.3
	Summer	20.6	47.7	48	6.90 ± 0.47	7.24 ± 2.03	8.9 ± 0.6	1.7 ± 0.4	18.6 ± 0.7	0.4 ± 0.0	34	63.1	2	15	0.82
	Autumn	8.7	39.1	39	6.26 ± 0.18	18.77 ± 1.64	11.0 ± 0.9	1.2 ± 0.2	9.3 ± 0.2	1.1 ± 0.0	18	53.1	4.2	10	2.5
St 2	Winter	11	14.4	15	6.11 ± 0.07	4.00 ± 0.80	10.5 ± 0.7	0.4 ± 0.1	10.1 ± 0.8	0.5 ± 0.0	9	33	1	10	0.8
	Spring	15.3	32.1	32	6.57 ± 0.08	1.91 ± 0.33	9.7 ± 1.0	0.5 ± 0.1	40.3 ± 1.9	< 0.2	11	59	1.7	9	1.3
	Summer	17.9	35.5	36	6.81 ± 0.49	1.58 ± 0.20	8.2 ± 0.3	0.9 ± 0.1	6.2 ± 1.0	0.8 ± 0.2	23	56.2	1.9	9.2	1.08
	Autumn	9.3	31.4	31	6.31 ± 0.00	3.79 ± 0.76	10.9 ± 0.7	1.2 ± 0.3	15.5 ± 1.1	0.8 ± 0.0	21	55.4	1.3	9	1.1
St 3	Winter	10.6	19.3	20	6.36 ± 0.11	2.60 ± 0.54	10.7 ± 0.0	0.3 ± 0.0	11.0 ± 3.0	0.3 ± 0.1	11	15	0.2	10.9	0.7
	Spring	15.3	31.8	32	6.54 ± 0.09	1.46 ± 0.33	8.4 ± 0.8	0.8 ± 0.1	40.3 ± 1.4	< 0.2	14	51	2	12	2.9
	Summer	20	38.3	38	6.47 ± 0.52	10.99 ± 1.87	8.5 ± 0.1	1.8 ± 0.3	21.7± 1.7	1.2 ± 0.2	31	61.6	2	12	0.85
	Autumn	9.7	28.2	29	6.07 ± 0.21	6.18 ± 1.12	11.3 ± 0.5	1.5 ± 0.2	24.8 ± 0.7	0.7 ± 0.2	21	55.4	3.1	10	1.1
St 4	Winter	10.5	20.3	21	6.41 ± 0.15	19.33 ± 2.65	10.8 ± 1.2	0.6 ± 0.2	10.7 ± 1.1	0.1 ± 0.0	22	14	0.5	10	0.7
	Spring	14.9	32.7	34	6.84 ± 0.23	2.13 ± 0.25	9.2 ± 0.7	0.8 ± 0.2	37.2 ± 1.1	< 0.2	9	45	1.2	11	1.2
	Summer	21.6	36.6	37	6.49 ± 0.56	0.74 ± 0.15	9.0 ± 0.2	< 0.2	9.3 ± 1.0	0.4 ± 0.1	28	60.2	< 0.2	14	0.81
	Autumn	8.8	29.3	30	6.23 ± 0.00	3.40 ± 0.08	10.0 ± 0.4	< 0.2	12.4 ± 0.9	0.8 ± 0.2	3	32.1	2.8	11	1.1
St 5	Winter	10.3	21.8	22	6.25 ± 0.03	6.17 ± 2.07	11.0 ± 2.0	1.2 ± 0.4	11.0 ± 1.7	0.5 ± 0.3	14	25	2.2	10.9	0.7
	Spring	14.5	36.3	37	6.69 ± 0.13	1.86 ± 0.86	9.9 ± 1.1	1.9 ± 0.5	40.3 ± 1.3	4.0 ± 0.5	12	49	1.8	11	1.4
	Summer	21.1	43.4	41	6.87 ± 0.35	4.50 ± 0.77	8.6 ± 0.4	1.3 ± 0.5	31.0 ± 2.2	1.6 ± 0.2	25	57.5	1	9.4	1.35
	Autumn	9.1	34.1	33	6.13 ± 0.15	5.27 ± 0.95	11.0 ± 0.8	1.2 ± 0.2	6.2 ± 0.5	0.8 ± 0.2	29	60.7	4	9	1.1

Our results, using physico-chemical variables as the principal parameter for classifying water quality, indicated exceptional water quality for the whole Boroa River basin according to the secondary environmental quality standard for the protection of Chilean continental waters (CONAMA 2004). The only parameter which exceeded the permitted limits for waters of exceptional quality was the apparent colour.

The first two axes of the principal component analysis (PCA), for the physico-chemical variables and the sampling stations, explained 57% of the accumulated variability, the first was related to the temperature and dissolved oxygen and the second to turbidity (Fig. 3).

The MDS analysis between sampling seasons found a similarity between the spring and summer seasons and between autumn and winter (Fig. 4).

INDEX BIOTIC

Over the course of the year the biotic water quality index (Hilsenhoff 1988) varied; for the coastal basin of the Boroa River water quality varied from excellent (< 3.5), *i.e.,* unlikely to present organic pollution, to good (4.51-5.50), *i.e.*, with some organic pollution probable. The highest values were recorded in winter at stations 3 and 4 (Table 5).

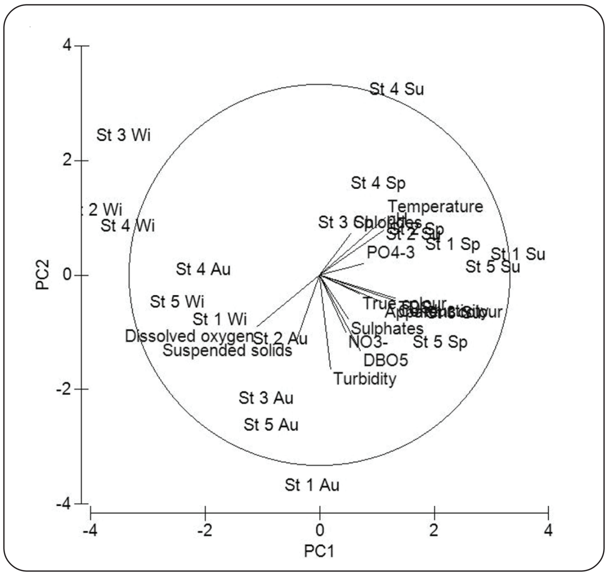

Figure 3. Results of the principal component analysis (PCA) for the sampling stations in the Boroa River basin during the 4 seasons of the year, based on the physico-chemical variables of the water (Wi: winter, Sp: spring, Su: summer, Au: autumn) / Resultados del análisis de componentes principales (PCA) de las estaciones de muestreo en la cuenca del Río Boroa durante las 4 estaciones del año, basados en las variables físico-químicas del agua (Wi: invierno, Sp: primavera, Su: verano, Au: otoño)

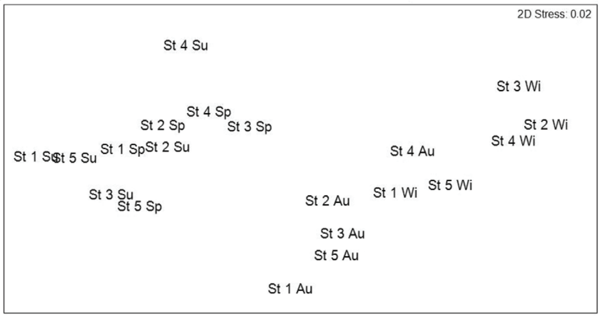

Figure 4. Results of the Multidimensional Scaling (MDS) for the sampling stations of the Boroa River basin during the 4 seasons of the year, based on the standardized physico-chemical variables and using Euclidian distances as measures of similarity (Wi: winter, Sp: spring, Su: summer, Au: autumn) / Resultados del Escalamiento Multidimensional (MDS) de las estaciones de muestreo de la cuenca del Río Boroa durante las 4 estaciones del año, basados en las variables físico-químicas estandarizadas y usando distancia Euclidiana como medida de similitud (Wi: invierno, Sp: primavera, Su: verano, Au: otoño)

Table 5. Modified Hilsenhoff biotic index values for the Boroa River at the 5 stations sampled during the 4 seasons of the year (E = Excellent, VG = Very good, G = Good) / Valores del Índice biótico de Hilsenhoff modificado para la cuenca del Río Boroa en las 5 estaciones de muestreo durante las 4 estaciones del año (E= Excelente, VG = Muy bueno, G = Bueno)

	Winter		Spring		Summer		Autumn	
Station 1	3.55	VG	3.32	E	3.12	E	4.51	G
Station 2	3.81	VG	3.87	VG	4.11	VG	4.4	VG
Station 3	5.15	G	4	VG	3.17	E	3.8	VG
Station 4	4.75	G	3.71	VG	3.65	VG	3.29	E
Station 5	-	-	4.3	VG	3.34	E	3.17	E

DISCUSSION

In the present study, 77 taxa of benthic macroinvertebrates were recorded from the Boroa River basin (12,910.73 ha). The basin had greater richness in comparison with other similar-sized basins located in southern Chile. For example, in the basin of the Peu Peu stream, 15,333.67 ha, 25 taxa were recorded in 5 stations (Leiva 2004); in the basin of the Cayucupil River, 12,200 ha, 36 taxa in 3 stations (Moya *et al*. 2009b). It may even be compared to a basin which is four times larger, the Damas River basin covering 51,400 ha, where 14 stations were sampled (Figueroa *et al*. 2003), showed the same number of taxa as was recorded in the Boroa basin. This high number of taxa may be due to the low level of anthropic intervention, since one of the factors causing the strongest negative effect on the abundance and diversity of aquatic invertebrates is the indiscriminate use of fertilizers, especially phosphate and nitrate, which contribute to an increase in eutrophication in the system, modifying biological conditions, reducing diversity and even causing the death of organisms (Arheimer *et al*. 2004, Alonso & Camargo 2005, Durán 2006, Martínez *et al*. 2007). The low levels of phosphates recorded in the Boroa River basin showed a negative correlation with abundance during the drier seasons, just as the low levels of nitrate showed a negative correlation with abundance in the wet seasons. However, Tawari-Fufeyin *et al*. (2008) described high levels of nutrients (phosphate and nitrate) in the rainy season corresponding with periods of high taxa richness in the Ossiomo River.

It is known that the abundance of many elevation-sensitive species diminishes from higher to lower elevations within basins (*e.g.*, Figueroa *et al*. 2003, 2007, Miserendino 2006). However, a few sensitive mayfly and stonefly species (Stoyanova *et al*. 2010) were also recorded in the lower parts of the basin. Giacometti & Bersosa (2006) in the Alambi River (Ecuador) and Bonada *et al*. (2000) in the Sant Cugat River basin (Spain) recorded communities of macroinvertebrates characteristic of clean waters in the lower-lying zones. These rivers have good self-cleaning mechanisms which allow survival of these communities. In the case of the Boroa River this situation was corroborated by the physico-chemical parameters, which indicated exceptional water quality throughout the basin. The most abundant taxa reaching the lowest areas were a gripopterygid stoneflies, *Limnoperla jaffueli*, and the Leptophlebiid mayflies *Nousia maculata* and *Meridialaris diguillina*. The latter order has been recorded in nearby water systems such as the Valdivia Coastal Reserve, in the Los Ríos Region (Guevara-Cardona *et al*. 2006) and the Itata River, Biobío Region (Habit *et al*. 1998), where it was the most dominant taxon.

The lower abundances of taxa recorded in spring may be due to factors related to the climate, physical habitat and the trophic bases of the water systems (Whiles *et al*. 2000), and an important factor to be considered is the season when insects emerge. In this respect there are records regarding bodies of water close to the Boroa River which show low synchronization in the flight season of Plecoptera (Caamaño 1985) and one Ephemeroptera (Gonser & Spies 1997); this conflicts with the results of Hollmand & Miserendino (2008), who mentioned that the majority of Plecoptera in southern Argentina presented defined periods of emergence (spring-summer), which agrees with our results.

In the Boroa River a significantly greater number of individuals and species were recorded in summer than in winter, in agreement with observations made in the rivers of Ecuador (Jacobsen & Encalada 1998, Giacometti & Bersosa 2006). Thus, the fact that a lower abundance of macroinvertebrates was recorded in winter may be due to the high rainfall, increased flow of the river and greater turbidity, the latter being one of the axes which contributed the most to explain the variability of the physico-chemical parameters. We suggest that this relationship should be studied further, with a closer analysis of the life cycles of aquatic insects.

One of the most commonly used biological methods employed to evaluate water quality is the use of benthic macroinvertebrates (Leunda *et al*. 2009), which allow for the detection of temporal episodes that occur during seasons and between sampling stations, that physico-chemical parameters cannot establish (Giacometti & Bersosa 2006, Figueroa *et al*. 2007). Thus, the combination of biological and physico-chemical methods proposed by Duran (2006) appears to be the best alternative for the determination of water quality. According to our physico-chemical analysis, the water quality results classified the Boroa River as of exceptional quality. Nevertheless, the apparent colour parameter in some stations exceeded the limit, possibly due to colloidal matter in suspension. The Biotic Index used proved to be a more accurate method than the one previously described, since it allowed us to define different water qualities that the physico-chemical method did not detect.

The variability of water quality given by the biotic index has been documented by various authors, and may be seasonal (Murphy 1978, Gratwicke 1999), or influenced by differences between sites (Zamora-Muñoz *et al*. 1995). Water quality values obtained through application the index biotic changed seasonally in the Boroa basin. In winter, physico-chemical parameters did not show any variation; however, the increased flow resulted in increased macroinvertebrate drift, contributing to the lower density, increasing the biotic index values and decreasing water quality, but always from very good to good. The opposite was found in summer, high abundance and diversity of taxa were recorded, with lower biotic index values indicating better water quality, ranging from very good to excellent. Thus, according to Linke *et al*. (1999), seasonality must be taken into account when using aquatic macroinvertebrates as bioindicators, since this phenomenon may influence the results of biomonitoring analyses. Certainly the seasonal variation in biotic index values in watersheds of southern Chile must be taken into account when designing water quality monitoring based on biotic indices.

Hopefully this investigation will inspire future workers to refine the application of biotic indices using benthic macroinvertebrates to monitor organic pollution in other river basins of southern Chile. This will require species level keys in the future to enable more sensitive biomonitoring studies. Periodic biomonitoring of organic contamination in river basins is needed to allow state environmental agencies to take steps to maintain the biotic integrity of streams and rivers in southern Chile.

ACKNOWLEDGMENTS

The authors wish to thank Raúl Arriagada and Eduardo Fernández for their assistance in the field; and Dr. Luciano Caputo for his comments on this work. This study was financed by FONDECYT Projects 1080317 and 1110798. The authors would like to thank the editor and two anonymous reviewers for helpful comments on an early version of this manuscript and assistance provided by DID (Dirección de Investigación de Desarrollo) of the Universidad Austral de Chile.

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Appendix 1. Tolerance values (TV) of benthic macroinvertebrates of the Boroa River, Chile used for the Family Biotic Index (FBI), according to Barbour *et al.* (1999) and Mandaville (2002) / Valores de tolerancia (TV) de los macroinvertebrados bentónicos del Río Boroa, Chile usados para el Índice Biótico de Familias (IBF), siguiendo a Barbour *et al.* (1999) y Mandaville (2002)

	Species	TV
Plecoptera		
Austroperlidae	*Klapopteryx armillata*	0
Grypopterygidae	*Notoperlopsis femina*	0
	Antarctoperla michaelseni	1
	Limnoperla jaffueli	2
	Perlugoperla personata	1
	Senzilloides panguipullii	1
	Aubertoperla illiesi	0
	Ceratoperla schwabei	1
Perlidae	*Kempnyella genualis*	0
Diamphipnoidae	*Diamphipnopsis samali*	0
	Diamphipnoa helgae	0
Notonemouridae	*Udamocercia* sp	0
Ephemeroptera		
Ameletopsidae	*Chiloporter eatoni*	2
Baetidae	*Andesiops peruvianus*	4
	Andesiops torrens	5
Leptophlebiidae	*Hapsiphlebia anastomosis*	2
	Meridialaris diguillina	4
	Nousia maculata	3
	Nousia delicata	3
	Penaphlebia chilensis	4
	Rhigotopus andinensis	2
Trichoptera		
Leptoceridae	*Brachysetodes* sp.	4
Hydrobiosidae	Hydrobiosidae	3
	Neochorema sinuatum	3
Sericostomatidae	*Parasericostoma ovale*	5
Hydropsychidae	*Smicridea annulicornis*	5
Ecnomidae	*Austrotinodes* sp.	5
	Ecnomidae	5
Chironomidae	*Corynoneura* sp.	4
	Eukiefferiella sp.	4
	Lopescladius sp.	5
	Orthocladius sp.	6
	Thienemanniella sp.	6
	Acricotopus sp.	10
	Pentaneura sp.	5
	Coelotanypus mendax	4
	Rheotanytarsus sp.	6
	Tanytarsus sp.	6
	Dicrotendipes sp.	8
	Podonomus albinervis	1
Collembola		
Collembola	Collembola	5
Odonata		
Gomphidae	*Neogomphus* sp.	5
Hemiptera		
Corixidae	*Corixa* sp.	4
Decapoda		
Aeglidae	*Aegla araucaniensis*	5
Parastacidae	*Samastacus spinifrons*	5
Acariformes		
Hydracarina n/i	Hydracarina	6
Torrenticolidae	*Torrenticola* sp.	6
Basommatophora		
Chilinidae	*Chilina dombeyana*	7

Received 15 September 2011 and accepted 19 January 2012