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Classification of the ecological quality of the Aysen and Baker Fjords (Patagonia, Chile) using biotic indices

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ABSTRACT

The AZTI's marine biotic index (AMBI), an ecological indicator for managing estuarine and coastal waters worldwide, was tested in two fjords in Chilean Patagonia. The Aysen Fjord (42° Lat. S) supports intensive salmon farming in coastal ecosystems, while the Baker Fjord (48° Lat. S) is currently just beyond the limit of the southern expansion of salmon concessions. The ecological status of the Aysen Fjord was classified as good, while the status of the Baker Fjord was classified as high and unbalanced. These differences were consistent with our expectations, illustrating the effect of local environmental conditions and human activities, combined with river inputs into semi-confined fjords. This method is appropriate for the evaluation of the ecological status of the fjords, but requires a sufficient amount of data for the robust environmental assessment as proposed by the Water Framework Directive (WFD).

1. Introduction

Macrobenthic invertebrate communities are broadly recognized as indicators for environmental impact on marine and estuarine systems (e.g. Borja et al., 2000; Diaz et al., 2004; Pinto et al., 2009; Van Hoey et al., 2010; Teixeira et al., 2012). Macrobenthic invertebrate communities are sensitive to accumulated organic matter and/or many contaminants, because they are relatively immobile residents in sediments (Pearson and Rosenberg, 1978; Teixeira et al., 2012). Univariate diversity indices such as the Shannon diversity, species evenness and other community indices are insufficient to distinguish between the changes produced by natural disturbance and those produced by anthropogenic factors (Warwick and Clarke, 1993). Traditional environmental assessment studies on an undisturbed community at a particular locality and a disturbed one in another locality might show the same level of diversity (Muniz et al., 2005). These metrics are often non-intuitive, and confuse the interpretation of environmental impact and consequent effects, thus making marine quality monitoring difficult for non-scientists, regulators or policymakers (Muniz et al., 2005).

Development and testing of classification schemes and biotic indicators for assessing the ecological quality (EcoQ) of coastal waters have been a major investment in recent years (e.g. Simboura and Zenetos, 2002; Diaz et al., 2004; Pinto et al., 2009; Borja et al., 2008, 2009a, 2009b; Muxika et al., 2005, 2011). One extensively applied index that produces good results is the AZTI's marine biotic index (AMBI) developed by Borja et al. (2000). This index is based on the assignment of taxa observed during a survey to certain ecological groups. Five ecological groups, ranging from sensitive species to first-order opportunistic species, were established based on empirical data from various studies and also on general expert experience (Borja et al., 2000; Muxika et al., 2005, 2011). This index has been successfully applied for detecting and assessing different sources of impacts on marine systems worldwide, including also negative effects of aquaculture on benthic systems (e.g. Muxika et al., 2005; Tomassetti et al., 2009; Forchino et al., 2011).

A new index, the Multivariate-AMBI (M-AMBI) was recently developed in response to the Water Framework Directive (WFD 2000/60/EC) requirements, to include additional metrics addressing benthic community integrity, and parameters that better define environmental quality of marine systems (Borja et al., 2004, 2009a; Muxika et al., 2007; Pinto et al., 2009). The M-AMBI is an extension of the AMBI (Muxika et al., 2007), which incorporates species richness and Shannon–Wiener ($\log_2$) diversity values. The M-AMBI is the outcome of inter-calibration efforts among members of the WDF to develop common methodologies. It has been applied to various systems outside Europe, e.g. in the United States, where it was demonstrated to provide consistently high agreement with local indices (Borja et al., 2008).

Salmon aquaculture in coastal marine systems may be a major potential source of environmental impact, including organic enrichment of sediments, reduced availability of oxygen, reduction

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of the biodiversity and changes in the community structure of the benthic macrofauna (e.g. Brown et al., 1987; Gowen and Bradbury, 1987; Weston, 1990; GESAMP, 1991; Macleod et al., 2004; Wildish et al., 2005; Edgar et al., 2005; Kalantzi and Karakassis, 2006; Wildish and Pohle, 2005; Hargrave et al., 2008; Hargrave, 2010; Neofitou et al., 2010; Villnäs et al., 2011). These effects are clear examples how farmed production can exceed local carrying capacity, i.e. the ability of the ecosystem to assimilate the waste generated. The subsequent ecological changes limit the long term viability of continuous aquaculture at any given locality (MacLeod et al., 2004; Wildish and Pohle, 2005). The impacts from organic enrichment are reduction in diversity, increase in opportunistic and pollution tolerant species and finally the complete absence of sensitive taxa (Brown et al., 1987; Gowen and Bradbury, 1987; Weston, 1990). The same stressors but also the release of hydrogen sulfide gas and accumulation of organic matter are also recognized as mechanisms responsible for the deterioration of the health of farmed fish and reduction or even loss of production (GESAMP, 1991; Hargrave et al., 2008).

Chilean salmon aquaculture began in the early 1980s, and production has grown exponentially to the presently second largest salmon producer worldwide after Norway (Buschmann et al., 2009). Even though it may be argued that the Chilean salmon industry has reached consolidation levels, its growth has not been without consequences, such as the 2007 outbreak of infectious salmon anemia (ISA). As part of the recovery pattern, the industry is looking for new areas in southern Chile, including pristine Patagonian coastal fjords. In the remote Aysen Region of southern Chile (45°S), salmon production meanwhile has advanced and contributes 80% to Chile's total salmon output – two decades ago aquaculture was almost non-existent there (Buschmann et al., 2006). The Baker Fjord is currently just beyond the limit of the southern expansion of salmon concessions. Future salmon concessions, however, are also planned for currently unoccupied channels and fjords in as far as the twelfth region of Chile (52°S), a likely result of the combined effects of growth and recovery of the industry (Gonzalez de la Rocha, 2008).

There are in fact comprehensive laws and regulations dealing with coastal areas and aquaculture, including environmental regulations from the National Marine Fisheries Service (SERNAPESCA), specific dispositions derived from Environmental Regulation for Aquaculture (RAMA, 2001) and the Secondary Environmental Quality Standards (SEQSs) for protection of marine and estuarine regions. In addition, an integrated coastal zone management (ICZM) has also been established, but this concept of integrated assessment is not widely applied (Tironi et al., 2008, 2010; Barton and Fløysand, 2010). The regulatory framework in Chile has not developed the sophistication to monitor, evaluate and manage impacts in an effective manner as comparable to elsewhere in the world. In general, environmental regulations are not based on empirical assessment of impacts (Buschmann et al., 2009). Investigations on the effects of salmon farming on the benthic systems, especially in southern Chile, have been restricted to only few locations (Soto and Norambuena, 2004; Mulsow et al., 2006; Quiroga, 2009). The regulatory system requires a shift from local or regional-based regulations to ecosystem-based environmental management (Soto et al., 2008; Aguilar-Manjarrez et al., 2010), based on models applied also in other regions of the world.

This study tests the application of AMBI and M-AMBI in the Aysen and Baker Fjords in Chilean Patagonia. The general goal is to classify the ecological status of the soft-bottom macrobenthic communities and sediment properties. The results will provide a proper baseline for future monitoring studies in Chilean coastal waters.

2. Methods

Sediment conditions and macrobenthic communities were studied at six stations in the Aysen Fjord (Fig. 1) in October 2009 and five stations in July 2010. In the Baker Fjord, three stations were sampled in June, September and November 2008 and in February 2009. The stations were placed along the fjords in order to sketch the ecological processes taking place in the study area. Macrofaunal samples were taken using a 0.1 m^2 van Veen grab. Faunal groups not properly sampled by this method such as nematodes and foraminifers were not included in the analysis. In soft-sediment studies a single grab will represent only a small fraction of the species diversity at a site because of small-scale spatial variability. Hence 2–6 replicate grabs from each benthic station were pooled for the data analyses of species abundance and biomass (Ellingsen, 2002). The sediment samples were sieved through a 500-μm mesh size screen and the biological material was fixed in a 10% buffered formaldehyde–seawater solution.

Sediment samples were collected from independent replicates using a gravity corer (ID = 50 mm). The whole samples were kept frozen (−20 °C) prior to analyses of total organic matter (TOM), total organic carbon (TOC), chlorophyll (Chl-a) and phaeopigments (Phaeop). Sediment grain size analyses were performed on the top 5 cm of the sediment cores using nested geological sieves following the procedures described by Folk (1974). Total organic matter (TOM) was obtained by means of the calcination method using a muffle furnace (Luczak et al., 1997). Total organic carbon (TOC) content of the surface sediments was performed on freeze-dried and homogenized sediment samples, decalcified with 1 N HCl, dried on a hot plate at 40 °C, and measured in a CHN elemental analyzer (LECO, model Truspec CHN).

Chl-a and Phaeop (i.e. phytopigment degradation product) were analyzed fluorometrically (Montani et al., 2003). Pigments registered with this method are denominated chloroplastic pigment equivalents (CPE, Gutiérrez et al., 2000). Independent replicates using a gravity corer were analyzed for total sulfide content with an Ag/Ag sulfide electrode and a combination reference electrode against a sodium sulfide standard (Hargrave et al., 1995; Wildish et al., 2001). Sediment redox potential (Eh_{NHE}) was measured for the 0–2 cm surface sediment layer using a platinum standard combination electrode with a calomel internal reference (SG™, Mettler Toledo). Carbon stable isotope ratio ($\delta^{13}C$) was analyzed at the CCHEN Laboratory, Chile (Silva et al., 2011a).

The benthos was preserved in the laboratory in 70% alcohol. The organisms were later extracted, counted and identified to the highest possible taxonomic level (usually species). The similarity matrix was calculated using the Bray Curtis index. Hierarchical clustering analysis and similarity profile permutation test (SIMPROF) were used to delineate macrobenthic assemblages of respective sampling stations (Clarke et al., 2008). The permutation test SIMPROF (5% level) was used to determine clusters with a significant internal structure. SIMPER analyses were performed to describe the contribution of species to similarities within and dissimilarities between station groups. Analyses were done using PRIMER-6 software (Plymouth, UK; Clarke and Gorley, 2006).

The macrobenthic community was classified following Ortiz and Quiroga (2010) and Quiroga et al. (2012). Taxon/species abundance data were used to calculate AMBI and M-AMBI using AMBI software (version 4.1, http://ambi.azti.es, Borja et al., 2000; Muxika et al., 2011). Taxa were classified into five ecological groups:

- *Ecological group I (EGI)* Species very sensitive to organic enrichment, present under unpolluted conditions (initial state). These include suspension-feeders, less selective carnivores and scavengers.

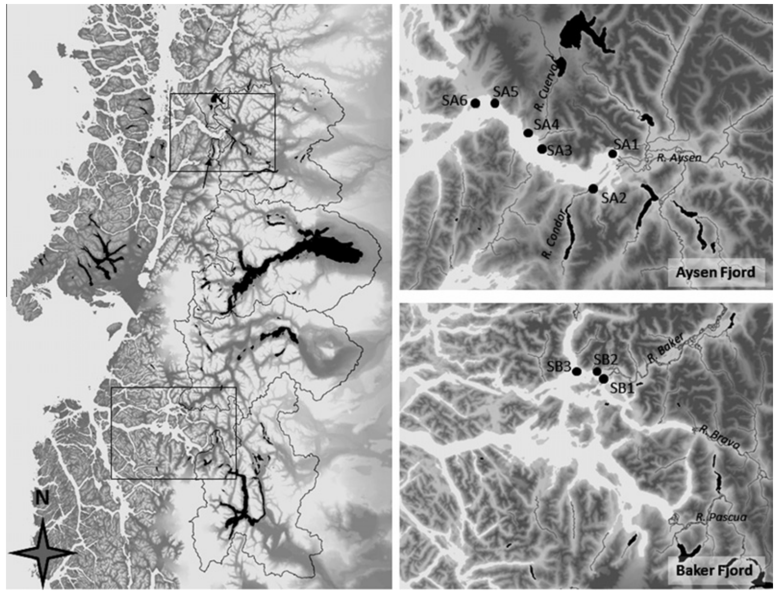


Fig. 1. Study area and sampling stations for the Aysen Fjord (SA) and Baker Fjord (SB), Chilean Patagonia (45°14′S–47°47′S).

- *Ecological group II (EGII)* Species indifferent to enrichment, always present in low densities with non-significant variation over time.
- *Ecological group III (EGIII)* Species tolerant to excess organic matter enrichment. These species may be present under normal unimpacted conditions, but their populations are stimulated by organic enrichment (slightly unbalance conditions). Examples include surface deposit-feeders species such as spionidae.
- *Ecological group IV (EGIV)* Second-order opportunistic species (slightly to pronounced unbalanced conditions). Examples include small-sized polychaetes and sub-surface deposit- feeders such as cirratulids.
- *Ecological group V (EGV)* First-order opportunistic species (pronounced unbalanced conditions). Typical taxa include deposit-feeders which proliferate in sediments under reducing conditions.

The distribution of these ecological groups as a function of their sensitivity to pollution stress results in a biotic index consisting of seven levels (Borja et al., 2000): normal (0), undisturbed (<1.2), slightly disturbed (1.2–3.3), moderately disturbed (3.3–5), heavily disturbed (5–6) and extremely disturbed (>6) and azoic sediments (7). The reference values adopted for the M-AMBI calculation were described by Teixeira et al. (2012). Five categories on this M-AMBI scale were established by the European Water Framework Directive intercalibration exercise: high quality (>0.77), good (0.77–0.53), moderate (0.53–0.38), poor (0.38–0.20), and bad (<0.20).

Both Fjords receive significant freshwater inflow from the Aysen and Baker Rivers, presenting a possible challenge in the implementation of the standard methodologies. The characterization of the riverine export of suspended sediments and organic matter to the Aysen and Baker Fjords was based on monthly grab samples bracketing the respective sampling periods. Water samples were filtered and dried on glass fiber filters and the suspended sediments were weighed, while organic matter was processed as described above. The suspended sediment concentration and fine organic matter concentration (FPOM) were generated using hourly discharge data (Dirección General de Aguas, Gobierno de Chile, www.dga.cl; Quiroga et al., 2012).

The relationships between biotic indices (i.e. AMBI, BI and M-AMBI) and environmental variables (i.e. TOM, TOC, CPE, Chl-a content and sulfide content) were calculated using an analysis of covariance (ANCOVA). This analysis was proposed in order to distinguish the differences between fjords influenced by freshwater input from rivers, because strong trends between indices and environmental covariates may be evident but obscured by fjord differences. The ANCOVA was carried out using the Statistica 7.0 StatSoft, Inc. software for combined data sets (i.e. Aysen and Baker Fjords).

3. Results

Aysen Fjord sediments were generally classified as sand-clays, with <5.0% of TOM content, but at stations SA1, SA3 and SA4, the slightly higher TOM content ranged from 5.57% to 11.90% (Table 1). TOC and Chl-a were lower at station SA5 (0.5–3.4% TOC, 79.7 $\mu g\ g^{-1}$ Chl-a) than the station SA1 (10.5–10.6% TOC, 715.8 $\mu g\ g^{-1}$ Chl-a). In addition, CPE varied between 10.8% at station SA6 and 25.1% at station SA1. In the Baker Fjord, surface sediment was less heterogeneous, characterized by consistently low TOM (<3.0%) and TOC (<0.86%). Chl-a content varied between 31.4 $\mu g\ g^{-1}$ at station SB3 (June 2008) and 127.0 $\mu g\ g^{-1}$ at station SB1 (February 2009).

In the Aysen Fjord the carbon stable isotope composition ($\delta^{13}C$) of the surface sediment samples varied between −24.7‰ at station SA6 and −27.9‰ at station SA3 (Table 1). In the Baker Fjord, the carbon stable isotope composition of the sediment was highest at station SB1 (−27.1‰) and lowest at station SB3 (−26.4‰), indicating a high contribution of terrestrial organic matter into the head of the fjords (Table 1).

In October macrofauna density in the Aysen Fjord was highest at station SA1 (5010 ± 1859 ind. m^{-2}) and lowest at SA4 (896 ± 297 ind. m^{-2}), while in July the highest macrofauna density occurred at station SA3 (10603 ± 3591 ind. m^{-2}) and the lowest at SA1 (2875 ± 297 ind. m^{-2}; Table 1). We did not observe any significant differences in macrofauna densities between October and July stations (ANOVA; $p > 0.05$). In the Baker Fjord the macrofauna density tended to increase with increasing distance from the head to the mouth of the fjord (Table 1). Highest densities occurred at the outer station SB1 (14034–17800 ind. m^{-2}), intermediate densities at SB2 (650–6641 ind. m^{-2}) and the lowest at SB3

Table 1
Benthic sediment parameters and macrobenthos sample information. TOM: Total organic matter, TOC: Total organic carbon, Chl-a: Chlorophyll-a, CPE: Chloroplastic pigment equivalents.

Location	Station	Sampling date	Latitude (°S)	Longitude (°W)	Depth (m)	Sand (%)	Clay (%)	TOM (±S.D) (%)	TOC (%)	Chl-a (±S.D) (μg g^{-1})	Chl-a in CPE (%)	Sulfide (μM)	Eh$_{NHE}$ (mV)	Species number	Mean density (ind. m^{-2})
Aysen Fjord															
Bahía Acantilada	SA1	16.10.2009	45°23′45	72°48′25	47	65.9	33.4	10.63 ± 0.54	3.36	–	–	393.3	38	15 ± 1	5010 ± 1859
Caleta Bluff	SA2	16.10.2009	45°28′21	72°52′37	26	97.3	1.7	4.17 ± 0.52	1.24	–	–	119.9	80	29 ± 2	3875 ± 573
Río Cuervo	SA3	15.10.2009	45°21′14	73°03′35	50	89.3	10.0	9.52 ± 0.76	2.82	–	–	42.8	41	23 ± 1	2943 ± 410
Punta Tortuga	SA4	15.10.2009	45°19′28	73°05′36	35	80.6	19.2	11.52 ± 0.46	2.56	–	–	89.2	79	16 ± 4	896 ± 297
Caleta Pérez	SA5	14.10.2009	45°14′34	73°13′36	36	88.7	11.2	3.12 ± 0.14	0.53	–	–	98.7	208	28 ± 1	4902 ± 1278
Cinco Hermanas	SA6	14.10.2009	45°16′03	73°14′37	50	77.3	21.9	2.13 ± 0.16	1.05	–	–	39.2	189	26 ± 6	4107 ± 1444
Bahía Acantilada	SA1	26.07.2010	45°23′45	72°48′25	47	49.6	49.9	10.47 ± 0.14	3.14	715.75 ± 238.37	25.1	–	35	17 ± 1	2875 ± 389
Rio Cuervo	SA3	26.07.2010	45°21′14	73°03′35	50	79.0	19.7	11.90 ± 0.38	2.48	261.57 ± 163.70	14.6	–	–	24 ± 1	10603 ± 3591
Punta Tortuga	SA4	25.07.2010	45°19′28	73°05′39	35	80.4	17.1	5.57 ± 0.03	1.54	132.40 ± 87.57	13.9	–	159	26 ± 6	9822 ± 2161
Caleta Pérez	SA5	24.07.2010	45°14′34	73°13′36	36	56.8	43.0	2.02 ± 0.02	0.59	79.67 ± 12.86	10.9	–	184	19 ± 1	4292 ± 929
Cinco Hermanas	SA6	24.07.2010	45°16′03	73°14′37	50	84.8	13.5	1.90 ± 0.03	0.55	140.50 ± 12.94	10.8	–	84	29 ± 2	6300 ± 676
Baker Fjord															
Rio Baker	SB1	29.06.2008	47°47′44	73°35′25	40	87.67	0.48	3.38 ± 0.04	0.74	70.39 ± 0.49	39.1	–	–	22 ± 8	14034 ± 8439
Rio Baker, Bajo Pisagua	SB2	29.06.2008	47°47′05	73°35′21	30	83.78	0.41	3.50 ± 0.04	0.86	65.81 ± 2.05	34.2	–	–	29 ± 1	10184 ± 1016
Rio Baker, Bajo Pisagua	SB2	08.09.2008	47°47′19	73°35′51	29	77.99	0.50	3.38 ± 0.02	0.64	44.75 ± 11.20	27.5	–	–	14 ± 4	650 ± 567
Rio Baker	SB1	28.11.2008	47°47′53	73°35′05	48	86.57	1.11	4.29 ± 0.08	0.84	120.51 ± 15.07	41.0	–	–	23 ± 3	12684 ± 4504
Rio Baker, Bajo Pisagua	SB2	28.11.2008	47°47′10	73°35′23	40	96.52	0.12	3.71 ± 0.05	0.78	98.01 ± 13.53	44.9	–	–	25 ± 4	6084 ± 1895
Rio Baker	SB1	24.02.2009	47°47′53	73°35′05	54	–	–	3.41 ± 0.03	0.68	127.02 ± 10.50	49.7	–	–	28 ± 3	17831 ± 9054
Rio Baker, Bajo Pisagua	SB2	24.02.2009	47°47′10	73°35′23	48	–	–	3.09 ± 0.08	0.62	67.74 ± 14.78	35.0	–	–	26 ± 3	6641 ± 629
Punta Raúl	SB3	28.06.2008	47°46′27	73°39′35	34	95.04	0.06	3.03 ± 0.06	0.48	31.44 ± 7.03	25.7	–	–	14 ± 5	744 ± 925
Punta Raúl	SB3	08.09.2008	47°46′48	73°39′29	66	89.79	0.13	3.31 ± 0.08	0.48	57.30 ± 4.54	31.3	–	–	6 ± 2	95 ± 49
Punta Raúl	SB3	26.11.2008	47°46′27	73°39′36	60	90.98	0.04	3.35 ± 0.11	0.45	51.77 ± 2.61	26.0	–	–	9 ± 3	144 ± 88
Punta Raúl	SB3	23.02.2009	47°46′27	73°39′15	58	39.60	0.50	3.37 ± 0.01	0.43	35.02 ± 7.67	23.9	–	–	7 ± 3	82 ± 37

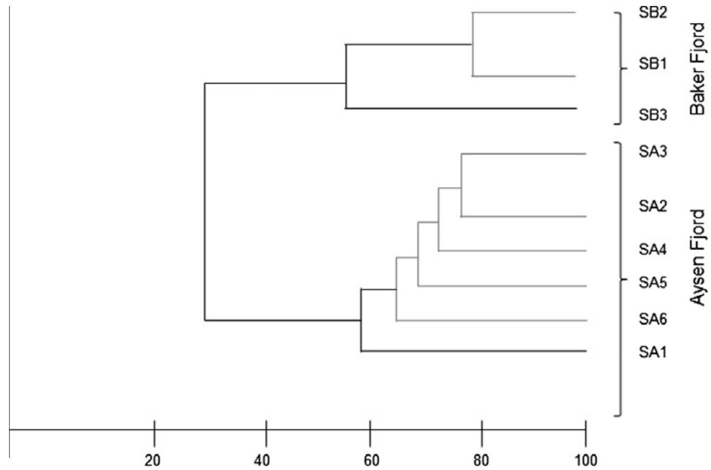
Location	Station	Sampling	d^{13} C (‰) date	Alloch. (%)
Aysen Fjord				
Bahia Acantilada	SA1	26.07.2010	−27.72	96.5
Caleta Bluff	SA2	16.10.2009	−25.95	74.4
Río Cuervo	SA3	26.07.2010	−27.93	99.1
Punta Tortuga	SA4	25.07.2010	−27.48	93.5
Caleta Pérez	SA5	24.07.2010	−25.65	70.6
Cinco Hermanas	SA6	24.07.2010	−24.69	58.6
Baker Fjord				
Rio Baker	SB1	03.08:2010	−27.08	88.5
Rio Baker, Bajo Pisagua	SB2	05.08.2010	−26.74	84.3
Punta Raúl	SB3	03.08.2010	−26.39	79.9

Table 2
Pooled data of species composition, mean density, standard deviation (SD), and percent of total macrofauna for the whole study period.

Taxa		Aysen Fjord			Baker Fjord			
		Mean density		%		Mean density		%
		(ind. m^{-2})	SD			(ind. m^{-2})	SD	
Polychaeta	*Capitella* sp A	1839.3	2034.4	36.4	*Aricidea antarctica*	3815.0	5338.4	45.6
	Prionospio sp A	557.3	527.6	11.0	*Levinsenia antarctica*	1486.6	1800.4	17.8
	Aphelochaeta sp A	460.1	509.2	9.1	*Capitella* sp A	1067.7	1090.5	12.8
	Aricidea antarctica	274.8	214.4	5.4	*Aphelochaeta marioni*	243.6	380.7	2.9
	Paramphinome australis	179.2	205.6	3.5	*Cossura* sp A	217.9	225.3	2.6
	Cossura sp A	179.1	356.0	3.5	*Aglaophamus peruana*	158.9	194.7	1.9
	Ampharete kerguelensis	154.8	288.7	3.1	*Prionospio* sp A	87.3	110.6	1.0
	Aglaophamus peruana	119.8	123.3	2.4	Lumbrineridae sp A	50.9	61.4	0.6
	Exogone sp A	111.5	231.4	2.2	*Sphiophanes soederstroemi*	43.9	87.2	0.5
	Lumbrineridae sp A	93.0	96.3	1.8	Terebellidae sp A	40.0	57.1	0.5
	Levinsenia antarctica	60.3	68.9	1.2	Hesionidae sp A	38.8	56.3	0.5
	Sabellidae sp A	41.8	94.3	0.8	*Leanira quatrefagesi*	38.4	44.5	0.5
	Hesionidae sp A	41.4	42.9	0.8	*Paramphinome australis*	35.4	50.9	0.4
	Polynoidae sp A	37.1	62.2	0.7	*Terebellides* sp A	23.9	37.7	0.3
	Glycera sp A	36.4	75.5	0.7	*Lumbrineris cingulata*	23.0	27.2	0.3
	Dorvilleidae sp A	31.3	75.7	0.6	*Pettiboneia* sp A	19.1	30.2	0.2
	Pettiboneia sp A	21.5	64.8	0.4	*Ninoe falklandica*	18.9	23.6	0.2
	Terebellidae sp A	19.2	35.1	0.4	Sabellidae sp A	13.9	31.9	0.2
	Ampharetidae sp A	18.6	37.0	0.4	Ampharetidae sp A	7.5	11.8	0.1
	Nereidae sp A	16.9	18.2	0.3	*Maldane sarsi*	5.5	12.9	0.1
	Maldanidae sp A	11.1	18.1	0.2	Ophelidae sp A	5.0	9.5	0.1
	Cistenides ehlersi	10.9	14.7	0.2	*Ammotrypane* sp	4.5	9.3	0.1
	Leanira quatrefagesi	10.8	22.4	0.2	*Nereis* sp A	2.7	7.3	<0.1
	Terebellidae sp B	8.3	19.3	0.2	Polynoidae sp	2.7	5.9	<0.1
	Ninoe falklandica	7.3	27.1	0.1	*Nereis eugeniae*	2.3	8.7	<0.1
	Phyllodocidae sp A	5.8	11.4	0.1	*Artacama valparaisiensis*	1.6	5.0	<0.1
	Terebellides sp A	4.0	13.2	0.1	*Aphelochaeta* sp B	1.3	8.1	<0.1
	Sphaerodoridae sp A	2.1	6.1	<0.1	Gonianidae sp A	1.3	3.8	<0.1
	Gonianidae sp A	1.5	5.4	<0.1	*Laonice weddellia*	0.7	2.6	<0.1
	Artacama valparaisiensis	0.6	2.7	<0.1	*Prionospio ehlersi*	0.5	4.0	<0.1
	Sternaspis scutata	0.4	1.7	<0.1	Glyceridae sp A	0.5	3.0	<0.1
	Orbiniidae sp A	0.4	1.7	<0.1	*Schistomeringos longicornis*	0.4	1.9	<0.1
					Sphaerodoridae sp A	0.4	1.9	<0.1
					Drilonereis sp A	0.2	1.3	<0.1
					Phyllodocidae sp A	0.2	1.3	<0.1
					Magelonidae sp A	0.2	1.3	<0.1
					Pilargidae sp A	0.2	1.3	<0.1
					Pisionidae sp A	0.2	1.3	<0.1
Crustacea	Ostracoda sp A	120.5	173.1	2.4	Pseudocumatidae sp A	20.2	79.0	0.2
	Cumacea sp A	37.2	72.9	0.7	Ostracoda sp B	16.8	28.9	0.2
	Amphipoda unidentified B	14.4	26.1	0.3	Phoxocephalidae sp A	15.2	19.7	0.2
	Phoxocephalidae sp A	13.5	26.7	0.3	Amphipoda unidentified A	8.9	18.7	0.2
	Cumacea sp B	10.2	46.0	0.2	*Cirolana albinota*	2.7	12.4	<0.1
	Amphipoda unidentified C	3.5	11.9	0.1	Amphipoda unidentified B	0.9	3.5	<0.1
	Amphipoda unidentified A	3.4	8.7	0.1	Ianiridae sp A	0.7	3.2	<0.1
	Cirolana albinota	2.4	9.0	<0.1	Bodotriidae sp A	0.5	2.3	<0.1
	Gomeza serrata	0.9	3.1	<0.1	*Gomeza serrata*	0.2	1.3	<0.1
Mollusca	*Thyasira patagonica*	237.0	233.9	4.7	*Thyasira patagonica*	680.7	795.6	8.1
	Macoma sp A	136.4	109.1	2.7	*Macoma* sp A	27.5	63.0	0.3
	Yoldiella sp A	9.3	21.7	0.2	*Yoldiella* sp A	20.0	29.8	0.2
	Bivalvia unidentified A	9.1	27.6	0.2	Scaphopoda sp B	11.3	28.0	0.1
	Nuculidae sp A	2.7	8.5	0.1	*Eunucula* sp A	2.0	7.2	<0.1
	Tindariidae sp A	1.1	3.8	<0.1	*Cuspidaria* sp A	0.2	1.3	<0.1
	Carditidae sp A	0.4	1.7	<0.1	*Chaetoderma* sp A	0.2	1.3	<0.1
	Caecum chilensis	0.4	1.7	<0.1	Bivalvia unidentified B	0.2	1.3	<0.1
	Scaphopoda sp A	0.4	1.7	<0.1				
Others	Nemertea sp A	74.9	67.8	1.5	Nemertea sp A	48.2	110.2	0.6
	Priapulidae sp A	12.7	19.6	0.3	Nemertea sp B	40.7	54.0	0.5
	Sipuncula sp A	3.2	14.9	0.1	Priapulidae sp A	4.5	8.9	0.1
	Nemertea sp B	2.6	12.2	0.1	Nemertea sp C	2.9	7.3	<0.1
	Ophiuroidea sp A	1.7	5.9	<0.1	Nemertea sp F	2.1	11.2	<0.1
	Holothuroidea sp A	1.3	3.5	<0.1	Sipuncula sp A	1.1	5.6	<0.1
	Schizasteridae sp A	0.6	2.7	<0.1	Nemertea sp D	0.5	2.3	<0.1
	Priapulidae sp B	0.4	1.7	<0.1	Nemertea sp E	0.2	1.3	<0.1
					Schizasteridae sp B	0.2	1.3	<0.1

(82–744 ind. m^{-2}). These differences among stations were significant (ANOVA, $p < 0.05$ and *a posteriori* test).

A total of 58 and 64 macrofauna species/groups were recorded in Aysen Fjord and Baker Fjord, respectively (Table 2). In general, polychaetes were dominating (>38 species), followed by crustaceans (>9 species) and molluscs (>9 species), including species of the genus *Chaetoderma* and of Scaphopoda. The community in the Aysen Fjord was dominated by seven polychaete species



		SA1	SA2	SA3	SA4	SA5
Aysen Fjord (68.76% similarity)	SA2	*Levinsenia antarctica* *Aphelochaeta* sp A *Phoxocephalidae* sp A				
	SA3	*Sabellidae* sp A *Aphelochaeta* sp A *Aglaophamus peruana*	*Phoxocephalidae* sp A *Aglaophamus peruana* *Maldane sarsi* *Thyasira patagonia* *Cossura* sp A *Sigalonidae* sp A			
	SA4	*Aglaophamus peruana* *Ampharete kerguelensis* *Terebellidae* sp A	*Aglaophamus peruana* *Maldane sarsi* *Exogone* sp A	*Aglaophamus peruana* *Sigalonidae* sp A		
	SA5	*Aglaophamus peruana*	*Aglaophamus peruana* *Paramphinome australis*	*Paramphinome australis* *Exogone* sp A	*Exogone* sp A	
	SA6	*Ampharete kerguelensis* *Exogone* sp A	*Ampharete kerguelensis*	*Exogone* sp A *Cossura* sp A	*Exogone* sp A	*Glycera* sp A *Pettiboneia* sp A

		SB1	SB2
Baker Fjord (64.57% Similarity)	SB2	*Aricidea antarctica* *Levinsenia antarctica*	
	SB3	*Aricidea antarctica* *Levinsenia antarctica* *Capitella* sp A	*Aricidea antarctica* *Levinsenia antarctica* *Thyasira patagonia* *Capitella* sp A

Fig. 2. Cluster diagram using the Bray-Curtis similarity index (Gray lines indicate groups of samples not separated by SIMPROF at $P < 0.05$), and results of SIMPER analysis (Macrobenthic species are grouped in relation to their contribution to average dissimilarity).

(*Capitella* sp A., *Prionospio* sp A, *Aphelochaeta* sp A., *Aricidea antarctica*, *Paramphinome australis*, *Cossura* sp A, and *Ampharete kerguelensis*) constituting 72% of the macrofauna, while the Baker Fjord polychaetes contributed 76% to total macrofauna, with *A. antarctica, Levinsenia antarctica* and *Capitella* sp A being the most important species.

The cluster analysis of macrobenthic data and SIMPROF test ($p < 0.05$) separated the benthos of the two fjords into two different groups (Fig. 2). Top discriminators for the average assemblage dissimilarity (71.86%) between Aysen and Baker Fjord were *L. antarctica, T. patagonica, Capitella* sp A. *antarctica, Cossura* sp A., *Aphelochaeta* sp A., *Prionospio* sp A., *P. australis, L. quatrefagesi and A. peruana*. In the Aysen Fjord, we distinguished two subgroups with several stations located along the fjord (stations SA2, SA3, SA4, SA5 and SA6) apart from station SA1 closest to the head of the fjord. In the Baker Fjord one subgroup included stations from the estuary entrance (SB1 and SB2) and separated them from the outer station SB3. In the Aysen Fjord, *L. antarctica* was the most discriminating but not the most abundant species at station SA1, followed by *Aphelochaeta* sp A, *Ampharete kerguelensis* and *Exogone* sp A. These species contributed almost 68.76% to the average within group similarity. In the Baker fjord the average within group similarity was 64.57% with the polychaetes *A. Antarctica and L. antarctica,* being the most common species at stations SB1 and SB2; high densities of *T. patagonica* made the station SB3 a bit different from both other stations.

In the Aysen Fjord across all sampling periods four stations were classified as moderately impacted and two stations as slightly impacted (Table 3). The AMBI showed a pattern of higher values (more degraded) in the middle of the fjord (station SA2 to SA5). All stations were evaluated as good quality, except for SA6 which was ranked as high, driven by the distinctively lower contribution of Ecological Group V (EGV < 10.6%) species. Diversity values did not exhibit significant differences among stations (Kruskall–Wallis test, $p > 0.05$), but the species richness was very high at station SA6 (Kruskall–Wallis test, $p < 0.05$). Throughout the sampling periods all three stations in the Baker Fjord were classified as only slightly impacted (Table 3). The AMBI values varied between 1.5 at station SB1 and 2.4 at station SB2 (see Table 3). The two inner fjord stations SB1 and SB2 were classified aś high qualitý, SB3 as good quality. The diversity and species richness were similar at SB1 and SB2 (no significant difference, Kruskal–Wallis test, $p > 0.05$), at SB3 the species richness appeared lower but the diversity slightly higher. Biological parameters derived from M-AMBI and BI were consistent with environmental data, as indicated by the ANCOVA analysis (Table 4). TOC, TOM and total sulfide were correlated to M-AMBI ($p < 0.001$). In addition, Chl-a content, CPE and Eh_{NHE} was also related to BI ($p < 0.05$), suggesting that the quality of the organic matter may be related to the presence of specific macrobenthic species or functional groups (i.e. sub-surface deposit feeders).

4. Discussion

The macrobenthic communities in Chilean fjords, which only recently have been studied (Montiel et al., 2011; Quiroga et al., 2012) are very rich and diverse. Infauna organisms are the most important contributors in these ecosystems, especially small-bodied

Table 3
AMBI, M-AMBI and results of overall ecological status for sampling stations.

Station	Aysen Fjord						Baker Fjord		
Location	SA1 Bahia Acantilada	SA2 Caleta Bluff	SA3 Rio Cuervo	SA4 Punta Tortuga	SA5 Caleta Perez	SA6 Cinco Hermanas	SB1 Rio Baker	SB2 Bajo Pisagua	SB3 Punta Raul
Ecological group I (%)	28.9	9.1	5.7	3.7	12.4	13.3	57.4	29.4	13.8
Ecological group II (%)	12.3	10.4	12.4	11.8	13.3	32.3	8.9	19.4	29.1
Ecological group III (%)	33.8	31.2	22.2	16.2	17.1	18.8	19.8	23.9	37.3
Ecological group IV (%)	2.2	15.0	16.2	9.0	9.4	25.1	4.9	6.8	7.5
Ecological group V (%)	22.8	34.4	43.4	59.2	47.8	10.6	8.9	20.5	12.3
Mean AMBI	2.7	3.8	4.0	3.9	3.9	2.8	1.5	2.4	2.1
Disturbance classification	Slightly impacted	Moderately impacted	Moderately impacted	Moderately impacted	Moderately impacted	Slightly impacted	Slightly impacted	Slightly impacted	Slightly impacted
BI[a] from Mean AMBI	2.0	3.0	3.0	3.0	3.0	2.0	2.0	2.0	2.0
Benthic community health	Unbalanced	Transitional to pollution	Transitional to pollution	Transitional to pollution	Transitional to pollution	Unbalanced	Unbalanced	Unbalanced	Unbalanced
Richness	24.0	35.0	35.0	33.0	39.0	46.0	53.0	50.0	36.0
Diversity (Shannon)	3.4	3.6	3.1	2.6	3.0	4.0	2.3	3.2	3.8
Not assigned taxa (%)	1.6	3.1	2.1	1.6	5.4	8.6	0.2	0.7	4.5
M-AMBI (0–1)	0.8	0.8	0.7	0.7	0.7	1.0	0.9	0.9	0.8
Ecological status	Good	Good	Good	Good	Good	High	High	High	Good

[a] BI: biotic index.

Table 4
Results of ANCOVA analysis with fjord as a covariate comparing sediment parameters and biotic indices.

Dependent variable	Independent variables	Effect	Wald statistic	*p* Value
M-AMBI	Total organic matter	Fjord	559.25	$p < 0.001$
	Total organic carbon		238.96	$p < 0.001$
	Total sulfide		13.45	$p < 0.001$
BI	Chlorophyll-a content	Fjord	134.61	$p < 0.001$
	CPE		116.08	$p < 0.001$
	Eh_{NHE}		6.09	$p < 0.05$

polychaetes (Paraonidae, Capitellidae and Cirratulidae) with continuous year-round breeding, short life-spans, and fast turnover rates. This has a strong regulating effect on the standing stock in these communities (Quiroga et al., 2012). Earlier studies comparing areas potentially impacted by salmon farms and unimpacted control sites (Soto and Norambuena, 2004) showed significant differences in species richness, but the low species richness in the control sites compared to our studies also suggest that this parameter might have been underestimated by these authors.

The type and spatial scale of ecological impact from coastal marine aquaculture depends on the method of aquaculture, the intensity of production, and the biological, chemical and physical characteristics of the coastal area (Wildish and Pohle, 2005). Although a single index may provide a rapid overview of the status of benthic environments, the use of M-AMBI is necessary in order to obtain an adequate description of the benthic community health. In terms of the ecological status at our study sites, we found the stations in the Aysen Fjord to be classified as good status, with station SA6, the outermost station located within the boundary of a national monument (without salmon farming activity), to be least disturbed; this site might hence be considered as a potential reference site. These results may be associated with the heavy salmon production activity in the Aysen Fjord (Soto and Norambuena, 2004; Tironi et al., 2008, 2010). In fact, organic enrichment associated with salmon production may cause changes of the community structure towards low-diversity benthic communities (Villnäs et al., 2011). In contrast the inner stations SB1 and SB2 in the Baker Fjord were classified to have a high ecological status and the outer station SB3 was classified as good. The health status of the benthic community in the Baker Fjord was classified as unbalanced, possibly related to high freshwater input from glacial river discharge (Quiroga et al., 2012).

The differences between the two fjords and the spatial patterns within each fjord are consistent with our expectations, but they also illustrate the potential confounding effect of local physical and environmental conditions, in this case the effects of major river inputs to semi-confined fjord ecosystems. Collectively these results reflect the riverine input of terrestrial organic matter, particularly evident near the head of both fjords. The station SA1 in the Aysen Fjord was clearly influenced by high fluvial input of organic matter (96.5%) comparable to what has been reported also for other rivers in the region (Silva et al., 2011a; Quiroga et al., 2012). The percentages of allochthonous organic matter were similar to other terrestrial influenced estuarine and fjord systems, such as Chiloe (Chile), Norway and Alaska (Silva et al., 2011a; Vargas et al., 2011 and references cited herein). It is also important to note that the suspended sediment concentration in the Baker River is about 25 times higher than in the Aysen River, however, the concentration of fine particulate organic matter (FPOM) is 4 times lower than in Aysen River (Fig. 3). The overall concentration of organic matter loaded from Aysen River is therefore approximately 100 times greater. Discharge into the two rivers is similar, except for the late summer and early fall (February until April), where glacial meltwater influx is higher in the Baker Fjord. Over an annual time scale, both rivers probably are influenced by terrestrial organic matter subsidy along their respective fjords, but the source is totally different in these rivers: high riverine loading of FPOM in the Aysen Fjord versus larger zone of light limitation by suspended sediments in the Baker Fjord. In addition, the timing of pulses from the two rivers also is rather different (Fig. 3), with distinct summer peaks of suspended sediment and organic matter in the Baker River, whereas both parameters in the Aysen River evidenced a clearly higher concentration level from early austral spring to late summer (August–February). The combination of timing and concentration of riverine subsidies may therefore have a complex interactive effect on the respective availability of primary production rates and thus the transport of allochthonous and autochthonous organic matter to benthic sediments.

There is a clear necessity to test and validate the AMBI and M-AMBI also in other similar sites within a similar wide range of environmental conditions, especially with respect to coastal

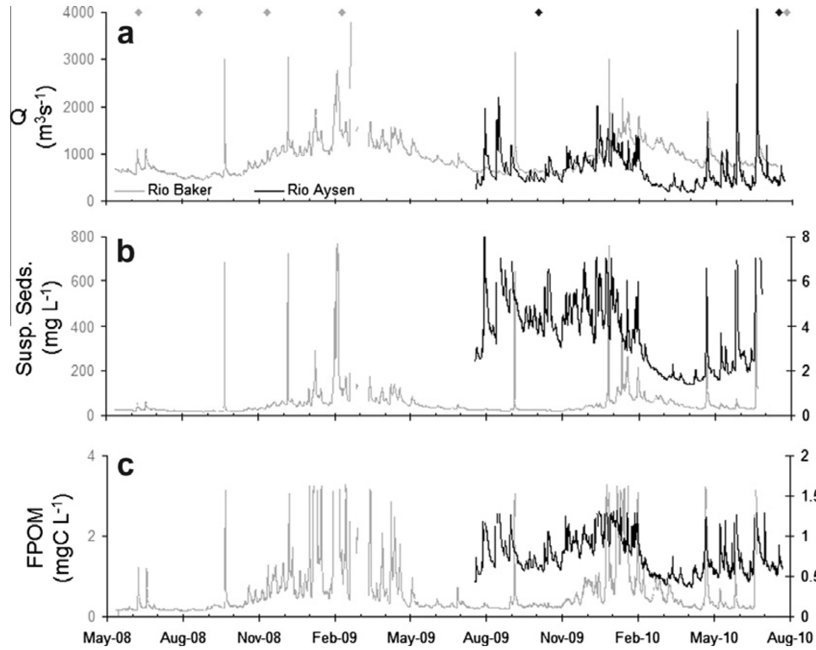


Fig. 3. Time series data and modeled fluxes in Baker and Aysen Rivers during the study periods; (a) Discharge for the two rivers, symbols (♦) show sampling dates; (b) modeled time series of suspended sediment concentration, based on primary sediment bearing tributaries and (c) modeled time series of the concentration of fine particulate organic matter.

riverine input, in order to establish a well-defined limitation of the influence and the applicability of the metrics. This in turn will facilitate interpretation of the biotic indices necessary for management decisions of the various coastal and fjord ecosystems. It is well known that a significant part of the OM is supplied by river discharge and coastal erosion (McLeod and Wing, 2009; Silva et al., 2011a; Quiroga et al., 2012). The terrestrial organic matter is deposited in sediments, and distributed by complex local hydrographic conditions thus enhancing the species sensitivity levels, which may lead to changes in their geographical distribution limits as has been already documented for other locations (Teixeira et al., 2012). Moreover the marine Patagonian coastal ecosystem is characterized by highly complex and diverse habitats that support high levels of biodiversity (Arntz and Ríos, 1999; Montiel et al., 2011) but unusual and unaccountable patterns in community composition. The AMBI assumes that each macrobenthic species is associated with a single ecological group (e.g., Borja et al., 2000; Teixeira et al., 2012). Analysis of similarity indicated that our results are consistent with the model proposed by Borja et al. (2000), as reflected by the high contribution (47.3%) of the polychaete *Capitella* sp. A in those stations classified as good ecological status. The macrobenthic fauna not assigned to any ecological group varied between 0.2% and 8.6% in this study (Table 3), which is acceptable for this analysis. The overall generality of AMBI and M-AMBI depends on the incorporation of observations from different habitats such as estuaries, fjords and closed bays under a broad range of temporal and spatial dynamics. This potentially renders cumbersome environmental assessment for large complex fjord ecosystems, suggesting that integration of an efficient sampling network with spatial analysis tools as e.g. GIS may be an essential aspect of aquaculture planning, as proposed also by FAO (Aguilar-Manjarrez et al., 2010; Silva et al., 2011b).

Chilean Patagonia is the world's largest fjord system, also considered as one of the most pristine ecosystems in the world (Mittermeier et al., 2003). The region is also under heavy pressure from the hydroelectric, tourism and salmon aquaculture industries, probably resulting in new and interacting stresses, pressures, and negative impacts on these ecosystems at complex spatial and temporal scales. Chilean government agencies often bear the dual responsibility of promoting increased economical development ignoring, however, at the same time environmental protection. Technical baseline data for decisions regarding appropriated areas for aquaculture are lacking totally. A proper management approach for ecosystems including human dynamics is essential to mitigate the environmental impact on coastal fjord ecosystems (Buschmann et al., 2009; Barton and Fløysand, 2010). In the case of salmon production this impact to some degree may be mitigated by means of the incorporation of an ecosystem approach for aquaculture (Soto et al., 2008; Aguilar-Manjarrez et al., 2010). The necessary foundation for mitigation and ecosystem management depends on the ability to define both, the reference conditions and the degree of stresses, as was tried in this study. These data thus provide a first baseline to classify Chilean fjords using the M-AMBI index. Our results suggest that such data are appropriate for the evaluation of Chilean fjord systems under different use, and they give further evidence that metrics based on general ecological principles are independent of regional geography (Borja et al., 2003, 2009a, 2009b; Muxika et al., 2005, 2007). Nevertheless, this approach requires a sufficient amount of data for the robust environmental quality assessment and the appropriate set of metrics established through regional public policy, as proposed by the Water Framework Directive (Borja and Muxika, 2005).

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