Investigaciones Marinas
ISSN: 0716-1069
spalma@ucv.cl
Pontificia Universidad Católica de Valparaíso
Chile

Rudolph, Anny; Aguirre, Gabriela; Moscoso, Julio; Silva, Nelson; Ahumada, Ramón
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Investigaciones Marinas, vol. 35, núm. 2, 2007, pp. 53-61
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Ecotoxicological quality in sediments of Reloncaví and Corcovado gulfs, Chile

Anny Rudolph[1], Gabriela Aguirre[1], Julio Moscoso[1], Nelson Silva[2] & Ramón Ahumada[1]
[1]Departamento de Química Ambiental, Universidad Católica de la Santísima Concepción Casilla 297, Concepción, Chile
[2]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

ABSTRACT. A study of the ecotoxicological quality of surface sediment was carried out on samples collected in the main fjords and interior channels between Reloncaví and Corcovado gulfs. The surface sediment samples were taken with a box corer and analyzed for organic matter content and grain size. Dissolved oxygen was measured in water samples taken 10 m off the bottom. The ecotoxicological evaluation of the sediments was performed using fertilization assays with gametes of *Arbacia spatuligera* (EPA/600/4-91/003). A fertilization rate of 95%, similar to that of the controls, was achieved. Therefore, the sediments at the sampled stations located in the middle of the channels were classified as non-toxic. Only the sediment at Station 26 (*i.e.*, sector Dalcahue) was classified as “slightly toxic” (90 ± 4.1%). The samples were taken from the central zones of the studied channels and fjords. Since toxicologically these central areas are not polluted, any possible effects of aquaculture activities are expected to be more local in scope.

Key words: sediment quality, fertilization assays, pollution, aquaculture, southern Chile.

Calidad ecotoxicológica de los sedimentos entre los golfos Reloncaví y Corcovado, Chile

RESUMEN. Mediante el análisis de muestras de sedimento superficial, tomadas en los principales fiordos y canales interiores entre el golfo de Reloncaví y golfo Corcovado, se realiza el estudio de su calidad ecotoxicologica. Las muestras superficiales de sedimentos se obtuvieron con un box corer. Se analizó el contenido de materia orgánica y textura de los sedimentos, y oxígeno en la columna de agua disuelto de muestras de agua tomadas a 10 m del fondo. Además, se realizó la evaluación ecotoxicológica de los sedimentos mediante pruebas de fecundación con gametos de *Arbacia spatuligera* (EPA/600/4-91/003), resultados que indican que sobre el 95% de ovas fueron fecundadas, valor semejante a los controles, lo que permite clasificar a los sedimentos de las estaciones ubicadas en el centro de los canales como no tóxicos. Sólo el sedimento de la estación 26 (*i.e.*, sector Dalcahue) mostró un 90 ± 4,1%, lo que se clasificaría como sedimento “levemente tóxico”. Las muestras fueron recolectadas en las zonas centrales de los canales y fiordos muestreados. Se concluye que estas zonas, desde el punto de vista toxicológico, no están contaminadas por lo que se estimó que un posible efecto de las actividades de la acuicultura sería más localizado.

Palabras clave: calidad de sedimentos, ensayos de fecundación, contaminación, acuicultura, zona austral, Chile.

Corresponding author: Anny Rudolph (annyr@ucsc.cl)

INTRODUCTION

The marine sediments are an environmental matrix (Elderfield, 1978) in which the stratigraphic disposition conforms a record of the processes and balances that occur in the surface layers (Stumm & Morgan, 1981; Colombo *et al.,* 1996). The chemical composition of the sediment depends on the mineralogical constitution of the mother rocks that originate it; the grain size, composition, and chemical characteristics of the environment in which it is deposited; the advective processes of the site; and anthropogenic contributions (Ahumada, 1998).

If the fraction of the natural or anthropogenic material that is input into an aquatic system is "trapped" in the sediments – and depending on its chemical quality – it can alter and/or change the substrate composition and may leave a record of such activity in the sediment column. In order for such a change to be recorded, it must be of a certain magnitude, persist over time, and occur in the absence of physical or biological disturbances (Ahumada, 1998). An analysis of critical variables (*i.e*., organic matter content, molecular markers) per strata in this matrix can show the history of the recent changes (Ponce-Velez & Botello, 1991), revealing the average production at a site and/or additional input generated by the use of the coastal area.

Non-specific toxicity assays offer one way to study the quality of recent sediments (Cairns & Pratt, 1989; Chapman, 1995). Such tests use the first centimeters of the surface sediment. Generally, chemical analyses of individual toxic compounds are not sufficient for inferring the potential ecological risk of a possibly polluted matrix since they do not allow evaluations of the combined action of all the chemical compounds present in the sediment at a given site, including their bio-availability (Chou *et al.,* 2003). Toxicity assays, on the other hand, integrate these effects, making this the recommended methodology for evaluating risks in areas adjacent to or near possible pollution sources. Moreover, these assays can be used as an early warning system to indicate changes in aquatic environments; they can be performed quickly and are useful for evaluating the effectiveness of implemented mitigation measures (Wong *et al*., 1999).

Given the advantages of analyzing the sediments as an environmental matrix (*i.e*., a historical record), a wide variety of toxicological tests has been developed. These tests range from evaluations of measurements of lethal and sub-lethal responses to estimates of alterations in the structure and functioning of benthic communities (Duffus, 1983; Van Gestel *et al*., 2001).

The last decade has seen a greater emphasis on toxicity tests using the more sensitive life stages of organisms, including, for example, procedures with amphipods, sea urchins, and polychaetes (Kast-Hutchenson *et al*., 2001; Amin & Comoglio, 2002). Fertilization tests with *Arbacia punctulata* gametes (EPA/600/4-91/003) have been standardized by USEPA (1988) as a type of non-specific toxicity assay. Excellent results have been achieved in Chile with the species *A. spatuligera*, which is easy to manipulate and whose analysis requires little time (Riveros *et al*., 1996; Larraín *et al*., 1999; Arévalo *et al*., 2001). Knowledge on the biology of the species (*i.e.,* feeding regimen studies, availability, acclimatization) has been furthered (Silva *et al*., 2004).

Most of the country's aquaculture centers are found in the channels and fjords between Puerto Montt and the Boca del Guafo, placing a heavy demand on this coastline. For example, 74 farming centers were registered in 1995 whereas, in 2003, 267 fish farms, 254 shellfish farms, and 273 algae farms were registered (SERNAPESCA, 2004).

Fish farming requires the artificial feeding of a large amount of fish. This, in turn, generates organic waste (fecal fish pellet, uneaten food) that is deposited on the sediment (Buchmann, 2005). A series of chemical compounds (*i.e*., antibiotics) used to avoid diseases in the farmed species are also added to the mix along with those agents used to limit the adherence of undesirable organisms to the facilities (*i.e*., antifoulings) (Alvial, 1993; Kelly *et al*., 1996, Buschmann, 2005). Some of these compounds are absorbed by particulate organic matter so that, when this sediments, the compounds are also carried to the bottom (Chou *et al.,* 2003).

The objective of this study was to carry out standardized, high sensitivity toxicity assays (*i.e*., fertilization tests with gametes of *Arbacia spatuligera*) in order to analyze the toxic quality of the sediments in some fjords and channels from Puerto Montt to Boca del Guafo.

MATERIALS AND METHODS

Sediment sampling was done during the CIMAR 10 Fiordos cruise between 17 August and 6 September

2004, on board the oceanographic vessel AGOR "Vidal Gormaz". Samples were taken at three stations in each of the seven sections of the study area between Puerto Montt (41.5º) and Boca del Guafo (43ºS) (Fig. 1; Table 1).

The same stations were used for water column dissolved oxygen samples. These were taken at standard depths and up to 10 m from the bottom with Niskin bottles attached to a rosette. Dissolved oxygen was determined according to the Winkler method as modified by Carpenter (1965).

The sediment samples were extracted with a box corer and three replicates were taken of the first three cm of the sediment. The samples were stored in polyethylene containers, labeled, and frozen at -20ºC for later organic matter, grain size, and toxicology analyses.

The organic matter content in the sediments was determined gravimetrically using the technique of weight loss on ignition (Byers *et al.*, 1978). The grain size (granulometry) was determined according to the Udden-Wenthworth classification through wet sieving.

The toxicity tests of the sediment were performed following the specifications of the method EPA/600/4-91/003 (USEPA, 1988) and modifications introduced by Zúñiga (1999). Basically, this method consists of producing an artificial fertilization using spermatozoids exposed for 60 min to an elutriate prepared with the sediment being studied; the fertilization is then compared with positive and negative controls that are performed simultaneously. The elutriate was obtained through the Dinnel & Strober (1985) methodology, that is, by agitating 50 g of sediment in a Heidolph Unimax 2010 at 5 rpm with 50 mL of filtered, aerated seawater for 10 min; this was later left in the cold (4ºC) for 12 h to separate the liquid phase (elutriate) from the sediment.

For the sea urchin test, specimens were collected by scuba divers in an area with low anthropogenic impact. The sea urchins were acclimated for a week at 13 ± 2ºC in 120-L glass aquariums with daily water changes, constant aeration, and food. The gametes were obtained by injecting a KCl 0.5 M solution into the perivisceral region of each specimen; these were used to prepare spermatic solutions of $7 \cdot 10^7$ sperm·mL^{-1} and $2 \cdot 10^3$ eggs·mL^{-1}.

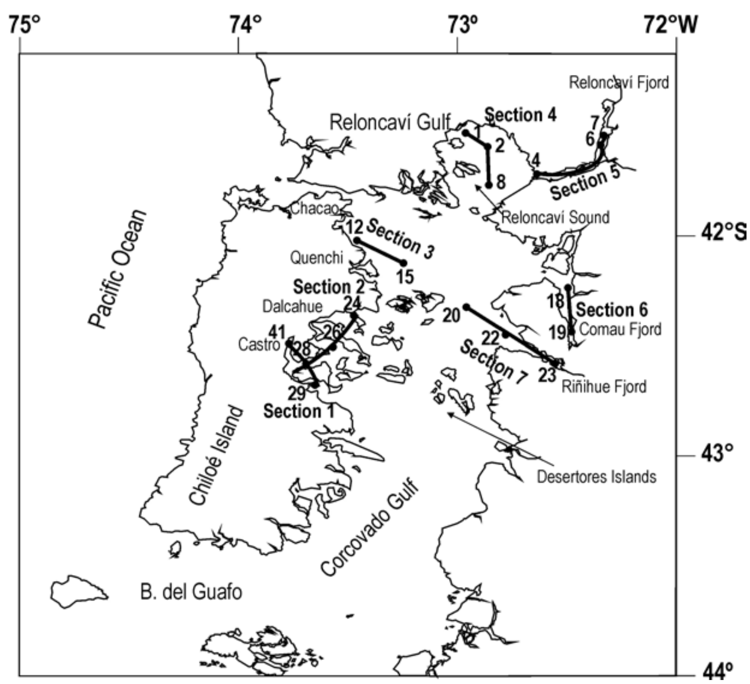

Figure 1. Study area, to indicate location of sections and sampling stations analyzed during CIMAR 10 Fiordos Cruise (August-September 2004).

Table 1. Geographical position, depth, water column dissolved oxygen, saturation percentage near the bottom (≈ 10 m), and sediment organic matter registered on CIMAR 10 Fiordos Cruise, 2004.

Station	Latitude (S)	Longitude (W)	Depth (m)	Sediment organic matter (%)	Dissolved oxygen ($mL \cdot L^{-1}$)	Saturation %
Section 1 Castro						
Sta. 41	42º 24.92	73º 44.54	18	5.87	5.61	88.2
Sta. 28	42º 34.92	73º 44.64	60	3.32	5.84	91.49
Sta. 29	42º 43.02	73º 35.52	145	5.39	5.82	91.32
Section 2 Dalcahue						
Sta. 24	42º 21.42	73º 29.58	108	5.22	5.77	90.6
Sta. 26	42º 30.06	73º 33.42	72	1.56	5.98	98.8
Sta. 28	42º 34.92	73º 44.64	60	3.32	5.84	91.5
Section 3 Chacao						
Sta. 11	-	-	-	Rocky bottom	-	-
Sta. 12	41º 59.04	73º 28.14	138	2.19	5.38	85.2
Sta. 15	42º 06.24	73º 14.94	192	7.05	5.18	91.5
Section 4 Reloncaví Sound						
Sta. 1	41º 31.44	72º 55.55	285	8.64	3.95	48.1
Sta. 2	41º 37.26	72º 50.52	298	6.16	3.87	61.8
Sta. 8	41º 45.12	72º 50.28	207	2.95	3.88	62.3
Section 5 Reloncaví Fjord						
Sta. 7	41º 33.00	72º 20.04	496	6.04	2.99	48.1
Sta. 6	41º 35.52	72º 20.46	155	6.49	2.73	43.9
Sta. 4	41º 43.02	72º 36.96	452	5.2	3.69	67.4
Section 6 Comau Fjord						
Sta. 17	42º 08.45	72º 45.08	363	6.37	3.60	-
Sta. 18	42º 13.87	72º 29.80	475	7.49	3.63	58.6
Sta. 19	42º 26.64	72º 26.10	80	7.28	3.96	63.5
Section 7 Riñihue Fjord						
Sta. 20	42º 26.64	72º 26.10	238	7.28	5.29	-
Sta. 22	42º 27.00	72º 45.30	257	7.85	5.05	80.2
Sta. 23	42º 32.70	72º 34.98	254	3.89	4.65	74.5

The experimental procedure consisted of preparing three series of tubes with: 5 mL of sediment elutriate from the sea urchin extraction site (*i.e.*, negative control), 5 mL of increasing concentrations of copper sulfate between 3.6 and 100 $\mu g \cdot L^{-1}$ (*i.e.*, positive control), and 5 mL of sediment elutriate from the study area. The sperm suspension (100 μL) was added to each of these series and then incubated for one hour at 13 ± 2ºC. After this, 1 mL of the egg solution was added and the tubes were again incubated for 15 min, during which time the fertilization took place. The assay was ended by adding 1 mL formaline at 10%. One hundred eggs were counted under a microscope (100X magnification) and the fertilized eggs, identified by the fertilization membrane, were expressed as a percentage.

The sensitivity of the toxicity assay was evaluated in function of the positive and negative controls through the application of the statistical package TOXSTAT (Gulley *et al.*, 1988). The EC_{50} was calculated through the probit method (Finney, 1971). Normality was analyzed with the Shapiro-Wilk test and the homogeneity of variance of the data with the Bartlett test. Later, the parametric Dunnett test was used to compare the percentages of fertilization for the sediment samples with respect to the controls (statistical package Statistica version 6.0, StatSoft. Inc; 2001).

RESULTS

The sampling carried out in the central sectors of the analyzed fjords and channels used shallow stations of around 18 m depth and deep stations up to 496 m (Fig. 1). The location of the sections, the sampling depths, and the results of the chemical characteristics of the sediment and near-bottom water at each station are presented in Table 1. The sections at Reloncaví Sound, Reloncaví and Comau fjords had the lowest near-bottom oxygen concentrations, with saturation values between 43.8 and 98.8% (Table 1).

The toxicity assays with the elutriate of the sediment samples presented fertilization percentages similar to the negative control, *i.e.*, > 95% (Table 2). Station 26 (section 2, sector Dalcahue) was an exception; there, the fertilization percentage was 90.0 ± 4.1. This 72-m-deep station had low organic matter content in the sediment (*i.e.*, 1.56%) and high oxygen throughout the water column (> 5 mL·L^{-1}), which does not explain the low percentages of fertilization success observed in this sector.

Table 2. Percentages of fertilization using sediment elutriates in fertilization assays with gametes from *Arbacia spatuligera,* evaluated by section in the study area (n = 8).

Station	Percentage of fertilization	Standard deviation
Section 1 Castro		
Sta. 41	98.75	1.83
Sta. 28	98.75	1.28
Sta. 29	99.0	1.81
Section 2 Dalcahue		
Sta. 24	99.50	0.93
Sta. 26	90.00	4.14
Sta. 28	98.75	1.28
Section 3 Chacao - Quemchi		
Sta. 11	-	-
Sta. 12	99.0	2.14
Sta. 15	98.25	2.43
Section 4 Reloncaví Sound		
Sta. 1	96.75	1.03
Sta. 2	97.38	3.54
Sta. 8	99.0	0.98
Section 5 Reloncaví Fjord		
Sta. 7	99.0	1.77
Sta. 6	97.88	2.03
Sta. 4	94.75	2.38
Section 6 Comau Fjord		
Sta. 17	-	-
Sta. 18	98.75	1.83
Sta. 19	99.12	1.46
Section 7 Riñihue Fjord		
Sta. 20	100	0
Sta. 22	98.25	2.92
Sta. 23	99.88	0.35

DISCUSSION

The particulate organic matter input into the ocean, for example through sewage emissions or, as in this study, from fish feeding activities in aquaculture, accumulates in the sediments, although not necessarily constituting a toxic component. Nonetheless, its accumulation and oxidative processes produce changes in the dissolved oxygen content of the interstitial water and in the overlying water column, also altering sediment grain size, absorption of chemical species, and the distribution of benthic species sensitive to the lack of dissolved oxygen. It is possible that suboxic conditions generate an additional stress on the toxic effect of the sediment components. Thus, the toxicity of the sediments depends on numerous factors and not only on the suboxia or anoxia associated with the substrate. Such an alteration is referred to as "non-specific toxicity".

According to Silva *et al*. (1997), Silva & Calvete (2002), and Valdenegro & Silva (2003), none of the fjords or channels in southern Chile presents an anoxic water column. These authors only observed suboxic conditions (1.5-3 mL·L^{-1}) in the deep areas at the heads of most of the channels and fjords along the continental edge. For Reloncaví Sound (section 4), Reloncaví Fjord (section 5), and Comau Fjord (section 6), the lowest values of dissolved oxygen fluctuated between 2.73 and 3.96 mL·L^{-1} in the deep basins (> 250 m) and these did not necessarily present the greatest percentages of organic matter (Table 2). Oxygen in the surface waters was always near the saturation level (90-110%). Silva *et al*. (1998a) found that, to the north of Boca del Guafo, the low dissolved oxygen contents observed in the bottom samples taken from deep channels and fjords were due to restricted circulation caused by topographic effects, specifically shallow sills and constrictions generated by groups of islands (*e.g*., the Desertores

Islands). Furthermore, the contribution of organic matter from the more productive estuarine areas should be considered.

The low concentrations of dissolved oxygen in these channels were detected in the early 1970s during the oceanographic cruise Hudson 70 (NODC, 1994), which was performed nearly 15 years prior to the inception of large marine farming companies. Hence, in spite of the current low concentrations of dissolved oxygen in the study area (2-3 mL $O_2 \cdot L^{-1}$), it is possible to sustain a diversity of benthic life in the sediment (Clavert *et al.,* 1996; Hagerman *et al.,* 1996).

The organic matter content fluctuated between 2.95 and 8.64%, with a total average of 5.88 ± 1.88 and a coefficient of variation (CV) of 32% for an n = 20. However, when the values lower than 4% were eliminated, the organic matter averaged 6.90 ± 0.92 with a CV of 13.27% (n = 14). This average value is similar to the base line concentration for the microbasins in this zone and to that reported for cruises carried out in 1995 (Silva *et al*., 1998b). The sediments in this area have average organic matter values as compared with the semi-enclosed areas of the highly productive Chilean coast such as Concepción Bay (*i.e*., 12-18% organic matter) (Rudolph *et al*., 2002) and with areas that maintain a high exchange with the adjacent oceanic zone such as Corcovado Gulf (*i.e*., 1-2% organic matter) (Silva *et al*., 1998b; Silva & Prego, 2002).

Concentrations of organic matter over 10% require large amounts of dissolved oxygen, thereby decreasing the concentrations in the water overlying the sediment and generating a reducing environment that favors the formation of organic macromolecules, with a great affinity for trace metals (chelation) and xenobiotics (absorption) (Birch *et al*., 2001; Lopes & Furlong, 2001; Hites *et al*., 2004; Monteiro & Roychoudhury, 2005).

When chemical compounds are deposited on the sediments, their elutriates release soluble chemical species (organic and/or inorganic) when the oxidation conditions of the water column change; these chemical species can form potential toxins for organisms. Toxicity tests were performed with the sediments from different basins in the fjords and channels in which marine farming facilities are located in order to establish the sedimentary condition or degree of toxicity.

The toxicity assays showed high percentages of fertilization, implying that the sediments in the studied areas do not contain concentrations of chemical compounds that impede fertilization according to the tests performed (Table 2). The evaluation criteria used to determine the sediment quality in function of the fertilization percentage of *A. spatuligera* gametes (Table 3) revealed "non-toxic" sediments that behaved similarly to the control (*i.e*., 95-100% fertilization). Only the sediments at Station 26 (section 2) had lower fertilization rates (90 ± 4.1%; n = 8) indicating toxicity; the sediments at this site were classified as "slightly toxic" (Table 3).

The information on the toxicological quality of the sediments in the study area was compared with the work of Aguirre (2004), who studied bay and port sediments along the Chilean coastline using the same methodology to determine toxicity and with similar assay sensitivity. The comparison suggests that the sediment in the middle of the analyzed channels and fjords corresponds to a non-toxic or low alteration zone (Fig. 2), similar to the sediments in Coliumo, an area subjected to minimal alteration (Fuentes-Ríos *et al*., 2005; Altamirano-Chovar *et al*., 2006). Nevertheless, this evaluation was based on one type of toxicity assay: the fertilization of sea urchin gametes. In order to accept or reject a working hypothesis that implies a possible alteration produced by aquaculture activities, future research

Table 3. Quality criteria of sediment as a function of the percentage of fertilization obtained using the *Arbacia spatuligera* gamete assay (Aguirre, 2004).

Scale	Quality criteria (% success in the fertilization)	Characteristic
1	100 - 95	No toxic, behavior of the controls
2	94 - 85	Slightly toxic
3	84 - 75	Toxic
4	74 - 55	Very toxic
5	< 54	Polluted

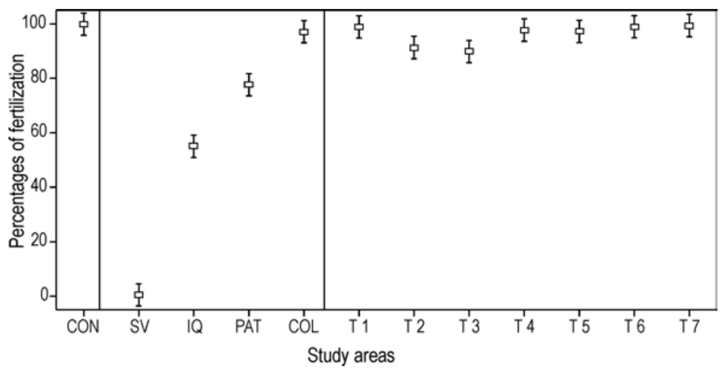

Figure 2. Comparative chart showing mean percentages of eggs fertilized in elutriates from test and control (CON) sediments from the ports of San Vicente (SV), Iquique (IQ), Patache (PAT), and Coliumo (COL, reference sector). Information from Aguirre (2004) and sections in the present study (T1 to T7).

should include assays with organisms that represent different levels of the trophic chain (Duffus, 1983; Van Gestel *et al*., 2001).

Finally, another element that should be kept in mind in terms of the results obtained herein is that the CIMAR 10 Fiordos sections were designed to detect mesoscale alterations, expecting to find evidence of alterations or spatial gradients from the sources of origin towards the center of the fjords, channels, and major basins of neighboring fjords. The results obtained on this scale do not indicate evidence of alterations and, therefore, the impact of the aquaculture activities could be more local than the spatial coverage of this study. However, the topographic characteristics of the zone (*i.e*., restricted circulation zones, deep basins) and the results of the chemical characteristics of the near-bottom water (*i.e*., low dissolved oxygen ≤ 3.5 $mL{\cdot}L^{-1}$) and the sediments (*i.e*., total organic matter ≈ 6.5%) correspond to restricted systems with high sedimentation and important sources of natural organic matter (*i.e*., autochthonous productivity), added to the allochthonous material introduced by rivers and anthropogenic activities (cities, aquaculture), which correspond to zones of high environmental risk.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Finance for financing this research through the Comité Oceanográfico Nacional, Project CONA-C10F 04-15.

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Received: 5 July 2006; Accepted: 31 August 2007