Bull. Environ. Contam. Toxicol. (2006) 77:171–178

DOI: 10.1007/s00128-006-1047-2

Bulletin of Environmental Contamination and Toxicology

Differential Sensitivity to Human Influence in Juvenile *Semimytilus algosus* (Gould, 1850) (Mollusca: Mytilidae) from Four Coastal Sites in South-Central Chile

C. Altamirano-Chovar,[1] A. Rudolph,[1] R. D. Sepúlveda[2]

[1] Department of Environmental Chemistry, Faculty of Sciences, Universidad Católica de la Santísima Concepción, Box 297, Concepción, Chile
[2] Institute of Ecology and Evolution, Universidad Austral de Chile, Box 567, Valdivia, Chile

Received: 31 October 2005/Accepted: 2 June 2006

The heavy metals constitute one of the major groups of pollutants monitored in the coastal zone (Ardiles and Inda 2000). Their distribution within an environment depends on factors such as general and local circulation patterns, sediment composition, and local human activities (Grecco et al. 2003). The metals are categorized as oligoelements, with natural concentrations in the ppb range.

Although at low concentrations many of these metals are indispensable for metabolic functioning in a wide variety of species, at high concentrations ranging to two orders of magnitude above natural concentrations, numerous of the heavy metals show varying risks of toxicity. Under some conditions, certain heavy metals may become accumulated in biological systems, based on their capacity for transference and biomagnification in trophic webs. The preceding has resulted in the development of technical methods for analysis of their bio-availability, and of the different chemical forms in which they may be found in environmental matrixes, including water, sediments, and organisms. A major line of research in this area has been the establishment of baseline values of heavy metal content in environmental matrixes in support of environmental monitoring programs for assessment of heavy metal pollution (Román et al. 1994). It is assumed that the response of an organism to a stimulus may be modified within the environment both by biotic and abiotic factors.

In the present study we have employed the capacity of a small, fast-growing mussel species, *Semimytilus algosus,* as an indicator of resistance to cadmium insult in relation to nearness to human industrial or other activity. *S. algosus* is a normally populous filter feeding bivalve on the coast of south-central Chile, although showing manifest declines in its population of some coastal areas. This mussel is distributed from Manta, Ecuador (00°56´S; 80°44´W) to the Gulf of Arauco, Chile (37°02´S, 73°32´W) (Guzmán et al. 1998). Of primary importance was determination of the range of tolerance of this species to Cd as a pollutant. Marine bivalves have traditionally been used as bio-indicators in ecotoxicological studies, given their ubiquitous presence in marine environments, and their capacity for filtering large volumes of water and sedimentary materials, in some cases concentrating pollutants in their tissues (Goldberg 1980, Llanes-Baeza and González 2002).

Correspondence to: A. Rudolph

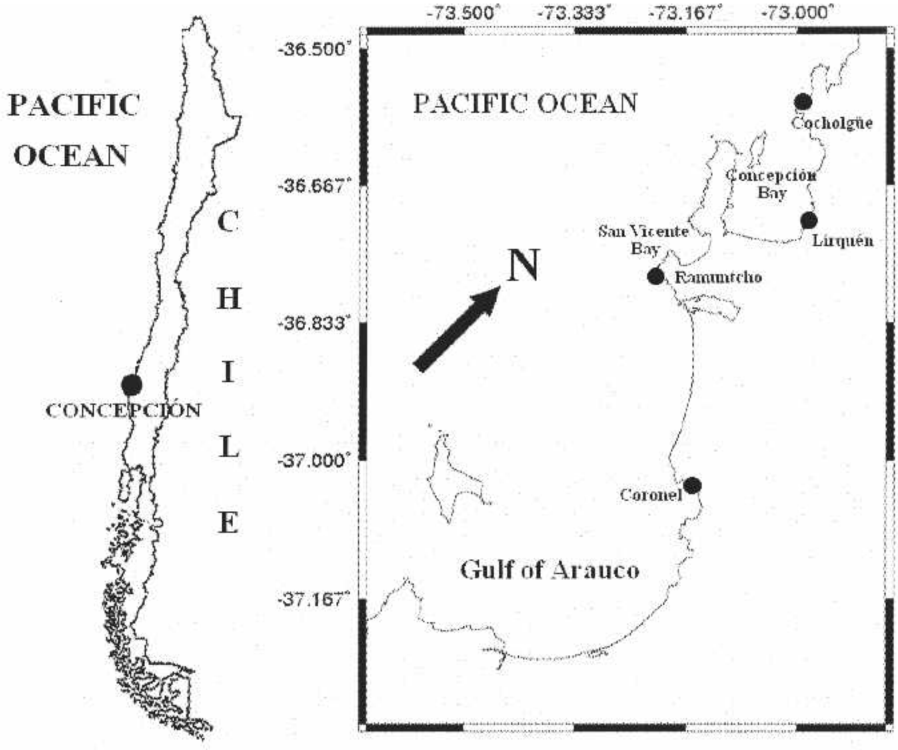

Figure 1. Map of the study area, to show beach collection sites for juvenile *Semimytilus algosus* during May 2003. The Coronel and Lirquén beaches were not far from industrial activities.

The hypothesis to be tested in the present study is that tolerance to Cd as a toxicant is comparatively greater among individuals of *S. algosus* inhabiting rocky shores exposed to man's activities than among individuals of the same species occurring far removed from man's activities.

MATERIALS AND METHODS

Mussels were collected at four beaches in central-southern Chile (Fig.1). The sampling sites differed in their degrees of exposure to the open sea and in their degrees of exposure to human activity in each area. The individual characteristics of each site are outlined as follows:

Cocholgüe beach is located at the northern extremity of Concepción Bay. It is a beach exposed to the ocean and subject to strong wave activity. Rocks on the beach are heavily populated by *S. algosus*. The main activity in the area is artisanal fishing, and there is no manifest evidence of industrial activity. It was assumed that the background Cd values at this site were similar to the "unpolluted".

Lirquén beach is in the SE sector within Concepción Bay, and has a low abundance of *S. algosus*. There are port facilities nearby, active in the loading of forestry and agricultural products. The Cd concentration in the water and organism tissue in this area has been reported (Table 1).

Ramuntcho beach is in the SE sector of San Vicente Bay. It is small and protected and is well populated by *S. algosus*. There is no industrial activity in the area, and low Cd background values were assumed as for Colchogüe.

Coronel beach is located within Coronel bay, and has a low abundance of *S. algosus*. The main industrial activities in the area are commercial fishing and coal mining. Cadmium in the seawater of this area has been reported (Table 1).

Juvenile *Semimytilus algosus* (Gould, 1850) (1.5 ± 1 cm) were hand-collected from natural populations in the four study areas cited above. The mussels were cleaned and distributed into 600 ml glass vessels with 10 mussels per system. Seawater in the systems was obtained distant from any industrial activity at Lenga (36°45´S; 73°08´W) and was filtered to 0.45μ. Acclimation of the organisms to conditions in the laboratory proceeded for 10 days, at 11 ± 2 °C, salinity = 33 psu, dissolved oxygen = 8 mg/L, pH = 8.4 and with a 12:12 hr photoperiod. Once in the lab, the mussels were fed *ad libitum* with an artificially culture of *Dunaliella salina*. Every 48 hr all treatments were cleaned and the toxicant concentration was return to the initial condition. During acclimation and experimentation the water was constantly aerated to maintain oxygen saturation. Survival of *S. algosus* in the tanks was 100% prior to beginning the experiments.

The treatments, in triplicate, included exposing the mussels to four (nominal) Cd concentrations, including 0.0 (control), 1.6, 6.4, and 12.8 μg/L. Organisms were monitored for 312 hr at 24 hr intervals. Death of the mussels was noted as gaping of the valves and lack of any response to mechanical stimulus. Test solutions were prepared from ($CdSO_4$, $8H_2O$) (Merck Co.).

Table 1. Mean cadmium concentrations in representative matrixes in areas related to sampling sites in the present study.

Matrix	Concentration	Location	Reference
Water column	2.10 ug/L	Concepción Bay	Carrera et al. (1993)
	0.10 ug/L	Concepción Bay	Balabanoff et al. (1980)
	0.59 ug/L	Concepción Bay	Gutiérrez (1989)
	4.88 ug/L	Coronel	Arcos et al. (1993)
	0.045 ppb	Lirquén	Gutiérrez (1989)
	0.016 ppb	Gulf of Arauco	Gutiérrez (1989)
Sediment	2.20 mg/Kg	Concepción Bay	Carrera et al. (1993)
	6.27 mg/Kg	Concepción Bay	Salamanca et al. (1988)
	2.24 mg/Kg	Gulf of Arauco	Salamanca et al. (1988)
Tissue			
A. ater	3.48 mg/Kg	Lirquén	Chiang (1989)
P. purpuratus	3.62 mg/Kg	Lirquén	Chiang (1989)

Data analysis: The Trimmed Spearman-Karber method from the Toxstat (Hamilton et al. 1977) program was used to calculate the lethal concentration of the toxicant (LC_{50}). Factorial ANOVA was applied with repeated measurements (SS type III), using site and concentration of toxicant as orthogonal factors and the time as the repeated measurement. Mortality of the *S. algosus* (%) was transformed to $y = \arcsin \sqrt{x/100}$ to comply with assumptions of normality, homoscedasticity, and sphericity (Zar 1999).

RESULTS AND DISCUSSION

The control organisms showed behaviour identical to that observed during their acclimation to laboratory conditions, including normal ventilation with valves semi-open, mantle extended, exhalant siphon extended, rapid adherence to the walls of the test vessel, and response to mechanical stimulation.

The mussels exposed to different concentrations of Cd showed sublethal reactions to the toxicant, including release from the substrate, closing of the valves, production of bubbly exudates and/or spontaneous spawning. These responses increased progressively beginning after 96 hr exposure, depending on the concentration of Cd in solution.

The Table 2 presents the different responses of mussels to the toxicant. The three factors showed significant differences both at spatial and temporal levels. The results of the factorial statistical analysis showed a significant interaction between the site and the toxicant concentration, demonstrating in this way that the mortality of mussels is the result of the joint effect of both factors. Likewise, the temporary factor showed significant interaction with the spatial effects, reason why it is possible to demonstrate that mussels' mortality is the result of combined effects among the collection site, the toxicant concentration and the exposure time.

Table 2. Results of 2-way repeated measures ANOVA, using type III sum of squares, for the effects of site (random), concentration of toxicant (fixed), and exposure time to the toxicant (repeated measurement).

Source of variation	SS	df	MS	F	P
Site (S)	14.376	3	4.792	162.140	<0.001
Concentration (C)	25.143	3	8.381	13.074	$=0.001$
S×C	5.769	9	0.641	21.690	<0.001
Residual	0.946	32	0.030		
Exposure time (T)	38.147	13	2.934	379.069	<0.001
T×S	5.887	39	0.151	19.498	<0.001
T×C	14.045	39	0.360	46.521	<0.001
T×S×C	3.108	117	0.027	3.432	<0.001
Residual	3.220	416	0.008		

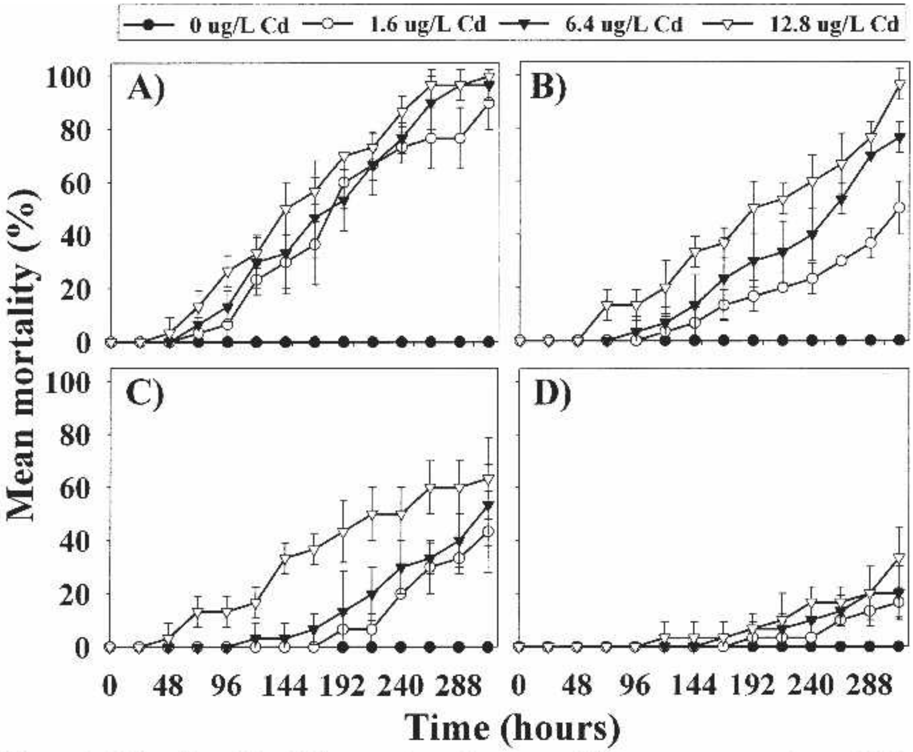

Figure 2. Mortality (%) of *Semimytilus algosus* at different concentrations of Cd (ug/L). Sites A) Ramuntcho and B) Cocholgüe had no evident human alteration, and C) Lirquén and D) Coronel were near industrial activities.

The most sensitive mussels were those collected at Ramuntcho and at Lirquén. In these tests, the first mortalities were observed after 48 hr of exposure, as shown in Figures 2A and 2C respectively. In tests with mussels collected at Cocholgüe, the first mortality was observed at 72 hr into the experiment (Fig. 2B). In contrast, mussels collected at Coronel only began to show mortality after 120 hr of exposure (Fig. 2D).

The 312 hr LC_{50} for Cd in these mussels was 1.2 ug/L for specimens obtained from Ramuntcho; 1.6 ug/L for those from Cocholgüe and 5.38 ug/L for those from Lirquén. The LC_{50} for mussels from Coronel could not be determined within the time period of the observation. The effect of the graded Cd concentrations at 312 hr exposure was greater in mussels from Ramuntcho and Cocholgüe with mortalities of 90% and 80% respectively (Fig. 2A and 2B); the mussels from Lirquén and Coronel, had mortalities of 60% and 30%, respectively.

It is concluded that *S. algosus* juveniles collected from sites exposed to potential sources of pollution were significantly, and variably more tolerant to Cd than were those from sites distant from human influence. This response was probably conditioned by the degree of human alteration encountered by the mussels within their habitat.

At the polluted sites, the toxicant/organism relation could induce a series of adaptive processes which would ultimately be expressed as mechanisms of resistance to the toxicant (Viarengo 1985, Anisimova et al. 1993, Montuelle et al. 1994, Moraga et al. 2003). The resistance to heavy metals could result from exposure of the mussels to sublethal pollution in the form of chronic low concentrations of toxicant over long periods of time (Daka and Hawkins 2004). For example, low metal concentrations can induce synthesis of metallothioneins or similar, which play an important role in the metabolism and in the detoxification of metals (Bebianno and Langston 1992). In fact, metal-binding proteins (similar to metallothioneins) has been found in several tissues of *Mytilus galloprovincialis* and *M. edulis* exposed to cadmium in laboratory conditions (Viarengo et al. 1985, Nolan and Duke 1983a, b), in samples collected from contaminated sites (Pavicic et al. 1985) and also in organisms exposed to contaminated sediments (Geffard et al. 2002).

An alternative explication is that mussels inhabiting polluted areas are the products of a genetic selection process in which only the Cd resistant individuals survived out of large cohorts of mussels produced in the region. A test of this hypothesis in future research should be made using mass culture techniques with wild mussels, with Cd bioassays applied to determine differential survival of the larvae which might indicate a genetic component in Cd resistance. These methods, however, require a highly specialized laboratory infrastructure, not available to us at this time.

REFERENCES

Anisimova L, T Siunova, A Boronin (1993) Resistance to metals gram negative bacteria isolated from sewage and soils of industrials regions. Microbiology 62:505-508

Arcos D, L Furet, F Carrasco, S Núñez, F Vargas (1993) Eutroficación en el ambiente marino de Chile Central: Efectos inducidos por la evacuación de residuos industriales líquidos. Invest Mar 21:51-74

Ardiles V, J Inda (2000) Evaluación de la contaminación de las Bahías Flamenco, Inglesa, Salado y Huasco (III Región), utilizando dos metodologías. Resúmenes XX Congreso Ciencias del Mar. Universidad Católica de la Santísima Concepción. Concepción p 77

Balabanoff L, I Gamé, R Hernández, G Navarrete, R Valdebenito (1980) Determinación de BOD, COD, NO_3^-, NH_4^+, PO_4^{3+}, Cl^-, $S^=$, SiO_2, Fe, NO_2^-, Mn, Cd, Zn, Pb, Cr, As, Sb, Bi, en agua, sedimento y material biológico. Subproyecto N°2 del Proyecto "Evaluación del grado de contaminación del litoral de la VIII región". Convenio Serplac VIII región – Universidad de Concepción. Tercer informe de avance, pág. 35

Bebianno MJ, WJ Langston (1992) Cadmium induction of metalothionein synthesis in Geffard A, Geffard O, His E, Amiard JC 2002. Comp Biochem Physiol 103C:79-85

Carrera ME, V Rodríguez, R Ahumada, P Valenta (1993) Metales trazas en la columna de agua y sedimentos blandos en Bahía Concepción, Chile. Determinación mediante voltametría de redisolución. Rev Biol Mar Valparaíso 28:151-163

Chiang J (1989) Metales pesados en sedimentos y organismos marinos provenientes de la zona norte, centro y centro-sur de Chile. Rev Pacífico Sur (Número especial):205-215

Daka E, SJ Hawkins (2004) Tolerance to heavy metals in *Littorina saxatilis* from a metal contaminated estuary in the Isle of Man. J Mar Biol Ass UK 84:393-400

Geffard A, O Geffard, E His, JC Amiard (2002) Relationships between metal bioaccumulation and metallothionein levels in larvae of *Mytilus galloprovincialis* exposed to contaminated estuarine sediment elutriate. Mar Ecol Prog Ser 233:131-142

Goldberg ED (1980) The surveillance of coastal marine water with bivalves. The Mussel Watch. Analytical techniques in environmental chemistry. J Albaiges (Ed):3733-3866

Grecco LE, AO Marcos, EA Gómez, S Botté, J Marcovecchio (2003) Natural and anthropogenic input of heavy metals in sediments from Bahía Blanca Estuary (Argentina). J Coastal Res, Special Issue 39:1-5

Gutiérrez F (1989) Diagnóstico de la contaminación marina en el Pacífico Sudeste por metales pesados, pesticidas y eutroficación. Informe. Doc. CPPS/COI/PNUMA (OCA)-PSE Cónsul.2., Comisión Permanente del Pacífico Sur 58 pp + tablas

Guzmán N, S Saá, L Ortlieb (1998) Catálogo Descriptivo de los Moluscos Litorales (Gastropoda y Pelecypoda) de la zona de Antofagasta, 23°S (Chile). Est Oceanol 17:17-86

Hamilton MA, RC Russo, RV Thurston (1977) Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ Sci Technol 11(7):714-719; Correction (1978), 12(4): 417

Llanes-Baeza C, N González (2002) Evaluación de los mejillones *Mytilopsis sallei* (Reclúz) y *Brachidontes exustus* (Linné) como bioindicadores de materia orgánica en la bahía de Chetumal, México. Universidad y Ciencia 18:29-41

Montuelle B, X Latour, B Volat, A Gounet (1994) Toxicity of heavy metals to bacteria in sediments. Bull Environ Contam and Toxicol 53: 753-758

Moraga R, C Merino, MA Mondaca (2003) Resistencia a metales pesados en bacterias aisladas de la bahía Iquique. Invest Mar 31: 91-95

Nolan CV, EJ Duke (1983a) Cadmium accumulation and toxicity in *Mytilus edulis*: involvement of metallothioneins and heavy molecular weight proteins. Aquat Toxicol 4:153-163

Nolan CV, EJ Duke (1983b) Cadmium-binding proteins in *Mytilus edulis*: relation to mode of administration and significance in tissue retention of cadmium. Chemosphere 12:65-74

Pavicic J, M Skreblin, I Kregar, M Tusek-Znidaric, and P Stegar (1985). Formation of inducible Cd-binding proteins similar to metallothioneins in selected organs and life stages of *Mytilus galloprovincialis*. Lucerne 11-13 669-705

Román G, A Rudolph, R Ahumada (1994) Estudio estacional de toxicidad por cadmio en *Choromytilus chorus* (Molina 1782). Bol Soc Biol Concepción 65:23-30

Salamanca MA, L Chuecas, F Carrasco (1988) Heavy metal content and distribution in surface sediments from three areas of the chilean coast. Gayana Miscelánea 9:1-16

Viarengo A, S Palmero, G Zanicchi, R Capella, R Vaissiere, M Orunesu (1985) Role of metallothioneins in Cu and Cd accumulation and elimination in the gill and digestive gland cells of *Mytilus galloprovincialis*. Lam Marine Environ Res 16:23-36

Zar JH (1999) Biostatistical analysis. Prentice Hall, New Jersey