Bull. Environ. Contam. Toxicol. (2002) 69:393–400

DOI: 10.1007/s00128-002-0075-9

Bulletin of Environmental Contamination and Toxicology

Copper Acute Toxicity Tests with the Sand Crab *Emerita analoga* (Decapoda: Hippidae): A Biomonitor of Heavy Metal Pollution in Chilean Coastal Seawater?

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Received: 15 August 2001/Accepted: 9 March 2002

The study of Chilean sandy beach organisms is today limited, in spite of the large distribution of some species along ca. 4.600 km of coast (Fernández et al. 2000). In general, the Chilean coastal marine environments induce an increasing interest to developing new methods and programs for ecotoxicological assessment, especially using native species (Zúñiga et al. 1995; Riveros et al. 1996; Larraín et al. 1999; Hernández et al. 2000). As a first approach, it is important to develop studies that permit the identification and future use of biomonitoring species.

Emerita analoga (Stimpson 1857) is a small suspension-feeder that lives in high-energy coastal beaches (Barron et al. 1999a). This species shows a bipolar geographical distribution between North and South American coasts, being absent in equatorial areas (Efford 1976). In North America it is found from Karluk (Alaska) to Magdalena Bay (California), and in South America from Salaverry (Perú) to the Estrecho de Magallanes (Chile), crossing through the Atlantic and extending their distribution throughout to False Bay (Argentina) (Efford 1976). The wide geographical distribution could be a consequence of the *E. analoga* having a zoea stage with a long persistent time in the plankton going from several weeks to months and a megalope stage living several weeks in the plankton (Efford 1970). This crustacean species could be an important potential ecological receptor of pollutants, especially because is one of the most abundant species in the intertidal zone and is an important source of food for coastal birds and fishes (Dugan et al. 1994, 1995). In the coast of California, *E. analoga* may reach densities higher than 52.000 ind m^{-2} (Dugan et al. 1995). This situation has also been reported for Chilean sandy beaches (Hernández et al. 1998; Jaramillo 1987, 1994).

Populations of *E. analoga* have been employed for environmental quality biomonitoring (Wenner 1988) and this species can also accumulate heavy metals and petroleum hydrocarbons from the environment (Burnett 1971; Rossi et al. 1978; Wenner 1988). Taking into account results from cadmium acute toxicity, Hernández et al. (2000) have suggested that *E. analoga* could be a good bioindicator for monitoring Chilean coastal environments. In spite of the high number of toxicity test studies with crustaceans, the sand crab has received a limited attention in the ecotoxicological field (Barron 1999a).

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Siegel and Wenner (1984) described abnormal reproduction of *E. analoga* near a nuclear power plant and Boese et al. (1997) reported that this species showed an intermediate sensitivity when was exposed to fluoranthene and cadmium compared to six other marine crustacean species.

The main objective of this work was to estimate the sensitivity of *E. analoga* to copper through a 10-day acute toxicity test under controlled laboratory experimental conditions and to discuss the potential use of this species for biomonitoring heavy metal pollution in Chilean coasts. Copper was used as a reference toxicant because during the last decade there was an increased interest to quantify copper effects in Chilean marine organisms and to relate them to seawater and sediment copper concentrations (Salamanca and Camaño 1994; Zúñiga 1998). Recently, the Chilean government has developed a particular interest in establishing environmental quality criteria. It is evident that the first step to fulfil it is to have quantitative scientific results with standardised toxicity tests using native species.

MATERIALS AND METHODS

E. analoga, sediment and seawater, were collected from Pingueral beach (36° 30' S; 72° 54' W) in December 1999. Pingueral is situated at 46 km to the North of Concepción and was selected as collection site because of its low degree of anthropic intervention and exposure to the open sea. Seawater and sediment (passed through a 4 mm screen sieve in the field to remove debris and indigenous organisms) were taken from the collection site and transported to the laboratory for toxicity tests. Sand crabs were collected during low tide and transported to the laboratory at densities lower than 100-ind m^{-2} in boxes of 5 L with oxygenated seawater. Acclimation was done for 10 days at 15 ± 1 °C in a 50 L aquarium with filtrated seawater over 5 cm of sediment. Photoperiod was 16:8 (using a tungsten lamp of 10-20 $\mu E/m^2/s$). Seawater was replaced each 48 h and sand crabs were fed with ground fishmeal (0,5 g d^{-1}). Individuals with erratic behaviour during acclimation (*e.g.* not buried or with bad swimming) were discarded.

The experimental design corresponded to a modification of ASTM standard toxicity tests with amphipods (ASTM 1990), adapting the experimental conditions to *E. analoga*. The sand in the toxicity tests showed a medium size particle of 0.65 mm and an organic matter content of 0.49% (determined following Buchanan 1984). At the moment of the organism collection, seawater showed a mean temperature of 15°C, a mean salinity of 30 ppt, a mean pH of 7.90 and a mean dissolved oxygen concentration of 8.9 mg L^{-1}.

Mean size of experimental organisms was between 8-10 mm with a mean weight of *ca.* 0,1 g. The experimental chambers were charged with not more than 1 g of live individuals per litre of seawater. Individuals of this size are sexually immature, characteristic diminishing possible variations associated to the sex. The experiments were made in static chambers with an exposure time of 10 d. A random experimental design was used with 4 replicates per treatment and 10 individuals per chamber.

Nominal concentrations of Cu^{+2} were 0 ppm (control) and 0.5, 0.75, 1.0, 2.0, 4.0, 6.0 and 10.0 ppm. Experimental copper concentrations were prepared from a stock solution of copper chloride Tritrizol Merck dissolved in filtrated seawater (0.45 μm). Toxicity tests were made in 1 L glass chambers, using a sand-substrate of mean size of 0.65 mm, free of organic matter. Elimination of organic matter was made through calcination at 550°C during 3 h and washing with filtrated seawater to eliminate ashes. Then, the sediment was dried at 70°C for 48 h. Thus, the ASTM method (ASTM 1990) was modified eliminating the highest possible quantity of pollutants from experimental sediments before starting the toxicity tests, which is very difficult to manage in sediment toxicity tests (Ditsworth et al. 1990).

Taking into account that *E. analoga* is a benthic suspension-feeder, the metal was put in the seawater column, leaving the sediment practically free of pollutant and avoiding copper uptake from the sediment, which made the organisms more sensitive during the development of the tests (DiToro et al. 1990).

Toxicity tests were static and executed in a room under a controlled temperature to 15 ± 1°C and a photoperiod similar to acclimation. Experimental chambers were disposed in a random distribution and were continuously oxygenated during the 10-day tests. Mortality was quantified daily, using as a criterion for death when an individual mechanically stimulated did not show mobility. The LC_{50} was calculated using the maximum likelihood Probit method (Barron et al. 1999b).

RESULTS AND DISCUSSION

The results showed that *E. analoga* have moderate sensitivity to dissolved copper in seawater. Mortality was not found in the control treatments after 10-days exposure. After 24 h first acute toxicity was detected at copper concentrations of 4.0 ppm or higher (2.5 % mortality) (Figure 1). 100% mortality was reached after 48 h at copper concentrations of 10.0 ppm. After 10-days exposure, copper concentrations of 0.5, 1.0 and 2.0 ppm showed lower mortalities than higher copper concentrations. In the lowest copper concentration (i.e., 0.5 ppm) first acute effects were detected after 9 days of exposure.

Daily LC_{50} estimates were between 8.6 ±1.1 ppm at 24 h and 2.4 ± 0.3 ppm at 72 h. From 96 h to 10-day exposures, LC_{50} values progressively decreased from 2.0 ± 0.2 ppm to 1.2 ± 0.1 ppm (Figure 2). No results on copper toxicity tests for *E. analoga* have been reported before this work. However, comparing obtained LC_{50}-24h with previous literature values for another 32 coastal marine species of North America (Gauthier and Early 1998), the sensitivity of *E. analoga* to copper is relatively low. This species can be located in the upper logarithmic quartile of species sensitivity (1 – 10 ppm), only being overpassed by *Rangia cuneata.*

Similar to the coast of California (Barron et al. 1999a), *E. analoga* could be used as a heavy metal biomonitoring species to assess environmental quality of sandy beaches along the Chilean coasts. However, owing to the differences in climatic and

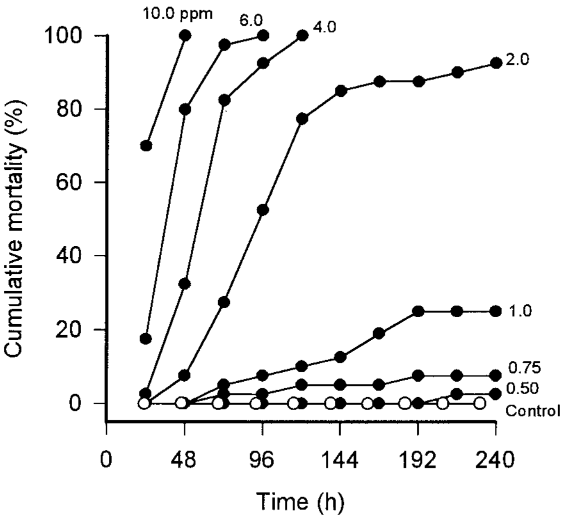

Figure 1. Cumulative mortality (%) of juvenile sand crabs (*Emerita analoga*) from Southern Chile exposed to different dissolved copper concentrations in seawater.

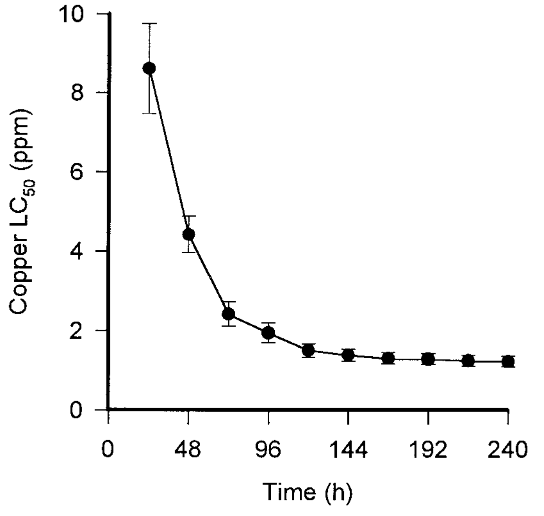

Figure 2. Variation in copper LC_{50} (mg/L) with juvenile sand crabs (*Emerita analoga*) from Southern Chile. Vertical bars show confidence intervals at 95%.

oceanographic characteristics between the two sites, it is likely that the specific-pollutant responses of *E. analoga* will show local variations related to site-specific population dynamics. Regarding this point, to compare results with populations obtained in different localities along the coast of Chile, it could be a requisite to verify the local-specific population sensitivity through the comparison of the toxicity responses with a reference toxicant, using similar standard experimental conditions.

According to Chuecas (1998), biomonitors or bioindicators are organisms employed to quantify pollutant abundance or bioavailability through the pollutant measurement in their tissues. According to assumptions of Phillip (1990), *E. analoga* fulfils four fundamental requisites to be a biomonitoring species: a) sessile or sedentary being representative of any sand beach study area; b) tolerate high levels of pollutants (e.g., heavy metals), wide range of salinity (i.e. can be found in estuaries) and adequate species for laboratory studies (e.g., toxicity tests and perhaps in studies of pollutant kinetics); c) abundant in the study area, easily identifiable, can be sampled in high numbers and provide sufficient soft tissues for pollutant analyses, and d) resistant to manipulation stress in laboratory studies and perhaps to the field transplants. Currently, no information is available for this species dealing with the relationship between environmental concentrations and soft tissues concentrations of pollutants.

E. analoga is an important ecological link having high densities in sandy beaches of high energy and is an important food resource for coastal birds and fishes (Jaramillo 1994). Otherwise, sand crabs have the capacity to bioaccumulate different pollutants from the environment (Wenner 1988). The low sensitivity of *E. analoga* to copper and cadmium and their potential capacity to accumulate heavy metals, makes it an adequate biomonitoring species to assess the bioavailability of heavy metals, such as been postulated for Chilean mussel bivalves (Valdovinos et al. 1998). Crustacean species more used in toxicity tests or monitoring programs in North America are *Mysidopsis bahia* and *Holmesimysis costata*, and are representative of estuarine and subtidal coastal seawaters. However, these species do not have the ecological relevance that *E. analoga* shows in sandy beaches (Barron 1999a).

Several behavioural ecological aspects of *E. analoga* indicated that this is an adequate biomonitoring species. Their post larval stages show a limited and aggregated spatial distribution in the intertidal (Cubit 1969). Hydrocarbon slides produced by oil-spills sometimes cover the sandy beach sediments, resulting in a potential risk for crab populations, because the individuals have a vertical migration with tides through a continuous process of emergence and boring (Cubit 1969).

In addition to the uptake of pollutants from the pore water phase of sediments, the characteristic of suspension feeding (Efford 1976) permits to *E. analoga* the possibility to incorporate pollutants through the food. The establishment of biomonitoring species to assess the Chilean sandy beach "health" and in a larger scale the entire Chilean coast is a priority necessity for the environmental marine risk assessment of coastal ecosystems. The development of accurate and standardised sediment toxicity tests and the field use of sediment bioaccumulation studies (Burnett 1971; Rossi et al. 1978; Wenner 1988) through one or more common Chilean intertidal species could become strong tools for the assessment and monitoring of coastal anthropogenic impacts.

Acknowledgments. We thank Dr. R. Swartz, Dr. W. Pelltier of U.S. EPA, Dr. L. Chuecas, D. Stead by support in methodological aspects. Finally, we thank DIUC of the Universidad de Concepción for their help during the research. This work was financed by the Programa FONDAP en Oceanografía y Biología Marina #3.

REFERENCES

ASTM (1990) Standard guide for conducting 10-days static sediment toxicity tests with marine and estuarine amphipods. American Society for Testing and Materials ASTM Designation E 1367-90: 24 pp

Barron MG, Podrabsky RS, Ogle RS, Dugan JE, Flicker RW (1999a) Sensitivity of the sand crab *Emerita analoga* to a weathered oil. Bull Environ Contam Toxicol 62: 469-475

Barron MG, Podrabsky RS, Ogle RS, Dugan JE, Flicker RW (1999b) Sensitivity of the mysid *Mysidopsis bahia* to a weathered oil. Bull Environ Contam Toxicol 62: 266-271

Boese Bl, Lamberson JO, Swartz RC, Ozretich RJ (1997) Photo-induced toxicity of fluoranthene to seven marine benthic crustaceans. Bull Environ Contam Toxicol 32: 389-393

Buchanan JB (1984) Sediment analysis. In: Holmes NA and AD McIntyre (eds) Methods for the study of marine benthos: 41-65. Blackwell Scientific Publications, Norfolk.

Burnett R (1971) DDT residues: Distribution of concentration in *Emerita analoga* (Stimpson) along the coastal California. Science 174: 606-608

Chuecas L (1998) Programa de monitoreo versus programa de biomonitoreo en el ambiente acuático. In: Arcos D (ed) Minería del Cobre, ecología y ambiente costero: 407-450. Editora Anibal Pinto S.A., Chile

Cubit J (1969) Behavior and physical factors causing migration and aggregation of the sand crab *Emerita analoga* (Stimpson). Ecology 50: 118-123

DiToro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mayr SM, Redmond MS (1990) Toxicity of cadmium in sediments: The role of acid volatile sulphide. Environ Toxicol Chem 9: 1487-1502

Ditsworth GR, Schultz DW, Jones JKP (1990) Preparation of benthic substrates for sediment toxicity testing. Environ Toxicol Chem 9: 1523-1529

Duggan JE, Hubbard DM, Wenner AM (1994) Geographic variation in life history in populations of the sand crab *Emerita analoga* Stimpson, on the California coast: relationships to environmental variables. J Exp Mar Biol Ecol 181: 255-278

Duggan JE, Hubbard DM, Page HM (1995) Scaling population density to body size: tests in two soft sediment intertidal communities. J Coastal Res 11: 849-857

Efford IE (1970) Recruitment to sedentary marine populations as exemplified by the sand crab, *Emerita analoga* (Decapoda, Hippidae). Crustaceana 18: 293-308

Efford IE (1976) Distribution of the sand crabs in the genus *Emerita* (Decapoda, Hippidae). Crustaceana 30: 169-183

Fernández M, Jaramillo E, Marquet P, Moreno C, Navarrete S, Ojeda P, Valdovinos C, Vásquez J (2000) Diversity, ecology and biogeography of Chilean benthic near-shore ecosystems: An overview and guidelines for conservation. Rev Chilena Hist Nat 73: 797-830

Gauthier R, Early P (1998) Copper regulatory status and status of water effects ratio: Navy perspective. In: Seligman PF and A Zirino (eds) Chemistry, toxicity and bioavailability of copper and its relationship to regulation in the marine environment: 10-17. Space and Naval Warfare Systems Center, Technical

Document 3066, San Diego
Hernández C, Contreras SH, Gallardo JA, Cancino JM (1998) Estructura comunitaria a lo largo de una playa arenosa de Chile central: Lenga, Bahía San Vicente. Rev Chilena Hist Nat 71: 303-311
Hernández C, Yánez R, Rudolph A (2000) Toxicity response of *Emerita analoga* (Stimpson, 1857) collected from beaches of South Central Chile. Bull Environ Contam Toxicol 65: 567-572
Jaramillo E (1987) Sandy beach macroinfauna from the Chilean coast: zonation patterns and zoogeography. Vie et Milieu 37: 165-174
Jaramillo E (1994) Patterns of species richness in sandy beach of South America. South African J Zool 29: 227-234
Larraín A, Riveros A, Silva J, Bay-Schmith E (1999) Toxicity of Metals and Pesticides Using the Sperm Cell Bioassay with the Sea Urchin *Arbacia spatuligera*. Bull Environ Contam Toxicol 62: 749-757
Lewis AG, Moffet J, Dibacco C, Levin L (1998) Copper interactions with biota. In: Seligman PF and A Zirino (eds) Chemistry, toxicity and bioavailability of copper and its relationsip to regulation in the marine environment: 28-31. Space and Naval Warfare Systems Center, Technical Document 3066, San Diego
Luoma SN, Steinert S, Streib-Montee R, Weis P (1998) The effects of copper on marine organisms In: Seligman PF and A Zirino (eds) Chemistry, toxicity and bioavailability of copper and its relationship to regulation in the marine environment: 36-39. Space and Naval Warfare Systems Center, Technical Document 3066, San Diego
Mohan, CV, Menon NR, Gupta TRC (1984) Acute toxicity of cadmium to six intertidal invertebrates. Fish Technol 21: 1-5
Moffett J, Paquin P, Mayer L (1998) Copper bioavailability. In: Seligman PF and A Zirino (eds) Chemistry, toxicity and bioavailability of copper and its relationship to regulation in the marine environment: 32-36. Space and Naval Warfare Systems Center, Technical Document 3066, San Diego
Phillips DJH (1990) Use of macroalgae and invertebrates as monitors of metal levels in estuaries and coastal waters. In: Furness RW and PS Rainbow (eds) Heavy metals in the marine environment: 81-99. CRC Press Inc. Boca Raton, Florida
Riveros A, Zuñiga M, Larraín A, Becerra J (1996) Fertilization relationships of the Southeastern Pacific sea urchin *Arbacia spatuligera* and environmental variables in polluted coastal waters. Mar Ecol Prog Ser 134: 159-169
Rossi SS, Rommel GW, Benson AA (1978). Hydrocarbons in sand crabs (*Emerita analoga*) from southern California. Chemosphere 2: 131-141
Salamanca MA, Camaño A (1994) Historia de la contaminación por metales traza en dos áreas costeras del norte y centro-sur de Chile. Gayana Oceanol 2: 31-48
Siegel SR, Wenner AM (1984) Abnormal reproduction of the sand crab *Emerita analoga* in the vicinity of a nuclear generating station in southern California. Mar Biol 80: 341-345
Valdovinos C, Figueroa R, Cid H, Parra O, Araya E, Privitera S, Olmos V (1998) Transplante de organismos bentónicos entre sistemas lénticos: ¿Refleja la biodisponibilidad de metales traza en el ambiente? Bol Soc Chilena Quim 43: 467-475

Wenner AM (1988) Crustaceans and other invertebrates as indicators of beach pollution. In: Soule DF and GS Kleppel (eds) Marine Organisms as Indicators: 199-229. Springer-Verlag, New York

Zuñiga M, Roa R, Larraín A (1995) Sperm cell bioassays with the sea urchin *Arbacia spatuligera* on samples from two polluted Chilean coastal sites. Mar Pollut Bull 30: 313-319

Zuñiga, M (1998) Ecotoxicología de metales en el ambiente acuático: Una revisión actualizada. In: D. Arcos (Ed.), Minería del cobre, ecología y ambiente costero. Editorial Anibal Pinto SA Chile :349-376