

## **Sensitivity of *Ampelisca araucana* Juveniles (Crustacea: Amphipoda) to Organic and Inorganic Toxicants in Tests of Acute Toxicity**

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Toxicity bioassays are valuable tools to evaluate the degree of pollution of interstitial waters and sediments (Kemp and Swartz 1988; Williams 1992; Di Toro et al. 1991). Sediment-dwelling organisms are frequently used as test specimens in these procedures. Among these, adult and pre-adult stages of infaunal amphipods are preferred. The amphipod *Ampelisca araucana* is a soft sediment dweller, commonly found in soft sediments along the central Chilean coast (González 1991), previously used to assess toxicity in marine sediments in San Vicente Bay (Larrain *et al.* 1998(a)). It has a semiannual life history, with oviparous females found year-round (Carrasco and Arcos 1984). This provides an excellent opportunity to test recently eclosed juveniles, easily obtained in the laboratory from gravid females, collected in the field. Lower developmental stages of aquatic animals are generally more sensitive than advanced stages in ecotoxicological evaluations (Mayer and Ellersieck 1986). However, being a too delicate larva could be an inconvenience when resistance to handling and the artificial laboratory conditions needed for a robust experimental design are required (Larrain *et al.* 1997). Considering that water is a direct way of contaminant exposure of infaunal organisms, mainly as interstitial water, we designed this study to test the capacity of *A. araucana* juveniles to survive without sediment for the extent of an acute toxicity bioassay and to measure with this method their sensitivity to organic and inorganic toxicants dissolved in sea water.

### **MATERIALS AND METHODS**

Samples of soft sediment were taken with a Beckman dredge (0.027 m<sup>2</sup> mouth area) in Coliumo Bay (36° 31' S; 72° 55' W) and transported to the Laboratory of Bioassays at the University of Concepción at 13° C. Oviparous females were collected from the sediment using a 1000 µm mesh-size sieve, transferred to a 10 L white plastic recipient and fed regularly with the microalgae *Dunaliella tertiolecta* and *Isochrysis galbana*. In a 48 hour period, sufficient juveniles hatch to provide the 120 experimental specimens necessary for each bioassay.

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The compounds used in the bioassays were Pentachlorophenol, 2,4-D,  $\text{CuSO}_4$  and  $\text{K}_2\text{Cr}_2\text{O}_7$ . All experiments were performed at 13° C for a 48 hour period with a photoperiod regime of 16/8 L/D. Glass (20 mL) and plastic (40 mL) test chambers were used for the organic and inorganic toxicants, respectively. Each experiment consisted of 5 treatments plus a control, with 4 replicates and 5 organisms in each one.

In order to establish the concentrations of PCP and potassium dichromate to be used, a dilution series of 0.1, 1, 10, 100 and 1000 mg/L was prepared. A similar set of concentrations was prepared for  $\text{CuSO}_4$ , in  $\mu\text{g/L}$ . These were used as preliminary values, based on sensitivities established for comparable organisms (Larrain *et al.* 1999). PCP testing was performed with the following concentrations: 0.01, 0.1, 1, 10, 100 mg/L; 0.0125, 0.025, 0.05, 0.1 mg/L and 0.0625, 0.125, 0.25, 0.5, 1 mg/L. 2,4-D and potassium dichromate were 50, 100, 200, 400, 800 mg/L and 38.5, 55, 78.5, 112, 160 mg/L respectively. Copper concentrations as  $\text{Cu}^{++}$  ion were 62.5, 125, 250, 500, 1000  $\mu\text{g/L}$ .

During the test, the animals were not fed. At the end of the bioassay, dead individuals were counted and the proportion of dead amphipods was used as response variable to estimate the LC50 through Probit analysis (US EPA 1988).

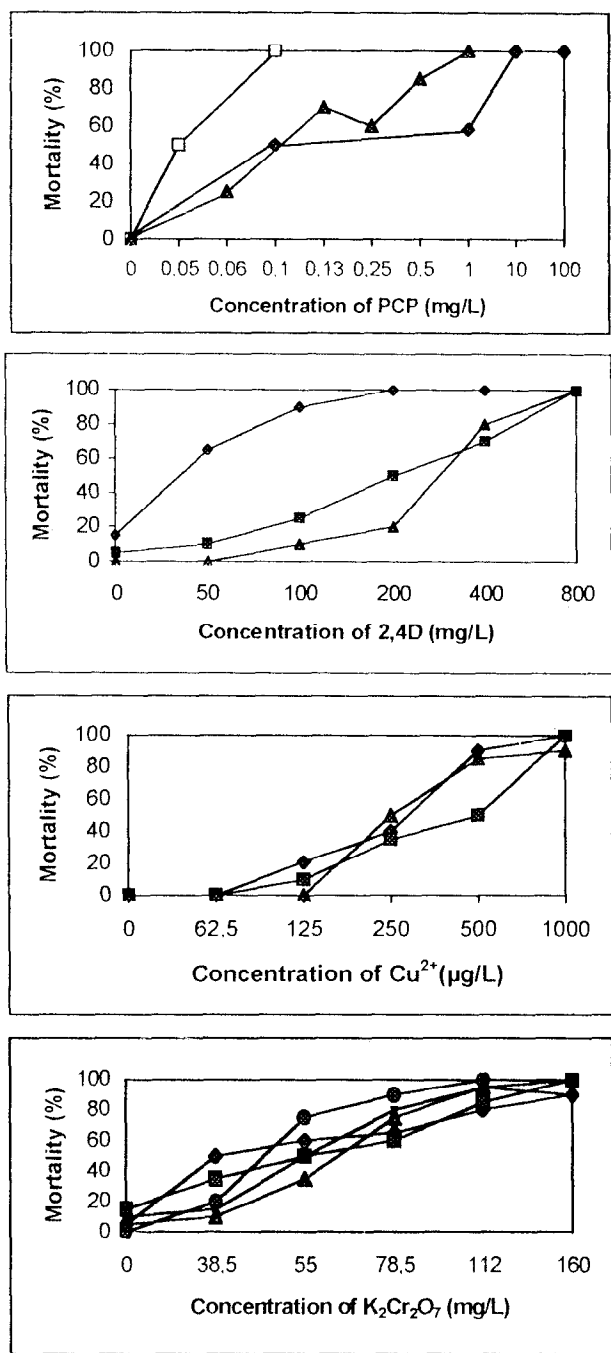
## RESULTS AND DISCUSSION

All controls resulted in low mortalities, under 20%, which indicates that the juveniles of this species can be maintained without sediment for at least the time needed in an acute toxicity test (Fig. 1).

The mean LC50, 48 hrs of the fungicide PCP in *A. araucana* juveniles was 0.09 mg/L  $\pm$  0.045 S.D. The mean LC50 of the herbicide 2,4-D was 91.2 mg/L  $\pm$  40.88 S.D.  $\text{CuSO}_4$  and  $\text{K}_2\text{Cr}_2\text{O}_7$  showed LC 50s of 305.5  $\mu\text{g/L}$   $\pm$  58.21 S.D. and 56.89 mg/L  $\pm$  9.76 S.D., respectively. The response curve of this marine amphipod to these chemical compounds is shown in Fig. 1.

Early developmental stages are, in general, more sensitive to stress conditions than adult forms. Juveniles of *Ampelisca araucana* in ecotoxicity test should reduce the risk of permitted acceptance of maximum concentrations of toxicants that might be unarmful to adults but deleterious to young. This amphipod has juveniles of a size that is easy to handle and resist well the absence of a sediment to bore in, along the two days of an acute test. Twenty eight percent of the tests performed had some level of mortality in the controls, but it was not significant.

Compared to other species from the coast of Chile that were exposed to the same substances in toxicity bioassays, juveniles of *A. araucana* were less sensitive to metals and more sensitive to organic compounds.



**Figure 1.** Mortality of two days old *A. araucana* exposed for 48 hrs to concentration gradients of different toxicants.

The mean LC50 of  $K_2Cr_2O_7$  found in this study was 56.89 mg/L. The same toxicant required 25 mg/L at 13° C to kill 50% of a population of the harpacticoid copepod *Tisbe longicornis* (Larrain *et al.* 1998(b) and 117 mg/L to inhibit to 50% the fertilization rate of the sea urchin *Arbacia spatuligera* (Larrain *et al.* 1999). With  $Cu SO_4$ , the mean LC50 for the amphipod was 305.5 µg/L, whereas the same response in *Tisbe longicornis* was obtained at 185 µg/L (unpublished data) and the EC50 in *Arbacia spatuligera* at 17.2 µg/L (Larrain *et al.* 1999). The response of *A. araucana* to the herbicide 2,4-D revealed a mean LC50 of 91.2 mg/L and for the fungicide PCP, 0.09 mg/L. Although there are no toxicity evaluations for this organic compound on *T. longicornis*, the fertilization test with *Arbacia* gametes provided EC50 values as high as 205.4 mg/L for 2,4-D and 4.2 mg/L for PCP (Larrain *et al.* 1999).

Studies on other species have shown similar sensitivities for these toxicants in 48 hours of exposure. In adult *Tisbe holothuriae*, the EC50 of Cu and Cr was 370 µg/L and 370 mg/L, respectively (Verriopoulos and Dimas 1988). The LC50 of pure PCP estimated from adult daphnids, *Daphnia magna*, *D. pulex* and *D. galeata* were 1.78, 4.59 and 0.51 mg/L, respectively (Stephenson *et al.* 1991).

These results suggest that *A. araucana* juveniles are relatively sensitive to chemicals of inorganic and organic nature, and therefore convenient as test organisms for pollution studies of the interstitial water of sediments. This agrees with the studies of Kemp and Swartz (1988) Dewitt *et al.* (1989) and Di Toro *et al.* (1990), which indicate that amphipods are useful organisms for toxicity assessment of sediments.

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