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Sensitivity of the Meiofaunal Copepod *Tisbe longicornis* to $K_2Cr_2O_7$ Under Varying Temperature Regimes

A. Larrain, E. Soto, J. Silva, E. Bay-Schmith

Laboratorio de Bioensayos, Departamento de Zoología, Universidad de Concepción, Casilla 2407-10, Concepción, Chile

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The Chilean coastal environment is increasingly used as a sink for effluents from industrial, mining, port and urban activities, as a direct result of an increased economic activity. Consequently, there is a growing need to develop standard bioassays to assess the effect of this use on coastal communities. Zúñiga *et al.* (1995) and Riveros *et al.* (1996) demonstrated the usefulness of a sea-urchin sperm cell bioassay to assess the degree of pollution of the water column from several locations on the most polluted coastal region in central Chile. Larrain *et al.* (in press) evaluated sediment status within the same region using the epibenthic amphipod *Ampelisca araucana.* Moore and Bett (1989) concluded that a better estimation of the real extent of pollution in the sedimentary habitat may be achieved by considering meiofaunal organisms, and especially copepods, for these groups have a greater sensitivity than macrofaunal organisms. Harpacticoid copepods of the genus *Tisbe* are particularly useful as bioassay organisms due to their high abundance within the sediment, wide geographic distribution, short lifecycles, and their amenability to laboratory culture and handling (Williams 1992). In the laboratory however, several experimental factors may affect survival of *Tisbe.* For example, Williams and Jones (1994) found lower survival in *Tisbe battagliai* reared at higher temperatures and lower food concentrations. These factors should be taken into account when assessing sensitivity of *Tisbe* copepods in culture. Therefore, in this paper we report results from acute bioassays using the meiobenthic copepod *Tisbe longicornis* and the metal toxicant $K_2Cr_2O_7$. The experiments were conducted within a range of temperature regimes that encompassed thermal variations in natural communities, in order to assess the use of *T. longicornis* as an indicator of pollution status in the meiofaunal communities of coastal environments off central Chile. $K_2Cr_2O_7$ is used as a standard procedure and reference toxicant in the literature. We applied it in a series of bioassay species in our Laboratory.

Correspondence to: A. Larrain

MATERIALS AND METHODS

To establish a laboratory population of *T. longicornis,* samples of silt-clay sediments were taken at Coliumo Bay (36°50'S; 72°55'W), central Chile, and adult copepods were collected from the sediment using a 250 µm mesh-size sieve. The collection site is a small (ca. 7 km^2), unpolluted bay with a maximum depth of 18 m at the mouth, whose water column temperature fluctuates between 12° and 17° in any normal (non El-Niño) year. In the laboratory, individuals were placed in culture chambers (33 L glass aquaria) at 13°C with constant and gentle aereation, and were fed twice per week 200 mL of an equal mixture of the microalgae *Dunaliella tertiolecta* and *Isochrysis galvana.* Water culture consisted of filtered (0.5 µm) seawater from Coliumo Bay, changed every 15 days. At about 21 days, females were fully ovigerous and larvae started to hatch. This population has been successfully maintained in the laboratory for almost 2 years. Adult copepods 15 days old (about 0.6 mm length) were collected from the laboratory population and used for bioassaying.

We employed a completely random design for all bioassays, with seawater with similar characteristics as in the culture chambers. Three temperature levels (13°, 16.5° and 20°C; using a Tectron-100 thermic bath) were used with five concentration levels of $K_2Cr_2O_7$ at each temperature, depending on the results of preliminary tests aimed at detecting the range of activity of the toxicant, and one control level of zero toxicant concentration. Each experimental unit consisted of 5 individuals placed in 50 mL plastic chambers with 10 mL of seawater plus the toxicant at the specified concentration. The bioassays lasted 48 hours, during which individuals were not fed and were kept at on ambient photoperiod (16:8). There were 4 replicates per treatment, and each bioassay was repeated 6 times to evaluate the reproducibility of the results. At the end of the tests, dead individuals were counted, and the proportion of dead copepods was used as response variable for probit analysis (US EPA 1988) to estimate LC50.

RESULTS AND DISCUSSION

Little variability was observed between the replicates of the bioassays under similar conditions of temperature and toxicant concentration (each panel of Fig. 1). Sensitivity increased with temperature (Fig. 1). At 13°, 16.5° and 20°C 100% mortality was observed at 100, 50 and 22 ppm, respectively. The overlap of 95% confidence intervals for LC50 suggest significant differences in almost all replicates at the three temperature levels (Fig. 2). Variability, measured as the amplitude of the 95% confidence intervals, also differed among the 3 temperatures considered: variability decreased with increasing temperature (Fig. 3). We have studied the sensitivity of *Tisbe longicornis* with $K_2Cr_2O_7$ and Cu++ (unpublished data). Both its sensitivity and the consistency of its response to these toxicants suggest it is a convenient organism for bioassaying. Besides, it is abundant and occupies a basal

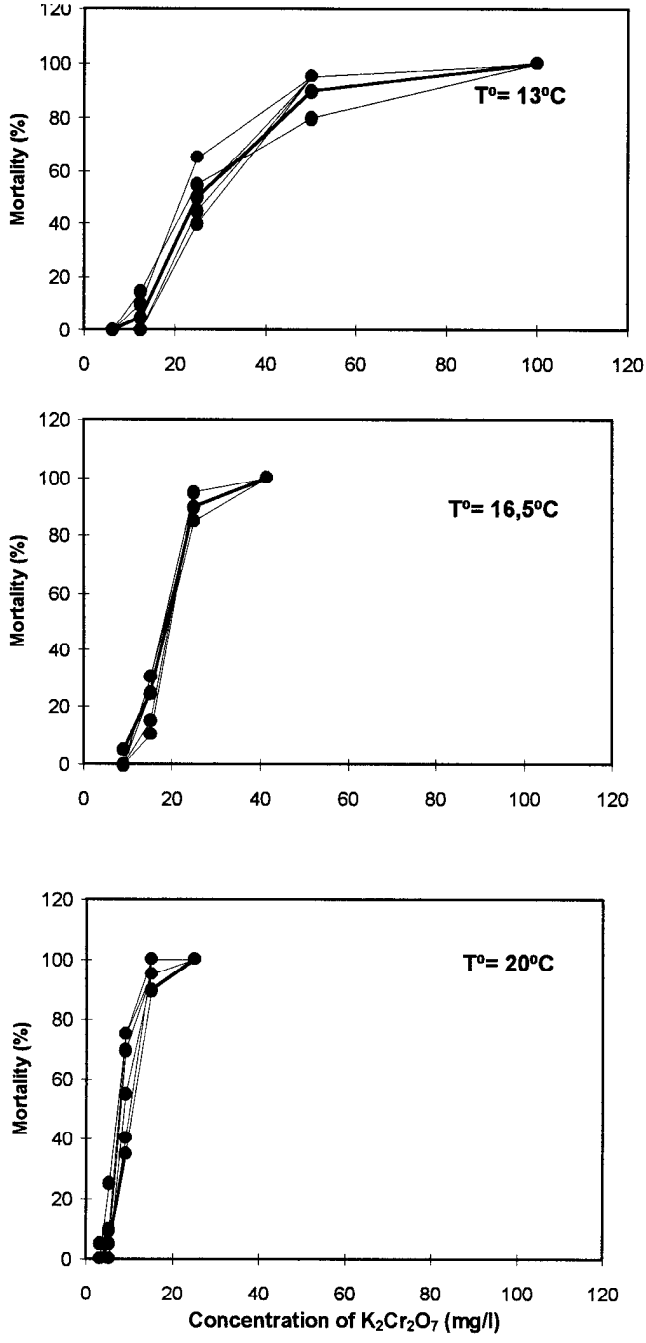

Figure 1. The effect of $K_2Cr_2O_7$ on survival of the meiofaunal copepod *Tisbe longicornis* under three temperature regimes. Each panel show six repetitions of the bioassays.

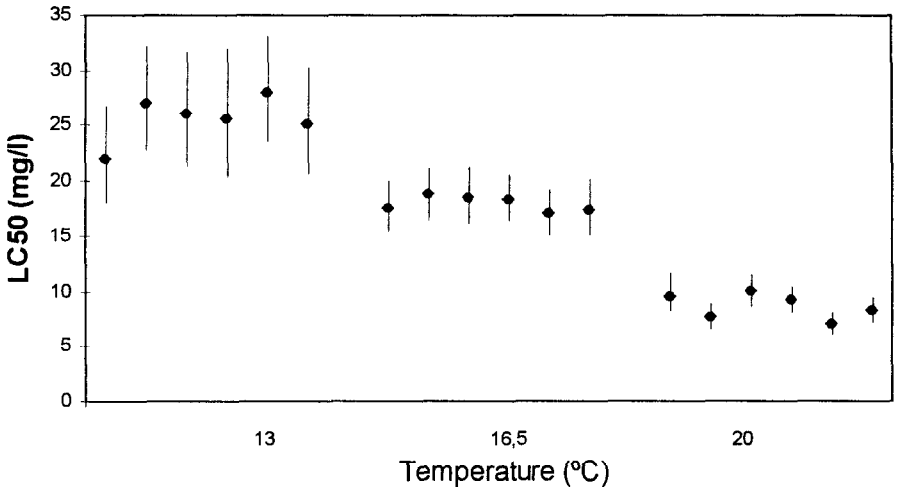

Figure 2. Estimated LC50s of $K_2Cr_2O_7$ on the meiofaunal copepod *Tisbe longicornis* under three temperature regimes, and confidence intervals.

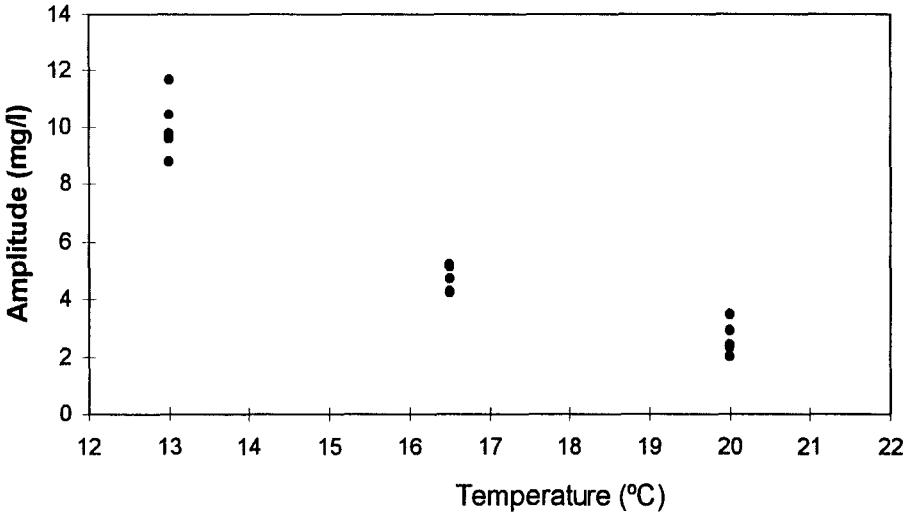

Figure 3. Statistical variability in LC50 estimation of $K_2Cr_2O_7$ on the meiofaunal copepod *Tisbe longicornis* under three temperature regimes.

central Chile. It is easy to collect and to establish permanent laboratory populations, it has a short life cycle with abundant offspring, is easy to handle in the laboratory. These features have been proposed as fundamental to consider a candidate organism as appropriate for establishing pollution standards (Persoone and Janssen 1993; Widdows 1993). However, the response of *T. longicornis* to the metal toxicant tested depended on temperature, to the point that LC50 decreased by more than half and the amplitude of the 95% confidence interval decreased to one fifth with an increase in temperature from 13° to 20°C. Williams and Jones (1994) observed a similar, strong effect of temperature on survival of laboratory reared *T. battagliai* Other experiments using different species of copepods also suggest that temperature is among the most important environmental factors that influences sensitivity to various toxic compounds (Verriopoulos et al. 1981, Lethinen et al. 1984, Cooney et al. 1984, Maruno et al. 1992, Damgaard and Davenport 1994). In another study, toxicity of chromium to the marine planktonic copepod *Acartia clausi* increased with temperature (Moraitou-Apostolopoulou and Verriopoulos 1982).

Variations in environmental conditions (pH, temperature, salinity) affect the sensitivity of individuals to toxic chemicals. Hence, when evaluating the potential effects of pollutants, it is important to know how changes in test conditions influence toxicity. Since temperature is one of the environmental conditions standardized in lethal and sub-lethal toxicity tests, it follows that the temperature used for bioassaying with *T. longicornis* must be tuned to thermal levels observed at the sites in which pollution status is to be evaluated.

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