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Effects of Temperature on the Acute Toxicity of Heavy Metals (Cr, Cd, and Hg) to the Freshwater Crayfish, *Procambarus clarkii* (Girard)

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The discharge of heavy metals by industry represents a serious water pollution problem due to the toxic properties of these metals and their adverse effects on water quality.

Chromium, an essential trace element for humans and animals, is involved in normal carbohydrate metabolism (Mertz 1969); however, it is toxic at high concentrations. There is no evidence that cadmium and mercury are biologically essential but their toxicity for organisms is well known. Both cause toxic effects at low concentrations to most organisms, especially in combination with other environmental variables such as temperature (Green et al. 1976).

Lake Albufera and the surrounding rice field waters are subjected to very heavy loads of sewage and toxic industrial residues (including heavy metals) from the many urban and wastewaters in this area (Roselló 1983). In 1978, the American red crayfish *Procambarus clarkii* appeared in Lake Albufera and in the surrounding rice fields. Without adequate sanitary control, the crayfish is presently being fished commercially for human consumption.

The purpose of the present study is to evaluate the degree of toxicity of various heavy metals (chromium, cadmium and mercury) to freshwater crayfish *Procambarus clarkii* at various temperatures.

MATERIALS AND METHODS

Adult intermolt specimens of the crayfish *Procambarus clarkii* were collected in Lake Albufera (Valencia, Spain) and taken immediately to the laboratory where they were maintained to 300-L aquaria and for 15 days, at 20°C with a daily diet of pork liver. Those specimens are known to contain chromium and cadmium residues after 15 days in tap water (Hernandez et al. 1986; Diaz Mayans et al. 1986).

Groups of ten crayfish were kept in tap water at several metal concentrations, each group in a 15-L experimental aquarium. Ten more crayfish used as controls were kept in 15-L clean water. Only crayfish weighing between 15 and 20 g were used.

Desired chromium concentrations were obtained by addition of appropiate amounts of stock solutions, which were prepared using tap water and $Na_2CrO_4.4H_2O$ (E. Merck).

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Reagent grade $CdCl_2 \cdot H_20$ and $HgCl_2$ (E. Merck) were made up to a stock solutions of 1 mg Cd^{++}/mL and 0.1 mg Hg^{++}/mL, respectively. Aliquots of these solutions were added to each test aquaria to achieve the appropiate concentrations.

Water quality in each aquaria was monitored daily for changes in pH and oxygen concentration, which ranged from 7 to 7.5 and 80% to saturation, respectively. Other variables were determined at the beginning of each experiment: alkalinity, hardness and chloride concentration, which ranged from 3.8-4.4 mmol/L, 180-300 mg $CaC0_3$/L and < 0.1 mg/L, respectively.

All tests have been conducted under "static conditions". In preliminary experiments the most suitable metal concentration ranges for acute toxicity tests were determined. Toxicity tests were carried out in a thermostated (± 1°C) water baths. No food was added during the experiments to avoid adsorption and/or chemical interactions of metal ions. Animals to be used in the toxicity tests were acclimated to the test conditions for at least two days before they were exposed to the metal. During acclimation and toxicity test period all aquaria were aerated. The 96 h period was that are recommended in the literature (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975).

The lack of movement by the pleopods and antennae when gently prodded was used as the criterion for animal death. Although pleopod movement is often taken by workers as the sole criterion, our experience is that it is not always reliable; pleopods may remain stationary for considerable periods in animals close to death whereas antennal movement can often still be stimulated. Animals were observed twice every day. Death animals were removed after observation in order to avoid cannibalism.

The percentages of mortality were calculated in each concentration after 96 h of exposure and converted to probits (Fisher and Yates 1963); the metal concentrations were converted to Logs. The concentrations causing 50 % mortality of the test animals, the LC50's and their 95 % confidence limits and the slope of the probit line were calculated using the method of Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

Preliminary experiments on the toxicity of chromium showed that after 96 h a concentration of 0.5 g Cr(VI)/L caused the death of only 40% of the population. Such elevated concentrations did not occur in the natural medium (Rosello 1983) and we did therefore not perform the acute toxicity for chromium.

Table 1. Slopes and intercepts of the individual regression lines for mercury and cadmium (Y= % mortality, X=Log. concentration in mg/L).

Mercury	Cadmium
20°C: $y = 4.50\,x - 7.85$; $r^2 = 0.94$	20°C: $y = 2.96\,x - 0.23$; $r^2 = 0.99$
24°C: $y = 2.34\,x - 0.94$; $r^2 = 0.99$	24°C: $y = 5.24\,x - 3.08$; $r^2 = 0.99$
28°C: $y = 2.71\,x - 0.81$; $r^2 = 0.98$	28°C: $y = 2.15\,x + 2.28$; $r^2 = 0.97$

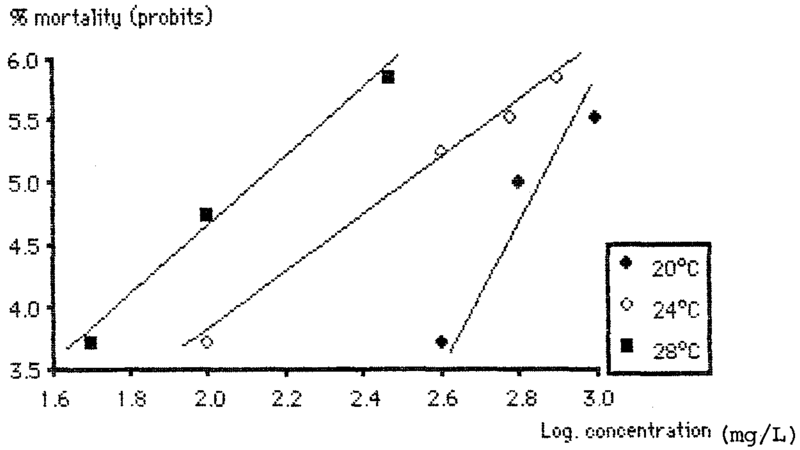

Figure 1. Mortality/Mercury-concentration relations for *Procambarus clarkii* at 20, 24 and 28°C.

A summary of the acute toxicity results for cadmium and mercury at various temperatures is given in the Table 2, showing the 96-h LC50 values with 95% confidence limits. It must be emphasized that all LC50's are based on the initial amount of metal added to the dilution water and that after 96 hours the concentrations may have been somewhat less than those indicated.

Figures 1 and 2 show the % mortality (converted to probits) after 96 h of mercury and cadmium exposure with respect to the metal concentrations at three temperatures (20, 24 and 28°C). Regression curves were fitted by the least squares method. Slopes and intercepts of the individual regression lines is given in the Table 1.

The responses of crayfish to mercury and cadmium was further investigated with respect to different exposure times (Figures 3 and 4). In general the increase in percent mortality was related to both time and metal concentration, with the highest mortality ocurring after 48 h of metal exposure. However, in the case of mercury at the highest temperatures (24 and 28°C), the highest mortality occurred between 24 and 72 h for 24°C and between 24 and 48 h for 28°C.

Table 2. The 96-h LC50 values (mg/L) and 95% confidence limits for mercury and cadmium at 20, 24 and 28°C with *Procambarus clarkii*. Each 96-h LC50 value represents the mean of 3 replicates.

Temperature (°C)	Mercury	Cadmium
20	0.79 (0.58 - 1.08)	58.5 (41.8 - 81.9)
24	0.35 (0.21 - 0.56)	34.8 (28.1 - 43.2)
28	0.14 (0.08 - 0.23)	18.4 (10.7 - 31.6)

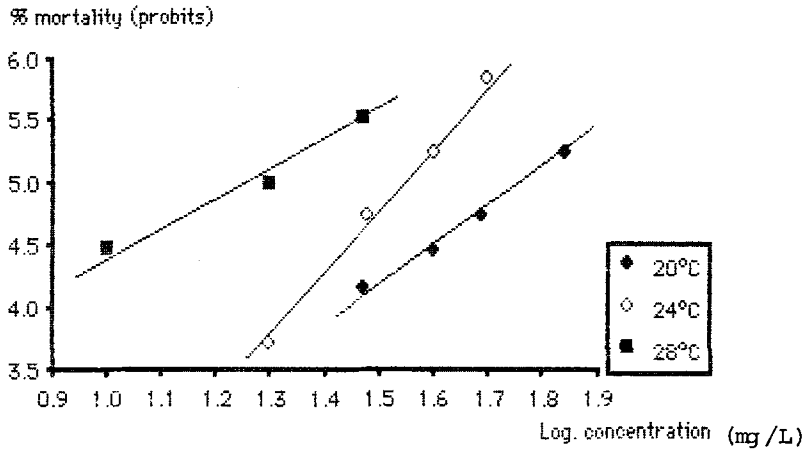

Figure 2. Mortality/Cadmium-concentration relations for *Procambarus clarkii* at 20, 24 and 28°C.

Results from this series of 96 hr-LC50 tests suggest that mercury has the most toxic effect on the *Procambarus clarkii*, followed by cadmium and chromium. These findings are in agreement with other authors. For example, Eisler and Hennekey (1977) reported the acute toxicity of cadmium, chromium, mercury, nickel and zinc to six estuarine macrofaunal species. In general, the rank order of toxicity of metals tested was Hg » Cd » Zn › Cr › Ni.

Ahsanullah (1982) studied the acute toxicity of chromium and mercury to the amphipod *Allorchestes compresa*. His results suggest that mercury has the most toxic effect, followed by chromium. Papathanassiou (1983) studied the effects of cadmium and mercury ions upon the longevity of *Palaemon serratus* and suggested that mercury is more toxic than cadmium.

Our results compared with literature data presented by Ahsanullah (1982) and Lake et al. (1979) show that *Procambarus clarkii* has very high LC50 for all heavy metals tested, except for mercury; that *Orconectes limosus* shows a 20°C 96-h LC60 = 1 mg Hg/L (Doyle et al. 1976); and that in the case of cadmium *Uca pugilator* present a 20°C 96-h LC50 = 6.6 mg Cd/L (O'Hara 1973). In comparison with the other metallic salts commonly found in polluted waters, mercuric chloride is far more toxic than all of them (Cairns et al. 1975).

In the present study we have found that the toxic effects of cadmium and mercury increased in parallel to the increase temperature. The effect of temperature on the mercury toxicity was more marked than in the cadmium toxicity. So when the temperature increases from 20 to 24°C the mercury LC50 decreased 56 % whereas the cadmium LC50 decreased 40%, and, when the temperature increased from 24 to 28°C, the LC50 decrease was 60% and 47% for mercury and cadmium, respectively.

A marked influence of temperature on the toxicity of heavy metals has been demonstrated for different aquatic animals. In general, the higher the temperature is, the more toxic the compounds will be (Cairns et al. 1975).

The relationship between heavy metals toxicity and temperature variation illustrates that physiological stresses lower the tolerance of organisms to

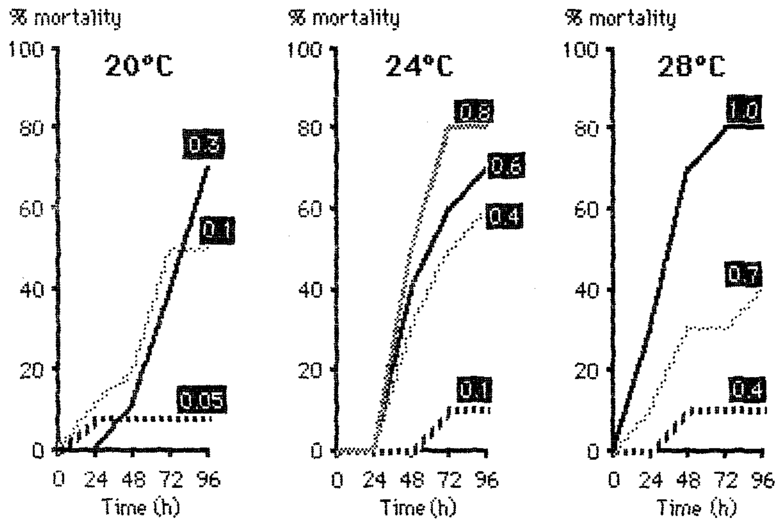

Figure 3. Percent mortality versus exposure time for crayfish exposed to various concentrations (black squares) of mercuric chloride (mg/L) at three temperatures.

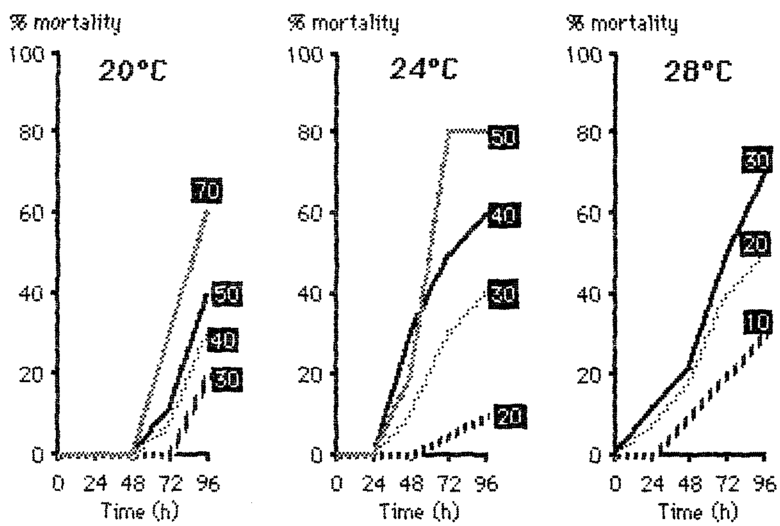

Figure 4. Percent mortality versus exposure time for crayfish exposed to various concentrations (black squares) of cadmium chloride (mg/L) at three temperatures.

environmental pollutants (O'Hara, 1973).

In conclusion, the *Procambarus clarkii* from Albufera Lake, present a high resistance to the heavy metals pollution. In previous reports, we have shown that this crayfish accumulated great amounts of chromium (Hernandez et al., 1986) and cadmium (Diaz Mayans et al. 1986) after exposure to these metals. The importance of metallothioneins in the detoxification events of heavy metals is well known (Ridlintong and Fowler 1979; Engel y Brouwer 1984). These kinds of mechanisms are probably related to the resistence and accumulation ability of heavy metals in *Procambarus clarkii*.

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