

Effect of Chromium and Cadmium on the Thermal Tolerance of the Prawn *Macrobrachium rosenbergii* Exposed to Hard and Soft Water

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In recent years there has been considerable research conducted on the effects of sublethal concentrations of heavy metals on the physiology of decapod crustaceans. Among these findings it has been reported that exposure to sublethal concentrations of Cr and Cd affect respiration in Callinectes similis (Ramirez *et al.* 1989), that Cd affects the osmoregulatory ability of C. sapidus (Frankard and Robertson 1979) and that there is a pattern for the accumulation and elimination of Cr in Xantho hidrophilus (Paternac and Legović 1986).

Some authors have demonstrated that tolerance to temperature, measured as the Critical Thermal Maximum (CTM) may be useful in evaluating the effects of sublethal concentrations of contaminants on poikilothermal aquatic organisms (Carrier and Beitinger 1988; Chagnon and Hlohowskyj 1989; Watenpough and Beitinger 1985). The usefulness of CTM in these studies is based on the hypothesis that it is an indicator of the general physiological state of the organism, which is altered in the presence of a contaminant (Paladino *et al.* 1980).

The Malay prawn Macrobrachium rosenbergii has been introduced into numerous countries of the world for the purpose of aquaculture. The great variety of environments into which this species has been introduced has resulted in the animals being frequently cultivated in water of a different origin as well as in the presence of sublethal levels of contaminants. Taking this into account, and considering that Cr and Cd are widely distributed environmental contaminants, this study was planned to define the effect of sublethal concentrations of Cr⁶⁺ and Cd²⁺ on the thermal tolerance of M. rosenbergii in freshwater with different levels of hardness.

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MATERIALS AND METHODS.

Juveniles (320) *M. rosenbergii* weighing between 2.4 and 5.16 g weight were obtained from a commercial culture farm. The animals were placed in eight 100-L glass tanks at a density of 0.8 animals/L. During 8 days of acclimation the prawns were kept at $20 \pm 1^\circ\text{C}$, 6.3 ± 0.6 mg O_2/L , 138 mg CaCO_3 of hardness, pH 7.5 and were fed a commercial balanced feed (shrimp feed of 35% of protein from Purina[®]) at a daily ratio of 10% of their body weight. Animals were kept randomly in two eighteen-tank groups: one had hard water (137.2 ± 0.3 mg CaCO_3/L) and the other soft water (47.7 ± 0.2 mg CaCO_3/L). The two types of water were prepared with reconstituted distilled water (EPA 1989). The animals remained in the new water for 96 hr before being exposed to the contaminants. Fifty percent of the water was changed daily to avoid the accumulation of metabolic products. Eighteen tanks of each water hardness were randomly chosen for the exposure of the prawns to two sublethal levels of Cr^{6+} and Cd^{2+} , with controls for each metal and hardness. The sublethal concentrations of metals used, 0.02 and 0.05 mg Cd^{2+}/L as CdSO_4 and 0.35 and 0.7 mg Cr^{6+} as $\text{K}_2\text{CrO}_4 \cdot \text{H}_2\text{O}$, were previously calculated by Ramirez (1992). The CTM Was measured after exposure times of 24, 48 and 96 hr.

The CTM was measured according to Cox (1974). The end point was defined as the loss of equilibrium when the prawns had been stimulated by high temperature and began to swim vigorously in the experimental device. This criterion was well defined and repeatable. In accordance with the CTM method, a 100% survival was observed for a group of 10 prawns that, without a contaminant, were rapidly placed in water at acclimation temperature at time which CTM was obtained. That temperature marked the limit between the tolerance and resistance zones which was defined as CTM by Hutchinson (1961) and to assure that the criterion used is adequately applied. One-liter containers were placed in a bath heated with a 1000 watt heater. Temperature in the containers was raised $1.2^\circ\text{C}/\text{min}$ until the end point was reached. One animal was placed in each container with the same type of water of each treatment. All containers were aerated to avoid stress caused by a decrease in oxygen concentration and by temperature stratification. The difference between the CTM of each concentration of each treatment and exposure time was calculated using an ANOVA and a Duncan's multiple range test (Zar 1974).

RESULTS AND DISCUSION

The criterion of loss of equilibrium to measure the CTM for a benthic species makes it possible to study the effects of contaminants in other species of ecological or aquacultural interest. It was possible to see here how the activity of the prawns increased with an increase in

temperature. This was also related to an increase in the speed of movement along the bottom followed by vigorous swimming. The point at which the CTM was determined was related to a sudden abdominal contraction followed by the loss of equilibrium.

A significant decrease in the CTM was observed in soft water (Table 1) only during the 48 hr exposure in 0.02 mg/L Cd^{2+} ($33.8 \pm 0.01^\circ\text{C}$) with respect to the control group ($34.6 \pm 0.2^\circ\text{C}$) ($p < 0.05$). No significant differences were observed between the experimental and control groups after 96 hr of exposure ($p > 0.05$). The CTM progressively increased for Cd-exposed animals from 24 to 96 hr ($p < 0.05$). In hard water, a lower CTM was noted with an increase in the concentration of Cd^{2+} and exposure time (Table 1). The CTM decreased from 35.8°C (control) to 33.8°C (0.05 mg/L Cd^{2+}) after 24 hr and from 35.4°C (control) to 34.6°C (0.05 mg/L Cd^{2+}) after 96 hr ($p < 0.05$). A decrease in CTM was observed at 0.02 mg/L Cd^{2+} from 35.5°C after 24 hr to 34.9°C after 96 hr ($p < 0.05$). Although the CTM was greater after 48 hr than observed at either 24 or 96 hr, a significant decrease from 36°C (control) to 35.5°C (0.05 mg/L Cd^{2+}) was observed ($p < 0.05$).

The different responses of the animals exposed to Cd^{2+} in hard and soft water could be related to the mechanisms for the uptake of Ca^{2+} in different types of water. Due to reduction of uptake of calcium in low-hardness water, Brown et al. (1991) reported that juvenile M. rosenbergii tend to retain Ca^{2+} when external levels are low, depositing Ca^{2+} in the carapace and increasing intermolt periods. If Cd^{2+} competes with Ca^{2+} and there is less uptake of Ca^{2+} in soft water, then the limited response noted here might be related to a slower intoxication by Cd^{2+} in this type of water. In contrast, the intoxication by Cd^{2+} in hard water, where the uptake of Ca^{2+} is greater (Greenaway 1985), might be quicker due to the uptake mechanisms of Cd^{2+} via competition by Ca^{2+} .

The absence of more significant results on the effects of Cd^{2+} in hard water might be related to the chelation function of CaCO_3 (EPA 1984a) together with the possible accumulation of Cd^{2+} in the exoskeleton followed by depuration through an increase in the molt frequency (Brown et al. 1991), or with metabolic compensation characteristics of crustaceans (Ramirez et al. 1989).

The effect of Cr^{6+} on the CTM was more prominent in soft water than in hard water (Table 2). In soft water a decrease in the CTM occurred in prawns exposed to Cr^{6+} after 24 and 96 hr of exposure as compared to controls at that time period ($p < 0.05$). There was 80% mortality of prawns at 0.7 mg/L Cr^{6+} after 96 hr. There were no differences between the CTM in the control and experimental groups with respect to exposure time ($p > 0.05$) (Table 2).

Table 1. Critical Thermal Maximum [mean \pm S.D. and number (N)] of Macrobrachium rosenbergii exposed to sublethal concentrations of Cd^{2+} (mg/L) in soft (A) and hard water (B).

		HOURS		
		24	48	96
<hr/>				
A				
Control	34.0 ± 0.2 ^{a,a} (10)	34.6 ± 0.2 ^{a,a} (8)	34.9 ± 0.3 ^{a,a} (7)	
0.02	33.9 ± 0.1 ^{a,a} (10)	33.8 ± 0.1 ^{b,a} (10)	35.5 ± 0.2 ^{a,b} (7)	
0.05	33.8 ± 0.1 ^{a,a} (9)	34.1 ± 0.1 ^{a,a} (10)	35.6 ± 0.2 ^{a,c} (7)	
B				
Control	35.8 ± 0.2 ^{a,a} (10)	36.0 ± 0.1 ^{a,a} (10)	35.4 ± 0.2 ^{a,a} (10)	
0.02	35.5 ± 0.3 ^{a,a} (10)	36.1 ± 0.2 ^{a,b} (10)	34.9 ± 0.2 ^{b,c} (10)	
0.05	33.8 ± 0.2 ^{b,a} (8)	35.5 ± 0.2 ^{b,b} (8)	34.6 ± 0.2 ^{b,b} (8)	

Values within a column without a letter in common are significantly different. The first letter indicates a difference between concentrations and the second one a difference between hours.

In hard water Cr^{6+} caused a decrease in the CTM after 48 hr at both concentrations and did so after 96 hr only at 0.7 mg/L Cr^{6+} . This decrease was from 35.3°C (control) to 34°C (0.35 mg/L Cr^{6+}) after 48 hr and from 35.4°C (control) to 33.9°C (0.7 mg/L Cr^{6+}) after 96 hr ($p < 0.05$; Table 2).

The different responses of the animals exposed to Cr^{6+} in the two types of water could be related to the physiological action mechanisms of the metal as well as to experimental conditions. With respect to this, EPA (1984b) indicated that the toxic effect of Cr^{6+} may increase up to 10 times in soft water causing normally sublethal concentrations to affect the survival of exposed organisms. Both Cr^{6+} concentrations were sublethal in hard water. In soft water however, 50% mortality of the animals occurred at 96 hr in 0.35 mg/l Cr^{6+} and 20, 40 and 80% mortality occurred in 0.7 mg/l Cr^{6+} at 24, 48 and 96 hr, respectively. Black et al. (1973) suggested that CaCO_3 is a chelator of heavy metals

Table 2. Critical Thermal Maximum [mean \pm S.D. and number (N)] of Macrobrachium rosenbergii exposed to sublethal concentrations of Cr⁶⁺ (mg/L) in soft (A) and hard water (B).

	HOURS		
	24	48	96
<hr/>			
A			
Control	36.1 ± 0.2 ^{a, a} (9)	35.6 ± 0.2 ^{a, a} (9)	35.6 ± 0.2 ^{a, a} (10)
0.35	34.9 ± 0.4 ^{b, a} (9)	36.3 ± 0.5 ^{a, b} (9)	34.2 ± 0.6 ^{b, a} (5)
0.7	35.3 ± 0.2 ^{c, a} (8)	34.5 ± 0.5 ^{a, a} (6)	----
B			
Control	35.5 ± 0.4 ^{a, a} (10)	35.3 ± 0.3 ^{a, a} (10)	35.4 ± 0.1 ^{a, a} (9)
0.35	35.3 ± 0.4 ^{a, a} (10)	34.0 ± 0.3 ^{b, b} (10)	35.1 ± 0.2 ^{a, a} (10)
0.7	36.0 ± 0.3 ^{a, a} (9)	34.6 ± 0.3 ^{b, b} (10)	33.9 ± 0.2 ^{b, c} (10)

Values within a column without a letter in common are significantly different. The first letter indicates a difference between concentrations and the second one a difference between hours.

in freshwater ecosystems. The relationship between hardness and the toxic activity of Cr⁶⁺ observed in this study confirms the role that CaCO₃ plays in the prevention or magnification of the effects of this contaminant.

The changes in the CTM of M. rosenbergii reflect the general physiological state of the organisms. Prosser (1991) proposed that thermal stress affects the nervous system and reduces the integrity of the neural membranes. This alteration is frequently accompanied by a decrease in the concentration of oxygen in the tissues. The metabolic incapacity of the prawns exposed to Cr⁶⁺ (Ramirez et al 1989), together with the effects of thermal stress, might have accelerated the alteration of the functions of the prawns' nervous system and, thus, provoked the decrease in CTM observed in this study.

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