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Oxygen Uptake by Excised Gills of *Procambarus clarkii* (Girard) from Albufera Lake of Valencia, Spain, under Heavy Metal Treatments

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The American red crayfish Procambarus clarkii, originally from Louisiana (USA) was introduced in Spain in the 70's in the Guadalquivir River swamps (Librero 1980). It appeared first ramdomly and in a more regular basis afterwards since 1978 in the Albufera Lake south of Valencia and in the surrounding rice fields. Recently the crayfish is being fished commercially and destined to human food.

On the other hand, Albufera lake and surrounding rice fields waters are being subjet since the last three decades to very heavy load of sewage, toxic industrial residues including heavy metals and pesticides from the many urban and industrial stlements in the zone (Dafauce 1975).

There are studies on the toxicity and deleterious effects of heavy metals and/or their accumulation on crayfish. Instead there are very few dealing with the physiological disturbances produced by them or their derivatives on marine and freshwater crustaceans. The effects of cadmium on crab and crayfish have been studied by Collier et al (1973) and Dickson et al (1982). Depledge (1984a,b) studied the effects that mercury has on the endogenus rhytms and on circulatory and respiratory activity changes in crabs. In relation with the effect of chromium, Sather (1966) studied the effect that it has on the metabolism of crayfish, and Doughtie, Rao (1984) studied the ultrastructural changes that it produces in several tissues of shrimps.

In the present study we have investigated the effect that heavy metals (Chromium, Cadmium and Mercury) have on the oxygen uptake by excised gills of Procambarus clarkii (Girard) coming from the Albufera Lake (Valencia).

MATERIALS AND METHODS

Adult intermolt specimens of the crayfish Procambarus clarkii were collected in Albufera Lake (Valencia, Spain) and carried immediately to the laboratory where they were transferred into 300-L aquaria for 10 days and maintained before treatment at 19.5 °C with a daily diet of pork liver. Before the experimental treatment the animals were maintained in tap water.

Fifty crayfish ranging in weight 17.5 to 34.8 g were divided into five groups of 10 animals each. Crayfish of groups A,B,C and D were kept in 15-L experimental aquaria containing 0.003, 0.032, 1, and 10 mg/L of cadmium as $CdCl_2$ (E.Merck) respectively, during 96 h at 19.5°C. Group E served as control.
The same procedure was followed for treatment with chromium and mercury. Fifty crayfish weighing 15.8-32.2 g were treated with 10, 37, 135, and 500 mg/L of chromium as Na_2CrO_4 (E.Merck) and fifty crayfish weighing 15.4-35.3 g were treated with 0.1, 0.4, 0.8, and 1.5 mg/L of mercury as $HgCl_2$ (E.Merck).
After 96 h, the animals were transferred to clean aquaria and kept for additional 5 h in tap water. The gills were dissected out and then assayed for oxygen consumption rates. Gill-tissues respiration rates were determinated for individual crayfish using a Gilson (Gilson Medical Electronics Inc.) differential respirometer. According to Dickson and Franz (1980) procedure, all gill filaments from the left side of the each crayfish (control and treated) were placed as a set in individual respiration flasks, in order to avoid the differences between anterior and posterior gill filaments (Engel et al 1975). Respirometer flasks (15 mL) were filled with 5 mL of water at 7.3 mgO_2/L (Winkler method) and 0.5 mL of 30% KOH was added to the center wells for CO_2 absorption.
After 10 min equilibration period, oxygen consumption was recorded every 15 min for a period of 60 min at 19.5°C. After the measurements were done, tissues were removed from the flasks and oven-dried to constant weight at 80 °C. The results of respiratory measurements are reported as μLO_2 consumed/hr/mg dry tissue weight.

RESULTS AND DISCUSSION

Hexavalent chromium at concentration of 500 mg/L produced mortality rates of 80% after 96 h exposure and the treated animals were not considered for oxygen consumption measurements. Gill tissue oxygen uptake rates of crayfish treated with 10, 37, and 135 mg/L of Cr VI are show in Table 1.
The calculated oxygen consumption rates for each experimental period (15, 30, 45, and 60 min), were analyzed statistically using the ANOVA test. In no cases significant differences were found ($p > 0.05$). However oxygen consumption rates of the gill tissue decreased as the chromium concentration increased.

Cadmium at concentration of 10 mg/L produced mortality rates of 50% after 96 h exposure and was not considered for oxygen uptake measurements. The gill tissue oxygen uptake rates of crayfish treated with 3.2, 32, and 1000 $\mu g/L$ of cadmium, are show in Table 2.
As it was in the case with chromium treatment, in the cadmium case there were not significant differences in the oxygen uptake rates ($p > 0.05$) between treatments within the times used, although, there is a trend showing higher oxygen consumption rates of gills from animals treated with the lower Cd doses,

diminishing when the concentration is higher than 1 mg/L, all referred to control values,

After 72 h exposure, 1.5 mg/L of mercury produced mortality rates of 100%. Table 3 show the gill tissue oxygen uptake rates of crayfish treated with 0.1, 0.4, and 0.8 mg/L of mercury. Oxygen consumption rates of gill tissue went up as the mercury concentration increased and this held for all time measurements. However there were no significant differences when analyzed by the ANOVA test ($p>0.05$).

Table 1. Gill tissue oxygen uptake rates (µL/mg) of crayfish treated with some concentrations of chromium for 60 min at 15 min intervals. Each value represents the mean±SE of 10 crayfish.

Time (min)	Chromium (mg/l)			
	0	10	37	135
15	0.53 ± 0.08	0.51 ± 0.08	0.46 ± 0.11	0.46 ± 0.23
30	0.85 ± 0.18	0.84 ± 0.19	0.74 ± 0.23	0.65 ± 0.21
45	1.22 ± 0.29	1.13 ± 0.21	1.07 ± 0.25	0.95 ± 0.30
60	1.64 ± 0.35	1,44 ± 0.25	1.38 ± 0.24	1.15 ± 0.28

In order to avoid the variability in oxygen uptake rates associated with animals movements when analyzed in whole crayfishes we have used excised gills for our experiments. There is a close correlation between whole animal and gill tissue respiration rates. Dickson and Franz (1980), indicate that gill tissue respiration rates are comparable to whole animal respiration studies in surface and cave crayfish.

Table 2. Gill tissue oxygen uptake rates (µL/mg) of crayfish treated with some concentrations of cadmium for 60 min at 15 min intervals. Each value represents the mean±SE of 10 crayfish, except for 3.2 µg/L group (n=9).

Time (min)	Cadmium (µg/l)			
	0	3.2	32	1000
15	0.42 ± 0.08	0.44 ± 0.12	0.49 ± 0.11	0.39 ± 0.15
30	0.75 ± 0.14	0.82 ± 0.24	0.82 ± 0.13	0.64 ± 0.21
45	1.14 ± 0.19	1.21 ± 0.35	1.20 ± 0.19	0.88 ± 0.29
60	1.52 ± 0.22	1.61 ± 0.48	1.58 ± 0.25	1.17 ± 0.83

Collier et al (1973) showed a great individual variation in the oxygen consumption rates of whole animals (mud crab) while oxygen uptake rates of the gill tissue alone was usefull for determining cadmium effects.

We have determined oxygen uptake over a period of 60 min with 15 min intervals and found neither losses on the isolated gill capability for oxygen uptake nor decrease in the oxygen uptake rates by the gill associated with the water diminution in oxygen concentration during the experiment. This is due, probably, to the water saturation in oxigen at the beginning of the experiment.

In our experience,the isolated gill oxygen uptake rates decreased in parallel to the increase in chromium concentration, but the differences were not stadistically significant.
In crayfish treated with Cr VI, the gills concentrate the metal in higher proportion than other organs (Sather 1966, Dickson et al 1979). Moreover, structural alterations of the gill tissue, due to Cr VI, have been observed (Doughtie and Rao 1984). It seems logical to assume a decrease on the oxygen uptake by the gills associated with chromium treatment. It has to be noted that chromium is so far considered as a not very toxic metal (Mertz 1969). In our experiments, a value of 500 mg/L of chromium was necesary to cause death in 80% of animals under treatment.

Table 3. Gill tissue oxygen uptake rates (μL/mg) of crayfish treated with some concentrations of mercury for 60 min at 15 min intervals. Each value represents the mean±SE of 10 crayfish, except for 0.4 mg/L (n=9) and 0.8 mg/L (n=8).

Time (min)	Mercury (mg/l)			
	0	0.1	0.4	0.8
15	0.30 ± 0.05	0.36 ± 0.10	0.37 ± 0.11	0.38 ± 0.06
30	0.55 ± 0.09	0.61 ± 0.13	0.64 ± 0.16	0.73 ± 0.12
45	0.79 ± 0.19	0.90 ± 0.19	1.00 ± 0.21	1.08 ± 0.17
60	1.03 ± 0.17	1.17 ± 0.21	1.34 ± 0.31	1.41 ± 0.22

In relation with cadmium effects on gill tissue respiration rate, we found that the oxygen uptake rates increased in gill tissue of crayfish treated with low concentrations of cadmium (3.2 and 32 μ g/L), but decreased when metal concentration was of 1 mg/L.
It has been stated that gill tissue ATP, total adenylate concentrations, and respiration rates of excised gill tissues were significantly lower of day 7 in crayfish exposed to 5 and

10μg Cd/L (Dickson et al 1982). In other crustaceans, exposures to low Cd concentrations were followed by gill respiration rates above control values (Thurberg et al 1977).
The increase in oxygen uptake rates showed by isolated gills from crayfish treated with 3.2 and 32 μg Cd/L could be due to the metal action upon the cellular energetics metabolism; so, our results will be in accordance to those of Byczkowski and Sorenson (1984) showing micromolar concentrations of cadmium estimulate respiration in liver mitochondria, whereas higher concentrations inhibit respiration. Low concentrations of cadmium after 4 d treatment caused an increase in ATP and total adenylates in clam tissues (Giesy et al 1983) and this could indicate concomitant increase in the respiration rate.
Giesy et al (1981) found that when freshwater crayfish (Procambarus pubescens) where exposed to 10 and 30μg Cd/L, the adenylate energy charge was significantly lower relative to control organisms after 2, 7, and 14 days exposure, but no after 5 days where those levels were greater than control.

Mercury showed the highest toxic effects on Procambarus clarkii out of the three metals tested. Mercury at concentration of 1.5 mg/L induced death of 100% of animals exposed. The gill tissue oxygen uptake rates increased in parallel to the concentration of mercury. Those results disagree with the accepted general idea about the mercury capability to decrease the crustacean metabolism. Cardiac activity and oxygen consumption where depressed in Carcinus following exposure to the Hg ions(Depledge 1984b). In the crab Uca, when larvae or adults are exposed to mercury pollution, locomotor activity, respiration and osmorregulation are depressed (Vernberg et al 1974). However following exposure to low level (0.05 mg Hg/L) the circulatory activity is increased and the crab motility became higher than in controls, although in higher concentration (0.1 mg Hg/L), locomotor, cardiac and respiratory activity were depressed (Depledge 1984a).
The mechanism implied in the changes of circulatory and respiratory activity remain unknown, but is assumed that there exists some damage at membrane and mitochondrial levels (Thurberg et al 1977; Byczkowski and Sorenson 1984).

The presence of significant decreases in gill tissue oxygen uptake rate and respiration activity in other crustaceans may indicate differences in toxicity due to species sensitivity, seasonal, or environmental parameters.

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