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Effects of Sublethal Exposure to Lead on Levels of Energetic Compounds in *Procambarus clarkii* (Girard, 1852)

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Lead is neither essential nor beneficial to living organisms; all existing data show that its metabolic effects are adverse. Lead is toxic to all phyla of aquatic biota. Most of the lead discharged into surface water is rapidly incorporated into suspended and bottom sediments (Eisler 1988). The American red crayfish, Procambarus clarkii (Girard, 1852), lives in a wide range of environmental conditions that include highly polluted waters (Del Ramo et al. 1988; Madigosky 1991). Lead present in lake sediments can be available to aquatic animals such as P. clarkii because it is a detritivor and burrow into the sediment. In fact, we found remarkable levels of lead in tissues of P. clarkii caught in Albufera Lake and kept 15 days in clean water (e. g. 223 μg/g dry weight in gills). Furthermore, P. clarkii has a high capacity for lead accumulation from water; and gills were the most important tissue of lead accumulation (Pastor et al. 1988).

Among effects that contaminants have on the physiology of the organisms, energetic state variables are important, since they will alter both survival and reproduction. Hepatopancreas is a major site for the energetic reserve in crayfish (Huner et al. 1990) and is a site of lead accumulation, although metal concentration in this organ is not as high as gills (Pastor et al. 1988).

The purpose of this study was to examine changes in energy reserves in hepatopancreas and gills of the crayfish P. clarkii, in response to sublethal exposure to lead. Gills are directly exposed to contaminants in the environment, and they are the first organ showing alterations by the action of the contaminants. Hepatopancreas was also chosen due to both, its relevance in the energetic metabolism and its role in heavy metal detoxification mechanisms.

MATERIALS AND METHODS

Adult intermolt specimens of Procambarus clarkii were collected from Lake Albufera (Valencia, Spain) and transferred to the laboratory and placed into 300-L aquaria. They were held for 15 days without food at 22°C. Afterwards, they were fed pork liver *ad libitum*. Three days after feeding crayfish ranging in weight from 20.5 to 32.3 g were randomly separated into two groups.

In the present study we used a sublethal lead concentration, but this was near the LC50 value (96 hr LC50= 127 mg/L at 22°C) (Pastor et al. 1988). We did not observe mortality during the experiment.

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One group was kept in clean water (control) and the second group was exposed to 100 mg Pb/L as $Pb(NO_3)_2$. Lead exposure was carried out in a semistatic way, removing lead solutions every day. Crayfish were not fed during lead exposure. After 12, 48, and 96 hr of lead exposure, animals from each experimental group were transferred to clean water and kept there for one additional hour. Gills and hepatopancreas were removed by dissection. During the test temperature was 22°C (±0.2), pH= 7.9 (±0.2), hardness 250 (±30) mg/L as $CaCO_3$.

A 0.5 gram sample of tissue was homogenized in 3 mL of ice-cold 6% aqueous perchloric acid solution. Following homogenization, a 50 µL aliquot was removed and added to 950 µL of 1N NaOH. It was stored at -20°C and was analyzed for total protein.

McKee and Knowles (1986) method was used with several modifications to isolate lipids. The homogenate was centrifuged for 10 min at 10000 x g at 4°C. Lipid material in the pellet was extracted twice with 2 mL of a mixture of 2:1 chloroform:methanol. The combined supernatant was brought to a constant volume, washed for 24 hr with a 0.9% aqueous sodium chloride solution, and the resulting organic phase was analyzed for lipids by the phosphovanillin method (Boehringer Mannheim kit nº 124303).

The protein samples in 1 N NaOH were resolubilized by incubating at 100°C for 60 min and analyzed by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Glycogen content in both hepatopancreas and gills was estimated via the method of Seifter et al. (1950). Samples for glycogen were homogenized in both 60 % KOH and 30 % KOH, and incubated for 30 min at 100 °C. Glycogen was purified by precipitation with 80 % ethanol, centrifugated, redissoluted with distilled water and diluted glycogen was measured by the anthrone method (Seifter et al. 1950).

Caloric contents (cal/mg) were calculated by assuming caloric values of 9.5 cal/mg for lipids, 4.3 cal/mg for glycogen, and 4.1 cal/mg for proteins (Graney and Giesy 1986).

Two-way analysis of variance was used to determine treatment and time effects on the mean of the various parameters studied. Mean separation was accomplished with Fisher PLSD test. The significance level was of ≤ 0.05.

RESULTS AND DISCUSSION

In both lipid content and glycogen content in gills, statistically significant differences between means corresponding to metal-treated and control groups were found due to time, but not due to treatment (Figure 1A and 1C).

Mean protein concentration of lead-exposed animals was slightly lower than that in control animals, but this difference was not statistically significant. There was no significant effect over time (Figure 1B).

The over-all effect is reflected in the caloric content (Figure 1D). This parameter has been proposed as an index that offers an integrated parameter of the energetic reserves. Mean values of caloric content in gills of lead-exposed animals were slightly lower than that in control animals, but this difference was not statistically significant.

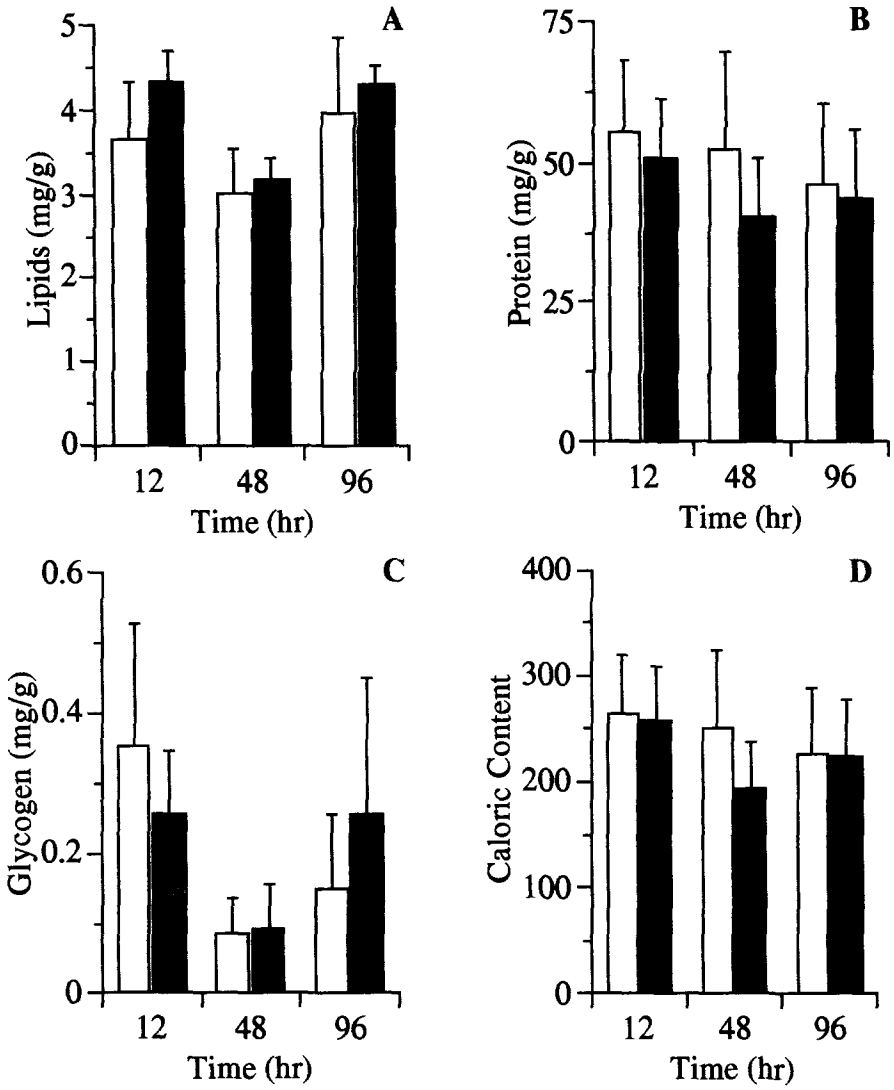

Figure 1. Lipids (A), protein (B), glycogen (C), and caloric contents (D) in gills of control (white bars) and lead-exposed (black bars) crayfish. Values represent means ± S.D. (n= 8).

In summary, after 96 hr of lead-treatment a slight decrease in protein content and an increase in lipid concentration was observed, but there were no significant differences in mean values from the control animals.

The increase in lipid/protein ratio can be reflected in the effect of structural damage caused by lead, which has also been described in previous works (Torreblanca et al. 1987; Rubio et al. 1991).

Lead exposure resulted in depleted levels of total protein, total lipids and glycogen in hepatopancreas. As a result caloric content was also diminished. Mean values of

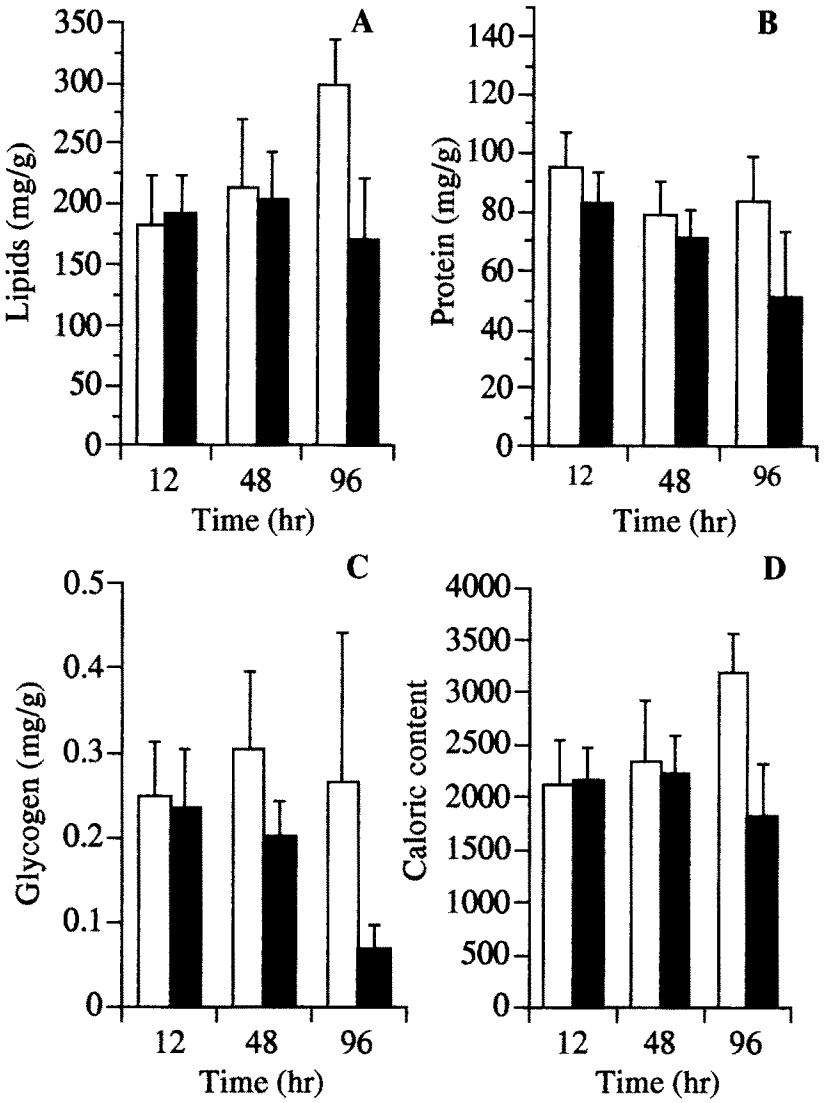

Figure 2. Lipids (A), protein (B), glycogen (C), and caloric contents (D) in hepatopancreas of control (white bars) and lead-exposed (black bars) crayfish. Values represent means ± S.D. (n= 8).

lipid, protein and glycogen levels decreased significantly over time in the crayfish exposed to lead (Figures 2A, 2B, and 2C).

Lipid concentration in control animals increased over time, while in treated animals it decreased. At 96 hr, protein, lipid and glycogen contents were significantly lower in treated crayfish when compared to control animals. In control animals the caloric content increased over time, while in animals exposed to lead it decreased (Figure 2D). There was no significant change in the caloric content due to time, but there was due to treatment. At 96 hr, caloric content was significantly lower in treated crayfish when compared to control animals.

The trend to increase caloric concentration with time in control animals may be the result of assimilation of the food ingested just before starting the experiment. In this case, lead exposure interfered with the replenishment of reserves in the hepatopancreas. This effect can be due to the general energetic cost of combating chemical toxicants in the hepatopancreas and other tissues (Calow 1991). The possibility of a direct effect of lead on some metabolic pathways cannot be rejected since it is known that lead alters the functionality of many enzymes. Organic reserves of hepatopancreas are required for molting and reproduction (Armitage et al. 1972; Huner et al. 1990), and sublethal exposure to lead may have adverse effects on these important processes.

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