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Copper Accumulation in the Crayfish (*Orconectes rusticus*)

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NEHRING (1976) investigated the use of aquatic insects as biological monitors of heavy metal pollution. He concluded that some aquatic insect species could be good biological monitors if they fulfill three important criteria: (1) they must be more tolerant of heavy metals than most fish; (2) they must accumulate metals in proportion to the concentrations present in their environment; and (3) they must accumulate the metals in a relatively short time period.

Most aquatic insects, however, spend only part of their life cycle in water and are, therefore, unavailable at certain times during the year. Further, aquatic insects can avoid high metal concentrations by releasing from the substrate and drifting downstream. For these reasons, non-burrowing crayfish species may be more desirable as biological monitors. They are present in streams and lakes year around and they are more stationary than aquatic insects.

The purpose of this study was to determine whether or not the crayfish, O. rusticus could fulfill NEHRING's (1976) criteria for a good biological monitor of heavy metal pollution. Since there is some evidence that the cupric ion is the most toxic form of aqueous copper (BIESINGER & CHRISTENSEN 1972, MERLINI & POZZI 1977), crayfish-accumulated copper was compared to both total and cupric copper in the culture water.

MATERIALS AND METHODS

Adult crayfish, O. rusticus, were collected from Indian Creek near Reily, Ohio. Copper dilutions were prepared with a solution of $CuSO_4{:}5H_2O$ in pond water which had been prefiltered through a 0.45 mm Millipore filter. All studies were conducted with one batch of pond water. Prior to copper addition, the pond water was analyzed for alkalinity, hardness, total copper, pH, dissolved oxygen, orthophosphate, chloride, sodium, potassium, iron, and nitrate.

Freshly-collected crayfish were acclimated to 25°C in pond water for 24 h prior to exposure. Solutions of 0.0, 0.1, 0.5, 1.0, 2.0, and 3.0 ppm total copper were prepared. One set of dilutions was analyzed for cupric ion concentration after 24 h. ANDREW et al. (1977) have suggested that 24 h is adequate time for copper equilibrium to occur. Cupric ion measurements were made with a Model 94-24 Orion Cupric Ion Electrode and a Model 90-02 Orion Double Junction Electrode connected to a Model 407A Orion Specific Meter. Electrodes were

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anchored in a light-tight box to minimize ambient light fluctuations and were calibrated with standard cupric ion concentrations prepared from double-distilled water with 0.002M KNO_3 added to adjust ionic strength.

After copper equilibrium was established, crayfish were individually exposed in 500 mL of each concentration. An attempt was made to attain an even size and sex distribution among the beakers. After 48 h, the crayfish were removed from the beakers, dried to constant weight at 105° C, and digested in a concentrated nitric and perchloric acid solution (ANONYMOUS 1960). Total copper of the digestate was determined by atomic-adsorption spectrophotometry.

The above procedure was repeated on three separate occasions. On the first occasion, 3 organisms were used per concentration. In the second and third tests, 4 animals per concentration were used. Duncan's new multiple range test showed no significant differences ($p = 0.95$) between tests (DUNCAN 1955). Consequently, all data were combined in the final analyses. All of the 24 animals in the second test were disarticulated after the drying steps so that differences in copper accumulation between the claws, thorax, and abdomen could be determined. Total accumulated copper was determined in these animals by combining the results from the component parts.

Data from the crayfish accumulation study were analyzed using the least squares procedure. This analysis showed the extent of the relationship between the dependent variable (crayfish-accumulated copper) and the independent variables (total and cupric copper). One experimental unit was represented by one crayfish.

RESULTS

All crayfish survived the 48 h exposure period and all, except two, were included in the final analysis. One control animal was discarded because it molted during the exposure period. Two other animals, at the 0.1 ppm level, were discarded because they were mistakenly placed in the same beaker.

A directly proportional relationship ($R^2 = 0.75$) was found between copper concentrations in crayfish tissue and total copper concentrations to which the crayfish were exposed. Copper concentrations in crayfish tissue were regressed on total copper in the culture water. The mean concentrations of Cu in the control animals was 120 ppm. This was not significantly different from a mean ppm for 7 freshly-caught animals ($P = 0.95$).

Duncan's new multiple range test revealed no significant differences ($P = 0.95$) between mean copper, concentrations of control animals and those of crayfish exposed to 0.1 ppm of total copper (Table 1). All other total copper concentrations produced significantly higher concentrations of copper in test animals than in controls. There was no difference, however, between animals exposed to 1.0 and

0.5 ppm of total copper. The amounts of copper found in the animals exposed to (0.0 and 0.1), (0.5 and 1.0), (2.0), and (3.0) ppm of total copper were significantly different from each other (Table 1). Crayfish-accumulated copper was also directly proportional to probe measured free copper (R^2 = 0.71). A student t-test revealed no significant differences (P = 0.95) in accumulated copper between male and female crayfish. Another t-test showed no difference (P = 0.95) between the dry weight of an animal and accumulated copper.

Analyses of body parts show that copper is accumulated in all areas of the crayfish body (Table 2). Accumulation occurs in all of these parts with about the same reliability. The coefficients of determination for the regressions of claws, thorax, and abdomen on exposure to copper concentrations were 0.68, 0.70, and 0.84, respectively. The thorax had the highest mean initial copper and there was no difference between initial copper levels in the claws and thorax. The thorax and abdomen accumulated about the same amount of copper at each exposure level. The claws accumulated the least amount of copper at any exposure level (Table 2).

Cupric Ion Measurements

The cupric ion electrode often displayed a Nernstian response down to 20 ppb of total copper in standard solution. Below this concentration, the electrode response became non-linear. STIFF (1971) observed similar electrode behavior at this low level of total copper. On some occasions, however, the Nernstian response was observed only down to 100 ppb in the standards. Electrode responses from copper additions to the pond water had lower slopes and intercepts than responses in which copper was added to distilled water. Unaltered pond water produced a millivolt reading between -35 and -40 on the specific ion meter. Distilled water produced a reading between +20 and +40 MV.

Eight different concentrations of $NaHCO_3$ were prepared and measured on the specific ion meter to determine if natural bicarbonate in the pond water could be the cause of this discrepancy. A linear relationship (R^2 = 0.98, Slope = -31.73) was found between the log of the bicarbonate concentrations (x-axis) and the millivolt readings (y-axis).

Addition of 60 ppm of bicarbonate, which was equivalent to the bicarbonate concentration of pond water dropped the electrode potential to only -12 MV. A maple leaf was then brought into the lab and squeezed into the flask. This was done to test the effects of organic matter on the electrode response. Within 5 min, the potential had reached -65 MV and was still dropping. No discoloration or precipitate formation occurred in the pond water following the addition of organic matter. In separate experiments, sodium and potassium nitrate were added to pond water and distilled water. No change in potential occurred as a result of these additions. Calcium and magnesium additions were not tested against electrode response since these elements would probably cause a slight rise in potential.

TABLE 1. Probe-measured, accumulated, and actual mean body concentrations of copper in crayfish

	Exposure Level (ppm)					
	0	0.1	0.5	1.0	2.0	3.0
Cu++ (ppm)	0.0	0.0	0.002	0.004	0.007	0.01
Actual Mean body copper (ppm)	120	130	170	180	220	250
Duncan Grouping@	A	A	B	B	C	D
Accumulated Copper (ppm)*	0	10	45	56	96	130
Micrograms of Copper Removed from beakers#	0	7.8	30	46	71	86

* Obtained by subtracting 120 from all mean body concentrations.

Obtained by multiplying accumulated copper by the mean weight of animals exposed at each concentration.

@ Means with the same letter are not significantly different according to Duncan's new multiple range test, DUNCAN (1955).

TABLE 2. Mean Concentrations of copper in crayfish body parts at different copper exposure levels.

Copper Exposure level (ppm)	Mean Copper in claws (ppm)	Mean Copper in thorax (ppm)	Mean Copper in abdomen (ppm)
0	77	180	85
0.1	89	210	120
0.5	120	250	130
1.0	120	280	160
2.0	150	300	210
3.0	170	350	260
	91*	170*	170*

* Total increase in tissue copper at the 3 ppm exposure level.

DISCUSSION

Although a direct proportional relationship between environmental and crayfish-accumulated copper has been demonstrated, more studies will be necessary to develop this organism as a biological

monitor. Crayfish must be tested for their ability to proportionally concentrate other metals such as chromium, zinc, lead, and mercury. Accumulation rates and retention times must be established for each of these metals. It would be important to know whether a crayfish exposed to different environmental concentrations of a metal for longer periods of time would continue to show different body burdens of these metals.

The form(s) of copper which are biologically accumulated by the crayfish remain unknown. If cupric ion is the only accumulated form of copper, then equilibria would have to shift in order to supply enough cupric ion to account for the total amount of copper accumulated by the crayfish. For example, the total amount of probe-measured cupric ion in the beakers at 3.0 ppm total copper was 5 µg (10 µg/L x 0.50 = 5 µg). The crayfish at this exposure level, however, extracted an average of 86 µg of copper from the water. This amount of accumulation would require 19 times the level of cupric ion initially present in solution. The rate at which cupric ion levels can be re-established is not known under the conditions stated. It would be useful to explore equilibrium rates for copper speciation in natural aquatic systems.

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