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Short Communication

COPPER TOXICITY TO *PARATYA AUSTRALIENSIS*. IV. RELATIONSHIP WITH ECDYSIS

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Abstract—Postmolt individuals of the freshwater prawn *Paratya australiensis* were more sensitive to copper toxicity than individuals at other stages of the molt cycle. In contrast to other reports, molting frequently was not increased by exposure to sublethal concentrations of copper.

Keywords—Copper toxicity Ecdysis *Paratya australiensis*

INTRODUCTION

Paratya australiensis is widely used in toxicity testing in Australia [1–5]. Toxicity data can be evaluated only in terms of the biology of the test species, and because the physiology of crustaceans is intrinsically linked with the molt cycle, the sensitivity to toxicants may also vary with molt stage. Four main molt stages may be distinguished—postmolt, intermolt, premolt, and ecdysis [6]. The period around ecdysis is one of considerable physiological activity, involving the mobilization of lipid reserves, formation of the new cuticle, and uptake of water and ions to expand the body volume [7–9]. It is therefore not surprising that many crustaceans show a greater tendency to die from natural causes at this time [7,10].

Lake et al. [1] found that postmolt *P. australiensis* were more sensitive to cadmium exposure than intermolt and premolt animals. With this result in mind, we analyzed the molt stage and toxicity data from a series of copper toxicity tests [3–5] to evaluate the relative sensitivities of the molt stages to copper.

Because the molt cycle of crustaceans is under hormonal control [11] and environmental stresses are known to affect hormonal activity [6], it is possible that copper exposure could influence the molt cycle of *Paratya*. The data of Skidmore and Firth [2] indicated that copper and zinc at subacute concentrations stimulated molting in *P. australiensis*, the prawn *Macrobrachium* sp., and the crayfish *Cherax destructor*, whereas higher lethal concentrations were inhibitory. However, they did not attempt to experimentally evaluate this indication directly. We investigated the effect of copper exposure on molting by recording the number of molts occurring at each test concentration of approximately 30 LC50 tests, as well as conducting an experiment specifically to investigate the influence of copper on the molt period of *P. australiensis*.

METHODS

Results discussed in this paper were all obtained from 96-h LC50 tests carried out to evaluate the influence of water chemistry on copper toxicity. Tests were done in Melbourne tap water, and details of the test protocols have been published elsewhere [3–5].

Changes in sensitivity at ecdysis

At the end of each toxicity test, live and dead prawns from each concentration were preserved separately in 70% ethanol and scored for molt stage, using the hardness of the integument and the state of the setation [12]. Within each experiment, the data for the concentrations in which mortality was ≤50% were pooled, and values of N (the number of postmolt organisms), X (the proportion of the total number of organisms that were postmolt organisms), and Y (the number of dead postmolt organisms) were calculated. The probability of obtaining a Y value greater than or equal to the calculated Y was then estimated for the particular N and X, using statistical tables for the cumulative binomial distribution [13]. Where mortality was >50%, data were not included in the analysis because any change in the sensitivity of postmolt individuals might have been masked by the high death rate. Corresponding analyses were carried out for intermolt and premolt *Paratya*, except that

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in most cases the probability of obtaining a Y value less than or equal to the calculated Y was estimated.

The effect of copper exposure on molt period

The number of molts that occurred at each test concentration in the tests conducted by Daly et al. [3–5] were recorded for each experiment. The number of "prawn days"—the number of days each prawn survived during the test summed over all the prawns exposed at that concentration—was calculated. Prawns that died after molting were considered to have survived for the full length of the test (either 6 or 9 d), because they would not have molted again within the test period, even if they had survived. The number of molts per prawn day was calculated for each concentration and compared with the control value, using a test for the equality of two proportions. It was assumed that there was random assortment of molt stages in each concentration at the beginning of the experiment.

The effect of copper on molt period was also tested experimentally. Forty acid-washed plastic containers were set up, each containing 1.5 L of aerated laboratory tap water at 15°C and one adult *Paratya*. The experiment was run as a static test in which water was changed twice per week, and the prawns were fed boiled lettuce leaves.

Each animal was allowed to molt three times to establish the length of two complete molt cycles, and the date of ecdysis was recorded. The test containers were checked for exuviae at least daily. Ten days after the third molt (about 0.4 of the molt cycle), each prawn was exposed to one of three copper concentrations—0, 15, or 40 μg/L Cu^{2+}—and then allowed to molt two more times. The concentrations of copper approximated the 96-h LC50 value to test acute effects, and a value less than half the 96-h LC50 value to test for subacute effects. Twenty containers were used for the 40-μg/L copper concentration and 10 for each of the control and 15-μg/L concentrations. Mortality occurred in some containers, particularly in the 40-μg/L concentration, before the five molts were completed. In these cases the molts that had been completed were included in the results, the test solution was changed, and another prawn was placed in the container. The procedure was then repeated for these animals, except that they were allowed to complete only one full molt cycle before being exposed to the test conditions.

The data for each of the control, 15- and 40-μg/L Cu^{2+} were pooled, and the mean molt period was calculated at each condition and compared using a Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks [14].

Table 1. The relationship between molt stage and sensitivity of *P. australiensis* to copper

Molt stage	No. of tests	Higher mortality than expected ($p < 0.05$)	Lower mortality than expected ($p < 0.05$)
Postmolt	20	7	0
Intermolt	21	0	6
Premolt	21	0	7

RESULTS

Changes in sensitivity at ecdysis

In 7 out of 20 tests (Table 1), postmolt *Paratya* suffered significantly higher mortality ($p < 0.05$) than that expected by their frequency in the test population. In no tests did postmolt *Paratya* suffer less than expected mortality. In contrast, intermolt and premolt prawns did not show higher than expected mortality in any tests but had significantly lower mortality than expected in 6 and 7 tests, respectively, of a total of 21.

Effect of copper on molt period

Of a total of 160 comparisons, in only seven did the number of molts per prawn day differ significantly from the control value (two-tailed test, $p < 0.05$). There is considerable natural variation in the molt period of *P. australiensis* (Table 2), with a mean of 25 d. The molt periods for the control, 40- and 15-μg/L Cu^{2+} were not found to be different ($d.f. = 2$, $H = 1.06$, $p \gg 0.05$). The statistical power of the comparisons between 15-μg/L Cu^{2+} and the control and 40-μg/L Cu^{2+} and the control were 80 and 13%, respectively.

DISCUSSION

The heightened sensitivity of postmolt *Paratya* to copper could have several possible explanations. It is possible that the prawns have a greater uptake of copper immediately postecdysis due to increased body permeability (e.g., [9,14]), increased metabolic rate [8,10,15], and/or increased intake of water

Table 2. Results of molting experiment with *P. australiensis* in Melbourne tap water at 15°C

Copper concentration (μg/L)	Mean molt period (days)	SD (days)	Range (days)
0	25	5	18–36
15	23	2	20–27
40	24	5	13–30

and ions to expand the body volume [6,8]. Alternatively, the combined effects of the stress of ecdysis and copper toxicity may be synergistic. Copper has been shown to cause transient inhibition of osmoregulation in fish [16,17], and if the same phenomenon occurs in *Paratya* it could be lethal at times such as ecdysis, when osmoregulation is most critical. Lake et al. [1] also found evidence of increased mortality in postmolt *Paratya* to cadmium, which they attributed to similar causes.

Skidmore and Firth [2] suggested that molting was enhanced at subacute concentrations of copper and zinc, and inhibited at concentrations above the LC50. The suggestion was based on the higher proportion of postmolt individuals found dead at subacute concentrations, compared with that at concentrations above the LC50. However, a higher proportion of dead postmolt individuals is to be expected at sublethal concentrations for two reasons. First, as we have shown, postmolt individuals are more sensitive than prawns in other stages of the molt cycle; therefore, a higher proportion of them will occur in lower concentrations. Second, at higher concentrations there is a higher mortality and a shorter effective exposure period; thus, there will be relatively fewer molting individuals. When this shorter exposure period is taken into account, as was done by comparing results in terms of prawn days, there are very few cases (7 of 160) in which the difference between the control and the copper-exposed molting frequency is significant.

There are obvious limitations in using the molt data from toxicity tests to assess the effect of copper exposure on the molt cycle of *Paratya*. It was necessary to assume that there was a random assortment of molt stages in each concentration at the beginning of the experiment, and the data are not in the most appropriate form for extracting the required information. For these reasons, the experiment was specifically carried out to test whether or not copper induced molting, thus reducing the molt cycle. The experiment confirmed the conclusions drawn from the toxicity data—that the molt cycle is not reduced by sublethal concentrations of copper.

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