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Copper Uptake and Regulation in a Copper-tolerant Decapod *Cambarus bartoni* (Fabricius) (Decapoda, Crustacea)

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Large amounts of acid forming sulphur dioxide, and "heavy metals" (specific gravity > 5, Niebor and Richardson 1980), including copper, are continuously being released into the environment by mining and smelting operation at Sudbury, Ontario, Canada (see references in Bagatto & Alikhan, 1987). Consequently, a number of lakes in this region have become acidic and metal stressed.

Earlier work by Bagatto and Alikhan (1987) showed that Orconectes virilis and Cambarus bartoni (Astacidae, Decapoda), from three lakes in the Sudbury region (Ramsey Lake in Sudbury, Joe and Nelson Lakes in Chelmsford) were tolerant to copper, cadmium and nickel. Furthermore, tissue concentrations of these metals in the crayfish were related to the distance of the habitat from the emission site.

In the current study the uptake and accumulation of copper by various tissues of a copper-tolerant crayfish, Cambarus bartoni, were monitored in the laboratory to ascertain the dynamic nature (i.e., the pattern in time) of responses of crayfish to increased levels of these two metals in the water.

MATERIALS AND METHODS

Intermoult adult Cambarus bartoni were trapped along rocky shores of Joe Lake (46° 44' N 81° 01' E) in Chelmsford, Ontario, 30 km north of Sudbury, Ontario. All animals were acclimated to laboratory conditions for a period of 2 wk. This acclimation also insured that

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all crayfish were post moult before they were used.

Concentrated copper sulphate (BDH Chemicals, Toronto, Ontario) stock solution was diluted to 0.125, 0.25 and 0.5 mg Cu L^{-1}, and delivered at the rate of 1 mL min^{-1} through a modified (for details, see Zia 1987) continuous-flow mini-diluter system, described by Benoit et al. (1982). The flow rate to each of the 8-L experimental chambers amounted to 50 (±0.5) mL min^{-1}, with the chamber displacement occurring at every 2 h and 40 min.

All experiments were performed under L12:D12 at 18°C (± 2°C) water temperature. Charcoal filtered and well aerated tap water (mean pH 6.74, Cu contents = 139 ± 5.8 µg Cu L^{-1}) was used as the dilution medium. Crayfish were provided with HiProMin fish food (containing 19.82 ± 0.77 µg Cu g^{-1} dry wt - Hartz Canada Inc., St. Thomas, Ontario) ad libitum.

Six animals (three males and three females) were removed at the start (day 1) and the end of the exposure week 1, 2, 3 and 4 for metal analysis.

Each crayfish was thoroughly rinsed, and its wet weight (g) and carapace length (mm) were determined to approximate their age-class (see Zia 1987 for details) before dissection. The exoskeleton, gills, hepatopancreas, alimentary canal, abdominal muscles, and remaining viscera (reproductive and excretory organs, nervous system, etc.) were placed on aluminium foil cups and oven dried at 80°C for 24 h to determine their dry wt. Tissue samples, along with procedural blanks, for analysis by Perkin-Elmer 703 Atomic Absorption Spectrophotometer, were digested in boiling aqua-regia {3 mL concentrated nitric acid : 1 mL concentrated HCl (BDH Chemicals, Toronto, Ontario)}, diluted to 20 mL with 1 M nitric acid and analyzed for copper by the flame method. The sensitivity for copper amounted to 0.077 µg mL^{-1}.

Statistical analyses of data were performed with the aid of a DEC-VAX/VMS computer, using $SPSS^{x}$ software (Statistical Package for Social Sciences, Chicago, Illinois). All data were checked for normality (Kolgomorov-Smirnoff test) and homogeneity of variance (Bartlett-Box F test), and they were log transformed, where necessary. As metal accumulation in males and females did not show any significant differences ($P > 0.05$), the data for the two sexes were pooled prior to analysis. An initial four-way ANOVA evaluated effects on metal levels of exposure time, treatment, sex and tissue. Within time and treatment, tissue metal

accumulations were compared using one-way ANOVA with Duncan's Multiple Range Test (applied only when $P < 0.05$). Regression analysis for metal levels in whole crayfish, as affected by exposure time during each treatment, was performed with the aid of an Apple Macintosh XL computer, using StatView software (Brain Power Inc., Calabasas, California).

RESULTS AND DISCUSSION

Tables 1 and 2 summarize data on copper concentrations in whole crayfish and their various tissues. It is evident from these data that copper is taken up from the surrounding medium and accumulated in various tissue by the crayfish. Furthermore, the increase in tissue copper stores in whole crayfish was related to the copper concentrations of various solutions, as well as to the exposure time. In general, tissue copper concentration at the end of a 4-wk exposure period was highest in crayfish exposed to 0.5 mg Cu L^{-1}, and lowest in those in the control (Table 2). The difference in copper

Table 1. Mean concentration of copper ($\mu g\ g^{-1}$ dry wt) in whole crayfish Cambarus bartoni at different exposure times (wk) in various treatments.

Exposure time (weeks)	Copper concentrations after exposure to mg Cu L^{-1}			
	0.5	0.25	0.125	Control
0	$148^{a,1*}$ (109, 203)**	$148^{a,1}$ (109, 203)	$148^{a,1}$ (109, 203)	$148^{a,1}$ (109, 203)
1	$237^{a,1}$ (151, 372)	$33^{a,2}$ (86, 206)	$150^{a,1,2}$ (91, 248)	$99^{a,2}$ (81, 121)
2	$204^{a,b,1}$ (147, 282)	$166^{a,1}$ (113, 242)	$165^{a,1}$ (112,243)	$106^{a,2}$ (80, 141)
3	$284^{b,1}$ (207, 391)	$155^{a,2}$ (118, 204)	$171^{a,2}$ (95, 307)	$150^{a,2}$ (93, 241)
4	$296^{b,1}$ (177, 493)	$218^{a,1,2}$ (156, 305)	$190^{a,2}$ (152,237)	$130^{a,2}$ (78, 217)

*Average of six crayfish in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.
**95% confidence limits.

concentration in crayfish in the control and those exposed to 0.125 and 0.25 mg Cu L^{-1} was not significant at the 5% level.Highest tissue copper concentration among various treatments was observed in the hepatopancreas and gills and the lowest in the exoskeleton, muscles and the viscera (Table 2).

Copper has been shown to be a regulated metal in several marine and freshwater decapods (Bryan 1968). This, according to Bonaventura and Bonaventura (1980), may be related to its essential biochemical role in the formation of the respiratory protein, haemocyanin. As this process appears to be regulated by the

Table 2. Uptake and accumulation of copper by various tissues of Cambarus bartoni at the end of a 4-wk exposure to various copper solutions.

	Copper concentration (µg g^{-1} dry wt)(1) after exposure to mg Cu L^{-1}			
Tissue	0.5	0.25	0.125	Control(2)
Exoskeleton	$116^{a,1}$ (71, 190)**	$85^{b,1}$ (57, 126)	$78^{b,1}$ (48, 129)	$54^{c,1}$ (62)* (31, 95)	^{c,1} (48,81)
Gills	$1167^{a,2}$ (788, 1729)	$909^{b,2}$ (739, 1118)	$571^{c,2}$ (515, 633)	$368^{d,2}$ (420)* (219,617)	^{e,2} (323,547)
Hp.***	$1494^{a,3}$ (885, 2522)	$2346^{b,3}$ (1438,3826)	$2185^{b,3}$ (1547,3087)	$1778^{a,3}$ (496)* (1016,3113)	^{c,2} (249,991)
Gut****	$328^{a,4}$ (230,468)	$360^{a,4}$ (266, 489)	$387^{a,4}$ (272, 550)	$311^{a,4}$ (337)* (160, 602)	^{a,3} (220, 517)
Muscles	$99^{a,1}$ (77, 128)	$129^{b,5}$ (96, 174)	$125^{b,5}$ (63, 249)	$88^{c,5}$ (143)* (34, 228)	^{d,4} (87,544)
Viscera	$276^{a,5}$ (145, 526)	$158^{b,6}$ (110, 225)	$185^{c,6}$ (86,398)	$92^{d,5}$ (109)* (34,228)	^{d,5} (87, 137)

*µg Cu g^{-1} dry wt at the start (day 1, week 1) of the exposure.
**95 per cent confidence limits.
***Hepatopancreas.
****Alimentary canal.
(1) Average of 6 replicates in each case. Means within each row followed by the same letter, and within each column followed by the same number are not significantly different at 5% level.
(2) 139 ± 5.8 µg Cu L^{-1}.

hepatopancreas, it was not surprising that most of the copper accumulation in the crayfish, in the present study, was observed inside the hepatopancreatic tissue. Similar findings in crayfish exposed to copper solutions are reported by Bryan (1968) and Bagatto and Alikhan (1987).

In agreement with the findings of Mierle (1981) with algae, hepatopancreatic copper in the crayfish, in the present study, significantly increased with increase in exposure time (Table 1). Thus, highest copper concentrations were observed in crayfish exposed to 0.25 mg Cu L^{-1} for 4 wk, and lowest in the control. This confirms the contention of Dallinger (1977) that limits within which copper is regulated, are closely adjusted to average copper concentration from all available sources in a particular habitat. However, differences in tissue copper concentration in crayfish exposed to 0.5 mg Cu L^{-1} (the highest dose) and 0.25 mg Cu L^{-1} (the intermediate dose) were not significant at $P > 0.05$, suggesting that copper was excreted by the crayfish exposed to the higher dose. Ogura (1959) found copper granules in the faecel material in Procambarus clarkii, indicating that hepatopancreatic cells were discharging vesicular copper into the alimentary canal. Similar observations concerning the excretion of zinc from zinc-loaded freshwater crayfish, Austropotamobius pallipes, have been reported by Bryan (1968).

Significant increase in copper concentrations in control animals in the present study may be due to high levels of copper (139 ± 0.77 μg L^{-1}) in the Sudbury tap water (see Materials and Methods).

The gill tissue,in the present study, contained a substantial amount of copper, and increases in copper stores of this tissue were also related to the exposure rate, and the exposure time (Table 1). According to Bryan (1968), trace metals enter the body of an aquatic invertebrate through two entry points:gills and the buccal cavity. In the present study, all animals were provided with an identical diets and therefore the increase in copper stores could only be due to the additional copper entering through gills. Ghate and Mulherkar (1979) reported distention of gill plates, and vacuolation and necrosis of the gill tissue after chronic exposure to copper sulphate of two species of freshwater prawns, Canidina and Macrobranchium,, and they concluded that gills were major sites for the storage of copper salts.

Gills are primary respiratory organs, and, as a consequence, they receive large volumes of haemolymph for oxygenation. The presence of high concentrations of copper (Table 2) in the gill tissue may also be related to large amounts of haemocyanin (the copper containing respiratory protein) dissolved in the haemolymph. Copper concentrations in the gill tissue increased by 747 $\mu g\ g^{-1}$ dry wt on exposure to 0.5 mg Cu L^{-1}. However, the histochemical examination of this tissue did not show the presence of definite copper granules. Sprauge (1964) suggests that excessive mucous, secreted by fish gills in response to toxic concentrations of heavy metals, may precipitate metals out and thus protect gill tissues from toxic effects. Secretion of protective mucous was also observed by Murti and Shukla (1984) during copper exposure in the shrimp, Macrobranchium lamarrei. It is possible that the increase in copper concentrations in this tissue is a reflection of the chelation of this metal with mucous secreted by gills, or due to adsorption of the metal on the cell surface. It is also feasible that copper in this tissue forms a part of the copper binding proteins, reported by Viarengo et al. (1980) in the gills Mytilus galloprovincialis, exposed for 24 to 48 h to copper compounds. However, further detailed study with copper radionucleotides will be required to support this contention.

Exoskeleton and remaining viscera (containing excretory and reproductive organs, nervous tissue, etc.) showed a minimum increase in their copper stores attributable to various treatments (Table 2). Copper concentrations in exoskeleton and remaining viscera at the beginning of the exposure period were in close approximation to those reported by Bagatto and Alikhan (1987) for the same species. Bryan (1968) reported higher copper and zinc concentrations in pigmented (melanin containing) tissues. In crustacean species, pigmented tissue lying below the cuticle may act as one of the sites for copper deposition. Weiser (1968) contends that freshwater decapods are similar to terrestrial isopods in that they, under low exposures, accumulate copper in their hepatopancreas, but shift the copper burden at higher exposure rates to the exoskeleton. However, it is not clear whether crayfish can make use of the copper stored in the exoskeleton. According to Bagatto and Alikhan (1987), exoskeletal copper storage may act as a sink for the excretion of excessive amounts of this metal during the moult cycle.

Copper concentrations in the digestive gut tissue (Table 2) and the abdominal muscles (Table 2) remained more or less constant throughout the experimental period. This

is not surprising since these tissues are not considered to be specific physiological sites for the storage of copper.

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