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Regulation of Essential Heavy Metals (Cu, Cr, and Zn) by the Freshwater Prawn *Macrobrachium malcolmsonii* (Milne Edwards)

K. Vijayram,[1] P. Geraldine[2]

[1]Research Department of Zoology, Periyar E.V.R. College, Tiruchirappalli 620 023, India
[2]Department of Animal Science, Bharathidasan University, Tiruchirappalli 620 024. India

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Despite the low concentrations of heavy metals in the surrounding medium, aquatic organisms take them up and accumulate them in their soft tissues to concentrations several fold higher than those of ambient levels (Bryan 1979; Rainbow *et al.* 1990). Knowledge of accumulation patterns of a particular trace metal is a prerequisite for understanding the significance of an observed metal concentration in a particular animal, especially from the aspect of biomonitoring. Many marine invertebrates accumulate heavy metals without any regulation and the accumulation necessarily being associated with mechanisms to store the metals in a detoxified form. Two detoxification mechanisms have been described, both of which may occur in one specimen. Heavy metals can either be bound up in insoluble metalliferous 'granules' (Mason and Nott 1981), or are bound to soluble metal-binding ligands, such as metallothioneins (Roesijadi 1992). Some marine decapod crustaceans have an innate ability to regulate the internal concentrations of essential but potentially toxic metals within a constant level, presumably to meet their metabolic demands (Rainbow 1985, 1992). However, at present, there is no such information relating to freshwater decapod crustaceans, especially shrimps which occupy a totally different environment.

Macrobrachium malcolmsonii (Milne Edwards), a potential aquaculture species for freshwater is found in abundance in one of the major Indian rivers, the Cauvery. In the present study, an attempt was made to determine whether the freshwater prawn, *M. malcolmsonii,* is able to regulate the three essential elements, copper, chromium and zinc, over a wide range of dissolved concentrations. These three metals were chosen because the Cauvery River receives pollutants containing these metals (Vijayram *et al.* 1990).

MATERIALS AND METHODS

Juvenile *M. malcolmsonii* (0.9 to 1.2 g in weight; 70-75 mm in length) were collected from the Cauvery River (Tamilnadu, India). The prawns were acclimated to city tap water for ten days before the study was conducted.

Twelve groups, each containing twenty prawns, were used for experiments. Each group was exposed for 22 days (intermoult period of *M. malcolmsonii)* to one of a number of metal concentrations in water of 160 mg/l total hardness at

Correspondence to: K. Vijayram

29°C ± 1°C under a 12 : 12 light - dark regime with continuous aeration. They were fed *ad libitum* with chopped mussel which did not have detectable amounts of Cu, Cr or Zn. The experimental media were changed daily.

The following series of experiments were performed:
1. Copper experiments: Four groups each containing twenty prawns were exposed to nominal concentrations of 9.6, 96, 137 and 240 µg Cu l^{-1}, respectively.
2. Chromium experiments: Four groups each containing twenty prawns were exposed to nominal concentrations of 12.9,129,184 and 323 µg Crl^{-1}, respectively.
3. Zinc experiments: Four groups each containing twenty prawns were exposed to nominal concentrations of 26, 260, 373 and 653 µg Zn l^{-1}, respectively.

These series ofconcentrations of Cu, Cr and Zn were chosen since they represented 1/100th, 1/10th, 1/7th and 1/4th of the 96-hr LC_{50} values of Cu, Cr and Zn, respectively Wijayram 1994).

4. Control experiments: Three groups, each containing 20 prawns, were maintained under exactly identical conditions as the exposed prawns except that there was no exposure to heavy metals; one group served as control for one series of experiments.

Sampling was done on days 1, 8, 15 and 22; on each occasion, five prawns were removed from each of the groups used in the heavy metal experiments and from the control groups. From each prawn, hepatopancreas, gill and muscle were dissected out, weighed and digested with concHNO_3(AR grade, BDH Ltd) at 100°C in a teflon tube. For metal extraction, from these samples, the methyl isobutyl ketone (MIBK) extraction method was used. Digests were made up to 5 ml with double distilled water and analyzed for total Cu, Cr and Zn content by flame atomic absorption spectrophotometry (Varian Techtron, AA-6D Spectrophotometer with background correction). The levels of detection of the instrument used were: Cu 0.003 µg ml^{-1}, Cr 0.005 µg ml^{-1} and Zn 0.0015 µg ml^{-1} The standard solutions for reference and calibration for Cu, Cr and Zn were prepared from stock solutions of electrolyte copper, chromium trioxide, and the zinc metal, respectively. The percentage recoveries of the standard solutions were 96, 95 and 98 for Cu, Cr and Zn respectively.

Statistical comparisons were made using analysis of variance (Sokal and Rohlf 1981). The raw data of prawn tissue metal concentrations were also subjected to linear regression analysis. The Student's 't' test was employed to test the significance of regression coefficients (Sokal and Rohlf 1981). All prawn tissue metal concentrations were expressed as µg g^{-1} wet weight.

RESULTS AND DISCUSSION

Data relating to the tissue Cu concentrations in control and exposed prawns after 22 days exposure (Table 1) were analyzed by the *a posteriori* sum of squares simultaneous test procedure (Sokal and Rohlf 1981). Significant differences were noted between the tissue Cu concentrations in controls and those in prawns held at different exposure levels. Regression analysis confirmed that in all three tissues there was a significant ($P<0.05$ and $P<0.01$) relation between body Cu concentrations and dissolved concentration of Cu (Table 2). These data do not provide evidence for the regulation of Cu uptake by the prawn.

Table 1. Levels of copper in the tissues of *M.malcolmsonii* exposed to sublethal concentrations of copper (Values expressed as µg g^{-1} wet weight of tissue are mean ± SEM of five observations). Values in parenthesis are percentage change over control.

Tissue	Days	Control	Nominal exposure levels µg l^{-1}			
			9.6	96	137	240
Hepatopancreas	1	4.74 ± 0.27	5.26 ± 0.12 (+11)	6.07 ± 0.18 (+28)	6.68 ± 0.11 (+41)	10.5 ± 0.85 (+122)
	8	4.43 ± 0.25	5.45 ± 0.24 (+23)	10.1 ± 0.64 (+129)	13.8 ± 0.34 (+212)	29.5 ± 0.84 (+564)
	15	4.62 ± 0.19	6.34 ± 0.34 (+37)	12.4 ± 0.93 (+168)	28.0 ± 0.64 (+506)	42.3 ± 2.51 (+815)
	22	4.51 ± 0.26	6.80 ± 0.33 (+50)	16.0 ± 0.85 (+255)	40.0 ± 0.66 (+787)	56.1 ± 4.10 (+1145)
Gill	1	3.93 ± 0.09	4.40 ± 0.45 (+12)	7.39 ± 0.07 (+88)	8.09 ± 0.32 (+106)	14.4 ± 1.30 (+263)
	8	3.89 ± 0.08	4.82 ± 0.38 (+24)	7.55 ± 0.13 (+94)	12.05 ± 0.45 (+208)	20.3 ± 1.74 (+421)
	15	3.90 ± 0.11	5.25 ± 0.67 (+35)	10.1 ± 0.24 (+159)	20.9 ± 0.75 (+436)	32.6 ± 3.08 (+340)
	22	4.01 ± 0.16	5.69 ± 0.54 (+42)	12.8 ± 0.64 (+218)	32.2 ± 1.06 (+702)	45.2 ± 3.12 (+1026)
Muscle	1	3.22 ± 0.07	3.25 ± 0.12 (+1)	3.82 ± 0.09 (+19)	4.28 ± 0.32 (+33)	9.92 ± 0.09 (+208)
	8	3.01 ± 0.07	3.34 ± 0.09 (+11)	5.48 ± 0.04 (+82)	6.08 ± 0.18 (+102)	12.2 ± 0.28 (+304)
	15	2.88 ± 0.08	3.54 ± 0.09 (+23)	6.68 ± 0.16 (+132)	9.16 ± 0.24 (+218)	18.4 ± 0.34 (+540)
	22	2.90 ± 0.09	3.89 ± 0.08 (+34)	8.27 ± 0.28 (+185)	14.0 ± 0.39 (+384)	20.6 ± 0.55 (+610)

Table 2. Regression equations for effect of experimental days (X) on copper concentration (Y) in tissues.

Tissue	Nominal exposure levels μg l^{-1}	't' value	Regression equation (Y on X)
Hepato-pancreas	9.6	9.58**	Y = 5. 057 + 0.079 X
	96	4.34*	Y = 5.871 + 0.458 X
	137	11.53**	Y = 3.365 + 1.630 X
	240	10.97**	Y = 10.04 + 2.136 X
Gill	9.6	6.41**	Y = 4.336 + 0.062 X
	96	10.10**	Y = 6.375 + 0.268 X
	137	8.67**	Y = 4.953 + 1.160 X
	240	14.09**	Y = 10.85 + 1.500 X
Muscle	9.6	16.84**	Y = 3.159 + 0.030 X
	96	8.52*	Y = 3.672 + 0.208 X
	137	12.40**	Y = 3.083 + 0.461 X
	240	6.82*	Y = 8.997 + 0.546 X

* $P < 0.05$ ** $P < 0.01$

Based on the concentrations of Cr noted in the hepatopancreas, gill and muscle *of M. malcolmsonii* (Table 3), it would appear that dose-and time-dependent accumulation of metal occurred. An *a posteriori* analysis of variance of the data showed significant differences between the tissue concentration of chromium in controls and that in test prawns exposed to different concentrations of the metals. A linear regression bordering significance ($P < 0.05$ and $P < 0.01$) could be plotted ofchromium uptake rate versus all the tissue chromium concentrations (Table 4). The data do not suggest the existence of a regulatory mechanism for Cr in the prawn.

Table 4. Regression equations for effect of experimental days (X)on chromium concentration (Y) in tissues

Tissue	Nominal exposure levels μg l^{-1}	't' value	Regression equation (Y on X)
Hepato-pancreas	12.9	6.421*	Y = 9.163 + 0.145 X
	129	8.050**	Y = 14.63 + 1.214 X
	184	9.864**	Y = 15.08 + 2.804 X
	323	9.322**	Y = 25.06 + 3.643 X
Gill	12.9	5.921*	Y = 6.673 + 0.042 X
	129	10.932**	Y = 17.200 + 0.309 X
	184	11.654**	Y = 16.440 + 1.114 X
	323	13.508**	Y = 26.870 + 1.644 X
Muscle	12.9	15.770**	Y = 5.062 + 0.102 X
	129	14.945**	Y = 6.665 + 0.638 X
	184	16.381**	Y = 6.096 + 1.088 X
	323	13.624**	Y = 6.930 + 1.630 X

* $P < 0.05$ ** $P < 0.01$

Table 3. Levels of chromium in the tissues of *M.malcolmsonii* exposed to sublethal concentrations of chromium (Values expressed as µg g^{-1} wet weight of tissue are mean ± SEM of five observations). Values in parenthesis are percentage change over control.

Tissue	Days	Control	Nominal exposure levels µg l^{-1}			
			12.9	129	184	323
Hepatopancreas	1	8.72 ± 1.01	9.24 ± 1.32 (+6)	15.0 ± 0.99 (+72)	17.8 ± 1.22 (+104)	20.4 ± 1.85 (+134)
	8	8.80 ± 0.93	10.4 ± 1.54 (+18)	25.2 ± 1.06 (+186)	36.5 ± 3.01 (+315)	62.9 ± 4.73 (+615)
	15	8.69 ± 0.84	11.4 ± 2.01 (+31)	33.7 ± 2.52 (+288)	59.4 ± 3.54 (+584)	87.2 ± 4.33 (+903)
	22	8.45 ± 0.97	12.3 ± 2.11 (+46)	40.5 ± 3.11 (+379)	75.6 ± 4.22 (+842)	97.3 ± 4.52 (+1051)
Gill	1	6.83 ± 0.77	7.10 ± 0.84 (+4)	17.7 ± 2.00 (+159)	19.1 ± 1.86 (+179)	24.3 ± 0.96 (+256)
	8	6.46 ± 0.51	7.24 ± 0.53 (+12)	19.5 ± 2.18 (+202)	24.3 ± 2.44 (+276)	46.0 ± 1.89 (+612)
	15	6.19 ± 0.32	7.49 ± 0.69 (+21)	21.6 ± 2.77 (+249)	30.6 ± 2.85 (+394)	52.2 ± 2.52 (+744)
	22	6.06 ± 0.47	8.00 ± 0.62 (+32)	24.2 ± 2.92 (+300)	43.0 ± 3.09 (+609)	60.6 ± 3.22 (+900)
Muscle	1	4.92 ± 0.11	5.02 ± 0.66 (+2)	6.59 ± 0.64 (+34)	7.82 ± 0.82 (+59)	9.0 ± 0.64 (+101)
	8	5.60 ± 0.09	6.10 ± 0.42 (+9)	12.3 ± 0.94 (+119)	13.8 ± 1.09 (+146)	18.1 ± 1.08 (+223)
	15	5.62 ± 0.13	6.58 ± 0.90 (+17)	17.3 ± 0.85 (+207)	22.5 ± 1.85 (+301)	31.1 ± 2.42 (+453)
	22	5.84 ± 0.25	7.24 ± 0.31 (+24)	19.8 ± 1.08 (+239)	30.3 ± 2.33 (+418)	43.6 ± 2.86 (+646)

Table 5. Levels of zinc in the tissues of *M.malcolmsonii* exposed to sublethal concentrations of zinc (Values expressed as µg g^{-1} wet weight of tissue are mean ± SEM of five observations). Values in parenthesis are percentage change over control.

Tissue	Days	Control	Nominal exposure levels µg l^{-1}			
			26	260	373	653
Hepatopancreas	1	18.6 ± 2.15	18.6 ± 1.29 (+0)	19.34 ± 1.34 (+4)	19.53 ± 1.92 (+5)	30.32 ± 2.52 (+63)
	8	19.1 ± 2.32	19.48 ± 1.82 (+2)	20.49 ± 2.22 (+7)	20.82 ± 1.25 (+9)	92.83 ± 4.56 (+386)
	15	18.8 ± 3.19	19.55 ± 1.61 (+4)	20.49 ± 2.54 (+14)	20.87 ± 1.46 (+11)	171.50 ± 6.89 (+812)
	22	18.9 ± 2.01	20.03 ± 3.00 (+6)	21.55 ± 1.94 (+14)	21.92 ± 2.01 (+16)	217.5 ± 6.54 (+1051)
Gill	1	13.4 ± 1.84	13.94 ± 1.11 (+4)	16.03 ± 1.82 (+19)	16.01 ± 0.94 (+19)	42.34 ± 2.11 (+216)
	8	14.3 ± 1.52	15.04 ± 1.79 (+2)	16.02 ± 1.36 (+12)	16.00 ± 1.11 (+12)	78.91 ± 3.54 (+451)
	15	14.6 ± 1.52	15.05 ± 0.99 (+3)	16.20 ± 1.19 (+11)	16.50 ± 1.42 (+13)	115.8 ± 3.98 (+693)
	22	14.8 ± 1.10	15.98 ± 2.01 (+8)	16.58 ± 1.19 (+12)	16.72 ± 2.09 (+13)	150.5 ± 5.64 (+917)
Muscle	1	10.8 ± 0.89	10.8 ± 0.52 (+0)	11.34 ± 0.34 (+5)	11.45 ± 0.97 (+6)	16.64 ± 1.84 (+49)
	8	12.2 ± 0.99	12.2 ± 1.11 (+0)	13.05 ± 1.52 (+7)	12.93 ± 1.22 (+6)	26.18 ± 2.52 (+114)
	15	12.4 ± 0.85	12.65 ± 0.89 (+2)	13.39 ± 1.13 (+8)	13.39 ± 0.94 (+8)	35.98 ± 2.54 (+190)
	22	12.1 ± 1.07	12.46 ± 0.24 (+3)	13.09 ± 0.94 (+8)	13.43 ± 1.55 (+11)	47.07 ± 3.01 (+289)

Data on the Zn concentrations in tissues of control and test prawns at different exposure levels (Table 5) were also analyzed by the *a posteriori* method. No significant differences were observed between tissue concentrations of Zn in controls and those in prawns exposed to Zn levels up to 373 µg l^{-1}. However, significant differences were seen between the tissue Zn levels of controls and those of test prawns exposed to concentration exceeding 373 µg l^{-1}. A significant regression ($P < 0.01$) could be plotted between Zn concentrations in the three tissues of test prawns and exposure to Zn concentrations exceeding 653 µg l^{-1} (Table 6).

Table 6. Regression equations for effect of experimental days (X) on zinc concentration (Y) in tissues.

Tissue	Nominal exposure levels µg l^{-1}	't' value	Regression equation (Y on X)
Hepato-pancreas	26	3.33	Y = 18.698 + 0.062 X
	260	3.24	Y = 19.278 + 0.095 X
	373	2.81	Y = 19.488 + 0.098 X
	653	10.94**	Y = 22.860 + 9.146 X
Gill	26	3.39	Y = 13.995 + 0.087 X
	260	2.53	Y = 15.907 + 0.026 X
	373	3.14	Y = 15.875 + 0.038 X
	653	12.43**	Y = 37.520 + 5.162 X
Muscle	26	3.43	Y = 11.135 + 0.078 X
	260	2.16	Y = 11.804 + 0.079 X
	373	3.02	Y = 11.749 + 0.091 X
	653	12.69**	Y = 14.450 + 1.468 X

** $P < 0.01$

Tissue levels of non-essential metals such as Cd, Pb, Ni are apparently not regulated by marine decapods (Rainbow 1985; Depledge and Rainbow 1990). In contrast, marine decapod crustaceans appear to regulate the tissue levels of essential heavy metals within certain constant limits (Chan and Rainbow 1993). Based on the results of the tests using Cu, Cr (Table 2, 4), it appears that *M. malcolmsonii* is unable to regulate these essential heavy metals, irrespective of the concentrations to which exposed. On the contrary, *M. malcolmsonii* appears to possess a physiological mechanism that regulates tissue Zn concentration within certain limits, provided the dissolved Zn concentration is below a threshold value (Table 6). When the regulatory mechanism collapses down at Zn exposures above this threshold concentration, net Zn is accumulated in proportion to the concentration of Zn. In *M. malcolmsonii* regulation of Zn may be achieved by changes in the rate of Zn excretion, which then matches the rate of Zn uptake. Under extreme conditions, when the concentrations of Zn to which the prawn is exposed is very high, the rate of Zn excretion cannot match the high rate of Zn uptake, and net accumulation of Zn results. Marine prawns are believed to regulate tissue Zn concentrations within levels which approximate the theoretically estimated Zn requirements for enzymes and, possibly, stabilization of the respiratory pigment haemocyanin molecule (White and Rainbow 1985). However, this aspect is yet to be ascertained in *M. malcolmsonii*.

The results of the present study suggest that *M. malcolmsonii* is unable to regulate tissue concentrations of Cu and Cr, in contrast to marine decapod

crustaceans. Hence, it is reasonable to postulate that there is considerable interspecific variability in the rate of uptake of individual metals (Timmermans and Walker 1989; Rainbow and Dallinger 1993). Finally, since net accumulation of Cu and Cr occurs in *M. malcolmsonii,* this prawn may be useful as a biological monitor for these metals since it fulfills the necessary prerequisites of a biomonitor (Phillips 1980). It is hoped that further studies will confirm the utility of *M. malcolmsonii* as a biomonitor for certain heavy metals.

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