Bull. Environ. Contam. Toxicol. (1995) 55:81–88

Environmental Contamination and Toxicology

Toxic Effects of Dissolved Copper on *Penaeus merguiensis* and *Penaeus monodon*

M. Ahsanullah, W. Ying

Australian Nuclear Science and Technology Organization, Private Mail Bag 1, Menai, New South Wales 2234, Australia

Received: 12 May 1994/Accepted: 14 December 1994

Dissolved metals are taken up by marine organisms either through gills (including absorption through general body surface) or diet. Some metals such as copper, iron and zinc are essential for the normal growth and development of organisms. However, metals may act as enzyme inhibitors if their concentrations are sufficiently different from the requirement of an organism (Moore 1985). Such concentrations of dissolved metals, therefore, can lead to either a toxic effect or inhibition of growth (Bernard 1977).

The impact of increased environmental pollution on the banana prawn, *Penaes merguiensis*, and the leader prawn, *P. monodon*, has recently given rise to concern (Darmono and Denton 1990; Ahsanullah and Ying 1993). Elevated copper concentrations have been found in some areas of the marine environment in Australia and Papua New Guinea, such as the Gulf of Papua (Baker et al. 1990), where *P. merguiensis* and *P. monodon* constitute a large part of the total yield of prawns. Denton and Burdon-Jones (1982) examined the toxicity of metals, including copper, to *P. merguiensis* and reported that with increasing salinity or decreasing temperature the 96-hr LC_{50} for copper increased. Metal concentrations in *P. merguiensis* and *P. monodon* from the Townsville region of Australia were also reported by Darmono and Denton (1990). However, no attempt was made to assess the sublethal effect of copper on these two species.

The purpose of this study was to assess the effects of dissolved copper on *P. merguiensis* and *P. monodon* using the parameters of acute toxicity, inhibition of growth and copper bioaccumulation.

MATERIALS AND METHODS

Juveniles of *P. merguiensis* (total length 1.5-2.5 cm, app. 4-wk old) and *P. monodon* (total length 2-3 cm, app. 4-wk old) were obtained from Gold Coast Marine Hatchery, Beenleigh, Queensland, Australia, and were transferred

Correspondence to: M. Ahsanullah

to two 200-L glass tanks and held in the laboratory for two weeks at 27°C. Seawater flowed through the tanks and prawns were fed on commercial food which contained 38% protein, 5% fat, 3% fiber, 12% moisture, 14% ash and 1 µg/g copper during the holding period. The salinity of the seawater used was 20°/oo, obtained by mixing 32°/oo seawater from Cronulla (NSW, Australia), with fresh water from the Woronora Reservoir (NSW, Australia). For the acute toxicity test with *P. merguiensis*, acid-washed plastic cups containing 500 ml of seawater (as control) and five concentrations of copper ranging from 0.3 to 2.0 mg/L were used. The experimental media were renewed after 48 hr. The duration of the acute toxicity test was 96 hours. Fifteen prawns were exposed to each copper concentration and control. Only one prawn was placed in each cup to avoid the problem of cannibalism. In the tests with *P. monodon*, a continuous flow system was established instead of semi-static test used for *P. merguiensis*. In this system six 80-L glass tanks (74 x 35 x 30 cm) were used for the control and the five different copper concentrations. Each tank contained fifteen 800-ml plastic cups and 40 L of water. Each cup containing a prawn was capped, while their sides and base were perforated with numerous small holes. Thus, each prawn was individually isolated but equally exposed to the test solution. Test solutions were continuously pumped at a rate of 20 ml/min into the tanks from six 225-L plastic storage drums containing various concentrations of copper and the control seawater. The water in the tanks was maintained at a constant level by a siphon system. Air was pumped into the tanks to maintain dissolved oxygen at 79-90% of saturation level. Observations on the mortality of the test animals were made at least twice a day. Prawns were not fed during the period of the test. The Spearman-Karber analysis was employed to calculate 96-hr LC_{50} values (Hamilton et al. 1977).

In a sublethal toxicity experiment nominal copper concentrations of 50, 100, 150 and 200 µg/L were used . Ten 80-L glass tanks were prepared and 60 L of test solution was introduced into each of these tanks. Twenty *P. merguiensis* and twenty *P. monodon* were introduced into each tank and the forty prawns were not isolated from each other. Dissolved oxygen in each tank was maintained at 80-90% of the saturation level. Prawns were fed on a measured amount (0.45g per tank) of the commercial food, administered twice a day, throughout the experimental period. The duration of the experiment was two weeks. Prawns were weighed individually at the beginning and end of the experiment. Experimental media were not renewed during the course of the experiment. Test concentrations were analyzed at the beginning and throughout the experimental period. Water samples were taken from tanks and filtered immediately through a 0.45 µm membrane.

Anodic stripping voltammetry (ASV) was employed for the analysis of these samples. After exposure, prawns were removed from the experimental tanks and transferred to clean seawater for one day to eliminate their gut contents. They were then washed with high-purity water obtained from a Milli-Q water purification system (Millipore Corporation, USA) and oven dried at 80°C. Dried whole individual *P. merguiensis* from each experimental tank were divided into two size groups: 0.01-0.03g and 0.03-0.05g, and *P. monodon* into three groups: 0.02-0.04g, 0.04-0.05g and 0.05-0.07g. Prawns from each group were pooled together, weighed, and digested

in a mixture of concentrated nitric acid and perchloric acid and evaporated to near dryness. The residue was redissolved in 15 ml of 5% diluted nitric acid. Copper was determined using inductively coupled plasma atomic emission spectrometry (ICPAES). The detection limit, calculated as 3 times the SD of the blanks, was 0.02 µg/L. A standard reference of oyster tissue (1566a) from the US National Institute of Standards and Technology was used for quality assurance. The analyzed copper concentrations of this reference were within the certified values.

RESULTS AND DISCUSSION

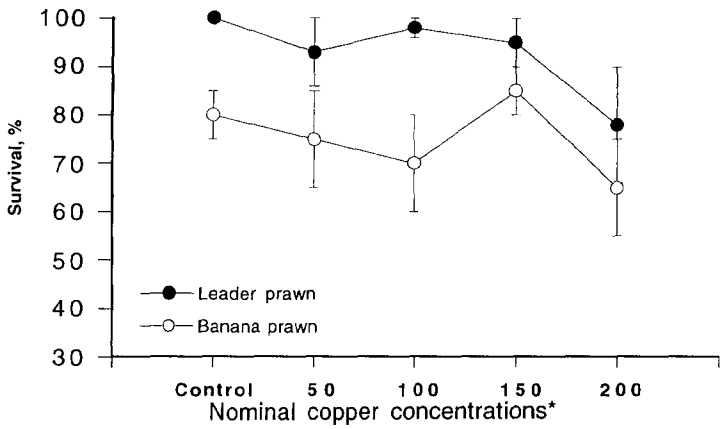
The 96-hr LC_{50} and its 95% confidence limits of dissolved copper for *P. merguiensis* were 0.38 and 0.21-0.68 mg/L, respectively. As *P. monodon* mortality was less than 50% in copper concentrations ranging between 0.5-2.5 mg/L, the Spearman-Karber analysis could not be applied in the calculation of the LC_{50} (i.e., the LC_{50} value for *P. monodon* was more than 2.5 mg Cu/L). Thus, there was no acute toxic effect of dissolved copper on *P. monodon* in seawater at or below 2.5 mg Cu/L.

Table 1. Dissolved copper concentrations (µg/L) in sublethal toxicity test tanks.

Days	Tanks									
	Control		50		100		150		200	
	1	2*	1	2*	1	2*	1	2*	1	2*
0	<1	<1	38	38	84	84	140	140	190	190
2	<1	<1	37	33	67	65	118	120	156	157
4	<1	<1	35	31	66	65	107	109	144	147
9	<1	<1	37	30	65	64	103	105	133	131
12	<1	<1	32	29	45	48	86	84	123	122
14	<1	<1	32	24	43	47	82	81	122	119

*2 was the duplicate of tank 1.

Based on the 96-hr LC_{50} values and their 95% confidence limits of dissolved copper especially for *P. merguiensis*, the maximum copper concentration used in the sublethal toxicity experiment was limited to less than 200 µg/L. Measured dissolved copper concentrations are shown in Table 1. Dissolved copper concentrations decreased with time during the two-weeks exposure as the experimental medium was not renewed. At the end of the experiment, approximately 40% of the introduced dissolved copper was absorbed/adsorbed by the tank walls, particulates of food, feces and uptake by prawns during the 2-weeks exposure. Average concentrations of dissolved copper (mean ± SD) were <1, 33 ± 4, 62 ± 14, 106 ± 21, 145 ± 25 µg/L for control and the nomimal concentrations 50,100,150, and 200 µg/L, respectively. There were no significant differences in copper concentrations between the tanks and their duplicates [the maximum differences were less than 4 µg/L (Table 1)]; the exception being the two tanks containing the nominal concentration of 50 µg/L in which on days



*For measured copper concentration see Table 1

Figure 1. Survival of *Penaeus merguiensis* and *Penaeus monodon* exposed to copper for 2 weeks.

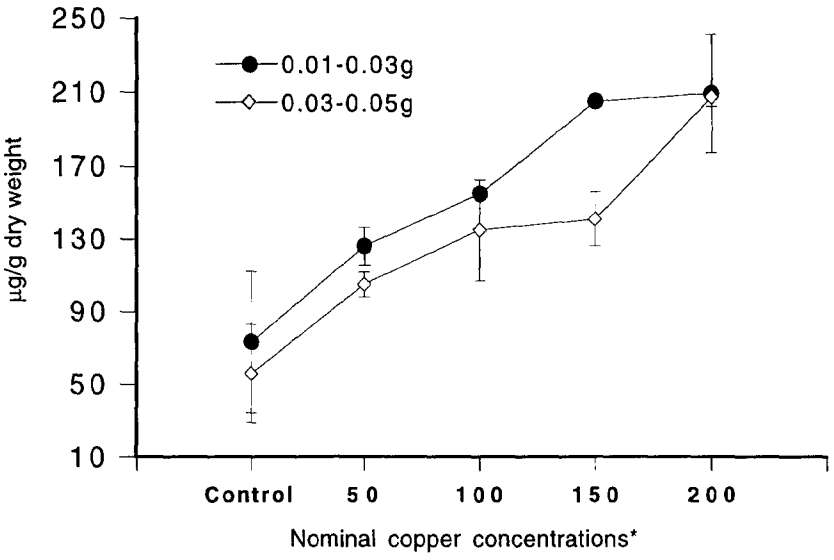
9 and 14, differences between Cu concentrations in the two tanks were 7 and 8 µg/L, respectively.

After 2-weeks exposure the survival percentage of animals in the control tanks for *P. monodon* and *P. merguiensis* was 100% and 80%, respectively, while survival rate for prawns in the highest Cu concentration (200µg/L) decreased (Figure 1). The survival of *P. merguiensis* was less than that of *P. monodon* in all test solutions.

Table 2: Average growth, measured as the wet weight gain(g), in *Penaeus merguiensis and P. monodon* exposed to copper for two weeks.

Species	Tank concentration				
	Control	50	100	150	200
P. merguiensis					
	0.064 **a**	0.051 **a**	0.037 **c**	0.027 **c**	0.039 **c**
	0.066 **a**	0.053 **c**	0.029 **b**	0.044 **b**	0.017
P. monodon					
	0.049 **b**	0.044 **c**	0.037	0.024	0.019
	0.061 **a**	0.066 **c**	0.050 **b**	0.027 **c**	0.044 **c**

Note : Two data points for each species and each concentration represent duplicate tanks. **a**= significant growth ($P<0.001$, t test) **b**= significant growth ($P<0.01$, t test) **c**= significant growth ($P<0.05$, t test)



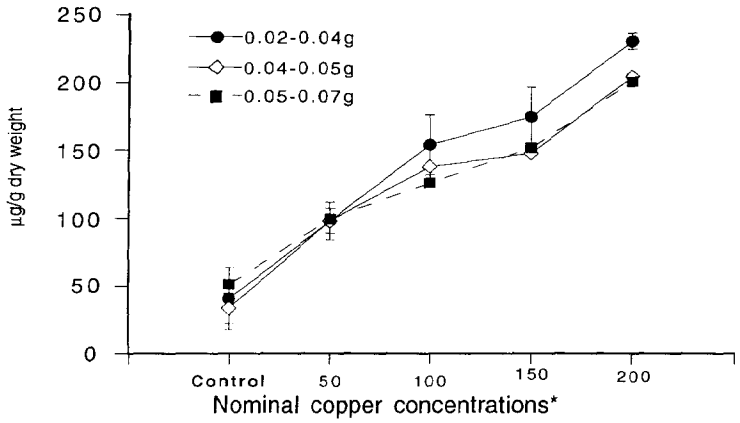
*For measured copper concentration see Table 1

Figure 2. Copper concentrations in tissues of *Penaeus merguiensis* exposed to copper for 2 weeks. (error bar = 1SE)

The data on average increased weight of prawns during the experimental period are summarized in Table 2. Analysis of variance (ANOVA) indicated that dissolved copper significantly affected the growth of *P. merguiensis* ($P<0.05$) but not of *P. monodon*. The Fisher's Protected Least Significant Difference (PLSD) test indicated that there was no significant effect of copper on the growth of *P. merguiensis* in 50-µg/L tanks while those in 100, 150 and 200-µg/L tanks grew significantly slower than those in control tanks ($P<0.05$). *P. monodon* grew in all copper concentrations at the same rate as it did in control solutions.

Both species of prawns accumulated copper during the experimental period. Copper concentrations of prawns after two-weeks exposure are shown in Figs. 2 and 3. With increasing dissolved copper concentrations in test solutions, copper concentrations accumulated by prawns increased and there were significant correlations between the two variables (Table 3). ANOVA showed that smaller *P. merguiensis* (0.01-0.03g dry weight) accumulated significantly more copper than those weighing between 0.03-0.05g ($P<0.05$) (Fig. 2). For *P. monodon* copper concentrations in the smallest group (0.02-0.04) were significantly greater than those in the other two groups (ANOVA, $P<0.05$), but there were no significant differences in copper concentrations between the 0.04-0.05g and 0.05-0.07g groups (Fig. 3).

The 96-hr LC50 for *P. merguiensis* for dissolved copper obtained in this study was 0.38 mg/L at 27°C and 20°/oo salinity. This value is similar to that



*For measured copper concentration see Table 1

Figure 3. Copper concentrations in tissues of *Penaeus monodon* (error bar = 1SE)

Table 3. Correlation coefficient for Cu concentration in *Penaeus merguiensis* and *Penaeus monodon* and Cu concentration in water.

Species	Group	R*	P
P. merguiensis	0.01-0.03g	0.97	<0.01
	0.03-0.05g	0.96	<0.01
P. monodon	0.02-0.04g	0.98	<0.005
	0.04-0.05g	0.96	<0.01
	0.05-0.07g	0.99	<0.005

R*=regression coefficient

recorded by Denton and Burdon-Jones (1982) and Ahsanullah et al. (1989), who reported 96-hr LC_{50} values of 0.53 mg/L at 20°/oo salinity and 30°C and 0.38 mg/L at 20°/oo salinity and 20°C, respectively. The slight discrepancy between these earlier test results and those of the present study may be due to the differences in salinity and temperature, the age difference of prawns used and differences between the water samples used (water and samples were filtered in the present study, as opposed to the study of Ahsanullah et al. (1989), while data on salinity were not reported by Denton Burdon-Jones (1982). The 0.21-0.68 mg/L values of 95% confidence limits and for the 96-hr LC_{50} in this study suggest that in sublethal studies for *P. merguiensis* it would be best if copper concentrations were maintained at <200 µg/L. For *P. monodon* the 96-hr LC_{50} was more than 2.5 mg Cu/L. This result suggests that this species is more resistant to the toxicity of copper than is *P. merguiensis* . It is worth noting that the test systems for these two species were different, one being semi-static in which experimental

media were renewed after 48 hr and the other being continuously flowing. However, this difference would not significantly affect copper toxicity. Studies in this laboratory with *P. monodon* using a similar system and copper concentrations to those used in this experiment for *P. merguiensis* showed that survival for *P. monodon* was more than 50% in 2.0 mg Cu/L (*unpublished data*).

The cannibalistic behavior of both *P. monodon* and *P. merguiensis* was observed in the laboratory during the acclimation period. In previous studies, Ahsanullah and Ying (1993) observed that the survival rate of *P. merguiensis* in control tanks in a 6-week test period during which prawns were not individually separated was 62%. Florence et al. (1994) suggest a survival rate of 56-76% in control tanks for *P. monodon* in a 30-d exposure to nickel-ore contaminated sediments. In the present study, the survival was 100% and 80% for *P. monodon* and *P. merguiensis*, respectively, in the control tanks. This suggests that while both species practice cannibalism, losses for *P. merguiensis,* due to the cannibalism, were more than those for *P. monodon*.

Results of the present study show that dissolved copper significantly affected the growth rate of *P. merguiensis* and there was a significant difference between the growth rate in 100,150 and 200 µg/L, and the controls. However, the effect of copper on the growth of *P. monodon* was not significant ($P>0.05$). For *P. merguiensis* a correlation coefficient of -0.81 ($P<0.005$) was calculated for weight increase and mean copper concentration during the experimental period, with a linear regression equation of:

Weight increase of individual *P. merguiensis* (g) = 0.059-0.00024 Cu (µg/L)

Based on this equation, 25 µg Cu/L would result in a loss of 10% growth over a 2-wk period.

Some crustacean species can regulate the concentrations of copper in their body and, as such, the concentrations of Cu may vary greatly between individual animals. Variations between individuals may be related to the molt-intermolt cycle (e.g., Phillips 1980; White and Rainbow 1984; Nugegoda and Rainbow 1988; Ahsanullah and Williams 1991; Ying et al. 1994). However, Alliot and Frenet-Piron (1990) reported that there was a similar annual profile pattern between copper concentrations in water and in a random group of the shrimp *Palaemon serratus* collected from the harbor of La Trinite-sur-Mer, France. Alliot and Frenet-Piron (1990) indicated copper was not regulated by *P. serratus* in the field situation. Darmono and Denton (1990) reported metal levels in various tissues of wild *P. merguiensis* and cultured *P. monodon* and found that *P. merguiensis* contained higher concentrations of copper in the hepatopancreas (81-199 µg/g wet weight) than did *P. monodon* (16 µg/g wet weight). The results of the present study confirm that copper is accumulated by *P. merguiensis* and *P. monodon,* and although,in the present study, data on Cu distribution in various tissues were not collected, Ahsanullah and Ying (1993) have shown that copper was accumulated in both cephalothorax and abdominal

flesh of *P. merguiensis*. The prawns in the study by Ahsanullah and Ying (1993) were exposed to the copper concentrations ranging between 0.40-0.55 mg/L, which are high and are rarely found in the natural environment. In the study by Ahsanullah and Ying (1993) when *P. merguiensis* were exposed to lower copper concentrations of 0.16 mg/L for six weeks, there was no accumulation of copper in abdominal flesh.

Acknowledgments. We thank Dr. I. Wood of Ok Tedi Mining Ltd, Dr. R. Smith of BHP Engineering, Dr P Airey and Mr. M. Gannon of Australian Nuclear Science and Technology Organisation for their useful comments.

REFERENCES

Ahsanullah M, Williams AR (1991) Sublethal effects and bioaccumulation of cadmium, chromium, copper and zinc in the marine amphipod *Allorchestes compressa*, Mar Biol 108:59-65

Ahsanullah M, Ying W (1993) Toxicity and bioaccumulation of copper from Ok Tedi Copper Mine ore bodies by the banana prawn *Penaeus merguiensis*. Investigation Report submitted to Ok Tedi Mining Ltd.

Alliot A, Frenet-Piron M (1990) Relationship between metals in sea-water and metal accumulation in shrimps. Mar Pollut Bull 21:30-33

Baker EK, Harris PT, Beck RW (1990) Cu and Cd associated with suspended particulate matter in Torres Strait. Mar Pollut Bull 21:484-486

Bernard M (1977) Chemical contamination of culture media: assessment, avoidance and control. In: Kinne O (ed) Marine Ecology, Vol III, part 3. Jon Wiley and Sons, NY, p 1459-1499

Darmono D, Denton GRW (1990) Heavy metal concentrations in the banana prawn *Penaeus merguiensis*, and the leader prawn, *P. monodon*, in the Townsville region of Australia. Bull Eviron Contam Toxicol 44:479-486

Denton GRW, Burdon-Jones C (1982) The influence of temperature and salinity upon the acute toxicity of heavy metals to the banana prawn (*Penaeus merguiensis* de Man). Chem Ecol 1:131-143

Florence TM, Stauber JL, Ahsanullah M (1994) Toxicity of nickel ores to marine organisms. Sci Total Environ 148:139-155

Hamilton MA, Russo RC, Thurston RV (1977) Timmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714-719

Moore MN (1985) Cellular responses to pollutants. Mar Pollut Bull 16:134-139

Nugegoda D, Rainbow PS (1988) Zinc uptake and regulation by the sublittoral prawn *Pandalus montagui* (crustacea: decapoda). Estuar Coast Shelf Sci 26:619-633

Phillips DJH (1980) In: Quantitative aquatic biological indicators. Applied Science Publishers Ltd, Essex, England, pp 389-392

White SL, Rainbow PS (1984) Regulation of zinc concentration by *Palaemon elegans*. Mar Ecol Prog Ser 16:135-147

Ying W, Batley GE, Ahsanullah M (1994) Metal bioavailability to the soldier crab *Mictyris longicarpus*. Sci Total Environ 141:27-44