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Toxicity of copper sulfate for survival, growth, molting and feeding of juveniles of the tiger shrimp, *Penaeus monodon*

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Abstract

The aim of this study was to determine the acute and chronic toxicity of copper sulfate for juveniles of *Penaeus monodon*. The 96-h LC50s (median lethal concentrations) of copper on juvenile *P. monodon* (0.63 ± 0.13 g) were 3.13 and 7.73 mg/l in seawater of 15‰ and 25‰, respectively. The mortality rates of *P. monodon* juveniles (0.19 ± 0.02 g) following exposure to 0 (control), 0.45, 0.90, 1.80 and 4.50 mg/l copper after 30 days was 0%, 0%, 5.6%, 22.2% and 55.6%, respectively. After 30 days of exposure, the body weight and total length of shrimps exposed to copper at 0.90 mg/l and higher was significantly lower ($P < 0.05$) than those in the control. The 30-day EC50 (concentration that reduced weight gain by 50% of that of the controls) and 60-day EC50 were 2.82 and 1.89 mg/l copper, respectively. The ratio of carapace length to total length of shrimps exposed to copper at 0.90 mg/l was significantly higher ($P < 0.05$) than the controls. Following exposure to copper as low as 0.90 mg/l, *P. monodon* shortened the time to the first molt, and decreased its growth and molting frequency. Following exposure to copper as low as 5.0 mg/l, *P. monodon* juveniles (6.25 ± 0.09 g) decreased their feeding. The MATC (maximum acceptable toxicant concentration) was 0.45 mg/l copper based on the growth and molting of shrimps weighing 0.18–1.03 g, and was 1.0 mg/l copper based on the feeding of shrimps weighing 6.25 g.

Keywords: *Penaeus monodon*; Copper sulfate; Lethal; Sub-lethal; Growth; Molting; Feeding

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1. Introduction

Tiger shrimp (*Penaeus monodon*) and kuruma shrimp (*P. japonicus*) are the most common penaeid shrimps currently being cultured commercially in Taiwan and other Pacific rim countries (Chen, 1990). The mass mortality of *P. monodon* due to vibriosis and several virus diseases, including MBV (monodon baculovirus) and WSV (white spot virus), has resulted in great losses for commercial shrimp production in south Asian countries. As a result, chemical treatment for maintaining optimal water quality has gained attention.

Copper sulfate is commonly applied to shrimp ponds to eradicate filamentous algae. The application of copper sulfate in ponds is also very effective in reducing the abundance of phytoplankton, including *Microcystis* and other blue-green algae. These synthesize and excrete objectionably-flavored compounds, such as geosmin, in pond water. Fish and shrimp acquire an off-flavor when held in water containing blue-green algae. The application rate of copper sulfate varies from 0.025 to 2 mg/l and is directly related to total alkalinity (Boyd, 1990). Shrimp farmers often apply excess amounts of copper sulfate in pond management. Therefore, the concentration of copper sulfate remaining in water and its toxicity is of primary concern.

The toxicity of copper to teleosts has been studied extensively by many workers and has been summarized by Sorensen (1991). Copper concentrations ranging from 0.6 to 2.4 mg/l have been reported as 96-h LC50 (median lethal concentration) values. The toxicity of copper sulfate on penaeid shrimps has been studied on *P. japonicus* (Bambang et al., 1995) and *P. monodon* (Guo and Liao, 1992). This paper provides information on the acute and chronic toxicity of copper sulfate to *P. monodon* juveniles.

2. Materials and methods

Three experiments with copper sulfate were conducted. The first experiment was a study of the acute toxicity of copper sulfate for juveniles of *P. monodon*. The second experiment evaluated chronic effects on growth and molting, and the third studied the effect on feeding.

2.1. Animals and seawater

P. monodon juveniles obtained from a private nursery located in Iilan, Taiwan, were shipped to the laboratory and acclimated in 25‰ seawater for 1 week before bioassay testing. They were then held in 25‰ seawater. The average wet weight of shrimps was 0.63 ± 0.13 g, 0.19 ± 0.02 g and 6.25 ± 0.09 g for the first, second and third experiment, respectively. Seawater pumped from the Keelung coast adjacent to the university was diluted with dechlorinated municipal water and filtered through sand and gravel filters by air-lift. The first experiment was conducted at 15‰ and 25‰ seawater, and the second and third experiments were conducted in 25‰, which is an isosmotic point for *P. monodon* juveniles (Ferraris et al., 1986). The shrimps used in the first experiment were divided into two groups and the salinity for one group was decreased at 3‰ per day with dechlorinated municipal water until a salinity of 15‰ was reached. This

resulted in one group at 15‰ and another group at 25‰ salinity and these were then acclimated for 1 week prior to the experiment.

2.2. Test solution

Copper test solutions were prepared by dissolving 5 g of copper sulfate in 20 ml of distilled water to prepare 1000 mg/l copper stock solution and then diluted with seawater to make 0, 1, 3, 6, 9, 12 and 15 mg/l copper at 15‰, and 0, 8, 11, 14 17, 20 and 23 mg/l copper at 25‰ as test solutions for the first experiment. Concentrations of 0, 0.45, 0.9, 1.8, 4.5 mg/l copper at 25‰ were used as the test solutions for the second experiment; and concentrations of 0, 1, 5, 10 and 20 mg/l copper at 25‰ were used as the test solutions for the third experiment.

2.3. Lethal effect of copper

Short-term median lethal concentration (LC50) toxicity tests were carried out according to the method described by Buikema et al. (1982) and the American Public Health Association et al. (1985). Shrimps were taken from the holding tanks and transferred to each solution. Bioassay experiments to establish tolerance limits were conducted in 20-l polyethylene tanks containing 10 l of test solution. Each tank contained 10 shrimp, and the water was aerated continuously by an air stone. Each test solution was renewed daily, in accordance with the static renewal method for toxicity tests (Buikema et al., 1982; American Public Health Association et al., 1985). There were triplicates for each test solution with a total number of 30 juveniles (10 per replicate) for each test solution. During the experiments, the shrimps were fed a commercial diet (37% protein, 4.4% fat, 1.0% fiber and 13% ash, designed for *P. monodon* by Tairown Products, Taiwan) twice a day (09:00 and 21:00 h) at 5% of body weight per day. Water temperature was maintained at 26 ± 1°C, dissolved oxygen was 6.09 ± 0.6 mg/l and the pH ranged for 8.06 to 8.24.

Observations were usually made at 24-h intervals up to 96 h. Death was assumed when shrimps were immobile and showed no response when touched with a glass rod. The concentration response of test organisms was determined for LC50 of copper with a computer program (Trevors and Lusty, 1985).

2.4. Effect of copper on growth

Shrimps were taken from the holding tank and individually housed in a cylindrical cage (10 cm diameter and 30 cm long) made of plastic screens (2×3 mm mesh size). Each tank contained 20 l of test solution and six cages, and was aerated with an air stone. There were triplicates for each test solution with a total number of 18 juveniles (six per replicate) for each test solution. The shrimp were also fed the commercial diet described above.

Bioassay tests were conducted using the static renewal method with test solutions renewed daily. Dead juveniles and uneaten feed were removed every day in the afternoon (14:00–16:00 h) when the water was renewed. Exuviae (molted exoskeletons) were also removed daily when they were found.

Observations were made every morning at 11:00 h, and the number of surviving animals was recorded every 15 days. During the experiment, the water temperature, pH level and dissolved oxygen averaged 25.5 ± 0.2°C, 8.13 ± 0.4 and 6.47 ± 0.3 mg/l, respectively. The wet weight, total length and carapace length of shrimps were measured at 15-day intervals for 75 days. Each individual shrimp was netted, placed on gauze to remove excess water and then weighed. Both wet weight gain and length increase of shrimp were computed. The relationship between copper concentration (as X) and wet weight gain or length increase (as Y) after each 15 days of observation were computed.

2.5. Effect of copper on feeding

The feeding tests were conducted in 8-l polyethylene tanks containing 2 l of test solution. Each test solution was conducted in five replicates with one shrimp in each replicate. The shrimps were fed individually with the same feed as above. After 1.5 h of feeding, unconsumed feed was pipetted, placed on filter paper and dried to a constant weight. The feeding rate was expressed as $(A - B) \times 100/C$, where A was the amount of feed supplied (g), B was the amount of feed (g) remaining after 1.5 h feeding, and C was the wet weight of shrimp (g).

2.6. Statistical analysis

All data were subjected to a one-way analysis of variance (Steel and Torrie, 1980). If a significant difference was indicated at the 0.05 level, then Duncan's multiple range test was used to identify any significant difference between treatments (Duncan, 1955).

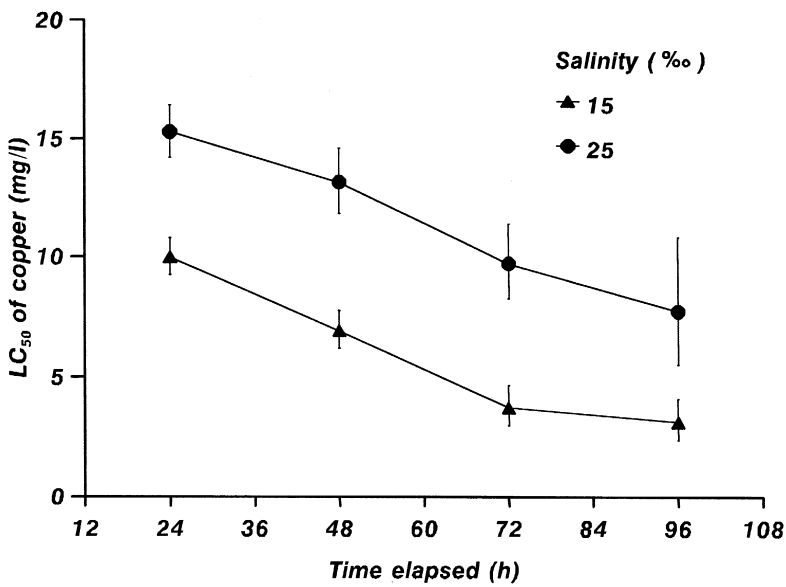

Fig. 1. LC50 values (95% confidence limits) versus time of copper (mg/l) for *P. monodon* juveniles.

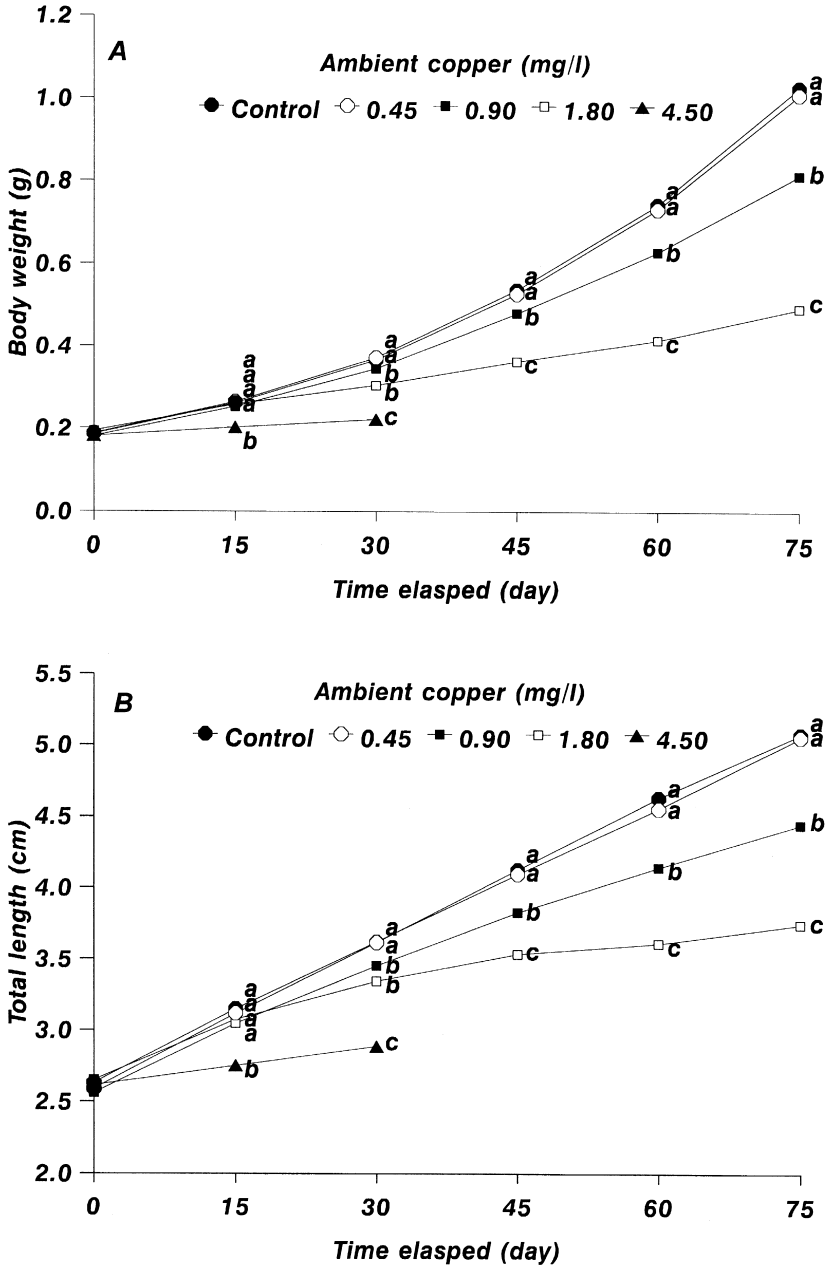

Fig. 2. Mean (SE) body weight (A) and total length (B) of *P. monodon* juveniles versus time of exposure to different concentrations of copper. Values in the same time period having different letters are significantly different ($P < 0.05$).

3. Results

3.1. Lethal effect of copper sulfate

All shrimps survived in the controls. All shrimps exposed to 12 and 15 mg/l copper at 15‰ died by 72 and 48 h, respectively, and when exposed to 23 mg/l copper at 25‰ they died by 48 h. However, no mortality occurred among the shrimps exposed to 1 mg/l copper at 15‰ for 96 h, and among those exposed to 8 mg/l copper for 12 h. The LC50 values of copper and their 95% confidence limits at different time periods are presented in Fig. 1. The lethal effect of copper was greater at the lower salinity. The 24-, 48-, 72- and 96-h LC50 of copper for *P. monodon* juveniles was 9.98, 6.92, 3.72 and 3.13 mg/l copper at 15‰, and 15.29, 13.15, 9.70 and 7.73 mg/l at 25‰, respectively.

3.2. Effect of copper on growth

All shrimps survived for 75 days in the controls and in 0.45 mg/l copper. However, all shrimps exposed to 4.50 mg/l copper were dead after 45 days. Mortality rates at 0.90 and 1.80 mg/l copper were 22.2% and 50% after 75 days of exposure, respectively.

The growth in wet weight and total length of shrimps exposed to each test solution is shown in Fig. 2. After 15 days the weight of shrimps exposed to 4.50 mg/l copper was significantly lower ($P < 0.05$) than those exposed to 1.80 mg/l copper and lower. The weight of shrimps exposed to 0.90 mg/l copper was significantly lower ($P < 0.05$) than controls and those exposed to 0.45 mg/l copper after 45, 60 and 75 days (Fig. 2A).

Table 1
The linear regression of various copper concentration (mg/l) as X versus weight gain (g) or length increase (cm) as Y, and the EC50 (concentration that reduced weight gain or length increase by 50% compared to controls) for *P. monodon* exposed to different concentrations of copper. $Y = A + BX$, where Y is weight gain or length increase, and X is copper concentration

Time elapsed (days)	A	B	r^2	EC50 (mg/l)
Weight gain (g)				
15	0.082	−0.013	0.965*	3.367
30	0.188	−0.036	0.974*	2.822
45	0.372	−0.104	0.932*	1.893
60	0.597	−0.195	0.940*	1.635
75	0.900	−0.319	0.951*	1.508
Length increase (cm)				
15	0.560	−0.089	0.952*	3.339
30	1.040	−0.172	0.994**	3.049
45	1.585	−0.375	0.979*	2.174
60	2.119	−0.624	0.979*	1.760
75	2.620	−0.821	0.960*	1.677

* Denotes significant level at 95%.
** Denotes significant level at 99%.

Table 2
Mean (SE) carapace length, total length and the ratio of carapace length to total length of *P. monodon* juveniles exposed to different concentrations of copper for 75 days

Copper (mg/l)	Carapace length (cm)	Total length (cm)	Ratio of carapace length to total length	*n*
Control	1.104[a] (0.167)	5.076[a] (0.250)	0.218[b] (0.021)	18
0.45	1.088[a] (0.193)	5.056[a] (0.213)	0.216[ab] (0.019)	18
0.90	0.984[ab] (0.109)	4.446[b] (0.265)	0.222[a] (0.025)	14
1.80	0.887[b] (0.095)	3.748[c] (0.273)	0.237[a] (0.028)	9

Values in the same column having different letters are significantly different ($P < 0.05$).

After 15 days the total length of shrimps exposed to 4.50 mg/l copper was significantly lower ($P < 0.05$) than those exposed to 1.80 mg/l copper (Fig. 2B). The maximum acceptable toxicant concentration (MATC) was 1.80 and 0.45 mg/l copper after 15 and 30 days of exposure, respectively.

The linear regressions of weight gain and length increase versus concentration of copper are given in Table 1. The average final weight after 75 days of the control shrimps was 5.5 times their initial weight (0.19 g). The average final weight of the shrimps exposed to 1.80 mg/l copper was 2.6 times their initial weight. The 30- and 60-day EC50 (concentration that reduced weight gain by 50% compared to controls) was 2.82 and 1.64 mg/l copper, respectively. The average total length of the control shrimps after 75 days was 1.96 times their initial total length (2.59 cm). The average total length of the shrimps exposed to 1.80 mg/l copper was 1.41 times their initial total length. The

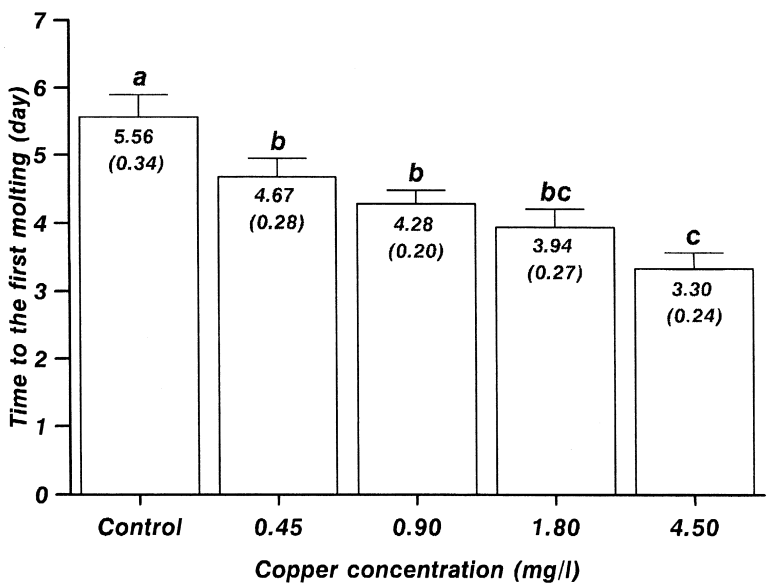

Fig. 3. Effects of different concentrations of copper (mg/l) on the time to the first molting of *P. monodon*. Values are mean (SE). Bars with the same letter are not significantly different ($P > 0.05$).

Table 3
Number of *P. monodon* juveniles molting, and the mean molting frequency of shrimps exposed to different concentrations of copper for 75 days

Copper (mg/l) frequency	Number of shrimp with molting frequency of			Mean molting frequency	*n*
	6	7	8		
Control	2	12	4	7.11[a]	18
0.45	1	12	5	7.22[a]	18
0.90	5	9	0	6.64[b]	14
1.80	6	3	0	6.32[b]	9

Values in the same column having different letters are significantly different ($P < 0.05$).

30- and 60-day EC50 was 3.05 and 1.76 mg/l copper, respectively. It appears that copper concentrations affected reduction in weight slightly more than reduction in total length.

The carapace lengths of shrimps exposed to 1.80 mg/l copper were significantly lower ($P < 0.05$) than those exposed to 0.45 mg/l copper and controls (Table 2). The ratio of carapace length to total length of shrimps exposed to 0.90 mg/l copper was significantly higher ($P < 0.05$) than that in control shrimps.

Following exposure to copper concentrations of 0.45 mg/l and above, the time before the first molt in *P. monodon* was shortened significantly (Fig. 3). *P. monodon* exposed to copper at 0.90 and 1.80 mg/l experienced a significant ($P < 0.05$) decrease in molting frequency (Table 3). Mean molting frequency decreased from 7.11 for control the shrimps to the 6.32 for shrimps exposed to 1.80 mg/l copper. The MATC was 0.45 mg/l copper as determined from the mean molting frequency of *P. monodon* after 75 days.

3.3. Effect of copper on feeding

The amount of feed the shrimps consumed declined with increasing copper concentration (Table 4). The feeding rates of shrimps exposed to 10 and 20 mg/l copper was significantly lower ($P < 0.05$) than that of those exposed to 5 mg/l copper, which was

Table 4
Effects of copper on feed intake and feeding rate (%) of *P. monodon* juveniles after 1.5 h of exposure to different concentrations of copper

Copper (mg/l)	Weight (g)	Feed intake (g)	Feeding rate (%)	*n*
Control	6.282[a] (0.095)	0.074[a] (0.011)	1.174[a] (0.160)	5
1	6.332[a] (0.047)	0.064[a] (0.009)	1.003[a] (0.129)	5
5	6.221[a] (0.072)	0.032[b] (0.010)	0.507[b] (0.155)	5
10	6.187[a] (0.137)	0.009[c] (0.002)	0.139[c] (0.038)	5
20	6.206[a] (0.106)	0.002[c] (0.001)	0.036[c] (0.008)	5

Values in the same column having different letters are significantly different ($P < 0.05$).

significantly lower than controls and shrimps in 1 mg/l copper. The MATC was 1 mg/l copper as determined from the effect of copper on feeding of *P. monodon*.

4. Discussion

In kuruma shrimp (*P. japonicus*), the tolerance of copper increased with developmental stage and salinity (Bambang et al., 1995). The 96-h LC50 of copper for juveniles was 1.20 and 2.05 mg/l at 17‰ and 37‰, respectively. The present study also demonstrated that the tolerance to copper decreased when the shrimps were in lower salinity, with 96-h LC50 of 3.13 and 7.73 mg/l at 15‰ and 25‰, respectively. These differences between the 96-h LC50 of copper for *P. japonicus* juveniles (13 g) and *P. monodon* juveniles (0.63 g) is considered to be mainly due to size and salinity differences.

Copper sulfate at 0.084 mg/l (which is equivalent to 0.033 mg/l copper) is used to reduce *Microcystis* blooms in ponds (Boyd, 1990). This concentration of copper did not significantly affect the growth of juvenile *P. monodon* over 75 days in the present study. However, following 10 days of exposure to copper as low as 0.06–0.10 mg/l, zoeal larvae of sand shrimp (*Metapenaeus ensis*) experienced a decrease in both growth and survival (Wong et al., 1995).

The toxic action and the physiological effect of copper on fish had been widely studied and is summarized by Taylor et al. (1995). The primary effect of copper in fish is in the gills (Evans, 1987), which are structurally damaged (e.g., Kirk and Lewis, 1993; Wilson and Taylor, 1993). This damage includes collapse of lamellae, lifting of lamellar epithelium away from pillar cells and swelling of epithelial cells. In addition, there is disturbance of ion regulation and respiratory gas exchange as a consequence of structural disruption of gill epithelium in copper-exposed fish (Lauren and McDonald, 1985; Wilson and Taylor, 1993). Gill damage was also observed in 15.9-mg/l-copper-exposed *P. monodon* (Guo and Liao (1992).

Thurberg et al. (1973) reported that, following 48 h exposure to copper in the range of 0–40 mg/l, the shore crab (*Carcinus maenas*) decreased its hemolymph osmolality with an increase of copper exposure, and that the reduction was most obvious when the crabs were exposed to low salinity. Bjerregaard and Vislie (1986) reported that following 6 days of exposure to copper of 0.5, 1.0 and 10 mg/l, *C. maenas* decreased its hemolymph osmolality, Na^+, K^+ and Cl^- concentrations. However, Boitel and Truchot (1989) reported that following 18 days of exposure to 1.0 mg/l copper, *C. maenas* experienced metabolic acidosis without marked changes of hemolymph ions. Exposing *P. japonicus* to copper concentrations of 0.5, 1.0 and 1.5 mg/l at full strength seawater (37‰) and diluted seawater (17‰), Bambang et al. (1995) reported that both hypo-osmoregulatory capacity and hyper-osmoregulatory capacity were significantly reduced after 4 days of exposure. Further research is needed to study osmotic and ionic regulation in penaeid shrimps under copper stress.

Increased hemoglobin, higher hematocrit levels and elevated numbers of erythrocytes have been observed in brook trout (*Salvelinus fontinalis*) after 21 days of copper exposure (McKim et al., 1970). Copper elevated oxygen consumption in brown trout (*Salmo trutta*) (Beaumont et al., 1995) and bluegill (*Lepomis macrochirus*) (O'Hara, 1971), and decreased oxygen consumption in common carp (*Cyprinus carpio*) (De

Boeck et al., 1995). There is no effect on the oxygen consumption of *C. maenas* after 48-h exposure to 40 mg/l copper (Thurberg et al., 1973). Further research is needed to study the oxygen consumption and oxygen affinity in penaeid shrimps exposed to sub-lethal levels of copper.

Molting of decapod crustaceans is affected by extrinsic factors such as temperature, salinity, light intensity and pollutants, and also by intrinsic factors such as nutritional state and hormones (reviewed by Kleinholz, 1985). Exposure to ammonia and nitrite in juvenile *P. monodon* resulted in an increase in molting frequency (Chen and Chen, 1992; Chen and Lin, 1992). Exposure to 0.5 mg/l saponin in *P. japonicus* decreased the time to the first molting and molting frequency (Chen and Chen, 1996). Similar responses were observed in the present study, which indicated that the time to the first molting was inversely related to ambient copper concentration. It is known that external factors like light and temperature, which stimulate the central nerve system and cause the secretion of hormones, would affect the molting cycle of decapod crustaceans (Wassenberg and Hill, 1984). However, the shrimps in the present study were tested in a controlled light/dark (L:D 12:12) and temperature (25.5 ± 0.2°C) environment. It would be interesting to study the cause of why shrimps exposed to copper experienced a reduction in the time before first molting and a decrease in molting frequency.

Based on the 96-h LC50 (3.13 and 7.73 mg/l) and an empirical application factor of 0.1 (Sprague, 1971), the "safe levels" are 0.31 and 0.77 mg/l copper for juveniles weighing 0.63 g at 15‰ and 25‰, respectively. The threshold concentration that produces statistically significant deleterious effects is commonly expressed as the MATC (Wickins, 1976; Chen and Chen, 1996). The MATC is 0.45 mg/l copper based on the weight, length, growth factor and molting frequency of shrimps weighing 0.18–1.03 g, and is 1.0 mg/l copper based on the feeding of shrimps weighing 6.25 g. Our study reveals that *P. monodon* exposed to a concentration of copper lower than the "safe level" may exhibit different degrees of chronic response, knowledge of which would be useful in pond management during shrimp farming.

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References

American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1985. Standard Methods for the Examination of Water and Wastewater. 16th edn. American Public Health Association, Washington, DC, 1268 pp.

Bambang, Y., Thuet, P., Charmantier-Daures, M., Trilles, J.-P., Charmantier, G., 1995. Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate (Crustacea, Decapod). Aquat. Toxicol. 33, 125–139.

Beaumont, M.W., Butler, P.J., Taylor, E.W., 1995. Exposure of brown trout, *Salmo trutta*, to sub-lethal copper concentration in soft acidic water and its effect upon sustained swimming performance. Aquat. Toxicol. 33, 45–63.

Bjerregaard, P., Vislie, T., 1986. Effect of copper on ions and osmoregulation in the shore crab *Carcinus maenas*. Mar. Biol. 91, 69–76.

Boitel, F., Truchot, J.-P., 1989. Effects of sublethal and lethal copper levels on hemolymph acid–base balance and ion concentrations in the shore crab *Carcinus maenas* kept in undiluted sea water. Mar. Biol. 103, 495–501.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. Birmington Publishing, Birmingham, AL, 482 pp.

Buikema, A.L. Jr., Niedertehner, P.R., Cairns, J. Jr., 1982. Biological monitoring: Part IV. Toxicity testing. Water Res. 16, 239–262.

Chen, L.C., 1990. Aquaculture in Taiwan. Fishing New Books, Oxford, 273 pp.

Chen, J.C., Chen, S.F., 1992. Effects of nitrite on growth and molting of *Penaeus monodon* juveniles. Comp. Biochem. Physiol. 101C, 453–458.

Chen, J.C., Chen, K.W., 1996. Effects of saponin on survival, molting and feeding of *Penaeus japonicus* juveniles. Aquaculture 144, 165–175.

Chen, J.C., Lin, C.Y., 1992. Effects of ammonia on growth and molting of *Penaeus monodon* juveniles. Comp. Biochem. Physiol. 101C, 449–452.

De Boeck, G., De Smet, H., Blust, R., 1995. The effect of sublethal levels of copper on oxygen consumption and ammonia excretion in the common carp, *Cyprinus carpio*. Aquat. Toxicol. 32, 127–141.

Duncan, D.B., 1955. Multiple-range and multiple *F* test. Biometrics 11, 1–42.

Evans, D.H., 1987. The fish gill: site of action and model for toxic effect of environmental pollutants. Environ. Health Perspect. 71, 47–58.

Ferraris, R.P., Parado-Estepa, F.D., Ladja, J.M., De Jesus, E.G., 1986. Effect of salinity on the osmotic, chloride, total protein and calcium concentrations in the hemolymph of *Penaeus monodon* (Fabricius). Comp. Biochem. Physiol. 83A, 701–708.

Guo, J.J., Liao, I.C., 1992. Toxicity of copper sulfate to juvenile grass prawn, *Penaeus monodon*. COA Fish. Ser. 33, 1–7.

Kirk, R.S., Lewis, J.W., 1993. An evaluation of pollutant induced changes in the gills of rainbow trout using scanning electron microscopy. Environ. Technol. 14, 577–585.

Kleinholz, L.H., 1985. Biochemistry of crustacean hormones. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea, Integument Pigments and Hormonal Process vol. 9 Academic Press, New York, pp. 464–522.

Lauren, D.J., McDonald, D.G., 1985. Effects of copper on branchial ionoregulation in the rainbow trout, *Salmo gairdneri* Richardson: modulation by water hardness and pH. J. Comp. Physiol. 155B, 635–644.

McKim, J.M., Christensen, G.M., Hunt, E.P., 1970. Changes in the blood of brook trout (*Salvelinus fontinalis*) after short-term and long-term exposure to copper. J. Fish. Res. Board Can. 27, 1883–1889.

O'Hara, J., 1971. Alterations in oxygen consumption by bluegills exposed to sublethal treatments with copper. Water Res. 5, 321–327.

Sorensen, E.M.B., 1991. Metal Poisoning in Fish. CRC Press, Boca Raton, FL, 361 pp.

Sprague, J.B., 1971. Measurement of pollutant toxicity to fish. Bioassay methods for acute toxicity. Water Res. 5, 245–266.

Steel, R.G.D., Torrie, J.H., 1980. Principle and Procedure of Statistics. McGraw-Hill, New York, 633 pp.

Taylor, E.W., Beaumont, M.W., Butler, P.J., Mair, J., Mujallid, M.S.I., 1995. Lethal and sublethal effects of copper upon fish: a role for ammonia toxicity? In: Taylor, E.W. (Ed.), Toxicology of Aquatic Pollution Physiological, Cellular and Molecular Approaches. Cambridge Univ. Press, Cambridge, UK, pp. 85–113.

Thurberg, F.P., Dawson, M.A., Collier, R.S., 1973. Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. Mar. Biol. 23, 171–175.

Trevors, J.V., Lusty, C.W., 1985. A basic microcomputer program for calculating LD50 value. Water, Air, Soil Pollut. 24, 431–442.

Wassenberg, T.J., Hill, B.J., 1984. Moulting behaviour of the tiger prawn *Penaeus esculentus* (Haswell). Aust. J. Mar. Freshwater Res. 35, 561–571.

Wickins, J.F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture 9, 19–37.

Wilson, R.W., Taylor, W.W., 1993. The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. J. Comp. Physiol. 163B, 38–47.

Wong, C.K., Cheung, J.K.Y., Chr, K.H., 1995. Effects of copper on survival, development and growth of *Metapenaeus ensis* larvae and postlarvae (Decapoda: Penaeidae). Mar. Pollut. Bull. 31, 4–12.