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# Effect of sublethal copper on growth efficiency of the shrimp, *Metapenaeus dobsoni* (Miers)

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**Abstract**

Energy utilization and growth are regarded as important biological indices to interpret stress effects in the case of aquatic organisms. In this investigation, an attempt is made to elucidate the effect of copper at sublethal concentrations, on the energy conversion and growth efficiency of a commercially important penaeid shrimp *Metapenaeus dobsoni*. Juvenile *M. dobsoni* were exposed to 0.05 and 0.15 mg Cu $l^{-1}$. The animals were fed *ad libitum* on fish meat or clam meat. Animal tissue, faecal pellets and feed were analyzed for CHN and the calorific values were calculated employing a stoichiometric model. Growth rates of animals maintained under controlled conditions and those exposed to 0.05 mg Cu $l^{-1}$ were not significantly different suggesting tolerance of the animal to low concentration of copper in the culture media. The differences between the assimilation rates for different treatments were also not statistically significant even though variations were noticed in the feeding rates of animals exposed to the heavy metal. Animals exposed to the higher concentration of copper (0.15mg Cu $l^{-1}$) resulted in lesser growth efficiency. The differences in gross and net growth efficiency were statistically significant. It was observed that the energy utilization for maintenance was increased in the case of *M. dobsoni* exposed to copper. Such enhanced energy expenditure led to reduction in somatic growth.

## Introduction

Studies on bioenergetics lead to a better understanding of the energy distribution for maintenance, growth and reproduction in organisms. Hence, this has been considered as an effective tool to understand the effect of chronic stress from pollutants. Since growth is a fundamental component of physiological fitness, estimation of growth rates serve as an important index for analyzing pollutant effects (Widdows, 1985). Measurements for several individual bioenergetic rate functions like feeding, food absorption, respiration, excretion and growth, ultimately provide an estimate of the overall growth efficiency (Johns and Miller, 1982).

Attempts have been made by several investigators to study the bioenergetics of marine organisms. However, information on the energetics of crustaceans is limited and study on energy budgets and growth as indicators of stress effects in shrimps is minimal. Johns and Miller (1982) used bioenergetics to investigate the mechanisms of pollutant toxicity in crustacean larvae and reported 11 to 27% reduction in the net growth efficiency. Additional studies on the energetics of penaeid shrimp are those of Sumitra-

Vijayaraghavan *et al.* (1981) on *Metapenaeus dobsoni* and Bautista (1986) on *Penaeus monodon*. Reduction in growth as a result of copper toxicity has been reported in *P. japonicus* (Liao and Hsieh, 1988). Liao and Hsieh (1990) studied toxicity of copper, cadmium and zinc in juvenile *Macrobrachium rosenbergiii* and reported conspicuous reduction in both growth and survival. A few notable contributions on the adverse effects of copper on the bioenergetics of lower crustaceans are on cladocerans (Koivisto *et al.*, 1992) and *Daphnia* spp. (Soundrapandian and Venkataraman, 1990; Zhu *et al.*, 1992). Toxic effects of copper have also been demonstrated in *Holmesimysis costata* (Martin *et al.*, 1989), *Artemia salina* (Rao and Latheef, 1989), *Balanus eburneus* (Weis and Weis, 1992) and *Gammarus pulex* (Maund *et al.*, 1992). Giudici and Migliore (1988), Giudici *et al.* (1988) and Giudici and Guarino (1989) studied copper toxicity to crustacean isopods *Asellus aquaticus* and *Idothea baltica* and observed significant reduction in body growth as a result of chronic exposure to the heavy metal. The toxicity of heavy metals to crustaceans is known to vary from one ontogenic stage to another (Giudici and Guarino, 1989; Liao and Hsieh, 1990). In this study, an attempt is made to assess the effects of copper on the rate of food intake, assimilation, and growth efficiency of a commercially important penaeid shrimp, *M. dobsoni*, widely distributed in tropical waters.

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## Material and methods

Juveniles of *Metapenaeus dobsoni* [measuring 25-35 mm from the tip of rostrum to the tip of telson] were collected from an aquaculture farm at Vypeen ($76^0$ 10' E, $10^0$ 0' N); transported to the laboratory in oxygen-filled polyethylene bags and acclimatized to a salinity of 20 ± 2 ppt. The sublethal concentrations of copper were determined with reference to the $LC_{50}$ values for the species (Sivadasan *et al.*, 1986) and the shrimp were exposed to 0.05 and 0.15 mg Cu $l^{-1}$. Copper sulphate (AR) was used in the test medium as the copper source. The animals were exposed to copper in a semi flow-through system, for 15 days at room temperature (28 ± $2^0$C). During the course of the experiments, the shrimp (both control and copper-exposed) were fed *ad libitum* on fish meat (Tilapia) and clam (<0.002 mg Cu $g^{-1}$) in the first and second experiments respectively. In both the experiments 3 replicates were run simultaneously for each treatment and the controls (n = 10 animals/replicate). The medium was well aerated and 2/3rd of the test solution in each replicate was replenished every 24 h. A group of 30 animals belonging to the same size group as that of the experimental animals was used to determine the initial tissue weight and energy content (kJ). Left over feed and faecal matter were siphoned out and collected separately every 24 h, washed in distilled water to remove salt and dried at 90ºC to a constant weight. Animals collected at the end of the experiments were washed in distilled water and dried to a constant weight. During the experiment, when the rate of mortality exceeded 70% that experiment was abandoned. The mortality exceeded 70% (due to accidental failure of aeration) in one of the replicates of the control series of the second experiment only. Whenever the rate of mortality was less than 30% (3 animals / replicate), the dry weight was added to the final tissue weight. Mortality of 4 animals was recorded during the course of the experiment (2 each in the third replicate of the low level copper treatment and first replicate of the high level copper treatment of the first experiment). The weight of feed consumed and assimilated was calculated from the weight of feed given, feed left over and faeces.

Animal tissue, faeces and feed were analyzed for carbon, hydrogen and nitrogen in an elemental (CHN) analyzer. The ash content was estimated after combusting the tissue, faeces and feed in a muffle furnace (590ºC). A stoichiometric model (Gnaiger and Bitterlich, 1984) based on the CHN composition of the organic dry weight was used to estimate the calorific values. The model assumed all nitrogen to be protein-bound with a common residual water fraction of 0.06. Percent assimilation (based on food consumed and food absorbed), gross growth efficiency and net growth efficiency were calculated following the balanced energy equation of Winberg (1960). Growth per unit of ingested food is given as the gross growth efficiency and growth per unit of absorbed food as the net growth efficiency (Widdows *et al.*, 1980). Assimilation and growth efficiency were calculated after converting the organic carbon fraction of the tissues, faeces and feeds into kJ. The organic carbon fraction in ash-free biomass, $w_c$ [(g organic C)/(g $_{af}$W)], is calculated as:

$$w_c = \frac{{}_{tot}w_c - {}_{ash}w_c \times w_{ash}}{1 - w_{ash}}$$

where, ${}_{tot}w_c$ is the total carbon mass in the total dry biomass [(g total C)/(g $_d$W)], ${}_{ash}w_c$ is the inorganic carbon fraction in the ash [(g inorganic C)/ (g ash)], and $w_{ash}$ is the mass fraction of ash in the dry weight [(g ash)/(g $_d$W)]. Assimilation and growth efficiency were calculated as given below.

Assimilation efficiency (%) = A/C x 100; gross growth efficiency (%) = P/C x 100; and net growth efficiency (%) = P/A x 100; where C = food consumed, A = food assimilated and P = weight gained, in terms of energy. Means were calculated from 3 replicates. To test the significant difference between the means of the characters under study, for the different levels of copper treatments and the feeds, the two-way analysis of variance was carried out. The *post hoc* comparison between the means was carried out by the Least Significant Difference (LSD) method.

## Results

Mass fraction of ash (g/g $_d$W) and organic fraction (g/g $_d$W) of tissue, feed and faecal matter of *Metapenaeus dobsoni*, exposed to copper contaminated culture medium and fed on fish meat or clam meat, were estimated and the energy contents (kJ) of feed consumed and assimilated were calculated. Total energy content for the tissues of the control and the copper-exposed shrimp showed variation. Copper-exposed animals normally had decreased energy content. Carbon content and calorific values of the faeces were uniformly reduced. The mass fraction of ash in faeces showed little variation between treatments. The first experiment was performed exposing *M. dobsoni* to 0.05 and 0.15 mg Cu $l^{-1}$, feeding the animals *ad libitum* on fish meat. Rates of food consumption and assimilation and growth in terms of energy were estimated at the end of the experiment. Food consumption was reduced in the group of animals exposed to the higher concentration of copper, the mean value being 48.63 kJ as against 54.45 kJ in the control group. Maximum food consumption (56.28 kJ) was noticed in animals exposed to 0.05 mg Cu l-1. The energy equivalent of faeces was also maximum in the low level copper exposed animals, it being 3.04 kJ as against 1.93 kJ in the control and 1.59 kJ in animals exposed to 0.15 mg Cu l-1. Thus, in the low level copper exposed animals, a portion of the energy was lost through faeces whereas in those exposed to 0.15 mg Cu l-1, food assimilation was more thereby producing lesser faeces. However, the differences in the assimilation efficiencies among treatments were not statistically significant. The mean percentage assimilation was 96.45 in the control group, 94.59 in the low level and 96.73 in the high level copper exposed shrimp. The levels of the toxicant in the culture medium adversely affected the final tissue weight which was reduced from 15.62 kJ in the control group to 15.24 kJ in the shrimp exposed to the lower level of copper and 11.53 kJ in those exposed to the higher level of copper. Thus, variation in the growth rate between the shrimp

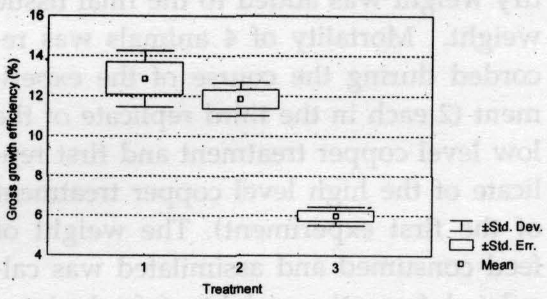


*Fig. 1a. M. dobsoni . Gross growth efficiency(%) when exposed to copper and fed on fish meat (1: control, 2 : 0.05 mg Cu $l^{-1}$, 3 : 0.15 mg Cu $l^{-1}$)*

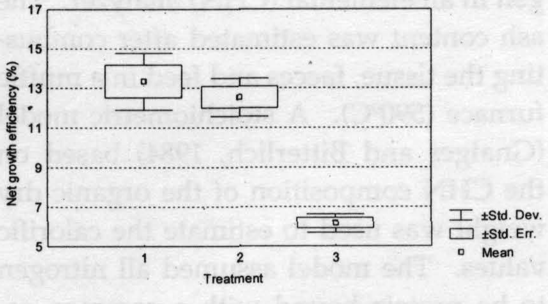


*Fig. 1b. M. dobsoni : Net growth efficiency (%) when exposed to copper and fed on fish meat (1 : control, 2 : 0.05 mg Cu $l^{-1}$, 3 : 0.15 mg Cu $l^{-1}$)*

exposed to 0.05 and 0.15 mg Cu $l^{-1}$ was much higher than the variation between those exposed to 0.05 mg Cu $l^{-1}$ and the control group. As a result the mean gross growth efficiency declined from 12.87% in the control group to 11.85% in the low level and 5.96% in the high level copper-exposed shrimp (Fig1a). A similar trend was noticed in case of the net growth efficiency also where the values declined from 13.34% in the control group to 12.53% and 6.16% in the low level and high level copper-exposed shrimp respectively (Fig1b). Analysis of the results showed statistically significant difference (P <0.01) in both the gross and net growth efficiencies between the control and the high level copper-exposed shrimp and also between the low and high level copper-exposed shrimp.

The second experiment was conducted exposing *M. dobsoni* to copper and using clam meat as feed. The energy contents of the feed consumed by the shrimp maintained in 0.05 and 0.15mg Cu $l^{-1}$ were comparable. However, the mean tissue weights gained in terms of energy were 21.42 kJ and 16.00 kJ, respectively. Loss of energy through faeces was higher in the 0.15 mg Cu $l^{-1}$ group (5.46 kJ) versus 2.93 kJ in the controls and 2.76 kJ in the 0.05 mg Cu $l^{-1}$ treatment. Mean values obtained for the gross growth efficiency and net growth efficiency in the treatments and the control are presented in Fig 2 (a & b). When the mean values for assimilation ranged from 92.24% to 96.08% among the different groups, values for the gross growth efficiency declined from 20.1% in the control to 19.74% and 11.93% in the shrimp exposed to 0.05 mg Cu $l^{-1}$ and 0.15 mg Cu $l^{-1}$, respectively. Thus, growth in terms of energy was reduced in the shrimp exposed to 0.15 mg Cu $l^{-1}$. A trend of decline was noticed in the case of net growth efficiency also, where the values decreased from 21.17% in the control group to 20.55% in the shrimp exposed to the lower level and 12.93% in those exposed to the higher level of copper. Thus the results showed nearly 50% reduction in both the gross and net growth efficiencies in the shrimp exposed to the higher dose of copper when

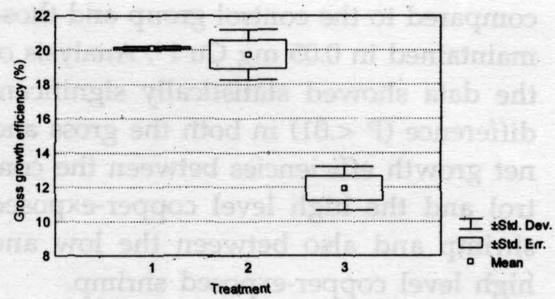


*Fig. 2a. M. dobsoni : Gross growth efficiency (%) when exposed to copper and fed on clam (1: control, 2 : 0.05 mg Cu- $l^{-1}$, 3 : 0.15 mg Cu $l^{-1}$)*

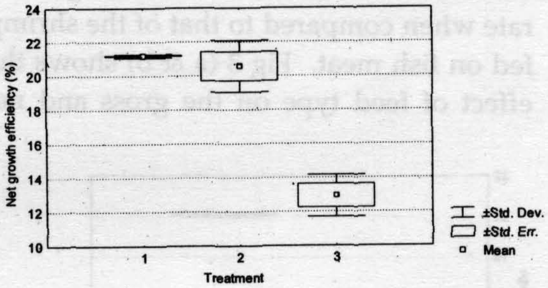


*Fig. 2b. M. dobsoni : Net growth efficiency (%) when exposed to copper and fed on clam (1 : control, 2 : 0.05 mg Cu $l^{-1}$, 3 : 0.15 mg Cu $l^{-1}$*

compared to the control group and those maintained in 0.05 mg Cu l$^{-1}$. Analysis of the data showed statistically significant difference (P <.01) in both the gross and net growth efficiencies between the control and the high level copper-exposed shrimp and also between the low and high level copper-exposed shrimp.

A study of the data clearly showed the influence of feed type on the growth rate of *Metapenaeus dobsoni*. The shrimp fed on clam meat showed a better growth rate when compared to that of the shrimp fed on fish meat. Fig 3 (a & b) shows the effect of feed type on the gross and net growth efficiencies of the control group of shrimp. The mean gross growth efficiency increased from 12.87% in the shrimp fed on fish meat to 20.1% in those fed on clam meat. In the case of net growth efficiency, the increase was from 13.34% to 21.17%. The effect of feed type was noticed in the copper-exposed shrimp also irrespective of the concentration of the toxicant present in the culture medium. In the low level copper-exposed shrimp, the mean values for the gross growth efficiency were 11.85% and 19.74% and those for the net growth efficiency were 12.53% and 20.55%, when fed on fish meat and clam

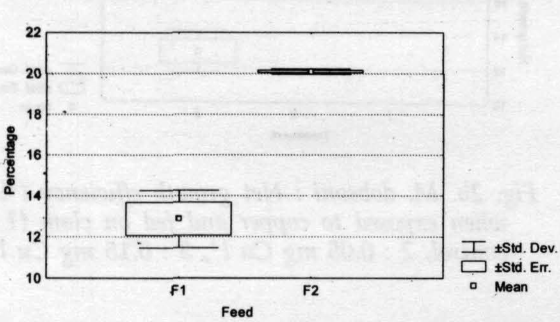


Fig. 3a. *M. dobsoni : Effect of feed on gross growth efficiency (%) in control animals. F1 : fish meat, F2 : clam*

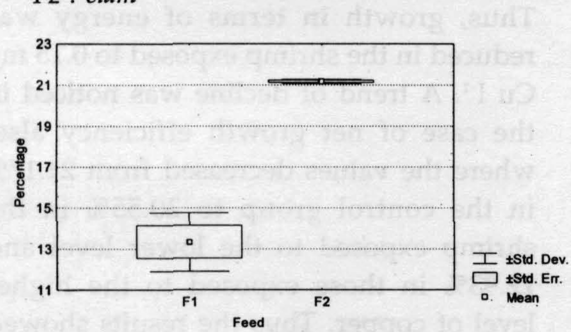


Fig. 3b. *M. dobsoni : effect of feed on net growth efficiency (%) in control animals. F1 : fish meat, F2 : clam*

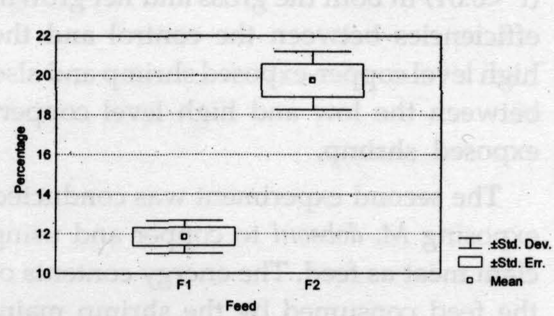


*Fig. 4 a. M. dobsoni. Effect of feed on gross growth efficiency (%) when exposed to 0.05 mg Cu l$^{-1}$. F1 : fish meat, F2 : clam*

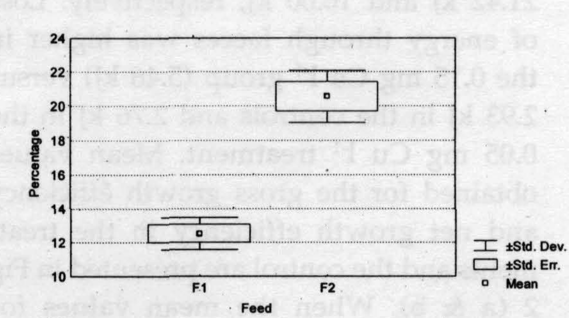


Fig. 4b. *M. dobsoni : Effect of feed on net growth efficiency (%) when exposed to 0.05 mg Cu l$^{-1}$. F1 : fish meat, F2 : clam*

respectively (Fig 4a & b). In the shrimp exposed to 0.15mg Cu $l^{-1}$, using clam as feed, also the mean values for the gross and net growth efficiencies (11.93% and 12.93% respectively) were much higher than the values (5.96% and 6.16% respectively) for those exposed to the same concentration of copper but fed on fish meat (Fig 5a & b). Thus the differences in the growth efficiencies noticed among treatments, were controlled not only by the heavy metal concentrations but also by the type of feed administered. The mean differences in the gross and net growth

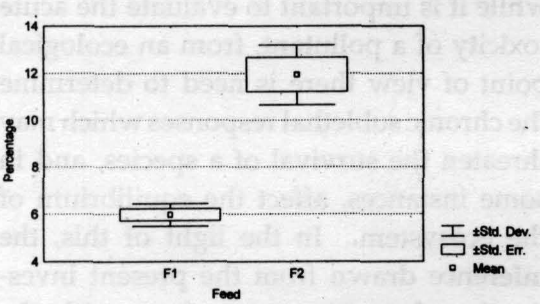


Fig. 5a. *M. dobsoni : Effect of feed on gross growth efficiency (%) when exposed to 0.15 mg Cu $l^{-1}$. F1 : fish meat, F2 : clam*

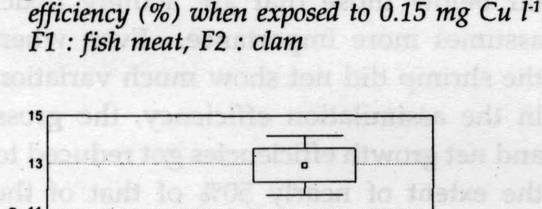


Fig. 5b. *M. dobsoni : Effect of feed on net growth efficiency (%) when exposed to 0.15 mg Cu $l^{-1}$. F1 : fish meat, F2 : clam*

efficiencies with reference to the variety of feeds for the shrimp belonging to the control group and those exposed to the different levels of copper were found to be statistically significant ($P < 0.01$).

## Discussion

An accurate understanding of food consumption, conversion and growth efficiency of marine invertebrates in their natural habitat is rather difficult since the types of food consumed and the quantities utilized cannot be accurately assessed. The bioenergetic models could also be influenced by the changing environmental parameters in general, and toxic conditions in particular. According to Rice (1990), a variation from the normal bioenergetic model on growth could be utilized in determining the separate effects of feeding rate, assimilation, activity or other variables of natural or anthropogenic origin. This would help to explain if deviation from a known growth pattern occurs due to natural causes, stress from xenobiotics, or both.

Rates of assimilation and gross and net growth efficiencies obtained for juvenile *M. dobsoni* in this study were comparable to those reported for the juveniles of the species by earlier workers (Sumitra-Vijayaraghavan *et al.*, 1981). Though the animals under stress from the toxicant showed comparatively higher consumption of food, this was not followed by equally efficient assimilation. However, the percentage assimilation efficiency was fairly high and comparable between treatments. The data further showed that the good rates of assimilation efficiency noticed in the shrimp exposed to copper, however, were not accompanied by enhanced growth rates. Reduction in the rates of gross and net growth efficiencies in these animals clearly indicated utilization of more energy for maintenance under stress from metal toxicity. In a similar study on the effects of copper, larval stages of rock crab *Cancer irroratus* exposed to 0.01 mg Cu $l^{-1}$ showed considerable reduction in the somatic growth (11 to 27% decrease in net growth efficiency) due to a significant increase in the maintenance cost (Johns and Miller, 1982). The authors felt that the mechanisms underlying such metabolic alterations are not well understood. Heath (1987) also reported that changes in the maintenance cost as a result of copper or any other metal are not well explained. The increase in the cost of maintenance as a result of copper toxicity would probably leave fewer calories for growth. Excretion of copper and repair of damaged tissues might contribute to such diversion of energy as might alterations in osmoregulation. Such pollutant-induced changes in the metabolic activities of the organisms could represent a general endogenous response to stress.

A significant observation made during the present study was that growth, in terms of energy, was comparable between *M. dobsoni* maintained under controlled conditions and those exposed to the lower concentration of copper (0.05 mg Cu $l^{-1}$). Thus, the shrimp seemed to be relatively tolerant to copper at low concentrations. However, exposure of the shrimp to 0.15mg Cu $l^{-1}$ resulted in highly reduced growth efficiency. Liao and Hsieh (1988) examined the toxicity of copper, cadmium and zinc in *Penaeus japonicus* and found that growth and survival of the shrimp in the test group were significantly lower than those in the control group. Maximum accumulation of the metals was often reached after 8 or 16 days of exposure. A similar study on the toxic effects of copper, cadmium and zinc in juvenile *Macrobrachium rosenbergii* also showed conspicuous reduction in growth and survival (Liao and Hsieh, 1990). Giudici *et al.* (1988) have rightly pointed out that while it is important to evaluate the acute toxicity of a pollutant, from an ecological point of view there is need to determine the chronic sublethal responses which may threaten the survival of a species, and in some instances, affect the equilibrium of the ecosystem. In the light of this, the inference drawn from the present investigation that copper interferes with the growth efficiency of *M. dobsoni* at levels far below those that are acutely toxic, assumes more importance. Even when the shrimp did not show much variation in the assimilation efficiency, the gross and net growth efficiencies got reduced to the extent of nearly 50% of that of the control. However, the effect of feed type on the growth efficiency of the animal is noteworthy. The present study clearly shows that, with the right type of feed, the growth rate can be greatly improved, even when the animals are under stress from the toxicant present at sublethal concentrations. According to Calabrese *et al.* (1984) the effects of sublethal levels of metals on marine animals may be concentration-dependent and these may not always be readily apparent, many requiring months before they become significantly measurable. The fact that the growth efficiency of the test animal, *M. dobsoni* is considerably lowered even at concentrations of copper that might appear safe in acute toxicity tests, is obviously a matter of concern.

## References


Bautista, M.N. 1986. The response of *Penaeus monodon* juveniles to varying protein / energy ratios in test diets. *Aquaculture*, **53** : 229-242.

Calabrese, A., J. R. MacInnes, D. A. Nelson, R. A. Greig and P. P. Yevich. 1984. Effects of long term exposure to silver or copper on growth, bioaccumulation and histopathology in the blue mussel *Mytilus edulis*. *Mar. Environ. Res.*, **11**:253-274.

Giudici, M.N. and S. M. Guarino. 1989. Effects of chronic exposure to cadmium or copper on *Idothea baltica* (Crustacea, Isopoda). *Mar. Pollut. Bull.*, **20** : 69-73.

----- and L. Migliore. 1988. Long term effect of cadmium or copper on *Asellus aquaticus* (L.) (Crustacea, Isopoda). In: Sladecek, V. (Ed), Congress in New Zealand, 1987. *Proc 1988. Verh. Int. Ver. Theor. Angew. Limnol / Proc. Int. Assoc. Theor. Appl. Limnol / Trav. Assoc. Int. Limnol. Theor. Appl.*, **23**:1660-1662.

-----,-----, C. Gambardella and A. Marotta. 1988. Effect of chronic exposure to cadmium and copper on *Asellus aquaticus* (L) (Crustacea, Isopoda). *Hydrobiologia*, **157**:265-269.

Gnaiger, E. and G. Bitterlich. 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. *Oecologia (Berl.)*, **62**:289-298.

Heath, A. G. 1987. Water pollution and fish physiology. CRC Press Inc. Boca Raton, Florida, 235 p.

Johns, D. M. and D. C. Miller. 1982. The use of bioenergetics to investigate the mechanisms of pollutant toxicity in crustacean larvae. In: Vernberg, W.B., Calabrese, A., Thurberg, F.P., Vernberg, F.J., (Eds), *Physiological Mechanisms of Marine Pollutant Toxicity*. Academic Press, New York : 261-288.

Koivisto, S., M. Ketola and M. Walls. 1992. Comparison of five cladoceran species in short and long term copper exposure. *Hydrobiologia*, **248** : 125-136.

Liao, I.C. and C.S. Hsieh. 1988. Toxicity of heavy metals to *Penaeus japonicus*; 1. Toxicities of copper, cadmium and zinc to *Penaeus japonicus*. *J. Fish. Soc. Taiwan*, **15** : 69-78.

----- and -----. 1990. Toxicity of three heavy metals to *Macrobrachium rosenbergii*. In: Hirano, R., Hanyu, I., (Eds), *Proceedings of the Second Asian Fisheries Forum, Tokyo, Japan*, 17-22 April 1989, 923-926 p.

Martin, M., J. W. Hunt, B. S. Anderson, S. L. Turpen and F. H. Palmer. 1989. Experimental evaluation of the mysid *Holmesimysis costata* as a test organism for effluent toxicity testing. *Environ. Toxicol. Chem.*, **8** : 1003-1012.

Maund, S.J., E.J. Taylor and D. Pascoe. 1992. Population responses of the freshwater amphipod crustacean *Gammarus pulex* (L.) to copper. *Freshw. Biol.*, **28** : 29-36.

Rao, V.N.R. and G. M. Latheef. 1989. Effect of copper on *Artemia salina* Linn. and of *Skeletonema costatum* (Grev.) as feed. *Comp. Physiol. Ecol.*, **14** : 41-48.

Rice, J.A. 1990. Bioenergetics modelling approaches to evaluation of stress in fishes. In: Adams, S.M., (Ed.), *Biological Indicators of Stress in Fish*. American Fisheries Symposium, **8** : 80-92.

Sivadasan, C.R., P. N. K Nambisan and R. Damodaran. 1986. Toxicity of mercury, copper and zinc to the prawn *Metapenaeus dobsoni* (Miers). *Current Sci.*, 55(7): 337-340.

Soundrapandian, S. and K. Venkataraman. 1990. Effect of heavy metal salts on the life history of *Daphnia similis* Claus (Crustacea: Cladocera). *Proc. of the Indian Acad. of Anim. Sci.*, 99 : 411-418.

Sumitra Vijayaraghavan, Joseph P. Royan and L. Krishna Kumari. 1981. Use of slaughter house waste as a feed for shrimps and prawns. *Indian J. Mar. Sci.*, 10 : 390-392.

Weis, J.S. and P.Weis. 1992. Construction materials in estuaries: Reduction in the epibiotic community on chromated copper arsenate (CCA) treated wood. *Mar. Ecol. Prog. Ser.*, 83 : 45-53.

Widdows, J. 1985. Physiological responses to pollution. *Mar. Pollut. Bull.*, 16 : 129-134.

-----, D.K. Phelps and W. Galloway. 1980. Measurement of physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay. *Mar. Environ. Res.*, 4 : 181-194.

Winberg, G.G. 1960. Rate of metabolism and food requirements of fishes. *Fish. Res. Bd. Can.* Translation Ser., 194 : 202 p.

Zhu, N., X. Xia, H. Wang and Z. Lin. 1992. Study on toxicity of wastewater from mining activities on some biota. *J.Environ. Sci.* (China), 4 : 116-123.