



Journal of
**Fisheries and
Aquatic Science**

ISSN 1816-4927



Academic
Journals Inc.

www.academicjournals.com

Toxic Effects of Copper on Bioenergetics and Growth Rates in Fingerlings and Adult Age of the Fish, *Mystus vittatus* (Bloch, 1794)

S. Subathra and R. Karuppasamy

Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract: The present study was performed to determine the toxic effect of copper (Cu) on various parameters of bioenergetics in different size of the fish, *Mystus vittatus*. The fingerlings (1.5±0.5 g) and adult (8±1 g) were subjected to their respective higher (0.75 and 1.91 mg L⁻¹) and lower (0.47 and 1.20 mg L⁻¹) sublethal concentration of Cu for a period of 60 days. The initial weight and energy content of both size group of test fish were estimated prior to the start of the experiment at each sampling period. Food consumption was monitored on a daily basis during the exposure period, while the final fish weight and energy content of both size groups of fish exposed to Cu were estimated at the end of exposure periods. The rate of feeding and absorption, the efficiency of absorption, the conversion and metabolic rates and growth rate of both the size groups under their respective higher and lower sublethal Cu concentration, led to decreasing trend with increasing periods of exposure. Of the two size groups, fingerlings seemed to be more sensitive to Cu poisoning than by the mature fish. The results obtained suggest that the inefficient feeding behaviour associated with impairment in absorption, conversion and metabolism. Ultimately it resulted in a severe suppression of the growth potential of *M. vittatus* to Cu.

Key words: *Mystus vittatus*, size groups, copper, feeding energetics, growth rate

INTRODUCTION

Environmental pollution is caused by heavy metals as a result of rapid industrialization (Joshi *et al.*, 2002). Copper sulfate (CuSO₄) is one of the most widely used heavy metal, as algacides for the control of phytoplankton in fish pond, reservoirs and lakes, as well as herbicides in aquatic weed control (Carbonell and Tarazona, 1993). The wide uses of high concentration of copper salts in fisheries and agricultural fields may create pollution problems to human health. Sadiq and Hussain (1997) reported that the concentration of copper in drinking water increased as a function of utility with copper pipe length. The Cu concentration rarely exceeds 50 nmol L⁻¹, in natural water, but in polluted water it may surpass 1 µmol L⁻¹ and makes it toxic (Hellawell, 1988). Environmental Bureau has adopted the copper limits recommended by the USEPA (1984) for the protection of aquatic life as 20 µg Cu L⁻¹.

The fishes are intimately associated with water and constitute an important food item in human diet. The heavy metal copper can be bioaccumulated by fish, either directly from the surrounding water or by ingestion of food (Saravana Bhavan and Geraldine, 2000). An important notion in understanding the Cu toxicity on fish is that when fish are exposed to Cu, one brings into play an unavoidable and incessant stressor and it has a renewed interest in the nutritional and toxicological effects of Cu on the bioenergetics in comparatively various size groups of fishes (Handey, 1996). However, recently Flik *et al.* (2002) have showed that Cu exposure has been known to reduce the growth of adult fish without affecting food consumption. In general, early developmental stages of animals are more sensitive to toxicant than are adults and in this regard fish are no exception. In fact, submicro molar

level of Cu can affect the development in young fish to adult (Stouthart *et al.*, 1996). On the other hand, this disproportionate sensitivity of young to adult fish may be attributed to their high surface to volume ratio and relatively high metabolic activity. But it remains to be determined the various parameters of bioenergetics of fish at what age it becomes more responsive to Cu, because the data on bioenergetic response to Cu has only been demonstrated separately either in adult or in fingerlings (Handey, 1996). Sublethal levels of a wide variety of toxicants have been found that the variability of fish growth is related to their size/age and period of exposure (Vosyliene *et al.*, 2003).

The researchers have therefore assessed the effects of Cu exposure on bioenergetics and growth rate in both fingerlings and adult fish of *Mystus vittatus* to monitor the metabolic response in respect to the age of fish.

MATERIALS AND METHODS

Experimental Animal

Healthy fingerlings (1.5 ± 0.5 g in weight and 3.1 ± 0.5 cm in length) and adult size (8 ± 1 g body weight and 9.1 ± 0.5 cm in length) of fish *M. vittatus* were collected from local freshwater bodies in and around Annamalai University, Annamalai Nagar. Fishes of fingerlings and adult age groups were separately maintained at $27 \pm 1^\circ\text{C}$ in a 1000 L tank continuously aerated and following dechlorinated tap water (pH 7.2 ± 7.4 ; hardness $185\text{--}200$ mg L^{-1} as CaCO_3 ; alkalinity $170\text{--}175$ mg L^{-1} as CaCO_3) at least 15 days prior to the experiments. Fishes were fed with small pieces of earthworm on alternate days and then water was renewed everyday. Feeding was suspended 24 h before the experiments.

Copper Toxicity

The heavy metal copper (Cu) in the form of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -AR grade) was used in the present study. The 96 h LC_{50} concentrations of copper sulfate was 18.62 mg L^{-1} for fingerlings and 47.86 mg L^{-1} for adult fish as calculated, by using probit analysis method (Finney, 1978). The fingerlings and adult stage of fish were exposed to their respective 1/40th and 1/25th of 96 h LC_{50} concentration of copper. The 1/40th 96 h LC_{50} of 0.47 and 1.20 mg L^{-1} (lower) and 1/25th 96 h LC_{50} of 0.75 and 1.91 mg L^{-1} Cu (higher) for chronic levels were used for the experiments to the fingerlings and the adult fish, respectively.

Feeding Experiment

Feeding parameters were conducted over a period of 60 days at chronic levels for both the fingerlings and adult size of *M. vittatus* exposed to their respective two different sublethal copper concentrations. The experiments were conducted in glass aquariums (100 L water capacity), each containing 20 fish in each age groups in 60 L of test solution. Twenty other fish in each age group were simultaneously used as control group. The experimental fish was fed with a daily ration weighed quantity of chopped earthworm pieces given at the *ad libitum* in a feeding tray twice a day. Food consumption was monitored on a daily basis during the exposure period, while the other parameters of bioenergetics and growth were assessed at each interval of 20, 40 and 60 days of exposure. The unconsumed feed was carefully removed using a pipette after 1 h of feeding and dried in a hot air oven at 80°C for two days, while the faeces were collected by filtering the entire medium. Growth of fish was determined by the Sacrifice method of Maynard and Loosi (1962) and the scheme of energy budget was derived by adopting the modified IBP formula (Vijayaraman, 1993), this is:

$$C = (P+E)+R+F+U$$

where, c is the food consumed; P is the growth; E is the exuvia; R is the material cost as heat due to metabolism; F is the faeces and U is the nitrogenous excretory product.

Statistical Analysis

Data analyses were carried out using the SPSS (statistical package for social sciences) statistical software, while the Tukey's two way analysis of variance (ANOVA) was applied to find the significant differences between days and concentrations among experimental groups at chronic levels.

RESULTS AND DISCUSSION

Heavy metals are known to changes in feeding parameters of fish (James *et al.*, 2003; Taylor *et al.*, 2000). The growth of fish is generally used as a sensitive and reliable endpoint in chronic toxicological investigations (De Boeck *et al.*, 1997). However, as the process of growth is, but a reflection of the effects of a toxicant on a number of different biochemical and physiological processes, which is often more informative to evaluate the effects on specific processes associated with bioenergetics, such as feeding, assimilation, excretion and metabolism (Saravana Bhavan and Geraldine, 2000). Rand and Peterocelli (1985) reported that growth is the culmination of many biochemical phenomena that occurs in a somewhat regulated pattern; therefore, contaminant intoxication should induce biochemical changes, especially in the skeletal system, before the reduction in growth of fish.

Table 1 and 2 show that the overall observed feeding parameters in both fingerlings and adult age of fish *M. vittatus* exposed to lower (0.47 and 1.20 mg L⁻¹) and higher (0.75 and 1.91 mg L⁻¹) sublethal concentrations of Cu during long-term exposure. At the end of all exposure periods, a two way ANOVA revealed a significant (p<0.05) reduction between periods and concentrations of Cu exposure for both feeding and growth parameters of each size groups. This reduction in feeding parameters was decreased with increasing concentration and period of Cu exposure in both size groups of fish. Similar results were found in Common carp (De Boeck *et al.*, 1997) and in *Perca fluviatilis* (Collvin, 1985) exposed to Cu. Also, Palanichamy *et al.* (1990) reported that the growth parameters in *M. vittatus* decreased with increasing concentration of chemical factory effluent.

Table 1: Bioenergetics and growth performance in fingerlings of *M. vittatus* exposed to sublethal concentration of Cu after low (0.47 mg L⁻¹) and high (0.75 mg L⁻¹) exposure

Parameters	Sublethal concentration of Cu (mg L ⁻¹)	Exposure periods (days)			Significant level (F-value)	
		20	40	60	Between days and concentrations	Interaction between days and concentrations
Feeding Rate (FR)	Control	45.07±0.092	46.10±1.101	47.12±0.452		
	0.47	44.31±0.351	43.30±0.952	42.28±0.955	140.400*	62.3**
	0.75	43.01±0.283	42.78±0.653	41.17±0.787	64.56**	
Growth Rate (GR)	Control	17.95±0.250	18.77±0.525	19.53±0.674		
	0.47	15.28±0.242	12.59±0.150	9.87±0.120	138.607*	14.96*
	0.75	12.04±0.169	10.67±0.122	9.28±0.105	153.65**	
Conversion Efficiency (CE) %	Control	39.82±1.598	40.71±1.570	41.45±1.630		
	0.47	34.84±1.023	29.08±0.750	23.30±0.910	114.407*	15.83*
	0.75	27.99±0.954	24.94±0.670	22.24±0.750	631.00**	
Absorption Rate (AR)	Control	30.75±1.405	31.55±0.975	33.78±1.450		
	0.47	25.74±0.765	23.72±0.672	18.70±0.725	190.556**	75.86**
	0.75	20.72±0.415	18.71±0.310	17.69±0.215	321.53**	
Absorption Efficiency (AE) %	Control	99.87±2.150	99.90±2.057	99.85±1.530		
	0.47	97.85±1.975	95.83±1.265	92.80±0.415	33.630*	11.32*
	0.75	94.78±1.750	92.76±1.542	81.07±0.415	28.21**	
Metabolic Rate (MR)	Control	12.08±0.150	17.22±0.520	20.25±0.600		
	0.47	11.46±0.205	11.13±0.315	8.83±0.415	73.195**	48.77**
	0.75	8.68±0.008	8.50±0.010	7.90±0.012	89.44**	

Values are mean±SD; Sample size (n) = 6; * Significant (p<0.05); ** Significant (p<0.01)

Table 2: Bioenergetics and growth performance in adult of *M. vittatus* exposed to sublethal concentration of Cu after low (1.20 mg L⁻¹) and high (1.91 mg L⁻¹) exposure

Parameters	Sublethal concentration of Cu (mg L ⁻¹)	Exposure periods (days)			Significant level (F-value)	
					Between days and concentrations	Interaction between days and concentrations
		20	40	60		
Feeding Rate (FR)	Control	50.42±1.060	51.61±1.980	53.65±1.715		
	1.20	48.68±0.150	45.36±0.519	43.74±0.932	108.450**	62.003**
	1.91	45.24±0.300	42.97±0.430	40.35±0.765	92.60**	
Growth Rate (GR)	Control	16.27±0.915	17.54±0.260	18.38±0.915		
	1.20	14.53±0.720	13.10±0.350	11.22±0.504	127.965*	38.63*
	1.91	12.94±0.790	11.36±0.135	10.38±0.560	31.35*	
Conversion Efficiency (CE) %	Control	32.26±1.623	33.91±1.260	34.26±1.055		
	1.20	29.85±0.500	28.88±0.950	25.94±0.923	121.441*	40.57*
	1.91	28.60±0.270	26.44±0.750	25.65±0.745	112.91**	
Absorption Rate (AR)	Control	25.74±0.750	27.78±0.630	29.89±0.632		
	1.20	23.73±0.625	21.71±0.520	18.69±0.436	113.726*	76.54**
	1.91	20.71±0.930	18.68±0.370	16.65±0.351	42.306*	
Absorption Efficiency (AE) %	Control	87.79±1.430	87.85±2.409	87.89±2.503		
	1.20	86.72±1.750	86.69±2.650	85.65±1.641	396.221*	121.60**
	1.91	84.65±1.950	83.63±1.995	83.58±1.710	62.53*	
Metabolic Rate (MR)	Control	9.47±0.105	10.76±0.190	11.51±0.861		
	1.20	9.21±0.060	8.39±0.130	7.47±0.635	98.930**	39.75*
	1.91	7.74±0.015	7.32±0.095	6.27±0.405	120.56**	

Values are mean±SD; Sample size (n) = 6; * Significant (p<0.05); ** Significant (p<0.01)

Feeding Rate

In the present investigation, the feeding rate was reduced significantly (p<0.05) in both size groups of fishes exposed to different sublethal concentrations of Cu over 60 days of exposure (Table 1 and 2). These results are very similar to the feeding rate of *Xiphophorus helleri* exposed to the Cu toxicity after longterm (140 days) exposure (James *et al.*, 2003). Ali *et al.* (2003) have reported that the lowered food intake of fish could be due to the effects of Cu on the central nervous system, which may lend support to the present findings. Lett *et al.* (1976) have inferred that the sublethal exposure of rainbow trout, *Salmo gairdneri* to Cu resulted in feeding interruption initially and growth reduction in due course. The rate of feeding and absorptions is significantly affected in fish *Heteropneustes fossilis* exposed to Cu (James *et al.*, 1995). Sarnowski (2004) revealed that the heavy metals might have disturbed the development of locomotors abilities that might also adversely affect the feeding abilities.

According to Kasthuri and Chandran (1997), food intake has been identified as a prime factor influencing growth by developing appetite, is found to be affected by toxicants. Besides appetite loss and changes in food intakes, metal-exposed fish some times spit or vomit taken up food (Handy, 1996). In the present observation, the growth rate directly depending on the rate of food consumption in both size groups. The increased relationship between the feeding rate and Cu concentration (data not published) suggests that Cu brings about growth reduction through lowered food consumption. Rubio *et al.* (2003) have observed reduced food catching efficiency of sea bass when fed with mixing of heavy metal toxicant. Similar findings have also been obtained by Rodgers and Beamish (1982) in *S. gairdneri* exposed to methylmercury and Collvin (1984) in *Perca fluviatilis* exposed to Cu.

Growth Rate

The growth rate in the experimental fishes of fingerlings and adult size *M. vittatus* exposed to lower and higher sublethal concentration of Cu over a long-term exposure (60 days) were significantly (p<0.05) reduced at all exposure periods as well as with time and concentration dependent

reduction in both groups of fish, compared to their respective control group (Table 1 and 2). The present result is equal to an earlier contribution devoted to the effect of Cu on growth performance of *Oreochromis niloticus* after chronic exposure (Ali *et al.*, 2003). According to Lett *et al.* (1976), the growth reduction in Cu exposed *S. gairdneri* is partly due to the increased metabolic rate and reduced food consumption. The present result closely coincides with the reported by Vijayaraman (1993) that the lesser rate of growth in all size groups of prawn exposed to heavy metals might have been due to the greater expenditure of energy towards their maintenance to overcome the stress caused by the heavy metals and to the fact that the food energy channeled towards fish growth would have minimized.

It is known that the Cu accumulation is related to reduction in growth rate of fish (Flik *et al.*, 2002). The relationship between concentration of Cu, period of exposure and growth rate are very similar to the report of Marr *et al.* (1996) which had similar scope and function but intercept exposed to Cu in rainbow trout *Concorhynchus mykiss*. The present observation supports the hypothesis of De Boeck *et al.* (1997) which states that the cessation of feeding accompanied by the catabolic effect of the catecholamines and corticosteroids on the stored energy reserves in the body tissue, results in reduced growth in stressed fish. According to Milliou *et al.* (1998), the reduced growth of *guppies* exposed to Cd is mainly induced by the secondary effects of Cd as a result of physiological stress. Decreased growth rate as a result of Cu exposed brook trout *Salvelinus fontinalis* (McKim and Benoit, 1971) Cr exposed *Perca fluviatilis* (Collvin, 1984) and Hg exposed *M. vittatus* (Sivakami *et al.*, 1995) have been reported.

Conversion Efficiency

The efficiency of food conversion is found to be lower ($p < 0.05$) in both fingerlings and adult age fish exposed to 0.47 and 1.20 mg L⁻¹ (lower) and 0.75 and 1.91 mg L⁻¹ (higher) concentration of Cu, respectively, for a long term. This reduction is directly proportional to the exposure periods which reach the maximum decrease of 49.49 and 25.13% at 60 days (chronic) of exposure, to higher concentration of Cu, respectively in fingerlings and adult age of fish (Table 1 and 2). Conversion efficiency in *M. vittatus* exposed to Cu may be due to the reduced intake of feed and increased cost of metabolism which is equal to the previous report of James *et al.* (2003) in Cu treated *X. hellari*. Furthermore, dietary enrichment of other essential amino acids (e.g., leucine, valine and lysine) has been shown to lead to depressed growth and food conversion in a number of fish species (Papoutsoglou *et al.*, 2005) and this lends support to the presently observed reduction of conversion efficiency in both size groups of *M. vittatus*.

The reduction in food consumption and the need for allocation of a greater proportion of absorbed food energy for various metabolic process associated with stress have possibly contributed to the reduction in the food conversion ratio (Saravana Bhavan and Geraldine, 2000). Similar findings have been noted in *C. striatus* exposed to DDI and methylparathion (Pandian and Bhaskaran, 1983). Lett *et al.* (1976) states that reduction in growth rate might also be due to the reduction of gross food conversion efficiency.

Growth reduction of fish is parallel to the observed reduced conversion efficiency in both sizes of fish i.e., growth is affected by the level of energy metabolism presumably standard metabolism, active metabolism or both and not simply a reduced food intake as has been a possible interpretation in previous studies (Graham *et al.*, 2000). Valente *et al.* (2001) have reported that feeding system depends on many factors including the fish's ability to operate the feeder, water temperature, photoperiod, reward level dietary energy content, stocking density and social dominance. Haniffa and Sundarvathanam (1983) have reported that growth rate and conversion efficiency of *O. mossambicus* decrease with increasing concentration of distillery effluents and this extends support to the present findings.

Absorption Rate and Efficiency

In the Cu treated fish of young and adult size groups, the rate and efficiency of food absorption are significantly ($p < 0.05$) reduced throughout the exposure periods of long-term treatment (Table 1 and 2). Similar results have been reported in *Macrobrachium Malcolimsonii* due to heavy metal toxicity (Vijayaraman, 1993). This lowered efficiency of food absorption may be due to retention of food for a longer period in the alimentary canal which might also have contributed to the reduction in food consumption. Further, results of the present study are in good agreement with those of Farman *et al.* (1980) who have reported that heavy metals inhibit the intestinal absorption in marine fishes, such absorption efficiency of digested food would have decreased for a unit time.

Another hypothesis that could explain the observed lower absorption is that the Cu could induce the alterations in protein conformation in the cell membrane, which could affect ATPase activity and it is known to aid the absorption of nutrients (Miller and Kinter, 1997). Muthukrishnan *et al.* (1986) report that the rate of feeding, absorption and conversion of nutrients in *C. carpio* decrease significantly when fed *ad libitum* and reared in media containing exposed to $HgCl_2$. Similar findings have also been obtained by Sivakami *et al.* (1995) in *M. vittatus* exposed to chromium and mercury.

Metabolic Rate

In the present study, the reduced rate of metabolism is observed in both fingerlings and adult fishes exposed to their respective sublethal concentrations of Cu for long term (60 days) treatment (Table 1 and 2). This reduced rate of metabolism throughout the periods of exposure might have been due to the lowered rate of food consumption which has resulted in impairment of growth. Thus, it appears that the copper-exposed fish of young and adult *M. vittatus* have spent more energy in sustaining their normal metabolism and may leave less available energy for growth. This effect has also been observed in copper exposed perch (Collvin, 1985) and *Coho salmon* (Buckley *et al.*, 1982).

The reduced metabolic rate of Cu treated fingerlings and adult fish show a maximum decrease of 60.98 and 45.53%, respectively in lower and higher concentration of Cu, at the end of exposure period (60th day) when compared to their control groups. Jezierska *et al.* (2006) state that reduction of feeding efficiency in metal intoxicated fish might have resulted in a decrease in activity due to reduced metabolic rate and disturbed functions of the fish organs. With close coupling between the metabolic rate and growth changes in the latter periods of metal exposure are to be expected when the metabolism is altered (Ali *et al.*, 2003).

In the earlier study, Waiwood and Beamish (1978) have demonstrated changes in the metabolic efficiency of Cu treated trout over a wide range of swimming speeds. Further, this study also provides evidence to suggest that basal metabolism is influenced by Cu in the experimental fishes which extend support to the present observations. It may also provide with a possible mechanism for the decline in energetic performance that observe cortisol-impairment. It has been hypothesized (Hontela, 1997) to be the result of chronically elevated basal cortisol secretion which would tend to increase catabolic processes thereby creating an energetic sink.

Further, the above observed results suggest that the sensitivity to Cu in young fingerlings is more evident than in adult stage of fish. Perhaps younger fish are less able to cope with increased energy demands associated with increased metabolism. Although, Cu sensitivity is known to decrease with increasing size, this increase in mass can probably explain only about one half of the reduced sensitivity based on the size (Howarth and Sprague, 1978). As pointed out earlier by Rand and Peterocelli (1985), it is also likely that the immature or young neonatal organisms in general are said to be more susceptible to chemical agents than the adult organisms. This may be due to differences in the degree of development of detoxification mechanisms between young and adult organisms.

ACKNOWLEDGMENTS

Authors are grateful to the Professor and Head, Department of Zoology and to the authorities of Annamalai University for providing the necessary facilities.

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