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Studies on Growth Responses of Fish During Chronic Exposures of Nickel and Manganese

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Abstract: Three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* were exposed to sub-lethal concentrations of nickel and manganese for 30 days. During these trials, fish were fed to satiation daily with the feed having Digestible Energy (DE) of 2.90 Kcal g^{-1} and 35% Digestible Protein (DP). 30-day sub-lethal exposure stress of metals to the fish caused no mortality. However, average weight, fork and total length increments of three fish species varied significantly during nickel and manganese chronic exposures. *Catla catla* were significantly more sensitive to manganese than nickel toxicity. During stress of both nickel and manganese, both *Labeo rohita* and *Cirrhina mrigala* exhibited decrease in their weights. However, this chronic stress did not exert any significant effect on feed intake of fish while the responses of three fish species were significantly variable. Chronic exposure of metals to both *Catla catla* and *Labeo rohita* exerted significant impact on the accumulation of nickel, followed by that of manganese in their bodies.

Key words: Growth, *Catla catla, Labeo rohita, Cirrhina mrigala,* nickel, manganese, stress, bio-accumulation

INTRODUCTION

Environmental contamination and exposure to heavy metals are the serious growing problem throughout the world. Human exposure to heavy metals has risk dramatically in the last fifty years as a result of an exponential increase in the use of heavy metals in industrial processes and products. In order to evaluate the acutely toxic effects, the quantitative parameters such as survival and mortality of fish are quite appropriate, whereas for long-term chronic effects, caused by sub-lethal concentration, the relative parameters are difficult to ascertain. As sub-lethal effect is in general more subtle and quantitative, it is difficult to monitor these at the population or community level, due to the complexity of an ecosystem and the specificity of the induced effect. Therefore, for the lower toxic concentrations, laboratory studies at the level of organism are indispensable for the identification of relevant effects. This avoids the complexity of population dynamics and focuses on the study of more specific and mechanistic action.

Growth is a simple and straight forward index of metal's effect because it integrates all the effects within the fish. A prerequisite for its use, however, is a meaningful experiment with growth itself, which is not a simple thing. Rainbow trout exposed to half the lethal concentration of copper showed an initial loss of appetite but compensated during a 39 day experiment so that their growth rate almost equaled to that of control. Furthermore, compensation was faster at a lower ration than at a higher on (Lett *et al*., 1986). Thus, a toxicologist gets into perplexing problems of interpretation. Problems involving not only ration, but appetite, acclimation and food conversion efficiency if it is particularly desired to understand how a toxicant effects growth (Nussey *et al*., 2000). Death is an easily detected deleterious response, where the criteria for death is usually lack of movement, for example no gill normal physiological processes are affected long before death of an organism and because death is too extreme a criterion for determining whether a substance is lethal or not, scientists had to search for physiological and biochemical indicators of health and sub-lethal toxicant effects. Previously, the metals toxicity studies were confined to salmonids (Bradley and Sprague, 1985; Cusimano *et al*., 1986) and some freshwater species like *Gasterosteus aculeatus*, *Rutilus rutilus*, *Perca fluviatilis* and *Leuciscus leucisus* under definite conditions (Svecevicius, 1999). The growth and bioenergetics of major carps under metal stress have never been studied. It is, therefore, imperative to study toxicity responses of fish to metal contamination along with growth and metal accumulation in their bodies. These data can be useful for future planning regarding sustainability of natural habitats for conservation of indigenous fish species.

MATERIALS AND METHODS

The experimental organisms were 90-day old fingerling *Catla catla, Labeo rohita* and *Cirrhina mrigala.* The fish fingerlings were kept under laboratory conditions in 500 liter tanks for two weeks prior to the experiment. Since the values for weight-length coefficient (condition factor) and ichthyopathological experiments, did not show any distinct pathological symptoms, it assure that fish were in good condition and good state of health. After acclimation period, fish were exposed to the following sub-lethal levels of metals (as pure chloride compounds) in tab water, using a static water system with continuous aeration under controlled laboratory conditions at room temperature:

	Sub-lethal metal concentration (mg L^{-1})	
Fish species	Nickel	Manganese
Catla catla	10.22	25.14
Labeo rohita	11.68	26.67
Cirrhina mrigala	13.30	33.31

The parameters viz. feed intake, increase or decrease in average weights, feed conversion ratios and condition factor (Kn) of three fish species (Carlander, 1970) were monitored during the study period. The exposure medium were continuously replenished and partly exchanged to maintain the above mentioned sub-lethal concentrations of metals for three fish species throughout the experimental period of 30 days for each trial. Hydro-chemical parameters such as temperature, dissolved oxygen, pH, electrical conductivity, total ammonia, chlorides, sodium, potassium and total hardness were monitored on daily basis at 10.00 and 18.00 h (AOAC., 1984). The concentration of metals in the test medium were measured by Atomic Absorption Spectrophotometry (Anonymous, 1989). During each trail, fish were fed to satiation daily at 9.00 and 17.00 h with the feed having Digestible Energy (DE) of 2.90 Kcal g^{-1} and 35% Digestible Protein (DP). These tests were performed in 50-liter glass aquarium. At the beginning and after growth trail of 30 days, the fish were analyzed for their metal concentrations (Anonymous, 1989). The data were analyzed (Steel and Torrie, 1986) by Analysis of Variance and Duncan's Multiple Range tests to compare different variables when Factorial ANOVA indicated significant differences. MSTAT-C package of the computer was used for statistical analyses of the data.

RESULTS

Fish growth during metal stress

***Catla catla*:** Table 1 shows average initial and final weight gains of *Catla catla* during sub-lethal chronic exposure for one month. This species of fish gained an average weight of 0.25g under nickel exposure while an average decrease of 0.22 g weight during manganese stress. The weight increments of fish during nickel and manganese stresses varied significantly. Average fork and total lengths of *Calta catla* increased during exposure to both metals. The difference among three fish species for increments in their fork and total lengths, exposed to all metals, varied significantly. Feed intake by the fish during chronic exposure of both metals did not change significantly during exposure of both the metals. The data on water quality parameters viz. dissolved oxygen, temperature, pH, electrical conductivity, total ammonia, carbon dioxide, chlorides, sodium, potassium, calcium, magnesium and total hardness, monitored on daily basis, for the test medium are presented in Table 2.

***Labeo rohita*:** During both nickel and manganese stress, *Labeo rohita* gave negative growth in terms of weight, fork and total length increments with statistical differences. This fish lost higher average weight of 0.72 g during manganese exposure. Feed intake by the fish, during sub-lethal chronic exposure of both metals, did not change significantly. The data on physico-chemistry of all test medium during chronic exposure of metals to this fish are presented in Table 2.

Cirrhina mrigala: During one month sub-lethal exposure period, *Cirrhina mrigala* lost their average weights of 2.96 and 3.90 g under nickel and manganese stress. The average fork and total length losses of fish followed almost the same trend as that of weight increments. Feed intake by the fish during chronic exposure of both metals differed non-significantly. The physico-chemistry of all test medium during chronic exposure of metals was determined on daily basis and averages for each of the variables are presented with ±SD in Table 2.

Condition factor of fish: Length-weight relationships in fish play an important role in fisheries investigation, because these relationships could be used as characters of differentiation of small taxonomic units like any other morphmetric relationship. In addition to the taxonomic character, it can help to determine the various events in the fish life history like metamorphosis and maturity. The coefficient of condition (Kn) can also be used to compare the relative heaviness and suitability of the environment for fish culture. Factors like environment, food and parasitization can affect the condition factor directly and also indirectly through changes in the average size and growth rate of fish. Data on the values of condition factor of three fish species before and after chronic metal stress

Table 1: Growth responses of fish during sub-lethal chronic exposure of metals

	Catla catla		*Labeo rohita*		*Cirrhina mrigala*	
	Nickel	Manganese	Nickel	Manganese	Nickel	Manganese
Exposure Conc.(mgL^{-1})	6.67	21.67	10.00	23.33	16.67	33.33
Fish survival rate (%)	100	100	100	100	100	100
Initial Av. fish weight (g)	3.78±0.97	3.78±0.97	2.83±1.02	2.83±1.02	7.45±2.27	7.45±2.27
Final Av. fish weight (g)	4.03±0.78	3.56±1.47	2.33±1.37	2.11±0.48	4.49±2.78	3.55±1.05
Weight increment (+ or -)	0.25a	-0.22b	-0.50c	-0.72db	-2.96e	-3.90f
Initial Av. fork length (mm)	67.33±6.47	67.33±6.47	60.00±6.38	60.00±6.38	87.50±13.08	87.50±13.08
Final Av. fork length (mm)	70.60±5.10	69.30±8.10	55.00±9.50	58.30±5.70	75.00±13.20	68.30±5.70
Fork length increment (+ or -)	3.27a	1.97b	-5.00d	-1.70c	-12.50e	-19.20f
Initial Av. total length (mm)	77.50±7.29	77.50±7.29	68.00±6.68	68.00±6.68	98.50±12.48	98.50±12.48
Final Av. total length (mm)	79.00±3.40	79.30±8.90	64.30±8.30	66.30±4.10	84.30±14.50	79.00±6.90
Total length increment (+ or -)	1.50 b	1.80a	-3.70d	-1.70c	-14.20e	-19.50f
Feed intake (g)	2.61±0.17a	2.64±0.21a	4.65±0.01a	4.64±0.24a	3.61±0.26a	3.59±0.18a
Fish Condition Factor (Kn)						
Before metal exposure	2.54±0.02a	2.59±0.08a	2.65±0.12a	2.67±0.09a	2.30±0.07a	2.33±0.01a
After metal exposure	1.89±0.11b	1.99±0.20b	1.72±0.22b	1.85±0.18b	1.17±0.11b	1.52±0.15b

(Means with similar letter in a single row and column are statistically similar at $p<0.05$)

Table 2: Physico-chemistry of test medium during chronic exposure of metals to the fish

	Catla catla		*Labeo rohita*		*Cirrhina mrigala*	
	Nickel	Manganese	Nickel	Manganese	Nickel	Manganese
Dissolved oxygen^{-1}	6.14±1.19	6.16±1.08	6.16±1.02	6.21±1.06	6.24±1.04	6.23±1.17
Temperature (°C)	23.71±2.09	23.68±2.10	23.63±2.15	23.79±2.18	23.67±2.14	23.79±2.12
pH	8.18±0.25	8.23±0.24	8.27±0.25	8.22±0.26	8.19±0.25	8.26±0.26
Electrical conductivity (mS cm^{-1})	1.80±0.06	1.80±0.06	1.80±0.05	1.81±0.06	1.79±0.05	1.80±0.05
Total ammonia (mg L^{-1})	4.76±1.61	4.11±1.40	3.40±0.98	3.89±1.07	3.13±1.19	3.83±1.26
Carbon dioxide (mg L^{-1})	0.00	0.00	0.00	0.00	0.00	0.00
Chlorides (mg L^{-1})	239.57±9.07	240.09±6.25	241.82±8.14	240.36±11.63	243.18±6.43	242.72±7.19
Sodium (mg L^{-1})	375.45±28.05	352.72±40.27	374.54±20.18	363.63±21.57	370.90±38.32	360.27±34.95
Potassium (mg L^{-1})	7.81±0.60	8.09±0.83	8.09±0.70	8.18±0.60	8.09±0.70	8.27±0.46
Calcium (mg L^{-1})	59.64±17.74	30.61±10.49	46.27±14.71	37.16±13.89	42.63±15.75	35.34±15.08
Magnesium (mg L^{-1})	35.13±14.94	44.61±8.56	37.87±14.61	44.26±13.09	31.39±12.15	45.16±12.78
Total hardness $^{-1}$	275.45±45.46	267.27±23.27	267.27±33.19	270.00±39.74	248.18±28.20	269.09±36.45

experiments were statistically analyzed to assess the suitability of test medium for fish. The condition factor of all the three fish species decreased significantly after chronic stress of both nickel and manganese, showing metal stress on the well-being of fish. However, manganese exerted non-significantly lesser stress to the fish than that of nickel as evident from the values of Kn (Table 1).

Metal accumulation in fish during chronic stress: Table 3 shows metal accumulation patterns in three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* before and after chronic exposure of nickel and manganese. A month long exposure of fish to the nickel sub-lethal level resulted in significantly higher nickel accumulation in *Catla catla* (3.95±3.16 ug g^{-1}), followed by the accumulation levels in *Labeo rohita* and *Cirrhina mrigala*. However, manganese accumulation was the maximum in *Cirrhina mrigala* (37.08±6.14 ug g^{-1}), followed by the accumulation levels in *Labeo rohita* and *Catla catla*.

Table 3: Metal concentrations (ug g^{-1}) in three fish species before and after chronic exposure of metals

	Metal concentration in fish body (ug g^{-1})	
Variable	Nickel	Manganese
Catla catla		
Before chronic exposure	1.14±0.99	1.50±0.36
After chronic exposure	5.09±2.21	11.53±0.96
Increase in metal concentration	3.95±3.16a	10.03±1.30c
Labeo rohita		
Before chronic exposure	0.94±0.40	3.39±0.59
After chronic exposure	3.45±3.50	23.92±3.99
Increase in metal concentration	2.51±3.11b	20.53±4.35b
Cirrhina mrigala		
Before chronic exposure	0.52±0.31	1.91±0.82
After chronic exposure	2.20±0.56	38.99±6.41
Increase in metal concentration	1.68±0.27c	37.08±6.14a

(Means with similar letter in a single column are statistically similar at $p<0.05$)

DISCUSSION

Growth is used as an index of metal's effect on fish because it integrates all the effects within the fish. Sub-lethal exposure stress of metals to the fish caused no mortality. However, average weight, fork and total lengths

of three fish species during these exposures varied significantly. *Catla catla* were significantly more sensitive to manganese than that of nickel. There were significant differences among three fish species for their feed intake during chronic exposure of metals. All the three fish species showed decrease in their wet weights except *Catla catla* under nickel exposure. The highest average weight loss of 3.90g was observed in *Cirrhina mrigala* during sub-lethal stress of manganese. However, during both nickel and manganese exposures, feed intake by all the three fish species did not change significantly. During chronic exposure of nickel, *Catla catla* accumulated significantly higher metal in its body, followed by that in *Labeo rohita* and *Cirrhina mrigala*. Manganese accumulation was significantly maximum in *Cirrhina mrigala*, followed by that in *Labeo rohita* and *Catla catla*. Heavy metal contamination stress usually showed depletion in food utilization parameters (Vincent *et al*., 2002). Any such disturbance could result in reduced fish metabolic rate and hence reduced growth (Sarnowski, 2003). Therefore, nutrition status, fish size and growth rate are considered while comparing whole-body as well as tissue heavy metal concentration data for bio-monitoring and risk assessment. During chronic metal stress, even though fish were significantly accumulating cadmium, growth reductions in bull trout occurred without any change in their feed intake (Hansen *et al*., 2002). However, feed intake by the fish reduced only at cadmium concentrations that also caused significant mortality in fish. The cadmium exposure concentration that resulted in reduced growth and survival in long-tem exposure of 0.786 ug L^{-1} was greater than the US federal aquatic life criteria value for the corresponding hardness. Metal contamination in yellow Perch has been shown to vary according to the water contamination and exposure period (Rajotte and Couture, 2002). Therefore, a first line of evidence for assessing potential metal effects on fish health appears in examining whether the metals of concern accumulate in target tissues during chronic exposure. Trace heavy metals viz. nickel, copper and zinc showed adverse effects on fish and among these copper was found to be the most active, followed by zinc and nickel (Khunyankari *et al*., 2001). Growth performance and bioaccumulation of copper in rainbow trout was both time and dose dependent along with reduced sensitivity to heavy metal with the passage of time (Eastwood and Counture, 2002).

A second line of evidence for examining metal-contaminated fish is their condition, using simple age, weight and length measurements from which growth rates and condition indicators are calculated. These measurements also provide estimates of recruitment and longevity of fish. The condition factor of all the three fish species decreased significantly after chronic exposure to both nickel and manganese, showing metal stress on the well-being of fish. However, manganese exerted non-significantly lesser stress to the fish than that of nickel as evident from the values of Kn. The condition factor values of fish after chronic stress differed from that before stress. However, after stress *Catla catla* and *Labeo rohita* exhibited comparatively better condition factor values than that of *Cirrhina mrigala*. Growth response, whole body cupper concentration and mortality of rainbow trout stressed with sub-lethal metal concentrations at normal water hardness of 100 mg L^{-1} (as $CaCO_3$) have been reported (Hansen *et al*., 2002). Fish growth and nitrogen incorporation were dose dependent and modeled as a function of metal exposure concentration and exposure duration. The same factors affecting longevity probably influenced the condition factor of fish, as reflected in growth rates and condition indicators of metal stressed fish. Growth rate has been estimated in a study using length-at-age relationships and consistently indicated that fish from the most polluted lakes demonstrated reduced growth relative to fish from reference (Rajotte and Couture, 2002; Eastwood and Counture, 2002). Similarly, condition factors derived from length and weight measurements (Fulton's Condition Factor) has also often indicated lower condition in polluted fish relative to reference fish (Eastwood and Counture, 2002; Levesque *et al*., 2002) that may be due to the mixture of metals present in the natural contaminated environments.

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