

Bioaccumulation of sodium, potassium, calcium and magnesium in *Rohu, Labeo rohita* (Ham.) fry

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ABSTRACT

The bioaccumulation of alkali and alkaline earth metals such as Na, K, Ca and Mg were studied in the fry of rohu, *Labeo rohita* (Ham.), a species of freshwater aquaculture importance in India. The experiments were conducted in laboratory conditions exposing the rohu eggs up to fry stage in one experiment; and fry to advanced fry stage in other experiment, to different concentrations of the salts. Sodium chloride (0.15, 1.5, 15, and 150 mg/l), potassium chloride (0.015, 0.15, and 1.5 mg/l), magnesium chloride (0.15, 1.5, 15, and 150 mg/l), and calcium chloride (0.15, 1.5, 15, and 150 mg/l) were used as test salts. The tests were conducted in 20-litre capacity glass aquaria stocked with 800 eggs up to fry stage; and in the second experiment with 60 numbers of fry up to advanced fry stage. Control animals were reared in the laboratory without addition of salts. The experiments were conducted in triplicate and each one continued for one-month duration. Normal rearing practices were followed and 50 per cent test solutions from each aquarium were replaced with fresh ones in every week. The bioaccumulations in wet weight basis in whole tissue of fry reared from eggs and in advanced fry exposed to respective salts at the end of the experiment were 100-572 and 91-147 ppm for sodium; 71-104 and 13-22 ppm for potassium; 16-32 and 24-48 ppm for calcium; and 210-660 and 150-300 ppm for magnesium respectively. In control the bioaccumulations for fry reared from eggs, and in advanced fry were 150-212 and 371-412 ppm for sodium; 85-99 and 80-85 ppm for potassium; 18-22 and 5.5-7.8 ppm for calcium; and 250-260 and 15 ppm for magnesium respectively. No definite relationship could be established between the alkali and alkaline earth metals concentration in aquatic environment and bioaccumulation in fish tissue, except sodium and calcium in fry stage.

Key words: *Labeo rohita*, aquaculture, sodium, potassium, calcium, magnesium and bioaccumulation.

INTRODUCTION

The dissolved solids of water mass influence the chemical density of the environment; abundance and composition of the biotic community¹. The alkaline and alkali earth metals are considered important in aquaculture ecosystem, but their amount if excess is harmful to the aquatic animals. Alkaline metals such as Li, Na, and K, and alkali-earth metal such as Be, Mg, Ca, Sr, Ba and Ra have an approximately equal toxicity for fish². Magnesium, calcium and potassium are more toxic to fish than sodium salts²⁻³.

It is well documented that metals and organic compounds can be accumulated by aquatic biota⁴. Bioaccumulation measurements refer to studies or methods monitoring the uptake and retention of these elements in organs and or tissues of organisms, such as fish⁵. This can only take place if the rate of uptake by the organism exceeds the rate of elimination⁶. There are five potential routes for an element to enter the fish: *via.*, food, non-food particles, gills, oral consumption of water and the skin. Once absorbed, it is transported by the blood to either a strong point (*i.e.*, bone) or to the liver for transformation and/ or storage. According to Heath

(1991)⁷, if the pollutant is transformed by the liver, it may be stored there or excreted in the bile or passed back into the blood for possible excretion by the gills or kidneys, or stored in fat, which is an extra-hepatic tissue. Therefore, the concentration found in different tissues after environmental exposure, for a specific time, depend on several dynamic processes all taking places concurrently.

MATERIAL AND METHODS

Test container

Glass jar tanks of 20 l capacity were used as test containers in the study. Before use, the jars were cleaned with laboratory detergents, then with 100% acetone and tap water. After each test, the containers were washed appropriately with acid to remove metals and bases; and detergents to remove organic compounds. Each of the test containers was provided with facilities of continuous aeration and covered with velon screen netting to prevent animals from jumping out.

Test concentration

While designing, minimum of three exposure (treatment) concentrations of a test substance and one control are required to conduct bioassay test and chronic exposure studies. In the present study the test organisms were exposed to a wide range of concentrations selected in logarithmic scale *i.e.*, sodium chloride (0.15, 1.5, 15, and 150 mg/l), potassium chloride (0.015, 0.15, and 1.5 mg/l), magnesium chloride (0.15, 1.5, 15, and 150 mg/l), and calcium chloride (0.15, 1.5, 15, and 150 mg/l). The range of concentrations selected was expected to produce both observed and no-effects.

Test solutions

The test solutions were prepared by dissolving the calculated amount of salts in the dilution water of the experimental aquaria.

Test procedure

Two experiments were conducted to evaluate the effects of calcium, magnesium, sodium and potassium on Indian major carp, rohu (*Labeo rohita*, Hamilton) egg and fry. The experiments were conducted in laboratory conditions exposing the rohu eggs up to fry stage in one experiment; and

fry to advanced fry stage in the other experiment, to different concentrations of sodium, potassium, calcium and magnesium salts. The tests were conducted in 20-litre capacity glass aquaria stocked with 800 eggs up to fry stage; and in the second experiment with 60 numbers of fry up to advanced fry stage. Control specimens were reared in the aquaria without addition of salts. Each concentration was tried in triplicate and the test was continued for one-month duration. Normal rearing practices were followed and 50 per cent test media was replaced with fresh ones in every week. During the experimentation period various observations were made in a specific manner and schedule in order to determine the various levels of accumulation of the test elements in fish.

Water quality management

Physico-chemical parameters such as temperature, dissolved oxygen, free carbon dioxide, pH, total hardness, total alkalinity, ammonia, nitrite, nitrate, phosphorus and dissolved organic matter of the test media were measured in every week following the standard laboratory methods prescribed in APHA (1998)⁸. Except pH and temperature all the other parameters were expressed in ppm.

Tissue collection

The fry (10-16 mm length and 0.006-0.017 g weight) and advanced fry (39-65 mm length and 0.373-1.864 g weight) were harvested from the experiment after one month of exposure to different concentrations of salt solutions in each experiment. From each concentration *i.e.*, each aquaria, fishes were selected randomly for required quantity of samples needed for bioaccumulation estimation.

Digestion and estimation

The wet weight of fish was estimated from each concentration and one gram tissue (whole body after removal of gut content) was taken and placed into digestion flasks and predetermined amount of concentrated nitric acid was added to the sample. The flasks were kept in the digestion chamber at 120°C until the vapor and the acid fluids inside the flask turned clear. After completion of the digestion process, the samples were cooled and filtered using Whatman filter paper No 42, and the final volume was made up to 25 ml with distilled

water. The samples were kept in airtight amber colored glass bottles for determination of the metal concentrations. The concentration of metals was determined using Systronics Flame Photometer (Model 128). Initially the Flame Photometer was calibrated using standard concentrations of sodium, potassium and calcium metal solution, and then reading was taken for samples. Magnesium was estimated by EDTA titration methods⁸.

All the data recorded from the experiments were tabulated and statistically analyzed.

RESULTS AND DISCUSSION

The bioaccumulation of sodium, potassium, calcium and magnesium in muscle tissue of the fry and advanced fry in two respective experiments of one month duration is presented in tables 1 and 2.

Experiment 1: Egg to fry

The sodium bioaccumulation in the tissue of the fry (104 ± 14.6 to 584 ± 10.4) was estimated lower in higher salt concentrations (Fig. 1). Accumulation of the magnesium was not reflected

Table 1: Accumulation of alkali and alkaline earth metals in muscle tissue of fry reared from egg stage in different test concentrations

Expt. Conc. (%)	Accumulation (ppm wet weight)			
	Sodium	Potassium	Calcium	Magnesium
0.001	-	109±30.3	-	-
0.005	-	133±52.2	-	-
0.01	365±40.4	-	32±1.0	633±60
0.02	584±10.4	-	23±0.5	645±15
0.025	-	88±1.	-	-
0.125	-	69±9.6	-	-
0.2	235±7.1	-	-	213±20.2
2.0	104±14.6	-	21.3±4.2	309±29
20	-	-	16.9±1.0	248±24.4
Control	199±44	92±4.9	20±1.4	255±3.5

Table 2: Accumulation of alkali and alkaline earth metals in tissue of advanced fry developed from fry stage in different test concentrations

Expt. Conc. (%)	Accumulation (ppm wet weight)			
	Sodium	Potassium	Calcium	Magnesium
0.002	-	17.3±1.6	-	-
0.015	96.3±2.2	21.1±0.2	-	-
0.02	-	17.7±1.0	-	185±37.8
0.15	99±7.1	13.7±0.5	31.1±3.5	140±17.3
1.5	217±25.2	22.2±2.1	47.5±0.6	290±±17.3
15	160±11	-	25±0.7	200±43.3
150	136±10	-	-	-
Control	393±18.5	82.8±2.5	6.7±1.2	15±0

proportionality in the tissue while exposed to higher concentrations of the test salts. Potassium accumulated in fry up to test concentration of 0.005 ppm and then decreased with increase of the salt concentration in the media. Accumulation of calcium (32 ± 1.0 to 17 ± 1.0) (Table 1 and Fig. 1) decreased from lower concentration to the higher concentration of exposure in case of egg developed to fry.

In general, at the end of the experiment, bioaccumulations in wet weight basis in whole tissue of fry reared from eggs exposed to respective salts were 100-572 ppm for sodium; 71-104 ppm for potassium; 16-32 ppm for calcium; and 210-660 ppm for magnesium. In control the bioaccumulation in fry reared from eggs was 150-212 ppm for sodium; 85-99 ppm for potassium; 18-22 ppm for calcium; and 250-260 ppm for magnesium.

Experiment 2: Fry to advanced fry

The bioaccumulation of sodium in fish tissue varied in different test concentrations and no definite relation was established between them. Bioaccumulation of calcium was increased up to 47.5 ± 0.6 while rohu seed was exposed to the test concentration of 0.15g/l and then after decreased with the increase of concentration. Magnesium and potassium did not follow any trend in their

accumulation in muscle tissue (Table 2 and Fig. 2).

The bioaccumulations in wet weight basis in whole tissue of advanced fry exposed to respective salts at the end of the experiment were 91-147ppm for sodium; 13-22 ppm for potassium; 24-48 ppm for calcium; and 150-300 ppm for magnesium. In control the bioaccumulation was 371-412 ppm for sodium; 80-85 ppm for potassium; 5.5-7.8 ppm for calcium; and 15 ppm for magnesium.

Physico-chemical properties of test solution

The water samples from different experimental aquaria had dissolved oxygen (DO) 4.0-7.6 mg/l, pH 6.5-7.6, total alkalinity 118-240 mg/l, total hardness 60-700 mg/l, conductivity 0.278-6.34 mili mho/cm, dissolved carbon dioxide 5-12 mg/l and chloride 40-4700 mg/l.

Metal distribution and accumulation in different tissues of fish varies depending on the sources, uptake, diet and or water borne exposure⁹. During early life, the yolk sac stage of fish is considered the most sensitive one, followed by the embryonic stage prior to completion of gastrulation¹⁰. Calcium deficiency includes swelling and poor hatchability of eggs and slow development;

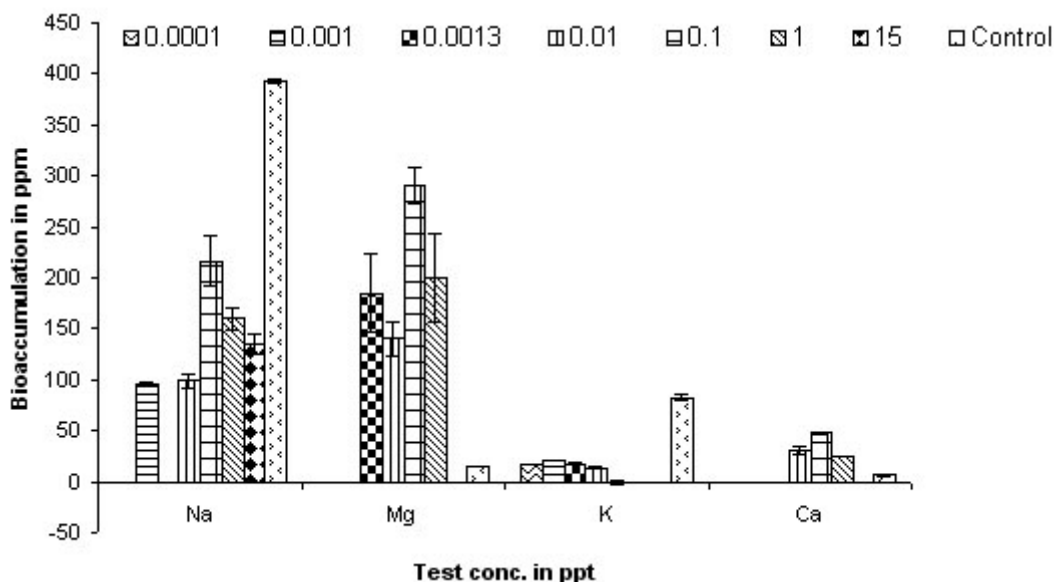


Fig. 1: Bioaccumulation of alkali and alkaline earth metals in rohu fry tissue reared from egg stage in different salt concentrations

lack of resistance and low survival of sac fry. Higher calcium concentration protects the fry from ammonia and metal ecotoxicosis. Magnesium is also essential in skeletal tissue metabolism and neuromuscular transmission¹¹. Freshwater fish derive magnesium ions by active uptake from the environment or from dietary sources. Fish eggs contain a significant amount of magnesium, and thought that some of it is associated with the yolk¹². Therefore, the yolk may serve as a magnesium source for the developing embryo during the early embryonic stage. In adult tilapia (*Oreochromis mossambicus*, Peters) a higher magnesium concentration in the ambient waters resulted in hypermagnesaemia and hypocalcaemia¹³⁻¹⁴. In the present experiment, all the metals were bioaccumulated more than the control in the fish tissue. The bioaccumulation was seen up to certain level and then after reduced with the increase of their salts in the aquatic environment.

Eggs were considered dead when parts of its content turned opaque and white, or when seen with structural damages, mainly vertebral deformities¹⁵.

In the present experiment, the sensitivity of rohu larvae soon after hatching was higher than the control which might be linked to the primary effect of sodium, potassium, calcium and magnesium on embryonic period or early larvae stages, and also to the chemical stress during hatching process. Early life stages of the teleosts are known to be the very sensitive parts of the life. The sensitivity first of all depends on the developmental stages and time of exposure¹⁶⁻¹⁷, physico-chemical characteristic of water, toxicant concentration and fish species¹⁸. In the present experiment the test specimen was exposed to the salts from the very beginning of egg stage to the advanced fry stage. The embryo disintegrated during cleavage and blastula stages when exposed to higher concentration of the salts. In some cases the larvae also deformed when exposed to the higher concentrations of salts.

When fish are exposed to elevated levels of metals in the aquatic environment, they can absorb the bioavailable ones directly from the environment *via.*, gills and skin, or through the ingestion of contaminated water and food. Metals

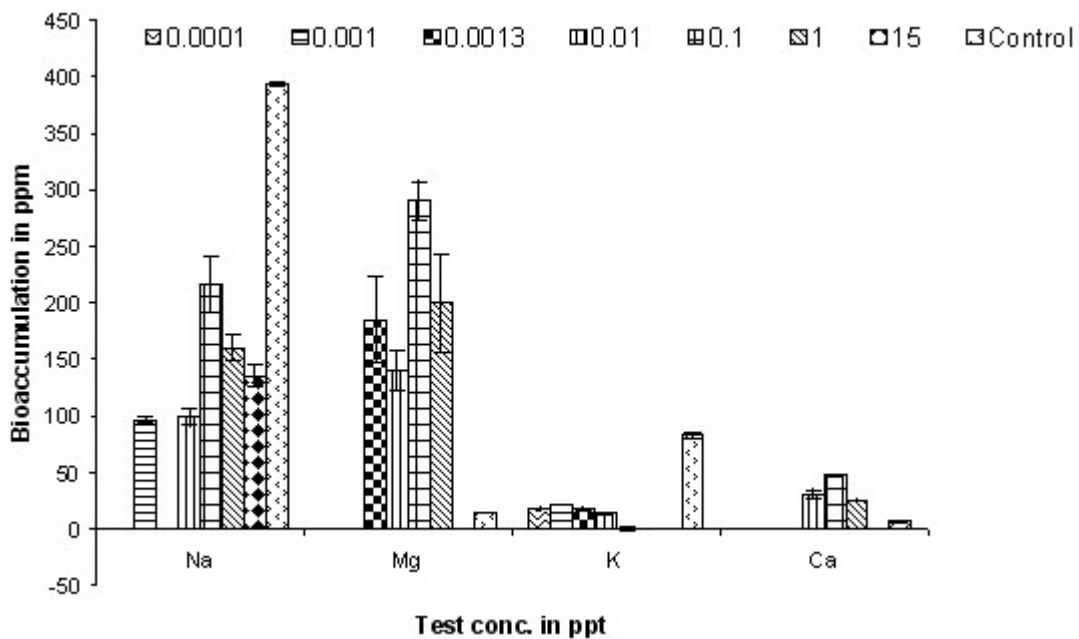


Fig. 2: Bioaccumulation of alkali and alkaline earth metals in advanced rohu fry reared from fry stage in different salt concentrations

in the fish are then transported by the bloodstream, which brings it into contact with the various organs and tissues¹⁹. Fish can regulate metal concentrations to a certain extent. The ability of each tissue to either regulate or accumulate metals can be directly related to the amount of the metal accumulated in the specific tissue. Furthermore, physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal²⁰.

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