Effect of salinity on growth, survival and biochemical alterations in the freshwater fish *Labeo rohita* (Hamilton 1822)

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ABSTRACT

Salinity tolerance in *Labeo rohita* (Hamilton 1822) fingerling was conducted for 96 h using a static, non-renewal system and LC₅₀ determined for 48 and 96 h exposure were 9.60 and 7.72‰ with standard deviation 9.27-9.93 and 7.41-8.03‰, respectively. For sublethal study, 0, 2.5, 3.5 and 4.5‰ were selected to assess the chronic effect of salinity on this species. At the end of 90 days of exposure, it was found that the average weight gain was maximum at 0‰ followed by 2.5‰, then 3.5‰ and the least at 4.5‰. Similarly, highest mortality (43.75%) was recorded in fishes exposed to 4.5‰ salinity and least (12.5%) at 2.5‰ at the end of 90 days of experiment. On termination of the experiment, ascorbic acid level was found significantly reduced (p<0.05) in brain, liver and muscle tissues in fishes exposed to salinity stress. Oxygen consumption rate of fish was maximum at 2.5‰ salinity which gradually decreased with increase in salinity and the lowest was observed at 4.5‰. From results of the study, it can be concluded that with increase in salinity, the survival rate, growth and tissue ascorbic acid level in *L. rohita* decreases. These results clearly indicated that *L. rohita* is vulnerable when exposed to higher salinity for longer duration.

Keywords: Ascorbic acid, *Labeo rohita*, Oxygen consumption, Salinity tolerance

Introduction

Salt affected soils are important ecological entity occupying 6% of the global land area and 2.6% of the geographical area in India. Indo-Gangetic Plain (IGP), which is the most fertile agricultural region in India also has around 3.095 million ha land is salt affected areas of which 12% exist in Bihar (Mandal and Sharma, 2006). Utilisation of these salt affected areas for productive purpose is the major challenge for the region. In this regard, inland saline aquaculture provides an opportunity for the diversification and expansion of agriculture through a potentially productive use of land that can no longer support standard agricultural enterprises (McDowall et al., 2016). Thus, aquaculture can be an adaptive approach to this environmental problem and can represent a potentially lucrative use for salt-affected land, with many economic, social and environmental benefits (Lymbery et al., 2007). However, selection of suitable fish species having tolerance to saline conditions is very important before venturing into aquaculture in these areas. This is mainly because higher salinity of water can act as an important stressor under natural as well as aquaculture conditions (Islam et al., 2014) and can directly influence the metabolism of fish, which ultimately affects the survival, growth, feed intake and even distribution of species (Garg, 1996; Sahoo et al., 2003; Kang’ombe and Brown, 2008; Akhtar et al., 2013; Mubarik et al., 2015).

Effects of salinity have been studied in several fish species but detailed work on effect of salinity on growth, survival and biochemical response on freshwater fish species are limited. *Labeo rohita* (Hamilton 1822), commonly known as ‘rohu’, is one of the Indian major carps (IMCs) used successfully in commercial aquaculture in the Indian subcontinent at least for the past six decades (Sahoo et al., 2015). It is considered to be a highly delicious fish species, has high market value and the fry and fingerlings of the species are easily available from freshwater finfish hatcheries across the country. Hence, a detailed study on the physiological responses and tolerance limit of this species to various salinity levels and an understanding on the potentiality of this species for culture in low saline waters could be highly beneficial. Therefore, the present study was undertaken with an objective to investigate the effects of shortterm as well as long-term exposure of the species to various salinity levels on growth, survival and selected biochemical parameters of rohu fingerlings under laboratory conditions.
Materials and methods

Experimental fish

Healthy fingerlings of *L. rohita* (14.93±0.39 cm and 23.1±1.86 g) were collected from the freshwater fish seed rearing farm of the ICAR-Research Complex for Eastern Region, Patna, Bihar. The fish were acclimatised to the laboratory conditions for a period of 15 days separately prior to the experimental trials.

Salinity tolerance trial

A static non-renewable salinity tolerance bioassay was conducted following standard methods (Reish and Oshida, 1987; APHA, 1998) to determine LC$_{50}$ of salinity for rohu fingerlings for 96 h. Brine solution was prepared using common raw salt and was used for preparing water with desired salinity (treatment) for the experiment. Initially a range finding test was conducted and based on the results, definitive test was conducted using 7 treatments, starting from 2‰ to 14% concentration (Table 1). The test was conducted in triplicates comprising 10 fishes each in 30 l test solution. In each tank, required volume of brine was added and thoroughly mixed and allowed to stand to 20 min before introduction of fishes. Simultaneously one control was also kept slightly away from the bioassay tanks to avoid contamination. No feeding was done and static nonrenewable method was followed. Round the clock aeration was provided in all the tanks using a portable aerator. Just before release of the fishes in the test solution, temperature, pH, dissolved oxygen and actual salinity were estimated (HANNA Multiparameter Analyser, HI 9829, Germany) from all the treatments and the parameters were found to be within the acceptable limits. Percentage mortality was recorded at 24, 48, 72 and 96 h intervals. Dead fishes were immediately removed from respective tanks. The data obtained from the experiment was subjected to probit analysis to estimate 96 h LC$_{50}$.

Based on the 96 h LC$_{50}$ value estimated for *L. rohita* fingerlings, three different sub-lethal salinities viz., 2.5, 3.5 and 4.5‰ and a control (0‰) were selected to assess the effect of salinity on the fishes. Water quality parameters were maintained at optimum levels throughout the experimental period. For preparation of water of required salinity, similar procedure as mentioned earlier was followed. Freshwater (0.22‰) was collected from a nearby bore well and stored in separate tanks (500 l) at least 3 days prior to use for preparation of brine and test solutions. For the experiment, 40 l of water was maintained in the experimental tanks throughout the experiment. Completely randomised design was followed and 64 fish were distributed in four different groups with two replicates per treatment. Feeding was done at the rate of 3% body weight per day. Standard pelleted feed with protein level 16.52±0.46% was used for feeding fishes. Uneaten food and faecal matter were removed on daily basis and complete water exchange was done once in a week and the study was conducted for a period of 90 days.

Monthly sampling was carried out to ascertain weight gain and survival of the fish using the following formulae:

\[
\text{Weight gain} (%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

\[
\text{Survival} (%) = \frac{\text{Number of fish survived after 90 days}}{\text{Initial number of fish stocked}} \times 100
\]

At the end of 90 days of experimental period, 6 fish per treatment (3 per tank) were sampled, anaesthetised with clove oil (50 μl l$^{-1}$) and tissue samples were collected from different organs for ascorbic acid analysis. For estimation, fresh tissues (kidney, brain and muscle) were used on wet weight basis. Ascorbic acid content was determined using 2, 4-dinitro-phenylhydrazine (DNPH) method following Roe and Keuther (1943). OD was measured in a UV-VIS spectrophotometer (E-Merck, Germany) at 540 nm.

Oxygen consumption rate (Debnath *et al.*, 2006) of fishes was estimated after 60 days of salinity exposure. The study was carried out after 96 h of weekly water exchange. Three fishes from each concentration were sampled after 24, 48, 72 and 96 h of salinity exposure and kept individually in a sealed glass chamber (2 l) having thick glass lid covering the top portion completely. After taking individual weight of fishes, each fish was kept inside the respective saline water for an hour. Also, the lid of the chamber was closed tightly and carefully to avoid air bubble inside the chamber. All four sides of the glass tank were covered with an opaque screen to minimise visual disturbances to the experimental fishes. The initial and final oxygen content was measured using digital oxy-meter 330 (sensitivity 0.01 mg O$_2$ mg$^{-1}$) (E Merck, Germany). The oxygen consumption rate was calculated as mg of oxygen consumed per kg of fish per hour.

Data analysis

The experimental results were subjected to one-way ANOVA using Statistical Package, SPSS version 11. For growth analysis, two-way ANOVA was conducted taking into consideration of weight gain in different salinities and monthly weight gain. Duncan’s multiple range test (DMRT) was carried out for *post hoc* comparison of means at 5% probability level.
Results and discussion

Acute toxicity is a reliable and important method for understanding the toxic effects of physiological stressors like salinity in a biological system. Median lethal concentration (LC$_{50}$) for 96 h is the most widely accepted assay for estimating acute toxicity of a particular substance. In the present study, data pertaining to effect of salinity on L. rohita are presented in Table 1. The LC$_{50}$ was calculated from probit analysis (Fig. 1) and found to be 9.60 and 7.72‰ corresponding to 48 and 96 h of exposure.

From the results it was observed that, the LC$_{50}$ of salinity stress decreased with increase in the time of exposure. Similar to the present study, Ghosh et al. (1973) reported that Catla catla and L. rohita fry and fingerlings tolerated 8‰ salinity without mortality, but survivability gradually decreased with increase in salinity. In another study, Devika et al. (2003) reported that, L. rohita survived in waters up to 8‰ salinity and beyond this, the fish showed signs of stress and mortality.

Based on the information received from salinity tolerance test, a sublethal study was initiated to record the impact of long term exposure of salinity on growth, mortality, tissue ascorbic acid levels and oxygen consumption in L. rohita. A 90 days’ experiment was conducted and data pertaining to growth expressed in percentage weight gain of L. rohita is illustrated in Fig. 2. From the figure it is clear that, average weight gain is maximum in control group followed by 2.5‰, then 3.5‰ and least in 4.5‰ at the end of 90 days of culture. Similarly, percentage weight gain was 32.2, 6.88, 4.02 and 3.70% respectively at 0; 2.5; 3.5 and 4.5‰ salinity levels (Fig. 2). Specific growth rate, daily growth rate and percentage growth rate (Fig. 3) also followed similar trend, highest in control followed by 2.5 and least in 4.5‰.

Growth rate of L. rohita showed decreasing trend with increasing salinity in the present study. Similar to the present study, decrease in specific growth rate and average daily gain have been recorded in L. rohita fingerling up to 8‰ salinity and the most preferred salinity for this species was found to be at 2‰ (Islam et al., 2014). Negative impact of salinity on growth have also been reported in L. rohita (Garg, 1996), Clarias batrachus (Sarma et al., 2013), Tilapia rendalli (Kang’ombe and

Table 1. Percentage mortality of L. rohita exposed to different salinity levels for a period of 96 h

| Treatment (%) | No. of fishes | % Mortality | % Total mortality |
|---------------|--------------|-------------|------------------|
|               |              | 24 h        | 48 h            | 72 h            | 96 h            |               |
| 2.00 and 4.00 | 30 each      | No mortality was recorded |             | 6.67           | 3.33           | 0.00          |
| 6.00          | 30           | 0.00        | 0.00            | 10.00           | 13.33           | 40.00         |
| 8.00          | 30           | 0.00        | 16.67           | 10.00           | 13.33           | 40.00         |
| 10.00         | 30           | 0.00        | 70.00           | 13.33           | 10.00           | 93.33         |
| 12.00         | 30           | 76.67       | 16.67           | 3.33            | 3.33            | 100.00        |
| 14.00         | 30           | 100.00      | 0.00            | 0.00            | 0.00            | 100.00        |
| Exposure     | LC$_{50}$    | Confidence level | Correlation coefficient (r) |
| 48 h          | 9.60‰       | 9.27 - 9.93‰ | 0.97             |
| 96 h          | 7.72‰       | 7.41 - 8.03‰ | 0.98             |
In the present study, survival rate of *L. rohita* was also affected at higher salinities and duration of exposure (Fig. 4). Mortality was highest in fishes exposed to 4.5‰ salinity and least in control group (only one fish died) at the end of 90 days’ exposure. No mortality was recorded in the first month of rearing. However, in a similar type of study on *L. rohita*, 100% survival rate of up to 6‰ salinity and 100% mortality beyond 10‰ salinity at 90 days of rearing have been reported (Islam et al., 2014). The difference in survival rate in contrast to the present study might be due to difference in size of fish used in the experiment, quality of feed used and experimental design adopted. Ghosh et al. (1973) reported that *C. catla* and *L. rohita* fry and fingerlings tolerated 8‰ salinity without mortality; however, survivability gradually decreased with increase in salinity. Low survival rates at higher salinity have been well documented in *C. carpio* (Mubarik et al., 2015) and *C. batrachus* (Sarma et al., 2013). In the present study, poor survival rate of *L. rohita* at higher salinity could be attributed to combined effect of confinement stress (Kang’ombe and Brown, 2008), low protein feed given during rearing period, reduced food intake (De Boeck et al., 2000) in addition to increased salinity stress.

Antioxidant vitamin C is well known for its major anti-stress role (Azad et al., 2007; Misra et al., 2007; Norouzitallab et al., 2009). In the present study, at the end of 90 days’ culture period ascorbic acid level was estimated in brain, liver and muscle tissues and it was found that ascorbic acid level was significantly reduced (p<0.05) in response to increase in salinity in brain, liver and muscle tissues (Fig. 5).

Ascorbic acid level was reduced by 11.0, 38.5 and 46.5% in brain; by 24.2, 38.0 and 36.4% in liver and 44.8, 55.6 and 53.8% in muscle tissue when exposed to 2.5, 3.5 and 4.5‰ salinity respectively. It appears that muscle is the most affected tissue followed by liver and brain. Similar results of decreasing ascorbic acid concentration in different organs with increasing salinity were also reported in case of *C. batrachus* when exposed to salinity and was attributed to stress mitigation effect of vitamin C (Sarma et al., 2013) or a defensive reaction of fish to combat stress (Madhuban and Kaviraj, 2009). In the present study, the reduction in ascorbic acid level might be due to high rate of utilisation of vitamin C at higher salinity levels. Vitamin C might have been used up for detoxification process (Mauck et al., 1978) or for preventing peroxidation of cells (Winston and Diguilo, 1987).
1991), which in turn might have caused a functional reduction in vitamin C content in different tissues causing possible cell injury. Reduction of vitamin C in fish due to pesticides exposure has already been reported (Madhuban and Kaviraj, 2009; Sarma et al., 2009).

In the present study, rate of oxygen consumption was also estimated to record if there is any change in the consumption rate when exposed to high salinity. Data pertaining to oxygen consumption of L. rohita exposed to different salinities is depicted in Fig. 6. It was found that oxygen consumption was significantly affected (p<0.05) with increase in salinity. The experiment was carried out in the laboratory within ambient water temperature of 28-30°C. Maximum oxygen consumption was recorded at 2.5‰ beyond which oxygen consumption decreased and lowest value was recorded at 4.5‰. Metabolism is a physiological process reflecting the energy expenditure of living organisms (Sarma et al., 2009). The metabolic rate of fish is usually indirectly measured as their rate of oxygen consumption (Kutty, 1981). In the present investigation, the rate of oxygen consumption in fish was significantly altered with increasing salinity. Oxygen consumption rate was increased above 0‰ and reached highest at 2.5‰, afterwards progressively decreased and reached lowest at 4.5‰. Similarly, there was behavioural alteration also. Fishes were very active, quickly responding to external stimuli at 2.5‰ while very lethargic and slow in response to external stimuli at 4.5‰. It is well known that with increase in temperature, oxygen consumption rate is increased (Das et al., 2005; Brahmane et al., 2014), however, there is paucity of information on direct effect of salinity alone on rate of oxygen consumption of freshwater species. Zheng et al. (2008) recorded lower oxygen consumption rate at 31‰ compared to 16‰ on M. miiuy. While in another study on oxygen consumption rate of Dicentrarchus labrax fingerlings, only slight variation was recorded between 3 and 45‰ at 20°C which was attributed to strong euryhaline nature of the species (Barnabe, 1990; Dalla et al., 1998).

The alterations in oxygen consumption in fishes at different salinities could be due to differences in energy required for osmoregulation or from changes in the spontaneous activity level of fish and changes in the routine metabolic rate (De Boeck et al., 2000). In the present study, oxygen consumption rate was lowest at 4.5‰ which could be attributed to energy conservation strategy adopted by the species to salinity mediated stress.

Based on the results of the present investigation, it can be summarised that, long term exposure to salinity can have significant impact in growth and physiology of L. rohita. The study clearly indicated that L. rohita can tolerate up to 2.5‰ salinity without any adverse effect on metabolism and growth. However, at higher salinity levels (3.5 and 4.5‰), growth, survival, tissue ascorbic acid levels as well as oxygen consumption were severely affected. Hence, it is not desirable to culture L. rohita at higher saline conditions. Being one of the most widely cultured freshwater species in India, studies to assess the impact of salinity on different life stages of this species is essential before introducing this species in low saline waters for farming purpose.

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Kamal Sarma et al. 46

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