**Full Length Research Paper**

**Growth, thermal tolerance and oxygen consumption in rohu, *Labeo rohita* early fry acclimated to four temperatures**

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Determination of early life stages of fish for thermal tolerance, optimum temperature for growth, rate of oxygen consumption is becoming essential for aquaculture industry in the climate change scenario due to long-term and short term (seasonal and diurnal) variability in temperature. Emphasis was made to understand the response of early of rohu (0.09 to 0.1 g) to thermal acclimation. Three hundred early fry stage rohu, *Labeo rohita* (initial weight 0.097±0.01 g) were equally distributed at four different temperatures (28, 30, 32 and 34°C) each with three replicates for a period of 40 days. Highest body weight gain was between 30 and 32°C and lowest feed conversion ratio (FCR) was at 30°C. The percentage weight gain and specific growth rate at 30°C were 382±8.01 and 0.88±0.03 % respectively, significantly higher than other acclimation temperatures. Thermal tolerance and oxygen consumption rate were analyzed to determine the temperature tolerance limits and metabolic activity at four acclimation temperatures. Critical thermal maxima (CTmax) was 42.86±0.04, 43.3±0.02, 44.45±0.02 and 45.42±0.03; critical thermal minima (CTmin) was 13.07±0.04, 14.35±0.02, 14.92±0.04 and 15.64±0.03 and oxygen consumption rate was 110.75±0.44, 126.57±0.60, 146.22±0.68, 166.47±0.86 mgO$_2$ kg$^{-1}$ h$^{-1}$ at 28, 30, 32 and 34°C respectively and increased with increasing acclimation temperatures. Oxygen consumption rate for four acclimatization temperatures increased significantly, 110.75±0.44, 126.57±0.60, 146.22±0.68, 166.47±0.86 mgO$_2$ kg$^{-1}$ h$^{-1}$ at 28, 30, 32 and 34°C respectively. Temperature preference of the early fry of rohu derived from relationship between acclimation temperatures and Q$_{10}$ values for 28 to 30°C, 30 to 32°C, 32 to 34°C were 1.94, 2.05, and 1.91 respectively. The optimum temperature range for growth was 30 to 32°C and Q$_{10}$ value was 32 to 34°C. Survival at different acclimation temperatures was between 98.7±2.31, 96.0±4.0, 93.3±2.31 and 94.7±4.62%, from lower to higher acclimation temperatures.

**Key words:** Acclimation temperature, critical temperature, critical thermal maxima (CTmax), critical thermal minima (CTmin), *Labeo rohita*.

**INTRODUCTION**

Fish inhabiting freshwaters are exotherms and cannot regulate body temperatures through physiological means (Moyle and Cech, 2004) as their body temperatures are very identical to the environment they inhabit. Temperature affects fish physiology in terms of thermal tolerance, growth, metabolism, food consumption,

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reproductive success and ability to maintain internal homeostasis due to variability in external environment (Fry, 1971). Temperature tolerance differs with species, acclimation temperature, acclimation duration and salinity (Ficke et al., 2007; Das et al., 2004; Das et al., 2005; Diaz et al., 2007; Manush et al., 2004). Laboratory quantification of lower and upper temperatures tolerance of aquatic animals is referred as critical thermal methodology (CTM) (Cowles and Bogert, 1944) in which fish is subjected to a continuous, constant increase or decrease in temperature till near-lethal or lethal endpoint is reached. CTM is the arithmetic mean of the thermal points at which locomotion of fish becomes disorganized and it loses the ability to escape the conditions that would result in death (Cox, 1974; Lowe and Vance, 1955). CTM is an ecologically relevant lethal index because fishes in nature encounter such temperatures either temporally or spatially as acute fluctuations outside of their limits (Brett, 1956; Hutchison, 1976). Acclimation response ratio (ARR) is an index of the magnitude of the thermal acclimation of the organism (Claussen, 1977; Re et al., 2005). In tropical freshwaters diurnal water temperature fluctuations approach their incipient upper thermal limits (United Nations Economic Commission for Asia and the Far East (1972); Irion and Junk (1997). Tropical fishes endure these temperatures (Milstein et al., 2000) but a small increase in temperature (1-2°C) in the region may cause the daily temperature maxima to exceed these limits, particularly for fish that currently exist in thermally marginal habitats (Roessig et al., 2004). In India freshwater aquaculture grew ten-fold from 0.37 million tonnes in the year 1980 to 4.04 million tonnes in 2010, with mean annual growth rate of over 6% (Handbook of fisheries and aquaculture). To sustain the aquaculture production in the climate change scenarios, information on fish physiological responses to various abiotic stress factors would help in better management of fishery and aquaculture resource. CTMax and CTMin are essential parameters of fish exposed to the cold winter and severe summer or drought months.

In the present study Labeo rohita early fry stage fish acclimated at 28, 30, 32 and 34°C under laboratory conditions were studied for their critical temperature tolerance limits, growth, feed conversion ratio and oxygen consumption rate.

MATERIALS AND METHODS

Experimental design

L. rohita spawn were procured from Khopoli Fish Farm, Government of Maharashtra and were hatched in CIFA portable hatchery/circular tanks at an ambient temperature of 28°C in the aquaculture laboratory, National Institute of Abiotic Stress Management, Baramati and acclimated for 20 days to recover from transportation stress. Before initiating the experiment 300 uniform sized fry were equally distributed between four treatments (28, 30, 32 and 34°C) with each replicated three times following a completely randomized design, with a stocking density of 25 fry/75 L water. Rearing conditions were kept uniform in the four experimental groups except water temperatures at 28, 30, 32 and 34°C.

Rearing for growth study

The temperatures were maintained at 28°C initially and were gradually increased by 1°C/day to 30, 32 and 34°C and were maintained for 40 days. Fish were fed for 40 day growth study. Photoperiod of 12 h light and 12 h dark was maintained with light exposure from morning 6 to evening 18 h. Dissolved oxygen level were maintained by aeration in all experiments. Ammonia and pH were monitored at regular interval (APHA, 1998) and maintained.

Feed and feeding

During the feeding trial fish were fed with pelleted feed containing 35% crude protein as recommended for L. rohita (Renukardhyay and Varghese, 1986). Initially rohu fry were fed twice a day (8 and 20 h) at 10% of body weight, which was determined periodically at ten day interval up to 40 days. Feed waste and fish excreta were removed daily before feeding. Every day, 50% water was exchanged with fresh chlorine free water. Fish were starved for a day prior to the assessment of growth, thermal tolerance and oxygen consumption.

Growth measurements

Growth rate of fish was measured in terms of percentage weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) as given below:

\[
\text{Percentage weight gain} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100
\]

\[
\text{Specific growth rate} = \frac{\text{final body weight-initial body weight}}{\text{duration of experiment (days)}} \times 100
\]

\[
\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given (dry weight)}}{\text{Weight gain (wet weight)}}
\]

\[
\text{Survival} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100
\]

Oxygen consumption

Rate of oxygen consumption was measured under identical conditions at 28, 30, 32 and 34°C acclimation temperature to estimate significant change in oxygen consumption at acclimation temperatures. Three replicates of six fish from each acclimation temperature were kept individually in sealed 5 L glass chambers. The glass chamber was made airtight after insertion of dissolved oxygen probe. The chamber was placed inside the temperature controlled aquaria at acclimation temperatures and the water was continuously circulated. The aquaria were covered with opaque screen to reduce stress due to visual treatment. The initial and final oxygen content was measured using Eutech cyberscan 600. Oxygen consumption was calculated as:

\[
\text{Oxygen consumption} = \frac{\text{Final oxygen concentration-Initial oxygen concentration}}{\text{weight of fish (kg)}} \times \text{Time (H)}
\]

Critical thermal tolerance

To estimate thermal tolerance, CTMax and CTMin of rohu fry, randomly selected six fish from each acclimation temperature were transferred to 52 L tank and maintained at acclimation temperatures.
of 28, 30, 32 and 34°C. Fish were exposed to a constant increase or decrease of temperature (0.3°C/min) till the loss of equilibrium (LOE), the designated end point for critical thermal maxima (CTMax) and critical thermal minima (CTMin) respectively (Paladino et al., 1980; Beiting et al., 2000) were observed. The fishes were rescued and recovered from the CTM experiments of the four acclimation temperatures. Beiting and McCauley (1990) have used the CTM method for analyzing the physiology of stress and adaptation in fish. The acclimation response ratio was calculated as stated by Claussen (1997) by dividing the tolerance change by the total change in acclimation temperature.

Statistical analysis

One way ANOVA was performed using the mean values of all parameters (SPSS, version 16.0). Duncan’s multiple range test (DMRT) was carried out for post hoc mean comparisons. Regression analysis was carried out to know the relationship between acclimation temperatures with growth, CTMax, CTMin and oxygen consumption.

RESULTS

Water quality parameters of rearing tanks of four acclimation temperatures were maintained for dissolved oxygen (DO), pH, and ammonia (mg L⁻¹). The DO levels decreased significantly with increasing water temperatures. Hydrogen ion concentration increased with increase in temperatures. Ammonia was monitored for accumulation of toxic nitrogenous waste products from fish metabolism in all acclimation tanks. In these experiments all parameters were maintained at optimum level with only variable of acclimation temperature.

Growth of Labeo rohita fry raised at four acclimation temperatures is presented in Table 2. It was observed that at 30°C acclimated rohu fry gained highest body weight (%) along with the highest specific growth rate, followed by 32°C and lowest by 34°C. FCR was significantly different at 30°C than 28, 32 and 34°C. Fry survival at all acclimation temperatures was similar and was not lethal to the rohu fry at the experimental acclimation temperatures. Preferred temperature was estimated using Q10 relationship with acclimation temperature which is considered to coincide with optimum temperature for growth (Brett, 1971; Kellogg and Gift, 1983). Preferred temperature is the point at which Q10 value starts to decrease with increase acclimation temperature (Kita et al., 1996). The final preference temperature for L. rohita fry was found to be 30°C based on the Q10 value and growth data indicated that optimum temperature range for rohu fry was 30°C. The estimation of Q10 suggests the optimal temperature requirements for fish. Highest body weight gain (%) and lowest FCR at 30°C indicates that temperature of 30°C is optimum for growth in early fry of L. rohita.

CTMax and CTMin increased significantly (p<0.05) with increasing acclimation temperatures (Table 1). The fish recovered the CTM temperatures completely. The fry at higher acclimation temperature exhibited higher CTMax and CTMin values. At 0.3°C min⁻¹ heating and cooling rate, CTMax ranged from 42.86±0.03 to 45.42±0.03 and CTMin ranged from 13.07±0.04 to 15.64±0.03 in 28-34°C acclimation temperatures. Both CTMax and CTMin regression analysis showed a positive relationship to acclimation temperature (CTMax = 30.89 + 0.42 × Acclimation temperature; P = 0.001, r² = 0.96 and CTMin = 1.63 + 0.41 × Acclimation temperature, P = 0.001, r² = 0.96). The average ARR for CTMax and CTMin was 0.43 for early fry of L. rohita at the range of 6 degree differential in acclimation temperature. Thermal tolerance polygon for early fry of L. rohita was 178.74°C² at 28 to 34°C acclimation temperatures used in the experiments (Figure 1).

The increase in oxygen consumption rate was significant to the increase in acclimation temperature (p<0.05) (Table 1). Mean oxygen consumption at 28, 30, 32 and 34°C were 110.75±0.44, 126.57±0.60, 146.22±0.68, 166.47±0.86 mg O₂ Kg⁻¹ h⁻¹ respectively. Q10 values were estimated and extrapolated as 1.94 (between 28 and 30°C), 2.05 (between 30 and 32°C) and 1.91 (between 32 and 34°C) (Table 1). The temperature and oxygen consumption regression model established was oxygen consumption = -152.06+9.34 × Acclimation temperature, P = 0.001, r² = 0.99.

DISCUSSION

In the present study early fry stage fish of initial weight 0.09 to 0.11 g were studied at acclimation temperatures of 28, 30, 32 and 34°C. The CTMax and CTMin values get influenced by rate of temperature change, size of fish, condition factor (K) of the fish and water toxicity (Baker al., 2003; Beitinger et al., 2000) was observed. The final preference temperature is the point at which Q10 value starts to decrease with increase acclimation temperature (Kita et al., 1996). The final preference temperature for L. rohita fry was found to be 30°C based on the Q10 value and growth data indicated that optimum temperature range for rohu fry was 30°C. The estimation of Q10 suggests the optimal temperature requirements for fish. Highest body weight gain (%) and lowest FCR at 30°C indicates that temperature of 30°C is optimum for growth in early fry of L. rohita.

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Figure 1. Thermal tolerance polygon of early fry of *Labeo rohita* over four acclimation temperatures (28, 30, 32 and 34°C). The area of thermal tolerance polygon was calculated as 178.74°C².

Table 1. Thermal tolerance (CTmax and CTmin), oxygen consumption and Q₁₀ value of *Labeo rohita* fry acclimated at four different temperatures (28, 30, 32 and 36°C).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acclimation temperatures (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28</td>
</tr>
<tr>
<td><strong>CTmax</strong></td>
<td>42.86±0.03ab</td>
</tr>
<tr>
<td><strong>CTmin</strong></td>
<td>13.07±0.04ab</td>
</tr>
<tr>
<td>Oxygen consumption (mg O₂ kg⁻¹ h⁻¹)</td>
<td>110.75±0.44a</td>
</tr>
<tr>
<td><strong>Q₁₀ value</strong></td>
<td>1.94 (between 28 and 30°C), 2.05 (between 30 and 32°C), 1.91 (between 32 and 34°C)</td>
</tr>
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</table>

Different superscripts (a,b,c,d) in the same row indicate significant difference (p<0.05) amongst different acclimation temperature.

Table 2. Growth parameters and survival of *Labeo rohita* fry reared at four temperatures (28, 30, 32 and 34°C).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acclimation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28</td>
</tr>
<tr>
<td><strong>Initial weight (g)</strong></td>
<td>0.10±0.01</td>
</tr>
<tr>
<td><strong>Final weight (g)</strong></td>
<td>0.37±0.03a</td>
</tr>
<tr>
<td><strong>Weight gain (%)</strong></td>
<td>286.6±45.6a</td>
</tr>
<tr>
<td><strong>Specific growth rate (%/day)</strong></td>
<td>0.69±0.08a</td>
</tr>
<tr>
<td><strong>Feed conversion ratio</strong></td>
<td>1.32±0.51a</td>
</tr>
<tr>
<td><strong>Survival (%)</strong></td>
<td>98.7±2.31</td>
</tr>
</tbody>
</table>

Superscripts indicate significant differences (p<0.005).

been observed that the oxygen-consumption rate of silver carp fry was 5 to 10 times greater than those of summer fingerlings and 15 to 20 times greater than those of 2-year-old fingerlings (NACA, 1989). Regression models though showing positive relation were different due to lower initial weight of rohu early fry for, CTMax (CTMax = 30.89 + 0.42 × Acclimation temperature: CTMax = 41.94 + 1.03 × Acclimation temperature) and CTMin (CTMin = 1.63 + 0.41 × Acclimation temperature: CTMin = 11.01 + 0.86 × Acclimation temperature). Regression model for oxygen consumption (Oxygen consumption = -152.0 + 9.340 × Acclimation temperature; oxygen consumption = 44.40 + 11.59 × Acclimation temperature) differ which may be due to weight dependent oxygen consumption rates of fish. Highest body weight gain and lowest FCR observed at 30°C correspond to the highest body weight.
gain and lowest FCR at 31°C as observed by Das et al. (2005). Acclimation response ratio, an index of the magnitude of thermal acclimation of organism (Claussen, 1997) is dependent on geographical temperature gradient (Herrera et al., 1998). It has been observed that tropical species have higher ARR values than temperate regions (Herrera et al., 1998; Re et al., 2005; Rodriguez et al., 1996) which is due to adaptation of a species to fluctuating temperature seasonally and over long terms. ARR differed from that reported by Chatterjee et al. (2004) which may be due to early life stage of fish. Thermal tolerance polygons provide important insights into fish ecology and distribution and are used to identify temperature related survival tactics (Bennett and Beitinger, 1997) and is also a useful comparative index of eurythermicity between species (Eme and Bennett, 2009).

These findings present the impact of rearing temperatures on the early fry stage of L. rohita. The early fry stage fish are very susceptible to change in environmental conditions and determination of their thermal tolerances, growth and oxygen consumption may help aquaculture industry to effectively manage fisheries and aquaculture of L. rohita for its growth trait.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

**REFERENCES**


