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Thermal tolerance, growth and oxygen consumption of *Labeo rohita* fry (Hamilton, 1822) acclimated to four temperatures

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Abstract

A 30 day feeding trial was conducted using a freshwater fish, *Labeo rohita* (rohu), to determine their thermal tolerance, oxygen consumption and optimum temperature for growth. Four hundred and sixteen *L. rohita* fry (10 days old, 0.385 ± 0.003 g) were equally distributed between four treatments (26, 31, 33 and 36 °C) each with four replicates for 30 days. Highest body weight gain and lowest feed conversion ratio (FCR) was recorded between 31 and 33 °C. The highest specific growth rate was recorded at 31 °C followed by 33 and 26 °C and the lowest was at 36 °C. Thermal tolerance and oxygen consumption studies were carried out after completion of growth study to determine tolerance level and metabolic activity at four different acclimation temperatures. Oxygen consumption rate increased significantly with increasing acclimation temperature. Preferred temperature decided from relationship between acclimation temperature and Q_{10} values were between 33 and 36 °C, which gives a better understanding of optimum temperature for growth of *L. rohita*. Critical thermal maxima (CTMax) and critical thermal minima (CTMin) were 42.33 ± 0.07, 44.81 ± 0.07, 45.35 ± 0.06, 45.60 ± 0.03 and 12.00 ± 0.08, 12.46 ± 0.04, 13.80 ± 0.10, 14.43 ± 0.06, respectively, and increased significantly with increasing acclimation temperatures (26, 31, 33 and 36 °C). Survival (%) was similar in all groups indicating that temperature range of 26–36 °C is not fatal to *L. rohita* fry. The optimum temperature range for growth was 31–33 °C and for Q_{10} values was 33–36 °C.

Keywords: *Labeo rohita*; Temperature; Dissolved oxygen; pH; Ammonia; Growth; Oxygen consumption; Thermal tolerance

1. Introduction

Freshwater aquaculture in India is dominated by carp (*Labeo rohita, Catla catla* and *Cirrhinus mrigala*) (Cyprinidae), which contribute about 87% of the total freshwater production (ICLARM, 2001). *L. rohita* is a major carp, widely cultured throughout India owing to its high commercial value. Growth rate is one of the most important parameters determining the economic efficiency of commercial fish culture, which is influenced by several biotic and abiotic factors (Brett and Groves, 1979). Temperature is a major factor, which directly influences metabolism affecting all physiological processes in ectotherms such as food intake, metabolism and nutritional efficiency (Brett, 1979; Burel et al., 1996). Thus, water temperature directly affects the growth of fish (Smith, 1989). Therefore, knowledge of

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suitable temperatures at which fish have a faster growth rate is very important for effective management of aquaculture systems (Cui and Wootton, 1988). Improved growth and survival were recorded in composite culture of Indian Major Carps when water temperature was 28 °C (Chakraborty et al., 1976). However, carps are reported to thrive well between 18.3 and 37.8 °C (Jhingran, 1975). Therefore, our present investigation was designed to delineate the positive effect of temperature on growth and metabolism of *L. rohita*.

Temperature tolerance of fish is dependent upon acclimation temperatures. Indian carp are eurythermal (Kasim, 2002). Work on fingerlings indicates that *C. mrigala* is the most tolerant species followed by *L. rohita* and *C. catla* and the zone of thermal tolerance was reported as 744.8 $°C^2$ (*L. rohita*) 728.8 $°C^2$ (*C. catla*) and 801.8 $°C^2$ (*C. mrigala*), respectively, over acclimation range of 12–40 °C (Das et al., 2004).

Metabolism is also dependent on acclimation temperature, acclimation period and species (Das et al., 2004; Manush et al., 2004). The metabolic rate of fish is indirectly measured as their rate of oxygen consumption (Brett, 1964, 1979; Kutty, 1968, 1981). Optimum temperatures can be estimated indirectly based on the relationship between oxygen consumption and acclimation temperature (Kita et al., 1996).

Hence, present study was designed to assess thermal tolerance, growth, feed conversion efficiency and oxygen consumption rate of *L. rohita* fry acclimated at 26, 31, 33 and 36 °C under laboratory conditions.

2. Material and methods

2.1. Experimental design

L. rohita (10 day old fry: 15 ± 1.3 mm, 0.385 ± 0.003 g) were procured from Khopoli fish farm, Government of Maharashtra, India, and were kept in circular tanks (500 L) at ambient temperature (26 °C) in the aquaculture wet laboratory, Central Institute of Fisheries Education, Mumbai, India, for 15 days to recover from transportation stress. Prior to commencement of growth study, 416 uniform sized fry were equally distributed between four treatments (26, 31, 33 and 36 °C) with each replicated four times following a completely randomized design, in plastic pools (100 L) with a stocking density of 26 fry/75 L water. Uniform rearing conditions were maintained in all the experimental groups except for the water temperatures (26, 31, 33 and 36 °C).

2.2. Rearing for growth study

Initial water temperature was maintained at 26 °C and the temperatures were gradually increased by 1 °C/day to the target temperatures (31, 33 and 36 °C) and were maintained for 30 days. Fish were fed for another 30 days growth study. A fixed photoperiod of 12L:12D (Light: Dark) was maintained with light exposure from 6 to 18 h. Aeration was provided in all the experimental containers to maintain the dissolved oxygen level. Other water quality parameters, pH, ammonia-N, nitrite-N and nitrate-N, were monitored at every 5 days interval (APHA, 1998) and maintained at the optimum rearing conditions for *L. rohita*.

2.3. Feed and feeding

Pelleted feeds (35% crude protein) as recommended for *L. rohita* (Renukardhyay and Varghese, 1986) were used during the feeding trial. Initial feeding was done twice a day (8 and 20 h) at 10% of the body weight and gradually decreased based on their body weight (assessed at each 10 days interval) up to 30 days. Siphoning of waste feed and faecal materials were done each day before dispensing the feed. Water exchange was carried out up to 50% of water with fresh chlorine free water every day. Fish was starved for a day prior to the assessment of standing stock, thermal tolerance test and rate of oxygen consumption.

3. Growth

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

$$\text{Percent weight gain} = \frac{\text{Final wt.} - \text{Initial wt.}}{\text{Initial wt.}} \times 100,$$

$$\text{Specific growth rate} = \frac{\text{Final body wt} - \text{Initial body wt}}{\text{Duration of experiment (days)}} \times 100,$$

$$\text{FCR} = \frac{\text{Feed given (dry wt)}}{\text{Weight gain (wet wt)}},$$

$$\text{Survival} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100.$$

3.1. Oxygen consumption

Rate of oxygen consumption was measured under similar conditions at four different acclimation temperatures (26, 31, 33 and 36 °C), movement of the fish was not restricted. Hence, any significant differences in oxygen consumption between treatments must be due to the acclimation status. Six fish from each treatment (total 24 fish) were kept individually in sealed glass chambers (5 L). An opening in the lid was fitted with a

gasket to ensure an air tight seal permitting the insertion of a dissolved oxygen probe. A magnetic stirrer was used to maintain a constant water circulation. The chamber was placed inside the thermostatic aquarium at their respective temperatures for an hour to prevent the temperature loss from jar. All four sides of the aquarium were covered with opaque screen to minimize the visual disturbances of the experimental animal. The initial and final oxygen content was measured using a digital oxymeter 330 (Merck, Germany, sensitivity 0.01 mg O_2 mg L^{-1}). Oxygen consumption was calculated as

$$\frac{\text{Final oxygen concentration} - \text{Initial oxygen concentration}}{\text{Weight of fish (Kg)} \times \text{Time (H)}}.$$

3.2. Thermal tolerance

Thermal tolerance was assessed at the end of feeding trial by randomly selecting six fish per treatment, i.e., 6 for CTMax and 6 for CTMin. Fish were transferred from rearing tanks to different aquaria (52 L water capacity) maintained at acclimation temperatures (26, 31, 33 and 36 °C) with minimum disturbance. Fish were exposed to a constant rate 0.3 °C min^{-1} of either increasing or decreasing temperature from 26, 31, 33 and 36 °C until the onset of loss of equilibrium (LOE), the designated end point for critical thermal maxima (CTMax) and critical thermal minima (CTMin), respectively (Paladino et al., 1980; Beitinger et al., 2000). All the fish were rescued and recovered after transfer to ambient temperature from the endpoint of CTM trial. This technique has been critically evaluated by numerous workers (Hutchinson, 1976) and is well established as a powerful tool for studying the physiology of stress and adaptation in fish (Beitinger and McCauley, 1990).

3.3. Statistical analysis

Mean values of all the parameters were analyzed by one way analysis of variance using statistical software (SPSS, version 11.0). Duncan's multiple range test (DMRT) was carried out for post hoc mean comparisons. Regression analysis was carried out to know the relationship between water temperatures with other water quality parameters.

4. Results and discussion

4.1. Water quality parameters

Water quality parameters of rearing tanks maintained at four temperatures (26, 31, 33 and 36 °C) are presented in Table 1. Dissolved oxygen concentration decreased significantly ($p<0.05$) with increasing water temperatures. Hydrogen ion concentration (0.03×10^{-7}–0.05×10^{-7} at 26 °C, 0.04×10^{-7}–0.07×10^{-7} at 31 °C, 0.10×10^{-7}–0.16×10^{-7} at 33 °C and 0.47×10^{-7} –0.50×10^{-7} at 36 °C) increased with increasing water temperatures. Regression model was established between water temperature and dissolved oxygen (DO) = 11.06–0.15 × acclimation temperatures ($p = 0.001$, $r^2 = 0.88$). Nitrite-N and nitrate-N concentration increased with increasing acclimation temperatures ($p<0.05$). Regression models are represented as nitrite-N = 0.0219 + 0.0386 × acclimation temperatures ($p = 0.001$, $r^2=0.94$) and nitrate-N = 0.0081 + 0.0204 × acclimation temperature ($p = 0.001$, $r^2 = 0.99$), respectively. Ammonia-N did not differ significantly between 31 and 33 °C and also between 33 and 36 °C. The regression model is established as ammonia-N = 0.07 + 0.0445 × acclimation temperature ($p = 0.001$, $r^2 = 0.96$). Results indicate that all the water quality parameters are closely related with water temperature.

Ammonia is the primary nitrogenous waste product of carp. It also reaches water from fish excreta, uneaten food and from microbial decay of nitrogenous compounds. High stocking density and uneaten food increases the ammonia levels when the dissolved oxygen is low (Merkens and Downing, 1957). Toxic concentrations of ammonia for short-term exposure are between 0.6 and 2 mg L^{-1} (EIFAC, 1973), which is higher than the present findings. In our experiment, we maintained a

Table 1
Water quality parameters of experimental container rearing fishes at different acclimation temperatures (26, 31, 33 and 36 °C)

Parameters	Acclimation temperatures (°C)			
	26	31	33	36
Dissolved oxygen (mg L^{-1})	$6.90^{a}\pm0.03$	$6.54^{b}\pm0.0$	$6.18^{c}\pm0.04$	$5.30^{d}\pm0.09$
Ammonia-N (mg L^{-1})	$0.10^{a}\pm0.008$	$0.17^{bc}\pm0.01$	$0.21^{cd}\pm0.01$	$0.24^{d}\pm0.01$
Nitrite-N (mg L^{-1})	$0.06^{a}\pm0.003$	$0.09^{b}\pm0.006$	$0.14^{c}\pm0.004$	$0.17^{d}\pm0.007$
Nitrate-N (mg L^{-1})	$0.03^{a}\pm0.004$	$0.04^{ab}\pm0.008$	$0.07^{bc}\pm0.005$	$0.09^{c}\pm0.004$

Different superscripts (a, b, c, d) in the same row indicate significant difference ($p<0.05$) (overall mean values) amongst different acclimation temperatures by using one way ANOVA. Values are expressed as mean ± SE ($n = 4$).

low stocking density and continuous aeration in order to avoid any confinement stress and ammonia accumulation in rearing tanks. Bacterial oxidation of ammonia results in the formation of nitrite and nitrate. The nitrite and nitrate levels were within the permissible limits for warm water fish (Boyd, 1982). However, these parameters were maintained to the optimum during the growth study.

4.2. Thermal tolerance

CTMax and CTMin were increased significantly ($p<0.05$) with increasing acclimation temperatures (Table 2). CTMax and CTMin values are also influenced by a variety of factors, rate of change of temperature used the size and condition factor (K) of the animals (Baker and Heidinger, 1996), as well as by the presence of toxic chemicals (Beitinger et al., 2000). In the present study, water quality was maintained, parameters were maintained at optimum level. This way acclimation was the only variable treatment. After each CTM test, all fish were completely recovered. It was found that fish exposed to higher acclimation temperature showed higher CTMax and CTMin values. Regression analysis showed a positive relation (CTMax $= 41.94 + 1.03 \times$ acclimation temperature, $P = 0.001$, $r^2 = 0.79$). Similarly, regression model between CTMin and acclimation temperature established as CTMin $= 11.01 + 0.86 \times$ acclimation temperature, $p = 0.001$, $r^2 = 0.96$. The results indicate that there is strong relation between acclimation temperatures and thermal tolerance (CTM) level. In the present study, it is interesting to note that *L. rohita* fry exhibits exceptionally high CTMax values in comparison to adult fish of same species in the tropical region. However, similar CTMax values 44.6 °C in *Cyprinodon macularis* (Lowe and Heath, 1969), 45.1 °C in *Cyprinodon variegates* (Bennett and Beitinger, 1997), and 45.4 °C was recorded in *Cyprinodon artifans* (Heath et al., 1993). Higher value of CTMax was noticed in *L. rohita* fry than the reported value for fingerlings of this species (Das et al., 2004). Present result indicates that small-size fish are more temperature tolerant than bigger fish even though bigger fish are less sensitive to temperature fluctuations (Rodnick et al., 2004). Similar observation was also made by Herrera et al. (1998) for *Macrobrachium rosenbegji* between post larvae and juveniles.

4.3. Oxygen consumption

Oxygen consumption rate increased significantly ($p<0.05$) with increasing acclimation temperature (Table 2). Mean oxygen consumption rates (routine) at 26, 31, 33 and 36 °C were 58.02, 66.04, 76.28 and 93.27 mg $O_2\ kg^{-1}\ h^{-1}$, respectively. Q_{10} values were estimated and extrapolated as 1.29 (between 26 and 31 °C), 2.05 (between 31 and 33 °C) and 1.95 (between 33 and 36 °C) (Table 2). Regression model between the temperature and oxygen consumption was established as oxygen consumption $= 44.40 + 11.59 \times$ acclimation temperature, $P = 0.001$, $r^2 = 0.96$.

4.4. Growth

Growth of *L. rohita* fry raised at different culture temperatures is presented in Table 3. Highest body weight gain (%) and SGR was found at acclimation temperature of 31 °C, followed by 33 °C with the lowest value obtained at 36 °C. FCR was similar at 31 and 33 °C but was significantly lowered ($p<0.05$) at 26 and 36 °C. Survival was similar in all the groups reared at different temperatures.

The preferred temperature is considered to coincide with the optimum temperature for growth (Brett, 1971; Kellog and Gift, 1983). Preferred temperature can be estimated from the relationship between Q_{10} and acclimation temperature. Kita et al. (1996) stated that preferred temperature is the point where Q_{10} value starts to decrease with increasing acclimation temperatures, which corresponds to the optimal temperature for

Table 2
Thermal tolerance (CTMax and CTMin), oxygen consumption and Q_{10} value of *Labeo rohita* fry acclimated at four different temperatures (26, 31, 33 and 36 °C)

Parameters	Acclimation temperatures (°C)			
	26	31	33	36
CTMax	$42.33^{a} \pm 0.07$	$44.81^{b} \pm 0.07$	$45.35^{c} \pm 0.06$	$45.60^{c} \pm 0.03$
CTMin	$12.00^{a} \pm 0.08$	$12.46^{b} \pm 0.04$	$13.80^{c} \pm 0.10$	$14.43^{d} \pm 0.06$
Oxygen consumption (mg $O_2\ kg^{-1}\ h^{-1}$)	$58.02^{a} \pm 0.24$	$66.04^{b} \pm 2.10$	$76.28^{c} \pm 1.43$	$93.27^{d} \pm 0.24$
Q_{10} value	1.29 (between 26° and 31 °C) 2.05 (between 31° and 33 °C) 1.95 (between 33° and 36 °C)			

Different superscripts (a, b, c, d) in the same row indicate significant difference ($p<0.05$) amongst different acclimation temperatures. Mean values are expressed as mean ± SE ($n = 6$).

Table 3
Growth parameters and survival of *L. rohita* reared at four temperatures (26, 31, 33 and 36 °C)

Parameters	Acclimation temperatures (°C)			
	26	31	33	36
Initial weight (g)	9.8 ± 0.12	10.36 ± 0.18	10.01 ± 0.14	9.99 ± 0.04
Final weight (g)	15.3^{a} ± 0.26	18.84^{b} ± 0.21	17.38^{c} ± 0.35	14.31^{a} ± 0.05
Weight gain (%)	57.51^{a} ± 2.71	80.72^{b} ± 5.58	76.69^{b} ± 2.9	44.25^{c} ± 1.96
Specific growth rate (%/day)	0.65^{a} ± 0.02	0.89^{b} ± 0.05	0.81^{b} ± 0.02	0.52^{c} ± 0.02
Feed conversion ratio	1.32^{a} ± 0.02	1.01^{b} ± 0.01	1.02^{b} ± 0.04	1.59^{c} ± 0.02
Survival (%)	100 ± 0.00	100 ± 0.00	98.71 ± 2.22	98.76 ± 2.13

Different superscripts (a, b, c, d) in the same row indicate significant difference ($p < 0.05$). Values are expressed as mean ± SE ($n = 4$).

growth. Thus, the final preferred temperature may be estimated indirectly from the relationship between oxygen consumption and acclimation temperature (Kita et al., 1996). In our study, the final preference temperature for *L. rohita* fry was found to be between 33 and 36 °C based on the Q_{10} value. Data from the growth study revealed that optimum temperature range for growth was 31–33 °C. This result indicates that the preferred temperature estimated from Q_{10} values of oxygen consumption nearly matches the optimum temperature for growth of *L. rohita*. Q_{10} values could have been more precise had the temperature range be narrower. Thus, estimation of Q_{10} and thermal optima estimation can serve as a preliminary and convenient method to screen candidate species used for aquaculture before a growth study is being performed.

Highest body weight gain (%) and lowest FCR were registered at 31 °C followed by 33 °C, but were not significantly different ($p \geqslant 0.05$). This indicates that temperature range from 31 to 33 °C may be regarded as optimum for better growth in *L. rohita* fry. Optimum temperature for growth of *L. rohita* was in the range of 31–33 °C, which nearly matches the optimum derived from Q_{10}.

Survival did not differ among rearing temperatures, indicating that temperature range of 26–36 °C was not lethal to *L. rohita*. Within this temperature range, the growth rate was optimum between 31 and 33 °C, which may be species specific. Findings of the present investigation will help for effective management strategies of temperature for *L. rohia* rearing and aquaculture in field conditions.

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