

Thermal tolerance, oxygen consumption and haemato-biochemical variables of *Tor putitora* juveniles acclimated to five temperatures

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Abstract A 30-day acclimation trial was conducted using *Tor putitora* to elucidate its thermal tolerance, oxygen consumption, haemato-biochemical variables and selected enzymatic activities at five acclimation temperatures (AT). Juveniles of *T. putitora* were randomly distributed among five treatment groups (20, 23, 26, 29 and 32 ± 0.5 °C). There was a significant curvilinear increase in critical thermal maxima (CT_{max}) ($y = -0.0693x^2 + 1.7927x + 34.628$, $R^2 = 0.996$) and lethal thermal maxima (LT_{max}) ($y = -0.1493x^2 + 2.3407x + 35.092$, $R^2 = 0.991$) with increasing AT. The oxygen consumption rate increased significantly with increasing AT. The Q_{10} values were 1.16 between 20 and 23 °C, 3.09 between 23 and 26 °C, 1.31 between 26 and 29 °C and 1.76 between 29 and 32 °C of AT. The acclimation response ratios were ranged between 0.37 and 0.59. Catalase, superoxide dismutase and ATPase activities were increased linearly in liver, gill and kidney, while brain acetylcholine esterase activity decreased linearly with increasing AT. Blood glucose remained unchanged up to AT of 26 °C and increased significantly at

AT of 29 and 32 °C. Haemoglobin content was increased linearly with increasing AT. The highest WBC count was observed at 20 °C, and no significant changes found till AT of 26 °C and significantly decreased at 32 °C. Total serum protein and globulin were significantly decreased with increasing AT. Highest values were observed at 20 °C and remained consistent till 26 °C, then decreased significantly. There was no significant change in A/G ratio through the AT 20–29 °C and increased significantly at 32 °C. The increase in CT_{max}, LT_{max} and oxygen consumption rate with increasing AT may suggest that the thermal tolerance of *T. putitora* is dependent on its prior thermal exposure history, and it could adapt to higher AT by altering its haemato-biochemical variables.

Keywords Acclimation temperature · Catalase · CT_{max} · LT_{max} · Oxygen consumption · *Tor putitora*

Introduction

Tor putitora commonly known as ‘golden mahseer’ is an important cyprinid fish endemic to Asia with natural distribution encircling the trans-Himalayan region and thrives well in the temperature range of 20–25 °C. They are attractive as sport fish as well as have tremendous aquaculture potential (Ingram et al. 2005). In recent years, due to anthropogenic pressure, pollution, environmental degradation and indiscriminate

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fishing, the population of golden mahseer in the natural water bodies had declined to an alarming level (Nautiyal 1994). It is now identified as a critically endangered species as depletion of mahseer populations has been reported from various parts of Asia (Hussain and Mazid 2001). Another important threat to the natural stocks of this species is the rising temperature due to global warming which is likely to further affect its productivity in wild populations as well as in aquaculture systems.

Water temperature is considered to be the most important factor in the development and growth of fish. As fish is an ectotherm, any alterations in the water temperature have noticeable and direct effect on various physiological processes and behavioural activities (Brett and Groves 1979). The survival and growth of fish are immediately affected by temperature fluctuations in their environments (Manush et al. 2004). All teleostean fish species have developed their own specific behavioural and physiological adaptive mechanism to cope up with temperature fluctuations (Prosser and Heath 1991). These adaptive capabilities enable them to survive through acclimation and adaptation to stressful temperature conditions (Hazel and Prosser 1974). However, temperature beyond optimum limits of a particular species adversely affects the health of aquatic animal due to metabolic stress and increases oxygen demand and susceptibility to diseases (Wedemeyer et al. 1999). The measurement of the metabolic rate is an effective tool to determine the effects of environmental factors such as the temperature on the organism (Manush et al. 2004). The oxygen consumption is closely related to the metabolic work and the energy flow that the organism can use for the homeostatic control mechanisms (Salvato et al. 2001; Das et al. 2005). 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 (Kutty 1981).

Changes in concentration and enzymatic activities often directly reflect cell damage in specific organs (Casillas et al., 1983). Measurement of acetylcholinesterase activity in fish brain is proven to be a specific tool to identify stress (Svobodova et al. 1994). Higher temperature induces oxidative stress in fish (Cadenas 1989). Fish possess a defence mechanism consisting of antioxidant enzymes like catalase and superoxide

dismutase, which play an important role in protecting cells against oxidative damage (Akhtar et al. 2010). Therefore, changes in the activities of these enzymes can be used as possible stress biomarkers in different aquatic organisms (Akhtar et al., 2010). ATPase activity is sensitive to changes in environmental variables like temperature (Morrison et al. 2006) to meet the increased energy demand by fish.

Haemato-immunological parameters including blood glucose levels are increasingly used as indicators of physiological stress response to endogenous or exogenous changes in fish (Santos and Pacheco 1996). A reduced white blood cell count is usually used a biochemical indicator of immunosuppression caused by various stressors (Kopp et al. 2010). Similarly, the concentration of serum proteins especially albumin and globulin is used as basic index for the health status of fish (Buchanan et al. 1997).

The likely effects of global warming make us to put important efforts to define thermal tolerance, temperature adaptation of fishes and their consequences on fish health. Therefore, thermal tolerance studies have gained significant attention of scientists to understand the impact of changes in temperature on animals, including fish as well. However, to the best of our knowledge, nothing is known about the adaptive responses of endangered golden mahseer, the king of Indian game fish to temperature variations. In this context, the present experiment was conducted to elucidate the thermal tolerance, oxygen consumption and some enzymatic responses as well as the effect of different acclimation temperatures on haemato-immunological variables of golden mahseer, *T. putitora* juveniles at different acclimation temperatures to assess its adaptive response at higher acclimation temperatures.

Materials and methods

Experimental design and rearing

Juveniles of *T. putitora* (13.62 ± 2.15 g, average weight \pm SE) were collected from fish ponds (temperature 19.5 °C) of Directorate of Coldwater Fisheries Research (DCFR), Bhimtal, Uttarakhand, India, and transferred to a rectangular FRP tank (2,000 L) in the wet laboratory of DCFR with sufficient aeration. The seasonal temperature of the pond ranged from 12 to 24 °C. The fish were disinfected by giving a salt

treatment (1 % NaCl w/v) for 5 min and KMnO₄ (5 ppm) dip treatment and then acclimatized at 20 °C for 10 days. After acclimatization, fishes were randomly distributed in five acclimation temperature groups in triplicates following a completely randomized design. The first group was maintained at temperature of 20 °C. For other four groups, the temperatures were gradually increased by 1 °C/day to the test temperatures (23, 26, 29 and 32 ± 0.5 °C) by using 150 W thermostatic heaters (General Trading Company, Mumbai, India) connected to individual temperature controllers. The acclimation was continued for 30 days. Fish were fed with pelleted feed (35 % crude protein) at the rate of 3.0 % wet body weight (as feed intake was observed to be nearly 3 %) twice daily at 10.00 and 17.00 h. The uneaten feed (if present) and faecal matters were removed by siphoning each day before dispensing the feed, and about 20 % water of the tanks was exchanged with preheated (as per test temperatures) water. Sufficient aeration was provided throughout the experimental period to maintain the dissolved oxygen level. Other water quality parameters like pH, ammonia-N, nitrite-N and nitrate-N were monitored at every 7-day interval. The fish were weighed at the beginning (13.62 ± 2.15 g, average weight ± SE) and the end (15.81 ± 1.27 g, average weight ± SE) of the acclimation trial.

Thermal tolerance

Thermal tolerance (CT_{max} and LT_{max}) was assessed using the critical thermal methodology (CTM) as described previously by Paladino et al. (1980). For this, twenty fish were maintained in each acclimation temperature for 30 days. At the end of 30-day acclimation trial, the fishes were transferred into thermostatic aquaria (Nirmal International, New Delhi, India, 40 L water capacity) for CT_{max} and LT_{max} study. The fishes were deprived of feed for 1 day before performing the thermal tolerance study. The temperature of water in the thermostatic aquaria was maintained similar to the acclimation temperature. Dissolved oxygen concentration was maintained at 6.4 ± 0.5 mg/L throughout the temperature tolerance study by continuous aeration. Water temperature in the aquarium was increased at a constant rate of 0.3 °C/min until loss of equilibrium, which was designated as the CT_{max} (Beitinger et al. 2000). The lethal thermal maxima (LT_{max}) were determined by

further increasing the temperature until the opercular movements were ceased (Kita et al. 1996). This technique has been critically evaluated by numerous workers (Debnath et al. 2006; Chatterjee et al. 2010; Akhtar et al. 2011).

Acclimation response ratio (ARR) was determined as the change in the CT_{max} per degree change in acclimation temperature defined by Claussen (1977).

Temperature quotients (Q_{10}) were calculated by using the following formula (Das et al. 2004).

$$Q_{10} = (\text{Rate 2}/\text{Rate 1})^{10/(\text{Temp 2}-\text{Temp 1})}$$

where Rate 1 = the oxygen consumption rate at temperature 1; Rate 2 = the oxygen consumption rate at temperature 2; Temp 1 = the lower of the two temperatures used to determine oxygen consumption. Temp 2 = the upper of the two temperatures used to determine oxygen consumption.

Final preferred temperature was estimated from expected relationships between acclimation temperature and oxygen consumption. The final preferred temperature was determined by using the point where a drop in the Q_{10} becomes apparent (Kita et al. 1996).

Oxygen consumption

Rate of oxygen consumption of fishes at different acclimation temperatures (20, 23, 26, 29 and 32 ± 0.5 °C) was measured at their respective temperatures following the methods of Das et al. (2004) with some modifications. Twenty fish from each acclimation group were kept individually in narrow necked glass flasks (4 L). The flask was sealed, and an opening in the lid was made for permitting the insertion of a dissolved oxygen probe ensuring that the seal is airtight. The chamber was placed inside the thermostatic aquarium at their respective temperature for an hour to prevent the temperature loss from flask. The aquarium was covered with opaque screen to minimize the visual disturbances of the experimental animal. The initial and final oxygen concentration was measured using a portable digital oximeter Oxi 315i (Weilheim, WTW, GmbH, Germany; sensitivity ± 0.02 mg O₂/L).

Oxygen consumption (mg O₂/kg/h) was calculated as

$$\frac{\text{Final oxygen concentration} - \text{Initial oxygen concentration}}{\text{Weight of fish (kg)} \times \text{Time (h)}}$$

Enzyme assays

At the end of the acclimation trial, six fish from each acclimation temperature group were killed and different tissues like liver, gill, kidney and brain were dissected out. Separate homogenates were prepared for each tissue. Tissues were homogenized with chilled 0.25 M sucrose solution using a mechanical tissue homogenizer (Remi, Mumbai, India). The homogenized samples were centrifuged ($8,000 \times g$ for 10 min); supernatants were collected and stored at $-20\text{ }^{\circ}\text{C}$ for subsequent enzyme assays. Acetylcholine esterase (AChE) was assayed from brain of *T. Putitora* by the method of Augustinsson (1957). Catalase activity was estimated according to the method of Takahara et al. (1960). The SOD activity was estimated by the method of Misra and Fridovich (1972). Adenosine triphosphatase activity was determined by Post and Sen (1967) method. Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow (1925).

Haematological studies

At the end of the acclimation trial, six fish from each temperature were taken out and the body surface was cleaned with blotting paper. Blood was collected by puncturing the caudal vein using a medical syringe (No. 23), which was previously rinsed, with 2.7 % EDTA solution (as anticoagulant) and shaken gently in order to prevent haemolysis of blood and were used for the estimation of blood glucose, haemoglobin percentage and total leucocyte count.

The haemoglobin percentage was determined by estimating cyanmethemoglobin using Drabkin's fluid provided in the kit following the manufactures protocol (Qualigens, Mumbai, India). The final concentration was calculated by comparing with standard cyanmethemoglobin (Qualigens, Mumbai, India). Total leucocyte was counted in a haemocytometer using leucocyte diluting fluid supplied with the kit (Qualigens, Mumbai, India). Blood glucose was estimated by the method of Nelson and Somogyi (1945).

Serum immunological studies

Six fishes from each group were anaesthetized with clove oil (50 $\mu\text{L/L}$), and blood was collected without anticoagulants from the caudal vein and allowed to

clot for sometime till the straw colour serum separated out. This serum was then stored at $-20\text{ }^{\circ}\text{C}$ for further analysis.

Serum protein was estimated by biuret and BCG dye binding method (Reinhold 1953) using the kit (total protein and albumin kit, Qualigens, Mumbai, India). Albumin was estimated by bromocresol green binding method (Dumas et al. 1971). The absorbance of standard and test was measured against the blank in a spectrophotometer at 630 nm. Globulin was calculated by subtracting albumin values from total serum protein. A/G ratio was calculated by dividing albumin values by globulin values.

Statistical analysis

As data were normal and homoscedastic, the mean values of all parameters were subjected to one-way analysis of variance to study the treatment effects. Duncan's multiple range tests were used to determine the significant differences between the means. Comparisons were made at 5 % probability level. Regression analysis was carried out to know the relationship between acclimation temperatures with other studied parameters. All the data were analysed using statistical package SPSS (Version 19).

Results

Water quality parameters

There was a significant linear decrease in pH ($y = -0.124x + 10.69$, $R^2 = 0.949$) and dissolved oxygen ($y = -0.101x + 8.967$, $R^2 = 0.982$) with increasing acclimation temperature (Table 1). This decrease in pH and dissolved oxygen is in agreement with Das et al. (2005). Other water quality parameters viz. ammonia-N, nitrite-N and nitrate-N were significantly increased as acclimation temperature increased from 20 to 32 $^{\circ}\text{C}$ (Table 1). The lowest values were observed at 20 $^{\circ}\text{C}$ and reached to maximum at 32 $^{\circ}\text{C}$. The linear regression models were fit with the acclimation temperature and ammonia-N ($y = -0.152 + 0.102x$, $R^2 = 0.820$); nitrite-N ($y = -0.145 + 0.10x$, $R^2 = 0.866$) and nitrate-N ($y = 0.718 + 0.128x$, $R^2 = 0.658$). Results indicated that all the measured water quality parameters were closely associated with acclimation temperature.

Thermal tolerance

The upper thermal tolerance of *T. putitora* juveniles was assessed at the end of the 30-day acclimation trial in terms of CT_{max} and LT_{max} . There was a significant curvilinear (second polynomial) increase in CT_{max} ($y = -0.0693x^2 + 1.7927x + 34.628$, $R^2 = 0.996$) and LT_{max} ($y = -0.1493x^2 + 2.3407x + 35.092$, $R^2 = 0.991$) with increasing acclimation temperature (Fig. 1a). A positive correlation was observed between CT_{max} and LT_{max} ($R^2 = 0.98$). Results indicated that *T. putitora* juveniles, which naturally thrive well in a temperature range of 20–25 °C, when exposed to higher acclimation temperature (up to 32 °C) showed higher CT_{max} and LT_{max} values.

Oxygen consumption

The rate of oxygen consumption (mg O₂/kg/h) of *T. putitora* juveniles increased significantly with increasing acclimation temperature ($y = 10.542x + 41.458$, $R^2 = 0.961$) (Fig. 1b). The lowest rate was observed at 20 °C and highest at 32 °C. There were 4.67, 46.63, 55.98 and 79.68 % increase in oxygen consumption rate at 23, 26, 29 and 32 °C, respectively, compared to oxygen consumption rate at 20 °C. The temperature co-efficient (Q_{10}) values for oxygen consumption and acclimation response ratio (ARR) of *T. putitora* are shown in Fig. 1c. Q_{10} values were 1.16 (between 20 and 23 °C), 3.09 (between 23 and 26 °C), 1.31 (between 26 and 29 °C) and 1.76 (between 29 and 32 °C of acclimation temperature). The ARR values were ranged between 0.37 and 0.59 (Fig. 1c).

Enzymatic responses

AChE activity (Fig. 1-D) in brain decreased linearly ($y = -3.568x + 25.77$, $R^2 = 0.940$) with increasing acclimation temperature.

Catalase and SOD activities in all the measured tissues were significantly increased with increasing acclimation temperature (Table 2). Catalase activity was increased linearly in liver ($y = 11.84x + 4.82$, $R^2 = 0.94$), gill ($y = 3.21x + 2.59$, $R^2 = 0.98$) as well as in kidney ($y = 8.34x - 2.39$, $R^2 = 0.95$) with increasing acclimation temperature. Similar trends were observed in the activity of SOD in different tissues.

There was significant linear increase in ATPase activity in liver ($y = 12.91x - 2.89$, $R^2 = 0.89$), gill ($y = 20.06x + 33.17$, $R^2 = 0.95$) and in kidney ($y = 20.49x + 52.51$, $R^2 = 0.96$) with increasing acclimation temperature (Table 3).

Haematological parameters

There were significant effects of acclimation temperature on blood glucose, total leucocytes and haemoglobin content of golden mahseer juveniles. Blood glucose (Fig. 2a) remained significantly unchanged up to the acclimation temperature 26 °C and increased significantly at acclimation temperature of 29 and 32 °C by 37 and 50 %, respectively, compared to 20 °C (Fig. 2a). Haemoglobin content was also elevated significantly due to increasing acclimation temperature (Fig. 2b). The highest WBC count (Fig. 2b) was observed at 20 °C of acclimation temperature, and no significant change was found till

Table 1 Water quality parameters of rearing tanks during experimental trial at different acclimation temperatures

| Acclimation temperature (°C) | Dissolved oxygen (mg L ⁻¹) | Ammonia-N (mg L ⁻¹) | Nitrite-N (mg L ⁻¹) | Nitrate-N (mg L ⁻¹) | pH |
|------------------------------|--|---------------------------------|---------------------------------|---------------------------------|---------------------------|
| 20 | 6.97 ^d ± 0.12 | 0.01 ^a ± 0.01 | 0.05 ^a ± 0.006 | 3.20 ^a ± 0.21 | 8.10 ^b ± 0.35 |
| 23 | 6.33 ^c ± 0.09 | 0.11 ^a ± 0.01 | 0.07 ^a ± 0.006 | 3.60 ^{ab} ± 0.15 | 7.87 ^b ± 0.12 |
| 26 | 6.23 ^b ± 0.09 | 0.13 ^a ± 0.01 | 0.12 ^b ± 0.012 | 4.13 ^{bc} ± 0.32 | 7.63 ^b ± 0.32 |
| 29 | 6.10 ^b ± 0.06 | 0.18 ^b ± 0.01 | 0.13 ^b ± 0.015 | 4.70 ^c ± 0.12 | 7.20 ^{ab} ± 0.35 |
| 32 | 5.70 ^a ± 0.06 | 0.23 ^c ± 0.02 | 0.17 ^c ± 0.009 | 4.57 ^c ± 0.35 | 6.57 ^a ± 0.15 |
| <i>P</i> values | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| <i>R</i> ² | 0.982 | 0.820 | 0.866 | 0.658 | 0.949 |

Mean values bearing different superscripts (a, b, c and d) under each column vary significantly ($P < 0.05$). Data expressed as mean ± SE, $n = 12$

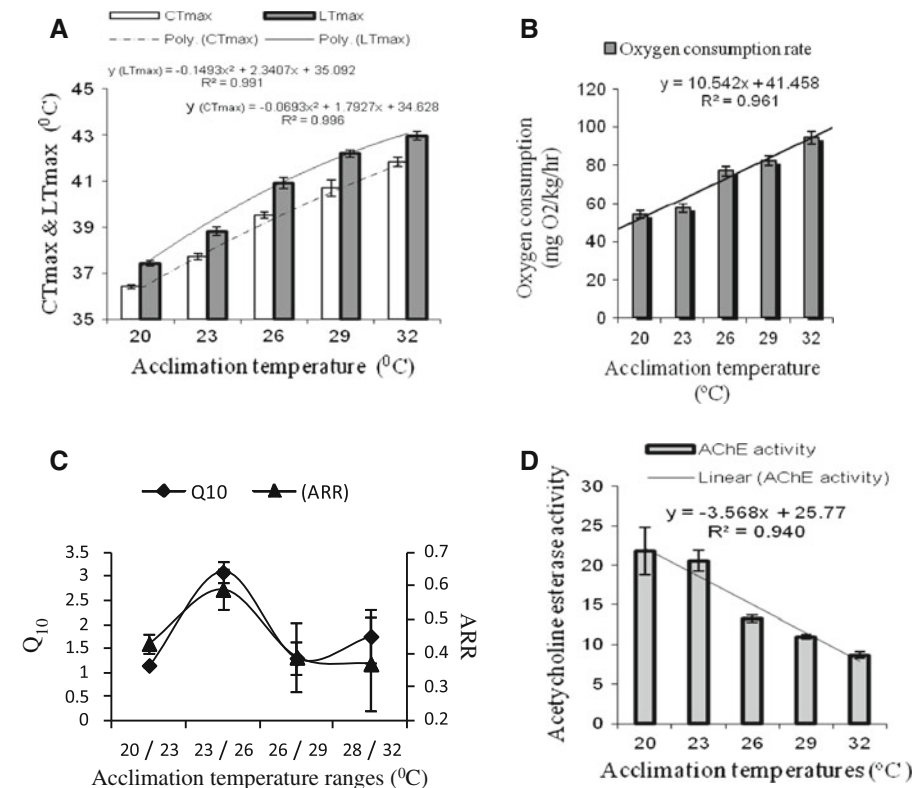


Fig. 1 Effect of acclimation temperatures on thermal tolerance, oxygen consumption, acclimation response ratio (ARR), Q_{10} and acetylcholine esterase activity of golden mahseer, *T. puiitora* juveniles. **a** Thermal tolerance (CT_{max} and LT_{max};

$n = 20$); **b** oxygen consumption rate ($n = 20$); **c** acclimation response ratio (ARR) and temperature co-efficient (Q_{10}) of oxygen consumption; **d** acetylcholinesterase activity ($n = 6$). Data expressed as mean \pm SE)

Table 2 Effects of acclimation temperature on antioxidative enzyme (catalase and superoxide dismutase) activity in different tissues of golden mahseer, *T. puiitora* juveniles

| Acclimation temperature (°C) | Catalase | | | Superoxide dismutase (SOD) | | |
|------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
| | Liver | Gill | Kidney | Liver | Gill | Kidney |
| 20 | 18.40 ^a \pm 2.42 | 5.26 ^a \pm 1.27 | 9.52 ^a \pm 1.18 | 9.00 ^a \pm 0.48 | 16.12 ^a \pm 0.38 | 13.45 ^a \pm 0.66 |
| 23 | 24.22 ^a \pm 3.17 | 8.89 ^{ab} \pm 1.26 | 11.82 ^a \pm 0.75 | 9.79 ^a \pm 0.69 | 18.2 ^b \pm 0.93 | 15.30 ^b \pm 0.28 |
| 26 | 46.18 ^b \pm 1.29 | 13.42 ^{bc} \pm 3.2 | 18.72 ^b \pm 0.75 | 12.92 ^{ab} \pm 0.34 | 19.67 ^c \pm 0.27 | 17.46 ^c \pm 0.36 |
| 29 | 46.34 ^b \pm 4.34 | 15.59 ^c \pm 1.0 | 31.85 ^c \pm 1.66 | 16.23 ^{bc} \pm 1.32 | 21.65 ^d \pm 1.66 | 20.33 ^d \pm 0.56 |
| 32 | 66.52 ^c \pm 0.58 | 17.95 ^c \pm 1.44 | 41.21 ^d \pm 1.74 | 20.36 ^c \pm 2.65 | 21.26 ^d \pm 0.41 | 19.25 ^d \pm 0.33 |
| <i>P</i> values | 0.001 | 0.004 | 0.001 | 0.001 | 0.001 | 0.001 |
| <i>R</i> ² | 0.94 | 0.98 | 0.95 | – | – | – |

Mean values bearing different superscripts (a, b, c and d) under each column vary significantly ($P < 0.05$). Data expressed as mean \pm SE, $n = 6$

Catalase: m moles H₂O₂ decomposed/min/mg protein at 37 °C

SOD: μ mole/mg protein/min at 37 °C

Table 3 Effects of acclimation temperature on adenosine triphosphatase (ATPase) and activity in different tissues of golden mahseer, *T. putilora* juveniles

| Acclimation temperature (°C) | ATPase | | |
|------------------------------|----------------------------|------------------------------|------------------------------|
| | Liver | Gill | Kidney |
| 20 | 15.19 ^a ± 2.34 | 61.95 ^a ± 1.20 | 80.66 ^a ± 2.25 |
| 23 | 20.52 ^{ab} ± 1.18 | 67.74 ^a ± 3.77 | 88.25 ^a ± 3.42 |
| 26 | 25.61 ^b ± 1.74 | 84.80 ^a ± 1.82 | 107.20 ^a ± 5.91 |
| 29 | 55.78 ^c ± 2.59 | 112.25 ^{ab} ± 10.52 | 133.23 ^{ab} ± 10.90 |
| 32 | 62.13 ^d ± 1.77 | 139.93 ^b ± 9.28 | 160.64 ^b ± 8.81 |
| <i>P</i> values | 0.001 | 0.032 | 0.029 |
| <i>R</i> ² | 0.89 | 0.95 | 0.96 |

Mean values bearing different superscripts (a, b, c & d) under each column vary significantly (*P* < 0.05). Data expressed as mean ± SE, *n* = 6

ATPase: µg of phosphorus released/mg protein/min at 37 °C

26 °C. Then, WBC count was significantly decreased by 31 % at acclimation temperature of 32 °C.

Serum immunological parameters

Significant effect of acclimation temperature was evidenced in all the measured serum parameters of *T. putilora* juveniles. Total serum protein (Fig. 2c) was significantly decreased with increasing acclimation temperature. The highest value was observed at 20 °C and statistically remained consistent till 26 °C followed by a drastic decrease. A similar trend was observed in serum globulin levels (Fig. 2c). Serum albumin levels were significantly decreased with increasing acclimation temperature (Fig. 2-D). There was no significant change in A/G ratio through the

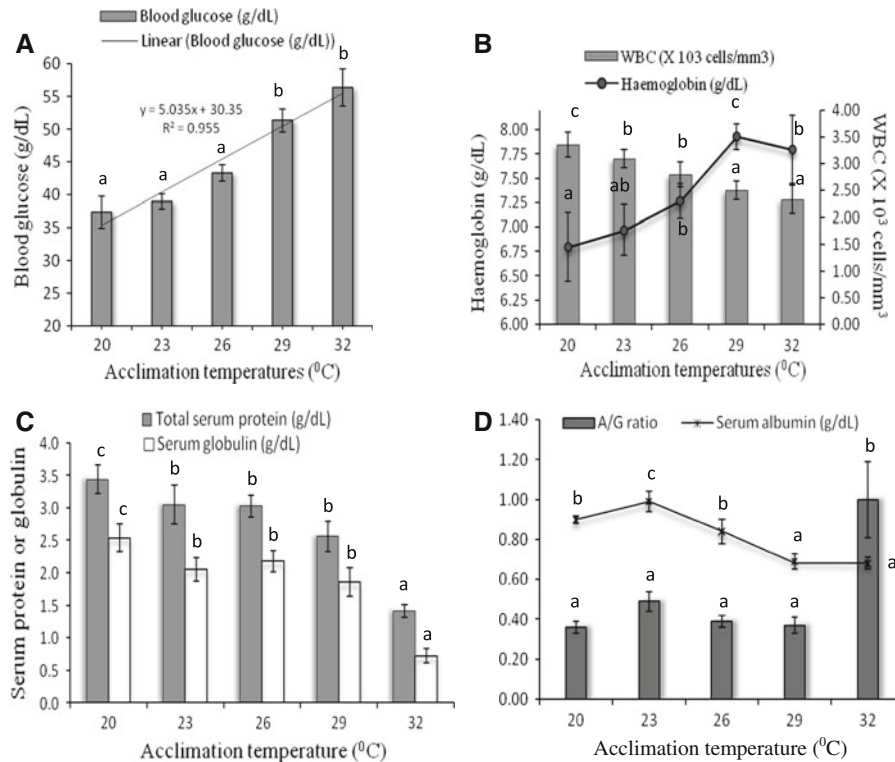


Fig. 2 Effect of acclimation temperatures on haemato-immunological variables of golden mahseer, *T. putilora* juveniles. **a** Blood glucose (g/dL); **b** total leucocytes (WBC) count ($\times 10^3$ cells/mm³) and haemoglobin (Hb) levels (g/dL); **c** total serum protein (g/dL) and serum globulin (g/dL); **d** serum

albumin (g/dL) and A/G ratio. Different superscripts (a, b and c) in each series under each panel signify statistical differences (ANOVA, Duncan’s test, *P* < 0.05). Data expressed as mean ± SE) (*n* = 6)

acclimation temperatures 20–29 °C and increased significantly at 32 °C (Fig. 2).

Discussion

As fish is ammonotelic, ammonia is the main nitrogenous excretory product of fish (Wilkie 2002). The metabolic rate of fish increases when water temperature increases which may result in higher ammonia excretion. In the present study, the higher ammonia–N levels found at higher acclimation temperatures might be due to increased metabolic rate as evidenced by higher oxygen consumption rate. The toxic concentrations of ammonia for short-term exposure are between 0.6 and 2.0 mg L⁻¹ (EIFAC 1973), which is much higher than our present findings. Although nitrite and nitrate levels were increased with increase in acclimation temperatures, their levels were within the permissible limits (<0.3 and 2–5 mg L⁻¹, respectively) of fish (Boyd 1982).

The increase in LT_{max} values with increase in acclimation temperature confirms that the thermal tolerance is largely dependent on fish's prior thermal exposure history or acclimation. Consequently, typical seasonal acclimation allows fish to be more tolerant to higher temperatures in summer than in winter (Bevelhimer and Bennett 2000). In the present study, the differences between the LT_{max} and acclimation temperatures decrease with the increase in acclimation temperature which suggests that even though *T. putitora* is capable of acclimating to rising temperature, there would be a threshold where the increases in acclimation temperature cannot allow *T. putitora* juveniles to cope up with higher temperature. The CT_{max} is considered as an indicator of the thermal tolerance of aquatic organisms (Paladino et al. 1980; Beitinger et al. 2000), which allows the identification of the temperature at which the loss of equilibrium occurs. For this reason, CT_{max} is being used as an efficient tool to evaluate the requirements at physiologic and ecological levels of the aquatic organisms by which they can find such temperatures under natural conditions (Das et al. 2004; Re et al. 2006). The direct relationship between thermal tolerance (CT_{max}) and acclimation temperature has been reported in many aquatic species (Das et al. 2004, 2005) as obtained in the present study for *T. putitora* juveniles. In addition to the acclimation temperature, a variety of factors like

size and condition factor (Baker and Heidinger, 1996), presence of toxic chemical (Beitinger et al. 2000) and species (Das et al. 2004) also influence the thermal tolerance limits. However, in the present study, the size of the *T. putitora* juveniles was kept uniform (15.81 ± 1.27 g, average weight ± SE) and water quality parameters were within the optimum range, and hence, the variation in CT_{max} and LT_{max} values could be mostly due to acclimation temperatures.

Metabolism is a physiological process reflecting the energy expenditure of living organisms. The rate of oxygen consumption is usually considered as an indirect measure of metabolic rate of fish (Kutty 1981). Also, the oxygen consumption is closely related to the metabolic work and the energy flow that the organism can use for the homeostatic control mechanisms (Salvato et al. 2001; Das et al. 2005). In the present investigation, oxygen consumption rates were significantly increased with increasing acclimation temperatures signifying increase in the total aerobic metabolism with rise in temperature as suggested by Kutty and Mohamed (1975). Similar findings were also reported by Das et al. (2004) in Indian major carps. This indicates that oxygen consumption of *T. putitora* is dependent on acclimation temperatures.

Many studies (Kita et al. 1996; Das et al. 2004, 2005; Debnath et al. 2006; Gonzalez et al. 2010) suggest that the point where the Q₁₀ for oxygen consumption starts to decrease with increasing acclimation temperatures also corresponds to the optimal temperature for growth. Thus, the final preferred temperature might be estimated indirectly based on the relationship between oxygen consumption and acclimation temperature (Kita et al. 1996). In the present investigation, although the lowest Q₁₀ value for oxygen consumption of *T. putitora* was observed between 20 and 23 °C but started to decrease at 26 °C which could be considered as the optimum temperature for *T. putitora* juveniles. The observed optimum temperature based on the Q₁₀ value also nearly corresponds to the preferred temperature (20–25 °C) of *T. putitora* in their natural environment. Our results are in agreement with the earlier reports in fish (Das et al. 2004, 2005) where Q₁₀ value significantly decreased at acclimation temperature beyond optimum range of the species studied. We also determined the acclimation response ratio (ARR) of *T. putitora* juveniles as defined by Claussen (1977) as the change in CT_{max} with change in the acclimation temperature.

ARR is considered as a measure to indicate the physiological response of aquatic organisms to a change in the temperature (Díaz et al. 2002; Gonzalez et al. 2010). A lower ARR value signifies for species of temperate climates or colds and higher values for tropical and subtropical species, demonstrating that the values are dependent of the geographic zone where the organisms dwell (Díaz et al. 2002; Re et al. 2006). It has been argued that for the species which dwell in cold and temperate regions experience gradual long-term temperature fluctuations and get sufficient time to make metabolic adjustments resulting in a no substantial shifts in their tolerance ranges, while, on the other hand, subtropical and tropical species which experience their greatest fluctuations of temperature over short periods have broad ranges of tolerance to adjust the comparatively rapid changes in water temperature (Díaz et al. 2002; Re et al. 2006; Gonzalez et al. 2010). In the present investigation, the ARR values were comparatively lower (0.37–0.59) than the tropical species as reported by Gonzalez et al. (2010).

A variable thermal environment can induce thermal stresses to aquatic animals (Brian et al. 2001) and potentially affects the enzyme activity and antioxidant defence system in aquatic organisms (Abele et al. 1998; Pörtner 2002). Higher temperature is reported to increase reactive oxygen species release and enhances the risk of oxidative damage (Abele et al. 1998). Most living systems adapt to oxidative stress by increasing their antioxidant potential which seems to be the most important effective protection against oxidative stress (Hermes-Lima 2004). Increased availability of anti-oxidative enzymes like superoxide dismutase and catalase is believed to minimize oxidative stress (Pörtner 2002). They directly detoxify harmful reactive oxygen species and oxidative damage to cellular components. In the present study, SOD and catalase activities in liver, gill and kidney tissues of *T. putitora* were significantly higher at higher acclimation temperatures which is a clear indication of higher magnitude of oxidative stress in these groups. Our results are in congruent with the findings of Dong et al. (2008) who found elevated activities of catalase and SOD in sea cucumber, *Apostichopus japonicus* exposed to higher temperature. Lushchak and Bagnyukova (2005) also observed that a sharp increase in temperature resulted in oxidative stress in goldfish. The uniform increase in SOD and catalase in all the measured tissues of *T. putitora* juveniles

possibly protected the increased ROS production at higher acclimation temperatures.

ATPase is a membrane-bound enzyme, accountable for the transport of ions through the membrane and instant release of energy (Chatterjee et al. 2010). In the present study, the ATPase activity increased uniformly in all the measured tissues of *T. putitora* with the increase in acclimation temperature. This shows that increased temperature caused ion regulatory impairment which ultimately resulted in increased energetic demand. The increased ATPase activity in the present study might have hydrolysed high-energy phosphate (ATP) resulting energy release and that energy must have been utilized to maintain ionic gradient across the membrane. These results are in agreement with the findings of earlier investigation in Atlantic teleost *Trematomus bernacchii*, acclimated at +4 °C resulted in a doubling of gill ATPase activity relative to −1.5 °C (Morrison et al. 2006). Similarly, cold acclimation also caused an increased ATPase activity in *Cyprinus carpio* and *Rutilus rutilus* (Heap et al. 1985) and in *Barilius bendelisis* (Kapila et al. 2009). ATPase activity of myofibrils from cold adapted fish species was considerably higher at low temperatures than for tropical species (Johnston et al. 1975). Our results are further corroborated by the findings in *Oreochromis mossambicus*, in which confinement stress was shown to increase the intestinal and renal ATPase activity (Nolan et al. 1999). Similarly, Chatterjee et al. (2010) reported a rise in ATPase activity of *Labeo rohita* fry when transported at high stocking density and longer duration.

Acetylcholine is a neurotransmitter found in the nerve synapse, and the enzyme AChE breaks the compound to prepare for new nerve conduction (Chatterjee et al. 2010). Various studies have confirmed that AChE enzyme gets inhibited by various toxicants (da Fonseca et al. 2008; Akhtar et al. 2010). Akhtar et al. (2012b) reported a decrease in AChE activity in *L. rohita* fingerlings exposed to an elevated temperature of 33 °C compared to its counterpart at ambient temperature (26 °C). It has been reported that low temperatures reduce the activity of acetyl cholinesterase (Wood et al. 1999). Kapila et al. (2009) observed increased activity of AChE in *Barilius bendelisis* exposed to low temperature. In agreement with the above studies, the present results also evidenced that AChE activity decreased significantly with the increase in acclimation temperature indicating

an inhibitory effect of higher acclimation temperature on AChE activity.

The measurement of blood glucose level is an effective method to evaluate the effect of different stressors. The elevation of blood glucose levels in fish by both corticosteroids and catecholamines makes it the ideal parameter to study the secondary stress response, on activation of direct sympathetic (Chromaffin tissue) as well as humoral (internal tissue) pathways (Wedemeyer and Mcleay 1981). Ample literature exists on the rise of glucose level on application of various stressors (Das et al. 2005; Akhtar et al. 2012a). In the present study, blood glucose remained significantly unchanged up to the acclimation temperature 26 °C and increased significantly at acclimations temperatures of 29 and 32 °C by 37 and 50 %, respectively, compared to 20 °C indicating stress in these higher temperature reared groups. These observations are in agreement with our previous findings (Akhtar et al. 2012a) where blood glucose levels of *L. rohita* fingerlings were increased at higher rearing temperature (33 °C) compared to control group reared at ambient temperature (20 °C).

Leucocytes (WBC) play an important role for enhancing non-specific or innate immunity, which is considered as an indicator of the health status of fish (Roberts 1978). Generally, at higher temperatures, the WBC counts decrease (Verma et al. 2007). The significant decrease in WBC at the acclimation temperature of 32 °C observed in the present study is in agreement with Verma et al. (2007) who found reduction in WBC in *Cyprinus carpio* advanced fingerlings on exposure to elevated temperatures and Akhtar et al. (2012a) in *L. rohita* fingerlings. However, Alexander et al. (2011) did not observe any significant change in WBC count of *L. rohita* fingerlings reared at 26 and 32 °C. Haemoglobin content was also elevated significantly due to increase in acclimation temperatures and was in congruent to Akhtar et al. (2012a).

Proteins are the most important compounds in the serum, and its concentration is used as a basic index for the health status of fish (Buchanan et al. 1997). Among the serum protein, albumin and globulin are the major proteins, which play a significant role in the immune response (Kumar et al. 2007). Serum globulin consists of several components like α -, β - and γ -globulin. Increase in the total serum protein, albumin and globulin levels is thought to be associated with a stronger innate immune response in fishes

(Wiegertjes et al. 1996). At higher temperatures, albumin and A/G ratio are reported to decrease (Verma et al. 2007; Akhtar et al. 2012a), which is in agreement with our results as decrease in total serum protein, serum albumin, serum globulin and A/G ratio was found at higher acclimation temperature groups.

The present study concludes that long-term changes in temperature lead ectothermal organisms including fish to exhibit acclimatory responses. The increase in CT_{max} and LT_{max} values with increase in acclimation temperature evidenced in the present study may suggest that the thermal tolerance of *T. putitora* is dependent on its prior thermal exposure history. The increased activities of anti-oxidative enzymes observed at higher acclimation temperatures seemed to be an important effective protective mechanism against oxidative stress. Moreover, the results obtained from the present study suggest that *T. putitora* juveniles could adapt to higher acclimation temperatures by altering haemato-biochemical variables and fatty acid composition. This physiological mechanism may facilitate *T. putitora* to respond the threat of increasing temperatures due to global warming.

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