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Effects of elevated water temperature on tolerance and stress in Chocolate mahseer *Neolissochilus hexagonolepis*: implications for habitat restoration and conservation

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The water temperature beyond tolerable limits adversely affects the growth and reproductive competence of teleost fish. The effect of such temperature rise will be more prominent in hill stream fishes like Chocolate mahseer because of their evolution in hilly environments and adaptation to low temperature. Here we examine the thermal tolerance, oxygen consumption and stress response in this fish species acclimatized at three different temperatures (24°C, 27°C and 30°C) for 45 days. The study reveals that CT_{max} and LT_{max} increased significantly ($P < 0.05$) with increasing acclimation temperature. Similarly, oxygen consumption rate at 24°C, 27°C and 30°C was 74.61 ± 2.11, 94.32 ± 2.33 and 122.54 ± 2.01 mg O_2 kg^{-1} h^{-1} respectively. Further, among the three acclimated temperatures tested, fishes reared at 24°C when subjected to thermal tolerance test encountered more stress (glucose level: 11.6 ± 1.14 mmol/l) than other groups (27°C: 9.22 ± 0.22 mmol/l; 30°C: 7.4 ± 0.89 mmol/l). Results suggest that water temperature of 31°C and beyond in natural water bodies of Meghalaya might create physiological stress in Chocolate mahseer, which in the long run may affect its reproductive performance. Therefore, we recommend adoption of proven *in situ* and *ex situ* conservation approaches to safeguard the species.

Keywords: Chocolate mahseer, climate change, thermal stress, teleost.

THE rising global temperature is receiving much attention because of its tremendous potential to disrupt ecosystems[1]. According to the latest reports, 'recent years have been among the warmest since 1860' and 'global mean temperature has increased by between 0.3°C and 0.6°C since the late nineteenth century'[1]. The increase of a few degrees in water temperature can set off ecological changes that would affect most forms of aquatic life. For example, fishes, as poikilotherm, are extremely sensitive to environmental changes and in particular to surrounding temperature. In general, temperature affects virtually all

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biochemical and physiological activities of the fishes. Thus, it should be viewed as an environmental resource, which evokes multiple effects on the fishes[2]. Long-term changes in temperature lead ectothermic animals to display accliamatory responses, which may include enzymatic changes thought to mitigate the effect of temperature on the metabolism[3]. Rising temperature up to a certain limit favours aquaculture by increasing growth rate and reducing the time to attain maturity. On the contrary, temperature beyond optimum limits of a particular species adversely affects the health of aquatic animals by increasing metabolic rates and subsequent oxygen demand. It also assists proliferation, invasiveness and virulence of bacteria and other pathogens that cause a variety of pathophysiological disturbances in the host[4].

Chocolate mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839; Figure 1) is a freshwater fish that inhabits hill streams and rivers of India, Bangladesh, Nepal, Myanmar, Indonesia and China[5]. In India, the species is distributed in water bodies located at 50–2000 m amsl in Arunachal Pradesh, Assam, Bihar, Jharkhand, Meghalaya, Nagaland, Uttar Pradesh and West Bengal[5–8]. It is an omnivorous, opportunistic feeder and feeds on green filamentous algae, aquatic insects, small fish, weeds and mollusks[5]. It is an outstanding game and food fish[5–7]. In Meghalaya, the species is popular as a sport fish and provides recreation to anglers and significantly contributes to the ecotourism[8]. During the last decade, it was observed to be supporting a substantial natural fishery in the major riverine and lake ecosystems of the Meghalaya[8]. However, due to the steadily declining population in natural water bodies of Meghalaya and other habitats of distribution across the country[7,8], recently, the fish has been declared to be Near Threatened (NT) by the IUCN Red List[9]. This may be attributed to various factors like habitat loss, pollution and steadily increasing water temperature in the region. Considering the potential of Chocolate mahseer in aquaculture and allied activities, we studied the temperature tolerance, oxygen consumption and stress response in this fish species acclimatized at three different temperatures (24°C, 27°C and 30°C).

Chocolate mahseer (mean body weight ± SE: 30.51 ± 0.52 g) were caught from Umiam river and brought to the wet laboratory of the Fisheries Division, ICAR Complex for NEH Region, Barapani, Meghalaya, in aerated open containers. The fishes were acclimated for 30 days to wet laboratory conditions prior to the experiment. During this period, fishes were fed with supplementary feed twice daily till satiation.

Figure 1. Chocolate mahseer (*Neolissochilus hexagonolepis*): the near threatened. Scale bar = 2 cm.

Acclimation of fishes (10/tub at each temperature, i.e. 24°C, 27°C and 30°C) was carried out separately in different insulated plastic tubs of 20 l water capacity at a stocking density of 0.7 kg of fish/m^3. Temperature in the experimental tanks was gradually increased at the rate of 1°/day from ambient temperature (17°C) to reach the test acclimation temperatures of 24°C, 27°C and 30°C, and maintained for a period of 45 days prior to the critical temperature experiment.

Acclimated fishes were subjected to constant rate of increase in temperature at the rate 1.0 ± 0.5°C/min until loss of equilibrium (LOE) was reached, which was designated as critical thermal maxima (CT_{max})[10,11]. This technique has been critically evaluated by numerous workers[3,11,12] and is well established as a powerful tool for studying the physiology of stress and adaptation in fishes[13,14]. A similar experimental set-up was used for performing lethal temperature maxima (LT_{max}) tests to know the lethal tolerance limit in relation to acclimation temperatures (24°C, 27°C and 30°C). LT_{max} was determined by observing the cessation of operculum movement[15].

A set of 15 acclimated fishes (5/tub at 24°C, 27°C and 30°C) were randomly chosen for the test. Each fish was individually kept in a sealed glass chamber (5 l capacity) with thick glass lid, cut to cover the top portion completely. An opening in the lid fitted with a gasket to ensure an airtight seal permitted the insertion of a dissolved oxygen probe[11]. The chamber was placed inside the insulated tanks at their respective temperatures for 1 h. Oxygen consumption at the end of the acclimation period (45 days) in different acclimation temperatures was measured using a digital oxymeter 330 (Merck, Germany, sensitivity 0.01 mg O_2 mg l^{-1}).

Five fishes each from thermal stressed and non-stressed (control) group were anesthetized in MS-222 (tricaine methanesulfanate) once they reached CT_{max} and LT_{max}. Blood was collected from each fish by cutting the caudal peduncle. The blood samples were left at room temperature for 1 h and then stored at 4°C overnight. The blood was centrifuged at 3000 rpm over 10 min for the collection of serum. The aliquots of serum were used for glucose analysis.

The serum sample (20 μl) was added to 2000 μl glucose reagent in a test tube. The content was mixed and incubated for 10 min at 37°C. A quantity (20 μl) of glucose standard solution was also mixed with 200 μl of glucose reagent and incubated for 10 min at 37°C. The absorbance of the standard solution and those of the samples were measured against absorbance of the reagent blank in a UV spectrophotometer at 505 nm (ref. 16).

Statistical analysis of CT_{max}, LT_{max}, rate of oxygen consumption and blood glucose estimation was carried out using one-way analysis of variance (ANOVA via SPSS 11.0 for Windows). Duncan's multiple range test was carried out for post hoc mean comparisons ($P < 0.05$).

Temperature adaptation is an essential physiological event in the life of poikilothermic animals and is strongly dependent on acclimation phase and temperature of their surrounding environment[15]. Here we observed that the Chocolate mahseer reared constantly at 24°C and then subjected to thermal tolerance test, encounter more stress (glucose level: 11.6 ± 1.14 mmol/l) and attain CT_{max} and LT_{max} at comparatively lower temperature than their counterparts reared at 27°C and 30°C.

Water quality parameters of experimental tanks are presented in Table 1. We observed that dissolved carbon dioxide concentration increased significantly with increasing water temperature. However, there was no significant difference in pH and other parameters, irrespective of the rearing temperatures between 24°C and 30°C. Generally, bacterial oxidation of ammonia which gets accumulated in water from excreta, uneaten food and high stocking density results in the formation of nitrate[17]. The excess accumulation of nitrate in the culture tank causes stress to the fish. In our study, the nitrate levels were within the permissible limits for fish[18]. Thus, stress to the animals due to such toxicants and overcrowding during the study period was overruled.

CT_{max} and LT_{max} increased significantly with increasing acclimation temperature (Table 2). For example, fishes acclimatized at 24°C attain CT_{max} at 31 ± 0.11°C, whereas fishes from the 30°C group attain CT_{max} at 36 ± 0.22°C. This might be due to the fact that thermal tolerance is largely dependent on prior exposure history or acclimation of the fish. As a result, typical seasonal acclimation allows the fishes to be more tolerant to higher temperature in summer than in winter[19]. Further, young Chocolate mahseer exhibit low CT_{max} values in comparison to several other species of fish such as *Cyprinodon macularis* (CT_{max}: 44.61°C)[20], *Cyprinodon variegates* (45.1°C)[21] and *Cyprinodon artifans* (45.4°C)[22]. This indicates that fishes at a young age are more temperature-tolerant than adults, though bigger fishes are less sensitive to temperature fluctuations[23]. Similar observations were also reported in carp[11,24], *Pangasius pangasius*[25] and *Macrobrachium rosenbegii*[26]. From the present study it can be concluded that there is a strong positive relationship between acclimation temperature and thermal tolerance level in the teleosts.

Metabolism is a physiological process indicating the energy expenditure of living organisms[3]. The rate of metabolism in fishes is usually measured by their rate of oxygen consumption[11,26]. In the present study, oxygen consumption rates were found to increase with increasing temperature (Table 3). Mean oxygen consumption rate at 24°C, 27°C and 30°C was 74.61 ± 2.11, 94.32 ± 2.33, and 122.54 ± 2.01 $mgO_2\ kg^{-1}\ h^{-1}$ respectively. Higher oxygen consumption by Chocolate mahseer at the highest acclimatized temperature suggests that the hill stream fish might spend more energy to cope with the stress caused by elevated water temperature and increased physiological activities. Therefore, if the surrounding water temperature rises in nature, the fish might require more food to fulfil the energy requirement for growth and reproduction[3,27,28].

Generally, under stress condition, body of the fish emits immediate responses recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system and the release of the stress hormones, cortisol and catecholamines (adrenaline and epinephrine) into the blood stream by the endocrine system[29]. Secondary responses occur as a consequence of the released stress hormones[30], causing changes in the blood and tissue chemistry, e.g. an increase of plasma glucose[31,32]. In the present study, we

Table 1. Water quality parameters in experimental tanks

	Acclimation temperature (°C)		
Water quality parameters	24	27	30
pH	7.24 ± 0.6	7.0 ± 0.5	7.6 ± 0.7
Alkalinity ($mg\ l^{-1}$)	45 ± 5.6	35 ± 7.1	35 ± 4.3
Hardness ($mg\ l^{-1}$)	50 ± 9.2	46 ± 8.1	45 ± 8.8
Chloride ($mg\ l^{-1}$)	30 ± 2.1	30 ± 2.5	30 ± 2.2
Free CO_2 ($mg\ l^{-1}$)	25 ± 2.9	28 ± 8.1	32 ± 1.5
Nitrate ($mg\ l^{-1}$)	0.017 ± 0.001	0.011 ± 0.001	0.029 ± 0.002
Phosphate ($mg\ l^{-1}$)	0.101 ± 0.012	0.110 ± 0.011	0.135 ± 0.001

Values are presented as mean ± SE of replication.

Table 2. Critical temperature (CT_{max} and LT_{max}) of Chocolate mahseer acclimated at 24°C, 27°C and 30°C

	Acclimation temperature (°C)		
Parameters	24	27	30
CT_{max}	31^a ± 0.11	33^b ± 0.16	36^c ± 0.22
LT_{max}	36^a ± 0.07	38^b ± 0.04	41^c ± 0.01

Superscripts with different letters (a, b, c) in a row indicate significant differences ($P < 0.05$). Values are expressed as mean ± SE ($n = 5$).

Table 3. Rate of oxygen consumption ($mg\ O_2\ kg^{-1}\ h^{-1}$) with acclimation (A) and without acclimation (B) in Chocolate mahseer at three test temperatures (24°C, 27°C and 30°C)

	Acclimation temperature (°C)		
Treatments	24	27	30
A	74.61^a ± 2.11	94.32^b ± 2.33	122.54^c ± 2.01
B	77.57^a ± 1.91	108.01^b ± 1.72	126.11^c ± 2.91

Superscripts with different letters (a, b, c) in a row indicate significant differences ($P < 0.05$). Values are expressed as mean ± SE ($n = 5$).

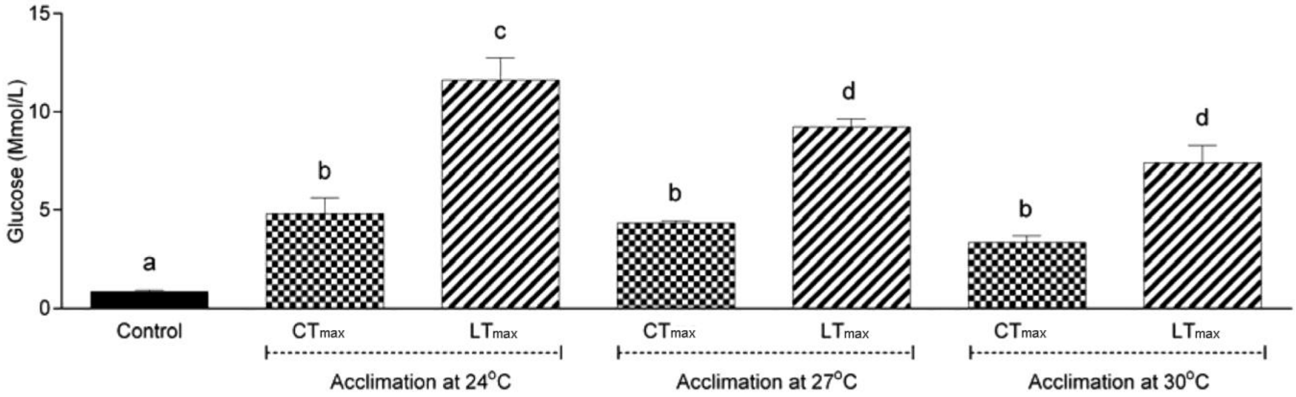

Figure 2. Changes of plasma glucose in Chocolate mahseer during the thermal tolerance test. Columns with different letters (a, b, c, d) vary significantly ($P < 0.05$).

examined the thermal stress response in Chocolate mahseer and observed that among the three acclimated temperature regimes, fishes reared at 24°C when subjected to thermal tolerance test encountered more stress (glucose level: 11.6 ± 1.14 mmol/l) than other groups (27°C: 9.22 ± 0.22 mmol/l; 30°C: 7.4 ± 0.89 mmol/l; see Figure 2). These results suggest that if there is a sudden increase in water temperature in natural water bodies, the fishes might undergo severe physiological stress and this may affect their reproductive performance, as reported in Pejerrey (*Odontesthes bonariensis*)[33]. This may be particularly true in the case of fishes originating from temperate and sub-temperate regions owing to their evolution in hilly environment where low temperature is conducive for optimum growth.

This study reported the relative thermal tolerance of Chocolate mahseer. Although hill stream fishes are believed to be less tolerant to high temperature owing to their evolution in hilly environments, there have been no earlier studies on this high-valued fish species regarding the level of thermal tolerance and adaptability at elevated water temperature. Fish species like bluefin tuna (*Thunnus orientalis*), Alabama shad (*Alosa alabamae*), Siberian sturgeon (*Acipenser baerii*) and Chinese paddlefish (*Psephurus gladius*) that are heading towards extinction due to a combination of stressors like climate change, pollution and habitat loss[34,35], the Chocolate mahseer population should also be protected.

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Implications of fossil valleys and associated epigenetic gorges in parts of Central Himalaya

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Conventionally, epigenetic gorges in tectonically active orogen are attributed to the bedrock geometry and original valley configuration. Because they are invariably associated with fossil valleys containing appreciable sediment succession, it is argued that the older river course was abandoned due to accelerated sedimentation (landslides or widespread fluvial aggradations) as a result of which rivers were forced to occupy the new course (epigenetic gorge). Thus it can be suggested that fossil valleys and gorges are the outcome of the climate–tectonic interaction. The present study is therefore undertaken in the monsoon-dominated and tectonically active inner Lesser Central Himalaya to understand the role of climate and tectonics in their evolution. Preliminary observations in three river valleys indicate that their locations (epigenetic gorges) are structurally controlled (independent of lithology). However, the abandonment of old river course (fossil valleys) was caused due to the accelerated sedimentation (climatically induced). Chronology of the fill sediment indicates that old river course abandonment occurred during the early Holocene climatic optimum (15–9 ka), whereas the incision leading to the epigenetic gorge formation began after 9 ka.

Keywords: Climate, epigenetic gorges, fossil valleys, incision, tectonics.

IN a tectonically active orogen, where rivers are actively incising, fossil valleys and associated epigenetic gorges are common features. The term 'epigenetic' refers to the secondary nature of the bedrock gorges, which occur after the formation of the original gorge and are the result of lateral shifting of the channel by landslide debris, alluvial fans or widespread fluvial aggradation[1–6]. There are few studies from the northwest and Central Himalaya where their formation mechanism climate–tectonic significance has been discussed[4,6,7]. The formation mechanism of epigenetic gorges and associated fossil valleys relies upon the bedrock geometry and original valley configuration. The bedrock geometry affects the location and lateral mobility of an incising channel, whereas original valley shape determines rates of bedrock incision[8]. The rate of bedrock incision associated with an epigenetic gorge can

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