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Thermal tolerance limit and oxygen consumption rates of *Labeo gonius* (Hamilton, 1822) fingerlings acclimated to four different temperatures

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Abstract
An experiment was conducted to evaluate the thermal tolerance limit and oxygen consumption rates of *Labeo gonius* (6.23±0.19 g) fingerlings acclimated to four different temperatures (27, 30, 33 and 36 °C) for a period of 60 days. Critical thermal maxima, CT_{max} (40.97±0.05, 41.89±0.05, 42.69±0.04 and 43.05±0.05) and critical thermal minima, CT_{min} (12.72±0.04, 13.62±0.04, 13.99±0.05 and 14.39±0.021) increased significantly ($p<0.05$) with increasing acclimation temperatures. Similarly, Lethal thermal maxima, LT_{max} (41.63±0.04, 42.41±0.05, 42.99±0.02 and 43.42±0.04) and Lethal thermal minima, LT_{min} (12.24±0.03, 13.25±0.03, 13.68±0.05 and 13.97±0.04) increased significantly ($p<0.05$) with increasing acclimation temperatures. A thermal tolerance polygon over the range of 27 to 36 °C had a calculated area of 256.24 °C^2. Oxygen consumption rate increased significantly ($p<0.05$) with increasing acclimation temperatures. Similarly, oxygen consumption rates also significantly ($p<0.05$) differed without acclimating at different temperatures. Temperature quotient (Q_{10}) was found to be 1.72 for acclimated fish and 2.23 for non-acclimated fish. From this study, it is evident that thermal tolerance limit of *L. gonius* is comparable to Indian major carps, indicating their potential for diversifying freshwater aquaculture.

Keywords: *Labeo gonius*, thermal tolerance, oxygen consumption, aquaculture

Introduction
Temperature is the major factor, which directly influences metabolism affecting all physiological processes in ectotherms [1, 2]. Physiological and behavioural activities of fish are affected due to variations in the water temperature because they cannot maintain their body temperatures differently from their surrounding environment. Higher temperature can alter the physiological functions such as thermal tolerance, growth, metabolism, food consumption, reproduction and ability to maintain internal homeostasis [3]. Water temperature influences oxygen concentration, metabolism, reproduction and growth of fishes [4, 5]. The United States National Research Council proposed that the global mean air temperature may increase by 1.5-4.5 °C in the next half century [6]. Rising temperatures up to certain limit favour aquaculture by reducing the time required to produce marketable sized animals. On the contrary, temperature adversely affects the health of the animal by increasing metabolic rate and subsequent oxygen demand, invasiveness and virulence of bacteria and other pathogens [7]. All teleostean species have developed their own specific adaptive mechanism, both behavioural and physiological, to cope up with temperature fluctuations [8]. These adaptive capabilities enable them to survive through acclimation and adaptation to stressful temperature conditions [9]. Induction of stress by environmental temperature variations determines whether an organism adapts to changed conditions and survives or suffers from physiological disturbances [10]. Knowledge of such temperature changes has relevance for fish in natural water bodies and in aquaculture especially in a scenario of climate change [11, 12]. Therefore, researchers have been making continuous attempts to define the thermal tolerance of various species of aquaculture importance.

In India, freshwater aquaculture is dominated by Indian major carps of Cyprinidae family, which contributes around 85% of the total freshwater production. However, the country is blessed with 15-20 varieties of minor and medium carps that have a high potential for freshwater aquaculture [13]. These carp species can be considered as alternative to the cultivable major carp species, for diversification in freshwater aquaculture. In India, attempts have also been made for diversification of the carp polyculture system through inclusion of new candidate species [14, 15]. *Labeo gonius* is a medium carp [16] of Cyprinidae family, commonly known as 'Kuria labeo' or 'Gonius' and Khursa bata. It is one of the important medium-sized carp species once well distributed in the warm water rivers of South-east Asian countries. In India, it is distributed in Assam, West Bengal, Orissa, Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh and Punjab in the major freshwater rivers, reservoirs, lakes, jheels and tanks [13]. The species is well distributed in North-eastern states of India [17, 18, 19, 20]. The fish has been identified as an important species for diversification of aquaculture practices in India [20].

Thermal tolerance limit of Indian major carps has been well defined by several workers [21, 22, 23]. However, no attempt has been made so far to evaluate the thermal tolerance limit of the *L. gonius* species. Considering the future prospects and growing demands of aquaculture sector, we investigated the thermal tolerance and oxygen consumption rates of *L. gonius* fingerlings acclimated to four different temperatures (27, 30, 33 and 36 °C) for a period of 60 days. Basic idea behind the study was to establish it as an alternative candidate species for freshwater aquaculture in different regions.

Materials and Methods

Site of the experiment

The experimental setup was maintained in the wet laboratory of Aquaculture Division of the ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Mumbai, India. Subsequent laboratory analysis was carried out in Fish Nutrition, Biochemistry and Physiology Division of ICAR-CIFE.

Experimental animal

Animals used for experimental purpose were fingerlings of *L. gonius*. The fish fingerlings (6.0-7.0 g) were procured from Jungalbalahu fish farm, Nagaon District of Assam, India (N 26º 12.520′ and E 92º 28.892'). The fishes were transported in 10L polythene packing with sufficient oxygen (10 fish in each pack) by air. On reaching the wet lab, they were carefully transferred to a circular tank (1000 L capacity) of flat bottom and were left undisturbed for the whole night. In order to ameliorate the handling stress, the fish were given a mild salt treatment the next day followed by antibiotic treatment using oxytetracycline at the rate of 15 mg l^{-1} [24] for three days in the same tank. Water exchange (40-50%) was carried out at every alternate day to maintain conducive water quality in the rearing tank. The stock was acclimatized to the laboratory condition with proper aeration facility before commencing the experiments. During the period, fishes were fed with a control diet containing 35% crude protein.

Acclimation of experimental animals

Fishes of uniform size were acclimated at 27, 30, 33 and 36 °C in 85-L plastic tubs to assess the thermal tolerance limit. Temperature in each treatment was maintained using temperature controller fitted with sensors (General Trading Corporation, Mumbai, India). Fishes were fed twice daily with a control diet and water exchange (25-50%) was carried out on every day to maintain water quality. During the acclimation period dissolved oxygen (DO) concentration was maintained by continuous aeration through centralized air blower. Acclimation of fish was carried out at 1 °C per day over ambient water temperature (27 °C) to reach the test temperatures and maintained for a period of 60 days. The acclimation procedure followed in the present study was based on the earlier investigations carried out on carps [21, 22, 23]. Therefore, we assume that the fish were completely acclimated prior to the tests. The fish were deprived of feed for one day before performing the thermal tolerance study.

Thermal tolerance limit and polygon

Thermal tolerance limit of *L. gonius* fingerlings was assessed after acclimated to four different temperatures (27, 30, 33 and 36 °C) following the critical thermal methodology (CTM) as described previously [22, 23, 25]. CTM was used to estimate thermal tolerance as the mean temperature at which fish exposed to slow, constant changes in water temperature reach a predefined nonlethal (but near lethal) end point [26]. Lethal temperature was determined to know the thermal limit at which mortality occurs. Twelve fish (six for CT_{min} & LT_{min} and six for CT_{max} & LT_{max}, separately) were randomly selected from each acclimated temperature and were shifted to separate thermostatic aquaria (Suan Scientific Instruments & Equipments, Kolkata, India, 52L water capacity, sensitivity ± 0.2 °C) for thermal tolerance study. The temperatures of the water in the thermostatic aquaria were maintained similar to the experimental groups. A 2-HP air blower was used to provide continuous aeration and dissolved oxygen concentration was measured at 5.8±0.5 mg l^{-1} throughout the thermal tolerance study.

Water temperature in the aquarium was increased/ decreased at a constant rate of 0.3 °C/ min, until loss of equilibrium (LOE) was reached, which was designated as the CT_{max} / CT_{min} [27]. The lethal thermal maxima (LT_{max}) / lethal thermal minima (LT_{min}) were determined by further increasing/ decreasing the temperature until the opercula movements were ceased [28, 29]. This technique has been critically evaluated by numerous workers [22, 23] and is well established as a powerful tool for studying the thermal tolerance in fishes [27]. A partial thermal tolerance polygon was generated from the CT_{max} and CT_{min} data by plotting acclimation temperatures (°C) on the X-axis and thermal tolerance limit (°C) on the Y-axis. The area of thermal tolerance was calculated from the polygon and expressed as °C^2.

Oxygen consumption rates and temperature quotients (Q_{10})

Oxygen consumption rates were measured in a static respirometer chamber, using a separate group of fish acclimated to different temperatures (27, 30, 33, and 36 °C), following the method adopted by earlier investigations [22, 23]. It was used to estimate the metabolic activity of the fish. Briefly, six fish per treatment, acclimated to a particular temperature were placed individually into a sealed glass chamber (5L) with 6.4 mm thick glass lid, cut to cover the top portion completely. An opening in the lid fitted with a gasket to ensure an air-tight seal permitted the insertion of a DO probe. The chamber was placed inside the thermostatic aquarium set at the respective test temperatures. All four sides of the aquarium were covered with opaque screens to minimize visual disturbances of the experimental fish. The oxygen consumption experiment was carried out for an hour. The initial and final oxygen contents in the static respirometer

were measured using a digital oxy-meter 330 (sensitivity 0.01 mg l^{-1} O_2, E-Merck, Germany) and the oxygen consumption rates for individual fish were expressed as mg O_2 kg^{-1} h^{-1}.
Similarly another set of 24 fish, maintained at an ambient temperature, was subjected to an increase in water temperature to reach the test temperatures (27, 30, 33 and 36 °C) in thermostatic aquaria separately, so as to delaminate the effect of an acute increase of temperature *vs*. acclimation procedure on oxygen consumption rate. The temperature quotients (Q_{10}) were calculated to assess the effect of acclimation on oxygen consumption rate by using the formula [30]:

$$Q_{10} = (\text{Rate2} / \text{Rate1})^{(10/\text{Temp2}-\text{Temp1})}$$

Statistical analysis

Statistical analyses for the acclimation temperature dependent relationships of CT_{Max}, CT_{Min}, LT_{Max}, LT_{Min} and oxygen consumption rate were performed using one-way analysis of variance (ANOVA) via SPSS 16.0 (SPSS, Chicago, IL, USA). Duncan's multiple range tests were used to compare the differences among treatment means at $p<0.05$. Oxygen consumption values were mass-adjusted considering mass exponent of 0.80 and reported at standard temperature and pressure [31].

Results

Thermal tolerance limit and polygon

Thermal tolerance limit of *L. gonius* fingerlings acclimated to four different temperatures is given in Table 1. Thermal tolerance limit of the fingerlings is widely influenced by the acclimated temperatures. Critical thermal maxima, CT_{max} and critical thermal minima, CT_{min} increased significantly ($p<0.05$) with increasing acclimation temperatures. Similarly, Lethal thermal maxima, LT_{max} and Lethal thermal minima, LT_{min} increased significantly ($p<0.05$) with increasing acclimation temperatures. A thermal tolerance polygon over the range of 27 to 36 °C had a calculated area of 256.24 °C^2 (Fig. 1).

Oxygen consumption rate

The oxygen consumption rates of *L. gonius* fingerlings with and without acclimatization (mg O_2 kg^{-1} h^{-1}) are presented in Table 2. Oxygen consumption rate increased significantly ($p<0.05$) with increasing acclimation temperatures. Oxygen consumption rates also differ significantly ($p<0.05$) without acclimating at different temperatures.

Temperature quotient (Q_{10})

Temperature quotients (Q_{10} values) of *L. gonius* fingerlings acclimated to four different temperatures is presented in Table 3. Temperature quotient (Q_{10}) was found to be 1.72 for acclimated fish and 2.23 for non-acclimated fish over the range of acclimation temperatures (27-36 °C). Final preferred temperature estimated from the Q_{10} value (1.87) was between 27-30 °C and (2.21) 30-33 °C.

Discussion

Thermal tolerance limit and polygon

Thermal tolerance limit of the *L. gonius* fingerlings is widely influenced by the acclimated temperatures. With increasing acclimation temperatures, mean values of CT_{max}, CT_{min}, LT_{max} and LT_{min} of *L. gonius* fingerlings increased significantly ($p<0.05$). It is obvious from the results that the thermal tolerance of fishes is dependent on the thermal exposure history before the experiment, which is regarded as acclimation. The strong relationship between the acclimation temperatures and the thermal tolerance level of *L. gonius* fingerlings supports evidence that temperature adaptation is an essential physiological phenomenon in fishes and is dependent on the acclimation temperature [32]. These results are in agreement with the findings of earlier investigations on Indian major carps (*L. rohita, C. catla and C. mrigala*) advance fingerlings [22], Rohu (*L. rohita*) fry [23] and Rohu (*L. rohita*) and common carp (*Cyprinus carpio*) early fingerlings [21]. Fish have specific behaviour in response to thermal acclimation, beyond which breakdown occurs, leading to stress and production losses due to anorexia, disease outbreak and ultimately death. It was reported that the thermal tolerance study is considered as a useful approach in assessing the impact of climatic change and culture potential of any candidate fish species. From this preliminary study, it is evident that thermal tolerance limit of *L. gonius* is comparable to Indian major carps, indicating their potential for diversifying freshwater aquaculture.
The area of the tolerance zone is a useful index of thermal tolerance [33]. Thermal tolerance polygon of *L. gonius* fingerlings over the range of acclimatization temperatures (27-36 °C) was calculated as 256.24 °C^2. There is no parallel report on CTM test in this species. Indian carps are eurythermal in nature due to their capacity to tolerate wider range of temperature [34]. Data extracted from earlier investigation in Indian major carps (*L. rohita, C. catla, C. mrigala*) fingerlings [21, 22], reveals the similar zone of thermal tolerance polygon. Results of the present findings also indicated that the thermal tolerance zone of *L. gonius* fingerlings is within the range of Indian major carps, which support its tolerance capacity to temperature variations.

Oxygen consumption rates

Oxygen is inevitable in maintaining physiological process of an organism. Rate of oxygen consumption increased significantly with increasing acclimation temperatures ($p<0.05$). Oxygen consumption is an index of metabolism in freshwater fish [35] and is dependent on the acclimation temperatures [36]. In fishes, the metabolic responses that are quantified in terms of oxygen consumption show a linear correlation to temperature due to its direct effect on the kinetics of the enzyme reactions involved [9, 37]. In aquatic ecosystems, oxygen plays a central role in the survival of fishes. Absolute values of oxygen consumption rate in the present study were comparable to earlier investigation in Indian major carps [22], early fingerlings of *L. rohita* and *C. carpio* [21] and *Carassius carassius* and *C. auratus* [38].

Temperature quotient (Q_{10}) value

Temperature quotient (Q_{10}) was found to be 1.72 for acclimated fish and 2.23 for non-acclimated fish over the range of acclimation temperatures (27-36 °C), which indicates that acclimation procedure has played vital role to maintain the homeostasis of *L. gonius* over the test temperatures. Final preferred temperature estimated from the Q_{10} value was between 27-30 °C (1.87) and 30-33 °C (2.21). So, culturing the species <33 °C and >27 °C will be an ideal condition for better growth and maximization of yield. The point where the Q_{10} for oxygen consumption starts to decrease with increasing acclimation temperatures also corresponds to the optimal temperature for growth [29, 39]. Thus the final preferred temperature may be estimated indirectly based on the relationship between oxygen consumption and acclimation temperature (Kita *et al*., 1996). It was reported that the preferred temperature coincides with the optimum temperature for growth [28, 29, 39]. The final preferred temperature for Indian major carps is in the range of 31-33 °C [22] and 29-34 °C in *L. rohita* [40], which is comparable to the *L. gonius* species. They also hypothesized that the point at which Q_{10} diminishes related to acclimation temperatures corresponds to the optimal temperature for growth, because the decrease in Q_{10} indicates that the metabolism of the species has decreased and that more energy for growth is available, similar to that obtained for *L. gonius* exposed to different acclimation temperatures.

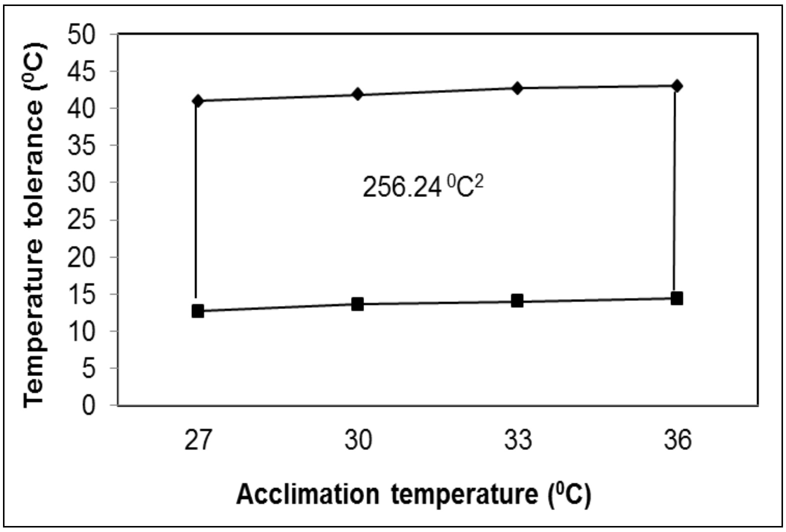

Fig 1: Thermal tolerance polygon of *L. gonius* fingerlings over four acclimation temperatures (27, 30, 33 and 36 °C) using CT_{max} and CT_{min} values.

Table 1: Thermal tolerance limit of *L. gonius* fingerlings acclimated to four different temperatures

Acclimated temperature	Parameters			
	CT_{Max} (°C)	LT_{Max} (°C)	CT_{Min} (°C)	LT_{Min} (°C)
27 °C	40.97^{d}±0.05	41.63^{d}±0.04	12.72^{d}±0.04	12.24^{d}±0.03
30 °C	41.89^{c}±0.05	42.41^{c}±0.05	13.62^{c}±0.04	13.25^{c}±0.03
33 °C	42.69^{b}±0.04	42.99^{b}±0.02	13.99^{b}±0.05	13.68^{b}±0.05
36 °C	43.05^{a}±0.05	43.42^{a}±0.04	14.39^{a}±0.02	13.97^{a}±0.04
Mean ± SE	42.15±0.17	42.61±0.14	13.67±0.13	13.29±0.14

Values in the same column with different superscript differ significantly ($p<0.05$); Data expressed as Mean ± SE, n=6

Table 2: Oxygen consumption rates in acclimated and non-acclimated condition of *L. gonius* fingerlings at four different temperatures

Temperatures	Oxygen consumption rates (mg O_2 kg^{-1} h^{-1})	
	Acclimated group	Non-acclimated group
27 °C	72.78^{d} ±1.42	73.67^{d} ±2.85
30 °C	87.75^{c} ±2.97	93.84^{c} ±2.35
33 °C	111.31^{b} ±2.40	120.30^{b} ±3.47
36 °C	119.37^{a} ±2.46	157.23^{a} ±3.20
Mean ± SE	97.80 ±4.02	111.26±6.69

Values in the same column with different superscript differ significantly ($p<0.05$); Data expressed as Mean ± SE, n=6

Table 3: Temperature quotients (Q_{10} values) of *L. gonius* fingerlings acclimated to four different temperatures

Temperatures	Temperature differences	Q_{10} values
27-30 °C	3 °C	1.87^{b}
30-33 °C	3 °C	2.21^{c}
33-36 °C	3 °C	1.26^{a}
27-36 °C (Acclimated)	9 °C	1.72^{A}
27-36 °C (Non-acclimated	9 °C	2.32^{B}

Different superscript letters in the same column indicate significance difference

Conclusion

Thermal tolerance limit of the *L. gonius* fingerlings are significantly influenced by the acclimation temperatures. Till now there are no studies or reports available on the thermal tolerance limit of the species although they are being considered as a candidate species for aquaculture in India. Therefore, the present findings on the thermal tolerance limits may help to assess the culture potential of this species in various agro-climatic regions. Thermal tolerance study is a useful tool in assessing the impact of climatic change and potential culture of any candidate fish species. From this study, it is evident that thermal tolerance limit of *L. gonius* fingerlings is comparable to Indian major carps, indicating their potential for diversifying aquaculture. However, more research in future is required to be carried out at wide acclimation temperatures to know the complete zone of thermal tolerance, which may strengthen the present findings. These basics information on the thermal tolerance limit of *L. gonius* species will be definitely useful to establish the species on commercial scale. This may also open a direction for exploring more diversified species for freshwater aquaculture.

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