

Note

Assessment of *Osteobrama belangeri* (Pengba), a high-value medium carp endemic to North-east India for thermal tolerance limits

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Abstract

Critical thermal maxima (CT_{max}), lethal temperature maxima (LT_{max}), opercular respiration rate (ORR - movement of operculum per minute), temperature quotient (Q_{10}) and stress markers of *Osteobrama belangeri* (Pengba), a high-value medium carp endemic to hilly ecosystems of North-east India, were examined with the aim of understanding its aquaculture potential and management requirements in the event of a temperature rise. Fish (7.82±0.35 cm/4.64±0.36 g) were acclimatised at 20, 25 and 30°C in insulated tanks (10 fish per tank) for 30 days. They were then exposed to a water temperature increase (@ 1°C day⁻¹) using a locally-fabricated thermostat and assessed for CT_{max} , LT_{max} and ORR. As the acclimation temperature increased from 20 to 30°C, CT_{max} , LT_{max} and ORR of the fish significantly increased. Regression analysis indicated a strong positive correlation between acclimation temperatures and CT_{max} ($y = 0.22x + 33.55$, $R^2 = 0.97$) and LT_{max} ($y = 0.27x + 32.86$, $R^2 = 0.99$). Fish blood samples were collected at the endpoints of CT_{max} and LT_{max} and analysed for serum alkaline phosphatase (ALP) activity. The results indicated that the fish experienced maximum stress at an acclimation temperature of 30°C (ALP: 38.7 U l⁻¹), followed by 25°C (35.6 U l⁻¹) and 20°C (31.7 U l⁻¹). The results suggested that a water temperature increase above 30°C can impair the physiology of *O. belangeri* and consequently, their reproductive competence. Therefore, in such events, proper management and additional care are necessary to protect them from extinction.



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Introduction

Climate-induced perturbations have garnered significant attention in fisheries in recent decades due to their tremendous potential to disrupt ecological balances in aquatic ecosystems and biological processes in fish and other aquatic life (Beniston, 2003). According to a wide range of climate models, our planet's average temperature could increase by 1.1 to 5.4°C by 2100 (Herring, 2021). Such an increase can lead to ecological imbalances. Fish, being cold-blooded animals (poikilothermic), are highly sensitive to temperature changes; temperature alterations virtually affect all their physiological and biochemical activities. In the event of long-term temperature changes, fish develops adaptive responses by modifying their biochemical, enzymatic, immunological and haematological

responses (Sarma *et al.*, 2010). Rising temperatures up to a certain limit benefit fish by augmenting their growth rate and reducing the time required to attain maturity. However, if temperatures exceed the threshold limits, it incites stress by increasing their metabolic rates and subsequent demand for oxygen (Wedemeyer, 1999). This limit varies from fish species to species, depending on their habits and habitats (Chatterjee *et al.*, 2004).

Osteobrama belangeri (Valenciennes, 1844), is a medium-sized carp endemic to the rugged ecosystems of North-east (NE) India. Popularly recognised as "Pengba" in NE India, this species commands high consumer demand and attains a competitive market price ranging from ₹650-800 per kg. Its demand lies in the distinctive flavour and lipo-protein textures it offers. The marketable size of this species ranges from 100 to 500 g.

The migratory pattern of *O. belangeri* involves a journey from the Chindwin River in Myanmar to the upstream regions of the Imphal River in Manipur, specifically for breeding in floodplains during the early monsoon season. This herbivorous fish primarily feeds on algae, soft aquatic plants, zooplankton, diatoms, among other sources. In the past, *O. belangeri* was abundant in Loktak Lake and neighbouring areas of Manipur. However, over the recent decade, a drastic decline in its natural population has occurred due to dam construction, habitat degradation and invasive species (Vishwanath and Shantakumar, 2007). Consequently, it has been categorised as “near threatened” in the IUCN Red List of Threatened Species (Vishwanath, 2010). To amplify mass-scale public awareness and conservation efforts, this fish was appointed as the ‘State fish’ of Manipur, aiming at aquaculture diversification and species conservation.

Recognising the significance of *O. belangeri* in regional aquaculture, a pioneering initiative was launched in 2011 by ICAR Research Complex for NEH Region, Meghalaya to introduce the fish to Meghalaya. This endeavour proved successful when the fish were effectively induced to breed in captivity in July 2014, thriving under mid-hill conditions at an elevation of 900 m above mean sea level (MSL), aimed at diversifying hill aquaculture (Das and Singh, 2017). Numerous comprehensive studies have been conducted concerning various aspects of this species. These studies encompass induced breeding methodologies utilising carp pituitary extract (Reddy, 2000), Ovaprim™ (Singh and Basudha, 2007), Ovatide™ (Devi et al., 2009), Wova-FH (Behera et al., 2010) and Gonopro FH (Das and Singh, 2017). Additionally, investigations have delved into its dietary preferences and feeding habits (Basudha and Vishwanath, 1999; Ramesh et al., 2014), morphological and molecular characterisations (Maisnam et al., 2017), polyculture potentials with other fish species (Yengkokpam et al., 2019; Das et al., 2020) and nutritional considerations (Basudha and Vishwanath, 1993; Surjobala et al., 2020). Surprisingly, despite the extensive research, no prior attempts have been made to evaluate the thermal tolerance limits of *O. belangeri* across different aquatic ecosystems. This remains an unexplored avenue deserving further investigation.

The exploration of thermal tolerance is a crucial approach in understanding physiological stress and adaptation in fishes (Beitinger and McCauley, 1990). Numerous researchers have critically evaluated this technique (Chatterjee et al., 2004; Das et al., 2004; Manush et al., 2004; Debnath et al., 2006; Sarma et al., 2010; Majhi and Das, 2013; Majhi et al., 2013; Das and Majhi, 2014), establishing that thermal adaptation is a fundamental physiological process in the life of fish and is highly dependent on the acclimation phase. The growth, survival and overall well-being of fish are contingent on the temperature of their environment (Debnath et al., 2006). When the environmental temperature surpasses their tolerance limits, it causes physiological distress, making it imperative to understand the tolerance limits of fish. These tolerance limits vary from one fish species to another.

O. belangeri flourishes in hill aquatic ecosystems and it is believed that hill-origin fishes exhibit lower tolerance to high temperatures due to their evolution in colder climates. Their immunity and disease resistance capacity might be suppressed in hills due to prolonged cold climates. The effects of climate variability on the productivity and reproductive competence of fish are particularly pronounced in hill ecosystems. Given the lack of information regarding the temperature tolerance limits of *O. belangeri*, a fish of growing importance in hill aquaculture in NE India, the present study was conducted with the aim of assessing the thermal tolerance and respiration rates of fish acclimatised to varying water temperatures.

The experimental specimens *O. belangeri*, measuring 7.82 ± 0.35 cm in length and 4.64 ± 0.36 g in weight, were sourced from the ponds of the institute farm at ICAR Research Complex for the North-Eastern Hill (NEH) Region, located in Meghalaya. A preparatory acclimation phase of 7 days was instituted under controlled wet laboratory conditions prior to the commencement of the experiment. During this acclimation period, the fish were provided with pellet feed *ad libitum* twice daily. Acclimation of the fish was conducted at three distinct water temperatures: 20, 25 and 30°C. This acclimation was executed within insulated thermocol boxes, each with a capacity of 40 l, at a stocking density of 10 fish per tank. The water temperature was gradually elevated at a rate of 1°C per day from the ambient temperature of 17°C to attain the targeted acclimation temperatures. These temperatures were then sustained for a period of 30 days before assessing their critical temperatures. Dissolved oxygen concentration was meticulously maintained at ≥ 5 mg l⁻¹ through continuous aeration. The fish were starved for a duration of 24 h prior to undergoing critical thermal maxima (CT_{max}) and lethal thermal maxima (LT_{max}) tests.

During the CT_{max} test, acclimated fish were subjected to a constant rate of water temperature increase at 1°C min⁻¹ until they reached a state of loss of equilibrium, which signified the critical thermal maxima (CT_{max}) (Beitinger et al., 2000). Following the test, the fish were carefully transferred back to their respective acclimation temperatures and all of them successfully recuperated to their normal state of being. The behavioural responses of the fish during the critical thermal test were diligently recorded. A comparable experimental set up was instituted to evaluate the lethal temperature maxima (LT_{max}) of the fish, gauged by monitoring the cessation of operculum movement (Kita et al., 1996). For the assessment of alkaline phosphatase (ALP) activities at CT_{max} and LT_{max}, a total of 10 fish were selected. Five fishes were subjected to thermal stress, while the other five were maintained as a control group with no thermal stress applied. All the fish were anaesthetised using MS 222 (Tricaine methane sulfonate) to facilitate the collection of blood samples *via* caudal peduncle puncture. The samples were left at room temperature for an hour and then stored overnight at 4°C. The samples were centrifuged at 3000 rpm for 10 min and serum was separated and analysed for alkaline phosphatase activity (Kind and King, 1954).

The opercular respiration rate (ORR) of fish for each unit of temperature increase was noted by observing their operculum movement per minute and then temperature quotient (Q₁₀) was calculated using the formula: $Q_{10} = (K_2 / K_1)^{10 / (T_2 - T_1)}$, where, K₁ and K₂ are respiration rates estimated based on the operculum movement per minute and T₁ and T₂ are the changes in water temperature (°C).

All statistical analyses were conducted employing one-way analysis of variance (ANOVA) through SPSS version 21.0 for Windows. Subsequently, Duncan's multiple range test (DMRT) was executed to facilitate *post hoc* mean comparisons, maintaining a significance level of $p \leq 0.05$.

Each fish species possesses a distinct thermal preference limit, surpassing which induces thermal stress and disrupts their usual functions crucial for growth and overall well being (Beitinger et al., 2000). Fish exhibit specific behaviours in response to thermal acclimation and when this acclimation is exceeded, it leads to breakdown, causing stress and resulting in production penalties such as anorexia, disease outbreaks and ultimately, mortality. The objective of the current study was to evaluate CT_{max}, LT_{max}, ORR, Q₁₀ and enzymatic stress in *O. belangeri*, aiming to ascertain its potential as a candidate species for diversifying mid-hill aquaculture in the region. The findings revealed that

fish reared at 20°C and subsequently subjected to the thermal tolerance test experienced more thermal stress (ALP activity: 14.9 U l⁻¹). Moreover, they reached CT_{max} and LT_{max} at comparatively lower temperatures than their counterparts reared at 25°C (ALP activity: 14.6 U l⁻¹) and 30°C (ALP activity: 13.8 U l⁻¹). This highlights that thermal adaptation is a crucial physiological process in the life of poikilothermic animals, significantly influenced by the acclimation phase and the temperature of their habitat (Kita *et al.*, 1996).

The results concerning CT_{max} and LT_{max} are graphically depicted in Fig 1, illustrating a clear trend. The mean CT_{max} values (38.06±0.06°C, 38.83±0.06°C and 40.26±0.08°C) demonstrated an upward trajectory with the escalation of acclimation temperatures (20, 25 and 30°C). Likewise, the mean LT_{max} values (38.30±0.15°C, 39.73±0.06°C and 41.03±0.08°C) increased in correspondence to the rise in acclimation temperatures. The regression analysis underscored a robust positive correlation between CT_{max} ($y=0.22x+33.55, R^2=0.97$) and LT_{max} ($y=0.27x+32.86, R^2=0.99$) values of the fish and the respective acclimation temperatures. This dependency on acclimation and life history is in line with the fact that thermal tolerance is substantially influenced by these factors. Seasonal acclimation enables fishes to exhibit higher tolerance to elevated temperatures during summer compared to winter (Bevelhimer and Bennett, 2000). A prior study by Das and Majhi (2014) showcased that elevated water temperatures effectively reduce stress and enhance somatic growth in *Channa stewartii*. These outcomes align with earlier research in a similar vein, encompassing chocolate mahseer (Majhi and Das, 2013), Indian major carps (Das *et al.*, 2004; Chatterjee *et al.*, 2004), *Pangasius pangasius* (Debnath *et al.*, 2006) and *Macrobrachium rosenbergii* (Manush *et al.*, 2004). Moreover, in this experiment, *O. belangeri*, at its current size, displayed a lower CT_{max} compared to other species such as *Cyprinodon macularis* (CT_{max}: 44.6°C), *Cyprinodon variegatus* (45.1°C) and *Cyprinodon artifans* (45.4°C) (Heath *et al.*, 1993; Bennett and Beiting, 1997). This implies that young fish exhibit greater thermo-tolerance than their adult counterparts, although larger fish are more susceptible to temperature fluctuations (Rodnick *et al.*, 2004).

The ORRs of fish are visually depicted in Fig. 2, demonstrating a notable increase as the temperature escalates (Fig. 1). Specifically, the mean ORR at 20, 25 and 30°C stood at 116.7, 113.4 and 132.2 operculum movements per minute, respectively. Oxygen consumption serves as a crucial indicator of metabolism in freshwater fish (Kutty and Mohamed, 1975) and is intrinsically linked to acclimation temperatures (Kita *et al.*, 1996). The elevation in ORR with increasing temperature indicates a decline in dissolved oxygen levels in the water, compelling the fish

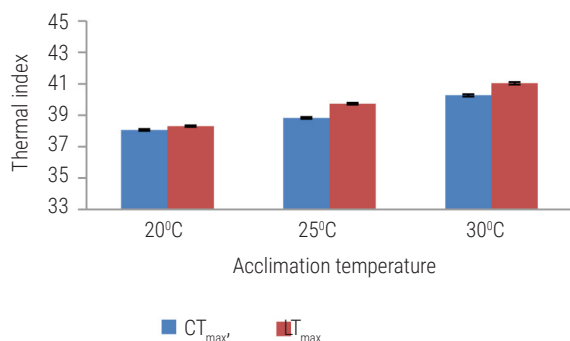


Fig. 1. Thermal tolerance (CT_{max} and LT_{max}) of *O. belangeri* under different acclimation temperatures (°C)

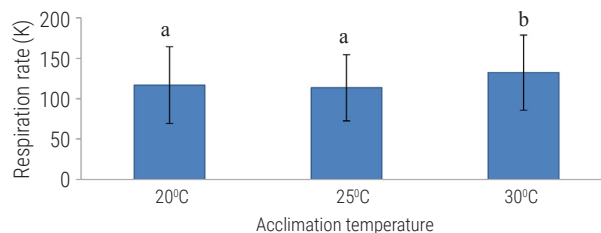


Fig 2. Opercular respiration rate (ORR) in *O. belangeri* during the thermal tolerance test. Columns with different letters (a, b) indicate significant difference (p ≤ 0.05)

to exert more energy in response to the strain induced by heightened water temperature and increased physiological demands (Majhi and Das, 2013). Studies by Sarma *et al.* (2010) and Kutty and Mohamed (1975) affirm that in natural settings, as water temperature rises, fish necessitate more food to meet the energy requirements for growth and reproduction. The recorded respiration rates in this study closely align with prior research on *C. punctatus* by Tantarapale *et al.* (2012). Tantarapale *et al.* (2012) also noted that operculum beats are contingent upon the size of the fish and thermal stress, generally observing higher operculum beats in small fish compared to the respiration rates of larger fish.

The temperature quotient (Q10) for *O. belangeri*, calculated at different acclimation temperatures, is visually represented in Fig. 3. Within an acclimation temperature range of 20 to 25°C, the Q10 was computed to be 0.95, while in the range of 25 to 30°C, the Q10 was determined to be 1.35. This outcome underscores the significant role of the acclimation phase in maintaining the fish's homeostasis across varying test temperatures. Comparatively, in a study by Das *et al.* (2020) on *Labeo gonius*, another endemic medium carp gaining importance in hill aquaculture in NE India, the reported Q10 value was 1.72. This value was derived under acclimation conditions ranging from 27 to 36°C, suggesting differential temperature sensitivities and responses between these closely related species.

Fish demonstrate a spectrum of responses when faced with stressors. The initial response involves the central nervous system perceiving a departure from the norm (Randall *et al.*, 1992), followed by secondary responses that encompass the release of stress hormones like cortisol and catecholamines into the bloodstream by the endocrine system (Barton and Iwama, 1991). These hormonal secretions lead to shifts in blood and tissue chemistry, including an upswing in blood enzymatic activities (Barton and Iwama, 1991; Begg and Pankhurst, 2004), coupled with alterations in behaviour.

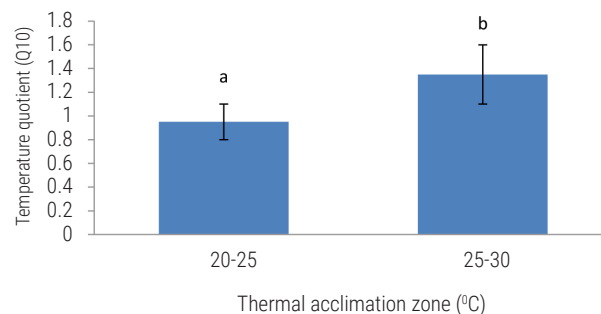


Fig 3: Temperature quotient of *O. belangeri* under different acclimation zones. Columns bearing different letters indicate significant difference (p ≤ 0.05).

In this experimental context, fish acclimated to 30°C exhibited behavioural responses characterised by disorganised swimming, heightened agitation and concerted efforts to escape when the water temperature surged to 40°C. Conversely, fish acclimated to 20°C displayed rigidity of pectoral fins, lethargic movements, and settling at the tank's bottom when the water temperature exceeded 35°C. Fish acclimated to 25°C showcased commonplace behavioural shifts, including suspension with head elevated and tail lowered, spiraling movements, cloudiness of eyes, gasping, sporadic movements, open-mouthed stance and contorted body, as the temperature surpassed 38°C.

The assessment of ALP activity, a pivotal indicator of heat stress in fish (Islam et al., 2020), was conducted in this study under varying acclimation temperatures and the results are depicted in Fig. 4. Among the subjected acclimation temperatures, fish reared at 30°C and subjected to the thermal tolerance test encountered heightened stress levels (ALP activity: 50.8 U l⁻¹ at LT_{max} and 38.7 U l⁻¹ at CT_{max}) in comparison to the other groups (25°C: 49.2 U l⁻¹ at LT_{max} and 35.6 U l⁻¹ at CT_{max}; 20°C: 45.5 U l⁻¹ at LT_{max} and 31.7 U l⁻¹). These findings signify that an abrupt surge in water temperature in natural aquatic habitats could induce profound physiological stress in fishes, potentially impacting their reproductive prowess (e. g., *Odontesthes bonariensis*) (Ito et al., 2008). This phenomenon holds particular significance for fish species originating from temperate and sub-temperate regions due to their evolutionary history in hilly terrains where lower temperatures are conducive to optimal growth.

The water quality parameters for the experimental tanks are outlined in Table 1, revealing a significant elevation in carbon dioxide content with escalating acclimation temperatures. However, no noteworthy differences were observed in the levels of pH and other water quality

Table 1. Water quality parameters (mean ±SD) in the experimental tanks

Water parameters	Acclimation temperature (°C)		
	20	25	30
Dissolved oxygen (ppm)	4.5±0.2	4.2±0.3	3.5±0.2
pH	7.24±0.5	7.2±0.6	7.3±0.6
Total alkalinity (ppm)	45±6.6	46±7.5	45±6.5
Hardness (ppm)	55±6.6	52±7.2	56±8.5
Chloride (ppm)	32±4.2	30±4.5	32±3.5
Carbon dioxide (ppm)	12.2±2.5	15.4±3.2	17.5±2.6
Nitrate (ppm)	0.02±0.01	0.03±0.01	0.03±0.01
Phosphate (ppm)	0.11±0.02	0.12±0.01	0.13±0.02

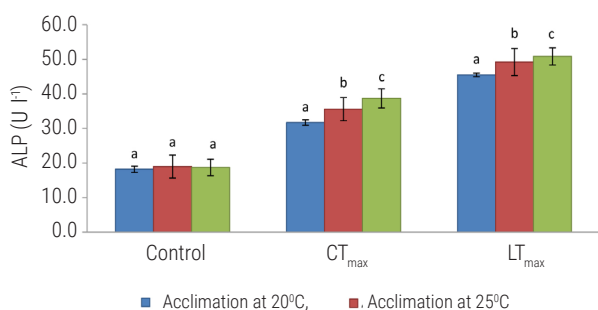


Fig. 4. ALP activity (U l⁻¹) in *O. belangeri* under different acclimation temperatures. Columns with different letters (a, b, c) indicate significant difference (p≤0.05).

parameters. It is essential to note that excessive nitrate accumulation in water can induce stress in fish. Ammonia, stemming from fish excretions, leftover food and deceased fish, accumulates in the water and transforms into nitrate through bacterial oxidation (Merkens and Downing, 1957). In our investigation, nitrate levels remained within permissible limits for fish, aligning with earlier findings by Majhi and Das (2013). Consequently, stress on the fish attributed to such toxicants during the study period was negligible.

In this comprehensive exploration of *O. belangeri*'s thermal tolerance and physiological responses, crucial insights have been gained, illustrating the influence of acclimation temperature on various parameters. The positive correlation between CT_{max} and LT_{max} with acclimation temperatures underscores the vital role of acclimation phases in shaping thermal tolerance. Concurrently, the rise in ORR signifies an augmented energy demand to cope with elevated water temperatures. Behavioural responses observed during thermal stress reveal distinct patterns, providing significant understanding of the coping mechanisms of *O. belangeri*. The assessment of ALP activity, a crucial heat stress indicator, underlines the physiological stress experienced by the fish, particularly those acclimated to higher temperatures. These findings bear vital implications for aquaculture and conservation efforts. Effective temperature management in aquaculture systems is imperative to maintain optimal conditions for growth and reduce stress. Additionally, in the face of climate change, proactive conservation strategies are crucial to safeguard the natural habitats of *O. belangeri* and related species. Further research should delve into the long term effects of thermal stress on reproductive performance and the overall ecological impact of thermal changes in aquatic ecosystems. Overall, understanding the thermal responses of *O. belangeri* is essential for sustainable aquaculture practices and effective conservation measures, crucial for the preservation of this species and the aquatic ecosystems it inhabits.

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