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Short Communication

Effect of temperature on body weight, metabolism and thermoregulation of air breathing catfish *Heteropneustes fossilis*

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Heteropneustes fossilis commonly known as singhi, air breathing catfish, is a potential candidate species for aquaculture in the climate change scenario where abiotic stressors are likely to play a major role in determining the quality of water and fish habitat. *H. fossilis* possesses an accessory respiratory organ for direct aerial respiration by surfacing the water and gulping atmospheric air which is stored in the respiratory organ. This unique character makes the fish culturable in derelict water, swamps, wetlands and backwaters of reservoirs which are low or deficient in dissolved oxygen (Nayak *et al*., 2000). Temperature is generally considered as amajorabiotic factor influencing behavior, physiology and distribution of aquatic ectotherms (Brett, 1971). Determining thermal limits of fish in aquatic environments and their responses in terms of acclimation and adaptation is of vital importance for management of water quality for aquaculture production. Critical thermal methodology (CTM) (Beitinger and Bennett, 2000) estimates thermal tolerance by exposing fish to a constant rate of increasing water temperature that is slow but fast enough to prevent thermal acclimation during the trail until a predefined, repeatable upper endpoint is reached that is near lethal but nonlethal. CTM assesses interaction of thermal stress and other stressors in the environment. The fish respiration method, oxygen consumption rate (OCR) is correlated with change in environmental conditions because of its relation to metabolism and energy flow necessary to maintain homeostasis (Salvato *et al*., 2001).In mustard, Islam *et al*., 2019, observed that thermal unit indices influence attainment of different phenophases and growth indicators suggesting its usage in growth prediction in mustard varieties. In larger animals, the habitat temperature plays an important role in determining occurrence of respiratory illness and diarrhoea as shown in jersey crossbred calves reared in indoors enclosures versus rearing in semi covered houses with provision of open spaces (Rai *et al.,* 2020). Present study was carried out to evaluate the effects of acclimation temperatures on thermal tolerance, oxygen consumption and growth of *Heteropneustes fossilis*.

Heteropneustes fossilis were collected from Bhima river at Ujani reservoir and transported to the experimental site in portable FRP tanks at 28°C and acclimated for 30 days to recover from transportation stress. The fish of length varying between 8.53-8.58 cm were equally distributed between four treatments (28, 30, 32 and 34°C) with each replicated three times following a completely randomized design, with a stocking density of 12 fish/50L water. Rearing conditions except water temperatures were kept uniform in all four experimental groups. The water temperatures were maintained at 28°C initially and gradually increased by 1°C per day to 30, 32 and 34°C and maintained for 40 days. Fish were fed for 40 days growth study, photoperiod of 12 hrs light and 12 hrs dark was maintained with light exposure from 600 hrs to 1800 hrs. Dissolved oxygen level was maintained by aeration in all experiments at 5 mg L^{-1}. Fish were fed with pelleted feed containing 35% crude protein, twice a day (8 and 20 h) at 10%of body weight, which was determined periodically at ten-day interval up to 40 days. Feed waste and fish excreta were removed daily before feeding. Fifty percent water was exchanged with fresh water every day. Fish were starved for a day prior to the assessment of growth, thermal tolerance and oxygen consumption. Growth rate of fish was measured as percentage weight gain. Due to air breathing behavior of *H. fossilis*, rate of oxygen consumption was measured from water at 28, 30, 32 and 34°C. To determine the oxygen consumption rates three replicates of six fish from each acclimation temperature were kept individually in sealed five-liter glass chambers. The glass chamber was made airtight after insertion of dissolved oxygen probe. The chamber was placed inside the aquaria at acclimation temperatures and the water was continuously circulated. The aquaria were covered with opaque screen to reduce stress due to visual treatment. The initial and final oxygen content was measured usingEutechcyberscan600. The temperature quotient (Q_{10}) was measured to analyze the effect of acclimation temperature on oxygen consumption rate (Kita *et al.,* 1996). Six fish from each experimental temperature were randomly selected to determine thermal tolerance CTmax and CTmin. Fish were exposed to a constant increase or decrease in temperature (0.3°C/min) till loss of equilibrium (LOE) of singhi was observed (Beitinger and Bennett,2000). The fish recovered the critical thermal method (CTM) temperatures completely. Thermal tolerance scope at each acclimation temperature was calculated by subtracting CTmax from CTmin. ANOVA was performed using the mean values of all parameters (SPSS, version 16.0). Duncan's multiple range test (DMRT) was carried out for post hoc mean comparisons. Regression analysis was carried out to know the relationship between acclimation temperatures with growth, CTmax, CTmin and oxygen consumption.

Growth of *H. fossilis* reared at four acclimation temperatures is presented in Fig.1. Highest body weight gain, 15.72±0.15% was observed at acclimation temperature of 30°C, which was significantly higher than 8.51±0.27, 5.54±0.07, and 4.69±0.07% at 28, 32 and 34°Ctemperatures respectively. Q_{10} values were estimated and extrapolated as 0.83 (between 28 and 30°C), 2.40 (between 30 and 32°C) and 0.73 (between 32 and 34°C) (Fig.2). Preferred temperature was estimated to be in between 30-32°C using the point at which the Q_{10} value starts to decrease with increase in acclimation temperature (Kita *et al*., 1996). CTMax and CTMin increased significantly ($p<0.05$) with increasing acclimation temperatures (Fig.2). At heating and cooling rate of0.3°C min^{-1}, CTMax ranged from 40.51±0.01 to 43.12±0.03 and CTMin ranged from 13.63±0.05 to 15.85±0.05for28-34°C acclimation temperatures. The temperature tolerance scope at 28, 30, 32 and 34°C acclimation was 26.88°C, 27.1°C, 28.24°C and 27.27°C, respectively with maximum at 32°C acclimation temperature. Both, CTmax and CTmin regression analysis showed a positive relationship to acclimation temperature (CTmax=27.25+0.47*Acclimation temperature, P=0.001, r^2=0.93 and CTMin= 3.48+0.36*Acclimation temperature, P= 0.001, r^2=0.94). Thermal tolerance polygon of *H. fossilis* was 164.24°C^2 at 28-34°C acclimation temperatures used in the experiment. The oxygen consumption rate increased from150.10±0.20at 28°C to 191.81±0.86mg $O_2kg^{-1}h^{-1}$ at 34°C showing a significant rise in requirement of oxygen at elevated temperatures (Fig.2). The acclimation temperature and oxygen consumption regression model established was oxygen consumption (OCR) = 133.13+15.19*Acclimation temperature, P= 0.001, r^2= 0.93.

Debnath *et al*., (2006) estimated thermal tolerance of yellowtail air breathing catfish *Pangasiuspangasius* at 30, 34 and 38°C acclimation temperatures and reported CTmax of 42.68, 43.67 and 44.05°C and CTmin of 12.37, 14.48 and 17.22°C respectively. Sarma *et al*., (2010) analyzed the effect of acclimation temperature on an air breathing fish *Anabas testudineus*. The CTmax reported was 40.15, 41.40 and 41.88°C while CTmin was 12.43, 13.06 and 13.94°Cat acclimation 25, 30 and 35°C temperature. The CTmax, CTmin and oxygen consumption increased significantly with increase in acclimation temperatures. Vasal and Sundarraj, (1978) determined the maximal and minimal lethal temperatures and thermal preference of *H. fossilis* during summer and winter. Lethal temperature minimum and maximum during summer and winter were observed to be 7.9 and 39.8°C and 4°C and 37.7°C respectively. Summer acclimation at 28°C resulted in the fish selecting a temperature range of 31.3 to 32°C, similarly winter acclimation at 16°C resulted in fish selecting a temperature range of 15 to 35°C. In the present study, *H. fossilis* thermal tolerance increased with risein acclimation temperatures showing a direct relation between acclimation temperatures and thermal tolerance. *H. fossilis* CTmax of 41.25°C at 30°C and 43.12°C at 34°C of acclimation temperature exhibited similarity to CTmax of 41.40°C at 30°C acclimation temperature of *A. testudineus* and CTmax of 43.67°C at 34°C acclimation temperature of *P. pangasius*, respectively. CTmin data suggests decrease in tolerance to cold as observed by 2.22°C decrease in lower temperature tolerance from 28 to 34°C acclimation. Oxygen consumption rates of *H. fossilis* increased from 150.10 to 191.81mg $O_2 kg^{-1} h^{-1}$ at 28-34°C acclimation temperatures exhibiting a strong relationship of increase in metabolic activity with increasing acclimation temperatures suggesting an increase in total aerobic metabolism with rise in temperature. The thermal tolerance polygons provide information on tolerance end points, serve as an index of eurythermicity between species, define intrinsic thermal tolerance zones independent of historical thermal exposure, upper and lower temperature tolerance zones gained due to life history exposure to temperatures (Beitinger and Bennett, 2000).The thermal tolerance polygon of *H. fossilis* at acclimation temperatures of 28-34°C, in a acclimation temperature range of 6°C was 164.24°C^2exhibiting narrow polygon to 278.30°C^2 of *A. testudineus* at acclimation temperatures of 25-35°C, 231°C^2 of *P. pangasius* at acclimation temperatures of 30-38°C, the Q_{10} value decreased at 30-32°C acclimation temperatures suggesting 30°C as preferred temperature.

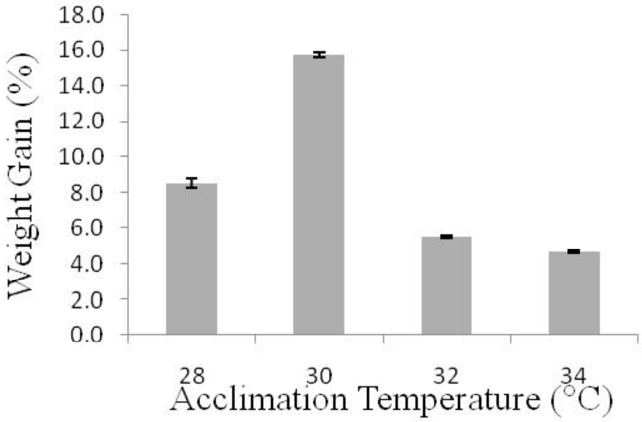

Fig. 1: Weight gain (%) of *Heteropneustes fossilis* acclimated at 28, 30, 32, 34°C

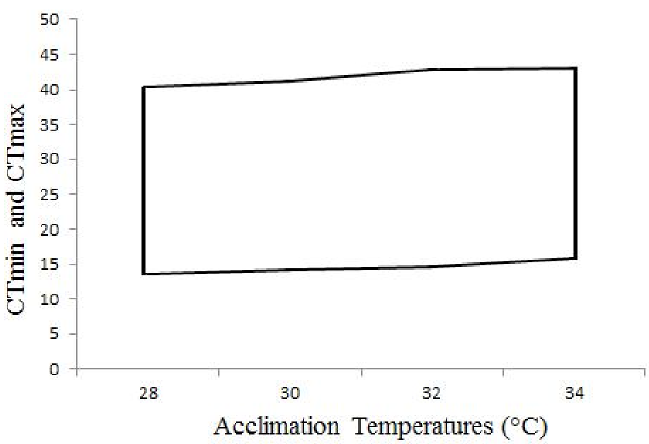

Fig. 2: Thermal tolerance polygon based on CTmax and CTmin of *Heteropneustes fossilis* acclimated 28, 30, 32, 34°C. The area of thermal tolerance polygon was calculated as 164.24°C^2

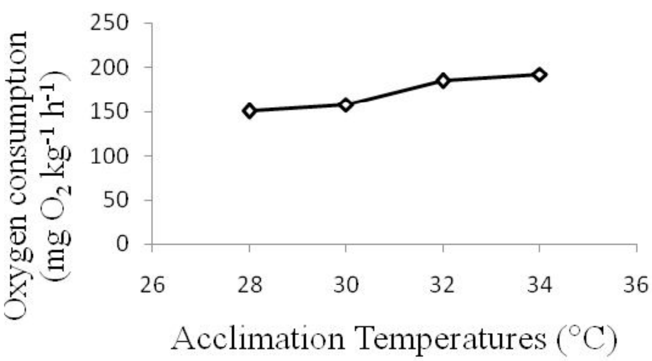

Fig. 3: Oxygen consumption (mg O_2 kg^{-1} h^{-1}) of *Heteropneustes fossilis* acclimated 28, 30, 32, 34°C

Thermal tolerance scope of catfish *P. pangasius* acclimated at 30, 34 and 38°C were 30.31, 29.19, 26.83°C, *A. testudineus* acclimated at 25, 30 and 35°C was 27.72, 28.34, 27.94°C, and *H. fossilis* acclimated at 28, 30, 32 and 34°C exhibited a thermal tolerance scope of 26.88, 27.10, 28.24 and 27.27°C. Thermal tolerance scope of *H. fossilis* was 3.21 and 1.66°C lower than *P. pangasius* at acclimation temperature of 30 and 34°C respectively and 1.24°C lower than *A. testudineus* at acclimation of 30°C.Thus, *P. pangasius*, *A. testudineus* and *H. fossilis* exhibit decrease in temperature stress tolerance window by 3.48, 0.62 and 0.39°C suggesting species specific variability in temperature tolerance window from broad to narrow.

In conclusion, this study suggests that 30°C is optimum temperature for growth of *H.fossilis*. Acclimation temperature of 30°C exhibited highest growth indicated by the highest body weight gain in comparison to the acclimation temperatures of 28, 32 and 34°C. The Q_{10} values suggests that the preferred temperature for optimum growth ranges between 30-32°C. This result suggests that water temperature affects the growth rates and knowledge of the optimum temperature of 30°C for faster growth and is essential for implementing suitable management for rearing and growth of *H. fossilis*. Thermal experiments on *H. fossilis* suggested that increase in acclimation temperature increases the thermal tolerance but decreases cold tolerance. The habitat temperature determines the rate of metabolism as observed in *H. fossilis* exhibiting increase in oxygen consumption with rise in acclimation temperatures. The thermal tolerance scope remains relatively constant over an acclimation temperature range of 6°C from 28 to 34°C.Preferred temperature of *H. fossils* lies between 30-32°C suggesting that water temperatures in aquaculture ponds in Western Maharashtra region are conducive for its culture. The air breathing behavior and thermal tolerance capacity of *H. fossilis* makes it a potential candidate for aquaculture. Further research on genetic responses to increasing temperature is essential in *H. fossils* and other air breathing catfish.

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