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Effect of Water Temperature on the Physiological Responses of Asian Catfish *Clarias batrachus* (Linnaeus 1758)

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Abstract

Temperature plays significant roles in the life of poikilothermic aquatic animal fish. The physiological responses of *Clarias batrachus* (Linnaeus 1758), magur (80.60±5.34 g) at different temperatures were observed. Fish were acclimated at 25 °C for 21 days and were exposed at 10, 15, 20, 25, 30 and 35 °C temperatures. Blood and muscle samples were analysed after 12 h and 7 days of reaching the assigned temperatures. Significantly ($P<0.05$) higher mortality of fish was found in 10 °C treatment compared to the other groups. At 10 °C, 50% fish died within 5 days. Serum protein was significantly ($P<0.05$) higher in fish exposed at 15 °C compared to the other treatments. Myeloperoxidase and reduced glutathione were significantly ($P<0.05$) higher in fish exposed at 30 °C and 25 °C, respectively compared to the other groups. Nitric oxide synthase was significantly ($P<0.05$) higher in fish maintained at 25 °C and 30 °C compared to the other treatments after 12 h and 7 days of exposure, respectively. Thiobarbituric acid reactive substance was significantly ($P<0.05$) higher in fish exposed at 10 and 15 °C compared to others after 12 h and 7 days of exposure, respectively. Lowering of temperature adversely affected physiological processes of magur.

Introduction

Among the various physical factors influencing the aquatic environment, temperature is of great importance and it plays a critical role in the life of aquatic poikilotherms. Temperature is considered as an abiotic master factor (Fry 1971; Brett 1979). Physiological processes of fish such as food consumption, digestion, immunity, etc. are influenced by water temperature. Five major effects of temperature on fish physiological processes are controlling, masking, limiting, directing and acting as a lethal agent (Fry 1947). Temperatures beyond the optimal limit of a particular species adversely influence fish health by increasing the metabolic rate, oxygen consumption and the invasiveness and virulence of pathogens, which in turn may cause a variety of pathophysiological disturbances that can lead to the death of the species (Gordon 2005; Dalvi et al. 2009). Low environmental temperatures show profound immunosuppressive effects on ectothermal animals like fish (Bly et al. 1986).

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The Asian catfish, *Clarias batrachus* (Linnaeus 1758), popularly known as magur, is a highly priced food fish and is widely distributed throughout the Indian sub-continent. In spite of its high market demand, culture of this species is not commercialized. The flesh of magur has been reported to contain 78.5% water, 15% protein, 1.3% mineral, 1.0% lipid, 4.8% carbohydrate, 0.21% calcium, 0.0007% iron and 0.011% vitamin and the energy content of 86 Kcal per 100 g flesh (Roy and Mollah 2009). The twin qualities, such as rapid growth and high marketability, makes magur a good candidate species for aquaculture production (Singh et al. 2009). Therefoe, fish farmers in various parts of India are interested to culture this species. Though magur is hardy in nature and tolerates adverse conditions (Kumari et al. 2006), it is essential to understand the physiological response of this economically important species to thermal fluctuations in the pond environment during various seasons in different geographic regions of India. A wide variation in water temperatures are observed in Indian sub-continent, showing diurnal and seasonal variations (Jain and Kumar 2012). Magur shows increased susceptibility to various pathogens in different seasons (Kumari et al. 2006). The lethal temperatures for *C. batrachus* ranged from 9.4-12.8 °C depending on their thermal history (Pardue 1970). Shafland and Pestrak (1982) found that the lethal temperature of magur was 9.4 °C. Dehadrai et al. (1985) reported that magur grew well up to 32 °C temperature and were seen to be under stress at 35 °C; mortality of fish started at 38 °C.

Haematological and biochemical parameters are important tools that can be used as effective and sensitive indices to monitor physiological and pathological changes in a particular fish species. Myeloperoxidase is an important enzyme most abundantly present in neutrophils. It has antimicrobial activity; it utilises hydrogen peroxide during respiratory burst and produces hypochlorous acid (Dalmo et al. 1997). Nitric oxide synthase (NOS) are a family of enzymes that catalyse the production of cellular signalling molecule nitric oxide which plays a vital role in many biological processes. The inducible isoform iNOS produces large amount of nitric oxide as a defence mechanism. The young fish use innate mechanisms during the first weeks/months of their development and this may protect farm fishes against pathogens at an early age (Rombout et al. 2005).

All aerobic organisms have well developed antioxidant system like glutathione and other thiols and an array of glutathione-dependent enzymes. The environmental stress resulting from temperature variation modulates the antioxidants and induces production of reactive oxygen species leading to lipid peroxidation (Flanagan et al. 1998; Guderley 2004). Lipid peroxidation is a well-established mechanism of oxidative damage caused by ROS such as superoxide (O_2^-), OH and hydrogen peroxide (H_2O_2), and the measurement of malondialdehyde (MDA) provides a convenient index of lipid peroxidation (Devasena et al. 2001). Lipid peroxidation in biological membranes causes impaired membrane function, structural integrity and inactivation of several membrane-bound enzymes (Goel et al. 2005). Induction or suppression of antioxidants has been recognized as one of the most important responses of the animals under stressful condition (Abele and Puntarulo 2004; Bocchetti et al. 2004). Effect of water temperature fluctuations on the antioxidant profile of fish is an important area for research as fish are often exposed to such environmental condition in

nature. However, less information is available regarding the influence of environmental temperature on the physiology of tropical freshwater fishes, especially for magur (Wilson et al. 2010). Therefore, the present investigation was undertaken to study the physiological responses of *C. batrachus* to a wide range of water temperatures, which the species faces in different parts of India.

Materials and Methods

Experimental system and culture of fish

Clarias batrachus, magur (80.60±5.34 g) were brought from Chatterjee Brothers' Fish Farm, Mogra, West Bengal in October 2011. Fish were kept in outdoor condition for 24 h and then randomly distributed in glass aquaria (50 L each) maintained in the wet laboratory facility. The stocking density was 1 fish·10 L^{-1}(5 fish·aquarium^{-1}). Each aquarium was connected with a filtration unit (Sera fil bioactive 130, Germany) and a cooling/heating (HAILEA Chiller HC-300A, China/ Sera Aquarium Heater 300, Germany) unit. This aquarium setup helped to maintain desirable temperature in the aquarium. The used water from the fish culture unit was first circulated into the filtration unit, then to cooling or heating unit and finally into the fish culture unit. Fish were acclimated at ambient temperature (25±2 °C) for 21 days to avoid transportation and handling stress. Fish were fed daily with feed (prepared in laboratory) containing 40% protein at the rate of 5% of body weight throughout the study period. The total amount of feed was divided in two instalments, and were given at 9:00 am and 5:00 pm. Constant aeration was provided to maintain high oxygen level (5.01-5.86 mg·L^{-1}) and for the continuous mixing of water.

Temperature range

Fish acclimated at 25 °C were exposed to six different temperature regimes namely 10, 15, 20, 25, 30 and 35 °C. Three replicates were used for each temperature and 25 °C temperature was considered as ambient temperature (control). Two groups were maintained above this temperature and three groups were below this temperature. Experimental temperatures were achieved by increasing or decreasing the water temperatures at a rate of 2 °C·day^{-1}. This required 2.5 (20 °C and 30 °C) - 7.5 (10 °C) days to reach different assigned temperatures.

Sample collection

Two fish were collected from each tank after 12 h of achieving the assigned temperature to study the immediate effect of stress after exposure. From the remaining three fish, two fish were collected after 7 days to study the effect of chronic stress. Therefore, a total of six fish were used for all biochemical assays (two fish per replicate x three replicates) from each temperature group in each sampling day. A total of two samplings were conducted in all treatments, except for the 10 °C treatment. In 10 °C treatment, after first sampling remaining fish died. Fish were taken out from the

experimental tank, and anaesthetized with MS-222 (Sigma, U.S.A.). Blood and tissue (muscles) samples were collected and stored at -80 °C for further study.

Biochemical assays

Total serum protein and muscle protein were determined by the method of Lowry et al. (1951) using Microplate Reader (BioTek, Synergy HT, NY, U.S.A.). The absorbance was recorded at 750 nm. Bovine serum albumin (BSA, HiMedia, India) was used for the preparation of standard curve.

Serum myeloperoxidase activity was measured according to the method of Quade and Roth (1997). In brief, 90 µL Hank's balanced salt solution (HBSS, without Ca^{2+} or Mg^{2+}) was added to 10 µL of serum in 96-well microplate. A volume of 35 µL of 20 mM 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB, Genei, India) and 5 mM H_2O_2 were added into each well. After 2 min of incubation at 25 °C, 35 µL of 4M sulphuric acid was added to stop the reaction. HBSS was used as blank. Optical density was measured at 450 nm using Microplate Reader.

Nitric oxide synthase (NOS) level of muscles was measured using the method of Lee et al. (2003). Tissue weighing100 mg was homogenized with 1 mL of chilled phosphate buffer (pH 7.4) and centrifuged at 10,500 x g for 20 min. The supernatant was immediately used for assay. A volume of 100 µL of supernatant was mixed with equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid) and incubated at room temperature for 10 min. The absorbance was recorded at 540 nm using Microplate Reader. The nitrite concentration was determined from the nitrite standard curve and expressed as mol mg^{-1} tissue.

Thiobarbituric acid reactive substance (TBARS) was measured following the method of Ohkawa et al. (1979), in which MDA, the end product of lipid peroxidation reacts with thiobarbituric acid (TBA). One gram of muscle tissue was homogenized in 9 mL of KCl (1.15%) and the sample was incubated in 0.6% TBA (pH 3.5) containing SDS (0.45%) for 1 h at 100 °C. After cooling, the sample was centrifuged at 825xg for 15 min and the absorbance of the supernatant was measured at 532 nm using Microplate Reader. The standard was prepared using 1, 1, 3, 3-tetramethoxy propane and the result was expressed as µmol MDA mg^{-1} protein.

Reduced glutathione (GSH) was determined using the method of Jollow et al. (1974). One gram of muscle tissue was homogenized in 10 mL phosphate buffer (0.1 M, pH 7.4) and was centrifuged at 10,500 x g for 20 min; the supernatant fraction was treated as post-mitochondrial supernatant (PMS,10%). This post-mitochondrial supernatant was precipitated with 4% sulfosalicylic acid (Himedia, Mumbai, India) in 1:1 ratio. The samples were kept at 4 °C for 1 h and then centrifuged at 1,500 x g for 15 min at 4 °C. The supernatant was used for GSH assay. The assay mixture contained supernatant, 0.1 M phosphate buffer (pH 7.4) and 5-5-dithiobis-2-nitrobenzoic acid. Optical density was measured at 412 nm using UV-visible spectrophotometer (Shimadzu 1601, Japan). The GSH value was expressed as mM of GSH mg^{-1} tissue.

Fish mortality

Dead fish from individual aquarium was removed and mortality of fish was recorded regularly.

Statistical analysis

The experimental data were expressed as mean±standard error (SE). One-way analysis of variance (ANOVA) and Duncan's multiple range test, DMR (Montgomery 1984) were used to find out the difference among various treatments and sampling days. In all cases, 0.05 was used as the level for accepted significance.

Results

Fish mortality

Fish mortality was seen when the water temperature reached 10 °C. A total of fifty percent of the fish died within 5 days of exposure at 10 °C. Therefore, there was no fish for second sampling which was scheduled after 7 days of reaching the assigned temperature. There was no mortality of fish in the other treatments.

Biochemical assays

Serum protein level was significantly ($P<0.05$) higher in fish exposed at 15 °C compared to others after 12 h and 7 days of reaching the assigned temperature (Fig. 1). Minimum serum protein level was found in fish exposed at 30 °C temperature after 12 h of exposure, the level was 14% lower compared to the fish exposed at acclimation temperature 25 °C. After 7 days of exposure, lowest serum protein level was recorded in fish exposed at 25 °C temperature. There was no significant ($P>0.05$) difference in the muscle tissue protein level of fish exposed at six different temperatures after 12 h and 7 days of reaching assigned temperatures. The muscle tissue protein concentrations ranged from 0.060 to 0.067 $mg \cdot mg^{-1}$ tissue in various treatments.

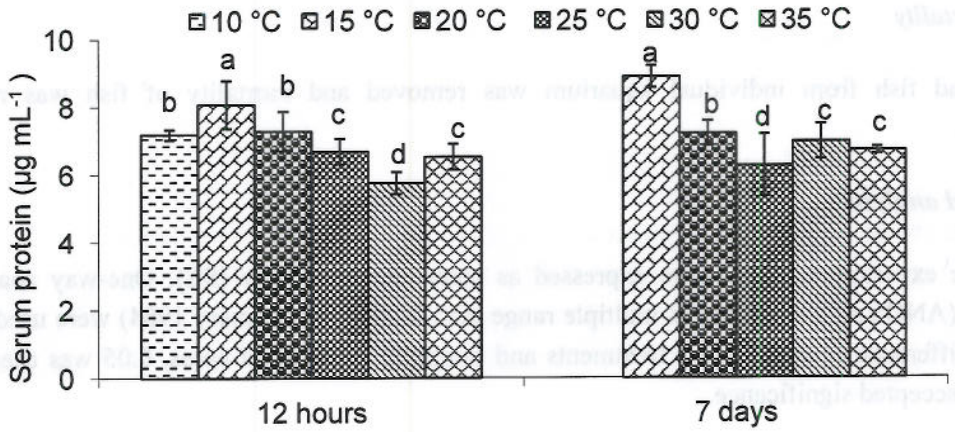

Fig.1. Effect of acclimation temperature on the serum protein level of *C. batrachus*. Bars with different superscripts are significantly ($P<0.05$) different (n=6).

Serum myeloperoxidase level was significantly ($P<0.05$) higher in the fish exposed at 30 °C compared to the other temperature groups, except the fish exposed at 25 °C on day-7 (Fig. 2). Fish exposed at 30 °C showed 30% and 3.5% higher myeloperoxidase levels compared to the fish exposed at 25 °C after 12 h and 7 days of exposure, respectively. Myeloperoxidase level reduced gradually with the decrease of temperature from the acclimation temperature of 25 °C. After 12 h of exposure, minimum level was found in fish exposed at 10 °C. A 4-24% reduced myeloperoxidase level was found after 12 h of exposures at 20-10 °C compared to the fish exposed at 25 °C. Whereas after 7 days of exposure at 20-15 °C myeloperoxidase was reduced by 12-40% compared to the group exposed at 25 °C.

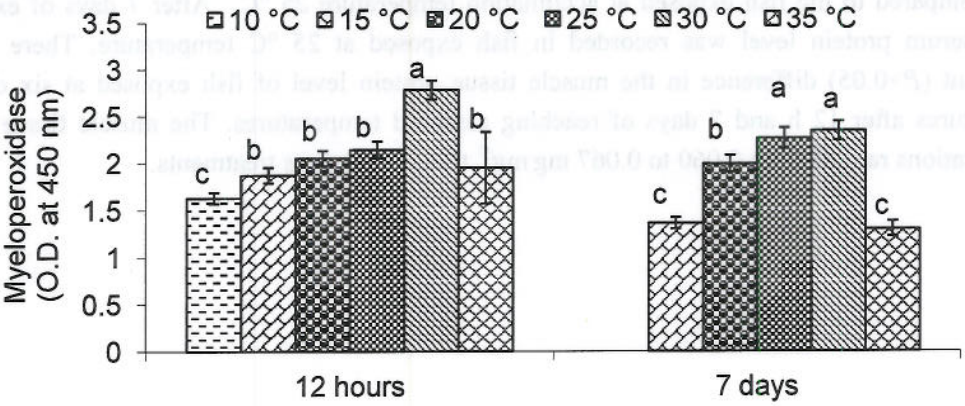

Fig. 2. Myeloperoxidase level of *C. batrachus* exposed to six different temperatures. Bars with different superscripts are significantly ($P<0.05$) different (n=6).

Nitric oxide synthase level was significantly ($P<0.05$) higher in fish maintained at 25 and 30 °C compared to the other treatments after 12 h and 7 days of exposure, respectively. Nitric oxide synthase level reduced when the water temperature decreased from 25 °C. A 24-42% reduced nitric oxide synthase level was found in fish after 12 h of exposures at 10-20 °C compared to the fish exposed at 25 °C. There was 14-41% reduction in nitric oxide synthase level in fish exposed at 30 and 35 °C compared to the fish exposed at 25 °C after 12 h of exposure. The level increased significantly ($P<0.05$) in each group after 7 days of exposure compared to the respective 12 h exposed group (Fig. 3).

TBARS level was significantly ($P<0.001$) higher in magur at 10 °C and 15 °C temperatures after 12h and 7 days of exposure, respectively compared to other treatments. TBARS level was significantly ($P<0.05$) lower in fish at 15 °C temperature after 7 days of exposure compared to the 12 h exposed fish in the same treatment (Fig. 4).

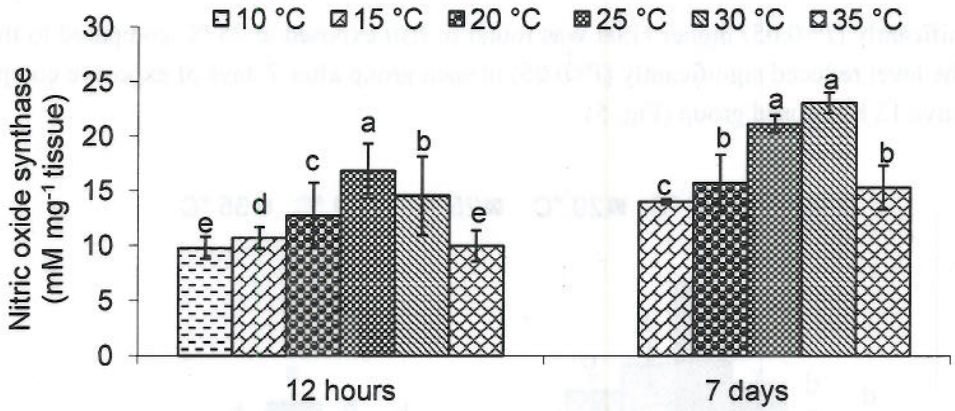

Fig. 3. Effect of acclimation temperature on the nitric oxide synthase of *C. batrachus*. Bars with different superscripts are significantly ($P<0.05$) different (n=6).

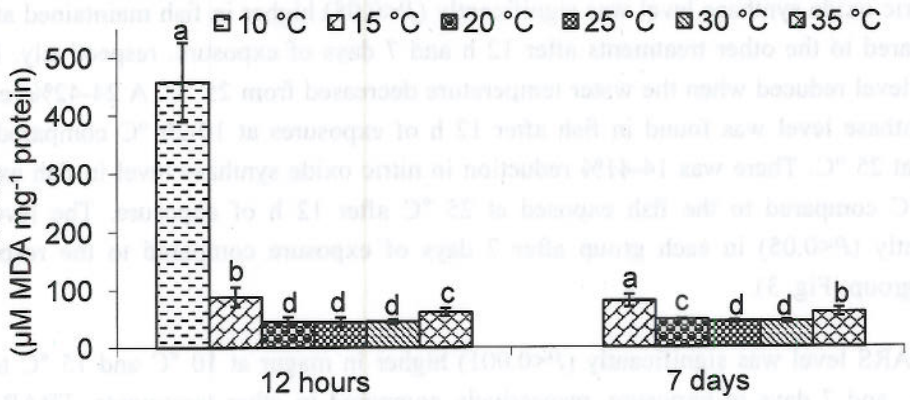

Fig. 4. Thiobarbituric acid reactive substance (TBARS) found in *C. batrachus* after exposure to six different temperatures. Bars with different superscripts are significantly ($P<0.05$) different (n=6).

Significantly ($P<0.05$) higher GSH was found in fish exposed at 25 °C compared to the other groups. The level reduced significantly ($P<0.05$) in each group after 7 days of exposure compared to the respective 12 h exposed group (Fig. 5).

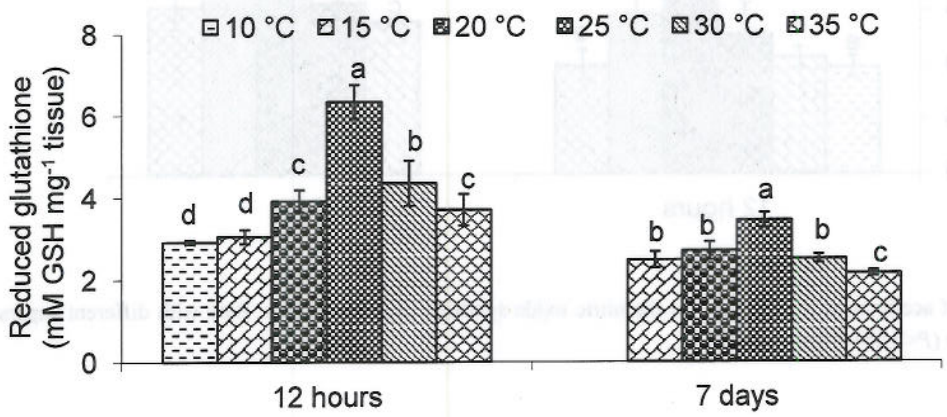

Fig. 5. Effect of acclimation temperature on reduced glutathione (GSH) of *C. batrachus*. Bars with different superscripts are significantly ($P<0.05$) different (n=6).

Discussion

Temperature of the aquatic environment is important for ensuring survival, distribution and normal metabolism of fish. The failure to adapt to temperature fluctuations results in fish mortality (Forghally et al. 1973). In the present study significantly higher mortality of magur was recorded at 10 °C treatment compared to the other temperatures. This confirmed the findings of Shafland and Pestrak (1982) that the lethal temperature for magur was 9.4 °C in Florida. Temperature beyond

optimum limits adversely affects the health of the animals due to metabolic stress, which have suppressive effects on growth, reproduction and immune capacity (Cnaani 2006). In the present investigation, the study of biochemical parameters in magur has revealed information on the physiological changes occurring in fish in response to decrease or increase of water temperature from the acclimation temperature of 25 °C. Higher level of serum protein in fish exposed at lower temperatures (20-10 °C) than those in the control (25 °C) indicated temperature-induced stress in fish after 12 h of exposure.

Serum myeloperoxidase level was 30% and 3.5% higher at 30 °C compared to the fish exposed to the acclimation temperature of 25 °C after 12 h and 7 days of exposures, respectively. Whereas 9-42% reduced myeloperoxidase level was found in fish exposed to 35 °C compared to the latter group (25 °C). Earlier study showed that temperature above 35 °C was stressful for magur (Dehadrai et al. 1985). Myeloperoxidase content of *C. batrachus* showed a seasonal pattern with the lowest significant values recorded in the coldest months (mean temperature range 19-24 °C) and highest values during the rest of the period with water temperature above 28 °C (Kumari et al. 2006). Myeloperoxidase is a microbicidal enzyme (Dalmo et al. 1997). Its elevated amount results in the destruction and elimination of invading pathogens (Yano 1992). Significantly ($P<0.05$) higher myeloperoxidase levels were found in fish exposed at 25 and 30 °C temperatures in the present study. Higher values of myeloperoxidase in magur at 25 and 30 °C indicate well-developed immune status in *C. batrachus* to overcome adverse environmental conditions.

A decreasing trend of nitric oxide synthase was found with the decrease of water temperature from the acclimation temperature of 25 °C. The immunologically "non-permissive" temperature has been established as 4 °C in salmonids, 14 °C in carp and 22 °C in channel catfish (Bly and Clem 1992). The innate immune response of channel catfish *Ictalurus punctatus* (Rafinesque 1818) was influenced by low temperature (10 °C) as phagocytic activity of neutrophils was reduced (Ainsworth et al. 1991). Clem et al. (1984) and Bly and Clem (1991) demonstrated that exposure to non-permissive temperature (<17 °C) resulted in immunosuppression. Temperature below or above the thermal limit can induce alterations to the fish immune response (Watts et al. 2001). In the present study lowering of values after 7 days of exposure showed the long term effect of stress in magur.

The increase in lipid peroxidation under the influence of lower temperature and disturbance of antioxidant balance as seen in the present study may result in additional stress in magur. Significantly higher lipid peroxidation was found in *Channa punctata* (Bloch 1793) exposed at 32 °C compared to the control group kept at 20 °C (Kaur et al. 2005).

In the present study glutathione level in magur reduced in both conditions of decrease or increase of temperature compared to the control group (25°C). In *C. punctata*, elevated temperature (32 °C) resulted in decreased glutathione content in tissue (Kaur et al. 2005). In stress, thiols (glutathione) are modulated by the cells, as they are the first to be used in cellular defence against stress (Meister and Anderson 1983; Dickinson and Forman 2002).

Temperature adaptation is an essential physiological phenomenon and is strongly dependent on acclimation episode and temperature of the environment (Das et al. 2004). In the present study, magur were acclimated at 25 °C and then exposed to a range of temperatures 10-35 °C. Being a tropical fish, magur showed better adaptability towards higher temperatures (>25 °C) compared to the lower temperatures (<25 °C). Martins et al. (2011) found that vaccinated channel catfish were severely impacted by low temperature, either at constant 15 °C or at 15-25 °C cycling temperature. The water temperatures below 25 °C affected the physiological responses of magur. In the present investigation, most of the parameters indicated that temperature between 25-30 °C is favourable for magur.

Conclusion

The lowering of temperature from the acclimation temperature adversely affected physiological processes of *C. batrachus*. Our results suggest that water temperature should not fall below 15 °C in the culture pond of magur. The lower thermal limit for magur has been observed as 10 °C in the present study.

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