P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-6* ISSUE-4* July-2021
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Metabolic Responses to Acute Hypoxia in Two Catfishes *Clarias batrachus* and *Mystus seenghala* with Different Respiration Patterns

Paper Submission: 15/07/2021, Date of Acceptance: 25/07/2021, Date of Publication: 26/07/2021

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Abstract

Hypoxia in fishes is a most important aquatic phenomenon in a tropical country like India. It may be a naturally occurring phenomenon due to biological and physical factors or may be caused due to anthropogenic activities around the water bodies. In order to compare the metabolic responses to acute hypoxia, experiments were carried out to find enzyme activities, protein profiling. Experiments on blood metabolites were also performed to find out intermediary metabolic products arise due to experimentally provoked hypoxia. At the end of this study we are able to find out that *Clarias batrachus* is more efficiently coped to the experimentally provoked hypoxia than the *Mystus seenghala.*

Key Words: Hypoxia; SDS-PAGE; LDH; MDH; Protein bands.

Introduction

Vertebrates try to maintain oxygen delivery in the conditions of hypoxia. If oxygen delivery is compromised and tissue oxygen levels fall then energy expenditure is reduced and anaerobic metabolism is up-regulated (Boutilier *et al*., 1988). Fish reduce energy expenditure during hypoxia by inhibiting feeding and reproduction, moving to a lower temperature and reducing swimming activity. These energy savings are considerable and genes associated with aerobic metabolism are down-regulated, probably in response to the reduction in aerobic energy expenditure. Anaerobic metabolism (mostly LDH activity) is up-regulated to maintain function in the face of limitations in aerobic energy production (Kumar 2018; Kumar 2019 and Kumar 2021). The liver plays a central role in these responses, but studies of the effects of hypoxia on liver cellular changes in vivo are rare compared with in vitro studies.

Hypoxia in fishes is a most important aquatic phenomenon in a tropical country like India. It may be a naturally occurring phenomenon due to biological and physical factors (Rosenberg *et al.*, 1991; Pihl *et al*., 1992; Hobak and Barnhart, 1996) or may be caused due to anthropogenic activities around the water bodies.

Even though most of the enzymes involved in glucose metabolism have been detected in fish, the regulation of carbohydrate metabolism differs in some aspects from that of mammals and its importance as a metabolic fuel in fishes is not fully understood (Kumar *et. al.,* 2015).

Review of Literature

Effect of oxygen deficiency on fish had drawn the attention of scientists as early as the 1920s and extensive literature is available on fish during that period. Story of studies of adaptations of fish to low oxygen was extended by investigation undertaken in swamps (Carter and Beadle, 1931). A comprehensive study has been made on a number of freshwater, estuarine and marine fishes by Davis (1975) to record the minimum oxygen requirements for survival and growth of fishes. Greaney *et al.,* (1980); Taylor and Miller, (2001); Pichavant *et al.,* (2003) studied the effects of chronic (weeks of) hypoxia on oxygen carrying capacity.

Dunn & Hochachka (1986) and Dalla Via *et al*. (1998) observed in their studies that a metabolic reorganization takes place as a result of hypoxia that tends to follow one of two generalized patterns: (i) either the rate of anaerobic ATP production increases (Pasteur effect) or (ii) the ATP rate declines (metabolic depression). Chabot and Dutil, (1999) and Pichavant *et al.,* (2000) studied the effects of chronic (weeks of) hypoxia on food intake.

Greaney *et al.,* (1980); Taylor and Miller, (2001); Pichavant *et al.,* (2000) studied the effects of chronic (weeks of) hypoxia on oxygen carrying capacity. Weber & Kraemer (1983) described that feeding and growth (Cech *et al.,* 1984; Bejda *et al.,* 1992; Secor & Gunderson, 1998; Taylor & Miller, 2001) are reduced in fishes when exposed to chronic hypoxia (≤3.0 mg O_2l^{-1}).

Dunn & Hochachka (1986) and Dalla Via *et al*. (1994) observed in their studies that a metabolic reorganization takes place as a result of hypoxia that tends to follow one of two generalized patterns: (i) either the rate of anaerobic ATP production increases (Pasteur effect) or (ii) the ATP rate declines (metabolic depression). Metabolic correlation and comparative study in various fishes with different respiratory patterns were performed by (Kumar *et al.,* 2015[1], Kumar 2015[2]; Kumar 2016; Kumar 2018, Kumar 2019 and Kumar 2021).

Aim of the Study

This study aims to analyze the comparative responses of aerobic and anaerobic enzyme activities, protein profiling and blood metabolites to different degrees of hypoxia in two different catfishes, *Clarias batrachus* and *Mystus seenghala*.

Materials and Methods

Live specimens (6 fishes) of *Clarias batrachus* and *Mystus seenghala (80-90 g 20-24 cm),* were procured from a local market and were acclimatized at normoxia (7.2±0.3 mg/L, DO), at least for a month in tanks of 100 L capacity filled with 25 L of water at 25±3°C. They were fed once a day with processed feed of goat liver or flesh and soybean powder. Feeding was stopped 48 h before the start of the experiment.

All the fishes were held for 12 hrs duration of experimentally provoked hypoxia at three different levels:

1. 65%-40%Oxygen saturation or 5.0±0.3 mg/l to 3.5±0.3 mg/l O_2 (Slight Hypoxia)
2. 40%-20% Oxygen saturation or 3.5±0.3 mg/l to 1.5±0.1 mg/l O_2 (Moderate Hypoxia) and
3. Below 20%Oxygen air saturation or ≤1.5±0.1 mg/l O_2 (Severe Hypoxia)

Three separate experiments were carried out in the closed respirometer (without access to air). Decrease in dissolved oxygen (DO) was accomplished by bubbling nitrogen directly into the water of the experimental tank, or into the reservoir that supplied water to the respirometer. DO probe (WTW, CellOx 325) and pH meter (pH electrode; WTW, SenTix® 41-3) were installed to record dissolved oxygen (DO) and pH.

Malate dehydrogenase (MDH; E.C. 1.1.1.37) activity was determined by conversion of oxaloacetate to malate (Somero and Childress 1980). Lactate dehydrogenase (LDH, EC 1.1.1.27) activity in the cell free extracts of muscle, liver, heart and brain was measured by a NADH linked optical assay following the method of Horecker and Kornberg (1948).

The SDS-PAGE was carried out according to Laemmli (1970) in Mini-PROTEAN Tetra System of BIO-RAD using a 5% (w/v) separating gel. After electrophoresis the gels were stained with coomassie blue R-250 for Visualization of the proteins. Molecular of the protein bands was determined with reference to standards (Genei Marker, PMW).

Observation

Lactate dehydrogenase (LDH) activity in *Clarias batrachus*

Highest LDH activity was observed in muscle and lowest in liver during normoxia. Significant changes (p≤0.05%) in LDH activities were observed between normoxia and severe hypoxia in muscle and heart (Fig 1).

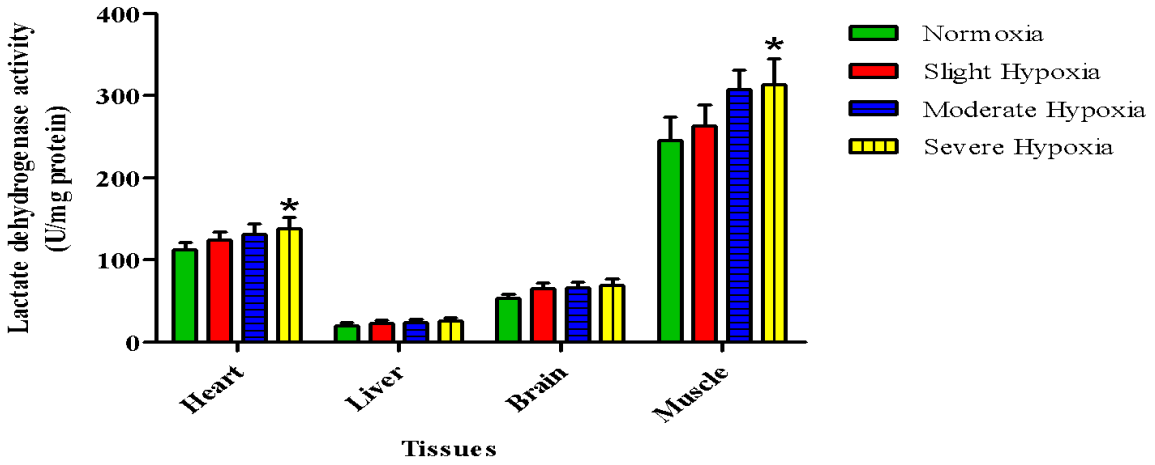

Figure-1: Mean specific activity of lactate dehydrogenase (LDH) enzyme (U/mg protein) in liver, brain, heart and muscle of *Clarias batrachus* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents significant differences (*p*<0.05) between normoxia and severe hypoxia.

Lactate dehydrogenase (LDH) activity in *Mystus seenghala:*

Highest LDH activity was observed in muscle followed by liver and heart. Lowest LDH activity was observed in the brain. Significant changes (p≤0.05%) in LDH activities were observed between normoxia and severe hypoxia in muscle and liver (Fig 2).

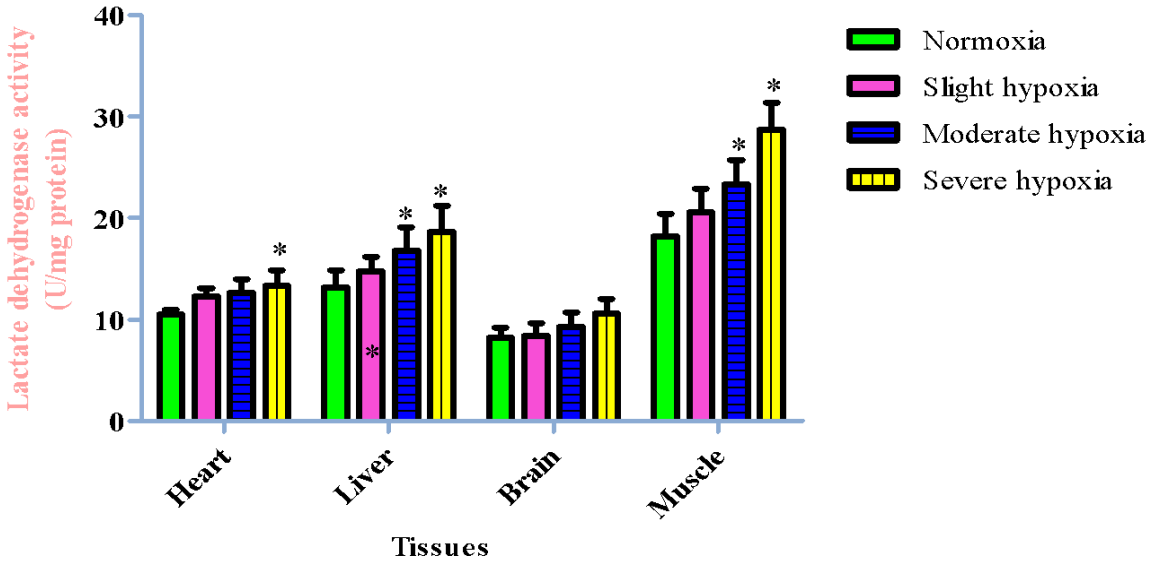

Figure-2: Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Mystus seenghala* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents significant differences (p<0.05) between normoxia and different stages of hypoxia.

MDH activity in *Clarias batrachus*

Highest MDH activity was observed in heart followed by liver and lowest in muscle during normoxia. Maximum decrease in MDH activity was found in the heart (25.42%) during severe hypoxia. Significant changes (p≤0.05%) observed between normoxia and severe hypoxia in heart and liver (Fig. 3).

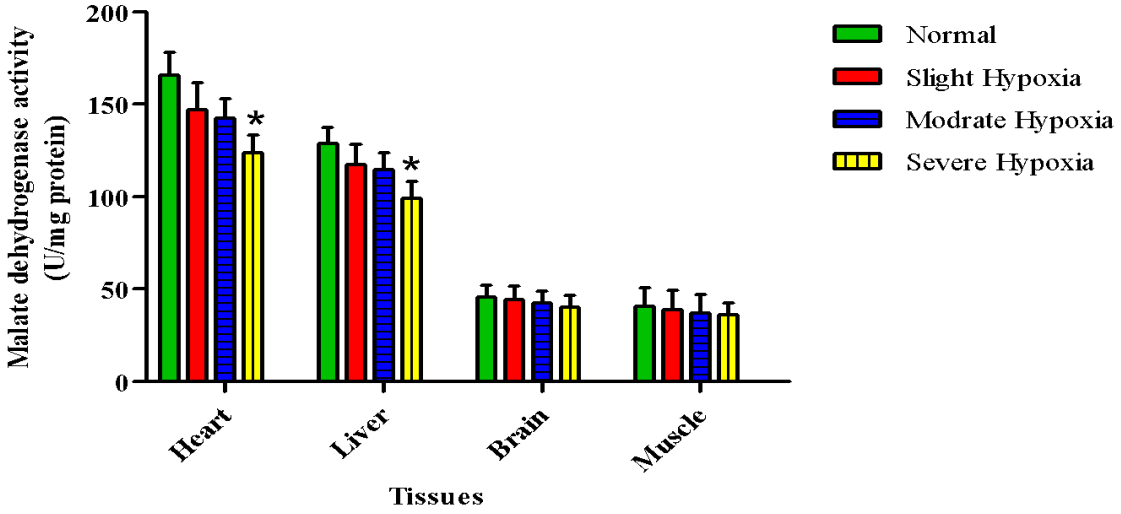

Figure-3: Mean specific activity of Malate dehydrogenase (MDH) enzyme (U/mg protein) in heart, liver, brain and muscle of *Clariasbatrachus* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6.) Asterisk (*) represents significant differences (p<0.05) between normoxia and severe hypoxia.

MDH activity in *Mystus seenghala*

Highest MDH activity was observed in heart and muscle followed by liver and. Lowest MDH activity was observed in the brain. The enzyme activity was decreased in muscle and brain tissue at slight and moderate hypoxia but significant changes were not

reported. Significant changes (p≤0.05%) observed between normoxia and severe hypoxia in all the four tissues (Fig. 4).

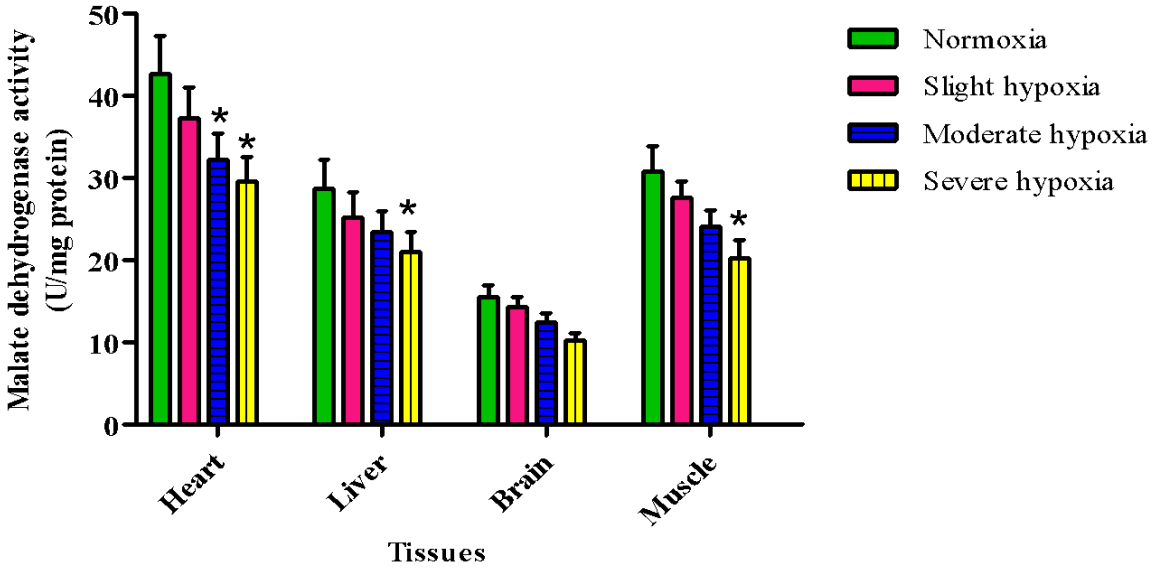

Figure-4: Mean specific activity of malate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Mystus seenghala* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents significant differences (*p*<0.05) between normoxia and different stages of hypoxia.

SDS-PAGE analysis in Clarias batrachus

In hypoxia heart 17.3kDprotein bands were absent and 44.0kD extra protein bands were found (Table 5). In hypoxic liver extra protein band of 72.4kD mol. wt. was present while 29.1kD, 38.4kD and 44.6kD mol. wt. proteins were absent. In hypoxia brain 20.6kD, 34.1kD, 54.2kD, 60.5kD and 70.4kD mol. wt. protein bands were absent while extra protein band having mol. wt. 44kD was observed. In hypoxia muscle protein band of 44.3kD mol. wt. was absent (Fig.5).

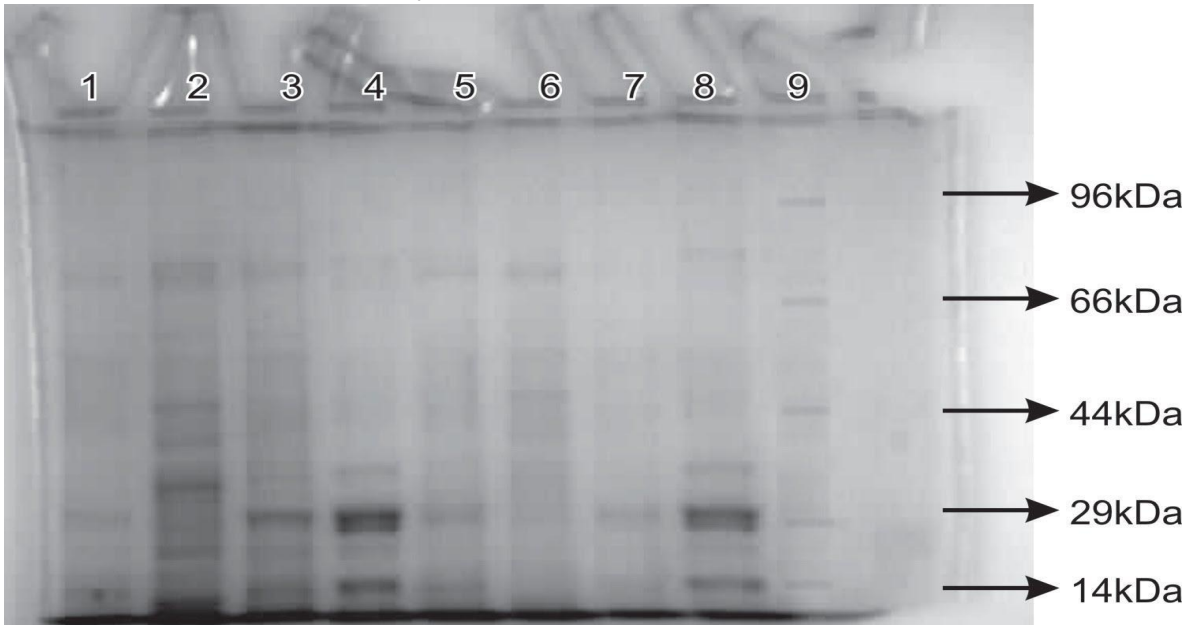

Figure-5: SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Clarias batrachus*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle,

lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia muscle and lane 9: mol. wt. Marker (Sigma wide range marker).

SDS-PAGE analysis in *Mystus seenghala*

In hypoxic brain one extra protein band of 14.0kD mol. wt. was found while 36.0kD and 48.2kD protein bands were absent. In hypoxia muscle 36.0kD protein band was absent and no other changes were observed. In hypoxia heart no change in protein banding pattern was observed. In hypoxic liver one extra protein band of 96.0kD was found while no other changes in protein bands were observed (Fig 6).

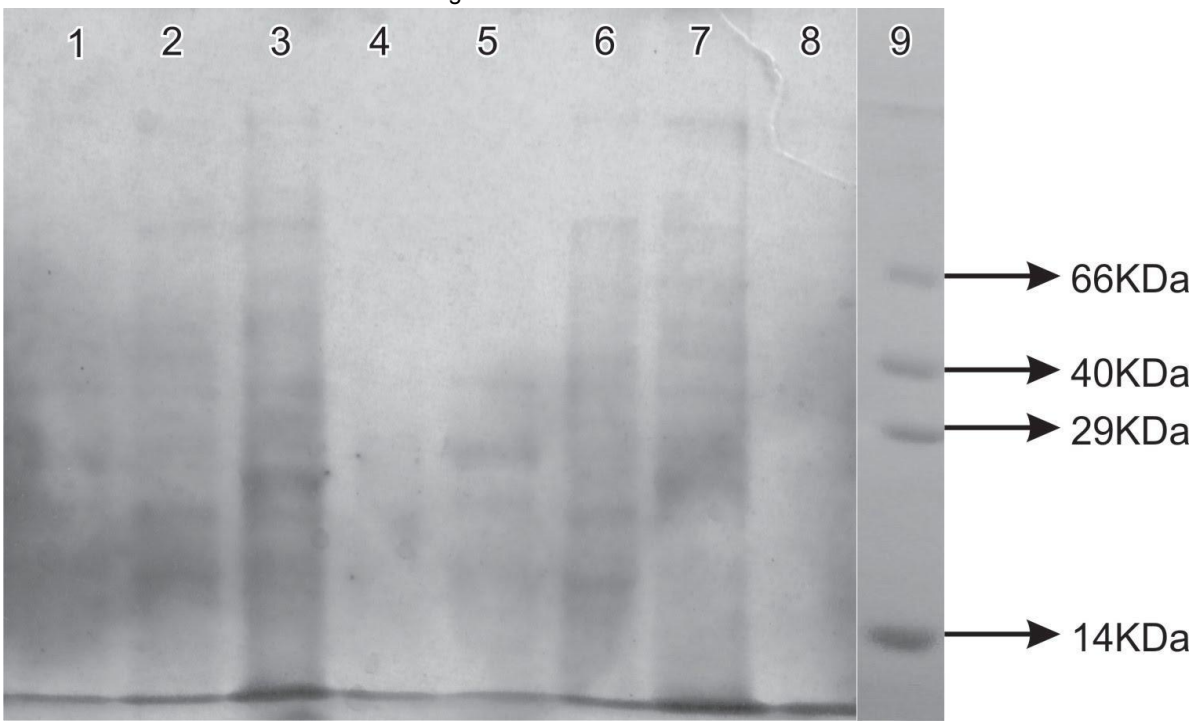

Figure-6: SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Mystus seenghala*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle, lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia muscle and lane 9: mol. wt. Marker (Sigma wide range marker).

Discussion

Heart, liver and brain are known as aerobic tissues which normally tend to avoid anaerobic accumulation of lactate while muscle is known as anaerobic tissue. Therefore the LDH level is adjusted in these aerobic tissues according to the degree of exposure to hypoxia (Almeida-Val *et al*., 2000). The LDH levels found to be observed in different fish species in an investigation has been found to support this observation (Kumar *et al.,* 2015[1], Kumar 2015[2]; Kumar 2016; Kumar 2018; Kumar 2019 and Kumar 2021).

The LDH level in *C. barachus* shows more significant changes than the *M. seenghala*. These results, in combination with the absence of lactate accumulation in white muscle, indicate anaerobic metabolism is only beginning to be employed to supplement energy demands at the level of moderate hypoxia, and metabolic depression is an effective way of conserving ATP until fishes faced with almost anoxic conditions (Kumar *et al.,* 2015[1], Kumar 2015[2]; Kumar 2016; Kumar 2018; Kumar 2019 and Kumar 2021). In other studies with different degrees of hypoxia exposure, levels of lactate increased to a greater extent in blood and white muscle (Richards *et al*., 2007; Wood *et al*., 2007) than in the current study.

Higher LDH activity in *C. batarachus* after hypoxia denotes an increase in anaerobic metabolism as a source of energy while lower level of LDH in *M. seenghala* shows decreased level of anaerobic respiration during hypoxia. No significant LDH activity occurs in *M. seenghala* while it is found in *C. batrachus.* Lactate produced under hypoxia may be transferred to the blood and other tissues and even kept to be oxidized after return to normal conditions. The drop in rate of increase in lactate observed in severe hypoxia in all tissues except for muscle may be due to aquatic surface respiration (ASR) that these fishes perform, especially after moderate hypoxia (Rantin & Kalinin, 1996).

The specific activities of enzymes of glycolysis (LDH) and gluconeogenic (MDH) were found to be tissue specific and species specific too. Strongly suppressed by hypoxia, the white muscles reflected decreased energy demand of the tissue during sustained hypoxia. In contrast, several enzyme specific activities were higher in liver tissue after exposure to hypoxia, suggesting increased capacity for carbohydrate metabolism.

The activity of gluconeogenic enzyme (MDH) was observed to be lower in liver tissue in decreasing order in both the fishes. The decreased activity of this enzyme in the liver is known to be coupled with increased protein catabolism in skeletal muscle (Martinez *et al*., 2006).

Increased levels of glycolytic (LDH) enzymes in the muscles have been correlated with burst swimming capacity of fish (Somero and Childress, 1980; Pelletier *et al*., 1993). In white muscle anaerobic pathways support burst swimming activity (Almeida-Val *et al*., 2000). In case of air-breathing fish *Clarias batrachus* it can be correlated with frequent movement of fish to the surface at the onset of hypoxia. The reduced level of LDH under the condition of sustained hypoxia can be attributed to the constant "surfacing behaviour" of the fish when negligible movement is observed.

Enzymes MDH is known to catalyse the reversible oxidation of malate to oxaloacetate requiring NAD^+ as a cofactor. Found both in cytoplasm and mitochondria, the two forms are recorded to play roles in the gluconeogenesis, lipogenesis, in malate-aspartate shuttle during aerobic glycolysis and in the Kreb's cycle (Almeida-Val *et al*., 2000). Increase in MDH levels in the liver observed in the present investigation is suggestive of a role in increased glycogen synthesis as the liver is the known organ of gluconeogenesis. Its increased level in the heart is also significant as the heart is an organ which depends on glucose as an important metabolic fuel. Its increased levels in the brain are probably due to an increase in oxidative powered capacity of this organ during conditions of long lasting stress.

In *C. batrachus*, a facultative air breather, the hypoxia was found to be associated with activation of anaerobic respiration in response to oxidative stress caused by hypoxia which was reflected by increased levels of LDH in muscle and liver and decrease in MDH levels in heart and liver after exposure to different durations of experimentally provoked hypoxia (Kumar *et al.,* 2015[1], Kumar 2015[2]; Kumar 2016; Kumar 2018; Kumar 2019 and Kumar 2021). These physiological alterations are accepted to be correlated with its capacity to tolerate hypoxic conditions as observed earlier in *C. batrachus* (Tripathi *et al*, 2013).

In *Clarias batrachus* there are more protein bands found in heart and liver than the brain and muscle during hypoxia which shows more metabolically active tissues. While in *M. seenghala* there are less protein bands found in hypoxia heart and muscle tissue than the liver and brain during hypoxia. These results of protein metabolism of *Clarias batrachus* in comparison to *M. seenghala* shows more metabolically activeness of the fish.

Conclusion

Because the different tissues of *Clarias batrachus* have more active aerobic enzymes (MDH) and anaerobic enzymes (LDH) and also more metabolically active protein bands than the *Mystus seenghala.* On the basis of above results and discussion we can say that the former is more tolerant to graded hypoxia than the latter.

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