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Comparative Account of Metabolic Responses to Acute Hypoxia in Two Catfishes *Heteropneustes fossilis* And *Mystus seenghala* With Different Respiration Patterns

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In spite of several studies, not much attention has been directed towards the role of their enzymes in the metabolic adjustments to hypoxia and comparing the same between fishes of two different respiratory habits. The present work aims to analyze the metabolic adjustment to different degrees of hypoxia in two Cypriniform groups which present different respiratory patterns and make a comparative study between the two.

Because of their rich evolutionary history and current ecological diversity of these specific groups of teleosts the study of the fishes or this group has been particularly informative in elucidating the responses of animals to hypoxia.

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

Introduction

The first responses of fishes to environmental hypoxia are always related to respiratory and circulatory changes. Respiratory adaptations are well documented for trout and carps (Jones *et al*., 1970). Many studies have been conducted by submitting the organisms, especially fishes to hypoxia in order to study intermediary metabolic processes.

Suppression of the activity, rate of metabolism is an essential survival strategy in many hypoxia adapted animals (Nilsson & Lutz, 1993). Metabolic depression and change in enzyme activities have been recorded in many fish species (Hochachka and Guppy, 1987; Nilsson & Lutz, 1993; Greaney *et al*., 1980; Van den Thillart and Smith, 1984; Storey, 1988). By reducing their metabolic rate during hypoxia, fish delay the depletion of glycogen stores as well as the accumulation of toxic levels of lactate in the body. Changes in enzyme profiles in response to hypoxia have been undertaken in different fishes, air breathing and water breathing both (Shouberidge & Hochachka 1983; Claireaux and Dutil 1992; Sebert *et al*., 1993; Almeida-Val*et al*., 1995). However studies on exposure of fishes acclimated to different dissolved oxygen concentration did not give a single answer for enzyme responses (Shaklee *et al*. 1977; Almeida-Val and Hochachka 1993; Almeida-Val *et al*., 1995). The effect of hypoxia on enzyme activities of fish, acclimated to different temperatures has also been undertaken (Hochachka & Somero, 1973; 1984; Panepucci *et al*., 2000). It has been observed that ectothermic organisms like fish (Armoured fish, *Rhinelepis strigosa,* a facultative air breather) use biochemical strategies to obtain metabolic homeostasis during variation in dissolved oxygen content. Some of the important responses observed may be enumerated as following: Changes in enzyme activities (Greaney *et al.,* 1980; Van den Thillart and Smit, 1984; Storey, 1988), metabolic depression (Hochachka& Guppy, 1987; Nilsson and Lutz 1993) and the metabolic rate has been observed to fall upto 20-30% in anoxic condition in *Carassius auratus* (Van Waversveld *et al.,* 1989) which has been calculated by heat production.

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.

The team of Almeida-Val has contributed significantly to the understanding of roles of aerobic and anaerobic enzymes in conditions of hypoxia by studying effect of selected stresses including hypoxia on Amazon fish species at tissue enzyme levels and have concluded significantly by correlating oxidative enzyme levels with growth rate of fish. They have not only studied the metabolic correlates of hypoxia between air versus water breathers (Almeida-Val and Hochachka, 1995) but have made comparative investigation between two air-breather of same environment (Amazon Basin) (Dunn *et al.*1983; Hochachka *et al.* 1978). Although a number of enzymes have been attempted as parameters of various functions (Tripathi *et al.*, 2013), LDH and MDH are the most commonly studied glycolytic and gluconeogenic enzymes. Metabolic correlation and comparative study in various fishes with different respiratory patterns were performed (Kumar 2015; Kumar 2016; Kumar 2019; Kumar *et al.,* 2020 and Kumar 2021[1]; Kumar 2021[2]).

Aim of the Study

This study aims to analyze the comparative responses of aerobic and anaerobic enzyme activity and protein profiling to different degrees of hypoxia in two different catfishes, *Heteropneustes fossilis* and *Mystus seenghala* .

Materials and Methods

Live specimens (6 fishes) of *Heteropneustes fossilis* 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 the 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

LDH activity in *Heteropneustes fossilis*

Significant change (p≤0.05%) in LDH activities were observed between normoxia and moderate and severe hypoxia in muscle and in heart it was found between normoxia and severe hypoxia (Fig. 1). No pronounced change was observed in LDH activity in liver and brain during different time duration of hypoxia.

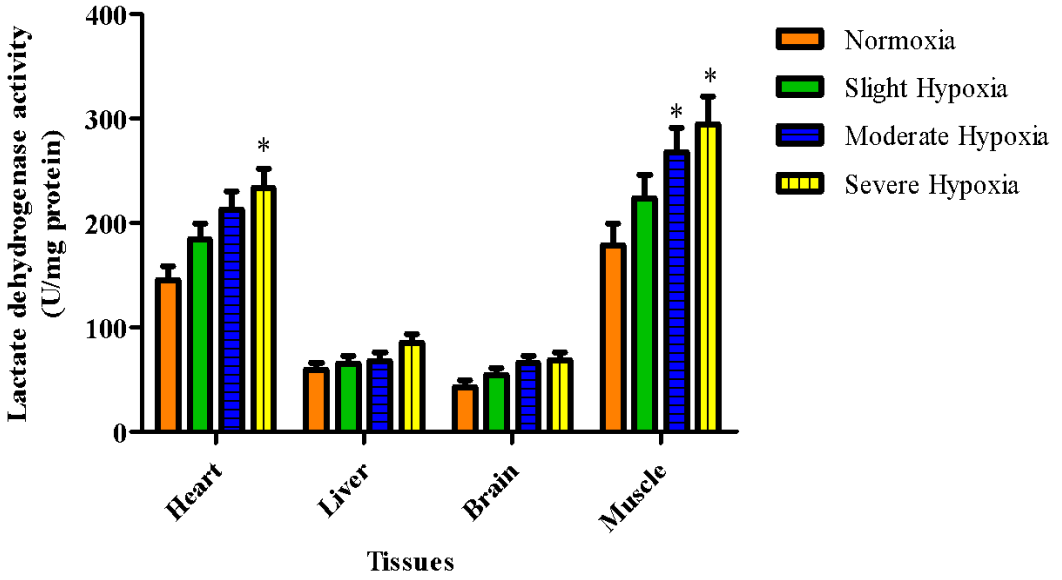

Figure-1: Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Heteropneustes fossilis* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents significant differences (p<0.05) between normoxia and 72 hours of hypoxia.

LDH activity in *Mystus seenghala*

During slight hypoxia maximum increase in LDH activity was observed in heart (16.88%) followed by muscle (12.78%) and liver (11.74%). During moderate hypoxia maximum increase in LDH activity was observed in muscle (27.70%) followed by liver (27.19%) and heart (20.49%). Maximum percentage increase in LDH activity was found in muscle (58.52%) followed by liver (41.21%) and brain (28.48%) during severe hypoxia. Significant changes (p≤0.05%) in LDH activities were observed between normoxia and severe hypoxia in muscle and liver (Fig 2).

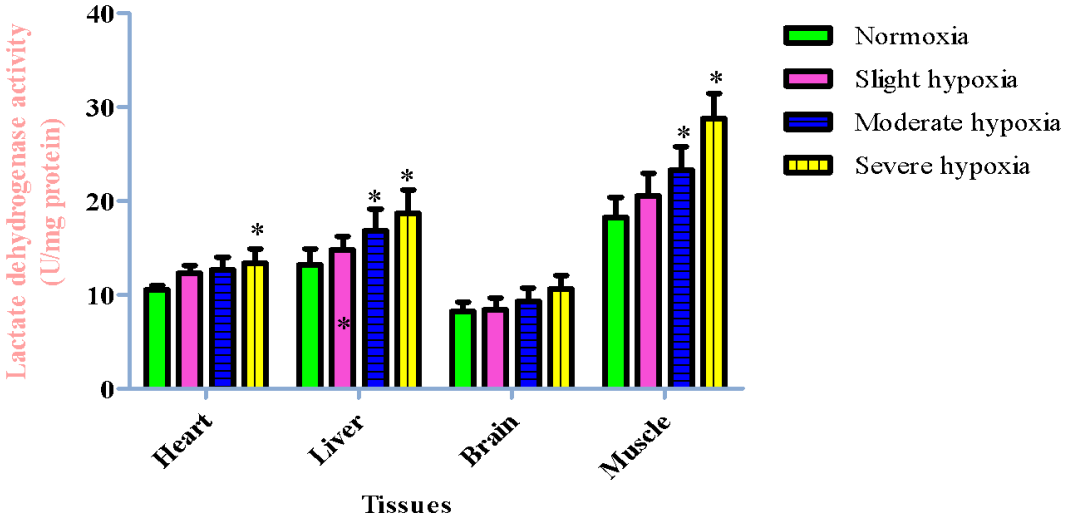

Figure-2: Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Mystus seenghala* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents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

MDH activity in *Heteropneustes fossilis*

Highest MDH activity was observed in heart followed by brain and lowest in muscle during normoxia. MDH activity in different tissues did not show significant differences between normoxia and slight and moderate hypoxia. Significant changes (p≤0.05%) observed between normoxia and severe hypoxia in heart, liver and muscle (Fig.3).

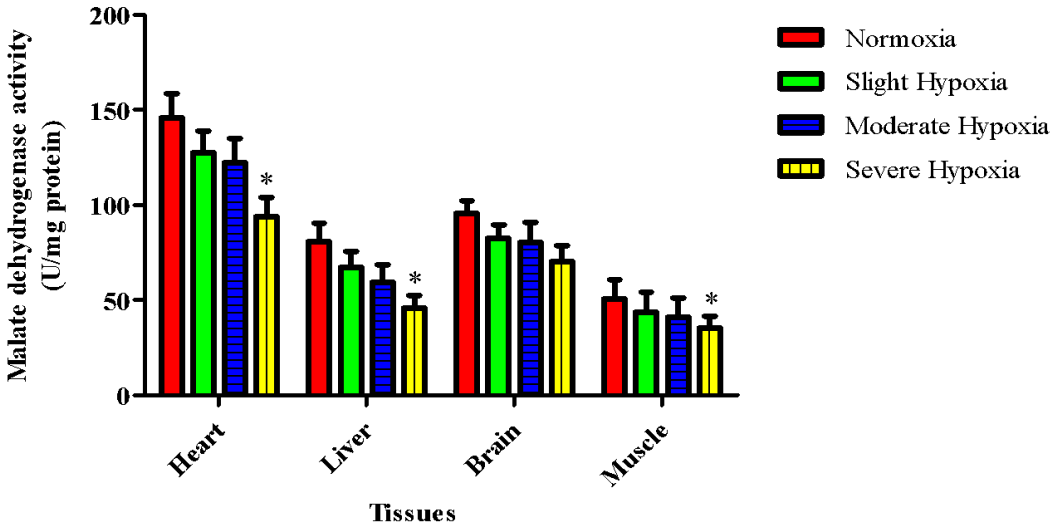

Figure-3: Mean specific activity of malate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Heteropneustes fossilis* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents significant differences (*p*<0.05) between normoxia and different periods of 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. Significant changes (p≤0.05%) observed between normoxia and severe hypoxia in all the four tissues (Fig. 4).

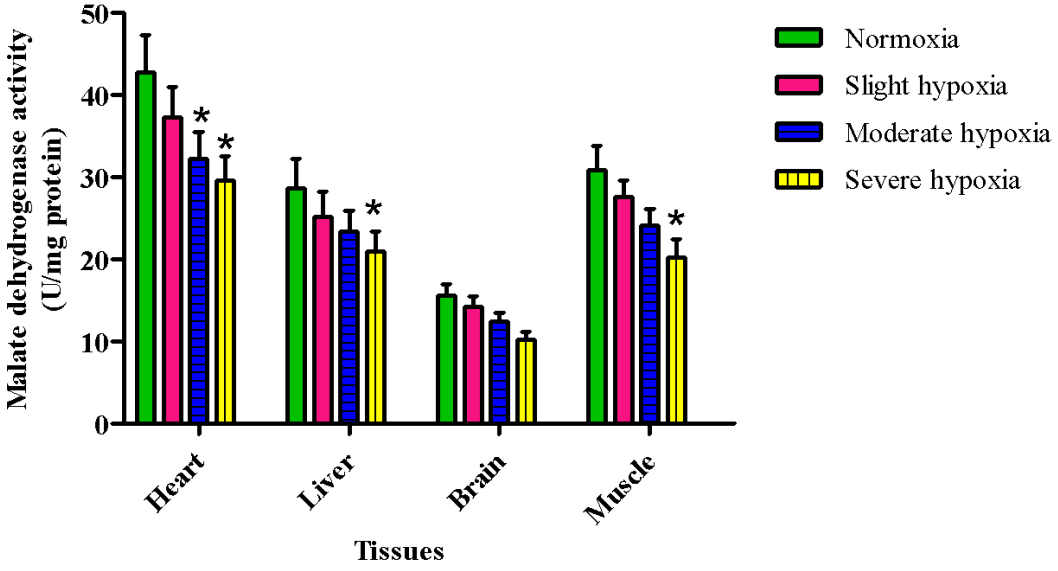

Figure-4: Mean specific activity of malate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Mystus seenghala* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents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

SDS-PAGE analysis in *Heteropneustes fossilis*

In hypoxia heart 35.1kD and 66.8kD protein bands were absent (Table 6). In hypoxia liver two extra protein bands of mol. wt. 45.8kD and 58.4kD were present while 36.1kDprotein band was absent. In hypoxia the brain extra protein bands having mol. wt. 20.7kD, 32.6kD, 60.2kD and 72.6kD were observed while 14.3kD and 36.0kD proteins were absent. In hypoxia muscle extra protein bands having mol. wt. 35.4kD and 45.3kD were present (Fig. 6).

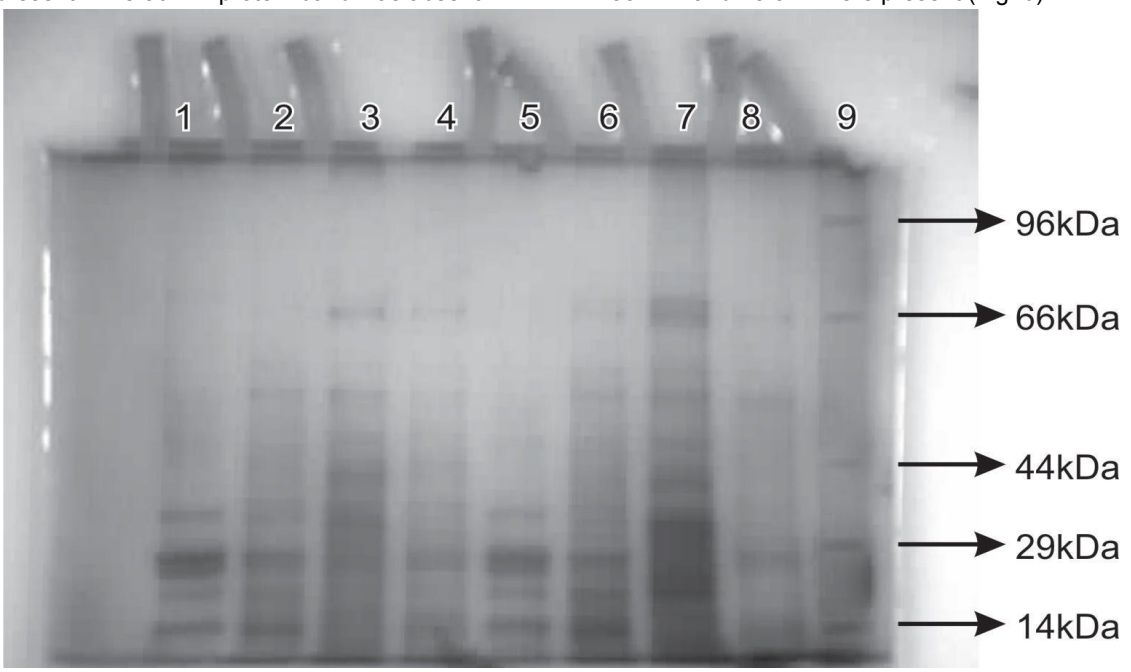

Figure-5: SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Heteropneustes fossilis*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle, lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia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

SDS-PAGE analysis in *Mystus seenghala:*

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

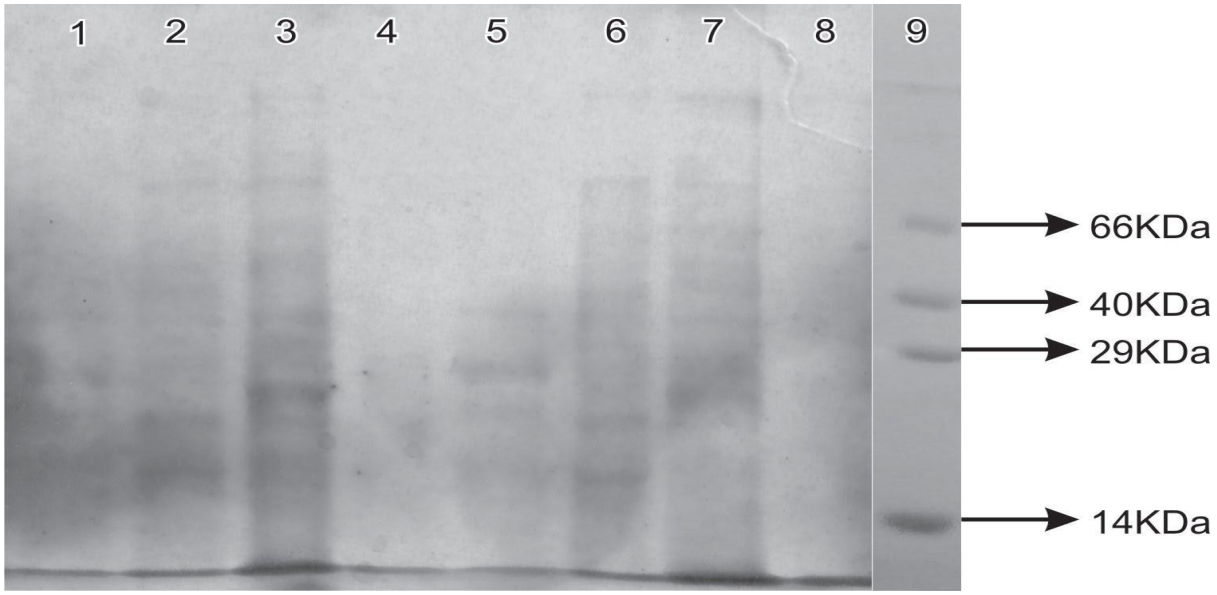

Figure-6: SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Mystus seenghala*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle,

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

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 (Kumar 2015; Kumar 2016; Kumar 2019; Kumar *et al.,* 2020). Hypoxia exposure showed lesser effect on enzymes in heart and brain as compared to white muscle and liver. It was most probably due to preferential perfusion of heart and brain during hypoxia by oxygen. The LDH levels observed in different fish species in an investigation has been found to support this observation (Kumar 2015; Kumar 2019; Kumar *et al.,* 2020 and Kumar 2021[1]; Kumar 2021[2]).

The LDH level in *H. fossilis* shows more significant changes than the *Mystus 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 this level of oxygen deprivation, and metabolic depression is an effective way of conserving ATP until fish are faced with almost anoxic conditions (Kumar 2015; Kumar 2019; Kumar *et al.,* 2020 and Kumar 2021[1]; Kumar 2021[2]).

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).

In *Heteropneustes fossilis* 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 *Mystus 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 *Heteropneustes fossilis* in comparison to *Mystus seenghala* shows more metabolically activeness of the fish.

Conclusion: Because the different tissues of *Heteropneustes fossilis* has more active aerobic enzymes (MDH) and anaerobic enzymes (LDH) and also more metabolically active protein bands than the *Mystus seenghala* we can say that the *Heteropneustes fossilis* is more tolerant to graded hypoxia than the *Mystus seenghala.*

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