P: ISSN No. 0976-8602 RNI No. UPENG/2012/42622 VOL.-10, ISSUE-3, July 2021
E: ISSN No. 2349-9443 Asian Resonance

Comparative Account of Metabolic Responses to Acute Hypoxia in Two Fishes *Heteropneustes fossilis* and *Cyprinus carpio* With Different Respiration Patterns

Paper Submission: 04/07/2021, Date of Acceptance: 15/07/2021, Date of Publication: 24/07/2021

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Abstract

The metabolic adjustments to different levels of hypoxia have been studied in fish of two different respiratory habits. The same environmental pressure that caused the development of different air breathing mechanisms is considered to induce long and short term adaptation mechanisms in water breathing fishes too. The adaptations have resulted due to the complex interaction of biochemical, physiological and molecular characteristics. Besides catfish groups, there are few other air breathing fishes in which roles of the enzymes in the metabolic mechanisms/ adjustments to different degrees of hypoxia have been studied. 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 if intermediary metabolic products arise due to experimentally provoked hypoxia. At the end of this study we are able to find out that *Heteropneustes fossilis* is more efficiently coped to the experimentally provoked hypoxia than the *Cyprinus carpio.*

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

Introduction

Water breathing catfishes are supposed to be hypoxia tolerant fish and are often found in hypoxic environments. Similarly there are carps belonging to Cypriniformes which are known to survive highly polluted or oxygen deficient waters such as *Cyprinus carpio*. Emphasis on physiological adaptation to environmental changes in the catfish species is a rather recent development like that of their use in aquaculture (Das and Ratha, 1996). Some of the physiological adaptive mechanisms of catfishes in general and air-breathing catfishes in particular in response to physico-chemical factors, other than hypoxia such as light, temperature, ammonia, xenobiotics or other pollutants are also on record.

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 concentrations did not give a single answer for enzymes 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.

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.

Bushnell *et al.,* (1984) investigated the effect of chronic hypoxia on fish swimming performance and metabolism. The effect of hypoxia on swimming activity of fishes was supported by Dahlberg *et al.,* (1968), Bushnell *et al.,* (1984). 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 $O_2 l^{-1}$).

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.,* (2003) studied the effects of chronic (weeks of) hypoxia on food intake. Metabolic correlation and comparative study in various fishes with different respiratory patterns were performed (Kumar 2016; Kumar 2017; 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 with two respiration patterns in fishes, *Heteropneustes fossilis* and *Cyprinus carpio.*

Materials and Methods

Live specimens (6 fishes) of *Cyprinus carpio* and *Heteropneustes fossilis (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.

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). Malate dehydrogenase (MDH; E.C. 1.1.1.37) activity was determined by conversion of oxaloacetate to malate (Somero and Childress 1980).

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. Molecularity 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 was observed between normoxia and moderate and severe hypoxia in muscle and in heart it was found between normoxia and severe hypoxia (Fig. 1).

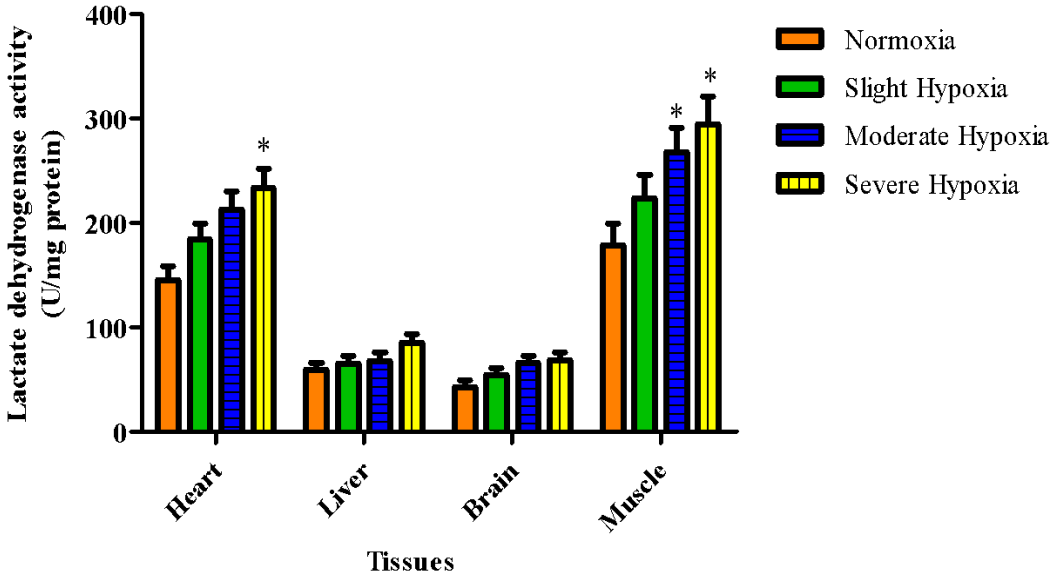

Figure-1: Mean specific activity of lactate dehydrogenase (LDH) enzyme (U/mg protein) in muscle, brain, heart and liver of *Heteropneustes fossilis* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk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

LDH activity in *Cyprinus carpio*

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 heart (Fig 2).

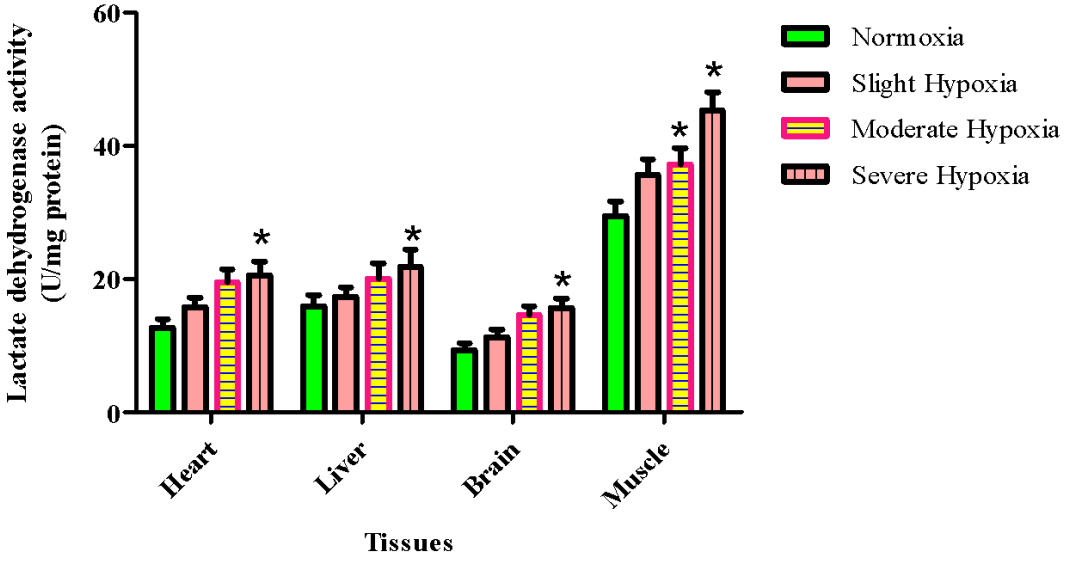

FIGURE-2: Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in muscle, brain, heart and liver of *Cyprinus carpio* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents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

MDH activity in *Heteropneustes fossilis*

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

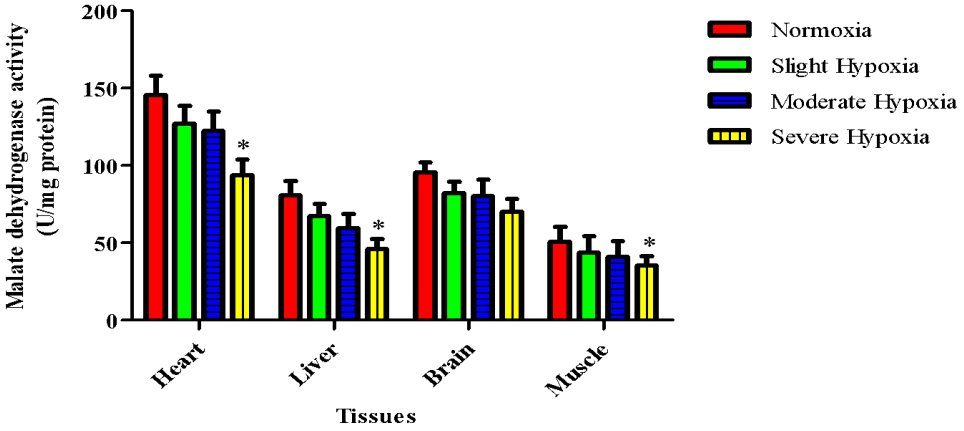

Figure-3: Mean specific activity of malate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Heteropneustes fossilis* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*)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

MDH activity in *Cyprinus carpio*

MDH activity was observed to be decreased in all these tissues taken for observation during all hypoxia period. Significant changes (p≤0.05%) were observed between normoxia and moderate and severe hypoxia in brain and liver while in heart it was observed between normoxia and severe hypoxia only (Fig. 4).

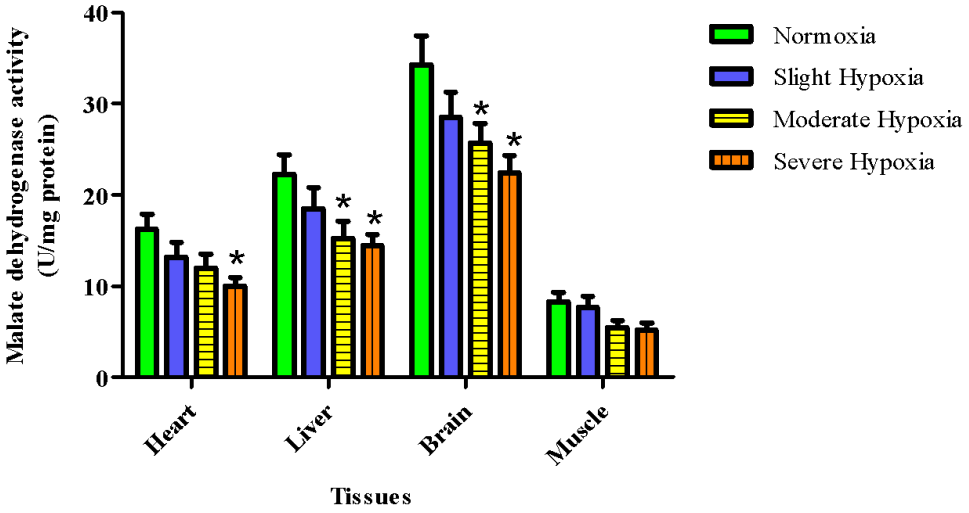

Figure-4: Mean specific activity of Malate dehydrogenase (MDH) enzyme (U/mg protein) in heart, liver, brain and muscle of *Cyprinus carpio* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*)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

SDS-PAGE analysis in *Heteropneustes fossilis*

In hypoxic heart 35.1kD and 66.8kD protein bands were absent. 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 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. 5).

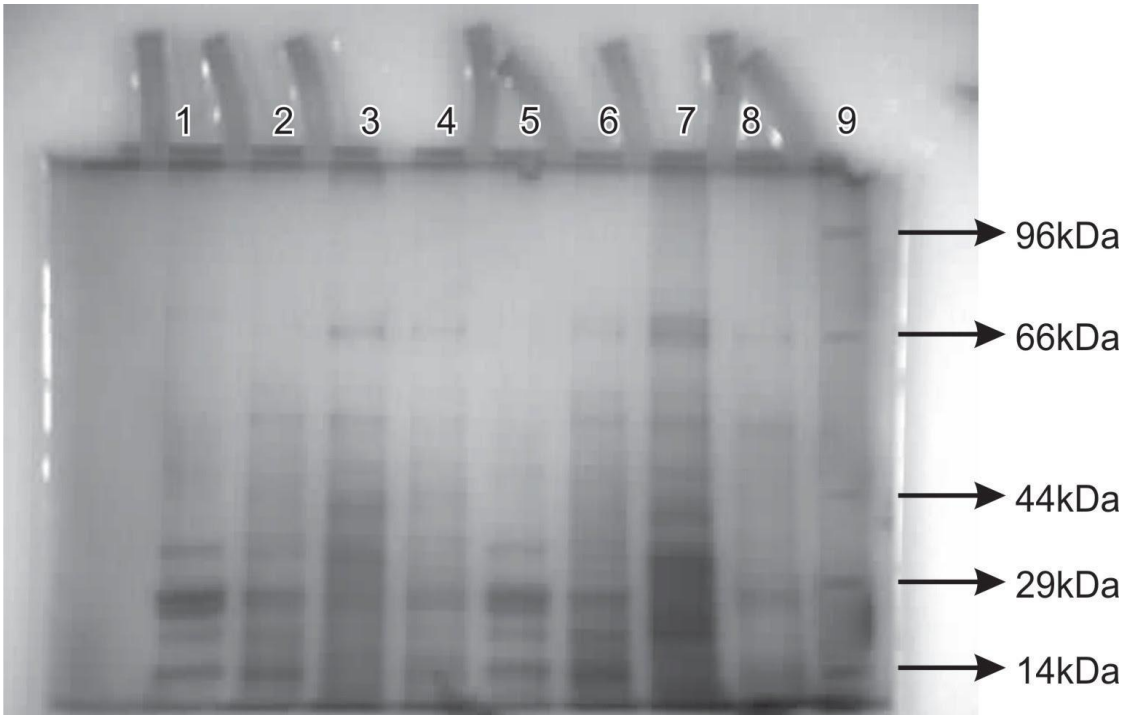

Figure-5: SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Heteropneustes fossilis*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle, lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia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

SDS-PAGE analysis* in *Cyprinus carpio

In hypoxia heart 29.5kD and 55.7kD protein bands were absent and 96.7kD extra protein bands was present (Table 6). In hypoxia liver extra protein bands of mol. wt. 29.4kd, 40.8kD and 66.2kD were found while 14.1kD, 23.4kD, 45.5kD and 90.2kD protein bands were absent. In hypoxia brain protein bands having mol. wt. 44.5kD and 50.4kD were absent. No pronounced change in muscle protein banding pattern was observed during hypoxia when compared with normoxia (Fig. 6).

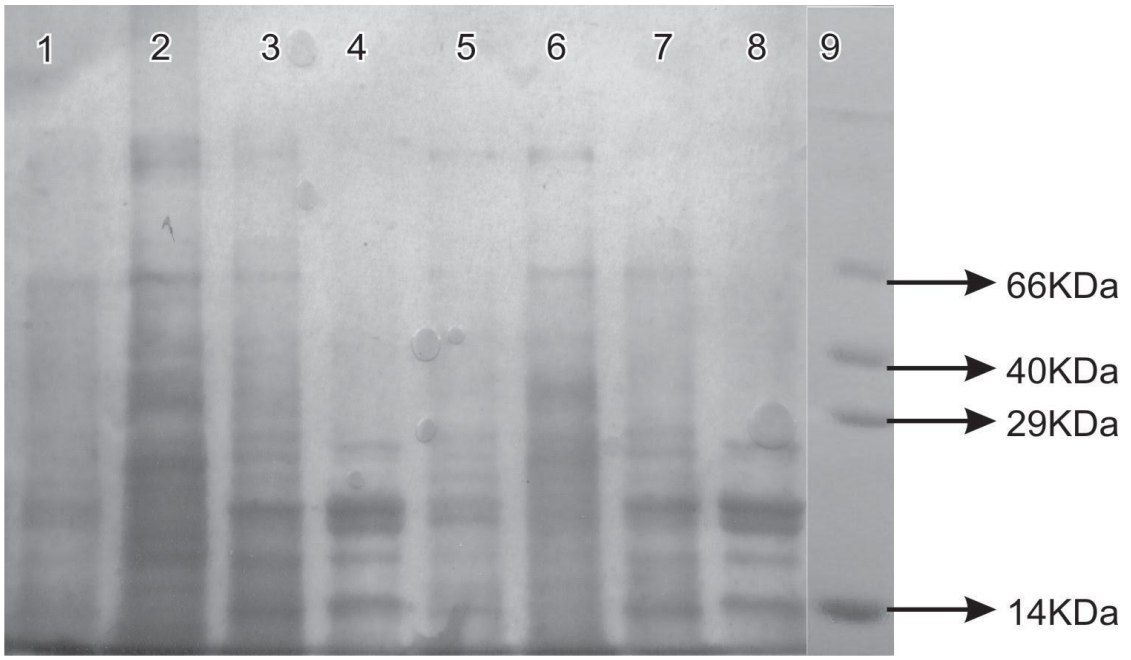

Figure-6: SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Cyprinus carpio*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle, lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia muscle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

Discussion

Heteropneustes fossilis showed higher LDH activity after exposure to different ranges of hypoxia. In *Cyprinus carpio,* the magnitude of increase in LDH activity decreases with increasing hypoxia. This shows less efficiency of this species against severe hypoxic conditions. Results observed in *C. carpio* suggest that non-air-breathing fishes found in Indian fresh water do not depend upon anaerobic respiration in hypoxic conditions. These fishes have less degree of hypoxia tolerance as compared to air-breathing catfishes.

At very low oxygen concentrations, many fishes rely on an increase in anaerobic carbohydrate metabolism to supplement aerobic energy production (Van den Thillart & Van Waarde, 1985; Virani & Rees, 2000). In some species, this shift is correlated with increased tissue LDH activity (Greaney et al., 1980; Van den Thillart & Van Waarde, 1985). In *Heteropneustes fossilis* maximum percentage increase in LDH activity was found in muscle (64.70%) followed by heart (60.21%). Significant changes (p≤0.05%) were observed in LDH activities between normoxia and severe hypoxia in muscle and heart.

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 2016; Kumar 2017; Kumar *et al.,* 2020 and Kumar 2021[1]; Kumar 2021[2]).

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 *C. carpio* there are fewer protein bands found in hypoxia heart and muscle tissue than in the liver and brain during hypoxia. These results of protein metabolism of *Heteropneustes fossilis* in comparison to *C. carpio* showed 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 *Cyprinus carpio,* we can say that the *Heteropneustes fossilis* is more tolerant to graded hypoxia than the *Cyprinus carpio.*

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