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# Effect of Hypoxia and Metabolic Adaptations in Common Carp, *Cyprinus Carpio*

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**Abstract**

Hypoxia is a frequently occurring environmental phenomenon in the freshwater and even coastal system of a tropical country like India. It may be naturally occurring phenomenon due to biological and physical factors or may be caused due to anthropogenic activities around the water bodies. In tropical aquatic system such as India, dissolved oxygen in the water of pools, swamps, lakes and rivers may change radically, from almost 100% saturation or sometimes hyper saturation at noon to zero at night of the same day. Sharp seasonal fluctuations also occur frequently occurring. Low oxygen concentration occurs in a wide range of aquatic systems and range in temporal frequency, seasonality and persistence. These have always been naturally occurring low oxygen habitat but anthropogenic activities related primarily to organic and nutrient enrichment have led to increase in hypoxia and anoxia both in fresh as well as marine system. Freshwater systems are more frequently faced with low oxygen condition and fishes in a tropical country like India are quite frequently exposed to this. The general public is aware of the results of hypoxia as the phenomenon of “Fish Kills” occurring frequently in natural waters.

**Keywords:** Hypoxia; SDS-PAGE; LDH; MDH; Glucose; Lactate; Protein

**Introduction**

Dissolved oxygen is one of the most important environmental factors to sustain lives of fish and other aquatic organisms which rely on aquatic respiration alone. In oxygen deficient environment the supply of oxygen is less than required or consumption exceeds supply. Dissolved oxygen in such condition can decline from the levels required by most animal lives generating hypoxic condition.

The link between hypoxia and fish responses combines behavioural and physiological strategies that can mitigate the effect of exposure to hypoxia. It may limit the energy budget or scope of growth and activity of an organism. In general the responses may be manifested at three levels:

1. Behaviour to avoid hypoxic areas, utilize well aerated micro environments or reduce activity (Kraemer, 1987; Van den Thillart *et al.,* 1994; Dalla via *et al.* 1998; Wannamaker and Rice; 2000)
2. Physiological and morphological adjustments that improve the oxygen extraction and delivery to tissues (Jensen *et al.,* 1993; Sollid *et al.,* 2003)
3. Biochemical changes that increase the capacity of tissues to function and survive at low oxygen (Hochachka, 1980; Van den Thillart and Van Waarde, 1985; Hochachka and Somero, 2002).

Animals exposed to periods of hypoxia show adaptations at the behavioural, morphological and physiological all the three levels. At physiological level, fish commonly resort to one of the two strategies:

1. Maintenance of low levels of activity which is fueled by anaerobic metabolism
2. Depression of metabolism accompanied by decreasing ATP producing and consuming processes (Boutilier 2001; Lutz and Nilsson 1997).

In order to survive this important environmental parameter air-breathing fishes, like other fish species, require a series of coordinated metabolic adjustments aimed to balance overall suppression of systemic ATP demand along with a proportional increase in the fraction of remaining metabolism that is supported by anaerobic glycolysis alone. This has been

observed in case of Amazon fish species in response to seasonal variance in oxygen availability in the Amazonian water bodies (Almeida Val *et al.,* 2000). Experiments framed in laboratory on these fish species have been observed to tolerate the hypoxia by reducing their standard metabolic rates (Muusez *et al.,* 1998). The fish in such condition have been observed to down regulate the absolute enzyme levels and up-regulate the tissues glycolytic capacity.

**Review of Literature**

Effect of oxygen deficiency on fish had drawn the attention of scientists as early as 1920s and extensive literature is available on fish during that period (Gardner, 1926). 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.

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). Chabot and Dutil, (1999) and Pichavant *et al.,* (2000, 2001) studied the effects of chronic (weeks of) hypoxia on food intake, whereas effect of hypoxia on reproduction has been studied by Wu *et al.,* (2003).

**Aim of the Study**

Because of the link between urbanization and increased anthropogenic activities and the increase in their adverse effect on aquatic system there is a need to understand the mechanisms behind the observed effect of hypoxia and improved hypoxia tolerance. The present piece of work aims to analyze the response of protein profiling, enzyme assay and blood parameters to different degrees of hypoxia in *Cyprinus carpio*.

**Materials and Methods**

Live specimens (6 fishes) of *Cyprinus carpio (80-100 g, 14-16 cm 0-90 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 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). Protein concentration of different tissues was estimated by Folin-phenol method (Lowery et al., 1951). SDS-PAGE technique was carried out in Mini-PROTEAN Tetra System of BIO-RAD using a 5% (w/v) separating gel (4).

**Observation**

**LDH activity**

**Table 1**. Mean specific activity of lactate dehydrogenase (LDH) enzyme (Units/mg proteins) in different tissues of *Cyprinus carpio* subjected to slight, moderate and severe hypoxia for same time duration (12h)

| Tissue | Normoxia | Slight Hypoxia | Moderate Hypoxia | Severe Hypoxia |
|---|---|---|---|---|
| Heart | 12.60±1.35 | 15.77±1.4 | 16.50±1.85 | 17.60±2.06 |
| Liver | 15.92±1.6 | 17.25±1.51 | 20.02±2.34 | 21.76±2.56 |
| Brain | 9.31±0.96 | 11.23±1.18 | 13.56±1.38 | 14.60±1.45 |
| Muscle | 29.41±2.16 | 35.61±2.32 | 37.13±2.45 | 45.29±2.64 |

Highest LDH activity was observed in muscle followed by liver and heart. Lowest LDH activity was observed in brain (Table 1). LDH activity was observed to be increased in all these tissues taken for observation in all hypoxia period. During slight hypoxia maximum increase in LDH activity was observed in heart (25.15%) followed by muscle (21.08%) and brain (20.62%). During moderate hypoxia maximum increase in LDH activity was observed in brain (45.64%) followed by heart (30.95%) and muscle (26.19%). During severe hypoxia maximum increase in LDH activity was observed in brain (56.82%) followed by muscle (54.04%) and heart (39.68%). Significant changes (p≤0.05%) in LDH activities were observed between normoxia and severe hypoxia in muscle and heart (Fig 1).

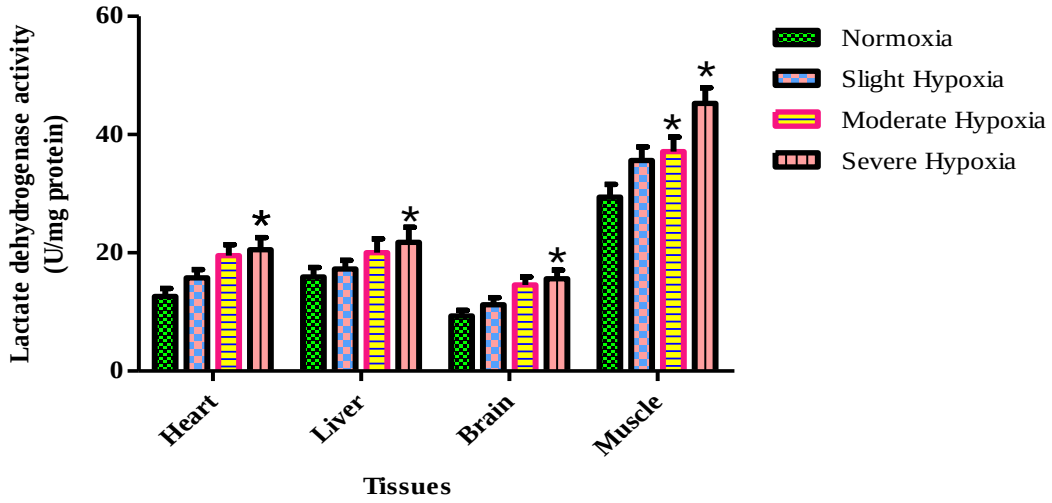


**Figure-1:** Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Cyprinus carpio* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*)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

**MDH activity in *Cyprinus carpio***

**Table-2**: **Mean specific activity of malate dehydrogenase (MDH) enzyme (Units/mg proteins) in different tissues of *Cyprinus carpio* subjected to slight, moderate and severe hypoxia for same time duration (12h)**

| Tissue | Normoxia | Slight Hypoxia | Moderate Hypoxia | Severe Hypoxia |
|---|---|---|---|---|
| Heart | 16.21 ±1.7 | 13.19 ±1.56 | 11.91±1.62 | 10.02 ±0.89 |
| Liver | 22.29 ±2.1 | 18.47 ±2.3 | 15.23±1.89 | 14.42 ±1.2 |
| Brain | 34.23 ±3.2 | 28.53 ±2.75 | 25.64 ±2.16 | 22.40 ±1.93 |
| Muscle | 8.29 ±0.99 | 7.69 ±1.2 | 5.4 ±0.83 | 5.19 ±0.76 |

MDH activity was observed to be decreased in all these tissues taken for observation during all hypoxia period. Highest MDH activity was observed in brain followed by liver and heart. Lowest MDH activity was observed in muscle (Table 2). During slight hypoxia maximum decrease in MDH activity was observed in heart (18.63%) followed by liver (17.13%). During moderate hypoxia maximum decrease in MDH activity was observed in muscle (34.86%) followed by liver (31.67%) and heart (26.53%). During severe hypoxia maximum decrease in MDH activity was observed in heart (38.18%) followed by liver (35.30%) and brain (35.56%). 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. 2)

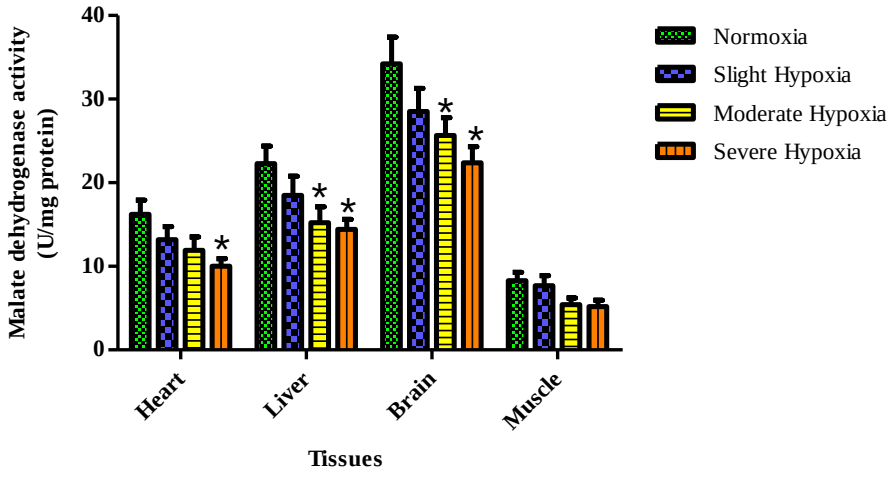


**Figure-2:** Mean specific activity of Malate dehydrogenase (MDH) enzyme (U/mg protein) in heart, liver, brain and muscle of *Cyprinus carpio* exposed to varying oxygen concentration i.e. different

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.

**Protein content in *Cyprinus carpio***

**Table-3**: Determination of protein content (mg/gm wet weight) in different tissues of *Cyprinus carpio* subjected to slight, moderate and severe hypoxia for same time duration (12h)

| Tissue | Normoxia | Slight Hypoxia | Moderate Hypoxia | Severe Hypoxia |
|---|---|---|---|---|
| Heart | 25.2±2.34 | 18.3±2.12 | 20.4±1.91 | 16.5±1.72 |
| Liver | 34.2±3.1 | 36.4±3.4 | 32.5±2.7 | 26.2±2.3 |
| Brain | 28.25±2.7 | 27.4±2.5 | 25.5±2.4 | 13.5±1.3 |
| Muscle | 22.9±2.1 | 18.7±1.7 | 13.5±1.3 | 10.1±1.1 |

Highest protein content was observed in liver followed by brain and heart and lowest in muscle during normoxia (Table 3). Protein content was observed to be decreased in all the tissues except in liver during slight hypoxia suggest that the metabolism during hypoxia is depressed. Maximum decrease in protein content was found in muscle (55.89%) followed by heart (34.52%) and liver (23.39%) during severe hypoxia. During slight hypoxia maximum decrease in protein content was observed in heart (48.08%) and muscle (27.05%) followed by liver (14.07%). No pronounced changes were observed in brain during this period. During moderate hypoxia maximum decrease in protein content was observed in muscle (55.89%), heart (19.04%) and brain (9.84%) followed by liver (4.97%). Protein content in different tissues did not show significant differences between normoxia and slight and moderate hypoxia except in muscle between normoxia and moderate hypoxia. Significant changes (p≤0.05%) were observed between normoxia and severe hypoxia in muscle and brain (Fig. 3).

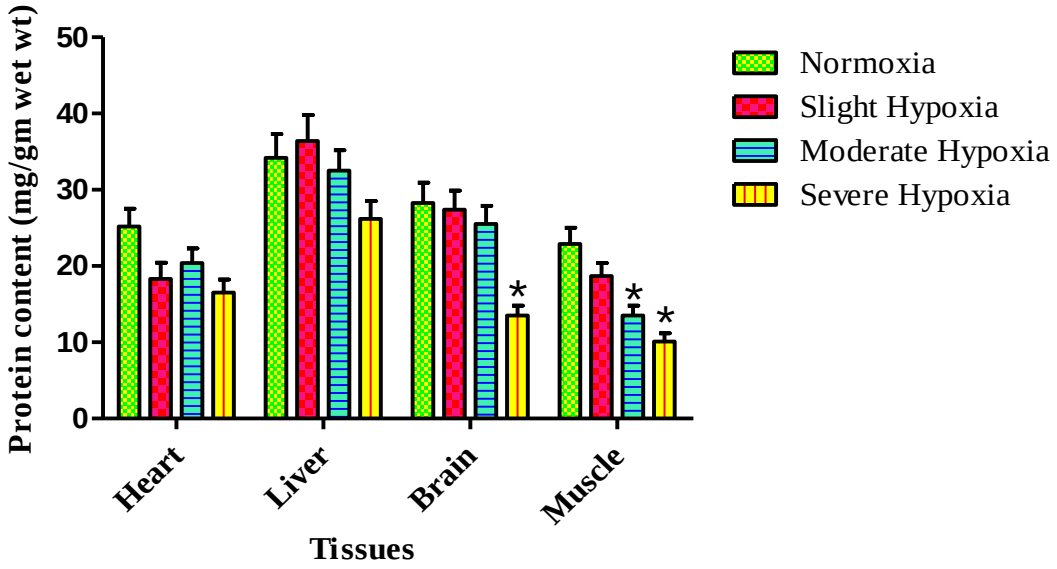


**Figure-3:** Mean protein content(mg/gm wet weight) in heart, liver, brain and muscle of *Cyprinus carpio* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (Values are means±s.e.m.,n=6). Asterisk (*) represents significant differences ($p$<0.05) between normoxia and different range of hypoxia.

***SDS-PAGE analysis* in *Cyprinus carpio***

**Table-4:** Molecular weight (kDa) of protein/peptide bands obtained from different tissues of *Cyprinus carpio* subjected to hypoxia for same time duration (12h)

| Lane 1 NH | Lane 2 NL | Lane 3 NB | Lane 4 NM | Lane 5 HH | Lane 6 HL | Lane 7 HB | Lane 8 HM |
|---|---|---|---|---|---|---|---|
| 14.7 | 14.1 | 14.1 | 14.8 | 14.7 | - | 14.1 | 14.8 |
| 20.3 | 20.8 | 20.8 | 20.7 | 20.3 | 21.8 | 20.8 | 20.7 |
| 29.5 | 23.4 | 29.4 | 29.4 | - | 29.4 | 29.4 | 29.4 |
| 32.0 | 32.1 | 32.1 | 36.3 | 32.0 | - | 32.1 | 36.3 |
| 36.1 | 36.2 | 36.2 | | 36.1 | 36.2 | 36.2 | |
| 40.8 | 44.5 | 44.5 | | 40.8 | 44.5 | - | |
| 55.7 | 56.4 | 50.4 | | - | 56.4 | - | |
| 66.5 | 90.2 | 66.2 | | 66.5 | 66.2 | 66.2 | |
| | 96.5 | 96.5 | | 96.7 | 96.5 | 96.5 | |

Marker protein in lane-9 as shown in figure-5. NH-Normoxia Heart; NL-Normoxia Liver; NB-Normoxia Brain; NM-Normoxia Muscle; HH-Hypoxia Heart; HL-Hypoxia Liver; HB-Hypoxia Brain; HM-Hypoxia Muscle.

In hypoxia heart 29.5kD and 55.7kD protein bands were absent and 96.7kD extra protein bands was present (Table 18). 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. 4).

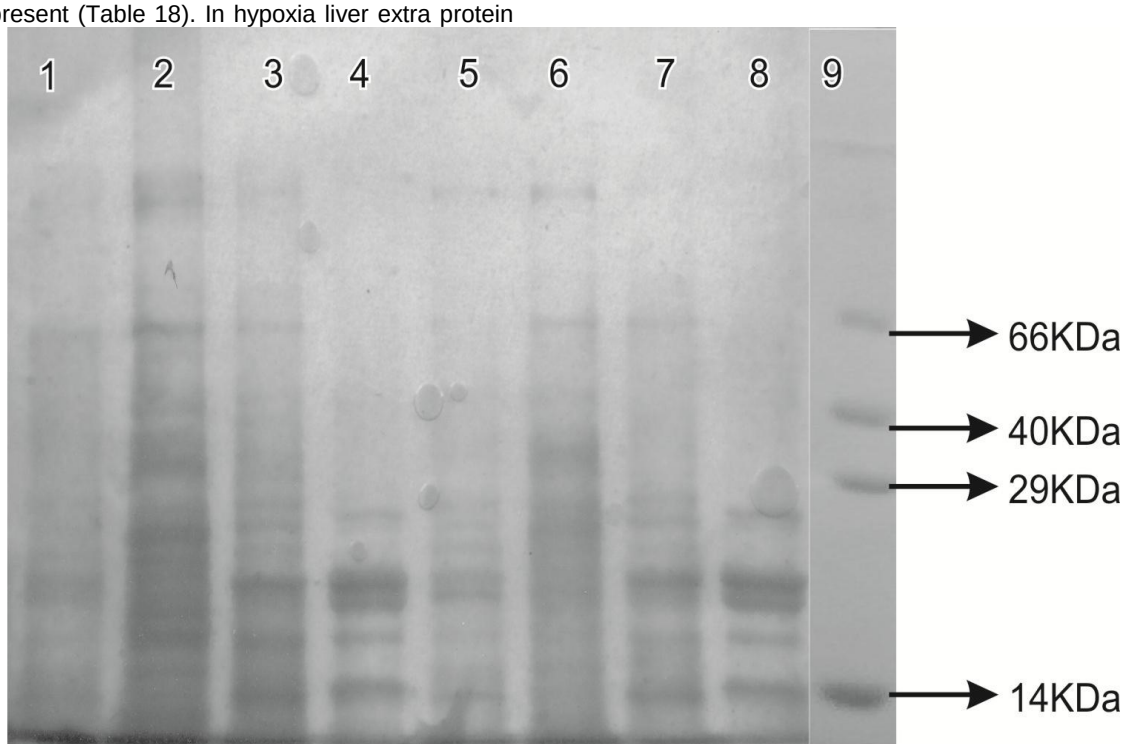


**Figure-14:** SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Cyprinus carpio*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle, lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia muscle and lane 9: mol. wt. Marker (Sigma wide range marker).

**Discussion**

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 enzymes specific activities were higher in liver tissue after exposure to hypoxia, suggesting increased capacity for carbohydrate metabolism. 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. Its worth noticing that the blood that goes to brain and eye in these catfishes has to pass through carotid labyrinth of these fishes which is a known chemosensory organ. This structure is known to be involved in skimming and checking of blood for enzymes and oxygen both (Munshi and Hughes, 1987)

The activity of gluconeogenic enzyme MDH was observed to be lower in liver tissue in decreasing order in all the four fish observed. The decreased activity of this enzyme in liver is known to be coupled with increased protein catabolism in skeletal muscle (Martinez *et al*., 2003).

Increased levels of glycolytic 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* and *Heteropneustes fossilis,* it can be correlated with frequent movement of fish to the surface at the onset of hypoxia (A. Kumar and A. Gopesh 2015; A. Kumar 2015; A. Kumar 2018; A. Kumar 2019; A. Kumar, A. Gopesh and S. Sundram 2020). 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.

Brain, liver and heart are known as aerobic tissues which normally tend to avoid anaerobic accumulation of lactate. Therefore the LDH level is adjusted in these tissues according to the degree of exposure to hypoxia (Almeida-Val *et al*., 2000). The LDH levels observed in different catfishes (A. Kumar and A. Gopesh 2015; A. Kumar 2015; A. Kumar 2017; A. Kumar 2018; A. Kumar 2019; A. Kumar, A. Gopesh and S. Sundram 2020) has been found to support this observation. Specific activities of glycolytic enzyme in muscle have earlier been correlated with the burst

swimming activity of fish in response to various stresses in Atlantic Cod *Gadus morhua* (Somero and Childress, 1980; Pelletier *et al*, 1993).

Thus it can be assumed that the oxygen uptake from the air was not sufficient to sustain complete aerobic metabolism at this aquatic tension and that the fish was metabolically in a hypoxic condition during which anaerobic glycolysis was activated. At this stage LDH activity in oxidative tissue like liver was observed to be increased. Higher LDH activities have been observed earlier in liver and gill under hypoxic stress in *C. batrachus* by Tripathi *et al*. (2013). Present investigation also reported increased LDH activity in liver. Role of higher LDH activity in liver and gill tissue under hypoxic stress is attributed to the clearance of blood lactate and provision of glucose for metabolism by extra hepatic tissues such as heart and brain, which are important organs involved in the maintenance of homeostasis of animals (Martinez *et al*., 2003).

According to Walton & Cowery (1982), carbohydrate metabolism is not believed to be a major energy source in fish, but it is reasonable to assume that its importance increases during hypoxia because of activation of anaerobic glycolysis.

By contrast, the common carp *Cyprinus carpio*, which maintains low levels of activity during hypoxia/anoxia exposure, exhibits a depression in protein synthesis of approximately 35% in heart and 55% in muscle, 25% in liver tissue, but no significant depression in the brain (Smith *et al*., 1996).

**Conclusion**

Acute hypoxia in *Cyprinus carpio* (water breathing) induced an increase in blood glucose levels. Except by the brain, where we observed a suppression of LDH and an increase in MDH, no other enzyme change was observed. Based on the results obtained, it can suggest that *Cyprinus carpio* is not tolerant to acute hypoxia, but could be tolerant to graded hypoxia; otherwise it would not be able to remain in places with low oxygen levels.

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