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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Blood Metabolic Adaptations in Non Air-Breathing Catfish *Mystus seenghala*

Ajay Kumar
Assistant Professor,
Deptt. of Zoology,
Government Degree College,
Bahua Dehat, Fatehpur, U.P.
India

Abstract

Aquatic organisms which are frequently exposed to hypoxia show adaptations at behavioural, morphological and physiological levels. To assess the effect of hypoxia at physiological level, change in protein profiling and blood metabolites in selected tissues of cat fish, *Mystus seenghala* was undertaken. Fish were exposed to experimentally provoked hypoxia for different duration and were sacrificed to study the effect of hypoxia on selected blood parameters and protein profiling in heart, liver, brain and muscle. Significant changes were recorded. The observations indicate that different tissues respond differently to the stress of hypoxia and the blood parameters respond in a tissue specific manner.

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: Protein Profiling, Blood Metabolites, Catfish, Hypoxia, Glucose, Lactate.

Introduction

Throughout the world large areas of fresh and coastal waters are becoming polluted that lack sufficient oxygen, one of the basic building blocks of life. This condition is called "Hypoxia". Hypoxia means "low oxygen" in aquatic ecosystems, low oxygen usually meaning a concentration of less than 2-3 mg of O_2/litre of water (Mg/l) in marine and 4-5 mg O_2/l in fresh water systems. These waters are usually acidic, rich in CO_2 and H_2S (Almeida-Val and Hochachka, 1995) but are observed to be varied in oxygen concentrations. Hypoxia is frequently accompanied by hypercapnia (elevation of CO_2 in water) acidification of the body tissue, including blood (Burnett and Stickle, 2001)

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.

Animals exposed to periods of hypoxia show adaptations at the behaviour, 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 fuelled by anaerobic metabolism
2. Depression of metabolism accompanied by decreasing ATP producing and consuming processes (Boutilier, 2001; Lutz and Nilsson 1997).

Effect of Hypoxia

Hypoxia can have profound effects on different organisms. In fishes the effect differs in fishes of different respiratory habits. So far the ultimate effect observed at individual and population levels can be enumerated as:

1. Reduced fish growth rates, limiting or productive habitat, increase in mortality of young fish (Burnett and Stickle, 2001).
2. Decrease in feeding habit (Burnett and Stickle, 2001).
3. Decrease in growth rate (Almeida-Val *et al.*, 2000).

4. Increase in ventilation rate (Somero and Childress, 1980; Pelletier *et al.*, 1993).
5. increase in flow of blood on respiratory surfaces
6. switching from aerobic to anaerobic metabolism
7. reduction in their overall metabolism
8. inhibition of immune responses (Thomas *et al.* 2003) causing greater mortality
9. negative effect on reproduction (Wu *et al.,* 2003; Braun *et al.*, 2006).
10. The role of apoptosis in response to hypoxia has also been studied *in vitro* in other Vertebrates.

On the whole hypoxia may limit the energy budget or scope of growth and overall activity of an organism.

Review of Literature

The low oxygen is a major stress in the environment was inferred by the extensive researches of Jones (1952). Kutty (1968) and 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), Kutty (1968), Bushnell *et al.,* (1984). Dutil and co-workers (2007) investigated swimming performance of fishes during different periods of hypoxia. Greaney *et al.,* (1980); Taylor and Miller, (2001); Pichavant *et al.,* (2003) studied the effects of chronic (weeks of) hypoxia on oxygen carrying capacity.

Dreidzic & Hochachka (1975) explored the possibility of accumulation of lactate in carp and revealed that the low rate of lactate accumulation in the carp white muscle during hypoxia could be explained by the existence of alternative anaerobic pathways to glycolysis in this tissue, as occurs in the muscles of numerous facultative anaerobes and diving mammals (Magnum & van Winkle, 1973; Hochachka, Owen, Allen &Whittow, 1975). In these animals, during anaerobiosis, carbohydrates and amino acids are utilised simultaneously with the production of a variety of end products (Hochachka, 1975). The coupling of two mitochondrial energy yielding reactions to glycolysis allows both an increase in the high energy phosphate equivalents and the maintenance of redox balance within the cell (Hochachka, Fields & Mustafa, 1973; Hochachka, 1975). However, no evidence has been found for the accumulation of multiple anaerobic end products in the white muscle of either the mirror (Driedzic &Hochachka, 1975) or crucian carp (Johnston, 1975a).

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).

The aquatic organismsnot having the option to escape from low oxygen concentrationsare known to adapt other mechanisms (Boutilier, 2001). For these species, the major metabolic exercise is to decrease the capacity for ATP production via oxidative phosphorylation, leaving animals to rely on anaerobic glycolysis and/or fermentation to maintain energy equilibrium (referred to as the Pasteur effect).

However the heart and brain are affected less when exposed to hypoxia and the rate of glucose utilization by the teleost brain is thought to be limited by hexokinase activity alone (Soengas and Aldegunde, 2002).

The protein synthesis is one of the major energy consuming processes, accounting for 18-26% of cellular energy expenditure (Hawkins, 1991). But Guppy *et al.,* (1994) observed that the down regulation of protein turnover is one of major contributing factors to the depression in ATP turnover and metabolic depression at the whole animal level.

Jibb and Richards, (2008) found in their studies that depressing metabolic rate during hypoxia is a key mechanism for the conservation of endogenous substrates thereby extending the amount of time that can be spent under oxygen limiting conditions.

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:

1. 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 and blood parameters to different degrees of hypoxia in Cypriniforms, mainly catfishes, which present different respiratory patterns.

Materials and Methods

Live specimens of *Mystus seenghala (80-90 g 14-16 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 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-breath) for collection of different tissues. 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 temperature.

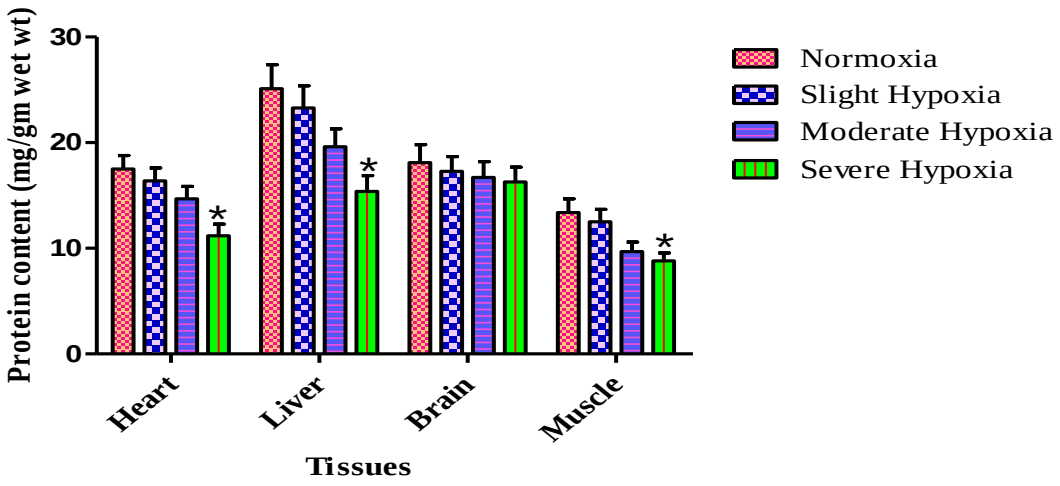
Protein Content in *Mystus seenghala*

Table-1: Determination Of Protein Content (Mg/Gm Wet Weight) In Different Tissues Of *Mystus seenghala* Subjected To Slight, Moderate And Severe Hypoxia For Same Time Duration (12h)

Tissue	Normoxia	Slight Hypoxia	Moderate Hypoxia	Severe Hypoxia
Heart	17.5±1.29	16.4±1.21	14.7±1.17	11.2±1.08
Liver	25.1±2.3	23.3±2.1	19.6±1.7	15.4±1.5
Brain	18.1±1.7	17.3±1.4	16.7±1.5	16.3±1.4
Muscle	13.4±1.3	12.5±1.2	9.7±0.9	8.8±0.75

Highest protein content was observed in liver and brain followed by heart and lowest in muscle during normoxia (Table 1). Protein content was observed to be decreased in all the tissues suggest that the metabolism during hypoxia is depressed in *Mystus seenghala.* Maximum decrease in protein content was found in liver (38.64%) followed by heart (36.00%) and muscle (34.32%) during severe hypoxia. During slight hypoxia maximum decrease in protein content was observed in liver (7.17%) and muscle (6.71%) followed by heart (6.28%). No pronounced changes were observed in brain during this period. During moderate hypoxia maximum decrease in protein content was observed in muscle (27.61%) and liver (21.91%) followed by heart (16.00%). Protein content in different tissues did not show significant differences between normoxia and slight and moderate hypoxia. Significant changes ($p \leq 0.05\%$) observed between normoxia and severe hypoxia in liver, muscle and heart (Fig. 1).

Figure-1: Mean Protein Content (Mg/Gm Wet Weight) in Heart, Liver, Brain and Muscle of *Mystus seenghala* 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 *Mystus seenghala*

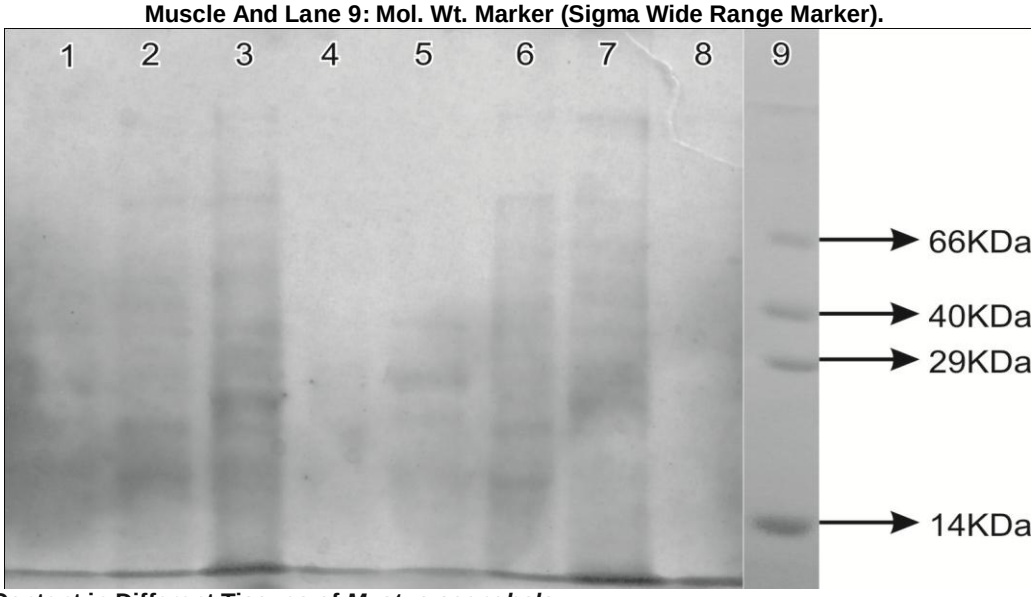
Table-2: **Molecular Weight (Kda) of Protein/Peptide Bands Obtained From Different Tissues of *Mystus seenghala* subjected to Hypoxia For Same Time Duration (12h)**

Lane 1 NH	Lane 2 NL	Lane 3 NB	Lane 4 NM	Lane 5 HH	Lane 6 HL	Lane 7 HB	Lane 8 HM
29.5	29.5	29.5	29.5	29.5	29.5	***14.0***	29.5
32.0	36.0	***36.0***	36.0	32.0	36.0	29.5	-
36.1	44.1	40.1	44.1	36.1	44.1	40.1	44.1
44.8	50.8	44.8	60.8	44.8	50.8	44.8	60.8
	55.2	***48.2***			55.2	50.5	
	66.5	50.5			66.5	55.4	
		55.4			***96.0***	66.2	
		66.2				96.5	
		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 no change in protein banding pattern was observed (Table 2). In hypoxia liver one extra protein bands of 96.0kD was found while no other changes in protein bands were observed. In hypoxia brain one extra protein bands 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 (Fig 2).

Figure-2: 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).



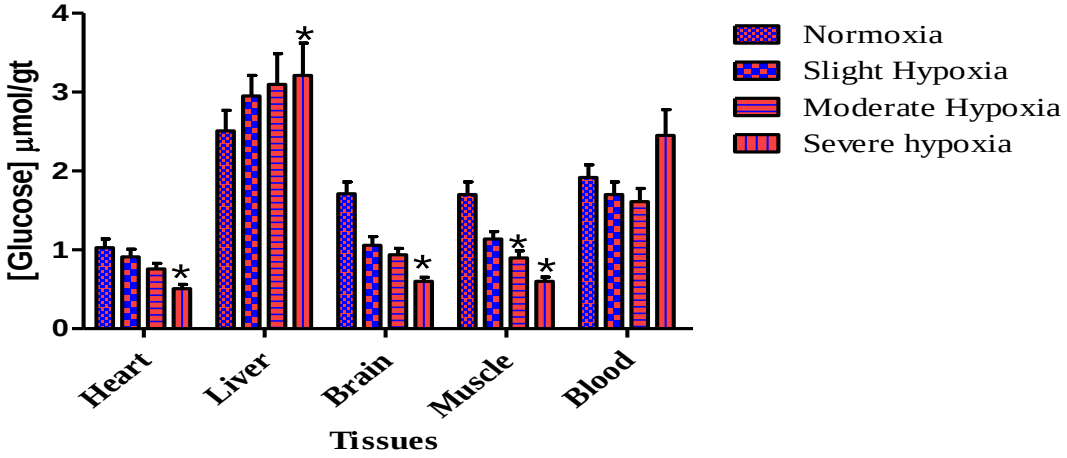
Glucose Content in Different Tissues of *Mystus seenghala*

Table-3: Determination of Tissue Glucose Content in Different Tissues of *Mystus seenghala* Subjected to Slight, Moderate and Severe Hypoxia For Same Time Duration (12h)

Tissues	Normoxia	Slight Hypoxia	Moderate Hypoxia	Severe hypoxia
Heart	1.03±0.11	0.91±0.10	0.76±0.069	0.51±0.053
Liver	2.51±0.26	2.95±0.26	3.10±0.39	3.21±0.090
Brain	1.71±0.15	1.06±0.11	0.94±0.08	0.60±0.050
Muscle	1.70±0.16	1.14±0.09	0.90±0.088	0.60±0.056
Blood	1.92±0.16	1.70±0.16	1.61±0.17	2.45±0.330

Highest glucose content was observed in liver followed by blood and Brain and lowest glucose content was observed in Heart (Table 3). During slight hypoxia liver showed increasing trend while other tissues showed decreasing trend. Maximum decrease was observed in brain (38.01%) followed by muscle (32.94%). At the moderate hypoxia stage, maximum decrease in glucose content was observed in muscle (47.05%) followed by brain (15.67%). During severe hypoxia increase in glucose content was observed in liver (27.88%) and blood (27.60%) while it was observed in decreasing trend in brain (38.38%), muscle (32.41%) and heart (50.48%). Significant changes ($p \leq 0.05\%$) were observed between normoxia and severe hypoxia in heart, liver, brain and muscle (Fig. 3)

Figure-3: Glucose Concentrations in Different Tissues of *Mystus seenghala* Submitted to Normoxia and Different Periods of Hypoxia. Error Bars are Within Limits of Symbols When Not Visible. Values are Means ± SD, N = 6. * P< 0.05.



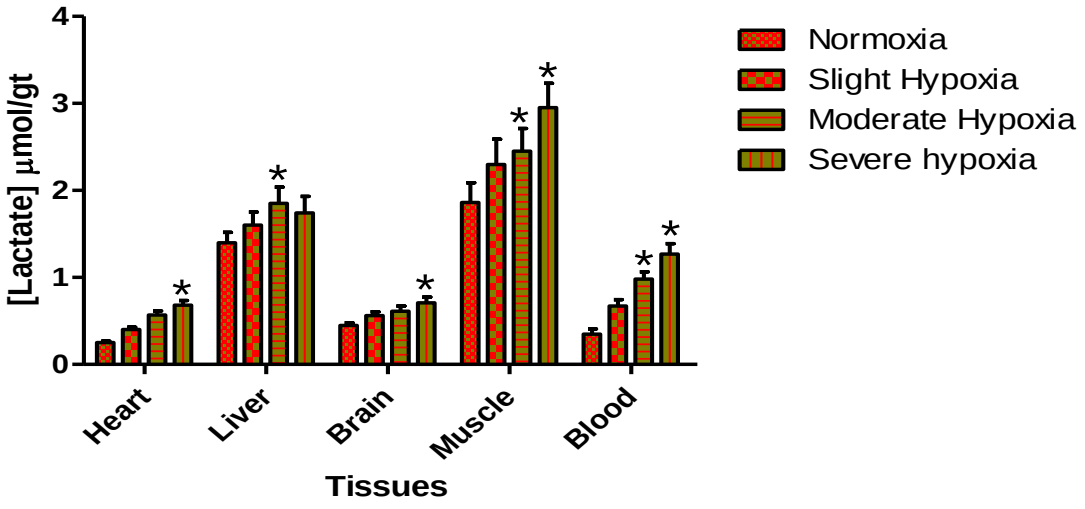
Lactate content in different tissues of *Mystus seenghala*

Table-4: Determination of Tissue Lactate Content in Different Tissues of *Mystus seenghala* Subjected To Slight, Moderate and Severe Hypoxia for Same Time Duration (12h)

Tissues	Normoxia	Slight Hypoxia	Moderate Hypoxia	Severe hypoxia
Heart	0.25±0.017	0.400±0.029	0.57±0.045	0.68±0.055
Liver	1.40±0.120	1.600±0.15	1.85±0.190	1.74±0.190
Brain	0.45±0.030	0.560±0.046	0.61±0.060	0.71±0.065
Muscle	1.86±0.230	2.300±0.29	2.45±0.260	2.95±0.280
Blood	0.35±0.060	0.670±0.074	0.98±0.085	1.27±0.120

Highest lactate content was observed in muscle followed by liver and brain and lowest lactate content was observed in heart (Table 4).During slight hypoxia all tissues showed increasing trend in lactate accumulation as the fish rely mostly upon anaerobic respiration for its energy requirements and metabolic depression. Maximum increase was observed in blood (91.42%) and heart (60.00%) followed by muscle (23.65%). At the moderate hypoxia stage, maximum increase in lactate content was observed in blood (nearly 3-fold) followed by heart (2.2-fold). During severe hypoxia maximum increase in lactate content was observed in blood (3.6-fold). Significant changes (p≤0.05%) were observed between normoxia and moderate and severe hypoxia in all the tissues (Fig. 4).

Figure-4: Lactate Concentrations in Different Tissues of *Mystus seenghala* Submitted to Normoxia and Different Periods of Hypoxia. Error Bars are Within Limits of Symbols When Not Visible. Values Are Means ± SD, N = 6. * P< 0.05.



Discussion

In fish, increase in blood glucose level and decrease in liver glycogen level, are one of the first signs of stress and carbohydrate metabolism (Wepener, 1990). Stress response in fish is generally characterized by an increase in adrenalin causing mobilization of liver glycogen into blood glucose (Swallow and Flemming, 1970). Cortisol lowers the liver glycogen and increase in blood glucose during stress. Metabolic consequence of cortisol impairment may be a reduced capacity to mobilize liver glycogen stores (Hontela *et al*. 1995).

Carbohydrate metabolism mainly concerns to fulfill demands of animals by its aerobic and anaerobic segments (Nelson and Cox, 2002). The lactate levels acts as an index of anaerobiosis, which was beneficial for animal in tolerating hypoxic condition.

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 four fish species in present investigation 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).

These catfishes are well known for their “surfacing behaviour” under environmental conditions. The reduction of routine spontaneous activity during hypoxia most probably saves energy expenditure which is one of the adaptive strategies adopted by these fishes in hypoxia. When allowed to air-breath the fishes were seen hanging onto the surface, constantly for a definite period depending upon the weight of the fish. After this fish started setting down

to bottom ultimately dying if hypoxia still persisted (Gopesh, 1983). *Cyprinus carpio* and *Mystus seenghala* showed restlessness initially and then succumbed to prolonged hypoxia. Both these fishes did not show "surfacing behaviour" as these are commonly known as non air-breathing fish species.

Among catfishes *C. batachus* and *H. fossilis* showed higher LDH activity after exposure to different range of hypoxia (A. Kumar 2016). In *Mystus seenghala,* magnitude of increase in LDH activity decreases with increasing hypoxia level. This shows less efficiency of this species against severe hypoxic condition. This level of increase in LDH activity in *Mystus seenghala* is most probably due to the fact that it did not rely upon anaerobic respiration liker air-breathing catfish.

Glucose and lactate changes during hypoxia are showed in Fig2-3 in all the *Clarias batrachus*. Blood did not show significant change in glucose concentrations during hypoxia, which explains the increases and decreases of this metabolite within the tissues only. Liver showed a sharp decrease after four hours of hypoxia and subsequent recuperation, probably due to ASR. The lack of glucose increase in liver supports the conclusion that glycogenolysis was not activated in the slight and severe hypoxia but that glucose was consumed to be re-established to normal values after this period. Muscle, heart, and brain showed significant increases in glucose after severe hypoxia probably due to glycogenolysis activation.

Dunn & Hochachka (1986, 1987), both, reported an increase in glucose after hypoxia in trout *Salmo gairedeneri*. 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 its role in activation of anaerobic glycolysis activation.

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 (Kumar A. 2017), but no significant depression in the brain (Smith *et al*., 1996). Similar to the common carp, *M. seenghala* exhibited tissue specific depression in protein synthesis when exposed to acute hypoxia exposure. Rates of protein synthesis in liver, heart and muscle were depressed by 30–40%, whereas rates of protein synthesis in the brain were depressed only by 17%. Thus, our results reinforce the idea that fish need to maintain protein synthesis in the brain to prevent damage to neural tissue, and to sustain appropriate brain functions so that predators can be effectively avoided. One of the suggested mechanisms controlling the depression of protein synthesis, and therefore depression of metabolic rate, is a decrease in pH (Hochachka and Somero, 2002; Richards *et al*., 2007). The reduction of protein synthesis has been linked to an increase in recombinant elongation factor 2 kinase (EF2K) caused by exposure to low pH (Dorovkov *et al*., 2002)

Conclusion

There is no significant lactate accumulation in white muscle after slight hypoxia. According to Jorgensen & Mustafa (1980) significantly higher values of lactate in muscle are only registered after 21 hours of hypoxia in flounder *Platichtys flesus*. The other tissues and blood show a significant increase in lactate after up to moderate hypoxia and then a drop after severe hypoxia (Kumar A. & Gopesh A. 2015; Kumar A. 2016; Kumar A. 2017). Increase in lactate after hypoxia denotes a increase in anaerobic metabolism as a source of energy. 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; Rantin *et al*., 1998). Muscle and brain do not show variations between hypoxia and normoxia. Farrel & Steffensen (1987) estimated that blood lactate oxidation can fuel approximately 20% of cardiac aerobic metabolism at rest and 100% after exercise, which is consistent with findings of Milligan & Girard (1993), showing that blood lactate is a preferred substrate for cardiac muscle metabolism.

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