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Innovation The Research Concept

Metabolic Responses to Acute Hypoxia in Two Fishes *Clarias batrachus* and *Cyprinus carpio* with Different Respiration Patterns

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Abstract

Acute hypoxia in their environment induces differential responses that result from both their specific respiration mode and their metabolic adjustments. The same environmental pressure that covered the development of different air breathing mechanisms is considered to include long and short term adaptation in fishes. These adaptations are the results of adjustments in their behavioural, physiological, biochemical and molecular characteristics which enable them to survive episodes of hypoxia. 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 *Cyprinus carpio.*

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

Introduction:

Acute hypoxia exposure has been observed to induce differential responses that result from both their specific mode of respiration and metabolic adjustments. No attention has been directed towards the comparison of the enzyme activities in their differential metabolic adjustment to hypoxia as a stress, between water-breathing carp and catfish on one hand and air-breathing and non-air-breathing catfishes, on the other. Present work is a comparative investigation on the differential response of two selected enzymes in these groups of fishes to experimentally provoked hypoxia (Kumar 2016).

Ratio between anaerobic and aerobic enzymes as LDH/CS is restricted in air breathing fishes. Heart oxidative enzymes of different Amazon fish generally present low activities compared with temperate teleost fish (Driedzic and Almeida-Val, 1996).

There is an extensive background of work, in general and in fish, in particular, using lactate dehydrogenase (LDH) (Wilson 1977; Graves & Somero 1982; Panepucci *et al*., 1984; 1987; Coppes & Somero 1990) and Malate dehydrogenase (cMDH) (Shaklee *et al*. 1977; Schwantes & Schwantes 1982 a, b; Farias & Almeida –Val 1992; Lin and Somero, 1995 a, b).

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; Hales & Able, 1995; 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 *et al.,* 2015; Kumar 2016; Kumar 2017; Kumar 2018 and Kumar 2021).

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, *Clarias batrachus* and *Cyprinus carpio*.

Materials and Methods

Live specimens (6 fishes) of *Cyprinus carpio* and *Clarias batrachus (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 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

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).

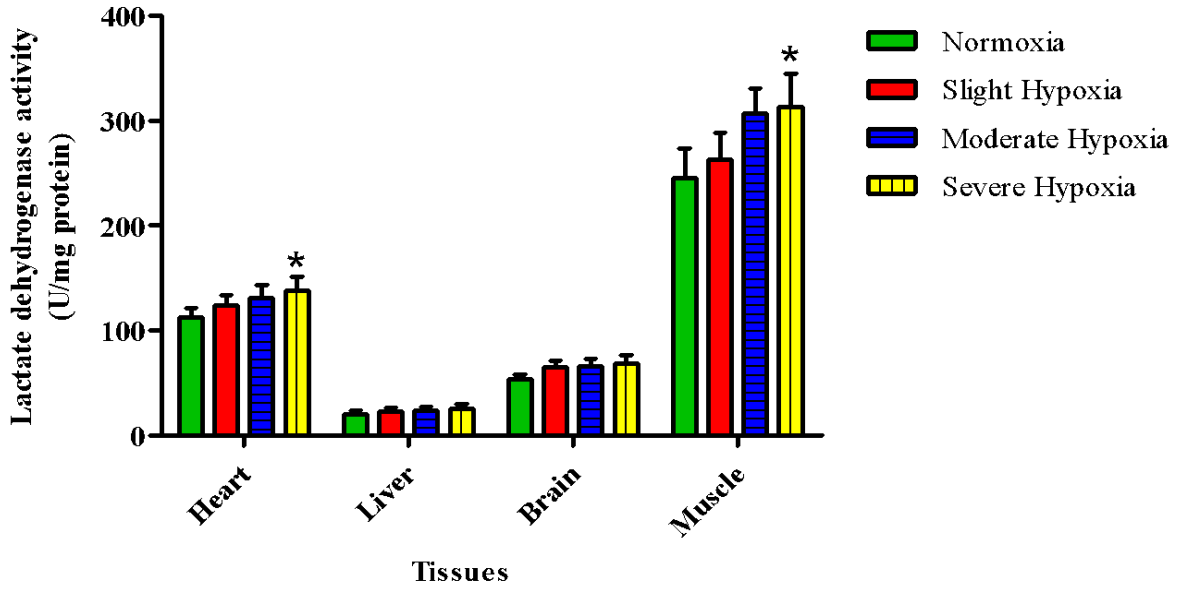

Figure-1: Mean specific activity of lactate dehydrogenase (LDH) enzyme (U/mg protein) in heart, liver, brain and muscle of *Clarias batrachus* exposed to varying oxygen concentration i.e. different hypoxia period for 12

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 *Cyprinus carpio*

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

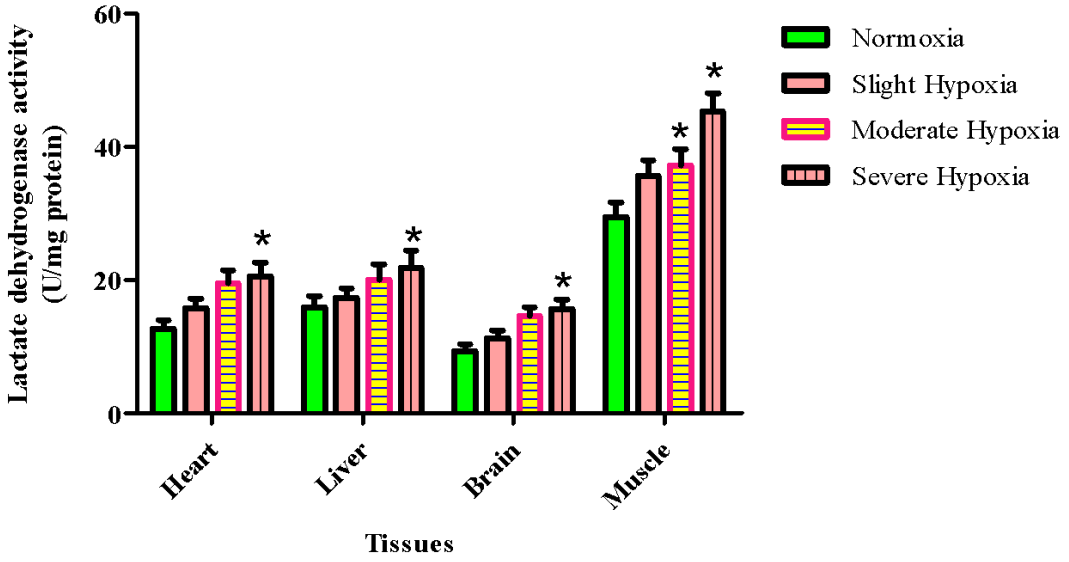

Figure-2: Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Cyprinus carpio* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*) represents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

MDH activity in *Clarias batrachus*

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

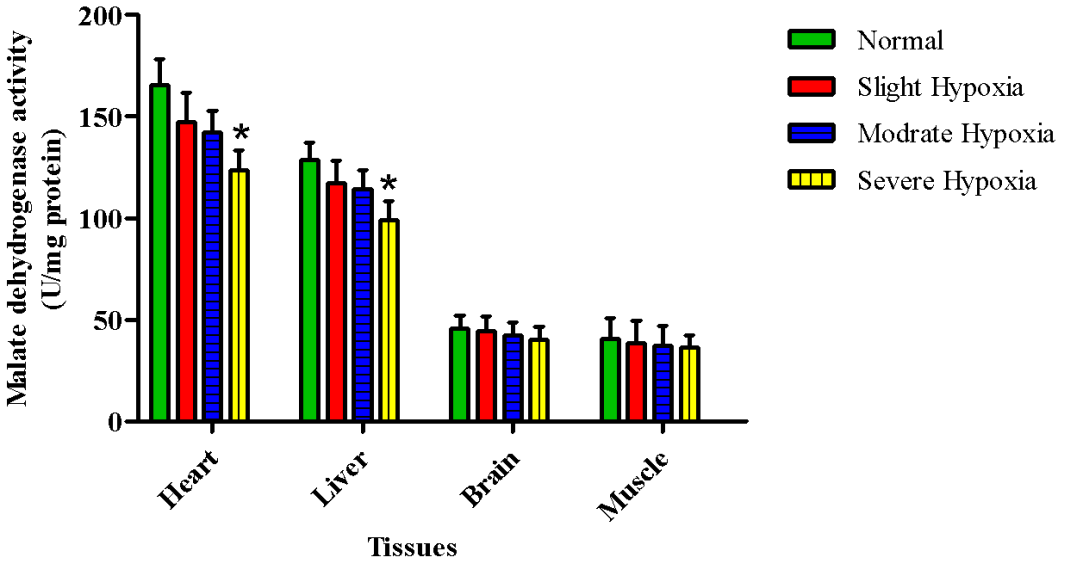

Figure-3: Mean specific activity of Malate dehydrogenase (MDH) enzyme (U/mg protein) in heart, liver, brain and muscle of *Clariasbatrachus* exposed to varying oxygen concentration i.e. different hypoxia period for 12

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 *Cyprinus carpio*

MDH activity was observed to be decreased in all these tissues taken for observation during all hypoxia periods. 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).

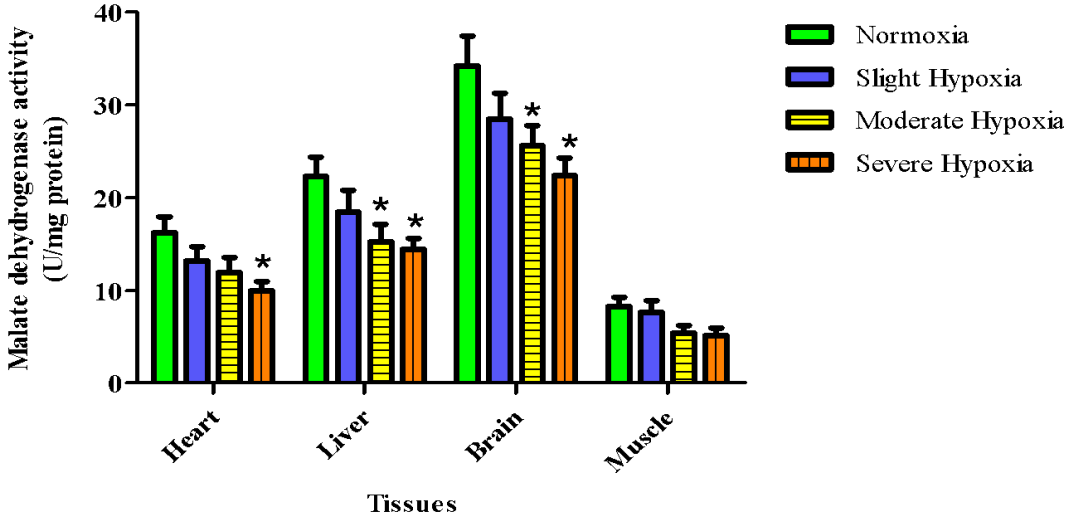

Figure-4: Mean specific activity of Malate dehydrogenase (MDH) enzyme (U/mg protein) in heart, liver, brain and muscle of *Cyprinus carpio* exposed to varying oxygen concentration i.e. different hypoxia period for 12 hours duration. (U, µmole substrate/min; Values are means±s.e.m., n=6). Asterisk (*)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

SDS-PAGE analysis in Clarias batrachus

In hypoxia heart 17.3kDprotein bands were absent and 44.0kD extra protein bands were found. 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 bands having mol. wt. 44kD was observed. In hypoxia muscle protein band of 44.3kD mol. wt. was absent (Fig. 5).

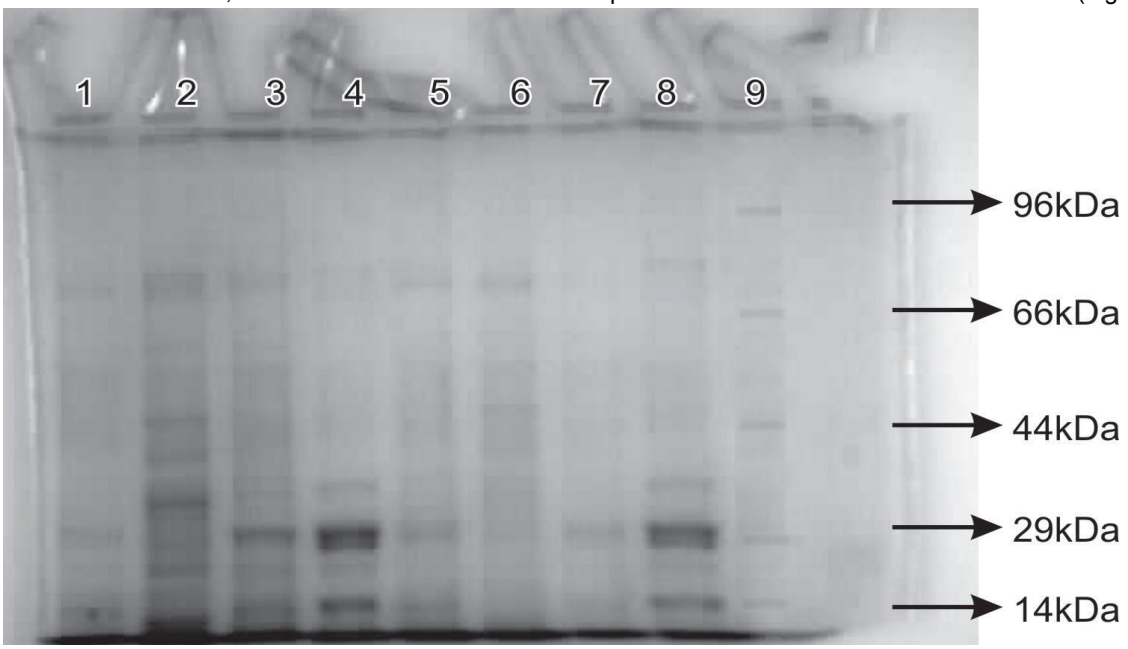

Figure-5: SDS-PAGE (Laemmli, 1970; 12% separating gel) profile of proteins of different tissues of *Clarias batrachus*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle, lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia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

SDS-PAGE analysis* in *Cyprinus carpio

In hypoxia heart 29.5kD and 55.7kD protein bands were absent and 96.7kD extra protein bands were 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).

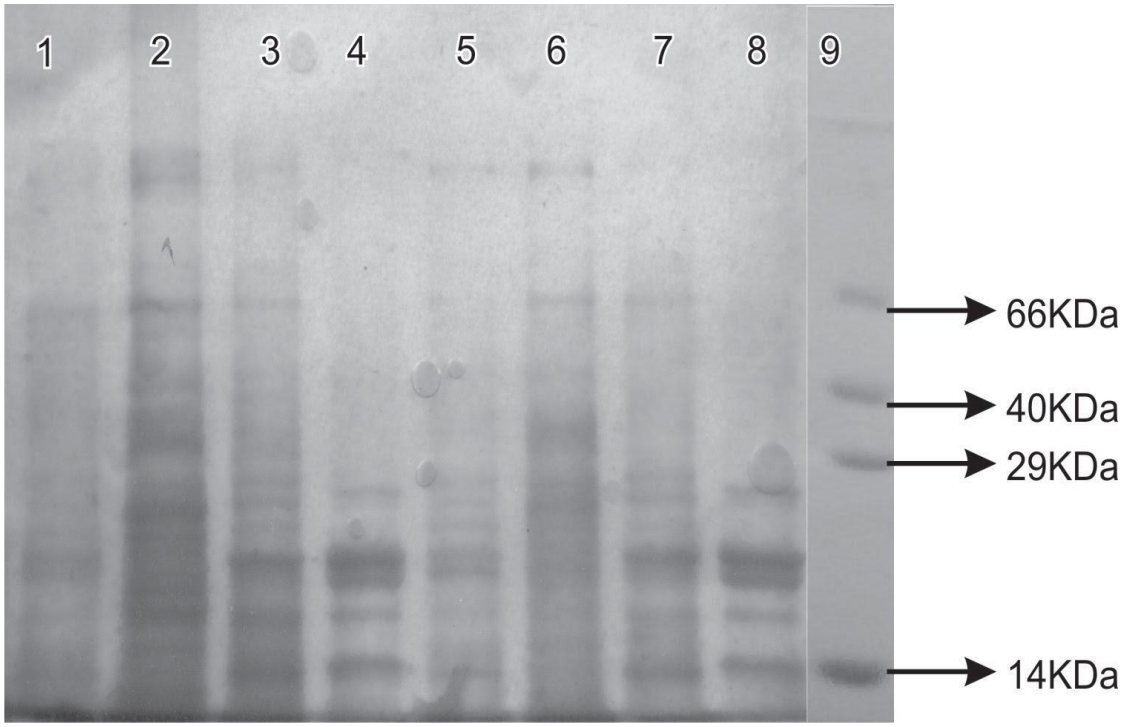

Figure-6: SDS-PAGE (Laemmli, 1970; 12% separating gel) analysis of proteins of different tissues of *Cyprinus carpio*. Lane 1: normoxia heart, lane 2: normoxia liver, lane 3: normoxia brain, lane 4: normoxia muscle, lane 5: hypoxia heart, lane 6: hypoxia liver, lane 7: hypoxia brain, lane 8: hypoxia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

Discussion

Enzymes MDH is known to catalyse the reversible oxidation of malate to oxaloacetate requiring NAD^+ as a cofactor. Found both in cyptoplasm 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; Kumar 2017; Kumar 2018 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 *Cyprinus carpio* 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 *Cyprinus carpio* shows more metabolically activeness of the fish.

Conclusion

Because the different tissues of *Clarias batrachus* 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 former is more tolerant to graded hypoxia than the latter.

References

1. *Almeida-Val, V.M.F., Val, A.L. Duncan, W.P., Souza, F.C.A., Paula-Silva, M.N. and Land, S. (2000). Scaling effects on hypoxia tolerance in the Amazon fish Astronotus ocellatus (Perciformes: Cichlidae): contribution of tissue enzyme levels. Comp. Biochem. Physiol. 125B, 219-226.*
2. *Bejda A.J., B.A. Phelan, and A.L. Studholme (1992) The effect of dissolved oxygen on the growth of young-of-the-year winter flounder, Pseudopleuronectes americanus. Environ Biol Fishes 34:321–327.*
3. *Bushnell, P. G., Steffensen, J. F. and Johansen, K. (1984). Oxygen consumption and swimming performance in hypoxia-acclimated rainbow trout Salmo gairneri. J. Exp. Biol. **113**, 225-235.*
4. *Coppes, Z. L. &Somero, G.N., 1990, Temprature differences between the M_{41} lactate dehydrogenase of stenothermal and eurythermal Sciaenid fishes. J. Exp. Zool., 254: 127- 131.*
5. *Carter, G. S. & L. C. Beadle, (1931).The fauna of the swamps of the Paraguayan Chaco in relation to its environment. II. Respiratory adaptations in the fishes. Journal of the Linnean Society of London (Zoology) 37: 327-366.*
6. *Cech J, Mitchell S, Wragg T (1984) Comparative growth of juvenile white sturgeon and striped bass: effects of temperature and hypoxia. Estuaries Coasts 7:12–18*
7. *Chabot, D. and Dutil, J.-D. (1999). Reduce growth of Atlantic cod in nonlethal hypoxic conditions. J. Fish Biol. **55**, 472-491.*
8. *Dalla Via, J., Van den Thillart, G., Cattani, O. and Cortesi, P. (1998). Behavioural responses and biochemical correlates in Solea solea to gradual hypoxic exposure. Can. J. Zool.76, 2108-2113.*
9. *Davis, J. C. (1975). Minimal dissolved oxygen requirements of aquatic life with emphasis of Canadian species: a review. Journal of the Fisheries Research Board of Canada 32, 2295–2332.*
10. *Driedzic, W.R. and V.M.F. Almeida-Val. (1996). Enzymes of cardiac energy metabolism in Amazonian teleosts and the fresh-water energy metabolism in Amazonian teleosts and the fresh-water stingray (Potamotrygon hystrix). J. Exp. Zool., 274: 327-333.*
11. *Dunn, J. F. and Hochachka, P. W. (1986).Metabolic responses of trout (Salmo gairdneri) to acute environmental hypoxia. J. Exp. Biol. 123, 229-242.*
12. *Farias, I.P. & Almeida-Val, V.M.F., 1992, Malate dehydrogenase (sMDH) in Amazon cichlid fishes: Evolutionary features. Comp. Biochem. and Physiol., 103B: 939-943.*
13. *Greaney, G S., Place, A., Cashon, R., Smith, G., Powers, D. (1980) Time course of changes in enzyme activities and blood respiratory properties of killifish during long-term acclimation to hypoxia. Physiol. Zool. 53: 136-144*
14. *Graves, J. E. &Somero, G. N., 1982, Electrophoretic and functional enzymic evolution in four species of Eastern Pacific barracudas from different thermal environments. Evolution, 36: 97-106.*
15. *Horecker, B. L. & Kornberg, A. (1948). The extinction coefficients of the reduced band of pyridine nucleotides. J. Biol. Chem., 175. 385-390.*
16. *Kumar, A. and Gopesh A. (2015) Effect of Hypoxia and Energy Conservation Strategies in the Air-Breathing Indian Catfish, Clarias batrachus. Natl. Acad. Sci. Lett. (March–April 2015) 38(2):135–137*
17. *Kumar, A. (2016). Metabolic correlates of Hypoxia in certain Fishes with different respiratory patterns. D. Phil. thesis. University of Allahabad, Allahabad, U P India-211002.*
18. *Kumar, A. (2017). Interdemic variation in haematocrit and lactate dehydrogenase in the Indian cyprinid Cyprinus carpio in conditions of hypoxia; Remarking An Analisation VOL-2 ISSUE-6 September-2017*
19. *Kumar, A. (2018) Hematological and blood metabolites adaptations in catfish Clarias batrachus in the conditions of hypoxia; Remarking An Analisation VOL-3 ISSUE-9 December-2018.*
20. *Kumar, A. (2021) Effect of hypoxia and metabolic adaptations in common carp, Cyprinus carpio. Shrinkhala Ek Shodhparak Vaicharik Patrika. Vol. 8, Issue 8, April 2021.*
21. *Laemmli, U.K., (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227, pp.680-685.*
22. *Lin, J. &Somero, G.N., 1995a, Thermal adaptation of cytoplasmic malate dehydrogenase of eastern pacific barracuda (Sphyraenassp.): The role of differential isoenzyme expression. J. Exp. Biol., 198: 551-560.*
23. *Lin, J. &Somero, G.N., 1995b, Temperature-dependent changes in expression of thermostable and thermolabileisozymes of cytosolic malate dehydrogenase in the eurythermal goby fish Gillichthys mirabilis. Physiol. Zool., 68(1): 114-128.*
24. *Pichavant, K., J. Jerson-Le-Ruyet, N. Le Bayon, A. Severe, A. Le Roux, L. Quemener, V. Maxime, G. Nonnotte, and G. Boeuf (2000). Effects of hypoxia on growth and metabolism of juvenile turbot. Aquaculture 188(1-2): 103-114*
25. *Panepucci, L., Schewantes, M. L. & Schwantes, J.R. & Schewantes A. R., 1984, Loci that encode the lactate dehydrogenase in 23 species of fish belonging to the order Cypriniformes, Siluriformes, and Preciformes; Adaptive features, Comp. Biochem. Physiol., 77B: 867-876.*
26. *Secor, D.H. and T.E. Gunderson. (1998). Effects of hypoxia and temperature on survival, growth, and respiration of juvenile Atlantic sturgeon, Acipenser oxyrinchus. Fish. Bull. 96: 603-613.*
27. *Shaklee, J.B., Christiansen, J.A. Sidell, B.D., Prosser, C.L. & Whitt, G.S., 1977, Molecular aspects of temprature acclimation in fish:*

Contributions of changes in enzymes patterns to metabolic reorganization in the green sun fish. J. Exp. Zool., 201:1-20

28. *Schwantes, M. L.B. & Schwantes, A. R.,1982a, Adaptive features of ectothermic enzymes-I. Temperature effects on the malate dehydrogenase from a temperate fish Leiostomus xanthurus. Comp. Biochem. Physiol., 72B: 49-58.*
29. *Schwantes, M.L.B. & Schwantes, A. R., 1982b, Adaptative features of ectothermic enzymes-II. The effects of acclimation temperature on the malate dehydrogenase of the Spot, Leiostomus xanthurus.Comp. Biochem. Physiol., 72B: 59-64.*
30. *Somero, G. N. & Childress, J. J. (1980). A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-size fish. Physiological Zoology 53, 322–337*
31. *Taylor, J.C. and J.M. Miller (2001).Physiological performance of juvenile southern flounder, Paralichthys lethostigma, in chronic and episodic hypoxia. J. Exp. Mar. Biol. Ecol. 258: 195-214.*
32. *Tripathi R. K.; Mohindra V.; Singh A.; Kumar R.; Mishra R. M. and Jena J. K. (2013) Physiological responses to acute experimental hypoxia in the air-breathing Indian catfish, Clarias batrachus J. Biosci. 38(2), June 2013, 373–383.*
33. *Weber, J. M. & Kramer, D. L. (1983). Effects of hypoxia and surface access on growth, mortality, and behaviour of juvenile guppies, Poecilia reticulata. Canadian Journal of Fisheries and Aquatic Sciences 40, 1583–1588.*