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Interdemic Variation in Haematocrit and Lactate Dehydrogenase in the Indian Cyprinid *Cyprinus Carpio* in Conditions of Hypoxia

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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 anaerobic and aerobic enzyme activity and blood parameters in selected tissues of carp fish, *Cyprinus carpio* was undertaken. Fish were exposed to experimentally provoked hypoxia for different duration and were sacrificed to study the effect of hypoxia on selected enzymes activities 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 enzyme activity respond in a tissue specific manner.

Keywords: Hypoxia, LDH, Blood Parameters, Carp fish

Introduction

Hypoxia is a frequently occurring environmental phenomenon observed in the freshwater and even coastal system of a tropical country like India. It may be naturally occurring phenomenon due to various biological and physical factors (Rosenberg *et al.*, 1991; Pihl *et al*., 1992; Hobak and Barnhart, 1996; Fu *et al*., 1999) or may be caused due to anthropogenic activities around the water bodies.

In tropical aquatic systems, dissolved oxygen in the water of ponds, 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 are also recorded to occur frequently.

It was therefore aimed to undertake investigation on the enzyme activities in response to experimentally provoked hypoxia in carp fish, *Cyprinus carpio* and correlate with the energy conservation strategies of the fish which is known to tolerate long periods of hypoxia.

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.

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_2l^{-1}).

Investigations undertaken on the fish by workers abroad like Wilson (1977), Graves and Somero (1982), Panepucci *et al.* (1984, 1987), Copes and Somero (1990) revealed that LDH in fishes is usually encoded by three loci, one expressed principally in skeletal muscle (LDH-A), another in heart muscle (LDH-B) and a third one in the eye (LDH-C).

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.

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. How these stresses (Hypoxia, temperature, hypercapnea, starvation etc.) in the present references affect the fish species of different respiratory habits.
2. If prolonged exposure to sub-lethal conditions can actively import coping mechanisms that are useful for the survival of these fishes.
3. The mechanisms behind the observed effect of hypoxia and improved hypoxia tolerance.

The present piece of work aims to analyze the response of enzyme LDH and haematological parameters to different degrees of hypoxia in Cypriniforms, mainly carpfish, which present different respiratory patterns.

Materials and Methods

Live specimens of *Cyprinus carpio (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. 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).

Heparinized blood was used for erythrocyte counts, haemoglobin estimation and haematocrit (Hct) evaluation. Erythrocyte count was made with the help of Neubaur's haemocytometer using standard diluents. Haemoglobin was estimated by the method of Blaxhall and Daisley (1973). [Hct] was determined following centrifugation of microhematocrit capillary tube filled with blood, at 10,000 rpm for 5 min (Assendelft and England 1982). Erythrocytic indices like mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) Mean cell haemoglobin concentration (MCHC) was measured by Wells and Weber (1991).

LDH activity

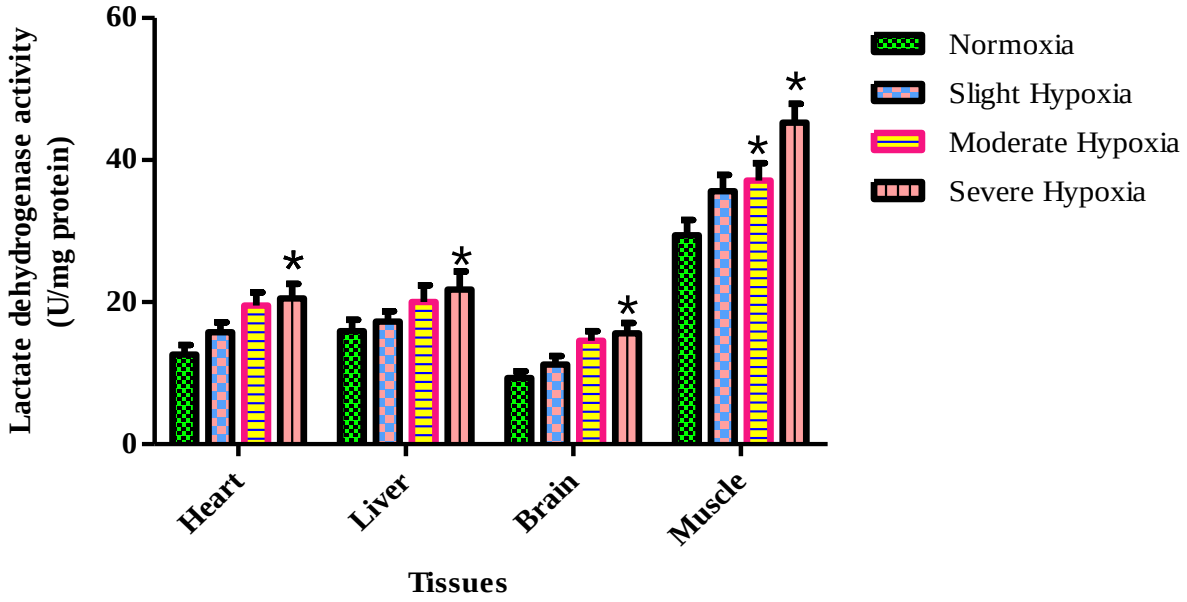
Highest LDH activity was observed in muscle followed by liver and heart. Lowest LDH activity was observed in brain (Table 1). LDH activity was observed to be increased in all these tissues taken for observation in all hypoxia period. During slight hypoxia maximum increase in LDH activity was observed 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).

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 or 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

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



Haematological changes

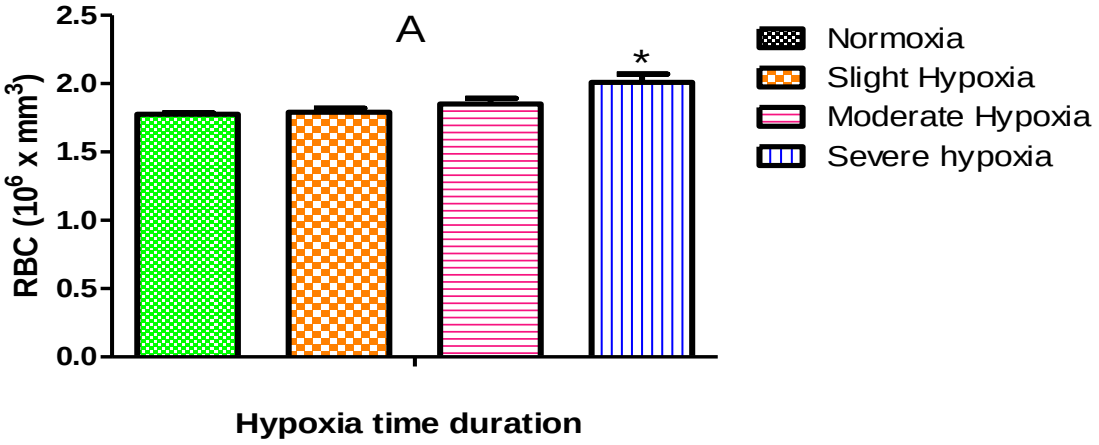
There was slight changes were observed in blood parameters during different stages of hypoxia. No significant (p≤0.05%) changes were observed in all blood parameters during different stages of hypoxia (Table 2). An increase (0.788%) in RBC content was observed during slight hypoxia. At moderate (4.22%) and severe (13.17%) hypoxia an increase in RBC content was observed in more magnitude than the slight hypoxia. Blood haemoglobin (Hb %) was increased during all stages of hypoxia. It was increased (1.15%) at slight hypoxia and increased (5.74%) at moderate hypoxia. As water oxygen level was decreased at severely level Hb content in blood was increased (16.37%). Haematocrit (Hct %) value increased 10.86% at slight hypoxia, 15.35% at moderate hypoxia and 18.23% at severe hypoxia. Other haematological parameters like MCH and MCV were also increased at all the three stages of hypoxia. MCHC decreased at slight hypoxia level but increased at moderate and severe hypoxia level.

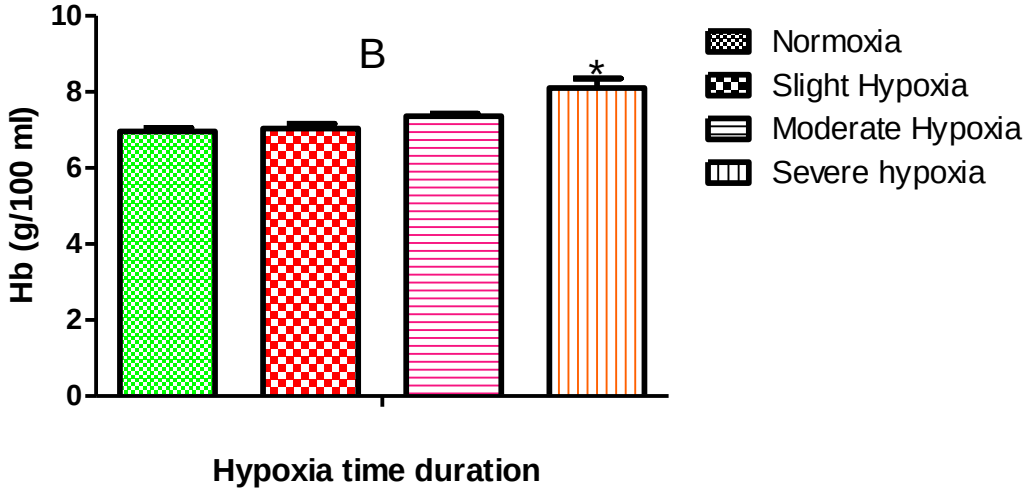
Table-2
Haematological Changes in *Cyprinus Carpio* Exposed to Different Level of Hypoxia. Values are Mean of Three Replicates±Standard Error of Mean.

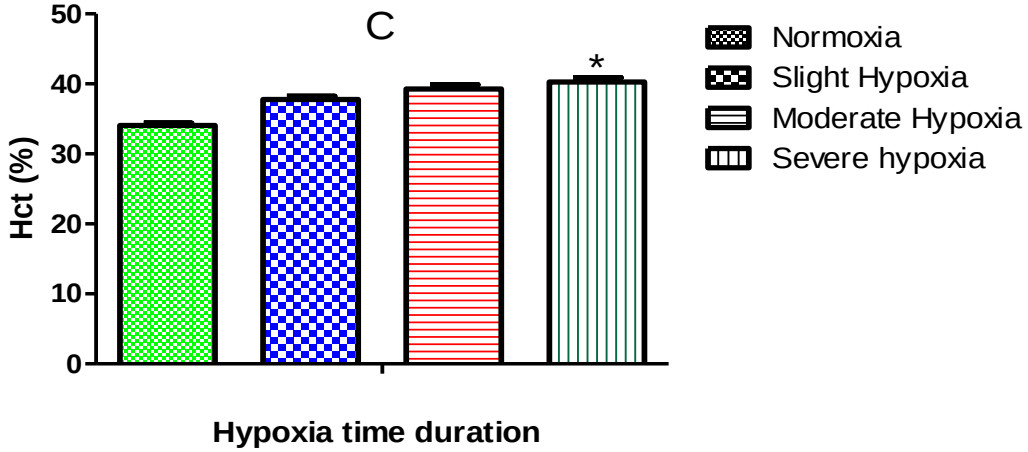
	RBC (10^6×mm^3)	Hb(g/100ml)	Hct (%)	MCV (fl/cell)	MCH Pg/cell	MCHC (%)
Normoxia	1.776±0.0258	6.96±0.09	34.06±0.35	192.1±3.24	39.33±1.07	20.27±0.28
Hypoxia						
Slight hypoxia	1.790±0.005	7.04±0.12	37.76±0.48	197.26±3.89	40.09±1.10	20.26±0.23
Moderate hypoxia	1.85±0.018	7.36±0.07	39.29±0.59	203.28±4.15	41.12±1.24	20.43±0.37
Severe hypoxia	2.01±0.031	8.1±0.25	40.27±0.62	212.45±4.23	42.52±1.33	21.11±0.42

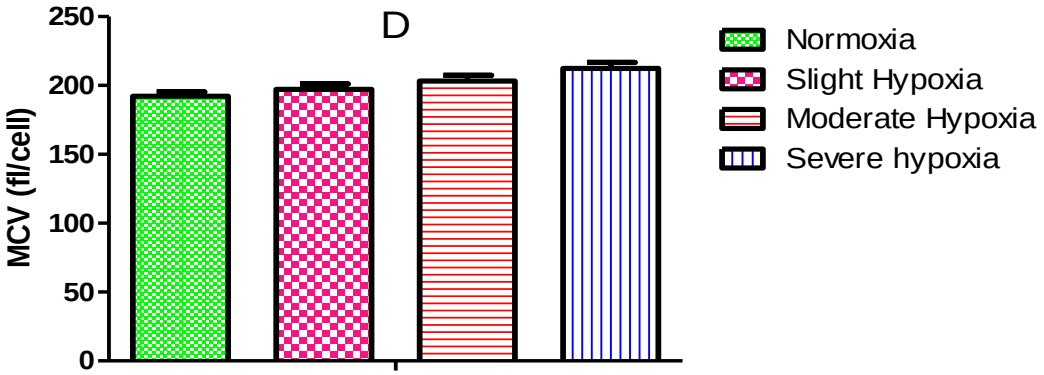
Figure-2

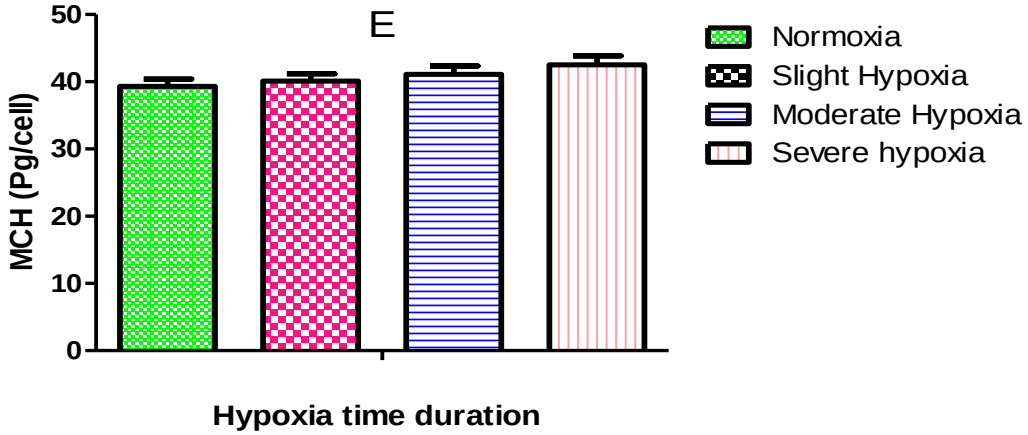
Haematological Parameters in Blood of *Cyprinuscarpio* Exposed to Varying Oxygen Concentration I.E. Different Hypoxia Stages For 12 Hours Duration.(A) Rbcs (10^6×Mm^3), (B) Hb (Gm/100 Ml), (C) Hct (Per Deciliter), (D) MCV (Fl/Cell), (E) MCH (Pg/Cell) And (F) MCHC (Gm/Decilitre).Asterisk (*) Represents Significant Differences (*P*<0.05) Between Normoxia And Different Hypoxia Stages.

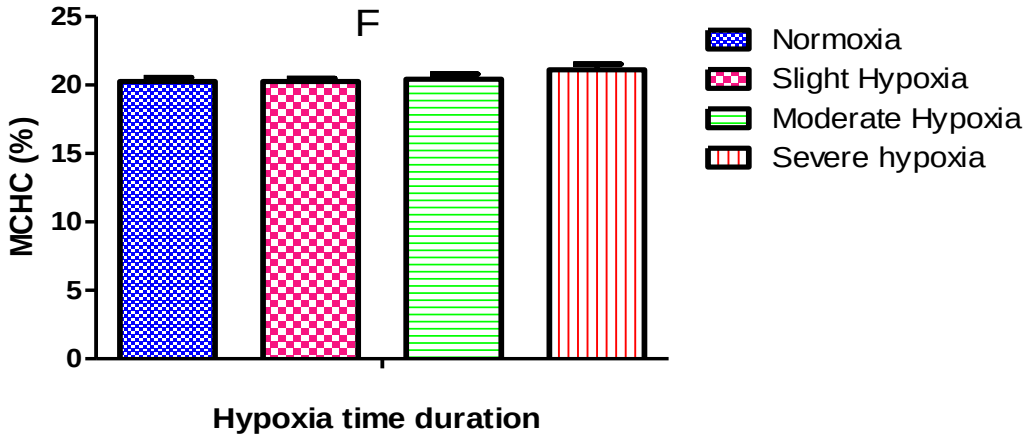












Discussion

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 *Cyprinus carpio* in present investigation has been found to support this observation.

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).

Measurements of maximal LDH activity in tissues from a diverse array of fish species have shown that the amount of enzyme varies considerably, both within tissues and among species. In white skeletal muscle, LDH activity, expressed as units per gram tissue, increases with body size,

nutritional status and locomotor activity (Somero & Childress, 1980; Sullivan & Somero, 1983; Almeida-Val *et al*., 2000; Martinez *et al*., 2003). In *Cyprinus carpio*, the activity of LDH in white skeletal muscle is positively correlated with body mass and falls within the range of activities measured in other species of similar size. There is also considerable interspecific variation in LDH activity in liver, heart and brain (Greaney *et al*., 1980; Somero & Childress, 1980; Kleckner & Sidell, 1985; Kolok, 1992; Almeida-Val *et al*., 2000). Values from *Cyprinus carpio*, especially those obtained after laboratory maintenance fall in the upper end of the range reported for these tissues. Liver, heart and brain LDH activities in *Cyprinus carpio* were not significantly related to body mass, consistent with the lack of a mass effect on LDH activity in non-muscle tissue reported by Tripathi *et al.* (2013). In contrast, a positive relationship exists between body mass and LDH activity in liver, heart and brain from *Astronotus ocellatus* (Agassiz), which correlates with greater hypoxia tolerance in larger fish (Almeida-Val *et al*., 2000).

Acute hypoxia in *C. carpio* (water breathing) induced an increase in blood glucose levels. Except by the brain, where we observed a suppression of MDH and AChE (A. Kumar., 2016) 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.

The level of metabolic depression achieved by *Cyprinus carpio* is similar to that of goldfish *Carassius auratus* and crucian carp *Carassius carassius*, both of which decrease metabolic rate by approximately 70% under anoxia (Van Waversveld *et al*., 1989).

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). 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).

Extended holding of large mouth bass, *Micropterus salmoides* at low DO induced an improved ability to transport oxygen in blood relative to fish held at higher oxygen concentrations. Concentrations of both Hct and Hb were significantly higher in *Micropterus salmoides* held at low oxygen for 50 days relative to fish held at higher oxygen. Hct is the percentage of packed red blood cells relative to the whole volume of blood, but does not account for the size or number of erythrocytes. Hb is a quantification of the O_2 binding protein found in red cells, whereas MCHC is a measure of the Hb in a given volume of packed erythrocytes (Houston, 1990). Increases in Hct and/or Hb are typically caused by an increase in the production of erythrocytes, swelling of the erythrocytes, or a combination of both. These changes are typically a result of catecholamine releases that induce the release of erythrocytes from the spleen (Jensen *et al*. 1993), or acidosis in the blood, which alters the affinity of Hb to bind oxygen, and can stimulate an increase in erythrocytes (Wells, 2009).

Increases in Hb and Hct concentrations between the air-breathing and non air-breathing groups during an oxygen challenge may have been driven by the release of erythropoietin, the hormone responsible for synthesizing erythrocytes and releasing erythrocyte stores from the spleen. This is evidenced by the increase of erythrocytes numbers (i.e., increase in Hct and Hb) without increasing the amount of Hb per cell volume (i.e., no change in MCHC). This is only offered as a potential mechanism as erythropoietin was not quantified. Rainbow trout (*Oncorhynchus mykiss*) subjected to sustained hypoxia (maximum 216 h) had persistent increases in erythropoietin, as well as increased Hb levels (Lai *et al*.2006), thereby providing an improved ability for oxygen uptake. Additionally, long-term exposure to hypoxia increases both Hb and Hct concentrations for numerous fish species, both air and water breathers (Scott and Rogers, 1981; Tun and Houston, 1986; Petersen and Petersen, 1990 and Timmerman and Chapman, 2004). These changes typically confer an increase in oxygen-binding affinity or increased substrata for oxygen binding on the erythrocyte, improving performance of fish in low oxygen conditions.

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. 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, specially 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.

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. *Blaxhall, P. C. and Daisley, K. W. 1973.Routine haematological methods for use with fish blood .J. Fish Biol. 5:771-781.*
4. *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.*
5. *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*
6. *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.*
7. *Dorovkov, M. V., Pavur, K. S., Petrov, A. N. and Ryazanov, A. G. (2002).Regulation of elongation factor-2 kinase by pH. Biochemistry 41, 13444-13450.*
8. *Fu XW, Nurse C, Wang YT, Cutz E. (1999).Selective modulation of membrane currents by hypoxia in intact airway chemoreceptors from neonatal rabbit. J Physiol 514:139–150.*
9. *Graves, J. E. & Somero, G.N., 1982, Electrophoretic and functional enzymic evolution in four species of Eastern Pacific barracudas from differential thermal environments. Evoluion, 36: 97-106.*
10. *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*
11. *Hoback, W. W. and Barnhart, M. C. (1996).Lethal limits and sublethal effects of hypoxia on the amphipod Gammaruspseudo limnaeus .J. Nth. Amer. Benth. Soc. 15, 117-126.*
12. *Hochachka, P. W. and Somero, G. N. (2002). Biochemical Adaptation. Princeton: Princeton University Press.*
13. *Houston AH (1990) Blood and circulation. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 273–334*
14. *Horecker, B. L. & Kornberg, A. 1948. The extinction coefficients of the reduced band of pyridine nucleotides. J. Biol. Chem., 175. 385-390.*
15. *Jensen, F. B., Nikinmaa, M. and Weber, R. E. (1993). Environmental perturbations of oxygen transport in teleost fishes: causes, consequences and compensations. In Fish Ecophysiology (ed. J. C. Rankin and F. B. Jensen), pp. 161-179. London: Chapman & Hall.*
16. *Jones, D. R., Randall, D. J., Jarman, G. M. (1970). A graphical analysis of oxygen transfer in fish. Respir. Physiol. 10: 285-298*
17. *Kleckner, N. W. &Sidell, B. D. (1985). Comparison of maximal activities of enzymes from tissues of thermally acclimated and naturally acclimatized chain pickerel (Esoxniger). Physiological Zoology 58, 18–28*
18. *Kolok, A. S. (1992). Morphological and physiological correlates with swimming performance in juvenile largemouth bass. American Journal of Physiology 263, R1042–R1048.*
19. *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.*
20. *Lai JCC, Kakuta I, Mok HOL, Rummer JL, Randall D (2006) Effects of moderate and substantial hypoxia on erythropoietin levels in rainbow trout kidney and spleen. J Exp Biol 209:2734–2738*
21. *Martı´nez, M., Guderley, H., Dutil, J.-D., Winger, P. D., He, P. & Walsh, S. J. (2003).Condition, prolonged swimming performance and muscle metabolic capacities of cod Gadus morhua. Journal of Experimental Biology 206, 503–511.*
22. *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.*
23. *Panepucci, L., Schewantes, M. L. &Schwantes, J.R. &Schewantes A. R., 1987, Biochemical and physiological properties of the lactate dehydrogenase allozymes of the Brazilian teleost, Leporinusfriderici, Anostomidae, Cypriniformes., Comp. Biochem. Physiol., 87B:199-206.*
24. *Petersen JK, Petersen GI (1990) Tolerance, behavior and oxygen consumption in the sand goby, Pomatoschistus minutes (Pallas), exposed to hypoxia. J Fish Biol 37:921-933*
25. *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*
26. *Pihl, L., Baden, S. P., Diaz, R. J. and Schaffer, L. C. (1992).Hypoxia-induced structural changes in the diet of bottom-feeding fish and crustacea. Mar. Biol. 112, 349-361.*
27. *Richards, J.G., Wang, Y.S., Brauner, C.J., Gonzalez, R.J., Patrick, M.L., Schulte, P.M., Chippari-Gomes, A.R., Almeida-Val, V.M. and*

Val,A.L. (2007).Metabolic and ionoregulatory responses of the Amazonian chiclid, Astronotus ocellatus, to severe hypoxia. J. Comp. Physoil. B 177, 361-374.

28. *Rosenberg, R., Hellman, B. and Johansson, B. (1991).Hypoxic tolerance of marine benthic fauna. Mar. Eco. Prog. Ser. 79, 127-131.*
29. *Scott AL, Rogers WA (1981) Haematological effects of prolonged sublethal hypoxia on channel catfish Ictalurus punctatus (Rafineque). J Fish Biol 18:591–601*
30. *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.*
31. *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*
32. *Sullivan, K. M. & Somero, G. N. (1983). Size- and diet-related variations in enzyme activity and tissue composition in the sablefish, Anoplopoma fimbria. Biological Bulletin 164, 315–326.*
33. *Timmerman CM, Chapman LJ (2004) Behavioral and physiological compensation for chronic hypoxia in the sailfin molly (Poecilia latipinna). Physiol Biochem Zool 77: 601–610*
34. *Tun N, Houston AH (1986) Temperature, oxygen, photoperiod, and the haemoglobin system of the rainbow trout, Salmo gairdneri. Can J Zool 64:1883–1888*
35. *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.*
36. *Van den Thillart, G. & van Waarde, A. (1985). Teleosts in hypoxia: aspects of anaerobic metabolism. Molecular Physiology 8, 393–409.*
37. *Virani, N. A. & Rees, B. B. (2000). Oxygen consumption, blood lactate and inter individual variation in the gulf killifish, Fundulus grandis, during hypoxia and recovery. Comparative Biochemistry and Physiology 126A, 397–405.*
38. *Van Waversveld, J., Addink, A. D. F., Van den Thillart, G (1989).Simultaneous direct and Indirect calorometry on normoxic and anoxic goldfish. J. exp. Biol. 142: 325-335*
39. *Weber, J. M. & Kramer, D. L. (1983).Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, Poecili areticulata.Canadian Journal of Fisheries and Aquatic Sciences 40, 1583–1588.*
40. *Wilson, T.L.,1977, Interrelations between pH and temperature for the catalytic rate of the M4 isozyme of lactate dehydrogenase (EC 1.1.1.27) from goldfish (Carassiusauratus). Arch Biochem Biophys., 179: 378-390.*
41. *Wells RMG (2009) Gas transport and hemoglobin function: adaptations for functional and environmental hypoxia. In: Richards JG, Farrell AP, Brauner CJ (eds) Fish physiology: hypoxia, 1st edn. Academic Press, New York, pp 255–299*