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SHORT COMMUNICATION

# Effect of Hypoxia and Energy Conservation Strategies in the Air-Breathing Indian Catfish, *Clarias batrachus*

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**Abstract** Fish in a tropical country like India are frequently exposed to different duration of hypoxia. The effect of hypoxia on the physiology of fish, air-breathing catfish *Clarias batrachus* were exposed to different duration of hypoxia and its effect on activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were studied in four tissues (heart, liver, brain and muscle). The specific activity of LDH increases in all tissues, which reflects towards onset of anaerobic respiration and decrease in energy demand in all these tissues. In contrast, MDH specific activities were decreased significantly in heart, suggesting involvement of strong aerobic respiration in heart during hypoxia. The present investigation revealed that during hypoxia enzyme activities responded in a tissue-specific manner in the fish *C. batrachus* reflecting the balance of energetic demands, metabolic role and oxygen supply of particular tissues.

**Keywords** Catfish · Hypoxia · Surfacing behaviour · LDH · MDH

Hypoxia is an environmental stressor, caused by normally large temporal and spatial variations in oxygen content of water [1]. Animals are known to adopt different mechanisms to tolerate hypoxia. Many of these responses are behavioural, including surface breathing, reduced activity, and/or increased ventilation rate [2]. Maintenance of low levels of activity is fuelled by anaerobic metabolism and decrease in metabolism accomplished by decreasing enzyme activity and consuming processes [3, 4]. In addition to these responses, some species have evolved additional physiological or molecular mechanisms and the capacity to undergo sustained metabolic depression or to up-regulate anaerobic glycolysis [5].

Lactate dehydrogenase (LDH, lactate; NAD-oxidoreductase, EC 1.1.1.27) and malate dehydrogenase (MDH, L-malate: NADH oxidoreductase, EC 1.1.1.37) are among the most extensively studied enzymes [6–17]. LDH is a glycolytic enzyme [6–12] whereas MDH is an enzyme involved in gluconeogenesis and lipogenesis and in the malate–aspartate shuttle during aerobic glycolysis [13–17]. With an aim to investigate the effect of hypoxia on the metabolism of LDH, an enzyme of anaerobic respiration and MDH, an enzyme of oxidative respiration was undertaken on an air-breathing catfish *Clarias batrachus*.

Experiments were set for determination of enzyme activity at different duration of hypoxia on normal healthy specimens of *C. batrachus*. Each fish (52.00 ± 2.3 g, 19.2 ± 0.2 cm) was introduced in 5 l glass jar and the lid was then sealed with melted wax. Fish were allowed to stay in the jar undisturbed and constantly observed for behaviour pattern. The fish were taken out at 24, 48 and 72 h of hypoxia and were dissected quickly to take out muscle, heart, brain and liver from it and processed for different observation specifically.

LDH activity in cell free extracts of muscle, liver, heart and brain was measured by a NADH linked optical assay following the method of Horecker and Kornberg [18]. MDH activity was determined by conversion of oxaloacetate to malate. Enzyme activity was expressed in μmole $min^{-1}$ mg $protein^{-1}$. The molar extinction coefficient of

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NADH at 340 nm ($6.22 \times 10^3$ $M^{-1}$ $cm^{-1}$) was used to calculate the enzyme activity.

The data were expressed as mean ± SE values and analyzed using one-way ANOVA followed by Tukey's post hoc test to determine homogenous subsets. In all cases, α level of 5 % ($p \leq 0.05$) was selected to signify differences.

Experiments performed on specimens of *C. batrachus* exposed to hypoxia showed significant differences in enzyme activity from the fishes in normoxia at 25 °C. A marked pattern of behaviour was observed in accordance with the physiological changes observed during different periods of hypoxia. Another significant observation from present investigation has been the recording of tissue specific response to hypoxia.

LDH activity was observed to be increased in the heart after 24 h and was recorded to go down up to nearly normal condition after 48 h of hypoxia. No pronounced change was observed in LDH activity in liver and brain during different periods of hypoxia. In muscle, activities were almost 50 % higher than in heart. Significant changes in LDH activities were observed between normoxia and 72 h of hypoxia in muscle and heart when the fish were found in moribund condition (Fig. 1).

After 24 h of hypoxia MDH activity was observed to be decreased in heart and liver while it remained unchanged in brain and muscle. It was observed to be increased slightly as compared to normal condition in heart and liver after 48 h exposure of hypoxia. Further decrease was observed in MDH activity between 72 h of hypoxia and normoxia in heart and liver. The enzyme activities remained unaffected in brain and muscle tissues at this stage also (Fig. 2).

In the present investigation undertaken on catfish *C. batrachus* significant changes in the activities of two selected enzymes LDH and MDH were observed in response

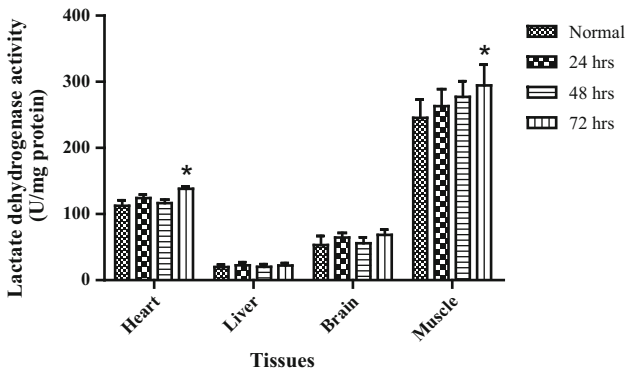


**Fig. 1** Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Clarias batrachus* exposed to hypoxia for 24, 48 and 72 h. (U, µmole substrate/min; Values are mean ± SD, n = 6). *Asterisk* (*) represents significant differences ($p < 0.05$) between normoxia and 72 h of hypoxia

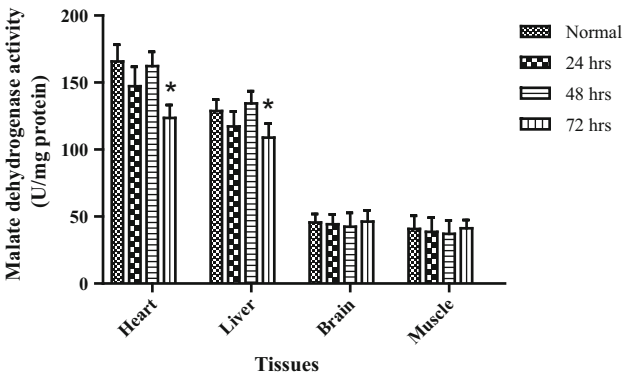


**Fig. 2** Mean specific activity of malate dehydrogenase enzyme (U/mg protein) in heart, liver, brain and muscle of *Clarias batrachus* exposed to hypoxia for 24, 48 and 72 h. U, µmole substrate/min; Values are mean ± SD, n = 6. *Asterisk* (*) represents significant differences ($p < 0.05$) between normoxia and 72 h of hypoxia

to experimentally provoked hypoxia. The level of LDH, a glycolytic enzyme showed increase in muscles at the initial stages of hypoxia. This fluctuation seems to be related with the onset of anaerobic pathways. It may also be correlated with up and down movement of fish in experiment at the onset of hypoxia. The high LDH accumulation in the muscle is in accordance with the behavioural response observed after which the fish resumes "surfacing behaviour" utilising the residual air present at the surface. Specific activities of glycolytic enzyme in muscle have been correlated with the burst swimming activity of fish in response to various stresses in Atlantic Cod *Gadus morhua* [17, 19]. Close to normal levels of LDH recorded in brain, liver and heart indicated towards tendencies of these aerobic tissues to avoid anaerobic respiration [16]. These tissues are known to regulate LDH level according to available environmental oxygen, so that less of lactate accumulates in these tissues.

Higher levels of MDH have been recorded in heart and liver in *C. batrachus*. The role of this enzyme in the metabolism is to supply intermediary metabolites (oxaloacetate) for the Kreb's cycle used as source of carbon in oxidative metabolism [20]. Thus, the higher levels of MDH observed in heart reflects the role of this enzyme for cardiac tissues after 48 h of hypoxia. This pattern is similar for liver which shows role of MDH in gluconeogenesis [13, 20].

Present investigations clearly support the earlier observation undertaken on other teleosts [6–16] and air-breathing fish *C. batrachus* [21]. *C. batrachus* was observed to undergo a series of coordinated metabolic adjustments which aims at balancing an overall suppression of systemal ATP demand along with proportionate increase in fractions of remaining metabolism that is supported by anaerobic glycolysis alone [6, 10]. One of the major responses to

hypoxia has been recorded to be an increase in anaerobic ATP production via glycolysis [17, 19]. Number of observations are on record which revealed that exposure to hypoxia increase the activities of glycolytic enzymes that presumably augment the capacity of fish tissues for anaerobic energy production [9, 20]. Although there is an extensive background of work in general and specific properties of LDH [6, 9] a single answer for enzyme responses has not been reached [10, 12] which needs to be addressed with more in depth targeted investigations.

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