Physiological responses to acute experimental hypoxia in the air-breathing Indian catfish, *Clarias batrachus* (Linnaeus, 1758)

Ratnesh Kumar Tripathi[1], Vindhya Mohindra[1,*], Akanksha Singh[1], Rajesh Kumar[1], Rahasya Mani Mishra[2] and Joy Krushna Jena[1]

[1]*National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow 226 001*

[2]*Awadhesh Pratap Singh University, Rewa 486 001*

**Corresponding author (Fax, +91-522-2442403; Email, vindhyamohindra@gmail.com; vmohindra@nbfgr.res.in)*

With an aim to study the mechanism of adaptation to acute hypoxic periods by hypoxia-tolerant catfish, *Clarias batrachus*, the mass-specific metabolic rate (VO_2) along with its hematological parameters, metabolic response and antioxidant enzyme activities were studied. During progressive hypoxia, *C. batrachus* was found to be an oxyconformer and showed a steady decline in its aquatic oxygen consumption rate. When *C. batrachus* was exposed for different periods at experimental hypoxia level (0.98±0.1 mg/L, DO), hemoglobin and hematocrit concentrations were increased, along with decrease in mean cellular hemoglobin concentration, which reflected a physiological adaptation to enhance oxygen transport capacity. Significant increase in serum glucose and lactate concentration as well as lactate dehydrogenase activity was observed. Antioxidant enzymes were found to operate independently of one another, while total glutathione concentration was unaffected in any of the tissues across treatments. These observations suggested that hypoxia resulted in the development of oxidative stress and *C. batrachus* was able to respond through increase in the oxygen carrying capacity, metabolic depression and efficient antioxidant defense system to survive periods of acute hypoxia.

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1. Introduction

Hypoxia is an environmental stressor, caused by normally large temporal and spatial variations in oxygen content of water (Damotharan *et al.* 2010) and influences fish behaviour, survival, growth and reproduction (Wilhelm-Filho *et al.* 2005; Braun *et al.* 2006). Some fish species have evolved the ability to survive low oxygen exposure. However, the extent of tolerance varies among species, depending on severity and duration of hypoxia. A simple metric that is commonly employed to determine the hypoxia tolerance in these fishes is the determination of whole animal O_2 consumption rate (VO_2), which is thought to reflect the ability of an organism to extract O_2 from the environment to maintain routine metabolic rate as dissolved oxygen (DO) decreases. A low critical oxygen tension (pCrit) is associated with greater hypoxia tolerance presumably because of improved O_2 uptake and transport to tissues at low water oxygen. Consequently, pCrit has been employed routinely as an important measure of hypoxia tolerance in aquatic organisms including fishes (Speers-Roesch *et al.* 2012).

Under hypoxic conditions animals adopt different mechanism to tolerate hypoxia. Many of these responses are behavioural, including surface breathing, reduced activity, and/or increased ventilation rate (Timmerman and Chapman 2004). Hematological parameters are considered as pathophysiological indicators and are closely related to the response of fish to environmental and biological factors (Fernandes and Mazon 2003). In addition to these responses, some species have evolved additional physiological or

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molecular mechanisms, and the capacity to undergo sustained metabolic depression or to up-regulate anaerobic glycolysis (Hochachka and Somero 2002). Despite the pathway (aerobic or anaerobic) adapted by the fish, partially reduced oxygen intermediates, free radicals or 'reactive oxidant species' (ROS) are generated, which can cause oxidative stress, damaging lipids, proteins and nucleic acids (Lushchak and Bagnyukova 2007; Mustafa *et al*. 2011). Oxidative stress develops as a consequence of disturbance between generation and elimination of ROS with certain physiological consequences (Lushchak 2011). The antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) act to eliminate these ROS produced within the cell (Lushchak 2011), while the processes of gene expression, apoptosis and signalling are affected by glutathione level within cell and tissue (Trachootham *et al.* 2008).

Species from the family of Clariidae are known for their air-breathing capabilities (Graham 1997) and their ability to survive the adverse conditions of frequent oscillations in oxygen content in their habitat, as they use air-breathing mechanisms to avoid hypoxia (de Graaf and Janssen 1996). The Indian catfish, *Clarias batrachus,* commonly known as 'Mangur' is a freshwater air-breathing teleost species, endemic to the Indian subcontinent and has a fairly common distribution in freshwaters of the plains throughout India (Chonder 1999). It inhabits wetlands, swamps, rivers ponds and tanks, and is well adapted to adverse ecological conditions, such as dissolved oxygen changes in the same habitat during different seasons of the year (Saha and Ratha 2007; Mohindra *et al.* 2012). *C. batrachus,* known to be hypoxia-tolerant, is a facultative air breather at normoxia (Munshi and Ghosh 1994), and it was hypothesized for the present study that the hypoxic conditions will be associated with activation of anaerobic respiration and oxidative stress in *C. batrachus.*

The present study was undertaken to determine the mass-specific metabolic rate (VO_2) of *C. batrachus*, and to study the mechanism of adaptation to acute hypoxic periods, its hematological parameters, metabolic response and antioxidant enzyme activities were explored.

2. Materials and methods

2.1 *Fish maintenance and acclimatization to normoxic conditions*

Live fishes (30–80 g, 16–20 cm) were collected from commercial catches at Lucknow (26° 55′ N, 80° 59′ E), Uttar Pradesh, India, and were acclimatized at normoxia (5.00±0.1 mg/L, DO), at least for a month in tanks of 100 L capacity filled with 25 L of water at 22±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.

2.2 *Determination of gill ventilation rate, mass-specific metabolic rate (VO_2) and critical oxygen tension (pCrit) under hypoxic conditions*

2.2.1 *Acute experimental hypoxic conditions:* In this study, a specially designed closed respirometer made of glass was used, of 7 L capacity with an inlet for the air. A submersible pump was installed inside to circulate the water and an aqua heater (LifeTech) to maintain the temperature inside. Facilities to record dissolved oxygen (DO) and temperature (DO probe; WTW, CellOx 325) and pH (pH electrode; WTW, SenTix® 41-3) were installed. The experiments were set up in triplicate: fishes (47.00±2.0 g, 18.1±0.1 cm) were kept in respirometer, completely filled (without access to air) with water (5.00±0.1 mg/L, DO at 25.0°C), individually. The decrease in DO (due to fish own respiration) was recorded at 15 min intervals, for a minimum of 16 h duration or until the fish suffocates, and the data was used to calculate mass-specific metabolic rate (VO_2) as described below.

2.2.2 *Gill ventilation rate:* The behaviour of the fishes during progressive hypoxia was recorded with video camera (Cyber-shot, DSC HX 200 V, Sony), and the number of gill strokes (ventilator frequency) per min for each fish were counted at an interval of every 30 min until the suffocation point. Individual gill strokes were easily recognized as flowing movements of the operculum as described by Dean (1912).

2.2.3 *Mass-specific metabolic rate (VO_2) and critical oxygen tension (pCrit):* VO_2, oxygen consumption rate during free movement under experimental conditions (Saint-Paul, 1984), was determined following Cech (1990):

$$VO_2 = (((DO_i - DO_f) \times V)/(M \times T))(mg\,O_2/kg/h$$

where DO_i=initial DO concentration, DO_f=final DO concentration, V=volume of respirometer in liters, M=weight of fish in kg and T=time in hours.

For the determination of pCrit, we used individual fish VO_2 values, and then individual data points were averaged, and plotted against 16 different DO concentration values, spaced evenly every 1 h. The XY scatter plot was generated to describe the relationship between VO_2 and DO with linear regression modeling using Excel (MS Office 2003), to obtain regression equation and r-squared values of the analysis.

2.3 *Exposure of fish to experimental hypoxia and sample collection*

The best fit curve to describe the relationship between VO_2 and DO was a straight line for pooled values with simple linear regression modeling (refer Results); the experimental hypoxic level of 0.98±0.1 mg/L DO (2.39±0.24 kPa) was

selected, which was quite below the threshold reported in previous studies for closely related air-breathing catfish species. For the experiments, 21 acclimatized fishes (31.0±1.2 g, 17.60±0.33 cm) were divided into seven groups of three fishes each. First (Normox) group was kept under normoxia (5.00±0.1 mg/L DO at approximately 25.0°C) in partially filled respirometer with an opened air inlet to have access to air and under constant aeration, as described above. For the experimental hypoxia, three fishes were held in the closed respirometer (without access to air-breath), and decrease in DO was due to fish own respiration until experimental hypoxic level (0.98±0.1 mg/L DO), at which DO was further maintained by intermittent aeration. Samples of groups, named PH (progressive hypoxia), were taken when DO reached at experimental hypoxic level and for others (H1 to H12) after 1, 2, 3, 6 and 12 h intervals at experimental hypoxia.

After exposure to hypoxic conditions as well as for normoxia, blood was collected from caudal vein with non-heparinised syringes, and about 100 μL of whole blood was quickly transferred to EDTA vials for hemoglobin and hematocrit determination, and the remaining was allowed to clot at 37°C for 30 min, followed by centrifugation at 5000 rpm for 5 min and serum was separated (Dacie and Lewis 1991). Further, liver, muscle and gill tissues were excised after the animals were euthanized and flash-frozen in liquid nitrogen. Care was taken for the whole operation to last no longer than 5 min.

2.4 *Blood parameters*

Hemoglobin [Hb] and hematocrit [Hct] were determined from whole blood, while lactate and glucose were measured from serum. [Hct] was determined following centrifugation of microhematocrit capillary tube filled with blood, at 10,000 rpm for 5 min (Assendelft and England 1982). Cyanmethaemoglobin method (Dacie and Lewis 1991) was used to determine [Hb]. Mean cell haemoglobin concentration (MCHC) was calculated from ratio of [Hb] to fractional [Hct] (Wells and Weber 1991).

Serum glucose was determined by autozyme STAT glucose kit (Accurex Biomedical Pvt Ltd, India) based on an enzymatic method that uses glucose oxidase and peroxidase reaction to form a red quinoneimine dye that absorbs maximum at 505 nm, measured with Microlab® 300 semiautomated analyser (Vilat Scientific, Dieren, the Netherlands). The intensity of the colour complex was directly proportional to the concentration of glucose (mg/dL).

Quantitative determination of lactate was done using LO-POD enzymatic colorimetric kit (Ref: 1001330 Spinreact, Spain). The kit utilizes peroxide function of aminophenazone and chlorophenol, which forms a red quinone compound, reducing H_2O_2 formed initially by the oxidation of lactate to pyruvate. The absorbance of the red colour compound measured at 505 nm with Microlab® 300 semiautomated analyser (Vilat Scientific, Dieren, the Netherlands) was proportional to the lactate concentration (mg/dL).

2.5 *Biochemical assays*

Prior to assays, liver, muscle and gill tissues were weighed and mechanically disrupted by a teflon pestle motor driver under ice-cold bath. Homogenization was performed in appropriate volume of homogenization buffer (pH 7.0) as recommended in the kits used, for different assays as given below.

2.5.1 *Total glutathione:* Total glutathione (GSH) concentration was determined in serum, liver, gill and muscle tissues. Tissue lysates were centrifuged at 10,000*g* for 15 min at 4°C, and the supernatant was incubated on ice until analyses. All the samples were deproteinized before assay and serum samples were concentrated by lyophilization. Liver and gill tissue lysates were used after appropriate dilutions (liver 1:10; Gill 1:5 times), while serum and muscle samples were not diluted. The assay of total glutathione was done using Cayman's GSH Assay kit (Cat no. 703002, Cayman Chemical Company, MI), following the manufacturer's protocol. The assay utilized enzymatic recycling method with glutathione reductase. The sulphhydryl group of GSH reacted with DTNB, producing TNB. The mixed disulphide GSTNB produced concomitantly was reduced to GSH by glutathione reductase to recycle GSH. The concentration of GSH in the sample was measured as the absorbance of TNB, which provides an accurate estimation of GSH (μM) in the sample. Measurement of the absorbance of TNB was performed at 405–414 nm with Synergy™ HT ELISA reader (BioTek Instruments, Inc USA).

2.5.2 *Enzyme assays:*

2.5.2.1 Lactate dehydrogenase (LDH; EC 1.1.1.27): Lactate dehydrogenase (LDH) activity was determined in serum, liver, gill and muscle tissues. All tissue lysates were centrifuged at 10,000*g* for 15 min at 4°C, and the supernatant was incubated on ice until analyses. Supernatant of all tissue samples were diluted (1:10 times) for LDH activity assay. LDH is an oxidoreductase that catalyses the inter-conversion of lactate and pyruvate. Colorimetric kinetic determination of LDH activity was determined using QuantiChromTM Lactate Dehydrogenase Kit (Cat No. DLDH-100, BioAssay Systems, USA), following the manufacturer's protocol. The assay was based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm, which was determined with Sun Rise A 5082 ELISA plate Reader (TECAN, Salzburg, Austria). The intensity of the purple colour formed is directly proportional to the enzyme activity. LDH activity was reported in international units (μmol NADH oxidized per min)

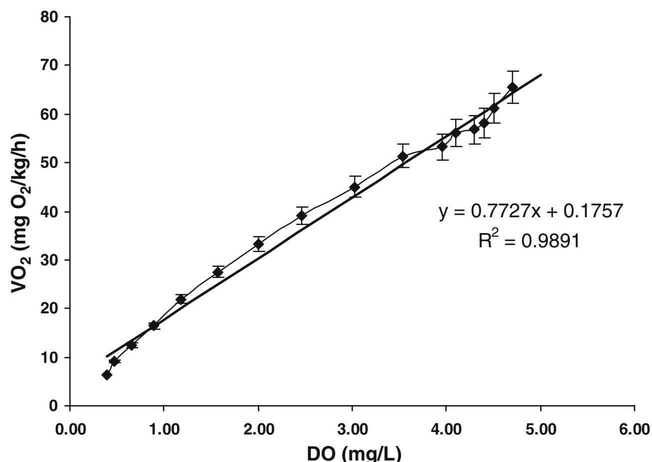

Figure 1. Representative graph showing VO_2 versus $[O_2]$ curve for *Clarias batrachus* respiration with declining dissolved oxygen concentration. Values are expressed as mean±standard deviation.

per milligram protein, after normalization with estimated total protein in milligrams in the respective tissues.

2.5.2.2 Superoxide dismutase (SOD; EC 1.15.1.1): Superoxide dismutase (SOD) activity was determined in serum, liver, gill and muscle tissues. All tissue lysates were centrifuged at 1500*g* for 5 min at 4°C, and the supernatant was incubated on ice until analyses. Only serum samples were diluted (1:5 times) for SOD activity assay. The activity of SOD was measured using colorimetric measurement Assay kit (Cat no. 706002, Cayman Chemicals Ltd, USA), following the manufacturer's protocol. The assay utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine absorbing maximum at 460 nm, which was determined with Sun Rise A 5082 ELISA plate reader (TECAN, Salzburg, Austria). SOD activity was reported as units per milligram protein.

2.5.2.3 Catalase (CAT; EC 1.11.1.6): Catalase (CAT) activity was determined in serum, liver, gill and muscle tissues. All tissue lysates were centrifuged at 10,000*g* for 15 min at 4°C, and the supernatant was incubated on ice until analyses. The supernatant of all the tissue samples were not diluted for CAT activity assay. The colorimetric measurement of CAT activity was done using catalase assay kit (Cat no. 707002, Cayman Chemicals Ltd, USA), following the manufacturer's protocol. The kit utilizes the peroxide function of CAT for determination of enzyme activity. The enzyme reacts with methanol in the presence of H_2O_2, forming formaldehyde, which upon oxidation with purald (chromagen) forms a purple colour bicylic heterocyclic compound showing absorbance at 540 nm, which was determined with Sun Rise A 5082 ELISA plate Reader (TECAN, Salzburg, Austria). Activity was reported in units per milligram protein.

2.6 *Statistical analysis*

All results were expressed as mean±standard deviation (SD) and all the values from hypoxia treated samples were compared with that of normoxic conditions. Data were analyzed for homoscedasticity of variance (Levene's test) and these requisites were achieved by log-transformation. The effect of hypoxia on parameters determined at a single time point (such as those obtained following terminal sampling: hematocrit, hemoglobin, serum lactate, serum glucose, total glutathione, superoxide dismutase, catalase and lactate dehyderogenase activity) were analyzed using one-way ANOVA (with control values from fish exposed to normoxic conditions). When differences were indicated, Tukey's *post hoc* test was used to determine homogeneous subsets. In all cases, α level of 5% ($p<0.05$) was selected to signify statistically significant differences. All statistical tests were performed with SPSS software (version 12.01, 2003).

Table 1. Quantitative estimation of hematocrit (Hct), hemoglobin (Hb) and mean corpuscular hemoglobin concentration (MCHC) in blood of *Clarias batrachus* subjected to progressive and acute hypoxia without access to air (at 0.98 mg/L≈18 mmHg) for different time intervals

Treatments	Time period at pCrit (h)	Hct (%)	Hb (gm%)	MCHC (gm%)
Normox		25.0±4.00^{a}	9.83±0.55^{a}	39.8±4.24^{a}
PH	0	18.3±4.73^{a}	8.43±1.10^{a}	47.2±7.12^{a}
H1	1	18.0±2.00^{b} (–28.0%)	9.50±1.81^{a}	53.5±9.81^{c} (+34.4%)
H2	2	20.3±3.21^{a}	7.93±1.31^{b} (–17.3%)	39.0±1.92^{a}
H3	3	22.0±3.61^{a}	8.83±1.38^{a}	40.2±0.78^{a}
H6	6	32.2±0.76^{b,c} (+28.8%)	10.6±0.35^{c} (+8.0%)	32.9±0.42^{b} (–17.2%)
H12	12	41.0±4.00^{c} (+64.0%)	13.5±1.05^{c} (+36.9%)	32.9±0.65^{b} (–17.3%)

[Hct], [Hb] and MCHC values are mean±SD, n=3; statistical differences among treatments are indicated by dissimilar letters. Values (%) in brackets indicate increase (+) or decrease (–) in experimental hypoxia as compared to normoxia. Superscripts a, b, c indicate homogenous subsets (Tukey's *post hoc* test).

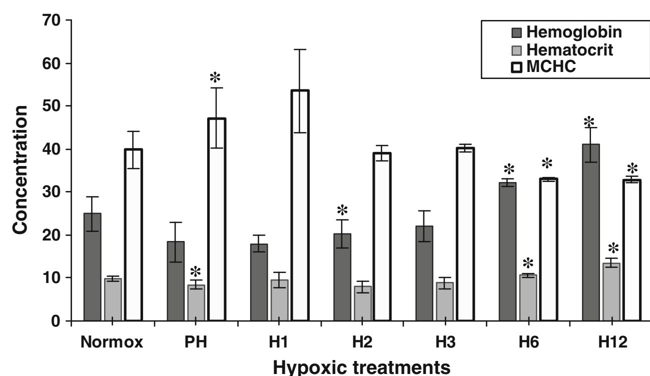

Figure 2. Hematological parameters of *Clarias batrachus,* mean hemoglobin concentration (gram per deciliter), mean hematocrit concentration (per deciliter) and mean cell hemoglobin concentration (gram per deciliter) in normoxia (Normox), progressive hypoxia (PH) and after 1 h (H1), 2 h (H2), 3 h (H3), 6 h (H6) and 12 h (H12) at 0.98±0.1 mg/L, dissolved oxygen. Mean±standard deviation. Asterisk (*) represents significant differences ($p<0.05$) between normoxia and acute hypoxia.

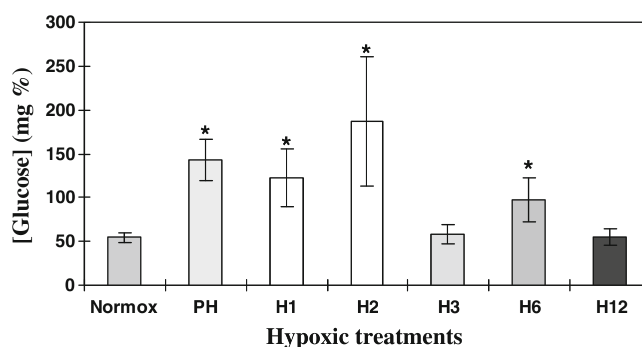

Figure 3. Mean serum glucose concentration (milligram per deciliter) of *Clarias batrachus,* under normoxia (Normox), progressive hypoxia (PH) and 1 h (H1), 2 h (H2), 3 h (H3), 6 h (H6) and 12 h (H12) at 0.98±0.1 mg/L, dissolved oxygen. Mean±standard deviation. Asterisk (*) represents significant differences ($p<0.05$) between normoxia and acute hypoxia.

3. Results

3.1 *Responses to acute experimental hypoxia*

It was found that in most of the period during experiment, fishes became quiescent and sedentary without noticeable activity; however, intermittently they exhibited vigorous movements while coming to the upper portion of respirometer to air-breath. Furthermore, no significant difference ($p>0.1$) was observed in gill ventilation rate (frequency of the gill strokes) between normoxia (58.50±2.12 strokes/min) and under progressive-hypoxia-exposed fishes (59.22±7.62 strokes/min). However, fish attempt to air-breath, i.e. trying to come up to the surface, was observed at 1.6 mg/L DO (3.98 kPa) under progressive hypoxia.

Table 2. Quantitative estimation of glucose and lactate concentration in serum of *Clarias batrachus* subjected to progressive and acute hypoxia without access to air (at 0.98 mg/L≈18 mmHg) for different time intervals

Treatments	Serum glucose (mg%)	Serum lactate (mg%)
Normox	54.4 ± 7.63^{a}	46.0 ± 0.27^{b}
PH	$143\pm23.4^{b,c}$ (+)	129 ± 0.25^{c} (+)
H1	$123\pm33.3^{b,c}$ (+)	89.0 ± 0.29^{b}
H2	$187\pm73.6^{c,d}$ (+)	72.0 ± 0.35^{b}
H3	58.3 ± 10.7^{a}	56.0 ± 0.10^{b}
H6	97.3 ± 25.7^{b} (+)	18.0 ± 0.33^{a} (–)
H12	55.3 ± 9.29^{a}	46.3 ± 0.35^{b}

Serum glucose and serum lactate values are mean±SD, n=3; statistical differences among treatments are indicated by dissimilar letters. Whereas (+) or (–) represents significant increase or decrease in experimental hypoxia as compared to normoxia. Superscripts a, b, c indicate homogenous subsets (Tukey's *post hoc* test).

3.2 *Determination of mass-specific metabolic rate (VO_2) and pCrit*

There was a steady decline in mass-specific metabolic rate (VO_2) of *C. batrachus* (figure 1) with decreasing DO concentration, where no point of sharp decrease in VO_2 with varying DO was observed. When calculated from start of the experiment to 16 h or till suffocation of fish, VO_2 reduced from 65.6±3.52 mgO_2/kg/h at 4.8±0.1 mg/L (11.99±0.24 kPa) to 6.45±1.35 mgO_2/kg/h at 0.37±0.1 mg/L (0.87±0.24 kPa) at 25°C. The best fit curve to

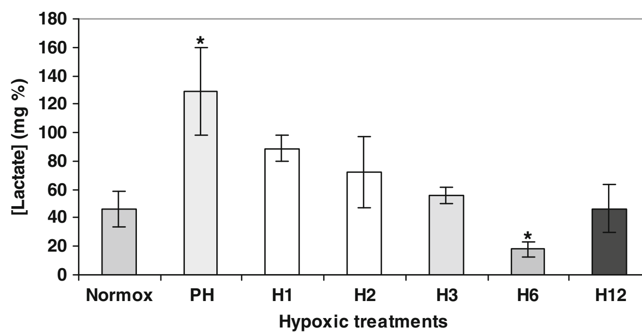

Figure 4. Mean serum lactate concentration (milligram per deciliter) of *Clarias batrachus,* under normoxia (Normox), progressive hypoxia (PH) and 1 h (H1), 2 h (H2), 3 h (H3), 6 h (H6) and 12 h (H12) at 0.98±0.1 mg/, dissolved oxygen. Mean±standard deviation. Asterisk (*) represents significant differences ($p<0.05$) between normoxia and acute hypoxia.

describe the relationship between VO_2 and DO was a straight line for pooled values with simple linear regression modeling ($y=0.7727x+0.175$; $R^2=0.989$). Furthermore, there was no statistical support for justifying two lines in the plot (i.e. no breakpoint where a regulation pattern was superseded by a conforming pattern) that can permit us to calculate pCrit.

3.3 *Hematological and blood metabolite levels*

[Hct] and [Hb] were found to be significantly increased ($p<0.01$), with respect to normoxic control groups by 1.64- and 1.37-fold, respectively, following 12 h exposure at experimental hypoxic level, while MCHC significantly decreased by 1.20-fold ($p<0.05$) following 6 and 12 h of hypoxia exposure (table 1). The blood metabolite levels suggested a particular trend under experimental hypoxia (figure 2). Significant increase in serum glucose levels were observed for group PH (2.63-fold) and after 1 h (2.26-fold; $p<0.01$), 2 h (3.44-fold; $p<0.001$) and 6 h (1.79-fold; $p<0.05$) (table 2) at experimental hypoxic level (figure 3). Serum lactate concentration initially increased significantly (2.80-fold; $p<0.05$) following PH and 1 h of hypoxia, while it decrease at 6 h (2.56-fold; $p<0.05$) and stabilized at its initial level at 12 h at experimental hypoxic level (figure 4).

3.4 *Lactate dehydrogenase activity*

LDH activity ranged from 9.63 to 104 U/mg protein in the gill and from 12.9 to 27.8 U/mg protein in the liver (table 3). It was significantly higher in gill tissue at PH (4.66-fold) and 12 h (3.36-fold) and in liver at 12 h (2.15-fold) of hypoxia (figure 5). There was no significant change observed in other tissues.

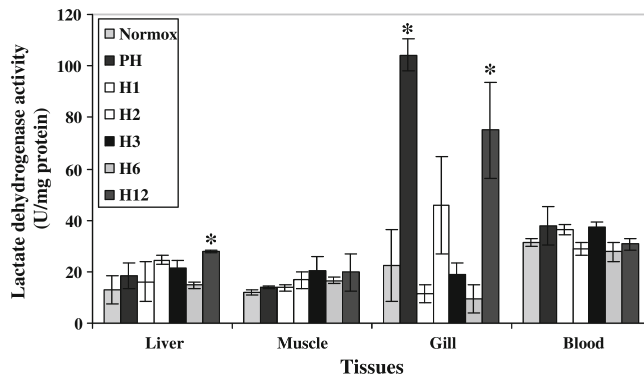

Figure 5. Mean specific activity of lactate dehydrogenase enzyme (U/mg protein) in liver, muscle, gill, and blood of *Clarias batrachus* in normoxia (Normox), progressive hypoxia (PH) and after 1 h (H1), 2 h (H2), 3 h (H3), 6 h (H6) and 12 h (H12) at 0.98±0.1 mg/L, dissolved oxygen. Mean±standard deviation. Asterisk (*) represents significant differences ($p<0.05$) between normoxia and acute hypoxia.

3.5 *Total glutathione*

Total glutathione concentration ranged from 138 to 204 μM/mg protein in liver tissue, 4.66 to 69.6 μM/mg protein in muscle and 34.7 to 103 μM**N**mg protein in gill tissue (table 4). Total glutathione level did not change significantly in any tissue, except for muscle, where a significant increase (5.95-fold; $p<0.05$) was observed at 12 h of hypoxia (figure 6), in comparison to normoxia. In serum, glutathione activity was below detection limit.

3.6 *Superoxide dismutase activity*

SOD activity ranged from 0.18 to 0.92 U/mg protein in liver, 0.99 to 2.97 U/mg protein in muscle, 0.14 to 3.26 U/mg

Table 3. Mean specific activity of lactate dehydrogenase (LDH) enzyme in different tissues of *Clarias batrachus* subjected to progressive and acute hypoxia without access to air (at 0.98 mg/L≈18 mmHg) for different time intervals

	Lactate dehydrogenase enzyme (Units/mg protein)			
Treatments	Liver	Muscle	Gill	Serum
Normox	12.9 ± 5.30^{a}	11.8 ± 1.00^{a}	$22.3\pm14.0^{a,b}$	$31.2\pm1.39^{a,b}$
PH	$18.5\pm5.06^{a,b}$	13.8 ± 0.43^{a}	104 ± 6.28^{d} (+)	37.9 ± 7.67^{b}
H1	$16.1\pm7.68^{a,b}$	13.7 ± 1.37^{a}	11.4 ± 3.42^{a}	$36.3\pm1.98^{a,b}$
H2	$24.6\pm1.59^{a,b}$	16.8 ± 3.34^{a}	$45.8\pm18.8^{b,c}$	$28.8\pm2.39^{a,b}$
H3	$21.2\pm3.35^{a,b}$	20.6 ± 5.23^{a}	$18.9\pm4.53^{a,b}$	$37.3\pm1.99^{a,b}$
H6	14.8 ± 1.40^{a}	16.7 ± 1.46^{a}	9.63 ± 5.45^{a}	27.7 ± 3.61^{a}
H12	27.8 ± 0.57^{b} (+)	19.8 ± 7.23^{a}	$75.0\pm18.8^{c,d}$ (+)	$30.7\pm2.32^{a,b}$

Mean specific activity values of LDH, are mean±SD, $n=3$; statistical differences among treatments are indicated by dissimilar letters. Whereas (+) represents significant increase in experimental hypoxia as compared to normoxia. Superscripts a, b, c, d indicate homogenous subsets (Tukey's *post hoc* test).

Table 4. Total glutathione (GSH) concentration in different tissues of *Clarias batrachus* subjected to progressive and acute hypoxia without access to air (at 0.98 mg/L≈18 mmHg) for different time intervals

Treatments	Total GSH (μM/mg protein) Liver	Muscle	Gill	Blood
Normox	196±43.4^{a}	11.7±4.98^{a}	72.9±34.4^{a}	bd
PH	274±72.3^{a}	6.58±4.18^{a,b}	204.4±121.5^{a}	bd
H1	138±38.2^{a}	4.98±1.36^{a}	34.7±6.54^{a}	bd
H2	205±36.7^{a}	7.78±5.28^{a,b}	79.3±22.9^{a}	bd
H3	159±28.5^{a}	4.66±0.32^{a}	87.5±22.8^{a}	bd
H6	162±45.7^{a}	46.9±9.59^{a,b}	42.3±6.80^{a}	bd
H12	252±66.1^{a}	69.6±11.63^{b} (+)	103±15.1^{a}	bd

Total glutathione concentration values are mean±SD, n=3; statistical differences among treatments are indicated by dissimilar letters. Whereas bd represents the concentration below detection limit of assay. Whereas (+) represents significant increase in experimental hypoxia as compared to normoxia. Superscripts a, b indicate homogenous subsets (Tukey's *post hoc* test).

protein in gill and 0.14 to 0.65 U/mL in serum (table 5). After PH, the highest mean SOD activity was observed in gill tissue, which was significantly increased (6.15-fold; p<0.05), while it was significantly decreased (p<0.05) in liver (PH; 3.84-fold, 1 h; 5.13-fold, 2 h; 2.49-fold, 6 h; 4.85-fold and 12 h; 1.61-fold) and serum (1 h; 3.07-fold, 2 h; 2.69-fold, 3 h; 2.39-fold and 6 h; 2.15-fold), as compared to normoxic control groups (figure 7). There was no difference in muscle SOD activity at any of the time interval.

3.7 *Catalase activity*

CAT activity ranged from 10.9 to 55.9 U/mg protein in serum, 111 to 303 U/mg protein in gill, 25.4 to 69.2 U/mg protein in muscle and 419 to 559 U/mg protein liver (table 6). Significant increase (p<0.05) in catalase activity was detected in gill (3 h; 1.93-fold, 6 h; 1.64-fold and 12 h; 1.38-fold), muscle (6 h; 2.64-fold) and serum (6 h; 2.70-fold) (figure 8).

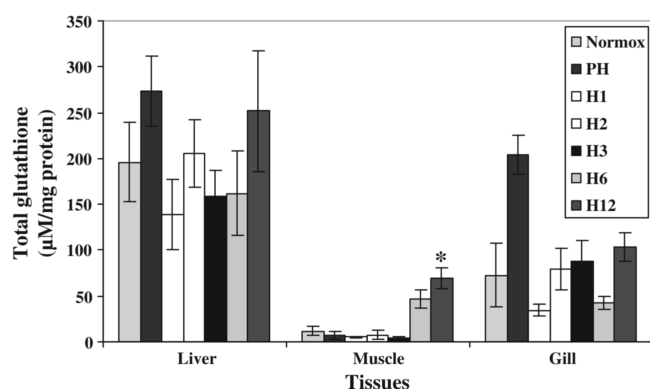

Figure 6. Total glutathione concentration (μM/mg of protein) in liver, muscle and gill of *Clarias batrachus* in normoxia (Normox), progressive hypoxia (PH) and after 1 h (H1), 2 h (H2), 3 h (H3), 6 h (H6) and 12 h (H12) at 0.98±0.1 mg/L, dissolved oxygen. Values are expressed as mean±standard deviation. Asterisk (*) represents significant differences (p<0.05) between normoxia and acute hypoxia.

4. Discussion

In the present study, it was observed that in the facultative air-breathing catfish, *C. batrachus*, oxygen consumption rate decreased linearly with decrease in environmental oxygen, and it was not able to regulate oxygen consumption in hypoxic conditions, thus indicating that *C. batrachus* can be classified as an oxyconformer (Portner *et al.* 1985). In previous reports, oxyconforming response to lowering oxygen levels have been reported in fishes like *Galaxias maculatus* (Urbina and Glover 2012) and *Acipenser naccarii* (McKenzie *et al.* 2007) as well as in a sea worm, *Sipunculus nudus* (Portner *et al.* 1985). Furthermore, suppression of rate of metabolism is an obligate survival strategy in many hypoxia/anoxia-adapted animals (Nilsson and Lutz 1993). By reducing their metabolic rate during hypoxia, animals delay the depletion of glycogen stores as well as the accumulation of toxic levels of lactate. This is also supported by the observation that frequency of gill strokes remained constant in both normoxic and progressive hypoxia conditions. It was suggested that in the scaleless fish, skin may also play an important role in oxygen uptake in the face of decreasing oxygen environment, and cutaneous oxygen uptake may have an important role in shaping the oxygen consumption response to hypoxia (Urbina *et al.* 2012). This may be true in case of *C. batrachus*, as rich vascularisation of the tissues underlying the operculum epithelium was observed, which may assist in accessory cutaneous respiration to cope with the oxygen-depleted conditions of swamps and ponds, which it inhabits (Garg and Mittal 1990). McKenzie *et al.* (2007) suggested that a fish species, under static conditions, acts as

Table 5. Mean specific activity of superoxide dismutase (SOD) enzyme in different tissues of *Clarias batrachus* subjected to progressive and acute hypoxia without access to air (at 0.98 mg/L≈18 mmHg) for different time intervals

Treatments	SOD activity (Units/mg protein)			
	Liver	Muscle	Gill	Blood
Normox	0.92±0.32^{b}	0.99±0.39^{a}	0.53±0.19^{a,b}	0.43±0.14^{b,c}
PH	0.24±0.15^{a} (–)	1.16±0.84^{a}	3.26±1.15^{c} (+)	0.65±0.14^{c}
H1	0.18±0.07^{a} (–)	2.13±0.32^{a}	0.14±0.07^{a}	0.14±0.03^{a} (–)
H2	0.37±0.17^{a} (–)	2.00±0.19^{a}	0.89±0.12^{a,b,c}	0.16±0.01^{a} (–)
H3	0.54±0.15^{a,b}	2.97±1.37^{a}	0.97±0.19^{a,b,c}	0.18±0.03^{a} (–)
H6	0.19±0.05^{a} (–)	2.15±0.89^{a}	1.02±0.02^{b,c}	0.20±0.04^{a} (–)
H12	0.57±0.21^{a} (–)	2.32±0.88^{a}	1.43±0.30^{b,c}	0.32±0.14^{a,b}

SOD activity values are mean±SD, n=3; statistical differences among treatments are indicated by dissimilar letters. Whereas (+) or (–) represents significant increase or decrease in experimental hypoxia as compared to normoxia. Superscripts a, b, c indicate homogenous subsets (Tukey's *post hoc* test).

an oxyconformer and if it is allowed to swim, may act as an oxy-regulator; as swimming might make a significant contribution to oxygen uptake due to ram respiration. In the present study, the chosen volume of the respirometer was large enough for the fish to swim about; however, swimming activity, except occasionally, was not observed during the decreasing environment oxygen, even though it became active upon initial stage of experiment, when not allowed to air-breath and then the fish calmed down. The reduction of routine spontaneous activity in hypoxia saves energy expenditure, and may be an adaptive strategy. Also, it has been shown previously that change in water parameters (e.g. pH and dissolved CO_2) potentially associated with the use of closed respirometry have no effect on pCrit (Henriksson *et al.* 2008) and

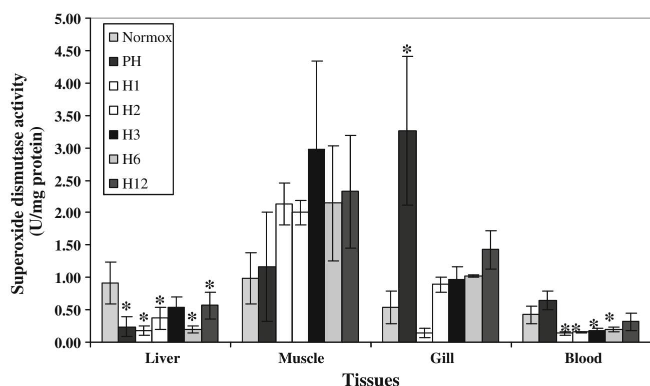

Figure 7. Mean specific activity of superoxide dismutase enzyme (Units/mg protein) in liver, muscle, gill, and blood of *Clarias batrachus* in normoxia (Normox), progressive hypoxia (PH) and after 1 h (H1), 2 h (H2), 3 h (H3), 6 h (H6) and 12 h (H12) at 0.98±0.1 mg/L, dissolved oxygen. Mean±standard deviation. Asterisk (*) represents significant differences (p<0.05) between normoxia and acute hypoxia.

oxygen consumption (Ishimatsu *et al.* 2008) in fish. It has also been observed that both closed and semiclosed respirometry gave identical results in determination of oxygen consumption (Urbina and Glover 2012), which suggest that alterations in dissolved CO_2 levels observed in the present study were unlikely to have influence on the observations made.

In the present study on *C. batrachus*, an increase in [Hb] and [Hct] and decrease in MCHC in hypoxic conditions and its weak correlation (r=–0.80), with mean values of [Hct] after 6 and 12 h exposure to experimental hypoxia, suggested the possibility that oxygen carrying capacity of the blood might be enhanced by bringing more red blood cells into circulation. These cells are most likely released from the spleen upon adrenergic and/or cholinergic stimulation (Nilsson and Grove 1974). These hormones serve to increase the transfer of oxygen across the gills and the transport of oxygen, in the blood, to actively metabolizing tissues. During environmental hypoxia, catecholamines are mobilized into the blood when the arterial oxygen content significantly decreases (Perry and Reid 1994). Evidence from teleost fish suggests that the release of red blood cells via splenic contraction does occur in response to elevated catecholamines (Nilsson *et al*. 1975). Splenic contraction has been well characterized in fishes in response to hypoxia (Lai and Todd 2006). However, the ability of the spleen to act as a RBC reservoir in air-breathing fish has not been examined.

The level of blood lactate concentrations is routinely used as an indicator of the extent of anaerobic metabolism. In the present study, when exposed to progressive hypoxia up to experimental hypoxia level without access to air, the serum lactate levels of *C. batrachus* were significantly increased as compared to normoxic conditions. Thus, it can be assumed that the oxygen uptake from the air was not sufficient to sustain complete aerobic metabolism at this aquatic oxygen tension

Table 6. Mean specific activity of catalase (CAT) enzyme in different tissues of *Clarias batrachus* subjected to progressive and acute hypoxia without access to air (at 0.98 mg/L≈18 mmHg) for different time intervals

Treatments	Catalase activity (Units/mg protein)			
	Liver	Muscle	Gill	Blood
Normox	$390 \pm 26.6^{a,b}$	26.2 ± 4.40^{a}	$157 \pm 79.4^{a,b}$	$20.7 \pm 2.41^{a,b,c}$
PH	443 ± 32.8^{b}	25.4 ± 4.12^{a}	111 ± 48.6^{a}	10.9 ± 2.98^{a}
H1	$421 \pm 44.7^{a,b}$	28.6 ± 7.08^{a}	$194 \pm 34.0^{b,c}$	$23.0 \pm 4.48^{a,b}$
H2	419 ± 12.4^{a}	36.4 ± 5.34^{a}	142 ± 51.1^{a}	$30.3 \pm 17.3^{c,d}$
H3	$452 \pm 59.2^{a,b}$	49.9 ± 9.17^{a}	303 ± 48.1^{d} (+)	$33.2 \pm 4.48^{b,c,d}$
H6	559 ± 15.7^{b}	69.2 ± 10.0^{b} (+)	$257 \pm 32.0^{c,d}$ (+)	55.9 ± 28.7^{e} (+)
H12	423 ± 86.1^{b}	53.9 ± 25.3^{a}	$217 \pm 28.1^{c,d}$ (+)	$33.2 \pm 15.7^{c,d}$

CAT activity values are mean±SD, $n=3$; statistical differences among treatments are indicated by dissimilar letters. Whereas (+) represents significant increase in experimental hypoxia as compared to normoxia. Superscripts a, b, c, d, e indicate homogenous subsets (Tukey's *post hoc* test).

and that this fish was metabolically in a hypoxic condition and anaerobic glycolysis was activated. Simultaneously, at this stage, serum glucose as well as LDH activity in oxidative tissues, liver and gills, at 12 h at experimental hypoxia level, were found to be significantly increased. The most striking result from this study was that serum lactate concentration peaked at reaching experimental hypoxia level through progressive hypoxia and then showed a decreasing trend, when oxygen was stabilized at this level. The only two pathways to clear lactate are oxidation and gluconeogenesis, i.e. lactate is converted back into pyruvate and either oxidized to CO_2 and H_2O or incorporated into glycogen, causing serum lactate to decrease. However, the activation of lactate oxidation and glucose production during hypoxia, in the face of metabolic depression shown by *C. batrachus,* is not clear. Martinez *et al.* (2006) demonstrated in isolated rat hepatocytes, that under experimental hypoxic conditions, the expression of gluconeogenic enzymes is stimulated via activation of phosphoenolpyruvate carboxykinase by hypoxia-inducible factor 1α (HIF-1α). This suggestion gets support from the fact that during the present study also, expression of HIF-2α, a paralogue of HIF-1α, has been found to be up-regulated during hypoxia (data not shown). However, more work is needed to elucidate whether this same mechanism also activates gluconeogenesis in this fish. Consequently, the role of higher LDH activity in liver and gill tissues, in this fish under hypoxic stress, can be in the clearance of blood lactate and the provision of glucose for metabolism by extra-hepatic tissues such as heart and brain, which are important in the maintenance of an organism's homeostasis (Martinez *et al.* 2011).

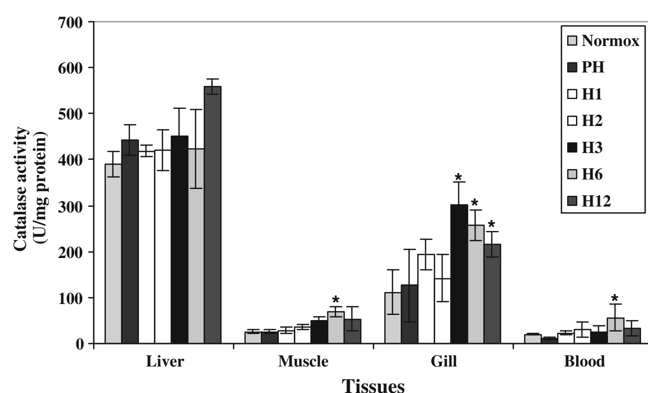

Figure 8. Mean specific activity of catalase enzyme (U/mg protein) in liver, muscle, gill, and blood of *Clarias batrachus* in normoxia (Normox), progressive hypoxia (PH) and after 1 h (H1), 2 h (H2), 3 h (H3), 6 h (H6) and 12 h (H12) at 0.98±0.1 mg/L, dissolved oxygen. Mean±standard deviation. Asterisk (*) represents significant differences ($p<0.05$) between normoxia and acute hypoxia.

Reactive oxygen species (ROS) are products of partial reduction of molecular oxygen which may be produced by inefficient mitochondrial respiration and/or specialized systems, designed to produce ROS and can be up-regulated in hypoxic environments or exposures to metal ions (Staples and Buck 2009; Lushchak 2011; Mustafa *et al.* 2012). In order to detoxify these ROS, the antioxidant enzymes that make up the antioxidant defense system are expected to increase under hypoxia as evident from various studies on fish, and turtles (Lushchak *et al.* 2005; Baker *et al.* 2007). However, no such pattern was observed in the present study, nor has correlative activity among CAT and SOD been observed in other studies (Oruc and Uner 2000). SOD activity was high only in gill as an initial response. As gills are the primary tissue to capture available oxygen for an air-breathing fish, kept submerged under hypoxia, in order to eliminate ROS initially produced, SOD activity was induced followed by that of CAT activity in later hours, which may be relevant adaptive mechanism against post hypoxic ROS insult. However, liver and serum had shown a decrease in SOD activity, similar to that reported for spot (Cooper *et al.* 2002) and turtle *Trachemys scripta elegans* (Willmore and Storey 1997). Total glutathione, an antioxidant (Wilhelm-Filho *et al.* 2005) and indicator of redox state, was

unaffected in different tissues of *C. batrachus*. Unaltered level of glutathione were also reported for Nile tilapia during hypoxia/reoxygenation (Welker *et al.* 2012), which may indicates that redox state of cells is not affected irrespective of duration of hypoxia (Lushchak and Bagnyukova 2006).

In conclusion, on the basis of the results presented here, it was found that the *Clarias batrachus*, an air-breathing fish, is an oxyconformer, and the exposure of fish to different periods of experimental hypoxia resulted in adjustment of oxygen carrying capacity, metabolic depression and antioxidant defense system. These physiological alterations might be correlated with its capacity to tolerate hypoxic conditions. Although these broad outlines of adaptation for hypoxic survival are recognized through this study, understanding of signals involved in these interrelated processes needs to be further explored.

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References

Assendelft OW and England JM 1982 *Advances in hematological methods—the blood count* (Boca Raton, FL: CRC Press)

Baker PJ, Costanzo JP and Lee RE Jr 2007 Oxidative stress and antioxidant capacity of a terrestrially hibernating hatchling turtle. *J. Comp. Physiol. B.* **177** 875–883

Braun N, Lima RL De, Moraes B, Loro VL and Baldisserotto B 2006 Survival, growth and biochemical parameters of silver catfish, *Rhamdia quelen* (Quoy & Gaimard, 1824), juveniles exposed to different dissolved oxygen levels. *Aqua. Res.* **37** 1524–1531

Cech JJ 1990 Respirometry; in *Methods of fish biology* (eds) CB Schreck and PB Moyle (Bethesda MD: American Fisheries Society) pp 335–362

Chonder SL 1999 *Biology of finfish and shellfish* (Howrah, India: SCSC Publisher) pp 303–313

Cooper RU, Clough LM, Farwell MA and West TL 2002 Hypoxia-induced metabolic and antioxidant enzymatic activities in the estuarine fish *Leiostomus xanthurus*. *J. Exp. Mar. Biol. Ecol.* **279** 1–20

Dacie SIV and Lewis SM 1991 *Practical haematology 7th edition (London, Melborne and New York: J.A. Chuchill Ltd. Livingstone)*

Damotharan P, Perumal NV, Arumugam M, Vijayalakshmi S and Balasubramanian T 2010 Seasonal variation of physico-chemical characteristics in point calimere coastal waters south east coast of India. *Middle East J. Sci. Res.* **64** 333–339

Dean B 1912 Additional notes on the living specimens of the Australian lungfish *Ceratodus forsteri*, in the collection of the Zoological Society of London. *Proc. Zool. Soc. London*. **82** 607–612

de Graaf G and Janssen H 1996 *Artificial reproduction and pond rearing of the African catfish, Clarias gariepinus in sub-Saharan Africa*. (Amsterdam: Nesfisco Foundation)

Fernandes MN and Mazon AF 2003 Environmental pollution and fish gill morphology; in *Fish adaptations* (eds) AL Val and BG Kapoor (Enfield: Science Publishers) pp 203–231

Garg TK and Mittal AK 1990 The epidermal and inner epithelial lining of the operculum in *Clarias batrachus* Clariidae, Siluriformes. *Jap. J. Icthy*. **37** 149–157

Graham JB 1997 *Air-breathing fishes: Evolution, diversity and adapatation* (San Diego, CA: Academic Press)

Henriksson P, Mandic M and Richards JG 2008 The osmo-respiratory compromise in sculpins: impaired gas exchange is associated with freshwater tolerance. *Physiol. Biochem. Zool.* **81** 310–319

Hochachka PW and Somero GN 2002 *Biochemical adaptation: mechanism and process in physiological evolution* (Oxford: Oxford University Press) p 466

Ishimatsu A, Hayashi M and Kikkawa T 2008 Fishes in high-CO_2, acidified oceans. *Mar. Ecol. Prog. Ser.* **373** 295–302

Lai AY and Todd KG 2006 Hypoxia-activated microglial mediators of neuronal survival are differentially regulated by tetracyclines. *Glia* **53** 809–816

Lushchak VI 2011 Environmentally induced oxidative stress in aquatic animals. *Aquat. Toxicol.* **101** 13–30

Lushchak VI and Bagnyukova TV 2006 Effects of different environmental oxygen levels on free radical processes in fish. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **144** 283–289

Lushchak VI and Bagnyukova TV 2007 Hypoxia induces oxidative stress in tissues of a goby, the rotan *Perccottus glenii*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **148** 390–7

Lushchak VI, Bagnyukova TV, Lushchak OV, Storey JM and Storey KB 2005 Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp *Cyprinus carpio* tissues. *Int. J. Biochem. Cell Biol.* **37** 1319–1330

Martinez ML, Landry C, Boehm R, Manning S, Cheek AO and Rees BB 2006 Effects of long-term hypoxia on enzymes of carbohydrate metabolism in the Gulf killifish, *Fundulus grandis*. *J. Exp. Biol.* **209** 3851–3861

Martinez ML, Raynard EL, Bernard BR and Chapman LJ 2011 Oxygen limitation and tissue metabolic potential of the African fish *Barbus neumayeri*: roles of native habitat and acclimatization. *BMC Ecol.* **11** 2

McKenzie DJ, Steffensen JF, Korsmeyer K, Whiteley NM, Bronzi P and Taylor EW 2007 Swimming alters responses to hypoxia in the Adriatic sturgeon *Acipenser naccarii J. Fish Biol.* **70** 651–658

Mohindra V, Tripathi RK, Singh A and Singh B 2012 Molecular characterization and expression analysis of a novel cystatin-like gene in a hypoxia-tolerant Indian catfish, *Clarias batrachus* [Linnaeus, 1758]. *Fish Shellfish Immunol*. DOI: 10.1016/j.fsi.2012.11.018

Munshi JSD and Ghosh TK 1994 Metabolic wheel hypothesis as applied to air-breathing fishes of India; in *Advances in fish*

biology (ed) HR Singh (Delhi: Hindustan Publishing Corporation) pp 70–78

Mustafa SA, Al-Subiai SN, Davies SJ and Jha AN 2011 Hypoxia-induced oxidative DNA damage links with higher level biological effects including specific growth rate in common carp, *Cyprinus carpio* L. *Ecotoxicol.* 20 1455–1466

Mustafa SA, Davies SJ and Jha AN 2012 Determination of hypoxia and dietary copper mediated sub-lethal toxicity in carp, *Cyprinus carpio*, at different levels of biological organization. *Chemosphere* **87** 413–422

Nilsson GE and Lutz PL 1993 Role of GABA in hypoxia tolerance, metabolic depression and hibernation–possible links to neurotransmitter evolution. *Comp. Biochem. Physiol. C* **105** 329–336

Nilsson L, Kogure K and Busto R 1975 Effects of hypothermia and hyperthermia on brain energy metabolism. *Acta Anaesth. Scand.* **193** 199–205

Nilsson S and Grove DG 1974 Adrenergic and cholinergic innervation of the spleen of the cod: *Gadus morhua*. *Eur. J. Pharmacol.* **28** 135–143

Oruc EO and Uner N 2000 Combined effects of 2, 4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of *Oreochromis niloticus*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **127** 291–296

Perry SF and Reid SG 1994 The effects of acclimation temperature on the dynamics of catecholamine release during acute hypoxia in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **186** 289–307

Portner HO, Heisler N and Grieshaber MK 1985 Oxygen consumption and mode of energy production in the intertidal worm *Sipunculus nudus* L: Definition and characterization of the critical PO_2 for an oxyconformer. *Res. Physiol.* **59** 361–377

Saha N and Ratha BK 2007 Functional ureogenesis and adaptation to ammonia metabolism in Indian freshwater air-breathing catfishes. *Fish Physiol. Biochem*. **33** 283–295

Saint-Paul U 1984 Physiological adaptation to hypoxia of a neotropical characoid fish *Colossoma macropomum*, Serrasalmidae. *Env. Biol. Fishes*. **11** 53–62

Speers-Roesch B, Brauner CJ, Farrell AP, Hickey AJR, Renshaw GMC, Wang YS and Richards JG 2012 Hypoxia tolerance in elasmobranchs. II. Cardiovascular function and tissue metabolic responses during progressive and relative hypoxia exposures. *J Exp. Biol.* **215** 103–114

Staples JF and Buck LT 2009 Matching cellular metabolic supply and demand in energy-stressed animals. *Comp. Biochem. Physiol. A* **153** 95–105

Timmerman CM and Chapman LJ 2004 Behavioral and Physiological Compensation for Chronic Hypoxia in the Sailfin Molly (*Poecilia latipinna*). *Physiol. Biochem. Zool.* **77** 601–610

Trachootham D, Lu W, Ogasawara MA, Valle NRD and Huang P 2008 Redox regulation of cell survival. *Antioxid. Redox Signal* **10** 1343–1374

Urbina MA and Glover CN 2012 Should I stay or should I go? Physiological, metabolic and biochemical consequences of voluntary emersion upon aquatic hypoxia in the scaleless fish *Galaxias maculatus*. *J. Comp. Physiol. B* DOI: 10.1007/s00360-012-0678-3

Urbina MA, Glover CN and Forster ME 2012 A novel oxyconforming response in the freshwater fish *Galaxias maculatus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **161** 301–306

Welker AF, Campos EG, Cardoso LA and Hermes-Lima M 2012 Role of catalase on the hypoxia/reoxygenation stress in the hypoxia-tolerant Nile tilapia. *Am. J. Physiol. Regu. Physiol.* **302** 1111–1118

Wells RMG and Weber RE 1991 Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* Walbaum? An interpretation of recent data based on blood viscosity measurements. *J. Fish. Biol.* **38** 53–65

Wilhelm-Filho D, Torres MA, Zaniboni-Filho E and Pedrosa RC 2005 Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, *Leporinus elongates* Valenciennes, 1847. *Aquaculture* **244** 349–357

Willmore WG and Storey KB 1997 Antioxidant systems and anoxia tolerance in a freshwater turtle *Trachemys scripta elegans*. *Mol. Cell Biochem.* **170** 177–185

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