

HYPOXIA TOLERANCE IN AMAZON CICHLIDS

Adriana R. Chippari-Gomes
(PhD Graduate Program - INPA)
Laboratory of Ecophysiology and Molecular Evolution (LEEM)
National Institute for Research in the Amazon (INPA)
Avenida André Araujo, 2936, Manaus, Brasil. CEP 69083-000
Phone (55) 092 643-3191 Fax (55) 092 643-3186
e-mail: chippari@inpa.gov.br

Maria de Nazaré Paula-Silva (LEEM-INPA)
Adalberto Luís Val (LEEM – INPA)
José Eduardo P.W. Bicudo (USP São Paulo – SP)
Vera Maria F. Almeida-Val (LEEM-INPA)

Abstract

Amazon cichlids are amongst the most diverse Neotropical fish groups. They are considered a plastic group from ecological, genetic, and evolutionary point of view. Their radiation may be considered as the result of environmental adaptations they developed during their evolutionary history. Among these adaptive traits, their ability to survive environmental hypoxia and anoxia has been described in several genera and seems to reflect their ecological preferences. The present paper compares behavioral, physiological and biochemical responses of four cichlid species exposed to acute hypoxia.

Introduction

Neotropical cichlids are advanced teleosts and occur in South and Central America and in the south part of North America. Recent reports have described them as a monophyletic clade retaining a fast rate of molecular evolution based upon a significantly higher level of genetic variation compared to their African counterparts (Farias et al., 1999). This group has always been considered a plastic group (Stiassny, 1991). Most authors consider their facility to adapt to heterogeneous habitats, as well as their fast adaptive radiation, as a cause of its numerous speciation events (Fryer and Iles, 1972; Kornfield, 1979, 1984; Stiassny, 1991).

Metabolic adjustments to extremely variable environments have been described as a complement of such phenotypic plasticity, particularly when fishes are exposed to hypoxia, a common event in Amazon water bodies (Almeida-Val et al., 1993; Val and Almeida-Val, 1995; Almeida-Val et al., 1995). The isozymic tissue distribution of Lactate Dehydrogenase (LDH: E.C. 1.1.1.27) represents species' adaptive tolerance to hypoxia and is adjustable to hypoxia exposure (Almeida-Val et al., 1995; 1999).

During our last expedition to Anavilhanas Archipelago in December 1999, our main goal was to establish hypoxia tolerance in cichlids and verify the presence of patterns among species. The present paper compares the preliminary data obtained with four cichlid species found in abundance at that time.

Material and Methods

Animals

Species were captured in Anavilhanas Archipelago at Negro River in the proximities of IBAMA (Brazilian Environmental Agency) floating station (Amazonas, Brazil). INPA's Research Vessel Amaná II was anchored at 2°43' S and 60°45' W during the whole expedition period (December 1-10, 1999). Dissolved oxygen was monitored at the locality of capture and during different periods of the day. At that time, the amount of dissolved oxygen in the water was not a limiting factor (5.6 ± 0.115 mg/L O₂). The following species were studied: *Heros sp* (body mass 170.0±17.5g, ventricle mass 0.057±0.005g); *Uaru amphiacanthoides* (body mass 218.25±23.3g, ventricle mass 0.067±0.007g); *Satanoperca jurupari* (body mass 181.9±12.4g, ventricle mass 0.091±0.008g) and *Geophagus altifrons* (body mass 202.1±14.3g, ventricle mass 0.058±0.005g). Specimens were kept in outdoor tanks for 24 hours prior to the experimental procedure. After this period, three groups of two specimens (total 6) were exposed to acute hypoxia ($P_{wO_2} \cong 43$ mmHg) (N₂ flushing) in experimental polyethylene aquaria (56 L) at room temperature (26.0±2.0°C). The period of time the animals supported before losing equilibrium was measured for each animal. Oxygen contents were monitored during the whole experiment using a Digital acid-base analyzer, attached to a PO₂ module Radiometer, PHM72-Mk2. During the experimental period, we measured VO₂ and opercular movements. For control purposes, each experiment was also repeated with animals exposed to normoxia ($P_{wO_2} \cong 160$ mmHg). All experimental procedures took place at INPA's Research Vessel Amaná II, in December 1999, when river water levels were low.

Blood sample

Immediately after the experiments, blood was collected from the caudal vein into heparinized syringes, transferred to Ependorff tubes and kept on ice. Blood parameters: hematocrit, hemoglobin concentration, and red blood cell counts (RBC) were estimated by classical methods. Plasma glucose was estimated using enzymatic method with commercial kit (Doles®).

Tissue preparation

Immediately after blood collection, animals were killed with a sharp blow to their head followed by severing of the spinal cord, as recommended by animal care associations. Tissues (skeletal and heart muscles) were then excised and promptly frozen in liquid nitrogen. Samples were transferred to laboratory and stored at a - 73°C before analysis. Skeletal and heart muscles were homogenized by hand in test tubes with glass tube rods in 4 vol. (skeletal muscle) or 9 vol. (heart muscle) of medium containing 75 mM TRIS, 1 Mm EDTA, and 1 mM DTT at pH 7.6. The homogenates were centrifuged at 18,000 g for 30 minutes at 4°C in a Sorvall RC5B centrifuge. Supernatants (tissue extracts) were used to estimate enzyme activities and electrophoresis. All assays were performed directly on total extracts or dilutions of total extracts.

LDH activities

Maximum activity of lactate dehydrogenase (LDH; E.C. 1.1.1.27) were determined at 25°C using a Genesys 2 spectrophotometer. Assay conditions followed those described in Almeida-Val et al. (1995). All enzyme assays had a final cuvette volume of 1 mL, and were measured at 340 nm. Composition of the assay media was 0.15 mM NADH, 1 mM KCN and 50 mM imidazole (pH 7.4). The reaction was initiated with the addition of 1 mM pyruvate, or with the inhibitory concentration (10 mM). Enzyme activities were expressed as moles of pyruvate converted per minute per gram of wet tissue.

Data analysis

The results are expressed as means \pm SEM. Statistical differences between groups were tested with Student's *t*-test or by one-way ANOVA (Sigma Stat). Significance of difference was accepted when $P < 0.05$.

Results and Discussion

VO₂ and Opercular movements

Among all analyzed species, *S. jurupari* had no tolerance to acute hypoxia. The remaining three species tolerate hypoxia for 60 minutes, or more (43 mmHg) (table 1 and figure 1).

Table 1. Opercular movements and time to reach disequilibrium of four Amazon cichlids submitted to normoxia and acute hypoxia.

SPECIES (TIME TO REACH DISEQUILIBRIUM)	Opercular movements (mov/min)	
	Normoxia	Hypoxia
Heros sp (60 min)	^a 78.7 ± 1.3	45.8 ± 6.7*
Uaru amphiacanthoides (85–6.7 min)	^{ab} 70.3 ± 5.3	30.2 ± 6.3*
Satanoperca jurupari (< 20 min)	^{ab} 72.2 ± 6.5	50.5 ± 4.4*
Geophagus proximus (67.5–12.2 min)	^b 62.4 ± 2.0	37.0 ± 3.7*

Note: Values are given as means ± SEM (mov/min). * Represents significant difference between treatments (normoxia and hypoxia). Different letters mean significant differences between means.

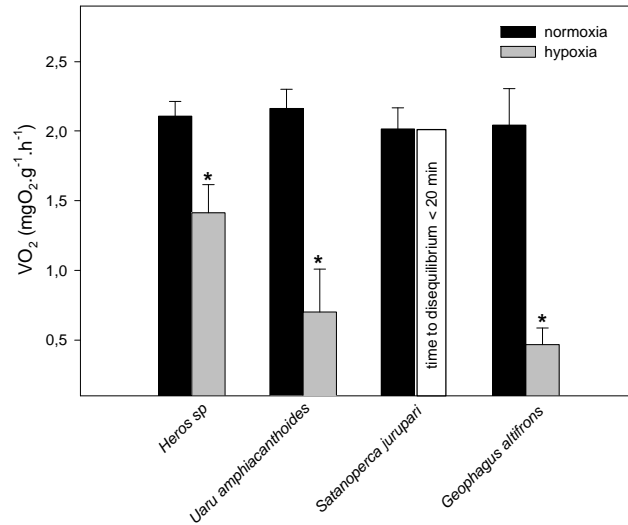


Fig. 1. VO₂ of four Amazon cichlids submitted at normoxia and acute hypoxia. Symbol (*) represents significant difference for each species in different condition.

Cichlids constitute a group of water-breathing fishes, which are, in the great majority, adapted to hypoxia and anoxia. Most species in this family are considered to inhabit shallow and hypoxic várzea lakes (shallow waters) in the Amazon basin. Such behavior requires a good tolerance to short and long-terms hypoxia episodes (Junk et al., 1983). As already shown for African cichlids (Verheyen et al., 1994), our data are consistent with the hypothesis that, among this family, there are two groups: those that are hypoxia-tolerant and those that are hypoxia-non-tolerant. Regarding the studied species, *Satanoperca jurupari* is the only species considered to be hypoxia-non-tolerant. However, there is no noticeable pattern in the responses among the remaining species.

Hematological parameters

Satanoperca jurupari exposed to hypoxia presented an increase in hematocrit and RBC, suggesting a red blood cell release into this species' circulating blood. The remaining 3 species showed no hematological changes ($P>0.05$) (table 2).

Table 2. Hematological parameters in four Amazon cichlids exposed to hypoxia (N) and hypoxia (H).

		Ht (%)	Hb (g/dL)	RBC $\times 10^6 \cdot \text{mm}^{-3}$	Glucose (mg/dL)
<i>Heros sp</i>	N	^a 28.3 \pm 2.0	4.9 \pm 0.3	^{ab} 1.7 \pm 0.2	8.8 \pm 2.4
	H	25.4 \pm 2.3	^{ab} 5.5 \pm 0.4	1.8 \pm 0.2	9.0 \pm 1.0
<i>U. amphiacan toides</i>	N	^{ab} 25.3 \pm 1.5	5.1 \pm 0.4	^{ab} 1.7 \pm 0.1	8.7 \pm 1.8
	H	28.8 \pm 2.0	^a 5.8 \pm 0.5	2.1 \pm 0.2	6.8 \pm 1.9
<i>S. jurupari</i>	N	^b 21.3 \pm 1.0	4.7 \pm 0.5	^b 1.3 \pm 0.1	6.2 \pm 1.0
	H	26.9 \pm 1.6*	^b 4.3 \pm 0.2	1.6 \pm 0.1*	8.6 \pm 1.4
<i>G. proximus</i>	N	^a 27.3 \pm 1.5	6.0 \pm 0.4	^a 2.3 \pm 0.2	12.8 \pm 2.41
	H	27.3 \pm 2.2	^{ab} 5.4 \pm 0.5	2.0 \pm 0.1	12.3 \pm 2.3

Note: Symbol (*) represents significant difference for each species in different conditions, and letters represent significant difference among species in same condition.

LDH activity levels

The exposure to hypoxia induced a decrease in muscle LDH levels from *Heros sp* and an increase in LDH levels of *S. jurupari*. On the other hand, muscle LDH from both *U. amphiacanthoides* and *G. altifrons* did not respond to acute hypoxia exposure. Pyruvate inhibition rates responded accordingly to these changes (table 3). LDH from *Heros sp* was not inhibited by high pyruvate concentrations in both normoxia and hypoxia groups, indicating the reliance of this tissue on anaerobic glycolysis. The same result could be observed in *U. amphiacanthoides* and *Geophagus altifrons*. On the other hand, there was an increase in inhibition rate in muscle LDH from *S. jurupari* exposed to hypoxia (table 3) showing that the activation of anaerobic metabolism is impaired in this tissue when pyruvate levels increase up to 10 mM.

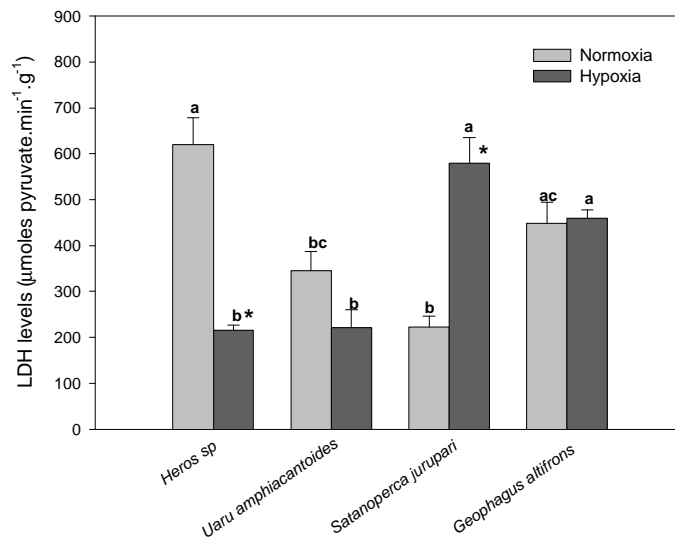


Figure 2. LDH levels in muscle from analyzed species at different experimental conditions. Symbol (*) represents significant difference for each species in different condition.

Table 3. Enzyme inhibition rates obtained between white muscle (WM) and heart (H) LDH activities at 1 and 10 mM pyruvate concentrations.

SPECIES	Normoxia		Hypoxia	
	WM	H	WM	H
Heros sp	0.87±0.11	0.74±0.05	0.34±0.03	0.86±0.11
Uaru amphiacanthoides	0.54±0.02	0.59±0.22	0.54±0.04	0.96±0.01
Satanoperca jurupari	0.74±0.08	1.48±0.27	2.84±0.48	1.24±0.22
Geophagus proximus	0.75±0.08	1.18±0.09	0.70±0.01	1.57±0.34

Heart LDH levels of *U. amphiacanthoides* and *S. jurupari* responded to hypoxia exposure in a different way. *Uaru amphiacanthoides* presented an increase in LDH levels indicating that this species' heart activates anaerobic glycolysis, while *S. jurupari* decreases anaerobic metabolism in this organ revealing its metabolic suppression (figure 3). There was no inhibition in LDH levels when 10 mM pyruvate concentration was used in heart of *Heros sp* and *Uaru*

amphiacantoides, while heart LDH from *S. jurupari* and *G. altifrons* is inhibited in all analyzed situations (table 3).

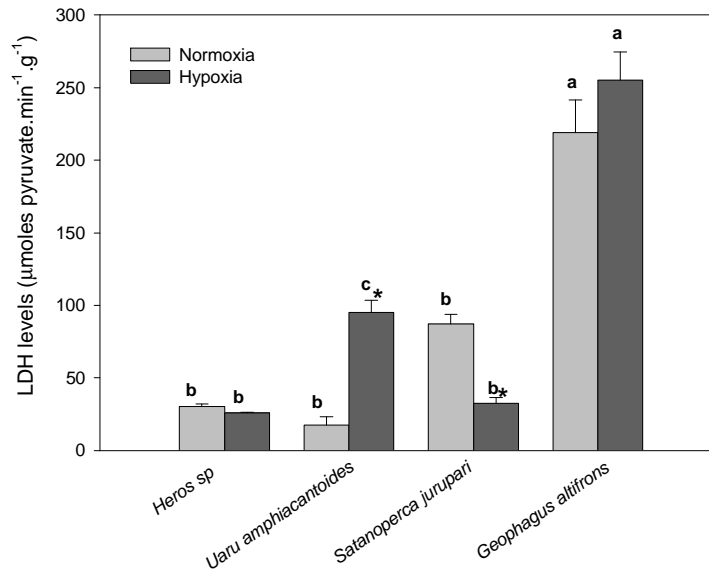


Figure 3. LDH levels in heart from analyzed species at different experimental conditions. Symbol (*) represents significant difference for each species in different condition. Letters represent differences among species at same experimental procedure.

Cichlid's responses to hypoxia have been related to their preferential habitats and to their ability to regulate tissue expression of the enzyme lactate dehydrogenase (Almeida-Val et al., 1995; 1999). Except by *S. jurupari*, all species regulated their metabolism to tolerate acute hypoxia exposure. Studies with lacustrine and riverine African cichlids also suggested that the species are adapted to tolerate hypoxic environments, and their ability to regulate metabolic rates under gradually increasing hypoxia differs markedly among species

(Verheyen et al., 1994). While the experimental design for the present work differs from that used with African cichlids, our data also reveal that acute hypoxia exposure induces metabolic responses that differ markedly among species. In addition, the regulation of LDH levels is also species-specific. Unfortunately, the molecular mechanisms responsible for induction of anaerobic metabolism in the tissues under hypoxia have not been clarified yet.

Considering the myriad of adaptive characteristics already described in fish, particularly tropical species, several options of morphological and anatomical adaptations are linked to metabolic adjustments (reviewed in Val and Almeida-Val, 1995; Almeida-Val and Hochachka, 1995). Amazon fish are supposed to either activate anaerobic metabolism or suppress oxidative metabolism when exposed to hypoxia (Almeida-Val et al., 1993). Among cichlids, these two possibilities are considered based on LDH isozymic distribution in heart of several species according to its preferential habitats (Almeida-Val et al., 1995).

This is the first comparative study about hypoxia tolerance in neotropical cichlids. Thus, more data will be needed before we define patterns of metabolic responses in such group. The present data, plus other enzyme assay measurements (not shown), suggest that there is a species-specific response to hypoxia among cichlids, which may follow species adaptation to preferential habitat.

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