

**METABOLIC RESPONSES TO ACUTE HYPOXIA
IN TWO AMAZONIAN CATFISHES WITH DIFFERENT
RESPIRATION PATTERNS**

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Abstract

Amazon fishes are amongst the most diverse groups of freshwater fish in the world. Among them, several groups developed air-breathing mechanisms to avoid low oxygen constraints, which are common in Amazon waters. The order Siluriformes includes catfishes and armored catfishes and is one of the most diverse groups in the Amazon with more than 1200 species described. Such diversity is reflected also in feeding habits, fish size, swimming performance and breathing patterns, among others. The facultative air-breathing habit in the family Loricariidae (armored catfishes) is an adaptation to low oxygen environments. Fish of this family is capable to use its stomach as a gas exchanger. Water breathing catfishes, which are more active fishes, in contrast, are not supposed to be hypoxia tolerant fishes but are often found in hypoxic environments. The present work comparatively analyses hypoxia tolerance and metabolic differences between a facultative air-breather and a water-breather fish species belonging to order Siluriformes. Acute hypoxia exposure induces differential responses that result from both their specific respiration mode and

metabolic adjustments. The inability of the water-breather species to cope with hypoxia and adjust its metabolism does not conform to their ability to survive hypoxia environment at Varzea lakes. Further studies are needed before we can have a clear picture.

Introduction

A bimodal breathing system may be defined as the one in which an organ or organs of the animal at a given stage of life history utilizes both water and air in its gas exchange mechanisms (Mittal et al., 1999). The same environmental pressure that caused the development of different air-breathing mechanisms is considered to induce long- and short-term adaptations in Amazon fishes. These adaptations are the result of adjustments in biochemical, physiological and molecular characteristics and enable the fish of the Amazon to survive episodes of hypoxia. Among other fish groups, many loricarioids (Amazonian armored catfishes) have developed mechanisms to breathe air. Rapp Py-Daniel (2000) suggested that these mechanisms are related to the encapsulation of their swim bladder. Near 50% of loricarioids are air breathers. Interestingly, the neotropical groups that breathe air have their stomach or intestine different from the African/Asian siluriforms, which, in turn, have pharyngeal sacs modified for breathing function. The family Pimelodidae (neotropical catfishes) has water-breathing habits and a broad distribution in the Amazon basin. Besides the importance of these two siluriform groups, not much attention has been directed towards the role of their enzymes in the metabolic adjustments to hypoxia. This work is intended to analyze the metabolic adjustments to acute hypoxia presented by two siluriforms species, which present different respiration patterns.

Material and methods

The animals were captured in Anavilhanas archipelago, Negro river (December, 1999). The animals were kept in outdoor tanks for 24 hours prior to the experimental procedure. Three groups of two specimens were exposed to acute hypoxia ($P_{wO_2} \cong 43\text{mmHg}$) (N_2 bubbling) in experimental polyethylene aquaria (56L) at room temperature ($26.0 \pm 2.0^\circ\text{C}$). 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 PO_2 module Radiometer, PHM72-Mk2. During the experimental period, we estimated VO_2 and opercular movements. The experimental control group was exposed to normoxia ($P_{wO_2} \cong 160\text{mmHg}$).

Immediately after loss of equilibrium, fish were removed and blood was collected from caudal vein into heparinized syringes. The blood was immediately transferred to Eppendorf tubes and centrifuged at 3,000 rpm for 10min. Plasma was used to estimate lactate levels, using the Sigma Chemical Co. kit 260-110. Immediately after blood collection, animal was weighed and killed (following Animal Care Association). Tissues (white muscle, heart, liver and brain) were then excised and promptly frozen in liquid nitrogen. Enzyme activities (PFK, PK, LDH, CS and MDH) were determined at 25°C using a Genesis spectrophotometer. Assay conditions followed those described in Driedzic and Almeida-Val (1996). The results are expressed as means \pm SEM. Statistical differences between groups were tested with Student's *t*-test. A significant difference was accepted when $P < 0.05$.

Results and Discussion

Glyptoperichthys gibbiceps is a facultative air-breather (personal observations; L. Rapp Py-Daniel, personal communication), which lives near the bottom of lakes and streams and feed algae, detritus and small live animals. *Calophysus macropterus* is a water-breathing fish and presents a more active life style compared to *G. gibbiceps*. Furthermore, as regard as feeding behavior, *C. macropterus* is opportunistic, feeding animal and vegetal items according to their availability. Despite these differences, their metabolic rates are similar ($P=0.084$): *G. gibbiceps* breathes $2.105 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, while *C. macropterus* breathes $3.041 \text{ mgO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at normoxia.

Calophysus macropterus presents higher absolute enzyme levels compared to *G. gibbiceps* (Table 1). We may also suggest, based on enzyme levels that *C. macropterus* relies its metabolism on glycolysis much more than *G. gibbiceps*. The ability to regulate glycolysis may be derived from enzyme levels of tissues and organs and the life styles and respiration pattern of the fish being analyzed (Almeida-Val, et al. 1999). Hochachka (1996) had already suggested that genes for glycolytic enzymes are thought to be regulated by being linked to common inducing or repressing signals, during long-term evolutionary processes. Earlier comparisons of the metabolic profiles of Amazon fishes and North temperate teleosts (Almeida-Val et al., 1993; Almeida-Val and Hochachka, 1995; Driedzic and Almeida-Val, 1996) revealed that (i) in general, Amazon fishes down-regulate enzymes, and (ii) an up regulation of glycolytic capacity may be observed in both anaerobic and aerobic tissues, regardless of respiration type.

When it comes to a closely related species comparison, as the ones in the present paper, a new picture emerges: metabolic profiles do reflect life styles and breathing patterns, which in turn allow the species to differential environmental adjustments, accordingly. During the previous Fish Biology Congress, we have suggested that different species of the family cichlid present different responses to hypoxia, which could be related to their preferential habitats and to their ability to regulate LDH expression (Chippari-Gomes et al., 2000). Such differential responses to hypoxia could rely also on different metabolic profiles, as it turned out to be true (Chippari-Gomes, PhD Thesis, in preparation). However, the differences between enzyme levels of *C. macropterus* and *G. gibbiceps* (Table 1) do not reflect their ability to deal with hypoxia.

Table 1. Glycolytic enzymes values ($\mu\text{moles substrate}\cdot\text{min}^{-1}\cdot\text{gram wet tissue}^{-1}$) obtained for each species at normoxia (first row) and at acute hypoxia (bottom). \downarrow and \uparrow represent, respectively, decrease and increase in acute hypoxia exposure ($P<0.05$). **(a)** Represent equal enzyme level in both species ($P<0.05$). **WM** white muscle, **H** heart, **L** liver and **B** brain.

Tissue	<i>G. gibbiceps</i>			<i>C. macropterus</i>		
	PFK	PK	LDH	PFK	PK	LDH
WM	13.19	39.24	53.73	17.44	54.70	566.16
	13.56	42.41	45.77	16.71	54.16	555.23
H	19.96	27.88	21.63	22.66	57.208	76.43
	19.98	27.33	23.54	24.56	57.34	79.21
	a			a		
L	9.03	42.86	34.83	17.36	84.52	944.22
	10.9	72.40	36.17	19.05	80.80	1110.5
B		\uparrow				
	2.10	71.12	40.45	5.59	122.22	1294.5
	0.37	53.92	43.90	2.37	116.35	1938.4
	\downarrow	\downarrow		\downarrow		\uparrow

Most Amazon fish may be considered tolerant to hypoxia (Almeida-Val et al., 1999). The great majority of siluriforms inhabits Varzea lakes, which are poor in oxygen. These species are tolerant to hypoxia or developed mechanisms to breathe air. The air-breather *G. gibbiceps* survived acute hypoxia for 80 minutes, while *C. macropterus* last no longer than 20 minutes at $Pw_{O_2} \cong 43\text{mmHg}$.

Table 2. Comparative values of Citrate Syntase (CS) and Malate Dehydrogenase (MDH) enzymes, expressed as $\mu\text{moles substrate}\cdot\text{min}^{-1}\cdot\text{gram wet tissue}^{-1}$ for each species and their respective change (bottom) after acute hypoxia exposure. \downarrow and \uparrow represent, respectively, decrease and increase in acute hypoxia exposure ($P<0.05$). **(a)** Represent equal enzymatic level in both species ($P<0.05$). **WM** white muscle, **H** heart, **L** liver and **B** brain.

	<i>G. gibbiceps</i>		<i>C. macropterus</i>	
	CS	MDH	CS	MDH
WM	3.11	9.41	4.23	16.85
	6.04	11.58	4.4	15.97
	\uparrow a	\uparrow	a	
H	79.64	351.12	32.773	494.42
	84.75	224.19	50.77	446.73
		\downarrow	\uparrow	
L	24.54	420.32	57.22	686.72
	27.46	265.43	19.57	616.68
		\downarrow	\downarrow	
B	10.56	140.23	9.02	181.43
	8.78	79.22	6.70	181.24
	\downarrow	\downarrow	\downarrow	

Glyptoperichthys gibbiceps presented suppression in VO_2 after 80 minutes of acute hypoxia exposure. However, opercular movements exhibit no change in both species (figure 1). Acute hypoxia exposure also resulted in reduced blood lactate and increased blood glucose levels in *G. gibbiceps* (figure 2). Again, for this species, glycolytic enzymes did not exhibit differences in muscle levels, while suppression occurred in the species' brain. There was an increase in liver PK levels that could account for increased glucose levels in this species. The

oxidative enzymes (MDH and CS) increased in white muscle and decreased in other organs, reflecting a general suppression in the metabolism. Thus, the overall data suggest that there is no activation of anaerobic metabolism and hypoxia tolerance in this species. This is probably due to suppression of aerobic metabolism, which is based at low rates utilizing the oxygen stored at its air-breathing organ (its stomach). Furthermore, we observed that after hypoxia exposure fish lies at the bottom of the aquaria remaining quiet until oxygen returns to normal levels.

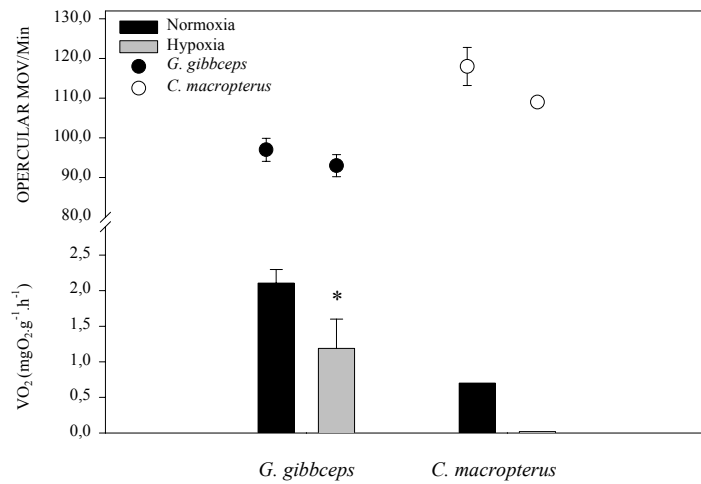


Figure 1 – Effect of acute hypoxia on *G. gibbeceps* and *C. macropterus* in opercular movement and VO₂.

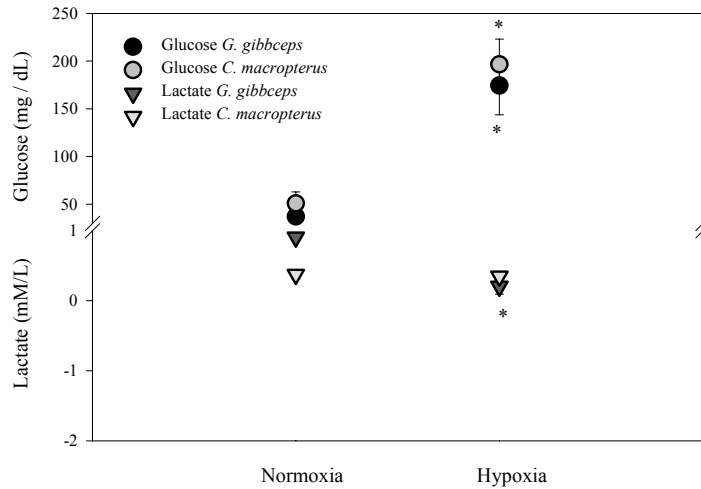


Figure 2 - Effect of acute hypoxia on *G. gibbiceps* and *C. macropterus* in blood glucose and lactate.

When the animals are about to lose equilibrium, they try to reach the aquaria surface, struggling, and immediately show apnea. Other studies of Amazonian facultative air-breathers indicate an increase in air-breathing frequencies, what results in avoidance of anaerobic metabolism activation. However, in the present device, fish access to surface was denied, impairing them to gulp air at the water surface layer.

Many Amazon water-breathing fish present metabolic adjustments when exposed to hypoxia. Acute hypoxia in *C. macropterus* (water breathing) induced an increase in plasma glucose levels. Except by the brain, where we observed a suppression of PFK and CS, no other enzyme change was observed. Based on our results, we can suggest that *C. macropterus* 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 as it does in Varzea lakes all year long (NPL, personal observations).

The preferential habitats of Amazonian fishes are supposed to be related to species hypoxia tolerance (Almeida-Val et al., 1995). Current knowledge about this issue is based on field observations of different Varzea systems (Junk et al., 1983) or for specific fish groups as electric fishes (Crampton, 1998), or fieldwork coupled with enzyme distribution as observed in cichlids (Almeida-Val et al., 1995). However, based on the present data we may suggest that this may not be the case for all fish groups. Some catfishes, which are water-breathers and vigorous swimmers, may occur in low oxygenated places even being unable to breathe air or quickly suppress their metabolism. So, further experiments, exposing *C. macropterus* to graded hypoxia, are needed to investigate which mechanisms allow this species to survive and acclimatize to natural hypoxic environments.

Acknowledgments

The present work was supported by INPA (grant # 1-3140) and CNPq-PNOPG (grant # 400030/99-3). NPL is the recipient of a PhD fellowship from CNPq. ALV and VMFAV are the recipients of research fellowships from CNPq.

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