

ECOLOGICAL IMPLICATIONS OF METABOLIC PROFILES OF HEART AND SKELETAL MUSCLES OF FISHES OF THE AMAZON

Vera Maria Fonseca de Almeida e Val
INPA-Aquaculture, Alameda Cosme Ferreira, 1756
69.083-000 Manaus, AM, Brazil
Phone: +55 92 643-3191 Fax: +55 92 643-3186
e-mail: veraival@cr-am.rnp.br

Lucia Luiza Ladewig de Paneppucci
Associate Researcher - CNPq
Department of Physiological Sciences - UFSCar

Hypoxia defense strategies in fish of the Amazon

As a result of oxygen shortage in Amazon waters, fish have developed a series of adjustments to improve oxygen transfer to tissues at different levels of biological organization. Among fishes of the Amazon we can find examples of ethological, morphological, anatomical, physiological, metabolic and molecular adjustments to survive chronic hypoxia. The result of such adjustments is a variety of respiration types, life styles, and preferential habitats, reflecting the enormous phenotypic plasticity of the group.

Development of air breathing habit occurred in several groups of Amazon fishes. The advantages of using air as a source of oxygen include independence from oxygen fluctuations in water bodies, reduction in energy costs in pumping an oxygen rich fluid, and the decreased size of the pump needed. The difficulties include disturbance of hydrostatic balance, exposure to predation, and exposure to frequent temperature changes. Thus, the solution of air-breathing was tried independently many times during the course of evolution (reviewed by Val & Almeida-Val, 1995). The main changes that allowed Amazon fishes to breathe air include the vascularized swim-bladder as in *Arapaima gigas* (obligatory air-breather) and *Hoplerythrinus unitaeniatus* (facultative air-breather), the vascularized stomach and intestine as in the species of the genus *Dora*, *Callichthys*, *Hoplosternum*, *Lipossarcus*, and *Ancistrus*, among others, and the pharyngeal and mouth diverticula as in *Hypopomum*, *Electrophorus*, and *Synbranchus*. In addition *Lepidosiren paradoxa*, a true lungfish, also occur in the Amazon.

The development of other strategies to improve oxygen uptake of water also appeared independently in several groups of fishes of the Amazon. Many fish species, belonging to different groups present the aquatic surface respiration (ASR) which allows them to explore the oxygen-rich surface layers of the water. Among them, the best examples are *Colossoma macropomum* and *Brycon melanopterum* since they improve this adaptive trait developing a morphological adaptation: the expansion of the lower lips (reviewed by Val, 1986).

A third type of defense strategy against hypoxia appears in some groups of water-breathing fishes. That is the decrease in the metabolic rates, decreasing their energy demands and increasing their energy yields, when exposed to severe hypoxia. Many cichlids present this type of strategy.

Among them *Cichlasoma amazonarum* survives long-term severe hypoxia depressing its hole metabolism (Almeida-Val *et al.*, 1995).

Along with these strategies, air-breathing fish developed metabolic-level adjustments. We have suggested two major types of enzyme-level adjustments: (i) a down regulation of absolute activities of enzymes of aerobic and anaerobic pathways; and (ii) an up-regulation of glycolytic-oxidative capacities on a tissue-by-tissue basis (Almeida-Val & Hochachka, 1995). However, in a recent study of heart enzymes from strictly water-breathing fish we observed that they also present a down-regulation of absolute activities of enzymes from aerobic and anaerobic pathways (Driedzic & Almeida-Val, 1996).

Considering oxygen limitation as a selective driving force during the evolution, and considering that suppression in energy demands implies in suppression of absolute enzyme activities, we have analysed the enzyme levels in heart and skeletal muscles from tropical fishes presenting different ecophysiological requirements. This paper presents an initial evaluation of metabolic profiles of heart and skeletal muscles from phylogenetic related fishes inhabiting the Amazon basin and Paraná basin (south region of Brasil). These species were chosen considering their migration habits, their ecophysiological requirements, and their different habitats.

Enzyme profiles of selected tropical fish species

The enzyme levels (*in vitro* maximal activity levels) for heart and skeletal muscles from selected tropical fishes are described in the table. The enzyme absolute activities for fishes analysed in the present study were estimated as described by Driedzic & Almeida-Val (1996). Pyruvate inhibition rates were estimated using 10mM pyruvate as high concentration. Results are shown as μ moles of substrate converted per minute per gram of wet tissue (U/gwt). Data for *Colossoma macropomum* heart muscle are from Driedzic & Almeida-Val (1996). *Prochilodus nigricans* and *Colossoma macropomum* occur in the Amazon basin and *Prochilodus scrofa* and *Piaractus mesopotamicus* occur in the Paraná basin; they belong to the same family, Prochilodontidae and Serrasalminidae, respectively, and are known to present migration habits for spawning.

Table 1. Enzyme levels of selected enzymes in heart and skeletal muscle of selected tropical fish. CS, citrate synthase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; m, muscle; h, heart.

Species/Tissues		CS	MDH	LDH low	LDH high	LDH/CS	MDH/LDH	Pyr inhibition
<i>Prochilodus nigricans</i> *	m	14	300	1,396	2,540	100	0.2	0.5
	h	14	1,079	1,335	877	95	1.5	1.5
<i>Prochilodus scrofa</i> **	m	29	491	1,040	3,077	36	0.5	0.3
	h	106	3,507	1,120	2,997	10	1.2	0.4
<i>Colossoma macropomum</i> *	m	-	593	2,653	2,626	-	0.2	1.0
	h	0.4	289	573	-	1,432	0.5	-
<i>Piaractus mesopotamicus</i> **	m	17	284	3,163	5,944	350	0.09	0.5
	h	16	1,628	4,344	3,268	272	0.4	1.3

* Amazon basin species ** Paraná basin species

As many other large characins, *Prochilodus* and *Colossoma* school and migrate in the Amazon waters (Goulding, 1980; Ribeiro, 1983). These species spawn in the confluence of “black” and “white” rivers, and then spent animals migrate back to the “black” rivers, dispersing across the flooded forest. The fertilized eggs are passively carried downstream in the “white” water river. Larval development takes place in the floodplain areas, a nutrient-rich water system. As the water level falls, the preadult animals school and migrate upstream through the “white” water river and then along the first “black” water tributary that they find. After a long migration, these animals disperse across the flooded forest, previously occupied by the adults, where they have plenty of food (see Val & Almeida-Val, 1995 for detailed information). Thus, this fish species of the Amazon migrate continuously and for long distances requiring a muscle designed for endurance. These kind of muscles should be powered by mitochondrial-based metabolism as suggested by Hochachka (1994).

Spawning migration of *Prochilodus scrofa* and *Piaractus mesopotamicus* is quite different. During the breeding season these species, among several others, swim upstream and migrate long distances facing many obstacles including differences in river bed levels, some times “jumping” water falls (Almeida-Val, personal observations). This kind of exercise requires a burst performance muscle (as when aruanã jumps out of the water to catch its prey) that is powered by anaerobic glycolysis (Hochachka, 1994).

Absolute enzyme activities of skeletal muscles of these four species (table), however, suggest that *Prochilodus scrofa* and *Piaractus mesopotamicus* present higher oxidative powered muscles, as indicated by citrate synthase (CS) and malate dehydrogenase (MDH) activities. Interestingly, lactate dehydrogenase (LDH) are present in higher levels also in *Prochilodus scrofa* and *Piaractus mesopotamicus* when compared to the Amazon fishes. In general there is a trend to down regulate absolute activities of enzymes from aerobic and anaerobic pathways. This has been observed in Amazon air- and water-breathing fishes (see also Driedzic & Almeida-Val, 1996). On the other hand, an up regulation of glycolytic power in muscles of Amazon fishes suggests that anaerobic glycolysis is probably the main energy source for white muscle as well as for *Piaractus mesopotamicus*. Similar rates of LDH/CS are found only in muscles of burst swimming fish as tuna (Hochachka *et al.*, 1979) and aruanã (Hochachka *et al.*, 1978).

Heart enzyme levels deserve a special attention. While *Prochilodus nigricans* and *Piaractus mesopotamicus* present CS values that fit in the regular range described for teleosts, *Colossoma macropomum* heart CS values are too low, increasing enormously its glycolytic power (LDH/CS > 1,000); and *Prochilodus scrofa* heart CS activity is an order of magnitude higher than any other teleost (Driedzic, 1992), reaching values four times higher than tuna’s CS heart levels and equivalent only to birds and mammals heart CS (Hochachka *et al.*, 1988). The energy required for migration, the ecophysiological characteristics, and the hypoxia acclimatization of these species are not enough to explain such extremely low and high oxidative hearts. Fuel preferences and ultrastructure evaluations are needed.

Currently, the organisms living in the Amazon waters, present the plasticity that allowed them to adapt to their environment. Fish of the Amazon can be considered as a plastic and experimental group from the evolutionary point of view, so can fish of the Paraná basin. Among them, specific diversity is reflected also in diversity of strategies to survive environmental constraints. Other enzymes from glycolytic metabolism and lipid metabolism have been analysed in these four species and will be further addressed.

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