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Research Article

Metabolic adjustments in *Satanoperca* aff*. jurupari* (Perciformes: Cichlidae)

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Abstract

In this paper, we describe the enzyme levels and isozyme distribution in skeletal and heart muscle of *Satanoperca* aff*. jurupari*, (Cichlidae, subgroup Geophaginae). LDH and CS were measured in skeletal and heart muscle. Starch gel electrophoresis was used to determine the isozyme/allozyme patterns in different tissues; LDH, MDHs, PGM, PGI, ADH, G-6-PDH and SOD were screened for the numbers of *loci*, presence of alleles, and tissue specificity. The LDH/CS ratio in heart and skeletal muscle were 173.36 and 6.1, respectively, indicating anaerobic metabolism in the former and aerobic metabolism in the latter muscle. No inhibition by pyruvate (based on the ratios of LDH activity with 1 mM and 10 mM pyruvate) was detected in heart and skeletal muscle, indicating the presence of physiological plasticity in heart muscle. The heart can cope with anaerobic metabolism for short periods of hypoxia such as occurs in nature. Isozyme patterns for most of the enzymes analyzed were similar to the general patterns for advanced teleosts. *S.* aff. *jurupari* had no reduced LDH-B* expression in heart muscle, but the, MDHs-B* *locus* was duplicated, as reported for most Amazon cichlids species. Only three out of the 13 *loci* analyzed (PGM, PGI and SOD) were variable. These results are consistent with the metabolic profile and life style of the most cichlids. A low genetic variability may be a counterpart for plasticity, and may be guaranteed by the regulation of invariable structural genes.

Key words: cichlid, plasticity, metabolism, enzymes, Amazon.

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Introduction

Neotropical cichlids are among the most advanced teleosts in South American. Recent reports have described this group as a monophyletic clade with a fast rate of molecular evolution based upon a significantly higher level of genetic variation compared to their African counterparts (Farias *et al*., 1999). Cichlids have always been considered a plastic group, because of their ability to adapt to heterogeneous habitats and their fast radiation, factors which have contributed to numerous speciation events in the group (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 fish are exposed to hypoxia, a common event in Amazon water bodies (Almeida-Val *et al*., 1993, 1995; Val and Almeida-Val, 1995). The isozyme distribution of lactate dehydrogenase (LDH) in tissues of neotropical cichlids is indicative of a species' adaptive tolerance to hypoxia, with the levels of this enzyme varying according to the degree of exposure to hypoxia. Based on the tissue distribution of LDH, *Satanoperca* aff*. jurupari* is considerable a species that does not tolerate hypoxia (Almeida-Val *et al*., 1995).

In contrast to other vertebrate lineages, tolerance hypoxia in fish probably has multiple independent evolutionary origins, particularly in view of the various adaptive responses of fish, especially tropical species to low oxygen availability (reviewed in Val and Almeida-Val, 1995; Almeida-Val and Hochachka, 1995). All cichlids are water-breathing teleosts. The species *Astronotus ocellatus* is considered to be hypoxia tolerant since it may survive anoxia at 28 °C for 6 h (Muusze *et al*., 1998). Most Amazon cichlid species inhabit shallow hypoxic varzea lakes in the Amazon basin, and hence require good tolerance to short and long-term hypoxia (Junk *et al*., 1983). Experiments in our laboratory have shown that hypoxia tolerance increases as *A. ocellatus* reaches adulthood (Almeida-Val *et al*.,

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1999). *Cichlasoma amazonarum*, known locally as acará-comum, occurs in all types of environments and its heart LDH distribution varies according to the oxygen availability in the environment (Almeida-Val *et al*., 1995). This plasticity of cichlid species allows them to visit low oxygen environments while searching for food and reproduction sites.

As part of a wide-ranging study to determine the metabolic preference of several cichlid species and to assess their preference for habitats in the Amazon floodplain, as well as their hypoxia tolerance, we have examined the metabolic preferences and isozyme patterns of cardiac and skeletal muscle in *S.* aff. *jurupari.* The kinetic properties of LDH (pyruvate inhibition) and the activities of citrate synthase are described along with tissue isozyme distribution of several *loci*.

Material and Methods

S. aff. *jurupari* (n = 15, body weight 90.2 ± 6.8 g, length 14.6 ± 0.3 cm) were captured near the confluence of the Rio Negro and Rio Solimões (Figure 1). The fish were killed immediately with a sharp blow to the head followed by severing of the spinal cord, as recommended by standard ethical procedures. Tissues (skeletal and heart muscle, liver, eye, and brain) were then excised and promptly stored at -80 °C in the Laboratory of Ecophysiology and Molecular Evolution at INPA.

Tissue preparation

Tissues were homogenized in ice-cold 50 mM phosphate buffer, pH 7.0, with a Potter-Elvejhem homogenizer.

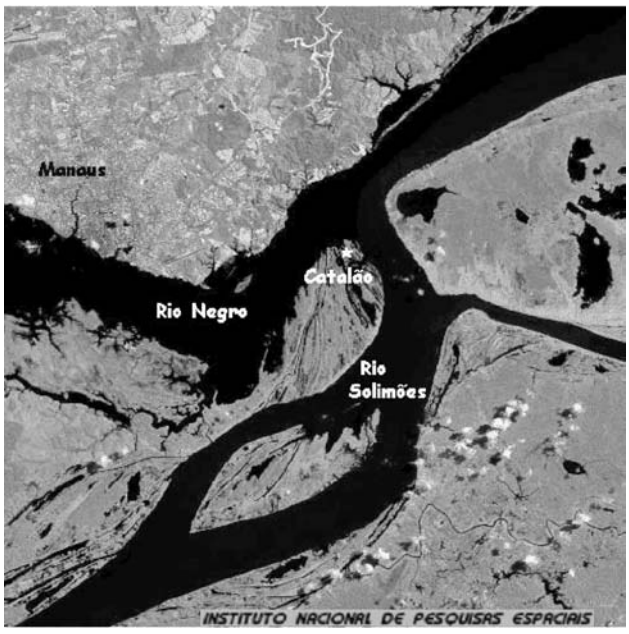

Figure 1 - Satellite image obtained from INPE/MCT, showing the sampled area (white asterisk) in the Lago do Catalão region (59°54'29" N, 3°9'47" S).

The tissue to buffer muscle ratio was 1:4 (w:v) for skeletal muscle, liver, brain and eye, and 1:9 for heart. The homogenates were centrifuged at 18,000 g for 30 min at 4 °C in a Sorvall RC5B centrifuge. The resulting supernatants were used for enzyme activities and for electrophoresis.

Enzyme assays

Maximal activities were determined at 25 °C using a Genesys 2 spectrophotometer, and techniques reviewed in Driedzic and Almeida-Val (1996). Lactate dehydrogenase (LDH; E.C. 1.1.1.27.) enzyme activities were measured by following the oxidation of NADH at 340 nm (mM extinction coefficient = 6.22) and citrate synthase (CS; E.C. 4.1.3.7.) enzyme activities were determined based on the reduction of free coenzyme A with DTNB (5,5'dithiobis(2-nitrobenzoic acid)) at 412 nm (mM extinction coefficient = 13.6). The assay conditions were based on well-established protocols for fish tissues (Sidel *et al*., 1987; Moon and Mommsen, 1987; Singer and Ballantyne, 1989). LDH assays were developed using buffer containing 0.15 mM NADH, 1 mM KCN and 50 mM imidazole, pH 7.4 at 25 °C. The reactions were initiated by adding 1 mM or 10 mM pyruvate. The CS assays were done using the buffer containing 0.4 mM acetyl CoA, 0.25 mM DTNB and 75 mM Tris, pH 8.0 at 25 °C. The reactions were initiated by adding 0.5 mM oxaloacetate.

Electrophoresis

Electrophoresis was done in horizontal gels containing 13% (w/v) corn starch prepared according to Val *et al.* (1981). All electrophoresis was done at 4 °C, at a voltage of 5 V/cm for 15-18 h, using an EPS 500-400 Pharmacia power supply (Uppsala, Sweden). Electrophoresis was used to study the LDH, MDHs (E.C. 1.1.1.37.; malate dehydrogenase (soluble fraction)), ADH (E.C. 1.1.1.1.; alcohol dehydrogenase), PGM (E.C. 2.7.5.1.; phosphoglucomutase), PGI (E.C. 5.3.1.9.; phosphoglucose isomerase), G-6-PDH (E.C. 1.1.1.49.; glucose-6-phosphate dehydrogenase) and SOD (E.C. 1.15.1.1.; superoxide dismutase) enzyme systems. The staining solutions were prepared according to recipes described in Allendorf *et al*. (1977) and modified by Leitão (1998).

Results

Electrophoretic patterns

The isozyme patterns revealed that most of the enzymes analyzed were similar to the general patterns described for advanced teleosts. A diagrammatic representation of the isozyme patterns is shown in Figure 2. Only three out of the 13 *loci* analyzed were variable (four alleles in PGM-1*, two alleles in PGI-B* and two alleles in SOD-1*) (Table 1). Allele frequencies were not determined because of the small number of analyzed individuals.

Table 1 - Tissue expression, *loci* and alleles of isozyme systems in *Satanoperca* aff. *jurupari*.

Enzyme (polymer)	*Loci*	Main tissue expression	Alleles
Lactate dehydrogenase (tetramer)	LDH-A* LDH-B* LDH-C*	Skeletal muscle (major) Heart muscle (major) Eye (major)	
Malate dehydrogenase (dimer)	MDHs-A* MDHs-B_1* MDHs-B_2*	Liver (major) Skeletal muscle (exclusive) Skeletal muscle (exclusive)	
Alchool dehydrogenase (monomer)	ADH*	Liver (exclusive)	
Phosphoglucomutase (monomer)	PGM-1*	Liver (major)	PGM-1_{60} PGM-1_{100} PGM-1_{150} PGM-1_{190}
Phosphoglucose isomerase (dimer)	PGI-A* PGI-B*	Liver (major) Skeletal muscle (exclusive)	 PGI-B_{80} PGI-B_{100}
Glucose-6-phosphate dehydrogenase (tetramer?)	G6PDH-1* G6PDH-2*	Skeletal muscle	heart
Superoxide dismutase (dimer)	SOD-1*	Liver (major)	SOD-1_{71} SOD-1_{100}

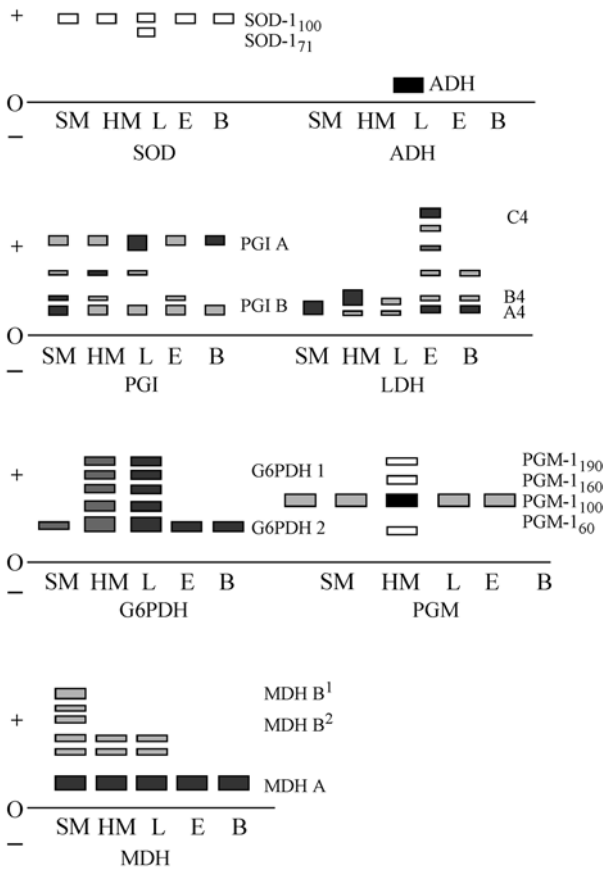

Figure 2 - Schematic representation of the isozyme systems of *Satanoperca* aff. *jurupari* obtained in horizontal starch gel electrophoresis. SM - skeletal muscle, HM - heart muscle, L - liver, E - eye, and B - brain. Open bars represent variant alleles. O - origin.

Most of the isozyme systems had distinguishable electrophoretic patterns which could be used for the determination of LDH, MDH, ADH, PGI, and SOD. However, PGM and G-6-PDH showed poor resolution. *S.* aff. *jurupari* showed no reduction in LDH-B* expression in heart muscle, as already described (Formiga-Aquino *et al*., 2000). The LDH-A* *locus* was the only gene expressed in skeletal muscle and appeared along with LDH-B in all other tissues analyzed; the LDH-C* *locus* was detected mainly in the eye. The presence of the LDH-C* *locus* in brain was suggested by the appearance of isozymes formed by a combination of LDH-B and C subunits (Figure 2). As reported for most Amazon cichlid species the MDHs-B* *locus* was duplicated in *S.* aff. *jurupari*; MDHs-B* *loci* (1 and 2) occurred exclusively in skeletal muscle while the MDHs-A* *locus* occured in all other tissues, particularly in liver. The ADH isozyme occured exclusively in liver, while SOD predominated in liver, but was also present in other tissues.

Metabolic profile

The enzyme activities of heart and skeletal muscle (Table 2) revealed a highly anaerobic skeletal muscle whereas the heart had a lower anaerobic power capacity, *i.e.* a predominately oxidative tissue. Pyruvate inhibition ratios, determined as the ratio between the LDH activities with a 1 mM pyruvate and 10 mM pyruvate, were lower than 1, indicating no inhibition at high pyruvate concentrations in heart and skeletal muscle (Table 2). Thus, heart muscle was able to cope with anaerobic metabolism for during short periods of hypoxia.

Discussion

The genetic variability in natural populations varies according to the position of the species in vertebrate groups, population size, and environmental conditions (reviewed by Nei, 1987). Among fish groups, the cichlids are considered advanced teleosts since they belong to the order Perciformes and the superorder Acanthopteygii (Nelson, 1994). The family Cichlidae occurs throughout the neotropics, Africa, Madagascar, and India.

Based on mitochondrial DNA analysis, the Geophaginae, the subgroup that includes *S.* aff. *jurupari*, has the highest evolutionary rate of all cichlids (African and neotropical) (Farias *et al*., 1999). As stated above, the Cichlidae is one of the most successful groups among the Perciformes, both ecologically and evolutionarily (Stiassny, 1991). The plasticity of this group and the high rates of speciation are some of the reasons for this success.

Table 2 - Enzyme levels in skeletal and heart muscle of. *Satanoperca* aff. *jurupari*.

Enzyme	Skeletal muscle	Heart muscle
LDH (Pyruvate 1 mM)	222.8 ± 21.8	57.4 ± 4.9
LDH (Pyruvate 10 mM)	335.3 ± 27.0	171.1 ± 11.4
Pyruvate inhibition ratio	0.66	0.34
Citrate synthase (CS)	1.3 ± 0.2	9.4 ± 1.4
LDH/CS ratio	173.4	6.1

Note: Enzyme activity is expressed as μmol $min^{-1}g^{-1}$ wet weight. Results are presented as mean values SEM (n = 15). The pyruvate inhibition ratio was calculated as the ratio of activities measured with 1 mM and 10 mM pyruvate. A ratio > 1 indicates inhibition.

However, the small number of variable *loci* detected in the present work (3 out of 13) contrasts with the observations of other studies. Two main arguments may be considered to explain this discrepancy: first, despite the high morphological variability among African cichlids from Lake Victoria, the genetic distances between species are very small, based on the percent age of substitutions per allozymes *locus* (Sage *et al*., 1984), and the substitution rates at mitochondrial *loci*, are higher than for most nuclear DNA *loci* (reviewed by Sültmann and Mayer, 1997). Second, fish mitochondrial DNA (particularly that of Perciformes) has a nucleotide substitution rate three to five times lower than that of mammals (Cantatore *et al*., 1994). According to Nei (1987), intra*locus* variance may be caused by the limited number of individuals sampled at each *locus* and can be reduced by increasing the number of *loci* examined. Unfortunately, the number of *loci* and individuals examined in the present work were both lower than those recommended for calculating the rate of heterozygosity which could be used for comparison with other fish species. Increasing the number of *loci* examined and the number of individuals sampled for this and other neotropical cichlid species will be necessary, in order to assess the heterozygosity of structural genes (isozymes/allozymes) among neotropical cichlids and other fish groups.

Isozymes (duplicated structural genes) are good markers for assessing the genetic and evolutionary position of fish groups (reviewed by Whitt, 1987). The presence of a duplicated gene pattern in the MDHs-B* *locus* has been described for most species of neotropical cichlids examined so far (Farias and Almeida-Val, 1992; Monteiro *et al*., 1991) and is considered to be indicative of the monophyly of the group.

Isozyme tissue specificity may indicate the degree of differentiation between duplicated genes (Whitt, 1987) and, in the case of *S.* aff. *jurupari*, most isozyme systems showed electrophoretic patterns similar to other cichlid species, as well as to species considered phylogenetically advanced (Kettler and Whitt, 1986; Whitt, 1987; Almeida-Val and Val, 1993). The LDH isozyme system illustrates these observations. The reduction in heteropolymer formation involving different gene products (*i.e.*, subunits A and B) is considered to reflect the high degree of divergence between *loci*. This property is frequently observed in advanced teleosts (reviewed in Almeida-Val and Val, 1993). The absence of heteropolymers involving LDH-A* and LDH-B* products in *S.* aff. *jurupari* confirmed its phylogenetic position as an advanced teleost.

Variations in the *loci* expression of LDH isozymes are related to tissue metabolic preferences and environmental adaptive traits in Amazon fishes (Almeida-Val *et al*., 1993). The high speciation rates (*i.e.*, adaptive radiation) observed in this group and its plasticity have been cited to explain phenotypic changes in isozyme tissue distribution among species and in the same species in different environmental conditions (Almeida-Val *et al*., 1995). *S.* aff. *jurupari*, can't cope with long-term exposure to hypoxia. However, the metabolic profile observed here was consistent with some degree of hypoxia tolerance, even in aerobic heart muscle.

The heart anaerobic rate (LDH/CS ratio) determined here is amongst the lowest such rate described for hearts from tropical and temperate region fish (Almeida-Val and Hochachka, 1995; Val and Almeida-Val, 1995; Driedzic and Almeida-Val, 1996). However, heart LDH showed no inhibition at substrate concentrations considered inhibitory for most aerobic tissues, thus indicating that this species may cope with short episodes of moderate hypoxia.

Comparison of the absolute enzyme rates among species indicated that *S.* aff. *jurupari* may be a sluggish fish which also depends on oxidative metabolism. The LDH rates for both muscles were below the average rates for Amazon air- and water-breathing fish species (Hochachka and Hulbert, 1978; Hochachka *et al.*, 1978a,b,c,; Hochachka, 1980; Driedzic and Almeida-Val, 1996; Almeida-Val and Farias, 1996). *A. ocellatus*, a related species, is also considered a sluggish, territorial fish, and the same LDH rates are observed in its heart muscles (Driedzic and Almeida-Val, 1996). However, in contrast to *S.* aff. *jurupari*, the heart LDH isozyme distribution in *A. ocellatus* is compatible with hypoxia tolerant fishes.The hypoxia tolerance of *A. ocellatus* increases as it grows. This adaptive trait is conferred by an increase in the anaerobic power as a result of increased LDH rates in most tissues (Almeida-Val *et al*., 1999). The CS rates differ between these two species, with *S.* aff. *jurupari* having a more oxidative heart, compatible with Amazon air-breathing fishes (Almeida-Val and Hochachka, 1995), as well as with northen temperate and Antarctic teleosts (Driedzic and Almeida-Val, 1996). The results obtained here for *S.* aff. *jurupari* are consistent with the metabolic pattern of a territorial and moderate life style fish, common to most cichlids. The low genetic variability may be compensated for by a certain degree of plasticity which may be guaranteed by the regulation of invariable structural genes, as already ob-

served in another cichlid, *C. amazonarum* (Almeida-Val *et al*., 1995).

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