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Karyological, biochemical, and physiological aspects of *Callophysus macropterus* (Siluriformes, Pimelodidae) from the Solimões and Negro Rivers (Central Amazon)

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Abstract

Karyological characteristics, i.e., diploid number, chromosome morphology and nucleolus organizer regions (NORs), biochemical characteristics, i.e., electrophoretic analysis of blood hemoglobin and the tissue enzymes lactate dehydrogenase (LDH), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), and phosphoglucose isomerase (PGI), and physiological characteristics, i.e., relative concentration of hemoglobin and intraerythrocytic concentrations of organic phosphates were analyzed for the species *Callophysus macropterus* collected from Marchantaria Island (white water system – Solimões River) and Anavilhanas Archipelago (black water system – Negro River). Karyological and biochemical data did not reveal significant differences between specimens collected at the two sites. However, the relative distribution of hemoglobin bands I and III (I = 16.33 ± 1.05 and III = 37.20 ± 1.32 for Marchantaria specimens and I = 6.33 ± 1.32 and III = 48.05 ± 1.55 for Anavilhanas specimens) and levels of intraerythrocytic GTP (1.32 ± 0.16 and 2.76 ± 0.18 for Marchantaria and Anavilhanas specimens, respectively), but not ATP or total phosphate, were significantly different, indicating a physiological adaptation to the environmental conditions of these habitats. It is suggested that *C. macropterus* specimens from the two collecting sites belong to a single population, and that they adjusted some physiological characteristics to adapt to local environmental conditions.

Key words
- *Callophysus*
- Fish
- Amazon
- Population genetics
- Adaptation

Introduction

The Amazon region presents a wide variety of aquatic environments separated by geographical barriers that limit gene flow among individuals. Although it has been postulated that this has produced a high intraspecific heterogeneity in fish populations, few studies have been conducted on this topic. Not only do different environments present adaptive challenges, but also any single environment can have wide fluctuations that additionally challenge its inhabitants. The capacity of organisms to adapt to such unstable environments may be linked to genetic and biological variability. However,

physiological plasticity at the individual level also permits exploitation of different environments. Presently, little is known about environmental effects on the distribution and abundance of populations versus biochemical and physiological variability.

The species of fish chosen for this study belongs to the family Pimelodidae, genus *Callophysus*, which is monotypic. *Callophysus macropterus*, locally known as "piracatinga", is found dispersed across the Amazon basin and in the Orinoco River, being commercially exploited in both systems. *Callophysus macropterus* is representative of a migratory species of catfish (Siluriformes) and is abundant in a wide variety of environments. Thus, it is a convenient subject for studies of population structure and adaptive responses to different environmental conditions.

Karyotype and structural and functional properties of isozymes, allozymes, and hemoglobin among other proteins have been helpful for the evaluation of evolutionary aspects and population structures of fishes (1-6). In addition, it is believed that an examination of the physiology of hemoglobin-oxygen affinity and its allosteric modulators, the intraerythrocytic phosphates, is important to characterize the adaptive capacity of fish populations (7). Thus, the application of cytogenetic, biochemical, and physiological methods to assess population differences provides an objective approach to probing responses to environmental heterogeneity. The present study uses these tools to examine differences and similarities in *C. macropterus* in order to assess how this species has responded to two different environments. Is this ability to exploit two environments due to genetic-biochemical differences or to a physiological adaptive response?

Material and Methods

Callophysus macropterus specimens were obtained from the Solimões River, near Marchantaria Island (60°00'W 3°15'S), and from the Anavilhanas Archipelago, Negro River (60°45'W 2°43'S), during the low-water season.

Chromosome analyses

Forty live specimens from Marchantaria Island and 15 from the Anavilhanas Archipelago were studied. Chromosome preparations were obtained from kidney cell suspensions by the air-drying method of Bertollo and co-workers (8), which was modified by using 0.025% colchicine and an exposure time of 45-90 min. The nucleolus organizer regions (NORs) were identified by silver staining according to the technique of Howell and Black (9). Chromosome morphology was determined on the basis of arm ratios, as recommended by Levan and co-workers (10).

Isozyme analyses

Samples of skeletal muscle, liver, heart, eye, and brain were routinely obtained from the fish (110 Marchantaria Island specimens and 43 Anavilhanas Archipelago specimens), kept first in an ice-salt mixture (-17°C) during transport to the laboratory and then stored at -20°C until the time for analysis. Samples were homogenized for 10 s at 4°C using a Sorvall Omnimixer in phosphate buffer, pH 7.0 (1/1, w/v), and centrifuged at 27,000 *g* for 30 min at 4°C in a refrigerated centrifuge. The supernatants were used for electrophoresis.

Horizontal starch gels were prepared (13%, w/v) according to Val and co-workers (11). For lactate dehydrogenase (LDH, E.C. 1.1.1.27), phosphoglucose isomerase (PGI, E.C. 5.3.1.9), and alcohol dehydrogenase (ADH, A.C. 1.1.1.1) the buffer system was prepared according to Boyer and co-workers (12). For malate dehydrogenase (MDH, E.C. 1.1.1.27) the buffer system was Tris-citrate (13). Electrophoresis was carried out for 14

h at 4°C by applying 6 V/cm with a Pharmacia EPS 500/400 power supply.

Gels were stained according to procedures outlined by Shaklee and co-workers (14) for LDH, by Shaw and Prassad (15) for MDH, by DeLorenzo and Ruddle (16) for PGI, and by Brewer (17) for ADH. The nomenclature specified for alleles at each locus is according to the International Biochemical Commission.

To test the observed genotypic distributions against the expected Hardy-Weinberg distribution, a G-test (18) was used. A contingency χ^2 test (19) was used to determine the significance of inter-sample homogeneity in allele frequencies.

Measurement of hemoglobin

Blood samples from 30 Marchantaria Island specimens and 21 Anavilhanas Archipelago specimens were collected from the caudal vein into heparinized syringes immediately upon capture and kept on ice until processing. Plasma was removed from blood, and after washing three times with ice-cold saline solution (0.9%), the red blood cells (RBC) were lysed with 5 mM Tris-HCl, pH 8.0 (1 part RBC to 5 parts Tris) and frozen at -20°C. The stroma was eliminated by centrifugation at 20,000 *g* for 20 min at 4°C in a Sorvall RC-5B refrigerated centrifuge and the hemolysates were stored at 4°C and used for electrophoresis.

The electrophoretic patterns of hemoglobin were obtained by starch gel electrophoresis (20), and with agar-starch on microscope slides according to Araújo and coworkers (21), modified by Machado (22) using corn starch (13 g/100 ml) (11). The same buffer system was used for both media: Tris-borate-EDTA (0.9 M Tris, 0.2 M boric acid, and 0.02 M EDTA acid, pH 8.6, diluted 1/40 in water). Borate buffer, 0.35 M, pH 8.6, for the starch gel and 0.035 M, pH 8.6, for the agar-starch were used in electrode vessels. Electrophoresis was carried out at 4°C with a Pharmacia EPS 500/400 power supply.

The starch gels were stained with amido black 10B for total proteins and with benzidine for hemoglobin. The agar-starch-coated slides were stained only with amido black 10B. The relative concentration of each hemoglobin fraction was determined on agar-starch-coated slides using an Argos 7/8 densitometer. The *t*-test was used to determine significant differences between sample hemoglobin concentrations.

Measurement of intraerythrocytic phosphates

Blood samples of various specimens of *C. macropterus* were pooled. The plasma was removed and the phosphates were extracted with 0.6 M perchloric acid. The extracts (N = 6 for both collecting sites) were chromatographed using a Dowex 1 x 8 resin, 400 mesh, in a 28 x 1 cm column according to Bartlett (23). The method described by Bartlett (24) was used to determine if inositol polyphosphate was present.

Results

Karyotypes and nucleolus organizer regions

The karyotype of *C. macropterus* obtained at the two sites was characterized by a diploid number of 2n = 50 chromosomes, 22 metacentric (pairs 1-11), 18 submetacentric (pairs 12-20) and 10 acrocentric (pairs 21-25) chromosomes (Figure 1). The fundamental number was 90 for all specimens analyzed. No significant karyotypic differences were observed between animals from the two collecting sites. A single pair of NOR-bearing chromosomes was detected, coinciding with a secondary constriction located on the short arms of an acrocentric chromosome pair, the 22nd pair in the complement. The NORs were heteromorphic. No differences were observed with

respect to gender or collection site.

Isozymes

LDH was monomorphic in all specimens of *C. macropterus* caught at the two sites. The LDH distribution in the tissue suggests that this enzyme is coded by two co-dominant loci (LDH-A* and LDH-B*) that characterize subunits A and B, respectively. The enzyme is a tetramer and is present as five isozymes (A_4, A_3B_1, A_2B_2, A_1B_3, B_4) with typical binomial distribution. As expected, isozyme A_4 predominated in skeletal muscle and isozyme B_4 predominated in heart muscle (Figure 2).

MDH was present in two forms, the mitochondrial (m-MDH) and the cytosolic or soluble form (s-MDH) (Figure 2). s-MDH was characterized by three anodic bands suggesting a dimeric composition coded by two gene loci: MDH-A* and MDH-B*. These isozymes were tissue specific; in other words, isozyme A_2 predominated in the liver and B_2 in muscle. MDH, like LDH, revealed a monomorphic pattern in specimens from the two collecting sites.

The ADH enzyme was detected only in the liver (Figure 2) and showed a single cathodic migration band for all samples. The electrophoretic pattern was monomorphic and similar for the individuals from both sites.

The PGI enzyme was characterized by three symmetrically distributed electrophoretic bands, indicating its dimeric composition as expressed by two co-dominant gene loci. Loci PGI-A* and PGI-B* codify the isozymes A and B, respectively. These isozymes are distributed in the different tissues in the following way: isozyme A_2 is predominant in liver, eye, and brain and B_2

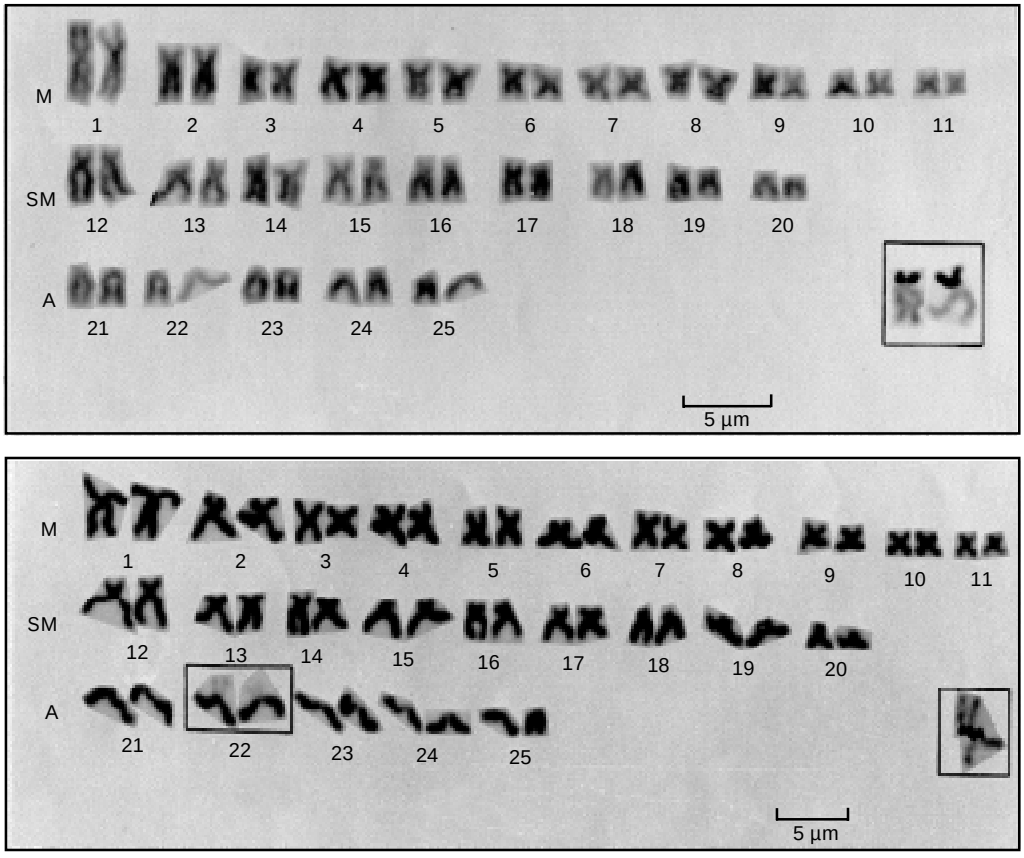

Figure 1 - Karyotype of *Callophysus macropterus* (2n = 50) collected from the Solimões River, near Marchantaria Island (top), and from the Negro River, near Anavilhanas Archipelago (bottom). M, Metacentric; SM, submetacentric; A, acrocentric.

in muscle. The heterodimer AB appears in the heart, together with a moderate presence of homodimer A_2. Four alleles were detected for PGI in the two populations: A-100, A-120, B-100, and B-450. All possible patterns were found in the two populations (Figure 3).

Allele analysis showed no significant difference between heterozygotes from the two collecting sites (Table 1). The genotypic distribution was found to be the same for the individuals collected from both sites. The χ^2 values were not significant (P>0.05), also when the data from the two sites were combined.

The contingency test (Table 2) shows the gene frequency of the alleles present in both populations. The genetic composition of the specimens captured from Marchantaria Island site was not significantly different (P>0.05) from the genetic composition of specimens collected from the Anavilhanas Archipelago.

Hemoglobin electrophoresis

Starch gel electrophoresis of the hemolysates of *C. macropterus* from the two sites revealed three hemoglobin bands, each band presenting identical electrophoretic mobility for all analyzed specimens. In agar-starch, a variation was observed in the relative concentration of the three bands of hemoglobin. The mean concentration of band I was significantly higher (*t*-test, P<0.001) in the animals collected from Marchantaria Island. The concentration of band II did not differ significantly between the individuals from the two sites. The mean concentration of band III was significantly higher in the animals from Anavilhanas Archipelago (*t*-test, P<0.001). These results are summarized in Figure 4.

Intraerythrocytic phosphates

No qualitative differences were observed in the chromatographic patterns of intraerythrocytic phosphates between specimens from the two collecting sites. Inositol polyphosphate was not detected in the erythrocytes of *C. macropterus*. The phosphates primarily detected were: ADP, ATP, GTP, and Fe-GTP. There was, however, a quantitative difference in GTP between samples from the two sites (Figure 5). GTP concen-

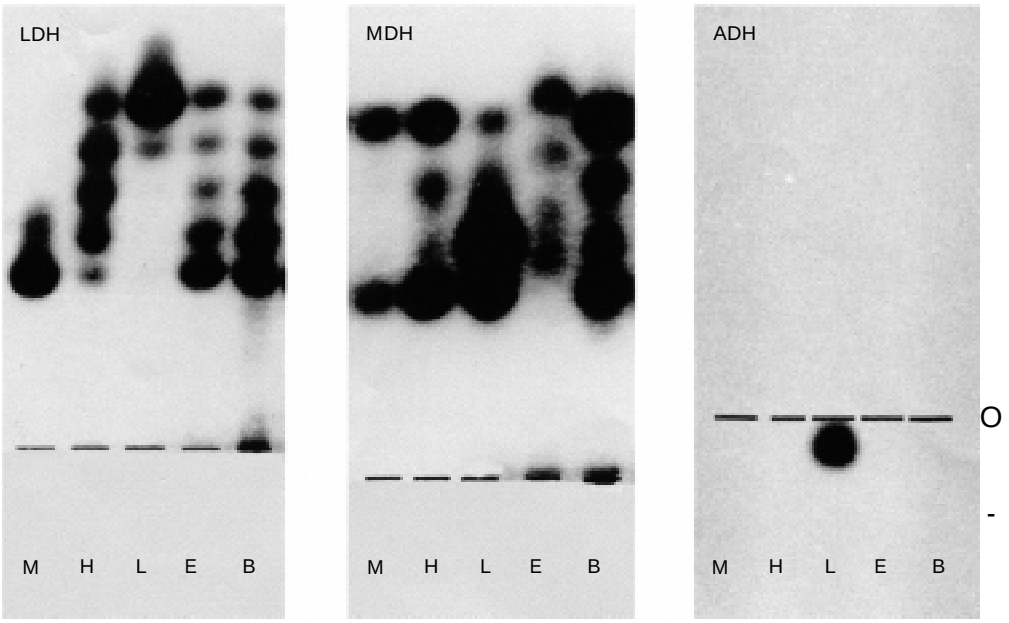

Figure 2 - Distribution of isozymes of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and alcohol dehydrogenase (ADH) in skeletal muscle (M), heart (H), liver (L), eye (E), and brain (B) of *Callophysus macropterus*. O = Origin.

trations for samples from Anavilhanas Archipelago were significantly higher ($P<0.05$) than those for samples from Marchantaria Island. Concentrations of inorganic phosphorus and ATP were not significantly different. ATP and GTP ratios also differed. The ATP/GTP ratio was less than 1 for the individuals from Anavilhanas Archipelago and higher than 1 for the individuals from Marchantaria Island.

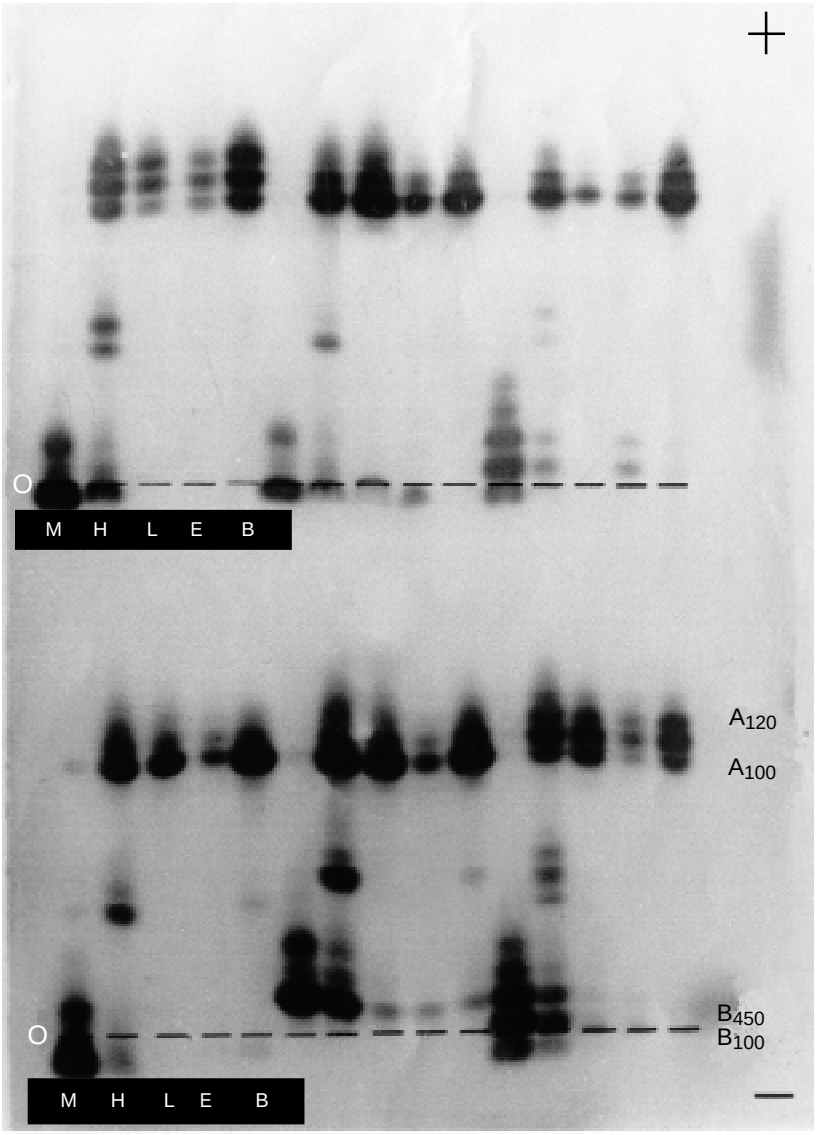

Figure 3 - Distribution of isozymes and allozymes of phosphoglucoisomerase (PGI) in muscle (M), heart (H), liver (L), eye (E), and brain (B) of *C. macropterus* specimens collected from the Solimões River, near Marchantaria Island (top), and from the Negro River, near Anavilhanas Archipelago (bottom). Four allelic forms were found in animals from both sites (A_{100}, A_{120}, B_{100}, and B_{450}). O = Origin.

Discussion

Based on karyotypic observations of some fish species of the family Pimelodidae, we may state that populations of the same species having different karyotypes can be found within the same hydrographic basin, as reported for species of *Rhamdia* (25,26). Such differences have been related to supernumerary chromosomes that may confer adaptive advantages. However, the species studied here, *C. macropterus*, also a member of the Pimelodidae family, exhibits a single karyotype for the animals from both collecting places.

It is important to note here that out of the 258 South American species of the Pimelodidae family, only about 20 had their karyotypes studied. Currently available information indicates that the Pimelodidae family has a diploid chromosome number varying from 2n = 46 in *Pimelodella* sp (27) to 2n = 63 in *Rhamdia hillari* (26), with most of the analyzed species having 2n = 56 (28).

Most of the pimelodids possess NORs located on the short arm of a single chromosome pair (29). In *C. macropterus* they were found on the short arms of the 22nd pair of acrocentric chromosomes. The heteromorphism in NOR size found in *C. macropterus* was closely similar to that found in *Pseudoplatystoma fasciatum* and *P. tigrinum*, showing a convergent pattern among these species. The NOR heteromorphism in *C. macropterus* is not associated with the collecting sites. The lack of karyotypic variation suggests a high similarity among animals from the two environments.

A pattern of five LDH isozymes closely similar to that found for *C. macropterus* has been described for other species of catfish: *Pseudoplatystoma tigrinum*, *Brachyplatystoma filamentosum*, *Hoplosternum littorale* (30), *Pimelodus maculatus* (31), *Ictalurus* sp, *I. nebulosus*, *I. marmoratus* and *I. punctatus* (32). There are interspecific differences in LDH electrophoretic mobility

Table 1 - Test of Hardy Weinberg equilibrium for PGI-A and PGI-B of *Callophysus macropterus* from Marchantaria Island and Anavilhanas Archipelago.

Site	Genotype			Allele frequencies		d.f.	χ^2	P
	A_{100}	$A_{100}A_{120}$	A_{120}	A_{100}	B_{120}			
Marchantaria	92 (92.16)	8 (7.68)	0 (0.16)	0.96	0.04	1	0.174	0.7-0.5
Anavilhanas	35 (34.81)	2 (2.15)	0 (0.03)	0.97	0.03	1	0.04	0.95-0.9
Total	127 (126.26)	10 (10.52)	0 (0.22)	0.96	0.04	1	0.249	0.9-0.8
$\Sigma\chi^2$						2	0.219	0.9-0.8
Heterozygosity							0.030	0.9-0.8

Site	Genotype			Allele frequencies		d.f.	χ^2	P
	B_{100}	$B_{100}B_{450}$	B_{450}	B_{100}	B_{450}			
Marchantaria	37 (37.82)	49 (47.35)	14 (14.82)	0.615	0.385	1	0.120	0.8-0.7
Anavilhanas	13 (11.89)	16 (18.17)	8 (6.94)	0.567	0.433	1	0.525	0.5-0.3
Total	50 (49.65)	65 (65.65)	22 (21.70)	0.602	0.398	1	0.013	0.95-0.9
$\Sigma\chi^2$						2	0.645	0.5-0.3
Heterozygosity							0.632	0.5-0.3

Table 2 - Contingency analyses of the homogeneity of PGI-A and PGI-B alleles of *Callophysus macropterus* from Marchantaria Island and Anavilhanas Archipelago.

Site	n	Genotype		Allele frequencies	d.f.	χ^2	P
		A_{100}	A_{120}				
Marchantaria	100 (185.40)	184 (14.60)	16	200	1	0.145	0.8-0.7
Anavilhanas	37	70 (68.60)	4 (5.4)	74	1	0.391	0.7-0.5
Total	137	254	20	274	1	0.536	0.5-0.3

Site	n	Genotype		Allele frequencies	d.f.	χ^2	P
		B_{100}	B_{450}				
Marchantaria	100 (120.44)	123 (79.56)	77	200	1	0.137	0.8-0.7
Anavilhanas	37	42 (44.56)	32 (29.44)	74	1	0.369	0.7-0.5
Total	137	165	109	274	1	0.506	0.5-0.3

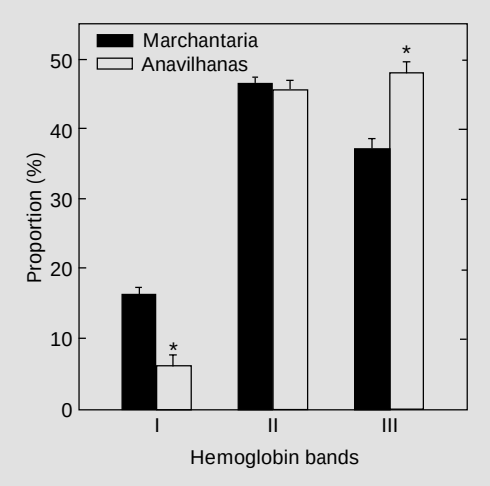

Figure 4 - Relative concentration (%) of electrophoretic hemoglobin bands of *Callophysus macropterus* collected from the Solimões River, near Marchantaria Island (filled columns), and from the Negro River, near Anavilhanas Archipelago (open columns). Asterisks indicate significant differences (P<0.05) between specimens from different collecting sites (*t*-test).

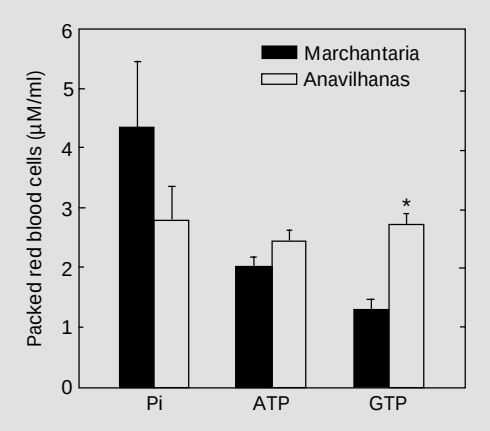

Figure 5 - Inorganic phosphate (Pi), adenosine triphosphate (ATP) and guanosine triphosphate (GTP) in red blood cells of *Callophysus macropterus* collected from the Solimões River, near Marchantaria Island (filled columns), and from the Negro River, near Anavilhanas Archipelago (open columns). Asterisk indicates significant differences (P<0.05) between specimens from different collecting sites (*t*-test).

among these species, i.e., each species displays its own pattern, but intraspecific variations are not observed. The findings for ADH and MDH were similar.

MDH isozymes displayed a clear difference in tissue specificity. Isozyme A_2 was predominant in liver and B_2 in white muscle, also reported for other fish species. De Luca and co-workers (33), for example, described a distribution pattern for *Astyanax fasciatus* that was bi-directionally divergent for s-MDH. The physiological significance of the distribution of MDH isozymes in the different tissues is not yet clear.

For PGI, the PGI-A locus was considered monomorphic even though the variant allele A_{120} was present at very low frequency (<0.05). According to Richardson and co-workers (2), it is not common to employ a heterogeneity index if the rare allele frequency is less than 0.2. These rare alleles are maintained in populations by a balance between mutation and selection (34); in other words, the selection coefficient is the same as the mutation coefficient.

Based on the analysis of PGI-B, a single variant, one may suggest that individuals collected from Marchantaria Island and the Anavilhanas Archipelago probably belong to the same population unit ($X_{heterog} = 0.632$; P>0.05). However, these results do not necessarily mean that there is only one population. It is difficult to state that the two samples really belong to one population, because only a low proportion of loci are being evaluated and according to King and co-workers (35), only differences (not similarities) between populations can be suggested based on electrophoretic analysis of the enzymes. Thus, the frequency values cannot be considered to be positive evidence of genetic flow between the populations analyzed.

Riggs (36) pointed out that hemoglobin components with different functional properties and/or with different relative concentrations could serve different physiological needs in variable environments. Such changes in the relative concentration of the hemoglobin bands can be considered a response to environmental variability (37). The present study shows significant differences in the mean concentration of hemoglobin bands in *C. macropterus*, which may be related to the condition of each environment where the animals were collected.

The majority of Amazon fishes have ATP and GTP in their red blood cells and the concentration of these phosphates varies according to the natural oscillations of oxygen availability (38). In *C. macropterus*, the ATP/GTP ratio was less than 1.0 for the individuals captured at Anavilhanas Archipelago and greater than 1.0 for the samples from Marchantaria Island, showing that these animals exhibit different physiological responses to

the predominant conditions of each environment. This capacity to adjust intraerythrocytic concentrations of organic phosphates, as well as the differences found in hemoglobin bands lead us to suggest that fish of this species may be responding to environmental specificity (pH, dissolved oxygen, temperature, etc). Moreover, this capacity not only allows the animals to exploit those habitats but also to remain there. Population plasticity, which is a consequence of physiological flexibility at the individual level, not only permits the exploitation of different environments, but also provides the ability to adapt to fluctuations within each environment.

The fact that *C. macropterus* is a migratory fish can explain the genetic similarity between the samples obtained from the two sites. The physicochemical characteristics of the water do not provide a barrier to prevent genetic free-flow among individuals of this species. Thus, the presence of *C. macropterus* in the two different environments can be attributed to physiological plasticity and suggests that this fish species can disperse in large areas.

The effective management of a species depends upon knowledge of their adaptive limitations (39). This present study is a first attempt to examine differences and similarities in one species of fish found in two differing environments to probe its adaptive capability. However, further studies are needed to describe the relationship between these physiological changes and environmental O_2 availability, pH, temperature, nutrition, activity, etc. Such studies are necessary to elucidate how the distribution of fish populations takes place, how fish abundance is regulated, and the limitations for each species in different habitats. This information will provide a basis for adequately managing fishing resources.

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