

# Genetic variability of *Prochilodus lineatus* (Characiformes, Prochilodontidae) in the upper Paraná river

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## ABSTRACT

The genetic variability of the "curimba", *Prochilodus lineatus*, from three locations in the Paraná river basin, was investigated by starch gel electrophoresis. A total of 160 specimens were analyzed for 19 enzymes, 12 of which permitted successful interpretation of electrophoretic patterns. Eighteen loci were identified and six of them proved to be polymorphic (*EST-1\**, *EST-2\**, *IDH-1\**, *PGM-1\**, *PGM-2\**, *LDH-2\**). Mean heterozygosity was considered high (13%) by comparison with the literature. A low level of differentiation was found among subpopulations, with mean  $F_{ST} = 0.018$ . Values of genetic distance and genetic identity suggest that, at least along this stretch of the river, *P. lineatus* comprises a single breed with high gene flow. This analysis has important implications for fishery management, aquaculture, and conservation of the stocks.

## INTRODUCTION

The "curimba" or "curimbatá", *Prochilodus lineatus* (Valenciennes, 1836) (= *P. scrofa* Steindachner, 1881), is an iliophagous characiform which is endemic in the Paraná and Paraguai river basins. This species shows migratory behavior and population stratification in terms of distribution of body length classes and extent of sexual maturation (Toledo-Filho, 1983; Gomes *et al.*, 1989). The "curimba" is conspicuous among the migratory species of the Paraná river basin and is considered to be the fourth most important species in fish landings in the Itaipu reservoir (Agostinho *et al.*, 1994). There is a clear economic interest in this species and consequently a need for fishery management.

The management of a fishery requires some knowledge of the population structure, including the possible existence of genetically distinct populations, which can be assessed by electrophoresis of enzymes and DNA (Allendorf and Utter, 1979; Allendorf *et al.*,

1987). Genetic management tends to order the exploitation, avoiding gene pool erosion and warranting food production in the face of human population growth (Foresti *et al.*, 1992). The preservation of the environmental patchiness of a foodplain is vital to the maintenance of biological and genetical diversity; however, this maintenance has been endangered by reservoir construction. The Paraná river basin is one of the most intensively dammed. By the end of the twentieth century it is expected that 69 hydroelectric dams will be built in the Brazilian portion of the basin alone. Only 230 km of the original 809 km of the upper Paraná river in the Brazilian portion are now flowing. The construction of the Ilha Grande reservoir will wipe out the last lotic stretch of the upper Paraná river (Agostinho *et al.*, in press).

The dams themselves may be formidable barriers to the dispersal of many freshwater organisms, especially migratory ones. Beyond the impacts caused by the flux control, they compromise the survival, mating success, and gene flow that can alter the gene frequencies of the species. Even recognizing the potential of extrinsic barrier impacts on the freshwater

species and the strong need for genetic data as an aid in management, the studies of genetic structure have been almost totally ignored in Brazil. The objectives of the present study were to quantify the genetic variability and genetic distance within sampled subpopulations using enzyme electrophoresis, to inform about some fishery strategies for the management of "curimba" from the high Paraná river and to alert about the importance of heterogeneous environment preservation in floodplain ecosystems for the maintenance of high levels of genetic variability.

## MATERIAL AND METHODS

Adult individuals of *P. lineatus* were fished with nets in November 1993 and May 1994 from three sampling sites in the Paraná river basin, i.e., Paraná, Baía and Ivinheima rivers (Figure 1). Samples of liver and muscle were removed from fresh fish, frozen in liquid nitrogen, and stored at -20°C. The tissues were homogenized in Eppendorf tubes with CCl<sub>4</sub> and 0.02 M Tris/HCl buffer, pH 7.5 at 1:1:2 concentrations, respectively, using plastic sticks. All homogenates were centrifuged at 25,000 rpm for 30 min in a refrigerated centrifuge with temperature ranging from 1 to 5°C, and stored at -20°C until the time for electrophoresis. The supernatants were processed within two weeks of their preparation. The buffer system used was 0.125 M Tris/0.0375 M citrate, pH 8.0, modified from McAndrew and Majumdar (1983). The standard histochemical staining procedures were based on Aebersold *et al.* (1987). The interpretation of enzymatic patterns concerning the quaternary structure of enzymes, was based on information from Ward *et al.* (1992). Nomenclature of gene loci followed the recommendations of Shaklee *et al.* (1990). In this paper, the loci were designated by the abbreviations of enzyme names in italicized capital letters, followed by an italic Arabic number and an asterisk. The locus and allele that coded the least anodal isozyme were designated 1 and A, respectively.

The Biosys-1 program (Swoford and Selander, 1981) was used for statistical analysis. Variability was estimated by the 95 and

99% criterion of polymorphic loci and by the unbiased mean heterozygosity (Snedecor and Irwin, 1933). Polymorphic loci were submitted to chi-square tests for deviation from Hardy-Weinberg equilibrium, with correction for small samples (Levene, 1949). The differentiation within and among subpopulations was estimated by the contingency test, F-statistics (Wright 1951, 1965; Nei 1977, 1978) and statistics of Nei (1978). The significance of  $F_{IS}$  was tested by the formula of Li (1955):  $\chi^2 = N \cdot F_{IS}^2$ , with significance at the 5% level. The  $F_{IT}$  values were tested using the significance method of Brown (1970) in which  $|F_{IT}| \cdot \sqrt{N}$  should be greater than 1.96 for significance at the 5% level (N is the sample size). The  $F_{ST}$  values were tested statistically at the 1% level of significance to determine whether or not they were significantly different from zero, using the procedure of Workman and Niswander (1970), where  $\chi^2 = 2N \cdot F_{ST}$  had  $s - 1$  degrees of freedom, N is the number of fish sampled and s is the number of stocks.

## RESULTS

Twelve of the 19 enzymes studied showed satisfactory resolution using liver extract (Table I), resulting in a total of 31 alleles distributed among 18 loci, all of them with anodal migration, except for the *LDH-1\** locus. Enzymatic patterns of muscle extracts were not analyzed due to the poor resolution, except for LDH.

EST - Two zones of esterase activity were observed for this monomeric enzyme and were assumed to represent the expression of two loci. The most anodal

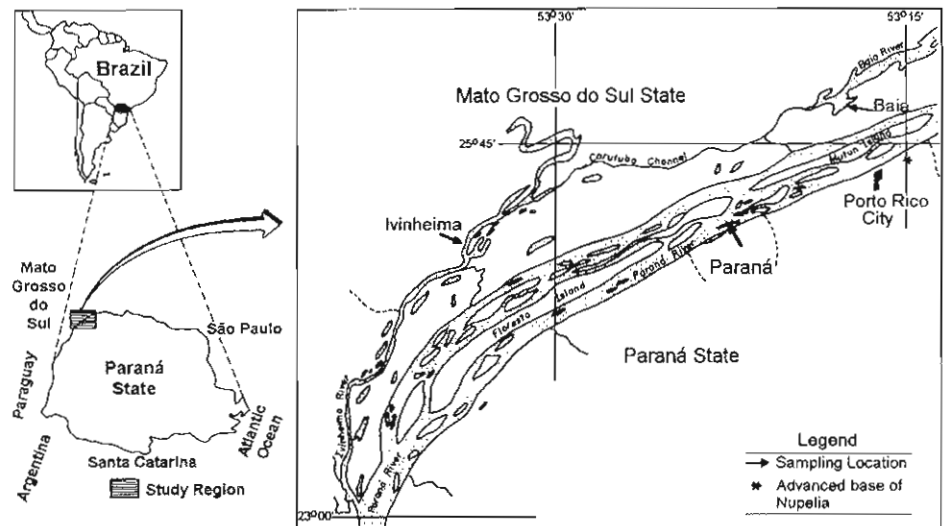


Figure 1 - Map of sampling locations.

**Table I** - Names, abbreviations and numbers for the assayed enzymes in *Prochilodus lineatus* subpopulations. The names and numbers follow IUBNC (1984). E.C. = Enzyme number.

| Enzyme name                        | Abbreviation and E.C. |
|------------------------------------|-----------------------|
| Alcohol dehydrogenase              | (ADH 1.1.1.1)         |
| Esterase                           | (EST 3.1.1.1)         |
| Phosphoglucomutase                 | (PGM 2.7.5.1)         |
| Glycerol-3-phosphate dehydrogenase | (G3PDH 1.1.1.8)       |
| Glutamate dehydrogenase            | (GLUDH 1.4.1.2)       |
| 3-Hydroxybutyrate dehydrogenase    | (HBDH 3.1.1.30)       |
| L-Iditol dehydrogenase             | (IDDH 1.1.1.14)       |
| Isocitrate dehydrogenase           | (IDH 1.1.1.42)        |
| L-Lactate dehydrogenase            | (LDH 1.1.1.27)        |
| Malate dehydrogenase               | (MDH 1.1.1.37)        |
| Superoxide dismutase               | (SOD 1.15.1.1)        |
| Xantine dehydrogenase              | (XDH 1.2.1.37)        |

locus (*EST-1\**) was represented by four distinct isozymes, indicating the existence of four different alleles; two alleles were observed at the *EST-2\** locus (Figure 2).

**IDH** - The dimeric structure of IDH suggests that it is coded for by a single polymorphic locus, in which four alleles were observed. In the "curimba", the heterodimers IDH-AC and IDH-BD had the same mobility as the homodimers IDH-BB and IDH-CC, respectively (Figure 3).

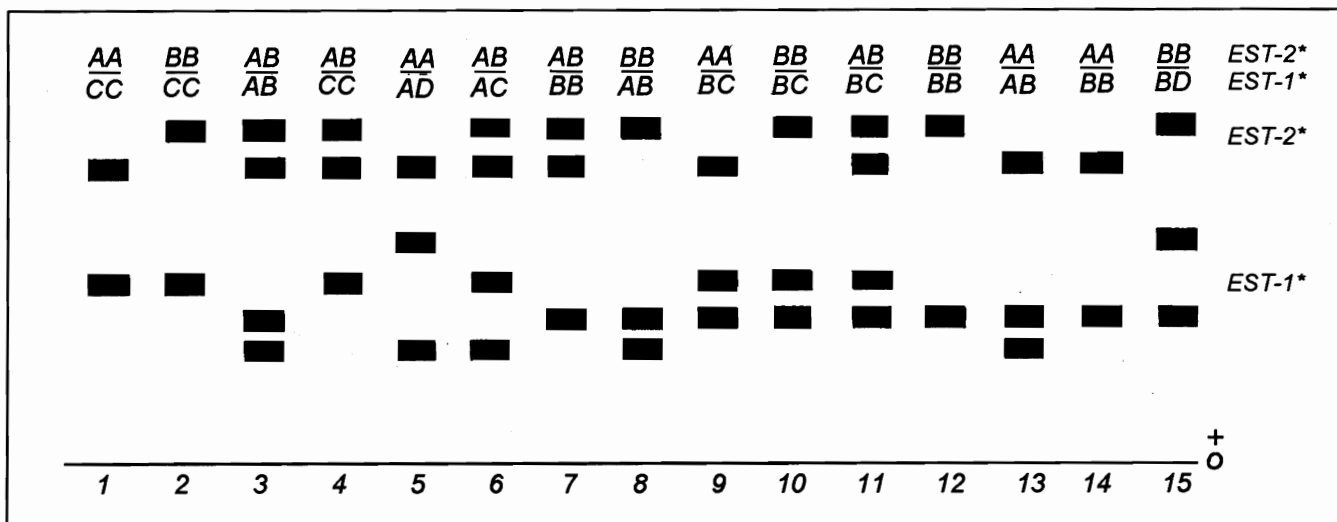
**LDH** - This tetrameric enzyme has been shown to be coded for by two loci in "curimba" (Toledo-Filho and Ribeiro, 1977 and Fenerich-Verani *et al.*, 1990a). The A and B loci described in the above reports correspond to the *LDH-1\** and *LDH-2\** loci in our nomenclature, respectively. In the present study, the most common

phenotype was five-banded. Only two different five-banded heterozygotes with different electrophoretic mobility at the *LDH-2\** locus were observed, suggesting the existence of three alleles. The cathodal monomorphic locus *LDH-1\** was expressed only in skeletal muscle (Figure 4).

**PGM** - In the "curimba", this monomeric enzyme is represented by three zones of activity, which were assumed to represent the expression of two loci. In the least anodal and intermediate zones of activity typical monomeric enzyme phenotypes (two-banded heterozygotes) were found. These zones were attributed to expression of the *PGM-1\** and *PGM-2\** loci, respectively. Three alleles were observed for both loci. The third most anodal zone showed three and five-banded phenotypes, which are characteristic of a tetrameric enzyme coded for by two monomorphic loci (Figure 5).

**MDH** - This dimeric enzyme has been reported to be coded for by three loci in the "curimba" (Fenerich-Verani *et al.*, 1990b). The *mMDH*, *sMDH-A\**, and *sMDH-B\** loci reported by these investigators correspond to the *mMDH-1\**, *sMDH-2\**, and *sMDH-3\** loci, respectively, in our nomenclature. The *sMDH-2\** locus is predominant in liver extracts. All individuals analyzed were shown to be homozygotes. According to Fenerich-Verani *et al.* (1990), the least anodal isozyme may correspond to alcohol dehydrogenase (ADH) expression (Figure 6).

**ADH, G3PDH, GLUDH, HBDH, SOD and XDH** were all represented by a single band. Each one of these enzymes was designated as if coded for by one locus. **IDDH** was represented by two invariable bands, suggesting the existence of two monomorphic loci. The gels of the Paraná river subpopulation did not have



**Figure 2** - Diagrammatic representation of the 15 EST phenotypes detected at the *EST-1\** and *EST-2\** loci of *Prochilodus lineatus* liver. Capital letters above = genotypes for each locus.

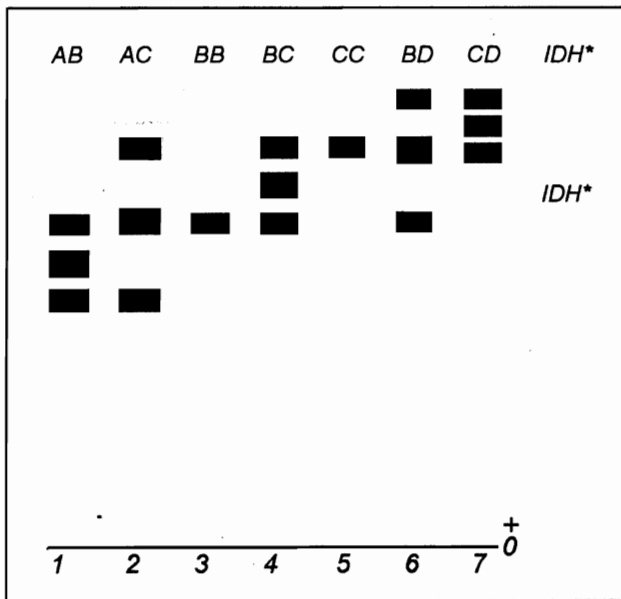


Figure 3 - Diagrammatic representation of the seven IDH phenotypes detected at the IDH locus of *Prochilodus lineatus* liver.

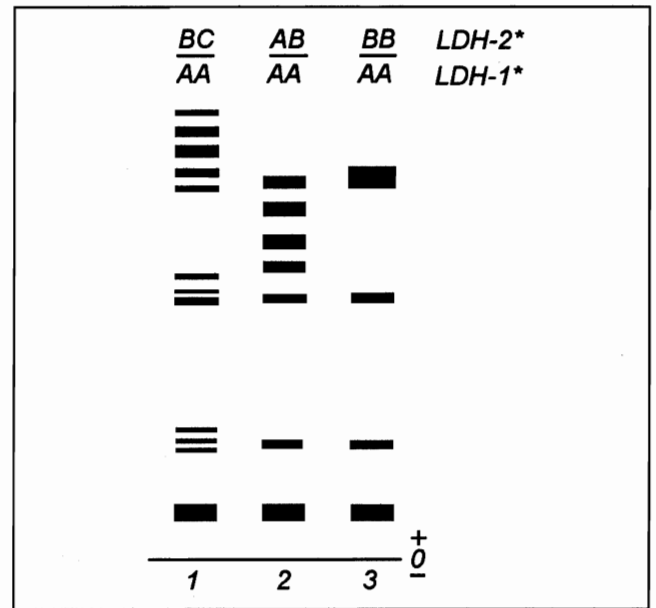


Figure 4 - Schematic representation of the three LDH phenotypes detected at the LDH-1\* and LDH-2\* loci of *Prochilodus lineatus* liver.

good definition compared to the other sampling sites. The allele frequencies of each subpopulation and total population (pooled data), and the chi-square contingency test are given in Table II. Only the *PGM-2\** locus demonstrated significant heterogeneity among subpopulations ( $P < 0.05$ ).

The Hardy-Weinberg equilibrium test displayed significant departures from expected genotypic proportion ( $P < 0.05$ ) at the *EST-2\** locus of the Paraná river subpopulation, and at the *EST-1\** and *EST-2\** loci of the total population (Table III). Since the band patterns of the gels for the Paraná river subpopulation did not show good definition, the statistical analysis for the total population was performed excluding allele frequencies of *EST-1\** and *EST-2\** loci, resulting in nonsignificant values (0.818 and 0.404, respectively).

Estimates of genetic variability for each subpopulation and for the total population were calculated on the basis of mean heterozygosity and polymorphism (Table IV). Although expected heterozygosity values for each locus were variable (0 to 0.61), all the subpopulations proved to be similar, in terms of heterozygosity and polymorphism.

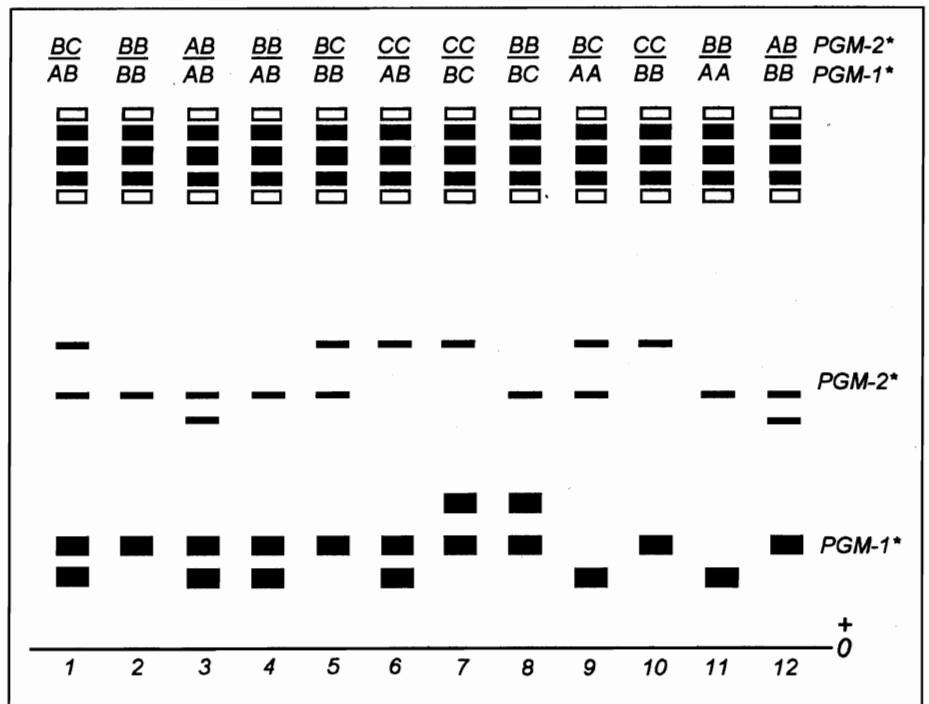
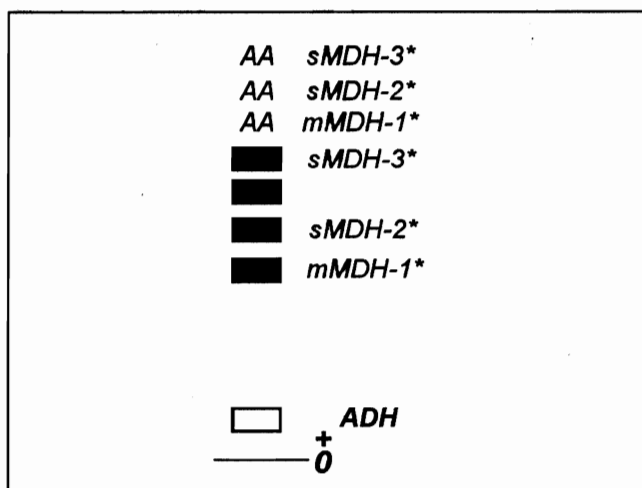


Figure 5 - Schematic representation of the twelve PGM phenotypes detected at the *PGM-1\** and *PGM-2\** loci of *Prochilodus lineatus* liver.

The statistics of Nei (1978), displayed in Table V, revealed the high genetic homogeneity of the samples with a small differentiation between Paraná river and Baía river subpopulations. Population differentiation was also examined by calculating  $F_{IS}$ ,  $F_{IT}$  and  $F_{ST}$  values for each locus and the mean value across all loci (Table VI).  $F_{IS}$  values correspond to mean deviation from random mating within subpopulations,  $F_{IT}$  values correspond to mean deviation from random



**Figure 6** - Schematic representation of the MDH phenotype detected at the *mMDH-1\**, *MDH-2\**, and *MDH-3\** loci of *Prochilodus lineatus* liver.

mating over all subpopulations and  $F_{ST}$  values correspond to the measure of the degree of genetic differentiation among subpopulations. The mean values demonstrated the low level of inbreeding and the high homogeneity of the subpopulations sampled. The results of the Wright's F-Statistics performed without *EST-2\** from the Paraná river subpopulation demonstrated a small variation for mean values of  $F_{IS}$  and  $F_{IT}$ , but  $F_{ST}$  remained constant.

The  $F_{IS}$  values for each subpopulation tested by the formulae of Li (1955) were significantly different from zero ( $P < 0.05$ ) at the *EST-1\**, *EST-2\** and *PGM-2\** loci from the Paraná river subpopulation, exactly the loci in which bands had low definition. The  $F_{IT}$  values for *EST-1\** and *EST-2\** were significantly different from zero ( $P < 0.05$ ) using the method of Brown (1970). None of the  $F_{ST}$  values tested by the procedures of Workman and Niswander (1970) were significantly different from zero ( $P < 0.05$ ).

## DISCUSSION

The levels of genetic variability and polymorphism reported in this study for the "curimba" can be considered high when compared to the mean values obtained by Nevo (1978) for 51 teleost species ( $H = 0.051$  and  $P = 0.152$ ) or the mean heterozygosity values calculated by Powell (1975) and Ward *et al.* (1992) for marine and freshwater fishes (0.058 and 0.051, respectively). High levels of heterozygosity and polymorphism might be expected for species of fish occurring over a broad range of patches in a river (Zimmerman, 1987), and showing a large population size, due to the lower levels of inbreeding.

**Table II** - Allele frequency estimates and contingency chi-square analysis for *Prochilodus lineatus* from three sampling sites in the upper Paraná river. P = Probabilities relative to contingency chi-square. N = Sample size. Total = Allele frequencies for pooled data.

| Locus           | Allele | Paraná | Baía  | Ivinheima | P     | Total |       |
|-----------------|--------|--------|-------|-----------|-------|-------|-------|
| <i>ADH-1*</i>   | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
|                 | N      | 50     | 60    | 50        |       | 160   |       |
| <i>EST-1*</i>   | A      | 0.085  | 0.100 | 0.097     | 0.673 | 0.093 |       |
|                 | B      | 0.610  | 0.483 | 0.629     |       | 0.578 |       |
|                 | C      | 0.268  | 0.383 | 0.258     |       | 0.299 |       |
|                 | D      | 0.037  | 0.033 | 0.016     |       | 0.029 |       |
| N               |        | 41     | 30    | 31        |       | 102   |       |
| <i>EST-2*</i>   | A      | 0.561  | 0.567 | 0.463     | 0.368 | 0.524 |       |
|                 | B      | 0.439  | 0.433 | 0.537     |       | 0.476 |       |
| N               |        | 33     | 30    | 41        |       | 104   |       |
| <i>G3PD-1*</i>  | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| N               |        | 50     | 60    | 50        |       | 160   |       |
| <i>GLUDH-1*</i> | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| N               |        | 50     | 60    | 50        |       | 160   |       |
| <i>HBDH-1*</i>  | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| N               |        | 50     | 60    | 50        |       | 160   |       |
| <i>IDDH-1*</i>  | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| N               |        | 50     | 60    | 50        |       | 160   |       |
| <i>IDDH-2*</i>  | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
|                 | N      | 50     | 60    | 50        |       | 160   |       |
|                 | A      | 1.000  | 1.000 | 1.000     |       | 1.000 | 1.000 |
|                 | N      | 50     | 60    | 50        |       | 160   |       |
| <i>IDH-1*</i>   | A      | 0.085  | 0.033 | 0.021     | 0.077 | 0.044 |       |
|                 | B      | 0.488  | 0.650 | 0.542     |       | 0.570 |       |
|                 | C      | 0.415  | 0.300 | 0.396     |       | 0.362 |       |
|                 | D      | 0.012  | 0.017 | 0.042     |       | 0.023 |       |
| N               |        | 41     | 60    | 48        |       | 149   |       |
| <i>LDH-1*</i>   | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| N               |        | 50     | 60    | 50        |       | 160   |       |
| <i>LDH-2*</i>   | A      | 0.000  | 0.000 | 0.010     | 0.354 | 0.994 |       |
|                 | B      | 0.990  | 1.000 | 0.990     |       | 0.003 |       |
|                 | C      | 0.010  | 0.000 | 0.000     |       | 0.003 |       |
| N               |        | 50     | 60    | 50        |       | 160   |       |
| <i>mMDH-1*</i>  | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| <i>sMDH-2*</i>  | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| <i>sMDH-3*</i>  | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| N               |        | 50     | 60    | 45        | 1.000 | 155   |       |
| <i>PGM-1*</i>   | A      | 0.351  | 0.225 | 0.194     | 0.091 | 0.253 |       |
|                 | B      | 0.638  | 0.750 | 0.796     |       | 0.731 |       |
|                 | C      | 0.011  | 0.025 | 0.010     |       | 0.016 |       |
| N               |        | 47     | 60    | 49        |       | 156   |       |
| <i>PGM-2*</i>   | A      | 0.045  | 0.024 | 0.015     | 0.010 | 0.029 |       |
|                 | B      | 0.875  | 0.690 | 0.794     |       | 0.788 |       |
|                 | C      | 0.080  | 0.286 | 0.191     |       | 0.183 |       |
| N               |        | 44     | 42    | 34        |       | 120   |       |
| <i>SOD-1*</i>   | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
| N               |        | 50     | 60    | 50        |       | 160   |       |
| <i>XDH-1*</i>   | A      | 1.000  | 1.000 | 1.000     | 1.000 | 1.000 |       |
|                 | N      | 50     | 60    | 50        |       | 160   |       |

The methodological error hypothesis seemed to explain the divergence of allele frequencies expected by Hardy-Weinberg equilibrium observed in *EST-2\** of the Paraná river subpopulation. Many of the significant deviations occurred only in those enzymes subjected to relatively rapid loss of activity. Electrophoretic analysis

**Table III** - Probability values of the chi-square test for Hardy-Weinberg equilibrium in *Prochilodus lineatus*. Total = Pooled data.

| Locus  | Paraná | Baía  | Ivinheima | Total         |
|--------|--------|-------|-----------|---------------|
| EST-1* | 0.052  | 0.976 | 0.780     | 0.041/0.818*  |
| EST-2* | 0.006  | 0.901 | 0.148     | 0.017/0.404** |
| IDH-1* | 0.512  | 0.898 | 0.578     | 0.599         |
| LDH-2* | 1.000  | -     | 1.000     | 1.000         |
| PGM-1* | 1.000  | 0.975 | 0.986     | 0.874         |
| PGM-2* | 0.051  | 0.999 | 1.000     | 0.418         |

\*Analysis without EST-1\* from the Paraná river subpopulation;

\*\*analysis without EST-2\* from the Paraná river subpopulation.

**Table IV** - Estimates of genetic variability in *Prochilodus lineatus*. Observed heterozygosity ( $H_0$ ), expected heterozygosity ( $H_E$ ), proportion of polymorphic loci by the 95% criterion (0.95) and 99% criterion (0.99). SEM = Standard error of the mean for heterozygosity.

| Locus    | Paraná<br>$H_E/H_0$ | Baía<br>$H_E/H_0$ | Ivinheima<br>$H_E/H_0$ | Total       |
|----------|---------------------|-------------------|------------------------|-------------|
| EST-1*   | 0.554/0.341         | 0.619/0.533       | 0.537/0.419            | 0.569/0.422 |
| EST-2*   | 0.500/0.758         | 0.499/0.467       | 0.503/0.634            | 0.501/0.625 |
| IDH-1*   | 0.590/0.634         | 0.490/0.400       | 0.554/0.542            | 0.543/0.510 |
| LDH-2*   | 0.020/0.020         | 0.000/0.000       | 0.020/0.020            | 0.012/0.013 |
| PGM-1*   | 0.474/0.468         | 0.389/0.367       | 0.332/0.367            | 0.403/0.397 |
| PGM-2*   | 0.229/0.159         | 0.446/0.429       | 0.338/0.353            | 0.347/0.308 |
| Mean     | 0.132/0.132         | 0.136/0.122       | 0.127/0.130            | 0.132/0.126 |
| SEM      | 0.053/0.058         | 0.054/0.048       | 0.051/0.052            | 0.052/0.051 |
| P (0.95) | 0.278               | 0.278             | 0.278                  | 0.278       |
| P (0.99) | 0.333               | 0.278             | 0.333                  | 0.278       |

**Table V** - Nei's (1978) genetic distance (below diagonal) and genetic identity (above) among *Prochilodus lineatus* subpopulations.

| Subpopulations | Paraná | Baía  | Ivinheima |
|----------------|--------|-------|-----------|
| Paraná         | ****   | 0.996 | 0.999     |
| Baía           | 0.004  | ****  | 0.999     |
| Ivinheima      | 0.001  | 0.001 | ****      |

**Table VI** - Summary of F-statistics for all *Prochilodus lineatus* subpopulations.

| Locus   | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---------|----------|----------|----------|
| EST-1*  | 0.232    | 0.242    | 0.013    |
| EST-2*  | -0.255   | -0.243   | 0.009    |
| EST-2** | -0.103   | -0.102   | 0.011    |
| IDH-1*  | 0.025    | 0.039    | 0.015    |
| LDH-2*  | -0.010   | -0.005   | 0.005    |
| PGM-1*  | -0.015   | 0.008    | 0.022    |
| PGM-2*  | 0.059    | 0.094    | 0.037    |
| Média   | 0.013    | 0.031    | 0.018    |
| Média*  | 0.043    | 0.061    | 0.018    |

\*Analysis without EST-2\* from the Paraná river subpopulation.

performed by Lavery and Shaklee (1989) and on "corvina" by Maggioni (1992) demonstrated loss of activity of EST.

Only the PGM-2\* locus, which demonstrated an increase in the homozygous frequencies, showed significant allele divergence among the three subpopulations. This divergence can be explained by the Wahlund effect, since the mean expected heterozygosity among the three subpopulations was lower than the mean expected heterozygosity in the total population. The occurrence of a selective event cannot be evaluated, because the fitness of the different isozymes is unknown. In this way, we cannot say whether or not isozyme-environment interaction can yield a high level of homozygotes.

Mean genetic distance and genetic identity demonstrated the high level of subpopulation similarity. These values were lower than those reported by Shaklee *et al.* (1982) for conspecific populations of a large number of marine and freshwater species, whose mean values were  $D = 0.05$  and  $I = 0.97$ . The small genetic distance, the low inbreeding values ( $F_{IS}$  and  $F_{ST}$ ) and differentiation level ( $F_{ST}$ ) measured for *P. lineatus* clearly reflect the ample dispersal of this species and its panmictic behavior. The gene flow is sufficient to maintain the high level of genetic homogeneity. The high level of genetic homogeneity may be explained as a result of substantial gene flow between subpopulations, from the viewpoint of a neutral model. From the viewpoint of an adaptive selection model, the possibility of balanced selection may be considered. Although selectionists and neutralists have different explanations concerning allele divergence between subpopulations, they agree that the level of heterozygosity is correlated with the size of the population.

Although these results are still preliminary, they strongly suggest that the subpopulations comprise a single stock. Thus, the use of a single exploitation model is possible, considering that overexploitation of one of the subpopulations can be felt by the total population.

The large number of barriers in the Paraná river compromise the genetic variability of migratory species since upstream flow through the barriers is not possible. Occasional gene flow downstream is possible when fish can stay alive after barrier transposition. There is no transposition of "curimbas" across the Itaipu reservoir barrier, a fact that could alter the allele frequencies of populations. Avise and Felley (1979), examining *Lepomis macrochirus* populations from 64 localities distributed evenly among eight reservoirs and two drainages, did not find any evidence of inbreeding within localities, but allele frequencies among localities

were often heterogeneous. According to Zimmerman (1987), the isolation of populations can make them more susceptible to stochastic processes and inbreeding that can shape their genome. Galhardo and Toledo-Filho (1988), in a genetic-biochemical study of transferrin of "curimba", observed that the natural population of the Mogi-Guaçu river was in Hardy-Weinberg equilibrium, while the cultivated population of the Paraibuna reservoir showed deviations due to the high level of inbreeding. The increase of homozygosity does not mean the extinction of the population, but it could affect exploitation. Comparative studies between natural and cultivated species have demonstrated that species with high H values show high additive genetic variance in quantitative traits, such as growth rate, reproductive success or resistance to disease (Allendorf and Ryman, 1987; Skaala *et al.*, 1990; Hindar *et al.*, 1991; Reina *et al.*, 1994). Leberg (1990) verified that a 25% reduction in heterozygosity led to a 56% reduction in population size, in *Gambusia holbrooki*.

The high levels of heterozygosity and polymorphism reported for the "curimba" indicate that this area is adequate to collect founder stocks. According to Toledo-Filho *et al.* (1992), founder stocks should be formed from wild stocks of the same basin since they show lower levels of inbreeding and higher biological potential to adapt to reservoir conditions.

The maintenance of environmental heterogeneity in the Paraná river is important for the preservation of aquatic organisms, since this would guarantee the reproductive success of species such as *P. lineatus* (Agostinho, 1992; Vazoller, 1992; Agostinho *et al.*, in press; Gomes and Agostinho, in press) and, consequently, stability of the gene pool. The results obtained in this study show the need for maintenance of the flowing stretch of the Paraná river, and therefore the cost-benefit ratio for the Ilha Grande hydroelectric scheme should be reconsidered in relation to the loss to fisheries and the effects on biological diversity.

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## RESUMO

A variabilidade genética das subpopulações de curimba, *Prochilodus lineatus*, coletadas em três localidades da bacia do rio Paraná, foi analisada pela eletroforese em gel de

amido. Um total de 19 sistemas enzimáticos foram analisados, em 160 indivíduos, dos quais 12 apresentaram padrões eletroforéticos interpretáveis geneticamente. Dos 18 loci identificados, seis mostraram polimorfismo (*EST-1\**, *EST-2\**, *IDH-1\**, *PGM-1\**, *PGM-2\**, *LDH-2\**). A heterozigose média de 13% foi considerada alta quando comparada com os dados da literatura. Um baixo nível de diferenciação foi encontrado entre as subpopulações, com  $F_{ST} = 0,018$ . Os valores de distância e identidade genética sugerem que, ao menos neste trecho da planície de inundação, *P. lineatus* representa uma única unidade reprodutiva com alto fluxo gênico. Esta análise tem importantes implicações para o manejo de pesca, piscicultura e conservação dos estoques.

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