Genetic variability in a *Leporinus lacustris* Campos, 1945 (Osteichthyes: Anostomidae) population from *Lagoa do Carão* (Upper *Paraná* River floodplain), Brazil

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ABSTRACT. A *Leporinus lacustris* population from the floodplain of Upper *Paraná* River was analyzed for genetic diversity by allozyme data. Specimens were sampled in Southern Brazil, at *Lagoa do Carão* (22°44'S/53°17'W) in the floodplain of Upper *Paraná* River. A total of thirty *loci* were identified in sixteen enzymatic systems (AAT, ACP, ADH, EST, GDH, G_3PDH, G_6PDH, GPI, IDHP, L-IDDH, LDH, MDH, MDHP, PER, PGM, and SOD), on 15% corn starch gel electrophoresis. Proportions of polymorphic *loci* were estimated as 26.67%. Expected heterozygosity was estimated as 0.0806 ± 0.0313, which was lower than previous estimates for *L. friderici*, *L. elongatus* and *L. obtusidens* from the Tibagi River, a tributary of the *Paraná* River basin. The low heterozygosity of the *L. lacustris* analyzed population could be attributed to the sedentary habit of this species.

Key words: allozymes, genetic variability, *Leporinus lacustris,* heterozygosity, *Paraná* River.

RESUMO. Variabilidade genética em uma população de *Leporinus lacustris* Campos, 1945 (Osteichthyes: Anostomidae) da Lagoa do Carão (planície de inundação do alto rio Paraná), Brasil. A variabilidade genética de *Leporinus lacustris* foi estimada a partir de uma população coletada na lagoa do Carão (22°44'S/53°17'W), na planície de inundação do alto rio Paraná. Foram identificados trinta locos em dezesseis sistemas enzimáticos analisados (AAT, ACP, ADH, EST, GDH, G_3PDH, G_6PDH, GPI, IDHP, L-IDDH, LDH, MDH, MDHP, PER, PGM, e SOD), por eletroforese em gel de amido de milho 15%. A proporção de *loci* polimórficos foi estimada em 26,67%. A heterozigosidade média esperada foi estimada em 0,0806 ± 0,0313, a qual foi menor que as estimadas anteriormente para *L. friderici*, *L. elongatus* e *L. obtusidens* do rio Tibagi, um tributário da bacia do rio Paraná. A baixa heterozigosidade da população de *L. lacustris* analisada pode ser atribuída aos hábitos sedentários desta espécie.

Palavras-chave: alozimas, variabilidade genética, *Leporinus lacustris* , heterozigosidade, rio Paraná.

Introduction

In Central and South America, the genus *Leporinus* of the Anostomidae family comprises 87 fish species (Garavello and Britski, 2003). The species *Leporinus amblirhynchus, L. elongatus, L. friderici, L. lacustris, L. macrocephalus, L. microphthalmus, L. obtusidens, L. octofasciatus* and *L. striatus* were described in the Upper *Paraná* River basin (Agostinho and Júlio Jr., 1999). For almost twenty years, the Nupelia research center, at the State University of *Maringá*, state of Paraná, Brazil, has studied the ecology of fish species in the Upper *Paraná* River floodplain. *L. friderici* and *L. lacustris* are among the ten most abundant dwelling in the floodplain (Luiz *et al*., 2004).

Genetic variability knowledge of natural populations is important for planning effective conservation programs capable of assuring their long-term maintenance and evolution. The genetic variability of *L. elongatus, L. friderici, L. obtusidens*, *Schizodon intermedius* and *S. nasutus* from *Tibagi* River, a tributary of the *Paraná* River basin, was analyzed by allozyme (Chiari and Sodré, 1999) and by RAPD markers (Chiari and Sodré, 2001). In previous studies, Renno *et al.* (1989, 1990) analyzed the genetic variability of *L. friderici, L. granti, L.* aff.

steyermarki, and *L. lebailli* populations from French Guiana.

While the species analyzed by Chiari and Sodré (1999, 2001) live in lotic environments and are short-distance migrators, *L. lacustris* is typical of lentic environments and has been characterized as a sedentary fish species (Suzuki *et al.*, 2004). *Lagoa do Carão* has lower oxygen levels, higher *a*-chlorophyll concentration, lower electric conductivity and pH, more variable temperatures, and higher nitrogen concentration as compared to lotic environments (Thomaz *et al.*, 2004). *L. lacustris* has been described as an herbivorous species in the Upper *Paraná* floodplain (Hahn *et al.,* 2004), where it reaches up to 19 cm length and reproduces from September to March (Vazzoler *et al.*, 1997).

This work aimed at studying the genetic variability of a *L. lacustris* population from the upper *Paraná* River floodplain and at comparing it to that previously estimated for other *Leporinus* species from the other localities of *Paraná* River basin (Chiari and Sodré, 1999). Data may contribute to understanding the relationship among living habits, environmental characteristics, and genetic diversity in this species.

Material and methods

From March to August 2002, 30 adult specimens of *Leporinus lacustris* were sampled by using gillnets, in *Lagoa do Carão* (22°44'S/53°17'W) at the floodplain of Upper *Paraná* River basin. *Lagoa do Carão* is a lagoon connected to the *Baia* River, which is a tributary of *Paraná* River (Figure 1). Immediately after capture, tissues of liver, white skeletal muscle, gill, stomach, gonad, eye, kidney, and heart were removed from each specimen and frozen in liquid nitrogen. Tissues were homogenized with plastic sticks in 1.5 mL microcentrifuge tubes in the presence of Tris/HCl 0.02 M, pH 7.5 buffer (1:1 v:w). Carbon tetrachloride (CCl_4) was added to homogenized liver samples (1:2 v:v) because liver tissues contain large amounts of fat (Pasteur *et al.*, 1988). Homogenized samples were centrifuged at 45.114 x *g* for 30 minutes, at temperatures ranging from 1°C to 5°C. Supernatant fractions were submitted to horizontal electrophoresis in 15% corn starch gel (Val *et al.*, 1981).

All the removed tissues were submitted to electrophoresis to visualize isozyme expression patterns and choose which tissues were suitable for population analysis.

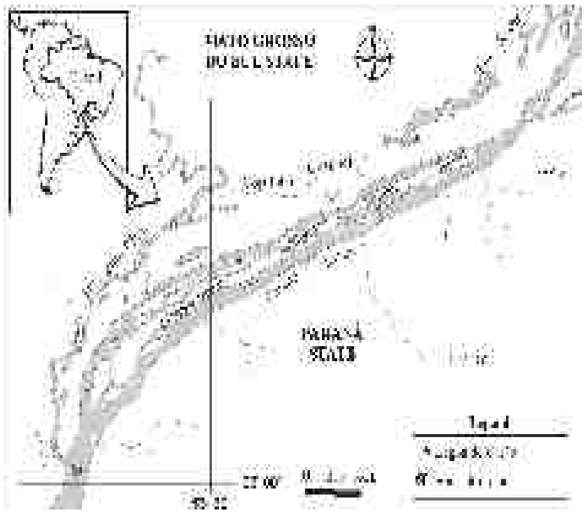

Figure 1. Geographic localization of Lagoa do Carão in the Upper Paraná Rover floodplain.

Sixteen enzymatic systems, Acid phosphatase (ACP; E. C. 3.1.3.2), Alcohol dehydrogenase (ADH; E. C. 1.1.1.1), Aspartate aminotransferase (AAT; E. C. 2.6.1.1), Esterase (EST; E. C. 3.1.1.1), Glycerol-3-phosphate dehydrogenase (G3PDH; E. C. 1.1.1.8), Glucose 1-dehydrogenase - NAD+ (GCDH; E. C. 1.1.1.118), Glucose-6-phosphate dehydrogenase (G6PDH; E. C. 1.1.1.49), Glucose-6-phosphate isomerase (GPI; E. C. 5.3.1.9), L-Iditol dehydrogenase (L-IDDH; E. C. 1.1.1.14), Isocitrate dehydrogenase - NADP+ (ICDH; E. C. 1.1.1.42), L-Lactate dehydrogenase (LDH; E. C. 1.1.1.27), Malate dehydrogenase (MDH; E. C. 1.1.1.37), Malate dehydrogenase - NADP+ (MDHP; E. C. 1.1.1.40), Peroxidase (PER; E. C. 1.11.1.6), Phosphoglucomutase (PGM; E. C. 5.4.2.2), Superoxide dismutase (SOD; E. C. 1.15.1.1) were analyzed (Table 1).

Buffer composition, enzymatic systems and electrophoretic conditions are shown in Table 1. Standard procedures of histochemical staining were used to visualize specific enzymes according to Aebersold *et al.* (1987). Genetic interpretation of gels was based on the quaternary structure of the enzymes, according to Ward *et al.* (1992). Enzyme nomenclature followed the proposal of Murphy *et al.* (1996). Data were analyzed using the software Popgene 1.31 (Yeh and Boyle, 1997). Genetic variability was estimated by frequency of polymorphic *loci* (*loci* with more than one allele) and Nei's unbiased heterozygosity (He) or genetic diversity (Nei, 1978). Genotypic frequencies were tested for Hardy-Weinberg equilibrium, using χ^2 test. The data were compared to those of Chiari and Sodré (1999) by means of t-test (Nei, 1987).

Results

A total of 30 enzymatic loci were identified for *L. lacustris* specimens sampled in *Lagoa do Carão* at the Upper *Paraná* River floodplain. Table 2 shows the results of tissue expression of each enzyme loci, regarding both number and intensity of bands. One can observe that the *loci* expression of five enzymatic systems was restricted to liver (ADH, GDH, G3PDH, IDDH, and SOD). In Figures 2 and 3, electrophoretic patterns of eleven enzymatic systems in seven different tissues of *L. lacustris* are shown. It is possible to observe the number and color intensity of each band. Electrophoretic patterns of enzymatic systems of *Leporinus lacustris* in stained starch gel were similar to those previously reported for other *Leporinus* species (Chiari and Sodré, 1999) and for other species of fishes from the Upper *Paraná* River

Table 1. Experimental conditions used in electrophoresis of 14 enzyme systems of Leporinus lacustris from the Upper Paraná River floodplain.

Enzyme	Tank Buffer	Gel Buffer	Migration Time	References
AAT, G_6PDH	Tris-maleate- EDTA 0,1-0,1-0,01 M pH 7,4	Tris-maleate- EDTA 0,01-0,01-0,001 M pH 7,4	14 hours	Shaw and Prasad (1970)
ACP, EST	Tris-HCL 0,02 M pH 7,5	Tris-HCL 0,002 M pH 7,5	5.5 hours	Ruvolo-Takasusuki *et al.*, (2002)
ADH, SOD	Tris-borate-EDTA 0,18-0,1-0.004M pH 8,6	Tris-borate-EDTA 0,045-0,025-0.001M pH 8,6	14 hours	Boyer *et al.*, (1963)
GPI, LDH	Tris-citrate 0,135-0,043M pH 7,0	Tris-citrate 0,009-0,003M pH 7,0	14 hours	Shaw and Prasad (1970)
G_3PDH, GDH, IDDH, IDHP, MDH, MDHP, PER, PGM	Tris-citrate 0,135-0,043M pH 8,0	Tris-citrate 0,009-0,003M pH 8,0	14 hours	Shaw and Prasad (1970)

Table 2. Tissue expression of different enzymatic *loci* identified in *Leporinus lacustri*s from *Lagoa do Carão* (Upper *Paraná* River floodplain).

Locus	eye	gill	liver	heart	White muscle	Red muscle	stomach
Aat	+	+	++	+++	+	+++	++
Acp-1	+	++	+	-	-	-	-
Acp-2	-	++	+++	-	-	-	-
Adh-1	-	-	+++	-	-	-	-
Est-1	+++	+++	+++	+	+	++	+++
Est-2	++	++	++	+	-	+	++
Est-3	-	+	+	-	+	++	-
Gdh-1	-	-	++	-	-	-	-
Gdh-2	-	-	++	-	-	-	-
G3pdh-1	-	-	++	-	-	-	-
G3pdh-2	-	-	++	-	-	-	-
G6pdh-1	-	-	++	-	-	-	-
G6pdh-2	+	+++	+++	+	-	-	++
Gpi-A	++	++	++	++	-	++	+++
Gpi-B	++	++	+	++	+++	+++	+
Iddh-1	-	-	+++	-	-	-	-
Iddh-2	-	-	+++	-	-	-	-
Idhp-1	++	++	+++	-	-	-	++
Idhp-2	++	++	-	++	+	+++	+++
Ldh-A	+	+	+	++	+	+	+
Ldh-B	+++	+++	-	++	-	+	+++
sMdh-A	++	++	+++	++	+	+++	+++
sMdh-B	+	+	-	++	++	+++	-
MMdh	++	++	++	++	+	+++	+++
Mdhp-1	+	-	+++	++	+	++	-
Mdhp-2	+	+	_	++	+	+	+
Per-1	+	+	+	+	+	+	+
Per-2	++	++	++	++	_	+	+
Pgm-1	++	++	+++	+++	++	++	++
Sod-1	-	-	++	-	-	-	-

- = no expression; + = low expression; ++ = intermediate expression; +++ = high expression

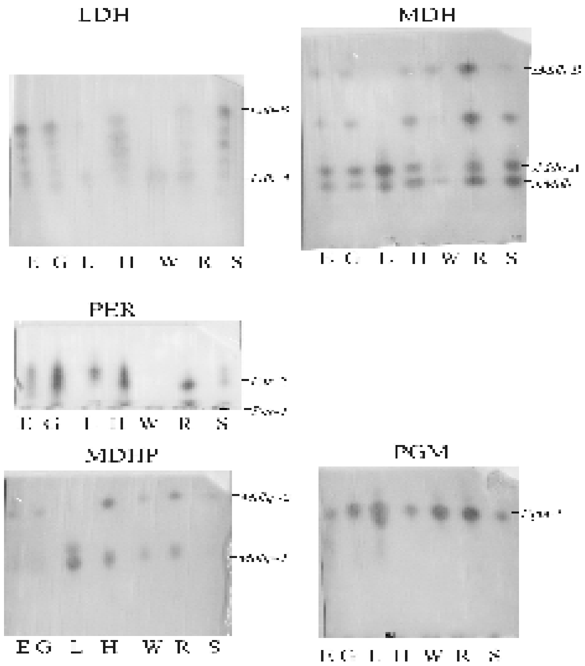

Figure 2. Tissue distribution of the expression of different loci identified in *Leporinus lacustris* from the Upper Paraná River. AAT=Aspartate aminotransferase; ACP=Alkaline phosphatase; EST=Esterase; G6PDH=Glusose-6-phosphate dehydrogenase; GPI=Glucose phosphate isomerase; and IDHP=Isocitrate dehydrogense; E=eye; G=gill; L=liver; H=heart, W=white muscle; R=red muscle; S=stomach.

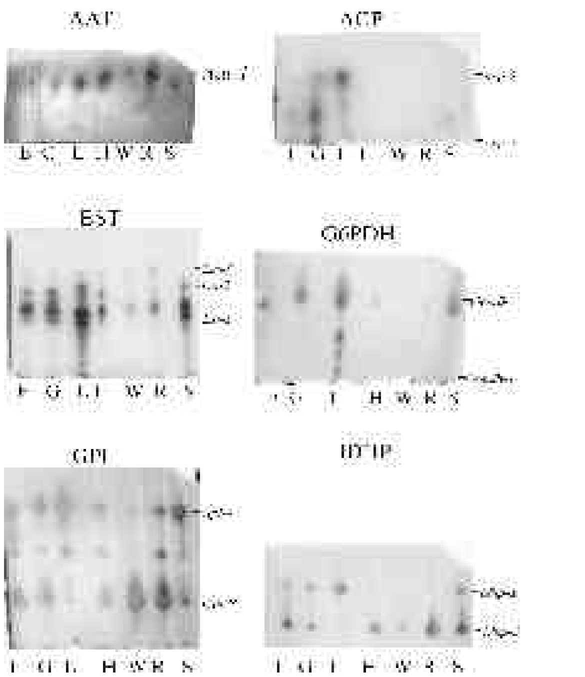

Figure 3. Tissue distribution of the expression of different loci identified in *Leporinus lacustris* from the Upper Paraná River. LDH=Lactate dehydrogenase; MDH=Malate dehydrogenase; MDHP=Malate dehydrogenase; NADP= dependent; PER=Peroxidase; PGM=Phophoglucomutase; E=eye; G=gill; L=liver, H=heart; W=white mescle; R=red muscle; S=stomach.

floodplain (Zawadzki *et al.*, 2000, 2001, 2004a, 2004b; Peres *et al.,* 2002). Eight loci (26.67%) were polymorphic (Table 3) and all of them were in Hardy-Weinberg equilibrium. The observed (ho) and expected (he) heterozygosity for each *locus*, and the mean of observed (Ho) and expected heterozygosity (He) for overall *loci* are shown in Table 3. Means of observed and expected heterozygosity were estimated as Ho = 0.0623 ± 0.0281 and He = 0.0806 ± 0.0313 which was significantly lower than that estimated by Chiari and Sodré (1999) for *L. friderici* (t_{47} = 4.28; P< 0.001), and for *L. elongatus* (t_{46} = 4.40; P < 0.001) but was not significantly different from estimated for *L. obtusidens* (t_{46} = 0.62; P> 0.05). Data revealed an excess of homozygotes in the analyzed sample of *L. lacustris* because observed heterozygosity (Ho) was lower than expected heterozygosity (He), which resulted in a fixation index of 0.227.

Discussion

The electrophoretic patterns of the same enzymatic systems analyzed in this work and in Chiari and Sodré (1999) for *Leporinus* species were similar. Unfortunately, in their work, Chiari and Sodré (1999) analyzed only five systems just in heart, muscle and liver and they did not provide gel photos. The enzyme systems EST, IDHP, LDH, and MDH of *Leporinus* species showed the same number of bands and the same number of *loci*. For PGM and G-3-PDH systems there are some differences. Chiari and Sodré (1999) identified three loci for PGM system while this research has identified only one in *L. lacustris*. Figure 1 of Chiari and Sodré (1999) shows that *PGM-3** locus has higher color intensity than *PGM-1** and *PGM-2* loci*. These weaker bands may be due to sub-bands formation instead of locus expression. Richardson *et al.* (1986) accounted for this fact for PGM enzymes. Another possibility is different times of gel staining. In our experiment the staining solution was washed away as soon as the bands suggested avoiding gel darkening, while in Chiari and Sodré (1999) experiment, the gel was stained for a longer time allowing the disclosure of other loci with weaker expression. Chiari and Sodré (1999) identified four G-3-PDH loci for *L. friderici* and three for *L. elongatus* and *L. obtusidens*, while only two loci were detected in *L. lacustris.* G-3-PDH enzyme has dimeric quaternary structure (Ward *et al.*, 1992) and are coded by two loci with heterodimer formation, resulting in a three band pattern. Then the band identified as *G-3-PDH-1** locus by Chiari and Sodré (1999) may be a sub-band, and the *G-3-*

PDH-3 locus may be a heterodimer between *G-3-PDH-2** and *G-3-PDH-4* loci*.

Table 3. Allele frequencies (freq.), observed (ho) and expected (he) heterozygosities for each locus, mean observed (Ho) and expected (He) heterozygosities for all loci, frequency of polymorphic *loci* identified in *Leporinus lacustris* from *Lagoa do Carão* (Upper *Paraná* River floodplain).

Locus-Allele	N	Freq.	ho	he
Aat-1-a	30	1.0000	0.0000	0.0000
Acp-1-a	30	1.0000	0.0000	0.0000
Acp-2-a	30	1.0000	0.0000	0.0000
Adh-1-a	30	1.0000	0.0000	0.0000
Est-1-a	30	1.0000	0.0000	0.0000
Est-2-a	30	0.6833	0.4667	0.4428
Est-2-b	30	0.3000		
Est-2-c	30	0.0167		
Est-3-a	30	1.0000	0.0000	0.0000
Gdh-1-a	30	1.0000	0.0000	0.0000
Gdh-2-a	30	1.0000	0.0000	0.0000
G3pdh-1-a	30	1.0000	0.0000	0.0000
G3pdh-2-a	30	0.9667	0.0667	0.0644
G3pdh-2-b	30	0.0333		
G6pdh-1-a	30	1.0000	0.0000	0.0000
G6pdh-2-a	30	1.0000	0.0000	0.0000
Gpi-B-a	30	0.3667	0.3000	0.4850
Gpi-B-b	30	0.6167		
Gpi-B-c	30	0.0167		
Gpi-A-a	30	0.9833	0.0333	0.0328
Gpi-A-b	30	0.0167		
Iddh-1-a	30	1.0000	0.0000	0.0000
Iddh-2-a	30	1.0000	0.0000	0.0000
Idhp-1-a	28	1.0000	0.0000	0.0000
Idhp-2-a	28	0.9643	0.0714	0.0689
Idhp-2-b	28	0.0357		
Ldh-A-a	30	1.0000	0.0000	0.0000
Ldh-B-a	30	1.0000	0.0000	0.0000
mMdh-a	30	1.0000	0.0000	0.0000
sMdh-A-a	30	1.0000	0.0000	0.0000
sMdh-B-a	30	1.0000	0.0000	0.0000
Mdhp-1-a	30	0.7667	0.0000	0.3578
Mdhp-1-b	30	0.2333		
Mdhp-2-a	30	1.0000	0.0000	0.0000
Per-1-a	30	1.0000	0.0000	0.0000
Per-2-a	30	1.0000	0.0000	0.0000
Pgm-1-a	30	0.7167	0.3000	0.4061
Pgm-1-b	30	0.2833		
Sod-1-a	27	0.3519	0.6296	0.5590
Sod-1-b	27	0.0926		
Sod-1-c	27	0.5556		
Mean	29.5		0.0623	0.0806
SE	0.19		0.0281	0.0313

The number of polymorphic loci is 8 (26.67 %); N = number of analyzed individuals.

Genetic variability within the analyzed *L. lacustris* population from *Lagoa do Carão* was estimated as 26.67% of polymorphic *loci.* The expected heterozygosity mean (He = 0.081 ± 0.031) was higher than the average 0.051 described for 195 species of fishes from several localities of the world (Ward *et al.,* 1992). According to Ward *et al.* (1994), the values of expected heterozygosity mean estimated for 107 species of fishes, analyzed by starch gel electrophoresis, ranged from 0 to 0.05 for 54% of the studied species, from 0.05 to 0.10 for 30%, from 0.10 to 0.15 for 12%, and higher than 0.15 for 4% of them. Although the expected heterozygosity estimated for *L. lacustris* could be considered high, as compared to Ward *et al.* (1994) data, it was lower than those estimated for other *Leporinus* species. In previous studies by Chiari and Sodré (1999), the expected heterozygosity was estimated for *L. friderici* (0.132 ± 0.046), *L. elongatus* (0.142 ± 0.054), and *L. obtusidens* (0.090 ± 0.042). The expected heterozygosity mean of the *L. lacustris* population was 0.0806 ± 0.0313, which was significantly lower than that estimated for *L. friderici* (t_{47} = 4.28; P< 0.001), and for *L. elongatus* (t_{46} = 4.40; P < 0.001) but was not significantly different from estimated for *L. obtusidens* (t_{46} = 0.62; P> 0.05). Therefore, the *L. lacustris* data were more similar to *L. obtusidens* than to the two other studied *Leporinus* species.

Possibly, the lower genetic variability detected in *L. lacustris* could be primarily attributed to an isolation of this population in its environment. Data show that *L. lacustris* is a sedentary species (Suzuki *et al.*, 2004), which could lead to isolation and endogamy. Low allozyme variability has been described for other sedentary Loricariids, such as *Hypostomus* (Zawadzki *et al.* 2004b), *Loricariichthys* (Zawadzki *et al.*, 2000), and *Neoplecostomus* (Zawadzki *et al.*, 2004a) living on lotic environments. In contrast, the genetic variability of *Hoplias malabaricus,* another sedentary species of upper *Paraná* River floodplain, was estimated as He = 0.14 ± 0.04 both in lentic and lotic environments (Peres *et al.*, 2002). Sedentary habits may enable *L. lacustris* species to avoid high environmental heterogeneity, which would lead to a kind of isolation and high levels of endogamy, with a consequent decrease in the heterozygosity.

Further studies are necessary for a better understanding of the interactions among genetic variability of tropical and sub-tropical fish species with their unique ecosystems and reproductive habits.

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