Research Article

Reproductive strategies and genetic variability in tropical freshwater fish

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Abstract

We estimated the genetic variability of nine fish species from the Brazilian upper Paraná River floodplain (*Astyanax altiparanae*, *Hoplias malabaricus*, *Leporinus lacustris*, *Loricariichthys platymetopon*, *Parauchenipterus galeatus*, *Pimelodus maculatus*, *Rhaphiodon vulpinus*, *Roeboides paranensis* and *Serrasalmus marginatus*) based on data for 36 putative allozyme *loci* obtained using corn starch gel electrophoresis of 13 enzymatic systems: aspartate aminotransferase (EC 2.6.1.1), acid phosphatase (EC 3.1.3.2), esterase (EC 3.1.1.1), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glucose-6-phosphate isomerase (EC 5.3.1.9), Iditol dehydrogenase (EC 1.1.1.14), isocitrate dehydrogenase – NADP$^+$ (EC 1.1.1.42), L-lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37), malate dehydrogenase-NADP$^+$ (EC 1.1.1.40), phosphoglucomutase (EC 5.4.2.2) and superoxide dismutase, (EC 1.15.1.1). The mean expected heterozygosity varied from zero to 0.147. When data from the literature for 75 species of tropical fish were added to the nine species of this study, the heterozygosity values differed significantly among the groups of different reproductive strategies. The highest mean heterozygosity was for the non-migratory without parental care, followed by the long-distance migratory, and the lowest mean was for the non-migratory with parental care or internal fecundation.

Key words: genetic variability, heterozygosity, Paraná River, polymorphism, reproductive strategies, tropical fish.

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Introduction

The reactions that species offer to environmental selective pressures is reflected in their physiological strategies, which can reduce maintenance energy, increase the efficiency of energy acquisition and, consequently, optimize reproductive efficiency. The success of such strategies may be estimated by the individual ability of being genetically present in the offspring (Agostinho and Júlio Jr., 1999).

In fish, reproductive strategies are extremely diversified, encompassing variations in the way they meet and attract partners, in spawning locations and parental care (Wootton, 1990). External fertilization and embryonic development are the dominant pattern for the majority of teleost fish, although internal fertilization and external embryonic development occurs in some Brazilian freshwater species such as *Parauchenipterus galeatus*. Blumer (1982) described parental care as any type of investment that enhances the survival probability of offspring (reproductive success) and which vary from pre-fertilization activities such as nest building (*e.g.* in *Hoplias malabaricus*) to mouth brooding and live-bearing.

Adaptation to heterogeneous environments depends upon the genetic variability of species, reduction in genetic variability resulting in reduced feeding and reproductive performance leading to inefficient exploration of the habitats (Kirpichnikov, 1992). Several authors have tried to explain the mechanisms which maintain genetic variability in natural populations. Nevo (1988) analyzed the relationship between heterozygosity and ecological and biological features such as climate (arctic, temperate and tropical), geographic range (wide, narrow, endemic), habitat type (underground, terrestrial, aquatic), habitat range (specialist, generalist) aridity (arid, sub-arid, sub-humid, mesic with a moderate or well-balanced moisture supply) and territoriality (territorial, non-territorial) in 1,111 species (vertebrates, invertebrates, and plants) and concluded that environmental heterogeneity is the major factor in maintaining and structuring genetic diversity in natural populations. Ward *et al.* (1994) compared the heterozygosity of marine, freshwater and anadromous (fish which live mostly in the sea but breed in fresh water) fish and concluded that marine fish have greater genetic variability than the other types. Heithaus and Laushman (1997) investigated the effects of ecology, life history and water quality on genetic variation

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of three stream-dwelling fish species and revealed that the genetic variability tends to decrease as species become more ecologically specialized.

Allozyme electrophoresis has been extensively used to estimate genetic variability in natural populations (Nevo, 1988; Van Der Bank *et al.*, 1989; Ward *et al.*, 1994) and our study employed this technique to estimate the genetic variability of nine species of fish from the Brazilian upper Paraná River floodplain and, jointly with literature data, verify the relationships between genetic variability and reproductive strategies.

Material and Methods

From March to December, 2002 in the Brazilian upper Paraná River floodplain, nine species from the most abundant fish with different reproductive strategies were sampled from Baía River (22°44' S, 53°17' W) and Paraná River (22°46' S, 53° 15'22" W) (Figure 1). The species sampled were *Astyanax altiparanae* Garutti & Britski 2000, *Hoplias malabaricus* (Bloch 1794), *Leporinus lacustris* Campos 1945, *Loricariichthys platymetopon* Isbrücker & Nijssen 1979, *Parauchenipterus galeatus* (Linnaeus 1766), *Pimelodus maculatus* Lacépède 1803, *Rhaphiodon vulpinus* Agassiz 1829, *Roeboides paranensis* Pignalberi 1975 and *Serrasalmus marginatus* Valenciennes 1847. A total of 267 specimens were sampled.

Immediately after capture, white skeletal muscle, gill, heart and liver tissue were removed from each specimen and frozen in liquid nitrogen. Tissues were homogenized with
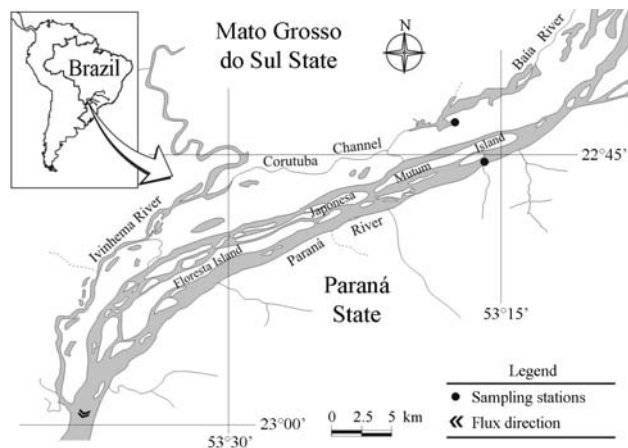

Figure 1 - Paraná River floodplain. Sample localities are indicated by a black circle.

plastic sticks in 1.5 mL microcentrifuge tubes containing Tris/0.02 M HCl (1:1 w:v) buffer, pH 7.5. Carbon tetrachloride (CCl_4) was added to the homogenized liver samples (1:2 v:v) due to the large amounts of fat present in the tissues (Pasteur *et al.*, 1988). The homogenized samples were centrifuged at 45,114 x g for 30 min at temperatures between 1° and 5 °C and the supernatants submitted to horizontal electrophoresis in 15% corn starch gel (Val *et al.*, 1981).

We evaluated 16 enzymatic systems (Table 1), enzyme nomenclature following the proposals of Murphy *et al.* (1996). Electrophoreses conditions were according to the following authors: Boyer *et al.*, (1963) for SOD; Ruvolo-Takasusuki *et al.*, (2002) for ACP and EST; Shaw

Table 1 - Electrophoretic conditions for 16 enzymatic systems analyzed for species from the upper Paraná River floodplain. The enzymes were run for 14 h, except for α- esterase (EST) EC 3.1.1.1 and acid phosphatase (ACP) EC 3.1.3.2, which were both run for 5.5 h.

Enzymes (abbreviation) and enzyme commission number	Tissue	Buffer*
Aspartate aminotransferase (AAT) EC 2.6.1.1	Liver	I
Alcohol dehydrogenase (ADH) EC 1.1.1.1	Liver	II
α- Esterase (EST) EC 3.1.1.1	Liver	III
Acid phosphatase (ACP) EC 3.1.3.2	Liver	III
Glucose dehydrogenase (GCDH) EC 1.1.1.118	Liver	IV
Glycerol-3-phosphate dehydrogenase (G3PDH) EC 1.1.1.8	Liver	IV
Glucose-6-phosphate isomerase (GPI) EC 5.3.1.9	Heart, liver and muscle	IV
Glucose-6-phosphate dehydrogenase (G6PDH) EC 1.1.1.49	Liver	I
L-Iditol dehydrogenase (L-IDDH) EC 1.1.1.14	Liver	IV
Isocitrate dehydrogenase -NADP$^+$ (IDHP) EC 1.1.1.42	Heart, gill and liver	IV
L-Lactate dehydrogenase (LDH) EC 1.1.1.27	Heart	IV
Malate dehydrogenase -NAD$^+$(MDH) EC 1.1.1.37	Gill, heart, liver and muscle	IV
Malate dehydrogenase -NADP$^+$(MDHP) EC 1.1.1.40	Heart and muscle	IV
Peroxidase (PER) EC 1.11.1.7	Liver	IV
Phosphoglucomutase (PGM) EC 5.4.2.2	Liver and heart	IV
Superoxide dismutase (SOD) EC 1.15.1.1	Liver	II

*I = Tris/EDTA/Maleate pH 7.4 (Shaw & Prasad, 1970); II = Tris/EDTA/borate pH 8.6 (Boyer *et al.*, 1963); III = Tris-HCl pH 7.5 (Ruvolo-Takasusuki *et al.*, 2002); IV = Tris/citrate pH 7.0 (Shaw and Prasad, 1970).

and Prasad (1970) for AAT, G3PDH, G6PDH, GPI, IDDH, IDHP, LDH, MDH, MDHP and PGM. Standard histochemical staining procedures were used to visualize specific enzymes (Aebersold *et al.*, 1987). Genetic interpretation of the gels was based on the quaternary structure of the enzymes (Ward *et al.*, 1992). Data were analyzed using the POPGENE program version 1.31 (Yeh *et al.*, 1997). Genetic variability was estimated using Nei's unbiased heterozygosity (He) or gene diversity (Nei, 1978). The observed (Ho) and expected (He) heterozygosities for each putative *loci* and the overall loci means were also calculated. Genotypic frequencies were tested for Hardy-Weinberg equilibrium using the chi squared (χ^2) test.

The species were organized in three distinct groups of reproductive strategy according to Vazzoler and Menezes (1992): group 1 (G1), containing the long-distance (> 100 km) migratory species *P. maculatus* and *R. vulpinus*; group 2 (G2), containing the sedentary or short-distance (< 100 km) migratory species *A. altiparanae*, *L. lacustris*, *R. paranensis* which provide no parental care; and group 3, containing the sedentary or short-distance migratory species *H. malabaricus*, *L. platymetopon*, *P. galeatus, S. marginatus* with internal fertilization or which provide parental care. Since it is unlikely that the species analyzed by us were representative of all tropical fish, we added data from the literature for a further 75 tropical fish species (61 from Brazil and 14 from Africa) for which the heterozygosity values had been estimated using allozyme data (Table 2), hence the total number of species analyzed was 84.

The significance of differences between groups was evaluated using a null models analysis of variance and the EcoSim 7 program (Gotelli & Entsminger, 2006).

Results

Genetic variability

The calculated genetic variability values for each species are presented in Table 3, from which it can be seen that the greatest frequency of polymorphic *loci*, average number of alleles per locus and heterozygosity were obtained for *A. altiparanae*, followed by *H. malabaricus*. No polymorphic locus was detected in *R. vulpinus* for the 29 loci analyzed. Except for *P. maculatus* the expected heterozygosity were higher than obtained heterozygosity, which indicates, on average, an excess of homozygotes for all loci.

All loci were in Hardy-Weinberg equilibrium (HWE) for *L. lacustris, L. platymetopon, P. maculatus, R. vulpinus* and *R. paranensis*. The loci not in HWE were *G6pdh-1, Iddh-1* and *Mdhp-1* for *A. altiparanae, Gdh-1* for *S. marginatus, Gdh-2* for *P. galeatus, Adh-1, G6pdh-1, Gpi-A, Gpi-b, sMdh-B* and *Sod-1* for *H. malabaricus*.

Heterozygosity and reproductive strategies

The lowest heterozygosity values in our sample of nine species were for the G1 long-distance migratory

species (*R. vulpinus* = 0 and *P. maculatus* = 0.011), which therefore also presented the lowest group average (He = 0.005). The highest average heterozygosity (He = 0.110) occurred in the G2 sedentary or short-distance migratory species without parental care, with *A. altiparane* having the highest heterozygosity (0.152) of all the species analyzed. The G3 sedentary or short-distance migratory species with internal fertilization or parental care exhibited intermediate heterozygosity (0.067).

When we considered the nine tropical fish species analyzed by us plus the 75 species from the other studies we found that the number of species in each reproductive strategy group and the average heterozygosity (He) ± the standard error for each group was as follows: G1 = 7 (Ho = 0.064 ± 0.021), G2 = 8 (He = 0.081 ± 0.016) and G3 = 69 (He = 0.046 ± 0.002) (Table 3, Figure 2), with He varying from zero to 0.142 for G1, from 0.006 to 0.152 for G2, and from zero to 0.143 for G3 (Tables 2 and 3). These data show that the highest average heterozygosity was presented by the G2 sedentary or short-distance migratory species without parental care, the G1 long-distance migratory species had and intermediate average heterozygosity and the G3 species with parental care or internal fertilization presented the lowest average heterozygosity.

A Shapiro-Wilk test for normality showed that the He values were not normally distributed (W = 0.9199, p < 0.0001) and the Levene test for homogeneity of variances showed a significant value (F = 4.843, p = 0.0103), indicating that the variances were heterogeneous. We conducted ANOVA but since the assumptions of normality and homogeneity of variances were not met by the data the significance of the ANOVA was tested by null models using the EcoSim 7 programs. The ANOVA detected a significant difference ($F_{2,81}$ = 4.02; p < 0.05) between the three reproductive strategy groups.
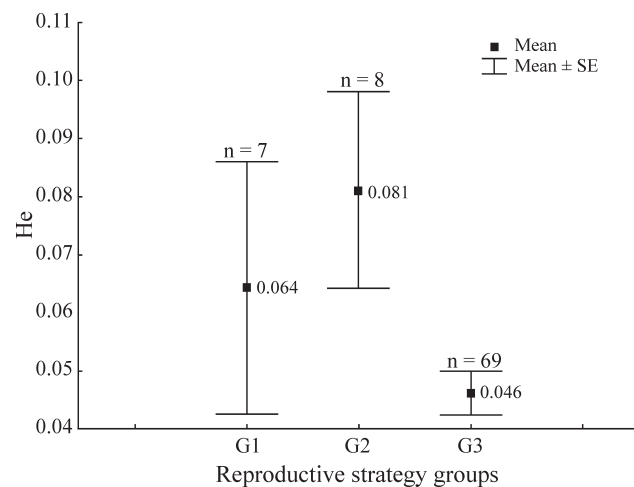

Figure 2 - Mean heterozygosity values for each reproductive strategy group of tropical fish species (Brazil and Africa). Group 1 (G1) = migratory species; Group 2 (G2) = short-distance migratory or sedentary species without parental care; and Group 3 (G3) = short-distance migratory or sedentary species with parental care or internal fertilization.

Table 2 - Heterozygosity (He) values estimated for 75 tropical fish species from Brazil and Africa based on enzymatic data. Group 1 = Migratory species, Group 2 = sedentary species or short-distance migratory without parental care; Group 3 = sedentary species or short-distance migratory with internal fertilization or parental care. Africa = Af, Brazil = Br.

Reproductive strategy group, species (country of isolation, reference*)	Heterozygosity	Reproductive strategy group, species (country of isolation, reference*)	Heterozygosity
Group 1		Group3 (continued)	
Leporinus elongatus (Br, 12)	0.142	*Hypostomus* sp1 (Br, 4)	0.042
Leporinus obtusidens (Br, 12)	0.090	*Hypostomus* sp1 (Br, 9)	0.028
Pinirampu pirinampus (Br, 11)	0.043	*Hypostomus* sp1 (Br, 5)	0.107
Prochilodus lineatus (Br, 16)	0.132	*Hypostomus* sp1 (Br, 8)	0.143
Rhinelepis aspera (Br, 5)	0.032	*Hypostomus* sp1 (Br, 6)	0.060
		Hypostomus sp2 (Br, 9)	0
Group 2		*Hypostomus* sp2 (Br, 8)	0.059
Iheringichthys labrosus (Br, 11)	0.083	*Hypostomus* sp2 (Br, 5)	0.062
Leporinus friederici (Br, 12)	0.132	*Hypostomus* sp2 (Br, 4)	0.009
Pimelodus ortimani (Br, 15)	0.024	*Hypostomus* sp2 (Br, 6)	0.072
Pimelodus sp (Br, 15)	0.006	*Hypostomus* sp3 (Br, 5)	0.048
Schizodon nasutus (Br, 12)	0.092	*Hypostomus* sp3 (Br, 8)	0.091
		Hypostomus sp3 (Br, 6)	0.053
Group 3		*Hypostomus* sp3 (Br, 4)	0.032
Chetia flaviventris (Af, 1)	0.015	*Hypostomus* sp4 (Br, 4)	0.080
Clarias gariepinus (Af, 2)	0.042	*Hypostomus* sp5 (Br, 4)	0.099
Crenicichla iguassuensis (Br, 3)	0.033	*Hypostomus* sp6 (Br, 4)	0.021
Crenicichla sp (Br, 3)	0.063	*Hypostomus* sp7 (Br, 4)	0.063
Hemichromis elongatus (Af, 1)	0.020	*Hypostomus ternetzi* (Br, 5)	0.069
Hisonotus sp (Br, 4)	0	*Loricariichthys anus* (Br, 13)	0.023
Hypostomus albopunctatus (Br, 5)	0.031	*Loricariichthys platymetopon* (Br, 13)	0.050
Hypostomus ancistroides (Br, 5)	0.082	*Loricariichthys* sp (Br, 13)	0.035
Hypostomus ancistroides (Br, 4)	0.052	*Megalancistrus parananus* (Br, 5)	0.019
Hypostomus boulengeri (Br, 6)	0.078	*Neoplecostomus paranensis* (Br, 14)	0
Hypostomus cochliodon (Br, 6)	0.070	*Neoplecostomus* sp (Br, 14)	0.030
Hypostomus cochliodon (Br, 5)	0.039	*Oreochromis andersonii* (Af, 1)	0.019
Hypostomus aff *commersoni* (Br, 7)	0.044	*Oreochromis macrochir* (Af, 1)	0.031
Hypostomus commersoni (Br, 5)	0.088	*Pterygoplichthys anisitsi* (Br, 5)	0.032
Hypostomus aff *derbyi* (Br, 5)	0.037	*Pterygoplichthys anisitsi* (Br, 6)	0.038
Hypostomus derbyi (Br, 7)	0.011	*Serranochromis angusticeps* (Af, 1)	0.013
Hypostomus hemanni (Br, 8)	0.068	*Serranochromis carlottae* (Af, 1)	0.031
Hypostomus cf *latirostris* (Br, 6)	0.029	*Serranochromis condringtonii* (Af, 1)	0.035
Hypostomus latifrons (Br, 6)	0.070	*Serranochromis giardi* (Af, 1)	0.013
Hypostomus microstomus (Br, 5)	0.023	*Serranochromis macrocephalus* (Af, 1)	0.021
Hypostomus margaritifer (Br, 10)	0.061	*Serranochromis robustus* (Af, 1)	0.018
Hypostomus margaritifer (Br, 5)	0.106	*Serranochromis thumbergi* (Af, 1)	0.018
Hypostomus myersi (Br, 7)	0.017	*Tilapia rendalli* (Af, 1)	0.031
Hypostomus paulinus (Br, 9)	0.027	*Tilapia sparrmanii* (Af, 1)	0.032
Hypostomus regani (Br, 6)	0.033		
Hypostomus regani (Br, 4)	0.056		
Hypostomus regani (Br, 5)	0.098		

*References (see reference list for full references): 1. Van der Bank *et al.* (1989), 2. Van der Bank *et al.* (1992), 3. Renesto *et al.* (2001), 4. Zawadzki & Renesto (unpublished data), 5. Zawadzki *et al.* (2005), 6. Renesto E. (unpublished data), 7. Zawadzki *et al.* (1999), 8. Zawadzki *et al.* (2004)a, 9. Paiva *et al.* (2005), 10. Zawadzki *et al.* (2002), 11. Almeida & Sodré (1998), 12. Chiari & Sodré (1999), 13. Zawadzki *et al.* (2000), 14. Zawadzki *et al.* (2004)b, 15. Renesto *et al.* (2000), 16. Revaldaves *et al.* (1997).

Table 3 - Allele frequencies at 36 loci of nine fish species from the Paraná River floodplain. Bold type numbers are loci not in Hardy-Weinberg equilibrium. The nine species were: *Astyanax altiparanae* (Apar), *Leporinus lacustris (*Lcut), *Loricariichthys platymetopon* (Lpty), *Pimelodus maculatus* (Pmct), *Rhaphiodon vulpinus* (Rvlp), *Roeboides paranensis* (Rprn), *Serrasalmus marginatus* (Smgt), *Parauchenipterus galeatus* (Pglt) and *Hoplias malabaricus* (Hmal). Numbers in boldface type indicate the *loci* which are not in Hardy-Weinberg equilibrium.

Locus	Alleles	Apar	Lcut	Lpty	Pmct	Rvlp	Rprn	Smgt	Pglt	Hmal
Aat-1	a	1.000	1.000	1.000	1.000	1.000	0.965	1.000	1.000	1.000
	b						0.035			
Aat-2	a				1.000	1.000	1.000	0.033	1.000	1.000
	b							0.967		
Acp-1	a		1.000	1.000	1.000	1.000	0.967	1.000	1.000	1.000
	b						0.033			
	c	1.000								
Acp-2	a		1.000			1.000			1.000	
Adh-1	a		1.000	1.000	1.000	1.000	0.200		1.000	**0.729**
	b						0.800			**0.271**
Adh-2	a								1.000	
Est-1	a	0.065	1.000	1.000	1.000	1.000	0.817	0.017	1.000	0.090
	b	0.903					0.150	0.766		0.455
	c	0.032					0.033	0.217		0.455
Est-2	a	0.984	0.683	1.000	1.000	1.000	1.000	1.000	1.000	
	b	0.016	0.300							
	c		0.017							
Est-3	a		1.000	1.000	1.000	1.000		1.000	0.783	
	b								0.217	
Est-4	a					0.983				
	b					0.017				
Gdh-1	a		1.000	0.167	0.033	1.000	1.000	**0.034**	1.000	1.000
	b			0.833	0.967			**0.828**		
	c							**0.138**		
Gdh-2	a		1.000			1.000			**0.200**	
	b								**0.767**	
	c								**0.033**	
G3pdh-1	a	0.919	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	b	0.081								
G3pdh-2	a	1.000	0.967	1.000	1.000				1.000	
	b		0.033							
G6pdh-1	a	**0.134**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**0.833**
	b	**0.775**								**0.167**
	c	**0.091**								
G6pdh-2	a		1.000			1.000				
Gpi-A	a	0.113	0.983	1.000	0.933	1.000	1.000	1.000	0.950	**0.750**
	b	0.339	0.017		0.067				0.050	**0.250**
	c	0.161								
	d	0.258								
	e	0.097								
	f	0.032								
Gpi-B	a	0.968	0.367	0.850	1.000	1.000	0.850	0.983	1.000	**0.208**
	b	0.032	0.617	0.117			0.133	0.017		**0.479**
	c		0.017	0.033			0.017			**0.313**
Iddh-1	a	**0.581**	1.000	1.000	1.000	1.000		1.000	1.000	
	b	**0.339**								
	c	**0.080**								
Iddh-2	a		1.000	1.000				1.000		
Idhp-1	a	1.000	1.000	1.000	1.000	1.000	1.000	0.833	1.000	1.000
	b							0.167		
Idhp-2	a	0.032	0.964	1.000	1.000	1.000	0.933	1.000	0.983	1.000
	b	0.968	0.036				0.067		0.017	
Idhp-3										1.000
Ldh-A	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-B	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
mMdh-1	a	0.984	1.000	1.000	1.000			1.000	1.000	1.000
	b	0.016								

Table 3 (cont.)

Locus	Alleles	Apar	Lcut	Lpty	Pmct	Rvlp	Rprn	Smgt	Pglt	Hmal
sMdh-A	*a*	1.000	1.000	0.100	1.000	1.000	1.000	1.000	1.000	1.000
	b			0.900						
sMdh-B	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.983	**0.604**
	b								0.017	**0.396**
Mdhp-1	*a*	**0.800**	0.767	1.000	1.000	1.000	0.933	1.000	1.000	0.938
	b	**0.200**	0.233				0.067			0.063
Mdhp-2	*a*	**0.484**	1.000	1.000	1.000	1.000	1.000		1.000	1.000
	b	**0.516**								
Per-1	*a*		1.000	1.000	1.000	1.000		1.000	1.000	1.000
Per-2	*a*		1.000	1.000	1.000	1.000			1.000	1.000
Per-3	*a*			1.000						
Pgm-1	*a*	1.000	0.717	1.000	0.967	1.000	0.467	1.000	0.067	1.000
	b		0.283		0.033		0.533		0.433	
	c								0.500	
Sod-1	*a*	1.000	0.352	1.000	1.000	1.000	1.000		0.633	**0.318**
	b		0.093						0.333	**0.0682**
	c		0.555						0.033	
Sod-2	*a*				0.983	1.000			1.000	
	b				0.017					
Number of specimens (N)		31	30	30	30	42	30	30	30	24
Average number of alleles per locus (K)		1.8	1.3	1.1	1.2	1.0	1.4	1.3	1.3	1.4
polymorphic loci frequency ($P_{0.99}$)		0.524	0.267	0.107	0.172	0	0.381	0.217	0.226	0.333
Average observed heterozygosity (Ho)		0.068	0.062	0.006	0.011	0	0.052	0.039	0.039	0.048
Average expected heterozygosity (He)		0.152	0.081	0.026	0.011	0	0.084	0.045	0.062	0.137
Standard deviation (SD)		0.225	0.171	0.077	0.028	0	0.142	0.108	0.154	0.220
He standard error (SE)		0.041	0.031	0.014	0.005	0	0.025	0.020	0.028	0.045

Discussion

The genetic variability estimated in nine fish species from the upper Paraná River floodplain using Nei's gene diversity (He) varied from zero to 0.1518 with an average of 0.066, very near to the average of 0.051 described for 195 piscine species from several world-wide localities reviewed by Ward *et al.* (1992).

It has been generally accepted that populations of long-distance migratory species have higher genetic variability than non-migratory species because of their higher gene flow. Although this is true for several *taxa* (Ward *et al.*, 1992), our data are not in agreement with this hypothesis. In our study, no polymorphic locus was found in a sample of 29 analyzed *loci* of *R. vulpinus*, a long-distance migratory species. This is surprising, since other cases of absence of genetic variability have been found in two sedentary species, *Neoplecostomus paranensis* from the upper Paraná River (Zawadzki *et al.*, 2004b) and *Hypostomus* sp. 2 from the third-order Paraná River tributary Ribeirão Maringá (Paiva *et al.*, 2005). Furthermore, *P. maculatus*, the other long-distance migratory species analyzed in our study, showed low genetic variability (He = 0.011), contrasting with the value of He = 0.132 estimated for other migratory species such as *Prochilodus lineatus* (Revaldaves *et al.*, 1997) and *Leporinus friderici* (Chiari and Sodré, 1999).

The genetic variability estimated by us for *S. marginatus* (He = 0.045) and *P. galeatus* (He = 0.062) were intermediate compared to the average of 49 freshwater fish species (He = 0,046) estimated by Ward *et al.* (1994). On the other hand, our estimated values for *A. altiparanae* (He = 0,152), *H. malabaricus* (He = 0,137), *R. paranensis* (He = 0,084) and *L. lacustris* (He = 0,081) were high compared to the average found by Ward *et al.* (1994).

In the nine species analyzed by us the average heterozygosity of species with different reproductive strategies was not statistically different when compared amongst each other, which could lead to the conclusion that reproductive strategies are not related to genetic variability. However, our results could have been biased because the group of migratory species analyzed by us had only two species, so to compensate for this we carried out an analysis of our set of nine species combined with data on 75 tropical fish species from the literature. When the data for the 84

tropical fish species were analyzed, the relationship between heterozygosity and reproductive strategies were changed and there was a significant difference between the reproductive strategies groups (Figure 2). With this analysis, the average heterozygosity values which differed most were those between the G3 parental care group with and the G2 group without parental care. The heterogeneity of the heterozygosity variances between groups may have been due to the number of species analyzed, since the minimum and maximum heterozygosity values were similar among the groups.

The number of surviving offspring is likely to be greater in species with parental care than in species without parental care (Vazzoler, 1996), and species exhibiting parental care tend to be less variable. This can be observed in *L. platymetopon*, which brood their fry inside the male's mouth and is the most abundant species of the Paraná River floodplain. In species without parental care, few offspring are likely to survive, and a greater genetic variability is important to face environmental challenges.

Nei (1987) pointed out three main factors related to heterozygosity level are the quaternary structure of proteins, the molecular weight of the protein subunit and the species population size. In addition to these factors, we found that parental care is another factor that should be taken into account to maintain gene diversity. However, since a large number of factors influence the amount of genetic variability, we believe that further studies will be necessary to elucidate the relationship between genetic variability and ecological features of fish species.

Acknowledgments

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