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Research Article

Genetic characterization of the Neotropical catfish *Pimelodus maculatus* (Pimelodidae, Siluriformes) in the Upper Uruguay River

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Abstract

Freshwater fish present unique challenges when one attempts to understand the factors that determine the structure of their populations. Habitat fragmentation is a leading cause of population decline that threatens ecosystems worldwide. In this study, we investigated the conservation status of genetic variability in the Neotropical catfish (*Pimelodus maculatus*). Specifically, we examined the structure and genetic diversity of this species in a region of the Upper Uruguay River fragmented by natural barriers and dams. There was no genetic structure among the four sites analyzed, indicating the existence of only one population group. A combination of environmental management and genetic monitoring should be used to minimize the impact of impoundment on panmitic populations of migratory fish species.

Key words: conservation genetics, dams, habitat fragmentation, microsatellite.

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Introduction

Investigations into the mechanisms involved in creating population genetic structure are an important aspect of wildlife management because genetic variation is the means whereby a given species responds and adapts to a changing environment (Oliveira *et al.*, 2009). One of the primary impacts of many human activities is habitat fragmentation (Templeton, 2001), especially for rivers, in which damming inhibits migratory behaviour and decreases environmental cues for spawning, and can lead to substantial reductions in gene flow within and between some river systems (Yamamoto *et al.*, 2004; Dudgeon *et al.*, 2006; Helfman, 2007; Barletta *et al.*, 2010; Coleman *et al.*, 2010; Hugueny *et al.*, 2011).

The hydropower potential of Brazilian rivers is large and currently includes more than 988 hydroelectric dams (ANEEL, 2012). Dams restrict the free movement of aquatic animals by preventing them from reaching upstream habitats (Benke, 1990; Dynesius and Nilsson, 1994) and by interfering with the migratory behavior of freshwater fishes (Pringle *et al.*, 2000). Such interference can lead to a reduction in population size and increase the probability of differentiation because of genetic drift (Heggenes and Røed, 2006; Dehais *et al.*, 2010).

The structuring of populations in reduced and sometimes isolated groups has an impact on the erosion of genetic variation and increased inbreeding, which are factors of paramount importance in conservation programs (Frankham *et al.*, 2002; Allendorf and Luikart, 2007). In populations with restricted gene flow, the changes in allele frequencies due to genetic drift are inversely related to population size and are compounded by the number of generations of isolation (Falconer, 1989). In river systems, fish can form a panmictic population or genetically differentiated populations with sufficient gene flow to maintain the integrity of the metapopulation (Piorski *et al.*, 2008). McGlashan and Hughes (2000) identified freshwater fish with high levels of genetic differentiation among populations from different river systems. For many freshwater fish, physical barriers (natural or man-made), reproductive behavior and ecological characteristics exert a large influence on genetic structuring (Avise and Felley, 1979; Holderegger and Wagner, 2006; Barthem *et al.*, 1991; Godinho *et al.*, 2007a; Vergara *et al.*, 2008).

The family Pimelodidae is one of the largest families of Neotropical catfish (Pinna, 1998). This family includes the yellow-mandi catfish, or mandi-pintado (*Pimelodus maculatus*), which is a small abundant catfish that is important in regional fishing (Zaniboni-Filho and Schulz, 2003).

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The Upper Uruguay River has its headwaters in the Serra Geral mountains near the southern coast of Brazil, where it is known as the Pelotas River, and continues along the border between the Brazilian states of Santa Catarina and Rio Grande do Sul. The Upper Uruguay River is formed from a series of pools and rapids that become an obstacle to fish movement during periods of drought (Eletrosul/CNEC, 1990). These rapids were flooded by the reservoir created by the Itá hydroelectric dam in 1999 (Zaniboni-Filho and Schulz, 2003). Since then, four additional hydroelectric dams have been constructed on the Upper Uruguay River, bringing the current total to five dams.

Many studies have suggested that anthropogenic disturbances that cause habitat fragmentation are responsible for genetic variation among and within populations of freshwater fish (Neraas and Spruell, 2001; Pamponet *et al.*, 2008; Esguícero and Arcifa, 2010). Such variation has been studied by a variety of techniques, including microsatellites, which have become one of the most popular markers for making inferences on population genetics because they are abundant, widely distributed in the genome and highly polymorphic (O'Connell and Wright, 1997; DeWoody and Avise, 2000; Wu and Drummond, 2011).

The aim of this study was to investigate the genetic diversity in *P. maculatus* in the Upper Uruguay River with particular reference to the genetic differentiation among populations resulting from natural barriers and isolation by hydroelectric dams.

Materials and Methods

Sampling and DNA extraction

The Upper Uruguay River is formed by rapids, with the Augusto César Gorge (1,493 km from the river mouth) located just below the confluence with the Peixe River being an important obstacle; in this stretch, the river drops 8 m in only 7 km (Eletrosul/CNEC, 1990). Several hydroelectric dams have been constructed on this river, including the Itá dam (built in 2000, with a flooded area of 141 km^2) in the middle section of the river, the Machadinho dam (built in 2002, with a flooded area of 56.7 km^2), upstream of Itá dam in the middle segment of the river, and the Barra Grande dam (built in 2005, with a flooded area of 94 km^2) located at the head of the river.

Two hundred and ten specimens of *P. maculatus* were collected from 2007 to 2009 at different locations along the Upper Uruguay River during all seasons (Figure 1). All samples were collected after construction of the dams and were obtained from four sites: BG – upstream of the Barra Grande dam (n = 51), MA – downstream of the Barra Grande dam and upstream of the Machadinho dam (n = 60), IT – downstream of the Machadinho dam and upstream of the Itá dam (n = 48) and DI – downstream of the Itá dam (n = 51) (Table 1). Genomic DNA was extracted from fin clips using the proteinase K/phenol-chloroform protocol (Sambrook *et al.*, 2001).

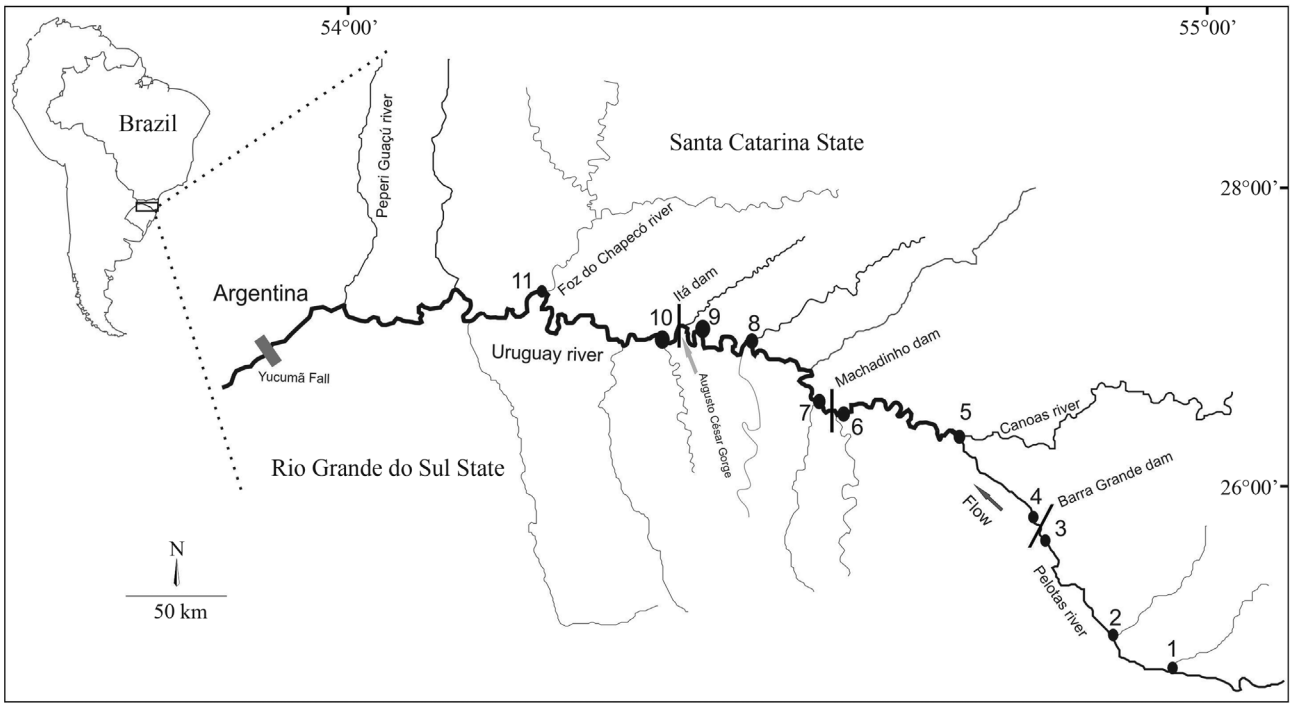

Figure 1 - Samples were obtained from four regions in Upper Uruguay River Basin: BG – upstream of the Barra Grande dam (sites 1-3; n = 52), MA – downstream of the Barra Grande dam and upstream of the Machadinho dam (sites 4-5; n = 60), IT – downstream of the Machadinho dam and upstream of the Itá dam (sites 7-9; n = 48) and DI – downstream of the Itá dam (sites 10 and 11; n = 50).

Table 1 - Sampling sites for *Pimelodus maculatus*, including sample sizes per site (N) and localization.

Study site	ID	Specific collecting location	N	Localization	
BG	1	Pelotas River	23	28°16'05.25" S	50°41'47.34" W
	2	Downstream Vacas Gordas River	6	28°02'28.54" S	50°28.71" W
	3	Immediately upstream of Barra Grande dam	22	27°57'58.54" S	51°01'59.14" W
MA	4	Downstream of Barra Grande dam	20	27°32'16" S	51°51'24" W
	5	Downstream Canoas River	23	27°35'59,7" S	51°23'28,9" W
	6	Immediately upstream of Machadinho dam	17	27°31'25" S	51°47'05" W
IT	7	Downstream Machadinho dam	21	27°31'37" S	51°47'06" W
	8	Itá reservoir	13	27°22'43" S	51°59'18" W
	9	Immediately upstream of Itá dam	14	27°17'10" S	52°20'27" W
DI	10	Immediately downstream of Itá dam	12	27°05'54" S	53°01'02" W
	11	Downstream Chapecó River	39	27°06'10" S	53°24'07" W

Microsatellite amplification and genotyping

Eight polymorphic loci (*Pmac01, Pmac02, Pmac03, Pmac06, Pmac07, Pmac08, Pmac10* and *Pmac11*) were selected from 11 previously reported microsatellites (Paiva and Kalapothakis, 2008). PCR amplifications of each microsatellite locus were done using an MJ Research PTC-100 thermal cycler with the assay conditions described by these authors for each locus. The PCR products were separated by electrophoresis in 4% polyacrylamide gels with a 10-bp DNA ladder (Gibco BRL) and stained with silver (Creste *et al.*, 2001). DNA fragments stained with silver were illuminated with a white light transilluminator and analyzed manually twice. All gels were documented with a scanner.

Data analysis

Prior to statistical analyses, Micro-Checker (Oosterhout *et al.*, 2004) was used to test for scoring errors due to null alleles, stuttering or large allele dropout. Genetic diversity was measured by the number of alleles per locus (*A*), observed heterozygosity (*H*o) and expected heterozygosity (*H*e) and assignment tests were calculated with GenAlex 6.41 (Peakall and Smouse, 2006). Allelic richness (R_A) and inbreeding coefficient (F_{IS}) were calculated with FSTAT 2.9.3 (Goudet, 2002). Departures from Hardy-Weinberg equilibrium expectations (HWE), genotypic linkage disequilibrium and the number of migrants per generation (*Nm*) were calculated with GenePop (Barton and Slatkin, 1986).

The occurrence of genetic structuration among groups was investigated with pairwise F_{ST} (Weir and Cockerham, 1984) in FSTAT. However, since the traditional F_{ST} may have undesirable attributes in some situations when estimated from highly polymorphic markers such as microsatellites (Jost, 2008; Heller and Siegismund, 2009) we also calculated a recently developed alternative measure, D_{EST} (Jost, 2008), using the software SMOGD 1.2.5 (Crawford, 2010).

Two contrasting Bayesian clustering methods were used to examine population genetic structure without allocating individuals to populations prior to analysis: STRUCTURE ver. 2.2 (Pritchard *et al.*, 2000) and SAMOVA ver. 1.0 (Dupanloup *et al.*, 2002). Based solely on genetic data, STRUCTURE identifies the number of distinct clusters, assigns individuals to clusters and identifies migrants and admixed individuals. To determine the number of populations (*K*) within the complete data set, two independent simulations for K = 1-6 with 100,000 burn-in iterations and 500,000 data iterations were run. Analysis was done using the admixture model of population structure (*i.e.*, each individual derives some fraction of its genome from each of the *K* populations) and allele frequencies correlated among populations (*i.e.*, allele frequencies in the different populations are likely to be similar due to factors such as migration or shared ancestry) (Pritchard *et al.*, 2000). The estimation of *K* based on maximum posterior likelihood ln (*K*) is an approximation and, in many empirical data sets, may continue to increase once the true number of populations is reached; therefore, an alternative method to estimate *K* based on the change in likelihood (ΔK) was also applied (Evanno *et al.*, 2005).

By using genetic data and geographic coordinates, SAMOVA (spatial analysis of molecular variance) defines groups of populations that are geographically homogenous and maximally differentiated from each other (Dupanloup *et al.*, 2002). To determine the number of groups within the complete data set, 100 simulated annealing processes for each K = 1-11 (sample sites; Table 1) were run on a sum of square size differences distance matrix.

The genetic structure and the possible existence of natural groups of populations beyond the molecular partitions between and within sites, as well as variations among individuals, were examined through analysis of molecular variance (AMOVA) performed with ARLEQUIN version 3.11 (Excoffier *et al.*, 2005), with 1,023 permutations to test for significance. The analysis of the four groups was

defined based on the fragmentation caused by the dams (Table 1).

Results

Genetic variability

All of the microsatellites analyzed were highly polymorphic and the number of alleles per locus ranged from five (*Pmac*8) to 25 (*Pmac*2) (mean = 15.81). The expected and observed heterozygosities ranged from 0.629 (*Pmac*8) to 0.948 (*Pmac*2) and from 0.385 (*Pmac*8) to 0.978 (*Pmac*7), respectively. All populations exhibited high levels of allelic richness (R_A) that ranged from 4.98 (*Pmac*8) to 24.0 (*Pmac*2) (Table 2). There was no linkage disequilibrium for the pairs of loci analyzed, indicating that the observed frequency of the combination of alleles for a pair of loci was similar to the expected frequency. The F_{IS} index suggested a heterozygote deficiency in the four populations analyzed (Table 2). Significant departures from Hardy-Weinberg equilibrium ($p < 0.0055$, adjusted using the Bonferroni correction) were detected at the population level for 11 loci. Estimates of the occurrence of null alleles, as checked with the program Micro-Checker, indicated that null alleles might be present at nine cases in which departure from Hardy-Weinberg equilibrium was identified (Table 2).

Population genetic structure

The average differentiation among the study populations was low for F_{ST} (0.0229) and moderate for D_{EST} (0.255). The genetic differentiation among populations estimated by the F_{ST} pairwise comparisons index ranged from 0.004 to 0.0352; these values were not significant, indicating the absence of genetic differentiation among the groups analyzed (Table 3). The differentiation index D_{EST} had the highest values, which ranged from 0.129 (between BG and DI) to 0.3307 (between MA and IT).

The values for the gene flow parameter *Nm*, which was measured based on the observed private alleles (Barton and Slatkin, 1986), were high, with an average of 2.86 migrants per generation. Determination of the distribution of molecular variation by AMOVA revealed that most of the total genetic variance was also found within individuals (Table 4). SAMOVA revealed that there was no genetic structure among the individuals of *P. maculatus* from the Upper Uruguay River. Analysis of the entire dataset using STRUCTURE indicated that the most likely value for $K = 2$ based on the highest mean estimated log probability (Pritchard *et al.*, 2000). However, with the Evanno correction, the highest value of ΔK for the entire data set revealed a lack of structure among the groups ($\Delta K = 1$) (Figure 2).

Discussion

Microsatellite analysis

The overall genetic variation in *P. maculatus* of the Upper Uruguay River basin was high (*H*e = 0.629 to 0.948)

Table 2 - Genetic variation in populations of *Pimelodus maculatus*.

Allele	Parameter	Collection site			
		BG	MA	IT	DI
Pmac1	N	51	60	47	50
	A	14	15	15	16
	H_O	0.653	0.766	0.680	0.659
	H_E	0.877	0.849	0.8572	0.881
	R_A	13.142	13.721	14.414	14.433
	F_{IS}	0.264	0.106	0.216	0.245
	HWE	0.0000*$^+$	0.1206	0.0154	0.0003*$^+$
Pmac2	N	48	58	37	41
	A	21	17	25	23
	H_O	0.787	0.948	0.902	0.837
	H_E	0.937	0.920	0.934	0.945
	R_A	20.138	15.844	22.211	24.000
	F_{IS}	0.171	-0.021	0.047	0.101
	HWE	0.0000*$^+$	0.7254	0.051	0.0083
Pmac3	N	51	59	46	50
	A	17	15	17	17
	H_O	0.820	0.877	0.911	0.755
	H_E	0.9178	0.891	0.916	0.933
	R_A	16.056	13.732	17.145	18.529
	F_{IS}	0.117	0.025	0.017	0.201
	HWE	0.0073	0.3877	0.432	0.0000*
Pmac6	N	51	59	42	49
	A	19	17	18	19
	H_O	0.787	0.903	0.857	0.846
	H_E	0.925	0.907	0.926	0.919
	R_A	18.161	15.215	18.215	17.538
	F_{IS}	0.160	0.014	0.085	0.092
	HWE	0.0011*$^+$	0.0982	0.0213	0.0009*
Pmac7	N	51	60	48	48
	A	18	20	20	19
	H_O	0.961	0.775	0.88	0.977
	H_E	0.914	0.896	0.924	0.928
	R_A	16.988	16.202	17.893	19.290
	F_{IS}	-0.042	0.147	0.058	-0.068
	HWE	0.8721	0.0156	0.1373	1.000
Pmac8	N	51	60	48	48
	A	6	5	6	6
	H_O	0.384	0.650	0.617	0.500
	H_E	0.6280	0.678	0.693	0.636
	R_A	5.692	4.976	5.977	5.995
	F_{IS}	0.396	0.05	0.122	0.192
	HWE	0.000*$^+$	0.2129	0.1007	0.0007*
Pmac10	N	51	60	47	50
	A	20	19	21	21
	H_O	0.862	0.758	0.729	0.829
	H_E	0.924	0.898	0.912	0.932
	R_A	16.459	15.947	18.583	19.865
	F_{IS}	0.077	0.164	0.211	0.121
	HWE	0.0971	0.0008*$^+$	0.000*$^+$	0.000*$^+$
Pmac11	N	51	60	48	50
	A	10	10	12	12
	H_O	0.826	0.666	0.760	0.702
	H_E	0.834	0.833	0.854	0.867
	R_A	9.565	9.590	11.764	11.456
	F_{IS}	0.019	0.208	0.12	0.201
	HWE	0.6216	0.007	0.0025*$^+$	0.0075

A, mean number of alleles per locus; F_{IS}, coefficient of inbreeding shown for individual loci and null allele ($^+$); H_O and H_E, observed and expected heterozygosities, respectively; HWE, Hardy-Weinberg equilibrium; R_A, allelic richness. *Significant deviations from Hardy-Weinberg equilibrium (Bonferroni correction: $0.0055 < \alpha < 0.05$).

Table 3 - Values of F_{ST} (below diagonal) and D (above diagonal) for pairwise comparisons of the four sites sampled.

	BG	MA	IT	DI
BG	-	0.2723	0.2686	0.1293
MA	0.0300	-	0.3307	0.2611
IT	0.0294	0.0352	-	0.2383
DI	0.0040	0.0231	0.0150	-

Significance level: 0.05.

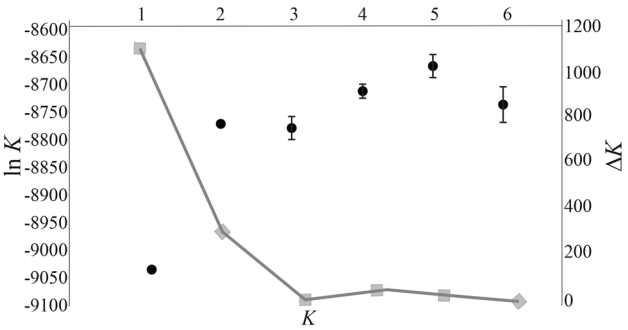

Figure 2 - STRUCTURE results for maximum likelihood ln K (•) and ΔK (–), for one to six populations (K). Maximum peaks in K were at $K = 1$.

when compared with other with other species of Siluriformes (*He* = 0.055 to 0.861 (Abreu *et al.*, 2009) and 0.310 to 0.942 (Pereira *et al.*, 2009)). The loci investigated showed a high level of polymorphism (15.81 alleles/locus). Genetic diversity was high and similar to that commonly found in wild fish populations (Martins *et al.*, 2003; Pereira *et al.*, 2009; Garcez *et al.*, 2011).

Tests for stutter miscall and allelic dropout in MICROCHECKER were not significant in any of the samples. The occurrence of null alleles seen here is a common problem with microsatellite markers and may be explained by the low efficiency of hybridization of the primers used to amplify some loci, possibly because of point mutations in one or more annealing sites of these primers (Callen *et al.*, 1993; O'Connell and Wright, 1997; Dakin and Avise, 2004) or because of failures associated with manual genotyping.

Population structure

Our analysis revealed high genetic diversity and no population structuring. Estimates of population differentiation are crucial for understanding the connectivity among populations and provide an important tool for developing conservation strategies (Balloux and Lugon-Moulin, 2002). The populations of *P. maculatus* showed extremely high levels of diversity, with low F_{ST} values (mean = 0.0229) (Table 3). The low F_{ST} values and individual-based Bayesian clustering seen here did not detect any genetic structure among the four populations. An equivalent comparison done with the estimator of actual differentiation D_{EST} indicated a higher level of differentiation among these populations (0.129 to 0.3307).

D_{EST} is considered to be particularly suitable for estimating population structure with microsatellites markers, especially in situations where within-locus genetic diversity is high, as is often the case for highly polymorphic microsatellite data (Hedrick, 2005; Jost, 2008). In general, D_{EST} estimates tend to be 3-4 times higher than their F_{ST} equivalents, with a near-perfect correlation between both estimates (Ensing *et al.*, 2011). According to Jost (2008), F_{ST} statistics tend to underestimate true levels of population differentiation compared to Jost's D and related statistics because they detect smaller levels of genetic differentiation in relation to D_{EST}. Bird *et al.* (2011) recommended that researchers apply both a xation index and an index of genetic differentiation to their datasets. Our data set provides an opportunity to compare those two statistics.

Habitat fragmentation is one of the most common outcomes of environmental changes induced by human activities (Frankham *et al.*, 2002; Fabry *et al.*, 2008; Tuomainen and Candolin, 2011). However, our analysis of the genetic structure of populations of *P. maculatus* revealed that despite the existence of seasonal natural barriers, the methods used were unable to differentiate the populations of *P. maculatus* genetically. AMOVA has been widely used for hierarchical analysis of the genetic differentiation among populations (Dupanloup *et al.*, 2002; Pereira *et al.*, 2009; Coleman *et al.*, 2010; Wollebaek *et al.*, 2011). Here, AMOVA indicated that the vast majority of variance occurred within populations and not between them. These results agree with those obtained by Population Assignment

Table 4 - Uruguay River AMOVA summaries under the standard model followed by the microsatellite model (SMM), as implemented in Arlequin 3.1.0.2 (Excoffier *et al.*, 2005). The data show the degrees of freedom (df), sum of squared deviation (SSD), variance component estimates, the percentage of total variance that each component contributed, the fixation index and the probability of obtaining by chance alone a more extreme variance component than the observed values (*p*-value). The *p*-values were derived from significance tests (1023 permutations) calculated with the distance method (F_{ST}) in Arlequin 3.11.

Source of variation	df	SSD	Variance component	Percentage variation	Fixation index	*p*-value
Among groups	3	37.657	0.0521	1.44	0.0144	0.030
Among populations within groups	4	26.724	0.0539	1.49	0.0151	0.000
Within individuals	210	620.000	2.9523	97.07	0.1777	0.000
Total	419	1.504.44	361.213			

using GenAlex which, based on individuals of known origin, found that many individuals belonging to a given population were attributed to other populations that were different from the previously established one. SAMOVA and STRUCTURE established that the populations were genetically homogeneous. The absence of genetic structure in a population has also been observed for pacu (*Piaractus mesopotamicus*) (Calcagnotto and DeSalle, 2009; Iervolino *et al.*, 2010) and the genus *Prochilodus* (Sivasundar *et al.*, 2001).

High levels of genetic diversity are often observed in migrating fish with large populations because, in large populations, high rates of migration minimize the effects of genetic drift (Santos *et al.*, 2007). *Pimelodus maculatus* is a lateral migratory species that leaves the main river to spawn in tributaries (Zaniboni-Filho and Schulz, 2003). However, there are reports of migrations occurring over long distances. The reproduction of Neotropical migratory fish occurs during the rainy season, when the fish travel to tributaries to spawn (Agostinho *et al.*, 2003), and these migrations can be regarded as a homogenizing agent among populations (Turner *et al.*, 2004). *Pimelodus maculatus* produces small floating eggs that can be passively carried to the main channel, thereby mixing larvae from different tributaries. In addition, during the rainy season, natural barriers are frequently submerged by the high water level, thus facilitating the migration of fishes.

The level of gene flow was high, with an average of 2.86 migrants per generation, indicating that some gene exchange occurred among all of the sampled populations before construction of the dams. According to Nei (1987), *Nm* values above 1 indicate that genetic flow constitutes a positive factor against genetic differentiation among populations (Spieth, 1974). These results are consistent with the values found by Pereira *et al.* (2009). When populations of *Pseudoplatystoma corruscans* in the La Plata Basin were analyzed, these authors found $Nm = 2.65$ and the occurrence of low to moderate genetic structure. In contrast, Abreu *et al.* (2009) reported $Nm = 0.841$ for this same species, with pronounced genetic differentiation. The gene flow data observed here for *P. maculatus* were supported by the F_{ST}, AMOVA and STRUCTURE analyses, with no significant differences among the populations. Another important factor related to the homogeneity of these fish populations could be the ability of some individuals to migrate further than others. The unique reproductive characteristics of these fish affect the potential for gene flow among populations because of the possibility of contact among different regions; this contact involves crossing physical barriers that in turn facilitates genetic exchange among populations.

High gene flow has previously been reported for populations of *P. maculatus* from the lower and middle Tietê River (6.480), the lower and upper Tietê River (4.596) and the middle and upper Tietê River (4.332), with important differences among the populations of these three regions (Almeida and Sodré, 1998). However, Almeida *et al.* (2003) found structuring among populations in the upper, middle and lower Paranapanema River, with gene flow estimates of 4.464, 2.173 and 1.877, respectively. According to these authors, the differentiation of *P. maculatus* populations in the Paranapanema River most probably reflected the existence of many waterfalls, some of which are more than 60 m in height. The level of gene flow may be the most important determinant of population structure because it defines the extent to which each local population is an independent evolutionary unit (Slatkin, 1995).

According to Zaniboni-Filho and Schulz (2003), before the construction of dams, the Upper Uruguay River had a bed composed of a sequence of pools and rapids that, in periods of drought, became an obstacle for fish to swim through. However, we found no significant differences between individuals from below and above the Augusto César gorge. Currently, the population of *P. maculatus* in the Upper Uruguay River basin is separated by five hydroelectric dams, with no possibility for interaction among the populations. Given the natural isolation imposed by rivers and the small size of most populations, freshwater fish species would be expected to show higher levels of subdivision and genetic differentiation among populations (Ward *et al.*, 1994; Neraas and Spruell, 2001). However, high levels of gene flow ensure short genetic distances and help to maintain homogenous populations; this results in limited evolutionary differences between regions while promoting relatively high genetic variability. Ramella *et al.* (2006) reported high genetic diversity among individuals of *P. maculatus* based on an analysis using RAPD markers.

The Upper Uruguay River is continually being fragmented by the construction of hydroelectric dams, resulting in the isolation of different populations of fish species. Yamamoto *et al.* (2004) reported that habitat fragmentation, such as caused by dams, can altering the genetic variability of many freshwater fish species in which the effects of isolation on genetic variation and population differentiation have been studied (Hansen and Mensberg, 1998, Neraas and Spruell, 2001). The risks associated with population fragmentation are directly associated with the level of gene flow among the fragmented populations (Frankham *et al.*, 2002), such that the lower the flow between populations the greater the risk of losing variability.

The ability to identify and define biological populations is crucial for taking informed decisions on conservation and management (Waples and Gaggiotti, 2006). The results described here provide important information for the conservation management of *P. maculatus* populations, for which we found that a naturally occurring obstacle and the time of isolation since construction of the dams were not sufficient to result in genetic differentiation of the populations in the Upper Uruguay River. Future studies may provide additional information on the magnitude of the im-

pact caused by hydroelectric dams on the genetic structure of this species.

In populations with a restricted gene flow, changes in the allele frequencies caused by genetic drift are inversely related to population size and can be compounded by the number of generations of isolation (Falconer, 1989). Consequently, migration corridors between populations separated by dams may be valuable for sustaining their evolutionary potential (Wollebaek *et al.*, 2011).

The effects of existing dams on the fish community need to be understood in order to make better informed decisions about future projects. *Pimelodus maculatus* is an abundant migratory species in which genetic flow maintains the samples in panmixy, including geographically distant populations. Because the genetic effects of population isolation may be similar across species the results from this study should prove useful to a variety of ecosystem managers. Studies of the genetic structure of freshwater fish should be requested by IBAMA, the Brazilian federal environmental agency, as a prerequisite before the construction of dams in Brazilian rivers.

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