GENETIC DIVERSITY OF WILD POPULATIONS AND BROODSTOCKS OF CURIMBA FOR RESTOCKING PROGRAMS IN THE TIETÊ, GRANDE, PARDO AND MOGI-GUAÇU RIVERS (BRAZIL)

Nelson Mauricio LOPERA-BARRERO[1]; Silvio Carlos Alves Dos SANTOS[2]; Maria Del Pilar RODRIGUEZ-RODRIGUEZ[3]; Darci Carlos FORNARI[4]; Camila ZANCHETA[5]; Angela Rocio POVEDA-PARRA[1]; Carlos Antonio Lopes de OLIVEIRA[5]; Ricardo Pereira RIBEIRO[5]

ABSTRACT

Prochilodus lineatus is a tropical fish that in recent years as shown a decrease in the number of wild populations. Restocking programs have been developed as a conservation method, and the genetic monitoring of populations and broodstocks is important to ensure the viability of such programs. The objective of the current study was to evaluate the genetic diversity of wild samples (WSamp) and broodstocks of *P. lineatus* in restocking programs in the Tietê, Grande, Pardo and Mogi-Guaçu Rivers (São Paulo - Brazil) using microsatellite markers. High intra-population genetic variability was observed. The number of alleles per locus ranged from three to seven, and was detected the differentiation of alleles among WSamp and the broodstocks. This differentiation was confirmed using a dendrogram analysis. Positive values of the FIS indicated the presence of endogamy in seven of 10 WSamp. The AMOVA and FST indicated small and moderate genetic differentiation among these WSamp and high genetic differentiation in comparison to the broodstocks. This differentiation was confirmed by the values of the genetic distance, genetic identity and number of migratory individuals. The results indicated that there was high intra-population genetic variability, genetic similarity in WSamp and broodstocks and differentiation between WSamp and broodstocks. We intend that this work could be used to assist restocking programs in of *P. lineatus* in the studied region and serve as a model of monitoring for other programs conducted in Brazil.

Keywords: *Prochilodus lineatus*; genetic differentiation; genetic conservation; microsatellites

DIVERSIDADE GENÉTICA DE POPULAÇÕES NATURAIS E ESTOQUES DE REPRODUTORES DE CURIMBA PARA PROGRAMAS DE REPOVOAMENTO DOS RIOS TIETÊ, GRANDE, PARDO E MOGI-GUAÇU (BRASIL)

RESUMO

Prochilodus lineatus é um peixe tropical que nos últimos anos tem apresentado uma redução no número de populações selvagens. Programas de repovoamento foram desenvolvidos como método de conservação, e o monitoramento genético das populações e reprodutores é importante para assegurar a viabilidade de tais programas. O objetivo do estudo foi avaliar a diversidade genética de amostras selvagens (WSamp) e reprodutores de *P. lineatus* do programa de repovoamento nos rios Tietê, Grande, Pardo e Mogi-Guaçu (São Paulo - Brasil), utilizando marcadores microssatélites. Alta variabilidade genética intra-populacional foi observada. O número de alelos por loco variou de três a sete e foi detectada a diferenciação dos alelos entre WSamp e os reprodutores. Essa diferenciação foi confirmada por meio da análise do dendrograma. Os valores positivos da FIS indicaram a presença de endogamia em sete das 10 WSamp. A AMOVA e o FST indicaram pequena e moderada diferenciação genética entre as WSamp e alta diferenciação genética em comparação com os reprodutores. Esta diferenciação foi confirmada pelos valores de distância e

Artigo Científico: Recebido em 25/08/2014 – Aprovado em 13/05/2015

[1] *Departamento de Zootecnia, Programa de Pós-Graduação em Ciência Animal, Universidade Estadual de Londrina (UEL). Rod. Celso Garcia Cid, PR 445, km 380 – Campus Universitário – CEP: 86051-980 – Londrina – PR – Brazil. e-mail: angelapovedaparra@hotmail.com; nmlopera@uel.br (corresponding author)*

[2] *Hidrelétrica AES Tietê – Promissão. Rod. BR 153, km 139 – Promissão – SP – Brazil. e-mail: Silvio.Santos@aes.com*

[3] *Departamento de Zootecnia, Universidade Federal dos Vales de Jequetinhonha e Mucuri (UFVJM). Rod. BR 367, Km 583, 5000 – Campus JK - CEP: 39100-000 – Diamantina – MG – Brazil. e-mail: rodrigpilar@gmail.com*

[4] *Genetic Fish Rise - Primavera do Norte – Sorriso – MT – Brazil. e-mail: darci.peixegen@gmail.com*

[5] *Departamento de Zootecnia, Programa de Pós-Graduação em Zootecnia, Universidade Estadual de Maringá (UEM). Av. Colombo, 5.790 – Jardim Universitário – CEP: 87020-900 - Maringá – PR – Brazil. e-mail: camilazancheta@hotmail.com; caloliveira@uem.br; rpribeiro@uem.br*

identidade genética e pelo número de indivíduos migrantes. Os resultados indicaram que houve alta variabilidade genética intra-populacional, similaridade genética em WSamp e reprodutores e diferenciação entre WSamp e reprodutores. Pretende-se que este trabalho possa ser usado para auxiliar os programas de repovoamento de *P. lineatus* na região estudada e sirva como modelo de monitoramento para outros programas realizados no Brasil.

Palavras-chave: *Prochilodus lineatus*; conservação genética; diferenciação genética; microssatélites

INTRODUCTION

The Tietê, Grande, Pardo and Mogi-Guaçu Rivers are important hydrological resources in São Paulo State (Brazil) because they are water sources and supply labor work and food to the established rural communities along the rivers. According to MARCENIUK *et al.* (2011), the headwaters of the Tietê River alone are habitat to at least 56 fish species (7 orders, 16 families and 40 genera). Despite this diversity, these rivers have been subject to modifications from a variety of human activities. According to BUCKUP *et al.* (2007) and AGOSTINHO *et al.* (2008), water pollution, alluvial sedimentation, deforestation, habitat destruction, overfishing, introduction of exotic species and dam building are the main threats to the wild populations of freshwater fish in Brazil.

The threat from hydroelectric power plants merits highlighting. Dams allow for local and regional energy development but cause serious and irreversible biological and ecological modifications by changing the natural hydrological regime of these rivers and predation (AGOSTINHO *et al.*, 2008; 2012). All of these modifications, therefore, have changed the habitat quality and dynamics of the biota decreasing the wild populations of migratory fishes and/or reducing their genetic variability (LAROCHE and DURAND, 2004). This result has increased the risk of extinction for at least 10 species that have been evaluated in the headwaters of the Tietê River (MARCENIUK *et al.* 2011).

Pioneering studies by GODOY (1975) and TOLEDO-FILHO (1981) had already verified the decrease of curimba, *Prochilodus lineatus* (Valenciennes 1836, Prochilodontidae, Characiformes) wild populations in Mogi-Guaçu, Pardo and Grande Rivers, mainly due to human activities that produce environmental change and reducing food availability. These migratory species are part of several South American hydrographic basins and are an important part of the Brazilian fishery economy (GARCEZ *et al.*, 2011). In the last decade, despite the population and genetic alterations that wild stocks are showing (RAMELLA *et al.* 2006; RUEDA *et al.* 2013), this species is the main fishery resource in these water sources.

One of the strategies to increase the stocks of these species has been restocking programs. Although these restocking programs have been conducted for several decades in Brazil and for more than 15 years in the Tietê, Grande, Pardo and Mogi-Guaçu Rivers, there is a gap in the research about their genetic and environmental efficiency (AGOSTINHO *et al.*, 2005; POVH *et al.*, 2010). Some of these programs were unsuccessful because of broodstock mismanagement (BORREL *et al.*, 2007; LOPERA-BARRERO, 2009) and the absence of monitoring of the wild populations. These factors can lead to endogamy depression (AGUIAR *et al.*, 2013), which reduces the performance and survivorship and increases the viral susceptibility of the released animals. Therefore, information must be collected to achieve the best conservation responses and to determine the risks and actions needed to improve the success of these programs.

Thus, there is a need for the genetic evaluation of wild populations and broodstocks used in restocking programs. For the neutral nature, microsatellite markers are one of the best techniques for genetic evaluations, and they are useful in collecting information on the diversity and genetic structure of various wild fish and other populations (AUNG *et al.*, 2010; LOPERA-BARRERO *et al.*, 2010a; POVH *et al.*, 2011; PETERSEN *et al.*, 2012; ABDUL-MUNEER, 2014).

The objective of the current study was to evaluate the genetic diversity of wild samples and broodstocks of *P. lineatus* from the restocking programs in the Tietê, Grande, Pardo and Mogi-Guaçu Rivers using microsatellite markers.

MATERIAL AND METHODS

Sample collection

Samples of the caudal fins of *P. lineatus* were collected from 10 sampling sites in the reservoir of the Hydroelectric power plants (HPP) Bariri – Tietê River (BAR), Nova Avanhandava – Tietê River (NAV), Mogi-Guaçu – Mogi-Guaçu River (MOG), Caconde – Pardo River (CAC), Euclides da Cunha – Pardo River (EUC), Limoeiro – Pardo River (LMO), Barra Bonita – Tietê River (BAB), Ibitinga – Tietê River (IBI), Promissão – Tietê River (PRO) and Água Vermelha – Grande River (AGV), and is supervised by the AES company of Brazil. Samples were also collected from four broodstocks raised in the HPP of Promissão (fish farm) that have been used in the restocking program conducted by the AES (Figure 1, Table 1). These broodstocks were the results of individuals that had been collected from the Parana River 15 years previously and new individuals that were occasionally added from fish collected in the Tietê and Pardo Rivers. The restocking program has been realized for more than 20 years (more than 100,000 *P. lineatus* fingerlings annually) (Personal communication).

Figure 1. Mapping the sampling locations. 1, HPP Bariri – Tietê River (BAR); 2, HPP Nova Avanhandava – Tietê River (NAV); 3, HPP Mogi-Guaçu – Mogi-Guaçu River (MOG); 4, HPP Caconde – Pardo River (CAC); 5, HPP Euclides da Cunha – Pardo River (EUC); 6, HPP Limoeiro – Pardo River (LMO); 7, HPP Barra Bonita – Tietê River (BAB); 8, HPP Ibitinga – Tietê River (IBI); 9, HPP Promissão – Tietê River (PRO); 10, HPP Água Vermelha – Grande River (AGV); 11, Broodstock A (BSA); 12, Broodstock B (BSB); 13, Broodstock C (BSC); and 14, Broodstock D (BSD).

DNA extraction and quantification

The DNA extraction followed the NaCl based protocol found in the study of LOPERA-BARRERO *et al.* (2008). DNA was quantified in a Shimadzu spectrophotometer (UV 1601, Columbia, USA) with an absorbance of 260 nm. The samples were diluted to 10 ng μL^{-1}. The quality of the DNA was assessed by electrophoresis in agarose gel at 1%, using a buffer of TBE 1X (500 mM^{-1} Tris-HC1, 60 mM^{-1} boric acid and 83 mM^{-1} EDTA) for 1 h at 70 V. The gel was examined under UV radiation after exposure to ethidium bromide (0.5 $\mu g\ mL^{-1}$) for 1 h. Images were then photographed using Kodak® EDAS (Kodak 1D Image Analysis 3.5, New York, USA).

Table 1. Characteristics of 10 wild samples and four broodstocks of *Prochilodus lineatus*.

WSamp and Broodstocks	Codes	Sampling size	Operating year	Latitude	Longitude
HPP Bariri	BAR	30	1965	22º09'11''S	48º45'08''W
HPP Nova Avanhandava	NAV	30	1982	21º07'05''S	50º12'02''W
HPP Mogi Guaçu	MOG	30	1999	22º22'46''S	46º54'01''W
HPP Caconde	CAC	30	1966	21º34'36''S	46º37'27''W
HPP Euclides da Cunha	EUC	30	1960	21º36'11''S	46º56'56''W
HPP Limoeiro	LMO	30	1958	21º37'30''S	47º00'34''W
HPP Barra Bonita	BAB	30	1963	22º31'10''S	48º32'00''W
HPP Ibitinga	IBI	30	1969	21º45'33''S	48º59'26''W
HPP Promissão	PRO	30	1975	21º17'52''S	49º47'20''W
HPP Água Vermelha	AGV	30	1978	19º52'04''S	50º20'43''W
Broodstock A	BSA	30	1997	21º17'52''S	49º47'20''W
Broodstock B	BSB	30	1997	21º17'52''S	49º47'20''W
Broodstock C	BSC	30	1997	21º17'52''S	49º47'20''W
Broodstock D	BSD	30	1997	21º17'52''S	49º47'20''W

Amplification and electrophoresis

The DNA was amplified to the reaction final volume of 20 µL, using a solution of 1 X buffer of Tris-KCl, 2.0 mM^{-1} of $MgCl_2$, 0.8 μM^{-1} of each primer (Forward and Reverse), 0.4 mM^{-1} of each dNTP, 1 U de Platinum Taq DNA Polymerase and 20 ng of DNA. First, the DNA was denatured at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing for 30 s with a different temperature for each primer (Table 2), extension at 72 °C for 1 min, and the final extension at 72 °C for 10 min. Four loci described by HATANAKA *et al.* (2002) – *Pl01* - and YAZBECK and KALAPOTHAKIS (2007) – *Pli30*, *Pli43*, *Pli60* - were amplified (Table 2).

The amplified samples were submitted to electrophoresis in 10% polyacrylamide gel (acrylamide: bisacrylamide – 29: 1), denatured (6 M urea), and conducted in buffer TBE 1X (90 mM^{-1} of Tris-Borate and 2 mM^{-1} of EDTA) at 320 V and 250 mA for 7 h. The microsatellite alleles were visualized using silver nitrate following the modified BASSAM *et al.* (1991) method. The gel was submitted to the fixing solution (10% ethanol and 0.5% acetic acid) for 20 min, stained (6 mM^{-1} of silver nitrate) for 10 min, visualized (0.75 M of NaOH and 0.22% of phormol-40%), and photographed using the Nikon CoolPix® (5200, New York, USA).

Data analysis

The allele size was calculated with Kodak EDAS-290, using DNA ladders (Invitrogen, USA) of 10, 50 and 100 bp. The type and size of the alleles in the populations of *P. lineatus* (50 – 300 bp) were organized in data matrices using the microsatellite loci, which were analyzed with computer software to estimate the parameters of intra and inter-population genetic diversity.

Table 2. Characterization of the four loci used in this study.

Loci	Repeat motifs	Primer sequence 5′ – 3′	Ta (°C)*
Pl01	$(AATTT)_{10}$	F: TGACTGTGAACACGGTCACGC R: ACACAGTAGAACATACCTCTG	60
Pli30	$(GTCT)_n$ Complex core. 116 bp long	F: GATGTCGGTTCTTGTACAGTGGTG R: AGCTGCTGAGGATTCTGGGTCAC	68
Pli43	$(GT)_{13}$	F: AGTCCACTCCTTAGGCGAGTGAG R: ATAGACGGGCATGTGTCACAGCT	60
Pli60	$(GA)_{13}$	F: GCTAGGACGGTTAGCGTCCACTG R: CGACACGTACATCATTACCTCGG	69

**Ta = annealing temperature.*

The number of alleles, the observed (*Ho*) and expected (*He*) heterozygosity, the test of Hardy-Weinberg equilibrium (HWE), the migratory number (Nm) and the inbreeding index (F_{IS}) of WRIGHT (1978) were calculated for every locus using the GENEPOP 4.0.6 Software (ROUSSET, 2008). In the HWE test, the deficiency or excess of heterozygotes at the loci was based on the Fisher Exact Test calculated by a Markov chain analysis (Markov chain length: 100,000; dememorizations: 10,000). The allelic frequency, distance (GD) and genetic identity (GI) were calculated using the PopGene 1.31 Software (YEH *et al.*. 1999). The allelic richness (A_R) was calculated using the FSTAT 2.9.3.2 Software (GOUDET, 2002). The presence of null alleles was verified using the Micro-Checker 2.2.3 Software (VAN OOSTERHOUT *et al.*, 2004). The values of fixation index (F_{ST}) (WEIR and COCKERHAM, 1984), the linkage disequilibrium and the Analysis of Molecular variance (AMOVA) were calculated using the Arlequim 3.1 Software (EXCOFFIER *et al.*, 2005). In these final analyzes, the wild samples (WSamp) and broodstocks were discriminated by all of the combinations allowed (91 combinations of populations). Using the discriminatory method for the FST values, we followed WRIGHT (1978), where the values from 0.00 to 0.05; 0.051 to 0.15; 0.151 to 0.25 and > 0.25 indicate small, moderate, high and highest genetic differentiation, respectively. An unweighted pair-group mean analysis (UPGMA) tree was constructed based on Nei's genetic distance (NEI, 1978) of pairwise locations using MEGA Software, version 5.0 (TAMURA *et al.*, 2011). The reduction of the effective population size was calculated with Bottleneck Software 1.2.02 (CORNUET and LUIKART, 1996) using the I.A.M. (infinite allele model) and S.M.M (step-wise mutation) mutation models, and the Wilcoxon sign-rank test. The significance of *P*-values was adjusted following Bonferroni sequential corrections for multiple simultaneous statistical tests (RICE, 1989).

RESULTS

A total of 44 alleles were detected for the four loci examined in 420 individuals from 10 wild samples (WSamp) and four broodstocks. These loci were polymorphic and the amplified microsatellite alleles were consistent and reproducible with sizes ranging from 50 bp (*Pl01*) to 300 bp (*Pli*30). The locus with the highest number of alleles was *Pli30* (14 alleles), followed by *Pli60* (11 alleles), *Pl01* (10 alleles) and *Pli43* (9 alleles) (Table 3). The average number of alleles in each locus and samples ranged from 4.25 (BSC) to 6.25 (MOG and EUC).

Alleles with a low frequency (lower than 0.1) were observed in all of the WSamp, but the highest number was found in BAR (10 alleles), EUC (10 alleles) and LMO (9 alleles). The lowest allelic frequency (0.0167) was found at the *Pl01* locus (100 bp) in BAR and IBI, and the highest frequency (0.5714) was observed at the *Pli43* locus (210 bp) in the IBI. All of the broodstocks had low frequency alleles, with the highest numbers in BSD (five alleles) and BSB (four alleles). The lowest frequency (0.0167) was observed at the *Pl01* (50 bp) and *Pli30* (130 bp) loci in BSA, and the highest frequency (0.5000) was at the *Pli43* locus (110 bp) for all broodstocks (Table 3). Neither the presence of null alleles nor linkage disequilibrium was observed.

The observed heterozygosity (*Ho*) differed from the expected heterozygosity (*He*) at loci in all of the WSamp and broodstocks ($P<0.01$). These data were characterized by the Hardy-Weinberg disequilibrium (HWD). The average of *He* and *Ho* at each locus had values from 0.683 (*Pli43*) to 0.789 (*Pl01*: 0.738; *Pli30*: 0.789; *Pli60*: 0.744) and from 0.671 (*Pli30*) to 0.845 (*Pl01*: 0.692; *Pli43*: 0.793; *Pli60*: 0.845), respectively. At *Pli30* (BSC), *Pli43* (BSA, BSB, BSC and BSD) and *Pli60* (AGV, BSA and BSC), there was 0.990 and 1.000 observed heterozygosity. According to research in *Prochilodus* wild populations (HATANAKA *et al.* 2006; CARVALHO-COSTA *et al.* 2008; RUEDA *et al.* 2011) the average *Ho* was high (> 45%) for all groups (WSamp and broodstocks) featuring a high genetic variability intra-population. However, in comparison, the lowest values were observed in CAC (0.723), EUC (0.548), LMO (0.476), BAB (0.669) and PRO (0.728). The allelic richness (A_R) was different between groups ($P<0.05$), and it was lower in the broodstocks than in the WSamp after the Bonferroni adjustment (Table 4).

Table 3. Characterization of loci investigated and frequency of alleles in *Prochilodus lineatus* Wsamp and Broodstocks.

Loci	A[1]	S[2]	Populations									
			BAR	NAV	MOG	CAC	EUC	LMO	BAB	IBI	PRO	AGV
Pl01	10	210	0.03	0.07	0.05	0.02	0.06	0.04	0.05	0.05	0.08	0.04
		198	0.18	0.11	0.07	0.04	0.19	0.15	0.02	0.13	0.03	0.09
		188	0.13	0.26	0.25	0.26	0.15	0.25	0.36	0.32	0.42	0.24
		170	0.43	0.26	0.34	0.24	0.25	0.29	0.12	0.28	0.28	0.46
		110	0.20	0.28	0.12	0.28	0.27	0.25	0.45	0.20	0.17	0.13
		100	0.02	0.02	0.16	0.17	0.08	0.02	---	0.02	0.03	0.04
			BSA	BSB	BSC	BSD						
	04	91	0.12	0.20	0.17	0.26	---	---	---	---	---	---
		80	0.25	0.45	0.45	0.26	---	---	---	---	---	---
		73	0.45	0.33	0.23	0.29	---	---	---	---	---	---
		50	0.18	0.02	0.15	0.19	---	---	---	---	---	---
			BAR	NAV	MOG	CAC	EUC	LMO	BAB	IBI	PRO	AGV
Pli30	07	300	0.03	0.02	0.05	0.03	0.09	0.06	0.25	0.39	0.15	---
		270	0.45	0.18	0.18	0.32	0.05	0.02	0.07	0.06	0.17	0.22
		240	0.02	0.33	0.08	---	0.09	0.15	0.04	0.21	0.08	0.11
		210	0.35	---	0.20	0.40	0.31	0.07	0.36	0.11	0.28	0.17
		190	0.03	0.26	0.20	0.10	0.11	0.26	0.09	0.13	0.15	0.20
		188	0.07	0.11	0.20	0.10	0.22	0.33	0.12	0.06	0.10	0.15
		170	0.05	0.09	0.08	0.05	0.11	0.11	0.07	0.04	0.07	0.15
			BSA	BSB	BSC	BSD						
	07	200	0.30	0.30	0.32	0.22	---	---	---	---	---	---
		180	0.28	0.17	0.32	0.29	---	---	---	---	---	---
		164	0.18	0.18	0.12	0.22	---	---	---	---	---	---
		160	---	0.13	0.05	0.05	---	---	---	---	---	---
		150	0.13	0.13	---	0.08	---	---	---	---	---	---
		143	0.08	0.03	0.05	0.05	---	---	---	---	---	---
		130	0.02	0.05	0.13	0.10	---	---	---	---	---	---
			BAR	NAV	MOG	CAC	EUC	LMO	BAB	IBI	PRO	AGV
Pli43	06	210	0.07	0.13	0.15	0.21	0.02	---	0.25	0.57	0.46	0.50
		192	0.28	0.35	0.22	0.11	0.27	0.10	0.37	0.07	0.24	0.15
		190	0.25	0.07	0.21	0.21	0.46	0.43	0.19	0.25	0.20	0.12
		140	0.17	0.25	0.19	0.36	0.14	0.44	0.10	0.11	0.06	0.18
		130	0.23	0.20	0.12	---	0.09	0.02	0.08	---	0.04	0.06
		120	---	---	0.10	0.10	0.02	0.02	---	---	---	---
			BSA	BSB	BSC	BSD						
	03	110	0.50	0.50	0.50	0.50	---	---	---	---	---	---
		100	0.15	0.15	0.13	0.07	---	---	---	---	---	---
		90	0.35	0.35	0.37	0.43	---	---	---	---	---	---
			BAR	NAV	MOG	CAC	EUC	LMO	BAB	IBI	PRO	AGV
Pli60	06	228	0.03	0.24	0.14	0.21	0.02	0.04	---	0.07	0.35	0.15
		204	0.21	0.09	0.21	0.11	0.09	0.50	---	0.24	0.23	0.38
		188	0.19	0.07	0.12	0.21	0.26	0.19	0.35	0.21	0.15	0.25
		156	0.29	0.39	0.23	0.36	0.41	0.27	0.39	0.17	0.27	0.22
		140	0.21	0.11	0.12	0.10	0.06	---	0.19	0.19	---	---
		134	0.07	0.09	0.16	---	0.15	---	0.06	0.12	---	---
			BSA	BSB	BSC	BSD						
	05	150	0.37	0.42	0.45	0.42	---	---	---	---	---	---
		130	0.18	0.19	0.29	0.20	---	---	---	---	---	---
		121	0.20	0.17	0.02	0.12	---	---	---	---	---	---
		110	0.13	0.19	0.25	0.22	---	---	---	---	---	---
		100	0.11	0.01	---	0.05	---	---	---	---	---	---

For the population names see Table 1; [1] Numbers of alleles; [2] Size of alleles (bp).

Table 4. Statistics of microsatellite loci of *Prochilodus lineatus* Wsamp and broodstocks**.**

Locus	BAR	NAV	MOG	CAC	EUC	LMO	BAB	IBI	PRO	AGV	BSA	BSB	BSC	BSD
Pl01														
Ho[1]	0.70	0.81	0.67	0.59	0.84	0.61	0.57	0.70	0.50	0.73	0.83	0.83	0.66	0.65
He[2]	0.73	0.78	0.78	0.78	0.81	0.78	0.66	0.77	0.73	0.72	0.69	0.65	0.70	0.75
F_{IS}[3]	0.04	-0.03	0.14	0.24	-0.04	0.21	0.14	0.09	0.32	-0.02	-0.19	-0.27	0.05	0.13
HW[4]	**	NS	**	**	NS	**	**	**	**	NS	NS	NS	**	**
N_A[5]	6.0	6.0	6.0	6.0	6.0	6.0	5.0	6.0	6.0	6.0	4.0	4.0	4.0	4.0
A_R[6]	5.4	5.6	5.9	5.4	5.9	5.5	4.5	5.4	5.8	5.8	3.9	3.5	4.0	4.0
Pli30														
Ho	0.72	0.33	0.70	0.76	0.51	0.44	0.60	0.69	0.76	0.70	0.73	0.70	0.99	0.76
He	0.68	0.78	0.84	0.72	0.82	0.79	0.78	0.78	0.83	0.84	0.78	0.82	0.77	0.81
F_{IS}	-0.06	0.58	0.17	-0.05	0.37	0.44	0.23	0.11	0.08	0.16	0.06	0.15	-0.29	0.06
HW	NS	**	**	NS	**	**	**	**	**	**	**	**	NS	**
N_A	7.0	6.0	7.0	6.0	7.0	7.0	7.0	7.0	7.0	6.0	6.0	7.0	6.0	7.0
A_R	6.2	5.6	6.9	5.7	6.9	6.5	6.8	6.8	6.9	5.9	5.5	6.7	5.8	6.8
Pli43														
Ho	0.73	0.93	0.96	0.72	0.39	0.42	0.79	0.75	0.68	0.76	0.99	0.99	0.99	1.00
He	0.78	0.76	0.83	0.74	0.69	0.62	0.76	0.60	0.70	0.70	0.61	0.61	0.60	0.56
F_{IS}	0.06	-0.22	-0.15	0.02	0.44	0.33	-0.04	-0.24	0.02	-0.09	-0.64	-0.64	-0.66	-0.78
HW	**	NS	NS	**	**	**	NS	NS	**	NS	NS	NS	NS	NS
N_A	5.0	5.0	6.0	5.0	6.0	5.0	5.0	4.0	5.0	5.0	3.0	3.0	3.0	3.0
A_R	4.9	4.9	5.9	4.9	5.2	4.3	4.9	3.9	4.8	5.0	3.0	3.0	2.9	2.9
Pli60														
Ho	0.89	0.96	0.89	0.80	0.43	0.42	0.70	0.93	0.96	0.99	0.99	0.92	0.99	0.96
He	0.80	0.77	0.83	0.76	0.74	0.65	0.69	0.82	0.74	0.73	0.77	0.73	0.66	0.73
F_{IS}	-0.12	-0.25	-0.06	-0.05	0.42	0.35	-0.02	-0.12	-0.31	-0.37	-0.30	-0.27	-0.51	-0.32
HW	NS	NS	NS	NS	**	**	NS	NS	NS	NS	NS	NS	NS	NS
N_A	6.0	6.0	6.0	5.0	6.0	4.0	4.0	6.0	4.0	4.0	5.0	5.0	4.0	5.0
A_R	5.8	5.9	5.9	4.9	5.7	3.8	3.9	5.9	4.0	4.0	4.9	4.6	3.6	4.9
Mean														
Ho	0.76	0.76	0.80	0.72	0.54	0.47	0.66	0.76	0.72	0.80	0.89	0.86	0.91	0.84
He	0.75	0.77	0.82	0.75	0.76	0.71	0.72	0.74	0.75	0.74	0.71	0.70	0.68	0.71
F_{IS}	-0.01	0.01	0.02	0.04	0.29	0.33	0.07	-0.04	0.03	-0.08	-0.26	-0.25	-0.35	-0.22
HW	NS	**	**	**	**	**	**	NS	**	NS	NS	NS	NS	NS
N_A	6.0	5.7	6.2	5.5	6.2	5.5	5.2	5.7	5.5	5.2	4.5	4.7	4.2	4.7
A_R	5.6	5.5	6.2	5.3	5.9	5.0	5.0	5.5	5.4	5.2	4.3	4.4	4.1	4.6

*For the population names see Table 1; [1] observed heterozygosity; [2] expected heterozygosity; [3] inbreeding index; [4] equilibrium test of Hardy-Weinberg; [5] number of alleles per locus; [6] Allelic richness. NS = Not significant; ** P<0.01 after Bonferroni adjustment (nominal a = 0.05).*

The average negative values of the F_{IS} index from BAR, IBI, AVG, BSA, BSB, BSC and BSD (from -0.019 to -0.354) showed the absence of endogamy. Conversely, the positive values from NAV, MOG, CAC, EUC, LMO, BAB and PRO (from 0.014 and 0.336) showed the presence of heterozygote deficiencies, and these data were characterized by a HWD in all cases (Table 4). The results, obtained using the I.A.M. and S.M.M mutation models, indicate the presence of a drifting, which is evidence of a recent bottleneck in the NAV, MOG, CAC, EUC, LMO, BAB and PRO.

Based on the AMOVA, the highest genetic variability (P<0.05) was observed within the individuals denoting a small and highest genetic differentiation among WSamp, broodstocks and WSamp vs. broodstocks (Table 5). The results from the distance and genetic identity analysis indicated the genetic similarity of the WSamp. The values ranged from 0.112 (MOG x EUC) to 0.603 (NAV x EUC) and from 0.547 (NAV x EUC) to 0.892 (MOG x EUC) for distance and identity, respectively (Table 6).

The F_{ST} from all combinations of WSamp (45 combinations) ranged from 0.011 (MOG x EUC) to 0.151 (NAV x EUC), which is evidence of small to moderate genetic differentiation. Similarly, the results from the Nm analysis indicated high gene flow among the populations, with values from 2.97 and 12.91 individuals per generation (Table 6).

Table 5. AMOVA (analysis of molecular variance), F_{ST} and Wright classification for the *Prochilodus lineatus* WSamp and broodstocks.

	VC[1]	**PV[2]**	***F*$_{ST}$[3]**	**Wright[4]**
WSamp				
AS[5]	0.10424	6.43	0.0643	Moderate
AIS[6]	0.09489	5.85		
WI[7]	1.42236	87.72		
Total	1.62149	100		
Broodstocks				
AS	0.01270	1.20	0.0120	Small
AIS	0.22809	21.61		
WI	1.27083	77.19		
Total	1.51162	100		
WSamp vs. Broodstocks				
AS	0.29492	16.58	0.1658	High
AIS	0.04091	2.30		
WI	1.52484	85.72		
Total	1.86067	100		

[1] Variance components; [2] Percentage variation; [3] Fixation index; [4] Wright classification; [5] Among samples; [6] Among individuals within samples; [7] Within individuals.

Table 6. Statistics used in the combination of *Prochilodus lineatus* Wsamp and broodstocks.

Groups	**BARxNAV**	**BARxMOG**	**BARxCAC**	**BARxEUC**	**BARxLMO**	**BARxBAB**	**BARxIBI**	**BARxPRO**
F_{ST}[1]	0.019	0.026	0.040	0.020	0.098	0.115	0.071	0.066
Wright[2]	Small	Small	Small	Small	Moderate	Moderate	Moderate	Moderate
GI/GD[3]	0.752/0.285	0.875/0.134	0.860/0.151	0.841/0.173	0.679/0.388	0.754/0.283	0.632/0.459	0.731/0.314
Nm[4]	5.55	11.25	8.80	8.03	3.76	4.97	3.62	4.87
Groups	**BARxAGV**	**BARxBSA**	**BARxBSB**	**BARxBSC**	**BARxBSD**	**NAVxMOG**	**NAVxCAC**	**NAVxEUC**
F_{ST}	0.052	0.285	0.285	0.283	0.267	0.030	0.117	0.151
Wright	Moderate	Highest	Highest	Highest	Highest	Small	Moderate	Moderate
GI/GD	0.776/0.254	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.871/0.138	0.757/0.279	0.547/0.603
Nm	5.64	1.29	1.25	1.20	1.30	11.23	5.73	2.97
Groups	**NAVxLMO**	**NAVxBAB**	**NAVxIBI**	**NAVxPRO**	**NAVxAGV**	**NAVxBSA**	**NAVxBSB**	**NAVxBSC**
F_{ST}	0.081	0.073	0.097	0.052	0.093	0.163	0.264	0.266
Wright	Moderate	Moderate	Moderate	Moderate	Moderate	High	Highest	Highest
GI/GD	0.717/0.332	0.741/0.300	0.674/0.395	0.779/0.250	0.763/0.271	0.000/0.000	0.000/0.000	0.000/0.000
Nm	4.43	4.97	4.28	6.13	5.66	1.58	1.34	1.28
Groups	**NAVxBSD**	**MOGxCAC**	**MOGxEUC**	**MOGxLMO**	**MOGxBAB**	**MOGxIBI**	**MOGxPRO**	**MOGxAGV**
F_{ST}	0.263	0.046	0.011	0.018	0.033	0.042	0.019	0.013
Wright	Highest	Small	Small	Small	Small	Small	Small	Small
GI/GD	0.000/0.000	0.865/0.145	0.892/0.112	0.830/0.186	0.779/0.250	0.778/0.251	0.875/0.133	0.887/0.114
Nm	1.38	10.09	12.91	7.14	6.13	6.59	10.41	11.21
Groups	**MOGxBSA**	**MOGxBSB**	**MOGxBSC**	**MOGxBSD**	**CACxEUC**	**CACxLMO**	**CACxBAB**	**CACxIBI**
F_{ST}	0.241	0.241	0.244	0.327	0.096	0.103	0.053	0.146
Wright	High	High	High	Highest	Moderate	Moderate	Moderate	Moderate
GI/GD	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.781/0.248	0.702/0.354	0.751/0.286	0.751/0.286
Nm	1.57	1.52	1.46	1.10	6.14	4.08	4.98	3.02
Groups	**CACxPRO**	**CACxAGV**	**CACxBSA**	**CACxBSB**	**CACxBSC**	**CACxBSD**	**EUCxLMO**	**EUCxBAB**
F_{ST}	0.020	0.046	0.277	0.277	0.279	0.288	0.051	0.034
Wright	Small	Small	Highest	Highest	Highest	Highest	Moderate	Small
GI/GD	0.726/0.320	0.703/0.352	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.813/0.207	0.862/0.149
Nm	4.86	4.45	1.31	1.28	1.22	1.32	6.26	8.38
Groups	**EUCxIBI**	**EUCxPRO**	**EUCxAGV**	**EUCxBSA**	**EUCxBSB**	**EUCxBSC**	**EUCxBSD**	**LMOxBAB**
F_{ST}	0.088	0.072	0.084	0.271	0.260	0.277	0.276	0.124
Wright	Moderate	Moderate	Moderate	Highest	Highest	Highest	Highest	Moderate
GI/GD	0.670/0.400	0.721/0.327	0.682/0.382	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.610/0.495
Nm	4.16	4.88	4.28	1.35	1.31	1.25	1.35	5.75

Table 6. (cont.) Statistics used in the combination of *Prochilodus lineatus* Wsamp and broodstocks.

Groups	**LMOxIBI**	**LMOxPRO**	**LMOxAGV**	**LMOxBSA**	**LMOxBSB**	**LMOxBSC**	**LMOxBSD**	**BABxIBI**
F_{ST}	0.106	0.057	0.072	0.205	0.183	0.214	0.187	0.042
Wright	Moderate	Moderate	Moderate	High	High	High	High	Small
GI/GD	0.655/0.422	0.663/0.411	0.760/0.274	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.766/0.267
Nm	3.51	3.61	4.88	1.18	1.15	1.37	1.19	5.17
Groups	**BABxPRO**	**BABxAGV**	**BABxBSA**	**BABxBSB**	**BABxBSC**	**BABxBSD**	**IBIxPRO**	**IBIxAGV**
F_{ST}	0.057	0.097	0.263	0.264	0.265	0.238	0.073	0.040
Wright	Moderate	Moderate	Highest	Highest	Highest	High	Moderate	Small
GI/GD	0.804/0.218	0.678/0.388	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.868/0.141	0.848/0.165
Nm	6.06	3.84	1.22	1.19	1.14	1.23	9.02	7.83
Groups	**IBIxBSA**	**IBIxBSB**	**IBIxBSC**	**IBIxBSD**	**PROxAGV**	**PROxBSA**	**PROxBSB**	**PROxBSC**
F_{ST}	0.230	0.327	0.303	0.290	0.023	0.215	0.167	0.244
Wright	Highest	Highest	Highest	Highest	Small	High	High	High
GI/GD	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.887/0.114	0.000/0.000	0.000/0.000	0.000/0.000
Nm	1.28	1.25	1.20	1.29	11.03	1.30	1.27	1.21
Groups	**PROxBSD**	**AGVxBSA**	**AGVxBSB**	**AGVxBSC**	**AGVxBSD**	**BSAxBSB**	**BSAxBSC**	**BSAxBSD**
F_{ST}	0.264	0.218	0.218	0.249	0.221	0.004	0.016	0.018
Wright	Highest	High	High	High	High	Small	Small	Small
GI/GD	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.000/0.000	0.962/0.039	0.935/0.067	0.977/0.023
Nm	1.31	1.28	1.25	1.20	1.29	27.94	20.07	13.27
Groups	**BSBxBSC**	**BSBxBSD**	**BSCxBSD**					
F_{ST}	0.009	0.015	0.006					
Wright	Small	Small	Small					
GI/GD	0.968/0.032	0.968/0.033	0.975/0.025					
Nm	21.68	22.58	25.14					

For the population names see Table 1; [1] fixation index; [2] genetic differentiation of WRIGHT (1978); [3] genetic identity and genetic distance; [4] migratory number.

When assessing the broodstock values, the results of the distance (from 0.023 to 0.067) and genetic identity (from 0.935 to 0.977) analyzes indicated high genetic similarity. A similar pattern was observed in the results from the AMOVA for the broodstocks, with small differentiation between broodstocks (F_{ST} = 0.0120) (Table 5). The F_{ST} from all combinations of broodstocks (six combinations) had values ranging from 0.004 (BSA x BSB) to 0.018 (BSA x BSD), which indicates a small genetic differentiation. Similarly, the results from the Nm analysis indicated a high gene flow among the groups, with values ranging from 13.27 to 27.94 individuals per generation (Table 6).

Finally, when comparing WSamp with broodstocks, the results of the distance and genetic identity indicated high genetic differentiation, which confirms the results from the allelic frequencies. Based on the AMOVA, the highest genetic variation (p<0.05) was observed within groups where F_{ST} value of 0.1658 indicated high genetic differentiation (Table 5). Conversely, for all combinations (40 combinations), the FST values ranged from 0.163 (NAV x BSA) to 0.327 (MOG x BSD), suggesting a high and highest genetic differentiation. Similarly, the results from the Nm analysis indicate a low gene flow among the WSamp and broodstocks, with values ranging from 1.10 to 1.58 individuals per generation. The dendrogram from the UPGMA confirmed these results, indicated two specific clusters: one for the WSamp and the other for the broodstocks (Figure 2).

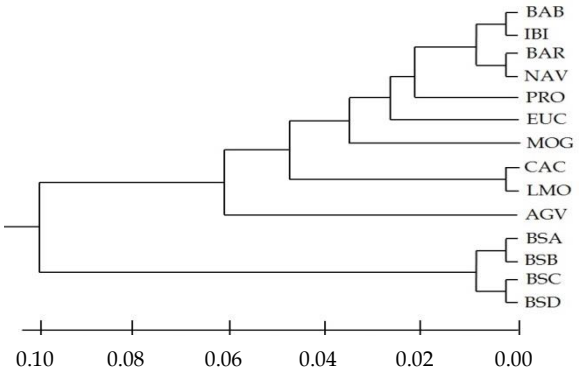

Figure 2. The unweighted pair-group mean analyzes (UPGMA) tree from ten WSamp and four broodstocks of *Prochilodus lineatus*. For the population names see Table 1.

DISCUSSION

Genetic variability

The loci were highly polymorphic, and they amplified the microsatellites alleles consistently. According to FOPP-BAYAT *et al.* (2010), a high degree of polymorphism indicates informative loci that can be used in population studies. The 44 alleles observed were distributed among several loci (*Pli30*: 14 alleles, *Pli60*: 11 alleles, *Pl01*: 10 alleles and *Pli43*: 9 alleles). The number of alleles from *Pl01* was similar to the results reported by HATANAKA *et al.* (2006). Otherwise, *Pli30*, *Pli43* and *Pli60* contained fewer alleles than reported by YAZBECK and KALAPOTHAKIS (2007) (Table 3). This difference may be a result of different geographic location of each sample (i.e., different rivers) because each sampling site has a pool of adapted alleles that are specific to each region or location. This condition has been observed in several researches (BRADIC *et al.*, 2012; OROZCO BERDUGO and NARVÁEZ BARANDICA, 2014). Other factors, such as the difference in length of repeat microsatellite (microsatellite with longer repeats showed higher polymorphism) and the methods used to detecting polymorphism (automatic DNA sequencer vs. polyacrylamide gel) may cause this difference (GOLDSTEIN and SCHLOTTERER, 1999). Allele number is positively related to sample size (GOLDSTEIN and SCHLOTTERER., 1999; MU *et al.*, 2011), however, according to HALE *et al.* (2012), 25 to 30 per individuals population is enough to accurately estimate allele frequencies.

The difference (P<0.01) between *Ho* and *He* in most of the wild samples (WSamp) and broodstocks was characterized by deficits of heterozygotes, according to the Hardy-Weinberg equilibrium (Table 4). These deficits are typical in fish and may arise from Wahlund effect, endogamy or bottleneck effect (CAMP, 1998; GARCÍA DE LEÓN *et al.*, 1997; O'CONNELL and WRIGHT, 1997). Despite the presence of alleles with a low frequency (BAR, LMO, EUC, BSB and BSD) and the deficit of heterozygotes, the genetic variability was high in all groups (WSamp and broodstocks). The average *Ho* values were high for the WSamp and broodstocks, indicating high intra-population genetic variability. In similar work, HATANAKA *et al.* (2006) and CARVALHO-COSTA *et al.* (2008) used microsatellite markers to assess populations of *Prochilodus argenteus* and *Prochilodus costatus* collected in the Três Marias dam (São Francisco River, Brazil) the average *Ho* and *He* value were high (>0.450) for all groups (WSamp and broodstocks), which is a characteristic of high intra-population genetic variability, despite the heterozygosity deficit detected in four of the six loci.

Comparatively, the lower *Ho* values were detected in three WSamp from the Pardo (CAC, EUC and LMO) and two WSamp from the Tietê River (BAB and PRO), which was in agreement with the presence of low-frequency alleles (EUC and LMO), with deviation in HWE (heterozygous deficiency) and with the positive values of F_{IS}. All samples taken from the old HPPs (in operation since 1958 and 1975) had lower genetic variability (CAC, EUC, LMO and BAB). The same results could be explained by the reduction in the number of individuals in the WSamp (the bottleneck effect). This reduction was potentially influenced by: 1) the impacts of dams and HPP on wild populations of migratory fish that prevented the movement of individuals to appropriate locations during the reproduction cycle and the fragmentation of the aquatic habitat by the power plants (DEHAIS *et al.*, 2010). Studies on the migratory performance of *Prochilodus* indicate that this genus of fish can migrate distances of 400 to 600 km (AGOSTINHO *et al.*, 1993; ESPINACH-ROS and DELFINO, 1993). GODINHO and KYNARD (2006) analyzed the migration of *P. argenteus* downstream in the HPP Três Marias in the São Francisco River, Minas Gerais State, Brazil, and detected a maximum migratory distance of 127 km. In addition, a study of *P. lineatus*. PESOA and SCHULZ (2010) found an individual displacement of 102.7 km in the Sinos River, Rio Grande do Sul State, Brazil. This fragmentation and the geographic isolation affect the gene flow, thus causing smaller populations and leading to the erosion and loss of genetic variability. These effects are also exacerbated by the number of generations that have become isolated (FALCONER, 1989; RIBOLLI *et al.*, 2012). 2) Overfishing may be another factor that has a significant influence on the number of individuals in these populations, exacerbating the genetic bottleneck and reducing

the genetic variability. 3) The Tietê, Grande, Pardo and Mogi-Guaçu Rivers have faced several environmental changes, including pollution, riverbed siltation, deforestation along river banks, habitat destruction and introduction of exotic species. These changes in habitat quality have also affected the food chain, which can cause mortality and reduce the effective size of the population and consequently the genetic variability. 4) All of these factors that impact the population can also interact to cause a greater impact than they would alone.

The allelic richness, which characterizes the number of segregating alleles in each population (CABALLERO *et al.*, 2010) was lower in the broodstocks than in the WSamp. This result indicates the presence of historical fluctuations in broodstock size, which was likely influenced by the founder effect. This result was not unexpected because the genetic background of broodstocks is characterized by lower genetic variability than wild populations (FOPP-BAYAT, 2010) possibly caused by founder effect (ALLENDORF and PHELPS, 1980; LACY, 1987) and inappropriate reproductive management (POVH *et al.*, 2011). Thus, reproductive management is of fundamental importance to preserve the genetic diversity of juveniles used in restocking programs (LOPERA-BARRERO, 2009).

The WSamp of BAR, IBI and AVG and the broodstocks - BSA, BSB, BSC and BSD, showed the absence of endogamy based on the F_{IS} value. In the broodstocks, this result confirms the role of the founder effect in causing the lower allelic richness. Otherwise, we found the presence of endogamy in the NAV, MOG, CAC, EUC, LMO, BAB and PRO. The deficit of heterozygotes at one or more loci and the losses alleles indicate null alleles (AUNG *et al.*, 2010), Wahlund effect, or both (HATANAKA *et al.*, 2006) in addition to the presence of the Bottleneck effect (GONZÁLEZ-WANGUEMERT *et al.*, 2012). The analysis by the Micro-Checker 2.2.3 did not find the presence of null alleles, which indicates that the null alleles had no influence. The Wahlund effect, defined as deviations from HWE than may arise from inadvertent grouping of multiple populations into one, or from analyzing a large number of related individuals (HARTL and CLARK, 2007; O´LEARY *et al.*, 2013), did not influence the values found because the samples were taken from geographically different locations along river fragments that had been caused by the presence of HPP. However, we found that these populations are in drifting, which shows evidence of a recent bottleneck, i.e., a significant reduction in the size of the population in a short time. Usually, a bottleneck shows an excess of homozygotes (NA-NAKORN and MOEIKUM, 2009), causing allele losses, increases in the genetic drift and reductions in genetic variability. Thus, the deficit of heterozygotes from NAV, MOG, CAC, EUC, LMO, BAB and PRO and the lower average of *Ho* from CAC, EUC, LMO, BAB and PRO was the consequence of bottlenecks.

Genetic structure

The analysis of the presence and distribution of alleles indicates genetic similarities among WSamp from the Tietê, Grande, Pardo and Mogi-Guaçu Rivers. Similarly, the values of distance and genetic identity highlight the genetic similarities among these populations, as confirmed by the AMOVA (F_{ST} = 0.0643) and by the values of F_{ST} and Nm for all the combined populations (Table 6). The dendrogram highlights one cluster for the WSamp and another for broodstocks. The cluster of the WSamp also highlighted genetic relationships among the different rivers. The WSamp BAB, IBI, NAV and PRO (Tietê River) formed a small group, followed by EUC and MOG (Pardo and Mogi-Guaçu Rivers, respectively). Another group was formed by CAC and MOG (Pardo River) and finally AGV (Grande River). This explains the small and moderate genetic differentiation found through the F_{ST}.

There are no previous studies on microsatellites of *P. lineatus* in this region to provide a comparison. There are only two studies presented by ALMEIDA *et al.* (2003) and GARCEZ *et al.* (2011) used RAPD and PCR-RFLP markers, respectively. In the study of ALMEIDA, who analyzed populations of *Pimelodus maculatus* in the upper, middle and low Tietê River was observed genetic similarity of the populations (using genetic diversity, gene flow and genetic identity) as a result of the movement of fishes through sluiceways built to allow the movement of boats through the BAB, BAR, IBI, PRO and

NAV HPP (hydroway Tietê-Paraná rivers). Similarly, when analyzing wild populations of *P. lineatus* of HPP located in Mogi-Guaçu and Grande rivers, GARCEZ found no significant differentiation between populations (using F_{ST}, Mantel test, AMOVA, nucleotide and haplotype diversities).

BARROCA *et al.* (2012) analyzed wild populations of *P. costatus* downstream and upstream of the Gafanhoto and Cajuru dams in the Pará River – Minas Gerais, Brazil (built in 1940) and found genetic differentiation when comparing these populations. The explanation of this differentiation could be the lack of migration because of the dam, restocking since 1983, the length of the river, or all these factors. These results obtained in the present study could be explained by three hypotheses: 1) the continuous movement of fishes between the rivers could explain the genetic similarity, since that the Mogi-Guaçu River is upstream of the Pardo, which itself is upstream of the Grande River. The Grande River flows into the Paraná River, which also receives water from the Tietê River (which is some kilometers away). 2) Subpopulations may have been formed, clustering new groups with similar genetic pools in each reservoir after building the HPP in the rivers studied (Pardo = three HPP - Limoneiro, Euclides da Cunha and Caconde; Grande = eight HPP - Peixoto, Estreito, Jaguara, Igarapava, Volta Grande, Porto Colômbia, Marimbondo and Agua Vermelha; Mogi-Guaçu: HPP Mogi-Guaçu; Tietê = five HPP – Nova Avanhandava, Bariri, Barra Bonita, Ibitinga and Promissão), which impeded the natural gene flow. Currently, there is link only between the HPP of the Tietê (BAB, BAR, IBI, PRO and NAV) throughout artificial sluiceways. 3) The continuous restocking of these populations for more than 15 years in the sampling areas may have homogenized the genetic pool of these wild populations.

On the first hypothesis, the pioneering study by TOLEDO-FILHO *et al.* (1987) revealed that the range of *Prochilodus scrofa* population in the Grande river basin corresponds to 1072 km of the river extension, being reported migratory distances 400-600 km for *Prochilodus* genus in other basins (AGOSTINHO *et al.*, 1993; ESPINACH-ROS and DELFINO, 1993). However, other researches has shown that *P. lineatus* has a mixed performance migratory (resident and migratory fish), with a small migration of 102-127 km (GODINHO and KYNARD, 2006; PESOA and SCHULZ, 2010). Moreover, TOLEDO-FILHO *et al.* (1987) in the Grande river basin observed that four dams were barriers to migration (Socorro, Salto de Pinhal, HPP Mascarenhas de Moraes and HPP Limoeiro) and two dams (Itaipava and Emas Novas) equipped with passage ladders, allowed the movement upstream. Thus, despite the connection among the rivers, the migratory performance of *P. lineatus* populations is being affected. Therefore, the genetic similarity among the Tietê, Grande, Pardo and Mogi-Guaçu rivers cannot be completely explained by this hypothesis.

For the second hypothesis, it is important to highlight that several studies analyzing the genetic impact of dams and HPP on wild populations of fish strongly demonstrate the creation of genetic structuration, which is reinforced by the generations under isolation. For example, YAMAMOTO *et al.* (2004) evaluated the genetic differentiation of 11 wild populations of *Salvelinus leucomaenis* in Japanese Rivers that were affected by dams and observed the genetic differentiation among all population combinations and the reduction in genetic variability in proportion to the time under isolation. Similarly, ESGUÍCERO and ARCIFA (2010) analyzed the morphological characteristics of *Salminus hilarii* upstream and downstream of the Gavião Peixoto dam on the Jacaré-Guaçu River, Brazil (built in 1913), and reported fragmentation and interpopulational structuration. BARROCA *et al.* (2012) analyzed wild populations of *P. costatus* downstream, between and upstream of the Gafanhoto and Cajuru dams in the Pará River (built in 1940) and detected genetic differentiation, suggesting a small amount of or no gene flow between the dams. In contrast, RIBOLLI *et al.* (2012) analyzed the impact of three dams built in 2000, 2002 and 2005 on the genetic variability of 11 wild populations of *P. maculatus* in the Alto Uruguay River and found low differentiation and genetic structure. Considering the results from these studies and that BAR, NAV, CAC, EUC, LMO, BAB, IBI, PRO and AGV have been operating for more than 30 years, the formation

of subpopulations and genetic population structuration are to be expected. Therefore, the genetic similarity among the Wsamp of the current rivers cannot be sustained using the second hypothesis, although historical similarities may persist over time, influenced by the size of subpopulations, the number of generations after the separation and the breeding with individuals from other subpopulations (natural movement or restocking). Thus, the influence of sluiceways could be the explanation for the genetic similarity among the Wsamp of the Tietê River (BAR, NAV, BAB, IBI and PRO). Similarly, the latest HPP (MOG = established 14 years ago) could be the only one without such structuration.

Finally, studies have noted that a well-run restocking program can effectively increase the number of fish in wild populations (BLANCO GONZALEZ *et al.*, 2009; LOPERA-BARRERO *et al.*, 2010a; BARROCA *et al.*, 2012). Additionally, reports from ARAKI and SCHMID (2010), MARIE *et al.* (2010) and SATAKE and ARAKI (2012) have shown that the genetic homogeneity of wild populations is possible after generations of restocking. Despite these results, the lack of genetic similarity among the WSamp and the broodstocks evaluated in the current study showed that this hypothesis was not sustained by itself. It is likely that the broodstock sampling was not representative of the sampled wild populations, because the ancestries were collected from the Paraná River (not studied in the current work), with later addition of individuals from Tietê and Pardo Rivers. Thus, it is believed that a combination of all these factors (historical movement of *P. lineatus* in the investigated region, the presence of sluiceways and restocking) is the best explanation for the current results and the genetic similarity of the Tietê, Grande, Pardo and Mogi-Guaçu rivers.

In the four current broodstocks, we also found genetic similarities after analyzing the presence and distribution of alleles, AMOVA, genetic distance and identity, F_{ST} and Nm values for all of the population combinations, which were considered to be only one genetic cluster. This result could have been expected because the broodstocks were formed using the same ancestries collected from the Paraná River and had individuals added from the same populations from the Tietê and Pardo Rivers. Other factors, such as breeding systems (i.e., small groups of broodstocks, sexual proportion of reproductive dominance, endogamy, high individual variation during reproduction) and size fluctuations across time can result in changes to and losses of allelic diversity (BORREL *et al.*, 2007; FOPP-BAYAT *et al.*, 2010; LOPERA-BARRERO *et al.*, 2010b; POVH *et al.*, 2010), which could be the case in the current broodstocks.

Genetic conservation

Information about the variability and genetic structure of wild populations is essential to reduce the genetic decline, improve conservation programs such as restocking, and guarantee the survivorship of ichthyologic resources. According to GARDNER *et al.* (2010), insights into restocking programs along with study of the ecology and monitoring of released stocks is important to verify the potential of biological and genetic impacts of broodstocks on wild populations.

The correct use of restocking programs requires several conditions. First, establish broodstocks with genetic variability must be established to increase the initial genetic variability through the choice of individuals that better represent the genetic pool of the wild populations, offer an important basis to formulate reproductive strategies (BORREL *et al.*, 2007; LOPERA-BARRERO, 2009), and minimise adaptation to captivity (WILLIAMS and HOFFMAN, 2009). Mating related individuals can reduce the effective size of the broodstocks, which favours endogamic depression (GONZÁLEZ-WANGUEMERT *et al.*, 2012).

The genetic diversity in the wild populations is the raw material for maintaining species diversity and evolutionary capacity, which allows adaptation to environmental changes (SANFORD and KELLY, 2011). Thus, the wild populations must also be genetically analyzed. If individuals are released into the wild, these individuals must be genetically representative of these populations (LOPERA-BARRERO, 2009). Therefore, genetic adaptation to captivity must be minimized by reducing the number of generations in captivity (WILLIAMS and HOFFMAN, 2009)

and modifying reproductive management to preserve the genetic variability (RODRIGUEZ-RODRIGUEZ *et al.*, 2010).

CONCLUSION

The genetic variability in the WSamp and broodstocks was high. Within all ten HPP and four broodstocks, there was genetic similarity observed in contrast to the differentiation that was found between both. The combination of historical movement of *P. lineatus* in the investigated region, the presence of sluiceways and restocking is the best explanation for the genetic similarity in the WSamp. Genetic monitoring using molecular markers of broodstocks, progenies and wild populations is fundamental to maintain the genetic variability and prevent inbreeding effects in populations. Therefore, the current experiment study has great importance in assist restocking programs of *P. lineatus* in the Tietê, Grande, Pardo and Mogi-Guaçu rivers, and serve as a model of monitoring for other programs conducted in Brazil.

ACKNOWLEDGEMENTS

We are grateful to AES Tietê Company and ANEEL (National Electric Energy Agency) by supported this experiment.

REFERENCES

ABDUL-MUNEER, P.M. 2014 Application of microsatellite markers in conservation genetics and fisheries Management: recent advances in population structure analysis and conservation strategies. *Genetics Research International*, *1*: 1-14.

AGOSTINHO, A.A.; VAZZOLER, A.E.A.M.; GOMES, L.C.; OKADA, E.K. 1993 Spatial distribution and behaviour of *Prochilodus scrofa* during its life cycle, in the high Parana river floodplain and Itaipu reservoir, Parana, Brazil. *Revue d'Hydrobiologie Tropicale*, *26*(1): 79-90.

AGOSTINHO, A.A.; THOMAZ, S.M.; GOMES, L.C. 2005 Conservation of the biodiversity of Brazil's inland waters. *Conservation Biology*, *19*(3): 646-652.

AGOSTINHO, A.A.; PELICICE, F.M.; GOMES, L.C. 2008 Dams and the fish fauna of the Neotropical region: impacts and management related to diversity and fisheries. *Brazilian Journal of Biology*, *68*(4): 1119-1132.

AGOSTINHO, A.A.; AGOSTINHO, C.S.; PELICICE, F.M.; MARQUES, E.E. 2012 Fish ladders: safe fish passage or hotspot for predation? *Neotropical Ichthyology*, *10*(4): 687-696.

AGUIAR, J.; SCHNEIDER, H.; GOMES, F.; CARNEIRO, J.; SANTOS, S.; RODRIGUES, L.R.; SAMPAIO, I. 2013 Genetic variation in native and farmed populations of Tambaqui (*Colossoma macropomum*) in the Brazilian Amazon: regional discrepancies in farming systems. *Anais da Academia Brasileira de Ciências*, *85*(4): 1439-1447.

ALLENDORF, F.W. and PHELPS, S.R. 1980 Loss of genetic variation in a hatchery stock of cutthroat trout. *Transactions of the American Fisheries Society*, *109*(5): 537–543.

ALMEIDA, F.S.; SODRÉ, L.M.K.; CONTEL, E.P.B. 2003 Population structure analysis of *Pimelodus maculatus* (Pisces, Siluriformes) from the Tietê and Paranapanema Rivers (Brazil). *Genetics and Molecular Biology*, *26*(3): 301-305.

ARAKI, H. and SCHMIT, C. 2010 Is hatchery stocking a help or harm? Evidence, limitations and future directions in ecological and genetic surveys. *Aquaculture*, *308*(1): 2-11.

AUNG, O.; NGUYEN, T.T.T.; POOMPUANG, S.; KAMONRAT, W. 2010 Microsatellite DNA markers revealed genetic population structure among captive stocks and wild populations of mrigal, *Cirrhinus cirrhosus* in Myanmar. *Aquaculture*, *299*(1-4): 37-43.

BARROCA, T.M.; SANTOS, G.B.; DUARTE, N.V.R.; KALAPOTHAKIS, E. 2012 Evaluation of genetic diversity and population structure in a commercially important freshwater fish *Prochilodus costatus* (Characiformes, Prochilodontidae) using complex hypervariable repeats. *Genetics and Molecular Research*, *11*(4): 4456-4467.

BASSAM, B.J.; CAETANO-ANOLLÉS, G.; GRESSHOFF, P.M. 1991 Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, *196*(1): 80-83.

BLANCO GONZALEZ, E.; MURAKAMI, T.; YUNEJI, T.; NAGASAWA, K.; UMINO, T. 2009

Reduction in size-at-age of black sea bream (*Acanthopagrus schlegelii*) following intensive releases of cultured juveniles in Hiroshima Bay, Japan. *Fisheries Research*, *99*(2): 130–133.

BORREL, Y.J.; CARLEOS, C,E,; ASTURIANO, J.F.; BERNARDO, D.; VÁZQUEZ, E.; CORRAL, N.; SÁNCHEZ, J.A.; BLANCO, G. 2007 Use of microsatellites and a combinatorial optimization approach in the acquisition of gilthead seabream (*Sparus aurata* L.) broodstocks for hatcheries. *Aquaculture*, *269*(1-4): 200-210.

BRADIC, M.; BEERLI, P.; GARCÍA-DE LEÓN, F. J.; ESQUIVEL-BOBADILLA, S.; BOROWSKY, R. L. 2012. Gene flow and population structure in the Mexican blind cavefish complex (*Astyanax mexicanus*). *BMC Evolutionary Biology*, *12*(9): 2-16.

BUCKUP, P.; MENEZES, N.; GHAZZI, M. 2007 *Book of freshwater fish species in Brazil*. Rio de Janeiro: Museu Nacional. 195p.

CABALLERO, A.; RODRÍGUEZ-RAMILO, S.T.; ÁVILA, V.; FERNÁNDEZ, J. 2010 Management of genetic diversity of subdivided populations in conservation programmes. *Conservation Genetics*, *11*(2): 409-419.

CAMP, 1998 Report of the workshop *Conservation Assessment and Management Plan (CAMP) for freshwater fishes of India*. Coimbatore: Zoo Outreach Organization (ZOO) and National Bureau of Fish Genetic Resources (NBFGR). 137p.

CARVALHO-COSTA, L.F.; HATANAKA, T.; GALETTI JR, P.M. 2008 Evidence of lack of population substructuring in the Brazilian freshwater fish *Prochilodus costatus*. *Genetics and Molecular Biology*, *31*(1): 377-380.

CORNUET, J.M.; and LUIKART, G. 1996 Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, *144*(4): 2001-2014.

DEHAIS, C.; EUDELINE, R.; BERREBI, P.; ARGILLIER, C. 2010 Microgeographic genetic isolation in chub (Cyprinidae: *Squalius cephalus*) population of the Durance River: estimating fragmentation by dams. *Ecology of Freshwater Fish*, *19*(2): 267-278.

ESGUÍCERO, A.L.H. and ARCIFA, M.S. 2010 Fragmentation of a Neotropical migratory fish population by a century-old dam. *Hydrobiologia*, *638*(1): 41-53.

ESPINACH-ROS, A. and DELFINO, R. 1993 Status of fisheries in the River Plata basin in Paraguay, Bolívia, Argentina and Uruguay. FAO Fisheries Information, *490*: 36-51.

EXCOFFIER, L.; LAVAL, G.; SCHNEIDER, S. 2005 Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*: 47-50.

FALCONER, D.S. 1989 *Introduction to Quantitative Genetics*. 3rd edition. New York: Longman. 438p.

FOPP-BAYAT, D. 2010 Microsatellite DNA variation in the Siberian sturgeon, *Acipenser baeri* (Actinopterygii, Acipenseriformes, Acipenseridae), cultured in a Polish fish farm. *Acta Ichthyologica et Piscatoria*, *40*(1): 21–25.

FOPP-BAYAT, D.; JANKUN, M.; KUZMINSKI, H. 2010 Genetic characterization of Polish cultured brook trout, *Salvelinus fontinalis* (Mitchill), based on microsatellite DNA analysis. *Archives of Polish Fisheries*, *18*(2): 93-99.

GARDNER, M.J.; COTTINGHAM, A.; PHILLIPS, N.M.; HESP, S.A.; CHAPLIN, J.A.; JENKINS, G.I. 2010 *Biological performance and genetics of restocked and wild Black Bream in the Blackwood river estuary*. Report to the West Australian Fish Foundation. Australia: Project Funded by the Department of Fisheries. 60p.

GARCEZ, R.; CALCAGNOTTO, D.; ALMEIDA-TOLEDO, L.F. 2011 Population structure of the migratory fish *Prochilodus lineatus* (Characiformes) from rio Grande basin (Brazil), an area fragmented by dams. *Aquatic Conservation*, *21*(3): 268-275.

GARCÍA DE LEÓN, F.J.; CHIKHI, L.; BONHOMME, F. 1997 Microsatellite polymorphism and population subdivison in natural populations of Europe sea bass *Dicenbrachus labrax* (Linnaeus, 1758). *Molecular Ecology*, *6*(1): 51-62.

GODINHO, A.L. e KYNARD, B. 2006 Migration and Spawning of Radio-Tagged Zulega *Prochilodus argenteus* in a Dammed Brazilian River. *Transactions of the American Fisheries Society*, *139*(3): 1-10.

GODOY, M.P. 1975 *Peixes do Brasil*. Subordem Characoidei. Bacia do rio Mogi-Guassu. Piracicaba: Editora Franciscana. p.219-397.

GOLDSTEIN, D.B. and SCHLOTTERER, C. 1999 *Microsatellites: Evolution and Applications*. Oxford: Oxford University Press. 368p.

GONZÁLEZ-WANGUEMERT, M.; FERNÁNDEZ, T.V.; PÉREZ-RUZAFA, A.; GIACALONE, M.; D'ANNA, G.; BADALAMENTI, F. 2012 Genetic considerations on the introduction of farmed fish in marine protected areas: The case of study of white seabream restocking in the Gulf of Castellammare (Southern Tyrrhenian Sea). *Journal of Sea Research*, *68*: 41-48.

GOUDET, J. 2002 *FSTAT: A Program to Estimate and Test Gene Diversities and Fixation Indices* (version 2.9.3.2). Lausanne, R: Department of Ecology and Evolution. Available on: <http//www.unil.ch/izea/softwares/fstat.html> Access at: 13 Mar. 2014.

HALE, M.L.; BURG, T.M.; STEEVES, T.E. 2012 Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *PLoS ONE*, *7*(9): 1-10.

HARTL, D.L.; CLARK, A.G. 2007 *Principles of Population Genetics* (Fourth edition). Sunderland Massachusetts: Sinauer Associates. 565p.

HATANAKA, T.; HENRIQUE-SILVA, F.; GALETTI JR, P.M. 2006 Population substructuring in a migratory freshwater fish *Prochilodus argenteus* (Characiformes, Prochilodontidae) from the São Francisco River. *Genetica*, *126*(1-2): 153-159.

HATANAKA, T.; HENRIQUE-SILVA, F.; GALETTI JR, P.M. 2002 A polymorphic, telomeric-like sequence microsatellite in the Neotropical fish *Prochilodus*. *Cytogenetic and Genome Research*, *98*(4): 308-310.

LACY, R.C. 1987 Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, selection, and population subdivision. *Conservation Biology*, 1(2): 143–158.

LAROCHE, J. and DURAND, J.D. 2004 Genetic structure of fragmented populations of a threatened endemic percid of the Rhône river: Zingel asper. *Heredity*, *92*(4): 329-334.

LOPERA BARRERO, N.M. 2009 Conservation of *Brycon orbignyanus* natural populations and stocks for their reproductive, genetic, environmental sustainability: A model for species threatened with extinction. *Ciencia e Investigación Agrária*, *36*(2): 191-208.

LOPERA-BARRERO, N.M.; POVH, J.A.; RIBEIRO, R.P.; GOMES, P.C.; JACOMETO, C.B.; LOPES, T.S. 2008 Comparison of DNA extraction protocols of fish fin and larvae samples: modified salt (NaCl) extraction. *Ciencia e Investigación Agrária*, *35*(2): 65-74.

LOPERA BARRERO, N.M.; RIBEIRO, R.P.; POVH, J.A.; SIROL, R.N.; MANGOLIN, C.A. 2010a Genetic evaluation of pacu (*Piaractus mesopotamicus*) natural populations and from the broodstocks of a stock enhancement program using microsatellite markers. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *62*(4): 954-963.

LOPERA BARRERO, N.M.; RODRIGUEZ-RODRIGUEZ, M DEL P.; RIBEIRO, R.P.; POVH, J.A.; VARGAS, L.; STREIT JR, D.P.; FORNARI, D.C. 2010b Reproductive contribution of a *Brycon orbignyanus* (Cuvier and Valenciennes, 1850) offspring in the semi-natural reproductive system using microsatellite markers. *Zootecnia Tropical*, *28*(3): 403-411.

MARCENIUK, A.P.; HILSDORF, A.W.S.; LANGEANI, F. 2011 The ichthyofauna from the headwaters of the rio Tietê, São Paulo, Brazil. *Biota Neotropica*, *11*(3): 217-236.

MARIE, A.D.; BERNATCHEZ, L.; GARANT, D. 2010 Loss of genetic integrity correlates with stocking intensity in brook charr (*Salvelinus fontinalis*). *Molecular Ecology*, *19*(10): 2025-2037.

MU, X.D.; HU, Y.C.; WANG, X.J.; SONG, H.M.; YANG, Y.X.; LUO, J.R. 2011 Genetic variability in cultured stocks of *Scleropages formosus* in Mailand China revealed by microsatellite markers. *Journal of Animal and Veterinary Advances*, *10*(5): 555-561.

NA-NAKORN, U. and MOEIKUM, T. 2009 Genetic diversity of domesticated stocks of striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878), in Thailand: relevance to broodstock management regimes. *Aquaculture*, *297*(1-4): 70-77.

NEI, M. 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89*(3): 583-590.

O'CONNELL, M. and WRIGHT, J.M. 1997 Microsatellite DNA in fishes. Reviews in Fish *Biology and Fisheries*, *7*(3): 331-363.

O´LEARY, S.J.; HICE, L.A.; FELDHEIM, K.A.; FRISK, M.G.; MCELROY, A.E.; FAST, M.D.; CHAPMAN, D.D. 2013 Severe Inbreeding and Small Effective Number of Breeders in a Formerly Abundant Marine Fish. *PLoS One*, *8*(6): 1-8.

OROZCO BERDUGO, G. and NARVÁEZ BARANDICA, J. C. 2014 Genetic diversity and population structure of bocachico *Prochilodus magdalenae* (Pisces, Prochilodontidae) in the Magdalena River basin and its tributaries, Colombia. *Genetics and Molecular Biology*, *37*(1): 37-45.

PESOA, N.A. and SCHULZ, U,H. 2010 Diel and seasonal movements of grumatã *Prochilodus lineatus* (Valenciennes 1836) (Characiformes: Prochilodontidae) in the Sinos River, Southern Brazil. *Brazilian Journal of Biology*, *70*(4): 1169-1177.

PETERSEN, R.S.; GARCIA, J.E.; MELLO, G.; LIEDKE, A.M.R.; SINCERO, T.C.M.; GRISARD, E.C. 2012 Análise da diversidade genética de tilápias cultivadas no estado de Santa Catarina (Brasil) utilizando marcadores microssatélites. *Boletim do Instituto de Pesca*, *38*(4): 313-321.

POVH, J.A.; RIBEIRO, R.P.; SIROL, R.N.; STREIT JR, D.P.; MOREIRA, H.L.M.; SIEWERDT, F.; LOPERA BARRERO, N.M.; MANGOLIN, C.A.; VARGAS, L. 2010 Microsatellite analysis of the parental contribution of *Piaractus mesopotamicus* to the production of offspring in the semi-natural system of reproduction. Brazilian *Archives of Biology and Technology*, *53*(2): 389-396.

POVH, J.A.; RIBEIRO, R.P.; LOPERA BARRERO, N.M.; JACOMETO, C.B.; VARGAS, L.; GOMES, P.C.; LOPES, T.S. 2011 Microsatellite analysis of pacu broodstocks used in the stocking program of Paranapanema river, Brazil. *Scientia Agricola*, *68*(3): 308-313.

RAMELLA, M.S.; KROTH, M.A.; MEURER, S.; NUÑER, A.P.O.; ZANIBONI FILHO, E.; ARISI, A.C.M. 2006 Genetic variability in four fish species (*Pimelodus maculatus*, *Prochilodus lineatus*, *Salminus brasiliensis* and *Steindachneridion scripta*) from Uruguay River basin. *Brazilian Archives of Biology and Technology*, *49*(4): 589-598.

RICE, W.R. 1989 Analyzing table of statistical tests. *Evolution*, *43*(1): 223-225.

RIBOLLI, J.; MELO, C.M.R.; ZANIBONY-FILHO, E. 2012 Genetic characterization of the Neotropical catfish *Pimelodus maculatus* (Pimelodidae, Siluriformes) in the Upper Uruguay River. *Genetics and Molecular Biology*, *35*(4): 761-769.

RODRIGUEZ-RODRIGUEZ, M DEL P.; LOPERA-BARRERO, N.M.; RIBEIRO, R.P.; POVH, J.A.; VARGAS, L.; SIROL, R.N.; JACOMETO, C.B. 2010 Genetic diversity of piracanjuba used in stock enhancement programs with microsatellite markers. *Pesquisa Agropecuaria Brasiliera*, *45*(1): 56-63.

ROUSSET, F. 2008 Genepop 2007: a complete re-implementation of the GENEPOP software for Windows and Linux. M*olecular Ecology Resources*, *8*(1): 103-106.

RUEDA, E.; SOMMER, J.; SCARABOTTI, P.; MARKARIANI, R.; ORTÍ G. 2011 Isolation and characterization of polymorphic microsatellite loci in the migratory freshwater fish *Prochilodus lineatus* (Characiformes, Prochilodontidae). *Conservation Genetics Resources*, *3*(4): 681-684.

RUEDA, E.C.; CARRIQUIRIBORDE, P.; MONZÓN, A.M.; SOMOZA, G.M.; ORTÍ, G. 2013 Seasonal variation in genetic population structure of sábalo (*Prochilodus lineatus*) in the Lower Uruguay River. *Genetica*, *141*(7-9): 401-407.

SANFORD, E. and KELLY, W. 2011 Local adaptation in marine invertebrates. *Annual Review of Marine Science*, *3*: 509–535.

SATAKE, A. and ARAKI, H. 2012 Stocking of captive-bred fish can cause long-term population decline and gene pool replacement: predictions from a population dynamics model incorporating density-dependent mortality. *Theoretical Ecology*, *5*(2): 283-296.

TAMURA, K.; PETERSON, D.; PETERSON, N.; STECHER, G.; NEI, M.; KUMAR, S. 2011 MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, *28*(10): 2731-2739.

TOLEDO-FILHO, S.A. 1981 *Biologia populacional do curimbatá (**Prochilodus scrofa**) Steindachner, 1881 (Pisces, Prochilodontidae) do rio Mogi-Guaçu:*

Aspectos quantitativos. São Paulo. 171p. (Tese de Livre-Docência, USP).

TOLEDO-FILHO, S.A.; GODOY, M.P.; SANTOS, E.P. 1987 Delimitacão populacional do curimbatá, *Prochilodus scrofa* (Pisces, Prochilodontidae) do Rio Mogi-Guaçu, Brasil. *Revista Brasileira de Biologia*, *47*(4): 501–506

VAN OOSTERHOUT, C.; HUTCHINSON, W.F.D.; WILLS, P.M.; SHIPLEY, P. 2004 Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3): 535-538.

WEIR, B.S. and COCKERHAM, C.C. 1984 Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6): 1358-1370.

WILLIAMS, S.E. and HOFFMAN, E.A. 2009 Minimizing genetic adaptation in captive breeding programs: A review. *Biological Conservation*, *142*(11): 2388-2400.

WRIGHT S. 1978 *Evolution and genetics of population*. Chicago, IL: University of Chicago Press. 590p.

YAZBECK, G.M. and KALAPOTHAKIS, E. 2007 Isolation and characterization of microsatellite DNA in the piracema fish *Prochilodus lineatus* (Characiformes). *Genetics and Molecular Research*, *6*(4): 1026-1034.

YAMAMOTO, S.; MORITA, K.; KOIZUMI, I.; MAEKAWA, K. 2004 Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: Spatial–temporal changes in gene frequencies. *Conservation Genetics*, *5*(4): 529-538.

YEH, F.C.; BOYLE, T.Y.Z.; XIYAN, J.M. 1999 *PopGene Version 131: Microsoft Window-based freeware for population genetic analysis*. Alberta, AB: University of Alberta and Center for International Forestry Research. Available at: <www.ualberta.ca/~fyeh/> Access on: 13 Mar. 2014.