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Genetic monitoring by rapd markers for repopulation programs of *Salminus brasiliensis* (Pisces, Characiformes)

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ABSTRACT. The preservation of the genetic variability of hatchery stocks used to supplement natural populations is a priority. Current study employed RAPD markers to examine the genetic diversity of *dourado* samples from the middle Paranapanema river and from the broodstock used in the stock enhancement program of the Aquaculture and Hydrobiology Station at the Salto Grande Hydroelectric Power Plant. Nineteen RAPD primers were analyzed, which generated 299 bands and the latter were used for genetic analysis. The percentage of polymorphic fragments was higher in stock captured in the Paranapanema river than from fry stocks. The genetic diversity was lower in the broodstock than in natural population. Amova results showed that most inter-population genetic variation lay within stocks (83.9%) and not between them (16.1%). Moderate genetic differentiation (F_{ST} = 0.16) was reported. Nevertheless, differentiation decreased when the four fry stocks were mixed and analyzed as a single population (F_{ST} = 0.07). Instead of releasing each lot separately into the water, mixing specimens produced in the various fry stocks before releasing them in the river would be more feasible. The restocked population will have a genetic structure closer to natural populations.

Keywords: *dourado*, genetic variability, hatchery stocks, fry stocks.

Monitoramento genético utilizando marcadores RAPD em programas de criação em cativeiro de Dourado

RESUMO. Conservar a variabilidade genética dos estoques de alevinos de peixes para suplementar populações naturais é uma prioridade. O objetivo deste estudo foi examinar a diversidade genética por meio dos marcadores de RAPD de amostras de peixes reprodutores de dourado do médio Paranapanema (população natural) e alevinos usados no programa de repovoamento da estação de aquicultura da UHE de Salto Grande. Foram analisados 19 *primers* de RAPD, que geraram 299 bandas, as quais foram utilizadas para as análises genéticas. A porcentagem de fragmentos polimórficos foi mais elevada na população natural do que nos estoques. A diversidade genética também foi mais baixa nos alevinos. Os resultados da Amova mostraram que a maior parte de variação genética interpopulacional está dentro dos estoques (83,9%) e não entre eles (16,1%). Foi observada uma diferenciação genética moderada (F_{ST} = 0.16). Esta diferenciação diminuiu quando os quatro estoques de alevinos eram misturados e analisados como uma única população (F_{ST} = 0.07). Em vez de liberar cada lote separadamente no ambiente, seria melhor misturar todos os indivíduos produzidos nos vários estoques de alevinos antes de liberá-los no rio. Desta maneira, a população reintroduzida terá uma estrutura genética mais perto da população natural.

Palavras-chave: dourado, variabilidade genética, estoque de matriz, estoque de alevinos.

Introduction

The construction of dams requires the adoption of procedures to reduce the impact on fish populations during and after reservoir formation. Several strategies have been employed to preserve fauna in rivers divided by dams. Some of these measures have been tested worldwide, or rather, the construction of elevators and ladders for fish transport, the vehicular transport of fish from the area below the dam to the reservoir or even the implantation of hatcheries to reproduce native species for future repopulations. The construction of stairs and repopulation programs are the most utilized (POMPEU et al., 2012).

Fish are among the most important natural resources in the world and their great biodiversity not only supplies nutritional needs through commercially exploited species, but also plays an important ecological role, as is widely recognized in aquatic conservation and in aquatic ecosystem

management and control (ORMEROD, 2003). According to Vieira and Pompeu (2001), many people think that the greater the amount of fish in the watercourse, the better its quality. The relationship between quantity and environmental quality triggers petitions to add fish stocks to aquatic systems characterized by few species, poor fishing results or reduced stocks (VIEIRA; POMPEU, 2001). Some environmental agencies frequently adopt fishing results as a mitigating measure and/or an observation of impact. Most releases of hatchery fish by government agencies occur within the native ranges of the taxonomic species and are in section of the river in which wild populations are still present. Consequently, interactions occur between hatchery fish and wild populations, with potential viability issues (KOSTOW, 2009).

Aquaculture practices may inadvertently decrease the genetic variability present in farmed stocks by the selection and breeding of related specimens or by the use of a small number of parents as broodstock. In this case, unless genetic records are maintained, there is a great probability of increased inbreeding (BARROSO et al., 2005; MACHADO-SCHIAFFINO et al., 2007). Research has demonstrated that the ecological effects of hatchery programs may significantly reduce wild population productivity and abundance even where genetic risks do not occur (KOSTOW, 2009).

Salminus brasiliensis (Cuvier, 1816), popularly called *dourado*, belongs to the Characidae family and is one of the most commercially and ecologically important species in its native regions (La Plata, São Francisco, Magdalena and the periphery of Amazon/Orinoco basins) (LIMA et al., 2003). These predatory, ichthyophagous fish, the largest Neotropical characiforms, are one of the main targets of professional and amateur fishing in South America (LIMA et al., 2003). The *Salminus brasiliensis* has lately occurred with some small abundance in the Paranapanema river basin down- and midstream from the hydroelectric plants (HEPs), especially in the Capivara reservoir (downstream from HEP Canoas I) and in certain large tributaries (SIROL; BRITTO, 2005). Several studies suggested that this species has been vulnerable to overexploitation, habitat degradation (ZAYAS; CORDIVIOLA, 2007) and to fragmentation due to dam construction (AGOSTINHO et al., 2003).

Random amplified polymorphic DNA (RAPD) analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete genome regions with short oligonucleotide primers of arbitrary sequence (WILLIAMS et al., 1990). The method is simple and quick to perform, and most importantly, no prior knowledge of the genetic make-up of the organism in question is required. RAPD has been successfully used in fishery management and conservation genetics of wild populations (ALMEIDA et al., 2003; BÁRTFAI et al., 2003; LOPES et al., 2007).

Public opinion has still failed to question which species are being used in restocking programs, the genetic structure of these species, or about fish species that became or will be extinct due to the release of farmed fish among wild ones. Current study evaluates comparatively the population structure of one lot of *dourado* matrixes collected in the Paranapanema river, corresponding to a wild population of *Salminus brasiliensis*, and four lots of fry stock produced from matrixes at the Salto Grande HEP breeding program on the Paranapanema river. Genetic variability within and among the different stocks was evaluated by RAPD to highlight the tasks necessary in the future for better hatchery management and species conservation.

Material and methods

Specimens of *S. brasiliensis* were collected in the fish ladders of Canoas I (50º 31'W and 22º 56'S) and Canoas II (50º 15'W and 22º 56'S) HEPs, in the Middle Paranapanema river, with reservations as to the spawning period from September to March (Figure 1). Approximately 250 specimens were obtained from 2001 to 2003, and maintained in a lake at the Hydrobiology and Aquaculture Station of Salto Grande Hydroelectric Plant in Salto Grande, São Paulo State, Brazil. These fish were representatives of the natural population (matrix stock). Table1 shows the number of males and females taken from stock for fry production.

Table 1. Number of males and females, number of loci obtained, genetic variability ($\bar{P}$ = proportion of polymorphic fragment) and index of allelic fixation F_{ST} for stocks of *S. brasiliensis*. L1, L2, L3 and L4 = stocks of fry fish.

Stock	Males	Females	N. of loci	$\bar{P}$	F_{ST}
L1	19	9	299	44.85	0.15640
L2	16	06	299	36.14	0.17252
L3	12	05	299	40.00	0.17120
L4	12	04	299	40.98	0.16315
Matrix			299	48.02	0.14118

Breeders for fry production were selected from the fish population kept in the lake. Breeder selection criteria were secondary sexual characteristics described by Woynarovich and Horváth (1983). The extract of carp pituitary gland was used for the matrixes' hormonal inducement, following Woynarovich and Horváth (1983), with regard to the morphophysiological state of the animals and extract application. Hormone dose consisted of 5 mg extract kg^{-1} for females and 1 mg kg^{-1} males (total dose).

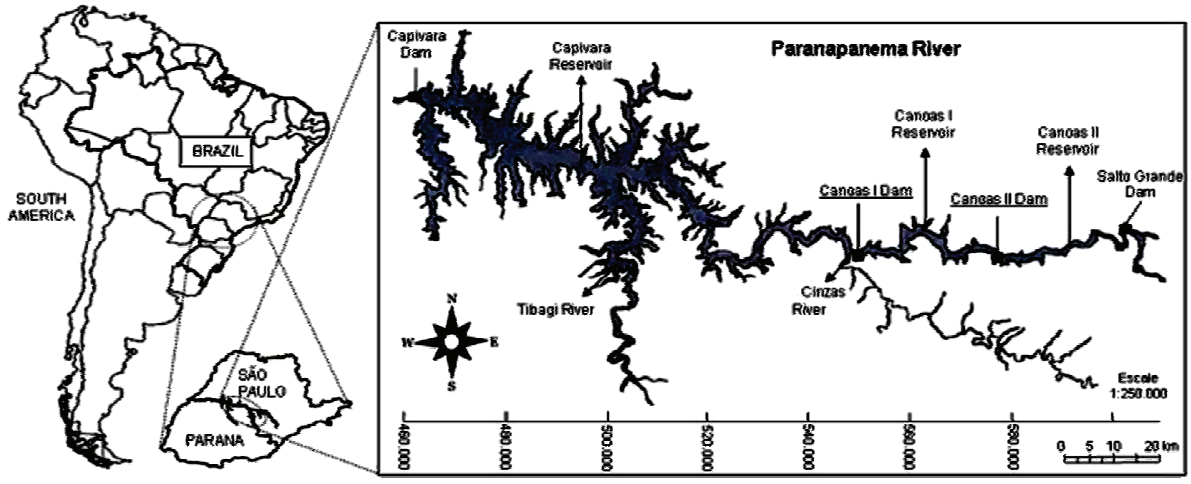

Figure 1. Partial view of the Paranapanema river and its principal affluents (rivers Tibagi and Cinzas). Featured are the two collection sites: HEP Canoas I and HEP Canoas II.

Egg laying was natural and occurred in a circular tank measuring approximately 10 m in diameter and 1 m deep, with complete water exchange every two hours and egg collection undertaken from beneath, provided with a 200 liter conic incubator for temporary storage. The eggs were afterwards transferred to different incubators and the larvae transferred to tanks. Table 1 describes the number of females and males used to produce the four fish fry stocks analyzed (denominated L1, L2, L3 and L4).

The males and females selected from each reproduction were separated in another tank so that the same genitors would not be used in the production of each fry stock for repopulation.

The adipose fin was obtained from each specimen and stored in 70% ethanol alcohol at -20ºC for later DNA extraction. Most of the collected specimens were released at the same site, while others were labeled and conserved in the Zoology Museum of the State University of Londrina, Londrina, Paraná State, Brazil (MZUEL4986, 4987 and 4988).

DNA extraction and Random Amplified Polymorphic DNA (RAPD) analysis

DNA was extracted from the adipose fin according to procedures described by Almeida et al. (2001). The 60 different decamer oligonucleotides used as random primers in RAPD screening were purchased from Operon Technologies Ltd. Nineteen (OPC 2, 11; OPX 1, 6, 7, 9, 12, 13, 14, 17, 18, 19; OPAM 4, 7, 9, 10, 11, 13, 16) were selected, owing to the number of bands obtained and their ability to produce consistent fragment patterns. Amplification reactions were performed in a total volume of 15 µL containing 10-15 ng of template DNA, 0.25 µmol primer, 3.5 mM $MgCl_2$, 250 µmol dNTP, and 1 unit of *Taq* DNA polymerase (Biotools) in the supplied reaction buffer. Control reactions were run with all components except genomic DNA. Reaction runs were performed in a PTC-100 thermal cycler (MJ Research), and consisted of an initial denaturation for 5 min. at 92°C, followed by 40 thermal cycles of 40 at 92; 90 at 40; 120 s at 72°C; and a final cycle of 5 min. at 72°C.

RAPD products were resolved by electrophoresis at 3 V cm^{-1} in 1.4% agarose gels, containing TBE buffer (0.89 M Tris, 0.89 M boric acid and 2 mM EDTA, pH 8.3) diluted 1:20 (v:v). Gels were stained with ethidium bromide and documented by Electrophoresis Documentation and Analysis System (EDAS 290 - Kodak). PCR reactions were standardized and repeated twice to avoid limitations caused by RAPD-PCR method, such as sensitivity to reaction conditions, contamination and occasional non-reproducible fragments. Only those bands clearly distinguishable and routinely repeatable were selected for genetic analysis.

Data analysis

Intrapopulation and interpopulation analyses were the two types of analysis carried out. Twenty-three specimens from each fish fry stocks and 16 specimens of *S. brasiliensis* from the lake population (matrix stock) were used. Twenty-three specimens were collected from the matrix stock but only 16 produced amplification products.

Two interpopulational analyses were performed: the first analysis compared each of the four fish fry stocks with the matrix stock; the second analysis compared all individuals of the fry stocks, considered as a single group, with the matrix stock.

The RAPD profile for each primer was determined by comparing directly the amplified DNA electrophoresis band patterns produced with the primer. Data obtained were analyzed as binary variables (band presence or absence). RAPD polymorphisms were analyzed according to the following presuppositions: 1) bands from different loci do not comigrate; 2) each locus is a two-allele system in which only one allele is amplifiable; and 3) alleles arise from identical mutations, among and within individuals (LYNCH; MILLIGAN, 1994). The scores obtained using all primers in the RAPD analysis were then pooled for the construction of a single data matrix.

The following parameters were calculated by software TFPGA 1.3 (MILLER, 1997): genetic variability was estimated by the proportion of polymorphic loci ($\bar{P}$), using the 95% criterion, and genetic distance (D) (NEI, 1978). Correction was performed as described by Lynch and Milligan (1994).

Arlequin 3.0 (EXCOFFIER et al., 2005) determined the distribution of genetic differentiation by F_{ST} estimates among populations and molecular variance analysis (Amova). The significance of these tests was verified by the random permutation method, with 1000 and 10,000 permutations, respectively.

The magnitude of genetic differentiation among the stocks of *S. brasiliensis*, was determined by scale proposed by Wright (1978), where F_{ST} values from 0 to 0.05 indicate little genetic differentiation; from 0.05 to 0.15 moderate differentiation; from 0.15 to 0.25 high differentiation; above 0.25 very high differentiation.

Inter-stock genetic similarity dendrograms were constructed by Jaccard (J) coefficient and UPGMA cluster analysis algorithm by NTSYS-PC 2.10 (ROHLF, 2008).

Results and discussion

Repeated amplifications with the primers were reproducible and generated good quality banding patterns of sufficient variability and with intense stained fragments sharply separated from other bands.

The analysis of RAPD electrophoresis profiles for the 19 primers yielded 299 fragments, of which 174 (58.19%) were polymorphic. Figure 2 shows a RAPD electrophoresis profile for one of the selected primers. The number of fragments per primers ranged between 11 and 23, whereas the size of the amplified products was between 250 and 2400 bp.

The determination of the genetic variability of the matrix group used for repopulation programs is a fundamental tool for the genetic conservation of a species (SIROL; BRITTO, 2005; POVH et al., 2008). Another indispensible strategy is to assure that genetic variability is maintained in fry lots to be released into the river. Consequently, the genetic monitoring of repopulation programs is essential for genetic conservation. Molecular markers are efficient instruments for this purpose, providing genetic management in hatcheries by minimizing endogamy and maximizing genetic variability (BARROSO et al., 2005; KOSTOW, 2009; MACHADO-SCHIAFFINO et al., 2007). Specimens of *S. brasiliensis* in current trial revealed good population variation when estimated by genetic parameters.

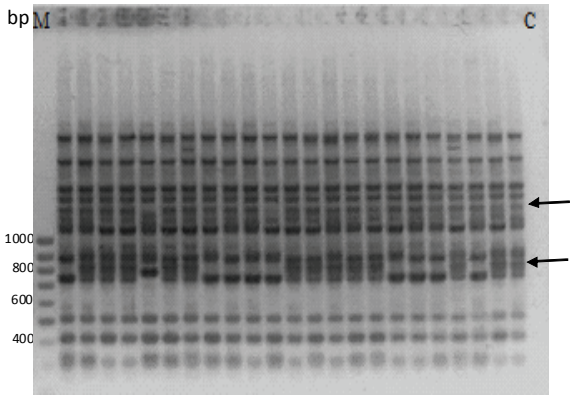

Figure 2. Random-amplified polymorphic DNA (RAPD) gel profiles showing banding pattern of *S. brasiliensis* for fry fish stock L2 with primer OPAM 9. M = 100 bp DNA ladder Biotools, C = negative control. Arrows indicate some polymorphic bands.

When the rates of genetic variability of fry lots were compared (Table 1) with the number of males and females used as breeders, L1, with 19 males and 9 females, demonstrated the highest variability value ($\bar{P}$). A reduction in genetic variability was also observed when comparing the matrix stock ($\bar{P}$ = 48.02%) with fry stocks ($\bar{P}$ < 44.85%). Long-term population persistence partially depends on the maintenance of the evolutionary potential which, in turn, requires genetic variation (MACHADO-SCHIAFFINO et al., 2007). A reduction in genetic variability may result in an inefficient repopulation program (low fry survival in the aquatic environment) and may lead towards irreversible genetic impact on natural populations (LOPERA-BARRERO et al., 2008).

In spite of the reduction in genetic variability observed in fry stocks, rates were quite similar to the mean of $\bar{\bar{P}}$ (43.49%) reported by Lopes et al. (2007) for *S. brasiliensis*. The specimens mentioned in the above paper were collected in the fish ladders at the Canoas Plant on the river Paranapanema. This is actually the same site where specimens, representing the natural population and belonging to the matrix lot of the present study, were gathered.

According to Sofia et al. (2006), estimates of genetic variability using RAPD fingerprints may produce

biased results in samples with less than 10 specimens. Although using a smaller number of individuals to matrix lot (N = 16), a high number of loci (299) was analyzed to reduce possible bias in the analysis, as suggested by Nei (1978).

A reduction in the number of polymorphic loci may be correlated to the limited number of wild specimens and/or populations used to create hatchery stocks (MACHADO-SCHIAFFINO et al., 2007). According to Toledo-Filho et al. (1992), after reduction of a natural population (stock donor) to a lower (founder stock) containing N individuals, the amount of genetic variability that remains may be expressed by the formula 1-(1/2N). Applying this formula to present trial, a decrease in genetic variability for fry stocks L1 and L2 by 2% and for L3 and L4 by 3% is expected.

The production of fry with high genetic variability depends entirely upon a breeding stock with the same characteristic. Inadequate reproductive management may promote reduction in genetic variability (MOREIRA et al., 2007). According to Sirol and Britto (2005), conventional fertilization techniques, such as those that involve the gamete extrusion of a few breeders for fertilization, may trigger a reduction in the variability of offspring, while natural techniques of induced reproduction with a significant lot of breeding stock, such as the one utilized in current study, normally do not present this problem. Nevertheless, this methodology does not exclude the necessity of validating the genetic variability of fry. In current study, the mean rate of genetic variability ($\bar{P}$ = 40.49%) for fry stocks was slightly lower, but the rates obtained were quite similar to those reported by Lopes et al. (2007) for this species.

The fixation index F_{ST} is a convenient and widely used measure of genetic differences among subpopulations. The identification of causes underlying a particular rate of F_{ST} observed in natural population is often difficult (HARTL; CLARK, 1997). F_{ST} rates among individuals of each stock (Table 1) were significant and indicated a moderate to high genetic differentiation (> 0.14), according to the scale by Wright (1978). The lowest F_{ST} rate was observed in the matrix lot (representing the natural population) and the highest value was in L2. The datum corroborates with the genetic variability rates observed, or rather, high for the matrix stock and low for L2 fry stock. L1 was the fry stock with the lowest F_{ST} rate and coincided with the highest observed genetic variability rate and the highest number of breeding stock used.

Genetic differentiation rates ranged from moderate to high (between 0.118 and 0.191) in the pair-wise comparison of F_{ST} rates (Table 2). The highest rates of genetic differentiation (F_{ST}) were observed in the comparison between fry stock of L2 and the matrix stock. These fry stocks presented the lowest rates of genetic variability in the intrapopulation analysis and the highest F_{ST} rates

Table 2 shows that L1 had the lowest rate registered when fry and matrix stocks were compared (F_{ST} = 0.144). Such a low genetic differentiation rate could have been due to the fact that L1 stock was obtained with the greatest number of breeders, presenting the highest rate in genetic variability and the lowest F_{ST} rate in intrapopulation analysis. Genetic distance analyses (D) corroborate the data described above and reveal that greater genetic distance occurred between the matrix stock and the fry stocks.

Table 2. Genetic distances (below) and population pair-wise F_{ST} (above) among five stocks of *Salminus brasiliensis*. L1, L2, L3 and L4 = stocks of fry fish, M = matrix stock.

Stocks	L1	L2	L3	L4	M
L1	----	0.1179	0.1295	0.1418	0.1437
L2	0.0470	----	0.1822	0.1725	0.1907
L3	0.0460	0.0523	----	0.1593	0.1906
L4	0.0435	0.0507	0.0436	----	0.1746
M	0.0768	0.0818	0.0728	0.0723	----

The results of Amova for the first interpopulation analysis showed that the greatest part of the genetic variation is contained within (83.88%) the stocks and not among them (16.12%) (Table 3).

Table 3. Analysis of molecular variance (AMOVA), F_{ST} and genetic distance (D) for stocks of *Salminus brasiliensis.* d.f. = degree of freedom, (P = F_{ST} P rate).

Analysis	Source of variation	d.f.	Sum of squares	Variance components	% of variation	F_{ST}	D
Stocks	Among Groups	4	259.27	4.43993	16.12	0.1612	0.076
	Within Groups	42	970.64	23.11058	83.88	(p = 0.00)	
	Total	46	1229.91	27.55052	100		
Matrix stock X Fry fish stock	Among Groups	1	40.87	1.41250	7.17	0.0718	0.052
	Within Groups	30	548.25	18.27500	92.83	(p = 0.00)	
	Total	31	589.12	19.68750	100		

These results were expected for natural populations and were similar to those reported by Lopes et al. (2007) for *S. brasiliensis*, where the highest variation percentage was within the groups (98.22%) and not among them (1.78%). Studying natural populations of *Piaractus mesopotamicus* in the river Paranapanema and restocking populations,

Povh et al. (2008) obtained Amova rates in which the highest variation percentage was within each group (84.2%) and not among the groups (15.8%), or rather, rates which were similar to those in current study.

In the second interpopulation analysis in which joint grouping of all fry lots was compared to the matrix stock, a reduction was observed in genetic differentiation and percentage of among-stock variation (Amova), as well as in genetic distance (Table 2). According to the scale proposed by Wright (1978), rates of genetic differentiation indicated from high (F_{ST} = 0.1612) to moderate (F_{ST} = 0.072) differentiation. The percentage of variation among stocks was from 16.12 to 7.17%, with genetic distance rates between 0.07 and 0.05.

The dendrogram of genetic similarity (Figure 3) corroborated the genetic distance data, in which a tendency toward the grouping of individuals of each stock could be observed. Basavaraju et al. (2007), studying common carp stocks, observed that the data set-generated dendrogram clearly showed grouping of samples according to their origin.

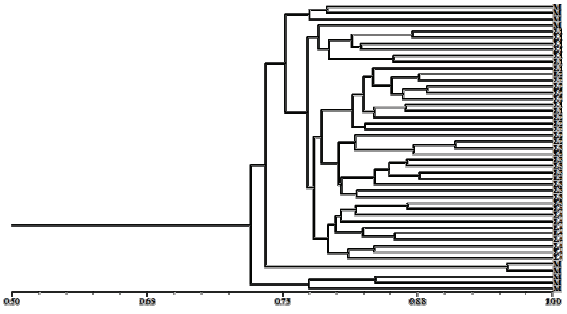

Figure 3. Upgma dendrogram representing genetic relationship, by Jaccard coefficient, among stocks of *S. brasiliensis* based on randomly amplified polymorphic DNA data. L1, L2, L3 and L4= stocks of fry fish, M = matrix stock.

Such data could serve as a base for the planning of fry release procedures. Instead of releasing each lot separately into the environment, as occurs in many hatchery stations, it would be better to mix all individuals produced in the various fry stocks before releasing them into the river. The restocked population will thus have a genetic structure closer to the natural population.

The constant genetic monitoring of the natural populations of threatened species, as is the case with *S. brasiliensis*, is required. In fact, it is also fundamental for the management of hatchery stocks maintained over long periods (RAMELLA et al., 2006).

However, the massive releases of hatchery-produced fish have raised concern on their genetic effects on wild populations at two levels: (1) hatchery fish may have a reduced genetic variability which may eventually lower the genetic diversity in the population into which they are released; and (2) genetic viability of wild populations may be eroded by the transplantation of non-native fish or their hatchery-derived offspring (PEREIRA et al., 2010). It is therefore necessary to have adequate knowledge on the genetic population structure, which may be done by molecular genetic analysis prior to any restocking or stock enhancement project.

S. brasiliensis is a highly prized fish for commercial and sport fisheries throughout its distribution. Commercial catches and trophy size, however, have been decreasing in the Paraná river basin (RESENDE, 2003) and in the river Paranapanema (SIROL; BRITTO, 2005) since the late 1940s, despite efforts to regulate sport and commercial fisheries.

According Lopes et al. (2007), some findings indicate that natural populations of *S. brasiliensis* come from the Capivara reservoir, downstream the HEP Canoas I, and receives on its left bank two large affluents, the rivers Tibagi and Cinzas. Hoffmann et al. (2005) report that, after the construction of the Canoas dams, only the stretch of the Capivara Reservoir between the river Cinzas and Canoas I HEP has lotic waters required for the sexual stimuli in the reproduction of *S. brasiliensis.* The specimens of *S. brasiliensis* present in the Capivara Reservoir are mainly from the river Cinzas, a fact verified by Hoffmann et al. (2005). These researchers made seasonal collections between 2001 and 2004 at four different sites in this reservoir. The species was found only at a site close to the mouth of this river. Consequently, specimens from the rivers Cinzas and Tibagi may be used, if necessary, to maintain natural stocks of *S. brasiliensis* with the genetic characteristics identified in this study.

Besides monitoring the genetic structure and variability of fish populations present in the aquatic environment, such factors as the preservation of riparian forests, the protection and inspection of rivers, the control of fishing, and the participation of public and private entities and society as a whole are of paramount importance for the conservation of the ecosystem and the success of restocking programs (VIEIRA; POMPEU, 2001).

Although one major drawback of RAPD methodology is the impossibility of determining whether a specimen is homozygous or heterozygous in its dominant traits, results show that the technique is actually a simple, rapid and cost-efficient approach for acquiring information about general genetic variability within and among wild and farmed populations of *S. brasiliensis*. Moreover, although the present analysis provides primary

genetic data on *S. brasiliensis*, the results may be a worthy guide for future fish breeding policies.

Conclusion

The genetic variability observed in the fry stocks were quite similar to natural population.

Mixing all individuals produced in the various fry stocks before releasing them into the river is more feasible.

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References

AGOSTINHO, A. A.; GOMES, L. C.; SUZUKI, A. I.; FERREIRA JULIO, H. JUNIOR. Migratory fishes of the upper Paraná river Basin, Brazil. In: CAROLSFELD, J.; HARVEY, B.; ROSS, C. A.; BAER, A. (Ed.). **Migratory fishes of south America**: biology, fisheries, and conservation status. Ottawa: International Development Research Center/The World Bank, 2003. p. 19-98.

ALMEIDA, F. S.; FUNGARO, M. H. P.; SODRÉ, L. M. K. RAPD and isoenzyme analysis of genetic variability in three allied species of catfish (Siluriformes: Pimelodidae) from the Tibagi river. **Journal of Zoology**, v. 253, n. 1, p. 113-120, 2001.

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

BARROSO, R. M.; HILSDORF, A. W. S.; MOREIRA, H. L. M.; CABELLO, P. H.; TRAUB-CSEKO, Y. M. Genetic diversity of wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiformes, Characidae, Bryconiae) using microsatellites. **Aquaculture**, v. 247, n. 1/4, p. 51-65, 2005.

BÁRTFAI, R.; EGEDI, S.; YUE, G. H.; KOVÁCS, B.; URBÁNYI, B.; TAMÁS, G.; HORVÁTH, L.; ORBÁN, L. Genetics analysis of two common carp broodstocks by RAPD and microsatellite markers. **Aquaculture**, v. 219, n. 1/4, p. 157-167, 2003.

BASAVARAJU, Y.; PRASAD, D. T.; RANI, K.; KUMAR, S. P.; NAIKA, U. D.; JAHAGEERDAR, S.; SRIVASTAVA, P. P.; PENMAN, D. J.; MAIR, G. Genetic diversity in common carp stocks assayed by random-amplified polymorphic DNA markers. **Aquaculture Research**, v. 38, n. 2, p. 147-155, 2007.

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

HARTL, D. L.; CLARK, A. G. **Principles of population genetics**. Sunderland: Sinauers Associates, Inc., 1997.

HOFFMANN, A. C.; ORSI, M. L.; SHIBATTA, O. A. Diversidade de peixes do reservatório da UHE Escola de Engenharia Mackenzie (Capivara), rio Paranapanema, bacia do alto rio Paraná, Brasil, e a importância dos grandes tributários na sua manutenção. **Iheringia. Série Zoologia**, v. 95, n. 3, p. 319-325, 2005.

KOSTOW, K. Factors that contribute to the ecological risks of salmon and steelhead hatchery programs and some mitigating strategies. **Reviews in Fish Biology and Fisheries**, v. 19, p. 9-31, 2009.

LIMA, F. C. T.; MALABARBA, L. R.; BUCKUP, P. A.; SILVA, J. F. P.; VARI, R. P.; HAROLD, A.; BENINE, R.; OYAKAWA, O. T.; PAVANELLI, C. S.; MENEZES, N. A.; LUCENA, C. A. S.; MALABARBA, M. C. S. L.; LUCENA, Z. M. S.; REIS, R. E.; LANGEANI, F.; CASSATI, L.; BERTACO, V. A.; MOREIRA, C.; LUCINDA, P. H. F. Genera Incertae Sedis in Characidae. In: REIS, R. E.; KULLANDER, S. O.; FERRARIS, C. J. (Org.). **Check list of the freshwater fishes of south and Central America**. Porto Alegre: Edipucrs, 2003. p. 106-156.

LOPERA-BARRERO, N. M.; RIBEIRO, R. P.; VARGAS, L.; POVH, J. A.; GOMES, P. C.; MANGOLIN, C. A.; BOSO, K. M. O.; GUALDA, T. Genetic characterization of *Prochilodus lineatus* (Valenciennes, 1836) (Characiformes: Prochilodontidae) stocks, used in stocking programs: importance for the ichthyofauna conservation and ecosystem. **Bioscience Journal**, v. 24, n. 4, p. 86-93, 2008.

LOPES, C. M.; ALMEIDA, F. S.; ORSI, M. L.; BRITTO, S. G. C.; SIROL, R. N.; SODRÉ, L. M. K. Fish passage ladders from Canoas Complex - Paranapanema river: evaluation of genetic structure maintenance of *Salminus brasiliensis* (Teleostei: Characiformes). **Neotropical Ichthyology**, v. 2, n. 2, p. 131-138, 2007.

LYNCH, M.; MILLIGAN, B. G. Analysis of population structure with RAPD markers. **Molecular Ecology**, v. 3, n. 2, p. 91-99, 1994.

MACHADO-SCHIAFFINO, G.; DOPICO, E.; GARCIA-VAZQUEZ, E. Genetic variation losses in Atlantic salmon stocks created for supportive breeding. **Aquaculture**, v. 264, n. 1/4, p. 59-65, 2007.

MILLER, M. P. **Tools for population genetic analyses (TFPGA) 1.3**: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author, 1997.

MOREIRA, A. A.; HILSDORF, A. W. S.; SILVA, J. V.; SOUZA, V. R. Variabilidade genética de duas variedades de tilápia nilótica por meio de marcadores microssatélites. **Pesquisa Agropecuária Brasileira**, v. 42, n. 4, p. 521-526, 2007.

NEI, M. Estimation of average heterozygosity and genetic distance from a small number of individual. **Genetics**, v. 89, n. 3, p. 583-590, 1978.

ORMEROD, S. J. Current issue with fish and fisheries: editor's overview and introduction. **Journal of Applied Biology**, v. 40, n. 2, p. 204-213, 2003.

PEREIRA, J. C.; LINO, P. G.; LEITÃO, A.; JOAQUIM, S.; CHAVES, R.; POUSÃO-FERREIRA, P.; GUEDES-PINTO, H.; NEVES, S. M. Genetic differences between wild and hatchery populations of *Diplodus sargus* and *D. vulgaris* inferred from RAPD markers: implications for production and restocking programs design. **Journal of Applied Genetics**, v. 51, n. 1, p. 67-72, 2010.

POMPEU, P. S.; AGOSTINHO, A. A.; PELICICE, F. M. Existing and future challenges: the concept of successful fish passage in South America. **River Research and Applications**, v. 28, n. 4, p. 504-512, 2012.

POVH, J. A.; RIBEIRO, R. P.; SIROL, R. N.; STREIT JUNIOR, D. P.; LOPERA-BARRERO, N. M.; VARGAS, L.; GOMES, P. C.; LOPES, T. S. Diversidade genética de pacu do rio Paranapanema e do estoque de um programa de repovoamento. **Pesquisa Agropecuária Brasileira**, v. 43, n. 2, p. 201-206, 2008.

RAMELLA, M. S.; KROTH, M. A.; MEURER, S.; NUÑER, A. P. O.; ZANIBONI FILHO, E.; ARISI, A. C. M. 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**, v. 49, n. 4, p. 589-598, 2006.

RESENDE, E. K. Migratory fishes of the Paraguay-Paraná Basin, excluding the upper Paraná Basin. In: CAROLSFELD, J.; HARVEY, B.; ROSS, C. A.; BAER, A. (Ed.). **Migratory fishes of south America**: biology, fisheries, and conservation status. Ottawa: International Development Research Center/The World Bank, 2003. p. 99-156.

ROHLF, F. J. *NTSYS-pc.* **Numerical taxonomy system version 2.2**. Setauket: Exeter Publishing, Ltd., 2008.

SIROL, R. N.; BRITTO, S. G. Conservação e manejo da ictiofauna: repovoamento. In: NOGUEIRA, M. G.; HENRY, R.; JORCIN, A. (Org.). **Ecologia de Reservatórios**: Impactos potenciais, ações de manejo e sistemas em cascata. São Carlos: Rima, 2005. p. 275-284.

SOFIA, S. H.; SILVA, C. R. M.; GALINDO, B. A.; ALMEIDA, F. S.; SODRÉ, L. M. K.; MARTINEZ, C. B. R. Population genetic structure of *Astyanax scabripinnis* (Teleostei, Characidae) from na urban stream. **Hydrobiologia**, v. 553, n. 1, p. 245-254, 2006.

TOLEDO-FILHO, S. A.; ALMEIDA-TOLEDO, L. F.; FORESTI, F.; GALHARDO, E.; DONOLA, E. **Conservação genética de peixes em projetos de repovoamento e reservatórios**. São Paulo: USP, 1992. (Cadernos de Ictiogenética).

VIEIRA, F.; POMPEU, P. S. Peixamentos uma alternativa eficiente? **Ciência Hoje**, v. 30, n. 175, p. 28-33, 2001.

WILLIAMS, J. G. K.; KUBELIK, A. R.; LIVAK, K. J.; RAFALSKI, J. A.; TINGEY, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Research**, v. 18, n. 22, p. 6531-6535, 1990.

WOYNAROVICH, E.; HOVÁTH, L. **A propogação artificial de peixes de águas tropicais**. Brasília: FAO/Codevasf, 1983. (Manual de Extensão 5).

WRIGHT, S. **Evolution and the genetics of populations**. London: The University of Chicago Press, 1978.

ZAYAS, M. A.; CORDIVIOLA, E. The conservation state of characidae fish (Pisces: Characiformes) in an area of the Plata Basin, Argentina. **Gayana**, v. 71, n. 2, p. 178-186, 2007.

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