Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

dos Anjos dos Santos, Carlos Henrique; de Brito Leitão, Marco Aurélio; Fernandes Silva de Sousa, Carolina; Ximenes Santana, Givanildo; Paula-Silva, Maria de Nazaré; Fonseca de Almeida-Val, Vera Maria

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Acta Scientiarum. Biological Sciences, vol. 34, núm. 2, 2012, pp. 191-197
Universidade Estadual de Maringá
.png, Brasil

Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v34i2.7149

Genetic variability of wild and captivity populations of *Colossoma macropomum* (Cuvier, 1818)

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ABSTRACT. Tambaqui (*Colossoma macropomum*) is among the most important fish species of the Amazon and one of the most cultivated in Brazil. In the present work we have evaluated the genetic variability of wild and captivity populations of *C. macropomum*. Enzymatic markers were used to estimate the genetic variability of 41 specimens from a wild group; and 30, 33 and 45 from three captivity groups, which came from Pentecostes (Ceará State), Jaboticabal (São Paulo State) and Itacoatiara (Amazonas State), respectively. Nine isoenzymic systems were used to evaluate the genetic variability of these populations. Using zimogram data we obtained the polymorphism level, allele number, allelic frequency, observed and expected heterozigosity, Wright F statistics ($F_{IS,}$ F_{ST}), genetic distance, level of similarity and group analysis. The isoenzymic data showed that, from the nine systems, six presented polymorphic loci (*Fbp-2*, *G6pdh-2*, *G6pdh-3*, *Pgi-1*, *Pgi-2* and *Pgm-1*). The populations from Pentecostes and Jaboticabal presented loss of genetic variability and low heterozigosity, compared to the wild population and to the artificial population acquired at Itacoatiara fish farm. Based on these results and on fish farmer information we could consider the population from Itacoatiara as recently derived from a wild population. Concluding, we suggest that the artificial populations of tambaqui, which contain animals originated from this funding population at Pentecostes, should be renewed with the introduction of a new group of individuals with genetic variability equivalent to the wild population.

Keywords: conservational genetics, isozyme, fish management, tambaqui.

Variabilidade genética de populações selvagens e de cativeiro de *Colossoma macropomum* (Cuvier, 1818)

RESUMO. O tambaqui é uma espécie de peixe bastante importante na região amazônica e uma das espécies mais cultivadas no Brasil. O objetivo deste trabalho foi avaliar a variabilidade genética do *Colossoma macropomum* de cativeiro e selvagem, utilizando marcadores isoenzimáticos. Utilizamos 41 indivíduos de uma população da natureza e 30, 33 e 45 de populações de cativeiro de Pentecoste, Jaboticabal e Itacoatiara, respectivamente. Nove sistemas isoenzimáticos foram utilizados para verificar a variabilidade genética do tambaqui, bem como os níveis de polimorfismos, números de alelos, freqüências alélicas, heterozigosidade observada e esperada, estatística F de Wright (F_{IS} e F_{ST}), distância e similaridade genética, e análise de agrupamento. Das nove isoenzimas analisadas apenas seis sistemas apresentaram polimorfismo (*Fbp-2*, *G6pdh-2*, *G6pdh-3*, *Pgi-1*, *Pgi-2* and *Pgm-1*). Verificamos que as populações de Pentecostes e Jaboticabal estão com falta de heterozigotos e apresentam-se estruturadas geneticamente em relação às populações de Itacoatiara e da Natureza que apresentam excesso de heterozigotos e não são estruturadas. Concluímos que os indivíduos de Itacoatiara são provenientes de populações selvagens e sugerimos que se realize uma renovação dos plantéis de tambaqui de Pentecostes e Jaboticabal, com o intuito de recuperar a variabilidade genética perdida.

Palavras-chave: conservação genética, isoenzimas, manejo, tambaqui.

Introduction

Fish culture is a branch of agribusiness and has been growing in Brazil. The reason for this phenomenon is the decrease of environmental stocks of the main species with commercial value. Within the natural species found in the Brazilian watershed, we can emphasize the tambaqui (*Colossoma macropomum*), a species that can reach 30 kg and has been cultivated in several Brazilian regions (VAL; HONCZARIK, 1995).

There are evidences that wild populations of tambaqui are depleted by the excessive fishing effort and not by their habitat reduction. The idea of creating fish artificially became stronger since the

environmental stocks of these commercially valuable fishes decreased by overfishing (VAL; ALMEIDA-VAL, 1995). This idea has been contributing for the raise of aquaculture intake in the global production of fish and is becoming an alternative to the increase of food production of aquatic origin (ARBELÁEZ-ROJAS et al., 2002).

However, if previous genetic planning is not included for the activity, aquaculture process will not result in the desired genetic integrity of captivity populations, and some management will be required to allow genetic breeding to the stocks. To achieve good results and genetic improvement of the progeny, high genetic variability is needed in founder populations, assuring the existence of sufficient variety that will account for survival and improvements of consecutive generations.

Studies comparing genetic variability of artificial and natural populations of fish are required to understand how the variations on mating choices, survival, reproduction, and growth rate contribute to alterations in genetic and genotypic frequency, and if these alterations improve fish adaptation to changing environments. Almeida-Val and Farias (1996) suggested that the environmental pressure that was imposed to this and other species along their evolutionary history, gives this group a high genotypic variability and, consequently, phenotypic plasticity.

Studies based on protein markers are among the first methodologies used to investigate genetic variability between the 70s and 80s, when few polymorphic loci were discovered (PARKER et al., 1998). A number of allozyme surveys examined the amount of variation in different populations of the same or related species (HEDRICK, 2005). Isozymic markers were widely applied to detect genetic variability losses of fish artificial populations compared to natural populations (RYMAN; STAHL, 1980). Protein markers were also used in phylogenetic studies (TRINGALI et al., 1999) and to detect natural fish population variability (CALCAGNOTTO; TOLEDO-FILHO, 2000).

According to Allendorf and Ryman (1987) and Allendorf et al. (1987), these markers require a great extent of loci to be used in the study of population variability, since the occurrence of mutational events on the genetic material may not always result in alterations of protein structure. Amino acid replacements in the sequence of a protein may not result in changes of its electrophoretic mobility, underestimating allele variability levels (SOLFERINI; SCHEEPMAKER, 2001). However, these systems have been recently used as powerful tools in the analysis of population genetic structure and ecological mechanisms of dispersal, isolation and recolonization in wild fish (PHILLIPS et al., 2009).

Considering the decrease in the survival of artificial populations of tambaqui in the South of Brazil, this study has aimed to analyze the genetic variability of wild and captivity populations of *Colossoma macroporum*.

Material and methods

Wild population of *Colossoma macropomum* was collected in the Lake Catalão, nearby the city of Manaus – AM (41 specimes – wild populations). Captivity populations were acquired from Departamento Nacional de Obras Contra as Secas - DNOCS, Pentencoste, Ceará State, (30 specimens – Pentecoste populations); Centro de Aquicultura da Universidade do Estado de São Paulo – Caunesp, Jaboticabal, São Paulo State, (33 specimens – Jaboticabal populations); and Amazon-Fish, Itacoatiara, Amazonas State, (45 specimens – Itacoatiara populations).

The specimens were collected and conserved in liquid nitrogen at the Laboratory of Ecophysiology and Molecular Evolution from National Institute for Amazon Research (LEEM-INPA) in Manaus, Amazonas State, Brazil. Tissue samples as muscle, liver and heart were homogenized with a plastic stick in propylene tubes (1.5 mL) with 100 μL of Tris-HCl 0.02 M, pH 7.5 buffer and centrifuged at 44,000 *g* at 5°C during 30 min. Due to the presence of a great amount of fat in the liver, 100 μL of carbon tetrachloride (CCl_4) was added to the tubes (PASTEUR et al., 1988).

Gels were prepared with 13% corn starch (VAL et al., 1981). Two buffer solutions were used: Tris 0.135 M/Citric acid 0.043 M pH 7.0 (TC) (SHAW; PRASAD, 1970) and Tris 0.18 M/Boric acid 0.1/EDTA 0.004 M pH 8.6 (TBE) (BOYER et al., 1963). The gel was made with TC diluted 1:15 and TBE diluted 1:4. The enzyme extract was applied to the gel using Kodabromide (Kodak®) paper strips (4 mm x 8 mm) soaked with the samples. A voltage gradient of 200 V was applied to the gel during 16 hours (TC buffer), and a voltage gradient of 400 V was applied to the gel during 10 hours for TBE buffer. After electrophoreses, the gels were horizontally sliced lengthwise into two slabs, which were incubated with specific staining solutions according to Murphy et al. (1996) (Table 1). Allele and loci designations followed the International Union of Biochemistry and Molecular Biology (IUBMB, 1992) nomenclature.

Table 1. Buffer systems used for separation of enzymes.

Enzymes (abbreviation)	EC[a]	Tissue[b]	Buffer
Alcohol dehydrogenase (*Adh*)	1.1.1.1	L	TBE
Malate dehydrogenase -NAD$^+$ (*Me*)	1.1.1.40	L	TC
Fructose-1,6-bisphosphatase (*Fbp*)	3.1.3.11	L	TC
Glucose-6-phosphate dehydrogenase (*G6pdh*)	1.1.1.49	L	TBE
L-Lactate dehydrogenase (*Ldh*)	1.1.1.27	H, M	TC
Malate dehydrogenase (*Mdh*)	1.1.1.37	H, L, M	TC
Glucose-6-phosphate isomerase (*Pgi*)	5.3.1.9	H, L, M	TC
Phosphoglucomutase (*Pgm*)	5.4.2.2	H, L, M	TC
Superoxide dismutase (*Sod*)	1.15.1.1	H, L, M	TBE

[a]EC: Enzyme Commission number; [b]H: Heart; L: Live; M: Muscle.

Genetic variability was estimated using Nei's (1978) average heterozygosity (He and Ho). Wright's F-statistics was calculate using the methods described by Weir and Cockerham (1984). The homogeneity of allele frequencies between populations was checked through contingency chi-squared tests. Unbiased genetic identity and genetic distance were also calculated according to Nei (1978). All estimates were calculated using Biosys-1 software (SWOFFORD; SELANDER, 1981).

Results and discussion

The especimen's isozymic profiles were analyzed through corn starch gel electrophoresis using nine enzymatic systems (Table 1). Table 2 shows the allelic frequencies obtained for the 18 loci to the population from each locality. Despite the small quantity of isoenzyme systems analyzed, the loci/allele numbers were adequate for the population analysis and genetic parameters obtained. The electrophoretic banding pattern was similar to that described by Limeira et al. (2009) in two populations of *Rineloricaria* for the average polymorphism and allelic frequency.

Six, out of 18 loci (33.3%) exhibited polymorphism (*Fbp-2*; *G6pdh-2*; *G6pdh-3*; *Gpi-1*; *Gpi-2* and *Pgm-1*) in the wild population; three (16.7%) in the Pentecostes population (*Fbp-2*; *Gpi-2* and *Pgm-1*); three (16.7%) in Jaboticabal population (*Fbp-2; G6pdh-3* and *Gpi-1*) and six (33.3%) in Itacoatiara population (*Fbp-2*; *G6pdh-2*; *G6pdh-3*; *Gpi-1*; *Gpi-2* and *Pgm-1*) (Table 3).

Low polymorphism rate was also detected as the number of alleles per locus (Table 3). We did not detect deviation from Hardy-Weinberg equilibrium at any of the polymorphic loci in *C. macropomum* populations (Table 4).

Mean value for observed and expected heterozigosities for all loci of *C. macropomum* were: Wild population, Ho = 0.125 and He = 0.120; Pentecoste, Ho = 0.053 and He 0.106; Jaboticabal, Ho = 0.059 and He = 0.118; and Itacoatiara, Ho = 0.134 and He = 0.131. Wright's F statistics (F_{IS} and F_{ST}) for estimating the excess of heterozygotes and population structure (WRIGHT, 1978) was calculated for all loci. Mean values were F_{IS} = -0.04 and F_{ST} = 0.07 for wild population; F_{IS} = 0.50 and F_{ST} = 0.62 for Pentecoste; F_{IS} = 0.50 and F_{ST} = 0.56 for Jaboticabal; and F_{IS} = -0.02 and F_{ST} = 0.02 for Itacoatiara. Wright`s F statistics shows that populations of Pentecost and Jaboticabal lack heterozygotes ($F_{IS} > 0$) and show high structure ($F_{ST} > 0.25$). However, we found that the wild and Itacoatiara populations presented excess of heterozygotes ($F_{IS} < 0$) low levels of structure ($F_{ST} < 0.05$).

Table 2. Allelic frequency for all *Colossoma macropomum* populations.

Loci	Alleles	Wild population Lake Catalão	Pentecostes	Artificial populations Jaboticabal	Itacoatiara
Adh – 1	100	1.000	1.000	1.000	1.000
Me – 1	100	1.000	1.000	1.000	1.000
Me – 2	100	1.000	1.000	1.000	1.000
Me – 3	100	1.000	1.000	1.000	1.000
Fbp – 1	100	1.000	1.000	1.000	1.000
Fbp – 2	100	0.648	0.621	0.661	0.733
	90	0.352	0.379	0.339	0.267
Ldh – 1	100	1.000	1.000	1.000	1.000
Ldh – 2	100	1.000	1.000	1.000	1.000
G6pdh – 1	100	1.000	1.000	1.000	1.000
G6pdh – 2	100	0.712	1.000	1.000	0.567
	110	0.288	0.000	0.000	0.433
G6pdh – 3	100	0.846	1.000	0.889	0.733
	90	0.154	0.000	0.111	0.267
G6pdh – 4	100	1.000	1.000	1.000	1.000
Mdh – 1	100	1.000	1.000	1.000	1.000
Mdh – 2	100	1.000	1.000	1.000	1.000
Gpi – 1	100	0.731	1.000	0.729	0.810
	80	0.269	0.000	0.271	0.190
Gpi – 2	100	0.865	0.981	1.000	0.810
	120	0.135	0.019	0.000	0.190
Pgm – 1	100	0.648	0.304	1.000	0.632
	105	0.135	0.696	0.000	0.368
Sod – 1	100	1.000	1.000	1.000	1.000

Table 3. Genetic variability of the 18 studied loci in *Colossoma macropomum* populations (mean ± standard error).

Population	# individuals by loci	# alleles by loci	% of polymorphic loci
Wild	30.7 ± 1.1	1.3 ± 0.1	33.3
Pentecostes	26.0 ± 1.5	1.2 ± 0.1	16.7
Jaboticabal	26.8 ± 1.3	1.2 ± 0.1	16.7
Itacoatiara	25.1 ± 1.6	1.3 ± 0.1	33.3

Table 4. Chi square contingency analysis for polymorphic loci: *Fbp-2*, *G6pdh-2*, *G6pdh-3*, *Gpi-1*, *Gpi-2* and *Pgm-1*.

Loci	Alleles number	Chi square	DF[a]	P[b]
Fbp–2	2	1.844	3	0.60541
G6pdh–2	2	30.398	3	0.00000
G6pdh–3	2	9.462	3	0.02374
Gpi–1	2	16.904	3	0.00074
Gpi–2	2	15.081	3	0.00175
Pgm–1	2	62.844	3	0.00000
Total		136.532	-	0.00000

[a]DF: Degrees of Freedom; [b]P: Chi square probability.

Nei's genetic identity and distance values (NEI, 1978) values are presented in table 4. Figure 1 shows

UPGMA dendrogram using Nei's (1978) similarity indices for analyzed populations.

Table 5. Identity (above diagonal) and genetic distance (below diagonal) of Nei (1978) among four samples of *Colossoma macropomum*.

Population	Environment	Pentecoste	Jaboticabal	Itacoatiara
Wild population	-	0.984	0.971	1.000
Pentecoste	0.017	-	0.991	0.976
Jaboticabal	0.030	0.009	-	0.963
Itacoatiara	0.000	0.024	0.038	-

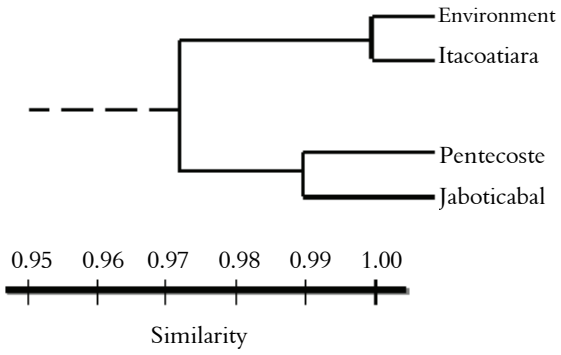

Figure 1. UPGMA dendrogram from the similarity indices of Nei (1978) for the four *Colossoma macropomum* populations.

According to Chaplin et al. (1998) and Hovgaard et al. (2006) enzyme polymorphic loci are successfully used to differentiate populations from diverse fish species. The efficiency in using isoenzyme as genetic markers and as a tool for diagnosis, identification and limitation of fish stocks or any other live stock has also been recently reaffirmed in literature (PHILLIPS et al., 2009; SILVA et al., 2008).

Table 2 shows the allelic frequency of nine isoenzyme systems. In the present study, only six, out of the analyzed 18 loci were polymorphic. *Fbp-2* presented polymorphism in all analyzed populations of Tambaqui; *G6pdh-2* presented monomorphism in Pentecostes and Jaboticabal populations; *G6pdh-3* and *Gpi-1* were monomorphic for Pentecostes specimens and the *Gpi-2* and *Pgm-1* were monomorphic for Jaboticabal specimens. These data indicate a loss of genetic variability in artificial populations that may be the result of an extensive period of population isolation from nature and a high degree of inbreeding. This may explain these findings with such a small amount of variable loci.

We also verified that the number of alleles per locus was practically the same in all populations. It has been observed that the wild and Itacoatiara populations present the same polymorphism index and that the Pentecostes and Jaboticabal populations present similar values indicating similar origin for the two groups (Table 3). According to Avise and Aquadro (1982) populations differing at the level of 10% generally represent distinct species.

The isoenzyme systems can reveal the differential expression of duplicated loci among different tissues and in different developmental stages of the animal. Therefore, the same isoenzyme system can show different patterns of enzymatic activity in each cell when present. According to Fisher and Whitt (1978) these differences cause regulation on gene's tissue and ontogenetic expression. Regulatory gene variation may be operative in determining the number of molecules of enzyme in a cell. Higher or lower levels of isozymes are also related to different metabolic activity of the tissues, as well as to the respective subcellular compartmentalization of enzymes (ZAWADZKI et al., 2001).

The genetic variability analysis revealed the occurrence of a wide variation in heterozygosity when tambaqui populations are compared to the average heterozygosity for freshwater fish (He = 0.051) established by Ward et al. (1992). Wild population (0.125), Pentecostes (0.053), Jaboticabal (0.059) and Itacoatiara (0.134) presented higher values of heterozygosity. According to Ward et al. (1994) the values of expected heterozygosity for 107 species of fishes analyzed by starch gel electrophoresis, range from 0 to 0.05 in 54% of them, from 0.05 to 0.10 in 30%, from 0.10 to 0.15 in 12%, and higher than 0.15 in 4% of them. Tambaqui wild population follow in the 4% of highest heterozigosity levels. The loss presented in captivity populations is directly related to the size of breeding stocks and to the increased inbreeding in fish farms along the generations. Thus, the time of isolation of these populations (number of generations since the beginning of that stock) contributed to the loss of heterozigosity. It is important to emphasize that the population from Jaboticabal has been funded by the Pentecostes DNOCS population.

We consider the isolation time (ZAWADZKI et al., 2005) and the decrease in population size the main cause of the increase in the rate of inbreeding and in the consequent loss of genetic variability of populations of Pentecoste and Jaboticabal.

According to Frankham et al. (2004) captive populations of threatened species lose genetic diversity due to the bottleneck at found, small subsequent population size and because Ne (population effective size) is lower than N (population size). Consequently, loss of genetic

diversity is minimized by using an adequate number of founders, minimizing the number of generations by breeding from older animals, or using cryopreservation, and maximizing both the population size and Ne/N ratio.

We suggest either a replacement or renovation in the parental couples of Pentecostes and Jaboticabal fish stocks as the best way to recover their genetic variability. Populations utilized in fish culture, or restocking programs, must be founded from a sufficiently bigger number of specimens (ALLENDORF; RYMAN, 1987) in order to avoid an important decrease in Ne. However, big populations originated from small groups or from unequal progenitor contribution can exhibit a low Ne (BRISCOE et al., 1992), and low Ne is probably amongst the most important causes of genetic variability loss, resulting in genetic drift (FRANKHAM, 1996).

The genetic effect of stock formation in fish culture has been documented in several fish species and has shown that even for stocks formed by 100 specimens, no population will absorb more than 45% of these specimens' contributions without suffering a drastic and potentially dangerous fall in the genetic variability (TANIGUCHI, 2003). Consequently, isolated populations, especially small ones, may have their heterozygosity eroded by inbreeding process or by chance (SOLÉ-CAVA, 2001).

The values of genetic distance and genetic identity among tambaqui populations (Table 4) showed that the individuals from the Itacoatiara population probably came from the environment population (wild population) or were recently founded by adults from nature. This assumption is based on the fact that the values of genetic distance and genetic similarity between these populations showed no difference (genetic distance = 0 and genetic similarity = 1.0). A similar pattern was also observed for Pentecostes and Jaboticabal populations and we can presume that both belong to the same founder stock.

Population genetic analysis, using different types of genetic molecular markers, complemented with other fishery biology approaches, is urgently needed for the current management of captivity populations and for future health of cultured fish generations.

Our following plans are to keep searching for appropriate enzyme or isozyme variant alleles, which can conveniently diagnose tambaqui and its genetic stocks, to help in the long-term management of this species in the intensive and extensive aquaculture activities.

Conclusion

Isoenzymes *Fbp-2*, *G6pdh-2*, *G6pdh-3*, *Gpi-1*, *Gpi-2* and *Pgm-1* showed to be efficient in detecting genetic variability loss in captivity tambaqui populations compared to wild population.

The specimens from Pentecostes and Jaboticabal presented loss of genetic variability, what requires an immediate replacement or renewal of these stocks.

This study showed that the population of Itacoatiara was founded recently from wild and variable specimens and that the number of found stocks is, probably, bigger than the number of founding stocks of the artificial populations here studied.

Acknowledgements

This research had financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa da Amazônia (FAPEAM) through project PRONEX (no. 503849/2004-9), and was granted by CNPq to Carlos Henrique dos Anjos dos Santos (PhD scholarship) and Vera Maria Fonseca de Almeida e Val (research fellowship). The authors thank DNOCS (Pentecoste, Ceará State), CAUNESP (Jaboticabal, São Paulo State) and fish farm Itacoatiara (Amazonas State) for the samples of *Colossoma macropomum*.

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Received on May 26, 2009.
Accepted on May 18, 2010.