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LOSS OF GENETIC DIVERSITY AMONG PIRARUCU BROODSTOCKS IN FISH FARMS: A PILOT STUDY

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ABSTRACT - Molecular markers can be used to monitor genetic variability in cultivated fish stocks with the aim of developing better aquaculture techniques and increasing productivity. In view of the above, the objective of the present work was to evaluate the genetic variability in pirarucu broodstocks in fish farms in the southeast and northeast of the of Pará state, Brazil. The samples were genotyped using four microsatellite markers, and genetic diversity indices were calculated for each broodstock. A total of 16 alleles were identified from the four loci tested in the 33 pirarucu samples, with means of 3.00, 2.75, and 2.25 alleles per locus in broodstocks from Tucumã, Parauapebas, and Moju municipalities, respectively. The Parauapebas broodstock had the highest mean observed heterozygosity. In the broodstocks from Tucumã and Parauapebas, the markers AgCAm16 and AgCAm18 were not at Hardy-Weinberg equilibrium. This pilot study showed that there were considerable losses of genetic variability in pirarucu breeding centers in the municipalities of Tucumã, Parauapebas and Moju compared to the variability in the natural populations of this fish species.
Keywords: *Arapaima gigas*, aquaculture, molecular markers, microsatellites, population genetics.

PERDA DE DIVERSIDADE GENÉTICA ENTRE ESTOQUES DE REPRODUTORES DE PIRARUCU: UM ESTUDO PILOTO

RESUMO - Os marcadores moleculares podem ser usados para monitorar a variabilidade genética em estoques de peixes cultivados com o objetivo de desenvolver melhores técnicas de aquicultura e aumentar a produtividade. Diante do exposto, objetivou-se com o presente trabalho avaliar a variabilidade genética em reprodutores de pirarucu em pisciculturas no sudeste e nordeste do estado do Pará, Brasil. As amostras foram genotipadas usando quatro marcadores microssatélites e os índices de diversidade genética foram calculados para cada estoque de reprodutor. Foram identificados um total de 16 alelos nos quatro locos testados nas 33 amostras de pirarucu, com médias de 3,00, 2,75 e 2,25 alelos por locus em estoques de reprodutores dos municípios de Tucumã, Parauapebas e Moju, respectivamente. O estoque de reprodutores de Parauapebas apresentou a maior heterozigosidade média observada. Nos estoques de Tucumã e Parauapebas, os marcadores AgCAm16 e AgCAm18 não estavam em equilíbrio de Hardy-Weinberg. Este estudo piloto mostrou que houve perdas consideráveis de variabilidade genética nos centros de reprodução de pirarucu nos municípios de Tucumã, Parauapebas e Moju em comparação com a variabilidade nas populações naturais desta espécie de peixe.
Palavras-chave: *Arapaima gigas*, aquicultura, marcadores moleculares, microssatélites, genética de populações.

INTRODUCTION

The pirarucu (*Arapaima gigas*) is among the 15 most intensively farmed fish species in Brazil. Production has taken a leap in recent years with 189.265 thousand tons produced in 2020 (IBGE, 2020), contributed to the growth of Brazilian fish farming. Fish breeding farms play key roles in maintaining genetic variability, which is fundamental to preventing excessive endogamy and the consequent loss of the adaptive capacity that forms the basis for breeding programs (AMARAL et al., 2019).

Molecular markers can be used to monitor genetic variability in cultivated fish stocks with the aim of developing better aquaculture techniques and increasing productivity (DUDU et al., 2015; FAZZI-GOMES et al. (2021b); LOPERA-BARRERO et al., 2015). Our area of interest, the Carajás region (Southeast mesoregion, Pará state), has not implemented any type of strategic plan for the sector; therefore, we hypothesized that the pirarucu stocks there would have low genetic diversity. We expected the genetic diversity of *A. gigas* broodstocks to be lower than that of wild populations because, although previous studies by our group have found moderate genetic differentiation and high genetic variability among natural *A. gigas* populations in the Lower Amazon region (FAZZI-GOMES et al., 2017), studies of cultivated broodstocks of other Amazonian fish species have generally reported reduced genetic variability (LOPERA-BARRERO et al., 2015; AGUIAR et al., 2019). Therefore, the objective of this study was to assess the genetic variability of pirarucu broodstocks in breeding farms in the Southeast and Northeast

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mesoregions of Pará state, Brazil, using microsatellite markers.

MATERIALS AND METHODS

The procedures adopted in this study were approved by the Ethics Committee of the Federal Rural University of Amazonia (23084.000623/2018-32).

A total of 33 samples, which were each composed of a small fragment of the caudal fin, were collected from pirarucu broodstocks in fish breeding farms in municipalities in both the Southeast mesoregion of Pará [Tucumã (n = 10) and Parauapebas (n = 11)] and the Northeast mesoregion of Pará [Moju (n = 12)]. The tissue samples were preserved in 95% ethanol and subsequently stored at -20 ºC. DNA extraction was performed using the protocol described by Fazzi-Gomes et al. (2021a). The DNA concentration in the samples was calculated based on the absorbance index (A) of the bases at 260 nm using a NanoDrop™ ND-1000 spectrophotometer (Thermo Scientific) and standardized to a concentration of 5 ng μL^{-1}.

The samples were genotyped using four microsatellite markers (AgCAm16, AgCAm15, AgCAm18, and AgCTm3) from the genotyping system developed by Hamoy et al. (2008). After this, 1 µL of each PCR product was added to 8.5 µL of highly deionized (Hi-Di) formamide (Applied Biosystems) and 0.5 µL of Gene Scan 500 LIZ (Applied Biosystems), and then visualized in the Applied Biosystems 3130 Genetic Analyzer automated capillary sequencer. Individuals were genotyped using the Gene Mapper 3.7 program (Applied Biosystems). Genetic diversity indices were then calculated. The observed (H_o) and expected heterozygosity (H_e) and possible deviations from Hardy-Weinberg equilibrium were determined for each microsatellite locus using the Arlequin 3.5 program, followed by the Bonferroni correction (RICE, 1989) of the p-values found. The number of alleles per locus (N_A) was calculated using the Fstat version 2.9.3.2 program. The interpopulation genetic differentiation among broodstocks was verified by calculating FST statistics (WEIR and COCKERHAM, 1984) in the Arlequin 3.5 program (EXCOFFIER; LISCHER 2009).

RESULTS AND DISCUSSION

A total of 16 alleles were found for the 4 microsatellite loci tested in the 33 pirarucu broodstock samples analyzed. The *Agcam16* and *Agcam18* loci were not at Hardy-Weinberg equilibrium (after Bonferroni correction - $p<0.005$) due to there being an excess of heterozygotes in the broodstocks from Tucumã and Parauapebas, respectively. The Parauapebas broodstock had the highest mean observed heterozygosity value (H_o = 0.75), followed by that in Tucumã (H_o = 0.7) and Moju (H_o = 0.66). Regarding mean expected heterozygosity values, those of the broodstocks from Tucumã (H_e = 0.6) and Parauapebas (H_e = 0.52) were the highest, followed by that of Moju (H_e = 0.47). The broodstock with the highest mean number of alleles per locus was Tucumã (N_A = 3), followed by Parauapebas (N_A = 2.75) and Moju (N_A = 2.25).

The analysis of the interpopulation genetic differentiation among the three broodstocks showed moderate values for the differentiation between Moju and Parauapebas (FST = 0.08020) and Moju and Tucumã (FST = 0.09332), and little differentiation between Parauapebas and Tucumã (FST = 0.02079). The decrease in genetic diversity is evident in the number of alleles per locus (N_A) calculated, as the Moju broodstock had a mean N_A of 2.25, followed by that of the broodstocks from Parauapebas, with a mean N_A of 2.75, and from Tucumã, with a mean N_A of 3. Several studies have analyzed natural pirarucu populations, such as Hamoy et al. (2008), who found they had a mean N_A of 6.25, and Fazzi-Gomes et al. (2017), who found they had a mean N_A of 6.5 and 6.75 in populations from the Curuai and Paru lakes, respectively (Figure 1).

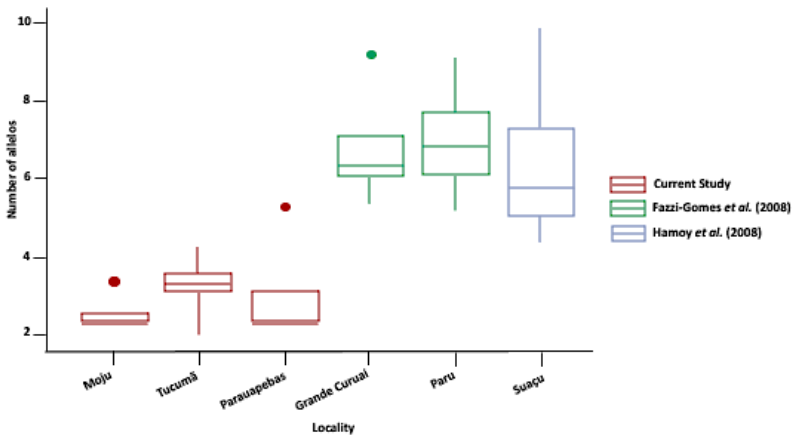

FIGURE 1 - Comparative boxplot for the mean numbers of alleles per locus (mean NA) in pirarucu populations from Moju, Tucumã, and Parauapebas (broodstocks) and data from the literature for natural populations.

This loss of genetic variability in pirarucu broodstocks is likely related to the low number of animals used for mating in breeding farms. According to Lima et al. (2015), breeding in fish farms is totally random, but information on the lineages of the animals used is fundamental to avoid consanguineous couples, such as mating between siblings, and thus to ensure a higher economic yield, reduce mortality, and increase the

production efficiency of the offspring, as well as to maintain genetic diversity.

The founder effect is another factor that could influence the loss of genetic variability in broodstocks, since the small samples of founders used cannot represent the true genetic diversity in natural populations (COLLEY and FISCHER, 2013). Considering the FST values found, the population differentiation results of this study agreed with the standards proposed by Hartl and Clark (2010) and showed that the Moju broodstock is moderately differentiated from those in Parauapebas and Tucumã. This moderate differentiation could be related to the different origins of the breeders used in these broodstocks. Similarly, the low differentiation among breeders in the broodstocks from Tucumã and Parauapebas could be explained by the fact that these breeders came from the same or a similar founder stock. Moreover, these two municipalities are located close together (265 km apart), so there is no isolation by distance (AGUILLON et al., 2017), which can also explain the lack of population differentiation between them.

Hardy-Weinberg equilibrium was not observed in this study due to an excess of heterozygous individuals in the analyzed broodstocks, which may have occurred due to the type of microsatellite markers used, since the use of dinucleotide markers often leads to PCR artifacts, known as stutter bands (QUÍLEZ et al., 2014), which impair differentiation between heterozygotes and homozygotes. On the contrary, tri- and tetranucleotide microsatellite markers result in fewer stutters.

The next challenge will be to increase the number of individuals analyzed to obtain more accurate information, and to perform more robust analyses using larger numbers of molecular markers. In addition, it is necessary to develop new microsatellite markers belonging to other classes than those used herein, such as tri- and tetranucleotide markers, to obtain more accurate and reliable genetic data. These efforts will contribute to the implementation of breeding and genetic improvement programs aimed at increasing the productivity of local and national aquaculture operations.

CONCLUSIONS

This pilot study showed that there have been considerable losses of genetic variability in pirarucu breeding farms in the municipalities of Tucumã, Parauapebas, and Moju compared to the variability in the natural populations of this fish species.

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