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EVOLUCIÓN

GENETIC VARIABILITY AND REPRODUCTIVE CHARACTERISTICS OF ZEBRAFISH (*Cyprinidae Danio rerio*) STOCKS

Variabilidad genética y características reproductivas de poblaciones de pez cebra (*Cyprinidae Danio rerio*)

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ABSTRACT

Specimens of cultured zebrafish acquired from different fish farms in Brazil may show genetic variability and alteration in allele frequency due to genetic drift and selective pressure in a captive environment, resulting in the differentiation of productive and reproductive characteristics. The aim of this study was to evaluate the genetic variability and reproductive characteristics of 180 zebrafish specimens from six Brazilian fish farms. A deviation from the Hardy-Weinberg equilibrium was observed in all evaluated stocks. Differentiation among stocks was observed in the amount of genetic variability with respect to observed heterozygosity and the inbreeding coefficient (F_{IS}). Genetic distance between stocks was determined through the F_{st} index, and the formation of four distinct groups was observed by plotting the dendrogram based on Nei's genetic distance. Differences were observed among reproductive parameters, such as the average number of eggs per female and hatchability. This second parameter proved to be related to the level of inbreeding of the population, whereas this effect was not observed for spawning frequency. We conclude that zebrafish stocks from the 6 different Brazilian fish farms present significant genetic and phenotypic variability. The genetic structure affects fecundity and should be considered when carrying out work where reproductive rates are evaluated.

Keywords: Animal model, consanguinity, DNA, female, fish larvae.

RESUMEN

Especímenes de pez cebra adquiridos en diferentes piscifactorías pueden mostrar variabilidad genética y alteración en la frecuencia de los alelos debido a la deriva genética y presión selectiva llevada a cabo en un ambiente cautivo, lo que resulta en la diferenciación de las características productivas y reproductivas. Este estudio busco evaluar la variabilidad genética y las características reproductivas de 180 especímenes de pez cebra adquiridos de seis piscifactorías brasileras. Hubo una desviación en el equilibrio de Hardy-Weinberg en todas las poblaciones evaluadas. Se encontró diferenciación en términos del grado de variabilidad dentro de las poblaciones, en vista de los resultados de la heterocigosidad observada y el coeficiente de endogamia (F_{is}). La distancia genética entre ellos se verificó usando el índice F_{st}, y se observó la formación de cuatro grupos distintos al trazar el dendrograma basado en la distancia genética de Nei. Se observó una diferencia en relación con los parámetros reproductivos, como el número promedio de huevos por hembra y la incubabilidad. Este segundo parámetro demostró estar relacionado con el nivel de consanguinidad de la población, y este efecto no se verificó para la frecuencia de desove. Se puede considerar que las existencias de pez cebras de diferentes lugares tienen variabilidad genética y fenotípica. La estructura genética influye principalmente en la fertilización y debe tenerse en cuenta al realizar trabajos donde se evalúan los índices reproductivos.

Palabras Clave: Modelo animal, consanguinidad, ADN, hembra, larvas de peces.

INTRODUCTION

Danio rerio (Hamilton, 1822), popularly known as zebrafish, is widely used in scientific research due to its genetic similarity with humans (Vilella et al., 2008; Howe et al., 2013) and its attributes that favor its use and maintenance in laboratories (Best et al., 2010). Under controlled environmental conditions, mainly water temperature (26-28 °C) and photoperiod (14 h light/10 h dark cycle), the zebrafish can reproduce throughout the year, with weekly spawning producing more than 700 eggs per female (Mizgirev and Revskoy, 2010). Its eggs, embryos and larvae are transparent, which allows the monitoring of all initial stages of development (Goessling and Sadler, 2015).

Zebrafish specimens used in studies may originate from lines produced in laboratories, such as the AB and TU lines, maintained and made available by the Zebrafish International Resorce Center (ZIRC) (Nasiadka and Clark, 2012). Methodologies such as transgenesis and mutagenesis are also applied to produce individuals with specific genetic and phenotypic characteristics (Santoriello and Zon, 2012). However, the use of individuals from fish farms or pet stores for research is still common in many scientific areas (Vignet et al., 2013).

The zebrafish naturally inhabits the Asian continent (Menon, 1999) but is now widespread around the world (Kinth et al., 2013) due to its use in research or as an ornamental fish. Individuals from different regions may present genetic and phenotypic variations, which results in the formation of subpopulations in both natural and captive environments (Brown et al., 2012). Studies show that zebrafish from different subpopulations vary in terms of growth (Meyer et al., 2013), behavioral (Vignet et al., 2013), physiological (Monroe et al., 2016) and genetic characteristics (Coe et al., 2009). Among the latter, the level of inbreeding is one key parameter that differs between stocks (Nakadate et al., 2003).

The mating of related individuals generates offspring with higher homozygosity, resulting in an increase in phenotype characteristic of recessive alleles (Willoughby et al., 2015). The decrease in genetic variability is common in individuals kept in captivity, such as in fish farms or laboratories, due to the use of few breeders for the formation of offspring that all share common ancestry. Phenotypes resulting from recessive alleles are associated with reduction of production parameters, such as diminished growth rate and survival and increases in body deformities (Kause et al., 2005). In the case of zebrafish, the relation between genetics and phenotypic characteristics should be analyzed, since these factors can affect research using this animal model as a tool for studying the pathogenesis of human diseases (Brown-Peterson et al., 2011).

Based on this, the present study sought evidence of the existence of genetic and reproductive differences in zebrafish stocks from six Brazilian fish farms. We hypothesized that fish have genetic variability between and within stocks, especially in relation to the inbreeding index. We also hypothesized that these stocks have distinct reproductive characteristics with respect to fertility and hatchability.

MATERIALS AND METHODS

Animals and Ethics Committee

All experiments were carried out in accordance with the regulations established by the Ethics Committee of the State University of Maringá (Brazil), with approval of the project provided under protocol number 6359231115.

The study was conducted with zebrafish animals purchased from six Brazilian fish farms from three different states. The stocks were identified according to their place of origin: one from Paraná (PR), four from São Paulo (SP-1, SP-2, SP-3, and SP-4) and one from Minas Gerais (MG) (Fig. 1).

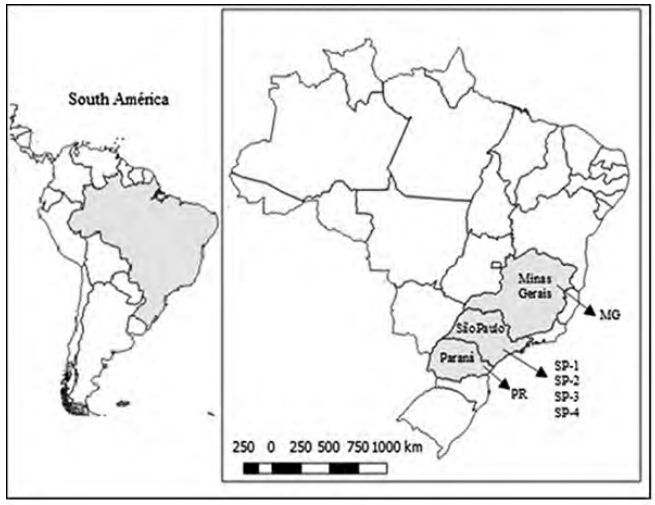

Figure 1. Source maps of zebrafish stocks.

Analysis of genetic variability and structure

For determination of genetic diversity and variability, the caudal fin of 30 fish of each origin was collected, totaling 180 specimens. Sample collection and genetic analysis took place in January 2016.

DNA extraction was performed according to the methodology described by Lopera-Barrero et al. (2008). Subsequently, total DNA was measured with a PICODROP® spectrophotometer (Picodrop Limited, Hinxton, UK), and the sample concentration was standardized to 10 ng/μL. The integrity of the DNA was evaluated on a 1 % agarose gel stained with SYBR SafeTM DNA Gel Stain (Invitrogen, Carlsbad, CA) by electrophoresis conducted in 0.5X TBE buffer (250 mM Tris-HCL, 30 mM boric acid, and 41.5 mM EDTA)

for 2 h at 90 volts. The gel was visualized using a transilluminator, and the image was captured by the software L-PIX HE (Loccus Biotechnology, São Paulo, BR).

Then, the amplification of DNA samples was performed using 13 μL of Mix and 2 μL of genomic DNA (20 ng) in a total final reaction volume of 15 μL. The mix was prepared with 1.5 μL buffer (1x), 0.45 μL MgCl2 (1.5 mM), 1.2 μL dNTPs (0.2 mM), 0.3 μL of each primer (0.2 mM), 0.1 μL Taq DNA polymerase (0.5 U per reaction), 2 μL of genomic DNA, and 9.15 μL ultrapure H2O. The PCR amplification protocol consisted of the following steps: initial denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C (temperature for all primers) for 1 min, extension of 72 °C for 1 min 30 s; and finally, the last extension cycle of 72 °C for 10 min. This process was carried out in a Veriti® thermocycler (Applied Biosystems®, Austin, TX, USA). In the amplification process, ten microsatellite primers (Z-160, Z-5033, Z-4188, Z-5395, Z-1531, Z-4425, Z-4003, Z-4586, Z-5649, Z-1402) specific for *D. rerio* described by Shimoda et al. (1999) were tested.

Amplified samples were subjected to 10 % denaturing polyacrylamide gel electrophoresis conducted in a buffer at 180 V for 7 h. For visualization of the microsatellite alleles, the gels were stained with silver nitrate and subsequently photographed. Allele size was calculated using 100 bp DNA ladder (Invitrogen, Carlsbad, CA).

Evaluation of reproductive performance

The reproductive analysis took place from February to March 2016. During this period, the fish were kept in individual aquaria with 750 mL of water, constant aeration and a 14 h light/10 h dark photoperiod. They were fed twice daily with commercial diet (Bernaqua), containing 57 % crude protein and granulometry of 300 to 500 µm. Mating was performed at eight-day intervals for six weeks, using fived couples from each stock. Specimens for reproductive experiments were six months old and had an average weight and total length of 0.455 ± 0.10 g and 14.64 ± 3.21 mm, respectively.

A ratio of 1:1 (male:female) was used between couples from the same origin, selected according to their sexual dimorphism. They were kept isolated in individual tanks and only placed in the same breeding tanks on the afternoon prior to the scheduled breeding, where they remained until mind-morning of the following day, when they were relocated in their individual aquariums. When the spawning occurred, the eggs were collected, counted, and stored in structures separated according to the couple. On the third day after fertilization, larvae counting was performed. Thus, the reproductive parameters evaluated were spawning frequency (SF), total number of eggs (TE), average number of eggs (AE) per female and hatchability (HA).

Statistical analyses

The genetics parameters evaluated were the number of alleles (N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e) by locus and stock. Likewise, the Hardy-Weinberg equilibrium (H-W) and the inbreeding coefficient (F_{IS}) were determined. F_{ST} index analysis was performed while taking into account the level of genetic differentiation—small (0.00 - 0.05), moderate (0.05 - 0.15), high (0.15 - 0.25) and very high (> 0.25)—as defined by Wright (1978). They were analyzed using the software GenAlEx 6.5 (Peakall and Smouse, 2012). A dendrogram was created by the UPGMA method using Nei's genetic distance, with the packages adegenet (Jombart, 2008) and poppr (Kamvar et al., 2015) (R Foundation for Statistical Computing Vienna, 2011).

The means of the reproductive parameters were submitted to analysis of variance (ANOVA) and Tukey's test at 5 % significance. All statistical analyses were performed using SAS program (Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, USA).

Table 1. Allele characteristics of six microsatellite loci amplified. NA: average number of alleles

Locus	Allele size (pb)	NA	Allele frequency	Allelic diversity (Gst)
Z-5395	170-200	7	0.019/0.167/0.094/0.325/0.214/0.139/0.042	0.075
Z-160	205-270	9	0.069/0.039/0.200/0.003/0.447/0.153/0.042/0.042/0.044/0.003	0.157
Z-4003	130-255	15	0.003/0.011/0.061/0.053/0.203/0.031/0.128/0.064/0.114/0.061/0.194/0.056/0.017/0.003/0.003	0.044
Z-4425	125-255	17	0.197/0.058/0.064/0.092/0.039/0.008/0.028/0.019/0.017/0.011/0.044/0.028/0.158/0.003/0.142/0.014/0.078	0.111
Z-4188	180-270	15	0.011/0.033/0.039/0.053/0.219/0.056/0.072/0.008/0.169/0.161/0.008/0.169/0.161/0.008/0.081/0.064/0.019/0.006	0.129
Z-5649	135-185	10	0.011/0.069/0.072/0.117/0.128/0.131/0.197/0.164/0.067/0.039	0.099

Table 2. Genetic structure of six microsatellite loci of zebrafish stocks acquired in 6 different Brazilian fish farms ("stocks").

Stocks	Variables	Locus						Mean
		Z-5395	Z-160	Z-4003	Z-4425	Z-4188	Z-5649	
SP-1								
	Na	3	6	9	5	6	5	6
	Ho	0.37	0.60	0.83	0.77	0.33	0.27	0.53
	He	0.60	0.72	0.74	0.74	0.79	0.76	0.72
	H-W	0.00	0.00	0.00	0.00	0.00	0.00	
	F_{IS}	0.38	0.18	-0.12	-0.04	0.58	0.64	0.27
SP-2								
	Na	6	4	10	6	9	4	6.5
	Ho	0.67	0.43	0.67	0.37	0.53	0.53	0.53
	He	0.76	0.58	0.82	0.72	0.76	0.74	0.73
	H-W	0.11	0.00	0.03	0.00	0.00	0.00	
	F_{IS}	0.12	0.26	0.18	0.49	0.30	0.28	0.27
SP-3								
	Na	5	4	12	5	8	4	6.3
	Ho	0.63	0.37	0.73	0.40	0.30	0.33	0.46
	He	0.68	0.63	0.88	0.74	0.74	0.59	0.71
	H-W	0.00	0.01	0.20	0.00	0.00	0.00	
	F_{IS}	0.07	0.41	0.17	0.46	0.59	0.44	0.36
SP-4								
	Na	7	3	8	9	7	7	6.8
	Ho	0.60	0.37	0.77	0.40	0.33	0.27	0.45
	He	0.82	0.51	0.84	0.80	0.77	0.82	0.76
	H-W	0.00	0.03	0.23	0.00	0.00	0.00	
	F_{IS}	0.27	0.27	0.08	0.50	0.57	0.67	0.39
MG								
	Na	7	3	8	11	4	8	6.8
	Ho	0.43	0.53	0.63	0.73	0.63	0.27	0.54
	He	0.81	0.40	0.81	0.88	0.61	0.84	0.72
	H-W	0.00	0.26	0.00	0.00	0.00	0.00	
	F_{IS}	0.47	-0.32	0.22	0.17	-0.03	0.68	0.19
PR								
	Na	4	6	9	8	8	8	7.1
	Ho	0.43	0.23	0.63	0.60	0.40	0.30	0.43
	He	0.65	0.77	0.84	0.76	0.79	0.84	0.77
	H-W	0.02	0.00	0.02	0.00	0.00	0.00	
	F_{IS}	0.34	0.70	0.25	0.21	0.49	0.64	0.49

Na, Number of alleles; Ho, Observed heterozygosity; He, Expected heterozygosity; H-W, Hardy-Weinberg equilibrium (*p*-value), F_{IS}, Inbreeding coefficient.

RESULTS

Genetic variability analysis

Of the ten primers used in the study, only six allowed amplification of the samples and data analysis (Table 1). The marker Z-1402 presented only nonspecific bands, whereas Z-5033, Z-1531 and Z-4586 did not allow clear visualization of the alleles, even after performing tests in the amplification protocol.

The Z-4003 locus stood out as being the most polymorphic, presenting the highest values for the evaluated genetic parameters. For SP-1 and PR, deviation from Hardy-Weinberg equilibrium was observed in all evaluated loci ($p < 0.05$). No deviation was observed for the Z-160, Z-5395, and Z-4003 loci for the stocks MG, SP-2 and SP-3, and SP-4, respectively. Although fish from PR showed greater inbreeding, the FIS index showed a deficit of heterozygotes in all stocks evaluated (Table 2).

Regarding FST, all comparisons among the six groups were different ($p < 0.01$). The SP-1 stock showed high differentiation from SP-2 and SP-3, which were acquired from the same state. The other comparisons show a moderate genetic differentiation, with FST values ranging from 0.05-0.15 (Table 3).

Table 3. Estimates of genetic differentiation (F_{st}) among the six stocks of zebrafish acquired in 6 different Brazilian fish farms (SP-1, SP-2 etc.).

	SP-1	SP-2	SP-3	SP-4	MG	PR
SP-1						
SP-2	0.189					
SP-3	0.188	0.054				
SP-4	0.124	0.127	0.099			
MG	0.125	0.113	0.136	0.070		
PR	0.132	0.133	0.098	0.084	0.133	

The genetic identity was visualized by the UPGMA dendrogram obtained from Nei's genetic distance, where the formation of four groups among the stocks was determined (Fig. 2).

Evaluation of reproductive performance

Among all six stocks, the average spawning frequency per female was 2.57 ± 1.33 times over the six-week study period, with a maximum of four spawnings. The average number of eggs per female in each spawning was 261 ± 118 and hatchability was 57 ± 25 %.

Among the six stocks, SP-1 had the highest values for the reproductive parameters evaluated, standing out statistically in the total number of eggs and spawning frequency (Fig. 3a-3c). On the other hand, PR presented the lowest values of several reproductive parameters, differing statistically from SP-1 in the average number of eggs per female and total number of eggs (Fig. 3a-3d). However, these stocks did not differ in terms of spawning frequency (Fig. 3c). Also, there was no statistical difference in hatchability between stocks (Fig. 3b), which ranged from 17 % (PR) to 66 % (SP-1).

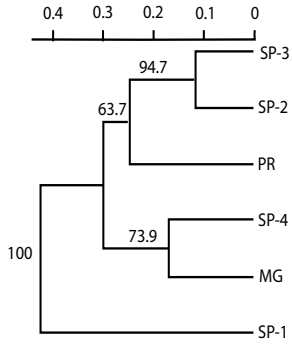

Figure 2. Dendrogram construction based on Nei's genetic distance (UPGMA) between six stocks of zebrafish acquired in different Brazilian fish farms.

DISCUSSION

Genetic variability

The PR stock had the highest number of alleles (7.1); however, lower heterozygosity was observed, indicating a lower genetic variability of this stock compared to the others, due to a high prevalence of homozygous alleles. The analysis of heterozygosity is the basis for stock assessment, and the higher the index, the greater the genetic differentiation within a population (Moreira et al., 2007). The results of the present study for the number of alleles and for the heterozygosity corroborate the results of other studies that evaluated zebrafish specimens produced in fish farms (Gratton et al., 2004; Coe et al., 2009). However, these values were higher than observed in the isogenic lines standardized and consolidated in research, such as the AB and TU (Willoughby et al., 2015), and were lower than those values for wild specimens collected in their natural environment on the Asian continent (Gratton et al., 2004).

The occurrence of greater genetic polymorphism in natural populations is expected, as crosses are usually randomized, and the possibility of inbreeding is lower. On the other hand, the process of domestication and the production of captive animals causes a reduction in the genetic variability of the stocks, which is reflected in the decrease in allelic richness and heterozygosity. This decrease in variability has been confirmed in several commercially produced fish species, for example, *Oreochromis niloticus*, *Colossoma macropomum*, *Brycon orbignyanus* (Fessehaye et al., 2009; Rodriguez-Rodriguez et al., 2010; Lopera-Barrero et al., 2015)a través de marcadores

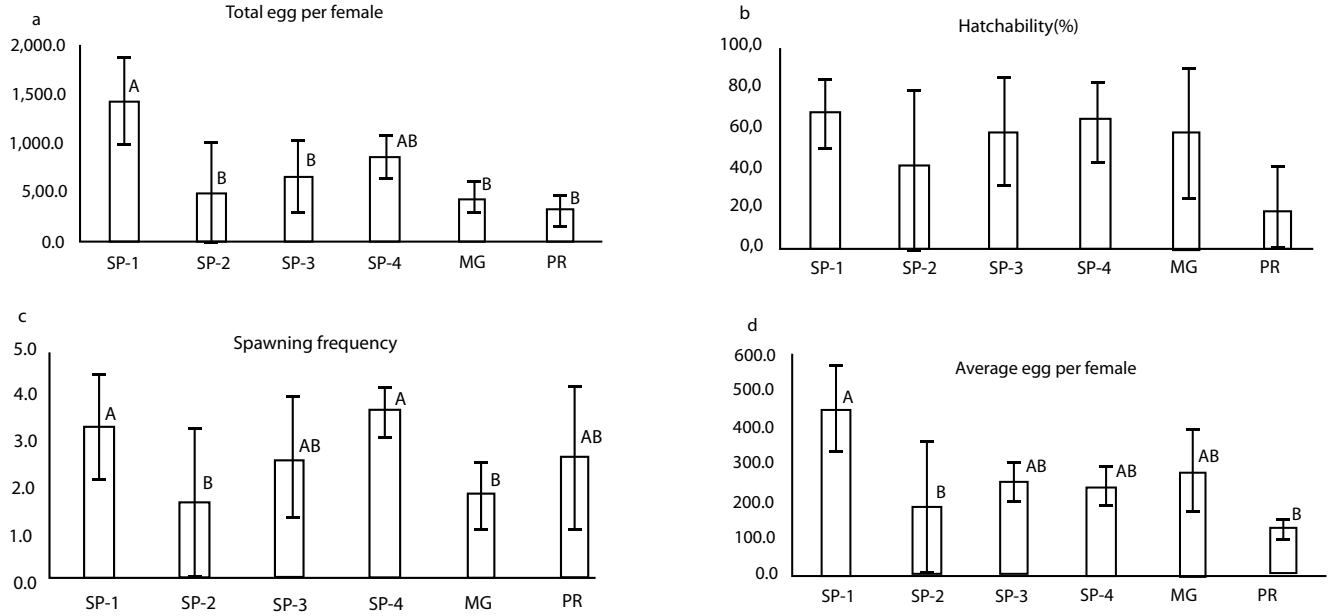

Figure 3. Total number of eggs **(a)**, hatchability (%) **(b)**, spawning frequency **(c)** and average egg per female **(d)** of zebrafish stocks. The data are expressed as the means ± SD. Significant differences ($p < 0.05$) are indicated by superscript letter.

microsatélites. Se analizaron muestras de 44 reproductores, de 70 larvas y de 69 alevinos, con la amplificación de cinco loci descritos para Brycon opalinus. El número de alelos, la heterozigosidad observada (Ho and is usually caused by a lack of control of inbreeding among the breeding groups, including the use of few breeders with high kinship among them, as well as failure to separate parents from offspring over the course of production (Silva et al., 2016) key for the survival of innumerous ecologically or economically important fish species. Among these species are Neotropical silversides (*Atherinella brasiliensis*).

A deviation from Hardy-Weinberg equilibrium was identified in most loci in all stocks. According to Waples (2015), the deviation from Hardy-Weinberg equilibrium can be attributed to selection or genetic drift, which promotes a change in allele frequencies over generations. Populations with low numbers of breeders tend to suffer more directly the consequences of genetic drift. Moreover, deviation in allele frequency is commonly observed in animals kept in captivity, especially in small stocks, or when strong selection occurs. The founder effect (establishment of a new population by a small number of parents) can also accelerate a decrease of variability through genetic drift (Santos et al., 2016), which leads to loss or allele fixation. One of these factors, or a combination of them, might be responsible for the deviation from Hardy-Weinberg equilibrium in the six stocks studied.

The results of the F_{IS} Fixation Index (Coefficient of inbreeding) were in accordance with the observed heterozygosity values, where SP-3, SP-4, and particularly PR were more inbred. Some species of fish have a delicate larval phase, with high mortality contributing to a decrease in genetic variability (Rodriguez-Rodriguez et al., 2010)a través de marcadores microsatélites. Se analizaron muestras de 44 reproductores, de 70 larvas y de 69 alevinos, con la amplificación de cinco loci descritos para Brycon opalinus. El número de alelos, la heterozigosidad observada (Ho) because fewer individuals are likely to become parents, causing a genetic bottleneck. This fact is evident in the zebrafish, which has a larval phase with high mortality (Mizgirev and Revskoy, 2010). Despite this, being a prolific species, only few individuals are required to meet the production demand, especially when considering that research laboratories usually do not need large numbers of individuals to carry out individual experiments.

Therefore, the use of few broodstocks to produce offspring in each generation contributes to a gradual decrease in the genetic variability of the stock and, consequently, to a decrease in heterozygosity and increase of inbreeding. Under laboratory conditions, a decrease in genetic variability and alteration of zebrafish stock genotypes is more significant because many research institutions have a limited number of breeders and limited availability of housing structures for individuals, preventing the maintenance of many diverse breeding individuals to produce new offspring.

Although inbreeding is an important factor in some studies, inappropriate reproductive management is a risk to the maintenance of genetically diverse laboratory stocks. In this sense, Brown et al. (2012) reported on the substructure of zebrafish populations, such as AB and TU, distributed among different laboratories, distinguishing both the genotype and the phenotype.

The genetic distance between stocks were also evaluated, demonstrated by the FST Index, and genetic distance by dendrogram. The results showed different degrees of genetic substructure between stocks. SP-2 and SP-3 showed lower

genetic differentiation, which suggests a possible common origin, followed by MG and SP-4, which are from different Brazilian states.

Differentiation between genetic groups may reveal different degrees of adaptability to environmental challenges (Frankham et al., 2008). Therefore, for laboratory animals used for certain studies (ecotoxicology, e.g.), it is important to have the same phenotypic and genotypic variations as wild populations (Brown et al., 2012). However, from another perspective, uniformity in the phenotype and genotype (isogenic animals) are necessary in medical studies, for example, when testing the effect or dose of a medication.

Reproductive performance

Variation existed in the reproductive parameters among stocks, mainly in average number of eggs per female and spawning frequency. The total number of eggs was related to the average number of eggs released per female and the frequency of spawning. The average weekly spawning of zebrafish is 200 - 300 eggs per female (Menon, 1999), indicating that the fish evaluated in the present study were within the range described in the literature. The PR stock, with lower observed heterozygosity and F_{IS}, produced a lower average number of eggs (118), furthermore characterized by decreased hatchability (17 %).

Studies have shown that inbreeding decreased the gonadosomatic index and the number of eggs of female Coho salmon (*Oncorhynchus kitsutch* Walbaum, 1792) (Gallardo et al., 2004) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (Su et al., 1995), respectively. However, these species have reproductive characteristics that differ from those of zebrafish, which is an iteroparous fish with multiple spawning events and asynchronous oocyte development.

In a study carried out with zebrafish, which evaluated the reproduction of full siblings, half-siblings, and unrelated individuals, the adverse influence of inbreeding was observed only for the number of fertilized eggs produced, but not for the average number of eggs (Mrakovcic and Haley, 2004). Research has shown a greater effect of inbreeding on the gonadosomatic index of males (Mrakovcic and Haley, 2004) and on the quantity and quality of fish sperm (Mehlis et al., 2012; Langen et al., 2017a; Langen et al., 2017b).

The lower quality of the semen in inbred animals negatively affects the fertilization success and consequently the hatching rate. Monson and Sadler (2010) showed a lower number of embryos (fertilized eggs) from inbred mattings among zebrafish laboratory lines (AB and TU). Embryos and larvae of zebrafish are widely used in studies related to embryology and toxicology, as well as in studies where procedures such as transgenesis and mutagenesis are performed (Mizgirev and Revskoy, 2010). In these studies, the hatchability is an important parameter evaluated (Bai et al., 2010; Pamanji et al., 2016; Liang et al., 2017). The results obtained in this study show the need to use individuals from only one location to avoid or reduce the effects of genetic and phenotypic variation on the results of the reproductive parameters. However, this practice is difficult when using specimens from pet shops, which in turn can buy animals from various producers.

The spawning frequency varied among the stocks but did not correlate with the genetic variability data. In a study evaluating Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758), Fessehaye et al. (2009) concluded that kinship had no significant effect on the occurrence of spawning. The large variation in spawning frequency warrants further studies to assess how different environments, densities, sex ratio and lineages may affect reproductive parameters in laboratories. More investigations to analyze how genetic variability can affect other reproductive variables are also needed.

The growing demand of zebrafish as an animal model makes it necessary to increase knowledge about the breeding conditions and genetic parameters of each lineage. The results of the present work will be useful to guide and improve fish breeding practices, to meet quality criteria of research centers using zebrafish and to establish standards to define the genetic and reproductive characteristics of experimental stocks.

CONCLUSION

Zebrafish stocks from different breeding centers in Brazil present genetic variability and divergence as well as distinct reproductive characteristics. When studies are conducted with zebrafish, these variations need to be considered to better meet the quality objectives and generalizability criteria and correctly interpret the results of the studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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