Revista de Ciências Agroveterinárias
Universidade do Estado de Santa Catarina (UDESC)

Research Article

Genetic characterization of four generations of *Oreochromis niloticus* subjected to individual selection in southern Brazil

Caracterização genética de quatro gerações de Oreochromis niloticus submetidas de seleção individual no sul do Brasil

Bruno Corrêa Silva*(ORCID 0000-0002-1478-3777), Adriana Pereira(ORCID 0000-0002-8997-7111), Haluko Massago(ORCID 0000-0003-3627-054X), Keny Henrique Mariguele(ORCID 0000-0001-5344-2302)

Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Itajaí, SC, Brazil. *Author for correspondence: brunosilva@epagri.sc.gov.br

Submission: November 20th, 2024 | Acceptance: March 06th, 2025

ABSTRACT

The process of artificial selection can lead to a rapid decrease in genetic variability across generations, consequently reducing the response to selection in a breeding program. Therefore, understanding the genetic characteristics of the breeding stock is essential, making it important to assess inbreeding and allele frequency changes over generations. In this context, the present study performed the genotypic characterization of four generations of Nile tilapia subjected to individual selection for final weight, aiming to evaluate inbreeding and changes in allele frequencies across generations. The first four generations of GIFT tilapia from the Epagri breeding program were characterized using 11 microsatellite markers. There was only a 5.2% reduction in the total number of alleles across generations. The average number of effective alleles per marker remained similar across generations (4.07 ± 0.4 for G1; 3.88 for G2; 3.86 for G3; and 3.87 for G4). Overall, observed heterozygosity was higher than expected heterozygosity, leading to F_{IS} values of -0.042, 0.027, -0.042, and -0.017 for G1, G2, G3, and G4, respectively. Therefore, individual selection did not result in significant losses of genetic variability across generations; however, significant changes in allele frequencies were observed at some *loci*, which genetically differentiated the generations from one another. Genotypes clustered by the Bayesian method identified 9 groups in G1, 16 groups in G2, and 12 groups in both G3 and G4. Thus, using the microsatellite markers, it was possible to genetically characterize the breeding stocks of four generations of the Epagri tilapia breeding program, and an adequate maintenance of genetic variability in the stock was observed, allowing for the program's continuity. However, important changes in allele frequencies were also detected in the evaluated markers as a result of the applied selection.

KEYWORDS: Tilapia. Microsatellite markers. Breeding program. Inbreeding. Allele frequency.

RESUMO

O processo de seleção artificial pode levar a uma rápida diminuição da variabilidade genética através das gerações e, consequentemente, reduzir a resposta à seleção no programa de melhoramento. Portanto, o conhecimento das características genéticas do plantel de reprodutores é fundamental, tornando importante avaliar a consanguinidade e as mudanças alélicas ao longo das gerações. Sendo assim, o presente estudo realizou a caracterização genotípica de quatro gerações de tilápias-do-nilo submetidas à seleção individual para peso final, a fim de avaliar a consanguinidade e alterações nas frequências alélicas ao longo das gerações. As quatro primeiras gerações de tilápia GIFT do programa de melhoramento da Epagri foram caracterizadas por 11 marcadores microssatélites. Houve uma redução de apenas 5,2% no número total de alelos ao longo das gerações. O número médio de alelos efetivos por marcador permaneceu semelhantes entre as gerações (4,07 ± 0,4 para o G1; 3,88 para o G2; 3,86 para o G3; e 3,87 para G4). Em geral, a heterozigosidade observada foi superior à heterozigosidade esperada, levando a valores de F_{IS} de -0,042, 0,027, -0,042 e -0,017 para G1, G2, G3 e G4, respectivamente. Portanto, a seleção individual não causou perdas significativas de variabilidade genética ao longo de gerações; no entanto, houve mudanças significativas nas frequências alélicas de alguns *loci,* que

diferenciaram geneticamente as gerações entre si. Os genótipos agrupados pelo método Bayesiano permitiram identificar 9 grupos no G1, 16 grupos em G2, e 12 grupos para G3 e G4. Assim, através dos marcadores microssatélites utilizados, foi possível caracterizar genotipicamente os plantéis de reprodutores de tilápia de quatro gerações do programa de melhoramento da Epagri, e verificou-se uma adequada manutenção das variabilidades genéticas do plantel para continuidade do programa. Contudo, foi possível também detectar mudanças importantes nas frequências alélicas dos marcadores avaliados, resultante da seleção aplicada.
PALAVRAS-CHAVE: Tilápia. Marcadores Microssatélites. Programas De Melhoramento. Endogamia. Frequência Alélica.

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* Linnaeus 1758 (Osteichthyes: Cichlidae), stands out as the tropical species with the greatest potential for continental aquaculture, given its fast growth, easy adaptation to farm conditions, and its meat quality. World production of tilapia grew by 34.2% between 2011 and 2022, reaching 5.3 million tons (FAO 2024). Brazil is the world's fourth largest producer, following China, Indonesia, and Egypt. Brazilian tilapia production reached 579 thousand tons in 2023, a 5.28% increase over the previous year. The southern region of Brazil, which has a subtropical climate, accounts for 45.35% of this production (PEIXE-BR 2024).

The Genetically Improved Farmed Tilapia (GIFT) strain is widely distributed across the globe (PONZONI et al. 2011). In Brazil, the primary tilapia strains used in aquaculture are the Chitralada (Thai) strain and those derived from GIFT, such as Supreme, AquaAmerica, GIFT-UEM, GIFT-Epagri, among others (BARROSO et al. 2016). The GIFT strain was originally selected for growth in tropical Asian countries, with a focus on selecting individuals better adapted to these conditions (BENTSEN et al. 2017). Over time, this strain has been introduced to several countries, where further improvements were made by selecting individuals suited to local environments.

In Brazil, breeding programs for this strain began in 2005 following the introduction of 30 GIFT tilapia families from Malaysia. This led to the establishment of a tilapia breeding nucleus in Maringá, Paraná state (OLIVEIRA et al. 2015). In 2011, Epagri, a public institution focused on rural technology research and development in Santa Catarina state, initiated a breeding program with individual tilapia selection using the GIFT strain as the base population (SILVA et al. 2020). The aim of this program was to select tilapia with enhanced growth performance under the rearing conditions of southern Brazil, characterized by a subtropical climate (SILVA et al. 2023).

Epagri has been distributing this improved genetic material to fingerling producers. Between 2013 and 2022, more than 140,000 broodstocks were delivered (SILVA et al. 2023). However, the artificial selection process can rapidly reduce genetic variability across generations. Reduced genetic diversity may negatively affect selection response in breeding programs and lead to declines in zootechnical performance (ALI et al. 2024). Therefore, continuous monitoring of heterozygosity and the inbreeding coefficient in generations of Nile tilapia subjected to selection is essential to ensure the maintenance of genetic diversity and the sustainability of aquaculture (MAMOON et al. 2024).

Given these challenges, molecular tools play a key role in assessing the genetic impact of selective breeding. Microsatellite markers (simple sequence repeats – SSR) are widely employed to evaluate genetic diversity, determine kinship relationships, and monitor allele frequency changes in fish populations (ROMANA-EGUIA et al. 2004, HASSANIEN & GILBEY 2005, MOREIRA et al. 2007, BRIÑEZ et al. 2011, PETERSEN et al. 2012, RODRIGUEZ-RODRIGUEZ et al. 2013, ZHU et al. 2017, MONTOYA–LÓPEZ et al. 2019, SILVA et al. 2020, AHMED et al. 2023, TINE et al. 2023, UKENYE & MEGBOWON 2023, MAMOON et al. 2024). These markers are particularly valuable for breeding programs as they enable the detection of genetic drift, the estimation of effective population size, and the identification of changes in genetic structure over multiple generations (JOSHI et al. 2017, UKENYE & MEGBOWON 2023).

Despite the recognized importance of SSR markers in genetic monitoring, few studies have systematically evaluated the effects of individual selection on genetic variability in Nile tilapia. The implementation of breeding programs in southern Brazil, where temperature fluctuations can impose selective pressures distinct from those in tropical regions, raises questions about the genetic consequences of long-term selection. Understanding how allele frequencies shift over generations and whether genetic variability is maintained or diminished is essential for optimizing selection strategies and ensuring the genetic resilience of farmed tilapia populations (AHMED et al. 2023, MAMOON et al. 2024).

Therefore, the present study conducted a genotypic characterization of four generations of Nile tilapia subjected to individual selection using SSR markers. The aim was to assess whether there was a decrease in genetic variability and changes in allele frequencies across generations, providing insights into the genetic consequences of selection for growth in ponds under suboptimal temperature conditions, contributing to the optimization of breeding strategies in southern Brazil.

MATERIALS AND METHODS

GIFT tilapia from Epagri's breeding program were used in this study. All procedures followed the standards and guidelines of the Animal Maintenance Ethics Committee and were approved by CEUA/IFC (No. 224/2017) and CEUA/Epagri (No. 004/2020).

Epagri established a Nile tilapia breeding program focusing on individual growth selection, utilizing specimens from nine GIFT strain families sourced from the State University of Maringá (UEM). These animals were obtained between 2009 and 2013, initially forming nine distinct genetic stocks, which were used to establish Generation 1 (G1). In Generation 2 (G2), nine genetic stocks were maintained, while in Generations 3 (G3) and 4 (G4), seven stocks were preserved. Each stock consisted of 29 to 40 females and 12 to 26 males. Crossbreeding was performed by pairing females from one stock with males from a different stock, selected based on genetic distance as determined by genotyping with microsatellite markers. These crossbreeding events were conducted in 50 m² ponds, generating new stocks for the next generation of selection.

Approximately 20 days after mating, 10,000 to 25,000 larvae per stock were

collected, followed by nursery rearing in 50 m² ponds. When the fish reached an average weight of 2 g, a random sample of 1,000 to 1,200 individuals per stock was selected and transferred to 50 m² ponds for growth selection. Each generation was managed separately, with individual stocks undergoing the full selection process. During the growth selection phase, bi-weekly biometric assessments were conducted to monitor fish growth and adjust feeding rates accordingly. Water quality parameters, including temperature, dissolved oxygen, transparency, ammonia, nitrite, and alkalinity, were measured weekly to maintain optimal conditions.

All management practices followed Epagri's guidelines for tilapia production in ponds (SILVA et al. 2024). Upon reaching 280 to 310 days of age, all fish were weighed, and the individuals with the highest final weights from each stock were selected for further breeding. The number of selected males and females, as well as the number of genotyped individuals per generation, is shown in Table 1.

Table 1. Number of animals used for selection, number of selected animals, and number of genotyped animals in each generation of individual selection.

Generation	Nº animals used for selection	Nº selected animals		Nº genotyped animals	
		Male	Female	Male	Female
G1	9.000	116	261	45	90
G2	10.294	232	360	135	135
G3	7.000	139	269	70	140
G4	7.329	140	282	112	168

The animals selected for final weight in each generation were sampled for genotypic characterization using microsatellite markers. A caudal fin sample, approximately 2.0 cm² in size, was collected from each individual and preserved in 70% ethanol at -20 ºC. DNA extraction followed a modified protocol by ALJANABI & MARTINEZ (1997), which involves high concentrations of NaCl. The modifications included a one-hour incubation in lysis buffer and DNA precipitation using isopropanol. DNA quality and quantity were measured using a Microvolume Spectrophotometer (Jenway, UK). After quantification, working solutions were prepared at a concentration of 20 ng/µL for subsequent analyses.

Eleven SSR loci were amplified using specific primers (Table 2). The selection of SSR markers was based on literature reports indicating their association with traits such as weight gain, cold tolerance, and immune response (CNAANI et al. 2003, CNAANI et al. 2004). Additionally, markers showing high polymorphism in previous studies on Nile tilapia were included (HASSANIEN & GILBEY 2005, ROMANA-EGUIA et al. 2005, MELO et al. 2006, MOREIRA et al. 2007, PETERSEN et al. 2012).

The M13(-18) tail sequence was added to the 5' end of each forward primer to enable automated genotyping with fluorescent dyes, following the protocol by SCHUELKE (2000). Three different fluorophores (6-FAM, VIC, and PET) were used for primer labeling. The PCR reaction mixture consisted of 40 ng of DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 µM forward primer, 0.4 µM forward primer with the M13 tail, 0.4 µM reverse primer, and 1.0 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), in a final volume of 25 µL.

Table 2. SSR markers used with their sequence and annealing temperature.

Primer	Annealing temperature (ºC)	Sequence	Genbank
UNH 104	56	F- GCAGTTATTTGTGGTCACTA R- GGTATATGTCTAACTGAAATCC	G12257
UNH 108	56	F- GGGATCAGCTGTTAAGTTT R- TGAGTTGATTATTAATTTCTGA	G12261
UNH 160	56	F - CCATTGGCTCTTACATC R- GATAGCATTTCTGTAGTTATGG	G12312
UNH 208	56	F – CTTCTTGGCCTACAATTT R - CAGATGGGTGATAGCAA	G12359
UNH 222	56	F-CTCTAGCACACGTGCAT R-TAACAGGTGGGAACTCA	G12373
UNH 848	54	F – TCCCCCGTAATAAATTAAACCA R - GCCTGTGAATAACAATGTATTTCCT	G68186
UNH 868	56	F – TCCTTGTTCAGACCTTGTGG R - AGCCAGGCTGAAAGGAAATA	G68199
UNH 879	56	F – GCATAAGGTGACTGGCTGGT R - ACAAAGGGGTCCTGCAATTT	G68206
UNH898	56	F – GATGTCCCCACAAGGTATGAA G - TAATCCACTCACCCCGTTTC	G68215
UNH 952	56	F – CAGACTGATGGCACAGAGGA R - TCTGCAATAGTGGCCATGAA	G68249
UNH 998	58	F – TCAATTGGTTTTACAGGAACACA R - GCTGAGGTCAGCTTACATGTCT	G68277

The PCR reactions were conducted using a Veriti thermocycler (Applied Biosystems, Carlsbad, CA, USA) under the following conditions: initial denaturation at 95 ºC for 5 minutes; 30 cycles of 45 seconds at 95 ºC, 45 seconds of annealing at the locus-specific primer's annealing temperature, and 1 minute at 72 ºC; followed by 8 cycles of 45 seconds at 95 ºC, 45 seconds at 53 ºC, and 1 minute at 72 ºC. A final extension step was carried out at 72 ºC for 30 minutes. Amplified fragment analysis was performed using an ABI3500 automated genetic analyzer with a 50 cm capillary, POP7 polymer, and GeneScan 500 LIZ as the size standard (Applied Biosystems). Amplification profiles were recorded in base pairs (bp) according to the genotyping results provided by the GeneMapper 5.0 software (Applied Biosystems).

The software GeneAlEx 6.5 (PEAKALL & SMOUSE 2006) was used for statistical analysis of genotypic characterization, allele frequency and number of alleles per *loci*, observed heterozygosity (Ho) and expected heterozygosity (He), Hardy-Weinberg equilibrium (HWE), inbreeding coefficient (F_{IS}) and fixation index (F_{ST}). Effective number of alleles (Ae) was calculated using the following formula: $Ae = 1/\Sigma xi^2$, where xi is the frequency of each allele per *loci*. Analysis of Molecular Variance (AMOVA), with 9,999 random permutations, and Principle Coordinate Analysis (PCoA), based on Nei's genetic distances, were also performed using software GenAlEx 6.5. A dendrogram derived from Ward's method using Euclidean distance was constructed using software Past 3.15 (HAMMER et al. 2001).

A Bayesian model implemented by the software Structure 2.3.4 (PRITCHARD et al. 2000, FALUSH et al. 2003) was applied to infer the number of population groups. The population inference produced was performed without incorporating predefined population information for the mixed model and with correlated frequency.

Population number analysis (K) was performed for values ranging from 1 to 30 with ten independent chains, each chain having a length of 50,000 iterations, followed by 100,000 repetitions of the Markov chain Monet Carlo (MCMC) method.

RESULTS AND DISCUSSION

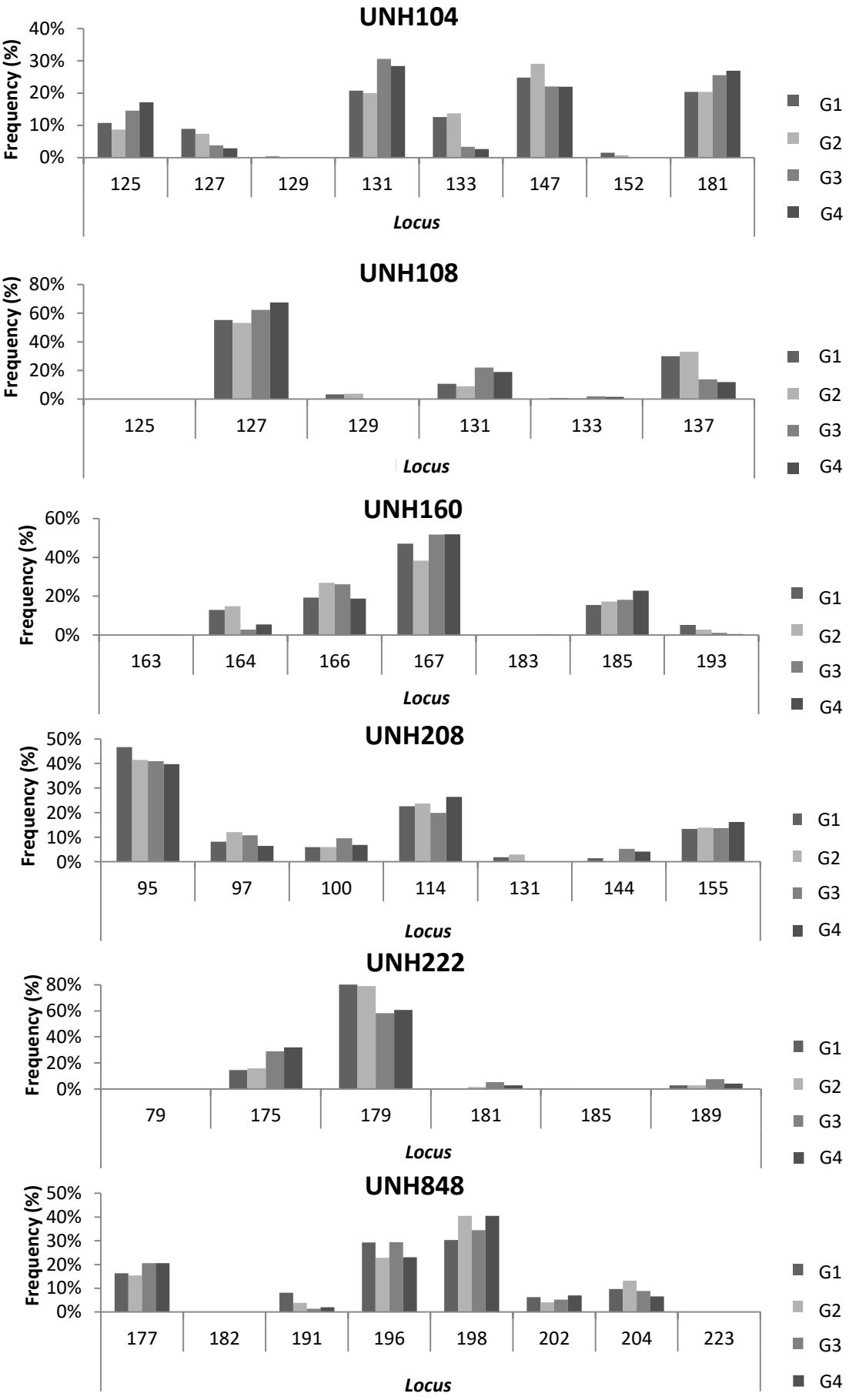
According to AMOVA, of all the available genetic variability, 1% is among generations and 99% is within generations (Table 3). The fact that genetic variability is significantly greater within generations than among generations is expected by the adopted mating system. Since G1 was the base population of G2, which in turn was the G3 and G4-forming population, and there was no introduction of new genetic material from the outside between generations.

Table 3. Molecular analysis of variance (AMOVA) for Nile tilapia stocks from GIFT strain.

Sources of Variation	df	SS	MS	Estimation of variation	Variation Percentage
Among generetions	3	78.64	26.21	0.051	1%
Within generations	1784	6907.27	3.87	3.872	99%
Total	1787	6885.91		3.923	100%

All markers were polymorphic, with at least four different alleles. The most frequent allele in G1 and G2 was UNH 222/179, while in G3 and G4, it was UNH 108/127, with values of approximately 0.80 to 0.60 (Figure 1). There was only a 5.2% reduction in the total number of alleles across generations, with G1 presenting 77 alleles for the 11 SSR markers, and G4 presenting 73 alleles. Among the identified alleles in this study, about 26% of alleles had a frequency below 0.1. RODRIGUEZ-RODRIGUEZ et al. (2013) performed the genetic characterization by five SSR markers in four generations of GIFT tilapia and did not observe allele losses over the generations but did observe several alleles with frequencies below 10% (0.100).

The microsatellite marker UNH 898 exhibited the highest total number of alleles (ranging from 10 to 11) and the highest number of effective alleles (6.18 to 7.28) among the analyzed *loci*, indicating its high polymorphism and potential utility in assessing genetic diversity within the studied populations. Its elevated effective allele count suggests that this marker captures a substantial portion of the genetic variability, making it particularly valuable for genetic monitoring in selective breeding programs. However, a study analyzing O. niloticus populations from Egyptian fish farms (MAMOON et al. 2024) reported a significantly lower number of effective alleles for this same marker (Ae = 1.12), highlighting the importance of population-specific evaluations when selecting markers for genetic assessments.


UNH104
Frequency (%)
40%
30%
20%
10%
0%
125
127
129
131
133
147
152
181
Locus
G1
G2
G3
G4
UNH108
Frequency (%)
80%
60%
40%
20%
0%
125
127
129
131
133
137
Locus
G1
G2
G3
G4
UNH160
Frequency (%)
60%
40%
20%
0%
163
164
166
167
183
185
193
Locus
G1
G2
G3
G4
UNH208
Frequency (%)
50%
40%
30%
20%
10%
0%
95
97
100
114
131
144
155
Locus
G1
G2
G3
G4
UNH222
Frequency (%)
80%
60%
40%
20%
0%
79
175
179
181
185
189
Locus
G1
G2
G3
G4
UNH848
Frequency (%)
50%
40%
30%
20%
10%
0%
177
182
191
196
198
202
204
223
Locus
G1
G2
G3
G4

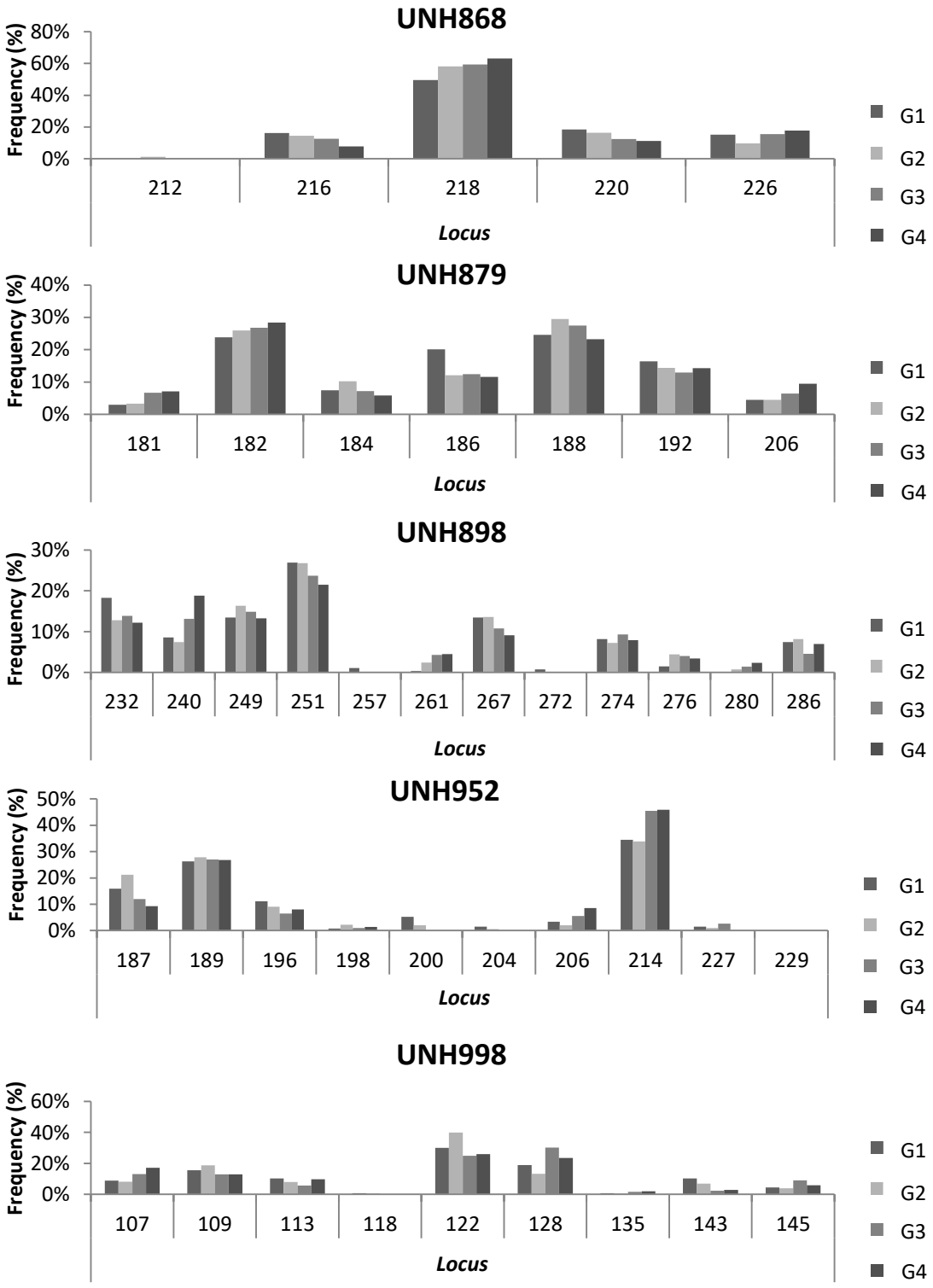


Figure 1. Allele frequency histograms by locus - UNH104, UNH108, UNH160, UNH208, UNH222, UNH 848, UNH868 UNH879, UNH898, UNH952 and UNH998 for the four generations (G1, G2, G3 and G4) of Nile tilapia (*Oreochromis niloticus*) from the GIFT strain, derived from the Epagri breeding program.

The average total number of alleles per marker was 7.0 ± 0.6 for G1, 6.8 ± 2.0 for G2, 6.1 ± 0.6 for G3, and 6.7 ± 1.7 for G4. The number of effective alleles averaged 4.1 ± 0.4 for G1, 3.9 ± 1.4 for G2, 3.9 ± 0.5 for G3, and 3.9 ± 1.7 for G4 (Table 4). The average observed and expected heterozygosities were 0.737 and

0.709 in G1, 0.680 and 0.702 in G2, 0.730 and 0.706 in G3, and 0.702 and 0.695 in G4, respectively. These results correspond to F_{IS} values of -0.042 for G1, 0.027 for G2, -0.042 for G3, and -0.017 for G4 (Table 4).

Table 4. Number of genotyped individuals (N), Number of total and effective alleles per *loci* (A and Ae), observed heterozygosity (Ho), expected heterozygosity (He), Hardy-Weinberg equilibrium (HWE) and coefficients of inbreeding (F_{IS}) for the three generations (G1, G2, G3 and G4) of the tilapia GIFT strain (*Oreochromis niloticus*) from the Epagri breeding program.

Loci		G1	G2	G3	G4	Mean ± standard deviation
UNH104	N	135	270	209	280	
	A	8	7	6	6	-
	Ae	5.51	5.05	4.32	4.30	-
	Ho	0.874	0.859	0.770	0.704	-
	He	0.821	0.804	0.770	0.769	0.802±0.040
	F_{IS}	-0.068	-0.071	-0.003	0.083	0.791±0.013
	HWE	59.91***	59.85***	54.47***	51.06***	-0.015±0.036
UNH108	N	135	270	209	278	
	A	5	5	4	5	-
	Ae	2.46	2.49	2.20	1.98	-
	Ho	0.519	0.626	0.608	0.518	-
	He	0.595	0.599	0.546	0.496	0.568±0.029
	F_{IS}	0.125	-0.047	-0.115	-0.046	0.559±0.024
	HWE	9.66ns	22.15*	10.31ns	16.38ns	-0.021±0.051
UNH160	N	135	270	209	280	
	A	5	5	5	7	-
	Ae	3.31	3.68	2.71	2.79	-
	Ho	0.711	0.563	0.632	0.654	-
	He	0.701	0.730	0.632	0.642	0.640±0.031
	F_{IS}	-0.019	0.227	-0.001	-0.019	0.676±0.023
	HWE	36.61***	146.08***	31.46***	38.04*	0.047±0.060
UNH208	N	135	270	209	278	-
	A	7	6	6	7	-
	Ae	3.36	3.75	4.02	3.78	-
	Ho	0.748	0.670	0.799	0.741	0.740±0.026
	He	0.705	0.735	0.753	0.374	0.732±0.010
	F_{IS}	-0.065	0.086	-0.064	-0.008	-0.013±0.035
	HWE	70.68***	56.43***	50.40***	38.50*	
UNH222	N	134	270	209	280	
	A	5	5	4	5	-
	Ae	1.45	1.54	2.32	2.11	-
	Ho	0.351	0.348	0.703	0.539	-
	He	0.311	0.352	0.571	0.527	0.485±0.085
	F_{IS}	-0.131	0.008	-0.235	-0.024	0.440±0.064
	HWE	4.20ns	1.73ns	35.76***	8.12ns	-0.095±0.055
UNH879	N	134	268	209	280	
	A	7	7	7	7	-
	Ae	5.16	4.92	5.17	5.38	-
	Ho	0.806	0.817	0.880	0.836	-
	He	0.809	0.798	0.808	0.816	0.835±0.016
	F_{IS}	0.000	-0.025	-0.092	-0.027	0.808±0.004
	HWE	71.87***	41.16**	116.75***	76.47***	-0.036±0.020
UNH898	N	134	2690	209	279	-
	A	11	10	10	10	-
	Ae	6.18	6.52	7.11	7.285	-
	Ho	0.791	0.770	0.799	0.817	0.794±0.010
	He	0.841	0.848	0.861	0.864	0.854±0.068
	F_{IS}	0.056	0.091	0.070	0.053	0.068±0.009

	HWE	172.03***	230.17***	378.50***	248.96***	
UNH998	N	135	269	209	280	-
	A	9	9	8	8	-
	Ae	5.51	4.31	5.02	5.47	-
	Ho	0.956	0.684	0.746	0.836	0.805±0.059
	He	0.822	0.768	0.803	0.819	0.803±0.012
	F_{IS}	-0.167	0.110	0.068	-0.023	-0.003±0.061
	HWE	122.29***	103.92***	107.21***	126.90***	
UNH952	N	135	269	209	280	
	A	9	10	7	6	-
	Ae	4.35	4.05	3.31	3,28	-
	Ho	0.719	0.706	0.622	0.800	-
	He	0.773	0.755	0.700	0.696	0.712±0.036
	F_{IS}	0.067	0.062	0.109	-0.151	0.731±0.019
	HWE	100.71***	148.84***	94.82***	45.42***	0.022±0.059
UNH868	N	135	269	209	280	
	A	5	5	4	4	-
	Ae	3.03	2.53	2.45	2.22	-
	Ho	0.785	0.643	0.641	0.607	-
	He	0.672	0.605	0.594	0.551	0.669±0.040
	F_{IS}	-0.172	-0.064	-0.083	-0.103	0.606±0.025
	HWE	21.44*	12.82ns	12.39ns	16.96**	-0.106±0.024
UNH848	N	135	269	209	280	-
	A	6	6	6	8	-
	Ae	4.46	3.83	3.87	3.71	-
	Ho	0.844	0.796	0.833	0.675	0.878±0.039
	He	0.776	0.741	0.742	0.732	0.749±0.010
	F_{IS}	-0.089	-0.076	-0.123	0.076	-0.053±0.044
	HWE	39.01***	57.38***	37.59**	30.31ns	
Loci mean	N	135	270	209	280	-
	A	7.00	6.82	6.09	6.64	-
	Ae	4.071	3.88	3.86	3.85	-
	Ho	0.737	0.680	0.730	0.702	0.712±0.020
	He	0.709	0.702	0.706	0.695	0.704±0.019
	F_{IS}	-0.042	0.027	-0.042	-0.017	-0.019±0.014

ns Not significant, *P<0.05, **P<0.01, and ***P<0.001

The observed allele counts and heterozygosity levels provide crucial insights into the genetic diversity and stability of the tilapia populations across four generations subjected to individual selection. The slight reduction in the total number of alleles from G1 to G3, followed by a modest increase in G4, may indicate a potential genetic bottleneck effect mitigated by selection strategies or broodstock management. The maintenance of a relatively stable number of effective alleles across generations suggests that genetic drift has not drastically reduced functional genetic variation (ROBLEDO et al. 2018, LIND et al. 2020).

The observed and expected heterozygosity values, which remained relatively stable across generations, suggest that genetic diversity has been maintained, an essential factor in preventing inbreeding depression and ensuring the long-term adaptability of the population. The F_{IS} values, mostly near zero or slightly negative, indicate a balanced genetic structure with minimal inbreeding or an excess of heterozygotes in some generations (GJEDREM et al. 2012, NGUYEN et al. 2022). These findings reinforce the importance of continuous genetic monitoring to sustain diversity levels and optimize selective breeding strategies for Nile tilapia.

Hardy-Weinberg equilibrium (HWE) was observed for the following markers: UNH 108 (in G1, G3, and G4), UNH 222 (in G1, G2, and G4), UNH 868 (in G2 and G3), and UNH 848 (in G4). The deviations from Hardy-Weinberg equilibrium (HWE) in these markers across generations highlight potential selection pressures, non-random mating, or genetic drift acting on these *loci* (LIND et al. 2020).

The markers with the lowest F_{IS} values were UNH 222, UNH 848, and UNH 868. In contrast, the markers UNH 160, UNH 898, and UNH 952 exhibited higher positive F_{IS} values, indicating lower heterozygosity at these *loci*. Despite a 5.2% reduction in the number of alleles across generations, the average heterozygosity remained stable, and the FIS results demonstrated no significant loss of genetic variability in the population over the generations. This stability is likely due to the fact that the lost alleles were of low frequency within the population. Similarly, RODRIGUEZ-RODRIGUEZ et al. (2013) reported that heterozygosity was preserved across four generations of GIFT tilapia, aligning with our findings that there was no significant genetic variability loss in the loci analyzed within the breeding program. Furthermore, ROMANA-EGUIA et al. (2005) observed that one generation of mass selection in a tilapia population maintained the heterozygosity index without increasing inbreeding at the loci analyzed.

Genotype clustering in G1, G2, G3, and G4 revealed 9, 16, 12, and 12 groups, respectively, with corresponding FST values of 0.1752, 0.2320, 0.4763, and 0.2654 (Figure 2). These findings indicate that genetic differentiation between groups increased over generations, particularly from G1 to G3, as evidenced by the higher F_{ST} values. The increase in the number of groups identified by the Bayesian model in G2, G3, and G4 compared to G1 suggests a greater genetic structuring within the population over time. This could be attributed to the effects of individual selection, which may have led to the emergence of distinct genetic clusters within the population. Importantly, the absence of a significant increase in inbreeding across generations, as supported by the stable or slightly increasing F_{ST} values, indicates that the breeding program successfully maintained genetic diversity and minimized inbreeding. This is consistent with studies emphasizing the importance of managing genetic structure and diversity in selective breeding programs to avoid inbreeding depression and ensure long-term sustainability (LIND et al. 2020, NGUYEN et al. 2022).

The comparison of the characterization data from this study with previous studies conducted in Brazil on GIFT tilapia is particularly insightful, as the base population for these studies originates from the same source—30 families introduced to the country in 2005 (OLIVEIRA et al. 2015). The divergence lies in the methods used to manage and develop this genetic material in different regions of the country. PETERSEN et al. (2012) evaluated the genetic variability of GIFT tilapia produced in Santa Catarina and reported a total of 12 alleles per locus for the markers UNH104, UNH108, and UNH160, a higher number compared to the 4 to 11 alleles identified for these markers in the present study. This reduction in the number of alleles is likely attributed to the selective breeding process applied to the population in our facility over the past six years.

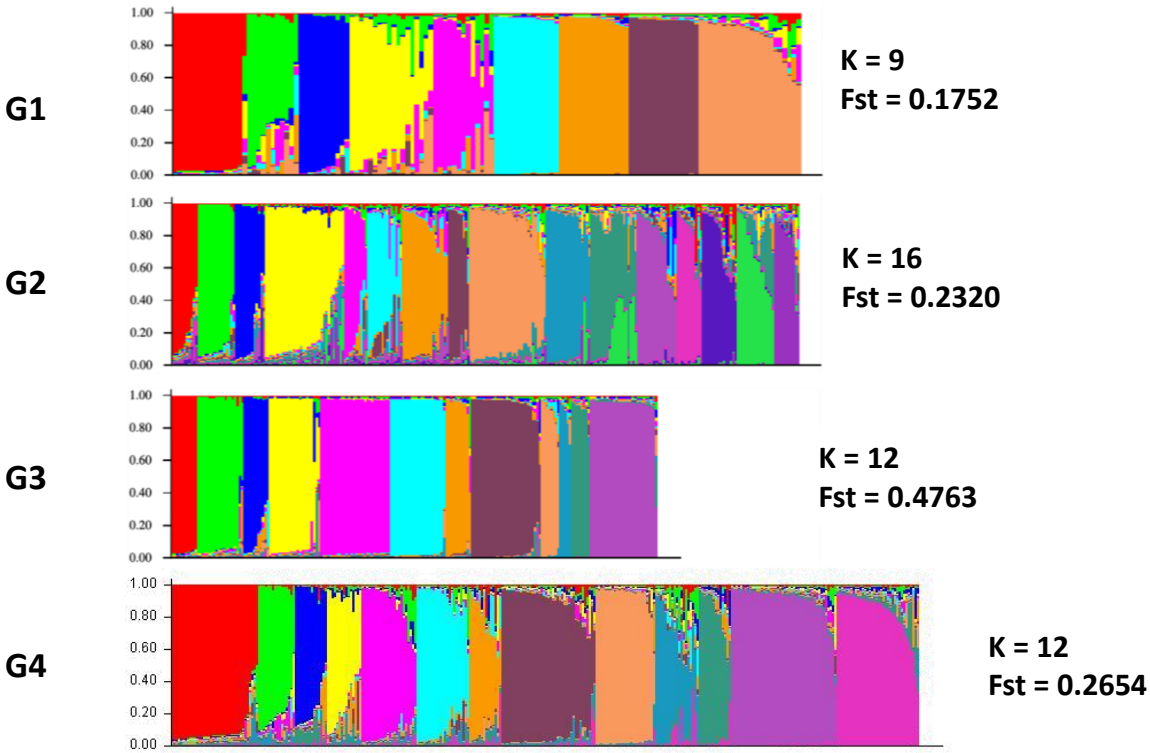


Figure 2. Bayesian clustering analysis of four generations (G1, G2, G3, and G4) of *Oreochromis niloticus* from the Epagri breeding program. The analysis identified genetic structuring within the population, revealing the formation of distinct genetic groups across generations (K). Each color represents a different genetic cluster, illustrating allele frequency shifts due to selection. The F_{ST} values for each generation indicate the degree of genetic differentiation.

In contrast, other studies conducted in Brazil with smaller sample sizes and fewer markers reported an average number of alleles per locus for the GIFT strain ranging from 4.2 to 6.1, which is lower than the values observed in this study (RODRIGUEZ-RODRIGUEZ et al. 2013, BAGGIO et al. 2016, DIAS et al. 2016). Generally, these studies reported lower observed heterozygosity (Ho) levels, resulting in positive F_{IS} values for the analyzed populations. For instance, RODRIGUEZ-RODRIGUEZ et al. (2013) reported a positive F_{IS} value of 0.281, attributing the reduced heterozygosity observed in their broodstock to the selection process applied. Other studies evaluating GIFT strain stocks in Brazil have documented F_{IS} values of 0.16 and 0.35 (PETERSEN et al. 2012, DIAS et al. 2016). In contrast, BAGGIO et al. (2016) observed a F_{IS} value of -0.042 for GIFT tilapia stocks, which is consistent with the values found in our study. Similarly, studies on the genetic characterization of farmed tilapia in other parts of the world using microsatellite markers have generally reported lower Ho values compared to expected heterozygosity (He) and positive F_{IS} values (ROMANA-EGUIA et al. 2004, BRIÑEZ et al. 2011, GU et al. 2014, ZHU et al. 2017, MONTOYA-LÓPEZ et al. 2019, MAMOON et al. 2024).

Among the markers used in this study, some are already known to be regions of the genome associated with phenotypic traits of commercial interest, that is, quantitative traits *loci* (QTL). According to CNAANI et al. (2004), the markers UNH848, UNH868, and UNH898 are associated with tilapia weight characteristics

and together explain 37.9% of the variation of this trait. In Figure 1, it is possible to observe, throughout the generations, important changes in the frequencies of some alleles in all of these markers, which is expected since the selection is made for the weight gain. For the UNH848 marker, it was possible to observe a significant reduction over the generations in the 194 bp allele frequency, while for the UNH868 marker, it was possible to observe a reduction for the 225 bp allele. On the other hand, we observed a significant increase in the 223 bp allele of the UINH868 marker, as well as in the 245 bp, 264 bp, and 278 bp alleles of the UNH898 marker (Figure 1). However, the negative F_{IS} values for the UNH 848 and UNH 868 markers indicate that they still have high heterozygosity for these *loci*, and consequently it is still possible that there is genetic variability for the weight gain trait.

For the UNH879 marker, which is associated with cold tolerance (CNAANI et al. 2003), the largest change in allele frequencies observed over the generations was a reduction in allele 192 bp frequency and an increase in alleles 186 bp and 212 bp. According to studies by our group, cold-tolerant tilapias showed higher frequency of the 186 bp allele and a lower frequency of the 192 bp allele than the cold-sensitive tilapias (SILVA et al. 2021). This may be an indication that tilapia selection in southern Brazil, submitted to more severe winters, may select animals more cold-tolerant of the subtropical climate.

This observation aligns with the findings of BEHRENDS et al. (1996), who demonstrated that selecting cold-tolerant individuals resulted in offspring with superior growth performance at suboptimal temperatures of 21 °C. The heritability of cold tolerance in tilapia has been well-documented (THODESEN et al. 2013, NITZAN et al. 2016), confirming that this trait can be inherited and that significant genetic gains can be achieved through selective breeding programs. Furthermore, other studies have identified microsatellite markers associated with cold tolerance (ZHU et al. 2015, HUI et al. 2022). Collectively, these markers can be used to explain a greater proportion of the variability in cold tolerance and can be integrated into marker-assisted selection strategies to enhance breeding programs.

In addition to the results already discussed in this study, genetic diversity studies within the stock used in a breeding program allow for the efficient targeting of mating for the formation of next-generation families, as used in the Epagri program. As an example, a study conducted in Egypt with tilapia, also from the GIFT strain, observed higher performance in mating among populations that had greater genetic distances (ALI et al. 2017). For the population of this study, SILVA et al. (2019) reported a genetic gain of 8.4% in the final weight between generations G1 and G2; and SILVA et al. (2023) reported a superior growth between G4 to G1 for 33.9%.

CONCLUSION

This study successfully characterized the genetic diversity of four generations of *Oreochromis niloticus* subjected to individual selection in Epagri's breeding program using 11 microsatellite markers. The results indicate that, despite a modest reduction in the total number of alleles (5.2%), genetic variability within the population was effectively maintained. This stability is evidenced by the consistent number of effective alleles across generations and the overall balance between observed and

expected heterozygosity. Furthermore, the low F_{IS} values suggest that inbreeding was successfully controlled, reinforcing the sustainability of the selective breeding strategy implemented.

However, significant changes in allele frequencies were observed at specific *loci*, particularly in markers associated with economically important traits such as growth and cold tolerance. These findings highlight the impact of selection on the genetic structure of the population and underscore the importance of continuous genetic monitoring to prevent the loss of genetic diversity and ensure a sustained response to selection over time. Bayesian clustering analysis further revealed genetic differentiation across generations, suggesting the emergence of distinct genetic groups, likely reflecting selection-driven shifts in specific genomic regions. Overall, this study demonstrates that implementing individual selection while maintaining genetic variability and guiding mating decisions based on genetic distance—determined through microsatellite markers—effectively mitigates inbreeding risks. Future research should explore the integration of genomic selection and functional genomics to further enhance breeding efficiency and ensure the long-term sustainability of farmed Nile tilapia populations.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis: Bruno Corrêa da Silva, Adriana Pereira, Haluko Massago and Keny Henrique Mariguele; investigation: Bruno Corrêa da Silva and Haluko Massago; resources and data curation: Bruno Corrêa da Silva, Adriana Pereira and Keny Henrique Mariguele; writing-original draft preparation: Bruno Corrêa da Silva; writing-review and editing: Bruno Corrêa da Silva and Keny Henrique Mariguele; visualization: Adriana Pereira ad Haluko Massago; project administration: Bruno Corrêa da Silva. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was supported by Foundation for Research and Innovation of the State of Santa Catarina (FAPESC) between Project number 2017TR1709 and Project number 2023TR001541.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

ACKNOWLEDGEMENTS

We would also like to thank the employees Silvio Demarch Filho, Leandro Bortoli, Silvano Garcia, João Burg, Emidio Sant-Anna de Lara, and Karla Geovana Weiss de Souza for their support.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

AHMED SM et al. 2023. Population structure and genetic diversity of Nile tilapia (*Oreochromis niloticus*) using microsatellite markers from selected water bodies in southwest Ethiopia. Veterinary Medicine and Science 9: 2095-2106.

ALI FS et al. 2017. Genetic improvement of farmed Nile tilapia (*Oreochromis niloticus*) through selective breeding in Egypt. International Journal of Fisheries and Aquatic Studies 5: 395-401.

ALI FS et al. 2024. Genetic Diversity Assessment and Association Analysis of Body Characters of the Nile tilapia (*Oreochromis niloticus*) using AFLP. Egyptian Journal of Aquatic Biology & Fisheries 28: 843 – 858.

ALJANABI SM & MARTINEZ I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25: 4692-4693.

BAGGIO RA et al. 2016. Identifying Nile tilapia strains and their hybrids farmed in Brazil using microsatellite markers. Pesquisa Agropecuária Brasileira 51: 1744-1750.

BARROSO R et al. 2016. Gerenciamento genético da tilápia nos cultivos comerciais. Palmas: Embrapa Pesca e Aquicultura. Disponível em: https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1036709. Acesso em: 15 out. 2019.

BEHRENDS LL et al. 1996. Cold tolerance in maternal mouthbrooding tilapias: heritability estimates and correlated growth responses at suboptimal temperatures. In The Third International Symposium on Tilapia in Aquaculture 41: 257.

BENTSEN HB et al. 2017. Genetic improvement of farmed tilapias: response to five generations of selection for increased body weight at harvest in *Oreochromis niloticus* and the further impact of the project. Aquaculture 468: 206-217.

BRIÑEZ BR et al. 2011. Genetic diversity of six populations of red hybrid tilapia, using microsatellites genetic markers. Revista MVZ Córdoba 16: 2491-2498.

CNAANI A et al. 2003. Detection of a chromosomal region with two quantitative trait loci, affecting cold tolerance and fish size, in an F2 tilapia hybrid. Aquaculture 223: 117-128.

CNAANI A et al. 2004. Genome-scan analysis for quantitative trait loci in an F2 tilapia hybrid. Molecular Genetics and Genomics 272: 162-172.

DIAS MA et al. 2016. Evaluation of the genetic diversity of microsatellite markers among four strains of *Oreochromis niloticus*. Animal Genetics 47: 345-353.

FALUSH D et al. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567-1587.

FAO. 2024. The State of World Fisheries and Aquaculture 2024 – Blue Transformation in action. Rome. Disponível em: https://doi.org/10.4060/cd0683en.

GJEDREM T et al. 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture 350-353: 117-129.

GU D et al. 2014. Genetic diversity of invasive Oreochromis spp. (tilapia) populations in Guangdong province of China using microsatellite markers. Biochemical Systematics and Ecology 55: 198-204.

HAMMER Ø et al. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 9.

HASSANIEN HA & GILBEY J. 2005. Genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*) revealed by DNA microsatellites. Aquaculture Research 36: 1450-1457.

HUI AI et al. 2022. Mapping QTL for cold-tolerance trait in a GIFT-derived tilapia line by ddRAD-seq. Aquaculture 556: 738273.

JOSHI D et al. 2017. Microsatellite markers and their application in fisheries. International Journal of Advances in Agricultural Science and Technology 4: 67-104.

LIND CE et al. 2020. Selective breeding in fish and conservation of genetic resources for aquaculture. Reviews in Aquaculture 12: 969-983.

MAMOON A et al. 2024. Investigating Nuclear DNA Microsatellites in the Nile Tilapia (*Oreochromis niloticus*): Insights into Association Genetics. Egyptian Journal of Aquatic Biology & Fisheries 28: 661–675.

MELO DC et al. 2006. Caracterização genética de seis plantéis comerciais de tilápia (*Oreochromis*) utilizando marcadores microssatélites. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 58: 87-93.

MONTOYA-LÓPEZ AF et al. 2019. Genetic diversity of four broodstocks of tilapia (*Oreochromis* sp.) from Antioquia, Colombia. Revista Colombiana de Ciências Pecuárias 32: 201-213.

MOREIRA AA et al. 2007. Variabilidade genética de duas variedades de tilápia nilótica por meio de marcadores microssatélites. Pesquisa Agropecuária Brasileira 42: 521-526.

NGUYEN NH et al. 2022. Genetic improvement of Nile tilapia (*Oreochromis niloticus*) in Malaysia: Current status and future prospects. Aquaculture Reports 23: 101031.

NITZAN T et al. 2016. Maternal effects in the inheritance of cold tolerance in blue tilapia (*Oreochromis aureus*). Environmental Biology of Fishes 99: 975-981.

OLIVEIRA CALD et al. 2015. Avaliação genética de tilápias-do-nilo durante cinco anos de seleção. Pesquisa Agropecuária Brasileira 50: 871-877.

PEAKALL R & SMOUSE PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

PEIXE-BR. 2024. Anuário 2024 Peixe BR da Piscicultura. Disponível em: https://www.peixebr.com.br/anuario-2024/. Acesso em: 10 out. 2024.

PETERSEN RL et al. 2012. Análise da diversidade genética de tilápias cultivadas no estado de Santa Catarina (Brasil) utilizando marcadores microssatélites. Boletim do Instituto de Pesca 38: 313-321.

PONZONI RW et al. 2011. Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the WorldFish Center with the GIFT strain. Reviews in Aquaculture 3: 27-41.

PRITCHARD JK et al. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

ROBLEDO D et al. 2018. Applications of genotyping by sequencing in aquaculture breeding and genetics. Reviews in Aquaculture 10: 670-682.

RODRIGUEZ-RODRIGUEZ MP et al. 2013. Caracterização genética de gerações de tilápia GIFT por meio de marcadores microssatélites. Pesquisa Agropecuária Brasileira 48: 1385-1393.

ROMANA-EGUIA MR et al. 2004. Genetic diversity in farmed Asian Nile and red hybrid tilapia stocks evaluated from microsatellite and mitochondrial DNA analysis. Aquaculture 236: 131-150.

ROMANA-EGUIA MR et al. 2005. Genetic changes during mass selection for growth in Nile tilapia, *Oreochromis niloticus* (L.), assessed by microsatellites. Aquaculture Research 36: 69-78.

SCHUELKE M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233-234.

SILVA BC et al. 2019. Ganho genético após uma geração de seleção individual para peso final e variáveis morfométricas em tilápia. Revista de Ciências Agroveterinárias 18: 103-110.

SILVA BC et al. 2020. Genetic characterization of selected Nile tilapia in Santa Catarina. Semina: Ciências Agrárias 41: 1739-1754.

SILVA BC et al. 2021. Cold tolerance and performance of selected Nile tilapia for suboptimal temperatures. Aquaculture Research 52: 1071-1077.

SILVA BC et al. 2023. Tilápia Epagri SC 04. Florianópolis: Epagri. Disponível em: https://sistemas.epagri.sc.gov.br/semob/consulta.action?subFuncao=consultaPublicacoesDetalhe&cdDoc=57664. Acesso em: 10 out. 2024.

SILVA BC et al. 2024. Monocultivo de tilápia em viveiros escavados em Santa Catarina. Florianópolis: Epagri. 126p. (Epagri. Sistemas de Produção 52).

THODESEN J et al. 2013. Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth, pond survival and cold-water tolerance of blue tilapia (*Oreochromis aureus*) after four generations of multi-trait selection. Aquaculture 396: 32-42.

TINE M et al. 2023. Genetic Characterization of Nile Tilapia (*Oreochromis niloticus*) Strains from Senegal for Sustainable Local Aquaculture Production. Open Journal of Genetics 13: 1-22.

UKENYE EA & MEGBOWON I. 2023. Comparison of genetic diversity of farmed *Oreochromis niloticus* and wild unidentified tilapia (Wesafu) using microsatellite markers. Biodiversitas Journal of Biological Diversity 24: 2953-2957.

ZHU HP et al. 2015. Screening and identification of microsatellite markers associated with cold tolerance in Nile tilapia *Oreochromis niloticus*. Genetics and Molecular Research 14: 10308-10314.

ZHU WB et al. 2017. High genetic diversity and differentiation in three red tilapia stocks revealed by microsatellite DNA marker analysis. Aquaculture International 25: 1997-2006.