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Genetic Traceability of European Sea Bass (*Dicentrarchus labrax*) and Gilthead Seabream (*Sparus aurata*) for Technological Advancements in Breeding Management

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Abstract

This study evaluated the genetic variability and traceability potential of farmed European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) populations from a fish farm located in Petrosino (Marsala, Sicily) (FAO 37), using microsatellite markers. A total of 64 *D. labrax* and 63 *S. aurata* individuals were genotyped with species-specific multiplex panels (9 and 10 loci, respectively). High levels of polymorphism were observed in both species, with an average of 12 alleles per locus in *D. labrax* and 9.1 alleles per locus in *S. aurata*. Mean observed heterozygosity (Ho) was 0.530 in *D. labrax* and 0.459 in *S. aurata*, while expected heterozygosity (He) reached 0.762 and 0.702, respectively. The fixation index (F) indicated moderate heterozygote deficiency in both populations (0.320 in *D. labrax* and 0.352 in *S. aurata*). Significant deviations from Hardy–Weinberg equilibrium were detected at most loci in both species, suggesting non-random mating, genetic drift, or population substructure. The probability of identity (PI) values across loci confirmed the high discriminatory power of the microsatellite panels, supporting their suitability for individual identification and genetic traceability applications in aquaculture. Overall, the results highlight that, despite substantial genetic variability, the observed heterozygote deficiency and deviations from equilibrium may reflect suboptimal breeding management practices. These findings underline the importance of implementing regular genetic monitoring and integrating molecular tools into broodstock management to maintain genetic diversity, reduce inbreeding, and support sustainable aquaculture production.

Keywords: genetic traceability; Mediterranean aquaculture; breeding management

Key Contribution: This study evaluated the genetic structure of farmed European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). populations using microsatellite markers to support genetic traceability and sustainable aquaculture. High allelic richness was observed in both species, but significant deviations from Hardy–Weinberg equilibrium and elevated fixation indices indicated a considerable risk of inbreeding. The

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results highlight the need for continuous genetic monitoring and the integration of advanced genomic tools to preserve genetic diversity, prevent genetic depletion, and ensure the long-term sustainability of Mediterranean aquaculture.

1. Introduction

European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) are two of the most important species in Mediterranean aquaculture, widely farmed for their economic value in the seafood industry. These species have been the focus of extensive research due to their commercial importance and the need to optimize breeding programs for sustainable production [1]. Effective genetic management in aquaculture is essential for enhancing productivity, improving disease resistance, and for preserving genetic diversity [2]. However, uncontrolled breeding and the lack of proper genetic monitoring can lead to inbreeding depression, loss of genetic diversity, and reduced overall farm productivity [3].

In this context, genetic traceability plays a fundamental role in maintaining the integrity of breeding programs by ensuring that only genetically superior individuals are selected for reproduction and by monitoring the genetic diversity of farmed populations. Its incorporation into breeding management is essential for the long-term sustainability of aquaculture, as it facilitates the identification of desirable traits such as growth rate, disease resistance, and reproductive success [4,5]. Moreover, it helps mitigate issues such as genetic erosion, which may arise when breeding stocks are overexploited or inbred. In parallel, the use of molecular tools such as microsatellites and single nucleotide polymorphisms (SNPs) enables breeders to perform marker-assisted or genomic selection, thereby supporting more informed decisions and promoting healthier and more productive fish populations [6–8].

The application of genetic traceability in aquaculture has become increasingly relevant in the context of European Union regulations on sustainable aquaculture and the protection of native and economically important species. This includes monitoring the genetic structure of farmed populations and ensuring traceability to prevent hybridization between wild and farmed stocks, which could potentially lead to the loss of local genetic diversity [9]. Microsatellites are widely used in aquaculture for assessing genetic diversity, managing breeding programs, and ensuring genetic traceability in farmed populations. They also support the management and conservation of economically important species by helping evaluate genetic variability within both wild and farmed populations, which is essential for preventing inbreeding and maintaining healthy genetic diversity [10].

Moreover, microsatellites are essential tools for genetic traceability in breeding management practices. Due to their high polymorphism, microsatellites allow for the identification of family lines and the tracking of individual genealogy within a selective breeding program [11]. Studies on species such as European sea bass and gilthead seabream have shown that microsatellite analysis enables monitoring of genetic diversity in both farmed and wild populations, identifying potential signs of genetic depletion due to intensive selection [12–15]. Additionally, microsatellites are used for tracing parentage and managing controlled breeding programs, thereby avoiding inbreeding and ensuring that selection is performed among genetically distinct individuals. In European sea bass they assist in tracing breeders and managing genetic quality in breeding programs [16].

Another key application of microsatellites in aquaculture is population differentiation. Microsatellite-based techniques allow for the identification of distinct genetic groups within a species and the monitoring of changes in genetic structure in response to artificial selection or resource management practices [8,17]. Finally, microsatellites are also used to

differentiate between wild and farmed populations, preventing unintentional hybridization. They are instrumental in understanding the genetic structure and long-term effects of selective breeding in species such as *Perca fluviatilis* [18].

The objective of this study was to assess genetic variability in farmed populations of European sea bass and gilthead seabream using microsatellite markers, with the aim of establishing a reference database to support breeding management, improve selection efficiency, and contribute to the long-term sustainability of aquaculture programs.

2. Materials and Methods

2.1. Sampling and DNA Extraction

A total of 64 individuals of European sea bass and 63 individuals of gilthead seabream, reared in ground tanks of a fish farm located in Petrosino (Marsala, Sicily), were randomly sampled as part of the farm's normal operations using non-lethal procedures. Small portions of the caudal fin were excised using sterile scissors and collected in sterile tubes containing 100% ethanol for preservation.

The tissue samples were homogenized, and DNA was extracted using a 2% CTAB lysis buffer (1.4 M NaCl, 2% (*w*/*v*) CTAB, 0.1 M Tris-Base, 0.015 M EDTA) with an automatic extractor (ALLSHENG Auto-Pure96, Shanghai, China). Extracted DNA was quantified using a Nanodrop spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, DE, USA) and stored at −20 °C.

2.2. Primer Selection and Multiplex Polymerase Chain Reaction (PCR)

Based on previous studies, ten microsatellite markers were selected for gilthead seabream [13] (Table 1), and nine markers for European sea bass [14] (Table 2).

Table 1. List of the 10 molecular markers selected from Navarro et al. [13], for the analysis of the species gilthead seabream: locus name (Locus), primer sequence (Sequence), annealing temperature (Ta), amplified fragment length (Size range).

Locus	Sequence	T_a	Size Range	Reference *
SauK140INRA	F: CATGGCAACGGGGTAGGT R: GTATGCTTGCAAGTGTATGTGTGTC	53	66–100	Launey et al. (2003) [19]
SauI47INRA	F: GCCAGCCAGGGTACGTC R: GTTTCCATATCATTACACTGTGGCTA	53	88–106	Launey et al. (2003) [19]
SauH98INRA	F: AACTTAGCAGGGGGAGGCTA R: GTTTCACAAGTGTGCTGAAAGATTACTGT	53	108–122	Launey et al. (2003) [19]
SauG46INRA	F: CAAGGTGTCGCCGTGTCT R: GTTTACTGCCAAGGTCAGAGTCACA	53	110–134	Launey et al. (2003) [19]
PaGA2a	F: GACAGAGAGGGAGTGGATGTG R: GTTTATCATCATCATCATCAGTCAGAGC	58	114–142	Adcock et al. (2000) [20]
PbMS2	F: CTCGCGGTAGTTGTTCACAGAGC R: GCAACAATGGAGGGTTCGTCATC	58	126–165	Stockley et al. (2000) [21]
SaGT32	F: GAACACACACGCTGCATACAC R: GTTTGTTGAGGAGGTCAGAGAGGATAATG	58	146–182	Batargias et al. (1999) [22]
Sal12	F: GTAACAATGGTAACGGGGAGTG R: GTTTACCAGACAGCAAAACTGGAT	58	220–264	Brown et al. (2005a) [23]
SauD69INRA	F: GTGTGAAATGGCGTTGATCC R: GTTTGCTGGATTTGAAACTCTGCTAAC	53	222–268	Launey et al. (2003) [19]
SauD182INRA	F: GTCTCACACCAGATTCAAGACAGTA R: GTTTACTTCTGTGTTTCATCTTCATCTCG	58	250–270	Launey et al. (2003) [19]

* Repeat motif and number of alleles are reported in the references [19–23].

Table 2. List of the 9 molecular markers selected from Chistiakov et al. [14], for the analysis of the species European sea bass (*Dicentrarchus labrax):* locus name (Locus), primer sequence (Sequence), annealing temperature (Ta), amplified fragment length (Size range).

Locus	Sequence	T_a	Size Range	Reference *
DLA0008	F: AAGCTATCTGATCTCGCTTG R: ACGTGATTAAGTGTTTGTGAG	56	236–298	Kotoulas et al. (2003) [24]
DLA0119	F: GCAGGTTCAAATTATTTTTGCTC R: TCCTCCTTTTGCTTGCTAGG	54	219–261	Chistiakov et al. (2004) [14]
DLA0016	F: GTGACCGCAGATGAAGAAC R: ACTGTGGGCTCATAAACATC	54	228–258	Chistiakov et al. (2004) [14]
DLA0020	F: GTCTAATGAGCAGTGGAGCAG R: GCATGTTAGATCCACCTCTTTC	56	153–175	Chistiakov et al. (2004) [14]
DLA0105	F: GAGGCTGTATGCTGTTGCAG R: ACCCATGCATAAGGTCAGTG	56	138–172	Chistiakov et al. (2004) [14]
DLA0145	F: CCCACAATAGATTCAAATAG R: CACACATGCAATTATACTG	54	152–188	Chistiakov et al. (2004) [14]
DLA0248	F: TGCATGATGATGTGTGAGCA R: TGGCAGGCTAAAACCTCAAG	54	111–127	Chistiakov et al. (2004) [14]
DLA0228	F: CCAATGTTTTCATCCCCTCA R: TTGCTGCTTGTGAAGTGACC	54	86–98	Chistiakov et al. (2004) [14]
DLA0244	F: ACTGAAAGCACAGCCTGGTT R: CCCCCATCCAATACACTCAC	54	100–104	Chistiakov et al. (2004) [14]

* Repeat motif and number of alleles are reported in the references [14,24].

DNA fragments were amplified using multiplex PCR with a commercially available kit (Type-it Microsatellite PCR Kit, Qiagen, Hilden, Germany) according to the manufacturer's protocol. The PCRs were conducted on a thermal cycler (Applied Biosystems, San Diego, CA, USA). For the microsatellites of the European seabass, the PCR reaction was set up in a final volume of 20 µL, containing 10 µL of Master Mix, 2 µL of primer mix, 4 µL of DNA, and sterile water added to bring the volume to the final amount. Amplification was performed using the "microsat fish" program, which involves an initial denaturation step at 95 °C for 5 min, followed by 35 cycles consisting of denaturation at 95 °C for 40 s, annealing at 53 °C for 50 s, and extension at 72 °C for 1 min. At the end of the cycles, a final extension was performed at 60 °C for 30 min. The samples were then kept at 10 °C until recovery.

For the PCR of the gilthead seabream, the microsatellites were organized into two groups, based on the optimal annealing temperature of the different loci. Group 1 included loci amplified at an annealing temperature of 53 °C, namely: SAU K140, SAU L47, SAU H98, SAUG46, SAL12, and SAUD69.

Group 2, on the other hand, comprised loci requiring a higher annealing temperature of 58 °C, namely: PAGA2, PBMS2, SAGT32, and SAUD182. For both groups, the PCR protocol included an initial denaturation step at 95 °C for 5 min, followed by 35 cycles consisting of denaturation at 95 °C for 40 s, annealing for 50 s at the group-specific temperature, and extension at 72 °C for 1 min. Amplification concluded with a final extension at 60 °C for 30 min, followed by holding at 10 °C until sample retrieval. The PCR reaction for microsatellite amplification was set up in a final volume of 20 µL, containing 10 µL of Mastermix (2x), 0.5 µL of forward primer (20 µM), 0.5 µL of reverse primer (20 µM), 3 µL of DNA, and sterile water added to reach the final volume.

Genetic Analyzer (Applied Biosystems, San Diego, CA, USA) with the GeneScan-500 ROX Size Standard (Applied Biosystems, Foster City, CA, USA). Genotype profiles were generated and visualized using GeneMapper ID 5.0 software (Applied Biosystems).

2.3. Statistical Analysis

The results were recorded by tabulating the peak patterns observed at each locus for both species. Statistical analysis of the data obtained from the microsatellite markers was performed using GenAlEx software v 6.5 [25]. This software was employed to obtain genetic data such as the number of effective alleles (NE), the number of alleles (NA), allele frequency, observed and expected heterozygosity (HO and HE, respectively), locus identity probability (PI), locus exclusion probability (PE) and Fixation Index (F). Deviations from Hardy–Weinberg equilibrium (HWE) were assessed using the R package pegasv.1.3 [26]. Genotypic data were converted into locus format using the genind2loci function, and exact tests for Hardy-Weinberg Equilibrium were performed using the HWE.test function. Statistical significance was evaluated based on *p*-values obtained from the tests, and to account for multiple comparisons across loci, a Bonferroni correction was applied.

3. Results

3.1. Results for Gilthead Seabream

The 10 microsatellite loci selected in this study for gilthead seabream were employed to assess the genetic diversity of a population of 63 individuals from the Sicilian farm investigated (Na > 4). The information for the 10 microsatellites analyzed by GenAlEx is provided in Table 3 and Figure 1. All microsatellite loci were polymorphic, with a total of 91 alleles, averaging 9.1 alleles per locus, and a range from 5 alleles (SauI47INRA) to 14 alleles (PbMS2) per locus. The number of effective alleles (NE) differed from the total number of alleles (NA), ranging from 1.72 (SauH98INRA) to 9.295 (PbMS2), with an average of 4.113 alleles per locus. The Ne/Na ratio averaged 0.451, ranging from 0.172 (SauH98INRA) to 0.663 (PbMS2). The mean observed heterozygosity (Ho) and expected heterozygosity (He) were 0.459 and 0.702, respectively. The PbMS2 marker exhibited the highest heterozygosity level (0.889), while the SauH98INRA marker had the lowest heterozygosity (0.238). The fixation index (F), which measures population differentiation due to genetic structure and is often assessed using genetic polymorphism data such as single nucleotide polymorphisms (SNPs) or microsatellites (SSRs), was 0.352. Allele frequencies are reported in Figure 2. All loci deviated significantly from Hardy-Weinberg equilibrium (HWE) ($p < 0.01$), except for SauI47INRA, as shown in Table 4.

Table 3. Number of samples (N), Number of expected alleles (Na), Number of effective alleles (Ne), Ne/Na ratio, Information Index (I), Observed heterozygosity (Ho), Expected heterozygosity (He) and partial heterozygosity (UHe), Fixation Index (F) for the 10 microsatellite markers adopted for gilthead seabream genotyping.

Locus	N	Na	Ne	Ne/Na	I	Ho	He	UHe	F
SauK140INRA	63	8	4.425	0.553	1.664	0.444	0.774	0.780	0.426
SauI47INRA	63	5	2.086	0.417	0.834	0.460	0.521	0.525	0.116
SauH98INRA	63	10	1.727	0.172	1.037	0.238	0.421	0.424	0.434
SauG46INRA	63	7	2.795	0.399	1.369	0.302	0.642	0.647	0.530
SauD69INRA	63	11	3.767	0.342	1.692	0.429	0.735	0.740	0.417
PaGA2a	63	7	3.713	0.530	1.505	0.413	0.731	0.737	0.435
PbMS2	63	14	9.295	0.663	2.394	0.889	0.892	0.900	0.004
SaGT32	63	11	4.808	0.437	1.889	0.286	0.792	0.798	0.639
Sal12	63	11	5.145	0.467	1.855	0.698	0.806	0.812	0.133
SauD182INRA	63	7	3.364	0.480	1.406	0.429	0.703	0.708	0.390
Average		9.1	4.113	0.451	1.565	0.459	0.702	0.707	0.352

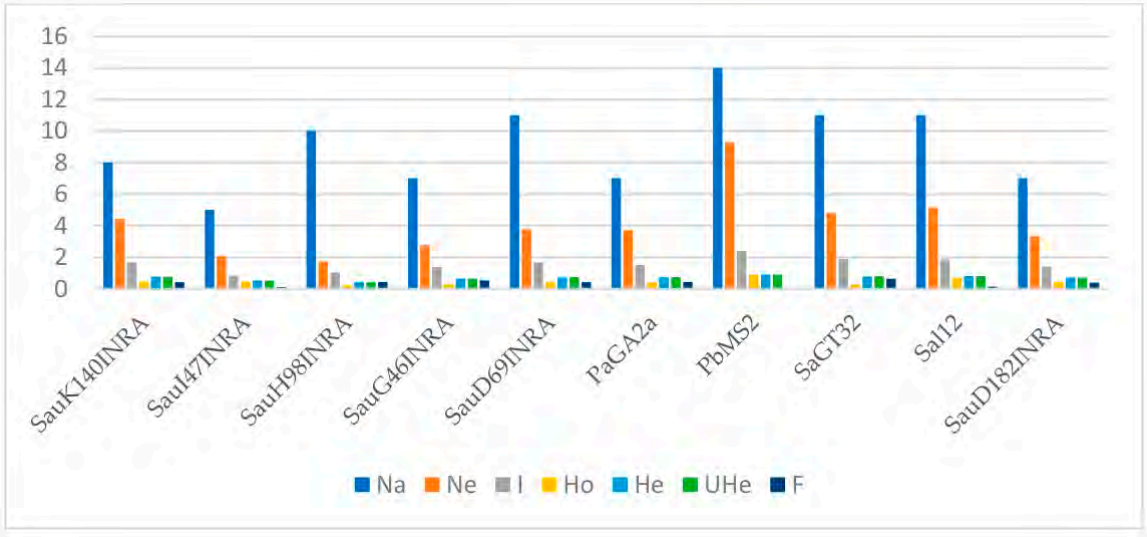

Figure 1. Allelic parameters for each locus in European sea bass.

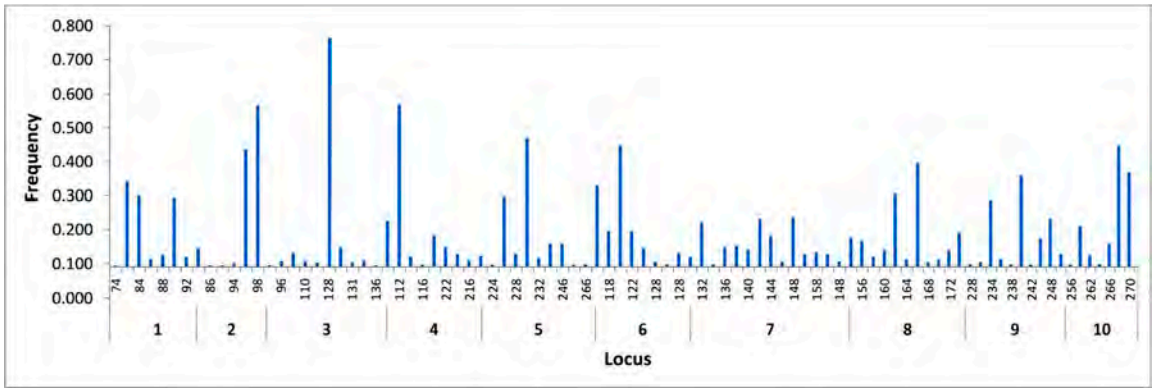

Figure 2. Allele frequency for each of the 10 microsatellite loci analyzed in the population of gilthead seabream.

Table 4. Hardy-Weinberg equilibrium of the analyzed loci for gilthead seabream *(Sparus aurata)* and their *p* values.

Locus	Prob	*p* Value
SauK140INRA	<0.001	**
SauI47INRA	0.952	ns
SauH98INRA	<0.001	**
SauG46INRA	<0.001	**
SauD69INRA	<0.001	**
PaGA2a	<0.001	**
PbMS2	<0.001	**
SaGT32	<0.001	**
SaI12	<0.001	**
SauD182INRA	<0.001	**

ns = not significant; ** = *p* significant.

3.2. Results for European Sea Bass

The 9 microsatellite loci analyzed in this study for European sea bass were used to assess the genetic diversity of a population of 64 individuals from the Sicilian farm investigated (Na > 2). The information for the 9 microsatellites analyzed by GenAlEx is provided in Table 5 and Figure 3.

Table 5. Number of samples (N), Number of expected alleles (Na), Number of effective alleles (Ne), Ne/Na ratio, Information Index (I), Observed heterozygosity (Ho), Expected heterozygosity (He) and partial heterozygosity (UHe), Fixation Index (F) for the 9 microsatellite markers adopted for European sea bass genotyping.

Locus	N	Na	Ne	Ne/Na	I	Ho	He	UHe	F
DLA0248	64	10	4.303	0.430	1.718	0.484	0.768	0.774	0.369
DLA0020	64	6	3.239	0.539	1.358	0.641	0.691	0.697	0.073
DLA0008	64	21	7.522	0.358	2.420	0.578	0.867	0.874	0.333
DLA0228	64	10	4.618	0.461	1.741	0.281	0.783	0.790	0.641
DLA0105	64	14	4.234	0.302	1.867	0.656	0.764	0.770	0.141
DLA0119	64	20	8.039	0.401	2.462	0.578	0.876	0.883	0.340
DLA0244	64	3	2.030	0.676	0.755	0.109	0.507	0.511	0.784
DLA0145	64	14	4.956	0.354	1.945	0.906	0.798	0.805	−0.135
DLA0016	64	13	5.051	0.388	1.982	0.531	0.802	0.808	0.338
Average		12	4.888	0.434	1.805	0.529	0.761	0.768	0.320

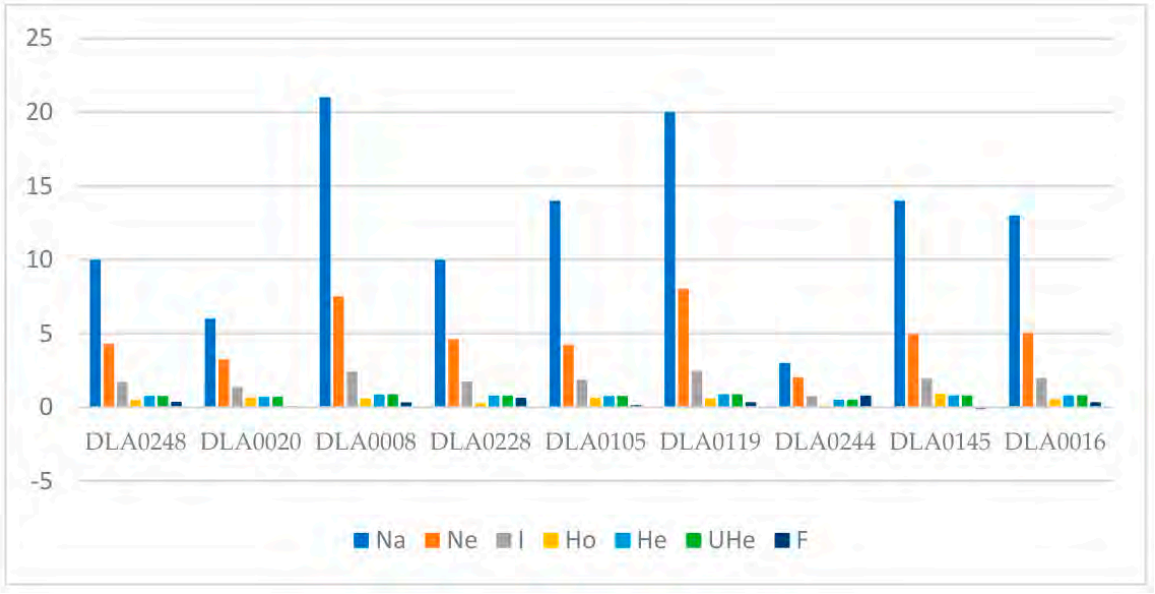

Figure 3. Allelic parameters for each locus in gilthead seabream.

All microsatellite loci evaluated were polymorphic, with a total of 111 alleles, averaging 12 alleles per locus, and a range from 3 alleles (DLA0244) to 21 alleles (DLA0008) per locus. The number of effective alleles (Ne) differed from the total number of alleles (Na), ranging from 2.03 (DLA0244) to 8.04 (DLA0119), with an average of 4.88 alleles per locus. The Ne/Na ratio averaged 0.434, ranging from 0.302 (DLA0105) to 0.676 (DLA0244). The mean observed heterozygosity (Ho) and expected heterozygosity (He) were 0.530 and 0.762, respectively. The DLA0119 marker exhibited the highest heterozygosity level (0.876), while the DLA0244 marker showed the lowest heterozygosity (0.507). The fixation index (F), which measures population differentiation due to genetic structure and is often evaluated using genetic polymorphism data such as single nucleotide polymorphisms (SNPs) or microsatellites (SSRs), was 0.320. Allele frequencies are reported in Figure 4. All loci deviated significantly from Hardy-Weinberg equilibrium (HWE) ($p < 0.01$), except for DLA0145, as shown in Table 6.

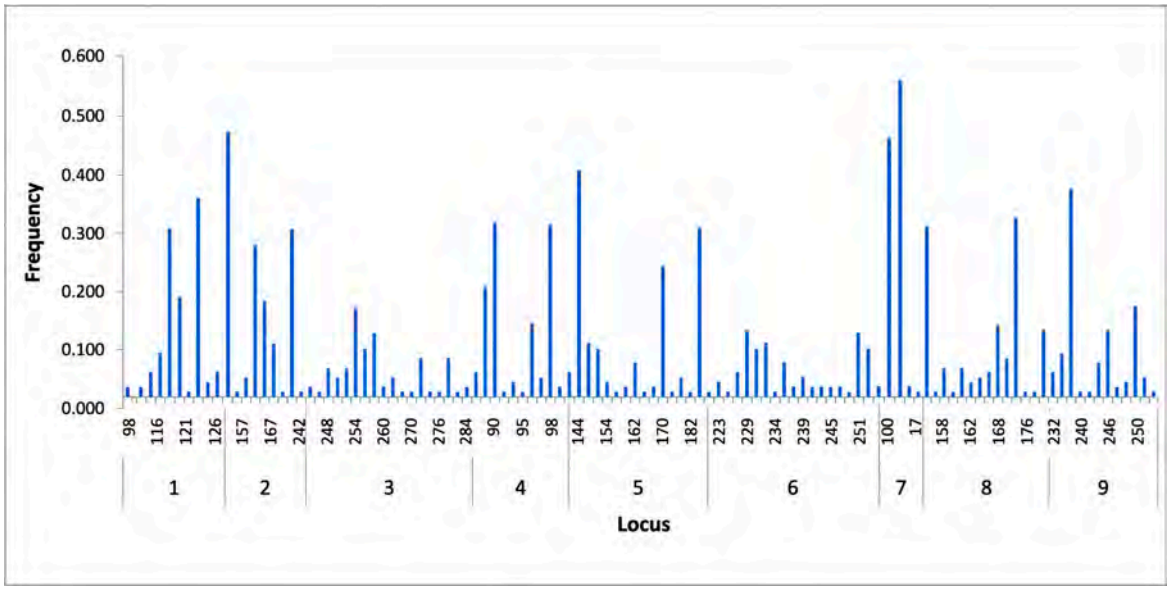

Figure 4. Allele frequency for each of the 9 microsatellite loci analyzed in the population of European sea bass.

Table 6. Hardy-Weinberg equilibrium of the analyzed loci for European sea bass and their *p* values.

Locus	Prob	*p* Value
DLA0248	<0.001	**
DLA0020	0.036	**
DLA0008	<0.001	**
DLA0228	<0.001	**
DLA0105	<0.001	**
DLA0119	<0.001	**
DLA0244	<0.001	**
DLA0145	0.924	ns
DLA0016	<0.001	**

ns = not significant; ** = *p* significant.

4. Discussion

4.1. Genetic Diversity and Population Structure in Gilthead Seabream

The analysis of microsatellite loci in gilthead seabream from the Sicilian farm indicate substantial genetic diversity within the population. The average number of alleles per locus (9.1) and the total number of alleles (91) highlight the polymorphic nature of the loci examined, which is crucial for maintaining the genetic health of the population. These findings are consistent with previous studies in farmed gilthead seabream, which also reported high genetic variability in farmed populations [12].

The observed heterozygosity (Ho = 0.459) was lower than the expected heterozygosity (He = 0.702), suggesting that the population may experience some level of inbreeding or genetic drift, as has been observed in other aquaculture species [27]. Notably, the fixation index (F = 0.352) indicates moderate population differentiation, which could be due to genetic structure within the farm or between the farmed and wild populations. This is particularly important for understanding the long-term sustainability of the farmed population and its potential impact on wild gene pools, especially if genetic introgression occurs [28].

The significant deviations from Hardy–Weinberg equilibrium observed in both species should be interpreted in the context of aquaculture breeding practices. Although individ-

uals were reared within the same tanks, mating is not random in farmed conditions. In particular, reproduction is typically characterized by a limited number of broodstock contributing disproportionately to each cohort. Furthermore, the presence of multiple full-sib and half-sib families within the same tank can generate a hidden population substructure (Wahlund effect), which leads to an apparent deficit of heterozygotes and significant deviations from Hardy–Weinberg expectations. This phenomenon is well documented in aquaculture species where offspring from few parental crosses are subsequently mixed during rearing [29,30]. The obtained results therefore likely reflect practical breeding management conditions, including uncontrolled mating, unequal parental contribution, and the absence of pedigree-based selection strategies. The low Ne/Na ratio (0.451) further suggests that the population may be at risk of genetic bottlenecks or inbreeding depression, which could compromise its long-term viability and adaptability to environmental changes.

Consistent with findings from similar studies in other aquaculture species that reported comparable levels of genetic diversity and heterozygosity, these results emphasize the importance of continued genetic monitoring and management practices to prevent the loss of genetic diversity in farmed populations [16,27]. Furthermore, the high heterozygosity at certain loci (e.g., PbMS2) supports the potential of microsatellites as valuable markers for selection in breeding programs aimed at enhancing genetic diversity and disease resistance in aquaculture [8].

4.2. Genetic Diversity and Structure in European Sea Bass

The analysis of microsatellite loci in the European sea bass population from the Sicilian farm revealed a considerable level of genetic diversity. The 9 loci were all polymorphic, with a total of 111 alleles, an average of 12 alleles per locus, and a range of 3 alleles (DLA0244) to 21 alleles (DLA0008) per locus. This indicates a high level of genetic variation, which is crucial for maintaining population adaptability and resilience, as previously observed in similar farmed fish populations [14].

The number of effective alleles (Ne), which accounts for the genetic contribution of each allele to the overall population structure, ranged from 2.03 (DLA0244) to 8.04 (DLA0119), with an average of 4.88 alleles per locus. The Ne/Na ratio averaged 0.434, which is consistent with patterns observed in other farmed fish populations where effective allelic diversity is typically lower than total allelic diversity due to factors such as inbreeding or genetic drift [8].

A heterozygous deficiency was observed at several loci, specifically in *S. aurata* (SaGT32, SauH98INRA, SauG46INRA) and *D. labrax* (DLA0244, DLA0228). The number of effective alleles (Ne), which is influenced by expected heterozygosity, exhibited a highly skewed distribution when compared to the observed number of alleles (Na). According to Carolino et al. [31], the Ne/Na ratio reflects the allele distribution in relation to the total number of alleles at a given locus. A high Ne/Na ratio suggests the presence of a diverse range of alleles at a specific locus, whereas a low Ne/Na ratio indicates a prevalence of a limited number of alleles despite a high total count of alleles. This pattern was observed for the DLA0016 marker in *D. labrax* and the SauH98INRA marker in *S. aurata*. These findings suggest that the allele frequencies at these loci are highly variable.

The DLA0119 marker exhibited the highest level of heterozygosity (Ho = 0.876), suggesting a high degree of genetic variability at this locus, while the DLA0244 marker showed the lowest heterozygosity (Ho = 0.507), indicating a possible reduction in genetic diversity at this locus.

The observed heterozygosity (Ho = 0.530), as observed for *S. aurata* and other studies, was lower than the expected heterozygosity (He = 0.762), indicating potential inbreeding or genetic drift, which could be attributed to limited breeding practices or the small effective

population size [14,16]. The fixation index (F = 0.320) indicates moderate genetic differentiation within the population, which could reflect population structure or limited gene flow between subgroups within the farm population [28,32]. Furthermore, the significant deviation from Hardy-Weinberg equilibrium (HWE) for all loci, except DLA0145, suggests potential violations of random mating, which could be caused by non-random mating, population substructure, or selection pressures [17]. Such deviations warrant further investigation into the breeding practices and management strategies to ensure the maintenance of genetic diversity and minimize the risk of inbreeding.

4.3. Implications for Genetic Management and Traceability

These results underscore the importance of continued genetic monitoring and management practices to maintain the long-term health and sustainability of farmed *S. aurata* and *D. labrax* populations, ensuring they remain genetically robust and adaptable to environmental and selective pressures.

The implementation of genetic traceability in aquaculture, particularly for species such as European sea bass and gilthead seabream, faces several key challenges. One primary issue is the high genetic variability among populations, which complicates the development of reliable genetic markers for traceability. As observed in this study, the number of alleles per locus varied widely, and deviations from Hardy-Weinberg equilibrium (HWE) suggest population structure or non-random mating, which can further complicate genetic analysis [17]. These complexities require careful marker selection, especially since farmed populations may show different genetic patterns from wild populations [28].

Additionally, selective breeding in aquaculture can reduce effective population size and increase inbreeding, as indicated by the lower Ne/Na ratios (0.434 for *D. labrax*). This reduction in diversity can hinder accurate genetic traceability [32].

Therefore, our results confirmed that microsatellite markers are valuable tools for assessing genetic diversity and traceability. Genetic traceability plays a crucial role in enhancing breeding management in aquaculture, especially for species such as European sea bass (and gilthead seabream). By monitoring genetic diversity, traceability ensures the preservation of genetic integrity, optimizes genetic improvement efforts, and prevents genetic crossover between wild and farmed populations.

For breeding programs, genetic traceability helps maintain genetic diversity and avoid inbreeding. In this study, both species showed high allelic diversity, with gilthead seabream having 91 alleles and European sea bass 111 alleles. By tracking these alleles over generations, breeding programs can preserve genetic health and avoid genetic bottlenecks [32].

Genetic traceability also prevents the introgression of wild alleles into farmed populations. The observed genetic differentiation (F values of 0.352 for gilthead seabream and 0.320 for European sea bass helps maintain distinct genetic lines for farmed stocks, avoiding negative impacts on wild populations [28]. Furthermore, selective breeding can benefit from insights into loci under selective pressure, as indicated by heterozygosity levels in this study, allowing farms to optimize traits like growth and disease resistance. Beyond these general considerations, the practical implementation of genetic traceability in aquaculture systems requires the integration of molecular tools into routine farm management workflows. A first step involves the establishment of a reference genetic database of broodstock individuals, obtained by genotyping all breeders using a standardized panel of microsatellite markers. This enables accurate parentage assignment of offspring and the identification of family structure within production cohorts. Subsequently, offspring batches should be periodically sampled and genotyped to monitor the contribution of individual breeders and detect potential imbalances in reproductive success. This information can be directly used to optimize mating schemes, for example by implementing rotational crosses, factorial

mating designs, or by excluding overrepresented parental lines in subsequent breeding cycles. Such strategies are essential to maintain an adequate effective population size and reduce inbreeding. The integration of genetic data with farm management records (e.g., spawning events, tank allocation, and growth performance) would further enhance decision-making processes, enabling the development of more efficient and sustainable breeding programs. Overall, the routine application of genetic traceability requires relatively low additional costs once genotyping platforms are established, and represents a feasible strategy for improving both genetic management and product traceability in commercial aquaculture systems.

5. Conclusions

Farmed populations of European sea bass and gilthead seabream still retain good genetic diversity, but signs of heterozygote deficiency, Hardy–Weinberg equilibrium deviations, and moderate fixation indices reveal that inbreeding and genetic drift are already occurring. Left unmanaged, these factors threaten to reduce long-term adaptability and productivity.

To ensure sustainable aquaculture, farms must integrate routine genetic monitoring with improved breeding strategies. Key actions include tracking inbreeding levels at each generation, maintaining a large and balanced broodstock, and using controlled mating strategies. Additionally, implementing genetic traceability tools for accurate parentage assignment, screening new broodstock before introduction, and preventing escape events are crucial steps to preserve genetic diversity and protect wild populations.

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