Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.134473 2025, 24(6) 1305-1326

Research Article

Analysis of genetic diversity and population structure in a domesticated Asian seabass (*Lates calcarifer*) population from Iran using microsatellite markers

Nazemroaya S.[1]*, Sadr A.S.[1], Torfi Moozanzadeh M.[2], Youneszadeh Feshalami M.[1], Houshmand H.[1], Ahahangarzadeh M.[1], Zabayeh Najafabadi M.[2], Hafezieh M.[3], Mirbakhsh M.[3], Golshan M.[3], Sharifian M.[3], Khosraviani K.[4]

1 Aquaculture Research Center -South of Iran, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran
2 Bandar-e Imam Khomeyni Marine Fish Research Station, Aquaculture Research Center -South of Iran, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran
3 Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
4 Department of Fisheries, College of Natural Resources and Marine Science, Tarbiat Modares University, Noor, Iran
*Correspondence: s.nazem@areeo.ac.ir

Keywords

Genetic variation,
Genetic differentiation,
Founder population,
Lates calcarifer

Article info

Received: January 2025
Accepted: April 2025
Published: November 2025

Abstract

This study aimed to assess the population structure and genetic diversity of captive *Lates calcarifer*. The investigation included three cultured cohorts initially imported in 2016, 2018, and 2019 (hereafter termed 96, 98, and 99, respectively) as fry to a Marine Fish Research Station (MFRS) at 12 gene loci. The results indicated that all microsatellite markers were polymorphic in all populations. Across three cohorts, the average allele number was 18.5 per locus with nearly half (49%) of 65 unique alleles detected in cohort 99. The average observed alleles (Na), allele richness (Ne), and Shannon Information index (I) in the whole population were 6.25, 4.22, and 1.54, respectively. The mean observed heterozygosity (Ho) in all three cohorts was obtained as 0.733, which accorded with expected heterozygosity (He). The highest and lowest observed alleles were detected in 99 and 96, respectively. The highest mean Na (7.5), Ne (5.02), and Shannon index (1.72), and lowest mean Na (5.33), Ne (3.67), and I (1.39) were observed in cohorts 99 and 96, respectively. Stock 98 represented the Na, Ne, and I as 5.92, 3.97, and 1.5, respectively. The maximum and minimum Ho belonged to 98 (0.787) and 96 (0.675) cohorts, respectively. The fixation index (F) was low in three cohorts and even showed a negative value in 98 (-0.069). Wright's *F*-statistics, including F_{IS}, F_{IT}, and F_{ST}, estimations were evaluated as -0.01, 0.05, and 0.06, illustrating the non-inbreeding coefficient within the population and overall population and the low coefficient of co-ancestry, respectively. Chi-square tests revealed that all 12 markers conformed to Hardy-Weinberg equilibrium (HWE) in the 98 cohorts. In contrast, cohorts 96 and 99 deviated meaningfully from HWE in one locus (*Lca*098) and two loci (*Lca*130 and *Lca*137) (p<0.01), respectively. Nei's standard genetic distance and Pairwise F_{ST} distance between the three cohorts showed the lowest and highest genetic distance between pair cohorts 96-98 and 96-99, respectively. Analysis of molecular variance determined that between-population component of genetic variance (3%) was firmly lower than the within-population component (97%), resulting in meaningful genetic differentiation between populations (F_{ST} = 0.033) (p<0.01). Based on the population genetic structure analysis, under the admixture model, the most negligible probability is associated with K=2 in seabass cohorts, meaning two significant distinct genetic structures. We found that these populations in the MFRS may create a base population, a prerequisite for further selective inbreeding programs.

Introduction

Over the past decade, Asian seabass (*Lates calcarifer*) has emerged as a promising candidate in Iran mariculture industry, thanks to its ability to tolerate a wide range of salinity levels and various culture conditions (Mozanzadeh *et al.*, 2021). These favorable traits could significantly boost the production of this species, contributing to both domestic and regional markets while enhancing national food security (Garlock *et al.*, 2022). To achieve this goal, the Iranian Fisheries Scientific Research Institute (IFSRI) aimed for continuous mass production of fry and sought to reduce reliance on imported fry and brooders. Consequently, thousands of Thai Asian seabass fry were introduced to the Marine Fish Research Station (MFRS) in Bandar-e-Imam Khomeini in three different cohorts during the years 2016, 2018, and 2019 (the terms 96, 98, and 99 are solely used when referring to cohorts introduced in mentioned years, respectively, in the current study) and cultures until they reached sexual maturation (Torfi Mozanzade *et al.*, 2023). In aquaculture, many fish species are known for their mass spawning and high fertility, and even a few breeding individuals can yield large offspring populations that can meet the demand for fish farming. However, inbreeding and genetic drift incidents are inevitable in captive brooders with small effective population sizes, skewed parental contributions in propagation, and mass larval mortalities, resulting in reduced genetic diversity and genetic variation of traits in the long term, accordingly, decrease in fitness, and finally lower productivity in aquaculture (Jenkins *et al.*, 2020; Kobayashi and Kijima, 2010; Duncan *et al.*, 2013; Hillen *et al.*, 2017). Moreover, the success of genetic improvement is hampered by the reduction in genetic diversity due to the founder effect, resulting from using few individuals in propagation (Kivisild, 2013; Sánchez-Velásquez *et al.*, 2022). As cited in the literature review, a hatchery can continuously boost farming efficiency and productivity through broodstocks management, reproduction control, and genetic improvement (Duncan *et al.*, 2013), where investment in genetic broodstocks management has led to enhancing progeny survival and reducing inbreeding (Conrad *et al.*, 2013). According to the goal of a hatchery, employed genetic management strategies that may either preserve genetic diversity or avoid high inbreeding rates in captivity consider methods, including molecular relatedness estimates, pedigree-based crosses, minimal relatedness selection, and minimal kinship selection (Fisch *et al.*, 2015). Fish farmer's role in implementing these strategies is crucial, as proper genetic management can be achieved by conserving genetic diversity and minimizing inbreeding when the pedigree is unavailable (Sánchez-Velásquez *et al.*, 2022).

Molecular data is utilized to determine the relationship between the founders of captive populations and reconstruct the pedigrees when they are not present (Russello and Amato, 2004). The use of genetic markers made the identification of stock structure and genetic variation in populations easier in the 1990s. All genetic marker applications in fisheries biology

rely on detecting genetic variation among individuals. The discovery of genetic differentiation would suggest that source groups have distinct stocks and should be classified as distinct units or stocks (Abdul-Muneer, 2014). Researchers have employed different markers in genetic diversity studies based on the facilities, budget, goals, and novelty. Cuéllar-Pinzón *et al.* (2016) reviewed genetic analyses for 168 marine species and found that microsatellites were the most versatile marker employed. Microsatellites, with 81% of all genotyping methods, were the dominant markers for the first two decades after exploration, while their use decreased to 18% in recent years and rapidly replaced with novel and compound techniques (Kanaka *et al.*, 2023). However, they still seem to be the precise and preferred markers for genetic diversity in cultured stocks and natural populations because of being highly polymorphic, multi-allelic, highly reproducible, having good genome coverage, viable genotyping techniques, and being high-throughput systems that have cost-effective approaches (Choi *et al.*, 2018; Sánchez-Velásquez *et al.*, 2022; Zhang *et al.*, 2022; Wenne, 2023). Microsatellites have been characterized and developed by many researchers for Asian seabass to support population genetic variation and structure studies, parentage studies (Zhu *et al.*, 2010; Senanan *et al.*, 2015; Singh *et al.*, 2023; Wong *et al.*, 2023) and identifying markers linked to growth (Wang *et al.*, 2006; Joerakate *et al.*, 2018) during two past decades.

In this research, we aimed to investigate three key aspects of hatchery broodstocks of Asian seabass: 1) genetic diversity, 2) inbreeding coefficient, and 3) population structure. We focused on broodstocks originating from three different cohorts and analyzed them using 12 microsatellite loci at the Marine Fisheries Research Station (MFRS), which is the primary scientific center for marine fish research in Iran. This study is the first of its kind conducted on Asian seabass stocks in Iran. Our findings are crucial for establishing a founder population, which is a prerequisite for future breeding programs.

Material and methods

The introduced Asian seabass fry cohorts 96, 98, and 99 were bred separately in 5-ton tanks until they reached sexual maturity. After two years, the survived brooders were PIT tagged (tags dimensions: 2.12 × 12 mm from ICAR Company, MJ Utrecht, The Netherlands). A total of 120 *Lates calcarifer* specimens were collected, 40, 36, and 44 individuals for 96, 98, and 99, from three different cohorts. Caudal fin tissue was preserved in 70% ethanol and stored at -20 °C for further genomic DNA extraction using Animal Tissue DNA Isolation Kit (DENAzist Asia, Iran) with some modifications. DNA quantity and quality were evaluated with an ND-3800-OD Nano DOT Microspectrophotometer (Hercuvan Lab System, Malaysia) and checking on a 1.5% agarose gel, respectively, then adjusted to a final concentration of 30 ng/µL. Multi-locus genotypic data were obtained by amplifying the following 12 microsatellite markers, including *Lca*220, *Lca*098, *Lca*193, *Lca*074, *Lca*062, *Lca*137, *Lca*040, *Lca*130, *Lca*064, *Lca*063, *Lca*147, *Lca*411, which showed the highest polymorphism and heterozygosity (Zhu *et al.*, 2010) in multiplex reactions (Table 1).

Table 1: Characterization of 12 microsatellites of Asian seabass (*Lates calcarifer*).

Primer name	sequence	Motif	Allele number	Size (bp)	Fluorescent Type
*Lca*193-F	CAGTCGGGCGTCATCA TCCCACCCCAGTCCAGAAA	(TG)18	14	244–310	5′6-FAM
*Lca*193-R	GTTT TACCAGAGCCTGAAACACAGTAGG				
*Lca*098-F	CAGTCGGGCGTCATCA CAAAGGGGCCACTGCACATAAT	(TG)14	12	195–237	5′6-FAM
*Lca*098-R	GTTT CTCCAGCTCACCCAGGTTCACT				
*Lca*220-F	CAGTCGGGCGTCATCA ATGGCTGTGAAAAGACTGGTATCT	(TG)11	11	124–144	5′6-FAM
*Lca*220-R	GTTT CGCCCCTCACTCAACAGAG				
*Lca*137-F	CAGTCGGGCGTCATCA CGCCTTAAATCTCTACGCTCTGG	(TG)17	10	238–274	5′ -PET
*Lca*137-R	GTTT TCGCATGCTGTAATTAAGGTGGTA				
*Lca*062-F	CAGTCGGGCGTCATCA AGGATGGCACGCTGAAACTATCG	(CA)23	12	200–226	5′ -PET
*Lca*062-R	GTTT ATAAGCTTGTACAGGGGCTGAGTGC				
*Lca*074-F	CAGTCGGGCGTCATCA CATCATTTACACTCTGTTTGCCTCAT	(CA)13	6	158–170	5′ -PET
*Lca*074-R	GTTT GACAGACAGGTGTTTTAGCCTATTTG				
*Lca*064-F	CAGTCGGGCGTCATCA CCCACGGTTTATTTATCTGTCATTATC	(AC)21	11	276–304	5′ -VIC
*Lca*064-R	GTTT AGGCATATGCACCTCACAAGAGTG				
*Lca*130-F	CAGTCGGGCGTCATCA GAGGCTCCCAATCCCAACAA	(TG)19	10	194–242	5′ -VIC
*Lca*130-R	GTTT GGAGGCAGACGAGGAAGGAA				
*Lca*040-F	CAGTCGGGCGTCATCA TGAGGAAGCATCAGCCTGTAATCA	(GT)15	6	124–138	5′ -VIC
*Lca*040-R	GTTT CAGGACGCAAACACTGAAAT				
*Lca*411-F	CAGTCGGGCGTCATCA GTGGTGCAGCGGTTGCTCTC	(CA)21	8	249–275	5′ -NED
*Lca*411-R	GTTT CCGACTCATGCTGCTTTTCGTAAT				
*Lca*147-F	CAGTCGGGCGTCATCA TGCCCCTAATGTATTCTTTCCACT	(TC)35	16	164-226	5′ -NED
*Lca*147-R	GTTT GCTCCCACCTCTCATTCATTATTC				
*Lca*063-F	CAGTCGGGCGTCATCA CAAGATGTTGCTGATATCATTGTCT	(GT)17	7	130-158	5′ -NED
*Lca*063-R	GTTT AGATGGCTCTCCACCAGATTAT				

The PCR amplification and genotyping process was carried out with utmost precision, ensuring the accuracy of the genetic data. The total volume of the PCR amplification reaction was 25 µL; each reaction contained 12.5 µL of 2× Ampliqon

Taq DNA Polymerase Master Mix (Denmark), 1 µL of DNA (concentration 30 ng/µL), 1 µL of fluorescent forward/reverse primers, and PCR water for the remainder. PCRs were conducted on Corbett Research (CG1-960) Palm cycler with the following cycling conditions: 94°C for 5 min; 30 cycles of 94°C for 30 s, 55°C for 40 s, and 72°C for 50 s; and finally, 72 °C for 10 min. The PCR products were genotyped based on capillary electrophoresis using Applied Biosystems™ 3500 XL Genetic Analyzer (Thermo Fisher Scientific) equipped with Gene Mapper 3.5 software. The GenescanLiz500 size standard was used to determine the PCR product size.

The fsa files obtained from the sequenced microsatellite fragments were analyzed in the Geneious IR9 software under the Microsatellite plugin (Kearse *et al.*, 2012). The obtained genotypes were saved as an Excel file and were used to analyze the following statistics in different software. MICRO-CHECKER 2.2.3 software was used to check the frequency of null alleles (Van Oosterhout *et al.*, 2004). Different genetic diversity parameters, including the number of alleles (NA), number of effective alleles (Ne), expected heterozygosity (He), observed heterozygosity (Ho), Shannon's information index (I), polymorphic information content (PIC), analysis of molecular variance (AMOVA, 10000 permutations, significance: $p<0.05$), Hardy–Weinberg Equilibrium (HWE), and principal coordinates for three cohorts were estimated using GenAlEx 6.5 (Peakall and Smouse, 2012). The pairwise population genetic differentiation index (F_{ST}) and Nei's genetic distance were calculated using FSTAT 2.9.4 software (Goudet, 2002). Wright's definition was used to analyze the F_{ST}, where 0–0.05, 0.05–0.15, 0.15–0.25, and >0.25 infer minor, moderate, significant, and great genetic differentiation, respectively. MEGA 6 was used to construct an unweighted pair group method with the UGMA tree based on Nei's genetic distance (Tamura *et al.*, 2011) among the individuals belonging to three cohorts. Bayesian clustering program STRUCTURE 2.3.2 (Pritchard *et al.*, 2010) was used to estimate the most likely population structure based on microsatellite data for the three cohorts followed by an admixture model, with a priori population information, using a burn-in period of 500,000 MCMC and 1,000,000 subsequent MCMC repeats for each K value between 1 and 10 for four iterations. The convergence of the mean estimate of the ln probability of ΔK was established to ascertain the accurate number of clusters K (Pritchard *et al.*, 2000). Structure Harvester was run to determine the statistically most supported number of clusters using the ΔK metric (Earl and vonHoldt, 2012).

Results

Micro-Checker results did not reveal any evidence of scoring alleles because of stuttering, either for large allele dropout or the presence of null alleles. The results indicated that all microsatellite markers were polymorphic in all Asian seabass populations, and ranged from seven (*Lca*074) to fourteen (*Lca*220, *Lca*067, and *Lca*147) per locus. Across three cohorts at 12 microsatellite loci, 128 alleles were found with an average value of 10.67 alleles per locus. The highest and lowest average

number of different alleles (Na), Effective Alleles number (Ne), and Shannon Information index (I) belonged to *Lca*063 with 8.67, 5.80, and 1.91, and *Lca*074 with 3.67, 2.23, and 0.94, respectively. The *Lca*193 and *Lca*040 showed the maximum and minimum observer heterozygosity (Ho), with a value of 1.00 and 0.29, respectively. The Shannon Information index results at the loci level mirrored those from Ne and He in terms of trend, except for Ho. Nearly half (49%) of 65 unique alleles were detected in cohort 99. Moreover, the *Lca*137 locus had exclusively two unique alleles in cohort 99. The overall average observed alleles (Na), allele richness (Ne), and Shannon Information index (I) within three cohorts were 6.25, 4.22 per locus, and 1.54, respectively. The overall mean observed heterozygosity (Ho) in all three cohorts was obtained as 0.73, corresponding with the mean expected heterozygosity (He=0.73). Wright's F-statistics, including F_{IS}, F_{IT}, and F_{ST}, estimations were evaluated as -0.01, 0.05, and 0.06, illustrating the non-inbreeding coefficient within the population, overall population and low coefficient of co-ancestry, respectively (Table 2). This detailed genetic diversity and population structure analysis provides a comprehensive understanding of the genetic makeup of the cohorts.

Table 2: Characteristics and genetic diversity indices for twelve microsatellite loci in *Lates calcarifer*.

Locus	N	Na	Ne	I	Ho	He	F_{IS}	F_{IT}	F_{ST}
Lca220	14	7.33	4.88	1.76	0.80	0.79	-0.01	0.03	0.04
Lca098	13	7.00	4.79	1.69	0.74	0.78	0.05	0.10	0.05
Lca193	8	4.33	2.84	1.15	0.29	0.61	0.53	0.57	0.09
Lca074	7	3.67	2.23	0.94	0.82	0.55	-0.49	-0.45	0.03
Lca062	9	6.33	4.81	1.68	0.84	0.79	-0.06	-0.04	0.02
Lca137	8	6.00	4.25	1.57	0.61	0.76	0.20	0.25	0.07
Lca040	8	5.67	4.04	1.49	1.00	0.74	-0.36	-0.28	0.06
Lca130	12	6.67	4.41	1.61	0.71	0.76	0.06	0.16	0.10
Lca064	9	5.00	3.10	1.31	0.81	0.68	-0.20	-0.16	0.03
Lca063	14	8.67	5.80	1.91	0.79	0.81	0.02	0.07	0.05
Lca147	14	7.67	4.87	1.69	0.84	0.75	-0.11	-0.01	0.09
Lca411	9	6.67	4.62	1.69	0.53	0.78	0.32	0.35	0.05
Average	10.42	6.25	4.22	1.54	0.73	0.73	-0.01	0.05	0.06
SE	0.79	0.41	0.29	0.08	0.05	0.02	0.08	0.08	0.01

N, number of alleles, Na, Average number of different alleles/ locus; Ne, number of effective Alleles (Ne), He, expected heterozygosity; Ho, observed heterozygosity; F_{IS}, inbreeding coefficient of an individual relative to the subpopulation; F_{IT}, inbreeding coefficient of an individual relative to the total population, F_{ST}, inbreeding coefficient between population

The highest and lowest observed alleles were detected in 99 (with 90 alleles) and 96 (with 64 alleles), respectively. The highest mean Na (7.5), Ne (5.02), and Shannon index (1.72), and lowest mean Na (5.33), Ne (3.67), and I (1.39) were observed in cohorts 99 and 96, respectively. Cohort 98 represented the Na, Ne, and I as 5.92, 3.97, and 1.5, respectively. The cohorts with the highest and lowest Ho were 98 (0.79) and

96 (0.68), respectively, while the Ho in 99 was 0.73. The fixation index (F_{IS}), showing the inbreeding coefficient, was low in three cohorts and even showed a negative value in cohort 98 (-0.07), indicating that excess heterozygosity resulted in negative F_{IS} values. The deviation from Hardy–Weinberg equilibrium, a significant finding, was observed at some loci in cohorts. Chi-square tests revealed that all of the 12 markers conformed to Hardy-Weinberg equilibrium (HWE) in the 98 cohort, while cohorts 96 and 99 deviated meaningfully from HWE in one locus (*Lca*098) and two loci (*Lca*130 and *Lca*137) (p<0.01), respectively (Table 3).

Table 3: Genetic diversity statistics of three *Lates calcarifer* cohorts at 12 microsatellite loci.

Cohort		Locus													
		*Lca*220	*Lca*098	*Lca*193	*Lca*074	*Lca*062	*Lca*137	*Lca*040	*Lca*130	*Lca*064	*Lca*063	*Lca*147	*Lca*411	Average	SE
96.00	Na	8.00	4.00	4.00	4.00	5.00	5.00	4.00	4.00	4.00	9.00	6.00	7.00	5.33	0.51
	Ne	5.26	3.39	2.30	2.33	4.44	3.17	2.82	2.99	2.99	6.45	2.86	5.00	3.67	0.38
	I	1.87	1.29	1.03	1.00	1.54	1.33	1.16	1.19	1.19	2.00	1.37	1.75	1.39	0.09
	Ho	1.00	0.50	0.30	0.80	0.60	0.30	1.00	0.70	1.00	0.80	0.60	0.50	0.68	0.07
	He	0.81	0.71	0.57	0.57	0.78	0.69	0.65	0.67	0.67	0.85	0.65	0.80	0.70	0.03
	F	-0.23	0.29	0.47	-0.40	0.23	0.56	-0.55	-0.05	-0.50	0.05	0.08	0.38	0.03	0.11
	HWE	ns	**	ns	ns	ns	*	ns	*	*	ns	ns	*		
98.00	Na	7.00	7.00	3.00	4.00	7.00	6.00	6.00	8.00	5.00	7.00	5.00	6.00	5.92	0.42
	Ne	4.91	4.76	2.05	2.19	4.50	4.91	5.06	4.63	3.00	3.38	3.68	4.63	3.97	0.31
	I	1.74	1.72	0.83	0.98	1.69	1.67	1.69	1.77	1.30	1.56	1.43	1.65	1.50	0.09
	Ho	0.78	1.00	0.11	0.67	1.00	0.89	1.00	0.89	0.89	0.67	1.00	0.56	0.79	0.08
	He	0.80	0.79	0.51	0.54	0.78	0.80	0.80	0.78	0.67	0.70	0.73	0.78	0.72	0.03
	F	0.02	-0.27	0.78	-0.23	-0.29	-0.12	-0.25	-0.13	-0.33	0.05	-0.37	0.29	-0.07	0.09
	HWE	ns	ns	*	ns	ns	ns	ns	*	ns	ns	ns	ns		
99.00	Na	7.00	10.00	6.00	3.00	7.00	7.00	7.00	8.00	6.00	10.00	12.00	7.00	7.50	0.67
	Ne	4.48	6.21	4.17	2.18	5.50	4.65	4.25	5.63	3.32	7.56	8.07	4.25	5.02	0.49
	I	1.67	2.05	1.58	0.85	1.81	1.71	1.62	1.87	1.43	2.16	2.28	1.66	1.72	0.11
	Ho	0.64	0.73	0.45	1.00	0.91	0.64	1.00	0.55	0.55	0.91	0.91	0.55	0.73	0.06
	He	0.78	0.84	0.76	0.54	0.82	0.79	0.76	0.82	0.70	0.87	0.88	0.76	0.78	0.03
	F	0.18	0.13	0.40	-0.85	-0.11	0.19	-0.31	0.34	0.22	-0.05	-0.04	0.29	0.03	0.10
	HWE	ns	*	ns	*	ns	**	ns	**	ns	ns	ns	ns		

Na, allele number; Ne, effective number of alleles; He, expected heterozygosity; Ho, observed heterozygosity; F, fixation index; HWE, deviation from the Hardy-Weinberg equilibrium (ns, non-significant; *: p<0.05; **: p<0.01, ***: p<0.001).

The genetic differentiation between cohorts, as analyzed using F_{ST} and Nei's genetic distance calculated from a pairwise comparison, is shown in Table 4. The statistical significance found in all pairwise F_{ST} estimates (p<0.05) underscores the importance of this differentiation. The Nei's standard genetic distance and Pairwise F_{ST} distance between the three cohorts showed the lowest genetic distance between pair cohorts 96-98 (with values of 0.199 and 0.034, respectively) and the highest genetic distance between pair cohorts 96-99 (with values of 0.337 and 0.049, respectively).

Furthermore, the Nei's and F_{ST} distance between pair cohorts 89-99 were calculated as 0.337 and 0.045, respectively (Table 4).

Analysis of molecular variance (AMOVA) revealed that the between-population component of genetic variance (3%) was firmly lower than the within-population component (97%), indicating that variation within individuals was the main source of genetic variation. This resulted in a small but meaningful genetic differentiation between populations (F_{ST}=0.03, p<0.01) (Table 5; Fig. 1).

Table 4: Pairwise population matrices Nei's genetic distance (below the diagonal) and F_{ST} values using all loci (above the diagonal) among three fish cohorts of *L. Calcarifer*.

Stocks	96	98	99
96	0.000	0.034	0.049
98	0.199	0.000	0.045
99	0.337	0.329	0.000

Table 5: Summary analysis of molecular variance (AMOVA).

Source	df	SS	MS	Est. Var.	Percentage of variation	*F*-Statistics values	*p*- value
Among populations	2	16.21	8.11	0.16	3%	F_{ST}=0.03	0.00
Among individuals within populations	27	132.92	4.92	0.27	6%	F_{IS}=0.06	0.03
Within individuals	30	131.50	4.38	4.38	91%	F_{IT}=0.09	0.00
Total	59	280.63		4.81	100%		

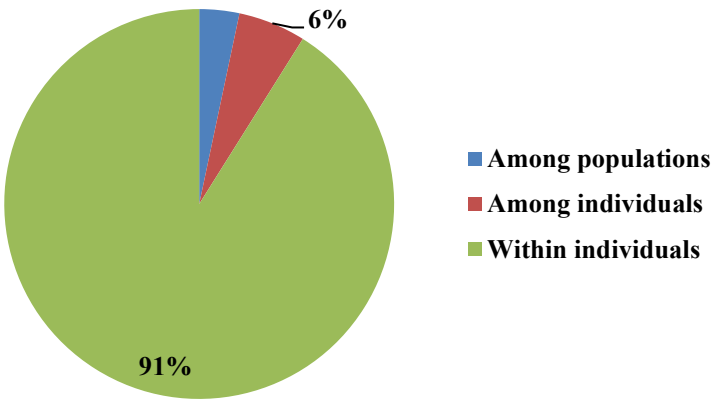

Figure 1: Percentage of molecular variance among *L. calcarifer* populations and individuals, and within individuals.

Principal coordinates analysis of three *L. calcarifer* cohorts revealed genetic structure among samples (Fig. 2). The distribution pattern of the samples shows a large amount of genetic mixing and similarity between them. However, the cohort 96 was close to the 98. These cohorts are closer genetically compared to 99. Cohort 99 is relatively distant from the other cohorts. Based on STRUCTURE Bayesian model-based clustering algorithm to analyze population genetic structure, under the admixture model, the Delta-K value in seabass cohorts was highest when

K equaled 2, meaning that the optimal number of populations of the three *L. calcarifer* cohorts was two (Fig. 3). Individuals from 96 and 98 were assigned to Cluster 1, while Cluster 2 exclusively consisted of 99 individuals (Fig. 4). The admixture analysis findings were in line with those of the STRUCTURE analysis (Fig. 5).

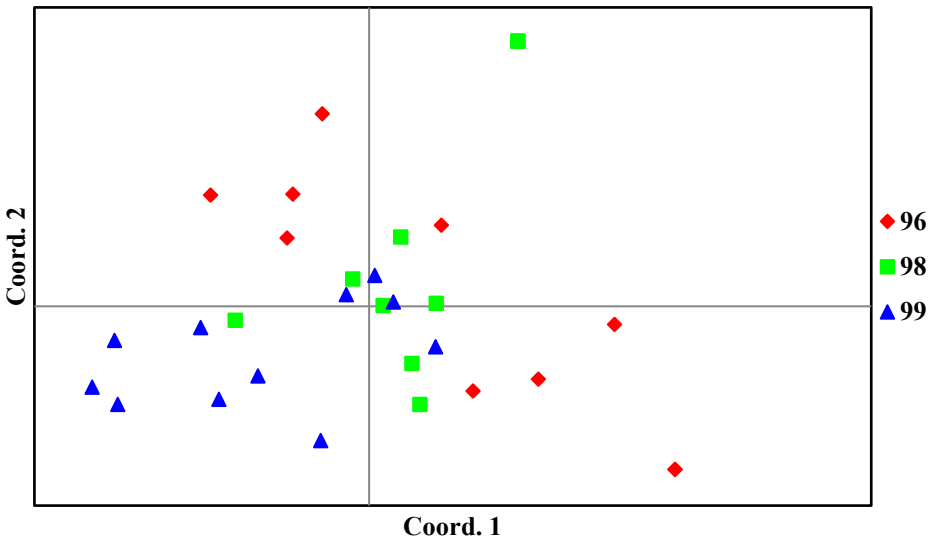

Figure 2: Genetic scatter plots of *L.calcarifer* exhibited by principal coordinate analysis (PCoA).

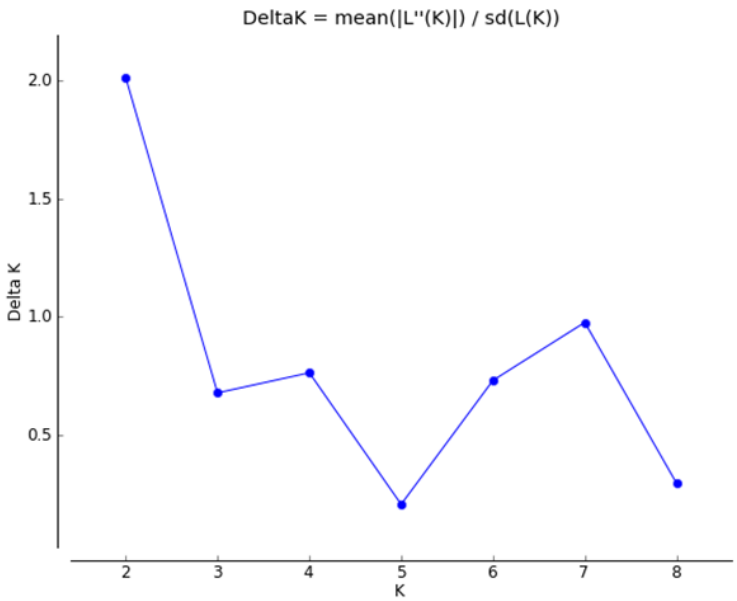

Figure 3: Relationships between the number of clusters (K) and the corresponding Delta K statistics from structure analysis.

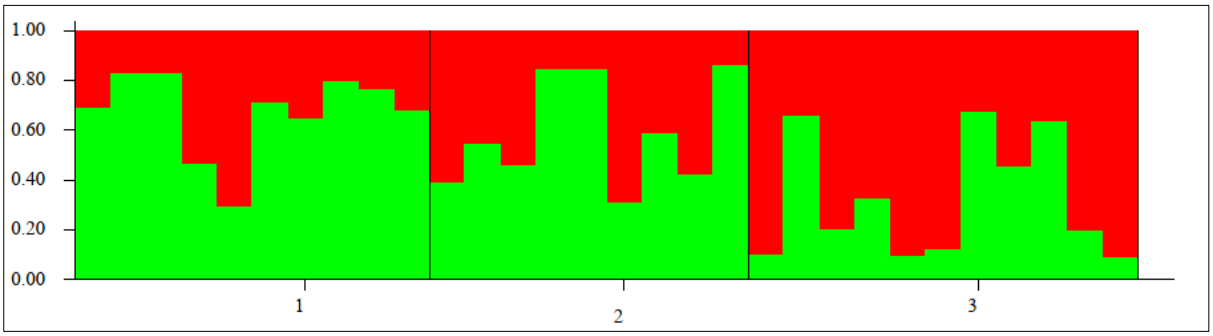

Figure 4: STRUCTURE genetic cluster analysis for *L. calcarifer* populations (K=2), 1: cohort 96, 2: cohort 98, and 3: cohort 99.

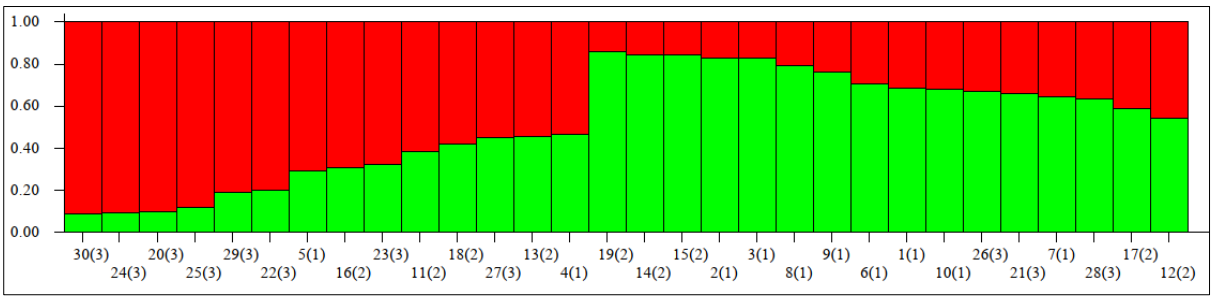

Figure 5: Admixture plots for two population of *L. calcariferi* (Selected samples).

Discussion

In our study, 12 standard sets of microsatellite loci described by (Zhu *et al.*, 2010) were tested. All loci were successfully amplified. An analysis of Asian bass genetic diversity and population structure was powerfully accomplished through these 12 microsatellite markers with high polymorphism. A total of 128 alleles were found, representing the allelic diversity. Across three different cohorts of Asian seabass broodstocks kept at the MFRS, the average allelic number was 10.67 alleles per locus, which is lower than the value of 20.6 alleles per locus in other marine fishes (DeWoody and Avise, 2000) and that calculated in the study of Yue *et al.* (2009) in Asian seabass (15 alleles/ locus). By contrast, it is relatively comparable to that indicated in anadromous fish with 11.3 alleles per locus (DeWoody and Avise, 2000), thin lip grey mullet, *Chelon ramada*, (a Catadromous species) with 11 alleles per locus (Pereira *et al.*, 2023) while higher than that in Singh *et al.* (2023) who found an average value of 8.19 in Asian seabass. Prasertlux *et al*. (2025) encountered 3.429–11.571 alleles per locus in five domesticated populations and two commercial stocks of Asian seabass. According to our results, the three cohorts exhibit a proper level of allelic diversity. Cohort 99, with a 7.5 average number of alleles, is more meaningfully diverse than the other two cohorts, 96 (5.33) and 98 (5.92). Likewise, the average number of alleles ranged from 6.385 to 7.077 in Asian seabass populations gathered from commercial catches of three major landing sites in India (Singh *et al.*, 2023). In contrast, the average alleles number was lower than that encountered in the Southeast Asian seabass population, with a value of 13.28 (Yue *et al.*, 2009). The loss

of functional genetic variation, which can be detected by reducing the number of observed alleles at microsatellite loci, is a phenomenon that should be prevented primarily in aquaculture (Lind *et al.*, 2009). To illustrate the importance of this phenomenon, a notable case was found in Asian seabass of different lines where the average number of alleles reduced during four generations of selection from 10 to 7.1 (Wong *et al.*, 2023). However, the number of alleles is often not comparable due to the size of the samples and the general inconsistency of data from different studies (Yue *et al.*, 2009). The allele richness (Ne) parameter for measuring allele number without considering sample size (El Mousadik and Petit, 1996) represented that this parameter in cohort 99 (5.02) is higher than the two other cohorts (96= 3.67 and 98= 3.97). The high allelic richness, indicating a high effective population size (Lind *et al.*, 2009), provides an accurate genetic diversity estimation in candidate populations for conservation or selection programs (Diz and Presa, 2009). In the current study, the average number of alleles per locus (6.25) and the average number of effective alleles (4.22) exhibiting allelic richness indicated moderate genetic diversity across all cultured cohorts at the MFRS. However, Yue *et al.* (2009) found that the wild Asian seabass population owned a notable allele richness than the cultured one, ranging from 7.60 to 8.5 and 3.72 to 7.89 in wild and farmed populations, respectively, allele richness was not satisfying in cohorts 96 and 98, and accorded with a low range of farmed populations in the mentioned study, and across all cohorts, it mirrored the average range of the cultured population. In the present study, the allele richness is now in the cultured Asian seabass population range. However, mating between genetically related brooders might lead to a reduction in the next generations. It is worrisome since maintaining and increasing the genetic diversity of broodstocks is crucial to guarantee the sustainability of future breeding programs. In a similar case in Asian seabass of different lines, Wong *et al*. (2023) noticed a gradual decrease in allelic richness from F0 (Ar=9.12) to F4 (Ar=6.28), but the decrease was not statistically meaningful. However, they considered this insignificance meaningless because an allele cannot be recovered when it diminishes in one generation.

The optimal parameter for assessing population genetic variation is genetic heterozygosity, defined by the proportion of heterozygous genotypes at every microsatellite locus (Du *et al.*, 2022). Heterozygosity represents a broad spectrum of genotypes as a response to adaptability in changing environmental conditions. It might show its advantages in more heterozygous individuals at substantial characteristics such as growth, fertility, and disease resistance (Beardmore *et al.*, 1997). The observed heterozygosity being close to the expected heterozygosity suggests that the population is in equilibrium. Population size significantly affects observed heterozygosity, while expected heterozygosity could represent more accurately the level of genetic diversity, which is only affected by inheritance (Du *et al.*, 2022). If the observed heterozygosity is lower than predicted, anticipating some

level of inbreeding is possible. Calculating higher heterozygosity than predicted might be the cause of an isolate-breaking effect or the mixing of two previously isolated populations (Kanaka *et al.*, 2023). In this study, the observed and expected heterozygosity are equal (0.73) in the population in MFRS and are higher than the average and almost close to the average value calculated for wild marine fish (0.79) (DeWoody and Avise, 2000), exhibiting that the population is in equilibrium. The amount of observed and expected heterozygosity in the 96, 98, and 99 cohorts is higher than the average, reflecting a high level of genetic diversity (Du *et al.*, 2022), and also consistent with the results of a previous study on Asian sea bass (Yue *et al.*, 2009). Cohort 98 had a higher observed heterozygosity than the other cohorts, which also was slightly above the expected heterozygosity. Higher observed than expected heterozygosity can occur in admixed populations, where the recent progeny of individuals was derived from several separate stocks, leading to the merging of genetic variants and increased genetic diversity (Boca *et al.*, 2020). The higher observed heterozygosity than expected in cohort 98 can be due to the use of diverse lineage in their reproduction (brooders purchased from different Southeast Asia regions in the private sector hatchery) in Iran. Yue *et al.* (2009) found that various populations of Asian seabass have different genetic diversity, and the genetic diversity of the Australian wild population was less than that of the Southeast Asian wild population. Despite the low allelic richness, the expected and observed heterozygosity was remarkably preserved in the current study. Wong *et al.* (2023) came across a result that was similar to ours. The quantity of genetic variability can be determined by measuring heterozygosity across loci as a general indicator. When the number of marker loci is significant enough to account for a large portion of genome polymorphisms, the relationship between heterozygosity at a set of loci and genomic heterozygosity is highly correlated (Dewoody and Dewoody, 2005; Miller *et al.*, 2014). Since we employed the markers characterized by Zhu *et al*. (2010) in this study, by comparing the observed and expected heterozygosity between both studies, we determined that the amount of observed heterozygosity in four loci (Lca193, Lca137, Lca130, and Lc411) are less than its value in the mentioned study. However, it is almost equal or much more in the rest of the loci. Additionally, the expected heterozygosity for most of the loci in our study is either greater or equal to what they observed. Since proper broodstock management for operations like breeding and selection requires an understanding of genetic diversity, the amount of heterozygosity showed that although the Asian seabass stocks in our study are cultured fish, they maintain good genetic diversity for further operations.

In population genetics studies, the Shannon index enables differentiation between populations with identical numbers of alleles, when in some populations, only a few common alleles dominate loci. In contrast, in others, all alleles contribute more evenly to variation. The Shannon index is more susceptible to losing rare variants (e.g., caused by genetic

bottlenecks) than heterozygosity, providing more information than AR or merely estimating allele numbers (Sherwin *et al.*, 2017). Unlike heterozygosity, which has a limit of one for each allele, the maximum value of Shannon's index in molecular studies is equal to ln(n) (Konopiński, 2020), ranging from zero (without diversity) to infinity. The average Shannon's information index in all cohorts is 1.54, demonstrating the diversity in the population. The highest and lowest Shannon diversity index was observed in cohorts 99 and 96, with the values 1.723 and 1.395, respectively. The highest Shannon index was obtained at Lca063 locus (2.222), with the highest number of alleles and effective alleles. In contrast, the lowest Shannon index was found at Lca074 locus (1.044), where it gains the lowest alleles number and effective alleles. These values support the maximum and minimum range calculated for the Shannon variation. The changes in Shannon's information index value at the level of populations and loci indicate the effectiveness of microsatellite markers in revealing the differences. Thus, the markers employed in this investigation are practical for evaluating genetic diversity in Asian sea bass. The trends of Shannon index results for cohorts 96 and 99 were similar to those derived from allele richness and expected and observed heterozygosity and correlated positively. In cohort 98, the trends of Shannon index results only were correspondent to those obtained from allele richness and the expected heterozygosity, and vice versa differed from observed heterozygosity because the observed heterozygosity in cohort 98 was estimated more than the expected heterozygosity.

The F index, a pivotal tool in our comprehension of inbreeding, plays a significant role in indicating an increase in allelic fixation and a decrease in heterozygosity. Its importance cannot be overstated in our research.

The fixation indices for each homozygous genotype are determined based on the fixation indices for the heterozygous genotypes (Nagylaki, 1998). The F index value is meagre in all three cohorts and negative (-0.07) in the 98. The negative value of this index in the 98 is because the observed heterozygosity is more significant than expected (Pudovkin *et al.*, 1996). It's crucial to note that founder effects and bottlenecks, as highlighted by Yue *et al.* (2009), are common in cultured populations and can lead to a decline in genetic diversity. They pointed out that rare alleles at polymorphic DNA marker loci often become infrequent in populations that encounter a reduction in effective population size, and the absence of rare alleles has a more significant consequence on expected heterozygosity at mutation-drift equilibrium than observed heterozygosity, leading to an overabundance of the latter.

Hardy-Weinberg Equilibrium (HWE) is a powerful tool for estimating the number of homozygous and heterozygous variant carriers based on their allele frequency in non-evolving populations. Genotyping errors can be detected by noticing deviations from HWE in large population databases, leading to excessive heterozygote excess. However, it might also be a sign of natural selection since the

occurrence of a variant causing recessive disease should be less expected in a homozygous state in the population. In contrast, it may reach high allele frequency in a heterozygous state, mainly if they are favorable (Abramovs *et al.*, 2020). In addition, other reasons may reject a test of HWE, including mutation, migration, selection, and population substructure (Laird and Lange, 2011). The Chi-square tests revealed that all 12 markers conformed to HWE in the 98 cohorts. In contrast, cohorts 96 and 99 deviated meaningfully from HWE in one locus (Lca098) and two loci (Lca130 and Lca137) (p<0.01), respectively. In all three loci in two cohorts, 96 (Ho: He, 0.5: 0.775 in Lca098) and 99 (Ho: He, 0.545: 0.822 in Lca130 and Ho: He, 0.636: 0.764 in Lca137) that departed from HWE, the observed heterozygosity is lower than the expected heterozygosity. Therefore, in the current study, the genotypic error causing the increase of observed heterozygosity was not the reason for the departure from the Hardy-Weinberg equilibrium in these loci. Natural or biological causes, including genomic deletions, population stratification and inbreeding, may cause heterozygosity loss, leading to HWE departure (Chen *et al.*, 2017). In this study, the reduction of heterozygosity in three loci that can lead to departure from the Hardy-Weinberg equilibrium is also consistent with the positive values of F_{IS} in the same loci. In this study, the reduction of heterozygosity in three loci that can lead to departure from the HWE is also consistent with the positive values of F_{IS} in the same loci. Zhu *et al.* (2006) found that the wild populations of Asian seabass were in HWE but were not about the Singapore breeding populations in some loci. In our study, all three cohorts showed overall HWE equilibrium, and this indicates that the reproduction process in the private section hatchery, where the brooders of this study had been imported in the initial form of fry from Thailand, did not lead to deviating from the HWE.

Researchers use various methods to determine the differentiation and genetic structure of populations. The most common approaches rely on F-statistics, which require establishing population boundaries at the beginning of the study. Introduced by Wright (1951), F-statistics were developed to characterize population structure in natural populations and quantify genetic drift and allelic diversity. Different F-coefficients, such as F_{IT}, F_{IS}, and F_{ST}, account for gene correlation across all populations, within the population, and within the total population, respectively. F_{ST}, for example, estimates inter-specific genetic divergence, F_{IS} describes inbreeding within taxa, and F_{IT} represents overall inbreeding for a species. Heterozygosity (H) and genetic drift play significant roles in F-statistics, with inbreeding leading to sub-structuring and decreased heterozygosity. Genetic drift and inbreeding are interconnected processes and can be expressed in terms of H (Kanaka *et al.*, 2023). According to the Wright (1965)' criterion, the F_{IT} value ranges from 0 (no isolation) to 1 (total isolation). However, lower values (such as 0.5) may indicate complete isolation among populations and a tendency toward polymorphism in loci. In this study, the F_{IT}, or total inbreeding coefficient, for Asian seabass cohorts was calculated as 0.049,

which indicates that there is no overall inbreeding and that the population is not isolated in terms of inbreeding. Wright (1965) pointed out that when F_{IS} is 0, there is neither inbreeding nor outbreeding, suggesting random mating and adherence to HWE. An F_{IS} equal to 1 indicates complete inbreeding, while an F_{IS} of -1 signifies complete outbreeding. In the current study, the average value of the F_{IS}, which is the inbreeding coefficient within the population, was estimated at 0.005, indicating that inbreeding in these populations is insignificant and the population is in equilibrium. The F_{ST} value is also significant. When F_{ST} is 0, it means no population structure or differentiation; values between 0 and 0.5 indicate low population differentiation, whereas an F_{ST} of 1 implies complete differentiation of the population. In our study, the average value of the F_{ST} that estimates the amount of genetic differences between cohorts was 0.033. This value does not indicate complete differentiation but does not imply a lack of differentiation. It shows significantly little difference between cohorts.

In the context of Analysis of Molecular Variance (AMOVA), the total variance is divided into distinct components, each depicting the proportion of the total variance at different hierarchical levels, including within and among populations. Thus, variance component ratios can be employed to describe population structure (Yue *et al.*, 2009).

The analysis of molecular variance (AMOVA) in the current study revealed that 97% of the genetic variance exists within populations, while only 3% exists between populations. This result indicates a small but significant genetic differentiation between populations, with an F_{ST} value of 0.033 (p<0.01). Additionally, a study by Yue *et al.* (2009) found that within-population genetic differentiation accounted for 18% of the total, while between-population differentiation accounted for 88.9% in different populations of Asian sea bass. It is important to note that the difference between our study and Yue et al.'s study is significant, as their study included populations from three geographical regions: Singapore, Australia, and Thailand, whereas all three cohorts in our study originated from the same geographical region in Southeast Asia (Thailand). This is likely the reason for the low value of the genetic variance and the amount of between-population diversity in our study.

Moreover, research by DeWoody and Avise (2000) has determined that marine fishes show higher genetic diversity and lower genetic differentiation than freshwater fishes. This is attributed to the large population size and high gene flow in marine environments and the small size of the effective population and limited gene flow in freshwater populations. As emphasized by Hartl and Clark (1997), although the amount of F_{ST} obtained is low, it can still be meaningful.

Genetic distance measures the variations in the genetic makeup between two populations or closely related species. It is calculated by analyzing the frequency of genetic variants at multiple loci within each population. This measurement is commonly used to construct phylogenetic

trees for populations and estimate how much they have diverged from each other. Nei's and F_{ST} genetic distance matrices are two examples of commonly used measures. Nei's distance, also known as the standard genetic distance, is widely cited. It considers the effects of mutation and genetic drift, and its estimated value is proportional to evolutionary time (Nei, 2013). In the current study, Nei's standard genetic distance and pairwise F_{ST} distance between the three cohorts showed the lowest and highest genetic distance between pair cohorts 96-98 and 96-99, respectively. It is worth noting that both cohorts 96 and 98 were obtained from the same breeding farm in two different years, so this result is not unexpected.

In this study, we employed one of the most cited tools for defining population structure, the STRUCTURE software with the ΔK method (Stankiewicz *et al.*, 2022). This Bayesian clustering program does not make assumptions about the grouping of individuals at the start of the study. It helps reduce errors in defining population boundaries (Latch *et al.*, 2006) to examine the genetic structure of seabass populations. This analysis with the admixture model revealed two distinct genetic structures, denoted as K=2. The results indicated that all populations were mixtures of two clusters: one consisting of 96 and 98 individuals and the other exclusively comprised of 99 individuals. These findings accord with the truth that the different origins of the initial brooders may have led to the divergence of the two clusters in the population analysis of the *L. calcarifer*. However, Stankiewicz *et al.* (2022) suggested a combination of visual inspection of STRUCTURE plots and calculation of the alternative estimators at various thresholds in addition to ΔK to conclude population structure better.

These results are consistent with the Principal Coordinate Analysis (PCoA). The distribution pattern of the samples shows moderate genetic mixing and similarity among them. However, cohort 96 was genetically close to 98, with both being closer to each other than 99. Cohort 99 is relatively distant from the other cohorts.

As highlighted by Duan *et al.* (2023), the significance of genetic monitoring cannot be overstated. It provides crucial information that is instrumental in developing effective management strategies for breeding programs. This underscores the importance of our collective efforts in this field and the potential impact of our work on the future of seabass populations.

Earlier research has indicated that both wild stocks and most cultured populations of Asian sea bass in Thailand exhibit reasonable genetic diversity, providing valuable genetic resources for the future genetic improvement of this species (Yue *et al.*, 2009; Senanan *et al.*, 2015). The group spawning method, the highly productive population, and the lack of an optimal breeding program reducing diversity are standard breeding methods for Asian sea bass (Senanan *et al.*, 2015). These protocols allow the creation of Asian sea bass founder stocks with relatively high diversity.

Conclusions

The results of this study are crucial for choosing new broodstocks to support the sustainable development and growth of

aquaculture. Population structure analysis and principal coordinate analysis (PCoA) revealed that the three cohorts of *L. calcarifer* were divided into two subpopulations. Although the genetic diversity in population 99 is higher than in 96-98, the overall genetic diversity in the current study is satisfactory and comparable to previous studies. In our study, the observed proper level of diversity may be associated with the management strategies employed for the broodstock two generations ago in Thailand, especially concerning the initial brooders purchased by the private sector in Iran. It could also stem our brooders, the offspring of these Thai brooders. The significant genetic heterogeneity discovered among different stocks enables the creation of a new breeding population by crossing these stocks, resulting to increase in the genetic diversity of the base population. Consequently, these MFRS stocks could serve as a base population, a prerequisite for further selective inbreeding programs.

Acknowledgments

We thank the Iranian Fisheries Science Research Institute (IFSRI) for funding (No. 2-74-12-006-000041) for this study. The authors are also grateful for the assistance made by the staff of Bandar-e-Imam Khomeini Marine Fish Research Station (MFRS).

Conflicts of interest

The authors report no conflicts of interest.

References

Abdul-Muneer, P.M., 2014. Application of microsatellite markers in conservation genetics and fisheries management: recent advances in population structure analysis and conservation strategies. *Genetics Research International,* 2014, 691759. DOI:10.1155/2014/691759

Abramovs, N., Brass, A. and Tassabehji, M., 2020. Hardy-Weinberg Equilibrium in the Large Scale Genomic Sequencing Era. *Frontiers in Genetics,* 11, 210. DOI:10.3389/fgene.2020.00210

Beardmore, J.A., Mair, G.C. and Lewis, R.I., 1997. Biodiversity in aquatic systems in relation to aquaculture. *Aquaculture Research,* 28(**10**), 829-839. DOI:10.1046/j.1365-2109.1997.00947.x

Boca, S.M., Huang, L. and Rosenberg, N.A., 2020. On the heterozygosity of an admixed population. *Journal of Mathematical Biology,* 81(**6**), 1217-1250. DOI:10.1007/s00285-020-01531-9

Chen, B., Cole, J.W. and Grond-Ginsbach, C., 2017. Departure from Hardy Weinberg equilibrium and genotyping errors. *Frontiers in Genetics,* 8, 300354. DOI:10.3389/fgene.2017.00167

Choi, N., Seo, D., Manjula, P. and Lee, J.H., 2018. Genetic diversity studies using molecular genetic markers. *Journal of Animal Breeding and Genomics,* 2(**1**), 21-27. DOI:10.12972/jabng.20180017

Conrad, J.L., Gilbert-Horvath, E.A. and Garza, J.C., 2013. Genetic and phenotypic effects on reproductive outcomes for captively-reared coho salmon, *Oncorhynchus kisutch*.

Aquaculture, 404-405, 95-104. DOI:10.1016/j.aquaculture.2013.04.013

Cuéllar-Pinzón, J., Presa, P., Hawkins, S.J. and Pita, A., 2016. Genetic markers in marine fisheries: Types, tasks and trends. *Fisheries Research,* 173, 194-205. DOI:10.1016/j.fishres.2015.10.019

DeWoody, J. and Avise, J., 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology,* 56(**3**), 461-473. DOI:10.1111/j.1095-8649.2000.tb00748.x

Dewoody, Y.D. and Dewoody, J.A., 2005. On the estimation of genome-wide heterozygosity using molecular markers. *Heredity,* 96(**2**), 85-8. DOI:10.1093/jhered/esi017

Diz, A.P. and Presa, P., 2009. The genetic diversity pattern of *Mytilus galloprovincialis* in Galician Rías (NW Iberian estuaries). *Aquaculture,* 287(**3**), 278-285. DOI:10.1016/j.aquaculture.2008.10.029

Du, J., Hou, C., Chen, X., Xiao, J., Gul, Y. and Wang, H., 2022. Morphometric analysis and fluorescent microsatellite markers to evaluate the genetic diversity of five populations of *Penaeus japonicus* in China. *Aquaculture and Fisheries,* 7(**3**), 321-327. DOI:10.1016/j.aaf.2020.10.005

Duan, B., Kang, T., Zhang, C., Wan, H., Mu, S., Guan, Y., Liu, W., Li, Z., Tian, Y., Ren, Y. and Kang, X., 2023. Identification of candidate genes associated with body weight in swimming crab (*Portunus trituberculatus*) according to conjunctive analyses of BSA-seq and RNA-seq. *Aquaculture Reports,* 32, 101732. DOI:10.1016/j.aqrep.2023.101732

Duncan, N.J., Sonesson, A.K. and Chavanne, H. 2013. 2 - Principles of finfish broodstock management in aquaculture: control of reproduction and genetic improvement. In*:* Allan, G. and Burnell, G. (eds.). Advances in Aquaculture Hatchery Technology. Woodhead Publishing. Sawston, Cambridge, UK. pp 23-75. DOI:10.1533/9780857097460.1.23

Earl, D.A. and vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources,* 4(**2**), 359-361. DOI:10.1007/s12686-011-9548-7

El Mousadik, A. and Petit, R.J., 1996. High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. *Theoretical and Applied Genetics,* 92(**7**), 832-839. DOI:10.1007/BF00221895

Fisch, K.M., Kozfkay, C.C., Ivy, J.A., Ryder, O.A. and Waples, R.S., 2015. Fish hatchery genetic management techniques: integrating theory with implementation. *North American Journal of Aquaculture,* 77(**3**), 343-357. DOI:10.1080/15222055.2014.999846

Garlock, T., Asche, F., Anderson, J., Ceballos-Concha, A., Love, D.C., Osmundsen, T.C. and Pincinato, R.B.M., 2022. Aquaculture: The missing contributor in the food security agenda. *Global Food Security,* 32,

100620. DOI:10.1016/j.gfs.2022.100620

Goudet, J., 2002. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9. 3.2). Avaiable at: https://www2.unil.ch/popgen/softwares/fstat.htm

Hartl, D.L. and Clark, A.G. 1997. Principles of population genetics. Oxford University Press. Sunderland, MA, USA. 672P.

Hillen, J.E.J., Coscia, I., Vandeputte, M., Herten, K., Hellemans, B., Maroso, F., Vergnet, A., Allal, F., Maes, G.E. and Volckaert, F.A.M., 2017. Estimates of genetic variability and inbreeding in experimentally selected populations of European sea bass. *Aquaculture,* 479, 742-749. DOI:10.1016/j.aquaculture.2017.07.012

Jenkins, S.F., Ishengoma, E. and Rhode, C., 2020. A temporal assessment of family composition and genetic diversity in a commercial cohort of Dusky Kob, *Argyrosomus japonicus*, across the production cycle. *Aquaculture,* 516, 734640. DOI:10.1016/j.aquaculture.2019.734640

Joerakate, W., Yenmak, S., Senanan, W., Tunkijjanukij, S., Koonawootrittriron, S. and Poompuang, S., 2018. Growth performance and genetic diversity in four strains of Asian sea bass, *Lates calcarifer* (Bloch, 1790) cultivated in Thailand. *Agriculture and Natural Resources,* 52(**1**), 93-98. DOI:10.1016/j.anres.2018.05.015

Kanaka, K.K., Sukhija, N., Goli, R.C., Singh, S., Ganguly, I., Dixit, S.P., Dash, A. and Malik, A.A., 2023. On the concepts and measures of diversity in the genomics era. *Current Plant Biology,* 33, 100278. DOI:10.1016/j.cpb.2023.100278

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics,* 28(**12**), 1647-9. DOI:10.1093/bioinformatics/bts199

Kivisild, T. 2013. Founder Effect. *In:* Maloy, S. and Hughes, K. (eds.). Brenner's Encyclopedia of Genetics (Second Edition). Academic Press. San Diego, United State. pp 100-101. DOI:10.1016/B978-0-12-374984-0.00552-0

Kobayashi, T. and Kijima, A., 2010. Effects of Inbreeding Depression in Pacific *Abalone Haliotis Discus Hannai*. *Journal of Shellfish Research,* 29(**3**), 643-649. DOI:10.2983/035.029.0313

Konopiński, M.K., 2020. Shannon diversity index: a call to replace the original Shannon's formula with unbiased estimator in the population genetics studies. *PeerJ,* 8, e9391. DOI:10.7717/peerj.9391

Laird, N.M. and Lange, C. 2011. The fundamentals of modern statistical genetics. Springer, New York. 226 P. DOI:10.1007/978-1-4419-7338-2

Latch, E.K., Dharmarajan, G., Glaubitz, J.C. and Rhodes, O.E., 2006. Relative performance of Bayesian clustering software for inferringpopulation substructure and individual assignment at low levels of population differentiation. *Conservation Genetics,* 7(**2**), 295-302. DOI:10.1007/s10592-005-9098-1

Lind, C.E., Evans, B.S., Knauer, J., Taylor, J.J.U. and Jerry, D.R., 2009. Decreased genetic diversity and a reduced effective population size in cultured silver-lipped pearl oysters (*Pinctada maxima*). *Aquaculture,* 286(**1**), 12-19. DOI:10.1016/j.aquaculture.2008.09.009

Miller, J.M., Malenfant, R.M., David, P., Davis, C.S., Poissant, J., Hogg, J.T., Festa-Bianchet, M. and Coltman, D.W., 2014. Estimating genome-wide heterozygosity: effects of demographic history and marker type. *Heredity,* 112(**3**), 240-247. DOI:10.1038/hdy.2013.99

Mozanzadeh, M.T., Safari, O., Oosooli, R., Mehrjooyan, S., Najafabadi, M.Z., Hoseini, S.J., Saghavi, H. and Monem, J., 2021. The effect of salinity on growth performance, digestive and antioxidant enzymes, humoral immunity and stress indices in two euryhaline fish species: Yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*). *Aquaculture,* 534, 736329. DOI:10.1016/j.aquaculture.2020.736329

Nagylaki, T., 1998. Fixation indices in subdivided populations. *Genetics,* 148(**3**), 1325-32. DOI:10.1093/genetics/148.3.1325

Nei, M. 2013. Genetic Distance. *In:* Maloy, S. and Hughes, K. (eds.). *Brenner's Encyclopedia of Genetics (Second Edition).* San Diego: Academic Press. DOI:10.1016/B978-0-12-374984-0.00615-X

Peakall, R. and Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28(**19**), 2537-9. DOI:10.1093/bioinformatics/bts460

Pereira, E., Mateus, C.S., Alves, M.J., Almeida, R., Pereira, J., Quintella, B.R. and Almeida, P.R., 2023. Connectivity patterns and gene flow among *Chelon ramada* populations. *Estuarine, Coastal and Shelf Science,* 281, 108209. DOI:10.1016/j.ecss.2022.108209

Prasertlux, S., Chaimongkol, A., Chaimongkol, J., Janpoom, S., Tang, S., Rongmung, P., Ittarat, W., Ninwichian, P., Songsangjinda, P., Sakamoto, T. and Khamnamtong, B., Sae-Lim, P., Klinbunga, S., 2025. Genetic diversity, heritability, and estimated breeding values for growth of domesticated Asian seabass *Lates calcarifer* from Thailand. *Aquaculture and Fisheries*, 10(**4**), 576-585. DOI: 10.1016/j.aaf.2024.04.006

Pritchard, J.K., Stephens, M. and Donnelly, P., 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics,* 155(**2**), 945-959. DOI:10.1093/genetics/155.2.945

Pritchard, J.K., Wen, W. and Falush, D., 2010. Documentation for structure software: Version 2.3. University of Chicago, Chicago, USA. 1- 37.

Pudovkin, A.I., Zaykin, D.V. and Hedgecock, D., 1996. On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics,* 144(1), 383-7. DOI:10.1093/genetics/144.1.383

Russello, M.A. and Amato, G., 2004. Ex situ population management in the absence of pedigree information. *Molecular Ecology,* 13(**9**), 2829-2840. DOI:10.1111/j.1365-294X.2004.02266.x

Sánchez-Velásquez, J.J., Pinedo-Bernal, P.N., Reyes-Flores, L.E., Yzásiga-Barrera, C. and Zelada-Mázmela, E., 2022. Genetic diversity and relatedness inferred from microsatellite loci as a tool for broodstock management of fine flounder *Paralichthys adspersus*. *Aquaculture and Fisheries,* 7, 664-674. DOI:10.1016/j.aaf.2021.06.008

Senanan, W., Pechsiri, J., Sonkaew, S., Na-Nakorn, U., Sean-In, N. and Yashiro, R., 2015. Genetic relatedness and differentiation of hatchery populations of Asian seabass (*Lates calcarifer*) (Bloch, 1790) broodstock in Thailand inferred from microsatellite genetic markers. *Aquaculture Research,* 46(**12**), 2897-2912. DOI:10.1111/are.12442

Sherwin, W.B., Chao, A., Jost, L. and Smouse, P.E., 2017. Information Theory Broadens the Spectrum of Molecular Ecology and Evolution. *Trends in Ecology & Evolution,* 32(**12**), 948-963. DOI:10.1016/j.tree.2017.09.012

Singh, R.K., Kumar, R., Bhordia, A., Mandal, S., Kantharajan, G., Chandran, R., Ajithkumar, T.T., Mohindra, V. and Lal, K.K., 2023. Development of genome-wide novel microsatellite markers and genetic variability analysis of Asian seabass, *Lates calcarifer* (Bloch, 1790). *Aquaculture International,* 31(**5**), 3055-3070. DOI:10.1007/s10499-023-01116-5

Stankiewicz, K.H., Vasquez Kuntz, K.L., Group, C.M. and Baums, I.B., 2022. The impact of estimator choice: Disagreement in clustering solutions across K estimators for Bayesian analysis of population genetic structure across a wide range of empirical data sets. *Molecular Ecology Resources,* 22(**3**), 1135-1148. DOI:10.1111/1755-0998.13522

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology Evolution,* 28(**10**), 2731-9. DOI:10.1093/molbev/msr121

Torfi Mozanzade, M., Zabaieh Najafabadi, M., Houshmand, H., Ahangarzadeh, M., Osoli, A., Saghavi, H., Mehrjooian, S., Seyed Morezaei, S.R., Hosseini Malayeri, S.J., Hafezieh, M. and Hosseinzadeh Sahafi, H., 2023. Broodstocking and propagation techniques of Asian sea bass (*Lates calcarifer*) larvae under captivity conditions. *Iranian Scientific Fisheries Journal,* 32(**5**), 1-14. DOI:10.22092/ISFJ.2024.130595. (in Persian)

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P., 2004.

Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* 4(**3**), 535-538. DOI:10.1111/j.1471-8286.2004.00684.x

Wang, C.M., Lo, L.C., Zhu, Z.Y. and Yue, G.H., 2006. A genome scan for quantitative trait loci affecting growth-related traits in an F1 family of Asian seabass (*Lates calcarifer*). *BMC Genomics,* 7(**1**), 274. DOI:10.1186/1471-2164-7-274

Wenne, R., 2023. Microsatellites as molecular markers with applications in exploitation and conservation of aquatic animal populations. *Genes,* 14(**4**), 808. DOI:10.3390/genes14040808

Wong, J., Sun, F., Wang, L., Yang, Z.T., Wen, Y.F., Pang, H.Y., Lee, M., Yeo, S.T., Liang, B., Chen, K., Jiang, J.H. and Yue, G.H., 2023. Changes in genetic diversity of Asian seabass in a 20-year breeding program. *Aquaculture,* 575, 739738. DOI:10.1016/j.aquaculture.2023.739738

Wright, S., 1951. The genetical structure of populations. *Annals of Eugenics,* 15, 323–354. DOI:10.1111/j.1469-1809.1949.tb02451.x

Wright, S., 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution,* 19, 395–420. DOI:10.1111/j.1558-5646.1965.tb01731.x

Yue, G.H., Zhu, Z.Y., Lo, L.C., Wang, C.M., Lin, G., Feng, F., Pang, H.Y., Li, J., Gong, P. and Liu, H.M., 2009. Genetic variation and population structure of Asian seabass (*Lates calcarifer*) in the Asia-Pacific region. *Aquaculture,* 293(**1-2**), 22-28. DOI:10.1016/j.aquaculture.2009.03.053

Zhang, L., Mou, C., Zhou, J., Ye, H., Wei, Z., Ke, H., Huang, Z., Duan, Y., Zhao, Z., Zhao, H., Li, H., Du, J. and Li, Q., 2022. Genetic Diversity of Chinese Longsnout Catfish (*Leiocassis longirostris*) in Four Farmed Populations Based on 20 New Microsatellite DNA Markers. *Diversity,* 14(**8**), 654. DOI:10.3390/d14080654

Zhu, Z.Y., Grace, L., Lo, L.C., Xu, Y.X., Felicia, F., Renee, C. and Yue, G.H., 2006. Genetic analyses of Asian seabass stocks using novel polymorphic microsatellites. *Aquaculture,* 256(**1**), 167-173. DOI:10.1016/j.aquaculture.2006.02.033

Zhu, Z.Y., Wang, C.M., Lo, L.C., Lin, G., Feng, F., Tan, J., Chou, R., Lim, H.S., Orban, L. and Yue, G.H., 2010. A standard panel of microsatellites for Asian seabass (*Lates calcarifer*). *Animal Genetics,* 41(**2**), 208-12. DOI:10.1111/j.1365-2052.2009.01973.x