

Article

Does the Artificial Enhancement and Release Activity Affect the Genetic Diversity of Marbled Rockfish *Sebastes marmoratus* in Zhoushan Waters?

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Abstract: Artificial enhancement and release activity is an important method in the restoration of fishery resources. In order to understand the possible genetic effect of hatchery-released populations on wild populations during the artificial enhancement and release activities of *Sebastes marmoratus* in Zhoushan waters, we utilized mitochondrial DNA control region sequences to examine the genetic diversity in four *S. marmoratus* populations, including one farmed population, one released population and two wild populations. A total of 68 haplotypes from 123 individuals were detected, including 3 shared haplotypes. Haplotype diversity ranged from 0.944 to 0.980, with a mean of 0.966. The nucleotide diversity ranged from 0.020 to 0.025, with a mean of 0.022. Analysis of Molecular Variance (AMOVA) indicated that the primary genetic variation occurs within populations and the index of genetic differentiation between populations (F_{ST}) among the four populations showed no differentiation. The results indicate that the current artificial enhancement and release has not impacted the *S. marmoratus* population in Zhoushan waters. Continued long-term monitoring is essential to protect the high-quality germplasm resources of *S. marmoratus*.

Keywords: *Sebastes marmoratus*; artificial enhancement and release; genetic diversity; mitochondrial DNA control region

Key Contribution: This article discusses the influences of genetic diversity of *Sebastes marmoratus* caused by the artificial enhancement and release activity in Zhoushan water and provides data on future artificial enhancement and release activity.



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1. Introduction

Artificial enhancement and release involve the use of artificial methods to introduce aquatic animals, including parents and fertilized eggs, into natural waters. This practice aims to bolster population numbers, restore the structure of aquatic ecosystems, and ultimately enhance fishery resources, improve water quality, and maintain ecological equilibrium [1]. Widely adopted globally, it serves as an effective strategy for replenishing fishery resources and enhancing productivity [2]. The inception of artificial enhancement and release dates back to the early 20th century when countries such as the United States, the United Kingdom, and Norway pioneered these activities, with China following suit in the 1970s and remarkable results of marine fishery resource enhancement of China has been achieved in the last 50 years [3]. The stocking and release areas in China are mainly

concentrated in the nearshore harbors, bays, and tidal flats of the Bohai Sea, Yellow Sea, East China Sea, and South China Sea. In the Yellow Sea and Bohai Sea, the execution of *Penaeus chinensis* artificial enhancement and release initiatives has successfully mitigated the fishing pressure on the natural resources [2]. In the East China Sea, the implementation of artificial enhancement and release activities for *Sepiella maindron* has played a role in the restoration of *S. maindron* resources in the northern waters of Zhejiang province [4]. Despite its positive impact on supplementing resources and boosting production capacity, the burgeoning practice of artificial enhancement and release has prompted increased scrutiny regarding its effects on wild populations in restocking areas. Consequently, evaluating the efficacy of restocking has become a paramount concern. This evaluation, centered around the artificial enhancement and release activities, delves into the economic, ecological, and social benefits within a defined timeframe post-release, constituting the significant aspect of artificial enhancement and release [5]. Marking techniques emerge as the primary method for assessing the effectiveness of artificial enhancement and release. In the context of fish as the subject of application, marking technology falls into the following four categories: physical markers, chemical markers, biological markers, and molecular markers [6]. Molecular markers, as exemplified by the exploration of Laikre et al. [7], prove invaluable in analyzing genetic considerations. They address questions concerning the potential for gene exchange during large-scale reproduction of fishery resources, potential loss of original heritage in wild populations, transmission of mutation characteristics and habitat adaptability, and alterations in community composition and structure.

The Zhoushan Fishing Ground is situated at the estuaries of the Yangtze River, Qiantang River, and Yongjiang River, precisely at the pivotal intersection of the Taiwan Warm Current and the Yellow Sea Cold Water Mass. Due to its unique geographical location and abundant nutrient content, the Zhoushan Fishing Ground has become an ideal habitat for the growth and reproduction of various fish species [8,9]. In recent years, the intensive development of the fishing industry and increased fishing activities have led to a significant depletion of fisheries resources in the Zhoushan Fishing Ground, resulting in a sharp decline in the population numbers of economically valuable fish species. With the continuous increase in fishing pressure, some crucial fishery resources face the threats of endangerment and overfishing, including economically vital species such as *Sebastes marmoratus*, Cuvier, 1829 [10]. The marbled rockfish, *S. marmoratus*, belongs to the class Osteichthyes, order Scorpaeniformes, and family Scorpaenidae. It is a typical warm-water demersal fish [11], mainly distributed in the warm water zone of the central and southern coasts of the Northwest Pacific Ocean [12], especially in the Bohai sea, the Yellow sea, the East China sea and the South China sea in China. The *S. marmoratus*, renowned for its delicious and nutritious flesh, holds significant economic value, showcasing typical characteristics of the Scorpaenidae family [13]. The *S. marmoratus* typically reaches a size of 15 to 20 cm, although some individuals are known to grow larger, reaching 30 to 40 cm [14]. It tends to have a sedentary lifestyle, with a relatively small range of activity, usually within one kilometer [15]. However, with the changing seasons, it exhibits slight migratory behavior over short distances, following certain seasonal patterns. During the cold seasons of late autumn and early winter, it tends to migrate towards shallow water areas, while in spring and summer seasons, it returns to deep-water areas [16]. The *S. marmoratus* stands out due to its unique reproductive strategy of ovoviviparity. In this distinctive method, the eggs are fertilized within the mother's body, retained, and then develop in the reproductive system of the mother [17]. This reproductive adaptation provides an advantage for its adaptability in specific ecological environments. *Sebastes marmoratus* is also a typical reef-based fish in the Zhoushan sea area and plays a crucial role as one of the primary breeding species in the construction of marine ranches [18]. In recent years, Zhejiang province has actively promoted the release of *S. marmoratus* in the waters of Zhoushan. In order to carry out artificial enhancement and release more effectively in the Zhoushan sea area without causing adverse effects on the ecosystem, Hao et al. [19] conducted an estimation of the ecological carrying capacity for *S. marmoratus* using the ecopath model.

The determined ecological carrying capacity for *S. marmoratus* in the Zhoushan sea area was found to be 0.00795 t/km². Consequently, it is recommended that the stocking quantity for *S. marmoratus* should be 11,582,000 individuals. According to the research of Chen et al. [20], approximately 706,000 *S. marmoratus* were released in the past five years.

In order to assess whether the artificial enhancement and release activities affect the genetic diversity of *S. marmoratus* in Zhoushan waters, we used the mitochondrial DNA control region as a marker to analysis the genetic diversity and structure of the two natural wild populations (Shengsi and Dongtou) to assess whether the artificial enhancement and release activity (Dongji) is affected by the introduction of farmed populations (Xixuan). The objective of this study could potentially hide or maintain the natural signal of local genetic variability of *S. marmoratus*. Understanding the genetic diversity within the region has significant implications for the advancement and management of aquatic organisms, aiding in the mitigation of resource decline and promoting the sustainable development of fishery resources.

2. Materials and Methods

2.1. Experimental Animals and Sampling

A total of 123 samples were collected from four locations (Shengsi (30), Dongji (32), Xixuan (31) and Dongtou (30)). Among these locations, samples from Shengsi and Dongtou were obtained through sea fishing, as no proliferation or release activities of *S. marmoratus* were detected in these areas. Therefore, these samples are referred to as wild populations, abbreviated as SS and DT, respectively. Samples from Dongji were also obtained through sea fishing, but artificial enhancement and release activities of *S. marmoratus* were recorded in this area, making it a mixed population, abbreviated as DJ. The samples from Xixuan originated from a local aquaculture farm and serve as the primary source of most of the released *S. marmoratus* fingerlings, thus referred to as the farming population, abbreviated as YZ (Table 1, Figure 1). All experimental samples were approved by the State Oceanic Administration of China and Ethics Committee of Zhejiang Ocean University, and the procedures were conducted in accordance with national laws and regulations. The muscle tissues from samples were collected and saved in absolute alcohol with −20 °C.

Table 1. The sampling details for four populations of *Sebastiscus marmoratus*.

Sampling Site	Abbreviation	Coordinate	Sampling Date
Shengsi	SS	30°42' N 122°29' E	August 2023
Dongji	DJ	30°20' N 122°36' E	May 2023
Xixuan	YZ	29°53' N 122°18' E	November 2022
Dongtou	DT	27°50' N 121°14' E	August 2023

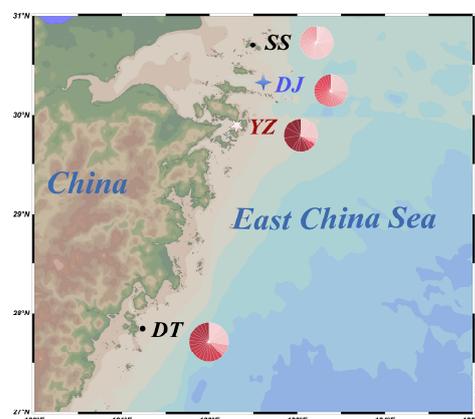


Figure 1. The picture shows the sampling locations along the coast of China. SS stands for Shengsi, DJ stands for Dongji, YZ stands for Xixuan and DT stands for Dongtou. The circle and blue characters

represent the wild population, the four-pointed star and red characters indicate the mixed population, and the five-pointed star represents the farmed population. The pie chart represents the haplotype of this group, and the different colors represent the different haplotypes.

2.2. DNA Extraction and Polymerase Chain Reaction

The genomic DNA of each sample was extracted using the salt-extraction procedure [21]. Subsequently, the extracted DNA was tested on 1.5% agarose gel, and the samples that passed the examination were stored in a $-20\text{ }^{\circ}\text{C}$ freezer for future use. The primers used were as follows: DL-S: 5'-CCCACCACTAACTCCCAAAGC-3' (forward) and DL-R: 5'-CTGGAAAGAACGCCCGGCATG-3' (reverse) [22]. The polymerase chain reaction (PCR) was carried out in a 25 μL volume, consisting of 12.5 μL of Taq MasterMix (Beijing Com Win Biotech Co., Ltd., Beijing, China), 1 μL of the forward primer, 1 μL of the reverse primer, 1 μL of genomic DNA, and 10 μL of ddH₂O. The PCR protocol included initial denaturation for 3 min at 94 $^{\circ}\text{C}$, followed by 40 cycles of 30 s at 94 $^{\circ}\text{C}$ for denaturation, 40 s at 52 $^{\circ}\text{C}$ for annealing, 45 s at 72 $^{\circ}\text{C}$ for extension, and a final extension for 10 min at 72 $^{\circ}\text{C}$. The amplification products were detected and observed using 1.5% agarose gel, and subsequently sent to TSINGKE Biotech Co., Ltd. (Hangzhou, China) for bidirectional sequencing.

2.3. Data Analysis

After obtaining COI genetic sequences through PCR amplification, we first utilized the BLAST function on the online NCBI platform (<http://www.ncbi.nlm.nih.gov/>) for homology comparisons to ensure the accuracy of the sequences. Subsequently, we employed BioEdit v 7.2.6.1 software [23] for preliminary analysis. Through meticulous screening, potential errors arising from sequencing inaccuracies were meticulously filtered out. We selectively chose fragments displaying standard peak profiles and devoid of interference, converting them into FASTA files. Following this, MEGA-X software [24] was employed for precise sequence comparison and end-trimming, laying a robust foundation for subsequent data processing. This step is crucial to guarantee the high quality and reliability of the obtained sequence data, providing a dependable basis for further genetic analysis and subsequent research. Subsequently, the DnaSP 6 software [25] was employed to differentiate haplotypes and calculate various classical genetic parameters, such as haplotype diversity (h) and nucleotide diversity (π). For a more in-depth exploration of genetic variation, we utilized Arlequin v 3.5.2.2 software [26] to conduct Analysis of Molecular Variance (AMOVA), calculate the genetic differentiation index (F_{ST}), and perform neutrality tests among the four populations. Subsequently, a haplotype network diagram was constructed using Network 10.2.0.0 software [27], providing a visual representation of the relationships among the haplotypes. Using the MEGA-X software, we crafted phylogenetic trees that offer a holistic view of the evolutionary relationships among the analyzed sequences. To further probe the intricate patterns of gene flow, we used both R Studio and the online tool divMigrate (<https://popgen.shinyapps.io/divMigrate-online/>, accessed on 27 November 2023) to investigate the current dynamics of genetic exchange among diverse populations.

3. Results

In this study, sequences of 123 samples of *S. marmoratus* were aligned and processed, resulting in a 458bp control region sequence used for genetic analysis. The average content of A, T, C, G bases in these sequences was 35.53%, 27.71%, 19.93% and 16.83%, respectively. The content of A + T (63.24%) was significantly higher than that of G + C (36.76%), showing obvious base composition bias. Among the 123 individuals, a total of 68 different haplotypes were identified, with the SS population, DJ population, DT population, and YZ population demonstrating 22, 26, 16, and 23 haplotypes, respectively. Haplotype 6, Haplotype 7, Haplotype 18 and Haplotype 22 are shared haplotypes among the populations. Haplotype 4 is shared by the SS, DJ and DT population, excluding the YZ population. Haplotype 12 is shared by the SS, DJ and YZ population, excluding the DT population (Table 2). In

this study, the haplotype diversity (Hd) across the four populations ranged from 0.944 to 0.980, with an average haplotype diversity of 0.966, indicating a high level ($Hd > 0.5$). The mutation sites ranged from 44 (DJ and SS) to 46 (DT), and the average nucleotide diversity (π) for all populations was 0.022, ranging from 0.020 to 0.025, also at a high level ($\pi > 0.005$). Among the four populations, the DJ population exhibited the highest Hd , while the YZ population showed the lowest. In terms of Pi , the YZ population had the highest, while the SS population had the lowest (Table 3).

Table 2. Distribution of mitochondrial DNA control region haplotypes in four *S. marmoratus* populations.

Haplotype	SS	DJ	DT	YZ	Accession Numbers	Haplotype	SS	DJ	DT	YZ	Accession Numbers
Hap_1	1				PP700307	Hap_35		1			PP700341
Hap_2	1				PP700308	Hap_36		1		1	PP700342
Hap_3	1				PP700309	Hap_37		1			PP700343
Hap_4	4	1	3		PP700310	Hap_38		1			PP700344
Hap_5	1				PP700311	Hap_39		1			PP700345
Hap_6	5	3	2	3	PP700312	Hap_40		1			PP700346
Hap_7	4	4	3	5	PP700313	Hap_41		1			PP700347
Hap_8	1				PP700314	Hap_42			1	1	PP700348
Hap_9	1				PP700315	Hap_43			1		PP700349
Hap_10	1				PP700316	Hap_44			1		PP700350
Hap_11	1				PP700317	Hap_45			1		PP700351
Hap_12	1	2		1	PP700318	Hap_46			1		PP700352
Hap_13	1				PP700319	Hap_47			1		PP700353
Hap_14	1				PP700320	Hap_48			1		PP700354
Hap_15	1	1			PP700321	Hap_49			1		PP700355
Hap_16	1				PP700322	Hap_50			1		PP700356
Hap_17	1				PP700323	Hap_51			1		PP700357
Hap_18	1	1	2	1	PP700324	Hap_52			1		PP700358
Hap_19	1				PP700325	Hap_53			1		PP700359
Hap_20	1				PP700326	Hap_54			1		PP700360
Hap_21	1				PP700327	Hap_55			1		PP700361
Hap_22	1	1	1	1	PP700328	Hap_56			1		PP700362
Hap_23		1			PP700329	Hap_57			1		PP700363
Hap_24		1			PP700330	Hap_58			1		PP700364
Hap_25		1			PP700331	Hap_59			1		PP700365
Hap_26		1			PP700332	Hap_60				1	PP700366
Hap_27		1			PP700333	Hap_61				2	PP700367
Hap_28		1			PP700334	Hap_62				1	PP700368
Hap_29		1			PP700335	Hap_63				1	PP700369
Hap_30		1			PP700336	Hap_64				4	PP700370
Hap_31		1			PP700337	Hap_65				3	PP700371
Hap_32		1			PP700338	Hap_66				2	PP700372
Hap_33		1			PP700339	Hap_67				2	PP700373
Hap_34		1			PP700340	Hap_68				1	PP700374

Table 3. Genetic diversity analysis of four *S. marmoratus* populations.

Population	Sample Size	S	h	Hd	K	π	PiJC
SS	30	44	22	0.961	9.211	0.020	0.020
DJ	32	44	26	0.980	9.698	0.021	0.022
YZ	31	45	16	0.944	11.669	0.025	0.026
DT	30	46	23	0.979	10.230	0.022	0.023
Total	123	73	68	0.966	10.240	0.022	

S, mutation sites; h, haplotypes numbers; Hd, haplotype diversity; K, average number of pairwise divergences; π , nucleotide diversity; PiJC, nucleotide diversity with JC.

The genetic distance within populations ranged from 0.021 to 0.026, while the distances between populations ranged from 0.021 to 0.025 (Table 4). Based on the *S. marmoratus* population origin, the *S. marmoratus* population was divided into several gene pools for AMOVA analysis (Table 5). The four populations were analyzed as one gene pool and the results showed that the intra-population variation accounted for 99.56% of the overall variation. The four populations were analyzed as two gene pools (SS and DT form one group, and DJ and YZ form another group) and the results showed that the intra-population variation accounted for 99.34% of the overall variation. The four populations were analyzed as three gene pools (SS and DT form one group, DJ is one group and YZ is the other) and the results showed that the intra-population variation accounted for 99.16% of the overall variation. Based on the above results, it can be concluded that genetic variation is primarily derived from interpopulation. Notably, in this experiment, the F_{ST} values between populations, excluding the YZ population, were negative. However, all average F_{ST} values between populations were less than 0.05, indicating no significant differences among the four populations. Nm values also supported the same results (Table 6, Figure 2). In the exploration of gene flow dynamics across the four distinct *S. marmoratus* populations, we utilized three distinct thresholds (0, 0.35, and 0.6) to construct directional relative migration networks. A careful examination of these visual representations highlights varying intensities of gene exchange among the populations. Notably, the gene flow from SS to YZ exhibits an asymmetric pattern, demonstrating greater strength compared to the flows from YZ to DJ and YZ to DT. This observed migration pattern strongly suggests an elevated level of genetic exchange between the SS and YZ populations, emphasizing their pronounced genetic similarity.

Table 4. Genetic distance of four *S. marmoratus* populations.

Population	SS	DJ	DT	YZ
SS	0.021			
DJ	0.021	0.022		
DT	0.022	0.022	0.023	
YZ	0.024	0.024	0.025	0.026

Above the diagonal is the distance within the population, and below the diagonal is the distance between populations.

Table 5. AMOVA analysis of *S. marmoratus* populations.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
One gene pool (SS, DJ, DT, YZ)				
Among populations	3	17.365	0.022 Va	0.44
Within populations	119	607.245	5.103 Vb	99.56
Two gene pools (SS, DT) (DJ, YZ)				
Among groups	1	7.214	0.035 Va	0.68
Among populations	2	10.151	−0.001 Vb	−0.02
Within groups	119	607.245	5.103 Vc	99.34
Three gene pools (SS, DT) (DJ) (YZ)				
Among groups	2	14.948	0.133 Va	2.58
Among populations	1	2.417	−0.090 Vb	−1.74
Within groups	119	607.245	5.103 Vc	99.16

Table 6. F_{ST} and N_m analysis of four *S. marmoratus* populations.

Population	SS	DJ	DT	YZ
SS		inf	inf	7.001
DJ	−0.006		inf	17.493
DT	−0.017	−0.018		21.527
YZ	0.034 *	0.014	0.012	

Taking the diagonal as an example, F_{ST} is below the diagonal and N_m is above the diagonal. “*” indicates the P is less than 0.05. and the “inf” stands for infinity.

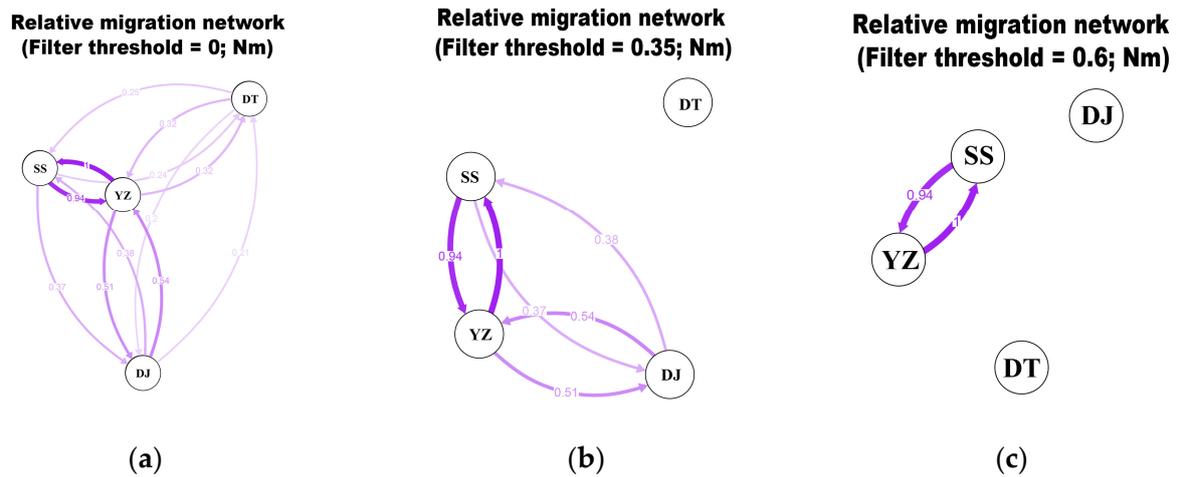


Figure 2. Directional relative migration networks of *S. marmoratus* populations constructed with online software divMigrate using N_m values above 0 (a), 0.35 (b) and 0.6 (c).

In addition, on the foundation of a star-like topological structure, haplotypes are predominantly distributed among three branches, namely Haplotypes 6, 7 and 18. Haplotype 4 is exclusive to the SS, DJ, and DT populations, while it is absent in the YZ population. No significant differences in haplotypes within these three branches were observed across the four populations (Figure 3). The phylogenetic tree based on the neighbor-joining method revealed a similar result with a star-like topological structure with no significant geographic agglomeration in the four populations. Based on population origin, the haplotypes from wild individuals tend to cluster together, as do those from individuals in the release area (Figure 4). The results of the neutral tests showed slight differences between the two methods. For Tajima’s D test, the calculated D values were −0.632, −0.412, −0.443, and 0.133 for the SS, DJ, DT, and YZ populations, respectively, with all p -values greater than 0.05. In Fu’s FS test, the calculated F values were −6.966, −11.579, −7.465, and −0.008 for the SS, DJ, DT and YZ populations, respectively. Tajima’s D value of the YZ population was greater than 0, while the other result of the neutral test was less than 0 (Table 7). Overall, the SS, DJ and DT populations exhibited significant negative selection.

Table 7. Neutral selection analysis of four *S. marmoratus* populations.

	Statistics	SS	DJ	DT	YZ	Mean	s.d.
Tajima’s D test	Tajima’s D	−0.632	−0.412	−0.443	0.133	−0.338	0.329
	p -value	0.308	0.362	0.351	0.609	0.408	0.136
Fu’s FS test	FS	−6.966	−11.579	−7.465	−0.008	−6.505	4.799
	p -value	0.013	0.002	0.007	0.525	0.137	0.259

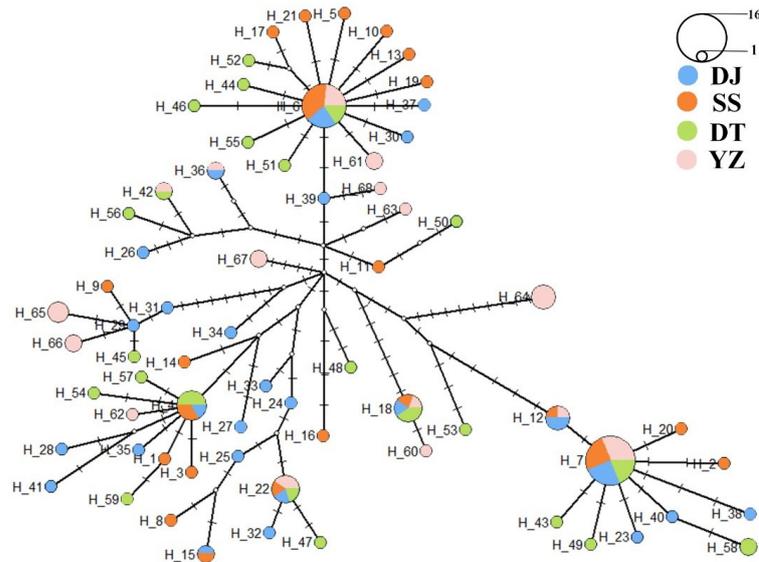


Figure 3. A star-like topology of four *S. marmoratus* populations. Different colors stand for different populations and the size of the circle indicates the number of this haplotype.

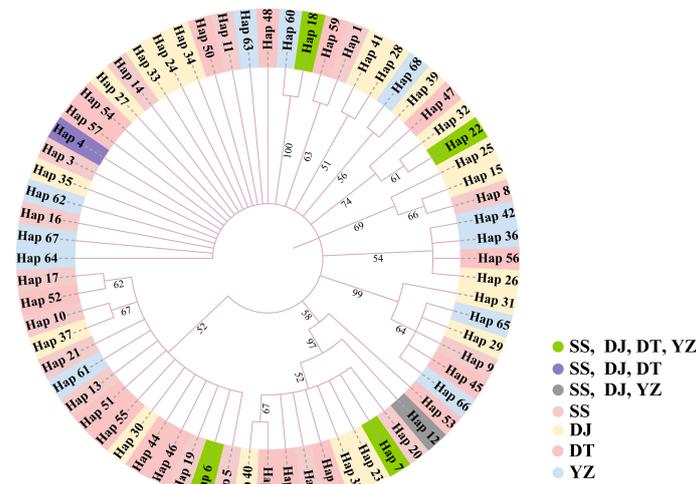


Figure 4. The neighbor-joining tree based on the haplotype of four *S. marmoratus* populations.

4. Discussion

Currently, numerous marine fish are being augmented and released through stocking programs aimed at enhancing their biomass. However, with the widespread implementation of these activities, concerns have arisen about the potential impact of this process on the genetic diversity of released species. Studies have indicated that releasing large quantities of hatchery-reared juveniles may have detrimental effects on the genetic diversity of wild populations [7]. Araki and Schmid [28] conducted an in-depth review of 266 peer-reviewed academic papers. The findings revealed that in the field of aquaculture, the practice of releasing hatchery-reared fish fry into natural environments often leads to adverse effects on local wild fish populations. However, it is noteworthy that in specific contexts, the impact of such stocking activities on the genetic diversity of local fish populations is not always evident. In fact, in some cases, these releases have even shown positive effects on the abundance of fish populations. The investigation conducted by Berejikian et al. [29] exemplifies this phenomenon, illustrating that the introduction of hatchery-reared steelhead salmon into river ecosystems led to a notable augmentation in their spawning numbers at specific locations. This deliberate release strategy was found to significantly enhance the reproductive success of the steelhead salmon in their natural habitat, consequently

exerting a favorable influence on the broader river ecosystem. Likewise, as indicated by Agnalt [30], European lobsters that underwent hatchery rearing displayed spawning levels comparable to their wild counterparts upon release. The methodology involving hatchery rearing followed by release was observed not only to sustain spawning levels but also to fortify the indigenous European lobster resources. This approach, thereby, makes a positive contribution to the equilibrium and sustainability of the ecosystem.

From the point of view of genetic diversity, haplotype diversity of *S. marmoratus* ranged from 0.944 to 0.980, and the average nucleotide diversity ranged from 0.020 to 0.025. Both of these parameters indicate that *S. marmoratus* has a high level of genetic diversity, indicating a long evolutionary history between its divergent lineages or having undergone secondary contact [31]. Similar results were found by Liu et al. [32] and they also used the mitochondrial DNA (mtDNA) control region as a molecular marker to explore the genetic diversity of *S. marmoratus* in the northwestern Pacific Ocean. Based on the data of Liu et al. [32], the haplotype diversity of *S. marmoratus* in Zhoushan was 0.9601 ± 0.0238 , and nucleotide diversity was 0.0219 ± 0.0116 , which is similar to our results. Furthermore, after conducting a BLAST comparison on the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 22 April 2024), our study identified 16 haplotypes (including shared haplotypes: Haplotypes 4, 6, 7, 12, 18 and 22) that are identical to those found in the study by Liu et al. The emergence of additional haplotypes may be attributed to recent enhancement and release activities. Based on the AMOVA analysis and haplotype phylogenetic tree, intra-population variability is similar to the inter-population variability. The populations we investigated, especially the mixed population where released individuals often originate from fish farms, may have originated from a relatively limited number of individuals. This could result in a founder effect, where the genetic diversity within the released population and among different populations becomes more similar due to their shared genetic origin. On a larger scale, as observed in our study, the genetic diversity of *S. marmoratus* exhibited a high haplotype diversity and low nucleotide diversity in the northwestern Pacific [32]. This pattern may be explained by the effects of glacial cycles. Due to the harsh glacial environment, *S. marmoratus* faced species extinction in many areas, but some individuals survived due to their distribution in the East China Sea basin. During the post-glacial period, as the environment became more favorable, *S. marmoratus* populations began to expand outward, resulting in the genetic diversity observed today [33–35]. Xu et al. [36] used single-nucleotide polymorphisms (SNPs) to conduct PCA and topology analyses, indicating that the East China Sea glacial refugium, particularly the Okinawa Trough, serves as the center of diversity and origin for *S. marmoratus*.

On the foundation of a star-like topological structure, no significant differences in haplotypes within these three branches were observed across the four populations and shared haplotypes were excited among the four populations (Haplotypes 6, 7, 18 and 22). More interestingly, Haplotype 4 is exclusive to the SS, DJ, and DT populations, while it is absent in the YZ population. Additionally, YZ exhibits the lowest haplotype diversity among the four populations, paired with the highest nucleotide diversity. Tajima's D test for neutral selection further reveals distinctions in YZ, suggesting positive selection effects. In summary, differences exist between the cultured population and others, likely attributed to unique environmental conditions, the specificity of the number and source of parents [37]. Therefore, considering the specific environment of the farm, Haplotype 4 might not be suitable for survival and could have been eliminated. Furthermore, based on genetic distance, the SS and DT populations are the closest because they are both wild populations. And compared with other populations, the genetic distance of DJ and YZ population is closer. This observation may be linked to the artificial enhancement and release activities of *S. marmoratus* [38]. Notably, there was an absence of stocking in the SS and DT regions, and DJ was the primary zone for the proliferation and release activities of *S. marmoratus*. *Sebastes marmoratus* is an ovoviviparous organism, and its strong site fidelity and sedentary behavior make gene flow exchange challenging. The released individuals introduce genes from the breeding farm and facilitate genetic exchange between the YZ and

DJ populations. The study's findings indicate genetic diversity disparities between cultured and other populations, but this difference has not yet reached a significant level [31]. This implies a potential shared evolutionary ancestor between the SS and DT populations, resulting in their relatively close genetic distance. Influenced by the artificial enhancement and release activities of *S. marmoratus*, the DJ population occupies an intermediate position between the other two populations. For a comprehensive understanding, a deeper analysis of the impact of stocking activities on the genetic structure of *S. marmoratus* populations was undertaken, revealing novel insights in F_{ST} and gene flow analyses. The F_{ST} values between the YZ population and others range from 0.011 to 0.034, generally insignificantly small, except for the SS population where F_{ST} is both small and significant. Conversely, F_{ST} values between the DJ population and the SS and DT populations are less than 0, with Nm values showing a similar pattern. This indicates that, despite differences, the cultured population has not differentiated from others thus far. Directional relative migration networks illustrate the strongest gene flow from SS to YZ, attributed to the parental generation of the YZ population originating from the SS population. The phylogenetic tree based on haplotypes revealed no significant geographic agglomeration among four populations and the inter-population haplotypes were intermixed. Individuals of the same origin coming together and individuals of releasing interspersing within may be due to the fact that samples from the DJ population were obtained from sea fishing, encompassing both wild individuals and released individuals. AMOVA analysis similarly maintains that genetic variation primarily occurs within populations, suggesting no significant differentiation among the four populations.

In this study, we conducted a thorough and detailed analysis of the genetic diversity within the breeding population, released mix population, and wild population of *S. marmoratus* in the Zhoushan Sea area. The results indicate that there were no significant differences in genetic diversity observed among four populations. We further delved into the genetic differentiation among the four populations under consideration but found no conspicuous signs of genetic divergence. Consequently, we reasonably infer and conclude that the breeding and stocking activities of *S. marmoratus* in the Zhoushan Sea area have not resulted in significant adverse effects on genetic diversity. Leber et al. [39] suggested that the management and monitoring of the impact of hatchery-reared fish on natural populations are very important. We speculate that this phenomenon may be attributed to two reasons. Firstly, the duration of *S. marmoratus* enhancement and stocking activities in the Zhoushan Sea area has been relatively short. According to the report of Chen et al. [20], large-scale analyses of *S. marmoratus* breeding and releasing activities have only been carried out in Zhoushan waters in recent years. A similar phenomenon has been observed in the restocking activities of *Sebastes schlegelii* [40]. The results showed that compared with wild populations, the genetic diversity of released populations of *S. schlegelii* did not significantly decrease in the short term, and the genetic diversity index of recaptured populations did not change significantly. Wang et al. [40] shared a comparable perspective and speculated that short-term restocking activities would not significantly impact the genetic diversity of wild populations. Secondly, the selection of broodstock is crucial for producing individuals genetically related to local adaptation. As indicated by the director of the breeding farm and research and our study, the parental stock of *S. marmoratus* originates exclusively from the local area. This approach has significantly minimized the adverse effects of breeding and releasing activities on the genetic diversity of local species. As the global trend of releasing hatchery-reared fish for stock enhancement becomes increasingly widespread, more scholars are delving into the potential impact of hatchery-reared juveniles on the genetic diversity of wild populations. The prevailing consensus among most researchers is that the local origin of hatchery fish broodstock is a key determinant of this impact [41,42]. Therefore, we believe it is essential to conduct genetic analysis on the species intended for release before initiating any enhancement and release activities. This will aid in selecting suitable individuals for release, thus mitigating the potential negative impact of such activities on the genetic diversity of local species.

5. Conclusions

The genetic diversity observed in the *S. marmoratus* broodstock population, the stocked population, and the marine fishery population was high, with no discernible genetic differentiation. This indicates that the current stock enhancement and release activities have not adversely affected the genetic diversity of *S. marmoratus* in the Zhoushan Sea area. This phenomenon may be linked to factors such as the quantity and duration of release, as well as the number and origin of the parent group. For future stocking activities, it is crucial to consider these factors, emphasizing the need for careful attention to the number and origin of the population involved and the ongoing monitoring of genetic diversity.

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