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Genetic Evaluation of Black Sea Bream (*Acanthopagrus schlegelii*) Stock Enhancement in the South China Sea Based on Microsatellite DNA Markers

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Abstract: This is the first genetic evaluation of hatchery-based stock enhancement of black sea bream (*Acanthopagrus schlegelii*) in the South China Sea after a two-year monitoring period. In this study, microsatellite DNA markers were used to calculate the contribution rate and analyze genetic changes before and after stock enhancement. Two out of one hundred and sixty nine individuals from three recaptured populations were assigned to broodstock with a contribution rate of 1.18%, revealing that the hatchery-released juvenile fish could survive in the natural environment and had a positive effect on population replenishment in wild black sea bream abundance. However, we found that the release population had the lowest genetic diversity and significant genetic differentiation from other populations. In addition, genetic diversity detected in the recaptured population was lower than that in the wild population, and their genetic differentiation reached a significant level. Our results suggested that releasing cultured black sea bream juveniles with low genetic quality might be genetically harmful for the maintenance of wild genotypes. Therefore, it is necessary to assess the genetic variation of the hatchery population before implementing a stock enhancement and establish a long-term evaluation for monitoring the genetic effect caused by releasing this fish species.

Keywords: *Acanthopagrus schlegelii*; hatchery-released effect; genetic diversity; population structure; fish stock recovery

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1. Introduction

Since fishery resources play a role in global food security, concerns about overfishing are increasing. As a survey showed, about 89.5% of the wild fish populations are partly or fully overexploited worldwide [1]. Stock enhancement via artificially producing fish juveniles and releasing them into the wild environment is an effective method for addressing the deterioration of fishery resources [2]. In addition to directly increasing the biomass of wild fish stocks, stock enhancement helps local stocks to maintain self-sustainable development [3]. Stock enhancement programs have been widely applied for decades over 300 species, such as flathead grey mullet (*Mugil cephalus*), silver carp (*Hypophthalmichthys molitrix*) and Korean rockfish (*Sebastes schlegelii*) [4–7]. In China, about 95 billion individuals composed of marine fish, shrimp and scallops were released into coastal regions during 2004–2013 [7]. Nevertheless, negative genetic effects of hatchery-released juveniles on natural populations have been reported in some fish species. For example, the population structure of silver carp (*H. molitrix*) in the Yangtze River was changed by the hatchery-released population [4]. In a study of amago salmon (*Oncorhynchus masou ishikawae*), introgression via stocked fish changed the phenotype in indigenous populations [8]. As reported in an evaluation of the stock enhancement red sea bream (*Pagrus major*), the long-term extensive hatchery release program caused a decline in the genetic diversity of

wild populations in Kagoshima Bay [9]. Therefore, genetic evaluation is urgently needed to preserve the genetic diversity and maintain the population structure of the natural populations to ensure a workable and responsible stock enhancement program [10]. Microsatellite DNA markers have been shown to be superior in tracing the pedigree in aquaculture, analyzing genetic diversity and population structure [11], which were commonly used as efficient tools for genetic evaluation of stock enhancements [12–14].

Black sea bream (*Acanthopagrus schlegelii*) (Bleeker, 1854) is a warm temperate demersal fish, inhabiting reefs or sand mud substrate near the offshore area in the coastal waters of the Northwest Pacific, ranging from the South China Sea to the coastal waters of Japan and Korea [15,16]. According to the observation, black sea breams are opportunistic feeders that prey on crustaceans, shellfish and seaweeds, and they prefer to move around rather than stay at a restricted site if there is a short supply of food [17]. A release–recapture experiment in Daya Bay showed that black sea bream spread radially along the inner and outer directions of the bay after release, and the farthest distance was about 70 km, indicating that black sea bream may be of strong locomotion ability [18]. With great commercial and recreational importance, it has been overfished over the past few decades, as evidenced by decreasing catches and declining sizes at sale [19]. As a result, stock enhancements have been implemented in several regions, including Japan [20,21], Korea [22] and China [16,23]. Genetic evaluation studies of black sea bream stock enhancement in Japan suggested that it was necessary to routinely monitor the genetic effects of stock enhancement since they found potential harmful effects on the genetic composition of the wild population [20]. The evaluation in Jeju (Korea) suggested that intensive breeding practices for stock enhancement may have resulted in a further decrease in genetic diversity [22]. Therefore, population genetic evaluation on black sea bream was essential to analyze the changes in genetic diversity and population structure before and after stock enhancement and further to provide basic information for its management [24]. In China, black sea bream was listed as an important species for stock enhancement, with a large number of individuals released into the wild environment annually. Nevertheless, it was surprising that there was no research regarding the genetic effects of black sea bream stock enhancements. A previous study in the Pearl River Estuary (Guangdong, China) did not evaluate the genetic changes of natural populations before and after stock enhancement because they did not recapture samples [16]. Thus, it is necessary to monitor the genetic effects of black sea bream stock enhancement in China, for the purpose of regional conservation assessments and regular monitoring of the fisheries [25].

In this study, microsatellite DNA markers were used to calculate the contribution rate of the stock enhancement in Daya Bay, a semi-closed bay located in the north of the South China Sea. Moreover, we examined the genetic variability and divergence of the wild black sea bream population before and after the enhancement. We expected to facilitate the genetic management of stock enhancement for black sea bream by providing useful genetic data.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

Among factors affecting the efficacy of stock enhancement, the selection of release area is considered to be the most critical factor [26]. In general, the ideal release and recapture spots are the areas where the majority of fish inhabit. As a result, in this study, sample collections were conducted in the northwest sea area of Daya Bay (Figure 1), where most of wild black sea breams inhabit with abundant seaweeds [27]. The black sea bream broodstock population (BP) comprised 29 males and 25 females collected from Daya Bay using trawl nets and transferred to the hatchery (Marine Fisheries Development Center of Guangdong Province, Huizhou, China) in January 2017 (Figure 1), reared in a concrete-walled pond with aquaculture setting [28]. After seed production, fertilized eggs were collected and incubated at 18–28 °C in a pond with seawater. Feeding started on the seventh day after hatching and occurred four times a day according to hatchery

operations. Later, 200 individuals (about 30 mm of total length) were randomly sampled in March 2017 as the released population (RP) from the approximately 30 thousand hatchery-reared juveniles released into Daya Bay. Hatchery-reared juveniles were released at an offshore coast next to the hatchery. In order to compare the status of the natural population before and after artificial releasing, 48 wild black sea breams were collected as the wild population (WP) in Daya Bay before the release of the RP. In seeking food supply, plenty of released juveniles migrated to the area highlighted by dashed circle (Figure 1), where three recaptured populations were collected using trawl nets: R1706 was the first batch of recaptured population consisting of 50 samples, collected in June 2017; the second batch samples were recaptured in November 2018 as R1811 with 79 individuals; the last recaptured population contained 40 black sea breams, sampled in December 2018 and named R1812.

The pectoral fin and muscle tissue of each sample was stored at −20 °C for DNA extraction using a DNA extraction kit (Tiangen, Beijing, China). The concentration of the DNA samples was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) to determine the quality.

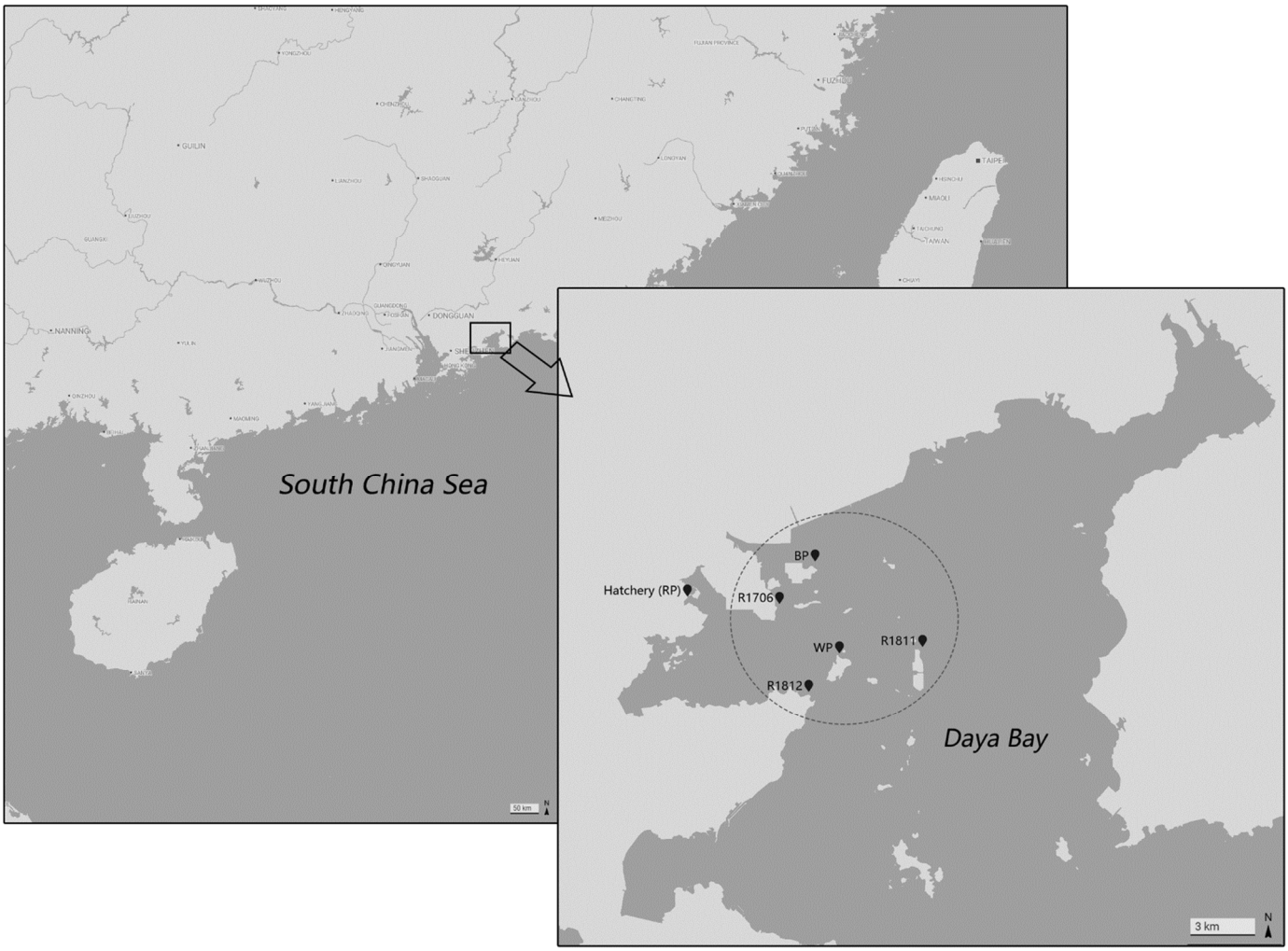

Figure 1. Sampling location (created with Datawrapper). Dashed circle is the area where a majority of wild black sea breams inhabit Daya Bay; BP, broodstock population; RP, released population; WP, wild population; R1706, R1811 and R1812 are three recaptured populations of black sea bream.

2.2. Genotyping and Calculation of the Contribution Rate

Genotyping was performed using seven microsatellite loci developed in our previous research [28] (GenBank: No. MH782241-MH782243, No. MH782245-MH782248). The polymerase chain reaction was carried out in a final volume of 20 μL containing: 100 ng of template DNA, 0.2 μM of each primer (synthesized by Tsingke, Beijing, China), 10 μL of 2 × Taq PCR StarMix with Loading Dye (GenStar, Beijing, China) and 3 μL of deionized water. The PCR program was as follows: initial denaturation at 95 °C for 10 min, followed by 35 cycles at 95 °C for 30 s, 52 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 5 min. Alleles were analyzed on the ABI3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with GeneScan LIZ 500 as a size standard. Allele length was detected using GeneMapper v.4.0 (ThermoFisher Scientific, Waltham, MA, USA). The contribution rate of the released population (the proportion of the released individuals or their offspring in recaptured populations) was an important index to evaluate the effect of stock enhancement, which was analyzed using Cervus v.3.0.3 [29].

2.3. Genetic Diversity and Population Structure

Population genetic diversity was assessed by the number of alleles (Na), the number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) using GenAlEx v6.502 [30] and polymorphism information content (PIC) of each microsatellite locus using Cervus v3.0.3 [29]. The allele number was influenced by the size of different samples, so we calculated the allele richness (Ar) using FSTAT v2.9.4 [31]. Differences in genetic diversity parameters within populations were conducted by the pairwise Wilcoxon signed-rank test (SPSS, IBM, Armonk, NY, USA).

Genetic differentiation among populations was estimated with pairwise *Fst* values and significance tests of pairwise *Fst* were computed using a permutation with 10,000 replicates using Arlequin v3.11 [32] with sequential Bonferroni correction at the significance level of 0.05. In addition, factorial correspondence analysis (FCA) based on gene frequency was conducted using Genetix v4.05 [33]. We also used Structure v2.3.4 [34] to visualize the genetic divergence among samples. This program was used to infer the number of putative clusters (K) and assign individuals into corresponding clusters. We performed this analysis under the admixture model and using 10^5 iterations after a 10^5 burn-in length with K ranging from 1 to 6, and each K performed 5 runs. The most likely K value was inferred by calculating ΔK using Structure Harvester [35]. Clumpp v1.1.2 [36] was used to analyze the results of the former calculation and the genetic structure stacked bar chart was drawn using distruct v1.1 [37].

3. Results

3.1. Contribution Rate of Stock Enhancement

Based on the parentage analysis among three recaptured populations and the broodstock using software Cervus, there were two recaptured individuals related to the broodstock (Table 1). One recaptured individual was found in R1706, which was allocated to a pair of parents with the contribution rate of 2%; the other was in R1812, allocated to a single dam with the contribution rate of 2.5%, indicating that it might have been bred by a wild sire and the allocated dam. In addition, the contribution rate of R1811 was 0%. The total contribution rate of the stock enhancement is: 2/(50 + 79 + 40) × 100% = 1.18%.

Table 1. Positive result of parentage analysis of recaptured black sea bream samples.

Recaptured Sample	Broodstock	Mismatched Loci	Confidence
R1706-042	♀23-♂17	0	*
R1812-040	♀03	0	*

Mismatched loci: microsatellite loci of recaptured sample which was different from it of broodstock. *: the parentage relationship is significant with a 95% confidence.

3.2. Genetic Diversity within Populations

The genetic diversity at each microsatellite locus for each population is presented in Table 2. The seven microsatellite loci showed high polymorphism in every population (Mean PIC > 0.5) [38]. As expected, the wild population collected before release (WP) had the highest genetic diversity (Ar: 7.73, Ho: 0.60, He: 0.66), while the hatchery-reared released population (RP) had the lowest (Ar: 4.55, Ho: 0.46, He: 0.59). Moreover, the discrepancy of genetic parameters between BP and RP was statistically significant (Wilcoxon signed-rank test, $p < 0.05$). Further investigation revealed a reduction in allele richness and heterozygosity in three recaptured populations (R1706, R1811 and R1812) (mean Ar: 6.26, mean Ho: 0.50 and mean He: 0.61) compared to WP, showing there may be a negative effect ($p < 0.05$) on genetic diversity of population in the wild after release.

Table 2. Summary genetic statistics of microsatellite loci in black sea bream.

Locus	Parameter	BP *n* = 54	RP *n* = 200	R1706 *n* = 50	R1811 *n* = 79	R1812 *n* = 40	WP *n* = 48
M320	Na	4.00	3.00	2.00	2.00	2.00	3.00
	Ne	1.47	1.42	1.08	1.03	1.10	1.24
	Ho	0.17	0.04	0.04	0.03	0.10	0.17
	He	0.32	0.30	0.08	0.02	0.10	0.19
	PIC	0.30	0.26	0.07	0.03	0.09	0.18
	Ar	3.98	2.74	2.00	1.76	2.00	3.00
M414	Na	5.00	4.00	5.00	5.00	4.00	7.00
	Ne	2.81	2.43	2.64	2.63	2.61	2.68
	Ho	0.76	0.71	0.64	0.58	0.55	0.65
	He	0.64	0.59	0.62	0.62	0.62	0.63
	PIC	0.58	0.50	0.55	0.55	0.54	0.56
	Ar	5.00	3.96	4.80	4.51	4.00	6.50
M448	Na	15.00	10.00	15.00	14.00	13.00	14.00
	Ne	8.03	6.56	9.73	7.90	9.28	9.52
	Ho	0.89	0.77	0.74	0.80	0.98	0.75
	He	0.88	0.85	0.90	0.87	0.89	0.89
	PIC	0.86	0.83	0.89	0.86	0.88	0.89
	Ar	14.06	8.38	14.35	11.96	13.00	13.75
M473	Na	7.00	3.00	5.00	6.00	5.00	12.00
	Ne	2.33	2.17	2.78	2.63	2.62	3.88
	Ho	0.63	0.50	0.68	0.52	0.58	0.85
	He	0.57	0.54	0.64	0.62	0.62	0.74
	PIC	0.54	0.48	0.58	0.58	0.56	0.72
	Ar	6.66	3.00	5.00	5.01	5.00	11.60
M478	Na	10.00	8.00	10.00	13.00	10.00	5.00
	Ne	5.02	3.36	3.95	3.96	4.64	3.79
	Ho	0.76	0.52	0.74	0.66	0.83	0.50
	He	0.80	0.70	0.75	0.75	0.78	0.74
	PIC	0.78	0.66	0.71	0.71	0.76	0.69
	Ar	9.20	6.18	9.16	10.14	10.00	5.00
M417	Na	4.00	3.00	3.00	3.00	4.00	7.00
	Ne	2.89	2.26	2.53	2.27	2.75	3.96
	Ho	0.48	0.32	0.50	0.33	0.33	1.00
	He	0.65	0.56	0.61	0.56	0.64	0.75
	PIC	0.59	0.49	0.53	0.48	0.57	0.71
	Ar	4.00	3.00	3.00	3.00	4.00	6.67

Table 2. *Cont.*

Locus	Parameter	BP n = 54	RP n = 200	R1706 n = 50	R1811 n = 79	R1812 n = 40	WP n = 48
M454	Na	7.00	7.00	7.00	6.00	6.00	8.00
	Ne	2.97	2.33	4.01	4.34	3.48	3.34
	Ho	0.37	0.45	0.22	0.29	0.38	0.25
	He	0.66	0.57	0.75	0.77	0.71	0.70
	PIC	0.62	0.50	0.71	0.74	0.67	0.65
	Ar	6.72	4.60	6.92	5.88	6.00	7.61
Mean	Na	7.43	5.43	6.71	7.00	6.29	8.00
	Ne	3.65	2.93	3.82	3.54	3.78	4.06
	Ho	0.58	0.46	0.51	0.47	0.53	0.60
	He	0.65	0.59	0.62	0.60	0.62	0.66
	PIC	0.61	0.53	0.58	0.56	0.58	0.63
	Ar	7.09	4.55	6.46	6.04	6.29	7.73

BP, broodstock population; RP, released population; WP, wild population; R1706, R1811 and R1812 are three recaptured populations of black sea bream. Na: number of alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphism information content, Ar: allele richness.

3.3. Genetic Differentiation among Populations

Pairwise Fst analysis showed that RP was significantly divergent from other populations, even from its broodstock population (BP), after Bonferroni correction for multiple comparisons (Table 3, Fst: 0.057–0.068, $p < 0.001$). A significant differentiation was observed between WP and three recaptured populations, meaning that the genetic structure of natural black sea bream population was changed after the stock enhancement. The pairwise Fst index of three recaptured populations was less than 0.05 with no statistical difference (after Bonferroni correction, $p > 0.001$). The results of FCA further supported the significant genetic differentiation in terms of allele frequency between populations (Figure 2), which divided six populations into four clusters (cluster 1 for BP, cluster 2 for RP, cluster 3 for WP and cluster 4 for three recaptured populations). Additionally, in simulations of the Bayesian approach with the software Structure, the ΔK clearly suggested two or three clusters were the likely population genetic group (Figure 3). When K = 2, the cultured populations (BP and RP) were assigned into one group, whereas the natural populations (WP and three recaptured populations) showed identical genetic properties (Figure 4). When K = 3, BP and RP were further divided into two groups on the bias of K = 2 (Figure 4).

Table 3. Pairwise Fst between six populations of black sea bream.

	BP	RP	R1706	R1811	R1812	WP
BP	\	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
RP	0.014	\	0.000 *	0.000 *	0.000 *	0.000 *
R1706	0.036	0.068	\	0.126	0.054	0.000 *
R1811	0.031	0.068	0.006	\	0.324	0.000 *
R1812	0.018	0.057	0.010	0.005	\	0.000 *
WP	0.030	0.061	0.015	0.026	0.022	\

Value below the diagonal is the pairwise Fst, value about the diagonal is the p value, * means significant difference after Bonferroni correction ($p < 0.001$).

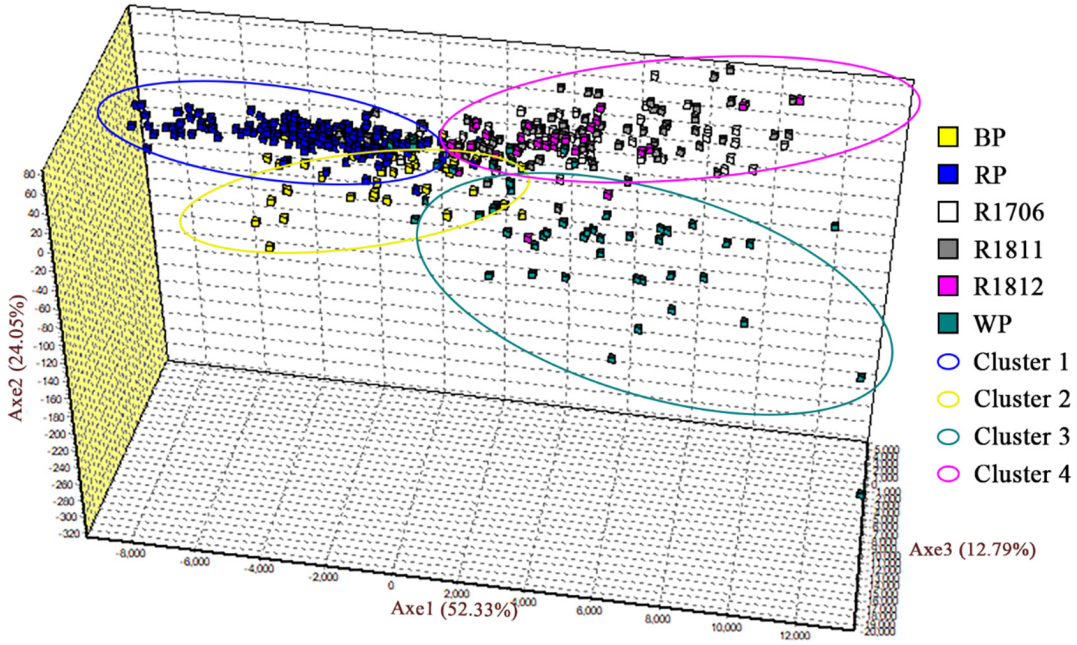

Figure 2. FCA result of six black sea bream populations, which divided them into four clusters.

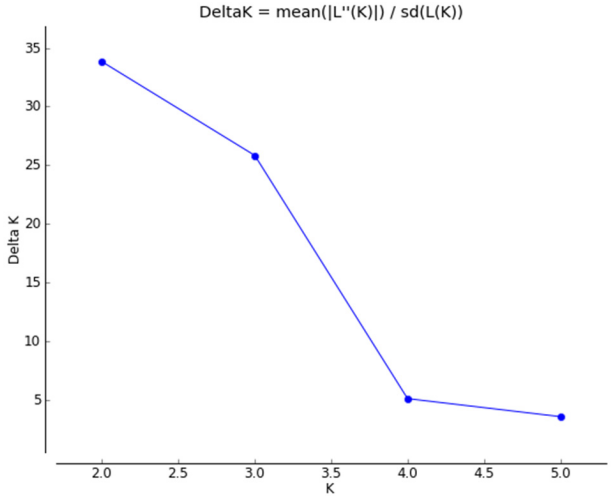

Figure 3. Result of Structure. ΔK value at K = 2 and K = 3 were relatively higher than other K value, indicating that K = 2 or 3 were the options to divide six black sea bream populations.

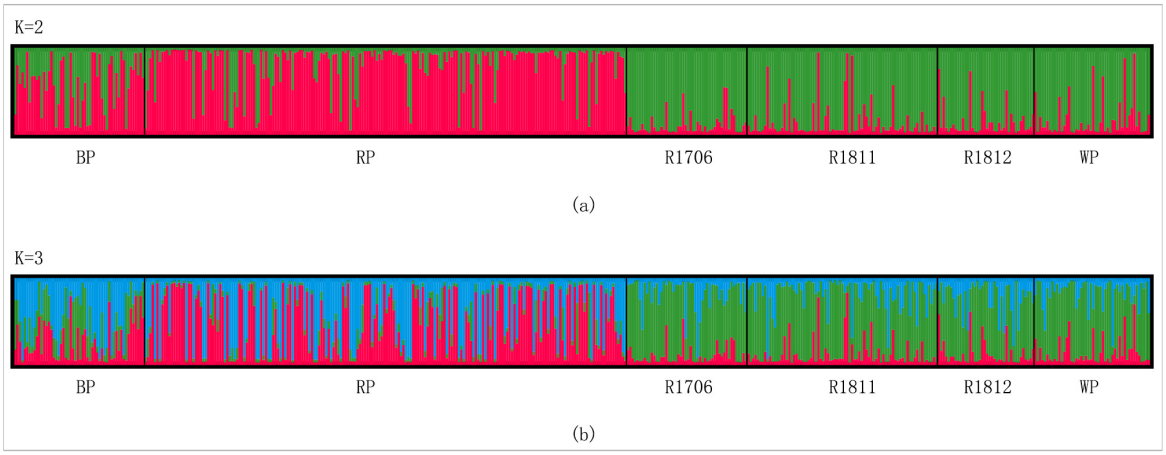

Figure 4. Results of structure analysis based on microsatellite loci. Each individual is represented by a vertical line, which is colored according to the assigned groups at estimated K = 2 (**a**) and K = 3 (**b**).

4. Discussion

4.1. The Contribution Rate of the Stock Enhancement

The contribution rate of the released population (the proportion of released individuals and their potential offspring in recaptured populations) is an important parameter to reflect the adaptation of released individuals to the wild environment. In this study, we released black sea bream juveniles with body lengths of 30 mm into Daya Bay and recaptured three batches in two years. The contribution rates of each batch ranged from 0% to 2.5%, and the total contribution rate of the stock enhancement was 1.18%, which was relatively small compared with other recapture investigations of black sea bream [20,21,23,39]. For example, a former recapture investigation of black sea bream in Daya Bay using plastic oval tags reported that the recapture rates of three batches varied between 2.49% and 7.76% within seven months after releasing juveniles with 50–95 mm body lengths [23]. Because of a shorter recapture timescale and larger body size of juveniles, the mortality rate of released black sea bream decreased, and therefore the contribution rate rose. In addition, various release areas of stock enhancements would result in different contribution rates on account of geographical conditions, food abundance and the amount of wild population before stock enhancement. Daio Bay is a small bay located in Hiroshima, Japan, where the released population may not be able to spread as far as in Daya Bay. As a result, the contribution rates of stock enhancement for black sea bream in Daio Bay (Hiroshima, Japan) were much higher. For instance, in a stock enhancement carried out in Daio Bay from 2000 to 2001, 12.5 and 13.5% of captured samples were identified as hatchery reared in 2003 and 2004, respectively [20]. Moreover, Jeong et al. [21] reported that the contribution rate of the released population of black sea bream in Daio Bay was up to 58.8%, owing to a shorter recapture timescale: 100 days after release.

Mortality usually happens in released populations after they are released into the natural environment, which would greatly affect the contribution rate of stock enhancement. The physiological condition of released fish and their ability to adapt to the natural environment are major factors of a stock enhancement [40]. Black sea bream showed strong adaptability to the natural environment and there were no reports of any signals of high mortality. In this study, no evidence showed the mortality of the released population, but there was a recaptured individual bred by a wild sire and a hatchery-reared dam, indicating that some of the released individuals could adapt to the wild environment and had gene flow with the wild population. As one study showed, differences in the growth pattern between wild and hatchery black sea bream did not exceed 40 days [41]. In addition, the growth speed of the released black sea bream was relatively slow in the early release period (about 30 days) but increased afterwards [27]. All these studies led to a conclusion that released black sea bream only required a short amount of time to adapt to the natural environment, ensuring a relatively low mortality rate in stock enhancement. Hatchery-reared populations of different species vary greatly in their ability to adapt to the wild environment. An assessment of a red sea bream (*P. major*) stock enhancement program in Japan found that there were no released fish in the recaptured populations, which may be related to the failure of the released population to adapt to the natural environment, causing a great number of deaths [42]. Likewise, the recapture rate of the hatchery gilthead sea bream (*Sparus Aurata*) of 15 g ranged from 0% to 0.1% in the bay of Cádiz [43]. In order to ensure a higher livability and increase the contribution rate of a stock enhancement, juveniles should be reared to a longer body length before release, such as juveniles at 40 mm in Hiroshima Bay [20]. However, longer cultivation means greater costs. A study about the viability of releasing 20 mm body length individuals in Daio Bay found that no significant difference in the growth rate was observed between different body lengths of black sea bream [39]. Combined with our result that 30 mm body length juveniles were able to adjust to the wild environment and have gene flow with natural individuals, we suggested that releasing juveniles with a no less than 20 mm body length would be more suitable for stock enhancements, which may improve the effectiveness of stock enhancement as well as maintain the cost of hatchery.

4.2. Genetic Evaluation of the Stock Enhancement

Genetic diversity is related to the survival, adaptation and even evolutionary potential of a species [44]. A decrease in genetic diversity caused by released populations could lead to a series of problems, such as trait deletion, the high-level expression of harmful genes, resulting in a low survival rate of a population, poor fertility and weak environmental adaptability [45]. In addition, stock enhancements need to be aware of the change in the population structure of the target species, as the introduction of released population might have negative effects [46]. In this study, we detected a decline in genetic variation in three recaptured populations compared to their wild counterparts, showing there may be a negative effect caused by the hatchery-reared released population with the lowest genetic diversity (Table 2). Moreover, there is no difference in genetic diversity between recaptured populations sampled in 2017 and 2018, indicating that the population genetic characteristics of natural black sea bream were stable in a two-year period after release. As a result, the decrease in genetic diversity of wild black sea breams was probably due to hatchery-reared population with the lowest genetic diversity rather than a genetic diversity decrease occurring in the entire wild black sea bream population in Daya Bay. With respect to genetic divergence among populations, pairwise F*st*, FCA and Structure analysis (Table 3, Figures 2 and 4) consistently supported the significant differentiation between the released population and the wild populations. Significant differentiation between three recaptured populations and the wild population was also detected, indicating that the hatchery-reared released population may have influenced the original population structure of the wild population. According to our results, there was a great loss of genetic diversity in the hatchery-reared released population and a significant genetic differentiation between the wild population (F*st* = 0.061, $p < 0.001$), which may be the key factor causing the genetic differences between the wild population and three recaptured populations. Likewise, genetic variation losses in hatchery-reared populations and genetic differentiation between hatchery-reared populations and wild populations were common in fish species such as Atlantic salmon (*Salmo salar L.*) [47], Asian seabass (*Lates calcarifer*) [48] and grass carp (*Ctenopharyngodon idella*) [49]. As a consequence, releasing a mass of hatchery juveniles with reduced genetic variation and significant genetic differentiation might affect the genetic composition of the wild populations. Therefore, stock enhancements should be cautiously conducted, and a long-term evaluation mechanism should be established after stock enhancements.

For the purpose of avoiding the situations mentioned above, efforts should be made in seed production, especially focusing on the broodstock. The incorporation of alleles from a population in the gene pool of another genetically distinct population is a threat to the genetic integrity of natural populations [50]. The significant differentiation of population structure in different geographical areas was observed in black sea bream (divergence between western Japan and South Korea) [51], showing that broodstock should be selected in a native population. However, genetic differentiation was detected between the broodstock population and the wild population in this study (F*st* = 0.030, $p < 0.001$), although all of the broodstock individuals were collected from the same location of the wild population. We considered that it was related to artificial selection during pedigree construction, which showed the importance of assessing genetic differentiation before seed production. In aquaculture practice, genetic differentiation is usually influenced by a random genetic drift [52], due to the unequal sex ratio and the bias reproductive success of broodstock [53]. Therefore, we suggested that broodstock should be selected according to the mating system and reproductive success of black sea bream in order to avoid biased reproductive contribution. Expanding the number of native breeders and collecting fertilized eggs at several times could also help in decreasing the genetic differences and conserving a larger genetic resource of natural populations [54,55]. Moreover, substantial loss of genetic variation can happen after just one generation [56]. In order to maintain the genetic diversity of the released population, it was necessary to assess the genetic variation between broodstock and offspring. In this study, there was a significant genetic

differentiation between the broodstock population and the released population, with a loss of genetic diversity in the latter. Consistent with our former study in groupers, it was mainly due to the founder effects caused by using a small number of breeding individuals and random genetic drift in the hatchery [57]. Using a limited number of broodstock individuals, unequal contributions of broodstock individuals to broods, and inbreeding in broodstock would reduce genetic variability, change genetic composition, and increase the genetic load in the released population [58]. In a study of the common crab (*Portunus trituberculatus*), the genetic diversity of the released population was significantly lower than the natural populations when its broodstock was only nine individuals, while when there were fifty broodstock individuals, the genetic diversity was in line with the natural populations [59]. Therefore, we suggest building a broodstock with a sufficient number to maintain the genetic diversity of the released population.

To sum up, technicists should pay attention to broodstock management to maintain a high genetic diversity of populations and low genetic divergence between hatchery-reared released populations and natural populations [60]. It was necessary to calculate the variation in genetic diversity and examine the genetic differentiation between released populations and natural populations before stock enhancement in order to avoid releasing unsuitable juveniles.

5. Conclusions

To our knowledge, this is the first genetic evaluation of hatchery-based stock enhancement of black sea bream in the South China Sea. Our research demonstrated that the total contribution rate of the stock enhancement was 1.18%, which may have a positive effect on recovering the natural population. However, there was a decline in the genetic diversity of three recaptured populations compared to the wild population and a significant genetic differentiation between them, indicating the hatchery-reared released population may be genetically harmful in the stock enhancement because of its lowest genetic diversity and significant differentiation from the natural population. Thus, attention should be paid to produce a suitable released population for stock enhancement, including expanding the number of broodstock, characterizing the genetic diversity and analyzing the population structure between natural populations ahead of release. Meanwhile, a long-term evaluation mechanism should be established after stock enhancement, and population recapture should be carried out regularly to evaluate the genetic effects.

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References

1. Pauly, D.; Zeller, D. Comments on FAOs State of World Fisheries and Aquaculture (SOFIA 2016). *Mar. Policy* **2017**, *77*, 176–181. [CrossRef]
2. Kitada, S. Economic, ecological and genetic impacts of marine stock enhancement and sea ranching: A systematic review. *Fish Fish.* **2018**, *19*, 511–532. [CrossRef]
3. Araki, H.; Schmid, C. Is hatchery stocking a help or harm?: Evidence, limitations and future directions in ecological and genetic surveys. *Aquaculture* **2010**, *308*, S2–S11. [CrossRef]
4. Fang, D.A.; Luo, Y.T.; Xu, D.P.; Yang, X.W.; Wang, X.H. Relationship between genetic risk and stock enhancement of the silver carp (*Hypophthalmichthys molitrix*) in the Yangtze River. *Fish Res.* **2021**, *235*, 9. [CrossRef]
5. Leber, K.M.; Arce, S.M. Stock enhancement in a commercial mullet, *Mugil cephalus* L., fishery in Hawaii. *Fish. Manag. Ecol.* **1996**, *3*, 261–278.
6. R.L., W.; D.M., B. An Evaluation of Present Techniques for the Enhancement of Fisheries. Available online: www.fao.org/3/W8514E/W8514E01.htm (accessed on 6 July 2021).
7. Wang, L.J.; Wu, Z.H.; Wang, Y.J.; Liu, M.X.; Song, A.H.; Liu, H.J.; You, F. Genetic Assessment of a Black Rockfish, *Sebastes schlegelii*, Stock Enhancement Program in Lidao Bay, China Based on Mitochondrial and Nuclear DNA Analysis. *Front. Mar. Sci.* **2020**, *7*, 11. [CrossRef]
8. Kawamura, K.; Furukawa, M.; Kubota, M.; Harada, Y. Effects of stocking hatchery fish on the phenotype of indigenous populations in the amago salmon *Oncorhynchus masou ishikawae* in Japan. *J. Fish Biol.* **2012**, *81*, 94–109. [CrossRef]
9. Hamasaki, K.; Toriya, S.; Shishidou, H.; Sugaya, T.; Kitada, S. Genetic effects of hatchery fish on wild populations in red sea bream *Pagrus major* (Perciformes, Sparidae) inferred from a partial sequence of mitochondrial DNA. *J. Fish Biol.* **2010**, *77*, 2123–2136. [CrossRef]
10. Ortega-Villaizán Romo, M.D.M.; Aritaki, M.; Taniguchi, N. Pedigree analysis of recaptured fish in the stock enhancement program of spotted halibut *Verasper variegatus*. *Fish. Sci.* **2006**, *72*, 48–52. [CrossRef]
11. Norris, A.T.; Bradley, D.G.; Cunningham, E.P. Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture* **1999**, *180*, 247–264. [CrossRef]
12. Wang, W.; Lyu, D.; Wang, M.; Liu, K.; Kong, J.; Shan, X.; Jin, X. Research in migration route of hatchery released Chinese shrimp (*Fenneropenaeus chinensis*) in the Bohai Bay using method of SSR marker. *Acta Oceanol. Sin.* **2020**, *39*, 76–81. [CrossRef]
13. Hsu, T.H.; Huang, C.W.; Lee, H.T.; Kuo, Y.H.; Liu, K.M.; Lin, C.H.; Gong, H.Y. Population genetic analysis for stock enhancement of silver sea bream (*Rhabdosargus sarba*) in Taiwan. *Fishes* **2020**, *5*, 19. [CrossRef]
14. Taylor, M.D.; Becker, A.; Quinn, J.; Lowry, M.B.; Fielder, S.; Knibb, W. Stock structure of dusky flathead (*Platycephalus fuscus*) to inform stocking management. *Mar. Freshw. Res.* **2020**, *71*, 1378–1383. [CrossRef]
15. Buxton, C.D.; Carpenter, K.E.; Mann, B.Q.; Pollard, D.; Russell, B.; Liao, W.; Shao, K. *Acanthopagrus schlegelii*. The IUCN Red List of Threatened Species. Available online: https://dx.doi.org/10.2305/IUCN.UK.2014-3.RLTS.T170153A20567110.en (accessed on 31 August 2021).
16. Shan, B.; Liu, Y.; Song, N.; Ji, D.; Yang, C.; Zhao, Y.; Gao, T.; Sun, D. Genetic diversity and population structure of black sea bream (*Acanthopagrus schlegelii*) based on mitochondrial control region sequences: The genetic effect of stock enhancement. *Reg. Stud. Mar. Sci.* **2020**, *35*, 101188. [CrossRef]
17. Tsuyuki, A.; Umino, T. Spatial movement of black sea bream *Acanthopagrus schlegelii* around the oyster farming area in Hiroshima Bay, Japan. *Fish. Sci.* **2017**, *83*, 235–244. [CrossRef]
18. Liu, Y.; Yang, C.; Shan, B.; Sun, D.; Liu, S.; Li, T.; Lliu, M.; Xie, Q. Investiqation of a mark-recapture method of black poray, *Acanthopaqrus schlegelii*, in Daya Bay usinq plastic oval tags. *J. Fish. Sci. China* **2019**, *26*, 63–70. (in Chinese). [CrossRef]
19. Law, S.-W. *The Biology and Fisheries Status of Seabreams (Family: Sparidae) in Hong Kong and Adjacent Waters*; The University of Hong Kong: Hong Kong, China, 2017.
20. Gonzalez, E.B.; Nagasawa, K.; Umino, T. Stock enhancement program for black sea bream (*Acanthopagrus schlegelii*) in Hiroshima Bay: Monitoring the genetic effects. *Aquaculture* **2008**, *276*, 36–43. [CrossRef]
21. Jeong, D.-S.; Gonzalez, E.B.; Morishima, K.; Arai, K.; Umino, T. Parentage assignment of stocked black sea bream *Acanthopagrus schlegelii* in Hiroshima Bay using microsatellite DNA markers. *Fish. Sci.* **2007**, *73*, 823–830. [CrossRef]
22. An, H.S.; Hong, S.W.; Lee, J.U.; Park, J.Y.; Kim, K.-K. Genetic diversity of wild and farmed black sea bream populations in Jeju. *Anim. Cells Syst.* **2010**, *14*, 37–44. [CrossRef]
23. Yan, L.; Changping, Y.; Binbin, S.; Dianrong, S.; Shengnan, L.; Teng, L.; Manting, L.; Qijian, X. Investigation of a mark-recapture method of black porgy, *Acanthopagrus schlegelii*, in Daya Bay using plastic oval tags. *J. Fish. Sci. China* **2019**, *26*, 63–70. [CrossRef]
24. Yamashita, Y.; Sanchez, G.; Kawai, K.; Tomano, S.; Fujita, H.; Umino, T. The role of the isolation of the marginal seas during the Pleistocene in the genetic structure of black sea bream *Acanthopagrus schlegelii* (Bleeker, 1854) in the coastal waters of Japan. *PeerJ* **2021**, *9*, e11001. [CrossRef]
25. Law, C.S.W.; Sadovy de Mitcheson, Y. Reproductive biology of black seabream *Acanthopagrus schlegelii*, threadfin porgy *Evynnis cardinalis* and red pargo *Pagrus major* in the northern South China Sea with consideration of fishery status and management needs. *J. Fish Biol.* **2017**, *91*, 101–125. [CrossRef] [PubMed]
26. Dinis, M.T. Practical flatfish culture and stock enhancement—Edited by H.V. Daniels and W.O. Watanabe. *J. Fish Biol.* **2012**, *81*, 1145–1146. [CrossRef]

27. Lin, J.; Chen, T.; Chen, L.; Guo, J. The techniques of *Sparus macrocephalus* tagged and released in Daya Bay. *J. Fish. China* **2001**, *25*, 79–83.
28. Wang, X.; Liu, S.; Yang, Y.; Wu, L.; Huang, W.; Wu, R.; Li, G.; Zhang, H.; Meng, Z. Genetic evidence for the mating system and reproductive success of black sea bream (*Acanthopagrus schlegelii*). *Ecol. Evol.* **2020**, *10*, 4483–4494. [CrossRef]
29. Kalinowski, S.T.; Taper, M.L.; Marshall, T.C. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **2007**, *16*, 1099–1106. [CrossRef] [PubMed]
30. Peakall, R.; Smouse, P.E. genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **2005**, *6*, 288–295. [CrossRef]
31. Goudet, J. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9. 3. Available online: http://www2.unil.ch/popgen/softwares/fstat.html (accessed on 31 August 2021).
32. Eecoffier, L.; Lischer, H.E.L. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **2010**, *10*, 564–567. [CrossRef]
33. Belkhir, K. GENETIX 4.05, logiciel Sous Windows TM Pour la génétique des Populations. Available online: http://www.genetix.univ-montp2.fr/genetix/genetix.html (accessed on 14 March 2021).
34. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef]
35. Earl, D.A.; VonHoldt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
36. Jakobsson, M.; Rosenberg, N.A. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **2007**, *23*, 1801–1806. [CrossRef]
37. Rosenberg, N.A. distruct: A program for the graphical display of population structure. *Mol. Ecol. Notes* **2004**, *4*, 137–138. [CrossRef]
38. Botstein, D.; White, R.L.; Skolnick, M.; Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **1980**, *32*, 314–331.
39. Umino, T.; Hayashi, M.; Miyatake, J.; Nakayama, K.; Sasaki, T.; Okada, K.; Nakagawa, H. Significance of release of black sea bream at 20-mm size on stock enhancement in Daiô Bay, Hiroshima. *Suisanzoshoku* **1999**, *47*, 337–342.
40. Carson, E.W.; Bumguardner, B.W.; Fisher, M.; Saillant, E.; Gold, J.R. Spatial and temporal variation in recovery of hatchery-released red drum *Sciaenops ocellatus* in stock-enhancement of Texas bays and estuaries. *Fish Res.* **2014**, *151*, 191–198. [CrossRef]
41. Nakagawa, H.; Umino, T.; Hayashi, M.; Sasaki, T.; Okada, K. Changes in biochemical composition of black sea bream released at 20 mm size in Daio Bay, Hiroshima. *Suisanzoshoku* **2000**, *48*, 643–648.
42. Perez-Enriquez, R.; Takemura, M.; Tabata, K.; Taniguchi, N. Genetic diversity of red sea bream *Pagrus major* in western Japan in relation to stock enhancement. *Fish. Sci.* **2001**, *67*, 71–78. [CrossRef]
43. Sánchez-Lamadrid, A. Effectiveness of releasing gilthead sea bream (*Sparus aurata* L.) for stock enhancement in the bay of Cádiz. *Aquaculture* **2004**, *231*, 135–148. [CrossRef]
44. Ryman, N.; Utter, F. *Population Genetics & Fishery Management*; The Blackburn Press: Caldwell, ID, USA, 1987; Volume 1990, pp. 141–159.
45. Asahida, T.; Shinotsuka, Y.; Saitoh, K.; Tsuzaki, T.; Aritaki, M.; Yamashita, Y. Parental Contributions in a Japanese Flounder Hatchery Inferred from Mitochondrial DNA Haplotypes. *J. World Aquac. Soc.* **2004**, *35*, 199–208. [CrossRef]
46. Ward, R.D. The importance of identifying spatial population structure in restocking and stock enhancement programmes. *Fish Res.* **2006**, *80*, 9–18. [CrossRef]
47. Machado-Schiaffino, G.; Dopico, E.; Garcia-Vazquez, E. Genetic variation losses in Atlantic salmon stocks created for supportive breeding. *Aquaculture* **2007**, *264*, 59–65. [CrossRef]
48. Yue, G.H.; Zhu, Z.Y.; Lo, L.C.; Wang, C.M.; Lin, G.; Feng, F.; Pang, H.Y.; Li, J.; Gong, P.; Liu, H.M.; et al. Genetic variation and population structure of Asian seabass (*Lates calcarifer*) in the Asia-Pacific region. *Aquaculture* **2009**, *293*, 22–28. [CrossRef]
49. Liu, F.; Xia, J.-H.; Bai, Z.-Y.; Fu, J.-J.; Li, J.-L.; Yue, G.H. High genetic diversity and substantial population differentiation in grass carp (*Ctenopharyngodon idella*) revealed by microsatellite analysis. *Aquaculture* **2009**, *297*, 51–56. [CrossRef]
50. Létourneau, J.; Ferchaud, A.L.; Le Luyer, J.; Laporte, M.; Garant, D.; Bernatchez, L. Predicting the genetic impact of stocking in Brook Charr (*Salvelinus fontinalis*) by combining RAD sequencing and modeling of explanatory variables. *Evol. Appl.* **2018**, *11*, 577–592. [CrossRef]
51. Jeong, D.S.; Umino, T.; Kuroda, K.; Hayashi, M.; Nakagawa, H.; Kang, J.C.; Morishima, K.; Arai, K. Genetic divergence and population structure of black sea bream *Acanthopagrus schlegeli* inferred from microsatellite analysis. *Fish. Sci.* **2003**, *69*, 896–902. [CrossRef]
52. Wade, M.J.; McCauley, D.E. Extinction and recolonization: Their effects on the genetic differentiation of local populations. *Evolution* **1988**, *42*, 995–1005. [CrossRef]
53. Li, J.; Wang, G.; Bai, Z. Genetic variability in four wild and two farmed stocks of the Chinese freshwater pearl mussel (*Hyriopsis cumingii*) estimated by microsatellite DNA markers. *Aquaculture* **2009**, *287*, 286–291. [CrossRef]
54. Fraser, D.J. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evol. Appl.* **2008**, *1*, 535–586. [CrossRef] [PubMed]

55. Orita, R.; Nagano, Y.; Kawamura, Y.; Kimura, K.; Kobayashi, G. Genetic diversity and population structure of razor clam Sinonovacula constricta in Ariake Bay, Japan, revealed using RAD-Seq SNP markers. *Sci. Rep.* **2021**, *11*, 9. [CrossRef]
56. Porta, J.; Maria Porta, J.; Cañavate, P.; Martínez-Rodríguez, G.; Carmen Alvarez, M. Substantial loss of genetic variation in a single generation of Senegalese sole (*Solea senegalensis*) culture: Implications in the domestication process. *J. Fish Biol.* **2007**, *71*, 223–234. [CrossRef]
57. Wang, L.; Meng, Z.; Liu, X.; Zhang, Y.; Lin, H. Genetic diversity and differentiation of the orange-spotted grouper (*Epinephelus coioides*) between and within cultured stocks and wild populations inferred from microsatellite DNA analysis. *Int. J. Mol. Sci.* **2011**, *12*, 4378–4394. [CrossRef] [PubMed]
58. Bert, T.M.; Crawford, C.R.; Tringali, M.D.; Seyoum, S.; Galvin, J.L.; Higham, M.; Lund, C. Genetic Management of Hatchery-Based Stock Enhancement. In *Ecological and Genetic Implications of Aquaculture Activities*; Bert, T.M., Ed.; Springer: Dordrecht, The Netherlands, 2007; pp. 123–174.
59. Yang, S.; Song, N.; Zhang, X.; Wang, Y.; Wang, S.; Gao, T. Genetic diversity of swimming crab (*Portunus trituberculatus*) from four broodstock populations in stock enhancement inferred from mitochondrial control region. *J. Fish. China* **2014**, *38*, 1089–1096.
60. Doyle, R.M.; Perez-Enriquez, R.; Takagi, M.; Taniguchi, N. Selective recovery of founder genetic diversity in aquacultural broodstocks and captive, endangered fish populations. *Genetica* **2001**, *111*, 291–304. [CrossRef] [PubMed]