Acta Oceanol. Sin., 2021, Vol. 40, No. 6, P. 41–49
https://doi.org/10.1007/s13131-020-1697-7
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

Parentage determination of black sea bream (*Acanthopagrus schlegelii*) for stock enhancement: effectiveness and loss of genetic variation

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Received 28 November 2019; accepted 3 June 2020

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Abstract

The stock enhancement programs for black sea bream *Acanthopagrus schlegelii* have been conducted in China for a few years. However, little information has been reported concerning the effectiveness and genetic effect of black sea bream stock enhancement. In order to detect the contribution of released individuals in Zhujiang River Estuary (ZRE) and Daya Bay (DB), six microsatellite markers were used to identify the hatchery-released individuals. In addition, this pedigree of hatchery populations (broodfish and hatchery-released offspring) was traced to detect the number of effective parents (N_e), the inbreeding coefficient and the decrease of genetic variability in the reproduction. The pedigree reconstruction showed that at least 69 (out of 93) broodfish had offspring. The estimated N_e was 54.8, consequently the inbreeding coefficient was 0.91%. The genetic diversity of hatchery-released offspring was lower than that in that of broodfish (heterozygosity alleles, 0.727–0.774), some alleles (number of alleles, 61–69) and genetic variance were lost during reproduction. It was observed that wild samples had higher levels of genetic diversity compared with hatchery populations as well as recaptured samples in releasing area. A total of 128 hatchery-released black sea bream were identified among 487 recaptured samples in ZRE, while a total of 15 samples were identified among 96 samples in DB. In summary, there was a high survival of released fish. Nevertheless, the results provided evidence to consider a loss of genetic variation in hatchery-released stock and a negative genetic effect of the stock enhancement.

Key words: *Acanthopagrus schlegelii*, stock enhancement effectiveness, microsatellite maker, genetic effect, pedigree of hatchery populations

Citation: Shan Binbin, Liu Yan, Song Na, Yang Changping, Liu Shengnan, Gao Tianxiang, Sun Dianrong. 2021. Parentage determination of black sea bream (*Acanthopagrus schlegelii*) for stock enhancement: effectiveness and loss of genetic variation. Acta Oceanologica Sinica, 40(6): 41–49, doi: 10.1007/s13131-020-1697-7

1 Introduction

Global fisheries landings have declined due to over-fishing, of the total number of stocks assessed in 2013 based on the Food and Agriculture Organization of the United Nations (FAO)’s analysis, fully fished stocks accounted for 58.1% (FAO, 2016). Stocking of hatchery-raised fish into natural environments has widely been acknowledged as an intuitive approach to enhance the exploitable resources. It is a popular tool to augment the natural supply of juveniles and optimize harvests by overcoming recruitment limitation (Bell et al., 2008). In rencent decades, stock enhancement programmes have succeeded in many countries (Jenkins et al., 2004; Sekino et al., 2005; Cheng et al., 2014). However, many programmes cannot offer general conclusions about the effectiveness of stock enhancement. In order to examine the contribution of released individuals in natural population, some released juveniles were tagged external. Nevertheless, the loss of tags often limits the usefulness of assessment of the contribution of released individuals. In addition, it was reported that the

Foundation item: The Science and Technology Project of Guangdong Province under contract No. 2019B121201001; the National Key R&D Program of China under contract No. 2019YFD0901301; the Fund of China-Vietnam Joint Survey on Fish Stocks in the Common Fishing Zone of the Beibu Gulf; the Central Public-interest Scientific Institution Basal Research Fund, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science under contract No. 2021SD14; the China-ASEAN Maritime Cooperation Fund (China-Vietnam Fishery Stock Enhancement and Conservation in Beibu Gulf); the Hainan Provincial Natural Science Foundation of China under contract No. 320QN358.

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hatchery-reared individuals have slower growth rates compared to their wild counterparts (Støttrup et al., 2002). Therefore, it is necessary to estimate the ration of hatchery-released individuals in natural population.

Besides the effectiveness of stock enhancement, concerns about the genetic effects of releases hatchery-reared individuals into wild populaitons have attracted much attention when evaluating the stock enhancment programmes (Sekino et al., 2002; Hamasaki et al., 2010; Escalante et al., 2014). Concern was often expressed about genetic changes in hatchery-reared populations resulting from domestication selection for survival in the hatchery environment (Waples, 1999). If the hatchery-reared juveniles have been released and interbreed with the wild populations, the genetic diversity of wild populations may be altered after hatchery individuals added to those populations. The genetic effects are irreversible and long-term that the wild population may change to an admixed population (wild, hatchery and products of their hybridization, if hatchery released and wild individuals interbreed), the genetic damage may not be expressed for several generations and may be difficult to identity (Campton, 1995). Generally, many enhancement hatcheries get broodstock continually from the wild and do not typically use hatchery-reared progeny to propagate the next generation, the effect of artificial selection through hatchery was likely to be low (Hedgecock and Coykendall, 2007). What is noteworthy is that fish are highly fecund, it is possible to obtain high numbers of offspring with only a few breeders. There is potential for genetic deterioration of the broodstock if small number of effective breeding fish were kept within the hatchery (Hedgecock and Sly, 1990; Smith and Conroy, 1992). Therefore, a sufficient number of broodstock is an effort to prevent the reduction of genetic diversity. Unfortunately, it was reported that the effective parents contributing to the hatchery production juveniles were less the actual broodfish. Hara and Sekino (2003) found that only 57% of the 14 broodstock parents contributed to the production of offspring in Japanese flounder *Paralichthys olivaceus*. Similarly, the ratio was less than 40% in red sea bream *Pagrus major* (Perez-Enriquez et al., 1999). The gene pool inherited by the offspring was only a small part of the wild populations, and the offspring showed a reduced genetic variability under the circumstances. If the genetic diversity of the hatchery-reared organisms is low, their release will put the genetic variability of the wild population at risk through inbreeding. The limited number of effective breeders is no doubt one reason for this (Allendorf and Phelps, 1980; Iguchi et al., 1999). Thus, it is important to fully understand the number of effective broodstock, which contributed to the next generation (Gall, 1987).

Black sea bream, *Acanthopagrus schlegelii*, is an important commercial and sport fishing species widely distributed throughout coastal areas of China. Hatchery release of *A. schlegelii* in China began in the early 1980s since the wild individuals were depleted (Zhong et al., 1998). Millions of *A. schlegelii* juveniles were released in most coastal cities over the country, and many stock enhancement programs have recovered the depleted wild populations and increased the annual commercial catch. Assessment of the success of the *A. schlegelii* stock enhancement in terms of the survival of released individuals in China has primarily consisted of releases-recovery of juveniles marked with external tags (Lin et al., 2001). However, it is reported that released juveniles were often too small for tags to retained. In addition, due to the increase of artificial fry production, the potential genetic impact of the release of hatchery-reared fish on the wild fish stocks is a growing concern. Most hatchery stocks typically show reduced genetic variability with natural populations, which may possibly result in the loss of disease resistance ability or other capabilities to adapt to new environment (Allendorf and Phelps, 1980). Considering that the large number hatchery-released *A. schlegelii* annually, it was surprising that no information regarding the effectiveness and genetic evaluation was available from stock enhancement in China.

Microsatellite DNA markers were widely used in studies of pedigree tracing in hatchery-reared population, identification of hatchery-released individuals and interactions between wild and hatchery fish (Karlsson et al., 2008; Borrell et al., 2011; Gonzalez et al., 2013). Here, microsatellite DNA markers were used to for parentage studies in a set of samples of broodstock and offspring derived from an *A. schlegelii* stock enhancement. This study aimed to assess effective breeding numbers (N_e) and inbreeding coefficients in *A. schlegelii* hatchery-released populations of the stock enhancement programme. In addition, this study monitored the effect of the *A. schlegelii* stock enhancement program in the Zhujiang River Estuary (ZRE) and Daya Bay (DB) within a year after the program.

2 Materials and methods

2.1 *Broodfish and offspring*

Broodfish of the released juveniles comprised 54 females and 39 males. All broodfish originated from wild captives in the waters surrounding releasing area. The broodfish were maintained in a culture pond, then all fertilized eggs were collected and incubated for around 72 h. Newly hatched larvae were transferred and maintained in a pond until they reached a release size of approximately 30 mm. After spawning activity, standard length and body weight were recorded for each broodfish, and the muscle tissue from all broodfish (females and males) were sampled. In addition, 141 hatchery-reared offspring were collected prior to releasing activity. All samples were stored in 95% ethanol.

2.2 *Release and recapture*

The offspring were released in the ZRE and DB in 2015 (Table 1). In order to detect the effect of the stock enhancement, after the release, a total of 692 specimens were collected by angling or trawling in the release sea area monthly from August 2015 to April 2016 (Table 2). The collected specimens comprised wild *A. schlegelii* and hatchery-released *A. schlegelii*. Standard length and body weight were recorded for each sample. Muscle tissue from all samples were collected and stored in 95% ethanol. Figure 1

Table 1. Information about the releasing

Releasing date (yy/mm/dd)	Releasing site	Releasing number	Body length/mm	Body weight/g
2015/05/22	ZRE	73 000	30.43	0.71
2015/06/29	ZRE	17 000	40.42	2.02
2015/06/29	DB	18 000	40.42	2.02
2015/07/29	DB	5 500	80.78	17.97

Note: ZRE, Zhujiang River Esturay; DB, Daya Bay.

Table 2. Sample information of *A. schlegelii*

Recapture time (yy/mm/dd)	Recapture site	Number of total samples	Number of genotyped individuals
2015/08/16–2015/08/24	ZRE	172	96
2015/08/03–2015/08/26	DB	123	96
2015/09/04	ZRE	103	97
2015/10/01	ZRE	203	203
2015/12/01	ZRE	42	42
2016/03	ZRE	28	28
2016/04	ZRE	21	21
Total	–	692	583

Note: ZRE, Zhujiang River Esturay; DB, Daya Bay; –, no data.

showed the geographical positions of samples screened in this study. Total genomic DNA was extracted from ethanol preserved muscle tissue using a standard phenol-chloroform procedure.

2.3 ***Microsatellite genotyping and analysis***

Six dinucleotide microsatellite loci were analyzed: Asca1, Asca3, Asca4, Asca6, Asca16 and Asca17 (Jeong et al., 2003, 2007). PCR was performed in 25.15 μL reaction mixture containing 17.5 μL of ultrapure water, 1 μL of forward primer, 1 μL of reverse primer, 2 μL of dNTPs, 2.5 μL of 10×PCR buffer, 0.15 μL of rTaq and 1 μL of DNA template. PCR was carried out in the Eppendorf thermal cycler under the following conditions: 5 min initial denaturation at 94°C, and 35 cycles of 45 s at 94°C for denaturation, 45 s at an annealing temperature, and 45 s at 72°C for extension, and a final extension at 72°C for 10 min. Amplification products were separated and visualized on an ABI Prism 377 DNA sequencer (Applied Biosystems). Microsatellite alleles were scored using GeneMarker v2.2 (SoftGenetics, USA).

2.4 ***Data analysis***

Cervus 3.0 software (Marshall et al., 1998; Kalinowski et al., 2007) was used to perform parentage assignments. Firstly, the program analyzed the allele frequency, and calculated the polymorphic information content (PIC) and exclusionary power. The exclusionary power is defined as the probability of not excluding an unrelated candidate parent pair from parentage of a given offspring at one locus (non-excluding unrelated candidate parent pair, NE-PP). According the six loci, the combined NE-PP was the probability that the set of loci will not exclude a pair of unrelated candidate parents from parentage. Secondly, the simulation of parentage analysis was carried out to estimate critical values of the log-likelihood statistics LOD or Delta, so that the confidence of parentage assignments made using the parentage analysis module can be evaluated statistically. The confidence level was 80% (relaxed)–95% (strict) in present study. Finally, the hatchery-reared juveniles collected before releasing were assigned to the most likely candidate parent pair with sexes known. The result of pedigree information of broodfish and juveniles was used to quantify the number of actual parents (N_a), i.e., those parents that have been matched with at least one offspring were considered as actually reproduced and contributed genetic material to the releasing juveniles. Then, N_e was estimated after correcting the effects of different sex ratio in the breeders with $N_e = 4N_f \times N_m / (N_f + N_m)$, where N_f and N_m are the number of females and males, respectively (Perez-Enriquez et al., 1999). Moreover, a

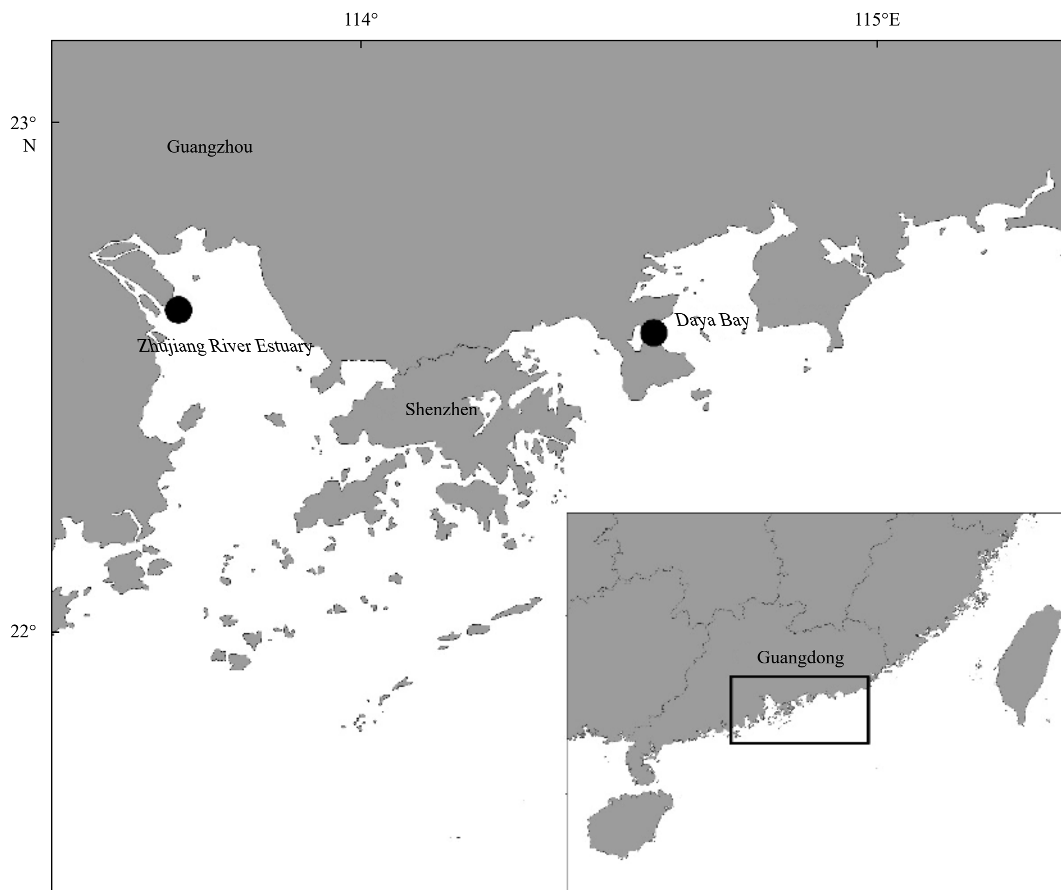


Fig. 1. The approximate location of sampling localities.

correction of N_e due to a nonrandom distribution of family size by parents was applied by using the final effective population sizes ($N_e^*=8N_e/(4+V_{kf}+V_{km})$), where V_{kf} and V_{km} are the variances in family size for females and males, respectively. Finally, the inbreeding coefficients (ΔF) were calculated based on the formula $\Delta F=1/2N_e^*$ (Franklin, 1980). To detect the relationship between body length and contribution of the breeder, this study compared the body length and the offspring number of breeders. In order to document the survival of hatchery-released *A. schlegelii*, 583 recaptured samples (96 in DB and 487 in ZRE) were identified under the same process.

The POPGENE software was used to calculate number of alleles (N_A), observed heterozygosity (H_o), expected heterozygosity alleles (H_E) and Shannon Index (I). Allelic richness (A_R) values were obtained by the software FSTAT 2.9.3, the FSTAT 2.9.3 was also used to estimate the population differentiation among populations (F_{st} values). To determine significance levels of F_{st}, multilocus genotypes were randomized between pairs of samples (1 000 permutations), then significance after Bonferroni correction was calculated (Rice, 1989). GENEPOP 3.4 was used to test deviations from Hardy-Weinberg equilibrium (HWE) expectation (Raymond and Rousset, 1995). Moreover, the null allele was checked by Micro-Checker 2.2.3 (Van Oosterhout et al., 2004).

3 Results

3.1 *Parentage assignments and effective breeders number estimation*

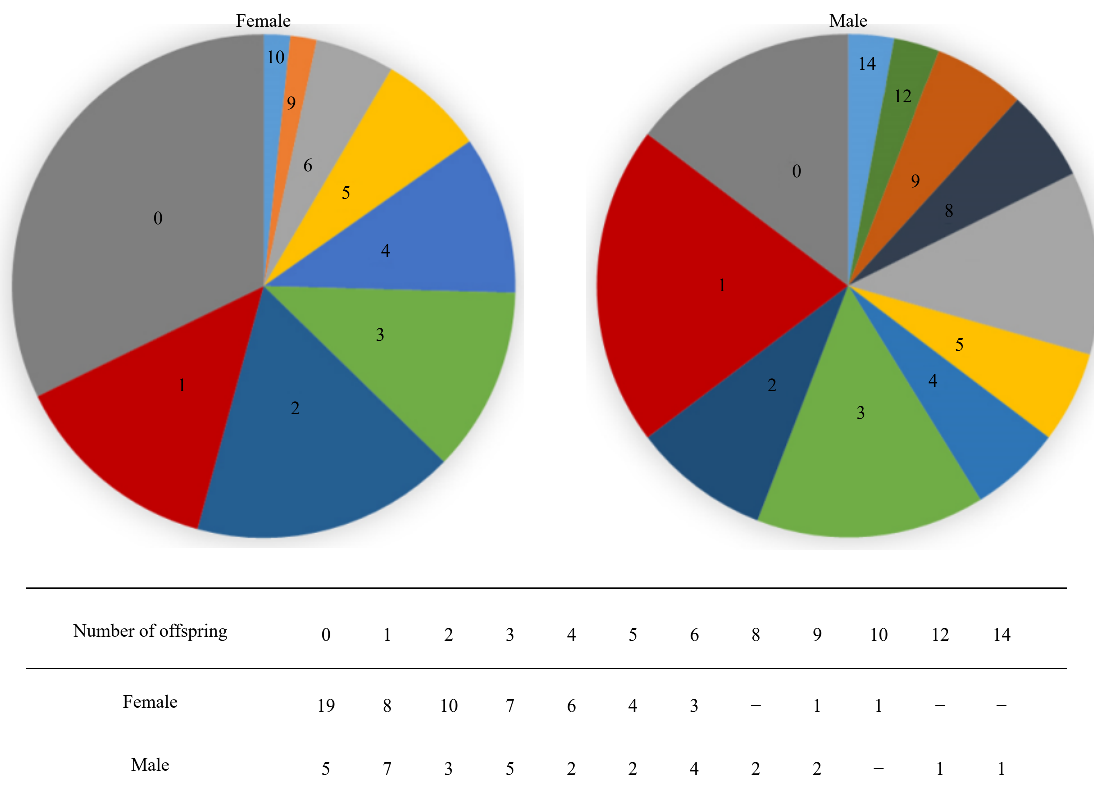
Genetic data was acquired from a total of 93 broodfish (59 females, 34 males) and 141 hatchery-reared offspring. The PIC values were range from 0.426 to 0.878, the average value was 0.724. Furthermore, the combine NE-PP value approximated to 0 (0.000 03), showed a high discrimination power (Table 3). Finally, almost all offspring (130/141, 92.2%) were assigned to a parental couple without uncertainty, the 11 offspring were assigned to multi-couples and then discarded.

There were 40 out of 59 females (67.8%) together with 29 out of 34 males (85.3%) contributed to the progenies. Therefore, N_a counted from the pedigree analysis was 69 individuals (74.2% of the broodstock). N_e (67.3) was calculated for the correction of unequal number of females and males. In order to eliminate the bias in different family sizes, the N_e^* dropped to 54.8. As a result, ΔF was 0.91%. In addition, the significant unequal contribution in breeders were observed. The number of offspring that the actual breeder contributed to was ranged from 1 to 14 (Fig. 2). The number of offspring and standard length of broodstock (both fe-

Table 3. Parameters of microsatellite loci in identification of hatchery-released individuals and parentage assignments

		Locus						Mean	Combine NE-PP
		Asca1	Asca3	Asca6	Asca4	Asca16	Asca17		
Parentage assignments	NE-PP	0.577	0.088	0.398	0.251	0.077	0.101	–	0.000 03
	PIC	0.426	0.867	0.576	0.742	0.878	0.857	0.724	–
Identification of hatchery-released individuals	NE-PP	0.339	0.066	0.251	0.208	0.148	0.079	–	0.000 013
	PIC	0.639	0.886	0.69	0.775	0.808	0.877	0.779	–

Note: PIC, polymorphic information content; NE-PP, average non-exclusion probability for a candidate parent pair.



Number of offspring	0	1	2	3	4	5	6	8	9	10	12	14
Female	19	8	10	7	6	4	3	–	1	1	–	–
Male	5	7	3	5	2	2	4	2	2	–	1	1

Fig. 2. The distribution of the broodfish's contribution to offspring.

male and male) were shown in Fig. 3. Obviously, there was no significant association between the standard length and the contribution to progenies.

3.2 *Identification of hatchery-released individuals*

The discrimination power of microsatellites for parentage analysis was high, the combined non-exclusion probability reached approximately 0 using only six loci (Table 3). PIC with 0.779 on average at all loci could also confirm the proper assignment of the progeny. Genetic data were acquired from a total of 93 broodfish and 583 recaptured samples.

The results of the identification analysis were shown in Table 4. A total of 143 hatchery-released *A. schlegelii* (24.5%) were identified among the 583 recaptured samples. Altogether, 15 of 143 hatchery-released *A. schlegelii* were in DB, only one batch of samples was collected in DB, the rate of hatchery-released was 15.6%. For samples in ZRE, the rate of released individuals was 26.2% (Table 4).

In order to investigate growth difference between released individuals and other *A. schlegelii*, the histograms of standard length were charted (Fig. 4). There was no obvious difference of increased weight between recaptured hatchery-released and recaptured non-hatchery-released *A. schlegelii*. In addition, the significance test of standard length between released stock and others was analyzed, and no significant difference was observed.

3.3 *Genetic diversity and difference*

Genetic diversity measured as number of alleles (A), allele richness (A_R), expected H_E and I were presented in Table 5. The results showed both broodfish and hatchery-released fish had significantly lower genetic diversity compared with recaptured samples. In order to compare the genetic diversity of the population in enhancement area before and after releasing, the hatchery-released fish were removed from the recaptured population (wild fish in recaptured fish in the ZRE and wild fish in recaptured fish in DB, WZRE and WDB). The WZRE and WDB have a higher genetic diversity than hatched fish in the ZRE and DB, respectively. The recaptured samples (ZRE and DB) certainly revealed a lower of genetic diversity compared with non-hatchery-released fish (WZRE and WDB). In addition, the genetic diversity of recaptured fish in the ZRE was higher than that in the DB.

In present study, the broodfish showed a higher genetic variation than hatchery-released fish. There was a reduction in genetic variation in progeny. The total number of alleles fell from 69 to 61, a total of 8 alleles were lost during the reproduction. Almost all the lost alleles were rare alleles, in which the frequencies in broodstock were low. After the corresponding adjustment due to differences in sample sizes, the A_R values estimated for the hatchery-released offspring (9.285) substantially reduced compared with that estimated for the broodstock and wild population. In addition, the expected H_E and I also showed that the genetic diversity of hatchery-released offspring was significantly lower than those estimated based on the microsatellite genotypes for the broodstock and wild populations.

Significant HWE departure were observed in recaptured sample depending on the loci. By contrast, the data of six loci did not show any significant HWE departure for broodfish. The results from Micro-Checker showed there was no null allele detected in hatchery-released fish and samples collected in the DB. Nevertheless, the presence of null alleles was detected in brood-

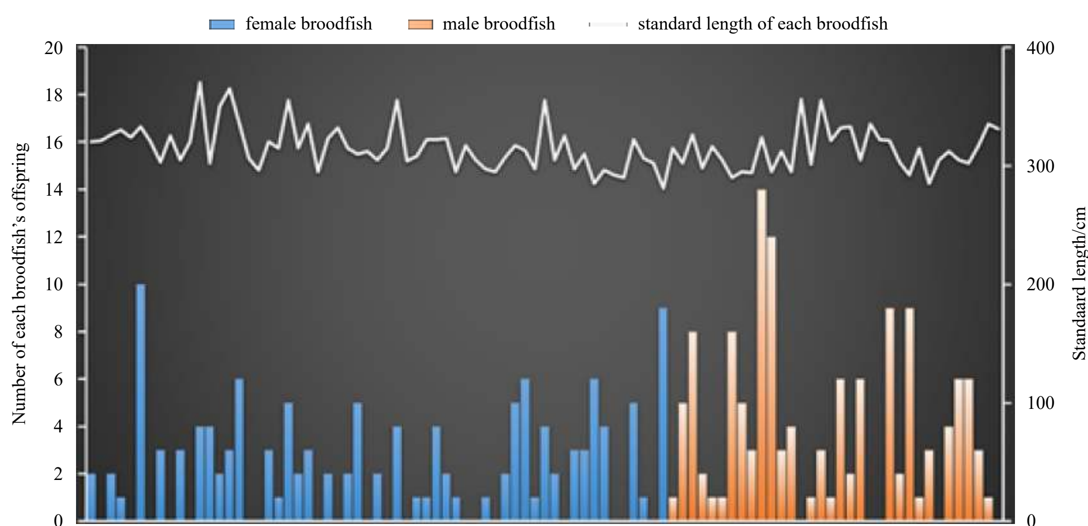


Fig. 3. The numbers of offspring and body length of breeders.

Table 4. The quantity and percentage of released-recaptured *A. schlegelii* in samples

Recapture date (yy/mm/dd)	Recapture site	Number of genotyped individuals	Number of released-recaptured fish	Rate/%
2015/08/16–2015/08/24	ZRE	96	33	34.4
2015/08/03–2015/08/26	DB	96	15	15.6
2015/09/04	ZRE	97	21	21.6
2015/10/01	ZRE	203	53	26.1
2015/12/01	ZRE	42	11	26.2
2016/03	ZRE	28	6	21.4
2016/04	ZRE	21	4	19.0
Total	–	583	143	24.5

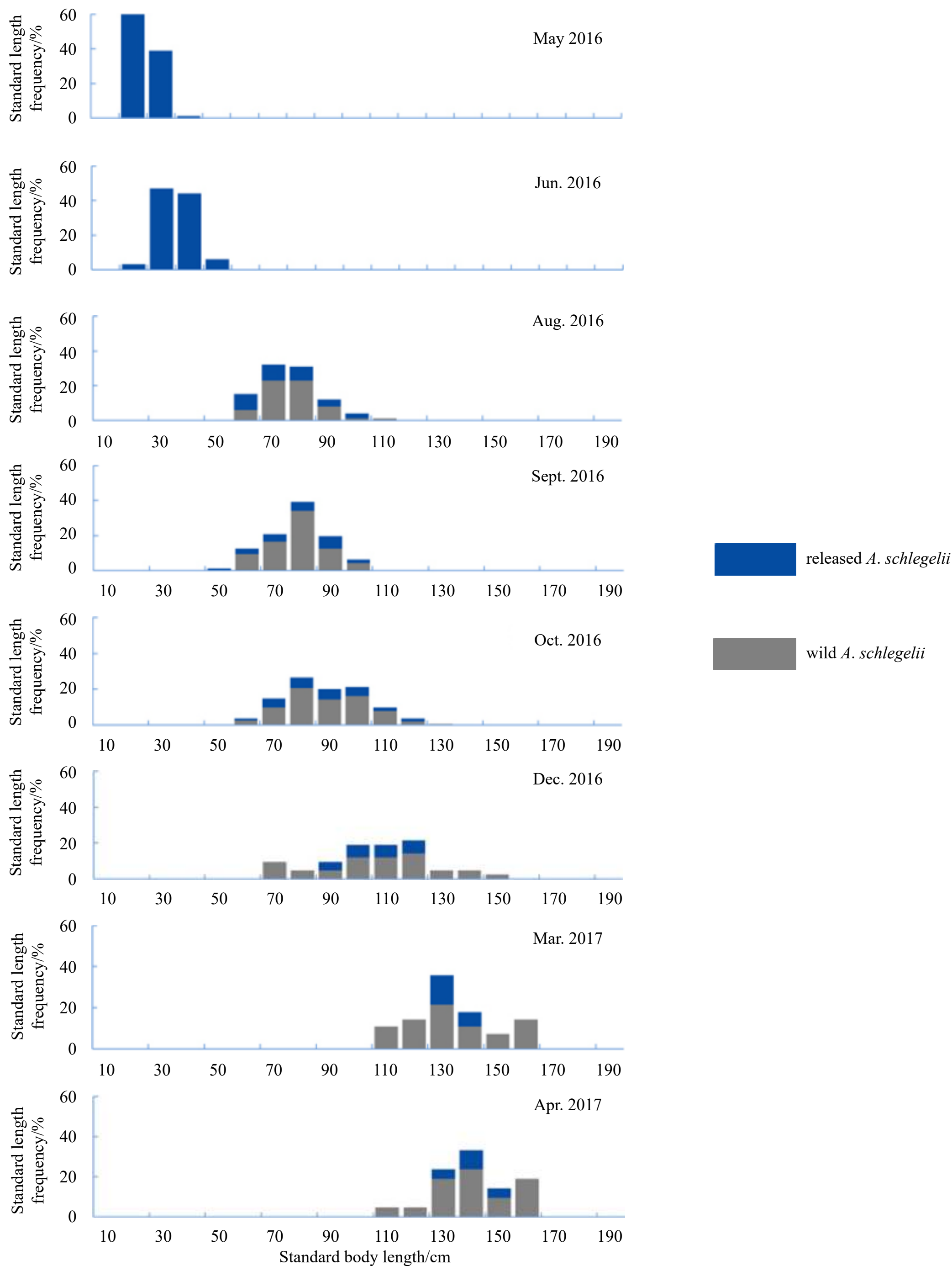


Fig. 4. Standard length frequency distribution of *A. schlegelii.*

fish at one locus (Asca 16) and samples collected in ZRE at four loci (except Asca 4 and Asca 17).

Low microsatellite pairwise F_{ST} values (–0.005 to 0.049) were observed among the populations, suggesting that there was high homogeneity between the different samples (Table 6). Except WZRE vs RZRE (recaptured fish in ZRE) and WDB vs. RDB (recaptured fish in DB), all pairwise F_{ST} values were statistically significant (P<0.05). The F_{ST} values estimated between broodstock and offspring was low (0.004) with statistically significant. Similarly, same information could be obtained after Nei's distances analyses (Table 6) among samples (0.002–0.138) (Nei, 1978). There was no significant genetic difference among broodfish, hatchery-released offspring and wild population.

4 Discussion

The black sea bream has a great commercial value in fishing industry and a long history of stock enhancement which carried out in most of coastal city in China. The use of aquaculture for stock enhancement of black sea bream, by releasing hatchery-reared juveniles, is now an intuitive approach to enhance the exploitable resources. However, for many stock enhancement species, the low genetic diversity in hatchery-reared populations has been recorded by many researchers (Smith and Conroy, 1992; Sekino et al., 2002; Hamasaki et al., 2010). In order to minimize

Table 5. Genetic variability in broodfish, hatchery-released fish, and recaptured fish

	n	A	A_R	H_E	H_o	I	Fis	HWE
Broodfish	93	69	11.108	0.774	0.754	1.882	0.027	NS
Hatchery-released fish	141	61	9.285	0.727	0.758	1.706	−0.046	–
WZRE	359	115	15.119	0.820	0.743	2.139	0.096	–
RZRE	487	115	14.469	0.803	0.740	2.071	0.083	–
WDB	81	59	9.755	0.733	0.787	1.628	−0.117	–
RDB	96	59	9.461	0.724	0.780	1.605	−0.113	–

Note: RZRE, recaptured fish in the Zhujiang River Estuary; WZRE, wild fish in recaptured fish in the Zhujiang River Estuary; RDB, recaptured fish in the Daya Bay; and WDB, wild fish in recaptured fish in the Daya Bay. A, number of alleles; A_R, allele richness; H_E, heterozygosity alleles; I, Shannon index; HWE, deviations from Hardy-Weinberg equilibrium; n, sample size; H_o, observed heterozygosity; Fis, fixation index; NS, $P < 0.05$; $^*P < 0.05$; –, no data.

Table 6. Estimates of F_{ST} (below diagonal) and *genetic distance* (above diagonal) value

	Broodstock	Hatchery-reared fish	WZRE	RZRE	WDB	RDB
Broodstock	–	0.026	0.081	0.061	0.105	0.097
Hatchery-released fish	0.004*	–	0.097	0.074	0.118	0.108
WZRE	0.025*	0.034*	–	0.003	0.138	0.132
RZRE	0.015*	0.022*	0.001*	–	0.120	0.113
WDB	0.039*	0.049*	0.041	0.034*	–	0.002
RDB	0.035*	0.044*	0.040*	0.032*	−0.005	–

Note: RZRE, recaptured fish in the Zhujiang River Estuary; WZRE, wild fish in recaptured fish in the Zhujiang River Estuary; RDB, recaptured fish in the Daya Bay; and WDB, wild fish in recaptured fish in the Daya Bay. $^*P < 0.05$; –, no data.

the deleterious genetic effects of inbreeding, low genetic diversity and small population sizes in the hatchery-released offspring, the detailed information on the parental contribution is essential for stock enhancement.

4.1 *Number of effective breeders and inbreeding coefficients*

In order to preserve the genetic diversity of the wild population, special attention is necessary in the reproduction of hatchery-released juvenile. According to the previous studies, the cause of reduction of genetic variability in hatchery-reared individuals was inferred to be a result of the number of actual parents contributing to the offspring being less than of the total broodstock or natural (Taniguchi et al., 1983; Gonzalez et al., 2010). Taniguchi et al. (1983) reported the numbers of contributing parents in two first generation *A. schlegelii* populations were estimated to be as small as 16 and 26, although the number of parents held in a hatchery was 102. The parents contributing to the hatchery production of black sea bream juveniles were first estimated by Taniguchi et al. (1983), it reported the actual number of broodfish was less than 30%. After estimating the stocked black sea bream in Hiroshima, Jeong et al. (2007) reported that only 32 in 2000 and 30 in 2001 out of the 51 broodstock contributing to the mating process. Gonzalez et al. (2010) changed the *A. schlegelii* broodstock management that collected eggs at several timings over a single night, and greatly contributed to increase the effective number of breeders from 37 to 88 in actual 143 breeders. The result of present was better than previous studies, the actual number of breeders detected in present work was 69 out of the total breeders (74.2%).

The number of effective breeders in small populations (i.e., the effective population size), is an essential information for evaluation of the rate of inbreeding (Gall, 1987). According to present study, after the correction of unequal number of different sexes in breeders and nonrandom distribution of family size, N_e^* was 54.8, decreased by 41.1% compared to the total breeders. Although N_e^* is much smaller, the value is slightly higher than the minimum 50 suggested by FAO to maintain genetic diversity in short-term programs (Meffe, 1986). The small number of effective breeders obtained in present study caused a final inbreeding depression of 0.91%. In order to loss of variation and conserve rare alleles that might be the basis of future adaptation, Franklin (1980) suggested hatchery managers should try to protect against inbreeding from exceeding 1% per generation. In summary, the high N_e^* in present study, together with low-level inbreeding coefficients, was a consequence of the large number of parents. Beside the larger number of breeders, the fertilized eggs collection strategy (multiple times in 72 h) was contribution to preserve the gene pool of broodsfish.

4.2 *Effect of the A. schlegelii stock enhancement program*

The microsatellite makers in present study proved highly effective in discriminating hatchery-released from wild black sea bream. The percentage of hatchery-released individuals in our study ranged from 15.6% in the DB to 26.3% in the ZRE. The percentage was higher than the results of the reported black sea bream enhancement program in Japan (Gonzalez et al., 2008). Considerably higher rates of hatchery-released in study may for the following key reasons: (1) The larger body length of the hatchery-released juveniles was contributed to the survival of the juveniles. It was conducive for released juveniles to escape the predations and survive in the competition with wild individuals. (2) Furthermore, abundant artificial reefs in the ZRE and DB play a crucial role as shelters, protecting released individuals from destructive fishing methods (i.e., trawling). Black sea bream likes habitat around reefs, the particular environment of releasing water area maybe contributed to prevent the releasing individuals from dispersing to open waters. The percentage in the DB was significantly lower than that in the ZRE, it may result in the difference releasing quantity.

4.3 *Genetic effects of the stock enhancement*

In order to decrease the genetic effects of the stock enhancement, it is recommended to minimize the differences between hatchery-reared stocks and wild populations. Interest has been directed toward genetic monitoring in production of juveniles. Allendorf and Phelps (1980) recognized three indicators to meas-

ure the loss of genetic variation in a hatchery strain: a reduction in the proportion of polymorphic loci, in the number of alleles per locus and in the heterozygosity. Genetic assessment of hatchery strains of black sea bream has been undertaken using several kinds of molecular markers. Taniguchi et al. (1983) examined hatchery-reared black sea bream based on 42 isozymes, the results showed genetic variability of the hatchery stocks, indicated by the proportion of polymorphic loci, number of alleles per locus, and heterozygosity, was lower than that in wild population. Jeong et al. (2007) examined that the loss of alleles during black sea bream reproduction accounted for 16.9% based on the microsatellite maker. In present study, due to the absence of many broodfish, some alleles and genetic variance were lost during reproduction. The genetic diversity was slightly lower in offspring compared with broodstock. Furthermore, the genetic diversity observed in microsatellite analysis of hatchery populations (broodfish and offspring) was significantly lower than that in wild population. The lower genetic diversity of released offspring was most caused by the low genetic diversity of the broodstock. The juveniles with lower genetic diversity have been released in enhancement area. Therefore, after the released individuals joined the natural populations, the mixed population showed a decline in genetic diversity.

If there was a genetic drift in the reproduction, a significant genetic difference could be estimated between breeders and progeny. Borrell et al. (2011) detected the genetic distances between *Engraulis encrasicolus* progeny and breeders were longer than that among wild samples. The result observed in present is in contrast with other study, low F_{ST} and genetic distance values were estimated between the breeders and offspring. It demonstrated that there was no pronounced population genetic differentiation. In addition, there was no significant genetic difference between the hatchery populations and natural populations. After the releasing, no substantial genetic changes were observed.

5 Conclusions

Stock enhancement releasing is a contentious topic in fisheries management (Molony et al., 2003). In summary, the results of this study showed that hatchery-released *A. schlegelii* has high survival rates, and becomes a part of the *A. schlegelii* stock in enhancement area. Nevertheless, the low number of broodfish that contributed to the offspring led to a reduction of genetic variance in hatchery population. Furthermore, the hatchery-reared stock showed a lower genetic heterozygosity and diversity than wild population. The possible negative impact of stocking was a loss of genetic diversity in stocked populations in the ZRE and DB. The results obtained here could be considered to preserve and monitor the gene pool of the natural stocks after releases.

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