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Parentage assignment of stocked black sea bream *Acanthopagrus schlegelii* in Hiroshima Bay using microsatellite DNA markers

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ABSTRACT: The genetic contribution of 51 broodstock, comprising 29 females and 22 males, reared at Hiroshima City Marine Products Promotion Center for the production of stocked black sea bream was monitored during two consecutive years using seven microsatellite DNA loci. The high discrimination ability of these markers was reflected in the polymorphic identification content ($PIC = 0.831$), the exclusion probability ($Q \sim 1$), and the low probability of identity index ($I = 3.635^{-10}$). The total number of breeders contributing to the mating process was estimated at 32 (62.7%) in 2000 and 30 (58.8%) in 2001. On pedigree reconstruction, 69.3% of the offspring were successfully assigned to a single broodstock pair. Loss of alleles accounted for 16.9% during seed production; nevertheless, 90.9% of males and 69.0% of females participated in the mating process. Based on microsatellite genetic tagging, 58.9% of the fish sampled during the two months after release were identified as hatchery stock, presenting no significant differences from wild conspecifics in either fork length or body weight.

KEY WORDS: ***Acanthopagrus schlegelii*, black sea bream, genetic tagging, microsatellite DNA, parentage analysis, stock enhancement.**

INTRODUCTION

Black sea bream *Acanthopagrus schlegelii* is widely distributed from the South China Sea to the coastal waters of Japan and Korea. In Japan, black sea bream is an important resource for sport and commercial fisheries, with around 6 million fingerlings artificially stocked annually.[1] Although survival and acclimation of released fingerlings into the natural environment were well documented,[2–4] knowledge concerning genetic aspects is still limited.

Many authors warned of the reduction in genetic variability derived from limited numbers of breeders under hatchery conditions.[5–9] The genetic drift might induce a smaller growth and survival, as well as increase the susceptibility to diseases in offspring.[10] Recent studies assessing the genetic diversity of stocked sparidae have demonstrated the strength of microsatellite DNA over previous techniques using proteins.[11–13] Moreover, the high polymorphism screened by microsatellites results is very useful to examine consanguineous strains. Parentage assignment using a few numbers of microsatellite DNA markers has been evidenced in some of the most important fish species for stocking and cultivation.[8,14–16]

In Hiroshima Prefecture, western Japan, the catch of black sea bream accounted for 15% of the total black sea bream catch in Japan. Hiroshima Bay is known as the main fishing ground with 60% of the catch in the Prefecture. The recent increment in the volume of landings of this fish seems to be linked to the intensive stocking practices in the area since 1982. Therefore, genetic monitoring of released black sea bream is necessary to preserve and manage the genetic resources efficiently.

Two main objectives were achieved in the present study. First, in order to assess the genetic variability between breeders and stocked black sea

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Table 1 Core repeats, primers sequences and annealing temperature of microsatellite loci in black sea bream

Locus	Core repeat sequences	Primer sequences (5′ to 3′)	Tm (°C)	GenBank accession No.
ACS3	$(CA)_{16}$	F: GGACCCATATGTCTGTCAAA R: TTTATGAGCGACCCTGAGTC	57	AB095008
ACS4	$(GT)_{12}$	F: TTTACACACCGGGAGCTCAA R: GTAAAGATCCATGGAGGTGC	57	AB095009
ACS6	$(GT)_{12}$	F: GTCTGACATCATGCTCTGAG R: ACAACCGTCTTCTTCTTACG	56	AB095010
ACS9	$(CA)_{21}$	F: GAACTGACAGTAGTGACTGG R: GTGCTGCAGTTTATATCTGC	56	AB095011
ACS16	$(CA)_{28}$	F: ACCAAGGACCCTTGTGAATG R: GCATGTCTGCAGCAACAGCA	53	AB095012
ACS17	$(CA)_{24}$	F: ACAGGCGCATGTGTTTATGC R: ATCTCTCCGTATCTTCCTCC	64	AB095013
ACS21	$(CA)_{12}$	F: CGGAAAGGAAGCAGCTCTTG R: AACTGTTAGCCAGGGTCAGC	64	AB095014

Tm, annealing temperature.

bream juveniles and its potential effect on natural populations, the contribution of the broodstock was assessed by tracing the pedigree of fingerlings during two consecutive years based on microsatellite DNA markers. Second, the effectiveness of the stocking program during the critical postrelease period was investigated by identifying stocked juveniles at a small cove of Ninoshima Island in Hiroshima Bay by using microsatellite-based genetic tagging.

MATERIALS AND METHOD

Broodstock and offspring

This study was conducted under standard seed production and stock enhancement procedures. The broodstock comprised 29 dams and 22 sires originating from wild captives, and hatchery strains. Fish were accommodated at Hiroshima City Marine Products Promotion Center in 2000 and 2001. The same breeders were maintained during the two consecutive years of study without individual replacement or mortality. Sex of each breeder was checked by spermiation or oviposition after squeezing the abdomen gently in June 2000. All broodstock presented longer than 330 mm in body length, rejecting any possibility of sex change in this protandrous hermaphrodite species.

According to routine seed production, the mesocosm spawning method was used to collect fertilized eggs in one night during the spawning peak in May 2000 and 2001. Larvae were reared under routine hatchery condition. For pedigree tracing, 70 offspring in 2000 and 110 in 2001 of 3 months of age were randomly collected just before release. In addition, 20 000 juveniles were stocked in Ninoshima Island in 2001 and then subjected to a release–recapture experiment for the stock separation study in the present work.

DNA extraction and detection of microsatellite loci

The pectoral fin from each of the 51 breeders was partially clipped, and inserted into 95% ethanol for DNA extraction in 2001. Muscle tissue of offspring and wild fish were stored at –80°C. DNA extraction was carried out by the standard SDS–phenol–chloroform procedure.[17] Microsatellite analysis was performed using seven loci of dinucleotide (CA/GT) repeats described in Table 1. Besides three loci, *ACS3*, *ACS6*, and *ACS9* isolated in a previous study,[11] four microsatellite loci, *ACS4*, *ACS16*, *ACS17*, and *ACS21* were newly isolated. Briefly, genomic DNA was digested with *Sau* 3AI and DNA fragments ranging 400–600 bp were selected on agarose gel. The size-selected fragments were ligated into the *Bam*HI site of pUC18 plasmid vector. Positive transformations were screened by the chemiluminescence method with $(CA)_{10}$ synthetic biotinylated oligonucleotide. Positive clones were sequenced using an Abiprism 377 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

In every set of primers, the 5′end of the reverse primer was labeled with biotin. Polymerase chain reactions (PCRs) were prepared using *Taq* polymerase (TaKaRa, Shiga, Japan) and carried out in a GeneAmp PCR System 9700 instrument (Applied Biosystems). The PCR program consisted of one denaturation at 95°C for 1.5 min followed by 35

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cycles of 30 s at 95°C, 1 min at annealing temperatures given in Table 1, 30 s at 72°C, and a final extension of 2 min at 72°C. Each PCR product was electrophoresed in 8% polyacrylamide gel (7 M urea, 8% polyacrylamide). Afterwards, amplified DNA fragments were transferred to a nylon membrane, and detected by chemiluminescence (Sequencing High-Cycle, Toyobo, Osaka, Japan) with pUC18 ssDNA as sequencer ladder.

Genetic variability

The genetic variability among black sea bream samples was assessed through the number of alleles per locus and the observed and expected heterozygosities (*Ho*, *He*) using GENEPOP v3.4 software.[18] Deviations from Hardy–Weinberg equilibrium (HWE) at each locus were estimated using a test analogous to Fisher's exact test, with a modified version of the Markov chain method (10^5 steps and 10^4 dememorizations),[19] performed with the Arlequin v2.0 package.[20] A linkage disequilibrium test of non-random association of alleles at different loci and allele frequency at each locus was tested by analysis of molecular variance (AMOVA).[20]

Microsatellite discrimination power

The discrimination ability of microsatellite DNA markers for pedigree reconstruction was assessed estimating the polymorphic information content (*PIC*) in the broodstock[21] by using Cervus 2.0 software.[22] The probability of excluding a parental pair from a randomly chosen offspring given by the exclusion probability (Q)[23] was also calculated. In addition, in order to avoid offspring–broodstock mismatching, the probability of identity index (I) applied to red sea bream *Pagrus major* [24] was determined.

Parentage matching and pedigree analysis

Parentage assignment was partly supported by PAPA software.[25] The best match between the genotypes of the offspring and the broodstock at all loci was confirmed by manual operation. The effective number of parents (*Ne*) was estimated after correcting the effects of different sex ratio in the breeders,[26] and family size differences,[27] resulting in $Ne^* = 8 \times Ne/(4 + V_{kf} + V_{km})$, where V_{kf} and V_{km} are the variances in family size among females and males, respectively. The inbreeding coefficient (F) is expressed as $F = 0.5 \times Ne^*$.

Stock separation

From the release–recapture experiment on 29 July 2001, 20 000 juveniles were stocked at a small cove (Daio Bay) in Ninoshima Island at Hiroshima Bay. A map showing the locality of releasing site has been presented elsewhere.[2] In order to assess the recapture rate of released juveniles, 199 fish were collected using a throw net at the releasing area from 30 July–28 September. Stock discrimination between wild and hatchery fish was conducted using five microsatellite DNA markers (*ACS3*, *ACS4*, *ACS6*, *ACS9*, and *ACS16*). Variations in fork length (FL, mm) and body weight (BW, g) between release–recaptured and wild fish were analyzed.

RESULTS

Genetic variability and pedigree analysis

On changes in genetic variability between the broodstock and their offspring in 2000 and 2001 (Table 2), the number of alleles per locus in the present study ranged 7–18. The broodstock averaged 13.3, with mean values of 11.1 and 11.0 in 2000 and 2001 offspring, respectively. *Ho* averaged 0.846, 0.844, and 0.854 for the same three groups, revealing deviations from HWE in the offspring of 2001 at two loci. Despite no significant difference ($P < 0.05$) in allele frequencies, loss of alleles through seed production was detected 16.9% on average.

The discrimination power of microsatellites for parentage analysis, the exclusion probability, reached approximately 1 using only five loci (Table 3). *PIC* with 0.831 on average at all loci could confirm the proper assignment of the progeny. In addition, the probability of finding two offspring sharing the same genotype at all loci is limited to less than 1×10^{-9} ($I = 2.980 \times 10^{-10}$, $I = 4.290 \times 10^{-10}$, in 2000 and 2001, respectively).

The pedigree analysis successfully assigned 69.3% of the offspring (69.7% in 2000 and 69.0% in 2001) to a single parental couple (Table 4). The reconstructed mating model (Fig. 1) compiles the total of 36 and 40 families identified in 2000 and 2001, respectively. Female #23 mated seven males in 2000, and male #49 constituted eight families each year; these fish were the most active breeders.

The number of contributors was estimated at 32 (62.7%) in 2000 and 30 (58.8%) in 2001. Progenies from 20 dams (69.0%) and 20 sires (90.9%) were identified within the two years studied. Balance in the sex proportion among breeders was observed; however, differences in the family sizes caused the final effective population sizes (*Ne*) to be reduced to 19.8 (38.1%) and 8.9 (70.3%) in 2000 and 2001,

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Table 2 Genetic diversity of broodstock and offspring in 2000 and 2001 at seven loci

	ACS3	*ACS4*	*ACS6*	*ACS9*	*ACS16*	*ACS17*	*ACS21*	Mean
Broodstock								
Sample size	51	51	51	51	51	51	51	
No. of allele	18	8	8	18	15	14	12	13.3
Observed (H_o)	0.882	0.745	0.784	0.922	0.823	0.902	0.863	0.846
Expected (H_e)	0.844	0.816	0.800	0.915	0.896	0.897	0.828	0.857
(H_o/H_e)	1.045	0.913	0.980	1.008	0.918	1.006	1.042	0.987
2000 offspring								
Sample size	68	70	69	67	67	66	66	
No. of allele	14	7	7	16	14	11	9	11.1
Observed (*Ho*)	0.758	0.894	0.864	0.924	0.818	0.833	0.818	0.844
Expected (*He*)	0.857	0.834	0.795	0.881	0.857	0.886	0.773	0.840
(*Ho*/*He*)	0.884	1.072	1.087	1.049	0.954	0.940	1.058	1.004
2001 offspring								
Sample size	106	106	109	106	106	106	106	
No. of allele	14	8	7	14	13	12	9	11.0
Observed (*Ho*)	0.853	0.873	0.794	0.892	0.902	0.922	0.745	0.854
Expected (*He*)	0.833	0.831	0.781	0.853	0.858	0.886	0.783	0.832
(H_o/H_e)	1.024*	1.050	1.017	1.046	1.051	1.041	0.951*	1.027

*Departure from Hardy–Weinberg equilibrium at $P < 0.05$.
Allele frequencies presented in Appendix.

Table 3 Microsatellite discrimination power expressed by the exclusion probability (*Q*), polymorphic information content (*PIC*) and probability of identity index (*I*)

	Broodstock		Offspring	
Locus	*Q*	*PIC*	*I* (2000)	*I* (2001)
ACS3	0.8803	0.823	3.637×10^{-2}	4.601×10^{-2}
ACS4	0.8085	0.783	5.221×10^{-2}	5.359×10^{-2}
ACS6	0.7811	0.760	7.285×10^{-2}	7.755×10^{-2}
ACS9	0.9524	0.899	2.699×10^{-2}	3.975×10^{-2}
ACS16	0.9632	0.873	3.866×10^{-2}	3.585×10^{-2}
ACS17	0.9194	0.878	2.628×10^{-2}	2.118×10^{-2}
ACS21	0.8440	0.801	7.857×10^{-2}	7.433×10^{-2}
2 loci	0.9771			
3 loci	0.9950			
4 loci	0.9998			
5 loci	~1			
6 loci	~1			
7 loci	~1			
Mean		0.831	2.980×10^{-10}	4.290×10^{-10}

respectively. These values resulted in a final 2.5% of inbreeding depression in 2000 and 5.6% in 2001 (Table 4).

Stock separation

A total of 199 previously released black sea bream juveniles were recaptured between 30 July and 28 September at Daio Bay in Ninoshima Island. On 30 July (first day after release, DAR), 76.9% of the samples represented stocked specimens. This percent changed to 25% on 7 August (ninth DAR), 70% on 22 August (24th DAR), 67.3% on 5 September (38th DAR), and 60% on 28 September (61st DAR). Consequently, stocked individuals accounted for 58.8% of the catch during the two months following the release date.

On the FL distribution of wild and hatchery stocks fish, no significant differences in either frequencies ($P < 0.01$, F-ratio) or lengths ($P < 0.01$, Student's *t*-test) between hatchery and wild groups were evidenced (Fig. 2). In addition, the weight–length relationships indicated similar patterns ($P < 0.01$) for both groups: $BW = 0.016 \times FL^{3.059}$ ($r^2 = 0.983$) and $BW = 0.017 \times FL^{3.065}$ ($r^2 = 0.967$) in wild and hatchery stock, respectively (Fig. 3).

DISCUSSION

The proper management of individuals as broodstock is essential for stock enhancement. The Hiroshima City Marine Products Promotion Center used 51 black sea bream as broodstock to produce juveniles for stocking at Hiroshima Bay, releasing around 1 million seedlings annually. To understand the importance of this resource in the economy of the Prefecture, it is necessary to assess the contribution of the breeders as well as monitor possible effect of releasing their juveniles.

Using seven microsatellite DNA markers, 69.3% of the offspring were properly assigned to their true parents. The value is similar to the 73.5% reported for *Pagrus major* with only four microsatellite

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Table 4 Results of pedigree tracing and estimation of the inbreeding coefficient in 2000 and 2001

Matching to	Offspring 2000	Offspring 2001
One parental couple	46 (69.7%)	69 (69.0%)
Two or more parental couples	13 (19.7%)	23 (23.0%)
One parent alone	6 (9.1%)	6 (6.0%)
One sex couple	1 (1.5%)	2 (2.0%)
Number of families observed	36 (5.6%)	40 (6.3%)
Number of contributors		
Female (Nf)	15 (51.7%)	14 (48.2%)
Male (Nm)	17 (77.2%)	16 (72.7%)
Total	32 (62.7%)	30 (58.8%)
Ne (unequal sex proportion) = $4 \times Nf \times Nm/(Nf + Nm)$	31.9	29.9
Variance of offspring per parent		
Per female (V_{kf})	3.9	13.1
Per male (V_{km})	5.0	9.8
Ne^* (unbalanced family size) = $8 \times Ne/(4 + V_{kf} + V_{km})$	19.8	8.9
Inbreeding coefficient ($F = 0.5 \times Ne^*$)	0.025	0.056

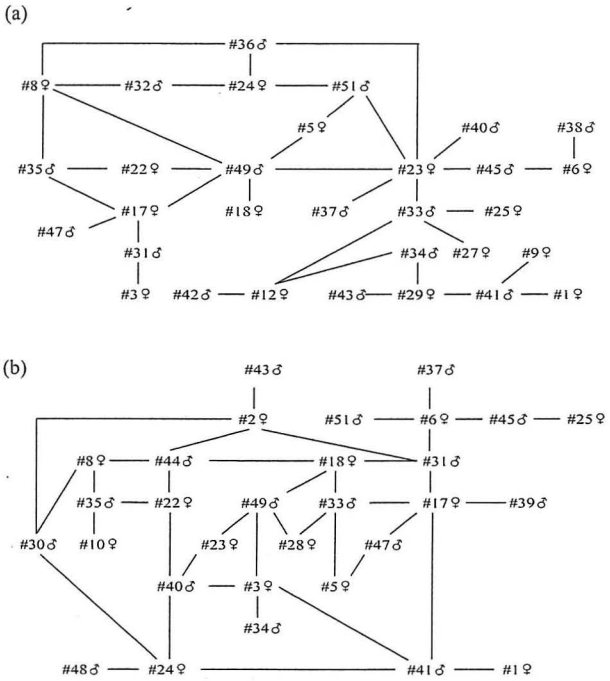

Fig. 1 Reconstructed mating model of candidate parent black sea bream in (a) 2000 and (b) 2001 using seven microsatellite loci. Specimen numbers for individual fish are shown.

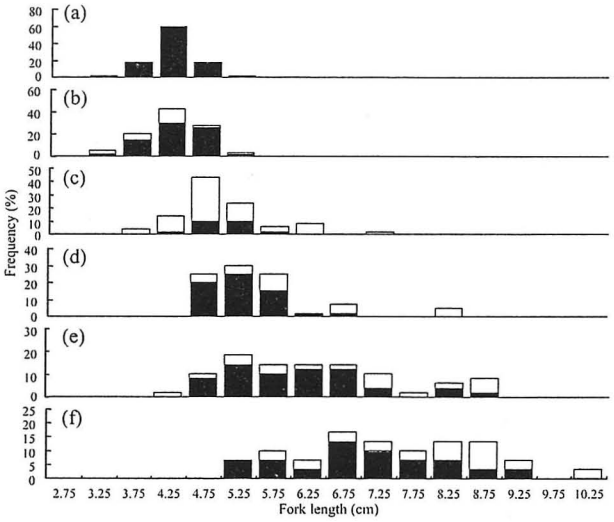

Fig. 2 Changes in fork length (cm) distribution between hatchery (■) and wild (□) black sea bream for (a) hatchery stock, and on (b) 30 July 2001, first day after release (DAR), (c) 7 August, eighth DAR, (d) 22 August, 23rd DAR, (e) 5 September, 37th DAR, and (f) 28 September, 61st DAR.

DNA markers.[15] However, the success in matching broodstock–offspring of these two sparidae are lower than those reported in Atlantic halibut *Hippoglossus hippoglossus* or Japanese flounder *Paralichthys olivaceus*.[8,16] In both flatfish species, the number of locus and alleles scored fewer mating broodstock, and were similar to those obtained in the present study. Despite the strong discrimination ability screened by the seven loci, the use of extra loci with hyperpolymorphism is necessary in order to assign all offspring to a single parent couple.

In 2000, 36 families were comprised of 62.7% (32 fish) of the breeders, rising to 40 families from 58.8% (30 fish) in 2001. Offspring were assigned to 90.9% of the sires, in contrast to only 69% of the dams within the two years. In Japanese flounder, 50 and 57% of the broodstock were observed to contribute into the mating process.[7,16] However, a single sire contributed to 99% of offspring production.[16]

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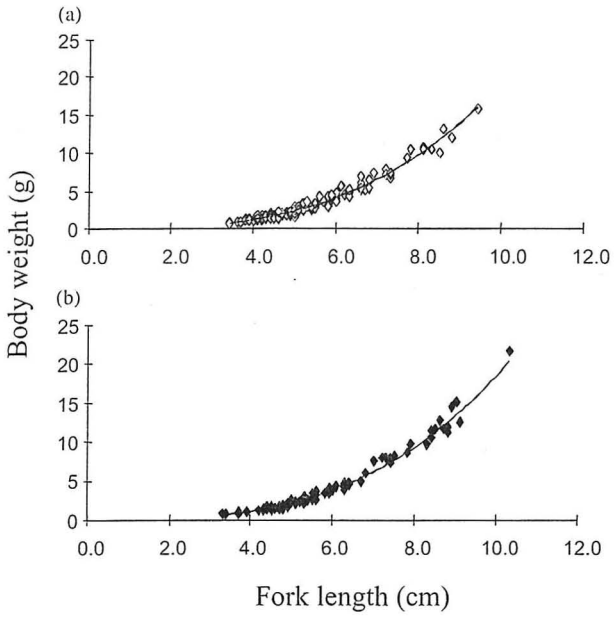

Fig. 3 Relationships between fork length (cm) and body weight (g) of (a) wild (◇) and (b) hatchery (◆) black sea bream after release. Wild $BW = 0.016 \times FL^{3.059}$, $R^2 = 0.983$; hatchery $BW = 0.017 \times FL^{3.065}$, $R^2 = 0.967$.

Working on black sea bream, Taniguchi *et al.*[5] reported that only 15–25% of the 102 breeders contributed to the first offspring generations based on the analysis of 42 isozyme loci. In their results,[5] the percent of candidates for mating was much lower than that observed in the present study. An explanation for the difference in contribution rate might be reflected in the discrimination power of the markers between isozyme and microsatellites. Other factors may be attributed to difference in background and rearing techniques of breeders. For instance, Taniguchi *et al.*[5] observed the mating process using only wild captive black sea bream. In contrast, our observation was conducted with a mix of hatchery and wild captive fish. Further, imperfect dietary or nutritional conditions for broodstock in the 1980s might have negative effects for reproduction.

The proportion of candidate breeders for reproduction in the present study is also much higher than the 30% reported in red sea bream.[15] This difference may be reflected in mating behavior in sparidae species. Mating in red sea bream takes place in the evening, postponed to around midnight in black sea bream. Nocturnal mating might have the advantage of reducing artificial stress in hatchery conditions.

Variations in the family sizes reduced the final effective population sizes (*Ne*) to 19.8 and 8.9 in 2000 and 2001, respectively. These values are much lower than the minimum 50 suggested by FAO[28] to maintain genetic diversity in short-term programs. Using 250 individuals as broodstock, the final $Ne^* = 63.7$ that was scored in red sea bream[15] reflects the necessity of using large numbers of breeders to maintain genetic diversity. At the other extreme, $Ne^* = 3.3$ in Japanese flounder,[16] confirming the negative consequences of mating low numbers of individuals and the potential risk of genetic drift in case of stocking juveniles into natural conditions.

The small effective population sizes obtained for black sea bream caused a final inbreeding depression of 2.5 and 5.5% in 2000 and 2001, respectively. This problem was already detected in the three flatfish species, Atlantic halibut,[8] Japanese flounder,[16] and barfin flounder.[29] Alarcón *et al.*[12] remarked that protandric hermaphrodite species tend to present lower effective population sizes, increasing the inbreeding depression. In red sea bream, the final inbreeding level using 250 breeders was 0.8%,[24] suggesting that few broodstock, in addition to founder effects, are the main factors producing inbreeding problems observed in black sea bream.

Norris *et al.*[6] referred to the small population size and poor management practices as the main factors to explain the loss of genetic diversity in hatchery strains. Taniguchi *et al.*[5] pointed out the loss of genetic diversity detected by heterozygosity levels through parentage analysis using allozyme markers.[5] However, parameters for loss of genetic diversity using microsatellite markers may be reflected in the allelic diversity. Black sea bream presented similar levels of heterozygosity as other marine fish species,[7,13,30] and remarkable losses of alleles (e.g. 16.9%) in the present study was evident with unchanged heterozygosity. In contrast to red sea bream,[24] using the same one-day egg collection method, no reduction in the frequencies of the major alleles was observed. Although it should be carefully monitored, this finding might indicate there are no limitations of this methodology in our target species.

In the present study, average recapture rate accounted for 58.8% of the catch during the two months after release at Daio Bay in Ninoshima Island. This level had already been documented using external tags.[2,3] However, the rates in the two initial recapture trials fluctuated from 25% at the ninth to 76.9% at the first DAH. We observed that after release, hatchery fish formed their own school separate from the wild fish. This behavior might lead to maldistribution and distortion in recapture rates between wild and hatchery fish. Recapture rates stabilized after the 24th DAR (e.g. 70% at 24th, 67.3% at 38th, and 60% at 61st DAR), suggesting the schools blended when the hatchery fish acclimated to the new environment.

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In Japan, great effort has been made to improve hatchery production, despite scarce data available regarding the effectiveness of the stocking programs.[31] In Kagoshima Bay,[1,31] stocked red sea bream represented 54% of the commercial landings. Imai[32] reported only 8–12% of specimens were hatchery stock in Kanagawa Prefecture. However, there is evidence that released red sea bream could not be identified in Kochi Prefecture.[24] In Australia, 12% of sampled *Acanthopagrus butcheri* was identified as hatchery origin.[33] Very low recapture rates were also reported in *Sparus aurata*, the main sparidae species for aquaculture in Mediterranean countries.[34] The congruence of several factors such as good release techniques, fast metabolic adaptation, species ability to remain in the releasing and/or birth place, low fishing pressure, and high nutritional levels at the release area seem to explain the high survival of black sea bream in Hiroshima Bay. The similarities in the growth and size distribution between released and wild fish may partly support these hypotheses.

This experience demonstrates that black sea bream represents a very interesting species for stocking, with fast acclimation to the natural environment. Alarcón *et al.*[12] found that the success of gilthead sea bream *Sparus aurata* for aquaculture resided in the high levels of heterozygosity derived from the combination of molecular, demographic, and evolutionary factors. The results obtained in the present study might also confirm the success of black sea bream for domestication. However, the loss of alleles and the inbreeding problems derived from low numbers of breeders suggest the necessity to reconsider the stocking strategy. Increasing the number of breeders, collecting eggs from different days, and a posterior long-term genetic monitoring assessment might contribute to reduce the genetic drift. It would help to understand the reproductive behavior of this species in order to properly manage the fishery of this important resource in Hiroshima Bay. Considering the importance of stocking programs in Japan and the low number of successful cases reported,[1] the results obtained in this study are a useful insight into black sea bream.

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APPENDIX

Allele frequencies of broodstock and offspring in 2000 and 2001 at seven loci

ACS3				*ACS4*				*ACS6*				*ACS9*				*ACS16*				*ACS17*				*ACS21*			
bp	Br	2000	2001	bp	Br	2000	2001	bp	Br	2000	2001	bp	Br	2000	2001	bp	Br	2000	2001	bp	Br	2000	2001	bp	Br	2000	2001
81	0.010	0	0.014	63	0.078	0.081	0.151	96	0.108	0.132	0.137	73	0.010	0.007	0.009	91	0.010	0.007	0.028	126	0.049	0.017	0.063	88	0.098	0.114	0.083
83	0.020	0.066	0.033	65	0.010	0	0.009	98	0.343	0.338	0.382	77	0.029	0.037	0.024	93	0.108	0.132	0.118	140	0.010	0	0.010	90	0.176	0.182	0.201
87	0.039	0.037	0.009	67	0.059	0.081	0.061	100	0.049	0.066	0.104	79	0.039	0	0	95	0.137	0.162	0.118	142	0.049	0.059	0.043	94	0.088	0.045	0.054
89	0.020	0.044	0.024	69	0.196	0.184	0.179	102	0.186	0.191	0.132	81	0.137	0.191	0.146	97	0.206	0.235	0.217	144	0.118	0.119	0.077	96	0.069	0.068	0.064
91	0.049	0	0.042	71	0.167	0.243	0.156	104	0.118	0.125	0.075	83	0.029	0	0	99	0.020	0.029	0.019	146	0.118	0.127	0.115	98	0.098	0.091	0.108
93	0.343	0.294	0.340	73	0.304	0.162	0.250	106	0.176	0.140	0.160	85	0.098	0.066	0.160	101	0.098	0.074	0.047	148	0.147	0.186	0.159	100	0.010	0.008	0
95	0.137	0.118	0.156	75	0.147	0.184	0.170	108	0.010	0.007	0	87	0.020	0.059	0.028	103	0.078	0.007	0.038	150	0.108	0.093	0.091	102	0.333	0.417	0.382
97	0.039	0.051	0.028	77	0.039	0.066	0.024	114	0.010	0	0.009	89	0.098	0.103	0.175	105	0.069	0.044	0.066	152	0.010	0	0	104	0.010	0	0
99	0.098	0.140	0.142									91	0.176	0.235	0.255	107	0.029	0.029	0.047	154	0.020	0.051	0.063	110	0.069	0.061	0.093
101	0.049	0.118	0.066									93	0.069	0.059	0.047	109	0.069	0.029	0.019	156	0.010	0	0	112	0.010	0	0.005
103	0.010	0.022	0.042									95	0.078	0.074	0.061	113	0.010	0	0	158	0.069	0.059	0.043	118	0.010	0	0.010
105	0.010	0	0									97	0.010	0.015	0	115	0.127	0.206	0.245	162	0.147	0.136	0.139	122	0.029	0.015	0
111	0.078	0.066	0.080									99	0.029	0.037	0.005	121	0.020	0.029	0.014	166	0.137	0.136	0.188				
113	0.010	0	0									101	0.049	0.066	0.042	123	0.010	0.007	0	172	0.010	0.017	0.010				
115	0.020	0.015	0.019									105	0.010	0.007	0	125	0.010	0.007	0.024								
123	0.010	0.007	0									109	0.039	0.007	0.009												
125	0.049	0.015	0.005									113	0.069	0.007	0.005												
127	0.010	0.007	0									121	0.010	0.029	0.033												

Br, Broodstock; 2000, offspring in 2000; 2001, offspring in 2001.

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