

## **Studies on genetic evaluation of black sea bream *Acanthopagrus schlegeli* in stock enhancement**

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The utility of microsatellite (MS) loci was examined as a useful genetic marker and the genetic assessment in black sea bream released into Hiroshima Bay, whether the release project would be effective in fishery management.

### **1. Genetic variability between wild and hatchery-stock using DNA fingerprinting**

Genetic variability based on the band sharing indices (BSI) was higher in wild population collected from Miyajima and Ninoshima Islands, than those from hatchery-stocked seeds order of the full-sib progeny. The BSI of hatchery-stocked offspring was slightly higher than those of wild population. However, an obvious difference in the BSI observed between hatchery-stocked seeds and full-sib progeny. These results suggested that influence of genetic variability in stocking seeds might be maintained in Ninoshima Island.

### **2. Isolation of MS loci**

Eleven MS loci were isolated from the muscle and caudal fin of black sea bream. Allelic inheritance mode of MS was examined using the single spawning pair and their juveniles. *Acs1\** and *Acs8\** loci had only one genotype. *Acs5\** locus ( $p > 0.05$ ,  $\chi^2 = 7.815$ ,  $df = 3$ ) did not fit the Mendelian inheritance, the other loci showed hetero-genotype and well followed the Mendelian inheritance.

### **3. Genetic diversity and structure**

Four MS loci, *Acs1\**, *Acs3\**, *Acs4\** and *Acs9\**, were used to investigate genetic diversity and population structure in black sea bream collected from six locations (western Japan and southern Korea) in addition to hatchery-stock and post stock population at Hiroshima Bay. The genetic variation of these loci in eight populations revealed high variability of heterozygosity (0.521 to 1.000). In the wild population, mean number of alleles per locus was from 10.8 to 13.5 and the expected heterozygosity was from 0.774 to 0.826, while hatchery-stock population and post stock population were 10.0 and 11.2, 0.776 and 0.775, respectively. According to the Nei's genetic distance and statistics  $F_{ST}$ , geographical separation was not found among the western Japan populations. The relatively small genetic distance between hatchery-stock population and post stock population showed possibilities as a genetic marker of black sea bream.

### **4. Pedigree tracing of a hatchery stock**

The mean observed heterozygosity was not different between breeders and offspring. The mean number of alleles per locus decreased less than 16.5%. For 69.3% (69.7% in 2000 and 69.0% in 2001) of the offspring one, parental couple was determined by pedigree tracing analysis. The actual contribution rate was 69.0% for females and 90.9% for males over two years, however more than 30% of females did not participate to spawning in either year. The effective number of contributors ( $Ne^*$ ) was 19.8 in 2000 and 8.9 in 2001. These  $Ne^*$  gave an inbreeding coefficient ( $F$ )

of 0.025 and 0.056.

### **5. Stock separation using MS DNA**

Totally 199 samples, 117 (58.8%) black sea bream were adjudged to be hatchery-stock collected from Ninoshima area. MS markers as a genetic tag are useful for the estimation of recovery from the wild environment. Application of the MS marker showed the same growth rate of hatchery stock with the wild population in Hiroshima Bay.

Consequently, MS-DNA loci proved potential tools as a great genetic marker for the genetic assessment of black sea bream released into Hiroshima Bay. Genetic structure of black sea bream in western Japan was not found geographically separated population.

As the mass production of black sea bream offspring for release project, the heterozygosity was not different between breeders and offspring. And heterozygosity as a genetic indicator to assess genetic variability was shown genetic similarity with the wild population, indicating that there was no influence on genetic differentiation of wild population in Hiroshima Bay. However, the mean number of alleles per locus decreased less than 16.5%. Therefore, black sea bream produced from the limited number of parents were released into the natural environment might induce depletion of genetic diversity in wild population.

In pedigree tracing analysis, the actual contribution rate was 69.0% for females and 90.9% for males over two years. More than 30% of females did not participate throughout two years. Therefore, the number of broodstock, egg collection frequency during spawning season and induction of new broodstock should be considered to conserve genetic variability of wild population.

Totally 117 (58.8%) black sea bream were adjudged to be released stock among 199 samples caught from Hiroshima Bay, indicating effectiveness of stock project.

In the studies, MS maker showed the potential tool for genetic evaluation of black sea bream offspring. Therefore, all of the stocked offspring for stock enhancement program should be done with the appreciation of genetic divergence.

**Key words:** black sea bream, *Acanthopagrus schlegeli*, microsatellite, genetic diversity, stock enhancement