

Managing the Genetic Resources in the Intensive Stock Enhancement Program Carried out on Black Sea Bream in Hiroshima Bay, Japan

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1. Introduction

The establishment of sustainable fishery management strategies requires detailed characterization of the stocks, including their genetic diversity and structure (Allendorf and Ryman, 1987; Ryman, 1981). Traditionally, the large population size, wide distribution and the potential high mobility during the pelagic eggs and larval phase was presumed to explain the lack of genetic differentiation and population structure found in marine species (Hauser & Carvalho, 2008; Ward et al., 1994). Natural selection and high gene flow were considered the main evolutionary forces affecting genetic structure of marine organisms. However, recent genetic studies on marine fish have evidenced population structure at different geographical and temporal scales (Knutsen et al., 2003; Umino et al., 2009; Watts et al., 2010). This information is especially relevant to preserve the genetic identity of wild stocks and minimize the negative genetic interaction between wild and hatchery specimens from escapees and stock enhancement programs (Blanco Gonzalez & Umino, 2009; Glover et al., 2010). Imprecision or lack of genetic information may not only exacerbate problems that affect yields of fisheries but also erode the gene pool and the potential adaptive response of the stock in an irretrievable manner (Laikre et al., 2010; Reiss et al., 2009; Ward, 2006). Despite its importance for conservation and management, it has not been until recently that marine stock enhancement programs started integrating genetic analysis and monitoring data prior to, during and after release (Bert et al., 2001; Blanco Gonzalez et al., 2008a). Meanwhile, our knowledge about the genetic resources of commercially-farmed stocks for their identification in case of escapees is still very scarce (Glover et al., 2010; Svåsand et al., 2007).

Microsatellite DNA markers generally exhibit high levels of genetic polymorphism and are a priori presumed to behave as neutral markers, i.e. the effects of selection are neglected. Consequently, genetic changes among stocks will be explained by their origins and population demography processes; mainly by gene flow and genetic drift, and mutation to some extent (Luikart et al., 2003). Microsatellites have been the most common genetic marker employed in stock enhancement programs, being extensively used for delineating the genetic diversity and population structure of the species prior to and after the release

(Blanco Gonzalez & Umino, 2009; Jeong et al., 2003; Perez-Enriquez et al., 2001), assigning individuals to baseline hatchery stocks (Blanco Gonzalez et al., 2008a; Ortega-Villaizán et al., 2005) or tracing the pedigree to manage the captive broodstock (Blanco Gonzalez et al., 2010; Jeong et al., 2007; Perez-Enriquez et al., 1999).

Black sea bream *Acanthopagrus schlegelii* is an important commercial and sport fishing species in Japan and represent one of the most intensively stocked fish in the country with millions of juveniles released annually. The largest releases have been conducted in Hiroshima Bay (Fig. 1) which is also the primary fishing area in the country (Blanco Gonzalez et al., 2008b). The stock enhancement program in this bay started in the early 1980s after a drastic reduction of landings during the previous two decades, when fishing yields dropped from about 500 mt in 1960s to 150 by the end of 1970s. Juveniles for stocking were usually originated from fertilized eggs produced in one night during the spawning peak and collected by tank overflowing. They were reared in indoor tanks and released at 3 cm in total length. Initially, released juveniles were identified marking their otoliths with alizarin-complexon and by ventral fin-clipping (Nakagawa et al., 2000; Umino et al., 1999; Yamashita et al., 1997). Using non-genetic markers, it was possible to investigate the acclimation process (Nakagawa et al., 2000; Yamashita et al., 1997), migration (Anonymous, 1987) and optimum size-at-release (Umino et al., 1999). Later on, the development of microsatellite DNA markers (Jeong et al., 2003, 2007) has contributed to address fundamental questions regarding the effects of the releases on the genetic resources of the natural stock.

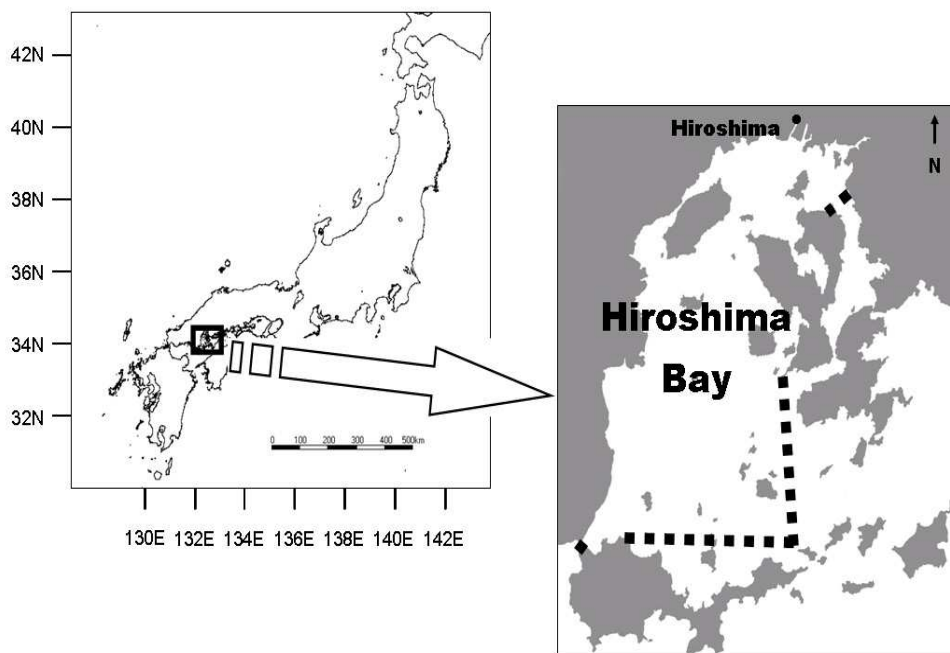


Fig. 1. Map of Hiroshima Bay.

In addition to providing a better estimation of the contribution to the fishing yield; microsatellite genotyping helped to characterize the genetic architecture of black sea bream and minimize the potential deleterious effects of fish releases by improving broodstock management. This paper reports on the progress of using microsatellite DNA markers to gain understanding of the genetic implications of the stock enhancement program carried out on black sea bream in Hiroshima Bay.

2. Genetic risks associated to releases

Stock enhancement programs have been implemented as a mean to recover depleted populations from many taxonomic groups worldwide (Blanco Gonzalez et al., 2008b; Laikre et al., 2010). However, the limited number of breeders reared to produce the offspring for release and the differential population origins have raised awareness about harmful loss of genetic diversity and changes in allele composition that large-scale releases may cause on the native stocks (Allendorf and Ryman, 1987; Laikre et al., 2010; Ryman, N. 1981). The relative large population size and higher dispersion reported in marine fish compared to anadromous and freshwater species favors gene flow and reduces genetic differentiation between stocks (DeWoody & Avise, 2000; Ward et al., 1994). Hence, freshwater and anadromous species are more vulnerable to the harmful genetic effects of large-scale releases (Cross, 1999; Hauser & Carvalho, 2008). Nevertheless, genetic risks on marine species should not be underestimated.

2.1 Genetic diversity among-populations

The first step to be accomplished before implementing a stock enhancement program is to delineate the genetic architecture of the recipient population (Allendorf & Ryman, 1987; Ryman 1981). Whenever possible, the broodstock should be of local origin. Non-local breeders may carry alleles that were previously absent in the wild. Interbreeding of their offspring with wild specimens can replace the original gene pool by genotypes that are locally non-adapted; thus, affecting survival, growth or disease resistance, and ultimately compromising the viability and productivity of the stock (Cooke & Philipp, 2006; Laikre et al., 2010). Sometimes, interbreeding of genetically divergent stocks may result in hybrid vigor in the F1 generation (Shikano & Taniguchi, 2003) that may evolve into outbreeding depression and breakdown of co-adapted gene complexes after the second generation (Cross, 1999).

2.2 Genetic diversity within populations

The broodstock should comprise enough specimens to accurately represent the genetic identity of the stock to be enhanced (Allendorf & Ryman, 1987; Taniguchi, 2003). This number is crucial because the gene pool present in the broodstock will determine the maximum genetic diversity that may be inherited by the offspring (Allendorf & Ryman, 1987). Therefore, efforts should be directed towards avoiding loss of genetic diversity or changes in the genetic composition caused by genetic drift, selection due to domestication and inbreeding depression.

2.2.1 Genetic drift

Genetic drift is a stochastic process that changes allele frequencies in the next generation. The loss on genetic variation may be reflected in the heterozygosity, proportion of

polymorphic loci and number of alleles per locus (Allendorf & Phelps, 1980) and will increase exponentially to the reduction on the population size (Nei et al., 1975). Ideally, in order to maximize the transference of the gene pool to the offspring and minimize a potential reduction on fitness, broodstock management should aim at keeping the sex ratio between male and female at 1:1 and ensuring that all breeders kept in the hatchery contribute equally to the offspring (Allendorf & Ryman, 1987). Under this ideal situation, the effective number of breeders (N_b) will double the broodstock size, while skewed sex ratio or variance in family sizes will reduce N_b . Allendorf & Ryman (1987) reported that keeping N_b at 50 will lead to the loss of approximately 10 % of the genetic variation after ten generations, a fact that may have important deleterious effects on survival and growth (Falconer, 1981). In hermaphroditic species, including some sea breams, collecting mature breeders will prevent from posterior undesirable sex changes that may skew the proportion of males and females (Cross, 1999).

2.2.2 Domestication

Domestication selection tends to favor genetic profiles better adapted to the hatchery conditions. Good broodstock management practices contribute to maximize the transference of genetic information to the offspring; however, domestication selection may change or reduce the genetic variability at some loci and erode the adaptive potential of the stock (Taniguchi, 2004). In stock enhancement programs, it is noteworthy to consider that the direction and intensity of local selective forces between hatchery and wild environments are likely to differ; hence, the longer the fish are reared in captivity the greater effects of domestication selection will be (Araki & Schmid, 2010; Bekkevold et al., 2006; Milles & Kapauscki, 2003). Good survival and fitness under hatchery conditions may evolve into poor performance once the juveniles are released into the wild. In this regard, most of studies suggest that unintentional selection domestication produces negative effects on fitness-related traits including survival, morphology, behavior, response to predation or disease resistance, and that ultimately can compromise the reproductive success and the viability of the stock (see reviews by Araki & Schmid, 2010; Fraser, 2008; Reisenbichler & Rubin, 1999; Thorstad et al., 2008).

2.2.3 Inbreeding depression

Inbreeding depression represents the reduction on fitness produced by breeding related specimens. Initially, the ratio of homozygous genotypes will be augmented. Consequently, harmful recessive alleles that were unexpressed under heterozygosity will be exposed to selective forces that will reduce fitness. As relatives are more likely to carry the same rare deleterious alleles, inbreeding may increase the occurrence of harmful effects on fitness (Lynch, 1991). Several empirical studies on fish species have suggested inbreeding to be associated with morphological abnormalities, slow growth or low reproductive success (Araki et al., 2009; Kincaid, 1983; Shikano & Taniguchi, 2003). Chances of mating relatives will increase proportionally to the population size and scale of the releases. Hence, stock enhancement programs should keep a large wild-born broodstock and foster mating schemes that maximize N_b . Once released into the wild, in addition to contributing to the fishing yield, a portion of the offspring is expected to interbreed with their wild counterparts (Bell et al., 2008). Ryman & Laikre (1991)

warned that in a successful stock enhancement program, the increment in the portion of hatchery-reared offspring may reduce the total effective population (wild and hatchery) and favor inbreeding. However, in their review of marine fish stocking programs, Kitada et al. (2009) found no evidence of long-term negative effects of large-scale releases on fitness in the wild population. Furthermore, the minimal kinship approach (Doyle et al., 2001), collecting several batches of eggs over the spawning season (Nugroho & Taniguchi, 2004) or at different time intervals over a single night (Blanco Gonzalez et al., 2010) have proven promising results to increase N_b and minimize the loss of genetic diversity.

3. Genetic resources of Black sea bream in Hiroshima Bay

The success of any stock enhancement program greatly depends on the accurate identification and characterization of the genetic architecture in the population to be managed (Allendorf & Ryman, 1987; Ryman, 1981). Unfortunately, by the time the stock enhancement program in Hiroshima Bay started, information on the genetic resources of black sea bream in Japan was very scarce and limited to other regions (Sumantadinata & Taniguchi 1982; Taniguchi et al., 1982, 1983). In fact, it was not until 1997, when almost twenty million juveniles had been released already (Fig 2.), that the first genetic analysis of samples collected from Hiroshima Bay was carried out by minisatellites (Jeong et al., 2002). Consequently, due to the lack of genetic information prior to the commencement of the stock enhancement program and the large number of juveniles released, the evaluation of the genetic impact of the releases has focused on the stock inhabiting Hiroshima Bay at the time of the research rather than on the original native stock.

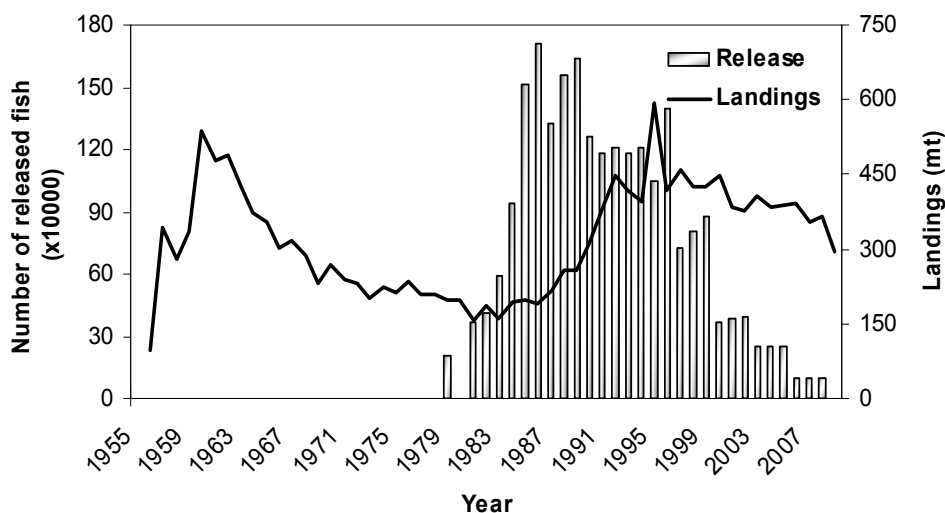


Fig. 2. Annual fluctuation in released and landed black sea bream in Hiroshima Bay.

In contrast to minisatellites, microsatellite markers estimate allele frequencies at a given locus (Estoup & Agners, 1998) hence are very useful for studies on population genetics. In order to characterize the gene pool of black sea bream in Hiroshima Bay and evaluate the effectiveness and genetic implications of the stock enhancement program carried out in the bay, eight highly polymorphic microsatellite markers were developed in our laboratory (Jeong et al. 2003, 2007). This set of microsatellites has been used at different stages of the stock enhancement program (Table 1).

3.1 Genetic diversity and population structure

Initially, four microsatellites were genotyped to assess the genetic divergence and population structure of black sea bream collected at six locations in Western Japan and Korea (Jeong et al., 2003). Despite the large stocking histories, the genetic diversity of the natural stock from Hiroshima Bay, expressed either as number of alleles per locus (6-20) or observed heterozygosity (0.65-0.96), was very high and similar to the wild stocks from other locations (Jeong et al., 2003).

Between 2000 and 2002, the offspring released by the Hiroshima City Marine Products Promotion Association (HCMPPA) were produced by only 51 breeders (Blanco Gonzalez et al., 2008a; Jeong et al., 2007). In spite of its small size, levels of genetic variation in the broodstock were similar to the natural stock from Hiroshima Bay (Table 1). In contrast, rare alleles at the loci presenting the highest polymorphism were missing in the offspring prior to the release (Jeong et al. 2003, 2007); warning about genetic drift associated to the differential contribution among parental fish, as reported during the seed production of other species commonly used in stock enhancement programs (Nugroho et al. 2000; Sekino et al., 2003; Sugama et al., 1988).

Sample origin	Hatchery (%)	Number of alleles (loci)	Observed Heterozygosity	Reference
Natural 2000	–	6-20 (4)	0.65-0.96	Jeong et al., 2003
Broodstock 2000-2002		8-18 (7)	0.74-0.92	Jeong et al., 2007; Blanco Gonzalez et al., 2008a
Pre-release 2000	100	6-16 (4)	0.57-1.0	Jeong et al., 2003
Pre-release 2000	100	7-16 (4)	0.76-0.92	Jeong et al., 2007
Pre-release 2001	100	7-14 (7)	0.74-0.92	Jeong et al., 2007
Post-release 2001	50*	7-17 (7)	0.52-1.0	Jeong et al., 2003
Post-release 2003	12.5	8-20 (6)	0.77-0.90	Blanco Gonzalez et al., 2008a
Post-release 2004	13.5	7-17 (6)	0.84-0.90	Blanco Gonzalez et al., 2008a
Post-release 2006	–	7-24 (6)	0.61-0.98	Blanco Gonzalez & Umino, 2009

* Proportion assumed based on tag-recapture data

Table 1. Studies on the genetic diversity of black sea bream in Hiroshima Bay

Once released, stock enhancement programs aim at maximizing fitness performance and minimizing negative genetic or ecological interactions between wild and hatchery fish. Several experiments using non-genetic markers indicated high survival and fast acclimation of black sea bream juveniles released in Hiroshima Bay (Ji et al., 2003; Nakagawa et al., 2000; Umino et al., 1999; Yamashita et al., 1997). However, limitation of food resources related to the stock enhancement program was suggested to explain the reduction on size-at-age recorded on the adults collected in 2000 compared to 1983 (Blanco Gonzalez et al., 2009). Genetically, levels of diversity 10 days after the release were similar to those observed in the natural stock (Jeong et al., 2003). Meanwhile, given the small sample sizes ($n = 10$ and 14 specimens in 2003 and 2004, respectively), the lower number of alleles scored in the fish collected three and four years after the release were not conclusive about any erosion due to fish stocking (Blanco Gonzalez et al., 2008a). In order to deduce the putative origin of the samples, a population assignment test was performed using WHICHRUN (Banks & Eichert, 2000) with a jackknife procedure; choosing the first natural stock collected in Hiroshima Bay whose genotype was characterized (Jeong et al., 2003) and the broodstock reared at the HCMPPA between 2000-2002 (Blanco Gonzalez et al., 2008a) as baseline populations (Fig. 3). Most of the fish collected in 2003 (82.5%) and 2004 (84.6%) were assigned to the broodstock; nevertheless, about 60% of them remained within the two dashed lines indicating deviation from equality by a factor of 10. Consequently, although these results supports the above mentioned idea of high survival and contribution of juvenile releases, the small differences in the genotype probability of the fish between the baseline populations requires a careful interpretation of the conclusions.

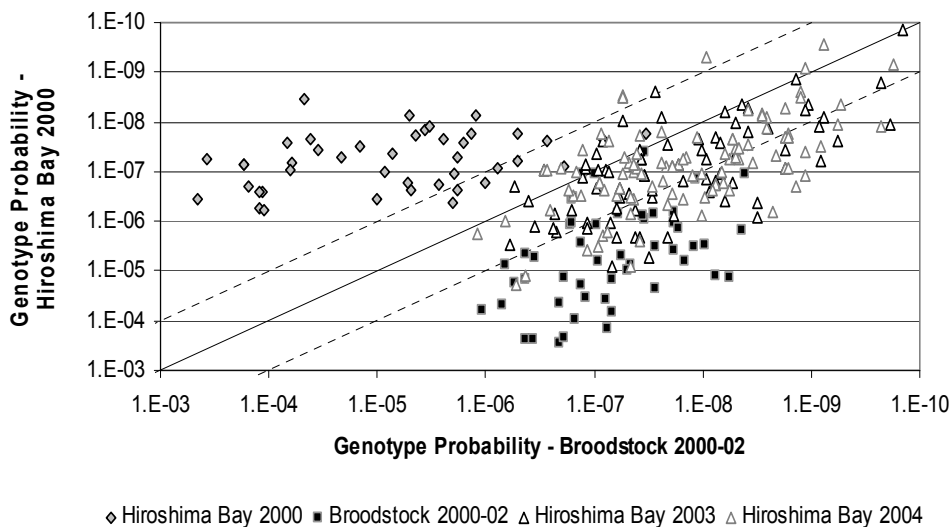


Fig. 3. Genotype assignment test to putative origin baselines (Hiroshima Bay 2000 and Broodstock 2000-02). The value of the X and Y coordinates represents the genotype probability of the fish collected in Hiroshima Bay in 2003 and 2004 for each baseline plotted on a log scale. The solid line represents equal probabilities of belonging to each baseline populations and separates the region of the graph where the probability is assigned to Hiroshima Bay 2000 (above) and Broodstock 2000-02 (below). The dashed lines deviate from equality by a factor of 10.

The genetic integrity of black sea bream within Hiroshima Bay and the existence of certain population structuring related to the stock enhancement program was evaluated genotyping samples collected from five locations at six microsatellites (Blanco Gonzalez & Umino, 2009). At Ninoshima, the location where stocking was most intense, the lowest number of alleles was scored. Moreover, an initial evaluation of the pairwise F_{ST} value suggested genetic differences between black sea bream from the western and eastern part of Hiroshima Bay. However, the differences disappeared once the analysis was performed standardizing the age class composition of the samples and black sea bream evidenced high genetic homogeneity among all locations, suggesting that the effects of the stock enhancement program were extensive over Hiroshima Bay.

3.2 Individual assignment and parental contribution

The polymorphism of the genetic markers determines their ability to correctly assign each offspring to a parental couple and facilitates the posterior identification once recaptured. The microsatellite DNA markers developed for black sea bream were highly polymorphic (Table 1) and exhibited high exclusion power and good performance for parentage analysis and pedigree reconstruction (Blanco Gonzalez et al., 2008a; Jeong et al., 2007).

The relevance of the origin and size of the broodstock to ensure a high N_b and low rate of inbreeding to preserve the native gene pools, contrasts with the high costs and space requirements of rearing a large number of breeders. In Hiroshima Bay, the broodstock kept at the HCMPPA never comprised more than 100 fish. Moreover, part of the fish was usually of hatchery origin and/or maintained to produce offspring for several years. During the period 2000-2002, the broodstock comprised 29 dams and 22 sires originated from wild captive and hatchery strains (Jeong et al., 2007). Genotyping seven microsatellites, the pedigree analysis conducted on the juveniles produced in 2000 and 2001 revealed that the proportion of breeders contributing to the offspring was 59% and 63%, respectively (Table 2), increasing to 76.5% combining both years (Jeong et al., 2007). This proportion is much larger than previously reported with isozymes, where only 15.7-25.5% of the breeders contributed to the first two generations of offspring (Taniguchi et al., 1983), likely related to the improvement in the markers performance and the broodstock management techniques. Jeong et al. (2007) found that the sex ratio among contributors maintained 1:1, however, the variance in family size due to the differential contribution among breeders reduced N_b to 20 in 2000 and 9 in 2001, and resulted in a high inbreeding coefficient, F (Table 2).

Management efforts to preserve the genetic integrity and maximize fitness performance of black sea bream should offset the potentially deleterious environmental and genetic effects (Allendorf and Ryman, 1987). Genetic drift has drastically reduced the genetic resources prior to the release (Jeong et al., 2007), eroding the adaptive potential of the juveniles. However, the large number of hatchery-reared fish identified two months (Jeong et al., 2007) and four years after the release (Blanco Gonzalez et al., 2008a) suggested no harmful effects on adaptation to the natural conditions. Moreover, rates of inbreeding of the latter group were similar to those observed before the release (Table 2) and the contribution of additional contributors was also detected (Blanco Gonzalez et al., 2008a). Although promising results were achieved, it is noteworthy drawing conclusions carefully because the small sample sizes analyzed may mask some underlying effects.

Origin	Size	Number of contributors (%)	Nb	F (%)	Reference
Pre-release 2000	70	32 (63)	20	2.5	Jeong et al. 2007
Pre-release 2001	110	30 (59)	9	5.6	Jeong et al. 2007
Post-release 2003	10	13 (26)	17	3.0	Blanco Gonzalez et al. 2008a
Post-release 2004	14	14 (28)	16	3.3	Blanco Gonzalez et al. 2008a

Table 2. Summary of parentage analysis conducted on black sea bream in Hiroshima Bay. Sample origin and size, loss of alleles, number of contributors, Nb, F and reference.

4. Conclusions

Microsatellite-based studies have provided insights into key aspects to preserve the natural gene pool and minimize any potential harmful genetic or ecological effects derived from the stock enhancement program carried out on black sea bream in Hiroshima Bay. The large number of juveniles released and their good acclimation to the natural environment have partly contributed to increase the fishing yields in the bay (Fig. 2); nevertheless, limitation for food was suggested to be responsible for the reduction in size-at-age observed two decades after the commencement of the stock enhancement program (Blanco Gonzalez et al., 2009). Interestingly, levels of genetic diversity in Hiroshima Bay were similar to other locations where stocking activities had never been conducted (Jeong et al., 2003) and black sea bream was suggested to comprise a large panmictic stock in western Japan. Reviewing the only two large-scale marine stock enhancement programs conducted worldwide over multiple generations and where data on the catches and genetic diversity of wild and hatchery-released fish have been monitored, Kitada et al. (2009) emphasized the importance of replacing the broodstock annually. The panmictic genetic structure, large population size and gene flow of red sea bream (*Pagrus major*) inhabiting Kagoshima Bay, Japan, was suggested to contribute to attenuate the genetic differentiation resulted from producing the hatchery-released offspring from the same small broodstock over several years. On the other hand, on Pacific herring (*Clupea pallasii*), they found no evidence of genetic erosion attributable to the stock enhancement programs, using a native broodstock with annual replacement. None of the programs showed any sign of fitness decline.

Genetic drift due to the limited number of breeders kept in the hatchery was identified as a major constraint to preserve the genetic resources of black sea bream in Hiroshima Bay (Blanco Gonzalez et al., 2008a; Jeong et al., 2007). The small broodstock reared at the HCMPPA provided a relatively good representation of the genetic diversity of the natural stock inhabiting Hiroshima Bay (Jeong et al., 2003); however, their differential contribution resulted in a very small Nb and high rates of inbreeding, warning about the risks of genetic erosion and the need to improve broodstock management practices (Jeong et al., 2007). In this regard, Blanco Gonzalez et al. (2010) demonstrated that a larger number of breeders and the collection of eggs at two-hourly intervals contributed to increase Nb and reduce the rate of inbreeding. The minimal kinship approach (Doyle et al., 2001) and the collection of

several batches of eggs at different days over the spawning season have also proven good results to preserve the genetic resources of stock enhancement programs on red sea bream (Nugroho & Taniguchi, 2004).

Parentage analysis confirmed that the released juveniles had good survival and growth (Jeong et al., 2007), and some of them reached maturity (Blanco Gonzalez et al., 2008a), suggesting that hatchery conditions had no negative effects on the posterior fitness performance in the natural environment. Moreover, despite the loss of genetic diversity observed before the release, black sea bream showed high genetic homogeneity within Hiroshima Bay and no sign of population structuring (Blanco Gonzalez & Umino, 2009). Given the large number of juveniles produced by a small number of parental fish and their high survival, interbreeding between natural and hatchery fish is likely to have taken place (Blanco Gonzalez et al., 2008a); hence, broodstock management strategies and genetic monitoring should aim at avoiding genetic swamping of the natural population (Ryman & Laikre, 1991).

The stock enhancement program conducted on black sea bream in Hiroshima Bay is expected to continue providing first-hand empirical information about the genetic and ecological implications of fish releases in marine ecosystems. In 2008, due to several socio-economical problems (Umino et al., 2011), the program was stopped. Therefore, future studies will deepen our understanding of the evolutionary processes underlying large-scale releases and how to preserve the genetic resources. Indeed, genetic diversity at selective markers should insight into the genetic component involved in juvenile survival and local adaptation.

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6. References

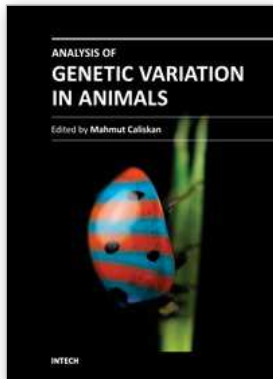
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Analysis of Genetic Variation in Animals includes chapters revealing the magnitude of genetic variation existing in animal populations. The genetic diversity between and within populations displayed by molecular markers receive extensive interest due to the usefulness of this information in breeding and conservation programs. In this concept molecular markers give valuable information. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in animals and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation in animals by presenting the thoughts of scientists who are engaged in the generation of new idea and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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