Stock enhancement program for black sea bream (*Acanthopagrus schlegelii*) **in Hiroshima Bay: monitoring the genetic effects**

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Abstract

Black sea bream have been intensively stocked in Hiroshima Bay since 1982. However, no information regarding the long-term effects resulting from the release of large numbers of juveniles on the wild population has been reported. We investigated the fate of black sea bream juveniles released at the sampling site from 2000 to 2001 using six microsatellite DNA markers. In 2003 and 2004 respectively, 12.5 and 13.5% of captured black sea bream were identified as hatchery reared. Variation in total length by age between the released and native stocks was not statistically significant. Low F_{ST} values confirmed high genetic similarity between wild and hatchery stocks. Pedigree reconstruction confirmed a small loss of genetic variability, with a final inbreeding rate for recaptured fish of around 3% per year, rising to 4% when both years were combined. There was high survival of released black sea bream juveniles. Some of these specimens reached maturity and may have hybridized with their wild counterparts, highlighting the need to monitor the genetic effects of releasing large number of juveniles.

Keywords: black sea bream, *Acanthopagrus schlegelii*, stock enhancement, genetic diversity, pedigree, microsatellite DNA

1. Introduction

Enhancement programs have been proposed as a means to increase the biomass of depleted fishery stocks (Tringali and Bert, 1998; Liao, 1999). The contribution of releasing fingerlings into natural environments has been widely examined through external tagging studies. However, the loss of such tags and the slower growth rates of released individuals compared to their wild counterparts often limits the usefulness of such studies for long term population assessments (Blankenship and Leber, 1995; Støttrup et al., 2002; Sánchez-Lamadrid, 2004).

Recently, concerns about the possible genetic effects of released have become a focal point when evaluating the effectiveness of enhancement programs. Of particular concern is the possibility of genetic drift derived from releasing hatchery reared fish (Tringali and Berth, 1998; Pérez-Enriquez et al., 1999; Ortega-Villaizán et al., 2006). The necessity of using large numbers of non-related wild individuals, with a proximate genetic composition to the wild stock, as breeders has been also emphasized (Taniguchi, 2004). Long-term monitoring was also proposed as a routine practice (Blankenship and Leber, 1995; Liao, 1999). Araki et al. (2007) reconstructed a three-generation pedigree for steelhead trout reared in captivity and subsequently bred in the wild. They reported a significant reduction in fitness, and warned of the risk of using multiple generations of hatchery reared broodstock to supplement natural populations.

Microsatellite DNA markers are acknowledged as very powerful genetic markers for studies of population genetics, evolutionary relationships, and gene mapping (Goldstein and Schlötterer, 1999). They are also suitable for monitoring changes in genetic diversity and tracing the pedigree in aquaculture operations (O'Connell and

Wright, 1997; Norris et al., 1999). The minute quantities of biological material needed, which minimizes trauma to the fish (Garcia de León et al., 1997), and their permanence as “tags”, means that the analysis of microsatellite DNA is a very useful tool for genetic studies.

Parentage assignment has been widely applied to high value commercial species under hatchery conditions by screening a small number of microsatellite DNA markers (Pérez-Enriquez et al., 1999; Norris et al., 2000; Sekino et al., 2003; Borrell et al., 2004; Jeong et al., 2007). However, the number of studies that have examined recaptured specimens is very limited. Low recapture rates have been attributed to fish mortality and dispersion (Ortega-Villaizán et al., 2005, 2006).

Black sea bream, *Acanthopagrus schlegelii*, are one of the main fishery resources in Hiroshima Bay, an area intensively subjected to intensive stocking since 1982. The effectiveness of the stock enhancement program for black sea bream in Hiroshima Bay is traditionally evaluated by measuring the increment in landings or by using external tags (Yamashita et al., 1997; Umino et al., 1999). DNA markers have been used to assess the genetic variability between wild and hatchery-stocked black sea bream (Jeong et al., 2002, 2003). Recently, after tracing the pedigree of the juveniles used in stocking, Jeong et al. (2007) evaluated their survival rate during the critical period following the release. However, no information regarding the long-term genetic impacts of releasing large numbers of fingerlings into the natural stock is available. Therefore, further studies are required in order to clarify growth and maturation rates of hatchery reared juveniles as well as their contributions to the future generations.

This paper represents the first study monitoring the effects of the stock enhancement program conducted in a sparid species by using microsatellite DNA markers. Moreover, considering that black sea bream in Hiroshima Bay become mature at 3 years of age (Kinoshita, 1936, 1939), these results suggest that some of the previously released juveniles may have become part of the spawning stock. This experience may be of high relevance for fishery management as it provides an insight of the possible genetic impact of released fish on future generations of wild stocks.

2. Materials and methods

2.1. *Fish rearing and recapture*

The broodstock comprised 29 dams and 22 sires (Br, *n*=51) originated from wild captives and hatchery strains reared at the Hiroshima City Marine Products Promotion Center (HCMPPC). Since the beginning of the enhancement program in 1982, the broodstock at the HCMPPC has been changed every three to four years.

Each year, fertilized eggs were collected on one night during the spawning peak in May, and reared in indoor tanks. The larvae were then transferred to net cages for intermediate culture at Daio Bay, a small cove off Ninoshima Island in the Seto Inland Sea of Japan (Fig. 1). Afterwards, a total of 20,000 juveniles, averaging 4 cm in total length (TL), were randomly selected for release at Daio Bay each year over a three year period (2000-2002).

During the spawning season, a total of 90 specimens in 2003 and 117 in 2004 were collected at the release site by angling (Table 1). Considering the release period, only individuals aged 1-3 years in the 2003 sample and 2-4 years in the 2004 sample

could be the previously released offspring. After aging the samples (scale-reading), the total number of potential hatchery reared offspring was adjusted to 80 and 104 for the 2003 and 2004 samples respectively. TL, body weight (BW), sex and gonadal weight (GW) were recorded for each specimen.

2.2. *DNA extraction and microsatellite PCR*

Muscle tissue from the specimens collected in 2003 and 2004 was prepared for DNA extraction by immersion in 95% ethanol and storage at 4ºC. DNA was obtained following the standard SDS-phenol/chloroform procedure described by Taggart et al. (1992). The amplification was performed by the PCR using six loci (*ACS*3, *ACS*4, *ACS*6, *ACS*9, *ACS*16, *ACS*17) isolated for the same species. The total reaction volume was 10 µl, according to the *TaKaRa Ex Taq*™ kit (TaKaRa BIO INC). The reaction was carried out at primer-specific annealing temperatures (see Jeong et al., 2007).

2.3. *Fluorescent genotyping*

Fragment separation of amplified DNA was performed on an ABI PRISM® 377 DNA Sequencer (Applied Biosystems) by adding 1 µl of PCR product to 2.25 µl of loading buffer GeneScan™ 400HD [ROX™ DYE] internal lane size standard (Applied Biosystems). The mixture was denatured for 3 min at 95ºC prior to electrophoresis for 3 hours onto a 36-lane 8% polyacrylamide gel. The fragments were analyzed with GeneScan™ Analysis Software v.3.1 (Applied Biosystems) following the Local Southern Method.

2.4. *Genetic diversity*

The number of alleles per locus (*A*), unique alleles (*A**), and expected and observed heterozygosity (*He* and *Ho*, respectively) were calculated as means for genetic variation between the breeders, and hatchery and wild populations of black sea bream at each locus in 2003 and 2004 using the Excel Microsatellite Toolkit and GENEPOP v3.3 software (Raymond and Rousset, 1995). The allelic richness (*Ar*) was also calculated in order to correct the variation in sample sizes by FSTAT (Goudet, 2001). The inbreeding coefficient F_{IS} (Weir and Cockerham, 1984) was also determined with this software. Departures from Hardy-Weinberg equilibrium (HWE) at each locus were checked using a test analogous to Fisher's exact test, with a modified version of the Markov chain method (10^5 steps and 10^4 dememorisations) (Guo and Thompson, 1992). The analysis was performed with the ARLEQUIN v3.0 package (Excoffier et al., 2006). Using the same program we assessed the genetic differentiation among all possible population combinations, comparing the significance of pairwise F_{ST} values (Reynolds et al., 1983; Slatkin, 1995) under the null hypothesis of no differences between the populations and random allelic permutation procedures. A Bonferroni adjustments to the *P*-values were applied whenever multiple tests were performed (Rice, 1989). The frequency of null alleles (*fn*) described by Summers and Amos (1997) was calculated with Cervus 2.0 software (Marshall et al., 1998).

The discrimination effectiveness of the microsatellite was assessed by the polymorphic information content (*PIC*) by using Cervus 2.0 software (Marshall et al., 1998). The probability of a multilocus genotype shared by two specimens was estimated

by the probability of identity index (*I*) (Paetkau and Strobeck, 1994). In addition, the exclusion probability (*Q*) (Villanueva et al., 2002) was also calculated.

2.5. *Stock separation and pedigree analysis*

Wild and hatchery origin individuals in 2003 and 2004 (W2003, W2004, H2003, H2004) were identified by comparing the allele sizes at a given locus between each specimen with all the 51 breeders using PAPA software (Duchesne et al., 2002). True parental couples were assigned whenever the couple crossing genotype coincided with that of the offspring at all loci. The same process was performed with all 184 samples selected as potential hatchery reared offspring.

The number of effective parents contributing to the mating process (*Ne*) was estimated. Corrections due to unequal sex ratio (Crow and Kimura, 1970) and family size differences (*Ne**) (Hill, 1979) were applied. Finally, the rate of inbreeding expressed by the inbreeding coefficient (*F*) was estimated as a function of its inbreeding effective population size $F=1/2Ne^*$.

3. Results

3.1. *Genetic diversity*

The results of the genotype analysis showed high levels of polymorphism, ranging from 5 alleles presented at loci *ACS*4 and *ACS*6 in H2003 to 20 alleles at locus *ACS*9 in W2003 (Table 2). The Br had the highest number of alleles at all loci (83 alleles, mean = 13.8). *Ar* evidenced the differences in sample size among groups. *A** were found at the most polymorphic loci, being absent at *ACS*4 and *ACS*6 except in the Br.

High levels of heterozygosity were observed, with mean *Ho* ranging from 0.80 to 0.93. Deviations from HWE were detected at most loci in W2004, with a final heterozygosity excess deduced from the mean negative value of F_{IS}. The presence of null alleles that produce departures from HWE was not detected (Table 3).

A statistically significant loss of *Ar* was observed in the recaptured offspring when compared to the breeders (15.5% and 14.5% in 2003 and 2004, respectively). However, the reduction in 2003 occurred primarily at the *ACS*4, *ACS*6 and *ACS*9 loci, while it was more pronounced at the *ACS*3, *ACS*16 and *ACS*17 loci in 2004. In contrast, a slight loss of *Ho* was detected in H2003. In 2004, the excess of heterozygosity observed in the offspring was statistically significant.

Low F_{ST} values were observed among the samples of black sea bream, suggesting that there was high homogeneity between the different groups (Table 4). W2004 had the highest F_{ST} values, which were significantly different from the offspring. The differences were also significant between wild and hatchery groups and among years. However, the differences between W2003 and the other samples were not statistically significant.

The highly polymorphic nature of the loci employed is essential for successful offspring-breeder assignment. In this regard, the mean *PIC* score (0.848) reflects the high discrimination power of the loci (Table 5). In addition, the proper assignment of the offspring to their true parents evidenced by the exclusion probability (Q=1) and the low probability of identity index (I=2.79E-05 in 2003 and I=2.82E-06 in 2004) confirm the strength of the microsatellites used.

3.2. *Pedigree analysis*

Stocked fish accounted for 12.5% and 13.5% of the samples in 2003 and 2004 respectively (Table 6). A total of 10 families from 26% of the breeders were observed in 2003, while 28% of the breeders contributed to 11 families in 2004. The proportion of breeders contributing over the two year period was 33%.

The largest family, female #10-male #34, comprised three offspring, one caught in 2003 and two more caught in 2004 (Table 7). In 2003, all families were represented in the wild sample by a single offspring. Female #10 mated with three males and was assigned to five offspring. Male #34 was the main contributor with eight offspring from five different females.

The low number of offspring that were assigned to their parents, combined with the differences in size between families resulted in an effective population size of 16.6 and 15.2 for 2003 and 2004, respectively. However, for the entire period it is reduced to 11.8. The final inbreeding rate was approximately 3% for each year, rising to 4% when both years were combined.

3.3. *Growth*

Most of the black sea bream identified as hatchery reared belonged to the youngest group of each year analyzed (Fig. 2). Excluding the absence of 3-year-old fish in 2004, the released specimens represented 10-17% of the samples caught for each age class.

The variation in TL by age for released and wild black sea bream is shown in Fig. 3. No statistically significant differences between the wild and hatchery stocks were observed.

4. Discussion

The primary fishing area for black sea bream in Japan is located in Hiroshima Bay, accounting for 10% of the landings. Black sea bream have been released intensively into Hiroshima Bay since 1982. Support for the effectiveness of the stock enhancement program is based on an increase in landings (Jeong et al., 2007). However, the potential harmful effects on the genetic diversity of the wild fish derived from releasing large numbers of hatchery reared juveniles have not been monitored to date.

4.1. *Genetic diversity*

This study revealed high levels of heterozygosity among groups, similar to that measured in our previous study (see Jeong at al., 2007), and in other marine teleost species (Bentzen et al., 1996; Hara and Sekino, 2003; De Innocentiis et al., 2004). In contrast to the stocked samples, the Br and each of the wild samples had similar numbers of alleles. The reduction of *Ar* in the offspring (15.5% and 14.5% in 2003 and 2004, respectively) was similar to the 17% reduction reported for juveniles before release in 2000 and 2001 (Jeong et al., 2007). The low pairwise F_{ST} values are in agreement with those previously reported in Hiroshima Bay (Jeong et al., 2003) and confirm the similarity between the groups. The deviations from HWE were not attributed to the presence of null alleles, but may be related to the rate of inbreeding and/or the presence of large numbers of alleles with a small sample size (Castro et al., 2006).

In spite of the theoretical strength of the low *I* score, a study carried out on lemon sharks using dual tagging (microsatellite and external tag) showed that this value may

decrease by several orders of magnitude whenever sibling individuals are analyzed (Feldheim et al., 2002). Given that our results suggest an inbreeding problem the probability of finding two individuals sharing the same genotype may be much higher than that expressed by *I*.

4.2. *Pedigree analysis*

Low effective population sizes, as well as inbreeding problems in restocking programs, have been two of the main concerns faced by both government officials and hatchery managers. In order to avoid or minimize genetic drift, pedigree tracing has been proposed as a key component of any program that produces offspring for stocking purposes (Taniguchi, 2004; Ward, 2006).

Umino et al. (1999) reported that the stocked group averaged 45.6% of wild-caught samples during a four-year study (1994-1997). That proportion was estimated to be 42.5% three months after the release (Yamashita et al., 1997). Recently, 58.8% of the black sea bream sampled 100 days after the release were identified as being of hatchery origin (Jeong et al., 2007). The juveniles are released at a very young age and a small size thus reducing the negative effects of hatchery rearing and shortening the time for adjustment. Furthermore, the fact that the juveniles were not fed before release might contribute to their adaptation to wild conditions during the critical period after release. Natural mortality, predation, fishing effort and migration are common factors affecting the dynamics of the wild stocks, and may explain why our catch was made up of 13% stocked black sea bream. In addition, a large number of fish from the youngest year class were assigned to one breeder, even though no couple partner was identified. It is possible

that these fish might be of wild origin; spawned by the first generation of hatchery reared fish that had matured and mated with wild mates. The unknown genotype of the wild counterpart would explain the absence of an identifiable parent.

The recapture rate of this study similar to that reported for black bream (*Acanthopagrus butcheri*) released at 14 months of age in Australia (Lenanton et al., 1999). In contrast, only 0.3% of released gilthead sea bream (*Sparus aurata*) were recaptured in Spain (Sánchez-Lamadrid, 2004). In Kagoshima Bay (Japan), market surveys assessing the specific external features of hatchery reared specimens of the two main marine species stocked in the country (see Kitada, 1999), estimated the recovery rates at 9% for red sea bream (*Pagrus major*) (Imai, 2005) and 1.5-3.5% for Japanese flounder (*Paralichthys olivaceus*) (Atsuchi and Masuda, 2004). However, none of the stocked red sea bream were identified following genotyping at 5 microsatellites loci (Pérez-Enriquez and Taniguchi, 1999). Recently, the contribution of the stock enhancement program to the total landings for red sea bream and Japanese flounder was estimated as 36-41% and 19-26%, respectively (Kitada and Kishino, 2006). Kitada and Kishino (2006) concluded that there is no link between stocking practices and the fluctuations observed in wild fish populations. Information on the contribution of stocked black sea bream to the total landings is not available. Nevertheless, the large number of juveniles released since the early 1980s, in combination with the high survival rates of stocked juveniles, and the fast growth rates found in the present study, suggest a similar pattern.

The fact that wild and recent reduction in the number of black sea bream released yearly into Hiroshima Bay has not been accompanied with a reduction in landings.

Instead, landings have remained at sustainable levels. The fact that wild and released fish successfully bred clearly counteracts the negative effects of releasing hatchery fish. The genetic profile of the spawning stock then is better adapted to the wild environment.

The main contributors in the present study (female #10 and male #34) differ from those reported prior to the release (Jeong et al., 2007). The proportion of breeders represented was similar among years, 25.5 and 27.5% in 2003 and 2004, respectively. Despite this, the low number of offspring assigned, and the fact that the largest families were produced from only two individuals, led to a slightly different *Ne**, 16.6 and 15.2 in 2003 and 2004, respectively. The final inbreeding rate, estimated in the juveniles before release, was between 2.5 and 5.6% (Jeong et al., 2007). The fluctuation in the inbreeding rate observed for black sea bream may reflect shifts in allele frequencies among generations (Laikre et al., 2002), confirming the necessity for long-term monitoring of the pedigree.

Brown et al. (2005) estimated the inbreeding rate per generation in a commercial stock of gilthead sea bream, using similar numbers of breeders, as 2.7-3.5%. In contrast, a study that reared 250 breeders, reported a much lower level (0.8%) of inbreeding for red sea bream (Pérez-Enriquez et al., 1999). The problems associated with using a small number of broodstock was also highlighted in Japanese flounder (Sekino et al., 2003) and barfin flounder (*Verasper moseri*) (Ortega-Villaizán et al., 2006), where the use of only 18 breeders resulted in a final *Ne** of 3.3 and 6.2, respectively.

In the future, studies that recapture a large number of samples are necessary to obtain accurate information on the genetic contribution of the broodstock to the stock enhancement program and the genetic effect on the wild population. Moreover,

histological studies, that use a larger number of juveniles that have reached maturity, should be performed to provide insights into the spawning events.

Conclusions

Black sea bream are a good species for stocking in Hiroshima Bay. The proportion of juveniles released during the previous three years accounted for 12.5 and 13.5% of the samples in 2003 and 2004, respectively. Moreover, considering that some of the specimens identified as being of hatchery origin were mature, they may have already mated with their wild counterparts, thus increasing the stock biomass. The studies on the genetic diversity of black sea bream in Hiroshima Bay are limited (Jeong et al., 2002, 2003, 2007). Information regarding the population size and the genetic structure of the native stock prior to the start of the stock enhancement program is not available. This lack of information means that careful examination of the potential changes in the genetic composition of the wild stock due to the intensive enhancement program is necessary, as was already noted by Ryman (1981, 1997). Given that some of the breeders reared at the HCMPPC may represent offspring from the original native population, releasing large numbers of offspring may contribute to the preservation of the original gene pool (see Ryman, 1981). Our results provide insight into the genetic effects of the introgression from released black sea bream in Hiroshima Bay. However, further studies that analyze larger sample sizes over longer periods of time are needed.

The low number of breeders that contributed to the offspring in the present study led to high levels of inbreeding. This highlights the problem of genetic drift, which can occur during intensive rearing when a small number of broodstock are used. Hatchery

practices should avoid changes in allele frequencies and conserve the genetic composition of the wild populations (Allendorf, 1993; Taniguchi, 2003). Without this, a reduction of fitness and disease resistance may be observed (Allendorf and Phelps, 1980).

Our results suggest that released black sea bream juveniles have high survival rates, reach maturity and become part of the spawning stock. Nevertheless, the small sample size of fish identified as hatchery reared, and the potential harmful effects on the genetic composition of the wild stock inhabiting Hiroshima Bay make it necessary to routinely monitor the genetic effects of the stock enhancement program.

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Figure Captions

Fig. 1. Map showing the location of the release and sampling site.

Fig. 2. Distribution of wild and hatchery specimens in 2003 and 2004.

Fig. 3. Variation in total length (TL) by age in wild and hatchery black sea bream.

Tables

Table 1. Sampling date, number of samples (n), age, total length (TL), body weight (BW) and number of potential offspring.

Table 2. Genetic variability of breeders (Br), offspring 2003 (H2003), offspring 2004 (H2004), wild 2003 (W2003), wild 2004 (W2004).at 6 microsatellite loci.

Table 3. Estimated frequency of null alleles (*fn*) at six loci. Br=breeders, H2003=offspring 2003, H2004=offspring 2004, W20003=wild 2003, W2004=wild 2004. NS=Not significant.

Table 4. F_{ST} values between breeders (Br), offspring 2003 (H2003), offspring 2004 (H2004), wild 2003 (W2003), wild 2004 (W2004). H=H2003+H2004, W=W2003+W2004, 2003=H2003+W2003, 2004=H2004+W2004 and ALL= H2003+H2004+ W2003+W2004.

Table 5. Exclusion probability (*Q*) and polymorphic information content (*PIC*) of the broodstock and probability of identity index (*I*) of the offspring in 2003 and 2004.

Table 6. Pedigree analysis results for the offspring of 2003 and 2004.

Table 7. Families composition in 2003 and 2004. A = Offspring of 2003, B = Offpring of 2004. Each letter represents one offspring assigned to their male and female parent.

Fig. 1

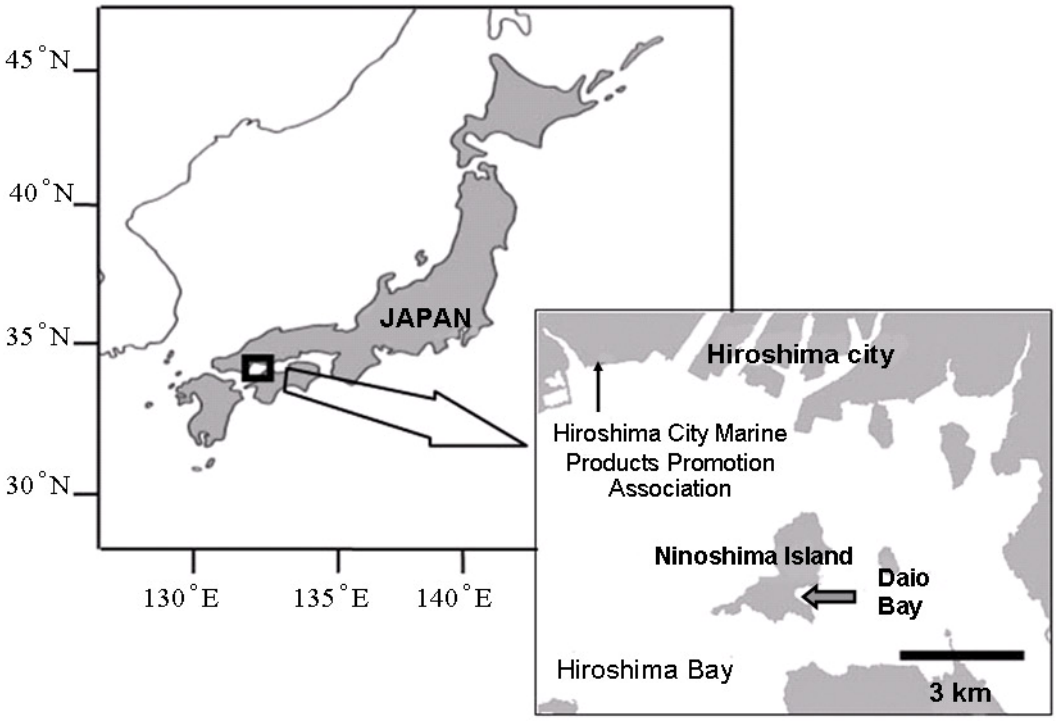
45 °N
40 °N
35 °N
30 °N
130 °E
135 °E
140 °E
JAPAN
Hiroshima city
Hiroshima City Marine
Products Promotion
Association
Ninoshima Island
Daio
Bay
Hiroshima Bay
3 km

Fig. 2

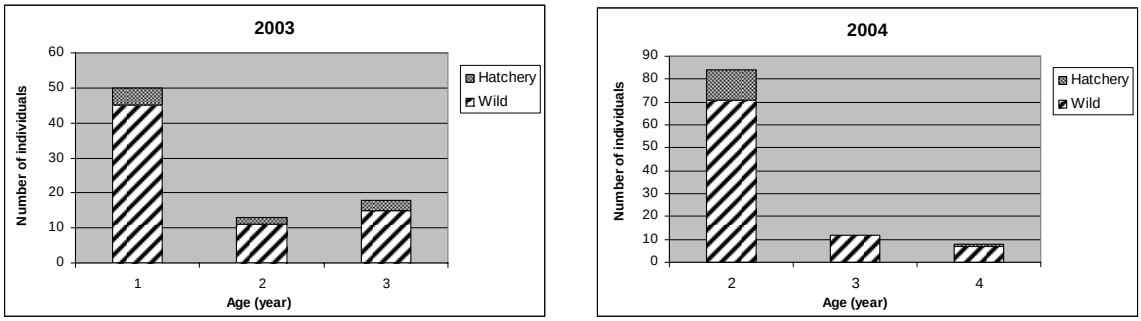
2003
Number of individuals
60
50
40
30
20
10
0
1
2
3
Age (year)
Hatchery
Wild
2004
Number of individuals
90
80
70
60
50
40
30
20
10
0
2
3
4
Age (year)
Hatchery
Wild

Fig. 3

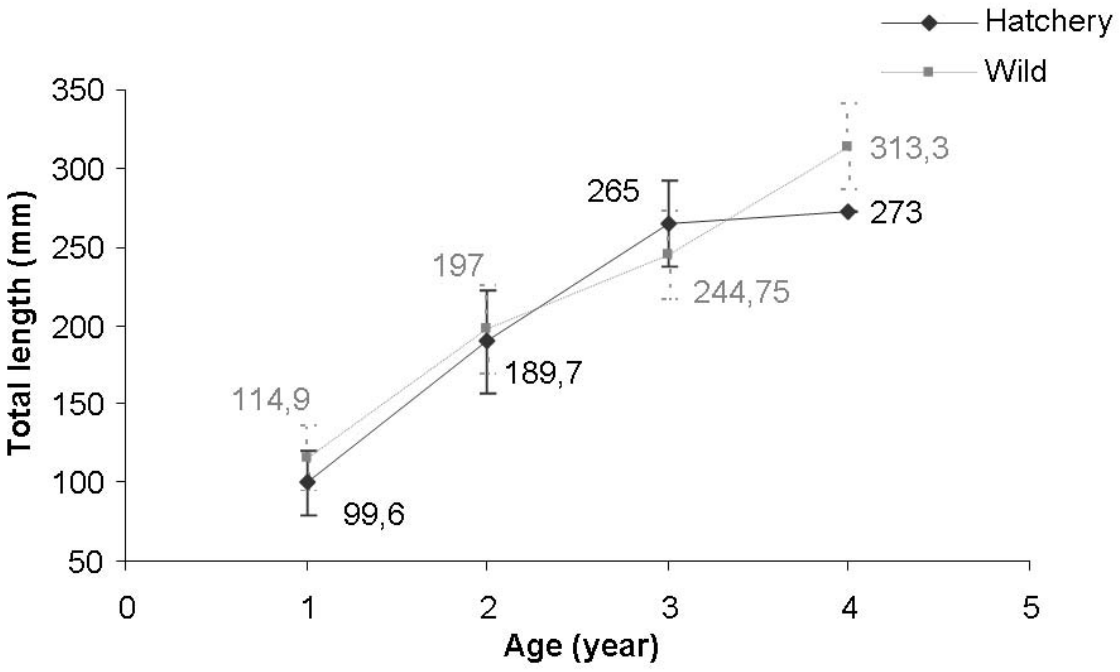
Hatchery
Wild
Total length (mm)
350
300
250
200
150
100
50
0
1
2
3
4
5
Age (year)
99,6
114,9
189,7
197
265
244,75
273
313,3

Table 1.

Date	n	Age (years)	TL (mm)	BW (g)	Pot. offspring
2003	90				80
05/21	10	1-6	125-370	35-829	6
05/23	44	1-4	74-348	7-715	40
06/01	6	1-4	120-310	29-816	4
06/18	10	1-3	108-240	34-385	10
06/25	11	1-3	70-240	14-425	11
07/12	9	1-2	104-159	43-110	9
2004	117				104
04/24	2	3-4	216-305	174-460	2
05/19	34	1-4	137-318	32-511	32
05/25	28	2-9	177-403	89-1100	21
06/02	9	2-6	190-345	117-595	7
06/18	26	1-5	150-345	47-550	24
06/23	18	2-3	180-254	92-222	18
Total	207	1-9	70-403	7-1100	184

Table 2.

Locus		Br	Hatchery		Wild	
			H2003	H2004	W2003	W2004
		n=51	n=10	n=14	n=70	n=90
	A	17	9	8	17	17
	Ar	9.3	9	7.0	8.8	9.5
*ACS*3	*A**	3	7	3	3	4
	Ho	0.88	0.90	0.86	0.91	0.90
	*F*is	-0.027	-0.218	-0.040	-0.068	-0.015*
	A	9	5	6	9	9
	Ar	6.7	5	5.9	7.3	6.9
*ACS*4	*A**	1	0	0	0	0
	Ho	0.74	0.80	0.93	0.81	0.82
	*F*is	0.097	0.007	-0.130	0.053	0.034*
	A	9	5	6	8	7
	Ar	6.8	5	6.7	5.9	6.2
*ACS*6	*A**	2	0	0	0	0
	Ho	0.74	0.70	0.93	0.67	0.83
	*F*is	0.107	0.131	-0.154	0.127	0.006
	A	18	8	10	20	16
	Ar	10.5	8	8.8	10.8	9.6
*ACS*9	*A**	3	5	3	3	1
	Ho	0.92	0.60	0.93	0.86	0.97
	*F*is	-0.011	0.200	-0.101	0.063*	-0.070*
	A	15	8	8	14	13
	Ar	9.1	8	7.5	8.9	8.2
*ACS*16	*A**	4	3	1	2	2
	Ho	0.82	0.90	0.93	0.89	0.96
	*F*is	0.079	-0.032	-0.094*	0.002*	-0.092*
	A	15	9	9	14	16
	Ar	9.2	9	7.8	8.9	9.0
*ACS*17	*A**	5	5	4	1	5
	Ho	0.90	0.90	1.00	0.87	0.90
	*F*is	-0.003	-0.038	-0.193	0.017	-0.007*
Mean						
A		13.8	7.3	7.8	13.7	13.0
Ar		8.6	7.3	7.4	8.4	8.2
*A**		3	3.3	1.8	1.5	2
Ho		0.83±0.08	0.80±0.13	0.93±0.04	0.84±0.09	0.90±0.06
*F*is		0.039	0.008	-0.119	0.030	-0.025*

*Departure from HWE at $P < 0.05$

Table 3.

Locus	Br	H2003	H2004	W2003	W2004
*ACS*3	-0.017	-0.159	-0.048	-0.036	-0.010
*ACS*4	0.051	-0.025	-0.077	0.023	0.016
*ACS*6	0.044	0.052	-0.090	0.070	0.001
*ACS*9	-0.009	0.089	-0.070	0.030	-0.037
*ACS*16	0.035	-0.044	-0.078	-0.003	-0.049
*ACS*17	-0.006	-0.053	-0.110	0.005	-0.007
6 LOCI	NS	NS	NS	NS	NS

Table 4.

	Br	H2003	H2004	W2003	W2004	H	W	2003	2004	ALL
	n=51	n=10	n=14	n=70	n=90	n=24	n=160	n=80	n=104	n=184
Br		0.003	-0.002	0.000	0.005	0.005	0.002	0.000	0.003	0.000
H2003			-0.007	0.007	0.015*					
H2004				0.003	0.014*					
W2003					0.006					
H							0.011**			
2003									0.004**	

*$P < 0.005$ after Bonferroni correction, k=10

**$P < 0.017$ after Bonferroni correction, k=3

Table 5.

	Broodstock		Offspring	
Locus	*Q*	*PIC*	*I* (2003)	*I* (2004)
ACS3	0.893	0.839	0.357	0.125
ACS4	0.823	0.793	0.167	0.123
ACS6	0.836	0.804	0.175	0.152
ACS9	0.948	0.895	0.363	0.118
ACS16	0.963	0.873	0.080	0.099
ACS17	0.922	0.881	0.092	0.103
2 loci	0.9812			
3 loci	0.9970			
4 loci	0.9998			
5 loci	1			
6 loci	1			
Mean		0.848	2.79E-05	2.82E-06

Table 6.

Matching to	Offspring		
	2003	2004	Combined
n	80	104	184
Released	10 (12.5%)	14 (13.5%)	24 (13.0%)
Wild	70 (87.5%)	90 (86.5%)	160 (87%)
Number of contributors	13 (25.5%)	14 (27.5%)	17 (33.3%)
Female (*Nf*)	8 (27.6%)	8 (27.6%)	10 (34.5%)
Male(*Nm*)	5 (22.7%)	6 (27.3%)	7 (31.8%)
Total number of families	10	11	19
Ne (unequal sex proportion) = 4*NfNm*/(*Nf*+*Nm*)	12.3	13.7	16.5
Variance of offspring per parent			
Per female(*Vkf*)	0.21	1.36	1.60
Per male(*Vkm*)	1.7	1.87	5.62
*Ne** (unbalance family size) = 8*Ne*/(4+*Vkf*+*Vkm*)	16.6	15.2	11.8
Inbreeding coefficient (*F* = 1/2*Ne**)	0.030	0.033	0.042

Table 7.

Female \ Male	#32	#34	#35	#39	#40	#47	#51
#1		A			B	A	
#6						B	A
#8				A			
#9		B					
#10		ABB		B		B	
#13					A		
#14		AB					
#25		A			B		A
#27				A	BB		
#28	B		BB				