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## Estimation of Actual Number of Parents Contributing to Seed Production of the Japanese Flounder

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### Summary

The actual number of contributing parents for three hatchery seeds ( $N_{cs}$ ) of the Japanese flounder were estimated based on the magnitude of random drift in allele frequencies at isozymic loci. The seeds were obtained from three different parental stocks and they were composed of the two or three offspring populations obtained at different times during the spawning season of their parental stocks. The  $N_{cs}$  for the seeds were from 90.3 to 126.4 and the values were reflected in the sizes of their parental stocks, respectively. Out of the eight offspring populations within the seeds examined, the actual number of parents contributing to four offspring populations ( $N_{co}$ ) indicated a much smaller parental stock size but the values of the remaining four almost reflected their actual parental stock size.

The genetic variation has been examined in many natural fish populations. Recently, determining the effect of releasing of hatchery fish on natural fish populations has become a major subject for fishery management. Therefore, the focus has shifted to hatchery populations.

Artificial propagation of the Japanese flounder (*Paralichthys olivaceus*) has been established in a number of hatchery stations in Japan and these hatchery fish have been released into natural waters to sustain the wild stock. Such a hatchery fish population are called the seeds in Japan. Releasing of the seeds might be the cause of the change in genetic variability by the founder and/or the bottle-neck effect depending on the restricted number of the parents kept in hatcheries. A decrease of genetic variability in the hatchery fish population in salmonids (1, 2), red seabream (3, 4), black seabream (5), and black rockfish (6) has been reported.

In the Japanese flounder, the screening of genetic variations at 12 isozymic loci revealed no differences in the amount of genetic variability between natural and seed populations on average (7). However, genetic variability of seed population fluctuated more than that of the natural population. The fluctuation

of genetic variability in the seed population was assumed to be promoted by the founder and/or the bottle-neck effect depending on the restricted number of the parents stocked in each hatchery station.

The hatchery seed of the Japanese flounder is produced from the stocked fish which have been collected from the natural populations and reared in the artificial pond on the land. The parental fish laid the eggs at several times during the long spawning season (two or three months). Therefore, the hatchery seed is composed of the offspring populations obtained at different spawning times. Liu *et al.* (8) revealed that the genetic composition of parental stock was not completely reflected in their offspring populations in the seed production. This fact suggests that all individuals in parental stocks do not contribute to their offspring populations.

This study focused on the offspring populations in the seed production of the Japanese flounder, and estimated the actual number of contributing parents for the seeds ( $N_{cs}$ ) and offspring populations ( $N_{co}$ ) based on the magnitude of random drift in alleles.

### Materials and Methods

The hatchery seeds of the Japanese flounder examined in this study are shown in Table 1. The seed of Miyagi 95 was produced in 1995 in Miyagi Prefectural Fisheries Experimental Station. The seed of Fukushima 95 and 96 were produced in 1995 and 1996 in Fukushima Prefectural Fisheries Experimental Station. The parental stocks were different from each other, and the parental numbers were from 84 to 200. The two or three offspring populations were

TABLE 1. *Collecting Data of Three Hatchery Seeds and Eight Offspring Populations of the Japanese Flounder Examined in This Study*

Seed	Parental stock size (No. of individuals)	Offspring population	Egg collecting date	Sampling date
Miyagi 95	84	A ( 99)	'95. 7. 7	'95. 10. 17
		B (100)	'95. 7. 14	'95. 10. 31
		C ( 99)	'95. 7. 21	'95. 10. 31
Fukushima 95	200*	A ( 99)	'95. 4. 7	'95. 11. 2
		B (100)	'95. 7. 3	'96. 3. 22
Fukushima 96	200*	A (100)	'96. 4. 19	'96. 11. 11
		B (100)	'96. 6. 14	'96. 11. 11
		C (100)	'96. 5. 14	'96. 1. 11

Figure in each parenthesis indicates number of individuals used electrophoretic analysis.

\* : These values were informed from the hatchery staff.

obtained at different spawning times (one week and three months) in each seed, and the 99 to 100 individuals were collected randomly from each rearing tank at 100–244 days after eggs were laid.

All samples were stored at  $-50^{\circ}\text{C}$  until electrophoretic analysis. The methods of starch-gel electrophoretic analysis and nomenclature of alleles at the 19 isozymic loci were the same as those described in previous paper (7). The actual number of parents contributing to each seed ( $N_{cs}$ ) was estimated by the following formula :

$$N_{cs} = q(1 - q) / 2\sigma\delta q^2$$

where  $q$  and  $\sigma\delta q^2$  is the mean and the variance of allele frequency at the locus showing variations of the offspring populations, respectively.

### Results

#### *Actual number of parents contributing to seeds*

Out of the 19 isozymic loci examined, the 11 loci ( $\alpha Gpd-1$ ,  $\alpha Gpd-2$ ,  $\alpha Gpd-3$ ,  $Fdp$ ,  $Idh-2$ ,  $Ldh-3$ ,  $Mdh-1$ ,  $Mdh-2$ ,  $Me$ ,  $Mpi$ , and  $Sod$ ) showed monomorphic in all offspring populations and the remaining eight loci ( $Aat-1$ ,  $Aat-2$ ,  $Gpi-1$ ,  $Gpi-2$ ,  $Idh-1$ ,  $Ldh-2$ ,  $6Pgd$ , and  $Pgm$ ) showed variation in at least one offspring population. Allele frequencies at the eight loci are shown in Table 2. In all offspring populations, only *Idh-1* locus showed polymorphic (0.95 criterion). At the other loci, the most frequent alleles were common in all offspring populations.

Within each hatchery seed, allele frequencies at several loci fluctuated among offspring populations. The difference of allele frequencies among offspring populations within each seed was examined by a *t*-test. The result showed that significant difference ( $p < 0.05$ ) was observed in at least one locus in all pairs of offspring populations within each seed. This significant fluctuation was considered to be caused by random drift in alleles of the parental stock in each seed production. It suggests that the actual number of parents for each offspring population determined by the magnitude of random drift are different from each other.

The actual number of parents contributing to each seed ( $N_{cs}$ ) calculated from the variance of allele frequencies at the locus showing variations of the offspring populations are shown in Table 3. The  $N_{cs}$  in each allele within one seed fluctuated largely, but the mean of these values was 90.3 to 126.4 and almost reflected the parental stock size in each seed, respectively.

#### *Actual number of parents contributing to offspring populations*

The values of  $N_{cs}$  almost reflected the parental stock size of each seed. This suggests that the mean of allele frequencies of the offspring populations is able to

TABLE 2. Allele Frequencies at Eight Loci in Offspring Populations Composing Three Hatchery Seeds of the Japanese Flounder

Locus	Allele	Miyagi 95			Fukushima 95		Fukushima 96		
		A (99)	B (100)	C (99)	A (99)	B (100)	A (100)	B (100)	C (100)
<i>Aat-1</i>	<i>B</i>	0.720	0.717	0.745	1.000	1.000	0.995	1.000	1.000
	<i>C</i>	0.280	0.283	0.255	0	0	0.005	0	0
<i>Aat-2</i>	<i>A</i>	0	0	0	0	0.005	0	0	0
	<i>B</i>	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000
<i>Gpi-1</i>	<i>B</i>	1.000	0.995	0.933	1.000	0.995	1.000	1.000	1.000
	<i>C</i>	0	0	0	0	0.005	0	0	0
	<i>D</i>	0	0.005	0.067	0	0	0	0	0
<i>Gpi-2</i>	<i>B</i>	0.970	1.000	0.929	0.940	0.950	0.990	1.000	1.000
	<i>C</i>	0.030	0	0.071	0.060	0.050	0.010	0	0
<i>Idh-1</i>	<i>A</i>	0.505	0.295	0.540	0.540	0.489	0.645	0.439	0.580
	<i>B</i>	0.495	0.705	0.460	0.460	0.511	0.355	0.561	0.420
<i>Ldh-2</i>	<i>B</i>	1.000	1.000	1.000	0.890	0.865	0.860	0.970	0.875
	<i>C</i>	0	0	0	0.110	0.135	0.140	0.030	0.125
<i>6Pgd</i>	<i>A</i>	0.690	0.543	0.897	0.860	0.820	0.835	0.838	0.857
	<i>B</i>	0.310	0.457	0.103	0.140	0.180	0.165	0.162	0.143
<i>Pgm</i>	<i>B</i>	0	0	0	0.105	0.005	0.010	0.015	0.005
	<i>C</i>	1.000	1.000	1.000	0.725	0.905	0.760	0.626	0.647
	<i>D</i>	0	0	0	0.170	0.090	0.230	0.359	0.348

Sample size of offspring population is indicated in each parenthesis.

TABLE 3. Actual Number of Parents Contributing to Three Hatchery Seeds ( $N_{CS}$ ) of the Japanese Flounder

Allele	Miyagi 95		Fukushima 95		Fukushima 96	
	$\sigma\delta q^2$	$N_{CS}$	$\sigma\delta q^2$	$N_{CS}$	$\sigma\delta q^2$	$N_{CS}$
<i>Aat-1<sup>B</sup></i>	$2.36 \times 10^{-4}$	419.9	$1.25 \times 10^{-5}$	119.6	$8.33 \times 10^{-6}$	119.8
<i>Gpi-1<sup>B</sup></i>	$1.39 \times 10^{-3}$	8.4	$1.25 \times 10^{-5}$	119.6	—	—
<i>Gpi-2<sup>B</sup></i>	$1.27 \times 10^{-3}$	12.9	$5.00 \times 10^{-5}$	519.8	$3.33 \times 10^{-5}$	44.9
<i>Idh-1<sup>A</sup></i>	$1.76 \times 10^{-2}$	7.0	$1.30 \times 10^{-3}$	96.0	$1.11 \times 10^{-2}$	11.1
<i>Ldh-2<sup>B</sup></i>	—	—	$3.13 \times 10^{-4}$	171.4	$3.56 \times 10^{-3}$	12.4
<i>6Pgd<sup>A</sup></i>	$3.16 \times 10^{-2}$	3.3	$8.00 \times 10^{-4}$	84.0	$1.42 \times 10^{-4}$	464.9
<i>Pgm<sup>B</sup></i>	—	—	$5.00 \times 10^{-3}$	5.2	$2.50 \times 10^{-5}$	198.0
<i>Pgm<sup>C</sup></i>	—	—	$1.62 \times 10^{-2}$	4.7	$5.19 \times 10^{-3}$	21.0
<i>Pgm<sup>D</sup></i>	—	—	$3.20 \times 10^{-3}$	17.7	$5.11 \times 10^{-3}$	21.0
$\overline{N}_{CS} \pm S.E.$	90.3 $\pm$ 82.4 (84)		126.4 $\pm$ 52.8 (200*)		111.6 $\pm$ 55.6 (200*)	

Parental stock size for each seed is indicated in each parenthesis.

\* : These values were informed from the hatchery staff.

TABLE 4. Actual Number of Parents Contributing to Offspring Populations ( $N_{co}$ ) in the Hatchery Seed of the Japanese Flounder

Seed	Offspring population	$V\Delta p$	$N_{co}$	$N_{cs}$	Size of parental stock
Miyagi 95	A ( 99)	0.008203	157.8		
	B (100)	0.056053	9.7	90.3	84
	C ( 99)	0.065378	8.2		
Fukushima 95	A (100)	0.009592	109.5		
	B (100)	0.009160	119.6	126.4	200*
Fukushima 96	A (100)	0.017453	40.0		
	B (100)	0.020701	31.7	111.6	200*
	C (100)	0.003590	-352.8		

Figure in each parenthesis indicates number of individuals used electrophoretic analysis.

\* : These values were informed from the hatchery staff.

approximate the allele frequency of their parental stock. Based on the magnitude of random drift in allele frequencies of the offspring populations from those of parental stock (mean of allele frequencies in the offspring populations), the actual number of parents contributing to each offspring population ( $N_{co}$ ) was estimated from following formula proposed by Taniguchi *et al.* (5):

$$N_{co} = (2N_s - 1) / 2(N_s V\Delta p - 1)$$

where  $N_s$  and  $V\Delta p$  stand for the number of samples used estimating allele frequencies in the offspring populations and the variance of standardized fluctuation from the allele frequencies of their parental stock, respectively.  $N_{co}$  estimated for the eight offspring populations were from -352.8 to 157.7 (Table 4). The  $N_{co}$  for the B and C offspring populations of Miyagi 95 and the B offspring population of Fukushima 96 ( $N_{co} = 8.2 - 40.0$ ) were much smaller than both of their parental stock size and the values of  $N_{cs}$ . But for the A and B of Fukushima 95, the values ( $N_{co} = 109.5$  and  $119.6$ ) almost reflected by both of their parental stock sizes and the values of  $N_{cs}$ . The much larger value than the parental stock size ( $N_{co} = 157.8$ ) in the A offspring population of Miyagi 95 seed and the negative value ( $N_{co} = -352.8$ ) in the C offspring population of Fukushima 96 seed were due to a small variance of standardized fluctuations ( $V\Delta p$ ). Allele frequencies of the two offspring populations were not different significantly from those of each parental stock (*t*-test,  $p > 0.05$ ). Therefore, the  $N_{co}$  of these offspring populations should be reflected by their parental stock size.

### Discussion

Liu *et al.* (7) revealed that genetic variability of the seed population of the Japanese flounder fluctuated more than that of the natural population and suggested that this fluctuation promoted by the founder and/or the bottle-neck effect depending on the restricted number of the parents stocked in each hatchery station. Concerning with this aspect, in the present study, the estimated  $N_{co}$  for four offspring populations were much smaller than their parental stock size. This result suggests that such offspring populations are produced by a few contributing individuals in the parental stock. This case might be caused in the earlier or later stages of the spawning season of the parental stock.

On the other hand, the  $N_{co}$  for some of the offspring populations were almost reflected by their parental stock size. This result suggests that almost of the individuals of the parental stock contributed to the offspring population. The occurrence of such a case might depend on the period of the height of prosperity of egg spawning of the parental stock.

The loss of genetic variation in seed production is concerned with the number of reproductive individuals in parental stock. Our results showed that the estimation of the number of reproductive individuals provides a mean to assess the possibility of the loss of genetic variation in seed production. This may prove to be extremely valuable in the management of seed production of the Japanese flounder. The effort to increase the actual number of parents should be needed of concern to the genetic management of seed production of the Japanese flounder. Regarding to this point, this study revealed that the  $N_{cs}$  for all examined hatchery seeds almost reflected their parental stock size, respectively. This suggests that the mixture of offspring populations promotes increasing the actual number of parents contributing to the seed. Therefore, the genetic management of seed production of the Japanese flounder, our present data suggests that the seeds should be produced mixing with many offspring populations obtained during the period of the height of spawning prosperity from a parental stock composed of many individuals from the natural population.

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