Reduction of Female Proportion in Lower Growing Fish Separated from Normal and Feminized Seedlings of Hirame Paralichthys olivaceus

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Each group of normal males and sex-reversed gynogenetic females (phenotypical male) of hirame Paralichthys olivaceus were crossed with normal females, and the obtained larvae and juveniles were reared at two different water temperatures of 18°C and 23-25°C (only normal generation) during the sex determination period. When lower growing fish (about 20 mm in total length) appeared in their original populations, these were separated and reared in other tanks. The populations reared at 18°C without growth retention indicated the primary genetic sex ratio, while the populations reared at high temperatures showed clearly lower proportions of females. The lower growing fish showed lower proportions than those in the original group, at both water temperatures. These results show that the sex ratio of a pond population is greatly affected not only by rearing water temperature, but also by the existence of lower growing fish.

Key words: female proportion, slower growing fish, stress, feminized seedling, flounder

Recent studies using a sex-reversed gynogenetic female crossed with a normal female suggested that the sex of hirame Paralichthys olivaceus is determined by the male hetero type (XY type) mechanism,1,2) in spite of a lack of sex chromosomes the same as in many other fishes.3) The sex ratio of hirame is greatly affected by environmental factors such as the rearing water temperature during the sex differentiation stage.2,4) The temperature sensitivity among races may also affect sex ratio (unpublished data).

It is well known that females grow much faster than males,4,5) and this fact should be put to practical use soon. However, for feminized seedling production on a commercial basis, a stable method of producing a high proportion of females must still be developed, because low proportions of females are sometimes observed even if fish are reared under suitable temperature conditions. This gynogenetic study of hirame found that the existence of lower growing fish greatly affects the sex ratio in addition to the above factors. This finding will be useful to produce a high proportion of females in feminized production.

Materials and Methods

For normal generation (N1, N2, N3, and N4 in Table 1), one normal male was crossed artificially with one normal female. For feminized seedling production (F1 and F2 in Table 1), four sex-reversed gynogenetic females (phenotypical male) were crossed artificially with one normal female. These experiments were performed from December 1992 to April 1993. Three lots (N1, N2, and N3) of fertilized eggs from normal generation were divided into two lots the day before hatching. The rearing temperature of each of the two lots was maintained at 18°C from the egg stage until the 90th day after hatching, then raised to 25°C (N1-18, N2-18, and N3-18, see Table 1). The temperatures of the others were maintained at 20°C until the 20th day after hatching, raised to 23°C until the 45th day after hatching, then maintained at 25°C after the 50th day following hatching (N1-23, N2-23, and N3-23, see Table 1). The rearing temperatures in the two lots (F1, and F2) of feminized seedling production were maintained at 17°C from the egg stage until the 20th day after hatching, then maintained at 18°C after the 25th day following hatching (F1-18 and A2-18, see Table 1).

100 l polycarbonate tanks and 500 l FRP tanks for feminized seedling production were used for the rearing experiments. The number of newly hatched fish stocked was 3,000 per 100 l. Rotifer Brachionus plicatilis, Artemia nauplius, and an artificial diet were used as foods. The rearing tanks were illuminated (800 lx, 12 h light and 12 h dark photoperiods).

The lower growing fish were separated 55-70 days after hatching from the original populations (about 30-40 mm in average total length) in the case of rearing at 18°C, and on the 45th day after hatching (about 40 mm in average total length) in the case of rearing at 23-25°C, because growth differed according to rearing water temperature. The separated lower growing fish, which were about 20 mm in total length, were fed on an artificial diet in the new tanks. The sex ratio was calculated following microscopic observation of gonads, which were sampled from 21 to 51 progeny per lot on the 120th day after hatching.

Results and Discussion

The growth of fish in the original group and the separated fish are shown in Fig. 1. Lower growing fish began to appear when the average total length in the original population reached about 20 mm. By then, hirame larvae had finished metamorphosis and had begun to settle on the bottom. Furthermore, when hirame juveniles were able to inhabit the bottom and ate freely, their body color changed to clear. However, in cases of overpopulation, the lower
growing fish had to swim continuously at the surface, and were unable to move to their demersal habitat. These lower growing fish showed a darker body color while swimming, but when they were transferred into a new tank, they immediately inhabited the bottom. Then they soon began to take food, and their body color became clear. They grew favorably and reached almost the same size as the fish in the original population by the 100th day after hatching. 20 mm to 50 mm in total length is considered to be the main sex differentiation period in hirame from histological studies6,7) and the author’s unpublished data, as shown in Fig. 1. This figure shows that the appearance period of the lower growing fish overlaps with the onset of sex differentiation. It is assumed that these fish were subjected continuously to great stress until they were separated from the original population and transferred to a new tank.

The proportion of females and survival rates in each experimental group are shown in Table 1. The decrease in survival rates was caused by a bacterial disease and other unknown diseases before the separation of lower growing fish from the original group. The survival rates at 18°C reared under normal generations were 1.8 to 19.1%, and within these, the proportion of females ranged from 43.1 to 54.1%. On the other hand, the survival rates at 23°C reared under normal generations were 5.7 to 18.3%, and within these, the proportion of females ranged from 14.3 to 26.5%. Thus, no correlation was found between survival rate and proportion of females, but the proportion of females of the latter groups was clearly lower than that of the former. These results agree with those of Yamamoto et al.2)
The lower growing fish reared at 18°C appeared at a density higher than 700 individuals per m², but those reared at 23°C did not appear at a lower density than 800 per m² and appeared at a density of 1,500 per m². Thus, the density at which lower growing fish appeared increased with an increase of rearing temperature, but the appearance of lower growing fish at the same temperature depended clearly on the rearing density.

The proportion of females in three lower growing fish (shown as -L in Table 1) taken from the original normal generation groups was 20.0% (N1-18-L) to 43.1% (N1-18) and 9.5% (N2-18-L) to 50% (N2-18) at 18°C, and 0% (N1-23-L) to 24.2% (N1-23) at 23°C, respectively. Thus the proportion of females in lower growing fish separated from the original groups was lower than the proportion in the original groups, at both examined temperatures. The proportion of females between original groups and lower growing fish was significantly different at 5% (Chi-square value 4.76), 1% (8.56), and 1% (7.81), respectively. The proportion of females in groups reared at 18°C without retention of growth is considered to indicate the primary genetic sex ratio, because it was around 50%. A possible cause of the low proportion of females in the lower growing fish is that the sex hormone was not secreted normally because they were subjected to great stress during part of the sex differentiation period, and this prevented their differentiation into females. But there is no evidence linking the normal secretion of sex hormone and stress. Endocrinological examination is necessary in future studies.

Furthermore, in the case of the feminized seedlings produced by crossing a sex-reversed gynogenetic female and a normal female, the trend of decreasing sex ratio in the lower growing fish was nearly the same as in the normal generation. The proportion of females was 23.3% in the mixed lower growing fish separated from the two feminized seedling groups in which the proportion of females was 80.4 and 90.0%. The proportion of females between the original groups and lower growing fish was also significantly different, at 1% (Chi-square value 25.47).

The proportion of females in the F1-18 group of feminized seedlings was lower than that in F2-18 because many lower growing fish appeared when they were fed an artificial diet and survived in large quantities as mentioned below. The figure on the left-hand side in Fig. 2 shows the proportion of females at each size class in the small size fish (F1-18*2) separated artificially from the group (F1-18) with 80.4% proportion of females. As shown in this figure, the proportion of females decreased with decreasing size class. The two figures on the right-hand side in Fig. 2 show the relationship between total length and proportion of females in groups (N3-18*, N4-18*) in which lower growing fish reared at 18°C did not appear because of their low rearing densities (see density in Table 1). The proportion of females, despite showing large variations, was not correlated with total length, and was distributed around 50%. This suggests that sex differentiation in these groups occurred according to primitive genetic sex determination, because the lower growing fish were able to grow when not subjected to stress under a reasonably low density. On the contrary, it was assumed that the low proportion of females in the group (F1-18) which contained many lower growing fish was caused by the many small fish which survived in spite of the separation.

The results of this study suggest the following two methods of improving feminized seedling production: 1) The rearing method should be selected such that lower growing fish do not appear. 2) Any lower growing fish which do appear should be separated and eliminated.

**References**