交雑魚マダイ×クロダイおよびマダイ×ヘダイの生殖腺成熟

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Gonadal Maturation of *Pagrus major* × *Acanthopagrus schlegeli* and *Pagrus major* × *Sparus sarba* Sea Bream Hybrids

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**Abstract**

Gonadal maturation in hybrids of red sea bream ♀ × black sea bream ♂ and red sea bream ♀ × silver bream ♂ reared for 4 years in net cages on the sea was compared with that of red sea bream, black sea bream, and gynogenetic diploid silver bream. The gonads of both hybrids were clearly immature in their appearance compared with those of the parent species during the same period. The gonado-somatic index of the three parent species peaked in April with average values higher than 8 whereas the values for hybrids never exceeded 1. Histological examination of the gonads revealed spermatogonia, spermatocytes, spermatids, and spermatogenesis in the males of both hybrids, but oocytes were not observed. From these results, it was made clear that both males and females of the two types of hybrids showed hybrid sterility.

As a link of breeding of cultured marine fishes, the authors produced hybrids from red sea bream, *Pagrus major* × black sea bream, *Acanthopagrus schlegeli* (afterwards called as Makuro-dai) and red sea bream × silver bream, *Sparus sarba* (afterwards called as Mahe-dai) and reported their growth, external morphology and environmental stress resistance

If we look at the hybrids thematologically, it is important that the both male and female of first filial generation (F₁) become matured and have fertil function in order to fix the species, and then it becomes possible to proceed further breeding by means of reciprocal crosses between the hybrid F₁ or F₂ and the parent fish species. However, in case of hybrids among such different species as salmon/trout, carp, crucian carp, goldfish, etc., of freshwater fishes, abnormality in gonadal maturation and the infertility have been reported. On the other hand, studies on maturation of hybrids among seawater fishes is extremely few until now. Therefore, in the present study, investigations were made on the aspects of gonadal maturation in hybrids Makuro-dai and Mahe-dai.

**Materials and Methods**

After young fishes in 71 days after hatching of both hybrids (Makuro-dai and Mahe-dai) that had been produced on March, 1990 in the previous paper, as well as those of parent fish species (red sea bream, black sea bream and gynogenetic diploid silver bream) were respectively accommodated in net cages that were installed on the sea, they were bred for 4 years, and employed as the test fishes.

Surveys of gonad were carried out once a month from February to June, 1994 and more than four fish

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Key words: Gonadal maturation, Hybrid, Red sea bream, Black sea bream, Silver bream
were respectively picked out from each net cage and employed. After the body weight and the fork length measured for employed individual bodies, gonad was taken out and the weight was measured, and the gonado-somatic index (GSI = 100 × gonad weight/body weight) was calculated. After the photograph of gonad was immediately taken, a part of it was fixed in a 10% neutral formalin solution, and embedded into paraffin by the usual method, and a 7-15 μm thick section was prepared. The section was double stained with hematoxylin-eosin and the histological observation was made by means of light microscope. The

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**Fig. 1.** Gonads of red sea bream × black sea bream (A), red sea bream × silver bream (B), red sea bream (C: top, ovary; bottom, testis), black sea bream (D: top, ovary; bottom, testis) and gynogenetic diploid silver bream (E, ovary) on April 7, 1994.
maturation stage of gonad was classified into I ~ IV stages according to the methods of Matsuyama et al. (1987)¹² and Matsuura et al. (1987)¹³, in which the stage I was prescribed as the immature period, and stage IV as the mature period. The average body weights of respective sample fishes used for gonad survey on April 7, 1994 were 1.136±0.218 g (n=5) for Makuro-dai, 1.338±0.231 g (n=5) for Mahe-dai, 2.752±0.331 g (n=5) for red sea bream ♂, 2.214±0.112 g (n=5) for red sea bream ♀, 456±236 g (n=3) for black sea bream ♂, 590±125 g (n=3) for black sea bream ♀, and 1,700±192 g (n=4) for silver bream ♂.

Results

The photos of gonad of respective test fishes on 7th of April, 1994 were shown in Fig. 1. It was observed that the gonads of Makuro-dai and Mahe-dai were clearly immature compared with those of red sea bream, black sea bream and silver bream at the same period. Further, they were all covered with a light colored fatty substance, and this tendency was more clearly observed in case of Mahe-dai. Furthermore, the discrimination between male and female was completely impossible from the appearance of respective gonad of the hybrids. More, according to the sample (the photo is omitted) on 15th of June which was 2 months later, the fatty substance that had covered the gonad turned brown in both hybrids, and most of gonads had hypertrophy or degeneracy.

Fluctuations of GSI of the hybrids and parent fish species were measured for 5 times from February to June and they were shown in Figs. 2 and 3. The GSI of all females of red sea bream, black sea bream and silver bream became to show the highest values in April. The mean values were 8.8±0.8 (n=5), 16.0±3.8 (n=3), and 20.1±5.0 (n=4), respectively. On the other hand, GSI of male in case of red sea bream were already higher at the time point of February (8.3±0.9, n=5), and these tendencies continued until April. GSI of male black sea bream showed maximum values (10.3±1.8, n=3) at April. In every parent fish species, the decrease of GSI was observed on and after May in which the water temperature exceeded 20°C. On the other hand, in both Makuro-dai and Mahe-dai

Fig. 2. Changes in the gonado-somatic index (GSI) of 4-year-old red sea bream, red sea bream × black sea bream and black sea bream during spawning season.
Fig. 3. Changes in the gonado-somatic index (GSI) of 4-year-old red sea bream, red sea bream × silver bream and gynogenetic diploid silver bream during spawning season.

Fig. 4. Histological sections of gonads of 4-year-old red sea bream × black sea bream (A, B) and red sea bream × silver bream (C, D) on April 7, 1994. Sc, spermatocytes; Sg, spermatogonia; St, spermatids; Sz, spermatozoa.
GSI showed lower values than 1 in most fish throughout the surveying period. However, each only one individual of Makuro-dai and Mahe-dai showed value of 4.1 and 5.2, respectively. The reason is not known at present.

In histological observations of gonad of females of red sea bream, black sea bream and silver bream, they all showed the maturation stage IV in April which was the central stage, and the peak of maturation stage and the peak of GSI coincided. On the other hand, in their males during March and May, the maturation stage IV continued in case of red sea bream, and the gonads in case of black sea bream were from the developmental period of maturation stage III to maturation IV. However, the gonads of Makuro-dai and Mahe-dai were always in an undeveloped period of maturation stage I, and the maturation did not proceed in every individual under the survey. From the histological feature of gonad of Makuro-dai, spermatogonia, spermatids and spermatogenesis were observed (Fig. 4-A, B). On the other hand, also in the gonad of Mahe-dai the each developmental stage of spermatogonia to spermatogenesis was observed (Fig. 4-C, D). However, the tissue like oocyte was not observed in any gonads of the hybrids.

**Discussion**

The relation between the maturation stage (I ~ IV) and GSI nearly coincided in case of red sea bream, black sea bream and silver bream, respectively, and the maturation of these fish species reached their peaks in nearly April.

On the contrary, the increase of GSI was not observed entirely throughout the survey period (from February to June) in case of the hybrids Makuro-dai and Mahe-dai. From the histological feature of gonad, spermatogonia, spermatids and spermiogenesis were observed, but it is estimated that they did not have reductive functions as males because GSI was lower than 1. The oocyte was not observed in any gonads.

According to Suzuki[5-11], in males of hybrids of many combinations in case of bitterling subfamily and pike gudgeon subfamily, although the formation of spermatocyte was observed, the sperm could not be formed because spermatogenesis was not performed normally. Further, hybrids between Carassius carassius auratus and Gnathopogon elongatus elongatus were all intersex, and neither testis nor ovary was formed even though they were at the maturation stage. As the cause that the hybrid sterility occurs, he estimate that there may be also an essential physiological factor besides the disharmony due to the difference between chromosome numbers of parent fish species.

The chromosome numbers of the hybrids, Makuro-dai and Mahe-dai; and the parent fish species are all 2n = 48 and the difference between the fish species is not observed*, and it is not considered that the cause of sterility in the hybrids lies in the difference of chromosome numbers. However, when the comparison was made with the case of hybrid from Japanese parrot fish, Opalates fasciatus ♀ × spotted parrot fish, O. punctatus ♂, in which the maturation is possible14,15, the difference of karyotype between both hybrids and the parent fish species in the present study is great*, and it is estimated that the different degree of karyotype gives some influences on the hybrid sterility.

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交雛魚マダイ×クロダイおよびマダイ×ヘダイの生殖腺成熟

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1990年3月に作出した交雛魚マダイ♀×クロダイ♂およびマダイ♀×ヘダイ♂を4年間飼育した後、2月から6月までの生殖腺成熟の様相を、同行様に4年間飼育した両親魚種（マダイ、クロダイおよびヘダイ）と比較した。その結果、両交雛魚から摘出した生殖腺は、外観的に同時期の両親魚種のそれらに比較して明らかに未成熟であった。両親魚種の生殖腺指数はいずれも4月に最大値となり、その平均値はいずれも8以上の高い値であった。交雛魚のそれは1以下であった。生殖腺組織像から両交雛魚とも精原細胞、精母細胞、精細胞および精子形成が観察できだが卵母細胞は認められなかった。以上より、両交雛魚は雌雄ともに生殖不全すなわち種仔不妊であることが明らかにされた。