

## PRODUCTION OF ALL-FEMALE DIPLOID AND TRIPLOID

### OLIVE FLOUNDER, *PARALICHTHYS OLIVACEUS*

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#### Introduction

Olive flounder (*Paralichthys olivaceus*) is the most important marine food fish in Korea which are produced both by capture and culture. One of major problems in olive flounder culture is sex-related dimorphism in growth rate where females grow much faster than males (Kim et al., 1994). When females reached to 600g of body weight which is near to marketable size, the average size of males is about half of their female progenies. Depends on this, production of all-female population of this species should improve the yields, and fish farmers much eager to culture the mono-sex, only females. Fortunately, olive flounder has the XX-XY based sex determination mechanism in which females are homogamety (Tabata, 1991).

All female populations can be produced by crossing phenotypic males (but genetically female) with normal females in species whose sex determination mechanism is female homogamety. Reversed phenotypic males can be traditionally obtained by hormonal sex reversal, however it inevitably requires the intensive labourious steps such as progeny test for selecting the reversed genetic females. Induced gynogenesis in species where female is homogametic, and masculinization of these gynogenetic females could exclude this time consuming proceduces because they are all genetically females. In addition, induced gynogenesis has been given much attention because of their potential interests such as the rapid establishment of pure inbred lines and accelerated elimination of recessive deleterious genes (Thorgaard, 1983; Quillet et al., 1991; Kim et al., 1993).

The object of this study is to develop all-female diploid and triploid populations by chromosome set manipulations including induced gynogenesis, and sex control techniques in order to enhance the productivity in olive flounder farms.

## Materials and methods

The artificial induced and multiple spawning in olive flounder were performed by employing the human chorionic gonadotropin (1~2 IU HCG/g BW) and carp pituitary (10 µg CP/g BW).

We developed the all-female olive flounder by chromosome set manipulation and sex reversal. Gynogenetic diploid flounders were induced by artificial insemination with UV-irradiated heterospecific sperm from several species of fishes and by applying the cold shocks. Gynogenetic diploid females (XX) were masculinized by physical treatment to phenotypically male (XX) which can be used for the production of all-female population.

Reproductive performances of induced gynogenetic male has been examined by cytological evaluations and fertilization trials. All female diploid and triploid populations were produced by crossing the induced gynogenetic diploid male (XX) with normal female (XX), and/or by cold shock treatment (Fig. 1).

## Results and discussion

### Induction of gynogenesis

Gynogenetic diploid females were induced using several UV-irradiated heterospecific sperms and cold shocks; the optimal UV dose was ranged 3,600 to 4,200 (erg/mm<sup>2</sup>), and treatment of 2°C for 45 mins, 2 mins after fertilization gave the best results for blocking 2nd polar body (Table 1 & 2).

### Masculinization of gynogenetic diploid females

Induced gynogenetic females were masculinized to phenotypic males by sex reversal. Masculinization of gynogenetic females was done by elevation of culture temperature in critical periods and also by other some physical methods without employing any steroids. These masculinization methods were patent pending to our country (93-18132, South Korea).

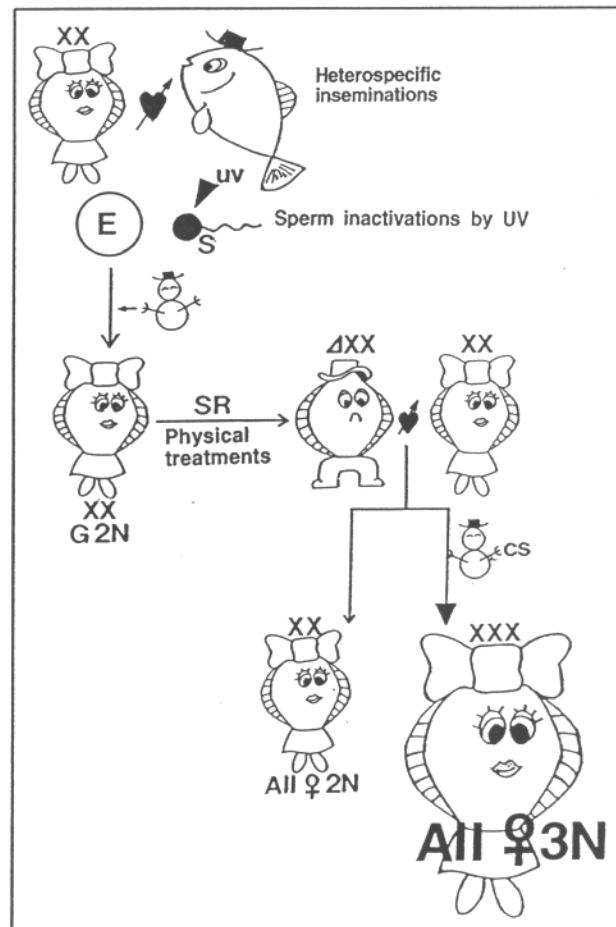


Figure 1. Experimental procedure for this study.

Table 1. Effects of ultraviolet irradiation for inactivation of spermatozoan DNA with several fish species

Species	Dose (erg/mm <sup>2</sup> )	Hatching rate (%)	Haploidy (%)
<i>Acanthopagrus schlegeli</i>	3,900	15.4	100
<i>Misgurnus mizolepis</i>	3,600	0	0
<i>Paralichthys olivaceus</i>	4,200	24.3	100

### Fertility of gynogenetic diploid males

Reproductive abilities of gynogenetic diploid males including the histological analyses of testis, cytological analyses of spermatozoa and fertilization trials with normal eggs were evaluated along with normal diploid males. Gonads of gynogenetic diploid males were histologically normal, and many spermatozoa were observed in their testis (Fig. 2). Mean number of spermatozoa from the control and gynogenetic diploid males were  $(2.58 \pm 1.17) \times 10^9$  and  $(2.42 \pm 0.79) \times 10^9$  cells per 1ml of milt, respectively ( $P > 0.05$ ). Amount of milt per kg body weight from the gynogenetic diploid male ( $20.6 \pm 12.9$  ml) was significantly higher ( $P < 0.01$ ) than that from the control male ( $8.3 \pm 5.4$  ml). Size and morphology of spermatozoa from the two experimental groups were not different from the control male ( $P > 0.05$ ). More than 80% of fertilization rates and hatching rates were observed when the eggs from the control were fertilized with the gynogenetic diploid male sperms (Table 3).

Table 2. Effects of cold shock (45 mins) on fertilized eggs of olive flounder, *Paralichthys olivaceus* for the induction of gynogenetic diploid

No. of eggs treated	Temp (°C)	Survival rate of embryos (%)	Gynogenetic diploidy (%)
1,500	0	59.2	37.7
1,500	2	68.8	62.5
1,500	4	70.6	28.7

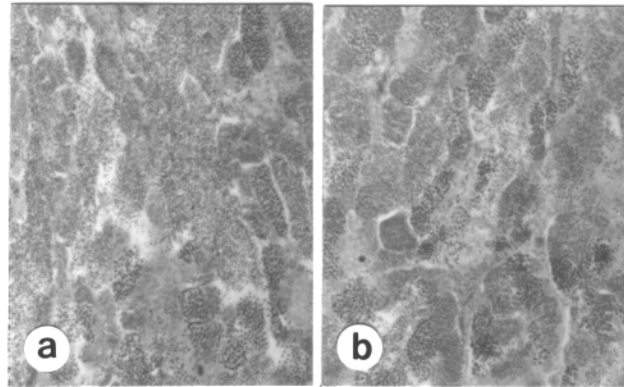


Figure 2. Transverse sections of control male and gynogenetic diploid male *Paralichthys olivaceus* gonads: (a) control testis; (b) gynogenetic diploid male testis

Table 3. Results of artificially fertilized with normal diploid female and gynogenetic diploid male olive flounder, *Paralichthys olivaceus*

Exp. Group	No. of eggs	Amount of milt used (ml)	Floating rate (%)	Fertilization rate (%)	Hatching rate (%)
1	120,000 (3)*	35.0 (2)*	41.9	84.0	78.5
2	450,000 (3)	33.0 (4)	66.7	94.0	92.0
3	360,000 (3)	19.0 (2)	40.3	70.3	81.9
4	1,405,000 (9)	23.0 (5)	36.7	70.7	84.0
5	660,000 (4)	14.8 (2)	50.8	80.2	89.2
6	780,000 (9)	14.8 (4)	31.4	88.6	76.4
Mean $\pm$ SD	636,000 $\pm$ 460,000	23.3 $\pm$ 8.1	44.6 $\pm$ 11.5	81.3 $\pm$ 8.8	83.7 $\pm$ 5.5

\* Number of fish used

### Induction of all-female diploid and triploid

Table 4 showed the sex ratios of progenies produced by crossing the gynogenetic males with normal females.

Table 4. Sex ratios of progeny by mating with gynogenetic diploid male and normal female in olive flounder, *Paralichthys olivaceus*

Exp. group	No. of fish observed	Sex		Percentage of female
		Female	Male	
Diploid				
I	60	60	0	100
II	70	70	0	100
Triploid				
III	60	60	0	100
IV	80	80	0	100
Total	270	270	0	100

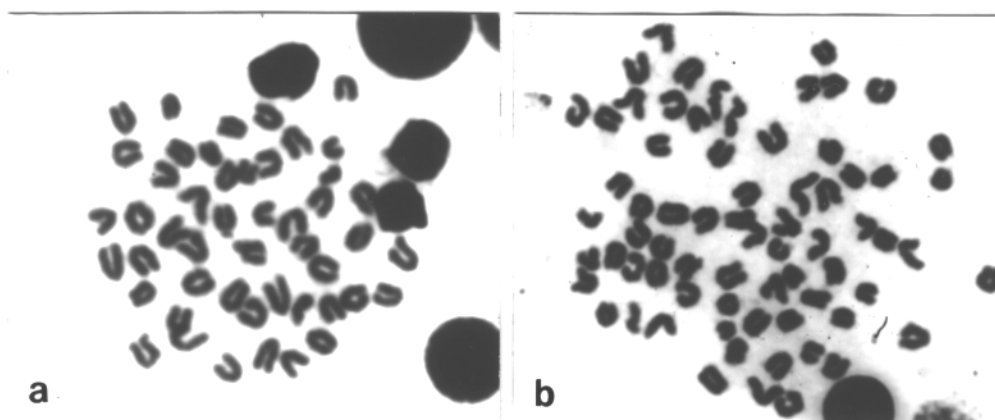


Figure 3. Metaphase spreads of diploid female (a) and artificial triploid female (b) *Paralichthys olivaceus*.

Complete population all-female was successfully obtained, and the all-female triploids were also successfully produced by cold shocks. The chromosome number of diploids and triploids showed  $2n=48$  and  $3n=72$ , respectively, and their karyotypes were consisted of all acrocentric chromosomes (Fig. 3). The gonads of 4-month-old triploids were sterile histologically (Fig. 4). Ongoing studies are producing the all-female flounder populations in a commercial scales, and are evaluating the performances of all-female populations and enhanced yields.

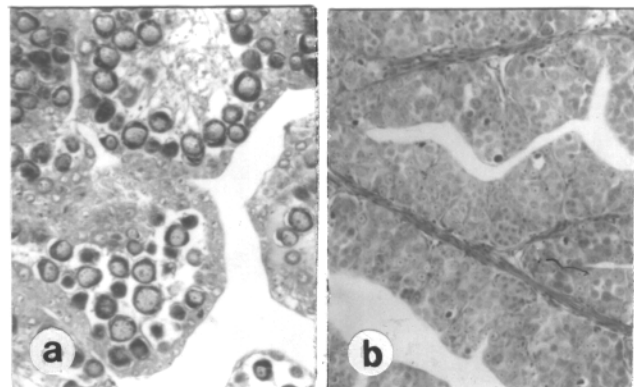


Figure 4. Transverse sections of diploid and triploid female *Paralichthys olivaceus* gonads: (a) diploid ovary; (b) triploid ovary

## References

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