Chapter 3
Grouper Aquaculture Research in Jeju Island, Korea

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Abstract

*Eleven grouper species inhabit in the coastal waters of Jeju Island, Korea, and spawn there in summer (June to August). Among these species, the seven-band grouper (*Epinephelus septemfasciatus*), longtooth grouper (*E. bruneus*), and red spotted grouper (*E. akaara*) have high economic value. Recently, to allow resource recovery and help the aquaculture industry, interest in the reproductive characteristics of groupers has increased. To develop aquaculture techniques for groupers, the Marine and Environmental Research Institute of Cheju National University, other related research institute of the Jeju Island, and private companies have examined (1) broodstock management and sexual maturation in rearing tanks, (2) the hormonal induction of sexual maturation and sex-change techniques, (3) cryopreservation of sperm, and (4) the production of fertilized eggs and rearing larvae.*

Introduction

In Korea, the commercial importance and peculiar reproductive features of groupers have attracted the interest of many investigators. Recent research on groupers has examined sexual characteristic and sex reversal (Lee *et al.* 1993; Hwang *et al.* 1998; Lee *et al.* 2002), maturation (Kim *et al.* 1997, Lee *et al.* 1998), and larval rearing (Song *et al.* 2005).

Eleven species of groupers are distributed in the Southern Sea near Jeju

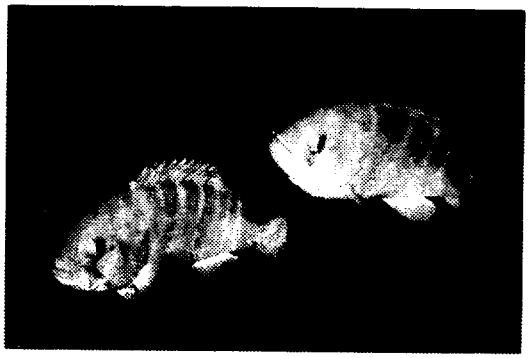

Figure 1. Seven-band grouper (*Epinephelus septemfasciatus*). Korean name: Neong-sung-eo; Local name (Jeju): Gu-moon-zaeng-i

Figure 2. Longtooth grouper (*Epinephelus bruneus*). Korean name: Ja-ba-ri; Local name (Jeju): Da-gum-ba-ri

Figure 3. Red spotted grouper (*Epinephelus akaara*). Korean name: Buk-ba-ri; Local name (Jeju): Buk-ba-ri

Island, including the seven-band grouper (*Epinephelus septemfasciatus*; Figure 1), longtooth grouper (*E. bruneus*; Figure 2), and red spotted grouper (*E. akaara*; Figure 3). Most inhabit rocky regions in the coastal waters at depths of 5-30 m, and the spawning season is June to August (Kim *et al.* 2005). These three grouper species have high economic value and great potential for cultivation in southern Korea.

Although grouper aquaculture began in the mid 1990s at the Marine and Environmental Research Institute (MERI) of Cheju National University, other related research institute of the Jeju Island and private companies, it can be considered to still be in its infancy. One of the difficulties in grouper aquaculture is obtaining mature broodstock, which is necessary to procure healthy fertilized eggs.

Broodstock Maintenance

MERI is located in southern Korea, where the Kuroshio Current flows into the Korea Strait (Figure 4). The ambient water temperature along the coast of Jeju Island ranges from 11 to 30°C (winter to summer). The water temperature in winter ranges from 11 to 15°C (December to April) and is unsuitable for the growth of groupers. Underground seawater available on Jeju Island maintains water temperatures of 16 to 18°C throughout the year, enabling the rearing water temperature to be maintained at about 17°C during the winter (salinity 33 ppt).

Grouper aquaculture on Jeju Island began in 1993. Broodstock were purchased from fishermen, anaesthetized with 200 ppm 2-phenoxyethanol, and

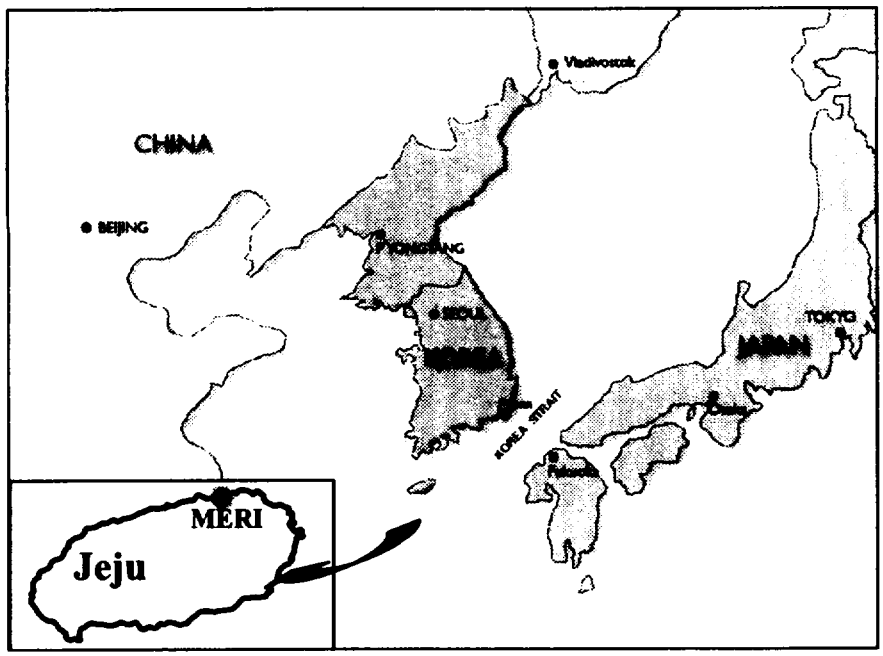

Figure 4. Location of Marine and Environmental Research Institute (MERI), Cheju National University.

tagged with a microchip for individual management. Total length (TL) and body weight (BW) were also measured (Figure 5). The fish were kept in indoor rearing tanks (50 to 70 T) at a stocking density of 6 to 8 kg m^{-2} in a flow-through system. The broodstock were fed moist pellet and sardines once a day at 2-3% BW, and with squid or minced fish mixed with vitamin and mineral mix as supplement diets during the spawning season.

Egg production by *E. septem-fasciatus* and *E. bruneus* were examined at MERI between 2000 and 2003. In 2000 to 2001, we successfully induced the natural spawning of *E. septem-fasciatus* from June to July, but the egg quantity was too small and could not be used for seed production. Since 2003, quantity of fertilized eggs has been increasing gradually with the artificial induction of sexual maturation and egg production.

Techniques for Induced Maturation

Induction of final maturation and ovulation

To confirm the maturity of females, ovary samples were obtained by biopsy. Small fragments of ovary were extracted by silicone tube inserted into the urogenital papilla of an anesthetized (200 ppm 2-phenoxyethanol) female. A female found with mature oocytes was given an injection of human chorionic gonadotropin (HCG) at doses of 500-1000 IU kg^{-1} BW (Figure 6).

In 2007, the total volume of stripped eggs was approximately 800 ml for *E.*

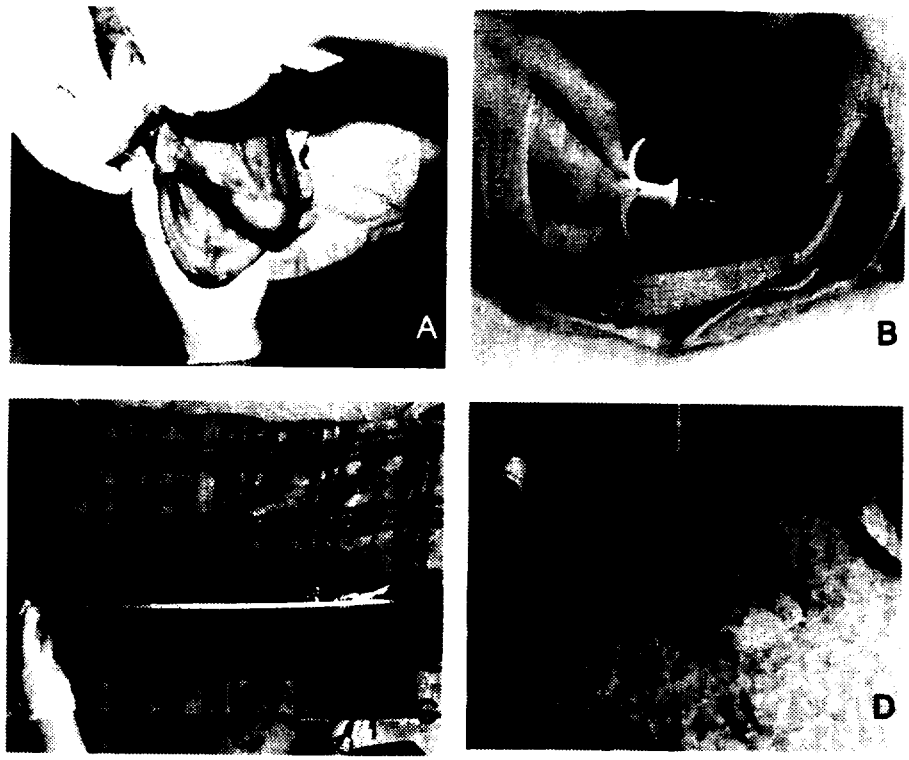

Figure 5. Broodstock management of groupers. (A) treatment of external wound; (B) tagging with microchip; (C) measurement; (D) disinfection.

septemfasciatus, 4,750 ml for *E. bruneus*, and 35 ml for *E. akaara* (Table 1).

Inducing sex change

Most groupers are protogynous hermaphrodites; they first mature sexually as females and change into males later. In nature, it is difficult to obtain sperm. Thus, in order to have a stable supply of sperm, it is necessary to masculinize young groupers using hormone treatment. We induced the masculinization of immature female *E. septemfasciatus* and *E. bruneus* by injecting cholesterol pellets containing 17α-methyltestosterone (MT) or inserting them into MT using a silicone tube. The initial control fish had immature ovaries

Table 1. Summary of grouper broodstock and production of fertilized eggs by artificial induction in 2007.

Experimental fish	No. of female	Body length (cm)	Body weight (kg)	Eggs collected (ml)	Floating eggs (ml)	Sinking eggs (ml)
Epinephelus septemfasciatus	5	41-66	2.8-5.9	800	600	200
E. bruneus	14	51-72	3.2-7.6	4,750	3,950	800
E. akaara	3	28-32	0.6-0.8	35	25	10

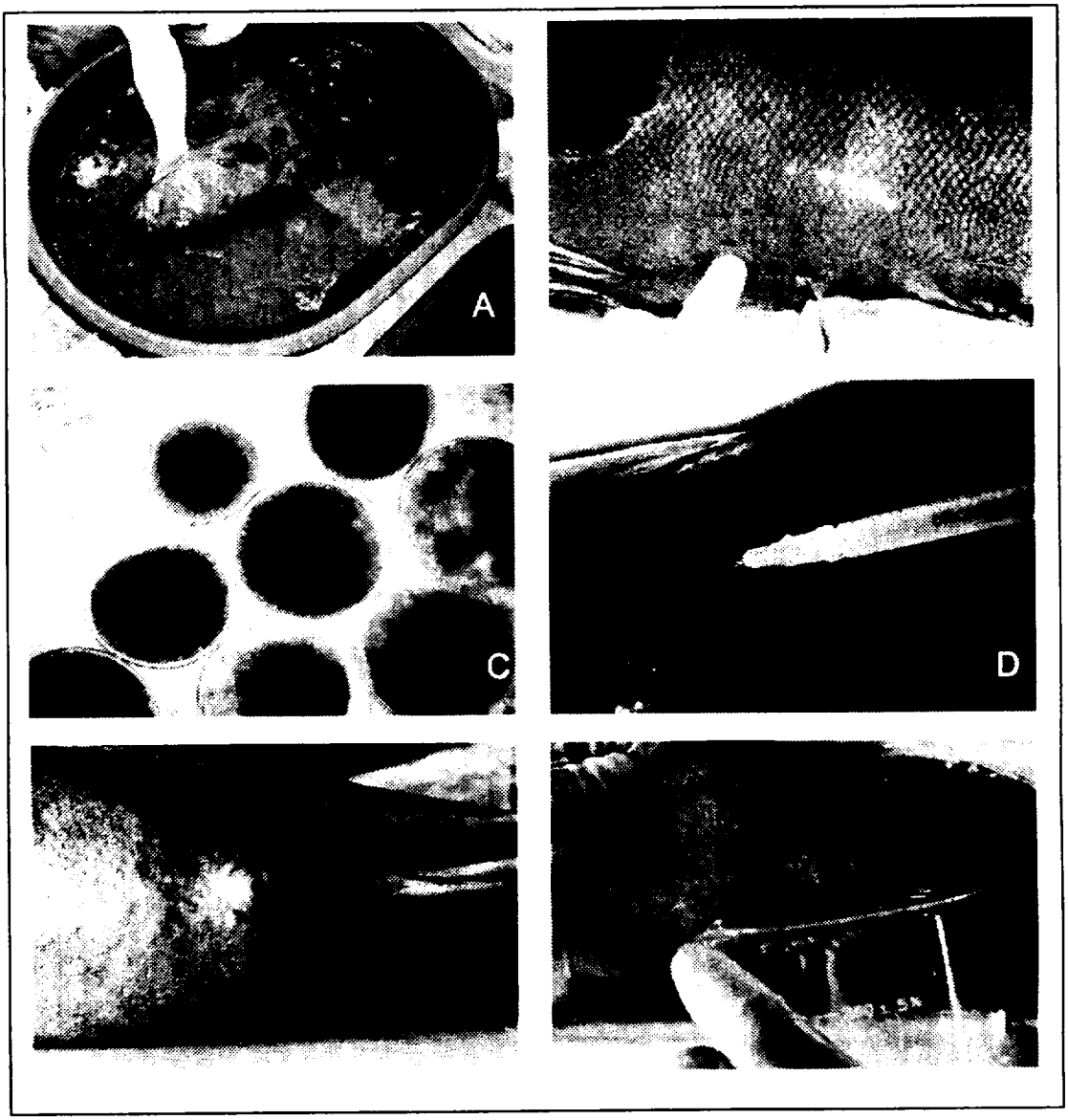

Figure 6. Examination steps for sexual maturation of seven-band grouper. (A) anaesthetization; (B) canulation; (C) yolk globule stage in the ovary; (D) hormone injection; (E) external feature of genital pore in mature female; (F) egg stripping.

containing perinucleolar oocytes. After 4 weeks, fish in the MT treatment group underwent spermatogenesis without producing spermatozoa. After 8 weeks, the MT treatment group produced spermatozoa, which were found in the efferent duct (Figure 7).

In 2002-2006, functional masculinization and sperm production were induced in 27 of 30 *E. septemfasciatus* and 13 of 20 *E. bruneus* (Table 2).

Cryopreservation of Sperm

Since there is an unbalanced sex ratio at sexual maturity in groupers, it is

Table 2. Induced masculinization of grouper with 17α-methyltestosterone from 2002 to 2006.

Experimental fish	No. of fish	Total length (cm)	Body weight (kg)	No. of functional male*
Epinephelus septemfasciatus	30	30-63	0.8-3.2	27
E. bruneus	20	50-68	2.6-4.3	13

* spermatozoa distributed in efferent duct.

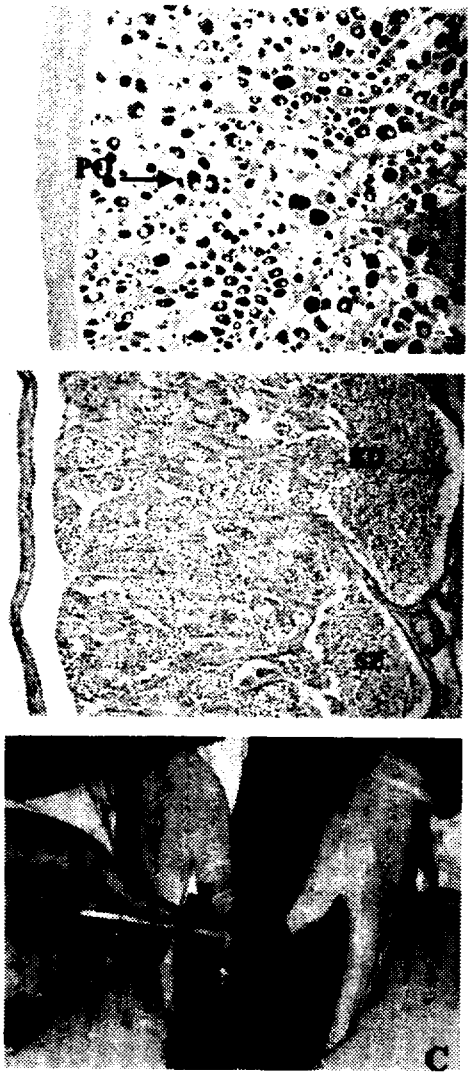

Figure 7. Gonad of seven-band grouper treated with 17α-methyltestosterone (MT). (A) initial control group; (B) MT treated group; (C) extraction of semen. ED: efferent duct, PO: perinucleolus oocyte, SZ: spermatozoa

difficult to obtain sperm under natural conditions. Consequently, the cryopreservation of sperm is an effective method for ensuring a continuous and stable supply of sperm. For the cryopreservation of grouper sperm, glucose is used as a diluent, and dimethylsulfoxide (DMSO) is used as a cryoprotectant. The method of sperm cryopreservation involves (1) collecting semen from functional males, (2) centrifugation of the fresh semen, (3) mixing of the cryoprotectant and diluent with the sperm pellet, and (4) storing the straws of sperm in liquid N_2 (–196°C). When performing artificial fertilization using frozen and thawed sperm, the fertilization and hatching rates were 66.7-99.6% and 79.3-96.6%, respectively, depending on the elapsed time (Figure 8).

Egg Collection and Incubation

The eggs are stripped from induced females, while sperm is stripped from functional males. Artificial fertilization involves mixing eggs and sperm in a ratio of 1×10^6 eggs to 0.1 ml sperm by dry method. After fertilization for 2 min, the fertilized eggs float on the water surface. These are then rinsed with filtered seawater for 1 min and transferred to incubation tanks. Mal-

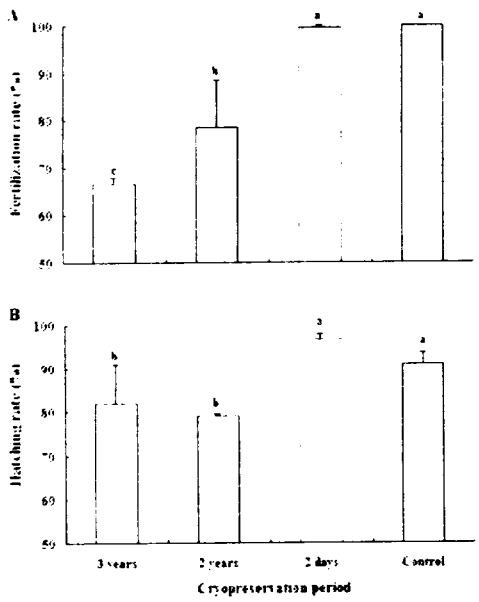

Figure 8. Ability of fertilization of frozen-thawed semen at different cryopreservation periods. (A) fertilization rate; (B) hatching rate. Different letters indicate significant differences ($P<0.05$).

formed and dead eggs are siphoned out (Figure 9).

Egg diameter and oil globule diameter are measured using a microscope and profile projector. The egg diameter of the fertilized grouper eggs ranged from 780-950 μm (Table 3). In fish, egg size is important for increasing the survival of larvae in nature, and survival depends on the size of the hatched larvae, reserves of endogenous nutrition, and initial feeding rate (Kayano and Wan 1996). Fertilized grouper eggs range from 700 to 900 μm in diameter (Kitajima *et al.* 1991; Lee *et al.* 1998) and the egg size and quality are influenced by broodstock feed and environmental factors (Rasem *et al.* 1997).

Table 3. Egg and oil globule diameters of groupers.

Experimental fish	Egg diameter (μm)	Oil globule diameter (μm)
Epinephelus septemfasciatus	790-890	170-230
E. bruneus	830-950	220-260
E. akaara	780-850	160-220

The fertilized eggs of *E. septemfasciatus* were incubated at 25°C, and the first cleavage took place about 1 h after fertilization. The fertilized eggs reached the morula, blastula, gastrula, and embryo stages about 4.5, 10.5, 14.0 and 17.0 h after fertilization, respectively. Hatching began about 35 h after fertilization (Figure 10).

Larval Rearing

The floating fertilized eggs were collected with a net and transferred to a 50-m^3 rearing tank. Hatching began approximately 35 h after fertilization at 25°C. The water temperature of the larval rearing tank was controlled by a boiler system.

The conditions of the rearing tanks were adjusted to 300-700 lux and a 14L:10D photoperiod. To prevent the mass death of the hatched larvae due to surface tension, an oil film was formed in the rearing tank using 0.1 ml m^{-2} of feed oil dropped onto the water surface of the tank every morning and evening from hatching to 3 days after hatching (DAH; Song *et al.* 2005).

After 3 DAH, the water was partially exchanged daily by draining through a 150 μm filter and then slowly replacing the water with fresh seawater to maintain good water quality. As the larvae grow,

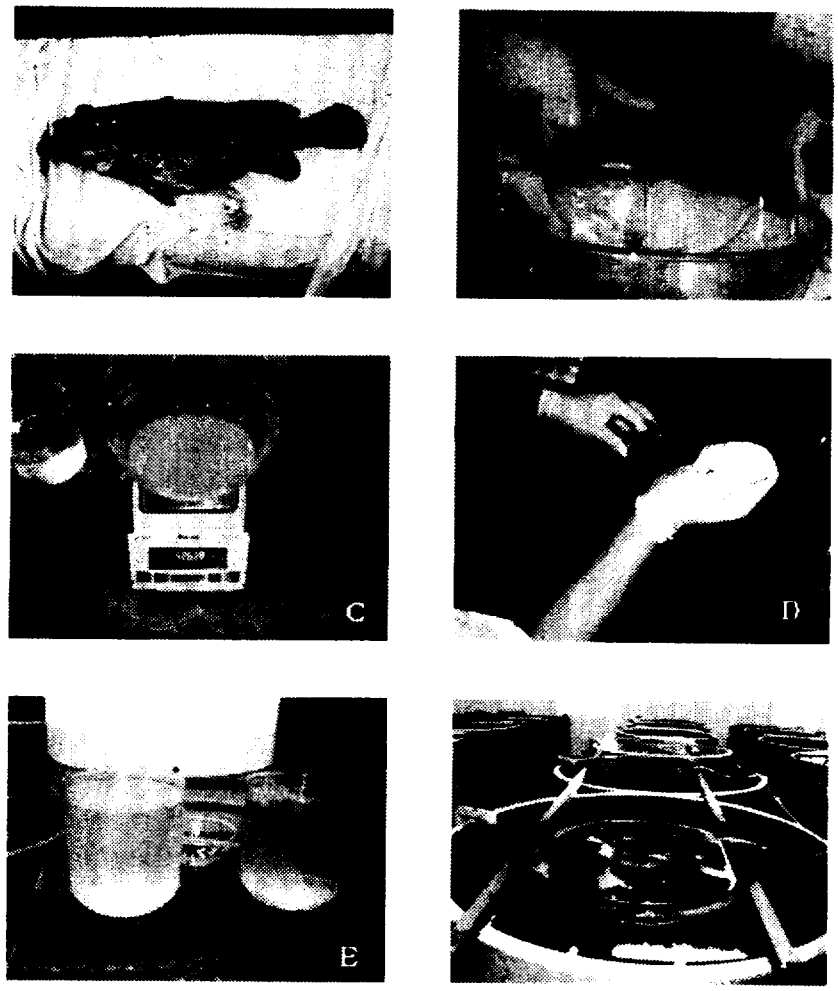

Figure 9. Artificial ovulation and fertilization. (A) anaesthetized mature female; (B) egg stripping; (C) egg weighing; (D) fertilization (10^6 eggs with 0.1 ml semen); (E) collection of floating eggs; (F) incubation and hatching of fertilized eggs.

the water exchange volumes were accordingly increased.

At 3 DAH, the larvae were fed rotifers (80-180 μm) at a density of 10 ind. ml^{-1}, and newly hatched *Nanno-chloropsis* sp. and *Artemia* (2-3 ind. ml^{-1}) were introduced at 16 DAH until 50 DAH. At 15 DAH, the larvae were gradually weaned onto artificial food (Figure 11).

The newly hatched larvae measured 2.0±0.2 mm TL. By 11 DAH, the larvae reached 4.1±0.1 mm TL and began to metamorphose. The rudiments of the second dorsal and pelvic spines appeared at 11 DAH. Both spines, a larval characteristic, began to elongate; the abdominal cavity was densely lined with melanophores, and the two spines appeared on the pre-operculum at 17 DAH. At 28 DAH, dorsal and anal fins with rudimentary rays began to develop. The appearance and extension of the dorsal and pelvic spines were similar to

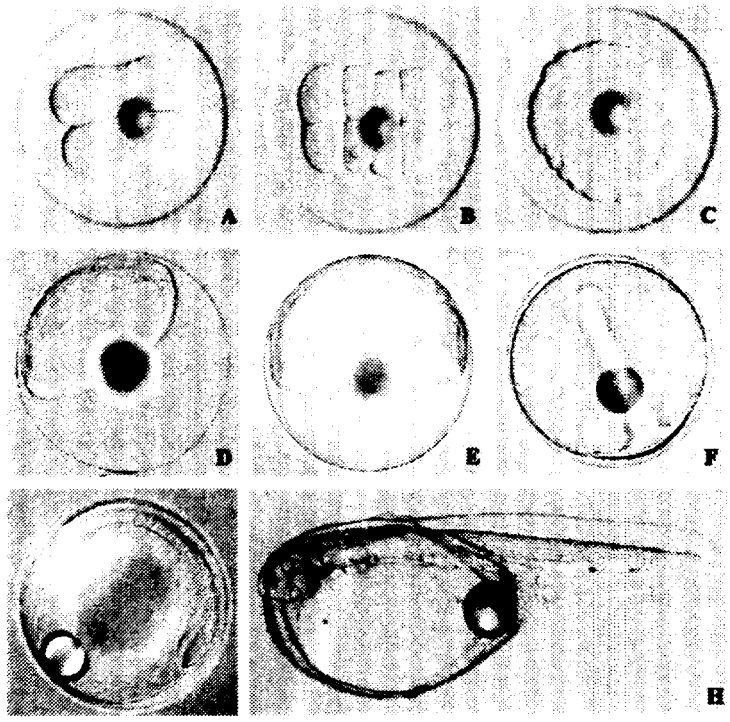

Figure 10. Embryonic development of grouper. (A) 4-cell stage; (B) 8-cell stage; (C) morula stage; (D) blastula stage; (E) gastrula stage; (F) embryo formation; (G) myotomes formation; (H) newly hatched larva.

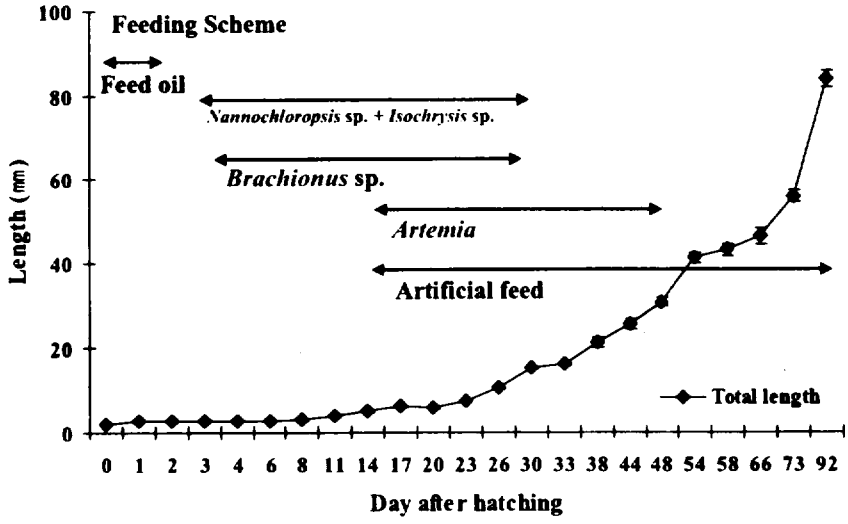

Figure 11. Growth rate and feeding schemes used for larval rearing of *Epinephelus bruneus*.

that in other groupers (Kitajima *et al.* 1991). At 50 DAH, the rays of all fins had differentiated completely and body color appeared. At this time, metamorphosis had finished and the larvae resembled adult fish. By 54 DAH, the larvae reached 41.1±1.2 TL and had completed metamorphosis. There-after, the larvae grew quickly and reached 93.8±2.0 mm TL at 92 DAH (Figure 12).

Production and Problems

Commercial seed production of groupers began in 2003 on Jeju Island and was first attempted in the southern coastal region of Korea in 2006. During the process of seed production, we disinfected the fertilized eggs with iodine, sterilized the culture water with an oxidant, and conducted size grading to reduce cannibalism at 50 DAH (Figure 13). About 50,000 fish were produced which represents 2-3% of the annual production of fertilized eggs. Despite of the precautionary measures and culture techniques applied, mass death still occurred mainly as a result of (1) surface tension, (2) failure to adapt to the initial food, (3) cannibalism, and (4) infection with viral nervous necrosis virus (VNNV). During larval and juvenile

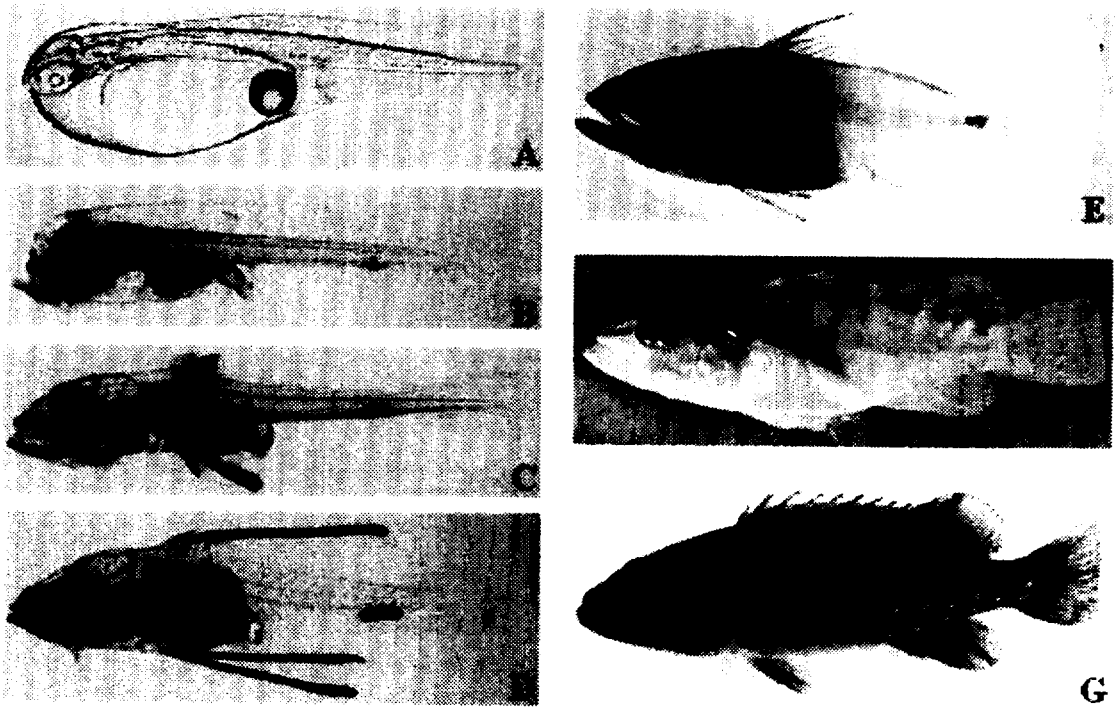

Figure 12. Development of larval and juvenile longtooth grouper in the laboratory. (A) newly hatched larva, 2.0 mm; (B) 4 DAH, 2.8 mm; (C) 11 DAH, 4.1 mm; (D) 28 DAH, 4.3 mm; (E) 38 DAH, 21.2.mm; (F) 50 DAH, 30.7 mm; (G) 92 DAH, 93.8 mm.

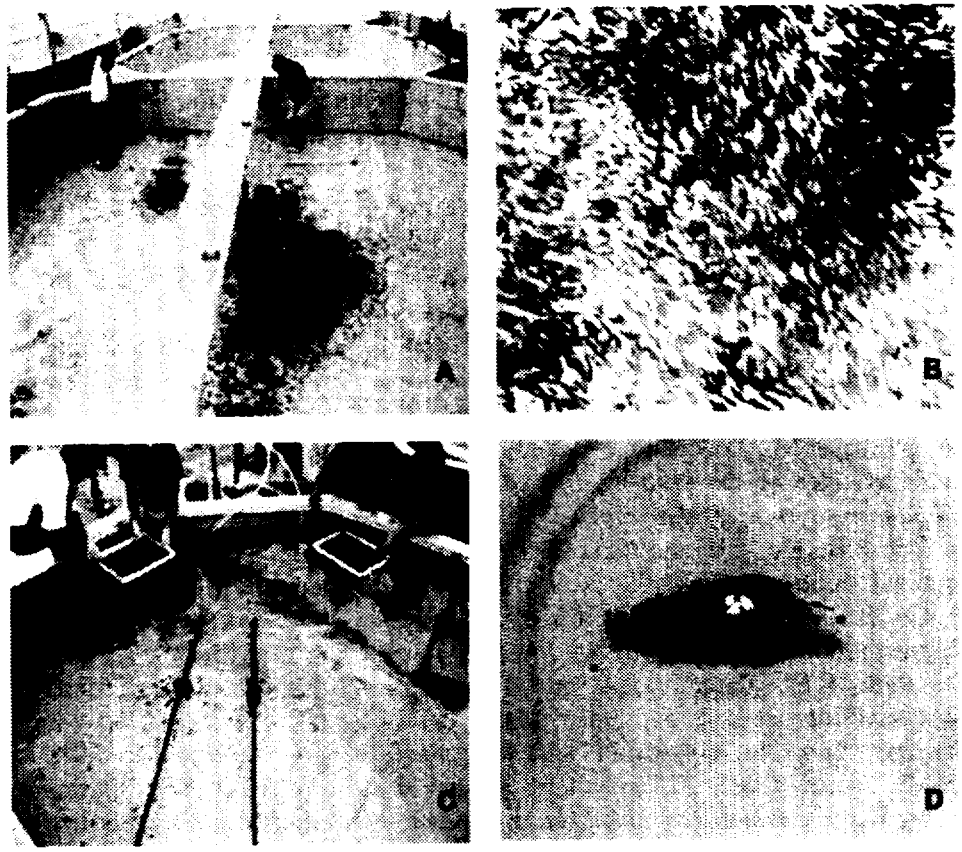

Figure 13. Seed production of and cannibalism in longtooth grouper. (A) larval rearing tank; (B) a school of larvae; (C) selection; (D) cannibalism.

cultures, external polymorphism reduced the commercial value of the fish.

In the future, to develop a grouper aquaculture on Jeju Island, MERI will (1) devise an environmentally friendly method of broodstock maintenance, (2) examine the production of healthy fertilized eggs, (3) develop a food that can also serve as immunostimulant, and (4) examine improved culture management methods for larvae and juvenile fish.

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