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A Review of the Culture of Grouper in Japan

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Feral grouper are useful as broodstock for inducing natural spawning in tanks. The degree of hatching of spawned eggs depends on the stocking density and the sex ratio of the spawners, and on the food items provided. Improving egg hatchability and achieving high survival are prerequisites for scale-up of production. Because grouper larvae have small mouths, unicellular algae and oyster trochophores are being examined intensively as initial food organisms. The use of oyster trochophores and diatoms improves the survival of metamorphosed fish in large scale tanks. Aquaculture ventures for grouper might be possible in areas where the water temperature is higher than 18 °C.

Tremendous strides have been made in the culture of marine fish larvae in the past two decades. Coupled with this, the development of techniques for naturally spawning brood fish and for the mass culture of food organisms has enabled numerous commercially important species to be grown in intensive aquaculture and used for restocking coastal waters. Serranid species are regarded as having aquaculture potential because of their scarcity in the wild and marketability in tropical and semi-tropical areas. Rearing trials for mass production of grouper are vigorously conducted in Japan.

The aim of this review is to describe the present status of natural spawning and larval rearing of Serranidae. The artificial rearing of Serranid fish was began by MITO et al. (1967) using *Epinephelus akaara*. At present, five species of Serranidae are being cultured for aquaculture experiments (Table 1). The most popular species reared for aquaculture purposes is *E. akaara*.

Broodstock maintenance

Spawner candidates of *E. akaara*, weighing from 300 to 500 g, are usually caught by angling and are maintained in settling tank of 1 to 40 m^3 capacity at a density of 1 to 2 individuals/m^3 (MIKI et al. 1984; WATANABE et al. 1985; HAMAMOTO et al. 1986a; NARITA et al. 1986). Water is continuously exchanged at rate of about five to twenty

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Table 1. Serranid fish cultured in Japan

Species	Broodstock	Egg collection	Tank cap. (m^3)	WT (°C)	Egg diameter (mm)	Larval size (mm)	Authors
E. akaara	Wild	Af[1]	0.5	26—31	0.7—0.77	1.45—1.56	MITO et al. (1967)
E. fasciatus	Wild	Ns[2]	8	23.6—26.3	0.77	2.1	MURAI et al. (1984)
E. microdon	Wild	Hi[3]	—	26.3—31.3	0.803	—	OKINAWA PFES (1984)
E. salmonoides	Wild	Ns	240	20—27	0.88—0.96	1.92—2.12	HAMAMOTO et al. (1986c)
E. moara	Wild	Ns	240	19—27	0.86—0.93	2.01	NAKAGAWA (1988)

Af[1], artificial fertilization; Ns[2], natural spawning; Hi[3], hormone injection

changes per day. The parental fish are fed various trash fishes, such as anchovy and sand lance, as well as *Euphausia*, short neck clams, squid and formula pellets prepared for sea bream and yellowtail (MIKI et al. 1984; NANBA and WADA 1984; MORIZANE et al. 1985; HAMAMOTO et al. 1986a; MATSUNAGA 1988). The fish usually show limited feeding behavior at temperatures below 10 °C. To reduce physiological stress shelter using concrete traps is provided in the tanks and sand is added (MIKI et al. 1984; NANBA and WADA 1984; HAMAMOTO et al. 1986a; HIROSHIMA PFES 1987; KAYANO and ODA 1987a).

HAMAMOTO et al. (1986a) studied sex reversal of *E. akaara* in captivity. According to their investigation, all specimens less than 21 cm standard length (SL) were females, and hermaphroditism was observed in specimens ranging from 22.0 to 32.5 cm SL. The ratio of females to males decreased after they reached about 25 cm SL, or about 500 g in body weight.

E. microdon and *E. malabaricus* are cultivated only in Okinawa island, a semitropical area (OKINAWA PFES 1984). KAKAZU and SINZATO (1988) tried to raise immature adults of *E. microdon* and *E. malabaricus*, weighing 765 to 928 g, which had been transported from the Philippines. Trash fishes such as flying fish, *Prognichthys agoo*, and *Tilapia* sp. were fed to parental fish stocked in a 4-m^3 tank.

Spawning and egg production

The spawning behavior of *E. akaara* has been observed at many fisheries stations to be fairly regular in terms of seasonal period and temperature. Spawning begins spontaneously in late June or early July when the temperature is about 20 °C, and continues until early September when the temperature is from 26 to 27 °C (UKAWA et al. 1966; MIKI et al. 1984; MORIZANE et al. 1984, 1985; NANBA and WADA 1984; WATANABE et al. 1985; HAMAMOTO et al. 1986a; KAYANO and ODA 1987a; MATSUMOTO 1988). In regard to spawning time, MIKI et al. (1984) reported that parental fish

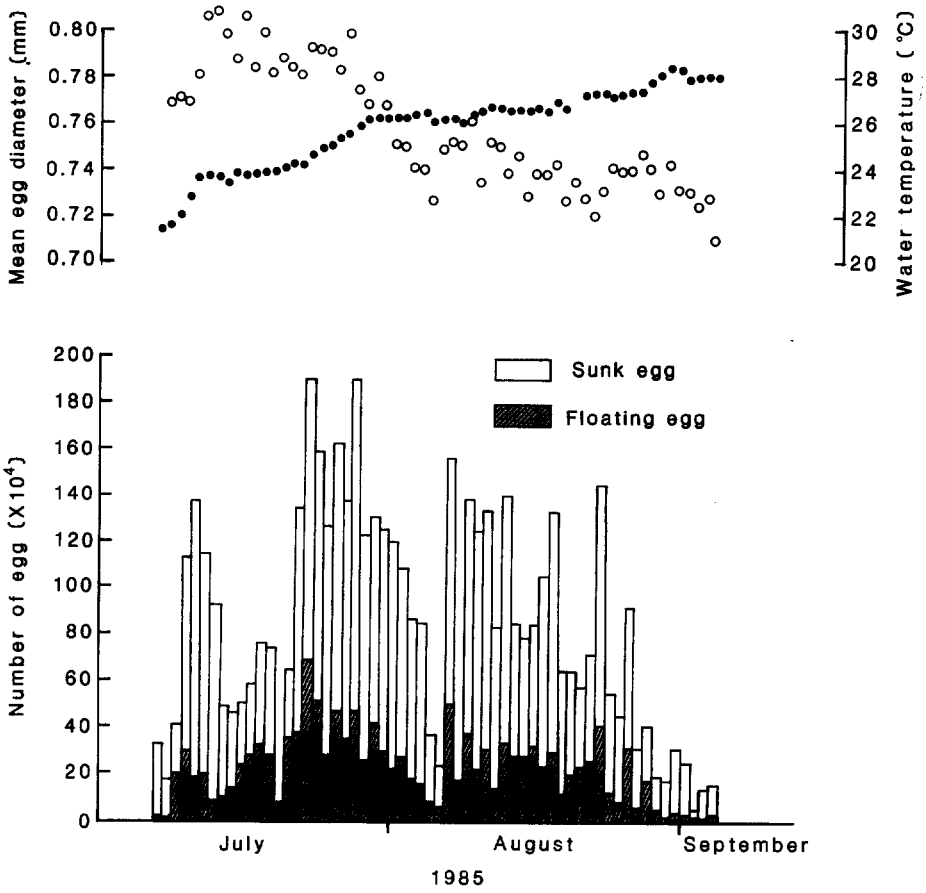

Fig. 1. Number of eggs collected per day, egg diameter (open circles) and water temperature (closed circles) during the period of natural spawning of *Epinephelus akaara*. Redrawn from HAMAMOTO et al. (1986a) with permission of the author.

released eggs between 10 and 11 p.m. almost every day during the spawning season. Under these environmental conditions, one female produces 830,000 to 1,250,000 eggs during the spawning season, lasting 50 to 60 days (MIKI et al. 1984; MORIZANE et al. 1985). However, the ratio of floating eggs to total eggs and the percent hatching is low compared with other marine species. MORIZANE et al. (1985) stressed that the use of various foods results in a high percentage of egg availability.

The size of naturally spawned eggs decreases as the temperature in the rearing tank increases (MORIZANE et al. 1984; HAMAMOTO et al. 1984; HAMAMOTO et al. 1986a, b, Fig. 1). Egg diameter ranges from a minimum of 0.7 mm to a maximum of 0.826 mm (UKAWA et al. 1966; MIKI et al. 1984; MORIZANE et al. 1984; WATANABE et al. 1985; HAMAMOTO et al. 1986b; KAYANO and ODA 1987b).

For *E. salmonoides*, one male (103 cm total length) and seven females (ranging

from 75 cm to 110 cm total length) were maintained as spawners in a 240-m^3 aquarium with other marine fishes. Natural spawning was observed over a period of 22 days, from April 22 to June 13, 1985, with temperatures ranging between 22.2 and 25.5 °C. The timing of spawning was very regular, from 17^{05} to 19^{45} in the evening. Spawned eggs varied from 0.88 mm to 0.96 mm in diameter, with the oil globule being 0.19 to 0.22 mm. No significant difference was observed in egg diameter during season. Incubation time was 32 hrs at 24.5 °C (HAMAMOTO et al. 1986c).

MURAI et al. (1984) conducted experiments on the natural spawning of *E. fasciatus*. Eighty-five parent candidates, (ranging from 18.3 to 36.7 mm in total length and 155 to 790 g body weight) were caught by angling in January, 1983 and maintained in a 80-m^3 circular concrete tank. Scad (*Decapterus muroadsi*) flesh (500 g) was fed daily to the 85 parental fish. Natural spawning occurred intermittently from April 27 to August 6, 1983. Temperature fluctuated between 23.6 and 29.2 °C, with an average of 26.3 °C during spawning. The diameter of eggs spawned on April 28 averaged 770 μm; the oil globule averaged 190 μm. The degree of hatching was 96.7% at maximum and 33.1% at minimum, with an average of 83.1%. Incubation took about 35 hrs at 23.1 to 25.9 °C. Newly hatched larvae were 2.1 mm in total length. No larval rearing trials were conducted for this species.

Since natural spawning for the broodstock of *E. microdon* did not occur, injections of Gonatropin (HCG) were made by the OKINAWA PFES (1984) on May 25, 1983, to obtain viable eggs. Spawning first occurred 3 days after HCG injection and continued until June 1, 1983. Manipulations of the hormone resulted in the release of 26 million eggs over days of spawning. A second trial of hormone injections resulted in the release of 15 million eggs from August 11 to August 14. Spawned eggs ranged from 788 to 814 μm in diameter. The degree of hatching varied from 8.9 to 89.2%, with an average of 39.6%, resulting 5.18 million larvae.

Larval rearing

At hatching, larvae of *E. akaara* range from a minimum of 1.45 mm total length to a maximum of 1.9 mm. The larvae hatch out 23 to 25 hrs after fertilization at 25.1 to 27 °C (MIKI et al. 1967). Unfed larvae can survive for 5 to 6 days after mouth opening at temperatures of 21 to 25.4 °C (KAYANO and ODA 1987b). In seawater of 34.8 ‰ diluted 20-60%, more than 50% of the newly hatched larvae survive to day 6 at a temperature of 21.8±0.2 °C (MORIZANE et al. 1984). The newly hatched larvae open their mouths at two days and have absorbed most of the yolk sac on the third

day after hatching (UKAWA et al. 1966). Spawned eggs and/or newly hatched larvae are maintained in tanks of various capacities, ranging from 0.5 m^3 to 150 m^3, with densities of 10,000 to 20,000 eggs/m^3 and 5,000 to 130,000 larvae/m^3 (NANBA and WADA 1984; JAPAN FARM. FISH. ASSOC. 1986; NARITA et al. 1986; KAYANO and ODA 1987b). For practical purposes, spawned eggs are counted volumetrically; relationships used to evaluate the daily amount of spawning in hatchery investigations are 2100 eggs/g (MIKI et al. 1984), 2700 eggs/g (KAYANO and ODA 1987b) and 3775 eggs/g or 3100 eggs/m*l* (MORIZANE et al. 1984).

Usually no special manipulation of light and temperature are used for the purpose of mass production. Artificial illumination of 200-2000 lux intensity is assumed to increase the feeding incidence of first feeding larvae (HAMAMOTO et al. 1986b). Larvae have been given various food items as their growth progresses, including oyster trochophores or diatoms from mouth opening to about one week after hatching, rotifers from mouth opening to about 3 weeks, and *Artemia* nauplii and granular pellets from 2 weeks to 4 or 5 weeks (HIROSHIMA PFES 1987; KAYANO and ODA 1987b; FUKUNAGA 1988). The use of phytoplankton, such as *Pavlova lutheri*, *Protogonyaulax affinis*, *Gymnodinium falcatum*, *G. splendens*, etc., as initial food organisms either solely or supplemented with rotifers, has been examined (HAMAMOTO and YOSHIMATSU 1984; ARAKAWA et al. 1985; MORIZANE et al. 1985; HAMAMOTO et al. 1986b; KAYANO and ODA 1986b). HAMAMOTO and YOSHIMATSU (1984) confirmed that grouper larvae ingested *Gymnodinium splendens*, but no effects on growth and survival potential were found. KAYANO and ODA (1987b) examined the rearing of first-feeding larvae with diatoms from natural blooms consisting primarily of *Skeletonema costatum*, *Chaetoceros* sp. and *Thalassiosira* sp. After 10 days, survival was 16 to 21% greater for other experimental lots. About 30,000 juveniles of 20 mm total length were produced by inoculating diatoms into the rearing tank. Tintinnids are also potential initial prey for larval grouper. Grouper larvae less than 4 mm digested a large number of Tintinnids, as well as oyster trochophores (HIROSHIMA PFES 1986). To date no cultured unicellular algae have been found useful as initial prey for grouper larvae on a practical level. Oyster trochophores are used as initial food for larvae, especially in small-scale culture. Because of the difficulty of obtaining in quantity, oyster trochophores can not be used for large-scale production.

Since *Artemia* nauplii are nutritionally deficient (WATANABE et al. 1983), an enrichment procedure is widely conducted to meet the nutritional requirement of juvenile grouper. It is possible to wean larvae at 25 to 30 mm with flesh of various fish, *Euphausia* and formula diets for sea bream. Concerning rearing water, MORIZANE et al. (1984, 1985) used 32% seawater diluted 50 to 80% to rear larvae beyond metamor-

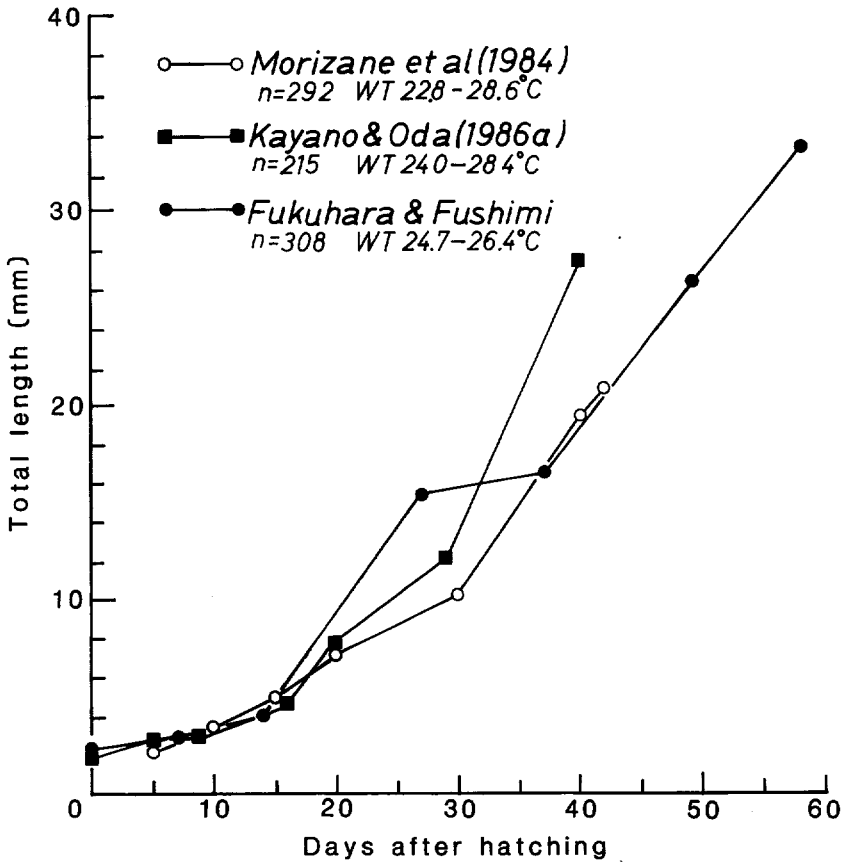

Fig. 2. Larval growth of laboratory-reared *Epinephelus akaara* larvae. Reproduced with permission of the authors.

phosis, achieving survival 75 to 80% greater than with other dilutions and with full-strength seawater.

Weanlings have been succeedingly transferred to floating net cages of 1.5- to 5-m^2 capacity (MORIZANE et al. 1985; HIROSHIMA PFES 1986, 1987) with stocking densities of 86 to 140 fish/m^3 (HIROSHIMA PFES 1986, 1987).

Elongated spines of the dorsal and ventral fins, unique morphological characters of Serranid fish, were observed in grouper from 3 to 19 mm standard length, and transformation from larvae to juveniles occurred at 7.3 to 9.6 mm SL (FUKUHARA and FUSHIMI 1988).

Fifteen rearing trials with newly hatched larvae of *E. microdon* were conducted using S-type rotifers at 5 to 10 ind./m*l* in 1- to 60-m^3 tanks (OKINAWA PFES, 1984). The major cause of high mortality was cannibalism among survivors after their settlement in the rearing tank. Growth of fry was retarded from January to March when temperatures as low as about 18 °C were recorded; steady growth was observed

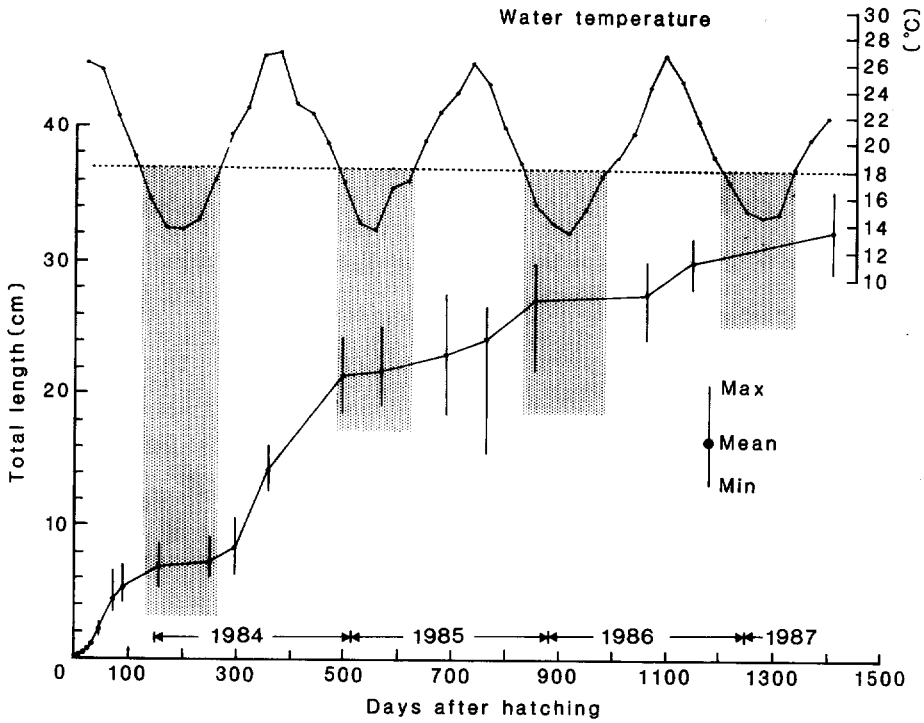

Fig. 3. Growth of reared *Epinepelus akaara* from hatching to age 4. Temperature represents the monthly average value. Shadowed areas indicate the season when the temperature is lower than 18 °C. Sample size for each measurement varies from 20 to 60 fish. Data source; MORIZANE et al. (1984) and TAKECHI and MORIZANE (unpublished).

with increasing temperature. Fry reached 21.6 cm total length and 203 g body weight about 15 months after hatching.

Larval growth

The early phase of larval growth is shown in Fig. 2. For each set of observations, growth is slow for about the first 2 weeks and increases fairly steadily thereafer. This may be attributable to increasing temperature and to the nutritional effects of weaning diets.

Larval survival from hatching to the weaning stage varies considerably in rearing trials; less than 1.0% survival has been recorded (ARAKAWA et al. 1985; MORIZANE et al. 1985; WATANABE et al. 1985; JAPAN FARM. FISH. ASSOC. 1986; KAYANO and ODA 1986a) as well as 2.9% to 8.1% survival (Table 2). A stable survival rate has not been attained in hatchery or fisheries center.

The overall growth of this species has been investigated in detail by MORIZANE et

al. (1984) and TAKECHI and MORIZANE (unpublished). Rapid growth occurs during the period when temperatures are higher than about 18 °C. Slower growth or cessation of growth occurs at temperatures lower than 18 °C (Fig. 3), as was reported for *E. microdon* in Okinawa. The growth increment during the warm season decreases as the fish progress from one to four years old.

Table 2. Rearing experiment on larval *E. akaara* in a large tank

Egg source	Tank. cap. (m^3)	Duration (day)	Number produced	Length at harvest (TL, mm)	Survival (%)	WT (°C)	Authors
Ns[1]	32	50	27,000	20.0	2.9	25.7—28	KAYANO & ODA(1987b)
Ns	75	27—31	28,932	15.6—19.7	3.98	25.9—27	HIROSHIMA PFES(1986)
Ns	60	36—40	83,000	19.8	8.1	25.6—26.5	FUKUNAGA(1988)

Ns[1], Natural spawning

Discussion

Feeding of various trash fishes and lowering the density of parent fish to 1-2 individuals/m^3 results in a higher percent hatching (MORIZANE et al. 1984, 1985; HAMAMOTO et al. 1986a; KAYANO and ODA 1986; WATANABE et al. 1987) than found previously here in Japan and other coutries (CHEN et al. 1977; HUSSAIN and HIGUCHI 1980; HUANG et al. 1987). WATANABE et al. (1985) state that hatching occurs when the spawners are fed a single food item and are maintained in captivity with an imbalanced sex ratio.

These findings suggest that feeding of a mix of fish flesh, low spawner density and a balanced sex ratio are major factors in obtaining viable eggs through natural spawning techniques for Serranid fish.

The hatchability of naturally spawned eggs is generally higher than that for eggs obtained from artificial fertilization or by hormone injection. Even for natural spawning of grouper, however, hatchability varies. This is partly because of the nutritional content of the feed used for broodstock culture. Studies of how to meet nutritional requirements of the broodstock are necessary for developing spawning technology.

Concerning the temperature of natural spawning, *E. akaara* begins to spawn at about 20 °C and finishes when the temperature drops below 26°C or 27°C (MIKI et al. 1984; NANBA and WADA 1984; MORIZANE et al. 1985; WATANABE et al. 1985, 1987; HAMAMOTO et al. 1986a). The temperature limit for active feeding is about 13 °C for spawners in tanks. These temperatures are also important in the handling of parental fish during the course of artificial propagation.

Although unicellular algae and molluscan larvae (oyster and mussel) are commonly used for rearing fish larvae with small mouths, including Serranid fish (FUKUHARA 1987), marked success has not been attained on a commercially viable scale. The most popular method is to use S-type rotifers and/or strained through a fine-mesh net. To improve the survival of first-feeding larvae, different food organisms, such as Tintinnids must be prepared in large quantities. The cause of low survival of first-feeding larvae, is considered to be poor feeding activity, which might be related to the quality of eggs and broodstock.

Cannibalism resulting in mass mortality has been observed for the early stages of *E. akaara*, 8 to 50 mm long (MORIZANE et al. 1984; KAYANO and ODA 1986a; NARITA et al. 1986), *E. microdon* (OKINAWA PFES 1984) and *E. moara* (NAKAGAWA 1988). No optimal procedure for avoiding cannibalism is yet known. Hanging synthetic filament in the rearing tank decreases cannibalism somewhat (JAPAN FARM. FISH. ASSOC. 1986; FUKUNAGA 1988). Cannibalism occurs partly because of the size variation of survivors after they are able to take non-living food. It is, therefore, important to identify and eliminate the causes of such variation. Generally, frequent feeding and reduced stocking density relieve the problem of cannibalism in metamorphosed fish.

Because retardation or cessation of growth occurs at low temperatures in *E. akaara* and *E. microdon*, aquaculture ventures should be conducted in area warmer than about 18 °C year-round. Restocking activities for these species might be beneficial in areas where the temperature is below the limit of growth of the grouper. The temperature of 18 °C appears to be an important environmental factor for determining the location of farming grounds for Serranid fish.

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ハタ類の種苗生産と飼育

福原　修

わが国におけるハタ類の養殖は，近年その資源量の不足と市場性から急激に注目されるようになった。種苗生産は，こうした背景のもとで飼育技術の確立のために努力が注がれているが，親魚の養成から産卵，仔魚飼育と養成などの諸側面で多くの困難な問題を有している。特に産卵親魚の管理，ふ化仔魚の初期飼育と養成を中心に既往の知見を整理し，今後の方向について若干の考察を加えた。