

OBSERVATION ON GONAD MATURATION OF THE FIRST GENERATION (F1) OF HUMPBACK GROUPEL, *Cromileptes altivelis*

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ABSTRACT

The purpose of this experiment was to know gonad maturation and spawning frequency of the first generation in humpback grouper, *Cromileptes altivelis*. This experiment had two groups of feed types (A and B). Both groups were put in circular concrete tanks with water volume of 75 m³ each. Each tank was stocked 40 fishes with body weight of 500–1,000 g/fish and total length of 28–38 cm. Group (A) was fed with trash fish, squid, and vitamin mix, while group (B) was fed with artificial diet. Both groups were fed once a day, in the morning. The water was exchange continuously, of 300%–500% per day. The result showed the fish spawn after 31 months rearing. During the experiment, the range of water temperature and salinity were 28.4°C–31.7°C and 30.2–34.4 ppt, respectively.

KEYWORDS: gonad, F1, humpback grouper

INTRODUCTION

Humpback grouper, *Cromileptes altivelis* is one of the serranid species having high market value. Humpback grouper with total length of 2 inches has been reared for ornamental fish, while the fish with 0.5 kg in body weight has been produced for consumption. Artificial seed production of humpback grouper has been successfully applied at Gondol Research Institute for Mariculture (GRIM) This species has an economic potential especially in Southeast Asia.

The technology of humpback grouper culture must be improved for sustainable seed production all the year. Therefore fish culture techniques have to be focused on broodstock management, gonadal maturation, egg handling, nutrition, disease, and genetic. Thus, both good quality and quantity of eggs can be obtained. Some experiments have been conducted related to improve gonad maturation of broodstock in fish culture such as improving nutrition and vitamin administration (Halver, 1976; Waagbo *et al.*, 1989; Azwar, 1997). In addition, the application of LHRH-a and 17 α methyltestosteron hormones for gonadal maturation in some marine fishes such as milkfish,

Chanos chanos, tiger grouper, *Epinephelus fuscoguttatus*, orange spotted grouper, *E. coiodes*, and humpback grouper, *C. altivelis* had been reported (Tamaru *et al.*, 1987; Prijono *et al.*, 1990; Tamaru, 1990; Makatutu *et al.*, 1997; Tridjoko *et al.*, 1997).

Although mass seed production of humpback grouper, *C. altivelis* has been successfully produced at GRIM and fish farming (backyard hatchery) in Gondol vicinity, but the survival rate was unstable. The survival rate of humpback grouper from 1997-2001 at GRIM varied between 10% and 58%. However, mass seed production is still hindered by disease viral nervous necrosis (VNN) low egg quality, limited frequency of spawning, and gonad maturation.

Grouper hatchery uses wild broodstock and the problem was difficult to obtain the number, age, and the same size. On the other hand, grouper population in the wild has been decreased due to over fishing. An alternative to solve the problem, is broodstock obtained from the first generation (F1). The purpose of this trial is to determine gonadal development and spawning frequency of the first generation of humpback grouper, *C. altivelis*.

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MATERIALS AND METHODS

Eighty fishes obtained from the first generation (F1) the range of body weighing 50—400 g and 28—38 cm in total length were used for this experiment. Fishes were divided into two groupes i.e fed with trash fish, squid, and vitamin mix (fortified natural diets, FND) and fed artificial diet (AD). All fishes were reared in circular concrete tanks with water volume of 75 m³ from February to December 2003. Each concrete tank was stocked 40 fishes. Fives samples were taken from the two groups observation of the gonad histology and blood, conducted every two months. All fishes were anaesthetized using 2-phenoxy ethanol at a concentration of 150 ppm, there after all of them were canulated for egg checking and sperm and then sperm were observed under a microscope. The observations included histological of gonad development, spawning frequency, growth, and blood profile. Water quality such as water temperature, PH, salinity, DO, phosphate, nitrite, nitrate, and ammonia were monitored weekly.

Blood Collection

One mL of blood was obtained from the caudal vein using 2.5 mL syringe containing anti-coagulant (Heparin leo, Pharmaceutical, Denmark) before taking blood sample, then the blood was transferred into capillary tube, and capillary tube was centrifuged with 12,000 rpm for 10 minutes and the percentage of hematocyte were counted.

Hemoglobin Observation

The blood was put into the blood chamber HB meter, then shaken using hemolysis applicator containing 10 ig sodium oxalate and 200 ig saponin. After that, the hemoglobin was counted using hemoglobinometer (IMI, Japan).

Counting of Total Erythrocyte and Total Leukocyte

Blood was taken by a pipette and added Rees-Eckert solution (sodium citrate, formaldehyde 3%—40%, and Brilliant cresyl blue) up to a scale of 101. After that, the pipette was shaken for three minutes and then kept for three minutes. For counting total leucocyte, blood was taken by pipette at a scale of 0.5 and an added Rees-Eckert solution up to a scale of 11. Both of erythrocyte and leukocyte were counted using 'Improved Neubauer'. Counting

of total erythrocyte and total leukocyte based on formula were as follows:

1. Total erythrocyte = $5 \text{ area} \times 10 \times 5 \times 200$ or $n \times 10^6/\text{mm}^3$
2. Total of leukocyte = $64 \text{ area} \times 200 \times 10$ or $n \times 10^3/\text{mm}^3$

RESULTS AND DISCUSSION

The result of the blood profiles of the first generation of humpback grouper are shown in Figure 1. All hemoglobin, hematocyte, total of erythrocyte and total of leukocyte of fishes fed with artificial diet (AD) have higher blood cells compared to those fed with trash fish (FND). We suggest that nutrition composition in the artificial diet is more complete than trash fish for stimulating to produce in blood cells. Klontz (1994) reported that the range of blood cells is still the normal standard value. Hemoglobin has a function for realizing oxygen over all cells and taking out carbon dioxide (Browmn, 1993).

Based on observation of the oocytes development, the oocytes found initially after the fish were reared in the tank for 4 months, both of two groups indicated the oocytes started to develop (Table 1). The fishes fed trash fish (FND) generate egg diameter ranged from 200 to 300 mm, while egg diameter of less than 200 mm was obtained from the fish group fed artificial diets. Oocytes with diameter of 200—300 mm was obtained from the fish weighted 730—1,120 g and 32.3—38.2 cm in TL. The largest oocytes diameter, more than 450 mm, was found in fish with body weight of 920 g and 36.7 cm in TL. One fish with body weight of 1.35 kg and 39.3 cm in TL at group (FND) showed sex change (transition) from female to male.

Mishima & Gonzares (1994) reported that gonadal maturation in humpback grouper, *C. altevelis* from the wild caught related to total length of their body, i.e., fish with 39.5 cm TL (immature), 40—50 cm TL (mature), 50—55 cm TL (transition of sex change), and more than 55 cm TL (male), respectively. In contrast, our observations showed that fish with body weight less than 2,5 kg and 40—50 cm in TL indicated male (Tridjoko *et al.*, 1996). Humpback grouper of the first generation (F1) reared for 10 to 18 months, did not spawn yet as reported by Subaidah *et al.* (2001) and Prisdimmingo *et al.* (2001). Result showed that both of two groups (FND and AD) indicated that

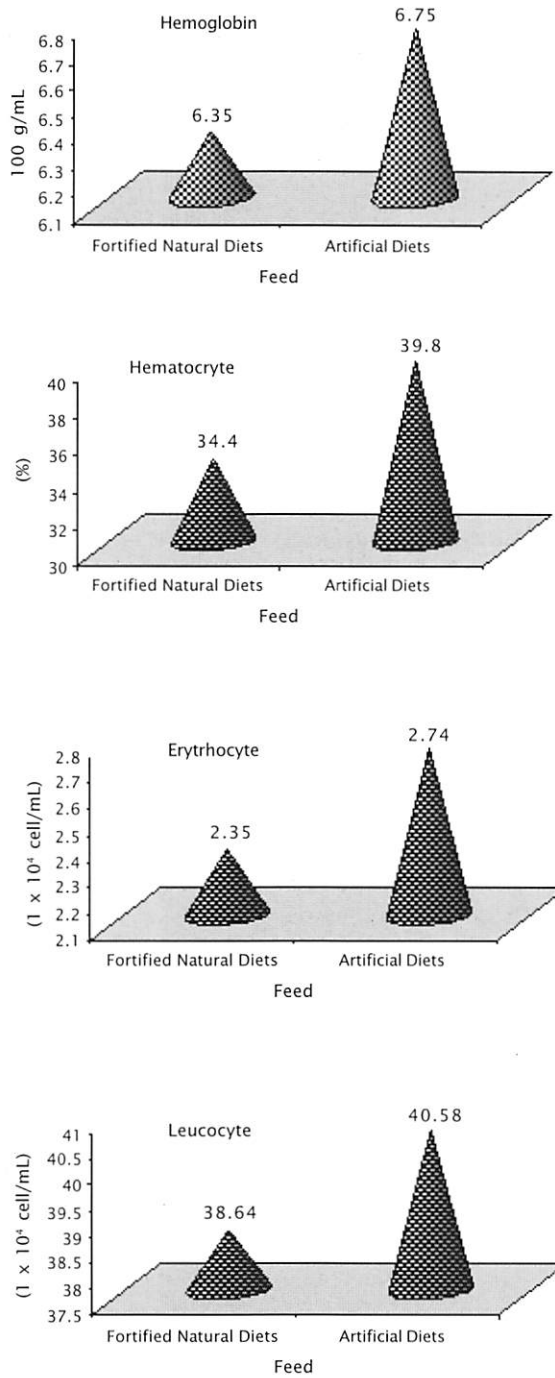


Figure 1. Profile of the blood cells between humpback grouper fed Fortified Natural Diets and Artificial Diets

Table 1. Observation of oocytes development in humpback grouper with different feed

Month	Total length (cm)		Body weight (g)		Oocyte diameter (μm)	
	FND	AD	FND	AD	FND	AD
February	-	-	-	-	-	-
March	-	-	-	-	-	-
April	-	-	-	-	-	-
May	28.0	26.0	540	400	200--300	< 200
June	32.3	27.1	730	485	> 450	< 200
July	34.5	30.0	815	520	> 450	200--300
August	35.8	29.2	935	660	> 450	200--300
September	38.2	31.5	1,120	767	> 450	300--450
October	39.3	36.3	1,350	868	?	300--450
November	43.5	36.7	1,976	920	♂	> 450
December	-	-	-	-	-	-

oocytes have developed and the fish reared in group (FND) could spawn. Thus, broodstock of the first generation have a good prospect of culture as spawner in the future.

According to the observed histological oocytes development (Figure 2) have indicated that fish with range of body weight of 1,100—1,262 g and 38.0—41.0 cm in TL in FND group produced a total number of eggs ranged of 107,504—318,520, while in B group produced a total number of eggs ranged 96,245—

110,000 with range of body weight of 650—850 g and 34.0—38.8 cm in TL (Table 2).

Histological of oocytes development in two groups (FND and AD) are shown in Figure 2 and 3. Histological of oocytes development showed that fishes reared in group (FND) were better than fishes reared in group (AD). Where oocytes have developed mature and ripe stage was found in group (FND) while oocytes developed to chromatin nuclear to yolk vesicle stage was found in group (AD). We suggested that

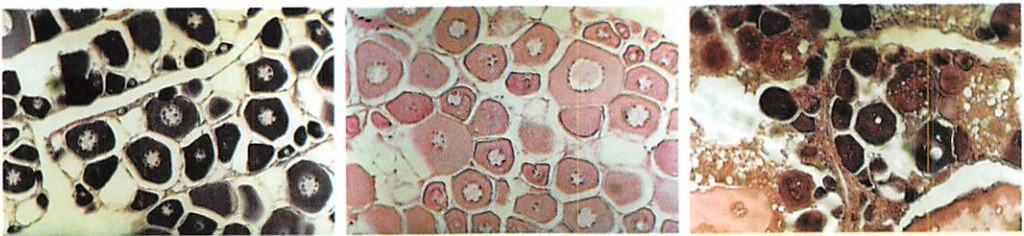


Figure 2. Histology of oocytes development of the fish fed with fortified natural diets

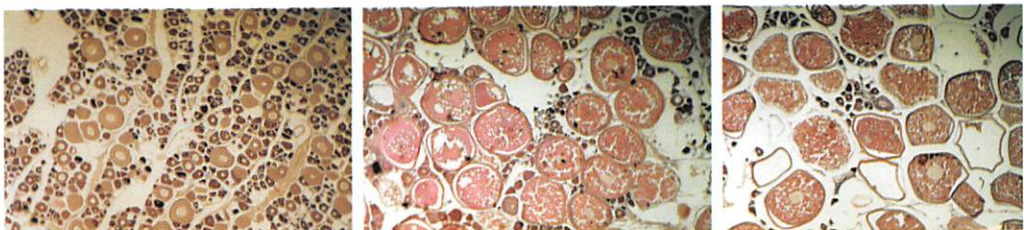


Figure 3. Histology of oocytes development of the fish fed with artificial diets

Table 2. Gonadal development observed in humpback grouper reared with different feed

Tank	Body weight (g)	Total length (cm)	Standard length (cm)	Gonad weight (g)	Number of egg
Fortified Natural Diets					
1	660	33.5	27.2	3.5	-
2	850	35.7	28.5	4.8	-
3	1,100	38.0	31.5	20.3	107,504
4	1,200	40.7	32.2	35.4	277,225
5	1,262	41.0	35.5	21.8	318,520
Artificial Diets					
1	450	30.7	24.5	5.5	-
2	520	32.4	28.9	4.2	-
3	650	34.0	27.2	4.3	96,245
4	830	34.2	28.0	4.5	-
5	850	38.8	29.0	4.7	110,000

fish fed with trash fish, squid and vitamin are better than fish fed with artificial diet stimulate gonadal maturation. Watanabe (1988) reported that squid protein source is important to gonadal maturation and eggs quality

Spawning frequency of the first generation of humpback grouper during the experiment is shown in Table 3. First, spawning occurred on 25th of July 2003 was found at group (FND) with total number of eggs of 55,000, but all the eggs were unfertilized. The range of eggs diameter were 160—178 mm. The second spawning occurred on 27th of June 2003 with total number of eggs were 410,000, fertilized of 10% and hatching rate of 42%. The range of eggs and oil globule were 738—890 mm and 163—180 mm, respectively. Total spawning frequency was eight times during the experiment with total number of eggs of 1,246,000 and the range of hatching rate of 0%—44%, where the fifth and the eighth spawned with hatching rate of 5 and 44%, respectively. No spawning during the experiment was found in group (AD).

Fluctuation in spawning frequency, egg number and fertilized egg during the spawning period was caused by gonadal maturation level between female and male were not simultaneously during breeding season. Moreover, the number of fishes having ripe egg stages a few. Tridjoko *et al.* (1996) reported that the ideal ratio between male and female is 1 (male): 2

(female). Werawatgoempa *et al.* (1997) reported that the oocytes contained different stages of development depending on the season, when they are in nonbreeding season contained 1-3 stages and the oocytes were mostly in stage 4-6 during the breeding season. Ours result showed that the oocytes stage were 5 and 6 oocytes *i.e.* at mature and ripe stages was found in group (FND) (Figure 2a,b). Our observation on oocytes development in group (B). showed that the oocytes stages were mostly in stage 1-3. Thus, fishes were reared in group (AD) did not spawn.

To sustain successful in aquaculture all the year depends upon broodstock quality such as number, age, body weight, and total length. Egg and sperm qualities are depending on food quality and food type. Egg quality is reflection of chemical composition in yolk-sac, affected by food nutrition (Watanabe, 1988). Hardjamulia (1988) reported that variation of egg size due to genetic, food, and environment. Gonadal weight will increase with increasing egg diameter and it will decrease in weight during spawning season (Effendi, 1977).

Water quality such as water temperature, pH, salinity, DO, phosphate, nitrite, nitrate and total ammonia are shown in Table 4. Water quality during experiment was still in the normal condition for rearing of humpback grouper (Tridjoko *et al.*, 1996).

Table 3. Spawning frequency of the first generation of humpback grouper reared with different feed

Variable	Rearing tank	
	FND	AD
Broodstock number (fishes)		
Initial	40	40
Final	25	30
Age (month)	37	26
Body weight (g)		
Started	500-1,000	450-630
End	805-1,976	712-925
Sacrificed	12	10
Spawned eggs	8	-
Number of eggs	1,246,000	-
Egg diameter (μm)	725-875	-
Oil globule diameter (μm)	158-176	-
Hatching rate (%)	0-44	-

Table 4. Water quality during the experiment

Parameter	Rearing tank	
	FND	AD
Temperature ($^{\circ}\text{C}$)	28.4-31.7	28.5-31.7
Salinity (ppt)	30.2-34.4	30.5-34.6
pH	6.5-8.0	6.5-8.0
DO (mg/L)	5.73-7.2	5.73-7.4
Phosphate (mg/L)	0.035-0.220	0.041-0.237
Nitrite (mg/L)	0.072-0.102	0.055-0.128
Nitrate (mg/L)	0.242-0.323	0.270-0.307
Ammonia (mg/L)	0.346-0.373	0.342-0.361

CONCLUSIONS

The first generation of humpback grouper reared in circular concrete tank with a water volume of 75 m³ for 31 months develops into naturally mature fish which ready to spawn. However, the egg quality is still low. Obviously, further research on broodstock nutrient requirement and hormone application is a necessity.

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