INDUCED FUCTIONAL MALE OF CORAL TROUT GROUPER (*Plectropomus leopardus*) USING 17α-METHYLTESTOSTERONE HORMONE

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ABSTRACT

The success of grouper seeds production is depends on the availability of qualified broodstock. The nature of grouper is protogynous hermaphrodite, causing difficulties to mantain female and a bit difficult to get male broodstock, one possibility to accelerate sex revers is by hormone manipulation. The aim of this experiment was to find effectiveness of 17α-methyl testosterone hormone to produce coral trout grouper (*Plectropomus leopardus*) functional male. The experiment was conducted in floating net cage by using 6 net cages with size of 2 m x 2 m x 2 m at density of 25 fish/cage, size of fish were 377.27±21.49 g. The fishes were treated by hormone implantation at concentration of 50 µg/kg body weight and without hormone implantation as a control with 3 replicates. The results showed that the highest concentration of testosteron in fish blood (1.144±0.135 pg/mL) was detected after four months of hormone treatment, but the concentration of testosteron in fish blood declined after 8th months of treatment. The treated fish with hormone grew faster than control. Based on histological analysis of gonad, female gonado somatic index was higher for treated fish compare to control. Its seems that hormone tratment lead to promote development of female maturity and than sex reverse into male become faster.

KEYWORDS: **induced, male, coral trout, *Plectropomus leopardus*, 17α-methyl testosterone, hormone**

INTRODUCTION

The culture of coral trout grouper (*Plectropumus leopardus*) have been developed since 2005 (Suwirya, 2005). The main constrain in expanding aquaculture of this fish is the availability of seed. At present the seed being used for culture mainly from wild caught fish.

Research on artificial seed production has been carried out in Gondol Research and Development Institute for Mariculture since 1995. The technology on larval rearing followed the method on humpback grouper and tiger grouper with minor modification.

Other constrain in preparing coral trout seed is maintaining broodstock. This fish is *protogenous hermaphrodites*, where their sex reverse from female to male after reaching certain age and size (Allsop & West, 2003) and due to a changes in social structures that occur in the population (Perry & Grober, 2003). Some time it is difficult to obtain male and female at the same time.

In order to accelerate sex reverse from female to male hormone induction is commonly applied. This hormone will induce or accelerate sex reverse from female to male or formed a

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male without passing the female stage. Several hormones commonly used in producing male fish such as testosterone (T), 11-ketotestosterone (11-KT), 17α-methyltestosterone (MT), and testosterone propionate (TP). Sex changes requires affectivity of hormones and timing of which depends on the type and dose of hormones and hormone delivery method (Yeh *et al.*, 2003a).

The used 17α-methyltestosterone have been applied to accelerate sex reversal of humpback grouper, *Cromileptes altivelis* (Tridjoko *et al.*, 2010). In the present study, the use of 17α-methyltestosterone is aiming to accelerate sex reverse of coral trout grouper (*Plectropomus leopardus*).

MATERIALS AND METHODS

Tested Animal was Coral Trout Grouper, *P. leopardus*

Research on production of functional male of coral trout grouper (*Plectropomus leopardus*) was conducted using 17α-methyltestosterone hormone implantation starting from March-October 2012 in floating net cages at Institute for Mariculture Research and Development, Gondol. Six net cages with size of 2 m x 2 m x 2 m were used for the experiments. Tested animal of 150 fishes with average weight and length were 377.27±21.49 g and 28.9±0,72 cm respectively.

The fish were treated by hormone implantation and without implantation as control. In the treated fish 50 ug/kg body weight hormone 17α-methyltestosterone was implanted intramuscularly. The hormone implantation was repeated every month. The number of fish per net cage was 25 fishes. The fish was fed with pellets twice a day at *ad libitum*. Every two weeks, the net was changes and at the same time, fish were bathed into fresh water for ± 5 minutes to eliminate parasites (*Neobenedenia* sp.) attached in the body of the fish.

Testosterone Levels

The blood samples were taken just before implantation, after 4 and 8 months of implantation. Prior to blood sampling, the fish was anesthetized using *2-phenoxyethanol* at a dose of 50 ppm. Blood was taken from the base of the caudal peduncle by using a heparinized syringe 5 cc. Testosterone content in the fish blood were analyzed. The blood was transferred into an 1.75 mL eppendorf tube and stored in styrofoam box with ice. To obtain blood plasma, eppendorf tube was placed in a tilted position at room temperature for 2 hours to separate (*cloting*) between serum, plasma, and other blood cells and then it was centrifuged at 5,000 rpm for 15 minutes. The obtained supernatant was taken carefully as much as 250 mL using a micro-pipette and then transferred into a new eppendorf tube and stored in freezer at minus 20°C for further analysis. Analysis of testosterone levels in the blood using the Elisa kit 11-Keto Testosterone EIA.

Gonado Somatic Index, Hepato somatic Index, and Gonad Maturity Level

At the end of the experiment, two fishes from each cage was taken randomly for blood analyses then dissected to remove gonad and liver. Each liver and gonad was weighed to determine the HSI (hepato somatic index = weight of liver/body weight x 100%) and GSI (gonado somatic index = gonad weight/body weight x 100%). The gonads were fixed using Bouin's solution for further histological analyzes to determine the stage of gonad maturation.

Haemocitology Variability

Haemocitology variability of coral trout grouper blood was observed after 4th and 8th monts hormone implementation. Haemocitology observation was conducted in accreditized laboratory at Research and Development Institute for Mariculture, Gondol.

Growth Pattern

Measurement of growth rate of fish was based on monthly observation by measuring the total length and body weight of each fish for each treatment and its replication. Growth rate was analyzed statistically using t-test ($P<0.05$).

RESULTS AND DISCUSSION

Testosterone Levels

The concentration of hormone testosterone in the blood of *P. leopardus* is presented in Table 1.

The testosterone content in the blood taken just before experiment started ranged from 0.8 to 1.0 pg/mL with average of 0.929±0.100. Base on this value, approved that all fishes before start the experiment were

Table 1. Concentration of testosteron in the coral trout grouper *P. leopardus* blood after treatments

Treatments	Concentration of testosterone (pg/mL) (month)		
	0	4	8
Without hormone implantation	0.926±0.125	1.144±0.135	0.868±0.494
With hormone implantation	0.932±0.075	1.407±0.262	0.501±0.076

female. This is consistent with the results of the study of Sembiring *et al.* (2011), which suggests that coral trout grouper blood containing testosterone levels above 700 pg/mL was positive male. Similar result was reported on catfish (*Clarias macrocephalus*) that male at the reproductive stage, the content of 11-KT testosterone was between 159-434 ng/mL (Fermin *et al.*, 1997).

At the fourth month of culture (three months after the first implantation), testosterone levels in blood of all treated fish markedly increased. However, the same condition also occurred in the control treatment. The increase of testosterone level as indicates of a process of sex reverse. However, blood analysis of the eighth month of culture, there were sharply declined of testosterone level in all treated and control fish, even lower than the value at the fourth month. This showed that the application of hormone implantation for three times with one month interval was not effective to maintain testosterone concentration in the liver. In other words, if treated fish was juvenile (immature), three consecutive month of hormone implantation was not enough to induce sex change. Based on gonad histology, the gonad development of all 6 randomly sampled of hormonal treated fish were reached IV stage of gonad maturation, while from 6 of control fish, only four fish reached IV stage of gonad maturation. Probably implanted fish with hormone grew and gonad developed faster than without hormone. Furthermore, the result emphasis that the sex change process has to pass through mature female then turn into male. But with the hormone implantation, the female productive period may be shorter so that the sex changes become faster. According to Yeh *et. al.* (2003b), on potato grouper, *Epinephelus tukula* by implanted with testosterone hormone on reproductive female, sex reverse process into male is happen rapidly.

Gonado Somatic Index, Hepato somatic Index, and Gonad Maturity Level

Gonado somatic index (GSI, %) was used for determination of the maturation stage. From 12 gonads of fish analyzed histological, the lowest GSI value was 0.35 in control fish and the highest was 4.80% in hormone treated fish with. In general, GSI value of ripe coral trout grouper is greater than 1.4%. Further gonad observation, showed higher fecundity of treated fish compared to control fish. In this study, two fish were matured, one fish with 1,080 g body weight (hormone treated fish) had 838,286 fecundity and the other was control fish with 923 g body weight and 416,491 fecundity. Treated fish with hormone tend to increase its GSI, on the other hand, its HSI value was decreased (Table 2). This is consistent with the results of Bayle (1952), Smith (1957) *in* Hajjej *et al.*, (2010) on tuna like fish, where the process of liver metabolism to induce gonad maturation was affect on reduction of liver weight or as a result of the accumulation of fat in the liver during the gonad maturation.

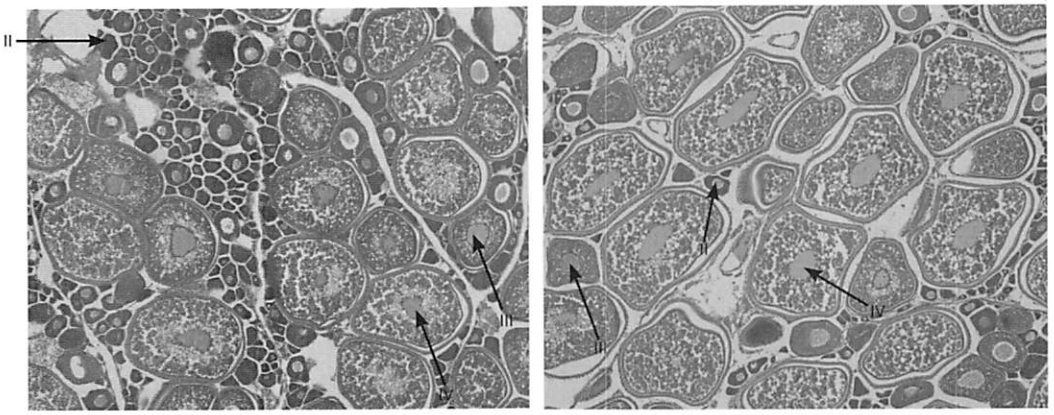
Gonad histology of coral trout grouper can be seen in Figure 1. Development of the gonad was better on hormone implanted fish than control. Histology slides showed that the implanted coral trout grouper 100% reached maturation stage IV while control fish, only 60% reached maturation stage IV. The result showed that treated fish with testosterone was accelerated growth rate and the gonad development compared to control fish. Perhaps treated fish was first affect on inducing its productivity, shorter female period then lead to sex reverse faster than control fish.

Growth

Fish weight on implantation of hormone treatment increased slightly faster than the control fish with weight gain per month of

Table 2. Body weight (g), gonado somatic index (GSI), and hepato somatic index (HIS), gonad maturation stage of coral trout grouper, *P. leopardus* at the end of the experiment

Treatments	Replication	Body weight (g)	GSI (%)	GMS	HSI (%)
Without hormone implantation	A	900	1.45	IV	1.60
		853	1.35	IV	1.08
	B	760	0.35	II	1.89
		882	1.70	IV	2.19
	C	780	4.29	IV	2.16
		923	0.94	II	1.85
With hormone implantation	A	820	1.93	IV	2.15
		885	3.44	IV	1.12
	B	1,080	4.80	IV	1.82
		825	3.73	IV	1.32
	C	1,080	2.66	IV	1.69
		898	1.47	IV	2.16



Without hormone implantation

With hormone implantation

Figure 1. Histological section of coral trout grouper, *P. leopardus* (HE x 100) gonad (I, II, III, and IV = stage of gonad maturation)

73.14 g and 72.96 g respectively (Figure 2). The weight gained is still possible to increase if the environmental conditions can be controlled. In culture period from June to July and August to September, the water temperature tend to decline and was affect on the growth of fish cultured. During this period, the weight gain of treated and control fish only had an average of 49 g and about 25 g consecutively. This value was very low compared to the other period of cultured which reached more than 115 g for treated fish and 121 g for the control.

Haemocytology Performance of Culture Coral Trout Grouper

Implantation of 17α-methyltestosterone affect on variability of blood haemocytology such as percentage of haematocrite, haemoglobine content, total number of erythrocyte and leucocyte. Control fish (without hormone implantation) showed lower variability of haemocitology compared to hormone implanted fish (Table 3). The same results were reported that aplication of chorionic hormone could accelerate the gonad maturation and spawning (Priyono *et al.*, 1990), and also affect on the variability of haemocitology (Secombes, 1988; Narnaware *et al.*, 1997).

The survival rate of coral trout grouper during the study was 100% for both hormone implanted treatment and control fish. No mortality during the culture period due to the carefully sampling and handling of fish so that fish got stress or injured.

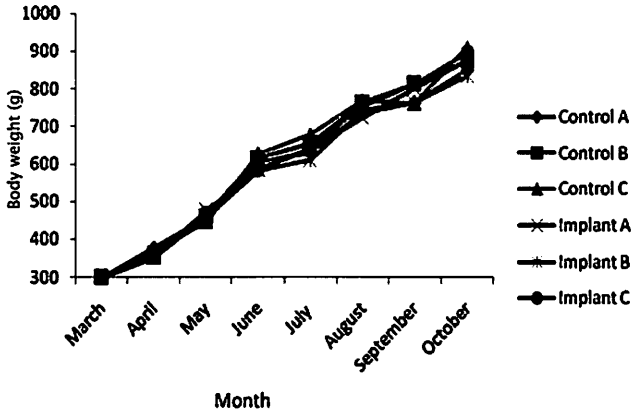


Figure 2. Mean weight gained (g) of coral trout grouper *P. leopardus* during the experiment

Table 3. Haemocytology performance (Haematocrite, Haemoglobine, Total erythrocyte and Leucocyte) of blood of coral trout grouper, *P. leopardus*

Treatments	Rearing period (month)	Haemocitology performance			
		Haematocrite (%)	Haemoglobine (g/100 mL)	Total erythrocyte (sel/mL)	Total leucocyte (sel/mL)
Without hormone implantation	4	41.5	7.75	3,130,000	61,500
	8	47.0	8.25	3,765,000	81,500
With hormone implantation	4	45.5	8.37	3,787,500	76,000
	8	57.0	8.75	4,000,000	84,000

CONCLUSIONS

Levels of testosterone in blood of coral trout grouper after treated by 17α-methyltestosterone was the highest (1.407±0.262 pg/mL), and then decreased in all fish at 8th month culture, the average of the levels of testosterone was 0.501±0076 pg/mL in hormone implanted fish and was 0.868±0.494 pg/mL for control fish.

Gonad maturation stage of hormone implanted fish was high and this emphasis that period of female productivity could be shorter then tend earlier to sex change.

The result showed that hormone implanted fish growth was faster and allometric positive with growth index of 3.1 compared to control fish which was slower and allometric negative with growth index of 1.3.

Implantation of 17α-methyltestosterone hormone at a dose of 50 mg/kg body weight of coral trout grouper not only affect on productivity but also haemocytology performance.

RECOMMENDATIONS

The application of 17α-methyltestosterone hormone implantation should be continued to determine the effectiveness of this hormone to induce sex change and functional male of coral trout grouper.

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