

## RECOVERY OF FEMALE'S GONAD AND HORMONAL CONTROL OF SPERM ON *Heterobranchus longifilis*

### *Recovery Gonad Betina dan Kontrol Produksi Sperma Secara Hormonal pada Ikan Heterobranchus longifilis*

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#### ABSTRACT

Two experiments were conducted in the IRD-Gamet Laboratory, Montpellier, France. The first experiment was to evaluate the recovery of *Heterobranchus longifilis* females' gonad after induced ovulation, under tropical condition. Eggs diameter was used as indicator of the recovery rate following by induced breeding, hatching rate and abnormality of larvae. The results showed that the recovery was about 28 – 35 days after ovulation. The second experiment was carried out to improve milt production by hormone treatments, volume of intra testicular sperm and the numbers of spermatozoa were observed. The results indicated that the treatments did not prove any significant differences.

Keywords: hormone, gonad, reproduction, *Heterobranchus longifilis*

#### ABSTRAK

Dua kegiatan penelitian dilakukan di Laboratorium IRD-Gamet, Montpellier, Perancis. Penelitian pertama dilakukan untuk mengetahui recovery gonad ikan *Heterobranchus longifilis* betina setelah ovulasi, di daerah tropis. Diameter telur digunakan sebagai indikator recovery gonad, diukur pada saat sekitar 28-35 hari setelah ovulasi. Penelitian kedua dilakukan untuk meningkatkan produksi sperma dengan memberikan perlakuan hormon. Volume semen dan jumlah spermatozoa dievaluasi. Hasil penelitian menunjukkan perlakuan tidak memberikan hasil yang signifikan.

Kata kunci: hormon, gonad, reproduksi, *Heterobranchus longifilis*

#### INTRODUCTION

Among catfish, *Heterobranchus longifilis* is originally from West Africa and considered as an economic important species. Due to the value and its popularity, the culture of this species is becoming more preferable. However, there is a limited supply of seed to the market.

Some studies have been carried out to develop the technique of artificial reproduction on *H. longifilis*, such as seasonally reproductive cycle and hormone induced ovulation of the female of this fish in tropical pond or pen culture (Legendre, 1986; Freund, *et al*, 1995); effect of varying latency

period on the quantity and quality of ova after human chorionic gonadotropin (hCG) induced ovulation (Legendre and Oteme, 1995) and effect of dietary lipids on growth, survival and fatty acid composition of fry (Legendre *et al.*, 1995). Although there are some studies on the reproduction of this fish, information about the recovery of *H. longifilis* after induced spawning is still limited. This information is important to be known as a base line data for providing schedule for fry production. Objective of the present study are to elucidate the recovery time of the female and to improve the production of milt of males by hormone induction.

## MATERIAL AND METHOD

### Experiment 1 (Female Gonad Development)

Five matured female and male sized 2.4 – 4.8 kg were used in the present study. Fish were fed 1-2% of the biomass with formulated feed containing 35% protein two times daily. The fish were reared in 2.8 m<sup>3</sup> fibreglass tanks. All females were artificially spawned by injection of 1500 iu of hCG/kg body weight. After spawning, a sampling of egg from each female were fertilized and incubated to evaluate the hatching rate, larvae quality, and percentage of normality.

Eggs were incubated in the 300 ml water in plastic container placed in the water-bath at 28 – 29 °C. Hatching rates were determined using three replications. Hatching rate and condition of larvae (normal and abnormal) were observed at 30<sup>th</sup> hour after fertilisation. All spawned female were recultured in fibre tank connect to a water recycling system at a controlled temperature of 28°C.

Observation on the eggs diameter was carried out every week up to mature eggs diameter for spawning ( $\phi > 1.3$ mm). Prior to collecting eggs, all female were anaesthetised by 0.4 ml of 2-phenoxyethanol per litre of water.

Eggs were collected by intra-ovarian biopsy with a plastic catheter external diameter 3.0 mm and internal diameter 1.5 mm. At every biopsy total number of 50 eggs was sampled and measured using binocular microscope at 25 magnifications. Data of eggs diameter was listed in tabulation system. If optimal diameter was reach ( $\phi > 1.3$ mm), the fish were induced for ovulation. Then collected eggs were fertilized. Proportion of normal and deformed larvae was determined. During the intra ovarian biopsy, 5 ml blood sample was also collected for the evaluation of concentration of vitellogenin. Blood was form the caudal vein. The blood was placed in 1.5 ml ependorf type tube and centrifuged for five minutes at 5,000 rpm. The supernatant was removed and stored frozen at – 20° C.

### Experiment 2 (Male Gonad Development)

Twenty-one used male of *H. longifilis* were divided in three groups; each group had seven fish and reared in fibreglass tank in a recirculation water system. Every three days the fish were induced with 0.2 ml kg<sup>-1</sup> ovaprim, 300 iu hCG per kg and 0.2 ml of 0.9 % of sodium chloride as control. After five injections all males were sacrificed, then testis was removed and weighted. Sperm was collected in a graduated tube to determine the volume. All sperm were stored at the refrigerator at 5°C.

During the sperm quantity (number spermatozoa/ml) observation, sperm were diluted in sodium chloride at rate 1 : 99 by volume. After diluting, one drop of sperm was put on slide glass of Thomas Haemocytometer and then counted. Sperm number was calculated using the formula:

$$N = N_c \times 0.4 \times 10^9$$

N : Total of spermatozoa /ml

N<sub>c</sub> : Number of spermatozoa counted (means small squares)

## RESULT AND DISCUSSION

### Experiment 1 (Female Gonad Development)

The result showed that 80% of the females presented eggs diameter between 1.44 - 1.60 mm (Table 1) at fourth – fifth sampling (28 - 35 days) after spawning with the average of hatching rate 81.2%, normal larvae 68.8%, abnormal 31.2%.

As shown in Table 1, recovery time of the female gonad after induced spawning relatively shorter than other catfishes. In the similar study done by Legendre and Oteme (1995) in Africa showed that recovery of this fish is less than a month. There is slightly different in the period of recovery in the present study compare to the previous study. The difference may be due to the fish handling. In the current study, observation of oocyte diameter and blood sample is conducted on the same fish. Meanwhile, the previous study blood collection is carried out

on the different fish. Thus the fish in the current study have on additional stress influencing the metabolism and gonad development.

### Experiment 2 (Male Gonad Development)

The results of the second experiment are listed on the Table 2 and 3.

Oteme et al. (1995) presented characteristics and morphological of testes and the sperm of the catfish *H. longifilis*, the coexistence in the lobules indicates that this species is able to perform continuous

reproduction. No seasonal trend was noticed in the development of gonadosomatic index (GSI) and in the quality of the sperm produced over a year's period. However, maximum sperm production was observed in April and September.

In the present observation, the males *H. longifilis* is produced maximum milt at November after hormone administration of ovaprim. The result showed that significant effect of hormone on quantity of sperm ( $P > 0.05$ ). Average volume of milt per kilo of body weight at ovaprim, hCG treatment and

Table 1. Maximum development of eggs diameter of *H. longifilis*

No	Weight of females (g)	First sampling (mm)	Seconds sampling (mm)	Third sampling (mm)	Fourth sampling (mm)	Fifth sampling (mm)	Sixth sampling (mm)
1	4 620	< 0.4	1.40	1.36	1.48	1.56	Induced
2	3 105	< 0.4	1.24	1.48	1.52	induced	-
3	2 375	< 0.4	1.24	1.28	1.28	dead	-
4	4 830	< 0.4	0.88	-	1.44	1.6	-
5	4 790	< 0.4	1.24	1.56	1.48	1.48	1.48

Table 2. Gonad observation after hormone treatments

Treatment	Average gonad weight (g)	Testes (g)	Sperm (ml)	GSI (%)	Sperm (ml/kg)
Ovaprim (n=7)	2.77	20.371	13.314	1.962	4.77 **
hCG (n=7)	2.79	15.686	8.229	1.571	2.84
Control (n=7)	2.87	16.857	8.971	1.704	2.93

Note: \*\* = significantly different

Table 3. Means of number spermatozoa after hormone treatments

Treatments	Number of spermatozoa/ml ( $\times 10^9$ )
Ovaprim (n = 7)	$5.45 \pm 1.28$
hCG (n = 7)	$4.66 \pm 1.25$
Control (n = 7)	$5.11 \pm 1.83$

control are 4.77; 2.84 and 2.93 ml respectively. Statistically, there was not significant different among hormone treatments on the concentration of spermatozoa (number of spermatozoa/ml).

### CONCLUSION

The results showed that the recovery of females' gonad after induced spawning was about 28 – 35 days after ovulation. The results indicated that the hormone treatment was significant different for volume of sperm but did not prove any significant difference for the number of spermatozoa.

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### REFERENCES

- Freund, F., G. Horstgen-Schwark, and W. Holtz. 1995. Seasonally of the reproductive cycle of female *Heterobranchus longifilis* In tropical pond. *Aquatic Living Resource*, 8: 297-302.
- Legendre, M. 1986. Seasonal changes in sexual maturity and fecundity, and HCG-induce breeding of catfish, *Heterobranchus multifilis* Val. (Clariidae), reared in the Ebrie Lagoon (Ivory Coast). *Aquaculture* 55: 201-213.
- Legendre, M, and Z. Oteme, 1995. Effect of varying latency period on the quantity and quality of ova after hCG induced Ovulation in the African catfish *Heterobranchus longifilis*. *Aquatic Living Resource*, 8:309-316.
- Legendre, M., N. Kerdchuen, G. Corraze, and P. Bergot. 1995. Larval rearing of African catfish *Heterobranchus longifilis* (Teleostei, Clariidae): effect of dietary lipids on growth, survival and fatty acid composition of fry. *Aquatic Living Resource*, 8:355-363.
- Oteme, Z.J and S. Gilles. 1995. Larval rearing of *Heterobranchus longifilis*: Evaluation of need for live prey. *Aquatic Living Resource*, 8: 351-354.