Reproductive Characteristics and Induced Breeding in *Heterobranchus longifilis*

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Abstract

Two experiments were conducted in the IRD-Gamet Laboratory, Montpellier, France. The first was to evaluate the recovery of Heterobranchus longifilis *female gonad after induced ovulation, under tropical condition. Oocyte 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 to 35 days after ovulation. The second was carried out to improve the milt production by hormone treatments. Volume of intratesticular sperm and the number of spermatozoa were observed. The results indicated that the treatmens did not prove any significant difference*.

Key words: *reproduction, HCG, induce breeding,* Heterobranchus longifilis

Introduction

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

Six matured female and male sized 2,4 - 5,5 kg are used in the present study. Fish are fed 1-2% of the biomass with formulated feed containing 35% protein two times daily, and reared in 2.8 m^3 of fibre-glass tanks. All females are artificially spawned by injection of 1500 IU of HCG/kg body weight. After spawning, a sampling of ova from each female is fertilized and incubated to evaluate the hatching rate, larvae quality, and percentage of normality.

Oocytes are incubated in the 300 ml plastic container of water kept in the water-bath at 28 – 29^oC. Hatching rates are determined using three replications. Hatching rate and condition of larvae (normal and abnormal) are observed at 30th hour after fertilisation. All spawned female are recultured in fibre tank connected to a water recycling system at a controlled temperature of 28^oC.

Observation on the oocytes diameter is carried out every week up to mature oocyte diameter for spawning (ϕ>1.3mm). Prior to collecting oocytes, all female are anaesthetised by 0.4 ml of 2-phenoxyethanol per liter of water.

Oocytes are 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 40-50 oocytes are sampled and measured using binocular microscope at 25-magnification (25X). Data of oocyte diameter is listed in tabulation system. If optimal diameter is reached (ϕ>1.3mm), the fish are induced for ovulation. Then collected ova are fertilized. Proportion of normal and deformed larvae are determined. During the intra ovarian biopsy 5 ml blood sample is also collected for the evaluation of concentration of vitellogenin. Blood is form the caudal vein. The blood is then placed in 1,5 ml ependorf type tube and centrifuged for five minutes at 5,000 rpm. The supernatant is removed and stored frozen at –20^{o}C.

Twenty-one used male of *H. longifilis* are divided in three groups; each group has seven fish and reared in fibreglass tank in a recirculation water system. Every three days all fish are induced with 0.2ml kg^{-1} ovaprim, hCG 300 IU kg^{-1} and 0.9% of sodium chloride 0.2 ml kg^{-1} as control. After five injections all males are sacrificed, testis is removed and weighted. Sperm is collected in a graduated tube to determine the volume. All sperm are stored at the refrigerator at 5^{o}C.

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

$$N = Nc \times 0.4 \times 10^{9}$$

N : Total of spermatozoa /ml
Nc : Number of spermatozoa counted (means small squares)

Result and Discussion

The result showed that 80% of the females presented oocyte diameter between 1.40-1.56 mm (Table 1) at 28-35 days after spawning with the average of hatching rate 81.2%, normal larvae 68.8%, and abnormal 31.2%.

Table 1: Development of average oocyte diameter (mm) of *H. longifilis* observe during the study

No	Tag Number	Weight of Female (g)	First Sampling (mm)	Second Sampling (mm)	Third Sampling (mm)	Forth Sampling (mm)	Fifth Sampling (mm)	Sixth Sampling (mm)
1	7196	4620	<0.4	0.50-1.40	1.36 (m)	1.48 (m)	1.56 (m)	Induced
2	5532	3105	<0.4	0.40-1.24	1.48 (m)	1.52 (m)	Induced	-
3	21469	2375	<0.4	0.40-1.24	0.4 -1.28	0.4 -1.28	Dead	-
4	3121	4830	<0.4	0.4 - 0.88	-	0.4 -1.44	0.48-1.6	-
5	5464	4790	<0.4	0.48 -1.24	0.40-1.56	1.48 (m)	1.48 (m)	1.48 (a)

Note: m = mode, a = atretic

From the 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.

Oteme *et al.* (1995) presented characteristics and morphological of testes and the sperm of the catfish *H. longifilis*, the coexistence in the lobules indicate that this species is able to perform continuous reproduction. No seasonal trend was noticed in the development of gonadosomatic index and in the quality of the sperm produced over a year's period. However, maximum sperm production was observed in April and September.

Table 2: Means of number spermatozoa after hormone treatments

Treatments Hormone	Means of N spermatozoa /ml (± SD) *)
Ovaprim (n = 7)	5,45 x 10^ 9 ± 1,28 [a]
HCG (n = 7)	4,66 x 10^ 9 ± 1,25 [a]
Vehicle (n = 7)	5,11 x 10^ 9 ± 1,83 [a]

*) Value followed by the same letter are not statistically different (P>0.05)

In the present observation, the male *H longifilis* 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), mean volume of milt per kilo of body weight at ovaprim, HCG treatment and 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.)

Table 3: Gonad scarified the and of experiment

Treatments	Initial B weight (g)	S.vehichle (g)	Testis (g)	Spermato (ml)	Vs + Testis	Collagen Testis	GSI (%)	Sperma tozoa ml/kg *)
Ovqprim	2,77 (1.8 - 3.8)	28.186	20.371	13.314	48.557	6.357	1.962	4.77 [a]
HCG	2.79 (1.5 - 3.7)	23.543	15.686	8.229	39.229	7.057	1.571	2.84 [b]
Control	2.87 (1.5 - 3.6)	29.286	16.857	8.971	46.143	7.329	1.704	2.93 [b]

N = 21 males

*) Value followed by the same letter are not statistically different (P>0.05)

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