COMPARATIVE STUDY OF THE EFFECTS OF HUMAN CHORIONIC GONADOTROPIN IN INDUCED SPAWNING OF CATFISH (*Clarias macrocephalus*)

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ABSTRACT

Ovulation in mammals is controlled by two hormones secreted by the Pituitary Gland, the Follicle Stimulating Hormone (FSH), and the Luteinizing Hormone (LH). FSH, which is secreted by the anterior lobe of the pituitary is responsible for the increase in the production of estrogenic hormone (estrone)* which stimulates Graafian follicles (ovarian follicles) to mature and ovulate. LH, on the other hand, organizes the corpus luteum** upon ovulation and causes the secretion of progesterone which stimulates the secretion of viscid glycogenic fluid for the nutrition of the embryo prior to the development of the vascular system. The mechanisms of these hormonal interaction and their effects on the estrous cycle in mammals operate in the principle of reciprocal action.

A parallel situation exists in lower vertebrates. In fishes, gonadal activities such as the transition from juveniles to sexual maturity and the seasonal spawning cycle are **pre-determined** by Gonadotropin secreted by the Meso-adenohypophysis lobe of the pituitary.

* Estrone — $C_{18}H_{24}O_3$

** Corpus luteum — made up of luteal cells containing a yellowwish fat-like substance surrounded by an enveloping membrane permeated by blood vessels.

Seasonal reproduction of fishes reveals two consecutive events:

1. The seasonal growth of the ovary and the transformation of the oocytes into yolk-laden primary oocytes which takes place during late preparatory and pre-spawning period and which depends upon the length of day and increasing temperature.
2. Maturation and spawning of yolk-laden primary oocytes which takes place during the spawning season.

The first event which involves seasonal growth of the ovary and oocytes occurs spontaneously. Maturation and spawning however, seemed to be affected by various environmental factors, such as rainfall and the availability of food, the absence of which inhibits this second phase of the cycle. This resorts to induced breeding techniques by the injection of pituitary hormones such as Human Chorionic Gonadotropin to stimulate the release of gametes from the ovary. Since Human Chorionic Gonadotropin is LH-like, this implies that a similar hormone might be secreted by the Meso-adenohypophysis of the fish pituitary.

INTRODUCTION

Human Chorionic Gonadotropin, commercially available in the Philippines under the brand APL (Ayerst), is a gonad-stimulating principle obtained from the urine of pregnant women. It is an amorphous powder and is freely soluble in water.* It is LH-like and was found to effectively induce spawning in *Clarias batrachus* (Ramaswani and Sundararaj, 1957; Ramaswani and Laksman, 1958, 1959; Sundararaj and Goswani, 1966). In Tanay Research Laboratory, experiments to artificially induce *Clarias macrocephalus* to spawn were conducted. In an attempt to conduct a comparative study of the dosage effect and to establish the most effective dose, variations in hormone dosage were made.

One of the weaknesses of this experiment, however, is the inability of the authors to determine the exact ages and stage of maturity of the breeders since the latter were procured from the consumers market. Thus, some of the breeders were over-ripe for breeding procedures.

* Ayerst literature

INDUCED SPAWNING PROCEDURE

1. Conditioning of breeders:

Conditioning the breeders is important to enable them to recover from stress and injury due to extensive handling, since they were obtained from the consumers market. For this purpose, the breeders were placed in a 2 m x 2 m x 1.20 m net cage set in the Reservoir Pond of the station. This net cage permits the total harvesting of breeders without subjecting them to extensive handling. In harvesting the breeder, the cage was simply lifted from the pond and from the net enclosure, the breeders were transferred to a plastic container and were taken to the concrete tanks of the hatchery for selection and segregation. (See Fig. 1)

2. Selection and segregation of breeders:

Sexing or sexual segregation is done by examining the external features of the breeders and taking note of the following:

1. Genital pore of the female is round or oval while the male has pointed genital papilla.

2. Female has bigger, distended abdomen.

3. Female is relatively bigger than the male.

Suitability of the breeders to respond to induced breeding techniques is also determined by the following:

1. The female should have a palpable, soft and distended abdomen.**

2. Female's genital pore should be moist and reddish, so with the male's genital papilla.**

3. Determination of hormone dose:

Female breeders were weighed individually to determine the dose of hormone to be injected, and were then placed singly in a 23-cm x 43 cm x 36 cm net container. (Fig. 2).

For experimental purposes, the breeders were divided into three groups and were injected with different base hormone doses.

For Group I (breeders Nos. 1-8), a base hormone dose of 2.5 iu/g was injected per breeder (Refer to Table 1).

For Group II (breeders Nos. 9-16), a base hormone dose of 3.0 iu/g. was used (Refer to Table 2).

** Hara, 1972

For Group III (breeders Nos. 17-22), a base hormone dose of 2.0 iu/g was used (Refer to Table 3).

Total Hormone Dose is obtained from the formula:

D_t = Base Dose x weight of the breeders (g)

where:

D_t = total hormone dose

Base dose in this experiment: 2; 2.5; 3 iu/g respectively.

4. Hormone injection:

APL is a white powder contained in a secule* containing 5,000 international units (iu). This is dissolved in a 5-cc of Ringer solution** such that 1 cc of the solution contains 1,000 iu.*** Initial injection of 1/3 of the total dose was administered intramuscularly, above the lateral line at the caudal peduncle using 1 cc syringe. (Refer to Tables 1, 2, and 3). After a lapse of six hours, a second injection of 2/3 of the total dose was again injected to the breeders at the same area, but this time on the opposite side. To avoid struggling during the process of injection, breeders were injected while wrapped inside a hand net.

After injection, the breeders were returned to the net containers and the containers tightly covered to prevent escape of breeders.

5. Stripping:

After 12-13 hours from the second injection, response to hormone injection could be observed by gently pressing the abdomen of the breeders. At this point, eggs oozed out of the genital pore. The breeder is placed in MS 222* solution and then dried with a piece of cloth and weighed. Stripping is done by two persons, one holding the caudal end and the other holding the head with the left hand while the fingers of the right hand pressed the abdomen at the area where the ovaries are located to extrude the eggs and these in turn were received by a dry, clean, petri disc. The eggs were weighed to determine the number of eggs/breeder. This is done using the formula:

* secule — special vial containing an injectable dry preparation (Ayerst)
** Ringer solution — NaCl 7.5 g.; KCl .2 g.; CaCl .35 g., dissolved in 1 liter of distilled H_2O
*** based on the general dilution formula
* MS 222 — anesthesizing material

Total Number of Eggs = weight of eggs x 500 eggs/g.
Total number of eggs/breeder is shown in Table 4.

Simultaneous with the stripping process is the preparation of the spermatozoan solution with which to fertilize the eggs. This is prepared by the following procedure:

1. Cut off the head of the male breeder.
2. Make a horizontal slit along the coelomic region.
3. The testes are taken out and washed in Ringer solution.
4. The testes are then cut and macerated in 50 cc of the same solution.

The resulting milky solution contains the spermatozoa. This solution was poured into the egg disc, and then mixed carefully using a feather. Small amount of water was added for washing. The fertilized eggs were then transferred to hatching trays inside an incubator. (Fig. 3)

6. Hatching Rate:

To determine the hatching rate, a 5 cc sample of eggs from each breeder was taken, counted and placed in floating net incubators (Fig. 4) properly marked. Hatching started 23-25 hours, and was completed after 36 hours. Fry from each net incubator were counted and hatching rate for each breeder determined by the formula,

$$H_r = \frac{\text{Number of Fry}}{\text{Number of Eggs}} \times 100\%$$

RESULTS

All the breeders responded to hormone injection although the second group which were injected with a base hormone dose of three iu/g responded earlier.

Breeders injected with a hormone dose of three iu/g gave the highest rate of hatching averaging 51.68%. This was followed by 2.5 iu/g base dose with average hatching rate of 49.68%. Breeders injected with 2.0 iu/g gave a very poor hatching rate at 29% average.

Rate of survival was also determined by:

$$R_s = \frac{\text{Number of eggs x Hr}}{\text{Number of eggs}} \times 100\%$$

where:

R_s = Rate of survival
H_r = Hatching rate

For rate of survival, Group I has the highest, averaging 53.96%; for Group II, 50.16% average and 31.96% for Group III.

Results of this experiment show the effectivity not of a single dose but rather of a dose range of 2.5 to 3.0 iu/g depending upon the degree of maturity of the breeder. A lower dose such as 2 iu/g gave very poor results, even abortive in some cases.

The significance of LH (APL being chiefly of this nature) therefore, in inducing fishes to breed, lies in its effect in stimulating ovulation and the release of gametes from nearly ripe gonads.

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Table 1. Shows the relationship between weight of breeders and hormone dose for Group I.

No.	Weight (g)	Total Dose(iu)	First Dose(iu)	Time(PM)	Second Dose(iu)	Time(PM)
1	197.4	493.50	164.50	3:40	329.00	9:45
2	214.0	535.00	178.33	3:45	356.67	9:50
3	253.0	632.50	210.83	3:50	421.67	9:55
4	223.50	558.75	186.25	3:55	372.50	10:00
5	298.9	747.25	249.08	4:00	498.16	10:05
6	178.0	445.0	148.33	4:05	296.67	10:10
7	258.0	645.00	215.00	4:10	430.00	10:15
8	254.9	637.25	212.42	4:15	424.83	10:20

* secule — special vial containing an injectable dry preparation (Ayerst).
** Ringer solution — NaCl 7.5 g; KCl .2 g; CaCl 35 g; dissolved in 1l H_2O.
*** based on the general dilution formula.

Table 2. Shows the relationship between weight of breeders and hormone dose for Group II.

No.	Weight (g)	Total Dose(iu)	First Dose(iu)	Time(PM)	Second Dose(Iu)	Time(PM)
9	299.4	898.20	299.40	3:00	598.80	9:06
10	256.3	768.90	256.30	3:05	512.60	9:08
11	245.6	736.80	245.60	3:06	491.20	9.10
12	255.8	767.40	255.80	3:10	511.60	9:15
13	256.6	769.80	256.60	3:15	513.20	9:20
14	205.7	617.10	205.70	3:20	411.40	9:25
15	235.0	705.00	235.00	3:25	470.00	9:30
16	197.5	592.50	197.50	3:30	395.00	9:35

Table 3. Shows the relationship between weight of breeders and hormone dose for Group III.

No.	Weight (g)	Total Dose(iu)	First Dose(iu)	Time(PM)	Second Dose(iu)	Time(PM)
17	221.3	442.60	147.53	4:25	295.06	10:30
18	229.7	459.40	153.13	4:30	306.26	10:35
19	191.5	383.00	127.67	4:35	255.33	10:40
20	184.5	369.00	123.00	4:40	246.00	10:45
21	224.5	449.00	149.67	4:45	299.33	10:50
22	270.0	540.00	180.00	4:50	360.00	10:55

Table 4. Shows the relative number of eggs calculated from the weight of eggs from each breeder.

Breeder No.	Weight of Eggs	Appr. Number of Eggs
1	35.9 g.	17,950
2	39.5 g.	19,750
3	37.0 g.	18,500
4	46.3 g.	23,150
5	29.8 g.	14,900
6	33.7 g.	16,850
7	28.7 g.	14,350
8	32.9 g.	16,450
9	63.1 g.	31,550
10	46.5 g.	23,250
11	38.9 g.	19,450
12	37.5 g.	18,750
13	48.0 g.	24,000
14	33.5 g.	16,750
15	31.6 g.	15,800
16	34.2 g.	17,100
17	32.9 g.	16,450
18	29.3 g.	14,650
19	30.2 g.	15,100
20	28.8 g.	14,400
21	21.4 g.	10,700
22	35.5 g.	17,750

Table 5. Relative hatching rate per breeder taken from the samples.

Breeder No.	Number of Eggs	Number of Frys	Hatching Rate
1	106	87	82.08%
2	227	211	92.95%
3	132	52	39.39%
4	175	156	89.14%
5	96	11	11.45%
6	183	130	71.10%
7	125	-0-	-0-
8	167	19	11.37%
9	124	25	20.16%
10	178	153	85.95%
11	204	121	59.31%
12	159	14	8.80%
13	191	117	61.25%
14	178	13	7.30%
15	179	144	80.44%
16	164	148	90.24%
17	197	56	28.42%
18	135	-0-	-0-
19	207	33	15.94%
20	152	74	48.68%
21	121	-0-	-0-
22	126	102	80.95%

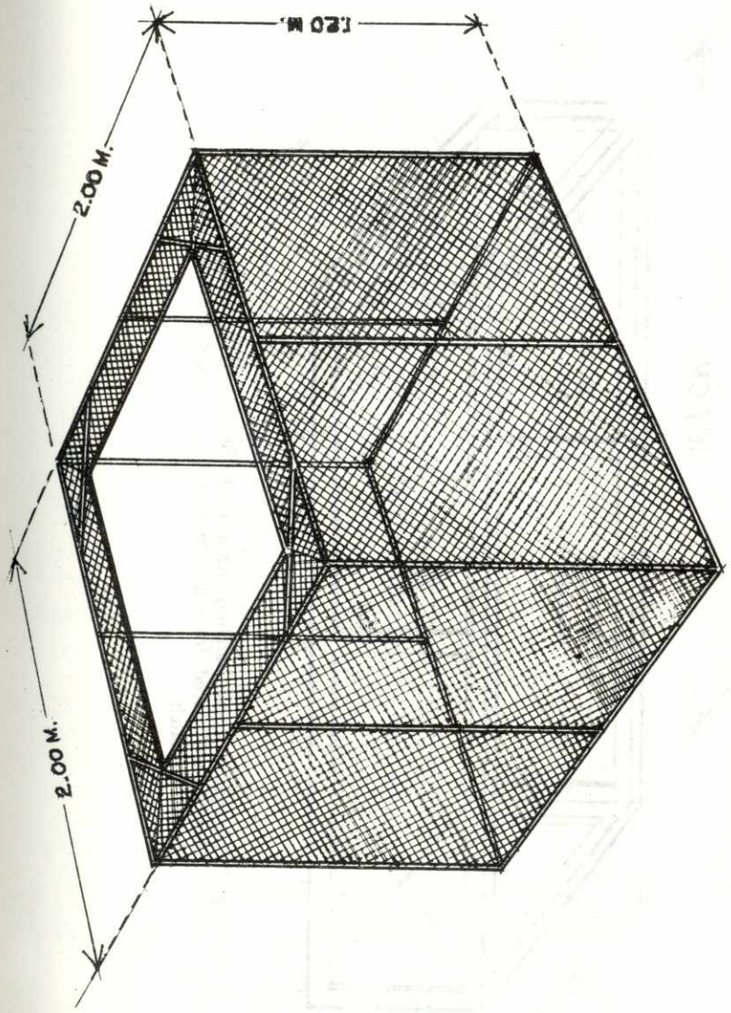

Fig. 1. The net cage made of 3/8" diameter iron frame with net enclosure of size 8 knots, twine 210/12, knotless netting, used in conditioning the breeders prior to hormone injection.

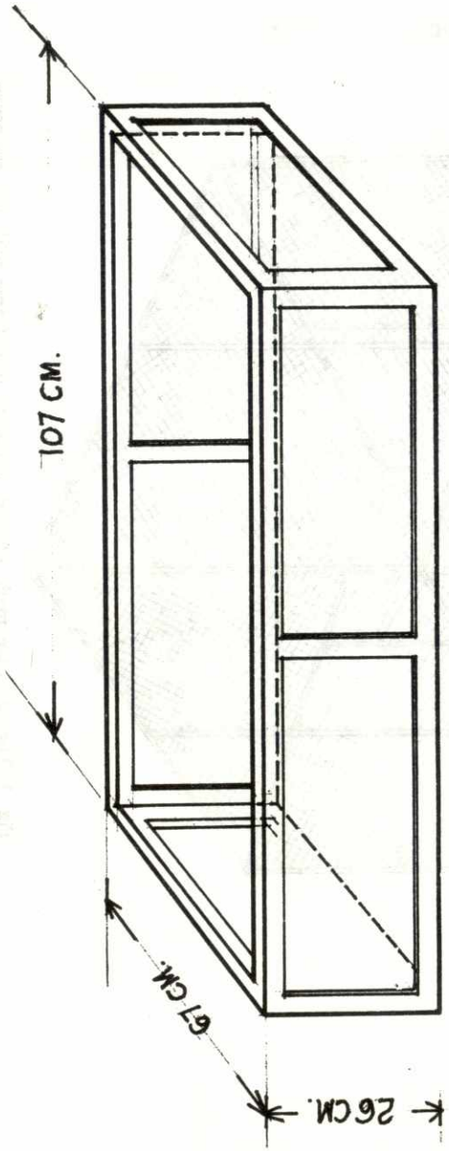

Fig. 3. Incubator where hatching trays made of framed *kakabans* (Arenga fibers) are placed for egg attachment and where the eggs are allowed to hatch.

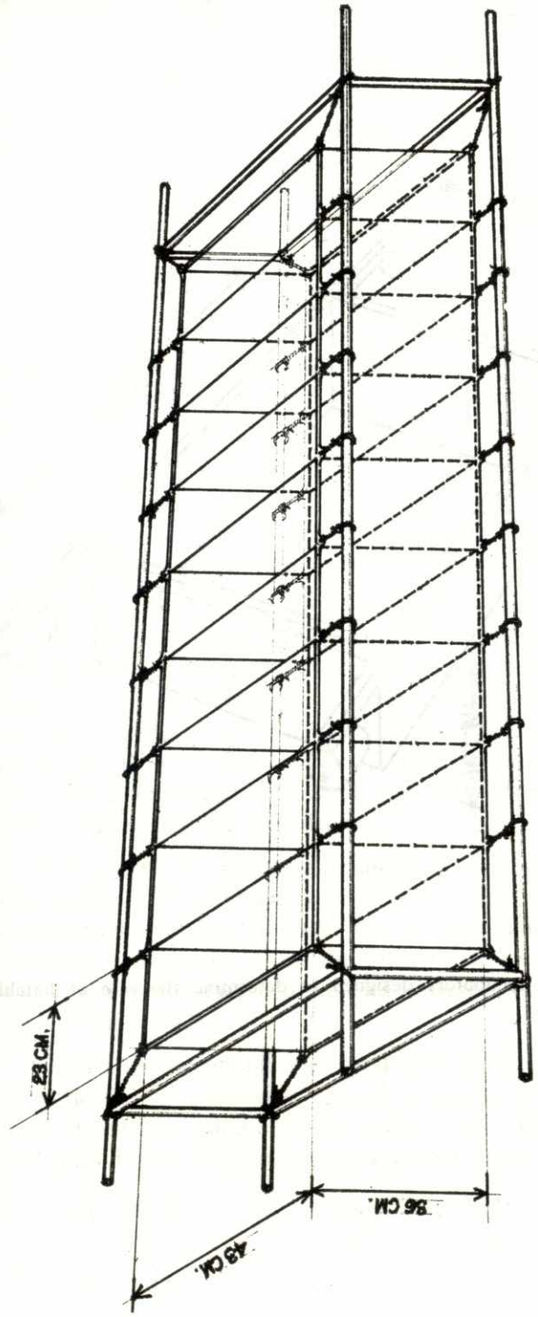

Fig. 2. Shows the linear arrangements and measurement of the net containers for Hito breeders.

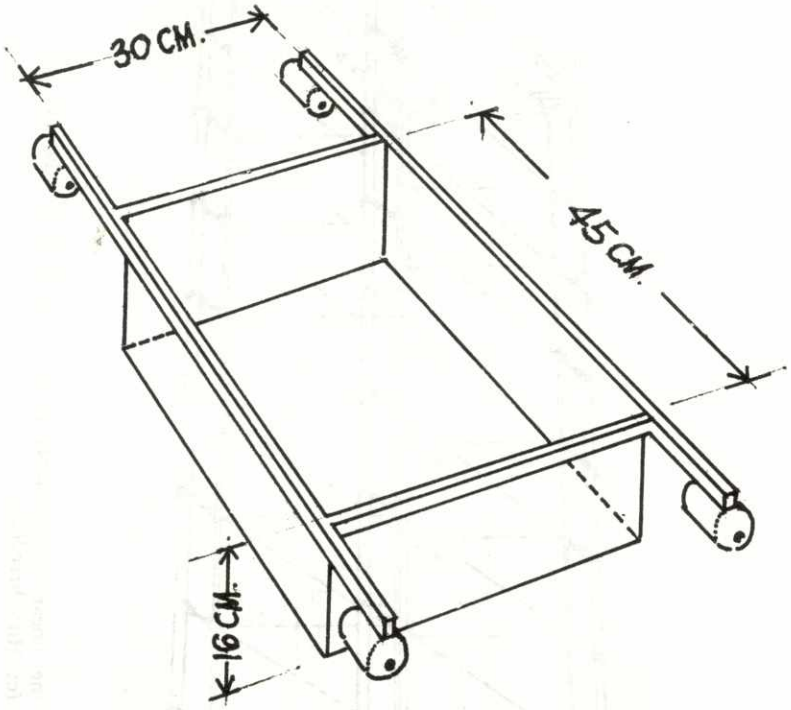

Fig. 4. Floating net incubators designed to determine the rate of hatching per breeder.