OBSERVATION ON THE HORMONE SPAWNING OF ASIATIC CARPS IN THE PHILIPPINES

By

THOMAS G. REYES
Supervising Fishery Biologist

Artificial spawning of bighead carp, Aristichthys nobilis, silver carp, Hypophthalmichthys molitrix, grass carp, Ctenopharyngodon idellus (C & V) through hormone injection have been successfully undertaken.

INTRODUCTION

Freshwater fish culture development carried out by the Philippine Fisheries Commission and UN/FAO in 1966-1969 gained wide recognition throughout the country. Production of more than three tons of fish per hectare was proven in an upland fish farm. Silver carps attained an average weight of 10 kgs/fish in 11 months, while bighead grew up to 25 kgs (Garcia's fish farm, Cuyapo, Nueva Ecija, 1971) in two years.

In 1968, the Philippine Fisheries Commission imported two million Chinese carp fingerlings from Taiwan worth $20,000 (U.S. $1 = ₱4.00 conversion) or ₱100,000 C & F Manila.

To assure adequate local supply of fish seedlings, and minimize dollar outflow, government and privately owned fish hatcheries were established.

Success was achieved in induced spawning of silver carp, *Hypophthalmichthys molitrix* and bighead carp, *Aristichthys nobilis,* in 1969, and grass carp, *Ctenopharyngodon idellus,* in 1971.

METHODS

The work was conducted at four private fish hatcheries[1] from 1969-1972. Breeders were reared in variable sizes of ponds from 2,000 square meters to two hectares, with a water depth of two to

[1] a. Celis Fish Hatchery, Dingle, Iloilo.
b. Garcia's Fish Hatchery, Cuyapo, Nueva Ecija
c. Central Luzon Fish Hatchery, Candaba, Pampanga.
d. Magiliw Fish Hatchery & Farm, Rosales, Pangasinan.

three meters. Broodstock used in hormonal treatment varied with the species; silver carp, 4-10 kg; bighead, 3-14 kg; grass carp, 3-7 kg; aged 1-2 years.

Hatchery facilities were composed of concrete hatching tanks (1.2 x 10 x .80 m) provided with 24 faucets for individual incubation bags. Modification and changes in the type and materials of bags (Fig. 1) had been done before a high survival of fry were attained. Measurement of bags varied with the size of the hatching tank:

a. The conical shaped-bag was made of fine mesh net with aluminum funnel at the bottom. Capacity per bag is 7,000-10,000 eggs. Problem: The circulation of eggs was limited due to its tapering shape, and the connection between the cloth, funnel and plastic tubing was loose. Mortality was high because eggs and newly-hatched fry passed through the stitches between funnel and cloth.

b. The cylindrical bag was made of plastic including the funnel. There was only a four-inch overflow below the top of the bag.

Problems:

1) Oxygen deficiency because water passing through the bag was not affected by the water outside the hatching tank.
2) Eggs carried by the agitation of water clogged the outlet thus, water flowed out on the top of the hatching bag. In this type of bag we found difficulties in regulating the water flow. Capacity per bag ranged from 10,000-15,000 eggs.

c. The design of the 3rd type was the same as that of b(1) except for the materials. The upper portion was made of fine mesh cloth sewed on the funnel-shaped plastic below, then attached to a wooden funnel by epoxy glue. Fry survival was 60-70%. Capacity was 15,000-20,000 eggs.

d. The latest type was used at the Magiliw Fish Hatchery at Rosales, Pangasinan. It is made of plain G.I. sheet. The upper portion was punched with one-inch holes along the circumference and plastered with fine mesh cloth on the inner part. Then, it was painted with epoxy paint to make the adhesion of cloth firm. The adhesiveness of the cloth to the G.I. sheet was lost after two spawning seasons. This type was found to be practicable to the workers insofar as cleaning, handling, and safekeeping is concerned. Rate of survival ranged from 75-85%.

The water was first pumped into a reservoir then distributed to each faucet passing through a plastic tubing to the incubation bags. Thus, upward movement of the water kept the demersal eggs in circulation. Water temperature ranged from 27-30°C.

Pituitary glands were extracted from donors (Cyprinus carpio), macerated in a tissue homogenizer and diluted with physiological saline solution and commercial hormone (synahorin). Mass treatment on selected spawners was based on mg/kg body weight of fish. Treatments were done intraperitoneally or intramuscularly two times at six to seven hours intervals.

Handstripped eggs (Fig. 2) were placed in an enameled or plastic basin and fertilized artificially by means of dry method.

RESULTS

a. *Hormonal treatment*

Results obtained in 1969 to 1972 regarding pituitary hormone injections of Asiatic carps revealed varying degrees of success and failures (Table 1). Spawning cannot be obtained if absorption of eggs has begun (Pickford and ATZ 1965).

Liu (1963), and Tang, et al. (1964), reported that injection with pituitaries combined with doses of chorionic gonadotrophin shows stronger action than that with fish pituitary alone for bringing about ovulation in Chinese carps and striped mullets. Similar results were obtained for silver carp, bighead, and grass carp. However, dosage varied depending on the physiological conditions of a particular group of fish. Sneed and Clemens (1959) stated that differences in response of individual fish could be best explained on the basis of a variability in physiological development of the recipients.

TABLE 1. RESULTS OBTAINED IN 1969 TO 1972 REGARDING HORMONE INJECTION OF ASIATIC CARPS

DATE OF TREATMENT	TEMPERATURE: OF WATER °C	RECIPIENT: SPECIES	NO. OF FISH TREATED	WT/FISH (kgs)	DONOR: SPECIES	NUMBER	WEIGHT (kgs)	DOSAGE/FISH: 1st	2nd	REMARKS
ILOILO HATCHERY										
1969-07-08	27-28°C	Silver Carp	4	3	Common Carp	8	1.4	40 R.U. synahorin*	40 R.U.synahorin	1-Complete ovulation 1 partial ovulation
1969-07-09	27-28°C	Silver Carp	4	4	Common Carp Silver Carp	10 4	0.4 1.0	4.2 mg. pit. gland: 30 R.U. synahorin 3 mg. Pit. gland	4.2 mg.pit. gland 30 R.U. synahorin 3 mg. pit. gland	2-ovulated
1969-07-25	27-29°C	Bighead	4	5	Common Carp	12	0.5	50 R.U. synahorin 6 mg. pit. gland	50 R.U. synahorin 3 mg. pit. gland	No ovulation
1969-07-27	23-29°C	Silver Carp	5	3	Common Carp Acetone dried-powdered form-pit.)			50 R.U. synahorin 5 mg. pit. gland	50 R.U.synahorin 5 mg. pit. gland	1-Ovulated
					-do-			40 R.U. synahorin 10 mg. pit. gland	20 R.U. synahorin 5 mg. pit. gland	3-Ovulated
1970-03-10	28-30°C	Silver Carp	4	6	Common Carp (fresh pit.) gland	15		50 R.U. synahorin 12 mg. pit.gland	50 R.U. synahorin 10 mg. pit. gland	2-Ovulated
1970-03-15	28-29°C	Bighead	2	9	-do-	12	0.5	50 R.U. synahorin 6 mg. pit. gland	50 R.U. synahorin 3 mg. pit. gland	No Ovulation
		Silver Carp	2	6	-do-	10	0.5	30 R.U. synahorin 2.2 mg. pit. gland	20 R.U. synahorin 2.3 pit. gland	No ovulation
1970-03-20	28°C	Silver Carp	4	2	-do-	10	0.8	30 R.U. synahorin 2.4 mg. pit. gland	30 R.U. synahorin 2.4 mg. pit gland	1-Ovulated
1970-04-02	29°C	Silver Carp	5	2	-do-	5	4.5	30R.U. synahorin 2.5 mg. pit. gland	20 R.U. synahorin 2.5 mg. pit. gland	1-Ovulated
1970-04-07	28-30°C	Silver Carp	5	2	Acetone dried pit. gland of common carp			30 R.U. synahorin 6 mg. pit. gland	30 R.U. synahorin 6 mg. pit. gland	All ovulated
1970-04-15	28-29°C	Silver Carp	5	3	-do-			50 R.U. synahorin 12 mg. pit. gland	50 R.U. synahorin 18 mg. pit. gland	Complete Ovulation
		Bighead	2	9	-do-			50 R.U. synahorin 16 mg. pit. gland	50 R.U. synahorin 10 mg. pit. gland	All ovulated
1970-04-25	27-28°C	Bighead	4	8	-do-					

* R. U. - Rabbit Unit
* Pit. - Pituitary gland

Table 1. Continued . . .

DATE OF TREATMENT	TEMPERATURE: OF WATER °C	RECIPIENT: SPECIES	NO. OF FISH TREATED	WT./FISH: (kgs.)	DONOR: SPECIES	NUMBER	WEIGHT (kgs)	DOSAGE/FISH: 1st	2nd	REMARKS
1970-05-11	28-29°C	Silver Carp	6	6	Acetone dried pit. gland of common carp			30 R.U. synahorin 10 mg. pit. gland	30 R.U. synahorin 10 mg. pit. gland	All ovulated
LIMJOCO'S HATCHERY Candaba										
1971-08-11	27°C	Silver Carp	4	5	Common Carp	3	3 kg & dried gl.	50 synahorin 16 mg. pit. gland	50 synahorin 18 mg. pit. gland	2-Ovulated
		Grass Carp	2	5	Common Carp	2	2	10 synahorin and 8 mg. gland	35 synahorin 8 mg. gland	No ovulation
Dr. Garcia's Farm Cuyapo N, Ecija										
1971-05-04	28°C	Silver Carp	2	10	Common Carp	5	10	25 synahorin 15 mg. gland	40 synahorin 20 mg. gland	Ovulated
		Bighead	2	10	Common Carp	6	12	35 synahorin 20 mg. gland	30 synahorin 16 mg. gland	Ovulated
1971-05-14	28°C	Bighead	2	14	Common Carp		dried gl.	35 synahorin 18 mg. gland	30 synahorin 20 mg. gland	Ovulated
Delgado's Farm Or. Mindoro										
1971-06-20	27°C	Grass Carp	2	7	Common Carp		dried gl.	35 synahorin 12 mg. gland	25 synahorin 9 mg. gland	No Ovulation
Cojuangco's Farm										
1971-06-22		Grass Carp	3	6	Common Carp		dried gl.	30 synahorin 10 mg. gland	40 synahorin 10 mg. gland	All Ovulated *1st successful attempt
Magiliw Fish Hatchery										
1972-05-03	28°C	Grass Carp	4	3	Common Carp		dried gl.	40 synahorin 6 mg. gland	35 synahorin 6 mg. gland	Ovulated
1972-06-04	27°C	Grass Carp	4	2.5	Common Carp	2	d.5	30 synahorin 4 mg. gland	30 synahorin 6 mg. gland	All ovulated
1972-06-07	20°C	Grass Carp	3	3	Common Carp	4	4	40 synahorin 6 mg. gland	35 synahorin 6 mg. gland	All ovulated

We support this analysis based on our latest results by obtaining a 95% positive response, (Table 1 on hormone injection of silver carps, bighead, and grass carp) depending on the skill of workers in the selection of spawners.

The dose of injection required to spawn a fish varies with the size and species of the recipient. Results showed (Table 1) that bighead carp, *A. Nobilis*, could be induced to spawn by giving four mg of acetone dried pituitary gland (Cyprinus carpio) per kilogram body weight of recipient (3-14 kgs) fish plus 90-100 rabbit unit of synhorin regardless of size. While for silver carp and grass carp, they need the same amount of pituitary gland but require 70-80 rabbit unit of synhorin.* Fresh pituitary gland extracted from common carp (Cyprinus carpio) of similar weight to that of the recipient plus a small amount of gonadotrophin were also found effective.

It was observed that female matures earlier than male. One-year old female responded to our treatment but problem on the availability of milt from the male was encountered.

On the second year of operation, it was found necessary for males to receive a single dose of pituitary gland at midnight or six hours before the female releases eggs. Usual ovulation took place 12 hours after the first injection. However, ovulation at 9-10 hours was rarely encountered. Similar to the report of Tang (1964), properly selected spawners that were treated with hormones released eggs through the genital papilla continuously (12-13 hours after the first injection) and after being lifted from the water. Occasionally, eggs were discharged upon slight pressure on the abdomen.

b. *Artificial Fertilization*

Artificial fertilization of eggs was done by dry method. At expected ovulation time, female was taken for stripping. Stripped eggs were placed in a dry basin and added to the milt (sperms) of the male. With the aid of sterilized chicken feather, the eggs were stirred carefully for one minute, then washed with clean water to remove the mucus, fluid and other excreta. Eggs were placed in a hatching

* Commercial hormone which is a mixture of chorionic gonadotrophin and mammalian hypophysical extract.

bag with circulating water at the rate of two to three gallons per minute per faucet.

Since this research was conducted in four hatcheries (Fig. 3) in different places and the writer came over only during the spawning season, there were discrepancies and inconsistencies* in the facilities especially in the water supply, hatching bags, and manner of aerating water and other hatchery equipment. Hence, there were hatchery problems encountered in various degrees.

There were various limiting factors observed responsible for the high mortality in the incubation of eggs and growing fry to fingerlings:

1) Low percentage of fertilization due to insufficient amount of milt (sperm) taken from the male carp.
2) Oxygen defficiency during incubation period. (Sources of water in these hatcheries were deep wells. Water was pumped into the reservoir). The aerator was only used at Magiliw Fish Hatchery, 1972. The critical stage in hatching starts one to seven hours from the time the eggs are released.
3) High CO^2 in the water destroyed the egg shells during the incubation process, thus, only 5-10% survival was attained.
4) Immature eggs — Small and greenish-colored eggs were released due to overdose. Tang (1964) cited in his report that the result of overdose of hormone injection was the release of a large portion of immature eggs.
5) Fungus infestation affected the eggs in its development stage. Davis (1953) considered the disease as caused most likely by amoeba and bacteria. On the other hand, Tang (1964), considered it as caused by protozoan parasites. The majority of eggs died during the developmental stages of blastula and gastrula. This happened three to six hours after the eggs were fertilized. Further study of this problem is still needed.

c. *Rearing fry to fingerling:*

Comparative trials between trough and suspended inverted mosquito net (hapa) method of rearing fry were closely observed. More favorable results were obtained in trough than in suspended nets.

* Construction of hatcheries were done without considering and consulting the person who operates the facilities.

Newly hatched fry numbering 20,000 were transferred in wooden troughs (1.2' x 8' x 10') and in a net (1 x 3 x 1 meters) in the pond. Daily supplemental feeds given for both medium were the same. After 15 days, evaluation was done, obtaining a 90% survival in the trough but only 10% in the suspended net.

The high survival rate in the trough could be attributed to constant flow of water and freedom from predation. Excreta were always removed, and constant temperature was maintained, unlike in the suspended net where fry were highly exposed to sunlight. However, the entrance of insects could not be prevented and the removal of excreta and excess food could be done.

Immediate transfer of fry to nursery pond after the absorption of yolk sac was tried but led to high mortality. At this stage, the fry were still vulnerable to predation. Predators that were observed present in the pond were back swimmers, copepods, water boatman and other insects that swarm at night and thrive in the water.

It was also observed that one-week is the right age for fry to be transferred to the nursery pond.

SUMMARY

1. The success obtained in hormonal treatment of Asiatic carps proves beyond reasonable doubt that hatchery operation in the Philippines will help increase our fish production.

2. The criteria in obtaining high rate of survival in hatching are:
   a. Clean and well aerated water;
   b. Minimal dose of hormone;
   c. Removal of dead eggs to prevent fungus infestation;
   d. Constant temperature.

3. The response to hormonal treatment varies with physiological development of the recipients and species of carps. Dose is 1-1.5 mg pituitary gland from the donor carp of same size, 50-90 rabbit units of synahorin.

4. Rearing fry in troughs is effective.

ACKNOWLEDGMENT

The writer is indebted to Director Felix R. Gonzales and former Commissioner Andres M. Mane of Philippine Fisheries Commission; Yun An Tang and James Anderson, UN/FAO experts; and Mrs. Medina Delmendo, Acting Chief, Freshwater Fisheries Division, for their valuable support and encouragement. Acknowledgment is also given to Messrs. Albert Celis, E. Garcia, C. Limjuco and Jimmy Estrella, owners of the fish hatcheries, for their great enthusiasm in the implementation of this work. Likewise, Messrs. A. Gatus, R. Pinto and E. Martinez have valuable contribution in the construction of facilities. A grant from UN/FAO/FFHC in connection with this work is highly acknowledged.

The author also wishes to extend great appreciation to the workers in the different fish hatcheries for their assistance during the conduct of this work.

LITERATURE CITED

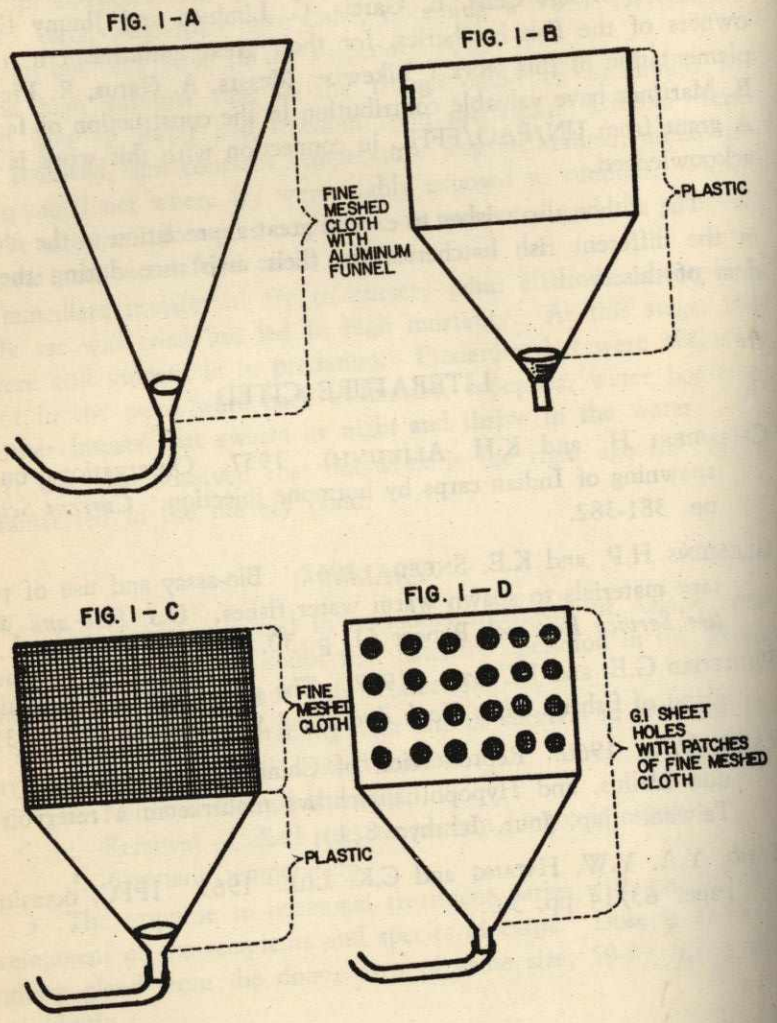
CHANDERI H. and K.H. ALIKUNHI. 1957. Observations on the spawning of Indian carps by hormone injection. *Current Science,* pp. 381-382.

CLEMENS H.P. and K.E. SNEED. 1962. Bio-assay and use of pituitary materials to spawn warm water fishes. *U.S. Fish and Wildlife Service Research* Report 61, p. 30.

PICKFORD G.E. and J.J. ATZ. 1957. The physiology of the pituitary gland of fishes. *New York Zoological Society,* New York, 631 p.

TANG, Y.A. 1960. Reproduction of Chinese carps, Ctenophoryngodon idellus, and Hypophthalmichthys molitrix in a reservoir in Taiwan. Jap. Jour. Ichthyo 8(1); 1-2.

TANG, Y.A. Y.W. HWANG and C.K. LIU. 1963. IPFC, occasional paper 63/14 pp. 3-6.



Figs. 1-A-1-D. MODIFICATION AND CHANGES IN THE TYPE AND MATERIALS OF HATCHING BAGS FOR ASIATIC CARP EGGS.

Fig. 2. Extraction of male milt of grass carp is being done through the dry method.

Fig. 3. Artificial fertilization of Asiatic carp eggs by dry method. Eggs flow freely 12 hours after hormone treatment.