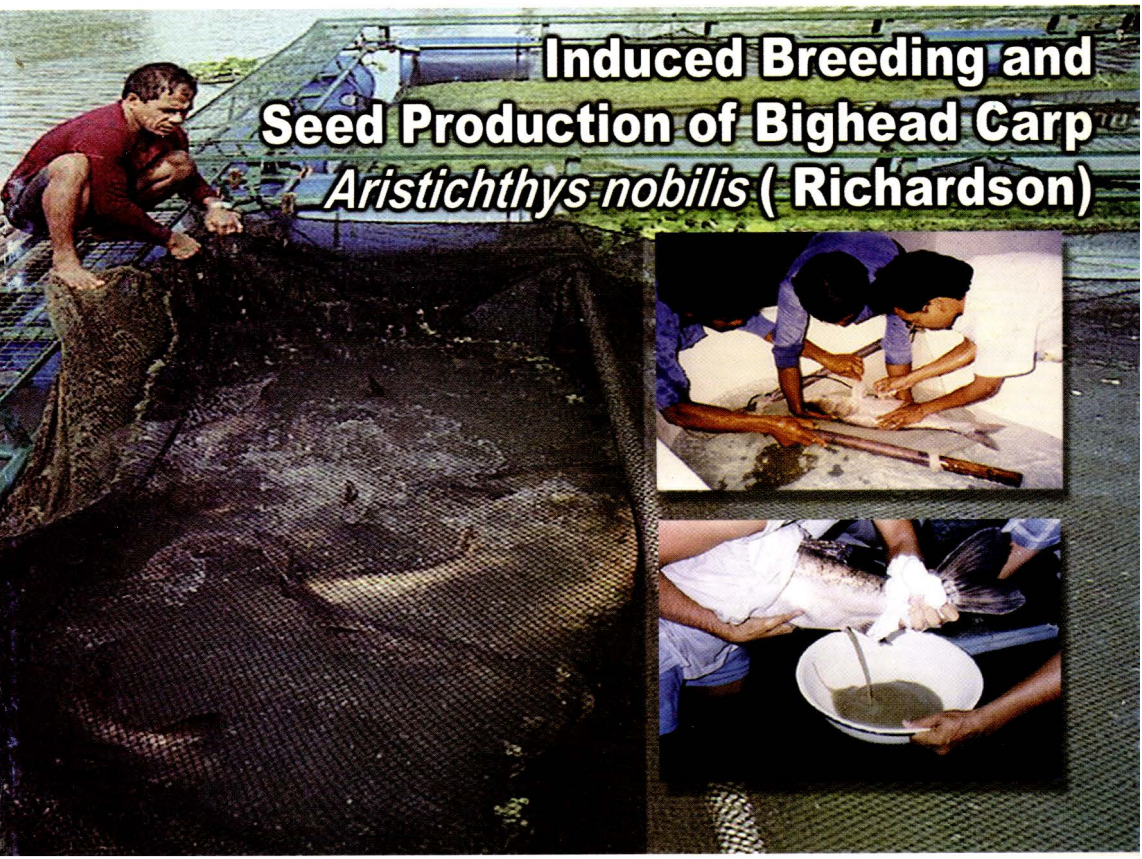
AQUACULTURE EXTENSION MANUAL NO. 33
JULY 2001

Induced Breeding and Seed Production of Bighead Carp *Aristichthys nobilis* (Richardson)



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Foreword

The Binangonan Freshwater Station (BFS) of SEAFDEC Aquaculture Department is among the pioneers in the research and development of the culture and artificial propagation of bighead carp in the Philippines. This is the third most popular species for freshwater aquaculture in the Laguna de Bay area. Bighead carp has become a popular fish in other parts of the country as well because of the need for cheap protein source and in support to the government's food security program. Researchers at BFS started work on Chinese carps in the late seventies. Shortly after the early research output on the bighead carp was released, private fish farmers in the Laguna de Bay region took interest in this new species as a potential alternative to mikfish and tilapia.

The publication of this manual on broodstock management and breeding of bighead carp is long overdue. It is a product of more than two decades of research and technology verification by the staff of SEAFDEC/AQD, its partner institutions, and private fish farmers. It is hoped that this manual will benefit aquaculturists who may want to go into production of bighead carp, as well as extension workers, technicians, teachers and students in the field of aquaculture.

Rolando R. Platon, Ph.D.
Chief, SEAFDEC AQD

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Introduction

The fast-growing bighead carp, *Aristichthys nobilis*, is a popular Chinese carp species in the Philippines, next to the common carp, *Cyprinus carpio*. Preliminary studies on Asiatic carps including bighead carp were conducted in 1966 by the former Philippine Fisheries Commission, now the Bureau of Fisheries and Aquatic Resources (BFAR). In 1968, the Commission imported two million carp fingerlings from Taiwan in conjunction with the launching of FAO-assisted Food for Hunger Campaign Program. This program has implemented research, training and extension activities to promote carp culture in the Philippines and helped establish government and private hatcheries to ensure a steady supply of carp fry and fingerlings. By the end of 1969, initial success was achieved in the artificial propagation of bighead carp in cooperation with the Central Luzon Fish Hatchery in Candaba, Pampanga and Celis Fish Hatchery in Dingle, Iloilo. The use of carp pituitary extract in induced spawning showed varying degrees of success and failure that highlighted the need for more studies on the artificial propagation of this species.

Bighead Carp, *Aristichthys nobilis* (Richardson)

Source: Freshwater Fishes in Malaysia (poster)
Jabatan Perikanan
Kementerian Pertanian Malaysia

In 1979, the SEAFDEC Aquaculture Department started work on different carp species. Significant findings on the culture, breeding, gonad maturation and rematuration of bighead carp in floating net cages in Laguna de Bay have accelerated the development of bighead carp culture in the Philippines. In recent years, bighead carp has gained acceptance as an important food fish in the country. Around Laguna de Bay, bighead carp is considered one of the most important freshwater fishes for culture because of its fast growth and high survival. Pen and cage culture of carp is sustained by the availability of juveniles as a result of improved hatchery technology. However, progress in large-scale production is being challenged by the present problems on genetic quality of broodstock. Hence, research studies on carp at SEAFDEC/AQD have now focused on the refinement of hatchery and broodstock management practices.

The advanced induced spawning technology for bighead carp developed at SEAFDEC/AQD through the years is presented in this manual. This handbook emphasizes the adoption of efficient carp hatchery techniques for optimal production of good quality eggs and juveniles. Proper transport and conditioning to minimize stress in broodfish, which are often neglected, are given importance. The induced spawning techniques are described in detail, which include injection protocols and computation of hormone requirements, hormone preparation, injection methods, stripping of fish, and egg incubation. Design of facilities and materials for egg incubation are presented, and a simple recirculating system for effective water quality management is illustrated to ensure successful hatchery operations.

Broodstock Management

Source of broodstock

Young bighead carp to be grown to broodstock size are generally obtained from reliable sources. Some hatcheries acquire adult bighead carp instead of younger fish to save on time. Potential broodstock are selected from fish grown in pens or cages. Age varies from 8-12 months with body weights ranging from 1-2 kg each. Others do practice broodstock movement in which bighead carp from one hatchery are borrowed and paired with those in another hatchery for induced spawning. This scheme is done in an attempt to minimize inbreeding.

Age at first maturity

Water temperature has the greatest influence on age at first maturity of bighead carp. It takes a longer time for bighead carp to mature in temperate countries than in the tropics. Under normal lake conditions in the Philippines, bighead carp attain sexual maturity at about 2 years of age when the fish weigh 2-3 kg each. With supplemental feeding, onset of gonad maturation can be advanced by several months. Male bighead carp attain first gonad maturity earlier than the females. However, female broodstock are usually bigger than males of the same age.

Desirable characteristics

The reproductive capacity of a fish is determined by its genetic makeup. However, fish farmers and hatchery operators usually rely on the external characteristics as practical indicators of a potential broodstock. Young fish to be reared to broodstock size should exhibit fast growth and have normal physical appearance and well-developed sex organs. The fish should be free of parasites and not manifest clinical signs of a

disease. A two-year old bighead carp (2-3 kg) usually produces 30,000 to 70,000 ovulated eggs per kg.

Fish that fail to mature after a reasonable length of time beyond the expected age at first gonad maturity are harvested as food fish.

Gonad maturation in ponds and lake cages

Bighead carp broodstock are traditionally grown in earthen ponds. Culture conditions should ensure fast growth of the fish. Proper pond preparation and water management, adequate food (natural and artificial), and optimum stocking density (1 fish/sq m, 2-3 kg/fish, with supplemental feeding) have to be considered.

In Laguna de Bay, young bighead carp are farmed in pens for about a year and then harvested and sold as food fish. However, some fish are grown to broodstock size for another year. The broodstock are maintained in cages rather than in pens. Usually, 25 fish are stocked in 5x10 sq m cage without supplemental feeding. The site for pens and cages should have a good water circulation.

Once the bighead carp in a batch attain first gonad maturity, fish with mature gonads can be observed year-round. Peak months for the occurrence of mature bighead carp in cages in Laguna de Bay vary from year to year depending on the lake conditions. For example, a high percentage of fish with mature gonads was observed from March to May in 1986, August to November in 1993, and May to October in 1994. As the fish grow older (e.g., 3-4 years of age), the percentage of fish with mature gonads at any given time increases.

Successive gonad rematuration in bighead carp has been observed to occur under lake conditions. Thus, multiple induced spawnings of a female bighead carp can be carried out in a one-year cycle.

Increase in chlorinity (salinity) in Laguna de Bay during saltwater intrusion from Manila Bay adversely affects gonad maturation of both male and female bighead carp stocked in pens and cages. The effect ranges from absence of gonad maturation to regression of the gonads. Thus, induced spawning of bighead carp from the lake is conducted before the expected saltwater intrusion which usually occurs in summer months (March-April) when the lake is at its lowest level. Induced spawning is also scheduled a few months after saltwater intrusion when rainy season sets in and the chlorinity of the lake water decreases. However, bighead carp fry have been observed to be tolerant to low levels of salinity in the nursery.

Facilities and Materials for Induced Spawning

The success of carp hatchery operations is influenced, in part, by good water supply, efficiency of the facilities and availability of materials used during induced spawning.

Water source and reservoir

An overhead water reservoir (e.g., 10-ton capacity) is needed especially in areas where deep-well water is being used. This ensures availability of cool and aerated water during conditioning and induced spawning of the broodstock.

Conditioning tank

This is a large circular concrete tank in which the broodstock are placed upon arrival at the hatchery until they are induced to spawn and stripped. The conditioning tank is usually 4-5 m in diameter and 1.2 m high and filled with water up to 1-m level. At least one pipe (2-inch dia) from which water flows at the surface is provided. The pipe is slightly flattened at the tip to produce a strong but fine stream of water (see arrow). Air stones are placed at the periphery of the tank for vigorous aeration.

Water in the conditioning tank flows in circular motion. Water can be drained from the bottom into a smaller tank where a submersible pump can be operated automatically to recirculate it. Water can also flow through continuously using a standpipe. A combination of recirculating and flow-through water is sometimes used.

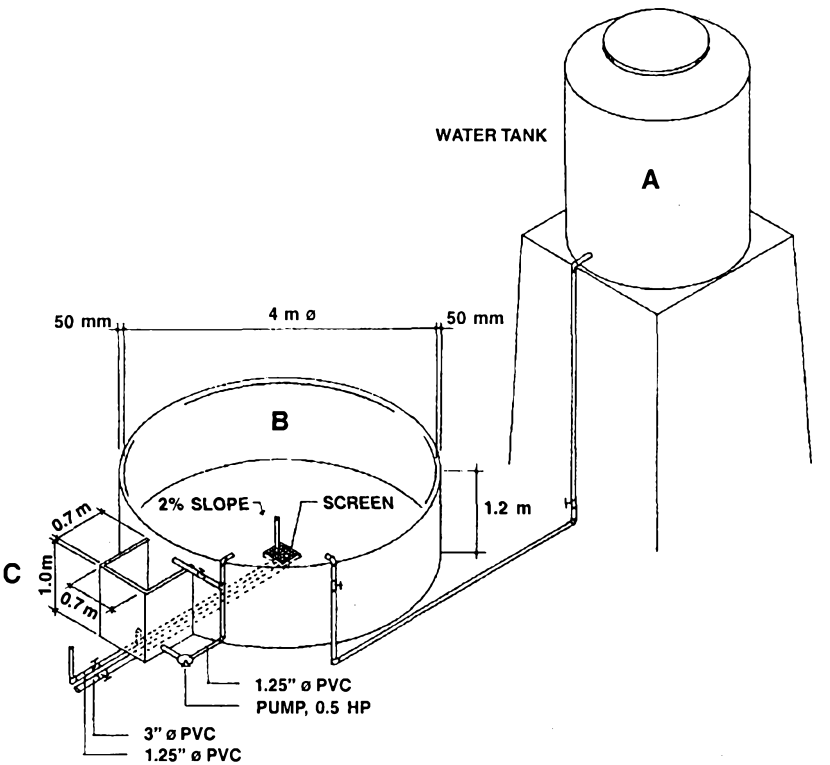


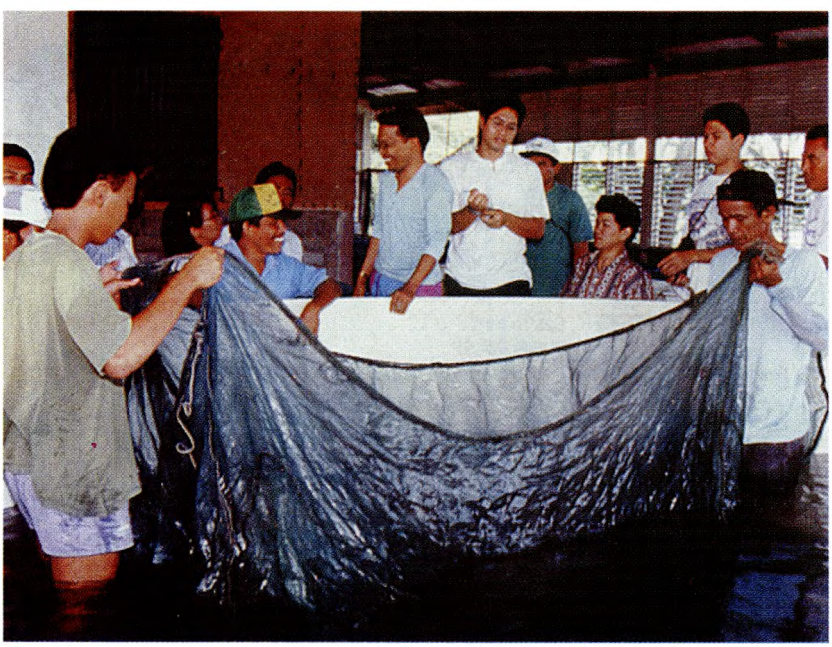
Figure 1. Lay-out of (A) elevated water reservoir, (B) conditioning tank, and (C) sump

Holding tanks

These are smaller multi-purpose tanks, usually one-ton capacity and preferably circular or oval. In these tanks, spent fish are allowed to recuperate before they are finally brought back to the broodstock cages or ponds.

Materials for handling, injecting, and stripping of the broodstock

Catching net. It is a fine-meshed net cage (5x5x2.5m) used to catch the broodstock to be injected and stripped. The edge of the side of cage that is dropped to the tank bottom is lined with metal sinkers to prevent escape of fish. The same side of the cage is then raised and hung at the rim of the conditioning tank. The technicians can get inside the net cage to catch the broodstock.



A **hammock** made of soft cloth is used to hold the fish while being injected or awaiting injection. It measures about 75 cm long and 100 cm wide, with a PVC pipe support at both ends. It is opened to form a V-shape when receiving the fish to be injected.

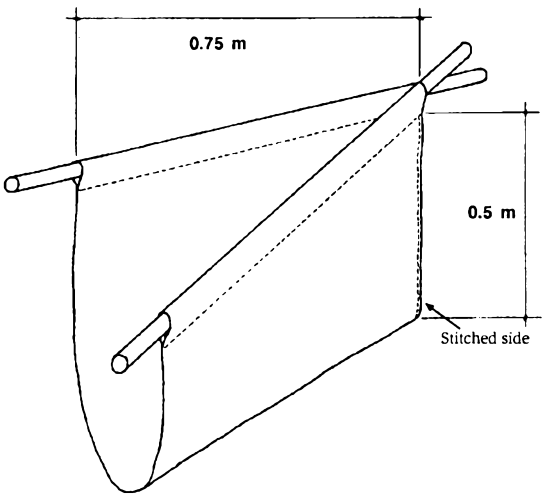


Figure 2. A cloth hammock

A **cotton sack** is used to hold the fish during stripping or transferring of broodstock. It is open at both ends but can be closed using drawstrings. It should be large enough to hold the whole fish. When ready for stripping, the broodfish is placed inside the sack head first towards the closed end. The drawstring is loosened when the fish is released into the water also head first. The use of this sack is optional when fish are handled by skilled technicians.

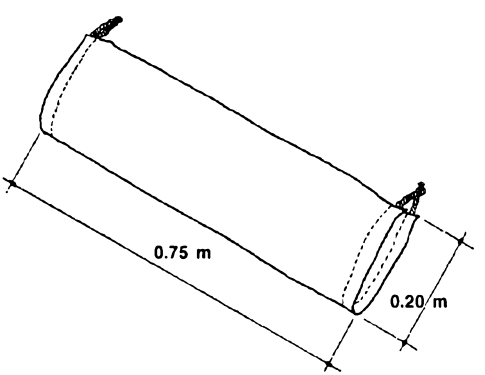


Figure 3. A cotton sack

Hormones used for induced spawning of bighead carp are commercially available LHRH-a (luteinizing hormone-releasing hormone analogue) and HCG (human chorionic gonadotropin). Depending on the protocol for hormone-induced spawning, a dopamine antagonist (e.g., pimozide or domperidone) is sometimes used.

Triple distilled water or normal saline is used as solvent in LHRH-a and HCG preparations. Pimozide and domperidone require **dimethyl sulfoxide** and **propylene glycol** as solvents.

Saline water (0.6% NaCl) is used to dilute milt or is added to the milt and eggs following dry fertilization.

Syringes with needles (3- and 5-ml capacity; gauge 23 needle) are used in hormone preparation and injection of fish.

Cotton towels are used to wipe dry the fish before stripping.

Enamel basin (30 cm dia) is used to contain stripped eggs and milt. Several of dry basins are needed especially if stripping is done one after the other.

Large **chicken feathers** are used to mix the eggs and milt. The feathers should always be dry before use.

Broodstock Selection

Marking the broodstock

Bighead carp are marked in order to distinguish individual fish. A number (or letter) is etched on the dorsal part of the head using a lead pencil. Markings are renewed every 1-2 months.

Selecting a mature fish

Females that have soft palpable abdomen with swollen and reddish genital papilla are candidates for induced spawning. When turned ventral side up or taken out of the water and held on its tail, the contour of the ovaries can be seen to extend anteriorly.

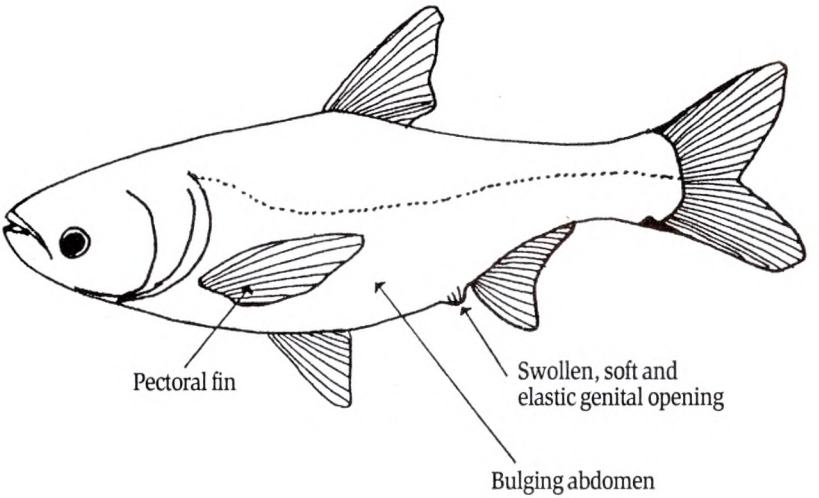


Figure 4. Distinguishing features of a gravid female bighead carp

Females with extremely distended abdomen are not chosen for induced spawning as this may indicate regression of the ovary.

A more accurate method to determine readiness of a female fish for induced spawning is through an ovarian biopsy as shown in picture below.

A polyethylene tube (2-mm inner diameter) is carefully inserted into the urogenital pore to draw out some eggs from the ovary by aspiration or suction. Good quality eggs are usually grayish to greenish and the size is fairly uniform. A clearing agent (85 ml of 95% alcohol, 10 ml formalin (40% formaldehyde), and 5 ml glacial acetic acid) is used to determine the maturity of the eggs based on the position of the nucleus. Aspirated eggs are placed in the clearing solution and observed under a dissecting microscope after 3-5 minutes. Maturing to mature eggs have off-center or migrating nucleus and will most likely respond to the prescribed hormone treatment. Overly mature or degenerating eggs do not have a clearly visible nucleus.

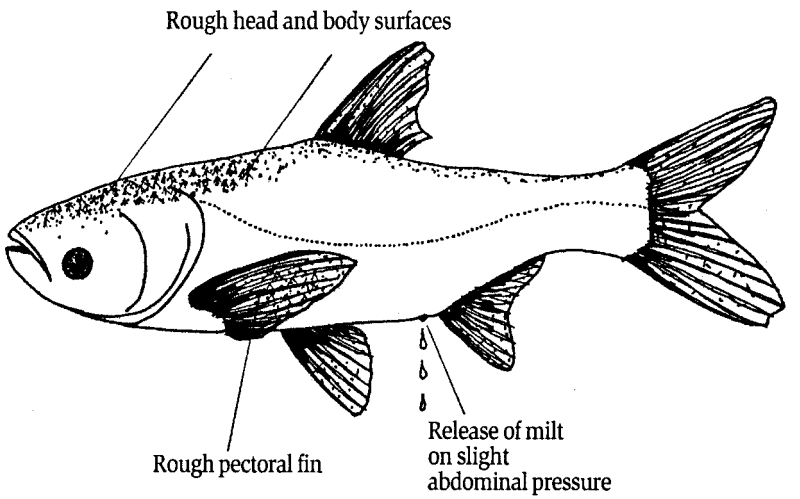


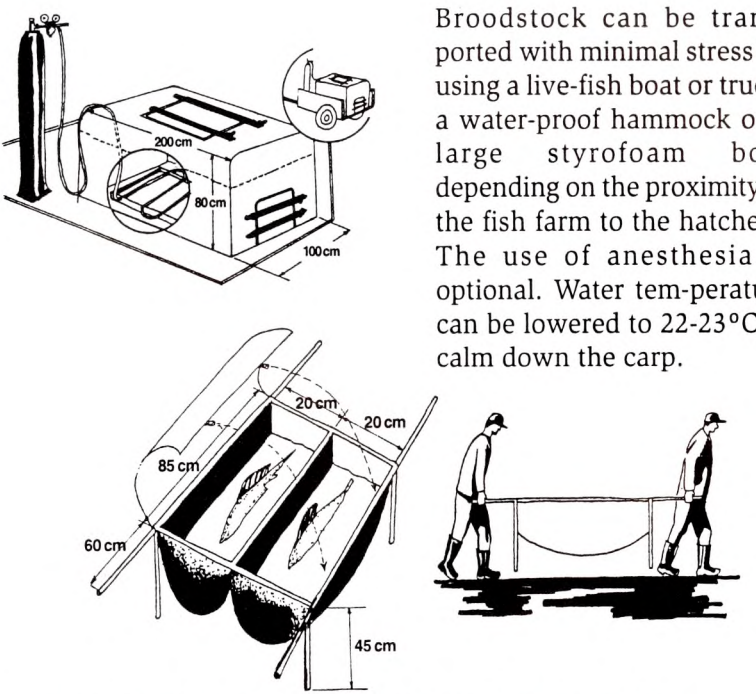
Figure 5. Distinguishing features of a mature male bighead carp

Males with rough or callous pectoral fins (particularly along the big rays and the side close to the body) and whose head and body surfaces are rough to touch (fine, sand paper-like) are usually selected for induced spawning. The males also express milt when a slight pressure is applied on the abdomen.

Two to three males are used for every female fish in order to ensure a high fertilization rate. As much as possible, the source of males is different from that of the females.

Transport of Broodstock

Soon after the fish are selected for induced spawning, they are transported to the hatchery.



Broodstock can be transported with minimal stress by using a live-fish boat or truck, a water-proof hammock or a large styrofoam box, depending on the proximity of the fish farm to the hatchery. The use of anesthesia is optional. Water tem-perature can be lowered to 22-23°C to calm down the carp.

Figure 6. Methods of transporting bighead carp broodstock

Conditioning of Broodstock

As soon as the broodstock arrive in the hatchery, they are weighed and then placed in the conditioning tank. The sex, identification number and corresponding weight of the fish are recorded. Ovarian biopsy may also be done and the fixed eggs examined under a dissecting microscope.

Bighead carp broodstock receive hormone injections after about 24 hours of conditioning in the tank. This enables the fish to recover from any handling and transport stress, and to adjust to the new environment. A shorter conditioning time is possible if the injection protocol uses dopamine antagonist. Broodstock are not fed during conditioning.

It is important to monitor water temperature (not to exceed 30°C) and dissolved oxygen levels (at least 6 mg/l) during the conditioning period.

Induced Spawning

Bighead carp, like the other Chinese carps, breed in nature during monsoon months when water level is high and river current strong. Bighead carp do not spawn naturally in captivity and in static water conditions. Thus, they have to be induced to spawn by hormone injection.

Action of exogenous hormones

The LHRH-a mimics the function of a gonadotropin releasing hormone produced naturally by the hypothalamus. Gonadotropin releasing hormone stimulates the pituitary gland to produce endogenous gonadotropins. The HCG simulates the pre-ovulatory surge of gonadotropin which is responsible for the final maturation of the ova and ovulation in fish. The release of gonadotropin in fish is inhibited by dopamine, a substance also produced by the hypothalamus especially when the fish are stressed. Dopamine antagonist

such as pimozide (PIM) or domperidone (DOM) is used to block the inhibition of dopamine. Moreover, dopamine antagonists have been reported to potentiate the activity of gonadotropin analogues.

Injection protocols and computations

Several induced spawning protocols have been tested and reported for the bighead carp (See Appendix I). While the broodstock are in the conditioning tank, the hormones needed by the fish are computed and prepared based on the injection protocol to be used.

The double-injection protocol using LHRH-a and HCG is still commonly used in the Philippines. The females receive two injections; the males are injected once during the second injection of the females or sometimes twice just like the females. Two ways of determining the volume of hormone preparations to be injected using a double-injection protocol are shown in Appendix II.

The Linpe method of induced spawning of bighead carp using single injection is being used at times in some private hatcheries. It involves the simultaneous injection of LHRH-a and a dopamine antagonist particularly domperidone (DOM).

	LHRH-a	DOM
Dose	50 µg LHRH-a /kg fish	5 mg DOM/kg fish
Concentration	50 µg/ml	5 mg/ml
Injection volume	1 ml/kg fish	1 ml/kg-fish

Although commercial preparations of combined gonadotropin releasing hormone analogue and DOM (e.g., Ovaprim* and Ovatide*) have been developed, they are not commercially available in the Philippines.

* *Mention of trade names does not mean endorsement of the products.*

DOM can be purchased in tablet form (Motillium*) from commercial drug stores. Each tablet contains 10 mg DOM. Tablets are ground finely using a mortar and pestle, dissolved in dimethyl sulfoxide (DMSO), and suspended in propylene glycol. The computation for the amount of hormones to be injected to the bighead carp is similar to the examples given for the double-injection protocol (Appendix II).

Hormone preparation

Inducing hormones are prepared at one time for the whole batch of broodstock in the conditioning tank. At least two syringes with needles are used for one kind of hormone: one for the solvent and the other for the hormone in solution. This will prevent contamination of the solvent. Hormone preparations are properly labeled to include the concentration and date.

LHRH-a. The ampules are opened by filing the 'neck' and breaking off the tip. With the use of a syringe with needle, a known partial amount of solvent is carefully drawn out and added to one ampule at a time. The hormone in pellet form quickly dissolves. The solution is transferred to a bigger covered bottle with the use of another syringe with needle. The rest of the solvent is used to rinse the ampules and added to the bigger bottle containing the hormone in solution. The desired volume of hormone can be drawn out and injected to the fish.

HCG. It comes in convenient vials with metal-sealed rubber stopper. When HCG is available at 5,000 IU/vial, it is not necessary to transfer the HCG solution to a bigger bottle. The exact amount of solvent (1.25 ml in this case) is added to each vial. Otherwise, the procedure for the preparation of LHRH-a solution will be followed.

DOM. The preparation of dopamine antagonist needs careful attention. The solvent is a combination of dimethyl sulfoxide (DMSO) and propylene glycol (1:9 v/v or 10%

of the solvent is DMSO and 90% is propylene glycol). After determining the amount of dopamine antagonist needed, DMSO is added with constant stirring until DOM completely dissolves. Then propylene glycol is added. The final concentration should be 5 mg DOM/ml. This preparation is quite viscous.

Injecting the bighead carp

Fish may be injected intramuscularly or intraperitoneally. Intramuscular injection is given near the base of the dorsal fin and just above the lateral line.

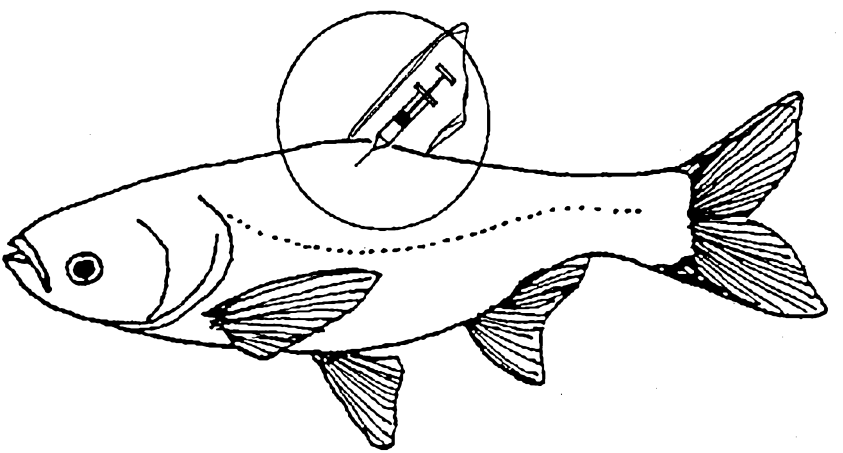


Figure 7. Intramuscular injection method

Intraperitoneal route is appropriate for larger injection volumes because, unlike the muscle tissue, the peritoneal cavity has more space to hold fluids. Intraperitoneal injection is usually given at the base of the pectoral fin. In this case, fish are injected carefully to ensure that vital organs are not punctured. The size of the syringe with needle should be just enough for the volume of hormone to be injected. The fish should be handled gently and properly to minimize stress and loss of scales or mucus.

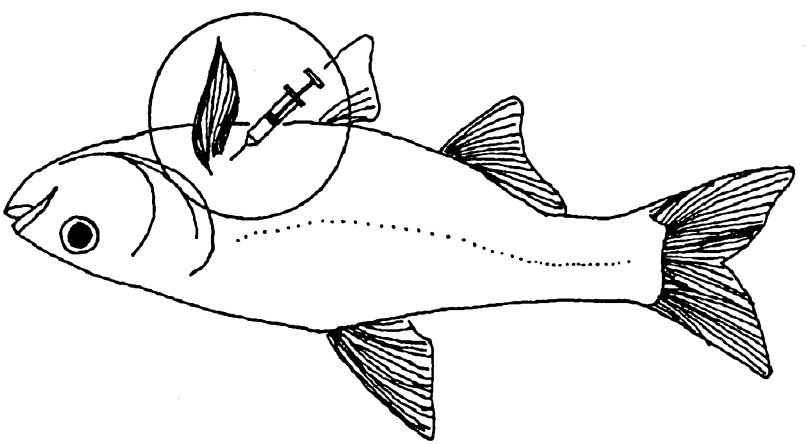


Figure 8. Intraperitoneal injection method

- The broodstock are gathered at one area of the conditioning tank with the use of a fine-meshed catching net. Since each fish is marked, the pre-determined volume of hormone to be injected to the fish can be drawn out immediately from the bottle.

- Partially immersed in water, each fish is positioned ventral side up in the hammock. With the pectoral fin slightly lifted, the fish is injected at a 45-degree angle near the base of the pectoral fin. The injection site is massaged (preferably with cotton wet in alcohol) following withdrawal of the needle.

- When receiving two injections, fish should be injected at one side each time (e.g., left side only for the first injection and right side only for the second injection).

Stripping of Fish

The broodstock in the conditioning tank have to be observed even before the expected time of spawning. This is because bighead carp may release eggs spontaneously in the tank, which is not encouraged because fertilization of eggs is very low.

Spawning is impending when fish are seen swimming in pairs. Sometimes, a male fish bumps its head on the abdomen of the female fish. This occurs usually 5-12 hours after the second injection. The fish are then gathered at one area of the conditioning tank using a fine-meshed catching net. A soft, swollen belly of a female fish held upside down indicates that eggs have been ovulated and the fish is ready for stripping.

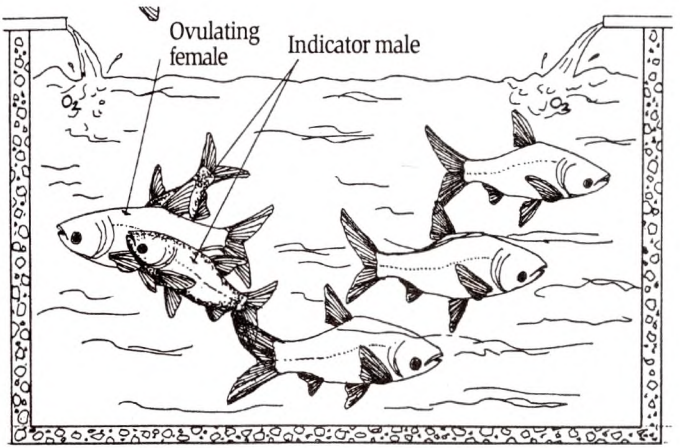


Figure 9. Characteristic swimming behavior of broodstock ready for stripping

- The male and female fish are held individually in a hammock in preparation for stripping.
- Before stripping, the fish body is wiped dry with a cotton towel.
- The tail is held firmly with one hand (usually the left hand) while the head is held close to one's body by the right arm. The fish is stripped by the right hand.

- The female fish is stripped first, followed by the male.
- Gentle massage is applied on the abdomen of fish from anterior to posterior direction with the free hand (usually the right hand) to express the eggs or milt which are then collected in a white enamel basin. (For males, urine is first gently squeezed out of the urogenital pore before milt is collected.)
- Milt is then poured over the eggs and mixed quickly in circular motion using a fowl feather. This allows dry fertilization to take place. Stripping and mixing should be done quickly to ensure fertilization of many eggs.
- Saline water (0.6 % NaCl) is then added and mixing is continued using a feather or by swirling the basin. The saline water makes the sperm motile for a longer period of time.
- Then, the eggs are washed several times using the incubation water before they are finally placed in the hatching tanks. (See section on Incubation of Eggs)

  Spent fish are placed in a holding tank where they are allowed to recover from handling stress. Fish are then returned to the broodstock cages or ponds during cooler times of the day or late in the afternoon.

Facilities and Materials for Egg Incubation

The following have to be ready when induced spawning is scheduled in the hatchery:

Recirculating water system for incubation

Water reservoir. This tank contains hard water for incubation. (See section on Preparation of Hard Water). Volume of water in the reservoir is about 10 times the total volume of the incubation tanks. The water is aerated.

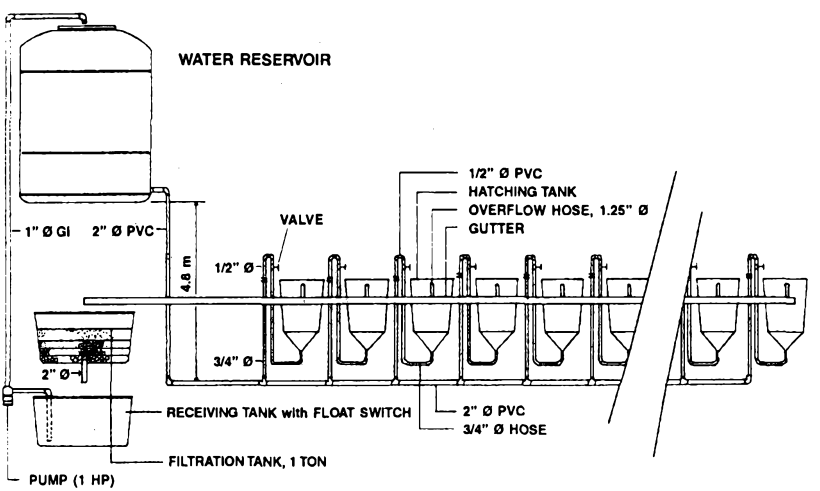


Figure 10. Incubation set-up with a recirculating water system

Filtration tank. It is usually a one-ton tank with layers of sand, gravel and charcoal from top to bottom and fine-meshed net in between layers. This receives the water coming out of the hatching tanks. Filtered water will then flow by gravity to a receiving tank.

Receiving tank or sump. This is a one-ton tank that receives filtered water which is automatically pumped back to the overhead water reservoir when the tank becomes full. The pump's capacity is 1/2 HP.

Incubation/Hatching tanks - Stainless steel, conical hatching tanks may be used in the hatchery. Each hatching tank has a capacity of 135 liters of water. The tank has a metal screen at the full-capacity level so that excess water will flow out to the filtration tank by gravity without losing the eggs or larvae.

Each tank is provided with a valve in order to regulate water flow through a 0.75-inch hose attached to the bottom. Water flows by gravity from the reservoir to the hatching tanks.

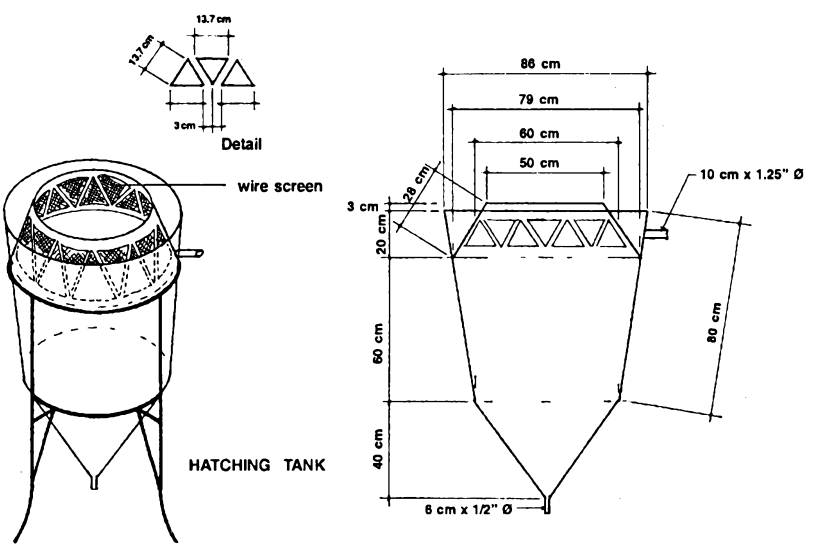


Figure 11. Details of a bighead carp incubator

Preparation of hard water for incubation

It may be necessary to prepare "hard water" for incubating eggs depending on the water source. Well water usually has a hardness of up to 50 mg/l expressed as $CaCO_3$. However, the water used to incubate carp eggs should have a hardness ranging from 300 to 500 mg/l expressed as $CaCO_3$.

In the preparation of the "hard water", the total volume needed by the recirculating system for incubating eggs (i.e., the reservoir, receiving tank and hatching tanks) has to be considered. For a total water volume of 10 tons, 1 kg of $MgCO_3$ and 2 kg of $CaCl_2$ are dissolved in a small amount of water and added to the receiving tank or sump. The freshly prepared "hard water" can stay in the receiving tank of the recirculating system until after the second injection. A few hours before the expected time of stripping, the prepared "hard water" is then pumped from the sump to the reservoir, down to the hatching tanks and eventually back to the sump. When the solution is completely mixed after running through the incubation system, the set-up is ready to receive the eggs for incubation.

It is best to analyze the water hardness and make necessary adjustments before use because technical grade chemicals may contain inert materials. Some are also lost to surfaces of the recirculating system.

Incubation of Eggs

The fertilized eggs are incubated in cone-shaped incubation or hatching tanks. Eggs are stocked up to 1,000/liter. Since bighead carp eggs tend to sink, water flow into the tanks has to be controlled such that water current gently keeps the carp eggs afloat. Too strong water flow will mechanically break the egg membrane or cause the eggs to stick to the wire screen at the water outlet.

Water inflow should be checked frequently throughout the incubation period. When the eggs are hatched and the larvae are at the free-swimming stage, water current has to be reduced.

Egg incubation takes 18-24 hours until hatching. About 6 hours post incubation, fresh tap water may be introduced into the receiving tank to replenish part of the water in the incubator or hatching tanks and slowly reduce water hardness. When water flowing out of the hatching tanks becomes bubbly before the expected hatching time, more fresh water can be introduced continuously into the receiving tank. By the time hatching of eggs is complete, water should have been totally replaced. Water replenishment is necessary to improve water quality and enhance hatching of eggs.

The carp larvae remain in the hatching tanks until the yolk sac has been absorbed indicating the onset of exogenous feeding. This usually lasts for about 3 days post incubation at 27°C.

Larval Rearing

Four to five days after hatching, bighead carp larvae are harvested from the incubators and stocked in the nursery tanks or large basins with mild aeration at 20-30 individuals per liter. Most private hatcheries feed the larvae with homogenized hard-boiled chicken egg yolk or mashed hard-boiled egg yolk which has passed through a 70-micron sieve as initial source of nourishment. The form and particle size of the feed have to be considered for initial feeding of the bighead carp larvae. Sometimes, feeding with rotifers (*Brachionus*) is done until days 6-7.

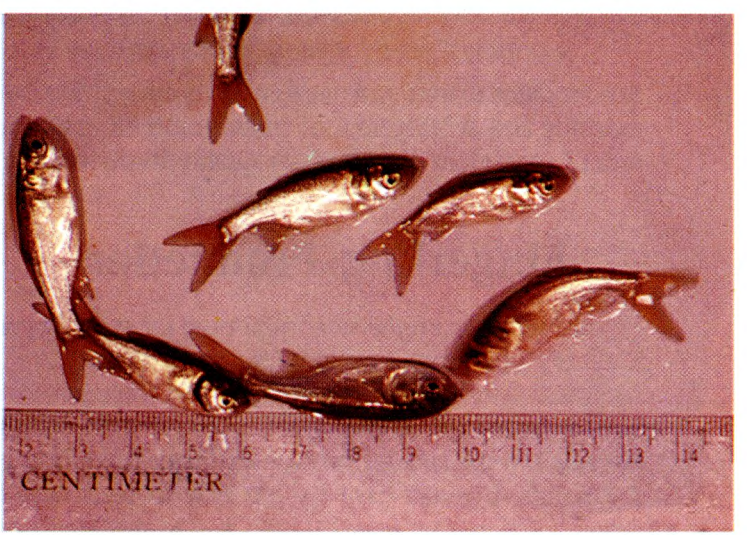
Newly hatched *Artemia* nauplii may also be given to the carp larvae on the 5th or 6th day of rearing. However, since *Artemia* cysts are incubated for 24 hours in marine water, thorough washing of the nauplii with fresh water is necessary prior to feeding to the larvae. Moreover, *Artemia* cysts are incubated as required for a day's feeding. Bigger size zooplankton such as the cladoceran *Moina macrocopa* can be fed following *Artemia*, lasting until the end of a 2-week larval rearing period. Combining artificial feeds with particle size of 70-80 microns has been shown to enhance growth and survival of bighead carp fry.

Accumulated wastes and uneaten foods are siphoned out every morning and at least 50% of water volume is replenished, in order to maintain a good water quality in the rearing tanks.

Fingerling Production

Fry-to-fingerling production is optional for some bighead carp hatchery operators since not many of them have nursery facilities. After two weeks of rearing in the nursery tanks or basins, fry are transferred to fertilized ponds or floating hapa net cages in the lake for further rearing to fingerling stage. In the ponds, bighead carp fry are able to feed with ease on larger zooplankton, insect larvae, and formulated diets. Fish

can be given supplemental feeds at 2-3% of the body weight daily. To maintain a high plankton count in the ponds, it is best to apply chicken manure as fertilizer at a rate of 50 tons/ha. Feeding on zooplankton can enhance the growth and survival of bighead carp fry and fingerlings. Rearing period for fry up to fingerling stage (3-5 cm) may last for 4-6 weeks.



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Appendix I

Table 1. Hormones and dosages in the induced spawning of bighead carp

Water temp (°C)	Hormone injection(s)*	Time to ovulation after injection	Source
27-30	**Female:** 1st injection: 20 µg LHRH-a/kg body weight 2nd injection, 8 hours after the 1st injection: 1800 IU HCG/kg 1 **Male:** 1st injection: none 2nd injection: 900 IU HCG/kg body weight	6-8 hours	Santiago et al. (1991)
25-30	**Female:** 1st injection: 20 µg LHRH-a/kg body weight 2nd injection, 12 hours after the 1st injection: 2000 IU HCG/kg **Male:** 1st injection: 20 µg LHRH-a/kg body weight 2nd injection: 1000 IU HCG/kg	5-8 hours	Santiago and Gonzal (2000)
25-30	**Female:** 1st injection: 180 IU HCG/kg body weight 2nd injection, 6 hours after the 1st injection: 20 µg LHRH-a/kg; 1620 IU HCG/kg **Male:** 1st injection: 10 µg LHRH-a/kg body weight or 900 IU HCG/kg 2nd injection: none	7-11 hours	Fermin (1991)
25-30	**Female:** 1st injection: 7.5 µg LHRH-a/kg body weight plus 1.5 mg DOM/kg 2nd injection, 6 hours after the 1st injection: 67.5 µg LHRH-a/kg body weight plus 13.5 mg DOM/kg **Male:** 1st injection: 10 µg LHRH-a/kg body weight or 900 IU HCG/kg 2nd injection: none	6-10 hours	Fermin (1991)
20 -30	One injection** 5 mg DOM + 50 µg LHRH-a/kg body weight or 5 mg DOM + 20 µg sGnRH-a/kg	8-12 hours 8-12 hours	Lin and Peter (1996)

* Abbreviations: LHRH-a, luteinizing hormone-releasing hormone analogue
HCG, human chorionic gonadotropin
DOM, domperidone (a dopamine antagonist)
sGnRH-a, salmon gonadotropin releasing hormone analogue

** The Linpe method uses a combination of LHRH-a and DOM given in one injection.

Appendix II

Procedures for determining the volume of hormones to be injected to the bighead carp broodstock. (For simplicity, two males will be paired with one female bighead carp.)

Double-injection protocol:

	Dose	
	1st injection	**2nd injection***
Female	20 µg LHRH-a/kg fish	2000 IU HCG/kg
Male	20 µg LHRH-a/kg fish	1000 IU HCG/kg

* given 12 hours after the 1st injection

Procedure 1 (refer to Table 2):

- Record the body weight of individual female and male fish (see column A and column B).
- Based on the prescribed doses, compute the amount of hormones needed by each fish (see column C and column E).

First injection

for female or male fish:

Weight of fish x 20 µg LHRH-a/kg = Amount of LHRH-a per fish

Column B x 20 = Column C

Second injection

for female fish:

Weight of female x 2000 IU HCG/kg = Amount of HCG per female

Column B x 2000 = Column E

for male fish:

Weight of male x 1000 IU HCG/kg = Amount of HCG per male

Column B x 1000 = Column E

(Note: The dose for male fish is only one-half of that of the female.)

- Obtain the sum of hormone requirements and decide how many vials or ampules of hormones and how much solvent to use.

***Note:** Commercial LHRH-a usually comes in 250 µg/ampule. LHRH-a concentration of 40 µg/ml will give the right volume to be injected to each fish. HCG is usually available at 5,000 IU/vial. HCG solution may be prepared at 4,000 IU/ml.*

Assuming 250 µg LHRH-a per ampule and a total requirement of 468 µg LHRH-a, two ampules and 12.5 ml of solvent will be used to give a final con-centration of 40 µg LHRH-a per ml.

Volume of solvent for LHRH-a
= (2 x 250 µg) ÷ 40 µg LHRH a/ml
= **12.5 ml**

Seven (7) vials of HCG and 8.75 ml of solvent (1.25 ml/vial) will be used to obtain 4,000 IU HCG/ml.

Volume of solvent for HCG
= (7 x 5,000 IU) ÷ 4,000 UI /ml
= **8.75 ml (or 1.25 ml/vial)**

- Calculate the volume of hormone to be injected to each fish on the first injection (see column D) and on the second injection (see column F).

Volume of hormone = Amount of hormone per fish ÷ concentration

First injection:

*Column C ÷ (40 µg LHRH-a/ml) = ml of LHRH-a per fish (**see column D**)*

Second injection:

*Column E ÷ (4,000 IU HCG/ml) = ml of HCG per fish (**see column F**)*

Table 2. Results of computations using procedure 1

			First injection	Second injection	
	Fish weight (kg)	Amount of LHRH-a (µg/fish)	Volume of LHRH-a (ml)*	Amount of HCG (IU/fish)	Volume of HCG (ml)**
A	B	C	D	E	F
Female					
1	2.2	44	1.10	4400	1.100
2	3.1	62	1.55	6200	1.550
3	3.5	70	1.75	7000	1.750
Male					
10	2.1	42	1.05	2100	0.525
11	2.5	50	1.25	2500	0.625
12	2.8	56	1.40	2800	0.700
13	2.2	44	1.10	2200	0.550
14	2.7	54	1.35	2700	0.675
15	2.3	46	1.15	2300	0.575
Grand Total	23.6	468		32,200	

* LHRH-a concentration is 40 µg/ml.

** HCG is 4,000 IU/ml.

***Procedure 2** (refer to the double-injection protocol given above and Table 3):*

This also requires preparation of LHRH-a at a concentration of 40 µg LHRH-a/ml and HCG at 4000 IU/ml.

The following injection volumes are suitable for the bighead carp:

0.5 ml LHRH-a/kg female or male fish

0.5 ml HCG/kg female; 0.25 ml HCG/kg male

(Note: This difference in injection volume would allow preparation of just one concentration of HCG for both females and males.)

- Record the body weight of individual female and male fish (see column A and column B).
- Obtain the sum of body weight of all fish for computation of LHRH-a requirements. Obtain also the sum of body weight of female fish and male fish, separately, for computation of HCG requirements.

Amounts of hormones to be prepared in solution:

(23.4 kg females and males) (20 µg LHRH-a/kg)
= 468 µg LHRH-a

[(8.8 kg females) (2000 IU HCG/kg)] + [(14.6 kg males) (1000 IU HCG/kg)] = **32,200 IU HCG**

- Determine the number of ampules of LHRH-a and vials of HCG to be used. The amount of solvent to be used is likewise determined.

If LHRH-a is available at 250 µg per ampule, two ampules will be used with 12.5 ml of solvent to produce 40 µg LHRH-a/ml.

Since HCG comes in 5000 IU per vial, seven vials should be used with 8.75 ml of solvent (or 1.25 ml per vial) to produce 4,000 IU HCG/ml.

- For the first injection, compute the volume of LHRH-a preparation to be injected to the female and male fish (see column C).

First injection:

Weight of fish x injection volume = volume of LHRH-a

or

Column B x 0.5 ml LHRH-a/kg fish = Column C

- For the second injection, compute the volume of HCG preparation to be injected to the female and male fish (see column D).

Second injection:

Weight of fish x injection volume = volume of HCG.

Column B x 0.5 ml HCG/kg female = Column D
Column B x 0.25 ml HCG/kg male = Column D

(Take note that the volume (and dose) for the male fish is one-half of that of the female fish.)

Table 3. Results of computations using procedure 2

		1st injection	2nd injection
Fish number	Weight (kg)	Volume of LHRH-a (ml)/fish*	Volume of HCG (ml)/fish**
A	**B**	**C**	**D**
Females			
1	2.2	1.10	1.100
2	3.1	1.55	1.550
3	3.5	1.75	1.750
Total, females	8.8		
Males			
10	2.1	1.05	0.525
11	2.5	1.25	0.625
12	2.8	1.40	0.700
13	2.2	1.10	0.550
14	2.7	1.35	0.675
15	2.3	1.15	0.575
Total, males	14.6		
Grand total	23.4	11.7	8.05

* LHRH-a concentration is 40 µg/ml.

** HCG concentration is 4,000 IU/ml.

Note: Regardless of injection protocol, the volume of hormones to be prepared is normally in excess of what is actually needed to give allowance to spillage or other losses.

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About SEAFDEC

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in December 1967 for the purpose of promoting fisheries development in the region. Its member countries are Japan, Malaysia, the Philippines, Singapore, Thailand, Brunei Darussalam, the Socialist Republic of Vietnam, Union of Myanmar, and Indonesia.

Representing the Member Countries is the Council of Directors, the policy-making body of SEAFDEC. The chief administrator of SEAFDEC is the Secretary-General whose office, the Secretariat, is based in Bangkok, Thailand.

Created to develop fishery potentials in the region in response to the global food crises, SEAFDEC undertakes research on appropriate fishery technologies, trains fisheries and aquaculture technicians, and disseminates fisheries and aquaculture information. Four departments were established to pursue the objectives of SEAFDEC.

- The Training Department (TD) in Samut Prakan, Thailand, established in 1967 for marine capture fisheries training
- The Marine Fisheries Research Department (MFRD) in Singapore, established in 1967 for fishery post-harvest technology
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines, established in July 1973 for aquaculture research and development
- The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia, established in 1992 for the development and management of the marine fishery resources in the exclusive economic zones (EEZs) of SEAFDEC Member Countries.

SEAFDEC/AQD is mandated to:

- promote and undertake aquaculture research that is relevant and appropriate for the region
- develop human resources for the region
- disseminate and exchange information on aquaculture