Aqua-Internship Program
Asia Link project

Faculty of Fisheries

Bangladesh Agricultural University, Mymensingh

Seed Production Technique of Indigenous Magur (*Clarias batrachus*) and Shing (*Heteropneustes fossilis*) Through Induced Breeding

Name of Intern : Md. Rajib Sharker

Name of MS Supervisor : Professor Dr.Md. Mukhlesur Rahman Khan
Fisheries Biology and Genetics Department

Name of Field Supervisor : A.K.M. Nurul Haque
Owner of the Farm

Name of Aqua-Industry : Brahmaputra Fish Seed Complex

INTRODUCTION

Bangaladesh is a land of high potential water resources. There are 260 freshwater fish species, 24 freshwater prawns, 475 marine fish species, 36 marine or brackish water shrimps and 16 exotic species available in this country (DoF, 2008). At least 55 species of catfishes belonging to 35 genera have been recorded in Bangladesh (Rahman, 2005). In Bangaladesh fish contributes 63% of total animal protein supply. Total catfish production in inland water is 85,869 metric ton (DoF, 2008). Due to natural and man made hazards, biodiversity of fish and other aquatic organisms in open water have been declining so much and with such rapidity that the aquatic animals, especially fish are unable to cope with (Mollah, 2005). That's why the dependency on hatchery produced fry has increased rapidly to protect the species from being extinct. Fin fish hatchery was first established in Jessore by Mohoshin Master in 1967. Since then the number of fish hatchery has increased uninterruptedly reaching over a thousand in 2010 to fulfill the ever increasing demand of the fin fish seeds for aquaculture industry of Bangladesh. There are 126 govt. hatcheries and rests are private hatcheries most of which are present in Jessore, Comilla and Mymensingh district. In Bangladesh both public and private hatchery produced around 423986kg hatchling (DoF, 2008). Brahmaputra fish seed complex is an established, popular and renowned hatchery in Mymensingh district. Total area of this hatchery is 80decimal in which there are three units such as catfish, koi and carp units and two overhead tanks. In catfish and koi unit there are 45 and 8cisterns respectively both large and small but in carp unit there are 34jars, 12cisterns and 4circular tanks. There are 70 ponds used as brood pond, culture and nursery pond. During breeding season carp spawn production cycle starts from Saturday and ends in Thursday by selling the spawn and catfish and koi spawns are produced interruptedly during this time. They sell catfish and koi by nursing in own pond. The availability of magur, shing, pabda has declined drastically from open waters such as rivers, haors etc and this fishes are rarely found, sold at an exorbitant price in the market. Artificial breeding is the most widely used way to increase their abundance. Brahmaputra hatchery use different types of hormones such as PG, HCG, Ovaprim etc. to induce magur, shing and pabda.

OBJECTIVES:

The present study has been planned to fulfill the following main objectives-

1. To know and practice the seed production technique of magur and shing by using different types of hormones.
2. To determine the effectives of different hormones for seed production of magur
3. To know the maintenance and hatchery operation technique.

MATERIALS AND METHODS

Seed production technique of magur (*Clarias batrachus*):

Indigenous magur is a tasty and nutritious fish. In past magur was easily found in natural waterbody. But day by day the abundance of this fish has been decreased. Around 1997 the owner of this farm first tried to culture them in confined waterbody. After doing various experiments and hard working finally he succeeded to develop the culture technique and to establish the commercial seed production technology and thus increase the availability of this fish in market.

In brief the seed production technology followed by this farm is given below:

Brood fish rearing:

Stocking density of magur is 50-100 broods per decimal. Magur become sexually mature at the age of 1yr when the weight is around 100g. 35% protein rich feed is used at the rate of 3% body weight.

Mature brood fish selection:

Breeding season of magur is very short (June to July). It is easy to identify mature male and female broods on the basis of secondary sexual characters. During breeding season females are easily identified by their soft and swollen abdomen due to presence of mature bulky eggs. On the other hand males are identified by their flat abdomen and long protruded genital papillae.

Fig 1: Mature male and female magur identification

Hormone extract preparation: Required amount of hormone is weighed by balance. Then ground by mortar and pestle manually with very small amount of water. Ground continuously until homogenous mixture is found. Maximum 1ml water is used per kg body weight of fish.

Fig 2: Preparation of hormone extracts

Then the solution is taken into the syringe and kept into water to remain cool.

Hormone is prepared immediate before injection.

Hormone injection: PG (pituitary gland) extracts, HCG (Human Chorionic Gonadotropin) mixed with PG and ovaprim mixed with PG solution are used as hormone to collect the eggs. Only female is treated with hormone to collect eggs and for collecting sperm untreated male is sacrificed. 27 females are divided into 3 treatments and marked as T_1, T_2 and T_3 having 9 females in each treatment in such a way that

Fig 3: Injected female and uninjected male in cistern

the average weight of 9 females under each

The females under each treatment are kept separately in different cisterns. The females under treatment T_1, T_2 and T_3 are treated with PG (pituitary gland) extracts, HCG with PG and ovaprim with PG solution respectively. One must give more attention to the maturity of fish rather than hormone dose. If fish become fully matured then small amount of hormone is required to ovulate the female.

If hormone dose is higher then seed quality is deteriorated.

In case of PG, for double dose treatment, first female is treated with 5mg PG/kg body weight and after 7-8hrs of first treatment second injection is given at the rate of 15mg PG/kg body weight of fish. Injection is given on the fleshy part of the dorsal side of female. Then they are kept into the cistern with continuous water circulation. After 20-24hrs of second injection, it is the time to collect the eggs from the female.

Single dose for HCG is used to ovulate the female at the rate of (5000IU HCG+5mg PG)/1.5kg female.

In case of ovaprim single dose is used at the rate of (200micro g ovaprim+5mg PG)/2kg female.

Egg collection: Eggs are collected from the fish by stripping. Till now it is not possible to induce the female to spawn naturally. In case of magur more pressure is needed than other fish. But one need to take care to that egg is not affected due to heavy pressure. In case of male it is impossible to obtain milt by stripping because of the lobular structure of

the testes. For collection of milt, the testes were dissected out from the body cavity and macerated in 0.9% salt solution.

At first eggs are collected in a bowl from the female and at the same time as soon as possible male is sacrificed and sperm are collected.To ensure the maximum fertilization rate sperm suspension is mixed with eggs by gently stirring with a feather in bowl. The whole activities will be finished within 1-1.5min.

If fully matured male and female are used then the male female ratio is 1:6.

Collected eggs are kept into seperate cistern and small rectangular cistern is better than large cistern. The length of the cistern is 8 feet, width 4 feet and water depth is 3 inch. Due to stickiness of the eggs these are carefully kept into the cistern with the help of feather in such a way that the eggs are not attached together. Shower is given through the perforated PVC pipe to ensure the maximum oxygen supply.

Fig 6: Fertilized eggs in cisterns with continuous water supply

They have longer incubation period and is required 30-36hrs and during this longer time fungal infection may occur. Infected eggs are immediately taken out from the cistern otherwise all the eggs will be infected. Newly hatched larvae (1mm) move to the corner of the

Fig 7: Clustered hatchling of magur in cistern

cistern and clustered together. After 3days of hatching first feeding is given. Live zooplankton is used as food upto satiation level.

Live zooplankton is collected from the pond and after several washing they are used as food. Fry remain into the cistern for 2days then they are transferred into the nursery pond. Percent ovulation, fertilization and hatching rates are recorded to determine the effectiveness of hormone using the following formula:

$$\% \text{ ovulation} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \times 100$$

$$\% \text{ fertilization} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs (fertilized + unfertilized)}} \times 100$$

$$\% \text{ hatching} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs (fertilized + unfertilized)}} \times 100$$

Rearing in nursery pond: 15-20decimal rectangular sized pond is better for nursing of magur fry. For preparation of nursery pond at first pond is dried completely and then liming is done at the rate of 1kg per decimal and kept for 1-2 days after immediate tillage. Then watering is done by shallow machine and water depth is maintained between 1 and 1.5feet. All unwanted plants and animals are removed from the pond. Frog, snake and other feral animal are very harmful for nursing of fry. Further attention must be paid to protect the nursery pond from being entering the frog, snake etc. So small mesh sized net is used to surround the whole nursery pond. 5days old fry are stocked in nursery pond at the rate of 50-60gm per decimal. Hard boiled egg yolk and flower solution is used as feed. Blended hard boiled duck egg (20) are mixed with 1kg

Fig 8: Preparation of nursery pond

flower solution and then spread into the water 3 times per day. After 5-6 days nursery koi feed is used. Within 30 days fry become 3inch size.

Selling of fry: 3inch sized fry are sold at the rate of Tk. 2/piece. Three layered plastic bag of 30”x18” size are used. 250g fry is weighed which contain around 500 two inch sized fry are kept into plastic bag with oxygen (from oxygen cylinder) and water. The plastic bag is tightly packed to prevent the entry of air into the bag. The selling bag contain around 8-10L water, 250g fry and rest is oxygen. Before selling at least 2hrs conditioning is better. Fry remain reasonably better around 15hrs in that type of plastic bag during transportation.

Fig 11: Packing the bag tightly containing fry, water and oxygen

Precautions during seed production:

1. Matured broodfish must be selected otherwise all activities will be unsuccessful.
2. During stripping gentle pressure should be put also.

3. Egg density into the cistern should not be higher and they are carefully kept into the cistern in such a way that the eggs are not attached together.
4. Continuous water circulation should be ensured and 27-28^{o}c water temperature should be maintained.

Seed production technique of shing (*Heteropneustes fossilis*):

Natural abundance of shing is becoming decreased day by day due to environmental degradation. But around 1999 huge amount of fry of shing was produced firstly by the owner of this farm for commercial purpose to increase the availability of this species in market. Till then various experiments were conducted to protect this species from being extinct. At last in 2002 it was possible to produce huge amount of fry commercially by the farm owner.

In brief the fry production technique of shing of this farm is given below:

Brood fish rearing:

Shing become sexually mature at the age of 10-11 months. Healthy and disease free broods are stocked at the ratio of 50:50 (male: female) in separate pond and stocking density for shing is 200 broods per decimal respectively. Brood fish are fed with good quality feed at the ratio of 5% body weight which is prepared with 30% fish meal, 30% soybean meal, 30% wheat flour and 10% rice bran and vitamin.

Mature brood fish selection:

Shing has long breeding season extending from mid April to mid August Mature male and female broods are selected on the basis of secondary sexual characters. During breeding season females are easily identified by their soft and swollen abdomen due to bulky eggs and round and swollen genital papillae. Female is larger than the male. On the other hand males are identified by their flat abdomen and long protruded genital papillae.

Protruded genital papillae of male

Fig 12: Mature male shing

Hormone injection: For induced breeding matured male and female should be selected. Two types of hormones are used to induce the shing to breed such as PG (pituitary gland) extracts and HCG (Human Chorionic Gonadotropin) solution.

Using PG (pituitary gland) extracts: After selecting mature male and female PG extracts are used to induce them to breed. Single dose is used for both male and female. Female is treated with 30mg PG/kg body weight of fish and male is treated with 5-10mg PG/kg body weight of fish. Male and female ratio is 1:1. However maximum eggs will be found if the male and female ratio is 1.5:1, fertilization rate is higher.

Using HCG (Human Chorionic Gonadotropin) solution: Only female fish is treated with HCG solution and the dose is 5000IU HCG/2-3kg fish and male is treated with 5-10mg PG/kg body weight of fish.

At first female is treated with 2-3mgPG/kg body weight and injection is given in the soft pectoral fin base. After 6hrs of first injection again female is treated with 4-6mg PG/kg body weight and at the same time male is also treated with 4-6mg PG/kg body weight.

Injection is given on the fleshy part of the dorsal side and then they are kept into the cistern with continuous water circulation. They spawn naturally and fertilize the egg. If stripping is used for shing fertilization rate is not more than 5% which will never bring success to a commercial farmer. On the other hand to produce huge amount of fry for commercial purpose large number of fish is required for stripping but it is impossible to stripe huge amount of fish because it is time consuming.

Naturally egg collection is done by two ways such as

Using hapa

Using cistern

Using hapa: At first 1cm mesh size hapa made with polythene is used whose length is 12 feet and width is 8 feet. Then the hapa is set in the cistern in such way that the hapa remains 6inch away from the bottom. 3feet water depth is maintained and artificial aeration is used for continuous water circulation to ensure the maximum oxygen supply. Then the injected male and female are stocked into the hapa at the ratio of 1:1 (male: female). After 10-12hrs of second injection fish spawn naturally and eggs are fertilized by the sperm. They usually start spawning at mid night and complete in the morning. The eggs of these fish are slightly sticky. The eggs are settled down on the bottom of the cistern through the open space of the hapa. In the morning after complete spawning of eggs, spent fish with hapa is taken out from the cistern and at the same time fertilized eggs are collected from the bottom of the cistern through siphoning procedure by using small pipe into plastic bowl.

Using cistern: Injected male and female are kept into the cistern and after 10-12hrs of injection fish spawn naturally and eggs are gathered in the center of the cistern. Then the fertilized eggs are collected through siphoning procedure.

Using hapa is better than cistern because spawned male and female are collected easily from the hapa without affecting the fertilized eggs.Then the collected fertilized eggs are kept into the small cistern and 3-4inch water depth is maintained. Shower is given through the perforated PVC pipe to ensure the maximum oxygen supply. Around 18-24hrs is required for hatching of fertilized eggs. After 3days of hatching yolk sac is absorbed. After 2days of hatching first feeding is given to ensure the availability of food when yolk sac is absorbed. They are cannibalistic in nature and that is why feed is given at 3hrs interval.

Hard boiled egg yolk is used as feed, mixed with water and used upto satiation level. Ground small tubificid worm may also be used as feed. After 4-5days rearing of shing fry into the cistern then they are transferred into the nursery pond. But in case of pabda after first feeding they are transferred to the nursery pond.

Rearing in nursery pond:

For nursing of fry 15-20decimal pond area is better. At first pond is dried completely and 5-10kg cow dung per decimal is given in the pond then after immediate tillage 1kg lime per decimal is given and kept for 1-2 days. Then watering is done by shallow machine. Frog, snake and other discarded animal is very harmful for nursing of fry. It is a must to give more attention to protect the nursery pond from being entering the frog, snake etc. So small mesh sized net is used to surround the whole nursery pond. 5days old fry is stocked into the nursery pond at the rate of 60gm fry per decimal. 50% rice bran and 50% dried fish powder are mixed together and used as nursery feed at the rate of 20% body weight per day. They are nocturnal in habit and so feed is given 2 times at night. Fry is reared in the nursery pond for upto 40 days until the fry become 2inch size.

Selling of fry:

During selling the size of fry is 2inch and the selling rate of shing is Tk1.5/piece Three layered plastic bag of 30”x18” size are used. 250g fry is weighed which contain around 625 two inch sized fry are kept into plastic bag with oxygen (from oxygen cylinder) and water. The plastic bag is tightly packed to prevent the entry of air into the bag. The plastic bag contain around 8-10L water, 250g fry and rest are oxygen. Before selling at least 2hrs conditioning is better. Fry remain better around 15hrs in that type of plastic bag during transportation.

RESULTS AND DISCUSSION

For inducing ovulation in female magur three different hormones are used. Data representing the effects of three different hormones on ovulation of female fish and the rate of fertilization and hatching of eggs are presented in **Table 1**.

Table 1: Effect of different hormones on ovulation of females and fertilization and hatching of eggs of magur (*Clarias batrachus*)

Treat ments	Hormones	wt of female	Ovulation status of females (%)	Latency period (hr)	Average fertilization rate (%)	Average hatching rate (%)
T_1	PG 20mg PG/kg female	1kg	60	30-35	40.65±5.64	20.33±4.53
T_2	HCG (5000IU HCG+5mg PG)/1.5kg female.	1.5kg	80	20-24	60.47±2.45	50.65±3.35
T_3	Ovaprim (200micro g ovaprim+5mg PG)/2kg female.	2kg	60	20-24	50.35±1.39	40.25±1.52

ANOVA test for fertilization and hatching rates of magur

Parameters	Source of variation	Sum of squares	df	Mean square	F- ratio
Fertilization	Between groups	1800	2	900	368.85
	Within groups	58.67	24	2.44	
	Total	1858.67	26		
Hatching	Between groups	4200	2	2100	860.66
	Within groups	58.67	24	2.44	
	Total	4258.67	26		

ANOVA test showed significant ($p<0.01$) difference in fertilization and hatching rate for three different hormones while considering the ovulation rate where T_2 was significantly ($p<0.05$) higher than others. (HCG+PG) hormone is better to induce magur than other hormones. The ambient water temperature during incubation ranged between 24-27^{o}c. One study reported that PG and HCG are equally effective for induction of spawning in *Heteropnuestes fossilis* Gheyas *et.al.* (2000).

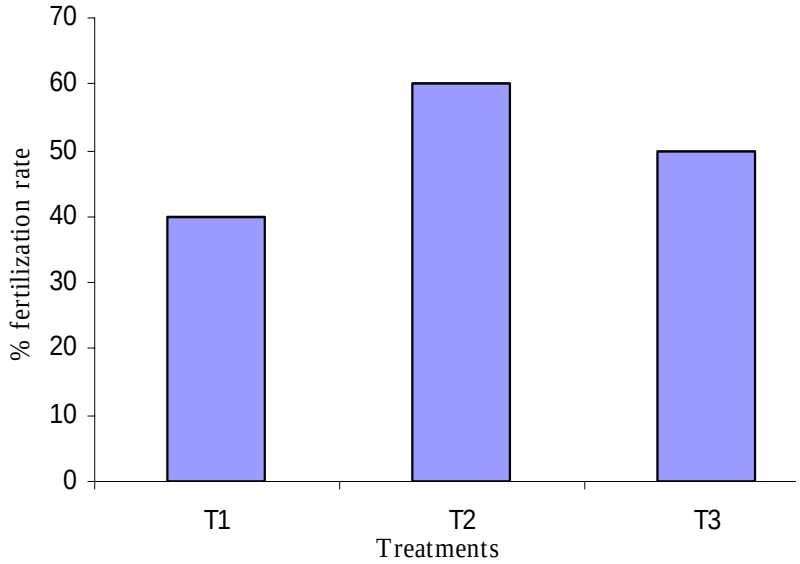

Fig 16: Percent fertilization rate in different treatments

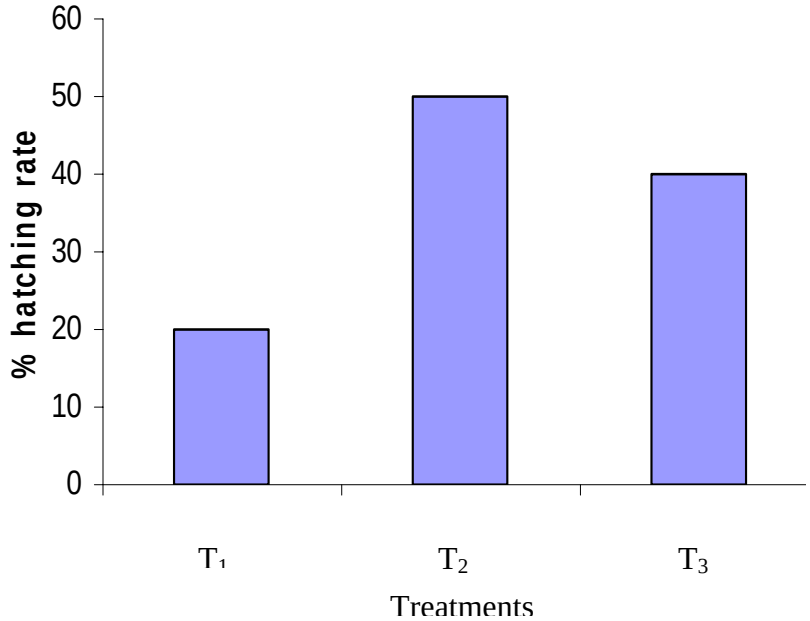

Fig 17: Percent hatching rate in different treatments

The ambient water temperature during incubation ranged between 24-27^{o}c. (HCG+PG) hormone is better to induce magur than other hormones. One study reported that PG and HCG are equally effective for induction of spawning in *Heteropnuestes fossilis* Gheyas *et.al.* (2000). It may be due to species variation and maturity. This hatchery use hard boiled egg yolk and flour for larvae rearing but another study reported that larvae fed mixed feed (live and artificial) showed significantly better growth Yasmin *et al*.(1998).

The production rate of magur, shing, pabda and koi in Brahmaputra fish seed complex in this breeding season are given below:

Fish species	Production (%)
Koi	80
Shing	10
Magur	8
Pabda	0.5

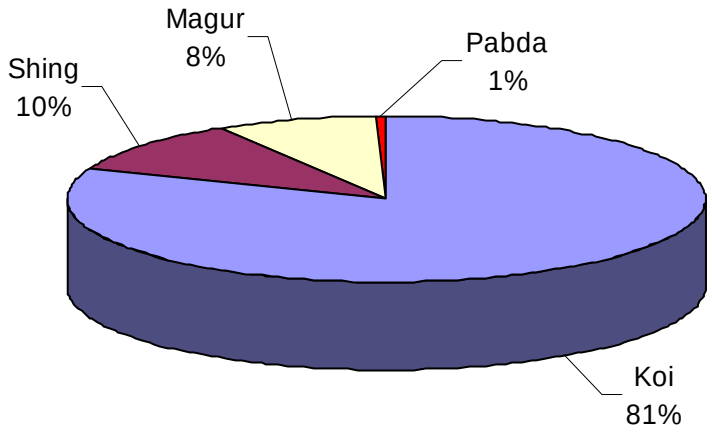

Fig: Percent production of koi, shing, magur and pabda

Conclusion

It is clear from the present study that the seed production technique of indigenous magur and shing is complicated. Mature fish identification and hormone selection is very much important for induced breeding. It is evident from the results and discussion section of the present study that the HCG mixed with PG is best for induced breeding of magur to maximize the ovulation, fertilization and hatching rates.

REFERENCES

DoF. 2008. Annual Report 2007-2008. Department of Fisheries, Bangladesh, Ministry of Fisheries and Livestock, 111pp.

DoF. 2008. Fisheries Statistical Yearbook of Bangladesh 2007-2008. Department of Fisheries, Bangladesh, Ministry of Fisheries and Livestock, 2-34pp.

Gheyas A.A., Akter R., Khan M.H.K., Mollah M.F.A. 2000. Effectiveness of carp pituitary gland extract and human chorionic gonadotropin in inducing spawning in shinghi (*Heteropnuestes fossilis*). Progress. Agric. 11(1&2): 169-173.

Gupta M V and Rab A (1994) Adoption and economics of silver barb *(Puntius gonionotus)* culture in seasonal waters in Bangladesh. Published by ICLARM, Metro Manila, Philippines, with financial assistance from USAID, Baridhara, Dhaka, Bangladesh.