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Study on Seed Production Technique of Indigenous Magur (*Clarias batrachus*), Shing (*Heteropneustes fossilis*) and Pabda (*Ompok pabda*) Through Induced Breeding.

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ABSTRACT
This is the report of three months (from 01.04.2010 to 30.06.2010) observation of different aspects of seed production (magur, shing, pabda) in Brahmaputra fish seed complex. Pabda is already declared as endangered fish (IUCN, 2000) and other species is going to be in vulnerable condition. In case of magur (Clarias batrachus) three different hormones under treatment T_1, T_2 and T_3 (PG, HCG mixed with PG, Ovaprim mixed with PG respectively) were used to determine the effectiveness of hormones on ovulation, fertilization and hatching of magur. 27 females were 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 the average weight of 9 females under each treatment remained approximately similar. The dose of hormones were 5mg PG/kg female (1st dose) and 15mg PG/kg female (2nd dose), (5000IU HCG+5mg PG)/1.5kg female, (200micro g ovaprim+5mg PG)/2kg female. Stripping of female was done and was sacrificed the male to collect milt and fertilized eggs were kept into cistern for hatching. Then the fry was transferred to the nursery pond after first feeding. Nursery pond preparation and breeding were done simultaneously. After rearing in nursery pond upto 2inch size fry, selling was done with different rate per piece. HCG mixed with PG was found to be effective hormone to maximize the ovulation, fertilization and hatching rate of magur. In case of shing, PG and HCG (30mg PG/kg and 5000IU HCG/2-3kg) and in case of pabda 6-9mg PG/kg of female was found effective.
Keywords: *Induced breeding, magur, shingh, pabda.*

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 [1]. At least 55 species of catfishes belonging to 35 genera have been recorded in Bangladesh [2]. In Bangaladesh fish contributes 63% of total animal protein supply. Total catfish production in inland water is 85,869 metric ton [3]. Due to natural and manmade 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 [4]. 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 [1]. 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, shing and pabda 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. In brief the seed production technology followed by this farm is given below:

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

Mature brood fish selection:

Breeding season of magur is very short (June to July)**.** During breeding season females were 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).

Hormone extract preparation: Required amount of hormone was weighed by balance. Then ground by mortar and pestle manually with very small amount of water. Ground continuously until homogenous mixture was found. Maximum 1ml water was used per kg body weight of fish. Then the solution was 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 were used as hormone to collect the eggs. Only female was treated with hormone to collect eggs and for collecting sperm untreated male was sacrificed. 27 females were 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 the average weight of 9 females under each treatment remained approximately similar. The females under each treatment were kept separately in different cisterns. The females under treatment T_1, T_2 and T_3 were treated with PG (pituitary gland) extracts, HCG with PG and ovaprim with PG solution respectively. In case of PG, for double dose treatment, first female was treated with 5mg PG/kg body weight and after 7-8hrs of first treatment second injection was given at the rate of 15mg PG/kg body weight of fish. Injection was given on the fleshy part of the dorsal side of female. Then they were kept into the cistern with continuous water circulation. After 20-24hrs of second injection, it was the time to collect the eggs from the female. Single dose for HCG was used to ovulate the female at the rate of (5000IU HCG+5mg PG)/1.5kg female. In case of ovaprim single dose was used at the rate of (200micro g ovaprim+5mg PG)/2kg female.

Egg collection: Eggs were collected from the fish by stripping. Till now it was not possible to induce the female to spawn naturally. In case of magur more pressure was needed than other fish. In case of male it was impossible to obtain milt by stripping because of the lobular structure of the testes (Fig. 2). For collection of milt, the testes were dissected out from the body cavity and macerated in 0.9% salt solution.

At first eggs were collected in a bowl from the female and at the same time as soon as possible male was sacrificed to collect the sperm. To ensure the maximum fertilization rate sperm suspension was mixed with eggs by gently stirring with a feather in bowl (Fig. 3). The whole activities were finished within 1-1.5min.

Collected eggs were kept into seperate cistern and small rectangular cistern was better than large cistern. Due to stickiness of the eggs, these were carefully kept into the cistern with the help of feather in such a way that the eggs were not attached together. Shower was given through the perforated PVC pipe to ensure the maximum oxygen supply (Fig. 4).

They have longer incubation period and was required 30-36hrs and during this longer time fungal infection may occur. Infected eggs were immediately taken out from the cistern otherwise all the eggs will be infected. Newly hatched larvae (1mm) move to the corner of the cistern and clustered together. After 3days of hatching first feeding was given. Live zooplankton was used as food upto satiation level.

Live zooplankton was collected from the pond and after several washing they were used as food. Fry remain into the cistern for 2days then they were transferred into the nursery pond.

Percent ovulation, fertilization and hatching rates were 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 (Fig. 5): 15-20 decimal rectangular sized pond is better for nursing of magur fry. For preparation of nursery pond at first pond was dried completely and then liming was done at the rate of 1kg per decimal and kept for 1-2 days after immediate tillage. Then watering was done by shallow machine and water depth was maintained between 1 and 1.5feet. All unwanted plants and animals were removed from the pond. Frog, snake and other feral animal were very harmful for nursing of fry. Further attention was paid to protect the nursery pond from being entering the frog, snake etc. So small mesh sized net was used to surround the whole nursery pond. 5days old fry were stocked in nursery pond at the rate of 50-60gm per decimal. Hard boiled egg yolk and flower solution was used as feed. Blended hard boiled duck egg (20) were mixed with 1kg flower solution and then spread into the water 3 times per day. After 5-6 days nursery koi feed was used. Within 30 days fry became 3inch size.

Selling of fry: 3inch sized fry were sold at the rate of Tk. 2/piece. Three layered plastic bag of 30"x18" size were used. 250g fry was weighed which contain around 500 two inch sized fry were kept into plastic bag with oxygen (from oxygen cylinder) and water. The plastic bag was tightly packed to prevent the entry of air into the bag. The selling bag contained around 8-10L water, 250g fry and rest was oxygen. Before selling at least 2hrs conditioning was better.

Seed production technique of shing (*Heteropneustes fossilis*) and pabda (*Ompok pabda*):

Natural abundance of shing is becoming decreased day by day due to environmental degradation. Once upon a time pabda was found in haor, baor, river etc. but day by day the abundance is decreased due to climatic change and destruction of breeding ground. Recently IUCN (5) has declared the pabda as endangered fish. Till then various experiments were conducted to protect this species from being extinct.

In brief the fry production technique of shing and pabda of this farm was given below:

Brood fish rearing:

Shing and pabda become sexually mature at the age of 10-11 months. Healthy and disease free broods were stocked at the ratio of 50:50 (male: female) in separate pond and stocking density for shing and pabda was 200 and 70-80 broods per decimal respectively. Brood fish were fed with good quality feed at the ratio of 5% body weight which was prepared with 30% fish meal, 30% soybean meal, 30% wheat flour and 10% rice bran and vitamin.

Mature brood fish selection:

Shing and pabda have long breeding season extending from mid April to mid August Mature male and female broods were selected on the basis of secondary sexual characters. During breeding season females were 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 were identified by their flat abdomen and long protruded genital papillae (Fig. 6).

In case of pabda females were easily identified by their soft and swollen abdomen due to bulky eggs and males were identified by their flat abdomen and rough pectoral fin (Fig. 7).

Hormone injection: For induced breeding mature male and female were selected. Two types of hormones were 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 were used to induce them to breed. Single dose was used for both male and female. Female was treated with 30mg PG/kg body weight of fish and male was treated with 5-10mg PG/kg body weight of fish. Male and

female ratio was 1:1. However maximum eggs were found if the male and female ratio was 1.5:1, fertilization rate was higher.

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

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

Injection was given on the fleshy part of the dorsal side and then they were kept into the cistern with continuous water circulation. They spawned naturally and fertilized the egg. If stripping was used for shing, fertilization rate was not more than 5%. On the other hand to produce huge amount of fry for commercial purpose, large number of fish was required for stripping but it was impossible to stripe huge amount of fish because it was time consuming. But in case of pabda all female did not ovulate naturally. Then eggs were collected very carefully from the unovulated female by stripping procedure. At the same time males were sacrificed to collect the testes and were macerated with 0.9% (physiological saline) salt solution. To ensure the maximum fertilization rate sperm suspension was mixed with eggs by gently stirring with a feather in bowl.

Naturally egg collection was done by two ways such as

Using hapa
Using cistern

Using hapa: At first 1cm mesh size hapa made with polythene was used whose length was 12 feet and width was 8 feet. Then the hapa was set in the cistern in such way that the hapa remains 6inch away from the bottom. 3feet water depth was maintained and artificial aeration was used for continuous water circulation to ensure the maximum oxygen supply. Then the injected male and female were stocked into the hapa at the ratio of 1:1 (male: female). After 10-12hrs of second injection fish spawned naturally and eggs were fertilized by the sperm. The eggs of these fish were slightly sticky. The eggs were 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 was taken out from the cistern and at the same time fertilized eggs were collected from the bottom of the cistern through siphoning procedure by using small pipe into plastic bowl.

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

Using hapa was better than cistern because spawned male and female were collected easily from the hapa without affecting the fertilized eggs. Then the collected fertilized eggs were kept into the small cistern and 3-4inch water depth was maintained. Shower was given through the perforated PVC pipe to ensure the maximum oxygen supply. Around 18-24hrs was required for hatching of fertilized eggs. After 3days of hatching yolk sac was absorbed. After 2days of hatching first feeding was given to ensure the availability of food when yolk sac was absorbed. Hard boiled egg yolk is used as feed, mixed with water and used upto satiation level. Ground small tubificid worm was also used as feed. After 4-5days rearing of shing fry into the cistern then they were transferred into the nursery pond. But in case of pabda after first feeding they were transferred to the nursery pond.

Rearing in nursery pond:

For nursing of fry 15-20decimal pond area was better. At first pond was dried completely and 5-10kg cow dung per decimal was given in the pond then after immediate tillage 1kg lime per decimal was given and kept for 1-2 days. Then watering was done by shallow machine. Frog, snake and other discarded animal was 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 was used to surround the whole nursery pond. 5days old fry was stocked into the nursery pond at the rate of 60gm fry per decimal. 50% rice bran and 50% dried fish powder were mixed together and used as nursery feed at the rate of 20% body weight per day. They were nocturnal in habit and so feed was given 2 times at night. Fry was reared in the nursery pond for upto 40 days until the fry became 2inch size.

Selling of fry:

During selling the size of fry was 2inch and the selling rate of shing was Tk 1.5/piece but the selling rate of pabda was Tk 2/piece. Three layered plastic bag of 30"x18" size were used. 250g fry was weighed which contain around 625 two inch sized fry were kept into plastic bag with oxygen (from oxygen cylinder) and water. The plastic bag was tightly packed to prevent the entry of air into the bag. The plastic bag contained around 8-10L water, 250g fry and rest was oxygen. Fry remained better around 15hrs in that type of plastic bag during transportation.

Shing culture is benefited in our country and widely practiced. But pabda culture in pond is not widespread in our country due to lack of huge amount of fry. If above technique is followed then it is confirm that the fry deficiency is minimized.

RESULTS AND DISCUSSION

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

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

Treatments	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 (200microg ovaprim+5mg PG)/2kg female	2kg	60	20-24	50.35±1.39	40.25±1.52

Fig 1: Mature male and female magur identification

Protruded genital papillae of male

Swollen abdomen of female

Fig 2: Sacrificing the male and stripping of female to collect sperm and egg respectively

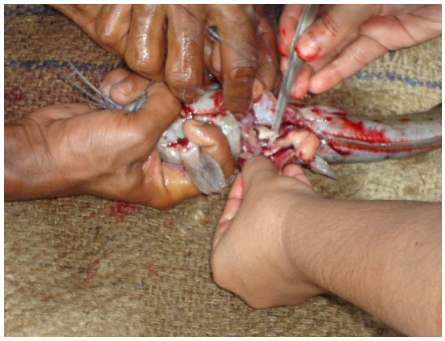
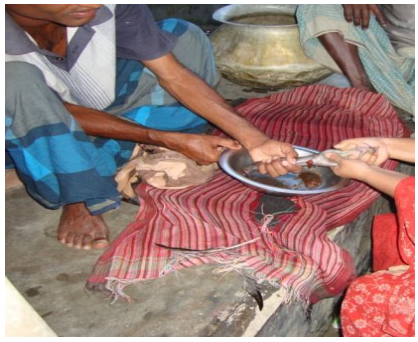
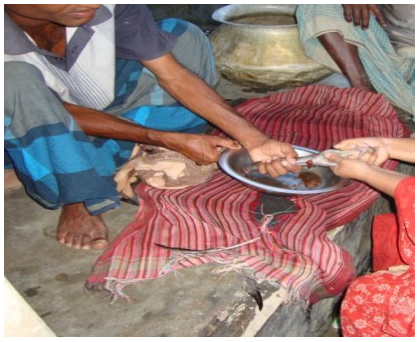




Fig 3: Fertilization of egg by gently stirring with a feather

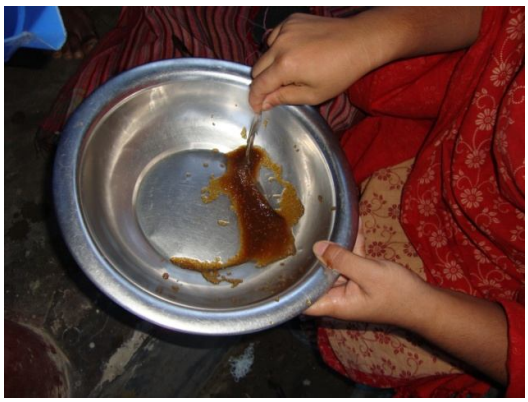


Fig 4: Fertilized eggs in cisterns with continuous water supply

Fig 5: Preparation of nursery pond

Fig 6: Mature male and female shing

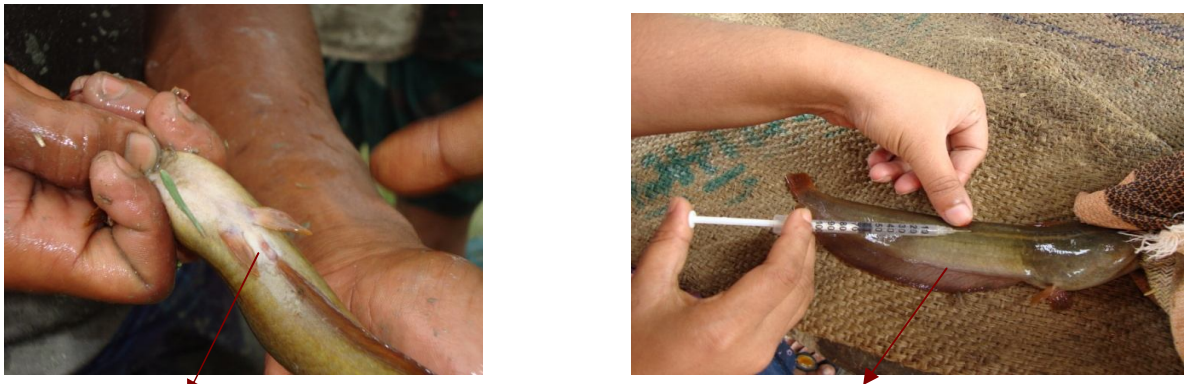


Fig 7: Mature female and male pabda

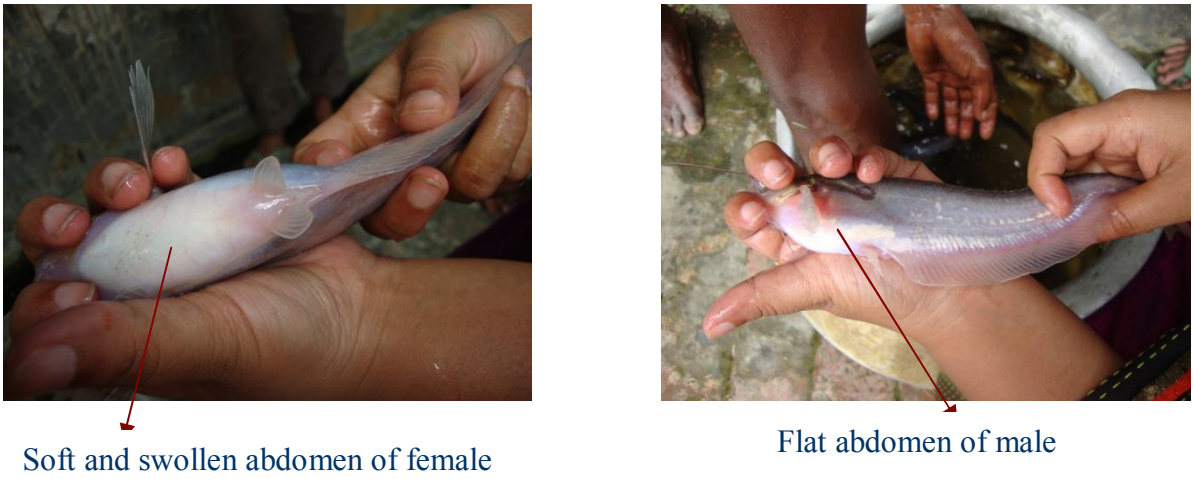
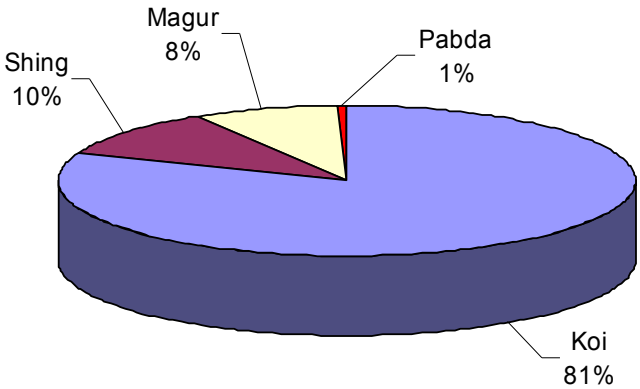


Fig. 8: Percent production of koi, shingh, magur and pabda



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 was 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 *Heteropneustes fossilis* Gheyas *et.al.* [6].

It may be due to species variation and maturity. We used 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*. [7].

The production rate of magur, shing, pabda and koi in Brahmaputra fish seed complex in this breeding season are given in Fig. 8.

It is clear from the present study that the seed production technique of indigenous magur, shing and pabda 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

1. DoF. (2008). Annual Report 2007-2008. Department of Fisheries, Bangladesh, Ministry of Fisheries and Livestock, 111pp.
2. Rahman, A.K.A., (2005).Freshwater fishes of Bangladesh. Zoological society of Bangladesh. Dhaka. 209-211pp.
3. DoF. (2008). Fisheries Statistical Yearbook of Bangladesh 2007-2008. Department of Fisheries, Bangladesh, Ministry of Fisheries and Livestock, 2-34pp.
4. Mollah, M.F.A. (2005). Domestication of riverine catfish *Rita rita* (Hamilton).Reports submitted to UGC, 12pp.
5. IUCN, Bangladesh., (2000). Bangladesher Bipanno Prani. IUCN Bangladesh. The World Conservation Union . 294pp.
6. 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 (*Heteropneustes fossilis*). *Progress. Agric*. 11(1&2): 169-173.
7. Yasmin A., Mollah M.F.A., Haylor G.S. 1998. Rearing of catfish (*Clarias batrachus* Lin.) larvae with live and prepared feeds. *Bangladesh J. Fish. Res.* 2(**2**): 145-150.
8. 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.