

Aqua-Internship Program
Asia Link project
Faculty of Fisheries
Bangladesh Agricultural University, Mymensingh

**Induced Breeding Technique of Indian Major Carps (Rui- *Labeo rohita*,
Catla- *Catla catla*, Mrigal- *Cirrhinus cirrhosus* and Kalibaush- *Labeo
calbasu*) and Thai Koi (*Anabas testudineus*)**



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Shambhuganj, Mymensingh



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A Report Approved
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A Report Submitted
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Background

Induced breeding is a technique whereby ripe brood fishes are stimulated by treating them with inducing agents to breed in captivity. The stimulation promotes a timely release of eggs and milts from ripe broods. The technique of induced or artificial propagation of fish using pituitary hormone first came into light in 1934 in Brazil by Von Thering *et al.* although it was started experimentally in 1931. In Indian subcontinent, however, the technique of induced breeding was first attempted by Hamid Khan in 1937 with *Cirrhinus cirrhosus*. In Bangladesh, Yusuf Ali was the first scientist to achieve success in induction of spawning of Indian major carps in 1965. Indian major carps are considered the best suitable carp species for their cultivable qualities. They contribute the largest share in the total aquaculture production of the country. In the statistical year 2007-2008, IMCs formed 21.37% of total aquaculture production of the country (FRSS, 2009). IMCs are native to the Indo-gangetic riverine systems of India. All of them naturally breed in rivers. Although they attain maturity in confined or stagnant (pond) waters, do not breed there and need inducement for spawning. Natural spawning of major carps usually coincides with the South-West monsoon in North-Eastern India and Bangladesh and lasts from May to August. Though the three IMCs viz. *Catla catla*, *Labeo rohita* and *Cirrhinus cirrhosus* belong to three different genera and differ not only in morphology but also in their habitat and feeding behavior, they exhibit considerable similarity in several details of reproductive biology such as sexual dimorphism, courtship, mating, spawning, spawning season etc. Availability of required quantity of fish seed of the desired species at the appropriate time is one of the prime factors that lead to success of aquaculture operation. Though remarkable success has been achieved over the years in spawning the carps, availability of seed of desired size still remains a constraint. The Freshwater Station of BFRI has developed the induced breeding technique of exotic Thai koi- a strain of koi (Kohinoor and Zaher, 2006). Koi fish inhabits the paddy fields, haors, *beels*, ponds and canals. It can withstand harsh environmental conditions such as low oxygen, wide range of temperatures and other poor water conditions. The species is considered as a valuable item of diet for sick and convalescents. It contains high amount of physiologically available iron and copper, which are essentially needed for hemoglobin synthesis. In international and domestic markets, koi has a great demand but the growth rate of our native koi is slow taking more time to attain marketable size. Presently, Thai koi is getting more popularity than our native strain as the growth rate of Thai koi is much higher. This fish attains 80-100 g size within 3-4 months. It was first brought in Bangladesh in 2000 by Talukdar Farm (Rupganj). In 2002, private entrepreneurs imported first growing koi strain seed from Thailand. Effective result was

obtained in induced breeding of Thai koi by Nurul Haque, Brahmaputra Fish Seed Complex (Shambhuganj, Mymensingh) in 2004.

Objectives

The present study was principally aimed to achieve the following objectives:

- To study the seed production technique of IMCs and Thai koi through induced breeding.
- To gain practical skills on induced breeding technique.
- To acquire practical knowledge on the production of all female Thai koi using sex-reversed (xx) male.
- To gain practical experience on nursing of Thai koi fry.

Description of activities:

Study area

The study was conducted in a fish hatchery named Brahmaputra Fish Seed Complex situated at Char Nilakshmia Union under Mymensingh Sadar Upazila of Mymensingh district from July to September 2010. The geographical location of the study area is shown in Figure 1.



Fig 1. Map showing the geographical location of the study area

The hatchery complex has two parts- one is the hatchery unit (80 decimal) where breeding activities are done and another is the culture unit (27 acre) where brood rearing, nursing and culture is done. Ground water is supplied to the hatchery unit from 2 overhead tanks. There are 70 ponds of which 9 are broodfish pond of carps.

Induced breeding of IMCs

Collection and stocking of broods

Brood fish were collected from wild source (the Halda, Jamuna and Padma). Generally, all the major carps attain sexual maturity in the second year and the males mature earlier than female. They are seasonal riverine spawners, spawning during the southwest monsoon months (April to August/September). They spawn in inundated shallow areas adjacent to the river during floods. They do not spawn in confined waters. Generally 600-700 kg fish of 2-3 years old are stocked in a 60 decimal pond before September.



Fig 2. Harvested broodstocks of IMCs

Management of broods

Water depth of brood fish pond should be 5-6 feet. Water exchange is done every 15 days interval to keep the fish disease free and to mature the fish earlier.

Feeding of broods

Feed should contain 25% protein. Protein content more than 25% degrades water quality. From September to December the mixture of equal amount of mustard oil cake and rice bran are applied by forming ball. Feed is given at the rate of 2% of their body weight. Special care is

taken from January by supplying balanced diet containing 50% rice bran, 10% wheat bran, 5% flour, 20% fish meal, 15% mustard oil cake and vitamin premix specially vitamin E.

Breeding

Ready to spawn male and female fish were harvested early in the morning and kept at separate tank with showering for 6 hrs. During conditioning no feed was provided to the broods and most of the foods of their stomach came out as faeces. Before injection the broods were kept on foam to minimize the stress and the eyes were covered so that the fish can not move. The broods were injected with PG extract at the pectoral fin bases at 45° angle (Figure 3a). After 6 hrs of first dose females received a second dose. Males received a single dose during the second dose of female. After ovulation eggs were collected from the female in a bowl by stripping i.e., by giving gentle pressure on the abdomen of female. Then milt was collected from the male by applying the same procedure. The eggs and milts were mixed by using a feather for fertilization. The fertilized eggs were transferred to the hatching jar (Figure 3b).



Fig 3. (a)Injecting of fish

(b) Hatching jar with incubated fertilized eggs

Selling of spawn

4 days old spawn were sold. Before selling the spawn were conditioned for 2 hrs in cistern (12ft × 8ft × 2.5ft) under shower. Then they were taken in glass nylon net to remove water. After water removal the spawn were measured with 250 ml beaker and kept in polythene bag (30 inch × 18 inch). Quickly O₂ was added to the bag from O₂ cylinder and the bag was tied using thin rope (Figure 4). The packet was two layered- an inner polythene layer and an outer plastic layer. Each packet contained-

- 8-10 L water
- 250 g carp spawn and rest of the part is O₂



Fig 4: Packing the bag tightly containing fry, water and oxygen

Induced breeding of Thai koi

Brood management

At first pond was dried. If drying is not possible then undesirable species is removed by netting or by using rotenone. After a week liming was done at the rate of 1 kg lime/decimal. Then fencing was done surrounding the pond using nylon net. Watering was done and water depth was maintained at 3-4 ft. 15-20 decimal ponds are good for brood rearing. Fast growing and healthy fish should be selected as brood. 100 g female broods were stocked at a density of 100-150 fish/decimal and 30-35 g sex-reversed male (xx) broods were stocked at a density of 1000-1500 fish/hapa (5 m × 2 m). Sex-reversed male broods were produced in 2008 which are now used to produce all female koi fry. They were fed with grower feed twice a day at the rate of 3% of their body weight.

Induced breeding

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 5 : Preparation of hormone extract

Injecting of Thai Koi

Mid February to July is suitable time for breeding of Thai koi. However, they can be bred throughout the year if managed properly. The selected breeders (male and female) were put in two separate cisterns of 12ft × 8ft each. The PG extract was administered into the muscular basal part of the pectoral fin (Figure 5a). After injection, both the males and females (about 500 fish) were shifted to spawning tank (12ft × 8ft × 3.5ft) at 1:1 ratio which was provided with artificial aeration. The spawners were removed from the spawning tank after 7-8 hrs when breeding activity was over.



Fig 6. Injecting a Thai koi with PG extract

The eggs of koi float on the surface of water. Fertilized eggs were then automatically transferred with water into hatching tank arranged in a series of 4-5 tanks adjacent to the breeding tank (Figure 5b). The ideal size of a hatching tank is 8ft × 5ft × 3.5ft and 5 hatching

tank is required for 1 breeding tank. Water depth was maintained at 2ft. The outlet for transferring eggs of each tank must be 2 inch lower from the previous. Oxygen was supplied by using aerator and water was supplied using a pipe deep into the tank.

After 2-3 days of hatching yolk sac was absorbed and boiled egg yolk of duck was served for first feeding. Boiled egg yolk was blended and screened by glass nylon net before applying. First feeding was given at morning and afternoon and then the fry were transferred to the nursery pond for nursing.

Nursing

Nursing can be done by two ways. Single stage rearing- using one pond and two stage rearing- using two ponds. The area of first pond is 15-20 decimal and second pond is 40-45 decimal. After drying, liming and fencing watering is done up to 3 ft depth and 100 g flour/decimal is mixed with pond water. This flour results in growth of plenty of zooplankton which is very essential for fry. After 2 days 0.2 ppm sumithion is added to the pond water. Another 2 days later fry are stocked. Fry obtained from 100-150 pair brood (5-10 lac) is stocked in a pond. The fry are fed with boiled egg. The whole egg is blended and screened by glass nylon net and then mixed with pond water. 10 eggs are enough for a 20 decimal pond. Feed is applied twice/trice a day. From third day 500 g boiled flour is mixed with 10 eggs and fed twice/trice a day. After 5-6 days fry are transferred into the 2nd pond. At this stage nursery feed is applied at the rate of 10 kg feed/4 lac fry. Before applying, the feed is soaked in water for 12 hrs. After 8-10 days the fry become about 1 inch size which are either sold or stocked.



Fig 7. Preparation of nursery pond

Selling of fry

Before selling the fry were conditioned for 3 hrs in a hapa. The fry were weighed using a balance and then packaged. The procedure of packaging is similar to that of carp spawn.



Fig 8. Conditioning of Thai koi fry

Culture

Culture can be done directly by using one pond or by using two ponds.

One pond using method: Using this method marketing can be done 10 days before using two ponds method. But here stock should count 20% less than the actual stock.

Two ponds using method: Here, first 1 inch sized fry are stocked in a small pond (2 thousands fry/decimal) and nursed for 20 days. After 20 days the fry are transferred to a second pond. Fry should be transferred at 11 AM.

Pond selection: Pond with good water holding capacity and free from flood is suitable for koi culture. Rectangular pond of 60-80 decimal is good. Water exchange facility should exist and water depth should be 4-5 ft.

Pond preparation: Culture can be done both in new and old pond. In case of new pond 10kg cowdung/decimal is applied and then tilled after adding little amount of water. In case of old pond, after drying 1kg/decimal lime is added and then tilled. If drying is not possible then rotenone is applied to remove undesirable species. Too much muddy pond is not suitable. Fencing is done surrounding the pond so that frog, snake etc. can not enter into the pond. After watering the pond upto 2 ft fry should be stocked. With the growing of fry water depth should increase upto 4-5ft.

Stocking density: Depending on water exchange facility 500-1000 fry can be stocked per decimal of pond area.

Stocking: After 4-5 days of watering stocking is done. 1 day after watering 100 g flour/decimal is added to the pond water to assist the growth of zooplankton. To reduce mortality during stocking the following is must-

- The fry should be released at evening
- Fry should be released into comparatively shallow water
- Before releasing fry should be conditioned properly with pond water
- The next day of fry releasing 500 g salt is added for 2 ft water depth. After 1 week a second dose of salt is applied.

Feeding: Floating feed is more effective for koi culture. The grower feed should contain at least 30% protein and starter and nursery feed should contain 35% protein. Feed is applied thrice in a day. Normally, they attain 90-100 g at 120 days. At this size they are sold. 100 kg fish/decimal can be produced in a season by culturing twice.

Disease problem

Only EUS is found to occur in koi. Normally, the fishes harvested for sampling is attacked by EUS. EUS can also occur as a result of frequent netting.

Prevention: To prevent EUS salt is applied to pond water at the rate of 1 kg salt/decimal. After a week a second dose of salt is applied. Normally EUS occurs in winter season. So, before winter season special care is taken-

- Water exchange should be done at least once in a week.
- 1 kg/decimal salt should apply at every 15 days interval.
- Stocking density should be reduced to 150-200/decimal.
- Sampling should be done every 15 days interval to check the health of fish.
- Frequent netting should not be done.

Marketing

Within 100-120 days marketing can be done. Feeding should be stopped at least 1 day before marketing. Fish weighing more than 85 g fetch high market price. Before marketing the fish should be conditioned in a hapa. 700-800 kg fish can be conditioned in a 15ft × 10ft hapa for 5-6 hrs (Figure 9). Then a solution of 500 g salt should be splashed in the hapa to avoid fungal infection. The fishes are then kept into drum for transportation. 30 kg fish can be transported in a drum in a 6-7 hrs journey.



Fig 9. Conditioning of Thai koi before marketing

Result and Discussion

Induced breeding of IMCs

Broodfish produced from the river Halda and Padma is very good whereas broodfish produced from the river Jamuna produce a number of abnormal fry (Khan *et al.*, 2006). Good results were obtained by stocking 2-3 species together i.e., catla with grass carp and Thai sarpunti, rui and mrigal with silver carp to utilize the feeds properly in all layers of the waterbody.

Selection or proper identification of mature and healthy male and female broodfish is an important step in any induced breeding programme. Khan and Mukhopadhyay (1975) stated that the success of induced breeding depends on the proper selection of broods. Identification of male and female broods was done on the basis of some external features known as secondary sexual characteristics presented in Table 1.

Table 1: Criteria for identification of male and female broods of IMCs

Male	Female
<ul style="list-style-type: none"> • Small in size • Abdomen normal, not bulky like female • Rough at the inner side of pectoral fin • By gentle pressure on abdomen whitish milt came out through the genital pore 	<ul style="list-style-type: none"> • Relatively larger in size • Abdomen bulgy, elastic and soft • Smooth at the inner side of pectoral fin • By gentle pressure on abdomen eggs came out through the genital pore

The dose of PG varies from species to species and also with season which is shown in Table 2. The dose of PG was very low because appropriate showering was done before injecting the fish and PG extract was prepared manually by mortar and pestle and was not centrifuged.

Table 2: Dose of PG extract required for precipitating ovulation in IMCs at different times

Month	Species	Dose for female (mg/kg)		Dose for male (mg/kg)
		1 st Dose	2 nd dose	
April, May	Rui	2	6	2
	Catla	2	4	2
	Mrigal	2	4	2
	Kalibaush	2	6	2
May, June	Rui	1	3	2
	Catla	1	2	2
	Mrigal	0.5	2	1
	Kalibaush	1	3	2
June, July, August	Rui	1	-	1
	Catla	1	2	1
	Mrigal	2	2	1
	Kalibaush	1	2	1

The sex ratio was 2:1 (female:male). Ovulation occurred after 6 hrs of injection i. e., latency period was 6hrs and after 18-20 hrs of fertilization the eggs hatched out. After 72 hrs of hatching first feeding was done. After giving the first feed once or twice the fry are usually sold.

Highest fertilization rate and hatching rate were around 95% and 90%, respectively in the month of May and highest survival rate was around 80%. The amount of fry produced also varies from month to month which was shown in Table 3.

Table 3: Production of major carp fry in different months

Month	Production (kg)
March	50
April	300
May	500
June	700
July	400
August	200

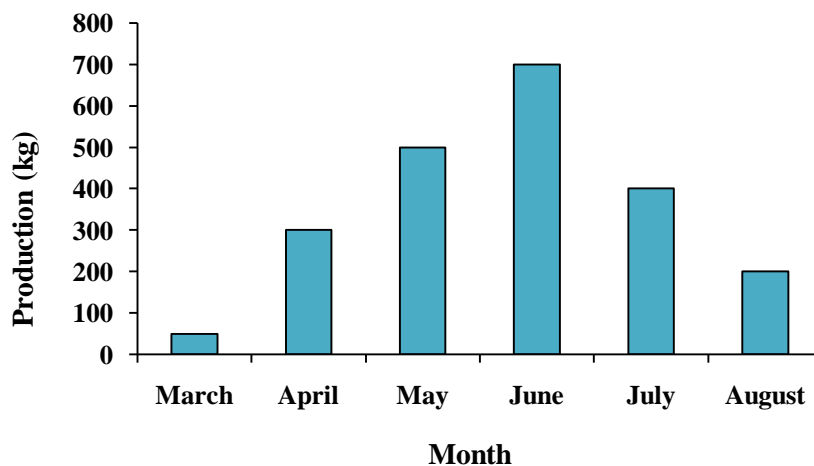


Fig 10. Monthwise production of carp fry

Induced breeding of Thai koi

The criteria for identification of male and female broods were presented in Table 4.

Table 4: Criteria for identification of male and female broods of Thai koi

Male	Female
<ul style="list-style-type: none">• Small in size (average wt. 30-35 g)• Abdomen normal, not bulky like female• Body slender• By gentle pressure on abdomen whitish milt came out through the genital pore	<ul style="list-style-type: none">• Relatively larger in size (average wt. 100 g)• Abdomen bulging, elastic and soft• Body robust• By gentle pressure on abdomen eggs came out through the genital pore

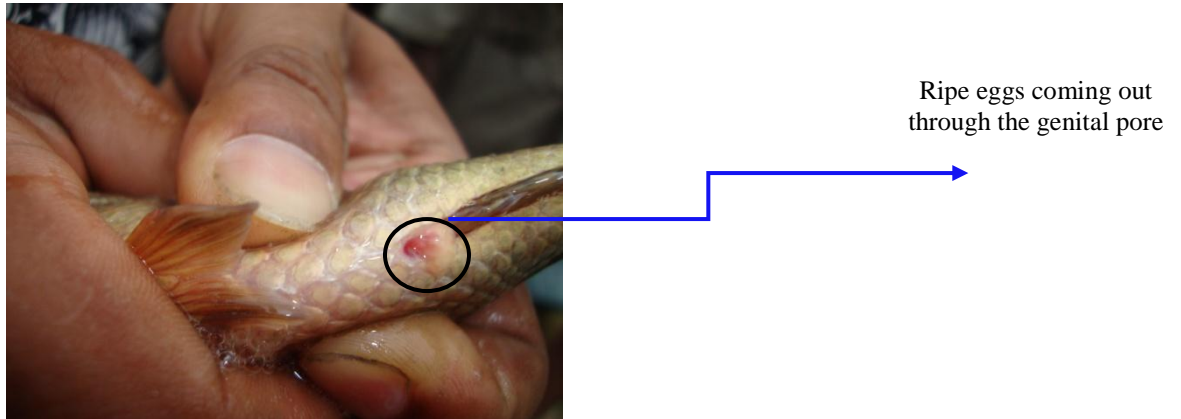


Fig 11. Ready to spawn female Thai koi

Both male and female received single dose of PG. The best result was obtained by using 6-8 mg/kg female and 2-3 mg/kg male in respect of fertilization and hatching.

Ovulation occurs after 7-8 hrs of injection and after 18-20 hrs of fertilization hatching occurs. Highest fertilization rate, hatching rate and survival rate were found around 80%, 75% and 60% respectively.

Fry production of Thai koi also varies from month to month which is shown in Table 5.

Table 5: Production of Thai koi fry in different months

Month	Production (lac)
February	5
March	20
April	30
May	20
June	20
July	15
August	10

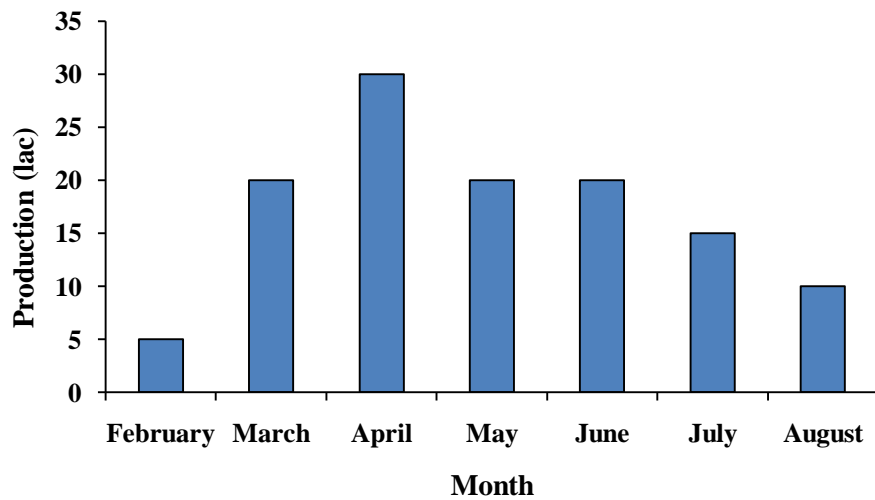


Fig. 12. Monthwise production of Thai koi fry

Two stage nursing is more profitable because by this method uniform size of fry can be obtained. During culture proper growth was obtained by applying the following feeding schedule -

- 1st 10 days 50% of body weight (crumble)
- 2nd 10 days 30% of body weight (crumble)
- 3rd 10 days 20% of body weight (starter)
- 4th 10 days 15% of body weight (starter)
- 5th 10 days 10% of body weight (starter)
- 6th 10 days 5% of body weight (grower)
- 7th 10 days 3% of body weight (grower) until marketing.

Conclusion

In conclusion, it may be suggested that more emphasis should be given to develop the technique of induced breeding of fish and popularize it all over the country, because of the fact that the first and foremost prerequisite for successful intensive fish cultivation and development of Inland Fisheries is an assured supply of pure quality fish seed. It is now used as a widely accepted means of artificial propagation to overcome constraints in fingerling supply particularly for species that do not breed in captivity.

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References

- FRSS, 2009. Fisheries Resources Survey System. Department of Fisheries (DoF), Bangladesh.
- Khan, H.A. and Mukhopadhyay, M., 1975. Production of some air breathing fishes by hypophysation. *J. Inla. Fish. Soc. India*, 5: 156-161.
- Khan, M.M.R., Alam, M.S. and Bhuiyan, M.M.H., 2006. Allozyme variation of hatchery and river populations of rohu (*Labeo rohita*, Hamilton) in Bangladesh. *Aquaculture Research*, 37: 233–240.
- Kohinoor, A.H.M. and Zaher, M., 2006. Fisheries Newsletter: 14 (1). Bangladesh Fisheries Research Institute.