A Seminar Paper on

From Hormone Injection to Fertilization: A Step-by-step Guide to Induced Breeding of Butter Catfish, *Ompok pabda*

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From Hormone Injection to Fertilization: A Step-by-step Guide to Induced Breeding of Butter Catfish, *Ompok pabda*[1]

by

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ABSTRACT

Ompok pabda is a freshwater endangered catfish native to some of the Asian countries including Bangladesh. The fish is very popular for its tender flesh and mild flavor. Its natural population is decreasing day by day due to climate issues and manmade reasons. As this species doesn't breed naturally under captive condition, induced breeding can be a solution in this case. At first broods are collected from natural habitat and fed with supplementary feed, live food containing 50-55% protein. First maturity founded at the length of 16.3cm and 17cm for male and female of *O. pabda* respectively and 30 – 40 g in weight. There is a strong correlation between female size and fecundity, with the average relative fecundity of 18,000-22,000 eggs/100 g fish. For induced breeding fish is stimulated by ovaprim, ovatide, sGnRH hormone. The stimulation promotes timely release of sperms and eggs from ripe gonads. Best result found for ovatide dose- female 0.9 ml/kg and male 0.3 ml/kg which resulted fertilization rate at 84% and hatching rate at 85%. The fully swollen fertilized eggs are round and brownish with average diameter of 1.05 mm. The yolk sac absorption period was found 3-4 days. The average length of the newly hatched larva was found 4.38 mm from 0-14 days. Hatching periods varied between 18 and 24 hours. Larval feeding with tubifex, *Moina*, *Artemia* nauplii, chicken liver, egg yolk showed better growth and survival. This paper is based on secondary information prepared to sum up all those previously documented information to establish a guide in the steps of induced breeding of *Ompok pabda*.

Keywords: *Ompok pabda*, Induced breeding, Broodfish**,** Hormone administration**,** Fertilization, Hatching

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Chapter I

INTRODUCTION

Ompok pabda, known as butter catfish is a well-known small indigenous fish species (SIS) of Bangladesh. This fish is also native to India, Afghanistan, Bhutan, Pakistan and Myanmar. It is a bony fish, belonging to the family siluridae of the order siluriformes is a freshwater catfish, locally known as "madhu pabda" (Viswanath *et al*., 2007). It is a delicious, tasty, nutritious catfish with high price because of few bones, bony structure and rich lipo-protein content (Banik *et al*., 2012). This fish is an excellent source of omega-3 and omega-6 fatty acids, vitamins and minerals, all of these nutrients are very essential for growing children, pregnant women and elderly people. Highest retail price was recorded for *Ompok pabda* in Bangladesh and its consumption was restricted among higher class and middle-class consumers only (Samad *et al*., 2010).

There was a time when pabda was commonly found in streams, rivers, canals, inundated fields, beels, jheels, reservoirs and tanks. However, various studies of recent times have shown that the population of pabda species has been declining tremendously for the last few decades. There are many reasons behind this declination like overexploitation, habitat shrinkage caused by massive siltation and wetland conversion, water pollution, climate issue and many more. *Ompok pabda* was categorized as Endangered (IUCN Bangladesh 2000). In the recent report- "Red List Species of Bangladesh" given by IUCN in 2015, *Ompok pabda* has been listed again as "Endangered" in Bangladesh and "Near Threatened" globally. So, this species is considered to be facing a very high risk of extinction in the wild. Artificial breeding can be used for seed production which is a great solution to prevent its extinction in nature.

Pabda has a high market demand for its good quality flesh and taste, less bone, excellent nutrient profile. The market price of is about 400-500 BDT/ kg (Size: 20-25 cm, Weight: 60-80 g) in Bangladesh. So, the poor people are hardly able to purchase this tasty fish at that price. Pabda has a great potentiality in our country for diversification. Day by day, its demand is also increasing as ornamental fish among aquarists.

Pabda doesn't breed naturally in captive and artificial rearing condition. Enhancing knowledge about its domestication culture system can increase its production and may upgrade its current "Endangered" situation. Induced breeding or artificial breeding is a technique by which mature fishes are prepared to breed in captive condition stimulating by applying hormone. The basic requirement of the controlled fish culture industry is the fish seed. Now short supply of quality seed and dependency on wild seeds, which is unreliable, time consuming and uneconomical are major constraints for culturing this fish. To overcome such problems, induced spawning is thought to be the only alternative method for quality seed supply and production (Sharma *et al*., 2010).

In Bangladesh, very limited research has been conducted to find out the exact pathway of induced breeding and culture of *Ompok pabda*. Till now artificial breeding information of pabda not that much enough compared to carp species. So, farmers are not so interested in culturing this because of the possibility of facing loss.

Now-a-days in the question of higher production and conservation purpose, captive breeding is one of the major steps suggested by experts. In case of any species proper knowledge on its feeding and reproductive biology is a must for successful captive breeding. Besides proper knowledge on the dose of inducing agent (e.g. hormone) is also mandatory. Till now few research works have been done on feeding, habit, reproductive biology, induced breeding of pabda fish. There is a lacking of consolidated report on these aspects.

The seminar paper aims to consolidate the already available information with pointing out the information lacking which will be helpful for its fishery and conservation management along with documentation of some possible strategies which can be considered for its availability in coming days.

Objectives

The specific objectives of this review paper are as follows:

1. To find out the steps of induced breeding of *Ompok pabda* sequentially
2. To highlight the importance of induced breeding of *Ompok pabda*

Chapter II

MATERIALS AND METHODS

This paper is entirely a review paper. So, this paper is mainly based on secondary information. Different published reports and articles are used to prepare this paper. Information has been assembled from various articles published in the journals, websites available on the online platform.

Constructive suggestions from my major professor and course instructors helped me to improve this paper. After the collection of all the related information, it was gathered and logically presented in the current form.

Chapter III

REVIEW OF FINDINGS

In this chapter, findings of different authors have been accumulated and discussed under different headings to ascertain the objectives of the paper.

For successful induced breeding program broodstock management, water quality management etc. are mandatory prior to hormone injection.

3.1 Broodstock management

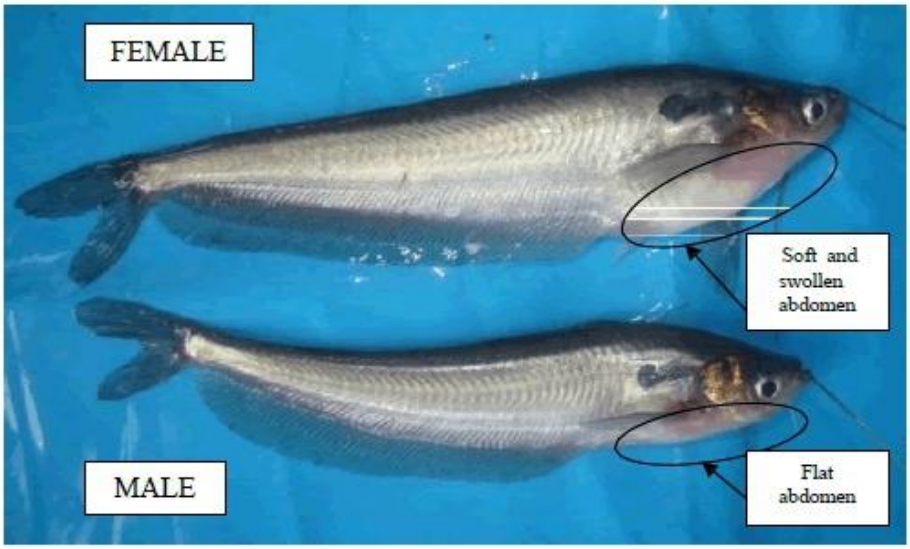
In captive culture, proper rearing of the brood fish is also essential. Selection of good quality brood fish is a prerequisite for successful breeding program. Superior type of brood fish enhances the breeding activities. If proper brood fish are used, fecundity, fertilization, hatching, larval survival rate will be increased and more viable fish seed will be produced.

3.1.1 Selection of breeders

At first, brood fishes of 1-2 years old are collected from rivers. During the breeding season, male and female pabda can easily identified by observing the secondary sexual characteristics. Female ascendency on male in the population of this fish species have reported by Banik *et al*. 2012.

Table 1: Selection criteria to select mature breeders of pabda (Bishwas *et al*., 2018)

Serial no.	Male	Female
1. Size	Small	Relatively large
2. Abdomen	Abdomen normal; not bulky like female	Abdomen bulging, elastic and soft
3. Body shape	Body slender, more transluscent and less pigmented	Body robust and pigmented
4. Pectoral fin	Serration of the pectoral spine is pronounced	Serration of pectoral spine is not pronounced
5. Genital Papilla	Elongated and pointed or conical shape	Large. Round and fleshy with reddish vent



Source: (Chattopadhyay, 2018)

Figure 1: Male and female brooder of *Ompok pabda*

3.1.2 Length, age at first maturity & fecundity

Banik *et al*., 2012 have reported 16.3cm and 17cm as length at first maturity for male and female of *O. pabda* respectively. Chakrabarty *et al*., 2007 have reported that *O. pabda* used to attain maturity at the end of first year of its life. Early maturation of males than females has been reported by all these researchers.

The fish attained maturity at the end of the first year. Males matured earlier than females, which became mature at 30 – 40 g in weight. Fertilization is external and spawning occurs once a year during the monsoon season (June – August) with a peak in July (Chakrabarty *et al*., 2007).

Qasim & Qayyum, 1963 have reported absolute fecundity range of 2,500-40,000 for *O. pabda* while Banik *et al*., 2012 have reported the range of 2,190-41,552 for the same. Chakrabarti *et al*., 2007 have documented relative fecundity range of 2,00,000- 2,50,000/kg of body weight for *O. pabda*.

Gonado Somatic Index (GSI) is the ratio of fish gonad weight to body weight.

GSI= (Weight of gonad/weight of body) × 100 (Parameshwaran *et al*., 1971)

Table 2: Fecundity and GSI of gravid female *Ompok pabda* (Roy *et al*., 2021)

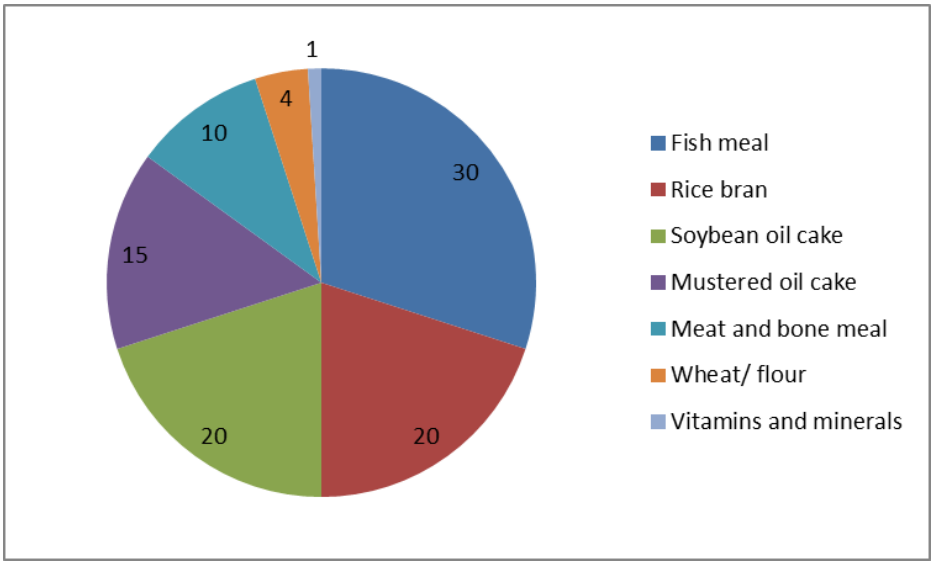
Serial no	Total Length (cm)	Body Weight (g)	Gonad Weight (g)	Fecundity	GSI
1	21.1	115.6	4.11	12526	3.56
2	21.8	113.7	5.03	15225	4.42
3	22.3	119.7	4.86	14847	4.06
4	22.0	115.6	5.13	12505	4.44
5	22.8	113.28	4.98	15771	4.40
6	22.9	113.15	5.01	16479	4.43
7	23.5	119.93	5.72	18088	4.77
8	22.8	112.6	4.67	15465	4.15
9	23.2	118.8	4.87	19626	4.10
10	22.4	115.6	5.18	18360	4.48

3.1.3 Feed Management

Pabda broodfish should be fed daily with a 55% protein pelleted feed. A mixture of mustard oil cake, fishmeal, rice bran and wheat flour can be pulverized and fed in the form of dough. Chakrabarty et al., 2007 have reported brooders were fed with supplementary feed, mixture of ground nut oil cake, rice bran and fish meal (1:1:0.5) fortified with vitamin and mineral mix at 10% of the body weight apart from the natural food organisms (insect larvae, small trash fish and prawn) present in the pond. He reported successful rearing of the brood stocks and thus these feed formulations can be used effectively in coming days.

Table 3: Ingredients used in supplementary feed for pabda brood (Hossain *et al*., 2017)

Ingredients	**Amount (%)**
Fish meal	30
Rice bran	20
Soybean oil cake	20
Mustered oil cake	15
Meat and bone meal	10
Wheat/ flour	4
Vitamins and minerals	1



Source: (Hossain *et al*., 2017)

Figure 2: Feed ingredients percentage for pabda brood

3.1.4 Conditioning of brood fish

Numerous studies have stated that brood fishes can be kept in earthern pond for rearing. Regular monitoring of brood fish is necessary. During breeding season broodfish are harvested from pond, then male female broodfish are kept separately in tank. Proper aeration is needed, for that constant water flow is maintained. Prior to fertilization careful handling is a crying need to avoid injury, secondary infection and stress (Bishwas *et al*., 2018). Conditioning of brood is needed to release stress. Selected male female fishes should be kept in separate tanks for about 6 hours prior to hormone induction. At this time constant water flow is vital to ensure proper aeration. In every step of handling, carrying, transferring of broodfish should be done very carefully. If the broodfishes anyhow feel stress the ovulation will be delayed or may even stop even after hormone injection. Feeding is prohibited during the period of conditioning.

3.2 Water Quality Management

Suitable physicochemical parameters are so important for broodfish rearing, otherwise they feel stress which can impact negatively on survivality, growth and reproduction. Broodstock matured at PH 7.4 – 7.8 (Chakrabarty *et al*., 2009)

Table 4: Water quality parameters suitable for pabda brood

Parameter	Banik *et al*., 2011	Hossain *et al*., 2017	Kohinoor *et al*., 2018	Hussan *et al*., 2020
Temperature (°C)	29 ± 2.20C	28±1.00	27.20±2.42	27.29 ± 1.78
pH	7.5 ± 0.92	7.03±0.15	7.78±0.34	7.41 ± 0.22
Dissolved Oxygen (mg/L)	7.0 ± 2.32	7.5±1.00	4.80±0.83	7.42 ± 0.18
Total Alkalinity (mg/L)	120 ± 27.10	-	143±11.12	153.33 ± 6.11
Ammonia-nitrogen (mg/L)	<0.1	-	0.028±0.022	

Table 4 shows that water parameters maintained in various study are almost the same

3.3 Induced spawning

3.3.1 Spawning Technique

For induced breeding, completely matured broodfishes are used-

i. Fully ripe female can be identified by rounder, fuller abdomen, reddish vent colour and rounded genital papilla
ii. Males are identified by an elongated and pointed genital papilla

3.3.2 Inducing agent

It is observed that both Carp Pituitary Extract (CPE) and Ovaprim hormone are quite effective for captive breeding of on *O. pabda*. As compared to the conventional hypopysation, which requires high quantity of CPE, Ovaprim is quite effective at low quantity for inducing breeding under captivity. For successful spawning and seed production of Pabda, Ovaprim can be a better option than Carp pituitary extract. Ovaprim not only induce complete spawning but fertilization, hatching and survival is more ensured than CPE (Chattopadhyay, 2018). Formulation of Ovaprim and Ovatid-

i. **Ovatide**- synthetic gonadotropin releasing hormone analogue (GnRH-a) + domperidone
ii. **Ovaprim**- salmon gonadotropin releasing hormone analogue (sGnRH-a) + domperidone (Basavaraja *et al*., 2013)

Table 5: Comparison of the efficacy between two common synthetic spawning agents, ovatide and ovaprim (Basavaraja *et al*., 2013)

Trial no.	Spawning agent	Number of broodfish		Doses (ml/kg)		Fertilization rate (%)	Hatching rate (%)
		Female	Male	Female	Male		
1	Ovaprim	4	2	0.8	0.3	82.4	71.6
	Ovatide	5	5	1.0	0.4	70	75
2	Ovaprim	2	3	0.9	0.3	86.5	83.5
	Ovatide	4	3	0.9	0.3	84	85

Table 5 reveals that for ovatide dose 0.9 ml/kg for female and 0.3 ml/kg for male comparatively higher fertilization rate and hatching rate can be gained

Table 6: Mostly used inducing agents for captive breeding of *Ompok pabda* in various research till now

Inducing agent	Dose for female (ml/kg)	Dose for male (ml/kg)	Reference
Ovaprim	1- 1.5	0.5-1.0	Chakrabarty *et al*., 2009 and Debnath *et al*., 2013
Ovaprim	0.5	0.5	Gupta 2015
Ovaprim	1.2	0.6	Chaturvedi *et al*., 2013
Ovaprim	1.0	0.5	Banik *et al*., 2012
Ovatide	1.5	0.75	Hussan *et al*., 2020
Ovatide	0.5	0.5	Sridhar *et al*., 1998
S-GnRHa (Ovupin)	0.5	0.25	Hossain *et al*., 2017
S-GnRHa	0.7	0.5	Raizada *et al*., 2013

From table 6 it is clear that ovaprim is mostly used inducing agent for captive breeding of pabda

3.3.3 Hormone administration

After gut evacuation, a single injection of inducing hormone (e.g. Ovatide, OvaFH, Gonopro, Ovasis) should be administered to all broodfish. Fish are injected intramuscularly above the lateral line towards the dorsal fin using a 1-ml syringe. The needle is inserted at an angle approximately 30° from the head (Figure 2). Injected spawners are kept separately in containers to avoid disturbances and self-injuries (Bishwas *et al*., 2018).

Source: (Chakrabarty *et al*., 2009)

Figure 3: Administering hormone to broodstock

3.4 Collection of egg and sperm

The latency period recorded for female brood is 10 hours after hormone injection. When the latency period ends female fish is stripped by gently pressing the abdomen with a thumb. Pressing is started from pectoral fin towards the genital papilla. Gently pressing is highly recommended to avoid the contamination of egg with blood. If the hormone treatment is accurate, ovulated eggs will flow easily in a thick mass from the genital vent. Ovulated eggs are brown and there are about 600 eggs in one gram of eggs. There is a strong correlation between female size and fecundity, with the average relative fecundity of 18,000-22,000 eggs/100 g fish. The mature ovary contains different sizes of eggs. Fully ripe ova are dull yellow/green and measure from 760 to 875 μm in diameter (Bishwas *et al*., 2018).

Source: (Bishwas *et al*., 2018)

Figure 4: Stripping ovulated eggs from pabda female broodfish

As being catfish pabda males cannot be stripped. For spawning purpose the male fish are sacrificed. The male fish is dissected and testes are removed to collect milt. Clean and dry cotton cloth is used to place the dissected testis. Then milt is squeezed out surgically from the testis.

3.5 Fertilization

Collected eggs and sperm are mixed gently for 1 minute. Feather is used for mixing purpose. After about one minute, fertilization has taken place and the sperm has lost its activity and fertilized eggs are ready for incubation in tanks (Bishwas *et al*., 2018). Fertilized eggs are washed thoroughly with clean water and then placed in tank for hatching with constant aeration (Chakrabarty *et al*., 2007). Fertilization was recorded in the range of 59– 82 % at water temperature range of 25-27°C (Hussan *et al*., 2020).

Source: (Bishwas *et al*., 2018)

Figure 5: Fertilization of eggs by activation of sperm with water

3.6 Egg incubation

Plastic tubs, cements tanks or aquaria can be used for egg incubation. Among these plastic tubs are inexpensive, easy to carry and clean. Well aerated flowing water is maintained to get better survival rate. Researchers have strongly suggested that the water of hatching tubs or cement cisterns must be clean and chlorine free.

3.7 Embryonic development

- 30 minutes after fertilization the blasto disc begins to form over the yolk, following first, second and third cleavage
- Sixty four cell stage was observed after 70 minutes post fertilization followed by morula stage in two hours
- Yolk plug stage appeared after five hours
- The cephalic and caudal end of the embryo had differentiated after 10 – 15 hours
- After 16 – 21 hours the gut had faintly appeared posterior to the yolk sac, leading to the anus, and movement of the embryo could be seen within the egg
- After 22 hours movements of the embryo were observed
- The eggs began to hatch after 23 hours (Chakrabarti *et al*., 2009)

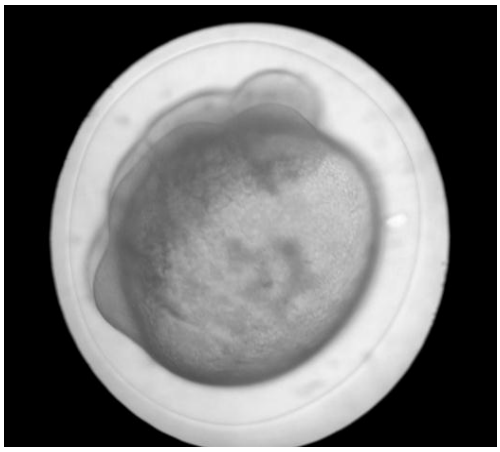


Figure 6 (a): Time- 1 hour and 10 minutes

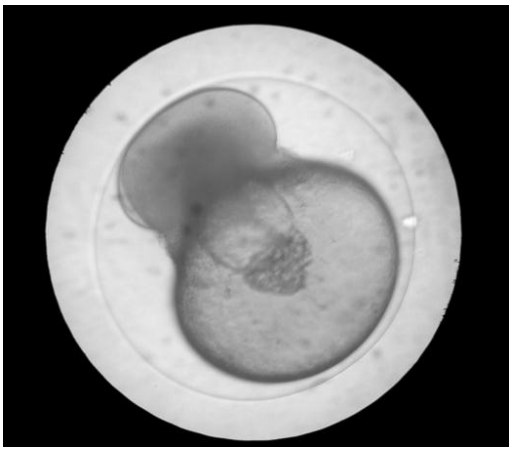


Figure 6 (b): Time- 1 hour and 42 minutes

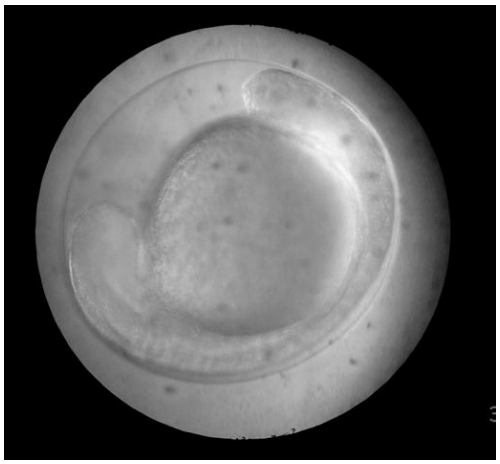


Figure 6 (c): Time- 11 hours and 40 minutes

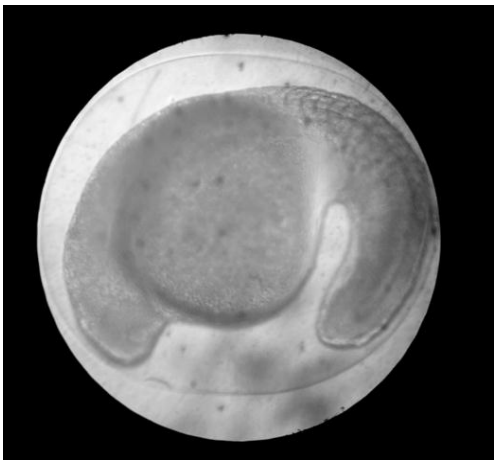


Figure 6 (d): Time- 15 hours

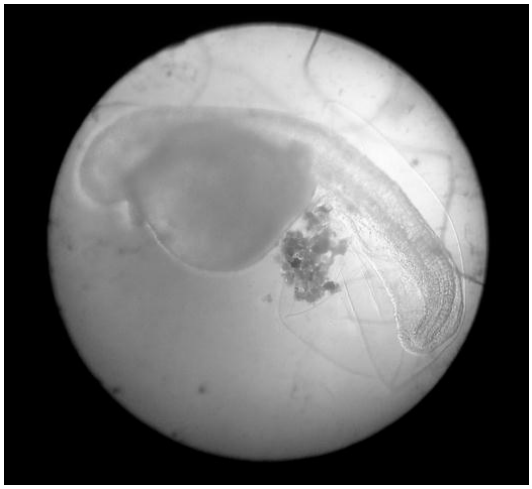


Figure 6(e): Gut begins to appear posterior to the yolk sac

Source: (Chakrabarti *et al*., 2009)

Figure 6 (a, b, c, d, e): Embryonic development of eggs of *Ompok Pabda*

Table 7: Measurement of different stage of embryonic and larval development (Roy *et al*., 2021)

Larval stage	Measured Length(mm)
0 hour	1.5 mm
6 hours	2.6 mm
12 hours	4 mm
24 hours	4.3 mm
36 hours	4.6 mm
48 hours	4.9 mm
60 hours	5.2 mm
72 hours	6 mm
7 days	7.5 mm
14 days	3.2 cm

3.8 Hatching

Hatching occurred at about 23-25 hours after spawning and the hatchlings were light yellow in colour. The survival of the hatchling varied from 51- 72% (Bhowmick *et al.*, 2000; Mukherjee and Das, 2001). Embryos hatch 22-24 h after fertilization at 28-30°C (Bishwas *et al*., 2018).

The hatching rate depends on the fertility of eggs. Low hardness and alkalinity is conducive for better embryonic development, leading to better hatching rate. Newly-hatched larvae possess a large yolk-sac that is absorbed within 2-3 days. Non-fertilized eggs and shells

remaining from hatched eggs must be removed immediately to avoid fouling of water. The screen and non-fertilized eggs should be removed from the hatching tub or cistern as soon as most eggs have hatched and fallen through the screen (Bishwas *et al*., 2018).

Source: (Chattopadhyay, 2018)

Figure 7: Fry of Pabda

3.8.1 Effect of temperature on hatching

The hatching period depends on temperature and species. While Sridhar *et al*. (1998) found the hatching period to be 24-25 hour, Mukherjee and Das (2001) observed it to be 22 h in *O. pabda*. Like the hatching period, the egg and embryo development also depend on temperature and species. *O. pabda* eggs taking 5 h to reach yolk-plug stage and 8 h to attain bean shaped- embryo (Chakraborty *et al*., 2007). It took 10 h to reach bean-shaped embryo at 28-29ºC, with the yolk-sac taking 3-4 h for absorption (Basavaraja *et al*., 2013).

3.8.2 Effect of light, darkness and stress conditions on spawning

There is no significant effect of light, darkness on induced spawning. If the brood pair doesn't breed even after induction of hormone, it should be assumed that the pair is in stressed condition or the dose of hormone is not sufficient to induce them.

3.9 Larval rearing

Only seed production is not all in induced breeding, proper rearing of seeds to get highest survivability is prime significance in reaching the ultimate goal. High mortality rate during the period of larval rearing is the most serious problem as has been reported for commercial production of this catfish species. Newly hatched larva can be reared in fiber glass tanks and

cement cisterns as well as in nurseries using pond water. Water levels were adjusted at different stages of rearing to minimize the stress to larva. Aquatic weeds such as *Hydrilla* can be provided to give cover for the larvae (Chakrabarti *et al*., 2009).

Source: (Bishwas *et al*., 2018)

Figure 8: Survival can be enhanced by providing hiding places to larval rearing units

Table 8: Suitable water parameters for larval rearing (Chakrabarti *et al*., 2009)

Parameters	**Range**
Temperature	25 ± 3° C
Dissolved oxygen	3 –5 mg/l
Alkalinity	120-150 mg/l
Water level	3 – 4 cm (gradually increased to 15 – 20 cm after one week)

3.9.1 Larval feeding

Various studies showed that better survivability and growth of larvae can be achieved when fed with live mass cultured zooplankton along with tubifex, *Moina*, *Artemia* nauplii. These food components can be supplied during the early larval stages to promote growth as well as to increase survivability. In fry stages and onwards, the fish can be fed with compound feed (rice polish, silk worm pupae and boiled egg) at 100% of body weight of the population (Gupta, 2018).

CHAPTER IV

CONCLUSIONS

On the basis of the findings of this review paper, the following conclusions are drawn-

Pabda fish farming is becoming popular even in our salinity prone areas like Khulna region. In a very recent report of The Daily Star (18 November, 2022) Md Alauddin Zoardar who is a pioneer of pabda fish farming in Khulna stated that in his second time cultivation of pabda fish about 85% of the fry survived and he sold for 650 taka per kg earning a profit of around 3 lakh. He also said that pabda farming had great potentiality in Bangladesh as they could be cultured thrice a year. But they have to buy pabda fry from Mymensingh hatcheries which is troublesome and not economic. This scenario is common in everywhere in Bangladesh. If all the hatcheries of our country get familiar with the induced breeding method of pabda, the production will be able to meet up the market demand of that fish. If the production increases, the price will decrease. In this article every step of induced breeding like broodstock management, water quality management, induced spawning, hormone administration technique, fertilization, egg incubation, hatching are discussed broadly which can encourage the hatchery owners by giving a complete guidance for pabda seed production.

As being endangered fish species, it is very necessary to develop captive breeding program and culture techniques for *Ompok pabda* to save them from extinction. In these cases, induction of spawning can be of great value. It has not been received much attention in aquaculture mainly due to non-availability of information regarding its breeding and culture technique. Pabda fish farming business requires comparatively less investment, but profit from this business is higher. At present pabda fish farming business can create a great opportunity for the people especially for the unemployed educated youth.

Finally, it can be concluded the findings of this article can help the hatcheries for better understanding of induced breeding protocol and motivate farmers a lot to culture *Ompok pabda*. That could fulfil the market demand and also serve as a tool to aid its conservation.

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