Original Research Article

Commonly Practiced Induced Breeding Techniques of Carp Species in Three Important Fish Hatcheries of Kurigram District, Bangladesh

ABSTRACT

The study was carried out to investigate the induced breeding practices and variations in effective hormone dosage used in induced breeding procedures in three different fish hatcheries of Kurigram District of Bangladesh. The hatcheries mainly produced carp fish seeds, including five native fish species such as Rui *(Labeo rohita)*, Catla *(Catla catla)*, Mrigal *(Cirrhinus mrigala*), Bata (*Labeo bata*), Punti (*Puntius sarana*) and four exotic fish species namely Silver carp (*Hypophthalmichthys molitrix*), Bighead carp (*Aristichthys nobilis*), Grass carp (*Ctenopharyngodon idella*) and Common carp (*Cyprinus carpio*). Exotic carps were the highest produced fish seeds (38%) in the Kurigram district, followed by major carps (35%), Bata (16%), Punti (6%) and others (5%). Three main types of inducing agents including PG (pituitary gland), HCG (human chorionic gonadotropin) and Ovupin (GnRHa injection) were used for induced spawning. The rate of 1st dose of injection of PG, HCG, and GnRHa varied from 0.5 to 2 mg/kg body weight, 100 to 200 IU/kg body weight, and GnRHa (only one dose) 0.2 to 0.4 ml/kg body weight, respectively. The rate of 2nd dose of injection of PG varied from 2 to 12 mg/kg body weight. The PG is usually primarily used for first and second doses; the ovulation period of different fish species varied between 12 to 72 hours, whereas the hatching rate ranged from 70% to 95%. Due to the variations in inducing agents and doses, different ovulation, fertilization, and hatching rates were observed.

KEYWORDS

Artificial spawning, Hatchery, Brood, Carp, Hormone, Bangladesh, PG, HCG

1. INTRODUCTION

In the fiscal year of 2019-20 [1] Bangladesh emerged as one of the leading fish-producing nations globally, with a total production of 45.03 lakh MT. The country is gifted with vast aquatic assets with a wide variety of aquatic species. The country's fisheries sector contributes 3.52% to the national GDP and 26.37% to the agricultural GDP, making it capable of being self-sufficient in fish production [1]. Bangladesh ranked third in the world in farmed fish production by the Food and Agriculture Organization in 2022, which is considered an exceptional accomplishment of the fisheries sector. Previously aquaculture practices in Bangladesh started with natural seeds, but due to various factors such as the devastation of habitats and breeding grounds, degradation of ecological balance, overfishing, the building of drainage structures, industrial pollutants, and agrochemicals it has now mostly been replaced by hatchery seed production.

The induced breeding or hypophysation technique has significantly transformed quality fish seed production and opened up vast opportunities for advancing the inland fisheries and aquaculture sector. Since 1975, Bangladesh has successfully embraced induced breeding techniques and cost-effective hatchery designs. Bangladesh has 103 government-run hatcheries and 963 private hatcheries, that highlighting the growing adoption of these techniques [2]. The country has accomplished self-reliance in carp seed production, although quality seeds remain limited in scale [3]. The principle of hypophysation technique involves stimulating ripe broods by pituitary or any other synthetic hormones to breed in captive conditions. It promotes the timely release of sperm and eggs, enabling seed incubation, hatching, and rearing under secure and weather-independent conditions.

The understanding of artificial breeding holds great significance as it enables the intensive production of certain species under controlled conditions, ensuring a reliable and consistent supply of high-quality fish seeds and pure spawn [4]. Pituitary gland (PG) extract and Human Chorionic Gonadotropic (HCG) hormone are commonly used as inducing agents. However, some other agents like Ovupin, Ovaprim etc. are also used for both Indian major carp and other species.

For successful induced breeding, proper hatchery and broodstock management is essential. But many problems exist in fish hatcheries, such as improper hatchery management, poor broodstock management, adverse selection, undesirable sizes of brood fish, inbreeding depression, lack of technical knowledge, lack of good quality fish seeds, inadequate brood fish, collection of broods from the same stock, water quality problem and inadequate supply of nutritive food for brood fish are the most common. However, day by day, the demand for fry and fingerlings is increasing as more farmers show interest in fish culture. So, there is a huge chance to develop this sector by developing a management system and reliable induced breeding technique. Our study was conducted to learn about the induced breeding technique in Kurigram, the basic strategy of brood fish and hatchery management, and the results according to different management techniques.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out between March and April of 2021 in three different fish hatcheries (Ma Matsya, M. Hossain, and Ananya Matsya) of kurigram town under Phulbari upazila (Fig. 1). Ma Matsya, M. Hossain and Ananya Matsya hatcheries are located in Chandrokhna, Shimulbari and Barabhita areas, respectively of Kurigram district.

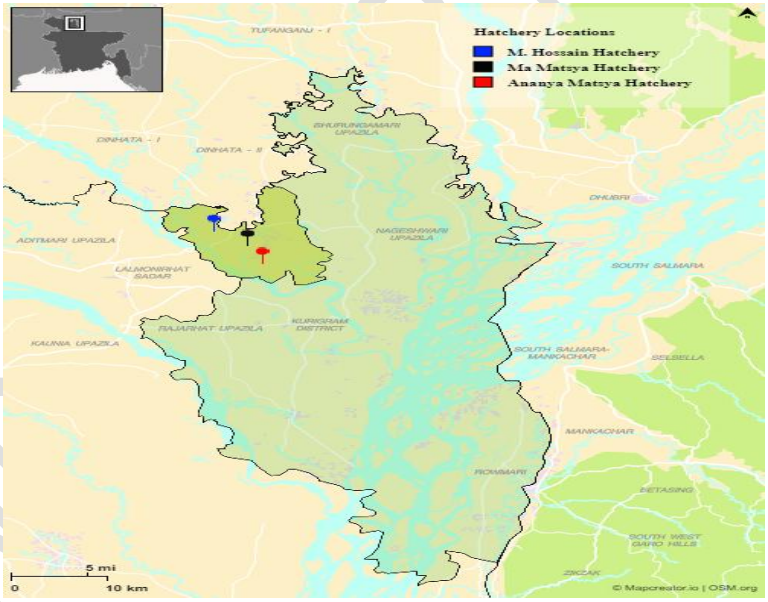

Fig 1. Observed fish hatcheries and their locations in Kurigram district.

2.2 Study Design

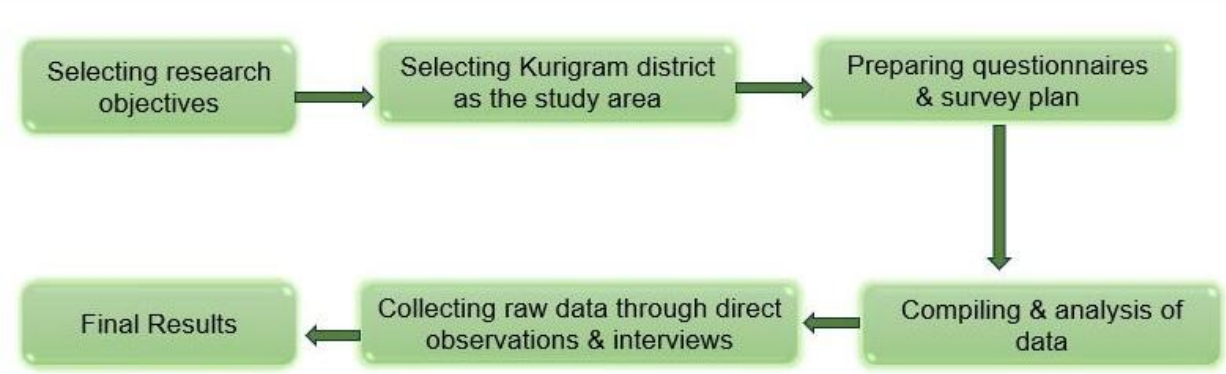

Fig 2. Conducted flow chart for the study

2.3 Data Collection

Primary data was collected through pre-arranged interviews of the operators and hatchery managers, observing the procedures, and surveying each study area. Additional pertinent secondary data and information were gathered from the District and Upazilla Fisheries Offices of the Department of Fisheries (DoF).

2.4 Data Processing and Analysis

The collected data were verified for trustworthiness and precision with the help of hatchery personnel. All the accumulated data were cautiously summarized and examined, and then, for further analysis, several pertinent tables were prepared.

3. RESULTS

3.1 Brood Stock Collection

Broods were primarily collected from natural sources such as different lakes, rivers, haors, baors, beels, jheels etc. The optimal time for brood collection usually falls between November to March. In this study, the hatcheries' broodstock sources are different (Table 1).

Table 1. Brood fish source of the hatcheries.

SL No.	HATCHERY NAME	BROOD FISH SOURCE
1.	Ma Matsya Hatchery	Collected from local pond.
2.	M. Hossain Hatchery	Carp- Jamuna and Halda river origin fish. Punti- China origin.
3.	Ananya Matsya Hatchery	Collected from local pond & river.

3.2 Brood fish selection for induced breeding

The success of induced breeding depends on adequately selecting the broods based on their peripheral characteristics. The mature adult males can be distinguished from the females by the presence of denticulation on the dorsal surface of the pectoral fin, which feels rough to the touch. On the other hand, the ripe females exhibit a soft and bulging stomach with a swollen pinkish genital opening.

The hatchery managers mainly selected the broods based on their weight and age (Table 2), as well as their physical characteristics including size, coloration and growth rate.

Table 2. Age and weight of selected brood fish

Type	Common Name	Scientific name	Weight	Age
Native carp	Rui	*Labeo rohita*	1-4 kg	2+
	Catla	*Catla catla*	2-6 kg	2+
	Mrigal	*Cirrhinnus mrigala*	1-4 kg	2+
	Bata	*Labeo bata*	0.2-0.8 kg	1+
	Sarputi	*Puntius sarana*	0.15-0.5 kg	1+
Exotic carp	Silver carp	*Hypopthalmicthys molitrix*	2-4 kg	2+
	Grass carp	*Ctenopharyngodon idella*	3-5 kg	2+
	Common Carp	*Cyprinus carpio*	2-5 kg	1+
	Big head carp	*Aristichthys nobilis*	2-4 kg	2+

3.3 Inducing Agents and Hormonal Dose Selection

In the examined hatcheries, mainly three types of inducing agents: pituitary gland (PG), Human Chorionic Gonadotropin (HCG), and Gonadotropin Releasing Hormone analogues (GnRHa) were used for induced breeding purposes, which were marketed as Ovupin (100 mg Domperidone + 0.2 mg S-GnRHa). Notably, each hatchery has a different selection of dosages and hormone choices depending on species and specific inducing agents utilized (Fig. 3).

Fig 3. Hormone preparation of PG and Ovupin in M. Hossain hatchery.

Ma Matsya Hatchery used 0.1 ml/kg as first dose of given solution for all carp species. Second dose is administered after 6 hours of first dose and it varies in Indian Carps (Rui, Mrigal, Puti, Bata, Common Carp) and Exotic Carps (Bighead, Grass carp, Common carp). Indian carps are given solution at either 0.6 ml/kg for female or 0.15 ml/kg for males. Whereas exotic carps are given solution at either 1.2 ml/kg or 0.15 ml/kg for males. Second dose of pituitary gland (PG) is given at 1 mg/kg for females and 1.5 mg/kg for males for every species. The dilution rate is ten individual PG, which is 30 mg, mixed with 3 ml of water (Table 3).

As for M. Hossain hatchery, three types of inducing agents are used: pituitary gland (PG), human chorionic gonadotropin (HCG), and Ovupin. For first dose in females, 0.5 to 2 mg/kg PG or 200 IU/kg HCG is used for both types of carps. Additionally, for second dose in females, 6 to 10 mg/kg PG is

administered in Indian carp and 6 to 12 mg/kg in exotic carp. Second dose of HCG level for females is 1200 IU/kg for Indian and exotic carp. As for males, 2 mg/kg PG, 200 IU/kg HCG, or 0.3 l/kg Ovupin is given as second dose (Table 4).

At the Ananya Matsya hatchery, only one dose is given to male carp species, whereas two are given to females. For Indian carp species, 1 to 2 mg/kg PG is applied for males and 1 to 2 mg/kg as first dose, followed by 6 to 8 mg/kg PG as second dose is applied for females. No PG is given to male Indian carp. Furthermore, 150 to 200 IU/kg HCG is used for male exotic carp. As for females, 100 to 150 IU/kg HCG and 8 to 12 mg/kg PG are given as first and second doses, respectively (Table 5).

Table 3: Inducing Agents & Hormonal Dose Selection of Ma Matsya Hatchery.

Ma Matsya Hatchery						
Species	1st Dose	2nd Dose (6 hour after 1st Dose)				
	Female	Female			Male	
	Given Solution (ml/kg)	PG (mg/kg)	Given Solution (ml/kg)	PG (mg/kg)	Given Solution (ml/kg)	PG (mg/kg)
Rui	0.1	1	0.6	6	0.15	1.5
Mrigal	0.1	1	0.6	6	0.15	1.5
Puti	0.1	1	0.6	6	0.15	1.5
Bata	0.1	1	0.6	6	0.15	1.5
Common Carp	0.1	1	0.6	6	0.15	1.5
Bighead	0.1	1	1.2	12	0.15	1.5
Grass Carp	0.1	1	1.2	12	0.15	1.5
Silver Carp	0.1	1	1.2	12	0.15	1.5
Dilution rate: 10 PG (30 mg) mixed with 3 ml water						

Table 4: Inducing Agents & Hormonal Dose Selection of M. Hossain Hatchery

M. Hossain Hatchery								
Species	1st Dose		2nd Dose (6 hour after 1st Dose)					
	Female		Female			Male		
	PG (mg/kg)	HCG (IU/kg)	PG (mg/kg)	HCG (IU/kg	OVUPIN (ml/kg)	PG (mg/kg)	HCG (IU/kg)	OVUPIN (ml/kg)
Bata	0.5-2.0	200	6-10	1200	0.6	2	200	0.3
Mrigal	0.5-2.0	200	6-10	1200	0.6	2	200	0.3
Sar Puti	0.5-2.0	200	6-10	1200	0.6	2	200	0.3
Rui	0.5-2.0	200	6-10	1200	0.6	2	200	0.3
Common Carp	0.5-2.0	200	6-10	1200	0.6	2	200	0.3
Silver Carp	1.0-2.0	200	6-12	1200	0.6	2	200	0.3
Bighead	1.0-2.0	200	6-12	1200	0.6	2	200	0.3
PG	PG is used on both 1st and 2nd Dosage.							
HCG	HCG is mainly used for 1st Dose and rarely used as 2nd Dose.							
OVUPIN	OVUPIN is used as 2nd Dosage or one single Dose.							

Table 5: Inducing Agents & Hormonal Dose Selection of Ananya Matsya Hatchery

Ananya Matsya Hatchery

Species	Male		Female		
	One Dose		1st Dose		2nd Dose
	PG (mg/kg)	HCG(IU/kg)	PG (mg/kg)	HCG(IU/kg)	PG (mg/kg)
Rui	1-2		1-2		6-8
Catla	1-2		1-2		6-8
Mrigal	1-2		1-2		6-8
Bata	1-2		1-2		6-8
Sarputi	1-2		1-2		6-8
Common Carp	1-2		1-2		6-8
Silver Carp		150-200		100-150	8-12
Grass carp		150-200		100-150	8-12
Bighead		150-200		100-150	8-12

3.4 Hormone administration

In all the observed hatcheries, two places were used to inject hormones into a fish to instigate hypophysation. The first method involved an intraperitoneal injection, which was administrated through the ventral bottom part behind either the pelvic fin (Fig. 4) or pectoral fin, and intra-muscular injections, typically performed on the dorsal part of the fish above the lateral line. During hypophysation, two dosage levels were commonly utilized: a preparatory dose and a decisive or final dose, usually with a time gap of 6 to 12 hours between the two injections. Generally, the preparatory dose constitutes 10-25% of the total. However, no preliminary doses were necessary for males.

Fig 4. Intra peritoneal hormone administration in M. Hossain hatchery.

3.5 Stripping

During stripping in three hatcheries, the technician employed gentle pressure using his thumb near the genital opening to squeeze out the eggs from the fish. For smaller fishes, the technician was captured by the hands and pressed near genital pores to release the eggs on a dry plastic bucket. In contrast, the larger fishes (over 4-5 kg) were laid on a cushioned table during the process. When ovulation occurs, a stream of eggs will emerge and the operator has messaged the abdomen of the fish from front to back to ensure all the eggs were stripped out (Fig. 5A). After the eggs were collected, the milt was squeezed out from the male fish into the same bucket similarly as the eggs. Two people were required for proper stripping, one holding the fish firmly and alternating between releasing eggs and milt (Fig 5B).

(A) Female (B) Male

Fig 5. Stripping of male and female brood fish in Ma hatchery.

3.6 Incubation and Hatching

The period that is needed to hatch out larvae from the fertilized eggs is called the incubation period, and it is conducted in Incubators. Funnel incubators were commonly used in all three hatcheries (Fig. 6). Incubation period depends on species and incubation temperature. According to Aktar *et al.,* 2015, incubation period of various fish species varies, ranging from a minimum of 12 to a maximum of 72 hours [5]. Valeta et al. (2013) also discovered that the incubation period tends to decrease with higher incubation temperatures [6]. Aeration is essential for the embryonic development of eggs just before the hatching of larvae; they need more oxygen because of wriggling activities to come out from the shell (Fig. 7). During incubation, egg density in the incubator influences both the hatching rate and embryonic development.

Fig 6. Funnel incubators in Anannya hatchery

Fig 7. Hatchlings produced in M. Hossain hatchery.

3.7 Ovulation rate

The calculation of ovulation rates was performed using the following formula:

Ovulation rate (%) = (No. of egg ovulated/ Total number of fish injected) × 100
The ovulation rate of the hatcheries was 95% for Ma Matsya Hatchery and 90% for both M. Hossain and Ananya Matsya Hatchery, respectively (Fig. 8). Ma Matsya Hatchery used PG at the rate of 7mg/kg body weight for Indian carps and 13mg/kg body weight for exotic carps. On the other hand, M. Hossain hatchery used PG (6.5-12/kg body weight for Indian carps, 7-14mg/kg body weight for exotic carps), HCG (200 IU/kg body weight for 1st dose), or Ovupin (0.6 ml/kg body weight for one single dose). Ananya Matsya Hatchery used PG (7-10 mg/kg body weight) and HCG (100-200 IU/kg body weight for 1st dose).

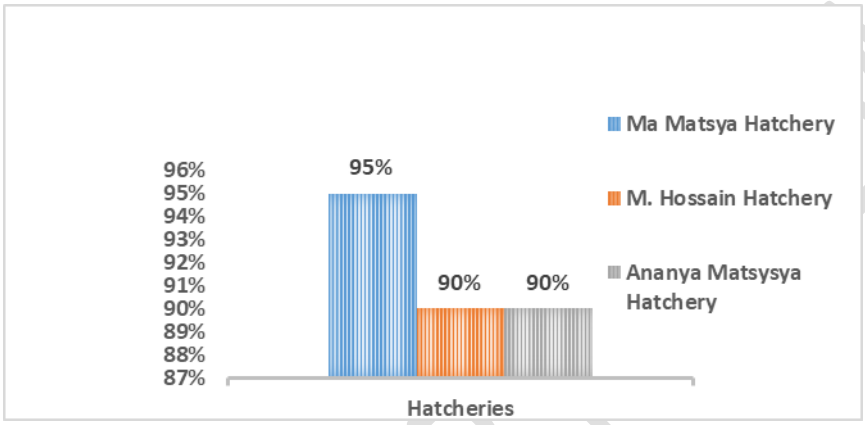

Fig 8. Ovulation rate of the hatcheries.

3.8 Fertilization rate

The fertilization rates were calculated as follows:

Fertilization rate (%) = (No. of fertilized eggs/ Total no. of eggs) × 100

The highest fertilization rate was observed in M. Hossain Hatchery (90-95%), followed by Ananya Matsysya Hatchery at 85-95%, and the lowest fertilization rate was observed in Ma Matsya Hatchery which is 80-90% (Fig. 9).

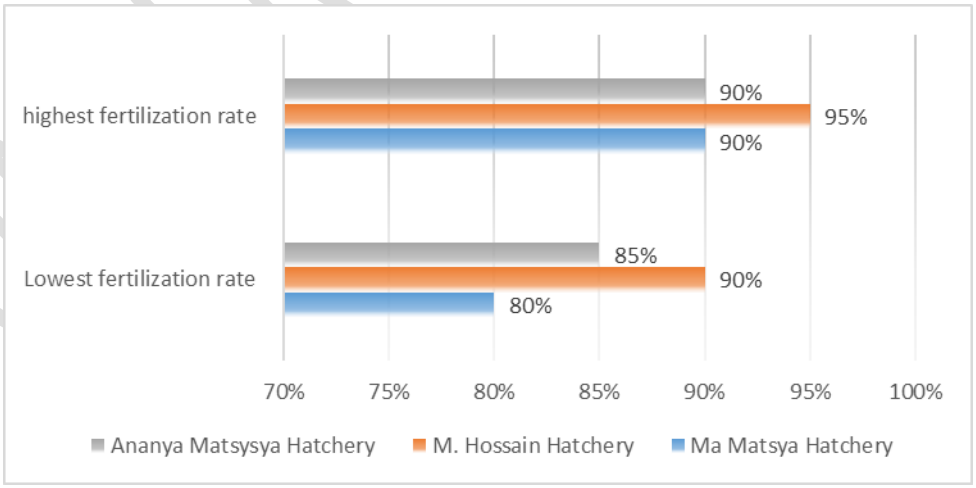

Fig 9. Fertilization rate of the hatcheries.

3.9 Hatching rate

The formula for the hatching rate is:

Hatching rate (%) = (No. of eggs hatched/ Total no. of fertilized eggs) × 100

During the study, the maximum hatching rate was observed in M. Hossain Hatchery, which was 85%-95%, following the hatching rates of Ananya Matsysya Hatchery and Ma Matsya Hatchery, which was 80%-90% and 70%-90%, respectively (Fig. 10).

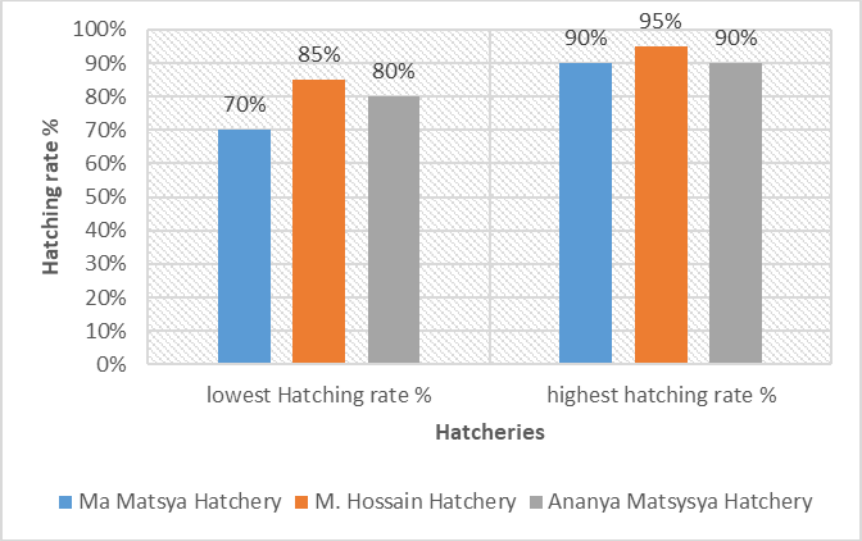

Fig 10. Hatching rate of the hatcheries

4. DISCUSSION

We observed the uppermost ovulation rate at Ma Matsya hatchery when they used 7mg PG/kg body weight for Indian carp and 13mg PG/kg for exotic carp species. Notably, deviations from these dosages of PG levels resulted in a decreased ovulation rate. Chaudhuri [7] found that induced breeding experiments yielded better results when the giver and receiver fishes belonged to the same species. Bhuiyan et al. [8] also discovered that the ovulation rate is highest at 7 mg PG/kg body weight, while higher (9mg PG/kg) or lower doses (5 mg PG/kg) led to a decreased rate. Fontenel [9], Atz and Pickford [10], Das and Khan [11], Chaudhuri [12], Mirza et al. [13], Alam and Bhuiyan [14] also implemented similar practices for induced breeding in various fishes.

On the other hand, Ovupin (0.6 ml/kg body weight) showed the best result in the best fertilization rates. Two different hormones for two doses (HCG 100-150 IU/kg body weight +PG 6-12 mg/kg body weight) showed better results than only using PG for two doses (1 mg+ 6-12mg/kg body weight).

Moreover, using ovupin gives the highest hatching rate than others. HCG+PG also gives better results than PG, but not as much as Ovupin. These findings align with the research of Yeasmin et al. [16] on common carp, where Ovaprim (similar to Ovupin) exhibited better fertilization and hatching rates compared to HCG and PG. A similar result was reported by Indira et al. [17] who observed better ovulation, fertilization, and hatching rates in Indian major carp treated with Ovaprim than those treated with PG.

In summary, the study highlights the ovulation, fertilization, and hatching rate of larvae primarily influenced by the hormone doses, brood stock mismanagement, quality of the brood, maturity of eggs,

seasonal variation, incubation process, water flow during incubation, quality of hatchery water and handling procedure of the broods. It was also detected that the fish bred successfully under artificial conditions when the appropriate hormonal injections were applied.

5. CONCLUSION

Based on the investigational study conducted on three important fish hatcheries in Phulbari Upazila of Kurigram District, Bangladesh, it was evident that fish hatcheries significantly impact inland fish production and aquaculture development. Induced breeding has proven to be a revolutionary technique in the fisheries sector worldwide. To ensure high-quality and abundant fish production, providing financial and material support, facilitating information sharing, and organizing workshops and seminars conducted by DoF and Universities is crucial. Additionally, there is a need to focus on developing hatchery facilities and further enhancing induced breeding techniques to foster the growth and progress of aquaculture in Bangladesh.

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