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Research Article

Brood Rearing and Dose Optimisation for Induced Breeding of Raikor, *Cirrhinus reba* (Hamilton, 1822)

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ABSTRACT

An experiment on brood rearing and induced breeding of the near threatened fish species Raikor, *Cirrhinus reba* using the pituitary gland (PG), was conducted from March to August 2020 at the Floodplain Sub-station of Bangladesh Fisheries Research Institute, Santahar, Bogura. Broods were collected and reared in the ponds of the hatchery complex. The total length (cm), body weight (g), gonad weight (g), and gonado-somatic index (%) of this species were measured during the rearing period. To standardize the breeding technique, a total of 90 brood fish of *C. reba* were treated with different doses of PG, specifically, 2.0, 4.0, 6.0 mg/kg body weight for females and 1.0, 2.0, 3.0 mg/kg body weight for males in different treatments, namely T_1, T_2, and T_3 respectively. A significant difference ($p<0.05$) was observed in fecundity, ovulation (%), and the fertilization rate (%) among the treatments. Based on the results, T_2 (4.0 mg/kg body weight for females, 2.0 mg/kg body weight for males) produced the most favorable results. The current observations could be applied to *C. reba* stimulated breeding for the advancement of hatchery formation. More research on the nursing, nurturing, and culture of the near- threatened *C. reba* at varied densities and feedings is necessary for their conservation and restoration.

Keywords: Induced breeding, embryology, gonado-somatic index, *C. reba*

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INTRODUCTION

Bangladesh, a self-sufficient country that ranks third in inland open-water capture production, fifth in world aquaculture production, and fourth in world tilapia production (FAO, 2022), is supplying a total output of 4.62 million metric tons per year (DoF, 2022). The Fisheries sector contributes up to 60% of animal protein consumption in Bangladesh (Sufian et al., 2017; DoF, 2022; Mustafi et al., 2022) and fish is the primary source of protein for most people (Haque et al., 2015).

The floodplain contains both persistent and temporary wetlands which are both highly important and vulnerable to various hydrological changes and threats (Maria et al., 2016; Mondal & Pal, 2017). Bangladesh has an extensive number of unique watercourses, including rivers, lakes, floodplains, estuaries, canals, *beels*, *haors*, and *baors* (IUCN Bangladesh, 2015; Suravi et al., 2017; Pandit et al., 2020) which occupies about 80% of the country's land area (Brammer, 1990) and is home to 65% of Bangladesh's inhabitants (di Baldassarre et al., 2014). Most of the rural populations in the floodplains rely on natural water sources for their livelihoods and agricultural activities (Pandit et al., 2022).

Among the 260 freshwater fish species (Rahman, 2005; DoF, 2022), about 64 and 27 freshwater species are under threatened and near-threatened conditions, respectively (IUCN Bangladesh, 2015). *Cirrhinus reba* (Hamilton,

1822) is a highly nutritious, near-threatened fish which is rich in protein, fat, vitamins, and minerals (Bogard et al., 2015) and is dispersed in India, Bangladesh, Pakistan, Nepal, Myanmar, and Thailand (Bhuiyan, 1964; Jayaram, 1981; Davis & West, 1993; Rahman, 2005; Talwar & Jhingran, 1991; Bogard et al., 2015). This delicious and popular fish (Reba carp) is locally known as Reba, Raik, Bata, Aikhor, Raikor, and Tatkini. In the past, Raikor was found in large quantities in natural water bodies in Bangladesh, but this fish species is also currently under threat, due to both human activities and ecological factors such as habitat destruction and loss, construction of flood protection embankments and roads, overfishing, water pollution, climate change, and the introduction of invasive alien species (Galib et al., 2009, 2013; Imteazzaman & Galib, 2013; Mohsin et al., 2009; Mia et al., 2017; Islam et al., 2019; Pandit et al., 2021; Talukder et al., 2021; Das et al., 2022a; Mia et al., 2022; Kamal et al., 2022; Kunda et al., 2022). In these circumstances, Bangladesh is working to develop artificial breeding, fry production, and farming management technologies through research to save this fish from extinction.

Production of *C. reba* from natural water bodies is decreasing day by day (Gupta & Banerjee, 2016). Artificial breeding can increase the number of fingerlings and fry, and the development of culture technology can offer cultivation of these fish in shallow waterways (Haque et al., 2023). This species has a high market price in the commercial market (Sultana et al., 2018), is locally available, is cultivable in small ponds, matures in 1 year, is easier for artificial breeding, has a prolonged breeding period, is delicious to eat, and is environmentally friendly. Numerous explorations on the biology of this species are available, such as on feeding (Naik et al., 2015, Lashari et al., 2010), life-history character (Hossain et al., 2013), and induced spawning (Sarkar et al., 2004). Lashari et al. (2007) sampled the gonado-somatic index (GSI) and reproductive success of *C. reba* in fishponds in the area of Jacobabad, Sindh, Pakistan. Although induced breeding techniques of this species have been attempted in some cases, the technique has not yet been standardized. The recording of reproductive capacity is a vital part of the fisheries realm because it has a direct impact on fish manufacturing (Mian et al., 2017; Shafi et al., 2012). The current study aims to understand *C. reba*'s brood-rearing process, determine the appropriate pituitary gland (PG) hormone dosage for induced breeding, know the fecundity and nursing of fry, and evaluate water quality factors.

MATERIALS AND METHOD

Experimental site

From March to August 2020, the study was conducted at the Bangladesh Fisheries Research Institute's floodplain sub-station at Santahar, Bogura (Figure 1).

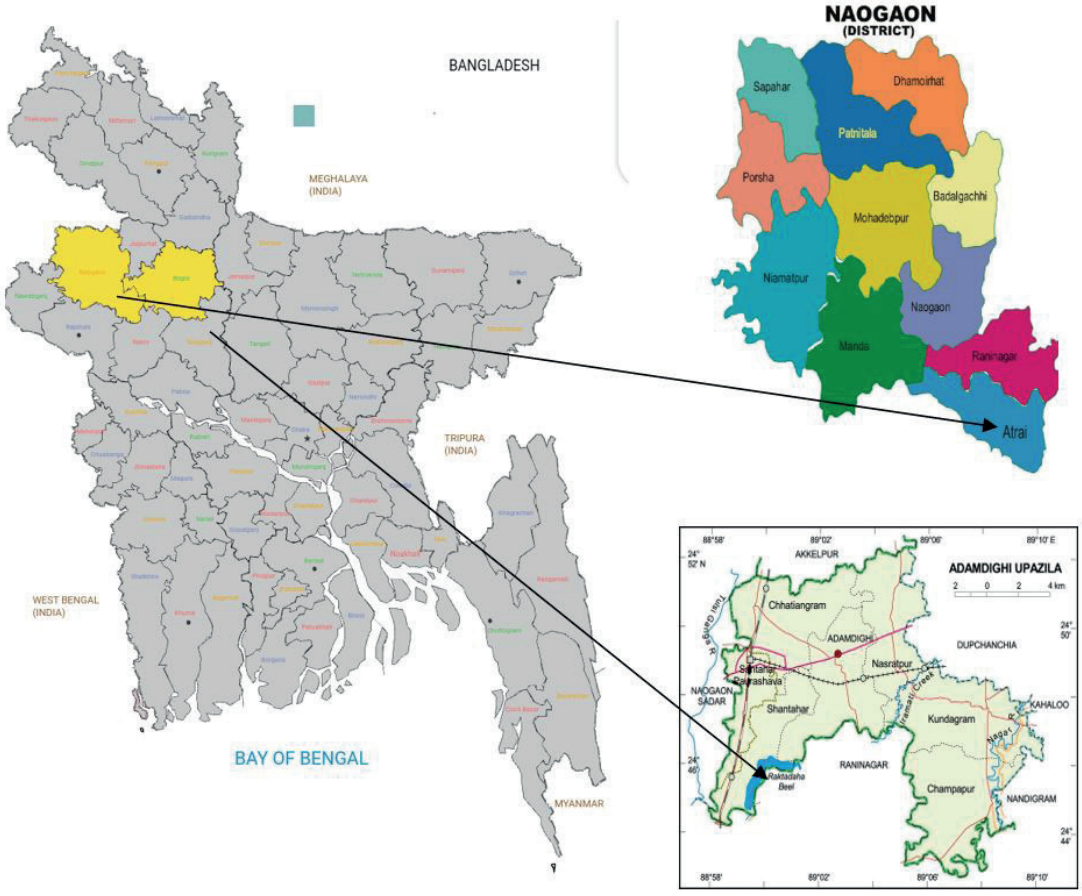

Figure 1. The location of sample collection and study areas. Collection of brood fishes.

The Raikor species is commonly found thriving in various aquatic environments such as rivers, canals, ponds, *beels*, and floodplains across Bangladesh, including the Ratargul swamp forest (Kunda et al., 2022), Kawadighi *Haor* (Kamal et al., 2022), Bookbhara *Baor* (Mohsin et al., 2009), Chalan *Beel* (Galib et al., 2009), Surma River (Mia et al., 2022), Choto Jamuna River (Galib et al., 2013), Juri River (Islam et al., 2019), Shari-Goyain River (Talukder et al., 2021; Das et al., 2022a), Dhanu River (Pandit et al., 2021), and Halti *Beel* (Imteazzaman & Galib, 2013). Full-grown, robust, and pathogen-free fish were collected from Bogura's Raktadaho *Beel* and Naogaon›s Little Jamuna River.

Pond preparation and brood nurturing

Prior to the start of the research, the ponds, which were 1.5m deep, were cleared of vegetation, dried, limed (250kg/ha), and manured with cow dung at a rate of 500kg/ha. Liming was done 4-5 times per year during pond preparation and during the culture period at 0.40-1.20kg per decimal. Fertilization was done prior to fish stocking and as needed during the culture period. Urea and TSP (Tripple Super Phosphate), as well as MP (Murate of Potash) and DAP (Di-ammonium Phosphate), were used as fertilizers at the study site. Underground water was used to meet the mandatory water depth of the fishpond.

Feeding

Following stocking, the fish were fed 10% of their body weight once daily in the morning in the form of dough balls. Supplemental feed included 60% rice bran, 30% mustard oil cake, and 10% flour.

Selection of broods for breeding

Healthy and disease-free Reba broods can be recognized by their external sexual characteristics. Male fish have tough, sandy scales on the flanks, neck area, and anterior dorsal side, whereas females have smooth scales. Males are longer and have larger pectoral fins than females. Males are distinguished by their flat abdomens and long, protruding genital papillae, whereas females have soft, oozing abdomens.

The brood fish conditioning and PG extract preparation

Broods were measured and placed in separate cisterns with continuous water flow for 8–9 hours to condition. A freshly prepared extract of widely viable wet pituitary glands was used to induce ovulation. The specified volume of PG was carefully measured using an electronic balance, and the PG was prepared for administration to the broods.

Experimental design

During the study period, nine fishponds were assigned for three treatments, each with three replicates, in a Completely Randomized Design (CRD) (Table 1).

Reba's gonads were accumulated between March and August of 2020. During the experiment, 50 samples were collected to calculate the GSI. The ovary was removed completely, weighed, and stored in 10% buffered formalin.

Histology of gonads

Throughout the experiment, both types of gonads were collected from the brood fish. Fish were dissected and arranged in a balanced buffered formalin solution. Specimens were prepared in a scaled alcohol and xylene installation before being encapsulated in paraffin columns in plastic cassettes. The conventional hematoxylin and eosin staining methodology described by Van-Dyk & Pieterse (2008) was used. Portions of 3-5 microns were cut using a servomotor microtome machine (Leica RM 2125), and photographs of the besmirched phases were picked with a composite photomicroscope.

Table 1. Designing the experiment.

Treatments	No. of fish	Replication	Sex ratio	PG dose (mg/kg)	
				M	F
T1	10	3	Male: Female=1:1	1	2
T2	10	3		2	4
T3	10	3		3	6

Gonado-somatic index (GSI)

Fecundity

The Von Vayer method was used to calculate fecundity. The gonads were picked out with scissors and the exterior connective tissues of the ovaries were removed. Blotting paper was used to absorb any excess moisture, and the ovaries were weighed with an electronic balance (Model FX- 300). Then, 10 mg from each gonad was precisely extracted. The overall egg count was determined by multiplying the overall average number of eggs counted by the total weight of the ovary.

Injecting PG extract into the experimental fish

To inject PG extract into the experimental fish, each Reba was carefully placed on a soft fabric piece. A graded 1-ml syringe was filled with the required amount of PG solution, which was injected intramuscularly under the fish's pectoral fin. A specific amount of the PG extract was injected into brood fishes according to the experimental protocol (Table 1).

Ovulation, fertilization, and embryonic development

Broods were separated into different aquifers based on intervention, and the breeding attitude was considered. During the rutting season, an ephemeral fountain was kept running smoothly to disperse the eggs and keep them from sinking to the bottom, as well as to keep the eggs aerated. The amount of hormone transfused affected the ovulation time. Both male and female sperm and eggs were forced to release and were fertilized 7-9 hours after injection. The fertilized embryo was then collected and incubated in a separate plastic container. Fertilized eggs swell, harden, and become sticky when they are exposed to water. Throughout the time of incubation, dead eggs were lifted from the water tanks every two hours. After the completion of the hatching process, the hatchlings were counted and recorded.

Tending of Reba fry

The ponds were drained and left to dry to eliminate rapacious and weed fishes. After drying and weed removal, 1kg/decimal lime was applied to the pond bottom. One day after liming, the ponds were

refilled with water to a depth of 1.0m and 2kg/decimal flour, 1.5kg/decimal mustard cake, 50gm/decimal yeast, and 250gm/decimal molasses were incorporated into the ponds three to four days before releasing the fry. Sumithion was used to control pest expansion. The spawn of the fish was not fed for the first day after release to allow them to adapt quickly to their changed surroundings. After three days, flour and hard-boiled eggs were added. Mustard oil cakes were used for 10–15 days, then again for 28–30 days.

Hydrological parameter monitoring

The temperature, transparency, dissolved oxygen (DO), pH, and ammonia were evaluated at monthly intervals in the morning. The water temperature was measured using a Celsius thermometer. To achieve transparency, a Secchi disc was used. The dissolved oxygen was measured using a portable DO meter (YSI digital DO meter). The pH of the pond water was measured using a pH meter (Digital pH meter). Ammonia levels were determined using an ammonia test kit.

Evaluation of growth parameters

The total length, body weight, and mortality were all tracked at regular intervals, and growth metrics were analyzed using the following equations:

(Mollah et al., 2008). where Wb = the total body weight (kg) of all fishes to be injected and Pt = the dose in mg of PG to be injected per kg body weight under a particular treatment.

The volume of extract (ml) = Wt (PG weight (mg)) × 1.0 (extract volume (ml) to be injected per kg body weight of fish) (Mollah et al., 2008).

*The Gonado-somatic Index (GSI%) = (Weight of gonad/Weight of fish) * 100*

Fecundity = (Number of eggs in the fraction×Total weight of ovary) / weight of the fraction

*Fertilization = (Number of fertilized eggs/Total number of egg counted)*100*

*Hatchability = ((Number of hatchlings (two days old)/(Total number of fertilized egg))*100*

*Survival rate = Number of fries harvested/Number of fries stocked)*100*

*Ovulation (%) = (Number of fish ovulated/Total number of fish injected)*100*

Observation of embryonic development

An optical micrometer, a stage micrometer, and a computer-linked microscope were used to examine the embryonic iterations of fertilized eggs. Collected specimens were fleetingly tainted with methyl orange and safranin to allow for a concise examination under an electronic microscope. This study outlined ten distinct embryonic developmental phases.

Statistical evaluation

All data were analyzed using one-way ANOVA (Analysis of Variance) followed by DMRT in SPSS (Statistical Package for Social Science) version 25.0. A one-way ANOVA was used to compare mean differences. The hypotheses of normal distribution and variance similarity were verified prior to conducting the analysis.

RESULTS AND DISCUSSION

The temperature (°C), pH, dissolved oxygen (mg/l), transparency (cm), and NH_3 (mg/l) were measured every month in the brooder pond and found to be within a suitable range (Table 2). The thermal range of water in three treatments was found to range between 28.00 and 31.50°C during the experimental period and was found to coincide with Ali et al. (2017), which is within the optimum range (26.06-31.97°C) for fish culture (Boyd, 1982). The fact that there were no clouds in the sky and a strong sun in July may have contributed to the highest temperature of 31.50°C that was recorded in T_1 in July and the lowest water temperature (29.0°C) ever recorded in T_1 in March. The most favorable range of pH needs to be between 6.5-9.0 for fishponds and aquatic life (Swingle, 1969); a value below 5.5 turns the water too acidic for fish (Makori et al., 2017), where our findings (6.9-7.6) are above the acidic criteria. Similar water quality has been maintained by Rahman et al. (2021) and Araf et al. (2021) during the captive breeding of freshwater fish.

The pH readings from this investigation were consistent with the findings of the authors mentioned above. In July, T_2 had the greatest DO level (6.82mg/l), whereas in August, it had the lowest dissolved oxygen content (5.29mg/l). The suitable dissolved O_2 level for fishponds should be greater than 5mg/l (Bhatnagar and Singh, 2010); Oxygen below 3mg/l is considered detrimental to fish progression (Ross et al., 2001). To support fish life in freshwaters, 5.0mg/l of dissolved oxygen is necessary, according to Chapman and Kimstach (1996). According to Mallasen et al. (2012), oxygen concentrations below 4.0mg/l are necessary for tropical fish growth. As a result, the experiment's DO values support the conclusions of the authors mentioned above. The ideal transparency coverage for fish farming is 15–40cm (Boyd, 1982). The current study's findings were consistent with those of Begum et al. (2017). The safe limit of ammonia concentration was not specified, but it was higher than the value of 0.012 mg/l generally cited by fish culturists, as mentioned by Meade, 1985. The current report's ammonia levels stayed within an acceptable range (>0.012mg/l).

Both histological and morphological changes were considered when identifying brood fishes. *C. reba* ovarian development was studied to determine the structure and timeline of germ cell growth and maturation stages. The yolk granule stage in the histological process indicates the ripening condition of brood fish. During July, the ovary had the late yolk granule stage (LYGS) and the testes had spermatozoa (SZ) and spermatids (ST) (Figure 2). To assess the maturity of fish gonads, morphological changes in the brood fish were observed. Fish length, gonad weight, and gonado-somatic index were measured monthly for the majority of the experiment. The mean values of these parameters varied significantly ($p<0.05$) between months, as shown in Figure 3. During the study period, there was a positive relationship between total length and body weight. The GSI values of female *C. reba* changed from 3.32±0.15 to 12.2±1.30% and in the male it was 1.68±0.02 to 3.4±0.03 with the change of months (Figure 3).

Table 2. Hydrological parameters (mean±SD) were assembled throughout the study period under various treatments from March to August 2020.

Months	Treatments	Temperature (°C)	pH	Dissolved oxygen (mg/l)	Transparency (cm)	NH_3 (mg/l)
March	T_1	29.0±2.15	7.6±.6	6.11±0.37	36.33±4.22	0.54±0.11
	T_2	30.12±0.9	7.1±0.5	6.55±0.40	35.9±2.58	0.49±0.09
	T_3	30.2±1.2	7.2±0.47	5.9±0.11	37.51±0.44	0.50±0.09
April	T_1	30.99±1.2	7.3±0.8	5.8±0.31	36.33±4.22	0.48±0.11
	T_2	29.23±0.7	7.6±0.4	6.4±0.31	35.6±2.5	0.49±0.12
	T_3	30.22±1.2	7.2±0.14	5.9±0.55	34.66±5.1	0.51±0.11
May	T_1	31.00±2.3	7.2±0.41	6.2±0.11	36.33±2.01	0.50±0.09
	T_2	29.88±2.1	6.9±0.42	5.81±014	33.99±4.22	0.54±0.12
	T_3	30.44±1.8	7.3±0.32	6.24±0.35	34.88±4.3	0.49±0.10
June	T_1	30.19±1.1	7.1±0.81	6.64±0.41	36.66±3.33	0.50±0.10
	T_2	31.00±1.0	6.9±0.28	5.64±0.55	36.33±3.18	0.48±0.07
	T_3	30.09±1.0	7.3±0.47	5.51±0.19	35.5±5.5	0.49±0.08
July	T_1	31.50±1.9	6.8±0.61	6.65±0.54	33.5±2.22	0.51±0.11
	T_2	29.91±1.7	7.5±0.21	6.82±0.71	36.33±2.89	0.50±0.12
	T_3	30.33±1.9	7.2±0.35	6.3±0.48	35.5±4.9	0.54±0.12
August	T_1	29.47±1.0	7.2±0.87	5.6±1.00	36.88±3.22	0.49±0.10
	T_2	31.14±1.9	6.9±0.17	5.29±0.87	34.5±2.8	0.50±0.13
	T_3	31.21±1.0	7.6±0.87	5.91±1.0	34.8±4.45	0.47±0.18

The average GSI of the fish is supposed to augment with the fish's ripeness, and it deters after spawning. The lowest GSI was found during the quiescent stage, but in females, it was found that the GSI was steadily augmented from March to May. Then it was hastily improved in June. The peak GSI was recorded in July for both females and males, indicating that July is the apex breeding season of *C. reba* (Figure 3). These values started to plummet in August, both in males and females. Male gonad weights ranged from 1.68 to 5.16g, with the largest weight being discovered in July and the smallest weight being discovered in March. The reproductive progress of the fish was studied by observing the GSI values as they started going up with fish progression, peaking during maximal ripening, and rapidly dropping after that when the species had been spent (le Cren, 1951).

The highest female GSI was 12.2±1.30% in July, while the lowest was 3.32±0.15% in March. During the study period, a rising trend in weight and GSI (%) was seen monthly with a significant difference (p<0.05). As a result, the gonad weight increased along with the GSI (%) of *C. reba*, reaching a high in July. Jewel et al. (2019), Akther & Akther (2011), Mathialagan and Sivakumar (2012), and Lashari et al. (2007) all came to more or less the same conclusions.

The fecundity was calculated using the Von Vayer method. Significantly different fecundities were estimated at 21,119.44±1,731, 37,805.44±1,509, and 29,468.22 ±1,784 in T_1, T_2, and T_3, respectively. The fecundity of *C. reba* was found to vary significantly (p<0.05) between regimens, and it was highest in T_2, followed by T_3 and T_1 (Table 3). The fecundity range during the research period was within the range of 20,722 to 211,200 eggs, according to Lashari et al. (2007), who previously studied the same species of fish in aquaculture farms in Pakistan.

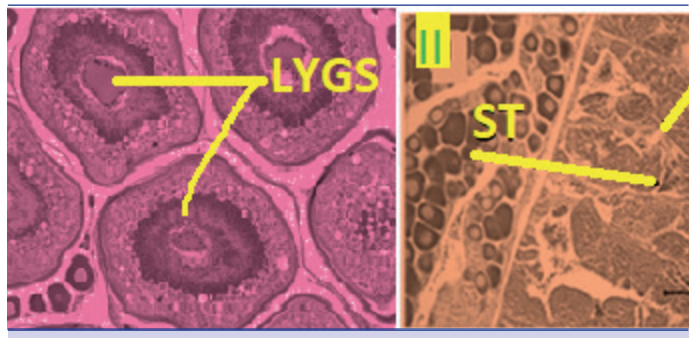

Figure 2. Histological section of *C. reba* showing (i) the ovary with the late yolk granule stage (LYGS) and (ii) the testes with spermatozoa (SZ) and spermatids (ST) stained with hematoxylin and eosin.

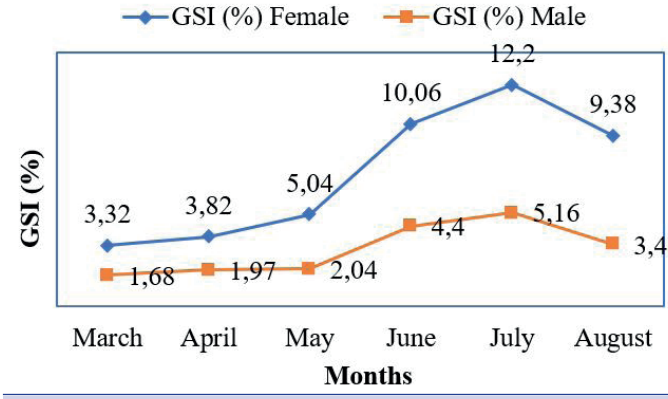

Figure 3. Monthly difference of the gonado-somatic index (GSI %) in males and females of *C. reba*.

From the Baigul Reservoir (U.P.), India, Khan (1986) reported greater fertility of *C. reba* at 22,356 to 437,400 eggs. Jewel et al. (2019) also discovered fecundity (11,542.10 to 53,602.28) with comparable outcomes. The discrepancies between the current findings and those of certain research articles result from elements that have an influence on fish productiveness, such as fish habitat and diet, as well as characteristics like fish size, age, and condition.

For three treatments, separate doses of the hormone were injected into broods. After injecting the proper dose of PG, 7-9 hours are needed for the latency period. The ovulation rate was 68.22±2.11, 75.25±2.43, and 71.12±1.69% in T_1, T_2, and T_3, respectively (Table 3). The significantly highest ($p<0.05$) ovulation rate was found in T_2 (75.25±2.43%) and the lowest was found in T_1 (68.22±2.11%).

It might be because the medication efficiently triggered eggs in the ovary to ovulate. Average fertilization rates (%) of *C. reba* were 60.25±3.05%, 88.88±5.17%, and 75.71±7.27 in T_1, T_2, and T_3, respectively, during the study period, and significantly ($p<0.05$) it was higher in T_2 followed by T_3 and T_1 where 2mg/kg for the male and 4mg/kg for the female of the PG hormone was used on *C. reba*. This was like the discovery made by Sarkar et al. (2004), who did captive breeding of *C. reba* using synthetic estrogen ovaprim and found the high side fertilization rate which ranged from 90 to 95%.

The hatching rate (%) of *C. reba* measured during the study period in T_1, T_2, and T_3 were 87.5±2.25, 88.23±3.27 and 86.67±4.15%, respectively (Table 3). The hatching rate (%) of *C. reba* was higher ($p<0.05$) in T_2 followed by T_1 and T_3. The survival rates of *C. reba* from hatchlings to fry in T_1, T_2, and T_3 were 75.57±2.27, 76.45±2.37, and 76.57±3.04%, respectively (Table 3). In this study, it was found that the survival rate of *C. reba* did not vary significantly ($p>0.05$) among the treatments. Significantly higher ovulation (%) was recorded from T_2 (75.25±2.43) followed by T_3 (68.22±2.11) and T_1 (71.12±1.69). The latency period in the present study was found to be 7-9 hours, whereas the latency period of *Heteropneustes fossilis* was 22–25 hours (Kohli & Goswami, 1987), that of *Clarias gariepinus* was 16–20 hours (Munshi & Hughes, 1991), and 30 hours for *C. stiriatus* (Marimuthu et al., 2001). The higher hatching rate (%) of *C. reba* was in T_2 (88.23±3.27) followed by T_1 (87.5±2.25) and T_3 (86.67±4.15). Das et al. (2022b) reported 61-86% hatching rate in *Nandus nandus* using a dose of PG hormone of 2mg/kg for males and 4mg/kg for females. Our findings comply with the above result. No significant ($p>0.05$) difference in the endurance rate of *C. reba* among the treatments was recorded. Sarkar et al. (2004) found that in the survival rates of *C. reba*, hatching started 10–12 hours after injection. The present findings of the survival rate of *C. reba* from hatchlings to fry were comparable to the findings of the above authors.

The embryonic developmental stages of this fish were observed (Figure 4). Reba's fertilized eggs (Figure 4b) demonstrate meroblastic cleavage within 30–35 min after fertilization (Figure 4c). By administering Gn-RH in *Mystus cavasius*, Ali et al. (2021) observed the first cleavage to a 32-cell formation in 00:35–2:20h, whereas the new research ascertained the first cleavage achieved a 32-cell formation in 00:30–3:20h. At 190–210 minutes after fertilization, the morula phase was acquired (Figure 4c). At that point, the blastoderm had dispersed well over the protein and the embryo was distinguishable. Ali et al. (2021) discovered the very same phase in *M. cavasius*, but much earlier, at 2:20h after fertilization. The same progression was discovered by Nesa et al. (2017) by administering PG extract to *M. cavasius*. The Blastula phase was found in the present experiment (3:50-4:20h after fertilization) (Figure 4d), but Ali et al. (2021) found 3:30-4:00h after

Table 3. Details of the induced breeding of *C. reba* through the use of different doses of PG.

Treatments	Mean brood weight (g)		Latency period (hr)	Hatching time (hr)	Fecundity (%)	Ovulation (%)	Fertilization (%)	Hatching (%)	Survival (%)
	Male	Female							
T_1	102±10.2	118±14.6	7	15	21119.44 ±1731^c	68.22±2.11^c	60.25±3.05^c	87.50 ±2.25	75.57±2.27
T_2	105±6.8	115±11.4	8	16	37805.44 ±1509^a	75.25±2.43^a	88.88±5.17^a	88.23±3.27	76.45±2.37
T_3	103±5.8	116±12.2	9	17	29468.22 ±1784^b	71.12±1.69^b	75.71±7.27^b	86.67±4.15	76.57±3.04

Mean values in a single row containing the same superscript letters do not differ significantly ($p<0.05$).

fertilization in *M. cavasius*. The C-shaped gastrula part (Figure 4e) starts at 6:0-6:40h post-fertilization, whereas Ali et al. (2021) recorded it at 5:0–5:30h after fertilization. The winding motion of the embryo was seen in current research as it unwinds from being encircled over the yolk realm. The tail had already become separate, but the neck remained attached to the yolk sac.

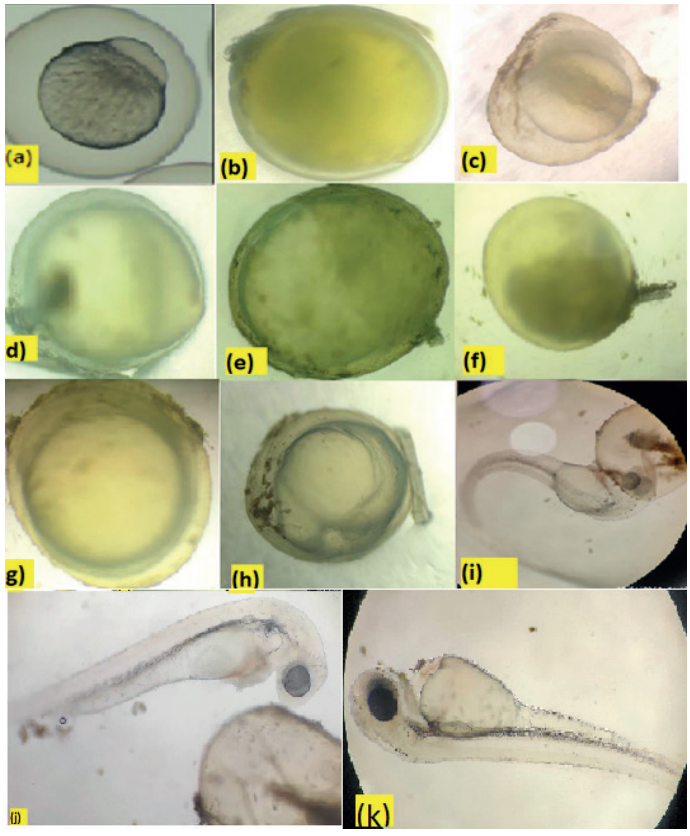

Figure 4. Embryonic developmental stages of *C. reba*: (a) unfertilized egg, (b) fertilized egg, (c) morula stage, (d) Blastula stage, (e) Gastrula stage, (f) Late gastrula stage, (g) Yolk plug stage, (h) Organogenesis, (i) Stage before hatching, (j) Hatching stage, and (k) Hatchling.

The rhythm of the tail is rapid just prior to starting the larval stage and beats around 50-60 times per minute. The larvae began hatching between 10:00 a.m. and 12:00 p.m. The small larvae were easily recognized by their distinct heads, trunks, and rear regions (Figure 3k). Hatching time in *M. cavasius* was 24–25h (Ali et al., 2021); in *Rita rita* hatching was started after 22h of incubation and completed after 30h of incubation at 35°C (Mollah et al., 2011); and in the *C. batrachus* hatching time varied between 23-26 hours at a temperature range of 31–37.5°C (Das, 2002).

CONCLUSION

The current findings could be applied to induce the breeding of *C. reba* to advance hatchery reproduction. This insight may be useful for sustainable strategic planning and maintenance of *C. reba*, which could have an important impact on mitigating the overall nourishment of Bangladesh's villagers. More exploration on nursing, nurturing, and culture of this near-threatened fish at various densities or feeding levels are needed at both on-station and on-farm stages in Bangladesh and throughout this territory to save these fish from extinction or to preserve and restore them.

Conflict of interest: The researchers acknowledge that they have no conflicts of interest.

Ethics committee approval: All procedures used in experiments involving humans and animals (fish) were following the ethical standards of the "Hajee Mohammad Danesh Science and Technology University, Dinajpur" Ethical Committee. All survey participants provided informed consent.

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