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RESEARCH ARTICLE

Spawning biology, breeding, and larval rearing techniques for *Xenentodon cancila* (Hamilton) for aquaculture and recreational use in Bangladesh: The first approach

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Abstract. The study examined *Xenentodon cancila* (Hamilton) breeding biology, reproduction, and larval rearing. In this study, gonadosomatic index (GSI) and fecundity were measured for breeding biology every 15 days. Nine doses of common carp pituitary gland (CPG), human chorionic gonadotropin (HCG), and Ovaprim for breeding were administered, except in the control treatment that was without hormones. Mustard oil cake (D1), powdered feed (D2), and one-day-old live tilapia (*Oreochromis* sp.) larvae (D3) were used to determine larval survival after 28 days. From the end of May to mid-July, the mean GSI value exceeded 15 ± 0.72%, peaking at 16.02 ± 1.28% in June. Mean fecundity ranged from 110 ± 13.34 to 2961 ± 318.57, peaking at the end of June 2019, which indicated that this was the peak breeding season for *X. cancila*. The best spawning was with CPG at 20 mg kg^{-1} fish body weight (BW) and Ovaprim at 1.0 ml kg^{-1} BW, but the control group (B) and the group administered 500 IU kg-1 HCG (H1) did not spawn. Latency and hatching periods were 19-21 and 260-288 h, respectively. The highest fertilization, hatching, and survival rates were 92 ± 4.63%, 69 ± 5.52%, and 66 ± 4.11% with 20 mg kg^{-1} CPG (C2), while the next closest value was recorded with 1.0 ml kg^{-1} Ovaprim (O2). After 28 days, D3 had the highest survival rate (47.5 ± 4.62%), followed by D2 (31 ± 2.42%), and D1 (18 ± 1.54%). The findings will be useful for hatcheries, fish farmers, the ornamental fish sector, and biodiversity protection.

Keywords: *Xenentodon cancila*, breeding performance, hormone, survival rate, larvae

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Introduction

Xenentodon cancila (Hamilton) is an indigenous fish species that is predominantly found in freshwater swift streams, rivers, ponds, lakes, and inundated areas throughout Bangladesh and in various habitats in India, Bhutan, Pakistan, Malaysia, Myanmar, Nepal, Cambodia, Indonesia, Sri Lanka, Thailand, Viet Nam, Laos, and Hawaii (Froese and Pauly 2012). In Bangladesh the common names of this fish include kaikka (Rahman 1989), kakila gar (Al-Mamun 2003), and kankely (Nath and Dey 1989). In the wild, *X. cancila* only consumes insects, tiny fish, and crustaceans; however, in captivity, it will consume live fish (Pethiyagoda 1991, Rainboth 1996). This fish is oviparous, and its eggs have surface tendrils that attach to substrates in the water (Breder and Rosen 1966). *X. cancila* is of both nutritional and medicinal importance. It contains high levels of protein (15%) and is used in traditional medicine to treat nocturnal enuresis in children. It is also in high demand among aquarium fish enthusiasts (Mohanty et al. 2018), and, thus, is a valuable species in the ornamental fish trade. Aquaculture in Bangladesh focuses on breeding and rearing ornamental fish for the ornamental trade, which is a growing industry with increasing demand. Domestically producing indigenous ornamental fish is a promising way to generate export earnings and enhance the country's socioeconomic well-being since ornamental fish keeping is very popular worldwide. So, in this case, this fish can play a unique role.

X. cancila is often caught in small quantities, along with other indigenous fish species. However, obtaining sufficient numbers of fry and fingerlings from natural waters for stocking ponds is a challenge. Moreover, the species is experiencing population declines in its native ranges. To address these issues, induced breeding and larval rearing techniques can be used to produce *X. cancila* fry for commercial and ornamental culture in Bangladesh. The propagation of this particular species has the potential to generate additional revenue streams for hatchery operators and ornamental fish traders. Moreover, it could also lead to the creation of employment opportunities for individuals residing in rural areas, specifically through the management of and cultivation of this species in nurseries. Augmenting the production of this particular species can potentially create an affordable protein source for individuals with limited resources. Additionally, it could have a positive impact on the preservation of fish biodiversity while supporting population sizes and safeguarding the species from extinction.

Implementing induced breeding techniques can potentially serve as a means to investigate the conservation of this fish species in both natural and cultural contexts, thereby mitigating the risk of extinction. Prior to initiating induced breeding, it is necessary to consider the breeding biology and physicochemical water parameters, breeding season, breeding behavior, and sexual characteristics (Paul et al. 2021). Many studies have reported various breeding seasons for *X. cancila* – May to September (Bano et al. 2012), June to July (Subba and Meheta 2012), June to August (Chakrabarti and Banerjee 2015), April to August with peaks in June (Mian et al. 2017, Islam and Dutta 2018). Reported gonadosomatic index (GSI) values range from 6.5 to 14.1 (Mian et al. 2017) and 0.912 to 10.87 (Borthakur 2018), while fecundity ranges are 750-2852 (Bhuiyan and Islam 1990), 300-2,234 (Mian et al. 2017), 144-194 (Borthakur 2018), to 779-2,099 (Islam and Dutta 2018). Gonadal maturity stage is reported to include six ovarian developmental stages (Subba and Meheta 2012) or three ovarian developmental stages (Bano et al. 2012). Induced breeding is a method of artificially inducing natural reproduction in mature fish in captivity using pituitary gland or synthetic hormones. Human chorionic gonadotropin (HCG) and carp pituitary gland (CPG) extract are the most common stimulating agents used in fish breeding. Additional agents that have been reported to induce responses include Ovulin, Ovaprim, Profasi, Pregnyl, and luteinizing hormone releasing hormone (LH-RH) (Minar et al. 2012). Hormonal stimulation aids the release of eggs and sperm at the appropriate times (Jhingran and Pullin 1985). *X. cancila* usually reproduces on its own, but it needs to be cared for in a certain way so that natural reproduction can happen, especially during the monsoon season. It is thought that *X. cancila* only lays eggs at certain times of the year in the wild, and to the authors' knowledge, it has never been successfully breed in captivity in Bangladesh. Prior research on *X. cancila* breeding biology is essential for efficient fisheries management and breeding programs. Therefore, the purpose of this study was to improve hormone-induced breeding methods for *X. cancila* and create management strategies for fry nurseries.

Materials and Methods

Study Area

The study was carried out at the Bismillah Fish Seed Production Center and Farm located in Nangalkot, Cumilla, Bangladesh, with the geographical coordinates of 23°10'N 91°12'E. *X. cancila* fry were obtained from the wild and subsequently introduced into an experimental pond located on the premises of the aforementioned farm for further processing. Mature fish were transported from the pond to the fish seed production facility and subjected to a bath treatment in 5 ppt saline water for approximately 30 minutes prior to subsequent processing.

Brood pond preparation, rearing, and collection

A brood pond with an area of 800 m^2 and a water depth between 0.7-1.0 m was prepared for the experiment. The pond was cleaned of predators and unwanted fish species by draining the water and drying, followed by an application of rotenone to kill any remaining unwanted fishes and aquatic insects. Aquatic vegetation was manually removed, and lime was applied at a rate of 1 kg per 40 m^2. After seven days, 7 kg of cow dung per was added as organic fertilizer. Additionally, 100 g of urea and 70 g of triple superphosphate (TSP) per 40 m^2 of the pond were also used as inorganic fertilizers. Successfully induced breeding requires the proper care of male and female brood fish. The fry were reared at a density of 0.62 fish m^{-2} in the brood rearing ponds. Live flying barbs (*Esomus danricus*) were supplemented in the brood fish diet at a rate of 3% of the total body weight of *X. cancila*. Regular monitoring of fish development was performed until the fish reached maturity. Healthy, sexually mature brood fish were chosen to ensure successful induced breeding. Secondary sexual traits identified mature males and females. Sixty healthy, active *X. cancila* brood fish were carefully collected from the brood pond for breeding at a 1:1 sex ratio.

Monitoring water quality parameters

Water quality parameters influence induced breeding and the rates of spawning, fertilization, and hatching. At four hours intervals, physicochemical water parameters including dissolved oxygen (mg l^{-1}), water temperature (°C), pH, and total dissolved solids (TDS) were measured in spawning tanks of every treatment and also in the pond in which the larvae were reared.

Experimental design

We chose CPG, HCG, and Ovaprim for the breeding program for *X. cancila* in Bangladesh due to their widespread availability, cost-effectiveness, and popularity among hatchery owners. Three distinct hormone types were utilized in the process of induced breeding of *X. cancila*, namely CPG from United Agro Fisheries Ltd, Bangladesh; HCG manufactured by Shandong Ginye Biotech Co., Ltd., Chaina; and Ovaprim (sGnRHa+ dopamine), which is a synthetic analog of gonadotropin-releasing hormone (GnRH) and a dopamine antagonist. GnRH plays a crucial role in regulating reproductive hormones in fish, while the dopamine antagonist helps to enhance the effectiveness of GnRH by blocking the inhibitory actions of dopamine. The manufacturer is Syndel, USA.

This study employed a fixed regimen of three doses for each type of hormone. For each hormone dose, three pairs of brood fish–three male and three female were selected, with a fixed sex ratio of 1:1 (M:F). In the study, the doses of CPG were 15 (C1), 20 (C2), and 25 (C3) mg kg^{-1} fish body weight (BW); HCG was 500 (H1), 1000 (H2), and 1500 (H3) IU kg^{-1} BW; and Ovaprim was 0.5 (O1), 1.0 (O2), and 1.5 (O3) ml kg^{-1} BW (see Table 1). A spawning pool was set up in a cement tank measuring 0.9 m × 1.8 m

Table 1
Hormone protocols for *X. cancila* induced breeding. Sex ration: 1:1, N = 3

Group/hormone	Treatment	Female weight (g)	Male weight (g)	Female dose	Male dose
Control					
	B	19.7±2.2	29.1±3.5	No	No
CPG (mg kg^{-1})					
	C1	21.3±1.7	27.4±2.8	15	8
	C2	19.1±2.1	26.8±4.2	20	10
	C3	21.2±1.6	28.2±3.2	25	12
HCG (IU kg^{-1})					
	H1	20.7±2.1	28.6±3.6	500	250
	H2	17.8±1.5	27.9±2.8	1000	500
	H3	18.8±1.3	27.4±3.1	1500	750
Ovaprim (ml kg^{-1})					
	O1	19.3±1.1	25.4±2.9	0.5	0.25
	O2	21.1±2.1	26.9±2.5	1.0	0.5
	O3	20.7±1.5	25.3±3.1	1.5	0.75

CPG = carp pituitary gland, HCG = human chronic gonadotropin

× 0.3 m (W × L × H). The water system's inlet and outlet were regulated. *Ipomea aquatic*, a type of emergent aquatic weed, was utilized as a breeding substrate in the spawning pool. In order to facilitate breeding, the brood fish were collected from the experimental pond in the early morning and then placed in a brood tank measuring 2.1 m × 3.0 m × 0.30 m for acclimation, which lasted for 12 h. The fish were anesthetized with a solution of 2 ml of 2-phenoxy ethanol in 20 l of water prior to injection with a 24-gauge needle in the upper dorsal region. A single dose of each hormone was administered to both male and female brood fish, but the doses for males were half those for the female.

Fry rearing in cages

A test period of 28 days was conducted for rearing fish in cage nets in a pond. Six cages were used for three different feeding treatments, and each treatment had two replications. The first feeding treatment was mustard oil cake feed (D1), the second was powdered complete feed (D2) with a crude protein content of 35%, and the third was one-day-old live larval tilapia (D3). The larvae, aged 2.5 d, were transferred into the silken cotton cages (1.2 × 1.8 × 0.6 m) for rearing. The stocking density was 400 larvae per cage. On the first day, the feed dose for each treatment was 15% of fish body weight, and the fish were fed two times daily. *Pistia stratiotes* was used in the cages to provide shelter for the larvae during the study period.

X. cancila GSI and fecundity

Information on the breeding biology of *X. cancila* was obtained by determining GSI and fecundity. Fish specimens were gutted with blunt instruments and soft brushes to avoid any accidental injury or cuts. Gonads were removed and preserved in a 10% formalin solution. A digital balance with an accuracy of 0.01g (EAGems-B00Z5KETD4) (Shimadzu UX320G) was used to weight the gonads. To calculate the GSI, the weight of each female fish was recorded, and the gonads were carefully removed and weighed on an electronic balance after drying off any extra fluid with paper. The GSI was calculated using the method by Afonso-Dias et al. (2005).

$$Gonadosomatic\ index\ (GSI) = \frac{gonad\ weight}{body\ weight} \times 100$$

The weights of entire ovaries were measured initially to estimate fecundity. Then samples were collected from the anterior, central, and posterior regions of the ovaries, and the total number of eggs from each sub-sample was counted. Behera et al. (2010) developed an equation to predict the total number of eggs in each subsample.

$$F = \frac{gonad\ weight \times number\ of\ eggs\ in\ subsample}{sub-sample\ weight}$$

Breeding parameters of *X. cancila*

Breeding performance of *X. cancila* was determined with the following equations,

a) Latency period (h): Time interval from the first injection to ovulation (Kucharczyk et al. 2005)

b) Number of female fish laying eggs (%) (Holden et al. 1971):

$$\frac{number\ of\ female\ fish\ laying\ eggs}{total\ number\ of\ female} \times 100$$

c) Fertilization rate (%) (Behera et al. 2010):

$$\frac{number\ of\ fertilized\ eggs}{total\ number\ of\ eggs} \times 100$$

d) Hatching rate (%) (Islam et al. 2011):

$$\frac{number\ of\ hatchlings}{total\ number\ of\ fertilized\ eggs} \times 100$$

e) Survival rate (%) (Alam et al. 2006):

$$\frac{number\ of\ hatching\ survivors}{total\ number\ of\ hatching} \times 100$$

Data analysis

All treatments were evaluated using means plus standard error (SE). SPSS 17.0 for Windows was used to statistically analyze the experimental calculations. One-way analysis of variance (ANOVA) was used to compare significant experimental observations. Duncan's New Multiple Range Test (DMRT) was used to analyze significant variations among means to determine significantly significant findings ($P < 0.05$).

Results

X. cancila gonadosomatic index and fecundity

Throughout the study period from October 2018 to September 2019, a total of 360 fish specimens were collected (15 specimens per sampling) from the experimental pond to determine GSI and fecundity. The GSI values of this species were negligible in this study from the end of November 2018 to the end of January 2019. The GSI value of *X. cancila* grew chronologically from the middle of February 2019 to the end of June, and then the value declined from the middle of July until the end of January (Fig. 1). From the end of May to mid-July, the mean GSI value was 15 ± 0.72%, and the highest mean value was 16.02 ± 1.28% in June (Fig. 1). These findings suggest that the highest incidence of spawning occurs during the month of June.

Due to the diminutive size of the eggs, the fecundity of *X. cancila* could not be observed with the naked eye from the beginning of October 2018 to the end of February 2019 (Fig. 2). Mean fecundity ranged from 110 ± 13.34 to 2961 ± 318.57, and the highest fecundity of 2961 ± 318.57 was observed at the end of June 2019, which may indicate that the optimum reproductive season for this species is June (Fig. 2).

X. cancila latency period

The findings indicated that the spawning period of *X. cancila* varied between 18-21 h across various treatments (Fig. 3). The study indicated that the latency period was comparatively longer with the treatment based on HCG, whereas it was relatively shorter with Ovaprim. The latency period in the B (control) and H1 treatments was 0 h, which meant that reproduction did not occur.

Number of female fish laying eggs

In June 2019, trials were conducted on *X. cancila* brood fish with the administration of hormones.

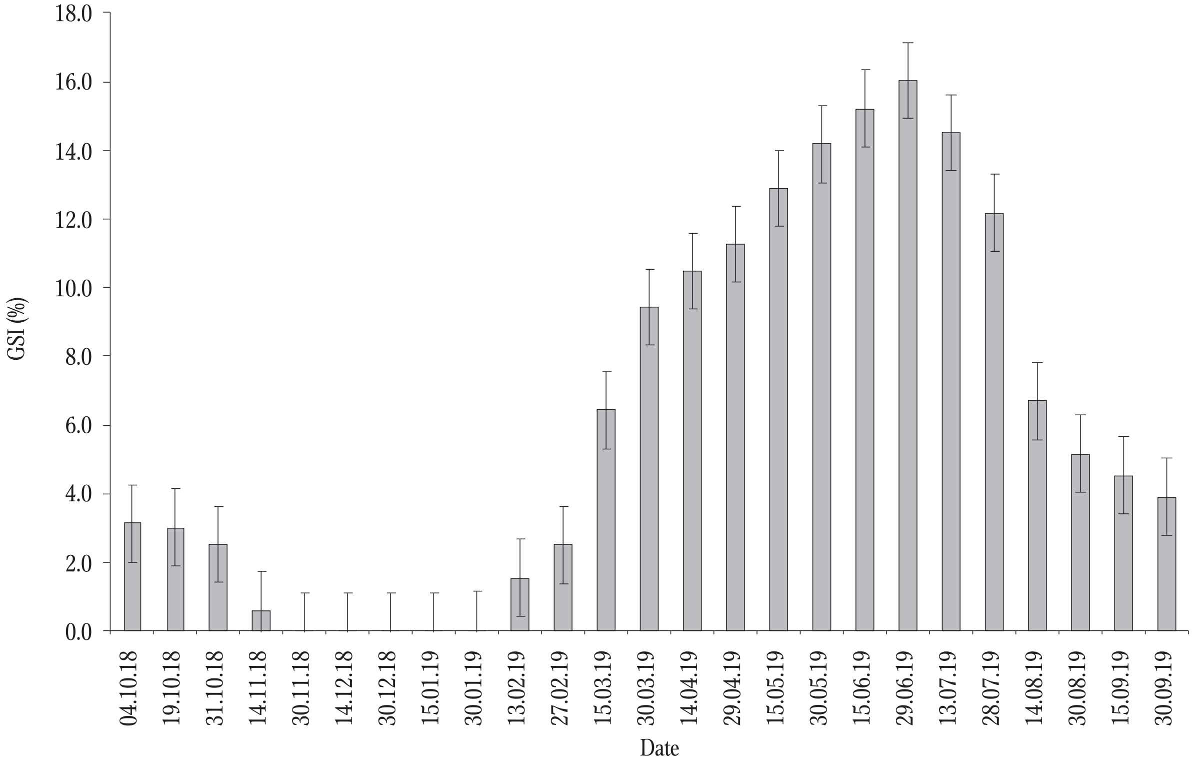

Figure 1. Average *X. cancila* GSI values throughout the study period.

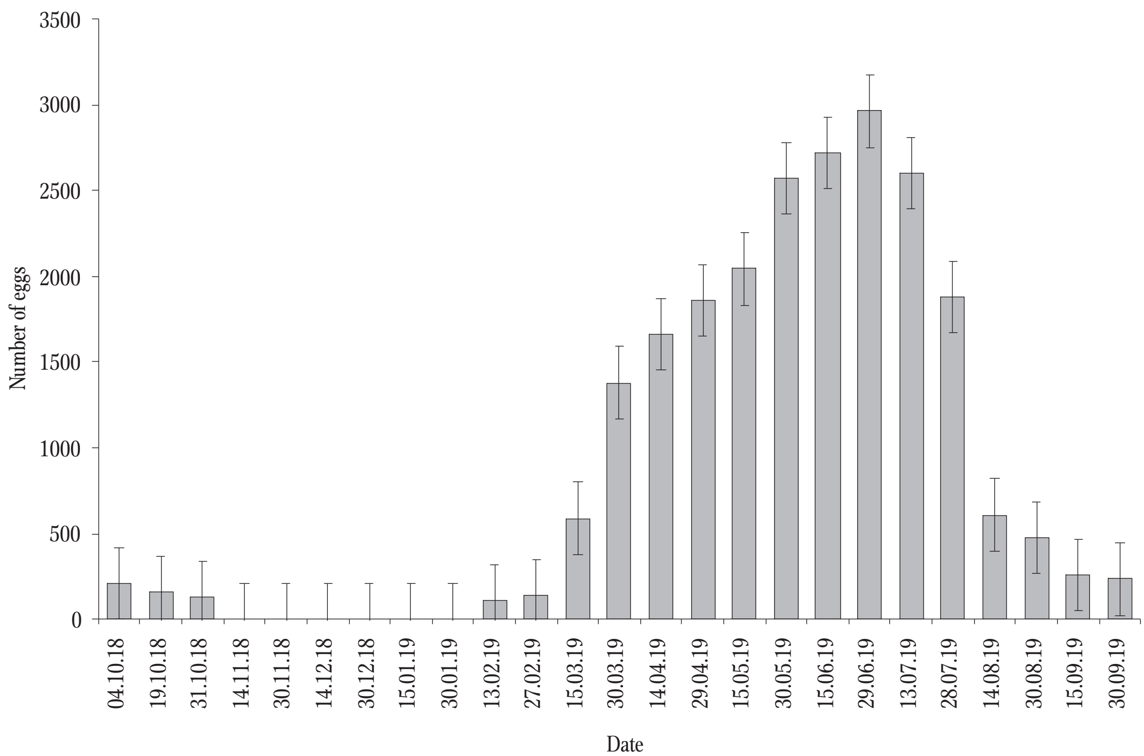

Figure 2. Average *X. cancila* fecundity in different months.

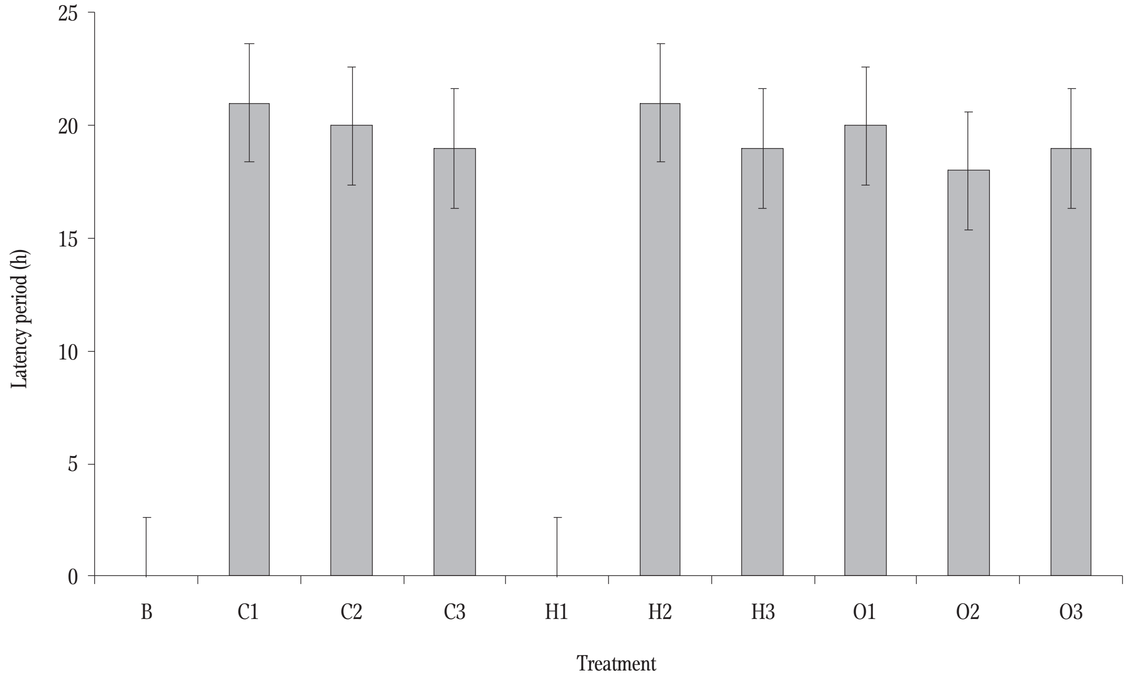

Figure 3. *X. cancila* latency period with different treatments.

CPG, HCG, and Ovaprim were administered in nine distinct treatments. The outcomes of these treatments were characterized by 0-90% female fish laying eggs. The percentages of female fish laying eggs in the different treatments were C2 (98%) and O2 (97%), followed by C3 (67%), O1 (60%), H3 (60%), C1 (33%), O3 (30%), and H2 (30%). The fish in treatments B and H1 did not spawn (Table 2).

X. cancila fertilization rates

To ensure the safety of the *X. cancila* eggs, the brood fish were removed from the spawning pool (incubator) after spawning was complete. The average fertilization rates ranged from 67 ± 2.31% to 92 ± 4.63% across different treatments conducted in June 2019 (Fig. 4). The doses of 20 mg kg^{-1} CPG (C2) and 1.0 ml

Table 2
Number of females laying eggs with different treatments in June 2019

Hormone Dose	Hormone Type	Treatment	No. of females laying eggs (%)	Remarks
Control	No	B	0	No visible eggs
15 mg kg^{-1}	CPG	C1	33	Visible eggs
20 mg kg^{-1}		C2	98	Visible eggs
25 mg kg^{-1}		C3	67	Visible eggs; 2 males and 1 female died
500 IU kg^{-1}	HCG	H1	0	No visible eggs
1000 IU kg^{-1}		H2	30	Visible eggs
1500 IU kg^{-1}		H3	60	Visible eggs
0.5 ml kg^{-1}	Ovaprim	O1	60	Visible eggs
1.0 ml kg^{-1}		O2	97	Visible eggs
1.5 ml kg^{-1}		O3	30	Visible eggs; 2 females and 3 males died

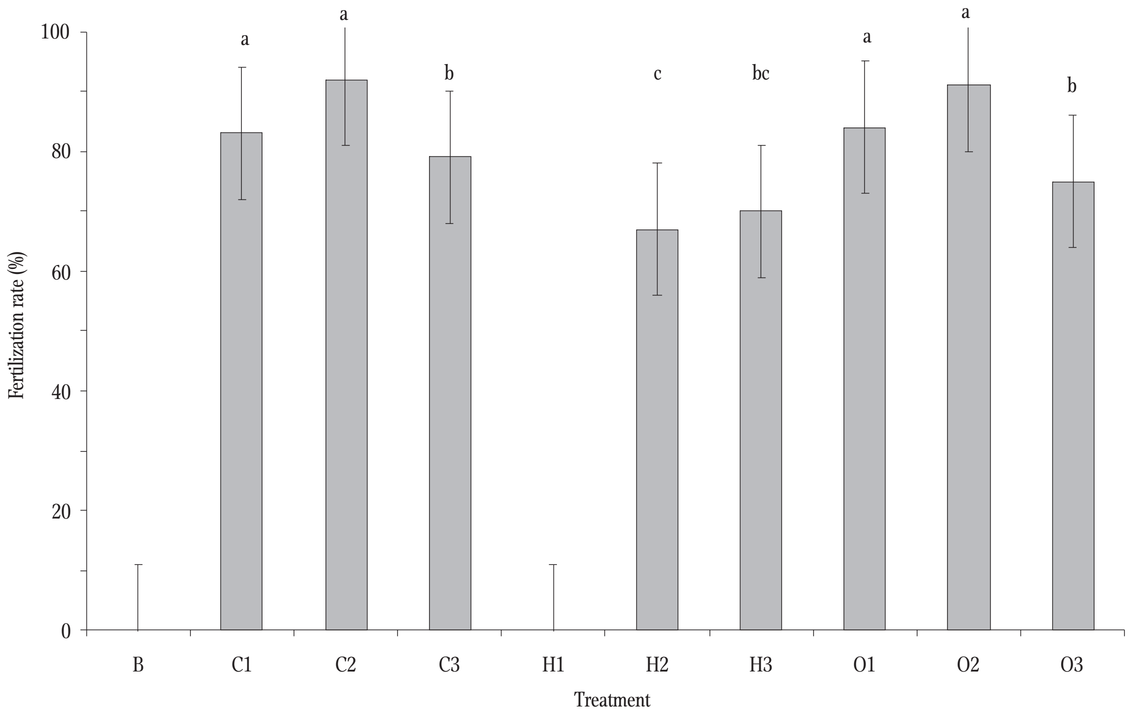

Figure 4. Fertilization rates among treatments. Treatments with different letter indices differ statistically (ANOVA, P < 0.05).

kg^{-1} Ovaprim (O2) were the most efficacious for *X. cancila* fertilization rates (Fig. 4), followed by O1 (84 ± 6.14%), C1 (83 ± 5.88%), C3 (79 ± 6.92%), O3 (75 ± 7.17%), H3 (70 ± 4.24%), and H2 (67 ± 6.82%). There were no significant (P > 0.05) differences among treatments C1, C2, O1, O2, and, C3, O3, H3, but there were significant differences between H2 and the other treatments.

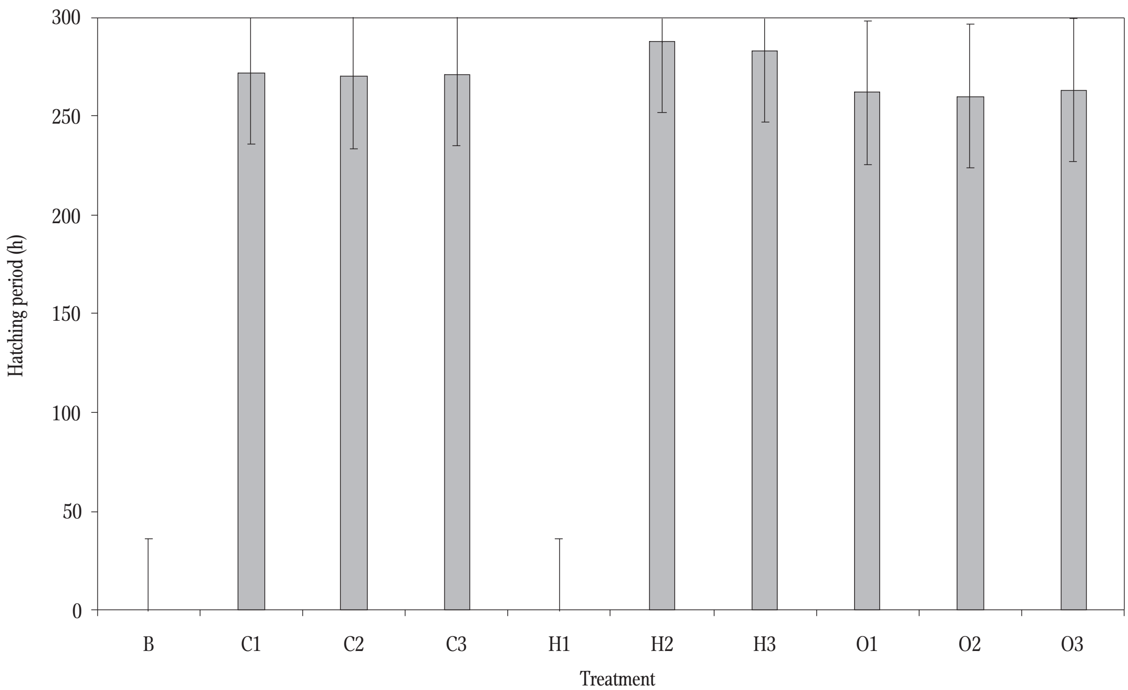

Figure 5. Hatching periods with different treatments.

X. cancila hatching period

The results of the experiment indicated that the mean duration of *X. cancila* hatching was between 260-288 h across various treatments (Fig. 5). The spawning behavior of the brood fish involved the deposition of eggs onto submerged aquatic vegetation to which they attached and underwent embryonic development prior to hatching. The present study found a significant reduction in the duration of the hatching period in the Ovaprim treatment group compared to the CPG and HCG treatment groups.

X. cancila hatching rate

The average *X. cancila* hatching rate ranged from 45 ± 2.08% to 69 ± 5.52% across different treatments (Fig. 6). Hatching rates in descending order were as follows: 69 ± 5.52% (C2); 68 ± 3.87% (O2); 63 ± 4.29% (O1); 57 ± 3.17% (C3); 56 ± 4.51% (C1); 52 ± 2.68% (O3); 51 ± 3.59% (H3); 45 ± 2.08% (H2). ANOVA showed a significant ($P < 0.05$) difference in nine doses of CPG, HCG, and Ovaprim (Fig. 6).

X. cancila larval survival rates in incubators

Throughout the study, the larvae in the incubator were not supplied with any feed. The *X. cancila* larvae were incubated for 2.5 days, and survival rates were monitored every four hours. The mean survival rates were from 42 ± 2.53% to 66 ± 4.11% across treatments (see Fig. 7). The CPG treatment (C2) at a dose of 20 mg kg^{-1} resulted in the highest survival rate observed. As anticipated, there was a negative correlation between the duration of time and the survival rate.

Incubator water quality parameters

During the experimental period, the ranges of water quality parameters in the incubators were as follows: temperature – 27.8 ± 0.76-28.9 ± 0.58°C; dissolved oxygen – 7.6 ± 0.21-7.9 ± 0.11 mg l^{-1}; pH – 7.1 ± 0.13-7.3 ± 0.13; TDS (ppm) 325 ± 12.2-376.54 ± 20.8 (Table 3).

X. cancila fry survival rate in cages

Each cage was stocked with 400 larvae (2.5 days old). During the larval rearing stage, the survival

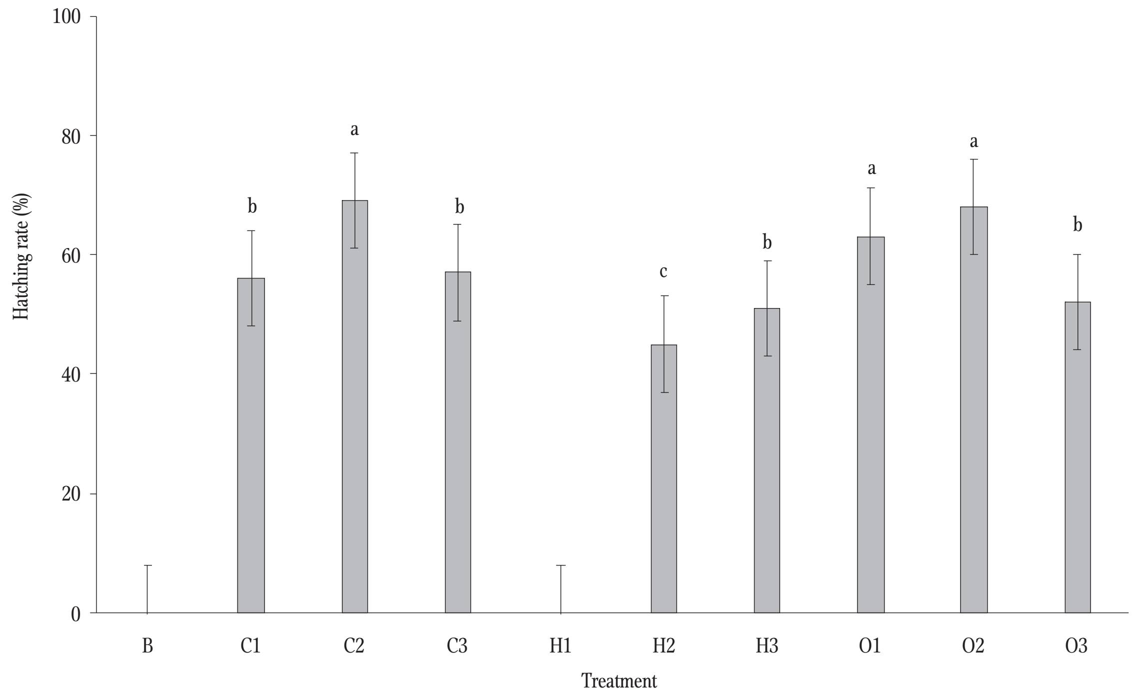

Figure 6. *X. cancila* hatching rates with different treatments. Treatments with different letter indices differ statistically (ANOVA, $P < 0.05$).

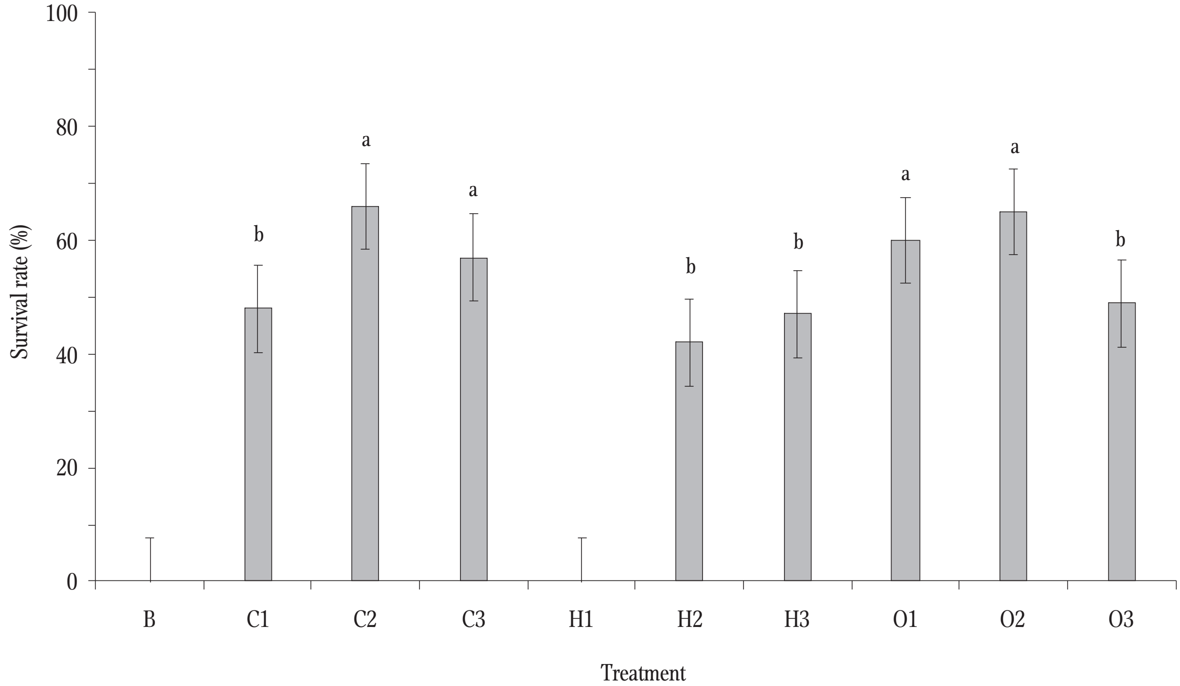

Figure 7. *X. cancila* survival rates with different treatments. Treatments with different letter indices differ statistically (ANOVA, P < 0.05).

Table 3
Incubator water quality parameters for *X. cancila* breeding

	Treatments									
Parameters	Cont. (B)	C1	C2	C3	H1	H2	H3	O1	O2	O3
Temp. (°C)	28.8±0.59	28.7±0.74	28.5±0.43	28.1±0.76	28.9±0.54	28.2±0.46	28.5±0.78	27.8±0.76	28.9±0.58	27.5±0.43
DO (mg l^{-1})	7.9±0.11	7.8±0.24	7.8±0.28	7.6±0.32	7.6±0.21	7.7±0.34	7.7±0.49	7.6±0.32	7.7±0.21	7.8±0.28
pH	7.1±0.13	7.2±0.18	7.2±0.13	7.3±0.21	7.2±0.19	7.3±0.27	7.3±0.15	7.2±0.21	7.3±0.19	7.3±0.13
TDS (ppm)	325±12.2	337±25.2	346.54±20.8	328.35±16.7	350.98±21.7	373.77±19.2	360.23±14.2	357.35±16.7	362.98±21.7	376.54±20.8

rates in each treatment were initially 100%. With time and as various feed types were administered in the different treatments, decreased survival rates were observed. Statistically significant differences (P < 0.05) were noted among the treatments. Following a 28-day period, treatment D3 had the highest survival rate (47.5 ± 4.62%), followed by D2 (31 ± 2.42%) and D1 (18 ± 1.54%). During the larval rearing period in the pond, the physicochemical parameters of the water were as follows: the water temperature averaged 29.6 ± 1.25°C, pH levels were 7.4 ± 0.31, and the dissolved oxygen (DO) concentration was 6.4 ± 0.83 mg l^{-1}.

Discussion

The aquatic biodiversity of Bangladesh is abundant, but some indigenous fish species are currently endangered by natural disasters and human activities, which makes them less available for consumption (Alam et al. 2015, Barman et al. 2021). This not only affects the nutritional value of these fishes for consumers, it also affects their value as ornamental fish. *X. cancila* fry and fingerlings are not sufficiently abundant in nature for culture and ornamental purposes, which is why it is important to optimize captive breeding and to develop fry rearing protocols for this species.

GSI and fecundity are crucial parameters in the study of the reproductive biology of fishes. GSI is an indicator of ovarian maturation and the spawning biology of fish species, making it an essential tool for assessing reproductive seasonality. The findings of the current study revealed that the highest GSI values were in June, while the lowest were in February, with no GSI values detected from November to January. These results are consistent with previous studies conducted by Rahman et al. (1997), Dey et al. (2016), and Mian et al. (2017). Gradual decreases in GSI values from August to January (winter season) in this study may be influenced by environmental temperature fluctuations (Pedro et al. 2020) and metabolic changes during the reproductive phase (Ladisa et al. 2022). However, GSI values gradually increased from February, peaking in June, which suggested that gonads were developing, and June is when most species begin their spawning seasons. While June had the highest peak GSI value, it was also higher in April, May, and July. Therefore, the spawning season for this fish is likely to be from April to July, as is confirmed by other researchers who report similar findings for this species (Bano et al. 2012, Subba and Meheta 2012, Chakrabarti and Banerjee 2015, Mian et al. 2017, Islam and Dutta 2018).

Fish fecundity is affected by several factors, including fish age, size, species, season, and food availability (Mekkawy and Hassan 2011). Fecundity was high in the peak season compared to other months, and similar findings are reported by Bhuiyan and Islam (1990), Mian et al. (2017), and Islam and Dutta (2018). High fecundity and GSI values are important parameters of breeding biology that can indicate appropriate breeding times, and this information can be applied in captive breeding of fish species.

Latency periods, fertilization rates, hatching rates, incubation periods, and survival rates depend on factors such as the doses and types of hormones administered (Paul et al. 2021), sex ratio (Paul et al. 2021, Yeasmine et al. 2021), and water physicochemical parameters (Basudha et al. 2017). The present study evaluated the most effective hormone for inducing *X. cancila* spawning. The results of the treatments with CPG, Ovaprim, and HCG differed (fertilization rates, hatching rates, and spawn survival rates) in the present study. Among the treatments, the best breeding parameters were noted with CPG (20mg kg^{-1}) and Ovaprim (1.0 ml kg^{-1}). In the control (B) and H1 (500 IU kg^{-1} HCG hormone) groups, no ovulation was noted in captivity. In this study, the *X. cancila* latency period was longer compared to other species such as *Labeo rohita* (Hamilton), *Hypophthalmichthys molitrix* (Val.), and *Cyprinus carpio* L. (Sharif et al. 2022). A comparison of results is presented in Table 4.

The incubation period of *X. cancila* was comparatively longer than that of other fish species. The current study revealed that the rate of embryonic development in fertilized eggs was comparatively slower in comparison to other cyprinid species like *C. carpio*. The fertilized eggs attached to submerged aquatic plants on which they developed. Throughout the developmental phase, a consistent flow of water was sustained in the cement tanks. Eggs were not collected in the present study to observe the embryonic development stages. The administration of Ovaprim and HCG resulted in variations in the duration of shorter or longer hatching periods, and the hatching periods for *X. cancila* were longer than those reported in previous studies conducted by Sridhar et al. (1998), Sayeed et al. (2009), Islam et al. (2017), and Paul et al. (2021).

Water temperature plays a critical role in egg incubation, and it influences embryo development, hatching times, hatching rates, and fish viability (El-Hakim and El-Gamal 2009, Sugiarto et al. 2015). Studies by Alikunhi et al. (1962) and Rana (1990) report a correlation between temperature and hatching time, while Hoar and Randall (1969) found an inverse relationship between temperature and hatching rate. The average temperature in the incubators during June (28.4 ± 0.27°C) was conducive to fish breeding, which is consistent with findings by Polo et al. (1991), Sugiarto et al. (2015), and Radonić et al. (2005). Dwivedi and Zaidi (1983) recommend maintaining DO concentrations between 5-6 mg l^{-1} in incubators for successful fish spawning, hatching, and spawn survival. In the current study, DO concentrations were kept above this range and were similar to

Table 4
Comparisons of breeding several fish species with hormones

Species	Hormone	Fertilization rate (%)	Hatching period (h)	Hatching rate (%)	Survival rate (%)	References
X. cancila	CPG, HCG, Ovaprim	67-92	260-288	45-69	42-66	Present study
Mystus vittatus	CPG, HCG, Ovaprim	38.9- 92.3	21-23	27.8-92.6	14-69	Paul et al. 2021
Puntius sarana	Ovatide	90.5	15-16	75.39	51-52	Udit et al. 2014
Lepidocephalicthys guntea	CPG	60-78	72	49-65	-	Sayeed et al. 2009
Mystus gulio	HCG	50-74	-	55-75	48-52	Alam et al. 2006
Hemibagrus menoda	Ovatide	90-97	21-22	76-95	80-85	Hasan et al. 2021
Cirrhinus reba and *Labeo bata*	CPG	87.88 and 81.47	-	86.19 and 75	-	Rahman et al. 2007
Channa punctatus	Ovaprim	78.3-97.6	-	84.3-96.3	-	Marimuthu and Haniffa 2010
Acanthocobitis botia	Ovaprim	48.57-73.13	-	23.43-54.13	28.15-62.58	Srivastava et al. 2014
Mastacembelus armatus	CPG	15-95	-	5-62	-	Ali et al. 2018
Macrognathus pancalus	Ovasis	84-96	-	86-92	-	Borah et al. 2020
Glossogobius giuris	CPG	51-92	35-48	52-91	-	Yeasmine et al. 2021

those in previous studies by Dey et al. (2015) and Basudha et al. (2017). The pH range in the present study was within standard parameters and was consistent with findings by Dey et al. (2015) and Basudha et al. (2017). However, the average TDS in the current study ranged from 325 ± 12.2 to 376.54 ± 20.8 ppm, which exceeded levels recommended by Davis (1993) and Bashuda et al. (2017).

When induced breeding of fish is successful, rearing larvae is a crucial strategy for effective fish farming (Mahfuj et al. 2012). The first feeding of larvae is important for growth and survival. For larval rearing, boiled egg yolk as an initial feed is applied for the first three to five days in most freshwater carp species in Bangladesh (Jhingran and Pullin 1985, Mumtazuddin and Khaleque,1987). After 28 days, the highest survival rate was noted when the larvae were fed one-day-old larval tilapia, followed by complete powder feed and mustard oil cake. A statistically significant difference (was noted in survival among the treatments. Different types of feeds, water quality, and live feed may affect larval survival rates. Egg yolk-fed *Lepidocephalichthys guntea* (Hamilton) larvae had the highest survival rate of up to 50% (Sayeed et al. 2009). Formulated diets may reduce water quality and tank water parameters, impacting early larval development and survival (Abi-Ayad and Kestemont 1994). Certain freshwater fish species (*L. rohita*, *C. carpio*, and *Ctenopharyngodon idella* (Val.)) were given artificial diets from the first feeding and had better survival and growth (Jhingran and Pullin 1985, Legendre et al. 1995, Carvalho et al. 2006). The combination of live and formulated feed has been found to yield superior larval survival of carnivorous species when compared to natural feed (Giri et al. 2002). Since *X. cancila* is classified as carnivorous, the larvae exhibited the highest rate of survival when fed live larvae in the present study.

Conclusion

X. cancila, a freshwater species of both ornamental and nutritional value, is facing extinction in

Bangladesh from natural and anthropogenic factors. It is crucial for government agencies, non-governmental agencies, and associated institutions to disseminate information to raise awareness about the importance of this species and factors that threaten it with extinction. Fry produced through induced breeding techniques can help protect this species against extinction in nature. The effectiveness of induced breeding depends on having full knowledge of a species' biology, breeding behavior, fecundity, fertilization, and the availability of competent technicians and proper hatchery facilities. Appropriate doses for female *X. cancila* were determined to be 1.0 ml kg^{-1} for Ovaprim and 20 mg kg^{-1} for CPG. Acceptable larval survival rates were noted with one-day-old live larval tilapia feed. Additionally, conducting regular research will lead to improved breeding and culture protocols for this species. Standardized doses and demonstration centers can further enhance breeding and hatching techniques for this species and meet more significant demands for quality seed in Bangladesh.

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