Turk. J. Fish.& Aquat. Sci. 25(6), TRJFAS26826
https://doi.org/10.4194/TRJFAS26826

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Optimizing Hormonal Induction and Water Quality Parameters for Enhanced Reproductive Success in Grass Carp (*Ctenopharyngodon idella*)

Shyamal Kumar Paul[1], Kazi Faridul Hasan[2], Nadia Sultana[3], Debasish Saha[1], Srijan Sarker[4], Bhakta Supratim Sarker[1,*]

[1]Department of Fisheries and Marine Science, Faculty of Science, Noakhali Science and Technology University, Noakhali 3814, Bangladesh.
[2]Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.
[3]Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.
[4]Department of Fisheries, Matsya Bhaban, Ministry of Fisheries and Livestock, GoB, Bangladesh.

How to Cite

Paul, S.K., Hasan, K.F., Sultana, N., Saha, D., Sarker, S. Sarker, B.S. (2025). Optimizing Hormonal Induction and Water Quality Parameters for Enhanced Reproductive Success in Grass Carp (*Ctenopharyngodon idella*). *Turkish Journal of Fisheries and Aquatic Sciences*, *25(6)*, *TRJFAS26826.* https://doi.org/10.4194/TRJFAS26826

Article History
Received 16 September 2024
Accepted 15 January 2025
First Online 07 February 2025

Corresponding Author
E-mail: sarkerbs@nstu.edu.bd

Keywords
Gonadosomatic index
Captive breeding
Ovulation
Fertilization
Hatching

Abstract

This study optimizes hormonal induction techniques and water quality parameters to improve reproductive success in Grass Carp (*Ctenopharyngodon idella*). Conducted over a year at Bismillah Fish Seed Production Center, Bangladesh, key reproductive indicators including gonadosomatic index (GSI) and hepatosomatic index (HSI) were monitored. Peak values were observed in March, with females showing a GSI of 21.48±2.69% and an HSI of 8.03±1.83%, and males at 1.37±0.38% (GSI) and 4.68±0.23% (HSI). Six hormonal treatments (CPG, hCG, Ovupin, and combinations) were tested. Ovulation rates ranged from 47% to 95%, with Ovupin inducing the shortest ovulation period (5.48 hours) and hCG the longest (7.18 hours). Fertilization rates varied from 65.74% to 93.48%, with CPG (T1) achieving the highest fertilization (93.48±5.83%) and hatching rate (89.36±7.26%). Larval survival rates after 48 hours were highest with CPG (73.9±3.79%) and lowest with hCG (32.5±2.14%). Optimal water quality parameters contributed to successful breeding. The study concludes that CPG and Ovupin + CPG are the most effective protocols for enhancing Grass Carp reproduction.

Introduction

The grass carp (*Ctenopharyngodon idella*), a widely cultivated herbivorous freshwater fish valued globally for aquaculture which was introduced to Bangladesh from Hong Kong in 1966 and Japan in 1979 to control aquatic weeds (Elder & Murphy, 1997; Rahman, 2005).However, its rapid growth rate and affordability soon made it a favorite among farmers and consumers alike (Rahman, 2005). To meet the rising market demand, hatchery operators turned to induced breeding techniques to produce viable fry efficiently.

Achieving successful induced breeding in *C. idella* requires a deep understanding of both biological and environmental factors. Critical aspects include the spawning season, maturity based on length and weight, and reproductive biology indicators such as the Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI), ovum diameter, and age at maturity. The breeding season of grass carp varies by region, occurring from April to June in Egypt (El-Gamal et al., 2021), in May in the USA (Carlos, 2016), from June to August in Japan (Shireman & Smith, 1983), and from March to May in Bangladesh (Talwar & Jhingran, 1991), with water temperature playing a pivotal role in these timings.

Water temperature is particularly crucial, with recommended ranges between 20 – 30°C for successful spawning and sexual maturity (Kucharczyk et al., 2016). Notably, grass carp can spawn at temperatures as low as 15°C (Cudemore & Mandrak, 2004), while optimal

spawning typically occurs above 27°C (Chilton & Muoneke, 1992). Grass carp generally reach sexual maturity within 2 to 4 years (Kamilov & Komrakova, 2003; Shireman & Smith, 1983), with lengths varying from 53 cm in males to 62 cm in females (El-Gamal et al., 2019). Sexual maturity often differs from maximum size or weight, making GSI and HSI vital measures for assessing breeding readiness (Bozkurt & Öğretmen, 2012; Kamilov & Komrakova, 2003; Elder & Murphy, 1997).

Induced breeding often involves the use of various hormones such as Carp Pituitary Gland (CPG) (Salman et al., 2023; Biswas et al., 2021; Jha & Neupane, 2019), Human Chorionic Gonadotropin (hCG) (Chakraborty, 2022; El-Gamal et al., 2019), LHRH-A (Jha & Neupane, 2019; FAO, 2009), and ovupin /ovaprim (S-GnRH analog and Dopamine antagonist) (Aktar & Islam, 2015; Rashid et al., 2014; Naeem et al., 2011). These hormones play a significant role in influencing spawning rate, latency period, fertilization rate, and larval survival (Chakraborty, 2022; Abilov et al., 2022; Paul et al., 2021). Influence of different hormones and their combinations on the final maturation of gametes and reproductive efficiency (Król et al., 2024; Nowosad et al., 2023; Kucharczyk et al., 2019). Additionally, environmental factors such as water temperature (Toomey et al., 2023; Shaddoud et al., 2023), dissolved oxygen (Rashid et al., 2014), pH (Sapkale et al., 2011; Gao et al., 2011), water hardness (Rach et al., 2010), and metal content (Gárriz & Miranda, 2020) also critically affect fertilization rates, hatching periods, and larval survival.

Successful induced breeding of *C. idella* requires careful consideration of breeding biology, hormone use, and water quality management. While the breeding season and hormone application are key determinants, water quality can be effectively managed in captivity. Despite the declining use of CPG in favor of synthetic hormones due to their ease of use and single-dose efficacy, CPG remains a reliable choice for producing viable fry. This study aims to compare the effectiveness of different hormonal treatments at various developmental stages of *C. idella* in Bangladesh, aiming to demonstrate in advance that similar studies have not been published before.

Material and Methods

Location, Duration, and Protocol for Reproduction

This study was conducted at the Bismillah Fish Seed Production Center and Farm in Nangalkot, Cumilla, Bangladesh, located at 23°10'N latitude and 91°15’E longitude. The research spanned from January to December, focusing on the measurement of GSI and HSI of both female and male *Ctenopharyngodon idella* and on conducting an induced breeding program in March. During the study, fish were stocked at a density of 1100 kg/ha in brood rearing ponds and fed a diet containing 20% protein at a rate of 3% of their body weight.

Measurement of Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI)

The GSI and HSI values are crucial indicators for determining the optimal timing for induced breeding. These indices were measured monthly for both male and female *Ctenopharyngodon idella* from January to December. Gonad and liver weights were measured using a digital balance accurate to 0.01g (Shimadzu UX320G). Fish were dissected, and internal organs were carefully removed with a soft brush and blunt forceps to avoid injury.

The GSI was calculated using the formula by Afonso-Dias et al. (2005):

$$GSI = \frac{\text{Gonad weight}}{\text{Fish weight}} \times 100$$

The HSI was calculated using the formula by Rajguru (1992):

$$HSI = \frac{\text{Liver weight}}{\text{Fish weight}} \times 100$$

Experimental Design

The experiment was designed to evaluate the effectiveness of six hormonal treatments (T1, T2, T3, T4, T5, and T6) on the induced breeding of grass carp, using different combinations of CPG (Carp pituitary gland), hCG (Human chorionic gonadotropin), and ovupin (Domperidon 100 mg and S-GnRH 0.2mg) (Table 1). CPG hormone was procured from United Agro Fisheries, Bangladesh, while hCG and ovupin were sourced from Hebei Norvka Biotech Co., Ltd., and Ningbo Sansheng Pharmaceuticals Ltd., China, respectively. The control group did not receive any hormonal treatment. In this experiment, a 1:2 (female: male) sex ratio was employed using 3-year-old brood fish. Broodstock were collected randomly from the available population to ensure a representative sample. While this resulted in some variation in weight ranges across treatments, it reflects natural population dynamics and logistical realities (Table 2). Each treatment was repeated to ensure precision.

Brood fishes were conditioned in a cemented circular tank at a water temperature of 26.5±0.8°C for 12 hours prior to hormonal administration. In each treatment, two females and four males were admired hormone (Table 1) for induced breeding of *Ctenopharyngodon idella*. The first dose was administered to the female at 6 pm, followed by a second dose 6 hours later at midnight. Males received their dose at the time of the female's second dose. Fish were kept together in a circular water tank after the second dose. Hormones were injected at a 45° angle beneath the pectoral fin using a 2 ml syringe (22-gauge). Eggs and sperm were collected through hand stripping, mixed using bird feathers, and fertilized eggs were

Table 1. Name of hormone and their composition, does for male and female for induced breeding of *Ctenopharyngodon idella*

Treatment	Hormone	Composition	Sex ratio (F : M)	Female		Male
				1st dose	2nd dose	One time
T1	Carp pituitary gland (CPG)	Carp pituitary homogenate	1 : 2	0.75 mg/kg	7 mg/kg	1 mg/kg
T2	ovupin	S-GnRHa+Domperidone	1 : 2	0.4 ml/kg		0.2 ml/kg
T3	Human chorionic gonadotropin (hCG)	Gonadotropin	1 : 2	50 IU/kg	150 IU/kg	80 IU/kg
T4	hCG + CPG	Gonadotropin+ Carp pituitary homogenate	1 : 2	50 IU/kg	6mg/kg	80 IU/kg
T5	ovupin + CPG	S-GnRHa+Domperidone+ Carp pituitary homogenate	1 : 2	0.1 ml/kg	6 mg/kg	1 mg/kg
T6	hCG + ovupin	Gonadotropin + S-GnRHa+Domperidone	1 : 2	50 IU/kg	0.3 ml/kg	0.2 ml/kg

Table 2. Average weight of female and male *Ctenopharyngodon idella*, along with the average weight of eggs and sperm obtained after stripping across different treatments

Treatment	Female				Male			
	Avg. weight of broods (kg)	Avg. weight of spawned broods (kg)	Avg. Eggs weight (kg)	Weight of eggs after striping (%)	Avg. weight of broods (kg)	Avg. weight of spawned broods (kg)	Avg. Sperm weight (kg)	Weight of sperm after stripping (%)
Control	3.28±0.71^{a}	3.28±0.71^{a}	0*	0*	2.62±0.46^{b}	2.62±0.46^{ab}	0*	0*
T1	3.49±0.60^{a}	2.76±0.09^{a}	0.73±0.05^{a}	20.92	2.48±0.51^{b}	2.416±0.11^{b}	0.064±0.006^{a}	2.58
T2	2.75±0.32^{b}	2.28±0.06^{b}	0.47± 0.06^{b}	17.09	2.87±0.78^{a}	2.786±0.09^{a}	0.084±0.009^{a}	2.93
T3	4.22±0.93^{a}	3.89±0.11^{a}	0.33±0.03^{b}	7.82	3.04±0.37^{a}	3±0.22^{a}	0.04±0.006^{b}	1.32
T4	3.65±0.48^{a}	3.24±0.12^{a}	0.41±0.06^{b}	11.23	2.89±0.53^{a}	2.864±0.28^{a}	0.036±0.008^{b}	1.25
T5	4.08±0.74^{a}	3.3±0.08^{a}	0.78±0.07^{a}	19.12	2.97±0.26^{a}	2.896±0.15^{a}	0.074±0.006^{a}	2.49
T6	2.89 ±0.29^{ab}	2.45±0.05^{b}	0.44±0.05^{b}	15.22	2.64±0.49^{b}	2.559±0.13^{b}	0.061±0.004^{a}	2.38

*Sperm collection was attempted in the control group using the same stripping process as other treatments, but it was unsuccessful, potentially due to the lack of hormonal stimulation.

transferred to a funnel-type incubator (1 kg of eggs per 50 liters of water). In the fertilization procedure, sperm and oocyte volumes were measured indirectly to ensure consistency across treatments by subtracting the broodstock weight before and after stripping. Sperm was then mixed directly with oocytes as shown in Figure 1c. The volume measurement process was standardized as described. The differences in fertilization rates across treatments are attributed to the hormonal treatments, as sperm and oocyte volumes were consistently controlled. In the control group, the stripping process was performed similarly to the hormonally treated groups. Water temperature, dissolved oxygen, pH, and TDS were monitored every 6 hours using a multiparameter (HACH, Model no. HQ30d) during the hatching process.

Preparation of Hormone for Induced Breeding

Wet carp pituitary glands were purchased, preserved in hermetically sealed vials, and prepared for injection. The glands were air-dried, weighed using an analytical electronic balance (College B204-S, Switzerland), homogenized, dissolved in distilled water, and centrifuged to obtain a clear supernatant for injection. The required dosage was calculated using the formula by Alam et al. (2006):

$$\text{Weight of PG (mg)} = \left(\frac{\text{Total body weight of fish (g)}}{1000}\right) \times \text{Pt}$$

Pt is the hormone dosage in mg per kg body weight.

Ovupin (100 mg DOM+ 0.2 mg S-GnRHa) and hCG were diluted in distilled water at 2cc/kg, and the appropriate dose was administered to male fish beneath the pectoral fin. The fish were then released into separate glass tanks with continuous air and water flow.

Breeding Parameter

The hand stripping method was used to collect eggs and sperm from the fish, except in the control treatment, where natural spawning was allowed. Ovulation rate was calculated using the following equation:

$$\text{Ovulation rate (\%100)} = \frac{\text{Number of fish ovulated}}{\text{Total number of fish injected}} \text{x100}$$

(Paul et al., 2021)

Eggs that did not survive displayed a white and opaque appearance within 8 to 10 hours post-fertilization. Conversely, successfully fertilized eggs remained transparent and showed embryonic development, including the formation of embryonic eyes within 20 minutes of fertilization (Figure 2). The fertilization rate was calculated using the formula:

$$\text{Fertilization rate (\%100)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \text{x100}$$

(Paul et al., 2021)

Hatching rates were determined by directly counting the number of hatched larvae and calculating the proportion relative to the total number of fertilized eggs:

a) Injection into the pectoral region

b) Colletion of eggs by hand stripping

c) Mixing of fish sperm by hand stripping

d) Funnel incubator for hatchling

Figure 1. Chronological breeding activities of *Ctenopharyngodon idella* (a to d) conducted in the hatchery.

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

(Islam et al., 2011)

The survival rate of larvae in the incubator was monitored over a period of two days, with counts taken at six-hour intervals. Newly hatched larvae were placed in a cylindrical white container with a capacity of 50 milliliters, and water from three bowls was evenly distributed for enumeration. The survival rate was calculated using the following formula:

$$\text{Survival rate (\%100)} = \frac{\text{Number of surviving hatchlings}}{\text{Initial number of hatchlings}} \times 100$$

(Alam et al., 2006)

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS (version 22.0 for Windows). The normality of the data was assessed using the Shapiro-Wilk test to ensure compliance with statistical assumptions. Percentage data were transformed using the arcsine square root method prior to analysis to address normality and homoscedasticity concerns. After statistical analyses, the percentage data were retransformed to their original scale for clarity in tabular and graphical presentations.

Pearson`s correlation was used to evaluate the strength and significance of the relationship. Significant differences among treatments were determined using one-way analysis of variance (ANOVA), followed by Duncan's New Multiple Range Test (DMRT) for post-hoc comparisons at a significance level of $P<0.05$.

Results

Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI)

GSI and HSI values of *C. idella* were measured monthly using five pairs of fish each time. The results revealed an inverse relationship (r=-0.05) between the GSI and HSI values for both female and male fish. Specifically, as the GSI value increased, the HSI value tended to decrease, indicating a disproportionate relationship between these indices.

For female fish, the highest GSI value (21.48±2.69%) was observed in March, which is indicative of peak reproductive readiness, while the

lowest GSI value (1.02±0.45%) occurred in September. Conversely, the highest HSI value for females (8.03±1.83%) was recorded in September, suggesting an accumulation of energy reserves during non-spawning periods. In male fish, the highest and lowest GSI values were 1.37±0.38% and 0.11±0.06%, respectively, with corresponding HSI values of 4.68±0.23% and 2.01±0.08% (Figure 3).

The GSI values for both females and males showed a gradual increase from November to March, aligning with the approach of the breeding season, and then gradually declined post-March. In contrast, the HSI values for both sexes increased from May to October, before declining gradually thereafter, reflecting the cyclical nature of energy storage and usage related to reproductive activities (Figure 3).

Quantity of Eggs and Sperm After Spawning

In all treatments, three-year-old broodstock (both female and male) were used for breeding purposes. Ovulation occurred 6-8 hours after the administration of the second hormone dose to the females. The stripping process in the control group was consistent with other groups; however, no sperm was obtained, likely because of the absence of hormonal stimuli.

The percentage of body weight loss in females due to egg release followed this order: T1 (20.92%) > T5 (19.12%) > T2 (17.09%) > T6 (15.22%) > T4 (11.23%) > T3 (7.82%). Similarly, the weight loss in males due to sperm release was observed in the following order: T2 (2.93%) > T1 (2.58%) > T5 (2.49%) > T6 (2.38%) > T3 (1.32%) > T4 (1.25%) (Table 2).

Figure 2. Fertilized and unfertilized eggs of *Ctenopharyngodon idella* were observed stacked (left image) and inside a clear jar (right image). A distinct difference was noted: fertilized eggs appeared transparent, while unfertilized eggs were opaque.

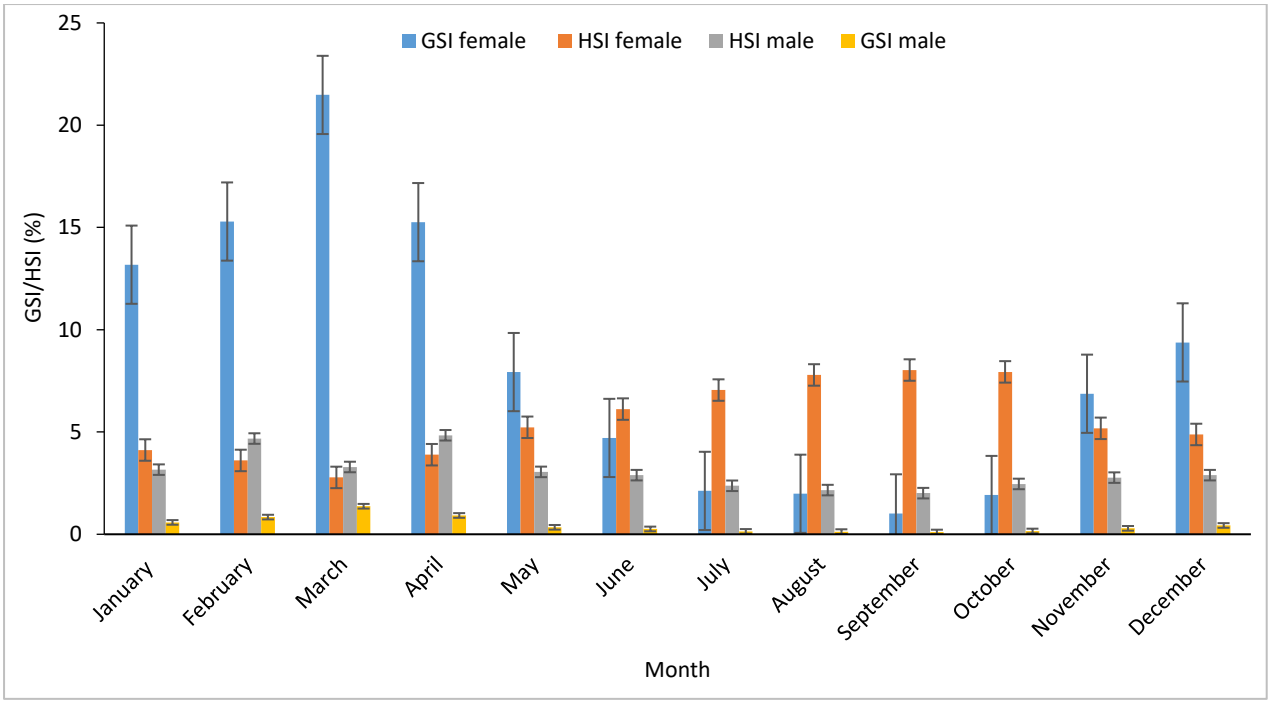

Figure 3. Monthly GSI and HSI values of *Ctenopharyngodon idella.*

Breeding Performance

In the control treatment, no ovulation occurred. The highest ovulation rates were observed in treatment T1 (95%), followed by T5 (92%), T2 (84%), T4 (79%), T6 (67%), and T3 (47%) (Table 3). The CPG (T1) and ovupin plus CPG (T5) treatments demonstrated the best ovulation performance. The duration of the latency period varied across treatments, with the shortest latency period recorded in T2 (5.48 hours), followed by T5 (5.58 hours), T1 (6.16 hours), T6 (6.48 hours), T4 (6.51 hours), and T3 (7.18 hours) (Table 3).

Regarding fertilization rates, the highest was observed in T1 (93.48±5.83%), followed by T5 (86.79±9.52%), T2 (82.17±10.05%), T4 (79.27±5.29%), T6 (70.11±4.95%), and T3 (65.74±8.16%) (Table 3). There was no fertilization was observed in the control treatment due to ovulation was absent. The ANOVA test revealed significant differences (P<0.05) in fertilization rates among the various hormonal treatments.

The hatching period also varied among treatments. The longest hatching period was found in T3 (27.16 hours), followed by T4 (26.13 hours), T6 (24.41 hours), T5 (23.04 hours), T1 (22.41 hours), and T2 (21.38 hours), with the shortest hatching period associated with the ovupin treatment.

For hatching rates, the highest rate was recorded in T1 (89.36±7.26%), followed by T5 (84.8±7.38%), T2 (79.17±8.47%), T4 (69.61±5.93%), T6 (62.73±7.29%), and T3 (39.53±6.24%) (Table 3). The ANOVA test indicated significant differences (P<0.05) in hatching rates among the treatments.

Survival Rate of Larvae in the Incubator

No food was provided during the assessment of larval survival rates in the incubator. After 48 hours, the ANOVA test indicated a significant difference (P<0.05) among the treatments. Duncan's Multiple Range Test (DMRT) revealed the following chronological order of survival rates: T1 had the highest survival rate at 73.9±3.79%, followed by T5 at 69.8±2.84%, T2 at 63.1±3.29%, T4 at 51.4±3.02%, T6 at 47.1±3.13%, and T3 at 32.5±2.14% (Table 4).

Water Quality Parameters in the Incubator

The water for all incubators was sourced from groundwater. The water parameters at the source showed a temperature range of 25.8 – 27.2°C, pH of 7.3 – 7.5, dissolved oxygen (DO) levels between 5.84 – 6.02 mg/l, and total dissolved solids (TDS) from 410 – 436 ppm.

Within the incubators, the water quality parameters were as follows: temperature ranged from 26.9±0.48°C to 28.2±0.68°C, pH varied between 7.4±0.04 and 7.6±0.03, DO ranged from 4.65±0.09 mg/l to 5.93±0.09 mg/l, and TDS ranged from 137±25 ppm to 210±26 ppm (Table 5).

Discussion

Reproductive Biology

Understanding the reproductive biology of *Ctenopharyngodon idella* is vital for optimizing breeding techniques in aquaculture. Key parameters such as GSI, HSI, fecundity, egg diameter, breeding season, genital color, and body color have been widely studied. These parameters are influenced by various factors, including water temperature, nutrition, and genetics (Paul et al., 2021; Manzoor et al., 2020; Lin et al., 2006).

Our study identified March as the peak breeding season, with the highest GSI values observed. These results are in agreement with previous research conducted in Egypt (El-Gamal et al., 2021) and Uzbekistan (Kamilov and Komrakova, 2003). However, they show notable differences compared to studies in Japan (Shireman and Smith, 1983), where breeding occurs from June to August, in the USA during May (Carlos, 2016), and from April to July (Elder and Murphy, 1997). The variation in breeding seasons is likely influenced by both environmental and genetic factors. This observation is consistent with the understanding that optimal environmental conditions are essential for successful reproduction (Bozkurt and Öğretmen, 2012; Kamilov and Komrakova, 2003). Table 6 presents a comparative analysis of reproductive parameters across various regions. For instance, the GSI percentages observed in Egypt (El-Gamal et al., 2019) similarly align with our results. The table emphasizes the variability in reproductive parameters under different environmental conditions, highlighting the need to adapt breeding practices to specific settings to optimize grass carp production in aquaculture.

Use of Inducing Agent for Breeding

The effectiveness of fish breeding can be significantly enhanced through the application of various inducing hormones, which are commonly used in aquaculture to manage and improve reproductive processes across different fish species. In Bangladesh, fish hatchery operators frequently employ inducing agents such as CPG for Indian, common, and Chinese carps; hCG for silver carp; and ovupin for catfish (Paul et al., 2021).

The success of induced breeding in cyprinid fishes depends on the effectiveness of different spawning agents and methods, a finding that aligns with previous studies (Kucharczyk et al., 2024; Kujawa et al., 2022; Nowosad et al., 2016; Kucharczyk et al., 2005; Kucharczyk et al., 1997) and is supported by this research.

The success of induced breeding relies on multiple factors, including the maturity of the broodstock, the sex ratio, the dosage and type of hormones used, the quality of the broodfish, and the physico-chemical properties of the water, including its exchange rate

Table 3. Ovulation rate, latency period, fertilization rate, hatching period and hatching rate of *Ctenopharyngodon idella* under different treatment

Treatment	Ovulation rate (%)	Latency period (hrs.) after final dose	Fertilization rate (%)	Hatching period (hrs.)	Hatching rate (%)
Control	No ovulation				
T1	95	6.16	93.48±5.83^{a}	22.41	89.36±7.26^{a}
T2	84	5.48	82.17±10.05^{b}	21.38	79.17±8.47^{b}
T3	47	7.18	65.74±8.16^{d}	27.16	39.53±6.24^{d}
T4	79	6.51	79.27±5.29^{bc}	26.13	69.61±5.93^{c}
T5	92	5.58	86.79±9.52^{a}	23.04	84.8±7.38^{a}
T6	67	6.48	70.11±4.95^{c}	24.41	62.73±7.29^{c}

Table 4. Survival rate of larvae in the incubator at six-hour intervals under different treatments

Hormone	Treatment	1st day (24 hrs.)				2nd day (24 hrs.)			
		1	2	3	4	5	6	7	8
CPG	T1	97±2.16	91±7.27	86±6.25	81±3.68	78±5.72	76±4.47	74±3.85	73.9±3.79^{a}
ovupin	T2	93±4.31	88±8.19	81±7.28	76±6.16	71±5.57	67±2.92	65±3.77	63.1±3.29^{b}
hCG	T3	89±9.07	78±9.34	65±5.79	57±5.39	51±6.15	43±3.64	36±2.72	32.5±2.14^{d}
hCG + PG	T4	91±7.75	85±8.26	77±4.15	69±5.28	61±4.58	58±4.52	54±3.26	51.4±3.02^{c}
ovupin + PG	T5	94±4.79	90±8.21	84±7.21	79±7.24	77±3.86	73±6.38	71±3.17	69.8±2.84^{ab}
hCG + ovupin	T6	87±8.42	81±9.41	71±6.69	65±4.62	62±5.11	55±4.61	51±4.47	47.1±3.13^{c}

Table 5. Physicochemical parameters of water in Incubator

Parameters	1st hour	After 6 hours	After 12 hours	After 18 hours	After 24 hours	After 30 hours	After 36 hours	After 42 hours	After 48 hours
Temperature (^{o}C)	27.6±0.52	27.7±0.71	27.3±0.53	26.9±0.48	27.9±0.61	27.7±0.27	27.1±0.49	27.2±0.43	28.2±0.68
pH	7.6±0.02	7.6±0.03	7.5±0.02	7.6±0.03	7.5±0.02	7.4±0.04	7.6±0.02	7.5±0.01	7.5±0.03
DO (mg/l)	5.43±0.14	5.79±0.17	5.48±0.11	5.93±0.09	5.29±0.14	5.02±0.12	4.72±0.06	4.65±0.09	4.73±0.10
TDS (ppm)	148±23	210±26	187±17	169±18	180±23	153±31	137±25	142±19	157±22

(Rahman et al., 2013). Our study evaluated the use of three types of hormones—CPG, hCG, and ovupin—in various combinations for inducing breeding in *C. idella*. We found that CPG yielded the highest percentage of eggs, a result consistent with findings by Salman et al. (2023) and Biswas et al. (2021). In contrast, our findings differ from those reported in Egypt (El-Gamal et al., 2019). Furthermore, studies in Egypt (El-Gamal et al., 2019) and India (Rashid et al., 2014) observed certain combination variations, where the use of hCG and ovatide resulted in higher success rates. Table 7 illustrates the differences in hormone doses and their effectiveness. For example, the CPG doses reported in Malaysia (Jhingran and Pullin, 1985) were higher than those applied in our study, which may account for the variations in breeding success. These results underscore the importance of optimizing hormonal treatments to suit specific regional conditions.

Evaluation of Breeding

The latency period can vary depending on the type and dosage of hormones used (Salman et al., 2023). In our study, we observed that ovupin, a synthetic hormone, resulted in a shorter latency period compared to hCG, which led to a longer latency period.

The fertilization and hatching rates are critical metrics in artificial breeding of *C. idella* and are influenced by the choice of inducing agents, such as hormones, water circulation, oxygen levels, and other physico-chemical parameters of the water (Paul et al., 2021; Karim et al., 2016; Rahman et al., 2013; Jhingran & Pullin, 1985). These rates are crucial for assessing the success of the breeding process and the quality of the offspring produced. Our study recorded shorter latency periods compared to those reported by Rashid et al. (2014), while aligning with the observations of Saidin et al. (1988) and Jhingran and Pullin (1985). Similarly, the fertilization rates achieved in our study (up to 93.48%) exceeded those reported in Nepal by Jha and Neupane (2019) but were consistent with findings from Egypt as documented by El-Gamal et al. (2019).

In this study, the survival rate of grass carp larvae was best with the CPG treatment and lowest with hCG. This suggests that while CPG may offer better outcomes for larval survival, the choice of hormone can significantly impact the efficiency of the breeding process.

Moreover, Table 8 presents a comparative analysis of breeding performance. For example, hatching period observed in Bangladesh (Biswas et al., 2021) was similar to our study, but hatching rates were higher, possibly due to differences in water management practices. These findings underscore the importance of fine-tuning breeding protocols to maximize efficiency. A limitation of this study is the variation in the weight range of breeders across treatments. While standardizing weight ranges could have provided more controlled

Table 6. A comparative study on the breeding biology and seasonality of *Ctenopharyngodon idella* across different countries.

Species	Breeding Season	GSI (%)	Fecundity (No.)	Gonad diameter (mm)	Country	Authors	Remarks
C. idella	Feb to April	15 - 21.5			Bangladesh	Present study	
C. idella	April- May	0.36±0.025 - 17.64±0.381			Egypt	El-Gamal et al., 2021	Similar to the present study
C. idella	April to May	0.264±0.08 - 2.79±0.3 (male)			Egypt	Sheha et al., 2021.	Similar to the present study
C. idella	April to June	0.3664±0.025 - 17.64 5±0.381	268372±10772 to 810990±21510		Egypt	El-Gamal et al., 2019	Similar to the present study
C. idella	May	11.50			USA	Carlos, 2016	Similar
C. idella		-	417867±36.274	1.04±0.028	Türkiye	Bozkurt and Öğretmen, 2012	Not study fecundity in the present study
C. idella	March to April	9 - 20	100000 - 990000	0.9 - 1.8	Uzbekistan	Kamilov and Komrakova, 2003	Similar
C. idella	April-July	4 - 22	85,528 eggs/kg		USA	Elder and Murphy, 1997	Similar
C. idella	June to August				Japan	Shireman and Smith, 1983	Dissimilar due to environmental condition and genetically

Table 7. A comparative study on the types and doses of various hormones used in different research studies

Species	Country	Hormone	Female dose (ml/kg) or (mg/kg)	Male dose (ml/kg) / (mg/kg)	Reference	Remarks
C. idella	Bangladesh	CPG, hCG, ovupin	See Table 1	See Table 1	Present study	
C. idella	Iraq	CPG, hCG and ovaprim	4 - 6 mg/kg, 700 - 1200 IU/kg, 0.4 - 0.6 ml/kg	-----	Salman et al., 2023	Different from the present study
C. idella	Bangladesh	hCG and GnRHa	0.3 - 0.8	0.1 - 0.2	Chakraborty, 2022	Not covered in this study
C. idella	Bangladesh	CPG	0.5 - 5.5	1 - 2	Biswas et al., 2021	Similar to the present study
C. idella	Nepal	LHRH-A	0.6µg/kg	0.15 µg/kg	Jha and Neupane, 2019	Not similar
C. idella	Egypt	CPG, hCG	3 - 5 , 750 - 1500 IU/kg	5	El-Gamal et al., 2019	Not similar to the present study
C. idella	Bangladesh	ovupin	0.5 - 0.9	0.2 - 0.4	Aktar and Islam, 2015	Similar to the present study
C. idella	India	ovatide	0.8	0.45	Rashid et al, 2014	Higher to the present study
C. idella	Pakistan	ovaprim-C	0.6	0.2	Naeem et al., 2011	Higher from the present study
C. idella	Worldwide	LRH-A	---	---	FAO, 2009	Not covered in this study
C. idella	Malaysia	CPG, hCG,	4 - 6	1 - 2	Saidin et al., 1988	Similar to the present study
C. idella	Malaysia	CPG	F: 5 - 6 , S: 5 - 6	5	Jhingran and Pullin, 1985	Higher from the present study
C. idella	China	LHRH-A	5 - 10	2.5 - 5		Not covered in this study
C. idella	India	CPG	F: 3 - 5, S: 7 - 10	2 - 3		Higher from the present study
C. idella	Thailand	CPG	F: 0.23 - 1, S: 1 - 3.4	0.23 - 1		Lower from the present study

Table 8. A Comparative study in the context of fertilization rate, hatching rate, and survival rate of *Ctenopharyngodon idella*

Species name	Country	Latency period (hrs.)	Ovulation rate (%)	Fertilization rate (%)	Hatching period (hrs.)	Hatching rate (%)	Survival rate (%)	Reference
C. idella	Bangladesh	5.48 -7.18	47 - 95	65.7-93.48	21.38-27.16	39.53-89.36	20.72-73.92	Present study
C.idella	Bangladesh	----	98%	----	----	94	----	Chakraborty, 2022
C. idella	Kazakhstan	----	----	72.5%	----	43.2%;	----	Abilov et al., 2022
C. idella	Bangladesh	----	----	93.43 ± 1.53%,	18-24	86.94±2.77%	----	Biswas et al., 2021
C. idella	Nepal	----	----	40 - 70	----	60 - 70	----	Jha and Neupane, 2019
C. idella	Hungry	----	70.5 - 87.3	----	----	----	----	Szabó et al., 2019
C. idella	Egypt	----	----	80.66% - 82.13%	----	82.31%-86.85%	----	El-Gamal et al., 2019
Indian major carps	Bangladesh	----	----	----	10 - 70	55-85	----	Aktar and Islam, 2015
C. idella	India	14 - 16		80.03	20-30	70.10	15.21	Rashid et al., 2014
C. idella	Pakistan	----	----	80.36	18-22	79.49%	----	Naeem et al., 2011
C. idella		6	60 - 80	----	----	----	----	Saidin et al., 1988
C. idella	India	5 - 6	----	15 - 80	----	----	----	Jhingran and Pullin, 1985

comparisons, the random collection of broodstock reflects practical constraints and the natural variation in the population. This approach ensures broader applicability of the findings under real-world conditions.

Effect of Physico- Chemical Parameters on Fish Breeding

The physico-chemical properties of water—such as dissolved oxygen (DO), pH, temperature, ammonia, nitrite, nitrate, alkalinity, hardness, total dissolved solids (TDS), and total suspended solids (TSS)—are crucial factors influencing ovulation, fertilization rate, hatching period, hatching rate, and larval survival (Stott & Cross, 1973).

Temperature significantly impacts fish egg and larval development. Optimal temperatures are critical for successful breeding. In Bangladesh, hatcheries typically operate within a temperature range of 22.8 – 23.1°C (Siddique et al., 2022), whereas our study maintained temperatures between 26.9±0.48°C and 28.2±0.68°C. Higher temperatures (> 30°C) can lead to shorter latency periods, hatching times, and reduced larval survival rates (Paul et al., 2021).

pH levels also affect breeding success. Extreme pH values—whether acidic or alkaline—can damage egg membranes and impede embryo development, leading to lower hatching success (Marimuthu et al., 2019). For instance, Jezierska and Witeska (1995) reported that common carp (*Cyprinus carpio*) larvae perished at a pH of 5.5. In our study, the water exhibited a mild alkaline nature, which did not noticeably affect hatching and survival rates.

Dissolved oxygen (DO) is essential for embryonic development and the metabolic processes within eggs. Low DO levels can cause hypoxia, impairing embryonic development and reducing hatching success (Qiang et al., 2019). After hatching, larvae rely on oxygen from the water, and inadequate DO can lead to physiological stress and increased mortality (Randall & Tsui, 2002). Anita and Dewi (2020) observed better hatching and survival rates at DO levels of 7 mg/L; however, our study recorded DO levels below this threshold.

Total Dissolved Solids (TDS) levels are also important, though the ideal TDS range varies by fish species and their natural habitats. Some species thrive in high TDS environments, such as brackish or saline waters, while others are adapted to low TDS, like freshwater environments (Boyd, 2015). Our study found TDS levels within the range commonly maintained in freshwater finfish hatcheries in Bangladesh (146 - 200 mg/L) (Mou et al., 2018), suggesting that the observed TDS levels were appropriate for the species studied.

Conclusions

Understanding the reproductive biology and effective breeding techniques for *Ctenopharyngodon idella* is crucial for advancing aquaculture practices and enhancing fish production. This study highlighted several key findings:

Reproductive Parameters

The GSI and HSI demonstrated significant fluctuations throughout the year, with peak GSI values observed in March, indicating the optimal breeding season for grass carp. The data also revealed that GSI and HSI are inversely related, with increased GSI corresponding to decreased HSI.

Breeding Performance

The use of different hormonal treatments (CPG, hCG, and ovupin) significantly influenced ovulation rates, fertilization rates, and hatching success. CPG treatment (T1) yielded the highest fertilization and hatching rates, with ovupin resulting in the shortest latency period. These findings align with previous research that emphasizes the effectiveness of CPG in enhancing breeding performance.

Larval Survival

The survival rate of larvae was highest with CPG treatment (T1), underscoring the importance of choosing the right hormonal inducer for optimal larval survival. Water quality parameters such as temperature, pH, dissolved oxygen (DO), and total dissolved solids (TDS) were well-maintained within acceptable ranges, contributing to successful hatching and larval development.

Physico-chemical Parameters

Water temperature, pH, DO, and TDS significantly impacted breeding outcomes. Optimal water conditions are critical for ensuring high fertilization rates, efficient hatching, and strong larval survival. Maintaining appropriate physico-chemical parameters is essential for maximizing aquaculture productivity.

In conclusion that the best performance of induced breeding of *Ctenopharyngodon idella* with carp pituitary gland (CPG) (T1). From the study, implement these findings can lead to improved breeding efficiency of *Ctenopharyngodon idella*, better larval survival rates, and overall advancements in aquaculture practices.

Ethical Statement

This study was conducted with the approval of the Ethical Review Committee of Noakhali Science and Technology University, adhering to the ethical guidelines and standards set by the committee. Ethical approval was obtained under reference number NSTU/SCI/EC/2024/264 to ensure that all procedures were conducted in accordance with ethical principles.

Funding Information

This work was funde by the National Agricultural Technology Programme (Phase II) of Bangladesh Agricultural Research Council, Farmgate, Dhaka, Bangladesh (Grant number CRG 553, Fiscal Year 2017-2018).

Author Contribution

Shyamal Kumar Paul: Conceptualization and design, Writing, Funding Acquisition; Kazi Faridul Hasan: Investigation, Data Curation, drafting of the paper; Nadia Sultana: Methodology, Data analysis, Drafting; Debasish Saha: Project Administration, Supervision; Srijan Sarker: Investigation, Resources, Data Curation; Bhakta Supratim Sarker: Visualization and interpretation of data, reviewing and revising the draft. All authors have reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors also wish to express their deep appreciation to the Md Shamsuddin Kalu, Bismilah fish seed production and farm, Langolcourt, Cumilla, Bangladesh for his operational and physical facilities.

References

Abilov, B., Nikolova, L., Isbekov, K., & Kulmanova, G. (2022). Application of carp pituitary and Nerestin in artificial reproduction of species of the family Cyprinidae. *Bulgarian Journal of Agricultural Sci*ence, *28*(1), 129–138. https://journal.agrojournal.org/page/en/details.php?article_id=4008

Afonso-Dias, I., Reis, C., & Andrade, J. P. (2005). Reproductive aspects of *Microchirus azevia* (Risso, 1810) (Pisces: Soleidae) from the south coast of Portugal. *Scientia Marina*, *69*(2), 275–283. https://doi.org/10.3989/scimar.2005.69n2275

Aktar, N. & Islam, N.M. (2015). Induced breeding practices of the fish hatcheries in the North-Western region of Bangladesh. *Journal of Aquaculture & Marine Biology*, *2*(6). https://doi.org/10.15406/jamb.2015.02.00048

Alam, M.J., Begum, M., Islam, M.A. & Pal, H. K. (2006). Spawning behavior and induced breeding of an esturine cat fish, *Mystus gulio* (Ham.). *Bangladesh Journal of Fishery Resources*, *10*(2), 101-109. http://hdl.handle.net/1834/34191

Anita, N. S., & Dewi, N. N. (2020). Evaluation of hatching rate, growth performance, and survival rate of cantang grouper (*Epinephelus fuscoguttatus* × *lanceolatus*) in concrete pond at Situbondo, East Java, Indonesia. *IOP Conference Series Earth and Environmental Science*, *441*(1), 012019. https://doi.org/10.1088/1755-1315/441/1/012019

Biswas, A., Choudhury, M., Farid, M. A., Rahman, M. A., & Rahman, M. A. (2021). Induced breeding of freshwater fishes and cost benefit analysis of a selected fish hatchery in Jashore, Bangladesh. *Annual Research & Review in Biology*, *36*(11) 15–25. https://doi.org/10.9734/arrb/2021/v36i1130446

Boyd, C. E. (2015). Water quality. In *Springer eBooks*. https://doi.org/10.1007/978-3-319-17446-4

Bozkurt, Y., and Öğretmen, F. (2012). Sperm quality, egg size, fecundity and their relationships with fertilization rate of grass carp (*Ctenopharyngodon idella*). *Iranian Journal of Fisheries Science*, 11(4), 755–764. https://jifro.ir/article-1-735-en.html

Carlos, A.C. (2016). Asian Carp reproductive ecology along the upper Mississippi River invasion front. A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE. Iowa State University, Ames, Iowa. https://dr.lib.iastate.edu/entities/publication/7c426fd9-8c6d-49b3-83fc-98f82f9db403

Chakraborty, B.K. (2022). Induced breeding practices of the freshwater fish hatcheries in Bangladesh. *International Journal* of *Oceanography* & *Aquaculture, 6*(2), 000225. https://doi.org/10.23880/ijoac-16000225

Chilton, E. W., & Muoneke, M. I. (1992). Biology and management of grass carp (*Ctenopharyngodon idella*, Cyprinidae) for vegetation control: a North American perspective. *Reviews in Fish Biology and Fisheries*, *2*(4), 283–320. https://doi.org/10.1007/bf00043520

Cudemore, B., and Mandrak, N. E. (2004). Biological synopsis of grass carp (*Ctenopharyngodon idella*). *Canadian Manuscript Report of Fisheries and Aquatic Sciences*, 2705, v+44p. https://publications.gc.ca/site/eng/9.832257/publication.html

Elder, H. S., & Murphy, B. R. (1997). Grass Carp (*Ctenopharyngodon idella*) in the Trinity River, Texas. *Journal of Freshwater Ecology*, *12*(2), 281–289. https://doi.org/10.1080/02705060.1997.9663535

El-Gamal, A. E., Darwish, S. T., & Sheha M. A. (2019). The Reproductive Biology of Grass Carp (*Ctenopharyngodon Idella*) Collected From Floating Cages in River Nile, Egypt. *East African Scholars Journal of Biotechnology and Genetics*, *1*(6), 149-157. https://www.easpublisher.com/get-articles/20

El-Gamal, A. E., El-Sayyad, H., Sheha, M. A., & Barakat, R. O. (2021). Histological and histochemical studies on the ovarian development of the grass carp, Ctenopharyngodon idella with special references to atretic phenomena. *Egyptian Journal of Aquatic Biology and Fisheries*, *25*(1), 477–492. https://doi.org/10.21608/ejabf.2021.145443

FAO. (2009). *Ctenopharyngodon idella*. In Cultured aquatic species fact sheets. Text by Weimin, M. Edited and compiled by Valerio Crespi and Michael New. CD-ROM (multilingual). https://www.fao.org/fishery/docs/CDrom/aquaculture/I1129m/file/es/es_grasscarp.htm

Gao, Y., Kim, S., & Lee, J. (2011). Effects of pH on Fertilization and the Hatching Rates of Far Eastern Catfish Silurus

asotus. *Fisheries and Aquatic Sciences*, *14*(4), 417–420. https://doi.org/10.5657/fas.2011.0417

Gárriz, Á. and Miranda, L.A. (2020). Effects of metals on sperm quality, fertilization and hatching rates, and embryo and larval survival of pejerrey fish (*Odontesthes bonariensis*). *Ecotoxicology*, 29, 1072–1082. https://doi.org/10.1007/s10646-020-02245-w

Howard, S. E., & Brian R. M. (1997). Grass Carp (*Ctenopharyngodon idella*) in the Trinity River, Texas. *Journal of Freshwater Ecology*, 12:2, 281-289. https://doi.org/10.1080/02705060.1997.9663535

Islam, S., Shah, S. and Rahi, L. (2011). Study of fecundity and induced breeding of *Mystus vittatus*. *Bangladesh Journal of Zoology*, *39*, 205-212. http://doi.org/10.3329/bjz.v39i2.10587

Jezierska, B., Witeska, M. (1995). The influence of pH on embryonic development of common carp (*Cyprinus carpio*). *Archives of Polish Fisheries*, 3(1): 85–94. https://doi.org/10.1016/0044-8486(95)91954-T

Jha, B.S., and Neupane, S. (2019). Induced breeding of grass carp *Ctenopharyngodon idella* (*Valenciennes*) rupandehi, Nepal. *Zoo-Journal*, *5*, 82-84. https://doi.org/10.3126/zooj.v5i0.34933

Jhingran, V.G. and Pullin, I.S.V. (1985). A hatchery manual for the common, Chinese and Indian major carps. *ICLARM Studies and Reviews*, *11*, 191 p. Asian Development Bank, Manila, Philippines and International Center for Living Aquatic Resources Management, Manila, Philippines. https://hdl.handle.net/20.500.12348/3520

Kamilov, B., and Komrakova, M. (2003). Growth and maturation of the grass carp, *Ctenopharyngodon idella*, in Uzbekistan. *Pakistan Journal of Zoology*, *35*(2), 115-118. https://www.cabdirect.org/cabdirect/abstract/20033175061

Karim, E., Hasan, J., & Hoq, E. (2016). Hormone Induced Artificial Breeding of a Commercially Important Marine Finfish, Striped Mullet (*Mugil cephalus* L.) in Bangladesh. *Turkish Journal of Fisheries and Aquatic Sciences*, *16*, 135-140. http://doi.org/10.4194/1303-2712-v16_1_14

Król, R., Nowosad, J., Kupren, K., Piech, P., Kujawa, R., Fopp-Bayat, D., Dąbrowski, M., Zielonka, L., Dariusz Kucharczyk, D. (2024). Effect of temperature and spawning agents on wild female asp (*Leuciscus aspius*) reproductive efficiency under controlled conditions. *Animal Reproduction Science*, 265, 107489. https://doi.org/10.1016/j.anireprosci.2024.107489.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Wyszomirska, E. (1997). Induced spawning in rudd (*Scardinius erythtrophthalmus* L.). *Polish Archives of Hydrobiology*, 44, 209–213.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Targońska-Dietrich, K., Wyszomirska, E., Glogowski, J., Babiak, I., & Szabó, T. (2005). Induced spawning in bream (*Abramis brama* L.) using pellets containing GnRH. *Czech Journal of Animal Science*, *50*(3), 89-95. https://doi.org/10.17221/4000-CJAS

Kucharczyk, D., Czarkowski, T., Nowosad, J., Targońska, K., Kupren, K., Wyszomirska, E., Roman Kujawa, R., László Horváth, L., Müller, T. (2016). Influence of temperature on successful European eel female maturation under controlled conditions. *Turkish Journal of Fisheries and Aquatic Sciences*,16: 477-482. https://doi.org/10.4194/1303-2712-v16_2_28

Kucharczyk, D., Nowosad, J., Kucharczyk, D.J., Kupren, K., Targońska, K., Wyszomirska, E., Kujawa, R. (2019). Out-of-season artificial reproduction of common dace (*Leuciscus leuciscus* L.) under controlled conditions. *Animal Reproduction Science*, 202, 21-25. https://doi.org/10.1016/j.anireprosci.2019.01.003.

Kucharczyk, D., Fopp, W., Wojtyszek, P., Nowosad, J., Kucharczyk, D.J., Drężek, J., Kołosińska, A., Omirzhanova, N., Dietrich, G.J. (2024). Artificial reproduction of wild and pond-cultured common tench (*Tinca tinca*) under controlled conditions, Animal Reproduction Science, Volume 261, 2024, 107411. https://doi.org/10.1016/j.anireprosci.2024.107411.

Kujawa, R., Piech, P., Nowosad, J., Kucharczyk, D. (2022). Comparison of different methods of wild vimba bream *Vimba vimba* (L.) spawning under controlled conditions. *Animal Reproduction Science*, 244, 107036. https://doi.org/10.1016/j.anireprosci.2022.107036.

Kumar, P., Biswas, G., Ghoshal, T. K., Kailasam, M., & Vijayan, K. K. (2018). Embryonic and larval developments of brackish water catfish, *Mystus gulio* (Hamilton and Buchanan, 1822) induced with human chorionic gonadotropin and consequent larval rearing. *Aquaculture Research*, 49(7), 2466–2476. https://doi.org/10.1111/are.13706

Lin, Q., Junyi, L., Gao, Y., Shen, L., Cai, J., Luo, J. (2006). The effect of temperature on gonad, embryonic development and survival rate of juvenile seahorses, Hippocampus kuda Bleeker. Aquaculture. 254. 701-713. https://doi.org/10.1016/j.aquaculture.2005.11.005.

Majhi, S.K., & Rasal, A.R. (2014). Comparative elevation in water temperature induces somatic growth and rapid proliferation of gonadal germ cells in three species of carp. *Turkish Journal of Fisheries and Aquatic Sciences*, *14*, 739-748. http://doi.org/10.4194/1303-2712-v14_3_16

Manzoor, P.S., Rawat, K.D., Tiwari, V.K., Poojary, N., Majeedkutty, A.B.R. (2020). Dietary lipid influences gonadal maturation, digestive enzymes and serum biochemical indices of *Cyprinus carpio* reared in biofloc system. *Aquaculture Research,* 51: 3244–3254. https://doi.org/10.1111/are.14659

Marimuthu, K., Palaniandya, H., Zainal, A. M. (2019). Effect of different water pH on hatching and survival rates of African catfish *Clarias gariepinus* (Pisces: Clariidae). *Aceh Journal of Animal Science*, *4*(2), 80-88. https://doi.org/10.13170/ajas.4.2.13574

Mou, M. A., Khatun, R. and Farukh, M. A. (2018). Water quality assessment of some selected hatcheries at shambhuganj Mymensingh. *Journal of Environmental Science & Natural Resources*, *11*(1&2), 235-240. https://doi.org/10.3329/jesnr.v11i1-2.43390

Naeem, M., Zuberi, A., Salam, A., Ashraf, M., Elahi, N., Ali, M., Ishtiaq, A., Malik, T., Khan, M.J., Ayaz, M.M., Iqbal, M.J., and Ahmad, B. (2011). Induced spawning, fecundity, fertilization rate and hatching rate of Grass carp (*Ctenopharyngodon idella*) by using a single intramuscular injection of ovaprim–Cat a fish hatchery Faisalabad, Pakistan. *African Journal of Biotechnology*, *10*(53), 11048-11053. https://doi.org/10.5897/AJB10.1481

Nowosad, J., Abdel-Latif, H.M.R., Łuczyński, M.J., Boroń, A., Kapusta, A., Kujawa, R., Przybył, A., Kucharczyk, D. (2023). Artificial reproduction of vimba bream (Vimba vimba) cultured generation F2 and eggs management under controlled conditions. *Aquaculture*, 575, 739741.https://doi.org/10.1016/j.aquaculture.2023.739741.

Nowosad, J., Kucharczyk, D., Targońska, K., Wyszomirska, E., Chwaluczyk, R., Kupren, K., (2016). The synergistic effect of temperature and hormonal stimulation on spawning efficiency of common barbel, Barbus Barbus L. Turkish Journal of Fisheries and Aquatic Sciences 16, 517–524. https://doi.org/10.4194/1303-2712-v16_3_04

Paul, S.K., Sarker, S., Sarker, B. S., Alam, M. M., Majumdar, P. R. (2021). Breeding biology and dose optimization for captive breeding of striped dwarf catfish, *Mystus vittatus*, using different hormones. *Iranian journal of fisheries*, 21(1), 104-121. https://doi.org/10.22092/ijfs.2022.125852

Qiang, J., Zhong, C.Y., Bao, J.W., Liang, M., Liang, C., Li, H.X., He, P., & Xu, P. (2019). The effects of temperature and dissolved oxygen on the growth, survival and oxidative capacity of newly hatched hybrid yellow catfish larvae (Tachysurus fulvidraco♀ × Pseudobagrus vachellii♂). *Journal of Thermal Biology*, *86*, 102436. https://doi.org/10.1016/j.jtherbio.2019.102436

Rach, J.J., Sass, G.G., James, A.L. & Mark, P.G. (2010). Effects of Water hardness on size and hatching success of silver carp eggs. *North American Journal of Fisheries Management*, *30*(1), 230-237. https://doi.org/10.1577/M09-067.1

Rahman, A.K.A. (2005). *Freshwater Fishes of Bangladesh*, 2nd ed., Zoological Society of Bangladesh, Dhaka, Bangladesh, xviii+394pp. https://en.bdfish.org/2010/11/book-freshwater-fishes-bangladesh/

Rahman, M.M., Hossain, M.Y., Hossain, M.I., Provhat, S.J., Islam, M.S., & Hossain, M.B. (2013). Induced Breeding of the Stinging Catfish, Heteropneustes fossilis: Comparison among Different Inducing Agents. *Turkish Journal of Fisheries and Aquatic Sciences*, *13*(3), 523-527. http://doi.org/10.4194/1303-2712-v13_3_17

Rajaguru, A. (1992). Biology of two co-occurring tonguefishes, *Cynoglossus arel* and *C. lida* (Pleuronectiformes: Cynoglossidae), from Indian waters. *Fishery Bulletin*, *90*(2), 328-367. https://fisherybulletin.nmfs.noaa.gov/content/biology-two-co-occurring-tonguefishes-cynoglossus-arel-and-c-lida-pleuronectiformes

Randall, D.J. and Tsui, T.K.N. (2002). Ammonia Toxicity in Fish. *Marine Pollution Bulletin*, 45, 17-23. https://doi.org/10.1016/S0025-326X(02)00227-8

Rashid, M., Balkhi, M.H., Naiko, G.A., Ahamad, T. (2014). Induced breeding of grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) using ovatide as synthetic hormone at national fish seed farm (Nfsf) manasbal, Kashmir, J&K. *Fisheries and Aquaculture Journal*, *5*(4), 1000110. https://doi.org/10.4172/2150-3508.1000110

Saidin, T. B., Ahmad, A. B. O., & Sulaiman, M. Z. B. (1988). Induced spawning techniques practised at Batu Berendam, Malaka, Malaysia. *Aquaculture, 74*(1-2), 23-33. https://doi.org/10.1016/0044-8486(88)90082-8

Salman, N. A., Al-Noor, S. S., Khalaf, K. A. (2023). Comparative study of hormonal stimulation of hCG, PG-extract and ovaprim on thermal accumulation period and egg production in grass carp *Ctenopharyngodon idella*. *International Journal of Aquatic Research and Environmental Studies*, *3*(2) 99-108. https://injoere.com/article-1-79-en.html

Sapkale, P.H., Singh, R.K. & Desai, A.S. (2011) Optimal water temperature and pH for development of eggs and growth of spawn of common carp (*Cyprinus carpio*). *Journal of Applied Animal Research*, *39*(4), 339-345. https://doi.org/10.1080/09712119.2011.620269

Shaddoud, R., Saad, A., and Badran, M. (2023). Induced spawning of grass *Carpctenopharyngodon* idella, using common carp pituitary extract with domperidone. *Asian Journal of Biology*, *17*(4), 19-30. https://doi.org/10.9734/AJOB/2023/v17i4328

Sheha, M. A., Darwish, S. T., El-Gamal, A. E., & Barakat, R. O. (2021). Histological studies on the testicular cycle of grass carp, Ctenopharyngodon idella in related to gonadotrophic cells activity of the pituitary gland. *Egyptian Journal of Aquatic Biology and Fisheries*, *25*(1), 141–160. https://doi.org/10.21608/ejabf.2021.145789

Shireman, J. V. and Smith, C. R. (1983). Synopsis of biological data on the grass carp, *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844). In: *FAO Fisheries Synopsis*, No. 135iv + 86pp. http://www.fao.org/3/a-ap938e.pdf

Siddique, M. A. B., Ahammad, A.K. S., Bashar,A., Neaz, A. H., Mahalder, B., Alam, M. M., Biswas, J. C., Haque, M.M. (2022). Impacts of climate change on fish hatchery productivity in Bangladesh: A critical review. *Heliyon*, *8*(2), e11951. https://doi.org/10.1016/j.heliyon.2022.e11951

Stott, B. & Cross, D. G. (1973). A note on the effect of lowered temperatures on the survival of eggs and fry of the grass carp *Ctenopharyngodon idella* (Valenciennes). *Journal of Fish Biology, 5*(6), 649-658. https://doi.org/10.1111/j.1095-8649.1973.tb04501.x

Szabó, T., Urbányi, B., Müller, T., Szabó, R., László Horváth, L. (2019). Assessment of induced breeding of major Chinese carps at a large-scale hatchery in Hungary. *Aquaculture Reports*, *14*, 100193. https://doi.org/10.1016/j.aqrep.2019.100193

Talwar, P. K. and Jhingran, A. G. (1991). *Inland Fishes of India and Adjacent Countries (Vol.* 1 and 2). Oxford & IBH Publishing Co. Pvt. Ltd., India. 1158pp. https://www.google.com.bd/books/edition/Inland_Fishes_of_India_and_Adjacent_Coun/UG90mc4yD50C?hl=en&gbpv=1

Toomey, L., Giraldo, C., Loots, C., Mahé, K., Marchal, P., & MacKenzie, K. (2023). Impact of temperature on Downs herring (*Clupea harengus*) embryonic stages: First insights from an experimental approach. *PLoS ONE*, *18*(4), e0284125. https://doi.org/10.1371/journal.pone.0284125

Venkatesh, B., Shetty, H.P.C. (1978). Studies on the growth rate of the grass carp *Ctenopharyngodon idella* (Valenciennes) fed on two aquatic weeds and a terrestrial grass. Aquaculture, 13(1), 45-53. https://doi.org/10.1016/0044-8486(78)90126-6