



Optimization of induced breeding, embryonic development and larval ontogeny of Gangetic catfish, *Mystus cavasius*: evaluating the efficacy of exogenous hormones on reproductive performance

Rohit Kumar¹ • Prem Kumar² • Debajit Sarma¹ • Kedar Nath Mohanta² • Sukham Munilkumar¹ • Ajit Kumar Verma¹ • Vinod Kumar Paswan¹ • Gokul S¹ • Atul Kumar Singh¹ • Satendra Kumar³ • Bijay Kumar Behera⁴

¹ Division of Aquaculture, ICAR- Central Institute of Fisheries Education, Mumbai 400 061, Maharashtra, India

² Division of Fish Nutrition, Biochemistry & Physiology, ICAR- Central Institute of Fisheries Education, Mumbai 400 061, Maharashtra, India

³ Division of Aquatic Environment & Health Management Division, ICAR- Central Institute of Fisheries Education, Mumbai 400 061, Maharashtra 400 061, India

⁴ National Fisheries Development Board, Hyderabad 500052, Telangana, India

Correspondence

Prem Kumar; Division of Fish Nutrition, Biochemistry & Physiology, ICAR- Central Institute of Fisheries Education, Mumbai 400 06, Maharashtra, India

 prem.cife@gmail.com

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Abstract

Gangetic catfish, *Mystus cavasius* (Hamilton, 1822) is a commercially important freshwater catfish widely distributed in Southeast Asia. However, commercial farming of this species is hindered due to the unavailability of hatchery produced seed. To address this issue, two separate experiments were conducted. In the experiment one, three doses of human chorionic gonadotropin (HCG; 5, 10, and 15 IU g⁻¹) and commercial hormone (CH; 5, 10, and 15 µl g⁻¹) were administered to optimize the hormone dose for induced breeding. In the experiment two, mature brooders were induced to spawn using the optimum dose of CH (10 µl g⁻¹) to study the embryonic development and larval ontogeny. The results revealed that the optimum doses of HCG and CH were 15 IU g⁻¹ and 10 µl g⁻¹, respectively. Embryonic development showed the appearance of morula, blastula, gastrula, neurula, somitogenesis, and hatching at 1:20, 1:50, 4:55, 8:15, 10:05 and 16:30 hr post spawning (hps), respectively. Newly hatched larvae measured 2.339±0.210 mm in total length. Larvae were characterized by the presence of an oval shaped yolk sac (volume: 0.221±0.114 mm³) devoid of oil globules. Major morphological features observed during larval ontogeny include the formation of chin barbels, caudal fin rays, notochord, mouth opening and anal pore opening at 6 hps, 18 hps, 1, 2, 3, day post hatching (dph), respectively. The findings suggested that CH at a dose of 10 µl g⁻¹ and HCG at a dose of 15 IU g⁻¹ were the effective doses for induced breeding and seed production of *M. cavasius*.

Keywords: embryonic development; hormones; induced breeding; larval ontogeny; *Mystus cavasius*

1 | INTRODUCTION

Aquaculture and fisheries together supply a significant quantity of aquatic products globally, with total aquatic animal production reaching 195 million tonnes of which 103 million tonnes were contributed by aquaculture (FAO 2026). Among the freshwater aquaculture groups, catfishes are considered potential candidate species for diversification and production expansion (Em *et al.* 2026). Current global catfish production is 7.28 million tonnes, which is comparatively lower than the total carp production (34.18 million tonnes) (FAO 2026). A major challenge to the development of catfish aquaculture is the inadequate availability of sufficient quantities of hatchery produced seed (Khan *et al.* 2022; Barasa *et al.* 2024). Reliable seed production and effective larval rearing are essential for the expansion of catfish aquaculture (Sarker *et al.* 2026).

Among the catfishes, Gangetic catfish, *Mystus cavasius* (Hamilton, 1822) is considered a promising species for aquaculture diversification (Khan *et al.* 2022; Sabuz *et al.* 2024). *Mystus cavasius* belongs to the family Bagridae and is widely distributed across South and Southeast Asia (Banu *et al.* 2020). Owing to its high nutritional value and strong consumer preference, this small indigenous fish species (SIFS) has high market demand and potential for aquaculture diversification (Harun-Ur-Rashid *et al.* 2021; Sabuz *et al.* 2024). Despite these attributes, the culture of *M. cavasius* remains largely dependent on wild seed resources, highlighting the need for reliable hatchery-based seed production. Only limited numbers of published literature are available on the captive breeding of *M. cavasius* using different inducing agents such as Ovupin (0.5 ml kg⁻¹, Ali *et al.* 2021), OvatideTM (0.2 ml kg⁻¹, Das *et al.* 2018) and pituitary gland extract (PGE) (10 mg kg⁻¹, Rahman *et al.* 2004). Moreover, there is no report available on the use of human chorionic gonadotropin (HCG) for induced breeding of this species.

Commonly used hormones for induced breeding in fish include PGE, HCG, and commercial formulations based on gonadotropin releasing hormone analogue (GnRHa) (Nica *et al.* 2022). Among these, PGE is a widely used inducing agent for catfish breeding (Szabo *et al.* 2023). However, its year-round availability is inconsistent, and preparation of its extract is time-consuming and cumbersome. Therefore, HCG and commercial hormones (CH) are widely used (Aktar *et al.* 2014). In the hypothalamus-pituitary-gonads (HPG) axis, HCG directly acts on the gonads (Phelps *et al.* 2010), whereas CH acts on the pituitary gland to stimulate the release of endogenous gonadotropin (Elakkanai *et al.* 2015). Successful and effective induced breeding and quality seed production depend on the dose and type of hormones (Singh *et al.* 2023). The use of suboptimal dose of hormone leads to an increased latency period, incomplete ovulation, poor gamete quality and non-synchronization in spawning, whereas supra-

optimal hormonal dose triggers hydration of premature oocytes and plugging of the genital vent and mortality (El-Hawarry *et al.* 2016). Response to hormonal induction is species-specific and it may vary with the maturity stages and environmental condition. Extensive research has been carried out on the induced breeding of commercially important catfishes through the use of different kinds and doses of hormones. The reported effective doses of CH for induced breeding in catfishes are 20 µl g⁻¹ in *Mystus gulio* (Kumar *et al.* 2021a), 1.5 ml kg⁻¹ in *Clarias batrachus* (Rajbongshi *et al.* 2020), 1.2 ml kg⁻¹ in *Ompok bimaculatus* (Mishra and Rawat 2017), 0.5 ml kg⁻¹ in *Heteropneustes fossilis* (Rahman *et al.* 2013). Similarly, the effective doses of HCG for induced breeding in catfishes are 10 IU g⁻¹ in *M. gulio* (Kumar *et al.* 2021), 6 IU g⁻¹ in *C. gariepinus* (RS Zidan *et al.* 2020), 3000–4000 IU kg⁻¹ in *C. batrachus* (Sahoo *et al.* 2008).

Successful induced spawning alone does not ensure reliable seed production of a fish species. Therefore, the knowledge of embryonic development and larval ontogeny are equally important to understand the embryonic developmental pattern, incubation period, time of first feeding and characteristic pigmentation patterns (Okomoda *et al.* 2017; Abhilash *et al.* 2025). With this background, the present study aimed to determine the optimal doses of HCG and CH for the induced breeding of *M. cavasius* and to generate a comprehensive database on its embryonic development and larval ontogeny.

2 | METHODOLOGY

2.1 Broodstock development

Broodstock of *M. cavasius* were raised in a cemented tank (5 m × 4 m × 1.5 m) with a 6–8 cm soil base at the wet laboratory of ICAR-Central Institute of Fisheries Education, Mumbai, India. Before stocking, the cement tanks were prepared following the standard protocol of pond preparation viz. draining, sun drying, water filling and liming at the dose of 250 kg ha⁻¹. In the month of March, advanced fingerling (500 numbers, mean weight: 5.56±1.25 g, mean length: 6.34±1.00 cm) were collected from the wild (Hooghly River, West Bengal, India) and transported in closed containers to the experimental site. Before transferring to the acclimation tank (5000 L FRP tank), fish were given 50 ppm formalin bath for 3 minutes to get rid of ectoparasites (Kumar *et al.* 2018). During this treatment no mortality was observed. After seven days of acclimation, around 300 healthy fish individuals were stocked in the well prepared broodstock holding cement tank, and reared for three months in natural photoperiod. During rearing, fish were fed twice daily to the satiation with a commercial pelleted feed containing 32% crude protein and 6% lipid.

2.1.1 Maturity assessment: After three months of rear-

ing, the maturity of the male fish was assessed by the presence of pointed and reddish genital papilla whereas the maturity of the females was assessed through ovarian biopsy (Kumar *et al.* 2021b).

2.1.2 Ovarian biopsy: A total of 30 female fish individuals were randomly collected from the broodstock rearing tank for ovarian biopsy. The ovarian biopsy was performed to collect the oocytes by using a sterile polyethylene cannula (diameter: 1.80 mm). Around 30 oocytes were immediately measured with help of a trinocular microscope (Radical RXLr-5, India) at 4X magnifications (mosaic 2.4).

2.1.3 Gonadal histology: To understand the gonadal tissue architecture of ovary and testis, six numbers of mature male and female fish were sampled. Fish were euthanized with overdose of MS-222 and dissected to collect the gonadal tissues. The collected gonads tissues (size, 6 × 6 mm) were fixed in 10% neutral buffer formalin (NBF) for a week. During fixation periods, NBF was changed on alternate days. Further, the fixed tissues were processed as per the procedure described by Pradhan *et al.* (2012). In brief, tissue preserved in NBF solution were rinsed overnight in tap water and dehydrated gradually in graded ethanol series (70%, 95%, and 100%), followed by xylene treatment and paraffin embedding. The embedded samples were sectioned at a thickness of 5 µm with aid of microtome. The tissue section were dewaxed in xylene, stained with haematoxylin-eosin (HE) stain, and mounted with DPX to observe under light microscope. Histological observation of tissue slides was made using a compound light microscope (Lawrence, LM521803S) and photomicrographs were analysed using microscope assisted software (Mosaic 2.4).

2.2 Experiment one: optimization of hormone dose

Prior to breeding, selected mature male and female brooders were disinfected with formaldehyde (50 ppm) for two to three minutes to get rid of ectoparasites. The induced breeding experiments were conducted during the peak spawning season (July) under a natural photoperiod (12L:12D). Two different inducing hormones (CH and HCG) at three different doses were evaluated in triplicates ($n = 3$). The first trial was conducted to optimize the dose of HCG, where females (27 individuals) were administered HCG at doses of 5, 10, and 15 IU g⁻¹ body weight and half the dose was given to males (54 individuals). In the second trial, females (27 individuals) were administered with CH at the doses of 5 µl g⁻¹ body weight (200 µg sGnRHa + 100 µg domperidone), 10 µl g⁻¹ body weight (400 µg sGnRHa + 200 µg domperidone), and 15 µl g⁻¹ body weight (500 µg sGnRHa + 250 µg domperidone). Simultaneously, males (54 individuals) received half the dose administered to females. After the administration of

hormone, fish (male and female sex ratio: 2:1) were carefully transferred to spawning tanks (capacity: 700 L) having provision of recirculatory aquaculture system (RAS). In each tank, 15 to 20 egg collectors (nylon fibre) were dispersed.

2.2.1 Breeding performance: Breeding performance in response to hormonal induction was assessed based on latency period, spawning, relative fecundity, fertilization rate, incubation period, hatching rate, and larval survival (Kumar *et al.* 2018, 2019).

Latency period (hr) = Time of injection (hr) – Time of spawning (hr)

Spawning response (%) = (Number of spawned fish / Number of injected fish) × 100

Relative fecundity (eggs g⁻¹ female body weight) = (total number of egg spawned / Weight of female g).

Fertilization rate (%) = (Number of fertilized eggs / Total number of eggs counted) × 100

Incubation period (hr) = Time period between fertilization and till hatching

Hatching rate (%) = (Number of hatched eggs / Total number of fertilized eggs) × 100

Larval survival (%) = (Live larvae number in sample / Total number of the larvae) × 100

2.3 Experiment two: embryonic development and larval ontogeny

To study the embryonic development and larval ontogeny, one more breeding trial was conducted using the optimum dose of CH (10 µl g⁻¹ body weight). In this experiment, 20 mature males and 10 females (male: female sex ratio 2:1) were selected and acclimatized in a breeding tank (700 L water volume, fitted with RAS) for 24 hr. Following acclimatization, the breeding trials were conducted according to the protocol described in the first experiment. After spawning, the egg collector containing adhesive eggs were carefully transferred to incubation tanks (500 L). To monitor embryonic development, approximately 30 to 50 eggs were sampled from the incubation tank. During the first hour, eggs were sampled at 10 minutes intervals and later at 45 minutes intervals to until hatching. Whenever distinct developmental changes were observed, images were captured with the aid of the microscope (Radical RXLr-5, India) at 4× or 10× magnifications (mosaic 2.4). Immediately after hatching, larvae were carefully shifted to larval rearing tanks, and fed with *Chlorella* sp. (density of 1–5 × 10³ cells ml⁻¹) and newly hatched *Artemia* nauplii. To assess the larval ontogeny, larvae were sampled at 6, 12, 18 hps and at 1, 2, 3, 4 and 5, days post hatching (dph) from the larval rearing tank. Microscopic observations focused on yolk sac utilization, organ development, morphological changes and initiation of exogenous feeding. Morphometric parameters includ-

ing total length (TL), and yolk sac volume were measured using image analysis software (mosaic 2.4). The volume of yolk sac (V) was calculated as: $V = \pi/6 * Lh^2$ (where 'h' is yolk sac height in mm and 'L' is yolk sac length in mm).

2.4 Physicochemical parameters of water

Physico-chemical parameters such as temperature (°C), dissolved oxygen (mg L^{-1}), and pH were measured with a multi-parameter meter (Hanna instrument, USA), whereas total hardness (mg L^{-1}), alkalinity (mg L^{-1}), total ammonia nitrogen (TAN mg L^{-1}) were measured according to APHA (2005). All the physicochemical parameters of water were recorded in broodstock rearing, induced breeding, embryonic development, and larval ontogeny tanks are presented in Table 4.

2.5 Data analysis

Comparison of all the variables of breeding performance was made using one-way analysis of variance (ANOVA). All the analyses were performed using SPSS (version 20.0) compatible for windows. MS-Excel was used to calculate the mean value and standard deviation of data generated from breeding, embryonic development and larval ontogeny.

3 | RESULTS

3.1 Maturity assessment

The mean oocyte diameter of mature females ranged between $850 \pm 12.50 \mu\text{m}$ (Figure 1A). The gonad histology observation of mature female and male are shown Figure 1B and 1C, respectively. Histology of ovarian and testis tissue indicates the synchronous type of oocyte development and the tubular type of testis.

3.2 Experiment one - Breeding performance

The breeding performance in terms of latency period (hr), spawning (%), relative fecundity, fertilization rate (%), incubation period (hr), fertilized egg diameter (μm), hatching rate (%) and larval survival (%) with CH and HCG are summarized in Table 1. Among CH groups, the amount of $10 \mu\text{L g}^{-1}$ body weight (T2) gave significantly ($p < 0.05$) higher relative fecundity (2282.33 ± 17.95), fertilization rate ($82.34 \pm 5.08\%$), hatching rate ($81.50 \pm 3.33\%$), and larval survival ($58.13 \pm 1.39\%$) compared to the other groups. The longest latency period (11.08 ± 0.04 hr) was recorded at the lowest dose ($5 \mu\text{L g}^{-1}$) and the shortest (9.90 ± 0.03 hr) at the highest dose ($15 \mu\text{L g}^{-1}$) of CH.

In the HCG groups, a dose of 15 IU g^{-1} body weight (T3) induced 100% spawning with significantly ($p < 0.05$) higher fecundity (2112.00 ± 33.17), fertilization rate ($74.33 \pm 3.76\%$), hatching rate ($81.40 \pm 2.91\%$), and larval survival ($53.47 \pm 2.08\%$) than the other lower doses. Similar to CH, the latency period decreased with increasing hormone dose, ranging from 12.58 ± 0.21 hr at 5 IU g^{-1} to

10.65 ± 0.18 hr at 15 IU g^{-1} . There was no significant ($p > 0.05$) difference in incubation time and fertilized egg diameter across all HCG and CH doses.

3.3 Experiment two- Embryonic development and larval ontogeny

3.3.1 Embryonic development: The specific characteristics of embryonic development of *M. cavasius* are summarized in Table 2 and FIGURE.1 (Figure 1D–1O). Fertilized eggs (mean diameter: $1035 \pm 15 \mu\text{m}$) were transparent, spherical, and enclosed by a gelatinous membrane, which imparted a slightly adhesive nature (Figure 1D). Unfertilized eggs were whitish and opaque. Around 10 to 15 min after fertilization, the fertilized eggs began to swell, resulted in a slight increase in egg diameter by 10%. Cell division was first observed 15 min after fertilization near the animal pole of the fertilized egg. First cleavage formed two blastomeres, and subsequent division led to formation of the 4 cell, 8 cell, 32 cell, and 64 cell stages (Figure 1E). After 64 cell stage, counting of blastomere cells became difficult. After 1.20 hps, the fertilized egg reached the morula stage, characterized by irregular division of blastomeres and formation of a ball-like structure over the top of yolk mass (Figure 1F).

The blastula stage was observed at 1:50 hps and lasted till 3:05 hps. The early blastula stage was characterized by the formation of a blastodisc composed of loosely arranged blastomere positioned over the yolk mass (Figure 1G). During the late blastula stage, a distinct yolk syncytial layer (YSL) was observed following the fusion of marginal blastomeres with yolk sac (Figure 1H). Gastrulation commenced at 4:55 hps with the proliferation and expansion of blastoderm cells toward the vegetal pole, indicating the onset of epiboly. Different levels of epiboly including 50%, 70%, and 90% are showed in Figure 1I–1K, respectively. Gastrulation was completed at 7:50 hps, which was characterized by the formation of germ ring and closure of the blastopore (1L).

Somitogenesis was characterized by the development of somite blocks in the embryonic axis (10.05 hps). Somites first appeared in the cephalic region and subsequently extended to caudal region. At the 3–4 somites stage, brain vesicle became deciphered (Figure 1L). At the 13–14 somite stage, the optic vesicle and notochord were clearly observed, and the first heart beat (70 min^{-1}) was recorded (Figure 1M). At the 25–26 somite stage, distinct brain parts including forebrain, midbrain and hind brain, were observed, together with detachment of caudal region from the yolk sac and the first muscular twitching at 8:05 hps. The rate of muscular twitching was $6\text{--}8 \text{ min}^{-1}$ (Figure 1N). Pre-hatching embryos exhibited vigorous contractions, particularly in the caudal region, and both radial and rotational movements were recorded. Caudal flexion occurred at an interval of approximately 38 sec. At this stage, the embryos exhibited an average heart

rate of 60 beats min⁻¹. Hatching commenced at 16:30 hps and completed within 30–40 min (Figure 1O).

3.3.2 Larvae ontogeny: During hatching, the tail emerged first through the chorion. The total length of newly

hatched larvae was 2.339±0.210 mm (Figure 1P). Details of larval development and organogenesis of from 0 to 5 day post hatching (dph) are summarized in Table 3 and Figure 1P–1X.

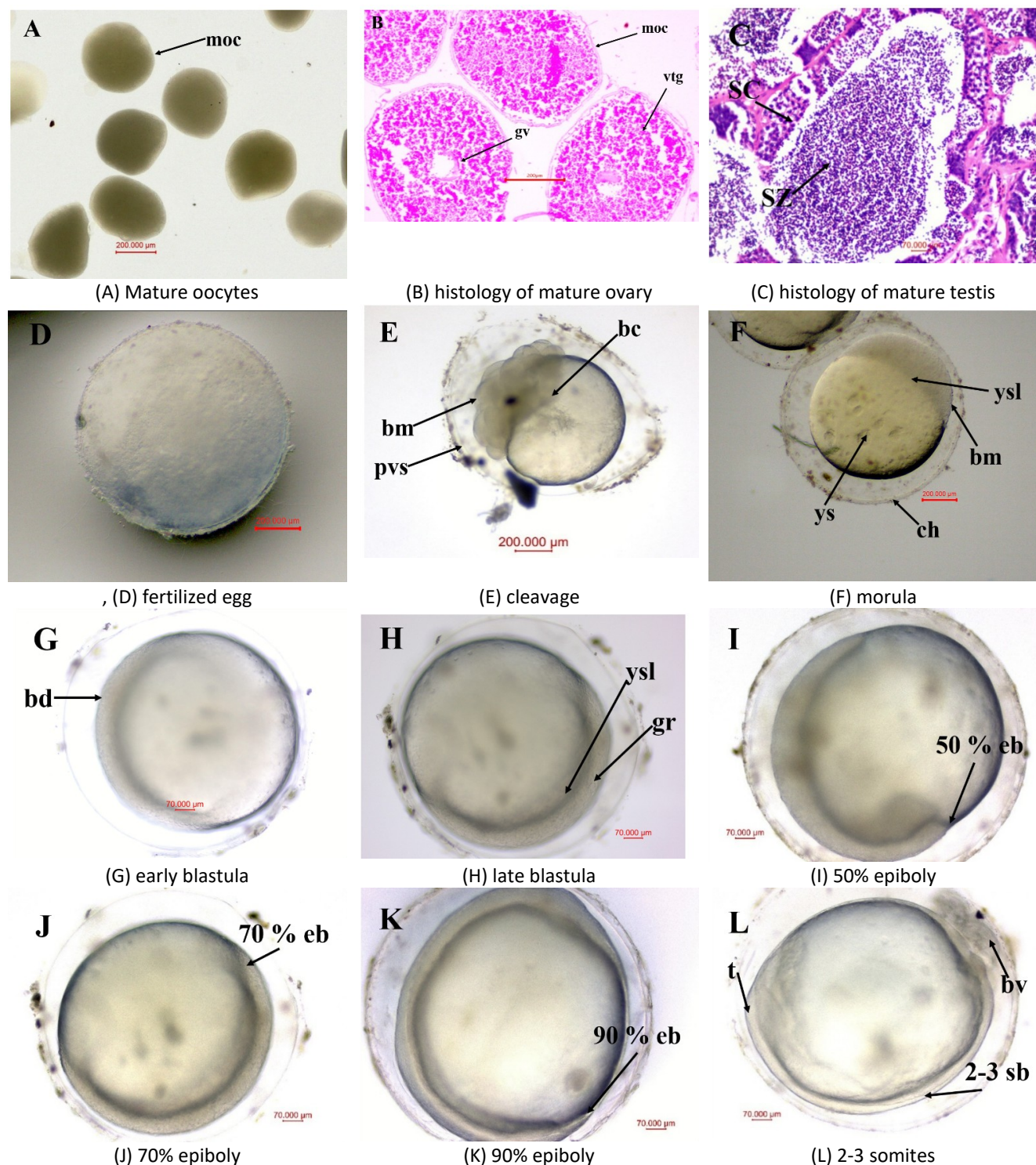


FIGURE 1 Mature oocytes, gonadal histology, embryonic development, and larval ontogeny of the Gangetic catfish, *Mystus cavasius*. Legends are at Page 6.

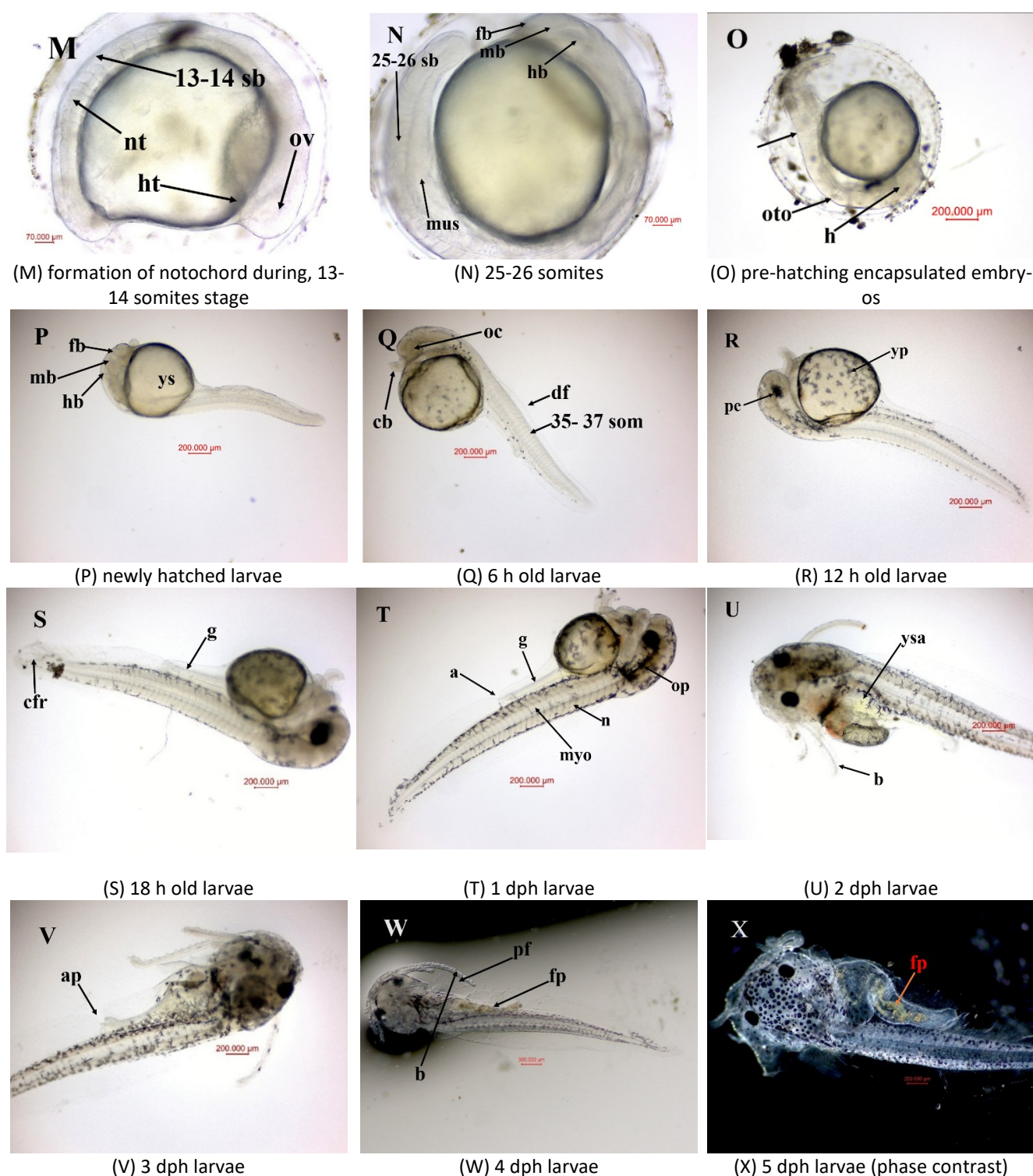


FIGURE 1 Continued.

*Abbreviations: a- anus, ap- anal pore open, bc- blastocoel, bd- blastoderm, b- barbels, bm- blastomere, bv- brain vesicle, ch- chorion, cb- chin barbel, cfr- caudal fin rays formation, df- dorsal fin fold, eb- epiboly, fb- forebrain, fp- Food particles, g- gut, gv- germinal vehicle, gr- germinal ring, gv- germinal vehicle, hb- hindbrain, h- head, ht- heart, mus- musculature, mb- midbrain, myo- myotomes, moc- mature oocytes, nt- notochord, ov- optic vesicle, oc- optic cup, oto- otolith, pf- pectoral fin, pe- pigmented eye, pvs- perivitelline space, sc- spermatocytes, som- somites, sz- spermatozoa, sb- somite block, vtg- vitellogenin, ysl- yolk syncytical layer, ys- yolk sac, yp- yolk sac pigmented, ysa- yolk sac absorbed.

TABLE 1 Effect of different doses of human chorionic gonadotropin (HCG) and commercial hormone (CH) on the breeding performances of Gangetic catfish, *Mystus cavasius*.

Treatment	Latency period (hr)	Spawning (%)	Relative fecundity (eggs g ⁻¹)	Fertilization rate (%)	Incubation period (hr)	Fertilized egg diameter (μm)	Hatching rate (%)	Larval survival (%)
CH								
5 μl g ⁻¹	11.08 ^c ± 0.04	41.66 ^a ± 8.33	2107.33 ^b ± 17.52	56.42 ^a ± 3.59	16.10 ± 0.15	1030.66 ± 0.88	47.10 ^a ± 2.49	54.13 ^b ± 1.70
10 μl g ⁻¹	10.15 ^b ± 0.03	100.00 ^c ± 0.01	2282.33 ^c ± 17.95	82.34 ^b ± 5.08	16.20 ± 0.06	1034.66 ± 1.45	81.50 ^b ± 3.33	58.13 ^b ± 1.39
15 μl g ⁻¹	9.90 ^a ± 0.03	66.66 ^b ± 8.33	1945.67 ^a ± 16.83	47.32 ^a ± 5.82	16.37 ± 0.03	1032.66 ± 1.76	51.40 ^a ± 1.82	25.87 ^a ± 2.58
p-value	<0.001	0.003	<0.001	0.006	0.221	0.216	<0.001	<0.001
HCG								
5 IU g ⁻¹	12.58 ^c ± 0.21	25.00 ^a ± 0.00	693.33 ^a ± 52.07	58.67 ^a ± 2.60	16.27 ± 0.12	1025.33 ± 1.45	63.30 ^a ± 6.82	28.53 ^a ± 1.19
10 IU g ⁻¹	11.35 ^b ± 0.05	58.33 ^b ± 8.33	1933.33 ^b ± 41.55	72.00 ^b ± 1.73	16.05 ± 0.03	1026.00 ± 1.15	77.70 ^b ± 5.65	46.93 ^b ± 1.96
15 IU g ⁻¹	10.65 ^a ± 0.18	100.00 ^c ± 0.00	2112.00 ^c ± 33.17	74.33 ^b ± 3.76	16.10 ± 0.03	1025.33 ± 1.20	81.40 ^c ± 2.91	53.47 ^c ± 2.08
p-value	<0.001	<0.001	<0.001	0.016	0.797	0.914	0.115	<0.001

Mean values were subjected to one-way analysis of variance (ANOVA). Different superscripts letters within the same column indicate significant differences ($p < 0.05$) among hormone dose treatments ($p < 0.005$). Values are expressed as mean ± SE ($n = 3$).

TABLE 2 Embryonic developmental stages, mean development time, and key morphological characteristics of the Gangetic catfish, *Mystus cavasius* (hps, hours post spawning).

Development stages	Average time period (hps)	Key characters
Fertilized eggs	00:00	Spherical in shape, transparent and adhesive in nature
Morula	1:20	Blastodisc formed with multiple blastomeres cells at the animal pole, large yolk mass present below the blastodisc within the chorion
Blastula		
Early blastula	1:50	Uncountable numbers of blastomere cells were deciphered
Late blastula	3:05	Blastocoel appear beneath the blastoderm and germ ring was visible
Gastrula		Characterized by movement of cells.
30% epiboly	4:55	Blastoderm cover 30% of the yolk sphere, Germ ring was more distinct and well defined
50% epiboly	5:40	Blastoderm cover 50% of the yolk sphere, Germ ring thickens.
70% epiboly	6:10	Blastoderm cover 70% of the yolk sphere.
90 % epiboly	7:20	Blastoderm cover 90% of the yolk sphere
Neurula	8:15	Formation of neural parts like brain was seen
Somitogenesis		Characterized by formation of somites
2-3 somites stage	10:05	Appearance of brain vesicle
13-14 somites stage	12:00	Optic vesicle, notochord, and first heart beat seen
25-26 somites stage	14:00	Myotomes stage, with distinct forebrain, midbrain, and hindbrain The caudal region became detached from the yolk sac, and the first muscular movements were observed. Muscular twitching was at the rate of 6–8 min ⁻¹ .
Hatching	16:30	Hatching completed within 30–40 minutes

TABLE 3 Total length, yolk sac volume, and key morphological characteristics of Gangetic catfish, *Mystus cavasius* larvae from 0 to 5 days post hatching (dph).

Larval age	Total length (mm)	Yolk sac volume (mm ³)	Specific characters
0 dph	2.339±0.210	0.221±0.114	Newly hatched larvae were un-pigmented and characterized by presence of single large yolk sac, without oil globules, as well-differentiated brain and a tubular heart (Figure 1P). 6 hps larvae are characterized by presence of chin barbels, rudimentary gut, 35–37 somites, dorsal fin fold, optic cup, and less melanophores (Figure 1Q). 12 hps larvae are characterized by presence of pigmented yolk sac, and pigmented eye (Figure 1R). 18 hps larvae had caudal fin rays (Figure 1S).
1 dph	2.883±0.031	0.121±0.047	Yolk sac was detached from ventral side of head, a higher concentration of melanophores was observed in the head region, and notochord was visible (Figure 1T).
2 dph	3.312±0.019	0.081±0.012	Mouth opened, yolk sac absorbed (Figure 1U).
3 dph	3.824±0.037	Nil	Melanophores were distributed across the body surface, with a higher concentration in the caudal and head regions. The barbels were elongated yolk sac was absorbed and anal pore opened (Figure 1V).
4 dph	4.468±0.054	Nil	Gut filled with food particles (Figure 1W).
5 dph	5.274±0.021	Nil	Barbels elongated, pectoral fin seen, and food particles present in the digestive tract (Figure 1X).

TABLE 4 Physio-chemical parameters recorded during broodstock rearing, induce breeding, incubation, and larvae ontogeny of the Gangetic catfish, *Mystus cavasius*.

Parameters	Broodstock rearing	Induce breeding	Embryonic development	Larval rearing
Temperature (°C)	26.50±2.10	27.05±1.50	27.20±1.10	27.77±0.14
Dissolved oxygen (mg L ⁻¹)	5.50±1.10	6.5±1.14	6.5±0.5	6.78±0.18
pH	7.20±1.10	7.90±0.05	7.80±0.5	7.85±0.5
Total hardness (mg L ⁻¹)	75.50±2.10	93±0.03	90.32±0.05	95.64±0.04
Total alkalinity (CaCO ₃ mg L ⁻¹)	98.38±2.10	111±0.02	105±0.01	113.26±0.02
Total ammonia nitrogen (mg L ⁻¹)	0.30±0.20	0.01±0.01	0.21±0.12	0.03±0.07

Mean values of all the assays were subjected to one-way analysis of variance. Values are expressed as mean ± SE (*n* = 3).

4 | DISCUSSION

4.1 Maturity assessment

Ovarian biopsy showed that the mature female had a mean oocyte diameter of 850±12.50 µm, which is close to the mature oocyte diameter of *M. gulio* (Kumar *et al.* 2018). Histological observation of the matured ovary showed that oocyte growth in *M. cavasius* was of the synchronous type, which is similar to that observed in most catfish species (Uno *et al.* 2025). Mature testis histology revealed the presence of large number of spermatozoa in the centre of the tubule and a few spermatocytes around its periphery, which is similar to the common pattern of testis histology in other teleosts (Mechaly *et al.* 2025).

4.2 Breeding performance

HCG and CH are commonly used hormones for fish oocyte

maturation, ovulation and spawning (Nargesi *et al.* 2023; Attia *et al.* 2025). The CH, consist of a GnRH analogue (GnRHa) and dopamine antagonist. The GnRHa acts on the pituitary gland to stimulate the release of gonadotropins from the pituitary gland. Further, CH allows greater integration of reproductive regulation and associated physiological processes due to its action at a higher level in HPG axis (pituitary gland). This gives sufficient time (longer latency period) to accomplish the reproductive physiological process of ovulation and spawning (Mechaly *et al.* 2024). Contrary to this, the HCG acts directly on the gonads by mimicking endogenous gonadotropins and typically results in a shorter latency period (Singh *et al.* 2023; Yaqoob *et al.* 2025). In the present study, the dose of CH (10 µl g⁻¹ body weight) induced 100% spawning in females, which resulted in superior breeding performance in terms of higher relative fecundity, fertilization rate,

hatching rate, and larval survival. These findings are consistent with earlier studies in *H. fossilis* (CH $0.4 \mu\text{l g}^{-1}$, Mahalder et al. 2023), *M. gulio* (CH $20 \mu\text{l g}^{-1}$, Kumar et al. 2021a), and *Pangassius pangasius* (CH $1.0 \mu\text{l g}^{-1}$, Fero-sekhan et al. 2015). However, both suboptimal ($5 \mu\text{l g}^{-1}$ body weight) and supra-optimal doses ($15 \mu\text{l g}^{-1}$ body weight) of CH resulted in poor reproductive performance. Supra-optimal doses of CH led to reduced breeding success, which might be due to excess and faster secretion of gonadotropins from pituitary gland. The excess gonadotropin would have hydrated the oocytes faster and plugged the genital pore (Black and Black 2013).

In the present study, the supra-optimal CH dose of $15 \mu\text{l g}^{-1}$ body weight resulted in lower fecundity, fertilization rate, hatching rate, and larval survival. The higher dose of CH would have triggered the release of immature oocytes, and the fertilization of immature oocytes would have negatively impacted the rate of fertilization and embryonic development. Furthermore, the reduced larval survival at higher CH doses could be attributed to the fertilization of physiologically immature oocytes, which lacked sufficient nutrient reserves for proper organogenesis. Similar observations were made in *Channa striata* (Bagra et al. 2024), *M. gulio* (Kumar et al. 2021a), and *C. batrachus* (Sahoo et al. 2008). This compromised the oocyte quality and led to reduced fertilization rates and poor larval survival. The dose of HCG at 15 IU g^{-1} body weight in female fish triggered the spawning of all females (100% spawning). This dose also resulted in significantly superior breeding performance in terms of shorter latency period ($10.65 \pm 0.18 \text{ hr}$), higher fertilization rate ($74.33 \pm 3.76\%$), hatching rate ($81.40 \pm 2.91\%$), and larval survival ($53.47 \pm 2.08\%$). Similarly successful induced breeding using the HCG has been reported in *M. gulio* (10 IU g^{-1} , Kumar et al. 2021a), *Silurus asotus* (10 IU g^{-1} , Kumakura et al. 2003), *Pseudoplatystoma fasciatum* ($5\text{--}10 \text{ IU g}^{-1}$, Leonardo et al. 2004), and *C. batrachus* (0.8 IU g^{-1} , Zairin Jr et al. 1992). The suboptimal HCG doses of 5 and 10 IU g^{-1} body weight resulted in lower spawning rates of 25.00% and 58.33%, respectively, which might be due to an insufficient quantity of gonadotropin required for captive spawning of *M. cavasius*. Consequently, these suboptimal doses were associated with longer latency periods, lower fertilization rates, reduced hatching, and decreased larval survival. This observation is supported by the findings of earlier researches (Black and Black 2013; Mandal et al. 2016; Quyen et al. 2025).

4.3 Embryonic development and larval ontogeny

Understanding the basic biology of embryonic and larval ontogeny is essential for effective feeding management and larval rearing, and it varies among fish species as well as with environmental conditions (Olaniyi and Omitogun 2014). In the present study, fertilized eggs were transparent, adhesive, and round in shape, which is similar to the

fertilized eggs of other catfish species such as *Horabagrus nigricollaris* (Abhilash et al. 2025); *H. fossilis* (Mahalder et al. 2023); *Silurus glanis* (Adakebaikie et al. 2025); *M. gulio* (Kumar et al. 2018) and *C. batrachus* (Rahman et al. 2011).

The mean diameter of fertilized egg of *M. cavasius* recorded in this study ($1.035 \pm 0.15 \text{ mm}$) was smaller than that of *H. fossilis* and *C. batrachus*, ($1.3\text{--}1.5 \text{ mm}$; Puva-neswari et al. 2009; Rahman et al. 2011), and *M. gulio* ($1.1 \pm 0.13 \text{ mm}$; Kumar et al. 2018). However, Rahman et al. (2004) reported that fertilized eggs of *M. cavasius* were $0.49\text{--}0.51 \text{ mm}$ in diameter and pinkish in colour. Such variation might occur as a complex result of surrounding ecosystem conditions. This suggests that environmental variation plays an important role in determining egg size and characteristics. In the current study, cleavage was of the meroblastic type, which was similar to that in other teleost (Blagrove et al. 2026). Half mulberry shaped, uncountable blastomere cells were noticed at the animal pole of the morula stage of embryonic development. This stage reached at 1:20 hps. In contrast, Rahman et al. (2004) reported that the morula stage reached 3:30 hps. Blastula started at 1:50 hps and ended at 3:05 hps, which is characterized by the formation of blastodisc YSL. Similar observation was made by Das et al. (2018); they noticed the blastula stage at 3:20 hps in *M. cavasius*. Transition from blastula to gastrula stage was characterized by the movement of embryonic cells, formation of germ ring, and the closer of blastopore. The closure of blastopore marked of end of gastrulation.

In this study, we observed that the movement of germ ring cells over the yolk sac, which completely covered the yolk mass at 7:40 hps. Contrary to this, in the same species gastrulation was recorded at 6:01 minutes (Das et al. 2018). In teleost, neurula stage characterized by the formation of neural parts such as head and tail bud (Kimmel et al. 1995). In the current study, the neurula stage was formed at 8:15 hr. Somitogenesis is characterized by formation of somatic block, notochord, and optic vesicle. Other characteristics of this stage include inception of heartbeat, segmentation of brain into forebrain, midbrain and hind brain, and muscular contractions. These characteristics are similar to those observed in other Siluriformes (Reis et al. 2025). The incubation period is strongly dependent on the temperature, being longer at lower temperatures and shorter at higher temperature (Rahman et al. 2011).

In the present study, the incubation period was 16:30 hr at $27.20 \pm 1.10^\circ\text{C}$, which was slightly lower than the observation of Rahman et al. (2004) ($19 \pm 2 \text{ hr}$ at $27\text{--}29^\circ\text{C}$), and higher than the observation of Das et al. (2018) (14 hr at $26 \pm 2^\circ\text{C}$) in the same species. In the present study, hatching initiated with vigorous tail twitching, leading to rupture of the egg membrane and subsequent emergence of tail-first of the larvae. This pattern is similar

to that observed in *M. gulio* (Kumar *et al.* 2018) and *H. fossilis* (Puvaneswari *et al.* 2009). The newly hatched larvae were un-pigmented with a single large yolk sac, inactive and remain settled at the bottom of the hatching tanks. As the larvae grew, the yolk sac was gradually utilized to meet the basic metabolic energy demand for growth and survival. In the present study, we observed that the yolk sac of *M. cavasius* was completely absorbed at end of the 2 dph. The current finding agrees with earlier reports of 2 dph in *M. cavasius* (Rahman *et al.* 2004; Das *et al.* 2018), *M. gulio* (Kumar *et al.* 2018), 72 hr in *H. fossilis* (Puvaneswari *et al.* 2009), and 72 hr in *C. batrachus* (Rahman *et al.* 2011). Notably, in the present study, *M. cavasius* larvae began exogenous feeding (36 hps), which is before the complete exhaustion of yolk sac. Such overlap between endogenous and exogenous feeding has also been documented in *M. gulio* (Kumar *et al.* 2018), *M. macropterus* (Wang *et al.* 1992), and *C. batrachus* (Landge 1995), indicating that the initiation of feeding prior to yolk exhaustion is a common strategy to improve the survival of larvae (Yúfera and Darias 2007; Pradhan *et al.* 2012).

5 | CONCLUSIONS

From the study it is concluded that *Mystus cavasius* can be successfully spawned in captivity through administration of either CH (10 µl g⁻¹) or HCG (15 IU g⁻¹). Newly larvae hatched out from the embryo after an incubation period of 16.30±0.30 hr at 27.20±1.10°C. The newly hatched larvae possessed an oval shaped yolk sac with a single oil globule. *Mystus cavasius* larvae are mixed feeding larvae, which began exogenous feeding before complete exhaustion of the yolk sac.

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ETHICAL APPROVAL

In this study, animals were handled in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA), functioning under the Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, ensuring ethical care and appropriate use (Approval No. 524/GO/ReRcBiBt/S/02/CCSEA).

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Rohit Kumar conducted this experiment, analysis, writing

original draft; Dr. Prem Kumar: Conceptualisation, Supervision and Writing review and editing; Dr. Debajit Sarma: Resources and Funding acquisition; Dr. Kedar Nath Mohanta: Writing – review and editing; Dr. Ajit Kumar Verma: Methodology; Dr. Sukham Munilkumar: Data curation; Vinod Kumar Paswan: Visualization; Gokul S: Formal analysis; Atul Kumar Singh: Software; Satendra Kumar: Validation; Bijay Kumar Behera: Writing review and editing.

AI-USE STATEMENT

The authors did not use any AI to generate, analyse, or interpret data, or to determine the study outcomes.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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- R Kumar**  <http://orcid.org/0009-0006-2331-0249>
- P Kumar**  <http://orcid.org/0000-0003-0668-1794>
- D Sarma**  <http://orcid.org/0000-0002-2425-7017>
- KN Mohanta**  <http://orcid.org/0000-0002-4476-8594>
- S Munilkumar**  <http://orcid.org/0000-0002-4476-8594>
- AK Verma**  <http://orcid.org/0000-0001-7466-1213>
- VK Paswan**  <http://orcid.org/0009-0007-0676-4860>
- Gokul S.**  <http://orcid.org/0009-0005-4090-9264>
- AK Singh**  <http://orcid.org/0009-0008-5442-4948>
- S Kumar**  <http://orcid.org/0009-0000-1305-2545>
- BK Behera**  <http://orcid.org/0000-0003-4960-9768>