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Research Article

Observation of Embryonic and Larval Developmental Stages in Endangered Nona Tengra (*Mystus gulio*) Induced with S-GnRHa

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MAH did field and laboratory works, report writing and reviewing literature. MAH instructed the field and laboratory assays, produced manuscript and revised for publication. AKMMH and BD helped in field and laboratory works. SM and MMI planned and conceptualized the methodology and supervised the works academically.

Keywords
Mystus gulio, S-GnRHa, Induced breeding, Embryonic and larval development

Abstract | *Mystus gulio* is a commercially important estuarine small catfish in Bangladesh and it is prone to decreasing due to natural and anthropogenic changes. Information of induced breeding of this catfish and its embryonic development could contribute to database and conservation approach. The S-GnRHa dose optimization and embryonic-larval development study were conducted in a freshwater condition in order specify optimal dose of synthetic hormones in induced breeding. The synthetic GnRHa was used as induction agent where male injected half of the doses of female and a control without S-GnRHa was assigned. The stages of embryonic development were observed under a photomicroscope. Average fertilization and hatching rates varied from 74.33% to 83.89% and from 72.33% to 85.11% respectively among different treatments and control females did not ovulate. The eggs of *M. gulio* was firmly adhesive and immediately after spawning the average diameter of fertilized eggs were 0.48±0.01 mm. After fertilization, the first cleavage stage of *M. gulio* occurred within 40 min. The distinctiveness of blastomeres was progressively dropped and morula, blastula, and gastrula stages were found at 3:40 h, 4:20 h and 5:00 h, respectively after fertilization. The larvae-initiated hatching at around 21:00 h after fertilization and total length of larvae were computed 1.11±0.01 mm while the temperature of water was 30±1°C. The present study revealed that application of S-GnRHa hormone at dose of 0.5 ml kg^{-1} body weight of female could be efficacious for production of *M. gulio* seed in freshwater successfully.

Novelty Statement | This research work is very first approach to breed *Mystus gulio* by using S-GnRHa in freshwater environment and to observe their early development stages.

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Introduction

Induced breeding is a method whereby using pituitary hormone or any other synthetic hormone ripe fish breeders are induced to breed in captivity (Bailung and Biswas, 2014; Kumar *et al*., 2018). Induced breeding technique is advantageous because it provides pure good quality spawn as well as ensures availability of fish seed, whereas availability of seed from natural source is contingent and depends on several environmental factors (Wahab *et al*., 2003; Mollah *et al*., 2008). Artificial propagation of breeding provides key information for the conservation of threatened species, management of cultivation and high productivity

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(Sahoo *et al.*, 2008; Dhara and Saha, 2013; Nakaghi *et al.*, 2014). Embryonic development observation will help to acquire knowledge on early biological characteristics of any fish which could be beneficial for hatchery manager and production programmers of this species (Kohinoor *et al.*, 1991; Bromage *et al.*,2001).

The *M. gulio* contributes a lot to coastal fishery both in commercial aspects and in providing food and nutrition for local community (Kumar *et al.*, 2018; Sakthivel *et al.*, 2013). It is a popular and commercial small indigenous fish species, so now a days its demand is rapidly increasing in Bangladesh (Gupta, 2014; Sarker *et al.*, 2002), but their seed is not available in anywhere. Some initial breeding trial had been approached previously only for brackishwater or salinity prone areas (Sarker *et al.*, 2002; Mijkherjee *et al.*, 2002; Alam *et al.*, 2006; Hossain *et al.*, 2014). Therefore, it is not cost effective to carry brackishwater far away from that of the coastal region for artificial breeding of *M. gulio* and using artificial saltwater in the hatchery might not be economically effective. However, beside the development of induced breeding and culture technique, it is obvious to draw out the embryonic development of *M. gulio* (Kimmel *et al.*, 1995; Korzelecka-Orkisz *et al.*, 2010; Olaniyi *et al.*, 2013). With a view to the management and conservation of *M. gulio*, and to establish seed production technique and culture potentials in freshwater, the present exploration has been held out using S-GnRHa in freshwater and embryonic stages of *M. gulio* were observed as well.

Materials and Methods

Study site and duration

The research was conducted for a three-month duration from June 2017 to August 2017 at a privately owned "Alalpur Hatchery and Fisheries Ltd.", Mymensingh. Major treatment and pond research work of induced breeding was performed at the hatchery facilities of Alalpur Hatchery while observation of embryonic development was carried out at the nearby laboratory facility of Fisheries Biology and Genetics laboratory in Bangladesh Agricultural University, Mymensingh.

Nurturing and selection of brood fish

The experimental pond was 0.2 acre (1 acre = 4046.86 m^2) having 0.9-1.1 meters in depth and brood fish was stocked at intensity of 2500-3000 per acre. During rearing period lime, fertilizer, and cow dung were properly applied. Urea and Triple Super Phosphate fertilizer were used at the level of 20 kg $acre^{-1}$ and 10 kg $acre^{-1}$ respectively at 15 days' interval, then lime was used at the rate of 100 kg/acre and 5-7 days after application of cow dung at 600-700 kg/acre rate for one time. Broodfish were fed with supplementary feed according to the description of Siddiky *et al.* (2015), briefly 5-6% of body weight with feed (moisture 10%, protein 62%, fat 8%, carbohydrate 20%, ash 15%, fiber 1.5%, calcium 2.1%, and phosphorus 2.5%) were given twice a day. Good appearing, healthy sexually mature, and ready to spawn broods were chosen as breeders. Adult male and female brood fishes were recognized by prominent secondary sexual features (Seethal *et al.*, 2016). The mature males were identified by their flat abdomens and protruded pointed genital opening, whereas the female's broods were distinguished by presence of puffy belly as well as round-enlarged urogenital opening. The weight of the matured male and female were ranged between 10-20 g and 17-30 g, respectively.

Hormonal induction of brood fish

The breeding induction were performed by using synthetic S-GnRHa commercially produced by Ningbo Sansheng Pharmaceutical Co. Ltd. and liquid solution were injected beneath the dorsal side and above the lateral line with a 1ml syringe. Then brood fishes were kept simultaneously into the breeding tank with continuous water showering. Each of the tanks were 1.5×0.5×1 m^3 in size and four treatments were assigned as T_1, T_2, T_3 and T_4 each with three replications as well (Table 1). The ratio of male and female was 1:1 in all treatments and fertilization were performed naturally.

Table 1: Induced breeding trial of *M. gulio* with S-GnRHa hormone.

Treatment	Hapa	Dose for female fish (ml kg^{-1}bw) body weight	Dose for male fish (ml kg^{-1}bw) body weight
T_1	H_1, H_2, H_3	0.25	0.125
T_2	H_1, H_2, H_3	0.5	0.25
T_3	H_1, H_2, H_3	1.0	0.5
T_4 (Control) (0.9% NaCl)	H_1, H_2, H_3	1	0.5

Identification of fertilized eggs

The embryonic development had started immediately after fertilization followed by the subsequent hormone treatment and the fertilized eggs appeared wet, bulging as well as somewhat transparent, however, the color of unfertilized eggs was whitish and opaque in appearance (Figure 1).

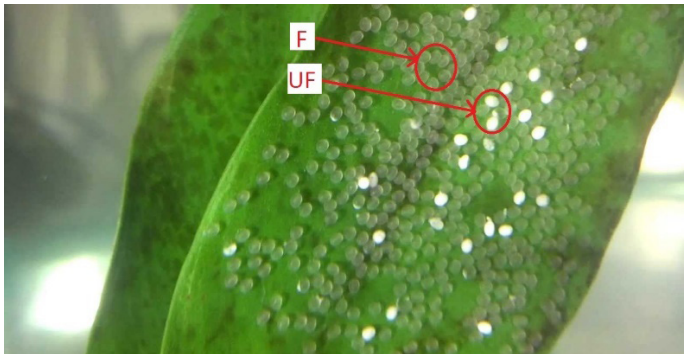


Figure 1: Fertilized (F) and unfertilized (UF) eggs of *M. gulio*.

Calculating the rate of ovulation, fertilization, and hatching

The ovulation rate was determined by using below formula from Legendrea *et al*. (2000).

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

A random sample of 100 eggs were taken from the hatching jar in a dish to determine the fertilization rate. and the rate was calculated by using the description of Unuma *et al*. (2004).

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of both fertilized and unfertilized eggs}} \times 100$$

To calculate the rate of hatching, 100 fertilized eggs were putted in a distinct jar with unceasing water supply. Hatchling numbers were counted manually immediately after completion of hatching and the hatching rate was assessed using following formula (Unuma *et al*., 2004):

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

Observation of embryonic development

The fishes were ovulated at about 7-8 hs of the hormone injection and a few eggs were plotted in a Petridish with water. Study of embryonic development was performed under a dissecting microscope and for that only the fertilized eggs were chosen which contained uniform as well as round yolk sphere with smooth perivitelline space. At regular interval, egg was monitored to document the timing of each embryonic development stage and each stage was captured by using a camera (Rigla-32, Optikam B3 Digital camera, Italy) attached with the microscope (OLYMPUS CX21). The diameter of egg was measured in mm scale by using live sample on "ImageJ" software. Embryonic stages were characterized by following the description of Kumar *et al*. (2018).

Data analysis

A one-way analysis of variance (ANOVA) was followed to determine the significance ($p<0.05$) level of hormonal treatments. The level of significance of the results was tested following Tukey HSD using SPSS (IBM version 26) programming and by using Microsoft Excel 2016.

Results and Discussion

Optimal dose of S-GnRHa and rate of ovulation, fertilization, and hatching

A synthetic gonadotropin-releasing hormone S-GnRHa was used at three distinct doses. For female, doses were 0.25, 0.5 and 1.0-ml kg-1bw, whereas for male were injected as 0.125, 0.25-, and 0.5-ml kg-1bw (Table 2). Among the doses, 0.5 ml kg^{-1}bw for female showed the maximum fertilization rate (83.89%) and hatching rates (85.11%), and this is statistically different from the other doses in female ($P<0.05$) (Table 2). All females were ovulated within 12 hs for all treatment tanks excluding control (Table 2) and the latency period was accounted as 7-8 h. All hormone dose recipient females spawned successfully and hatched between 18:00-21:00 h after spawning. However, statistically there was no significant difference in fertilization rate and hatching percentage among hormone treated groups. The highest fertilization rate was significantly different at $P<0.05$ in T2 (83. 89%) followed by T3 (78. 56%) and T1 (74.33%) as well as the highest hatching rate was significantly different at $P<0.05$ in T2 (85.11%) followed by T3 (74.66%) and T1 (72.33%) (Table 2).

Embryonic development

The fertilized eggs of *M. gulio* were firmly glued. Detailed discloser of embryonic development stages and time interval has been presented in Table 3. The embryonic development stages took place within the chorion and end up with hatching. Membrane of the ovum was disconnected from the rest of the ovum cell by a tiny perivitelline surface. The unfertilized eggs of *M. gulio* were globular, demersal, opaque, and whitish (Figure 2a). They were slightly adhesive. The mean size of unfertilized egg diameter was measured 0.48±0.00 mm. The color of fertilized eggs was brownish, and it appeared as spherical, demersal as well as adhesive to the substratum (Figure 2b). The common diameter of the fertilized egg was observed as 0.48±0.00 mm.

Table 2: Performance of different doses of S-GnRHa hormone on ovulation, fertilization, and hatching rate of *M. gulio*.

Parameter (Mean ± SD)	Treatment			
	T_1 (0.25 ml kg^{-1}bw)	T_2 (0.50 ml kg^{-1} bw)	T_3 (1.00 ml kg^{-1}bw)	T_4 (1.00 ml kg^{-1}bw) (.9%NaCl)
Total length (cm)	11.66 ± 0.75	12.5 ± 0.866	12.33 ± 1	12.00±.866
Body weight (gm)	20.89 ± 4.075	23.33 ± 2.5	22.66 ± 2.179	21.77±1.855
Ovulation rate (%)	100	100	100	-
Fertilization rate (%)	74.33 ±1.658^{c}	83.89 ±1.364^{a}	78.55 ±1.810^{b}	-
Hatching rate (%)	72.33 ± 1.5^{c}	85.11 ±2.088^{a}	74.66±2.121^{b}	-

Note: Values are means of data obtained ± Std. Deviation (mean ± SD) of determinations. Values in the same row with different superscripts are significantly different (P>0.05). The absence of superscripts indicates no significant difference between treatments.

Table 3: Different stages of embryonic development of the fertilized egg and their respective time in *M. gulio*.

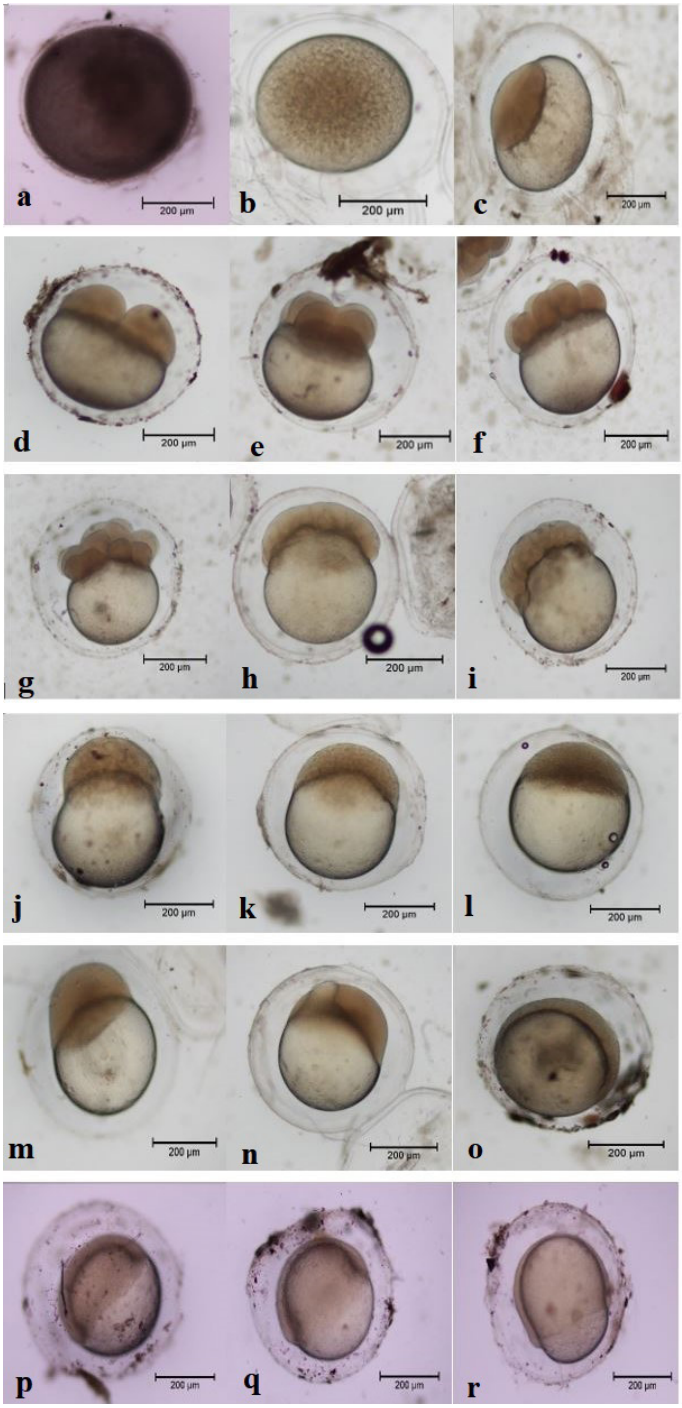
Time after spawning (h: min)	Development stage	Egg size (mm) (mean±sd)	Key description	Figure 2
	Unfertilized egg	0.486±0.0036	Opaque and whitish in color	a
00:00	Fertilized egg	0.486±0.0038	Spherical, demersal, adhesive, watery in color	b
00:15	Blastodics formation	0.488±0.00057	Blastodisc formed at the pole	c
00:40	2 cell stage	0.488±0.00057	First cleavage	d
00:55	4 cell stage	0.489±0.00057	Second cleavage	e
01:15	8 cell stage	0.489±0.00029	Third cleavage	f
01:40	16 cell stage	0.489±0.00034	Fourth cleavage	g
02:00	32 cell stage	0.490±0.00042	Fifth cleavage	h
02:30	Multi-cells	0.490±0.00057	Sixth cleavage	i-n
03:40	Morula stage	0.491±0.00079	Blastulation progresses to form a multicellular blastodisc.	o
04:20	Blastula stage	0.653±0.0021	A third of egg space occupied with blastoderm cells.	p
05:00	Gastrula stage	0.675±0.0023	Blastoderm spread on both the side which covering about 60-70% area and generating a thread like germinal ring	q-r
06:40	Yolk plug stage	0.702±0.0018	Yolk generation complete and cephalic region gets thicker.	s
08:00	Kidney shaped embryo	0.735±0.0025	Elongated embryo with distinct notochord.	u
10:15	Enlarged embryo	0.756±0.00094	The cephalic and caudal end become prominent and visible	v
11:15	Kupffer's vesicle formed	0.797±0.001	Observation of an oval area at the base of the caudal region forming kupffer's vesicle	w
12:30	Optic vesicle developed	0.898±0.0015	The tail becomes separated and optic vesicle fully built.	x
15:30	Rapid twisting movement	0.902±0.0025	Yolk mass segregated into yolk bulb and caudal region become highly active.	y
17:30	Fully active embryo	0.999±0.0015	The egg membrane become decomposed and lost its shape.	z
18:30	Just before hatching	1.00±0.0015	Embryo with the prominent eye and maxillary barbells.	i
21:00	Hatching	1.114±0.0026	Hatching of embryos start.	ii

Blastodisc stage was recognized on the presence of brownish spot on the animal pole in fertilized eggs which appeared within 00:15 h of post-fertilization. The cytoplasm of the egg cell was entirely segregated from the yolk and appeared as a distinct cap or blast disc at the animal (Figure 2c). The average diameter of this stage was 0.48±0.00 mm. At the initial of cleavage stage, the blastodisc was separated into doubled cells within 00:40 h after fertilization (Figure 2d) and the mean diameter was still 0.48±0.00 mm. The second cleavage (4-cell stage) appeared approximately 00:55h after fertilization and average diameter was 0.48±0.00 mm (Figure 2e). Third cleavage stage generated 8 blastomere cells within 1:15 h of fertilization and diameter was recorded as 0.48±0.00 mm (Figure 2f). Construction of blastomere cells increased as time progressed and attained 16 cells, 32 cells and multi cells within 1:40 h, 2:00 h and 2:30 h respectively after fertilization and mean diameter were observed 0.48±0.00, 0.49±0.00, and 0.49±0.00 mm, respectively (Figure 2g, h and i-n).

The morula stage characterized irregular and continuous cell divisions with numerous reduced sized cells. The cells were highly compacted, and thus, cell number counting was next to impossible. As blastomere cells were reduced in extent and the morula stage was attained within 3:40 h after fertilization and average diameter was recorded 0.49±0.00 mm (Figure 2o). Blastula stage of *M. gulio* appeared at about 4:20 h of post-fertilization. The blastula phase was typified by squeezing and compaction of the blast dermal cells (Figure 2p). The blastomeres cells were completely lost their distinctiveness and transferred in both the periphery of animal pole occupying 30% area over the yolk and mean diameter was recorded as 0.65±0.00 mm. In this study, blastomeres started to invade the yolk by proliferating across the yolk in shape of a thin sheet, then 5.00 h after fertilization the gastrulation ring was appeared. At this stage, the blastoderm further spread in both the side which covered about 60-70% area (Figure 2q) and poduce a structure of germinal ring (Figure 2r). The "C" shape embryonic stage appeared at gastrula stage (Figure 2r) and average diameter was observed 0.67±0.00 mm.

The earliest sign of the embryo was visible, and showed beginnings of optic cups after 10:15 h of fertilization (Figure 2v), and average diameter was observed 0.756±0.00094 mm. In about 11:15 h from fertilization, the somites started to appear and the head and tail end was differentiated (Figure 2w). At that moment, the terminal part of anterior and posterior region looked somewhat

round and somites number increased gradually (Figure 2x-z). In this stage, the average diameter was detected 0.898±0.0015 to 0.999±0.0015 mm. At the 15:30 h from fertilization, both tail, and head end were evidently noticeable. The embryo turns out to be lengthened circling around the yolk sphere. The cardiac beating was activated perfectly. Hollow pole of notochord surfaced and some of the embryos shown slight winding movement and the blood circulation could be observed (Figure 2i). After 18:30 h of fertilization, end point of both head and tail region were distinctly recognizable, embryo became elongated and it encircled the sphere of yolk as well as active heart beating continued.Hollow poleof notochord emerged and a few of the embryos showed unusual faint bending movement and the blood circulation was observable (Figure 2i).



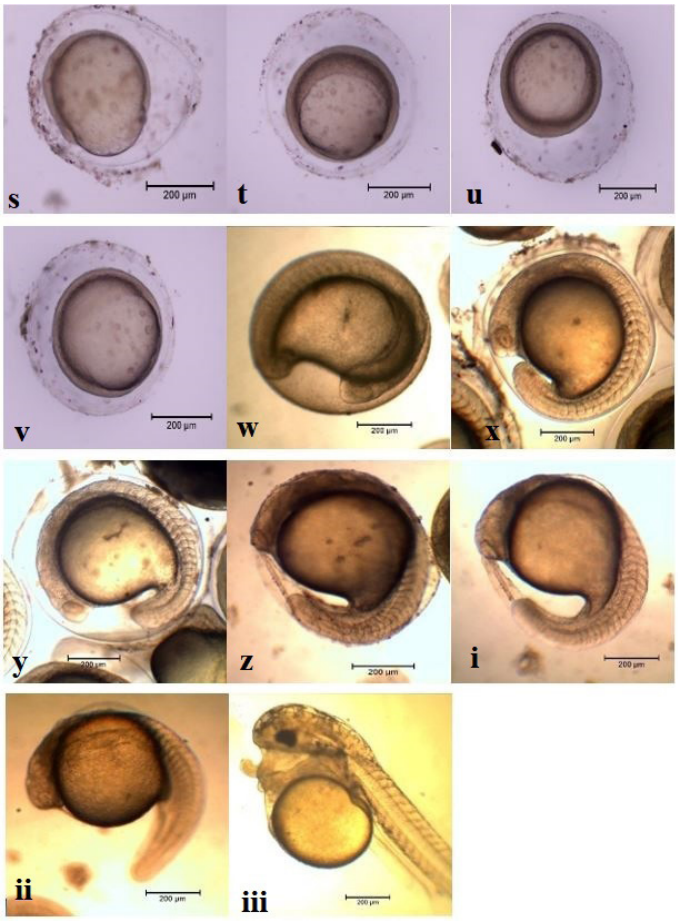


Figure 2: Development stages of *M. gulio* (a) Unfertilized egg, (b) Fertilized egg, (c) Blastodisc, (d) 2-cell stage, (e) 4-cell stage, (f) 8-cell stage, (g) 16-cell stage, (h) 32- cell stage, (i) 64-cell stage, (j) 128-cell stage, (k) 256-cell stage, (l) 512-cell stage, (m) Oblong stage, (n) Sphere stage, (o) Morula stage, (p) Blastula stage, (q) 60% epiboly, (r) 70% epiboly, (s) 80% epiboly, (t) 90% epiboly, (u) Epiboly complete, (v) Bud stage, (w) Somites formation, (x) Segmentation-1, (y) Segmentation-2, (z) Segmentation-3 (i) Just before hatching, (ii) Newly hatched, (iii) larvae 24 hr larvae.

The embryonic development progresses continued, and its movement became stronger gradually, and ultimately the embryo was able to be out of the encompassed membrane. Just before the hatching, embryo pounded speedily by its tail and it's became free, whereas yolk sac was attached to the head portion of embryo. The larvae started hatching at 21:00 h of fertilization. After hatching the larvae was straight and can be differentiated by head, trunk as well as tail and its length was 1.114±0.0026 mm (Figure 2ii).

The size of twenty-four-h larvae calculated typically 3.13±0.054 mm. Three sets of barbells arrived, and the maxillary couple was noticeably distinguished. The average lengths of 24 hs old larvae were 3.13±0.054 mm and three pair of barbells was emerged and among them, maxillary pair was distinctly visible. At this stage, total numbers of myotomes were 35-40 and mouth and anus of the larvae

had started to open as well. Lateral line of the larvae was discernible while the swim bladder as well as nostril also formed already (Figure 2iii-vii).

The latency period with S-GnRHa of *M. gulio* was begin at 7-8 h of hormone treatment and completed at 12 h of post hormone treatment. This finding was found to be little different to some previous research conducted with other hormone at different dosages as well as different environmental conditions (Alam *et al.*, 2006; Begum *et al.*, 2009). The duration of latency period were counted as 7–8 h for *Ompok bimaculatus* (Raizada *et al.*, 2013), 14- to 17h (Sahoo *et al.*, 2005) and 10-12 h (Müller *et al.*, 2020) for *Clarias batrachus* with carp pituitary (CPE) hormonal administration. Catfishes are seemed to have large gap in their latency period with synthetic hormonal injection (Sahoo *et al.*, 2005, 2008). The rate of fertilization have been accounted as 77.5 % with 0.6mg/Kg ovaprim treatment in *Mystus dibrugarensis* (Biswas, 2014), 77.33% with 5 μg g^{-1} of Human Chorionic gonadotropins in *M. gulio* (Kumar *et al.*, 2021). In current study, fertilization rates of T1, T2 and T3 groups were 74.33%, 83. 89% and 78.56%, respectively and this finding is coherent with the research of Alam *et al.* (2006) who found around 85% fertilization rate of *M. gulio* females with ovaprim induction. Some other studies in catfishes also found similar observation as 75% in *Pseudopimelodus charus*, and 80% in *Rhamdia quelen* with synthetic hormone induction (Sampaio and Sato, 2006). The highest and the lowest hatching rate were in present research recorded as 85.11% and 72.33% at the dose of 0.5 ml kg-1 bw and 0.25 ml kg-1bw of S-GnRHa hormone respectively. These figures are also supportive to previous study by Kumar *et al.* (2021) who observe hatching rates 71% for same species whereas Srivastava *et al.* (2012) found 55-60% hatching rate for female *Clarius batachus* with ovaprim treatment. The current study showed that the fertilized egg cell of *M. gulio* were strongly glued due to the presence of sticky jelly on the egg surface and brownish in color which aligned with the annotation from Arockiaraj *et al.* (2003), Puvaneswari *et al.* (2009), and Ferosekhan *et al.* (2015) for catfish ovum. The average thickness of the fertilized egg of *M. gulio* was noted as 0.486 mm in the present study. The average thickness of fertilized eggs of *M. cavasious* was 0.50 mm (Rahman *et al.*, 2004), 3.1 mm for *Pimelodus maculatus* (Sato *et al.*, 2003), 3.6 mm for *Zungaro jahu* (Nogueira *et al.*, 2012) and 1.0 to 1.3 mm for *Ompok pabo* (Sarma *et al.*, 2012). Therefore, egg diameter size is seeming to be species specific and varied among fish to fish.

A prominent reddish spot-on fertilized eggs of *M. gulio* had been reported by several researchers on different fish species (Rahman *et al.*,2004; Ferosekhan *et al.*, 2015). Rahman *et al.* (2004) reported 0.61 mm in diameter of blastodisc in *M. cavacius* which was lower than that of the present study as 0.48 mm. The cleavage stage of *M. cavasius* had attained after 00:45 h to 2:00 h (Rahman *et al.*, 2004) while this stage in *P. pangasius* occurred after 1:07-2:32 h (Ferosekhan *et al.*, 2015), 00:24 h to 1.1 h in *Botia lohachata* (Dey and Barat, 2015). The size of different cleavage stages were varied between 0.48-0.490mm in diameter at present study and this might be happened because of the variation of species and difference in environmental condition at the hatchery. Morula phase in the present study was emerged by 3:40 h post fertilization whereas same stage found at 3:40 h post fertilization in *M. cavasius* (Rahman *et al.*, 2004), at 03:43 h post fertilization in *Pangasius pangasius* (Ferosekhan *et al.*, 2015) and at 2h post fertilization in *Botia lohachata* (Dey and Barat, 2015). This difference may be because of environmental factors as well as different species. Blastula stage of *M. gulio* was appeared at 4:20 h post fertilization, with a mean diameter 0.65 mm in current research, whereas the same stage found at 3-3:30 h post fertilization in *O. pabo* (Chakrabarti *et al.*, 2009; Sarma *et al.*, 2012), 05:12 h post fertilization in *P. pangasius* (Ferosekhan *et al.*, 2015) and at 1.11-3.05 h post fertilization in *B. lohachata* (Dey and Barat, 2015). The "C" shape embryo stages have been reported at 07:27 h after fertilization in *P. pangasius* (Ferosekhan *et al.*, 2015), 3.05 to 6.33 h after fertilization in *B. lohachata* (Dey and Barat, 2015), 5:00 h after fertilization in *M. cavasius* (Rahman *et al.*, 2004) and in between 4 hs for Giant catfish *Heterobranchus bidorsalis* (Olaniyi and Omitogun, 2014). Dey and Barat (2015) reported that somites formed at 6.46 to 14.27 h post-fertilization, however, this stage appeared at 9:00-10:00 h in *O. pabo* (Sarma *et al.*, 2012), at 11:00 h in *P. sutchi* (Islam, 2005). The current research showed that at 11:15 h postfertilization, the somites started to appear and the head and tail end became differentiate. After 15:30 h of fertilization, end of both head and tail of embryo appeared to be distinctly recognizable. Embryo of *M. gulio* became elongated and it encircled the sphere of yolk as well as heart was actively beating. Ferosekhan *et al.* (2015) recorded this stage at 21:30 h post fertilization in *P. pangasius*, Rahman *et al.* (2004) at 15:00 h post fertilization in *M. cavasius,* Khan and Mollah (1998) at 18-22 h post fertilization in *Clarias gariepinus* and Kohinoor *et al.* (1997) at 14 h post fertilization in *O. pabo*. The morula, blastula, gastrula and neurula and organogenesis stages were found to be completed at 1:30, 3:00, 5:30, 7:30 and 17:15 h post-spawning respectively for induced breeding of *Mystus gulio* using human chorionic gonadotropin (Kumar *et al.*, 2018).

The current study appealed that the average hatching period in *M. gulio* accounted as 21 h after fertilization in between 29 to 31°*C* water temperature, whereas Alam *et al.* (2006) reported the hatching period of *M. gulio* was 18 to 20 h after fertilization, temperature range was 30.2 to 32.8°C and Rahman *et al.* (2004) stated the hatching period at 19 to 21 h after fertilization in *M. cavasius* at a water temperature 27-29.5°C. Ferosekhan *et al.* (2015)

found the hatching period at 25:27 h after fertilization in *P. pangasius* with a temperature range from 27.5 to 28.5°C. Incubation temperature and time of egg hatching was inversely correlated. Rahman *et al*. (2004) observed the barbell partially appeared of *M. cavasius* in 06 h old larvae and it took to 12 h for a clear appearance. The average length of one-day-old larvae was measured 3.13 mm and clearly visible 3 pairs of barbells appeared in current work. However, this is supportive to previous finding of the length of one-day-old larvae of *M. gulio* was 3.39-3.98 mm (Kumar *et al*., 2021; Rahman *et al*., 2004; Alam *et al*., 2006) and 2.0 mm in *Rita rita* (Mollah *et al*., 2011). Slight variation may be due to the quality and environments of brood stock.

Conclusions and Recommendations

Findings of present study revealed that *M. gulio* can be induced successfully by using 0.5 ml kg-1 dose of S-GnRHa hormone under captivity condition in freshwater. Further research is recommended for development and extension of mass breeding initiatives in freshwater condition with commercial aquaculture practices of this species.

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Conflict of interest

The authors have declared no conflict of interest.

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