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RESEARCH ARTICLE

The use of S-GnRHa (salmon gonadotropin releasing hormone analogue) in induced breeding and early embryonic development of Gulsha, *Mystus cavasius*

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ABSTRACT

The current study was carried out to optimize the dose of the synthetic hormone for induction, and to observe the embryonic and larval developmental in Gulsha, *Mystus cavasius*. Induced breeding was conducted by using Ovupin (S-GnRHa) hormone (each 1.5 ml vial contain 0.2 mg of an analogue of S-GnRHa) at four different doses i.e., 0.25, 0.5, 1.0- and 1.50-ml kg^{-1} body weight (BW) for females, and the half of these doses were applied to males. Among the applied doses, 0.5 ml kg^{-1} BW for female and 0.25 ml kg^{-1} BW for male provided the maximum fertilization (83.66%) and hatching (80.0%) rates. The eggs of *M. cavasius* were strongly adhesive, with covering on egg surface. The average diameter of fertilized eggs just after spawning was 85.58±5.87 µm. After fertilization, the first, second, and third cleavage stages occurred within 20-25, 35-40 and 60-65 min, respectively. The identity of blastomeres was gradually lost and appeared at 64-cell stage to 128-cell stage onwards. The 64-cell stage appeared at 150-160 min and the morula stage 3:00-3:20 h (blastomeres completely lost), respectively. The gastrula stage appeared at 5:0-5:30 h in which the blastoderm spread in both the sides covering about 60-70% area, together with a thread-like germinal ring. Afterward, twisting locomotion was recorded at 23:30 h. The larvae started hatching at 24:00 to 25:00 h. The barbells were partially visible when the larvae were 10-12 h of age. Finally, the yolk sac was fully absorbed in the end of Day 3.

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Introduction

Mystus cavasius (Order- Siluriformes), locally known as Gulsha, is a widespread catfish in Bangladesh (Chakrabarty & Ng, 2005; Rao, 2017) and it has been drawing interest due to its premium taste and nutritional prominence (Latif et al., 2018). The fish can easily be recognized by its elongated and compressed body, four pairs of barbells and a serrated spiny dorsal fin. Most of the catfish are highly preferred by local customers for their less intermuscular bone and flavor (Gupta, 2015; Jabed et al., 2020). Now, it is a vulnerable Small Indigenous Species (SIS) in Bangladesh (Iqbal et al., 2015; IUCN Bangladesh, 2015).

Aquaculture in Bangladesh is an increasing sector and its species ranges are expanding day by day (Alam et al., 2014; Jayasankar, 2018; Alam et al., 2020). Fry and fingerlings from the wild sources, i.e., rivers, lakes, etc. are not adequate and reliable (Verma et al., 2017; Mishra et al., 2018; Müller et al., 2020). That is why the number of hatcheries is also increasing to fulfil the demand of fry in aquaculture**.** The artificial propagation of Gulsha involves the injection of sexually mature females and males with synthetic hormones, with modifications, that have been used to spawn an entire range of catfish. Generally, catfish are injected with hormones under the peritoneal cavity (Bhenila & Biswas, 2014; Kumar et al., 2018; Kumar et al., 2021). Different hormones are used in different doses for different catfish species, which might have resulted in lower hatching and survival rates, and poor-quality fry and fingerlings. Generally pituitary gland (PG) extract was not used in the hatchery, especially for catfish as it is required in high amounts and the number of eggs ovulated is low (Mondol et al., 2014; Aktar, 2015). Moreover, the rearing techniques of embryonic, larval and fry stages of Gulsha are not well studied yet to increase the survival rates and produce quality fry and fingerlings. For artificial propagation studies; (i) it is important to identify the appropriate hormones and their dosage, (ii) to know the embryonic and larval stages of early fish development and (iii) to optimize the rearing techniques for fry to have quality seeds of Gulsha. For seed production, the early life history information should be known well to rear fry in the nursery ponds as it is related to maturity of gravid fish and yolk sac completion (Shehu et al., 2018; Zadmajid et al., 2019). In this study, the induced breeding techniques in hatchery condition for Gulsha were investigated to optimize dose of S-GnRHa and to observe the embryonic and larval developmental stages under microscopy.

Materials and Methods

Location of the Study

The experiments were conducted for a three-month period from June to August 2019 in two different locations: (a) the private hatchery at Alalpur, Mymensingh and (ii) the laboratory facilities of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. The induced breeding and fry rearing of *M. cavasius* were performed in Alalpur hatchery complex and the micrographs of embryonic development stages were taken at the Fisheries Biology and Genetics (FBG) Laboratory.

Designing the Experiments and S-Gnrha Injection

The cemented cisterns were washed properly prior to shifting the brood male and female for strictly maintaining hygienic and germ-free condition for spawning. The cemented cistern was washed by using lime and brushed. The male and female brood of *M. Cavasius* were collected from the hatchery pond for breeding. The brood fish were separated as male, and female based on their secondary sexual characteristics. Then, the selected brood fish were instantly transferred to the hatchery and located in the cistern for about 8h (from 8:30 am to 4:30 pm). They were kept under water showering that has both outlet and inlet facilities to induce the breeding conditioning. The dirt due to fish body mucous and the excreta of fish were washed out through the outlet because of continuous waterflow. The fish were left fasted while conditioning. A synthetic form of induced hormone, ovupin (S-GnRH) manufactured by Sansheng Pharmaceutical Co., Ltd, China, was purchased from the local market. Following the manufacture's instruction, sterilized distilled water was mixed with the hormone powder to make the solution for injection and each 1.5 ml vial contain 0.2 mg of an analogue of S-GnRHa (see Table 1 for the doses applied). After hormone preparation it was carefully taken into a 0.5 ml hypodermic syringe and injected intramuscularly in near the muscle area of lateral line. Injected fish were kept in the breeding cistern tanks with continuous water flow and monitored until they ovulate. Males and females were included in four different treatment groups, and in each group males and females were injected with specific doses of S-GnRHa (Table 1). For each treatment, there were three replications. The ratio of male to female was 1:1 in all treatments. There was no control group had been assigned as S-GnRHa hormone has been already used in induced breeding of *M. cavasius* and this research work was aimed at optimizing dose for commercial aquaculture.

Table 1. Induce breeding trial of *M. cavasius* with S-GnRHa hormone

Treatment	Hapa	Body Weight (g)		Dose (ml/kg) body weight	
		Female	Male	Female	Male
T1	H1, H2 H3	28.33±1.53	18.00±2.00	0.25	0.125
T2	H1, H2 H3	30.00±2.65	17.67±1.53	0.50	0 .25
T3	H1, H2 H3	30.00±0.82	18.33±1.70	1.00	0.50
T4	H1, H2 H3	26.00±3.61	16.67±1.53	1.50	0.75

Monitoring Embryonic and Larval Development and Water Quality Measurements

The hormone injected female and male fishes were kept into breeding cistern having a size of 1.5 × 0.5 × 1 m^3 with continuous water flow and once ovulation occurred, ovum was collected and transported to nearby facilities of the Bangladesh Agricultural University (BAU) for further analysis and observation of embryonic stages. Water temperature, pH and dissolved oxygen were analyzed on spot following standard methods (APHA, 1998). The developmental stage of the eggs was photographed under microscope (OLYMPUS CX21) with camera attachment (Rigla-32, Optikam B3 Digital camera, Italy). The water quality parameters i.e., water temperature, pH, and water transparency of the nursery pond were frequently recorded using Secchi's disk and measuring tape, pH paper, and thermometer.

Determination of Ovulation Rate, Fertilization Rate and Hatching Rate

The ovulation rate, fertilization rate and hatching rate was determined using the following equations (Eq. 1-3) from Legendre (2000), Rahman & Samat (2020) and Unuma et al. (2004), respectively.

$$Ovulation\ rate\ (\%) = \frac{Number\ of\ fish\ ovulated}{Total\ number\ of\ fish\ injected} \times 100 \quad (1)$$

$$Fertilization\ rate\ (\%) = \frac{Number\ of\ fertilized\ eggs}{Total\ number\ of\ eggs} \times 100 \quad (2)$$

$$Hatching\ rate\ (\%) = \frac{Number\ of\ eggs\ hatched}{Total\ number\ of\ fertilized\ eggs} \times 100 \quad (3)$$

Statistical Analysis

A one-way analysis of variance (ANOVA) implemented in MS Excel was used to determine whether there are any statistically significant differences between the different hormonal treatment groups. The level of significance of the results were tested following Duncan's and Turkey using IBM SPSS v27 program.

Results and Discussion

Artificial fertilization, together with embryonic development, was performed successfully, where 0.5 ml kg^{-1} BW for female and 0.25 ml kg^{-1} BW for male among four different doses showed the maximum fertilization and hatching rates, and significantly different ($P < 0.05$) from the doses of 0.25 ml kg^{-1} BW for female and 0.125 ml kg^{-1} BW for male (Table 1). Current study findings resembled to Mondol et al. (2014), where 0.5 ml kg^{-1} BW of ovupin for female was successful in carp seed production, whereas comparatively lower dose of 0.25 ml kg^{-1} of Ovaprim for females increased the effectiveness of spawning induction of blackfin sea bream, *Acanthopagrus berda* (Abbas et al., 2019). However, Araf et al. (2021) obtained relatively higher dosages of S-GnRHa i.e., 1-2.5 ml/kg body weight have been efficient in the spawning induction of air stinging catfish. All females were ovulated when treated with all the four different hormone doses. Mondol et al. (2014) also observed very similar results using ovupin in major carp species and Alam et al. (2006) using Ovaprim in estuarine catfish, *Mystus gulio*. Ali et al. (2014) obtained 82.67% ovulation rate with HCG and PG injection in *Heteropneustes fossilis*, El-Hawarry et al. (2016) got 70.76% with GnRHa plus domperidone (Dom) treatment in African catfish (*Clarias gariepinus*) and Hossen et al. (2021) acquired hatching success between 74.33-83.89% in *Mystus gulio* by using synthetic GnRHa. An account of 62% fertilization rate and 60% hatching rates have been reported for *M. cavasius* injected with 0.2 ml/kg BW ovatide (Das et al., 2018).

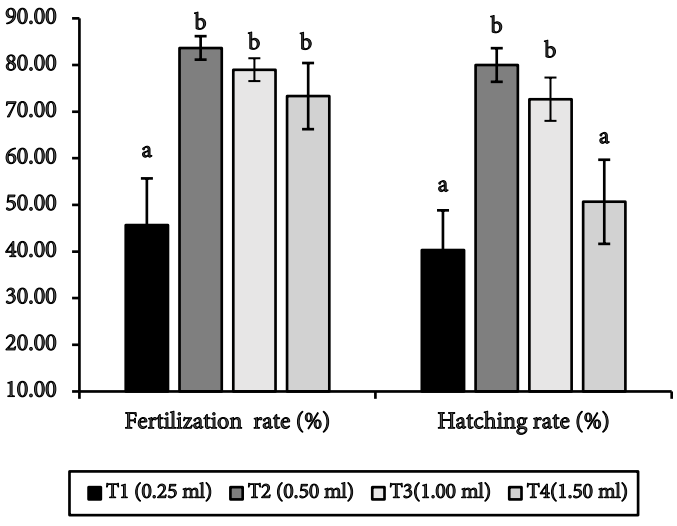

Figure 1. Effect of different doses of hormone on fertilization and hatching rate of *M. cavasius* (Y axes represent the percentages value of different parameters of Y axis; different letter in bar diagram refers the significant differences among the treatment)

Fertilization rate of eggs in different dosages i.e., 0.25, 0.5, 1.0 and 1.50 ml kg^{-1} BW were 45.66±10.01%, 83.66±2.51%, 79.00±3.00% and 73.33±7.09%, respectively (Figure 1). Mosha (2018) obtained highest fertilization rate of 87.34% in African Catfish (*C. gariepinus*) with Ovaprim introduction which was quite like our current study where the highest fertilization rate (83.66%), lowest as (45.66%) for the least hormone dose. Again, hatching rate of eggs were observed as 40.33±8.50, 80.0±3.60, 72.66±5.68 and 50.66±9.01 in respect to 0.25, 0.5, 1.0 and 1.50ml kg^{-1} BW hormone dose respectively (Figure 1). The highest hatching rate revealed 80.0±3.60 was quite lower that of Borah et al., (2020) found 92.49% in *M. pancalus* with Ovasis treatment. The T_2 treatment was found to be efficient in case of hatching and fertilization success.

Water quality profiles such as pH, temperature, and transparency of different treatments of *M. cavasius* ranged from 6.67-6.93, 29.33-30.33 °C, and 22.67-23.57 cm, respectively (Figure 2). Physico-chemical water parameters might play a significant role to improve fertilization rate and successful hatching. Current study findings denoted a good environment for successful induced breeding (Chand et al., 2011). In addition, optimum environmental conditions ameliorated the breeding performance in *Labeo bata* using Ovaprim and ovatide hormone (Behera et al., 2007), *M. pancalus* administrated with PG hormone (Alam et al., 2009) and in induced breeding of *A. testudineus* by using S-GnRHa (Rahman et al., 2021).

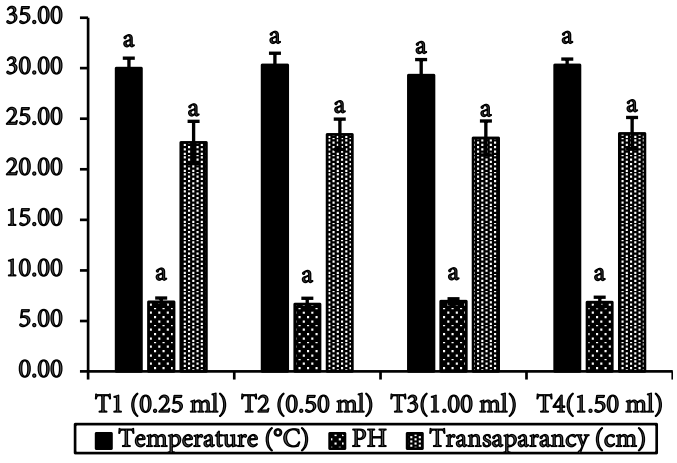

Figure 2. Physico-chemical water quality parameters of the breeding ponds

In our current study, the fertilized eggs of *M. cavasius* were strongly adhesive. Puvaneswari et al. (2009) reported that siluriform fishes showed adhesive forms of the eggs, for instance, fertilized eggs in *P. pangasius* are sticky and jellied in nature (Ferosekhan et al., 2015). Table 2 shows the embryonic development and interval time of observation in *M. cavasius*. The fertilized eggs (Figure 3b) of *M. cavasius* showed meroblastic cleavage in a first stage that divided the blastodisc into two blastomeres occurred within 20-25 min. after fertilization (Figure 3c). Nesa et al. (2017) obtained first cleavage in 00:21-2:09 h of post-fertilization to form 32-cell by administrating PG extract in *H. fossilis*, where the current study observed first cleavage up to 32-cell formation in 00:35-2:20 h. The morula phase was reached at 180-185 min. after fertilization (Figure 3i). At that stage, blastoderm spread over the yolk in and embryo became distinguished. Rahman et al. (2004) found the same stage of *M. cavasius,* however, quite earlier within 2:20 h after fertilization. Puvaneswari et al. (2009) and Nesa et al. (2017) also observed the same stage within 2:35 h of post-fertilization using Ovaprim and PG extract, respectively, in *H. fossilis*. Henceforth, blastula stage in the current study (3:30-4:00 h of post-fertilization) was illustrated as flattened and compacted blastodermic cell with occupied moderate surface (Figure 3j). Sarma et al., (2012) reported for *O. pabo* that blastula stage was observed in 3:30 h and one third of egg surface was coated by the blastoderm cells as well.

The gastrula phase arrived at 5:0-5:30 h after fertilization and traced through further expansion of the blastoderm taking around 60-70% surface (Figure 3k) and give rise to thread like germinal rings (Figure 3l). The "C" shape embryonic stage appeared at gastrula stage (Figure 3l). The head and tail regions become clearly prominent, and embryo appeared as encircled over the yolk sphere (Figure 3m). Ferosekhan et al. (2015) reported that the gastrula stage of *P. pangasius* appeared at 7:27 h, germinal ring sustained up to 8:00-9:00 h. This stage appeared at 9:00-10:00 h in *O. pabo* (Sarma et al., 2012) and at 11:00 h in *P. sutchi* (Islam, 2005). Twisting movement of *M. cavasius* embryo in the present study was observed through unfolded from encircled over the yolk sphere. Tail became free and the head left still adhere to yolk sac. The beating of tail become fast just preceding to hatching. Ferosekhan et al. (2015) reported that beating of tail was around 50-60 times per minute at 22:00-23:00 h post-fertilization. The larvae started hatching at 24:00 to 25:00 h. The tiny larvae were clearly distinguished with separated head, trunk, and tail region (Figure 3n). Hatching occurred at 24:00-26:00 h (25:27 ± 01:28 h) in *P. pangasius* (Ferosekhan et al., 2015), at 22 h in *R. rita* (Mollah et al., 2011), and at 26 h in *C. batrachus* (Das, 2002).

The larvae were 160.60±8.97 µm mm in measurement. The mouth of *M. cavasius* was not visible after hatching. After 2:30±3:30 h hatching, the fin fold was differentiated. A transparent thin blurred fin encircles over the caudal region and stretched up to the yolk sac (Figure 3q). This type of changes also identified in newly hatched larvae of *P. pangasius* by Ferosekhan et al. (2015), and in *P. sutchi* by Islam (2005).

Table 2. Observation of the embryonic development and interval time of *M. cavasius* (T_2 treatment group were considered for embryology study and three replicates were maintained in all cases)

Phase	Duration and size (mean ± SD)	Description	Figure No.3
Unfertilized Egg	99.17±4.29 µm	Opaque and whitish in color	(a)
Fertilized egg	85.58±5.87 µm	Transparent, adhesive, and watery in color	(b)
Cell division	35-40 min	Cleavage	
Two cells	20-25 min; 90.0±3.08 µm	Two cells over the yolk vesicle were identified.	(c)
Four cells	35-40 min; 85.76± 1.93µm	Four cells (blastomeres) were observed.	(d)
Eight cells	60-65 min; 98.82± 3.88µm	Eight cells were identified and looked like fingers.	(e)
Sixteen cells	90-100 min; 84.17±2.36 µm	Sixteen cells were observed (blastomeres were overlapping).	(f)
Thirty-two cell	120-140 min; 80.30±1.99 µm	Blastomeres were arranged in three layers with overlapping observation.	(g)
Multi-cell (64 and 128)	150-160 min; 82.94±2.08 µm	Cell proliferation numbers observed within the egg. Three layers were observed.	(h)
Morula	180-185 min.; 86.11±1.13 µm	Cleavage resulted into 64 and 128 cells. Look likes flowery.	(i)
Blastula	3.30-4.0 h; 84.70±2.11 µm	The blastomeres covered about 30-40% area and become compacted.	(j)
Gastrula	5-5.30 h; 90.0±0.76 µm	Blastoderm spread in both the side which covered about 60-70% area and thread line germinal ring appeared.	(k and l)
Head and tail bud formation	8.30-9.0 h; 77.64±1.93 µm	Head and tail visible	(m)
Just before hatching	23:30 h; 78.53±1.88 µm	Embryo encircled the whole yolk looking "c" shaped. Twisted movement of caudal region was started to unfold from the yolk sac.	(n)
Newly hatched larvae	24-25 h; 160.60±8.97 µm	Head, tail, and yolk sac were clearly identified as well as straight body observation.	(o and p)

Table 3. Observation of the larval development of *M. cavasius.* (T_2 treatment group were considered for embryology study and three replicates were maintained in all cases)

Age of larvae	Length (mm) of body and yolk sac	Characteristics	Figure No. 3
5 h	3.0±0.2 mm and 75 µm	Heart pumping identified. Yolk sac remained compact and eye yet identified.	q
10-11 h	3.3±0.1 mm and 66 µm	Eye clearly identified and melanophores were present around the yolk sac and brain region. A tubular heart appeared.	r
24 h	Yolk size 61.76±6.09 µm	Gradually reduced yolk from the yolk sac. Eye and anus identified. Blood circulation system fully functional.	s
4 days old	-	Yolk sac completely absorbed. Larvae freely swimming and started to eat natural food.	t

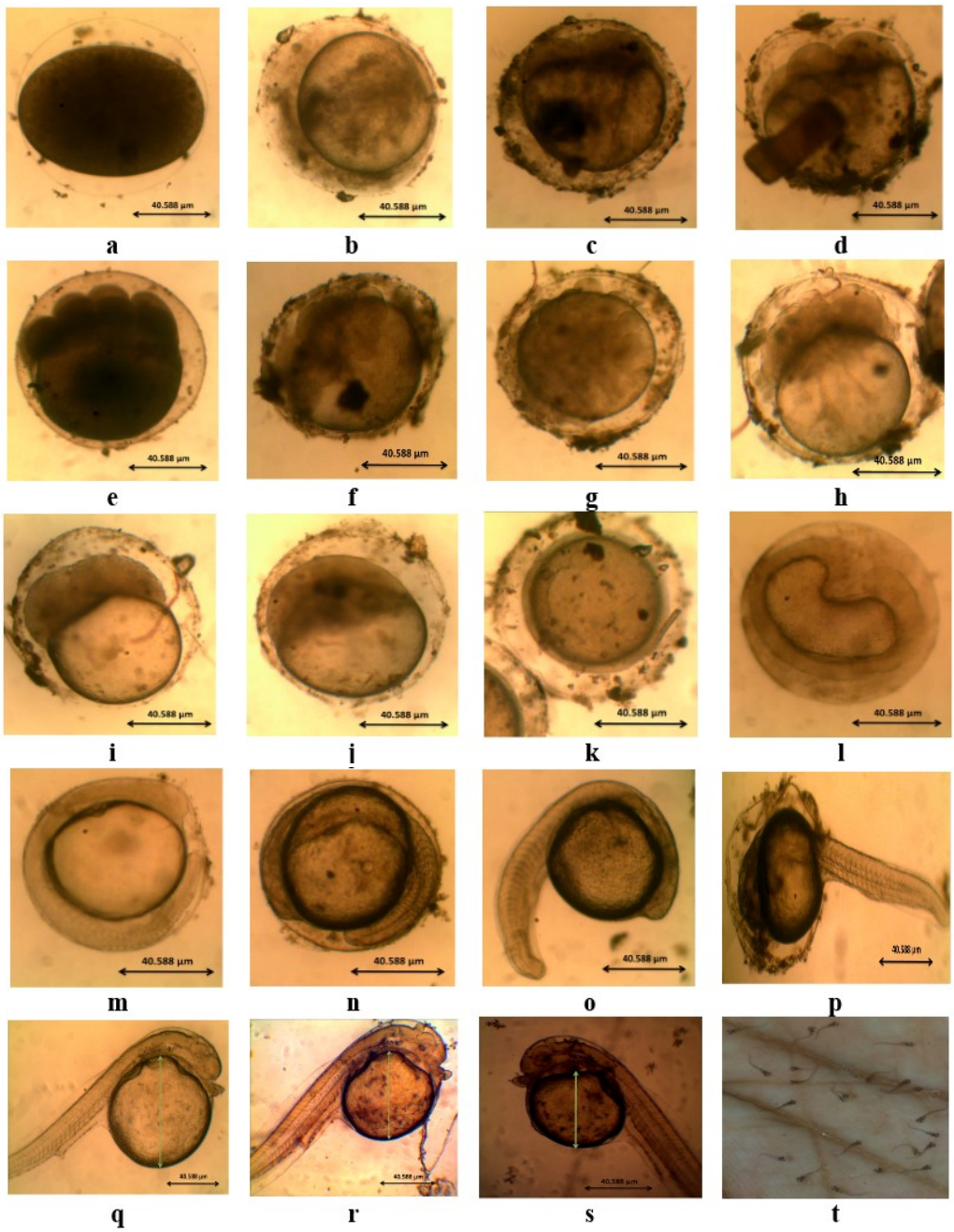

Figure 3. The Embryonic development of *M. cavasius*: (a) unfertilized egg; (b) fertilized egg; (c) two cell; (d) four cell; (e) eight cell; (f) sixteen cell; (g) thirty-two cell stage; (h) multi-cell (about 64); (i) multi-cell (about 128) stage; (j) morula stage; (k) blastula stage; (l) gastrula stage; (m) head and tail stage; (n) just before hatching; (o) newly hatched larva; (p) newly hatched larva (advanced stage); (q) Five hours after hatching; (r) ten hours after hatching; (s) twenty-four hours old larvae; (t) four days old larvae.

The position of the 5 h old larva's mouth and the eyes were non pigmented (Figure 3q). At that stage, a dorso-ventrally irregular fin appeared, and the larvae tried to start swimming. The swollen yolk gradually elongated when they were 10-11 h old, besides some melanophores appeared on the head region and around the yolk sac (Figure 3r). Afterwards, the barbell was partially observed. Rahman et al. (2004) reported that the barbell of *M. cavasius* was found in 6-12 h old larvae. The 24 h old larva with a reduced yolk sac appeared with dark pigmented anterior part of the head and prominent eyespot, meanwhile, two pairs of barbells were also noticed. The circulatory system was reported to be fully operating (Figure 3s). With the increase of age and time interval, gradual reduction of yolk sac was observed, which got completely absorbed at the end of third day (Figure 3t). The alimentary canal was also appeared at the age of three or four days and the larvae started having natural food items except yolk sac like as small zooplankton as feed. Rahman et al. (2004) reported that the yolk sac of *M. cavasius* was fully absorbed by 48 h old larvae. The round, dense yolk sac become reduced as the hatchling continued to grow in age and absorbed completely at the end of 72 hours of life before they take external foods (Islam, 2005; Ferosekhan et al., 2015).

Conclusion

The results of the current study suggests that a dose of 0.5 mg/kg body weight S-GnRHa application to females would be efficient for successful breeding induction in *M. cavasius* and better initiation of larval and embryonic growth. However, designing an experiment with a larger sample size is required for analyzing the further effects of this dose in aquafarms.

Compliance With Ethical Standards

Authors' Contributions:

This research includes MSc thesis work of first author. The chronology of author list reflects the contrition level of different author. However, the last author will be treated as team leader and principal supervisor as per institutional policy.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was duly compiled with all sorts of regional, institutional and national animals ethics clearance.

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