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A Study on Induced Breeding and Early Embryonic Development of the Tengara Catfish, *Mystus tengara* (Hamilton, 1822) in Captivity

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ABSTRACT

This study presented the first comprehensive report on the induced breeding and embryonic development of *Mystus tengara*. Matured brooders were induced with synthetic hormone (GONOPRO-FH) at a dosage of 0.5ml/ kg body weight for males and 1ml/ kg body weight for females in each trial. The trial conducted at a water temperature of 29ºC yielded the highest fertilization rate of 84% and hatching rate of 93% among the three trials. The fertilized eggs were 0.5mm in diameter, spherical, transparent, and highly adhesive. The zygote stage extended up to 00:15 hour post fertilization (hpf), followed by cleavage stage (00:15-01:20 hpf), blastula stage (01:40-03:25 hpf), gastrula stage (03:45- 05:24 hpf), segmentation stage (08:20-12:27 hpf) and pharyngula stage (13:09-14:10 hpf). Hatching occurs at 15:00 hpf, resulting in larvae with an average length of 1.62mm. The outcomes of this study hold significant implications for commercial fish farmers and breeders, facilitating the production of superior-quality seeds at reduced expenses. Furthermore, these findings contribute to both species' conservation efforts and advancements in species taxonomy.

INTRODUCTION

Mystus tengara (Hamilton, 1822) is a freshwater catfish distributed in India, Nepal, Bangladesh, and Pakistan **(Talwar & Jhingran, 1992)**. The species is locally known as 'Tengara' and has both food as well as ornamental value **(Gupta & Banerjee, 2013)**. The body of *Mystus tengara* is elongated and compressed, displaying a porcelain white color on the belly and flank, adorned with 4-5 horizontal bands ranging from dark brown to green-black. A distinct dark blotch is situated in proximity to the pectoral fin **(Gupta, 2015)**. In terms of sexual dimorphism, the female (Fig. 1) has a yellowish body tone and a wider body compared to the male. Males (Fig. 2) can be distinguished by the presence of a genital papilla near the anal fin and a greyish body tone with darker bands.

Fig. 1. Adult female *Mystus tengara* (Hamilton, 1822)

Fig. 2. Adult male *Mystus tengara* (Hamilton, 1822)

In West Bengal and Assam, the catfish such as *Mystus* are popular food fish due to their good taste and high nutrient profile **(Gupta & Banerjee, 2013)**. Commercial practices for the induced breeding of catfishes have also gained popularity in recent years due to their high market price, which ranges from 800 to 1,000 INR/kg **(Bailung & Biswas, 2014)**. Investigating embryogenesis plays a pivotal role in advancing conservation biology and the ornamental fish breeding sector **(Olufeagba *et al.*, 2015)**. Moreover, it contributes to the identification of chromosomal anomalies such as polyploidy **(Weber & Hostuttler, 2012; Najafpour *et al*., 2019)**.

However, information on the breeding of these species is limited, and there is no comprehensive data regarding their embryological development. Therefore, the present study aimed to provide detailed information on the captive breeding performance and embryonic development stages of the species, which will assist in their aquaculture, taxonomy, and conservation.

MATERIALS AND METHODS

Collection of specimens

A total of 40 adult *Mystus tengara* (24 males, and 16 females) with an average length of 7.3±0.5mm (Male) and 8.2 ±0.61mm (Female) and an average weight of 12.08± 1.14g (Male) and 13.61± 0.74g (Female) were collected from Kapla eel, Barpeta, Assam, India (26°20′13.8″N 91°13′26.9″E). The live specimens were then transported to the Aquaculture and Biodiversity Center of Gauhati University, Assam in well-aerated plastic bags with minimal stress.

Brooders management

The collected fish were acclimatized in a glass aquarium (121 x 45.5 x 45.5cm^3; 250.5L). The water quality parameters were maintained within the optimum range: the temperature at 27-29ºC, pH at 7.2-7.4, dissolved oxygen at 6.5-7mg/ L, ammonia at

0.01mg/ L, and conductivity at 193.2µS/ cm. PVC (Poly Vinyl Chloride) pipes and earthed pots were provided as the *Mystus tengara* is sensitive to light and prefers hiding space. The brooders were fed live Tubifex and chopped earthworm twice daily, at 10:00 and 16:00 hours of Indian Standard Time (IST), at a rate of 2-3% of their body weight.

Induced breeding

Prior to induced breeding trials, an FRP (Fiber Reinforced Plastic) hatchery of diameter 148cm was cleaned, disinfected, and filled with water up to 10cm. In the months of June and July 2023, three pairs (Male and female) of matured brooders were selected from the stocking tank for induced breeding. Then, selected fish were administrated a single dose of intramuscular injection of synthetic hormone (GONOPRO-FH) at 0.5ml/ kg body weight for the males and at 1ml/ kg body weight for females, respectively. A total of three breeding trials (T1, T2, and T3) were conducted and after hormonal injections, the fish were transferred to the FRP hatchery, and a continuous shower was provided. Spawning occurred 7 to 8 hours post-induction at a water temperature of 26 to 29°C, with an average fertilization rate of 80.66%.

Embryology

After successful spawning, a few fertilized eggs were collected from the FRP hatchery and were studied under the Labomed CZM4 stereo-zoom microscope at regular intervals of 5min. Photographs of the embryonic developmental stages were taken with the help of a Sony IMX766 OIS 50-megapixel camera, and stages were classified following **Kimmel *et al.* (1995)**. The fertilization and hatching rate were calculated for each breeding trial by following the formula described by **Unuma *et al.* (2004)**:

$$Fertlization\ rate(\%) = \frac{No.of\ fertilized\ egg}{Totalno.of\ both\ fertilized\ unfertlizedegg} \times 100$$

$$Hatching\ rate(\%) = \frac{No.of\ egg\ hathed}{Totalno.of\ fertilized\ egg} \times 100$$

RESULTS

The breeding trials achieved a 100% success rate upon conducting three experimental trials viz. T1, T2 & T3. It was observed that after 5 hours of hormonal injection, males became active and started to chase the females. The female laid the eggs uniformly throughout the FRP tank 7-8h post-induction, and the eggs remained attached to the substratum of the FRP tank. Detailed information on all breeding trials is provided in Table (1).

Table 1. Detailed information of the breeding trials of *Mystus tengara* (Hamilton, 1822)

Trials	**T1**		**T2**		**T3**	
Brooders	Male	Female	Male	Female	Male	Female
Total length (cm)	7.5±0.87	8.3±1.6	7.0±0.69	8.9±1.5	7.4±1.1	8.5±0.8
Total weight(gm)	12±0.46	13.74±0.6	12.66±1.45	14.1±0.87	11.6±1.53	13±0.75
Latency (hr:m)	8:00		7:30		7:00	
Fertilization rate (%)	78		80		84	
Hatching rate (%)	88		91		93	

The embryonic developmental stages were divided into seven major groups: zygote, cleavage, blastula, gastrula, segmentation, pharyngula, and hatching. The overall developmental stages of *Mystus tengara* are illustrated in Figs. (3, 4, and 5), while a summary of the embryological developmental stages is presented in Table (2).

Table 2. Embryonic developmental stages of *Mystus tengara* (Hamilton, 1822)

Period	**Stages**	**Time (h:m)**	**Description**	**Fig.**
Zygote	1-cell	00:00	Separation of chorion and newly fertilized egg	3(a)
Cleavage	2-cell	00:15	Formation of 2 blastomeres	3(b)
	8-cell	00:25	Formation of 8 blastomeres	3(c)
	16-cell	00:35	Formation of 16 blastomeres	3(d)
	64-cell	01:20	Arrangement of 64 blastomeres into 3 tiers	3(e)
Blastula	256-cell	01:40	256 blastomeres arrange themselves into layers	3(f)
	1 k cell	02:50	Cleavage in irregular planes gives rise to 1k cells	3(g)
	30% epiboly	03:25	Epiboly starts and the cells of the blastula cover 30% of the yolk	3(h)
Gastrula	50% epiboly	03:45	The blastoderm margin covers 50% of the animal pole	3(i)
	Germ ring	03:50	A germ ring appears at the thickening of the blastoderm margin after more than	3(j)

			50% epiboly	
	60% epiboly	04:15	Blastoderm covers by 60% of the yolk	3(k)
	70% epiboly	04:45	Blastoderm covers by 70% of the yolk	3(l)
	90% epiboly	05:13	Blastoderm covers by 90% of the yolk	4(a)
	Bud stage	05:24	Tail bud starts to appear	4(b)
	2-somite	08:20	Somitogenesis begins with 2 somites	4(c)
	4-somite	08:44	4 somites visible with head and tail buds	4(d)
Segmentation	6-somite	09:13	Optic primordium appears in the presumptive head region	4(e)
	10-somite	10:08	10 somites visible in this stage	4(f)
	12-somite	10:23	12 somites visible in this stage	4(g)
	18-somite	11:15	Tail elongation occurs and the tail end starts to dissociate from the yolk	4(h)
	23-somite	12:27	Optic vesicle clearly visible	4(i)
Pharyngula	Pharyngula 1	13:09	Tail dissociates further as it extends	4(j)
	Pharyngula 2	14:10	Tail contraction and heart beating begins	4(k)
Hatching	Hatched larva	15:00	Slightly curved immobile larva with yolk sac	5(a)
	Day 1 larva	36:00	Larva with a pair of prospective barbels can be seen	5(b)

Developmental stages

Fertilized eggs

The fertilized eggs are spherical, transparent, and highly adhesive, with an average diameter of 0.5mm.

Zygote period (00:00 - 00:10 hpf)

The zygote period (Fig. 3a) extends from 00:00 to 00:10 hours post-fertilization (hpf). During this period, the cytoplasm begins to move toward the animal pole, separating the blastodisc from the yolk.

Cleavage period (00:10 - 01:20 hpf)

The cleavage period is characterized by incomplete meroblastic division of the blastodisc, extending from 00:10 to 01:20 hpf. This period is further divided into six stages based on cell number.

- **2-cell stage (00:10 hpf)**
  The cleavage furrow is vertical, starting from the animal pole and moving toward the vegetal pole. The two blastomeres formed are of equal size and indistinguishable from each other (Fig. 3b).
- **8-cell stage (00:20 hpf)**
  Eight cells are formed by the incomplete division of the four cells, occurring in two planes on either side of the first cleavage plane (Fig. 3c).
- **16-cell stage (00:35 hpf)**
  Sixteen cells are formed through division in two parallel planes on either side of the second cleavage plane (Fig. 3d).
- **64-cell stage (01:20 hpf)**
  The sixth cleavage occurs in the horizontal plane, resulting in 64 cells organized into two layers of 32 cells each (Fig. 3e).

Blastula period (01:40 - 03:25 hpf)

During this period, the blastodisc begins to transform into a ball-like structure. At a later stage, epiboly starts, which continues into the gastrulation period.

- **256-cell stage (01:40 hpf)**
  This stage marks the beginning of blastulation, with blastomeres arranged in layers in an unordered fashion (Fig. 3f).
- **1K-cell stage (02:50 hpf)**
  Approximately 1,000 cells form an irregular margin around the yolk sac after the 10th cleavage cycle (Fig. 3g).
- **30% Epiboly (03:25 hpf)**
  The blastoderm forms a layer of uniform thickness, covering 30% of the distance of the yolk along the animal-vegetal axis (Fig. 3h).

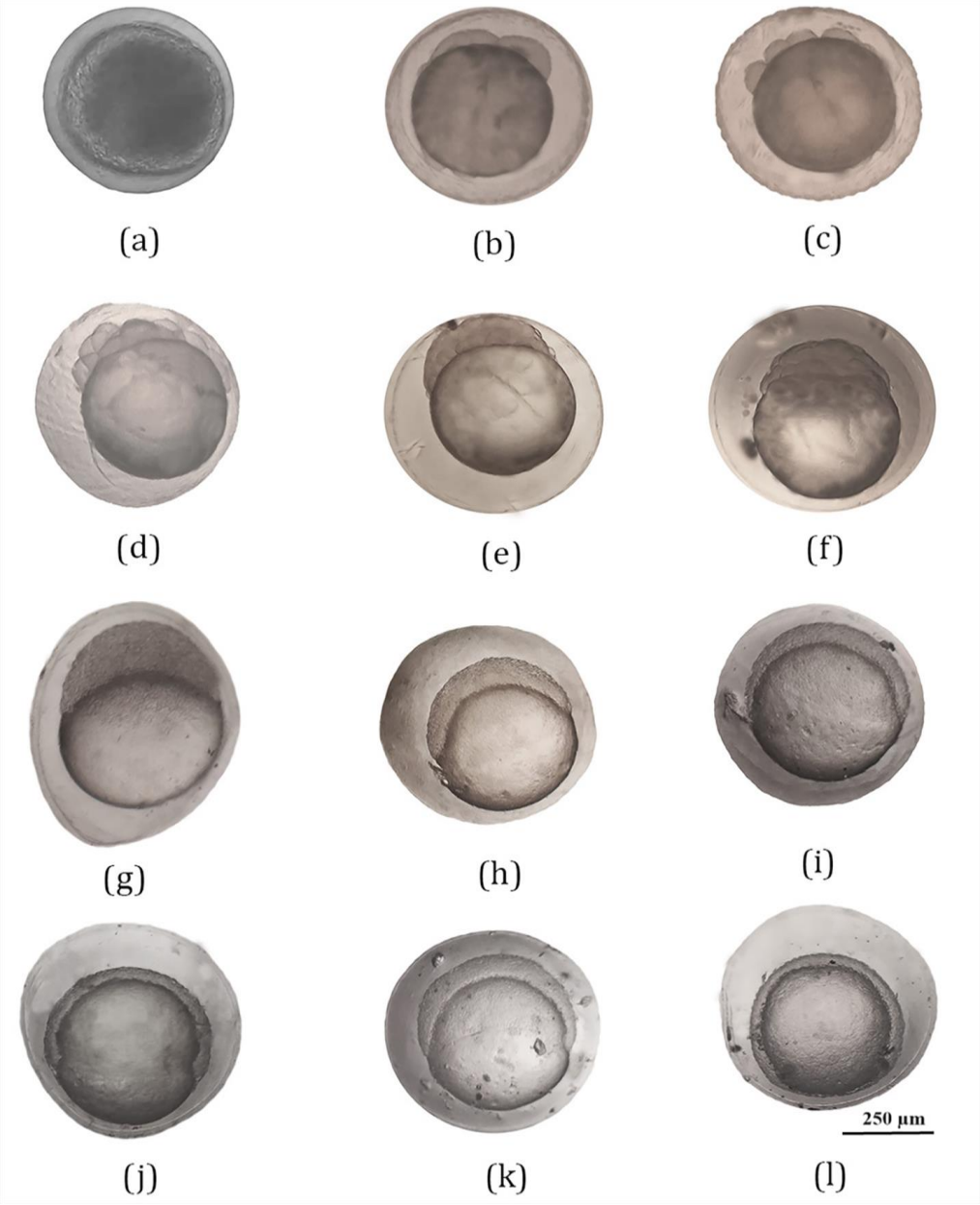

Fig. 3. Embryonic development in *Mystus tengara* (Hamilton, 1822): (a) Fertilized egg; (b) 2-cell stage; (c)8-cell stage; (d) 16-cell stage; (e)64-cell stage; (f) 256-cell stage; (g) 1K- cell stage; (h) 30% epiboly; (i) 50% epiboly; (j) Germ ring; (k) 60% epiboly; (l) 70% epiboly. (Scale bar: 250µm)

Gastrulation period (03:45- 05:24 hpf)

50% epiboly (03:45 hpf): During 50% epiboly, involution occurs which marks the beginning of gastrulation. The blastoderm covers 50% of the animal pole-vegetal pole distance (Fig. 3i).

Germ ring stage (03:50 hpf): A thickened annulus is formed at the margin of the blastoderm. The germ ring forms as a uniform structure, and it covers the entire circumference of the blastoderm. In this stage, the blastoderm still covers 50% of the animal pole-vegetal pole distance (Fig. 3j).

60% epiboly (04:15 hpf): The blastoderm is seen to have covered 60% of the distance between the animal pole and the vegetal pole (Fig. 3k).

70% epiboly stage (04:45 hpf): In this stage, 70% of the distance between the animal pole and the vegetal pole is covered by blastoderm (Fig. 3l).

90% epiboly stage (05:13 hpf): The whole yolk is covered by the blastoderm, leaving only a small portion of the yolk in the vegetal pole (Fig. 4a).

Bud stage (05:24 hpf): The bud stage signals the completion of epiboly with blastoderm covering the whole yolk. A distinct tail bud is developed at the posterior region of the embryonic axis. A thickening near the animal pole can be seen to be developed in the form of the prospective head of the embryo (Fig. 4b).

Segmentation period (08:20- 12:27 hpf)

During this period, the anterior-posterior axis and dorso-ventral axis become prominent. This segmentation period is marked by rapid morphogenetic movement leading to somitogenesis, rudimentary organ formation, elongation of the tail, etc. The formation of the furrow in the trunk defines the posterior margin of the first somite, and rapid formation of the somite takes place along the anterior-posterior axis.

2-somite stage (08:20 hpf): The formation of two somites is seen in the prospective trunk region in this stage (Fig. 4c).

4- somite stage (08:44 hpf): Tail bud thickens and four numbers of somites are seen (Fig. 4d).

6-somite stage (09:13 hpf): This stage is characterized by the formation of optic primordia along with the formation of six numbers of somites. The yolk sac becomes bean-shaped (Fig. 4e).

10- somite stage (09:43 hpf): The presence of otolith marks this stage along with the presence of ten numbers of somite. The yolk sac becomes more curved (Fig. 4f).

12-somite stage (10:08 hpf): Optic vesicle becomes prominent along with elongation of embryo due to growth of tail rudiment (Fig. 4g).

18-somite stage (10:23 hpf): The developing 18-somites now take a V-shaped appearance. Four subdivisions of the brain viz. telencephalon, diencephalon, midbrain, and hindbrain become discerned (Fig. 4h).

23-somite stage (11:15 hpf): Tail begins to straighten out due to constriction of yolk. All the somites in the trunk portion are in V-shaped appearance (Fig. 4i).

Pharyngula period (13:09-14:10 hpf)

Pharyngula-1 (13:09 hpf): During the earlier period of this phase, the lengthening of the embryo entirely wraps the yolk mass. The embryo now has a well-developed notochord and the somites extend up to the tail end (Fig. 4j).

Pharyngula-2 (14:10 hpf): The embryo displays rapid bursts of contraction in the trunk and tail portion (Fig. 4k).

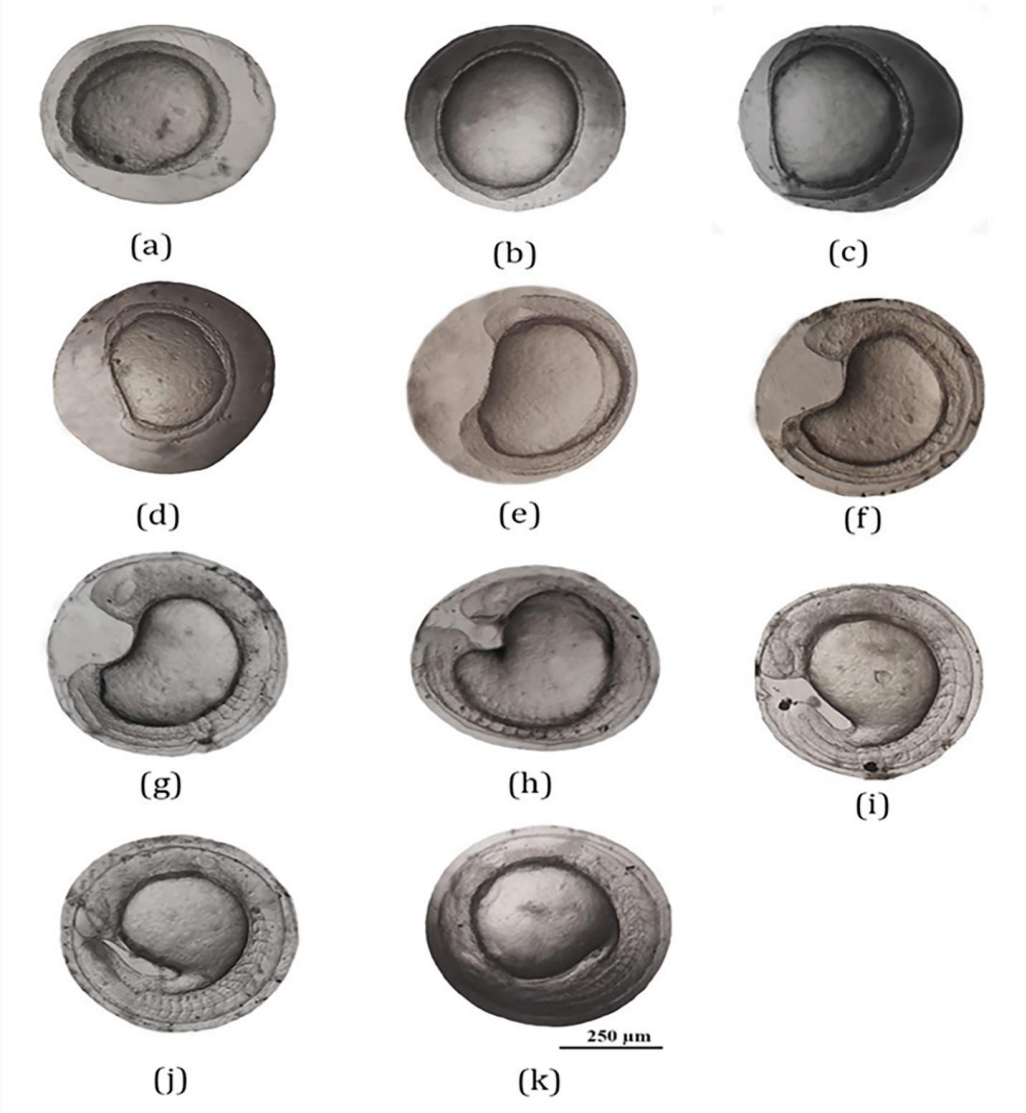

Fig. 4. Embryonic development in *Mystus tengara* (Hamilton, 1822): (a) 90% epiboly; (b) Bud stage(c) 2-somite stage; (d) 4-somite stage; (e) 6-somite stage; (f)10-somite stage; (g)12-somite stage; (h) 18-somite stage; (i)23-somite stage; (j) Pharyngula-1 stage; (k) Pharyngula-2 stage. (Scale bar: 250µm)

Hatching (15:00 hpf): The hatching of the embryo took place after 14:00-15:00 hours post-fertilization. The newly hatched larva is immobile, devoid of pigmentation, transparent, and comma-shaped with a spherical yolk sac (Fig. 5a).

Fig. 5. Larval development of *Mystus tengara* (Hamilton, 1822): **(a)** Just hatched larva; **(b)** 1-day old larva (Scale bar: 500µm)

DISCUSSION

This study provides the first detailed report on the induced breeding of *Mystus tengara* giving insights into early embryonic development of the species. In this study, we successfully bred *M. tengara* in three breeding trials by using synthetic hormone (GONOPRO-FH) at 1ml/ kg body weight. Successful induced breeding of other *Mystus* sp. like *Mystus gulio*, *Mystus vittatus,* and *Mystus dibrugarensis* has been achieved in the past with hormone doses of 0.8mg/ kg (carp pituitary hormone) **(Islam & Rahi, 2011)**, 0.8ml/ kg (Ovaprim) **(Bailung & Biswas, 2014)**, and 0.5ml/ kg(S-GnRH) **(Hossen *et al.*, 2021)**, respectively. Spawning takes place 7-8h post induction in *Mystus tenraga* in a water temperature of 26-29ºC which was also found in *Mystus gulio*, as reported by **Hossen *et al.* (2021)**. However, it was found to be 5-8h in case of *Mystus dibrugarensis* **(Bailung & Biswas, 2014)** in a water temperature of 27-29ºC.

The eggs of *Mystus tengara* are highly adhesive, and transparent in nature similar to that of *Mystus gulio* (**Hossen *et al.*, 2021)** but devoid of color. On the other hand, *Mystus cavasius's* eggs which are slightly pinkish in color **(Rahman *et al.*, 2004)**. The egg diameter of *Mystus tengara* is 0.5mm on average, closer to that of *Mystus cavasius* (0.5mm) and *Mystus gulio* (0.48mm) **(Rahman *et al.*, 2004; Hossen *et al.*, 2021)**. The fertilization rates of *Mystus tengara* for all three trials are 78, 80, and 84%, respectively, which are similar to that of *Mystus vittatus* (80%) as reported by **Islam *et al.* (2011)**; in *Mystus dibrugarensis* (77.54%) **(Bailung & Biswas, 2014)**, and in *Mystus gulio* (83.89±1.364%) **(Hossen *et al.*, 2021)**. Likewise, the hatching rate of all three trials are 88, 91, and 93%, respectively. In comparison to the *Mystus tengara,* the hatching rate of *Mystus vittatus* was only 56% **(Islam & Rahi, 2011)**.

According to **Piferrer *et al.* (2009)** and **Weber and Hostuttler (2012)**, the timing of cleavage provides vital information which is critical in studying chromosomal manipulation. In the case of *Mystus tengara*, the first cleavage was evident after 00:15 hpf. Comparing this with other *Mystus* sp. which took a longer time to achieve first

cleavage as shown by 00:45 hpf for *Mystus cavasius* **(Rahman *et al.*, 2004)** and 00:40 hpf for *Mystus gulio* **(Hossen *et al.*, 2021)**. In the case of Cyprinids also the time of first cleavage has been found to be delayed, such as 00:45 hpf for *Danio rerio* **(Kimmel *et al.*, 1995)**. Yolk extension as observed by **Kimmel *et al.* (1995)** in *Danio rerio* during somitogenesis was not spotted in the development of *Mystus tengara* in this study.

The hatching time of *Mystus tengara* has been found to be between 14:00 and 15:00 hours post-fertilization, which is significantly earlier than that of cyprinids such as *Danio rerio*, where hatching occurs at 48 hpf **(Kimmel *et al.*, 1995)**, and *Pethia shalynius*, which hatches at 26 to 27 hpf **(Nath *et al.*, 2021)**. In comparison to *Mystus cavasius* and *Mystus gulio*, which have hatching times of 19 and 21 hpf, respectively **(Rahman *et al.*, 2004; Hossen *et al.*, 2021)**, the hatching time of *Mystus tengara* is much earlier at 14 to 15 hpf.

The newly hatched larvae of *Mystus tengara* measure 1.62mm in length, which is comparable to *Mystus cavasius* (1.28mm) and *Mystus gulio* (1.14 mm) as reported by **Rahman *et al.* (2004)** and **Hossen *et al.* (2021)**, respectively. The larvae are transparent, slender, and devoid of pigmentation, similar to the findings reported by **Rahman *et al.* (2004)** in *M. cavasius*, **Bhattacharya *et al.* (2005)** in *P. conchonius*, and **Nath *et al.* (2021)** in *Pethia shalynius*.

Given that *Mystus tengara* has both food and ornamental value, information on its induced breeding and embryonic development is essential for conservation efforts and commercial breeding purposes. This study aimed to provide a baseline for further research on larval rearing and to develop a standardized breeding protocol for farmers.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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