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Embryonic development of banded gourami, *Colisa fasciata* in captive condition

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Abstract
The experiment was conducted to provide information on the embryonic development of banded gourami, *C. fasciata* under controlled aquarium condition. The process of embryonic development was divided into seven phases- the zygote, cleavage, morula, blastula, gastrula, segmentation and hatching periods. At fertilization, the eggs were 0.35-0.50 mm in diameter which displayed total length of 0.50-0.70 mm, 22 hours after fertilization. First cleavage was recorded within 35 minutes after fertilization and the embryonic rudiments of developing eggs appeared at 21-22 hours. Cleavage resulted into 64-128 blastomeres and the crown of the blastoderm starts spreading over the yolk in the form of a thin layer in morula stage. In gastrula, blastoderm covered 3/4th of the yolk and embryonic shield was clearly visible. The embryo shows conspicuous muscular contractions and the beating heart at 22 hours after fertilization. This embryonic studies support phylogenetic development by presenting supportive proofs to determine an organism's ancestral forms.

Keywords: *Colisa fasciata*, embryonic development, embryo, blastomere

1. Introduction
The banded gourami, *Colisa fasciata* [1] is the largest gourami in its genus. The fish is a tropical labyrinth fish native to Bangladesh, Eastern India, Myanmar, Nepal and Pakistan and transported around the world [2]. It is locally known as "khailsha" and found all freshwater areas of Bangladesh [3]. It commonly inhabits freshwater pools, ditches, ponds, marshes [4] and rivers as well as lakes with vegetation [5]. The fish is highly esteemed as food [6] and fetching BDT 300-550 per kg. The fish playing an important role in meeting the nutritional requirements of poor fishers in Bangladesh and its neighboring countries like India, Myanmar and Sri Lanka [7]. The species have also gained importance for its ornamental value as an indigenous aquarium fish but yet Bangladesh has to import from India [8]. The species has also ecological value as controlling Malaria, Chikungunya etc due to feeding on mosquito larvae. The species is declining rapidly from our natural habitat due to drastic reduction of the natural feeding and breeding ground as a result of human intervention and modification of its habitat [9]. Over exploitation and applying pesticides and insecticides in nearby agricultural fields are the main reason to be the threatened for ornamental fishes [10]. At present *C. fasciata* is under 'least concern' category in the IUCN red data book [11].
Information on embryonic development is crucial and important for fish seed production and commercial aquaculture [12, 13]. However, embryonic development in fishes depends on many environmental factors and embryonic stages started with the fertilization which occurs inside the chorion and end at hatching [14]. Embryology is the science that deals with the development of the organism from the zygote to the completion of its bodily structure and embryo is a stage of an organism before birth or hatching. How a one- cell fertilized egg or zygote is able to develop into a complete animal, usually within a relatively short time is an amazing and fascinating biological process. Conserving fish is only possible when the life cycle is fully understood. Therefore, considering the above facts, the study of embryonic development of the freshwater *C. fasciata* has been made to understand the early development. Embryonic studies support the phylogenetic development by presenting supportive proofs to determine an organism's ancestral forms.
There were several studies have been reported on gourami fishes from India like biology and fishery of *Colisa fasciata* [7], Captive breeding and embryonic development of *C. sota* [15], captive breeding of *Trichogaster labisua* [16], breeding and early development of *T. trichopterus* [17] and breeding and embryonic development of *T. lalius* [18].

However, single experiment was done in Mymensigh, Bangladesh on the embryonic and larval development of *T. fasciata* [9]. No other study has been reported on the embryonic development stages of the locally available *C. fasciata* from South-West Bangladesh. Therefore, the present study was undertaken to ascertain and observe the different larval development stages of *C. fasciata* to standardize the breeding techniques, to understand the biological or developmental clock and to know the early life story stages of *C. fasciata* in relation to various time intervals.

2. Materials and Methods

2.1 Study Site

The experiment was conducted in the laboratory of Fisheries and Marine Bioscience Department, Jessore University of Science and Technology, Jessore, Bangladesh. The aquarium set up, water supply facilities and other things related to this research work were assured.

2.2 Collection of egg sample

Broods were collected from the nearby baor named Majdhar baor in Barobazar, Jessore. Fishes were kept in water jar and immediately brought back to the laboratory of Fisheries and Marine Bioscience. The brood fish were acclimatized and reared in the aquarium with the formulated diet for three months. Water level was maintained about 40 cm in the aquarium and during rearing water hyacinth and some stones was supplied at the bottom. Healthy and active brood fishes were selected and kept in a separate aquarium. Continuous air flow was provided in the aquarium by using an aerator. Before conducting the breeding programme the male and female ratio were maintained 1:1 and the breeding was performed by using different hormone PG, HCG and Ovaprim. The buoyant, non-adhesive fertilized eggs were collected by a fine mesh net for the further microscopic study. The water parameters such as water temperature, pH and DO were measured everyday during observation of eggs.

2.3 Observation of embryonic development

Early developmental stages of *C. fasciata* were studied up to 22 hours starting after egg fertilization. Approximately 10-15 fertilized eggs were collected from the aquarium. The embryonic development progresses were observed under photographic microscope. Fertilized eggs were observed in every 15 minutes until morula stage and later on eggs were checked in every 1 hour interval until hatching. For the confirmation of each developmental stage, six eggs were examined. The embryonic developmental stages of *C. fasciata* were observed by using a photographic microscope (Carl Zeiss Microscopy GmbH, S.N. MKG 8639, Germaney) and photographs were taken for each stage of the development. The diameters of the eggs were measured.

3. Results

3.1 Embryonic development

The study of one type of evidence of evolution is called embryology, the study of embryos. An embryo is an unhatched animal in its earliest phase. The embryonic stage may refer to different stages in eggs. The physico-chemical condition such as temperature, dissolved oxygen and pH of water in experimental aquaria under different treatments were ranged from 26 to 29.1º C, 3.5 to 5.0 ml/l and 7.09 to 8.89 respectively. The average temperature, pH and DO were 27.48 º ± 0.82, 8.31± 0.45 and 4.21± 0.36 respectively. The developmental stages with their major characteristics are mentioned in Table 1. Stages of embryonic development of eggs varies according to different species can be summarized as follows-

Unfertilized egg: The unfertilized eggs of *C. fasciata* were floating, opaque and whitish in color (Fig. 1 a). Unfertilized eggs measured 0.25-0.35 mm in diameter.

Fertilized egg: The fertilization of the eggs took place as soon as the sperm enters into the eggs. The fertilized egg capsule was non adhesive, spherical in shape, transparent, off white in color and the eggs range in diameter from 0.35-0.50mm (Fig. 1 b). Fertilized eggs had a brownish spot (blastodisc) on one pole and easily recognizable with the naked eye.

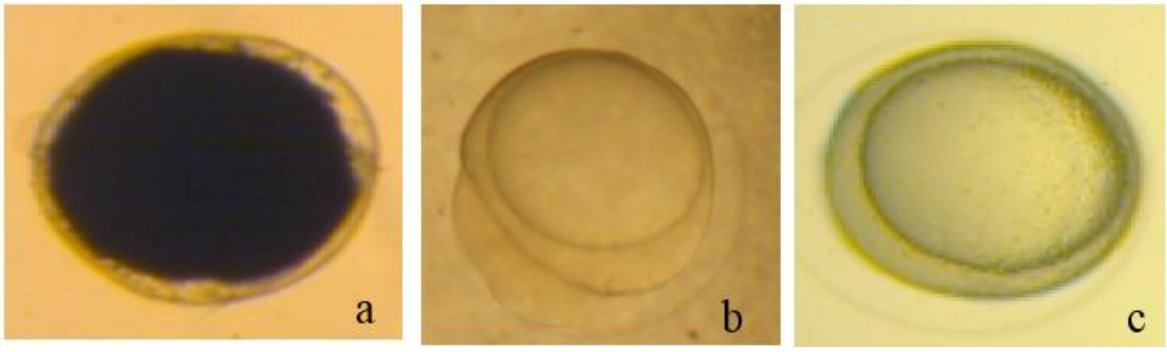
Two celled stage: The first cleavage started by dividing the blastodisc into two blastomeres. This event appeared in 25-30 minutes after fertilization (Fig. 1 c) with meroblastic type. Two blastomeres were observed at the animal pole containing only half size of the original cell.

Four celled stage: Four equal blastomeres resulting from the second mitosis appeared on a vertical plane, happened in 35-50 minutes after fertilization (Fig. 1 d). Each blastomere was smaller in size and fat globules were observed in the yolk**.**

Eight celled stage: This stage showed that eight blastomeres were seen after 1-1.20 hours of fertilization (Fig. 1 e). The blastomeres were smaller and equal in size.

Sixteen celled stage: The sixteen celled stage (Fig. 1 f) was developed within 1.30- 1.45hours.

Thirty-two celled stage: The 32- celled stage (Fig. 1 g) was appeared after 1.45- 2.15 hours following fertilization. The blastomeres became overlapping due to the limited confined space within the capsule. Blastoderm divided into 32-cell and the 32 blastomeres were already formed. The cells became smaller and were arranged irregularly.



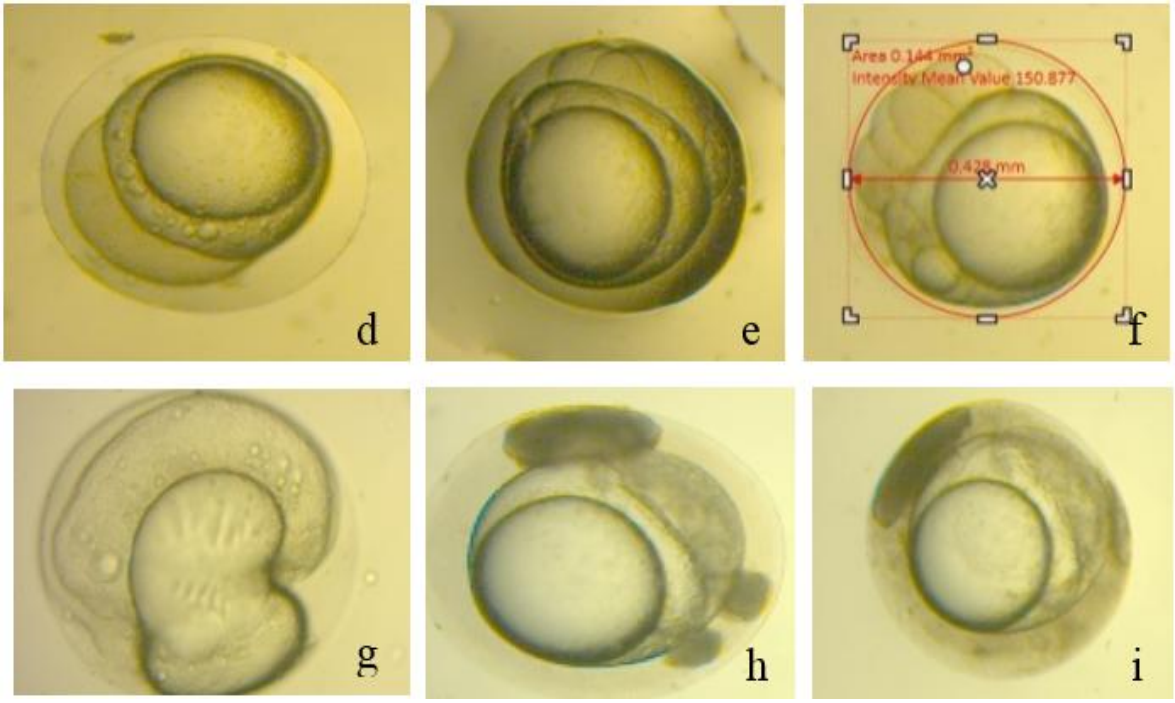


Fig 1: Unfertilized and fertilized eggs with developmental stages up to cleavage phase. a) unfertilized egg, b) fertilized egg, c) two celled stage, d) four celled stage, e) eight celled stage, f) sixteen celled stage, g) thirty two celled stage, h) 3-oil droplet stage and i) 1-oil droplet stage.

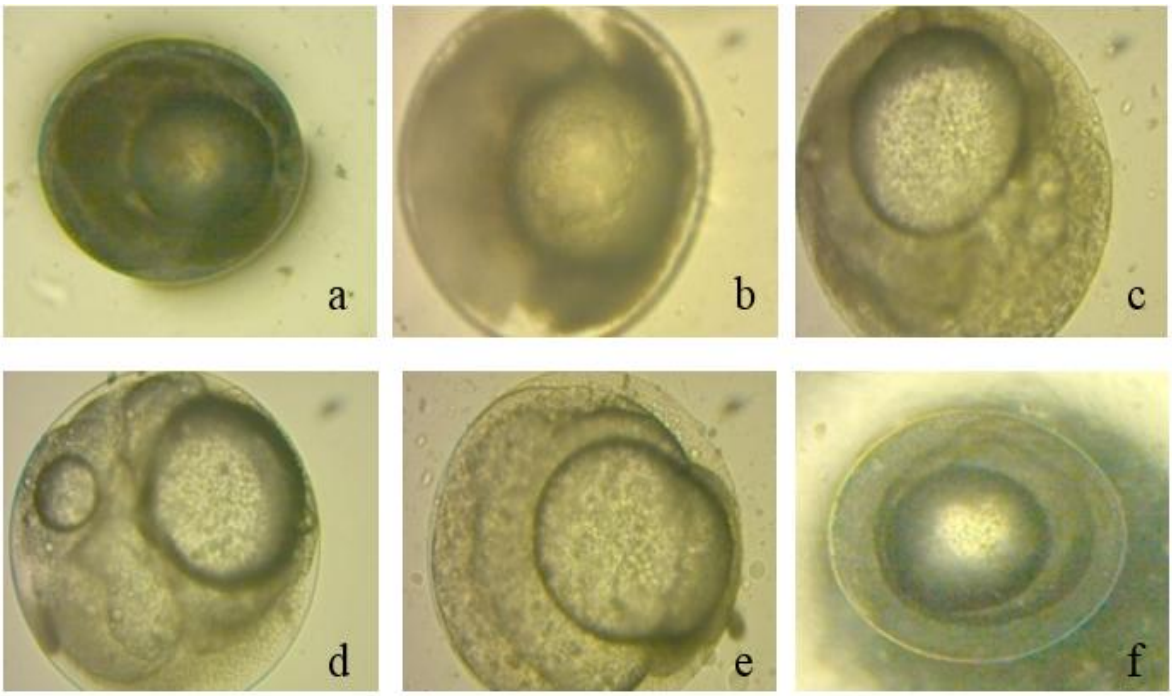
3-oil droplet formation: 3 -oil droplet (Fig. 1 h) formed at this stage after 2.30- 3.0 hours of fertilization. The eggs were 0.50-0.70mm in diameter.

1-oil droplet formation: The egg was measured 0.50-0.70mm in diameter. 1-oil droplet (Fig. 1 i) formed after 3.0-3.30 hours of fertilization.

Morula stage: Morula stage is shown in (Fig. 2 a). As successive cleavages occurred, the blastomeres were decreased in size and the morula stage was reached between 3.30-4.0 hours following fertilization. At this stage, the crown of the blastoderm starts spreading over the yolk in the form of a thin layer and the anterior and posterior ends of the embryo became differentiated. The egg was measured 0.50-0.70 mm.

Early blastula: The blastula (Fig. 2 b) is an early stage of embryonic development in animals. At this stage the egg was 0.50-0.70 mm in diameter. It is also called blastophere occurred in the vegetal pole at 4.0-4.30 hours after fertilization. At this moment, the crowded cells expanded over the yolk and the blastomeres were divided asynchronously.

Late blastula: The late blastula stage measured 0.50- 0.70 mm in diameter consists of a multicellular blastomere and fully completed at around 4.30 to 5.30 hours subsequent to fertilization (Fig. 2 c).



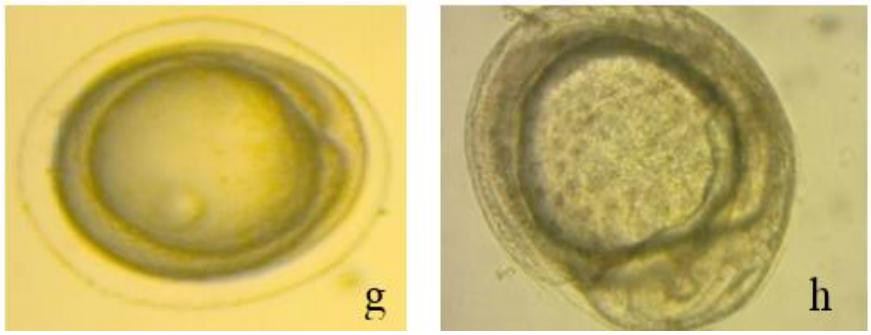


Fig 2: Embryonic development stages of *C. fasciata.* a) morula stage, b) early blastula, c) late blastula, d) early gastrula, e) late gastrula, f) embryonic shield formation, g) yolk plug stage and h) pre-hatching.

Early gastrula stage: Following the morula stage the blastoderm started invading the yolk by spreading over the yolk in the form of a thin layer (Fig. 2 d). Gastrulation resulted within 5.30-6.30 hours of fertilization. Eggs were measured 0.50-0.70 mm in diameter.

Late gastrula stage: In this stage, blastoderm covered 3/4th of the yolk (Fig. 2 e) was observed after 6.30- 7.0 hours after fertilization. The formation of embryonic shield noticed in this stage. The diameter of the eggs was 0.50-0.70 mm.

Embryonic shield formation: Embryonic shield (Fig. 2 f) was clearly visible after 8.0- 8.30 hours of fertilization. The egg size was 0.50-0.70 mm in diameter.

Yolk plug stage: At this stage, yolk invasion was completed by gradual spreading over the germ layer and was observed after 14- 16 hours after fertilization. Rudimentary head and tail were formed, and differentiated from the yolk (Fig. 2 g). The diameter of the eggs was still remain same size as earlier stages.

Just before hatch: Initially the head and tail end of the embryo was differentiated. The embryo was elongated and encircled the yolk materials. Both tail and head ends were clearly differentiated and the beating heart was visible. Heart rudiment and gill rudiment appeared one by one. The embryo further elongated and was gradually differentiated (Fig. 2 h). The body was transparent having no muscular structure and the tail gradually became detached from the yolk mass. This stage was obtained in 21-22 hours. Embryo started an occasional twisting movement inside the egg and continuously beat the egg shell by the caudal region especially around the middle part of the body. The embryos ruptured the egg shell by the continuous movement. Hatching continued for 2.30 hours because the entire embryo did not hatch out at a time.

Table 1: Summary of embryonic development events of *Colisa fasciata*

Name of stages	Sub-stage	Time (hr:min)	Diameter (mm)	Developmental features
Unfertilized egg		0.00	0.25-0.38	Opaque, buoyant, slightly elongated and whitish in color.
Fertilized egg		0.00	0.35-0.50	Transparent, non-adhesive, spherical and off-white in color.
Clevage	2 cells	25-30	0.35-0.50	First cleavage dividing the blasto disc into two blastomeres.
	4 cells	35-50	0.35-0.50	Second cleavage blastodisc divided via meridional cleavage to form four equal cells.
	8 cells	1-1.20	0.35-0.50	Third cleavage
	16 cells	1.30-1.45	0.35-0.50	Fourth cleavage
	32 cells	1.45-2.15	0.35-0.50	Fifth cleavage
3 oil droplet		2.30-3.00	0.50-0.70	3 oil droplet formed on the 3 sides of the yolk.
1 oil droplet		3.00-3.30	0.50-0.70	1 oil droplet formed on 1 sides of the yolk.
Morula		3.30-4.0	0.50-0.70	Cleavage resulted into 64-128 blastomeres and the crown of the blastoderm starts spreading over the yolk in the form of a thin layer.
Blastula	Early blastula	4.0-4.30	0.50-0.70	Blastomeres continued to divide but they were less synchronously.
	Late blastula	4.30-5.30	0.50-0.70	Spherical in shape, epibolic cells increase.
Gastrula	Early gastrula	5.30-6.30	0.50-0.70	Blastoderm cells begin to spread over the yolk.
	Late gastrula	6.30-7.0	0.50-0.70	Blastoderm covered 3/4th of the yolk and embryonic shield was clearly visible.
Embryonic shield formation		8.0-8.30	0.50-0.70	Embryonic shield was clearly visible.
Yolk plug stage		14- 16	0.50- 0.70	Yolk invasion was completed by gradual spreading over the germ layer.
Just before hatch		21-22	0.50- 0.70	The embryo shows conspicuous muscular contractions and encircled the whole yolk.

4. Discussion

In the present study, the embryonic development of *C. fasciata* is stated with developmental sequence from egg up to the end of embryonic development in controlled conditions. As the egg size depends on several factors, for example, species variation, broodstock age, broodstock size, feed and water quality. Temperature is the prime factor that influences the development of fish embryo. It is generally thought that higher temperature increase the development rate within certain limits. In the present study, the recorded average temperature was 27.48 ± 0.82º C that was quite optimum compare to other such studies like 27.8-28.9 ºC [15], 26 ± 0.1 ºC [9], 27.8-28.9 ºC [17] and 29.15 ± 0.95 ºC [18] reported in breeding of different gourami fishes in India and Bangladesh.

The characteristics of the unfertilized and fertilized eggs of *C. fasciata* were similar to [9] and [18] who noticed in *T. fasciata* and *T. laius* respectively. The diameter of the unfertilized and fertilized eggs of *C. fasciata* was also similar to [9]. In the present study it was recorded 0.25-0.35 mm and 0.35-0.50 mm of the unfertilized and fertilized eggs of *C. fasciata.* [9] reported for the same species as 0.20-0.30 for unfertilized eggs and 0.30-0.60 for the fertilized eggs. This is the confirmation of the egg characteristics of *C. fasciata*. However, larger egg size reported in other gourami species like 0.50-0.68 mm for honey gourami, *C. sota* [15], 0.60-0.69 mm for dwarf gourami, *T. lalius* [18], and 1.37-1.45 mm in nest building gourami, *T. trichopterus* [17]. The diameter of the fertilized eggs increased during morula stage and which remained almost the same size till hatch. The diameter during hatching in the present study recorded 0.50-0.70 mm whereas in case of *T. trichopterus* it was 2.5-2.75 mm [17].

The results of the present study have shown that the time of hatching out was 23 to 24 hours. The hatching was 22-24 hours for *Tricogaster fasciata* [9], 24.4 hours for dwarf gourami [18]**,** 23-24 hours and 24 hours for *Trichogaster trichopterus* [17]. However, a bit higher time reported for honey gourami [15] and giant gourami [19] showed that the time of hatching out was 28-30 hours and 26 hours respectively. This variation with the present study may be due to species variation or other water quality parameters.

In *C. fasciata,* it is showed that the two cell, four cell, eight cell, sixteen cell and thirty two cell stage were found in 25-30 minutes, 35-50 minutes, 1- 1.20 hours, 1.30-1.45 hours, 1.45-2.15 hours after fertilization. The present findings are more or less similar to [9] and noticed the same series at 25-30 minutes, 50 minutes, 1.10 hours, 1.30 hours, 2 hours. In *T. lalius*, the first cleavage occurred within 15 minutes and completed within 2 hours [18]. On the other hand, first cell division started in very late in case of *C. sota* where it took 16-20 hours [15].

It was shown in the present study that morula and blastula stage were appeared at 3.30- 4 hours and 4- 4.30 hours after fertilization. However, earlier morula and blastula formation noticed in *T. fasciata* [9] and it was 2-3 hours and 3.30 hours respectively. The more or less similar time reported in other species like 4.10 hours after fertilization in spiny eel, *M. aculeatus* [20]. In case of *Nandus nandus*, blastula was found 3.30±0.15 hours after fertilization [21], while in *Ompok pabo* this stage was found 3.30 hours after fertilization [22].

As teleost gastrulation is morphologically characterized by the presence of a germ ring [23]. In this study, gastrulation was observed at 5.30- 6.30 hours after fertilization. In *Heteropneustes fossilis* this stage noticed at 6.30 hours of fertilization [24] and in *Nandus nandus* also noticed at 6.30±0.15 hours [21] and at 5 hours of fertilization in *T. fasciata* [9], which was within the range of the present findings. However, in case of *T. lalius* gastrula occurred in 7-11 hours [18] and in *Mastacembelus pancalus* this stage was occurred in very late at 21 hours after fertilization [25].

In the present findings it was found that pre-hatching stage was near to 21- 22 hours with the head, primordial fin, tail, heart vibration, conspicuous muscular contraction and demonstration of twisting movements inside the egg capsule. The similar hatching was found in *T. fasciata* described by [9], in *C. sota* reported by [15] and in different fish species described by [24, 26].

5. Conclusion

Embryonic development of *C. fasciata* was studied in relation to various time intervals. The embryonic technique discussed in the present paper can be considered for further development of the breeding and rearing technique of the species. The techniques of embryonic development of ornamental fish may reduce the uncertainty and unavailability of fish seed/fry and may increase the large scale production for export purpose.

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