



Larval rearing and observation of larval development stages of *Colisafasciata* in captive condition

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Abstract

The early development information is a necessity to reproduction and rearing of larvae of any fish in the hatchery. The larvae of the *Colisa fasciata* were reared and examined the stages of larval development in captive conditions. The survival rate of larvae also checked in different water sources and an improved survival rate was observed in rainwater (39%) than the pond and supplied tap water. The main aspects of the larval period were yolk absorption, active body movements, the start of the exogenous feeding, body pigmentation, organogenesis, and fins differentiation. New hatchlings were 1.05 mm long with a large yolk sac, devoid of mouth and pigmentation. The development consequences happen sharply within the first 4-5 days and then it was slowly developing all other characteristics. Eyes became pigmented within 1st day of hatching and the pigmentation in the body also started from the same period. The pectoral fin buds appeared on the 2nd day while the yolk sac gradually reduced and larvae started a short span of swimming. The yolk sac was completely absorbed on the 4th day after hatching and started exogenous feeding. The development of notochord exhibits from 16th days and the larvae resembled the adult in its external features and were completed metamorphosis within 21st days of post-hatching. However, the development of some organs (like barbs) showed in later and further development of the existing organs showed until the 40th days of post-hatching. These findings could be used as baseline information to the hatchery operator to rearing of the larvae properly which will be encouraged to large-scale seed production of the fish in the near future.

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Introduction

The giant or banded gourami, *Colisa fasciata* (Bloch and Schneider, 1801) is the largest gourami in its genus. Gouramis commonly known as labyrinth fishes belong to the suborder *Anabantoidei*. It is locally known as “kholisa” and one of the common species of Small Indigenous Species (SIS) of the family Belontiidae found in Asia. *C. fasciata* is an important edible freshwater fish species of Bangladesh. It is one of the most favorite, commercially important and highly demanded fish due to its high nutritive and market values, palatability, and ability to tolerate adverse water quality conditions (Hamilton, 1822). The fish is found in swamps, ponds, ditches, marshes (Rahman, 1989), and the shallow margins of rivers covered with thick vegetation and weeds (Menon, 1999). Since it is indigenous species, it can be cultured in the pond, canal, beel (seasonal flood land), etc. if it is known details early development biology.

There is no sufficient information on the early development of the fishes. So it is necessary to undertake a proper study to characterize its various stages of larval development to understand the biological clock and cultural techniques of the species (Rahman *et al.*, 2009). Further, studies on larval development are imperative and consequential to the successful rearing of larvae for large scale seed production and aquaculture (Khan and Mollah, 1998; Rahman, 2004). So, it was felt necessary to study and characterize its various larval development stages of the species. There is some research on the biology and breeding have done on some kholisa fishes (*Tricogaster pectoralis*, Amornsakun *et al.*, 2004; *Colisa fasciatus*, Barman *et al.*, 2013; *Trichogaster trichopterus*, Bindu *et al.*, 2014; *Tricogaster fasciatus*, Hossen *et al.*, 2014; *Colisasota*, Mitra *et al.*, 2006) but no such details study on the rearing and larval development of the experimental species particularly in the Bangladesh context. Therefore, the present study has been conducted on the larval rearing as well as the observation of the larval development stages of *C. fasciata*.

Materials and methods

Study site

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

Experimental design

The experiment was designed into two distinct segments; i) larval rearing and ii) examine larval development progress. For larval rearing, there were three treatments designed according to different water sources (Table 1) and each treatment had one more replication. The glass aquariums (tanks; 20 liters each) were used for the rearing which was stocked at the same density (Table 1). In each tank, water hyacinth was placed as a substrate. The larval developments were observed under an electric microscope in every 24 hours' interval from each of the rearing tanks.

The water parameters such as pH, DO, the temperature was recorded twice in a day. The water was exchanged about 25% and wastes were siphoned out by siphoning tube every day. pH and DO was measured by a pH meter (EZODO, 7200; Taiwan) and DO meter (LTLutron YK-22DO; Taiwan) respectively.

Larval rearing and observation of development

Larvae were collected from the same laboratory which was induced by using hormone HCG. By using a glass jar and scoop net larvae were separated from their parents as soon as they hatch. From the third day of stocking when noticed yolk sac was absorbed, boiled egg yolk was supplied as feed. The boiled egg yolk was homogeneously mixed with water by filtering with a cotton net before supply. Larval development stages of *C. fasciata* were observed and taken photographs in once a day under an electric microscope (BoEco, Germany). The pictures were also taken by a digital camera (Sony, Model DSC-W520) from the eyepieces of the microscope. Larval development stages were characterized

according to their ontogenetic development and followed mostly to Barman *et al.*(2013), Binduet *al.*(2014), Hossein *et al.*(2014), Martell *et al.* (2005) and Mitra *et al.*(2006).

Results

Water parameters

The physico-chemical condition such as temperature, pH, and dissolved oxygen of rain, pond, and tap water in an experimental aquarium under different treatments were ranges between 26-30°C, 7-7.5, and 5-6.30mg/l which are shown in the Table 2. The mean values of water parameters were not significantly ($P < 0.05$) different among the treatments.

Larval rearing and survival rate of *C. fasciata* in different water sources

Hatching started at 22h after the completion of egg deposition. The newly hatched larvae remained in the bubble nest or attached to plant leaves or walls of the breeding aquaria (Fig.1). The staying behavior of the *C. fasciata* showed differences in different water rearing medium. In the pond water, larvae often remain in the bottom, in rainwater they often remain in the top of the water column, but in tap water, they remain mostly top and middle and sometimes in the bottom. The larvae showed more activity in the night than day time and they like to live in dark and less aerated water condition.

Table 1. The experimental design for different treatment for larval rearing of *Colisafasciata* with their stocking density.

Treatments	Water sources	Stocking density
T1	Rain water	100/tank
T2	Pond water	
T3	Supplied tap water	

The larvae were surviving in different rate in the entire experimental aquarium and kept them for 40 days. The survival rate differed significantly ($P > 0.05$) in different water sources.

The highest average survival rate was observed in T1 (39%) which was filled with rainwater followed by the T2 (pond water; 33%) and T3 (supplied tap water; 27%) respectively.

Larval development stages

The larval development stages with their visible characteristics are described below and mentioned briefly in Table 3. The development consequences happened quickly within the first 4-5 days and then slowly changed in the further development.

Newly hatched larvae: 1.05 mm in length

The embryo hatched out within 23-24 h. The color of larvae was somewhat black just after hatching which gradually changed to brownish color. Newly hatched larvae were laterally compressed and elongated. The entire head region attached to the yolk sac. Large yolk sac attached to the body.

The hatchlings had un-pigmented eyes and the mouth were closed still without distinct fins (Fig. 2A). The larvae could not swim but float passively in the water and irregularly appeared upside-down movement.

1st days old larvae: 1.17-1.25 mm in length

Eyes became pigmented and body pigmentation has started in the posterior part of the yolk. Several melanophores were scattered over the surface of the body and the yolk sac. The color of the larvae was brownish. A full complement of fins is rarely present, but a primordial fin fold was well developed. The mouth and anus were not yet opened (Fig. 2B).

2nd days old larvae: 1.35-1.41 mm in length

The larvae were increased in size by 1.35-1.41 mm in average length. More pigmentation started appearing on the body at this stage. Eyes became distinct and the size of the yolk sac gradually reduced. The pectoral fin buds appeared and melanophores were more concentrated at the head region than the body (Fig. 2C). The larvae started a short span of swimming at this stage.

Table 2. Water quality parameters of different treatment aquarium during rearing of *C. fasciata* larvae.

Water parameters	T1(Rain water)	T2(Pond water)	T3(Tap water)
Temperature(°C)	28.8 ± 0.67	28.3 ± 0.44	27.7 ± 0.69
pH	7.2 ± 0.19	7.3 ± 0.34	7.33 ± 0.20
DO(mg/l)	6.23 ± 0.14	6.17 ± 0.22	5.89 ± 0.17

3rd days old larvae: 1.55-1.60 mm in length

The mouth became terminal and the vertebral column well defined. Eyes and pupils were more prominent; melanophores still appeared from head to tail. The yolk sac reduced and notices dorsal fin bud (Fig. 2D).

4th- 5th days old larvae: 1.67-1.75mm in length

The pectoral fin buds enlarged and at the same time dorsal, caudal and ventral fins appeared as a continuous transparent flap with the slight

demarcation of fins. The yolk sac absorbed completely and the mouth became upturned (Fig. 2E). At this stage, the larvae became free swimming and the mouth started functioning.

6th – 7th days old larvae: 1.75-1.89mm in length

Eyes and pupils were more prominent. Pectoral fins were increased in size on both sides (Fig. 2F). Melanophores increased in numbers and appeared throughout the body (head to tail).

Table 3. The prime distinguishing characteristics in different time intervals of *C. fasciata* larvae in the aquarium.

Day	Characteristics
Just after hatching	Brown colored larvae with large yolk sac.
1	Functional heart with oval shaped large yolk sac.
2	Development of eyes and pectoral fin with comparatively small yolk sac.
3	Reduced yolk sac, prominent eyes and pupils. Dorsal fin formed.
4-5	Clear mouth and operculum, head and tail somewhat distinguished. Completely absorbed yolk sac.
6-7	Clear pectoral fin and complete melanophore.
8-9	Prominent mouth cleft, development of caudal fin rays.
10-15	Distinct air bladder and well developed jaws.
16-20	Developed notochord, head and body clearly distinguished.
21	Metamorphosis complete.
22-29	Pigmented eyes and prominent gills. Development of intestine and gut.
30-39	Clearly distinguished upper and lower jaw, caudal fin and barb.
40	All characteristics of a large kholisa fish.

8th – 9th days old larvae: 1.90- 2mm in length

Eyes and pupils were very clear and large. The brain lobe was clearly shown (Fig. 2G).

10th – 15th days old larvae: 2.19-3.10mm in length

The average length of larvae was 2.19-3.10mm. Air bladder appeared and well-developed jaws were shown. The body become elongated and compressed at the caudal region. The caudal fin was small (Fig.

2H).

16th – 20th days old larvae: 3.60-5.00mm in length

Mouth cleft was prominent and the upper jaw and lower jaw fully distinguished (Fig. 2I). Very well developed air bladder and spiky notochord were shown (Fig. 2J). Dorsal fin and anal fin extended to the caudal fin and appeared of the caudal fin rays (Fig. 2K).

21st day's old fry: 5.30-5.66 mm in length

The body of the larvae enlarged. The intestine, gill, gut, notochord, and all the fins were fully distinguished as adult *C. fasciata* (Fig. 2L). Larvae were increased by 5.30-5.66 mm in length. All the organs were clearly distinguished as their parents.

22nd-40th day's old fry: 5.88-6.78 mm in length

Body color of the larvae turned brownish to somewhat whitish. All the fins and rays developed. The larvae showed distinguishable caudal and dorsal fin (Fig. 2M) and also noticed the barbels. All characteristics of a large Kholisa fish and all the organs were developed as an adult on 40th days (Fig. 2N).

Discussion*Water parameters and survival rate*

The present experiments succeeded the rearing of larvae up to 40 days and onward. The second best survival days reported from North-East India and it was 30 days (Barman *et al.*, 2013). The other reported a very short time for other kholisa species like 15 days for *C. sota* (Mitra *et al.*, 2006) and 2 days for *T. fasciata* (Hossen *et al.*, 2014).

The recorded water parameters in rearing system (Temp=26-30°C, PH= 7- 7.5, DO= 5-6.5mg/l) in present experiments was quite optimum for the species according to the other such reported. In the present experiment, it was found that the survival rates up to 40 days in rainwater, pond water, and tap water were 39%, 33% and 27% respectively.

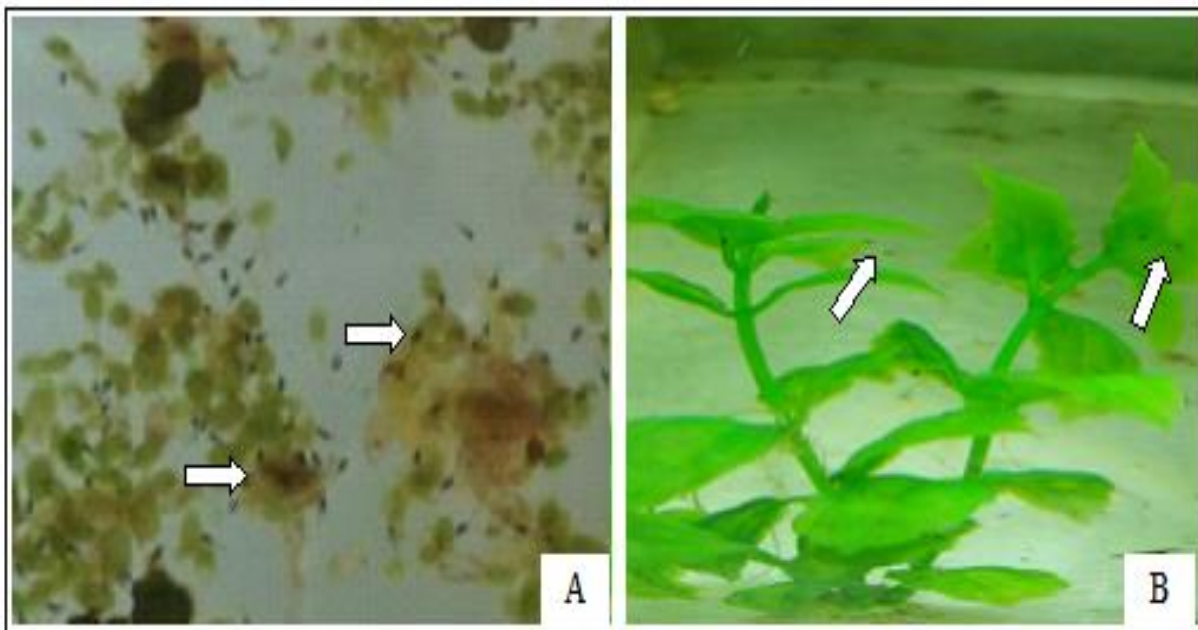


Fig. 1. Larvae that were attached to the floating leaves (A) and to the aquatic plants (B).

This finding is more or less similar to Mitra *et al.* (2006) in *C. sota* and showed that the survival rate was 30% at 27.8°C, 31% at 28.7°C, 32% at 28.9°C, 35% at 28.5°C. This apparent deviation in rainwater, pond water, and tap water may be the water quality parameters.

Though there was no significant ($P < 0.05$) deviation of water parameters among three different water sources maybe the lower pH and higher DO in rainwater could be the trigger for the higher survival of larvae.

Larval development

The basic larval development sequences were more or less similar to other studies on the kholisa fishes (*Tricogaster pectoralis*, Amornsakunet *et al.*, 2004; *Colisafasciatus*, Barman *et al.*, 2013; *Trichogaster trichopterus*, Binduet *et al.*, 2014; *Tricogaster fasciata*, Hossen *et al.*, 2014; *Colisasota*, Mitra *et al.*, 2006).

Though the symmetrical morphological development noticed in many kholisa fishes, different species showed in different time intervals after hatch.

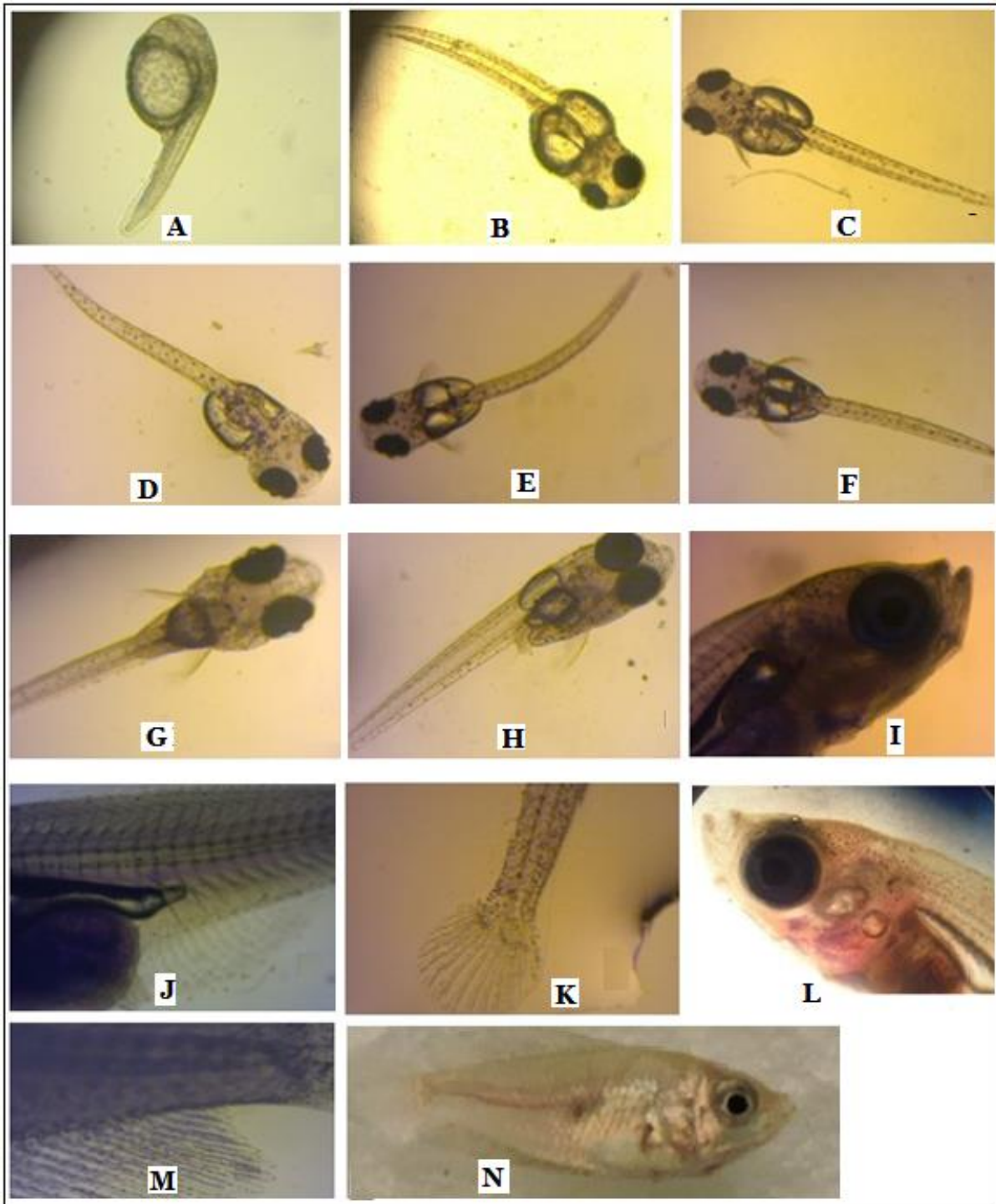


Fig. 2. Larval development stages of *C. fasciata*. A: Newly hatched larvae; B: 1st days old larvae; C: 2nd days old larvae; D: 3rd days old larvae; E: 4th days old larvae; F: 6th days old larvae; G: 13th days old larvae; H: 15th days old larvae (Inset=caudal fin); I- J- K: 16-20th day's old fry; L: 21st days old fry; M: 36th days old fry (inset=barbs); N: 40th days old fry.

The hatching time noticed within 23-24 hours of spawning at average $28.26 \pm 0.60^\circ\text{C}$ which is similar to Hossenet *al.*(2014) and Barman *et al.*(2013). They also reported 22-24 hours at $26 \pm 1^\circ\text{C}$ and 28°C for *T. fasciata* and *C.fasciatus* respectively.

The just-hatched larvae in the present study were somewhat black to brownish color which was differed from Hossenet *al.* (2014) and Mitraet *al.* (2006) where they found transparent color except the eyes. The entire head region attached to the large yolk sac.

A similar finding also found in *T. fasciata* (Hossen *et al.*, 2014) and in *C. sota* (Mitra *et al.*, 2006).

The large ovoid yolk sac was attached to the body in just after hatching which is also common in other kholisa fishes like in *C. sota* (Mitra *et al.*, 2006) and in *C. fasciatus* (Barman *et al.*, 2013). The length of the newly hatched larvae in the present study was smaller than others who reported on kholisa fishes. In the present study, it was recorded 1.05 mm while 1.7-2.0 mm recorded by Barman *et al.* (2013) in *C. fasciatus* and 3.02-0.06 mm recorded by Hossen *et al.* (2014)

in *T. fasciata*. This size variation may be due to different species, different broods' sizes or due to different geographical locations.

Though there was no published report on the brood size and larval length until their juvenile stages, it may compare to *T. fasciata* (Hossen *et al.*, 2014) that grows up to 4.53 ± 0.12 mm at 48-50 hours and in the case of the present species, it was only 1.35-1.41 mm. However, it was noticed that more than 6 mm in size during observation on the 40th day in the present experiment.

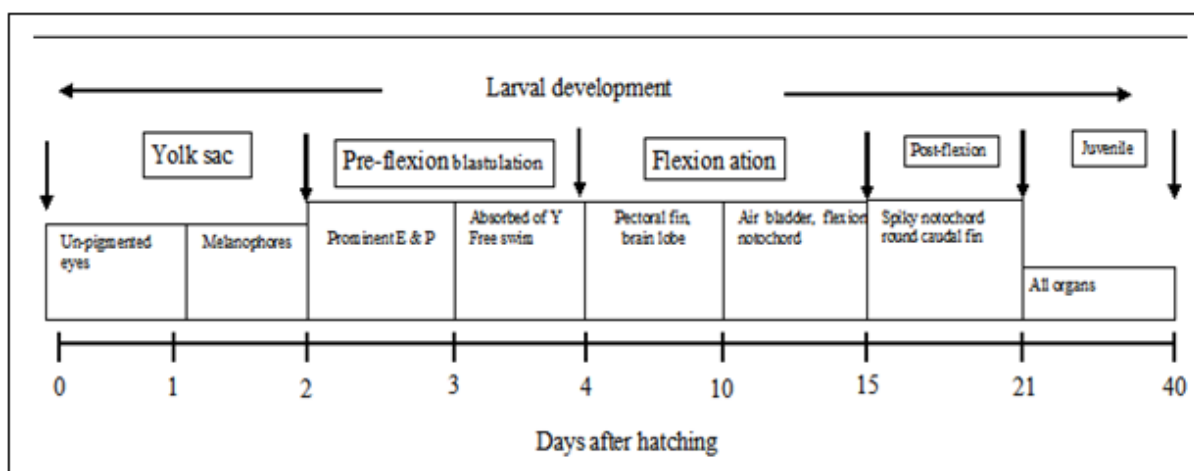


Fig. 3. The larval development consequences with different time intervals of post-hatching. E and P= Eyes and Pupils; Y=yolk sac.

It was observed that within the first two days of hatching, the larvae were inactive due to large yolk sac and they started to swim freely on the day 4th. A similar swimming pattern showed in *C. sota* (Mitra *et al.*, 2006). However early swimming showed in *T. fasciata* (Hossen *et al.*, 2014) and they noticed free swimming within 2nd day. This feature is directly related to the reduction of yolk sac and exogenous feeding. In the present findings, it was noticed that the yolk sac depleted on the 4th day and the larvae started to feed exogenous feeds. As, many fish larvae contain two kinds of energy reserves, yolk and oil globule (Bjelland and Skiftesvik, 2006), but *C. fasciata* had only yolk sac. This finding is similar to *T. fasciata* (Hossen *et al.*, 2014) and *C. sota* (Mitra *et al.*, 2006). In the present study it was observed that, after 2nd day of hatching, the pigmentation appeared on the body. The mouth could be differentiated ventrally to

the head region; eyes became distinct and the size of the yolk sac gradually reduced. The pectoral fin was elongated in shape and clearly visible and actively used for free motion on the 4th day that is in agreement with other studies as *T. fasciata* (Hossen *et al.*, 2014) and *C. sota* (Mitra *et al.*, 2006). Metamorphosis was completed at 21 days and the length reached about 5.30- 5.66 mm. Dorsal, anal and caudal fin were clearly differentiated in this stage. Marimuthu and Haniffa (2007) observed in case of *C. striatus* that metamorphosis was completed in 20th days and all the organs clearly distinguished as like their parents, which is similar to the present study.

Conclusion

The early development pursuance happens sharply within the first 4-5 days and yolk-sac absorbed

completely within these days and started to feed exogenous feed. The larvae become assemble like adult fish within 21 days and hence this period would be the larval stages of the fish. The present findings may provide a basis for further studies to find out the complete life history of *C. fasciata* and detailed studies may be helpful for large-scale seed production in the country.

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