International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **7(6),** 1-8, June **(2018)** *Int. Res. J. Biological Sci.*

Embryonic and larval development of mirror carp (*Cyprinuscarpio* var. *specularis*)

Dil Afroza Khanom, Ananna Das, Md. Abu Sayed Jewel*, Md. Ayenuddin Haque and Md. Delwer Hossain
Department of Fisheries, University of Rajshahi, Rajshahi-6205, Bangladesh
jewelru75@yahoo.com

Available online at: www.isca.in, www.isca.me
Received 28th January 2018, revised 15th May 2018, accepted 8th June 2018

Abstract

Embryonic and larval development stages of Mirror carp, Cyprinuscarpio var. specularis were studied at Fish seed multiplication farm, Puthia, Rajshahi, Bangladesh from November 2016 to April 2017. Brood fishes (body weight 2.5-3kg and 1.2-1.5kg, for male and female, respectively) were collected from the hatchery pond, and two separate dozes of pituitary hormone were used for induction of reproductive. Spawning occurred between 8-8.5h after the 2nd injection. The fertilized eggs were adhesive, round in shape and yellowish to whitish in color and its diameter was 1.10±0.02mm. The stages of embryonic development observed with zygote, followed by cleavage, early morula, blastula, gastrula, segmentation, pharyngula and until hatching. After 45min of fertilization first cleavage was observed, whereas two cells, four cells, eight cells, and sixteen cells stages were found after 45min, 60min, 90min, and 2h of fertilization, respectively. The early morula, blastula, gastrula, segmentation and pharyngula stages were visualized after 3, 7, 10, 13, 22h of fertilization, respectively. Hatching observed after 47h of fertilization and newly hatched larva measured 2.5±0.03 mm in total length. Larval development was observed until 2 days after hatching. Yolk sac was greatly reduced 2days after hatching and the larvae started swimming freely. The study concluded that embryonic developmental cycle completed onset of fertilization within 44.5h and larval developmental cycles completed from onset of hatching within 2 days.

Keywords: Embryonic development, larval development, fertilization, cleavage, *cyprinuscarpio* var. *specularis.*

Introduction

Mirror carp or Koi Carp *(Cyprinuscarpio* var. *specularis*) popularly known as carpio fish is an exotic fish in Bangladesh. It was first introduced in Bangladesh by Department of Fisheries (DoF) in 1960, which is native to other Asian countries, especially China and Japan. The fish is belonged to Cyprinidae family. It has the ability to adapt well in adverse environmental conditions and it known to has faster growth rate and better food value, which make it a popular cultivable species in Bangladesh. It also showed good performance in final production at high stocking density[1]. In commercial aquaculture of Bangladesh, spawning of mirror carp is conducted in a process called hypophysation where inducing agent such as pituitary extract is administrated in the fish. The environment of Bangladesh is favourable for culture of mirror carp where not only ponds, beels, rivers and canals are the main habitats but also cultivation of this species in rice fields during *boro* and *amon*se as on is also practiced[2]. Being a major cultivable species its full potential remains to realize due to the insufficient fry availability in aquaculture operation. Sometimes altered and abnormal development patterns occurred during embryonic development that causes a huge loses in fry availability.

Knowledge about fish development process is essential for life history studies and culture practices[3] that will provide better understanding of biological aspects together with dietary needs and habitat selectivity[4,5]. Therefore, studies on embryonic and early larval development are essential for successful rearing of larvae, large-scale seed production, conservation and for commercial fish farming[6,7]. There are 5 basic stages of life stages of fish which are Embryonic Phase, Larval Phase, Fry Phase, Ripe Phase and Senescent Phase. According to Mahapatra and Krishna[8], the embryonic phase includes fertilized egg, cleavage, morula, blastula, gastrula, embryonic body formation, optic vesicle and auditory vesicle formation, blastopore closing, tail formation and hatching stages. Followed by embryonic phrase, the hatchlings undergo organogenesis and the larval stage is ended with the appearance like their parents. Every stage of embryonic and larval development is very much sensitive and critical.

Therefore, embryonic and larval development studies are essential in the context of sustainability in fisheries production by reducing fry mortality. Although embryonic Larval and development of *Barbodes gonionotus*[9], *Cyprinus carpio*[10], *Rita rita*[11], *Labeo bata*[12] and *Mastacembelus pancalus*[13] have been reported in Bangladesh, information regarding embryonic and larval development of mirror carp (*Cyprinuscarpio* var. *specularis*) are very scare.

Therefore, the present study was to know the detailed embryonic and larval development of Mirror carp in hatchery condition.

Materials and methods

The experiment was conducted in Fish seed multiplication farm, Puthia, Rajshahi and Laboratory of Genetics and Biotechnology Department, University of Rajshahi from November 2016 to April 2017. The Institutional Animal Care and Use Committee, Institute of Environmental Science, University of Rajshahi, Rajshahi-6205, Bangladesh approved the study.

Broodstock selection, hormonal induction and spawning: Two doges of PG extract has been used to the hypophysation of brood Mirror carp where the weight of female and male brood fishes were 2.5-3kg and 1.2-1.5kg, respectively. 1st dose was injected to both female (2.5mg/kg) and male (1mg/kg) fishes at the same time. 2nd dose was given only to female fish at an interval of 9h and the dose was 8mg/kg of body weight (at temperature of 23±1°C). After 8.5h, fishes were stripped out for the collection of egg and milt in a plastic bowl and fertilization was done through mixing by shaking the bowl several times. After that, a fertilization (4g NaCl and 3g Urea per liter water) and tannin solution (0.5g/L water) were used to remove the stickiness of the eggs. Finally, the eggs were transferred into hatching jars with continuous water circulation.

Observation and measurement of eggs and larvae: Every ten minutes of interval, the fertilized eggs were observed to identify the developing stages up to morula stage. Followed by morula stage, the eggs were than observed at every one-hour of interval up to hatching. After 18 to 20h of hatching, the embryo started to twisting movement. All the sampled eggs and larvae were preserved in 0.1% formalin in plastic container. In the laboratory, egg and larval samples were taken in separate slides with the help of brush. Optika microscope was used to observe embryonic and larval developmental stages. At the same time, measurement (diameter and length of egg and larva) were taken using digital slide calipers. To describe each stage, 4-6 specimens were used.

Results and discussion

Embryonic development: The embryonic development of the Mirror carp was divided into six periods: zygote, cleavage, blastula, gastrula, segmentation, and pharyngula period. Each and Every steps were distinguished from other.

Unfertilized and fertilized eggs or zygote: The unfertilized eggs of *Cyprinuscarpio* var. *specularis* were spherical in shape. Eggs were yellowish white in color. The eggs were adhesive in nature. Unfertilized eggs ranged between 1.05-1.06mm in diameter. Fertilization of eggs took place as soon as the sperm enters into the eggs. The fertilized eggs were adhesive, demersal and round in nature. The eggs deposited singly and were highly adhesive throughout the incubation period. These became translucent as development progressed. The diameter of the fertilized egg capsule ranged between 1.08-1.11mm. 30 minold egg was yellowish white and diameter ranged between 1.41-1.42mm (Figure-1A and Table-1).

Cleavage: The first cleavage that divided the blastodisc into two blastomeresor 2 distinct equal size cells was observed at 45 min of post-fertilization (Figure-1B). The second cleavage completed (4-celled stage) at 60min after fertilization where the blastodisc was divided into 4 distinct cells (Figure-1C). 8-celled stage (Figure-1D) occurred at 90 minafter fertilization followed by 16-celled stage (Figure-1E) at 2h after fertilization. 64-celled stage (Figure-1F) was occurred at 3h after fertilization. The blastomeres at these stages were reduced in size. The cleavage planes were no longer regularly patterned as compared to 2-8 cell stage (Table-1).

Stages during the Morula Period: During the morula stage (at 4 h period), the blastomeres were further divided into many cells and accumulated around the animal pole, representing a flowery appearance (Figure-1G). The blastodermal cells (256-celled stage) appeared after 5h (Figure-1H). Here the eggs were appeared as smaller to the previous stage together with the increase in marginal cell was observed. Eggs were round shaped and yellowish white in color. After 6h late morula and 512 celled stage occurred (Figure-1I and Table-1).

Stages during Blastula Period: Shortening along the animal-vegetal axis of embryo was continued up to 7h and they appeared as approximately spherical in shape. This stage is called sphere stage (Figure-1J). After 8h the blastoderm was flattened down onto the yolk sphere and formed a dome shape structure (Figure-1K). In this stage, the egg capsule was yellowish white and round in shape. During blastula period, after the period of 9h, the embryonic shield was converted into a thickened margin of the blastoderm at 30% of the entire distance between the animal and vegetal poles(Figure-1L and Table-1).

Stages during Gastrula Period: Epiboly displaced the blastoderm margin to 50% of the distance between the animal and vegetal pole after 10h (Figure-1M) and after 11h the embryonic shield became more clearly visible and looks like a narrow steak (Figure-1N). Yolk became semi-lunar shape. The cell migration continued and covered 90% over the yolk sphere called as germ ring, giving a thread like appearance. This germ ring proceeded further with differentiation of slight broader at one end and narrow at other end during 12h indicating future cephalic region and tail, respectively. Body axis mostly encircled the vitelline sphere with well-differentiated head and tail, which looked like “C” shape (Figure-1O and Table-1).

Stages during Segmentation Period: After 13h of fertilization, eggs were slightly whitish in color. Tail and head region was slightly seen 15 and 17h after fertilization respectively. Slightly distinct head and tail region was seen with large yolk sac after 19h. Eyes were clearly seen with distinguishable head after 21 h. Large yolk sac was present (Figure-1P and Table-1).

Stages during Pharyngula Period: Head, eye and tail region was clearly seen with large yolk sac after 22h of fertilization. Oneday old embryo was observed with broadened cephalic

region with distinct fore brain. At 27.5h old embryo, tail was more elongated than before. Hatching started after 48h of fertilization. Egg diameter ranged between 1.84-1.85mm (Figure-1Q and Table-1).

Larval development: A larva (Latin; plural larvae) is a young form of animal where indirect development was observed through the process of metamorphosis. The larva looks completely different from the adult form.

Newly hatched larvae: Hatching occurred at about 48h after fertilization where the hatchlings appear as transparent and with an almost round yolk sac. Newly hatched larvae were slender, straight and transparent, gradually tapering towards the tail. The hatchlings ranged between 2.5 - 3mm in length (Figure-2A and Table -2).

1h Old Larvae: Notochord was seen. Dorsal and Ventral fin fold was clearly observed. Large yolk sac was present. The total length ranged between 3.2-3.4mm (Figure-2B and Table-2).

5h Old Larvae: The total length ranged between 3.5-3.6 mm. Large yolk sac was present. Dorsal and ventral fin fold was clearly observed (Figure-2C and Table-2).

9h Old Larvae: Fin folds were seen continuously around the tail. Total length of larvae ranged between 3.8-3.9 mm.Prominent notochord was found (Figure-2D and Table-2).

13h Old Larvae: The larvae increased 4-4.2mm in size. The fin folds were seen continuously around the tail region (Figure-2E and Table-2).

17h Old Larvae: Pegmentation was observed around the whole body. The larvae increased 4.2-4.4mm in size. Yolk sac was partially reduced than previous stage. The fin folds were slightly around the tail region (Figure- 2F and Table-2).

21h Old Larvae: Pectoral fins were slightly seen. The total length of the larvae was 4.5-4.6mm in size. Yolk sac and the fin folds around the tail region were more reduced than previous stage (Figure-2G and Table-2).

Table-1: Summary of Embryonic Development of *Cyprinuscarpio* var. *specularis.*

Periods (hours)	Developmental stages	Time After Fertilization (h)	Egg Diameter Range (mm)
Unfertilized egg	Egg gamete	00 min	1.05-1.06
Zygote (00-45 min)	Single cell	10 min	1.08-1.11
Cleavage (45 min-2.00h)	2-cell	45 min	1.52-1.55
	4-cell	60 min	1.55-1.57
	8-cell	90 min	1.56-1.58
	16-cell	2 h	1.56-1.58
	64-cell	3 h	1.56-1.58
Morula (2.00-6.00h)	128-cell	4 h	1.57-1.59
	256-cell	5 h	1.57-1.59
	512-cell	6 h	1.60-1.62
Blastula (6.00-9.00h)	Sphere	7	1.60-1.63
	Dome formation	8	1.62-1.63
	30%-Epiboly	9	1.63-1.64
Gastrula (9.00-12.00h)	50%-Epiboly	10	1.64-1.66
	Shield	11	1.64-1.67
	90%-Epiboly	12	1.65-1.67
Segmentation (12.00-21.00h)		13-21	1.66-1.74.
Pharyngula (21.00-48.00 h)		22-44.5	1.74-1.85

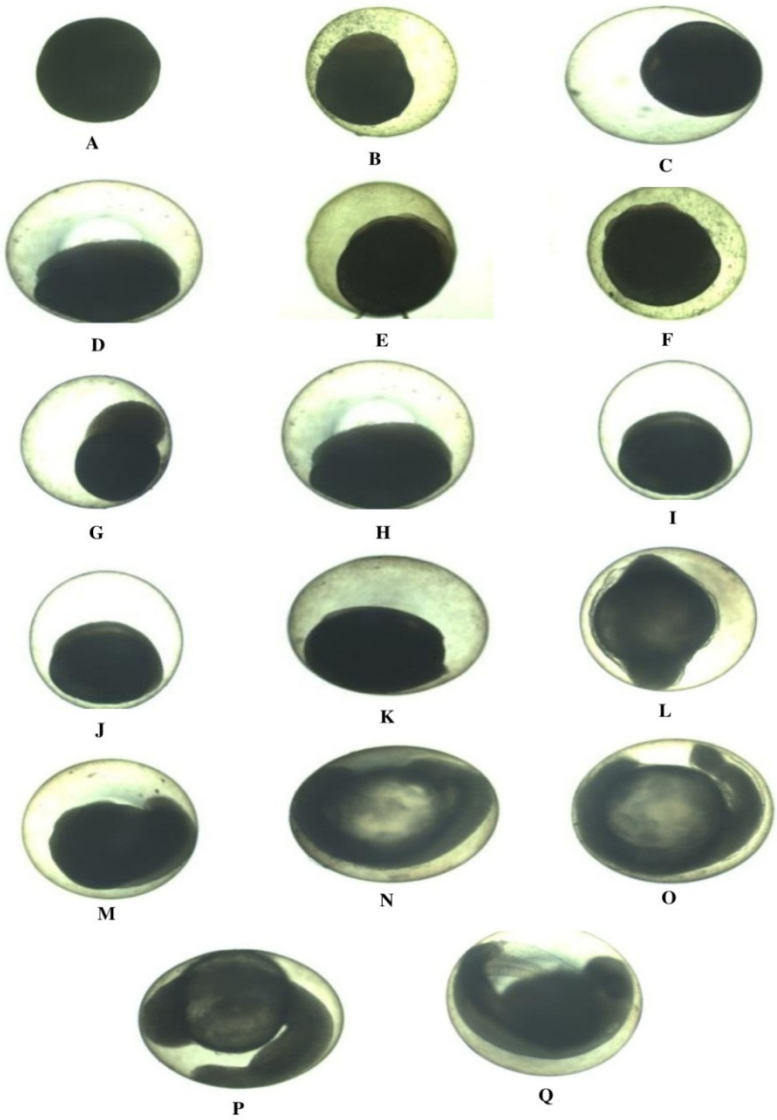

Figure-1: Embryonic development stages of *Cyprinuscarpio* var. *specularies*: Fertilized egg (A); Cleavage period (B-F); Morula period (G-I); Blastula period (J-L); Gastrula period (M-O); Segmentation period (P); Pharyngula period (Q).

25h Old Larvae: Pectoral fins were seen .Yolk sac was more reduced. The total length of the larvae was 4.8-4.9mm in size (Figure-2H and Table-2).

29h Old Larvae: Prominent pectoral fins were seen. At this stage, the length of the larvae was 5.1-5.2mm. Yolk sac as well as tail region fin folds were more reduced (Figure-2I and Table-2).

33h Old Larvae: The total length of the larvae measured between 5.3-5.4 mm. Pectoral fin more prominent (Figure-2J and Table-2).

37h Old Larvae: The eyes increased in size and pigmented. Pectoral fin more prominent. The larvae reached to 5.6-5.7 mm in size. Yolk sac was thinner (Figure-2K and Table-2).

41h Old Larvae: Pectoral fin well developed. Mouth cleft more prominent. The eyes increased in size. At this stage, the length of the larvae was 5.6-5.8mm. Tail region fin folds were more reduce (Figure-2L and Table-2).

45h Old Larvae: Mouth cleft was more prominent. Yolk sac greatly reduced. The larvae reached to 5.8-6 mm in size. Pectoral fin fold well developed.

Pelvic fins were seen (Figure-2M and Table-2).

49h Old Larvae: The larvae reached to 6-6.2mm in size. Yolk sac was greatly reduced. Pelvic fin was clearly visible.

The larvae were found swimming smoothly and feeding exogenously (Figure-2N and Table-2).

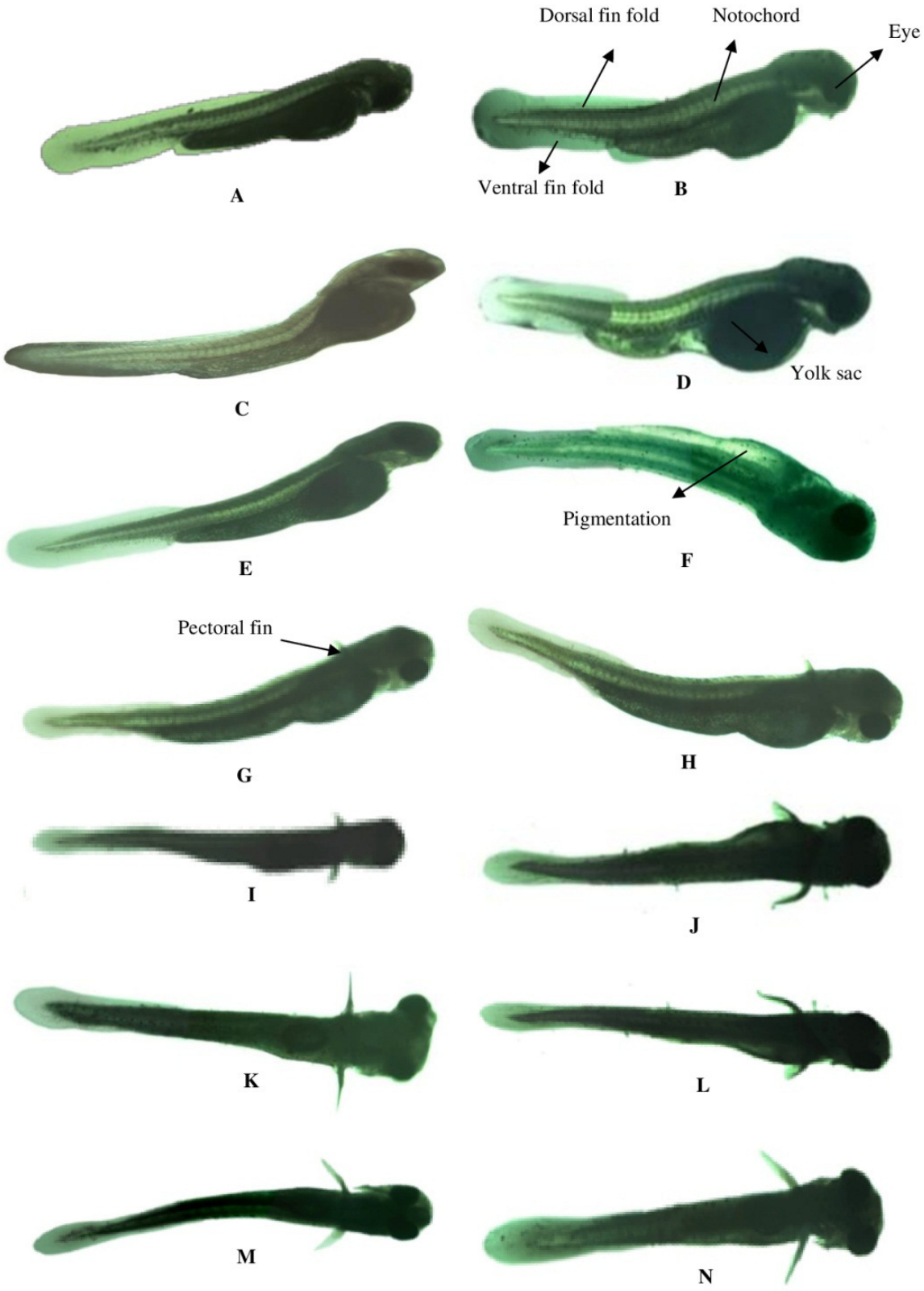

Figure-2: Larval developmental stages of *Cyprinuscarpio var. specularies*: (A) Newly hatched larva; (B) 1h old larva; (C) 5h old larva; (D) 9h old larva; (E) 13h old larva; (F) 17h old larva; (G) 21h old larva; (H) 25h old larva; (I) 29h old larva; (J) 33h old larva; (K) 371h old larva; (L) 41h old larva; (M) 45h old larva and (N) 49h old larva.

Table-2: Summary of Larval Development of *Cyprinuscarpio* var. *specularis.*

Time After Hatching (h)	Total Length Range (mm)	Observed Characteristics
1 hr	3.2-3.4	Notochord was seen. Dorsal and ventral fin fold was clearly observed. Large yolk sac was present.
5 h	3.5-3.6	Large yolk sac was present. Dorsal and Ventral fin fold was clearly observed.
9 h	3.8-3.9	Prominent notochord was found.
13 h	4-4.2	The fine folds were seen continuously around the tail region.
17 h	4.2-4.4	Pegmentation was observed around the whole body.
21 h	4.5-4.6	Pectoral fins were slightly seen.
25 h	4.8-4.9	Yolk sac was more reduced than before.
29 h	5.1-5.2	Prominent pectoral fins were seen. Yolk sac as well as tail region fin folds were more reduced .
33 h	5.3-5.4	Pectoral fin more prominent.
37 h	5.6-5.7	Yolk sac was thinner.
41 h	5.7-5.8	Mouth cleft more prominent. The eyes increased in size
45 h	5.9	Pelvic fins were slightly seen.
49 h	6-6.2	Yolk sac was greatly reduced. Pelvic fin was clearly visible. The larvae swim actively.

Discussion: Life starts with the unification of male and female gametes. As soon as a sperm fertilizes the egg, the zygote is formed and embryonic development starts and ends up at hatching. The hatchlings further undergo organogenesis, appear as like as their parents and brings the termination of larval stages[13]. The embryonic and larval development of mirror carp was observed by artificial fertilization. The fertilized eggs were round in shape, demersal and adhesive in nature. Nica et al.[3] and Haniffa et al.[14] also reported adhesiveness of egg in the same species as well. The color of the fertilized eggs was yellowish white, which also supports the findings of Haniffa et al.[14]. Average diameter of unfertilized and fertilized eggs was 1.05±0.02mm and 1.10±0.02mm, respectively. In case of fertilized eggs, the size was slightly larger for the same species as reported by Haniffa et al.[14]. This slight variation might be due to larger size of bloodstock that was used in the present study, which proofed by the findings of Bichi et al.[15] who reported that larger brood stock size produced larger eggs. The two-cell stage, four-cell stage, eight-cell stage and sixteen-cell stage of *Cyprinuscarpio* var. *specularis* were observed within 45, 60, 90 and 120min after fertilization, respectively, which was little bit of faster than the time reported by Ghosh et al.[10] for the same species. However, a more or less similar period was reported by Balon[16] where the same species taken a time of 30, 80, 100 and 120min to complete two-cell stage, four-cell stage, eight-cell stage and sixteen-cell stage, respectively. The blastula stagewas found within 7-9h after fertilization. The gastrula stage of *Cyprinuscarpio* var. *specularis* was found within 10-12h after fertilization. This stage was detected in the same species within 7.30-11.40h after fertilization by Haniffa et al.[14], in *B. gonionotus* within 5.00-6.40h after fertilization[9], in *P. sarana* within 8.0 to 10.40h after fertilization[17] and in *L. bata* within 8.30-12.00h[12] after fertilization. In the present study, hatching occurred 48h after fertilization at 29°C. Mihalache et al.[18], Ghosh et al.[10] and Haniffa et al.[14] noticed hatching at 50-58h, 75.20-80.30h and 71.20-73.30, respectively after fertilization in the same species, which take slight more time compared to the present study. In other species such as *B. gonionotus*[9], *Cirrhina mrigala*[19] and *L. bata*[12] hatching period was found within 13.20-14.00h, 16.00-19.00h and 18.00-20.00h, respectively after fertilization. This variation might be due to the species difference and temperature variation, which was previously reported by Udit et al.[20]. In the present study, the length of newly hatched larvae of *Cyprinuscarpio* var. *specularis* was 2.5±0.03mm, similar length was also found by Haniffa et al.[14] (2.7-2.9mm) and Ghosh et al.[10] (2.0-2.5mm) for the same species. Length of newly hatched larvae was noted 3.0 to 3.5mm in *P. sarana*[21] and 3.0-4.5mm in *Pangasius pangasius*[22]. Fin folds continuously around the tail were observed 13h of hatching which supports the findings (11-13h of hatching) of Ghosh et al.[10] but differs with Haniffa et al.[14] (6-8h of hatching). In the present study, larvae started swimming freely after 49h of

hatching which supports the findings of Mihalache et al.[18] who found that the larvae of same species started swimming freely after.

Conclusion

The study on embryonic and larval development of *Cyprinuscarpio* var. *specularis* definitely helps the breeders for its propagation under captive condition. The information generated in this study will also help the researcher those who are interested in the study of fish on embryonic and larval development.

Acknowledgement

We express our gratitude and profound regards to the personnel of Fish seed multiplication farm, Puthia, Rajshahi for their valuable cooperation.

References

1. Samad M.A., Khatun A., Reza M.S., Asrafuzzaman M. and Ferdaushy M.H. (2016). Effects of stocking density on growth, survival and production of mirror carp (*Cyprinuscarpio var. specularis*) spawn in nursery pond. *Asian J. Med. Biol. Res.*, 2(3), 429-435.

2. Islam S.S., Azam M.G., Adhikary S.K. and Wickramarachchi K.S. (2004). Efficiency of Integrated Rice, Fish and Duck Polyculture as Compared to Rice and Fish Culture in a Selective Area of Khulna District, Bangladesh. *Pakistan J. Biol. Sci.*, 7(4), 468-471.

3. Nica A., Cristea V., Gheorghe D., Hoha G.V. and Enache I.B. (2012). Embryonic and larval development of Japanese ornamental carp *Cyprinuscarpio* (Linnaeus, 1758). *SeriaZootehnie*, 58, 116-120.

4. Koumoundouros G., Divanach P. and Kentouri M. (2001). Osteological development of DentexdentexK (Osteichthyes: Spraidae): Dorsal, anal, paired fins and squamation. *Marine Biol.*, 138(2), 399-406.

5. Borçato F.L., Bazzoli N. and Sato Y. (2004). Embriogenesis and larval ontogeny of the" piau-gordura", Leporinus piau (Fowler) (Pisces, Anostomidae) after induced spawning. *Revista Brasileira de Zoologia*, 21(1), 117-122.

6. Rahman M.R., Rahman M.A., Khan M.N. and Hussain M.G. (2004). Observation on the embryonic and larval development of Silurid catfish, Gulsha (*Mystuscavasius*). *Pakistan J. Biol. Sci.*, 7(6), 1070-1075.

7. Honji R.M., Tolussi C.E., Mello P.H., Caneppele D. and Moreira R.G. (2012). Embryonic development and larval stages of *Steindachneridion parahybae* (Siluriformes: Pimelodidae) - implications for the conservation and rearing of this endangered Neotropical species. *Neotrop. Ichthyol.*, 10(2), 313-327.

8. Mahapatra B.K. and Krishna G. (2016). Embryonic and larval development of *Rasboradaniconius* (Hamilton): A potential indigenous ornamental fish of north-east India. *Int. J. Fish. Aqua. Stud.*, 4(6), 187-190.

9. Basak S.K., Basak B., Gupta N., Haque M.M. and Amin M.R. (2014). Embryonic and larval development of Silver barb (*Barbodesgonionotus*) in a mobile hatchery under laboratory condition. *European Sci. J.*, 3, 258-270.

10. Ghosh A.K., Biswas S., Sarder L., Sabbir W. and Rahaman S.M.B. (2012). Induced breeding, embryonic and larval development of Koi carp (*Cyprinuscarpio*) in Khulna, Bangladesh.Mesopot. *J. Mar. Sci.*, 27(1), 1-14.

11. Mollah M.F.A., Taslima K., Rashid H., Hossain Z., Sarowar M.N. and Khan M.R.K. (2011). Embryonic and larval development of critically endangered riverine catfish *Rita rita*. *Eurasia J.Biosci.*, 5, 110-118.

12. Miah M.I., Harun M.S., Rahman M.M., Haque M.R. and Hossain M.A. (2009). Study on the embryonic and larval development of an endangered species of Bata (*Labeobata*). *Int. J.Susta.Crop.Produ.*, 4(1), 72-82.

13. Rahman M.M., Miah M.I., Taher M.A. and Hasan M.M. (2009). Embryonic and larval development of Guchibaim, *Mastacembeluspancalus* (Hamilton). *J. Bangladesh Agri. Uni.*, 7(1), 193-204.

14. Haniffa M.A., Benziger P.S.A., Arockiaraj A.J., Nagarajan M. and Siby P. (2007). Breeding behavior and embryonic development of Koi Carp (*Cyprinuscarpio*). *Taiwania.*, 52(1), 93-99.

15. Bichi A.H., Isyaku S., Danba E.P., Kurawa I.A. and Nayawo A.A. (2014). Effect of brood stock size on egg fertilization, hatchability and fry survival rate of african catfish (*Clariasgariepinus*). *Bajopas*, 7(2), 150-154.

16. Balon E.K. (1995). The common carp, *Cyprinus carpio*: its wild origin, domestication in aquaculture, and selection as colored nishikigoi. *Guelph ichthyology reviews*, 3, 1-55.

17. Chakraborty B.K. (2004). Studies on the artificial propagation, larval rearing and culture of local sarpunti (*Puntiussarana*), dissertation. Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, 321.

18. Mihalache A., Opre A.L., Grecu I., Cristea V., Badalan C., Enache I. and Ion S. (2011). Artificial reproduction and embryonic development of the Japanese ornamental carp (*Cyprinuscarpio* Linnaeus, 1758). *J. Environ. Protec. Ecol.*, 12(4), 1835-1839.

19. Shoaib M. and Nasir M. (2013). Study on the embryonic and larval development of *Cirrhinamrigila* (F. Hamilton, 1822) in response of differential dosage of ovaprim. *Int. J. Biol. Res.*, 1(2), 95-103.

20. Thépot V. and Jerry D.R. (2015). The effect of temperature on the embryonic development of barramundi, the

Australian strain of *Latescalcarifer* (Bloch) using current hatchery practices. *Aquacult.Reports*, 2, 132-138.

21. Udit U.K., Reddy A.K., Kumar P., Rather M.A., Das R. and Singh D.K. (2014). Induced breeding, embryonic and larval development of critically endangered fish *Puntiussarana* (Hamilton, 1822) under captive condition. *J. Anim. Plant Sci.*, 24(1), 159-166.

22. Ferosekhan S., Sahoo S.K., Giri S.S., Saha A. and Paramanik M. (2015). Embryonic and Larval Development of Yellow Tail Catfish. *Pangasiuspangasius*. *J.Aquac. Res. Development*, 6, 6.