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Stages of lesser spiny eel, *Macrognathus aculeatus* in captive condition: embryonic development

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Abstract

The lesser spiny eel, *Macrognathus aculeatus*is one of the popular indigenous fish in Bangladesh but the biological information of the fish is rare. It is necessary to understand the embryonic development process to establish artificial propagation in captive condition. The embryonic development stages were examined in the aquarium condition and the embryogenesis is divided into seven major phases: Zygote phase, Cleavage phase, Morula phase, Blastula phase, Gastrula phase, Organogenesis phase and Hatching phase. The major phases also separated into different sub stages whenever possible according to their development characteristics. Fertilized eggs were adhesive, greenish and demersal with equal perivitelline space. The diameter of the fertilized eggs ranged between 0.85-1.00 mm. The perivitelline space and blastodisc formation occurred at 0.35 h after fertilization (AF). The first cleavage furrow noticed at the animal pole at 0.50 h AF. The further cell division like, four cells, eight cells, sixteen cells, thirty-two cells and multi cells stages were found at 1.05, 1.50, 2.30, 3.20 and 4.30 h of AF respectively. The morula, blastula, gastrula and organogenesis were visualized at 6.45, 10.5, 15 to 21, 24.5 to 32 h AF. The blastoderm covered almost 3/4th of the egg during 21.05 h AF. The first heartbeat noticed within about 31-32 h AF. At the same hours, the notochord in cellular structure was also visible. The head and tail ends of the embryo were clearly distinguished at 35.0 h AF. The hatching started 35 h AF and completed within 40 h at 29.11 ± 0.29 °C.

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Introduction

The lesser spiny eel, *Macrognathus aculeatus* (Bloch, 1786) locally known as tarabaim and one of the common species among eel fishes in Bangladesh. The fish is distributed in Bangladesh, China, India, Indonesia, Malaysia, Nepal, Singapore, Taiwan, Thailand, Viet Nam and West Africa (Rahman, 1989; Archarya and Iftekhar, 2000; Nguyen *et al*., 2011). It is an economically important species and preferred by the consumers of the Asian countries as a table fish due to its good taste and high protein contents. It plays an important role in controlling the population of harmful insects in the environment though its feeding habits. It also helps to control water pollution by eating detritus and occupies the third level in the food chain in waters (Rahman *et al*., 2009).

The species not yet reported as culturable species in Bangladesh because of unavailability of larvae and insufficient information about the embryonic and larval development. The fish is declining from our natural habitat as consider other eel fish like guchibaim (*M. pancalus*) in recent years due to various ecological changes in the aquatic ecosystem. Habitat modification, overexploitation, excess fertilizer and pesticides etc. are the main cause for warning natural breeding ground (Rahman *et al*., 2009) and decreasing the production of this species (Afroz *et al*., 2014). Besides these, the entire demand for the fish in the country is met though collection from the wild. The fish is under the category of 'near threatened' according to the assessment by the IUCN Bangladesh (2015). Thus, it is necessary to understand the details biology, especially on the embryonic development process to establish artificial propagation in captive condition and hence management of the species. There were several studies have been reported regarding the topic matter in and abroad on eel fishes like, on the development of Japanese eel, *Anguilla japonica* (Kiichiro *et al.,* 1975); development of eggs and larvae of pike eel, *Mureanesox cinereus* (Umezawa *et al.,* 1991); embryonic and larval development of tarabaim, *Macrognathus aculeatus* from India (Sahoo *et al*., 2007), from Mymensingh, Bangladesh (Farid *et al*., 2008); and embryonic and larval development of guchibaim (*Mastacembelus pancalus*)in Mymensingh, Bangladesh, (Rahman *et al*., 2009). But no studies have been conducted on the early development of the locally available *Macrognathus aculeatus* from south west part of Bangladesh. Therefore, it is necessary to undertake a comprehensive study to characterize its various stages of embryonic development for better understanding of the biological clock. This study was thus aimed to generate more information on the early life history, developmental stages of *Macrognathus aculeatus* in captivity and also to identify a specific stage of development during a particular time.

Materials and methods

Study site and preparation of aquarium

The experiment was conducted in the laboratory of the Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology, Jessore, Bangladesh. The experiment was conducted in rectangular glass aquaria (36 inch × 14 × 15 inch), each containing 30 liters of water. Water hyacinths were used as a source of plant and their roots are used as substrate to lay the eggs of the fishes. The water parameters such as pH, DO, temperature was recorded at each two-hourinterval. The water pH and DO were measured by a pH meter (EZODO, 7200, Made in Taiwan) and a DO meter (LTLutron YK-22DO, Made in Taiwan) respectively. The water temperature was also recorded by using the same DO meter.

Collection of eggs

Eggs of *M.aculeatus* were collected from the same experimental laboratory, which were produced by the induce breeding with Ovaprim hormone. The sticky eggs of the *M.aculeatus* were attached to the aquatic weeds and sometimes on the substrates. Eggs were collected softly either along with the roots of the water hyacinth or using a feather in each sampling.

Observation of embryonic development

The embryonic developments stages were observed and taken photograph with a photographic microscope (Carl Zeiss microscopy GmbH, S.N. MKG 8639, made by Germany).

Eggs were observed at every 5 to 10 minutes interval till the completion of morula and then after every one-hour interval until hatching. The oocytes and eggs at each sampling were measured using the same microscopic camera. The development stages and characteristics were confirmed by using different books and articles especially Sahoo *et al.* (2007), Farid *et al.* (2008) and Rahman *et al.* (2009).

Results

Aquarium environment

Water parameters are one of the critical factor for the spawning, embryonic development and hatching of any fish species. In the present study, the physico-chemical condition of spawning aquarium such as temperature, dissolve oxygen and pH were ranged from 28.5 to 30.2°C, 4.88 to 5.33 mg/L and 7.75 to 8.22 respectively (Fig. 1).

Characteristics of the egg

The greenish eggs were spherical, demersal and adhesive. It attached with the roots of water hyacinth in the aquarium. The unfertilized eggs were opaque while the fertilized eggs were greenish showed egg membrane and yolk (Fig.2A & 2B). The diameter of fertilized eggs increased to 0.85-1.00 mm from 0.80-0.85 mm of unfertilized eggs.

Table 1. Embryonic developmental stages with time and features of *M.aculeatus*.

Major phase	Sub-stages	Figure	Time (h/min)	Characteristics
Zygote	Unfertilized egg	Fig. 1	0.00	Eggs spherical, opaque, demersal
	Fertilized egg		0.30	Eggs greenish in color and adhesive, formation of perivitelline space
Cleavage	Blastodisc	Fig. 2	0.35	Formation of blastodisc at animal pole
	Two cell		0.50	Dividing blastodisc into two blastomere
	Four cell		1.05	Second division
	Eight cell		1.50	Formation of 8 blastomeres
	Sixteen cell		2.30	Attainment of 16 cell stage
	Thirty two cell		3.20	Formation of 32 blastomeres
	Multi cell		4.30	Quick succession transformed into 64, 128 cells
Morula		Fig. 3	6.45	Blastomeres were further reduced and accumulated at the animal pole
Blastula			10.50	The marginal blastomeres lost their boundaries and were compressed
Gastrula	Early gastrula		15.25	The blastoderm started invading the yolk by spreading over the yolk in the form of a thin layer
	Middle gastrula		19.00	The formation of germinal ring around yolk was clearly visible
	Late gastrula		21.05	Blastoderm covered 3/4 of the yolk
Organoge-nesis	Yolk plug stage	Fig. 4	24.50	Completion of yolk invasion. Appearance of rudimentary head and tail
	Somite stage		27.0-29.0	Anterior and posterior end was distinguished
	Segmentation		31-32	Notochord becomes visible. Both head and tail ends were clearly visible
Hatching		Fig. 5	35.0-40.0	twisting movement, embryos ruptured the egg shell

Embryonic development

The embryonic period starts when the egg is fertilized by a sperm and ends when the embryo has attained the generalized organ systems as they appear in common all the fish.

The fertilized eggs were hatched out within 35-40 hours after fertilization (AF).

The events in embryonic development and their respective time of observation in *M.aculeatus* are presented in Table 1 and Fig.2-6. The comparison of the embryonic development stages with other such studies are presented in Table 2.

Table 2. The comparison of embryonic development stages of *M.aculeatus* of present findings with other available findings.

Development stages	References			
	Sahoo *et al.*, (2007)	Rahman *et al.*, (2009)*	Farid *et al.*, (2008)	Present study (2016)
Blastodisc formation	0.30	0.30	0.15	0.30
Cleavage start	0.50	1.05	0.35	0.35
Complete cell division	-	5.20	5.15	4.30
Morula	4.10	10.40	7.20	6.45
Blastula	6.40	-	-	10.50
Gastrula	10.45	21.30	15.10	15-21
Yolk plug	25.30	31.30	30.15	24.50
Heart beat	-	32-33	-	31-32
Hatching	31.45	34-35	36-40	35.0-40.0

*Rahman *et al.* (2009) study on *Mastacembelus pancalus*.

Zygote phase

This phase is characterized with fertilized and the periviteline space formation. In the present study, it was noticed that the formation of blastodisc at the animal pole and formation of the perivitelline space at 0.30 h AF (Fig.2B). The egg membrane was fully separated by a thin space from the egg called perivitelline space which was equal all around and filled with yolk free clear fluid. Oil globules were visible in the yolk.

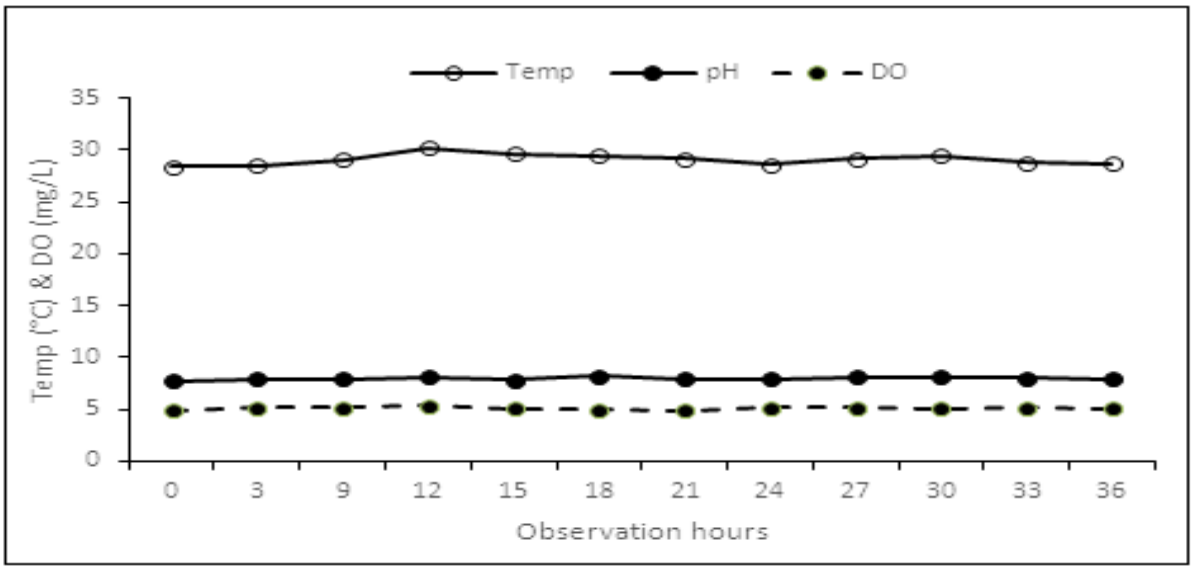

Fig. 1. The recorded water parameters (Temperature, DO and pH) during embryonic development of *M. aculearus.*

Clavage phase

The single cell stage became clear with the accumulation of cytoplasm over the animal pole as a protrusion at 0.35 h AF representing early blastodisc or germinal disc stage (Fig. 3A).

The first cleavage occurred within 0.50 h AF. The blastodisc was divided into two distinct cells by vertical cleavage (Fig. 3B). A second cleavage was at right angle to the first and observed forming four cells within 1.05 h (Fig. 3C). Further division of blastomere took place with the advancement of time to reach eight cell, sixteen cell, and thirty-two cell stage at 1.50, 2.30 and 3.20 h AF respectively (Fig. 3D, 3E, 3F).

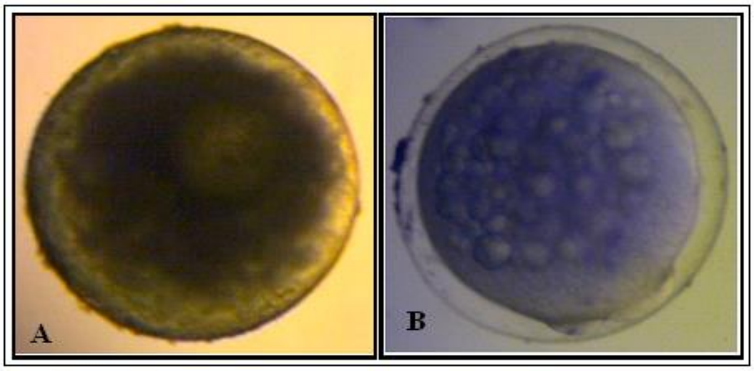

Fig.2. The unfertilized (A) and fertilized eggs (B) of *M. aculearus*.

After that quick succession took place and the thirty-two-celled stage transformed into 64, 128 celled stage and so on dividing geometrically. But this cell division occurred so quickly that it was not possible to observe or count the stages and hence referred as multi celled stage (Fig.3G). Eggs were measured still the same size within the range of 0.85 – 1.00 mm.

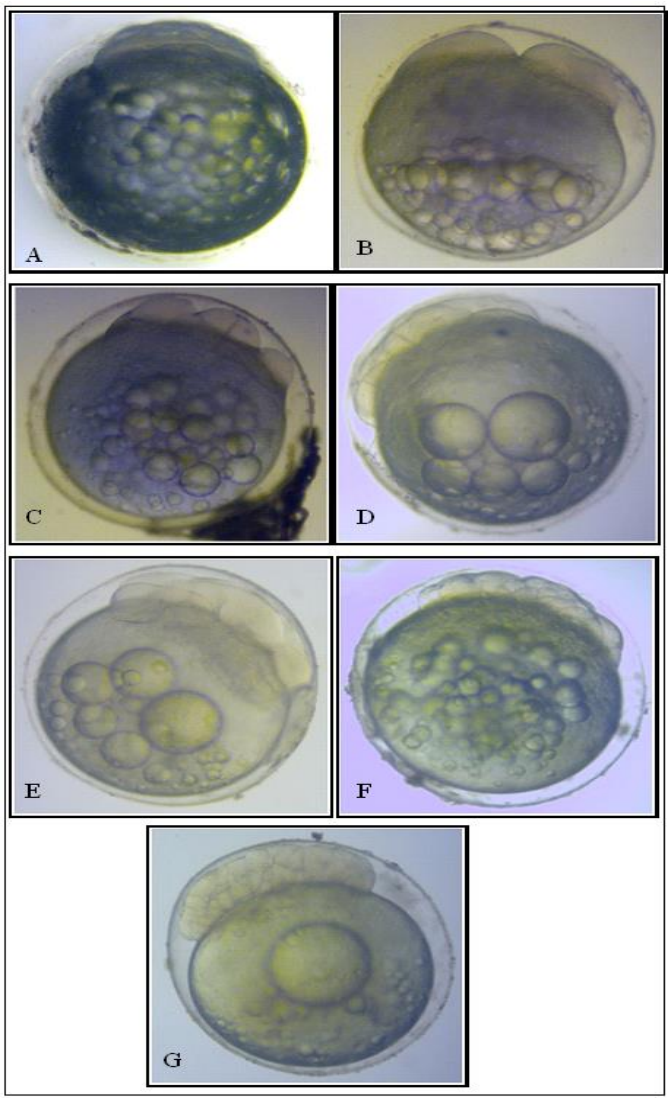

Fig. 3. The cleavage phase of *M. aculeatus*; Blastodisc (A) Two cell (B) Four cell (C) Eight cell (D) Sixteen cell (E) thirty-two cell (F) and Multi cell (G).

Morula stage

The blastomeres were further reduced in size and accumulated around the animal pole during the morula stage. It occurred at 6.45 h AF (Fig.4A).

Blastula stage

After morula, the developing embryo further divided into numerous cells and arranged in the form of a layer called blastoderm. Gradually, the blastoderm formed into several layers due to further cell division which is called blastodisc.

At this stage, a space was formed between yolk and blastoderm, which is called blastocoel. This stage of embryo is called 'blastula' (Fig. 4B) which observed at 10.50 h AF.

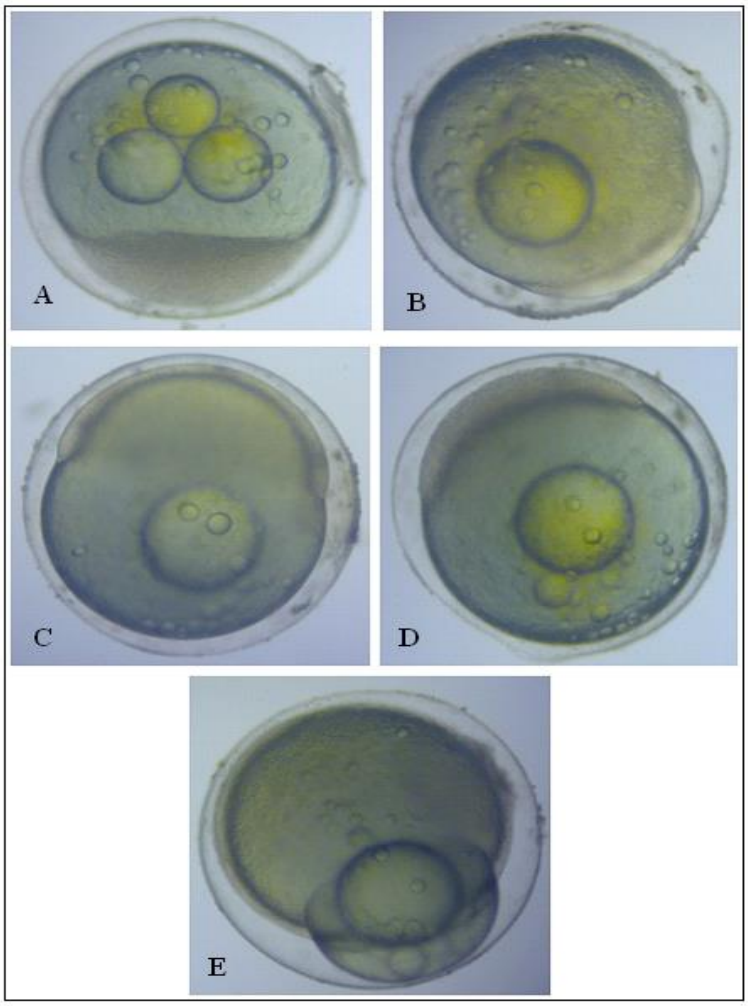

Fig. 4. The Morula (A), Blastula (B) and Gastrula phase (C, D, E) of *M. aculeatus*; Early gastrula (C), Middle gastrula (D), and Late gastrula (E).

Gastrula stage

The gastrulation phase is subdivided into three stages; like early gastrula, middle gastrula and late gastrula. The early gastrula recognized as the blastoderm started invading the yolk by spreading over the yolk in the form of a thin layer and which resulted within 15.25 h AF (Fig.4C). In the middle of gastrulation, a germinal ring around yolk was clearly visible and that about half of yolk was occupied by blastoderm (Fig. 4D).

The embryonic shield was clearly visible at late gastrulation and blastoderm covered ¾th of the yolk at 21.05 h AF (Fig.4E).

Organogenesis

The yolk invasion was completed by gradual spreading over the germ layer in the yolk plug stage (Fig. 5A). The rudimentary head and tail appeared in this stage within 24.50 h AF (Fig. 5B).

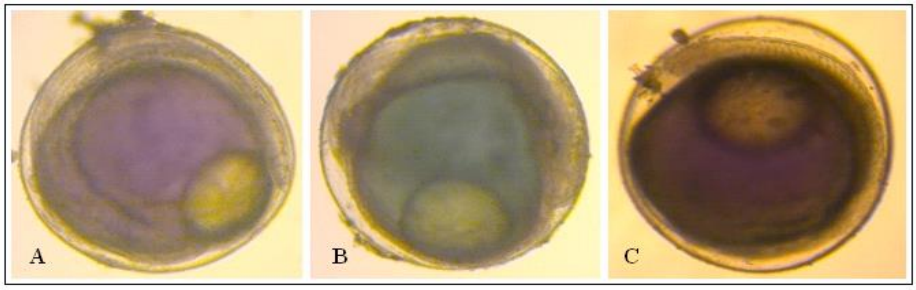

Fig. 5. The organogenesis phase of *M. aculeatus;* Yolk plug stage (A), Somite stage (B) and segmentation stage (C).

In further progress, the embryo was elongated and encircled the yolk materials and both tail and head ends were clearly differentiated (Fig. 5C). The first beating heart was visible at about 31-32 h AF and notochord in cellular structure became visible at the same time.

Hatching

In this phase, the embryo further elongated and gradually differentiated.The tail gradually became detached from the yolk mass (Fig. 6A). Embryo started occasional twisting movement. The embryos rupture the egg shell by the continuous movement. Larvae emerged with its tail portion first in 35.00 to 40.00 h AF (Fig.6B). Hatching continued for 5.00 h because the entire embryo did not hatch out at a time.

Discussion

Aquarium environment

The embryonic development of fishes is directly related to the water parameters particularly water temperature. The recorded water temperature in the present study was within 28.5 to 30.2°C which was close to other studies on eel fishes like 28-30°C (Das and Kalita, 2007), 28-29°C (Sahoo *et al.*, 2007), 27-28°C (Farid *et al.*, 2008) and 27-31°C (Rahman *et al.*, 2009). Thus, the water temperature recorded in the present study may be optimum range for the embryonic development of the eel fishes. The other parameters like pH and DO also under suitable range (7.75 to 8.12 and 5.25 to 5.75 mg/L). Das and Kalita (2003) successfully breed of *M. aculeatus* at the water pH 7.6-7.8 and dissolved oxygen 8-9 mg/L.

Characteristics of the egg

In the present study, the unfertilized eggs were opaque, demarsal and the fertilized eggs were round, greenish and adhesive which are supported by Farid *et al.* (2008). Though same characteristics of fertilized eggs showed in Mastacembelidae, different egg color such as transparent color observed in case of *M. pancalus* (Rahman *et al.*, 2009). The fertilized egg diameter in the present study recorded 0.85-1.00 mm which was larger than Farid *et al.* (2008). They recorded 0.70 mm of fertilized egg in *M. aculeatus*. However, further larger in size (1.20-1.40 mm) reported by Sahoo *et al.* (2007) in *M. aculeatus*.

Embryonic development

The cell division pattern and the further embryonic development stages were more or less similar to other studies of Mastacembelidae (Sahoo *et al.*, 2007; Farid *et al.*, 2008; Rahman *et al.*, 2009). However, differences noticed in case of time of development in different species and even within same species. The blastodisc at the animal pole in fertilized egg had noticed at 0.30 h AF which was similar to Sahoo *et al.* (2007) in *M. aculeatus* and Rahman *et al.* (2009) in *M. pancalus* but earlier occurrence within 0.15 h noticed in *M. aculeatus* (Farid *et al.*, 2008).

In the present study the two-cell stage, four-cell stage, eight-cell stage and sixteen-cell stage were found at 0.50, 1.05, 1.50 and 2.30 h AF. Farid *et al.* (2008) and Sahoo *et al.* (2007) found that these stages at 0.35, 0.45, 1.10, 1.35 h and 0.50, 1.30, 2.05, 2.50 h AF of the same species. These stages appeared in late in case of*M. pancalus* such as 1.05, 1.20, 2.20 and 3.10 h AF (Rahman *et al.*, 2009). The thirty-two and multi cell stage were found 3.20 h and 4.30 h AF which was more or less similar to Sahoo *et al.* (2007) who found that these stages at 3.30 h and 4.10 h AF in the same species.

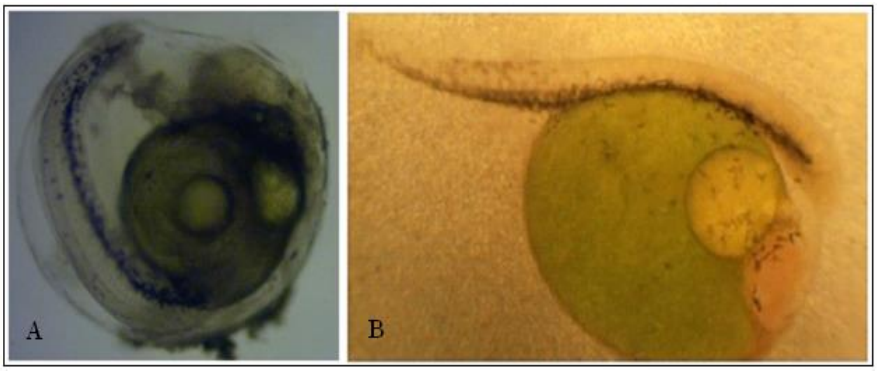

Fig.6. The just before hatch stage (A) and newly hatch larvae (B) of *M. aculeatus*.

In the present study, morula stage was found at 6.45 h AF which was different compare to other findings. Sahoo *et al.* (2007) noticed morula stage at 4.10 h AF. However, slow morula stage noticed (at 7.20 h AF) by Farid *et al.* (2008) in the same species. The morula stage appeared in very late in case of*M. pancalus* and it was within 10.40 h AF (Rahman *et al.*, 2009). The blastula stage was observed, in *M.aculeatus* at 10.50 h AF which was late compare to Sahoo *et al.* (2007) and they observed the same stage at 6.40 h AF.

The gastrula stage noticed in *M.aculeatus* at 15.25 hours after fertilization which was similar to Farid *et al.* (2008) who noticed this stage at 15.10 h AF. However, quick gastrula appeared (10.45 h) in *M.aculeatus* (Sahoo *et al.*, 2007) while late appeared (21.30 h) in *M. pancalus* (Rahman *et al.*, 2009).

In this experiment though gastrulation appeared in late compare to other but the yolk plug was attained 24.5 h AF which was earlier than other such reported like 30.15 h and 30.0 h AF reported by Farid *et al.* (2008) and Rahman *et al.* (2009). The heart rudiment was observed 31-32 at h AF. Rahman *et al.* (2009) observed it at 32-33 h AF in *M. pancalus*.

The hatching period of *M.aculeatus* completed within 35-40 h AF at a water temperature of 29.11 ± 0.29 °C which was more or less similar to Farid *et al.* (2008) who observed that the hatching period 36-40 h AF of the same species. Rahman *et al.* (2009) observed the hatching period 34-35 h AF in*M. pancalus*. Sahoo *et al.* (2007) recorded hatching time 31.45 h AF in *M.aculeatus*.

The development of embryo and hatching time in fertilized egg of most of the fishes are generally influenced by the temperature of water (Jhingran, 1983). Temperature is inversely proportional to the time of hatching (Alikunhi *et al.,* 1962; Mollah, 1983) and the hatching time is also species specific (Haor and Randall, 1969).

Conclusion

The fertilized eggs of the *M.aculeatus*are 0.85-1.00 mm in diameter. The first cleavage started within 1st h of hatching. After the incrimination of zygote, the embryo took 34 h to complete their development and started to hatch 35 h AF which completed within 40 h at 29.11 ± 0.29 °C. The present information could be helpful to biologist for the development artificial propagation of the species in aquarium condition.

Acknowledgement

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