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Larval rearing of lesser spiny eel, *Macrognathus aculeatus* in the captivity with emphasis on their development stages

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Abstract

The lesser spiny eel, *Macrognathus aculeatus* locally known as tara baim has been gaining importance for its food and ornamental values in Bangladesh. The species is declining rapidly from natural habitat and not yet reported as culture fish in the country due to mostly lack of hatchery-produced larvae. It is important to know breeding biology particularly larval development for their commercial seed production. In the present study, induced larvae were reared in the captive aquarium and their larval development stages were examined. The larvae were reared for 15 days in three treatments with three different sources of water. Though water parameters did not differ significantly among three treatments, the survival rate differed significantly ($P>0.05$). The highest average survival rate was observed in T_1 (42%) which was filled with rain water followed by the T_2 (pond water; 33.5%) and T_3 (supplied tap water; 19.5%) respectively. The larval development progresses were examined under electronic microscope and stages were categorized on the basis of their ontogenetic development with time. The development progress was broadly categorized into two-phase such as pre-larval (up to yolk absorption) and post-larval stage (up to metamorphosis). The larval development progress resembled to other eel fishes except some progress deviation with time. The newly hatched larvae were yellowish, transparent body with the total length of 2.08 ± 0.02 mm. The yolk sac was disappeared completely and started to exogenous feeding within 72 hrs of hatching but the larvae not yet fully metamorphosis resembled to Juvenile fish at 15 days of rearing.

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Introduction

The lesser spiny eel, *Macrognathus aculeatus* (Bloch, 1786) locally known as tara baim and one of the common species among Mastacembeliformes. It is widely distributed in Bangladesh, China, India, Indonesia, Malaysia, Nepal, Singapore, Taiwan, Thailand and Viet Nam and West Africa (Rahman, 1989; Archarya and Iftekhar, 2000; and Nguyen *et al.*, 2011). The fish, *M. aculeatus* is very popular and widely accepted in the Indian subcontinent due to its good taste, high market value, important production potentials and high protein contents. The caloric value of eel flesh is as high as 303 cal/100 g compared to 110 cal/100 g in other average fishes (Nasar, 1997). Huda (1959) reported that the fleshy edible of its body contain 5.3% fat and 74.2% water. *Macrognathus aculeatus* is a freshwater (also brackish water) fish, benthopelagic in nature and potamodromous (Riede, 2004). According to Bhuiyan (1964), the fish prefer freshwater and muddy parts of the small rivers, streams and canals. It plays an important role in controlling the population of harmful insects in the environment through 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).

Though there is no published data but considering other baim fish in the country, it may say that the availability of the fish has decreased sharply from natural habitat due to habitat destruction, overexploitation, as primes that reported for guchi baim (Afroz *et al.*, 2014). The natural breeding grounds of this fish are under threat due to drying up of the low lying areas and indiscriminate use of fertilizers and pesticides (Rahman *et al.*, 2009). Besides these, the entire demand for this fish in the country is met through collection from the wild which exaggerating the natural depletion of the species. The fish is under the category of 'near threatened' according to last assessment by IUCN Bangladesh (2015). The artificial propagation or proper management is essential to conserve the species. Thus, it is necessary to understand the details biology, especially on the larval rearing system and development process to establish artificial propagation in captive condition.

There are some research on the biology and breeding have done on eel fishes like spiny eel, *Mastacembelus pancalus* (Karim and Hossain, 1972; Hasan *et al.*,2016); peacock eel, *Macrognathus aculeatus* (Das and Kalita, 2003); barred spiny eel, *Macrognathus pancalus* (Suresh *et al.,* 2006) and Japanese eel, *Anguilla japonica* (Kiichiro *et al.* 1975). The other such studies on the development of larvae like *M. pancalus* (Rahman *et al.*, 2009; Afroz*et al.*, 2014), *M. aculeatus* (Farid *et al.*, 2008)and *Muraenesox cinereus* (Umezawa *et al.*, 1991)but no such details work on larval rearing and development of *M. aculeatus* except Farid *et al.* (2008). The changes of features during larval development and to understand the organogenesis are of crucial important, which are essential during the development of management and rearing technology of any new species for seed production. Studies on early larval development are important to the successful rearing of larvae for large scale seed production in aquaculture (Khan and Mollah, 1998; Rahman *et al.,* 2005). Therefore, it is necessary to undertake a comprehensive study to characterize its various stage of larval development for better understanding of the biological clock and rearing techniques of this species.

Materials and methods

Experimental design

The experiment was designed into two distinguish segments; 1) larval rearing and 2) larval development stages. The glass aquariums (20 liters) were used for the rearing. Larvae were reared in the three treatments such as rearing in rain water (T_1), rearing in pond water (T_2) and rearing in supplied tap water (T_3) (Table 1). The each treatment had one more replication. The larval development of *M. aculeatus* was studied in the laboratory of Department of Fisheries and Marine Bioscience, Jessore University of science and Technology, Jessore, Bangladesh. The aquarium set up, water supply facilities, working

space etc were assured before the breeding program.

Water quality measurement

The water parameters such as pH, DO, and temperature were recorded at each two hours interval during pre-larval stage and once daily during post-larval stage. 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 a DO meter (LTLutron YK-22DO Made in Taiwan). The water was exchanged at the rate of 20% daily and 50% within a week. The wastage was siphoned out daily by siphoning tube.

Broods collection, rearing and induced breeding

Brood fishes of *M. aculeatus* were collected from Joghati baor, Jessore. The fishes were brought back to laboratory, acclimatized and reared in the aquarium that was previously prepared. The aquariums were supported with stone, bamboo slit, and continuous air pumps etc. In addition, water hyacinth was supplied to hold the sticky eggs. Tubifex were used as feed daily accordance to their demand. The healthy and strong broods were selected from the conditioning aquarium and stocked at 1:1 for male and female in the breeding aquarium. The breeding was performed by administering the synthetic hormone Ovulin. Fish spawned after 18-20 hrs of inducing hormone. The eggs stick to the roots of the water hyacinth and hatch out at 35-40 hrs of spawning.

Larval rearing

Larvae were collected from the experimental aquarium by using glass jar. Larvae were acclimatized for short period of time and then stocked in the rearing aquarium at the rate of 100 larvae per aquarium (Table 1).

Larvae were feed regularly with boiled egg yolk from the 4th day onward when noticed the complete absorption of yolk sac. The larvae were reared for 15 days up to their larval development in three different sources of water (Table 1).

Larval development observation

Larval developmental stages were observed every 2 hrsup to 72 hrs starting from egg fertilization (Pre larvae) and every 12hrs interval during post larvae. Larvae were observed and taken photograph under photographic microscope (Carl Zeiss microscopy GmbH, S.N. MKG8639, made by Germany). The pictures also were taken sometime by using digital camera (Sony, Model DSC-W520), from the eyepiece of the microscope. The development stages and characteristics were recognized according to mostly Farid*et al.*, 2008, Rahman *et al.*, 2009 and Afroz*et al.,* 2014).

Statistical analysis

The result found in the study were subjected to statistical analysis, paired T-test that showed the significance (P>0.05) level of differences between the treatments. The statistical analysis was done with the aid of the Microsoft Excel.

Results

Larval rearing and survival rate of M. aculeatus in different water sources

Hatching started at 35 hrs after the completion of egg deposition and hatching competed within 40 hrs.

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

Treatments	Tank name	Water sources	Stocking density
T_1	R_1	Rain water	100 per tank
	R_2		
T_2	P_1	Pond water	
	P_2		
T_3	S_1	Supplied tap water	
	S_2		

The newly hatched larvae showed inactive and aggregated nature near the corners or walls of the breeding aquaria (Fig.1). However, after 4 days and onward they showed active and freely swimming all around the aquarium tank. The water parameters in the present study did not show any significant ($P<0.05$) different among the treatments (Table 2).

Table 2. Water quality parameters of different treatment aquarium during rearing of *M. aculeatus* larvae.

Water parameters	T_1(Rain water)	T_2(Pond water)	T_3(Tap water)
Temperature(˚C)	28.5 ± 0.61	28.0 ± 0.45	28.3 ± 0.48
pH	7.4 ± 0.13	7.6 ± 0.44	8.2 ± 0.21
DO(mg/l)	6.33 ± 0.16	6.31 ± 0.25	5.91 ± 0.12

(Mean ± SD). The water parameters did not showed significant different ($P>0.05$) among the treatments.

However, supplied tap water showed little bit harder than rain and pond water. The survival rate differed significantly ($P>0.05$) in different water sources after 15 days of rearing. The highest average survival rate was observed in T_1 (42%) which was filled with rain water followed by the T_2 (pond water; 33.5%) and T_3 (supplied tap water; 19.5%) respectively (Table 3).

Table 3. The survival rate of larvae of *M. aculeatus* in different water sources after 15 days of rearing.

Treatments	Initial stocking	Survival rate (%)	Average survival rate
T_1 Rain water	100	41.0	42.0%[a]
	100	43.0	
T_2 Pond water	100	32.0	33.5%[b]
	100	35.0	
T_3 Tap water	100	20.0	19.5%[c]
	100	19.0	

The values in different letter in the survival rate indicate significant differences ($P>0.05$).

Larval development stages

The development of the larvae was examined under electronic microscope. On the basis of morphological changes with time, the development stages of *M. aculeatus* were categorized into two stages such as 1. pre-larval and 2. post-larval stage. The distinguish characteristics developed for each stage are mentioned in Fig. 2, 3 and summarized in Table 4 and 5. The consequences of development with time also presented in figure 4.

Table 4. The developmental stages (summary) of Pre-larvae phase of *M. aculeatus* in the captive condition.

Age of larvae (hrs)	Mean length (mm)	Characteristics
Zero	2.08±0.02	Yolk sac attached to the body, yellowish color, transparent and showing internal organs. No visible eyes.
Two	2.12±0.04	Yolk sac still remained attached to the body and larvae slender
Four	2.16±0.01	Body becomes more transparent and takes cylindrical shape.
Six	2.21±0.02	The anterior part becomes more prominent and stronger.
Eight	2.35±0.06	The yolk sac partially reduced, tail becomes thickened, anus slightly visible.
Ten	2.50±0.03	A heart appeared, yolk sac reduced, eye, anus and intestine visible
Twelve	2.64±0.03	Chromatophores seen in the eye, ventral fin fold prominent.
Fourteen	2.77±0.03	Interior part of the yolk globular in shape

Eighteen	2.89±0.04	Newly chromatophores appeared above eyes, yolk sac became thin. Eyes became pigmented.
Twenty two	3.03±0.04	Operculum visible and myomeres partially visible.
Twenty eight	3.22±0.02	Visible air bladder and color changed to silver-yellowish color.
Thirty four	3.55±0.04	Brain lobe visible and mouth cleft formed.
Thirty eight	3.80±0.02	Mouth cleft more prominent and the eyes increased in size.
Forty four	4.10±0.02	Opercula fold appeared.
Fifty two	4.35±0.03	Prominent air bladder and gills.
Fifty eight	4.60±0.02	The appeared of pectoral fin bud and pigmented jaws.
Sixty four	4.85±0.01	Clearly visible pectoral fin and myomere.
Seventy two	5.06±0.01	Clearly visible brain lobe visible, yolk sac disappeared, larvae started feeding and swim actively.

Pre-larval phase

Pre larval phase recognized as starts from hatch and end with the absorption of yolk sac. This stage recognized up to72 hrs from hatch and the development progress were observed in every two hours. However, the development phases described with time when noticed recognizable characteristics.

Zero-hour larvae

Newly hatched larvae (2.08±0.02 mm) were yellowish color, transparent and large yolk-sac attach with the body. Hearts were functional in between head and the anterior margins of the yolk. No visible eyes noticed (Fig. 2A).

Table 5. The developmental stages (summary) of Post-larvae phase of *M. aculeatus* in the captive condition.

Age of larvae (day)	Mean length (mm)	Characteristics
Four day	6.30±0.48	Yolk sac was fully diminished, started to exogenous feeding
Five day	6.66±0.35	Prominent mouth cleft, upper and lower jaw, slightly visible anus.
Six day	7.44±0.54	Pigmented eyes, prominent gills, pectoral fin appeared
Seven day	8.06±0.43	Developed air bladder and clear visible of brain lobe
Eight day	8.64±0.66	Fully pigmented eyes, melanophores below the notochord
Nine day	9.10±0.36	Well developed intestine and anus
Ten day	10.20±0.87	Distinguished dorsal and caudal fin and slightly developed notochord
Eleven day	11.35±0.44	Eyes were heavily pigmented, auditory vesicle with otolith is distinguished
Twelve day	12.50±0.76	Elongated dorsal and ventral fins and depressed at the caudal end
Thirteen day	13.56±0.55	Operculum extend over gills
Fourteen day	14.26±0.33	Fully developed notochord and pigmented eyes
Fifteen day	15.05±1.02	Protrudedanus, small and rounded caudal fin

Two-hour larvae

The length of the larvae was2.12±0.04 mm. Yolk sac remained attached to the body. Melanophore bands appeared on posterior end of the body (Fig.2B).

Four-hour larvae

The body became more transparent and length was measured 2.16±0.02 mm. The yolk sac became partially decreased (Fig. 2C).

Six to eight-hour larvae

Melanophores appeared on the head. The anterior part began to thicken. The color of the yolk sac was brown yellowish and partially reduced. The anus bud is slightly visible (Fig. 2D).The length of the larvae measured to 2.35±0.06 mm.

Ten-hour larvae

A tubular pulsating heart appeared. Eye and anus

become slightly visible. Intestine was clearly visible and appeared the flexion notochord (Fig. 2E). The length of the larvae increased to 2.50±0.03 mm.

Twelve to eighteen-hour larvae

Melanophore bands were very much prominent at the posterior end of the body and also appeared above the eye and around the yolk sac. Brain did not differentiate from the body at 14 hrs larvae. The larvae were increased to 2.89±0.04 mm in 18 hrs and the eyes were slightly pigmented (Fig. 2F).

Twenty two to twenty eight-hour larvae

Eyes became pigmented and dark in color. Air bladder and mouth cleft visible in this stage (Fig. 2G). The anal became distinct and the color of larvae changed to silver- yellowish. The larvae were increased to 3.22±0.02 mm in size.

Fig. 1. The aggregated larvae of *M. aculeatus* in the aquarium corner (arrow) and the newly hatch larvae (inset).

Thirty two to forty four-hour larvae

The color of the larvae was whitish black. Eyes were increased in size and became densely pigmented. Opercula fold appeared (Fig. 2H). Brain lobe clearly visible and mouth cleft easily distinguished. The upper and lower jaws were fully formed. The larvae were increased to 4.10±0.02 mm in size.

Fifty two-hour larvae

The larvae were increased to 4.42±0.03 mm in size. Air bladder was distinct (Fig. 2I). A few black chromatophores were found in a row from the posterior to the auditory concentrations up to the base of the caudal fin.

Fifty eight-hour larvae

Eyes were fully pigmented(Fig. 2J). The yolk sac was reduced and showed slender like structure. Pectoral fin bud appeared. The length of the larva was 4.60±0.02 mm.

Sixty four-hour larvae

The larvae increased to 4.85±0.01mm in length. The brain lobe was fully visible. Gills were prominent. Air bladder was elliptical. Notochord was in an upward position only at the very terminal past. Pectoral fin folds became distinct and rudimentary rays developed in caudal fins (Fig. 2K).

Seventy-two hour larvae

Eyes were fully pigmented. Distinct black chromatophores were seen behind the eye (Fig. 2L). Dorsal and ventral fin folds were persistent. The larvae were silver blackish and transparent in color. Yolk sac completely disappeared and larvae had started feeding. Larva swims actively and reached 5.06±0.01 mm in total length.

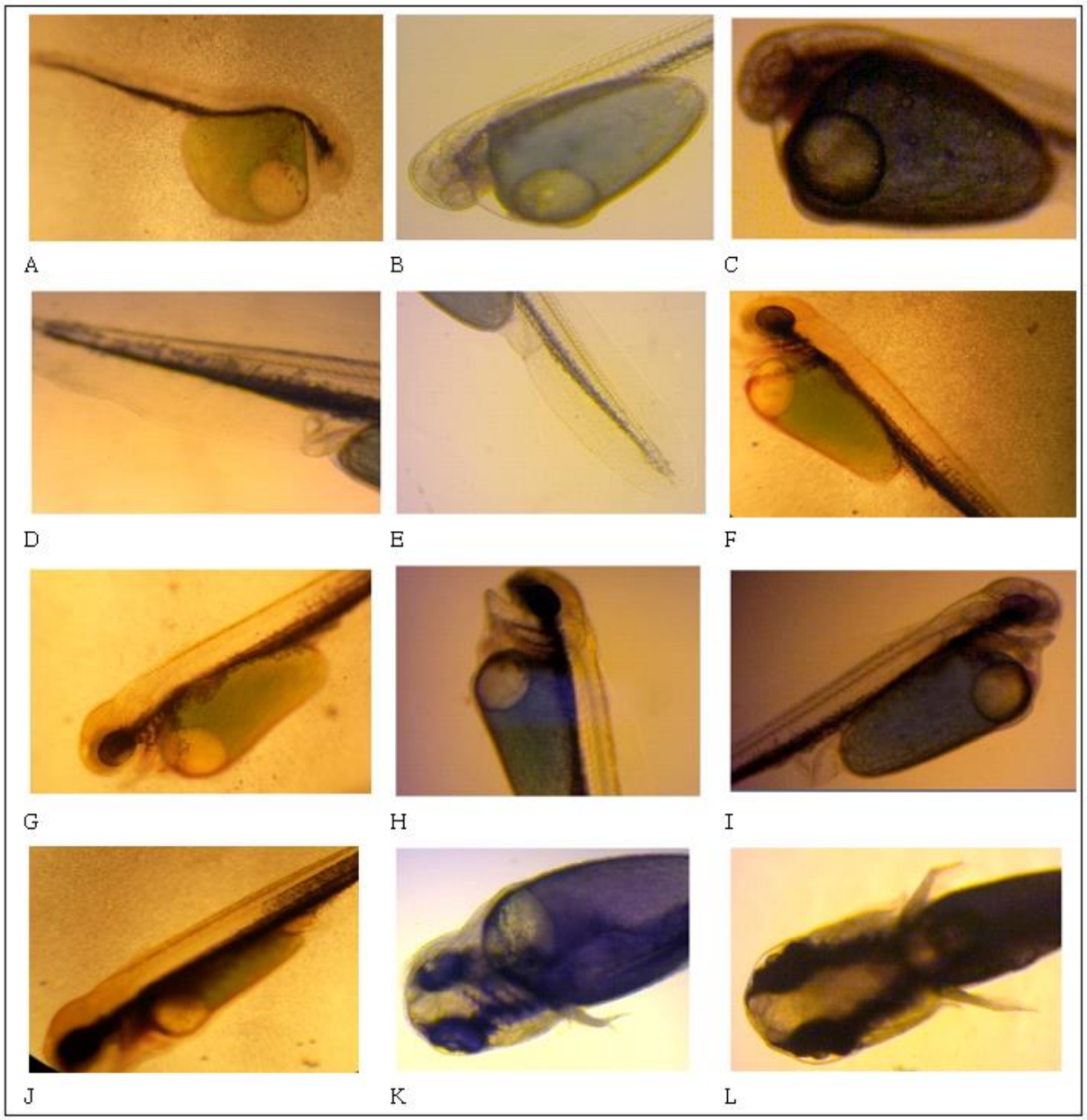

Fig. 2. Pre-larval development stages of *M. aculeatus*. A: Zero-hour post hatching larvae; B:Two-hour larvae; C: Four-hour larvae; D: Eight-hour larvae; E: Ten-hour larvae; F: Eighteen-hour larvae; G:Twenty-eight hour larvae; H: Forty-four hour larvae; I: Fifty one-hour larvae;J: Fifty eight-hour larvae; K: Sixty four-larvae andL:Seventy two-hour larvae.

Post-larval phase

Post larval phase starts after absorption of yolk sac and finished to the end of metamorphosis. Post larval phase were observed at every 24 hrs interval. The development features of the post larvae described for up to 15 days of larval rearing.

Four- days old larvae

This was the crucial period of the larval development. The yolk sac was fully diminished (Fig. 3A) and larvae started to exogenous feeding. The total length of the larvae was increased at 6.30±0.48 mm.

Five-six days old larvae

The total length of the larvae was 7.44±0.54 mm. They showed prominent mouth cleft, distinguished upper and lower jaw, and somewhat distinguished head and tail. Pigmented eyes, prominent gills and pectoral fin appeared (Fig. 3B).

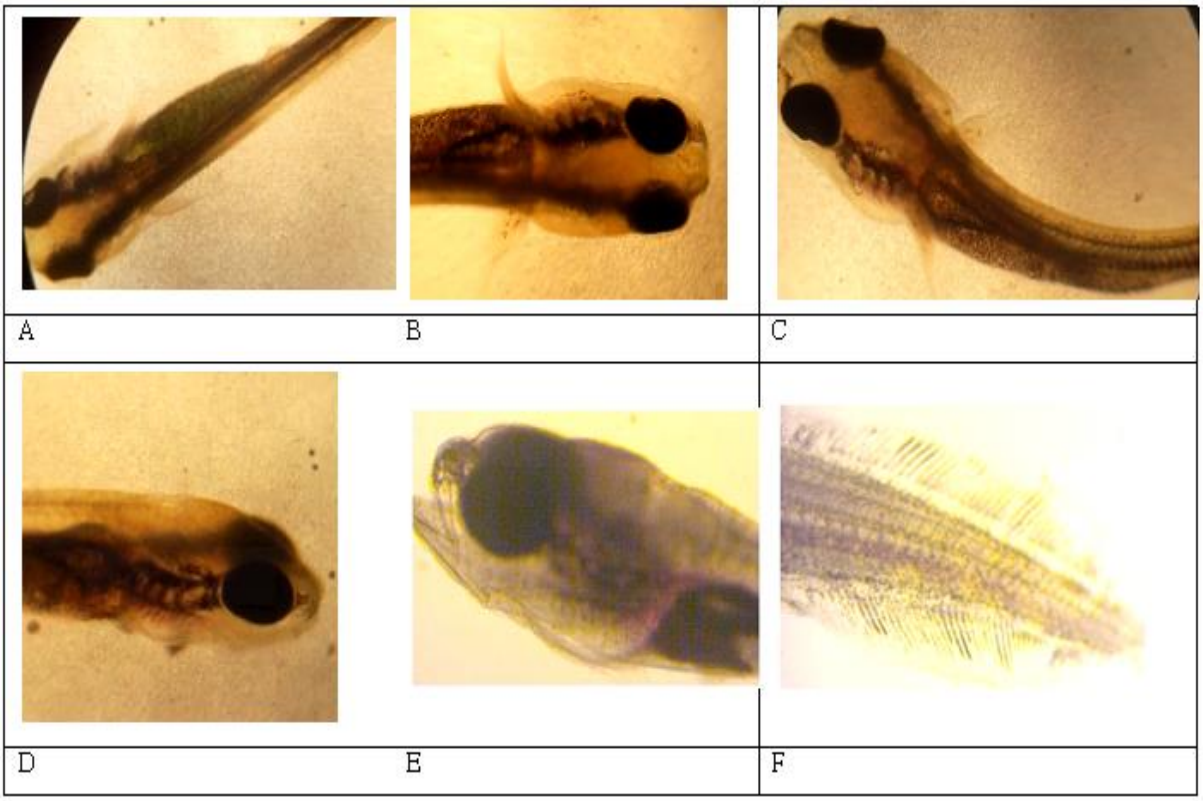

Fig. 3. Post-larval development stages of *M. aculeatus*. A: Four days old larvae; B:Six days old larvae; C: Eight days old larvae; D: Head portion of ten days old larvae; E: Head portion of twelve days old larvae; and F: Tail portion of fifteen days old larvae.

Seven-eight days old larvae

Total length of the larvae was 8.64±0.66 mm. Brain lobe was clearly shown. Body enlarged, intestine and gut were shown in this period (Fig. 3C).

Nine-ten days old larvae

Oil globules were shown on the head, dorsal and ventral side of the body (Fig. 3D).Well-developed intestine and anus, pronounced pectoral fin bud, distinguished dorsal fin and caudal fin and notochord slightly developed. Total length of the larvae reached 10.20±0.87mm.

Eleven-twelve days old larvae

Eyes were heavily pigmented, auditory vesicle with otolith is distinguished, dorsal and ventral fins is elongated and depressed at the caudal end. The length of the larvae was 12.50±0.76 mm (Fig. 3E).

Thirteen-fifteen days old larvae

Fully developed notochord, pigmented eyes, lens were distinguished (Fig. 3F). Few oil droplets were shown on the head. Operculum extends over gills. Anus was protruded. Caudal fin was small and rounded. The length of the larvae reached to 15.05±1.02 mm.

Discussion

Larval rearing

The present experiments first time succeed the rearing of larvae up to 15 days and onward of *M. aculeatus* in three different sources of water like rain water, pond water and supplied tap water. The recorded water parameters in rearing system (Temp=28-30°C, pH= 7.3- 8.5, DO= 5.5-6.5mg/l) in present experiments was quite optimum for the species according to the other such reported. Das and Kalita (2003) successfully breed of *M. aculeatus* at the water temperature between 28-30°C, pH 7.6-7.8 and dissolved oxygen 8-9 mg/L. In addition, it is mentioned that water temperature 27-28°C is suitable for *Macrognathus aculeatus* (Farid *et al.,* 2008) and 27-31°C is suitable for *M. pancalus* (Rahman *et al.*, 2009).

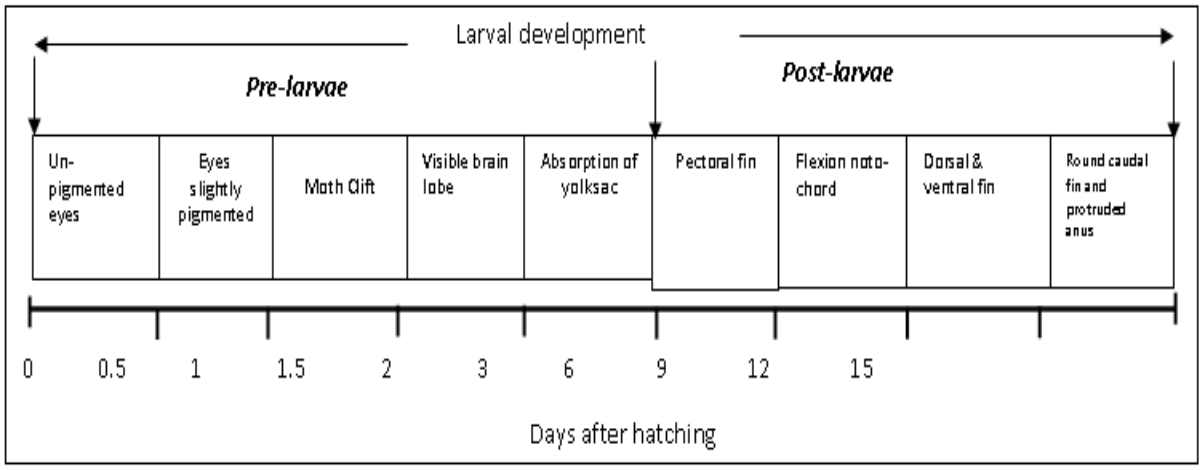

Fig. 4. The larval development consequences with different time intervals of *M. aculeatus*.

In the present experiment it was found that the survival rate up to 15 days in rain water, pond water and tap water were 42%, 33.5% and 19.5% respectively. This finding is more or less similar to Afroz *et al.* (2004) in *M. pancalus* and showed that the survival rate was 46.5%, 37.5% and 21.5% in rain water, pond water and tap water respectively. This apparent deviation in rain water, pond water and tap water may be due to the water quality parameters. Though, there was no significant ($P<0.05$) deviation of water parameters among three different treatments or sources of water but may be the lower pH and higher DO in rain water (T_1) could be the trigger for the higher survival of larvae.

Larval development

The general larval development sequences was observed under electronic microscope and their development progress showed more or less similar with other such studies like Farid *et al.* (2008), Rahman *et al.* (2009) and Afroz *et al.* (2014). However, some development features showed in different time intervals in the different species.

The newly hatch larvae of *M. aculeatus* were inactive, yellowish color with a large yolk sac attached to the transparent body. The similar findings reported for other eel fishes like *M. aculeatus* (Farid *et al.*, 2008); *M. pancalus* (Rahman *et al.,* 2009; Afroz *et al.*, 2014) and *Anguilla japonica* (Kiichiro *et al.* 1975). The length of newly hatched larvae of *M. aculeatus* was found to be 2.08±0.02 mm. Farid *et al.* (2008) recorded 1.70 ± 0.05 mm of the size of the newly hatch larvae for same the species. The very large hatchling of the same species like 4-5 mm recorded by Sahoo *et al.* (2007) from Indian water body. Kiichiro *et al.* (1975) recorded 2.9 mm of just after hatchling of Japanese eel, *Anguilla japonica*. This variation may be due to different species and geographical habitat. However, very close length like 2.10 ± 0.04 mm recorded by Rahman *et al.* (2009) in case of *M. pancalus* from Bangladesh water body. Thus it may say that the size of the newly hatch larvae of eel fish is about 2.00 mm in length.

The larval yolk sac partially reduced, eye and anus slightly visible within 8 -10 hrs in the present study which is similar to Farid *et al.* (2008) for the same species. However, these characteristics exhibited in late like 13 hrs (Rahman *et al.,* 2009) and 36-48 hrs in *M. pancalus* (Afroz *et al.*, 2014). The eyes started to pigment from 18 hrs of hatching which noticed at 24 hrs in the same species (Farid *et al.*, 2008). However, the eyes pigmentation started in very late (110-140 hrs) in *M. pancalus* (Afroz *et al.*, 2014). The color of the larvae changed to silver-yellowish after 28 hrs which noticed at 33 hrs in *M. pancalus* (Rahman *et al.,* 2009). However, the larvae again changed yellowish color to silver blackish color with 64 hrs which also reported by Farid *et al.* (2008) but they noticed this feature at 36 hrs of hatching. The

development of pectoral fin bud of *M. aculeatus* appeared at 51 hrs after hatching but Farid *et al.* (2008) found these developments in *M. aculeatus* within only 12 hrs of hatching.

The complete absorption of yolk sac, started to exogenous feeding and freely movement of the larvae noticed within 72 hrs (Fig. 5) which is almost similar to the findings to other such like Farid *et al.* (2008) reported within 66 hrs for *M. aculeatus* and Rahman *et al.* (2009) reported 67 hrs in case of *M. pancalus*. However, Afroz *et al.* (2014) reported the complete absorption of yolk sac at 80-90 hrs old larvae in *M. pancalus*. The timing of starting first feeding have evolutionary values and depends on the availability of the natural food. The synchronized activity of dependable community in nature is important. Perhaps these determine the different species to act differently. The larvae reached to 5.06±0.01 mm in total length within 72 hrs which is differed by Farid *et al.* (2008) and Rahman *et al.* (2009) who reported 6.0±0.09 mm and 5.50±0.02 mm for the species of *M. aculeatus* and *M. pancalus* respectively. Thus, the rate of development of the larvae varied from other species. This variation could be because of several environmental factors notably temperature. It is noticed that the higher the temperature the quicker was the larval development (Hoar and Randal, 1969).Though it noticed that the existence of the most of the organs like a juvenile fish of the species within 15 days but not yet metamorphosis and yet to complete larval phase. Das and Kalita (2003) reared up to 30 days but no information reported on their development stages. However, *M. pancalus* reported to rear up to 18 days (Rahman *et al.*, 2009) and 17 days (Afroz *et al.,* 2014) and they also reported as incomplete larval stages.

Conclusion

The better survival rate of *M.* aculeatus noticed in rain water condition than pond and supplied tap water. The newly hatched larvae were yellowish, transparent body with the total length of 2.08 ± 0.02 mm. The yolk sac was disappeared completely and started to exogenous feeding within 72 hrs of hatching but the larvae not yet fully metamorphosis resembled to Juvenile fish at 15 days of rearing. These findings may help to enrich the knowledge of biology and ecology of the fish, which also might help to start large scale seed production of the species *M. aculeatus*.

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