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Larval rearing and the observation of the development features in striped spiny Eel, *Mastacembelus pancalus* in captive condition

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Abstract
The larval were reared and examined the development features of the striped spiny eel, *Mastacembalus pancalus* in the aquarium condition. The PG induced larvae were reared in different water sources and recorded the average survival rate 31.5%. The transparent newly hatched larvae were 1.65±0.15 mm in length having with a large yolk sac and melanophores on the body. The yolk sac was completely disappeared on the 4th day and started to exogenous feeding. The pigmented eyes, gut and gills were prominent on the 5th day. The upper and lower jaw was well developed within 7-8 days. The dorsal fin and caudal fin were distinguished clearly within 9-11days and fins decorated with smooth fin ray within 18-25 days. Metamorphosis of the species completed in 35 days and the larvae resembled to their parents but the further development of the organs noticed until 45 days and onward while their length reached 18.21±1.65 mm.

Keywords: *Mastacembelus pancalus*, larval development, captive condition

1. Introduction
The striped spiny eel, *Mastacembelus pancalus* is one of the common fish species of the Mastacembelidae family which locally known as guchibaim. The fish is found in Asia and mainly to India, Pakistan, Bangladesh [1] and Nepal [2]. Bhuiya [3] described that this fish is highly estimated as food in Bangladesh. Though there are no available data on their ornamental value in Bangladesh but it gained ornamental value as an indigenous aquarium fish in India and is being exported to America, Europe, and other Asian countries [4, 5].
In the past, the fish was available in estuaries and freshwater habitat throughout Bangladesh [6]. But the availability of the guchibaim has decreased sharply from natural habitat due to mostly habitat destruction and overexploitation [7]. The natural breeding grounds of this fish are under threat because of drying up of the low lying areas and indiscriminate use of fertilizers and pesticides [8]. 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. Though in the last assessment of IUCN Bangladesh [9], the fish is under the category of 'least concern' but it was reported as 'critically endangered' by IUCN Bangladesh [10]. To conserve the species either it is needed for proper management of natural source or to introduce artificial propagation as well as culture. Whatever it is thought, it is necessary to understand the details biology, especially on the larval development process to establish artificial propagation in captive condition.
Notable works have been conducted on the biology and breeding on eel fishes like *Mastacembelus pancalus* [11, 12], *Mastacembelus armatus* [13], *Macrognathus aculeatus* [14], and *Macrognathus pancalus* [15]. The other such studies on the development of egg and/or larvae like *M. pancalus* [7, 8], *M. aculeatus* [16, 17], *Muraenesox cinereus* [18], *Anguilla rostrata* [19], *Mastacembelus mastacembelus* [20] but no such details work on larval development of *M. pancalus* except [7, 8]. However, consequences of the study are needed for the confirmation of the findings. It is essential to know the larval development features and organogenesis of any species for the development of management and larval rearing technology. Studies on early larval development are important and obligatory to the successful rearing of larvae for large scale seed production in aquaculture [21, 22]. Thus the present study was conducted to know the larval rearing technique in different sources of water and to understand their details larval developmental features.

2. Materials and Methods

2.1 Study site and preparation of aquarium

The experiment was conducted in the rectangular glass aquaria in the laboratory of department of Fisheries and Marine Bioscience, Jessore University of Science and Technology, Jessore, Bangladesh. The size of the each glass aquaria was 36 x 14 x 15 inch. As the fish larvae like to attach a root substrate, water hyacinths were used. The water parameters such as pH, DO, and temperature were recorded daily. 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).

2.2 Collection and rearing of larvae

The larvae of *M. panclaus* were collected from the same experimental laboratory, which were produced by the induce breeding with PG hormone. Broods were collected from the nearby natural habitat (Baor) with average body weight of 9g each. Broods were kept 1:1 ratio in aquarium for natural propagation after inducing. Fishes were spawned within 16-20 h after administration of hormone and hatched out at 36 h of spawning. The newly hatched larvae were collected from the experimental aquarium by using a glass jar and stocked in the rearing aquarium at the rate of 100 larvae per aquarium (Table 1). Larvae were feed regularly with boiled egg yolk once a day after the absorption of yolk sac. The boiled egg yolk was homogenously mixed with water by filtering with cotton net. The larvae were reared for 45 days and onward to observe their larval development in three different sources of water (Table 1).

Table 1: The experimental design for different treatment for larval rearing of *M. pancalus* 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		

2.3 Observation of the larval development

The larvae were examined at every 24 h at the same hour of a day. The larval developments stages were observed and taken photograph with a photographic microscope (Carl Zeiss microscopy GmbH, S.N. MKG8639, made by Germany). The larvae at each sampling were measured using the same microscopic camera. The pictures were also taken sometime by using a digital camera (Sony, Model DSC-W520), from the eyepiece of the microscope. The development stages and characteristics were confirmed by using different books and articles especially [7, 8, 16, 23].

2.4 Statistical analysis

The result found in the study was 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.

3. Results

3.1 Water environment and survival of larvae

The mean water temperature, dissolve oxygen (DO) and pH under different treatments were 29.27±0.40 °C, 5.20±0.50 mg/L and 7.76±0.32 respectively (Table 2). The recorded water parameters were not significantly (P>0.05) different between treatments. However, the survival rate was significantly (P>0.05) differ in different treatment and highest 41.5% recorded in rain water rearing (Table 3).

Table 2: Average water quality parameters of the different treatment aquarium during rearing of *M. pancalus.*

Water parameters	T_1	T_2	T_3
Temperature(ºC)	29.7± 0.41	29.3± 0.33	28.8± 0.54
pH	7.41± 0.13	7.75± 0.34	8.12± 0.42
DO (mg/l)	5.73± 0.32	5. 25± 0.12	4. 62± 0.18

Table 3: The survival rate of larvae of *M. pancalus* in different water sources.

Treatment	Stocking	Survival rate (%)	Average survival rate
T_1 Rainwater	100 100	40 43	41.5%[a]
T_2 Pond water	100 100	31 36	33.5%[b]
T_3 Tap water	100 100	22 17	19.5%[c]

The different subscript ([a, b, c]) in the average survival rate column indicate the significant (P>0.05) differences

3.2 Larval development stages

The larval development consequences (Table 4 and Figures 1 and 2) according to their morphometric progress in time are described below.

Day 0 (average length 1.65±0.15 mm)

The fertilized eggs were hatched out within 36-39 h. Larvae become brownish color, cylindrical and transparent body. Yolk sac was large and attached to the body (Fig. 1 a). The hearts of the larvae were functional in between head and the anterior margins of the yolk. Swimming movement was restricted and they were attached to the aquarium wall. Melanophores appeared on the body and yolk sac.

Day 1 (average length 2.21±0.45mm)

Yolk sac slightly decreased with the existence of eye position (Fig. 1 b). Melanophores appeared more prominent. Myomere was partially visible.

Day 2 (average length 2.54± 0.18 mm)

Yolk sac was convex interiorly in 2nd day, and become tubular due to absorption of the yolk sac interiorly (Fig. 1 c). The melanophores appeared around the yolk sac. The tail was thickened (Fig. 1 d).

Day 3 (average length 3.21±0.23 mm)

The pectoral fin bud slightly appeared. Mouth portion was clear. A few black chromatophores were found in a row from the posterior to the auditory concentrations up to the base of the caudal fin. Large black chromatophores were observed on the head. Eyes and anus were visible (Fig. 1 e).

Day 4 (average length 3.51±0.05 mm)

In the 4th day, yolk sac was fully diminished (Fig. 1 f). Mouth cleft was prominent. Head and tail somewhat distinguished. Larvae started to exogenous feeding and boiled egg yolk was fed to them as first feed.

Day 5-6 (average length 3.68±0.20 mm)

Eyes were more prominent and upper and lower jaw distinguished during the 5th to 6th day of larvae (Fig. 1 g). The

gut and gills were prominent and appearing of pectoral fin. Hatchlings showed actively free swimming.

Day 7-8 (average length 4.21±0.25 mm)

The pectoral fins were clearly distinguished; eyes were heavily pigmented during 7th and 8th days (Fig. 1 h). The dorsal fin appeared. The upper jaw and lower jaw were well developed and air bladder distinguished. Body enlarged, intestine, gut and few oil globules were shown in this period. Brain lobe was clearly shown. Melanophores were shown below the curve notochord and the thickened tail was more clear (Fig. 1 i).

Day 9-11 (average length 5.57 ± 0.63 mm)

Eye ball was large and distinct (Fig. 1 j). Intestine and anus were well developed (Fig. 1 k). Gill was clearly distinguished. Pectoral fin bud more pronounced. Dorsal fin and caudal fin distinguished, and notochord slightly developed (Fig. 1 l). Oil globules were shown on the head, dorsal and ventral side of the body. Body color became brown- yellowish. Dorsal and ventral fin fold is elongated and depressed at the caudal end.

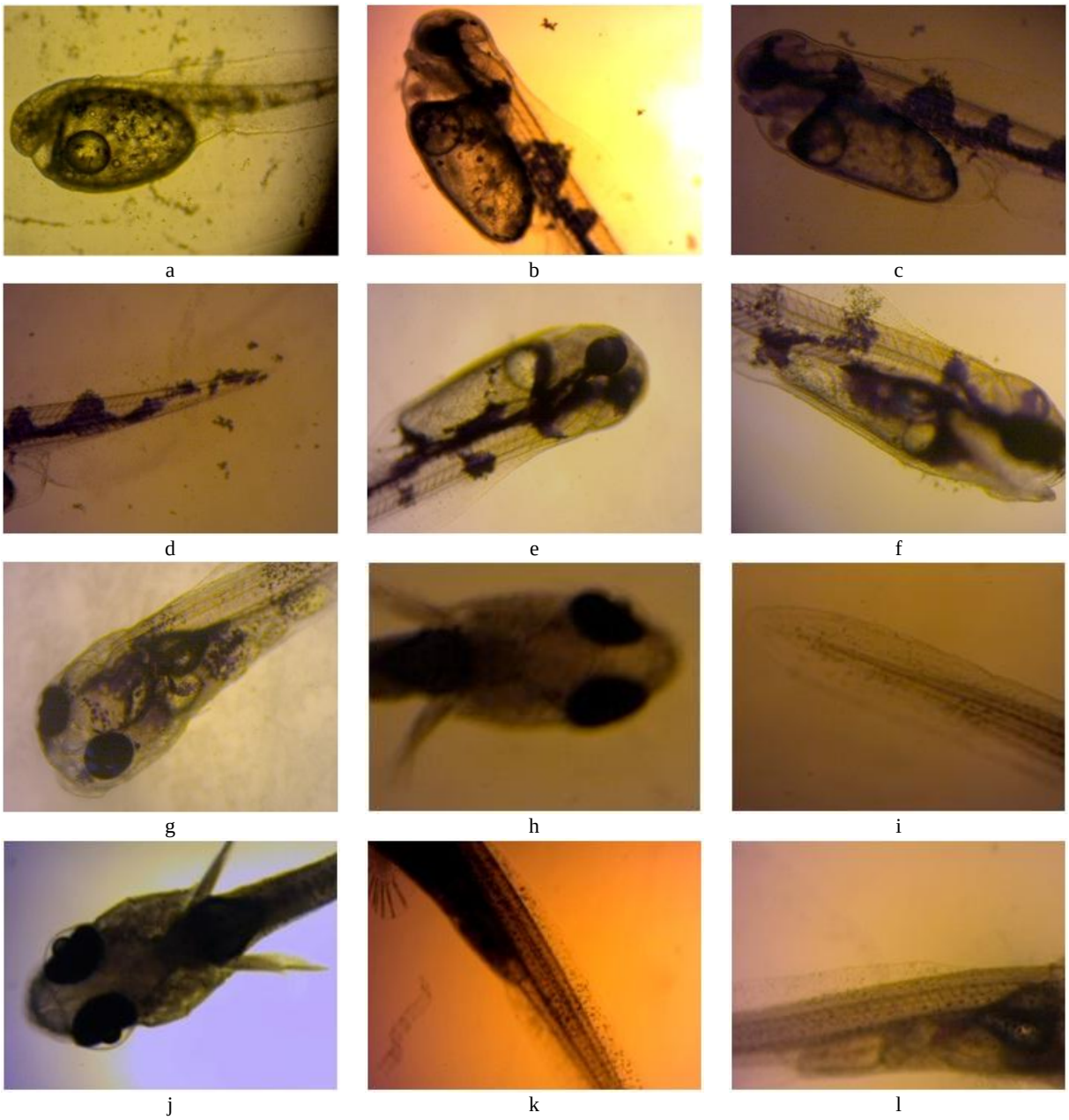

Fig 1: The larval development progress of *M. pancalus* up to first 11 days at captive condition. a) 0-days larvae, b) 1-days larvae, c-d) 2-days larvae, e) 3-days larvae, f) 4-days larvae, g) 5 to 6-days larvae, h-i) 7 to 8-days larvae, and j-k-l) 9 to 11-days larvae.

Day 12-16 (average length 6.68±0.37 mm)

During the 12th to 16th days, body become elongated and compressed at caudal region with rounded caudal fin. The lens was distinguished in the pigmented eyes (Fig. 2 a). Myomere (muscle band) was distinguished. Operculum extended over the gills (Fig. 2 b) and noticed of protruded anus.

Day 17-25 (average length 9.76±0.78 mm)

Spiky notochord was shown in these days of larvae. Air bladder was fully developed. Body compressed at the posterior end. Dorsal fin and anal fin extended to the caudal fin but not confluent with the caudal fin. Fins were contained with smooth fin ray (Fig. 2 c). The anterior part of the dorsal fin was narrower and had spine in 25th days old larvae (Fig. 2 d).

Day 26-35 (average length 14.21±1.73 mm)

Caudal, dorsal and anal fin rays were observed during 29th days. The anal and the dorsal fins were confluent with caudal by narrow flanges. The larvae moved actively. Pigmentation

was more pronounced throughout the head and body (Fig. 2 e). The number of rays increased in the body during 33rd days (Fig. 2 f) with the clear notochord (Fig. 2 g). The snout was elongated with well pronounced lobes at the trip (Fig. 2 h). All the organs clearly distinguished as their parents at the age of 35th days. The anterior spine was short, sharp and devoid of any inter-spinuous membrane (Fig. 2 i).

Day 36-45 (average length 18.21± 1.65 mm)

Caudal fin was rounded and spine fully developed in the dorsal fin at the age of 40th days. Fry displayed a caudal and anal fin with branched rays (Fig. 2 j), representing the full complement of rays (Fig. 2 k). At the 45th days, fry was elongated and provided with all morphological characters like an adult (Fig. 2 l).

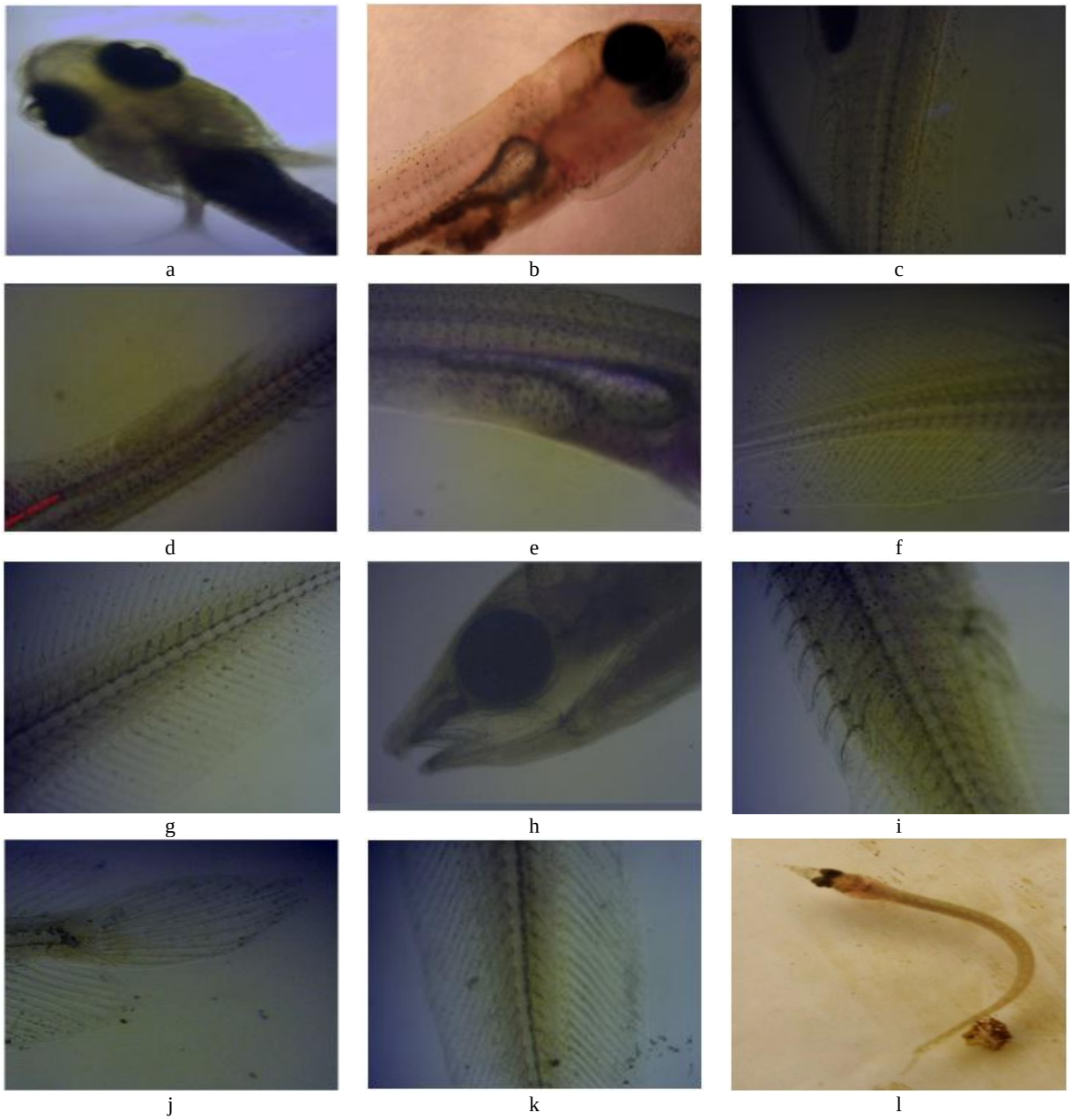

Fig 1: The larval development progress of *M. pancalus* from rearing of 12 days to 45 days at captive condition. a-b) 12 to 16-days larvae, c) 17-days larvae, d) 25-days larvae, e) 29-days larvae, f-g-h) 33-days larvae, i) 35-days larvae, j-k) 40-days larvae, and l) 45-days larvae.

Table 4: The summary of larval development stages with feature of *M. pancalus*

Larval age (days)	Characteristics
0	Brownish- yellowish color larvae, cylindrical and transparent body, large yolk sac.
1	Yolk sac slightly decreased. Prominent melanophores
2	Yolk sac convex interiorly. Thickened tail.
3	Clear mouth portion. Large black chromatophores on head.
4	Fully diminished yolk sac. Prominent mouth cleft. Larvae started to exogenous feeding.
5-6	Pigmented eyes. Appeared of pectoral fin, prominent gut and gills
7-8	Fully pigmented eyes, well developed jaws and visible of brain lobe.
9-11	Well-developed intestine and anus and gills.
12-16	Visible myomere (muscle band). Operculum extended over gills.
17-25	Spiky notochord. Fins contained smooth fin ray. Spine at dorsal fin.
26-35	More pronounced spiky fins. The snout was elongated with well pronounced lobes at the trip.
36-45	Development of most fin rays. Elongated fry alike an adult.

4. Discussion

4.1 Water parameter and survival of larvae

The recorded water parameters such as temperature, dissolve oxygen (DO) and pH under different treatments were 29.27±0.40 °C, 5.20±0.50 mg/L and 7.76±0.32 respectively that were quite optimum for rearing of *M. pancalus* larvae. Afroz *et al.* [7] and Rahman *et al.* [8] mentioned that suitable water temperature were 27-31 °C and 27-30 °C respectively for the rearing *M. pancalus* larvae. Das and Kalita [14] estimated that the water temperature between 28-30 ºC, pH 7.6- 7.8, dissolve oxygen 8-9mg/L could be suitable for *M. aculeatus*. *M. pancalus* accept pH between 6 and 8, but might do best in neutral to alkaline water [19]. It was stated that the higher temperature influences quicker embryonic and larval development of the species [24]. This experiment was done in June-August, when photoperiodic length was long and thus beneficial for larval development.

The present study was the first time succeeds to rear the larvae up to complete their larval development stages. They reared for 45 days and onward. Earlier [7] succeed to rear up to 17 days of the same species larvae. However, earlier mortality reported for the same and other eel fishes like larvae of the pike eel (*Muraenesox cinereus*) survived for 10 days [18], *M. aculeatus* for 4 days [14] and *M. pancalus* survived for 3 days [8].

Though there was no significance ($P>0.05$) difference among the water parameters in different treatments but significant ($P>0.05$) difference showed in survival rate among treatments. The average survival rate was 31.5% with the highest 41.5% in T_1 where larvae were reared in rain water for raring of 45 days. Afroz *et al.* [7] noticed average survival 35% on rearing of 15 days and all the larvae were died on the day of 17. In the present experiment first time succeed in rearing them up to juvenile and release them at the University pond complex.

4.2 Larval development

The newly hatched larvae of *M. pancalus* showed the typical features of the eel and the larval development sequences were more or less similar to other such studies like, *Muraenesox cinereus* [18], *Mastacembelus pancalus* [7, 8] and *Anguilla rostrata* [19].

The characteristics of the newly hatchling of the present study like brownish color larvae, cylindrical and transparent body with the large yolk sac attached to the body supported by the [7, 8]. The average length of the newly hatched larvae was 1.65±0.15 mm which was smaller than [8] who recorded 2.10±0.04 mm. There was also variation noticed in fertilized egg diameter in both study like in the present study, the egg diameter was 0.67±0.04 mm whereas 0.70±0.02 mm recoded by the [8]. However, very large newly hatch larvae (4-5 mm) were reported by Sahoo *et al.* [16] in case of *M. aculeatus* where egg size was 1.2 to 1.4 mm. Thus it may say that different species and size of eggs might be responsible for difference in larval length of fishes.

Umezawa *et al.* [18] reported that the newly hatched larvae of the pike eel showed eyes, anus, pectoral fin fold which did not noticed in the present study. Rahman *et al.* [8] observed operculum, air bladder and mouth cleft within 1-1.5 days. In the present study except the air bladder other organs were developed after 4 days of hatching. The yolk sac completely absorbed on day 4 in the present study which supported by [7] but quick absorption (within 3rd day) noticed by [8]. The eye started pigmented from 3rd day which fully pigmented by 7-8 days which strongly was supported by [7] but earlier pigmentation (within 3 days) noticed in *M. pancalus* [8] and in *M. cinereus* [18]. The most of the fin rays and the spiny notochord noticed within 9-11 days and 18-22 days of larvae which noticed 13-14 days and 15-16 days respectively by [7].

The first exogenous feeding period of any larvae is the critical characteristics to manage them and this mostly depends on the absorption of the yolk sac. In the present study the larvae of *M. pancalus* started first feeding on the 4th day after complete absorption of yolk sac which was similar to [7] and [14]. In the present study metamorphosis was completed in 35 days and the length reached about 14.21±1.73 mm. All the organs clearly distinguished as like as their parents in this stage. However, fry was much elongated and reached 18.21± 1.65 mm in length on the day of 45 of rearing.

5. Conclusion

The present study first time succeeds to rear *M. pancalus* up to their completion of larval development and onward. The larvae completely absorbed their yolk sac on 4th day and started to feed exogenous feed. The larval development completed within 35 days of rearing and they are ready to stock for culturing. These findings may help to fishery biologist to manage the fishery as well as further initiative to captive breeding and production of *M. Pancalus* larvae.

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