International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129
IJFAS 2014; 1(6): 163-167

www.fisheriesjournal.com
Received: 21-05-2014
Accepted: 10-06-2014

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Larval rearing of spiny eel, *Mastacembelus pancalus* in the captivity with emphasis on their development stages

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Abstract
The larvae of the spiny eel, *Mastacembelus pancalus* were reared and examined the development of larvae. Larvae survive up to 390 hrs of hatching and improved survival rate showed in rain water than pond and supplied tap water. The larval development progress were categorized into nine distinguish stages. In the first stage, larvae carried yolk sac on their body. In later stage, yolk sac was convex interiorly and become tubular. Yolk sac was completely disappeared in the third stage within 80-90 hrs of hatching and started to exogenous feeding. Eyes were fully pigmented at the fourth stage. In fifth stage, intestine and kidney were developed. Well-developed jaws were visualized in the sixth stage. In later stages, gut and airbladder were visualized. In the eight stage, distinct anal and dorsal fin were observed. At the last stage, spiky notochord was shown within 370-390 hrs of hatching but yet to complete larval stages.

Keywords: *Mastacembelus pancalus,* larval rearing, larval development**,** water quality.

1. Introduction
The Guchibaim (*Mastacembelus pancalus*) is one of the important eel fish in Bangladesh with great demand as good table fish. It is one of the common species of fishes of Mastacembelidae family found in Asia and known as striped spiny eel. *M. pancalus* usually lives beneath the mud, but during rains it is seen in the root of the water hyacinth or any other such substrate. It inhabits slow and shallow waters of rivers in the plains as well as estuaries and a variety of other freshwater habitats such as rivers, canals, beels and inundated fields in the past throughout Bangladesh [1, 2]. About 80% population is poor in the country and they depend on small size fish for their daily supply of animal protein as they are available at reasonable price [3]. Fish is the main source of protein in the diet of the people of Bangladesh because 60% of the animal protein comes from fish alone [4]. Earlier guchi baim was the dish item for poor, but now it belongs to middle and higher class people. The scenario has been changed not only for guchi baim but also for most of the small indigenous fishes of the country. Due to the unavailability in natural water body, the demand has increased many fold and obviously increased the price. Though there is no exact data, the availability of the guchi baim has decreased sharply and at present it is a very rare item in the locality. The guchi baim are harvested completely from wild source. However the harvested production is decreasing due to habitat modification, overexploitation, as primes. Thought the guchi baim is not in red listed by the IUCN [5], the species is critically endangered in Bangladesh [6]. To conserve the species either it is needed to 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 breeding and larval rearing. Notable works have been done on the larval development of some species of fishes in Bangladesh. But so far no works have been done on the larval development of *M. pancalus*. No doubt, we have no other choice to introduce indigenous species in a culture system to meet the demand of animal protein for the poor. Furthermore, the study will make encourage to scientist to put more attention to our local fisheries development and hence the national development. There are some research on the biology and breeding have done on some other eel fishes (*Mastacembelus pancalus,* Karim and Hossain [7]; *Mastacembelus armatus*, Serajuddinand Mustafa [8]; *Macrognathus aculeatus*, Das and Kalita [9]; *Macrognathus pancalus,* Suresh *et al.* [10]; *Mastacembelus pancalus,* Rahman *et al.* [11] and development of egg and larvae

(*Muraenesox cinereus*, Umezawa *et al.* [12]; *Anguilla rostrata*, Oliveira and Hable [13] but no such work for *M. pancalus*. The present study was the first time trial to rear in the control condition and to develop the larval developmental stages of the species.

2. Materials and Methods

2.1 Experimental design

The experiment was conducted in the laboratory of Department of Fisheries and Marine Bioscience (FMB), Jessore University of Science and Technology, Jessore, Bangladesh during July-August, 2012. The experiment was designed into two distinguish segments; i) larval rearing and ii) examine larval development progress. For the larval rearing, there were three treatments designed according to different water sources (Table 1) and each treatment had one more replication. The glass aquariums (tanks; 20 liters each) were used for the rearing which was stocked at same density (Table 1). In each tank, water hyacinth was placed as substrate. The larval developments were observed under an electric microscope in every 24 hours interval from the different rearing tanks.

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

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

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

2.2 Larval rearing and observation of development

Larvae were collected from the same laboratory which was produced by inducing first time in the country. By using a glass jar and scoop net larvae were separated from their parents as soon as they hatch. From the three day of stocking when noticed yolk sac was absorbed, boiled egg yolk was supplied as feed. The boiled egg yolk was homogenously mixed with water by filtering with cotton net before supply. The larval developments were observed under an electric microscope (BoEco, Germany). The pictures were taken by a digital camera (Sony, Model DSC-W520) from the eyepieces of the microscope. In addition, pictures were drawn manually by the microscopic observation. Larval development stages were categorized mostly according to their ontogenetic development and followed mostly to Umezawa *et al.* [12], John *et al.* [14], Unuma *et al.* [15], Rahman *et al.* [2] and Oliveira and Hable [13].

3. Results

3.1 Water parameter

The physico-chemical condition such as temperature, pH and dissolved oxygen of water in experimental aquaria under different treatments were ranged from 28.1 to 31.2 °C, 7.13 to 8.69, and 3.6 to 4.9mg/l respectively (Table 2). The mean values of water parameters were not significantly ($P<0.05$) different among the treatments.

Table 2: Water quality parameters (mean ± SD) of different treatment aquarium during rearing of *M. pancalus* larvae.

Water parameters	T1	T2	T3
Temperature (°C)	29.26 ± 0.768	29.28 ± 0.66	29.30 ± 0.77
pH	7.5 ± 0.17	7.86 ± 0.36	8.24 ± 0.20
DO (mg/l)	4.16 ± 0.15	4.14 ± 0.21	4.12 ± 0.14

3.2 Survival of *M. pancalus* in different water sources

The larvae were survived in the experimental aquarium for 17 days. There were about 50% mortality noticed on 5th days of rearing which turns to >80% during 14-15 days and rest were dead on 17th. However, the survival rate differed significantly ($P>0.05$) in different water sources. The mean higher survival of larvae was observed in T_1 (46.5%) which is filled with rain water followed by the T_2 (37.5%) and T_3(21.5%) respectively on the rearing day of 15th.

3.3 Larval development stages

There were nine development stages distinguished according to their morphometric progress in time (Figure 1 and Table 3). In the first stage just after hatch larvae were characterized with a large extruded yolk sac (Fig. 1A). Tail were thickened, melanophores appeared around the yolk sac. Myomere was partially visible in this stage. The larva, immediately after hatch, remains inactive, they tended to attach on the aquarium wall. The yolk sac convex interiorly and become tubular due to absorption of yolk sac interiorly in the second stage of development. The undeveloped notochord flexion was visible extending from the posterior portion of the brain to the end of the body within 36-48 hrs of hatch (Fig. 1B). The yolk sac has become more elongated and less deep. The head extends free

from the yolk sac as far back as the region of the heart. A series of melanophores were noticed near the margin of the dorsal fin fold as well as along the dorsal and ventral aspects of the body.

Table 3: The prime distinguishing characteristics of each development stages of larvae of *M. pancalus* in the aquarium

Stage No.	Hours after hatch	Distinguishing characteristics
01	06-12 hrs	Inactive larvae with large yolk sac
02	36-48 hrs	Yolk sac partially reduced and elongated.
03	80-90 hrs	Diminished yolk sac. Unpigmented eyes.
04	110-140 hrs	Start to pigment eyes. Prominent mouth cleft.
05	170-190 hrs	Fully pigmented eyes. Notocord clearly visible
06	210-230 hrs	Body laterally elongated. Eyes placed near each other.
07	270-290 hrs	Distinct pectoral fin, gut and airbladder.
08	320-340 hrs	Development of caudal and dorsal fin with soft rays
09	370-390 hrs	Distinct spiny notocord and most fin rays

In the later stage, yolk sac was absorbed completely within 80-90 hrs of hatch (Fig. 1C). The head and tail were somewhat distinguished with the prominent mouth cleft. The unpigmented eyes, anus, dorsal and anal finfold were visible in this stage. Larvae started free swimming and swam to the surface, where they seemed to gulp air, likely to fill the gas bladder. Swimming was somewhat restricted owing to the mass of yolk materials. Larvae of *M. pancalus* were first fed by boiled egg yolk once daily. The unpigmented eyes tuned to pigmented in the fourth stage (Fig. 1D). The pectoral fin bud, gut, air bladder was developed in this stage within 110-140 hours after hatching. Melanophores were showed below the curve of the notochord. Large satellite melanophores are scattered on the body at the bases of the fin folds. The head now has a very angular outline and the terminal mouth and jaws appear to be functional.

In the fifth stage, eyes were pigmented fully within 170-190 hrs of hatch (Fig. 1E). The eyes were increased in size, schedule and shifted to dorsally. Notochord was clearly visible in this stage and the large melanophores were appeared on the head. The body laterally schemed and more elongated in the sixth stage. The eyes shifted more dorsally and placed near of each other (Fig. 1F). Operculum extended over gills. Dorsal and anal finfold was elongated at the caudal end. Mandible was distinguished in this stage.

In the later stage, eye ball and pectoral finwere more clearly observed (270-290 hours after hatching). Gut and airbladder were compressed and well developed (Fig. 1G). Distinct caudal fin, anal fin and dorsal fin with soft fin rays were observed in 340 hours after hatching (Fig. 1H). The most fin rays were clearer in the observed last stage, where also noticed spiky notochord within 390 hrs of larval surviving (Fig. 1I).

4. Discussion

4.1 Water parameters

The recorded temperature in rearing system (27-30 ^{0}C) in present experiments was quite optimum for the species according to the other such reported. It is mentioned that water temperature was found 25-27 °C is suitable for *Macrognathus aculeatus*, 27-29 °C for *Mastacembelus erythrotaenia* [16], 22-25 ^{0}C is for survive of *Muraenesox cinereus* (see: Umezawa *et al.* [12] and 27-31 ^{0}C for *M. pancalus* (see: Rahman*et al.* [2]. The rate of larval development significantly depends on the ambient temperature [17]. The higher temperature influences quicker embryonic and larval development of the species [18]. The photoperiodic length was long during the experiment which was good as long photoperiod is always beneficial for larval development. The pH values in the present study were acceptable for the eel species. *M aculeatus, M. armatus, M. circumcinctus, M. erythrotaenia, M. siamensis, M. zebrius* were preferred P^H values 7-8 but more acceptable is 7.0 [16].

4.2 Rearing larvae of *M. pancalus*

Though the recorded water parameters did not differ significantly, the lower pH and higher DO in T_1 could be the trigger factors for the higher survival of larvae. The less pH recorded in the treatment T_1 than other two treatments. Though *M. pancalus* accept pH between 6 and 8, the neutral pH could be better for their survival. Most of the small indigenous species larvae showed better performance in neutral condition such as fire eel (6-7.5), zig-zag eel, tire track eel and spotted spiny eel (6-8) [16]. In addition, T_1 showed a bit higher dissolve oxygen than T_2 and T_3, which could be another factor for better survival.

In the present study, it was found that newly hatched larvae are not capable for effective swimming and feeding, and they were owing to attached to aquarium wall and the root of water hyacinth. Swimming was somewhat restricted owing to the mass of yolk materials. When yolk was fully absorbed, then hatchlings of *M. pancalus* showed free swimming on 5th days after hatching which was similar to *Muraenesox cinereus* (see; Umezawa *et al*. [12]. However, different time pattern noticed in other eel species such as *M. aculeatus* fry start free swimming 3 days after hatching [16] and *A. japonica* occasionally darting through water column from 6 days after hatching [13].

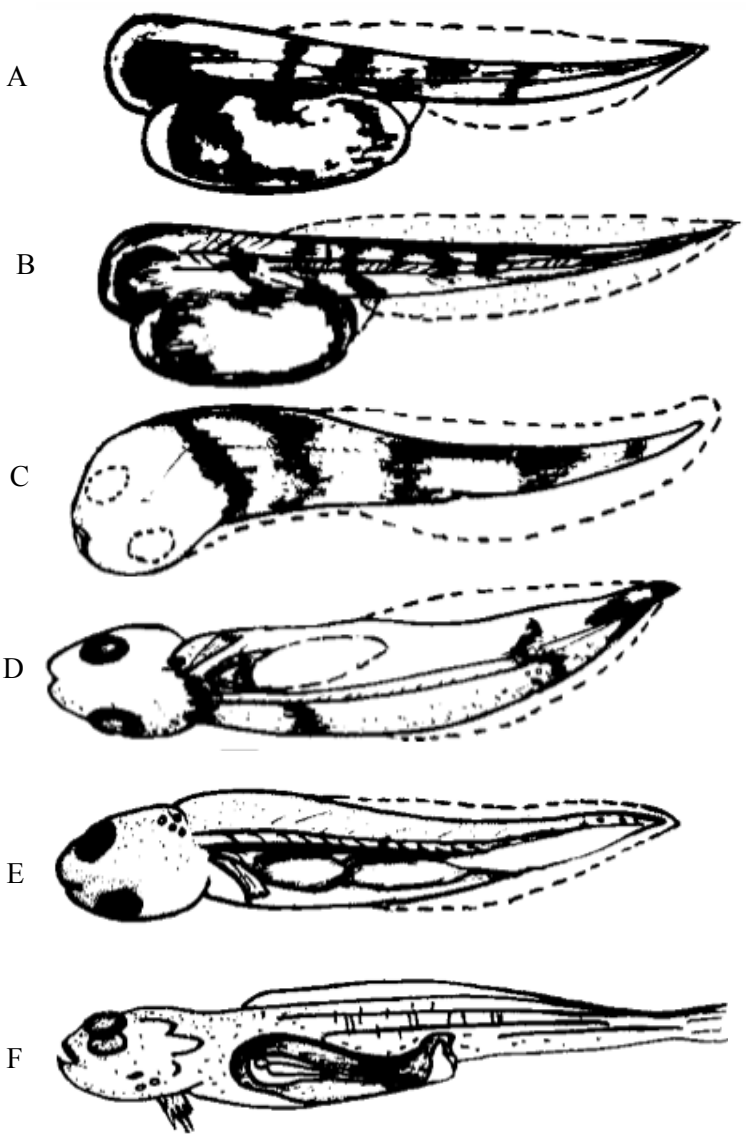
Larvae feeding is very crucial, once the yolk is absorbed, and then they fed zooplankton & boiled egg yolk [9]. In the present study, as yolk sac was absorbed by 90 hrs, so boiled egg was supplied on the 5th day evening. However, on the 12th day, tubifex was supplied to the larvae, but did not capable to ingest them and thus boiled egg yolk supplied until the end of the study. It is reported that fry of *Macrognathus pancalus* was started to feed with Artemia nauplii at 5-10 days old, moina species at 11-20 days old, blood worm at 21-35 days old,

tubifex species and blood worm (*Chilonomid* sp.) at 35-50 days old and small shrimp after 50 days [10].

Despite the relatively large fecundity and high hatching rate of *M. pancalus*, juvenile production was reduced by severe mortality during larval developmental stage. Mortality estimated as high as 90% larvae during the 16th day of rearing which turned 100% on the 17th day in all treatments. The similar survive rate reported for the larvae of Japanese eel which survived for 14 days [19, 20]. However, earlier mortality reported in other eel species like, larval of pike eel (*Muraenesox cinereus*) survived for 10 days [12], Japanese eel (*Anguilla japonica*) were survived in glass container only 3 days [15] and larvae of *Anguilla rostrata* were survived up to 6 days [13].

4.3 Development stages

The newly hatched larvae showed the typical feature characteristic of the eel [21, 22, 23, 24]. The basic larval development sequences were more or less similar to that known for other eel species*: Muraenesox cinereus* (see: Umezawa *et al.* [12], *Anguilla japonica* (see: Unuma *et al.* [15], *Angulla rostrata* (see: Oliveira and Hable [13].



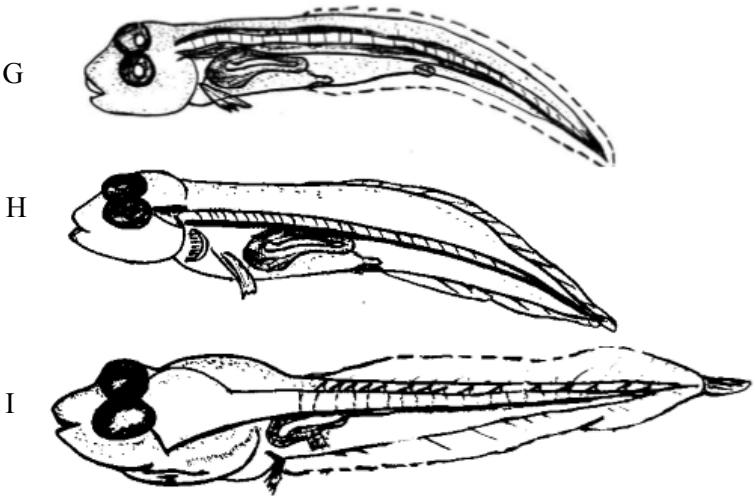


Fig 1: Larval development stages of *M. pancalus* in the captivity. A: 24 hours larvae; B: 48 hours larvae; C: 90 hours larvae; D: 140 hours larvae; E: 190 hours larvae; F: 230 hours larvae; G: 290 hours larvae; H: 340 hours larvae; I: 390 hours larvae.

Though the symmetrical morphological development noticed in many eel fishes, but different species showed in different time interval after hatch. The large ovoid yolk sac was attached to the body in just after hatching which is also common in other eel fishes like *Muraenesox cinereus* (see: Umezawa *et al*. [12], *Anguilla rostrata* (see: Oliveira and Hable [13]. The newly hatch larvae of pike eel showed eyes, anus, pectoral finfold (see: Umezawa *et al*. [12] which did not noticed in the present study. Rahman *et al.* [2] observed within 25-37 hours operculum, air bladder, pigmented eyes and mouth cleft. In the present study except airbladder other organs were developed after 80 hours of hatching. Rahman *et al.* [2] observed that at 36 hours of post hatching of *M. pancalus* pelvic fin fold were well developed, but in the present study showed different development pattern. Pelvic fins were not observed in *M. pancalus* and this findings also supported by Galib *et al.* [25] who reported that there were no pelvic fins of *M. pancalus.* In the present study, unpigmented eyes showed up to 110 hrs after hatching but Rahman *et al.* [2] found pigmented eyes within 51-60 hrs and in *M. cinereus,* this characteristics showed at 3 days old larvae (see: Umezawa *et al.* [12].

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. It is noticed that the yolk sac of larvae was completely disappeared within 90 hours after hatching which is similar to the larvae of *M. aculeatus*, in which yolk was fully absorbed in 96 hours after hatching [9]. However, earlier absorption reported by Rahman *et al*. [2] and they noticed complete absorption in *M. pancalus* within 72 hrs. The yolk sac fully depleted within 8 days after hatching in *Muraenesox cinereus* (see: Umezawa *et al.* [12], *Anguilla japonica* (see: Unuma *et al.* [15], *Angulla rostrata* (see: Oliveira and Hable [13] and started to exogenous feeding. Thus 80-90 hrs larvae of *M. panclus* can be called the "critical period" because the reserve of the yolk sac becomes exhausted and the fish is entirely dependent upon ingested food for nutrition.

Distinct caudal fin, anal fin and dorsal fin with soft fin rays was observed at 15 day's old larvae and spiky notochord was shown at 16 day's old larvae. Oil globules were completely disappeared. However, within 17 days of larvae culture the species not yet completed all the larval characteristics and not yet metamorphose. In similar study on *Anguilla japonica* (Japanese eel) hatched larvae survived for 14 days [19, 20]; *Muraenesox cinereus* (Pike eel) larvae survived for 10 days [12]; larvae of *Anguilla rostrata* (American eel) survived up to 6 days after hatching [13].

5. Conclusion

The present work generated some information on the early life history, developmental stages and commencement of fast feeding time for larval rearing. It is revealed that larval rearing of *M. pancalus* is sensitive to water quality. The study also establishes time distinctive stages of the larval development. It noticed that the larvae started to take an exogenous feed within 80-90 hours of hatching and most of the organs developed within 17 days but not yet completed larval phage.

6. Acknowledgements

This study is the part of the honor's thesis at Jessore University of Science and Technology, Bangladesh and partially funded by the University Grant Commission (UGC), Bangladesh.

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