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## Early embryonic and larval development of *Ompok pabo* with notes on its nursery rearing

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### ABSTRACT

Present study has been conducted to provide detailed information about the embryonic and larval development of *Ompok pabo*. The experiment was conducted in the breeding seasons from December, 2007 to November, 2010. Artificial breeding of *Ompok pabo* (Hamilton-Buchanan) were carried out at Goalpara College (Latitude 26° 10' 11" N and Longitude 90° 37' 37" E), Goalpara, Assam; India. Fertilized eggs were examined using a microscope till the end of larval developmental period to record each and every stage of embryonic and larval development.

**Key words:** embryonic development, *Ompok pabo*, breeding, larvae.

### INTRODUCTION

Embryonic development is a complex process in which cellular differentiation and proliferation occur simultaneously but at different rate [1]. Changes in the pattern of the entire structure of an organ or of specific organ in relation to the environment are decisive for evaluating the developmental pattern of a species [2]. *Ompok pabo* commonly known as 'pabo' is an Indian freshwater catfish with good market demands particularly in North-Eastern part of India. It is also a delicious, tasty, nutritious catfish having relatively few bones. However, over the last few decades, its wild population has undergone a steady decline mainly due to over exploitation, loss of habitat, disease, pollution, siltation, poisoning, dynamite and other destructive fishing [3]. The species has already been declared as an endangered species [4, 5]. In this context, captive breeding and the release of captive bred individuals into the wild are among the techniques used for conservation of rare and endangered fish species [6].

Early life history information is an essential requirement for optimization of mass seed production, culture and management [7], which is very much important to optimize larval growth and survival. This knowledge can then be used for the management and culture of the species in any environment. Embryonic development and larval development besides providing interesting information in itself are imperative and consequential to the successful rearing of larvae for large scale seed production [8]. According to [9], the development biology of *Ompok pabda* is an important aspect which may be considered as base line information for the researchers and fish culturists, who are engaged in the seed production of the species. Therefore, the present study has been conducted and it provides detailed information about the embryonic and larval development of *Ompok pabo*.

## MATERIALS AND METHODS

### Artificial breeding

The experiment was conducted in the breeding seasons from December, 2007 to November, 2010. Artificial breeding of *Ompok pabo* (Hamilton-Buchanan) were carried out at Goalpara College (Latitude 26° 10' 11" N and Longitude 90° 37' 37" E), Goalpara, Assam, India. Sexually matured fish weighing 22.19 gm to 25.20 gm were stocked in the earthen pond (area 120 m<sup>2</sup>). The fishes were fed with chopped earth worm, trash fish, live IMC fingerlings, formulated feed etc. Induced breeding operation was carried out following the methods of [10, 11 and 12]. Mature males and females (2:1) were selected based on the external morphological features [13]. Matured male fishes were identified by a pointed genital papilla, rough and serrated pectoral fin, freely oozing milt in slight pressure on the ventral side. In addition to that, maturity of the female was confirmed by relatively smooth pectoral fin, reddish genital papilla, soft and bulging abdomen etc. Maturity of female also can be determined by observing the demarcation line in two lobes of the ovary.

Brooders were collected from the stocking pond, segregated and stocked in two hapa (135 cm x 90 cm x 130 cm) fixed at the pond five hours earlier from injection. Both the male and female fish were artificially induced by intramuscular injection with 0.5 ml and 0.6 ml of ovatide per kg body weight respectively (**Fig. 1**). The injected females were stripped after 12 to 15 hours of injection by gently squeezing the abdomen to obtain eggs. After 11 to 12 hours of ovatide administration, the females were checked for their ovulatory response. The release of eggs through the genital pore performed by gentle pressure on the abdomen was considered as commencement of ovulation [14]. Eggs were collected in a clean plastic tray. The abdomens of induced males were dissected to remove the testes. Testes were then thoroughly macerated and sperm suspension was prepared with 0.9% saline solution. Sperm suspension was spread over the eggs and mixed gently with a feather for fertilization. After a while, they were washed thoroughly with clean water. Eggs were measured and transferred to flow through system which was pre arranged with four plastic tubs (40 liters in capacity) for incubation. After few minutes, the unfertilized eggs turned whitish; while the fertilized eggs remained transparent. The unfertilized eggs were removed carefully from the incubation tank. Fertilized eggs were examined using a microscope till the end of larval developmental period.

### Incubation of fertilized eggs:

Eggs were evenly spread on the surface of the tub. Water was sprinkled over the rearing tub by a small shower. To prevent the direct entry of plankton into the rearing system, a piece of plankton net was fixed at the tip of the of PVC pipe (inlet). During first five hours, water flow was maintained at about 0.5 l/minute to keep the eggs circulating around the incubation unit. The plastic tub was having an outlet guarded by a fine net. After five hours it was increased up to 1 l/minute to facilitate the removal of unfertilized eggs and to increase oxygen supply to the developing eggs. The unfertilized eggs were removed from each unit by a soft brush manually. At a regular interval, water was agitated to prevent the overlapping or clumping of eggs on the bottom.

Ten developing eggs were sampled at 5-10 minutes intervals until hatching and every 1 hour for the next period of larval development. After 22 to 24 hours, the hatched larvae were collected from the incubation tub and transferred to separate earthen tub of 40 liter capacity. Water quality of the incubation unit was estimated following the methods suggested by [15 and 16]. Egg diameter was calculated by taking average from the total length of ten randomly selected eggs. The observation of egg and larvae were carried out and photographs were taken subsequently.

## RESULTS AND DISCUSSION

### 1. Embryonic development:

The present observations on the morphology of fertilized eggs of *Ompok pabo* were more or less similar to those reported by [9 and 12]. The embryonic stage occurs inside the chorion and completes at hatching.

### Nature of egg:

Eggs were brownish in color and adhesive in nature (**Fig. 2**). Adhesive nature is similar to those of other catfish species such as *O. malabaricus* [17], *H. fossilis* [14] and *M. cavasius* [18]. The adhesive nature of eggs is an adaptation to prevent the flowing of eggs in the water currents and provide optimal oxygen supply [14]. Fertilized eggs were very transparent and had a reddish brown spot on one side which is easily recognizable with naked eye. But unfertilized eggs were creamy white in color and not transparent. The sizes of the fertilized eggs were 1.0 to 1.3 mm in diameter while the unfertilized eggs were 0.99-1.1 mm in diameter. The size of fertilized eggs was observed in *Heteropneustes fossilis* between 1.3 to 1.5 mm in diameter [14]. Variation in egg sizes might be related to the existence of different strains, conditions, and size of the female in wild condition [19]. Within 30 to 33 minutes after fertilization, all the eggs were swelled up uniformly with a small perivitelline space.



FIGURE 1. Hormone injection



FIGURE 2. Fertilized eggs



FIGURE 3. Blastodisc formation



FIGURE 4. Two cell stage



FIGURE 5. Four cell stage



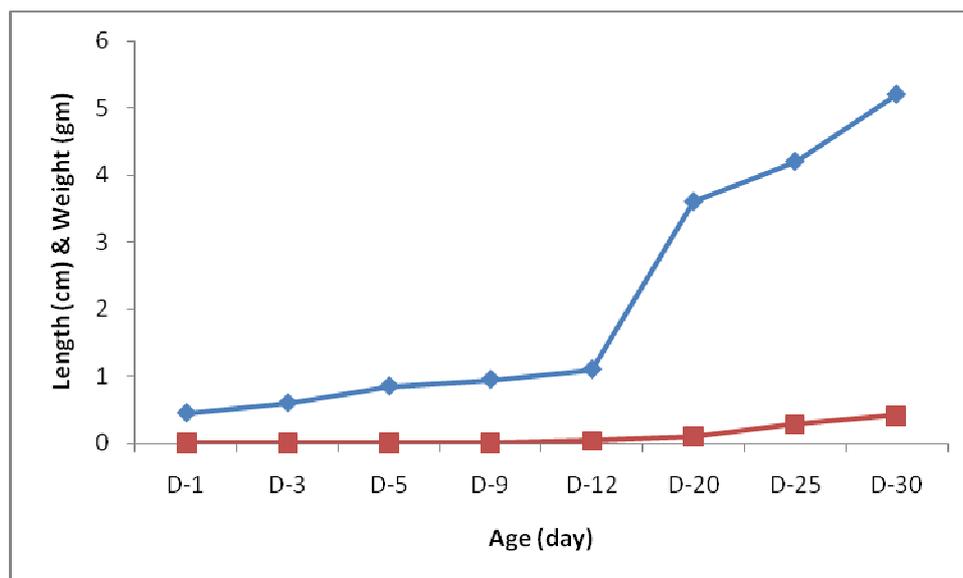
FIGURE 6. Sixty four cell stage

#### Formation of embryo:

The first cleavage took place about 36 minutes after fertilization with the formation of blastodisc as a crescentic light area over one end of the massive yolk (**Fig. 3**), which was divided into two blastomeres (**Fig. 4**). Chakrabarty *et al.* [9] observed first cleavage in about 30 minutes after fertilization followed by 16 celled stages in 70 minutes. After 10 to 12 minutes the second cleavage (four cell stage) took place (**Fig. 5**) and the third cleavage (eight cell stage) followed in another 12 minutes. The fourth cleavage (16 cell stage) was visible in next 8 minutes, followed by the fifth in another 4 to 5 minutes. In *Heteropneustes fossilis* after 90 minutes of fertilization, eggs reached the 16 celled stages [14]. However, the 64 cell stage was observed in 82 minutes (**Fig. 6**) after fertilization; while in *Ompok pabda* it was in 70 minutes after fertilization [9]. The morula stage was attained in 2:06 hours (**Fig. 7**). In the present observation, the blastoderm cells began involution over the yolk in 3:15 hours after fertilization. In *M. montanus*, the morula stage was visualized within 1.5 hour after fertilization [20]. Eggs were with a small perivitelline space. An advance stage (blastula) was observed in 3:30 hours; where one third of egg space was covered by the blastoderm cells. In *Ompok pabda*, the invasion of yolk by blastoderm cells began and one third of it

was covered in three hours [9]. Yolk plug stage was observed in progress in 4:20 hours (**Fig. 8**) and found distinct in 5 hours after fertilization. In the 6 hours, the yolk mass was completely covered by the blastoderm cells. At about 3.30 to 4 hours after fertilization, flattening of the cellular material occurred and the embryo attained the blastula stage [14]. A thick portion of the embryo was found at one side. This determined the cephalic region of the embryo. In *Mystus cavasius*, blastomeres started invading the yolk by spreading over the yolk in the form of a thin layer after 5 hours of fertilization [18].

**FIGURE 7. Morula stage****FIGURE 8. Yolk plug stage****FIGURE 9. Newly hatched larva****FIGURE 10. Haul of Pabo larvae****FIGURE 11. 20 days old larva****FIGURE 12. 25 days old larvae**

FIGURE 13. Growth of *Ompok pabo* larvae in nursery rearingTable 1. Embryonic development of *Ompok pabo*

Time after Spawning	Development stage	Key description
0 min	Fertilized egg	Eggs were adhesive, spherical, transparent and brownish in color. The diameter of the fertilized eggs varied from 1.0 mm-1.3 mm.
36 min	2 cell stage	First cleavage
46 to 48 min	4 cell stage	Second cleavage
60 min	8 cell stage	Third cleavage
1:08 hr	16 cell stage	Fourth cleavage
1:12-1:13 hr	32 cell stage	Fifth cleavage
1:22 hr	64 cell stage	Sixth cleavage
2:06 hr	Morula stage	Blastulation progresses to form a multicellular blastodisc.
3:30 hr	Blastula stage	A third of egg space covered by the blastoderm cells.
5:00 hr	Yolk plug stage	Yolk invasion complete and cephalic region gets thicker in size.
7:00-8:00 hr	Kidney shaped embryo	Elongated embryo with rudimentary notochord.
9:00-10:00 hr	Enlarged embryo	The cephalic and caudal end becomes almost differentiated.
11:00-12:00 hr	Kupffer's vesicle formed	An oval area is observed at the base of caudal region to form kupffer's vesicle.
13:0-14:00 hr	Optic vesicle developed	The tail rudiment gets separated and optic vesicle fully developed.
15:00-16:00 hr	Rapid twisting movement	Yolk mass differentiated in to yolk bulb. The caudal region at this stage found more active.
17:00-18:00 hr	Fully active embryo	The egg membrane becomes decomposed and lost its shape.
20:00-21:00 hr	Just before hatching	Embryo with prominent eye with rudiments of maxillary barbells.
22:00 hr	Hatchling	Hatching of embryos start.

Table 2. Physico-chemical parameters managed during experimental period

Sl. No.	Parameter	Incubation unit	Nursery rearing unit
1	Temperature ( $^{\circ}\text{C}$ )	$29.3 \pm 1.9$	$30.5 \pm 2.3$
2	pH	$7.9 \pm 0.8$	$8.1 \pm 1.2$
3	DO( $\text{mg l}^{-1}$ )	$10.28 \pm 2.4$	$9.76 \pm 2.1$
4	FCO <sub>2</sub> ( $\text{mg l}^{-1}$ )	$3.3 \pm 0.1$	$2.2 \pm 0.2$
5	Alkalinity( $\text{mg l}^{-1}$ )	$80 \pm 7$	$90 \pm 5$
6	Acidity( $\text{mg l}^{-1}$ )	$4.4 \pm 0.5$	$3.3 \pm 0.7$
7	Hardness( $\text{mg l}^{-1}$ )	$49 \pm 8.8$	$57 \pm 9.4$
8	Chloride( $\text{mg l}^{-1}$ )	$8.15 \pm 1.4$	$8.77 \pm 1.1$

**Differentiation of embryo:**

Observation made in 7:00-8:00 hours observed that the embryo became elongated. The rudimentary notochord was found to be laid in the embryo. Elongation of the embryo was completed and it was turned the shape of a kidney. Embryo was found enlarged in size and more elongated at 9:00 hours. In 9 to 10 hours, the cephalic and caudal ends were found differentiated in *Ompok pabo*, while in *M. cavasius* it was about 09:30 hours after fertilization [18]. In the 10:00 hours both the ends were found more distinct. The rudiments of optic vesicle were gradually formed. In the 11-12 hours embryo, an oval area was observed at the base of caudal region which will in later stage form

kupffer's vesicle. The cephalic end became prominent. Caudal end was almost free from the yolk mass. In the next 13-14 hour, the tail rudiments get separated and optic vesicle fully developed. Embryo was become more developed. Twisting movement of the developing embryo observed in 13 to 14 hours; however, in *Ompok pabda* it was observed in 14:00 hours [9]. The outer vitelline membrane was found gradually decomposed or dissolved at 15-16 hour. Twisting movement found rapid then earlier at this stage. According to [14], just 1-2 hour before hatching, the embryo of *H. fossilis* showed twisting movements inside the egg envelopes. Yolk mass differentiated in to yolk bulb. The caudal region at this stage was found more active. In 17-18 hours old embryo, the caudal region became very much elongated. The gut was faintly indicated at the posterior end of the yolk sac. The egg membrane became decomposed and lost its shape. The embryo started active movement within the egg membrane. The eye was found prominent in 19 hours stage. The cephalic region was well formed and became free from yolk mass. The rudiments of maxillary barbells were visible in 20:25 hours. In *Ompok pabda*, vigorous thrashing movements of the embryo were noticeable in 22 hours [9]. However, in the present investigation, frequent whipping motions of the embryo were observed in between 21 hours & 22 hours and finally the embryo hatched out in about 22 hours. The egg membrane gradually lost its shape. The temperature in the incubation unit at the time of hatching was recorded as 29.3°C. Mukherjee *et al.* [21] also reported similar hatching time in *Ompok pabo*. In *Clarias batrachus*, eggs normally hatch out within 26 hours after fertilization [22]. In *O. bimaculatus*, hatching occurred 24-25 hours after spawning and the hatchlings were light yellow in color [23]. Hatching period was varied from 17-21 hours in case of *M. cavasius* [18]. In *H. fossilis*, it was observed in 23-24 hours after fertilization [14]. Vijayakumar [17] reported hatching of embryo of *Ompok malabaricus* in 26-18 hours after spawning and the hatchlings were pale yellow in colour.

## 2. Larval development:

### Hatchling:

The newly hatched larva was slender, transparent, lack of mouth and pectoral fin (**Fig. 9**). Eye was distinct. Rudiments of maxillary and mandibular barbells were also noticed. The yolk sac was oval in shape and yellowish green in color. Body was observed without any pigmentation. The hatchlings were yellowish black in colour and measured 0.45 cm in length as well as 0.0075 gm in weight. The lengths of newly hatched hatchlings of *M. montanus* were  $3.0 \pm 0.1$  mm [20]. Observation made on the newly hatched larvae of *H. fossilis* recorded the length of  $2.5 \pm 0.2$  mm, which was transparent and faintly brown in color [14]. Mukherjee *et al.* [21] reported the length of 5-6 mm in *Ompok pabo*, while Rahman *et al.* [18] reported 2.59 to 2.62 mm in *M. cavasius*. Marimuthu and Haniffa [8] revealed that length variation in different species can be related to the size of eggs. According to Bagarinao and Chua [24], eggs diameters are positively correlated with larval length and weight at hatching. A small yolk sac was attached bellow the head region. The one day old larvae were very active and showed tail wagging movements. This activity might help in cutaneous respiration and also help to free the larvae from eggshell, which could be a potential site for bacterial growth and infection [25]. In *Ompok bimaculatus*, the newly hatched larvae swam very fast and rested on their lateral side due to their heavy yolk content [23]. It may mention that due to yolk content on the ventral side; larvae rested on their lateral side.

### Three days old larva:

Mouth was gradually developed and feeding commenced from three days old. The yolk sac was gradually disappeared from third day onwards. Chakrabarty *et al.* [13] observed absorption of yolk sac in *Ompok pabda* from three days. Pectoral fin was distinct at this stage. Tail and caudal fin formation were in progress. Both maxillary and mandibular barbells were formed. Mean length of the larvae were 0.55 to 0.65 cm.

### Five days old larvae:

Larva was observed well developed form at this stage. Mouth was fully developed and functional. Length was 0.85 cm in total length. The entire larvae were free swimming. Yolk substances were completely absorbed. The complete yolk sac absorption of *H. fossilis* was observed in third day when the larvae was measured an average length of  $5.0 \pm 0.2$  mm [14]. The yolk sac of *Mystus montanus* was fully absorbed only after 3<sup>rd</sup> day when the larvae reached a length of 5-5.5 mm [20]. The larva was free swimming and fully capable to capture food from 5<sup>th</sup> day onwards when the larvae measured 8.5 mm in length. This might be due to the development of caudal fin and pectoral fin. According to Brown [26], improvement in capture of prey could be due to the maturation of the visual system as well as improvement in locomotive ability.

### Ten days old larvae:

Larvae observed with prominent maxillary and mandibular barbells. They took the adult shape at this stage. Average total length (TL) measured was 0.95 to 0.98 cm. All the fins (caudal, pectoral, dorsal and anal fin) and both maxillary and mandibular barbells were found prominent in 10 days old larvae of *Ompok pabo*. Similar observations were also reported in *M. montanus* [20] and in *H. fossilis* [14].

**Twenty days old larvae:**

Head enlarged with developed and distinct eye observed (**Fig. 11**). Well developed eyes were observed in the 3 day old larvae of *H. fossilis* [14]. Improvement in capture of prey could be due to the maturation of the visual system as well as in the improvement in locomotive ability [26]. Body was having slight pigmentation of yellowish black in colour. Change of larval colour was also observed in *Mystus montanus* [20] in which larvae gradually changed to an orange color in the early post-larval stage. Similar color changes (purple red) were also noticed in *Channa striatus* larvae in the late post-larval stage [27]. Anal fin was well developed and caudal fin lobe distinct. Size of the larvae found as 3.6 cm in total length.

**Twenty five days old larvae:**

The length of 20 days old larvae measured an average of 4.2 cm. The larvae were showing active swimming and voracious feeding behaviors (**Fig. 12**). Larvae were morphologically similar to the adult in 25 days. In *M. montanus* voracious nature of feeding was observed in 20 days old larva [20]. Dorsal and caudal fin rays were prominent and without any pigmentation.

**Thirty days old larvae:**

Operculum was well formed with continuous up and down activity indicating functioning of gill for breathing. Arockiaraj *et al.* [20] reported fingerlings stage in this age in *M. montanus*. Dorsal region of the body found to be more pigment comparing to the other region. Mouth was fully functioning. In *Ompok malabaricus*, length attained at this stage was 5.2 cm (TL). After 30 days of hatching, juveniles were 22.2 mm in total length and resembled the adult with respect to all external characteristics indicating the end of the juvenile period [17]. At this stage larvae can be transferred to the nursery pond for further rearing.

**3. Nursery rearing**

Technology for breeding and seed production of *Ompok pabda* has already been developed by Chakrabarty *et al.* [13]. But there is no any standardize larval rearing as well as management technique for *Ompok pabo*. Mukherjee *et al.* [21] opined that the technique for induced breeding of Pabda (*Ompok pabo*) is comparable to the induced breeding of carp but special attention is needed in larval rearing, as large mortalities occur mostly after 24 hours from hatching.

The nursery rearing unit comprises an overhead tank, flow through system, water shower, rearing earthen tub etc. Double filtered pond water was also used for rearing. Water temperature, pH, DO, FCO<sub>2</sub>, alkalinity, acidity, hardness and chloride were maintained within the permissible limit as far as possible (**Table 2**). The foreign substance, dead larva (if any) and egg shells were siphoned out from the rearing tub. Feeding was not done for 72 hours after hatching as the larva contain a mass of yolk sac. In *Clarias batrachus*, the three-day old (4<sup>th</sup> day) larvae were fed with boiled hen egg yolk and zooplankton [22]. No food was given until the hatchling of *Clarias batrachus* reaches the spawn stage (yolk sac absorption) as mouth and other internal organs are fully developed only when the spawn stage is achieved [28]. This is in conformity of the present findings, where boiled chicken egg and filtered zooplankton were used as food. The yolk of an egg was grinded in tiny particles and given to the larva @ 50 – 60% of total body weight thrice daily (early morning, noon and evening time). From 9<sup>th</sup> day onwards hatchlings were fed mainly with chopped earthworms @ 8 – 10% of total body weight twice daily. Mixture of rice polish & mustard oil cake (1:1) was also used at the same rate. From 19<sup>th</sup> day, earthworms and dry fish powder was used and from 31<sup>st</sup> day, feeding was continued daily. The food items used were earthworms and raw small fishes such as *Rasbora* sp., *Puntius* sp., IMC fingerlings etc. Hossain *et al.* [29] observed that live feed like *Tubifex* are suitable for *C. batrachus* larvae. But in the present study live earth worm was used replacing *Tubifex* worm due to its high protein and lipid contents comparing to that of *tubifex* worm. The percentage composition of protein in earth worm is 67.68% [30]; while in live *Tubifex* worm it is 32.13% [31]. On the other hand, it can be easily cultured by vermicompost techniques. So, chopped earthworm was used as best food for *Ompok pabo* larvae in terms of growth and survival rate [3].

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