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Embryonic and Larval Development of Yellow Tail Catfish, *Pangasius pangasius*

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Abstract

The yellow tail catfish, *Pangasius pangasius* embryonic and larval development study was carried out. The eggs were adhesive and transparent in colour with equal perivitelline space. First cleavage appeared at 00:49 ± 00:02 h resulting two equal blastomers. The eight cells, thirty two cell and morula stage appeared at 01:30 ± 00:06, 02:04 ± 00:10 and 03:43 ± 00:33 h respectively. The blastomeres looked overlapped during these multi-cell stages and the size got reduced during morula stage onwards. The fertilized eggs took 09:29 ± 01:24 and 25:27 ± 01:28 h respectively for attaining "C" shape embryo and hatching. The transparent larvae were 3-4 mm in length with compact oval shape yolk sac of 1.4-1.6 mm in length at hatching. Heart beat was detectable (2-3 times per minute) in the newly hatched larvae, whereas the mouth, barbells or elementary canal were not visible. The mouth was clearly visible in one day old larvae, which remained opened and complete closing of mouth with jaw movement was noticed at the age of 11-12 dph (days post hatch). The fins were not seen during their early life due to the encircling of a uniform membrane from behind the dorsal side to the posterior part of yolk sac. This continuous membrane started disintegrating during 5-10 dph, within which the caudal, pelvic, pectoral and dorsal fin started appearing. The 11 dph larva possessed dorsal, pectoral; pelvic and caudal fin possessed 6-7, 6-7, 5-6 and 19-20 fin rays, respectively. The larvae were resembled just like adult fish at 12 dph.

Keywords: Catfish; Embryonic development; *Pangasius pangasius*; Fertilization; Larval development

Introduction

Members under Pangasidae family are in demand for aquaculture activities due to their growth potentiality. Their contribution to aquaculture production is exemplary in south-east Asian countries [1,2]. Many species are also under research to explore their growth potentiality with an aim to supply fish protein to the growing population. *Pangasius pangasius* is one such species, which is native to Indian sub-continent and is found in major river system [3]. The species is gradually declined in the natural water bodies due to over exploitation to satisfy the market demand as well as degradation of its native ground due to anthropogenic causes, which pushed it to an endangered species [4]. The information on the biology of this species has been compiled [5]. It is also considered as an excellent species among other Pangasids for mollusc control in the aquaculture ponds. In spite of its potentiality as a candidate species, enough attempts have not been made for its captive production. Recently few literatures have come up on its induced breeding as a sign of beginning for the domestication [6-8]. Our Institution is also in command to develop its package of practice through several projects. It is vital that potential endemic species be researched with the information on the embryonic and larval development. 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. The information on the embryonic and larval development is lacking in this catfish species. Considering the importance of *P. pangasius*, attempts were undertaken to study the detailed embryonic and larval history in the controlled condition.

Materials and Methods

Breeding protocol

The juveniles collected from river were maintained in the earthen pond till they attain maturity. The broods were selected for breeding operation during the month of July. The suitable females were selected on the basis of bulging abdomen and pinkish vent. Many occasion intra-ovarian oocytes were examined through catheter to see the uniformity of the oocyte size. Simultaneously the suitability of males was judged by seeing free oozing of milt. Ovaprim (sGnRHa + Domperidone) at the dosage level of 1.0 ml/kg body weight was injected to females for successful ovulation, whereas the males did not receive any hormone injection. The male and female were released separately in ferro-cement tanks (1.5 m diameter) with continuous aeration and showering. The tanks were well covered to avoid fish jumping and an outlet was provided for drainage of excess water. The females were found ready for stripping after 13-14 h of post injection. The eggs stripped from the females were mixed with the stripped milt of male for fertilization.

Observation of embryonic development

Few fertilized egg samples were collected in petridish to see the embryonic development under a dissecting microscope. The eggs containing uniform and round yolk sphere and smooth perivitelline space were considered for embryonic study. One of such egg was separated from each breeding attempt to study the embryonic development. The egg was kept in petridish and was put under water tap for continuous water exchange with an aim to provide oxygen to the developing embryo. Complete aeration was provided to all the tubs. Water temperature, pH, dissolved oxygen, nitrite-N and alkalinity were analyzed following standard methods [9]. Water quality parameters

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recorded during the egg development were as follows: dissolved oxygen between 5.8-6.7 mg L^{-1}, temperature 27.5 to 28.5°C, pH 6.9-7.8, nitrite 0.002-0.004 mg L^{-1} and alkalinity 122-130 mg L^{-1}. The egg is checked at regular interval to record the timing of embryonic development for each stage. The developmental stage of the egg was also captured under microscope with photographic attachment (CKS41, Olympus). A total of two observations were made from the eggs collected from two breeding operations in different times in the season to record the maximum variability of developmental timing. Compound microscope fitted with ocular micrometer was used to record the size of fertilized egg.

Observation of larval development

The total length and yolk sac biometry of newly hatched larvae was recorded using ocular micrometer fitted to a compound microscope and the weight was taken with the help of electronic balance (XS 105, Mettler Toledo). The morphological changes of larvae in each day were recorded through compound microscope.

Statistical analysis

All the data related to timing of embryonic development were expressed in hour: minute (mean ± SD). The mean and standard deviation values of timing of embryonic development were calculated by using Microsoft Excel 2013.

Results

Embryonic development and Egg development

The events in embryonic development and their respective time of observation in *P. pangasius* are presented in Table 1. The ovulated or fertilized eggs were round, adhesive in nature and looked transparent. The diameter of eggs just after stripping and after fertilization was 1.09-1.28 and 1.2-1.45 mm, respectively. The egg membrane was fully separated by a thin space from the egg called perivitelline space (0.07-0.09 mm), which was equal all around and filled with yolk free clear fluid (Figure 1a).

Cleavage stages (Single cell to Sixty four cell stage)

The fertilized eggs of *P. pangasius* showed meroblastic cleavage. An outgrowth appeared at the animal pole with the accumulation of yolk free cytoplasm called as blastodisc or germinal disc stage at 00:21 ± 00:01 h (Figure 1b). With the pass of time, the blastodisc became thick and a vertical line appeared over the blastodisc. The line moved down dividing the blastodisc into two blastomeres, representing two cell stages at 00:49 ± 00:02 h (Figure 1c). Further division of blastomere took place with the advancement of time to reach four (Figure 1d) and eight-cell (Figure 1e) stage at 01:07 ± 00:04 and 01:30 ± 00:06 h respectively. Sixteen (Figure 1f), thirty two (Figure 1g) and sixty four (Figure 1h) cell stage appeared at 01:51 ± 00:08, 02:04 ± 00:10 and 02:32 ± 00:20 h respectively. The blastomeres at these stages were unequal and reduced in size. The blastomeres were also overlapped and their boundaries were faintly visible.

Morula stage

This stage of development appeared at 03:43 ± 00:33 h. The blastomeres at this stage were divided into many cells. The small blastomeres resulted from repeated division of blastomers lost their boundaries and appeared compact. It looked just like a marigold flower at the animal pole (Figure 1i).

Blastula and gastrula stage

Blastula stage appeared at 05:12 ± 01:08 h. The flowery look of blastomeres at morula stage was compressed at this stage and the blastoderm occupied larger area. The blastomeres were completely lost their identity and moved in both the side of animal pole occupying 30-40% area over the yolk sphere (Figure 1j). Gastrula stage appeared at 07:27 ± 01:16 h (Figure 1k). The blastoderm over the vitelline sphere further spreaded in both the side compared to blastula stage, which covered about 60-70% area. The blastomeric cells over the yolk sphere were further compressed, giving an appearance of a thread line called as germ ring. One end of the germ ring observed to be little broad compared to the other end, indicating the future development of cephalic region. The "C" shape embryonic stage appeared at 09:29 ± 01:24 h (Figure 1l), where head and tail end was recognized. After another 6-7 h from this stage the differentiation of cephalic region, optic vesicle, dorsal fin fold and tail region were well marked. At this stage embryo appeared just like a miniature of fish larvae encircled over the yolk sphere.

Twitching and hatching

Twitching movement was observed at 21:30 ± 01:10 h of post fertilization. The embryo was fully formed with the clear differentiation of head and tail. The head portion was attached to yolk sac, whereas tail

Figure No.	Developmental stage	Time of appearance (h)	Characters
1a	Fertilization	00:00	Round, transparent and adhesive in nature
1b	Blastodisc	00:21 ± 00:01	An out growth over the vitelline sphere developed at animal pole
1c	Two cell	00:49 ± 00:02	First cleavage, where two cells over the yolk sphere was clearly visible.
1d	Four cell	01:07 ± 00:04	Second cleavage, where blastomeres were clearly visible and counted.
1e	Eight cell	01:30 ± 00:06	Third cleavage, where little overlapping of blastomeres was observed.
1f	Sixteen cell	01:51 ± 00:08	Fourth cleavage, where overlapping of blastomeres was observed and were placed in two rows.
1g	Thirty two cell	02:04 ± 00:10	Fifth cleavage where the blastomeres were visible in 2-3 layers.
1h	Sixty four cell	02:32 ± 00:20	Sixth cleavage, where overlapping of blastomeres was observed and were placed in 2-3 layers.
1i	Morula	03:43 ± 00:33	Blastodermal cells were very small due to repeated division and gave a flowery look at the animal pole.
1j	Blastula	05:12 ± 01:08	Difficult to recognize the blastomers. The blastoderm was compressed and occupied more than half area over the yolk sphere.
1k	Gastrula	07:27 ± 01:16	Thick layer of blastoderm or germinal ring occupied 3/4th area over the yolk sphere. One end of it was observed broad, which became the future cephalic part of the embryo.
1l	"C" shape embryo	09:29 ± 01:24	Embryo looked kidney shape over the yolk sphere with clear distinction of head and tail.
	Twitching	21:30 ± 01:10	Tail became free from yolk sphere and beat in quick succession to rupture the egg membrane.
1m	Hatching	25:27 ± 01:28	The larvae were transparent with straight body and having free swimming capability.

Time shown in the table for each developmental stage is the average value of two observations (Mean ± SD). Time is expressed as h:min ± h:min

Table 1: Different events of embryonic development of fertilized egg and their respective Time in *P. pangasius*.

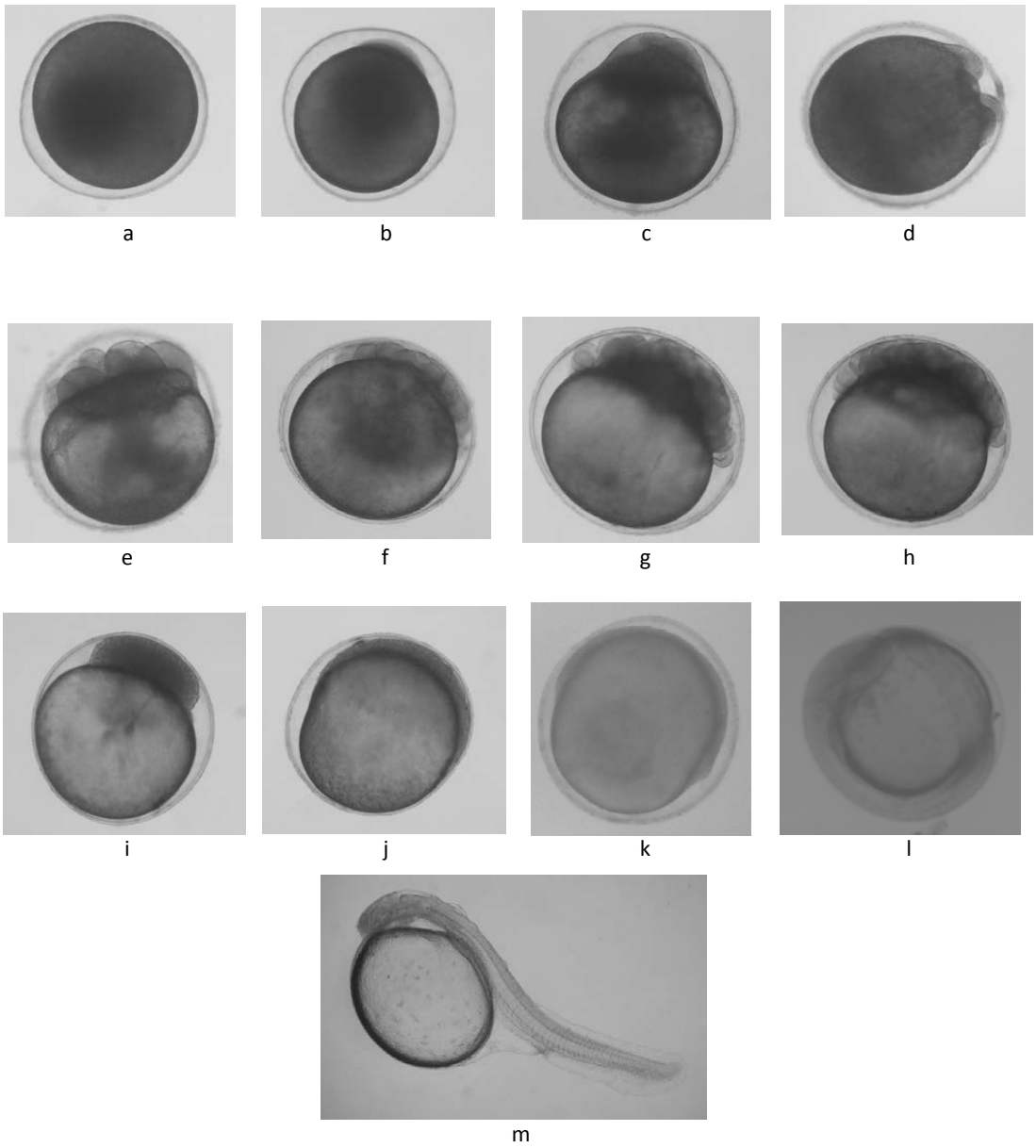

Figure 1: Embryonic development of *Pangasius pangasius* (a) fertilized egg (b) blastodisc (c) two cell (d) four cell (e) eight cell (f) sixteen cell (g) thirty two cell (h) sixty four cell (i) morula (j) blastula (k) gastrula (l) "C" shape embryo (m) hatchling.

became free. The beating of tail by the embryo just prior to hatching was very fast and continued for 7-10 second with a pause of 3-7.5 second. The larvae started hatching at 25:27 ± 01:28 h. The larvae remained dormant for few second just after hatching as if the embryo rested on the yolksac after which the activeness of larvae increased with the increase of tail movement. The tiny larvae were straight with the clear differentiation of head, trunk and tail (Figure 1m).

Larval development

The detail changes of morphometry in *P. pangasius* from the day of hatching to twelfth day of life are presented in Table 2. The larvae looked transparent with a straight body. The larvae were 3.0-4.5 mm in length bearing a round and compact yellowish yolksac, which were 1.4-1.6 mm in length and 1.2-1.4 mm in height. The mouth was not visible just after hatching, but the mouth cleft was clearly visible after 12-13 h of hatching. Gradual reduction of yolksac was observed with the increase of age, which got completely absorbed at the end of third day. The alimentary canal also was visible at this age and the larvae accepted live mixed zooplankton as feed during their rearing in hatchery condition. The heart beat was observed to be 2-3 times per second in the newly hatched larvae. The mouth remained opened in one day hatchling and the gap between the jaws was gradually reduced as the larvae became older. Complete closing of both the jaws was observed during 9-11 days of life. Development of pigments were observed on the body surface in this larvae, which was first appeared on head surface followed by over the belly as well as just below the dorsal side and finally at the base of pectoral spine. These black pigments gradually increase in size and merged, giving a deep grey coloration to the head surface and dorsal

Age of larvae	Length (mm)/ Weight (mg)	Description
0 dph	3.0-4.5/0.8-1.1 (Yolksac Length: 1.4-1.6 mm; Yolksac height: 1.2-1.4 mm)	Larvae look silvery. The eyes are very small and appear like black dot on the anterior part of head. Head is attached to yolksac, hence looks little bent at the anterior portion. Yolksac is round and compact. Mouth or mouth cleft is not distinguishable. Small cleft is visible at the posterior part of yolksac, indicating future anus. A continuous membrane encircled from just behind the yolksac and continues in ventral portion to dorsal side encircling tail region.
1 dph	3.5-4.8/0.9-1.2 (Yolksac Length: 1.2-1.5 mm; Yolksac height: 1.0-1.2 mm)	Mouth remains opened and lower jaw shows occasional vibration. Vent is clear. No change of continuous membrane from ventral to dorsal side is marked as also seen in the 0 day old larvae. Barbells are not appeared clearly, but a rudimentary bulging or elevation is marked below the lower jaw. Heart looks transparent, but beats are clear, which were 2-3 times per second.
2 dph	4-5/1.2-1.6 (Yolksac Length: 0.9-1.1 mm; Yolksac height: 0.6-1.0 mm)	Mouth opening increases. Small bristle like structure is visible in lower and upper jaw indicating its teeth. Sometimes lower jaw closes for a while. Barbells of lower jaw extend up to yolksac. Tail fin is broad like a sark tail and appearance of rays noticed. But the continuous membrane over the body persists.
3 dph	6-7/2.4-3.0 (Yolksac Length: 0.4-0.6 mm; Yolksac height: 0.2-0.3 mm)	The yolksac does not show any round shape. The yolk content in the yolksac reduced drastically. The continuous membrane on the dorsal side reduced in size and showed dissolving sign with a gap in the dorsal side of peduncle. Tail looks single without any lobe as found in two days larvae. Fin rays are visible but not prominent. Barbells of upper jaw start developing. The visibility of heart beat is reduced. Dorsal, pectoral and pelvic fins are not originated.
4 dph	7-8/2.7-3.2	Lower jaw is movable but still mouth remains opened. Upper jaw barbell is clearly visible. Degeneration of continuous membrane on the dorsal side noticed and an elevation of fin is observed on the dorsal side nearer to neck indicating the initiation of dorsal fin formation. But the membrane at the ventral portion remains continuous as before. No yolk material is seen and elementary canal is visible. Heart beat is not seen due to increase of thickness of body. Bifurcation of caudal fin is initiated and fin rays appeared.
5 dph	7.5-8.5/3.5-4.2	Lower jaw barbells are longer than the upper jaw barbells. Dorsal side looks uniform without any membrane. Elevation of fin still persists as observed on the dorsal side nearer to the neck. The continuous membrane beyond anus to caudal region persists but a depression is marked just before the tail region on the ventral side of caudal peduncle. Faint rays in ventral fin appeared. Bifurcation of caudal fin is clear with 17 rays. Few pigments appear on the upper surface of head.
6 dph	8-9/4.6-5.1	Eye is prominent, which is 0.2 mm in diameter. The pigments on the head are increased in number. The barbells are 2.8-4.7 mm in length. Caudal fin deeply forked with 17 rays, where the upper lobe is longer than the lower one. Rays appear in ventral fin, which are 15 numbers approximately.
7 dph	12-13/12.8-13.4	Snout looks round, but mouth remains opened. Elementary canal is coiled and extends up to vent. Pigments on the head surface expand in size. Pigments in less numbers also appear just below the dorsal side and over the belly. Ventral fin clearly demarcated with 17-19 rays. A membrane is visible in both side of operculum indicating future pectoral fin. Adipose tissue clearly visible.
8 dph	10-15/16-21	The visibility of elementary canal in the belly drastically reduced. The pigments on the above portion of belly increased in number. Dorsal fin grows bigger with faint rays, which are not countable. Pectoral fin is clear with 3-4 fin rays. Pelvic fin is not at all clear but two bulged membranous structures are found just before the anus.
9 dph	14-18/22-28	Complete closing of mouth is not found, but lower jaw movement is frequent. The teeth in lower and upper jaw are clearly visible. The pigments on the head fused in many places. Dorsal fin is clear with 5-6 fin rays. The ventral and tail fin possesses 25-27 and 17-18 rays respectively.
10 dph	15-19/26-32	Black patch on the head surface and above the belly is observed due to merge of pigments. Few dots are also seen below the base of pectoral fin. Heart beats 50-53 times per minute. Pelvic fin is clear with 3-4 rays. Pectoral fin well developed with 4-5 fin rays.The ventral fin well developed with 23-25 fin rays. Tail fin also possesses 18-20 rays.
11 dph	18-22/26-44	The ventral portion of larvae look silvery and the dorsal portion look deep grey. The dorsal, pectoral, pelvic, ventral and caudal fin possesses 6-7(1+5-6), 6-7, 5-6, 24-25 and 19-20 fin rays.
12 dph	21-28/37-46	The color of the larvae is same as seen at the age of eleven days. Mouth is completely closed with lower jaw movement. Few pigments still persist at the base of pectoral spine. Different morphological parts like fins, adipose tissue, barbells etc are prominent. The dorsal, pectoral, pelvic, ventral and caudal fin possesses 6-7(1+5-6), 6-7 (1+5-6), 6-7, 25-26 and 19-20 fin rays, respectively. It resembles with that of an adult *P. pangasius*. The larvae at this stage swim actively and accept plankton and compound feed during their rearing in the hatchery.

Table 2: Brief description and summary of morphometrical changes of *P. pangasius* during its larval development.

side of fish at the age of eleven days. A continuous membrane was found in the newly hatched larvae from its dorsal side to the posterior part of yolksac. This membrane lost its originality by self-dissolution with the pass of time and different fins were developed synchronously. The well identified caudal fin developed first followed by ventral fin, pectoral fin, dorsal fin and pelvic fin. These fins respectively possessed 19-20, 25-26, 6-7(1+5-6), 6-7(1+5-6) and 6-7 fin rays at the age of twelve days old larvae. By this time the larvae resembled an adult fish of *P. pangasius*.

Discussion

Embryonic development

The ovulated eggs (1.09-1.28 mm) of *P. pangasius* increased to 1.2-1.45 mm in size after incubation of fertilized eggs in hatchery, which might be due to hydration of the eggs. Swelling of egg from 1.0-1.3 to 1.3-1.6 mm in *Rita rita* [10] and 1.1-1.2 to 1.3-1.5 mm in *H. fossilis* [11] has also been documented. The fertilized eggs were strongly adhesive and found in clutch among the eggs during egg incubation in the hatchery. Many teleost under siluriformes show adhesive nature of the eggs [11-14]. This nature of eggs in *P. pangasius* could be due to sticky jelly like covering with radiating ridges on the egg surface as also reported in *Pseudobagrus ichikawani* [15]. The egg membrane got separated giving birth to the uniform perivitelline space. The yolk sphere pushed towards the vegetal pole as the embryonic development proceeded. This could be due to providing more space for the divisional activities of blastomeres at the animal pole. The clarity of blastomeres as in 2-4 cell stage was gradually reduced as the cleavage proceeded for 64 cell stage onwards. The identity of blastomeres was completely lost at morula and blastula stage. This loss of boundaries between the blastomeres might be due to repeated division and overlapping of cell resulting small and compact blastomeres at the animal pole as well as increased cellularity. This gradual change is a usual happening in the embryonic development of fertilized eggs in teleosts [12,16,17]. The sixty four cell stage and morula stage in the present study appeared at 2.53 h and 3.44 h respectively. In *P. sutchi* the 64 cell stage appeared earlier (1.35 h), whereas the morula stage appeared (2.1- 4 h) within

the similar time range as seen in *P. pangasius*. The time variation or similarity at the same stage of development between these two close species is acceptable as also observed in the developmental stages between *O. bimaculatus* and *O. pabo* [14,18]. Even though the gastrula stage appeared at 7.27 h, further events in the germinal ring continued for another 8-9 h resulting clear visibility of cephalic region, optic vesicle, dorsal fin fold and tail region. This stage appeared at 19 h in *P. pardalis* [19] at 9-10 h in *O. pabo* [14] and at 11 h in *P. sutchi* [20]. Beating of tail by the embryo (twitching) was approximately 50-60 times per minute at 22-23 h post fertilization. Hatching occurred at 24-26 h (25:27 ± 01:28 h) in *P. pangasius*, which is greatly varying with other siluriformes: 18 h in *P. corruscans* [21] 22 h in *R. rita* [10] and 26 h in *C. batrachus* [22]. These variations are mostly related to species variability and due to water temperature. Islam [20] reported the inverse relation of hatching in Thai pangas with temperature fluctuation. The eggs took another 2-3 h more for complete hatching in the present study.

Larval development

The newly hatched transparent larvae have straight body posture and anterior part of head looks bent due to attachment to the yolksac. This type of morphometry is also observed in newly hatched catfish larvae of *P. sutchi* and *Mystus cavasius* [12,20,22]. The larvae were reported to be 3.0-4.5 mm length compared to 4-5 mm in *O. pabo* and 7.8 ± 0.12 mm in *P. pardalis*. This variation is related to the egg size between the species. The egg size reported for *O. pabo* and *P. pardalis* were 1.0-1.3 and 2-3 mm, respectively [14,19,23] were also agreed with the positive correlation of egg size with the hatchling. The round and compact yolksac got reduced as the hatchling grows in age and complete absorption took place at the end of third day of life when the larvae were nourished with external feed. In agreement to our observation, Islam [20] in *P. sutchi* and Jumawan et al. [19] in sucker mouth catfish reported gradual reduction of yolksac till 4-7 days after which larvae were ready for external feed. The development of mouth or barbell was not found in the just hatched out larvae. The mouth was clear at first day of hatching with an opening between the two jaws. The ambiguity of mouth formation was also reported in *Hemibagrus nemurus* just after hatching [24] which also varies 8-10 h in *C. striatus* [13] and 3-4 h in *Heterobranchus longifilis* [25]. The alimentary canal was also visible at day four post-hatch, just after the day of yolk absorption. This indicated the exogenous feeding to the larvae during their rearing. This period varies in the fish species like 48 h for *H. longifilis* [25] and 72 h for *Clarias batrachus* [26]. The pigments are also seen on the body of hatchlings in the present study like few other catfish species [11,19]. The pigments merged during latter part of life (11-12 days) to give deep grey colour to the dorsal part of the body. The change of body color to orange in *M. montanus* [27] and purple red in *C. striatus* [28] has also been documented. The continuous membrane present just behind the posterior part of yolk sac to the dorsal side started disintegrating during the age of 5-10 days of life, within which the caudal fin, ventral fin, pectoral fin and dorsal fin started appearing. Similar continuous membrane over the body is also found in *P. sutchi* and *H. fossilis* [11,20]. But the complete appearance of fins in *H. fossilis* is within 2-6 days post hatch, which is much less compared to *P. pangasius*. The fry of 11-12 days resembles like an adult fish.

Conclusion

The present study summarized the embryonic and larval development of *P. pangasius*. These findings can also contribute for a better understanding of the embryonic development in other Pangasid catfishes. The observations on its larval development may provide a basis for further studies on its ontogeny. The information on mouth development, day at first feeding and free swimming behaviour may provide knowledge to develop key management during its hatchery production.

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