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Research Paper

OBSERVATION OF EMBRYOGENESIS OF STRINGING CATFISH (*Heteropneustes fossilis*) IN INTENSIVE CONDITION ALONG WITH DNA POLYMORPHISM

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Abstract

In this study induced breeding, embryonic chronology, larval development and DNA polymorphism of stinging catfish *Heteropneustes fossilis* were studied. Sexually matured male and female (2:1) was injected intramuscularly with pituitary gland (PG) hormone at the dose of 10 mg/kg and 70 mg/kg body weight of fish, respectively. All the brood fish were found to be ovulated after a period of 7-9 h of injection where natural fertilization occurred. The adhesiveness of the egg membrane became more apparent and had a reddish spot (blastodisc) on one pole. The incubation period was from 23-24 h at an average temperature of 29±1°C. The yolk absorption was completed within three days after hatching. The aerial breathing behavior of the larvae was observed 10th day after hatching. The larvae resembled the adult in its external features and were metamorphosed to young juveniles within 20th day post-hatching. Larval development was observed until 60 days and differentiation of larvae was checked through morphometric analysis. DNA polymorphism was assessed between the wild and experimental population of *H. fossilis* by RAPD assay considering three different primers (OPF-14, OPB-05 and OPB-19) and in total 84 DNA bands were found whereas 42 bands were recorded for each population. All markers were shown 100% polymorphism in both populations, but wild population shows higher polymorphic loci then the experimental population.

Key words: Induced breeding, embryonic chronology, larval development, DNA polymorphism, *Heteropneustes fossilis*.

INTRODUCTION

The stinging catfish (*Heteropneustes fossilis*) is very popular fresh water fish and high priced among other air-breathing catfish. It is considered as highly palatable, nutritional, tasty, fewer spines, less fat and well recommended as a diet for convalescing

patients [1]. It is hardy and amenable to a high stocking rate and utilizes atmospheric oxygen for respiration and thus makes them ideal fish species for aquaculture [2, 3, 4]. This fish is abundantly available in all types of open water systems of Bangladesh. But it may be threatened by over exploitation, ecological changes and subsequently habitat loss, and considered as least concern at present [5].

Aquaculture of this species will be helpful not only in increasing the overall production but also in the conservation of this important fish species. Very recently, the culture of *H. fossilis* has become popular, but fry produced in natural water is not enough to fulfill the demand for its culture. This fish breeds naturally during the rainy season in flooded rivers, inundated paddy fields and earthen ponds. The seed collection of this species from the wild is unreliable, time consuming and uneconomic for large-scale culture of this fish. To overcome these problems, induced spawning is thought to be the only alternative method for seed production and supply. Further, the rearing of the wild collected brood stock in captive conditions may not receive appropriate environmental cues for gonad maturation and spawning and it can cause reproductive development to be arrested in late vitellogenesis stage [6].

The understanding of the embryogenesis is a basic knowledge that is required to achieve success in improving the artificial production of any species and also improve their growth rate. A good knowledge of the embryogenesis will help to improve their culture practice, understand the species adaptations and improve artificial breeding techniques. Apart from these, understanding the biology of any species and the functional trends and environmental preferences of the different developmental stages was elucidated by using the knowledge of ontogeny [7, 8]. Embryonic development in fishes is dependent on many factors in addition to temperature, pH and **dissolved oxygen** [9]. The embryonic stages are considered to be very sensitive indicators of environmental disturbances [10]. Information on early embryonic, larvae develop and organogeny are of critical important in understanding the basis of biology of a particular species and their dietary needs as well as environmental preferences [7, 8]. Studies on embryonic and early larval development are imperative and consequential to the successful rearing of larvae for large scale **seed production** and aquaculture [11, 12].

Habitat degradation caused by natural and human interventions, injudicious application of pesticides in agricultural fields and release of industrial effluents have recently become great constraints for fish biodiversity in most aquatic ecosystems in Bangladesh. As a result, there is a declining trend in the availability of *H. fossilis* along with many others in natural water bodies of the country. Thus, it is a great concern whether the reduction in population size has had any impact on genetic variability of this species as there is an inverse relationship between the genetic variability and adaptability to environmental changes. Molecular markers are realistic and useful tools for the investigation and monitoring of genetic conditions both in natural populations and in captive stocks for conservation and stock improvement. RAPD fingerprinting technique is simple, fast, sensitive and allows the examination of genomic variation without prior knowledge of DNA sequences [13, 14], and therefore, RAPD assay was considered for observing DNA polymorphism of this fish while genetic variability is also termed as raw materials for selective breeding program. Therefore, the embryonic chronology, survival and larval development of *H. fossilis* were carried out in intensive

condition for improving its artificial propagation in controlled environments. In addition, DNA polymorphism of experimental larvae was compared with the wild population for observing genetic status of this fish.

MATERIALS AND METHODS

Experimental set up

This experiment was conducted in USDA Laboratory and Animal Biotechnology Laboratory of the Department Genetic Engineering and Biotechnology (GEB) at Shahjalal University of Science and Technology (SUST), Sylhet. The tank and aquarium set up, water supply facilities, working space etc. were assured before the breeding program. In this experiment, fiber glass aquarium was used for both spawning and rearing of fish. Two large size fiber glass aquariums (3×1.5×1.5ft) and including 8 small size glass aquariums (1.5×1.0×1.0ft) of the laboratory were used. The aquariums were supported with a filter, stones, continuous air-pump etc.

Important water quality parameters such as water temperature, pH and dissolved oxygen (DO) of experimental water were determined at weekly interval between 09.00 to 10.00 hrs. Temperature, dissolve oxygen and pH were measured using a Celsius Thermometer, a portable digital DO meter (MI 605, MARTINI) and pH meter (Hanna pH 300).

Brood fish collection and management

Brood fishes were collected from different market nearby the farm and reared in the large size fiber glass aquariums for optimization. Collected fish were identified by the study of morphological characteristics [15, 16]. The brood fishes were fed on farm made artificial balanced diet containing 30% protein. The brooders were reared for 4-5 days before onset of breeding practice with feeding at two times a day at the rate of 5-6% of the body weight. Water temperature was measured by thermometer and denoted as °C.

Induced breeding

Five pairs of brood fish were collected from the rearing fiber glass aquariums using a cast net in the morning between 8:00-9:00 am on the day of the breeding trials and immediately transferred to a circular tank in the Laboratory. Only conspicuous, healthy and uninjured fishes were selected for induced breeding. The male and female fish were determined by eye estimation based on the different criteria. The males and females were kept in separate tanks and continuous water flow was maintained to ensure sufficient aeration. However, no feeding was provided during the conditioning period. A continuous air flow was provided in the tanks by use of aerators. The sex ratio of the spawners was kept at 2:1 for male and female. The induced breeding was conducted by using commercially available pituitary gland (PG) at different doses and combinations. Three separate treatments were done with different combination of hormone (PG-PG) to male and female fishes with a single dose of injection. The hormone was administered intra-muscularly near dorsal fin and above the lateral line with the 1 ml syringe. For each treatment, 6 male and 3 female were used in a group. The male and female fishes were injected at 09.30 pm, 11:30 pm, and 11:45 pm before the day of breeding trail at the dose of PG 70 mg/kg, PG 10 mg/kg body weight of fish, respectively.

Spawning, fertilization and incubation

After injecting the hormone, both males and females were kept in the same spawning tank and fishes were allowed to release eggs and milt, fertilize naturally in the tank. All the brooders were found to be ovulated after a period of 7-9 h of injection. The brooders were then transferred from the holding tanks after the completion of ovulation. Eggs

were kept under shower of water by piercing a slender pipe. Thereafter, a continuous flow of water was maintained for aeration to ensure the environmental conditions were optimal for the hatching process.

Embryonic and larval development

Five developing eggs were sampled at 10 to 30 min intervals until hatching and every 4 h for the next three days and then only once a day until metamorphosis. In the present study, the developmental stages were divided into embryonic, larval and post larval development. The embryonic stage occurs inside the chorion and ends in hatching. The larval stage is characterized by nutritive contribution of the yolk sac and the stage ends when the larva becomes capable of exogenous feeding. The post larval stage begins immediately upon absorption of the yolk sac and was characterized by autonomous feeding. After the yolk sac absorption the larvae were fed with libitum twice in a day with zooplanktons where pond water was used. Developmental time from post fertilization was rounded to the nearest minute until the morula stage and then to the nearest hour. The age of the larvae was denoted as hour after activation. Measurements of egg diameter and standard length were made using an ocular micrometer. The total lengths (TL) of 20 randomly selected individual larvae were measured at each sampling time. The observation of eggs and larvae were carried out and photographs were subsequently taken using a binocular microscope (Labomoyed) till the end of the larval period.

Study of DNA polymorphism

In this experiment, 5 wild fish which was collected from a local market and 5 experimental fish were considered for genetic analysis. Liver tissue was isolated from each individual and washed them by using distilled water and 70% alcohol and preserved separately in 100% alcohol at -20^{0}C until DNA extraction. Genomic DNA was extracted through a short procedure [17] and the quality of DNA was checked by agarose electrophoresis on 1.2% agarose gel comparing with 1kb+ DNA ladder. The gel was run at 70 V for 40 minutes dying with ethedium bromide solution. Finally, photographs were taken by digital camera using gel documentation system. PCR amplification was performed with three arbitrary primers for detecting polymorphism of this experimental fish. The selected three primers such as OPF 14 (5'-TGCTGCAGGT-3'), OPB 05 (5'-TGCGCCCTTC-3') and OPB 19 (5'-ACCCCCGAAG-3') were adopted. PCR reactions were performed in a 15µl reaction mixture for each sample with 8µl of master mix (Promega Hot Start), 1µl of primer, 2µl of template DNA and 4µl deionized water. PCR reaction was conducted for pre heating 94˚c 3 minutes, denaturation at 94ºc for 1 minute; annealing temperature of selected three primers for this PCR was about 27-30ºc for 1 minute and 2 minutes for elongation or extension at 72˚c. A final step of 7 min for 72ºc was added to allow complete extension of the amplified fragments. The PCR was run for 35 cycles and the amplified PCR products from each sample were checked for banding pattern of DNA by the same as previous one while the molecular weight marker ranges from 75 bp to 20,000 bp (Generuler™).

RESULTS

Breeding behavior of *H. fossilis*

Injected fish was released into the breeding tank and breeding behavior was observed continuously. After 4 hours of injection the activities and movement of male fish was increased. The male started to move around the female and chase her. It started to nudge with its snout at the ventral region of the female fish. This activity was going for a long period. The activities of female were also increased. It started to move and stay at

middle of the water column. Suddenly it was seen that male fish quickly came to the female and the male nudge with its snout at the ventral region of the female. The female makes its body as "U" shaped and holds the head of the male inside its "U' shaped structure and on the bending condition the male brought the female at the surface of the water. Pressure was created on the ventral region of the female fish by the male fish with its snout. In this time the female released eggs at the surface of the water column and simultaneously the male ejaculated sperm. Then the eggs slowly fall down to the bottom of the tank. These activities were observed several times until the total eggs and milt was ejaculated in case of normal spawning allowed.

Embryonic development

(i) Fertilized egg

The diameter of the unfertilized eggs of *H. fossilis* was 1.1-1.2 mm. The stripped eggs were found transparent, spherical in shape, adhesive, brownish green in color and devoid of oil globules. Soon after insemination the ova began to swell leading to an increase in egg diameter. The size of fertilized eggs ranged between 1.3 to 1.5 mm. At one minute post-fertilization, the adhesiveness of the egg membrane became more apparent and the eggs adhered to the substratum. Fertilized eggs had a reddish spot (blastodisc) on one pole and readily recognizable with the naked eye (Plate 1a-c).

(ii) Formation of embryo

The first cleavage that divides the blastodisc into two blastomeres occurred within 15-20 min post-fertilization (Plate 1d). The second cleavage (4 cell stage) appeared 40 min after fertilization (Plate 1e) and the 8-cell stage was noticed within 75 min post-fertilization (Plate 1f). The 16 cell (Plate 1g) and 32 cell stages (Plate 1h) were observed at 90 and 120 min post-fertilization, respectively. After 64 cell stage of cleavage (Plate i), the blastomeres were decreased in size and the morula stage was reached between 2-2.30 h after fertilization (Plate j). At this stage the crown of the blastoderm starts spreading over the yolk in the form of a thin layer. At about 3.30-4h of fertilization, flattening of the cellular material occurred and the embryo attained the blastula stage (Plate k). After half an hour, the spread of blastoderm was evident and at 5h post fertilization, it was flattened on the top resulting in the formation of the germinal ring. The embryonic shield appeared within the next 2 h and by that time more than half a portion of the yolk was invaded and the head and tail ends of the embryo became clearly distinguishable. Gastrulation was in progress at approximately 6.30h after fertilization and the blastopore was evident (Plate l). In another 30 min, the yolk invasion was completed and the blastopore was seen almost closed.

(iii) Differentiation of embryo

Observation made at 8-10h post fertilization, revealed that the antero-posterior axis was distinguishable. The anterior protuberance formed a head fold and the posterior part elongated to form the tail fold. The maximum size of the coiled embryo at this time was 0.76 mm (Plate 1m). About 6-8 somites formed after 12h and the optic cups were clearly distinguished (Plate n). In about 13-15h from fertilization, more than three fourths of the egg peripherical space was occupied by the embryo and the number of mesodermal somites gradually increased from 8 to 12 and pigmentation was noticed in the somites (Plate o). At 17h the embryo, occupied the whole space inside the egg, the mesodermal somites ranged from 12- 15 in numbers (Plate p). Motility in the embryo was observed with 18-20 contractions per minute. In the 20h old embryo, 18-20 somites were observed (Plate q). The embryonic fin fold on the ventral side extended up to the 11th somite. In the 22h old embryos, the somites number increased to 22-25 and

the yolk was completed encircled by the embryo and the tail end was free from the first 2 somites and the embryo begins to twist itself, continuously beating against the inside of the eggshell by the caudal region, especially around the middle part of the body. Hatching took place about 23-24 h after fertilization (Plate r).

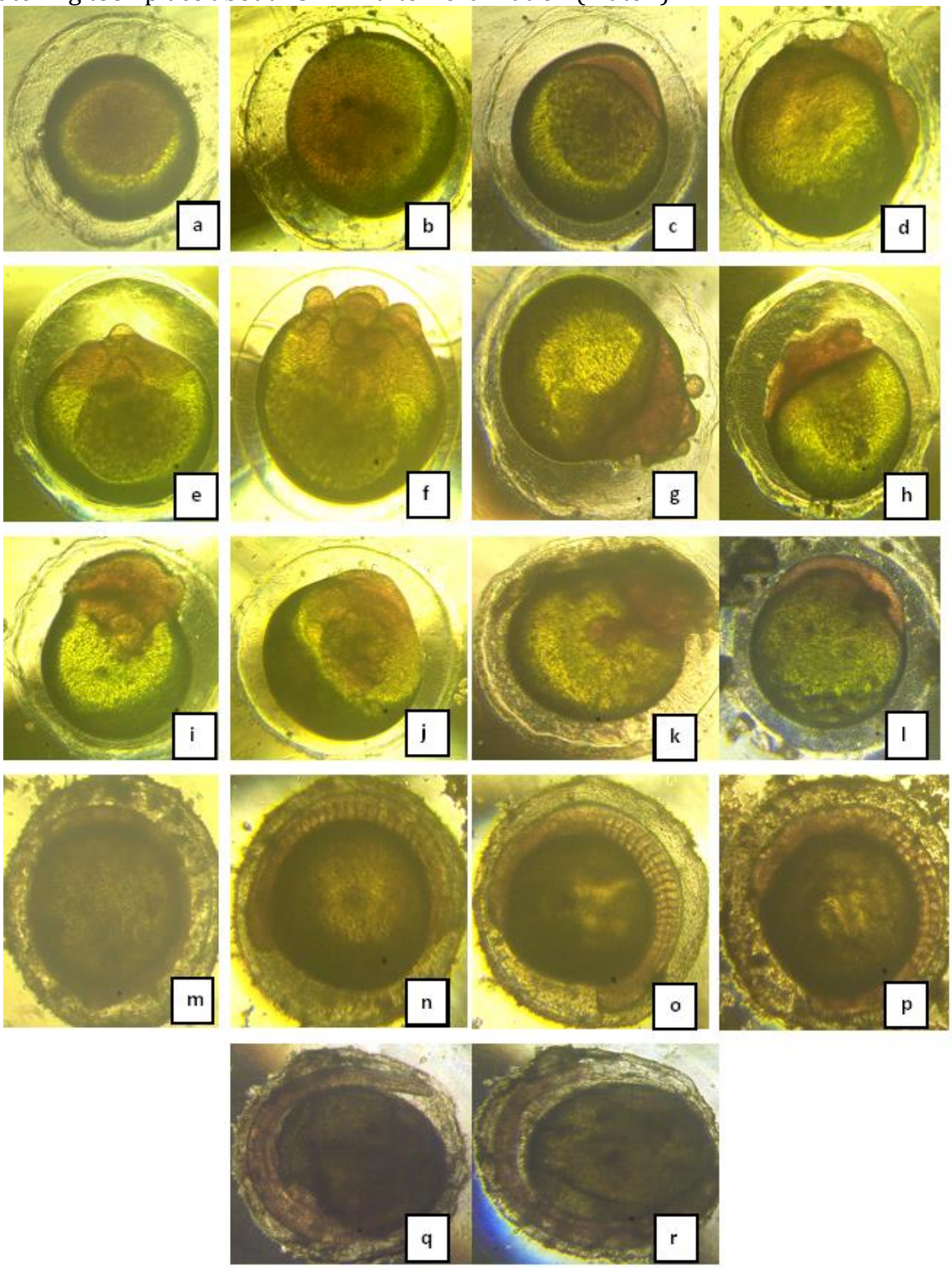

Plate 1: Stages of embryonic development of *H. fossilis*. (a) egg (b) zygote (c) fertilized egg (d) 2-cells stage (e) 4-cells stage (f) 8- cells-stage (g) 16-cells stage (h) 32-cells stage (i) 64-cell stage (j) Morula (k) blastula (l) gastrula (m) Embryonic shield (n) 12h old embryo (o) 15h old embryo (p) 17h old embryo (q) 20h old embryo (r) just before hatching

Larval and post larval development

(i) Hatchling

The newly hatched larvae were transparent and faintly brown in color and 2.7±0.2 mm in length with a laterally compressed body. The hatchlings had unpigmented eyes and were devoid of distinct mouths and fins. The yolk sac was oval in shape and pale greenish in color. A functional heart with blood circulation was observed. The larvae inhabited the bottom of the tank and swam with rapid movements using their tails.

(ii) 4-h old larva

The average length of the 4 h old larvae was found about 3.2±0.1 mm and brownish in color and the mouth has yet to develop. The heart becomes more distinct and circulation of body fluid was seen around the notochord in addition to the brain and yolk. The larvae were converged in a cluster and a few of them started swimming.

(iii) 8-h old larva

The average length of 8 h old larva was observed about 3.6±0.1 mm where the bulged yolk became gradually elongated at this stage. The larva displayed a dorsoventral unpaired fin. Internal body organs, like the heart and brain were clearly distinct. At this stage, the majorities of the larvae became active and moved to the water surface and were very sensitive to light.

(iv) 12-h old larva

The average length of 12 h old larva was seen about 3.7±0.1 mm. Heart activity was found clearly visible and blood circulation and pigmentation were increased.

(v) 16-h old larva

The average length of 16 h old larva was found about 3.9±0.1 mm. Dark pigmented and prominent eye spot appeared on the anterior part of the head. The mouth was not yet opened at this stage and the anal opening was still closed.

(vi) 24-h old larva

The average length of the 24 h old larva was measured at 4.0±0.2 mm with a reduced yolk sac. Dark pigmented and prominent eye spot appeared on the anterior part of the head and at this stage 32 myotomes were seen. The upper jaw and lower jaws were formed, the pectoral fin buds were seen as a small protuberance, and the alimentary tract was distinct. The heart was seen in front of the yolk and the blood circulatory system was fully functional.

(vii) 36-h old larva

The 36 h old larva attained an average length of 4.3±0.3 mm. The eyes were dark pigmented and spherical in shape. The pectoral fin was observed oval shaped with a membranous flap and actively used for free movement. The heart was distinctly visible, located behind the head and showed regular beats. The mouth was formed as a terminal opening and the vent just opened.

(viii) 48-h old larva

In this stage, the larva was seen 4.6±0.2 mm in length with a post anal length of 2.4 mm. The mouth cleft was well formed with a well developed lower jaw. The barbells became elongated and prominent around the mouth and the yolk reserve has further diminished in size. The pectoral fin has become paddle shaped with an undulating dorsal margin. The anal aperture and opercula were observed well formed and distinct. Blood circulation was observed in the opercula, heart and tail region. The alimentary canal became short, straight and distinct as well as the larva started feeding exogenously and a pouch like stomach was formed.

(ix) 3-d old larva

The larva was reached an average length of 5.0±0.2 mm with a post anal length of 2.7 mm and the body weight was found about 2.9±0.2 mg. The body was brownish in color and the mouth and anus were fully functional. The head was prominent and the eyes were further differentiated and pigmentation was appeared around the head and snout. The reserved yolk material was seen completely absorbed. The caudal fin was found with 5 rudimentary rays and the pectoral fins were observed vascularized. The larvae were exhibited vigorous movements to the water surface and occasionally sink to the bottom.

(x) 6-d old larva

The average length of 6 d old larva was measured at 5.8±0.15 mm in length and the body weight was observed about 3.8±0.2 mg. The entire body was brownish black in color with the pectoral and caudal fin rays clearly noticeable with eight rays observed in the caudal fin. Eyeballs were found large and distinct with groups of larvae assembling at the bottom of the aquaria.

(xi) 10-d old post larva

The average length of 10 days old larva was 8.90±1.0 mm with a post anal length of 4.2 mm and the body weight about 8.28±0.2 mg. Dorsal and anal fins were clearly demarcated and were almost separated from the caudal fin. The larvae showed frequent surfacing movements and the phenomenon of aerial breathing was observed and active swimming and foraging behavior of the larvae was noticed.

(xii) 15-d old post larva

The average length of larva was observed 10.1±1.2 mm with a post anal length of 6.4 mm and the body weight about 12.82±0.5 mg. The larvae were shown active swimming and voracious feeding behavior. A total of 8 caudal fin rays was distinguished, whereas, the ventral fin was clearly differentiated and in each pectoral fin, a stout spine was become distinguished. The body of the larvae was become opaque due to the accumulation of pigments.

(xiii) 20-d old post larva

The length of 20 d old larva was measured an average of 15.5±1.4 mm with a post anal length of 11.2 mm and the body weight about 17.38±0.5 mg. Pigmentation was appeared all over the body of the juvenile and at this stage, organogenesis was completed and the juveniles were morphologically similar to the adult except for their color patterns.

(xiv) 28-d old fry

The mean length was observed 22.00 ± 1.5 mm and the body weight about 25.74±1 mg. Fish changed their body color from light to dark reddish brown. The osseous plates appeared at this stage on the cephalic region.

(xv) 45-d old fish

The recorded mean length and weight were recorded 32.00 ± 2.00 mm and 30.92 ± 1.5 mg respectively. At this stage fish was observed elongated and provided with all morphological characters like an adult. The well formed osseous plates were recorded on the moderately sized dorso-ventrally flattened head. Two depressions were found on the head. The mouth was terminal, transverse and wide.

(xvi) 60-d old fry

The recorded mean length and weight were measured 45.00 ± 2.00 mm and 56.82 ± 3 mg respectively. At this stage fish was elongated and provided with all morphological characters like an adult. Occasionally came to surface water for engulfing air, frequent movement of fish was observed at night.

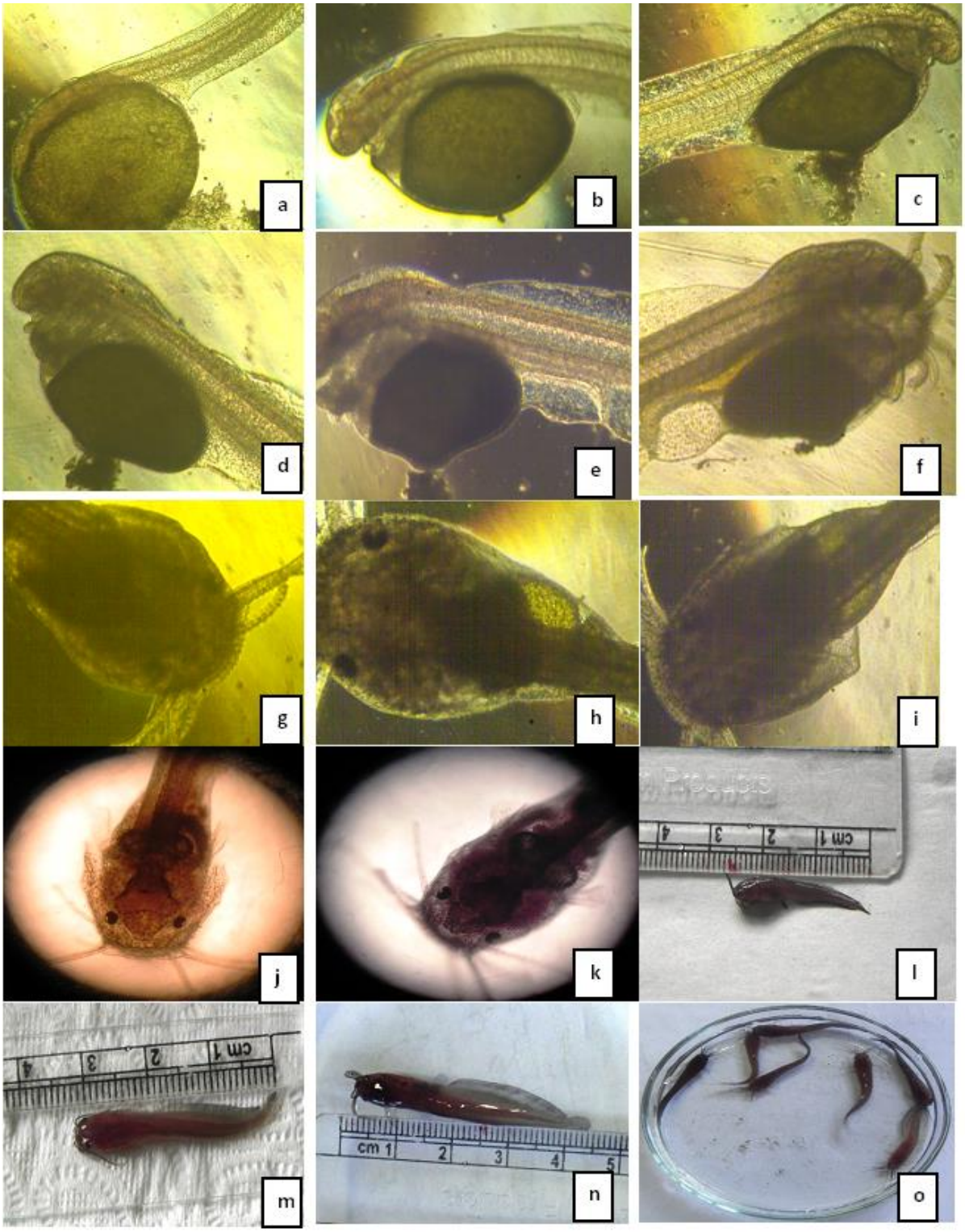

Plate 2: Phase of larval development of *H. fossilis*. (a) Newly hatched embryo (b) 4h old larva, (c) 12h old larva (d)16 h old larva (e) 24h old larva (f) 48h old larva (g) 3d old larva (h) 4d old larva (i) 6d old larva (j) 10d old larva (k) 15d old larva (l) 28d old larva (m) 45d old larva (m & n) 60 d old larva

Water quality parameters

To observe any appreciable change a large number of samples were analyzed for water quality parameters that might have occurred on response to case-pond aquaculture. The water temperature of experimental ponds varied from 20.10 to 32.20°C (27.7±0.734) during the study period. Dissolved oxygen was recorded at 7.30 to 8.30 am. The range of dissolved oxygen was recorded from 4.40 to 8.10 mg/l. The mean values of dissolved oxygen concentration were 6.86±0.140. The pH values of different treatments were found to be slightly alkaline ranging from 7.20 to 7.90 (7.66±0.034).

Analysis of DNA polymorphism

In this study, three RAPD markers were used to assess DNA polymorphism and each amplified banding profile was defined by the presence or absence of bands at particular positions on the gel where the scored as 1 if bands present or 0 if bands absent, separately for each individual and each primer. A total of 84 bands was detected among two populations of *H. fossilis* individual genotypes considering 5 individuals from each population (Table 1). In the experimental individuals highest number of bands (22) amplified by the primer OPF-14 and the lowest number of bands (5) amplified by the primer OPB-19. The highest number of bands (4.4) per sample was amplified from the primer OPF-14 and the lowest number of bands (1) per sample was amplified by the primer OPB-19. The primer OPB-05 amplified 3 numbers of bands per individual sample. For the wild population maximum bands (18) amplified by OPF-14 and lowest (10) by OPB-19. Maximum bands per sample (3.6) were amplified by OPF-14 and minimum (2) by OPB-19. In both populations an average number of DNA bands ware same but in case of polymorphic loci in wild population was found higher value. However, in all markers were shown 100% polymorphism in both populations.

Table 1: RAPD based analysis for ten individuals *H. fossilis*

Primer	Range of DNA Bands (bp) in T	Range of DNA Bands (bp) in W	Total No. of DNA Bands (T)	Total No. of DNA Bands (W)	Polymorphic loci (T)	Polymorphic loci (W)	Polymorphic loci (%)	Polymorphic loci (%)
OPF 14	71-423	71-455	22	18	13	12	100%	100%
OPB 05	71-423	71-404	15	14	11	8	100%	100%
OPB 19	280-571	348-1675	5	10	3	8	100%	100%
		Total	42	42	27	28		
		Average	14	14	9	9.33		

T= Tank population, W= Wild population

DISCUSSIONS

The Indian catfish or stinging catfish (*H. fossilis*) is a highly demanded freshwater fish species and has an excellent culture potential. Artificial propagation was successfully carried out in *H. fossilis* and the photographs of the embryological chronology were taken revealing the stages in the development of the organs and systems in the body. The latency period of eight hours reported in this work was quite short compared with the 18 to 24 hours and 21 to 24 hrs reported for the same species [3, 18]. These variations could be due to differences in the ripeness of eggs used or methods of

activation that were however not reported by the other authors. The shortness in the incubation period (22 hrs 8 mins) is an advantage for quick and mass artificial production of this species. The egg membrane was recorded fully separated from the egg and has a small perivitelline space which was filled with fluids and this fluid cushion may protect the eggs and the embryo from any external injury [19]. The fertilized egg diameter of *H. fossilis* ranged from 1.3 to 1.5 mm, which is more or less similar to the ranged between 1.4-1.6 mm [20]. The fertilized eggs of *H. fossilis* became adhesive, which is similar to those of other catfish species such as *C. batrachus, Mystus montanus* Jerdon and *Pangasiussutchi* [21, 22, 23]. In the present study the first cleavage occurred within 15-20 min and the 16-cells stage was reached in 90 min of post-fertilization whereas it was observed that the first cleavage, 16 cell and morula stages in *H. fossilis* were attained within 30, 70-80 and 100 minutes respectively [20]. In the present observation, the gastrula stage was reached in 7h after fertilization. Just 1-2h before hatching, the embryo of *H. fossilis* showed twisting movements inside the egg envelops where the similar hatching behavior was reported in the same fish by other study [20].
In this study, the incubation period lasted for 23-24h at a water temperature of 29ºC while it was observed 16-18h in *H. fossilis* in a previous study at a temperature of 26ºC [24]. The mouth was found to open at 36 h after hatching. Hatchling of *H. fossilis* commenced feeding at 48 h, whereas the first feeding in *H. longifilis* larvae commenced at 48 h after hatching [25]. The complete yolk sac absorption of *H. fossilis* was observed on the third day when the larvae measured an average length of 5.0±0.2 mm. Complete disappearance of the yolk materials was observed on the third day in *Clarias lazera* [26]. The utilization of atmospheric air and the aerial breathing habit was observed between 9th and 10th day after hatching. Similar reports were recorded in *C. striatus* [27]. Well developed eyes were observed in the 3 days old larvae of *H. fossilis*. The early development of the optic vesicles was an indication that the eye were functional before the hatchlings started active swimming and this will help to detect food well in advance of mouth formation, development of stomodium, and start of exogenous feeding. The early formations of the heart and rudimentary blood circulation at the embryonic shield stage were significant. This implies its functional significance as the network was laid before the embryo increased in size to allow for proper nutrient circulation in the embryo. Though the heart beat rate was low initially, the rate continued to increase as the embryo was maturing while similar observation has been reported in *Clarias gariepinus* [28]. The short embryonic or incubation period and fast sense organ development and air breathing habits starts at the 9-10th day after hatching in *H. fossilis* which suggest that it is a suitable species for small scale and commercial aquaculture. Fish farmers are less familiar with the culture of the catfish species because of the lack of breeding and feeding techniques and also non-availability of seeds from the wild [29]. Due to simple in operation, the RAPD fingerprinting technique has attracted widespread interests among the researchers. However, genetic characterization with RAPD markers has been constrained by the lack of complete genotypic information due to its dominant nature [30]. The RAPD technique has been found to be suitable for assessing DNA polymorphism between two populations of *H. fossilis* in the present study.
Analysis of three primers detected different levels of genetic diversity in each of the studied populations. A polymorphic locus contains more than one allele. The percentage of polymorphic loci was analyzed in the two different populations of *H. fossilis* in four different beel and one hatchery population of *H. fossilis* [31] whereas lower percentage polymorphic loci was recorded 68.5 and 74.7% respectively in cultured *Clarias gariepinus* species [32, 33], which did not corroborate with the current report of 100%

for the cultured and wild *H. fossilis* population. However, the present findings agree with the observation of Agbebi *et al*. [13]. This difference in polymorphic loci might be multifaceted which could include variant types of RAPD markers used. A total of 84 bands was produced in the two populations of which 100% were polymorphic while very low level of polymorphism (18.75%) were recorded at RAPD loci in two Indian populations of *H. fossilis* [14].

CONCLUSION

This study generated some information on the early life history of *H. fossilis* compared with previous finding. The result of the present study can be used as baseline information regarding the DNA polymorphism and population structure for the environmental adaptation and breeding program.

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