Abstract

Present study was conducted on induced breeding of commercially important Clarias batrachus due to the non-availability of its quality seeds from the natural resources for environmental degradation, shrinkage of natural breeding ground and illegal killing of juveniles and brood fishes. The aim of the study was to achieve success in fertilization and hatching using various doses of different inducing agents at different temperatures and latency periods followed by stripping method. In this study the developmental stages of fish (fertilized egg to 45th day old fish) were characterized chronologically. A trial was also made to optimize the survival rate of young developing fish upto 45th day by manipulating their feeding schedule and environmental conditions. The breeding experiments were done with pituitary gland extracts (40 and 120 mg/kg body weight for female and 25 and 50 mg/kg body weight for male) and Ovaprim (0.8 and 2.0 ml/kg body weight for female and 0.4 and 1.0 ml/kg body weight for male) at 26°C, 28°C and 30°C. The highest rates of fertilization (80%) and hatching (71%) of eggs were recorded in Clarias batrachus injected with carp pituitary gland extracts @ 50 mg/kg body weight of male and 120 mg/kg body weight of female at 28°C with a latency period of 15 hours. The fertilization and hatching rates were 77% and 65% respectively at 28°C at the higher doses of Ovaprim. The highest survival rate (82.5%) of developing fish was achieved supplying zooplankton as live feed upto 12th day followed by alternate supply of zooplankton, boiled egg with vitamin C and chopped tubifex from 13th to 45th day of rearing in indoor polyvinyl chloride tray with minimum fluctuation in temperature and dissolved oxygen.

Keywords: Pituitary gland extracts; Ovaprim; Clarias batrachus; Breeding; Fertilization; Hatching; Development

Introduction

The Asian catfish, Clarias batrachus (Linnaeus, 1758) commonly known as “magur” has a fairly common distribution in fresh and brackish waters of the plains throughout India. It has high commercial importance in India, Bangladesh, Thailand, Philippines, Myanmar and China due to its good taste, high protein (15.0%) and iron content (710 mg/100 gm tissue) with markedly low value of fat (1.0%) as well as therapeutic application [1-4]. The Central Board of fisheries, India suggested that emphasis should be given on catfish farming specially magur farming which has been identified as one of the potential national priority in Indian aquaculture [5]. In the natural environment, it spawns once a year [6]. The Spawning period is July-August [5,6]. The major constraint in the culture of magur is the non-availability of quality seeds from the natural resources due to environmental degradation for rapid industrialization and injudicious application of pesticides, shrinkage of natural breeding ground due to siltation, over exploitation and illegal killing of juveniles and brood fishes.

Therefore, it becomes necessary to take up breeding and larval rearing of magur in controlled conditions to meet the needs of small and medium sized entrepreneurs and local farmers. Induced spawning techniques for C. batrachus have been successfully used for seed production by few workers using various natural and synthetic agents like piscine pituitary gland extracts, Human Chorionic Gonadotropin (HCG), Ovaprim, Ovatide etc. [1,2,7-11]. Although induced breeding of C. batrachus is not a difficult task but main problem lies within the fertilization, hatching success and early development of the fish. Large scale mortality occurs in the early developing stage after absorption of yolk sac [12]. The reports on the breeding performance of C. batrachus by various stimulants using different doses are scanty [2,8-10,13,14]. The information on the effects of temperatures and the latency period on the breeding efficiency of C. batrachus are also very rare [10,11].

For expansion of catfish culture, knowledge of early development of young fish is imperative [15]. But there are sparse literature reports on the chronological early development of C. batrachus [16,17]. Moreover, high level survival of developing C. batrachus is another challenge for the hatchery operators and fish farmers as no standardized protocol of feeding to developing fish has so far been evolved.

In the present study, a trial was made for controlled breeding of Asian catfish Clarias batrachus at different temperatures using various doses of pituitary gland extracts and Ovaprim followed by stripping method to get the higher success rate in respect of fertilization and hatching. The latency period was also manipulated to get better performance in breeding in the present investigation. In addition, the developing young fish were also reared upto 45th day in indoor and outdoor conditions using live and artificial feed for optimizing their survivability.

Materials and Methods

Test organism

In the present study, disease free healthy gravid male (average length...
26 ± 1.5 cm and average weight 160 ± 5 gm) and female (average length 25 ± 2.7 cm and average weight 220 ± 7 gm) *Clarias batrachus* (Order: Siluriformes; Family: Claridae) were used for induced breeding. The selection of the gravid fish was made on the basis of external morphological features [6,18]. Gravid female fish were identified by the presence of soft swollen belly with short, round, button shaped and slit like genital papilla along with reddish vent (Figure 1). Prime maturity of the female was determined by examining the size uniformity of the eggs released by the gentle pressure on the swollen abdomen. Fully gravid males were identified by their slender and streamlined body with conical and elongated genital papilla having pointed reddish tip (Figure 2). They were collected from the local ponds and were acclimatized in the laboratory condition for 48 hours prior to breeding operation in the glass aquarium (120 cm×45 cm×45 cm) filled with unchlorinated, iron free tap water (pH 7.0 ± 0.13, free carbon dioxide 10.57 ± 2.1 mg/l, dissolved oxygen 5.85 ± 0.92 mg/l, total alkalinity 150 ± 5.07 mg/l as CaCO₃ and hardness 116 ± 6.70 mg/l as CaCO₃) having aeration facilities. During acclimatization male and female fishes were kept in separate aquaria and no feed was supplied.

### Inducing agents

Pituitary glands were collected from mature freshly dead carp fish and extracts as natural inducing agent were made following the method described by Bhowmick [19]. Ovaprim, a synthetic inducing agent is a product of Syndel Laboratories Ltd. Vancouver, Canada, marketed by Glaxo, India. It is a combination of 20 µg salmon gonadotropin releasing hormone analogue (D-Arg₆, Trp⁷, Leu 8, Pro⁹ Net) and 10 mg domperidone (as a dopamine receptor antagonist) dissolved in 1 ml calibrated quantities of non-toxic organic solvent [20,21].

### Breeding operations

The breeding operations were conducted at three different temperatures (26º, 28º and 30ºC). Pituitary gland extracts and ovaprim were separately administered to different sets of gravid fish. The doses of carp pituitary gland extracts were 40 and 120 mg/kg body weight for female and 25 and 50 mg/kg body weight for male. Ovaprim was administered at the rate of 0.8 and 2.0 ml/kg body weight for female and 0.4 and 1.0 ml/kg body weight for male. The doses of inducing agents were selected after a series of rough finding tests (data not shown). Inducing agents were administered to separate sets of brood fishes consisting of both sexes at 1:1 ratio. The required doses of pituitary gland extracts or Ovaprim was divided into two equal volumes and were administered intramuscularly as separate injections at the same time in left & right sides of the caudal peduncle region above the lateral line sense organ by hypodermic syringe with a small size needle (Beckton Dickinson needle No.24) at 45° angle. After injection, male and female fishes were kept separately in the water filled glass aquaria. After a gap of 15 hours of injection (latency period), the males were cut open and the testes were carefully removed in intact condition. Then the testes were cut into small pieces with scissor and squeezed properly mixing with 0.9% sodium chloride (NaCl) solution. The sperms remain in dormant condition in this suspension which was used immediately for fertilization of eggs. Simultaneously the female fish were stripped after the similar gap of 15 hours as latency period to release eggs into previously washed dry and clean enamel tray (Figure 3). For higher doses of pituitary gland extracts four additional latency periods (13, 14, 16 and 17 hours) were also maintained before stripping at 28ºC both for male and female fish (Table 2). For determination of total number

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**Table 1: Effects of stimulants on stripped out eggs of *Clarias batrachus* at 26º, 28º and 30ºC.**

| Latency period (Hours) | 26ºC | 28ºC | 30ºC |
|------------------------|------|------|------|
|                        | ± SD | ± SD | ± SD |

| Stimulant               | Fertilization Rate (%) | Hatching Rate (%) | Fertilization Rate (%) | Hatching Rate (%) | Fertilization Rate (%) | Hatching Rate (%) |
|-------------------------|-----------------------|------------------|-----------------------|------------------|-----------------------|------------------|
| Pituitary glands extract| 48% ± 4               | 45% ± 4          | 44% ± 4               | 47% ± 4          | 45% ± 4               | 47% ± 4          |
| Ovaprim                 | 46% ± 6               | 47% ± 5          | 45% ± 5               | 46% ± 5          | 46% ± 5               | 47% ± 5          |
|                         | ± SD                  | ± SD             | ± SD                  | ± SD             | ± SD                  | ± SD             |

**Table 2: Effects of latency period and temperature on the rate of fertilization and hatching of eggs of *Clarias batrachus* at higher dose of pituitary gland extracts (120 mg/kg body wt of female).**

Values (mean ± SD) within columns indicated by different superscript letters (a,b,c,d) and values (mean ± SD) within rows indicated by same superscript letter (m) are significantly different (DMRT, p<0.05).

**Table 3: Effects of different temperatures on breeding of *Clarias batrachus* using pituitary gland extracts and Ovaprim at different doses of pituitary gland extracts and Ovaprim at different doses.**

| Latency period (Hours) | 26ºC | 28ºC | 30ºC |
|------------------------|------|------|------|
|                        | ± SD | ± SD | ± SD |

| Stimulant               | Fertilization Rate (%) | Hatching Rate (%) | Fertilization Rate (%) | Hatching Rate (%) | Fertilization Rate (%) | Hatching Rate (%) |
|-------------------------|-----------------------|------------------|-----------------------|------------------|-----------------------|------------------|
| Pituitary glands extract| 42% ± 4               | 45% ± 4          | 43% ± 4               | 46% ± 4          | 44% ± 4               | 46% ± 4          |
| Ovaprim                 | 47% ± 5               | 46% ± 5          | 48% ± 5               | 47% ± 5          | 48% ± 5               | 47% ± 5          |
|                         | ± SD                  | ± SD             | ± SD                  | ± SD             | ± SD                  | ± SD             |

**Figure 1:** Ventral view of gravid female *C. batrachus* showing female genital papilla (FGP).

**Figure 2:** Ventral view of gravid male *C. batrachus* showing male genital papilla (MGP).
of stripped out eggs a small portion of egg mass was randomly collected in a clear petridish from each set. The total number of stripped out eggs in each set was calculated by multiplying the total number of eggs present in a small portion of collected egg mass with the total weight of stripped out eggs (g) and then it was divided by the weight of the collected egg mass (g). Freshly prepared milt suspension was then added gently to the eggs stripped after respective time of latency period by a dropper preferably within 2-3 minutes of stripping and thoroughly mixed with the help of disinfected bird's feather (Figure 4). A little freshwater was then added to activate the sperms. Then the tray was jerked gently for 2-3 minutes for proper mixing of eggs and milt. The fertilized eggs (Figure 5) were quickly washed and cleaned several times with fresh tap water for water hardening and to remove the residual milt and foam formed (Figure 6). Unfertilized eggs along with clotted blood and muscular dirt were discarded (Figure 5). For the assessment of fertilization percentage, a small portion of egg mass was randomly collected in a clear petridish filled with unchlorinated and iron free tap water for each set. The total number of eggs was counted in the collected egg mass with a magnifying glass. Then it was multiplied by one hundred and the resultant was divided by total number of collected eggs.

**Hatching operations**

A small scale pool was established under a shade by placing a set of polyvinyl chloride (PVC) trays (57 cm×37 cm×10.5 cm) on a platform in a flow through system of water fitted with aerator for hatching operation. Each tray was provided with two outlets at different levels. The outlets of the trays were connected to a common drainage pipe fitted at six inches down the tray. In addition, a white enamel tray (46 cm×30 cm×7.5 cm) was set within each PVC tray (Figure 7). Water
was supplied to the trays through the showers fitted with separate taps coming from a common galvanized iron (GI) pipe having 2 cm diameter. The common GI pipe was fixed at one foot above the trays which in turn was connected to the overhead tank. Water was released from overhead shower at a regular flow rate of about 1 to 1.5 l/minute. The outlets of all the trays were covered with bolting silk cloth (No.60) to prevent escape of eggs. The fertilized eggs were uniformly distributed in the enamel trays at the rate of 3000 ± 100 eggs per tray fitted within the PVC trays having regulated water flow and continuous aeration facilities. After hatching the hatchlings were counted by eye estimation method. The hatching rate was assessed by multiplying hundred with the total number of hatchlings obtained. Then the resultant was divided by the total number of fertilized eggs.

Rearing operations

The hatching unit was also used as the rearing unit, but the inner enamel tray was removed from the system (Figure 8). The water of each tray was aerated to maintain dissolved oxygen level above 5 mg/l throughout the hatching and rearing operations. Sufficient water level was also regulated during the experiment by opening and closing the outlets.

Hatchlings were reared in the same flow through system at the rate of 2000 ± 200 fish/tray up to 4th day and at the rate of 1000 ± 200 fish/tray up to 12th day until the young developing fish started frequent vertical movement. The developing fish were reared both in the indoor PVC tray and outdoor cement cistern (365 cm×117 cm×40 cm) at the rate of 500 nos./m² in a static and partial water replacement system during the period from 13th to 45th day. A regular water replacement (30-50%) was done in the morning to remove faecal matter, unused food materials and dead fish, if any. The water level was maintained in between 25-35 cm. in the cement cistern to avoid the exhaustion of developing fish during their vertical trip. Moreover, the young developing fish were treated with potassium permanganate solution at the rate of 1mg/l of water for 10 minutes once a week from the 10th day of rearing to avoid infection. The physicochemical parameters (temperature, pH, free carbon dioxide, total alkalinity and dissolved oxygen) of indoor and outdoor culture medium were recorded four times (04.00, 10.00, 16.00 and 22.00 hours) daily following the method described in APHA [22]. No feed was supplied up to 4th day of rearing due to nutritive contribution of yolk material. Finely sieved zooplankton were given as preferred live feed from 5th to 12th day of rearing at 4 hours interval. Three different sets of feeding trials were run using three different kinds of feed at the rate of 3% of their body weight at 8 hours interval during 13th to 45th day of rearing both in indoor and outdoor conditions. In Set I, the fish were fed with live zooplankton during rest of the rearing period. In Set II, the boiled and sieved hen’s egg with small amount of vitamin C was supplied as supplementary feed alternating with live zooplankton up to 20th day. The chopped tubificid worms were also used as feed followed by boiled hen’s egg with vitamin C and zooplankton during the period from 21st to 45th day of rearing in Set II. The worms before their use were treated with oxytetracycline at the rate of 250 mg/l of water for 15-20 minutes to make them pathogen free. The mixture of rice bran, mustard oil cake and trash fish at the rate of 1:1:1 ratio was powered and used as artificial feed in Set III during the period from 13th to 45th day of rearing.

For chronological developmental study, 30 developing eggs were sampled at every 1 hour interval until hatching and thereafter 30 developing fish were sampled at every 6 hours for the next four days and then only once a day until 45th day of development following the method of Puvaneswari et al. [15]. Measurements of egg diameter and standard length of fish were made using an ocular micrometer and other conventional methods. The total lengths of thirty randomly sampled individual fish were measured at each sampling time considering that the total length is the length from the tip of snout of fish to the end of caudal fin [23]. Similarly, total weight (wt.) of fish was measured by using electronic balance.

Statistical analyses

All values in the present experiment are expressed as the mean ± SD of three replicates and were statistically analyzed by one way ANOVA (analysis of variance) followed by DMRT (Duncan’s Multiple Range Test) to determine significant differences between the means [24].

Results

In the present study both male and female Clarias batrachus injected with booster doses (higher doses) of pituitary gland extracts (50 mg/kg body weight for male and 120 mg/kg body weight for female) or Ovaprim (1.0 ml/kg body weight for male and 2.0 ml/kg body weight for female) responded well in respect of optimum maturity of gonads and stripping property. The female fish showed smooth stripping and released fully developed eggs without cluster. Testes were fully developed and swollen. A significant amount of motile and viable sperms were obtained on squeezing the testes. Total number of eggs obtained after stripping of female injected with booster doses of pituitary gland extracts and Ovaprim at all the temperatures (at 26°, 28° and 30° C) were significantly higher (p<0.05) than the lower doses of respective stimulants (Table 1). In addition, in booster doses of pituitary gland extracts the egg production was also significantly higher than the booster doses of Ovaprim. In the lower doses of pituitary gland extracts (25 mg/kg body weight for male and 40 mg/kg body weight for female), stripping was smooth but ova were not developed fully in female. The ova came out in clusters. Testes were moderately developed but they were not properly swollen. At the lower doses of Ovaprim (0.4 ml/kg body weight for male and 0.8 ml/kg body weight for female), the stripping was not smooth and ova were not fully developed in female. The swollen and fully developed testes were not also observed at this dose. Total number of stripped out eggs at 28° and 30°C were significantly higher (p<0.05) than the number of eggs produced at 26°C.
injected with booster doses of pituitary gland extracts and Ovaprim (Table 1). But the number of stripped out eggs was not significantly varied (p<0.05) at different temperatures using the lower doses of pituitary gland extract and Ovaprim. At a particular temperature (26°C or 28°C or 30°C), the number of stripped out eggs significantly varied (p<0.05) in all the doses irrespective of the stimulants.

Smooth stripping and ova without clusters were recorded at higher dose of pituitary gland extracts (120 mg/kg body weight of female) when the latency period was maintained for 14 or 15 hours irrespective of temperatures. On the other hand, the stripping was not smooth and ova came out in clusters when stripping was made at 13 hours latency period. The ova lose their normal property in relation to shape, colour and hardiness when the fish were stripped at 16 hours and 17 hours slency periods. Significantly higher (p<0.05) fertilization and hatching rates were recorded at all the temperatures at 14 hours and 15 hours latency periods using higher dose of pituitary gland extracts (Table 2). The highest percentage of fertilization and hatching rates were observed at 28°C when stripping was done at 15 hours of latency period.

The rate of fertilization and hatching of eggs was significantly higher (p<0.05) at the higher doses of pituitary gland extracts (120 mg/kg) and Ovaprim (2.0 ml/kg) at all the temperatures (26°C, 28°C, 30°C) (Tables 3 and 4). However, the highest rate of fertilization (80%) and hatching (71%) was recorded at the higher doses of pituitary gland extracts at 28°C. On the other hand, in the lower doses of pituitary gland extracts and Ovaprim, the rate of fertilization and hatching were significantly reduced (p<0.05) irrespective of temperatures (Tables 3 and 4). Time required for hatching after fertilization was 30 ± 1.5 hours, 27 ± 0.5 hour and 25 ±1.0 hour at 26°C, 28°C and 30°C respectively irrespective of type and doses of the stimulants used.

The physicochemical parameters recorded from the rearing medium in the indoor PVC tray and outdoor cement cistern during rearing of young fish from 13th to 45th day are given in Table 5. A significant diurnal variation in temperature, free carbon dioxide and dissolved oxygen was recorded both in the indoor PVC tray and outdoor cistern (p<0.05). The temperature, free CO2 and dissolved oxygen of indoor PVC tray were also significantly varied from their respective values as observed in the outdoor cistern (Table 5). Consistently a higher level of dissolved oxygen concentration (5.90-6.41 mg/l) was recorded in the indoor rearing medium, but a high fluctuation in dissolved oxygen level (4.53-6.56) was observed in outdoor cistern. The diurnal fluctuations in temperature and free CO2 were also more acute in the outdoor cistern than indoor PVC tray. No significant variation was recorded in pH and total alkalinity in both the indoor and outdoor system.

Survival rates of young developing fish reared in indoor PVC tray and outdoor cement cistern with different sets of feeding trials were summarized in Table 6. Significantly higher (p<0.05) survival rates were observed in Set II over Set I and Set III both in indoor and outdoor rearing conditions. Significantly lower (p<0.05) survival rate of fish was recorded in Set III irrespective of culture conditions. The developing fish showed significantly higher (p<0.05) survival rate in all the Sets of indoor trials over their outdoor counterparts.

The developmental stages (fertilized egg to 45th day) of fish recorded in the present experiment are characterized chronologically:

**Fertilized egg**

- 0 hour: Egg was spherical, non-filamentous, demersal, adhesive, transparent and yellowish brown in colour with 1.80 ± 0.05 mm in diameter (Figure 5).

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**Table 3:** Effects of stimulants and temperature on fertilization rate of eggs of *Clarias batrachus*.

| Name of stimulant | Dose        | Mean No. of fertilized eggs and rate of fertilization (%) |
|-------------------|-------------|----------------------------------------------------------|
| Pituitary gland extract | Male | Female | 26°C | 28°C | 30°C |
| 25 mg/kg          | 40 mg/kg   | 1994± ± 29 | 46% | 2215± ± 30 | 50% | 2126± ± 35 | 48% |
| 50 mg/kg          | 120 mg/kg  | 4134± ± 51 | 76% | 4524± ± 33 | 80% | 4026± ± 58 | 72% |
| 0.4 ml/kg         | 0.8 ml/kg  | 1860± ± 53 | 41% | 2325± ± 37 | 45% | 2093± ± 36 | 45% |
| 1.0 ml/kg         | 2.0 ml/kg  | 3827± ± 40 | 72% | 4212± ± 37 | 77% | 3780± ± 31 | 70% |

**Table 4:** Effects of stimulants and temperature on hatching rate of eggs of *Clarias batrachus*.

| Name of stimulant | Dose | Mean No. of hatched eggs and rate of hatching (%) |
|-------------------|------|--------------------------------------------------|
| Pituitary gland extract | Male | Female | 26°C | 28°C | 30°C |
| 25 mg/kg          | 40 mg/kg   | 1525± ± 40 | 36% | 2319± ± 31 | 52% | 2014± ± 43 | 45% |
| 50 mg/kg          | 20 mg/kg   | 3590± ± 33 | 66% | 4032± ± 54 | 71% | 3411± ± 50 | 61% |
| Ovaprim           | 0.4 ml/kg | 0.8 ml/kg | 1555± ± 32 | 34% | 2163± ± 32 | 46% | 1840± ± 34 | 35% |
| 1.0 ml/kg         | 2.0 ml/kg  | 2870± ± 37 | 54% | 3546± ± 26 | 65% | 2825± ± 26 | 52% |

**Table 5:** Diurnal fluctuation of different physicochemical parameters of rearing medium in Indoor and Outdoor Experiments during rearing from 13th to 45th day.

| Times of day | pH | CO2 (mg/l) | DO (mg/l) | Total alkalinity (mg/l) |
|--------------|----|------------|-----------|------------------------|
| 04.00 h      | 7.6± ± 0.2 | 7.6± ± 0.6 | 7.6± ± 1.0 | 7.6± ± 1.5 | 7.6± ± 2.0 | 7.6± ± 2.5 | 7.6± ± 3.0 | 7.6± ± 3.5 | 7.6± ± 4.0 | 7.6± ± 4.5 | 7.6± ± 5.0 | 7.6± ± 5.5 |
| 10.00 h      | 7.6± ± 0.6 | 7.6± ± 1.2 | 7.6± ± 1.5 | 7.6± ± 2.0 | 7.6± ± 2.5 | 7.6± ± 3.0 | 7.6± ± 3.5 | 7.6± ± 4.0 | 7.6± ± 4.5 | 7.6± ± 5.0 | 7.6± ± 5.5 |
| 16.00 h      | 7.6± ± 0.6 | 7.6± ± 1.2 | 7.6± ± 1.5 | 7.6± ± 2.0 | 7.6± ± 2.5 | 7.6± ± 3.0 | 7.6± ± 3.5 | 7.6± ± 4.0 | 7.6± ± 4.5 | 7.6± ± 5.0 | 7.6± ± 5.5 |
| 22.00 h      | 7.6± ± 0.6 | 7.6± ± 1.2 | 7.6± ± 1.5 | 7.6± ± 2.0 | 7.6± ± 2.5 | 7.6± ± 3.0 | 7.6± ± 3.5 | 7.6± ± 4.0 | 7.6± ± 4.5 | 7.6± ± 5.0 | 7.6± ± 5.5 |

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eye and minute developing barbels. The body was more pigmented on the edge part especially on the cephalic region. The pigmentation appeared in rows over the body. Two fine red vessels appeared along the ventral side of the yolk sac. At day 3, the barbels were prominent and pectoral fin bud appeared. At day 4, the gill region became reddish in colour. The barbels were more prominent. The fish moved swiftly and preferred to hide in a darker corner of the tray (Figure 9).

5-9 day’s old fish

At day 5, the mean length was 8.75 ± 0.40 mm and mean weight was 9.09 ± 0.71 mg. Yolk sac was absent. Pigmentation appeared all over the body. The fish became reddish brown in colour. Head was provided with 4 pairs of distinct barbels. Pectoral fin with fin rays was well developed, dorsal and anal fins were continuous with caudal fin. The fish preferred to hide in the corner of the tray. On day 6, ventral fin buds were found. No significant change was recorded up to day 9 excepting length and weight.

10-15 days old fish

At day 10, the mean length and mean weight were 13.00 ± 1.00 mm and 25.71 ± 0.74 mg respectively. The body was light reddish brown in colour. The dorsal and anal fins were separated from the caudal fin. Well developed ventral fin and dorsal fin with 4 fin rays in each were noticed. No significant change was observed up to 15th day excepting length and weight. At day 14, fish started vertical movements and at day 15, both vertical and horizontal movements of the fish were noted (Figure 10).

16-27 days old fish

At day 16, the mean length of fish was 17.08 ± 1.05 mm and mean weight was 40.50 ± 0.80 mg. Caudal fin became homocercal and spine developed in the pectoral fin. At day 20, osseous plates on the cephalic region were distinctly observed in the fish body. No other significant morphological changes were recorded up to day 27 (Figure 11).

30 days old fish

The mean length was 38.00 ± 1.00 mm and mean weight was 397.00 ± 0.73 mg. Fish changed their body colour from light to dark reddish brown. The osseous plates appeared at this stage on the cephalic region (Figure 12).

45 days old fish

The recorded mean length and weight were 65.00 ± 3.00 mm and 1500 ± 27.50 mg respectively. At this stage fish was elongated and provided with all morphological characters like an adult. The fish was dark reddish brown in colour. 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. The fish preferred to stay in the resting state with an angle of 20º-60º touching the bottom with caudal fin during most of the vertical and horizontal movements.

Table 6: Survival rate (%) of developing Clarias batrachus in indoor and outdoor experiments during rearing from 13th to 45th day using various feed schedule.

| Feeding trial Set | Feed schedule | Survival rate (%) at Indoor condition | Survival rate (%) at Indoor condition |
|------------------|---------------|--------------------------------------|--------------------------------------|
| Set I            | Live zooplankton | 56.3± ± 0.27                       | 47.6± ± 3.86                        |
| Set II           | Alternate use of live zooplankton, boiled egg with little amount of vitamin C and chopped tubifex | 62.5± ± 2.25                       | 70.0± ± 1.69                        |
| Set III          | Finely powered rice bran, bran, mustard oil cake and trash fish mixture (1:1:1) | 51.9± ± 3.37                       | 44.1± ± 2.63                        |

Values (mean ± SD) within columns indicated by different superscript letters (a,b,c) and values (mean ± SD) within rows indicated by different superscript letters (m,n) are significantly different (DMRT, p<0.05).
carp pituitary gland extracts [6, 25,26] in Clarias sp. Spawning of C. batrachus at higher doses (100-150 mg/kg body weight of female) of carp pituitary gland extracts was also observed by Sidhimunika et al. [25], Khan and Mukhopadhyay [26]. Sahoo et al. [27] also used higher doses of inducing agents during late monsoon breeding operation to get higher success in induced breeding of Clarias batrachus.

In this investigation best result in respect of egg release, fertilization and hatching of egg was obtained in the fish treated with higher doses of pituitary gland extracts (Tables 1, 3 and 4). This is probably due to the proper activation of Gonadotropin hormone-II (GTH-II) cell present in the Pars distalis of the treated fish which in turn helps in the proper secretion of GTH-II. The secreted GTH-II binds to the specific receptor in the granulose cells of ovary or Leydig cells of testis and subsequently stimulates steroid hormone synthesis in these cells resulting in the better ovulation and spermatogenesis [28]. Another probable explanation for obtaining better results from fish injected with higher doses of pituitary gland extracts or Ovaprim is that the stimulants in higher doses stimulate the fish effectively by contracting the smooth muscles in the gonoduct of female before ovulation and thinning of semen just before spermatogenesis in male, culminating in spawning of fish [29]. The poor responses at lower doses of both pituitary gland extracts (40 mg/kg body weight for female) and Ovaprim (0.8 ml/kg body weight for female) may be due to insufficient secretion of gonadotropin leading to ovulation failure or blocking of ovipore by disintegrated ovarian tissue and egg bunches [10].

Zonneveld et al. [30] reported that right combination of the dose of inducing agent and the latency period plays the crucial role in the production of optimum quantity of eggs in catfish. Improper combination of these two may lead to breeding failure [11]. In present study, 14 to 15 hours latency period was most congenial for smooth release of fully matured eggs from the fish treated with higher dose of pituitary gland extracts leading to higher success in fertilization and hatching irrespective of all temperatures (Table 2). This result corresponds with the findings of Srivastava et al. [7]. They obtained better ovulation in C. batrachus using ovaprim (1.0-2.0 ml/kg body weight of female) with a latency period of 14-18 hours. Higher stripping responses in C. batrachus were also observed by Sahoo et al. [11] at 3000 and 4000 IU HCG in combination with 14-23 hour latency period. Comparatively poor breeding performance of C. batrachus at the latency period of 13 hours recorded in the present investigation may be due to inadequate release of gonadotropin and insufficient time for ovulation [11,31]. On the other hand lower success rate in fertilization and hatching of eggs during higher latency periods (16-17 hours) was probably due to over-ripe eggs which can not be ruled out by hormonal induction [27].

Highest rate of fertilization and hatching at 28°C in all treatments in the present study was may be due to the fact that this temperature played an important role to optimize all the physiological activities of the stimulant injected fish. Temperature specificity of different fish during sexual maturation and spawning was also recorded by many workers [32-36].

During rearing of young developing fish, higher survival rate was noticed in the smooth surfaced indoor PVC tray than the outdoor cement cistern. Marked diurnal fluctuations of temperature, free carbon dioxide and dissolved oxygen concentrations in the rearing medium of outdoor cistern exerts stress to the developing fish leading to increased rate of mortality. Consistency in higher level of dissolved oxygen concentration in the indoor rearing medium played the key role in maintaining higher survival rate of developing fish. High
hatchability and survival of developing young African catfish at higher concentration of dissolved oxygen were also observed by various workers [37,38]. Higher fluctuation in dissolved oxygen concentration in outdoor cistern was due to comparatively higher water temperature. Higher temperature decreases the dissolved oxygen concentration by reducing its solubility in water [39].

The developing C. batrachus showed better survival when they were fed with zooplankton as live feed alternately with boiled egg and chopped tubifex. This result corresponds with the findings of earlier workers [2,40].

The fertilized eggs of C. batrachus were adhesive in nature which was similar to those of other catfish species such as Mystus montanus, Pangasius satuli, Heteropneustes fossilis [15,41,42]. This represents an adaptation to prevent the flowing of eggs in the water currents and provide optimal oxygen supply [15]. The average diameter of fully swollen fertilized egg of C. batrachus as recorded in the present study was 1.80 ± 0.05 mm which was more or less similar in size (1.5-1.9 mm) as observed by the earlier workers [17,43,44]. In the present investigation, the fertilized eggs were yellowish brown in colour which corresponds with the findings of Khan [45] and Thakur et al. [44]. In this study, time required for hatching of eggs after fertilization was gradually decreased with increasing environmental temperature irrespective of type and doses of the stimulants. Zaki and Abdulla [46] and Herath [47] also observed shorter incubation period at higher temperatures. The present observation on the mean length of the newly hatched fish (4.10 ± 0.40 mm) corresponds with the results (4.5-4.7 mm) of Thakur [17]. The observation of Mookerjee and Mazumder [16] on length of newly hatched C. batrachus was slightly higher (5.8 mm) than our present records. Observation of Thakur [17] on the length and height of yolk sac (2.1 and 1.6 mm respectively) was similar to the present findings (2.1 and 1.5 mm respectively). The complete absorption of yolk sac in developing C. batrachus was recorded on 5th day in the present study which was similar with the observation of Thakur [17]. In this investigation, first vertical movement of developing fish was recorded on 11th day probably due to the formation of abdominal respiratory organ. Thakur [17] observed the vertical movement of fish in C. batrachus on 10th day. In the present study, fish showed active movement on 11th day onwards probably due to the development of extended and free caudal fin which was almost similar with the findings of Puvaneswari et al. [15] on Heteropneustes fossilis where free movement of the developing fish started on the 10th day. All morphological characters of the adult C. batrachus were distinctly observed in the young developing fish in the present study on 20th day. On the other hand, Thakur [17] stated that most of the adult characters in C. batrachus appeared by 15-20th day whereas Hossain et al. [2] observed the same on 18th day. This variability in appearing all morphological characters in C. batrachus may be due to availability of proper and adequate food with optimum physico-chemical properties of the rearing medium.

In the present study most successful results in respect of number of stripped out eggs, rate of fertilization and hatching were recorded in Clarias batrachus injected with carp pituitary gland extracts @ 50 mg/kg body weight of male and 120 mg/kg body weight of female at 28°C. The highest survival rate of developing fish can be achieved by applying zooplankton as live feed upto 12th day followed by alternate supply of zooplankton, boiled egg with vitamin C and chopped tubifex upto 45th day of rearing in indoor PVC tray. This breeding operation will provide some valuable information in the development of breeding technology of C. batrachus which will be very important for commercial hatcheries in respect of good quality seed collection. In addition, the chronological characterization of different developmental stages of the fish (fertilized egg to 45th day old fish) recorded in the present study will also help the fishermen to know the developmental pattern of C. batrachus and to take the proper management practices during rearing of their most sensitive earlier stages.

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