Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 11, pp.22208-22215, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

SPAWNING BIOLOGY, EMBRYONIC DEVELOPMENT AND REARING OF ENDANGERED LOACH, *BOTIA LOHACHATA* (CHAUDHURI) IN CAPTIVITY

***,[1]Arpita Dey, [2]Debapriya Sarkar and [1]Sudip Barat**

[1]Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, District- Darjeeling, Siliguri - 734 013, West Bengal, India
[2]Fishery Unit, Uttar Banga Krishi Viswavidyalaya, Pundibari-736165, District- Cooch Behar, West Bengal, India

ARTICLE INFO

Article History:
Received 16th August, 2015
Received in revised form
09th September, 2015
Accepted 28th October, 2015
Published online 30th November, 2015

Key words:

Botia lohachata,
Fecundity,
Gonado-Somatic Index,
Induced breeding,
Fertilization rate and Embryonic Development.

ABSTRACT

Botia lohachata or "Y- loach", an endangered and vulnerable fish, has both ornamental and edible value. Schistosomiasis or snail fever, a serious disease affecting human, domestic animals and wild animals is naturally controlled by the "Y-loach" in eating the freshwater snail and therefore plays a significant role in controlling the disease. The aim of the present study was, therefore, to breed this ornamental fish in captivity, study the embryonic development and conserve the fish in its natural habitat. The fecundity of females ranged from 3,731 to 23,120. The fish spawns in flowing water system at night. Embryonic and post embryonic development was recorded for 45 days. Each fish was given a dose of 0.025ml of WOVA-FH, a synthetic hormone, for induced breeding. The fertilized eggs measuring 0.9-1mm in diameter were observed to be demersal, nonadhesive and optically transparent. The average fertilization rate was found to be 95.98 %.The average Gonado-Somatic Index of female *Botia lohachata* was 24.46 and male 3.2. The embryos hatched after 14.30 h of fertilization from the chorion and measured 2.5 mm in total length. Correlation studies between total length, body weight, gonad weight, gonad length, fecundity and GSI were found to be significant ($p \leq 0.01$). The present work thus contributed to cover the deficient information for embryonic development of *Botia lohachata.* The embryonic development and captive breeding of this fish can play a great role in the conservation and its habitat protection.

***Citation*:** Arpita Dey, Debapriya Sarkar and Sudip Barat, 2015. "Spawning biology, embryonic development and rearing of endangered loach, *Botia lohachata* (Chaudhuri) in captivity", *International Journal of Current Research*, 7, (11), 22208-22215.

INTRODUCTION

Botia (loaches) is a genus of freshwater fish of the family Botiidae. *Botia lohachata* is an endangered species (CAMP, 1998) and vulnerable (IUCN, 2010) and has both ornamental and economical fish food value. The fishes are very colourful having bright bands, peaceful nature, lesser scales and barbles. The distribution of this tropical loach is native to India, Nepal, Bangladesh and Pakistan. *Botia lohachata* can reach an average length of 11cms and exist up to 10years. They lead a nocturnal life but adapt quickly in captive condition. They feed during the day time in captive condition, and prefer animal feeds like *Daphnia*, worms and Brine shrimp. They are benthic feeder but are also capable of feeding in mid and surface water. The "Y-loach", like many of its relatives, consumes some of the common aquarium type of snails. The fish is sometimes purchased mainly to get rid of infestation of snails in an aquarium.

***Corresponding author: Arpita Dey,**
Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, District- Darjeeling, Siliguri - 734 013, West Bengal, India

The "Y-loach" is a scavenger but does not eat fish wastes. Schistosomiasis, also known as "bilharzias", "bilharziosis" or "snail fever" is a serious disease affecting human and domestic animals as well as wild animals. The intermediate host of the parasite which causes the disease is a species of freshwater snails. The snail eating loach is one of the many natural controls of the freshwater snail population and plays its part in controlling the disease. "Y- loach" makes a cracking sound. This is either produced by forcing air through the gills and may be connected with feeding on the surface of the water or alternately produced by specialized teeth in the throat of the fish that appear to aid in the extraction of snails muscle from their shells.

Literature available on loaches are discrete .Information available show results on spawning biology and fecundity of *Cobitis taenia* (Juchno and Boron, 2006), fecundity of *Botia dario* (Hossain *et al*., 2007), spawning behaviour of *Sabanejewia vallachica* (Bohlen, 2008) and spawning biology of *Botia almorhae* (Joshi and Pathani, 2009) and diversity of loaches in Darjeeling, West Bengal (Acharjee, and Barat,

2014). No other literature is available as such either on behaviour, breeding or conservation aspects of loaches. These lacunae inspired us to investigate on behaviour, embryonic development, standardize artificial breeding, fry and fingerling rearing and conservation of the *Botia lohachata* which may contribute to some extent to the information database and conservation approach of the natural resource.

MATERIALS AND METHODS

Collection and experimental site

The collection of *Botia lohachata* weighing on an average from 2 gm to 4 gm were collected from sampling sites located at Bhelakopa, Dwitia Khanda of Cooch Behar district, West Bengal, India lying at $26^0$18'North latitude and $89^0$34'East longitude. After collection the fishes were oxygen packed in sterile polythene bags and kept in cartons for transport to the Fishery Wet Laboratory of Uttar Banga Krishi Viswavidyalaya, Cooch Behar. In the laboratory the fishes were transferred to suitable aquariums for regular rearing and maturation.

Water quality parameters for broodstock management

The water quality parameters are very important for the rearing and breeding of *Botia lohachata*. Fresh, dechlorinated and well aerated water was used for domestication of the fish in all the tanks. In all the experimental tanks for rearing and breeding pH, Specific conductivity and Total Dissolved Solids (TDS) were determined by using portable meter (Eutech). Total hardness (EDTA method) and Dissolved Oxygen (Winkler's method) were determined by Standard Methods (APHA, 2012). In the rearing tanks temperature of 27-32^0 C was maintained with the help of regulated water heaters (Thermostat). Scaling up of farming requires a constant supply of good quality fish seed, necessitating captive breeding, careful broodstock management and larval rearing. Broodstock can be managed in aquarium to promote gonad development. In the present study, the broodstock were fed with plankton, blood worm, tubifex and commercially available fish food at 5 – 10% of the total body weight per day. The fishes were monitored regularly for morphological indicators of maturation.

Gonado-somatic index(GSI) and Correlation analysis amongst length, weight, fecundity, gonad weight and gonad length

To determine the relation between gonad weight and body weight, Gonado-Somatic Index (GSI) was calculated. Correlation was determined by MS-Excel. Two lobes of the ovary from each sample fish were removed carefully by dissecting out the abdomen and dried off removing of excess moisture with blotting paper. Gonadosomatic index (GSI) expressed according to de Vlaming (1982) method for assessing the development of gonads was calculated as:

GSI = Weight of the ovary/ Total weight x 100

Induced Breeding

Forty (40) pairs of matured fish were injected at a dose of with synthetic hormone WOVA-FH (Biostadt India Limited, Mumbai) at the base of the pelvic fin. Prior to injection the fish were anesthetized with 2- phenoxy ethanol @ 2ml in 20 litres of water for easy handling for injection.

Fig A: Mature female *B. lohachata*

Fig B: Mature male *B. lohachata*

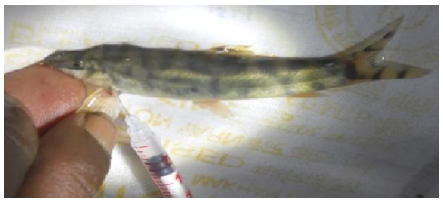

Fig C: Injected hormone in pelvic fin

Table 1. Details of experimental Set-up and design for induced breeding of *B. lohachata*

Experimental Set-up	Sex ratio	Experimental Design	Number of Fish	Dose of hormone (WOVA-FH)
A	1:1	500 litre aquarium with aeration.	10 pairs	0.025ml /fish
B	1:1	500 litre aquarium with aeration and shower.	10 pairs	0.025ml /fish
C	1:1	Chinese hatchery of diameter 2 m and height 0.5 m having running water facility increasing and decreasing speed of water flow of 1000 l^{-1} hr was maintained throughout.	10 pairs	0.025ml /fish
D	1:1	Chinese hatchery of diameter 2 m and height 0.5 m having running water facility increasing and decreasing speed of water flow of 5000 l^{-1} hr was maintained throughout.	10 pairs	0.025ml /fish

Fecundity and fertilization rate

Absolute fecundity was calculated according to the method of Hartman and Conkle (1960) using F=nG/g where, F is fecundity; n is mean numbers of eggs in all samples, G is weight of ovaries and g is weight of samples. Eggs were collected from three regions of the gonad like anterior, middle

and posterior. After 1 h of spawning 2 litre of water, eggs were collected from the hatchery and continued for 4 hours. Counting of the fertilized and unfertilized eggs were done. Fertilization rate was estimated by using the following formula (Udit *et al*., 2014).

Fertilization rate (%) = Fertilized eggs/ Total nunber of eggs in sample x 100.

Embryonic development and rearing of spawn

Egg samples on hatching were examined hourly and the developing stages were documented through micro-photograph. Eggs were collected as the fish were spawning. Initially photographs were continuously taken for the first two hours to capture the egg getting fertilized and the zygote stage undergoing cell division in the different stages and time frame. The standardization of rearing and culture of larvae and spawn to get healthy fingerlings were ranched in natural habitat. The yolk sac absorbed fry were harvested and stocked in well prepared cemented tank for further rearing. Feed was given twice daily. The monthly total length, body depth and body weight were taken. Before breeding season the adult fishes were ranched in different rivers of Terai, West Bengal, India.

RESULTS

Water quality for broodstock management

The water quality parameters are very important for the rearing and breeding of *Botia lohachata*. In all the experimental tanks for rearing and breeding pH was maintained in the range of 7.5 to 8.5, Total hardness in the range of 20-35 mg l^{-1}, Specific conductivity in the range of 110-180 µS, Total Dissolved Solid (TDS) in the range of 80-165 mg $l^{-1}$and Dissolved oxygen in the range of 7.6 - 8 mg l^{-1}. In the rearing tanks a constant temperature of 27-32^0 C was maintained with the help of thermostat. However, the temperature range can fluctuate between 27^{0}C- 32^{0}C. Broodstock was managed in aquarium to promote gonad development. Morphological indicator of maturated females was the development of enlarged belly which was lacking in males. During maturation, it was observed that males rise smaller in size than females. Males matured earlier than females. A common secondary sexual character was the brighter body colour of the male than that of the female fish. The dark bands on the skin of male were deep black during the breeding season, while such colour was absent in female. During the breeding period, the ripe male oozed out milt when slight pressure was applied on the vent. Eggs also oozed out with slight pressure on the belly of ripe female.

Gonado-Somatic Index and Correlation between length, weight, fecundity, gonad weight and gonad length

Spawning period was confirmed by the Gonado-somatic index (GSI). GSI increased from April to July. The average GSI of *Botia lohachata* for female was 24.46 and for male 3.2. Gonado-Somatic Index was higher in female than male. To establish the mathematical relationship between total length, body weight, gonad weight, gonad length, fecundity and Gonado-Somatic Index, Coefficient of Correlation (r) was done using the MS- Excel (Table 2).

Table 2. Correlation between the variables total length, body weight, gonad weight, gonad length, fecundity and Gonado-Somatic Index of the fish

Correlation between Characters	X	Y	r
Female and male Gonado-Somatic Index	Male GSI of the fish	Female GSI of the fish	0.889*
Fecundity and Gonad weight	Gonad weight of the fish	Fecundity of the fish	0.969*
Fecundity and total length	Fecundity of the fish	Total length of the fish	0.973*
Fecundity and body weight	Body weight of the fish	Fecundity of the fish	0.832*
Body weight and total length	Body weight of the fish	Total length of the fish	0.866*
Gonad weight and body weight	Gonad weight of the fish	Body weight of the fish	0.779*

* Show significance at p≤0.01

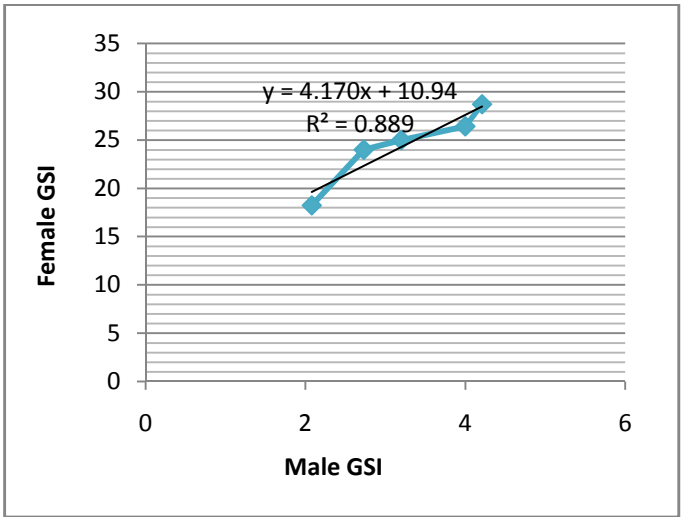

Fig.D: Relationship between Female and Male GSI

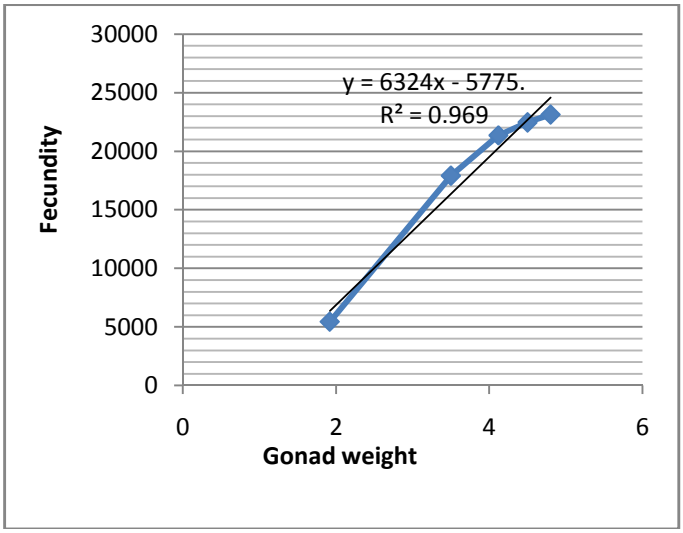

Fig.E: Relationship between Fecundity and Gonad weight

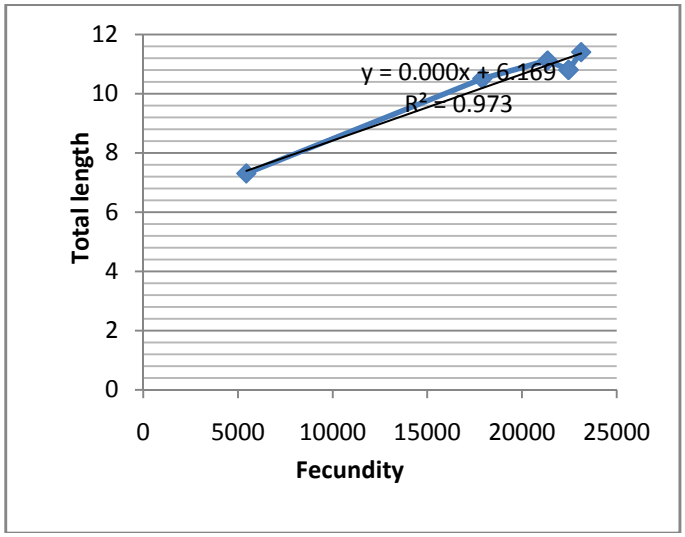

Fig.F: Relationship between Fecundity and Total length

The scatter diagram of female and male gonado-somatic index (**Fig. D**), fecundity and gonad weight (**Fig. E**), total length and fecundity (**Fig. F**), fecundity and body weight (**Fig. G**), total length and body weight (**Fig. H**) and body weight and gonad weight (**Fig. I**) showed a straight line relationship and is expressed as Y =a+bx, where, 'a' and 'b' are constants; X and Yare the variables. The coefficient of correlation (r) showed significance at p≤0.01.

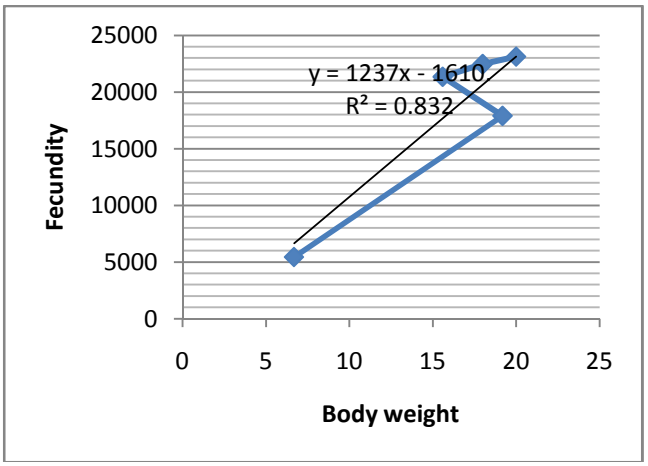

Fig.G: Relationship between Fecundity and Body weight

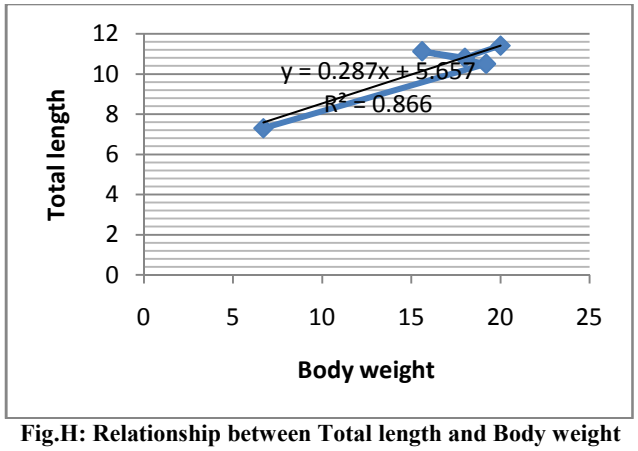

Fig.H: Relationship between Total length and Body weight

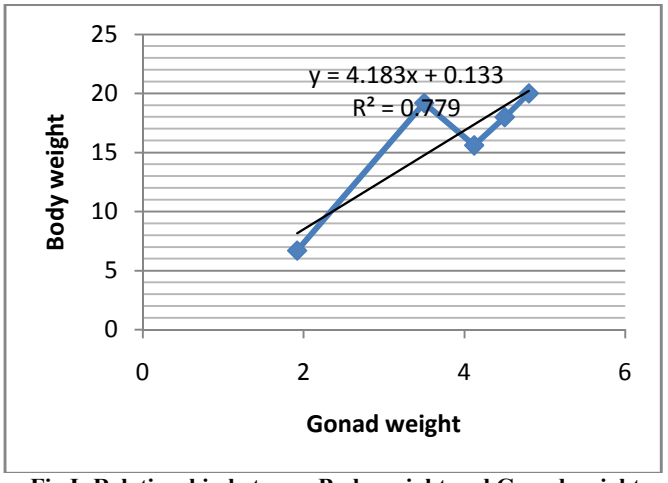

Fig.I: Relationship between Body weight and Gonad weight

Breeding in different Set-ups

Breeding experiments in captivity were conducted successfully for the ornamental fish *Botia lohachata* in May 2013 and May 2015 using synthetic hormone WOVA-FH. Fertilisation was external and spawning occured once a year during the monsoon months (May–August) with a peak in July. Observations were done at hourly intervals. Spawning pattern was observed in both male and female fish during the night. The male was found constantly hitting the female on the abdomen with its head while chasing her all around the aquarium. Cracking sound was heard every now and then. Females were being chased by more than one male at the same time and there was infighting amongst the males. Spawning commenced after half hour of chasing.

Fecundity and fertilization rate

The fecundity was estimated by random sampling method. The fecundity of females ranged from 3731 to 23120. On the basis of present experimental results, average fecundity was 15941. The fertilization rate was found to be 100% after one hour of spawning, 90% after two hours of spawning, 97.26% after three hours of spawning and 96.66% after four hours of spawning. The average fertilization rate was found to be 95.98.

Embryonic development

The embryonic development of *Botia lohachata* was divided into eight stages-Zygote, Cleavage Blastula, Gastrula, Segmentation, Pharyngula, Hatching and Early larval period. The recorded embryonic development stages are described as follows:

Zygote

The fertilized eggs were nonadhesive, whitish in colour and optically transparent. Fertilization activated the cytoplasmic movements (Dey *et al*., 2014). The yolkfree cytoplasm begins to stretch towards the animal pole gradually segregating the blastodisc from the vegetal cytoplasm. The diameter of the zygote was 0.9 to 1mm (Fig. 1).

Table 3. Summary of the different stages of breeding of *Botia lohachata* at different time schedules in the experimental Set-up A,B, C and D

Time Schedule	Set-up-A	Set-up -B	Set-up -C	Set-up -D
10p.m	Fishes cuddled up in a corner	Fishes cuddled up in a corner	Fishes were swimming against flow of water	Fishes were swimming against flow of water
11p.m	Same	Same	Same	Same
12p.m	Females swelled up but males were not active.	Females swelled up and slight actively could be seen in male	Females were being chased by the males at and at the same time there was in fighting amongst the male.	Females were being chased by the males at the same time the males were fighting with each other
1a.m.	Same	Same	Cracking sound was heard every now and then.	Cracking sound was heard every now and then Spawning had started; the paired fishes were swimming with the current and appearing at the surface clinging to each other hooked with the spine below the eyes which was producing the cracking sound.
2a.m.	Same	Males were chasing the females; but no spawning.	Spawning had started	All the fishes were spawning
3a.m.	Same	Same	Same	Same
4 a.m.	Same	Same	Same	Spawning stopped
5 a.m.	No spawning	No spawning	Spawning stopped	Spawning stopped

Cleavage

The first cleavage occurred 24 minutes after fertilization. The two blastomeres rounded in shape just after first cleavage. The blastomeres observed at the animal pole were only half the size of the original cell. After the first cleavage, the blastomeres divided synchronously at an interval of 4 to10 mins. Cleavage period was observed to be from 24min. to 1.1 hour in which the 64 cell stage was completed (Fig. 3-8).

Blastula

The blastula stage with the 128-cell stage ended with the commencement of the gastrula. Blastula was observed to be from 1.11 to 3.05 hours and completed 30% of the epiboly stage (Fig. 9-14).

Gastrula

In the gastrula period, extensive cell movement was observed including involution, convergence and extension, producing the three primary germ layers and the embryonic axis. Gastrulation began with cell involution at around 50% epiboly and completed the bud stage (Fig. 15-18). In the bud stage (Fig. 18), epiboly ended as the blastoderm completely covered the yolk plug. The tail bud then appeared as a distinct thickening at the caudal end of the embryo, near the site of yolk plug closure. Early polster was seen. Gastrula period was observed to be from 3.05 to 6.33 hours.

Segmentation

The segmentation period was characterized by the sequential formation of the somites, and lasted to just prior to hatching. During this period, the embryo elongated along the animal pole axis, the tail bud developed longer and rudiments of the primary organs became visible. Somites, formed in bilateral pairs as the developing embryo, extended posteriorly. Segmentation period was observed to be from 6.46 to 14.27 hours (Fig.19-23).

Pharyngula

During this period the embryo were bilaterally organized, with a well-developed notochord and a newly completed set of somites that extended to the end of a long post-anal tail. Body axis straightened from its early curvature. The yolk sac, circulation, pigmentation, and fins development continued. The nervous system was hollow. The head straightened out and lifted to the dorsal side. The brain was prominently sculptured. The blood flow was visible. Pigment formation began in cells of the pigmented retinal epithelium. The embryo continued to exhibit spontaneous side-to-side contractions involving the trunk and tail and the rate of contractions increased in bursts till the embryo hatched out of the chorion (Fig.24-26). The C-Shaped embryo elongated and gradually differentiated into a head and tail. The body formed into a C-shape (Fig. 27). The yolk was attached between the tail and head. Myotomes development was observed. The embryo started occasional movement. Twitching stage the tail got completely detached from the yolk. The yolk sac was restricted to the head region. The number of myotomes increased and the embryo became active and exhibited continuous twitching movement.

Hatching

Just after hatching from the chorion the larva at 14.30 h measured 2.5 mm (Fig.28). Head was slightly bent on the yolk, the eyes were large, yolk sac was present on the anterior-ventral side of the body and the heart and the optic vesicle were seen. They were responsive to stimulus and settled in the substrate (Fig. 28).

Larval development

The mouth appeared to be opened and slit like. After 22 h of hatching larvae started swimming and feeding. At first larvae were fed with *Paramecium* sp. then *Artemia* after 3days, the larvae consumed small sized zooplanktons (Fig.29-33). There was complete resorption of the yolk sac and minute pectoral fins were observed. The eyes were well set in the optic sockets.

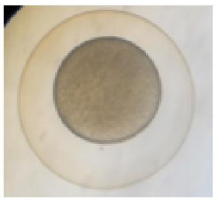

Fig.1-Single cell

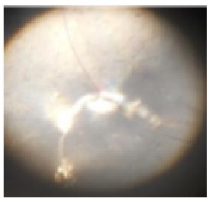

Fig.2-Sperm entry

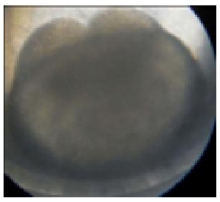

Fig.3-2 cell stage

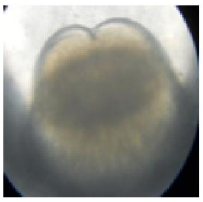

Fig.4- 4 cell stage

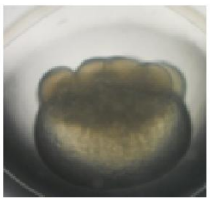

Fig.5-8cell stage

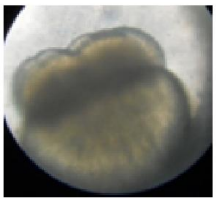

Fig.6-16 cell stage

Fig.7-32 cell stag

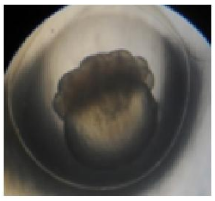

Fig.8-64 cell stage

Fig.9-128 cells stage

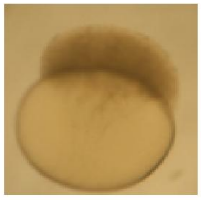

Fig.10-256 cells stag

Fig.11-512 cells stag

Fig.12-oblong stage

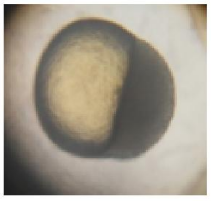

Fig.13-sphere stage

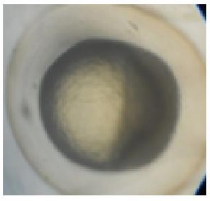

Fig.14-30% epiboly

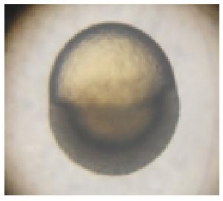

Fig.15-50% epiboly

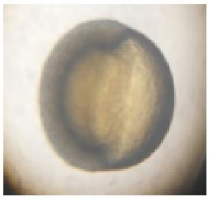

Fig.16-75% epiboly

Fig.17-90% epiboly

Fig.18-bud stage

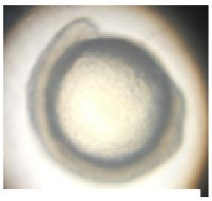

Fig.19-1, somite stage

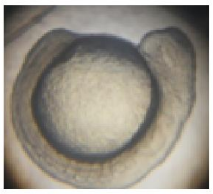

Fig.20-8,somite stage

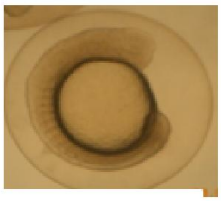

Fig.21-12,somite stage

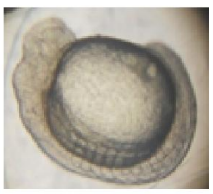

Fig.22-16,somite stage

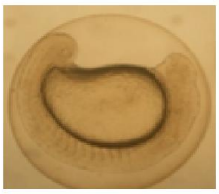

Fig.23-20,somite stage

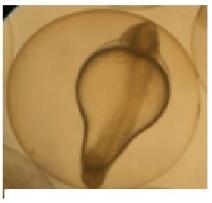

Fig.24-Pharyngula stage

Fig.25- Pharyngula stage

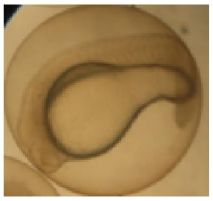

Fig.26-Before hatching, twitching

Fig.27-c-shaped embryo stage

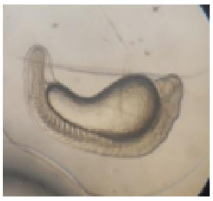

Fig.28-Newly hatched larva

Fig.29- take Paramecium

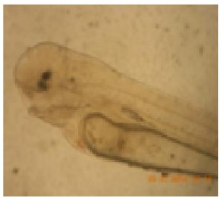

Fig.30- take Artemia

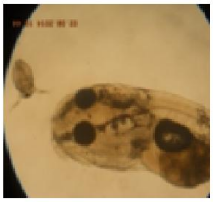

Fig.31- take zooplankton

Fig.32- 6 day old larva

Fig.33-7 day old larvae

Fig.34-10 day old larvae

Fig.35-20 day old larvae

Fig.36-45 day old fish

The dark lateral band was more prominently seen between the operculum and the caudal fin base. A 45-day old fish measured 3.5-4.0 cm (Fig.36) and completely resembled the adult fish in all its features and could be easily identified. The horizontal stripes matched the adult fish and attained the colourful pigmentation, which characterized it as an ornamental fish.

The present study was conducted to investigate and provide detailed information about the fecundity, fertilization rate, induced breeding and embryonic development of the endangered fish. The observations in the present study showed that males matured in April and the females in the last week of May. The results also revealed that WOVA-FH at 0.025ml per fish is sufficient to induce spawning in *B. lohachata*. The result of the findings further showed that B. *lohachata* could be successfully induced bred with WOVA-FH. The brood had was eaten their own eggs and there after stopped spawning, the brood fish were then removed from the breeding tanks.

The study of fecundity of *B. lohachata* revealed 3731 to 23120 with an average fecundity rate of 15941. The percentage of fertilization depended on the quality of brood stock. The average fertilization rate was 95.98. The low hatching rate may be attributed to hatching of eggs in confined water. The egg incubation period ranged between 14.00 and 14.30 hours and revealed that the fertilized eggs were transparent initially and the colour changed from whitish to creamy as the embryonic development proceeded. The fertilized eggs were small and then hardened the size of the eggs ranged between 1.27 to 1.39 mm.

DISCUSSION

Experimental Set-up A and B did not release any eggs but Set-up C and D released eggs. Best result was observed in Experimental Set-up D where flowing water was present. The study revealed that flowing water was essential for induced spawning of *Botia lohachata*. The spawning behaviour of *Botia lohachata* was similar to the Indian Major Carps like flowing water systems. The latency period was 4 to 5 hours in fish injected with 0.025ml WOVA-FH per fish. The latency period was very low in *Botia lohachata*. The latency period of *Puntius sarana* (Udit *et al*., 2014) was 8 to 9 hours after administration of inducing agent. The latency period of *Ompok pabda* (Purkayastha *et al*., 2012) was 6 to 8 hours of administration of Ovatide (synthetic hormone).

The fertilized eggs were transparent and unfertilized ones were opaque and white. Similar type of captive breeding, embryonic development, fecundity, fertilization rate and hatching rate were reported by Kimmel *et al.(*1995) on *Danio rerio*, Udit *et al*. (2014) on *Puntius sarana*, Dey *et al*. (2014) on *Devario aequipinnatus* and reproductive biology of *Ompok bimaculatus* (Malla and Banik, 2015).

The first cleavage occurred 24 min. after the eggs were fertilized of *Botia lohachata*. Udit *et al*. (2014) reported first cleavage occurred after 30 min. in *Puntius sarana*, Dey *et al.* (2014) after 45 min. in *Devario aequipinnatus* and Kimmel *et al. (*1995) after 40 min. in *Danio rerio.* Present study however reports that first cleavage occurred shortly after fertilization. The incubation period of *Botia lohachata* lasted for 14.00 to 14.30 hours. The incubation period was also lower than other species. The incubation period reported for *Danio rerio* was 48 hours (Kimmel *et al.,* 1995); in *Puntius sarana* 15-17 hours (Udit *et al*., 2014) and *Devario aequipinnatus* 36 hours (Dey *et al*., 2014). The incubation period of *Botia lohachata* was lesser than other ornamental and food fish.

Conclusion

Botia lohachata can be easily matured and bred successfully under captive conditions similar to that of carps. Brood stock management and hatcheries should be established for conservation, and ranching initiated for sustained natural recruitment of the species. Establishment of proper sanctuaries in selected areas of rivers, floodplain and reservoirs is recommended for conservation of this species. This study documents the breeding of ornamental fish *Botia lohachata* in captivity, with use of synthetic hormones, and embryonic and post embryonic development up to 60 days till it completely resembles the adult fish. The fry grew to about 12 to 15 mm in size in 18 days. The subject matter in this publication is useful for fish breeders, aquarium keepers and those involved with or interested in the study of fish larval and fry development.

Acknowledgements

The first author is grateful to the Mr. Kripan Sarkar (Rainbow ornamental fish farm, Jalpaiguri, West Bengal) and the associates Mr. Amar Chandra Dey and Mr. Sunil Barman for their valuable suggestions.

REFERENCES

Acharjee, M.L. and Barat, S. 2014. Loaches of Darjeeling Himalaya and adjoining areas of West Bengal: their prospects as Ornamental fish and constraints. *International Journal of Pure and Applied Bioscience,* 2:258-264.

APHA, AWWA, WEF, 2012. Standard methods for the examination of water and waste water, 22nd Edition. American Public Health Association, Washington.

Bohlen, J. 2008. First report on the spawning behaviour of a golden spined loach, Sabanejewia vallachica (Teleostei: Cobitidae). *Folia Zool.,* 57(1–2): 139–146.

CAMP, 1998. Conservation Assessment and Management Plan Workshops, (Ed) Sanjay Molur and Sally Walker. Published by Zoo Outreach organization. National Bureau of Fish Genetics Resources. Lucknow, India .

Dey,S., Ramanujam, S. N. and Mahapatra, B. K. 2014. Breeding and development of ornamental hill stream fish Devario aequipinnatus (McClelland) in captivity. *International Journal of Fisheries and Aquatic Studies*, 1(4): 01-07 .

Hartman, W.L. and C.Y. Conkle, 1960. Fecundity of red salmon at brooks and karluk Lakes, *Alaska. Fishery. Bull. Fish Wildli. Serv. U.S.A*, 180: 53-60.

Hossain, M.A, Khatum, M.R. and Hussain, M.A. 2007. On the fecundity and sex-ratio of *Botia dario* (Hamilton) (Cypriniformes :Cobitidae). *Univ. J. Zool. Rajshahi Univ.* Vol. 26:27-29.

Hossain, Q.Z., Hossain, M.A. and Parween, S. 2006. Breeding biology, captive breeding and fry nursing of humped featherback (Notopterus chitala, Hamilton-Bachanan 1822). Ecoprint, vol.13.

IUCN, 2010. Red List of Threatened Species [http://www.iucnredlist.org/apps/redlist/search].

Joshi, S.K. and Pathani, S.S. 2009. Spawning biology of a hill stream fish, *Botia almorhae* Day of Kumaun Himalaya, Uttarakhand, *Indian J. Fish*., 56(2) : 151-155.

Juchno, D. and Boron, A. 2006. Age, reproduction and fecundity of the spined loach *Cobitis taenia* L. (Pisces, Cobitidae) from Lake Klawoj (Poland). *Reproductive Biology*, 6, No. 2.

Kimmel, C.B., Ballard W.W., Kimmel, S.R., Ullmann, B, and Schilling, T. F. 1995. Stages of embryonic development of the zebrafish. *Dev Dyn* Vol 203: 253–310.

Malla, S. and Banik, S. 2015. Reproductive biology of an endangered catfish, Ompok bimaculatus (Bloch, 1794) in the lotic waterbodies of Tripura, North-East India. *International Journal of Fisheries and Aquatic Studies*, 2(4): 251-260.

Partridge, C., Cazalas, C.,Rozelle, J.,Hemming, J.and Boettcher, A. 2004. Small scale captive breeding of euryhaline pipe fish. *World Aquaculture*.

Purkayastha, S.,Sarma, S., Singh, A.S. and Biswas, S.P. 2012. Captive breeding of an endangered fish Ompok pabda (Hamilton-Bucanan) with ovatide from Guwahati, Assam. *Asian J. EXP. BIOL. SCI. VOL* 3(2):267-271.

Udit, U.K., Reddy, A. K., Kumar, P., Rather, M. A., Das, R. and Singh, D. K. 2014. Induced breeding, embryonic and larval development of critically Endangered fish puntius sarana (hamilton, 1822) under captive Condition. *The Journal of Animal & Plant Sciences*, 24(1): 159-166.

Vlaming, V.L. 1982. The effects of temperature and photoperiod on reproductive cycling in the estuarine gobiid fish (*Gillichthys mirabilis*). *Fish Bulletin,* 73: 1137–1157.

****