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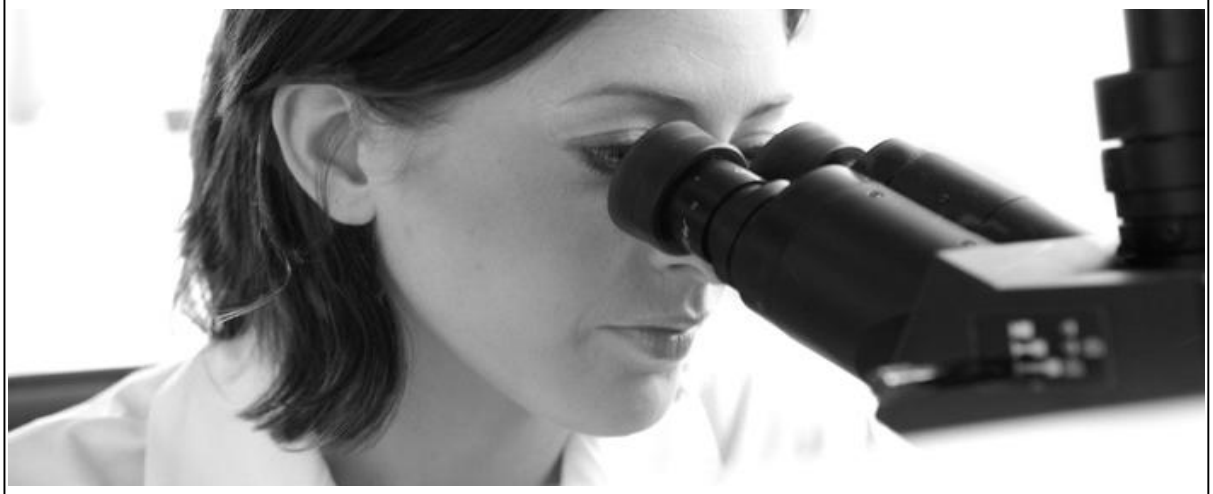

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RESEARCH ARTICLE

CAPTIVE BREEDING AND EMBRYONIC DEVELOPMENT OF AN ENDANGERED LOACH, BOTIA ALMORHAE (GRAY, 1831), IN COOCH BEHAR, WEST BENGAL, INDIA

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ABSTRACT

Botia almorhae, an endangered loach has both ornamental and edible value. The present study reveals the breeding of the ornamental fish in captivity, the embryonic development and conservation of the fish in its natural habitat. The fecundity of females ranged from 12632 to 22456. The fish spawns in flowing water system at night. Embryonic and post embryonic development were recorded for 7 days. Each fish was given a dose of 0.025ml of WOVA-FH, a synthetic hormone, for induced breeding. The fertilized eggs measuring 1-2 mm in diameter were observed to be demersal, non-adhesive and optically transparent. The embryos hatched after 15.30 -16.00 h from the chorion and measured 2.5 mm in total length. The present work thus contributed to the deficient information for *Botia almorhae.* Embryonic development and captive breeding of this fish can therefore play a great role in the conservation and habitat protection.

INTRODUCTION

Botia almorhae (Grey, 1831), commonly known as "Almorha loach" is an endangered (CAMP, 1998) and very rare loach in the Terai region of West Bengal. It is one of the most attractive loach found in India, Bangladesh and Nepal. *Botia almorhae* has high ornamental value and medium food fish. *Botia almorhae* is yellow body colour with black reticulated bands. *B. almorhae* feed on shrimp, snail, mosquito larva, *Tubifex*, *Daphnia* and so on. The most important thing for these loach is that they always have clean and well oxygenated water. Frequent water changes of about 25% per day is required for the Almorha loach. There is no literature on the embryonic development and artificial breeding of *B. Almorhae*, but only discrete information are available on loaches in general.

Some investigations have shown 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). These lacunae inspired us to investigate on the breeding behaviour, embryonic development and conservation of *Botia almorhae* which could 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 almorhae* weighing 2 gm to 4 gm were collected from sampling sites located at Bhelakopa, Dwitia Khanda of Cooch Behar 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 Wet Laboratory of Aquaculture and Limnology Research Unit, University of North Bengal. In the laboratory the fishes were transferred to suitable aquariums for acclimatization regular rearing and maturation.

Induced Breeding

Forty (40) pairs of matured fish were injected 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.

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Fig A Matured fish

Fig B Matured female fish

Fig C Matured male fish

Fertilisation was external and spawning occurred once a year during the monsoon season (May – August) with a peak in July. Observations were done at hourly bases. Spawning pattern was observed in both male and female fish during the night. The male was noticed to constantly hit the female on the abdomen with its head while chasing her all around the aquarium. Crackling sound was heard every now and then. Females were chased by more than one male at the same time and males were seen infighting.

The embryonic development of *Botia almorhae* was divided into eight stage-Zygote, Cleavage Blastula, Gastrula, Segmentation, Pharyngula, Hatching and Early larval period. The recorded embryonic development describe below:

Zygote period

The fertilized eggs were non-adhesive, whitish in colour and optically transparent. Fertilization activated the cytoplasmic movements. The yolk free cytoplasm begins to stream toward the animal pole gradually segregating the blastodisc from the vegetal cytoplasm. The diameter of the zygote was 1-2 mm (Fig. 1-3).

Table 1 Details of Experimental Set-up and design for induced breeding of *B. almorhae*

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 with increasing and decreasing speed of water flow at 1000 L^{-1} hr which 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 with increasing and decreasing speed of water flow at 5000 L^{-1} hr which maintained throughout.	10 pairs	0.025ml /fish

Fecundity

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.

Embryonic development

Egg samples in the aquarium were examined hourly and the developing stages were documented through micro-photograph. Eggs were collected as the fish spawned. Initially photographs were taken continuously for the first two hours to capture the eggs getting fertilized, the zygote stage, the cell division and then the different stages occurring at different time frames.

RESULTS

Breeding experiments in captivity were conducted successfully for the ornamental fish, *Botia almorhae,* during May 2014 and May 2015 with the use of synthetic hormone. Matured female could be easily recognized, by its rounded and swollen belly while the matured male by slimmer body and more colourful.

Cleavage period

The first cleavage occurred after 28 min of fertilization. The two blastomeres got rounded just after first cleavage. The two blastomeres observed at the animal pole were only half the size of the original cell. After the first cleavage, the blastomeres divided synchronously at intervals of 4-12 min. Cleavage period was observed to be 28min. -1.1 hours and completed the 64 cells stage (Fig. 4-9).

Blastula period

The blastula period began with the 128-cell stage and ended with the beginning of the gastrulation. Blastula period was observed to between 01.13to 03.10 hours and completed 30% of the epiboly stage (Fig. 10-18).

Gastrula period

In the gastrula period, extensive cell movements were 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 bud stage (Fig. 19-24). In the bud stage (Fig. 24) epiboly comes to a close as the blastoderm completely

covers 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 is seen. Gastrula period was observed to between 03.07 and 06.36 hours.

Segmentation period

The segmentation period was characterized by the sequential formation of the somites, and this period 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, extends posteriorly. Segmentation period was observed to be between 6.42 and 14.30 hours (Fig.25-33).

Pharyngula period

During this period, the embryo is a bilaterally organized, with a well-developed notochord and a newly completed set of somites that extend to the end of a long post-anal tail. Body axis gets straightened from its early curvature about the yolk sac, circulation, pigmentation, and fins begin their development. The nervous system is hollow. The head straightens out and lifts to the dorsal side. The brain is prominently sculptured. The blood flow is visible. Pigment formation begins in cells of the pigmented retinal epithelium. The embryo continues to exhibit spontaneous side-to-side contractions involving the trunk and tail and the rate of contractions increases in bursts till the embryo hatches out of the chorion (Fig.34-35). The embryo gets elongated and gradually differentiated into head and tail, and the body becomes into C-shaped. The yolk gets attached between tail and head. Myotomes development was observed. The embryo started occasional movement. As the twitching stage; the tail was completely detached from the yolk. The yolk sac was restricted to head region. The number of myotomes increased. The embryo became active and showed continuous twitching movement.

Hatching period

Just after hatching from the chorion, the larva at 14.40 h measured 2.5 mm (Fig. 36-38). The 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.

Larval development

The mouth appeared to be open and slit like. After 22 h of hatching larvae started swimming and feeding. Initially, the larvae fed on *Paramecium* then *Artemia*, and after 3days the larvae consumed small sized zooplankton (Fig.40-42). There was complete resorption of the yolk sac and minute pectoral fins were observed. The eyes were well set in the optic sockets. The dark lateral band was more prominently seen between the operculum and the caudal fin base.

The observations in the present study revealed, that males matured in April and females in the last week of May. The results had shown that WOVA-FH at 0.025ml per fish was sufficient to induce spawning in *B. almorhae* and that *B. almorhae* could be successfully induced bred with WOVA-FH. Further, the study on spawning fecundity of *B. almorhae* showed 12,632 to 22,456 numbers. The percentage of fertilization depends on the quality of brood stock. The low hatching rate may be attributed to hatching of eggs in confined water. In the present study, egg incubation period ranged between 15.30 and 16.00 hours and the colour of fertilized eggs was whitish and transparent initially and then changed to creamy as the embryonic development proceeded. The fertilized eggs were small and after hardening, the size ranged between 1.27 and 1.39 mm. The fertilized eggs were transparent and unfertilized ones were opaque and white. Similar type of embryonic development 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* by Malla and Banik (2015).

Fig1 Spawning in aquarium

Fig2 single cell

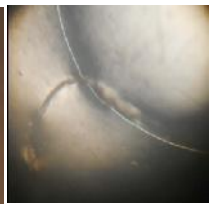

Fig3 Fertilized egg

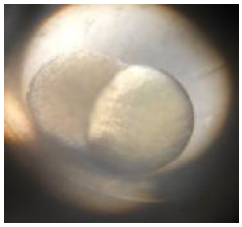

Fig4 Two cell stage

Fig5 Four cells stage

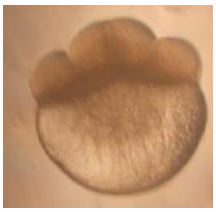

Fig6 8cells stage

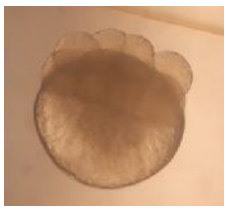

Fig7 16 cells stage

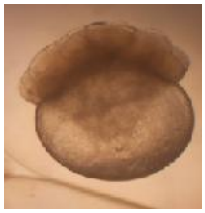

Fig8 32 cells stage

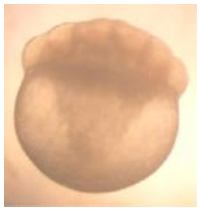

Fig9 64 cells stage

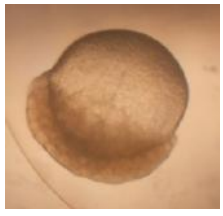

Fig10 128 cells stage

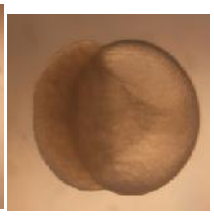

Fig11 256 cells stage

Fig12 512 cells stage

Fig131K cells stage

Fig14 oblong stage

Fig15 sphere stage



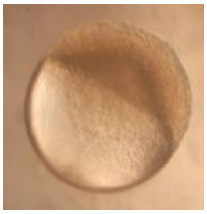

Fig16 sphere stage **Fig17** dome stage **Fig18** 30% epiboly



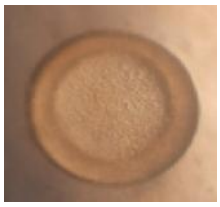

Fig19 50% epiboly **Fig20** 50% epiboly **Fig21** germ ring



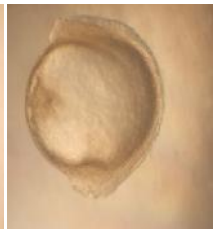

Fig22 75% epiboly **Fig23** 90% epiboly **Fig24** bud stage


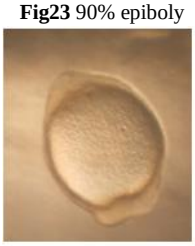
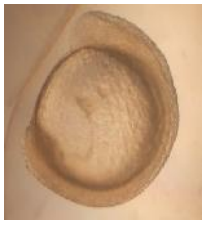

Fig25 1 somite stage **Fig26** 2, somites stage **Fig27** 3,somitesstage



Fig28 4, somites stage **Fig29** 7, somites stage **Fig30** 8,somites stage



Fig31 14, somites stage **Fig32** 16, somites stage **Fig33** 20, somites stage



Fig34 pharyngula stage **Fig35** pharyngula stage **Fig36** before hatching stage

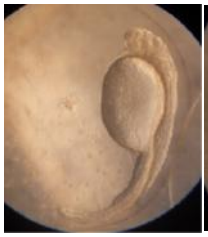
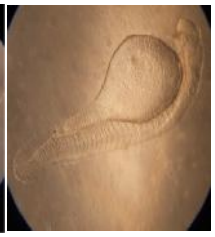


Fig37 C-shape embryo **Fig38** newly hatch larva **Fig39** 1, day old larva

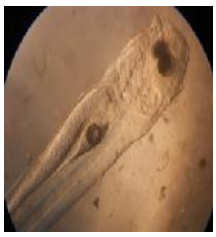
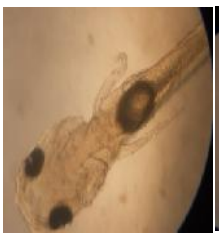


Fig40 2, day old larva **Fig41** 3, day old larva **Fig42** 7, day old larva

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 the flowing water was present. Present study revealed that flowing water was essential for induced spawning of *Botia almorhae*.

he spawning behaviour of *Botia almorhae* was similar with Indian Major Carps like flowing water systems. The latency period was between 05.00 to 05.30 hours in fish injected with 0.025ml WOVA-FH per fish. As studied by earlier workers, the latency period was very low in *Botia almorhae*. The latency period of *Puntius sarana* (Udit *et al*., 2014) was 8 to 9 hours of administration of inducing agent, and in of *Ompok pabda* (Purkayastha *et al*.,2012) it was 6 to 8 hours of administration of Ovatide, a 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 at 28 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 also revealed that first cleavage occurred after a short duration of fertilization. The incubation period of *Botia almorhae* lasted form 15.30 to 16.00 hours. The incubation period was also low from the others species. The incubation period reported for *Danio rerio* was 48 hours (Kimmel *et al.*,1995), *Puntius sarana* was 15-17 hours (Udit *et a*l.,2014) and *Devario aequipinnatus* was 36 hours (Dey *et al*.,2014). The incubation period of *Botia almorhae* had taken lesser time than other ornamental and food fishes.

CONCLUSION

Botia almorhae can be easily matured and bred successfully under captive condition similar to that of carps. It is first reported that the loach can breed in aquarium. 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 almorhae* in captivity with use of synthetic hormones and embryonic and post embryonic development up to 7 days. This study is useful for fish breeders, aquarium keepers and those involved in or interested in the study of fish larval and fry development.

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