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Research Article

Captive breeding and Early Development of Near Threatened Rainbow Snakehead (*Channa bleheri* Vierke, 1991)

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ABSTRACT

In the current study captive breeding, embryonic and larval development of Near Threatened (NT) (IUCN ver. 3.1) snakehead *Channa bleheri* Vierke, 1991 was carried out. Breeding of *C. bleheri* was tried under captivity by segregating paired males and females in aquariums. Three pairs of fishes bred successfully in the experiment. The fertilized eggs were non-adhesive, buoyant, and light yellow in colour with a diameter between 0.9 to 1.1 mm in diameter. The egg incubation period was between 32-34 hours. The fertilization and hatching rate was found between 84-87% and 78-84% respectively. Hatchlings appeared pale yellow in colour with well-defined yolk sac with transparent fin fold with fully functional heart measuring 2.8-3.2 mm long. Vigorous tail movement of larvae was seen with a well-defined yolk sac initially which got fully absorbed after 3 days. Post larval period of larvae started after yolk absorption and then the larvae started feeding exogenously. Aerial breathing started after 10 days and larvae reached a length of 1.2±0.27 cm. After 25 days larvae were seen to move in shoal and the length was 1.83±0.528 cm and resembles the adult fish in all aspects. Since the species is under constant threat due to its burgeoning demand in the ornamental fish trade, the culture protocol developed during the present investigation along with information on its rearing as well as embryonic and larval rearing will not only help in reducing pressure on the natural stocks but also pave way for the development of management and conservation strategies.

Key words: Captive breeding, *C. bleheri*, hatchlings, conservation

INTRODUCTION

Channa bleheri Vierke, 1991, an endemic snakehead from the upper Brahmaputra basin of Assam, is a colourful fish reported from certain pockets of Dibrugarh and Tinsukia districts of Assam and Dikrong River, Arunachal Pradesh, India (Vierke, 1991; Musikasinthorn, 2000; Courtenay Jr. & Williams, 2004; Vishwanath & Geetakumari, 2009). The fish is often harvested from its wild habitat as aquarium fish and traded in domestic and international ornamental fish markets (Praveenraj *et al*., 2019; Nayak *et al*., 2020; Harrington *et al*., 2022). Since, in India, harvesting of fish for the aquarium trade is entirely open access and unregulated (Raghavan *et al*., 2010); rampant exploitations have led to a rapid decline in its wild population and are currently assessed as Near Threatened (NT) in the IUCN Red List of Threatened Species (ver.3.1). Raghavan *et al*. (2013) enlisted *C. bleheri* as one of the endemic fishes which face a continuous threat from unregulated aquarium trade in India. The existence of unregulated and injudicious practice in the ornamental fish trade often damage these threatened fishes and their fragile ecosystems (Papavlasopoulou *et al*., 2013). To stop the further depletion of natural stocks of the species, immediate mitigative measures need to be implemented. Among the different measures available, captive breeding interventions have been regarded as a viable option to replenish many threatened fishes (Philippart, 1995; Sarkar *et al*., 2006).

Captive breeding operations in fishes are deemed important in management and conservation strategies as they provide critical life history information and supplement the restoration of extirpated populations (Rakes *et al*., 1999). The technique can contribute to conserving species that are faced with continuous extinction threats (Senanayake and Moyle, 1982; Philippart, 1995). Captive breeding interventions are a combination of several environmental signals that triggers the central nervous system to release the gonadotropin-releasing hormone (Yulintine *et al.*, 2017). It has always been a tedious task to breed fish in captivity as there is no natural environment to act as a stimulant. Among different species of *Channa*, versatility of breeding season is observed throughout the year leading to a paucity of specie-specific information on different species (Courtenay Jr. & Williams, 2004). Although there are many reports on the breeding of *Channa* species (Marimuthu *et al*., 2007; Hossain *et al*., 2008; Marimuthu *et al*., 2009; Marimuthu *et al*., 2011; Yulintine *et al*., 2017; Nayak *et al*., 2020) reports on breeding *Channa* species *sans* hormone induction is negligible. Among different attributes of captive propagation, embryonic and larval development is critical in understanding the basic biology, dietary pattern, and environmental preferences of fishes (Puvanesvari *et al*., 2009). Further, for large-scale seed production and successful larval rearing, understanding the development aspects of fishes is considered essential (Khan & Mollah, 1998; Rahman *et al*., 2004). Post yolk sac absorption, external feeding is deemed inevitable for

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further growth of larvae which can be achieved through external feeding interventions (Puvanesvari *et al.*, 2009). Another major constraint observed in the rearing of snakeheads and other fishes includes cannibalism which affects various age and size groups depending upon culture conditions and species (Smith & Reay, 1991; Kozłowski & Piotrowska, 2022). The phenomenon occurs mainly due to the unavailability of alternate food and size heterogeneity during culture that may be controlled through continuous sorting (Hecht & Appelbaum, 1988; Jensen, 1990). Variation in feeding preferences during their ontogeny makes it a herculean task for culturists in successful larval rearing of these species. It has been observed that due to a deficit in information on the culture techniques of snakehead fishes, there is a paucity of information to supplement large-scale seed production. With the increasing demand in the aquarium trade and threats to their natural habitats, sustainable utilization of *C. bleheri* is of utmost necessity to prevent the further decline of wild populations. Keeping this in view, the current investigation was carried out to evaluate the feasibility of captive rearing and monitoring the embryonic development and larval rearing of *C. bleheri* which would help address the existing threats constantly faced by the species.

MATERIALS AND METHODS

Collection of Specimen: Specimens of *C. bleheri* were collected from Maguri and Madhupur wetlands of Dibrugarh and Tinsukia district, Assam with the help of traditional gear 'Dingora' and 'Sepa' during September 2020, and reared in 'Ornamental Fish Breeding and Rearing Centre', Dibrugarh University, Dibrugarh. Rearing was done in glass aquariums measuring 2×1.5×1.5 ft with 127 L water capacity. The brooders were fed with live feed and commercial feed at the rate of 4-5 $g.kg^{-1}$ body weight. Commercial feed with 47% protein, 5% crude fat, 10% moisture, 17% ash content, 1% phosphorous, vitamin A 10,000 $IU.kg^{-1}$, vitamin D 2,500 $IU.kg^{-1}$, vitamin E 2,000 $IU.kg^{-1}$ and ascorbic acid 510 $mg.kg^{-1}$ was also used for feeding the brooders.

Monitoring water quality: Different water quality parameters were monitored regularly in the Freshwater Biology Laboratory, Department of Life Sciences, Dibrugarh University following APHA (2004).

Pairing of brooders: Four different aquariums sets were used for rearing brood specimens of *C. bleheri*. Each set contained 5 specimens. Male and female were segregated and reared separately i.e. two sets were male and two female. The identification of males and females was done based on observing the vent which is elongated in males and rounded in females. Apart from that pectoral fin in males appeared rough against smooth in females (Figure 1-4). Prior to breeding season, male fishes were brightly coloured as compared to female fishes. The breeding season was determined following Gogoi *et al.* (2013). The fishes of opposite sexes were introduced together in April 2021 and were observed to detect pairing in brooders. Three males and three females were introduced in 3 different sets of aquariums.

Figure 1. Elongated vent in male *C. bleheri*

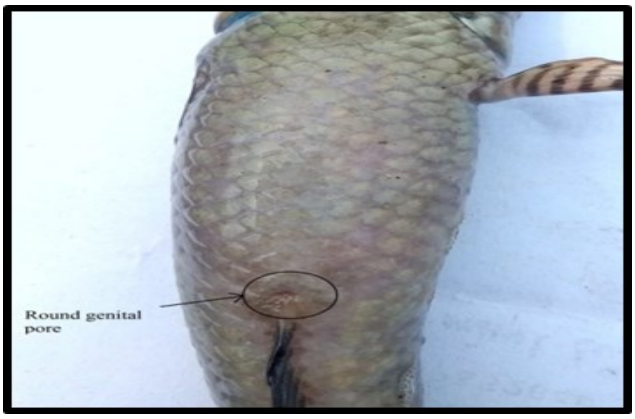

Figure 2. Elongated vent in male *C. bleheri*

Figure 3. Light colouration in female *C. bleheri* during breeding season

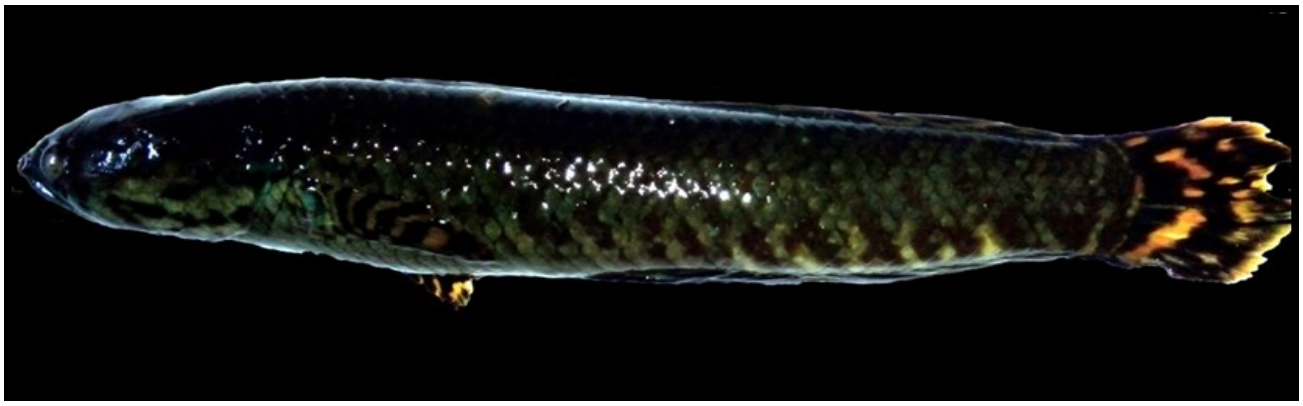

Figure 4. Dark colouration in male *C. bleheri* during breeding season

Release of eggs: After pairing, brooders were transferred to a different aquarium. Eggs were released within 10 days of the transfer. Length-weight of the brooders, fertilization rate, and hatching rate during the breeding operation was recorded (Table 1). Fertilisation rate and hatching rate were calculated by estimating the number of fertilised eggs as compared to total eggs while the latter was by the number of hatched eggs against total fertilised eggs.

Embryonic development study: After successful spawning, 20 developing eggs were sampled out and examined at 10 to 30 min intervals till hatching and after 4 h for the next 3 days followed by once a day until metamorphosis. Measurement of 10 specimens of different stages was taken and the average length was recorded each time. Some selective parameters like the appear

ance of germ ring, embryonic axis establishment, development of pigmentation, development of eyecups, tail separation from the yolk, and the number of myotomes or somites were taken for development study.

Larval developmental study: After yolk absorption, the larvae were fed twice at a fixed time with *Artemia* nauplii for 7 days. Stages of larval development and age were monitored at definite intervals. During each sampling, at least 5 to 10 eggs or larvae were measured. Diameter and length measurement was recorded under a microscope (LEICA DM 750). Observation of different egg and larval stages were captured to record a synchronous development in the early development of the fish. Larval rearing was carried out in aquariums similar to brooders' tanks. The leftover food and faeces were siphoned out the next morning. During rearing, the larvae

.were with *Artemia* nauplii for the first eight days post yolk-sac absorption. For the next 9^{th}-16^{th} day, the larval diet consisted of *Artemia* nauplii and egg yolk. From the 17^{th} day onwards, the larvae were fed with a high protein commercial diet containing 35-40 % protein, 3.5 -4% ash content, 8.2 % moisture content, and 7.5% fibre content manufactured by Aqua Paradise, Dibrugarh.

Commercial salt lake *Artemia* cysts were used for its culture under continuous 24-hour aeration, 25-30 ppt salt water, and 5 W bulb. Initially, 2 capsules measuring 1.28 g were cultured in 3 L of water, which was ultimately increased to 3.12 gm cyst in 5 L of water and fed twice a day at 10.00 h and 17.00 h. The cysts hatched in 24-28 hours and the nauplii (density 60,000-65000 L^{-1}) were collected by covering the surfaces of glass containers with black paper with a small area exposed to light which was finally siphoned out and poured into some freshwater tanks.

RESULTS

Successful breeding of *C. bleheri* was observed during the present investigation through habitat manipulation practices from late April to mid-May. The fish bred at a temperature range of 26-27ºC and at a slightly alkaline pH. The details of brooders, fertilisation and hatching rate as well as the abiotic factors recorded during the day of breeding are given in Table 1 and Table 2. Initial development of the egg after fertilization was observed after 20-30 mins. The fertilized egg then undergoes further development until hatching at 32-34 hours after fertilization. Post hatching, the larvae were seen accompanying their parents. After absorption of yolk sac, they fed readily with *Artemia*. In the later stages of they were to consume artificial diet mixed with *Artemia*.

Table 1. Length-weight of segregated pairs, fertilisation rate, hatching rate observed at the day of breeding.

Date	Length of brooders (cm)		Weight of Brooders (g)		Fertilisation rate	Hatching rate
	Male	Female	Male	Female		
28^{th} April, 2021	10.2	13.8	17.23	25.33	84%	78%
9^{th} May, 2021	10.4	14.2	16.78	27.84	80%	83%
12^{th} May, 2021	14.2	13.9	28.90	27.82	87%	84%

Table 2. Water parameters recorded on the day of breeding of *C. bleheri* without hormone stimulation

Date	Water Temperature(°C)	pH	Alkalinity (ppm)	Hardness (ppm)
28th April, 2021	26.2°C	7.3	45	41
9th May, 2021	26.5°C	7.3	48	39
12th May, 2021	27.7°C	7.2	52	44

Table 3. Different events of embryonic development recorded in *C. bleheri*

Time after spawning	Stage	Description
0 min	Fertilized egg	Fertilized eggs were non-adhesive, spherical, transparent, pale yellow in colour with a diameter between 0.9-1.1 mm in diameter (Figure 5)
20-30 minutes	Blastodisc formation	The first cleavage divides the blastodisc into two blastomeres (Figure 6).
45-60 minutes	4 celled stage	Second cleavage occurs (Figure 7)
60-70 minutes	8 celled stage	Third cleavage occurs (Figure 8)
90-120 minutes	16 celled stage	Fourth cleavage (Figure 9)
4-4.30 hour	Morula	Formation of multicellular blastodisc, progression of blastulation formation (Figure 10)
6-6.30 hour	Blastula	Formation of embryonic shield, half of the yolk gets invaded, differentiation of anterior and posterior end becomes evident (Figure 13)
8.30-9 hour	Gastrula	Invasion of yolk becomes complete, with formation of two-layered structure i.e. outer epiblast and inner hypoblast (Figure 14).
14-14.30 hour	Post- gastrula	Formation of germinal ring complete, embryonic shield gets established at this stage
18-18.30 hour	Early neurula	Cephalic regions becomes broader, forehead becomes distinct
22-22.30 hour	Neurula somite	Distinct embryonic rudiment, optic vesicles and two myotomes were demarcated.
23-23.30 hour	Late neurula	Formation of 6 myotomes, appearance of melanophores, notochord was formed, rudimentary heart appeared
25-26.30 hour	9-15 myotomes	Embryo occupied whole space in the egg, circulation of blood was observed, thickening of ectoderm to form eye lens (Figure 15).
27-28.30 hour	15-22 myotomes	Fully formed eye lens in the olfactory vesicle, heartbeat count at the rate 108-115/min, appearance of few melanophores over yolk sac (Figure 18).
31-31.30 hour	Pre -hatched embryo	Concretions on auditory vesicles, formation olfactory pits, colourless blood, heart beat at the rate 125-130/min, disappearance of Kupfer's vesicles (Figure 19).
32-34 hour	Hatching	Hatching of embryo starts (Figure 20)

Table 4. Different events of larval development recorded in *C. bleheri*.

Time after hatching	Description and behaviour
0 h (at hatching)	The newly hatched embryo appeared pale yellow in colour with well defined yolk sac with transparent fin fold that encircle the body. Heart was fully functional without mouth and anus. the average length of the larvae measured 2.8-3.2 mm long, vigorously moving its tail, lying inclined at the surface
4 h larvae	It measured 3-3.3 mm in length with unpigmented eyes and conspicuous depression identifies as position of mouth, larvae found in cluster (Figure 21).
8 h larvae	3.3-3.4 mm in length, brain and heart distinct, dorso-ventral unpaired fin was observed, sensitive to light and negatively phototrophic.
16 h larvae	The larvae at this stage measured 3.8-4 mm in length, pulsation of heart becomes clear, auditory capsule near eye prominent (Figure 22).
24 hour larvae	Invagination of buccal cavity was seen, pectoral fin and air bladder develops, larvae measured 4.1-4.3 mm, caudal din starts separating (Figure 23).
36 h larvae	Larvae measured 4.4-4.8 mm in length, rounded pectoral fin, less developed lower jaw, little yolk reserve remained (Figure 24).
48 h larvae	Average length 4.8-5.2 mm, mouth with fully developed lower jaw, yolk sac greatly decreased, larvae starts feeding exogenously, rudimentary gills appeared, larvae move in shoal clinging at the surface f the water for most of the time, paddle like pectoral fin
3 day old larvae	Eye balls move freely, body bilobed, yolk sac fully absorbed, larvae measured 5.2-5.6 mm in length, head becomes prominent
6 day old larvae	Larvae measured 0.65-7.2 mm in length. Head appeared black and pigmented from above with clear melanophores on dorsal side (Figure 25). Distinct and large eye balls could be seen at this stage. The pectoral and caudal fin appeared large and rounded and the fin rays could be clearly visible (Figure 26).
10 day old larvae	The larvae attained a length of 1.2±0.27 cm at this stage. The separation of rays was more prominent and counted 11 in both pectoral and caudal fins. Dorsal fin and anal fin could be seen developed and counted 21 on both the fins. Aerial breathing of the larvae could be seen at this stage (Figure 27)
25 day old larvae	The larvae at this stage measured 1.83±0.528 cm in length. The scales at the head became prominent at this stage and larvae could forage efficiently for food. Larvae tend to hide under surface of filter and in other artificial hideouts provided in the aquarium (Figure 29).

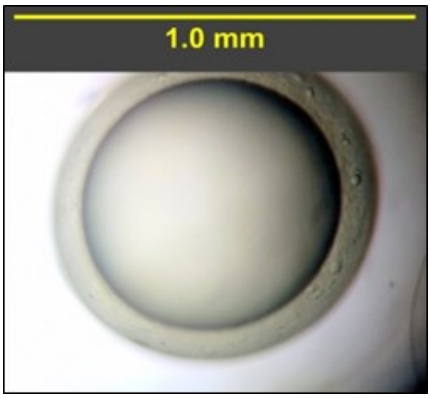

Figure 5. Fertilized egg

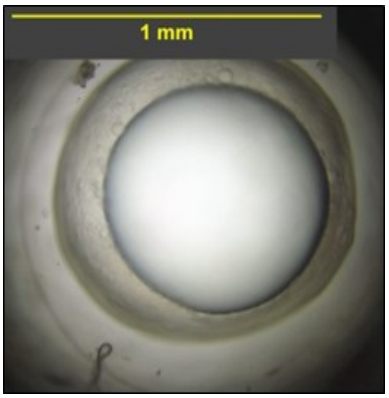

Figure 6. First cleavage stage

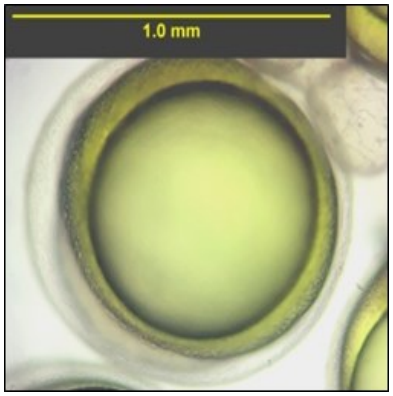

Figure 7. Second cleavage

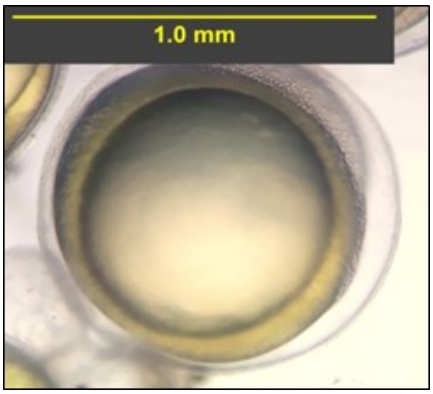

Figure 8. Third cleavage

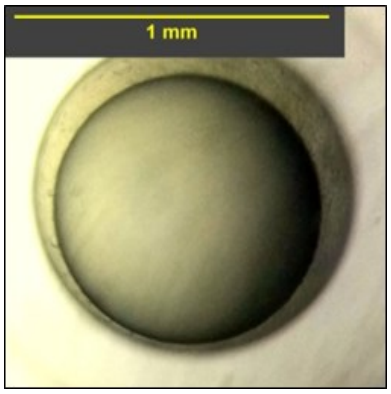

Figure 9. Fourth cleavage

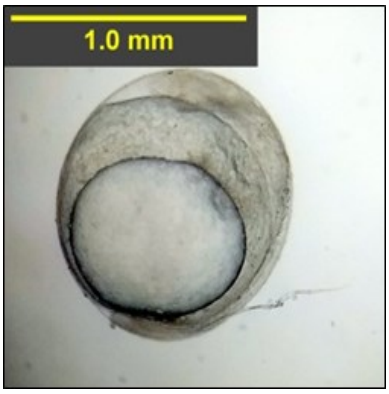

Figure 10. Morula stage

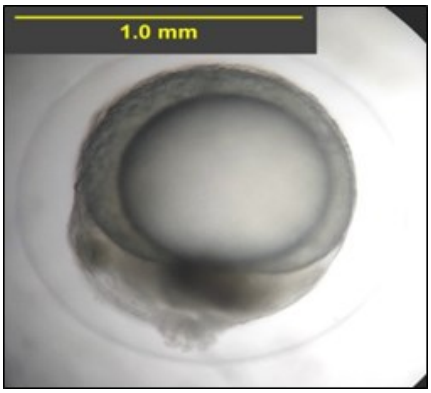

Figure 11. Multi cell stage

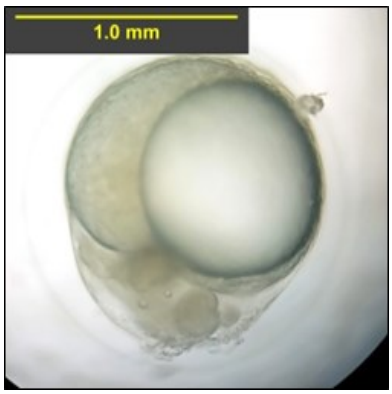

Figure 12. 14 hour old stage

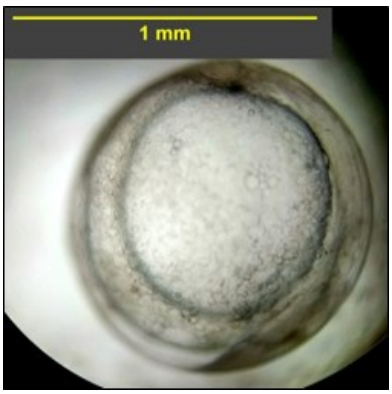

Figure 13. Blastula

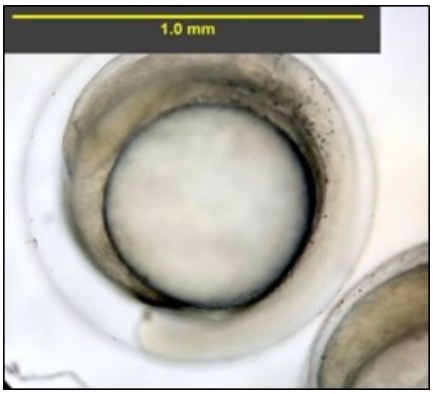

Figure 14. Gastrula Stage

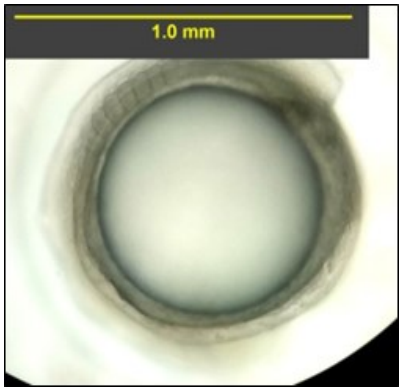

Figure 15. 9 Myotome Stage

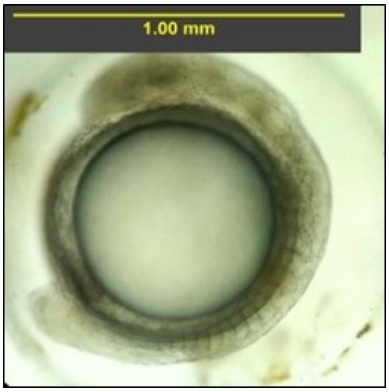

Figure 16. 16 Myotome stage

Figure 17. Yolk plug stage

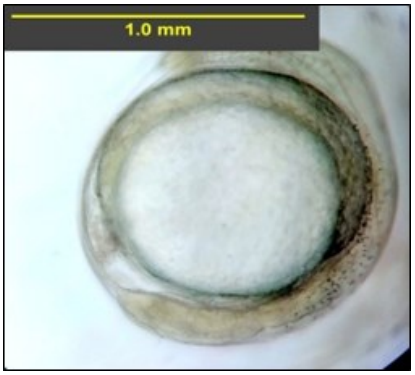

Figure 18. 22 Myotome stage

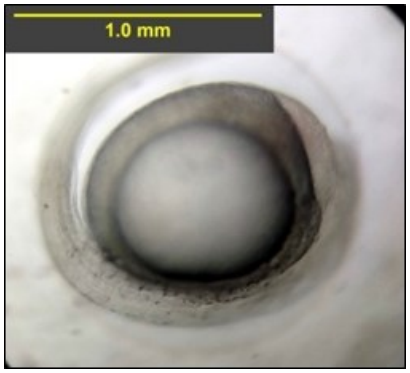

Figure 19. Pre-hatching

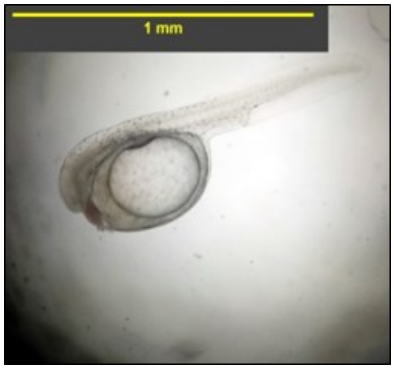

Figure 20. Newly hatched larvae

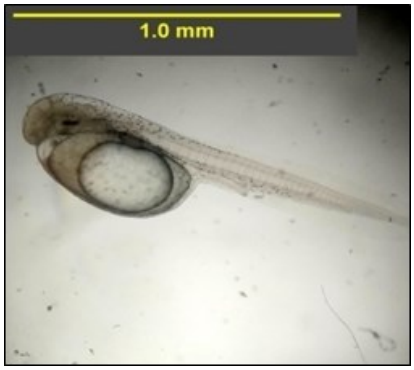

Figure 21. 4 hour old larvae

Figure 22. 16 hour old larvae

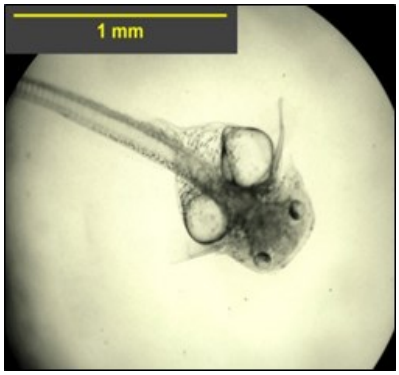

Figure 23. 24 hour old larvae

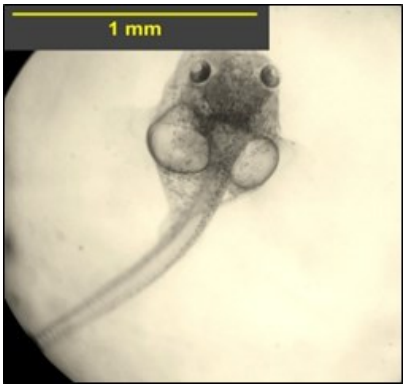

Figure 24. 36 hour old larvae

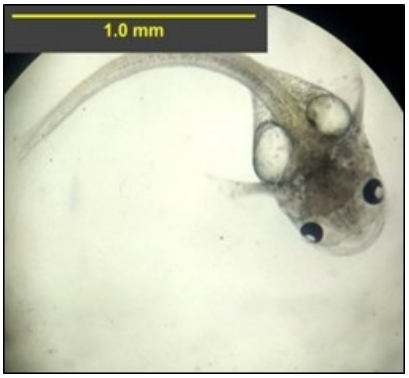

Figure 25. 3 day old larvae

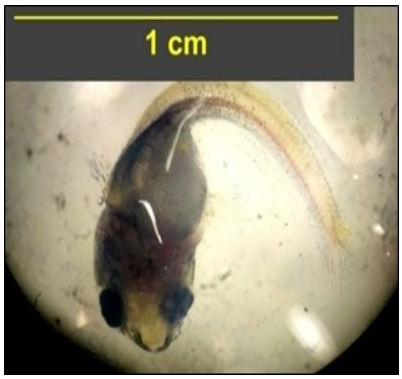

Figure 26. 6 day old larvae

Figure 27. 10 hour old larvae

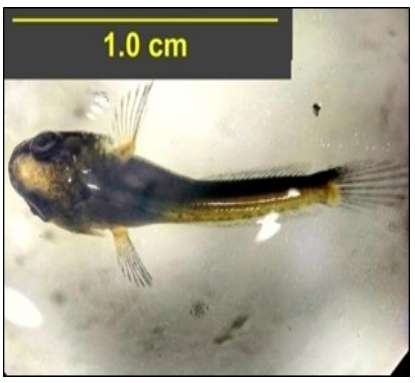

Figure 28. 15 day old larvae

Figure 29. 25 day old larvae

Figure 30. 40 day old larvae

DISCUSSION

The present investigation elucidated the efficacy of habitat manipulation techniques for captive propagation of *C. bleheri*. The findings bear significance as the species is reeling under constant pressure to meet its burgeoning demand in the ornamental fish industry. Successful spawning was observed in aquarium conditions that were created by simulating their natural habitats (Table 2). It was observed that maintenance of water quality during rearing environment plays a crucial role in successful breeding in captive conditions along with other biotic factors. The species bred in experimental setups *sans* any vegetation indicating that they were not mandatory to achieve successful spawning in aquariums. Similar findings have also been reported in other *Channa* species (Alikuhni, 1953; Parmeswaran & Murugeshan, 1976; Wee, 1982). Among the different water parameters considered in the study, the water temperature was found to play a pivotal role in inducing breeding in the species. The water temperature was maintained between 26-28°C during the breeding season, while during the whole culture, it was maintained between 25-29°C. Successful breeding of fishes in captivity has been reported to occur at 25-31°C (Khan, 1924; Marimuthu *et al*., 2007; Yulintine *et al*., 2017). As Channid species are believed to be very sensitive towards changes in water quality, utmost care was taken that water parameters to prevent drastic change during breeding. The brooders of *C. bleheri* showed marked reproductive behaviour during the rearing period. Males and females were seen pairing upon their release in the breeding tanks. Pairing in snakeheads is a significant reproductive behaviour shown by the species of *Channa* (Okada, 1960; Courtenay Jr. & Williams, 2004) which mostly culminates in successful breeding. Post pairing, the released eggs appeared like foam floating on the water surface and constantly guarded by the parents. It was observed that *C. bleheri* carried their eggs and their hatchlings in their mouth while experiencing external threats. Marked parental care behaviour has been reported among other Channid species as well.

Some *Channa* species have been reported to be mouth brooders while others vigorously guard their eggs and do not hesitate to attack even larger predators (Kottelat *et al*., 1993; Courtenay Jr. & Williams, 2004). The fertilization (87%) and hatching rates (84%) were found to be higher in females of larger sizes. In species such as *C. striata*, *C. marulius*, *C. argus*, etc. an increase in fecundity with body length has been reported (Quayyum and Qasim, 1962; Jhingran, 1984). Nayak *et al.* (2020) earlier reported 82% fertilization and 85% hatching rate in *C. bleheri* that were induced through the administration of synthetic hormone. In contrast, fertilization rate was 87% and hatching rate was 84 % during the present investigation thereby indicating a better breeding performance of *C. bleheri* when induced without hormone administration.

A common problem encountered in snakehead culture is cannibalism which often leads to a low survivability rate in newly hatched larvae (Ng & Lim, 1990; Qin *et al*., 1997). However, in our culture cannibalism was not observed. Sufficient external feeding supplied every day and keeping the parents together during the culture could be a plausible reason. Parents were seen to carry the larvae together as well as helping the larvae in their browsing ability which minimised the danger of cannibalism. Cannibalism is observed in cultures where larvae are kept separately or where they didn't accept the artificial diet (Qin *et al*., 1997; Haniffa *et al*., 2003; Purnamawati *et al.*, 2017). Apart from this, cannibalistic behaviour in fishes is also influenced by biotic factors such as fish size, stocking densities etc. as well as by abiotic factors such as feed ration, water temperature, lighting intensity, and tank shape and color etc. (Kestemont *et al*., 2003 ;Szczepkowski, 2009; Naumowicz *et al*., 2017). Hence, it is suggested that early segregation of the larvae from their parents should be avoided to avoid cannibalism in *C. bleheri*.

The development of eggs and larvae was also monitored during the present investigation. The stages observed during the development of *C. bleheri* were similar to

those found in other teleosts (Thakur *et al.*, 1974; Parameshwaran & Murugesan, 1976). In the present study, the first cleavage (2-celled stage) was found within 20-30 minutes followed by the second (4-celled stage), third (8-celled stage), and fourth (16-celled stage) cleavage in 45-60, 60-70 and 90-120 minutes post fertilization. However, species-specific variations in development stages have also been reported in some snakehead species. In *C. punctata* the first cleavage division was seen in 15 minutes and 15-20 minutes in *C. striata* (Haniffa *et al.*, 2003; Marimuthu *et al.*, 2007). Egg development reached the morula, blastula, and gastrula stages in 4-4.30 hours, 6-6.30 hours, and 8.30-9.00 hours respectively. In contrast to that, in *C. punctata* morula stage was observed in 2 hours, 3.15 hours, and 5 hours, while *C. striata* took 1.30-2 hours, 5-6 hours and 8-9 hours post-fertilization (Haniffa *et al.*, 2003; Marimuthu *et al.*, 2007). A decrease in the size of blastomeres was seen in *C. bleheri* as well as in all teleosts as cell division progresses (Mookerjee & Mazumdar, 1946).

The process of neurulation started after the axis formation. Early neurula was formed in 18-18.30 hours and late neurula in 23-23.30 hours followed by the formation of somites or myotomes. Somites numbering 6-9 were seen after 26 hours, 16-18 in 27 hours and 22-24 in 28 hours in *C. bleheri.* Neurulation in *C. striata* early initiated in 9.30-10 hours, late neurula in 13-14 hours, 10 somites in 15-16 hours, 15 myotomes in 17-18 hours, 22 myotomes in 20 hours (Marimuthu *et al*., 2007). The process of neurulation started in 9 hours in *C. punctata* followed by the formation of 6 somites in 14 hours, 13-15 in 18 hours, and 22 in 20 hours (Haniffa *et al*., 2003).

Variations in hatching in fishes have also been reported where the tail comes out first in some species, while the head comes out first in some (Mookerjee and Mazumdar, 1946; Thakur *et al*., 1974; Jacob, 2005). In *C. bleheri* tail comes out first followed by the head during hatching. Hatching was observed 32-34 hours post-fertilization at a temperature range of 26-29°C. The newly hatched larvae measured 2.8-3.2mm at hatching, 3-3.3mm after 4 hours, 3.3-3.4mm after 8 hours, 3.8-4mm in 16 hours, 4.1-4.3 after 24 hours, 4.4-4.8mm after 36 hours when the yolk reserve decreased to the minimum. In other teleosts, the length of larvae at hatching measured 4.09-4.9 mm in *H. fossilis,* 5.8 mm in *C. batrachus,* and 3.6 mm in *C. gariepinus* (Mookerjee & Mazumdar, 1950; Bruton, 1979; Ogunji & Rahe, 1999). Bagarinao *et al.* (1986) suggested that the difference in the size of eggs was responsible for such a length variation as egg diameters were positively correlated to the length and weight of larvae at hatching. Due to the phagocytic activity of the inner syncytial layer, the yolk is absorbed which is degraded to smaller substances to be easily carried by blood (Kamler, 1992). In the current study, the yolk was absorbed after 48 hours or 2 days and external feeding was given thereafter. The yolk sac attached to the larvae keeps them independent from external feeding and the time of yolk absorption varied among species which depends on temperature (Bagenal & Braum, 1968). In teleosts like *H. longifilis* the yolk sac absorbed fully in 55 hour (Ogunji & Rahe, 1999), on the 4th day in *Clarias lazera*, 3rd day in *Mystus macropterus*, 3rd day in *C. striata*, 3rd day in *A. testudineus,* 3rd day in *C. punctata*, and 3rd day in *Mystus montanus* (Ogunji & Rahe, 1999; Haniffa *et al.* 2003; Jacob, 2005; Marimuthu *et al.*, 2007). The length of *C. bleheri* larvae after yolk absorption measured 4.8-5.2 mm in length. Parmeswaran & Murugesan (1976) pointed out the length of different snakeheads after yolk absorption as 6.8-7.1 mm in *C. marulius*, 5.3-6.1 mm in *C. striata*, and 4.6-4.9 mm in *C. punctata.*

Bagenal & Braum (1968) reported catastrophic death of larvae during the 'critical period' of development i.e. time during which larvae shift to external feeding after yolk absorption. The unavailability of suitable feed at the beginning of external feeding was the major reason for mortality during this period. However, during our culture, no such causalities were found and larvae grew with a good survival rate. Meske (1984) reported a higher survival rate of larvae when fed with *Artemia.* Jacob (2005) found better growth and survival rate in fishes fed with *Artemia* as compared to larvae fed with egg yolk. In the present study, the larvae were initially fed with *Artemia* for 7 days followed by feeding them with *Artemia* and egg yolk for the next week. The larvae after yolk absorption measured 4.8-5.2 mm in length. *C. bleheri* started to feed readily on artificial feed after 15 days. The inclusion of egg yolk after the 9th day along with *Artemia* helped the larvae adapt to a non-motile diet. The larvae were fed twice a day at a fixed time so that they do not starve for food. A gradual mixing of motile and non-motile diets helped the larvae adapt to an artificial diet quickly in snakehead larvae (Qin *et al*., 1997). Longer rearing of high-density larvae without proper feeding has also been regarded as a major reason for cannibalism (Nickum & Stickney, 1993).

The development of the eye, fins, and mouth in larvae is very important as it will aid them to locate and forage for food sources easily. Eye lens developed fully in our culture after 27-28.30 hours post fertilization before hatching, pectoral and caudal fin developed fully after 24 hours of hatching, and full development of mouth after 48 hours or on the 2nd day of hatching. The development of these organs made the larvae swim efficiently and move toward their prey. Meanwhile, the eye appeared 6 hours post fertilization; fins appeared after 24 hours of hatching while the mouth developed fully on the 2nd day of hatching in *A. testudineus* (Jacob, 2005). In *C. punctata* eye formed 20 hours post fertilization, the fins after 10 hours of hatching, and the mouth on the second day of hatching (Haniffa *et al*., 2003). In *C. striata* eye developed fully 20 hours post fertilization, fins after 14 hours of hatching and 2nd day of hatching (Marimuthu *et al*., 2007). Aerial breathing of the larvae was seen after 12 days of hatching in our

culture, which was observed on the 10th day in *C. striata* (Marimuthu *et al*., 2007), 12-13 days in *A. testudineus* (Datta Munshi & Hughes, 1991), 14th day in *C. marulius* (Parmeswaran & Murugeshan, 1976), 5th day in *H. fossilis* and on 10th day in *C. batrachus* (Landge, 1995). The movement of larvae to capture their prey improved greatly after 15 days at length 1.3±0.34, while *C. striata* efficiently captured the prey after 10th day.

In conclusion, the present investigation demonstrated the efficacy of habitat manipulation in inducing breeding of *C. bleheri* reared in captivity. Temperature along with other water parameters played a crucial role in successful breeding even without administration of synthetic hormone. Novel findings during the study on rearing, diet and early development of the species will prove crucial for the species as the species is under increased conservation threat due to its demand in the ornamental fish industry. Moreover, the protocol highlighted will not help in formulating management and conservation strategies but will also aid those fish hobbyist to propagate the species without depending on natural stocks in near future.

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