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EARLY EMBRYONIC AND LARVAL DEVELOPMENT OF THREATENED HUMPED FEATHERBACK, *CHITALA CHITALA* (HAMILTON)

Durin Akhter Jahan[1]*, Joniara Rasid[1], Md. Mominuzzaman Khan[1] and Yahia Mahmud[2]
[1]Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh-2201, Bangladesh
[2]Bangladesh Fisheries Research Institute, Head Quarter, Mymensingh-2201, Bangladesh
*Correspondence: E-mail: durin-bfri@yahoo.com

ABSTRACT
The embryonic and larval development of humped featherback, *Chitala chitala* was studied from fertilization until metamorphosis. This study presents preliminary observations on the embryonic and larval development of *C. chitala* under laboratory conditions. The eggs were obtained through controlled breeding under captive condition. Fertilized eggs were adhesive, spherical and lacked oil globule in the yolk sphere with a diameter of 4.2 – 5.0 mm. At a temperature of 28-31°C, *hatching* was completed *within* 6-7 *days* of *fertilization.* The newly hatched larvae were 15 ±0.2 mm in length with large yolk-sac. The yolk-sac was completely absorbed by the larva 7-8 days after hatching. At the same time the digestive system became fully developed and the larvae searched for feeding. The study aims at a standard description of early developmental stages that will provide valuable information towards large scale seed production and aquaculture of this threatened fish species.

KEY WORDS: Embryo development, larva, metamorphosis, threatened, *featherback, Chitala chitala.*

INTRODUCTION

The Humped Featherback, *Chitala chitala* (Hamilton, 1822) locally known as 'Chital' is one of the most demanded freshwater fish in Bangladesh. It is popularly known as clown 'knife fish' (Notopteridae: Osteoglossiformes) commonly occurs in Bangladesh, India, Pakistan, Myanmar and Philippines (Bhuiyan, 1964; Talwar and Jhingran, 1991). In Bangladesh the species is available in freshwater bodies such as rivers, beels, canals, reservoirs, ponds etc. particularly in large rivers (Rahman, 1989; Talwar and Jhingran, 1991). The flesh is of good flavor and commercially important and has been prioritized as food, sport, aquarium, and highly priced cultivable fish (Sarkar *et al.*, 2006). Always this fish is marketed in fresh condition. A *traditional* Bengali recipe of '*fish* kofta' with *Chital* is very popular in our country. Recently abundance of this species in nature has declined due to heavy fishing pressure, habitat destruction, aquatic pollution and indiscriminate uses of pesticides. It is now a threatened species in Bangladesh based on the IUCN red list categories (IUCN, 2000). As a result breeding grounds are losing their suitability to be used by the species posing a great threat of extinction and till now it is not possible to collect the fry of this fish from the river system where they breed naturally. Information on early embryonic and larval development and organogeny is of critical importance in understanding the basic biology of a particular species and their dietary needs and environmental preferences (Koumoundouros *et al.*, 2001; Borcato *et al.*, 2004). Further, studies on embryonic and early larval development are imperative and consequential to the successful rearing of larvae for large scale seed production and aquaculture (Khan and Mollah, 1998; Rahman *et al.*, 2004).

Although *Chital* is a tasty, commercially important endangered fish of Indian subcontinent, published reports on its induced breeding, developmental biology and larvae rearing are quite scanty. Very limited research has been conducted on *C. chitala*. Earlier attempts have been made to study its reproductive biology (Kohinoor *et al.*, 2012), captive breeding (Hossain *et al.*, 2006; Sarker *et al.*, 2006), some of the biological parameters (Sarker *et al.*, 2008) and effects of climate change on the occurrence of *C. chitala* (Banik and Roy, 2014) of this fish have been investigated. The major concern of any seed production and hatchery system is to produce the maximum number of quality fingerlings from the available brood stock for aquaculture. The fry of chital is not available throughout the year for growing out in the farms for producing marketable size fish, which indicates significance for developing seed production technology of this species. So, it was felt necessary to study and characterize its various stages of embryonic and larval development to understand the biological clock of the species, identify the early life history stages and to detect first feeding time. The present work is the preliminary attempt and is expected to serve as a basis for further and more intensive future studies. An attempt was made to conduct this study to investigate and to provide the detailed information of the embryonic and larval development of *C. chitala*. This biological information will allow us to take appropriate measures for optimization of large scale seed production, culture and management of the species.

MATERIAL AND METHODS

This experimental works were carried out in the hatchery cum breeding field complex and the laboratory of the Freshwater Station (FS), Bangladesh Fisheries Research Institute (BFRI), Mymensingh, Bangladesh.

Controlled breeding in captivity:

Controlled breeding of *C. chitala* took place in the breeding field complex under captive condition. During early March, after preparing the experimental pond (following all scientific management measures including fertilization, liming, supplementary feeding and water management) GIFT tilapia broods had been stocked @ 10,000 ha^{-1} at sex ratio 2:1 (female: male) with SIS (Small Indigenous Species) of fish 25 $kgha^{-1}$. Auto-stocking tilapia and SIS provided adequate natural food to *C. chitala* to satisfy its voracious feeding habit on them (Agrawal and Tyagi, 1969; Sharma and Chandy, 1961). Brood tilapia and SIS were fed with supplementary feed containing 30% crude protein. Water was exchanged 10-15% fortnightly. The water quality parameters: pH 6.6-7.5, dissolved oxygen 5-6 mg/L and temperature 28ºC-31ºC were observed in the brood pond. In April 2013, the collected sexually matured brood fishes of *C. chitala* weighing from 2.5 kg to 4kg were then released into the earthen breeding pond (0.08 ha) at a sex ratio 1:2 (female: male). Matured male fish were identified by a slightly pointed genital papilla, and females by a swollen abdomen and a reddish swollen vent. In addition, the maturity of the female was confirmed by a slightly pressing the ventral side of the fish for oozing of eggs. Breeding pool was prepared during the third week of July, where wooden boards (27" × 7" × 1.5") were used as egg collector device. Approximately 48 to 72 h after breeding pool preparation, the substrates were checked for their ovulatory response. After fertilization, the swollen eggs attached readily to substrates (Figure 1). The fertilized eggs attached with substrate were immediately transferred to three cemented tank containing glass nylon hapa with continuous water supply throughout the incubation phase.

Figure 1. Deposited fertilized eggs on wooden substrate

Embryonic and larval development:

In the present study, the developmental stages were divided into embryonic and larval development. The embryonic stage occurs inside the chorion and ends in hatching. The larval stage is characterized by nutritive contribution of the yolk sac and the stage ends when the larva becomes capable of exogenous feeding. 24 h after collection, the unfertilized eggs turned whitish in color while the fertilized eggs were yellow in colour. The unfertilized eggs were removed carefully from the incubation tank. After that, the eggs were examined under a microscope to see whether the blastodisc had formed as an indication of successful fertilization. Twenty developing eggs were sampled at 12 h intervals until hatching and once a day until metamorphosis. Each sample was observed four times to identify the developmental stages (Haniffa *et al.*, 2003). The diameters of the eggs were measured by using an ocular micrometer. The hatching of fertilized eggs was completed within 6-7 days. The total lengths of 20 randomly selected individual larvae were measured at each sampling time (TL is the length from the tip of snout to the end of the caudal fin; Crawford, 1986). The observation of egg and larvae were carried out and photographs were subsequently taken using a compound binocular microscope (BX51, Olympus) till the end of the larval period.

RESULTS

Embryonic development

Unfertilized and fertilized eggs:

The unfertilized eggs of *C. chitala* were opaque, spherical and whitish in colour measuring 2.0 to 2.5 mm in diameter (Fig. 2a). While the fertilized eggs were *cream color*, transparent, spherical, and adhesive in nature (Fig. 2b).

Immediately after fertilization the diameter of the egg increased *owing* to slight swelling of the egg which ranged between 4.2 to 5.0 mm (Fig. 2c).

Formation of embryo:
The eggs of *C. chitala* reached slowly in their embryonic and larval stages. At day 1, the yolk sacs get compressed and the blastodisc had formed as an indication of successful fertilization (Fig. 2c). The egg shell of *C. chiala* was thick enough as a result; the early cleavage stages of embryonic development were not clearly visible under microscope at day 2. But the embryonic rudiments were noticeable 3 day after fertilization (Fig. 2d).

Table 1. Brief descriptions of the embryonic developmental stages of *C. chitala*

Developmental stage	Phase	Diameter mm	Time after fertilization (day)	Developmental Landmarks
I	Unfertilized egg	2.0-2.5	0	-Eggs opaque, spherical and whitish in color
II	Fertilized egg	4.2-4.5	0	-Eggs *cream color*, transparent, spherical, adhesive and lacked oil globule in the yolk sphere
III	Blastodisc formation	4.2-4.5	1	-Blastodisc was noticeable
IV	Early cleavage stages	4.2-4.5	2	-Not clearly visible
V	Embryonic rudiments	4.6-4.7	3	-The embryonic rudiments were noticeable
VI	Rudiments of notochord heart formation	4.6-4.7	4	-Rudiments of notochord appeared -Heart was vibrating actively and blood circulation was started -Movement of tail but head attached with egg capsule
VII	Eye formation and twisting movement	4.8-5.0	5	-Brain and eye formation -Twisting movement was noticeable
VIII	just before hatching	4.8-5.0	6	-Twisting movement become more vigorous and the embryo ruptured the egg capsule, started hatching.

When the embryo gets 4 day old the cephalic region becomes prominent and rudiments of notochord and the tubular heart was appeared underneath the head and the heart actively started the blood circulation (Fig. 2e). At day 5, the brain and optic bud were noticeable (Fig. 2f). At the same time, the yolk mass showed elongation and slow twitching movement was noticeable when the embryo was 5 day old. Before hatching (Fig. 2h), the embryo started vigorous twisting movement inside the egg and continuously beat the egg shell by the caudal region especially around the middle part of the body (day 6). This movement gradually became vigorous and the egg capsules were weakened. The incubation period was from 6-7 d at water temperature range of 28-31°C. However, it took about 7 days for the whole brood of egg to hatch out.

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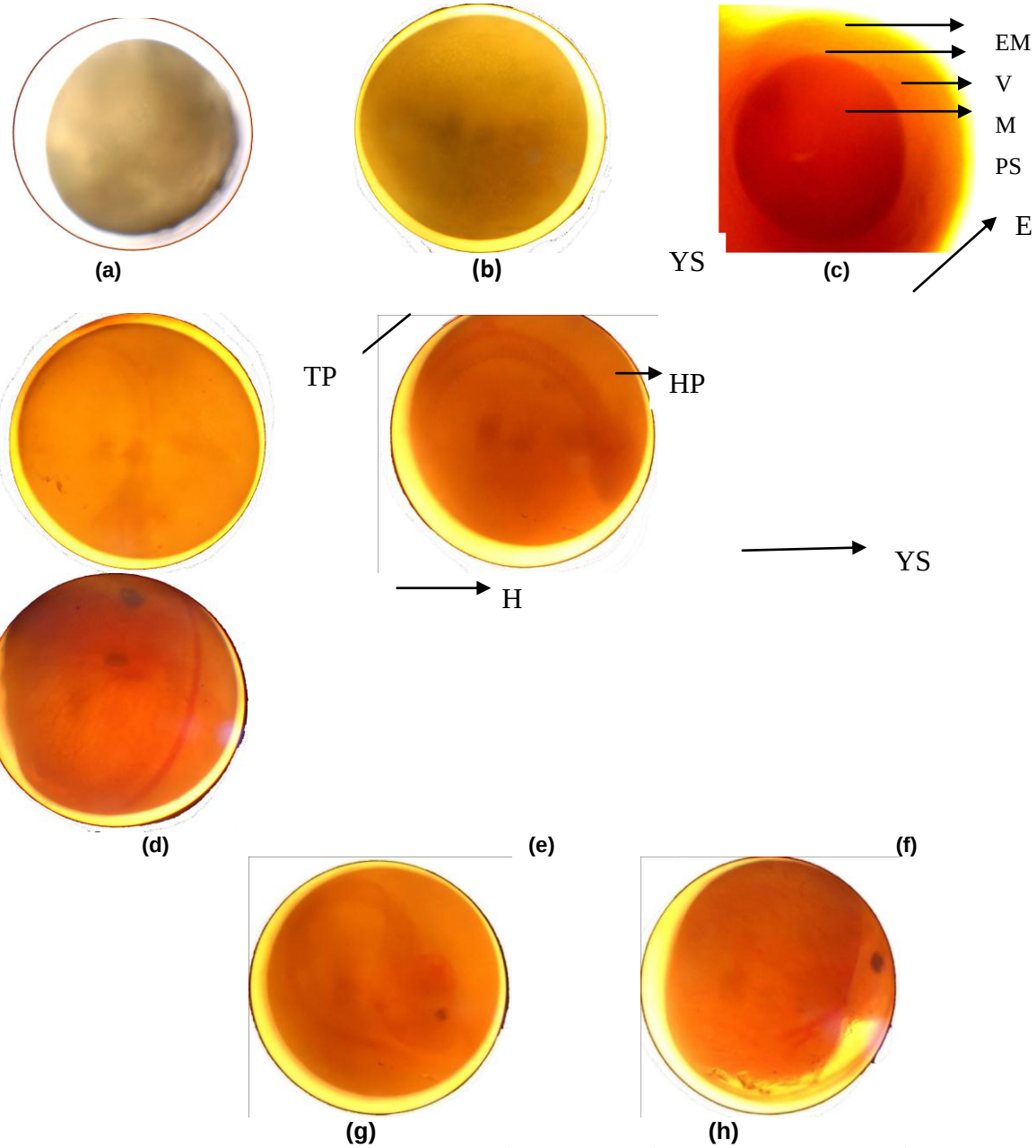

Figure 2. Embryonic developmental stages of *C. chitala* (a) unfertilized egg, (b) fertilized egg, (c) blastodisc formation stage (d) embryonic rudiments stage, (e) rudiments of notochord and starting of heart beat stage (f) eye formation (g) starting of twisting movement, (g) just before hatching. (EM- Egg membrane, VM- Vitelline membrane, PS- Perivitelline space, HP- Head portion, TP- Tail portion, H- Heart, Y- Yolk, YS- Yolk sac)

Larval development:

A larva (Latin; plural larvae) is a young form of animal with indirect development going through or undergoing metamorphosis (for example, insects, amphibians or cnidarians). The larva looks completely different from the adult form (Figure 3).

Newly hatched larva (1-2 d): The newly hatched larvae were transparent, faintly reddish in color, 15.5±0.2 mm in length and 0.05g in weight. The head and body of larvae were bent around the large yolk sac and attached to the substratum. The yolk sac was oval in shape and yellowish in color. The diameter of yolk sac was 4.85mm. A functional heart with blood circulation was noticed. The mouth was not developed but eye was clearly visible and notochord rudimentary.

3-4 d: The average length and weight of the larvae were 18.2 ± 0.2 mm and 0.08g ± 0.05 respectively. The diameter of yolk sac was 3.64 mm. Head still remain adhere with yolk sac. The heart becomes more distinct. Circulation of body fluid was seen around the notochord in addition to the yolk. The mouth appeared, upper and lower jaw were clearly visible. The pigmentation was dark in the anterior region and malanophores are scattered on the yolk sac. Three to four days after hatching, the caudal fin fold was noticed.

5-6 d: The larvae were 22.7 ± 0.1mm in length and 0.09 g ± 0.02 in weight. The bulged yolk became gradually elongated at this stage. Yolk sac became smaller. The diameter of the yolk sac was 2.37 mm at this stage. The alimentary canal became thicker and convoluted. Head well developed and notochord increased. A thin membranous fin fold surrounded the caudal region and extended up to yolk sac. At this stage larvae started to get detached from the hard substratum. The larvae were converged in a cluster and a few of them started swimming.

7-8 d: Average length and weight at this stage was 25.8 mm ± 1.09 and 0.10 g ± 0.05 respectively. Yolk sac was absorbed by most of the larvae. Body color of the larvae was light brown with clearly visible notochord with some dark pigmentation. All the fins like dorsal, pectoral and anal were clearly visible but rays were not cleared and ventral fins were rudimentary developed. Abdominal portion was more segmented than earlier stage. At the same time the digestive system became fully developed and the larvae searched for feeding.

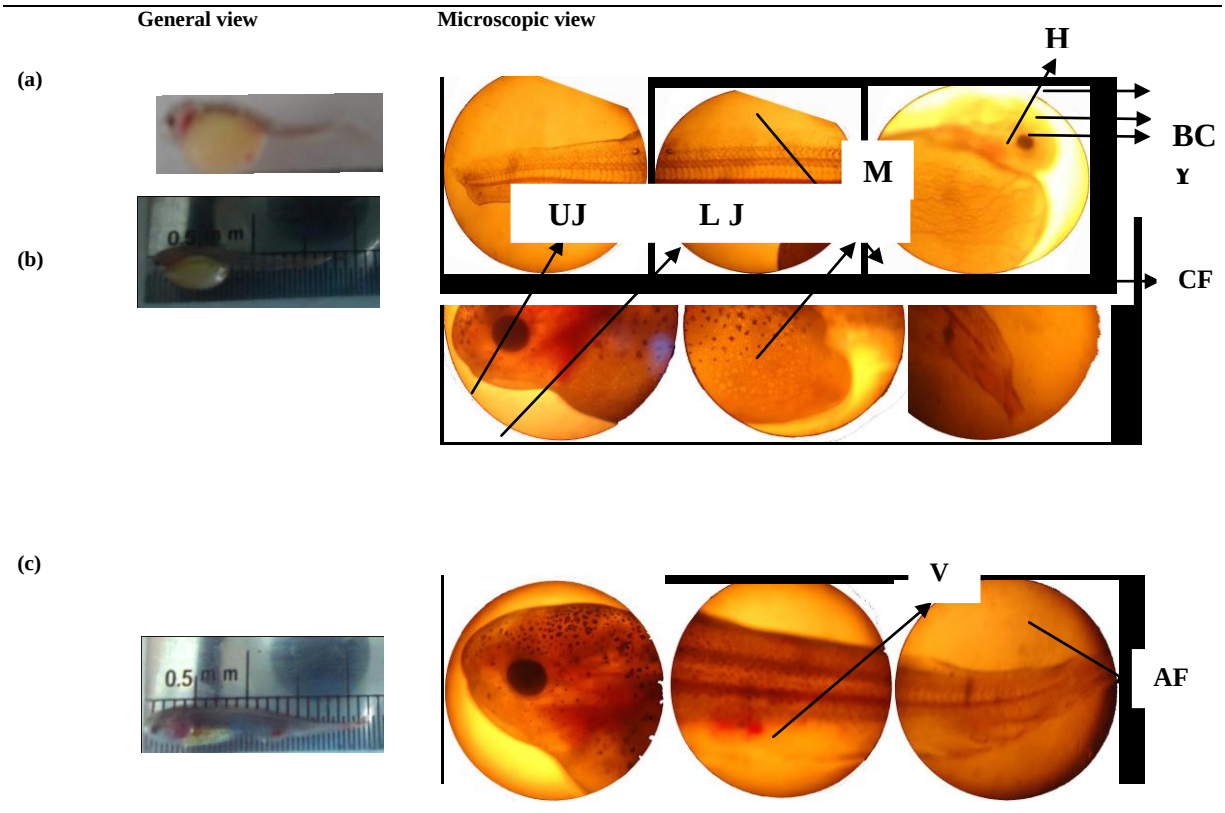

Figure 3. Larval development of *C. chitala* (a) Newly hatched larva; (b) 3-4 d old larva; (c) 5-6 d old larva (×40); (M-Myomere, H-Heart, E- Eye, BC-Blood capillaries, YS- Yolk sac, UJ- Upper jaw, LJ- Lower jaw, MP-Malanophores, CFB- Caudal fin bud, V- Vertebra)

DISCUSSION

This study confirms that *Chitala chitala* is a substrate spawner with cream colored egg envelope. Substrate spawning is also performed by other featherbacks; *Notopterus notopterus* (Srivastava *et al.*, 2012; Yanwirsal, 2013) and *Chitala ornata* (Smith, 1933). The eggs of *C. chitala* reached slowly in their embryonic and larval stages. The fertilized eggs of *Chitala* are adhesive and adhesiveness of eggs is the special character found in another featherback such as *Notopterus notopterus* (Yanwirsal, 2013; Srivastava *et al.*, 2012). Adhesive eggs are also found in other notopterid species such as *Chitala ornata* (Smith, 1933). In this study, the diameter of the fertilized eggs of *C. chitala* ranged between 4.2 and 5.0 mm whereas Bhuiyan (1964) and Rahman (1989) found eggs were 3-4.5 mm in diameter. However, Radheyshyam and Sarangi (2005) in India found fertilized eggs were 4.8-5.2 mm in diameter in the same species. In similar study, some researchers showed different egg size (3.5±0.5 mm) of *Notopterus notopterus* (Srivastava *et al.*, 2012). According to Yanwirsal (2013), newly spawned eggs of *N. notopterus* were 3.8–4.0 mm in diameter. These differences might be attributed to the species variation and brood size (Puvaneswari *et al.*, 2009).

In this study it has been shown that *C. chitala* hatched within 6-7 days after spawning at temperature of 28-31°C. Similar result was found in *Osteoglossum bicirrhosum* where hatching occurs around 7 days at 28 °C (Yanwirsal, 2013). However, In *Notopteru notopterus* hatching occurred around 5-6 days after spawning at 26±1 ^{o}C water temperature (Srivastava *et al.*, 2012). In other osteoglossids, hatching occurs in 7-14 days in *Scleropages leichardtii* (Lake, 1971, 1978), 12 days after spawning in *S. formosus* (Azuma, 1992), 5 days (Neves, 1962) and around 10–14 days in *Arapaima gigas* (Lake and Midgley, 1970). However the developmental stage at hatching is influenced by environmental factor such as temperature and oxygen conditions (Hamor and Garside 1979; Penaz *et al.*, 1983; Heming, 1982). In this study, free embryos of *C. chitala*, directly after hatching, measured around 15 mm in total length, which however, is longer to other notopterids, free embryos of 12 mm in *Chitala ornata* (Smith, 1933); around 10.5 mm (Yanwirsal, 2013) and 4mm (Srivastava *et al.*, 2012) in *N. notopterus*. The embryonic rudiment was observed within 3rd day post-fertilization in *C. chitala*, but a discrepancy of time was observed in case of *N. notopterus*, *O. bicirrhosum* which was within 4 days post-fertilization reported from Srivastava *et al.* (2012) and Yanwirsal (2013) respectively. This variation might be due to species difference.

CONCLUSION

Embryonic and larval stages of a fishes are the most delicate part their life. For successful rearing what should be done and when should be done is very important. *C. chitala* is a highly priced, delicious and well preferred food fish in Bangladesh. But their culture technique in controlled environment is not practiced. Chital was selected for this study because it is already declared as endangered fish, so an attempt should be taken to protect this species from being extinct. This study generated some information on the early life history and commencement of first feeding time for larval rearing. This unique research work will act as base line information for establishing the large scale seed production technique of *C. chitala* in near future.

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