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Article · July 2012

J. Exp. Zool. India Vol. 15, No. 2, pp. 425-430, 2012 *ISSN 0972-0030*

EMBRYONIC AND LARVAL DEVELOPMENT OF THREATENED BRONZE FEATHERBACK, *NOTOPTERUS NOTOPTERUS* (PALLAS)

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(Accepted 25 April 2012)

ABSTRACT – Fertilized eggs (egg capsule 3.5±0.5 mm and yolk sphere 2.5±0.5 mm) of *N. notopterus* were spherical, adhesive and lacked oil globule in the yolk sphere. On day 4, embryonic rudiment becomes distinct with 7-8 somites, the anterior protuberance formed head and the posterior part elongated in tail. Hatching took place on day 5-6 of fertilization at 26±1^{0}C water temperature. At this stage, embryo showed twitching movements, lashed out its tail, ruptured the egg capsule towards head region and emerged. During day 1-3, the head and body of larvae (4 mm) were bent around yolk sac, attached to the substratum by thread-like red coloured capillaries that disappeared on day 3. In 5-6 days old larvae larvae (8±0.5 mm; eye diameter 0.25 mm), head rudiment appeared, mouth opened, upper and lower jaws were clearly visible. In 7-8 days old larvae (size 9±0.5 mm, yolk sac 2.64 mm, head length 1.8 mm, eye diameter 0.35 mm), head and mouth were prominent, eyes clearly seen, notochord initially developed and alimentary canal thicker and convoluted. In 9-10 days old larvae (11±0.5 mm, head length 2.0, eye diameter 0.5 mm oval yolk sac 2.42 mm), head well-developed, notochord increased in length and anal fin slightly visible. On day 12-14, the larvae (light brown) measured 14±0.5 mm, completed yolk absorption, notochord clearly visible, dorsal, pectoral and anal fins were prominent except the ventral fin. Terminal mouth with nearly straight large cleft reaching mid-ventral orbit, eyes were shining, abdominal portion highly pigmented, granular structure appeared near the intestine, four gill arches in opercular cavity and air-bladder developed. Adult characters developed after day 28 of hatching, the pectoral, pelvic, dorsal and anal fins depicted 6-7, 3-4, 5-7 and 25-45 rays, transverse bars along the dorsal ridges relatively prominent, dark spots also developed on the whole body.

Key words **:** Fertilized egg, embryo, larval development, threatened, *Notopterus notopterus.*

INTRODUCTION

The oviparous bronze featherback (knife-fish), *Notopterus notopterus* (Family Notopteridae), a popular food fish with ornamental valuel, thrives well in freshwater rivers, ponds and lakes. It is a very hardy fish and can be easily reared in aquarium, stagnant water and aquaculture system on a variety of feeds. The bronze featherback is distributed in south and south-east Asian countries including Bangladesh, Malaysia, Thailand, Myanmar, Java, Sumatra and Borneo (Rahman, 1989). In India, it has been recorded (standard length of 60 cm under natural conditions) from Ganges, Brahmaputra, Mahanadi, Godavari, Krishna and Cauvery (Talwar and Jhingran, 1991; Sugunan and Sinha, 2001; Jayaram, 2010). The wild population of bronze featherback has declined in the past few years due to over-exploitation, pollution and habitat degradation (Palaniswami and Manoharan, 2010). Due to reduced abundance of *N. notopterus* in the wild, the fish has been categorically kept in the list of the threatened species of the country (CAMP, 1998; Mukherjee *et al*., 2002). Sarkar *et al* (2010) have also confirmed the threatened status of the fish in Gomti river. There exist some information on breeding, fecundity, induced spawning and egg incubation of *Notopterus chitala* (Singh *et al*, 1980; Hossain, 1999; Radheyshyam and Sarangi, 2005; Sarkar *et al*, 2006). Bronze featherback breeds naturally during June to August in rivers and ponds in India and there is a report on its natural breeding under captive conditions too (Haniffa *et al*, 2004). As artificial fecundation and ranching are the envisaged strategies for conservation and rehabilitation of endangered species (Minkley and Deacon, 1991; Maitland, 1993; Jensen, 1994; Pandey and Das, 2002; Das *et al*, 2006; Archdencom and Bonar, 2009; Shei *et al*, 2010), the technique of induces breeding in bronze featherback by using synthetic gonadotropin release hormone (sGnRH), ovaprim has recently been standardized (Srivastava *et al*, 2010). Since embryonic and larval development of *Notopterus notopterus* have not yet been studied (Parameswaran and Sinha, 1966; Chondar, 1999), an attempt was made to record the embryonic and larval development as a tool for identifying early life stages of the population of this species for *in situ* conservation.

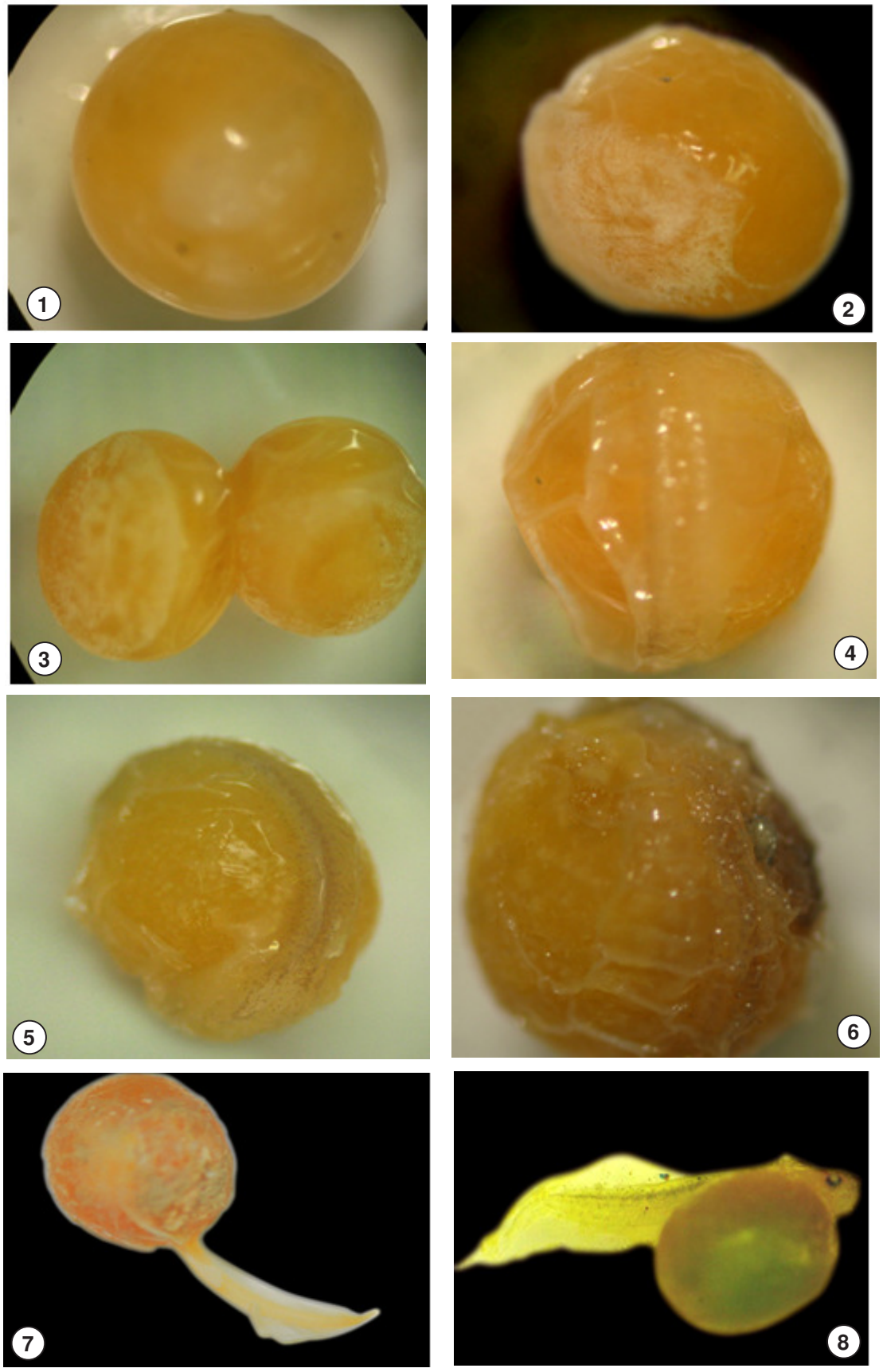
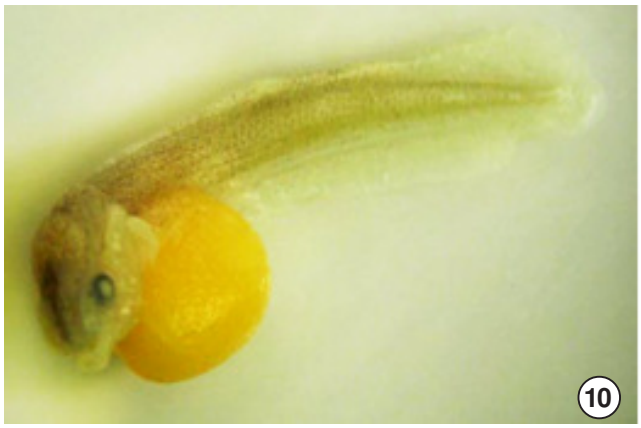
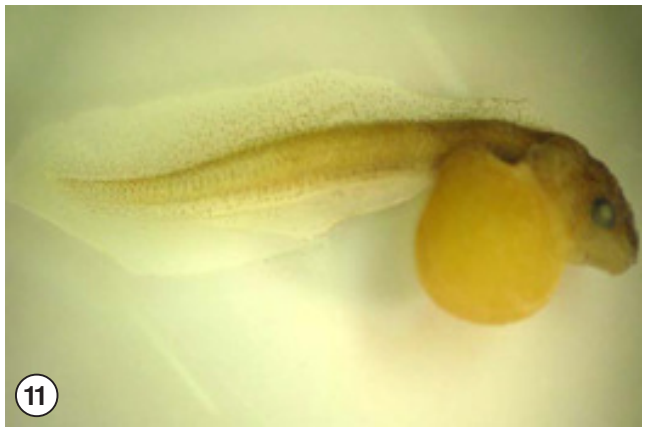
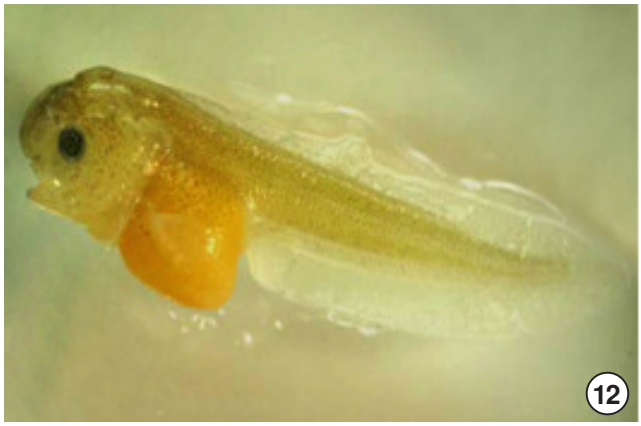
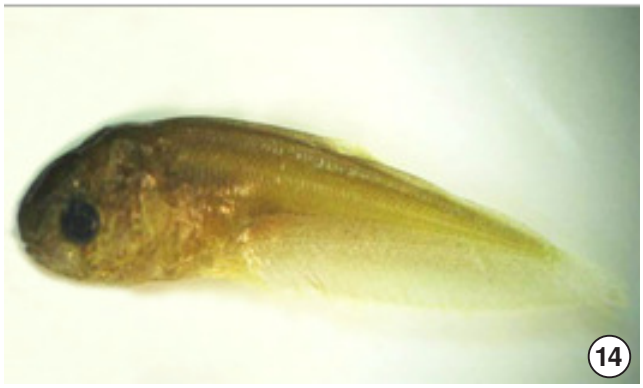


Fig. 1: Embryo just after fertilization. **Fig. 2:** Embryo on day one. **Fig. 3:** Two embryos on day two. **Fig. 4:** Embryo on day three. **Fig. 5:** Embryo on day four. **Fig. 6:** Embryo on day five. **Fig. 7:** Hatchling on day one. **Fig. 8:** Newly hatched larva.









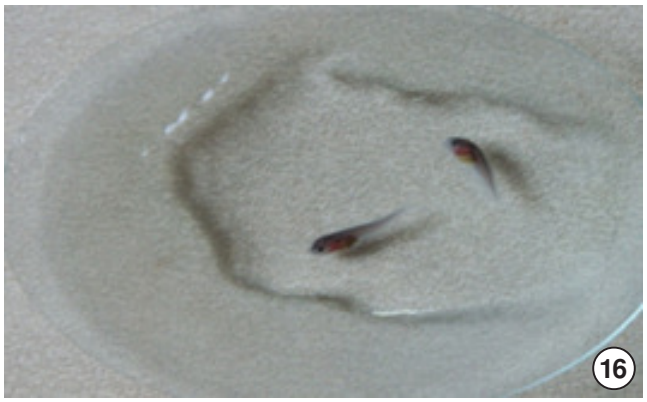


Fig. 9: Hatchling on day two. **Fig. 10:** Hatchling on day three. **Fig. 11:** Hatchling on day three. **Fig. 12:** Hatchling on day five.

Fig. 13: Hatchling after yolk absorption. **Fig. 14:** Hatchling on day ten. **Fig. 15:** Hatchling on day fourteen. **Fig. 16:** Twenty eight day old fingerling.

MATERIALS AND METHODS

Wild bronze featherback, *Notopterus notopterus*, collected from Daligunj and Khadra sampling stations of river Gomti at Lucknow, were induced bred by intramuscular administration of ovaprim @ 0.5 and 1.0 ml/kg body weight to male and female, respectively. After spawning, the developing eggs were carefully collected from the breeding compartments using dropper and transferred to plastic trough containing small quantity of water. The developing eggs were sampled hourly for the first 24 hours, every 3 hours for the next three days and then only once a day. The sampled eggs and larvae were fixed in 4% formalin solution for detailed observations. In the present study, the developmental stages were divided into embryonic, larval and post-larval development. The embryonic development started inside the chorion and completed at hatching. The developmental stages of fertilized eggs and larvae were recorded by using a trinocular microscope with photo-micrographic attachment (Nikon microscope, model Eclipse E400-UIII).

RESULTS AND DISCUSSION

Fertilized eggs

Fertilized eggs of *N. notopterus* were spherical, pale yellow and adhesive to the bottom. Due to the adhesive nature of the egg, considerable debris got adhered to the capsule. The egg capsule (3.5±0.5 mm) was transparent and yellowish white while the yolk was dark yellow. The yolk sphere (2.5±0.5 mm) contained no oil globule. They became translucent as the development progressed (Fig. 1).

Embryonic development

Though the eggs of *N. notopterus* were ejected in batches at irregular intervals during spawning but they did not develop at the same pace. However, the early larval development of the fish usually completed within 5-6 days. On day 1 of fertilization, germinal ring and embryonic shield were visible and the yolk was invaded (Fig. 2). The invasion of yolk and yolk stage were almost completed by day 2 (Fig. 3). On day 3, the myomeres and eye vesicles were demarcated (Fig. 4) while notochord became visible in the form of a tubular structure running longitudinally along the body of the embryo on day 4 which was little dilated at the head region and slightly tapering at the tail end (Fig. 5).

Early larval development

The details of the characters observed in the larval development have been summarized in Table 1. On day 5, the myomeres, head and tails were differentiated and the whole space inside the egg was occupied by the embryo, heart pulsation visible and separation of tail from the yolk initiated (Fig. 6). Before hatching, the developing embryo exhibited frequent twitching movements (after pause of about 30 seconds) which suddenly culminated with a violent jerk breaking the previtelline membrane and the hatchlings emerged out with tail first (Fig. 7). The newly hatched larvae with pigmented eyes measuring about 4±0.2 mm long were seen adhered to the yolk sac which was large and dark yellow (Fig. 8). During subsequent days (2-4), the larvae exhibited growth in size with narrow and elongated body as well as broader head in hind brain region (Fig. 9, 10, 11).

Size of the larvae on day 5-6 was 8±0.5 mm and eye diameter 0.25 mm with light yellowish yolk sac (2.64 mm). Body was deep yellow to dark pink in colour with rudimentary head, mouth opened, upper and lower jaws were clearly demarcated (Fig. 12). Larvae on day 7-8 measured 9±0.5 mm (TL), head length 1.8 mm, eye diameter 0.35 mm, yolk sac 2.42 mm with prominent head and mouth. Eyes were clearly seen, notochord developed whereas alimentary canal thickened and convoluted (Fig. 13). Larvae on day 9-10 measured 11±0.5 mm (TL), head length 2.0 mm, eye diameter 0.5 mm and lacked yolk, head well-developed, notochord increased and anal fin slightly visible (Fig. 14). Larvae on day 12-14 measured 14±0.5 mm and most of them completed yolk absorption, body colour light brown, eyes shining, air-bladder developed and notochord clearly visible (Fig. 15). By day 15, dorsal, pectoral and anal fins were clearly seen while ventral fin was not prominent. Terminal mouth with nearly straight large cleft reaching mid-ventral orbit while abdominal portion was highly pigmented than earlier stages and granular structure appeared near the intestine. The larvae possessed four gill arches in the opercular cavity.

Fingerling

Fingerling stage of larvae (above 27±0.5 mm) lasted from 25-30 days. After 28 day of hatching, the pectoral, pelvic, dorsal and anal fins showed 6-7, 3-4, 5-7 and 25-45 rays, respectively. Adult characters of the fish in all

Table 1: Characters on different developmental stages of *Notopterus notopterus*.

Stage	Age	Size (mm)
Just hatched	1st day	4.0±0.2
Hatchling	5th - 6th day	8.0±0.5
Early post-larva	7th-8th day	9.0±0.5
Late post-larva	9th - 10th day	11.0±0.5
Fry	12nd - 14 day	14.0±0.5
Fingerlings	25th - 30th day	27.0±0.5

respects were fully developed at this stage. Dorsal profile more humped than in the fry stage. Transverse bars along the dorsal ridges relatively prominent than earlier stages. Dark spots also developed on the whole body (Fig. 16).

Though a good number of reports are available on the early life stages of fishes and their characteristics (Kendall *et al*, 1984: Kimmel *et al*, 1995), great variations in size exists among species in young fishes before they become free living (after 3-5 days). Larval development has been studied in a number of teleosts inhabiting Indian waters-*Anabas testudineus* (Mookherjee and Mazumdar, 1946; Moitra *et al*, 1979), *Channa striatus* (Mukherjee *et al*, 1948, Alikunhi, 1953), *Clarias batrachus* (Mookherjee and Mazumdar, 1950; Thakur, 1980), *Channa punctatus* (Banerji, 1974), *Heteropneustes fossilis* (Thakur *et al*, 1974; Puvaneswari *et al*, 2009), *Clarias macrocephalus* (Moitra *et al*, 1987), *Mystus macropterus* (Wang *et al*, 1992), *Clarias gariepinus* (Haylor, 1992), *Brachydanio rerio* (Kimmel *et al*, 1995), *Mastacenbalus aculeatus* (Sahoo *et al*, 2007) and *Mugil cephalus* (Abraham *et al*, 1999). Time taken for early development of larvae varies in different species depending on the rearing water temperature- *Clarias mossambicus* 20-23 hrs, 27-30^{0}C- Greenwood, 1955), *C. macrocephalus* (20 hrs, 25-32^{0}C- Tongasanga *et al*, 1963), *H. fossilis* (18-24 hrs, 24-30^{0}C- Sundararaj and Goswami, 1969; Chaudhuri, 1971; Khan, 1972; Thakur *et al*, 1974), *Channa punctatus* (24-24.45 hrs, 26.5-28^{0}C- Banerjji, 1974), *Clarias. batrachus* (21-24 hrs, 26-29^{0}C- Thakur, 1980), *C. lazera* (24 hrs- De Kmipe and Micha, 1974), *Mystus seenghala* (>24 hrs, 28-30^{0}C-Singh *et al*, 1981). However, fertilized eggs of *Notopterus notopterus* took 5-6 days at temperature 26-28^{0}C. Similar observations were also observed in *N. chitala* too (27-28^{0}C 120±4 hrs-Hossain, 1999; Radheshyam and Sarangi, 2007). Embryonic and larval development studies are necessary for the successful rearing and seed production of the species (Abraham *et al*, 1999; Nayak *et al*, 2000). Since larval rearing remains the most critical phase in the success of seed production in air-breathing telosts, development of suitable protocol for the mass rearing of larvae is one of the important component for successful propagation and the problem appears to be due to relatively smaller size of the mouth and limited yolk reserves of the larvae (Shirota, 1970; Thakur *et al*, 1974; Khan and Mukhpoadhyay, 1975; Thakur, 1980; Bromage and Roberts, 1995; Nayak *et al*, 2000, 2004). Despite the success in artificial propagation of *N. notopterus* by induced spawning (Srivastava *et al*., 2010), refinement in the techniques of larval rearing is essential for conservation aquaculture of the species (Liao, 1993; True *et al*, 1996; Anders, 1998; Das *et al*, 2006).

ACKNOWLEDGEMENTS

We are grateful to the Director, NBFGR (ICAR), Lucknow and Principal, Govt. P.G. College, Satna for extending facilities to carry out the present investigation.

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