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Note

Observations on larval development of chocolate mahseer *Neolissochilus hexagonolepis* (McClelland, 1839)

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ABSTRACT

Present study embodied larval development of chocolate mahseer, *Neolissochilus hexagonolepis* in controlled conditions in the laboratory, from hatching till complete absorption of the yolk sac. Details pertaining to larval development of chocolate mahseers are not available, but are of obvious benefit for a better understanding of life history and for captive seed production and hatchery management programmes for aquaculture as well as for conservation purposes. The mature eggs and sperms were fertilized by dry stripping method. The fertilized eggs were then incubated in a flow through hatchery. The fertilized eggs were transparent, demersal, spherical, non-adhesive and brownish in colour with a diameter of 2.8 to 3.1 mm. Hatching started 40 h post-fertilization and was completed within 22 h at 22 ± 2 ^{0}C. Newly hatched larvae measured 8.5 mm in length and were devoid of mouth and pigmentation. The yolk sac was completely absorbed within 16 days post-hatching on attainment of 14.32 ±0.26 mm total length. At the same time the digestive system became fully developed and larvae started taking exogenous feed. The findings of the present study provide valuable information that may help establishing large scale seed production technique for chocolate mahseer.

Keywords: Development, Fertilization, Larval development, Morphometry, *Neolissochilus hexagonolepis*

Neolissochilus hexagonolepis (McClelland, 1839) commonly known as chocolate or copper mahseer is considered as one of the delicious food fish as well as game fish species of the Indian upland region specially of North-eastern Himalayas. Ogale (2002) described the mahseer as a sport fish that provide unparalleled recreation to anglers from all over the world. The species inhabits clear, fast flowing streams and rivers with stony, pebbly or rocky bottoms and considered as culture icon in the water bodies of North-east India. The culture of this species in large scale is still not common due to lack of adequate supply of seed and limited knowledge on the feeding and breeding requirements, among the farmers. In the natural water bodies, the population of this fish is decreasing due to various natural and anthropogenic factors (Sarma, 2009). Considering the importance of chocolate mahseer, early life history information is essential for optimisation of large scale seed production, culture and management (Puvaneswari *et al.,* 2009). Knowledge on early 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; Puvaneswari *et al.,* 2009). An understanding of normal larval morphology is critical, and can be used to evaluate culture conditions for mass production of high quality juveniles (Koumoundouros *et al.*, 1999). The transition from an endogenous to exogenous food supply is one of the most critical stages in the life history of any fish species. Information on larval stages are also indispensible in the study on ontogeny and phylogeny (Verreth *et al.,* 1992). Further, this information is also imperative in the successful rearing of larvae for large scale seed production and culture (Khan and Mollah, 1998; Rahman *et al.*, 2004; Puvaneswari *et al.,* 2009).

Breeding techniques are being developed for different species of mahseers (De Silva *et al.,* 2004; Ingram *et al.,* 2005; Sarma *et al*., 2010). Keshavanath *et al.* (2006) successfully induced maturity in Deccan mahseer (*Tor khudree*) under confinement and induced bred using carp pituitary and ovaprim. However, no systematic studies have yet been conducted on the developmental biology and culture of *N. hexagonolepis* (chocolate mahseer). The increasing demand of chocolate mahseer for sport and as a food fish, specially in Indian uplands necessitates concerted attempts to produce seed for angling and for aquaculture purposes using captive, pond reared broodstock. Therefore, in this study an attempt was

made to highlight some aspects of the larval development of the seed produced through pond reared broodstock of *N. hexagonolepis* in controlled lab-rearing condition and to describe the early life history stages of this species.

The study was carried out in the Hatchery Complex and Environmental Biology Laboratory of the Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand. The pond reared brooders (male and female fishes) were selected based on the external morphological features. The release of eggs through the genital pore following gentle pressure on the abdomen and appearance of chasing behaviour was considered as the commencement of ovulation. Eggs from ovulated females were stripped into circular plastic trays. Following ovulation, milt from mature male fish were mixed with the stripped eggs collected by dry stripping method. After 2 min of gentle stirring, the fertilized eggs were washed several times with freshwater to remove excess milt and blood clot. The eggs were then transferred to and spread as uniformly as possible in the trough of flow through hatchery with continuous water supply throughout the incubation phase. Two hours post-fertilisation, the unfertilized eggs turned whitish which were removed carefully from the hatching tank. Early developmental stages were studied under a stereomicroscope (Olympus) and photographs were taken till the end of the larval development. All the measurements were taken using scale reader (Sipcon, Profile projector SP-300). Five to six randomly selected larvae were used to describe each stage.

Larval development of *Tor* species (*T. khudree, T. tambroides* and *T. douronensis*) have been described previously (Kulkarni, 1971; Desai, 1972; Kulkarni, 1980; De Silva *et al.,* 2004; Keshavanath *et al.* 2006; Sarma, 2009) but nothing is known about the embryonic or larval development of chocolate mahseer. A short description of the morphometric changes occurring during the larval development of chocolate mahseer is depicted in Table 1, while the developmental stages are shown in Fig. 1. Unfertilized eggs were opaque, demersal, spherical and whitish in colour and the fertilized eggs were transparent, demersal, spherical and brownish golden in colour. The diameter of fertilized eggs ranged from 2.8 to 3.1 mm, which is more or less similar to those (2.0-3.08 mm) observed by De Silva *et al.* (2004). The egg membrane was separated from the rest of the egg by a small perivitelline space. Within 15 min of fertilisation, eggs swelled up with 20% increase in diameter. Subsequently, the egg diameter did not change over the remaining

Table 1. Morphometric changes during the larval development of chocolate mahseer (*N. hexagonolepis)* at 22 ± 2 ^{0}C

Time after hatching	Total length (mm)	Length of yolk sac (mm)	Developmental landmarks
0 h	8.41-8.68	5.93-6.01	Newly hatched larvae were straight, slender, transparent and gradually tapering towards the tail. Larvae silver in colour with laterally compressed body and the head very small. Yolk sac faintly brown in colour, composed of two well defined lobes attached to the body. Hatchlings had unpigmented eyes and were devoid of distinct mouth and fins. The larvae could not swim and passively floated in water.
12 h	8.87 - 8.94	5.72-5.83	Larvae silver in colour. Mouth yet to develop but a conspicuous depression was identified at the position of the mouth. A depression at the posterior end of the yolk sac was visible and was identified as anal pore. Eyes were unpigmented.
24 h	9.21-9.34	5.21-5.36	Bulged yolk became gradually elongated, with its size decreased considerably. Dark pigmented and prominent eye spot appeared on the anterior part of the head. Mouth clearly differentiated and melanophores/chromatophores appeared on the head, body and yolk sac. Ray like markings faintly noticeable at the caudal region. Blood vessels started appearing.
32 h	9.60-9.73	5.03-5.16	Larvae became darker in colour and more melanophores appeared on the head and body. Cardiovascular system and myomeres were visible. Fully formed upper and lower jaws were visible and gills were covered by the operculum. At this stage, the larvae became active and moved to the water surface and were sensitive to light.
44 h	9.71-9.96	4.5-4.74	Incipient dorsal fin appeared. Rudimentary rays appeared on the caudal fin. Eyes more melanised and increased in size. Myomeres were prominent and the length of yolk sac reduced. Blood circulation was well observed in the head, heart and tail region.
52 h	10.76-10.94	5.84-6.01	Yolk sac became slender but increased in length and thoracic region widened. Larvae grew both in length and girth.
112 h	11.18-11.25	4.82-5.10	Heart and dorsal fin became prominent. Alimentary canal appeared and yolk sac decreased in length. Eyes bulged and increased in size. Myomeres fully visible and thickened. Anal aperture and anal fin fold appeared.
142 h	11.67-11.82	4.02-4.16	Yolk sac further reduced in size. Anal fin developed. Caudal fin fully developed and fin rays were prominent. Myomere septae and vertebrae were fully developed.
16 days	14.32-14.58	Nil	Reserved yolk material completely absorbed and larvae were found swimming and feeding exogenously. Larvae morphologically similar to the adult except for their colour patterns.

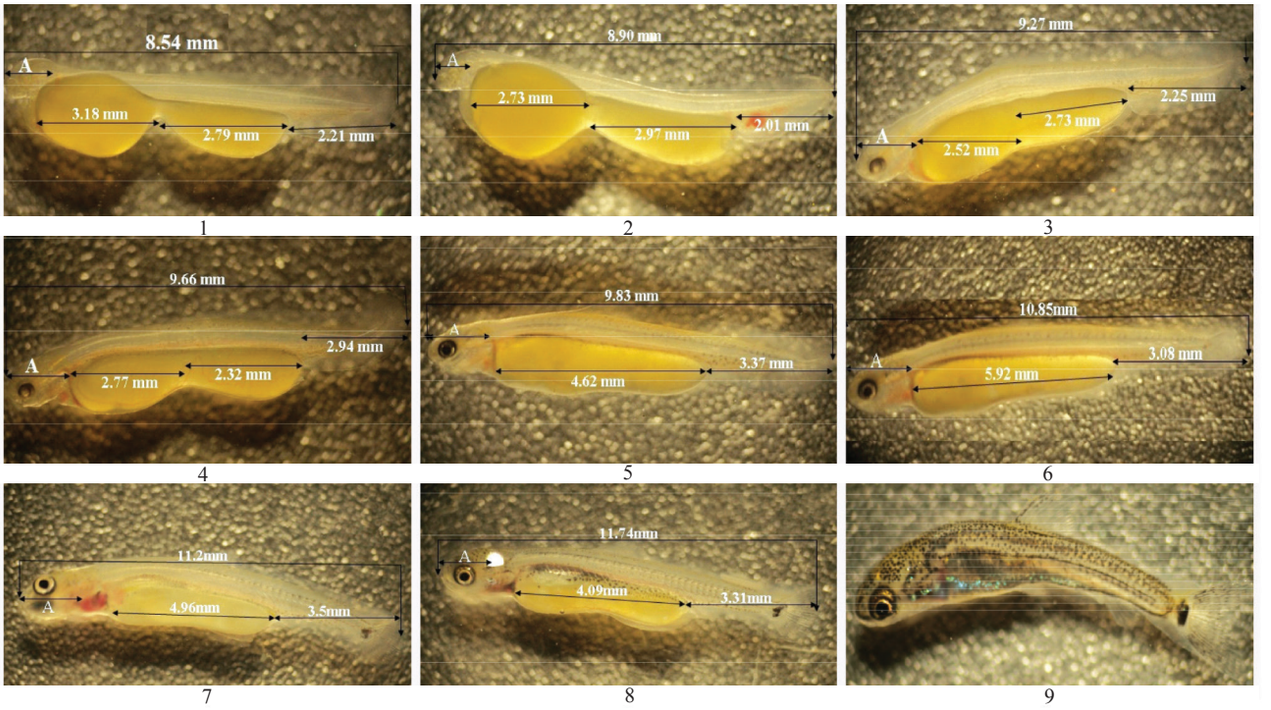

Fig. 1. Developmental stages of newly hatched larvae of chocolate mahseer (*N. hexagonolepis*) at 22 ± 2 ^{0}C. Larval developmental stages → 1) 0 h post-hatch (hph) ; 2) 12 hph; 3) 24 hph; 4) 32 hph; 5) 44 hph; 6) 52 hph; 7) 112 hph; 8) 142 hph and 9) 16 days post-hatch (dph). A - denotes head length.

incubation period as reported in other species of mahseer (De Silva *et al.,* 2004). Fertilized egg had a reddish spot (blastodisc) on one pole which is readily recognisable with the naked eye.

In *Tor tambroides* and *Tor douronensis,* hatching occurred 69-90 h post-fertilisation (hpf) and completed within 30 h (De Silva *et al.,* 2004). However, in the present study, hatching occurred at 40 hpf and completed within 22 h at temperature of 22 ± 2 ^{0}C. Before hatching, frequent embryonic twisting movements were observed as the embryo tried to rupture the perivitelline membrane. While hatching, the egg membrane was broken down from the caudal region and the hatchling emerged from the egg capsule with caudal region first. The newly hatched larvae were slender, transparent, silverish in colour and 8.5 mm in length with laterally compressed body. Ingram *et al.* (2005) observed more or less similar pattern in case of *T. tambroides* and *T. douronensis.* The larvae could not swim and floated passively in water with irregular upside down movement. The hatchlings had unpigmented eyes and were devoid of distinct mouth and fins as reported in other species of mahseer (De Silva *et al.,* 2004; Ingram *et al.,* 2005). As in other teleosts, chocolate mahsser also hatched with a quantity of yolk remaining attached to the hatchlings. The yolk sac was relatively large, elongate and bilobed and measured 5.93-6.01 mm in length. There was a noticeable constriction between the primary and secondary yolk sac as observed by De Silva *et al.* (2004) in *T. tambroides* and *T. douronensis.*

The primary yolk sac was larger in size, in both lateral and dorsal view as compared to the secondary yolk sac. Margins of both yolk sacs (lobes) were pigmented. During the yolk sac absorption period, larvae continued to grow in length, and the yolk sac declined in size. By 4 days post-hatch (dph), heart and dorsal fin became prominent. Alimentary canal, anal aperture and anal fin fold appeared and yolk sac decreased in length. Myomeres were fully visible and thickened. By 6 dph, rays of the caudal fin and internal organs including the liver and gall bladder were evident in some larvae. Myomere septae and vertebrae were fully developed. At this stage, melanophores had become denser and were spreading throughout the myomeres, along the notochord and laterally across the yolk sac. The reserved yolk material completely disappeared in 16 days after hatching on attainment of 14.45 mm in length and larvae were found freely swimming and feeding exogenously. At this stage the larvae were morphologically similar to the adult except for their colour patterns.

Further studies are needed on the factors affecting larval development in their natural environment.

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References

Borcato, F. L., Bazzoli, N. and Sato, Y. 2004. Embriogenesis and larval ontogeny of the Pirugordura, *Leporinus piau* (Fowler) (Pisces, Anostomidae) after induced spawning. *Revista Brasileira de Zoologia,* 21: 117-122.

De Silva, S. S., Ingram, B., Sungan, S., Tinggi, D., Gooley, G. and Sim, S. Y. 2004. Artificial propagation of the indigenous *Tor* species, empurau (*T. tambroides*) and semah (*T. douronensis*), Sarawak, East Malaysia. *Aquacult. Asia,* 4: 15-20.

Desai, V. R. 1972. Notes on the early larval stages of *Tor putitora* (Ham.). *J. Zool. Soc. India,* 24: 47-51.

Ingram, B., Sungan, S., Gooley, G., Sim, S. Y., Tinggi, D. and De Silva, S. S. 2005. Induced spawning, larval development and rearing of two indigenous Malaysian mahseer, *Tor tambroides* and *T. douronensis*. *Aquacult. Res.,* 36: 1001-1014.

Keshavanath, P., Gangadhara, B., Basavaraja, N. and Nandeesha, M. C. 2006. Induced breeding of pond raised mahseer, *Tor khudree* using carp pituitary and ovaprim. *Asian Fish. Sci.,* 19: 411-422.

Khan, M. M. R. and Mollah, M. F. A. 1998. Embryonic and larval development of African catfish, *Clarias gariepinus* (Burchell). *Bangladesh J. Fish.,* 21: 91-97.

Koumoundouros, G., Divanach, P. and Kentouri, M. 2001. Osteological development of *Dentex dentex* (Osteichthyes: Sparidae): dorsal, anal, paired fins and squamation. *Mar Biol*., 138: 399- 406.

Koumoundouros, G., Divanach, P. and Kentouri, M. 1999. Osteological development of the vertebral column and of the caudal complex in *Dentex dentex*. *J. Fish Biol.*, 54: 424- 436.

Kulkarni, C. V. 1971. Spawning habits, eggs and early development of Deccan mahseer, *Tor khudree* (Sykes). *J. Bombay Nat. His. Soc.,* 67: 510-521.

Kulkarni, C. V. 1980. Eggs and early development of *Tor* mahseer. *J. Bombay Nat. His. Soc.,* 77: 70-75.

Ogale, S. N. 2002. Mahseer breeding and conservation and possibilities of commercial culture. The Indian experience. *FAO Fisheries Technical Paper,* 431: 193-212.

Puvaneswari, S., Marimuthu, K., Karuppasamy, R. and Haniffa, M. A. 2009. Early embryonic and larval development of Indian catfish, *Heteropneustes fossilis*. *Eur. Asian J. Biosci.,* 3: 84-96.

Rahman, M. R., Rahman, M. A., Khan, M. N. and Hussain, M. G. 2004. Observation on the embryonic and larval development of silurid catfish, gulsha (*Mystus cavasius* Ham). *Pakistan J. Biol. Sci.,* 7(6): 1070-1075.

Sarma, D. 2009. Captive breeding and grow out of the golden mahseer. *Info Fish Int.,* 2: 26-29.

Sarma, D., Haldar, R.S., Das, P. and Mahanta, P. C. 2010. Management in seed production of golden mahseer, *Tor putitora* in hatchery conditions. *Aquacult. Asia,* 15(4): 11-14.

Verreth, J., Torreele, E., Sparzier, E. and Slurszen, A. 1992. The development of a functional digestive system in *Clarias gariepinus* (Burchell). *J. World Aquacult. Soc.*, 23(4): 286-998.

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