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Reproductive biology and artificial propagation of chocolate mahseer *Neolissocheilus hexagonolepis* (Mc Clelland) in Meghalaya, India

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ABSTRACT

The chocolate mahseer *Neolissocheilus hexagonolepis* (Mc Clelland) is a commercially important species of mahseer group which needs immediate attention due to its reduced abundance in Meghalaya. This fish is widely recognized as a sport fish by the anglers, as ornamental fish by the hobbyists and also a priced food fish. Fishes were found to mature from May onwards and gravid fishes were available in rivers from July to September. Females were larger in size when compared to males in the same age group. The sex ratio observed was 1 female: 1.26 male. Attainment of first maturity occurred when males were 9+ months and females were 11+ months old. The minimal length and weight at first maturity of female fishes were 17.8 cm and 70 g respectively whereas males attained maturity at an average length of 17.3 cm and weight of 50 g. The Gonado-somatic Index in ripe female was 16.19 and the average fecundity factor was 70.18. The fish was bred successfully in captive condition through stripping. Fertilization percentage of eggs ranged from 80 to 85. Incubation period varied between 81-97 hours and the percentage of hatching was 75-80. Yolk-sac absorption in larvae was complete in 6+ days after hatching. The spawn was stocked in glass aquaria and on an average, 85% survival was recorded after 15 days of rearing when they attained an average length of 2.084 cm and weight of 0.195 g. The fry was subsequently reared in cement cisterns @ 1 no./l and on an average, 92% survival was recorded after 60 days of rearing. During this period, the fish attained an average length of 4.82 cm and weight of 0.856 g.

Keywords: Chocolate mahseer, Meghalaya, Reproductive biology, Sexual maturity

Introduction

The fish species belonging to the genus *Tor* and *Neolissocheilus* under the family Cyprinidae are popularly known as 'Mahseer'. The fish inhabit the rivers and streams of 50 to 2000 meters above the mean sea level. Out of the seven species recorded and reported from North Eastern India (Ponniah and Sarkar, 2000; Vishwanath *et al*., 2007), the chocolate or copper mahseer, *Neolissocheilus hexagonolepis* (Mc Clelland) is found in almost all the major rivers and streams (Mahapatra *et al*., 2004a). This fish species is also found in the Cauvery River of Tamil Nadu (Jhingran, 1991). Besides, *N. hexagonolepis* occurs in Bangladesh, Burma, China and Nepal (Dasgupta, 1994).

The chocolate mahseer is a commercially important species and is widely recognized as sport fish due to its tremendous size and strength facilities (Marwein, 2000, Mahapatra *et al*., 2004b). *N. hexagonolepis* (maximum size 274.5 cm), are also now treated as ornamental fish in their juvenile stage (Mahapatra *et al*., 2003a; 2003b; 2004c; Biswas *et al*., 2007). The chocolate mahseer is a preferred food in the region and fetches Rs.150-200/- per kg (Mahapatra *et al*., 2004b). This species, commonly known as 'Kha saw' in Meghalaya was abundantly found in the rivers and reservoirs of the state until recent years. The reasons for the drastic decline in population of this important bio-resource have been evaluated (Marwein, 2000; Mahapatra *et al*., 2004b) and this species is believed to be under the threatened category (Menon, 1974; Sinha, 1994; Lakra *et al*., 2010).

In order to conserve the chocolate mahseer, it is imperative to study its biology and attempt its artificial propagation and rearing. Morphometric reports on the chocolate mahseer were published by several workers (Hamilton, 1822; Mc Clelland, 1839; Hora, 1940). The food and feeding habit, length-weight relationship, reproductive organs, spawning habit, early development and culture of this fish were also observed by some workers (Langdale Smith, 1944; Ahmed, 1948, Alikunhi, 1948; Ghosh and Zamadar, 2003). Success in artificial fecundation of golden mahseer collected from wild waters has been achieved at Bhimtal Research Centre of NRC-CWF (Joshi 1982, 1988; Desai, 1970; Sehgal, 1991; Sehgal and Malik 1991; Mohan *et al.*, 1994). The artificial propagation of *Tor khudree* was also reported by some workers (Kulkarni and Ogale, 1978).

The present study was envisaged to study the reproductive biology and artificial propagation of the chocolate mahseer *N. hexagonolepis*.

Materials and methods

Studies on reproductive biology of *Neolissocheilus hexagonolepis* was conducted from July, 2000 to August, 2005 in ICAR Fish Farm at Barapani, Meghalaya. A total number of 550 specimens were measured and weighed. Sexes were identified and the sex ratio was recorded. Observations were made on the maturity of fishes and 18 gravid females of length ranging from 22.5cm to 27.9 mm and weight from 138 to 155 g were considered for fecundity studies. The gonads were removed after careful dissection and weighed to the nearest milligram (mg). The gonads were then preserved in Gilson's fluid for further microscopic examination. The sex ratio was calculated. The size at first maturity was determined by regular checking of maturity changes throughout the year. The Ganado-somatic index (GSI) was calculated using the formula:

$$GSI = \frac{\text{Weight of the gonads}}{\text{Total wt. of fish - Wt. of gonads}} \times 100$$

For estimation of fecundity, ova were washed with tap water and kept on filter paper to dry. Four sub samples (air dried) were taken, counted separately for the number of ova present. The formula used for fecundity calculation was:

$$F = \frac{W}{W_1+W_2+W_3+W_4} \times (N_1+N_2+N_3+N_4)$$

Where F = Fecundity; W= total weight of the ovary; W_1, W_2, W_3 and W_4 = weight of each sub sample and N_1, N_2, N_3 and N_4 = ova number of each sub sample.

For captive breeding studies, the female brood fish weighed 200-300 g, while the males were of 50-100 g size. For artificial fecundation, stripping and fertilization was done subsequently. The rate of fertilization was estimated by keeping a sample of water hardened eggs in 5% glacial acetic acid solution for 24 hours where the viable eggs became translucent and the dead eggs gets cleared. Incubation was done in enamel trays with mild aeration. All the hatchery components were disinfected with 5% potassium permanganate solution before use. The non-viable eggs were culled daily after second day of fertilization. Occasional flushing of hatching stocks with malachite green @ 1: 2, 00,000 for 20 minutes was done as prophylaxis against fungal attacks and subsequent checking of mortality during incubation period. The spawn were reared in glass aquaria upto fry stage and the fry were subsequently reared in cement cisterns upto fingerling size. Water exchange was done every six hour intervals during

Table 1. Reproductive parameters of *Neolissocheilus hexagonolepis*

Particulars	Value	Remarks
Age at first maturity		
Male (months)	9+	Generally in 1+ years
Female (months)	11+	Generally in 1+ years
Length at first maturity		
Male (cm)	17.3	In pond condition
Female (cm)	17.8	In pond condition
Weight at first maturity		
Male (g)	50g	In pond condition
Female (g)	70g	In pond condition
Sexual characteristics		
Male	Silvery, smaller	Size difference in same age group according to sex.
Female	Yellowish, larger	
Sex ratio (female:male)	1: 1.26	In natural population
Gonado-somatic index (average)		
Mature female	10.65	
Ripe female	16.19	
The minimum number of ova produced by a female	8726	In female of 22.5cm and weight of 138 g.
Fecundity factor (average)	70.18	
Peak breeding time	August	Breeding season May to September in their natural habitat
Breeding type	Egg scattered	Generally lay eggs on sandy bottom
Nature of eggs	Non-adhesive	Initially mild stickiness was observed before water hardening.
Spawning periodicity	Multiple	Breeds more than one time in a single breeding season

incubation and hatching. In spawn and fry rearing tanks, water exchange was done at 7 days interval or when needed. A regular monitoring of water quality parameters was done following standard methods (Michael, 1990; APHA, 1998).

Results and discussion

The reproductive parameters of *N. hexagonolepis* is presented in Table 1. and stocking, culture and production details are reflected in Table 2. The physico-chemical parameters of water at the time of stripping, incubation, larval and fry rearing are presented in Table 3.

Table 2. Details of spawn and fry rearing of *N. hexagonolepis*

Items	GRT	CRT
Tank dimension (m)	1.22×0.48×0.48	2×1.5×0.5
Surface area (m^2)	0.59	3
Water depth (m)	0.1	0.2
Water volume (m^3)	0.059	0.6
Total no. of seed released	295	600
Rate of stocking		
No. /m^2	500	200
No. /m^3	5000	1000
No. /l	5	1
Culture period (days)	15	60
Length of fish		
At stocking (cm)	1.4	2.084
At end of culture (cm)	2.084	4.82
Weight of fish		
At stocking (gm)	0.022	0.195
At end of culture (gm)	0.195	0.856
Total no. recovered	250	552
Av. Survival (%)	85	92

GRT = Glass rearing tank, CRT = Cemented rearing tank.

Success in artificial fecundation was achieved without administration of pituitary gland extract or any other hormone. Details of artificial propagation as deduced from the present study are illustrated under separate heading. Principal operations involved for artificial breeding of chocolate mahseer includes collection of brood stock, stripping for artificial fecundation, fertilization, incubation and hatching, rearing of spawn and fry.

Selection of brooders

Domesticated brood stocks of the requisite type raised in running water channel were selected. Brooders especially females were kept under observation to detect the most appropriate time for stripping. Ripeness of the female was ascertained through softness of abdomen and its gravidness by exerting slight pressure on the belly to confirm free release of eggs. In the male fish, the readiness was confirmed by the jet flow of milt with gentle pressure near the vent (Fig. 1). Selected brooders were ripe and free oozing type for artificial fecundation. The ratio of the male and female brooders were 2:1.

Stripping and fertilization

Only ripe brooders were easily amenable to egg taking. The eggs were fertilized by dry method. The fully ripe eggs generally flow out under slight pressure on belly, which continued till all the ripe eggs came out. Sometimes, heavy pressure on the belly at the time of stripping resulted in oozing of blood due to injury of the internal organs. Soon after the eggs were stripped, the gravid male was stripped for free flowing milt which was spread over the eggs. The mixing of eggs and sperm was gently done using a fine soft brush and by moving the container sideways. Fertilization took place instantly after mixing of the two sex products. A little quantity of water was added and the eggs were allowed to remain in the same condition for sometime to ascertain complete fertilization. After about 2 minutes, the eggs were washed and excessive milt, blood and other extraneous substances, if any, were removed completely by changing the water repeatedly. The colour of the eggs of chocolate mahseer was golden to orange. The number of eggs received per kg body weight of fish through artificial fecundation varied between 15,000 -20,000/ operation. Generally fertilized eggs were 80-85%.

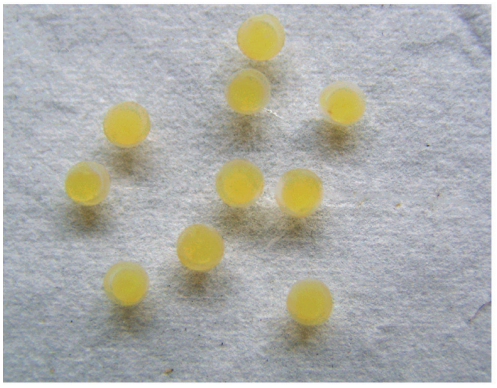
Table 3. Physico-chemical parameters of water at the time of stripping, incubation, larval and fry rearing of *N. hexagonolepis*

Parameters	Stripping -cum-fertilization	Incubation-cum-hatching	Spawn rearing	Fry rearing
Temperature (°C)	22 ± 1.5	22 ± 1.5	22.5 ± 1.5	21.5 ± 1.5
pH	6.8-7.5	6.5-7.0	6.8-7.0	6.8-7.1
Dissolved oxygen (ppm)	5.5 & above	6.0 & above	6.5 & above	6.5 & above
Hardness (ppm)	17.01	25.02	24.02	21.02
Alkalinity (ppm)	6.04	5.01	5.90	6.50

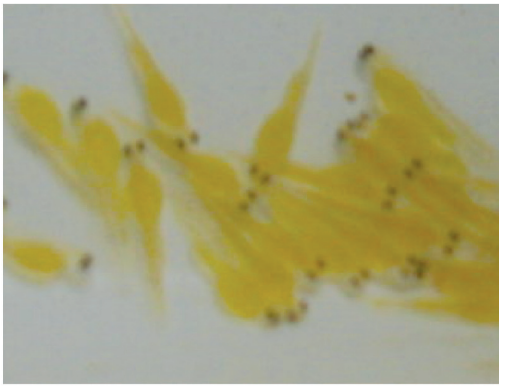
Females were larger in size when compared to males which belonged to the same age group. Sex ratio observed in the natural habitat was 1 female: 1.26 male. The age at first maturity in males was 9+ months while in female fishes it was 11+ months. The minimal length and weight at first maturity of female fishes were 17.8 cm and 70 g respectively whereas males attained maturity at an average length of 17.3 cm and weight of 50 g. The gonado-somatic index in ripe female was 16.19 and the average fecundity factor was 70.18.

Oozing male

Ripe female

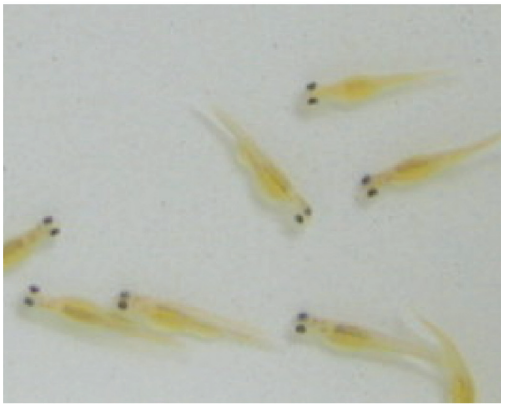


Incubated eggs - 2 days

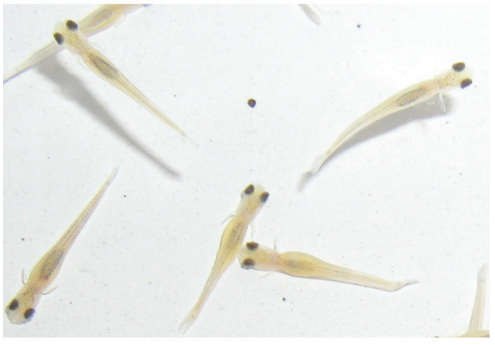


Hatchling - 2 days

Hatchling - 3 days



Hatchling -4 days



Spawn

Fry

Fig. 1. Different life stages of *N. hexagonolepis* obtained during artificial propagation

Incubation and hatching

The fertilized eggs of *N. hexagonolepis* were kept in hatching tray in a single layer having mild aeration facilities. Incubation period ranged from 81 to 97 hours. The newly hatched larvae measured about 8 mm in length. Percentage of hatching was 75 – 80. The yolk sac gradually decreased in size as the embryo grew. The yolk-sac absorption in larvae was completed in 6+ days after hatching (Fig. 1). The spawn stage *i.e.* the completely absorbed larva increased to 1.4 cm in length and 0.022 g in weight. At this stage, the larva started feeding with its mouth. Once the yolk-sac was completely absorbed and swim-up fry started moving freely, the larvae were shifted to glass aquaria for rearing which were provided with some pebbles.

Rearing of spawn and fry

The spawn was stocked in glass aquaria @ 5 per litre performed well and on an average 85% survival was recorded after 15 days of rearing. The fry was subsequently reared in cement cisterns for 60 days with a stocking density @1no./l upto fingerling size. The average survival percentage was 92. The larvae were maintained on planktonic diet for a period of one month. Subsequently, they were fed with rice bran and mustard oil cake in addition to a mixed culture of plankton.

One of the important ways for conservation of fish is through artificial breeding and propagation under controlled condition. The study of reproductive biology is considered to be one of the most fundamental aspects of applied fishery research. It is impossible to gain any real understanding of captive breeding under controlled condition without some apprehension of reproductive biology (Mahapatra, 1994).

The chocolate mahseer was found to mature from May onwards and gravid fishes were available during July - September in their natural habitat. This has given an indication that this species starts breeding during peak monsoon when hilly rivers are fully inundated with strong water current. In captive rearing of brood stock, the fish got matured both in pond and running water channel; but free oozing ripe brooders were recorded in running water channel. This may be due to the fact that water current with clear and oxygenated (>8p m) water triggered their gonads for peak maturity. Early embryonic development of the fertilized eggs and hatchling were quite slow and prolonged. This suggests the need for adequate care during hatching of eggs. Dissolved oxygen was also a limiting factor, especially during incubation and hatching where oxygen levels below 5 ppm hampered their hatching and survival.

The chocolate mahseer is a commercially important species and is widely recognized as a sport fish due to its tremendous size and strength facilities. It is highly esteemed by the anglers. But compared to the last two decades, the catch per unit effort (CPUE) has drastically decreased (Marwein, 2000; Mahapatra *et al*., 2004b). Recently, this fish species is popular as ornamental fish among hobbyists, both locally and globally (Mahapatra *et al*., 2003a, b). For sustainable supply and conservation of natural stock, captive breeding is an urgent need. Captive breeding and farming and also ranching in their natural habitat would probably help in the enhancement of their stock. This would help in the development of sport fishery, ornamental fishery and also the overall fish production of different north eastern hill states.

Since the population of chocolate mahseer was found to be declining gradually, our study may be relevant in conservation of this important fish species. The information from the present study would definitely help the breeders in the right direction for proper planning and development of artificial propagation techniques of chocolate mahseer under controlled conditions. There is strong need for intensive studies for mass scale seed production of this threatened species.

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