INDUCED BREEDING, EMBRYOLOGY AND REARING OF FRY OF DECCAN MAHSEER, TOR KHUDREE (SYKES)

KANGKU OLIVER N. SANGMA AND N. BASAVARAJA

Department of Aquaculture, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore - 575 002, India.

ABSTRACT

Mahseers are regarded as a food fish and an excellent sport fish and they once attracted the attention of best anglers and naturalists from world over. Of the seven species of mahseer belonging to the genus Tor in India, Tor khudree is the only important species available in streams and rivers of the Peninsular India and is known to grow to 120-150 cm (40-50 kg). Over the years, the natural stocks of T. khudree have depleted due to anthropogenic activities and hence several in situ and ex situ conservation strategies have been suggested to revive their stocks. The present study was undertaken with a view to throw more light on brood-stock care, induced spawning, egg incubation and fry rearing of the endangered T. khudree.

Feeding captive fish with a special brood-stock diet containing rice bran, oil cake, fish meal, and rice flour led to early sexual maturation and good spawning response. Soya-based extruded feed also yielded good results. Of the two spawning agents tested, ovaprim resulted in better spawning response (70%) than ovatide (37.5 %). The interval between injection and stripping of eggs ranged between 24 and 36 h at 20-23°C. The number of eggs obtained from ovaprim-injected (0.6 ml/kg) fish varied between 420 and 1,680 per female, while for ovatide-injected (0.6 ml/kg) fish it was 240-2,100 per female. The fertilization rate obtained for both the hormones was high (98-100%). Dry method of fertilization was found to be better than wet method, in terms of fertilization and hatching rates.

Interestingly, one wild-caught brood fish produced 3,240 eggs, without injection, accounting for nearly 13.27 % of eggs present in the ovary; the ovarian biopsy revealed oocytes at different stages of development, indicating T. khudree to be a batch spawner. Another wild female released 1080 eggs from two strippings when injected with ovaprim (0.6 ml/kg). The third female did not respond to injection.

The egg and embryonic developmental stages proceeded normally, but at a slower rate at 20-23°C (hilly area) and faster rates at 26-27°C (coastal area). Hatching periods were 80-112 and 63-70 h for the former and the latter, respectively. At the hilly region, the hatching rate was poor (13.99%), whereas it was significantly higher (42.8%) at the coastal region. However, there was no difference in the yolk-sac absorption period between the two regions.

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*Corresponding Author E-mail: n_b_raju@yahoo.com

The nursery rearing of the hatchery produced and wild fry indicated that the latter grew slightly faster than the latter. The results of this study will have important implications for induced breeding, aquaculture and conservation of T. khudree.

Key words : Brood-stock, breeding, embryology, hatching, fry rearing, Tor khudree

INTRODUCTION

India is blessed with some of the world's best game fishes like mahseers. The mahseers are regarded as a sacred fish by the Hindus. In the past, these game fishes had attracted the attention of the best anglers and naturalists from several parts of the world. Ogale (2002) described the mahseers as a sport fish that provide unparalleled recreation to anglers better than salmon. There are seven species of mahseer belonging to the genus Tor in India. They are: Tor tor, T. putitora, T. khudree, T. nelli, T. progenius, T. mussullah and T. mosal. Over the years, their natural stocks have depleted (National Commission on Agriculture, 1976; Ogale, 2000) due to anthropogenic activities and hence are considered threatened species. The mahseers, the heftly large freshwater fish of India, inhabit fast flowing streams and rivers of the hilly areas, with a temperature (optimum) range of 10-20°C, but some species can thrive and grow well in the plains and coastal regions at temperature ranging between 20 and 32°C (Bazaz and Keshavanath, 1993; Nandeesha et al., 1993; Basavaraja et al., 2002).

To conserve mahseer and develop sport fishing in India, the Government of India has been encouraging attempts to revive the fast dwindling stocks through the establishment of hatcheries. Investigations carried out by Tata Power Companies Limited (TPCL) at Lonavla, Maharashtra State, revealed the possibility of artificial propagation of mahseer (Kulkarni, 1971). Subsequently, several attempts were made for improving and refining methods for the breeding of several species of Tor in India, Nepal, Bangladesh and Malaysia (Mahata et al., 1993; Gurung et al., 2002; Ogale, 2002; Ingram et al., 2005). In India, a breeding technique, with or without hormone injection has been developed for T. khudree, T. putitora, T. tor and hybrid mahseer (Ogale and Kulkarni, 1987; Ogale, 2002). The brood fish for hatchery production of mahseer for stocking programmes or experimentation are derived from reservoirs, lakes or ponds (Kulkarni, 1971; Kulkarni and Ogale, 1991; Tripathi, 1977; Sehgal, 1999; Ogale, 2002).

Tripathi (1977), Joshi (1984) and Joshi et al. (2002) successfully hand-stripped domesticated or wild brood fish, without injecting hormones, while Nandeesha et al. (1993), Keshavanath et al. (2006) and Basavaraja et al. (2002) hand-stripped and fertilized eggs of pond-reared T. khudree following injection of synthetic hormones or pituitary extract. The credit for developing technique for spawning of mahseer in captivity goes to C. V. Kulkarni who helped establish the first mahseer hatchery at TPCL. Nandeesha et al (1993) were the first to achieve success in stripping and fertilizing eggs of pond-raised T. khudree. The

TPCL supplied about 95% of the country's mahseer fingerlings to different States of India and also to other countries where the fry are reared to fingerling stage and then stocked in natural water bodies to enhance mahseer stocks (Ogale, 2000). In addition, approximately 0.2 million mahseer fingerlings are released annually in the reservoir at TPCL and the mahseer population therein has increased substantially (Kulkarni and Ogale, 1995). The second mahseer hatchery was established at Harangi Fish Farm in Kodagu District of Karnataka, southern India, with a capacity of 1.0 million fry per year, in 1992. So far the hatchery has been able to produce only a few thousand fry of T. khudree and the same has been released in selected stretches of the river Cauvery (Nandeesha et al, 1993; Ogale, 2002). Attempts made during 2000-2004 on the breeding of pond-reared Deccan mahseer at Harangi with ready-to-use synthetic spawning agents helped produce a few thousand eggs as part of the studies on the fertility of the cryopreserved T. khudree spermatozoa (Basavaraja and Keshavanath, 2000; Basavaraja and Hegde, 2004; Basavaraja et al., 2006).The present study was undertaken with a view to develop a reliable method for spawning of captive as well as wild T. khudree brood fish, with an emphasis on comparison between two synthetic ovulating agents and the efficacy of wet and dry methods of fertilization.

MATERIALS AND METHODS

The present study was carried out at Harangi fish farm (a hilly area) and Fisheries College, Mangalore (a coastal area). Hatchery produced fingerlings were reared to adult stage and then were used as brood fish in this study.

Brood-stock care

The maturing adults (3-5 years) were separated sex-wise in May 2005 and stocked in 400m^2 rectangular ponds with a soil base of about 20 cm. They were fed with a feed comprising of groundnut oil cake (25%), fish meal (25%), rice bran (25%) and rice flour (25%) at 2-3% of body weight daily. Soya-based extruded feed (American Soybean Association, New Delhi) was fed to selected female fish at 3% body weight daily for 2 months prior to spawning. The ponds were manured once a month with cow dung (2,000-3,000 kg/ha). The fish was periodically checked for somatic and gonadal growth. Of the two trials conducted in this study, the first one was conducted in month of September, while the second trial was carried out in the month of October. During the course of the study, wild brooders were encountered in the nearby Harangi river, collected using a special type of bag net and hook and line, and used for artificial fecundation.

Injection of brooders

Two commercially available synthetic hormones, viz. ovaprim (Syndel Laboratories Canada; sGnRH-a + domperidone) and ovatide (Hemmo Pharma, Mumbai; mLHRH-a + domperidone). The sex was identified based on the morphological characters wherein the female showed

soft and swollen abdomen, while the male readily oozed out milt with gentle pressure on abdomen. Only single dose of the hormones was administered intramuscularly to both male (0.2-0.3 ml/kg body weight) and female (0.6 ml/kg body weight). The brooders were anaesthetized with quinaldine at 10 ppm for 1-2 minutes. The injected females and males were held separately in nylon hapas (rectangular box-shaped containers) with flowing water. The females were periodically checked for their readiness for stripping. The common water quality parameters were monitored during hatching and fry rearing.

Stripping operation and fertilization

Stripping operations were undertaken when the female showed signs of ovulation, generally at intervals of 6-12 hours following injection. This process was repeated several times till all the ovulated eggs were released. Males were also stripped in the same manner such that milt of one or two males was sufficient to fertilize each batch of eggs. The motility of spermatozoa was checked prior to fertilization. Dry and wet methods of fertilization were compared with two different milt volumes i.e. 250 and 500 µl. The colour of the freshly stripped eggs ranged from bright orange to pale orange. The latent period, fertilization rate, hatching rate and fry survival were recorded for both captive and wild brooders.

Egg incubation and hatching

The eggs were incubated at Harangi (temperature 20-23°C) and Mangalore (temperature 26-27°C). At Harangi, the eggs were incubated in rectangular wooden hatching trays with wire mesh at the bottom (150 cm x 20 cm x 10 cm), with running water. Some developing eggs were transported in 18 l capacity plastic bags with oxygen (1/3 water and 2/3 oxygen) by road (duration of journey 6 h) to the College Fish Farm. At Mangalore, the eggs were hatched in conico-cylindrical fiberglass tanks (80 l) with aeration (vortex blower). Important egg and embryonic developmental stages of T. khudree were recorded, both at Harangi and Mangalore.

Fry rearing

Fry rearing was carried out only at Mangalore in FRP tanks (80 l) with aeration. The swim-up fry were fed with egg yolk (a pinch for 200-300 fry) and sieved zooplankton (50 ml zooplankton for 346 fry; each ml contained 60 numbers) twice daily, morning and evening. This was continued for 14 days after which the fry were transferred to 25 m^2 manured concrete ponds at a stocking density of 0.1 million/hectare. In ponds, the fry were fed ad libitum with a feed containing fishmeal (25%), ground nut oil cake (25%), ragi flour (25%) and chicken egg yolk (25%) for 53 days.

Wild fry collection and rearing

When water flow in the Harangi river was minimal due to the closure of the crest gates of the Harangi reservoir, mahseer fry were located in the immediate downstream of the

reservoir. They were collected from the crevices of stones using plankton net and transferred to Mangalore after acclimatizing them for one day at Harangi. The wild fry were reared in FRP tanks for 3 days and then stocked in prepared nursery pond and fed with a diet as described above for hatchery-produced fry.

RESULTS

Breeding technique

In this study three trials were conducted on the artificial fecundation of T. khudree. The soybean-based extruded feed was given for about two months during pre-spawning season (July-August) and this resulted in a few females oozing out eggs with slight pressure on abdomen. However, they could not be stripped due to the inadequate technical staff at Harangi fish farm. After the feed was exhausted, the broodfish was fed with a diet formulated

Table 1. Spawning response, fertilization, hatching and fry survival rates of T. khudree injected with the two spawning agents

Trial	No. of females injected and weight (g)	Spawning Agent	No. of eggs obtained	Fertilization rate (%) (at Harangi)	Hatching rate (%) Harangi/ Mangalore	Fry survival (%) Harangi/ Mangalore
I	18 (150 - 1000)	Ovaprim/ ovatide	15,180	98 - 100	13.99/42.8	0.92/99.4
II	11 (300 - 1000)	Ovaprim/ ovatide	1090	77 - 98	Nil	Nil
III	8 (250 - 1000)	Ovaprim/ ovatide	817	90 - 98	Nil/0.24	Nil/100

Fry were reared in FRP tanks for 14 days and 23 days respectively, after which they were transferred to manured ponds, till the termination of nursery rearing.

using locally available ingredients, leading to results which were slightly inferior to that of extruded feed.

The response of fish to ovaprim was found to be better wherein 11 out of 20 injected females responded positively (Table 1). On the other hand, only 4 out of 17 females spawned when ovatide was injected. The latent period (interval between injection and stripping) ranged between 24 and 36 hours at 20-23°C. The number of eggs obtained from ovaprim-injected females varied between 420 and 1,680 per female, whereas for ovatide-injected fish it was 240-2,100 per female. The freshly stripped spermatozoa showed a motility rate of 95-100%

and duration of 1-2 minutes when activated with tap water.

Date of injection	Total number of females injected	Number of females responded	
		Ovaprim	Ovatide
Trial I	18 females	7	2
Trial II	11 females	1	1
Trial III	08 females	3	1

One freshly caught wild brood fish (probably a half spent fish), when stripped without injection, produced 3240 eggs which accounted for nearly 13.27% of the ova present in the ovary; the ovarian biopsy of the fish revealed oocytes at different stages of development, indicating T. khudree to be a batch spawner. Another wild caught female released 1080 eggs from two strippings when injected with ovaprim (0.6 ml/kg body weight). The third female did not respond to injection.

The colour of freshly stripped eggs varied from bright orange in farm-raised fish to pale orange in wild caught females. However, the developing good eggs were orange or lemon yellow in colour, while unfertilized eggs were opaque white and were periodically removed from the trays with a filler. The fertilization rates obtained for both the hormones were high (98-100%). The dry method of fertilization was found to be better than wet method at the tested milt concentrations. The egg and embryonic developmental stages proceeded normally, but at a slower rate at Harangi and faster rate at Mangalore.

Embryology

Important egg and embryonic developmental stages recorded are as follows:

Egg and embryonic stage	Hours after fertilization	
	At Harangi	At Mangalore
1. Blastodisc formation	2	-
2. Morula stage	5	4-5
3. Blastula stage	13	10
4. Gastrula stage	18	-
5. Yolk-plug stage	27	12
6. Embryo indication	37	-
7. Pea-shaped embryo	48	-
8. Twitching movement	55	33-34
9. Moderate twitching	63	55
10. Vigorous twitching	70	62
11. Hatching started	80	63
12. Hatching completed	112	70

The percentage of abnormal embryo was slightly higher at the hilly area than that of coastal region. The hatching periods were 80-112 and 61-70 hours at the former and the latter places, respectively. The hatching percentage was found to be very low (13.99) at Harangi, while it was high (42.8) at Mangalore.

The proportion of deformed fry observed at Harangi was high and they did not survive, whereas at Mangalore the number of deformed fry was almost nil (only one fry was found to be deformed). Only at Harangi, fungal growth on eggs was observed and the same was controlled by dipping eggs in a solution of $KMnO^4$ (3-5 ppm) for 5 minutes.

Wild fry (about 1-2 weeks old) of T. khudree collected from a stretch of the river adjacent to the fish farm from where the wild brooders were obtained were reared in nursery ponds parallel to that of hatchery-produced fry and the details are presented in Table 2. The length of newly hatched farm raised fry was 7.4 g (+/- 0.55). By 37 days, the fry attained a length of 5.63 cm and a weight of 2.03 g. The wild fry reached 22.7 mm (+/- 2.19 mm) after a rearing period of 20 days. By 67th day, the hatchery fry grew to 80.8 mm (weight: 6.39 g), while the wild fry attained 64.1 mm (weight: 3.02 g). The growth increment was found to be 1.05 mm/day and 106.31 mg/day during 60-day-rearing period.

Table 2. Length (mm) attained by T. khudree fry during nursery rearing

Days after hatching	**Hatchery produced fry (Mean ± SD)**	**Wild fry (Mean ± SD)**
First day	7.4 ± 0.55	Nil
Second day	9.0 ± 0.71	Nil
3rd day	9.4 ± 0.89	Nil
4th day	9.8 ± 0.45	Nil
5th day	10.4 ± 0.55	Nil
6th day	11.0 ± 0.71	Nil
14th day	18.0 ± 1.3	Nil
20th day	Nil	22.7 ± 2.19
37th day	56.3 ± 2.11 (wt. 2.03 ± 0.27g)	Nil
67th day	80.8 ± 4.08 (wt. 6.39 ± 0.91g)	64.1 ± 2.08 (wt. 3.02 ± 0.30g)
Survival (%) in FRP tanks	99.4	100

Important water quality parameters recorded during the study are given in Table 3. They were found to be within the permissible levels for mahseer hatchery, barring CO^2 which was marginally higher.

Table 3. Water quality parameters recorded at Harangi and Mangalore

Water quality parameter	**Harangi (in hatching trays)**	**Mangalore (in FRP tanks)**
Dissolved oxygen (mg/l)	9.2-10.4	5.2-6.8
Carbon dioxide (mg/l)	8.8-14.08	8.8-10.56
Total alkalinity (mg/l)	14-20	18-34
pH	6-6.5	6-6.5
Temperature (°C)	20-23	26-29

DISCUSSION

The results show that the soya-based diet stimulated gonadal development and spawning in T. khudree. However, the locally formulated feed was found to be slightly inferior to the extruded feed. Nandeesha et al. (1993) showed that feeding T. khudree with a quality feed (protein: 31.5%) led to successful gonadal maturation in the captive T. khudree. The results of the present investigation indicate that ovaprim works slightly better than ovatide in the induced breeding of T. khudree. Nandeesha et al. (1993) found no difference in latent period, spawning response, fertilization rate, hatching rate and fry survival between ovaprim and carp pituitary extract (CPE) in T. khudree, but suggested the use of ovaprim since it is injected only once to the female unlike CPE which is to be injected twice. Ingram et al. (2005) observed that ovaprim to be more effective in inducing ovulation in Malaysian mahseers than ovaplant, HCG and CPG. In India, ovaprim and ovatide are routinely used for the induced breeding of Indian and exotic carps and other fishes (Nandeesha et al., 1991; Francis et al., 1991; Pandey and Singh, 1997; Basavaraja et al., 1999). In this study, the injected females took a minimum of 24 hours (range: 24-36 hours) to ovulate (temperature: 20-23°C), which conforms to the results of Ingram et al. (2005) who found it to be about 30 hours. Interestingly, the wild brooder produced eggs without the injection of either ovaprim or ovatide. It is evident that wild brooders produced more egg than the captive brooders, due mainly to the larger size of the former. Kulkarni and Ogale (1978) reported that they were able to fertilize more than 0.5 million eggs of T. khudree caught from reservoir/lake at Lonavla. Kulkarni (1984) described the effective transportation of fertilized eggs in moist cotton wool, without water, by air. Our study revealed successful transportation of eggs and fry, including wild fry.

Dry method of fertilization was found to be better than wet method at both the milt volumes (250 and 500 µl), probably because of higher longevity of sperms in the former method. Our results also show generally high fertilization rates (77-100%), but hatching rates have been found to be low at Harangi which may be attributed to improper water supply during incubation. The hatch rates obtained at Mangalore were comparable with that (60%) reported by Nandeesha et al. (1993). For Malaysian mahseers, the hatching rate has

been found to be 0-35% (Ingram et al., 2005). Important egg and embryonic developmental stages studied in this study revealed that they proceeded normally; it was more so at Mangalore. Ingram et al. (2005) made a detailed study on the egg and embryonic stages of Malaysian mahseers. The hatching period was temperature dependent, with eggs taking more time to hatch at Harangi and less time at Mangalore due to higher temperature at the latter than the former. Nandeesha et al. (1993) observed similar hatching periods of 110-122 and 65-70 hours at Harangi and Mangalore, respectively. Ingram et al. (2005) reported hatching time of 69-90 hours at 26-30°C. The yolk-sac absorption period was also inversely proportional to water temperature. The period required for yolk-sac absorption in this study was almost same as that reported by Nandeesha et al. (1993). Ingram et al. (2005) observed it to be about 5-6 days. The results of the present study and our earlier studies indicate that T. khudree spawns generally towards the end of southwest monsoon (June-September), with maximum spawning during September-October. Most studies involving mahseers have reported that mahseer breed during July-October. However, mahseer is reported to breed several times in a year (Beavan, 1877; Nevill, 1915). In Malaysia, the mahseer are known to have a prolonged breeding season and are induced to spawn in November-December and again in April (Ingram et al., 2005).

The present study reports the first success on the stripping of wild brooders and location of spawning grounds of T. khudree in Karnataka.

It may be concluded that the soya-based extruded and the feed formulated using locally available feed ingredients could stimulate gonadal development in captive T. khudree. Ovaprim was found to be better than ovatide in stimulating ovulation. The successful location of the spawning grounds and wild fry availability would help increase T. khudree fry production in future.

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Corresponding Author :

Dr. N. Basavaraja,–Professor, Department of Aquaculture, KVAFSU, College of Fisheries, Kakanady, Mangalore-575002, Karnataka, India.
E-mail: n_b_raju@Yahoo.com