Reproduction and Seed Production of Sahar (*Tor putitora*) in Chitwan Nepal

Quality Seedstock Development/Study/13QSD02UM

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ABSTRACT

Sahar (*Tor putitora)* is a high-value indigenous riverine species of Nepal that is declining in its natural habitat and has been declared an endangered species. Limited seed production of this species in the temperate region has restricted its expansion in culture and rehabilitation in natural waters. An experiment was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, from August 2014 to April 2015 to explore and assess breeding performance of sahar in the Terai region of Nepal, which has a subtropical climate. Twenty-eight male (0.5–1.5 kg) and 35 female (0.8–2.5 kg) brood fish were reared in ponds at 1000 kg/ha and provided 35% protein feed at 3% body weight per day. Maturity was observed by sampling fish and applying pressure to the abdomen to express gonads every two weeks during the off-season; this frequency was increased to every third day as breeding season approached. One female sahar (3–5 years old) was ready for breeding in March when water temperature was 23.3–25.2 °C. In the same month, another female responded to an injection of inducing hormone (ovaprim) when the temperature was 25.3–28.7 °C. Males about 1–2 years old expressed milt in almost all months. Ova from mature females were obtained by simple hand stripping and fertilized with milt manually collected from males. The fertilized eggs were incubated in Atkin hatching trays. Survival and growth of fry were high and maturation details were similar to fish spawned under temperate conditions. This study demonstrated that natural and induced breeding and fry rearing is possible in the Terai region of Nepal. However, further studies on synchronization of breeding time and mass seed production are needed.

INTRODUCTION

Sahar (*Tor putitora*), also known as "mahseer," is an important fish species of the torrential water of the Himalaya. It is a popular, economically important, and high-value indigenous fish species. Sahar is important as a game and food fish and is widely distributed in rivers, streams, and lakes (Rai et al. 1997). The price of sahar in the Nepalese market is almost double that of commonly cultivated carps and tilapia. Sahar is still taken in capture fisheries in lakes and rivers, and no commercial cultivation has begun in Nepal. This species is declining from its natural habitat mainly due to urbanization, poaching, over-fishing, and ecological alterations of physical, chemical, and biological conditions in the natural environment (Bista et al. 2007). Hence, there is a need for conservation of this species. In recent years, success in artificial breeding at some research stations has provided the additional enthusiasm on developing sahar for commercial cultivation, as well as the rehabilitation in natural waters (Rai et al. 2006).

Attempts to culture and conserve sahar have been initiated in Nepal with major efforts to develop culture technology and propagate the species (Gurung et al. 2002, Joshi et al. 2002). This has led to better knowledge of spawning biology, ecology, and behavior, as well as preliminary growth performance in

captive conditions. Enhanced growth in tropical and subtropical ponds, as well as the recent breeding success in hatcheries, has raised new hopes on the prospects of sahar aquaculture in Nepal (Shrestha et al. 2005, Bista et al. 2001, 2007, Rai 2008). In addition to the culture of fish to adult size for consumption, these new developments can contribute to rearing individuals that can be stocked into natural waters to replenish populations there. Due to its omnivorous and predatory feeding, sahar has also proven to be a good candidate to co-culture with mixed-sex tilapia to control tilapia recruits and provide better size at harvest and yield of tilapia (Shrestha et al. 2011). Inclusion of sahar in polyculture of mixed-sex tilapia with carps has enhanced production in these ponds.

Sahar is known to be intermittent in spawning behavior. In Nepalese context, it can spawn year-round, except January, under cultured conditions. However, in natural waters, it spawns during the monsoon when rivers and streams are at peak flows. Sahar typically migrate a long distance from large rivers to streams for spawning. The Fisheries Research Center in Pokhara is the key center that produces sahar fry in limited quantity. Demand for sahar fry has increased for restocking in rivers and lakes, as well as for aquaculture production. Lack of availability of fish seed is a major bottleneck for commercial production and conservation.

OBJECTIVES

- To extend sahar breeding technology to Chitwan from work conducted in Pokhara;
- To develop protocols for sahar reproduction and mass-scale seed production in Chitwan;
- To establish nursing and rearing management practices of sahar fry in Chitwan; and
- To make sahar fry available for culture and restocking.

MATERIALS AND METHODS

The experiment for sahar breeding was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur, Chitwan, for nine months (1 August 2014 to 30 April 2015). For response studies, female sahar broods were transferred from the Fisheries Research Center, Pokhara, and reared at AFU. Male fish of more than one year age were collected from the aquaculture farm at AFU. Females of approximately 1.0–2.5 kg body weight were stocked in brood pond at the rate of 1,000 kg/ha. Brood fish were fed with 30%–40% protein diets. The feeding rate was 2%–5% body weight per day. Pond water quality parameters, such as temperature, pH, and dissolve oxygen, were measured every morning using Lutron Oxygen Meter DO-5510 and Lutron YK-21 PH model. Maturity of female fish was monitored at regular intervals. They were checked biweekly before the breeding season (May–August). The male broods were always found ripe with oozing milt after pressing their belly, but females were not ripe during May–August. As the breeding season approached during August– November and February–May, maturation testing was increased to every third day. Maturity observation was performed once a month for the remaining two months (December and January).

For maturity tests, fish collected from ponds were held in a hammock and readiness for spawning was examined by applying gentle hand pressure near the genital opening. Ripe males released milt, and females brood ova on 9 March. This female fish and two males were transported to the hatchery. The clean and dried female was stripped gently to empress eggs into a clean and dried bowl. Milt from both males was also collected in another bowl, then mixed with eggs for dry fertilization. The fertilized eggs were washed several times and spread in Atkin incubators by allowing one layer of eggs to settle on a single mesh screen in the flow- through system, and water flow was maintained at 7–9 L/min. The incubation trays were covered with a towel to reduce light levels in the tray. The eggs were observed after 24 hours during incubation, and unfertilized eggs were counted. Dead eggs were counted and removed each day to protect the healthy eggs from fungal infection. After four days (96 hours) hatching occurred and was completed within 24 hours when distinct eyes were seen in hatchling fish. Early hatched larvae had large amounts of yolk sac and settled around stones or near corners of the incubation tray. After

attaining free-swimming stage, the larvae were transferred into a tank of 2.5 m x 0.4 m x 0.3 m dimension.

On 26 March, another two female fish were injected with ovaprim hormone at the rate of 0.5 mg/kg body weight. Along with those females, four mature male fish were also kept in the spawning tank. After 26 hours, when the females were checked by pressing gently on the stomach, one female brood released eggs. Milt from two males was used to fertilize the eggs. Fertilized eggs were incubated as before, and a similar process was repeated. Hatching occurred after 60–72 h.

Reproductive parameters, such as relative fecundity, fertilization rate, hatching rate, and survival rate, were measured to analyze breeding performance. After fertilization, the total number of eggs and egg number per kg body weight were calculated. Egg size, mean weight of eggs and mean weight of swim-up fry were measured using an electronic balance. Fertilization rate, hatching rates, incubation period, hatchling survival rate, yolk absorption time, and time of hatchling to fry were also recorded.

The fry-rearing experiment was conducted for 45 days using two types of feed — commercial feed (T_1), and locally prepared feed (T_2) — for two batches of fry (from a naturally spawned brood and from a hormonally induced spawning). Each treatment was replicated three times. Sahar fry of 0.073–0.077 g body weight were stocked at 200 fry/m^2 in a hapa and water depth was maintained about 60 cm in each hapa. Fish fry were fed three times a day at 5% body weight throughout the experimental period.

RESULTS

Out of 35 female fish reared, one was observed overly matured on 21 November and another on 24 November 2014. On 8 December, two females were found overmatured, and, again on 9 December, two females showed overmaturation. Another female showed over maturation on 4 January 2015. In February, four females were overmature. In March, four females were overmature. In total, 15 female fish were found overmature during regular observations (Table 1).

Table 1. Result of maturity tests in different months from November 2014 to March 2015.

Date	Number of over mature females	Number of over spawned females	Water temperature of brood pond (°C)
21 November	1		21.2–23.0
24 November	1		19.7–22.8
8 December	2		18.2–21.2
9 December	2		17.5–20.5
4 January	1		15.5–17.2
21 February	1		19.1–21.3
24 February	2		19.4–22.0
27 February	1		20.6–22.8
4 March	2		22.5–26.0
7 March	1		22.2–25.4
9 March	1	1	23.3–25.2
27 March		1	25.3–28.7

One female was ready to spawn without injecting hormone (natural breeding) on 9 March 2015 when minimum temperature of water was 23.3°C and maximum was 25.2°C. Similarly, breeding of hormone-induced females occurred on 26 March when minimum temperature was 25.3°C and maximum was 28.7°C (Table 2). The total number of eggs obtained from the natural breeding female was 2585, while it was 4738 for hormone-induced breeding. Relative fecundity was 2,119 and 3,746 for natural and hormone-induced breeding, respectively. For natural breeding, 1g of ovulated eggs contained 94 eggs, while in hormone-induced breeding, 103 ovulated eggs were in 1 g of egg.

Fertilization rate of natural and hormone-induced breeding was 98% and 99%, respectively. The incubation period for natural and hormone-induced breeding was 96–104 h and 80–88 h, respectively. In the case of natural breeding, hatching rate was 95%, while that was 97% in hormone-induced breeding. Hatchling survival of natural breeding was 81%; whereas survival was 90% for hormone-induced breed hatchling (Table 2).

Newly hatched larvae were 9.4±1.2 mm long and 13.01±0.53 mg in weight. Similarly, newly hatched larvae of hormone-induced breeding were 8.9±0.7 mm long and 13.19±0.49, mg in weight (Table 3). It took 6 days to complete yolk sac absorption in natural breeding at 19.4–26.2 °C, but only 5 days to absorb yolk sac for induced larvae at 24.8–27.2 °C. Average yolk sac absorbed larvae from natural spawning were 11.5±0.5 mm long and 10.09±1.12 mg in weight. Similarly, average yolk sac absorbed larvae from hormone-induced breeding were 11.5±0.5 mm long and 9.87±1.41 mg in weight (Table 3).

Table 2. Breeding performance of sahar with and without induced maturation.

Description	Natural breeding	Induced breeding
Date	9 March	27 March
Temperature (°C)	23.3 –25.2	25.3–28.7
Female body weight (kg)	1.2	1.3
Male body weight (kg)	0.65, 0.80	0.72, 0.87
Total egg spawned	2585	4738
Egg number per kg body weight	2119	3746
Ovulated eggs per g	94	103
Fertilization rate (%)	98	99
Incubation period (hour)	96–104	80–88
Hatching rate (%)	95	97
Hatchling survival (%)	81	90
Yolk sac absorption period (days)	6	5
Time to swim-up fry (days)	17	17

Table 3. Mean and range of egg diameter, and length and weight (mean±SE) of larvae and fry from two breeding parents.

Parameter	Brood	
	Natural breeding	Induced breeding
Mean fertilized eggs diameter (mm)	2.9±0.2 (2.8–3.5)	3.1±0.3 (2.8–3.3)
Mean fertilized eggs weight (mg)	12.37±0.80 (11.57–13.17)	12.69±0.78 (11.97–13.47)
Mean newly hatched larvae length (mm)	9.4±1.2 (8.2–10.6)	8.9±0.7 (8.2–9.6)
Mean newly hatched larvae weight (mg)	13.01±0.53 (12.48–13.54)	13.19±0.49 (12.70–13.68)
Mean yolk sac absorbed larvae length (mm)	11.5±0.5 (11–12)	11.5±0.5 (11–12)
Mean yolk sac absorbed larvae weight (mg)	10.09±1.12 (8.97–11.21)	9.87±1.41 (8.46–11.28)
Mean swim-up fry (17 days) length (mm)	14.6±0.5 (14.08–15.12)	13.3±0.5 (12.8–13.8)
Mean swim-up fry (17 days) weight (mg)	20.96±1.08 (19.1–22.5)	14.04±0.40 (13.5–14.6)

During brood rearing, temperature ranged from 14.3°C to 38.2°C (Figure 1), DO from 1.4–13.5 mg/L, and average pH from 5.9 to 10.4. Natural spawning occurred when temperature was between 23.3°C and 25.2°C, while induced spawning occurred when temperature of pond was between 25.3°C and 28.7°C.

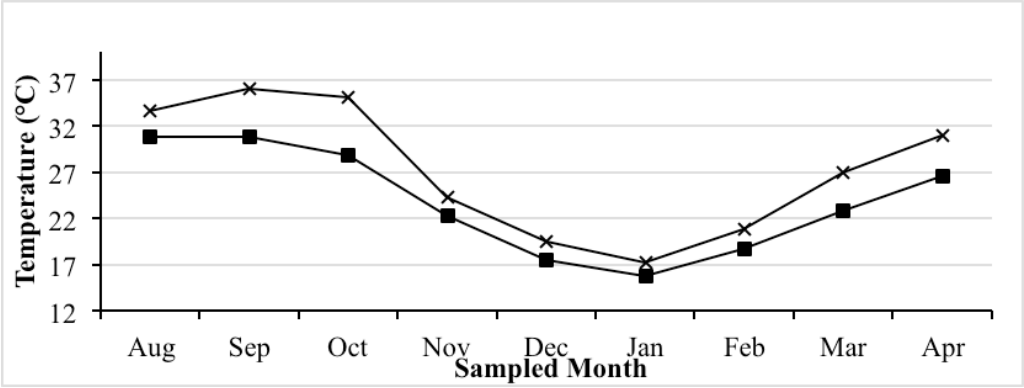

Figure 1. Average water temperature of the brood pond at morning and evening each month during brood rearing.

Growth parameters of swim-up fry after 17 days were obtained from two broods fed with two different types of feed (Table 4). In the case of fry obtained from natural breeding, there were no significant differences in mean initial weight, mean final weight, specific growth rate (SGR), daily weight gain (DWG), or survival rate for fry fed with two different types of feed. Similarly, in the case of fry from hormone-induced breeding, there was no significant difference in any growth parameters for fry fed with two different types of feed. Growth for both types of fry was linear over the rearing period (Figures 2 and 3).

Table 4. Growth performance of fry from two breeding parents fed with different feeds.

Brood	Natural breeding (B_1)		Induced breeding (B_2)	
Feed type	F_1	F_2	F_1	F_2
Mean initial wt. (mg)	76.47±7.65[a]	71.00±3.38[a]	80.83±4.14[a]	73.87±11.99[a]
Mean Final wt. (mg)	252.11±40.82[a]	330.88±39.49[a]	282.17±62.00[a]	272.79±12.73[a]
Culture days	40	40	40	40
SGR (%/day)	2.97±0.61[a]	3.84±0.25[a]	3.09±0.44[a]	3.29±0.30[a]
DWG (mg/fish/day)	4.39±1.17[a]	6.50±0.94[a]	5.03±1.46[a]	4.97±0.13[a]
Survival (%)	98.67±2.31[a]	98.00±1.80[a]	94.67±5.77[a]	95.17±3.33[a]
AFCR	1.7±0.2[a]	1.2±0.1[b]	1.8±0.4[a]	1.6±0.1[a]

Mean values with same superscript in the same row are not significantly different ($p<0.05$).

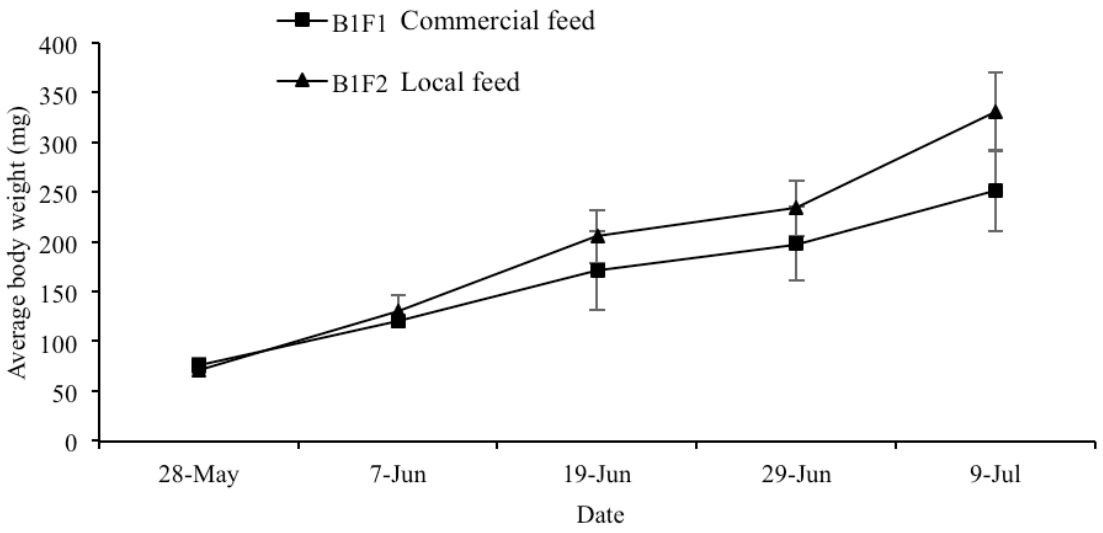

Figure 2. Growth of fry from natural breeding fed with two types of feed.

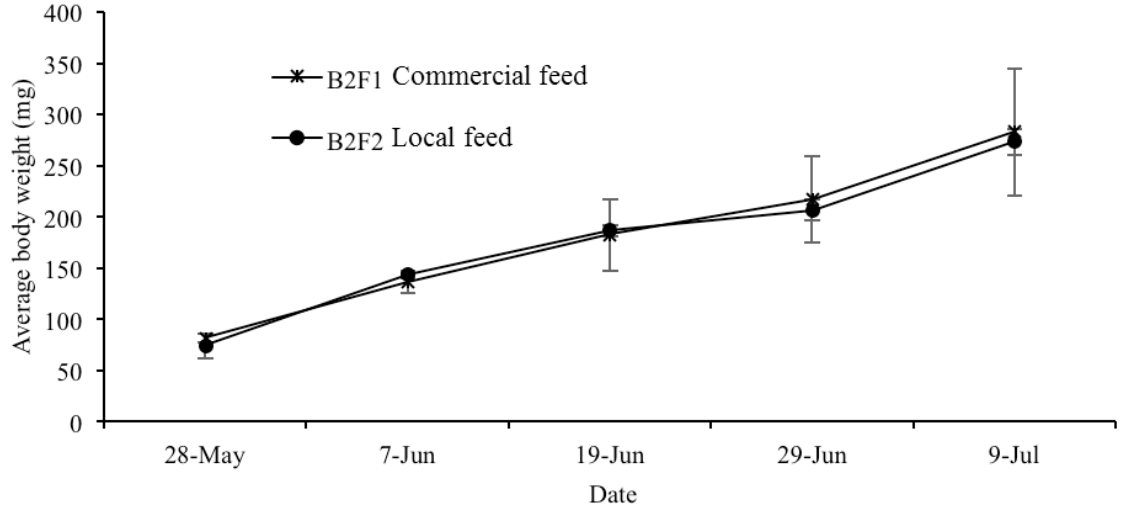

Figure 3. Growth of fry from induced breeding fed with two types of feed.

DISCUSSION

Regular maturity observation of sahar showed that getting the correct spawning time is critical for sahar breeding, and thus, examination of female fish for maturity must be done frequently. As reported by Bista et al. (2010), pond reared sahar show intermittent spawning characteristics, and determining optimum timing for egg stripping by frequent checking of brood fish may result in a spawning success rate of more than 50%. Although frequent examination of brood for maturity was done in this study, 15 females were found over mature, while successful breeding was attained only in 2 females. Overripe females were recorded even when temperature ranged between 15.5–28.7°C, from the last week of November to the last week of February. Bista et al. (2010) reported that spawning occurred when temperature ranged

between 26–27.4°C on one occasion, and 20–21°C on a second occasion in Pokhara, and there were more spawners in February and March compared to September and October. Pandey et al. (1998) reported successful spawning induced by hormonal injection when water temperature in the pond was 18–24 °C. However, the dose administered was lower (0.2 ml/kg body weight) than in this study. Bista et al. (2010) also reported that diameter and weight of fertilized eggs were 2.87±0.13 to 2.98±0.08 mm and 13.90±0.91 to 15.38±1.26 mg, respectively. They also reported an incubation period of 45–125 hours at water temperatures from 19–28 °C. The shorter incubation period for the second lot of eggs can be attributed to a higher temperature. The length and weight of newly hatched larvae were similar to data from Bista et al. (2010).

Female rearing was done in temperatures ranging between 14.3–38.2°C, DO ranging between 1.4–13.5 mg/L and average pH ranging between 5.9–10.4. There did not appear to be any stress of the female fish in response to these varying conditions. Our results for temperature at spawning were similar to Bista et al. (2010), who documented natural breeding in autumn when the temperature was 22–27°C, and in spring when the temperature was 19–25°C. Similarly, Pandey et al. (1998) documented induced breeding of sahar when temperature was 18–24°C. Bista et al. (2010) also reported that pH ranged from 7–9 and DO from 3–9 mg/L when natural breeding occurred.

There were no significant differences in mean final weight, SGR, DWG, and survival rate of fry from natural breeding when fed with commercial feed (F_1) and farm-made feed (F_2). Similarly, there were no significant differences in these values for fry from induced breeding when fed with two different types of feed. The DWG of fry were considerably lower than reported by other authors, such as FRC (1995, 0.02–0.03 g/day), Nepal et al. (1996, 0.52 g/day), Paudel (2003, 0.10–0.13 g/day), Acharya (2004, 0.32 g/day) and Bista et al. (2007, 0.18–0.28 g/day). Survival rate of fry during present study was considerably higher than values found by Paudel (2003, 33–51.3%) and Islam (2002, 83.8–89.4%), but similar values documented in FRC, Pokhara (1995, 90.4–92.1%), and Acharya (2004, 92%).

CONCLUSIONS

Breeding of sahar is possible in the subtropical climate of Nepal. The best temperature lies between 17.5°C and 28°C. During testing, we observed that maturity time is very short (12 to 24 h), and many fish were not found until they were overly mature. Testing should be conducted more frequently to find ripe female fish during the spawning interval. The success of hormone-induced spawning shows new hope for possible mass production of sahar seed.

QUANTIFIED ANTICIPATED BENEFITS

Sahar were successfully bred at the new site in Rampur, and, as a result, about 5,000 fry were produced. Five farms used sahar in carp polyculture trials during our on-farm trial with mixed-sex tilapia. We conducted a workshop on production of sahar seed with hatchery operators as well as development and research personnel to help extend this information to more users.

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