IMPROVING SEED PRODUCTION OF SAHAR (*TOR PUTITORA*) IN CHITWAN NEPAL

Quality Seedstock Development/Study/16QSD02UM

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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 using natural propagation has restricted its expansion in culture as well as rehabilitation in natural waters. We achieved success in artificial propagation of sahar using synthetic hormone. The breeding program was conducted at the Agriculture and Forestry University, Rampur, Chitwan and the Center for Aquaculture Research and Production, Kathar, Chitwan, Nepal during February to April 2017. Sixty five male (0.5-1.8 kg) and forty five female (1.1-2.1 kg) brood fish were reared in 200 m^2 earthen ponds at 1,000 kg/ha and transferred to 25 m^2 concrete tanks one month before the breeding season. Fish were fed with 32% crude protein feed at 3% body weight per day. Maturity was observed weekly by sampling fish and testing softness of the abdomen. Female brood fish with a soft and extended abdomen were injected with synthetic hormone (Ovaprim) at 0.5 mL/kg body weight. Males did not receive any hormone injection. After 24-26 hours of injection, ova from injected females were obtained by hand stripping and fertilized with milt collected from males. The fertilized eggs were incubated in Atkins hatching trays. Sixteen females were induced to spawn, and they produced 1630.80±184.30 (mean±SE) eggs per kg body weight. Mean hatching and larval survival rates were 78.4±1.9 and 74.7±1.1%, respectively. This study demonstrated that mass seed production and larval rearing of sahar is possible in the subtropical region of Nepal using induced breeding. Induced spawning reduces the number of over-matured females by synchronizing the stripping time of injected brood fish. Fry from these spawning were reared for 90 days in hapas, and their growth was about average for that size of fish (0.7 to 1 g/d) while their survival was very high (about 95%).

INTRODUCTION

Sahar (*Tor putitora*), also known as “mahseer,” is an important fish species from the glacial water of the Himalaya. It is distributed in Trans-Himalayan countries ranging from Afghanistan to Myanmar (Skene-Dhu 1923, MacDonald 1948, Day 1958, Desai 1994, Khan et al. 1994). Sahar is a well-known, indigenous game and food fish, economically important. Sahar is widely distributed in rivers, streams, and lakes (Rai et al., 1997). While it is still taken in capture fisheries in lakes and rivers, no commercial cultivation has begun in Nepal. This species is declining from its natural habitat mainly due to over-fishing and ecological alterations of physical, chemical, and biological conditions in the natural environment (Bista et al., 2007). In recent years, success in captive breeding at some research stations and induced breeding at subtropical climates in Chitwan, Nepal have provided the opportunity to develop sahar for commercial cultivation, as well as rehabilitation in natural waters (Jha et al., 2017).

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 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 mature year-round, except during January, under cultured conditions, but in natural waters, Sahar typically migrate a long distance from large rivers to streams for spawning during the monsoon (June to August), when rivers and streams are at peak flows. Programs of conservation for this fish have created increased demand for fry for restocking in rivers and lakes, as well as for aquaculture production. Insufficient availability of fish seed is a major bottleneck for commercial production and conservation.

OBJECTIVES

The overall objective of this project was to continue work to develop methods for controlled reproduction of sahar to provide seed for restocking or for aquaculture production. The specific objectives were:

1. To continue development of sahar breeding in Chitwan.
2. To improve protocols for manual detection of maturity and manual spawning of sahar.
3. To determine if gonadotropic hormone injection can be a feasible method to induce maturation of sahar.
4. To establish nursing and rearing management practices of sahar fry in Chitwan.

METHODS

The experiment for induced breeding of sahar was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur and Center for Aquaculture Research and Production (CARP), Kathar Chitwan, from September 2016 to December 2017 (Figure 1).

Figure 1. Ponds used for sahar experiments at CARP (left) and AFU (right).

Altogether 180 fish (70 females and 110 males) were reared as brood stock in a well prepared earthen pond of 200 m^2 size at CARP and in two concrete ponds each 25 m^2 in size at AFU. Females were 3 years old and approximately 1.0 kg or more body weight and males were 1+ years. They were stocked in brood ponds at the rate of 1,000 kg/ha. The male fish were always found ripe with oozing milt after pressing their belly. Brood fish were fed commercial pelleted floating feed with 32% protein at 3% of total biomass per day.

Among the 180 fish, fish were selected for spawning one month before the season. At Kathar, 30 females and 45 males were selected and kept in separate pond of 200 m^2 and 15 females and 20 males at Rampur were reared in concrete tanks of 25 m^2.

Maturity of female fish was monitored at regular intervals. They were checked biweekly before the breeding season. Male sahar can attain maturity in one year at a body size less than 50 g, but female fish first mature at the age of 3+ years. As the breeding season approached during August-November and February-April, maturity testing was increased to every third day.

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 ooze milt and ripe females release yellow orange eggs when stripped in this manner.

For hormonal induction of spawning, Ovaprim (Salmon Gonadotropin Releasing Hormone analogue with Domperidone) was applied at 0.5 ml/kg of female body weight as practiced in carp hatcheries in Nepal. Fully mature females with a soft belly and pinkish red vent but not ripe were induced to release eggs by hormonal injection. After 24-26 hours of injection, females were ready to spawn and eggs were stripped by pressure on the abdomen.

The females were wrapped with a soft towel for removing water from the body before stripping to avoid water drops mixing with eggs. The clean and dried females were stripped gently to collect eggs into a clean and dried bowl. Milt from males was collected in another bowl, then mixed well with eggs for dry fertilization. The fertilized eggs were washed several times and incubated in Atkins hatching trays by spreading one layer of eggs on a mesh screen in the flow-through system. Water flow was maintained at 7-9 L/minute. The incubation trays were covered with black plastic. The eggs were observed after 24 hours during incubation, and white eggs (unfertilized and dead) were counted and removed to protect healthy eggs from fungal infection. Depending on water temperature, hatching took place after 72-96 hours. Recently hatched larvae had large yolk sacs and settled around stones or near corners of the incubation tray. After attaining the free-swimming stage, larvae were transferred into a hapa of 3 m x 2 m x 1 m dimension.

Reproductive parameters, such as total number of eggs, egg number per kg body weight, fertilization rate, hatching rate, incubation period, and hatchling survival rate were also recorded to analyze breeding performance.

An experiment was conducted for 90 days (10 June – 9 September 2017) at AFU to evaluate growth performance of sahar fry. Fry were reared in fine meshed nylon hapas (2m x 1m x 1.2 m) suspended in a concrete pond. There were 4 treatments replicated thrice. The treatments were: 1) 5 fish/m^3; 2) 10 fish/m^3; 3) 15 fish/m^3; and 4) 20 fish/m^3. Stocking size of fish was 0.28-0.32 g. Fish were fed with commercial pelleted feed (32% protein content) at 5% of total biomass twice daily. The quantity of feed was adjusted fortnightly based on fish size. In situ temperature, pH, dissolved oxygen and transparency were measured weekly throughout the experimental period at 6.00-7.00 am.

RESULTS

Breeding of hormone-induced females occurred from 26 February to 4 April when water temperature ranged between 21.4°C and 28.5°C. After 24-26 hours of injection, ova from injected females were obtained by hand stripping. Sixteen females were induced to spawn and produced 2331.40±270.80 (mean±SE) eggs per female and 1630.80±184.30 eggs per kg body weight. There was a mean of 104.1±2.5 eggs per gram egg weight. Mean fertilization rate (%), incubation period (hour), hatching and larval survival rates were 96.8±1.5, 79-90, 78.4±1.9 and 74.7±1.1%, respectively (Table 1).

Table 1. Breeding performance of sahar produced by hormonal injection.

Parameter	Mean±SE
Breeding duration	2017-2-26 to 2017-4-9
Water temperature (°C)	21.4-28.5
Total no of female spawners	16
Mean weight of females (kg)	1.47±0.09
Mean weight of males (kg)	0.76±0.05
Mean total egg spawned (count)	2331.40±270.80
Mean egg number per kg body weight	1630.80±184.30
Mean egg number per gram egg weight	104.1±2.5
Mean fertilization rate (%)	96.8±1.5
Mean incubation period (hour)	79-90
Mean hatching rate (%)	78.4±1.9
Mean hatchling survival (%)	74.7±1.1

In addition to the 16 induced females, 6 naturally spawned females found during maturity testing of brood females released 115.0 g of ova. The total number of hatchlings produced from these spawnings was 8970, while 6727 fry resulted (75% survival). Regular observations from February to April revealed that only six females were overly mature and five females did not respond to hormonal injection (Table 2).

Table 2. Spawning response of sahar females that naturally matured.

Total number of spawners	6
Over mature females (no)	6
Total eggs spawned (g)	115.0
Hatchling production (no)	8970
Hatchling to fry (no)	6727

Annual water temperature of brood rearing ponds ranged from 16.2 to 31.2°C. The minimum temperature was recorded during January and maximum in July. During breeding season from September to November and February to April, the recorded temperature ranged from 19.2-26.2 and 20.4-29.5°C, respectively (Figure 2).

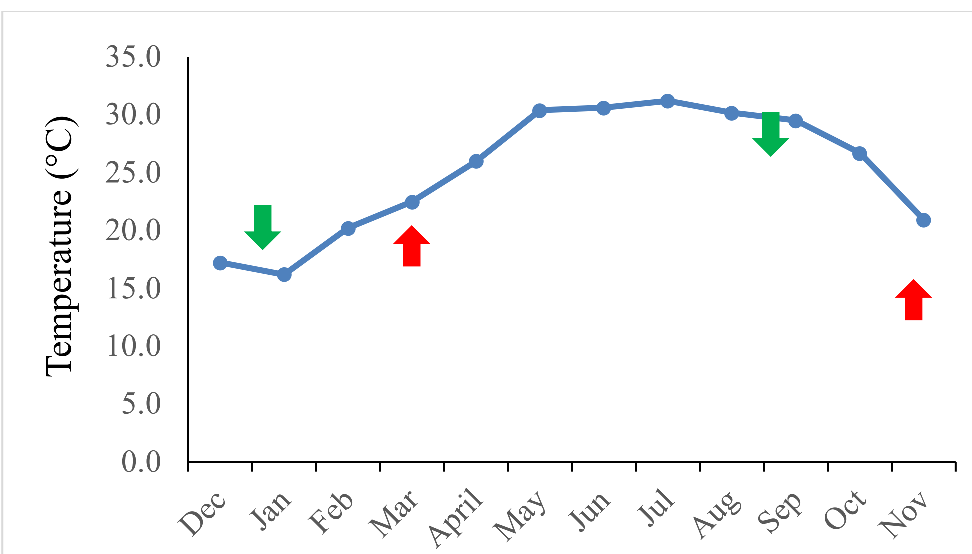

Figure 2. Annual water temperature of brood pond

Water temperature was recorded for the entire year in brood ponds. It ranged between 16.2-31.2°C. Spawning took place when temperature was between 20.2 to 26°C in February to April and 21 to 29.5°C in September to November. The first spawning occurred during a rising temperature phase while the second spawning occurred during falling temperature.

Growth and survival of sahar fry in different stocking densities indicated moderate growth rates and high survival (Table 3). Final harvest weight was significantly higher in T_1 than T_3 and T_4 ($p \leq 0.05$). There was no significant difference on survival among treatments. Growth rate of sahar fry was highest in the high density treatment, while condition factor was highest in the low density treatment (Table 4). Daily growth rate and condition factor were significantly higher in T_1 than T_3 and T_4 ($p \leq 0.05$). There was no significant difference in growth rate of fry under different treatments until 45 days growing time, but after that growth rate was significantly higher in T_1 than T_3 and T_4, where stocking density of fry was 3-4 times higher.

Table 3. Production and survival of sahar fry reared at different stocking densities

Parameter	Treatments			
	T_1 (5 fish/m^2)	T_2 (10 fish/m^2)	T_3 (15 fish/m^2)	T_4 (20 fish/m^2)
Mean stocking weight (g)	0.30±0.01^a	0.29±0.02^a	0.32±0.02^a	0.28±0.02^a
Stocking number (fish/hapa)	10.00±0.00	20.00±0.00	30.00±0.00	40.00±0.00
Average harvested weight (g)	6.13±0.33^a	4.34±0.98^{ab}	2.97±0.55^b	2.84±0.56^b
Harvest number (fish/hapa)	9.67±0.33	19.00±0.58	29.33±0.33	36.33±1.20
Survival (%)	96.7±3.33^a	95.0±2.89^a	97.8±1.11^a	90.8±3.00^a

Table 4. Growth rate of sahar fry reared at different stocking densities

Parameter	Treatments			
	T_1 (5 fish/m^2)	T_2 (10 fish/m^2)	T_3 (15 fish/m^2)	T_4 (20 fish/m^2)
Daily growth rate (g/day)	0.08±0.04^a	0.05±0.01^{ab}	0.04±0.01^b	0.03±0.01^b
Growth rate (% BW/day)	1.46±0.03^a	1.27±0.15^a	1.06±0.11^a	1.10±0.14^a
Condition factor	1.56±0.11^a	1.00±0.22^b	0.92±0.18^b	0.76±0.25^b

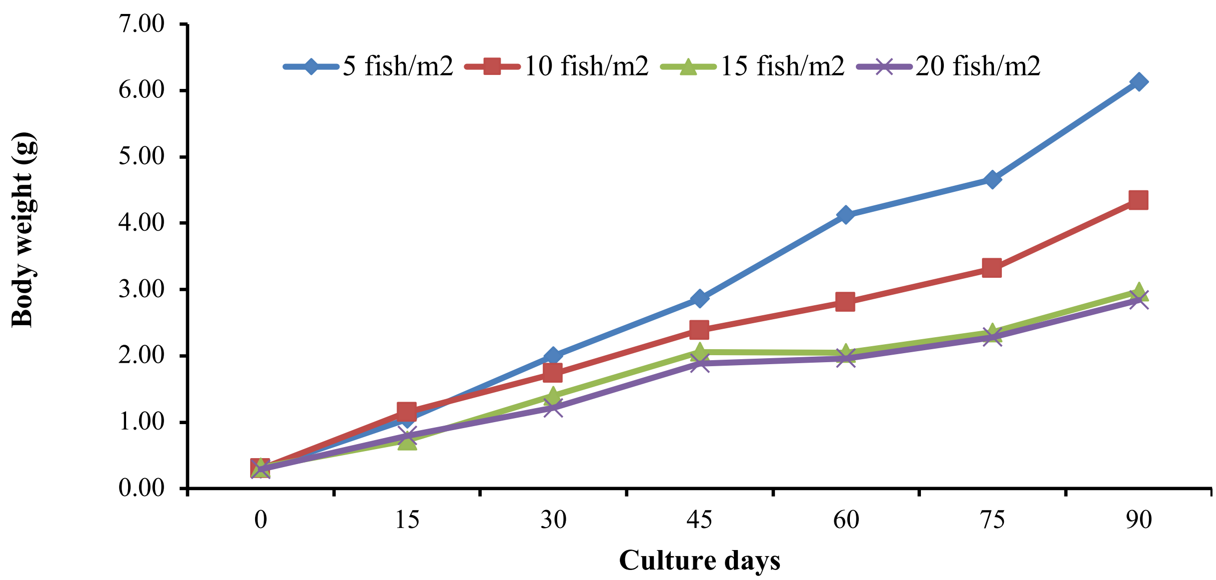

Figure 3: Size of fish over time in different treatments during the experimental period

Water temperature of the nursery pond ranged from 29.1-31.8°C during the experimental period, while mean dissolved oxygen was 7.7 (3.1-10.4), pH 8.4 (7.3-9.3) and Secchi disk depth was 30.5 cm (26.8-33.5).

Table 5. Mean and range of water quality parameters during experimental period

Parameter	Mean (Range)
Temperature (°C)	30.1 (29.1-31.8)
Dissolved oxygen (mg/L)	7.7 (3.1-10.4)
pH	8.4 (7.3-9.3)
Secchi disk depth (cm)	30.5 (26.8-33.5)

DISCUSSION

Sahar is known to be intermittent in spawning behavior in nature, and we found similar results in our rearing experiments. MacDonald (1948) was probably the first to mention that "…. Sahar is said to spawn three times in a season." Similarly, Desai (1994) noted breeding of sahar from July to March in a non-Himalayan River, Narmada, Madhya Pradesh, India. Pathani (1983) speculated there were at least four spawning acts on the basis of four different egg diameters and stages of development in mature ovary of mahseer (another name for sahar). Most findings indicate that sahar is a partial spawner due to the low number of eggs released during a single spawning event (Shrestha et al., 1990, Joshi, 1994, Shrestha, 1997). While partial or intermittent spawning characteristics may be important to produce more consistent overall reproduction in natural populations, they present a problem in using the fish for hatchery production. This can be overcome by regular inspection of the maturity status of females, but it is much more time consuming than using fish that spawn completely in one event and in synchrony. Partial spawning also results in fewer eggs from each spawn, limiting hatchery production of fry. Seed produced by artificial methods is important in helping to conserve and develop natural fisheries for this species, and successes of captive breeding are useful for aquaculture development. We demonstrated that mass seed production and larval rearing of sahar is possible in the subtropical region of Nepal by inducing maturation with a synthetic hormone. Induced spawning reduces the number of over-matured females found in captive populations by synchronizing the stripping time for injected fish.

Regular maturity observation at the correct spawning time is critical for sahar breeding, and thus, frequent examination of female fish for maturity is required. Low fecundity of this species, compared to other cultured carps, demands more female fish for mass seed production as well. As reported by Bista et al. (2010), pond reared sahar have intermittent spawning characteristics, and frequent checking of brood fish to determine optimum timing for egg stripping should result in a spawning success rate of more than 50%. Although we conducted frequent examination of brood fish for maturity, 6 females were found over mature, while successful breeding was attained by 6 females naturally and by 16 after hormone induction. Overripe females were recorded in our earlier study 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-March compared to September-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) reported an incubation period of 45-125 hours at water temperatures from 19-28°C, with the shorter incubation period attributed to higher temperature. Bista et al. (2010) 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) reported induced breeding of sahar when temperature was 18-24°C.

Growth and survival rate of fry reared from artificial spawning were comparable to other studies on this species. Daily weight gain of sahar fry was considerably lower than reported by Paudel (2003; 0.10-0.13 g/day), Acharya (2004; 0.32 g/day) and Bista et al. (2008; 0.18-0.28 g/day) but higher than values recorded in Pokhara (0.02-0.03 g/day). Growth was comparable to fry reared in cooler and warmer parts of Nepal by Bista et al. (2008, Table 6). Growth appeared to be density dependent, with the highest growth occurring at density of 5 fish/m^2 and growth declining at higher densities. This is somewhat surprising because all of these densities were below the densities commonly used for rearing carp fry or sahar fry in Nepal (Table 6). High survival rate even with high density is a positive sign for fry rearing and mass seed production of sahar, but more work is needed to clarify the role of density in the growth of sahar fry.

Table 6. Survival and growth of sahar fry and yearlings reared in farm ponds from locations in the hills and Terai. Within a group (fry or fingerlings), values with different superscripts in the same row are significantly different.

	Fry		Yearlings	
	Hill	Terai	Hill	Terai
Stocking, No./m^2	20	20	1	1
Initial size, g	0.42	0.42	3.6±1.2 [a]	3.6±0.74 [a]
Growing days	90	90	210	210
Survival %	87.3±5.2 [a]	91.6±7.6 [a]		
Final weight, g	7.9±0.9 [a]	9.7±1.7 [b]	39.0±5.8 [a]	60.8±10.4 [b]
Growth/day g	0.087	0.107	0.18	0.28

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