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

ASIA PROJECT: NEPAL
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Quality Seedstock Development/Study/16QSD02UM

Collaborating Institutions and Lead Investigators

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Objectives

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

Significance

Sahar (*Tor putitora*) is an economically important, high-value indigenous fish species in Nepal (Rai et al., 2007). The price of sahar in the Nepalese market is almost double the commonly cultivated carp and tilapia species. Sahar is still taken in capture fisheries in lakes and rivers, but no commercial cultivation has begun in Nepal. This species is declining from its natural habitat, mainly due to urbanization, illegal encroachment, over-fishing, and ecological alterations of physical, chemical, and biological conditions in the natural environment (Bista et al., 2008). The population status is considered endangered by the International Union for the Conservation of Nature (IUCN 2016) due to over harvest and habitat destruction.

Various national aquaculture plans for Nepal, most recently NARC (2010) and FAO (2013), have included the development of cold water systems for aquaculture in upland areas as a priority. In addition, seed production is also recognized as a major bottleneck to aquaculture development in Nepal. The potential culture systems for cool water areas include development of trout culture from imported fish and the use of indigenous fish like sahar for aquaculture. Rainbow trout culture is expanding in the country with government assistance, and all components of the culture business are developing. Culture of indigenous species is a high priority globally, as it reduces issues with invasive species introductions, is in harmony with local cultural needs, and increases the possible options for aquaculture production. However, for many indigenous species, particularly sahar, production of seed is very limited and is currently allocated mainly for restocking natural waters. This proposal seeks to produce large volumes of sahar seed and develop nursing and rearing techniques, so sahar culture can be extended from experimental farms to more commercial systems and help meet the need for additional restocking of natural waters. The latter target has clear impacts on environmental sustainability, while even commercial production for food would reduce pressure on wild stocks and enhance their survival.

Attempts to culture and conserve this species were initiated in Nepal with major efforts to develop culture technology and propagate the species (Gurung et al., 2001; Joshi et al., 2002). This has led to a better knowledge of spawning biology, ecology, and behavior of sahar, as well as preliminary growth performance in captive conditions. Enhanced growth in tropical and subtropical ponds and the recent breeding success in hatcheries have raised new hopes on the prospects of sahar aquaculture in Nepal

(Shrestha et al., 2005, 2007; 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, helping to halt the decline of this native species. Due to its omnivorous and predatory feeding, sahar has also proved to be a good candidate to co-culture with mixed-sex tilapia to control tilapia recruits in a pond 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 (Jaiswal, 2012).

Sahar is known to be intermittent in spawning behavior. Details from the propagation experiences in Pokhara indicate that it can spawn in most months, except January, but in natural waters, it spawns during the monsoon when rivers and streams are at peak flow. Sahar typically migrate a long distance from large rivers to streams for spawning. The Fisheries Research Center in Pokhara is the only location where fry are produced, and this production is still in limited quantity. Demand for sahar fry has increased for restocking in rivers and lakes and for aquaculture production. Lack of availability of fish seed is a major bottleneck for commercial production and conservation. Sahar breeding has been attempted in the Aquaculture and Fisheries Department of AFU in Rampur, Chitwan, and was successful in producing 250 fry during a practical class in 2010.

We conducted an experiment at the Department of Aquaculture and Fisheries, Agriculture and Forestry University, Rampur, Chitwan during the last experimental cycle of the AquaFish project to explore and assess breeding performance of sahar in the Terai region, 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 1,000 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 off season; the 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. 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 breeding and fry rearing is possible in the Terai region of Nepal.

In addition to natural spawning, attempts were made to induce three females to mature by injection of ovaprim. At 26 hours after ovaprim injection, some began to release eggs. Milt from two males was used to fertilize these eggs. Fertilized eggs were incubated as before, and a similar process was repeated. Hatching occurred after 96-104 hrs at 23-25 ^{o}C and 80-88 hrs at 25-29 ^{o}C.

While the previous experiments demonstrated that fry production can be successful in a sub-tropical climate, there remains a need for further studies on synchronization of breeding time and mass seed production. Maturity of female fish was monitored biweekly before the breeding season (May-August). Male fish were always found ripe with oozing milt after pressing their belly, but females were not ripe during May-August. In spite of frequent checking on maturity, only 1 out of 35 female fish reared was found at the right time to perform manual spawning, while 15 female fish were found to be over mature during regular observations. This indicates that females ripen very quickly and become overly mature rapidly after that. We need to either increase the frequency of maturity determination to daily during the spawning season, or determine improved ways to use ovaprim to induce maturation of premature fish.

This study is intended to refine the breeding technology, nursing, and fry production developed in the earlier experiment in order to increase availability of sahar fry for culture. While sahar are listed as endangered by IUCN, they are available for consumption and sport fishing in Nepal, and there are no restrictions to using them in aquaculture settings in the country.

Quantified Anticipated Benefits

The results of this study will further improve methods to produce sahar fry for restocking and will provide an additional fry for aquaculture in Nepal. We expect to improve seed production at the new site in Rampur and, as a result, should produce additional fry beyond our previous experiments. We hypothesize that induced maturation will be more successful than manual checking, and will also reduce effort since it will not be necessary to check for maturity on a daily basis. Availability of cultured sahar for restocking should help enhance sahar populations in natural waters. We expect to stock fry in at least five different natural waters as a result of our seed production. For Nepal, we intend to initiate outreach on production of sahar seed by conducting at least one workshop on our improved methods and results. Through this, we intend to train at least ten fish hatchery workers and ten women farmers in the induction technology.

One workshop with ten fish hatchery farmers and ten commercial fish farmers, in addition to government research scientists and extension officers being trained on sahar seed production. One report clarifying sahar production techniques. At least three graduate or undergraduate students trained on sahar reproduction techniques and research methodology.

Research Design and Activity Plan

Location

The Aquaculture Farm of AFU at Rampur and a private farm at the Center for Aquaculture Research and Production (CARP), Kathar, Chitwan, will be sites for testing of improved culture protocols.

Methods

Null Hypothesis. There are no differences in breeding and growth performance of sahar between the two spawning methods.

1.1 At least 30 female and 50 male fish (1-kg size or larger) will be maintained as brood stock in Chitwan.

1.2 Pond Facilities: earthen ponds of 200 m^2 and 4 concrete tanks at 50 m^2 each.

1.3 Culture Period: two years for mature brood maintenance and breeding.

1.4 Nutrient Input: daily feeding with locally made feed containing 35% protein at 2-3% BW.

1.5 Water management: maintain at 1.0-1.5 m deep. Water quality monitoring will be conducted using standard protocols, with monthly water sampling.

1.6 Over 600 sahar should be held for brood stock development in Chitwan ponds. We will monitor oocyte development (using cannulation on a subsample of about ten fish per month), gonadosomatic index (GSI) of brood stock fish that are stripped of eggs at maturation, and egg somatic index (ESI) for these same fish. Temperature and other conditions will be monitored over the brooding period. The most promising brood fish will be transferred to concrete tanks to allow for easier collection and regular determination of maturity.

1.7 Half of the females deemed to be near maturity will be injected with gonadotropic hormone (ovaprim/ovulin) at 5 mg/kg body weight. They will be held separately in small tanks and checked for maturity daily. Females with mature eggs will be used for manual spawning.

1.8 Half of the nearly mature females will continue to be held and checked daily for maturity. Those found with mature eggs will also be used for manual spawning.

1.9 Spawning will be done using the dry method, with eggs removed from females by pressure on the abdomen, then sperm from males using similar methods. Eggs and sperm will be mixed, and the fertilized eggs washed several times before moving them into Atkin incubators. One layer of eggs will be allowed to settle on a single mesh screen in the flow-through system. Water flow will be maintained at 7-9 L/minute. The incubation trays will be covered with a dark cloth to reduce light levels in the tray. The eggs will be observed 24 hours after initial incubation, and unfertilized eggs counted. Dead eggs will be counted and removed each day to protect healthy eggs from fungal infection. After 4 days (96 hours) hatching should occur over a 24-hour period, when distinct eyes will be seen in hatchling fish. After attaining free-swimming stage,

the larvae will be transferred into a nursing and feeding tank of 2.5 m x 0.4 m x 0.3 m dimension.

1.10 Fry will be reared in outdoor ponds, first by promoting natural feeds with fertilizer in the ponds, later using supplemental feed after fry have been successfully weaned.

1.11 Growth will be estimated over one year, with monthly sampling of fish size and apparent health for fingerlings raised in ponds.

2. Statistical Design:
Performance of sahar breeding (ESI, GSI, survival of fry, and growth of fry) will be compared between induced and naturally spawned fish.

3. Statistical Analysis: ANOVA or T-test as appropriate.

Trainings and Deliverables

Training: Ten government hatchery workers will be trained in a workshop on our new induction technology for sahar.
Ten private fish farmers will also be trained through a workshop on sahar breeding technology.
At least three graduate students will be trained on reproductive technology for seed production in aquaculture through involvement in this project.

Deliverables: One workshop on sahar induction technology.
One final report and hopefully one publication after the grant period on this technology.
One fact sheet on sahar production technology.

Schedule

Brood stock collection and maintenance will begin 1 May 2016, fry rearing is planned to occur from November 2016 through December 2017 (2 years of spawning seasons). Final report will be completed no later than 28 February 2018.