



# Induced Breeding and Larval Rearing of Snow Trout (*Schizothorax niger* Heckel) in Kashmir Himalaya with the Application of Ovatide

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## Abstract

Induced breeding was carried out successfully in *Schizothorax niger* with the application of synthetic hormone, ovatide. This is the first report of induced breeding of snow trout from Kashmir waters through use of any hormone preparation. The male and female brooders were injected with calculated doses of ovatide at the rate of 0.5 ml kg<sup>-1</sup> body weight of female brooders and 0.3 ml kg<sup>-1</sup> body weight of male respectively. The brooders responded well to the hormone and spawned within the breeding pool. A fertilization rate of 85% was achieved. Hatchability rate was around 65%. The juveniles were initially fed with skimmed milk powder and emulsified hen egg yolk in the ratio of 1:1, supplemented later with live zooplankton. On rearing, the fry attained a size of 25 mm and average body weight of 130 mg during a period of 90 days with a cumulative survival rate of 60% from fertilized eggs to swim up fry.

**Keywords:** Induced breeding, seed production, conservation, ovatide, *Schizothorax niger*

Received 11 May 2013; Revised 16 December 2013; Accepted 21 December 2013

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## Introduction

Snow trout, *Schizothorax niger* is endemic to Kashmir and found rarely in other parts of the world (Yousuf, 1996; Kullander et al., 1999). Snow trouts (Schizothoracids) are important food fishes distributed throughout aquatic bodies of Kashmir valley. The fish is found almost in every flat land

lake, wetlands and slow flowing zones of river Jhelum (Kullander et al., 1999; Bhat et al., 2010).

*Schizothorax niger* is the dominant lacustrine fish of Kashmir Himalayas and substantially contributes to fish catches among the Schizothoracids from the lakes of Kashmir Valley. Peak breeding season extends from last week of February to mid of April. Mature male brooders of *S. niger* develop white tubercles on the snout around peak breeding season. However, breeding of a fish as a general rule cannot be confined to some specific time schedule unless manipulated and is mostly governed by temperature of holding waters, photoperiod, nutritional status of brooders vis-à-vis maturity of gonads.

A number of inducing agents have been explored ranging from hCG, antiestrogens, GnRH and dopamine antagonists to steroids for inducing maturation and spawning in various fish species (Peter et al., 1986; Garcia-Abiadio et al., 1994). Ovaprim, a synthetic inducing agent of antagonist category, has also been successfully used for inducing spawning in various fishes by Nandeesh et al. (1990, 1991); Khan et al. (1992); Das et al. (1994); Roy (1996) and Singh (2000). Induced breeding of Indian coldwater fishes is difficult and is limited to few reports on Mahseer (Agarwal, 2001; Ogale, 2000). Also Raina (1992) and Ahmad et al. (2011) have worked on artificial fertilization by stripping the ripe brooders of coldwater species. Steroid hormone levels of progesterone and estradiol-17 β in the ovarian tissues of *Schizothorax niger* (Najar & Qadri, 1999; Najar, 2002; Najar et al., 2000, 2001 a, 2001 b) have been studied. But there are no reports available on induced breeding of Schizothorax fishes in Kashmir Himalaya.

Ovatide is cheap, highly potent and ready to use injectable solution containing a synthetic peptide as

an active ingredient analogous to gonadotropin releasing hormone (GnRH) with a dopamine antagonist. It promotes reproduction in fishes by inducing ovulation and spermiation. *S. niger* has shown decline in its size as well as population from the last three to four decades (Yousuf, 1996, Bhat et al., 2010). It has also been recently placed on the prioritized list of fishes for special attention by Directorate of Coldwater Fisheries Research (DCFR, 2013), India. Therefore, the objective of the present study was to standardize induced breeding of *S. niger*.

### Materials and Methods

Healthy ripe brooders were selected from natural waters and were acclimatized to hatchery conditions. After acclimatization, fishes were injected with ovatide (manufactured by Hemmo Pharmaceuticals Pvt. Ltd, Moses Road, Mahalaxmi Mumbai – 400011) at the rate of 0.5 ml kg<sup>-1</sup> body weight of female brooders and 0.3 ml kg<sup>-1</sup> body weight of male brooders during March and April. Male and female brooders were injected at the same time. Dose of ovatide had to be standardized starting from low dose of 0.1 ml kg<sup>-1</sup> to high dose of 1 ml kg<sup>-1</sup> of fish brooders. Brooders did not respond to low doses under temperate climate conditions of Kashmir. After increasing the dose rate, the optimum rate of 0.3 ml kg<sup>-1</sup> male brooders and 0.5 ml kg<sup>-1</sup> female brooders, the results were excellent. Higher rates had adverse effects on the male brooders as they got spent earlier and did not yield any milt at the time of stripping. Similarly, higher doses affected the female brooders, as they developed problem of bleeding at the time of stripping, so the appropriate dose was injected. Twenty five male brooders were used for fertilizing of ova from 45 females. For the experiment, 190 000 eggs were used; unfertilized ova became whitish and opaque and these had to be removed by siphoning and hand picking with sterilized forceps. The rate of fertilization was calculated by counting unfertilized ova as against fertilized ova in three samples that were spread in hatching tray under gentle flow of water. After injecting the brooders with the ovatide, the fishes were released in the breeding pool cylindrical in shape, having 2.15 m diameter, 0.9 m height and 3409 l capacity (operational capacity 2950 l) and maintained under the gentle flow of 0.35 – 0.42 l sec<sup>-1</sup> with circulating water having showers. The juveniles were initially fed with skimmed milk powder and emulsified egg yolk of hen in the ratio

of 1:1 at the rate of 10% body weight, later on supplemented with live zooplankton comprising of *Daphnia*, *Keratela* and *Moina*. The fry were subsequently fed with formulated feed containing about 40% made protein at the rate of 10 % body weight in the same way as has been followed by Agarwal (2001) (Table 1).

Water quality parameters, *viz.*, temperature (Celsius mercury thermometer calibrated up to 0.1°C), dissolved oxygen (Wrinkle's method modified, APHA 1998), pH (Microprocessor pH system-1011E), CO<sub>2</sub> (Mackereth et al., 1978), and conductivity (portable DB, 104 model) were checked throughout the experiment. Weight of brooder was taken using Sartorius digital balance, fecundity calculated by gravimetric method and percentage of fertilization and hatching percentage were calculated by the following formulae as per Wood (1930).

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs in a sample}}{\text{Total no. of eggs in a sample}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs in a batch}} \times 100$$

### Results and Discussion

The physico-chemical parameters of water of breeding and hatching tanks and rates of fertilization, hatchability and survival of *S. niger* are summarized in Table 1 (A & B). *S. niger* showed sexual dimorphism at the time of breeding. Male brooders developed rough and white tubercles at the snout and dark black colouration on the dorso-lateral sides. Female brooders developed protruding abdomen. Latency period under controlled conditions, when water temperature was low, was about 48 h. Initially after injecting the hormone, the brooders remained quiescent. However, after 12 h, brooders excited and exhibited courtship and after about 48 h, spawning occurred. Thirty percent brooders bred within the breeding pool, while rest of the brooders were in oozing condition and had to be stripped of ova and milt manually. The fertilized ova were harvested through the spawn collection troughs, cleared of faecal matter and were transferred to hatching pools. The spent brooders were removed from the breeding pool and released in the brood stock ponds. The brooders in oozing/running condition, which released eggs and milt on applying slight pressure on the abdomen, were stripped of

Table 1. Percentage composition of fish feed

| Ingredient                        | Inclusion Rate | Crude Protein | Crude Fibre | Calcium | Phosphorus | Total Ash |
|-----------------------------------|----------------|---------------|-------------|---------|------------|-----------|
| Fish meal (having 43.17% protein) | 57             | 24.98         | 1.94        | 3.84    | 0.91       | 21.86     |
| Soybean meal                      | 39             | 15.71         | 2.21        | 0.13    | 0.32       | 3.96      |
| Wheat flour                       | 02             | 0.21          | 0.19        | 0.02    | 0.04       | 0.31      |
| Vitamins & Minerals               | 02             | –             | –           | –       | –          | –         |

ova and milt manually in enameled trays. The milt and eggs were thoroughly mixed with the help of bird's feather. The fertilized eggs were water hardened for about half an hour. Excess milt was removed. The fertilized eggs were subsequently spread over hatching trays and maintained under gentle flow of water in the hatching troughs of the Flow-through-hatchery. The fecundity ranged between 15 000 to 18 000 eggs kg<sup>-1</sup> body weight.

The eggs of *S. niger* are non sticky. A fertilization rate of 85% was achieved through artificial fertilization by stripping male and female. However, fertilization rate was lower; about 80% within the breeding pool, where ova and eggs were released on their own after administration of synthetic hormone ovatide. The hatchlings remained quiescent for 2-4 days and after absorption of yolk sac, the hatchlings started swimming freely. The fry

attained a size of 25 mm and average body weight of 130 mg during a period of 90 days with a cumulative survival rate of 60% from fertilized eggs to swim up fry and reached a total body weight of 0.350 mg and total length of 314 mm after further 45 days.

Most of the fishes did not spawn in captivity and induced spawning through hormonal manipulation may be necessary. Failure of spawning after administration of gonadotropin in many species is due to the dopamine inhibitor activity (Peter et al., 1986; Nandeeshha et al., 1991). During the present investigation, ovatide was found successful for inducing fertilization in *S. niger* in circular hatchery conditions. This may be due to the salmon gonadotropin releasing hormone (sGnRH) and a dopamine inhibitor substance in the ovatide. In the present study, *S. niger* responded well to ovatide injection. Similarly Nandeeshha et al. (1990) presumed that carps having high dopamine activity responded only to higher doses of ovaprim. During the present investigation, a good fertilization percentage of 85% was achieved by dry method as the fish spawned within the pool although to a little extent only and had to be stripped of ova and milt manually. Agarwal et al. (2007) while working on induced breeding by using ovaprim on *Schizothorax richardsoni* have also found similar results. Ogale (2000) has also reported a high fertilization rate of 85- 100% in Mahseer. The higher rates of artificial fertilization during the present investigation may be related to the dry method of fertilization where the viability and motility of the spermatozoa remain high. However, the percentage of fertilization which was 80% in the present study has also been related with the maturity and weight of fish (Haque & Ahmed, 1991). Ovatide has the advantage that a single dose of this preparation brings the desired effect. Moreover, male and female brooders have to be injected at the same time. This saves a lot of labour

Table 2. Mean physico-chemical parameters of water in breeding and hatching tanks and rates of fertilization, hatchability and survival of *Schizothorax niger*

| Parameters                                 | Value               |
|--|---------------------|
| Transparency                               | 100%                |
| Water depth (meter)                        | 0.4                 |
| Temperature °C                             | 12-22 (Mean=16.5)   |
| pH   | 8.1-8.7 (Mean=8.4)  |
| Dissolved Oxygen (mg l <sup>-1</sup> )     | 8.4-11.5 (Mean=8.8) |
| Free CO <sub>2</sub> (mg l <sup>-1</sup> ) | 12.0-22.0 (Mean=16) |
| Conductivity (µS)                          | 35-42 (Mean=38.5)   |
| Rate of fertilization (Range) %            | 72-85               |
| Rate of hatchability (Range) %             | 59-65               |
| Survival rate from eggs to fry (Range) %   | 62-65               |

and stress caused to brooders in case two or more doses of hormone preparations have to be given to female brooders or male and female both. The juveniles responded well to the initial feed *viz.*, skimmed milk powder and emulsified egg yolk of hen in the ratio of 1:1. The advanced juveniles later on accepted the live zooplankton comprising of *Daphnia*, *Keratela* and *Moina* harvested from the adjoining ponds of the farm and the fry subsequently grew well with formulated feed having crude protein of about 40%, at the rate of 10% body weight. This is the first report of successful attempt on the induced breeding of *S. niger* through use of synthetic hormone preparation ovatide.

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