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Herbal Feed Additives for Gonadal Maturity and Breeding Performance of Female Snow Trout (*Schizothorax richardsonii*) in Captive Condition

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Abstract

Addressing the issue of gonadal maturity and breeding in captive condition for a commercially important indigenous Coldwater fish, snow trout, *Schizothorax richardsonii*, field study was conducted with dietary supplementation of the blend of Ashwagandha (*Withania somnifera*) root powder, dried powder of garlic (*Allium sativum*), and hing (*Ferula asafoetida*). Experimental fish of 3 years age were reared in FRP tanks (n = 4) of 1200 L capacity with stocking density of n = 60 in each tank and fed with control (CD) and experimental (TD) diets in duplicate at the feeding rate of 3–5% of their body weight twice in a day. Gonadosomatic index (GSI) and hepatosomatic index (HSI) were recorded for annual breeding cycle coupled with breeding indices in breeding season and regular monitoring of water quality parameters. In female stock, the GSI values ranges from 0.87 to 12.83 and 1.84 to 15.42 in control and treated groups, respectively. Two peaks of GSI values reflects the two breeding season of the species during the month of March and September. Similarly, HSI was lowest during the month of March and September in captive reared female fish and highest during the month of May to June and January to February, with similar trend in both, control treated and groups. In captive condition, 80% specimens were observed mature for spawning in treated group, while only 15% females were ready to spawn in control stock. The fecundity of the fish was observed in the range of 20210-23710 eggs/kg body weight with 80–94.2% fertilization rate. The egg size was observed in the range of 3.5–3.8 mm. The study revealed that 1.0% supplementation of blend of herbs in the diet is beneficial for gonadal maturity of females and better spawning of *S. richardsonii* in captive Coldwater condition.

Keywords: Blend of herbs, Fecundity, Gonadal maturity, Gonadosomatic index, Spawning

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Introduction

Schizothorax richardsonii (Gray, 1832), which is a member of the family *Cyprinidae* and subfamily *Schizothoracinae*, is an economically important, indigenous food fish, inhabiting stream and rivers of Himalayan and sub-Himalayan regions. This species is widely distributed in mountain regions of Asia-mainly the highlands (above an altitude of 670 masl) of Himalayan and central Asian.[1] In central Himalayas, *S. richardsonii* locally known as "Asela" and constitute major capture fishery in the Uttarakhand region. At present, it is realized that the population of this species in the natural habitat has declined significantly,[2-4] mainly due to overfishing, destructive fishing and increased human pressure and the species is now categorized under vulnerable category.[5] Since, this is a most preferable food fish in hill region; this species has aquaculture prospects and potential to be a candidate species for culture. On the other side, its decline wild population needs propagation in the natural cold water bodies of Indian Himalaya.[6-8] At present, snow trout culture and breeding are not in practice due to its slow growth and constraints in gonadal maturation under captivity. As a result, breeding and seed production of this species are a challenge in the hills, which necessitate the scientific approach for captive maturation and spawning. It is well accepted in the literature that broodstock nutrition has significant impact on reproductive performance.[9,10] Intensive feeding care is required for development of brooder in captive condition. Dietary compounds affect the endocrine system and consequently reproductive parameters, such as fecundity,[11] quality of gametes, embryos, and larvae.[9] In recent years, using of plant-based additives in aquaculture has been one of the methods used to promote weight gain, feed efficiency in cultured fish[12] and also helps to improve fertility.[13] Therefore, the present study was focused to evaluate the efficacy of herbal feed additives in gonadal maturity of female fish and spawning of snow trout in captivity.

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Materials and Methods

Experimental Site

The field study was carried out at ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand (Latitude 29° 21'N, Longitude 79° 34'E, 1370 masl) during May 2020 to April 2021.

Collection and Acclimation of Experimental Fish

Experimental female fish of the age of 3 years having average weight (167.6 ± 10.9 g) were procured from fish ponds of ICAR-Directorate

of Coldwater Fisheries Research, Bhimtal, acclimatized for 2 days, stocked in each tank and fed with experimental diets at the rate of 3–5% of their body weight with feeding frequency of twice in a day. Water quality parameters were monitored on daily (water temperature, pH, dissolved oxygen, and free carbon dioxide) and fortnightly (total alkalinity, nitrate, phosphate, and ammonia) basis. Different water quality parameters were estimated based on standard methods (APHA, 1998).[14]

Formulation of Experimental Diets

The main focus of the study was to evaluate the efficacy of herbal feed additives in gonadal maturity and captive breeding of snow trout (*S. richardsonii*). For the purpose, two diets were formulated, one as control diet or basal diet (Fish meal, 10%; Rice bran, 45%, mustard oil cake, 25%, and Soya bean oil cake, 25%) having 30% protein level and another as test diet having basal diet and 1% supplementation of the blend of tested herbs such as root powder of Ashwagandha, dried powder of garlic (*Allium sativum*), and hing (*Ferula asafoetida*).

Experimental Set-up

Experimental fish were reared in FRP tanks (n = 4) of 1200 L capacity. Each tank was stocked with 3 years old fish (n = 60) and fed with one control (CD) and one test diet (TD) in duplicate at the feeding rate of 3–5% of their body weight twice in a day.

Estimation of Reproductive Parameters

Three fish samples were randomly taken from each treatment tank, dissected using biological dissecting instruments, and ovaries were removed and weighed for the examination of Gonadosomatic index (GSI) and fecundity of female brood stocks.

GSI

GSI will be calculated by the following formula (Afonso-Dias *et al*., 2005)[15] on monthly basis.

$$\text{GSI} = \frac{\text{Weight of gonad (g)}}{\text{Total body weight (g)}} \times 100 \quad (1)$$

Hepatosomatic index (HSI)

The HSI was also calculated on monthly basis to observe the energy status for growth and gonadal development as liver is an important store of energy reserve in many fishes.[16,17] Female specimens were dissected and liver was taken out and weighed on a single pan electronic balance. HSI was calculated using following formula:

$$\text{HSI} = \frac{\text{Weight of liver (g)}}{\text{Total body weight (g)}} \times 100 \quad (2)$$

Determination of Breeding Indices

Effective fecundity of each female was determined by random sampling of eggs in 50 ml graduated measuring tube immediately after spawning. The total number of eggs in 10 ml was counted and multiplied with total volume of eggs released. The fertilization rate was determined by randomly taking a sample of 100 eggs in three replicates from the total eggs in a Petri dish. Fertilized eggs having intact nucleus were considered for calculating percentage of fertilization. The ova diameter was measured by keeping ten eggs in a row along with the measuring scale. The total length of eggs was divided by numbers of eggs to obtain mean diameter of each egg. Breeding indices were calculated using the following formulae:

$$\text{Fecundity} = \frac{\text{Number of stripped eggs}}{\text{Weight of ovulated fish}} \times 100 \quad (3)$$

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs in a batch}} \times 100 \quad (4)$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs in a batch}} \times 100 \quad (5)$$

$$\text{Survival rate (\%)} = \frac{\text{Total number of survived larvaeuntil day 7}}{\text{Total number oflarvae counted at day 1}} \times 100 \quad (6)$$

Results and Discussion

The present study reflects that female group of the *S. richardsoni* that received supplemented herbal feed diet gets maturity easily in captive condition, but only 15% females were reached at the peak of gonadal maturity in control stock without any supplementation of herbal feed. In general, GSI is considered as reliable estimate for gonadal maturity and spawning of any species. The GSI increased with the maturation of fish and reaches to its maximum at the peak period of maturity during the breeding season. GSI and volume of the gonad was suggested as indicators of gonadal state by Saksena (1987)[18] for Indian fresh water goby, *Glossogobius giuris*. The fecundity and GSI have also taken to assess the gonadal maturity in *Mystus gulio*,[19] in *Labeo dyocheilus*,[20] in *Labeo rohita*,[21] and in *Neolissocheilus hexagonolepis*.[22]

In the present study, changes in values of GSI were observed in females for 1 complete year (May, 2020–April, 2021) and depicted in Figure 1. In treated group, GSI increased significantly ($P < 0.05$). The GSI values of female snow trout were ranges from 0.87 to 12.83 and 1.84 to 15.42 in control and treated groups, respectively. GSI value attained two peaks in complete maturation cycle, during the month of March and September and reached

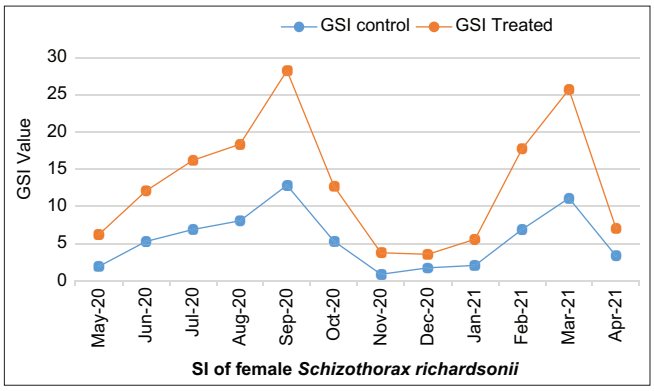

Figure 1: Monthly fluctuation in the Gonadosomatic index of *Schizothorax richardsonii* females

its lowest level during the month of May and December. An earlier report in *S. richardsonii* females,[23] documented a gradual increase in GSI reaching maximum during October–November and minimum during March–April. The results of the present study are in the conformity of the previous study conducted by Ciji *et al.* (2020).[24] Convict cichlid (*Cichlasoma nigrofasciatum*) fed a diet supplemented with 0.15 g essential oil of fennel fruits (*Foeniculum vulgare*) per kilogram of diet for 40 days presented an increase in GSI.[25] In contrast, Nile tilapia (*Oreochromis niloticus*) presented a decrease in GSI after 90 days of diet with extracts of *Azadirachta indica* leaves and *Carica papaya* seeds at 2 g per kilogram of diet.[26]

HSI has been often used as indicator of energy status in relation to gonadal development and growth of fish.[15,16,27] The correlative changes between liver weight and gonadal activity have been shown to be associated with energy requirement of the ovary for the development of oocytes.[28] Patil and Kulkarni (1996)[29] had also shown that GSI and HSI have relationship and this relationship is directly related to gonadal activity. In present study, HSI was the lowest during the month of March and September in captive reared female fish and the highest during the month of May to June and January to February, with similar trend in both, control treated and groups [Figure 2]. The female fish showed its minimum HSI (0.41–0.49%) in the month of September and March in control group and 0.50–0.54% in treated group, respectively. HSI differed significantly ($P < 0.01$) between control and treated fish and also varied significantly ($P < 0.01$) in relation to months in both the groups. The difference in HSI of both groups showed significant ($P < 0.05$) correlation between dietary herbal supplementation and reproductive status. It was observed that when the HSI values were at its minimal, the GSI values were highest and this condition suggests the point that the liver has a weight loss during reproduction which may indicate the mobilization of hepatic reserve for gonads maturation[30] and therefore, the same period might be the pre-spawning period of this fish.

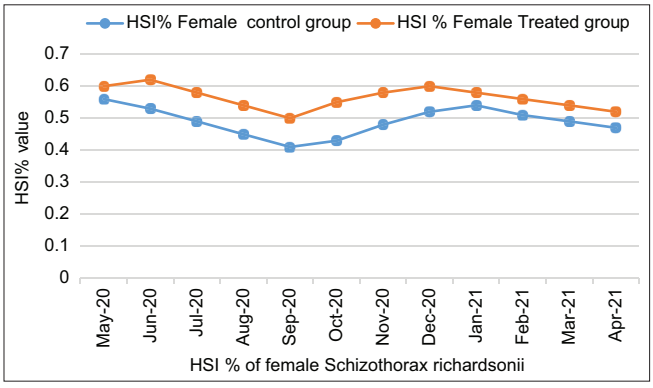

Figure 2: Monthly fluctuation in the hepatosomatic index of *Schizothorax richardsonii* females

During the present experimentation, captive reared females were used for spawning by dry stripping method and milt from the captive reared males was used to fertilize the eggs. Recorded breeding indices for both the groups, control and treated are summarized in Table 1. The fecundity was obtained relatively higher in treated group. Absolute fecundity increases with increase of fish weight and it was found relatively higher during the breeding operation in the month of September rather than in the month of March. Many studies have reported that fish fecundity increases with increasing brood size for both freshwater and marine fishes, for instance in rainbow trout.[31] In the present study, observed fecundity of the treated group was found in the range of 23640–23710 eggs/kg, which was 17.2% higher than the control group (20210–20228 eggs/kg) which is quite similar as reported by Pandey *et al*., (2010)[32] and Joshi *et al*. (2016).[33] The influence of herbal administration causing increase in fecundity has already been reported various authors (Canyurt and Akhan, 2008; Ciereszko and Dabrowski, 1993; Krol *et al*., 2006; Babu *et al*., 2009; Babu, 1999).[34-38] The increase in the fecundity of fish could be as a result of the presence of biflavonoid and xanthone in the herbs. Biflavonoid and xanthone are potent antioxidants which are capable of increasing the production of estrogen, the key hormone involved in the production and maturation of eggs in the ovary.[39]

The data obtained in the present study also showed the improvement in other breeding indices in the female fish fed with herbal supplemented diets. Treated group of female fish reflected better fertilization rate (94.2%) and larger size of ova (3.8mm). Emmerson (1980)[40] reported an enhanced rate in percentage of fertilization is due to nutritive feeding to brooders in the *Salmo trutta abanticus*. In treated group, hatching rate was observed up to 94% with 17.5% increase over the control group (81%) ($P < 0.05$). This showed a strong influence of the blend of herbal diets on enhancing the percentage of hatching. Babu *et al*., 2009[37] also reported enhanced quality of egg production and increase in the percentage of hatching with herbal maturation diet. Similar results were also reported by Lokman *et al*. (1998).[41] Babu (1999)[38] also reported increased level of hatching percentage with *Withania somnifera* supplementation in penaeid brood stock diet. This supplementation induced a decrease in alpha tocopherol concentration in the broodstock which helps to release the good quality of eggs that enhanced the level of percentage of hatching.

The water temperature during the captive rearing of brooders varied from 8.4 to 24.2°C. Dissolved oxygen value ranged from 6.6 to 8.8 mg/l, pH value from 7.2 to 7.4, free CO_2 from 0.0 to 1.9 mg/l, and total alkalinity from 82 to 157 mg/l. Ammonia and nitrate-N level was very much alike and ranged from 0.01 to 0.04 mg/l

Table 1: Comparative breeding indices of control and treated group

Date of stripping	*Control group*		*Treated group*	
Date of breeding operation	*23th Sept. 2020*	*25th March 2021*	*23 th Sept. 2020*	*25th March 2021*
Avg. weight of female fish (g)	160.66±5.94	158.44±3.18	182.75±16.82	168.52±15.57
Avg. weight of male fish (g)	89.28±9.71	75.93±0.58	92.16±4.58	87.29±9.54
Avg. number of eggs	3247±5.29	3205±165.42	4320±70.85	3995±57.74
Avg. ova diameter (mm)	3.42±0.23	3.27±0.23	3.8±0.15	3.5±0.18
Relative fecundity	20210±64.30	20228±37.27	23640±1120.06	23710±1132.09
Number of eggs/kg				
Fertilization rate (%)	86±2.00	80±2.00	94.2±3.61	90±3.46
Hatching rate (%)	89±4.36	81±2.65	94±4.00	87±2.00
Survival rate (%)	78±2.65	75±2.65	85±2.00	81±2.65

and 0.11 to 0.16 mg/l, respectively. Phosphate level was also in the range of 0.11–0.18 mg/l. Pandey *et al*. (2010)[32] observed similar results of water quality parameters during breeding of *S. richardsonii*. Recently, Abdel-Moniem *et al.* (2019)[42] reported that dietary implication of 2% garlic powder in the Nile tilapia (*Oreochromis niloticus*) brood stock diet has positive effects not only on the growth performance but also on reproductive parameters such as fecundity, hatching rate, and fry survival. Feeding with *W. somnifera, F. asafoetida* remarkably accelerated the spawning rate, fecundity, and larval quality in *Penaeus monodon*.[43] Similar results were also reported with the use of other herbs for improved reproductive performance in fish.[39,44-47] The present study also reflects a positive and significant impact of the dietary inclusion of blend of Ashwagandha (*W. somnifera*), garlic (*A. sativum*), and hing (*F. asafoetida*) on the gonadal maturity and spawning of snow trout under captive condition.

Conclusion

About 1% dietary supplementation with blend of herbs improves gonadal maturity of females and better spawning of *S. richardsonii* in captive Coldwater condition. This study also established the potency of blend of herbs as fertility enhancer in *S. richardsonii* brood stock and should be encouraged as it will minimize the dependence on synthetic drugs as fertility enhancing agents.

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