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Induced breeding of snowtrout (*Schizothorax richardsonii* -Gray), from Garhwal Himalaya (Uttarakhand, India) by pituitary gland extract.

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Abstract

Comparative breeding experiments were done in *Schizothoraxrichardsonii* by using pituitary gland extract (PGE) and stripping technique. Experiments were conducted for two successive years. PGE dose administered was 5mg/kg body weight to male fishes and 7 mg/kg to female fishes. Each dose was administered as two split doses 4 hours apart. For induced breeding, fecundity ranged from 5,200 to 13,542 eggs per female. In 15 sets of induced breeding experiments performed over two years, using PGE extract, fertilization success ranged from 78±1.98% to 76.7±2.18% and hatching success ranged from to 63.3±%3.05to 63.9±1.81%. Stripping experiments yielded similar results with their fertilization ranging from 67.7±3.48% to 64.4±2.67% and hatching ranging from 58.9±3.47% to 57.26±2.8%. Our results conclude that induced breeding is better than stripping and can be used effectively to breed *Schizothoraxrichardsonii*.

***Keywords**: induced breeding, Schizothorax, pituitary gland extract*

Introduction

*Schizothoraxrichardsonii*and other *Schizothorax* species (*S.plagiostomus* and *S.sinuatus*; Singh *et al*., 1987) are distributed in snow fed rivers of Himalaya including areas from Afghanistan, Pakistan, India, Burma, and China. This fish species is usually found above an altitude of 670 meters in the rivers and streams along the Himalayan range (Tilak and Sinha, 1975; Talwar, 1978). Raizada (1985) and Jhingran (1982) classified fishes of this subfamily as *Schizothoracine* under the family Cyprinidae and gave them a common name, snow trout. These fishes prefer rivers and streams having rapid water along with big pools and having water temperature range of 8– 22 degree centigrade (^{0}C). S*chizothorax*species is one of the main game and food fish of these rivers in Garhwal Himalaya and constitute about 85% of the total fish catch (mostly *S.richardsonii*) in upper stretches of Himalayan region of Uttarakhand. There is little information on population biology of snow trout of this region; however, Baloni and Tilak (1985) and Agrawal (1989) conducted investigations on spawning ground and fecundity of these fishes. In Garhwal Himalaya there has been no systematic and long term study to monitor the population of this native fish species (*Schizothorax* sp.). As there is no data available it is almost impossible to tell if there has been any change in population of this native fish species.

Population of these native snow trout are imperiled and if the situation is not yet alarming but will surely be the case after couple of years. Their population is rapidly declining primarily due to (1) the over exploitation of the fishery caused by poaching methods such as explosives, bleaching, poisoning, electrocuting, spearing which have destroyed brood stock, and (2) construction of roads and dams leading to siltation problem (3) Introduction of exotic carnivore fishes in rivers of Uttarakhand. In recent times, there has been construction of many hydroelectric projects in Garhwal Himalaya and it has been suggested that the population of this species would be threatened (Raina*et al*., 1985a: 1985b, 1986; Joshi, 1987). In Uttarkashi district, there are about five (05) major dams/barrages (Dabrani, Lohari-Nag Pala, Manari,

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Uttarkashi), either planned or functional, within a span of 100 kilometers on river Bhagirathi besides Tehri Dam (district Tehri). Many hydroelectric projects are also being constructed on river Alaknanda. It has been well documented that dams and barrages influence population dynamics of fishes as they cause huge fluctuation in water level as well as water flow patterns (Baxter, 1977, Ark *et al*., 2004, Gunkel*et al*., 2003, Tiemann*et al*., 2004, Merona*et al*., 2005, Lucas *et al*., 2009, New *et al*., 2009, Heppner *et al*., 2009). Both, river Bhagirathi and river Alaknanda have a very noticeable change in water level and water flow-rate due to these dams but no scientific study has been done so far documenting changes in population of native *Schizothorax* species. Moreover, feasibility studies regarding culturing this fish species in hatcheries using induced breeding technique and then releasing them are almost non- existent which may help in this native species conservation and development of commercial fisheries in Uttarakhand using this species. This is because most of the efforts are focused on fast growing fishes like carp (*Cyprinuscarpio*), while no attention has been paid to manage native fish populations.The present study was undertaken to evaluate the possibility of developing artificial propagation techniques and better fishery management and conservation of snow trout (*Schizothoraxrichardsonii*). The objective of the present work was to evaluate the efficacy of injecting PGE for inducing ovulation and artificial spawning (hypophysation) and compare it with generally used technique of stripping. Successful artificial propagation techniques would be helpful to conserve the native fish and convert an indigenous fish to a culturable fish for commercial uses. It will also generate fish farming in Uttarakhand as the hatcheries would provide the 'seeds' of an indigenous, economically important fish to interested farmers.

Material and Methods

Stripping Method- Brooders for stripping were caught from the Alaknanda River with the help of gill nets. Brooders of both the sexes were hand-stripped bank-side by applying slight pressure on the sides of the fish abdomen. First eggs were stripped out of a single female and collected in an enamel tray which was filled up-to 1/3rd with river water. Milt from 2 males was dropped directly right on top of eggs. Then eggs and milt were mixed with the help of bird's feather for 5 to 10 min. The excess milt was later washed off by changing the water in the trays. Total of 8 experiments was carried out in two years – 4 sets each year during breeding season.

PGE injection —PGE extract was prepared according to Chaudhri and Singh (1984) with some modifications. Briefly, pituitary gland was taken from fresh specimen (over 1 kg weight only) of Schizothroax species (*S.richardsonii* or *S. plagiostomus*) during winter months (Sept – Feb) and was preserved in absolute alcohol. The pituitary hormone extract was prepared by crushing the glands inside a tissue homogenizer and adding measured quantity of distilled water to it. The gland suspension was then centrifuged (8000 rpm for 8 minutes) and the supernatant were used for injection. The concentration of the extract was around 1–4 mg gland in 0.5 to 1 ml of water according to convenience of injection. The hormone was injected intramuscularly near the tail region of the fish. Induced breeding experiment was carried out on the bank of the Alaknanda River. Hapas (n = 15; 6 feet x4 feet x3 feet) were set in the shallow water of the river held the brooders. Each hapa had two male and one female brooder. These were taken in the first week of September and reared until the fishes were ready for spawning. Doses of PGE were given intramuscularly (with number 22 needle) to the brooders in the region of the caudal peduncle: 5mg/kg of body weight for each male and 7mg/kg for each female. The total dose was given in two split doses, 3-4 h apart. The injected fishes were returned to the hapas. Total of 8 induced breeding attempts were made in two years – 4 set of experiments each year.

Statistical Analysis: All comparative statistical analysis was done using Origin 8.6 software.

Results and Discussion

Stripping Method

Nine egg lots were generated by stripping the gametes together. The weight of the male fishes ranged from 125 to 1,025 gm while it was 500 to 1,225 gm for females. The number of eggs obtained by stripping varied from 2,592 to 8,768 while the percentage of fertilized eggs varied from 67.7±3.48% to 58.9± 3.37% and the

hatchingpercentage varied from 58.9±3.47 to 57.26±2.80% (Table 3a & 3b).

PGE injections

Of the 15 sets of hapas with *S. richardsonii* brooders, eggs were obtained in fourteen cases during the breeding season. The weight of the adult females ranged from 500 to 1,380 g and that ofmales ranged from 100 g to 1,000 g (Table 1 and 2). During experiments with PGE administration, the female *S. richardsonii* treated with 7mg/kg of PGE ovulated. Fecundity ranged from 5,200 to 13,542 eggs per female. Fertilization and hatching percentages ranged from 78.4±1.98% to 76.7±2.18% and 63.3±3.05% to 63.9±1.81%, respectively (Table- 1 and 2).

The percent fertilization (p>.002) and percent hatching (p>.02) was significantly higher in case of induced breeding method as compared to stripping method.Physico-chemical parameters of water measured during the course of study were: Water temperature varied from 15 to 19°C, while the atmospheric temperature was 12 to 20° C. Dissolved oxygen ranged from 8.2 to 9.8 mg/L and free CO_2 was 0.6 to 1.9 mg/lL respectively and pH varied from 7.6 to 7.8. (Table 4a & 4b) All measurements were made as per APHA 1992 and Goyal and Trivedi (1986).

Table 1: Details of induced breeding experimental setup for *S.richardsonii* during YEAR 1

Batch	brooders	Weight male fish (g)	Total dose of PGE (mg/ml)	Dose		Weight female fish (g)	Total dose of PGE (mg)	Dose		Response to treatment	Number of Eggs	Fertilization %	Hatching %
				1st	2nd			1st	2nd				
Batch 1	2 male	i. 900	4.5	1.5	3.0	1000	7.0	2.0	6.0	Ovulated	9924	78.6	61.5
	2 female	ii. 347	1.7	0.5	1.2	1210	8.47	2.4	3.0	Ovulated	11624	83.0	64.5
	2 male	i. 556	4.7	0.5	2.2	650	4.55	1.5	3.0	Ovulated	6754	65.9	63.76
	2 female	ii. 455		0.5	1.7	975	6.825	2.8	4.0	Ovulated	7222	73.5	63.83
			2.2										
	1 male	i. 500		0.5	2.0	765	5.355	2.3	3.0	Ovulated	7008	78.7	63.5
	1 female		2.5										
Batch 2	2 male	i. 700	3.5	1.5	2.0	1200	8.4	2.4	6.0	Ovulated	10,538	81.22	68.0
	1 female	ii 580	2.9	0.9	2.0								
Batch 3	2 male	i. 1000 ii. 950	5.0	2.0	3.0	950	6.65	2.6	4.0	Ovulated	6575	83.0	68.5
	2 female		4.7	1.7	3.0	1100		3.0	4.7	Ovulated	7400	83.6	69.7
	2 male		3.7	1.7	2.0	850	7.70	2.0	3.9	Ovulated	-	-	
	1 female	i. 750 ii. 400	2.0	0.5	1.5		5.65					-	
Batch 4	2 male	i. 700	0.5	0.2	0.2	1150	8.05	3.0	5.0	Did Not	-	-	-
	2 female	ii. 725	3.6	1.6	2.0	1100	7.7	2.7	5.0	Ovulate			
										Did Not ovulate	-	-	-
	3 male	i. 310	1.5	0.5	1.0								
	2 female	ii 556	2.7	0.8	2.0	1100	7.7	2.7	5.0	Ovulated	9998	85.2	72.6
		iii. 100	0.5	0.2	0.3	950	6.65	2.6	4.0	Ovulated	6840	71.4	37.6
												78.4 ±1.98	**63.3 ±3.05**

Table 2: Details of induced breeding experimental setup for *S.richardsonii* during – YEAR 2

Batch	brooders	Weight male fish (g)	Total dose of PGE	Dose 1st	Dose 2nd	Weight female fish (g)	Total dose of PGE (mg)	Dose 1st	Dose 2nd	Response to treatment	Number of eggs	Fertilization %	Hatching%
Batch 1	2 male 1 female	i. 450 ii. 825	2.2 4.1	1.0 1.0	1.3 3.2	1250 1250	8.75 8.75	3.0 3.0	5.7 5.7	Ovulated Ovulated	13261	75.4	59.9
	2 male 2 female	i. 1000 ii. 535	5.0 2.6	2.0 1.0	3.0 1.7	996 780	6.972 5.46	2.9 2.4	4.0 3.0	Ovulated Ovulated	13192 8949	72.52 74.0	63.8 68.2
Batch 2	2 male 1 female	i. 600 ii 425	3.0 2.1	1.0 1.0	2.0 1.2	1380 1100	9.66 7.7	3.6 4.7	6.0 5.0	Ovulated	7740	77.0	62.5
	3 male 2 female	i. 455 ii. 400 iii. 250	2.2 2.0 1.2	1.0 0.5 0.5	1.2 1.5 1.2						13542 9998	73.0 82.26	60.72 64.60
Batch 3	2 male 2 female	i. 950 ii. 700	4.7 3.5	1.7 1.5	3.0 2.0	765 1300	5.355 9.1	2.3 3.1	3.0 6.0	Ovulated Ovulated	5200 13000	82.5 62.26	69.2 51.38
	1 male 1 female	i. 900	4.5	1.5	3.0	500	3.50	1.5	2.0	Ovulated	5013	83.8	68.8
Batch	1 male 1 female	i. 840	4.2	1.2	3.0	700	4.90	4.9	3.0	Ovulated	5509	84.8	70.2
												76.7 ±2.18	**63.9 ±1.81**

Table 3a: Results from stripping on *S.richardsonii* YEAR 1

Batch	Experimental set of brooders	Number of eggs	% Fertilization	% Hatching
Batch 1	- 2 male + 1 female	4500	77.8	68.5
Batch 2	-1 male + 1 female	8208	64.3	55.5
Batch 3	- 2 male + 1 female	4695	62.1	52.5
Batch 4	- 2 male + 1 female	8442	66.8	59.3
			67.7±3.48	**58.9±3.47**

Table 3b: Results from stripping on *S.richardsonii* YEAR 2

Batch	Experimental set of brooders	Number of eggs	% Fertilization	% Hatching
Batch 1	2 male + 1 female	8768	70.39	65.7
Batch 2	2 male + 1 female	8158	68.4	60.2
Batch 3	2 male + 1 female 2 male + 1 female	8500 7212	67.2 59.8	58.3 52.0
Batch 4	1 male + 1 female	2592	56.5	50.1
			64.4±2.67	**57.26±2.8**

Our results and comparative analysis done during the investigations clearly show that, if used, PGE induced breeding of *Schizothoraxrichardsonii* would be advantageous over conventionally used technique of Stripping. Attempts to breed *Schizothorax* using induced breeding techniques were successful. In our induced breeding experiments fertilization ranged from 78.4±1.98% to 76.7±2.18% while hatching success was between 63.3±3.05% to 63.9±1.81%respectively. These results from induced breeding experiments were significantly higher than stripping experiments. Thus induced breeding method can be attempted to save the declining fish population due to dam construction and their by changing the characteristics of the ecological niches where these native fishes reside.Induced breeding has been carried out in other fish species also. The effective dose of the pituitary extract to precipitate ovulation varies in different fishes. Homoplastic and heteroplastic pituitary extract injections alone or in combinations with other synthetic hormones have been used by various workers to induce ovulation. Further, the number of injections required also varies (Rajayalakshmi*et al.* 1991). In induced spawning of sliver carp and grass carp fish pituitary hormone was administered in combination with Synarian human choronicgonodotropin (hCG) to the female breeders, while the male breeders received injection of pituitary hormone only (Joshi, 1981; Joshi and Khanna, 1983). In *Labeogonius* female breeder were injected with gonadotropin along with the fish pituitary extract and only pituitary injections was given to the male to fertilize the ovulated eggs (Desai *et al.* 1981, Joshi and Khanna, 1983). In the present experiment on *S.richardsonii* fish pituitary extract alone wasadministered to the male and female breeders for spawning and it resulted in successful ovulation. Pickford and Atz (1957) and Dodd (1960) have reported the finding of many investigators who have obtained negative results with human chorionic gonadotropin (hCG). These may be attributed either to the existence of phylogenetic specificity or more likely to seasonal unresponsiveness of the gonads. The timing of the experiment in relation to the spawning season is of great importance as suggested by the work of Ramaswami and Sundaraj (1957) who found that administration of hCG to gravid *Clariusbatrachus* was ineffective during May and June (pre-spawning season) whereas the same treatment induced optimum spawning in July and August (spawning period). Joshi and Khanna (1983) observed that in *L.gonius* the hCG alone as well as in combination period (last week of June and early July) in Nanaksagar reservoir. In *S.richardsonii* found in river Alaknanda around Srinagar Garhwal the pituitary gland extract (PGE) was quite effective during its peak spawning period (September to early November) – post monsoon time in river Alaknanda.Nandeesha*et al.* (1991) concluded that in economic terms, the use of ovaprim is advantageous. In trials on fish farm, the percentage of spawning success, the number of eggs obtained per kilogram of body weight of brooders, the fertilization rate and hatching percentage remained consistently higher with ovaprim as compared to crude extract of carp pituitary gland (CPE) or hCG treatment in almost all instances. Das *et al*. (1994) did induced spawning and hatching of *Puntisjavanicus* (Bleeker), injecting a single dose of ovaprim, an ovulation agent, resulted is complete spawning within 4 to 5 hours. Results of successful spawning through a single dose of ovaprim have been reported in several carp species in India (Nandeesha*et al*., 1990). Induced spawning in *Labeogonius* with 100 IU/day of chorionic gonadotropin occurred after 5 to 7 injections to female breeders that varied from 260 to 400 gm in their body weight and 296 to 318 mm in length (Joshi and Khanna, 1983).

Table 4a: Physico-chemical and meteorological parameters of breeding ground during year 1

Experiment Batch	Water temperature (degree C)	pH	DO (mg/l)	Free CO_2 (mg/l)
Batch 1	19	7.61	8.5	1.7
Batch 2	17	7.8	8.7	1.9
Batch 3	15	7.62	9.2	0.7
Batch 4	15.5	7.83	9.4	0.6

Table 4b: Physico-chemical and meteorological parameters of breeding ground during year 2

Experiment Batch	Water temperature (degree C)	pH	DO (mg/l)	Free CO_2 (mg/l)
Batch 1	18	7.6	8.2	1.9
Batch 2	17.5	7.64	8.8	0.7
Batch 3	17	7.68	9.5	0.6
Batch 4	16.5	7.82	9.8	0.9

In *Labeocalbasu* the dose of PG per kg body weight was given to the females in two split doses. The first dose of 2 mg / kg of body weight and the second dose was 3 mg/kg body weight while it was 2 mg/kg of body weight for the males (Jain et al 1985). As per our knowledge, probably, no induced breeding experiments have been done on *Schizothoraxrichardsonii*. Raizada (1985) made attempt on induced breeding on *S.plagiostomus* in Himachal Pradesh using homoplastic pituitary extract. He injected it in two doses at 5 mg/kg and 2 mg/kg body weight in females at an interval of 6 hours and 3 mg/kg and 2 mg/kg on males at same duration. But this approach of induced breeding was never applied for commercial hatcheries. Striping method has also been used in case of *Tor putitora,Torkhudree* and has given good results (Kulkarni and Ogale 1986; Tripathi, 1978)

Author concludes from two years of breeding experiments with *S.richardsonii* in Garhwal Himalaya that snow-trout is a post-monsoon breeder when the water is clear and the river water level is low. Low temperature and clear weather accelerates spawning and results were better. The percentage of fertilization and hatching is significantly higher when induced breeding technique is used as compared to stripping. Hence

by using induced breeding technique, hatcheries can produce seeds in large number that will help them financially. The author found that there has been rapid decline of this fish species in specific pockets along the dam sites, including spots where introductions of exotic species have been carried out, and serious attempts should be made to assess and monitor the population of this native fish species. Findings from our study would be implemented for hatcheries breeding Schizothorax species. Further experiments are also being carried out to modulate reproductive cycle of this fish species by controlling Light: Dark photo cycle, water temperature and water turbidity. This would help breed this fish faster.

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