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Induced Spawning of *Schizothorax zarudnyi* (Cyprinidae) By Using Synthetic Hormones (Ovaprim and HCG)

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Abstract: Snow trout, *Schizothorax zarudnyi* is native to Sistan district, Sistan and Baluchistan province, Southeast of I.R. Iran. In order to achieve the possibility of artificial breeding of *Schizothorax zarudnyi* using synthetic hormones (HCG and Ovaprim), this study was carried out. 97 wild fish (44 females and 53 males) were randomly allocated in five treatment groups. groups 1- 4 received 1.5 ml Ovaprim/Kg B.W., Ovaprim + HCG (1.2 ml/Kg + 5000 IU/Kg), Ovaprim + HCG (1.5 ml/Kg + 1300 IU/Kg), 2000 HCG mg/Kg respectively and the fifth NaCl 0.3 mg/Kg as control group. The results showed mean working fecundity for 1, 2, 3, 4 and 5 groups was 39531±7802, 18625±9704, 15682±5982, 0 and 0, respectively. Latency period was 36.2±3.77, 12.33±0.66 and 30.2±2.95, conversion percentage of dry egg to eyed egg was 88.97±0.9, 73.19±2.04 and 75.26±3.90, conversion percentage of eyed egg to larvae was 81.93±1.15, 75.67±0.19 and 71.03±1.03 for 1, 2 and 3 groups, respectively. There were significant differences in the fecundity, latency period and some other parameters among the treated groups using Ovaprim solely and the control group ($P < 0.05$). The results of this investigation clearly suggested that the Ovaprim is the most suitable for inducing spawning in Snow trout.

Key words: Induced spawning % *Schizothorax zarudnyi* % Sistan-Iran % Synthetic hormone

INTRODUCTION

Aquaculture industry has been rapidly progressed worldwide including Iran but many efforts are needed to transit aquaculture into sustainable developed era in Southeastern of Iran, especially Sistan district. Earlier approaches were mainly focused on introducing some non-native species mainly the common carp (*Cyprinus carpio)* silver carp (*Hypophthalmichthys molitrix),* grass carp (*Ctenopharyngodon idella),* bighead carp (*Aristichtbys nobilis)* and rainbow trout (*Oncorhynchus mykiss*) later.

Many fish populations have experienced drastic reduction in number worldwide, largely due to the adverse effects of the industries (including modern agriculture methods) and losing habitats. Programmed hatcheries management seems to be the most reliable means for replenishing natural stocks via captive breeding. The culture of schizothoracines is still in its experimental stage [1]. The genus *Schizothorax* has been reported from China, India, Afghanistan and some other countries [2] and some of *Schizothorax* species considered as cultural species because of their nutritional and trade values [1].

The population of snow trout (*Schizothorax zarudnyi*) has been dramatically decreased due to long-term drought (1997-2010) that resulted in destroying spawning niches, introducing Chinese carps to Hamoun Lake and its adjacent water bodies had affected snow trout habitats diversely. It seems that indigenous fish, the Snow trout might be classified as endangered species Sistan-Iran shows much promise on the grounds of its wide popularity properties and hardiness towards environmental conditions. Chinese carps have received relatively little attention locally despite their importance in other parts of Iran and many other countries.

Practically earlier attempts to induce spawning in *Schizothorax zarudnyi* failed to achieve acceptable results. (See aquaculture development in Sistan-Baluchistan, 2006). Those studies basically were focused on analyzing the snow trout overall breeding performance.

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Endemic fish species were considered suitable for aquaculture and snow trout as a suitable candidate regarding its palatability and nutritional values.

Some prerequisites for a suitable candidate are the ease in obtaining and raising fry or fingerlings, resistance to stresses and diseases and acceptable marketing value. In addition, no similar report has been published. Regarding to above mentioned criteria; we investigated the breeding performance of *Schizothorax zarudnyi* under partially controlled condition after inducing breeding using Ovaprim and/or HCG.

MATERIALS AND METHODS

Fish Collection: The brood fishes were captured during October to December 2010 from Chahnimeh's water reservoirs, Sistan-Iran, by local fishermen. In the same day, the fishes were transported to the Zahak Hatchery Complex in 1000 L containers fitted with oxygen diffusers. At the Hatchery, after disinfection, all fishes were transferred to an earth pond (0.35 ha) under natural conditions to pass three to five months before breeding season.

Selection and Handling: Selected females and males Snow trout were in mass 1328±45 and 632±17.6 g, respectively. After increasing the water temperature to about 14-18 degrees centigrade (middles of March 2010), 44 females and 53 males were selected for experiments. Females had soft, distended belly and pink-red genital papilla and males, which released milt when subjected to gentle pressure on the abdomen. Before the experiment, adult fish were transported to an indoor concrete tank with flowing water and a temperature of 14-18°C for 10-12 hours. Fishes were anaesthetized using 0.05-0.07 mg l^{-1} clove oil essence prior to handling.

Hormones: HCG (Pregnyl Chronic Gonadotropin) prepared by the Daroupakhsh Co., Tehran-Iran. Ovaprim (each vial contains 20 µg Salmon gonadotropin releasing hormone analog, 10 mg dopamine antagonist; domperidone), purchased from Syndel Laboratories, Ltd., Vancouver, Canada.

Hormonal Treatment: Fish were allocated into five groups: four experimental groups and a group as control. 24 hours after acclimation at 15-17°C, four treated groups received hormones Ovaprim, Ovaprim + HCG and HCG respectively via intraperitoneal injections at the base of the pectoral fins at the time interval shown in Table 2. The males (n=53) were allocated into five groups (Table 3) and received hormones synchronized to the 2nd female's injection for inducing spermiation.

Table 1: Weights and lengths of injected groups

Group	1	2	3	4	5 (control)
Weight (g)	1375±108.84	1463.33±72.81	1257.5±72.65	1050±86.60	1270±136.56
Length (cm)	50.67±1.26	50.75±0.97	48.58±0.78	45.75±1.88	49±2.19

Table 2: Dosage and hormonal preparations used for females inducing spawning in snow trout

			Injections dosage/kg b. w.				
Groups	Treatment	Fish No.	First	Second	Third	Fourth	Time interval
1	Ovaprim (ml/kg)	12	0.2	0.5	0.5	0.3	24h*
2	Ovaprim (ml/kg)	12	0.2	0.5	0.5	0.0	24h
	HCG (IU/kg)		1000.0	2000.0	2000.0	0.0	
3	Ovaprim (ml/kg)	12	0.2	0.5	0.5	0.3	24h
	HCG (IU/kg)		200.0	400.0	400.0	300.0	
4	HCG (IU/kg)	12	400.0	800.0	800.0	0.0	24h
5	NaCl (ml/kg)	8	0.3				

*Time interval between 3rd & 4th injections was 12hours

Table 3: Dosage and hormonal preparations used for males inducing spawning in snow trout

Groups	Treatment	Fish No.	Injections dosage/kg b. w.
1	Ovaprim (ml/kg)	15	0.3
2	Ovaprim (ml/kg)	15	0.3
	HCG (IU/kg)		1500.0
3	Ovaprim (ml/kg)	15	0.3
	HCG (IU/kg)		200.0
4	HCG (IU/kg)	4	500.0
5	NaCl (ml/kg)	4	0.3

Table 4: Effects of different doses of Ovaprim and HCG on snow trout spawning parameters

Groups	Injecteded females	Females spawned	Females weight (g)	Spawning rate (%)	Working fecundity	Relative fecundity	Mean volume of eggs/fish (ml)
1	12	10	1375±108.8	83.3	39531.25±7802.30^{b}	28410.61±4796.26^{b}	172.5±25.09^{b}
2	12	3	1463±72.8	25.0	18265.5±9704.69^{c}	11667.59±6282.52^{c}	246±28.21^{c}
3	12	5	1257.5±72.6	41.6	15682.33±5982.30^{c}	12931.4±4828.18^{c}	131.6±19.02^{c}
4	4	0	1050±86.6	0.0	0^{a}	0^{a}	0^{a}
5	4	0	1270±136.5	0.0	0^{a}	0^{a}	0^{a}

Groups designated by the same letter are not significantly different (P>0.05)

Effects of different doses of Ovaprim and HCG on snow trout spawning parameters

Groups	Egg diameter (mm)	Latency period (hrs)	% of dry egg to eyed egg	% of eyed egg to larvae	Larvae No.
1	1.72±0.104	36.20±3.77^{b}	88.93±0.9^{b}	81.93±1.15^{b}	34120±4562.84^{b}
2	1.45±0.012	12.33±0.66^{c}	73.19±2.04^{c}	75.67±0.19^{c}	40383.33±4508.35^{c}
3	1.57±0.124	32.80±2.20^{c}	75.26±3.9^{c}	71.03±1.03^{c}	20600±4008.14^{c}
4	0	0^{a}	0^{a}	0^{a}	0^{a}
5	0	0^{a}	0^{a}	0^{a}	0^{a}

Groups designated by the same letter are not significantly different (P>0.05)

Collection of Gametes and Incubation: Ripe gamete donors were anaesthetized in a solution containing 0.05-0.07 mg lG^{1} clove powder. Females were checked each 8 to 10 hours after second injections. Eggs were stripped into a plastic vessel and were fertilized using a "dry method" [3]. The eggs were stripped out in trays and the milt and eggs mixed using a feather. Generally the male: females ration considered 3:1.

All spawners were kept one week after gamete collection, all fishes closely monitored for survival rate.

Statistical Analysis: The results were analyzed by one way analysis of variance (ANOVA) using SPSS software (tenth version). Statistical differences between groups (spawning parameters) were analyzed using Tukey's multiple range test (P<0.05).

RESULTS

Based on abdominal size, shape and other related criteria all fishes identified at pre-ovulatory stage. The effects of hormonal treatments on reproduction parameters are shown in Table 4. Ten females of Snow trout treated triplet and quadruplet injections of Ovaprim responded to spawn successfully. Fishes receiving either NaCl (control group) or HCG (groups 4 and 5) did not ovulate. The number of positively responded fish of groups 1, 2 and 3 which treated using Ovaprim, Ovaprim + HCG (lower dose) and Ovaprim + HCG (higher dose) as fourth injections were ten, three and five of twelve females respectively. Of the five groups, group 1 (Ovaprim) was the only group that caused a significant difference (P<0.05) in spawning rate, in this group only two brood didn't spawned. There were no negative signs on fish bodies. There was significant difference (P<0.05) between group 1 and other groups in some parameters such as fecundity, volume of eggs, latency period, percent of egg conversion to larvae and larvae number obtained from fishes (P<0.05). Further, in group 2 and 3 some brood show undesirable symptoms such as color change, belly protuberant inordinately and egg stripping wasn't simply. The importance of these negative effects on broods seems highly dependent on the type of hormone used.

DISCUSSION

This study has been carried out to investigate the effect of pregnyl (Human chorionic gonadotropin, HCG) and Ovaprim (Salmon gonadotropin releasing hormone analog; dopamine antagonist, domperidone) on some spawning parameters after induction of spawning in snow trout *(Schizothorax zarudnyi)* using those preparations.

In cyprinids, the inhibitory action of dopamine on LH (luteinizing hormone) secretion is very strong and the use of anti-dopaminergic drugs combined with GnRH (gonadotropin releasing hormone) agonists is necessary to induce the ovulation in a sufficient percentage of brood stock [4, 5].

The use of various anti-dopaminergic drugs to potentiate the stimulatory action of GnRH or its analogues and induce ovulation in cyprinids fish is a well-known method in aquaculture [6, 7]. Starting in the early 1980s

when the phenomenon of dopaminergic inhibition of gonadotropin secretion in fish was discovered [8], up to now, a large number of reports have been published concerning the use of different GnRH analogues and different anti-dopaminergic drugs in inducing final maturation and spawning in numerous fish species [9-12].

The GnRH analouges, combined with strong dopamine antagonists, were generally very good in wild and cultured species stimulation [13-16]. However, discussion about these data is rather difficult because different forms of GnRH analogues have usually been used and also in different doses. Different forms of GnRH-a, sometimes from different sources, e.g. mammalian, fish, chicken, have different activities. Important differences were observed in latency time after the application of different spawning media. The shortest time between injections and ovulation was noted when Ovaprim with HCG was used as a spawning agent, in contrast to the fish stimulated with Ovaprim. The differences in latency period in females treated with HCG and Ovaprim may be explained by the fact that GnRH release from the pituitary and the ovarian in response to the released hormones is a sequential process, while in fish injected with HCG the ovarian in response to the exogenous GtH was a single process. The application of Ovaprim (GnRH-a with dopamine antagonist) induced high spawning rate in Snow trout. 83.3 percentage of females ovulated, which is much more than the other treated groups. The HCG is available more widely and in lower prices [17] but the percentage of ovulation using HCG and CPE was much lower.

Nandeesha *et al*., (1990) reported positive response of mrigal to Ovaprim at a dose of 0.3 ml kg^{-1} indicating a high potency of this drug in induced spawning. Comparative efficacy of pituitary gland and Ovaprim as inducing agent was studied on *Clarias batrachus* by Basu *et al.*, (2000). They stated that Ovaprim yielded better result with higher percentage of fertilization and hatching. Induction with Ovaprim yielded 80% fertilization of eggs and 60% of their hatching, whereas induction with pituitary gland extract resulted in 45% fertilization and 25% hatching. The hormonal dose of Ovaprim recommended for carp is 0.3 ml to 0.4 ml kg^{-1} [18]. However for the Snow trout in the present study a much higher dose of 1.5 ml kg^{-1} body weight made the best results.

As conclusion, administration of 0.20, 0.50, 0.50 and 0.3 mLkg^{-1} BW of Ovaprim was successful in inducing ovulation in the Snow trout, *Schizothorax zarudnyi*. The spawning rate, conversion of dry egg to eyed egg and conversion of eyed egg to larvae was 83.3%, 88.9% and 81.9% respectively and all rates for the five treatments were significantly different ($P < 0.05$). Unfortunately, the species population threading due to a combination of over-fishing, environmental degradation, spread of disease, pollution and lack of proper management. Therefore, we hope that the findings of the current study will be of help to the environmental and fishery officials, informing their Future decisions for their construction of the fish resources used for human consumption.

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