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Note

Advancement in spawning period of *Labeo dyocheilus* (McClelland, 1839) in the mid Himalayan regions by hormonal manipulation using Ovatide

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ABSTRACT

An attempt was made to advance the spawning period of *Labeo dyocheilus* (McClelland, 1839) by pretreatment with the synthetic gonadotropin releasing hormone analogue, Ovatide. Total 12 females and 24 males (1: 2 ratio) were used for breeding operations conducted during 30th May to 30th June 2016 comprising six sets. Female fishes of set I to IV were pretreated with intramuscular injection of the ovatide at the rate 0.3 ml kg^{-1} body weight twice at an interval of two weeks while males were injected @ 0.1 ml kg^{-1} body weight once before 15 days of induced breeding trial for achieving advance maturity and spawning during prespawning phase. Fishes of set V and VI were not subjected to any pretreatment with ovatide. On 30th of June all the six breeding sets were treated with ovatide for induced spawning. Female and males were injected at 0.7 and 0.3 ml kg^{-1} of body weight, respectively and left overnight in FRP tanks for spawning. Results indicated that in sets I to IV, fishes successfully spawned and fertilised eggs were collected in morning hours whereas fishes of set V and VI failed to spawn. Fertilisation and survival rates recorded were 79-82 and 67-71%, respectively. Results indicated that treatment of ovatide during prespawning phase is beneficial for advancing the gonadal maturity, successful induced spawning and prolonging the breeding period which may play a key role in production of substantial quantity of seed of *L. dyocheilus.*

Keywords**:** Advance spawning, *Labeo dyocheilus*, Mid Himalayas, Ovatide

Labeo dyocheilus (Mcclelland, 1839) is a fast growing, high valued cyprinid species and widely distributed in India (all along the Himalayas, Assam, Uttar Pradesh, Uttarakhand, West Bengal, Bihar), Bangladesh, Nepal (Talwar and Jhingran, 1991), Mekong and Bassac rivers of Mekong Delta, Vietnam (Tung *et al.,* 2006), Pakistan and Afghanistan (Dahanukar, 2010). Over the past 10 years its natural wild stocks have undergone a steady decline through uncontrolled and often indiscriminate fishing, increasing use of river water for irrigation, hydropower generation, municipal and industrial use as well as due to environmental pollution. It is listed among the 82 vulnerable freshwater fish species of India (CAMP, 1998) though IUCN (2013) has listed this species under the 'least concern' category. Recently, there have been efforts to develop controlled artificial reproduction of this species (Sarkar *et al*., 2001; Pandey *et al.,* 2011; Gupta, 2014). Fecundity of this fish is very high as compared to other cold water fishes (Sarkar *et al*., 2001, Pandey *et al*., 2011, Gupta *et al*., 2013), which reflects the importance of this fish to be incorporated into coldwater aquaculture sector as a new candidate species. Normally the induced breeding of the species is achieved from third week of July to second week of August in mid Himalayan region (Pandey *et al.*, 2011). In this short period, it is a great challenge to produce large quantum of seed to meet the demand for aquaculture of the species. Therefore, advancement of spawning period by hormonal manipulation will be advantageous for increasing its seed production. Kumar *et al*. (2007) reported advancement in gonadal maturity and spawning through hormonal manipulations in *Labeo rohita*. Manipulation of reproductive cycle by hormonal application has been tried in fishes using analogue of gonadotropin releasing hormone (GnRHa) alone or in combination with steroidal hormones (Crim, 1991). No attempt has been made so far on induction and advancement of ovulation in *L. dyocheilus* employing hormonal manipulation. Therefore, the present study was conducted with the objective to advance the spawning period of *L. dyocheilus* in the mid Himalayan region using the synthetic GnRH analogue, 'Ovatide'.

Healthy fishes of *L. dyocheilus* (200 - 540 g) were collected using cast net during morning hours from

Kosi River near Ramnagar, Uttarakhand (29°29.038'N; 79°08.777'E, 410 m MSL) and transported in live condition to ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal (29˚21´N; 79˚34´E, 1370 m MSL) in Kumoun region of Uttarakhand, India. The fish were disinfected with 5% $KMnO_4$ solution before stocking and then stocked in two cement tanks @ 0.20 kg m^{-3} in each tank (20 x 4.5 x 1.5 m); with boulders placed at the bottom of the tanks for facilitating growth of periphyton and also to provide hiding place for the fishes. Brood fishes were fed with formulated pelleted carp feed having 28% crude protein. Fishes were fed @ 2-3% of their body weight daily in two split doses (07:00 and 17:00 hrs.) and 50% water was exchanged fortnightly to maintain the water quality parameters under optimum levels for the brood stock.

Six breeding sets of *L. dyocheilus* were arranged during pre-spawning phase for hormonal manipulations as per the schedule given in Table 1. In breeding sets I to IV, two preparatory doses of ovatide was administered intramuscularly to female fish. First preparatory dose was given to female fish on 30 May 2016 while no hormone treatment was given to male fish at this time. On 15 June 2016, both female and males of each breeding set were injected with ovatide. On 30 June 2016, fishes of either sex were injected with the final dose of ovatide. In set V and VI no preparatory hormone doses were given to either sex and the brooders were directly given final hormone dose on 30 June 2016. The hormone doses administered are provided in Table 1. After hormone administration, both male and female fishes were kept overnight together for spawning, in FRP tanks (2 x 2 x 0.5 m) having continuously flowing water (1-2 l per min). After spawning, the males and females were removed from the spawning tank, eggs were collected by siphoning and incubated in portable FRP carp incubation pool developed by ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, Odisha, India. Latency period, fertilisation rate, hatching rate and survival rate were estimated as per Thomas *et al*. (2003).

$$\text{Fertilisation rate (\%)} = \frac{\text{No. of fertilised eggs}}{\text{Total no. of eggs}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{No. of hatchings}}{\text{Total no. of fertilised eggs}} \times 100$$

Physico-chemical parameters of water in the brood stock tanks were analysed following APHA (2005) during the experiment. Average water temperature, pH, alkalinity, total hardness and dissolved oxygen levels recorded were 18-22°C; 8.0-8.5; 52-58 mg l^{-1}, 65-70 mg l^{-1} and 6.4 -8.0 mg l^{-1} respectively.

Breeding performance of all the six breeding sets are presented in Table 2. In the set I to IV, successful spawning was achieved with 79-82% fertilisation rate whereas, no spawning was achieved in set V and set VI. The fertilised eggs were non-adhesive and demersal. Water hardening of the fertilised eggs took place in 4-5 h. Eggs were

Table 1. Schedule of hormone administration to the brood fishes

Breeding set	Date of hormone administration	Dose of Ovatide (ml kg body weight $^{-1}$)		Weight of fish (g)	
		Female	Male	Female (2 nos.)	Male (4 nos.)
I	30.05.2016 (1st Preparatory dose)	0.3	-	481 and 462	283; 217; 253 and 287
	15.06.2016 (2nd Preparatory dose)	0.3	0.1		
	30.06.2016 (Final dose)	0.7	0.3		
II	30.05.2016 (1st Preparatory dose)	0.3	-	486 and 456	261; 228; 241 and 272
	15.06.2016 (2nd Preparatory dose)	0.3	0.1		
	30.06.2016 (Final dose)	0.7	0.3		
III	30.05.2016 (1st Preparatory dose)	0.3	-	406 and 449	201; 219; 261 and 244
	15.06.2016 (2nd Preparatory dose)	0.3	0.1		
	30.06.2016 (Final dose)	0.7	0.3		
IV	30.05.2016 (1st Preparatory dose)	0.3	-	448 and 482	227; 269; 242 and 261
	15.06.2016 (2nd Preparatory dose)	0.3	0.1		
	30.06.2016 (Final dose)	0.7	0.3		
V	30.05.2016 (1st Preparatory dose)	-	-	381 and 464	214; 251; 219 and 291
	15.06.2016 (2nd Preparatory dose)	-	-		
	30.06.2016 (Final dose)	0.7	0.3		
VI	30.05.2016 (1st Preparatory dose)	-	-	473 and 521	264; 234; 301 and 281
	15.06.2016 (2nd Preparatory dose)	-	-		
	30.06.2016 (Final dose)	0.7	0.3		

Table 2. Breeding performance of *L. dyocheilus* in different breeding sets

Breeding set	Latency period (h)	Total eggs spawned (Nos.)	Fertilisation rate (%)	Incubation period (h)	Hatching rate (%)	Survival (%)	Remarks
I	11-13	319200	81±3.5	21-40	94±3.0	69±2.8	Successful spawning
II	12-13	303300	81±4.1	21-40	94±2.6	71±2.5	Successful spawning
III	11-12	296700	79±2.8	21-40	95±1.3	67±3.3	Successful spawning
IV	12-13	298300	82±5.3	21-40	94±3.1	68±4.9	Successful spawning
V	-	-	-	-	-	-	No spawning
VI	-	-	-	-	-	-	No spawning

transparent and average diameter of the fertilised eggs recorded was 3.8±0.41 mm and the incubation period was 21-40 h. Fishes of sets I, II, III and IV successfully spawned which indicated that the treatment of ovatide during pre-spawning phase not only advanced the gonadal maturity but also facilitated successful induced spawning of *L. dyocheilus* even during early spawning season. Vardia (1996) reported similar findings on advancement in maturity in Indian major carps using human chorionic gonadotropin (hCG). Similarly, Kumar *et al.* (2007) reported advancement in gonadal maturity in *Labeo rohita* with treatment of ovaprim during pre-spawning phase. Chalal *et al.* (2012) found advancement in ovarian maturity of *Schizothorax richardsonii* when subjected to treatment of pituitary gland extract (PGE). Gupta (2014) also successfully achieved breeding of *L. dyocheilus* in early spawning season by pre-treatment with ovaprim.

Fishes of set V and VI failed to spawn which indicated that *L. dyocheilus* was unable to breed in early spawning season without pre-treatment of ovatide during pre-spawning phase. Pandey *et al.* (2011) suggested that *L. dyocheilus* showed complete maturity by the 3rd week of July only and can be bred successfully only after that period. Failure of spawning in fishes of these sets indicated that induced spawning was not possible during early spawning season without hormonal pre-treatment.

The findings of the present study revealed that breeding of *L. dyocheilus* in early spawning season by advancing their maturity is very much beneficial for production of large quantum of seed in mid Himalayan region as it failitates extended breeding period from mid-June to last August as compared to normal breeding period *i.e.,* third week of July to last week of August. Breeding of *L. dyocheilus* in early spawning season may also be beneficial for nursery phase rearing of seed in the region as temperature favours the growth of larvae upto the month of September which otherwise starts to fall from October onwards and results in poor survival of larvae due to low temperature.

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