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Artificial Breeding, Gonadosomatic Index (GSI) and Fecundity of Captive Reared *Labeo gonius* in Coldwater Condition

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Abstract: *Labeo gonius*, an indigenous medium carp of the Cyprinidae family, is a potential species for polyculture with exotic carps in foothills and mid-latitudes. Apart from the plain and Tarai region, it is widely distributed all along foothill regions of Himalayan region in India with a great food value and fair market demand. An attempt was made to establish a breeding protocol and to assess the Gonadosomatic Index (GSI) and Fecundity of this hill aquaculture potent fish species. A total of 41 brooders were selected and injected intramuscularly with Ovatide@ 0.3, 0.5, and 0.7 ml/kg bw in females and 0.1, 0.2, and 0.3 ml/kg bw in males, and absolute fecundity were recorded between 72265 to 184322 ova with an average of 111330.8625±53449 ova per fish. Water temperature between 18-22 C was observed optimum for the egg incubation (48 h) with a higher hatching rate (82%) and better recovery of hatchlings. The spawning fecundity was observed as 203478 eggs/kg bw. GSI was observed by dissection of selected brooders which ranged from 3.324 ± 0.143 to 14.115 ± 1.214 which increased gradually from April to July and decreased in August in captive conditions. The highest value of GSI was recorded in July (14.115 ± 1.214) while the lowest was observed in January (3.324 ± 0.143). The single peak of GSI during July indicates the spawning season of *L gonius* in July-August. The present findings highlight the reproductive potential of *L. gonius* in coldwater conditions which enable the breeding and seed production aquaculture of this indigenous fish species in hills.

Keywords: *Labeo gonius,* Indigenous medium carp, Gonadosomatic Index (GSI), Fecundity, Breeding, Hill aquaculture

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Introduction

Labeo gonius, commonly known as 'Kuria labeo' or Gonius is an indigenous medium carp belonging to family Cyprinidae. It is widely distributed in Assam, West Bengal, Orissa, Uttar Pradesh, Uttarakhand, Bihar, Rajasthan, Madhya Pradesh, and Punjab in the major freshwater rivers, reservoirs, lakes, and tanks (Mohanta *et al*., 2008). The species has been reported from the Yamuna, Mahanadi, rivers of Kumaun Himalayas, river Kali, Rajasthan waters, and Kaziranga Wildlife

Sanctuary (Talwar and Jhingran, 1991; Chondar, 1999). CAMP report (1998) mentioned that the species is distributed in North-eastern states Assam, Meghalaya, Arunachal Pradesh, and Tripura. Freshwater fish culturing is an important sector of food production in Asia and throughout the world for raising the quality and quantity of domestic fish production for human consumption (Gjedrem *et al*., 2012). *Labeo gonius* is also found in the hill region of Uttarakhand, India as a candidate species with good consumer preference. Since the fish is an herbivorous bottom feeder, it can be cultured as a bottom-feeding substitute in composite fish culture. It is a highly fecund fish and may have prolonged breeding season. The species can be cultured in shallow water bodies. It is compatible with Indian major carps in composite fish culture. The fish is suitable for integrated fish culture system especially for paddy–cum fish culture. It is distinctly hardy in nature in comparison to other cultivable carp species and is suitable for culturing at high stocking density. It can be cultured in nonconventional aquaculture systems like pens and cages in open water bodies.

L. gonius breeds in riverine conditions during the monsoon season. In nature, it attains maturity after the third year of life span. The size at first maturity varies from 14-23 cm in males and 18-30 cm in females in different freshwater ecosystems. Peak maturity of this species is observed in May-June with an average size of 300-500 g. It is one such species that needs urgent attention for conservation, as the population of this commercially important species is declining from nature (CAMP, 1998).

Fish reproduction is a periodic phenomenon and is controlled by environmental (exogenous) as well as internal (endogenous) regulatory mechanisms. The act of breeding occurs under optimal environmental conditions that are favourable to the survival of the young ones. Environmental stimuli are detected by sensory organs, relayed to the brain-that triggers endogenous mechanisms into action. An endogenous mechanism is mediated through a cascade of various neurotransmitters and hormones secreted by tissues of a brain-hypothalamus-pituitary-gonadal axis. The secretions of the above axis are regulated through positive and negative feedback mechanisms involving specifically sensitive hormone receptors. The most important reproductive neuro-hormones are hypothalamic gonadotropin-releasing hormones (GnRH) and gonadotropin-release-inhibiting factors (hormones) (GRIF or GnRH) that regulate secretions of pituitary gonadotropin hormones (GtH) which in turn, regulate the synthesis of gonadal steroids responsible for the final maturation of gametes. An appropriate environmental stimulus may signal the arrival of optimal conditions for the fry, triggering spawning i.e. spermiation and ovulation. Fish in captivity may not always reproduce at the most favourable time. In this situation, hormones play a critical role in the reproductive processes. Hormone-induced spawning techniques influence this sequential mechanism at several steps, either by promoting or inhibiting the process. Induced reproduction in fish includes two main strategies. The first is the manipulation of the cultural environment to mimic important characteristics of the natural spawning environment of that particular fish. The second strategy is the administration of one or more naturally occurring reproductive hormones or their synthetic analogs in brood fish through injection or dietary methods. Both these strategies are commonly used, sometimes in conjunction with one another. Numerous hormones have been used to induce reproduction. However, recent researches and commercial aquaculture practices suggest the emergence of two lines of hormone-induced spawning as the best for successful breeding at the least expense.

Exogenous hormones such as pituitary gland extracts and others are commonly injected to mature brooders to induce breeding (Yaron, 2009). In India, a breeding technique of coldwater

fish species, with or without hormone injection has been developed for *Tor khudree*, *Tor putitora*, *Tor tor*, and hybrid mahseer (Ogale and Kulkarni, 1987; Ogale, 2002; Sangma and Basavaraja, 2010). Attempts have been made to breed Golden mahseer in the Kumaun region (Shyam Sunder, 1993; Ogale, 1997). Breeding of *Labeo dyocheilus* has been achieved using ovaprim in coldwater conditions under captivity (Pandey *et al.*, 2011). Captive breeding of *Channa aurantimaculata* in Assam is also described by Gogoi (2015). In recent years, induced breeding for qualitative and quantitative improvement of fish has been widely recognized as a popular technique for significant expansion of reproductive processes of domestic fishes (Dhawan and Kaur, 2004). Hence, the present study was undertaken to develop the induced breeding protocol and to assess the Gonadosomatic Index (GSI) and Fecundity of *Labeo gonius* in captivity and under coldwater conditions.

Materials and Methods

The experiments were conducted in the model fish hatchery of the Directorate of Coldwater Fisheries Research, Bhimtal (29^0 21'N and 79^0 34'E, Altitude 1370 msl), Uttarakhand, India. Male and female (n=41) brooders of *L. gonius* were collected from the wild and kept in the ponds. The fishes were reared in the cemented tank after disinfection in coldwater condition (8-23.5 C) and fed daily with conventional carp feed (protein level 24%) at 3% of their body weight. Fishes were dissected and gonad was taken out individually from male and female and weighed on a single pan electronic balance. GSI of female was calculated by using following formula.

$$GSI = \frac{Weight \text{ of gonad (g)}}{\text{Total body weigh t (g)}} \times 100$$

The brooders were selected based on their external characteristics such as females were selected on the basis of bulging abdomen, soft ventral abdominal region, smooth pectoral fin with reddish colour and oozing of eggs with gentle pressure on the abdomen while males were selected on the basis of roughness on pectoral fin and oozing of milt with gentle pressure on the abdomen.

The experiment was conducted with different body weight of brooders, varying between 450-512 g in female and 380-560 g in males. The selected brooders were injected intramuscularly with Ovatide@ 0.3, 0.5, 0.7 ml/kg bw in females and 0.1, 0.2 and 0.3 ml/kg bw in males. Immediately after hormone administration brooders were released into the breeding tank at 1:1 ratio of male and female.

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 were 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 only considered for calculating percentage of fertilization. The ova diameter was measured by keeping 10 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. The fertilization rate and hatching rate were calculated as follow:

$$\text{Fecundity} = \frac{\text{No. of stripped egg}}{\text{Weight of ovulated fish}}$$

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

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total No. of fertilized eggs in a batch}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Total number of survival larvae until day 7}}{\text{Total No. of larvae counted at day 1}} \times 100$$

One day after hatching, all the hatchlings were maintained in CIFA model FRP portable carp hatchery. Aeration was provided in the FRP tank and water was exchanged daily. The water quality parameters of brood stock pond and breeding

pond were analyzed as per APHA (1998) (Table 1).

Statistical analysis of the data were done by one way Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) to determine differences between the means taking at 1% ($P<0.01$) or 5% ($P<0.05$) significance levels.

Table 1: Physico-chemical parameters of broodstock pond and breeding pond

Parameters	Value	
	Broodstock pond	**Breeding pond**
Air temperature (C)	11-24.8	20±1.34
Water temperature (C)	8-22.8	18±1.46
pH	7.6-8.4	7.8±0.24
Dissolved oxygen (ppm)	6.2-7.4	7.2±0.80
Free CO_2	1.5-2.2	1.4±0.28

Results

Gonadosomatic index (GSI) increased gradually from April to July and decreased in the month of August in females in captive condition. GSI ranged from 3.324 ± 0.143 to 14.115±1.214 in captive-reared females. The spawning performance of *L. gonius* induced at different Ovatide dosages is presented in Table 2. Spawning was not noticed in low doses. Significantly, the highest ($P<0.05$) positive correlation was observed with bodyweight for egg size, fecundity, fertilization rate, and hatching rate. Female of average weight 492±30.0 g showed better breeding performance than the other two treatments.

Discussion

In the present study, a single intramuscular injection of synthetic hormone, Ovatide resulted in successful spawning of *L. gonius* without post-spawning mortality. Our findings corroborate with the GSI pattern reported by Pandey *et al*. (2011) in *L. dyocheilus*. A similar trend in GSI was also recorded by Gupta *et al*. (2013) in *L. dyocheilus* kept under captivity who observed normal ovarian development of fish. The greater value of GSI reflects the ovarian maturity in the fish (Mishra and Saksena, 2012). GSI can not only predict the breeding season but also indicate the maturity status and periodicity of spawning of a fish (Khanna, 2003). The highest value of GSI in the month of July clearly indicates the fish spawned once in a year with one spawning peak highest in the month of July. Results presented in the table show a sudden decrease in gonad weight from September to January as indicated by the decline of GSI after spawning. Results clearly indicate that female fish with greater weight had higher fecundity. A similar kind of observation was also recorded by Khan *et al*. (1992); and Gupta *et al*. (2013).

The gonadal development may be induced by increasing water temperature. The ovaries keep on growing and maturing from April to July of the following year. It does not spawn until the water temperature increases in April although the ovaries matured even during the cold season the fish did not begin to spawn until late spring. In this study, the breeding performance of *L. gonius* females after injection with Ovatide was considerably good. The use of Ovatide showed a 78% ovulation rate which was much better than the previous study by Behera *et al.* (2009) with a 55% of ovulation rate by using Ovaprim on the *T. tambroides* species. Similarly in other studies, Ovatide was found to be more effective and has successfully induced ovulation in several species such as *Labeo rohita* and *Cirrhina mrigala* as compared to Ovaprim (Dhawan and Kaur, 2004).

Ovulation is regulated by important endogenous hormones like a gonadotropin-releasing hormone, gonadotropic hormone, and gonadotropin inhibiting factor which are interdependent on each specific hormone or factor for proper regulation and corresponding signal mediation necessary for ovulation (Peter and Yu, 1997; Peter *et al*., 1998; Marimuthu *et al*., 2009).

Conclusion

Ovatide effectively stimulated fecundity in minor

Table 2: Spawning performance of *L. gonius* in relation to body weight of female at 18±1.46 C

Weight of female (g)	Weight of male (g)	Dose of Ovatide (ml/kg)		Latency period (h)	Spawning fecundity/kg bw	Fertilization rate (%)	Hatching rate (%)	Remark
		Female	Male					
482±16.0	463±12.4	0.3	0.1	Nil	No breeding	Nil	Nil	No spawning
567±28.0	474±16.2	0.5	0.2	15	48,740	92.6±4.6	88.4±1.2	Partial spawning
492±30.0	458±14.8	0.7	0.3	12	74,618	93.2±3.8	84.6±1.4	Complete spawning

carps. Different responses were observed in different doses in *Labeo gonius* under the influence of ovatide hormones at less temperature.

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