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Breeding performance of indigenous carp, *Labeo dero* in captivity under cold water condition of Uttarakhand, India

Authors Info

N.N. Pandey*, M. Gupta,
R. Singh, S. Ali, R.S. Haldar,
P. Kumar and A.K. Singh

Directorate of Coldwater Fisheries
Research (ICAR),
Bhimtal - 263 136, India

*Corresponding Author Email :
nityanfish@yahoo.co.in

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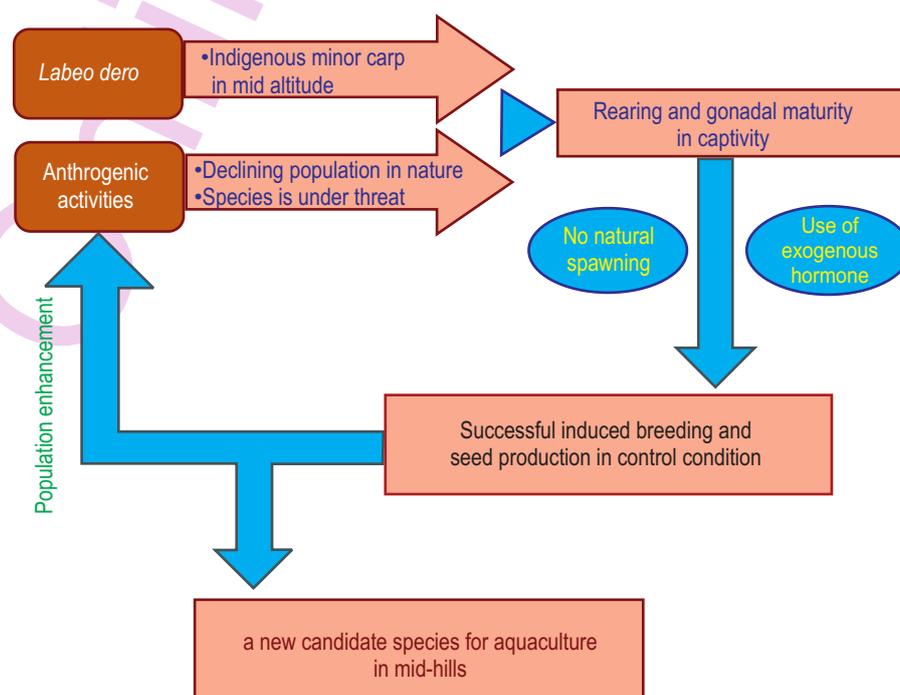
Abstract

Aim : To study the breeding performance of indigenous carp, *Labeo dero* in captivity under cold water condition.

Methodology : Rearing of adult fish in pond condition, observation of gonadal maturity and GSI, standardization of optimum dose of hormone, recording of breeding parameters such as fecundity, fertilization rate, hatching percentage, incubation period, egg size and hatchling size were studied.

Results : Single intramuscular dose of synthetic hormone, ovaprim @ 0.7 ml kg⁻¹ body weight for female and 0.3 ml kg⁻¹ body weight for male resulted in successful spawning of *L. dero* without post spawning mortality at 18°-22°C water temperature. Observed relative fecundity was 1,47,343 eggs kg⁻¹ with fertilization rate of 90% and hatching rate 78%. The average diameter of the fertilized egg was 1.6-2.8 mm.

Interpretation : Successful induced breeding of captive reared *L. dero* at 18°-22°C water temperature was observed. These findings would be helpful in seed production of *L. dero*, which can be used for natural stock augmentation and culture of this species.



Introduction

Labeo dero commonly known as 'Kalabans' in India, 'Gardi' and 'Kathalegi' in Nepal, 'Kursa' in Bangladesh, is one of the popular food fish and is widely distributed all along the foot hill regions of Himalayan ranges of India, Pakistan, Bangladesh, Nepal, Myanmar and China (Talwar and Jhingran, 1991; Mohindra et al., 2005). The fish is ordinarily white in colour with more elongated body and relatively small head. In India, it is common in the Gangetic belt and Indus river systems. Its maximum reported length is around 750 mm. It can be used in pond culture along with Indian major carps due to local market acceptance. The population of this species is struggling for its existence due to unplanned development projects of flood control and irrigation, dam construction, embankments, modification of river courses and other human activities and categorized as a vulnerable fish (Mahanta et al., 1994). Therefore, proper management initiatives of this species should be taken to save this fish in nature. To maintain the wild population of this fish in nature as well as its conservation, development of a suitable technology for captive breeding is urgently needed. Under captive condition, both, male and female attain gonadal maturity (Pandey et al., 2014), but do not spawn naturally.

Exogenous hormones such as pituitary gland extracts and others are commonly injected to mature brooders to induce breeding (Yaron, 2009). Due to the increasing cost of donor pituitary and cumbersome process, Human Chorionic Gonadotropin, Leutinizing Hormone Releasing Hormone and ovaprim are the alternatives of the pituitary extract (Haniffa and Sridhar, 2002). Ovaprim is a product that contains salmon gonadotropin releasing hormone analogue (sGnRH;D-Arg⁶,Pro⁹,Net) at a concentration of 20 µgml⁻¹ and dompridone, a dopamine antagonist at 10 mg ml⁻¹ (Hill et al., 2009). Dopamine antagonists are used for cessation of dopamine activity which acts as an inhibitory factor for the synthesis of gonadotropin (Naeem and Salam, 2005). Ovaprim is used to induce ovulation and spermiation in fish mostly by intramuscular or intraperitoneal route (Nandeeshia et al., 1990; Pandey and Singh, 1997; Raghav et al., 2012). In India, a breeding technique of cold water fish species, with or without hormone injection has been developed for *T. khudree*, *T. putitora*, *T. tor* and hybrid mahseer (Ogale and Kulkarni, 1987; Ogale, 2002; Sangma and Basavaraja, 2010). Attempts have been made to breed *T. putitora* (Golden mahseer) in the Kumaon region (Shyam sunder et al., 1993; Ogale, 1997). Breeding of *Labeo dyocheilus* has been achieved using ovaprim in cold water condition under captivity (Pandey et al., 2011). Captive breeding of *Channa aurantimaculata* in Assam is also described by Gogoi et al. (2016). There is only one report from the Nepal for semi artificial breeding of *L. dero* (Prasad, 2009). But no systematic research work has so far been undertaken on the breeding biology, breeding behavior and induced breeding of *L. dero*. Therefore, induced breeding approach was applied for successful spawning of captive reared brood fish. Hence, the

present study was undertaken to develop the induced breeding techniques of this fish in captivity and under cold water conditions

Materials and Methods

The experiments were conducted at ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, India. Immature fish of body weight 110-220 g were captured during January 2011 from the Kosi river, Ramnagar of Kumaon Himalayan region, India by local fishermen and transported to laboratory in 1000 l containers fitted with oxygen diffusers. The immature fish were reared for two year in the cemented tank under coldwater condition (8-23.5°C) and fed daily with conventional carp feed (protein level 24%) at 3% of their body weight. Fish were dissected and gonad was taken out individually from male and female and weighed on a single pan electronic balance to observe the maturity status. Gonadosomatic index of female were calculated from April to July.

After two years, the female brooders achieved 350 to 470 g weight and male achieved 210-250 g body weight and showed full maturity in pond environment with egg release and oozing milt from 3rd week of July to the end of August. Mature females were selected by their bulging soft abdomen, oval shaped reddish vent slit and smooth pectoral fins, while mature males were selected by pale reddish vent slit and rough pectoral fins. One day before the experiment, the brooders were selected and transferred to FRP tanks (2.5x2.5x.75m) of 3125 l capacity filled to a water level of 50 cm. Each breeding set consisted of two male and one female. Both the sexes of this species did not respond to natural spawning in captive condition. Hence, the selected females were randomly assigned to four treatment groups (T1, T2, T3 and T4) and were injected intramuscularly with ovaprim hormone @ 0.3, 0.5, 0.7, 0.9 ml kg⁻¹ body weight. All the males were injected with ovaprim hormone @ 0.2 ml kg⁻¹ body weight simultaneously with female. The hormone was administered during evening at 18:00 hrs.

Hormone treated fish were introduced overnight into the FRP tanks and left to spawn. Darkness was maintained in tank by covering the tank with green colour net. After spawning, the number of eggs and rate of fertilization were calculated. Fertilized eggs from the breeding tanks were transferred carefully to trays having flow through systems for incubation. Rate of fertilization, hatching, incubation period and survival of larvae was observed for each operation.

The effect of different temperature on egg incubation performance and survival were also observed to optimize suitable temperature for successful incubation. For the experiment, one hundred of fertilized eggs were randomly selected and kept in glass jar with 1 l of water. Water temperature was maintained at 16, 18, 20, 22 and 24°C each with three replicates. 16, 18 and 20°C were maintained by keeping the glass jar in BOD incubator. 24°C was maintained by electric heater controlled thermostatically and 22°C was the natural temperature of water at

the time of experiment. The fertilization rate and hatching rate were calculated. Physico-chemical parameters of water were analyzed following the protocols of APHA (2012). During the experiment, the average water temperature, pH and dissolved oxygen were recorded as 18-22°C, 8.4 -8.6 and 6.4 -8.0 mg l⁻¹, respectively.

Statistical analysis of data was 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. Results are presented as means ± standard deviation.

Results and Discussion

In both the sexes, Gonadosomatic index (GSI) increased gradually from the month of April to July and decreased in the month of August, which showed gonadal maturity in captive condition. GSI ranged from 3.324 ± 0.143 to 14.115±1.214 in captive reared females. Similar trend in GSI were recorded in *L. dyocheilus* (Singh *et al.*, 2008; Pandey *et al.*, 2011 and Gupta *et al.*, 2013b) and in *L. dero* (Pandey *et al.*, 2014) kept under captivity. In general, mature brooders did not show natural spawning in captivity, but, single intramuscular dose of synthetic hormone, ovaprim resulted in successful spawning of *L. dero* without post spawning mortality. Significantly, the highest (P<0.05) spawning fecundity was obtained in T3 (ovaprim 0.7ml kg⁻¹ body weight), than T2 (ovaprim 0.5 ml kg⁻¹ body weight). The highest fertilization rate and hatching rate was also observed in

T3 (ovaprim 0.7ml kg⁻¹ body weight) group (Table 1). Therefore, hormone dose (ovaprim) of 0.7 ml kg⁻¹ body wt. for females and 0.2 ml kg⁻¹ body wt. for males was found optimum for successful spawning of captive reared *L. dero*. Similar results of induce breeding were reported in *L. dyocheilus* (Sarkar *et al.*, 2004; Pandey *et al.*, 2011).

A positive correlation was observed between body weight of fish and breeding performance. Females having average body weight of 0.430±0.04 to 0.470±0.15 kg showed larger egg size, more fecundity, better fertilization rate and better hatching rate than the females of less body weight (0.350±0.08 to 0.390±0.06 kg) (Table 2). The latency period of *L. dero* ranged from 12-14 hrs at 18±1.5°C. These results are in conformity of *L. dyocheilus* administered with ovaprim (Pandey *et al.*, 2011). In the present study, relative fecundity was observed as 1,47,343±110 eggs kg⁻¹. However, Prasad (2009) reported higher relative fecundity of *L. dero* with wild brooders. The fertilization rate of 90±2.5% and hatching rate of 78±4.5% was recorded in present study, which is comparable with *L. dyocheilus* (Sarkar *et al.*, 2004; Singh *et al.*, 2008 and Pandey *et al.*, 2011) and *L. bata* (Hossain *et al.*, 2007). Prasad (2009) also observed fertilization rate and hatching rate in *L. dero*, which was approximately similar to this study.

Egg incubation performance of *L. dero* is presented in Table 3. It was found that optimum temperature range for the egg incubation in coldwater conditions was 18-22°C with incubation period of 20-29 hrs, which is almost similar to incubation period of *L. dyocheilus* (18-30 hrs) reported by Gupta *et al.* (2013a). Prasad

Table 1 : Spawning performance of *L. dero* induced at different Ovaprim dosages (ml kg⁻¹) at 18±1.5 °C

Treatment	Weight of female (kg)	Weight of male (kg)	Dose of Ovaprim (ml kg ⁻¹)		Latency period	Spawning fecundity/kg body weight	Fertilization rate (%)	Incubation period	Hatching rate (%)	Remark
			female	male						
T1	0.450±0.05	0.250±0.05	0.3	0.2	-	-	-	-	-	No spawning
T2	0.415±0.09	0.210±0.04	0.5	0.2	14-16	20,330±145 ^b	72±2.0 ^b	20-24 ^a	55±5.0 ^b	Partial spawning
T3	0.440±0.04	0.240±0.06	0.7	0.2	12-14	1,44,500±210 ^a	90±5.5 ^a	21-26 ^a	74±3.5 ^a	Complete spawning
T4	0.410±0.15	0.213±0.05	0.9	0.2	-	-	-	-	-	No spawning

Means with different superscript within the same group are significantly different (P< 0.05)

Table 2 : Breeding performance of *L. dero* in relation to body weight of females at 18±1.5 °C

Weight of female (kg)	Weight of male (kg)	Dose of Ovaprim		Egg size (mm)	Spawning fecundity/kg body weight	Fertilization rate(%)	Incubation period	Hatching rate (%)	Remark
		female	male						
0.350±0.08	0.260±0.05	0.7	0.2	1.6 ^b	51,650±145 ^b	69±2.5 ^b	21-26 ^a	55±4.0 ^b	Partial spawning
0.390±0.06	0.250±0.06	0.7	0.2	1.8 ^b	58,000±188 ^b	72±2.0 ^b	21-24 ^a	65±4.4 ^b	Partial spawning
0.430±0.04	0.250±0.05	0.7	0.2	2.8 ^a	1,34,500±210 ^a	92±5.5 ^a	21-26 ^a	74±3.5 ^a	Complete spawning
0.470±0.15	0.243±0.08	0.7	0.2	2.6 ^a	1,47,343±110 ^a	90±2.5 ^a	21-26 ^a	78±4.5 ^a	Complete spawning

Means with different superscript within the same group are significantly different (P< 0.05)

Table 3 : Egg incubation performance of *L. dero* at different temperature

Temperature (°C)	Incubation period		Hatching rate (%)	Survival rate (%)
	Hatching start(hr)	Hatching completed (hr)		
16	29	48	9±1.0 ^c	4.0±0.0 ^c
18	24	28	71±3.5 ^a	65±2.0 ^a
20	22	29	74±4.0 ^a	72±2.0 ^a
22	20	24	73±3.0 ^a	70±3.0 ^a
24	16	19	66±3.0 ^b	60±4.0 ^b

Means with different superscript within the same group are significantly different ($P < 0.05$)

et al. (2009) reported shorter incubation period (16-18 hrs) of *L. dero* at 24-26°C. Kikko et al. (2015) reported that lower incubation temperature resulted in smaller hatchling size and longer time to hatch.

The average size of one day hatchling was found to be 3.24±0.48mm, weighing 0.005 g. Yolk material was absorbed within 70-84 hrs of hatching at 22°C temperature and larvae started external feeding on 4th day. The survival percent of hatchling (72%) was comparatively higher than the survival percent (24.1%) as reported by Prasad (2009) in *L. dero*. The results of the present investigation demonstrate successful induce breeding of captive reared *L. dero* at 18-22°C water temperature.

These findings would be helpful for seed production of *L. dero* which can be used for natural stock augmentation and culture of this species. Finally, it is concluded that the success in seed production would be helpful for developing *L. dero* as a new candidate species for the coldwater aquaculture practice.

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