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Induction Spawning of Endangered Kuria *Labeo, Labeo gonius* (Hamilton-Buchanan, 1822) under Hatchery System

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Abstract

Efficacy of two inducing hormones (PG and Ovaprim) was conducted on induced breeding of endangered Kalibaus (*Labeo gonius*). Induced breeding of *L. gonius* was carried out from April to July, 2022. Male and female brood fish weighing between 0.85±0.43kg and 0.88±0.50 kg in case of male and 0.97±0.641.4kg to 0.99±0.802.5kg in case of female were selected for the induced breeding and sex ratio were maintained 1:1 for the breeding purpose. The experiment was designed with the month of April, May, June and July with PG and LHRH-A. Two hormonal sources were tested to evaluate their efficacy on ovulation, fertility and hatching rate of *L. gonius* under controlled conditions. Double doses of PG (an initial dose of 1.5mg/kg body weight and final dose of 4.0mg/kg body weight) and single dose of Ovuline® (LHRH-A) (0.4 -0.45ml/kg body weight) showed better results in case of females. Bothe PG and LHRH-A had shown better results of ovulation, fertility and hatching rate of *L. gonius* in the month of May-June. Males were administered with a single dose of Ovuline® (LHRH-A) 0.15 ml/kg body weight and PG 1.5 mg/kg body weight showed better results of spermiation. Highest GSI value (22.24%) and fecundity (300411±7.16) were found in 0.90±0.80kg body weighted fish and the lowest value of GSI (20.01%) and highest fecundity (380822±9.11) were found in 1.10±0.96kg body weighted fish. The highest fertilization rates (98.40±1.17%), hatching rates (88.03±2.76%) were recorded in treatment of PG and highest fertilization rates (98.64±0.84%), hatching rates (89.55a±0.88%) were recorded in treatment of LHRH-A. This experiment recommends Ovaprim and PG both are efficient for inducing ovulation, fertilization and hatching of *L. calbasu*. The hatchery operators may be used both PG and LHRH-A for induction of spawning better performance of *L. gonius* breeding.

Keywords: PG; LHRH-A; Ovulation; Fertilization; Hatchling; GSI

Abbreviations: IUCN: International Union of Conservation of Nature; HCG: Human Chorionic Gonadotropin; GSI: Gonado Somatic Index.

Introduction

Labeo gonius, (Hamilton-Buchanan, 1822) is an indigenous cyprinid of Bangladesh, which is locally known as Goni, Kurchi, Ghannya, Ghonia or Ghainna in different places of Bangladesh [1]. It is known for its excellent taste and market value. It is a freshwater fish species belonging to the family Cyprinidae under the order Cypriniformes. It is a popular food fish having good taste, less intramuscular bones and high protein content [2]. This fish species supports an important commercial fishery in rivers and reservoirs of different countries mainly in the Indian subcontinent [3-4]. This fish species was abundantly available in our open water system of rivers, streams, haors

and beels of Bangladesh [5]. The natural populations of this fish species has seriously declined due to overfishing, habitat degradation, aquatic pollution, dam construction and several other anthropological reasons which are affecting its feeding migration and spawning [6-9]. Like tropical cyprinids, it normally breeds in streams, rivers and floodplains. However, in very recent years, due to overfishing, prolonged drought, siltation, construction of flood control measures and drainage structure, habitat degradation, aquatic pollution, dam construction, and several other anthropological reasons this species is affecting its feeding migration and spawning [6-9]. These not only destroyed the breeding grounds but also caused havoc to the availability of brood fish including fry and fingerlings of open water. Recent studies suggest that worldwide 20% of all freshwater species are extinct, endangered or vulnerable [10]. International Union of Conservation of Nature (IUCN) Bangladesh Anwarul IM, et al. [11] enlisted *L. gonius* one of the endangered minor carp in Bangladesh.

Aquaculture contributes to national income, employment generation. Artificially produced fish seed at hatchery plays a role in socio economic development and food security of Bangladesh [12,13]. Artificially produced fish seed at hatchery supports the carp's culture of the country [14,15]. Many hormonal treatments such as carp pituitary extract (PG), human chorionic gonadotropin (HCG) or different luteinizing hormones have been used to induce spawning in different fish species.

Availability of *L. gonius* in indigenous waters, culture suitability with other carp species, great market demand and high nutritional quality makes it a good table fish [16,17]. Akhtar J, et al. [18] observed the effect of two inducing agents, PG and DOM+SGnRH on the induced breeding of *L. gonius*. The effect of ovaprim on the induced breeding of *L. gonius* is not yet observed. Besides, most of the hatchery operators in our country have no clear knowledge about effective hormone and its optimum dose. Considering the above facts, the present investigation was done to study the effective dose of Pituitary gland extract and ovaprim hormone on the breeding performance of *L. gonius*.

Therefore, it is the most important time to save the species from extinction through development of appropriate breeding, nursing and rearing techniques of spawn, fry and fingerlings of *L. gonius* [1]. This technology will prevent the fish from being extinct and at the same time, the general people will have the opportunity to catch and consume this delicious fish if the culture practice is developed in both closed and open water bodies. This fish has enormous aquaculture potential and it could be easily grown in fish ponds along with other polyculture species. In order to do so, a large quantity of fry and fingerlings would be required which could be met through successful rearing of fry and fingerlings.

Materials and Methods

The experiment was conducted in the Foijuddin hatchery, Sibpur, Gouripur, Mymensingh. The induced breeding experiments were conducted during April to July 2023. The mature male and female brood fishes were caught from the rearing pond with a seine net and they were placed in the separate breeding tank. The ripe fish were selected based on physical and visual examination of the pectoral fin, abdomen and genital opening [19]. Matured brood stocks of *L. gonia* were selected based on their maturity condition. Males oozing milt on slight pressing of abdomen was selected and female with distinct bulging of abdomen with a pinkish colour with egg. Intramuscular injection was done below the dorsal fin. Twenty four female fishes divided into eight groups were injected with PG extract Figure 1 and Ovuline® (LHRH-A) Figure 1 placed in separate spawning tanks at different times.

The doses of PG extract in females at 1.0 to 2.0 mg/kg body weights were required for first injection. At the time of 2nd injection male fishes were injected with PG extract at 2.0 mg/kg body weight and female fishes were injected with PG extract at 4.0-05 mg/kg body weight. Again Hormone Ovuline® (LHRH-A) at dose 0.3-0.4 ml/kg of female spawner and 0.15 ml/kg male was administered to the selected brood.

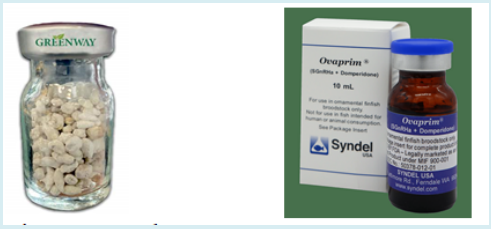

Figure 1: PG abstract and Ovuline® (LHRH-A).

Three male and three female fishes were released in the separate tank for one trail. Twelve trials (six for PG and six for Ovuline®, LHRH-A) were completed for identifying accurate hormone doses of *L. gonius* in the months of April, May, June and July. The weight of the fish ranged from 0.82kg to 0.90 kg male and 0.94kg to 1.01kg female was used in every trial. About 36 males and 36 females were used for breeding purposes. Breeding behavioral changes and spawning activities were observed up to ovulation time.

Eggs were fertilized by a dry stripping method. Female fishes were stripped to collect eggs in an enamel tray or plastic bowl. Milt from the male fish was collected by applying slight pressure on male's abdomen. The eggs and milt were mixed

thoroughly in the plastic bowl with a soft and clean feather. A few drops of water were added in the bowl and was stirred continuously for 5-6 mins. The eggs were washed several times with freshwater and swollen eggs were transferred to different hatching jars under continuous water circulating systems. The flow of water (600-800ml/min) in the jar was regulated during the incubation period. The eggs hatched out within 22 to 25 hrs at a temperature range of 26 to 31oC. After 22 to 25 hrs of fertilization, hatchlings started to come out from the egg shell and hatching was completed within 2.0 to 4.0 hrs. Unfertilized eggs and egg-shells were cleaned from the hatchling jar within an hour of hatching to protect larvae from fungal infection.

The GonadoSomatic Index (GSI) fertilization rate and hatching rate were calculated by the following formula:

$$Gonado\ Somatic\ Index\ (GSI) = \frac{Weight\ of\ gonad}{Weight\ of\ fish} \times 100$$ [20]

$$Fertilization\ rate = \frac{Number\ of\ fertilized\ eggs}{Number\ of\ total\ eggs} \times 100$$ [21]

$$Hatching\ rate = \frac{Number\ of\ hatchlings}{Number\ of\ fertilized\ eggs} \times 100$$

An early developmental stage of *L. gonia* was observed upto 68.0 to 72.0 hrs starting from egg fertilization as like *L. rohita*. The eggs were collected randomly from the hatching jar.

Boiled chicken egg yolk mixed with water and sieved through a glass nylon cloth. After hatching, the fine egg yolk emulsion was then spreaded in water to feed the hatchlings. Larvae from different pairs of parents were collected from hatching jars and released in the previously prepared different nursery ponds. The water temperature was recorded during the experimental period.

The data were analyzed by one way ANOVA using MSTAT Software (Version) followed by Duncan's Multiple Range Test to find out whether any significant difference existed among treatment means [22].

Results and Discussion

Gonadosomatic Index (GSI) and Fecundity: Estimation of gonadal maturity and spawning season of any species is possible by using the Gonadosomatic index of that species. The GSI value of *L. gonius* in this experiment was varied from 20.01to 22.40% and the obtained fecundity were varied from 300411±7.16 to 380822±9.11 (Table 1). Highest GSI value (22.40%) and fecundity (350561±7.76) were found in 0.92±0.88kg body weighted fish and the lowest value of GSI (20.01%) and fecundity (380822±9.11) were found in 1.10±0.96kg body weighted fish which is a very similar study of Chondar 1970. Mean ovarian weight of *L. gonius* was observed around 20.10% of the body weight. Mishra S, et al. [23] found that the ovarian weight was almost 20% of the body weight of full mature fishes. Reproductive cycle of the *L. gonius* is more or less similar to the study of the Mud eel, *Monopterus cuchia* [24,25].

Body wt. (kg)	Gonad wt. (g)	GSI (%)	Fecundity
0.90±0.80	200.23±1.70	22.24	300411±7.16
0.93±0.86	203.43±1.76	21.87	341544±8.19
1.10±0.96	220.08±1.66	20.01	380822±9.11
0.92±0.88	206.10±1.46	22.4	350561±7.76
0.95±0.86	200.48±1.38	21.1	351301±8.96
1.00±0.78	213.80±1.34	21.18	364110±9.24

Table 1: Gonadosomatic index and fecundity of *Labeo gonius*.

In this experience, it was found that *L. gonius* was bred in the month of April to July 2023 where May and June was the peak. Commencement of the breeding season for *L. gonius* as observed in the present investigation agrees with the report of Ahmed N [26] and Chakraborty BK, et al. [27]. Breeding of *L. gonius* was performed at an ambient water temperature of 26.0 to 28.8oC. This range of temperature is suitable for breeding of most indigenous small fishes [28]. *L. gonius* seemed to have a similar temperature requirement of Indian major carps. Male and female brood fish weighing between 0.82kg to 0.90 kg male and 0.94kg to 1.01kg female respectively, in good condition were selected for the induced breeding carried out during April to July 2023 [3].

Pertinent data regarding the time of injection and ovulation, fertilization rate, time of hatching, hatching rate and temperature are furnished in Table 2. In the present experiment, injection of pituitary extract of 2.0mg/kg body weight at first and of 4.0-4.5mg/kg body weight of second injection of the *L. gonius* showed better ovulation, fertility

and hatchability success [29-31]. In the case of male, the amount of PG required to promote spermatogenesis was found to be 1.5-2.5 mg/kg of body weight administered at the time of application of second injection to the females. Best spawning occurred in the month of mid-May to mid-June under dual hormonal regime at the PG dose of 2.0 and 4.0mg/kg body weight in the case of females.

Hormone	Months	Body weight		Doses of 1st injection (ml/kg or mg/kg)		Doses of 2nd injection ((ml/kg or mg/kg)		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr)	Hatching rate (%)	Incubation temperature (°C)
		Male (kg)	Female(kg)	Male	Female	Male	Female					
PG (Double dose)	April	0.85±0.43	0.98±0.80	-	2.0±0.01	2.0±0.02	4.5±0.05	6-8	82.02^{c}±3.02	18.0-24.0	73.22^{c}±3.86	26.2-29.4
	May	0.88±0.50	0.97±0.64	-	2.0±0.01	1.5± 0.01	4.0±0.03	5-6	98.10^{a}±1.17	18.0-22.0	88.33^{a}±2.76	
	June	0.87±0.34	0.99±0.80	-	2.0±0.01	1.5±0.01	4.0±0.02	5-6	98.44^{a}±1.02	18.0-22.0	88.80^{a} ±2.33	
	July	0.88±0.25	0.97±0.72	-	2.0±0.01	2.5±0.01	4.4±0.02	6-7	87.11^{b}±1.22	18.0-24.0	76.01^{b} ±3.03	

Table 2: Effect of different doses of hormone on the spawning of Labeo gonius.

Figures with different superscripts in the same column varied significantly (P < 0.01). In the months of early April and July, administration of PG extract in females at a dose of 2.0 and 4.5mg/kg body weight showed lower fertilization and hatching rate. Ovulation occurred after 6-8 hrs of 2nd injection and hatchings occurred after 16 to 18 hrs. of fertilization. Under the same PG doses highest fertilization and hatching rates were found to be 98.44±1.02% and 88.80±2.33%, respectively with significant differences with other doses. Thus the doses of PG have been optimized to 2.0 mg (Table 3).

Hormone	Months	Body weight		Doses of 1st injection (ml/kg or mg/kg)		Doses of 2nd injection ((ml/kg or mg/kg)		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr)	Hatching rate (%)	Incubation temperature (°C)
		Male (kg)	Female (kg)	Male	Female	Male	Female					
Ovuline® (LHRH-A)	April	0.82±0.44	0.96±0.72	-	-	0.15±0.01	0.50±0.02	6-9	81.10^{d}±2.02	18.0-24.0	72.04^{d}±1.06	26.2-28.8
	May	0.83±0.48	1±0.55	-	-	0.12±0.01	0.45 ±0.01	6-7	98.64^{a}±0.84	18.0-21.0	89.05^{a} ±0.88	
	June	0.9±0.44	1.02±0.83	-	-	0.12±0.01	0.44±0.01	6-7	97.18^{b}±1.02	18.0-22.0	86.11^{b}±1.68	
	July	0.86±0.28	0.98±0.82	-	-	0.15±0.01	0.50±0.02	6-9	87.13^{c}±1.80	18.0-24.0	77.11^{c}±2.02	

Table 3: Effect of different doses of hormone, Ovuline (LHRH-A)) on the spawning of Labeo gonius.

Figures with different superscripts in the same column varied significantly (P<0.01) and 4.0-4.5 mg/kg body weight at first and second injection, respectively, for female of *L. gonius* at an interval of 6 hrs, which was more or less similar to breeding of *Labeo rohita* and *Cirrhinus cirrhosus* Menon VR, et al. [32], *Cirrhinus reba* Hossain QZ [33] and *Puntius sarana* Chakraborty BK, et al. [27].The all fishes of *Labeo rohita* were ovulated administered with Ovaprim-C [34].

In the month of May, best spawning occurred at the dose of 0.45ml/kg body weight in case of female and 0.12ml ovaprim/kg bodyweight in case of male injected at the same time. In the month of April and July, with increase in the amount of hormone i.e. a dose of 0.50ml/kg body weight showed good fertilization and hatching rate. Ovulation occurred after 6.0-9.0 hrs of hormonal injection and hatchlings came out after 18 to 24 hrs of fertilization. In June better spawning occurred at the dose of 0.44ml/kg body weight in case of female and 0.12ml Ovulin (LHRH-A)/ kg body weight in case of male injected at the same time. Best fertilization and hatching rates were found to be at 98.64±0.96% and 89.05±0.88%, respectively in the month of 2rd week of May to 2nd week of June.

The result is in agreement with the work of Yeasmin SM, et al. [2] where all female brood fishes injected with ovaprim and the fishes were successfully spawned. The result of the current work was similar with the result found by Jamroz M,

et al. [35] when ovaprim-c was used for *L. gonius*. Naeem M, et al. [34] conducted an experiment on induced breeding of Silver carp (*Hypophthalmichthys molitrix*), where all the 30 female fishes were injected with Ovaprim-c at the rate of 0.6 ml/kg body weight and 100% ovulation were found.

Ovulin (LHRH-A) at 0.44 to 0.50 ml/kg body weight gave rise to complete ovulation of *L. gonius* in the stipulated time (6-9hr) which was very much similar to carp breeding [31]. The effective doses of Ovulin (LHRH-A) for induction of spawning have been optimized to 0.50-0.60 ml/kg body weight at single doses of injection for female of *Labeo calbas*u, which was more or less similar to breeding of *Catla catla, Labeo rohita and Cirrhinus cirrhosus* (0.40-0.50 ml, 0.30-0.40 ml and 0.25-0.30 ml/kg body weight), respectively [36]. A slightly increased amount of Ovulin (LHRH-A) as required in case of *L. gonius* seemed to be related with the species specificity phenomenon.

Better fertilization and hatching rates (98.44±1.02 and 98.64±0.84%) were found in fishes injected with PG and ovaprim. The fertilization rate and hatching rate of ovaprim treated fishes were not significantly (P>0.05) different from PG treated fish respectively with (2.0 mgPG/kg 1st dose and 4.0mgPG/kg 2nd dose; 0.45 ovaprim/kg). Behera BK, et al. [30] and Yeasmin SM, et al. [2] found that the rate of fertilization and hatching percentage are partially higher with Ovaprim at 0.45 ml/kg dose but the rates were decreased in 0.5ml/kg dose in induced breeding of common carp. Indira T, et al. [37] observed better ovulation, fertilization and hatching rates in the Indian major carp which were treated with Ovaprim than PG. Nandeesha MC, et al. [38] recommended ovaprim than PG hormone in the breeding of carps considering economic viability, farmer uses and ovulation, fertilization rate and hatching rate of carp fishes.

Labeo gonius is a seasonal breeder; it breeds in monsoon months [39-41]. The breeding season used to vary in different regions coinciding with the monsoon floods of those regions. Bhuiyan AS, et al. [42] have reported April-August as its breeding season while Kabir MA, et al. [43] have documented peak spawning in July in Bangladesh. Chakraboty BK, et al. [9] and Khan HA, et al. [44] noted that the success of induced breeding depends largely on proper selection of brood fishes, which has proved very true in the present experiment. Accomplishment of successful spawning depends on selection of suitable recipient fish at the proper stage of ovarian development and creation of congenial spawning conditions Nash CE, et al. [45] which is very accurate in the present experiment. The egg capsule and yolk sphere are yellowish brown in color. The ovulated eggs of *L. gonius* further increased around 0.2 mm in size after incubation of fertilized eggs in hatchery, which might be due to hydration of the eggs. The fertilized eggs were found in clutches among the eggs during egg incubation in the hatchery. The egg membrane got separated giving birth to the uniform perivitelline space. The yolk sphere pushed towards the vegetable pole as the embryonic development proceeded. This could be due to providing more space for the divisional activities of blastomeres at the animal pole. The clarity of blastomeres as in 2-4 cell stages was gradually reduced as the cleavage proceeded for 64 cell stages onwards. The identity of blastomeres was completely lost at the morula and blastula stage. After hatching 71-72hrs, yolk sacs were totally absorbed and the hatchlings were found to perform horizontal movement with sign of commencement of first feeding. Chicken egg yolk emulsion was fed for 200,000 hatchlings/one egg/day to meet up the dietary requirement. At that time the spawn was released in the nursery pond or sold to the nursery owner. The purpose of this was to start the alimentary canal functioning before transferring them in the nurseries (Price, 1989). The water temperature was the main key factor for ovulation and hatchling. Temperature was recorded 26.2-28.50C during the experimental period [46].

Conclusion

The findings of the present study show that both PG extract and Ovulin (LHRH-A) are equally effective in induction of spawning in *L. gonius* under controlled hatchery condition. The hatchery operators may use any of the two sources of reproductive hormones as per their choice. But considering the ease of hormone administration, cost and easy availability, both pituitary gland (PG) and Ovulin (LHRH-A) seems to be same advantageous for artificial propagation of *L. gonius*.

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