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Induced Breeding of Endangered *Labeo calbasu* (Hamilton, 1822) Under a Hatchery System

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Abstract

Experiment was conducted on the effectiveness of two inducing hormones (Ovaprim and PG) on induced breeding of *Labeo calbasu*, an endangered Kalibaus species. From April to July of 2022, *Labeo calbasu* were bred artificially. For the purpose of induced breeding, male and female brood fish weighing between 1.2 kg and 2.0 kg for the male and 1.4 kg and 2.5 kg for the female were chosen, and the sex ratio was maintained at 1:1. PG and LHRH-A were used in the experiment's design for the period of April, May, June, and July. Under controlled conditions, the impact of two hormones sources on *L. calbasu* ovulation, fertility, and hatching rate was studied. A single dose of Ovuline® (LHRH-A) (0.44 -0.50 ml/kg body weight) plus two doses of PG (an initial dose of 2.0 mg/kg body weight and a final dose of 4.0-4.5 mg/kg body weight) showed better results in this group of females. In the period of May and June, the ovulation, fertility, and hatching rate of *L. calbasu* were all better in both PG and LHRH-A. Ovuline® (LHRH-A) 0.15 ml/kg body weight and PG 2 mg/kg body weight provided as a single dose to males resulted in superior spermiation outcomes. Fish weighing 1.55±0.86 kg had the highest GSI value (22.82%) and fecundity (501301±9.96), whereas fish weighing 1.51±0.96 kg had the lowest GSI value (18.24%) and fecundity (350561±8.76). The treatment of PG showed the highest rates of fertilization (98.40±1.17%) and hatching (88.03±2.76%), whereas the treatment of LHRH-A showed the highest rates of fertilization (98.64±0.84%) and hatching (89.55a ±0.88%). According to the results of this experiment, PG and ovaprim are equally effective at causing *L. calbasu* ovulation, fertilization, and hatching. Both PG and LHRH-A could be applied by hatchery operators to enhance *L. calbasu* breeding performance by inducing better spawning.

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

Abbreviations: HCG: Human Chorionic Gonadotropin; GSI: Gonadosomatic Index.

Introduction

In India, Bangladesh, and Myanmar, *Labeo calbasu* is colloquially referred to as Calbasu/Kurcha/Kalabeinse, Kalibaus/Kalbasu, and Nga-nek-pya/Nga-noo-than/Nga-ong-tong/Nga-gyeen-boo, respectively Chondar SL [1]. After the three principal carps of India, *Labeo rohita, Catla catla,* and *Cirrhinus mrigala, L. calbasu* is the most significant species [1]. This species of freshwater fish is a member of the Cyprinidae family, which is a subfamily of the Cypriniformes order. In addition to being regarded as a good sport fish, this well-liked food fish has a good taste, few intramuscular bones, and a high protein content [2,3]. In

rivers and reservoirs throughout several nations, primarily in the Indian subcontinent, this fish species sustains a significant commercial fishery [1,4-6]. According to reports, fish has recently been introduced into India's decorative fish markets Gupta S, et al. [7] and is also being exported as native ornamental fish [8]. Due to overfishing, habitat degradation, aquatic pollution, dam construction, and other human-caused factors that affect the fish species' feeding, migrating, and spawning patterns, the species' natural populations have drastically decreased [9-11]. According to the IUCN Bangladesh 2000 Red List [12], it is listed as an endangered species in Bangladesh.

Aquaculture generates jobs and adds to the national income. Bangladesh's socioeconomic development and food security are influenced by fish seed that is artificially produced in hatcheries [13]. The nation's carp culture is maintained by artificially produced fish seed at hatcheries [14,15]. Several hormonal therapies, including human chorionic gonadotropin (HCG), carp pituitary extract (PG), and other luteinizing hormones, have been employed to stimulate spawning in several fish species.

The *Labeo calbasu* (Hamilton, 1822) is a good table fish due to its availability in native waters, adaptability for culture with other carp species, high nutritional value, and strong market demand [16,17]. The effects of two inducing agents, PG and DOM+SGnRH, on the induced breading of *L. calbasu* were studied by Akhtar J, et al. [18]. It has not yet been determined how ovaprim impacts *L. calbasu*'s induced breeding. In addition, the majority of hatchery operators in our nation lack a thorough understanding of the ideal hormone dosage. In light of the aforementioned information, the current study examined the effects of an effective dose of pituitary gland extract and ovaprim hormone on *L. calbasu* breeding performance.

There is 5.36g of oil from 65 cm of liver of *L. calbasu*. Vitamin "A" is present in its liver oil [19]. The market has a high demand for this fish. The study aims to determine the early life cycle from oocyte activation to the beginning of the fry under hatchery settings, as well as the technique of artificial propagation.

A sustainable induced breeding and nursery rearing technology can play a pivotal role in *L. calbasu* fry production for grow out culture. Artificial propagation technology of *L. calbasu* should be developed to protect this endangered important species. The goal of the current investigation was to determine how it would be to artificially breed using PG extract and LHRH-A inducing agents under controlled hatchery circumstances. Finally, the hatchery owner may use both of these PG extract and LHRH-A for artificial breeding.

Materials and Methods

The experiment was carried out in Sibpur, Gouripur, Mymensingh, at the Faizuddin Hatchery. The tests on induced breeding were place in April through July of 2021. Using a seine net, the adult male and female brood fish were removed from the raising pond and put in a separate breeding tank. The pectoral fin, belly, and genital entrance were examined physically and visually to determine which ripe fish were chosen [20]. *L. calbasu* mature brood stocks were chosen according to their maturity state. Men who oozed milk when their abdomens were lightly pressed were chosen, and women whose abdomens clearly buckled and were pinkish when they carried eggs were chosen. Below the dorsal fin, an intramuscular injection was administered. Twenty four female fishes divided into eight groups were injected with PG extract (Figure 1a) and Ovuline® (LHRH-A) (Figure 1b) placed in separate spawning tanks in different times.

The dose of PG extract in female at 1.0 to 2.0 mg/kg body weights was 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. Three male and three female fishes were released in the separate tank. Breeding behavioral changes and spawning activities were observed upto ovulation time.

Figure 1a: PG abstract.

Figure 1b: Ovuline® (LHRH-A).

Eggs were fertilized by 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 system. The flow of water (600-800 ml/min) in the jar was regulated during the incubation period. The eggs hatched out within 22 to 25 hrs at temperature range of 26 to 31oC. After 22 to 25 hrs of fertilization, hatchlings were 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 fertilization rate and hatching rate were calculated by the following formula:

[21].

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

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

An early developmental stage of *L. calbasu* was observed upto 68.0 to 72.0 hrs starting from egg fertilization. The eggs 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 pair of parents were collected from hatching jars and released in the previously prepared different nursery ponds. The water temperature was recorded during experimental period.

Statistical Analysis

The data were analyzed using one-way ANOVA with MSTAT Software (Version), followed by Duncan's Multiple Range Test to determine whether there was a significant difference between 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 Gonadosomatic index of that species. The GSI value of *L. calbasu* in this study was varied from 18.24 to 22.82% and the obtained fecundity were varied from 350561±8.76 to 501301±9.96 (Table 1). Highest GSI value (22.82%) and fecundity (350561±8.76) were found in 1.55±0.86 kg body weighted fish and the lowest value of GSI (18.24%) and fecundity (350561±8.76) were found in 1.51±0.96kg body weighted fish. Mean ovarian weight of *L. calbasu* was observed around 20.07% 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.

Body wt. (kg)	Gonad wt. (g)	GSI (%)	Fecundity
1.52±0.80	310.23±1.80	19.76	400411±8.06
1.53±0.86	345.43±1.66	21.57	441544±9.09
1.51±0.96	277.08±2.06	18.24	350822±10.01
1.50±0.88	288.10±1.26	19.34	350561±8.76
1.55±0.86	355.48±1.33	22.82	501301±9.96
1.54±0.78	329.39±1.26	21.71	484110±10.146

Table 1: Gonadosomatic index and fecundity of Kalibaus (*L. calbasu*)

L. calbasu was bred from April to July 2023, with the peak months being May and June. The start of the mating season for *L. calbasu* found in this study is consistent with the findings of Sah U, et al. [24] from Nepal. *L. calbasu* was bred at ambient water temperatures ranging from 26.0 to 28.8oC. This temperature range is ideal for growing most indigenous tiny fish [25]. *L. calbasu* appeared to have similar temperature requirements as Indian big carps. Male and female brood fish in good condition, weighing between 1.2 kg and 2.0 kg for males and 1.4 to 2.5 kg for females, were chosen for induced breeding from April to July 2023.

Table 2 contains relevant data on injection and ovulation times, fertilization rates, hatching times, hatching rates, and temperature. In the present investigation, injection of pituitary extract of 2.0mg/kg body weight at first and 3.0-5.0mg/kg body weight at second injection of *L. calbasu* resulted in enhanced ovulation, fertility, and hatchability success [26]. In the case of males, the amount of PG required to stimulate spermatogenesis was determined to be 1.5-2.5 mg/kg of body weight provided at the time of the second injection to females. Best spawning occurred between mid-May and mid-June under a dual hormonal regime with PG doses of 2.0 and 4.0mg/kg body weight in the case of females.

In the months of early April and July, treatment of PG extract to females at doses of 2.5 and 5.0mg/kg body weight resulted in reduced fertilization and hatching rates. Ovulation happened 6-8 hours after the second injection, and

hatching took place 16 to 18 hours following fertilization. The maximum fertilization and hatching rates at the same PG doses were 98.40±0.88% and 88.03±1.44%, respectively, with significant differences compared to other doses. Thus, the doses of PG were optimized to 2.0 mg and 4.0-5.0 mg/kg body weight at the first and second injections, respectively, for female *L. calbasu* at a 6-hour interval, which was more or less similar to breeding of *Labeo rohita* and *Cirrhinus cirrhosus* [27], *Cirrhinus reba* [28], and *Puntius sarana* [29].

Ovaprim-C was administered to all *Labeo rohita* fish to induce ovulation. The results are consistent with the work of Yeasmin SM, et al. [30], who injected all female brood fish with ovaprim and successfully spawned the fish. The current study yielded results similar to those obtained by Jamroz M, et al. [31] when ovaprim-c was utilized on *L. calbasu*. Naeem M, et al. [32] conducted an experiment on induced breeding of Silver carp (*Hypophthalmichthys molitrix*), in which all 30 female fish were injected with Ovaprim-c at a rate of 0.6 ml/kg body weight, and 100% ovulation was observed.

Ovulin (LHRH-A) was discovered to be a highly effective agent for induced spawning of *L. calbasu*. In the month of May, the best spawning occurred at a dose of 0.45ml/kg body weight in females and 0.15mlOvaprim/kg bodyweight in males injected simultaneously. In the months of April and July, increasing the amount of hormone, i.e. a dose of 0.50ml/kg body weight, resulted in improved fertilization and hatching rate. Ovulation occurred after 6.0-8.0 hours of hormonal injection, and hatchlings emerged between 18 and 24 hours following fertilization. In June, better spawning occurred at a dose of 0.46ml/kg body weight in females and 0.15ml Ovulin (LHRH-A)/kg body weight in males injected simultaneously. Fertilization and hatching rates were highest (98.64±0.96% and 89.55±0.88%, respectively) during the third week of May and the second week of June.

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 (g)	Female (g)	Male	Female	Male	Female					
PG (Double dose)	April	1.55±1.23	2.2±1.82	-	2.5	2	4.5	06-Aug	80.02^{c}±3.02	18.0-24.0	70.22^{c}±3.86	26.2-28.8
	May	1.6±1.50	2.08±1.64	-	2	2	4	05-Jun	98.40^{a}±1.17	18.0-22.0	88.03^{a}±2.76	
	June	1.66±1.34	2.02±1.80	-	2	2.5	4	05-Jun	98.04^{a}±1.02	18.0-22.0	87.10^{a} ±2.33	
	July	1.64±1.25	2.3±1.72	-	2.4	2.5	4.4	06-Jul	88.11^{b}±1.82	18.0-24.0	75.11^{b} ±3.33	
Ovuline® (LHRH-A)	April	1.5±1.22	2.16±1.32	-	-	0.15	0.5	06-Sep	81.10^{d}±2.02	18.0-24.0	72.03^{d}±1.06	26.2-28.8
	May	1.62±1.40	2.28±1.88	-	-	0.12	0.45	06-Jul	98.64^{a}±0.84	18.0-21.0	89.55^{a} ±0.88	
	June	1.63±1.44	2.22±1.72	-	-	0.12	0.44	06-Jul	97.88^{b}±1.02	18.0-22.0	86.01^{b}±1.68	
	July	1.68±1.55	2.4±1.82	-	-	0.15	0.5	06-Sep	87.83^{c}±1.82	18.0-24.0	78.01^{c}±2.08	

Figures with different superscripts in the same column varied significantly ($P < 0.01$).
Table 2: Effect of different doses of hormone on the spawning of *Labeo calbasu*.

Ovulin (LHRH-A) at 0.50 ml/kg body weight gave rise to complete ovulation in the stipulated time (13-14hr) which was very much similar to carp breeding [33]. 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 calbasu*, 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 Peter RE, et al. [34]. A slightly increased amount of Ovulin (LHRH-A) as required in case of *Labeo calbasu* seemed to be related with the species specificity phenomenon.

Comparatively better fertilization and hatching rates (98.64±0.84and 89.55±0.88%) were found in fishes injected with ovaprim. The fertilization rate and hatching rate of ovaprim treated fishes were not significantly ($P>0.05$) different than PG treated fish (98.40±1.17and 88.03±2.76% respectively with (2.0 mgPG/kg 1st dose and 4.0mgPG/kg 2nd dose). Yeasmin SM, et al. [30] 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. [35] observed better ovulation, fertilization and hatching rates in the Indian major carp which were treated with Ovaprim than PG. Nandeesha MC, et al. [33] recommended Ovaprim than PG hormone in the breeding of carps considering economically viability, farmer uses and ovulation, fertilization rate and hatching rate of carp fishes.

Labeo calbasu is a seasonal breeder that breeds during the monsoon season [36-39]. The breeding season used to vary among regions, coinciding with their respective monsoon floods. Khan H [40] identified July and August as the spawning months for *L. calbasu* in Punjab waters. According to Bhuiyan AS, et al. [41], the breeding season runs from April to August, while Kabir MA, et al. [42] found that peak spawning occurs in July in Bangladesh.

Khan HA, et al. [43] observed that the success of induced breeding is heavily dependent on adequate brood fish selection, which was confirmed in the current experiment. Successful spawning depends on selecting suitable recipient fish at the appropriate stage of ovarian development and creating conducive spawning conditions Nash CE, et al. [44], which is extremely correct in the current experiment. The egg capsule and yolk sphere are both yellowish brown in hue. The ovulated eggs of *L. calbasu* expanded in size by about 0.2 mm after fertilized eggs were incubated in a hatchery, which could be attributed to egg hydration. The fertilized eggs were discovered in a clutch among the eggs during egg incubation in the hatchery. The egg membrane split, resulting in a homogenous perivitelline area. The yolk sphere pushed towards the vegetable pole as embryonic development progressed. This could be owing to increased room for blastomere division at the animal pole. The purity of blastomeres in the 2-4 cell stage decreased considerably as cleavage progressed to the 64 cell stage. The individuality of blastomeres was totally lost during the morula and blastula stages. After 71-72 hours of hatching, the yolk sacs had been completely digested, and the hatchlings began to move horizontally, indicating the start of their first meal. To achieve the dietary requirement, 200,000 hatchlings were fed chicken egg yolk emulsion at one egg per day. The goal was to get the alimentary canal working before sending them to the nursery [45]. The water temperature was the most important element for ovulation and hatchling. During the experiment, temperatures ranged between 26.20-28.50 degrees Celsius [46,47].

Conclusion

A sustainable induced breeding and nursery rearing technology can play a key role in *L. calbasu* fry and fingerlings production for grow out culture. Artificial propagation and nursery technology of *L. calbasu* was developed to protect this endangered important Indian major carp. Therefore, the goal of the current study was to determine how simple it would be to artificially breed using both PG extract and LHRH-A inducing agents under controlled hatchery condition.

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