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International Journal of Oceanography & Aquaculture
ISSN: 2577-4050

Estimation of Fecundity and Artificial Propagation in a Hatchery System for *Puntius sophore* (Hamilton, 1822)

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Research Article
Volume 8 Issue 2
Received Date: May 15, 2024
Published Date: June 11, 2024
DOI: 10.23880/ijoac-16000319

Abstract

The main objectives of the present study were to identify the breeding season of Puntius sophore through observation of the Gonadosomatic index (GSI) and fecundity. GSI was calculated, and fecundity was determined by the gravimetric method. The highest value of GSI was 13.75% for the month of June. The highest fecundity (2194.03±30.80) was recorded in the month of June. The GSI values that the spawning season of Pool Barb starts from April to July, with a peak during the months of May to June. An experiment was conducted on the effectiveness of two inducing hormones (Ovaprim and PG) on the induced breeding of *P. sophore*. From April to July 2022, *P. sophore* was bred artificially. For the purpose of induced breeding, male and female brood fish weighing between 0.55 g and 0.70 g for the male and 5.44 g and 5.76 g 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 periods of April, May, June, and July. Under controlled conditions, the impact of two hormone sources on *P. sophore* ovulation, fertility, and hatching rate was identified. A single dose of Ovuline® (LHRH-A) (0.40–0.45 ml/kg body weight) plus two doses of PG (an initial dose of 2.0 mg/kg body weight and a final dose of 3.5–4.0 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 *P. sophore* were all better in both PG and LHRH-A. Ovuline® (LHRH-A) 0.12-0.14 ml/kg body weight and PG 1.2-2.0 mg/kg body weight provided as a single dose to males resulted in higher spermiation outcomes. Fish weighing 1.55±0.86 g had the highest GSI value (22.82%) and fecundity (501301±9.96), whereas fish weighing 1.51±0.96 g had the lowest GSI value (18.24%) and fecundity (350561±8.76). The treatment of PG showed the highest rates of fertilization (96.44±6.02%) and hatching (87.01±4.03%), whereas the treatment of LHRH-A showed the highest rates of fertilization (97.78±6.02%) and hatching (88.25±5.68%). According to the results of this experiment, PG and ovaprim are equally effective at causing *P. sophore* ovulation, fertilization, and hatching. Both PG and LHRH-A could be applied by the hatchery owners to enhance *P. sophore* breeding performance by inducing better spawning.

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

Abbreviation: GSI: Gonadosomatic Index.

Introduction

Puntius sophore is an important small indigenous species of Bangladesh, belonging to the family Cyprinidae of the order Cypriniformes. The common name of *P. sophore* is pool barb, spot fin swamp barb, or stigma barb, which has a wide geographical distribution in Bangladesh, India, Myanmar, Nepal, Pakistan, China, Bhutan, and Afghanistan [1,2]. The major habitats of *P. sophore* are rivers, streams, ponds, beels, floodplains, baors, and haors [3]. This fish is found in beels, ponds, rivers, floodplains, baors, haors, and everywhere in Bangladesh [4]. The fish is silvery, back gray-green to brownish, flanks with a somewhat bluish luster, and underside white [5,6]. This species matures in a year and breeds naturally in freshwater during monsoons [7]. The species has high economic value due to its nutritive status, ornamental value, and market demand for both fresh and processed products [8]. It has great importance for small-scale fishermen in Bangladesh [4,5]. Roos N, et al. [9] and Thilsted SH [10] reported that it is an important food resource and a crucial source of micronutrients that prevents malnutrition, vitamin and mineral deficiencies in rural communities, especially for vulnerable groups such as poor women and children in Bangladesh. Moreover, it has been used as an aquarium fish [11].

This species is also used to make fermented type products like Shidol. However, the abundance of *P. sophore* from natural resources is diminishing rapidly because of over exploitation and natural causes.

According to the IUCN [12], the status of freshwater fish species is threatened (25%), critically endangered (3%), endangered (12%), vulnerable (10%), near threatened (11%), least concerned (48%), and data deficient (16%). *Puntius sophore* is under the Least Concern category [12]. But 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 [13-16].

Knowledge of fecundity, the gonadosomatic index (GSI), and observations of gonadal development are important for the artificial propagation of *P. sophore*. The artificial breeding of a species is easier when knowing the gonadal development and spawning season of that species. Studies on reproductive physiology can provide important and basic information on the gonadal maturity, breeding potential, and breeding season of a species. Fecundity data as a biological parameter helps to assess the abundance, reproductive potential, and commercial potential of a fish stock. GSI helps in understanding the maturity stage and exact time of spawning [17]. Gonadal maturation represents a series of cyclic morphological changes where the gonads undergo gradual growth and ripening. Keeping in view the importance of the above biological parameters in determining reproductive success and formulating management strategies, this study will determine the fecundity, GSI, and ovarian development of P. sophore. Therefore, the target of the current study is to determine the GSI, fecundity, and artificial propagation of P. sophore. The goal of the current investigation is to determine how it would be possible to artificially breed using PG extract and LHRH-A-inducing agents under controlled hatchery circumstances.

Materials and Methods

Stocking and Collection of Brood

The fish were collected from the grow-out ponds at the Faizuddin Hatchery in Sibpur, Gouripur, and Mymensingh at a 15-day interval throughout the research period. The fish were sampled with a cast net.

At least 10 fish were captured, and live specimens were collected. The normal expected sex ratio of the *P. sarana* population was determined from the data of the gonadal sex differentiation study. The general morphology of gonads was studied, as well as the month-wise size, shape, and colour of male and female gonads during sample collection and preservation.

Gonadosomatic Index (GSI)

A gonadosomatic index is often used to determine the reproductive cycle of fish. The GSI is the measure of the relative weight of the gonad with respect to its total or somatic weight. The GSI increased with the maturation of fish and reached its maximum at the peak period of maturity. The GSI was calculated using the following formula [17]:

$$\mathrm{GSI} = \left(\text{Gonad weight} / \text{Body weight}\right) \times 100$$

Estimation of Fecundity

The gravimetric method [17] was used for the estimation of the fecundity of *P. sophore*. In this method, the ovaries were dissected out by a pair of scissors. The external connective tissues were removed from the surface of each pair of ovaries. The moisture of the ovaries was detached with the help of a blotting paper. The weight of the ovaries of each fish was documented with the help of a digital electronic balance. Then the ovarian part was taken distinctly from the anterior, middle, and posterior portions of each ovarian lobe. The number of mature and maturing eggs in each portion was found separately by actual counting. The mean number of eggs was determined and then multiplied by the total weight

of the ovary, which gave the total number of eggs, that is, the fecundity of the respective fish. This was done using the following formula:

$$F = N \times \text{Gonad weight}(g) / \text{Sample weight}(g)$$

where, F = Fecundity of fish; N = Number of eggs in the sample.

Induced Breeding

Induced breeding of *P. sophore* was designed in the months of April to July of 2023. The mature male and female brood fish were collected from the raising pond using a seine net and placed in a different breeding tank. The pectoral fin, belly, and genital entrance were examined physically and visually to determine which ripe fish were chosen [18-21]. The mature *P. shapore* broods were chosen according to their maturity state. Males who oozed milk when their abdomens were lightly pressed were chosen, and females whose abdomens clearly buckled and were pinkish when they carried eggs were chosen. Below the dorsal fin, an intramuscular hormone injection was administered. Twenty four female fish, divided into eight groups, were injected with PG extract and Ovuline® (LHRH-A) [19]. The fish are placed in separate spawning tanks at different times.

The dose of PG extract (Figure 1a) in females at 1.0 to 2.0 mg/kg body weight was required for the first injection. At the time of the second injection, male fish were injected with PG extract at 1.5 to 2.0 mg/kg body weight, and female fish were injected with PG extract at 3.5–4.5 mg/kg body weight. Again, Hormone Ovuline® (LHRH-A, Figure 1b) at doses of 0.2–0.4 ml/kg for female spawners and 0.15 ml/kg for males was administered. Three male and three female fish were released in a separate tank. Breeding behavior and spawning activities were observed up to the ovulation period.

Figure 1a: PG abstract.

Figure 1b: Ovuline® (LHRH-A).

The eggs were fertilized by the dry stripping method. Female fish were stripped to collect eggs in a plastic bowl. Milt from the male fish was collected by applying slight pressure to the abdomen. The eggs and milt were thoroughly mixed in the plastic bowl with a soft and clean feather.

A few drops of water were added to the bowl, which was stirred continuously for 4-5 minutes. The eggs were washed several times with freshwater, and the swollen eggs were transferred to different hatching jars under a continuous water circulation system. The flow of water (600–800 ml/min) in the jar was maintained during the incubation period. The eggs hatched out within 21 to 24 hours at a temperature range of 26 to 30°C. After 21 to 24 hours of fertilization, hatchlings started to come out of the egg shell, and hatching was completed within 2.0 to 4.0 hours. Unfertilized eggs and eggshells were removed 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 [22]:

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

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

An early developmental stage of *P. sophore* was observed up to 66.0 to 71.0 hrs starting from egg fertilization. Boiled chicken egg yolk was mixed with water and sieved through a glass nylon cloth. After hatching, the fine egg yolk emulsion was then spread in water to feed the hatchlings. Larvae from different pairs of parents were collected from hatching jars and released in the previously prepared nursery ponds. The water temperature was recorded during the experimental period.

Statistical Analysis

A one-way ANOVA with MSTAT Software (version) was used to evaluate the data, and Duncan's Multiple Range Test was then used to determine if there was a significant difference between treatments means [23].

Results and Discussion

Gonadosomatic Index (GSI) and Fecundity

An estimation of the gonadal maturity and spawning season of any fish species is possible by using the gonadosomatic index of that species. The GSI values of male and female *P. sophore* in this study varied from 0.10% to 0.34% and 10.05% to 14.30%, and the obtained fecundity varied from 2004.01±9.51 to 2218.41±23.02 (Table 1 & Figure 2). The highest GSI value (14.30%) and fecundity

(2218.41±23.02) were found in 5.66±0.06gm body weighted fish in the month of June, and the lowest values of GSI (10.05%) and fecundity (2004.01±9.51) were found in 4.38±0.07gm body weighted fish (Table 1 & Figure 2). The highest ovarian weight of P. sophore was observed around 0.81±1.01 g of body weight [21,24].

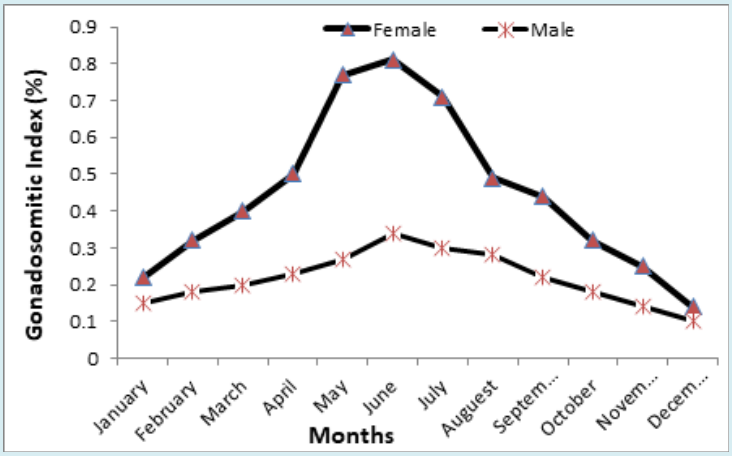

Figure 2: Gonadosomatic index of *Putius sophore*.

P. sophore was bred from April to July 2023, with the peak months being May and June. The start of the mating season for *P. sophore* found in this study is consistent with the findings of Hasan T, et al. [25]. *P. sophore* was bred at ambient water temperatures ranging from 26.0 to 28.5 °C.

Body Length (cm)	Body wt. (gm)	Gonad wt. (gm)	GSI (%)	Fecundity (# Number)
4.0-4.5	4.38±0.07	0.44±0.04	10.05	2004.01±9.51
4.6-5.0	4.40±0.03	0.49±0.05	11.14	2141.66±10.18
5.1-5.5	5.03±0.05	0.53±0.06	10.73	2159.30±11.55
5.6-6.0	5.22±0.04	0.58±0.07	11.11	2288.80±12.22
6.1-6.5	5.44±0.07	0.63±0.08	11.58	2304.44±20.34
6.6-7.0	5.53±0.04	0.71±0.08	12.84	2119.18±28.06
7.1-7.5	5.60±0.05	0.77±0.09	13.75	2194.03±30.80
7.6-8.0	5.66±0.06	0.81±1.01	14.3	2218.41±23.02

Table 1: Gonadosomatic index and fecundity of female *Puntius sophore*.

This temperature range is suitable for growing most indigenous small fish [26]. *P. sophore* appeared to have similar temperature requirements as minor 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 a pituitary extract of 2.0 mg/kg body weight at first and 3.0-4.0 mg/kg body weight at second injection of *P. sophorer* resulted in enhanced ovulation, fertility, and hatchability success [27].

In the case of males, the amount of PG required to stimulate spermatogenesis was determined to be 1.2–2.0 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 4.0 mg/kg body weight in the case of females.

In the months of early April and July, treatment of females with PG extract at doses of 2.0 and 4.0 mg/kg body weight resulted in reduced fertilization and hatching rates. Ovulation happened 6–8 hours after the second injection, and hatching took place 18-22 hours following fertilization. The maximum fertilization and hatching rates at the same

PG doses were 96.44±6.02% and 87.01±4.03%, respectively, with significant differences compared to other doses. Thus, the doses of PG were optimized to 2.0 mg and 3.5–4.0 mg/kg body weight at the first and second injections, respectively, for female *P. sophore* at a 6-hour interval, which was more or less similar to breeding of *Labeo calbasu*, *Labeo rohita*, *Cirrhinus cirrhosus*, *Cirrhinus reba*, and *Puntius sarana* [21,28-31].

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 (mg)	Female (mg)	Male	Female	Male	Female					
PG (Double dose)	April	0.55±0.03	5.53±0.04	-	2	2	4	7-Jun	80.12^{c}±4.02	18.0-24.0	70.11^{c}±3.66	26.2-28.2
	May	0.6±0.04	5.6±0.05	-	1.6	2	3.5	6-May	96.04^{a}±5.17	18.0-22.0	86.88^{a}±4.06	
	June	0.66±0.04	5.44±0.07	-	2	2	3.5	6-May	96.44^{a}±6.02	18.0-22.0	87.01^{a} ±4.03	
	July	0.64±0.05	5.66±0.06	-	1.2	2	4	6-6.5	88.01^{b}±5.82	18.0-24.0	74.31^{b} ±4.38	
Ovuline® (LHRH-A)	April	0.6±0.04	5.56±0.05	-	-	0.14	0.45	10-Aug	81.22^{c}±5.02	18.0-24.0	71.07^{d}±5.06	26.2-28.5
	May	0.65±0.05	5.66±0.06	-	-	0.12	0.44	10-Aug	97.64^{a}±6.84	18.0-21.0	86.61^{b} ±4.88	
	June	0.68±0.05	5.64±0.07	-	-	0.12	0.4	10-Aug	97.78^{a}±6.02	18.0-22.0	88.25^{a} ±5.68	
	July	0.7±0.06	5.76±0.08	-	-	0.14	0.45	10-Aug	85.83^{b}±7.82	18.0-24.0	77.51^{c}±6.08	

Table 2: Effect of different doses of hormone on the spawning of *Puntius sophore*.

Figures with different superscripts in the same column varied significantly ($P < 0.01$).

Ovaprim-C was administered to all *P. sophore* for inducing ovulation. The results are consistent with the work of Yeasmin SM, et al. [32], who injected all female brood fish with ovaprim and successfully spawned the fish. The current study yielded results similar to those obtained by Chakraborty BK, et al. [19] and Jamroz M, et al. [33] when ovaprim-c was utilized on *P. sophore*. Chakraborty BK, et al. [28] and Naeem M, et al. [34] conducted an experiment on induced breeding of *Labeo calbasu* and *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 the induced spawning of *P. sophore* (Table 2). In the month of May, the best spawning occurred at a dose of 0.44 ml/kg body weight in females and 0.12 ml/kg body weight in males injected simultaneously. In the months of April and July, increasing the amount of hormone, i.e., a dose of 0.45 ml/kg body weight, resulted in improved fertilization and hatching rates. 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 0.40 mL/kg body weight in females and 0.12 mL of ovulin (LHRH-A)/kg body weight in males injected simultaneously. Fertilization and hatching rates were highest (97.78±6.02% and 88.25±5.68%) during the first and second weeks of June.

Ovulin (LHRH-A) at 0.50 ml/kg body weight gave rise to complete ovulation in the stipulated time (08–10 hr), which was very similar to carp breeding [35]. The effective doses of ovulin (LHRH-A) for induction of spawning have been optimized to 0.40-0.45 ml/kg body weight at single doses of injection for females of *P. sophore*, which was more or less similar to breeding of *Catla catla*, *Labeo rohita*, *Cirrhinus cirrhosus*, and *Puntius sarana*, respectively [19,36,37].

Comparatively better fertilization and hatching rates (97.78±6.02 and 88.25±5.68) were found in fish injected with ovaprim. The fertilization rate and hatching rate of ovaprim-treated fish were not significantly ($P > 0.05$) different than PG-treated fish (96.44±6.02 and 87.01±4.03%), respectively, with 2.0 mg PG/kg 1st dose and 3.5 mg PG/kg 2nd dose. Yeasmin SM, et al. [32] found that the rate of fertilization and hatching percentage are partially higher with Ovaprim, but the rate of dose was decreased in induced breeding of common carp. Indira T, et al. [38] observed better ovulation, fertilization, and hatching rates in the Indian major carp that were treated with Ovaprim than PG.

Nandeesha MC, et al. [35] recommended Ovaprim over PG hormone in the breeding of carps, considering economically

viability, farmer uses and ovulation, fertilization rate, and hatching rate of carp fish.

Sophore is a seasonal breeder that breeds during the monsoon season [6,39-41]. The breeding season used to vary among regions, coinciding with their respective monsoon floods. Khan H [42] identified July and August as the spawning months for minor carp in Punjab's waters. According to Bhuiyan AS, et al. [43], the breeding season of minor carp and Puntius species runs from April to August, while Kabir MA, et al. [44] found that peak spawning occurs in June to July in Bangladesh.

The success of induced breeding is heavily dependent on adequate brood fish selection, which was confirmed in the current experiment [45]. Successful spawning depends on selecting suitable recipient fish at the appropriate stage of ovarian development and creating conducive spawning conditions, which is very similar to the current experiment [46]. The egg capsule and yolk sphere are both yellowish-brown in hue. The ovulated eggs of *P. sophore* expanded in size by about 0.15 mm after fertilized eggs were incubated in a hatchery, which could be attributed to egg hydration. The fertilized eggs were discovered in a Petridis 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 due 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 the blastomere was totally lost during the morula and blastula stages. After 68-72 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 [47]. The water temperature was the most important element for ovulation and hatchling. During the experiment, temperatures ranged between 26.2-28.5 degrees Celsius.

Conclusion

Study of fecundity, sustainable artificial propagation and nursery rearing technology will play a key role in *P. sophore* fry and fingerling production for growout culture. Artificial propagation and nursery rearing for *P. sophore* are being developed to protect from endangered status. 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 conditions to protect this species from extinction. The results of this experiment, PG and ovaprim are equally effective at causing *P. sophore* ovulation, fertilization, and hatching. So, the hatchery owners of Bangladesh can use both PG extract and LHRH-A inducing agents for artificial propagation.

Acknowledgements

The author acknowledge to Foijuddin Hatchery, Sibpur, Gouripur, Mymensingh for financial help and research facilities.

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