Original Research Article

Development of induced breeding technique for freshwater fish gobi, *Glossogobius giuris* (Hamilton, 1822) using pituitary gland (PG) extract

ABSTRACT

An attempt was made to establish induced breeding technique of freshwater fish gobi (*Glossogobius giuris)*, using the pituitary gland (PG) extract. Six experiments were conducted at the Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. First breeding trial was conducted in June using 40, 45, and 50 mg PG kg^{-1} body weight of the female fish. None of the fish was ovulated in the 1^{st} trial. Two more breeding trials were conducted in July and August, using 6 mg (T_1), 8 mg (T_2) and 10 mg (T_3) PG kg^{-1} body weight of female, and 3 mg (T_1), 4 mg (T_2) and 5 mg (T_3) PG kg^{-1} body weight of male fish. The single dose of 4 mg kg^{-1} body weight of PG was found to be effective for male fish in both months. The ovulation rates were recorded as 56.33±1.53, 82.67±2.52 and 75.33±1.53% in July and 58.00±2.65, 94.67±1.53 and 78.33±1.53% in August under the treatments T_1, T_2 and T_3, respectively. Hatching rates of eggs were observed as 52.00±4.36, 81.67±3.21 and 72.33±6.03% in July, and 54.67±3.23, 91.67±3.06 and 73.67±5.13% in August under the T_1, T_2 and T_3, respectively. Hatching time was ranged from 35 to 48 h and after the absorption of yolk sac (60-72 h), they were survived well when fed with tubificid worms and mixed zooplankton. The female treated with the dose of 8 mg PG kg^{-1} body weight in August showed the best performance so far as the ovulation, fertilization and hatching rates were concerned. The findings obtained from the present study reveals that induced breeding of *G. giuris*, using PG extract is successful for large scale production of quality seed in captive condition, which would further facilitate towards the aquaculture production and biodiversity conservation of this important fish species to a greater extent.

Keywords: Glossogobius giuris; PG doses; induced breeding; ovulation; fertilization; hatching.

1. INTRODUCTION

The freshwater gobi, *Glossogobius giuris* (Hamilton, 1822), locally known as baila or belia or bele fish, is belonging to the family Gobiidae of the order Perciformes. Under the family Gobiidae, 4 sub-families like Oxudercinae, Amblyopinae, Sicydiinae, Gobionellinae and Gobiinae are recognized. It contains 212 genera and 1875 species. The gobi (*G. giuris*) is native to fresh, marine and brackish water from the Red sea and East Africa through South Asia and the Indian Ocean to China, Australia and the islands of the Pacific Ocean. The populations of *G. giuris* are distributed mostly in tropical and subtropical areas. It is commonly found in estuarine areas and freshwater throughout Bangladesh, the Punjab, Ceylon, India, Myanmar, Malaysia and the Far East [1,2]. The fish is also available in freshwater haors, baors, beels, ponds, swamps, rivers and estuaries of Bangladesh [3]. This species is not yet found in IUCN Red list.

Glossogobius giuris, one of the small indigenous species (SIS), has a special preference in the diet of people of Bangladesh because of its special taste, low fat and high protein content [4]. It is generally found in canals, ditches and ponds and clear to turbid streams with rock, gravel or sand bottoms. *Glossogobius giuris* is the only species of genus *Glossogobius* available in Bangladesh [5]. The largest fish has been found to reach a length of 40 cm (standard length) and the smallest about 3 cm [6]. At times 2-3 adults of the species make a kilogram and normally 10-20 adult make a kilogram yet one should not be astonished if he finds 50-150 adult individuals fail to make a kilogram. The male and female are known to attain its first maturity at 11.4 and 10.3 cm length, respectively [7]. This species is very essential food fish, especially to the low-middle class and poor people, because of being relatively cheaper but sometimes very expensive to them [8]. Different kinds of delicious food items like “jhuri” can be made with the eggs of gobi (*G. giuris*) fish. Worldwide gobies are considered as a delicacy and precious food in some countries like Italy, India, Burma, Nepal and France [9]. The fish is highly esteemed as food and the price of larger fish is comparatively high than smaller ones. The price of large fresh fish is around Tk. 700-800 kg^{-1}. The majority of gobi fishes are being small that constitute a small fishery in Bangladesh but *G. giuris* which attains about a foot in length, is notable as it forms a fishery of some magnitude in the southern part of the country [8]. About 80% people of Bangladesh are poor and they depend on SIS (small indigenous species) for their daily supply of animal protein because they are available at reasonable price. Ahmed et al. [10] found high protein and low fat in the flesh of bele and considered as a very important food fish for containing all the nutrients. It has been treated as a minor commercial species for capture fishery [11]. Gobi contributes 1.93% of the total catch from Rajdhala beel in Netrakona district of Bangladesh as capture fishery and 1.09% in an average of the total capture from the river Padma [8,12]. However, there is no record on the total catch estimation of *G. giuris*.

As a commercially important fish, the production of *G. giuris* may be increased by culturing in the ponds. In Bangladesh, there are suitable waterbodies for culturing this species with other fishes or can be cultured as a single species in shallow waterbodies and will be exported as a delicious expensive fish. The natural environment of Bangladesh is also very favourable for its culture in

captivity. So, it is a suitable candidate for aquaculture as commercial farming. Before bringing this fish in aquaculture, it is needed to gather information about food and feeding behavior, reproductive biology, fecundity, breeding behaviour and season of this fish as well as to develop induced breeding technique for ensuring stockable-sized quality seeds. However, no systematic research work has so far been undertaken in Bangladesh on the induced breeding of gobi (*G. giuris*). Therefore, the present study has been carried out to find out the appropriate induced breeding technique for producing quality seeds. Thus, the present research bears practical importance and the findings may immensely be helpful for mass production of quality seeds of this species, which will not only facilitate to aquaculture production but also will save this tasty species from probable threat of extinction.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out in the Mini Hatchery and Breeding Complex of the Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh and the Government Fish Seed Multiplication Farm, Department of Fisheries, Shambhuganj, Mymensingh, Bangladesh.

2.2 The experimental fish

The sexually matured broods of gobi (*G. giuris*) was collected from the Mithamoin haor under Kishoreganj district of Bangladesh and stocked in the ponds for domestication, and were used for breeding purposes. Ready-to-spawn broods were selected and divided into different treatments having 6 females and 6 males in each treatment. All the fish under different treatments were treated with different PG doses.

2.3 Brood fish selection

A day before the breeding trial, good quality and healthy broods of *G. giuris* were caught randomly by reducing the water content from ponds. A total of 36 healthy, good and sexually matured male and female broods (18 males and 18 females) were selected and kept in 3 cisterns for breeding purpose. Selection and proper identification of brood fish is a vital step in any induced breeding technique. Identification of male and female broods was done on the basis of some external features known as secondary sexual characteristics and are shown in Table 1 and Figure 1. The mature females could easily be identified by their swollen abdomen and round or oval and swollen urogenital papillae. On the other hand, the mature males were identified by their flat abdomens and long protruded genital papillae.

Table 1. Secondary sexual characteristics used to select mature broods of gobi (*G. giuris*)

Characters	Female	Male
Body size	Smaller than male	Relatively larger than female
Pelvic fins during breeding season	Yellowish in colour	Black-yellowish in colour
Genital papilla	Short, fleshy, oval-shaped and prominent urogenital papillae	Sharp, straight and protruded urogenital papillae
Abdomen	Swollen, bulky and soft abdomen	Flat and thin abdomen, not bulky like female

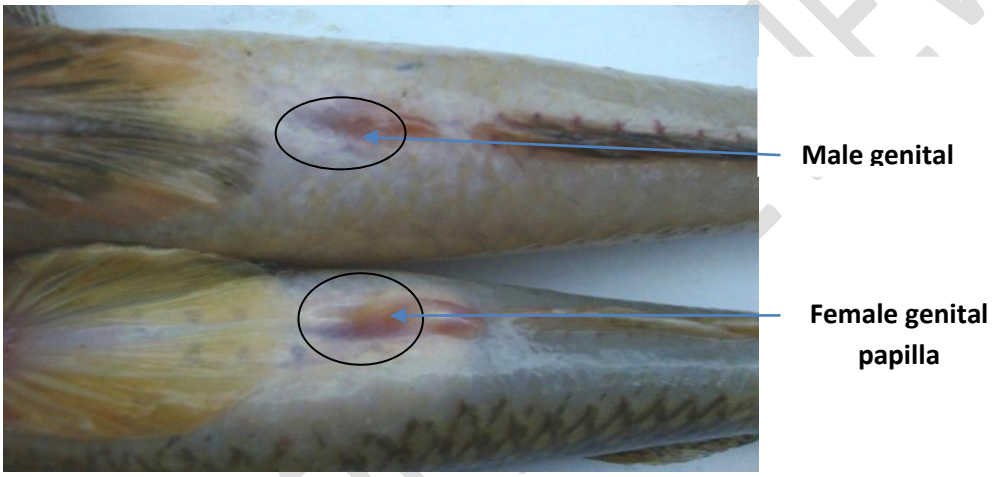

Fig. 1. Genital papillae of gravid male and female gobi (*G. giuris*)

2.4 Conditioning the brood fish

Selected female and male broods were weighed and kept in separate cisterns for 8-10 h for conditioning before treated with carp PG extract. During conditioning, continuous water flow was provided for aeration to ensure sufficient dissolved oxygen to the stocked brood fishes through porous PVC pipe, which was placed on the top of each cistern.

2.5 Experimental design

Three trials were conducted during the months of June, July and August to optimize PG doses for the induced breeding of *G. giuris*. In the first trial, nine females were divided into three treatments and designated as T_1, T_2, and T_3, having three females in each treatment. The females under each treatment were set-up under three replications indicated as R_1, R_2 and R_3 and kept in separate cisterns. The females under T_1, T_2, and T_3 were then treated with PG at the dose of 40, 45, and 50 mg kg^{-1} body weight, respectively during the month of June. Another two trials were conducted using 6 mg (T_1), 8 mg (T_2) and 10 mg (T_3) PG kg^{-1} body weight of female fish to develop induced breeding technique of *G. giuris* in July and August. In case of males, the PG doses of 3, 4, and 5 mg kg^{-1} body weight were used in T_1, T_2, and T_3, respectively.

2.6 Induction of ovulation

2.6.1 Source of pituitary gland (PG) and preparation of PG extract

For induction of ovulation, freshly prepared extract of commercially available dry carp pituitary glands (PG, available in the market) was used. To prepare the extract for injection, the required amount of PG was carefully weighed using an electronic balance. The required amount and volume of PG was calculated on the basis of body weight of all the fish of a particular treatment to be injected on a particular day using the standard formulae followed by Mollah et al. [13] as below:

$$\text{Weight (mg) of required amount of PG } (W_t) = W_b \times P_t$$

Where, W_b represents the total body weight (kg) of all fishes to be injected; and P_t represents the rate in mg of PG to be injected kg^{-1} body weight under a particular treatment.

The total volume of the extract required was calculated by the following formula:

$$\text{Vol. of extract (ml)} = W_t \times 1.0$$

Where, W_t represents the weight of PG (mg); and 1.0 represents the volume of the extract in ml to be injected kg^{-1} body weight of fish.

The weighed PG was homogenized with a small volume of distilled water and the homogenate was carefully transferred to a centrifuge tube by using distilled water to ensure complete transfer. The mixture was centrifuged for 6 min at 6000 rpm. The clear supernatant was transferred to a vial and was made pre-determined volume with distilled water. Based on the body weight of the gravid male and female, the required volume of PG extract was loaded in a graduated 1.5 ml hypodermic syringe.

2.6.2 Injecting the PG extract to the experimental fish

The selected broods of gobi fish were carefully put on a foam/sponge for injecting the PG extract. After that they were wrapped with a soft and soaked cloth and then the extract was injected intramuscularly to the fish under the pectoral fin (Figure 2). The dose was divided into two volumes (1st dose with 30% PG and 2nd dose with 70% PG) and injected to the females at 6 h interval. At 6-12 h post-injection, the abdomen of female was found to be swollen and soft (Figure 3). All the males were given a single dose during the 2nd injection to females. The physical injury and physiological pressure during harvesting, handling, transporting, injecting and holding the brood fish have harmful effects on spawning success. So, during handling, utmost care was taken and optimum water condition was maintained to minimize all kinds of stress.

Fig. 2. Injecting PG extract to the female gobi (*G. giuris*)

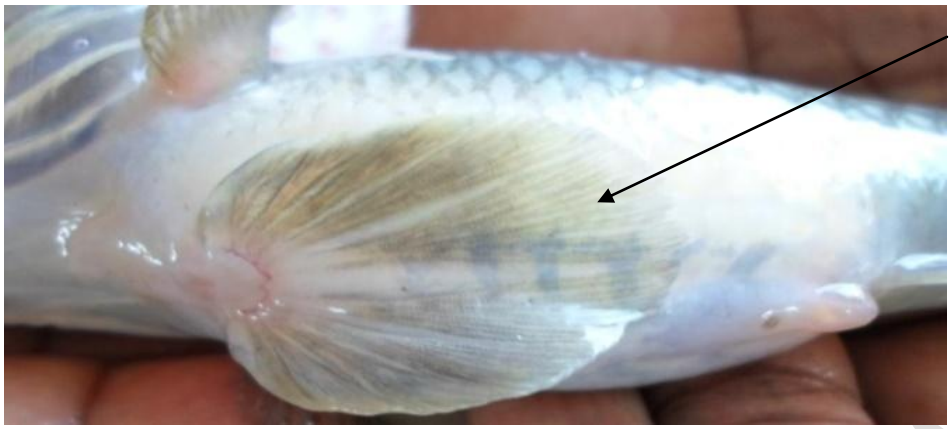

Fig. 3. Soft and swollen abdomen of female gobi (*G. giuris*) after PG injection

2.6.3 Ovulation, collection of eggs and fertilization with milt

After injection of PG, the males and females under each treatment were kept in different cisterns for stripping; the female was kept under observation to monitor if they exhibit any change in behaviour. During this period, a very close observation was done to see whether they exhibit pairing or courtship behaviour.

The females were checked every hour after 8 h of first injection by gently pressing their abdomen to ascertain the ovulation. A fish was considered to be ovulated when there were extrusions of a few eggs upon gentle pressure on the abdomen from anterior to posterior direction. The females upon ovulation were immediately stripped and eggs from each fish were collected in separate fertilization trays or petri dishes (Figure 4).

The male could not be stripped for the collection of milt. So, the testes from respective male were dissected out from its body cavity and were macerated in 0.85% NaCl solution. To affect and ensure fertilization, the sperm suspension was mixed with eggs by gently stirring with a feather (Figure 5) and water was added to the egg-sperm mixture to activate the sperms for fertilizing the eggs. Fertilized eggs were then washed several times with clean water to remove the excess milt, blood etc.

Fig. 4. Stripping of an ovulated female gobi (*G. giuris*)

Fig. 5. Fertilization processes of eggs with sperms of gobi (*G. giuris*)

2.7 Incubation and hatching of the fertilized eggs

The fertilized eggs of *G. giuris* were transferred to separate plastic bowls and trays for incubation. All the incubation bowls and trays received gentle shower through porous PVC pipes to ensure adequate aeration. When the fertilized eggs came in contact with water, they were started to swell and became stickier. Dead eggs were removed after every 3 h and their number was carefully recorded. Upon completion of hatching, the number of hatchlings was also counted and recorded.

2.8 Determination of fertilization and hatching rates

A batch of approximately 100 fertilized eggs was placed in each of 3 bowls of 1.25 L capacity to determine the fertilization and hatching rates of *G. giuris*. Soon after fertilization, the embryonic development started and the fertilized eggs assumed watery appearance or slightly transparent colour, while the unfertilized ones turned whitish and opaque as the time passes. Within 6 h of incubation, the numbers of fertilized and unfertilized eggs from each bowl were counted based on the colour and appearance of the eggs. The fertilized eggs began to change its size and colour from yellowish to watery and became transparent, while the unfertilized eggs turned opaque and whitish in colour. After completion of hatching, the numbers of larvae of each bowl were counted.

2.9 Indices of effectiveness of PG dose

The following parameters such as percent ovulation, percent fertilization and percent hatching were recorded as indices of the effectiveness of different PG doses:

Percent ovulation was calculated according to the formula used by Mollah et al. [13] as follows:

$$\text{Ovulation (\%)} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \text{ X } 100$$

Percentages of fertilization and hatching were calculated using the formulae followed by Mollah et al. [13] as below:

$$\text{Fertilization (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs (fertilized + unfertilized)}} \times 100$$

$$\text{Hatching (\%)} = \frac{\text{No. of hatchlings}}{\text{Total no. of fertilized eggs}} \times 100$$

For calculating the percent fertilization, a number of egg samples (about 50 eggs) were taken from each group and number of fertilized and unfertilized eggs was counted based on the change of colour and size of the eggs under a microscope (NOVEX-Holland).

2.10 Data analysis

Several indices of effectiveness of PG dose were analyzed by MS Excel computer package as descriptive values such as mean and percentage. The statistical data analysis was carried out with the aid of the computer software SPSS version 11.5.

3. RESULTS

Three trials were conducted for induced breeding of gobi (*G. giuris*) with different doses of PG extract during the months of May and June to August 2013, the detailed results of which are described below:

3.1 Optimization of PG doses

In order to standardize the PG dose for inducing ovulation in female *G. giuris*, three different doses of carp PG extract were used. Data representing the effects of PG doses on ovulation of female fish, and the rates of fertilization and hatching of eggs are presented in Table 2. For standardizing PG dose in female *G. giuris*, three different doses of PG viz., 40, 45 and 50 mg kg^{-1} body weight were used in treatments T_1, T_2, and T_3, respectively during the month of June. Two more breeding trials were conducted using 6 mg (T_1), 8 mg (T_2) and 10 mg (T_3) PG kg^{-1} body weight of fish in July and August. Data representing the effects of the PG doses on ovulation of female fish, and the rates of fertilization and hatching of eggs are presented in Table 2.

3.2 Trial 1

For standardizing PG dose in female gobi (*G. giuris*), three different doses of PG viz., 40, 45 and 50 mg kg^{-1} body weight were used in T_1, T_2 and T_3, respectively during the month of June. But no ovulation was occurred under this trial.

3.3 Trial 2 and 3

Two more trials conducted in July and August showed remarkable differences in effectiveness of three different doses of PG in inducing ovulation. Overall breeding activity including average ovulation, fertilization and hatching rates in all the trials are shown in Table 2 and Figure 6.

3.3.1 Ovulation rate

Ovulation rate showed marked differences in effectiveness among the three doses with months in inducing ovulation. Females did not respond with PG doses of 40, 45 and 50 mg kg^{-1} body weight in the

treatments T_1, T_2 and T_3, respectively during the month of June. However, another two breeding trials were conducted in July and August using 6 mg (T_1), 8 mg (T_2) and 10 mg (T_3) PG kg^{-1} body weight of female and 3 mg (T_1), 4 mg (T_2) and 5 mg (T_3) PG kg^{-1} body weight of male fish, respectively. Among the different treatments, average ovulation rates were recorded to be 56.33±1.53, 82.67±2.52 and 75.33±1.53% in July and 58.00±2.65, 94.67±1.53 and 78.33±1.53% in August under the treatments T_1, T_2 and T_3, respectively. The highest ovulation was observed as 94.67±1.53% in August under T_2, while the lowest value was recorded as 56.33±1.53% in July under T_1. However, ovulation rate under T_2 was significantly higher (P<0.05) than those of T_3 and T_1 in both July and August (Table 2 and Figure 6). Average latency periods were recorded as 25-35 h in July and August. In case of male fish, the PG dose (4 mg kg^{-1} body weight of fish) was found to be satisfactory in both months.

3.3.2 Fertilization rate

Average fertilization rates of eggs were recorded to be 51.33±1.54, 83.33±3.79 and 70.33±3.51% in July; 53.67.00±4.04, 92.33±2.52 and 74.00±3.61% in August at 6, 8 and 10 mg PG kg^{-1} body weight under the treatments T_1, T_2 and T_3, respectively. The highest fertilization was recorded as 92.33±2.52% in August under T_2 whereas the lowest value was recorded as 51.33±1.54% in July under T_1 (Table 2 and Figure 6). However, fertilization rate under T_2 was significantly higher than that of T_1 (P<0.05) in both July and August.

The embryonic period starts when the egg is fertilized by the sperm and involves a constant synthesis or the building up of those elements that are vital to the normal process in the development of individual. The eggs of *G. giuris* are dissimilar to those of many other fish species so far as the shape is concerned. Soon after fertilization, the eggs were swelled, extended longitudinally, and became adhesive and light brownish to transparent in colour. The swollen and long eggs were attached on some broken parts of earthen pots, which were used as shelters. The unfertilized eggs were whitish in colour and demersal, and also did not change in size and shape. The length of eggs just after fertilization was increased from 0.6 to 3.0 mm and the width from 0.3 to 0.5 mm, having tendency to attach on substrate or shelters provided. The development of *G. giuris* embryos occurred after 15 h post-fertilization at 27-29^{0}C.

3.3.3 Hatching rate

Average hatching rate of eggs were observed to be 52.00±4.36, 81.67±3.21 and 72.33±6.03% in July; 54.67±3.23, 91.67±3.06 and 73.67±5.13% in August under the treatments T_1, T_2 and T_3, respectively. The highest hatching was observed as 91.67±3.06% in August under T_2 and the lowest was recorded as 52.00±4.36% in July under T_1 (Table 2 and Figure 6). The hatching rate was significantly (P<0.05) higher in T_2 than those in all other treatments. Hatching time was ranged from 35 to 48 h in all treatments. Just after hatching, the larvae moved horizontally near the bottom. After 24-36 h, larvae were moved vertically from bottom close to the water surface and back quickly to the bottom again. Yolk sac absorption of the larvae was started after 40-48 h of hatching. Finally, after 48-72 h of hatching, larvae began to swim freely on a horizontal position.

Table 2. Effects of different PG doses on the ovulation response of female gobi *(G. giuris)*, and fertilization and hatching success of eggs

Trial and Month	Treatment	Weight of brood fish (g)		Dose of PG (mg kg^{-1})		Latency period (h)	Ovulation rate (%)	Fertilization rate (%)	Incubation period (h)	Hatching rate (%)
		Female	Male	Female	Male					
Trial 1 (June)	T_1	146.6±9.34	284.25±8.26	40	20	-	0	0	-	No ovulation occurred
	T_2	147.2±2.59	281.80±9.65	45	20		0	0	-	
	T_3	141.4±3.78	276.8±8.53	50	20		0	0	-	
Trial 2 (July)	T_1	141.60±6.11	275.4±3.97	6	3	25-35	56.33±1.53^{c}	51.33±1.54^{c}	35-48	52.00±4.36^{c}
	T_2	165.2±5.12	276.00±6.44	8	4		82.67±2.52^{a}	83.33±3.79^{a}		81.67±3.21^{a}
	T_3	159.4±3.44	278.00±7.45	10	5		75.33±1.53^{b}	70.33±3.51^{b}		72.33±6.03^{b}
Trial 3 (August)	T_1	168.40±5.03	283.40±9.24	6	3	25-35	58.00±2.65^{c}	53.67±4.04^{c}	35-48	54.67±3.23^{c}
	T_2	172.2±7.43	278.00±5.15	8	4		94.67±1.53^{a}	92.33±2.52^{a}		91.67±3.06^{a}
	T_3	173±3.74	274.80±5.31	10	5		78.33±1.53^{b}	74.00±3.61^{b}		73.67±5.13^{b}

***** For the female fish, the dose was divided into two volumes (1st dose 30% and 2nd dose 70%) and injected at 6 h interval, and the males were injected with single dose during 2nd injection to females. Mean values in the same column in each trial, having different superscripts are significantly different (P<0.05).

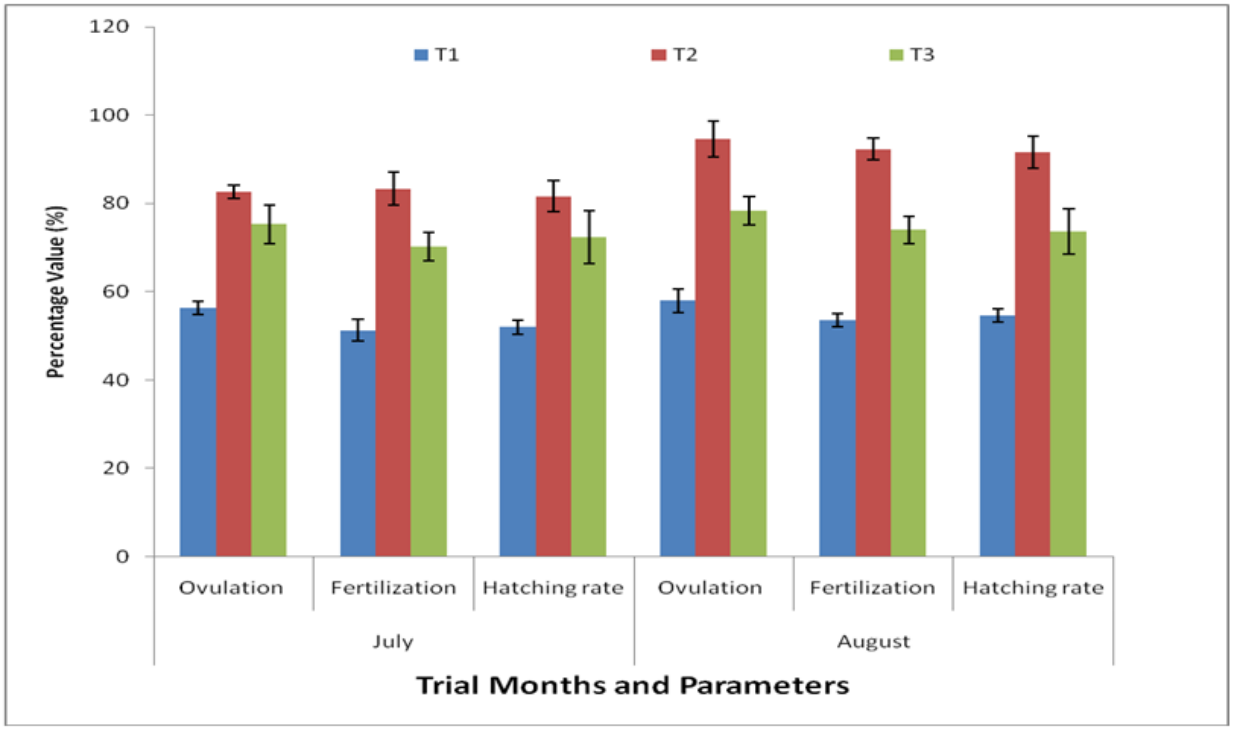

Fig. 6. Effects of different doses of PG extract on induced breeding (ovulation, fertilization and hatching) of gobi (*G. giuris*). Vertical bars of each parameters having different colours are significantly different (*P*<0.05)

4. DISCUSSION

Induced breeding trials of gobi *(G. giuris*) were conducted to standardize the dose of PG for successful ovulation. Three trials were carried out to develop an induced breeding technique of *G. giuris* with PG extract. Although many scientists [13-16] carried out experiments on some species with an aim to standardize the dose of PG for successful ovulation, there remains uncertainty among the doses reported by various workers. About the induced breeding of *G. giuris*, no information is available in Bangladesh or elsewhere in the world regarding the dose optimization and suitability of the inducing agents. So, it was felt extremely crucial to optimize a dose by which the induced breeding technique of the fish can be developed. However, very recently some of reproductive aspects of *G. giuris* were studied by Islam and Mollah [17], where the highest gonado-somatic index was observed in the month of July. They breed during the period of May to September with a peak in August. Having known this information, an experiment was conducted to find out a suitable dose of PG for induction of ovulation in *G. giuris* during the months of June to August 2013. The induction of ovulation and spawning in gobi using PG extract was done successfully. The PG doses of 40 to 50 mg kg^{-1} body weight was proved to be very high, which were selected on the basis of the preliminary work conducted by Islam and Mollah [17] to induce ovulation in the month of June. On the other hand, two breeding trials were conducted in July and August

using 6 mg (T_1), 8 mg (T_2) and 10 mg (T_3) PG kg^{-1} body weight of fish, where ovulation rates were recorded as 56.33±1.53, 82.67±2.52, 75.33±1.53% in July and 58.00±2.65, 94.67±1.53 and 78.33±1.53% in August under the treatments T_1, T_2 and T_3, respectively.

Fish breeding behaviour or courtship activity is a very important factor during the time of breeding. Spawning may be failed if any of the breeders do not show breeding behaviour [18]. Further, the rearing of the wild collected broodstock in captive conditions may not receive appropriate environmental cues for gonad maturation and spawning and it can cause reproductive development to be arrested in late vitellogenesis stage [19]. Many issues such as body size and shape, body colour, age, ripeness of female, appropriate dose of hormone, weather, rainfall, water flow, darkness, environmental factors, shelter, and activities of male are the causes involved in courtship behaviour for breeding. Male gobi *(G. giuris*) exhibited more aggressiveness and activity than female during pairing and spawning. Under the shelter, male came close to female, taking a side by side position and began to shake. After 10-12 h of PG administration, both male and female *G. giuris* exhibited breeding behaviour but males seemed more active in showing so than female by performing physical contacts. The aggressive male repeated its body movement and chased the female. Then mating was proceeded by courtship.

In the present study, the highest average fertilization rate (92.33±2.52%) was recorded during the month of August in T_2 where *G. giuris* broods were injected with a dose of 8 mg PG kg^{-1} body weight, whereas the lowest value (51.33±1.54%) was recorded during July in T_1, where the fishes were administered with 8 mg PG kg^{-1} body weight. Similarly, the highest hatching was observed as 91.67±3.06% during the month of August in T_2 and the lowest value was recorded as 52.00±4.36% during July in T_1. Incubation period ranged from 35-48 h in all treatments under the ambient water temperature of 27-29^{0}C. Though majority of the larvae were died within 7-8 days after hatching but they were survived when fed with tubificid worms and zooplankton. The fish treated with the dose of 8 mg PG kg^{-1} body weight in the month of August showed the best performance so far as the ovulation, fertilization and hatching rates were concerned, while the fish treated with 6 mg PG kg^{-1} body weight showed the lowest ovulation, fertilization and hatching rates of *G. giuris* eggs. Since fish of approximately similar size and maturity were used under the same management practices, the difference in the result obtained was only due to the variation in PG doses.

There are some factors responsible for the success of breeding. These are: good management, age and size of brood fish [20], feeding and manuring [21], doses of hormone used [22] and egg quality [21]. If the oocyte nuclei are found to be centrally located then such a fish would not respond to hormonal induced breeding treatment [23]. If the eggs are immature, fertilization does not occur. Pillay [24] opined that successful induced breeding depends on the appropriate dose of hormone administration; brood fish condition, sex ratio and even environmental conditions are equally important. It also depends on the maturation of fishes, especially the ripeness of oocytes in the female fish [[19,25].

The dose of hormone for induced breeding varies from species to species; some fishes need a very high dose, some species require a small dose and some require a moderate dose [26]. Islam et al. [27] used PG doses of 8 mg kg^{-1} body weight for female and 4 mg kg^{-1} body weight for male of *Mystus vittatus* and found 80% fertilization and 56% hatching rates. Khan and Mollah [14] induced *Pangasius pangasius* by injecting PG extract at the rate of 9, 10, 11 and 12 mg kg^{-1} body weight, which resulted 100% ovulation and the best result was obtained in case of fertilization and hatching rate of eggs when 10 mg kg^{-1} body weight of PG extract was used. Bhuiyan et al. [15] applied PG doses at the rate of 3, 6, 9, 12 and 15 mg kg^{-1} body weight, which induced 100% ovulation in *Puntius gonionotus*. They found that the dose of 6 mg kg^{-1} body weight was most efficient for induced breeding of *P. gonionotus* during the peak month of June. Saha et al. [28] worked on induced breeding of Thai koi, *Anabas testudineus* in different months of the breeding season under two sex ratios. They found better results in terms of fertilization (96.33%), hatching (89.67%) and survival (80.33%) rates in mid-April to May for the sex ratio of 1:2 (female: male), using the PG extract at 12 mg kg^{-1} body weight of female and half of the quantity of female for the male. Kohinoor *et al*. [29] successfully used PG extract at the rate of 8-12 mg kg^{-1} body weight for female *Anabas testudineus*. Kohinoor and Zaher [30] also stated that 10-12 mg kg^{-1} body weight of female was the best in considering the fertilization and hatching rates. The doses of PG extract for *G. giuris* were more or less similar to the dose used for *P. pangasius*, *P. gonionotus*, Thai koi and *Anabas testudineus*. On the other hand, Tan and Lam [31] administered HCG to ovulate marble gobi (*Oxyeleotris marmorata*) where over 90% eggs were fertilized of which >90% hatched subsequently and hatching time was extremely changeable, ranging from 2 to 5 days after fertilization at a temperature of 27^0C where larvae did not survive for more than a few days. In the present study rate of fertilization and hatching time of is more or less similar of marble gobi except the inducing agent.

Mollah [13] used four doses of PG viz., 80, 100, 120 and 140 mg kg^{-1} body weight in female *Rita rita* and found that 100 mg kg^{-1} body weight was effective for induction of ovulation, but lower and higher doses such as 80 and 100 mg kg^{-1} body weight had no effect on ovulation. This indicates that lower and higher dose was unable to induce ovulation. Similar findings were obtained in case of *G. giuris* where higher dose of PG such as 40 to 50 mg kg^{-1} body weight had no effect on ovulation. On the other hand, lower dose of 6 mg kg^{-1} body weight was not found to be sufficient for inducing the ovulation in *G. giuris*.

The latency period available in the literature is 6-25 h for *Channa punctatus* [32], 22-25 h for *Heteropneustes fossilis* [33], 14 h [34] and 16-20 h [35] for *Clarias gariepinus*. Average latency periods in case of *G. giuris* in the present study were recorded to be 25-35 h during July and August.

The results obtained from the present study indicated that the induced spawning of *G. giuris* was successful, using different doses of PG extract and in all trials, better performances in terms of ovulation, fertilization, and hatching rates of 94.67±1.53, 92.33±2.52 and 91.67±3.06%, respectively were found when used 8 mg PG kg^{-1} body weight under the treatment T_2.

5. CONCLUSION

From the above findings, it could be said that ovulation, fertilization, hatching and also survival rate of larvae of *G. giuris* varied mainly due to season, hormone dose and environmental factors as well as brood management, maturity of broods, quality of eggs, incubation temperature, oxygen supply through water flow during incubation and quality of hatchery water. Upon all considerations, injection of PG extract at a dose of 8 mg PG kg^{-1} body weight of female *G. giuris* in August showed better results. It was observed from the present investigation that the fertilization and hatching rate were commercially viable for the production of gobi (*G. giuris*) fish in captive condition. Overall, the present study concludes that induced breeding of gobi (*G. giuris*) is successful through PG extract for large scale production of quality seed for aquaculture production and conservation of this important fish species from extinction.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard, written ethical permission has been collected and preserved by the author(s).

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