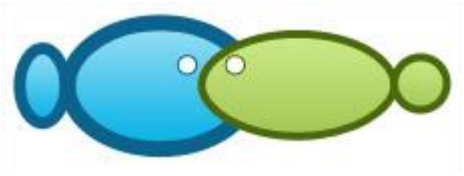

Efficacy of the reproductive hormone of pituitary gland for induced breeding of *Somileptes gongota* (Hamilton, 1822)

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Abstract. The endangered riverine catfish, locally known as "Pahari gutum" *Somileptes gongota* (Hamilton, 1822) is a very important small indigenous fish of Bangladesh. The efficacy of the reproductive hormone pituitary gland (PG) was examined for the ovulatory performance at three different doses, 10 (T1), 15 (T2) and 20 (T3) mg kg^{-1} body weight of reproductive adult fish. The experiment was conducted with 30 reproductive adults of *S. gongota* (15 females and 15 males) weighing on average 14.2±0.69 g males and 16.43±0.65 g females, to establish a suitable breeding technique. After two months of rearing, the matured females were injected a single dose, while the males were treated with half the dosage used for females. In the highest dose, almost 90% of fish spawned naturally, but, in other doses, partial natural spawning occurred. Average fertilization rates (%) were 80±5.48, 75±2.55 and 68±4.47%, while hatching rates were 72±4.42, 68±3.54 and 63±2.74% in T1, T2 and T3, respectively. Fertilization and hatching rates were significantly higher ($p<0.05$) in the highest dose. Hatching of fertilized eggs occurred between 23 and 24 h after incubation at 27 to 28°C, and the larvae started to feed within 72 h after hatching. The successful dose for the induction of breeding of *S. gongota* was 20 mg PG kg^{-1} body weight, which gave the best result of fertilization and hatching rates as well. This is considered as a landmark in the strategy of saving this endangered species by establishing a technique for mass production of fry.
Key Words: fertilization rate, hatching rate, latency period, ovulation rate, pahari gutum.

Introduction. Decline in fish catch due to overfishing and rampant killing of fish juveniles through destructive fishing gears have made natural fisheries a less profitable venture (Roy et al 2016). Alternatively, aquaculture practices have increased to meet the protein demand of the increasing population in Bangladesh. Major fish species cultured in Bangladesh are the Indian major carps (*Catla catla*, *Labeo rohita*, *L. calbasu* and *Cirrhinus cirrhosus*), *Cyprinus carpio* and tilapia (*Oreochromis niloticus*). However, more indigenous fish species should be cultured to ensure the sustainability of the aquaculture industry (Pennell et al 2001). Therefore, consistent efforts are needed to establish viable breeding and seed production techniques for the selected new species.

In Bangladesh, small fish from the natural water bodies such as rivers, canals, paddy fields, channels contribute with approximately 15.65% to the household income of fishermen (Roy et al 2016). As the demand and price of the small indigenous species (SIS), including *Somileptes gongota*, are high on the market, it is essential to produce adequate quantity through aquaculture that can support an alternate livelihood (Taslima & Mollah 2012). However, the major challenges for its aquaculture are paucity of wild juveniles and unavailability of hatchery produced juveniles. Thus, the development of successfully induced breeding techniques and mass production of fry at a commercial scale are crucial to protect this species from becoming extinct.

An important member of the freshwater fish fauna are the loaches (Family Cobitidae), which are poorly explored in Bangladesh. Loaches in Bangladesh are

represented by 6 genera (Rahman 1989). Most of them are of small size, inhabiting hill streams; a few are also found in rivers and swamps. Of these, the Gongota loach (*Somileptes gongota*, Hamilton, 1822), locally known as "Pahari gutum", belonging to the family Cobitidae, holds immense potential for the aquaculture sector in Bangladesh. It is known from Assam and Meghalaya to Uttar Pradesh along the base of the Eastern Himalayas in India. It inhabits generally muddy hill streams (Talwar & Jhingran 1991) and rivers of Mymensingh, Netrokona, Sylhet, Dinajpur, Rangpur and Rajshahi area in the rainy season (Bhuiyan et al 1992). This aquarium fish plays an important role in controlling the insect population (Rahman & Ruma 2007). Unfortunately, it has failed to draw the attention of the fishery biologists and only brief aspects of its biology are available (Shafi & Quddus 2001; Saha & Saha 2011). It is a very attractive fish and hobbyists like this fish for their excellent color and behavior. It is also very important from a nutritional point of view, especially for the rural communities of Bangladesh. The largest specimens had a recorded length of approximately 10 cm (Talwar & Jhingran 1991) and 130 mm of total length (Rahman 1989). The longest specimen from Barnai River, Rajshahi, had 6.5 cm (personal observation). The species is listed as an endangered species (IUCN-Bangladesh 2000). The Gongota loach is caught sporadically in the commercial fishery and, therefore, its availability is rather scarce in the fish markets. Thus, the increasing demand can only be met through aquaculture.

However, there is little information available on the broodstock development, induced breeding and fry production of *S. gongota*. Thus, successful artificial breeding in captivity will help to take steps on the way to restore the previous status of the biodiversity of the fish in the natural habitat and will also help aquarists. Keeping all this in mind, the present study has been conducted to induce the breeding of the *S. gongota* using different doses of the carp pituitary gland hormone (carp PG) to allow the optimization of the dose and to achieve higher ovulatory performance. These efforts could prevent the fish extinction; at the same time, rural people will have the opportunity to breed or consume the fish.

Material and Method

Broodstock collection and maintenance. The 25 pairs of Gongota loach used for this experiment were collected from the local rivers (Burikhora River, Tepa River and Barati River) of Nilphamari District in the Rangpur Division of Bangladesh. These fish were transported to the hatchery complex of the Department of Genetics and Fish Breeding, Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, during July 2019 and stocked in indoor tanks with provision of aeration and water flow-through system for acclimatization (Figure 1). After a brief quarantine in the indoor system, the fish were transported to an earthen pond. Ponds were equal in size, shape, basin formation, bottom type and depth (1.5 m) with an area of 40 m^2. The ponds were rectangular in shape, well exposed to sunlight, and a stocking density of 120 ind. per m^2 was maintained until breeding. The stocked fish were fed with a fishmeal based, formulated, sinking feed (30% crude protein; 7% lipid) at 5% of body weight per day, with two meals a day.

Water quality parameters were measured periodically and analyzed following standard procedures (APHA 2005). The water quality parameters, such as water temperature, pH, dissolved oxygen, total alkalinity, and total ammonia nitrogen are presented in Table 1.

Figure 1. Collection of *Somileptes gongota* fish from different local rivers of Nilphamari district, Bangladesh.

Table 1

Physico-chemical parameters of the water during the experimental period

Physico-chemical parameters	*Value (Mean±SE)*
Temperature (°C)	28±1
pH	7.6±0.2
Dissolved oxygen (mg L^{-1})	6.2±.23
Total alkalinity (mg L^{-1})	134-138
Total ammonia nitrogen (mg L^{-1})	0.02±0.01

Brood fish rearing and maturity study. Healthy and sexually mature adult fish were selected for breeding purpose. Mature males and females were identified on the basis of secondary sexual characteristics. The mature females were larger than males of similar age, and with a higher value of body depth. The males were identified by their flat abdomens and long protruding genital papillae (Chondar 1999). The females could be easily recognized by their soft and swollen abdomen, and the color of ovary was deep yellow. Deep yellow eggs would exit during gentle pressure on the abdominal region. The selected broods were kept in tanks for 6 h for conditioning, to adjust to the new environment. Male and female fish were kept in separate tanks and constant water flow was maintained to ensure proper aeration.

Application of PG to the brood fish. For spawning, the mature males and females were transferred to breeding tanks with continuous water flow for a period of 6-8 h. Afterwards, fish were shifted to a hapa made of synthetic nylon and provided with a continuous water flow. The ratio between males and females was 1:1. In this experiment, 15 pairs were used for induced breeding. Both males and females were administered a single dose at each trial. Three sets of doses were used: 20 & 10 mg PG kg^{-1} body weight (T1), 15 & 7.5 mg PG kg^{-1} body weight (T2), and 10 & 5 mg PG kg^{-1} body weight (T3), to identify the best dose of injections. Five females and five males were injected with each dose. Reproductive activities including nudging, dozing, circling, and ovulation and, finally, spawning were observed in the breeding tanks, and the spent fish were transferred to other tanks. A fish was considered to have ovulated when there was an

extrusion of a few eggs upon gentle pressure on the abdomen from an anterior to posterior direction.

Spawning induction and egg collection. In T1 and T2 treatments, the brood fish started to spawn after 7-8 h of injection, while in T3 the spawning started after 8-9 h. After nudging and circling, the male bent its body around the female. The genital pore of the male was brought near to the genital pore of the female. Eggs were ejected, and at the same time the male released the milt. The spawned eggs were collected by passing the surface water through an egg-collecting chamber fitted with a hapa of 500 µm. The eggs were sieved in the hapa, and the accumulated eggs were collected and treated with 20 ppm iodophore for 10 min for hatching. The transparent eggs were considered fertilized, whereas opaque eggs were considered dead and removed from the hapa. The fertilization rate was calculated with the following formula:

Fertilization rate = (Number of fertilized eggs / Number of total eggs) ×100

The fertilized eggs were then transferred into a hatching jar and maintained at a water temperature of 27-28°C and dissolved oxygen of 6-7 mg L^{-1} through continuous aeration. Approximately after 23-24 h of fertilization, larvae started to appear. The rate of hatching was calculated by the following formula:

Hatching rate = (Number of hatchlings / Number of total eggs) ×100

Statistical analysis. Comparison of treatment results was carried out using one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was performed to compare the different treatment means at a 5% level of significance. Statistical analysis was performed using Minitab version 17 software package.

Results and Discussion. The brood fish used in the breeding trials were in the same age group and the average body weight of male was 14±0.34 g in T1, 15±0.43 g in T2 and 13.6±0.29 g in T3. The average body weight of females was 16±0.5 g in T2, 17±0.44 g in T2 and 16.3±0.56 g in T3 (Table 2). Females were larger than males.

Detailed doses of PG used in the different trials are presented in Table 2. Though all the doses trialed resulted in ovulation, the dose of 20 mg kg^{-1} body weight of PG produced the best results in relation to fertilization and hatching rates. Ovulation occurred after 7-8 h of the injection at 27 to 28°C. The ovulation rate of females was 90% in T1. In T2, ovulation occurred after 7-8 h of the injection at and the rate was 75%, while in T3, ovulation rate was 66% and occurred after 8-9 h at the same incubation temperature of T2 (27°C). In T1, the fertilization rate was 80±5.48%, whereas in T2 and T3 the fertilization rates were 75±2.55 and 68±4.47%, respectively. Pal (2000) has studied the breeding of *Nandus nandus* at 28-29°C. Kohinoor et al (1991) accomplished induced breeding of *Anabas testudineus* at 27-30°C. In Bangladesh, the normal water temperature has 27-30°C from May to September. The indigenous fish of Bangladesh (including *S. gongota*) naturally breed at these temperatures.

Natural spawning was found successful at 20 mg PG kg^{-1} body weight in a single injection and completed without hand stripping. It was observed that both the females and males ejected eggs and milt at the contemporary times when the males were treated with a single injection of 10 mg PG kg^{-1} body weight (Figure 2). At doses of 15 and 10 mg PG kg^{-1} body weight, partial spawning was observed.

Table 2

Ovulatory performances of *Somileptes gongota* treated with carp pituitary galnd

Trial no.	*n*	*Weight of fish (g)*		*Number of injection or doses of PG (mg kg^{-1})*		*OR (%)*	*FR (%) (mean±SD)*	*IT (°C)*	*IP (hr)*	*LP (hr)*	*HR (%) (mean±SD)*
		Male	Female	Male	Female						
1	5	14 14.5 14.2 13.8 14	15.2 16 15.8 16.4 16.6	10	20	90	80±5.48^{a}	27-28	23-24	7-8	72±4.42^{a}
2	5	15 14.5 14.8 15.8 14.9	16.3 17 17.3 16.8 17.6	7.5	15	75	75±2.55^{ab}	27	23-24	7-8	68±3.54^{ab}
3	5	13.6 14.1 13.5 13.6 13.2	16.3 17.2 16 15.5 16.5	5	10	66	68±4.47^{b}	27	23-24	8-9	63±2.74^{b}

Note: OR - ovulation rate; FR - fertilization rate; IT - incubation temperature; IP - incubation period; LP - latency period; HR - hatching rate; n - number of fishes.

There was a significant difference ($p<0.05$) among the three doses in terms of fertilization and hatching rates. Fertilization and hatching rates at the dose of 20 mg PG kg^{-1} were significantly higher ($p<0.05$) than the lowest dose (10 mg PG kg^{-1}). No significant difference ($p>0.05$) was observed between T1 and T2, and T2 and T3 in the rate of fertilization and hatching (Figure 3). Spawning largely depends on the synchronization of ova and sperm release (Hoar & Randall 1969; Sayeed et al 2009).

Figure 2. Fry of *Somileptes gongota*.

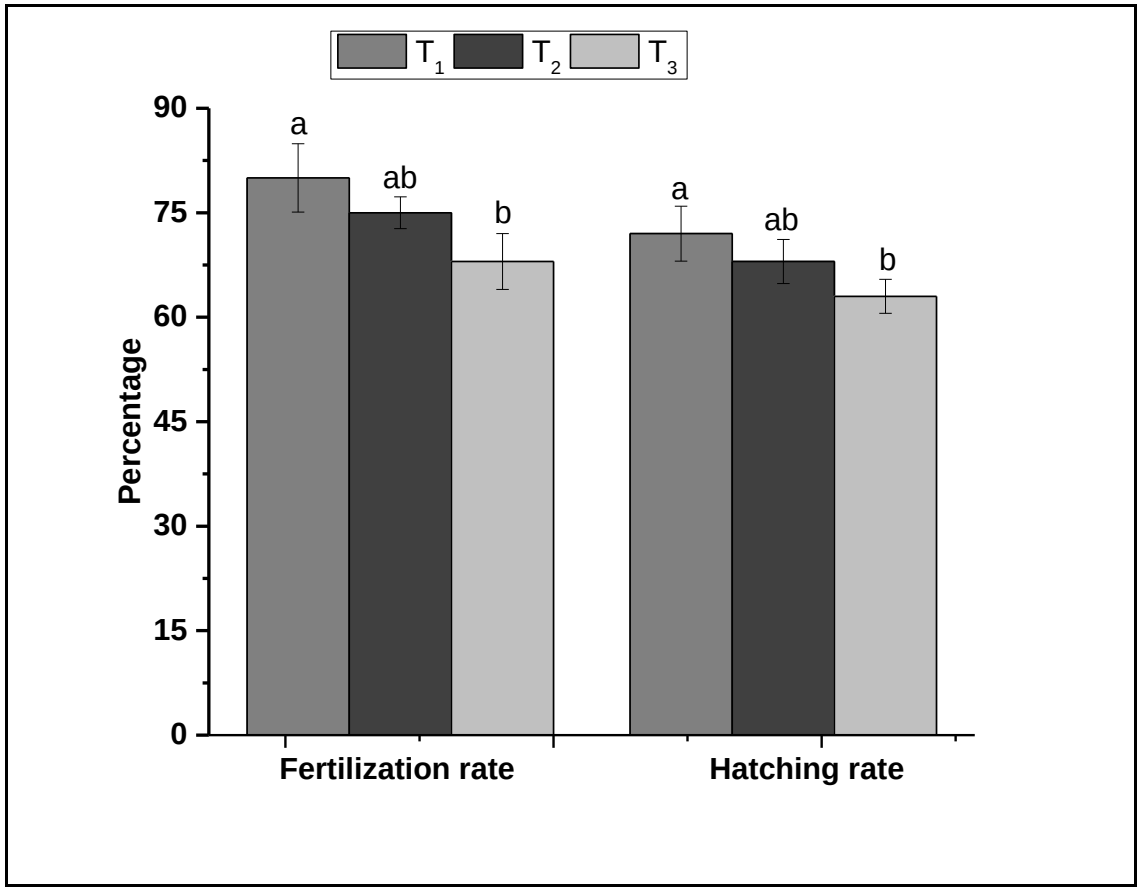

Figure 3. Fertilization and hatching rate (%) of *Somileptes gongota* over the course of three spawning trials.

In this trial, the successful dose was 20 mg PG gg^{-1} body weight. Sayeed et al (2009) administered a single dose at a rate of 10, 15 and 20 mg PG kg^{-1} body weight to females of *Lepidocephalichthys guntea* and half doses were applied to the males. They observed that at 20 mg PG kg^{-1} body weight, all fishes naturally spawned, whereas at 10 and 15 mg PG kg^{-1}, fish spawned only partially. However, they concluded that 10 and 15 mg PG kg^{-1} doses were best, based on the fertilization and hatching rates. Kohinoor et al (1991) applied a single injection at a rate of 8-12 mg PG kg^{-1} to the females of *A. testudineus* and 4 mg PG kg^{-1} body weight to the males. Ovulation occurred in all the injected females. The doses applied in the present study were more or less similar to those applied by Sayeed et al (2009) and higher than those used by Kohinoor et al (1991). This variation in doses might be due to the species variation. Thus, it may be said that every species is unique in their biological requirements.

Pal (2000) found that the fertilization and hatching rate of *N. nandus* were above 90% and above 80%, respectively. Kohinoor et al (1991) found that the fertilization and hatching rates of *A. testudineus* were 82±2 and 73±3%, respectively. Temperature is a very important factor for the incubation of eggs. The development of the embryo, the variability of hatching time of fertilized eggs and their viability are generally influenced by temperature (Hoar & Randall 1969; Rahman 1975; Jhingran 1983). Temperature is inversely proportional to the time of hatching (Alikunhi et al 1962; Mollah & Tan 1983; Rana 1990) and hatching success (Hoar & Randall 1969). In the present study, the incubation period of eggs was 23-24 h after fertilization, at 27-28°C temperature. Although no comparable data is available, several authors (Chakrabarty & Murty 1972; Thakur 1980; Haque 2007) observed that the incubation period of fertilized eggs of some fish lies between 18-32 h. Pal (2000) and Tarafder (2000) found that the incubation period of *N. nandus* was 18 to 20 h after fertilization, at 29±1°C temperature. Haque (2007) found that the incubation period of *Colisa fasciata* occurs within 18-22 h, at 27-

28°C in the same laboratory. Sayeed et al (2009) documented that the incubation period of *L. guntea* was 20-24 h after fertilization at 28°C.

Conclusions. This study presents a major success in the induced ovulation of *S. gongota* in Bangladesh. The carp pituitary gland dose at the rate of 20 mg kg^{-1} body weight produced the best results. Fertilization and hatching rates were high when the females were treated with a total dose of 20 mg kg^{-1} and males were treated with 10 mg kg^{-1} of carp pituitary gland. This is considered a landmark in the strategy of saving this endangered species by establishing a technique for mass production of the fry. Based on the information generated during this research program, causes of mass mortality of the larvae need to be considered and studied, so that a reliable technique of fry production can be established.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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