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Article

Efficacy of dry and wet carp pituitary gland (CPG) in the induced breeding of *Cyprinus carpio* var. *specularis* (Lacepède, 1803)

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Abstract: Fish hatcheries are generally regarded as the first step in the process of aquaculture development in any given nation. Fish breeding plays an important part in the process of supplying fry and fingerling to the farmers. The current study was conducted to identify the effectiveness of using dry and wet carp pituitary gland (CPG) in the induced breeding of mirror carp (*Cyprinus carpio* var. *specularis*), at Banchte Sheka fish hatchery Arabpur, Jashore from November to December 2021. The 1st dose of hormone, both for male and female brood fishes were injected with dry & wet PG at 0.8 mg PG/kg body weight. After 6 hours of the 1st dose, only female brood fishes were injected with the 2nd dose of hormone at 3 mg PG/kg body weight. After 6-7 hours of injection of 2nd dose, brood fishes became ready to spawn. The fertilized eggs were hatched after 42-48 hours. The ovulation rates were 100%. The fertilization rates were 88.3 % and 91.2% for wet and dry PG, respectively. The hatching rates were 84.2% and 87.5%, respectively. The survival rates were 79.5% and 83.3%, respectively. Therefore, dry CPG is highly recommended to hatchery owners considering the rate of fertilization, survival rate and availability. The information that has been offered here will be helpful to the owners of hatcheries when it comes to choosing the sort of pituitary gland to use in the breeding operation.

Keywords: dry PG; wet PG; ovulation rate; fertilization rate; survival rate

1. Introduction

Aquaculture practices in Bangladesh started with natural seed but now it is almost entirely (99.55%) replaced by hatchery produced seed, while supply of fingerlings is considered as a prerequisite for the development of aquaculture (Al-Asif *et al*., 2014; Ali *et al*., 2018; Faruk *et al*., 2018; Islam *et al*., 2017; Webber and Riordan, 1976). Since 1975, artificial fish breeding techniques and low cost hatchery designs have been successfully adapted in Bangladesh (Islam *et al*., 2016, 2017).

Mirror carp (*C. carpio* var. *specularis*) is a native carp of Europe (Islam *et al*., 2016; Wakida-Kusunoki and Amador-del-Ángel, 2011). In 1979, this carp was introduced to Bangladesh from Nepal for aquaculture purpose (Jewel *et al*., 2006; Mondol *et al*., 2006). The growth of this carp differs greatly in accordance with temperature,

availability of food, stocking density and supply of feed (Abdel-Aziz *et al*., 2021; Apon *et al*., 2019; Billah *et al*., 2020, 2019; Du *et al*., 2006; Kilambi and Robison, 1979; Maucieri *et al*., 2019; Yeasmin *et al*., 2018).
The rate of first dose injection of PG can be given up to 4 mg/kg for carp species and the ovulation period in different species from 1.5 to 6 hours (Minar *et al*., 2012). In some cases the fishes induced in pituitary extract might take 11.55 to 16.35 hours for vacating the ovaries (Rumondang *et al*., 2015), where fishes receives less stress than the synthetic induced hormones (Berezina and Fomina, 2021; Brzuska, 2001; El-Hawarry *et al*., 2016; Okomoda *et al*., 2017).
The regions of Jashore and Mymensingh in Bangladesh are home to the majority of the country's most important carp hatcheries (Al-Asif *et al*., 2014; Ali *et al*., 2018; Ali *et al*., 2016a; Faruk *et al*., 2018; Hossain *et al*., 2016; Rahman *et al*., 2016; Shabuj *et al*., 2016; Sharif and Al-Asif, 2015; Yeasmin *et al*., 2018, 2016; Zaman *et al*., 2017). Several of Bangladesh's fish hatcheries resort to the usage of synthetic hormones throughout the breeding process (Faruk *et al*., 2018; Islam *et al*., 2017; Rahman *et al*., 2018). The vast majority of hatcheries used the usage of Salmon gonadotropin hormone in order to release the eggs (Abit *et al*., 2021; Ali *et al*., 2016a; Ali *et al*., 2016b). The administration of synthetic hormones causes an increase in both the pre- and post-breeding stress levels of fish (Faught and Vijayan, 2018; Iwasa *et al*., 2017). The use of PG does not result in these kinds of issues (Chaudhuri, 1976; Hossain *et al*., 2013). Therefore, PG could be the answer to this problem. There has been a significant amount of study conducted on both PG and artificial hormones; however, there has been no research conducted comparing PG and artificial hormones (Al-Asif *et al*., 2014; Ali *et al*., 2018; Ali *et al*., 2016; Faruk *et al*., 2018; Hossain *et al*., 2016; Rahman *et al*., 2016; Shabuj *et al*., 2016; Sharif and Al-Asif, 2015; Yeasmin *et al*., 2018, 2016; Zaman *et al*., 2017). Therefore, the purpose of the current research was to evaluate the efficacy of employing dry and wet carp pituitary gland for induced breeding in terms of the ovulation rate, the fertilization rate, the hatching rate, and the survival rate of fish fingerlings.

2. Materials and Methods

2.1. Study area and duration

The experiment was conducted at a private hatchery of Arabpur (Banchte Shekha Hatchery), Jashore Sadar, Jashore from November to December, 2021 (Figure 1).

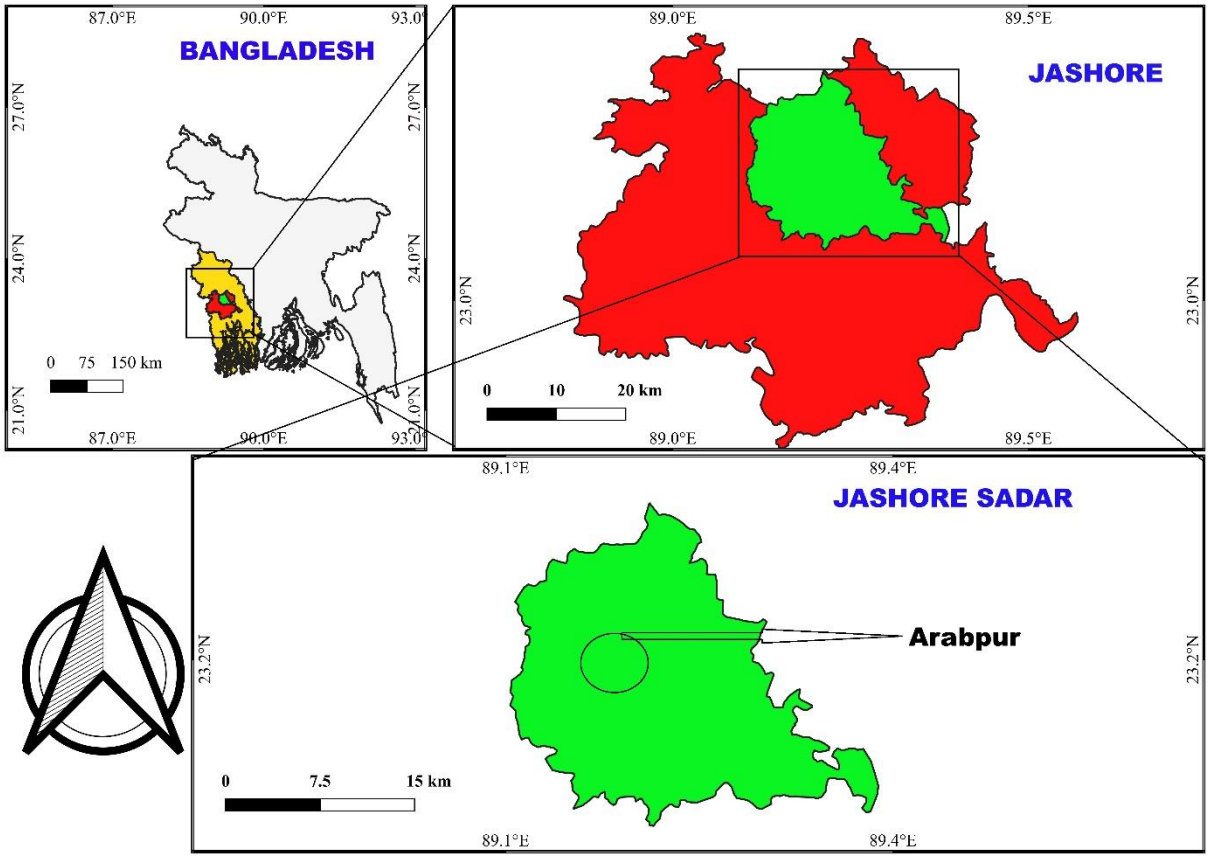

Figure 1. The location of the Banchte Shekha Hatchery, Arabpur, Jashore, Bangladesh.

2.2. Experimental fish and selection of broods

The common carp (*C. carpio* var. *specularis*) fish was used in the present study. The broodstock was reared in the pond of hatchery while before conducting the study the broods (males and females) were caught from the pond and acclimatize according to the hatchery protocols for the further operation. The selection criteria of the mature brood fishes of common carp (Table 1) was conducted following Islam *et al*. (2016).

Table 1. Selection criteria of the mature brood fish of common carp.

Male	Female
(a) Relatively small in size than the female.	Comparatively large in size than the male.
(b) Abdomen normal; not bulky like female.	Abdomen bulging, elastic and soft.
(c) Pectoral fins were rough.	Pectoral fins were slimy.
(d) Slightly protruding reddish vent seemed best criteria for male.	Small amount of eggs from the ovary with a small pressure were observed for maturity.

2.3. Conditioning of brood fish

Mature males and females from the brood rearing ponds were selected and immediately carried to the hatchery and kept in rectangular water tanks for about 15 hours for conditioning under water showering. No feed was supplied during the period of conditioning.

2.4. Collection and preparation of pituitary glands

Wet (PG), locally available (Fishtech Natural PG), processed and supplied by Fishtech Hatchery Limited were used as inducing agent which was collected from fish markets of Jashore and later on preserved in acetone. The PG was further processed in Fishtech PG Processing Laboratory at Jashore. The weighed dry and wet PG were transferred to a tissue homogenizer for thoroughly for crushing. The crushed PG then mixed with distilled water for dissolving and centrifuged with a hand centrifuge.

2.5. Weight of brood fish

Each single brood fishes were weighed before injecting 1st dose of hormone.

2.6. Hormone administration

The freshly prepared supernatant solution of hormone was then taken slowly in a syringe for injection. Brood fish was caught carefully by net and kept on sponge platform, while the whole body was covered by soft cloth. The dry PG and wet PG were then injected near the pectoral fin base in two groups of fishes and kept in two different tanks. The amount of dry PG solution for each fish was determined according to the body weight of the brood fish prescribed by Islam *et al*. (2016).

2.7. Estimation of ovulation rate

After 6 hours of 2nd hormone dose, female ovulation was occurred. The rate of ovulation was estimated by using the formula of Islam *et al*. (2016).

$$\text{Ovulation rate (\%)} = \frac{\text{Number of females ovulated}}{\text{Total number of females injected}} \times 100$$

2.8. Estimation of fertilization rate

The eggs and milts were mixed in a container for the fertilization process. The fertilized eggs were differentiated from the unfertilized ones after 15-20 minutes of stripping by the presence of "eye spot" and the swelling of the fertilized egg. The unfertilized eggs were white and opaque; while the fertilized eggs were transparent. The rate of fertilization was estimated by using the prescribed formula of Islam *et al*. (2016),

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs in sample}}{\text{Total number of eggs of sample}} \times 100$$

2.9. Estimation of hatching rate

Hatching rate was determined by the formula of Islam *et al*. (2016),

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchling in sample}}{\text{Total number of fertilized of sample}} \times 100$$

2.10. Estimation of survival rate

Survival rate was determined by the following formula of Rahman *et al*. (2016) and Islam *et al*. (2016),

$$\text{Survival rate } (\%) = \frac{\text{Number of survived fry in sample}}{\text{Total number of hatchlings in sample}} \times 100$$

2.11. Data analysis

The collected data from the hatchery operation were tabulated and analyzed using Microsoft package and Origin Pro 2020. The presented map was produced using QGIS desktop software.

3. Results

3.1. Weight of brood fishes

The average weight of male common carp fish for induce breeding was 2.13 kg, while the average weight of female was 2.88 kg. While the average weight of dry PG administrated female (3.25±0.25 kg) had more weight than the male (2.25±0.25 kg). On the other hand, the average weight of wet PG administrated female (2.50±0.50 kg) had more weight than the male (2.00±0.50 kg) (Table 2).

Table 2. The weight criteria of brood selection.

Sex	Body weight (kg)	Body weight (kg)
Male	2.25±0.25	2.00±0.50
Female	3.25±0.25	2.50±0.50

*Mean ± Standard error

3.2. Dry and wet PG dose used in induced breeding

The 1st dose of dry and wet PG were given both of male and female at the rate of 0.8 mg/kg body weight. After 6 hours, the 2nd dose was injected to the female at the rate of 6.5 mg/kg (Table 3).

Table 3. Doses and intervals of PG for male and female broods.

Sex	1st dry and wet PG dose (mg/kg)	Interval (hours)	2nd dry and wet PG dose (mg/kg)
Male (N=4)	0.8	6	-
Female (N=4)	0.8	6	3

After a period of 6-7 hours after the administration of the second dosage, the eggs and milts were collected using striping method. Brood fish were kept sex-separated in rectangular tanks with falling oxygenated water. For proper handling of common carp eggs, it is necessary to remove the adhesive substance which makes the eggs sticky. For this, a solution was made with water, to which 60 g of urea and 40 g of sodium chloride were added. The eggs and milts in the dish were mixed together with a soft brush for 40 minutes and then a solution containing urea and sodium chloride in distilled water was added to the mixture. After 40 minutes' milk was mixed with distilled water at a time 10 minutes and then eggs and milts was mixing with milk. Those solutions temporarily reduced the stickiness of the eggs and prolonged the fertilizing capacity of the milt. The collected eggs and milts then taken into a bowl and starrier for few minutes for making sure the fertilization process taken place. The fertilized eggs were then moved into incubation jars, where the hatching process takes place after a period of 42-48 hours following fertilization.

3.3. Ovulation rate

After 7 hours of 2nd hormone dose female ovulation was taken place, while we found 100% females were ovulated.

3.4. Estimation of fertilization, hatching and survival rate

The fertilization was higher in percentage in fishes where dry PG used (91.2%), followed by wet PG (88.3%). The hatching rate was found also higher in dry PG (87.5%), followed by wet PG (84.2%). On the other hand, survival rate was found higher in dry PG (83.3%) followed by wet PG (79.5%) (Figure 2).

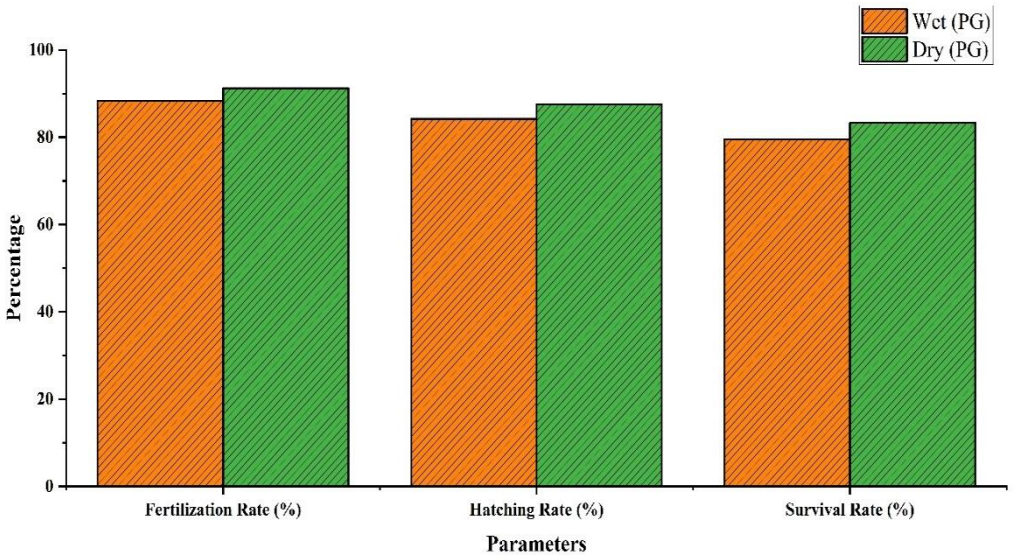

Figure 2. The fertilization, hatching and survival rate of common carp broods in hatchery setup.

4. Discussion

The induced breeding and fry production techniques of common carps viz. scale carp (*C. carpio* var. *specularis*), were observed at Banchte Sheka fish hatchery in Jashore. During the present study, the ovulation rate, fertilization rate, hatching rate and survival rate of common carps were investigated. The average weight of male common carp fish for induce breeding was 2.13 kg, while the average weight of female was 2.88 kg. While the average weight of dry PG administrated female (3.25±0.25 kg) had more weight than the male (2.25±0.25 kg). On the other hand, the average weight of wet PG administrated female (2.50±0.50 kg) had more weight than the male (2.00±0.50 kg). The study of Islam *et al*. (2016) reported the average weight of common carp male brood stock were 1.17±0.30 to 1.28±0.28 kg while the female brood body weight ranged between 1.27±0.18 to 1.72±0.30.

The present study stated that after 6 hours of the 1st dose, only female brood fishes were injected with the 2nd dose of hormone at 3 mg PG/kg body weight. After 6-7 hours of injection of 2nd dose, brood fishes became ready to spawn. The fertilized eggs were hatched after 42-48 hours Miah *et al*. (2008) suggested that, 1.0 mg PG/kg body wt. can be used in induced breeding of *Labeo bata*. Islam *et al*. (2016) reported that female fishes were injected with the second dose of PG at 4mg / Kg, the fertilized eggs were hatched after 42-48 hours and after 6-7 hours of second dose of PG injection, fishes become ready to spawn which is relevant to the present findings.

Minar *et al*. (2012) reported that, the ovulation period in different species from 1.5 to 6 hours while study of Yeasmin (2015) stated that after 6-7 hrs of injection ovulation occurred naturally and ovulation rate was 93.34%. The present study showed that, the present study showed that 6-7 hrs of injection ovulation was occurred and the ovulation rate was 100% which is more or less similar to the present study.

Yeasmin *et al*. (2018) showed that hatching rate was 87.33 ± 12.45%. The present study stated hatching rate was found also higher in dry PG (87.5%), followed by wet PG (84.2%); which was more than the previous study. On the other hand, survival rate was found higher in dry PG (83.3%) followed by wet PG (79.5%), which is relevant to present findings.

The present study showed that fertilization was higher in percentage in fishes where dry PG used (91.2%), followed by wet PG (88.3%). Ikechukwu *et al*. (2020) reported that, survival rates of fingerlings was 76.67% in pituitary. Jahan (2015) observed the induced breeding of carps- *Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosus*. The mean fertilization rates were 91±1%, 92±1 % and 91±1 % for rui; 92±1%, 94.33±2.08% and 91.33±1.53% for catla; 94.67±1.53%, 91.33±2.08% and 91±1% for mrigal in June, July and August, respectively Aliniya *et al*. (2013) and Yeasmin *et al*. (2018) showed that observed fertilization rate was 91% and 84.00 ± 6.89 %.respectively which is relevant to the present findings.

5. Conclusions

The most significant place that has been recognized as one of the most promising areas for the development of carp hatchery technologies is Jashore. The owners of the hatchery took great care to guard against the dangers of

inbreeding. The current study came to the conclusion that dry CPG produced the best results in terms of spawning, fertilization, hatching, and survival rate. As a result, the usage of this product is highly recommended to hatchery operators who are considering using it. For a better knowledge of the molecular level efficacy of this hormone, additional research on the manner of pituitary gland efficacy in both sexes of common carp or any other fish species can be examined.

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Data availability
All relevant data are within the article. The data of this current investigation will be available upon valid request by any authority from the corresponding author.

Conflict of interest
None to declare.

Authors' contribution
B.M. Newaz Sharif: conceptualization, methodology, data collection, analysis and manuscript writing; Antara Ghosh, Subrata Mondal, Mohammad Hasnal Alam, Md. Ariful Islam, Md. Shamsul Kabir : reviewing and editing; Md.Manjurul Karim: Supervision, reviewing and editing. All authors have read and approved the final manuscript.

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