Asian J. Med. Biol. Res. 2022, 8 (3), 146-153; https://doi.org/10.3329/ajmbr.v8i3.61444

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
https://www.ebupress.com/journal/ajmbr/

Article

Local and overseas carp pituitary gland in the induced breeding of *Cirrhinus mrigala* (Hamilton 1822)

Mohammad Hasnal Alam[1], Subrata Mondal[2], Md. Shamsul Kabir[1], Manjurul Karim[1], B. M. Newaz Sharif[3*], Md. Ariful Islam[4] and Md. Asrafur Rahman[4]

[1]Feed the Future Bangladesh Aquaculture and Nutrition Activity, WorldFish, Dhaka, Bangladesh
[2]Department of Fisheries and Marine Bioscience, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore-7408, Bangladesh
[3]Research and Development Department, Fishtech Hatchery Limited, Uttara-1230, Dhaka, Bangladesh
[4]Research and Development Department, Fishtech BD Limited, Uttara-1230, Dhaka, Bangladesh

*Corresponding author: B. M. Newaz Sharif, Research and Development Department, Fishtech Hatchery Limited, Dhaka, Bangladesh. Phone: +8801721881528; E-mail: shakilbau47@gmail.com

Received: 16 August 2022/Accepted: 11 September 2022/ Published: 15 September 2022

Abstract: Stimulating hormone for artificial fish breeding in hatchery industry is very crucial for hatching operation, healthy fry and fingerling production, while overall fish production of a country. The present study was conducted to assess the effectiveness of using local and overseas Carp Pituitary Gland (CPG) in the induced breeding of *Cirrhinus mrigala* at Jashore district from April to May 2022. The first dose of hormone, both for male and female brood fishes were injected with local and overseas CPG at 0.5mg/kg body weight. After six hours of the first dose, only female brood fishes were injected with the second dose of PG at 5 mg/kg body weight. After 6 hours of second dose CPG injection of local and overseas PGs, brood fishes started spawning. The ovulation rates for both local and overseas PGs were estimated 100%. The fertilization rates were 93.5 % and 92.6 % for local and overseas PGs; while the hatching rates were 91.2% and 89.5%, respectively. However, local CPG is highly recommended to hatchery owners considering the rate of fertilization, hatching rate and availability.

Keywords: local PG; overseas PG; ovulation rate; fertilization rate; survival rate

1. Introduction

Fish hatcheries are typically regarded as the initial stage in the development of aquaculture in any particular country. Fish breeding is critical in the process of supplying farmers with fry and fingerling (Sharif *et al*., 2022). Aquaculture techniques in Bangladesh began with natural seed but are now almost entirely supplanted by hatchery-produced seed, with fingerling availability considered a requirement for aquaculture growth (Al-Asif *et al*., 2014; Ali *et al*., 2018; Faruk *et al*., 2018; Islam *et al*., 2017; Webber and Riordan, 1976). Artificial fish breeding techniques and low-cost hatchery designs have been effectively implemented in Bangladesh since 1975 (Islam *et al*., 2016, 2017).

Carp growth in production facilities varies substantially depending on characteristics such as temperature, feeding frequency, stocking density, and feed supply (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).

In rare situations, fish produced in pituitary extract may take 11.55 to 16.35 hours to vacate the ovaries (Rumondang *et al*., 2015), with less stress than synthetically created hormones (Berezina and Fomina, 2021; Brzuska, 2001; El-Hawarry *et al*., 2016; Okomoda *et al*., 2017).

In Bangladesh, the bulk of the most significant carp hatcheries are located in the areas of Jashore and Mymensingh (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). A number of fish hatcheries in Bangladesh use synthetic hormones throughout the breeding process (Faruk *et al*., 2018; Islam *et al*., 2017; Rahman *et al*., 2018). To release the eggs, the great majority of hatcheries utilized salmon gonadotropin hormone (Abit *et al*., 2021; Ali *et al*., 2016a; Ali *et al*., 2016b).

In the aquaculture setup of India and Bangladesh, the two primary producing nations, mrigal is mostly farmed as a part of carp polyculture systems (Chwakravorty *et al*., 2019). Mrigal is also used in carp polyculture systems in Thailand, Vietnam, Pakistan, Myanmar, and Nepal (Hasanat *et al*., 2014).

Mrigal is typically raised with catla (*Catla catla*) and rohu (*Labeo rohita*), the other two primary carps found in India, while most carp farming in Bangladesh takes place in conventional ponds, with just around 16% of them being semi-intensive (Azim *et al*., 2001; Bais, 2018). The maturity of this species used attained in two years in captivity (Ujjania and Soni, 2018). Mrigal does not reproduce in ponds because it requires a fluvial habitat, and however, induced breeding has made captive breeding in hatcheries conceivable. Mrigal fish are extremely fecund, and fertility rises with age (Iqbal and Kausar, 2009; Sayeed, 2015).

Carp pituitary gland (CPG) is a fundamental element for induced breeding of different fishes in the hatcheries. CPG is used to inject hormone to the brood fishes for stimulating them to release mature egg and sperms in the confined waters. Utilization of CPG in the reputed hatcheries is a common practice due to the better performance of the off springs. But, there was no research conducted to compare the efficacy of local and overseas CPG in any Indian major carps. Therefore, the present study was conducted to compare the effectiveness of using local and overseas carp pituitary gland for induced breeding in terms of ovulation rates, fertilization rates, and hatching rates.

2. Materials and Methods

2.1. Study area and periods

The experiment was conducted at a hatchery (Banchte Shekha Hatchery) of Jashore from April to May, 2022 (Figure 1).

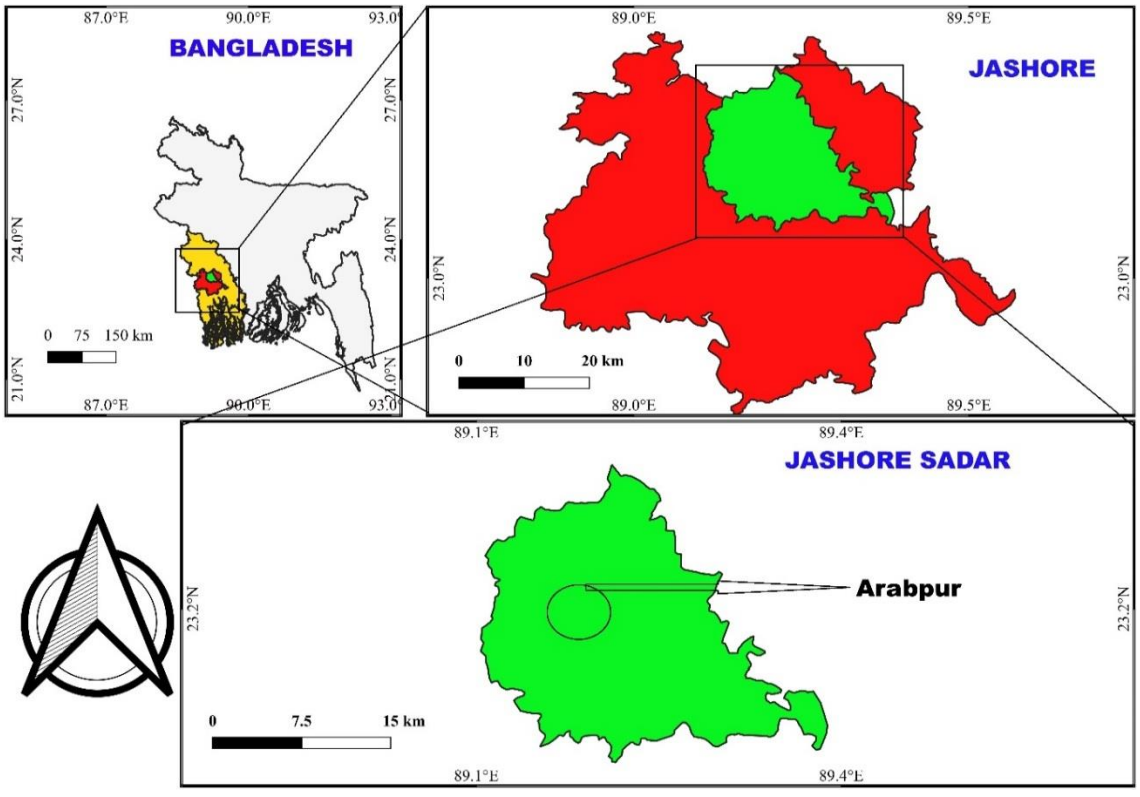

Figure 1. Study Area (adopted from Sharif *et al*., 2022).

2.2. Experimental fish and selection of brood stock

Cirrhinus mrigala was used as the experimental fish species for comparing the efficacy of local and overseas PG in the present study. The selection criteria of the mature brood *Cirrhinus mrigala* fish were followed from the guideline of Islam *et al.* (2016) (Table 1).

Table 1. Selection criteria of the *Cirrhinus mrigala* mature brood fish.

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

Locally available PG (Fishtech Natural PG), processed and supplied by Fishtech Hatchery Limited were used as inducing agent. The PG was collected from fish market and preserved in acetone. The overseas PG was also collected from the local market of Jashore city. The both PG were weighted by an analytical electronic balance. The weighed both of local and overseas a PG was transferred to a tissue homogenizer for thoroughly crushing. The crushed PG then diluted with distilled water to dissolve it and was centrifuged with a hand centrifuge for precipitations.

2.5. Hormone administration

The freshly prepared supernatant solution of local and overseas PG were taken slowly in a syringe for injection. Brood fish was caught carefully by net and kept in sponge were covered by soft cloth. Local and overseas PG were then injected near the pectoral fin base.

2.6. Weight of brood fish

Each single brood fish was weighed before injecting first dose of local and overseas PG.

2.7. Ovulation rate

After six hours of second 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 fertilized eggs were differentiated from the unfertilized ones 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),

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

2.9. Estimation of hatching rate

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

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

2.10. Data analysis

The collected data from the hatchery operation were tabulated and analyzed using Microsoft package and Origin Pro 2020.

3. Results

3.1. Weight of brood fishes

The average weight of male *Cirrhinus mrigala* administrated by local PG was 2.25±0.25 kg, while the average weight of female was 3.25±0.25 kg. The average weight of male *Cirrhinus mrigala* administrated by overseas PG was 2.65±0.55 kg, while the average weight of female was 3.00±0.60 kg (Table 2).

Table 2. The weight criteria for brood selection.

Species		Weight (Kg)	
Cirrhinus mrigala	**Type**	**Male**	**Female**
	Local	2.25±0.25	3.25±0.25
	Overseas	2.65±0.55	3.00±0.60

3.2. Local and overseas PG dose used in induced breeding

The first dose of local PG and overseas PG hormone, respectively, given both of male and female at the rate of 0.5 mg/kg body weight. After 6 hours, the second dose of PG injected to the female at the rate of 5 mg/kg (Table 3). After 6 hours of second dose of local and overseas PG, the eggs and milts were collected through hand stripping method. The ovulation rate for both local and overseas PGs administrated in *Cirrhinus mrigala* were 100%. The eggs and milts in the dish were mixed together with a feather for 1-2minutes. The fertilized eggs were transferred into incubation jars in which hatching occurs after 17-18 hours of fertilization.

Table 3. Doses of pituitary glands (PG) hormone for female and male broods.

Species	Sex	Respectively, 1st local and overseas PG Dose (mg/kg body weight)	Interval (hrs)	Respectively, 2nd local and overseas PG Dose (mg/kg body weight)
Cirrhinus mrigala	Male (N=4)	0.5	6	-
	Female (N=4)	0.5	6	5

3.3. Estimation of fertilization, and hatching rate

The fertilization was higher in percentage in fishes where local PG (93.50%) used followed by overseas PG (92.60%). On the other hand, the hatching rate was found higher in local PG (91.2%) and lower hatching rate was observed where overseas PG (89.5%) was used (Figure 2).

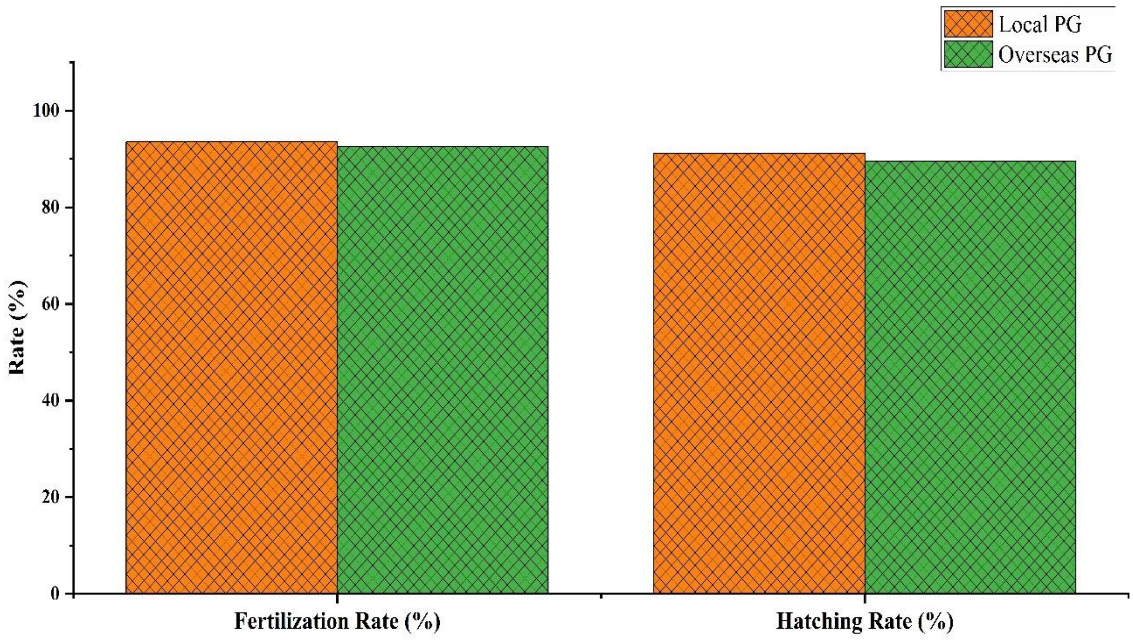

Figure 2. The fertilization, and hatching rate of *Cirrhinus mrigala* broods in hatchery setup.

4. Discussion

The induced breeding and fry production techniques of *Cirrhinus mrigala* observed at a fish hatchery in Jashore. During the present study, the ovulation rate, fertilization rate, and hatching rate of mrigal fish were investigated. The average weight of male *Cirrhinus mrigala* administrated by different PGs were 2.25±0.25 to 2.65±0.55 kg, while the average weight of female were 3.00±0.60to 3.25±0.25 kg. The sexual maturity of *Cirrhinus mrigala* was observed at the age of two and onwards, while the weight was estimated 644.26 to 4248.94 g (Ujjania and Soni, 2018). The current findings suggested that, these experimental fishes were sexually mature and as they were prepared for breeding standard feeding and nursing for the broodstock in hatchery was maintained.

The present study stated that after 6 hours of first dose, the second dose of PG injected to the female at the rate of 5 mg/kg (Table 3). After 6 hours of second dose of local and overseas PG, the eggs and milts were collected through hand stripping method. The ovulation rate for both local and overseas PGs administrated in *Cirrhinus mrigala* were 100%. 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.

The present study observed the ovulation rates were 100% for both local and overseas PGs while the result of Sharif *et al.* (2022) in common carb also found the similar study while comparing dry and wet PGs.

The fertilization was higher in percentage in fishes where local PG (93.50%) used followed by overseas PG (92.60%). Farid (2014) showed that the fertilization rate was 87.4%, and hatching rate was 84.16% in the induced breeding of common carp. Aliniya *et al.* (2013) observed that higher fertilization rate 91% was found when the 2 years old males were crossed with 3 years old female common carp ($P<0.05$). The study of Rokade *et al.* (2006) on induced breeding of major carp *Cirrhinus mrigala* suggested the fertilization rate of PG was 60% which was far lower than the present study, this could be the inability of cause spawning due to dopamine inhibitor which plays a crucial function in the production of gonadotropin (Peter *et al.*, 1987).

On the other hand, the hatching rate was found higher in local PG (91.2%) and lower hatching rate was observed where overseas PG (89.5%) was used. The study of Kumar *et al.* (2019) at a dosage of 0.25 to 0.50 ml ovaprim/kg body weight in *Cirrhinus mrigala*, the hatching percentage ranged from 60 to 95, with a high of 95%; this result was found relevant with this present study.

5. Conclusions

Jashore is a key location that has been identified as one of the most promising regions for the advancement of carp hatchery technologies. The hatchery's proprietors took considerable care to prevent the risks of inbreeding. The findings of the current study showed that local CPG generated the best spawning, fertilization, and hatching results. Therefore, hatchery owners who are thinking about using it are strongly advised to utilize this product.

Acknowledgements

The corresponding author would like to acknowledge the help of Dr. Abdulla-Al-Asif from Universiti Putra Malaysia (UPM) for offering insightful comments, directions and suggestions for fruitfully performing the investigation. The authors also would like to thank Fishtech Hatchery Limited and WorldFish, South Asia office for giving opportunity to conduct this study through the partnership with “Feed the Future Bangladesh Aquaculture and Nutrition Activity” funded by USAID. The corresponding author would like to thank, department of Fisheries and Marine Bioscience, Jashore University of Science and Technology.

Data availability

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

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

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