Iranian Journal of Fisheries Sciences 21(1) 104-121 2022
DOI: 10.22092/ijfs.2022.125852

## Research Article

# Breeding biology and dose optimization for captive breeding of striped dwarf catfish *Mystus vittatus* using different hormones

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Received: May 2021 Accepted: October 2021

**Abstract**

The striped dwarf catfish, *Mystus vittatus*, a small indigenous fish of Bangladesh is scarce facing anthropogenic interventions. The present study depicts the captive breeding of *M. vittatus* applying different stimulating hormones which might improve the production. For this purpose, 400 wild brood fish of *M. vittatus* were collected and some breeding parameters such as gonado-somatic index (GSI), hepato-somatic index (HSI) and fecundity were measured at 15 days interval at each month during a year. For captive breeding, the broods were kept in the containers dividing into two different groups (male: female sex ratio 1:1 and 1:2) treated with carp pituitary gland (CPG: 4 to 12 mg/kg), flash (S-GnRHa: 0.4 to 1.2 mL/kg) and CPG plus flash hormones (2+0.2 to 6+0.6 mg-mL/kg) by 15 different doses and planned with a single dose for male and female. A control unit with no hormone was designed for each sex ratio. For evaluating the breeding performance, fertilization rate, hatching rate and survival rate of larvae were compared. In addition, the water quality parameters (temperature, DO, TDS, and pH) of incubators were checked. Maximum GSI value (25.54±5.86%) was found in mid-July and minimum (0.11±0.01%) was in mid-October, whereas the HSI value was lowest in mid-July for both female (1.61±0.11%) and male (2.40±0.08%). The average fecundity was 16175±10803 from end-March to end-September whereas the highest and lowest values were 32794±1284 in mid-July and 2109±412 in mid-September. Based on the GSI and HSI values of male and female, mid-July is the spawning season of this species. The higher latency period (8-9 hrs.) was noted with CPG and lower (6-7 hrs.) with the CPG plus flash hormone. The highest fertilization rate (92.6±6.38%), hatching rate (78.4±5.73%) and survival rate (69±7.03%) were found with a dose of 3+0.3 mg+mL/kg (CD2) of fish at the sex ratio of 1:2 whereas the average water parameter of temperature, DO, pH and TDS were 29.72±0.30°C, 8.61±0.08 mg/L, 7.46±0.01, 180.49±18.53 mg/L. In captive conditions, the seed of *M. vittatus* can be mass-produced and helpful to aquaculture and conservation through this research.

**Keywords:** Captive breeding, Sex ration, Breeding biology, Fecundity, Hatching rate, Fertilization rate, *Mystus vittatus*

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[ DOR: 20.1001.1.15622916.2022.21.1.20.6 ]

## Introduction

*M. vittatus* belongs to the family of Bagridae and is considered as freshwater Small Indigenous Species (SIS) in Bangladesh. This fish was found in open and closed inland freshwaters like rivers, canals, beels (flood paddy field), haors (flood paddy field), ponds, ditches, and baors (oxbow lake) (Rahman, 1989; Talwar and Jhingran, 1991; Galib *et al*., 2009). Destruction of inland freshwater habitats due to manmade and natural activities such as industrial pollution and vigorous use of pesticides in agricultural land, urban development and so on and those activities drive to effect on fish biodiversity which leads to decline the fish population in the water bodies in Bangladesh (Suresh *et al*., 2006; Abujam and Biswas, 2011). Induced breeding might serve as an effective tool for better commercial production and viable fingerlings stocking in inland open water bodies can assist the conservation perspective. Before starting the captive breeding of fish species, knowledge of a fish's fecundity, gonado-somatic index, and hepato-somatic index are important for measuring the commercial stock as well as the life history, realistic culture, and current management (Lagler 1956, Doha and Hye, 1970). Many scientists have worked on this species especially on breeding biology (Bhat, 1971; Rao and Sharma, 1984; Sudha and Shkuntala, 1989; Hoque and Hossain, 1993), food and feeding habitat (Reddy and Rao, 1987; Shafi and Quddus, 2001; Chattopadhyay *et al*., 2014), length-weight relationship (Hossain *et al*., 2006; Tripathi *et al*., 2010; Victor *et al*., 2014), and induced breeding performance (Islam, 2011; Bhuiyan *et al*., 2018).

This species has high market value due to its taste in Bangladesh and the culture technique of this species is time demands. Before the culture of this species, the availability of fry needs to thrive. Now, fry of this species is difficult to collect from natural water bodies of Bangladesh for pond stocking. To meet the requirements of fry of *M. vittatus* can be managed through proper captive breeding technique. With this context of the previous study, the present experiment aimed to collect knowledge on breeding biology of *M. vittatus*, which could be useful in the future for management and conservation. Standardization of captive breeding will reduce the spawning interval and increase the yield of more seeds in a shorter period for commercial desire, and conserving the natural population.

## Materials and methods

### *Study period and area*

*M. vittatus* is locally known as Tengra or Guilla. This study has been carried out in a hatchery named “Bismillah Fish Seed Production Center and Farm” located at Nangolkot, Comilla, Bangladesh (23°10´N 91°12´E). This study has covered the subject of species collection, breeding biology, hormone preparation, dose optimization, breeding performance, and water quality parameters from January 2018 to July 2019. The reproductive induction with hormone has been taken in July, 2019.



[ DOR: 20.1001.1.15622916.2022.21.1.20.6 ]

*Experimental fish and sample*

About 400 breeders (male and female) were collected from wild sources and reared in the pond adjacent to Bismillah Fish Seed Production Center and Farm, Comilla, Bangladesh from January 2018 to December 2018. A 10 decimal pond was used for rearing the brood fish and fed with commercial feed (30% protein content with containing vitamin C&E) at the rate of 5% of body weight.

*Estimation of GSI (gonado-somatic index), HSI (hepato-somatic index) and absolute fecundity*

For investigation of breeding biology (GSI and HSI), *M. vittatus* were collected from the upper stream of Meghna River, Noakhali twice a month from January to December, 2018. A total of 288 males and 437 females were taken for the estimation of GSI, HSI and fecundity. Fish specimens were stored in 10% formalin solution and transported to the laboratory for further study shortly after being caught. The total length of each fish was then measured with a digital slide caliper with 0.01 mm accuracy (EAGems-B00Z5KETD4) and weighted with a digital balance with 0.01 g accuracy (EAGems-B00Z5KETD4) (Shimadzu UX320G). To prevent any unintended damaged or cut, the fish specimen was dissected and all internal organs were extracted with a soft brush and blunt forceps. The gonad and liver were separated and stored in levelled vials in a 10% formalin solution. The liver was then separated from the digestive tract. Digital balance was used to measure the weights of liver and gonad. For calculating the gonado-somatic index, the weight of individual fish (female) was measured and the gonads were removed carefully and weighed in an electronic after removing the excess moisture using a blotting paper. The gonado-somatic index was calculated using the formula of Afonso-Dias *et al*. (2005).

$$GSI = \frac{\text{Gonad weight}}{\text{Fish weight}} \times 100$$

HSI of female and male fish were studied separately to relate with GSI of female. HSI was calculated according to the formula of Rajguru (1992).

$$HSI = \frac{\text{Weight of liver}}{\text{Weight of fish}} \times 100$$

For fecundity estimation, at first, whole ovary weight was measured. Then three sub-samples were taken from three different positions of the ovary. Then the total number of eggs was counted from each sub-sample. The number of eggs from each sub-sample was estimated by the following equation of Behera *et al*. (2010):

$$F = \frac{\text{Gonad weight} \times \text{Number of eggs in sub sample}}{\text{Sub-sample weight}}$$

*Experimental design*

For induced breeding of *M. vittatus*, 96 males and 64 females were collected from the rearing pond and separated and released in water circulate containers for acclimatization before hormone administration. Two sex ratios (male: female- 1:1 and 1:2) were designed for this experiment with two different induced hormones; Carp pituitary gland (CPG) and Flash (S-GnRHa). In each sex ratio, a control experimental group was kept which was designed without any hormone administration for captive breeding. In the sex ratio of 1:1 (F: M),



[ DOR: 20.1001.1.15622916.2022.21.1.20.6 ]

two females and two males were used (P1 to P6, O1 to O6, and PO1 to PO6) and two females and four males were used in the sex ratio of 1:2 (C1 to C6, D1 to D6, and CD1 to CD6) in each treatment. For each sex ratio, the induced hormone of CPG, flash and CPG plus flash were planned for female and male with the dose of 4-12 mg/kg and 2-6 mg/kg, 0.4 -1.2 mL/kg and 0.2-0.6 mL/kg, 2+0.2- 6+0.6 mg+mL/kg and 1+0.1-3+0.3 mg+mL/kg body weight of fish (Table 1). Water quality parameters such as dissolved oxygen (DO), temperature, total suspended solids (TDS), and pH were measured every 6 hours for each incubator treatment during the captive breeding of *M. vittatus*.

**Table 1: Experimental design for captive breeding of *M. vittatus.***

| Sex ratio (F:M) and brood numbers | CPG | | | Flash | | | CPG and Flash | | |
|---|---|---|---|---|---|---|---|---|---|
| | Treatment | Dose (mg/kg) Female | Dose (mg/kg)-Male | Treatment | Dose (mL/kg)-Female | Dose (mL/kg)-Male | Treatment | Dose (mg+mL/kg)-Female | Dose (mg+mL/kg)-Male |
| | **Control** | | | **Without hormone dose administration** | | | | | |
| | P1 | 4 | 2 | O1 | 0.4 | 0.2 | PO1 | 2+0.2 | 1+0.1 |
| 1:1 & | P2 | 6 | 3 | O2 | 0.6 | 0.3 | PO2 | 3+0.3 | 1.5+0.15 |
| 2F: 2M | P3 | 8 | 4 | O3 | 0.8 | 0.4 | PO3 | 4+0.4 | 2+0.2 |
| | P4 | 10 | 5 | O4 | 1.0 | 0.5 | PO4 | 5+0.5 | 2.5+0.25 |
| | P5 | 12 | 6 | O5 | 1.2 | 0.6 | PO5 | 6+0.6 | 3+0.3 |
| | **Control** | | | **Without hormone dose administration** | | | | | |
| | C1 | 4 | 2 | D1 | 0.4 | 0.2 | CD1 | 2+0.2 | 1+0.1 |
| 1:2 & | C2 | 6 | 3 | D2 | 0.6 | 0.3 | CD2 | 3+0.3 | 1.5+0.15 |
| 2F: 4M | C3 | 8 | 4 | D3 | 0.8 | 0.4 | CD3 | 4+0.4 | 2+0.2 |
| | C4 | 10 | 5 | D4 | 1.0 | 0.5 | CD4 | 5+0.5 | 2.5+0.25 |
| | C5 | 12 | 6 | D5 | 1.2 | 0.6 | CD5 | 6+0.6 | 3+0.3 |

*Weight and length of brood fish*

Healthy mature males and females were selected based on their maturation length and weight. The maturation length and weight were estimated depending on the secondary sexual characteristics (body shape, coloration, size, fins and ovipositor). In this experiment, the length and weight of male and female brooders for each treatment were maintained near-equal (statistically similar). The range of average weight and length of female and male were 18.4± 1.93 to 24.5±2.63 g, 11.5±0.71 to17.2±2.11 g and 12.4±0.6 to 15.5±1.5 cm, 6.2±0.4 to 9.4±0.9 cm, respectively (Table 2).

*Collection and preparation of the hormone*

As an inducing agent, dry carp pituitary glands (CPG) were collected from the market in suitable condition and stored in airtight vials.



[ DOR: 20.1001.1.15622916.2022.21.1.20.6 ]

**Table 2: Weight and length of male and female for captive breeding of *M. vittatus.***

| Sex ratio (F:M) | Sex | CPG | | | Flash | | | CPG and Flash | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Treatment | Avg. Weight (g) | Avg. Length (cm) | Treatment | Avg. Weight (g) | Avg. Length (cm) | Treatment | Avg. Weight (g) | Avg. Length (cm) |
| 1:1 | | **Cont.** | | | **Female 21.8± 1.89g, 15.2±0.9 cm; Male: 15.3±1.93g, 7.9±1.2 cm** | | | | | |
| | Female | P1 | 23.2±0.45 | 14.1±1.1 | O1 | 22.3±1.54 | 14.1±0.9 | PO1 | 19.7±1.43 | 13.4±0.6 |
| | | P2 | 21.8±1.26 | 12.4±0.6 | O2 | 19.8±1.63 | 12.9±1.1 | PO2 | 20.6±1.92 | 13.8±0.8 |
| | | P3 | 18.4±1.93 | 14.4±1.2 | O3 | 20.2±1.17 | 14.2±1.3 | PO3 | 23.8±2.11 | 15.1±1.1 |
| | | P4 | 24.1±2.47 | 13.3±1.1 | O4 | 24.2±1.38 | 15.1±1.2 | PO4 | 24.5±2.63 | 15.5±1.5 |
| | | P5 | 21.7±1.79 | 13.5±0.8 | O5 | 21.9±1.03 | 13.6±0.7 | PO5 | 22.4±1.45 | 13.7±0.8 |
| | Male | P1 | 12.4±0.99 | 7.9±0.5 | O1 | 11.8±0.54 | 6.9±0.7 | PO1 | 13.4±0.72 | 7.5±0.6 |
| | | P2 | 15.2±1.23 | 8.1±1.1 | O2 | 13.9±0.83 | 7.4±0.9 | PO2 | 12.9±0.34 | 6.6±0.4 |
| | | P3 | 14.8±0.67 | 8.0±0.6 | O3 | 14.1±0.49 | 7.8±1.0 | PO3 | 14.7±0.81 | 8.1±0.6 |
| | | P4 | 17.2±2.11 | 9.4±0.9 | O4 | 13.9±0.73 | 7.1±0.5 | PO4 | 13.6±0.46 | 7.4±0.8 |
| | | P5 | 15.4±0.73 | 8.2±0.5 | O5 | 12.8±0.48 | 7.0±0.7 | PO5 | 14.5±1.1 | 8.3±0.4 |
| 1:2 | | **Cont.** | | | **Female 19.4± 0.69g, 14.1±0.7 cm; Male: 14.2±0.82g, 7.3±0.8 cm** | | | | | |
| | Female | C1 | 19.9±0.81 | 12.4±0.6 | D1 | 20.4±1.1 | 15.1±0.7 | CD1 | 22.4±1.1 | 14.1±1.0 |
| | | C2 | 20.5±0.56 | 13.7±0.9 | D2 | 19.7±0.9 | 13.6±0.6 | CD2 | 23.7±0.9 | 14.3±0.7 |
| | | C3 | 23.1±0.79 | 13.6±0.8 | D3 | 21.5±1.1 | 15.2±0.9 | CD3 | 21.1±0.9 | 13.5±0.9 |
| | | C4 | 22.7±0.45 | 14.1±0.7 | D4 | 22.2±0.8 | 14.9±0.7 | CD4 | 20.8±0.7 | 13.2±0.8 |
| | | C5 | 19.80±1.1 | 12.9±0.6 | D5 | 18.7±0.9 | 13.4±1.0 | CD5 | 22.9±1.0 | 14.2±1.2 |
| | Male | C1 | 12.9±0.76 | 7.5±0.7 | D1 | 13.2±0.62 | 7.7±0.9 | CD1 | 12.7±0.87 | 7.2±0.8 |
| | | C2 | 13.4±0.58 | 7.2±0.8 | D2 | 11.9±0.81 | 6.2±1.0 | CD2 | 12.3±0.53 | 6.9±0.7 |
| | | C3 | 14.2±0.92 | 8.0±0.6 | D3 | 12.6±0.78 | 6.9±0.7 | CD3 | 11.5±0.71 | 6.2±0.4 |
| | | C4 | 12.2±0.53 | 6.7±0.8 | D4 | 13.2±0.82 | 7.8±1.1 | CD4 | 13.20±1.0 | 8.1±1.1 |
| | | C5 | 12.6±0.72 | 7.1±1.0 | D5 | 12.7±0.43 | 7.3±0.8 | CD5 | 12.9±0.61 | 7.4±0.6 |

The pituitary glands were gently removed from the vial with a pair of forceps, dried for 2-3 minutes with filter paper, and then weighed using an analytical electronic balance (College B204-S, Switzerland). The weighed CPG was placed in a tissue homogenizer and crushed thoroughly. Then the crushed CPG was dissolved in 100 mL distilled water and centrifuged (Aei-04, Ajanta 3000 rpm) manually until precipitation. The freshly formulated hormone supernatant was then slowly collected into a 1 mL hypodermic syringe. The quantity to be weighed out was determined using the following formula based on the total body weight of all fish (Alam *et al*., 2006):

Weight of CPG (mg)=Wt. x Pt/1000

where, Wt. represents total body weight (g) of all the fishes to be injected and Pt, represents the rate in mg CPG to be injected/kg body weight under a particular treatment.

Flash hormone (100 mg Domperidone+0.2 mg S-GnRHa) is a synthetic hormone and diluted with distilled water in 2mL/kg and desired amount was taken in a 1 mL hypodermic syringe based on fish body weight. On the other hand, based on fish body weight, diluted flash and CPG hormone were mixed and taken into the syringe. After the preparation of hormone, a single dose was administrated for both male and female beneath the muscular portion of the dorsal fin at 12:15 AM. Hormone preparation and administration method were followed by the Sundararaj *et al*. (1972). The fish were released at a sex ratio of 1:1 and 1:2 in separate glass tanks with a continuous air and water flow system after hormone administration.



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*Breeding performance*

Latency period for females following the injection was calculated according to the method of Shirley and Allen (2021).

Dead eggs appeared whitish and opaque within 8 to 10 hours after fertilization, while translucent eggs containing embryonic eyes at the time of polar cap formation (about 20 minutes after fertilization) were considered fertilized. The fertilization rate was calculated according to the Behera *et al*. (2010).

$$F= \frac{\text{No of fertilized eggs}}{\text{Total number of eggs}} \text{ x } 100$$

Direct counting was used to determine the hatching rate. The percentage of dead eggs in each basin was determined after the larvae hatched. Several hours after the incubation processes, the percentage of hatched larvae was counted. The hatching rate was determined according to the method of Islam *et al*. (2011):

$$\text{Hatching rate} = \frac{\text{No of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

For three days, the survival rate was calculated using the direct counting oflarvae in each six hours interval. The hatchlings were first placed in a white funnel with a capacity of 50 mL. Hatchlings were gathered from three bowls of uniformly distributed water. The number of hatchlings was counted with the naked eye. In the formula, the average values of three bowls were used. The survival rate was calculated following the method of Alam *et al*. (2006):

$$\text{Survival rate} = \frac{\text{No. of hatchlings survive}}{\text{No. of hatchlings at the beggeining}} \times 100$$

*Statistical analysis*

In all of the treatments, the results were estimated as mean±standard error (SE). The experimental calculations were statistically analyzed using SPSS (Statistics 21) software tools (version 17.0 for windows). To compare substantial levels of variations between the experimental observations, a One-Way analysis of variance (ANOVA) was used. Duncan's New Multiple Range Test (DMRT) was used to evaluate significant differences among means for significant results ($p$<0.05).

**Results**

*Breeding biology of M. vittatus*

The GSI gradually increased from mid-April to end-July, the average value was 9.16±0.85 to 25.54±5.86% where the weight and length were 22.59±2.93 to 24.74±3.57g and 13.4±2.1 to 14.4±1.7cm, respectively. The highest GSI value of females was 25.54±5.86 in mid-July and the lowest was 0.11±0.01 in mid-October. After end-July, the GSI value of females was started gradually decrease that means *M. vittatus* reached peak breeding condition in the mid of July. Based on the HSI values of males, it was increased gradually from mid-



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January to end-March and mid-August to end-December and decrease from end-May to end-July. In males, the lowest value of HSI was 2.40±0.08% in mid-July while the highest in mid-January (7.77±0.76%) (Table 3). Thus, low hepatic activity was found during the month of mid-July for both males and females, which also suggests that mid-July is the breeding month of *M. vittatus*.

**Table 3: GSI and HSI values of *M. vittatus.***

| Month | No. of fish examined | Female | | | | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Avg. Body weight (gm) | Avg. Total length (cm)-F | Avg. Gonad weight (gm) | Avg. Fecundity (no.) | Avg. Weight of liver (gm) | Avg. GSI (%) | Avg. HSI (%) | Avg. Body weight (gm) | Avg. Total length (cm)- | Avg. Weight of liver (gm) | Avg. HSI (%) |
| Mid -January, 18 | 14F & 12M | 16.28± 2.83 | 10.2± 1.2 | 0.09± 0.01 | NC | 1.32± 0.31 | 0.55± 0.04 | 8.10± 1.28 | 10.04± 1.28 | 6.9± 0.82 | 0.78± 0.03 | 7.77± 0.76 |
| End January,18 | 18F & 08M | 16.93± 1.74 | 11.1± 0.9 | 0.10± 0.02 | NC | 1.14± 0.23 | 0.59± 0.07 | 6.73± 1.01 | 11.19± 1.76 | 7.2± 0.48 | 0.83± 0.05 | 7.42± 0.64 |
| Mid -February, 18 | 17F & 11M | 17.68± 3.19 | 11.2± 1.3 | 0.49± 0.07 | NC | 1.21± 0.16 | 2.77± 0.43 | 6.84± 0.98 | 9.85± 1.03 | 6.7± 0.93 | 0.64± 0.09 | 6.50± 0.32 |
| End February, 18 | 20F & 13M | 19.39± 2.37 | 12.3± 2.1 | 0.69± 0.09 | NC | 1.01± 0.18 | 3.56± 0.86 | 5.21± 1.12 | 12.14± 2.19 | 8.1± 1.0 | 0.81± 0.05 | 6.67± 0.53 |
| Mid- March,18 | 19F & 08M | 20.65± 1.96 | 12.5± 1.9 | 1.04± 0.04 | NC | 0.87± 0.09 | 5.03± 1.16 | 4.09± 0.91 | 10.86± 1.28 | 6.8± 0.67 | 0.76± 0.07 | 7.01± 0.61 |
| End March, 18 | 25F & 13M | 21.93± 4.29 | 13.1± 1.5 | 1.71± 0.14 | 6517± 734 | 0.81± 0.12 | 7.79± 1.43 | 3.69± 0.65 | 12.43± 1.42 | 7.3± 0.83 | 0.83± 0.04 | 6.68± 0.44 |
| Mid- April, 18 | 18F & 15M | 22.59± 2.93 | 13.4± 2.1 | 2.07± 0.28 | 7439± 490 | 0.76± 0.17 | 9.16± 0.85 | 3.36± 0.29 | 13.68± 1.94 | 7.7± 0.63 | 0.76± 0.08 | 5.56± 0.52 |
| End April, 18 | 17F & 12M | 23.16± 2.77 | 13.5± 3.4 | 2.32± 0.76 | 9371± 347 | 0.76± 0.19 | 10.02± 3.37 | 3.28± 0.18 | 15.19± 1.47 | 7.4± 0.59 | 0.79± 0.08 | 5.20± 0.39 |
| Mid- May, 18 | 21F & 17M | 23.62± 1.89 | 13.4± 2.6 | 3.51± 0.83 | 17521± 782 | 0.59± 0.09 | 14.86± 3.11 | 2.49± 0.17 | 12.64± 1.84 | 7.1± 0.83 | 0.52± 0.04 | 4.11± 0.08 |
| End May, 18 | 16F & 10M | 24.74± 3.57 | 13.9± 1.9 | 4.21± 0.74 | 23964± 920 | 0.51± 0.06 | 17.01± 2.95 | 2.06± 0.21 | 10.94± 0.94 | 6.9± 0.61 | 0.37± 0.06 | 3.38± 0.18 |
| Mid-June, 18 | 24F & 16M | 24.32± 2.61 | 14.2±2. 1 | 5.18± 0.98 | 25072± 1396 | 0.41± 0.04 | 21.29± 4.78 | 1.68± 0.17 | 14.18± 1.92 | 7.2± 0.73 | 0.41± 0.07 | 2.89± 0.05 |
| End June, 18 | 23F & 19M | 23.19± 1.98 | 14.4± 1.7 | 5.62± 0.93 | 28409± 1097 | 0.39± 0.03 | 24.23± 3.29 | 1.68± 0.09 | 10.31± 1.05 | 8.1± 0.48 | 0.29± 0.04 | 2.81± 0.06 |
| Mid July, 18 | 19F & 13M | 23.37± 3.29 | 13.4± 1.2 | 5.97± 1.41 | 32794± 1284 | 0.41± 0.04 | 25.54± 5.86 | 1.61± 0.11 | 12.94± 1.74 | 8.2± 0.73 | 0.31± 0.07 | 2.40± 0.08 |
| End July, 18 | 26F & 10M | 22.66± 2.42 | 13.2± 1.9 | 4.14± 1.29 | 29429± 1039 | 0.57± 0.04 | 18.27± 4.17 | 2.51± 0.25 | 09.86± 1.06 | 7.8± 0.44 | 0.34± 0.08 | 3.45± 0.17 |
| Mid-August, 18 | 14F& 12M | 21.83± 3.87 | 12.8± 1.4 | 2.99± 0.75 | 17184± 953 | 1.02± 0.27 | 08.69± 1.14 | 4.67± 0.58 | 11.35± 2.17 | 7.5± 0.67 | 0.61± 0,.05 | 5.37± 0.36 |
| End August,18 | 18F & 10M | 21.08± 2.81 | 12.1± 2.1 | 1.04± 0.16 | 6573± 259 | 1.28± 0.37 | 4.93± 0.95 | 6.07± 0.73 | 10.74± 1.83 | 7.7± 0.49 | 0.64± 0.07 | 5.96± 0.62 |
| Mid-September, 18 | 17F & 08M | 19.86± 3.62 | 13.4± 3.2 | 0.64± 0.06 | 2109± 412 | 1.59± 0.21 | 3.22± 0.78 | 8.01± 0.97 | 11.19± 1.29 | 7.2± 0.38 | 0.71± 0.06 | 6.34± 0.26 |
| End September, 18 | 13F & 11M | 18.86± 2.95 | 12.3± 2.7 | 0.15± 0.02 | NC | 1.55± 0.24 | 0.79± 0.08 | 8.22± 0.86 | 12.25± 2.08 | 6.6± 0.28 | 0.79± 0.09 | 6.45± 0.31 |
| Mid October, 18 | 16F & 14M | 17.92± 3.17 | 13.6± 2.2 | 0.02± 0.00 | NC | 1.56± 0.22 | 0.11± 0.01 | 8.71± 1.1 | 10.95± 1.74 | 6.9± 0.38 | 0.77± 0.04 | 7.03± 0.54 |
| End October, 18 | 12F & 10M | 17.12± 2.48 | 12.5± 1.8 | 0.001± 0.0 | NC | 1.54± 0.18 | 0.15± 0.00 | 8.99± 1.3 | 11.32± 1.59 | 7.4± 0.73 | 0.72± 0.06 | 6.98± 0.27 |
| Mid- November, 18 | 16F & 13M | 16.49± 4.11 | 13.1± 2.5 | Nil | NC | 1.51± 0.14 | 0.12± 0.00 | 9.15± 0.99 | 12.21± 1.24 | 7.3± 0.63 | 0.69± 0.07 | 6.16± 0.33 |
| End November,18 | 19F & 11M | 16.87± 3.92 | 12.5± 1.7 | Nil | NC | 1.52± 0.17 | 0.17± 0.00 | 9.01± 0.64 | 11.42± 1.83 | 7.5± 0.53 | 0.63± 0.03 | 5.52± 0.15 |
| Mid -December, 18 | 17F & 10M | 17.45± 2.74 | 11.9± 2.1 | Nil | NC | 1.48± 0.21 | 0.25± 0.00 | 8.48± 0.91 | 10.93± 1.15 | 7.1± 0.64 | 0.74± 0.06 | 6.77± 0.39 |
| End December,18 | 18F & 12M | 18.05± 4.08 | 12.7± 1.3 | 0.08± 0.00 | NC | 1.44± 0.31 | 0.44± 0.02 | 7.97± 0.67 | 11.06± 1.09 | 7.2± 0.48 | 0.69± 0.04 | 6.24± 0.21 |

Note: F= Female, M= Male, NC= Not countable

*Fecundity estimation*

In this experiment, the fecundity of *M. vittatus* was gradually increased from end-March to mid-July and decreased afterward. The highest and lowest fecundity was 32794±1284 in mid-July and 2109±412 in mid-September (Table 3). On the other hand, fecundity cannot count by the magnifying glass from mid-January to mid-March and end-September to end-December due to tiny eggs. The highest fecundity leads to the peak breeding time that was mid-July for the *M. vittatus.*

*Breeding performance*

The mode of ovulation was natural. The latency period varied among treatments



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($p$<0.05). The latency period of *M. vittatus* administrated by CPG, flash, and CPG plus flash were 8-9 hr, 7-8 hr, and 6-7 hr ($p$<0.05). In this experiment, the CPG had a longer latency period (8-9 hours) than the CPG plus flash hormone, which had a shorter latency period (6-7 hours). Among the treatments, the highest fertilization rate was observed as 87.3±7.23% (P4), 84.2±7.66% (O3) and 88.1±7.52% (PO2) in the sex ratio of 1:1 and 92.3±8.59% (C4), 90.5±7.12 (D3) and 92.6±6.38 (CD2) in the sex ratio of 1:2. No fertilization occurred in control treatment of both sex ratios. Fertilization rates were varied based on sex ratio and were found to be higher in the 1:2 sex ratio compare to the equal hormone dosage. The ANOVA test indicated that there was a significant ($p$<0.05) difference in fifteen doses of CPG, flash, and CPG plus flash hormone at the sex ratio of 1:1 and 1:2 in the view-point of fertilization rate. DMRT for fertilization rate revealed that significantly higher in chronological order was P4>P5>P3>P2>P1, C4>C5>C3>C2>C1, O3>O4>O2>O5>O1, D3>D4>D5>D2>D1, PO2>PO3>PO1>PO4>PO5, CD2> CD3>CD1>CD4>CD5. The embryos' activity was detected 20-23 hours after fertilization. The P1 and PO2 treatments had a longer hatching period (23 hours), while the C3, C4, D4, PO5, and CD1 treatments had a shorter hatching period (20 hours), and the other treatments had longer hatching periods (21-22 hours). In the sex ratio of 1:1, the highest and lowest hatching rates with CPG, flash, and CPG plus flash were 70.62±6.29% (P4), 71.5±5.38% (O3), 74.7±4.36% (PO2) and 32.5±4.77 (P1), 27.2±1.22% (O1), and 27.8±3.44% (PO5), respectively. On the other hand, the highest and lowest hatching rates were calculated as 76.4±6.37% (C3), 78.6±5.48% (D3), 78.4±5.73% (CD2) and 41.8±2.66% (C1), 36.7±2.10% (D1), 40.2±4.16% (CD5) in the sex ratio of 1:2. In the view-point of hatching rate, the ANOVA test revealed a substantial ($p$>0.05) difference in fifteen doses of CPG, flash, and CPG plus flash hormone at each sex ratio of 1:1 and 1:2. DMRT shows that the chronological order of the hatching rate was significantly higher: P4>P5>P3>P2>P1, C3>C4>C5>C2>C1, O3>O4>O2>O5>O1, D3>D4>D5>D2>D1, PO2>PO3>PO1>PO4>PO5, CD2>CD3>CD1>CD4>CD5. After 24 hours of hormone injection, some males and females died in some treatments, including PO4 (1 female and 1 male), PO5 (1 male and 1 female), and CD5 (2 male and 1 female) (Table 4).



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**Table 4: Breeding performance of *M. vittatus***

| Sex ratio (F:M) & Brood Number (F & M) | Treatment | Latency period (hr.) | Fertilization rate (%) | Hatching period (hr.) | Hatching rate (%) | Dead occurred (no.) |
|---|---|---|---|---|---|---|
| | | | **Carp Pituitary Gland (CPG) Hormone** | | | |
| **1:1 & 2F 2M** | Cont. | - | - | - | - | - |
| | P1 | 8-9 | 56.4±4.32$^{d}$ | 23 | 32.5±4.77$^{d}$ | x |
| | P2 | 8-9 | 61.9±5.21$^{c}$ | 21 | 46.7±5.59$^{d}$ | x |
| | P3 | 8-9 | 79.2±4.65$^{b}$ | 21 | 63.6±3.57$^{c}$ | x |
| | P4 | 7-8 | 87.3±7.23$^{a}$ | 22 | 70.2±6.29$^{a}$ | x |
| | P5 | 7-8 | 81.7±2.89$^{b}$ | 21 | 67.4±5.61$^{b}$ | x |
| **1:2 & 2F 4M** | Cont. | - | - | - | - | - |
| | C1 | 8-9 | 65.3±6.62$^{c}$ | 21 | 41.8±2.66$^{c}$ | x |
| | C2 | 8-9 | 72.3±4.22$^{c}$ | 21 | 59.7±5.19$^{b}$ | x |
| | C3 | 7-8 | 84.9±7.41$^{b}$ | 20 | 76.4±6.37$^{a}$ | x |
| | C4 | 7-8 | 92.3±8.59$^{a}$ | 20 | 75.9±3.72$^{a}$ | x |
| | C5 | 7-8 | 90.8±6.82$^{a}$ | 21 | 73.2±6.87$^{a}$ | x |
| | | | **Flash (S-GnRHa) Hormone** | | | |
| **1:1 & 2F 2M** | Cont. | - | - | - | - | - |
| | O1 | 8-9 | 39.8±2.89$^{d}$ | 22 | 27.2±1.22$^{d}$ | x |
| | O2 | 8-9 | 51.4±4.72$^{c}$ | 21 | 38.9±3.17$^{c}$ | x |
| | O3 | 8-9 | 84.2±7.66$^{a}$ | 22 | 71.5±5.38$^{a}$ | x |
| | O4 | 7-8 | 73.6±3.71$^{b}$ | 21 | 62.4±3.27$^{b}$ | x |
| | O5 | 7-8 | 42.9±3.65$^{c}$ | 21 | 32.8±1.59$^{d}$ | 2F |
| **1:2 & 2F 4M** | Cont. | - | - | - | - | - |
| | D1 | 8-9 | 44.3±4.67$^{d}$ | 21 | 36.7±2.10$^{c}$ | x |
| | D2 | 8-9 | 59.8±2.89$^{c}$ | 22 | 44.9±3.74$^{c}$ | x |
| | D3 | 7-8 | 90.5±7.12$^{a}$ | 21 | 78.6±5.48$^{a}$ | x |
| | D4 | 7-8 | 83.7±5.69$^{a}$ | 20 | 72.4±4.61$^{a}$ | 1F |
| | D5 | 7-8 | 63.4±4.36$^{b}$ | 22 | 59.9±3.25$^{b}$ | 2F |
| | | | **CPG and Flash Hormone** | | | |
| **1:1 & 2F 2M** | Cont. | - | - | - | - | - |
| | PO1 | 7-8 | 58.9±3.12$^{c}$ | 22 | 46.8±3.77$^{b}$ | x |
| | PO2 | 7-8 | 88.1±7.52$^{a}$ | 23 | 74.7±4.36$^{a}$ | x |
| | PO3 | 6-7 | 67.3±3.59$^{b}$ | 21 | 56.7±2.88$^{b}$ | x |
| | PO4 | 6-7 | 47.9±2.87$^{d}$ | 21 | 39.1±1.79$^{c}$ | 1F, 1M |
| | PO5 | 6-7 | 38.9±1.64$^{d}$ | 20 | 27.8±3.44$^{d}$ | 1F, 1M |
| **1:2 & 2F 4M** | Cont. | - | - | - | - | - |
| | CD1 | 7-8 | 68.4±3.55$^{c}$ | 20 | 51.1±3.92$^{b}$ | x |
| | CD2 | 6-7 | 92.6±6.38$^{a}$ | 21 | 78.4±5.73$^{a}$ | x |
| | CD3 | 6-7 | 77.3±4.39$^{b}$ | 21 | 68.7±3.11$^{a}$ | x |
| | CD4 | 6-7 | 56.9±4.61$^{c}$ | 22 | 47.9±5.78$^{c}$ | 1F |
| | CD5 | 6-7 | 48.2±2.98$^{d}$ | 21 | 40.2±4.16$^{c}$ | 2M, 1F |

*Survival rate of larvae in incubator*

For three days, the survival rate of larvae in incubators was measured at six-hour intervals. During the study period, no food was provided to the larvae in the incubator. At the sex ratio of 1:1 and 1:2 with administration of CPG, latest (after three days) survival rate of larvae were 47±2.18% (P4)> 41±3.75% (P5)>39±2.67% (P3)>17±3.06% (P2)>14±2.39% (P1) and 64±2.83% (C4)> 54±4.65% (C3)> 52±3.58% (C5)> 46±2.71% (C2)> 24±3.69% (C1). After three days, the survival rates of larvae were at the 1:1 and 1:2 sex ratio with Flash agent were 60±2.09% (O4)> 57±3.17% (O3)>46±5.83% (O5)>38±1.92% (O2)>29±2.39% (O1)



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and 65±2.99% (D4)> 63±4.37% (D3)> 53±6.01% (D2)> 52±3.61% (D5)> 47±2.77 (D1). Through CPG plus flash treatment, at the sex ratio of 1:1 and 1:2; the final survival rates (after 3 days) of larvae were 66±4.07% (PO2)> 51±1.87% (PO1)> 50±3.82% (PO3)> 44±4.68% (PO5)> 42±2.96% (PO4) and 69±7.03% (CD2)> 56±3.77% (CD1)> 55±4.78% (CD3)> 51±2.95% (CD5)> 50±5.16% (CD4) (Table 5).

**Table 5: The survival rate of larvae in the incubator.**

| | | | 13.07. 2019 | | | | 14.07.2019 | | | | 15.07.2019 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hormone | Sex (F: M) | Treatment | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| CPG | 1:1 | P1 | 73±2.44 | 66±3.53 | 49±4.12 | 38±3.67 | 32±2.59 | 29±3.59 | 23±3.11 | 22±2.87 | 21±1.87 | 19±2.51 | 17±2.43 | 14±2.39 |
| | | P2 | 80±.6.73 | 69±4.28 | 56±4.67 | 43±2.87 | 42±3.62 | 38±2.14 | 34±2.88 | 29±4.81 | 28±3.17 | 23±2.88 | 21±3.61 | 17±3.06 |
| | | P3 | 89±5.44 | 78±3.69 | 69±5.39 | 61±4.81 | 60±5.14 | 59±3.35 | 53±4.37 | 51±2.59 | 47±4.36 | 45±1.53 | 41±1.79 | 39±2.67 |
| | | P4 | 91±6.92 | 85±6.17 | 76±4.16 | 70±3.59 | 65±7.24 | 62±4.49 | 61±4.61 | 58±3.46 | 55±3.71 | 49±4.29 | 48±2.77 | 47±2.18 |
| | | P5 | 87±5.35 | 79±5.38 | 72±2.84 | 68±6.82 | 62±4.66 | 57±2.25 | 52±4.43 | 49±5.82 | 47±3.47 | 46±6.03 | 43±5.64 | 41±3.75 |
| | 1:2 | C1 | 72±4.27 | 63±3.32 | 54±5.17 | 51±3.94 | 46±3.24 | 41±3.28 | 38±5.18 | 34±3.19 | 33±2.99 | 29±4.15 | 25±2.87 | 24±3.69 |
| | | C2 | 82±7.15 | 77±4.16 | 70±3.89 | 66±4.55 | 62±4.83 | 60±5.17 | 57±4.45 | 54±4.27 | 51±3.13 | 50±3.73 | 48±4.11 | 46±2.71 |
| | | C3 | 87±5.92 | 81±4.24 | 77±4.91 | 74±6.16 | 73±3.76 | 71±7.07 | 68±3.32 | 66±4.57 | 62±5.27 | 59±4.27 | 57±2.81 | 54±4.65 |
| | | C4 | 95±6.34 | 92±3.58 | 87±3.82 | 84±5.73 | 81±3.59 | 79±2.87 | 74±5.59 | 71±5.11 | 67±4.41 | 66±5.22 | 64±3.76 | 64±2.83 |
| | | C5 | 89±6.29 | 82±4.17 | 75±4.28 | 71±4.33 | 68±5.27 | 66±3.13 | 61±2.86 | 59±4.84 | 58±3.38 | 55±1.94 | 53±2.19 | 52±3.58 |
| Flash | 1:1 | O1 | 77±4.11 | 71±3.26 | 69±3.19 | 62±2.69 | 57±5.18 | 55±5.19 | 51±3.38 | 46±5.17 | 41±4.44 | 38±3.72 | 32±2.56 | 29±2.39 |
| | | O2 | 81±7.48 | 77±4.11 | 72±5.87 | 69±6.13 | 66±4.29 | 59±4.47 | 53±5.45 | 51±3.18 | 49±5.39 | 45±5.51 | 41±3.52 | 38±1.92 |
| | | O3 | 93±8.36 | 88±4.23 | 85±6.11 | 81±2.49 | 77±5.58 | 74±5.27 | 71±5.66 | 69±6.02 | 68±3.32 | 63±4.28 | 59±2.99 | 57±3.17 |
| | | O4 | 91±6.82 | 87±3.18 | 82±3.57 | 76±3.15 | 73±5.39 | 70±5.14 | 66±3.84 | 65±4.17 | 63±4.75 | 62±3.37 | 61±4.14 | 60±2.09 |
| | | O5 | 86±5.99 | 82±3.35 | 78±4.29 | 72±4.29 | 68±4.72 | 65±3.36 | 60±4.96 | 58±3.09 | 53±4.21 | 51±4.29 | 49±2.07 | 46±5.83 |
| | 1:2 | D1 | 85±6.37 | 81±4.27 | 79±4.19 | 76±3.47 | 73±4.29 | 71±6.29 | 69±3.74 | 65±4.16 | 61±2.77 | 56±3.12 | 51±1.93 | 47±2.77 |
| | | D2 | 88±4.69 | 85±4.47 | 82±6.14 | 79±5.88 | 76±3.33 | 72±2.61 | 68±6.13 | 65±5.29 | 62±6.18 | 58±3.73 | 55±2.46 | 53±6.01 |
| | | D3 | 94±7.39 | 90±5.29 | 86±3.59 | 81±5.83 | 78±8.25 | 75±4.83 | 73±7.70 | 71±4.25 | 69±3.23 | 68±6.02 | 66±5.11 | 63±4.37 |
| | | D4 | 93±5.74 | 87±4.28 | 83±5.25 | 81±4.39 | 78±5.73 | 75±5.58 | 72±7.24 | 70±4.35 | 69±5.74 | 67±5.17 | 66±2.85 | 65±2.99 |
| | | D5 | 88±6.47 | 86±3.17 | 81±8.24 | 77±3.16 | 75±4.83 | 71±3.52 | 67±5.18 | 63±5.15 | 60±3.26 | 57±3.85 | 55±1.72 | 52±3.61 |
| CPG and Flash | 1:1 | PO1 | 84±5.66 | 77±3.32 | 71±5.18 | 70±6.29 | 67±6.46 | 63±4.66 | 61±4.74 | 60±2.63 | 59±4.81 | 55±5.77 | 53±2.71 | 51±1.87 |
| | | PO2 | 96±6.38 | 91±2.96 | 89±3.69 | 85±7.14 | 81±3.29 | 79±4.89 | 78±4.59 | 73±3.94 | 70±3.52 | 68±3.71 | 67±1.99 | 66±4.07 |
| | | PO3 | 83±4.76 | 80±5.38 | 76±4.82 | 71±5.48 | 68±6.78 | 63±8.12 | 59±4.65 | 58±5.12 | 57±5.27 | 53±6.06 | 51±1.42 | 50±3.82 |
| | | PO4 | 82±5.66 | 76±6.14 | 73±3.17 | 70±5.86 | 65±4.43 | 62±7.47 | 59±3.59 | 55±3.61 | 51±7.13 | 48±3.13 | 44±3.33 | 42±2.96 |
| | | PO5 | 84±7.59 | 80±4.26 | 77±3.59 | 72±4.49 | 69±7.75 | 65±3.85 | 61±7.29 | 59±4.28 | 55±2.63 | 51±5.56 | 47±2.49 | 44±4.68 |
| | 1:2 | CD1 | 86±6.14 | 84±4.89 | 80±6.38 | 78±5.87 | 74±6.16 | 70±4.59 | 67±4.48 | 65±5.18 | 61±1.96 | 58±3.19 | 56±1.92 | 56±3.77 |
| | | CD2 | 89±3.56 | 86±3.18 | 83±3.35 | 81±6.79 | 80±2.97 | 77±5.17 | 74±3.11 | 73±2.69 | 71±5.75 | 70±4.64 | 69±3.96 | 69±7.03 |
| | | CD3 | 90±6.58 | 88±4.82 | 84±7.29 | 80±4.18 | 78±5.72 | 74±4.26 | 71±4.87 | 69±4.48 | 63±4.28 | 60±3.73 | 57±2.15 | 55±4.78 |
| | | CD4 | 88±7.35 | 84±3.82 | 80±6.11 | 77±3.37 | 73±4.27 | 70±4.47 | 65±7.19 | 61±3.53 | 57±5.17 | 55±5.75 | 53±4.81 | 50±5.16 |
| | | CD5 | 87±4.62 | 83±4.72 | 79±4.25 | 75±4.43 | 71±7.13 | 69±3.47 | 66±2.63 | 63±5.19 | 60±4.09 | 56±2.67 | 53±3.77 | 51±2.95 |

*Water quality parameters*

During captive breeding of *M. vittatus*, water parameters such as pH, temperature, DO, and TDS were measured in the incubators of different treatments. For incubators, the water pH ranged from 7.42±0.03 to 7.56±0.03. The highest pH of the incubator was



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found in the treatment of D3 (7.56), whereas the lowest was found in P2 treatment (7.42). The water temperature ranged from 28.2±0.26 to 30.6±0.29°C. CD5 had the highest temperature (30.6°C) and PO3 had the lowest temperature (28.2°C) in the incubator. DO levels ranged from 7.69±0.27 (P5) to -9.81±0.11 mg/L (P4). The TDS ranged from 150±07 (O4) to 215±16 mg/L (C5) (Table 6).

**Table 6: Water quality parameters in the incubators.**

| Treatment | 13.07. 2019 | | | | 14.07.2019 | | | | 15.07.2019 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | Temperature (°C) | DO (mg/L) | TDS (mg/L) | pH | Temperature (°C) | DO (mg/L) | TDS (mg/L) | pH | Temperature (°C) | DO (mg/L) | TDS (mg/L) |
| Control | 7.47±0.02 | 29.2±0.69 | 9.01±0.14 | 152±11 | 7.48±0.01 | 29.2±0.54 | 8.45±0.06 | 178±13 | 7.51±0.02 | 29.4±0.30 | 8.74±0.22 | 184±17 |
| P1 | 7.45±0.01 | 29.2±0.48 | 8.72±0.09 | 167±14 | 7.46±0.01 | 29.1±0.34 | 9.11±0.09 | 190±10 | 7.42±0.03 | 29.6±0.43 | 8.48±0.15 | 188±19 |
| P2 | 7.47±0.03 | 29.6±0.49 | 9.02±0.11 | 159±12 | 7.44±0.02 | 28.9±0.55 | 8.87±0.06 | 187±06 | 7.52±0.02 | 29.3±0.65 | 9.01±0.16 | 178±23 |
| P3 | 7.44±0.01 | 29.9±0.82 | 8.86±0.10 | 152±12 | 7.48±0.01 | 29.4±0.67 | 8.56±0.10 | 174±09 | 7.43±0.04 | 29.9±0.23 | 8.83±0.27 | 199±29 |
| P4 | 7.45±0.02 | 30.5±0.05 | 8.67±0.15 | 167±15 | 7.52±0.03 | 28.8±0.39 | 9.81±0.11 | 168±13 | 7.54±0.04 | 29.6±0.41 | 7.99±0.31 | 189±17 |
| P5 | 7.54±0.03 | 30.3±0.65 | 7.92±0.11 | 170±19 | 7.49±0.02 | 29.6±0.61 | 8.92±0.09 | 206±12 | 7.55±0.01 | 29.2±0.40 | 7.69±0.27 | 188±11 |
| C1 | 7.54±0.03 | 30.2±0.46 | 8.12±0.08 | 158±13 | 7.43±0.01 | 29.7±0.38 | 9.56±0.23 | 188±10 | 7.50±0.02 | 28.9±0.43 | 9.01±0.33 | 205±10 |
| C2 | 7.49±0.04 | 30.5±0.52 | 8.56±0.07 | 161±11 | 7.44±0.03 | 29.7±0.48 | 8.95±0.19 | 159±09 | 7.49±0.02 | 29.6±0.49 | 8.49±0.09 | 189±09 |
| C3 | 7.43±0.02 | 30.4±0.47 | 8.59±0.09 | 163±14 | 7.45±0.02 | 29.7±0.65 | 8.11±0.09 | 180±08 | 7.51±0.01 | 29.9±0.32 | 9.24±0.11 | 188±15 |
| C4 | 7.43±0.01 | 30.4±0.55 | 8.12±0.11 | 168±12 | 7.44±0.04 | 30.0±0.47 | 8.05±0.17 | 208±11 | 7.40±0.03 | 30.1±0.41 | 8.92±0.21 | 201±10 |
| C5 | 7.44±0.04 | 29.9±0.37 | 7.98±0.12 | 154±14 | 7.44±0.02 | 30.0±0.37 | 8..87±0.15 | 215±16 | 7.43±0.03 | 30.3±0.27 | 7.99±0.18 | 207±13 |
| O1 | 7.44±0.03 | 29.8±0.42 | 8.48±0.09 | 163±15 | 7.45±0.02 | 30.1±0.66 | 8.48±0.22 | 177±13 | 7.47±0.03 | 30.2±0.30 | 8.39±0.13 | 189±18 |
| O2 | 7.45±0.04 | 30.4±0.68 | 8.77±0.05 | 155±16 | 7.45±0.03 | 29.8±0.72 | 8.13±0.13 | 162±09 | 7.42±0.01 | 30.5±0.26 | 8.42±0.27 | 179±10 |
| O3 | 7.45±0.04 | 30.5±0.57 | 9.13±0.06 | 153±09 | 7.47±0.02 | 29.4±0.28 | 8.27±0.09 | 203±11 | 7.43±0.02 | 30.4±0.27 | 9.01±0.23 | 200±08 |
| O4 | 7.45±0.02 | 30.4±0.45 | 8.65±0.08 | 150±07 | 7.43±0.03 | 29.3±0.62 | 8..11±0.08 | 156±15 | 7.48±0.02 | 30.4±0.34 | 7.94±0.19 | 201±17 |
| O5 | 7.43±0.05 | 29.6±0.55 | 9.53±0.09 | 162±06 | 7.46±0.04 | 28.9±0.28 | 8.46±0.17 | 185±10 | 7.51±0.03 | 29.7±0.15 | 8.53±0.18 | 207±07 |
| D1 | 7.44±0.04 | 29.6±0.61 | 8.45±0.09 | 167±10 | 7.40±0.01 | 28.8±0.39 | 7.98±0.11 | 214±15 | 7.45±0.01 | 29.7±0.21 | 8.49±0.12 | 188±09 |
| D2 | 7.47±0.03 | 29.9±0.34 | 8.73±0.11 | 155±08 | 7.41±0.01 | 28.6±0.44 | 8.42±0.19 | 198±09 | 7.47±0.01 | 29.2±0.24 | 8.68±0.14 | 194±12 |
| D3 | 7.46±0.04 | 30.5±0.58 | 8.83±0.15 | 163±06 | 7.52±0.02 | 28.9±0.52 | 8.08±0.10 | 187±07 | 7.56±0.03 | 28.9±0.28 | 8.38±0.18 | 196±10 |
| D4 | 7.45±0.04 | 30.3±0.47 | 9.37±0.09 | 152±11 | 7.48±0.02 | 29.6±0.47 | 8.46±0.08 | 182±12 | 7.45±0.02 | 29.6±0.24 | 9.32±0.09 | 201±15 |
| D5 | 7.44±0.03 | 30.2±0.52 | 8.87±0.14 | 159±10 | 7.42±0.03 | 29.9±0.29 | 8.17±0.06 | 184±11 | 7.49±0.02 | 29.5±0.32 | 7.94±0.08 | 204±10 |
| PO1 | 7.47±0.04 | 30.5±0.26 | 8.82±0.11 | 163±09 | 7.42±0.04 | 30.1±0.26 | 8.58±0.17 | 210±09 | 7.54±0.04 | 28.9±0.11 | 8.83±0.10 | 187±15 |
| PO2 | 7.44±0.03 | 29.7±0.31 | 8.83±0.13 | 158±05 | 7.44±0.03 | 30.0±0.56 | 8.22±0.16 | 186±12 | 7.54±0.02 | 28.6±0.27 | 8.69±0.13 | 183±12 |
| PO3 | 7.48±0.01 | 30.4±0.22 | 9.21±0.05 | 152±08 | 7.45±0.03 | 30.2±0.49 | 8.95±0.21 | 169±16 | 7.51±0.01 | 28.2±0.26 | 8.63±0.09 | 204±13 |
| PO4 | 7.45±0.05 | 30.5±0.45 | 8.75±0.09 | 157±04 | 7.46±0.02 | 30.2±0.64 | 8.23±0.18 | 205±10 | 7.48±0.01 | 28.5±0.18 | 8.73±0.16 | 200±11 |
| PO5 | 7.48±0.02 | 30.4±0.37 | 8.59±0.10 | 160±09 | 7.46±0.02 | 30.1±0.18 | 8.28±0.16 | 194±17 | 7.51±0.03 | 28.7±0.29 | 8.71±0.08 | 194±15 |
| CD1 | 7.47±0.02 | 30.3±0.29 | 8.65±0.16 | 162±07 | 7.44±0.03 | 30.2±0.35 | 8..11±0.09 | 179±12 | 7.49±0.01 | 28.9±0.17 | 8.89±0.11 | 196±09 |
| CD2 | 7.46±0.03 | 29.5±0.36 | 8.54±0.11 | 159±08 | 7.48±0.03 | 29.7±0.66 | 8.63±0.07 | 200±16 | 7.43±0.01 | 28.6±0.36 | 9.25±0.15 | 197±06 |
| CD3 | 7.44±0.01 | 29.7±0.41 | 8.65±0.09 | 156±08 | 7.52±0.02 | 29.7±0.24 | 8. 78±0.16 | 198±07 | 7.48±0.02 | 29.6±0.34 | 8.74±0.09 | 201±14 |
| CD4 | 7.45±0.03 | 29.8±0.52 | 8.74±0.08 | 162±10 | 7.49±0.01 | 29.7±0.51 | 8.17±0.18 | 186±12 | 7.42±0.03 | 29.9±0.26 | 8.62±0.08 | 200±12 |
| CD5 | 7.47±0.02 | 29.7±0.44 | 8.59±0.12 | 166±07 | 7.43±0.02 | 29.9±0.47 | 8.24±0.17 | 179±13 | 7.48±0.03 | 30.6±0.29 | 8.26±0.06 | 197±10 |

## Discussion

Breeding biology is the basic stool for successful captive reproduction of fish species. Gonado- somatic index (GSI) and hepatic-somatic index (HSI) indicate the breeding season of fish. The average range of GSI was from 0.11±0.01% to 25.54±5.86% from January to December whereas a higher GSI value (female) was calculated from the end-April to end-July but the highest in mid-July (25.54±5.86%). Bhuiyan *et al.* (2018) reported that the highest GSI value was calculated as 20.81±2.73% in July while the lowest GSI value (0.88±0.06%) was in December. On the other hand, the lower value of HSI (female) was found from mid-May to end-July, and the lowest value was 1.61±0.09% in mid-July. The average range of HSI value of male was 2.40±0.08-7.77±0.76% where the highest value was found in mid-January and lowest in mid-July. The GSI and HSI values indicated that *M. vittatus* may breed in the mid-May to end-July which is supported by another study (Basu *et al.*, 2013). For the species of *Ompok pabda*, the breeding season lasts from April to May in Bengal and Assam, and it lasts until the end of July in Assam (Chakraborty *et al.*, 2007). The GSI and HSI values may be varied due to environmental conditions, physico-



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chemical factors of water and geographical location, and length and weight of fish species. Banu and Ali (1992) reported that the peak spawning season of *Mystus tengra* to be in July. Faruq (1995) reported that the peak breeding season of *Heteropneustes fossilis* (Bloch), *Clarias batrachus* (Linnaeus), *Mystus cavasius* (Hamilton), and *M. vittatus* (Bloch) in June and July.

Mature eggs were laid down by a brood fish in their spawning season which can be determined by artificial stripping to estimate the fecundity. The spawning stock's reproductive ability is determined by fecundity. In this study, higher fecundity was found during the mid-July which can lead to the breeding season of *M. vittatus*. Bhuiyan *et al*. (2018) reported that the range of fecundity of *M. vittatus* was 13138±1365.94 to 25095.2±6792.5. Similarly, Islam *et al*. 2011 reported the fecundity of 18,210 to 44,620 that supports the present study. The variation of fecundity may depend on the environmental condition and location of the water body. In another species in the same genus of *Mystus* (*M. gulio*), the range of fecundity was 11887 to 21589 (Sarker *et al*., 2002) and 6,770 to 21,708 in *M. tengra* (Gupta and Banerjee, 2013).

The success of captive breeding depends on the maturity of brooders, sex ratio, hormone dose, type of hormone, brood quality, and physio-chemical parameters of water and water exchange rate (Afroz *et al*., 2014). In general, the captive breeding of catfish is virtuously responded with the synthetic hormone (S-GnRHa). The hormonal induction dose varies between species; some fish need a very high dose, some need a small dose and some need a moderate dose (Hoq, 2006). The sex ratio (F: M) was 1:1 and 1:2 in the present study. Ovulation, fertilization and hatching rates depend on the sex ratio, and more males can be influenced by the breeding parameters (Islam *et al*., 2011). The range of CPG, Flash and CPG plus flash was 2-12 mg/kg, 0.2-1.2 mL/kg and 1+0.1- 6+0.6 mg-mL/kg body weight. Some similar studies have given details in the context of the type of hormone, dose, and sex ratio.

The time delay interval for the injection of hormones and the first appearance of the eggs is the latency period. CPG plus flash hormone showed a shorter latency period (6-7 hr) compare to flash (7-8 hr) and CPG (8-9 hr) in the captive breeding of *M. vittatus*. Some similar studies showed the latency period of 6-10 hrs at different hormone doses (Bhuiyan *et al*., 2018), 6-8 hr at ambient temperature (Alam *et al*., 2006 and Begum *et al*., 2009), and lower than the finding of Islam *et al*. (2011), Mukherjee *et al*. (2002), and Kumar *et al*., 2018 at 28°C. In this experiment, the use of females early in maturity could create a longer latency period than that of females in late maturity.

The rates of fertilization and hatching reflect the well-being and efficiency of the brooders used during the reproduction processes. Certain factors are responsible for breeding success which includes good management of brood fish, age and size (Bromage, 1998), feeding and manuring (Springate *et al*. 1985), hormone dose (Nandeesha



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*et al*. 1990), and egg ripening efficiency (Springate *et al*. 1985). In many ways, determining the hatching rate of a fish is important for successful induced breeding. It can figure out how many fries can be produced from a given number of fish, as well as how many are lost and why. It can improve the hatchery products and, as a result, higher efficiency can be achieve. Hormonal dosages and sex ratio influenced the fertilization and hatching rate in this experiment. The low dosage of inducing hormone has resulted in late induction of species, whereas overdose has brought about in early milting. Among the doses, fertilization, hatching rate, and survival rate were found higher with the CPG plus flash hormone compare to the other hormone doses. The suitable hormone doses in triggering successful fertilization, hatching rate and survival rate were 3 mg CPG+0.3mL flash body weight/kg for both treatment of PO2 and CD2. In this study, the sex ratio affects the fertilization and hatching rate of *M. vittatus*. Data showed a higher fertilization and hatching rate with the 1:2 sex ratio based on the equal hormone dose. Fertilization and hatching rates were higher compared to the other findings of Alam *et al*. (2006), Islam *et al*. (2011), Bailung and Biswas (2014), Bhuiyan *et al*. (2018), and Kumar *et al*. (2018) that has shown in Table 7.

**Table 7: Comparative study in the context of the hormone, dose, and sex ratio of *M. vittatus*.**

| Species | Hormone | Dose (Female ) mg/kg or ml/kg | Dose (Male) mg/kg or ml/kg | Sex ratio (F:M) | References |
|---|---|---|---|---|---|
| *M. vittatus* | CPG | 6, 8 and 10 | 2, 4, 6 | 1:1 | Bhuiyan *et al.*, 2018 |
| | S-GnRHa | 0.5, 1.0 and 2.0 | | | |
| | CPG | 6, 8, 10, 12 | 3, 4, 5, 6 | 1:1, 1:2, 2:3 | Islam *et al*, 2011 |
| *Mystus dibrugarensis* | S-GnRHa | 1, 1.5 and 2.0 | | 1:2, 1:3 | Bailung and Biswas, 2014 |
| *M. gulio* | Ovaprium (S-GnRHa) | 1, 1.5 and 2.0 | | 1:1 | |
| | HCG | 10 IU/g | 5 IU/g | 1:2 | Kumar *et al*., 2018 |

The survival rate of hatching larvae depends on the higher and lower doses of hormone and this rate is the most important factor in fish production. In a three days trial in the incubator, the survival rate of *M. vittatus* was varied from 14.2±2.39 to 69±7.03%. This survival rate was within the range of other studies of Alam *et al*. (2006), Islam *et al*. (2011), and Kumar *et al*. (2018) (Table 8).



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**Table 8: Comparative study in the context of fertilization rate, hatching rate, and survival rate.**

| Species | Hormone | Fertilization rate (%) | Hatching rate (%) | Survival rate (%) | References |
|---|---|---|---|---|---|
| *M. vittatus* | CPG | 57-80 | 32-56 | 50-68 | Islam *et al.*, 2011 |
| | CPG and Flash | 68±8.89-83.33±1.67 | 10±2.9-69±1.0 | - | Bhuiyan *et al.*, 20018 |
| *Mystus dibrugarensis* | Ovaprium<br>HCG | Ovaprium<br>HCG | 80.7-84.7<br>50-74 | 71.3-72.7<br>55-75 | 55.5-67.3<br>48-52 |
| *M. dibrugarensis* | Ovaprium | 34.83-77.54 | 20.61-74.32 | - | Bailung and Biswas, 2014 |

Quality fish seed and fish production are heavily reliant on water quality, which has been identified as a critical factor in the success or failure of a fish culture operation (Mayer, 2006). Fertilization rate, quality of hatching rate, and survival rate of larvae influence by the water parameters (Alam *et al*., 2009). In this experiment the range of water quality parameters was within the ideal range of Bangladeshi fish hatchery and the values of temperature, pH, DO, TDS and ammonia were 28.5°C, 7.9 to 8.4, 6.8 to 7.8 mg/L , 146 to 200 mg/L and 0.04 to 0.06 mg/L, respectively in Bangladeshi fish hatcheries (Mou *et al*., 2018). According to Ahmed (1997), the minimum water quality for fish health should be 5 ppm, 6.7-8.6, <3 ppm, <0.02, and >20 ppm for DO, pH, free $CO_2$, ammonia, and alkalinity. The active spawning operation in the experimental tank coincided with the mean water temperature (25.33±0.88$^{o}$C), pH (7.30±0.05), and DO (11.13±0.41 mg/L) during the breeding season (Borah *et al*., 2020) which was a little difference from the present study.

Proper control of water parameters, the health of brooders and appropriate dose of hormone are the basis of successful induced breeding. Proper rearing and culture of this species will further increase the population. Dose optimization is an important aspect of the successful breeding program. CPG plus flash hormone gave better results in the case of fertilization, hatching, and survival rates. Among the 15 doses, the best result was found with the dose of 3 mg+0.3 mL (PO2 and CD2) for the successful breeding of *M. vittatus*. Between the sex ratio (1:1 and 1:2), the best performance was found with the sex ratio of 1:2 (F: M). The goal is that seed production of this species through captive breeding which can save *M. vittatus* from extinction and protection in nature by suitable management.

**Acknowledgment**

The authors would like to acknowledge the operational support of Bismillah Fish Seed Production Center and Farm, Langolcourt, Comilla, Bangladesh and also acknowledge the NATP-2, Bangladesh Agriculture Research Council, Dhaka, Bangladesh for the Research grants (NATP-2/PIU-BARC/Research CRG/2017/553) awarded during the study period.



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