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INDUCED BREEDING AND FRY NURSING OF *MYSTUS VITTATUS* (BLOCH 1797)

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Abstract: The work is related to optimization of PG dose and survivability of the spawn of *Mystus vittatus* in the tank nursery at Arabpur Fish Farm in Jessore, Bangladesh during 2000 to 2002. Trial doses of PG used for induced breeding of *M. vittatus* were 1 to 4 mg PG in the first dose and 4 to 8 mg in the second dose for female. On the other hand, the male fishes were administered only a single trial dose of 2, 3 and 4 mg PG. Induced breeding was successfully done in *M. vittatus* with 3 mg of PG kg^{-1} body weight in first dose and 6.0 mg PG in second dose for female and a single dose of 3 mg PG for male for virgin fish, F_1 and F_2 generation. The tank nursery performance of the fry was studied in regard to length, weight, production and survivability rate. Live animal feeds (rotifers) were used. The highest growth and survivability of the fish fry was obtained by using rotifers of 40 $cumm^{-1}$ and 20 $cumm^{-1}$, respectively.

Key words: Induced breeding, fry nursing, *Mystus vittatus*

Introduction

Bagrid species, though small in size, are commercially important. They are important as food fish, especially to the low middle class and poor people because of being comparatively cheaper and very tasty (Reddy, 1993). The Bagrid catfishes are widely distributed in Asia and Africa. They are mainly freshwater inhabitants, but one species i.e., *Mystus gulio* is partly marine. There are about 27 genera in Bagridae family occurring in the world, of which only 6 genera are reported from this subcontinent (Talwar and Jhingran, 1991). *Mystus* is one of the six genera that are found in abundance throughout Bangladesh.

On the average, the fish shows the highest feeding activity in the months of August to November. There are no marked differences of food choice of these fish. The fish feeds on whatever is available in the habitats. This fish is neither a true surface feeder nor a true bottom feeder since its food substances are distinguished through the different strata of the water bodies (Bhuiyan and Haque, 1984). The insect larvae dominate the food items and are followed by crustacean larvae, plant remains, rotifers and chironomid larvae, *Cypris*, besides sand and mud and unidentified food materials.

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Among many studies on different aspects of *M. vittatus*, the worth-mentioning include food and feeding habits of *M. vittatus* by Sharma (1978), biology by Bhatt (1971) and fecundity by Malhotra *et al.* (1979). The length-weight relationship and condition factor of *M. vittatus* from Bangladesh were studied by Hoque and Hossain (1993). However, little is known about spawning and breeding behaviour of the fish (Khan, 1924; Saigal and Motwani, 1962). Therefore, the present work was undertaken to observe induced breeding and nursery practices of *M. vittatus*.

Materials and Methods

***Species collection*:** A total of 200 pairs of mature healthy brood fishes were collected from the local market which were previously caught from Kopotakkha river and transported to Arabpur Fish Farm, Jessore where the experiment was conducted.

***Induced breeding*:** Eight trial doses for female and three for male were used to find out the breeding performance with pituitary gland (Table 1). The interval between first and second injection was 6 hrs for female but a single dose was administered to male during the first dose of female.

***Female and male brood separation*:** The fishes were separated by sex and kept in plastic bucket after applying the required doses of PG. The plastic bucket was filled up to 25 cm, continuous tap water for each plastic bucket with nylon mesh windows at three sides to discharge water. A mesh net was placed on the top of the plastic bucket to prevent the escape of fishes by jumping until the females received their second dose of PG.

***Hormonal extract preparation and injection*:** The acetone dried PGs were first placed on blotting paper so as to remove the excess acetone as much as possible followed by weighing with a sensitive scientific balance to pool the desired amount of PG. The pooled amount of PGs were finely crushed by tissue homogenizer and diluted with required amount (fish was injected with no more than 0.4 to 0.5 ml, to avoid injury) of distilled water. The solution was centrifuged to settle the tissue residue at the bottom and the resulting supernatant solution was then taken for injection with diabetic syringe. According to Rottmann *et al.* (1991), the concentration of hormone mixed in recommended dose, multiplied by the approximate weight of individual brood fish, was divided by the desired volume of the injection.

The volumes of injections were controlled at 0.1 to 0.2 ml for each parent fish. Various doses of PG were administered intramuscularly about 4 mm up from the lateral line longitudinal to the first spine of the dorsal fin. The second injection for female was administered at the opposite side of the first injection. In this regard, the fish was hold in one hand while administering the injection.

***Spawning in hapa*:** After second dose to female both male and female breeders were carefully transferred to spawning hapa (180 cm X 160 cm X 75 cm) constructed in a cistern (300 cm X 180 cm X 75 cm) having water shower on the top and flow from two sides at the total rate of 5.0 l sec^{-1} (gamete ratio of female and male was 1:1). The breeders were removed from the hapa after the ovulation (within 6 hrs was completed on the serrated leaves of date twigs provided earlier). The water quality parameters of hatching hapa was recorded as water temperature, 27-28 ºC; pH, 7.2-7.8; DO, 5.4-6.2 mg l^{-1}; total alkalinity, 380-410 mg l^{-1}; and total hardness, 450-510 mg l^{-1}.

Determination of percentage and duration of different stages: Fertilization percentage of eggs collected from the leaves of date twigs were determined under compound microscope after 4 hrs of spawning. Hatching rate was calculated by the following: number of hatchlings/number of fertilized eggs x 100. Deformities rates were also estimated by eye observation. The experiment was designed in eight independent treatments and there were three replicates in each treatment.

***Fry nursing*:** Nursery practices from yolk sac absorption to juvenile stages in tank nursery were done from 11 to 28 June, 2002. Physico-chemical parameters from the rectangular nursery tank were not measured because the exchange of water was done for several times.

***Nursery tank preparation*:** Rotifer was cultured in (15 cm X 10 cm X 5 cm) rectangular tanks so as to meet the nursery requirement regularly. The selected tanks were thoroughly cleaned and filled with filtered

freshwater for one feet and constantly aerated. The tanks were covered with closed mesh nets to avoid the interference of dragon fly and when necessary covered by white polythene especially during rainfall. The tanks were treated with mustard oil cake, triple super phosphate and urea at rate 75 mg l^{-1}, 7 mg l^{-1}, and 8 mg l^{-1}, respectively. These fertilizers were kept in a container for 12 hr with water for conditioning and the resulting extracts were used as fertilizer every morning at 10 am. After fertilization, phytoplankters taken from pond water (by filtering with 60 µm mesh cloth dominated by *Chlorella*) was inoculated as a food for rotifer culture. However, application of fertilizer was stopped for certain days to avoid excess bloom of plankters. Seeds of rotifer were collected from pond water by filtering through a 150 µm mesh sieve and inoculate into the tank. The population of rotifer started to grow massively after three days of fertilizer application and the spawn was released into the tank just after 7 days of first manuring.

Stocking of spawn: Rotifers multiplied rapidly, feeding on phytoplankton swam which were developed by utilizing fertilizers applied and the sunlight and organic matter which attained a peak density of 1,00,000 to 1,50,000 individuals l^{-1} in 5 to 7 days as measured with Sedgwick Rafters Counting Cell (S-R Cell). After attaining peak density, spawns (20 g) were stocked into the tank water and nursing was maintained for 10 days. The experiment was designed into three independent treatments and there were three replications in each treatment.

Post-stocking management: When rotifer population started to decline due to continuous feeding by the fry, half of the volume of culture water was removed from the bottom using filtered method by cloth and replaced with fresh filtered water.

The water was then fertilized for regeneration of plankton as described above. Depending on the quality and colour of tank water, application of fertilizer was started at a rate half of the initial dose after the stocking of spawn. Finally, the nursed fry of *M. vittatus* were released into the rearing pond to rear the fry until fingerling to brood fish stage.

***Statistical analysis*:** The data on breeding performance and nursery practices were normalized by arcsine transformation. Statistical analysis of the data for all the experiments were done by one way Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) to determine differences between the means taking at 1% (p<0.01) or 5% (p<0.05) significance levels (Gomez and Gomez, 1984).

Results

***Induced breeding*:** The breeding performance of *M. vittatus* was done in 2000, 2001 and 2002 for wild, F_1 and F_2 generations.

***Quantity of water hardened egg*:** Ovulation took place within 5:00 to 6:00 hrs after the second injection of female at 27-28 ºC in all the generations. Significant variation among the doses of PG was found in respect of quantity of water hardened eggs (Table 1). The highest number of eggs were found in T_5 for wild (20.27 g), F_1 (20.90 g) and F_2 (18.50 g) generations followed by T_4 (16.97, 14.90 and 17.50 g, respectively) in all the generations. While the lowest number of eggs were observed in T_3 (5.13, 5.83 and 6.17 g, respectively) preceded by T_6 (9.90, 10.13 and 14.43 g, respectively).

***Percentage of fertilization*:** Significant variation was found among the doses of PG in male (male, 3 mg) and female in respect of percentage of fertilized eggs (Table 1). The highest number of eggs were found to be fertilized in T_5 for wild (65.33%), F_1 (56.00%) and F_2 (80.67%) generations followed by T_4 (42.67, 37.67 and 67.33%, respectively) in all the generations. While the lowest number of fertilized eggs were observed in T_3 (10.33, 12.67 and 16.33%, respectively) preceded by T_6 (18.67, 22.33 and 44.33%, respectively).

***Percentage of hatching*:** The hatching of eggs took place between 19:00-20:00 hrs after ovulation at 27-28 ºC in all generations. The doses of PG significantly influenced the percentage of hatching of fertilized eggs in *M. vittatus* in 2000, 2001 and 2002 for wild, F_1 and F_2 generations,

respectively (Table 1). The highest number of eggs were found to hatch in T_5 for wild (60.67%), F_1 (51.33%) and F_2 (78.33%) generations, followed by T_4 (38.67, 33.67 and 63.33%, respectively) in all the generations. While the lowest number of hatching of eggs were observed in T_3 (6.67, 9.00 and 13.00%, respectively) preceded by T_6 (15.33, 18.00 and 40.67%, respectively).

Table 1. Breeding performance of *Mystus vittatus* in different years with the PG doses (in all experimental treatments, ten individuals of both female and male fish were used as brood fish).

Treatment	Egg (g)			Fertilization (%)			Hatching (%)			Deformities (%)			Spawn (g)		
	2000	2001	2002	2000	2001	2002	2000	2001	2002	2000	2001	2002	2000	2001	2002
T_1 (1+4;3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_2 (1+5;3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_3 (2+5;3)	5.13	5.83	6.17	10.33	12.67	16.33	6.67	9.00	13.00	6.33	6.67	7.33	0.83	1.27	1.96
T_4 (2+6;3)	16.97	14.90	17.50	42.67	37.67	67.33	38.67	33.67	63.33	4.33	5.67	4.67	15.91	12.21	27.06
T_5 (3+6;3)	20.27	20.90	18.50	65.33	56.00	80.67	60.67	51.33	78.33	3.67	3.33	3.33	29.66	25.92	35.19
T_6 (3+7;3)	9.90	10.13	14.43	18.67	22.33	44.33	15.33	18.00	40.67	7.67	6.67	7.33	3.63	4.35	14.35
T_7 (4+7;3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_8 (4+8;3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD	5.56	3.80	4.92	9.73	8.69	14.14	8.47	9.04	14.91	2.76	2.31	2.51	4.69	3.72	12.92
Level of significance	**	**	**	**	**	**	**	**	**	**	*	*	**	**	**

** Significant at 1% level; * Significant at 5% level; NS = Non significant. 2000 for Wild Fish; 2001 for F_1 generation; and 2002 for F_2 generation. T_5 means, Female: 1st dose 3 mg and 2nd dose 6 mg PG kg^{-1} body weight; Male: 3 mg PG kg^{-1} body weight i.e., (3+6;3)

***Percentage of deformities*:** The percentage of deformities was significantly affected by the doses of PG in *M. vittatus* in 2000, 2001 and 2002 for wild, F_1 and F_1 generations, respectively (Table 1). The highest number of hatchlings were found to be deformed in T_6 for wild (7.67%) but T_3 and T_6 for F_1 generation (6.67%) and T_3 and T_6 for F_2 (7.33%) generations followed by T_3 (6.33%) for wild and T_4 for F_1 (5.67%) and F_2 (4.67%) generations. While the lowest number of deformities were observed in T_5 (3.67, 3.33 and 3.33%, respectively), F_1 and F_2 generations.

***Quantity of spawn produced*:** The yolk sac absorption required from 24-26 hrs after hatching at the water temperature of 27-28 ºC in all generations. Significant variation among the doses of PG was found in respect of spawn production (Table 1). The highest quantity of spawn was found in T_5 for wild (29.66 g), F_1 (25.92 g) and F_2 (35.19 g) generations followed by T_4 (15.91, 12.21 and 27.06 g respectively) in all the generations. While the lowest amount of spawn was observed in T_3 (0.83, 1.27 and 1.96 g, respectively) preceded by T_6 (3.63, 4.35 and 14.35 g, respectively).

***Fry nursing*:** The nursery management of *M. vittatus* fry was studied in 18 to 28 June, 2002.

***Length*:** There was a significant variation among the treatments in relation to length of fry (Table 2). The longest fry (6.25 mm) was found in D_3 followed by D_2 (5.90 mm) and that was the lowest (5.70 mm) in D_1.

***Weight*:** The weight of fry significantly varied with the rate of feed used (Table 2). The highest weight of individual fry (0.739 mg) was found in D_3 (40 $cumm^{-1}$) followed by D_2 (0.669 mg) and that was the lowest (0.643 mg) in D_1.

Table 2. Growth, production and survivability of *Mystus vittatus* in tank nursery (Initial length and weight of *Mystus vittatus* spawn were 0.2025931 mg and 2.8 mm respectively after 26 hrs of hatching).

Treatment	Length (mm)	Weight (mg)	Production (kg 0.004 ha^{-1})	Survivability (%)
D_1	5.70	0.643	0.012	21.00
D_2	5.90	0.669	0.011	18.00
D_3	6.25	0.739	0.011	16.00
LSD	0.43	00	--	3.46
Level of significance	**	**	NS	*

** Significant at 1% level; * Significant at 5% level; NS =Non significant. Rate of rotifer as live feed used: D_1 = 20 cu mm^{-1}; D_2 = 30 cu mm^{-1} and D_3 = 40 cu mm^{-1}.

***Production*:** The effect of feed on the production of fry was non significant (Table 2). However, the production of fry was numerically the highest (0.012 kg 0.004 ha^{-1}) in D_1 and that was the lowest (0.011 kg 0.004 ha^{-1}) in D_2 and D_3.

***Survivability*:** The survivability rate of *M. vittatus* fry was significantly affected by different amount of feed used (Table 2). The highest survivability rates of fry (21.00%) was observed when 20 $cumm^{-1}$ of rotifers (D_1) was used followed by (18.00%) 30 $cumm^{-1}$ (D_2), while it was the lowest (16.00%) when 40 $cumm^{-1}$ rotifers was used (D_3).

Discussion

Induced breeding: Table 1 revealed that the treatment T_5 (3+6; 3 mg) was the optimum dose of PG for all the three generations (wild, F_1 and F_2) of catfish *M. vittatus* (fertilization and hatching range was 56.00-80.67% and 51.33-78.33%, respectively) followed by T_4 (2+6; 3 mg) and T_6 (3+7; 3 mg). The lowest results was obtained from T_3 (2+5; 3 mg) where the percentage of fertilization and hatching range was 10.33-16.33% and 6.67-13.00%, respectively. Though the T_5 for all the three generations showed the best breeding performance yet the treatment T_4, T_5 and T_6 did not vary widely in respect of production of eggs and spawn and percentage of fertilization and hatching in F_2 generation. This might be due to the range of 1st dose (2 to 3 mg) and 2nd dose (6 to 7 mg) for female influenced more or less similarly with favorable atmospheric and water conditions. Akhteruzzaman *et al.* (1991) reported that the females of *M. cavasius* weighing 40-100 g each were given single injection of 7-12 mg kg^{-1} body weight. Acetone dried pituitary gland and male fishes weighing 30-60 g each were given a dose of 4 mg $PGkg^{-1}$ body weight. The doses of pituitary ranged between 7 to 12 mg kg^{-1} body weight of the females and they responded equally to all the doses. The DNMRT analysis revealed that 10 mg and 10.5 mg of PG kg^{-1} have shown no significant difference. The rest doses were proved worst. It is clearly mentioned by Akhteruzzaman *et al.* (1991) that 10 mg PG kg^{-1} gave best result in respect of fertilization and hatching rate. But in the present study the PG doses of 3 and 6 mg (total 9 mg) gave the similar results with respect to the levels of spawning, fertilization and hatching of *M. vittatus.* The period of incubation (19-20 hrs) in the present study was found to be lower than *M. cavasius*, 30-32 hrs at a temperature ranging from 27-29 ºC. In all the cases, *M. cavasius* spawned within 7-8 hrs after injection but in *M. vittatus* took 5-6 hrs. This spawning performance indicated that the spawners might be at their optimal breeding condition.

Fry nursing: Special care was taken for *M. vittatus* fry in cemented rectangular tank to provide them sufficient rotifers cultured earlier. The post-larvae were fed on rotifers in semi-intensive care system reached a mean total length of 5.70-6.25 mm and weight of 0.643-0.739 mg with survival of 16-21.0% i.e., the production of 0.11-0.112 kg 0.004 ha^{-1} was obtained within 10 days. The survivability and production of fry was the highest in case of lower concentration of rotifers (20 cu mm^{-1}), while the growth (length and weight) of fry was maximum with higher concentration of rotifer (40 cu mm^{-1}). This might be due to the competition of food among the fry. The higher survivability gave the higher number of fry in F_1 which was resulted in higher competition for food and lower fry size. After 10 days of nursing the mouth opening became wider (almost half of the body length) to catch prey easily. This size is suitable for stocking in grow out ponds without additional care. Feeding of *Mugil cephalus* larvae was started on the third day after hatching, when the mouth became open and well formed.

The application of rotifers as mouth-opening stage feed and the larvae of *Artemia salina* and copepods for later stages are adequate for small- and middle-scale fry rearing (Guoxiong, 1997). Yamashita and Bailey (1989) reported an exponential gut evacuation pattern for 8-21 days old

pollock larvae under continuous feeding conditions at relatively high prey levels (2.0-11.4 rotifers ml^{-1}). It was intended to identify rates of survival and growth of newly hatched grass carp larvae (*Ctenopharyngodon idella*) in a hatchery using dry feed only until they reached a size which allowed stocking in growing ponds without additional nursing (Appelbaum and Uland, 1979).

Conclusion

Small indigenous fish species particularly those dwelling in close waters as well as open waters, have gradually become endangered. Induced breeding and fry nursing of *Mystus vittatus* in the present study are the first in Bangladesh to optimization of the pituitary dose with the mini cemented tank nursery. The results of the present study would undoubtedly play a significant role for future propagation and conservation of this highly valuable indigenous fish species of Bangladesh.

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