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Histological identification of gonadal maturation and induced breeding of tengra, *Mystus vittatus* (Bloch) for seed production

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Abstract. The study was conducted toidentify gonadal maturation and develop artificial breeding technique of striped dwarf catfish *Mystus vittatus*. Gonado-somatic index (GSI) was found to be highest as 20.81±2.73 and 6.06±2.96 for female and male respectively in the month of July.The mean fecundity was recorded between 13138±1365.94 and 25095.2±6792.5. Six developmental stages of ovary *viz*. chromatin nucleolar stage, early and late perinucleolar stage, yolk vesicle stage and early and late yolk granule stage; and four developmental stages of testis named spermatogonia, spermatocytes, spermatids and spermatozoa were observed. For induced breeding two inducing agents *viz*. PG extract and Flash (GnRHa) were used. Three different doses of PG such as. 2 and 6 mg (T_1); 4 and 8 mg (T_2) and 6 and 10 mg (T_3) per kg bodyweight of male and female respectively were tested to standardize the PG dose.T_1 provided best result in respect of ovulation of females, fertilization and hatching rates of eggs. In case of Flash, three different doses viz. 0.5 ml (T_1), 1 ml (T_2) and 2 ml (T_3) per kg bodyweightwere used for both male and female. The best result was obtained from T_2 and no significant difference ($p > 0.05$) in respect of ovulation, fertilization and hatching rates of eggs was observed among the treatments.
Key words: *Mystus vittatus*, Histology, Induced breeding.

Introduction

In Bangladesh, tengra, *Mystus vittatus* is usually found inmarginal vegetation in lakes and swamps with a mud substrate such as Chalan *beel* (Galib *et al*. 2009), and also found in flooded canals, *beels*, paddy and jute fields, streams, *haors*, oxbow lakes, ponds and rivers in rainy season. The fish is mainly plankton feeder with preference for zooplankton and feed mainly on shrimps, mollusks, fish copepods, cladocerans, rotifers, ostracods, insects, oligochaetes, chlorophyceae, bascillariophyceae and debris. Habitat degradation caused by natural and human interventions, injudicious application of pesticides in agricultural fields and release of industrial effluent have recently become great constraints for fish biodiversity in most aquatic ecosystems in Bangladesh. As a result, a remarkable reduction in abundance of *M. vittatus* has been observed in different closed water bodies. Tengra is a small indigenous species and it is categorized as least concern species (IUCN 2015), However, there is a risk for tengra to become threatened. Therefore, the wild tengra stock should be assessed and save them from the risk of being endangered through the development of artificial breeding

technique. So, this study was aimed to establish a breeding technique using different inducing agents through identification of peak breeding season by gonadal histological analysis so that, it can be used as a baseline to maintain the sustenance of the fish.

Materials and Methods

Experimental fish: Around 250 brood fish were collected from wild sources and reared in the pond adjacent to the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh during March 2013 to February 2014. Two ponds each having 2.5 decimal in size were used for rearing the brood fish. They were reared with commercial feed (Mega Feed) at the rate of 5-8% of body weight.

Estimation of gonado-somatic index (GSI): Fish samples were collected monthly from natural sources (Bramaputra river, *beels*) for twelve consecutive months and gonado-somatic index (GSI) wasdetermined to know the spawning frequency of fishes. The gonad of fish was dissected out and weighed. The GSI was calculated according to the following formula:

$$\text{GSI} = \frac{Gonadweight}{Bodyweight} \text{ X } 100$$

Fecundity was estimated by gravimetric method using the following formula:

$$\text{F} = \frac{\text{Gonad weight x N}}{\text{Sample weight}}$$

Where, F = Fecundity of fish

N = Number of eggs in sample

Histology of gonad: Gonads of female and male fish collected from natural sources were examined histologically to study the different maturation stages of ovary and testis. The ovary and testis were taken out very carefully and preserved in Blouin's fluid. The preserved samples were then taken out and arranged in a steel rack. Dehydration, clearing and infiltration processes of samples were carried out in an automatic tissue processor using a series of alcohol of increasing concentrations, three changes of xylene and finally through a series (three series) of molten wax. The samples were then embedded with melted wax. Paraffin embedded blocks was cut by microtome knife at 4-5 μm thickness. A suitable section was selected from the ribbon of section and picked up on a glass slide. To fix the section, the prepared slide was placed on a hot plate (20˚C) overnight. The sections were then cleared with xylene, rehydrated with alcoholic series and stained with hematoxylin and eosin stains. After staining the sections were mounted with Canada balsam and covered by cover slip. The prepared slides were then examined under a compound microscope to observe the monthly developmental variations. Photomicrographs of the stained sections were taken by using a compound photomicroscope.

Induced breeding trials of Mystus vittatus: Eighteen selected brood fish (9 females and 9 males) were kept in a cistern for about 6h for conditioning prior to injection and constant water flow was maintained to ensure proper aeration. Two trials were conducted for optimization of the dose of PG and Flash hormone respectively.

Trial I: Nine pairs of male and female were equally divided into three treatments and marked as T_1, T_2, and T_3. The pairs under each treatment were indicated as R_1, R_2 and R_3 and kept separate in bowl incubator. Each pair of the male and female under T_1, T_2, and T_3 were treated with PG extract at the dose of 2 and 6, 4 and 8, and 6 and 10 mg kg^{-1} body weight for male and female respectively.

Trial II: Six pairs of male and female were equally divided into three treatments and marked as T_1, T_2,and T_3. The pairs under each treatment were indicated as R_1 and R_2 and kept separate in bowl incubator. Each pair of the male and female under T_1, T_2, and T_3 were treated with Flash Hormone (GnRHa) at the dose of 0.5, 1.0 and 2.0 ml/kg body weight for both male and female broods.

Ovulation, fertilization and hatching of eggs: After injection the females were kept in same bowl and monitored them very closely to see any change in behaviour. Upon ovulation, females released eggs and males released sperm and thus fertilization occurred spontaneously. After fertilization of eggs both male and female fish were taken out from the bowl incubators. Upon completion of hatching, the number of hatchlings were counted and recorded. The following parameters were estimated as indices of the effectiveness of different PG and Flash doses:

Percent ovulation, fertilization and hatching:

$$\% \text{ Ovulation} = \frac{No.\ of\ fish\ ovulated}{Total no.\ of\ fish\ injected} \text{X } 100$$

$$\% \text{ Fertilization} = \frac{Number\ of\ fertilized\ eggs}{Total number\ of\ eggs\ (fertilized + unfertilized)} \text{X } 100$$

$$\% \text{ Hatching} = \frac{No.\ of\ eggs\ hatched}{Total no.of\ eggs\ (fertilized + unfertilized)} \text{X } 100$$

For calculating percent fertilization, a number of egg samples (about 50 eggs) were taken from each group and fertilized and unfertilized eggs was counted under a microscope.

Data analysis: The effects of hormones in different treatments of breeding trials were tested using a one-way analysis of variance (ANOVA) with the aid of the computer software Mstat and SPSS version 11.5.

Results and Discussion

For determining the peak spawning period of *M. vittatus,* gonado-somatic index and histological examinations of both ovary and testis were done. The gonado-somatic index

has been calculated from consecutive 12 month samples i.e. from March to February where a rise in the GSI values were observed from April to July. In case of female *M. vittatus,* the highest GSI value was found as 20.81±2.73 in July while the lowest GSI value (0.88±0.06) was found in December (Fig. 1). Similarly, in case of male highest GSI was recorded as 6.06±2.96 in July and lowest (0.07±0.06) in October (Fig. 2). These GSI values indicate that *M. vittatus* may breed in the month of June and July which agreed with the findings of Saxena (1972) for catfish *Rita rita*. Barua *et al*. (1986) reported a single spawning season of *Clarias batrachus* that existed from May to July. Banu and Ali (1992) reported the peak spawning season of *Mystus tengara* to be in July. Faruq (1995) worked on four species of catfish *viz. Heteropneustes fossilis, Clarias batrachus*, *Mystus cavasius* and *Mystus vittatus* and found their spawning peak in June and July. The mean GSI values in consecutive 12 months study period showed the existence of a single peak breeding season of *M.vittatus* during June and July. This fact was further supported by histological observations of monthly collected ovarian samples where a sudden increase in GSI value with a few maturing oocytes was also observed in October.

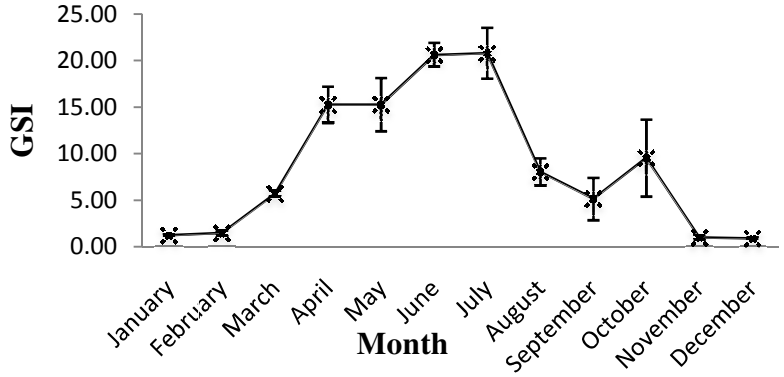


Fig. 1. Monthly mean of gonado-somatic index (GSI) of female *M. vittatus*. The bars indicate standard deviation of means.

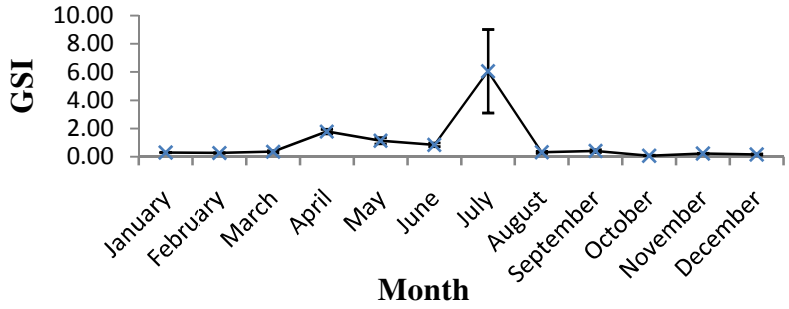
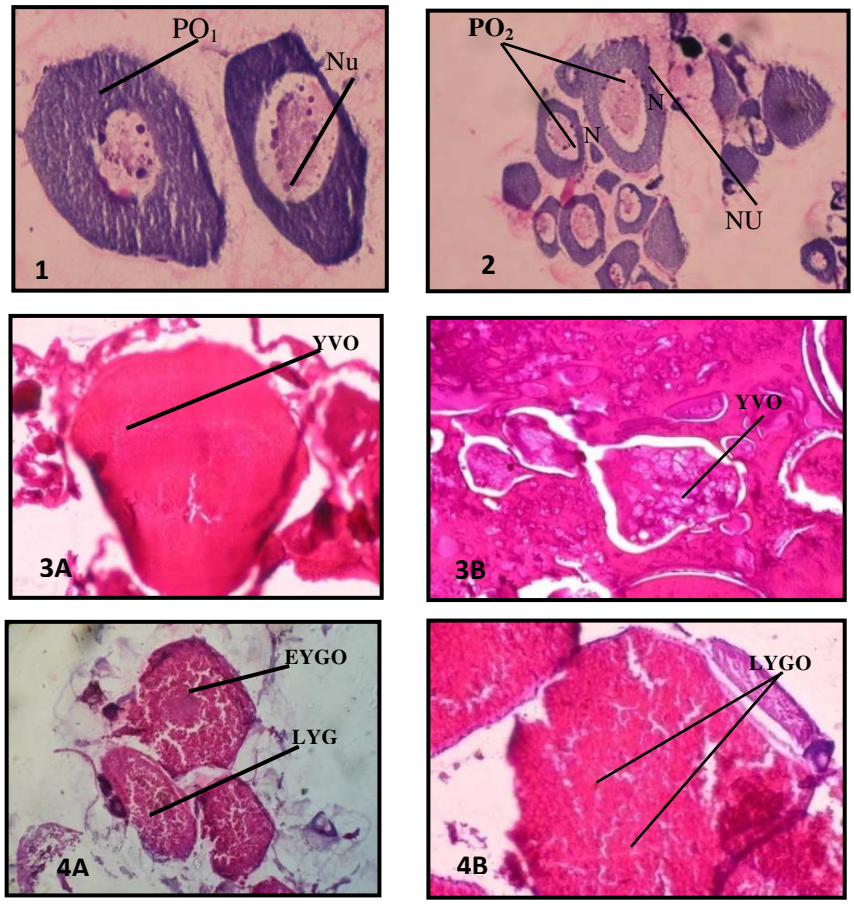
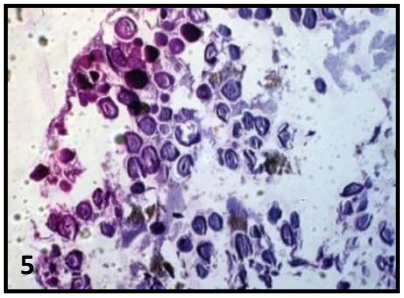
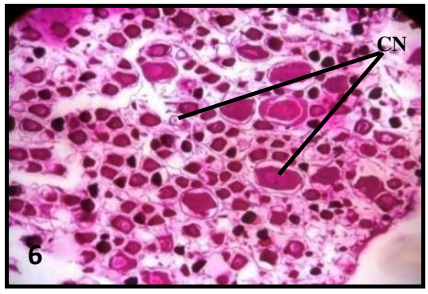


Fig. 2. Monthly mean of gonado-somatic index (GSI) of male *M. vittatus*. The bars indicate standard deviation of means.

Fecundity is used to assess the reproductive potential of the spawning stock. In the present study the mean fecundity of *M. vittatus,* varied from 13138±1365.94 to 25095.2±6792.5 for the fish measuring 10.72±0.35 to 13.24±0.40 cm in length and 13.52±0.89 to 26.41±2.11 g in weight (Data not shown). Islam *et al.* (2011) recorded the mean fecundity of *M. vittatus* as 33386. On the other hand, Gupta and Banerjee (2013) found fecundity of *Mystus tengara* ranged from 6,770 to 21,708 with an average of 13365.84±7260.4. Ovarian development of *M. vittatus* was examined to study the pattern and timing of growth phase and maturation stages of germ cells. Oocytes of immature stages (early and late perinucleolar stage oocytes) were found to be mostly available during January and March (Plate 1-2). Yolk-vesicle stage oocytes appeared in April and May (Plate 3A-3B). Early yolk granule stage oocytes appeared in June, and late yolk granule stage oocytes appeared in June and July (Plate 4A-4B), although some granular stage was found during September and October (Plate-5); chromatin nucleolar stage oocytes (CNO) appeared in November and December (Plate 6).

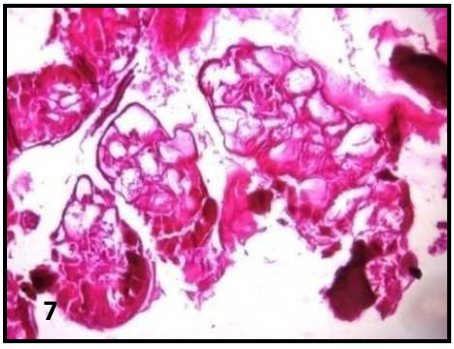


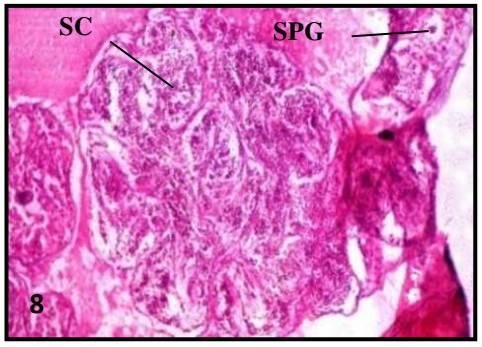


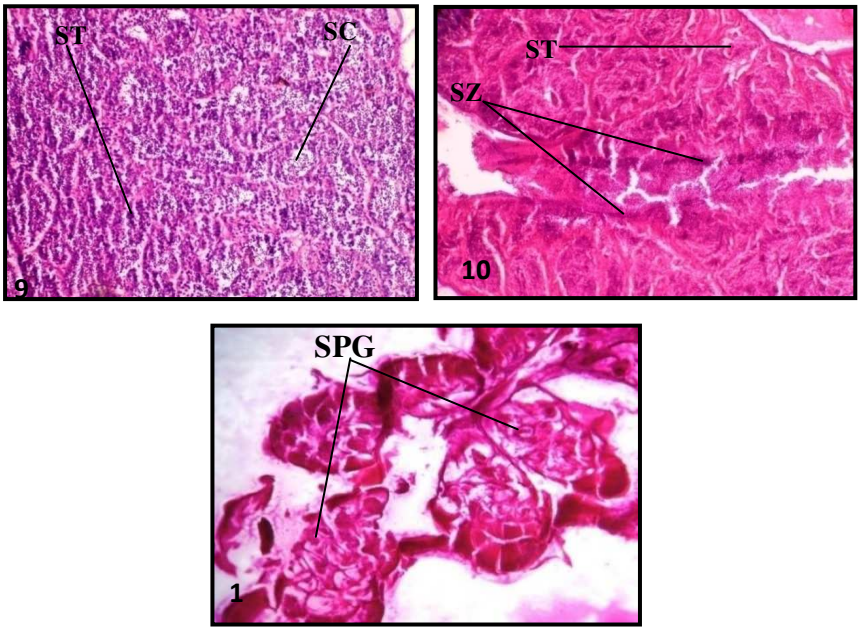


Plates 1-6 showing different developmental stages of ovary of *Mystus vittatus*: 1) Early perinucleolar stage in January and February, N= Nucleus with nucleoli (Nu), 2) Late perinucleolar stage in March and April; 3A-3B Yolk-vesicle stage in April and May; 4A-4B Early and Late yolk-granule stage in June and July; 5) Spent phase in August September and October; and 6) Chromatin nucleolar stage in November and December.

These observations agreed with the findings of Mollah (1986) for *Clarias macrocephalus*. The present study revealed four stages of spermatogenesis in *M. vittatus* which were identified as i) spermatogonia ii) spermatocytes iii) spermatids and iv) spermatozoa. Spermatogonia and spermatocytes appeared in during December and March, while spermatids and spermatozoa appeared in April through August. The resting phase of testis was found in September, October and November (Plates 7-11). More or less similar developmental stages of testis were reported by Mollah (1988) in *Clarias macrocephalus* and Guraya (1994) in salmonids. Mahmud *et al.* (2016) identified four stages of spermatogenesis in *Channa punctatus* and mature spermatozoa was highly abundant in June and July. From the observation of gonadal development in the present study, it may be stated that spawning of *M. vittatus* takes place from mid-May to July with peak in June and July.







Plates 7-11 showing different developmental stages of testis of *Mystus vittatus*:7) maturing testis in December and January, SPG=Spermatogonia; 8) Spermatocytes (SC)in February and March; 9) Spermatid in April and May, ST= Spermatid; 10) Spermatids and Spermatozoa in June, July and August; and 11) resting phase in September, October and November.

In the second experiment for optimizing the dose of PG and Flash (GnRHa), two trials were conducted on induced breeding of *M. vittatus*. However, the fish treated with the dose of 2 and 6 mgPG/kg body weight for male and female respectively showed the best performance as far as the ovulation, fertilization and hatching rates are concerned. There was no significant ($p>0.05$) difference in ovulation rates among the three treatments (Table I). Islam *et al*. (2011) tested four different doses of PG, *viz*. 6, 8, 10 and 12 mg/kg body weight for female and 3, 4, 5 and 6 mg/kg body weight for male *M. vittatus* and they found hormone doses at 4 mg/kg for male and 8 mg/kg for female provided best result in the sex ratio 2♂: 1♀. In the present experiment 1♂: 1♀ sex ratio was maintained as it was reported that there was a competition held between the males for mating with the females. Islam *et al*. (2011) reported 80% fertilization and 56% hatching for 4 and 8 mgPG/kg body weight for male and female *M. vittatus* respectively but in this study 69.31±6.96% fertilization and 10±2.9% hatching were obtained from PG applied at 4 and 8 mg/kg body weight for male and female respectively. In this study, though no significant difference was observed in fertilization rates of eggs among the three treatments but significantly ($p<0.05$) higher hatching (65±2.89%) was obtained from T_1 (2 and 6 mg PG/kg body weight for male and female respectively) (Table I). Mollah and Tan (1983) obtained 73.3% hatching of

Clarias macrocephalus eggs using 3.5-7.0 mg PE/100g body wt. This is attributable to the fact that at high doses of PG (8 and 10mg PG/kg body weight) fish getting weaker and releases less number of eggs. This might be a case of abortion rather than usual ovulation. Consequently, the fertilization and hatching rates were comparatively low. As all the fishes used in this experiment were approximately of similar size and maturity under the same environmental and management conditions, the differences obtained in the breeding trials were only due to the variation in PG doses.

Table I. Effect of different doses of PG on ovulation of females and fertilization and hatching rates of eggs in *Mystus vittatus*

Treat-ments	Replication	Dose of PG (mg/kg body weight of female)	Ovulation status		Latency period	Average fertilization rate (%)	Average hatching rate (%)	Remark
			Response	Average (%)				
T1	R1	6	+++		6-10 h			Good no. of larvae hatched
		6		100[a]		83.33±1.67[a]	65±2.89[a]	
	R2	6	+++		6-10 h			
	R3		+++		6-10 h			
T2	R1	8	+++		6-10 h			Few larvae hatched
		8		93.33±6.67[a]		69.31±6.96[a]		
	R2	8	++		6-10 h		10±2.9[b]	
	R3		+++		6-10 h			
T3	R1	10	+++		6-10 h			
		10		85.00±7.64[a]		68±8.89[a]	0	--
	R2	10	++		6-10 h			
	R3		++		6-10 h			

+++ Profuse ovulation and yielded sufficient number of ripe eggs. ++ Considerable ovulation, -- No ovulation.
Values in each column with different superscripts are significantly different ($p<0.05$).

In this experiment, among the three doses (0.5, 1 and 2 ml/kg) of the synthetic hormone Flash i.e. (GnRHa+domperidon), 1 ml/kg body weight provided the best result for both male and female respectively.Sridhar *et al.* (1998) used ovaprim at a dose of 0.5 ml/kg body weight to induce *Ompok bimaculatus*. In another experiment, Haniffa and Sridhar (2002) found 0.3, 0.5 and 0.7 ml/ kg body mass of ovaprim to be effective in *Heteropneustes fossilis*. On the other hand, Sharma *et al.* (2010) recommended 1 ml of ovatide/kg body weight for *Clarias batrachus* which is similar to the present study. Flash with the doses of 0.5 and 1 ml/kg body weight precipitated 100% ovulation whereas 2 ml/kg body weight of the same hormone caused 87.5±12.5% ovulation in *M. vittatus*. Similar to ovulation, variable fertilization (72.5±1.5%, 81.5±1.5%, and 68.5±8.5) and hatching (62.5±2.5%, 69±1.0% and 58.5±8.5%) rates of *M. vittatus* eggs were observed respectively from 0.5, 1.0 and 2.0 ml/kg doses of Flash. However, 1.0 ml/kg body weight of Flash resulted in highest fertilization and hatching rates among them (Table II). In *M. vittatus*, spawning took place within 6-10 h after injection and hatching within 24 h after injection (Table 2) while spawning and hatching of eggs of *O. bimaculatus* took 5-6 h and 24 h respectively after injection as reported by Sridhar *et al.* (1998). It has been observed that different doses of Flash gave comparatively better results than PG in *M. vittatus*.

Gonadal maturity, fecundity, month-wise gonado-somatic index (GSI) and histological study gave a clear indication that *M. vittatus* breeds during June and July in Bangladesh. The knowledge on breeding biology of *M. vittatus* obtained through the present experiment may be used as a baseline for further research to save this species from possible threat of extinction.

Table II. Effects of different doses of Flash on ovulation, fertilization and hatching of eggs of *Mystus vittatus*

Treat-ments	Replication	Dose of Flash (ml/kg body wt. of female)	Ovulation status		Latency period	Average fertilization rate (%)	Average hatching rate (%)	Remark
			Response	Average (%)				
T1	R1	0.5	+++	100[a]	6-10 h	72.5±1.5[a]	62.5±2.5[a]	Good no. of larvae hatched
	R2	0.5	+++		6-10 h			
T2	R1	1	+++	100[a]	6-10 h	81.5±1.5[a]	69±1.0[a]	Good no. of larvae hatched
	R2	1	+++		6-10 h			
T3	R1	2	++	87.5± 12.5[a]	6-10 h	68.5±8.5[a]	58.5±8.5[a]	Good no. of larvae hatched
	R2	2	+++		6-10 h			

+++ Profuse ovulation and yielded sufficient number of ripe eggs, ++ Considerable ovulation, -- No ovulation
Values in each column with different superscripts are significantly different (p<0.05)

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