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Dose optimization with synthetic hormone flash for induced spawning of *Shing (Heteropneustes fossilis)*

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Abstract

An experiment on induced breeding of *Heteropneustes fossilis* was carried out to determine the optimum dose of Flash hormone at the Bogura Matshya Khamar, Parulia, Palash, Norshindhi. The present study consisted of three treatments (T₁, T₂ and T₃) with nine replications of each. Forty five pairs of male and female were selected from the brood rearing ponds and the average body weight of the female and male were 100±10 g and 55±5 g respectively. To observe the effective dose for induced breeding, the females were injected at the rate of 0.5 (T₁), 0.45 (T₂) and 0.42 (T₃) ml Flash/kg body weight and correspondingly the males were administered a dose of 0.20 (T₁), 0.18 (T₂) and 0.17 (T₃) ml Flash/kg body weight in all treatments. The fertilization rate and hatching rate were determined. Then the hatchlings were reared in aquarium up to 7 days and survival rate was determined. The water temperature was recorded between 26 to 31°C during experimentation. Among the three treatments T₁ showed the best result in terms of fertilization rate (80.33%), hatching rate (71.67%) and survival rate (61.56%). The present findings can be used in induced breeding of *Heteropneustes fossilis* for the development of hatchery propagation.

Keywords: Dose optimization, Synthetic hormone flash, induce spawning, Shing

1. Introduction

Fisheries play a vital role in economic development, employment, human nutrition and for earning foreign exchange to develop our national economy. This sector contributes about 6.22% of the gross domestic product (GDP) and consist one forth (22.76%) of the total Agricultural production. About 2.46% foreign exchange come from this sector. Fish provide 60% of national protein consumption for the people of Bangladesh, DoF (2014) [5]. At least 55 species of catfishes belonging to 35 genera have been recorded in Bangladesh (Rahman, 2001) [17]. Fin fish hatchery was first established in Jessore by Mohoshin Master in 1967. Since then the number of fish hatchery has increased uninterruptedly reaching over a thousand in 2014 to fulfill the ever increasing demand of the fin fish seeds for aquaculture industry of Bangladesh. There are 124 government hatcheries and rests are private hatcheries most of which are present in Jessore, Comilla and Mymensingh districts. In Bangladesh both public and private hatchery produced around 423986 kg hatchling (DoF, 2014) [5]. It was reported that approximately 4689653 kg fry were produced from nurseries of Jessore during the year 2013 (Asif *et al.*, 2014) [1]. It is the second largest export industry after garments where 57% of the total exports of the fish and fisheries products and 97% of the shrimp produced are being exported. It contributes 4.37% to the Gross Domestic Products (GDP) and 23.37% to the agricultural sector (DoF, 2014) [5]. World food fish aquaculture production expanded at an average annual rate of 6.2 percent in the period 2000–2012, more slowly than in the periods 1980–1990 percent) and 1990–2000 (9.5 percent). Between 1980 and 2012, world aquaculture production volume increased at an average rate of 8.6 percent per year; World food fish aquaculture production more than doubled from 32.4 million tons in 2000 to 66.6 million tons in 2012 (FAO, 2014) [6]. At present Bangladesh is the fourth largest fish producing country in the world (FAO, 2014) [6]. *H. fossilis* (Bloch) locally known as "Shing" is an important air breathing catfish. Once Shing was very much abundant in almost all freshwater system in Bangladesh but in late 1980s, the catches of the fish have drastically declined from open waters due to various ecological changes in inland water bodies and thus has been recognized as an endangered species. Shing fishery contributed about 2.68% of total pond catch (DoF Statistical Year Book, 2012–13) [4]. The fish is now sold at an exorbitant price in the market. But in culture aspects, the growth rate of native strain is very slow in ponds ecosystem.

In order to fulfill ecological niche and other factor, as much as 12 exotic fish species had been introduced in our country for culture purpose (DoF, 2013) [4]. Report showed that Shing grows as much as 70- 130 g within 120days culture period. Culture potential of Shing has increased in various part of Bangladesh especially in Norshindhi due to its fast growth rate.

Ovaprim was useful in breeding of *Heteropneustes fossilis* with high percentage of fertilization, hatching, the mortality of the brooders was high but there was a concern over the possibility of toxicity caused due to high dose of Ovaprim (Bhattacharyya and Homechaudhuri, 2009) [2]. Artificial hypophysation of the *Heteropneustes fossilis* was first attempted by Khan and Mukhopadhyay (1972) [9]. While the

effect of Wova-FH on breeding was observed by Sarkar *et al.* (2003) [19].

Considering the importance of Shing the present work was undertaken to determine the optimum dose of the species to determine the optimum dose of “Flash (containing sGnRH + dopamin)” for induced breeding of Shing; and the fertilization, hatching and survivability rate of Shing.

Materials and Methods

Experimental site

The research work was conducted at the Bogura Matshya Khamar Complex, Parulia, Palash, Narshindhi (Figure 1). The experiment was performed during 1st August to 10th September 2015.



Fig 1: Arrow showing study area.

Facilities present in Bogura Matshya Khamar Complex

During the breeding period different infrastructure of the hatchery was used to complete the experiment such as rectangular tank, overhead tank etc. Apart from this there were a number of other infrastructures they are listed in the Table 1.

Table 1: Facilities available in Bogura Matshya Khamar Complex

Serial No.	Contents	Quantity
1.	Overhead tank	1
2.	Rectangular tank	19
3.	Circular tank	1
4.	Shallow machine	3
5.	Generator	0
6.	Shed	2
7.	Hand tube well	1
8.	Electric tubewell	1
9.	Deep tube well	2

Collection of fish

Two hundred and fifty five Shing were collected from the own brood pond. The collected fish were stocked in the rearing pond situated inside the hatchery complex, during 1st August to 10th September 2015.

Brood pond preparation

Brood fish is called as the heart of the hatchery and management practices of brood stock is the important step for good seed production. Artificial breeding mainly depends on sufficient amount of quality brood fishes. Brood stocks were managed scientifically so that mature broods could be obtained during the full breeding season. There were 4 brood ponds each having an average area of 30 decimal and the water depth between 1.0-1.5 meters. For preparation of the brood ponds, the usual practice was to eradicate the predator and the unwanted fishes by dewatering and drying. Rotenone and phostoxin were also used to remove the unwanted fish species and aquatic insects. Aquatic vegetation was removed manually. After cleaning the pond, lime was used at the rate of 1 Kg/decimal and 5-7 days after liming, cow dung was applied at the rate of 5-7 Kg/decimal as organic fertilizer. At the same time inorganic fertilizers such as Urea and TSP were also used at the rate of 150 g and 100 g per decimal respectively. Seven days after fertilization, fish were released in brood pond with intensive care. Feeding was done with formulated feed. In this formulated feed 20 -25% of protein level was maintained. The hatchery owners used aluminum pot to carry the brood fish from brood pond to hatchery unit.

Water quality Parameters

Water quality was measured by Eon,s and first care companies aqua parameter kit. Measured values shown in Table 2.

Table 2: Water quality parameter recorded during the breeding

Parameters	Range
Water pH	8- 8.5
Temperature	26-31 0C
Dissolve Oxygen (DO)	4.39 - 5.61mg/l
Free CO2	0mg/l
NH ₃ gas	0.01-0.02 mg/l

Selection of brood fish

Brood fishes were selected for brood stocking considering size, shape and color. Only the ready to spawn breeders were selected for breeding trials. The broods were easily identified on the basis of external feature of their abdomen and pectoral fins. Their enlarge abdomen with a softy touch (during the breeding season) enable them to be identified correctly. The average weight of the females and males were 100±10 gram and 55±5 gram respectively. Broods were selected on the basis of the criteria mentioned in Table 3. Ready to breed broods are also showed in Figure 2.



Fig 2: Broods are ready for breeding operation.

Table 3: Criteria followed to select mature breeders of Shing (*Heteropneustes fossilis*)

Female	Male
Relatively large in size	Small in size
Abdomen is swollen and soft	Slim and streamlined body
Round and blunt genital opening	Genital papilla elongated and pointed
Prominent reddish vent	Normal vent
Small amount of eggs from the ovary come out on gentle pressure	Whitis melt come out on gentle pressure in abdomen to be the best criteria for male

Conditioning

Mature males and females from the brood rearing ponds were selected and immediately carried to the hatchery. Male and female fishes were kept into separate hapa in rectangular tank for about 5 hours. They were subjected to induce the breeding condition under water showering. Water flow was maintained

to ensure sufficient aeration. No feed was provided during the period of conditioning.

Experimental design

Three treatments were considered for the experiment in each treatment nine replications were considered and total 90 fishes were used (Table 4).

Table 4: Experimental layout for dose optimization.

Treatment	Hapa	Replication	Dose (ml/Kg body weight)		Ratio/Number (Male: Female)
			Male	Female	
T1	Hapa-1	R ₁	0.2	0.5	1:1 (30: 30)
		R ₂			
		R ₃			
	Hapa-2	R ₁			
		R ₂			
		R ₃			
	Hapa-3	R ₁			
		R ₂			
		R ₃			
T2	Hapa-1	R ₁	0.18	0.45	1:1 (30: 30)
		R ₂			
		R ₃			
	Hapa-2	R ₁			
		R ₂			
		R ₃			
	Hapa-3	R ₁			
		R ₂			
		R ₃			
T3	Hapa-1	R ₁	0.17	0.42	1:1 (30: 30)
		R ₂			
		R ₃			
	Hapa-2	R ₁			
		R ₂			
		R ₃			
	Hapa-3	R ₁			
		R ₂			
		R ₃			

Collection and preparation of “Flash”

Locally available Flash was collected from market in preserved condition in airtight vials. At first, the required amount of Flash was taken in a small bowl by syringe which has a capacity of 1 ml (100 Unit). Then sufficient amount of water added to the Flash to dilute the solution.

Hormone administration

After preparation of Flash solution, brood fishes were caught carefully by net, and kept in sponge. Then the inducing agent was injected near the pectoral fin base. The amount of Flash solution for each fish was determined before injection according to the body weight of the broods. After injection male and female were kept in hapa where they released eggs automatically after 8-12 hours depending on the treated doses. For dose optimization fertilization rate, hatching rate and survival rate were determined.

Doses of Flash for male and female brood

The prepared doses of Flash solution for male and female broods were shown in Table 5.

Table 5: Doses of Flash for male and female broods of Shing under different treatments

Dose	T1	T2	T3
Male	0.2 ml/kg	0.18 ml/kg	0.17 ml/kg
Female	0.5 ml/kg	0.45 ml/kg	0.42 ml/kg

Dose of Flash was administered to the female and male broods in between 5:00 to 6:00 pm. Then the fishes were kept into hapa with proper aeration until they release their eggs.

Collection of fertilized eggs and transferring to hatching tank

After spawning the brood fishes were removed from the hapa by net and transferred into the ponds. The eggs were kept there to hatch.

Determination of fertilization rate

To determine the fertilization rate 100 eggs was taken in a Petridis from hatching jar. Then the eggs were observed under a magnifying glass and fertilized eggs were counted. The fertilized eggs are not transparent as the hatching egg. Their color is slightly brownish. The fertilized eggs were easily separated from the unfertilized eggs by the presence of transparent shell with gray spot within the eggshell, while the unfertilized eggs were opaque. The fertilization was determined by the following formula

$$\text{Fertilization rate (\%)} = \frac{\text{Number of Fertilized eggs} \times 100}{\text{Total no of eggs (fertilized + unfertilized)}}$$

Determination of hatching rate

Hatching was completed after 22±2 hours of. To determine hatching rate 100 fertilized eggs were collected in a tray and the total numbers of the hatchlings were counted by visual observations. The hatching rate was determined using the following formula:

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total number of fertilized eggs}} \times 100$$

First feeding

The yolk sac absorption was completed after 70±2 h of hatching. After 72 h of post-hatching boiled egg-yolk provided as first food for the hatchling of Shing at ambient temperature of 22 to 26 °C. At that time they were little bit of blackish and transparent in appearance.

Determination of survival rate

Newly hatched fry are observed for seven days to see survivability (Figure 6). All other conditions during the experimentation were maintained same. After completion of the experiment at 7th day (Figure 8), the number of total live larvae in the tray was counted separately for calculation of survival rate.



Fig 3: Fertilized Egg

Statistical analysis

The results found in the experiment were subjected to statistical analysis, ANOVA, (one way) that showed the significance (*P*<0.05) level of differences between the treatments. This statistical analysis was performed with the aid of the computer software SPSS version 16. Significant results (*p*<0.05) were further tested using Duncan’s New Multiple Range Test (DMRT) to identify significant differences among means.

Results

Management of brood Stock

At first fully matured and ready to spawn fishes were selected for induced breeding operation. All of the fish did not mature at a time. Fast growing fishes were found to mature in early breeding season followed by others. They were also fed with artificial diet during the period.

Breeding behavior

The breeding behavior was observed continuously after the hormone injection. Just after injection both the male and female shows normal activities and movement. At that time they stayed on the bottom at one corner of the tank. After 8-9 hours of injection the activities and movement of male fishes were increased. The male fish started to move around the female fish; after 9-10 hours of injection it started to nudge with its snout at the ventral region of female fish. At that time higher rate of opercula movements was observed in female fish. The activities of male and female fishers were observed several times. After that suddenly the male came quickly to the female and the male bents its body. Pressure was created on the ventral region of the male fish and the abdomen of the female. Eggs were ejected and at the same time male released milt. Male did not released sperm until the female ejected eggs. After releasing eggs and milt, brood fishes were transferred to the stocking pond.

Fish response to different doses

Response levels of Shing were also collected during the experimental period. Their ovulation and hatching time varied from one another in different doses. The following table shows variation toward different doses.

Table 6: Different doses of Flash used and their response to artificial breeding in different time

Time	Male & Female ratio	Sex	Dose of Flash (ml/kg)	Ovulation (hour after injection)	Hatching Time (h)
1 st August -10 th August, 2015	1:1	Male	0.2 ml/kg	12-14	19-20
		Female	0.5 ml/kg		
15 th August-25 th August, 2015	1:1	Male	0.18 ml/kg	12-14	20-22
		Female	0.45 ml/kg		
31 th August – 10 th September, 2015	1:1	Male	0.17 ml/kg	12-14	22-24
		Female	0.42 ml/kg		

Dose optimization with synthetic hormone Flash

Dose optimizations with Flash hormone for spawning of

female Shing were performed with different doses of Flash. Three different doses viz., 0.5, 0.45 and 0.42 ml Flash/kg body weight of fish were applied whereas each dose was consisted of one treatment e.g. T1, T2 and T3 respectively. Corresponding data representing the effects of Flash doses on fertilization rate, hatching rate and survival rate of Shing which are shown in Table 6 and various significant levels among them shown in Table 7.

Table 7: Performances of different doses of Flash on induced breeding of Shing

Treatment	Fertilization rate% M ± SE	Hatching rate% M ± SE	Survival rate% M ± SE
T1	80.33±1.75a	71.67±1.43a	61.56±1.65a
T2	71.97±1.98b	63.35±1.63ab	55.45±2.19ab
T3	63.56±0.65c	54.47±1.78b	46.78±2.65b

(M±SE); Values of the parameter in each column with different superscripts (a, b and c) differs significantly ($p < 0.05$)

Table 8: ANOVA table showing the level of significance in terms of different doses.

		Sum of Squares	df	Mean Square	F	Significance
Fertilization	Between Groups	574.546	2	281.765	15.786	.000
	Within Groups	480.788	24	20.046		
	Total	1055.529	26			
Hatching	Between Groups	285.876	2	151.879	4.654	.013
	Within Groups	856.654	24	39.654		
	Total	1142.530	26			
Survivability	Between Groups	393.675	2	202.876	4.765	.044
	Within Groups	1023.543	24	47.654		
	Total	1417.218	26			

Fertilization rate

From the experiment the fertilization rate were recorded as 80.33%, 71.97% and 63.56% in the treatments of T1, T2, and T3, respectively (Table 7 and Figure 7). The highest fertilization rate 80.33% was recorded in T1 whereas the lowest fertilization rate 63.56% was found in T3. The results from the ANOVA test indicated that there was a significant difference among three doses of Flash treatment whereas T1 was significantly ($p < 0.05$) higher than that of treatments T2 and T3 (Table 7).

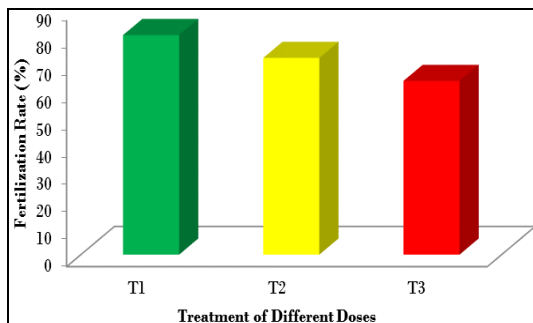


Fig 4: Comparison of fertilization rate (%) of Shing during breeding with different doses of Flash.

Hatching rate

The hatching rate was found 71.67%, 63.35% and 54.47% in treatments of T1, T2 and T3 respectively (Table 7 and Figure 8). The highest hatching rate was recorded 71.67% in T1 and the lowest hatching rate was recorded 54.47% in treatment T3. The result from the ANOVA test indicated that there was a significant difference among three doses of Flash. It was found that hatching rate in T1 was significantly ($p < 0.05$) higher than that of T3 but it was not significant in comparison with T 2 (Table 8)

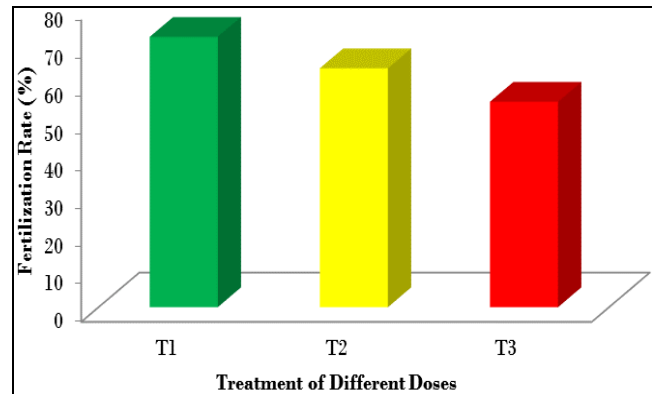


Fig 5: Comparison of hatching rate (%) of Shing during breeding with different doses of Flash.

Survival rate

The survival rate of Shing larvae those were produced by three different hormone doses treatments (Table 7 and Figure 9) were 61.56%, 55.45% and 46.78% in T1, T2 and T3 respectively after 7 days of experimental period. The results revealed that there was a difference among three doses of Flash and a significantly ($p < 0.05$) higher survival rate was observed in treatment T1 compared to the T3 but there was no significance difference among T2 and T3 respectively (Table 8).

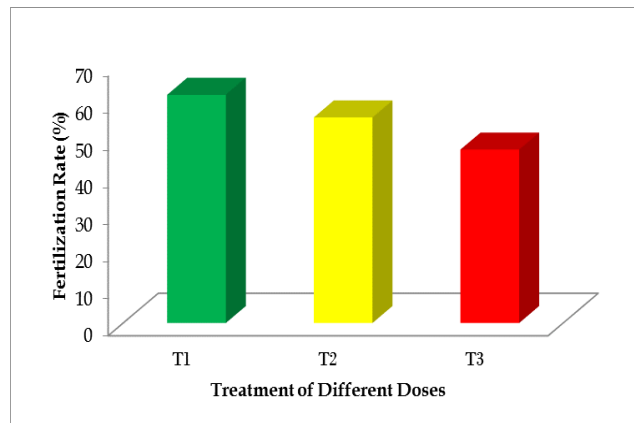


Fig 6: Comparison of survivability rate (%) of Shing during induced breeding with different doses of Flash.

Discussion

Heteropneustes fossilis is one of the most important catfish in Bangladesh. It has been drawing the attention of fish farmers in Bangladesh day by day due to its high market values, profitable culture and hardy nature. Artificial breeding of this species to obtain good quality fry become a necessary part of fry production in the hatchery. The present work on induced breeding of *H. fossilis* by using flash have been conducted to

develop a successful artificial breeding technique of the species, which will be helpful in producing good quality fry.

Several studies have been conducted on the growth performance of Shing in Bangladesh, but very limited study was carried out on the breeding technique relating this variety. Now a day, the fish *Heteropneustes fossilis* is considered the low supply, high demand and very expensive item (Samad *et al.*, 2013) [18]. The present work on induced breeding of Shing had been under taken to develop a successful artificial breeding technique in hatchery that would be tremendously helpful in farmer level and repopulate the natural habitat. Again Successful breeding technique depends on careful selection of brood stock and appropriate doses of induced hormone, which was suggestive of our findings.

The administration of the appropriate dose of hormone is the basic to the success of induced breeding; the condition of the brood fish and the environmental conditions are also equally important (Pillay, 1993) [16]. The success of induced spawning depends on a number of factors, which in most of the fishes are not clearly understood. The experiment was conducted to understand the response of spawners and possible outcome to different hormone doses.

Maintenance of temperature is very important in case of breeding purpose. Because at low temperature the eggs may fall in stressed condition. The embryo may be smaller due to loss of energy for the long stage in embryonic condition. For that reason the larvae may be deformed even normal hatching may become smaller and exhausted after hatching and ultimately nonviable.

The present experiment was conducted in the months of August to September which was considered the beginning of the breeding season of *Heteropneustes fossilis*. The pure strain of Shing was collected from the two different hatcheries in the months of June – July. Kohinoor *et al.* (1991) [11]. Suggested that the breeding season of 'Koi' is March to June. The breeding season of *Heteropneustes fossilis* lasts between the middle of April to the middle of June. In the laboratory, end of breeding was delayed as late as September (Thakur, 2003) [21]. The male and female ratio was maintained 1:1 which was suggested by Zworykin (2012) [22].

Temperature affect the ovulation period of the *Heteropneustes fossilis*. During the present study ambient temperature was in between 26–31 °C. Kohinoor *et al.* (1991) [11].

Accomplished induced breeding of *Heteropneustes fossilis* at the ambient temperature of 27-30 °C. However Thakur (2003) [21]. Reported that water temperature 28±1 °C and darkness is important environmental factors for successful spawning of *Heteropneustes fossilis*. The findings of the above authors agreed with the present study.

In present breeding trials the brood fishes were treated with Flash which was a synthetic gonadotropin releasing hormone. GnRH and sGnRHa successfully induce breeding in teleosts (Levavi-Sivan *et al.*, 2004) [12]. A surge of gonadotropin (Gill-H) associated with ovulation and its induction of female oocyte maturation by stimulating the synthesis of maturation inducing steroids (MIS) by the follicular cells (Goetz, 1983, Kime, 1993; Nagahama *et al.*, 1994) [8, 10, 15]. has been observed in several teleosts.

During the present experiment fertilization rates were found as 80.33%, 71.97% and 63.56%. Determination of fertilization and hatching rate of fish is important for various respects. It can determine the status of how many fry can be produced from a number of fish and how many are lost and why. It helps to improve the hatchery product and thereby production

(Misra, 1994) [14]. The best rates obtained for fertilization was 80.33% and the lowest rate 63.56%. This findings show that 0.5 ml Flash/Kg body weight is sufficient to achieve ovulation and good production of fry. Singh *et al.* (2012) [20]. Conducted similar breeding experiment of *Heteropneustes fossilis* with Ovaprim and gain best result at 0.3 ml/Kg body weight rate. Whereas, Malik *et al.* (2014) [13]. Conducted experiment on the koi carp using Ovaprim with a single dose of 0.2 ml/Kg for male and 0.3 ml/Kg for female brood fish. In this experiment he found 75.2% fertilization rate which is more or less similar to the current findings.

Hatching rates were observed as 71.67%, 63.35% and 54.47%. Ghosh *et al.* (2012) [7]. carried out similar breeding experiment in two seasons with Ovaprim where they found 42.78%, 44.60% and 55.00% hatching rate in the summer season, they had also used three treatment which was 0.5 (T1), 0.7 (T2) and 1.0 (T3) ml per Kg body weight. However, Singh *et al.* (2012) [20]. was able to breed *A. testudineus* using Ovatide where they found 48.7%, 69.2%, 92.3 and 83.5% hatching rate at a doses of 0.1, 0.2, 0.3 ml/Kg body weight and 30 mg CPE/Kg body weight. These findings differ from current experimental result. Above findings shows variation among the results. However, Chaudhuri (1960) [3]. had observed that induced breeding experiments yield better results when the donor and recipient fishes are of the same species. Again this variation may probably because they were treated in different doses of hormone treatments. Although some variations may arise due to the physiological differences of pair fishes and also for the experimental error. In order to reduce the experimental error, three replications were used in each treatment. This type of variation was also reported by different workers (Singh *et al.* 2012, Kohinoor *et al.* 1991) [20, 11].

It is very difficult to identify the reason for such different results. From the above discussion it can be said, that the fertilization, hatching and the survival rate of larvae differs mainly due to the hormone dose as well as quality of brood, seasonal variation, incubation density, water flow during incubation, quality of hatchery water and handling procedure of the broods, and the source of Flash. But upon all consideration Flash doses of 0.2 ml/Kg body weight for female and 0.13 ml/Kg body weight for male may be recommended for induced breeding of Shing in hatchery.

Conclusion

Studies on induced breeding of Shing were carried out at the Bogura Matshya Khamar, Parulia, Palash, Norshindhi. The experiment was performed during 1st August to 1st September 2015. The study was conducted to optimize the dose of Flash hormone for induced breeding of Shing and to determine the hatching rate, fertilization rate and survival rate of Shing. The present study consisted of three treatments with three replications was designed to optimize the suitable dose of Flash hormone for the best performance. Forty five pairs of male and female were selected as a ratio of 1:1 from the ponds and different doses of Flash were administrated to examine its effect. To observe the effective dose for induced breeding, the females were injected at the rate of 0.5 (T1), 0.45 (T2) and 0.42 (T3) ml/Kg body weight. On the other hand the males were administrated a dose of 0.2 (T1), 0.18(T2) and 0.17 (T3) ml/Kg body weight respectively. Then fishes were stocked in hapa. At 26 to 31°C water temperature the best result in terms of fertilization 80.33%, hatching 71.67% and survival rate 61.56% were found among the three different treatments.

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