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Determination of optimized dose with synthetic hormone for induced spawning of vietnamese koi (*Anabus testudineus*)

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ABSTRACT

An experiment on induced breeding of *Anabus testudineus* was carried out to determine the optimum dose of Flash hormone at the Reliance Aqua Farm hatchery, Boilor, Mymensingh. The present study consisted of three treatments (T_1, T_2 and T_3) 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 250±20 g and 200±15 g respectively. To observe the effective dose for induced breeding, the females were injected at the rate of 0.30 (T_1), 0.25 (T_2) and 0.20 (T_3) ml Flash/kg body weight and correspondingly the males were administered a dose of 0.13 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 30^0C during experimentation. Among the three treatments T_3 showed the best result in terms of fertilization rate (79.89%), hatching rate (66.22%) and survival rate (56.20%). The present findings can be used in induced breeding of *A. testudineus* for the development of hatchery propagation.

INTRODUCTION

Aquaculture is one of the fast growing sectors in Bangladesh. 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). The soil, water and climatic condition of Bangladesh are very favorable to inland fisheries, both open and closed waters as well as brackish water fisheries. Fish culture has been drawn a greater attention by the farmers in many parts of having a suitable natural habitat, tropical climate, productive and unplowed estuarine areas. 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, 2014). Through this consequence Vietnamese koi (*Anabus testudineus*) had been introduced in 2011 from Thailand by Sharnalata fish hatchery with the support from Innovision Consulting (Pvt.) Ltd and Swiss funded Katalysit for their high adaptability fast growing, nutritious value and a good market price (Torofdar, 2013). Report showed that Vietnamese koi grows as much as 250-300 g within 120 days culture period and the body color is almost similar to "Deshi koi" (Sarnalata Agro-Fisheries Ltd, 2013). Culture potential of Vietnamese koi has increased in various part of Bangladesh especially in Mymensingh due to its fast growth rate. Ovaprim was useful in breeding of *Anabas testudineus* 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). Artificial hypophysation of the *Anabas testudineus* was first attempted by Khan and Mukhopadhyay (1972) while the effect of Wova-FH on breeding was observed by Sarkar et al. (2005).Considering the importance of Vietnamese koi the present work was undertaken to determine

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the optimum dose of "Flash (containing sGnRH+dopamin)" for induced breeding of Vietnamese koi; and as well as having a clear view on ovulation, hatching and fertilization of the target species.

MATERIALS AND METHODS

Experimental site

The research work was conducted at the Reliance Aqua Farm hatchery, Boilor, Mymensingh. The experiment was performed during 10th January to 20th March 2015. The site was chosen as it was well equipped to conduct the research work.

Collection of fish

Hundred Vietnamese koi were collected from the two different hatcheries. The collected fish were stocked in the rearing pond situated inside the hatchery complex, during November – December 2014.

Brood pond preparation

There were 2 brood ponds each having an average area of 60 decimal and the water depth between 1.0-2.5 meters. 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 200 g and 100 g per decimal respectively.

Table 2
Water quality parameter recorded during the breeding period.

Parameters	Ranges
Water pH	7.8
Temperature	26-30^{0}C
Hardness of water	110 mg/l
Dissolve Oxygen (DO)	4.39 - 5.61 mg/l
Free CO_2	0 mg/l

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.

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 average weight of the females and males were 250±10 g and 300±15 g respectively.

Table 3
Criteria followed to select mature breeders of Vietnamese Koi (*Anabus testudineus).*

Male	Female
Small in size	Relatively large in size
Abdomen normal; not bulky like female	Abdomen bulging, elastic and soft
Body slender	Body robust
Whitis melt come out on gentle pressure in abdomen to be the best criteria for male	Small amount of eggs from the ovary come out on gentle pressure

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 with water showering and sufficient aeration. No feed was provided during the period of conditioning.

Breeding plan

For induced breeding of Vietnamese koi male and female broods were collected from the brood rearing ponds of the hatchery complex at 1:1 ratio of male and female. In the experiment "Flash (sGnRH+dopamin)" were used as inducing agent in both male and female fish. For female brood

Flash was induced at the rate of 0.2, 0.25 and 0.3 ml/kg body weight, which was considered as the treatment such as T_1, T_2 and T_3 respectively. For the male brood single doses of "Flash" 0.13 ml/kg body weight were injected. Each treatment considered for 9 replications. For each treatment 15 males and 15 females were placed in three different hapa containing 10 broods in each. So 90 brood fishes were injected for induced breeding that composed of 45 females and 45 males.

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 0.5 ml. 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 (Figure 3). 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 are shown in table 4.

Table 4
Doses of Flash for male and female broods of Vietnamese Koi under different treatments.

Dose	T_1	T_2	T_3
Male	0.13 ml/kg	0.13 ml/kg	0.13 ml/kg
Female	0.3 ml/kg	0.25 ml/kg	0.2 ml/kg

Dose of Flash was administered to the female and male broods in between 9 to 10.30 pm. Then the fishes were kept into hapa.

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 their to hatch.

Determination of fertilization rate

To determine the fertilization rate 100 eggs was taken in a Petridis from hatching jar. The fertilization was determined by the following formula

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

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 takes place after 70±2 h of hatching. After 72 h of post-hatching boiled egg-yolk provided as first food for the hatchling of Vietnamese koi at ambient temperature of 22 to 26^0C.

Determination of survival rate

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

Figure1
Three days old fry.

Figure 2
Seven days old fry.

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 programme 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.

Table 5
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)
20th February 2015	1:1	Female Male	0.3 0.13	10 - 12	22 - 24
12th March 2015	1:1	Female Male	0.25 0.13	10 - 12	21 - 24
22nd March 2015	1:1	Female Male	0.2 0.13	8 - 9	20 - 22

Physico-chemical condition of water

Physico-chemical condition such as temperature, dissolved oxygen and p^H of water in experimental tank under different treatments with different larval stocks of Vietnamese koi ranged between 26^0C to 30^0C, 4.39 to 5.61 mg/l and 7.9 to 8.15 respectively.

Dose optimization with synthetic hormone Flash

Dose optimizations with Flash hormone for spawning of female Vietnamese koi were performed with different doses of Flash. Three different doses viz., 0.2, 0.25 and 0.3 ml Flash/kg body weight of fish were applied whereas each dose was consisted of one treatment e.g. T_1, T_2 and T_3 respectively. Corresponding data representing the effects of Flash doses on fertilization rate, hatching rate and survival rate of Vietnamese koi which are shown in Table 6.

Table 6
Performances of different doses of Flash on induced breeding of Vietnamese koi.

Treatment	Fertilization rate% M ± SE	Hatching rate% M ± SE	Survival rate% M ± SE
T_1	68.78 ± 1.28^a	58.55 ± 1.95^a	47.00 ± 2.24^a
T_2	73.89 ± 2.01^b	60.88 ± 2.00^{ab}	51.66 ± 2.19^{ab}
T_3	79.89 ± 0.94^c	66.22 ± 1.93^b	56.20 ± 2.03^b

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

Fertilization rate

From the experiment the fertilization rate were recorded as 68.78%, 73.89% and 79.89% in the treatments of T_1, T_2, and T_3, respectively (Table 6). The highest fertilization rate 79.89% was recorded in T_3 whereas the lowest fertilization rate 68.78% was found in T_1. The results from the ANOVA test indicated that there was a significant difference among three doses of Flash treatment whereas T_3 was significantly (p<0.05) higher than that of treatments T_1 and T_2.

Hatching rate

The hatching rate was found 58.55%, 60.88% and 66.22% in treatments of T_1, T_2 and T_3 respectively (Table 6). The highest hatching rate was recorded 66.22% in T_3 and the lowest hatching rate was recorded 58.55% in treatment T_1. 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 T_3 was significantly (p<0.05) higher than that of T_1 but it was not significant in comparison with T_2.

Survival rate

The survival rate of Vietnamese koi larvae those were produced by three different hormone doses treatments (Table 6) were 47, 51.66 and 56.20% in T_1, T_2 and T_3 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 T_3 compared to the T_1 but there was no significance difference among T_1 and T_2 respectively.

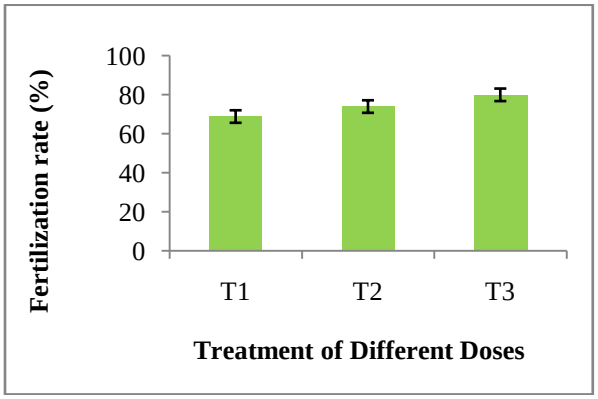

Figure3
Comparison of fertilization rate (%) of Vietnamese koi during induced breeding with different doses of Flash.

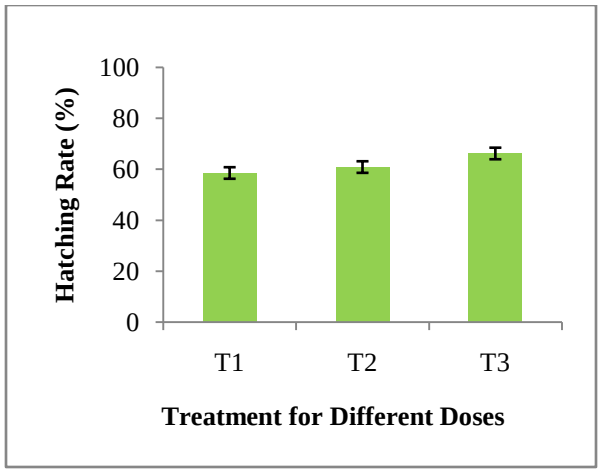

Figure 4
Comparison of hatching rate (%) of Vietnamese koi during induced breeding with different doses of Flash.

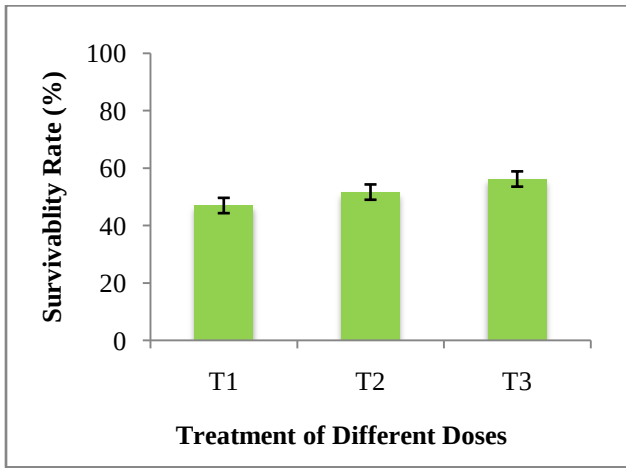

Figure 5
Comparison of survivablity rate (%) of Vietnamese koi during induced breeding with different doses of Flash.

DISCUSSION

Several studies have been conducted on the growth performance of Vietnamese koi in Bangladesh, but very limited study was carried out on the breeding technique relating this variety. Now a day, the fish *Anabus testudineus* (Bloch) is considered as an endangered species (Kohinoor, 2008).The success of induced spawning depends on a number of factors, which in most of the fishes are not clearly understood (Ram et al., 2001). The experiment was conducted to understand the response of spawners and possible outcome to different hormone doses.The present experiment was conducted in the months of February to March which was considered the beginning of the breeding season of *A. testudineus*. The pure strain of Vietnamese koi was collected from the two different hatcheries in the months of November – December. Kohinoor et al. (1991) suggested that the breeding season of 'Koi' is March to June. The breeding season of *A. testudineus* lasts between the middle of April to the middle of June. The male and female ratio was maintained 1:1 which was suggested by Zworykin (2012).Temperature affect the ovulation period of the *A. testudineus.* During the present study ambient temperature was in between 26 – 30°C. Kohinoor et al. (1991) accomplished induced breeding of *A. testudineus* at the ambient temperature of 27 - 30ºC. 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). 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 (Nagahama et al., 1994) has been observed in several teleosts. In this experiment male fishes were found to be responding by a single dose of 0.13 ml/Kg body weight and the females were injected at the rate of 0.3 (T_1), 0.25 (T_2) and 0.2 (T_3) ml/kg body weight. Rahman (2001) was able to breed *Ompok pabda* in hapa by administration of a single PG dose of 14 and 16 mg/Kg body weight for female and 12 mg/Kg body weight for the males. Also Akhteruzzaman et al. (1993) reported that female fishes weighing 55-110 g each were given single injection of 10-18 mg PG/ Kg body weight of acetone dried carp pituitary gland and male fishes weighing 45-90 g each were given a dose of 12 mg PG/kg body weight, ovulation occurred in all the injected females, he found best results using doses of 13 to 16 mg PG/ Kg body weight. However Hoque (1990) found that maturation of the ovary was achieved about one month earlier in silver carp *(Hypophthalmichthys molitrix)* and catla *(Catla catla).* Diets containing 1% vitamin premix and higher lipid levels showed better result in all aspects viz. selectivity, spawning success, fertilization and hatching rate. Singh et al. (2012)

conducted similar breeding experiment of *A. testudineus* with Ovaprim and gain best result at 0.3 ml/Kg body weight rate. Whereas, Malik et al. (2014) 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 58.55%, 60.88% and 66.20%. Ghosh et al. (2012) 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 (T_1), 0.7 (T_2) and 1.0 (T_3) ml per Kg body weight. However, Singh et al. (2012) 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. 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).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 Vietnamese koi in hatchery.

CONCLUSION

The highest percentage of fertilization rate, hatching rate and survival rate obtained in the present experiment may be considered as satisfactory however, there is scope for further improvement in the process. The present findings can be used in induced breeding of Vietnamese koi in the hatcheries. From the available references along with the present investigation on the induced breeding of the fish, higher percentages of fertilization and hatching were achieved from a comparatively lower dose of hormone. This can be considered as an effective means for commercial seed production in the hatcheries since cost and quality of hormone is one of the important key factors for a hatchery manager. The present study can throw light for further researches on the improvement of artificial breeding of the fish concerning the optimization of hormone application, optimization of the environmental parameters and other biological aspects.

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