



Effects of different age groups of female brood stocks on the reproductive performance of Catla (*Catla catla*)

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Abstract

The study was conducted with the doses 4, 5, 6 and 7 mg/kg body weight of Pituitary gland extract to determine the dose that will give the best result for induced breeding during March 2017 to July 2017 at Ma Fatema Hatchery, Jashore. The higher spawning fecundity were found in 5 mg/kg with value 760737±50169 contrary in 4, 6 and 7 mg/kg doses; the number were 684009±34818, 729355±65443 and 737826±25416, respectively. At the dose 5 mg/kg ovulation rate, fertilization rate, hatching rate, survival rate and deformity rate were found 100%, 88.64±2.09%, 86.09±2.58%, 87.78±3.27% and 3.45±1.42%, respectively. PG treated fishes showed best result at dose 5 mg/kg. Then 5 mg PG was used to find out the effects of different age groups of female broods. To determine the suitable age of broods three age groups were used. The mean weight of female broods of 2, 3, 4 years old were 3.22±0.39, 4.58±0.38 and 5.25±0.13 kg, respectively. The gonad somatic index were 24.12%, 21.32% and 20.04%; mean absolute fecundity 624016±34136, 726900±25805 and 767783±17917 in 2, 3 and 4 years female broods, respectively. In treatment 2, 3 and 4 years female broods' ovulation rate were 66.67%, 100% and 100%; fertilization rate were 75.64±2.12%, 85.88±3.00% and 89.84±3.49%, respectively. Hatching rate were 71.38±3.39%, 82.94±3.52% and 87.70±4.73%; Survival rate before start feeding were 74.09±3.33%, 78.52±4.31% and 88.12±3.38% in 2, 3 and 4 years age groups, respectively. Better breeding performance regarding fertilization and hatching rate was reported in 3 and 4 years female than 2 years age.

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Introduction

Carp denotes the leading species for aquaculture system in Bangladesh and the production of these species entirely depends on timely and sufficient amount of quality seeds. Individually carp species contribute 80% in overall annual production (DoF, 2018). The accessibility of fish fry is a necessary requirement for fish culture. The country is self-reliant in carp seeds production though quality fish seeds are produced in a narrow scale.

Catla is known as 'Catla' belongs to family Cyprinidae attains first maturity in the second year of life and in ponds it becomes mature when 22 months old (Alikunhi, 1957). Normal breeding of *Catla* does not happen within ponds, even the maturity of species; thus hormonal stimulation is required. Induced breeding of *Catla* has been providing for the entire seed demand in all of the countries. Culture of fish in Bangladesh introduced with natural seed but now it is highly (99.55%) replaced by hatchery manufactured seed (Minaret *et al.*, 2012).

Artificial propagation also known as hypophysation is accomplished with the association of reproductive hormones. Numerous hormones have been described to be broadly used to induce fish though the dosage used differs with fish breeders as no standard dose present for all fish species (Gomina, 2011). Only a dependable induced breeding and fry rearing procedure may confirm a steady source of quality fish seeds (Mollah *et al.*, 2008). Induced breeding through hormone usage and synthetic incubation of inseminated egg has advantages of enhanced percentage of fertilization and hatching, better situations for growth and survival of larvae to fingerling and better protection of larvae against hostile environmental condition and predators (Woynarovich and Horvath, 1980). Extensively cultured Indian major carps in inland waters like *Catla* generally do not breed in confined waters. They mature there, but only breed in the inundated shallow parts along the way of the rivers in rainy season which are their natural habitat. Induced propagation of Indian major carps has been flourished fruitfully by

administration of pituitary gland extract (PGE). The first time successful spawning of Indian major carps induced with pituitary extracts was carried out by Chaudhary and Alikunhi (1957).

Catla is utmost difficult to breed among the three Indian major carps as it needs proper eco-friendly environments for spawning. The losses of the spawn produced for the major carp species because of un-optimized conditions. Khan and Bhatti (1967) have worked on induced breeding of Indian Major Carps. Moshand Mlingi (2018) have observed the influence of variant doses of pituitary gland extract on African catfish (*Clarias gariepinus*). Miah *et al.* (2008) has worked on dose optimization with pituitary hormone for spawning of *Bata* (*Labeobata*) where he observed the effective dose for breeding. Above all, effect of different doses of pituitary gland extracts on breeding performance of *Catla* is not available.

Success of total action of induced breeding depends mostly on the appropriate selection of brood fishes (Khan and Mukhopadhyay, 1975). Fish husbandry business has been more concentrated on the quality of eggs or larvae than that of sperm, even the superiority of both gametes can influence pollination success and survival of fry (Rurangwa *et al.*, 2004). The healthy larvae are obtained from healthy gametes. The practice of improved quality gametes from fish brood stocks is of massive importance for confirming the production of viable larvae (Kjorsvik *et al.*, 1990). Age of fish impacts the quality of gametes and survival percentage of larvae. Mature and bulky females produced eggs with larger size compared to minor and younger ones (Gall, 1974). Usage of age oriented broodstock can ensure great quality eggs and larvae (Aliniya *et al.*, 2013). Maternal effects on the size of eggs and young fish have been considered in an evolutionary framework (Reznick, 1991). Ultimate variables that affect propagative possibility of fish stocks have found to differ with age, size and condition of spawning (Trippel, 1998).

Pitman (1979) worked on the impacts of female brood age and size of egg on development and mortality of

rainbow trout (*Oncorhynchus mykiss*). Similar study was accomplished by Shamspour *et al.* (2009) on 3 to 5 years old rainbow trout. Alp *et al.* (2003) studied on reproductive biology of brown trout (*Salmo trutta macrostigma*) in 2 and 5 years old. Kayam (2004) studied on the influence of mating different age groups of brood stocks on the reproductive performance of rainbow trout where he observed that among all the coupling combinations old female appear to be most productive. The present study is aimed at comparing the influence of varying dosage of pituitary gland extract on the number of eggs/spawning fecundity, ovulation, fertilization, hatching, survival and deformity rate of Catla and to evaluate the effects of different ages of female broods in respect of ovulation, fertilization, hatching and survival rate of Catla.

Materials and methods

Study area and duration of time

The experiment was conducted in the Ma-Fatema Hatchery, Chanchra, Jashore from March 2017 to July 2017 under induced spawning condition and a part of that research was also done in the laboratory of Fisheries and Marine Bioscience Department, Jashore University of Science and Technology.

Experimental fish

Total no. of the fish was thirty, consisting of 30 females and 42 males. Weight of test fish for female is 3.22 ± 0.39 Kg for two years, 4.58 ± 0.38 Kg for three years and 5.25 ± 0.13 Kg for four years, respectively.

Age determination

The broods of 2, 3 and 4 years ages were kept in separate brood ponds of Ma-Fatema hatchery. Age was determined on the basis of the hatchery owner's information.

Selection of brood fish

At first brood fish was selected randomly for dose optimization. To determine the age effect hatchery reared healthy, uninjured and fully mature carps of 2 years male and 2, 3 and 4 years female brood fishes

were selected.

Determination of sex

Better quality and properly matured male and female brood fishes were gathered according to Muir and Robert (1985).

Sex ratio

For induced breeding purposes and different age groups identifying, brood fishes were assembled from the brood rearing pond of the hatchery complex and kept in the concrete tank with 2:1 ratio of male and female.

Brood stock management

During the period of experiment, a special feed enriched with protein and vitamin E was formulated which enhanced the gonadal maturation of fishes.

Experimental design

Table 1 denotes different doses of pituitary gland extracts were used to breed female Catla whereas Table 2 remarks the breeding design with different ages of broods.

Collection and preparation of pituitary glands (PG) extract

The amount of PG to be weight out was calculated on the basis of the body weight of all the fishes using the following formula:

$$\text{Weight of PG (mg)} = \frac{\text{Wt} \times \text{Pt}}{1000}$$

Where, Wt represents total body weight (g) of brood fishes to be injected and Pt represents the weight (mg) of PG to be injected/kg body weight.

Administration of hormone

After conditioning up to 24 hours, the brood fishes were weighted and recorded. PG solution 2.0 mg was injected in female brood Intraperitoneally near the pectoral fin base in about 45° angles to the body surface of one side and second injection was given after 6 hours at 5.0 mg in the opposite side of the body where the first injection was administered. At that time 2.0 mg was injected to male.

Determination of Gonadosomatic Index (GSI)

Before the experiment, female brood fishes were collected and weighted (gm) from each age groups. They were sacrificed and gonad was collected. Water from gonad surface soaked with soft tissue and weighted. The gonadosomatic index of the female was calculated by using the following formula-

$$GSI(\%) = \frac{\text{Weight of gonad in gm}}{\text{Body weight of fish in gm}} \times 100$$

Estimation of fecundity

Gravimetric method was applied in the present study. The fecundity was obtained by using the following formula-

$$\text{Fecundity} = \frac{\text{Total gonad weight (gm)} \times \text{No. of eggs}}{\text{Weight of small portion of total gonad (gm)}}$$

Observation of ovulation success

Ovulation took place after 6 hours of final dose in injected females. The rate of ovulation was enumerated by using the following formula-

$$\text{Ovulation rate (\%)} = \frac{\text{Number of females ovulated}}{\text{Total number of females injected}} \times 100$$

Estimation of fertilization rate

The eggs were examined after 2 hours of mixing with sperm to determine the fertilization rate. The fertilized eggs were differentiated from the unfertilized ones as the fertilized eggs are transparent, non-adhesive, have the presence of "eye spot", round in shape while unfertilized eggs are white and opaque. For this purpose few eggs was taken in a petri-dish and observed under a magnifying glass. The rate of fertilization was estimated by using the following formulas-

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs in sample}}{\text{Total number of eggs in sample}} \times 100$$

Estimation of hatching rate

Hatchling rate was determined by the following formulas-

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings in sample}}{\text{Total number of fertilized eggs in sample}} \times 100$$

Determination of survival rate

After 72 hours the fry were feed with boiled eggs yolk grounded in a homogenizer and 72 hours of hatching the number of live fry was observed by taking sample in a pot (dish). Then survival rate was determined by using the following formula-

$$\text{Survival rate (\%)} = \frac{\text{Number of survived fry in sample}}{\text{Total number of hatchling in sample}} \times 100$$

Estimation of deformity rate

Deformed larvae were determined by visual observation. The larvae were kept in glass slide for observing external deformity and examined with the help of electronic microscope. The percentage of deformed fry was determined as follows:

$$\text{Deformity rate (\%)} = \frac{\text{Number of deformed fry in sample}}{\text{Total number of fry in sample}} \times 100$$

Data processing and analysis

Qualitative and quantitative analysis of all kinds of data were carried out, checked for reliability and accuracy. MS Excel was used to store all the data and Graph Pad Prism 6.0.5.0 version was used for the presentation of the graphs. Analysis of variance (one way), Tukey-kramer test for differences between means were used for analysis of different dose and age groups on fecundity, fertilization rate, hatching rate, survival rate and deformity rate using SPSS 16.0 evaluation version. The significance of the data from the effects of different ages was considered significantly different at $P < 0.05$. All the data were carefully summarized and scrutinized.

Results*Fecundity*

The higher spawning fecundity (number of eggs) was found in the case of 5 mg/kg dose and the value was 760737 ± 50169 . Contrary in 4, 6 and 7 mg/kg the values were 684009 ± 34818 , 729355 ± 65443 and 737826 ± 25416 , respectively (Fig. 1). There were no significant differences ($p < 0.05$) observed in the case of total number of eggs.

Ovulation rate of Catla (%)

The highest ovulation rate (100%) was recorded in 5

and 6 mg dose whereas, the lowest ovulation rate (33.33%) was recorded in 4 mg (Fig. 2).

Fertilization rate of Catla (%)

Average fertilization rates were recorded as 73.34±2.44, 88.64±2.09, 84.15±2.62 and 81.44±3.48% in 4, 5, 6 and 7 mg, respectively (Fig. 3). There was a significant ($P<0.05$) difference among four doses of PG extracts where treated with 5 mg

dose showed significantly ($P<0.05$) higher result.

Hatching rate of Catla (%)

Average hatching rates were recorded as 70.09±3.54, 86.09±2.58, 83.04±3.97 and 74.26±3.45 % in 4, 5, 6 and 7 mg dose, respectively (Fig. 4).

There was a significant ($P<0.05$) difference among four doses of PG extracts in respect of hatching rate.

Table 1. Different doses of pituitary gland extracts used to breed female Catla.

Treatments	1 st dose for female (mg/kg body wt.)	Time intervals (h) between two doses	2 nd dose for female (mg/kg body wt.)	Single dose for male (mg/kg body wt.)
4 mg	2.0	6	4.0	2.0
5 mg	2.0	6	5.0	2.0
6 mg	2.0	6	6.0	2.0
7 mg	2.0	6	7.0	2.0

Survival rate of Catla(%)

The survival rate of fry was observed after 4th day of hatching. The larvae those produced by four different doses of PG extracts in which result found in treatments were 72.08±3.32, 87.78±3.27, 82.38±4.96

and 76.26±3.78% in 4, 5, 6 and 7 mg, respectively (Fig. 5). There was a significant ($P<0.05$) difference among four doses of PG and a significantly ($P<0.05$) higher survival rate was observed in treatment 5 mg compared to the other three treatments.

Table 2. Breeding design with different ages of broods.

Serial No.	Replication	Male (age)	×	Female (age)
Treatment-1	3	Two years	×	Two years
Treatment-2	3	Two years	×	Three years
Treatment-3	3	Two years	×	Four years

Deformity rate of Catla(%)

Among four different doses the highest deformed larvae was observed in the dose 7 mg whereas the lowest was in 5 mg conducted dose (Fig. 6). The results indicated that there showed a significant difference ($P<0.05$) among doses of PG in the viewpoint of deformity rate.

Ovulation rate

The ovulation rate of 100% was achieved in 3 and 4 years female broods whereas 66.67% in 2 years age female broods (Fig. 7).

Fertilization rate at different treatments of ages

The mean rate of fertilization was found 75.64±2.12, 85.88±3.00 and 89.84±3.49% in 2, 3 and 4 years age, respectively (Fig. 8).

Hatching rate at different treatments of ages

Hatching started after 20±2 hrs mixing of eggs and sperms. The yolk sac absorption was observed after 70±2 hours of hatching. In 2, 3 and 4 years age female broods mean hatching rate was recorded as 71.38±3.39, 82.94±3.52 and 87.70±4.73%, respectively (Fig. 9).

Table 3. Common properties of female brood fishes at different ages.

Age (years)	Mean body weight (Kg)	Mean total weight of the ovary (gm) ±SD	Mean number of eggs per gram ±SD	Mean absolute fecundity ±SD	Mean relative fecundity (gm/kg)	GSI (%)
2	3.22±0.39	771±28.43	808±17	624016±34136	241±21.66	24.12%
3	4.58±0.38	973±15.28	747±15	726900±25805	213±14.19	21.32%
4	5.25±0.13	1051±10.41	730±8	767783±17917	200±2.65	20.04%

Survival rate at different treatments of ages

Survival rate was determined after 72 hours of hatching by taking sample. In 2, 3 and 4 years ages of female mean rates of survival were found 74.09 ± 3.33 , 78.52 ± 4.31 and $88.12 \pm 3.38\%$, respectively (Fig. 10).

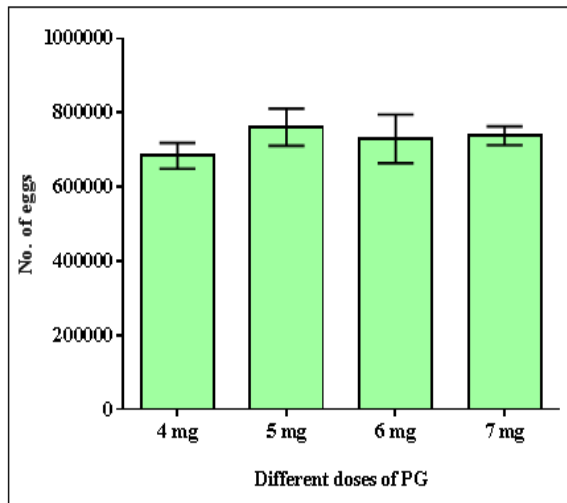


Fig. 1. Spawning fecundity (total no. of eggs) at different doses of PG extract.

Discussion

In the present study the highest ovulation rate (100%) was recorded in 5 mg/kg and 6 mg/kg treated dose of PG whereas the lowest value (66.67% and 33.33%) was found in 7 mg/kg and 4 mg/kg. In a higher dose 67% ovulation rate indicates that the brood fishes were highly mature in the better brood stock management practice with special diet.

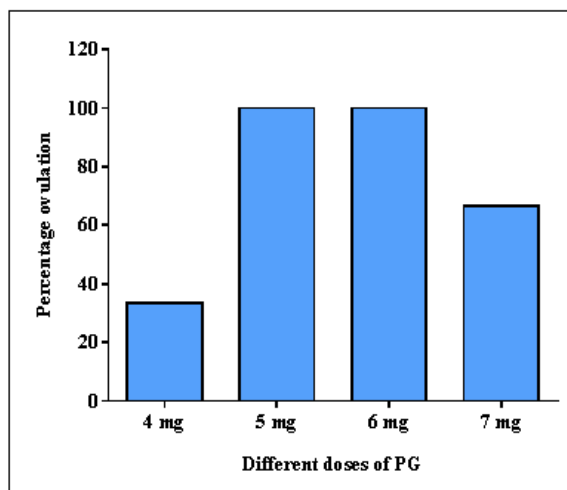


Fig. 2. Ovulation rate (%) at different doses of PG extract.

The results indicated that there was a significant ($P < 0.05$) difference among four doses of PG in the

viewpoint of ovulation rate and revealed that 4 mg/kg PG dose was significantly ($P < 0.05$) lower than 5, 6 and 7 mg but no difference between 5 mg and 6 mg while considering the ovulation rate. The ovulation rate increased in 5mg and 6mg/kg body weight but decreased in higher (7mg/kg) or lower doses (4mg/kg). The similar results was also observed by Ali *et al.* (2015); Minaret *et al.* (2012) and found that the best spawning occurred under the dose 5mg and 6mg PG/kg body weight in case of female. Yeasmin *et al.* (2013) conducted a work on *Cyprinus carpio* where all nine female fishes were injected with CPE at 5.0 mg/kg body weight and the ovulation rate were 100%. The ovulation rate of PG at the rate of 7mg/kg body weight showed decrease in ovulation.

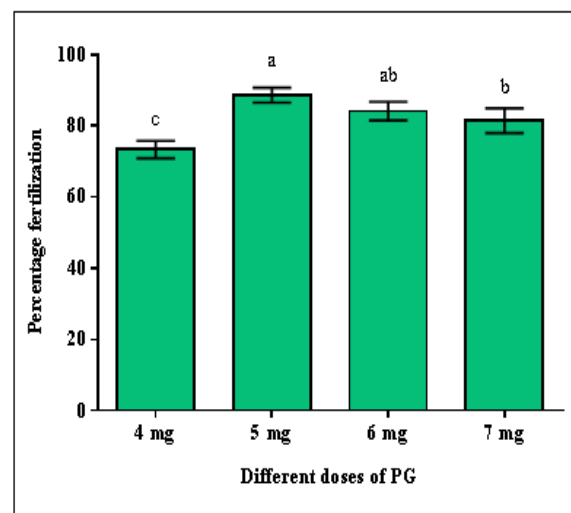


Fig. 3. Fertilization rate (%) at different doses of PG extract.

The spawning fecundity/total number of eggs was increased at 5 mg dose of 761110 ± 55424 whereas, comparatively lower number was observed in 4, 6 and 7 mg dose. This might be due to insufficient release of gonadotropin, agreeing to earlier studies (Van der Kraak *et al.*, 1983; Billardet *et al.*, 1984). The results indicated that as pituitary gland extract dosages increased, egg quantity was also increased. The explanation for obtaining better results from fish injected with higher doses of pituitary gland extracts is that, the stimulants in higher doses stimulate the fish effectively by contracting the smooth muscles in the gonads of female before ovulation and culminating in spawning of fish (Yeung *et al.*, 2006). The poor responses at lower doses or excessive higher

doses of pituitary gland extracts in female may be due to insufficient secretion of gonadotropin leading to ovulation failure or blocking of ovipore by disintegrated ovarian tissue and egg bunches (Smith, 1999).

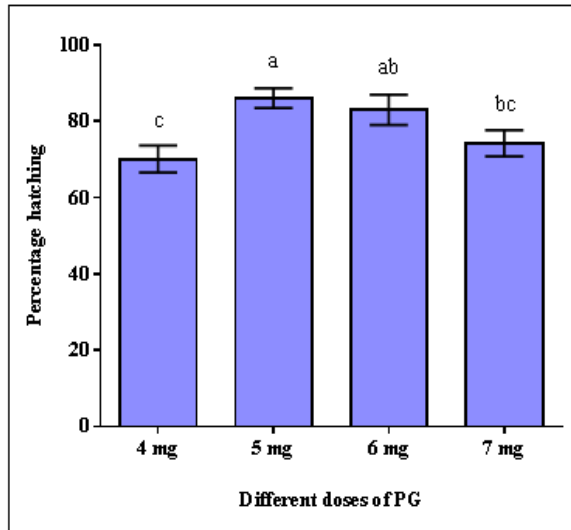


Fig. 4. Hatching rate (%) at different doses of PG extract.

The rate of fertilization was decreased in 4mg, 6mg and 7mg/kg body weight of PG dose. This result is in the agreement with the work of Ali *et al.* (2015) in induced breeding of Crucian carp when female response earlier. Higher and lower doses of PG, fertilization rate was also found to be lower. Higher dose of PG sometimes causes block in reproductive vein of female. On the other hand, fertilized eggs quality was very poor and they did not hatch which subsequently survive less. The result was in agreement with Miah *et al.* (2008) where they conducted experiment with PG on *L. bata*. More *et al.* (2010) also observed lower fertilization rate in higher doses of PG in Indian major carp.

It was found the best fertilization rate 88.64%, hatching rate 86.09% and survival rate 87.78% with the dose 5mg/kg. Similar findings were recorded by Ali *et al.* (2015); Kabir (2009); Jaman (2009) they were found the highest fertilization, hatching and survival in 5mg/kg body weight of crucian carp, Indian major carp and common carp, respectively. The highest fertilization rate was recorded as 93% in case of Rui at 5mg/kg body weight. Miah *et al.* (2008)

recorded similar type of result in bata when fertilization, hatching and survival rate was 84%, 85% and 84%, respectively. The present investigation demonstrated that the highest deformity rate (10.70±4.07%) was recorded in 7 mg/kg whereas the lowest value (3.45±1.42%) was found in 5 mg/kg dose of PG. Increasing PG doses above 5 mg led to over ripening of ova, which resulted in increased percent deformed larvae. On the other hand, the percentage of deformity was observed 6.29±2.19 and 5.24±2.18% in treatment 4 mg/kg and 6 mg/kg, respectively. Rahman *et al.* (2015) also found similar results on breeding performance of Bata where he reported that deformity rate was ranged from 6.50±2.65 to 11.75±3.59%. A significantly higher deformed larvae ($p < 0.05$) was observed at three doses compared to 5mg dose level. At lower or higher dose more deformity in larvae may be ascribed to the fertilization of un ripe or over ripe ova. Rao and Ram (1991); Goswami and Sarma (1997) also reported similar higher deformed larval production in *C. batrachus* at lower and higher dose of pituitary respectively during induced breeding.

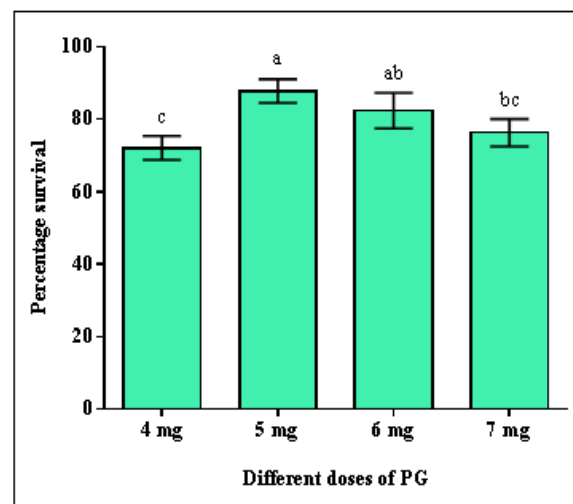


Fig. 5. Survival rate (%) at different doses of PG extract.

It was observed that successful spawning occurred among the age groups three and four compare to two years broods. It was observed that the ovulation rate starting from adolescent sterility rises rather rapidly to its highest point and gradually falls with advancing age to senile sterility reported by Rao (1961). Over the first two spawning seasons in female rainbow trout,

females produce better quality eggs in the second season (Brooks *et al.*, 1997) compare with first spawning season.

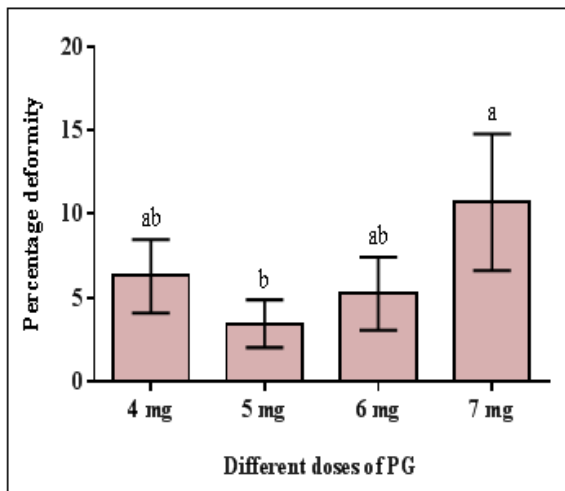


Fig. 6. Deformity rate (%) at different doses of PG extract.

Size of egg increased with increasing age. The highest number of eggs per gram ovary was observed in two years age female and the number of eggs per gm was 808 and lowest number of eggs was 730 in four years' female broods. The old females can produce larger eggs than the younger females. Similar results was also reported by Brooks *et al.* (1997) that larger and older females are believed to spawn larger eggs which show higher fertilization and hatching rates. Shamspour *et al.* (2009) observed in rainbow trout that larger fish produces bigger eggs. So, age is the factor for producing large size of eggs.

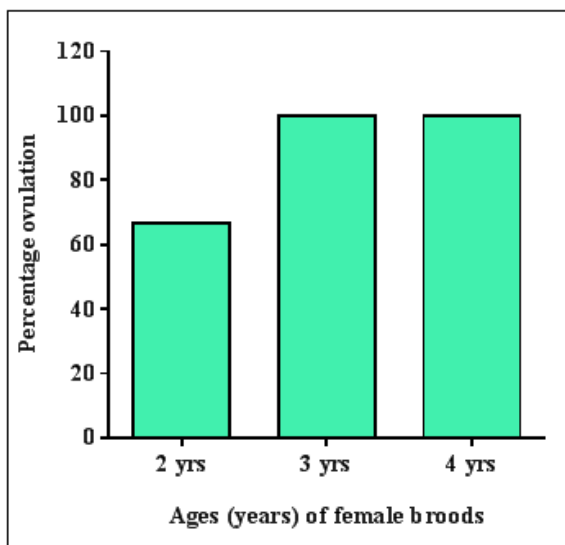


Fig. 7. Ovulation rate (%) at different ages of female broods.

Mean absolute fecundity was also greater in number of old female than Youngers. The study showed the highest absolute fecundity in four years age female and the number was 767783, in two years ago the number was 624016 and three years age female 726900. The study agreed with the result of Reznick *et al.* (2002) that fish fecundity increase with the age of female breeders. The findings was recorded by Zoharand Myloans (1987) studied on Indian major carps that fecundity of fish increased with the increase of weight of fish and gonad weight. Furthermore, Kjesbu *et al.* (1992) reported that density of eggs produced by second-time spawners, initially greater than density of eggs produced by the same females the year before.



Fig. 8. Fertilization rate (%) at different ages of female broods.

The fecundity of *C. catla* has been estimated from 2,30,831 to 30,77, 900 Natarajan and Jhingran (1963). According to Simpson (1951) the fecundity of an individual female varies according to many factors including age, size, environmental conditions that supports the present findings. Similar observations have been reported by various workers in different fish species like Siddiqui *et al.* (1976) in *Labeobata*, Faruq *et al.* (1996) in *Clariasbatrachus* and Narejo *et al.* (2002) in *Mastacembelusarmatus*. Relationships between potential fecundity and female age and/or size have been documented for many cod populations (Marteinsdottir *et al.*, 2000). When the egg size increases, the relative fecundity has also been reported to decrease with female age (Baum and

Meister, 1971). Same result was also recorded from the present study that relative fecundity was higher in the two years age female fish than the three and four years old broods.

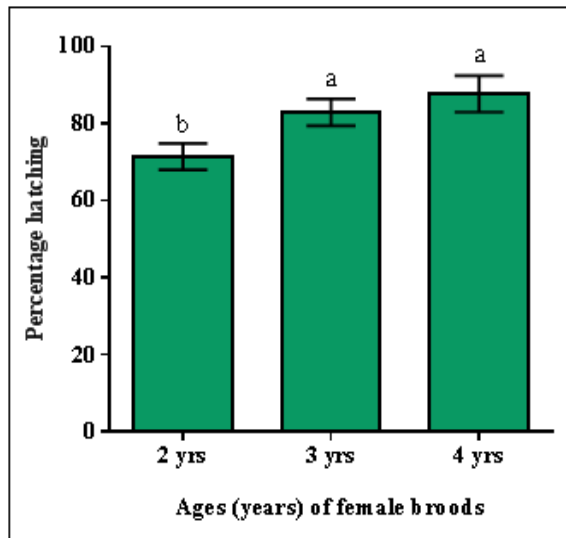


Fig. 9. Hatching rate (%) at different ages of female broods.

The relative fecundity for two, three and four years old female broods was 241, 213 and 200 gm/kg, respectively. The results showed that when the age was higher the rate of fertilization increased. The fertilization rate 89.84% and 85.88% were observed in 4 years and 3 years female broods, respectively and this two were higher than 2 years old female. This may be due to the size of eggs was bigger than the 2 years female. The age of fish is higher; the rate of fertilization is increased (Khara *et al.*, 2011). He also studied that oocyte quality related with its size and weight that can have a positive effect on fertility rate. Several studies shown that the smaller eggs of recruit spawners exhibit lower fertilization rates and greater mortality during incubation (Trippel, 1998). The study showed that comparatively higher hatching rates of 82.94% and 87.70% were found in 3 and 4 years female broods. The lowest hatching rate was 71.38% in 2 years female. This result is similar to the findings of Rahbar *et al.* (2011) that the age and size of brood fish have influence on the hatching and survival of larvae until the absorption of yolk sac stages found in Caspian brown trout (*Salmo trutta caspius*). The present study was agreed with Trippel *et al.* (1997a) that hatching success

appears to be significantly lower among eggs of first spawners compared with second or third time spawners. In the present study the highest survival rate 88.12% was found in 4 years female which is similar to the findings of Marteinsdottir and Steinarsson (1998) that the larger eggs are tended to be produced by the largest females of the third spawning stage. Larvae from the largest egg started to feed earlier than larvae from the smaller eggs.



Fig. 10. Survival rate (%) at different ages of female broods.

The study showed that survival rate was slightly reduced in 2 years and 3 years compared to 4 years female. The results agree with Marteinsdottir and Steinarsson (1998) who demonstrated that feeding success on day 3 also increased with egg size which influenced the findings of survivability increased with egg size.

Conclusion

PG at the dose of 5mg/kg showed the significantly good result ($P < 0.05$) ensuring quality eggs and more larvae in respect of ovulation (100%), spawning fecundity fertilization rate hatching rate, survival rate and deficient number of deformed larvae. Better breeding performance regarding fertilization, hatching and survival rate was found in 4 years female which showed higher level of significant difference ($P < 0.05$) than others age groups. Selection of four years female broods of Catla in the hatchery for better breeding performance is recommended for fish breeders in Bangladesh.

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