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Effect of formalin on fertilization, hatching rate of eggs of Thai Pangas (*Pangasius hypophthalmus*) and survival and growth performance of fry

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Key words: *Pangasius hypophthalmus*, formalin, fertilization, hatching, survival rate.

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Abstract

Eggs of matured Thai Pangas (*Pangasius hypophthalmus*) were ranging from 2500 to 4200g comparative to total body weight (TBW) which were treated with 0.5 ml and 1 ml formalin concentrations for 60 seconds to determine its efficacy and effect on fertilization, hatching of egg, survival and growth performance of fry in this present study. Each treatment was replicated three times in the experiment. The present research work was conducted at Mafatema fish hatchery, Chanchra, Jashore and laboratory of Fisheries and Marine Bioscience department, Jashore University of Science and Technology, Jashore, Bangladesh. In case of fecundity, mean fecundity did not differed significantly ($P>0.05$); 120580±9560, 134292±16035 and 117238±7986 per kg body weight for 0.5 ml (T_2), 1 ml (T_3) and control (T_1) group respectively. The highest fertilization rate (93.68±5.78) and hatching rate (88.12±9.34) were found in T_3 which were significantly different ($P<0.05$) with T_1 and T_2. This study revealed, T_3 showed the highest survival rate (92.50%) that is significantly different from T_1 and T_2. The present study showed that, the growth performance of *P. hypophthalmus* was found higher such as percentage of weight gain 95.26 ± 12.01 and specific growth rate 0.65 ± 0.06 in T_3 which were significantly higher than T_1 and T_2. Eggs of *P. hypophthalmus* treated with 1 ml diluted formalin concentration for 60 seconds showed better results in terms of fertilization and hatching rate of eggs, survival and growth performance of fry.

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Introduction

Bangladesh is blessed and enriched with vast water resources, which show a wide range of variation in nature. As a consequence, natural fisheries resources are scattered all over the country. Nowadays, due to the degradation of the ecological balance, changing catchments, construction of drainage structures and flood control, siltation, soil erosion, washing of industrial pollutants and agrochemicals, the capture fisheries in open waters of Bangladesh is under great danger. For that reason, aquaculture has been developed in this country, which mainly depends on fish hatcheries, a place for artificial breading, hatching and rearing through the early life stages of fish (Coche and Crespi, 2008).

P. hypophthalmus is highly fecund, seasonal spawner and breeds once a year in flooded rivers (Moses, 2016).Females fish attain maturity at the end of third year while male mature in two years (Griffith, 2010; Vidthayanon and Hogan, 2013). In Bangladesh, the induced breeding techniques are followed by the commercial hatcheries (Sharif and Asif, 2015 Ali *et al.*, 2016a; Ali *et al.*, 2016b; Shabuj *et al.*, 2016; Islam *et al.*, 2016; Hossain *et al.*, 2016; Islam *et al.*, 2017; Ali *et al.*, 2018).

Fungal infections on eggs causes disease problem which resulted into egg mortality, reduces hatching of fertilized eggs and survival of larvae (Jung *et al.*, 2004; Yeasmin *et al.*, 2016). The external surface of fish eggs is easily colonized by bacteria, such as *Flavobacterium* sp., *Psuedomonas* sp., *Aeromonas* sp. and *Vibrio* sp. (Miguez and Combarro, 2003; Madsen *et al.*, 2005; (Rahman *et al.*, 2017; Neowajh *et al.*, 2017)). Eggs are externally disinfected at the green and/or eyed stage to minimize the possibility of infection by bacteria, fungi or parasites. Formalin is widely used for treating fungal infections of fish eggs in intensive aquaculture operations. Formalin is a generic term which describes a solution of 37 % formaldehyde gas dissolved in water (Akpoilih and Adebayo, 2010). Solutions of formalin for use on fish should contain 10 to 15 % methanol which inhibits formation of par formaldehyde, a highly toxic compound. Formalin has long been used as traditional treatment for fish ecto-parasites. It is extremely effective against most protozoan as well as some monogenetic trematodes through bath, flush or flowing treatment methods (Jung *et al.*, 2004). Formalin is also one of the most commonly used chemical treatments for fungal control in fish hatcheries and effective in the control of fungus on eggs without adverse effect on hatchability and post-hatch survival as reported by Pedersen *et al.*, (2008). Egg disinfection is an important and routine bio-security practice among hatchery operators. Egg disinfection helps to prevent the transfer of external pathogens from brood stock to larvae and thus helps reduce the mortality associated with these pathogens. The use of formalin to treat fish eggs (*P. hypophthalmus*) before incubation has not been a common practice in Bangladesh by fish breeders and hatchery operators. Thai pangas (*P. hypophthalmus*) is one of the important aquaculture species in Bangladesh. It is widely cultured owning to its hardiness, fast growth and highly priced food fish. The efficacy of various disinfection methods has been studied using many different species of fish eggs (Salvesen *et al.*, 1997; Grotmol and Totland, 2000; Peck *et al.*, 2004). Formalin is widely used for treating fungal infection on fish eggs in intensive aquaculture operations to improve the hatchability and survival of larvae but there is problem of appropriate concentration of chemical and period of time the treated eggs are to be in contact with the chemical before incubation in order to reduce potential toxic effect on fish. The objective of this study therefore, was to assess the efficacy of formalin solution (0.5 ml and 1 ml) diluted at 99.5 ml and 99 ml respectively on treated eggs for 60 seconds post-fertilization.

Materials and methods

Sample size

Fifteen male (15) and fifteen female (15) broods ranging from 2500-4200 g total body weight (TBW) were collected from brood pond of Mafatema fish hatchery, Chanchra, Jashore from April to October, 2018.

Brood collection, conditioning and sex determination

Collected brood fishes (N=30) were kept separately in the rectangular tanks (1200 liter each) under showering condition to induce the breeding for 16 hours. They were maintained under optimum temperature and fed with 40 % crude protein commercial diet. The brood stocks were examined for gonad development according to the method of Blythe *et al.,* (1994) and reported by Yisa *et al.,* (2010). Males were examined for rigid and reddish infusion of the genital orifice and for females, genital orifice for reddish infusion, distension of the belly and release of eggs when gentle pressure was applied on the abdomen.

Hormone administration

Matured female brood fish were treated with a single dose of hormone (Ovaprim) according to the method of Goudie *et al.,* (1992) and hand stripped for eggs after a minimum latency period of twelve hours at water temperature of between 29-31°C.

Formalin treatment

Formalin solution was prepared by diluting 0.5 ml with 99.5 ml and 1 ml formalin into 99 ml distilled water respectively. After 6 hours of final dose, eggs and sperm are collected from the ovulated females and males by stripping the abdomen of the fishes with a gentle hand. Eggs are collected first into a plastic bowl. Then males are stripped over the same container. The eggs and sperms in the bowl are mixed together with a soft feather for 1 min. The fertilized eggs were differentiated from the unfertilized ones by the presence of "eye spot" and the swelling of the fertilized ones. The unfertilized eggs were white and opaque while the fertilized eggs were transparent. Sixty (60) fertilized eggs were divided into three equal portions and was used for the three treatments; T_1 (control), T_2 (0.5 ml) and T_3 (1 ml) for 60 seconds. Each treatment was replicated three times. Small quantity of saline solution was then poured onto the eggs to avoid sticking together. The fertilized eggs were rinsed with distilled water and taken to the funnel type incubator for incubation. When hatching was completed the hapa with un-hatched eggs and shells was lifted out of the incubation tank and washed. The hatchlings were collected in a pot (dish) and counted by visual observation using magnifying glass and recorded. 450 fries for each treatment at stocking rate of 150 fries per glass aquaria tank was reared for 8 weeks. After yolk absorption, the hatchlings were fed with de-capsulated artemia. Water quality parameters including temperature, Dissolved Oxygen, pH and conductivity were monitored and maintained at optimum level.

Fecundity, fertilization and hatching rate were determined according to method described by Oyelese, (2006) using the formula:

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

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized egg} \times 100}{\text{Total no. of egg}}$$

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

Mortality and survival rates were determined with the following formula:

$$\text{Mortality rate (\%)} = \frac{\text{Cumulative Mortality} \times 100}{\text{Total number stocked}}$$

$$\text{Survival rate (\%)} = \frac{\text{Cumulative Survival} \times 100}{\text{Total number stocked}}$$

The following parameters were used to evaluate growth of the *P. hypophthalmus* fry:

$$\text{Weight gain (\%)} = \frac{(FW - IW) \times 100}{IW}$$

Where, FW and IW designate average final and initial weight in g.

$$SGR = \frac{(\log W2 - \log W1) \times 100}{Tb - Ta}$$

Where, SGR = specific growth rate (% d−1), W2 = final live body weight (g) at time Tb (d), W1 = initial live body weight (g) at time Ta (d), Tb = time at final sampling, and Ta = time at initial sampling. The weights of the hatchlings were determined using sensitive electronic balance (P.E. mx Rady).

Data analysis

The results obtained in the experiment were subjected to statistical analysis. Qualitative and quantitative analysis of all kinds of data were carried out. MS Excel was used for presentation of the tables obtained from different types of data. One way analysis of variance (ANOVA) was done for the test of significance of fecundity, rate of fertilization and hatching rate of *P. hypophthalmus* using SPSS.

Results and discussion

The fecundity, fertilization and hatching rate of *P. hypophthalmus* were presented in Table 1.

Table 1. Mean Fecundity, fertilization rate and hatching rate of *P. hypophthalmus*.

Parameters	0.5 ml (T_2)	1 ml (T_3)	Control (T_1)
Fecundity	120580 ± 9560^a	134292 ± 16035^a	117238 ± 7986^a
Fertilization rate	76.45 ± 8.34^b	93.68 ± 5.78^a	69.35 ± 9.22^b
Hatching rate	69.48 ± 4.45^b	88.12 ± 9.34^a	62.56 ± 7.66^b

Table 2. Mean cumulative mortality, survival rates and percentages for *P. hypophthalmus* fry in control (T_1) and reared in tank for 8 weeks.

Initial Stock Per Tank 150				
Period (Weeks)	Mortality	% Cumulative Mortality	Survival	% Cumulative Survival
1	14	9.33	136	90.67
2	21	14.00	129	86.00
3	25	16.67	125	83.33
4	29	19.33	121	80.67
5	32	21.33	118	78.67
6	34	22.67	116	77.33
7	35	23.33	115	76.67
8	35	23.33	115	76.67
Mean		18.75		81.25

It showed that fecundity did not differed significantly ($P>0.05$) among the treatments. Highest (134292±16035) and lowest (117238±7986) fecundity was observed in T_3 and T_1 respectively. In the present study, among three treatments, T_3 showed the best result in case of fertilization rate (93.68±5.78) and hatching rate (88.12±9.34) shown in Table1. T_3 had significant difference ($P<0.05$) with T_1 and T_2 in respect to fertilization and hatching rate.

Table 3. Mean cumulative mortality, survival rates and percentages for *P. hypophthalmus* fry eggs treated at 0.5 ml formalin (T_2) and reared in tank for 8 weeks.

Initial stock per tank 150				
Period (Weeks)	Mortality	% Cumulative Mortality	Survival	% Cumulative Survival
1	10	6.67	140	93.33
2	16	10.67	134	84.00
3	19	12.67	131	87.33
4	21	14.00	129	86.00
5	23	15.33	127	84.67
6	25	16.67	125	83.33
7	26	17.33	124	82.67
8	27	18.00	123	82.00
Mean		13.92		86.08

The high dosage treatment of formalin showed a better result in fertilization and hatching rate of *Clarias gariepinus* (Yisa *et al.*, 2012, Akpoilih and Adebayo, 2010). Formalin is one of the most commonly used chemical treatments for fungal control in fish hatcheries and effective in the control of fungus on eggs and increasing egg's hatchability (Barnes *et al.*, 1998; Barnes *et al.*, 2000; Barnes *et al.*, 2003; Pedersoen *et al.*, 2008) described the microbial control and increased hatching percentages of the eyed eggs treated daily with formalin at the standard treatment level.

Table 4. Mean cumulative mortality, survival rates and percentages for *P. hypophthalmus* fry treated at 1 ml formalin (T_3) and reared in tank for 8 weeks.

Initial stock per tank 150				
Period (Weeks)	Mortality	% Cumulative Mortality	Survival	% Cumulative Survival
1	4	2.67	146	97.33
2	7	4.67	143	95.33
3	10	6.67	140	93.33
4	12	8.00	138	92.00
5	13	8.67	137	91.33
6	14	9.33	136	90.67
7	15	10.00	135	90.00
8	15	10.00	135	90.00
Mean		7.50		92.50

In the present study mean cumulative survival rate of *P. hypophthalmus* was 81.25%, 86.08% and 92.50% in T_1, T_2 and T_3, respectively shown in Table 2, 3 and 4. Highest mean cumulative survival rate (92.50%) was found in T_3 which was significantly different ($P<0.05$) from T_1 and T_2. This was also attributed to egg and milt quality and viability resulted in vigour hatchlings which increase chances of high survival. Formalin treated eggs showed the highest survival rate of *Channa striatus* (Milton *et al.,* 2015).

Table 4. Mean cumulative mortality, survival rates and percentages for *P. hypophthalmus* fry treated at 1 ml formalin (T_3) and reared in tank for 8 weeks.

Initial stock per tank 150				
Period (Weeks)	Mortality	% Cumulative Mortality	Survival	% Cumulative Survival
1	4	2.67	146	97.33
2	7	4.67	143	95.33
3	10	6.67	140	93.33
4	12	8.00	138	92.00
5	13	8.67	137	91.33
6	14	9.33	136	90.67
7	15	10.00	135	90.00
8	15	10.00	135	90.00
Mean		7.50		92.50

Highest specific growth rate (0.65±0.06) and weight gain percentage (95.26±12.01) were found in T_3 hown in table5. Weight gain percentage of T_3 and T_2 had significant differences with T_1. In case of SGR, T_3 had significant difference with T_1 and T_2. Fungi infection on the fry was reduced hence free from disease problem; this facilitated their growth rate (Yisa *et al.*, 2014).

Table 5. Growth of *P. hypophthalmus* observed in different treatments for 8 weeks.

Parameter	T_1	T_2	T_3
Mean initial weight	0.11 ± 0.008	0.11 ± 0.008	0.11 ± 0.008
Mean final weight	0.24 ± 0.06	0.29 ± 0.017	0.31 ± 0.09
Mean weight gain	0.12 ± 0.06	0.17 ± 0.07	0.24 ± 0.87
% weight gain	43.36 ± 7.06^b	79.57 ± 5.00^a	95.26 ± 12.01^a
SGR %	0.43 ± 0.03^b	0.44 ± 0.05^b	0.65 ± 0.06^a

Conclusion

Eggs of *P. hypophthalmus* treated with 1 ml diluted formalin concentration for 60 seconds in terms of fertilization, hatching, survival and growth performance was most effective and therefore recommended.

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