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Original Research Article

Effect of Chemical Disinfectant (formalin) on Hatching of Eggs of African Catfish (*Clarias gariepinus*), Survival and Growth Performance of Fry

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Keywords

Disinfectant; formalin; hatching; *Clarias gariepinus* egg;

ABSTRACT

Eggs of matured *Clarias gariepinus* size ranging from 550-800 g total body weight (TBW) were treated with 0.5 ml and 1 ml formalin concentrations for 60 seconds to determine its efficacy and effect on hatching, survival and growth performance of fry. Each treatment was replicated three times. The research was conducted at the Toxicology Unit at Fish Farm, Federal University of Technology, (FUT), Bosso Campus, Minna. Mean fecundity did not differed significantly ($P>0.05$); 138934^{a}, 110429^{a} and 123470^{a} for 0.5 ml, 1 ml and control among the treatments. Percentage fertilization and hatching differed significantly ($P<0.05$) between 1 ml, and 0.5 ml (64.43^{a} and 54.59^{b}) and (62.48^{a} and 42.59^{b}) respectively. The bred hatchlings were maintained for 8 weeks and total percentage mortality was 15, 13 and 20 for 0.5 ml, 1 ml and control respectively. Mean Total Body Weight (TBW) gain differed significantly ($P<0.05$) between 1 ml (3.27^{a}) and 0.5 ml (0.82^{b}). Treatment three (1 ml formalin solution) gave better result in terms of fertilization, hatching and growth performance. This shows that eggs treated with formalin at 1 ml concentration for 60 seconds is appropriate to disinfect eggs. The treatment is hence recommended for disinfecting *Clarias gariepinus* eggs before incubation.

Introduction

The demand for fish fingerlings for aquaculture is on the increase in Africa and has made hatchery propagation of culturable fish species important. Fungal infections on eggs causes disease problem which resulted into egg mortality, reduces hatching of fertilized eggs and survival of larvae Jung (2004). 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). Eggs are externally disinfected at the green and/or eyed stage to minimize the

possibility of infection by bacteria, fungi or parasites. 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, 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 (*Clarias gariepinus*) before incubation has not been a common practice in Nigeria by fish breeders and hatchery operators. African catfish (*Clarias gariepinus*) is specie with high economic value in Nigeria. 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) and 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 determine 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

Ten (10) samples, (5 males and 5 females) gravid *Clarias gariepinus* brood stock size ranging from 550-800 g total body weight (TBW) were procured from Tunga Mallam along Paiko road, Minna, Niger State. They were acclimatized for 2 weeks in holding indoor concrete tanks of 725.76 L water holding capacity of indoor hatchery Federal University of Technology, (F.U.T.), Bosso Campus Minna. 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. The selected samples were properly maintained separately before being used for breeding. 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 25-29^0 C. Formalin solution was prepared by diluting 0.5 ml with 99.5 ml and 1 ml formalin into 99 ml distilled water respectively. Twelve and half grams (12.5 g) of total eggs stripped were used to fertilize milt. Milt was obtained by sacrificing the male and testis removed,

cleaned with cotton wool to remove all the stained blood and then kept in a clean Petri dish and thereafter macerated to squeeze out milt. The milt and eggs were mixed together gently with a plastic spoon for 2-3 minutes. The 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). Treatment two and three (T_2 and T_3) were treated with 20 ml diluted formalin concentration for 60 seconds. Each treatment was replicated three times. Small quantity of saline solution was then pour onto the eggs to avoid sticking together. The fertilized eggs were rinsed with distilled water and taken to the incubator for incubation. Incubator made of net hapa with kakabams, placed inside glass aquarium tanks filled with clean water was used for the purpose. Fertilized eggs were spread in a monolayer on the kakabams in the incubator. Aeration was maintained by flow through system. The hapa was constructed from a coated nylon net with 1.5 mm mesh size. When hatching was completed the hapa with un- hatched eggs and shells was lifted out of the incubation tank and washed. 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 decapsulated artemia. Water quality parameters including temperature, Dissolved Oxygen, pH and conductivity were monitored and maintained at optimum level. Percentage mortality and survival rates were determined with the following formula:

$$\text{Percentage Mortality} = \frac{\text{Cumulative Mortality}}{\text{Total number stocked}} \times 100$$

And

$$\text{Percentage Survival} = \frac{\text{Cumulative Survival}}{\text{Total number stocked}} \times 100$$

(Iber Bergenal, 1978) as adopted by Yisa *et al.* (2010).

The weights of the hatchlings were determined using sensitive electronic balance (P.E. mx Rady).

Fecundity, percentage fertilization and hatchability were determined according to method described by (Oyelese, 2006) using the formulae:

$$\text{Fecundity} = \text{Total weight of stripped eggs} \times \frac{\text{Total No. of eggs in sub-sample}}{\text{Weight of eggs in sub-sample}}$$

$$\text{Percentage Fertilization} = \frac{\text{No. of fertilized eggs}}{\text{No. of eggs stripped}} \times 100$$

$$\text{Percentage Hatchability} = \frac{\text{No. of fry}}{\text{No. of fertilized eggs}} \times 100$$

One way Analysis of Variance (ANOVA) was used as statistical tool for the analysis. Data obtained were pooled from the replicate and mean values was calculated per treatment. Also Duncan Multiple range Test was used for mean separation. All differences in mean values of parameters were determined at P = 0.05 level of significance.

Results and Discussion

The fecundity, percentage fertilization and hatchability from the brood stocks *Clarias gariepinus* are presented in Table 1. It showed that fecundity did not differed significantly (P>0.05) 138934^a, 110429^a and 123470^a for 0.5 ml, 1 ml and control among the treatments. Results also showed that percentage fertilization and hatching differed significantly (P<0.05) between 1 ml and 0.5 ml concentrations (64.43^a and 54.59^b) and (62.48^a and 42.59^b) respectively. Table 2, 3 and 4 shows the cumulative mortality and survival rates that was recorded for 8 weeks of rearing. Cumulative mortality were15, 13 and 20 respectively for 05 ml,

Table.1 Mean Fecundity, % Fertilization and % Hatching of *Clarias gariepinus.*

Parameters	0.5 ml	1 ml	Control	±S.E.
Fecundity	138934.4[a]	110429.2[a]	123470.4[a]	6089.94
% Fertilization	54.59[b]	64.43[a]	64.35[a]	1.422
% Hatching	42.59[b]	62.48[a]	39.68[b]	1.453

Values carrying different superscript on the same row differed significantly from each other ($p<0.05$).

Table.2 Mean cumulative Mortality and Survival Rates and Percentages for *Clarias gariepinus* Fry Eggs Treated at 0.5 ml Formalin and Reared in Glass Aquarium Tank for 8 Weeks

Initial Stock Per Tank 150				
Period (Weeks)	Mortality	% Cumulative Mortality	Survival	% Cumulative Survival
1	5	3.33	145	96.60
2	9	6.00	141	94.00
3	11	7.33	139	92.60
4	12	8.00	138	92.00
5	13	8.70	137	91.13
6	14	9.30	136	90.60
7	15	10.00	135	90.00
8	15	10.00	135	90.00
Mean		7.83		90.00

Table.3 Mean cumulative Mortality and Survival Rates and Percentages for *Clarias gariepinus* Fry Treated at 1 ml Formalin and Reared in Glass Aquarium 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	90.33
4	11	7.33	139	92.67
5	12	8.00	138	92.00
6	13	8.67	137	91.33
7	13	8.67	137	91.33
8	13	8.67	137	91.33
Mean		6.92		93.08

Table.4 Mean cumulative Mortality and Survival Rates and Percentages for *Clarias gariepinus* Fry Control and Reared in Glass Aquarium Tank for 8 Weeks.

Initial Stock Per Tank 150				
Period (Weeks)	**Mortality**	**% Cumulative Mortality**	**Survival**	**% Cumulative Survival**
1	9	6.00	141	97.00
2	18	12.00	132	95.00
3	20	13.33	130	86.67
4	20	13.33	130	86.67
5	20	13.33	130	86.67
6	20	13.33	130	86.67
7	20	13.33	130	86.67
8	20	13.33	130	86.67
Mean		12.25		87.75

Table.5 Body Weight Gain of *Clarias gariepinus* Fry Treated Eggs with Formalin at 0.5 ml, 1 ml and Control Reared in Glass Aquarium Tanks for 8 Weeks.

Parameter TBW (g)	**Weeks**	**0.5 ml**	**1 ml**	**Control**
	1	0.29^{a}	0.28^{a}	0.24^{a}
	2	0.30^{a}	0.29^{a}	0.26^{c}
	3	0.45^{a}	0.46^{a}	0.41^{b}
	4	0.31^{a}	0.36^{a}	0.46^{a}
	5	0.35^{c}	0.48^{ab}	0.58^{a}
	6	0.48^{a}	2.52^{a}	2.76^{a}
	7	0.62^{c}	3.21^{b}	3.43^{a}
	8	0.82^{b}	3.27^{a}	3.46^{a}
	Mean	0.45	1.36	1.45

Values with different superscript on the same row differed significantly from each other ($p<0.05$).; TBW= Total Body Weight.

1 ml and control. Table 4 indicated highest mortality in treatment one (T_1) (control). Mean Total Body Weight (TBW) gain differed significantly ($P<0.05$) between 1 ml (3.27^{a}) and 0.5 ml (0.82^{b}) (Table 5). Percentage fertilization and hatching was highest in T_3 (64.43^{a} and 62.48^{a}. Akpoilih and Adebayo (2010) in their study effect of formalin on the hatching rate of eggs adverse effect on hatchability and post-hatch survival. The low mortality recorded and survival of larvae of the African catfish (*Clarias gariepinus*) using range finding and definitive tests obtained hatching rate of 65 % and 69 %. Similarly, Pedersen *et al.* (2008) reported that 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 in T_2 and T_3 was indicative of the fact that formalin has effectively reduced fungi

Infection on eggs and larvae of *Clarias gariepinus*. This result corroborated the report of Akpolilh and Adebayo (2010) where they recorded percentage survival to be high (85.53±9.56^a). This was also attributed to egg and milt quality and viability resulted in vigour hatchlings which increase chances of high survival (Yisa *et al.*, 2012). Mean Total Body Weight (TBW) was highest in T_3. Fungi infection on the fry was reduced hence free from disease problem, this facilitate their growth rate. Eggs of *Clarias gariepinus* 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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