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# Effects of formaldehyde, sodium chloride, potassium permanganate and hydrogen peroxide on hatch rate of African catfish *Clarias gariepinus* eggs

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#### Abstract

A study evaluating the effects of formaldehyde, sodium chloride, potassium permanganate and hydrogen peroxide treatment on the hatching success of *C. gariepinus* eggs was carried out from April to July 2006. Eggs were artificially fertilized, 50 counted and subjected to a static bath dip treatment in given concentrations of the above chemicals for either 15, 30 or 60-minute durations before being incubated at  $27 \pm 1$  °C for 24 h. Treatment efficacy was assessed by comparing the percent egg hatch in the treatment group to the untreated control group. Eggs treated with formaldehyde and sodium chloride at 250, 500 and 1000 ppm recorded greater mean percent hatch compared to the untreated controls. Likewise, percent hatch of eggs treated with hydrogen peroxide and potassium permanganate at concentrations ranging from 100–1000 ppm and 0.5–4.0 ppm respectively were greater relative to the untreated controls. The highest mean percent hatch recorded in the study was in eggs treated with 2 ppm potassium permanganate for 30 min (96.7%). Although formaldehyde and potassium permanganate gave the best performance, on the basis of safety concerns, ease of availability and cost, we recommend 1000 ppm sodium chloride treatment of catfish eggs for routine use by rural fish farmers to improve catfish egg hatchability. © 2007 Elsevier B.V. All rights reserved.

Keywords: Clarias gariepinus; Formaldehyde; Sodium chloride; Potassium permanganate; Hydrogen peroxide

# 1. Introduction

The African catfish *Clarias gariepinus* is highly appreciated as a good aquaculture species because of its resistance to diseases, ability to tolerate a wide range of environmental parameters and high stocking densities under culture conditions, relative fast growth rate, and good quality meat (Goos and Richter, 1996; Haylor, 1991; Hogendoorn, 1980; Huisman and Richter, 1987).

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Currently it is mainly grown in Africa and Europe. In Africa, however its full aquaculture potential has not yet been realized. To a large extent, the main constraint facing its culture in the continent has been the lack of adequate and reliable supply of catfish fry for stocking purposes.

Although *C. gariepinus* spawns naturally in flood plains during the rainy season, fry collection from the wild is unreliable and limited to the rainy season. The fish rarely spawns in captivity but induced breeding techniques have been perfected, adequately described and are routinely practiced in many hatcheries (Hogendoorn, 1979; Hogendoorn and Vismans, 1980). However the shortage of seed for stocking ponds continues to

Table 1 Chemical agents and their concentrations tested during the experiment

Compound	Range of concentrations tested (ppm)
<ul> <li>Formaldehyde (37% formaldehyde, 6–13% methanol)</li> <li>Sodium chloride (food grade evaporated granulated salt, fortified with potassium iodate)</li> </ul>	0 (control), 25, 50, 100, 250, 500, 1000, 2000, 4000, 10,000 0 (control), 25, 50, 100, 250, 500, 1000, 2000, 4000, 10,000
<ul> <li>Hydrogen peroxide (35% technical grade H<sub>2</sub>O<sub>2</sub> solution)</li> <li>Potassium permanganate (technical grade)</li> </ul>	0 (control), 5, 25, 50, 100, 250, 500, 1000, 2000 0 (control), 0.1, 0.25, 0.5, 1, 2, 4, 10, 20

persist in Africa. It is apparent that management protocols covering egg production, egg hatching, and particularly production techniques that enhance fry and fingerling survival need to be further simplified to ensure a sufficient supply of catfish seed.

Despite the high fecundity of C. gariepinus, the hatching rates of eggs in many hatcheries in Africa are erratic ranging from 8-70% depending on the degree of sophistication of management in the particular hatchery (de Graaf et al., 1995; Macharia et al., 2005). One probable cause of the erratic hatching is the parasitization of catfish eggs by aquatic fungi and bacteria. During artificial fertilization, a percentage of the eggs usually escape being fertilized. Unfertilized fish eggs are susceptible to fungal infection particularly from the family Saprolegniaceae (Post, 1987). During egg incubation, this fungi produces mycelia which grow and spreads from the nonviable to the healthy eggs suffocating them and causing mortality (Alderman and Polglase, 1984; Post, 1987). Indeed the problems associated with fungal infection on fish eggs are prevalent in hatchery rearing operations all over the world and in Europe and USA, the common practice is to routinely control them by using antifungal agents (Barnes et al., 1998; Barnes and Gaikowski, 2004; Celada et al., 2004; Gaikowski et al., 1998; Kitancharoen et al., 1998; Melendre et al., 2006; Rach et al., 1997).

In the present study the efficacy of some selected fungicides were evaluated in an attempt to establish simple, low-cost and low-technology method of improving the hatching rate of *C. gariepinus* eggs.

#### 2. Materials and methods

The experiment was performed at Moi University, Eldoret, Kenya from April 2006 to July 2006. Mature broodstock fish were seined from broodstock ponds at the Moi University Chepkoilel Fish Farm and ripe females and males (average weight  $220\pm0.1$  g) selected

Model parameter statistics fro	m the logistic regressio	n of the four test chemicals	
Chemical	Time (min)	Model	Parameter significance ( <i>P</i> -value)
Formaldehyde	15	Log $(\rho/1 - \rho) = 4.3444E-01 + 8.2777E-04 * C-4.5284E-07 * C^2 + 6.1653E-11 * C^3$	$\beta_0 (0.0000) \beta_1 (0.0000) \beta_2 (0.0001) \beta_3 (0.0016)$
	30	Log $(\rho/1 - \rho) = 4.8458E-01 + 6.7603E-04 * C-3.8559E-07 * C^2 + 5.1098E-11 * C^3$	$\beta_0 (0.0000) \beta_1 (0.0004) \beta_2 (0.0001) \beta_3 (0.0069)$
	60	Log $(\rho/1 - \rho) = 4.9823E-01 + 5.6109E-04 * C-3.3327E-07 * C2 + 4.3292E-11 * C3$	$\beta_0 (0.0000) \beta_1 (0.0069) \beta_2 (0.0324) \beta_3 (0.0107)$
Sodium chloride	15	Log $(\rho/1 - \rho) = 4.9102E-01 + 3.2882E-04 * C-1.3232E-07 * C^2 + 9.6648E-12 * C^3$	$\beta_0(0.0000) \beta_1(0.0000) \beta_2(0.0000) \beta_3(0.0000)$
	30	Log $(\rho/1 - \rho) = 4.4186E-01 + 3.2293E-04 * C-1.2887E-07 * C^2 + 9.4172E-12 * C^3$	$\beta_0 (0.0000) \beta_1 (0.0033) \beta_2 (0.0014) \beta_3 (0.0016)$
	60	Log $(\rho/1 - \rho) = 4.4520E-01 + 1.9785E-04 * C-9.1102E-08 * C^2 + 6.8430E-12 * C^3$	$\beta_0 (0.0000) \beta_1 (0.0408) \beta_2 (0.0108) \beta_3 (0.0100)$
Potassium permanganate	15	Log $(\rho/1 - \rho) = 4.6519E-01 + 2.2336E-01 * C-3.2481E-02 * C2 + 1.0535E-03 * C3$	$\beta_0$ (0.0000) $\beta_1$ (0.0000) $\beta_2$ (0.0000) $\beta_3$ (0.0000)
	30	Log $(\rho/1 - \rho) = 4.7027E-01 + 2.3121E-01 * C-3.5643E-02 * C2 + 1.1790E-03 * C3$	$\beta_0 (0.0000) \beta_1 (0.0000) \beta_2 (0.0000) \beta_3 (0.0000)$
	60	Log $(\rho/1 - \rho) = 4.9302E-01 + 1.5872E-01 * C-2.4486E-02 * C2 + 7.9947E-04 * C3$	$\beta_0$ (0.0000) $\beta_1$ (0.0000) $\beta_2$ (0.0000) $\beta_3$ (0.0000)
Hydrogen peroxide	15	Log $(\rho/1 - \rho) = 5.3118E-01 + 1.2784E-03 * C-1.3921E-06 * C^2 + 3.8143E-10 * C^3$	$\beta_0$ (0.0000) $\beta_1$ (0.0038) $\beta_2$ (0.0032) $\beta_3$ (0.0061)
	30	Log $(\rho/1 - \rho) = 5.2410E-01 + 1.0060E-03 * C-8.2651E-07 * C2 + 1.8693E-10 * C3$	$\beta_0 (0.0000) \beta_1 (0.0013) \beta_2 (0.0017) \beta_3 (0.0099)$
	09	Log $(p/1-p) = 5.5775E-01 + 1.0618E-03 * C-1.1668E-06 * C^2 + 3.1952E-10 * C^3$	$\beta_0(0.0000) \ \beta_1(0.0023) \ \beta_2(0.0037) \ \beta_3(0.0196)$

based on the method of Viveen et al. (1985) and transferred to the hatchery. They were then acclimated in hatching tanks for 1-day without feeding. To induce spawning, the selected female was injected with pituitary suspension from a sacrificed ripe male of similar size. After 12 h, the eggs were stripped into a dry bowl and fertilized with milt from a ripe male. One male was used to fertilize eggs from one female.

After fertilization, the eggs were randomly counted into equal lots of 50-eggs and each lot spread on  $5 \text{ cm} \times 5 \text{ cm}$  strips of mosquito netting where the eggs attached due to the natural adhesiveness of catfish eggs. Each strip containing eggs was then inserted inside individual 15 cm  $\times$  15 cm hatching bags made from fine meshed mosquito netting (mesh size 0.5 mm) which would prevent escape of any hatched larvae, and the mouth of each bag tightly tied. The individual hatching bags were randomly assigned in triplicate to static bath treatments of given concentrations of either formaldehyde, sodium chloride, hydrogen peroxide, potassium permanganate and a control (0 ppm) for 15, 30, or 60minute exposure periods (Table 1) before being transferred to randomized compartments of the incubation tank.

Borehole water supplying the incubation tank had the following characteristics: mean temperature  $27\pm1$  °C, total hardness as CaCO<sub>3</sub> 82.1 mg/L, pH 6.4–7.2, dissolved oxygen (DO) 6.6–7.5 and total ammonia nitrogen (TAN)  $0.28\pm0.06$  mg/L. Temperature and dissolved oxygen were measured using an oxygen–temperature meter (model 55,YSI,Yellow Springs Ohio, USA), pH measured using a pH meter (Hanna Instruments, Model 8519, USA) and TAN measured using the method of Boyd and Tucker (1992). The water flow rate was maintained at 1 liter per minute with aeration provided throughout the incubation and

Table 3

Mean ( $\pm$ SEM) percent hatch of *C. gariepinus* eggs in formaldehyde treatments

Concentration ppm	Exposure time		
	15 min	30 min	60 min
0 (control)	$43.3 \pm 1.9$	$44.4 \pm 2.9$	42.2±1.1
25	$38.9 \pm 4.0$	$44.4 \pm 2.9$	$42.2 \pm 9.9$
50	$40.0 \pm 3.3$	$52.2 \pm 9.9$	$53.3 \pm 8.8$
100	$60.0 \pm 9.9$	$62.2 \pm 4.8$	$64.1 \pm 5.9$
250	$72.2 \pm 4.0$	$70.0 \pm 3.8$	$78.9 \pm 2.9$
500	$72.2 \pm 9.9$	$68.8 \pm 2.9$	$71.0 \pm 5.8$
1000	$82.2 \pm 1.1$	$81.1 \pm 1.1$	$66.6 \pm 1.9$
2000	$78.9 \pm 2.9$	$71.1 \pm 4.0$	$64.1 \pm 6.9$
4000	$44.4 \pm 6.7$	$28.9 \pm 4.0$	$17.8 \pm 6.2$
10,000	$1.0 \pm 0.6$	$0.0\!\pm\!0.0$	$0.0 {\pm} 0.0$

Table 4
Mean (±SEM) percent hatch of C. gariepinus eggs sodium chloride
treatments

Concentration ppm	Exposure time		
	15 min	30 min	60 min
0 (control)	$43.3 \pm 5.1$	$42.2 \pm 8.0$	37.7±4.4
25	$36.7 \pm 3.8$	$33.3 \pm 3.3$	$48.1 \pm 6.9$
50	$35.6 \pm 8.9$	$43.3 \pm 9.6$	$31.1 \pm 10.6$
100	$61.2 \pm 1.1$	$55.6 \pm 2.9$	$58.9 \pm 6.8$
250	$62.2 \pm 7.2$	$53.3 \pm 8.8$	$47.8 \pm 7.8$
500	$70.0 \pm 5.1$	$58.9 \pm 4.8$	$53.3 \pm 8.8$
1000	$74.4 \pm 6.7$	$81.1 \pm 1.1$	$75.6 \pm 7.8$
2000	$56.7 \pm 10.7$	$48.9 \pm 6.8$	$41.1 \pm 7.8$
4000	$33.3 \pm 1.9$	$31.1 \pm 13.7$	$24.4 \pm 11.1$
10,000	$24.4 \pm 6.8$	$20.0 \pm 8.4$	$25.6{\pm}2.9$

hatching period. After 24 h the hatching bags were removed from the incubation tank, their mouths untied and hatched larvae enumerated by counting individual larvae inside the bags. Hatchability (% hatch) was calculated by dividing the number of larvae by the total number of eggs per lot and multiplying by 100 (i.e. larvae/ $50 \times 100$ ).

The data from our experiments consisted of the initial number of eggs in each lot and the number of fry that hatched at the end of the experiment. Since hatching success is a binary variable which should and follow a binomial distribution we performed a logistic analysis (Agresti, 1990) on the data by fitting the logistic model. The logit model is a general logistic model logit[ $\theta(x)$ ] =  $\log \left[\frac{\theta(x)}{1-\theta(x)}\right] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + ... + \beta_i x_i$  is a general logistic model, which takes the form  $\log \left[\frac{\rho}{1-\rho}\right] = \beta_0 + \beta_1 C + \beta_2 C^2 + \beta_3 C^3$  in dose response treatments; where  $\rho$  denotes the probability of survival,  $\beta_0$  is the intercept,  $\beta_1$  is the coefficient of concentration C,  $\beta_2$  is the coefficient of cubic response in C. We fitted the model using GENSTAT

Table 5

Mean (±SEM) percent hatch of *C. gariepinus* eggs in potassium permanganate treatments

Concentration ppm	Exposure time		
	15 min	30 min	60 min
0 (control)	37.8±2.9	34.4±2.2	36.7±1.9
0.1	$44.4 \pm 1.1$	$42.2 \pm 2.9$	$43.3 \pm 1.9$
0.25	$53.3 \pm 1.9$	$51.1 \pm 2.9$	$58.9 \pm 2.9$
0.5	$62.2 \pm 2.9$	$64.4 \pm 1.1$	64.4±2.9
1.0	$73.3 \pm 1.9$	$81.1 \pm 1.1$	$70.0 \pm 1.9$
2.0	$85.6 \pm 1.1$	$96.7 \pm 1.9$	$82.2 \pm 4.4$
4.0	$81.1 \pm 1.1$	$70.0 \pm 1.9$	65.6±2.9
10.0	$52.2 \pm 2.9$	$43.3 \pm 3.8$	$45.6 \pm 4.0$
20.0	$36.7 {\pm} 3.8$	$26.7 \pm 5.1$	$12.2 \pm 6.8$

Table 6 Mean (±SEM) percent hatch of *C. gariepinus* eggs in hydrogen peroxide treatments

Concentration ppm	Exposure time		
	15 min	30 min	60 min
0 (control)	$42.2 \pm 4.0$	$40.0 \pm 1.9$	41.1±2.9
5	$38.9 \pm 4.0$	$40.0 \pm 3.8$	$42.2 \pm 7.8$
25	$60.0 \pm 3.3$	$57.8 \pm 2.9$	$60.0 \pm 5.1$
50	$67.7 \pm 2.2$	$68.9 \pm 4.8$	$78.9 \pm 2.9$
100	$81.1 \pm 2.9$	$78.9 \pm 6.2$	$80.0 \pm 6.9$
250	$87.8 \pm 2.9$	$75.6 \pm 2.9$	$81.1 \pm 1.1$
500	$67.8 \pm 1.1$	$71.1 \pm 1.1$	$67.8 \pm 2.9$
1000	$85.6 \pm 2.9$	$86.3 \pm 3.3$	$72.2 \pm 4.1$
2000	$72.2 \pm 4.8$	$72.2 \pm 6.2$	$56.7 \pm 5.1$

(GenStat Release 4.24DE) statistical software program. Model fit was based on residual likelihood ratio chisquare statistic.

### 3. Results

The model parameter statistics are given on Table 2. Mean ( $\pm$ SEM) percent hatch of catfish eggs subjected to varying chemical treatments and exposure times are shown in Tables 3–6 while Figs. 1–4 present the predicted probability of hatch of the eggs.

As shown in Fig. 1, Tables 2 and 3 treatment of eggs with formaldehyde significantly affected the probability of egg hatch. The full logistic regression model fits the hatch data adequately. The mean percent egg hatch in treatment ranging from 250 to 2000 ppm was greater than in the control group. The exposure time of the eggs in the different formaldehyde treatments influenced the hatch performance in concentrations above 1000 ppm with the shorter exposure time (15 min) giving a higher percent hatch compared to the longer exposure time (60 min). Percent hatch was considerably lowered at



Fig. 1. Predicted probability of *C. gariepinus* hatch exposed to formaldehyde for 15, 30 and 60 min based on logistic analysis model.



Fig. 2. Predicted probability of *C. gariepinus* hatch exposed to sodium chloride for 15, 30 and 60 min based on logistic analysis model.

higher formaldehyde concentrations (>4000 ppm) with failure of egg hatching being observed in the 10,000 ppm formaldehyde treatment.

Logistic analysis revealed a significant effect in egg hatch based on sodium chloride treatment concentration (Fig. 2, Tables 2 and 4). Sodium chloride concentrations ranging from 100 to 1000 ppm resulted in greater percent hatch than the control group. The highest percent hatch (81.1%) was recorded at a concentration of 1000 ppm for 15 min exposure period. Percent hatch at 10,000 ppm was lower than that observed in 50, 25 and 0 ppm (control) treatment groups.

Treatment of the eggs with potassium permanganate significantly affected the probability of egg hatch (Fig. 3, Tables 2 and 5). The full logistic regression model adequately fits the hatch data. Increase in concentration of potassium permanganate resulted in increase in percent hatch of the eggs, and which were greater than control groups, until a concentration of 2 ppm was reached. Further increase in concentration beyond 2 ppm elicited a reduction in percent hatch. Time of exposure affected hatchability at potassium



Fig. 3. Predicted probability of *C. gariepinus* hatch exposed to potassium permanganate for 15, 30 and 60 min based on logistic analysis model.



Fig. 4. Predicted probability of *C. gariepinus* hatch exposed to hydrogen peroxide for 15, 30 and 60 min based on logistic analysis model.

permanganate treatment concentrations of over 4.0 ppm. Likewise, treating eggs with hydrogen peroxide significantly affected the probability of egg hatch (Fig. 4, Tables 2 and 6). The full logistic regression model fits the data. Best hatchability (86.3%) was obtained when eggs were treated with hydrogen peroxide concentration of 1000 ppm for 15 min.

## 4. Discussion

Chemicals are routinely used in Europe and North America for treating fungal infections of fish eggs in intensive aquaculture operations in an effort to improve the efficiency rates. This study was intended to assess, for the first time, the effect of four of the commonly used chemicals on the hatching success of African catfish eggs.

In the first experiment, we subjected catfish eggs to formaldehyde treatment concentrations between 0-10,000 ppm. The results show that concentrations of formaldehyde ranging from 250 to 2000 ppm gave hatching percentages of over 70%, which were higher than the results of the untreated control (0 ppm). The highest percent hatch (82%) was achieved with a 15 min dip in 1000 ppm formaldehyde solution. However treatment with formaldehyde at more than 4000 ppm reduced the hatching rates with no hatching being recorded at chemical concentration of 10,000 ppm. This could imply that the high concentrations (>4000 ppm) had a toxic effect on the catfish eggs. Since fungal infections were observed in all control egg treatments as a fluffy growth, it is probable that both chemical toxicity and fungal infection caused egg mortality resulting in the poor hatchability. Results from this study are in general agreement with the findings of other workers that dosages ranging from 250 ppm to 1500 ppm can be effective in treating or preventing fungal infections in fish eggs. Marking et al. (1994) and Schreier et al. (1996) reported that dosages of between 1000 to 1500 ppm applied daily or every other day for 15 min increased the hatching rate of rainbow trout (Oncorhynchus mykiss) eggs. Waterstrat and Marking (1995) tested different dosages of formalin on Chinook salmon eggs and found that concentrations of 500 ppm and 1000 ppm were most effective in controlling fungal infections. Froelich and Engelhardt (1996) observed that treating koi carp eggs with formalin at 250 mg/L significantly improved the hatch rate compared to the untreated controls with the highest hatch rate (98.8%) being achieved with a 60 min exposure of the eggs to the formalin concentration. Celada et al. (2004) documented that treatment of eggs of the astacid crayfish (Pacifastacus leniusculus) with 4500 ppm formaldehyde for 15 min every other day up to hatching increased the hatching rates of the eggs although this was disputed by Melendre et al. (2006) who reported that concentrations of 3000 ppm was the most effective.

The results from the sodium chloride experiment show that treatment of catfish eggs with salt concentrations ranging from 100 ppm to 1000 ppm improved the percent hatch compared to the untreated eggs. The best percent hatch was recorded in the 1000 ppm salt solution when the eggs were bathed for 30 min. Salt treatments at concentrations of more than 4000 ppm reduced the hatch rate. Improved hatch due to salt treatment at 0-5000 mg/L has also been recorded for channel catfish by Froelich and Engelhardt (1996). Phelps and Walser (1993) found that treating koi carp eggs with a salt concentration of 1000 and 2500 mg/l for a 60 min exposure duration significantly (P < 0.05) improved the hatch rate of the eggs compared with that of the control while a concentration of 5000 mg/l was toxic to the eggs. Schnick et al. (1989) reported that a 3000 ppm sodium chloride dip effectively removes protozoa from fish egg surfaces and limits any mycelial production that may lower egg hatching. They further said that higher concentrations of up to 10,000 ppm of sodium chloride could be used to treat eggs but only as a short dip of not more than 30 s. Celada et al. (2004) on the contrary found that concentrations of 30,000 ppm of salt applied three or two times a week did not prevent fungal growth on astacid crayfish eggs indicating that to be effective, very high salt concentrations are required.

Hydrogen peroxide has received attention for its control of several fish pathogens and is recommended as a general disinfectant in aquaculture for treating culture water and surface of tanks before introduction of fish (Avendano-Herrera et al., 2006). It is also one of the most effective antifungus therapeutants currently used by hatcheries to control fungal infection of fish eggs. The standard treatment regimen used by hatcheries is 500 mg/l hydrogen peroxide for 15 min daily. Results from the current study indicated that treating *C*.

gariepinus eggs with hydrogen peroxide concentrations of 100, 250, 500 and 1000 ppm improved egg hatch compared to the untreated controls. Furthermore, treatment concentrations of 100 and 250 ppm were efficacious for African catfish eggs although these were below the recommended minimal effective hydrogen peroxide concentration of 500 mg/l. Small and Wolters (2003) also reported significant increased percent hatch in channel catfish (Ictalurus punctatus) eggs treated with lower concentrations of hydrogen peroxide (70-250 mg/ 1). On the contrary, increased effectiveness of hydrogen peroxide at concentrations higher than 500mg/l has been documented in other studies. Rach et al. (1998) subjected eight species of warm- and coolwater fish eggs to hydrogen peroxide concentrations of 1000, 3000, and 6000 mg/l and in all instances the 1000 mg/l hydrogen peroxide concentration was the most effective and almost no eggs hatched if left untreated. In related experiments, Rach et al. (2004) observed that treatment of channel catfish eggs with hydrogen peroxide at either 500 or 750 mg/L significantly increased the percent hatch compared to the untreated control groups. In similar studies, Dawson et al. (1994), Marking et al. (1994) and Schreier et al. (1996) reported that doses of 500-1000 ppm of hydrogen peroxide controlled fungi and increased hatching rates of rainbow trout eggs. Kitancharoen et al. (1997a,b, 1998) and Barnes et al. (1998) likewise obtained improved hatching results when rainbow trout eggs were treated with concentrations of 1000 ppm hydrogen peroxide.

Potassium permanganate has been tested as a fungicide on eggs of several fish species. Effective dosages have been found to vary with the fish species tested. Liu et al. (1995) reported that doses of 25 and 75 ppm applied for 60 min every other day up to hatching time decreased hatching rates of chinese sucker (Myxocyprinus asiaticus) eggs while Marking et al. (1994) stated that concentrations of 50 and 100 ppm delayed hatching of rainbow trout eggs. Potassium permanganate treatments of 100 and 150 ppm controlled fungi but were toxic to the eggs (Melendre et al., 2006). The concentrations of potassium permanganate used in the present experiment were much lower and ranged from 0-20 ppm. The results show that treatments of catfish eggs with 1 ppm and 2 ppm potassium permanganate greatly improved the percent hatch compared to the controls. Potassium permanganate at 2 ppm treatment gave the best hatching performance with a 15 min exposure recording the highest hatching percentage (96.7%). Hogendoorn and Vismans (1980) found that higher concentrations of potassium permanganate usually damage egg membrane and chorion.

From the results of this study, formaldehyde, sodium chloride, potassium permanganate and hydrogen peroxide produced a concentration-dependent effect on the hatching of catfish eggs over the 24-hour incubation period. Highest hatching efficiencies were recorded in C. gariepinus eggs exposed to 2 ppm of potassium permanganate for 15 min. Higher concentration of formaldehyde, sodium chloride and hydrogen peroxides also gave good results. The antifungal treatment protocols employed were simple and should be easy for rural small-scale fish farmers to apply. However several factors that would preclude the use of some of the fungicides tested in this experiment need to be pointed out. Formaldehyde is expensive, difficult to store and is potentially carcinogenic (Melendre et al., 2006). Hydrogen peroxide is relatively expensive, not readily available and being a strong oxidizer needs to be handled with extreme care (Kitancharoen et al., 1997b) while potassium permanganate is toxic to fish eggs when used in high doses. Sodium chloride on the other hand is cheap and readily available in the rural areas making its utilization practical in small-scale hatchery operations.

The New Partnership for Africa's Development (NEPAD) in adopting the Millennium Development Goals (MDGs) has recognized the growth of the aquaculture sector as a key instrument for improving Africa's food security and nutritional status (Bene and Heck, 2005). The NEPAD "Fish for All "initiative therefore has resolved that important technical issues including fish seed supply require sound application in local contexts rather than high level innovations (Muir et al., 2005). In this regard, the result of this study supports the NEPAD initiatives as the simple egg treatment protocols can be applied in the rural setting.

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#### References

- Agresti, A., 1990. Categorical Data Analysis. Wiley, New York.
- Alderman, D.J., Polglase, J.S., 1984. A comparative investigation of the effects of fungicides on *Saprolegnia parasitica* and *Ocphonomyses astaci*. Trans. Br. Mycol. Soc. 83, 313–318.
- Avendano-Herrera, R., Magarinos, B., Irgang, R., Toranzo, A.E., 2006. Use of hydrogen peroxide against the fish pathogen *Tenacibaculum*

maritimum and its effect on infected turbot (Scophthalmus maximus). Aquaculture 257, 104–110.

- Barnes, M.E., Gaikowski, M.P., 2004. Use of hydrogen peroxide during incubation of landlocked fall Chinook salmon eggs in vertical-flow incubators. N. Am. J. Aquac. 66, 29–34.
- Barnes, M.E., Ewing, D.E., Cordes, R.J., Young, G.L., 1998. Observations on hydrogen peroxide control of *Saprolegnia* spp. during rainbow trout eggs incubation. Prog. Fish-Cult. 60, 67–70.
- Bene, C., Heck, S., 2005. Fisheries and the millennium development goals: solutions for Africa. NAGA, Worldfish Quarterly Center Q., vol. 28 (3 & 4), pp. 14–18.
- Boyd, C.E., Tucker, C.S., 1992. Water Quality and Pond Soil Analysis for Aquaculture. Alabama Agricultural Experiment Station. Auburn University, AL. 183 pp.
- Celada, J.D., Carral, J.M., Saez-Royuela, M., Melendre, P.M., Aguilera, A., 2004. Effects of different antifungal treatments on artificial incubation of the astacid crayfish (*Pacifastacus leniusculus* Dana) eggs. Aquaculture 239, 249–259.
- Dawson, V.K., Rach, J.J., Schreier, T.M., 1994. Hydrogen peroxide as a fungicide for fish culture. Bull. Aquac. Assoc. Can. 2, 54–56.
- de Graaf, G.J., Galemoni, F., Banzoussi, B., 1995. The artificial reproduction and fingerling of the African catfish, *Clarias* gariepinus (Burchell 1822) in protected and unprotected ponds. Aquac. Res. 26, 233–234.
- Froelich, S.L., Engelhardt, T., 1996. Comparative effects of formalin and salt treatments on hatch rate of koi carp eggs. Prog. Fish-Cult. 58, 209–211.
- Gaikowski, M.P., Rach, J.J., Olson, J.J., Ramsay, R.T., Wolgamood, M., 1998. Toxicity of hydrogen peroxide treatment to rainbow trout eggs. J. Aquat. Anim. Health 10, 241–251.
- Goos, H.J.Th., Richter, C.J.J., 1996. Internal and external factors controlling reproduction in the African catfish, *Clarias gariepinus*. In: Legendre, M., Proteau, J.P. (Eds.), The Biology and Culture of Catfishes. Aquatic Living Resources, vol. 9, pp. 45–58.
- Haylor, G.S., 1991. Controlled hatchery production of *Clarias gariepinus* (Burchell 1882): growth and survival of fry at high stocking density. Aquac. Fish. Manage. 22, 405–422.
- Hogendoorn, H., 1979. Controlled propagation of the African catfish, *Clarias lazera* (C&V), 1. Reproductive biology and field experiments. Aquaculture 17 (4), 323–333.
- Hogendoorn, H., 1980. Controlled propagation of the African catfish, *Clarias lazera* (C&V), 111. Feeding and growth of fry. Aquaculture 21, 233–241.
- Hogendoorn, H., Vismans, M.M., 1980. Controlled propagation of the African catfish *Clarias lazera* (C&V) II. Artificial reproduction. Aquaculture 21 (1), 39–53.
- Huisman, E.A., Richter, C.J.J., 1987. Reproduction, growth, health control and aquaculture potential of the African catfish *Clarias* gariepinus (Burchell, 1822). Aquaculture 63, 1–14.
- Kitancharoen, N., Ono, A., Yamamoto, A., Hatai, K., 1997a. The fungistatic effect of NaCl on rainbow trout eggs saprolegniasis. Fish Pathol. 32 (3), 159–162.
- Kitancharoen, N., Yamamoto, A., Hatai, K., 1997b. Fungicidal effect of hydrogen peroxide on fungal infection of rainbow trout eggs. Mycoscience 38, 375–378.

- Kitancharoen, N., Yamamoto, A., Hatai, K., 1998. Effect of sodium chloride, hydrogen peroxide and malachite green on fungal infection in rainbow trout eggs. Biocontrol Sci. 3 (2), 113–115.
- Liu, J.S., Chen, J.S., Yu, Z.Y., 1995. The effect of common fungicides on Chinese sucker (*Myxocyprinus asiaticus*) eggs. J. World Aquac. Soc. 26 (1), 84–87.
- Macharia, S.K., Ngugi, C.C., Rasowo, J., 2005. Comparative study of the hatching rates of African catfish (*Clarias gariepinus* Burchell 1822) eggs on different substrates. NAGA, World Fish Center Q., vol. 28 (3 & 4), pp. 23–26.
- Marking, L.L., Rach, J.J., Schreier, T.M., 1994. Evaluation of antifungal agents for fish culture. Prog. Fish-Cult. 56, 225–231.
- Melendre, P.M., Celada, J.D., Carral, J.M., Saez-Royuela, M., Aguilera, A., 2006. Effectiveness of antifungal treatments during artificial incubation of the signal crayfish eggs (*Pacifastacus leniusculus* Dana. Astacidae). Aquaculture 257, 257–265.
- Muir, J.F., Gitonga, N., Omar, I., Pouomogne, V., Radwan, I., 2005. Hidden harvests; unlocking the potential of aquaculture in Africa. Technical Review Paper. NEPAD-Fish for All Summit, 22–25 August 2005, Abuja, Nigeria.
- Phelps, R.P., Walser, C.A., 1993. Effects of sea salt on the hatching of channel catfish eggs. J. Aquat. Anim. Health 5, 205–207.
- Post, G., 1987. Textbook of Fish Health. Revised and Expanded Edition. T.F.H. Publications, Neptune City, New Jersey.
- Rach, J.J., Howe, G.E., Schreier, T.M., 1997. Safety of formalin treatment on warm- and coolwater fish eggs. Aquaculture 149, 183–191.
- Rach, J.J., Gaikowski, M.P., Howe, G.E., Schreier, T.M., 1998. Evaluation of the toxicity and efficacy of hydrogen peroxide treatments on eggs of warm- and coolwater fishes. Aquaculture 165, 11–25.
- Rach, J.J., Valentine, J.J., Schreier, T.M., Gaikowski, M.P., Crawford, M.P., 2004. Efficacy of hydrogen peroxide to control saprolegniasis on channel catfish (*Ictalurus punctatus*) eggs. Aquaculture 238, 135–142.
- Schnick, R.A., Meyer, F.P., Gray, D.L., 1989. A Guide to Approved Chemicals in Fish and Fishery Resource Management. University of Arkansas Cooperative Extension Service, Little Rock, AR. 22 pp.
- Schreier, T.M., Rach, J.J., Howe, G.E., 1996. Efficacy of formalin, hydrogen peroxide and sodium chloride on fungal infected rainbow trout eggs. Aquaculture 140, 323–331.
- Small, B.C., Wolters, W.R., 2003. Hydrogen peroxide treatment during egg incubation improves channel catfish hatching success. N. Am. J. Aquac. 65, 314–317.
- Viveen, W.J.A.R., Richter, C.J.J., van Oordt, P.G.W.J., Janssen, J.A.L., Huisman, E.A., 1985. Practical manual for the culture of African catfish *Clarias gariepinus*. The Netherlands Ministry for Development Co-operation, Section for Research and Technology. The Hague, The Netherlands. 122 pp.
- Waterstrat, P.R., Marking, L.L., 1995. Clinical evaluation of formalin, hydrogen peroxide, and sodium chloride for the treatment of *Saprolegnia parasitica* on fall Chinook salmon eggs. Prog. Fish-Cult. 57, 287–291.