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Full Length Research Paper

Tolerance of yolk sac and free swimming fry of African catfish (*Clarias gariepinus*, Burchell 1822) to chemotherapeutic doses of formalin

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This study reports tolerance levels and lethal concentrations of yolk sac and free swimming fry of African catfish (*Clarias gariepinus*) to different therapeutic doses of formalin. Comparative treatments were up to 2000 ppm for 15, 30 and 60 min exposure times and up to 150 ppm for 24 h exposure time for the second experiment on lethal concentrations. In both fry stages, higher survivals were recorded at short exposure times and at lower concentrations. The LC50 value for the yolk sac and free swimming fry was 130 and 90 ppm with total mortalities at concentrations beyond 150 and 125 ppm respectively. Although other concentrations and exposure times gave best performance in terms of survival, we recommend 15 min bath dip at 500 ppm for the yolk sac fry and 15 min at 600 ppm for the free swimming fry for routine use by rural fish farmers to reduce fry mortalities.

Key words: *Clarias gariepinus*, formalin, free swimming fry, lethal concentration, yolk sac fry.

INTRODUCTION

Based on production acreage, clariid catfishes have emerged as one of the most important groups of farmedcatfish in the world with the African catfish (*Clarias gariepinus)* being the main species cultured in Africa (Teugels, 1984). This fish is widely distributed throughout Africa and has long been considered as one of the most suitable species for culture (El Bolock and Koura, 1960; De Kimpe and Micha, 1974). Although *C. gariepinus* is widely cultured in South Africa and increasingly in Nigeria, it has not emerged as an important aquaculture species in Kenya because of inadequate supply of fingerlings as a result of massive larval mortality in the hatchery (Christensen, 1981; Macharia et al., 2005; Rasowo et al., 2007). This obstacle still exists despite the fact that protocols for controlled spawning and indoor rearing of *C. gariepinus* larvae have been established (El Bolock, 1976; Hogendoorn and Wieme, 1976; Hogendoorn, 1980; De Graaf and Janssen, 1996; Hecht et al., 1996). Moreover, the production systems in Kenya, especially the earthen ponds, which are the most common, are known to favour parasite growth due to overcrowding and suboptimal water quality (Kamata, 1966; Noga, 1996; Hoffman, 1999). This has increased their susceptibility to infection by disease-causing parasites which if left untreated, can result in 100% mortality especially in larvae (Noga, 1996; Hoffman, 1999). Very young fry (from 1 to 7 days post-hatch) can be infected with external ciliated protozoans, causing substantial mortalities (Hogendoorn, 1980a, b; Hogendoorn and Wieme, 1976; Hogendoorn and Vismans, 1980; Hogendoorn and Koops, 1983). The use of chemotherapeutics for the treatment of external ciliated protozoan parasites in food fishes has been tested (Bodensteiner et al., 1993, 2000; Straus, 1993; Thorburn and Moccia, 1993; Sanchez et al., 1997; Schlenk et al., 1998; Rach et al., 2000). Although, most of the therapeutic dosages used on clariid fishes were based on studies conducted on mature fish. Only a few studies have been conducted regarding the use of chemotherapeutics in the early life stages of clariidae (Bodensteiner et al., 1993, 2000; Straus, 1993; Thorburn and Moccia, 1993; Sanchez et al., 1997; Schlenk et al., 1998; Rach et al., 2000). The present study was designed to compare various treatment concentrations of

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formalin at different exposure times so as to determine the tolerance levels of yolk sac and free swimming fry exposed to a range of chemotherapeutic doses. A separate experiment determined the lethal concentration (LC50) of formalin for the two larval stages at an exposure time of 24 h. The procedure demonstrated here is simple and can be adopted by farmers with limited resources.

MATERIALS AND METHODS

The study was conducted at the Moi University, Chepkoilel (Eldoret, Kenya) campus fish hatchery from January to April 2009.

Spawning and fry production

Mature broodstock fish were seined from ponds at the Moi University Chepkoilel campus fish farm. Ripe females and males mean weight 300 - 500 g fish^{-1} were selected based on the protocol by Viveen et al. (1995) and transferred to the hatchery. They were acclimated in hatching tanks for one day without feeding. Whole pituitaries were removed from sexually mature males, homogenized immediately with absoluteacetone before the pituitaries were injected into the females. The dose was calculated on a 1:5:1 (donor: recipient weight basis). Due to high levels of aggression, broodstock females were separated from each other in the holding tanks using sturdy screens. Females with well developed eggs (1.1 - 1.4 mm diameter) were stripped 12 h after receiving a single dose of pituitary and kept at a temperature of 28°C. At this stage the eggs were hydrated and had gone through the process of ovulation. Holding the females in a head-up vertical position was a simple and reliable method of testing the readiness of the eggs for fertilization. By the time the eggs begun to run freely from the genital pore they were ready to be fertilized. To increase genetic variability, a minimum of two males were used to fertilize batches of eggs from females. To obtain adequate quantities of sperm, males were sacrificed and the testes removed. Fertilization was best effected by squeezing sperm directly onto the eggs, which had been stripped into a bowl, activated by water condition and thoroughly mixed using a bird feather. After fertilization, the eggs were spread on a slanting mesh net in an incubator for 12 h to facilitate hatching.

Experimental design

After hatching, equal lots of 50 yolk sac and free swimming fry were systematically counted in triplicate and placed in individual compartments in the glass aquaria for the formalin treatment at exposure times of 15, 30 and 60 min for experiment 1. Eight concentrations, that is, 0 (control), 50, 100, 250, 500, 1000, 1500 and 2000 ppm were used. A total of 72 compartments were used in experiment 1. The same procedure was applied for experiment 2 on acute toxicity but with seven concentrations that is, 0 (control), 25, 50, 75, 100, 125 and 150 ppm and exposure time of 24 h for both the stages of development. A total of 42 compartments were used for experiment 2. The yolk sac fry were not fed with any experimental diet because they could still survive on the yolk sac within the first few days after hatching. Free-swimming fry were fed with zooplanktons throughout the experiment. All fry in each tank were counted on the intended time basis, their survivals were recorded and daily rations readjusted accordingly.

Culture facilities

The culture units for the larvae consisted of compartmentalized glass tanks in a recirculating indoor system. The glass incubator tanks were provided with central drainage pipes surrounded by outlet pipes, perforated at the bottom, to facilitate cleaning and waste removal. The culture system was also provided with continuous aeration through an air compressor and immersion heaters, with thermostats, to maintain water temperature at 27 - 29°C. About 90% of the water was replaced daily by freshwater of similar temperature. Water quality variables, including temperature and dissolved oxygen (Oxygen-temperature meter-model 55, YSI, Yellow Springs Ohio, USA); transparency (Sechi disc) and pH (pH meter-Hanna Instruments, model 8519, USA) were monitored daily. The average values throughout the study period were recorded.

Data analysis

The data consisted of the number of larvae in each compartment that survived at the end of the experiment. Since the survival success is a binary variable which should follow a binomial distribution, a logistic analysis was performed (Agresti, 1990) on the data by fitting the logistic model. The logit model is a general logistic model as shown below:

$$\log it[\theta(\chi)] = \log\left[\frac{\theta(\chi)}{1-\theta(\chi)}\right] = \beta o + \beta_{1\chi 1} + \beta_{2\chi 2} + \ldots + \beta_{\chi i} \quad (1)$$

$$\log\left[\frac{p}{1-p}\right] = \beta o + \beta_1 C + \beta_2 C_2 + \beta_3 C_3 \quad (2)$$

It is a general logistic model, which takes the form of equation 2 in dose response treatments; where p denotes the probability of survival, β_0 is the intercept, β_2 is the coefficient of quadratic response in C and β_3 is the coefficient of cubed variable in C. We fitted the model using GENSTAT (GenStat Release 4.24DE) statistical software program. Model fit was based on residual likelihood ratio chi-square statistic having failed the Normality Test (Shapiro-Wilk).

RESULTS

Logistic analysis revealed a significant effect ($p < 0.05$) of formalin concentration on fry survival; (Figures 1, 2 and 3, Tables 1, 2, 3, 4, 5, 6 and 7). The model parameter statistics are given in Tables 5, 6 and 7. Mean (± SEM) percent survival subjected to varying concentrations of formalin and exposure times are shown in Tables 1, 2 and 3 while Figures 1, 2 and 3 present the predicted probability of survival of larvae. Table 4 depicts the optimal values in terms of concentration and survival success. As shown in Figures 1, 2 and 3, treating the catfish fry with formalin significantly affected the probability of fry survival. The full logistic regression model fits the survival data adequately.

Water quality parameters

To assess the kind of conditions the fishes and fry were

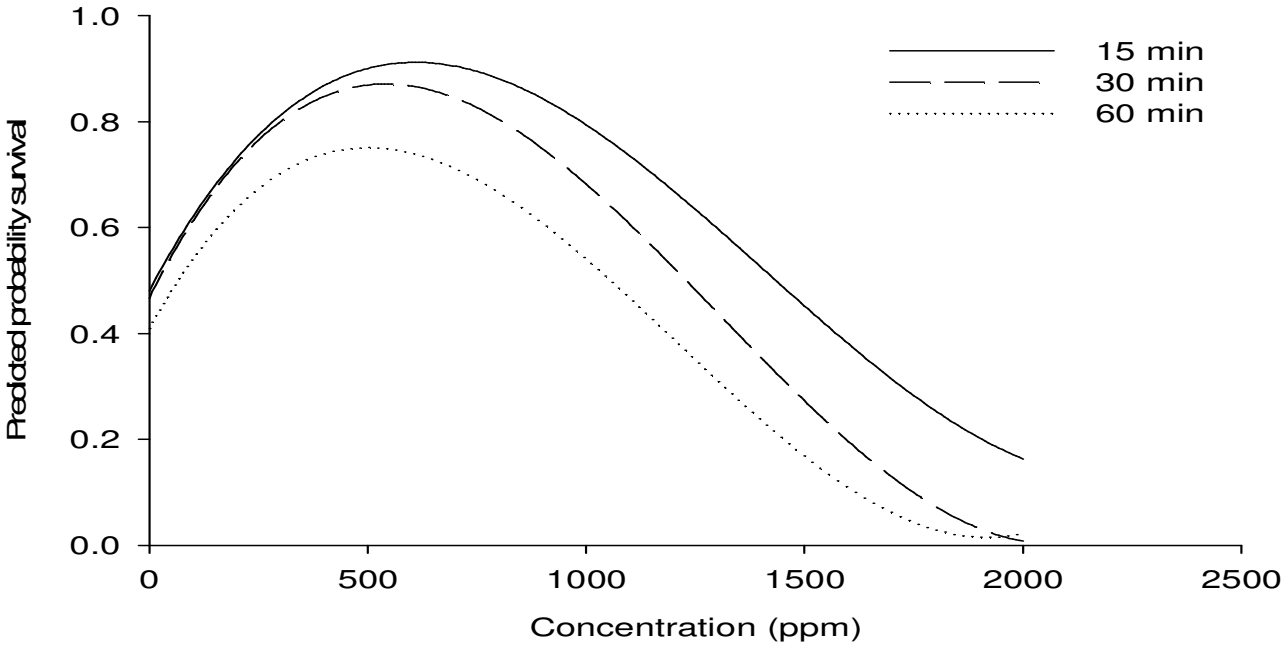

Figure 1. Predicted probability of yolk sac *Clarias gariepinus* survival exposed to formalin for 15, 30 and 60 min based on logistic analysis model.

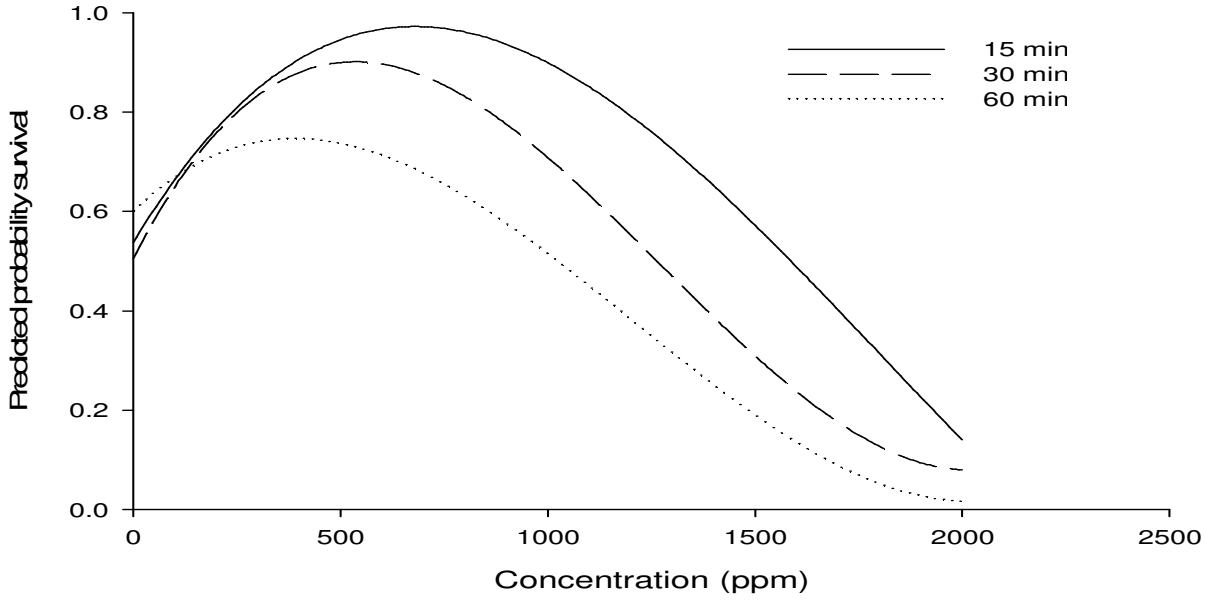

Figure 2. Predicted probability of free swimming *Clarias gariepinus* survival exposed to formalin for 15, 30 and 60 min based on logistic analysis model.

subjected to during the experiment optimum water quality were maintained throughout the experiment. Temperature, dissolved oxygen, pH and Total Ammonia Nitrogen (TAN) were 26 ± 2°C, 6.8 - 7.7, 6.6 - 7.3 and 0.27 ± 0.08 mg/l, respectively.

Yolk sac fry

Concentrations of formaldehyde ranging from 250 - 1000 ppm gave survival percentages of over 70%, which were higher than the untreated control (0 ppm). However

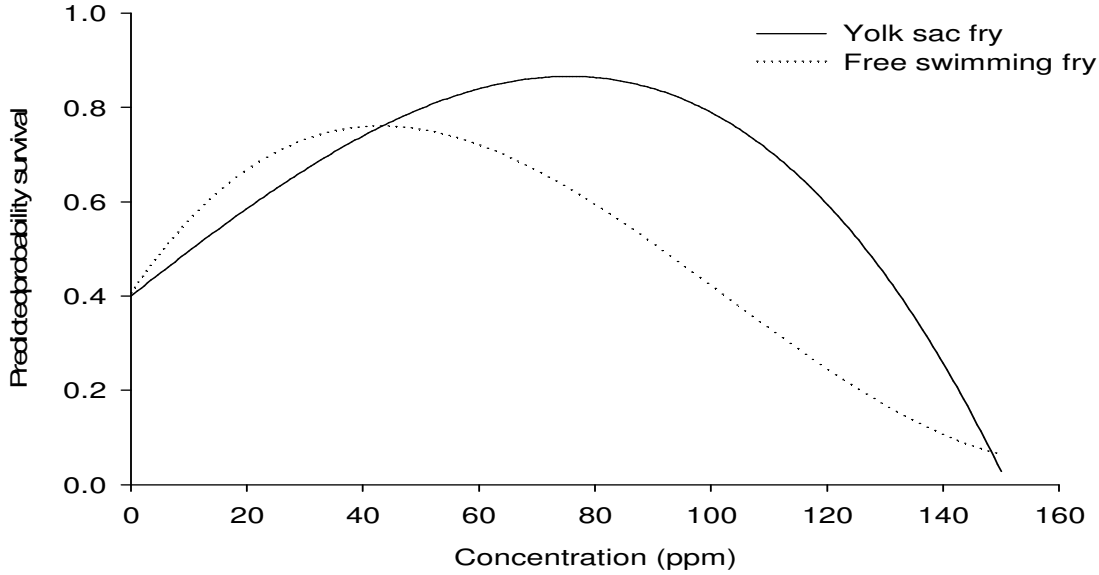

Figure 3. Predicted probability of *Clarias gariepinus* survival exposed to lethal concentration of formaldehyde for 24 h based on logistic analysis model.

Table 1. Mean (± SEM) percent survival of *Clarias gariepinus* in formalin treatments (Yolk sac fry).

Concentration (ppm)	Exposure time		
	15 min	30 min	60 min
0	22.7 ± 0.6	20.7 ± 0.6	14.3 ± 3.2
50	30.7 ± 0.6	27.3 ± 2.3	23.7 ± 0.6
100	29.7 ± 0.6	32.7 ± 1.2	30.7 ± 0.6
250	38.7 ± 0.6	40.7 ± 0.6	39.8 ± 1.5
500	44.3 ± 0.6	41.7 ± 0.6	32.0 ± 1.0
1000	40.7 ± 0.6	33.0 ± 1.0	23.7 ± 0.6
1500	22.0 ± 0.0	15.0 ± 2.0	12.3 ± 1.5
2000	8.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0

Table 2. Mean (± SEM) percent survival of *Clarias gariepinus* in formalin treatments (Free swimming fry).

Concentration (ppm)	Exposure time		
	15 min	30 min	60 min
0	27.0 ± 1.0	23.3 ± 1.2	24.3 ± 3.5
50	30.3 ± 0.6	27.7 ± 1.2	31.0 ± 1.0
100	35.0 ± 1.0	36.3 ± 0.6	39.0 ± 1.0
250	38.0 ± 0.0	39.3 ± 2.5	42.7 ± 2.5
500	46.0 ± 1.0	46.7 ± 1.5	32.0 ± 2.0
1000	48.7 ± 0.6	32.0 ± 2.0	23.7 ± 1.5
1500	26.0 ± 1.7	18.0 ± 5.6	12.3 ± 1.5
2000	7.7 ± 1.5	3.3 ± 1.5	0.0 ± 0.0

treatment with formaldehyde at more than 1000 ppm reduced the survival rate with total mortalities recorded at 2000 ppm concentration after 30 and 60 min exposure times respectively. Less than 20% of yolk sac larvae survived at 2000 ppm concentration Concentrations less than 250 ppm gave low survivals of less than 70%.

Table 3. Mean (±SEM) percent survival of *Clarias gariepinus* for lethal concentration of formalin treatments.

Concentration (ppm)	Free swimming fry	Yolk sac fry
0	24.0 ± 1.0	18.3 ± 1.5
25	27.3 ± 2.5	35.7 ± 1.5
50	37.0 ± 2.0	37.7 ± 1.2
75	40.3 ± 0.6	42.0 ± 1.0
100	23.3 ± 3.5	38.3 ± 1.2
125	0.0 ± 0.0	29.7 ± 1.5
150	0.0 ± 0.0	0.0 ± 0.0

Table 4. Summarized optimal data for yolk sac fry, free swimming fry and lethal concentration from the curves.

	Exposure time	Concentration (ppm)	Survival (%)
Yolk sac fry	15 min	500	90
	30 min	400	86
	60 min	300	75
Free swimming fry	15 min	600	94
	30 min	500	90
	60 min	350	74
	Lethal concentration		
Yolk sac fry	24 h	80	85
Free swimming fry	24 h	40	75

Generally, a 15 min bath dip gave the highest percentage of survivors compared to the other exposure times of 30 and 60 min. Survival for 60 min exposure treatment recorded was lower than at 15 and 30 min. The highest percent survival for 15 min was observed at 500 ppm where 90% of the larvae survived. For 30 min exposure time the highest percent survival was observed at 400 ppm where 86% of the fry survived. After a 60 min exposure the best survival (75%) was observed at 300 ppm.

Free swimming fry

The results for the free-swimming fry showed a similar trend as the yolk sac fry but differed slightly with increase in exposure time. The results showed that concentrations of formalin ranging from 250 - 1000 ppm gave survival of over 70%, which were higher than the untreated control (0 ppm). However treatment with formalin at more than 1000 ppm reduced the survival rate with total mortalities recorded at 2000 ppm concentration after 60 min exposure time. Less than 18% of the larvae at 2000 ppm concentration survived after 30 and 60 min exposure time respectively. Concentrations < 250 ppm gave low survivals of less than 70%. Meanwhile, the 15 min bath dip still gave the highest percentage of survivors compared to 30 and 60 min. The highest percent survival for 15 min was observed at 600 ppm where (94%) of the larvae survived. For 30 min exposure time the highest percent survival was observed at 500 ppm where 90% of the fry survived. For 60 min, there was a sharp decrease from 90 to 74% with the optimum survival observed at 350 ppm with 74% survival.

Lethal concentration

When both the yolk sac and free-swimming fry were subjected to acute toxicity concentrations for 24 h, the yolk sac stage tolerated better than the free swimming fry. The LC50 for the yolk sac was at 130 and 90 ppm for the free swimming fry. The highest predicted survival was 86% for the yolk sac fry and 81% for the free-swimming fry.

DISCUSSION

The interpretation of the tolerance levels of yolk sac and free swimming fry of *C. gariepinus* to therapeutic doses of formalin at varied exposure times is complex as the results are affected by many interrelated factors such as water quality, composition of feed for the free swimming fry and size of the ration (Hepher, 1988). In our study, water quality parameters were found to be similar in all compartments. The same feed was used and the ration was fixed similarly in all compartments. This study was

Table 5. Model parameter statistics from the logistic regression (yolk sac fry).

Yolk sac fry			
Chemical	**Time (min)**	**Model**	**Parameter significance (*p*-value)**
Formaldehyde	15	$\log(\rho/1-\rho)=4.8E\text{-}01 + 1.6E\text{-}02*C\text{-}1.6E\text{-}04*C^2 + 3.9E\text{-}08*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p < 0.001$) β_3 ($p < 0.001$)
	30	$\log(\rho/1-\rho)=4.7E\text{-}01 + 1.6E\text{-}02*C\text{-}1.9E\text{-}04*C^2 + 4.9E\text{-}08*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p < 0.001$) β_3 ($p < 0.001$)
	60	$\log(\rho/1-\rho)=4.1E\text{-}01 + 1.5E\text{-}02*C\text{-}1.9E\text{-}04*C^2 + 5.2E\text{-}08*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p < 0.001$) β_3 ($p < 0.001$)

Table 6. Model parameter statistics from the logistic regression (free swimming fry).

Free swimming fry			
Chemical	**Time (min)**	**Model**	**Parameter significance (*p*-value)**
Formaldehyde	15	$\log(\rho/1-\rho) = 5.4E\text{-}01 + 1.4E\text{-}02*C\text{-}1.3E\text{-}04*C^2 + 2.3E\text{-}08*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p < 0.001$) β_3 ($p < 0.001$)
	30	$\log(\rho/1-\rho) = 5.0E\text{-}01 + 1.6E\text{-}03*C\text{-}1.9E\text{-}06*C^2 + 5.0E\text{-}10*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p < 0.001$) β_3 ($p < 0.001$)
	60	$\log(\rho/1-\rho) = 6.0E\text{-}01 + 8.0E\text{-}04*C\text{-}1.2E\text{-}06*C^2 + 3.4E\text{-}10*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p < 0.001$) β_3 ($p < 0.001$)

Table 7. Model parameter statistics from the logistic regression (lethal concentration).

Lethal concentration			
Chemical	**Time (24 h)**	**Modl**	**Parameter significance (*p*-value)**
Formaldehyde	Yolk sac fry	$\log(\rho/1-\rho)=4.0\text{-}01 + 10.0E\text{-}01*C\text{-}1.2\text{-}03*C^2 + \text{-}4.6E\text{-}05*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p > 0.001$) β_3 ($p > 0.001$)
	Free swimming fry	$\log(\rho/1-\rho)=4.1E\text{-}01 + 1.6E\text{-}01*C\text{-}0.0E\text{-}02*C^2 + 6.0E\text{-}10*C^3$	β_0 ($p < 0.001$) β_1 ($p < 0.001$) β_2 ($p > 0.001$) β_3 ($p>0.001$)

intended to assess, for the first time, the effect of formalin, one of the commonly used chemical on the survival success of African catfish fry. In the first experiment, we subjected catfish fry to formalin treatment concentrations between 0 and 2000 ppm. The results are presented in Tables 1 to 7 and Figures 1 to 3 and explained in the results section. The increase in survival in both stages of fry development in the dome shaped curves (Figures 1 to 3) dignifies the range of tolerance levels from the controls the fry had before reaching the optimum survival. The optimum survival is shown at the peak of the eight dome shaped curves for each exposure time respectively Figures 1 to 3 and optimum results presented in Table 4. Moreover, when yolk sac fry were compared with free swimming fry without regard to concentrations, there were significant differences in tolerances among treatment groups. Survivors for the 15 and 30 min exposure time significantly varied with that of 60 min (Figures 1 to 2). The controls exposed for 15 and 30 min showed no difference between the life stages. Presumably lack of nutrition by the free-swimming stages was not yet a problem up to 30 min. In both treatment groups, the 60 min exposure time resulted into higher mortality than the 15 and 30 min exposure times. In Figure 3, free swimming fry experienced higher mortalities than the yolk sac fry. This may be due to (1) increased uptake of formalin due to a higher surface-area-to-volume ratio or (2) increased uptake of formalin through feeding and respiration by the delicate functional gills. Mortalities for the yolk sac might be due to permeability of the yolk sac that led to negative osmotic effects. According to Schreier et al. (1996); Sanchez et al. (1997) and Rothen et al. (2002), one possible explanation for the higher mortalities with formalin in the free-swimming fry

for the longer exposure time could be the difference in the development of the gastrointestinal tract. Yolk sac fry depend on the yolk for nutrition, hence acting as a formalin sink. The free-swimming fry by contrast, directly take water into the intestinal tract (and skin) while feeding (in this case zooplankton) and thus it can be hypothesized that the fry had quicker uptake and subsequently higher overall levels of formalin in the body. Another possible explanation is the difference in respiration. Rothen et al. (2002) argued that yolk sac fry respire primarily by diffusion of gases through the skin, whereas free swimming fry have functional gills that might be more affected by formalin leading to higher mortalities. This trend was clearly demonstrated in the acute toxicity tests in experiment 2. The yolk sac fry were better adapted than the free-swimming fry with their LC50 value at 130 and 90 ppm respectively. Other reasons as to why higher mortalities were observed in experiment 2 compared to experiment 1 as suggested by Howard (1989), Howard et al. (1991) and Rothen et al. (2002) include higher dehydration by formalin due to the longer exposure time of 24 h. The authors went further to assert that increase uptake of formalin due to the high surface area to volume ratio, increased permeability across the membranes of the larvae and hence creating osmolarity changes. Higher mortalities at higher concentrations and longer exposure times could also imply that a higher concentration was toxic and stressful to the larvae while very low concentrations and exposure times for both stages of fry development were not sufficient to treat the possibilities of fungal infections hence (Barnes et al., 1998; Rothen et al., 2002; Barnes and Gaikowski, 2004). Results from experiment 1 indicate that concentration at 2000 ppm was an overdose hence toxic whereas a concentration below 250 ppm was an under dose. Results of this study are in general agreement with the findings of other research works that dosages ranging from 50 to 600 ppm can be effective in treating or preventing fungal infections of fish larvae (Cochran and Cox, 1957; Barnes et al., 1998; ATSDR, 1999; Barnes and Gaikowski, 2004; Rothen et al., 2002; Van de Nieuwegiessen et al., 2008). Since increase in formalin concentration reduces the amount of dissolved oxygen, variations of the dissolved oxygen concentration and formalin concentration in the test solutions imply that yolk sac fry can defend themselves from toxic effects of formalin by reduced respiration rate as they respire through the skin. This does not apply to the free swimming fry which needs to consume water for them to respire through their delicate functional gills (Haylor, 1991, 1993; Schreier et al., 1996; Sanchez et al., 1997; ATSDR, 1999; Appelbaum and Kamler, 2000; Rothen et al., 2002). According to ATSDR (1999), Schreier et al. (1996), Sanchez et al. (1997) and Rothen et al. (2002) increase in formalin concentration reduces the amount of dissolved oxygen hence the higher mortalities observed at higher concentrations for both experiments in both stages of fry development. While the greatest mortalities occurred at 60 min, further studies need to consider the possibility of including an interaction term, that is, time X concentration into the model as well as examining the effects of these chemotherapeutics at 2 h to 2 days post treatment to verify the absence of significant long term effects. (We observed no significant mortalities up to 2 h for experiment 1 but did not include those observations in this paper because of the complicating effects of starvation and water quality after 2 h). This study focused on 15, 30 and 60 min intervals for experiment 1 and 24 h for experiment 2 to avoid the variables involved with feeding the fry and changes in water quality parameters.

Conclusion

In the present study, results from the controls had the lowest survival compared to the treatments, clearly indicating that the therapeutic dose treatment with formalin significantly reduced the fry mortalities. Although all life stages of the fry were tolerant of the therapeutic doses, their response showed great sensitivity to formalin. However, we encourage future research to examine the influence of water chemistry and accompanying zoospore concentration of *Saprolegnia* spp. on formalin efficacy for fungal growth.

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