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Short communication

Evaluation of sodium chloride (NaCl) for potential prophylactic treatment and its short-term toxicity to African catfish *Clarias gariepinus* (Burchell 1822) yolk-sac and swim-up fry

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ABSTRACT

Disease and parasite outbreaks cause mortality of fish, affecting mostly early stages of fish cycle. The outbreaks are usually prevented using a number of chemicals including salt (NaCl) for which protocols are lacking. The purpose of this study was to determine the efficacy of NaCl as a potential prophylactic treatment and its short term toxicity on African catfish (*Clarias gariepinus*) yolk-sac and swim-up fry during a 24 h exposure period. Three-day-old yolk sac and six-day-old swim-up fry ($n=50$ for each stage) were subjected to static bath dip treatment in increasing concentrations of NaCl (0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 4.0, and 10.0 g/L) for 15, 30 and 60 min. Toxic ranges were tested by exposing the fry stages to concentrations of 0.25, 0.5, 0.75, 1.0, 1.25, and 1.50 g/L NaCl for 24 h. Controls were not subjected to any concentration of NaCl. All experiments were executed in triplicate. Regardless of the concentrations and duration of exposure, survival of fish in NaCl treatment differed significantly ($P<0.05$) from the untreated controls. Effective concentration ranges of NaCl were 0.2–0.8 g/L and 0.2–1.0 g/L in the yolk sac and swim-up fry respectively at exposure duration of 15 min. No fry survival was recorded at a concentration of 10.0 g/L for either stage of development. The mean 24 h LC_{50} values for the yolk sac and swim-up fry were 0.61 and 0.70 g/L NaCl respectively. Sodium chloride may be used as prophylactic treatment in early stages of *C. gariepinus* but could be toxic at longer exposure times. However, empirical tests on efficacy of NaCl on pathogens are recommended.

1. Introduction

African catfish is one of the most important food fish species cultured both within and outside its natural range of tropical and subtropical environments (Adewolu et al., 2008). Its resistance to diseases, high fecundity and ease of larval production in captivity make it of commercial importance (Kestemont et al., 2007). However, there are still considerable challenges during larval production mainly due to outbreak of diseases and parasites in the culture units. Disease and parasite outbreaks are a significant constraint to the development of aquaculture worldwide (Subasinghe et al., 2001). To maintain healthy fry stocks, these outbreaks must be prevented and/or controlled. Many farmers attempt to control disease outbreaks and external parasitic infestations by prophylactic treatment with antibiotics, even when disease infections and external parasites are not evident (Holmström et al., 2003). Such practices result in development of antibiotic resistance (Cabello, 2006).

An alternative range of tested chemotherapeutics commonly used for treatment and prophylaxis of fungal infection and external parasites include formalin, common salt (NaCl), hydrogen peroxide (H_2O_2) and copper sulfate ($CuSO_4$) (Schreier et al., 1996). However concerns have been raised about safety of formalin (Marking et al., 1994; Rach et al., 1997), and toxicity of $CuSO_4$ (http://www.pesticideinfo.org/) and H_2O_2 to human health and environment (Watts et al., 2003). Therefore, NaCl remains the best alternative to control fungal outbreaks and external parasites. Moreover, NaCl has been tested and found to be effective as a prophylactic treatment against important protozoans, helminths and fungal pathogens (Noga, 2000; Schnick, 1988; Selose and Rowland, 1990).

Currently, only a few studies on the range of NaCl concentrations effective for prophylaxis and those that elicit short-term toxicity in early stages of fish have been carried out. This study was carried out to (i) determine the effective concentration ranges and duration of NaCl exposure that would serve as effective prophylactic treatment for *Clarias gariepinus* yolk-sac and swim-up fry and (ii) evaluate the

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potential short-term toxicity of prolonged exposure of *C. gariepinus* yolk-sac and swim-up fry to NaCl.

2. Materials and methods

This study was conducted at the hatchery of Fisheries and Aquatic Sciences Department, Moi University. The water source for this facility is tap water, and was stored in an intermediate holding tank for at least 48 h to remove chlorine before use in the experimental tanks. The water was continuously aerated and temperature controlled thermostatically at 27 ± 0.2 °C. One ripe female (258.2 g) and one mature male (262.2 g) *C. gariepinus* brood stock were seined from the ponds at the institution's research farm and transferred to the hatchery. Fry were obtained through pituitary injection, hand-stripping of eggs and artificial fertilization as detailed in de Graaf et al. (1995). Fry were transferred to flow-through nursery tanks maintained at 27 ± 0.2 °C and stocked at a density of 5 fry/L. The fry were fed live zooplankton (*Moina micrura*) ad libitum beginning 3 days post-hatch. Temperature and pH were measured with a portable thermometer and pH meter (Hanna instruments, Model 8519, USA). Dissolved oxygen (DO) was measured by oxygen meter (JENWAY 3405, Barloword Scientific Ltd, Essex, UK) and occasionally verified by Winkler method. The pH ranged from 6.9 to 7.3 and DO from 6.9 to 7.7 mg/L. Water flow through each nursery tank was maintained at 0.4 L/min to ensure a renewal rate of at least once every 2 h.

Two life stages of *C. gariepinus* were examined, namely, yolk sac fry (3-day-old) and free-swimming fry (6-day-old). The study comprised two experiments: a short-term efficacy test which measured survival after short term (15, 30 and 60 min) exposure to prophylactic concentrations, and a short-term toxicity test measuring survival after exposure of the three-day-old yolk sac and six-day-old free-swimming fry stages to NaCl for 24 h. Salt treatments were prepared by dissolving a weighed amount of NaCl (food grade, evaporated granulated salt, Kensalt™ Ltd. Company, Mombasa, Kenya) in distilled water to give the test concentrations. Designated salinities were verified by use of a salinometer (Model IC/SB-1 Salinity Cell, VA, USA). Salinity, osmolality and ions of the experimental waters are presented in Table 1. To evaluate prophylactic levels, groups of 50 fry of each stage were selected at random and exposed for 15, 30, and 60 min to static bath treatment of NaCl concentrations of 0 (control), 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 4.0 and 10.0 g/L. For the short-term toxicity test, groups of 50 fry of each stage were exposed to 0 (control), 0.25, 0.5, 0.75, 1.0, 1.25 and 1.50 g/L NaCl for 24 h following the OECD (1992) guidelines. All treatments were executed in triplicate. Test chambers were plastic beakers of 2 L capacity of test concentration. Treatments were allotted at random in the experimental units. Fry were placed in each plastic beaker approximately 20 min after the treatment chemicals were added by lowering a petri dish containing the test fry and a small amount of water into the beaker as gently as possible in order to minimize handling stress. At the end of each experiment, each beaker was examined with a flashlight to count the number of mortalities and the results recorded. Mortality was defined as enlarged, white opaque fry that were non-motile and did not respond to agitation with a plastic rod. Percent survival was calculated using the formula

$$\text{Percent survival} = \left(\frac{\text{Initial number of fry} - \text{Final number of fry}}{\text{Initial number of fry}}\right) * 100$$

Data on survival were presented as means (±SEM). Effects of exposure concentration and time of exposure was analyzed using two-way ANOVA. The short-term toxicity data consisted of the initial number of fish in each lot and the number that had died at the end of the experiment. Since survival or death is a binary variable which follows a binomial distribution, we performed a logistic analysis (Agresti, 1990) on the data by fitting the logistic model to evaluate toxic ranges of NaCl. We used the logit model, $\text{Log}\left[\frac{\rho}{1-\rho}\right] = \beta_0 + \beta_1 C + \beta_2 C^2 + \beta_3 C^3$, where ρ denotes the probability of survival, β_0 is the intercept, β_1 is the coefficient of concentration C, β_2 is the coefficient of quadratic response in C and β_3 is the coefficient of cubic response in C. All analyses were conducted using GENSTAT 10.0 statistical software program (Payne, 2009).

Table 1
Salinity, ions and osmolality measured in the sample of the control and experimental waters prepared.

Salt concentration (g/L)	Na^+ (mM)	Cl^- (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	Osmolality (mOsm/kgH_2O)
0.00	0.020	0.111	0.002	0.002	2.611
0.10	1.628	1.599	0.002	0.002	3.148
0.20	3.256	3.197	0.002	0.002	6.295
0.25	4.072	3.997	0.002	0.002	7.873
0.40	6.554	3.234	0.002	0.002	14.384
0.50	8.193	8.084	0.003	0.002	17.983
0.75	12.291	12.129	0.002	0.003	26.997
0.80	13.862	14.228	0.002	0.002	29.062
1.00	17.327	17.786	0.002	0.002	36.32
1.25	21.456	22.124	0.003	0.002	45.542
1.50	25.687	26.417	0.003	0.002	54.663
2.00	34.655	35.935	0.002	0.003	72.61
4.00	69.311	72.596	0.003	0.004	145.22
10.00	173.276	199.710	0.002	0.009	363.11

3. Results and discussion

When regular examination of fish is not undertaken, a number of parasites including protozoans, monogeneans as well as other pathogens can invade and hide in the external organs of the fish such as gills, skin, eyes, operculum, buccal epithelium and tongue (Lom and Dyková, 1992) and can cause mortality in fish if not eliminated (Scholz, 1999). Sodium chloride has been used effectively in aquaculture as anti-parasitic agent (Rach et al., 1997; Schelkle et al., 2011; Schreier et al., 1996). In *C. gariepinus* seed production, Rasowo et al. (2007) showed that NaCl was effective as statistic bath to improve egg hatchability. We further tested the prophylaxis of NaCl on two stages of *C. gariepinus* fry.

Survival of *C. gariepinus* fry exposed to varying concentration of NaCl for 15, 30 and 60 min is shown in Fig. 1. There were significant ($P<0.05$) interactions between NaCl concentration and exposure duration on fry survival. Generally, 15 min exposure resulted in higher survival of the yolk-sac and swim-up fry than 60 min exposures in all treatments. In addition, the survival of both the yolk-sac and swim-up fry in NaCl treatments ranging from 0.1 to 1.0 g/L was consistently higher than survival of fry in control treatment regardless of the exposure duration. Higher survival (90–95%) was recorded among yolk-sac fry exposed to 0.2 to 0.8 g/L of NaCl for 15 min than other treatments (Fig. 1a), which was similar to survival recorded during a 30 min exposure to 0.2 and 0.4 g/L NaCl. In the swim-up fry, exposure to 0.2 to 1.0 g/L NaCl concentrations for 15 min resulted in higher survival than other treatments and was similar to survival in concentration of 0.2 to 0.8 g/L NaCl after 30 min exposure (Fig. 1b). The observed low survival of fry in control is probably due to osmoregulatory disturbances since the body fluids of most freshwater fish are hyper-osmotic (osmolality of 260–330 mOsm/kgH_2O) with respect to their external medium (Souza-Bastos and Freire, 2009), which may cause mortality due to stress (Breves et al., 2010). However, parasitic infections and possible bacterial contamination are also possible causes of mortality and cannot be overlooked since in control, there was no anti-parasitic agent such as NaCl. However, NaCl was effective prophylactic treatment for both the three-day-old yolk sac and the six-day-old swim-up in concentrations ranging between 0.1 and 1 g/L. Francis-Floyd (1995) indicated that weak solutions containing 0.05 to 0.1 g/L salt may be used as a bath for