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BRIEF NOTES

Toxicity of Ammonia on *Oreochromis niloticus* Fingerlings

Charry Neleene L. Paracueles

School of Marine Fisheries and Technology
Mindanao State University at Naawan, 9023 Naawan, Misamis Oriental

Email: charryneleene86@gmail.com

ABSTRACT

Exposure of fish to ammonia has an acute toxic effect and certain high concentrations can cause massive mortalities in the cultured animals. This study was conducted in order to determine the 24 hour lethal concentration (LC50) of ammonia on *Oreochromis niloticus* fingerlings under local ambient condition. A total of 120 fingerlings with an average weight of 33.2 ± 0.23 g and an average length of 11-29 mm were used in the experiment. The test animals were distributed to 3L capacity plastic jars filled with 1L distilled water using a completely randomized design (CRD). The mean LC50 ammonia level is 4.10 ppm, a lethal concentration at which 50% of the fingerlings died. The computed mean LC25 is 2.025 ppm and mean LC75 is 8.31 ppm. The experiment showed that ammonia is very toxic to the fish with 100% mortality occurring after 1 hour and 2 hours of exposure to 20ppm and 15ppm, respectively. An average mortality of 83.3% occurred in 10ppm concentration after 4 hours of exposure. Behavioral responses to the intoxication include rapid swimming, rapid movements of the fins and operculum, efforts to gasp air from the surface of the water, and less mobility. External changes are increased secretion of mucous, darkening of the skin, and discoloration of the eyes. Results of this study have important implications on maintenance of water quality in aquaculture systems.

Keywords: Ammonia, toxicity, LC50, *Oreochromis niloticus*, aquaculture.

INTRODUCTION

Ammonia is a nitrogen waste or primary by-product of protein metabolism released by aquatic animals into the production pond environment (Wurts, 2003). Ammonia is known to be seriously toxic to aquatic organisms and is usually excreted by teleost fish through gills and urine as the main nitrogenous waste (Fallu, 1981). Short-term exposure to high concentrations or even to low ammonia levels for extended periods of time can be toxic effect on fish where its most targeted organ is the central nervous system. Ammonia concentrations are also closely linked to ph levels in the culture medium (Wurts, 2003).

The toxicity of ammonia in its un-ionized form NH_3 is ascribed to its ability to readily diffuse across the gill membranes due to its liquid solubility and lack of charge. As reported by other scientists, NH_3 in high concentrations could affect the growth of the fish, and promote histopatological alterations in several tissues, especially in the brain, and increasing cardiovascular activity (Marcon *et al.*, 2004).

Recent evidences show that primary causes of ammonia toxicity is the depolarization of neurons produced by the ionic form of ammonia (NH_4^+), which in turn leads to the excessive activation of *N*-methyl *D*-aspartate (NMDA) receptor, resulting in depletion of ATP. Thus, ammonia-intoxicated fish might experience convulsion and death symptoms (Marcon *et al.,* 2004). Nevertheless, studies on ammonia toxicity carried out in both cultured marine and freshwater fish indicate that its toxic effects differ significantly among species (El-Shafai et al., 2004; Marcon, *et.al.* 2004). The rainbow trout (*Oncorhynchus mykiss*) and mullet (*Mugil platanus*) were found to be highly sensitive to ammonia toxicity while the flathead minnow (*Pemiphales promelas)* and channel catfish (*Ictalurus puntatus*) are highly resistant to ammonia (EPA, 1998).

Nile tilapia (*Oreochromis niloticus*) is one of the fastest growing and hardiest species of fish in many aquaculture projects in the Philippines and many parts of the world. Effects of unionized ammonia toxicity on tilapia juvenile have been extensively studied with variable results. In their study on ammonia toxicity on *O. niloticus* fingerlings (Turkey) Karasu Benli and Koksal (2005) showed that the LC50 value between 7.40 ± 0.01 mg/L. El-Sherif and El-Feky (2008) also reported similar 48 hr-LC50 of 7.1 mg/l NH3- N for tilapia fingerlings (Egypt) while Daud *et al.(*1988) reported 48hr LC50 at 6.6 mg/l in hybrid red tilapia (*O. mossambicus* × *O. niloticus*). Information on median lethal concentration of ammonia on tilapia culture in local conditions, however, is not found in available literature.

This study was conducted in order to determine the mortality rate of tilapia fingerlings within 24 hours of exposure to different ammonia concentrations, to determine LC50 of ammonia using the Reed-Muench method, and to observe the behavioural changes of the tilapia fingerlings during the toxicity test. Results of this study will be useful in improving tilapia fingerling production through water quality monitoring and water management to maintain safe levels of ammonia in the culture environment.

METHODS

A. Test Animals

Nile tilapia fingerlings were obtained from the Mindanao State University College of Fisheries Hatchery (Fig. 1). A total of 120 fingerlings with an average weight of 0.28 g and lengths ranging from 11-29 mm were used as test animals in the ammonia toxicity experiments. Tilapia fingerlings were randomly distributed among 12 3L plastic jars at a stocking density of 10 fingerlings per jar.

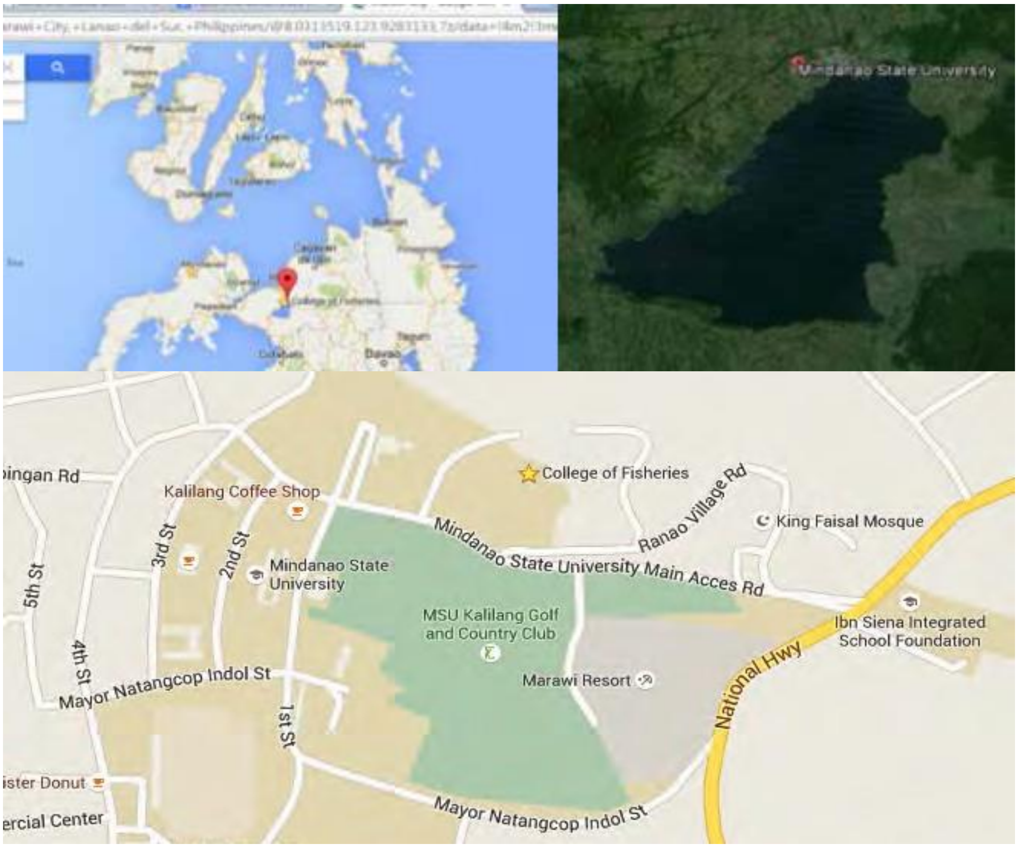

Figure 1. Location of College of Fisheries, MSU Marawi, near Lake Lanao in the ARMM region in Mindanao.

B. Test Chemical and Concentration

The toxicology experiment was made up of four treatments with three replicates each arranged in a completely randomized desgn (CRD). Four ammonia concentrations were used, namely, 25 ppm (Treatment A), 15 ppm (Treatment B), 10 ppm (Treatment C) and 0 ppm (Treatment D or Control). The ammonia was obtained from aqua ammonia or better known as ammonium hydroxide. Varying concentrations for the three treatments were obtained through dilution using of a prepared 0.2M stock solution with concentration equivalent to 3,408 ppm of ammonia.

Experimental Design, Measurements and Data Analysis

The fish behaviour was observed for 24 h at one hour intervals. Water temperature was measured with the use of a laboratory mercury thermometer. The fingerlings were given standard pellet diet throughout the experiment. Data were analyzed using One-Way Analysis of Variance (ANOVA) to determine any significant differences in toxicity effects on tilapia fingerlings among various ammonia concentrations.

RESULTS AND DISCUSSION

Lethal Concentrations of Ammonia

Calculated mean value of 24-h LC50 of ammonia on Nile tilapia fingerlings was 4.10 ppm while mean LC25 and LC75 were 2.03 ppm and is 8.31ppm, respectively. The LC50 is the median lethal concentration which indicates that 50% mortality in tilapia fingerlings can occur when exposed to 4.10 ppm concentration of ammonia for 24 hours. All test animals in Treatment A (20 ppm) died within the first hour of exposure, followed by Treatment B (15 ppm) wherein fifty percent (50%) of the test animals died after 2 hr. In Treament C (10 ppm), only one of the test animals survived after 4 h of exposure. No mortality was observed in the control group (Fig. 2). Analysis of Variance (ANOVA) showed that the different concentrations of ammonia had significantly different effects on mortality and survival of tilapia fingerlings (P value of $0.000 < 0.05$).

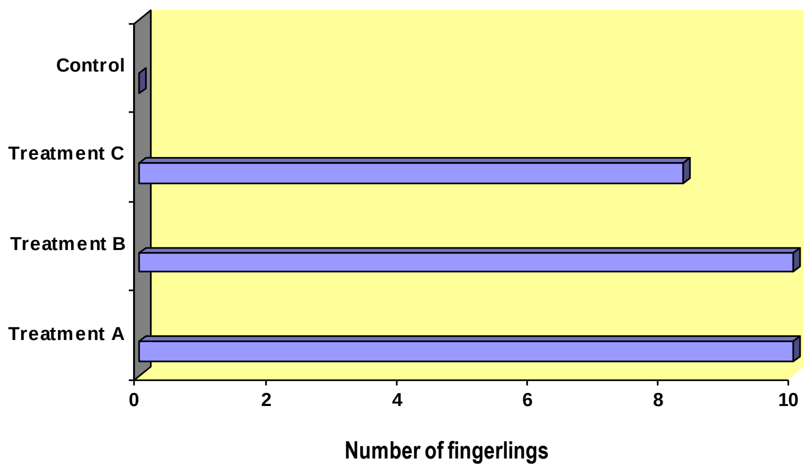

Figure 2. Average mortality in tilapia fingerlings subjected to different ammonia concentrations.

The effect of ammonia on the Nile tilapia fingerlings is fatal in this study, possibly due to its small size as compared to adults. Test animals with smaller size ranges are very sensitive to the intoxication of ammonia. Researchers have observed that the youngest fish (60-250 mg) are the ones which show the highest sensitivity to ammonia (Karasu Benli and Koksal, 2005). Results of the present study demonstrate that tilapia fingerlings are tolerant to ammonia at lower concentrations (i.e. below 4 ppm). It should be noted, however, that the mean LC50 value obtained in this study is lower than that obtained by other researchers (Daud et al., 1998; El-Sherif and El-Feky, 2008; Karasu Benli and Koksal, 2005).

Feeding rate, feed composition, fish metabolic rate, and the quantity of wasted feed affect the tank water quality of any culture experiment. Unconsumed feed will decompose within the system, resulting in further accumulation of ammonia-nitrogen and carbon dioxide in a culture tank, especially in an intensive system (Fallu, 1981; Marcon *et al.*, 2004). This reduces the oxygen content of the water which often results in detrimental impact on the health of the cultured organisms.

Ammonia build-up as by-product in a culture system is due to the excretion of total nitrogen ammonia (TAN) which consists of unionized ammonia (NH_3) and ionized ammonia (NH_4^+). Total nitrogen ammonia is excreted through the gills of fish as they assimilate feed and is also produced when bacteria decompose organic waste solids within the culture system. Fish when not immediately removed would become an ammonia contributor (Dening, 2007; Marcon et al., 2004; Karasu Benli and Koksal (2005). Ammonia, however, is volatile and it dissipates quite well in a tank or pond water which is highly aerated (e.g. flow through water system) or agitated, and if the water has high pH value (Dening, 2007).

The detrimental effect of ammonia can also be enhanced by the alkalinity of the solution such as ammonium hydroxide (NH_4OH) which when introduced to water increases pH value. Exposure of fish to alkaline conditions inhibits the rate of ammonia excretion, leading to ammonia accumulation and toxicity (Chew, *et al.*, 2002). An extremely high pH can damage developing juvenile fish, stripping it of its slime coat, oftentimes causing damage to outer surfaces like gills and eyes and eventually, death.

Behavior response of test animals

The first reactions of the fingerlings to ammonia were observed after 30 minutes of exposure to 20 ppm and 15 ppm ammonia, resulting in 50%-60% and 20% mortality, respectively. The fingerlings reacted to the presence of ammonia after 30 minutes of exposure, by swimming sideways, gasping for air at the water surface and rapid opening of the operculum and later died with their mouths open. Test animals of the control group remained at the bottom of the jar with normal movement of fins and operculum, and retained their normal skin coloration.

The skin and eye coloration of fingerlings exposed to ammonia became dark and there was a secretion of mucous on the body surface. On the other hand, in the case of the remaining test animals exposed to 10 ppm ammonia, the behavioral responses observed were minimal swimming activity, slow respiratory rate or opercular cover movement. After an initial color change, their skin coloration turned to normal (from dark to light), after several hours of adjustment.

Tilapia have physiological capabilities, which easily adapt themselves to different environmental conditions (Marcon, *et al.*, 2004). This cichlid species is very resistant to hypoxia and anoxia by depressing its metabolism, according to oxygen availability, to values below its standard metabolic rate. Under ammonia exposure of moderate concentration, this fish remains on the bottom of the aquarium for a long time, thus, reducing its ventilatory frequency and opercular movements. These behavioral strategies are similar to those found in fish under deep hypoxic and anoxic conditions, indicating that metabolic depression could be activated by fish to withstand acute ammonia toxicity.

IMPLICATIONS AND RECOMMENDATIONS

Tilapia is known to be a hardy species that can tolerate wide ranges of environmental conditions which makes it a popular species for aquaculture especially among poorer, developing nations. Results of this study, however, demonstrated that ammonia is acutely toxic on Nile tilapia juveniles, where high concentrations (10-20 ppm) can cause massive mortalities. This study also obtained a 24h LC5O of 4.10 ppm, lower than obtained by researchers in other countries. It is important to verify these values through confirmatory experiments, however, results point out the need for tilapia producers to be precautionary and implement regular monitoring of water ammonia levels and improved water maintenance practices.

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