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FULL LENGTH ARTICLE

Effect of prolonged ammonia toxicity on fertilized eggs, hatchability and size of newly hatched larvae of Nile tilapia, *Oreochromis niloticus*

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KEYWORDS

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Abstract The present study showed that a lethal dose of unionized ammonia (NH_3-N/l) ranged from 0.06 to 1.5 mg/l. LC50 value 96 h of unionized ammonia was recorded as 0.8 mg/l. The mortality rate reached 100% after exposure to 1.5 mg. Fertilization and hatching rates were more affected after exposure to high concentrations of ammonia (0.5–0.6 mg/l) ($p < 0.05$). The number of deformed eggs increased with increasing ammonia concentration. However, the lowest value of deformed eggs was less affected at low doses that ranged from 0.05 to 0.25 mg NH_3-N/l. Effects of high doses of ammonia (0.5–0.6 mg/l) were more pronounced on weights of larvae than those on lengths. There were no significant differences recorded in weight of larvae exposed to low doses of ammonia and those of control group ($p > 0.05$). The lowest value of survival rate was recorded after 60 days in groups of larvae that were exposed to high doses of unionized ammonia. Clinical signs such as malformation in yolk sac, spine curvature and darkening in eyes as well as skin were more pronounced after exposure to high doses of ammonia for a period of 60 days post-hatching.

Introduction

In natural surface water, ammonia occurs in two forms ionized ammonia NH_4^+ and unionized ammonia NH_3 (Paley et al., 1993). An important source of ammonia that enters the aquatic environments is agriculture run off (Brinkman, 2009). Ammonia is a well-known aquatic pollutant to fish. It is produced as an end product of nitrogenous metabolism (Evans and Pasnik, 2006). In addition, ammonia is more toxic to fishes at higher temperature and pH values. An increase in pH value causes a rise in the fraction of unionized ammonia and the water becomes toxic to fish. Water with a concentration less than 0.02 mg/l of ionized ammonia is safe for fish reproduction (EPA, 1999). Most fish species cannot tolerate high total ammonia concentration. An increased ammonia level in the environment either impairs ammonia excretion or causes a net uptake of ammonia from the environment (Randal and Tsui, 2002). Most lethal threshold concentrations have been

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reported in the range 0.2–0.4 mg/l NH_3-N (EIFAC, 1973). Furthermore, ammonia can act on fish nervous system and cause some symptoms such as hyperventilation, hyperexcitability and loss of equilibrium (Mckenzie et al., 2008). Little information is available on the effect of ammonia on yolk sea fry and larval stages and therefore further studies are needed. The purpose of the present study is to evaluate the effect of sub-lethal doses of ammonia NH_3-N on yolk sac fry and growth performance of larvae.

Materials and methods

Natural spawning of *Oreochromis niloticus* was carried out at hatchery of El-Serw Station Research for fishes. The experiment started on 4 June 2015 and lasted till the end of July. The total number of fish used during the experiment was 35 females and 15 males. The weight of females ranged from 130 to 150 g, however, the weight of males ranged from 150 to 170 g.

Preparation of broodstock fish for spawning

Both males and females were put in two separate cement ponds inside the green house hatchery at El-Serow fish farm. The fish were fed twice a day with an artificial food containing 25–30% proteins. The photoperiod was maintained on 18 L: 6 D cycle. In order to obtain the yolk sac fry at the appropriate time, males and females were mixed together for spawning. The fish spawned after two days. The fertilized eggs were collected using a fine meshwork. Some fertilized eggs were also collected from incubated females by passing a current of water either on mouth opening or gill arches. The total number of fertilized eggs used was 2500 and was put into a holding fiberglass aquarium, measuring 500 L capacity filled with dechlorinated tap water.

Experimental design

The experiment was carried out in six glass aquaria having a capacity of 50 L each with water depth of 24 cm. Another set of six glass aquaria were applied as a replicate. The chemical compound used was ammonium chloride (NH_4Cl). The concentration of this compound in each trial was evaluated and adjusted according to the sub-lethal dose used. Different parameters of water quality (pH, dissolved oxygen, temperature and hardiness) were measured using a modern remote sensory device "Automatic monitoring and control system" (AMCS) which was recently installed in hatchery of El-Serw fish farm. The concentration of sub-lethal dose of ammonia were evaluated in different preliminary trials and ranged from 0.05 to 0.60 mg/l. However the lethal concentration doses LC50 (96 h) were evaluated and ranged from 0.7 to 1.5 mg/l. The fertilized eggs were distributed in six glass aquaria (200 healthy fertilized eggs in each aquarium). The water in each aquarium was aerated using a small compressed air pump to maintain the oxygen concentration ranging from 6.5 to 6.9 mg/l and pH 7.6–8.0 throughout the period of experiment. The experimental doses of ammonia were applied as follows:

(1) The first glass aquarium was used as control without the addition of any treatment.
(2) The second glass aquarium, the sub-lethal dose used was 0.05 mg of NH_4Cl/l.
(3) The third glass aquarium, the sub-lethal dose used was 0.15 mg of NH_4Cl/l.
(4) The fourth glass aquarium, the sub-lethal dose used was 0.25 mg of NH_4Cl/l.
(5) The fifth glass aquarium, the sub-lethal dose used was 0.45 mg of NH_4Cl/l.
(6) The sixth glass aquarium, the sub-lethal dose used was 0.60 mg NH_4Cl/l.

We made the concentration of the ammonium stable throughout the trial by renewing half of the amount of water of the aquaria with fresh water containing the corresponding required ammonium concentration every three days.

At the beginning of the experiment, the deformed eggs and white floating eggs were removed and counted. This process continued daily till the end of the experiment (60 days post-hatching). The newly hatched fry weighed 0.05 mg and each was about 6.0 mm in length. The yolk sac was absorbed after 7 days after hatching, so the external food was supplied. This feed contained 35–40% protein. The frequency of feeding ranged from 4 to 5 times a day. In order to examine the condition of yolk sac fry in each treatment, the fertilized eggs were fixed in Serous fluid (600 ml of 95% alcohol + 300 ml of formaldehyde + 100 ml of acetic acid) according to methods of Szezerbik et al. (2008).

The fertilization rate was determined in each treatment according to the following equation:

$$\text{Fertilization rate} = \frac{\text{No. of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

The newly hatched fry rate was determined in each treatment according to the equation:

$$\text{Hatching rate} = \frac{\text{No. of hatching eggs}}{\text{No. of fertilized eggs}} \times 100$$

Samples collection

The samples of fish were collected after 10, 20, 50 and 60 days post-hatching. Lengths of larvae to the nearest "cm" and weights to the nearest "g" in each treatment were recorded. The condition factor in each treatment was evaluated using the equation:

$$\text{Condition factor} = \frac{W}{L^3} \times 100$$

where "W" is weight of fish and "L" indicates the total length of fish.

The survival rate was also calculated throughout the period of the experiment and compared to the control group. The following equation was applied.

$$\text{Survival rate} = \frac{\text{No. of survived fish}}{\text{Total number of fish}} \times 100$$

Histological observations

For each treatment, 5 newly hatched fry were collected and then fixed either in Bouin's solution or 10% neutral puffer formalin for a certain period according to the size of fry. Ovary