Acute Toxicity of NH_4Cl to Tambra Fish (*Tor Tambroides*) Fry

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Abstract

Ammonia is one of the nutrient wastes products which can be toxic to all vertebrates, including fish. Usually, ammonia toxicity is expressed as Total Ammonia Nitrogen (TAN, mgN/L). Tambra fish (*Tor tambroides*) is freshwater fish with important economic value in some areas in Indonesia. According to overfishing and forest degradation, the fish has become threatened. This research aimed to calculate the LC_{50} value of ammonia to Tambra fish (*Tor tambroides*) and estimate NOEC, LOEC, and MATC values of ammonia to Tambra fish (*Tor tambroides*). This study was conducted using the static test method with two steps. The first stage is a preliminary test, where the preliminary test is observed for 24 hours With five concentrations excluding control. Whereas in the second stage that is carried out, observation for 96 hours with four concentrations excluding control. The Probit test is Performed to obtain LC_{50} value results, then proceed with ammonia analysis using the Salicylate Test Kit method. The results showed that ammonia had acute toxicity with LC_{50}-96 hour value of NH_4Cl against Tambra fish fry (*Tor tambroides*) in the amount of 354.615 mg/L. Ammonia caused acute toxicity to Tambra fish fry (*Tor tambroides*).The LC_{50}-96 hour value was 354.615 mg/L in the form of Total Ammonia Nitrogen (TAN) or it was equivalent to un-ionized Ammonia (NH_3) of 9.8937 mg/L, while values of the Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) of ammonia on Tambra fish (*Tor tambroides*) were 578.24 mg/L and 280.18 mg/L, respectively.

Keywords: Ammonia, LC_{50}-96-hour, Tambra fish, toxicity

INTRODUCTION

Ammonia in the aquatic environment primarily comes from plants and animals excretion, a decomposition product of organisms and sewage, agricultural run-off, industrial wastes, and volcanic activity. Increasing ammonia concentration in freshwater can be toxic for aquatic organisms. Ammonia toxicity usually is expressed as total ammonia (TAN) in the aquatic environment (Randall & Tsui, 2002). The total ammonium is the total sum of ammonium (NH_4^+) and ammonia (NH_3). The latter is considered a product of body protein and nucleic acid biochemical degradation (Mardonesa *et al*. 2019). Increasing ammonia concentration in freshwater is harmful to aquatic organisms, such as fish. The toxic effect of ammonia on fish includes an imbalance of ionic regulation in blood plasma (Eddy, 2005), increased ventilation rates and gill epithelium damage, reduction of blood-carrying capacity (Russo, 1985), depletion of adenosine triphosphate

(ATP) in the brain depletion of adenosine triphosphate (ATP) in the brain (Camargo & Alonso, 2006), and the disruption of osmoregulatory and circulatory activity disrupting normal metabolic functioning of the liver and kidneys and the interruption of osmoregulatory and circulatory activity disrupting normal metabolic functioning of the liver and kidneys (Arillo *et al*., 1981; Tomasso *et al*., 1980).

Tambra (*Tor tambroides*), one of the mahseer fishes (Tor spp), is well-known as a large-bodied fish of Cyprinidae family. There are 20 species of this genus identified in the world. 4 live in Indonesian freshwater (Haryono & Tjakrawidjaja, 2006). Individual fish can reach over 50 kg, which has become popular as premier sport fishing (Pinder *et al*., 2019). Despite the high nutritional value and their ability to provide food security, Tambra also has high religious and cultural significance in several regions in Indonesia. For example, for Batak people in North Sumatra, the fish is used as a substitute for Batak fish (*Neolissochilus thieenmanni*) in many cultural ceremonies.

The fish have suffered population declines due to anthropogenic activities, such as pollution, habitat loss caused by deforestation and agricultural development, hydropower dan construction, and over-fishing. This species is also listed as endangered species by the International Union for Conservation of Nature (IUCN) (Pinder *et al*. 2019). Therefore, the conservation and protection of this species from harmful compounds such as ammonia is vital. To support the effort of Tambra fish conservation through its domestication, this research aimed to calculate the LC_{50} value of ammonia to Tambra fish (*Tor tambroides*) and estimate NOEC, LOEC, and MATC values of ammonia to Tambra fish (*Tor tambroides*). The novelty of this study is providing information of ammonia toxicity to the Tambra fish (*Tor tambroides*). Finally, we expect this study can contribute significantly to aquatic toxicity research.

METHODS

Time and Location

This research was conducted at the Aquatic Laboratory facility of Research Center for Limnology, Indonesian Institute of Sciences (LIPI) from July – September 2019.

Fish Handling

Tambra fish (*Tor tambroides*) fry, used for toxicity test was provided from Freshwater Research Unit, Ministry of Marine Affairs and Fisheries Republic of Indonesia. Eight hundred fish fry aged approximately one month with an average weight of 0.72 grams were acclimatized for two weeks before the test was conducted. During acclimatization and experiment, the fry was fed with a commercial fish diet. Fish were fed in the morning, and water quality and mortality were recorded during the acclimatization period in the afternoon. During the acclimatization period, the fish were put into a reservoir filled with water and aerated. The fish were acclimatized for seven days and fed. The purpose of this acclimatization is to adapt the fish to the experimental condition. A day before the toxicity test was conducted, fish stopped feeding.

Chemicals

To test for toxicity, we used anhydrous Ammonium Chloride (NH_4Cl, HIMEDIA) to make working ammonia concentrations during the toxicity test. Ammonium Chloride was diluted by distilled water for making an Ammonium stock solution (10.000 ppm). Agriculture lime ($CaCO_3$) was used for controlling water pH around seven during the toxicity test.

Toxicity Test

An acute toxicity test to determine the lethal concentration (LC_{50}) of ammonia to Tambra fish was conducted according to USEPA (2002)

with some modifications. The assay consisted of two steps, a range finding, and a definitive test. A series of ammonia concentrations were used in the range-finding test to determine Tambra fish's short-term lethal ammonia toxicity. 18 glass aquariums, volume 30 liter, filled with aged, aerated tap water as the diluent of ammonia. The test was ended in 48 hours, and the dead fish were recorded at the end of the test. Ammonia concentration during the toxicity test was measured as Total Ammonia Nitrogen (TAN). Total Ammonia Nitrogen (TAN) series in range-finding test were 0 mg/L, 25 mg/L, 5 mg/L, 50 mg/L, 100 mg/L, 400 mg/L and 800 mg/L, with 3 replications. Fish mortality records from the range-finding test were used to determine tentative LC_{50}. Tentative LC_{50} result from the range-finding test then was used to determine ammonia concentrations in the Acute definitive test.

Eight fish fry were used in each aquarium on the range-finding test, and fish mortality was recorded every 24 hours for 48 hours. Dead fish was removed immediately during observation. Fish mortality data from the range-finding test was calculated to determine the ammonia concentration sequence used in the definitive test.

Based on range-finding test result, TAN concentration sequence in definitive test were set up, 5 mg/L, 16 mg/L, 27 mg/L, 38 mg/L dan 50 mg/L, 3 replicates. Twenty fish in each aquarium were exposed in the toxicity test. Fish dead were recorded and removed every 24 hours. The definitive test was conducted for 96 hours (4 days). At the end of the test, fish mortality data were then used to calculate LC_{50}-96h. An acute toxicity test was conducted on static conditions without water renewal. Fish was not fed during the test. The test was valid when fish mortality in control was less than 10% of exposed fish.

Water Quality Parameters

Physico-chemical parameters measured during the toxicity test were temperature, pH, and Dissolved Oxygen (DO). Measurement was conducted using a portable water quality checker (WQC) HORIBA® U50. Physico-chemical parameters were measured every day at 10:00 am.

Analysis

Probit analysis was used to calculate LC_{50}-96h from fish mortality data. Probit analysis was established using software Simply Probit version 1.2 (PISCES Conservation Ltd, 2005).

No observed adverse effect concentration (NOAEC) and the lowest adverse effect concentration (LOAEC) were determined through one-way ANOVA followed by Tukey analysis in excel statistical package. The geometric mean of the NOAEC and the LOAEC was then calculated to provide Maximum Acceptable Toxicant Concentration (MATC). MATC is a value calculated through acute toxicity tests to help set water quality regulations to protect aquatic life (Rand & Gary, 1995).

RESULTS AND DISCUSSION

Acclimatization

Acclimatization aims to condition the Tambra fish (*Tor tambroides*) in the prepared aquarium so that the Tambra (*Tor tambroides*) fry can adapt to the new environment. Acclimatization lasted for seven days. During the acclimatization period, pH value and temperature were ranged between 6.8 – 7.5 and 26 - 27°C, respectively.

Based on Lesmana & Dermawan (2001), the optimum pH and temperature for Tambra fish fry were 7-7.5 and at a temperature of 25-27°C, respectively. Thus, acclimatization took place at the optimal pH and temperature for the growth of Tambra fish fry.

During the acclimatization, fish were fed by a commercial fish diet in the morning and the afternoon. Water quality and mortality were recorded during the acclimatization period. The mortality rate during the acclimatization period was 4%. This rate was below 10%, which was allowed during this period (APHA, 2000). A

day before the toxicity test was conducted, fish stopped from feeding (USEPA, 2002).

Range Finding Test

Tambra fish fry range-finding test showed that mortality was observed on 100 mg/L, 400 mg/L, and 800 mg/L of ammonia concentrations.

The relationship between NH_4Cl concentration and the number of Tambra fish deaths in this preliminary test for 48 hours can be seen Figure 1.

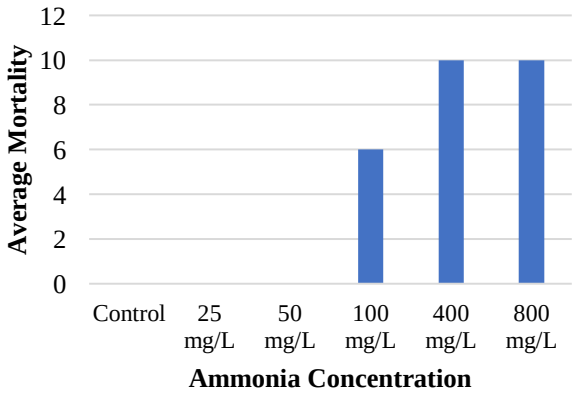

Figure 1. Range Finding Test Results

Based on Figure 1, it can be seen that the average fish mortality at concentrations of 0, 25, and 50 mg/L was zero, while at 100 mg/L, mortality was six fish. And the highest mortality occurred at concentrations of 400 and 800 mg/L, where all test animals were dead. However, in control, all of the test animals were alive. Based on the range-finding result, ammonia concentrations in the definitive test were determined. The concentrations were 67.81 mg/L, 107.69 mg/L, 280.18 mg/L and 578.34 mg/L, respectively.

Definitive Test

In the definitive test, observations were made for 96 hours to see fish mortality, and temperature and pH were measured. So that the LC_{50} value can be calculated, the graph of the relationship between NH_4Cl concentration and mortality of Tambra fish can be seen in Figure 2

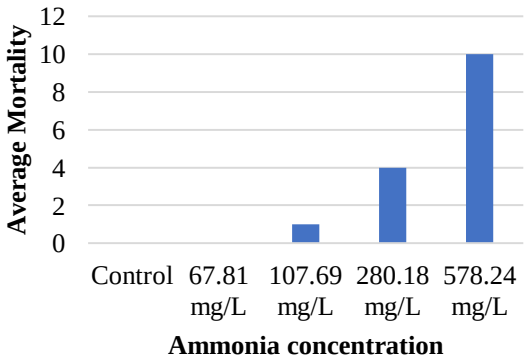

Figure 2. Definitive Test Result

Based on Figure 2, it can be seen that the average mortality of the test animals at the control and 67.81 mg/L were zero. At a concentration of 107.69 mg/L, there was the death of 1 test animal. At a concentration of 280.18 mg/L, 4 test animals died, and at a concentration of 578.24 mg/L, all test animals died. LC_{50} 96h from Probit analysis result for Tambra fish fry was 354.615 mg/L TAN. with lower confidence, and upper confidence limit was 228.650 mg/L and 549.975 mg/L, respectively. Un-ionized ammonia (NH_3-N) is considered as the most toxic fraction of TAN (USEPA, 1998). According to temperature and pH measurement during the acute definitive test, the TAN value was equal to 9.89 mg/L NH_3-N with a lower and upper confidence limit was 6.38 mg/L NH_3-N and 15.34 mg/L NH_3-N, respectively.

According to Rumampuk *et al.* (2010) and Yulaipi & Aunorohim (2013), the longer the exposure time of a toxicant to test animals followed by increasing concentrations during exposure will cause mortality on test animals. This is because the mortality of the test organisms during toxicity test is due to the damage of their respiratory organ (Robert, 1978).

Compared to some freshwater fish such as *Catla catla*, *Labeo bata, Cyprinus carpio,*

Oreochromis mossambica, Rasbora maninjau and *Gobiopterus brachypterus*, Tambra fish fry is more tolerant to TAN, because its LC_{50}-96h is higher than the LC_{50}-96h of those fish. Comparison of LC_{50}-96h from several freshwater fish is presented in Table 1.

Table 1. LC_{50} of Ammonia to several freshwater fish

No	Fish	LC_{50} ammonia	Source
1	*Catla catla*	15 mg/L TAN	Bhakta (2006)
2	*Labeo bata*	10 mg/L TAN	Bhakta (2006)
3	*Cyprinus carpio*	25 mg/L TAN.	Bhakta (2006)
5	*Oreochromis mossambica*	30 mg/L TAN	Bhakta (2006)
6	Elver (*Anguilla bicolor*)	467,4 mg/L TAN	(Yoga & Nursodiyanti, 2018)
7	*Rasbora maninjau*	29.9 mg/L TAN	(Yoga & Samir, 2020a)
8	*Gobiopterus brachypterus*	11.62 mg/L TAN	(Yoga & Samir, 2020b)

In determining the toxicity, data from toxicology test results are used to determine the concentration limit without effect called NOAEC (No Observed Adverse Effect Concentration). NOAEC is the highest concentration of the test substance that does not cause significant harmful effects on the test animals compared to the control in the toxicity test. LOAEC (The Lowest Observed Adverse Effect Concentration) is the lowest concentration of the test materialwhich has a significant effect on the test animals compared to the control in the toxicity test (Rand, 2008). The NOAEC and LOAEC values of Tambra fish for TAN are 280.18 mg/L and 578.24 mg/L, respectively. According to those values, the MATC of ammonia for Tambra fish is 402.51 mg/L.

Water quality during the toxicity test was still in the range of water quality requirements for Tambra fish (Lesmana & Dermawan, 2001). Water temperature, pH, and dissolved oxygen in the toxicity test tank are described in Table 2.

Table 2. Water Quality measurement result during Toxicity Tests

Parameter	Unit	Value
Temperature	°C	26.4 (25.3 – 27.5)
pH	-	7.6 (7.4 – 8.4)
Dissolved Oxygen	mg/L	4.96 (4.30 – 5.82)

CONCLUSION

Ammonia caused acute toxicity to Tambra fish fry (*Tor tambroides*). The LC_{50}-96 hour value was 354.615 mg/L in the form of Total Ammonia Nitrogen (TAN) or it was equivalent to un-ionized Ammonia (NH_3) of 9.8937 mg/L, while values of the Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) of ammonia on Tambra fish (*Tor tambroides*) were 578.24 mg/L and 280.18 mg/L, respectively. According to those values, the MATC of ammonia for Tambra fish is 402.51 mg/L.

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