Ammonia toxicity to endemic fish (*Rasbora maninjau*) of Lake Maninjau as a reference for water quality guidelines

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Abstract. Unionized ammonia (NH$_3$) is highly toxic to freshwater organisms. Yet, most of the available toxicity data on NH$_3$ were predominantly generated from temperate regions, while toxicity data on NH$_3$ derived from tropical species were limited. Bada (*Rasbora maninjau*) as an endemic fish in Lake Maninjau-West Sumatra, has an important economic value for local communities. They live in clearwater with sand to gravel lake/river beds. The catch of *R. maninjau* is decreasing along with the decline of the water quality. Nowadays, the water quality of Lake Maninjau is declining as the effect of organic wastes from domestic sources and floating cage aquaculture. Therefore, the information about toxicity of ammonia in fish is important as a management tool for fish conservation. This research was aimed to find the LC$_{50}$ value of ammonia in *R. maninjau*. A static acute toxicity test was conducted for 96 hours. Mortality was recorded for calculating LC$_{50}$-96h, No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). Values of LC$_{50}$-96h, NOEC and LOEC were 29.92 mg/L TAN or 0.7 mg/L NH$_3$, 27 mg/L TAN or 0.63 mg/L NH$_3$, and 38 mg/L TAN or 0.89 mg/L NH$_3$, respectively. Maximum Acceptable Toxicant Concentration (MATC) of ammonia to Bada was 32 mg/l TAN or 0.59 mg/l NH$_3$. The acute toxicity result showed that ammonia concentration affects the mortality of *R manijau* for 96 hours. The information about ammonia toxicity data will be used as a water quality guideline in Lake Maninjau.

1. Introduction
Lake Maninjau is a tectono-volcanic lake located in West Sumatra. The lake was a well-known tourist destination in the past. However, since floating fish cage was introduced in 1992 and then expanded in 1995 [1] the water quality of the lake tends to be degraded, because of nutrient load, which comes from excess fish food and fish wastes. The contribution of nutrients from the aquaculture has been counted around 400 tons/year, and about ninety-five percent of that were nitrogen and phosphorus [2].

In aquatic ecosystem, ammonia is considered one of the most toxic pollutants. It can be produced from natural decomposition of organic matter or excreted by aquatic organisms as a nitrogenous waste product. Anthropogenic activities also produce a lot of nitrogenous wastes which significantly deteriorate the water quality condition of freshwater. According to [3,4], enormous amounts of ammonia are produced by fertilizer production and usage, and animal husbandry, therefore ammonia can enter the aquatic environment through many sources such as sewage, animal and industrial wastes, and bacterial activity [5].
Elevated ammonia concentration in freshwater can cause toxic effects on aquatic organisms. Ammonia concentration in blood plasma of most of the freshwater fish is about 0.15–0.3 mmol L\(^{-1}\), elevation of its concentration in the body can cause an imbalance of ionic regulation [5]; proliferation in gill tissues, increased ventilation rates and damage to the gill epithelium; reduction in blood oxygen-carrying capacity due to progressive acidosis [6]; uncoupling oxidative phosphorylation causing inhibition of production and depletion of adenosine triphosphate (ATP) in the brain [7]; and the disruption of osmoregulatory and circulatory activity disrupting normal metabolic functioning of the liver and kidneys [8,9].

Ammonia toxicity, which usually expresses as total ammonia (TAN), in the aquatic environment increases with pH of the water [10]. 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 [11].

Bada (Rasbora spp.) belongs to the Cypriniformes order, Cyprinids family, and Rasbora genus. At least, four species of Rasbora can be found in Lake Maninjau. There are three species of Rasbora found in Lake Maninjau in 2017 which were \(R.\ cf.\ sumatrana\), \(R.\ lateristriata\), and \(R.\ argyrotaienia\). Moreover [12], \(R.\ maninjau\) as a new species of Rasbora in Lake Maninjau was found in 2014 [13]. This species also being an endemic species in Lake Maninjau. In spite of those, [14] recorded other species of Rasbora in their past observation, there were: \(R.\ daniconius\) and \(R.\ jacobsoni\).

Generally, Bada lives in shallow and clean water with rock bottom. Sulastri, et al. [15] said that Bada’s habitat is in the littoral zone with the rocky substrate. Djamanto, et al.[16] added that \(R.\ lateristriata\) usually spawns in running water with gravel or sandy bottom to ensure sufficient oxygen availability.

Single-species toxicity tests of single toxicant have been used to detect and assess the potential toxicological effects of chemicals on aquatic organisms. Data which generates from laboratory, single-chemical and single-species toxicity tests have been used to derived water quality guidelines in USA and Australia – New Zealand.

As part of National priority Research on Lake Restoration Technology to support aquaculture and tourism in Lake Maninjau, an effort to conserve Bada fish is through domestication. To support the domestication effort, therefore, this research was aimed to calculate LC\(_{50}\) value of ammonia to Bada fish (\(Rasbora maninjau\)), and to estimate NOEC, LOEC and MATC values of ammonia to Bada fish (\(Rasbora maninjau\)). Those values can be used for calculation of reference to water quality guidelines for ammonia.

2. Methods and Materials
2.1. Time and Location
Acute toxicity test was conducted in Unit for Technology Transfer of Lake Restorations Indonesian Institute of Sciences (LATPD LIPI) at Lubuk Basung, West Sumatra, Indonesia on March, 19th - 28th 2019.

2.2. Fish Handling
Bada fish used for toxicity test was provided from aquaculture facilities in LATPD LIPI. 800 fish were acclimatized for 2 weeks before the test was conducted. During acclimatization and experimentation, larvae were fed with a commercial fish diet. Fish were fed in the morning and in the afternoon, water quality and mortality were recorded during the acclimatization period. A day before the toxicity test conducted, fish stopped from feeding.

2.3. Chemicals
Ammonium Chloride (NH\(_4\)Cl, HIMEDIA) was used as a source of ammonia during toxicity test. Ammonium Chloride was diluted by aquadest for making an Ammonium stock solution (10,000 ppm). Calcium carbonate (CaCO\(_3\)) was used for controlling water pH around 7 during toxicity test.
2.4. Bioassay

Acute toxicity test to determine the lethal concentration (LC$_{50}$) of ammonia to Bada fish was run according to USEPA [17]. The assay was conducted in two steps, which were range-finding test and definitive test. In range-finding test, a series of ammonia concentrations were used to determine the short term lethal ammonia toxicity to Bada fish. 18 glass aquarium, volume 30 liter, filled with aged, aerated tap water as the diluent of ammonia. The test was conducted for 48 hours, and the dead fish were recorded at the end of the test. Ammonia concentration series in range-finding test were 0 mg/l, 0.5 mg/l, 5 mg/l, 50 mg/l and 500 mg/l, duplo. Fish mortality from the range-finding test was used to determine tentative LC$_{50}$. Tentative LC$_{50}$ result from a range-finding test was used to determine ammonia concentrations in the Acute definitive test.

On range-finding test, 8 fish were used in each aquarium and fish mortality was recorded every 24 hours for 48 hours. Dead fish were 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, ammonia concentration sequence in definitive test were 5 mg/l, 16 mg/l, 27 mg/l, 38 mg/l and 50 mg/l, 3 replicates. 20 fish in each aquarium was exposed to those ammonia concentrations. Dead Fish were recorded and removed every 24 hours. Fish mortality data then were used to calculate LC$_{50}$ 96h. Acute toxicity test was conducted on static conditions, without water renewal. Fish food was not allowed during the test. The test was valid when fish mortality in control is less than 10% of the amount of exposed fish.

2.5. 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) was calculated through one way ANOVA followed by Tukey analysis in Excel. NOAEC and LOAEC were used to calculate the Maximum Acceptable Toxicant Concentration (MATC). MATC is a value that is calculated through acute toxicity tests to help set water quality regulations for the protection of aquatic life. The MATC is reported as the geometric mean between the NOAEC and LOAEC [18].

2.6. 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 o’clock.

| Parameter           | Unit   | Measurement tool       |
|---------------------|--------|------------------------|
| Temperature         | °C     | Water Quality Checker  |
| pH                  | -      | Water Quality Checker  |
| Dissolved Oxygen    | mg/l   | Water Quality Checker  |

3. Results

The range-finding test result showed that ammonia concentration of 50 mg/l and 500 mg/l were very toxic to Bada. Those concentration killed all the fish in the test chambers since 24 hours exposure. (table 2) shows the mortality of Bada during range-finding test.
Table 2. Average Mortality of Bada (Rasbora maninjau) during Range-Finding Test

| TAN Concentration (mg/l) | Mortality (fish) |
|-------------------------|------------------|
|                         | 24 hours | 48 hours |
| 0                       | 1        | 1        |
| 0.5                     | 1        | 1        |
| 5                       | 1        | 1        |
| 50                      | 8        | 8        |
| 500                     | 8        | 8        |

The result of the range-finding test indicated that critical fish mortality occurred between 5 – 50 mg/l. Those concentrations then were considered to be the lowest and the highest concentration in the definitive test. The definitive acute toxicity test showed that LC50 96h of Bada for Total Ammonia Nitrogen was 29.92 mg/l with a lower confidence limit and the upper confidence limits were 27.13 mg/l and 32.84 mg/l, respectively. According to temperature and pH measurement during acute definitive test, that TAN value was equal to 0.7 mg/ NH3-N with lower confidence limit and upper confidence limit were 0.63 mg/l NH3-N and 0.77 mg/l NH3-N.

Table 3. Mortality of Bada during Definitive Toxicity Test 96 hours.

| TAN Concentration (mg/l) | Mortality (fish) |
|-------------------------|------------------|
|                         | 24 hours | 48 hours | 72 hours | 96 hours |
| 0                       | 1        | 1        | 1        | 1        |
| 5                       | 1        | 1        | 1        | 1        |
| 16                      | 1        | 1        | 1        | 1        |
| 27                      | 3        | 3        | 3        | 3        |
| 38                      | 12       | 12       | 12       | 12       |
| 50                      | 20       | 20       | 20       | 20       |

According to Bada mortality in definitive acute toxicity test, NOAEC and LOAEC values were 27 mg/l TAN (0.63 mg/l NH3-N) and 38 mg/l TAN (0.89 mg/l NH3-N), respectively (table 4). The MATC of ammonia to Bada was 32 mg/l TAN (0.59 mg/l NH3-N).

Table 4. One way ANOVA Result of Bada Mortality during Definitive Toxicity Test 96 hours.

| TAN Concentration (mg/l) | Mortality rate | annotation |
|-------------------------|----------------|------------|
| 0 (mg/l)                | 0.667          | a          |
| 5 mg/l                  | 0.667          | a          |
**Table 5.** Physico-Chemical Measurement during Toxicity Tests

| Parameter       | Unit  | Value              |
|-----------------|-------|--------------------|
| Temperature     | °C    | 26.29 (24.2 – 27.9) |
| pH              | -     | 7.65 (7.41 – 8.26)  |
| Dissolved Oxygen| mg/L  | 4.98 (4.31 – 5.82)  |

4. Discussion

Bada (*Rasbora maninjau*) is not very sensitive to total ammonia nitrogen (TAN) pollution. LC$_{50}$ 96h of Total Ammonia Nitrogen to Bada (*Rasbora maninjau*) was 29.92 mg/l. Compared to some freshwater fish such as *Catla catla*, *Labeo bata*, and *Cyprinus carpio*, the LC$_{50}$ 96h value of Bada is higher. However, that value was lower than LC$_{50}$ to elver and *Oreochromis mossambica* (table 6).

**Table 6.** LC$_{50}$ of Ammonia to Several Freshwater Fish

| No | Fish                  | LC$_{50}$ ammonia          | Source |
|----|-----------------------|-----------------------------|--------|
| 1  | Elver (*Anguilla bicolor*) | 467.4 mg/L TAN ; 0.466 mg/L NH$_3$-N | [19]   |
| 2  | *Catla catla*         | 15 mg/L TAN                  | [4]    |
| 3  | *Labeo bata*          | 10 mg/L TAN                  | [4]    |
| 5  | *Cyprinus carpio*     | 25 mg/L TAN                  | [4]    |
| 6  | *Oreochromis mossambica* | 30 mg/L TAN                  | [4]    |

Un-ionized ammonia (NH$_3$-N) is considered as the most toxic fraction of TAN [20]. LC$_{50}$ 96 h of NH$_3$-N for Bada (*Rasbora maninjau*), 0.7 mg/ NH$_3$-N, is comparable to *Oncorhynchus mykiss* of 0.291 mg/l NH$_3$-N [21], *Salmo trutta* was of 0.592 mg/l NH$_3$-N, *Prosopium williamsoni* was of 0.358 mg/l NH$_3$-N [22], and *Ictalurus punctatus* that was of 0.50 mg/l NH$_3$-N [11], which were originated from temperate region.

Compared to some ammonia quality standards, Bada is tolerable to un-ionized ammonia, because the MATC value, 0.59 mg/l NH$_3$-N, is higher than ammonia concentration for water quality criteria in Indonesia, ≤0.02 mg/l NH$_3$-N, Canada, 0.019 mg/l NH$_3$-N, USEPA, 0.02 mg/L NH$_3$-N. According to [23], MATC is useful for setting regulatory guidelines for the protection of aquatic life through water quality criteria in which only acute toxicity data exists.

Generally, physicochemical measurements during the test are fit to the range of Rasbora’s natural habitat. *Rasbora* spp. in Banjaram River (*R. aprotaenia, R. lateristriata, and R. argyrotaenia*) live in temperature between 23 to 30 degrees of Celsius [24]. Furthermore, *R. tawarensis* in Lake Laut Tawar live in neutral pH ranged from 7.74 to 7.83. While the measured DO is lower than in the natural habitat, 6.36 mg/l on the average, it did not significantly affect the result of this experiment which has
been seen by a result in the control (untreated) chamber.

5. Conclusions

- According to the result of the acute toxicity test to ammonia, LC$_{50}$ 96h of Bada (Rasbora maninjau) was 29.9 mg/L TAN (0.7 mg/L NH$_3$-N).
- Maximum Acceptable Toxicant Concentration (MATC) of ammonia to Rasbora maninjau was 32 mg/L TAN (0.59 mg/L NH$_3$-N)
- Bada (Rasbora maninjau) is relatively not sensitive to ammonia compared to freshwater fish from the temperate region.

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