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ACUTE TOXICITY OF AMMONIA AND NITRITE TO ANGEL FISH (*Pterophyllum scalare, Liechtenstein 1823*) AND THE EFFECT OF ERYTHROCYTE MORPHOLOGY

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ABSTRACT

Angelfish (*Pterophyllum scalare*, 1.54±0.03 g, n=500) were exposed for 24, 48, 72 and 96 hours to different concentrations NH_3-N (0.2-2 mg/L), and NO_2-N (1-35 mg/L) at pH 7.2±0.3. Using Probit analysis, 50% lethal concentrations (LC_{50}) and 95% confidence limits were calculated. The effects of ammonia (0.7 mg NH_3-N/L and 10 mg NO_2-N/L) nitrite to the red blood cells size were also investigated in this study. As a result of the study, 24, 48, 72 and 96 hours LC_{50} values of ammonia nitrogen (NH_3-N) for angelfish were determined as 0.99, 0.75, 0.65 and 0.58 mg/L; and nitrite nitrogen (NO_2-N) values were calculated as 29.38, 12.30, 7.98 and 6.28 mg/L. respectively. In the examination of blood smear, red blood cells were determined oval shaped (erythrocytes and its nuclei size), and their sizes were measured and evaluated as long and short axes. It was also determined that, exposed to ammonia and nitrite, erythrocyte morphology had changed that was statistically different from each other ($P<0.05$).

KEYWORDS:
Ammonia, Erythrocyte, Angelfish, Acute Toxicity Test, LC_{50}, *Pterophyllum scalare*

INTRODUCTION

Angelfish, (*Pterophyllum scalare*), is native to the Amazon basin, and distributed worldwide as aquarium fish [1]. This fish is one of the few fish species found in pet stores that originate from genuinely wild and captive stocks [2]. Angelfish prefers soft and low pH (6.5), and 27-30°C water temperatures [3]. The animals exposed to elevated concentrations of nitrogenous wastes increases as a result of high density aquaculture, particularly ammonia and nitrite [4-5].

In aquatic systems, ammonium may exist in both the un-ionized (NH_3 toxic) and the ionized (NH_4^+-non toxic) form. The toxicity of ammonium to aquatic organisms has primarily been linked to the un-ionized form [4-5-6-7]. The equilibrium between the two forms of ammonium is controlled primarily by pH and temperature [8]. If pH increases by one unit, the amount of toxic un-ionized ammonia increases about 10 times [9]. The average of the mean acute toxicity values for 32 freshwater species is 2.79 mg NH_3/L [10]. Ammonia is converted to nitrite, its intermediate oxidative product, which is still toxic to aquatic animals [11]. Elevated nitrite concentrations cause great problems in intensive culture of commercial fish species and ornamental fish [12-13].

Nitrite is an intermediate product in the oxidation of ammonium to nitrate. Nitrite is a well-known toxicant for fish. It is a disrupter of multiple physiological functions including ion regulatory, respiratory, cardiovascular, endocrine and excretory processes as well [14].

There are numerous studies on toxicity effects of ammonia and nitrite on a variety of fish [4-5-6-7-12-15-16-17-18], but not on angelfish. Vedel et al. [19] studied isolated and combined exposure to ammonia and nitrite in rainbow trout (*O. mykiss*).

The purpose of this study was to determine acute toxicity levels (LC_{50}) of un-ionized ammonia nitrogen and nitrite nitrogen in Angelfish, *P. scalare*. Furthermore, red blood cell (erythrocyte) sizes were examined.

MATERIAL AND METHOD

This study was conducted by RTEU Fisheries Faculty Aquarium Unit. Angelfish (*P. scalare*) of a standard length of 3.2±0.03 cm (2.2 - 4.1 cm) and average weight 1.54±0.03 g (0.83 - 2.55 g n=500) was used. They were produced at the aquarium unit of the institute from healthy fish (3-4 months old).

Glass tanks of 25 L capacity each were used with 15 L water volume. Tap water stored for at least 48 hours. Water temperature was 24.5±0.5°C; pH 7.2±0.2; dissolved oxygen 7.1±0.4 mg/L; nitrate <0.5 mg/L, nitrite, ammonia, carbon dioxide 0 mg/L, hardness 40±2.0 mg/L (as $CaCO_3$), alkalinity

23.2±1.8 mg/L (as $CaCO_3$), total chloride 10 mg Cl_2^-/L. No pH adjustment was applied.

Angelfish were exposed for 24, 48, 72 and 96 hours to different concentration of NH_3-N (0.2-2 mg/L), and NO_2-N (1-35 mg/L). The desired concentrations were obtained from stock solutions made with reagent grade sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) at pH 7.2. Using probit analysis, 50% lethal concentrations (LC_{50}) and 95% confidence limits were calculated individually for each experiment. The experiments were run separately with different groups of fish from the same acclimation glass aquaria, with both experiments having controls where no toxicant was added. The test concentrations and the controls were all conducted with ten fish (all treatments were conducted triplicate).

Toxicity trials were run in semi static system as mentioned in the publication OECD [20], where water and the toxicant were %50 replaced every day. Glass aquaria volume was 15 L, stocking density was equal to 10 fish per test glass aquaria and water was constantly aerated through air stones. Temperature was maintained at 24.5±0.5°C. During the trials, fish weren't fed. With the start of the experiment observations were made in three hours' time periods and dead fish were recorded and removed.

The concentration of un-ionized ammonia (NH_3) and un-ionized ammonia nitrogen (NH_3-N) were calculated according to Zhang et al. [21].

Fish were considered dead when they were motionless on the bottom, exhibited no opercular movement, and presented no response to mechanical stimuli. To evaluate toxicity on swimming behavior, the number of fish motion makes it look like feeding and the number of fish swimming erratically (circular swimming and loss of equilibrium) were observed.

Median lethal concentration (LC_{50}) and their respective confidence intervals (95%) after being exposed to either ammonia or nitrite during 24, 48, 72, and 96 h were calculated using the probit analysis in SPSS (version 13.0). Three preparations were prepared for each fish. Blood smears were air dried. May-Grunwalt Giemsa method was used for the staining of the blood samples. Erythrocytes measurements were observed according to the Protocol K [22] using Leica DM 750 with ocular micrometer.

Statistical analysis. The 24, 48, 72, 96 h LC_{50} values and their confidence intervals were evaluated from probit analysis using SPSS 13.0 for Windows. The effects of nitrite and ammonia on erythrocytes in each parameters observed, were made by one-way ANOVA followed by the Test of Tukey and Kruskal-Wallis.

RESULTS

Un-ionize Ammonia Toxicity. There was no mortality during the acclimation period before and after experiments and no control fish died during and after the toxicity tests. Juvenile angle fish mortality increased as the combination of ammonia concentration increased, LC_{50} values were calculated for 24, 48, 72, 96 hours as 0.986, 0.751, 0.654, 0.576 mg/L NH_3-N respectively (Table 1).

Nitrite Toxicity. The NO_2-N toxicity LC_{50} values were determined for 24, 48, 72 and 96h as 29.375, 12.291, 7.982 and 6.282 mg/L respectively (Table 2).

TABLE 1
LC_{50} values of ammonia nitrogen exposure to angelfish

pH Temp.	NH_3-N (mg L^{-1})			
	LC_{50} 24	LC_{50} 48	LC_{50} 72	LC_{50} 96
7.2±0.3 25±0.5 °C	0.986 (0.783-1.613)	0.751 (0.594-0.769)	0.654 (0.539-0.802)	0.576 (0.288-0.639)

TABLE 2
LC_{50} values of nitrite nitrogen exposure to angelfish under 7.2±0.3 pH and 25±0.5 °C conditions.

	LC_{50} 24 hour	LC_{50} 48 hour	LC_{50} 72 hour	LC_{50} 96 hour
NO_2-N (mg L^{-1}) Min. – Max.	29.375 (23.852-44.509)	12.291 (6.693-16.639)	7.982 (3.329-11.026)	6.282 (1.926-8.879)

Erythrocyte Measurements. Erythrocyte sizes were determined as long and short axis of cell and nuclei long and short axis length. The erythrocyte long axis of the control, nitrite and ammonia groups were determined as 12.625±0.131 µm, 12.374±0.151 µm, 12.193±0.126 µm respectively (Figure 1, P>0.05). Short axis length of the erythrocytes determined in the control, nitrite and ammonia groups as 6.605±0.067, 7.753±0.097, 7.959±0.081 µm respectively (Figure 2). According to statistical analysis, the data showed normal distribution, not showed covariance and that there are differences between them according to Kruskal-Wallis Tukey test (P<0.05). As a result of statistical analysis, long axis length of erythrocyte did not change but the size of the short axis and the nuclei was influenced. Compared by the long axis of erythrocytes, the data showed a homogeneous distribution and the covariance, there was not any difference according to One Way Anova test.

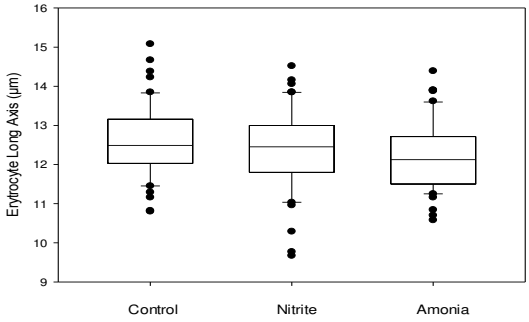

FIGURE 1
Long-axis length of angelfish erythrocyte (µm).

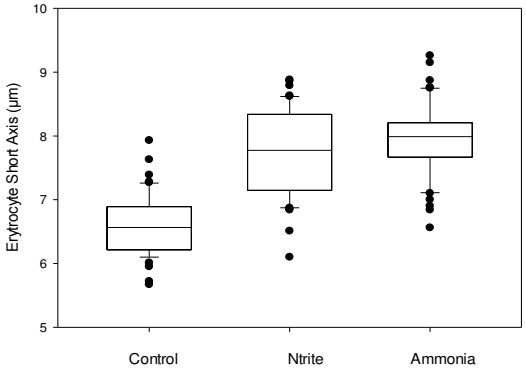

FIGURE 2
Short axis size of angelfish erythrocytes (µm).

Long axis lengths of nucleus of erythrocytes determined control, nitrite and ammonia group 5.728±0.048, 5.935±0.054 and 5.158±0.077 µm respectively (Figure 3). As a result of the analysis, the data showed normal distribution, but not showed covariance and to be important differences between them according to Kruskal-Wallis Tukey test (P<0.05).

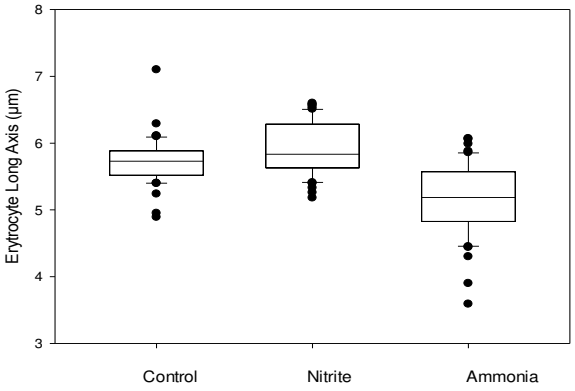

FIGURE 3
Long axis size of nucleus of angelfish erythrocytes (µm)

The short axis of erythrocyte nucleus of control nitrite and ammonia groups was measured as 3.134±0.034, 4.043±0.053 and 3.615±0.057 µm respectively (Figure 4). As a result of the analysis, the data showed normal distribution, but did not show covariance. According to Kruskal-Wallis Tukey test the differences between them were found to be important (P<0.05).

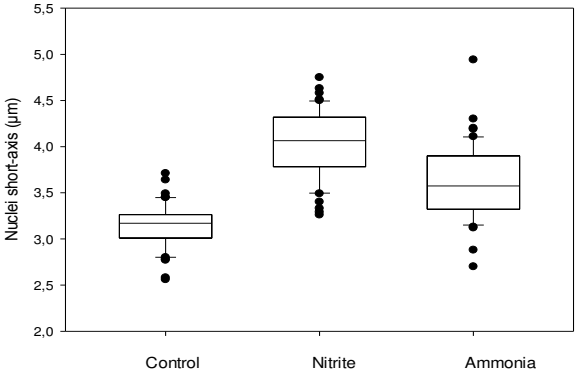

FIGURE 4
Short-axis size of nuclei of angelfish erythrocytes (µm)

DISCUSSION

Ammonia and nitrite are very important nitrogenous toxicant that can cause toxic effect on aquatic vertebrata such as fish [14-19]. Early reported researches on the toxic effects of ammonia to fishes implicated un-ionized ammonium (NH_3) as being toxic, and ionized ammonium (NH_4^+) was considered nontoxic or appreciably less toxic [4-19].

The toxicity of NH_3-N and NO_2-N to different fish species has been studied by several researchers. Some of them were related to the effects of acute

ammonia and nitrite was studied for 24, 48, 72 and 96 hours [5-7-15-16-21-23-24-25-26-27] on different fish species. Besides, some researchers investigated the chronic effects of ammonia and nitrite on growth and reproduction [4-6-18-28].

The estimation of the lethal limits of ammonia is hampered by a number of factors and is known to vary with species, other water-quality parameters, experimental methods, and the age, acclimation history and the condition of the test animals. For example, Weirich et al. [25] reported that, LC_{50} of NH_3-N (96 h) ranged from 0.32 to 0.60 mg/L for sunshine bass depending on calcium, and LC_{50} of NO_2-N (96 h) was 12.8 mg NO_2-N/L over all calcium concentrations. Howell and Baynes [29] mentioned that lethal limits for teleosts appear to range from about 0.07 mg NH_3-N/L for rainbow trout fry to 1.4 mg NH_3-N/L for juvenile striped bass (*Morone saxatilis*). In our study we estimated similar LC_{50} of NH_3-N (96 h) concentrations as found by Rodrigues et al. (2007) for juvenile cobia and Adelman et al. [30] for juvenile Topeka shiner. Our results indicated that the juvenile angle fish was more tolerant of un-ionize ammonia nitrogen than cardinal tetra [21] and pacu fish [15]. In our experiment we studied the toxic effect of NH_3-N and NH_3-N on angelfish (*P. scalare*), and determined 0.986 mg NH_3-N /L was effective in killing 50% of the angle fish within 24 hours and 0.576 mg NH_3-N/L killed 50% of the angle fish in 96 hours.

In this study we estimated 96 h LC_{50} concentration as 6.28 mg NO_2-N/L was toxic to juvenile angle fish. Higher LC_{50}s were reported by Lewis and Morris [31] for some warm water species and Dolezelova et al. [12] for zebrafish and guppy. The most sensitive fish was cardinal tetra with the LC_{50} of 0.34 mg NO_2-N/L [16] whereas in zebrafish the LC_{50} of 242.6 mg NO_2-N/L [12], in yellow catfish, the LC_{50} of 69-196 mg NO_2-N/L [21] , in juvenile cobia the LC_{50} of 210 mg NO_2-N/L [5]. It could be concluded that juvenile angle fish is similarly sensitive to nitrite nitrogen as pike-perch (LC_{50} 96h: 7.5 mg NO_2^--N/L [26]). Lewis and Morris [31] mentioned that, depending on the fish species, the addition of 1 mg/L chloride increases the 96 h LC50 by 0.29-to 2.0 mg/L NO2-N. In rainbow trout (pH: 7.7 and Cl^-: 10 mg/L), 3 mg NO_2^--N/L caused to death 50% of fish within 96 hours. As well as in studies on hot water fish 7 with 182 mg/L nitrite nitrogen killed of 50% of the fish on different types in 24-96 hours have been demonstrated [31]. These findings are similar to results of our studies.

Different from other studies, the morphology of red blood cells was investigated in this study. There are very limited data on red blood cells shape and size differentiation. Thurston et al. [28] reported that 0.06 and 0.4 mg NH_3/L can cause swelling and diminishing of red blood cells, irreversible blood damage. We also determined changes in the red blood cell shapes. The long axis of erythrocyte size did not show differences statistically between control and ammonia and nitrite experiments. On the other hand erythrocyte short axis and nuclei long and short axis of nitrite and ammonia groups were greater than the control statistically. This meant that erythrocytes were swollen.

The harmful effects of ammonia and nitrite on aquatic animals have attracted lots of attention in recent years. Especially in aquacultural facilities with RAS systems or aquarium systems, high levels of nitrite and ammonia have been found to cause intense physiological illness and mortalities. Determination of toxic effects of ammonia and nitrite on angelfish and calculation of LC_{50} values will be useful for aquarists.

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