

# Ammonia Toxicity to Four Freshwater Fish Species: Catla catla, Labeo bata, Cyprinus carpio and Oreochromis mossambica

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## Abstract

The experiment was conducted in order to determine the short term (96h) total ammonia tolerance level of four freshwater fish species to prevent their accidental mortality caused by ammonia toxicity. Fingerling (8±0.5g) of Catla catla, Labeo bata, Cyprinus carpio and Oreochromis mossambica were exposed to 5, 10, 15, 20, 25, 30 and 40mg/l concentration of total ammonia. Mortality of fishes was recorded at 12h interval in different treatments. Results obtained from the study revealed that 50% mortality of Catla catla, Labeo bata, Cyprinus carpio and Oreochromis mossambica was observed in 15, 10, 25 and 30mg/l respectively. It, therefore, can be concluded that LC<sub>50</sub> were 15, 10, 25 and 30 mg/l in Catla catla, Labeo bata, Cyprinus carpio and Oreochromis mossambica, respectively and which also clearly indicated that total ammonia tolerance level was maximum in Oreochromis mossambica which followed by Cyprinus carpio Catla catla and Labeo bata. Because of the accumulation of ammonia was lower in tilapia and higher in bata due to different enzymatic activity in its metabolic conversion process.

**Keywords:** Ammonia; Toxicity; *Catla catla*; *Labeo bata*; *Cyprinus carpio*; *Oreochromis mossambica*.

# 1. Introduction

Ammonia has been described as one of the most significant limiting factors for growth and survival of aquatic living resources among water quality criteria [1,2]. It is produced from different nitrogenous organic matters and inorganic compounds by bacterial decomposition as well as chemical transformation, which further converts into nitrite and nitrate compounds by own balancing system to reduce ammonia level in different aquatic environment. It is a well known aquatic pollutant and toxin of fish and it is also produced as an endproduct of nitrogenous metabolism. Ammonia and urea are the two major end-products of nitrogen metabolism in fish [3]. Most teleost fish are ammoniotelic and excrete ammonia as their principal waste product. Wood proposed that ammonia represents 92% and urea 8% of the N<sub>2</sub> excretion produced in carp (Cyprinus carpio) [4]. The two forms of ammonia in the environment are unionized and ionized ammonia, and unionized ammonia is toxic to fish because it can easily diffuse across gill membranes. Every aquatic life has a specific tolerance level of ammonia concentration. Knowing the concentration of ammonia that limit growth and impair respiration is useful for aquaculture management and may assist in the maximization of production [5]. A multiple increase of ammonia levels in water resulting in an imbalance within the aquatic environment of the ponds which affects badly on fish growth, exhibits crucial diagnostic sign for fish disease and causes severe mortality when concentration increased. It was found that slower growth of silver perch in ponds was associated with concentrations of un-ionized ammonia up to 0.65 mg/l. In most cases, due to significant increase of ammonia levels in the blood and consequently in ammonia autointoxication [6], toxic gill necrosis in carp [7] and haematological and enzymatic changes in Cirrhinus mrigal [8]. Besides these, ammonia toxicity causes sudden severe mortality during the rearing period of fingerling in the tank. In order to prevent accidental mortality by ammonia toxicity, the present study has been attempted to determine the short term tolerance level of ammonia for four freshwater fish species: Catla catla, Labeo bata, Cyprinus carpio and Oreochromis mossambica.

## 2. Materials and Methods

Experiment was conducted using different concentration of ammonia in glass aquarium to determine the short term (96 h) total ammonia tolerance level of four freshwater fish species. Ninety six glass aquariums (10 l) filled with tap water (pH 7.6, DO 3.1 alkalinity 190 mg/l and temperature 29.8°C) and grouped into four batches and each batch provided with anhydrous  $NH_4Cl$  (dried at 100°C) to make the concentration of 5, 10, 15, 20, 25, 30 and 40 mg/l ammonia and one control (C) in triplicate.

Water temperature and pH ranged from 28.5 to 31.75°C 7.6 to 8.1, respectively in all treatments



during the period of experimentation. The mean salinity of water was 0.47 ppt. The concentration of dissolved  $O_2$  and  $CO_2$  varied between 2.5 to 3 mg/l and 1.8 to 2.1 mg/l. The concentration of nitrite-N and nitrate-N ranged from 0.005 – 0.07 mg/l, and 0.01 – 0.03 mg/l, respectively.

Disease free healthy fingerlings (8  $\pm$  0.5 g) of catla (*Catla catla*), bata (*Labeo bata*), common carp (*Cyprinus carpio*) and Tilapia (*Oreochromis mossambica*) were procured from a local hatchery, acclimatized for one month and introduced @ 10 fishes/ aquarium in each treatment. Fish collection and preparation procedure was followed according to methods described by APHA [9].

Fish mortality was recorded at 12 h interval in different treatments. Water samples were collected from each of the treatment at fixed hour of the day (10.00 h) for examination of physico-chemical parameters (temperature, pH, CO<sub>2</sub>, dissolved O<sub>2</sub>, nitrite-N, nitrate-N and NaCl) at every 24 hours following the standard method APHA [9].

 $LC_{50}$  of total ammonia for fishes were analyzed by best fit regression using EXCEL statistical package.

#### 3. Results

#### 3.1 Fish mortality

Catla started mortality from 15 mg/l and  $LC_{50}$  was found in the 15 mg/l at 96 h No mortality was found in control, 5, 10 mg/l, whereas 70% mortality was observed in 20 mg/l and 100% fishes were died at 30 and 40 mg/l within 36 and 48 h period of the experimentation.

Bata started mortality from 10 mg/l did not showed mortality, whereas 70 and 80% mortality were found at 15 and 20 mg/l treatment but no fishes were survived at 25, 30 and 40 mg/l within 24 to 36 h.

No mortality was encountered in control, 5 and 10 mg/l, whereas 15 mg/l started mortality from 84 h in common carp. Lethal concentration 50 ( $LC_{50}$ ) was found at 25 mg/l showing 50% mortality of fish within 96 h. 100% mortality was showed in 40 mg/l within 48 h.

In tilapia, mortality was started at 25 mg/l from 72 h and lethal concentration was at 30 mg/l showing the 50% mortality of fish at 72 h period of examination. Mortality was not found in control, 5, 10, 15 and 20 mg/l, whereas 40 mg/l showed the 100% mortality.

#### 3.2 Behavior of fishes

No remarkable behavioural changes of fish were noticed when exposed to different concentration of ammonia nitrogen. The fishes exposed in control and lower concentration was very much active and behaves normally throughout the study. The opercular movement of fishes was reduced and later lethargic movements with frequent surfacing were noticed in higher ammonia concentration.

#### 4. Discussion

Results obtained from the study demonstrated that a distinct difference in total ammonia tolerance of four freshwater fish species.  $LC_{50}$  were 15, 10, 25 and 30 mg/l in catla, bata, common carp and tilapia, respectively which clearly indicated that ammonia tolerance level was maximum in tilapia which followed by common carp, catla and bata, respectively (Figure 1). Das et al. opined that the  $LC_{50}$  of total ammonia-nitrogen was 11.8 mg/l in *Cirrhinus mrigala* [8].



Figure 1. Relationship between fish mortality and ammonia concentrations showing the  $LC_{\rm 50}$  of four freshwater fish species employed



Despite the  $LC_{50}$ , from the critical observation of fish mortality data, it was obvious that effects of ammonia toxicity on bata started from 10 mg/l, on catla and common carp started from 15 mg/l and on tilapia started from 25 mg/l (Figure 1). Therefore, it may be concluded that the toxicity resistance ability was maximum in tilapia and minimum in bata. Though the ammonia toxicity on both catla and common carp showed in 15 mg/l but former one started from 48 h and later one started from 72 h. Due to the fact of period variation in ammonia toxicity of both fishes, it clearly indicated that toxicity resistance ability of common carp was more than that of catla.

The ammonia concentration in the blood plasma is increased from 5 to 10 times [10,11]. From this proposition, therefore, it may be implied from the study that blood ammonia level was high in the higher ammonia concentration due to accumulation. Total ammonia nitrogen accumulation in plasma ambient was dependent on ammonia concentrations [12]. High ammonia concentration causes respiratory problem, behavioral and physiological changes and can acts as a stressor on fish which ultimately resulting in mortalities due to acute toxicity. Reduced opercular movement and later lethargic movements with frequent surfacing of fishes were also due to severe respiratory stress due to ammonia toxicity in higher ammonia concentration.

Accumulated ammonia induced the activity of the enzymes of the ornithine-urea cycle for its metabolic conversion to urea which helped the freshwater fish toxaemia and to tolerate avoid hiah to concentrations of ammonia in the ambient medium [13]. The process of metabolic conversion differs in four fish species possibly for different enzymatic activity which resulting in the variation of accumulated ammonia concentration in tissue that leading to variation in  $LC_{50}$ . From this, it may be clearly concluded that the accumulation of ammonia was lower in tilapia and higher in bata showed  $LC_{50}$ 30 and 10 mg/l, respectively. Further study is needed to quantify the enzymatic activity and urea level under different concentration of ammonia in different fish species.

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#### References

- Russo R.C., Thurston R.V. (1991) Toxicity of ammonia, nitrite and nitrate to fishes. In: *Aquaculture and Water Quality* (Brune E., Tomasso J.R.), World Aquacultural Society Publication, pp. 58-89.
- [2] Tomasso J.R. (1994) Toxicity of nitrogenous wastes to aquaculture animals. *Rev. Fish. Sci.* **2**: 291-314.
- [3] Forster R.P., Goldstein L. (1969) Formation of excretory products. In: *Fish Physiology*, vol. 1 (Hoar W.S., Randall D.J.), Academic Press, New York, pp. 313–350.
- [4] Wood C.M. (1993) Ammonia and urea metabolism and excretion. In: *Physiology of Fishes* (Ewans D.H. ed.), CRC Press, Boca Raton, pp. 379-425.
- [5] Colt J., Armstrong D.A. (1981) Nitrogen toxicity to crustaceans, fish, and molluscs. *Bio-Engineering Symposium for Fish Culture*, 1: 34-37.
- [6] Svobodová Z., Pravda D., Palaâková J., et al. (1986) Jednotné metody hematologického vyseft.ování ryb. Edice metodik vúrh ve VodÀanech, VodÀany, 36 p.
- [7] Schreckenbach k., Spangenberg r., Krug s., et al.
  (1975) Die ursache Kiemennekrose. Z Binnenfisch, 22: 257-288.
- [8] Das P.C., Ayyappan S., Jena J.K., Das B.K., et al. (2004) Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of mrigal, Cirrhinus mrigala (Hamilton). Aquaculture Research, 35(2):134.
- [9] APHA (1995) Standard Methods for the Examination of Water and Wastewater (19th edn). American Public Health Association, Washington, DC, 1038 p.
- [10] Svobodová Z., Koláová J., Kouil J., Hamáâková J., Vykusová B., Kaláb P., et al. (1997) Haematological investigations in Silurus glanis L. females during preand postspawning period. *Pol. Arch. Hydrobiol.*, **44**: 67-81.
- [11] Svobodová J., Haluzík M., Bednářová V., Sulková S., Jiskra J., Nedvídková J., Kotrlíková E., Kábrt J., et al. (2001) Relation between serum leptin levels and selected nutritional parameters in hemodialyzed patients.(in Czech). *Vnitr Lek*, **47**: 594–598.
- [12] Ruyet J. Person-le, Lamers A., Roux A. le, Sévère A., Boeuf G., Mayer-Gostan N., et al. (2003) Long-term ammonia exposure of turbot: effects on plasma parameters. *Journal of Fish Biology*, **62**(4): 879.
- [13] Saha N., Ratha B.K. (1994) Induction of ornithineurea cycle in a freshwater teleost, Heteropneustes fossilis, exposed to high concentrations of ammonium chloride. *Comp. Biochem. Physiol.* **108**: 315-325.