**Bosmina fatalis** adapting to ammonia through oxidative stress and ribosome increase

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**ABSTRACT**

Ammonia is generated in large quantities during organic degradation, concluding that of cyanobacteria, which is toxic to aquatic animals. *B. fatalis* is a widespread cladoceran species found in freshwater. The present study aimed to explore the toxic responses of *B. fatalis* to ammonia. Our experiments showed that the 24, 48, 72 and 96 h LC50 values of NH3 on *B. fatalis* were 20.59, 13.06, 5.77 and 3.36 mg L⁻¹, respectively. A 14-days NH3 exposure study at 0, 0.22, 0.57 and 1.4 mg L⁻¹ were carried out. NH3 at low and medium doses (0.22 and 0.57 mg L⁻¹) displayed stimulative effects on the *B. fatalis* population and the body length of the newborns, while NH3 at high dose (1.4 mg L⁻¹) did not exhibit significant effect on the *B. fatalis* population and size. Transmission electron microscope (TEM) revealed significant increase in number of ribosomes at low doses, while the interior structure showed no marked change at high dose. The levels of indicators related to oxidative stress (ACP, SOD, CAT, GSH-Px and MDA) caused by NH3 first increased and then decreased with time in all the NH3-exposure groups. The mRNA expression of genes related to ribosomal structure (RPL12, RPL10, RPL10A, RPL27A and RPL19) and antioxidant stress (CAT, SOD and GSH-Px) was rapidly downregulated after NH3 exposure. Collectively, it is inferred that ammonia at a certain dose (≤0.57 mg L⁻¹) stimulates the growth and reproduction of *B. fatalis*, which may be its defensive reaction to ammonia.
1. Introduction

In recent years, ammonia has become a global concern due to its ubiquity in surface water and high toxicity (Park et al. 2018; Egnew et al. 2019). Waterborne ammonia (total ammonia nitrogen, TAN) generally exists in two forms: ionized ammonia ($\text{NH}_4^+$) and unionized ammonia ($\text{NH}_3$) (Constable et al. 2003). Particularly, the un-ionized form ($\text{NH}_3$) is highly toxic to most aquatic organisms (Sarma et al. 2003; Yang et al. 2012; Lyu et al. 2013). Previous studies have shown that NH$_3$ can damage to fish tissues, such as liver, gills, thyroid, kidneys, and spleen, leading to metabolic disorders and increased susceptibility to various diseases (Ackerman et al. 2006; Spencer et al. 2008). Additionally, high NH$_3$ concentration has been proved to impair the survival, reproduction, and growth of zooplankton (Leung et al. 2011; Cao et al. 2014).

*Bosmina* is a small filter-feeding zooplankton (about 0.254~0.319 mm) that is widely distributed in lakes and reservoirs of temperate and tropical regions (Goulden and Frey 1963; Havens and Decosta 1985; Stephen 2003). It can filter bacteria, organic debris and phytoplankton effectively, and plays an important role in the carbon cycle, nutrient mineralization, and energy flow (Huibin et al. 2015). Compared to other zooplankton, *Bosmina* has a hard shell and is well protected in the mud at the bottom of lake (Charles 1981). *Bosmina* can avoid the predation of invertebrates by changing the length of its antennae and spines, which can be used to trace the changes of zooplankton structure in the ecosystem to further understand the changes of eutrophication of a lake. Therefore, *Bosmina* is an ideal bio-indicator to study limnology and restoration ecology. (Ferdous and Muktadir 2009; Charles 1981; Alexander & Hotchkiss).

It have been reported that *Bosmina* is the dominant species in eutrophic water bodies, because *Bosmina* is too small to feed the larger cyanobacteria (Dao-Gui et al. 2013; Tao et al. 2013). With the spread of eutrophication, *Bosmina* may play a more important role in freshwater ecosystems. In eutrophic water bodies, a large amount of ammonia will be produced during the degradation of cyanobacteria, and ammonia concentration will be maintained at very high levels ($\text{NH}_3$ 0.55 mg·L$^{-1}$), which attained from NH$_4^+$ doses ($\text{NH}_4^+$ 9.34 mg·L$^{-1}$; pH 8, 23 °C) conversion according to the report (Lahti et al. 1997;
Wang et al. 2010). Therefore, the effect of ammonia on the B. fatalis, and its behavior in high ammonia environments is an interesting topic of study.

In the study, we investigated the toxic effects of ammonia on B. fatalis. Changes in growth and oxidative stress-related indexes such as acid phosphatase (ACP), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malonaldehyde (MDA) of B. fatalis were investigated after NH3 exposure. The ultrastructures of B. fatalis under different ammonia concentrations were observed using transmission electron microscopy (TEM). At the same time, the expression levels of genes related to ribosome structure and antioxidant were also detected. This experiment further explores the toxic responses of B. fatalis under different NH3 concentrations, and reveals the mechanism of B. fatalis’ adaptation to ammonia nitrogen.

2. Methods

2.1. Sample collection and maintenance

Previous study showed that B. fatalis is relatively abundant in summer and can be isolated from Lake Gaoyou (N32°42′, E119°25′) in quantity (Adamczuk et al. 2015). Therefore, the samples were collected in the summer of July 2019 from Lake Gaoyou (Jiangsu Province, China). One organism was chosen from the isolated samples, and monoclonal culture and amplification was performed on it in the aquatic laboratory of Yangzhou University, China. B. fatalis was maintained in 3000 ml water tanks in the laboratory and fed with Chlorella vulgaris at 25°C and under fluorescent light at 40 μmol photons m−2 s−1, with a light-dark period of 13:11 h.

2.2. Reagents and solution preparation

Analytical grade ammonium chloride (NH4Cl) (purity ≥ 99.8%) was obtained from Sigma (USA). The NH4Cl stock solution with an N concentration of 16000 mg·L−1 was prepared with double distilled water before the experiment and stored at 4°C until use. At the beginning of the test, the stock solution was diluted with double distilled water that pH was 7.6 (HI 8424 pH meter, Hanna corporation) to obtain the test solution exposed to NH3 at the required concentrations.

2.3. Calculation of molecular ammonia concentration

In order to find out whether actual NH3 concentrations in the culture solutions of different experimental groups show a certain gradient, we determined actual NH3 doses in culture solution from conversion of added NH4Cl stock solution by measuring water temperature, pH, and ammonia nitrogen of in each experimental group at 0, 6, 12 and 24 h, respectively. Ammonia nitrogen was determined using Nessler’s reagent spectrophotometry. Following which the NH3 concentration (mg/L) in the culture solution of each group was calculated using the following formula (Autian 1974).

\[
NH_3 = \frac{[NH_3 + NH_4^+]}{1 + 10^{(pKa - pH)}}
\]

The pKa was calculated from the equation proposed by Emerson et al. (Emerson et al. 1975):

\[
pKa = 0.09018 + 2729.92/T, \ (T \ \text{in K})
\]
2.4. Acute toxic test

Several wide-range test concentrations were used for the pretest prior to the formal test. The concentration gradient of the formal experiment according to the minimum entire-lethal concentration and the maximum zero-lethal concentration in the pre-experiment. The \( \text{NH}_4^+ \) exposure doses for \( B. \text{fatalis} \) were: 0, 5, 10, 20, 40 and 80 mg L\(^{-1} \). The calculated doses of \( \text{NH}_3 \) exposure for \( B. \text{fatalis} \) were: 0, 0.30, 0.59, 1.19, 2.37, 4.74 mg L\(^{-1} \). Chlorine was from removed the tap water used in the experiment by prolonged oxygenation. Each \( \text{NH}_3 \) exposure level was set in triplicate. The entire process was carried out in a six-hole plate with 15 \( B. \text{fatalis} \) and a 5 ml solution in each hole. The organisms was observed every 2 h using a stereomicroscope (ZEISS Discovery V12). The \( B. \text{fatalis} \) was considered dead when they showed no signs of movement. The dead \( B. \text{fatalis} \) were removed in time and their number were recorded after fixed intervals of at 24, 48, 72 and 96 h. We calculated percent mortalities in five \( \text{NH}_3 \) treatment groups for 24, 48, 72, and 96 h. \( \text{LC}_{50} \) values at 24 h, 48 h, 72 h, and 96 h were derived from regression of probit-transformed mortalities against log concentration. The safe concentration was calculated using the formula: \( \text{SC} = 0.1 \times 96 \text{h} \ \text{LC}_{50} \)

2.5. Chronic toxic test

Four levels of \( \text{NH}_3 \) (0, 0.22, 0.57, and 1.4 mg L\(^{-1} \)) were prepared based on the acute toxicity tests, \( \text{NH}_3 \) concentrations higher than those found in the environment were used to assess its adverse effects on \( B. \text{fatalis} \). During the test, \( B. \text{fatalis} \) were fed with the centrifuged \( \text{Chlorella} \) at cell concentration \( 1 \times 10^4 \) cells ml\(^{-1} \) every two days (Hanazato and Yasuno 1987). The survival rate and first breeding time of \( B. \text{fatalis} \) were monitored daily. Body length was measured from above the eye to the bottom of the tail-spine in all groups after 20-days exposure. Initially, 15 \( B. \text{fatalis} \) were placed in the control and treatment group in 100 ml beaker, the corresponding ammonia concentration water were replenished and the population numbers of \( B. \text{fatalis} \) were recorded on the seventh day. Additionally, the size of the first eggs and the first brood were recorded after the fixed intervals. The body length data were observed and photographed using microscope (CX21, Olympus Corporation), and measured using ruler of cellsens Dimension software.

2.6. \( B. \text{Fatalis} \) ultrastructural examinations by TEM

The whole body tissue of 15 \( B.\text{fatalis} \) from every treatment group was fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer at 4 \( ^\circ \)C for 12 h, and then washed thoroughly five times in a 0.1 M sodium phosphate buffer and fixed in 1% osmium tetroxide in 0.1 M sodium phosphate buffer under 4 \( ^\circ \)C. After washing, the tissue was dehydrated in graded alcohol and embedded with LR white resin. The blocks were cut on a slicer (ULTRACUT, Leica Microsystems Ltd). Semi -thin sections (1 um thick) were routinely stained with toluidine blue. Ultrathin sections (80 nm thick) were compared with uranyl acetate and lead citrate, and examined using TEM (HT7700, Hitachi) at 80 kV.

2.7. Antioxidant parameters analysis

Approximately 400 \( B. \text{fatalis} \) samples were collected from each beaker to determine the changes in the antioxidant parameters, which were measured using the whole-body homogenates. The entire operation was carried out on ice. The whole-body homogenates
were washed in 0.68% physiological saline and then dried with filter paper (Chen and Kou 1993). They were then placed in a Dounce homogenizer with 2 mL of 0.68% physiological saline solution and homogenized. This was followed by centrifugation at 2000 g of samples at 4 °C for 10 min to remove cellular debris. The supernatant was then eliminated for subsequent antioxidant parameter assays. We measured the ACP, SOD, CAT, MDA and GSH-Px levels. The protein concentration of the samples was determined using the Diagnostic Reagent Kit (Coomassie protein assay dye) and The diagnostic kit (Nanjing Jian Cheng Bioengineering Institute, China) was used to determine the ACP (U·mg⁻¹·protein), SOD (U·mg⁻¹·protein), CAT (U·mg⁻¹·protein), GSH-Px (mg·g⁻¹·protein), and MDA (nmol· mg⁻¹·protein).

### 2.8. RNA extraction, cDNA synthesis and quantitative real-time PCR of genes related to ribosomal structure and antioxidant stress after ammonia exposure

After 24 h of ammonia exposure for, the *B. fatalis* in the control and treatment groups was collected in a centrifuge tube with screen silk and temporarily stored in liquid nitrogen. The samples were quickly stored in a refrigerator at −80 °C after collection. Total RNA was extracted using a Trizol kit (Takara, Japan). We detected the RNA concentrations using Nanodrop 2000. The cDNA templates of *B. fatalis* were synthesized using M-MLV reverse transcriptase (Takara, Japan) according to the manufacturer’s instruction. Before reverse transcription, the genomic DNA was removed from total RNAs by DNase (Takara, Japan). The real-time quantitative PCR (qPCR) was used to analyze the gene expression profile of *B. fatalis* against ammonia exposure. 1 ul cDNA was added during qPCR experiment. β-tubulin gene was selected as the reference gene. The qPCR experiments were performed on CFX96 (Bio-Rad) with SYBR qPCR Mix. The ribosomal structure-related genes included RPL12, RPL10, RPL10A, RPL27A and RPL19. The antioxidant stress-related genes were including CAT, SOD and GSH-Px. The specific primer sequences were shown in Tab. 1. All primers were synthesized by the Nanjing Qingke Biological Company. The melting curve analysis was carried out in the range of 60-90 °C to detect a single amplified product. The relative expression levels were calculated using the 2⁻ΔΔCt method. Each sample had three replicates.

| Gene   | Name | Sequence          |
|--------|------|-------------------|
| RPL12  | F    | TTGGTCTCTTGGTTTG   |
|        | R    | CTTGTTGGTGAGTTT    |
| RPL10A | F    | CCCACAAGATTCCA     |
|        | R    | TTTCGCGCTTCACT     |
| RPL27A | F    | TAAGACATTCAAGTC    |
|        | R    | TTGCGACGATAAGT     |
| RPL19  | F    | TACGAAACGCAAGCC    |
|        | R    | CGAAGACAGCAGGAGGA  |
| RPS10  | F    | TCTACGATACCTCCTCCA |
|        | R    | GTGATGGCTCGATAGT   |
| CAT    | F    | AAAACCTGGCCATAGAAAGGCC |
|        | R    | TGAGTGAGAAGGAGAAGAAA |
| SOD    | F    | GAGCCGCAACCAAGGACAC |
|        | R    | CGCTCTGAGCAGAATTGC |
| GSH-Px | F    | GGCTGCTACGGGAGTAT  |
|        | R    | AACGTGATTGACGGAAAGG |
| β-tubb | F    | TTGAACCAATCATGAC    |
|        | R    | AAGCAGACAGACATGAC   |
2.9. Data analysis

All experimental data were analyzed using one-way analysis of variance (ANOVA). Tukey’s post-hoc test was used for the data with significant difference (p < 0.05). p values less than 0.05 were considered significant. SPSS statistical software 17.0 was performed for the analysis.

3. Results

3.1. LC50 experiment

Six NH₃ solution concentrations (NH₃ 0, 0.30, 0.59, 1.19, 2.37, 4.74 mg·L⁻¹) were selected for the acute toxic test. Exposure of *B. fatalis* to NH₃ at 24, 48, 72 and 96 h yielded LC₅₀ values of 20.59, 13.06, 5.77 and 3.36 mg·L⁻¹, respectively, indicating a significant decrease with time. The mortality of *B. fatalis* at different NH₃ concentrations in different time periods is shown in Figure 1. Safe concentration of NH₃ in *B. fatalis* is 0.34 mg·L⁻¹.

3.2. Chronic toxicity

After 20-days of exposure, the 0.57 mg·L⁻¹ NH₃ group showed a more significant effect on the *B. fatalis* body length than the control (p < 0.05) (Figure 2A). The initial number of *B. fatalis* was 15, after 7-days exposure, and the population of *B. fatalis* with different NH₃ concentration was shown in Figure 2B. The population of *B. fatalis* in the 0.22 and 0.57 mg·L⁻¹ NH₃ treated group was larger than the other groups (0.22 mg·L⁻¹ NH₃, p < 0.05, 0.22 mg·L⁻¹ NH₃, p < 0.01). When the exposure concentration was increased to 1.4 mg·L⁻¹, the growth rate began to decline. The difference in the *B. fatalis* population in 1.4 mg·L⁻¹ NH₃ and the control was insignificant. The sizes of newborns in the low and medium NH₃ doses were larger than those in the control and high-dose groups (p < 0.05) (Figure 2C). However, the difference in the first reproduction time and size of *B. fatalis* at different NH₃ concentrations was insignificant (p > 0.05) (Figure 2D and E).

![Figure 1](image-url)

*Figure 1*. Plot of measured 96 h LC₅₀ (points) of ammonia versus time. Data were fitted to a hyperbolic decay equation (line). Data were expressed as Mean ± SEM.
3.3. Transmission electron microscopic observation

1. NH₃ damage to the rough endoplasmic reticulum

The ribosomes of the rough endoplasmic reticulum in the intestinal parietal cells of *B. fatalis* in the control group were regular and dense, without exfoliation, and were located near the nucleus (Figure 3A). NH₃ at lower and high doses (NH₃ 0.22, 1.4 mg·L⁻¹) showed no significant effect on the rough endoplasmic reticulum (Figure 3B and C). However, in 0.22 mg·L⁻¹ NH₃ treatment group, the number of ribosomes in the endoplasmic reticulum increased obviously, and even more ribosomes were dispersed in the surrounding cytoplasmic matrix (red arrow). This phenomenon was not found in the highest concentration group.

2. NH₃ damage to mitochondria

The 0.22 and 1.4 mg·L⁻¹ NH₃ treated groups did not show any obvious effect on *B. fatalis* mitochondria compared with the control (Figure 3D–F). The number of mitochondria did not seem to make a significant difference (as indicated by the arrow), and the matrix was normal.

3.4. Molecular NH₃ calculation

The NH₃ concentration of were determined from the values of ammonia nitrogen, pH and temperature, and were observed to increase gradually over different time periods. From the beginning, the NH₃ concentration of formed an obvious gradient. At the 24 h mark, the NH₃ gradient was 0.029, 0.22, 0.57 and 1.4 mg·L⁻¹.

3.5. Effect of NH₃ on oxidative stress indicators

Overall, the levels of ACP, SOD, CAT and MDA first increased and then decreased in a time and dose-dependent manner. NH₃ promoted the activity of ACP, SOD, CAT and MDA at 6 and 12 h, but inhibited it at 24 h compared to the control group. However, GSH-Px activity increased significantly compared to the control at all time points (p < 0.05) (Figure 4A–E).
3.6. Different expression of genes related to ribosomal structure and antioxidant stress after NH₃ exposure

The defense mechanisms of *B. fatalis* after NH₃ exposure were also tested by conducting the qPCR, which detected the expression levels of different ribosomal structures and antioxidant stress related-genes (Figure 5). The relative expression levels were presented...
in the form of a heat-map, where the gene expression levels were displayed in different colors. In Figure 5, the red color represented expression levels in the control, and the green and blue color represented down-gene expression levels according to the legend on the right side of the figure. It was observed that after 24 h of exposure, the expression of ribosomal structure-related genes (RPL12, RPL10, RPL10A, RPL27A and RPL19) significantly decreased in all exposure groups. The expression of antioxidant stress-related genes (SOD, CAT and GSH-Px) also declined significantly compared to the control group (p < 0.05).

4. Discussion

In the present study, we investigated the toxic effects of ammonia on *B. fatalis*. *B. fatalis* can reproduce abundantly in eutrophic water, and is one of the dominant species (Figueroa-Sanchez et al. 2014; Jiang et al. 2014). In eutrophic water, NH₃ concentration is up to 0.55 mg·L⁻¹ (pH 8, 23°C), which is produced from NH₄⁺ conversion (Wang et al. 2010). This concentration is approximate to the middle doses in our experiment. Therefore, the set of ammonia concentrations used in the experiment has practical significance.

Previous studies have shown that NH₃ is toxic to zooplankton. Several studies have been carried out on the effects of NH₃ on zooplankton. For instance, *D. magna* showed a tolerance to a LC₅₀ of 2.94 mg·L⁻¹ NH₃ to at 48 h (pH 8.5, 20°C) (Gersich and Hopkins 1986). The 48 h LC₅₀ value for acute toxicity of NH₃ to *C.dubia* is 1.43 mg/L (Andersen and Buckley 1998). The 24 and 48 h LC₅₀ value for acute NH₃ toxicity to *Moina mongolica* is 9.89 and 7.52 mg/L, respectively (Yu-Xin 1996). In the present study, the lethal toxicity of ammonia nitrogen for *B. fatalis* at 24, 48, 72 and 96 h yielded LC₅₀ values of 347.4, 229.4, 93.3 and 56.7 mg·L⁻¹, respectively (Figure 1). Accordingly, the 24, 48, 72 and 96 h LC₅₀ of the unionized NH₃ for *B. fatalis* were 20.59, 13.07, 5.77 and 3.36 mg·L⁻¹ (pH
Thus, the tolerance of \textit{B. fatalis} to ammonia was higher than that of \textit{D. magna}, \textit{C. dubia} and \textit{M. mongolica}.

Reproduction and growth are the most important life activities of zooplankton, and environmental factors can change the status of zooplankton in water by affecting their reproduction and growth. The effects of NH$_3$ on the growth of most zooplankton have been well reported. NH$_3$ at 0.48 mg L$^{-1}$ significantly delayed the maturation time and decreased body size of \textit{Daphnia similis} (Lyu et al. 2013). At NH$_3$ 0.581 mg L$^{-1}$, it significantly delayed the time to the first clutch and first egg of \textit{D. magna} (Yang et al. 2012). Additionally, at 0.74 mg L$^{-1}$ NH$_3$ negatively affected the reproductive and growth performance of \textit{Brachionus calyciflorus} (Liang et al. 2020). All the above reports indicated that ammonia has negative effects on their development. Our study found that the growth and reproduction of \textit{B. fatalis} can be significantly promoted by ammonia at lower concentrations (0.22, 0.57 mg L$^{-1}$). Even in the high ammonia concentration (1.4 mg L$^{-1}$) groups, no inhibitory effect was found in the growth and reproduction of \textit{B. fatalis}. The present study showed that the body length and population of \textit{B. fatalis} increased significantly at low and medium NH$_3$ concentrations, the same goes for the body length of the newborn \textit{B. fatalis}, but time and body length of the first reproduction were not affected (Figure 2). We inferred that \textit{B. fatalis} population may rapidly increase by increasing the number of offspring per brood or broods under NH$_3$ exposure.

Ribosome is the main site of protein synthesis in cells, and plays a key role in reproduction, growth, antioxidant stress, etc (Kai-Chun et al. 2015). Ribosomal proteins can be used as candidate target genes for various environmental stresses (heavy metals, temperature, etc.) in aquaculture (Quinn et al. 2011; Mursalin Khan and Islam Sm 2015; Vieira et al. 2018), however, few studies have also shown the changes in ribosomal proteins with respect to varying NH$_3$ concentrations. The internal structure of \textit{B. fatalis} did not show significant damage under the different NH$_3$ treatment, and the internal ribosomes increased obviously in the low NH$_3$ groups (Figure 3). It is inferred that the ribosomal translation pathway may have changed, hence, we selected some important ribosomal protein genes (RPL12, RPL10, RPL10A, RPL27A and RPL19) for further study. The expression of these ribosomal structure-related genes significantly decreased in all the exposure groups. This was in contrast to the morphological observations and was possibly due to a delay in the gene expression or feedback regulation. Additionally, we believe that the increase in ribosomes under low NH$_3$ concentration is closely related to the promotion of growth and reproduction of \textit{B. fatalis}.

NH$_3$ is a neurotoxin that destroys the proteins in the body (Kosenko et al. 1999), causing severe oxidative stress, leading to excessive production of reactive oxygen species (ROS) (Murthy et al. 2001), changes in antioxidant and free radical concentrations and regulation of free radical scavenging enzyme systems, such as SOD, CAT and MDA (Trenzado et al. 2009; Hegazi et al. 2010). However, the activities of these enzymes are inhibited under severe oxidative stress, and oxidative damage leads to the loss of defense mechanisms (Zhang et al. 2004). In present study, the increase of ACP, SOD, CAT and GSH-Px in 0.22, 0.57, 1.4 mg L$^{-1}$ NH$_3$ groups at 6 h and 12 h indicated that NH$_3$ can induce the antioxidant defense of \textit{B. fatalis}. We can estimate the degree of cell oxidative damage by MDA, since it is the final product of lipid peroxidation (Shalata and Tal 1998). MDA increases in most species under stress induced by NH$_3$ (Jiang et al. 2012; Zhenjie et al. 2017; Huang et al. 2018). The increase in MDA in 0.22, 0.57, 1.4 mg L$^{-1}$ NH$_3$ groups at 6 h and 12 h suggested that the NH$_3$ induced antioxidant response was not sufficient to overcome the oxidative stress, while the decline of MDA at 24 h indicated that the oxidative stress response was slow down and \textit{B. fatalis} survived well in all groups.
Furthermore, antioxidant related genes were selected for further experiments. The transcription levels of SOD, CAT and GSH-Px were significantly down-regulated in all the NH₃ treated groups. The decrease in SOD, CAT and GSH-Px mRNA levels and enzyme activities may be due to an increase in ROS. Under severe oxidative stress, the excessive production of ROS may exceed the antioxidant capacity, resulting in a decrease in the antioxidant enzyme activity and transcription levels. This is consistent with the results of enzyme activity at 24 h, except for GSH-Px. In previous studies, the mRNA expression of the SOD and CAT genes were significantly regulated as environmental stress response under exposure to environmental pollutants (e.g., H₂O₂, temperature and MeHg) in marine rotifers, such as B. koreanus (Lee et al. 2017; Lee et al. 2019), B. plicatilis (Han et al. 2020), and B. calyciflorus (Yang et al., 2013). The results are similar to those of the present study.

5. Conclusion

Our study showed that certain-concentration of NH₃ (<1.4 mg·L⁻¹) did promote the development and reproduction of B. fatalis. This positive promoting effect was achieved by stimulating the number and function of ribosomes, and enhancing the antioxidative stress ability of B. fatalis. This study reveals the mechanism of B. fatalis adapting to NH₃. We find out that B. fatalis may be a promising organism that relieves NH₃ pollution in the water environment.

Author contribution

Wen Xue and Wenzhi Wei designed and conducted the experiment, Jiaqi Jin carried out the data analysis and chart drawing, Haokai Chen and Yingying Zhang helped data generation on gene expression, Feng Zhang and Daokai Yang helped in enzyme activity data collection. Wen Xue supervised and corrected the whole manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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