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The ammonia effects to the habitat requirements and adaptability of *Daphnia magna*

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ABSTRACT

In all of the pollutants in the Wenyuhe River Basin, ammonia is the most important one, which changed from 0.2 mg/L to more than 70 mg/L. In order to assess habitat requirements and adaptability to the aquatic environment, the effects of different concentration ammonia (0, 0.02, 2, 10, 20 and 70 mg/L) on *Daphnia magna* were tested. After 48-h, the immobility rate increased to about 60% in the exposure of 70 mg/L, which suggested that the acute toxicity occurred. Therefore, the 21-d effects of ammonia on F0 *D. magna* and the 10-d effects of ammonia on F1 *D. magna* were carried out in the treatments of 0, 0.02, 2, 10, and 20 mg/L. The results suggested that the body length, the dry weight, the mean spawning number, and the mean molting frequency of F0 changed from 3.17 ± 0.09 mm, 10.90 ± 0.10 mg, 125.50 ± 15.60 and 2.35 ± 0.11 to 2.85 ± 0.13 mm, 4.90 ± 0.60 mg, 88.60 ± 30.10, and 3.08 ± 0.33 respectively. The intrinsic rate of natural increase (r) decreased with increasing concentrations of ammonia nitrogen especially in those animals exposed to 20 mg/L. The body length, the dry weight, the mean spawning number, and the mean molting frequency of F1 changed from 2.47 ± 0.18 mm, 3.09 ± 0.13 mg, 16.50 ± 3.60, and 2.13 ± 0.10 to 2.64 ± 0.04 mm, 2.64 ± 0.33 mg, 0.00 ± 0.00, and 3.22 ± 0.12 respectively. These results showed that higher ammonia concentration affected the habitat requirements of *D. magna* significantly. Meanwhile, the body length difference between F0 and F1 suggested that continuous exposure to ammonia could improve the adaptability of the offspring.

Keywords: Ammonia; Habitat requirements; Adaptability; *Daphnia magna*

1. Introduction

Due to population growth and economic development, the discharge of increasing amounts of wastewater exceeds its self-purification capacity, which induces the serious pollution in the Wenyuhe River Basin [1]. The Wenyuhe River Basin, which is situated in the northeast part of Beijing, occupies a total area of 4,300 km^2, representing 26% of the territory of Beijing. The river basin, which includes the Wenyuhe River, the Qinghe River and the Longdaohe River, runs through Beijing, serves as the

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natural ecological barrier, and is of crucial importance in Beijing's overall development strategy. Therefore, the Wenyuhe River Basin holds an important concentration of industries, agricultural activities and urban areas, with an important demand of water [2]. Among the pollutants including ammonia, total nitrogen, total phosphorous and heavy metal [3,4], ammonia is the most important one [5], which changed from 0.2 mg/L to more than 70 mg/L during 2010 due to the discharge of the treated wastewater from Qinghe Wastewater Treatment Plant [6]. Ammonia can cause the inhibition of energy metabolism, and then induce the toxicity of different organs in aquatic organisms [7]. Therefore, it is necessary to assess the effects of ammonia on the habitat requirements and adaptability of aquatic organisms.

Daphnia magna, a small planktonic invertebrate crustacean (0.5–5.0 mm) with a short life cycle, is very sensitive to changes of the chemical composition in aquatic environments. The species has often been used in bioassays and environmental monitoring of aquatic systems due to the ease and economy of maintaining cultures [8,9]. *D. magna* as a widely distribution kind of invertebrate animals around the world, has been used for many years in standard tests of toxicity because of their high sensitivity, easy handling and high reproduction rate [10,11].

However, hardly any research was about the toxic effects of ammonia on *D. magna*. Therefore, in this study, the acute and chronic toxicity of ammonia on *D. magna* was tested to assess the effects of ammonia on its habitat requirements and adaptability.

2. Materials and methods

2.1. Test species

The experimental *D. magna* (24-h young) were cultured in our laboratory for more than 3 generations. The culture was maintained at 20 ± 2˚C under a 16-h light: 8-h dark photoperiod (illumination ranged between 3,000 and 4,500 lx). Culture medium was prepared according to the components of the Standard Reference Water (SRW) and *D. magna* were fed on *Scenedesmus obliquus* [12]. Before feeding *D. magna*, the culture medium of the algae was filtered and then diluted by SRW until the concentration reached 1 × 105 cells/ml. The quantity of the algae was about 1% beaker volume.

Before the exposure experiments, the gravid female *D. magna* already carrying eggs were taken out and cultured individually in 50-ml glass beakers of SRW until they oviposited. The healthy and uninjured neonates were taken and used for test.

2.2. Experimental setup

Ammonium chloride (NH_4Cl, analytical pure, CAS: 12125-02-9) was purchased from the Standard Sample Center of China. The acute test was performed in accordance with the standard protocol for *D. magna* acute test [13]. 48-h exposure experiments of ammonia (0, 0.02, 2, 10, 20 and 70 mg/L) were performed according to the ammonia concentration in the Wenyuhe River basin to make sure exposure concentration of the chronic experiment. Based on the acute test, 21-d chronic toxic test for F0 *D. magna* and 10-d chronic toxic test for F1 *D. magna* were performed in accordance with the standard protocol [14]. At last, the body length, the dry weight, the mean spawning number, the mean molting frequency were used to assess habitat requirements and adaptability of *D. magna* to ammonia. F1 *D. magna* was the first offspring of F0 *D. magna*. The intrinsic rate of natural increase (r) was discussed based on the individual survival according to the methods of Lotka [15].

During these studies, laboratory conditions were kept the same as in the culturing room. All determinations were repeated five times, and each time 10 individuals. *D. magna* were fed on *S. obliquus* three times a week. Before feeding *D. magna*, the culture medium of the algae was filtered and then diluted by SRW until the concentration reached 1 × 105 cells/ml. The quantity of the algae was about 1% beaker volume.

2.3. Statistical analysis

For the purpose of this study, all of the statistical data was treated by SPSS 13.0 with a 99% confidence limit. One-way analysis of variance was performed to compare the chronic toxicity of ammonia on *D. magna*.

3. Results and discussion

3.1. Acute toxicity results

The 48-h acute toxicity of ammonia on *D. magna* was shown in Table 1. In the treatments of 0, 0.02, 2, and 10 mg/L, the immobility rate of *D. magna* was no more than 1%. Even in 70 mg/L exposure, the immobility rate was about 60%, which suggested that ammonia was in a lower toxicity grade according to the toxicity of chemicals to the aquatic organism [16]. These results showed that there were no acute toxic effects on *D. magna* when the concentration was lower than 10 mg/L. Due to the acute toxicity of 70 mg/L ammonia, only 0, 0.02, 2, 10 and 20 mg/L were tested in the chronic toxicity experiments.

Table 1
The immobility rate of *D. magna* in different kinds of exposure

Concentrations (mg/L)	48-h immobility rate (%)	20-d immobility rate (%)
0	0	0
0.2	0	0
2	0	0
10	1	10
20	13	28
70	60	/

3.2. F0 chronic toxicity results

The results of 21-d chronic toxic test for F0 *D. magna* on the body length, the dry weight, the molting frequency and the mean spawning number were shown in Fig. 1. During this exposure experiment, no individuals died in the treatments of 0, 0.02, and 2 mg/L. But in 10 and 20 mg/L treatments, the immobility rates were about 10 and 28% respectively (Table 1). The 21-d chronic toxic effects of ammonia induced the decrease of the body length, the dry weight and the mean spawning number, and the increase of the mean molting frequency of *D. magna*. They changed from 3.17 ± 0.09 mm, 10.90 ± 0.10 mg, 125.50 ± 15.60 and 2.35 ± 0.11 in 0 mg/L to 2.85 ± 0.13 mm, 4.90 ± 0.60 mg, 88.60 ± 30.10, and 3.08 ± 0.33 in 20 mg/L respectively. The differences between the treatments of 0 and 20 mg/L were significant with $p < 0.01$.

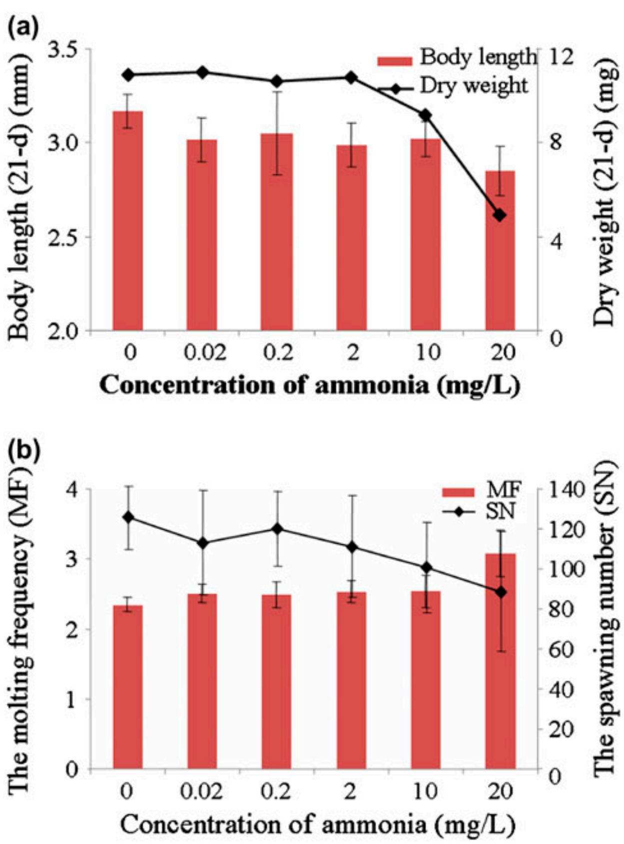

Fig. 1. The 21-d chronic toxicity of ammonia for F0 *D. magna*.

These results suggested that these parameters can be used as indicators in the assessment of the effects of ammonia on *D. magna*. The dry weight and the mean spawning number were more sensitive than the other two parameters. The reason for the differences of these indicators in different treatments might be that the ammonia exposure inhibit the energy metabolism of inside environment, and then induce the toxicity of different organs in aquatic organisms. 4 Therefore, some water body with ammonia (70 mg/L) in the Wenyuhe River basin might induce acute toxicity and induce no survival of *D. magna*, and other lower concentration ammonia might affect the habitat requirements of *D. magna*, which might induce the reproduction toxic effects (the mean spawning number). These results had been proved by the biodiversity research, which showed that there was no *D. magna* exist in the Wenyuhe River basin.

Table 2 shows that the average longevity of *D. magna* did not decrease significantly with increasing concentration of ammonia nitrogen except in the concentration of 20 mg/L ($p < 0.05$). In the treatments of control, 0.02 mg/L, 0.2 mg/L, and 2 mg/L ammonia, the time to first brood increased kept the same as each other, which was 6–7 days. However, in the high concentration exposures, it increased to about 9.4 days in 10 mg/L and 11 days in 20 mg/L ammonia with the significance $p < 0.05$. The total Number of offspring changed from 731 to 439. Exuviae number per adult also decreased as the concentrations of ammonia nitrogen increased in the medium. Significant differences ($p < 0.05$) between treated and control groups were found in the concentrations 20 mg/L.

3.3. F1 chronic toxicity results

In order to show the evidence for the improvement of the adaptability of the offspring of F0 *D. magna*, a 10-d chronic toxic test for F1 *D. magna* was carried out. Comparing with F0 *D. magna*, only the body length, the dry weight, the molting frequency and the mean spawning number were analyzed in F1 *D. magna*. The results showed that the body length, the dry weight, the mean spawning number, and the mean molting frequency of F1 changed from 2.47

Table 2
The 21-d chronic toxicity of ammonia for F0 *D. magna*

Ammonia (mg/L)	Average longevity (days)	Time to first brood (days)	Total number of offspring	Exuviae number per adult
Control	20.54 ± 0.677	6.20 ± 0.447	731	8.80 ± 0.255
0.02	20.18 ± 0.829	7.20 ± 1.304	567	8.56 ± 0.397
0.2	20.32 ± 0.700	6.00 ± 0.000	601	8.42 ± 0.217
2	20.46 ± 0.780	6.00 ± 0.000	645	8.28 ± 0.522
10	20.10 ± 0.418	9.40 ± 2.702*	502	8.26 ± 0.391
20	16.50 ± 2.715*	11.00 ± 0.707*	439	6.82 ± 0.554*

Values are Means ± SD, and $^{*}p < 0.05$.

± 0.18 mm, 3.09 ± 0.13 mg, 16.50 ± 3.60, and 2.13 ± 0.10 in 0 mg/L ammonia to 2.64 ± 0.04 mm, 2.64 ± 0.33 mg, 0.00 ± 0.00, and 3.22 ± 0.12 in 20 mg/L ammonia respectively (Fig. 2).

Different from the exposure of F0 *D. magna*, the body length of F1 *D. magna* was increased with the ammonia concentration increase, which suggested that the adaptability of the offspring increased. Ebert reported that pollutants could affect the reproduction of *D. magna*, and when aquatic environment quality improved, the offspring of *D. magna* would improve its adaptability [17]. However, the mean spawning number of F1 *D. magna* decreased to 0 in 20 mg/L ammonia, which suggested even the habitat requirements of F1 *D. magna* were affected significantly and no *D. magna* could exist in such polluted aquatic environment.

These results suggested that no evident chronic toxic effects be observed in less than 2 mg/L ammonia treatments. Meanwhile, due to the accumulation of different chemicals in *D. magna*, the chemical migration and transformation in test organisms should be investigated to discuss effects of the chemical accumulation in F0 *D. magna* on the energy metabolism, the organ toxicity, and the other toxicity effects of F1 *D. magna*.

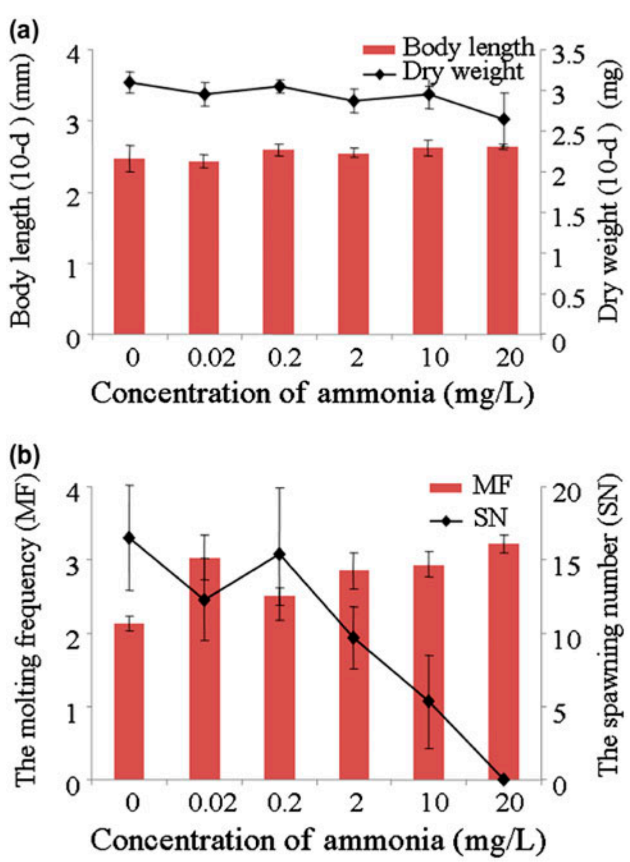

Fig. 2. The 10-d chronic toxicity of ammonia for F1 *D. magna*.

3.4. The intrinsic rate of natural increase

Fig. 3 shows the effect of ammonia nitrogen on the intrinsic rate of natural increase (r) of *D. magna*. The rate r decreased from 0.172 in control (0 mg/L) to

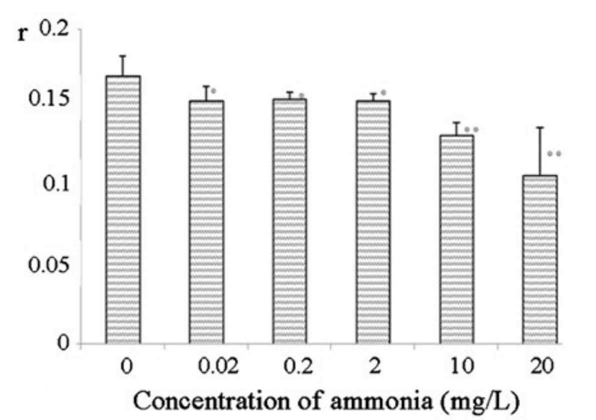

Fig. 3. The effect of ammonia nitrogen on the intrinsic rate of natural increase (r) of *D. magna*. $^{*}p < 0.05$, $^{**}p < 0.01$.

0.151 in 0.02 mg/L with the significance $p < 0.05$, and 0.113 in 20 mg/L with the significance $p < 0.01$. The results suggested that the intrinsic rate of natural increase (r) was sensitive to ammonia nitrogen.

The previous results on the toxicity effects on the intrinsic rate of natural increase (r) suggested that the reduction in the rate (r) may induce a consequence of chronic toxicant stress of ammonia nitrogen on *D. magna* [18].

4. Conclusions

From the study on the toxic effects of ammonia to the habitat requirements and adaptability of *D. magna*, it was concluded that:

(1) The reproduction and the growth could be effective indicators in the assessment of the effects of ammonia on *D. magna* and should be always considered in the assessment of the effect of ammonia on other aquatic organisms.
(2) Though ammonia is one kind of lower toxicity chemicals, even lower concentration ammonia might affect the habitat requirements of *D. magna*, which might induce the reproduction toxic effects.
(3) The offspring of F0 *D. magna* showed evident adaptability to ammonia exposure in the same pollutants without the assessment of the chemical accumulation, and the chemical migration and transformation in test organisms should be investigated.
(4) The habitat requirements of *D. magna* based on the reproduction and the growth could be affected by 0.2 mg/L ammonia significantly. Meanwhile, the body length difference between F0 and F1 suggested that continuous exposure to ammonia could improve the adaptability of the offspring.
(5) The ammonia concentration in the Wenyuhe River Basin, which changed from 0.2 mg/L to more than 70 mg/L could affect the habitat requirements of *D. magna*, and due to the 70 mg/L ammonia, no *D. magna* could alive in the Wenyuhe River Basin.

In the future, some efforts will be continued in the wild field to test the adaptability and the habitat requirements of *D. magna*, and extend this technique as a useful monitoring tool in the *in situ* monitoring of water quality.

Acknowledgments

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