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Acute Toxicity of Nitrite and Ammonia to *Daphnia similoides* of Different Developmental Stages: Using the Modified Gaussian Model to Describe

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Abstract To gain an insight into the tolerance of *Daphnia* to nitrite and ammonia, a modified Gaussian model was used to describe the trends of changes in LC50s of nitrite and ammonia to *Daphnia similoides* at different developmental stages. LC50s of NO_2–N and NH_3–N increased with age before maturation and then decreased at maturation. A modified Gaussian model provided an accurate fit for the changes in LC50s of NO_2–N and NH_3–N, in which the parameters have definite biological meanings. From this model, we can gain an insight into the maximum LC50 and the age that has the maximum LC50 and predict LC50s at any specific ages. We suggest that such a model might be used to describe the trend in acute toxicity of some other zooplankton species at different stages.

Keywords Acute toxicity · Modified Gaussian model · Nitrite · Ammonia · *Daphnia similoides*

Increasing concentrations of nitrite and ammonia in surface waters are becoming a worldwide concern. A surge in concentration of nitrite and ammonia usually occurs during the process of degradation of *Microcystis* blooms in some eutrophic lakes, such as Lake Taihu, the third largest freshwater lake in China, and one that has suffered frequent outbreaks of *Microcystis* blooms in recent years (Qin et al. 2007). The rapid increase in concentration of nitrite and ammonia in lake water may cause some acute toxicity to aquatic organisms, as both nitrite and ammonia are toxic to aquatic animals and can cause impairments of cerebral energy metabolism and damage to gill, liver, kidney, spleen, and thyroid tissues in fish, crustaceans, and mollusks (Colt and Armstrong 1981). It is well known that the tolerances of aquatic organisms to specific toxic chemicals are quite different across the developmental stages (e.g. Ostrensky and Wasielesky 1995; Zhao et al. 1997). However, no information is available on using models to describe the change of tolerance with age. *Daphnia* spp. has been widely used as a test species in ecotoxicological assays (e.g. Manning et al. 1996; Buratini et al. 2004; Cooman et al. 2005; Ra et al. 2008). Therefore, we selected *Daphnia similoides*, a predominant species of zooplankton in Lake Taihu, as a test organism to assess the acute toxicity of nitrite and ammonia to *Daphnia*. Then, we focus on using a modified Gaussian model to describe the trends of changes in LC50s of nitrite and ammonia to *D. similoides* at different developmental stages.

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Materials and Methods

The zooplankton species, *Daphnia similoides*, was originally isolated from Lake Taihu (119°53′45″–120°36′15″E, 30°55′42″–31°33′50″N), China and maintained in the laboratory for several months prior to this study. The zooplankton was cultured in beakers and fed with *Scenedesmus obliquus* as food at 25°C under fluorescent light at an intensity of 40 μE m^{-2} s^{-1} with a light–dark period of 12:12 h. Five different developmental stages (0.5, 1, 3, 5, and 7-day-old) of *D. similoides* were used in the experiments.

Nitrite and ammonia test solutions were prepared by dissolving sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) in freshwater. The concentrations of test solutions were set up according to a logarithmic contour interval (0.1). The concentrations of NO_2–N for test solution ranged from a minimum concentration of 12.78 to a maximum concentration of 202.90 mg L^{-1}. The concentrations of un-ionised ammonia nitrogen (NH_3–N) for the test solution ranged from a minimum concentration of 0.678 to a maximum concentration of 2.701 mg L^{-1}. NH_3–N concentrations were calculated using the general equation of bases (Albert 1973):

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

In fresh water, the calculation of *p*Ka is based on the equation developed by Emerson et al. (1975): $pKa = 0.09018 + 2729.92/T$, ($T = \text{Kelvin} = 273 + T$ °C). The nitrite concentration, ammonia concentration, pH level, and temperature in each beaker were measured daily by using a multi-parameter meter. When necessary the ammonia concentrations in the beakers were adjusted to the nominal concentration. Usually, for 48-h experiments, the test solutions in each beaker were replaced with fresh test solutions at 24 h.

Ten individuals were used per beaker and each concentration was run in eight replicates for a total of 80 animals per concentration. *D. similoides* exposed in freshwater without the addition of nitrite or ammonia were taken as controls. No food was supplied during the tests. The experimental conditions were identical to those used during culturing, reported above.

During the experiments, mortalities were recorded every 8 h. Dead individuals were confirmed under a microscope. Percent mortalities in each concentration were calculated for the 24- and 48-h time periods. LC50 values for the 24- and 48-h time periods were derived from regression of probit-transformed mortalities against log concentration.

All data were presented as mean ± 1 SE and were analyzed by one-way ANOVA ($\alpha = 0.05$) followed by Duncan's multiple range test. To reveal the trends of changes in LC50s of nitrite and ammonia with ages, the modified Gaussian model, $Y = a*\exp(-0.5*\text{abs}((X - X_0)/b)^c)$, was chosen to fit the data under different ages respectively, where Y is LC50 (mg L^{-1}), X is age (d), a is the maximum LC50 (mg L^{-1}), X_0 is the age when Y reaches the maximum value (d), and b and c are constants. All statistical analyses were carried out with SigmaPlot 11.0.

Results and Discussion

All individuals survived in the control solutions after 24 and 48 h exposure. The results of the LC50s and their 95% confidence intervals for NO_2–N and NH_3–N on different ages of *D. similoides* were summarized in Table 1. For every age, in both NO_2–N and NH_3–N, the 24 h LC50s were significantly higher than 48 h LC50s ($p < 0.05$).

For 24 h LC50s of NO_2–N, the LC50s of the 5 and 7-day-old individuals were significantly higher than those of 0.5 and 1-day-old animals ($p < 0.05$); similar results were detected in 48 h LC50s of NO_2–N. In addition, the 48 h LC50 of NO_2–N of the 3-day-old individuals was significantly higher than that of 0.5-day-old ($p < 0.05$). In general, both 24 and 48 h LC50 increased with age; but no significant difference was found between 5 and 7-day-old individuals ($p > 0.05$). The 95% confidence intervals (Table 1) were much wider for the LC50 values of NO_2–N, which may be due to the fact that nitrite is not very toxic to *D. similoides*. A modified Gaussian model provided an accurate fit for the changes in LC50 with age (Fig. 1). The maximum LC50s of NO_2–N at 24 h and 48 h were 156.99 and 63.09, respectively; the age that LC50s of NO_2–N at 24 and 48 h reached the maximum values were 6.25 and 5.74 days, respectively (Table 2).

For 24 h LC50s of NH_3–N, the LC50 of the 5-day-old individuals were significantly higher than those of 0.5, 1, and 7-day-old ones ($p < 0.05$); LC50 of the 3-day-old individuals was significantly higher than those of 0.5-day-old ones. For 48 h LC50s of NH_3–N, the LC50s of the 1, 3, 5, and 7-day-old individuals were significantly higher than

Table 1 LC50 (mg L^{-1}) of NO_2–N and NH_3–N for different ages of *Daphnia similoides* and their 95% confidence intervals

Age	LC50 of NO_2–N (95% confidence interval)		LC50 of NH_3–N (95% confidence interval)	
	24 h	48 h	24 h	48 h
0.5-day-old	48.99 (28.34–74.02)	18.75 (14.70–30.51)	1.30 (1.06–1.62)	0.98 (0.81–1.15)
1-day-old	52.73 (25.37–83.83)	28.78 (24.58–35.13)	1.48 (1.41–1.70)	1.22 (1.04–1.42)
3-day-old	88.78 (53.15–114.36)	38.65 (26.49–49.61)	1.71 (1.51–2.03)	1.31 (0.99–1.74)
5-day-old	142.99 (68.91–237.50)	59.22 (38.83–158.64)	1.75 (1.48–2.31)	1.36 (1.16–1.47)
7-day-old	150.90 (98.15–206.10)	54.76 (25.90–80.23)	1.46 (1.17–1.80)	1.24 (1.41–1.70)

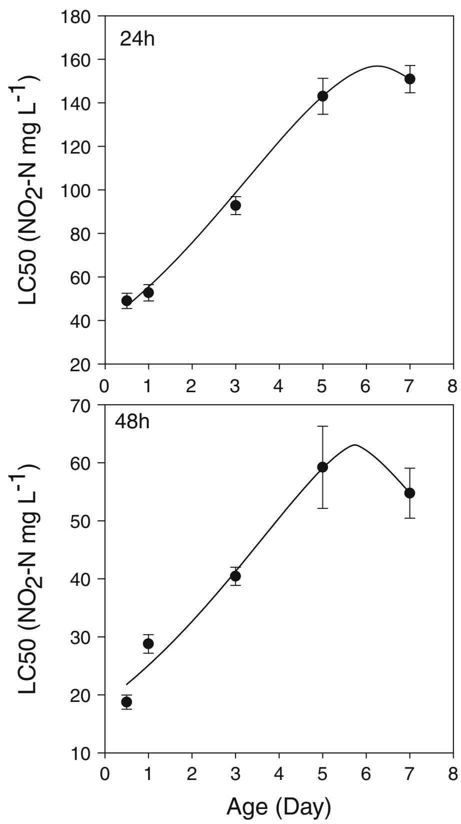

Fig. 1 Changes in LC50s of NO_2–N for *Daphnia similoides* at different ages fitted by modified Gaussian model. Vertical bars represent ± 1 SE

those of 0.5-day-old ($p < 0.05$); there is no significant difference among 1 to 7 days old ($p > 0.05$). A modified Gaussian model also provided an accurate fit for the changes in LC50 with age (Fig. 2). The maximum LC50s of NH_3–N at 24 h and 48 h were 1.74 and 1.33 mg L^{-1}, respectively; the age that LC50s of NH_3–N at 24 and 48 h reached the maximum values were 3.98 and 4.03 days, respectively (Table 2).

In the experiments, we used *D. similoides* at different ages; 0.5 and 1-day-old individuals can be taken as larval stage; 3 and 5-day-old can be taken as juvenile stages; whereas 7-day-old individuals have matured and start to reproduce. LC50s of NO_2–N increased with age and then decreased at the maturation, whereas LC50s of NH_3–N increased with age, reached a plateau at the juvenile stage and then decreased at the maturation stage. Here we first used a modified Gaussian model to fit the data. The modified Gaussian model provided an accurate fit for the changes in LC50s of NO_2–N and NH_3–N, in which the parameters have definite biological meanings. From this model, we can gain an insight into the maximum LC50 and the age at which this occurs. For example, the maximum LC50 of *D. similoides* was 156.99 mg L^{-1} in 24 h NO_2–N at an age of 6.25 days (Table 2). From Figs. 1, 2, the parameters of c in models of NO_2–N and NH_3–N are quite different, these indicate that the LC50 of NO_2–N has a triangle-like peak whereas the LC50 of NH_3–N has a square-like shape, which suggests different dynamics between LC50s of NO_2–N and NH_3–N: a small age range has the highest LC50 of NO_2–N (sharp peak) and the highest LC50s of NH_3–N occur over a larger range of ages (wide plateau). The model also can be used to predict the LC50 at a specific age.

In conclusion, a modified Gaussian model is suitable for describing the trend of change in tolerance of *D. similoides* to nitrite and ammonia; such a model might be used to describe the changing trends in acute toxicity of some other species at different stages.

Table 2 The parameters of age-course changes in LC50 fitted by modified Gaussian model $Y = a*\exp(-0.5*\mathrm{abs}((X - X_0)/b)^c)$

Chemical	Duration	Parameter	Coefficient	SE	*t*	*p*	*R*
NO_2–N	24 h	*a*	156.9850	6.9571	22.5648	0.0282	0.9993
		b	3.3962	0.4747	7.1539	0.0884	
		c	1.6852	0.3152	5.3463	0.1177	
		X_0	6.2453	0.2368	26.3701	0.0241	
	48 h	*a*	63.0949	9.9732	6.3264	0.0998	0.9898
		b	3.0765	1.0514	2.9260	0.2097	
		c	1.4161	0.7648	1.8516	0.3153	
		X_0	5.7385	0.3722	15.4176	0.0412	
NH_3–N	24 h	*a*	1.7357	0.0220	79.0552	0.0081	0.9972
		b	3.9940	0.2120	18.8399	0.0338	
		c	3.8575	0.9435	4.0886	0.1527	
		X_0	3.9770	0.0615	64.6834	0.0098	
	48 h	*a*	1.3349	0.0292	45.7184	0.0139	0.9901
		b	3.7399	0.1865	20.0525	0.0317	
		c	8.3448	2.7877	2.9934	0.2053	
		X_0	4.0317	0.1134	35.5538	0.0179	

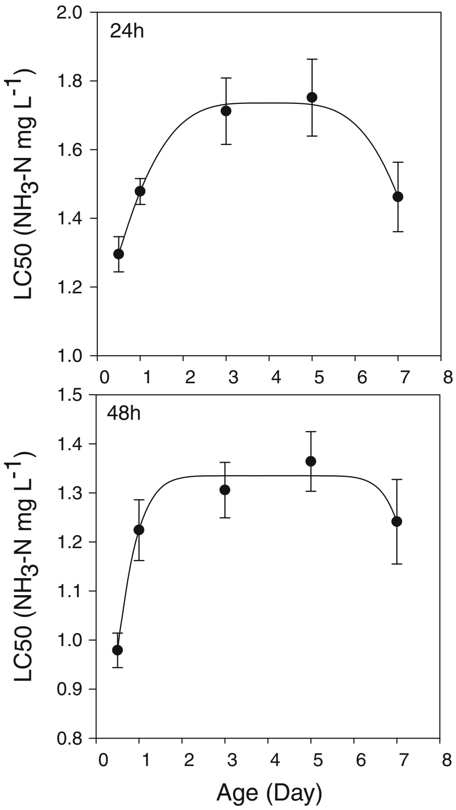

Fig. 2 Changes in LC50s of NH_3–N for *Daphnia similoides* at different ages fitted by modified Gaussian model. Vertical bars represent ± 1 SE

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