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Original Research

Effects of Tribromomethane on the Life-Table Demography of the Freshwater Rotifer *Brachionus calyciflorus* Pallas under Different Algal Densities

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Abstract

Toxic effects of tribromomethane (TBM), a most used drinking water disinfection byproduct, on rotifers are unknown. we investigated the effects of TBM (0, 0.001, 15, 30, 45, 60, and 75 mg/L) on the life table demography of the freshwater rotifer, *Brachionus calyciflorus,* fed with three densities (1.0, 2.0, and 4.0 × 10^6 cells/mL) of *Scenedesmus obliquus*. It showed that the TBM exposure decreased survivorship and fecundity of the rotifer and significantly affected the developmental stages and life table demographic parameters of *B. calyciflorus*. Compared with the control, treatments with 30-75 mg/L TBM significantly prolonged the juvenile period of rotifer under different algal densities. Treatments with 0.001 and 15 mg/L TBM significantly decreased the net reproductive rate of rotifer under the algal density of 1.0 × 10^6 cells/mL, while increased and did not affect it under the algal density of 2.0 and 4.0 × 10^6 cells/mL, respectively. These results demonstrate that TBM is toxic to *B. calyciflorus*, and the increased algal density can reduce the toxic effects of TBM on rotifers. The current safety limit of TMB in natural water could affect the community structure of organisms in the aquatic system.

Keywords: tribromomethane, *Brachionus calyciflorus*, chronic toxicity, algal density, life table demography

Introduction

One of the most adopted methods to inactivate coronaviruses (COVID-19) is to use chlorine-containing disinfectants. Unfortunately, as a result, large amounts of disinfection byproducts (DBPs) containing chlorine can enter the river through the sewage treatment system [1]. It is even worse that chlorination is still the most widely applied disinfection method to inactivate pathogenic microorganisms for water treatment. Trihalomethanes (THMs), e.g. trichloromethane (TCM), bromodichloromethane

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(BDCM), dibromochloromethane (DBCM), and tribromomethane (TBM), are dominant and well-known disinfection by products (DBPs). These products are formed when disinfectants, such as chlorine, react with natural organic matter in water [2]. Several THMs have been demonstrated to be mutagenic, genotoxic and/or carcinogenic in different test systems, raising an increasing concern about the association of THMs exposure with adverse developmental effects [3].

Bromide ions are ubiquitous in surface water, and their content varies from 0.003 to 2 mg/L depending on different water bodies [4]. About 10% of the bromide ions in raw water are transferred into brominated disinfection byproducts (Br-DBPs), including the above mentioned TBM [5]. Indeed, the average concentration of TBM could reach 0.001 and 0.003 mg/L in rivers and municipal pipe network [6-7], respectively. However, the maximum concentration of TBM in clear-wells of water plant using chlorine disinfection could reach to 75-95 mg/L [8].

Previous studies found that higher Br-DBPs formation due to the presence of bromine ions could increase the immobilization of *D. magna* [9-10]. The 96-h LC50 values of TBM to *Daphnia magna* and *Lepomis macrochirus* were 46 and 29 mg/L [11-12], respectively. The 48-h EC_{50} values of TBM on algal growth inhibition of *Dunaliella salina* was 0.24 mg/L [13]. The toxic effects of TBM on the growth and reproduction of aquatic organisms are found to be higher than other DBPs, which include TCM, dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) [14]. Unfortunately, the increasing seawater intrusion and human activities cause the increase of bromine content in surface water, intensifying the formation of Br-DBPs [15].

Rotifers are abundant and widely distributed in aquatic ecosystems and thus can play an essential role in the ecological processes of aquatic ecosystems [16]. Because of the rapid population turnover rate, rotifers contribute significantly to nutrient recycling in aquatic habitats. Therefore, if rotifer populations are adversely affected by a toxin, the function of aquatic ecosystems could be altered [17]. Rotifers have been used as model organisms to evaluate the toxicity of many environmental chemicals, including heavy metals [18], organic compounds [19], and nano-sized materials [20]. And increased supply of algal food could effectively alleviate the negative effects of toxicants to rotifers [21-22]. To the best of our knowledge, the effect of TBM on rotifers remains unknown, even though the toxicity of TBM on aquatic organisms has been noted [23].

Hence, to better understand the chronic effects of TBM on the dynamics of *Brachionus calyciflorus* and the influences of the algal density on the toxicity of TBM to rotifers, we evaluated the life table demography of the rotifer *B. calyciflorus* in response to different concentrations of TBM under three algal densities. This work is of significance to better predict the behavior of TBM in natural water bodies.

Materials and Methods

Rotifer Culture

We collected *B. calyciflorus* from incubation of dormant eggs selected from the sediment in Lake Jinghu (Wuhu City, China) and cultured them in EPA medium (96 mg/L of $NaHCO_3$, 60 mg/L of $CaSO_4$, 60 mg/L of $MgSO_4$ and 4 mg/L of KCl) at 25±1ºC by feeding the fresh green alga *Scenedesmus obliquus* for more than three months before experiments. Based on the morphological features, the rotifers were identified as *B. calyciflorus* s.s. according to Evangelia et al. [24]. The *S. obliquus* used in the laboratory were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China) and grown in HB-4 medium (200 mg/L of $(NH_4)_2SO_4$, 15 mg/L of K_2HPO_4, 15 mg/L of $CaCl_2$, 80 mg/L of $MgSO_4 \cdot 7H_2O$, 100 mg/L of $NaHCO_3$, 25 mg/L of KCl and 0.15 mg/L of $FeCl_3$). The culture of algal was kept under static-renewal conditions with a 16:8 h light:dark photoperiod at 130 lx at 25±1ºC in an illumination incubator, centrifuged in the exponential growth phase and re-suspended in distilled water at the desired densities, determined by direct counting with a hemacytometer. Rotifers were randomly divided into three groups and fed alga daily at the respective density of 1.0, 2.0, and 4.0×10^6 cells/mL for two weeks before our experiments.

Test Solutions

We purchased standard solutions of TBM (≥99%, CAS 75-25-2) from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). A stock solution was prepared by dissolving TBM in 100% methanol and diluted to the desired concentrations using EPA medium before. The measured concentrations of TBM in test solutions (Table 1) were determined using gas chromatography-mass spectrometry (GCMS-QP2020NX, Shimadzu, Japan).

Table 1. Nominal and measured concentrations of TBM.

TBM concentration (mg/L)						
Nominal	0.001*	15	30	45	60	75
Measured	0.011	18.3	29.8	43.8	73.3	78.2

*The detection limit of TBM were 0.01 mg/L

Life Table Experiments

In order to better reflect the toxicity of environmental concentrations, six concentrations (0.001, 15, 30, 45, 60, 75 mg/L) of TBM, a blank control, and a methanol control (methanol was the same as in 75 mg/L TBM) were chosen based on the concentrations of TBM in natural water bodies and special application scenarios mentioned above. The chronic test was carried out in 24-well plate at 25±1ºC without light, and each well contained one *B. calyciflorus* neonate (<4 h old) and 0.5 mL of the test solution with 1.0, 2.0, or 4.0 × 10^6 cells/mL *S. obliquus*. Ten neonates of each replicate and three replicates for each treatment were conducted independently. For the first 48 h, we checked rotifers every two hours and recorded the time of the first egg and the hatching. After 48 h, we checked rotifers every 8 h and the numbers of newborn rotifers and the initial rotifers that were still alive were recorded. If dead or newborn rotifers were found, they were removed. Every 24 h, the initial rotifers still alive were transferred into freshly prepared test solutions containing designated algae densities. The experiments were terminated when all initial rotifers died.

Developmental Stages and Life Table Demographic Parameters

We calculated the developmental stages according to Sha et al [25], which include juvenile period (JP, time from hatching to extrusion of the first egg), reproductive period (RP, time from extrusion of the first egg to extrusion of the last egg), post-reproductive period (PP, time from extrusion of the last egg to death), embryonic development (ED, time from extrusion of the first egg to hatching of the first juvenile), and average lifespan (LS, average survival time of rotifers). Age-specific survivorship (l_x), age-specific fecundity (m_x), life expectancy (e_0, life expectancy at hatching), generation time (T, $T = \Sigma l_x m_x x/R_0$), net reproductive rate (R_0, $R_0 = \Sigma l_x m_x$) and intrinsic rate of population increase (r_m, $r_m = lnR_0/T$), NOEC (NOEC, no-observed effect concentration), LOEC (LOEC, lowest-observed effect concentration), EC_{50} (EC_{50}, median effective concentration) were determined according to Pan et al. [26].

Statistical Analyses

All data were presented as mean±SE. We performed statistical analyses using SPSS S version 26.0 (SPSS Inc., Chicago, IL, USA). The normality and homogeneity of data were tested using Kolmogorov Smirnov and Levene's tests, respectively. We compared the differences between experimental and control groups using single-factor variance analysis (One-way ANOVA) and multiple comparisons (LSD test). Two-factor variance analysis (Two-way ANOVA) was used to analyze the significant effect of algal density, TBM concentration, and the interaction between algal density and TBM concentration on life table parameters. Regression analysis was performed to evaluate the relationship between TBM concentrations and parameters. Differences with p value<0.05 were considered significant.

Results and Discussion

Developmental Stages of *B. calyciflorus*

Previous studies found that JP of *B. calyciflorus* was prolonged in the treatments with bromate [27], fenitrothion [28], and dieldrin [29]. In the present study, under the algal densities of 1.0 × 10^6 cells/mL, compared with the control, JP was prolonged at 30-75 mg/L TBM (p<0.01), and both RP and LS were shortened at 30-75 mg/L TBM (p<0.01). Under the algal densities of 2.0 × 10^6 cells/mL, JP was prolonged at 45-75 mg/L TBM (p<0.05), RP was shortened at 75 mg/L TBM (p<0.01). Under the algal densities of 4.0×10^6 cells/mL, JP was prolonged at 45-75 mg/L TBM (p<0.05). These results indicate that rotifers maintain energy for survival by reducing investment in reproduction as a response to TBM exposure, especially under low algal densities (Fig. 1).

Effects of TBM on Survival and Reproduction of Rotifers

Previous studies revealed that TBM could significantly inhibit the survival of *Danio rerio* [23], *Daphnia magna* [11], and *Dunaliella salina* [13]. Here we showed that TBM of 30-75 mg/L significantly decreased the age-specific survivorship of rotifers under the algal of 1.0 × 10^6 cells/mL (p<0.05). TBM of low concentration significantly increased the age-specific survivorship of rotifers. In contrast, medium and high concentrations did not affect and increase them under the algal of 2.0 × 10^6 cells/mL, respectively (p<0.05). These results reveal that TBM at a specific concentration inhibits the survival of rotifers and thus affects the population growth of rotifers (Fig. 2).

Rifampicin and oxytetracycline were reported to be able to significantly increase the R_0 and r_m of rotifers at the concentrations of 2-6 mg/L and 30-90 mg/L, respectively [30-31], norfloxacin was also demonstrated to be able to promote the population growth of rotifers at a very low concentration (5 mg/L) [32]. Here we observed a similar hormesis effect of TBM on rotifers. TBM of 0.001 mg/L could significantly increase the R_0 under the algal densities of 1.0 and 2.0 × 10^6 cells/mL, respectively (p<0.05). TBM of 15 mg/L significantly increased the r_m under the algal densities of 2.0 × 10^6 cells/mL (p<0.05). In addition, we could find a clear dose-response relationship between TBM concentration and R_0 as well as r_m under three algal

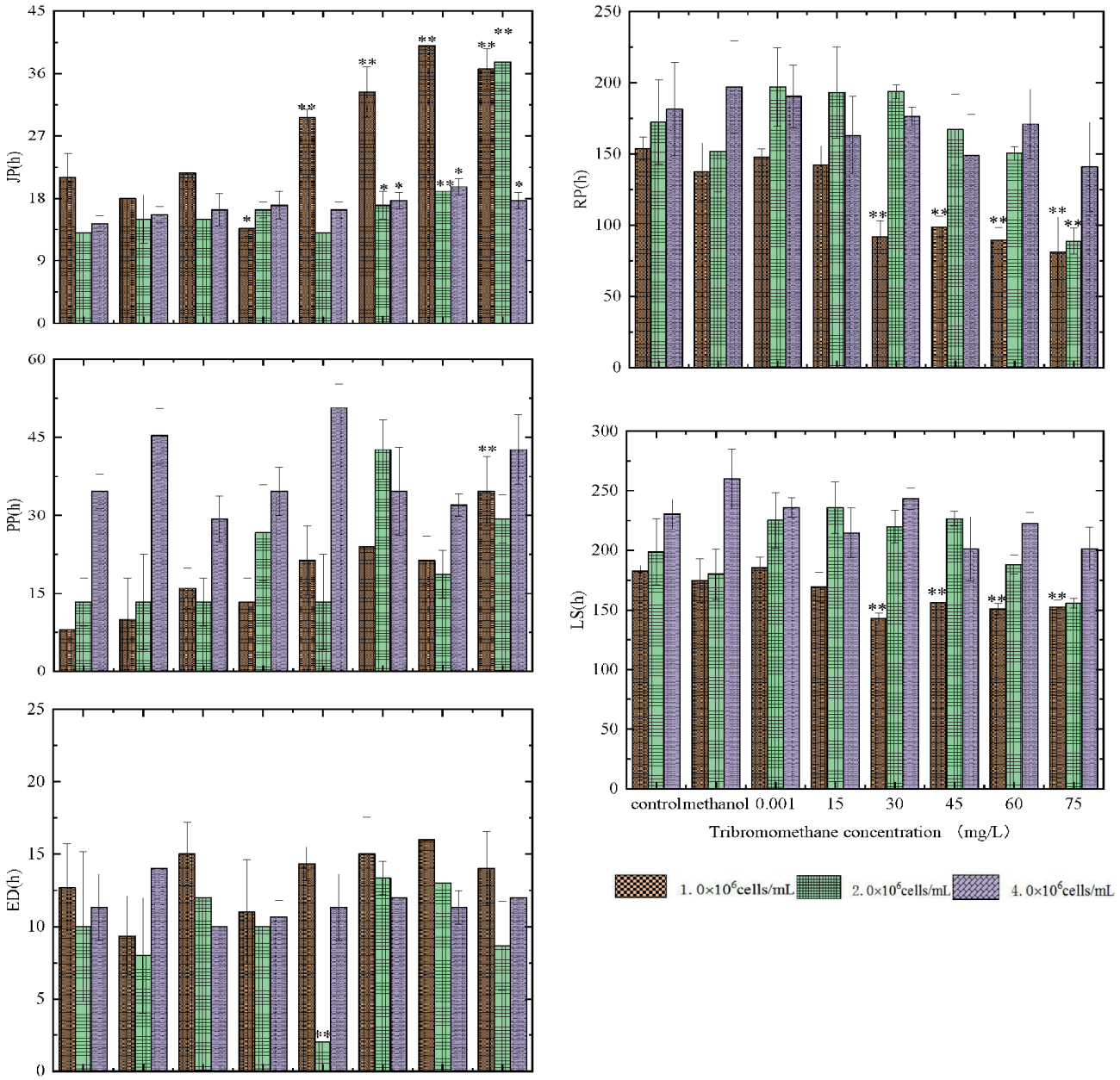

Fig. 1. Effects of TBM on the developmental stages of *B. calyciflorus*.
JP: juvenile period; PP: post-reproductive period; ED: embryonic development; RP: reproductive period; LS: life span; Significance levels: $^{*}p<0.05$, $^{**}p<0.01$

densities (Table 2) and the phenomenon of hormesis within a specific range. However, this growth promoting effect was not observed under 4.0 × 10^6 cells/mL of algal. These results demonstrate that the growth promoting effects of TBM on rotifers depend on its low concentration and the food level.

Combined Effects of Algal Density and TBM Concentration on Rotifers

Food level is an important factor affecting the survival and development of rotifers. The earlier studies reported that Ibuprofen [33], penicillin sodium [34] and oxytetracycline [31] had opposite effects on rotifers' life table parameters under different algal densities. Similarly, in the present study, at low algal density (1.0 × 10^6 cells/mL), TBM significantly affected rotifers e_0 and T, which was not the case at medium or high algal density (2.0 × 10^6, 4.0 × 10^6 cells/mL). Compared with medium and high algal density, the R_0 and r_m of rotifers were more significantly affected by TBM at low algal density (Table 3). There are several possible explanations for this phenomenon. Firstly, with increasing algae density, rotifers have more food to eat and produce sufficient energy to counter the toxicity of TBM. Secondly, the amount of toxin in each algal cell declines when algal density is medium or high, which can make rotifers' total intake of toxicants decrease.

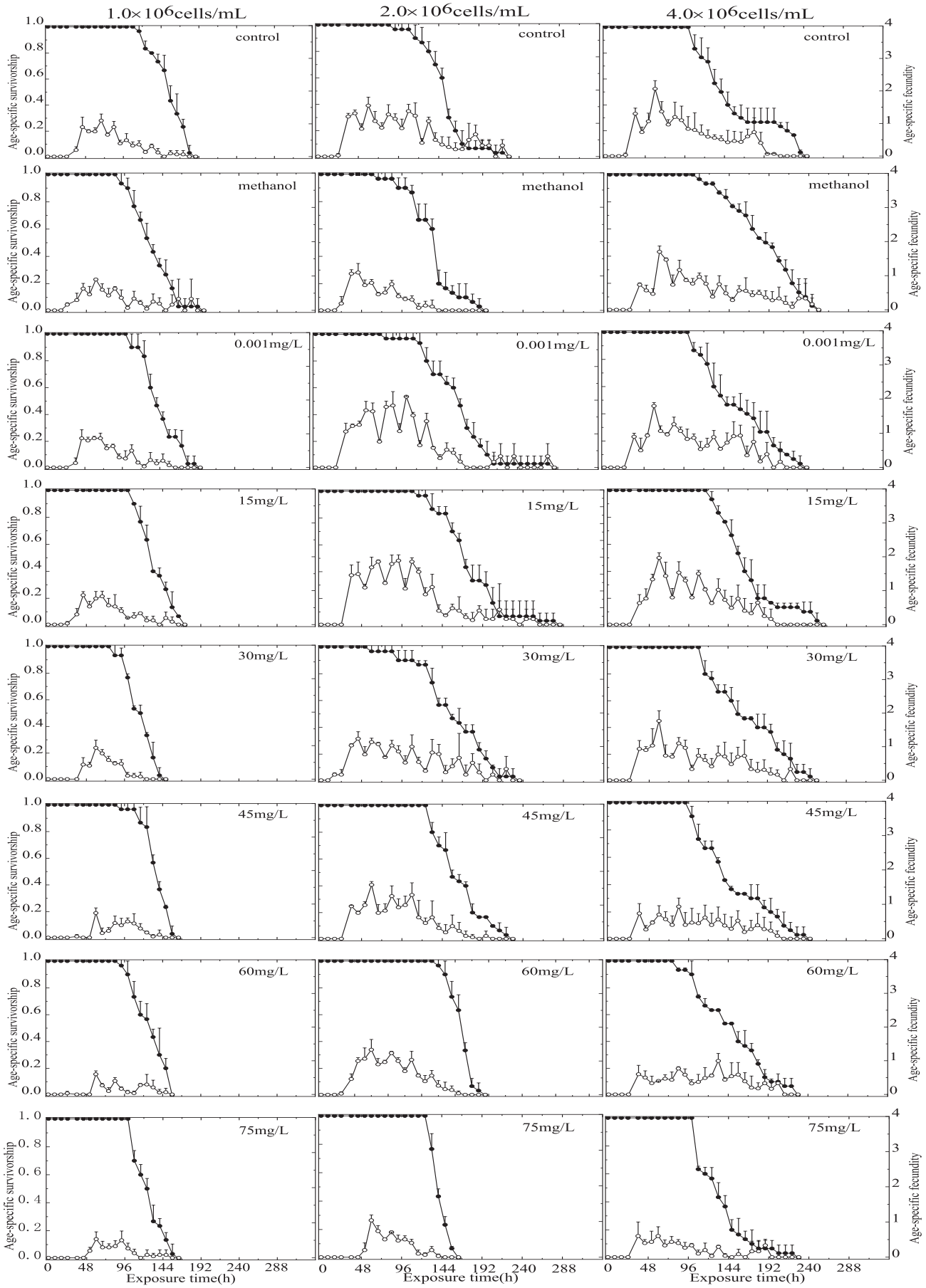

Fig. 2. Age-specific survivorship (filled circle) and fecundity (unfilled circle) of *B. calyciflorus* exposed to different concentrations at three algal densities.

Table 2. The relationships between life table demographic parameters of *B. calyciflorous* and TBM concentration at three algae densities.

Algal density (×10^{6}cells/mL)	Parameters	Regressive equation	Significant test
1.0	e_0	$y = 0.009x^2-0.897x+141.09$	$R^2 = 0.477$, $p<0.01$
	T	$y = 0.141x+73.11$	$R^2 = 0.280$, $p<0.05$
	R_0	$y = 0.001x^2-0.119x+6.957$	$R^2 = 0.912$, $p<0.01$
	r_m	$y = 0.00001x^2+0.3$	$R^2 = 0.801$, $p<0.01$
2.0	R_0	$y = -0.002x^2-0.001x+17.3$	$R^2 = 0.701$, $p<0.01$
	r_m	$y = -0.000006x^2+0.041$	$R^2 = 0.873$, $p<0.01$
4.0	e_0	$y = -0.008x^2+0.2x+159.12$	$R^2 = 0.321$, $p<0.05$
	R_0	$y = -0.001x^2-0.072x+15.1$	$R^2 = 0.677$, $p<0.01$
	r_m	$y = -0.000234x+0.039$	$R^2 = 0.668$, $p<0.01$

Table 3. Effects of TBM on the life-table demographic parameters of *B. calyciflorus* (mean±SE).

Parameters	Concentrations (mg/L)	Algal density (×10^6 cells/mL)		
		1.0	2.0	4.0
e_0(h)	Control	148.3±1.4	145.6±6.2	162.4±16.0
	Methanol	145.1±2.2	146.4±6.3	179.2±7.1
	0.001	137.3±2.5	162.1±9.3	153.7±4.7
	15	128.3±3.0**	169.9±4.0	162.4±13.3
	30	109.6±7.0**	151.2±13.4	162.9±7.2
	45	136.8±2.6	157.6±11.1	143.5±15.2
	60	121.6±4.0**	161.3±1.9	142.4±6.7
	75	119.7±4.6**	136.0±2.4	128.0±11.7
T(h)	Control	76.9±1.0	85.3±2.5	86.5±6.6
	Methanol	72.0±1.9	83.4±0.9	97.7±3.7
	0.001	73.6±1.8	89.1±2.2	91.6±0.4
	15	71.0±3.1	88.9±3.8	90.6±3.6
	30	69.1±1.7*	82.5±4.0	88.4±1.7
	45	89±0.4**	84.1±7.5	84.6±11.3
	60	84.7±3.7*	83.1±1.5	93.4±2.2
	75	80.3±2.3	81.6±2.0	71.8±5.2
R_0	Control	7.4±0.4	14.7±1.5	15.1±2.4
	Methanol	6.8±0.2	13.7±0.14	13.8±0.8
	0.001	6.3±0.2**	18.9±0.4*	13.7±0.7
	15	5.7±0.4**	20.6±0.8**	16.6±1.8
	30	4.2±0.3**	11.9±1.0*	12.4±1.3
	45	3.3±0.2**	13.0±1.2	7.6±2.0**
	60	2.2±0.1**	11.8±0.5*	6.8±0.5**
	75	2.7±0.2**	4.9±0.1**	4.3±0.6**

r_m	Control	0.029±0.001	0.04±0.001	0.040±0.001
	Methanol	0.026±0.001	0.035±0.001	0.035±0.001
	0.001	0.028±0.001	0.042±0.001	0.036±0.001
	15	0.03±0.004	0.044±0.001*	0.038±0.001
	30	0.021±0.001**	0.040±0.001	0.037±0.002
	45	0.014±0.001**	0.037±0.002	0.025±0.006**
	60	0.009±0.001**	0.035±0.002*	0.025±0.001**
	75	0.012±0.001**	0.020±0.001**	0.023±0.002**

Significantly different from the controls (p<0.05; **p<0.01)

Table 4. Effects of algal density and TBM concentration on life table demographic parameters of *B. calyciflorous* (two-way ANOVA).

Parameters	Sources	*SS*	df	MS		
e_0	Algal density (A)	8856.30	2	4428.15	20.8	p<0.01
	Concentration (B)	4280.14	6	713.36	3.351	p<0.01
	AB	4128.56	12	344.05	1.616	p>0.05
	Error	8941.55	42	212.89		
T	Algal density (A)	967.14	2	483.57	9.426	p<0.01
	Concentration (B)	548.96	6	91.49	1.784	p>0.05
	AB	1434.10	12	119.51	2.330	p<0.05
	Error	2154.59	42	51.3		
R_0	Algal density (A)	930.50	2	465.25	124.47	p<0.01
	Concentration (B)	743.33	6	123.89	33.15	p<0.01
	AB	199.27	12	16.31	4.44	p<0.01
	Error	156.99	42	3.74		
r_m	Algal density (A)	0.00	2	1.510^{-3}	132.90	p<0.01
	Concentration (B)	0.00	6	4.910^{-4}	44.59	p<0.01
	AB	4.7710^{-4}	12	4.010^{-5}	3.56	p<0.01
	Error	4.6810^{-4}	42	1.110^{-5}		

A low concentration of TBM (0.001, 15 mg/L) could significantly reduce the R_0 of rotifers under the algal densities of 1.0×10^6 cells/mL but increase the R_0 at the algal density of 2.0×10^6 cells/mL. Nevertheless, we did not observe any effects of TBM on the R_0 of rotifers if the algal density reached 4.0×10^6 cells/mL. These results showed the toxic effects of TBM on rotifers could be affected by algal density. The interaction of TBM concentration and algal density significantly influenced the T, R_0 and r_m of rotifers (p<0.05), suggesting that both TBM concentration and algal density play an essential role in rotifer population growth (Table 4).

It has long been proposed that r_m is one of the most effective indicators in life table experiments for monitoring environmental toxicity effects because r_m can explicitly express the survival rate and reproductive rate of each age in the population [35]. However, a recent study argued that the r_m is not necessarily the most sensitive index, and R_0 may have a lower LOEC value [27]. In our study, R_0 had the lowest LOEC under three algal densities, suggesting that R_0 could be the most sensitive endpoint. R_0 also had lowest EC_{50} under the algal densities of 4.0×10^6 cells/mL. This finding supports the hypothesis of taking R_0 as the experimental endpoint rather than r_m (Table 5).

According to the World Health Organization, the safety limit of TBM in natural water bodies is 0.1 mg/L [36]. However, our study shows that the survival, fecundity, and reproduction of rotifers can be affected when the concentration of TBM is 0.001 mg/L. Rotifers are major primary consumers in freshwater systems, play a significant role in energy conversion and

Table 5. The NOEC (mg/L), LOEC (mg/L) and EC_{50} (mg/L) of life-table parameters of *B. calyciflorous* under three algae densities.

Algal density (×10^6 cells/mL)	Parameters	NOEC	LOEC	EC_{50}
1.0	e_0	0.001	15	-
	T	15	30	-
	R_0	-	0.001	67.35
	r_m	15	30	-
2.0	e_0	-	-	-
	T	-	-	-
	R_0	-	0.001	61.52
	r_m	0.001	15	58.45
4.0	e_0	-	-	113.00
	T	-	-	-
	R_0	30	45	58.05
	r_m	30	45	70.51

-: By setting concentration limit, this parameter was not obtained

maintain the stability of aquatic ecosystem community structure and function. Therefore, at its current safety limit, TBM would profoundly impact the population growth of rotifers and, eventually, the balance of aquatic ecosystems.

Conclusions

The increased algal density can reduce the toxic effects of TBM on rotifers. Because R_0 had the lowest LOEC under three algal densities, we speculated that R_0 is the most sensitive to TBM. In addition, a significant dose-response relationship between TBM concentration and R_0 and r_m may exist under three algal densities. We concluded that the current safety limit of TMB in natural water could affect the community structure of organisms in the aquatic system and then disturb the ecological balance. However, these effects could be reversed by increasing algae densities in water.

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Conflict of Interest

The authors declare no conflict of interest.

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