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RESEARCH ARTICLE

Combined effects of oxytetracycline concentration and algal food level on the life-table demography of *Brachionus calyciflorus* (Rotifera)

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Abstract – Oxytetracycline (OTC) is commonly used for aquaculture and livestock, and its environmental concentration has increased to a considerable level and displays potential environmental risk. In the present study, the life-table demography of *Brachionus calyciflorus* exposed to sublethal concentrations (30.0, 60.0, 90.0, 120.0, 150.0 and 180.0 mg L^{-1}) of OTC was investigated at 1.0×10^6, 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *Scenedesmus obliquus*. The results showed that at each algal density, OTC concentration affected significantly life expectancy at hatching, net reproductive rate, generation time and intrinsic rate of population increase ($P < 0.01$), but did not affect proportion of mictic offspring of the rotifers ($P > 0.05$). Compared to the controls, and at 2.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 30.0–150.0 and 60.0–120.0 mg L^{-1} significantly prolonged life expectancy at hatching and generation time, treatments with OTC at 30.0–120.0 and 30.0–90.0 mg L^{-1} increased net reproduction rate and intrinsic rate of population increase, respectively, but the reverse was also true for those with OTC at 180.0 mg L^{-1}. Higher and lower algal densities decreased the magnitude of stimulatory effects of lower concentrations of OTC but enhanced that of inhibitory effects of high concentration of OTC on the survival, asexual reproduction and population growth of the rotifers. At the three algal densities, net reproduction rate was more sensitive to OTC than the other endpoints, and significant concentration-effect relationship existed between OTC concentration and each of all the life-table demographic parameters except the proportion of mictic offspring.

Keywords: Rotifer / antibiotics / algal density / chronic toxicity / life history

1 Introduction

Antibiotics are pharmaceuticals widely used not only for human and veterinary medication but also for livestock and aquaculture growth promotion (Sarmah *et al.*, 2006). After normal application, 50–90% of antibiotics and/or their metabolites are excreted from the body *via* feces or urine and enter the environment indirectly or directly (Schlusener and Bester, 2006). Contamination of water and soil systems with low concentrations of antibiotics may create bacteria resistant to antibiotics and then public health problems (Mulamattathil *et al.*, 2014; Poonia *et al.*, 2014). In recent years, the occurrence of antibiotics in aquatic ecosystems and their effects have received increasing attention by the scientific community (Sarmah *et al.*, 2006; Kümmerer, 2009; Aarestrup, 2012).

Oxytetracycline (OTC), originally isolated from *Streptomyces* in soil, is a broad-spectrum antibiotic against gram-negative and positive bacteria. Due to its low cost, high efficiency and practicability, OTC was reported as pharmaceutical of high usage and high potential risk for the aquatic environment in England, Korea and China (Jones *et al.*, 2002; Lee *et al.*, 2008; Ji *et al.*, 2012), and showed acute and chronic toxicity to organisms of different trophic levels, such as bacteria, algae, invertebrates and fish (Carvalho and Santos, 2016).

Zooplankton is frequently used to detect anthropogenic contamination because of their sensitivity to various toxicants and their important role in aquatic ecosystems. Among them, rotifers, especially *Brachionus calyciflorus* and *Brachionus plicatilis*, are frequently used in aquatic ecotoxicological studies (Snell and Janssen, 1995; Dahms *et al.*, 2011). With

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rotifers as test animals, Isidori *et al.* (2005) investigated the LC_{50} and EC_{50} values of OTC to *B. calyciflorus*, Göksan and Gökpinar (2001) studied the effects of OTC on the activity and mortality of *B. plicatilis*, and Rotman (2011) assessed the effects of OTC on population growth of *B. plicatilis*, but they did not deal with the effects on life-table demographic parameters which are the important theoretical bases to measure the effects of chronic exposure of populations to toxicants (Ferrando *et al.*, 1996).

Monogonont rotifers, such as *B. calyciflorus* and *B. plicatilis*, have a cyclical parthenogenesis mode of reproduction. Parthenogenesis dominates the monogonont life cycle where reproduction occurs in the absence of males (amicitc phase). After a period of clonal propagation, a sexual phase starts, induced by environmental factors such as population density, when some asexual females start to parthenogenetically produce sexual daughters that produce haploid eggs through meiosis which develop either into haploid males or, if fertilized, into resting eggs (Wallace *et al.*, 2006).

Food level is a key factor affecting not only the survival, reproduction and population growth of rotifers but also the toxicity of pollutants, and higher and lower algal densities enhance the negative effects of pollutants on the survival, reproduction and population growth of rotifers (Nandini and Sarma, 2000; Sarma *et al.*, 2001; Pickhardt *et al.*, 2002; Ramírez-Pérez *et al.*, 2004; Pan *et al.*, 2016). In the present study, we investigated the chronic toxicity of sublethal concentrations of OTC to *B. calyciflorus* in relation to *Scenedesmus obliquus* level, and compared the relative sensitivity of various endpoints to OTC exposure, with the aim of testing the following two hypotheses: (i) lower concentrations of OTC had stimulatory effects on the survival, asexual reproduction and population growth of *B. calyciflorus*, (ii) the sensitivity of life-table demographic parameters of *B. calyciflorus* to OTC varied with algal density, based on the effects of rifampicin on the survival, asexual reproduction and population growth of *B. calyciflorus* (Zhai *et al.*, 2016).

2 Materials and methods

2.1 Collection and culture of test animals

An individual of *B. calyciflorus* was obtained by hatching a resting egg collected from sediments of Lake Fengming (31°20′N, 119°21′E), identified morphologically under a microscope based on the taxonomic description by Koste (1978), and then clonally cultured under controlled laboratory conditions. Stock rotifer culture had been kept under static-renewal conditions with a 16: 8 h light: dark photoperiod at 130 lx at 25 ± 1 °C in an illumination incubator for over 3 months, with EPA medium (96 mg $NaHCO_3$, 60 mg $CaSO_4 \cdot 2H_20$, 60 mg $MgSO_4$ and 4 mg KCl per liter of distilled water; USEPA, 1993) as culture medium and the green algae *S. obliquus* as food. Before the experiments commenced, rotifers were cultured at 1.0×10^6, 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus* for at least 2 weeks. During acclimation, every 24 h residual food and resting eggs, if produced, in the bottom of each glass tube were eliminated, the culture medium was renewed, and the chosen density of *S. obliquus* was supplied. Algae were grown in a semi-continuous culture using HB-4 medium (Li *et al.*, 1959) renewed daily at 20%. Algae in exponential growth were centrifuged and resuspended in EPA medium.

2.2 Preparation of test solutions

Oxytetracycline hydrochloride (99.0% purity) used in the present study was purchased from Beijing Solarbio Technology Co. Ltd., China. Stock solution of 900 mg L^{-1} was carefully prepared by dilution of OTC in distilled water, and kept refrigerated and protected from light. Test solutions were prepared immediately before the beginning of the test by successive dilution of the stock.

2.3 Life table experiments

Based on the LC_{50}-value (34.21 mg L^{-1}) of Isidori *et al.* (2005), we selected seven toxicant concentrations (0 (control), 30.0, 60.0, 90.0, 120.0, 150.0 and 180.0 mg L^{-1}) for the life-table experiments. Considering the algal densities in eutrophic water bodies (Pickhardt *et al.*, 2002), we chose three densities (1.0×10^6, 2.0×10^6 and 4.0×10^6 cells mL^{-1}) of *S. obliquus*. For each toxicant concentration and food level combination, four replicates were set up. Life-table experiments were conducted in 8 mL glass cups each containing 5 mL test solution with the chosen density of algal food. We started the experiments by introducing 10 neonates (<4 h old) into each of the 84 test cups (seven toxicant concentrations × three food levels × four replicates). The experiments were conducted in darkness at 25±1 °C. During the experiments, every 12 h the number of initial individuals and the number of produced neonates (amictic and mictic) were counted under a microscope. Dead individuals were removed, and the neonates were moved into another fresh cup and cultured at the same conditions as stated above until they produced their eggs. Based on the egg type (amictic or mictic eggs), the female type of neonate (amictic or mictic female) was identified and the proportion of mictic offspring was calculated as the number of mictic offspring divided by the number of amictic and mictic offspring. Every 24 h the initial rotifers alive were transferred into freshly prepared test solution. The experiments were terminated when all initial individuals died (Birch, 1948).

Based on the data collected, age-specific survival (l_x) and age-specific fecundity (m_x) were constructed for each cohort using conventional life-table techniques (Poole, 1974), and life expectancy at hatching (e_0), generation time (T), net reproductive rate (R_0) and intrinsic rate of population increase (r_m) were calculated according to Krebs (1985) and Lotka (1913):

Net reproductive rate: $R_0 = \sum_0^\infty l_x m_x$

Generation time: $T = \frac{\sum l_x m_x x}{R_0}$

Intrinsic rate of population increase (r), first an approximation using: $r\text{-rough} = \ln R_0 / T$

For final calculation, we solved the equation: $\sum_{x=0}^{n} e^{-rx} l_x m_x = 1$

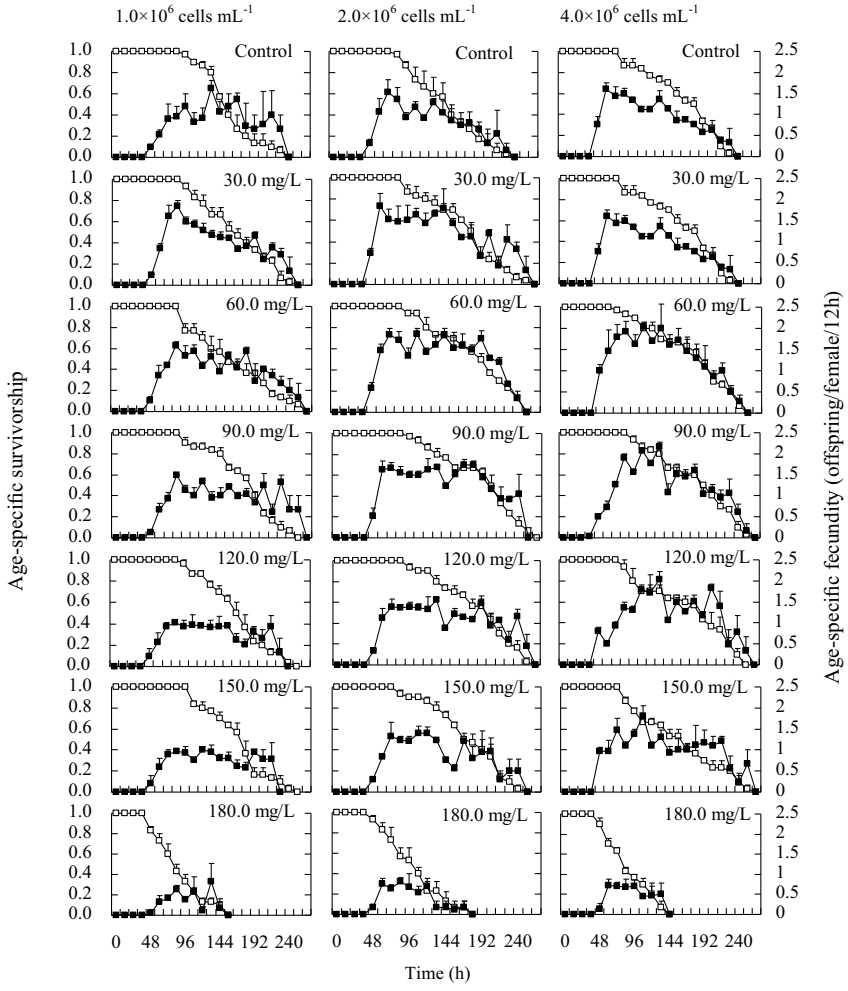

Fig. 1. Age-specific survivorship (unfilled square) and fecundity (filled square) of *B. calyciflorus* exposed to different concentrations of OTC and cultured at three food densities (mean ± standard error).

2.4 Statistical analyses

All statistical analyses were performed using SPSS 11.5. The Levene's test was performed to test the homogeneity of variances. Kaplan–Meier analyses were conducted to test for the differences in the age-specific survivorships of the rotifer cohorts among the seven groups at each food level. One-way analysis of variance (ANOVA) was conducted to identify the significant effect of OTC concentration on each of the life history variables of the rotifers cultured at each algal density, and two-way ANOVA was conducted to analyze the significant effects of toxicant concentration, food level and their interactions on each life-history variable. Multiple comparisons of the least significant difference were performed to determine which groups were significantly different among the seven groups at each food level. The relationships between OTC concentration and each of life-table demographic parameters were regressively analyzed. Results with P values of less than 0.05 were considered statistically significant.

3 Results

The age-specific survivorship and fecundity of *B. calyciflorus* exposed to different concentrations of OTC and cultured at three food densities are presented in Figure 1. Kaplan–Meier analyses revealed that OTC concentration significantly affected the survivorship of *B. calyciflorus* cultured at each algal density ($P < 0.05$). Compared with the controls, and at 1.0×10^6, 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus*, the rotifers exposed to OTC at 60.0–120.0, 30.0–150.0, and 90.0 and 120.0 mg L^{-1} survived longer, but the reverse was also true for those exposed to OTC at 150.0 and 180.0, 180.0, and 150.0 and 180.0 mg L^{-1}, respectively ($P < 0.05$). The OTC concentration at which the rotifers had a maximum fecundity increased from 30.0 to 30.0 and 60.0, and 60.0 and 90.0 mg L^{-1} when the algal level increased from 1.0×10^6 to 2.0×10^6 and 4.0×10^6 cells mL^{-1} (Fig. 1).

Figure 2 shows the selected life history variables of *B. calyciflorus* subjected to different concentrations of OTC and cultured at three food densities. OTC concentration

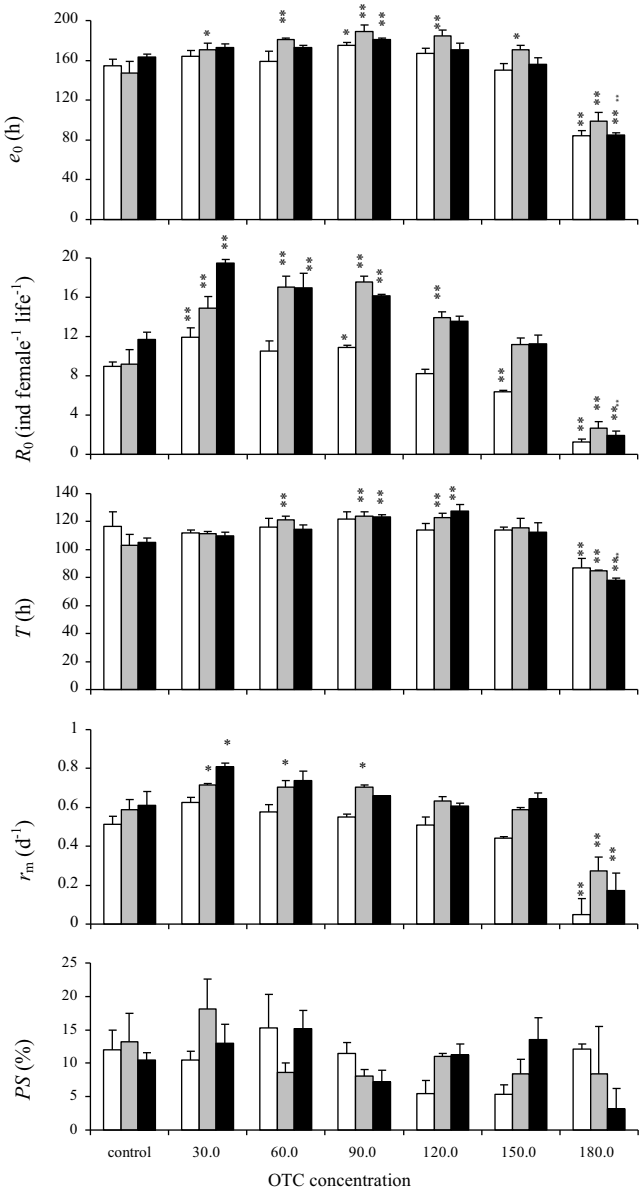

Fig. 2. Life expectancy at hatching (e_0), net reproduction rate (R_0), generation time (T), intrinsic rate of population increase (r_m) and proportion of sexual offspring (PS) of *B. calyciflorus* exposed to different concentrations of OTC and cultured at 1.0×10^6 (white bars), 2.0×10^6 (grey bars) and 4.0×10^6 (black bars) cells mL^{-1} of *Scenedesmus obliquus* (mean ± standard error). *Significant ($P < 0.05$) or **highly significant ($P < 0.01$) difference from the control at the same algal density.

significantly affected all the selected life-table demographic parameters ($P < 0.01$) except the proportion of mictic offspring ($P > 0.05$) of *B. calyciflorus* cultured at each algal density. Compared with the control, and at 2.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 30.0–150.0 mg L^{-1} significantly prolonged the life expectancy at hatching. However, at both 1.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus*, only the treatment with OTC at 90.0 mg L^{-1} significantly prolonged it. At the three food levels, treatment with OTC at 180.0 mg L^{-1} shortened the life expectancy at hatching (Fig. 2).

Compared with the control, and at 2.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 30.0–120.0 mg L^{-1} significantly increased the net reproduction rate, and that with OTC at 180.0 mg L^{-1} decreased it. However, at 1.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 30.0 and 90.0 mg L^{-1} significantly increased the net reproduction rate, and those with OTC at 150.0 and 180.0 mg L^{-1} decreased it. At 4.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 30.0–90.0 mg L^{-1} significantly increased the net reproduction rate, and that with OTC at 180.0 mg L^{-1} decreased it (Fig. 2).

Compared with the control, and at 2.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 60.0–120.0 mg L^{-1} significantly prolonged the generation time. However, at 1.0×10^6 cells mL^{-1} of *S. obliquus*, no treatment significantly prolonged the generation time. At 4.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 90.0 and 120.0 mg L^{-1} significantly prolonged the generation time. At the three food levels, treatment with OTC at 180.0 mg L^{-1} shortened the generation time (Fig. 2).

Compared to the control, and at 2.0×10^6 cells mL^{-1} of *S. obliquus*, treatments with OTC at 30.0–90.0 mg L^{-1} significantly increased the intrinsic rates of population increase. However, at 1.0×10^6 cells mL^{-1} of *S. obliquus*, no treatment significantly increased the intrinsic rate of population increase. At 4.0×10^6 cells mL^{-1} of *S. obliquus*, treatment with OTC at 30.0 mg L^{-1} significantly increased the intrinsic rate of population increase. At the three food levels, treatment with OTC at 180.0 mg L^{-1} decreased the intrinsic rate of population increase (Fig. 2).

Two-way ANOVA showed that food level had significant effects on the life expectancy at hatching, the net reproduction rate and the intrinsic rate of population increase ($P < 0.01$), OTC concentration had marked effects on the life expectancy at hatching, the net reproduction rate, the generation time and the intrinsic rate of population increase ($P < 0.01$), and the interaction between food level and OTC concentration had a significant effect on the net reproduction rate ($P < 0.01$) (Tab. 1).

A clear concentration-effect relationship existed between OTC concentration and life expectancy at hatching, net reproductive rate, generation time as well as intrinsic rate of population increase (Tab. 2).

4 Discussion

Under the chronic stress of antibiotics including streptomycin sulfate, tetracycline hydrochloride, tylosin tartrate and amoxicillin, the average lifespan and/or the life expectancy at hatching of *B. calyciflorus*, *B. plicatilis* and *B. havanaensis* significantly shortened (Araujo and McNair, 2007; González-Pérez *et al.*, 2016). However, lower concentrations of rifampicin markedly prolonged the average lifespan and the life expectancy at hatching of *B. calyciflorus* (Zhai *et al.*, 2016). Similarly, in the present study, OTC at 90.0, 30.0–150.0 and 90.0 mg L^{-1} significantly prolonged the life expectancy at hatching of *B. calyciflorus* cultured at 1.0×10^6, 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus*, respectively. In addition, OTC at 180.0 mg L^{-1} shortened the life expectancy at hatching.

Table 1. Effects of algal density and OTC concentration on life expectancy at hatching (e_0), net reproduction rate (R_0), generation time (T), intrinsic rate of population increase (r_m) and proportion of sexual offspring (PS) of *B. calyciflorous* (two-way ANOVA).

Parameter	Source	*SS*	d.f.	*MS*	*F*	*P*
e_0	Algal density (A)	1618.61	2	809.30	7.60	$P<0.01$
	OTC con. (B)	52 282.19	6	8713.70	81.84	$P<0.01$
	A × B	1476.43	12	123.04	1.16	$P>0.05$
	Error	4471.68	42	106.47		
R_0	Algal density (A)	271.94	2	135.97	74.11	$P<0.01$
	OTC con. (B)	1216.72	6	202.79	110.53	$P<0.01$
	A × B	99.14	12	8.26	4.50	$P<0.01$
	Error	77.06	42	1.84		
T	Algal density (A)	31.86	2	15.93	0.23	$P>0.05$
	OTC con. (B)	9647.32	6	1607.89	22.93	$P<0.01$
	A × B	789.26	12	65.77	0.94	$P>0.05$
	Error	2945.63	42	70.13		
r_m	Algal density (A)	0.27	2	0.13	24.19	$P<0.01$
	OTC con. (B)	1.80	6	0.30	54.38	$P<0.01$
	A × B	0.05	12	0.00	0.78	$P>0.05$
	Error	0.23	42	0.01		
PS	Algal density (A)	0.00	2	0.00	0.05	$P>0.05$
	OTC con. (B)	0.03	6	0.01	1.84	$P>0.05$
	A × B	0.05	12	0.00	1.59	$P>0.05$
	Error	0.11	42	0.00		

SS, sum of squares; d.f., degrees of freedom; *MS*, mean square; *F*, *F* ratio.

Table 2. Relationships between life expectancy at hatching (e_0, h), net reproduction rate (R_0, ind female^{-1} life^{-1}), generation time (T, h) and intrinsic rate of population increase (r_m, d^{-1}) of *B. calyciflorus* cultured at three algal densities and OTC concentration (x, mg L^{-1}).

Parameters	Regression equation	Significant tests
1.0×10^6 cells mL^{-1}		
e_0	$y=-0.006x^2+0.874x+146.819$	$R^2=0.773$, $P<0.01$
R_0	$y=-0.001x^2+0.073x+9.303$	$R^2=0.909$, $P<0.01$
T	$y=-0.002x^2+0.278x+111.3$	$R^2=0.447$, $P<0.01$
r_m	$y=-3.489\times10^{-5}x^2+0.004x+0.505$	$R^2=0.816$, $P<0.01$
2.0×10^6 cells mL^{-1}		
e_0	$y=-0.008x^2+1.308x+141.41$	$R^2=0.796$, $P<0.01$
R_0	$y=-0.001x^2+0.212x+9.37$	$R^2=0.914$, $P<0.01$
T	$y=-0.004x^2+0.633x+99.429$	$R^2=0.735$, $P<0.01$
r_m	$y=-3.356\times10^{-5}x^2+0.005x+0.586$	$R^2=0.822$, $P<0.01$
4.0×10^6 cells mL^{-1}		
e_0	$y=-0.007x^2+0.892x+156.143$	$R^2=0.854$, $P<0.01$
R_0	$y=-0.001x^2+0.152x+13.024$	$R^2=0.878$, $P<0.01$
T	$y=-0.004x^2+0.643x+99.059$	$R^2=0.707$, $P<0.01$
r_m	$y=-3.627\times10^{-5}x^2+0.004x+0.633$	$R^2=0.715$, $P<0.01$

The above stated results indicated that lower concentrations of rifampicin and OTC might have a stimulating effect on the survival of the rotifers, but higher concentrations of them might have a toxic effect.

As a consequence of chronic stress of streptomycin sulfate, tetracycline hydrochloride, tylosin tartrate and amoxicillin, a reduction in net reproduction rate was observed in *B. calyciflorus*, *B. plicatilis* and *B. havanaensis*

(Araujo and McNair, 2007; González-Pérez *et al.*, 2016). However, lower concentrations of rifampicin increased the net reproduction rate of *B. calyciflorus* cultured at 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus* (Zhai *et al.*, 2016). In the present study, OTC at 30.0 and 90.0, 30.0–120.0, and 30.0–90.0 mg L^{-1} significantly increased the net reproduction rate, but OTC at 150.0 and 180.0, 180.0 and 180.0 mg L^{-1} decreased the net reproduction rate of the rotifers cultured at 1.0×10^6, 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus*, respectively. It is worthy of further researching that how lower concentrations of OTC stimulate the reproduction of the rotifers.

Amoxicillin at 50–200 and 200 μg L^{-1} significantly shortened the generation time of *B. calyciflorus* and *B. havanaensis*, respectively (González-Pérez *et al.*, 2016). However, rifampicin at 2.0–10.0 and 10.0 mg L^{-1} significantly prolonged the generation time of *B. calyciflorus* cultured at 1.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus*, respectively (Zhai *et al.*, 2016). Similarly, in the present study, OTC at 60.0–120.0 and 90.0–120.0 mg L^{-1} significantly prolonged the generation time of the rotifers when they were cultured at 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus*, respectively. In addition, the present study also showed that OTC at 180.0 markedly shortened the generation time. The generation time is the average length of time between the birth of an individual and the birth of its own offspring. As such, it reflects changes in the time required to reach sexual maturity and the embryonic developmental time. The effects of sublethal concentrations of OTC on the time required to reach sexual maturity and the embryonic developmental time of the rotifers need investigation.

The results available now have showed that the effect of antibiotics on the intrinsic rate of population increase of rotifers varied with antibiotic species and concentration, rotifer species, and algal density (Wang et al., 2008; Rotman, 2011; González-Pérez et al., 2016; Zhai et al., 2016). Similarly, the present study showed that at 1.0×10^6 cells mL^{-1} of *S. obliquus*, OTC at 30.0–150.0 mg L^{-1} did not significantly affect the intrinsic rate of population increase of *B. calyciflorus*. However, at 2.0×10^6 and 4.0×10^6 cells mL^{-1} of *S. obliquus*, OTC at 30.0–90.0, and 30.0 mg L^{-1} increased significantly the intrinsic rate of population increase, respectively. At the three food densities, OTC at 180.0 mg L^{-1} significantly decreased the intrinsic rate of population increase of the rotifers.

Lower concentrations of OTC prolonged significantly the life expectancy at hatching and the generation time, and increased the net reproduction rate and the intrinsic rate of population increase of *B. calyciflorous*, which supported the hypothesis that lower concentrations of OTC had stimulatory effects on the survival, asexual reproduction and population growth of *B. calyciflorus*.

The inhibitory effects of higher concentrations of streptomycin sulfate, tetracycline hydrochloride, tylosin tartrate and amoxicillin on the survival, reproduction and population growth of rotifers are usually attributed to their toxicity (Araujo and McNair, 2007; González-Pérez *et al.*, 2016), and the stimulatory effects of lower concentrations of rifampicin were attributed to their inhibition of bacteria which might be harmful to rotifers (Zhai *et al.*, 2016). The mechanisms under the effects of sublethal concentrations of OTC on the survival, reproduction and population growth of *B. calyciflorus* might be the same as those above stated antibiotics.

Mictic female production is a prerequisite for resting egg production and is modulated by internal and external factors (Gilbert, 2004). Zhai *et al.* (2016) found that at the three algal levels, rifampicin at 2.0–10.0 mg L^{-1} significantly increased the proportion of mictic offspring of *B. calyciflorus*. Different from those results, the present study showed that at the three algal levels, OTC at 30.0–180.0 mg L^{-1} did not significantly affect the proportion of mictic offspring of *B. calyciflorus*.

The sensitivity of life-table demographic parameters of *B. calyciflorous* to antibiotics varied with not only antibiotic species but also algal density (Araujo and McNair, 2007; Zhai *et al.*, 2016). Identical results were obtained in the present study. At 1.0×10^6 cells mL^{-1} of *S. obliquus*, the net reproduction rate was the most sensitive to OTC contamination. At 2.0×10^6 cells mL^{-1} of *S. obliquus*, the life expectancy at hatching, the net reproduction rate and the intrinsic rate of population increase had the same sensitivity. At 4.0×10^6 cells mL^{-1} of *S. obliquus*, both the net reproduction rate and the intrinsic rate of population increase had the same sensitivity. These results supported the hypothesis that the sensitivity of life-table demographic parameters of *B. calyciflorous* to OTC varied with algal density.

5 Conclusion

At 2.0×10^6 cells mL^{-1} of *S. obliquus*, lower concentrations of OTC prolonged significantly the life expectancy at hatching and the generation time, and increased the net reproduction rate and the intrinsic rate of population increase, but higher concentrations of OTC shortened or decreased them. Both increase and decrease in algal density decreased the stimulatory effect of lower concentrations of OTC but enhanced the inhibitory effect of higher concentrations of OTC (Fig. 2). At the three algal densities, the net reproduction rate was the most sensitive to OTC, and significant concentration-effect relationships existed between OTC concentration and life expectancy at hatching, net reproductive rate, generation time as well as intrinsic rate of population increase. In natural water bodies, OTC pollution will change the dynamics patterns of *B. calyciflorous* population, the community structures and the functioning of ecosystems. The effect magnitude will vary with algal biomass in water bodies.

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