Bull Environ Contam Toxicol (2012) 88:20–24
DOI 10.1007/s00128-011-0450-5

Effect of *Microcystis aeruginosa* on the rotifer *Brachionus calyciflorus* at different temperatures

Xiong Zhang · Hong Geng

Received: 28 February 2011 / Accepted: 24 October 2011 / Published online: 6 November 2011

Abstract Rotifers are one of the smallest metazoans. They serve as a model organism for ecotoxicological studies. More than 60% of the lakes in China are increasingly eutrophic and they are susceptible to blooms of *Microcystis aeruginosa.* We investigated the effects of *M. aeruginosa* on the survival and reproduction of *Brachionus calyciflorus* using the life table method at different temperatures. The findings showed that concentration of *M. aeruginosa* significantly affected the intrinsic rate of increase (r_m), net reproductive rate (R_0), average lifespan (L) and offspring number ($p < 0.05$). Temperature also significantly affected the generation time (T), average lifespan (L) and offspring number ($p < 0.05$). Moreover, the interaction between temperature and concentration had statistically significant effects on offspring number ($p < 0.05$). *M. aeruginosa* suppressed the survival and reproduction of *B. calyciflorus*, particularly at a concentration of 10^6 cells/mL. The r_m values of the rotifers exposed at 10^6 cells/mL decreased more than 200% compared with those of the control group. However, at a lower concentration, 10^4 cells/mL, *M. aeruginosa* may supply appropriate nourishment to rotifers. In addition, at concentrations of 10^5 and 10^6 cells/mL, the inhibition of rotifers by *M. aeruginosa* heightened with increasing temperature.

Keywords *Brachionus calyciflorus* · *Microcystis aeruginosa* · Survival and reproduction · Temperature

X. Zhang · H. Geng (✉)
Key Laboratory for Microorganism and Bio-transformation, College of Life Science, South-Central University for Nationalities, Wuhan 430074, China
e-mail: genghong961007@yahoo.com.cn

With increasing human activities, the occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs, and recreational waters has become a worldwide problem. The eutrophication of lakes is one of the most serious environmental problems in China. More than 60% of the lakes have been reported to be eutrophic with the outbreak of cyanobacterial blooms in recent years (Gan et al. 2010). Such blooms are occurring annually in many aquatic systems in China, such as Lakes Dianchi, Chaohu and Taihu. *Microcystis aeruginosa* is usually the most dominant species (Liu et al. 2006; Gan et al. 2010) and it can produce different kinds of microcystins (Li et al. 2010), such as hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins. These toxins are harmful to a wide range of organisms (Gilbert 1996; Liu et al. 2006; Tilimanns et al. 2008).

Rotifers are common and important members of freshwater zooplankton communities. They have been used as a model organism for ecotoxicological studies. Cyanobacteria have been proposed to decrease the population growth of zooplankton in three basic ways: (1) by foming large colonial and filamentous morphologies (Trabeau et al. 2004), (2) by causing nutritional deficiencies (Geng and Xie 2008), and (3) by producing toxic secondary metabolites (Wilson and Hay 2007). Hansson et al. (2007) showed that the total zooplankton biomass was negatively correlated with microcystin concentrations, but rotifers exhibited a positive net response at the proper level of toxins. The results of the above-mentioned studies are on rotifers found in temperate climates. However, information about rotifers inhabiting subtropical waters is limited.

The occurrence of *M. aeruginosa* blooms are frequently related to environmental factors, such as temperature, light, pH and salt concentration. Temperature is an especially important factor in influencing the growth of

M. aeruginosa. Temperature has been shown to have a positive effect on the growth of *M. aeruginosa* (Jiang et al. 2008; Tonk et al. 2009). It has also been shown that the amount of toxin (Watanabe and Oishi 1985; Sivonen 1990; Tilimanns et al. 2008) and the type of microcystin produced by *M. aeruginosa* (Van der Westhuizen et al. 1986; Xu et al. 2010) varies with the temperature. To date, however, there have been very few experimental studies on the effects of temperature and other potential modifying factors on the responses of rotifers to *M. aeruginosa* (Gilbert 1996; Claska and Gilbert 1998; Zha et al. 2007; Soares et al. 2010). In the current study, we document not only the relationship between rotifers and *M. aeruginosa* but also the effects of temperature on the inhibitory capabilities of *M. aeruginosa*.

Materials and Methods

Brachionus calyciflorus used in the present study was collected from Lake Nanhu, Wuhan, China, and maintained in the laboratory for more than 6 months prior to the start of the investigation. To propagate the organism and to use it, Environmental Protection Agency (EPA) medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $MgSO_4$, 60 mg $CaSO_4$, and 4 mg KCl in 1 L of distilled water (Zha et al. 2007). Rotifers were cultured in EPA medium supplemented with 5.0×10^5 cells/mL of *Chlorella pyrenoidosa* at 25 ± 1°C in the dark.

The algae *C. pyrenoidosa* and *M. aeruginosa* were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. *M. aeruginosa* was batch cultured axenically in liquid BG-11 medium at 25 ± 1°C on a 12 L: 12 D photoperiod with dim light (~300 lx) in an illumination incubator. *C. pyrenoidosa* was cultured in HB-4 medium (Li et al. 1959; Geng and Xie 2008) under the same conditions with continuous aeration.

Experiments were conducted at three temperatures, 15, 25, and 35°C. Every temperature had one control group (0 cells/mL concentration of *M. aeruginosa*) and three treatment groups (10^4, 10^5, and 10^6 cells/mL), each with *C. pyrenoidosa* at a concentration of 5.0×10^5 cells/mL as a food source. Life table experiments were conducted in 24-well tissue culture plates. The diameter and volume of each well are 15.0 mm and 1.5 mL, respectively. Each group had 24 replications and these 24 neonates (≤2 h old) were randomly introduced into each well which contained 1 mL rotifer medium with the corresponding food combinations mentioned earlier. All initial parental females in these wells were transferred into fresh medium every 24 h. We recorded the numbers of original live individuals and neonates born once every 8 h until the death of the last animal among all groups. Based on the data collected, the demographic parameters were calculated and analyzed following the procedure of Hu and Xi (2008) where

l_x is the age-specific survival rate or the proportion of surviving individuals at age x to its original cohort;

m_x is the age-specific fecundity rate or the mean number of female offsprings produced in a unit of time by a female at age x.

The intrinsic rate of increase (r_m), net reproductive rate (R_0), and generation time (T) can be obtained using the following equations:

$$\sum_{x=0}^{n} e^{-r_m x} l_x m_x = 1; R_0 = \sum_{0}^{\infty} l_x m_x;\ T = \ln(R_0)/r_m$$

A two-way ANOVA was conducted to identify the significant effects of temperature, *M. aeruginosa* concentration, and temperature–concentration interaction on life history parameters in SPSS 18.0.

Results and Discussion

The effects of the four concentrations of *M. aeruginosa* on the age-specific survival rate (l_x) and fecundity (m_x) of *B. calyciflorus* at three temperatures are shown in Fig. 1. At the same temperatures, the higher the concentration of *M. aeruginosa,* the faster the l_x falls. In addition, the point at which the l_x begins to fall comes earlier along with the rise of the *M. aeruginosa* concentration. At any temperature, the l_x of rotifers exposed to 10^6 cells/mL of *M. aeruginosa* was extremely lower than that of rotifers exposed to the other three concentrations. Higher temperatures bring the rotifer fecundity forward. Rotifers begin to reproduce sooner as temperatures increase. Therefore, *B. calyciflorus* was strongly inhibited by *M. aeruginosa* (Geng and Xie 2008; Soares et al. 2010).

All the demographic parameters of the *B. calyciflorus* in the different conditions are shown in Table 1. At the temperatures tested, the R_0 and r_m values tended to decline when the concentration of *M. aeruginosa* increased. The rotifer population even appeared negative growth at a *M. aeruginosa* concentration of 10^6 cells/mL. The r_m values of the rotifers exposed at 10^6 cells/mL decreased more than 200% compared with those of the control group. When the temperature increased, the generation time was reduced. As determined by the two-way ANOVA, the concentrations of *M. aeruginosa* had statistically significant effects on r_m ($F = 8.874$, $p = 0.013$). Moreover, both temperature ($F = 140.150$, $p = 0.000$) and concentration ($F = 25.819$, $p = 0.000$) showed statistical significance on the average lifespan, but the interaction between temperature and concentration did not ($F = 1.869$, $p = 0.086$). Similarly, the two-way ANOVA showed that temperature ($F = 14.053$, $p = 0.000$), concentration ($F = 101.157$,

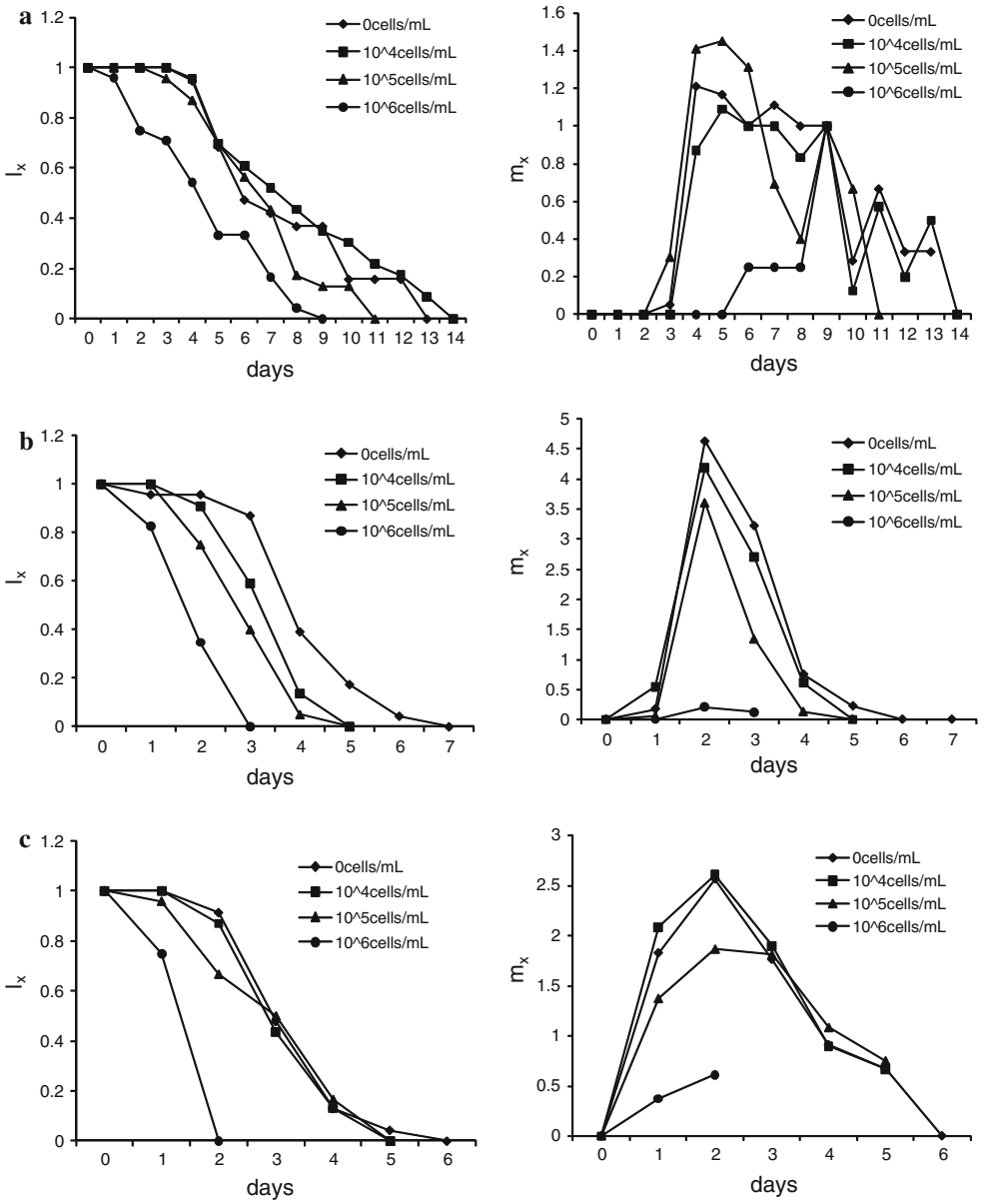

Fig. 1 Effect of *M. aeruginosa* and *C. pyrenoidosa* mixtures on the age-specific survival rate (l_x) and fecundity (m_x) of *B. calyciflorus* at three temperatures (**a** 15°C; **b** 25°C; **c** 35°C)

$p = 0.000$) and interaction between these two factors ($F = 4.064$, $p = 0.001$) had extremely significant effects on the offspring number.

To determine whether the change in temperature affected the ability of *M. aeruginosa* to negatively impact rotifers, we compared the r_m values of *B. calyciflorus* between the control and the treatment groups at different temperatures. At a low concentration (10^4 cells/mL) of *M. aeruginosa*, the inhibition of rotifers by *M. aeruginosa* was not always heightened with increasing temperature. In particular, at 35°C, the r_m value of the rotifers exposed at 10^4 cells/mL was greater than those of the control group. A possible reason may be that *M. aeruginosa* can supply appropriate nourishment to rotifers. However, at a higher concentration (10^5 and 10^6 cells/mL) of *M. aeruginosa*, the inhibition of rotifers by *M. aeruginosa* intensified with increasing temperature. In particular, as the concentration increased to 10^6 cells/mL, the inhibition grew more obvious. There are several possible reasons. Firstly, a temperature rise may improve the yield of toxins (Wicks and Thiel 1990; Davis et al. 2009; Al-Shehri 2010) and increase the rotifers' absorption of microcystin (Gilbert 1996; Hietala et al. 1997; Claska and Gilbert 1998). A number of studies (Wicks and Thiel 1990; Jiang et al. 2008; Tonk et al. 2009)

Table 1 Effects of different concentrations of *M. aeruginosa* with the same concentration of *Chlorella pyrenoidosa* on the life history parameters (mean ± SD) of *B. calyciflorus* at three temperatures

Temperature (°C)	Concentration (cells/mL)	R_0 (ind.)	Generation time (day)	r_m (day^{-1})	Average lifespan[a] (days)	Offspring number[b] (ind.)
15	0	3.88	5.58	0.24	7.74 ± 3.05	4.68 ± 2.75
	10^4	3.67	5.90	0.22	8.35 ± 3.30	4.43 ± 3.07
	10^5	3.85	4.90	0.28	6.96 ± 2.25	4.61 ± 2.23
	10^6	0.14	6.53	−0.31	4.83 ± 2.35	0.25 ± 0.53
25	0	7.74	2.28	0.90	4.39 ± 1.23	8.48 ± 3.15
	10^4	6.03	2.05	0.88	3.64 ± 0.85	7.59 ± 3.28
	10^5	3.29	2.11	0.56	3.20 ± 0.89	4.90 ± 1.94
	10^6	0.07	2.00	−1.31	2.17 ± 0.72	0.22 ± 0.52
35	0	5.16	1.60	1.02	3.57 ± 0.95	6.52 ± 2.31
	10^4	5.30	1.54	1.08	3.43 ± 0.90	6.91 ± 2.45
	10^5	3.65	1.72	0.75	3.29 ± 1.20	5.13 ± 2.86
	10^6	0.28	1.00	−1.27	1.75 ± 0.44	0.83 ± 0.82

[a, b] $n = 24$

also found that the cyanobacterial toxin concentrations were positively correlated with temperature. However, some conflicting reports showed that there was a slight increase in microcystin production at low temperatures and a slight decrease in toxicity at higher temperatures (Watanabe and Oishi 1985; Sivonen 1990; Gilbert 1996; Vezie et al. 1998). Secondly, as the present study has shown, the sensitivity of some zooplankton to a toxic cyanobacterium increases with increasing temperature. Thirdly, high temperatures seem to increase the ability of cyanobacteria to compete with algae for nutrients (Gilbert 1996; Claska and Gilbert 1998; Chu et al. 2007; JÖHnk et al. 2008). In nature, temperature can have much greater effects on the response of rotifers to *M. aeruginosa*. Previous laboratory investigations have shown that temperature rise can stimulate cyanobacteria growth (Van der Westhuizen et al. 1986; Dokulil and Teubner 2000; Tonk et al. 2009) and contribute to algal blooms (JÖHnk et al. 2008; Granéli et al. 2011). In addition, cyanobacteria are able to compete more successfully at higher temperatures, so they can predominate in phytoplankton communities (Tas et al. 2006; Al-Shehri 2010). Thus, in aquatic ecosystems containing cyanobacteria, temperature rise intensifies the inhibitory effect of cyanobacteria on rotifers and induces changes in the community structure of zooplankton.

From the present study, we conclude that *M. aeruginosa*, in combination with the green algae *C. pyrenoidosa* negatively impacted the survival and reproduction of planktonic *B. calyciflorus*. The life history parameters show that the survival and population growth of *B. calyciflorus* were significantly inhibited at high concentrations of *M. aeruginosa*. The inhibitory effects of *M. aeruginosa* on rotifer survival increases with increasing temperature. In addition, *M. aeruginosa* may actually supply a source of nutrients for the planktonic rotifers. In nature, the environmental factors are complicated, variable, and interinfluential. Thus, further studies are needed to determine the effects of cyanobacteria on zooplankton under different environmental factors.

Acknowledgments We thank Dr. Haiying Wang for her help in the alga cultivation and Dr. Richard McLaughlin and Jun Xu for reviewing this manuscript. This work was supported by the National Natural Science Foundation of China (No. 30700088, 31170439), the fund from the South-Central University for Nationalities (XJS09002), and the Special Fund for Basic Scientific Research of Central Colleges, South-Central University for Nationalities (No. CZY11008).

References

Al-Shehri AM (2010) Toxin-producing blooms of the cyanobacterium *Microcystis aeruginosa* in rainwater ponds in Saudi Arabia. Oceanol Hydrobiol St 39:171–187

Chu ZS, Jin XC, Iwami N, Inamori YH (2007) The effect of temperature on growth characteristics and competitions of *Microcystis aeruginosa* and *Oscillatoria mougeotii* in a shallow, eutrophic lake simulator system. Hydrobiologia 581:217–223

Claska ME, Gilbert JJ (1998) The effect of temperature on the response of Daphnia to toxic cyanobacteria. Freshw Biol 39:221–232

Davis TW, Berry DL, Boyer GL, Gobler CJ (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae 8:715–725

Dokulil MT, Teubner K (2000) Cyanobacterial dominance in lakes. Hydrobiologia 438:1–12

Gan N, Mi L, Sun X, Dai G, Chung F-L, Song L (2010) Sulforaphane protects Microcystin-LR-induced toxicity through activation of the Nrf2-mediated defensive response. Toxicol Appl Pharmacol 247:129–137

Geng H, Xie P (2008) Experimental studies on the effects of toxic *Microcystis aeruginosa* PCC7820 on the survival and

reproduction of two freshwater rotifers *Brachionus calyciflorus* and *Brachionus rubens*. Ecotoxicology 17:709–715

Gilbert JJ (1996) Effect of temperature on the response of planktonic rotifers to a toxic cyanobacterium. Ecology 77:1174–1180

Granéli E, Vidyarathna NK, Funari E, Cumaranatunga PRT, Scenati R (2011) Can increases in temperature stimulate blooms of the toxic benthic dinoflagellate *Ostreopsis ovata*? Harmful Algae 10:165–172

Hansson LA, Gustafsson S, Rengefors K, Bomark L (2007) Cyanobacterial chemical warfare affects zooplankton community composition. Freshw Biol 52:1290–1301

Hietala J, Lauren-Maatta C, Walls M (1997) Sensitivity of Daphnia to toxic cyanobacteria: effects of genotype and temperature. Freshw Biol 37:299–306

Hu HY, Xi YL (2008) Demographic parameters and mixis of three *Brachionus angularis* Gosse (Rotatoria) strains fed on different algae. Limnologica 38:56–62

Jiang Y, Ji B, Wong RNS, Wong MH (2008) Statistical study on the effects of environmental factors on the growth and microcystins production of bloom-forming cyanobacterium—*Microcystis aeruginosa*. Harmful Algae 7:127–136

JÖHnk KD, Huisman JEF, Sharples J, Sommeijer BEN, Visser PM, Stroom JM (2008) Summer heatwaves promote blooms of harmful cyanobacteria. Glob Change Biol 14:495–512

Li S, Zhu H, Xia Y, Yu M, Liu K, Ye Z, Chen Y (1959) The mass culture of unicellular green algae. Acta Hydrobiol Sin 4:462–472 (in Chinese with English abstract)

Li XQ, Yuan J, Yang F, Ti LH, Liang GY, Zhang J, Pu YP (2010) Environmental abundance and microcystin-LR production ability of toxic *Microcystis* in Nanquan region of Lake Taihu. J Southeast Univ 26:96–99

Liu Y, Xie P, Wu XP (2006) Effects of toxic and nontoxic *Microcystis aeruginosa* on survival, population-increase, and feeding of two small cladocerans. Bull Environ Contam Toxicol 77:566–573

Sivonen K (1990) Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. Appl Environ Microbiol 56:2658–2666

Soares MCS, Lurling M, Huszar VLM (2010) Responses of the rotifer *Brachionus calyciflorus* to two tropical toxic cyanobacteria (*Cylindrospermopsis raciborskii* and *Microcystis aeruginosa*) in pure and mixed diets with green algae. J Plankton Res 32: 999–1008

Tas S, Okus E, Aslan-Yilmaz A (2006) The blooms of a cyanobacterium, *Microcystis cf. aeruginosa* in a severely polluted estuary, the Golden Horn, Turkey. Estuar Coast Shelf Sci 68:593–599

Tilimanns AR, Wilson AE, Frances RP, Sarnelle O (2008) Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. Fund Appl Limnol 171: 285–295

Tonk L, Welker M, Huisman J, Visser PM (2009) Production of cyanopeptolins, anabaenopeptins, and microcystins by the harmful cyanobacteria *Anabaena* 90 and *Microcystis* PCC 7806. Harmful Algae 8:219–224

Trabeau M, Bruhn-Keup R, McDermott C, Keomany M, Millsaps A, Emery A, De Stasio B (2004) Midsummer decline of a Daphnia population attributed in part to cyanobacterial capsule production. J Plankton Res 26:949

Van der Westhuizen AJ, Eloff JN, Krüger GHJ (1986) Effect of temperature and light (Fluence Rate) on the composition of the toxin of the cyanobacterium *Microcystis Aeruginosa*(UV-006). Arch Hydrobiol 108:145–154

Vezie C, Brient L, Sivonen K, Bertru G, Lefeuvre JC, Salkinoja-Salonen M (1998) Variation of microcystin content of cyanobacterial blooms and isolated strains in Lake Grand-Lieu (France). Microb Ecol 35:126–135

Watanabe MF, Oishi S (1985) Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. Appl Environ Microbiol 49:1342

Wicks RJ, Thiel PG (1990) Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. Environ Sci Technol 24:1413–1418

Wilson AE, Hay ME (2007) A direct test of cyanobacterial chemical defense: variable effects of microcystin-treated food on two *Daphnia pulicaria* clones. Limnol Oceanogr 52:1467–1479

Xu Y, Wang GX, Yang WB, Li RH (2010) Dynamics of the water bloom-forming *Microcystis* and its relationship with physico-chemical factors in Lake Xuanwu (China). Environ Sci Pollut Res 17:1581–1590

Zha CW, Xi YL, Huang L, Zhao LL (2007) Effect of sublethal exposure to chlordecone on life history characteristics of freshwater rotifer *Brachionus calyciflorus* pallas. Bull Environ Contam Toxicol 78:71–75