Ann. Limnol. - Int. J. Lim. 48 (2012) 383–390
© EDP Sciences, 2012
DOI: 10.1051/limn/2012029

Available online at:
www.limnology-journal.org

Responses in population growth and reproduction of the freshwater rotifer *Brachionus calyciflorus* to microcystin-LR at different temperatures

Lin Huang[1,2], Yilong Xi[1]*, Xiaoping Xu[1] and Xinli Wen[1]

[1] Provincial Key Laboratories of Conservation and Utilization for Important Biological Resource in Anhui and Biotic Environment and Ecological Safety, College of Life Sciences, Anhui Normal University, Wuhu, Anhui 241000, China
[2] College of Biological and Pharmaceutical Engineering, WestAnhui University, Lu'an, Anhui 237012, China

Received 2 September 2012; Accepted 4 November 2012

Abstract – *Microcystis* blooms occur worldwide in eutrophic lakes. Microcystins (MCs) including microcystin-LR (MC-LR) released by *Microcystis* have adverse effects on aquatic organisms such as rotifers. To detect population growth and reproductive responses, the rotifer *Brachionus calyciflorus* was exposed to MC-LR at eight concentrations ranging from 0 to 200 μg.L^{-1} under different temperature (20, 25 and 30 °C), and population growth rate (*r*), ovigerous females/non-ovigerous females (OF/NOF) ratio, mictic females/amictic females (MF/AF) ratio, mictic rate (MR), and 7-d resting egg (7-d RE) production were investigated. The results showed that higher temperatures stimulate the population growth of *B. calyciflorus*. *B. calyciflorus* showed high tolerance to MC-LR at concentrations lower than 200 μg.L^{-1} under different temperatures. Compared to the control, MC-LR at all concentrations increased the *r* ($P < 0.05$), but decreased the OF/NOF and the MR of the rotifers at 30 °C ($P < 0.01$). A clear dose–response relationship existed between the *r*, the OF/NOF, and the MR of *B. calyciflorus* and MC-LR concentration at 30 °C, respectively. These sensitive parameters could be used to monitor the ecological effects of low concentrations of MC-LR in natural water bodies at the high temperature.

Key words: Rotifer / *Brachionus calyciflorus* / Microcystins-LR / Temperature / Reproduction parameters

Introduction

Toxic cyanobacterial blooms in freshwater rivers, lakes, reservoirs, and recreational waters have increasingly become a nuisance (Cohen, 1989; Chorus and Bartram, 1999; Carmichael, 2001; Paerl *et al.*, 2001; Vasconcelos and Pereira, 2001; Huisman *et al.*, 2005; Liu *et al.*, 2006; Vareli *et al.*, 2009; Ye *et al.*, 2009; Gan *et al.*, 2010). It is well known that many species of cyanobacteria are able to produce microcystins (MCs) (van Apeldoorn *et al.*, 2007), which can accumulate in the food chain (Xie *et al.*, 2005; Chen *et al.*, 2009a) and negatively affect aquatic organisms, animals, and human beings due to their potent hepatotoxicity and probable tumor promoters (Andersen *et al.*, 1993; Carmichael and Falconer, 1993; Chorus and Bartram, 1999; Matsunaga *et al.*, 1999; Carmichael *et al.*, 2001; Zimba *et al.*, 2001; Chen *et al.*, 2002, 2009b; Qiu *et al.*, 2007). To date, more than 80 structural analogues of MCs have been identified, and microcystin-LR (MC-LR) is the most frequently detected and the most toxic (Fastner *et al.*, 2002; Briand *et al.*, 2003; Zurawell *et al.*, 2004; Hoeger *et al.*, 2005; Blaha *et al.*, 2009). Thus, World Health Organization (WHO) established a provisional guideline value for MC-LR in 1 μg.L^{-1} of drinking water (WHO, 1998).

Zooplankton are mainly composed of protozoa, rotifers, cladocerans and copepods in freshwater bodies, and are the natural food link between the primary producers (*e.g.,* algae and bacterial) and secondary consumers (*e.g.,* insect larvae and fish fry) (Pennak, 1989; Nogrady *et al.*, 1993) and important in the maintenance of an ecological balance in freshwater ecosystems. Most planktonic rotifers have a cyclically parthenogenetic life cycle where asexual reproduction predominates, but there are periods where both asexual and sexual reproductions occur simultaneously. In monogonont rotifers, asexual reproduction in the absence of males is mixed with occasional bouts of sexual reproduction. Asexual (amictic)

*Corresponding author: ylxi1965@yahoo.com.cn

females are diploid and produce eggs mitotically, which develop into amictic females. Upon receiving the mictic stimulus, asexual females begin producing both sexual (mictic) and amictic daughters. Mictic females produce haploid eggs, if unfertilized, develop into haploid males. If fertilized, haploid eggs become diploid and develop into large, thick-walled resting eggs. After a period of dormancy that varies among species, resting eggs respond to species-specific hatching cues and hatch into amictic females, entering again into the asexual phase of the life cycle (Pourriot and Snell, 1983; Snell and Carmona, 1995).

It is well established that bloom-forming cyanobacteria are, in a general sense, of poor food quality for herbivorous zooplankton including cladocerans and rotifers (Porter and Orcutt, 1980), although the large variation in species-specific responses of zooplankton to cyanobacteria (Tillmanns *et al.*, 2008). Among the reasons for this poor quality, the toxicity of cyanobacterial compounds such as MCs has attracted the most interest (Porter and Orcutt, 1980; Lampert, 1987; DeMott, 1989). Rotifers, especially *Brachionus calyciflorus* and *Brachionus plicatilis*, have been widely recognized as bioindicators of water quality (Sladecék, 1983) and ideal bioassay animals in aquatic toxicology (Halbach *et al.*, 1983; Janssen *et al.*, 1993; Snell and Janssen, 1995). Although numerous studies have examined the effects of MCs on zooplankton, most investigations have focused on cladocerans, especially the genus *Daphnia* (Demott *et al.*, 1991; Lürling and van der Grinten, 2003; Ghadouani *et al.*, 2004; Chen *et al.*, 2005; Dao *et al.*, 2010; Yang *et al.*, 2011, 2012), few studies have focused on rotifers. Chen *et al.* (2002) found that 1–20 $mg.L^{-1}$ crude MC-LR has adverse effects on rotifer *B. plicatilis*. However, the effects of purified MC-LR on population growth and reproduction of rotifer *B. calyciflorus* have not been reported.

Temperature is one of important ecological factors. Generally, increase in temperature has a stimulating effect on the population growth of organisms including cyanobacteria and rotifers. Cyanobacterial blooms and MCs releasing are facilitated by high water temperatures and therefore benefit from climate warming (Codd, 2000; Jöhnk *et al.*, 2008; Paerl and Huisman, 2008). However the effect of increase in temperature on toxicity of MCs to population growth and reproduction of rotifers, such as *B. calyciflorus* has not yet been reported.

The main purpose of the present study was to assess the effect of MC-LR on population growth and sexual reproduction of *B. calyciflorus* under different temperatures by means of 3-day population growth, and 7-day resting egg (7-d RE) production tests, and to screen out sensitive endpoints which could be used to monitor the ecological effects of MC-LR on the population growth of rotifer *B. calyciflorus*.

Materials and methods

B. calyciflorus was isolated from the Lake Jiuliantang (31°33′N, 118°37′E) located in the center of Wuhu city in the east of China and then clonally cultured under controlled laboratory conditions. Stock rotifer cultures were kept under static-renewal conditions with natural illumination at 25 ± 1 °C in an illumination incubator for over 1 year. Rotifers were daily fed on *Scenedesmus obliquus* at 1.0–2.0 $\times 10^6$ $cells.mL^{-1}$. Before the experiments commenced, rotifers were cultured in EPA medium which was prepared by dissolving 96 mg of $NaHCO_3$, 60 mg of $CaSO_4$, 60 mg of $MgSO_4$, and 4 mg of KCl in 1 L distilled water (USEPA, 1985), and fed on 3.0×10^6 $cells.mL^{-1}$ of *S. obliquus* at 20 ± 1, 25 ± 1 and 30 ± 1 °C for at least 2 weeks, respectively. 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 distilled water and then stored at 4 °C. The density of the stock algal concentrate was estimated using haemocytometer.

MC-LR (purity at least 95% by HPLC) was purchased from Express Bio-technology Co., Ltd, Beijing, China, which was diluted with distilled water to stock solution of 100 $mg.L^{-1}$, then diluted to the desired concentrations using EPA medium.

Considering the range of dissolved MCs in natural waters in the world, from trace concentration to 200 $\mu g.L^{-1}$ (Sivonen and Jones, 1999), mostly from 0.1 to 10 $\mu g.L^{-1}$ (Lahti *et al.*, 1997; Lawton *et al.*, 1998), and the provisional guideline for MC-LR of 1 $\mu g.L^{-1}$ in drinking water (WHO, 1998), meanwhile, cyanobacterial blooms often development in warm seasons (Oliver and Ganf, 2000), seasonal increases in water temperature, and climate warming may exacerbate the impact of toxic cyanobacteria on rotifers (Gilbert, 1996), we selected test MC-LR concentrations were 0.001, 0.01, 0.1, 1, 10, 100 and 200 $\mu g.L^{-1}$ and temperatures were 20, 25 and 30 °C. Four replicates were carried out for each treatment. A control of EPA medium was also tested.

All the experiments were conducted in 8-mL glass chambers and started by introducing 10 neonates (< 3 h old) into each chamber containing 5 mL test solution and 3.0×10^6 $cells.mL^{-1}$ *S. obliquus*. Thereafter, the rotifers were cultured under static conditions with natural illumination at 20 ± 1, 25 ± 1 and 30 ± 1 °C in illumination incubators for 3 days, respectively. Then, the number of each type of rotifer females was counted following the methods described by Xi *et al.* (2007). All the rotifers were continually cultured for another 96 h to count the number of resting eggs (7-d RE) (Lu *et al.*, 2012). Meanwhile, the population growth rate (r, $r = (\ln N_t - \ln N_0)/t$, where N_0 and N_t are the initial and final population density, respectively, and $t = 3$), ovigerous females/non-ovigerous females (OF/NOF) ratio and mictic females/amictic females (MF/AF) ratio, and mictic rate (MR, the ratio of mictic females/total females), were calculated according to Radix *et al.* (2002). During the experimental period, the original rotifers were daily transferred into freshly prepared test solution containing 3.0×10^6 $cells.mL^{-1}$ *S. obliquus* with a micropipette.

Two-way analysis of variance (ANOVA) by SPSS 16.0, with MC-LR concentration and temperature as the

independent variable, and the *r*, OF/NOF, MF/AF, MR, or 7-d RE as the dependent variable, followed by Dunnett's test was conducted for pairwise comparisons of each MC-LR concentration and temperature relative to the control (Zar, 1999). Dose–response relationships between each of the reproduction parameters of *B. calyciflorus* and MC-LR concentration or temperature were calculated using regression analysis (Stephan and Rogers, 1985).

Results

Compared to the control, MC-LR concentration significantly affected the MF/AF, MR and 7-d RE at 25 °C, and the *r*, OF/NOF and MR of at 30 °C ($P < 0.05$), but did not affect the other production parameters of the rotifers ($P > 0.05$) (Fig. 1).

At 25 °C, MC-LR at 1 μg.L^{-1} increased the MF/AF, MR and 7-d RE of the rotifers ($P < 0.05$). At 30 °C, MC-LR at all concentrations increased the *r* ($P < 0.05$), but decreased the OF/NOF and MR of the rotifers ($P < 0.01$) (Fig. 1).

The results of the two-way ANOVA performed all the parameters of the rotifers subjected to MC-LR concentrations and temperatures are presented in Table 1. MC-LR did not affect the *r* ($P > 0.05$), but temperature and MC-LR × temperature interaction affected the *r* of the rotifers ($P < 0.05$). MC-LR, temperature and their interaction all affected the OF/NOF of the rotifers ($P < 0.01$). Temperature affected the MF/AF, MR, and 7-d RE of the rotifers ($P < 0.01$), but MC-LR and MC-LR × temperature interaction did not affect them ($P > 0.05$) (Table 1).

A clear dose–response relationship existed between the *r*, OF/NOF, and MR of the rotifer *B. calyciflorus* and MC-LR concentration at 30 °C, respectively (Table 2).

Discussion

Temperature is one of the important variables affecting the population growth and reproduction of rotifers including *B. calyciflorus*. Normally, within the range of tolerance levels, increase in temperature accelerates the reproduction and stimulates population growth of rotifers (Stelzer, 1998; Forbes and Calow, 1999; Pavón-Meza *et al.*, 2005; Geng and Zhu, 2008; Yin and Zhao, 2008). In nature, this relationship, higher temperatures promote the population growth of rotifers, is also existent (Andrew and Andrew, 2005; Wen *et al.*, 2011). In the present study, temperature also markedly increased the population growth rate of the rotifer *B. calyciflorus*, which supported the results of above-mentioned laboratory and field researches. In addition, temperature significantly affected OF/NOF, MF/AF, MR and 7-d RE of the rotifers.

MCs are mainly retained within the producer cells during *Microcystis* blooms development (Pflugmacher, 2004). In post-blooms, MCs are released into water after demise of *Microcystis* cells. The dissolved MCs

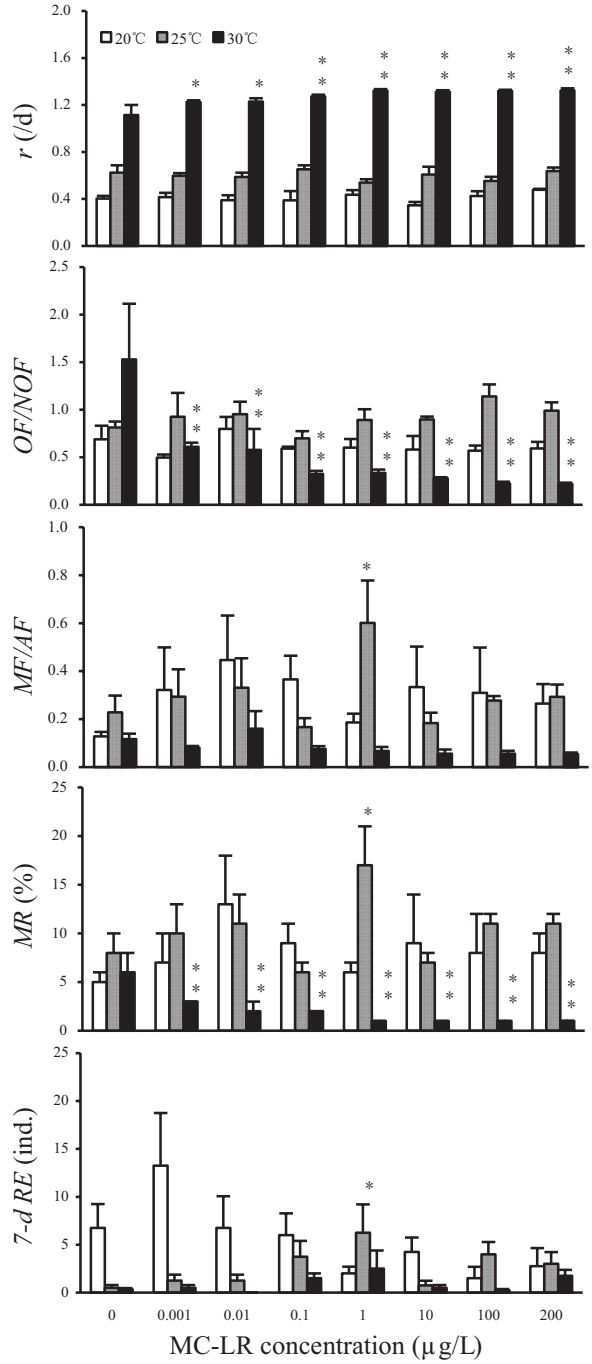

Fig. 1. Mean values ± standard error of population growth rate (*r*), ON/NOF ratio and MF/AF ratio, MR, and 7-d RE production of *B. calyciflorus* exposed to different concentrations of MC-LR at three temperatures. *Significant ($P < 0.05$), **highly significant ($P < 0.01$).

> 1800 μg.L^{-1} during the collapse of heavy blooms (Jones and Orr, 1994; Svrcek and Smith, 2004) can come in contact with a wide range of aquatic organisms including rotifers and have adverse effects on them (Chen *et al.*, 2002, 2009a, Cazenave *et al.*, 2006). MCs including MC-LR inhibit feeding, reduce growth, and increase mortality in cladocerans (DeMott *et al.*, 1991; Rohrlack

Table 1. Results of the two-way ANOVA performed population growth rate (r), OF/NOF ratio and MF/AF ratio, MR, and 7-d RE production of *B. calyciflorus* subjected to 8 MC-LR concentrations and three temperatures.

Source of variation	DF	SS	MS	F
r				
MC-LR	7	0.078	0.011	1.92 ns
Temperature	2	12.397	6.198	1075.45**
MC-LR × temperature	14	0.160	0.011	1.99*
Error	71	0.409	0.006	
OF/NOF				
MC-LR	7	1.788	0.256	3.55**
Temperature	2	2.751	1.376	19.13**
MC-LR × temperature	14	3.599	0.257	3.57**
Error	71	5.106	0.072	
MF/AF				
MC-LR	7	0.211	0.030	0.79 ns
Temperature	2	0.9341	0.467	12.25**
MC-LR × temperature	14	0.6081	0.043	1.14 ns
Error	71	2.707	0.038	
MR				
MC-LR	7	0.011	0.002	0.71 ns
Temperature	2	0.105	0.052	23.30**
MC-LR × temperature	14	0.045	0.003	1.42 ns
Error	71	0.160	0.002	
7-d RE				
MC-LR	7	95.945	13.706	0.51 ns
Temperature	2	323.896	161.948	6.08**
MC-LR × temperature	14	441.786	31.556	1.18 ns
Error	71	1891.667	26.643	

DF, degrees of freedom; SS, sum of squares; MS, mean square; F, F-ratio.
*Significant ($P < 0.05$), **highly significant ($P < 0.01$), ns: non-significant ($P > 0.05$).

Table 2. Relationships between population growth rate (r), OF/NOF ratio as well as MR of *B. calyciflorus* and MC-LR concentration at 30 °C.

Pollutant	Parameters	Regression equations	Significant tests
MC-LR	r	$Y=-0.0052x^2+0.0747x+1.0559$	$R^2=0.8991$, $P<0.001$
	OF/NOF	$Y=0.0563x^2-0.6223x+1.9178$	$R^2=0.8835$, $P<0.001$
	MR	$Y=0.0015x^2$-$0.0197x+0.0723$	$R^2=0.9032$, $P<0.001$

et al., 1999; Lürling and Van der Grinten, 2003; Ghadouani *et al.*, 2004; Wilson *et al.*, 2006).

Up to now, contrast with cladocerans, the researches about effects of purified MCs on rotifers are scarce (Chen *et al.*, 2002). Chen *et al.* (2002) studied the acute and chronic toxicity of MC-LR to the rotifer *B. plicatilis*. The results showed the 24-h LC_{50} value for MC-LR was 124.87 mg.L^{-1}, which indicated that rotifers showed rather strong tolerant capacity towards MC-LR. MC-LR at a range from 1 to 20 mg.L^{-1} suppressed the population growth of the rotifers and promoted the occurrence of male rotifers *B. plicatilis*. In the present study, we did not clearly find the deleterious effects of MC-LR on the rotifers *B. calyciflorus*. Notably, although MC-LR did not affect or slightly increase the population growth of the rotifers at two lower temperatures (20 and 25 °C), it significantly promoted the population growth of the rotifers at the high temperature (30 °C). There might be several reasons. Firstly, the MC-LR concentrations in our study (MC-LR: 0.001–200 μg.L^{-1}) are much lower than that in the study of Chen *et al.* (2002) (MC-LR: 1–20 mg.L^{-1}). A number of studies found that low concentrations of dissolved MCs had no harmful effects, on the contrary, stimulating effects on *Daphnia* (Chen *et al.*, 2005; Zhang *et al.*, 2008) and rotifers (Hansson *et al.*, 2007; Zhang and Geng, 2012). Secondly, increase in temperature may increase absorption of the rotifers to MCs (Gilbert, 1996; Hietala *et al.*, 1997; Claska and Gilbert, 1998), which may lead to stimulating effects of low concentrations of MC-LR on the population growth of the rotifers more obvious. Thirdly, the sensitivity of the rotifers to the MC-LR may increase with increasing temperature. Finally, there is a difference between the test rotifer species (Chen *et al.*, 2002; the present study). Thus, increase in temperature enhances the stimulating effects of MCs on rotifers and induces stronger competitive ability of rotifers during *Microcystis* blooms in aquatic ecosystems. The results of Liu *et al.* (2002) showed that *M. aeruginosa* blooms strongly suppressed the larger *Diaphanosoma brachyurum*, but enhanced the

development of the smaller cladocerans and rotifers. They thought that the smaller cladocerans and rotifers probably efficiently utilized organic matter from *M. aeruginosa* through the detritus food chain. However, in our opinions, MCs also probably acted important roles that stimulated the population growth of the smaller cladocerans and rotifers. It might be one of the reasons for zooplankton miniaturization, although it was needed for further researches, and may add to our knowledge on population and community dynamics among zooplankton in eutrophic aquatic ecosystems.

Reproductive functions are regulated by hormones and are consequently subjected to disruption by naturally occurring compounds acting as oestrogens. Among these compounds, toxins may act as endocrine disruptors, which modify the functioning of the endocrine system and induce reproductive disorders (Burkhardt-Holm, 2010). Some researchers found endocrine disrupting effects of cyanobacterial toxins such as nodularin-R and MC-LR on a transgenic human cell line MELN (Oziol and Bouaïcha, 2010) and MC-RR on *Daphnia magna* (Hulot *et al.*, 2012). The results of Hulot *et al.* (2012) showed that MC-RR reduced *D. magna* population growth rate and larger proportion of adults. In the present study, we found MC-LR at all concentrations significantly decreased the OF/NOF and MR of the rotifers *B. calyciflorus* at 30 °C. It might suggest reproduction function-disruptive effects of MC-LR on the rotifers *B. calyciflorus* as observed MC-RR on *D. magna* (Hulot *et al.*, 2012).

Rotifers, especially *B. calycifiorus* and *B. plicatilis*, are favored test animals in aquatic toxicology because of their small size, rapid reproduction, simplicity of culture, simple life cycle, global distribution and sensitivity to most toxicants (Snell and Janssen, 1995). Both *r* and *MR* were sensitive to most tested toxicants (Preston *et al.*, 2000; Radix *et al.*, 2002; Xi and Feng, 2004; Marcial *et al.*, 2005; Xi *et al.*, 2007; Ke *et al.*, 2009), and OF/NOF ratio in *B. calyciflorus* population was also a suitable endpoint for assessing the effects of ethinylestradiol, nonylphenol, dichlorvos, triazophos and chlorpyrifos (Radix *et al.*, 2002; Ke *et al.*, 2009). Similarly, in the present study, a clear dose–response relationship between the *r*, OF/NOF as well as MR of the rotifer *B. calyciflorus* and MC-LR concentration at 30 °C (Table 2) indicated that these sensitive parameters could be used to monitor the ecological effects of low MC-LR concentrations at the high temperature. In addition, Snell and Carmona (1995), Xi and Feng (2004), Marcial *et al.* (2005) and Xi *et al.* (2007) found that sexual reproduction was more sensitive than asexual reproduction to toxicants, in the present study, we also found similar results that MR was more sensitive than the *r* or OF/NOF to MC-LR at 30 °C (Table 2).

The interactions between temperature and toxicants such as pesticides and heavy metals usually change the tolerance capacity of zooplankton (Heugens *et al.*, 2001; Gama-Flores *et al.*, 2005; Nandini *et al.*, 2007), and regulate zooplankton population and community dynamics. Interactions of MC-LR and temperature on the population growth rate and the OF/NOF of *B. calyciflorus* detected in the present study may lead to changes in population structure of rotifers in freshwater bodies containing MCs.

Conclusion

Higher temperatures stimulate the population growth of the rotifer *B. calyciflorus*. *B. calyciflorus* showed a high tolerance to MC-LR at low concentrations (< 200 $\mu g.L^{-1}$), and the population growth of the rotifers was promoted exposed to different concentrations of MC-LR at 30 °C. This finding might be one of the reasons for zooplankton miniaturization in natural freshwater body containing MCs. In addition, a clear dose–response relationship existed between the population growth rate, OF/NOF, and MR of *B. calyciflorus* and the MC-LR concentration at 30 °C, respectively. These sensitive parameters could be used to monitor the ecological effects of low MC-LR concentrations in natural water bodies at the high temperature. During the collapse of heavy blooms, moreover, further studies are still needed to assess whether there are stimulating effects of MCs on the population growth and reproduction of *B. calyciflorus* under more environmental factors.

Acknowledgements. This work was supported by grants from the Natural Science Foundation of China (30470323 and 31170395), Foundation of Key Laboratory for the Conservation and Utilization of Important Biological Resources in Anhui province, Foundation for Young Talents in College of Anhui Province (2009SQRZ029), Natural Science Foundation for Young of Anhui Province (1208085QC59), and Natural Science Foundation of Education Department of Anhui Province (KJ2010B269).

References

Andersen R., Luu H.A., Chen D.Z.X., Holmes C.F.B., Kent M., Le Blanc M., Taylor F.J.R. and Williams D.E., 1993. Chemical and biological evidence links microcystins to salmon netpen liver disease. *Toxicon*, *31*, 1315–1323.

Andrew T.E. and Andrew J.A.M., 2005. Seasonality of rotifers and temperature in Lough Neagh,N. Ireland. *Hydrobiologia*, *546*, 451–455.

Blaha L., Babica P. and Marsalek B., 2009. Toxins produced in cyanobacterial water blooms – toxicity and risks. *Interdiscip. Toxicol.*, *2*, 36–41.

Briand J.F., Jacquet S., Bernard C. and Humbert J.F., 2003. Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems. *Vet. Res.*, *34*, 361–377.

Burkhardt-Holm P., 2010. Endocrine disruptors and water quality: a state-of-the-art review. *Int. J. Water Resources Dev.*, *26*, 477–493.

Carmichael W.W., 2001. Health effects of toxin-producing cyanobacteria: the cyano HABs. *Hum. Ecol. Risk Assess.*, *7*, 1393–1407.

Carmichael W.W. and Falconer I.R., 1993. Diseases related to freshwater blue green algal toxins, and control measures. *In:* Falconer I.R. (ed.), Algal Toxins in Seafood and Drinking Water, Academic, London, 187–209.

Carmichael W.W., Azevedo S.M., An J.S., Molica R.J.R., Jochimsen E.M., Lau S., Rinehart K.L., Shaw G.R. and Eaglesham G.K., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environ. Health Perspect.*, *109*, 663–668.

Cazenave J., Bistoni M.A., Zwirnmann E., Wunderlin D.A. and Wiegand C., 2006. Attenuating effects of natural organic matter on microcystin toxicity in zebra fish (*Danio rerio*) embryos – benefits and costs of microcystin detoxication. *Environ. Toxicol.*, *21*, 22–32.

Chen J., Zhang D., Xie P., Wang Q. and Ma Z., 2009a. Simultaneous determination of microcystin contaminations in various vertebrates (fish, turtle, duck and water bird) from a large eutrophic Chinese lake,Lake Taihu, with toxic *Microcystis* blooms. *Sci. Total Environ.*, *407*, 3317–3322.

Chen J., Xie P., Li L. and Xu J., 2009b. First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. *Toxicol. Sci.*, *108*, 81–89.

Chen W., Song L.R., Ou D.Y. and Gan N.Q., 2005. Chronic toxicity and responses of several important enzymes in *Daphnia magna* on exposure to sublethal microcystin-LR. *Environ. Toxicol.*, *20*, 323–330.

Chen Y., Wang J.Q., Wang Y. and Yu S.Z., 2002. Toxicity and population growth effects of microcystin on the rotifer *Brachionus plicatilis*. *China Environ. Sci.*, *22*, 198–201 (in Chinese with English abstract).

Chorus I. and Bartram J., 1999. Toxic Cyanobacteria in Water: a Guide to their Public Health Consequences, Monitoring and Management, E & FN Spon, London.

Claska M.E.and Gilbert J.J., 1998. The effect of temperature on the response of *Daphnia* to toxic cyanobacteria. *Freshwater Biol.*, *39*, 221–232.

Codd G.A., 2000. Cyanobacterial toxins, the perception of water quality, and the prioritisation of eutrophication control. *Ecol. Eng.*, *16*, 51–60.

Cohen P., 1989. The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.*, *58*, 435–508.

Dao T.S., Do-Hong L.C. and Wiegand C., 2010. Chronic effects of cyanobacterial toxins on *Daphnia magna* and their offspring. *Toxicon*, *55*, 1244–1254.

DeMott W.R., 1989. The role of competition in zooplankton succession. *In:* Sommer U. (ed.), Plankton Ecology: Succession in Plankton Communities. Springer-Verlag, Berlin, 195–252.

DeMott W.R., Zhang Q.X. and Carmichael W.W., 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnol. Oceanogr.*, *36*, 1346–1357.

Fastner J., Codd G.A., Metcalf J.S., Woitke P., Wiedner C. and Utkilen H., 2002. An international intercomparison exercise for the determination of purified microcystin-LR and microcystins in cyanobacterial field material. *Anal. Bioanal. Chem.*, *374*, 437–444.

Forbes V.E. and Calow P., 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology?*Environ. Toxicol. Chem.*, *18*, 1544–1556.

Gama-Flores J.L., Sarma S.S.S. and Nandini S., 2005. Interaction among copper toxicity, temperature and salinity on the population dynamics of *Brachionus rotundiformis* (Rotifera). *Hydrobiologia*, *546*, 559–568.

Gan N., Mi L., Sun X., Dai G., Chung F.L. and Song L., 2010. Sulforaphane protects microcystin-LR-induced toxicity through activation of the Nrf2-mediated defensive response. *Toxicol. Appl. Pharmacol.*, *47*, 129–137.

Geng H. and Zhu X.S., 2008. Effect of temperature on the experimental population of *Brachionus calyciflorus*. *J. South-Central Univ. National.*, *27*, 21–23 (in Chinese with English abstract).

Ghadouani A., Pinel-Alloul B., Plath K., Codd G.A. and Lampert W., 2004. Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria*. *Limnol. Oceanogr.*, *49*, 666–679.

Gilbert J.J., 1996. Effect of temperature on the response of planktonic rotifers to a toxic cyanobacterium. *Ecology*, *77*, 1174–1180.

Halbach U., Siebert M., Westermayer M. and Wissel C., 1983. Population ecology of rotifers as a bioassay tool for ecotoxicological tests in aquatic environments. *Ecotoxicol. Environ. Saf.*, *7*, 484–513.

Hansson L.A., Gustafsson S., Rengefors K. and Bomark L., 2007. Cyanobacterial chemical warfare affects zooplankton community composition. *Freshwater Biol.*, *52*, 1290–1301.

Heugens E.H.W., Hendriks A.J., Dekker T., van Stralen N.M. and Admiraal W., 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. *Crit. Rev. Toxicol.*, *31*, 247–284.

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

Hoeger S.J., Hitzfeld B.C. and Dietrich D.R., 2005. Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants. *Toxicol. Appl. Pharmacol.*, *203*, 231–242.

Huisman J., Matthijs H.P. and Visser P.M., 2005. Harmful Cyanobacteria, Springer, Berlin.

Hulot F.D., Carmignac D., Legendre S., Yéprémian C. and Bernard C., 2012. Effects of microcystin-producing and microcystin-free strains of *Planktothrix agardhii* on long-term population dynamics of *Daphnia magna*. *Ann. Limnol. - Int. J. Lim.*, *48*, 337–347.

Janssen C.R., Ferrando M.D. and Persoone G., 1993. Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus*: I. Conceptual framework and application. *Hydrobiologia*, *255/256*, 21–32.

Jöhnk K.D., Huisman J., Sharples J., Sommeijer B., Visser P.M. and Stroom J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biol.*, *14*, 495–512.

Jones G.J. and Orr P.T., 1994. Release and degradation of microcystin following algicide treatment of a *Microcystis aeruginosa* bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay. *Water Res.*, *28*, 871–876.

Ke L.X., Xi Y.L., Zha C.W. and Dong L.L., 2009. Effects of three organophosphorus pesticides on population growth and sexual reproduction of rotifer *Brachionus calycifiorus* Pallas. *Acta Ecol. Sin.*, *29*, 182–185.

Lahti K., Rapala J., Färdig M., Niemelä M. and Sivonen K., 1997. Persistence of cyanobacteria hepatotoxin microcystin-LR

in particulate material and dissolved in lake water. *Water Res.*, *31*, 1005–1012.

Lampert W., 1987. Laboratory studies on zooplankton-cyanobacteria interactions. *N. Z. J. Marine Freshwater Res.*, *21*, 483–490.

Lawton L.A., Cornish B.J.P.A. and MacDonald A.W.R., 1998. Removal of cyanobacterial toxins (microcystins) and cyanobacterial cells from drinking water using domestic water filters. *Water Res.*, *32*, 633–638.

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

Liu H., Xie P., Chen F.Z., Tang H.J. and Xie L.Q., 2002. Enhancement of planktonic rotifers by *Microcystis aeruginosa* blooms: an enclosure experiment in a shallow Eutrophic Lake. *J. Freshwater Ecol.*, *17*, 239–247.

Liu Y.M., Chen W., Li D.H., Shen Y.W., Li G.B. and Liu Y.D., 2006. First report of aphantoxins in China – water blooms of toxigenic *Aphanizomenon flos-aquae* in Lake Dianchi. *Ecotoxicol. Environ. Saf.*, *65*, 84–92.

Lu Z.H., Zhao B.K., Yang J.X. and Snell T.W., 2012. Effects of atrazine and carbaryl on growth and reproduction of the rotifer *Brachionus calyciflorus* Pallas. *J. Freshwater Ecol.*, *27*, 527–537.

Lürling M. and van der Grinten E., 2003. Life history characteristics of *Daphnia* exposed to dissolved microcystin-LR and to the cyanobacterium *Microcystis aeruginosa* with and without microcystins. *Environ. Toxicol. Chem.*, *22*, 1281–1287.

Marcial S.H., Hagiwara A. and Snell T.W., 2005. Effect of some pesticides on reproduction of rotifer *Brachionus plicatilis*. *Hydrobiologia*, *546*, 569–575.

Matsunaga H., Harada K.I., Senma M., Ito Y., Yasuda N., Ushida S. and Kimura Y., 1999. Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan: sudden appearance of toxic cyanobacteria. *Nat. Toxins*, *7*, 81–84.

Nandini S., Picazo-Paez E.A. and Sarma S.S.S., 2007. The combined effects of heavy metals (copper and zinc), temperature and food (*Chlorella vulgaris*) level on the demographic characters of *Moina macrocopa* (Crustacea: Cladocera). *J. Environ. Sci. Health Part A*, *42*, 1433–1442.

Nogrady T., Wallace R.L. and Snell T.W., 1993. Morphology and internal organization. *In:* Nogrady T. (ed.), Rotifera, Vol. 1: Biology, Ecology and Systematics, SPB Academic Publishing, The Hague, 142 p.

Oliver R.L. and Ganf G.G., 2000. Freshwater blooms. *In:* Whitton B.A. and Potts M. (eds.), The Ecology of Cyanobacteria, Kluwer Academic Publishers, Dordrecht, The Netherlands, 149–194.

Oziol L. and Bouaïcha N., 2010. First evidence of estrogenic potential of the cyanobacterial heptotoxins the nodularin-R and the microcystin-LR in cultured mammalian cells. *J. Hazard. Mater.*, *174*, 610–615.

Paerl H.W. and Huisman J., 2008. Blooms like it hot. *Science*, *320*, 57–58.

Paerl H.W., Fulton R.S., Moisander P.H. and Dyble J., 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *Sci. World J.*, *1*, 76–113.

Pavón-Meza E.L., Sarma S.S.S. and Nandini S., 2005. Combined effects of algal (*Chlorella vulgaris*) food level and temperature on the demography of *Brachionus havanaensis* (Rotifera): a life table study. *Hydrobiologia*, *546*, 353–360.

Pennak R.W., 1989. Freshwater Invertebrates of the United States. The Ronald-Press Co., New York.

Pflugmacher S., 2004. Promotion of oxidative stress in the aquatic macrophyte *Ceratophyllum demersum* during biotransformation of the cyanobacterial toxin microcystin-LR. *Aquatic Toxicol.*, *70*, 169–178.

Porter K.G. and Orcutt J.D., 1980. Nutritional adequacy, manage ability, and toxicity as factors that determine the food quality of green and blue-green algae for *Daphnia*. *In:* Kerfoot, W.C. (ed.), Evolution and Ecology of Zooplankton Communities, University Press of New England, New Hampshire, 268–281.

Pourriot R. and Snell T.W., 1983. Resting eggs of rotifers. *Hydrobiologia*, *104*, 213–224.

Preston B.L., Snell T.W. and Roberston T.L., 2000. Use of freshwater rotifer *Brachionus calycifiorus* in screening assay for potential endocrine disruptors. *Environ. Toxicol. Chem,*, *19*, 2923–2928.

Qiu T., Xie P., Ke Z., Li L. and Guo L., 2007. In situ studies on physiological and biochemical responses of four fishes with different trophic levels to toxic cyanobacterial blooms in a large Chinese lake. *Toxicon*, *50*, 365–376.

Radix P., Severin G., Schamm K.W. and Kettrup A., 2002. Reproduction disturbances of *Brachionus calyciflorus* (rotifer) for the screening of environmental endocrine disruptors. *Chemosphere*, *47*, 1097–1101.

Rohrlack T., Dittmann E., Henning M., Borner T. and Kohl J.G., 1999. Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*. *Appl. Environ. Microbiol.*, *65*, 737–739.

Sivonen K. and Jones G., 1999. Cyanobacterial toxins. *In:* Chorus I. and Bartram J. (eds.), Toxic Cyanobacteria in Water–a Guide to Their Public Health Consequences, Monitoring and Management, E&FN Spon, London, 41–111.

Sladecék V., 1983. Rotifers as indicators of water quality. *Hydrobiologia*, *100*, 169–201.

Snell T.W. and Carmona M.J., 1995. Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. *Environ. Toxicol. Chem.*, *14*, 415–420.

Snell T.W. and Janssen C.R., 1995. Rotifers in ecotoxicology: a review. *Hydrobiologia*, *313*/*314*, 231–247.

Stelzer C.P., 1998. Population growth in planktonic rotifers. Does temperature shift the competitive advantage for different species?*Hydrobiologia*, *387*/*388*, 349–353.

Stephan C.E. and Rogers J.R., 1985. Advantages of using regression analysis to calculate results of chronic toxicity test. *In:* Bahner R.C. and Hansen D.J.H. (eds.), Aquatic Toxicology and Hazard Assessment: Eight Symposium, American Society for Testing and Materials, Philadelphia, 328–339.

Svrcek C. and Smith D.W., 2004. Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. *J. Environ. Eng. Sci.*, *3*, 155–185.

Tillmanns A.R., Wilson A.E., Pick F.R. and Sarnelle O., 2008. Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. *Fundam. Appl. Limnology*, *171*, 285–295.

USEPA, 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwaater and Marine Organisms, *In:* Peltier W.H. and Weber C.I. (eds.), US Environmental Protect Agency, Washington, DC. EPA/600/4–85/013, 216 p.

van Apeldoorn M.E., van Egmond H.P., Speijers G.J.A. and Bakker G.J.I., 2007. Toxins of cyanobacteria. *Mol. Nutr. Food Res.*, *51*, 7–60.

Vareli K., Briasoulis E., Pilidis G. and Sainis I., 2009. Molecular confirmation of *Planktothrix rubescens* as the cause of intense microcystins-Synthesizing cyanobacterial bloom in Lake Ziros, Greece. *Harmful Algae*, *8*, 447–453.

Vasconcelos V.M. and Pereira E., 2001. Cyanobacteria diversity and toxicity in a wastewater treatment plant (Portugal). *Water Res.*, *35*, 1354–1357.

Wen X.L., Xi Y.L., Yang Y.F., Zhang X.A. and Zhang G., 2011. Temperature is the key factor controlling population dynamics of *Brachionus angularis* inLake Jinghu during summer and autumn. *J. Freshwater Ecol.*, *26*, 2277–2286.

WHO, 1998. Guidelines for Safe Recreational Water Environments: Coastal and Freshwaters, World Heath Organization, Geneva.

Wilson A.E., Sarnelle O. and Tillmanns A.R., 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. *Limnol. Oceanogr.*, *51*, 1915–1924.

Xi Y.L. and Feng L.K., 2004. Effects of thiophanate-methyl and glyphosate on asexual and sexual reproduction in the rotifer *Brachionus calycifiorus* Pallas. *Bull. Environ. Contam. Toxicol.*, *73*, 644–651.

Xi Y.L., Chu Z.X. and Xu X.P., 2007. Effect of four organochlorine pesticides on the reproduction of freshwater rotifer *Brachionus calyciflorus* pallas. *Environ. Toxicol. Chem.*, *26*, 1695–1699.

Xie L., Xie P., Guo L., Li L., Miyabara Y. and Park H., 2005. Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophicLake Chaohu, China. *Environ. Toxicol.*, *20*, 293–300.

Yang Z., Lü K., Chen Y. and Montagnes D.J.S., 2012. The interactive effects of ammonia and microcystin on life-history traits of the cladoceran *Daphnia magna*: synergistic or antagonistic?*PLoS ONE*, *7*, e32285.

Yang Z., Xiang F.H., Minter E.J.A., Lü K., Chen Y.F. and Montagnes D.J.S., 2011. The interactive effects of microcystin and nitrite on life-history parameters of the cladoceran *Daphnia obtusa*. *J. Hazard. Mater.*, *190*, 113–118.

Ye W.J., Liu X.L., Tan J., Li D.T. and Yang H., 2009. Diversity and dynamic of microcystin-producing cyanobacteria inChina's third largest lake, Lake Taihu. *Harmful Algae*, *8*, 637–644.

Yin X.W. and Zhao W., 2008. Studies on life history characteristics of *Brachionus plicatilis* O. F. Müller (Rotifera) in relation to temperature, salinity and food algae. *Aquat. Ecol.*, *42*, 165–176.

Zar J.H., 1999. Biostatistical Analysis, Vol. 4, Prentice Hall, New Jersey, 663 p.

Zhang S.Y., Cheng S.P., He F., Zhuang D.H. and Wu Z.B., 2008. Studies on the toxic effects of filtered cyanobacteria culture media of two different strains (microcystin-producing and microcystin-free) of *Microcystis Aeruginosa on Daphnia Magna*. *Acta Hydrobiol. Sin.*, *32*, 637–642 (in Chinese with English abstract).

Zhang X. and Geng H., 2012. Effect of *Microcystis aeruginosa* on the rotifer *Brachionus calycifiorus* at different temperatures. *Bull. Environ. Contam. Toxicol.*, *88*, 20–24.

Zimba P.V., Khoo L., Gaunt P., Carmichael W.W. and Brittain S., 2001. Confirmation of catfish, *Lctalurus punctatus* (Rafinesque), mortality from microcystins toxins. *J. Fish Dis.*, *24*, 41–47.

Zurawell R.W., Chen H., Burke J.M. and Prepas E.E., 2004. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. *J. Toxicol. Environ. Health Part B Crit. Rev.*, *8*, 1–37.