Experimental studies on the effects of toxic *Microcystis aeruginosa* PCC7820 on the survival and reproduction of two freshwater rotifers *Brachionus calyciflorus* and *Brachionus rubens*

Hong Geng · Ping Xie

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**Abstract** Blooms of *Microcystis aeruginosa* frequently occur in many eutrophic lakes in China; however, there is very little experimental study on the relationship between *Microcystis* and rotifers from Chinese waters. The effects of different concentrations of toxic *M. aeruginosa* PCC7820 on two common freshwater rotifers *Brachionus calyciflorus* and *B. rubens* were investigated in laboratory experiments. *B. calyciflorus* was able to utilize this strain of *M. aeruginosa* as a food source. However, *M. aeruginosa* suppressed the survival and reproduction of *B. calyciflorus* at the highest concentration (10⁶ cells/ml) probably due to the inadequate nutrition. *B. rubens* was inhibited by toxic *M. aeruginosa* PCC7820 and the inhibition increased with the increasing *Microcystis* concentration. Our study indicates that the two rotifers have different sensitivities to toxic *M. aeruginosa* and that toxic cyanobacteria may affect zooplankton community structure by differentially inhibiting the different zooplankton taxa.

**Keywords** *Microcystis aeruginosa* · *Microcystin* · *Brachionus calyciflorus* · *Brachionus rubens* · Survival and reproduction

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**Introduction**

Rotifers are among the most favorable test animals in aquatic toxicology (Snell and Janssen 1995). In recent years, there is an increasing trend in the use of rotifers as bioassay organisms for eco-toxicological studies. They are severely suppressed in zooplankton communities by large cladocerans, especially the large daphniids (Gilbert 1988). Thus, any factors that inhibit large cladocerans more severely than rotifers may increase the relative importance of rotifers, consequently affecting species composition of zooplankton. For instance, the presence of visually feeding, zooplanktivorous fish selectively eat large cladocerans favoring the development of rotifers (O’Brien 1987). It is widely known that blooms of cyanobacteria are often associated with drastic changes in zooplankton community structure, indicated by dominance of small cladocerans, rotifers or copepods (Edmondson and Litt 1982; Gilbert 1990; Fulton and Jones 1991; Hansson et al. 1998; Ghadouani et al. 2003). Laboratory investigations have revealed a number of mechanisms responsible for differential effects of cyanobacteria on various zooplankton species. Copepods are selectively feeders and can avoid ingesting potentially harmful cyanobacteria (DeMott and Moxter 1991). Cyanobacteria morphology (filament length or colony size) may interfere with feeding of large cladocerans (Gilbert and Durand 1990; Rohrlack 1999) and make cyanobacteria unable to be ingested by small zooplankton (Fulton and Pearl 1987; Rothhaupt 1991). In addition, toxins produced by cyanobacteria may have variable effects on different zooplankton taxa (DeMott et al. 1991; Gilbert 1994).

*Microcystis aeruginosa* is a common, bloom-forming species and can produce a kind of toxin named microcystins, which are microbial nonribosomally processed cyclic...
heptapeptides (Doekel and Marahiel 2001). Although numerous studies have examined the effects of *M. aeruginosa* on zooplankton, most investigations have focused on cladocerans, especially the genus *Daphnia* (Nizan et al. 1986; Lampert 1987; Reinikainen et al. 1994; Hietala et al. 1995; DeMott 1999; Rohrlack 1999; Ferrião-Filho et al. 2000; Chen and Xie 2003). Few studies are known about the rotifers (Fulton and Paerl 1987; Rothhaupt 1991; Smith and Gilbert 1995; Nandini and Rao 1998; Nandini 2000). Moreover, the results of above-mentioned studies on rotifers are most often limited to temperate climate, lacking information from subtropical waters. The results of studies conducted in one area may not be helpful in interpreting the observed patterns of zooplankton community structure in another area (Nilssen 1996; Lewis 1987; Williams 1994).

In China, blooms of *M. aeruginosa* frequently occur in many eutrophic lakes (Xie and Liu 2001), which have been a great nuisance to the whole aquatic ecosystem (Christensen 2006). Thus, we studied the impacts of cyanobacteria on zooplankton, most investigations have focused on cladocerans, especially the genus *Daphnia* (Nizan et al. 1986; Lampert 1987; Reinikainen et al. 1994; Hietala et al. 1995; DeMott 1999; Rohrlack 1999; Ferrião-Filho et al. 2000; Chen and Xie 2003). Few studies are known about the rotifers (Fulton and Paerl 1987; Rothhaupt 1991; Smith and Gilbert 1995; Nandini and Rao 1998; Nandini 2000). Moreover, the results of above-mentioned studies on rotifers are most often limited to temperate climate, lacking information from subtropical waters. The results of studies conducted in one area may not be helpful in interpreting the observed patterns of zooplankton community structure in another area (Nilssen 1996; Lewis 1987; Williams 1994).

In China, blooms of *M. aeruginosa* frequently occur in many eutrophic lakes (Xie and Liu 2001), which have been a great nuisance to the whole aquatic ecosystem (Christensen 1996; Lurling 2003). However, up to now, there have been very little experimental studies on the relationship between rotifers and *Microcystis* (Geng and Xie 2006). Thus, we studied the impacts of *M. aeruginosa* PCC7820 on the experimental populations of two common freshwater rotifers, *Brachionus calyciflorus* and *B. rubens*. The main purpose of the present study was to assess the effect of different concentrations of toxic *M. aeruginosa* PCC7820 in combination with the green alga *Scenedesmus obliquus* on survival and reproduction of the rotifers, so as to understand the affecting mechanisms of cyanobacteria on zooplankton community.

**Methods**

The rotifer species (mean adult lorica length ± SE, n = 50) used in the present study were *B. calyciflorus* (180 ± 3 µm) and *B. rubens* (140 ± 4 µm). They were both isolated from Lake Donghu and maintained in the laboratory for more than 6 months prior to the start of this investigation. Stock cultures were derived from a single individual. They were cultured in EPA medium (USEPA 1985) on *S. obliquus* and maintained at 25 ± 1°C on a phoptoperiod (16:8 L/D) with dim light (~300 lux) in an illumination incubator. Before the experiment commenced, the rotifers were fed on *S. obliquus* at the concentration of 5.0 × 10^5 cells/ml for at least 2 weeks.

*S. obliquus* was cultured in HB-4 medium (Li et al. 1959) at 25 ± 1°C on a 16 L: 8 D photoperiod. *M. aeruginosa* PCC7820 was cultured in BG11 medium (Stanier et al. 1971) under similar conditions but with a lower light intensity. The microcystins in this *M. aeruginosa* strain were extracted and analyzed using high performance liquid chromatography (HPLC) following the method described by Fastner et al. (1998) and Yokoyama and Park (2002). The microcystin-LR concentration was 3.16 µg/mg dry weight (SE = 0.26, n = 3). The two algae species were both provided by the Institute of Hydrobiology, Chinese Academy of Sciences. Algae in exponential growth were harvested by centrifugation and resuspended in EPA medium. Cell densities for *S. obliquus* and *Microcystis* suspensions were determined by direct hemacytometer count, and then diluted to the desired concentrations with EPA medium.

Population growth experiments were carried out in five treatments: unfed controls, fed *M. aeruginosa* (10^4, 10^5 and 10^6 cells/ml) alone and fed *S. obliquus* (5.0 × 10^5 cells/ml) alone. There were three replicates for each treatment, each replicate beginning with 14 (*B. rubens*) or 18 (*B. calyciflorus*) animals (0–6h age). The experiments were carried out in 5-ml beakers. Everyday the animals were transferred to fresh medium with appropriate food suspension and population size were noted.

Life-table experiments were conducted in 24-well tissue culture plates and initiated by introducing one neonate (0–6h age) into each well. Any neonates later proving to be mictic instead of amictic females were discarded. The treatments involved four concentrations of *Microcystis* (0, 10^4, 10^5, and 10^6 cells/ml) each with *S. obliquus* at a constant concentration of 5.0 × 10^5 cells/ml. The rotifers were observed every 8 h, and the numbers of original individuals alive and neonates produced were recorded. The initial parental females were transferred into fresh treatment media every 24 h. Mean lifespan and number of offspring produced per female were statistically compared using one-way analysis of variance (ANOVA).

All population growth and life-table experiments were conducted at 25 ± 1°C in darkness. To minimize evaporation, the beakers or plates were placed in a covered container which had its bottom covered with water. The container was placed on an orbital shaker rotating gently to prevent heterogeneity in the distribution of the algae.

Age specific survivorship (l_x) and fecundity (m_x) curves were constructed using conventional life-table techniques (Poole 1974). Net reproductive rate (R_0), generation time (T), life expectancy (e_0), and intrinsic rate of natural increase (r_m) were calculated according to Krebs (1985) and Lotka (1913).

**Results**

In the population growth experiment, *B. calyciflorus* populations differed in five different treatments (Fig. 1a). All animals in unfed controls died out within 3 days. However, there still had been survivals in other two *M. aeruginosa*-fed treatments before the termination of this experiment.
In other words, animals given *M. aeruginosa* alone persisted slightly longer than those given no food, although it showed very little reproduction and did not maintain a viable population, as it did in the *S. obliquus*-fed treatment. In contrast, in the *B. rubens* populations, rotifers cultured with *M. aeruginosa* alone died faster than the unfed controls (Fig. 1b).

The effects of *M. aeruginosa* PCC7820 on the age-specific survivorship (l_x) and fecundity (m_x) of the two rotifer species are presented in Fig. 2. For both rotifers, there were significant overall effects of *M. aeruginosa* on mean lifespan and offspring number (Table 1). However, *B. calyciflorus* was less sensitive to *M. aeruginosa*, and only at high concentration (10^6 cells/ml), both lifespan and offspring number were significantly reduced to 29 and 68% of the control value (Table 2). *B. rubens* was more susceptible to the presence of *M. aeruginosa* than *B. calyciflorus*, with both values reduced significantly at concentrations of 10^5 and 10^6 cells/ml. Moreover, the reduction in *B. rubens* was much greater than that in *B. calyciflorus*. At a concentration of 10^5 cells/ml, the mean lifespan and number of offspring of *B. rubens* were reduced to 38 and 67% of the control, respectively (Table 2).

All demographic parameters of the two rotifer species in the different treatments are showed in Table 3. When the concentration of *Microcystis* increased, the values of r_m, R_0 and e_0 reduced. This reduction was especially distinct at *M. aeruginosa* concentration of 10^6 cells/ml in *B. calyciflorus* and 10^5 and 10^6 cells/ml in *B. rubens*.

**Discussion**

The results of the present study show that *B. calyciflorus* cultured with *M. aeruginosa* alone survived better than those given no food. Although *B. calyciflorus* was unable to maintain population growth when fed *Microcystis* alone, we still observed egg production at high *Microcystis* concentration (10^6 cells/ml), indicating that this rotifer was able to utilize *Microcystis* as a food source despite its toxicity. This was also demonstrated at low *Microcystis* concentrations in life table experiments. DeMott et al. (1991) indicated that by providing some nutrition, even toxic cells could prolong survival when compared to starvation. Similar to our results, Fulton and Paerl (1987) observed that *B. calyciflorus* were able to resist *M. aeruginosa* toxins and to utilize *M. aeruginosa* at least as a supplementary nutritional source. Our previous study also testified this result (Geng and Xie 2006). However, there are also conflicting reports. Starkweather and Kellar (1987) found that *M. aeruginosa* NRC-SS-17 decreased both survivorship and reproduction of *B. calyciflorus*. Nandini and Rao (1998) and Nandini (2000) also reported that a strain of *M. aeruginosa* collected from a local pond was toxic to *B. calyciflorus* population. Thus, it appears that the effects of *M. aeruginosa* on rotifers depend on both *Microcystis* strains and rotifer clones.

In the present study, although *B. calyciflorus* was tolerant to *Microcystis* toxin to some extent, its survival and reproduction were adversely affected by toxic *M. aeruginosa* PCC7820 at the highest concentration (10^6 cells/ml). The possible reason may be the inadequate nutrition of *Microcystis*, which have been testified in some other studies (Arnold 1971; Fulton and Paerl 1987; Smith and Gilbert 1995; Ferrão-filho et al. 2000). In our population growth experiment, we found that animals cultured with *S. obliquus* survived and reproduced much better than those fed *Microcystis* alone, indicating the lower food quality in *Microcystis* comparing to green alga *S. obliquus*. 
Therefore, the most plausible explanation is that the proportion of nutritious green algae *Scenedesmus obliquus* in the diet might have decreased with an increase in the relative abundance of *Microcystis*. In other words, *Microcystis* ingested by *B. calyciflorus* could not compensate for the decrease of *S. obliquus* in terms of food availability.

This is also the first report on the effects of *M. aeruginosa* on the reproductive potentials \( r_m \) of *B. rubens*. There is only one study on the effects of *Microcystis* on this rotifer in terms of population size (Rothhaupt 1991),

### Table 1

<table>
<thead>
<tr>
<th>Rotifer</th>
<th>Parameter</th>
<th>df</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. calyciflorus</em></td>
<td>Lifespan</td>
<td>3</td>
<td>3.64</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Offspring number</td>
<td>3</td>
<td>4.18</td>
<td>0.009</td>
</tr>
<tr>
<td><em>B. rubens</em></td>
<td>Lifespan</td>
<td>3</td>
<td>11.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Offspring number</td>
<td>3</td>
<td>8.55</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Rotifer</th>
<th><em>Microcystis</em> concentration (cells/ml)</th>
<th>Life-span (days)</th>
<th>% Reduction</th>
<th>Offspring number</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. calyciflorus</em></td>
<td>0</td>
<td>5.87 ± 0.54 A</td>
<td>–</td>
<td>5.93 ± 1.38 A</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10^4</td>
<td>6.00 ± 0.48 A</td>
<td>–</td>
<td>6.67 ± 1.12 A</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10^5</td>
<td>6.00 ± 0.47 A</td>
<td>–</td>
<td>4.37 ± 1.04 A</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>10^6</td>
<td>4.15 ± 0.47 B</td>
<td>29</td>
<td>1.90 ± 0.74 B</td>
<td>68</td>
</tr>
<tr>
<td><em>B. rubens</em></td>
<td>0</td>
<td>7.44 ± 0.98 A</td>
<td>–</td>
<td>7.31 ± 1.99 A</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10^4</td>
<td>6.76 ± 0.84 A</td>
<td>9.1</td>
<td>8.35 ± 1.94 A</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10^5</td>
<td>4.62 ± 0.33 B</td>
<td>38</td>
<td>2.38 ± 0.63 B</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>10^6</td>
<td>2.84 ± 0.21 C</td>
<td>62</td>
<td>0.32 ± 0.23 C</td>
<td>96</td>
</tr>
</tbody>
</table>

The concentration of *Microcystis* ranged from 0 to \( 10^6 \) cells/ml while the concentration of *Scenedesmus* was kept constant at \( 5.0 \times 10^5 \) cells/ml. Means (±SE) followed by the same letter are not significantly different (Tukey’s HSD test, \( P < 0.05 \)).
showing that B. rubens cultured with Microcystis died faster than nonfed controls. Our results also found that M. aeruginosa PCC7820 was toxic to B. rubens. The increased inhibition at higher concentrations of Microcystis in the present study is consistent with the previous studies on other zooplankton species (Smith and Gilbert 1995; Ferrão-Filho et al. 2000).

In our study, B. rubens was more sensitive to toxic M. aeruginosa PCC7820 than B. calyciflorus, indicating the different sensitivities of different rotifers to toxic cyanobacteria. Because M. aeruginosa generally do not release extracellular toxins, only those zooplankton species that ingest Microcystis should be inhibited by their toxins. Therefore, the different susceptibilities of rotifers to M. aeruginosa may be partly due to their different tendencies to eat Microcystis. Unlike copepods and some other rotifers, Brachionus does not have the ability to select food based on its quality (DeMott 1986). Rothhaupt (1990) also found that Brachionus feed at high rates on polystyrene spheres that are comparable to feeding rates on algae of the same size and suggested that ingestion efficiencies are largely particle-size dependent, each rotifer strain having a relatively narrow range of effectively ingestible food items. B. rubens feeds more effectively on smaller particles <6 μm than B. calyciflorus. In our study, M. aeruginosa PCC7820 was unicellular with a diameter of 3–5 μm. So, B. rubens is likely to ingest Microcystis more efficiently than B. calyciflorus and, hence, to receive higher toxin concentrations in its tissues. However, in the natural waterbodies, Microcystis usually existing in the large, amorphous colonies might be too large for B. rubens to ingest. Therefore the inhibition might be reduced. We will discuss this point in the following paragraph. The other potential mechanism to explain the different susceptibilities may be the different sensitivities to cyanotoxins. Gilbert (1994) found that B. calyciflorus was the least sensitive to the soluble neurotoxic alkaloid anatoxin-a produced by Anabaena flos-aquae comparing to other three rotifer species.

Our results were compared with literature results for two studies with Daphnia that used the same strain of toxic algae (Table 4). The comparison of rm suppression in four zooplankton species suggests that the inhibition of two Daphnia species was greater than that of two Brachionus rotifers in the presence of M. aeruginosa. Previous laboratory investigations have also shown that large cladocerans are more inhibited by toxic cyanobacteria than smaller cladocerans and rotifers (Fulton and Paerl 1987; Fulton 1988; Gilbert 1990; Smith and Gilbert 1995). In field, such differential inhibition by toxic cyanobacteria could be much more pronounced. Toxic cyanobacteria occurring as large, amorphous colonies (Microcystis) or as

<table>
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<th>Table 3</th>
<th>The demographic parameters of Brachionus calyciflorus and B. rubens fed mixtures of Microcystis aeruginosa (0–10⁶ cells/ml) and a constant concentration of Scenedesmus obliquus (5.0 × 10⁵ cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotifer</td>
<td>Microcystis concentration (cells/ml)</td>
</tr>
<tr>
<td>B. calyciflorus</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
</tr>
<tr>
<td></td>
<td>10⁵</td>
</tr>
<tr>
<td></td>
<td>10⁶</td>
</tr>
<tr>
<td>B. rubens</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
</tr>
<tr>
<td></td>
<td>10⁵</td>
</tr>
<tr>
<td></td>
<td>10⁶</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Table 4</th>
<th>The intrinsic rate of growth (r_m) suppression of four different zooplankton species fed the mixtures of toxic Microcystis aeruginosa and nutritious, nontoxic algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zooplankton species</td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>D. pulex</td>
<td>24</td>
</tr>
<tr>
<td>D. magna</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>B. calyciflorus</td>
<td>25</td>
</tr>
<tr>
<td>B. rubens</td>
<td>25</td>
</tr>
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<td></td>
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</table>
long, aggregated or mucilage-coated filaments (*Anabaena*) would be too large for most rotifers to ingest, but could be eaten by cladocerans, especially large daphniids. Thus, toxic cyanobacteria should inhibit these daphniids more severely than rotifers. Since large cladocerans are superior competitors, capable of suppressing or causing extinction of rotifers through both exploitative and interference competition (see review in Gilbert 1988), the presence of these cyanobacteria in aquatic plankton communities could markedly affect zooplankton species structure by differentially inhibiting the daphniids and thereby favoring the rotifers.

**Conclusion**

The effects of toxic *M. aeruginosa* PCC7820 in combination with the green alga *S. obliquus* on the survival and reproduction of two freshwater rotifers *B. calyciflorus* and *B. rubens* were investigated in laboratory experiments. The results showed that *B. calyciflorus* cultured with *M. aeruginosa* alone survived better than those given no food. The life-table experiment also testified that *B. calyciflorus* was able to use this strain of *M. aeruginosa* as a food source. However, *M. aeruginosa* suppressed the survival and reproduction of *B. calyciflorus* at the highest concentration (10⁶ cells/ml) probably due to the inadequate nutrition. In contrast, *B. rubens* cultured with *M. aeruginosa* alone died faster than the unfed controls. The population of *B. rubens* was inhibited by toxic *M. aeruginosa* PCC7820, which was more severe with increased *Microcystis* concentrations. Our study showed that *B. rubens* was more sensitive to toxic *M. aeruginosa* PCC7820 than *B. calyciflorus*, indicating the different sensitivities of different rotifers to toxic cyanobacteria. Therefore, toxic cyanobacteria may affect zooplankton community structure by differentially inhibiting the different zooplankton taxa.

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