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Effect of Toxic *Microcystis aeruginosa* PCC7820 in Combination with a Green Alga on the Experimental Population of *Brachionus calyciflorus* and *B. rubens*

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The occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs, and recreational waters has become a worldwide problem (Paerl et al. 2001). Among the various cyanobacteria, *Microcystis aeruginosa* is perhaps the most common species and can produce a kind of hepatotoxin called microcystins (Christoffersen 1996, Oh et al. 2000). Microcystins are released into the water column during the collapse of toxic cyanobacterial blooms (Watanable et al. 1992) and can be harmful to many kinds of aquatic organisms including fish (Penaloza et al. 1990, Rabergh et al. 1991) and zooplankton (Fulton and Paerl 1987, Ferrao-Filho et al. 2000). In laboratory, most investigations, which have examined the effects of *M. aeruginosa* on zooplankton, are focused on cladocerans, especially the genus *Daphnia* (Nizan et al. 1986, Reinikainen et al. 1994, Hietala et al. 1995, DeMott 1999, Rohrlack 1999, Liu et al. 2005). Few studies are known about the rotifers (Fulton and Paerl 1987, Rothhaupt 1991, Smith and Gilbert 1995, Nandini and Rao 1998, Nandini 2000), and the results are conflicting. Moreover, the above-mentioned studies on rotifers are most often limited to temperate climate, lacking information from subtropical waters.

In China, blooms of *M. aeruginosa* frequently occur in many shallow eutrophic lakes (Xie and Liu 2001), and, up to now, there have been no experimental studies on the relationship between rotifers and *Microcystis*. Thus, in this study, we evaluated the impacts of *M. aeruginosa* on the experimental populations of two freshwater rotifers, *Brachionus calyciflorus* and *B. rubens*. The main purposes were to assess the effect of different concentrations of toxic *M. aeruginosa* PCC7820 in combination with the green alga *Scenedesmus obliquus* on population growth and body size of the rotifers, and to compare the different sensitivities of two rotifers to this *M. aeruginosa* strain.

MATERIALS AND METHODS

B. calyciflorus and *B. rubens* were collected from Lake Donghu and maintained in the laboratory for many generations prior to initiation of this experiment. A clone of each species was derived from a single female and cultured in EPA medium (USEPA 1985) on a diet of 5.0×10^5 cells/mL *S. obliquus* at 25±1℃ and 16L: 8D photoperiod.

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Axenic *M. aeruginosa* PCC7820 and the green algae *S. obliquus* were obtained from Institute of Hydrobiology, Chinese Academy of Sciences. *M. aeruginosa* PCC7820 was cultured in BG11 medium (Stanier et al. 1971) at 25±1℃ under a 16L: 8D photoperiod. In our study, this *M. aeruginosa* strain produced exclusively microcystin-LR, which was extracted and analyzed using high performance liquid chromatography (HPLC) following the method of Zheng et al. (2004). The microcystin-LR concentration was 3.16 μg/mg dry weight (SE=0.26, n=3). *S. obliquus* was cultured in HB-4 medium (Li et al. 1959) under similar conditions. Algae in exponential growth were concentrated by centrifugation and then resuspended in EPA medium. Algal concentrations were measured with a hemacytometer and diluted to the desired concentrations with EPA medium.

The experiments included four different treatments: Control- 5.0×10^5 cells/ml *S. obliquus*; M1- 10^4 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M2- 10^5 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M3- 10^6 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*. Each treatment had three replicates. Experiments were conducted in 20ml glass beakers containing 10ml food solution at 25±1℃ in incubator. Each beaker contains 10 neonates (<6h age) of one of two rotifer species. The animals were transferred daily to fresh medium with appropriate food suspension, and the numbers of all live individuals were recorded. The experiments were terminated after 14 days when populations had reached an asymptote.

For recording rotifer body size, 50-100 individuals of each treatment were selected randomly and preserved in 5% formalin when the experiments were terminated. However, individuals of *B. rubens* were not selected because this rotifer was unable to maintain the population at higher *Microcystis* concentrations. The lorical length (L, spines excluded) was measured using a calibrated ocular micrometer. The population growth rate (r) was calculated as following equation (Krebs, 1985): $r = (\ln N_t - \ln N_0)/t$, where t is the experimental period, N_t the population density after t days and N_0 the initial population densities. The L, r and the population density were statistically analyzed using one-way ANOVA and Tukey's tests.

RESULTS AND DISCUSSION

The population density and population growth rate of *B. calyciflorus* in different treatments are presented in Fig. 1. There was a significant impact of *Microcystis* concentration on both of the two characteristics (ANOVA, $P<0.001$). Post-hoc analysis showed that only the population in treatment M3 differed significantly from three other treatments ($P<0.05$, Tukey's test). The population of *B. calyciflorus* was not adversely affected by *Microcystis* at its concentrations of 10^4 and 10^5 cells/ml. Fulton and Paerl (1987) also found that *B. calyciflorus* was able to resist *M. aeruginosa* toxin and to utilize *M. aeruginosa* as at least a supplementary nutritional source.

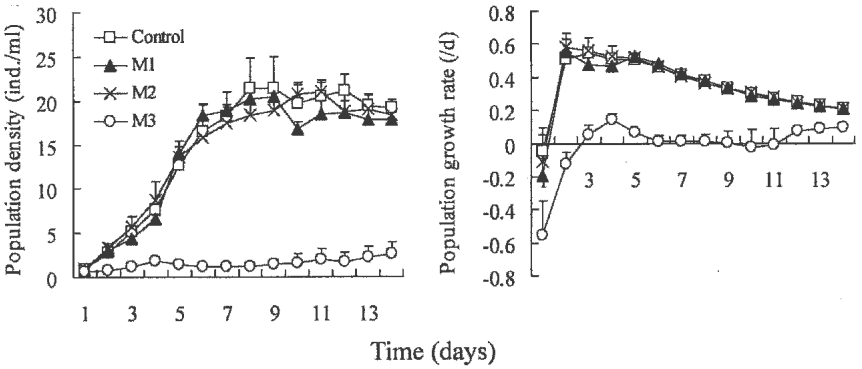

Figure 1. The population density and population growth rate of *Brachionus calyciflorus* in four different treatments. Control: 5.0×10^5 cells/ml *S. obliquus*; M1: 10^4 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M2: 10^5 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M3: 10^6 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*.

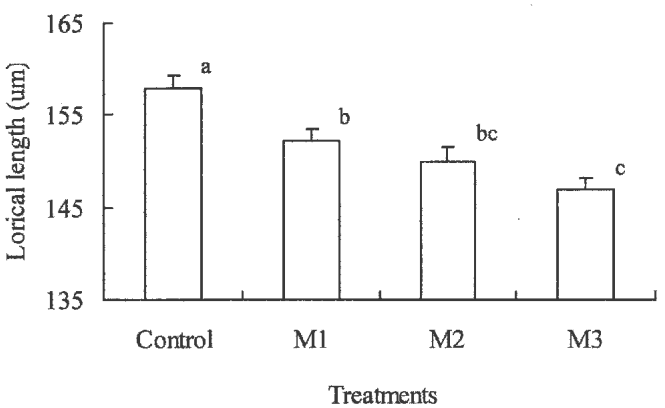

Figure 2. The lorical length of *Brachionus calyciflorus* in four different treatments. Control: 5.0×10^5 cells/ml *S. obliquus*; M1: 10^4 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M2: 10^5 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M3: 10^6 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*. (The same letters indicates that there are no significant differences among the corresponding treatments)

When the *Microcystis* concentration increased to 10^6 cells/ml, the population growth of *B. calyciflorus* was significantly inhibited, which was consistent with

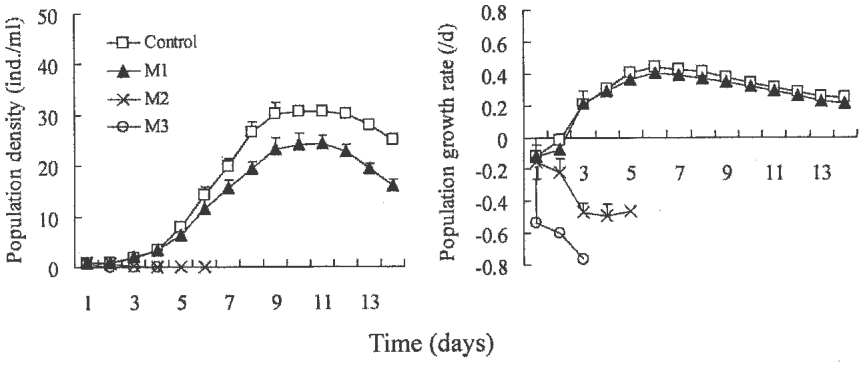

Figure 3. The population density and population growth rate of *Brachionus rubens* in four different treatments. Control: 5.0×10^5 cells/ml *S. obliquus*; M1: 10^4 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M2: 10^5 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*; M3: 10^6 cells/ml *M. aeruginosa* PCC7820+5.0×10^5 cells/ml *S. obliquus*.

the results of previous studies (Starkweather and Kellar 1987, Nandini and Rao 1998, Nandini 2000). There were two possible reasons. One is that the microcystins content increased with the increasing concentration of *Microcystis*, therefore, the inhibition on the population growth increased accordingly. Another plausible explanation is that the relative abundance of nutritious green algae *S. obliquus* in the diet might decrease with an increase of *Microcystis*. Some other studies have testified the inadequate nutrition of *Microcystis* (Arnold, 1971; Fulton and Paerl, 1987; Smith and Gilbert, 1995; Ferrão-filho et al., 2000). In other word, *Microcystis* ingested by *B. calyciflorus* could not compensate for the decrease of *S. obliquus* in terms of food availability.

The lorical length of *B. calyciflorus* was significantly influenced by the concentration of *M. aeruginosa* (ANOVA, $P<0.001$). The rotifers in control had the maximal lorical length among the four treatments. In addition, the lorical length of rotifers in treatment M1 was higher than that in treatment M3 (Fig. 2). The relationship between the lorical length (L, μm) and the concentration (X, $\times 10^4$ cells/ml) of *M. aeruginosa* could be described as the linear equation of $L= -0.0705X+153.71$ ($R^2 = 0.0401$, $P<0.01$). Similarly, Nandini and Rao (1998) showed that the size of *B. calyciflorus* was lower in the presence of *M. aeruginosa* at temperature of 30℃. Smith and Gilbert (1995) also found that the size at maturity in three species of *Daphnia* was significantly reduced by the toxic strains of *M. aeruginosa*.

The population growth of *B. rubens* was obviously inhibited in the presence of toxic *M. aeruginosa* (ANOVA, $P<0.001$). The animals in the treatments M2 and M3 couldn't maintain the population growth, and all the individuals died out after

5 and 3 days, respectively (Fig. 3). The population density and population growth rate of rotifers in the above two treatments were significantly lower than those in control and the treatment M1 ($P<0.05$, Tukey's test). The population density of rotifers in the treatment M1 was slightly lower than that in control, but no significant difference was observed. Our results found that *M. aeruginosa* PCC7820 was toxic to *B. rubens*, which was also indicated by the study of Rothhaupt (1991), showing that *B. rubens* cultured with *M. aeruginosa* died faster than unfed controls.

In our study, *B. rubens* was more susceptive to toxic *M. aeruginosa* PCC7820 than *B. calyciflorus*. 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 two rotifers to toxic *M. aeruginosa* may be partly due to their different tendencies to eat *Microcystis*. In this study, toxic *M. aeruginosa* PCC7820 was unicellular with a diameter of 3-5μm, which is readily ingestible by suspension-feeding rotifers. However, *B. rubens* could feed more effectively on smaller particles < 6μm than *B. calyciflorus* (Rothhaupt 1990). So, *B. rubens* is likely to ingest *Microcystis* more efficiently than *B. calyciflorus* and, hence, to receive higher toxin concentrations in its tissues. The other potential mechanism to explain the different susceptibilities may be the different sensitivities to microcystins. Gilbert (1994) found that comparing to three other rotifer species, *B. calyciflorus* was the least sensitive to the soluble neurotoxic alkaloid anatoxin-a produced by *Anabaena flos-qauae*.

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