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Effects of Toxic and Nontoxic *Microcystis aeruginosa* on Survival, Population-Increase, and Feeding of Two Small Cladocerans

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With increasing human activities, eutrophication in freshwater lakes has been accelerated worldwide in the past decades. The most serious problem of eutrophication is the occurrence of cyanobacterial bloom, which not only causes great nuisance to aquatic ecosystems (Falconer 1996; Lürling 2003), but also affects water aesthetic appearance and even human health (Meybeck et al. 1989; Paerl and Tueker 1995). *Microcystis*, inadequate in nutrition (Fulton and Paerl 1987; Smith and Gilbert 1995) is one of the most common groups of cyanobacterial blooms (Nandini 2000). Some strains of *Microcystis* can produce toxins named microcystins (MC). The microcystins,e specially the variant MC-LR, are believed to exert strong toxic effects on herbivorous zooplankton, causing increase in mortality, and reduction in reproductive output and feeding rate (Reinikainen et al. 1994; Laurén-Määttä et al. 1997). However, non-toxic *Microcystis* strains do not exert such harmful effects on cladocerans (Lürling 2003; Rohrlack et al. 2001).

The responses of larger cladocerans (mainly Daphnia spp.) to *Microcystis* have been intensively studied in the laboratory (DeMott 1999; Nizan et al. 1986), as they are a key component of freshwater food chains. However in field, *Microcystis* blooms usually cause decline in larger cladocerans but increase in small zooplankton such as rotifers and smaller cladocerans (Gilbert 1990; Fulton and Jones 1991; Hansson et al. 1998; Ghadouani et al. 2003; Gustafsson et al. 2004). In previous studies, it has been demonstrated that different smaller zooplankton show different responses to *Microcystis* blooms (Fulton and Pearl 1987; Smith and Gilbert 1995). It is necessary to study the effects exerted by toxic and non-toxic *Microcystis* on the smaller cladocerans.

MATERIALS AND METHODS

Two strains of *Microcystis aeruginosa* PCC7820 were used in our study, one contained microcystin-LR, called the toxic strain, and the other was a mutant strain of *M. aeruginosa* PCC7820 without microcystins, called the non-toxic strain. Toxic and non-toxic *M. aeruginosa* strains PCC7820 were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Before the experiment, the toxic and non-toxic *M. aeruginosa* strains PCC7820 were

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axenically cultured with BG11 medium in Erlenmeyer flask. The flasks were incubated on a rotating shaking device (60rpm) in 12L: 12D photoperiod at 25℃ ±0.5℃ in an incubator. The microcystins in the toxic *M. aeruginosa* PCC7820 were extracted and analyzed using high performance liquid chromatography (HPLC) following the method of Zheng et al. (2004). The content of microcystin-LR was 3.827 μg/mg dry weight, and no MC was detected in the non-toxic strain.

The green alga *Scenedesmus obliquus* was obtained from the Institute of Hydrobiology, CAS, and cultured with Shuisheng VI medium (Li et al. 1959) in 1 L Erlenmeyer flask. The flasks were also incubated on a rotating shaking device in 12L: 12D photoperiod at 25℃±0.5℃ in an incubator. The cladocerans, *Moina micrura* and *Ceriodaphnia cornuta*, were collected from Lake Donghu and Lake Chaohu, respectively, both of which are eutrophic lakes in China. The animals have been cultured in 1 L jars containing the M7 medium (Samel et al. 1999) with *S. obliquus* as food in laboratory for one year. All the animals used for survival and growth experiments came from clones established from a single parthenogenetic female.

In the survival experiments, ten juvenile animals (<12h old) were cultured in M7 medium with *M. aeruginosa* strains PCC7820 (toxic and non-toxic) at two concentrations: 1×10^4 cells/ml and 1×10^5 cells/ml in 250ml beakers with 200ml food. Each treatment lasted 10 days, and at each day the survivals were recorded and then transferred to another beaker with fresh food solution. Every experiment were carried out at 25℃±0.5℃ in an incubator and has three replicates.

In the growth experiment, ten juvenile animals (<12h old) were fed with mixed *M. aeruginosa* (toxic and non-toxic) and *S. obliquus* in five different proportions: Treatment A- Only *S. obliquus* with a concentration of 10^5cells/ml; Treatment B- 80% *S. obliquus* and 20% *M. aeruginosa* PCC7820 (8×10^4 cells/ml *S. obliquus*+2×10^4 cells/ml *M. aeruginosa* PCC7820); Treatment C- 50% *S. obliquus* and 50% *M. aeruginosa* PCC7820 (5×10^4 cells/ml *S. obliquus*+5×10^4 cells/ml *M. aeruginosa* PCC7820); Treatment D- 20% *S. obliquus* and 80% *M. aeruginosa* PCC7820 (2×10^4 cells/ml *S. obliquus*+8×10^4 cells/ml *M. aeruginosa* PCC7820); Treatment E- Only *M. aeruginosa* PCC7820 with a concentration of 10^5cells/ml. The total food concentration ata ll the treatments was kept constant at 1×10^5cells/ml. The experiment condition was the same with the survival experiment, and each experiment has three replicats. The experiments lasted 10 days and the survivals were recorded daily and transferred to another beaker with fresh food solution. Population increase rate (r) was calculated as follows: $r = (\ln Nt - \ln N_0)/t$, where t is the experimental period, Nt the number of animals in the beaker after t days and No the number of animals on the first day.

Feeding experiment is designed to test the inhibitory effects of *M. aeruginosa* PCC7820 on the feeding rate of two cladocerans. Ten adult animals of comparable body length were collected and were placed in 250ml beakers with 200ml food. In

this experiment, we also used the same five different food treatments (treatment A, B, C, D and E). Three replicate beakers per treatment were placed in the dark at 25℃±0.5℃ for 3 h. Initially and after 3h, algae concentrations were counted by haemacytometer under microscope. Clearance rate (*CR*, mL ind.$^{-1}$ h^{-1}) was calculated as follows: $CR= V(\ln C_0-\ln C_t)/(tN)$ (Peters 1984), where V is the the culture volume in mL （200ml）,t is the length of time the animals were allowed to feed (3h), N is the number of animals in the container, C_0 is the initial cell concentration and C_t is the final cell concentration after 3h. Variations in survival rate, population increase rate and clearance rate of cladocerans were analyzed by ANOVA in SPSS11.0.

RESULTS AND DISCUSSION

The results of the survival experiments are showed in Fig.1. Two-way ANOVA analyses indicated that both animal species (F= 6.572; P=0.05) and food type (F= 7.160; P=0.041) have significant effects on the survival rates of cladocerans. *M. micrura* showed a significantly lower survival in all the treatments than *C. cornuta*. This suggests that *M. micrura* was more sensitive to starvation and *Microcystis* than *C. cornuta*. Ferräo-filho et al. (2000) and Nandini (2000) also found the similar results. Romanovsky (1985) suggested that such difference may be associated with their different life histories. In this hypothesis, *Moina* belongs to the small-bodied, rapid-growing species. These species have maximum biotic potential and allocate much energy to reproduction, but are highly susceptible to starvation and to lower food quality. While *Ceriodaphnia* he suggested belongs to the small-bodied, but slow-growing species. These species are able to survive food depletion in disturbed environments due to a lower threshold food concentration, a low rate of juvenile growth and a slow population growth.

Bonferroni post hoc comparison revealed three homogeneous groups for the different food type: (1) 10^5cells/ml non-toxic *M. aeruginosa*, (2) 10^4cells/ml non-toxic *M. aeruginosa*, no food, (3) 10^4cells/ml toxic *M. aeruginosa* and 10^5cells/ml toxic *M. aeruginosa*. Both the survival rates of the two cladocerans were in the order of non-toxic *M. aeruginosa* > starved > toxic *M. aeruginosa*. The higher concentration of non-toxic *M. aeruginosa* PCC7820 resulted in higher survivorship, especially for *C. cornuta* which was observed to produce eggs at the end of the experiment. The median lethal time (LT_{50}, day) were also in the order of non-toxic *M. aeruginosa* > starved > toxic *M. aeruginosa* (Table. 1). This indicated that the mutant *M. aeruginosa* PCC7820 (no containing microcystins) in our study has no lethal effect on cladocerans, and the microcystins are the major cause of acute toxicity to cladocerans. Rohrlack et al. (2001) found that several daphnids fed with the mutant *M. aeruginosa* PCC 7806 survived either longer or as long as starving animals. Lürling (2003) found that the effect of non-toxic *Microcystis* on *D. magna* was comparable to no food treatment. In general, non-toxic *Microcystis* is believed to be nutritionally deficient.

The population growth rates of the two cladocerans significantly increased (One-

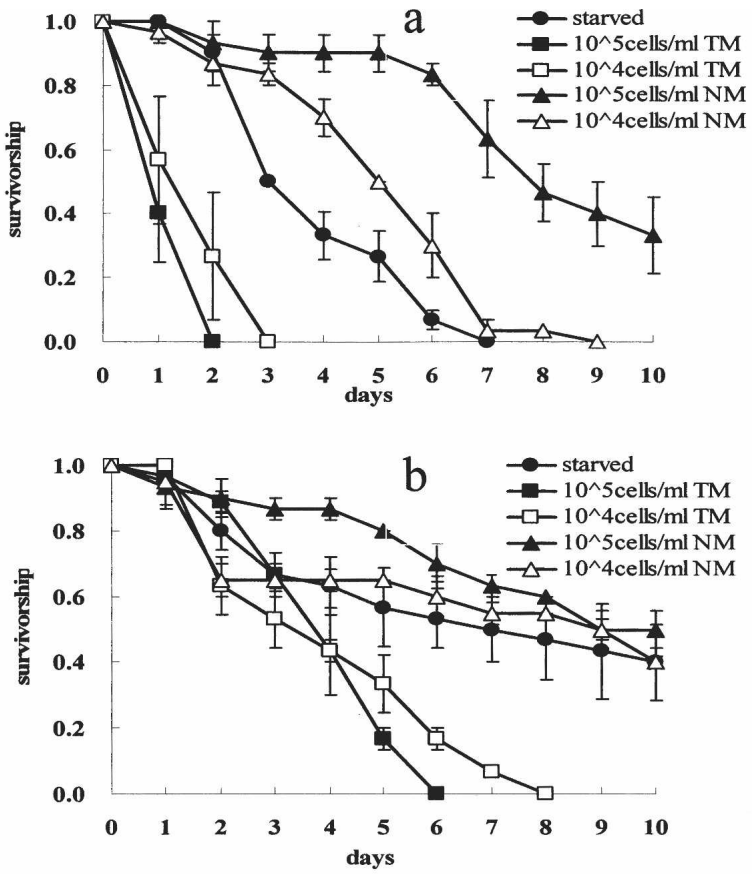

Figure 1. Survivorship of *M. micrura* (a) and *C. cornuta* (b) exposed to no food and single toxic or non-toxic *M. aeruginosa* PCC7820 with two different concentrations. TM: toxic *M. aeruginosa* PCC7820; NM: non-toxic *M. aeruginosa* PCC7820

Table 1. Median lethal time (LT_{50}, day) of two cladocerans in different treatments. The bracketed figures are 95% confidence limits.

Species	10^5cells/m NM	10^4 cells/m NM	Starved	10^4cells/m TM	10^5cells/ml TM
M. micrura	8.6(4.4-12.9)	4.9(3.6-10.3)	3.65 (2.3-4.9)	1.8(1.5-2.1)	0.98(0.7-1.3)
C. cornuta	9.8(3.6-16.2)	8.6(3.2-14.5)	7.2(5.6-9.3)	4.7(2.6-7.5)	3.7(1.6-5.7)

NM: non-toxic *M. aeruginosa*; TM: toxic *M. aeruginosa*

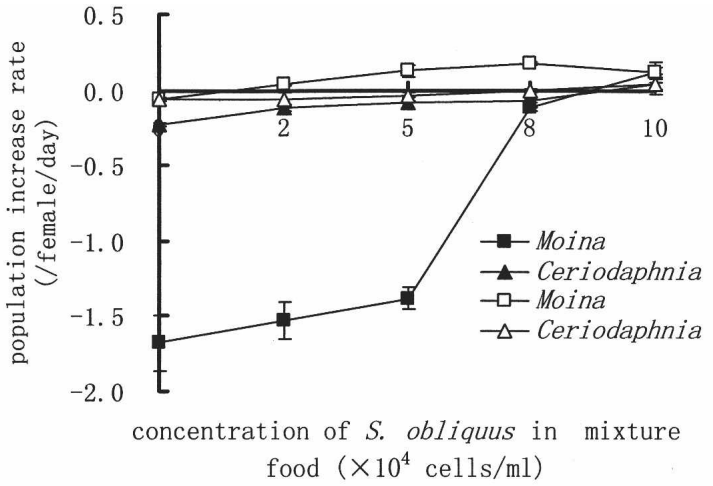

Figure 2. Relationship between the population growth rates of cladocerans and different combinations of *S. obliquus* and *Microcystis* in mixture food. The sum of *S. obliquus* and *M. aeruginosa* PCC7820 in each treatment was 1×10^5 cells/ml. Open symbols stand for mixture of *S. obliquus* and non-toxic *M. aeruginosa* PCC7820, and the black symbols for mixture of *S. obliquus* and toxic *M. aeruginosa* PCC7820.

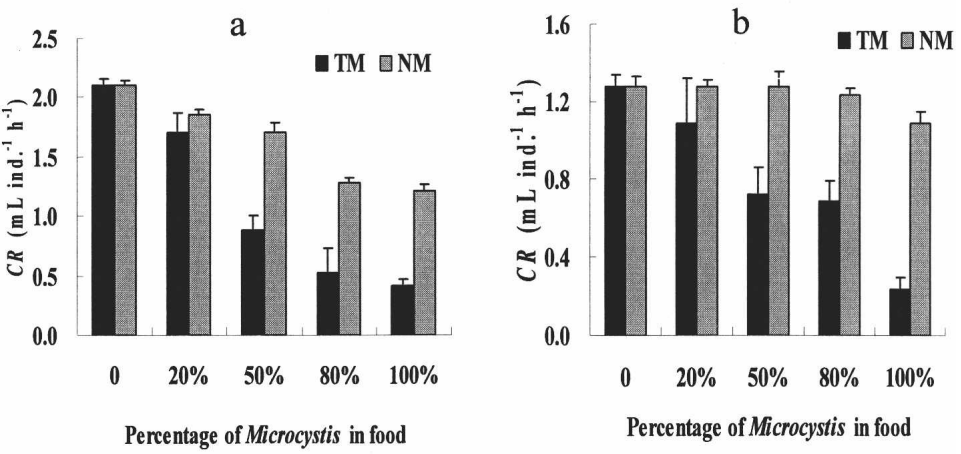

Figure 3.E ffects of toxic and non-toxic *M. aeruginosa* PCC7820 on the clearance rates of *M. micrura* (a) and (b) *C. cornuta*. TM: toxic *M. aeruginosa* PCC7820; NM: non-toxic *M. aeruginosa* PCC7820

way ANOVA, *Moina* with toxic strain: F=41.82, P<0.0001; *Moina* with non-toxic strain: F=7.18, P=0.005; *Ceriodaphnia* with toxic strain: F=15.07, P=0.0003, Fig. 2) with increasing proportions of *S. obliquus* in the mixed diet, except for that of *C. cornuta* in the treatment of non-toxic strain and *S. obliquus* (F=1.52; P=0.27). The population increase rate of *M. micrura* in the treatment of toxic *M. aeruginosa* and *S. obliquus* showed a sigmoid response (r^2=0.902) to the increasing *S. obliquus* concentration.

Scenedesmus is usually believed to have the ability to reduce toxic effects of toxic *Microcystis* on cladoceran, especially for *Daphnia* (Renikainen et al. 1994; Chen and Xie 2004). Our experiments on small cladocerans got a similar conclusion according to their significantly improved population increase rates when *S. obliquus* was added. All these results suggest that diet composition (containing *Scenedesmus* or not) is very important to the cladoceran survival, regardless of their body size. Naturally in field, there are plenty of quality foods (e.g., other green-algae, diatoms and bacteria) coexisting with *Microcystis* colonies. These quality foods can be effectively utilized by the cladocerans. But, colony of toxic *Microcystis* often clogs the pleopods of large cladocerans and causes its death after ingestion. For small cladocerans, such *Microcystis* colony is too large to be ingested (Fulton and pearl 1987). And the small-sized cladocerans may develop stronger tolerance against toxic *M. aeruginosa* than large-sized ones (Guo and Xie in press). These may be the main mechanisms explaining why small cladocerans can coexist with *Microcystis* blooms whereas larger cladocerans can not.

The clearance rates of *M. micrura* and *C. cornuta* are showed in Fig. 3. Fig 3a shows that food composition (two-way ANOVA, F= 7.917; P=0.035) and *M. aeruginosa* strains have significant effects (two-way ANOVA, F= 8.184; P=0.046) on the clearance rate of *M. micrura*. Thus, the clearance rates of *M. micrura* were influenced by the percentage of *M. aeruginosa* in the food. The higher percentage of toxic or non-toxic *M. aeruginosa* resulted in the remarkably decreased clearance rates. And the clearance rates of *M. micrura* were lower in the presence of toxic *M. aeruginosa* than that in the presence of non-toxic strain in the mixed diet. Bonferroni post hoc comparison revealed three homogeneous groups for the mixed food with different percentage of *M. aeruginosa*: 1) 0% and 20% *M. aeruginosa* treatments; 2) 50% and 80% *M. aeruginosa* treatments; 3) 100% *M. aeruginosa* treatments. From these results, we may propose that the feeding inhibition is not only a defense to microcystins but to some other compounds, which was corroborated by the studies of Rohrlack et al. (1999, 2001) and Lürling (2003). Further, we found that the feeding of *M. micrura* was inhibited more in the presence of toxic *M. aeruginosa* than that in the presence of non-toxic strain. This indicates that the microcystins are the major cause of the feeding inhibition of *M. micrura*.

Fig. 3b shows that the clearance rates of *C. cornuta* significantly decreased with the increasing toxic *M. aeruginosa* in the mixed food (F= 8.827; P=0.031), but not significantly decreased with the increasing non-toxic strain (P>0.05). The clearance rates were significantly higher in the mixed food containing non-toxic *M. aeruginosa* than that in the food containing toxic strain (F= 5.467; P<0.05).

From these results, it seems that microcystins were the cause of the feeding inhibition and no defense to other compounds occurred. However, Rohrlack et al. (2001) found that the microcystin-lacking cells of *M. aeruginosa* PCC7806 mutant were ingested at the same low rate as the microcystin-producing cell of the PCC7806 by the daphnids. The different results may due to the different experiment animals or some other mechanisms.

In conclusion, toxic *M. aeruginosa* exerted strong negative effects on the survival, population increase and feeding of the two cladocerans. *M. micrura* was more sensitive to starvation and *M. aeruginosa* than *C. cornuta*. *S. obliquus* can weaken the toxicity of *M. aeruginosa* to the cladocerans and can be effectively utilized as quality food. Microcystins are the major cause of the feeding inhibition of *M. micrura* and *C. cornuta*.

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