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Effect of Combinations of the Toxic Cyanobacterium *Microcystis aeruginosa* PCC7820 and the Green Alga *Scenedesmus* on the Experimental Population of *Daphnia pulex*

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In China, there are hundreds of shallow lakes, which serve great for fisheries and sightseeing. However, on account of the increasing human activities for decades, eutrophication has widely occurred. Blooms of cyanobacteria (blue - green algae) frequently occur in many eutrophic lakes (Xie and Liu 2001; Chen and Xie 2003), which have been a great nuisance to the whole aquatic ecosystem (Christoffersen 1996; Falconer 1996; Lürling 2003). *Microcystis aeruginosa* is frequently the dominant species (Lürling 2003). Such a kind of algae is inadequate in nutrition (Fulton and Paerl 1987; Smith and Gilbert 1995; Ferräo-filho et al. 2000; Nandini and Rao 1998) and can produce a kind of toxins named microcystins (MC), which are microbial nonribosomally processed cyclic heptapepetides (Doekel and Marahiel 2001). They, especially the variant MC-LR, are believed to exert strong toxic effects on herbivorous zooplanktons (DeMott et al. 1991; Reinikainen et al. 1994, 1995; Laurén-Määttä et al. 1997; Rohrlack et al. 1999). *Daphnia* are the intensively researched group, and feeding and growth inhibition by toxic cyanobacteria has been confirmed in laboratory studies (DeMott 1999; Nizan et al. 1986; Lampert 1987; Reinikainen et al. 1994; Hietala et al. 1995). The case in Chinese shallow lakes is rarely studied.

The purpose of this study is threefold. First, to find out the effects of a toxic cyanobacterium, *M. aeruginosa* strain PCC7820 at different concentration, on experimental population of the *D. pulex* from a Chinese fish pond; secondly, to test the inhibiting effect of *Scenedesmus obliquus* (green algae) on *M. aeruginosa* toxicity; thirdly, to compare the toxic effect of *M. aeruginosa* on *D. pulex* population at different temperature. The results are expected to help to understand the affecting mechanism of cyanobacteria toxicity on zooplankton.

MATERIALS AND METHODS

Pure *Microcystis aeruginosa* strain PCC7820 and the green algae *Scenedesmus obliquus* from Institute of Hydrobiology, CAS were fed during experiments. Prior to the experiment, *M. aeruginosa* strain PCC7820 was cultured with BG11 medium at 25±0.5℃ and under 12L: 12D photoperiod. Microcystin-LR content of *M. aeruginosa* strain PCC7820 was measured according to the method described by Fastner et al. (1998) and Yokoyama and Park (2002). *S. obliquus* was cultured

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with Shuisheng VI medium (Li et al. 1959) at 25±0.5℃ and with a 12L: 12D photoperiod.

D. pulex was collected from the Guanqiao pond with plankton net in April 2003. The Guanqiao pond (38°19'13"N, 117°52'50"E), near to Lake Donghu in Wuhan, China, is a small eutrophic crucian carp pond with dense *M. aeruginosa* blooms. In laboratory, *D. pulex* individuals were cultured by feeding *S. obliquus* under 20℃ and 12L: 12D photoperiod prior to experiments. Juveniles (<12h old) of *D. pulex* from the same adult female were used for experiments. The experiments were all conducted in 250ml beakers filled with 200ml food solution in incubators with a 12L: 12D photoperiod at 20℃ and 30℃. Each beaker contains 10 juveniles of *D. pulex*. Each treatment had three replicates. The animals were transferred daily to another beaker with fresh food solution, and their body length, individual numbers and the numbers of newborns were recorded. The experiment of each treatment lasted 10 days.

There were six different food treatments: Treatment A- *S. obliquus* with a concentration of 10^5cells/ml; Treatment B- *S. obliquus* with a concentration of 10^4cells/ml; Treatment C- 90% *S. obliquus* and 10% *M. aeruginosa* PCC7820 (9×10^4 *S. obliquus*+1×10^4 cells/ml *M. aeruginosa* PCC7820); Treatment D- 50% *S. obliquus* and 50% *M. aeruginosa* PCC7820 (5×10^4 *S. obliquus*+5×10^4 cells/ml *M. aeruginosa* PCC7820); Treatment E- *M. aeruginosa* PCC7820 with a concentration of 10^4cells/ml; Treatment F- *M. aeruginosa* PCC7820 with a concentration of 10^5cells/ml.

Population increase rate (r) was calculated as follows: $r = (\ln N_t - \ln N_0)/t$, where t is the experimental period, N_t the number of animals in the beaker after t days and N_o the number of animals on the first day. The population increase rate and the mortality of *D. pulex* were statistically compared using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Microcystin-LR concentration of *M. aeruginosa* strain PCC7820 was 0.306 μg/mg (dried weight). When fed with only *M. aeruginosa* PCC7820 (10^4 and 10^5cells/ml) at 20℃ and 30℃, *D. pulex* did not increase in body length (Fig. 1 E, F) and all the individuals died out within 4 days (Fig. 2 shows the population increase rate). In contrast, three *D. pulex* individuals remained alive after 10 days both at 20℃ and 30℃ when fed nothing. Such a result confirmed the toxic effect of *M. aeruginosa* PCC7820 on *D. pulex*, but no significant response of *D. pulex* populations to the toxin level of *M. aeruginosa* PCC7820 was observed. Whereas, *S. obliquus* was considered to be edible to *D. pulex*: Fig. 1A, B shows the body length increases of *D. pulex* and Fig. 2 shows the population increase at the level of 1×10^5 cells/ml (no population increase at the level of 1×10^4 cells/ml).

From a plentiful of literatures, we found *Daphnia* respond to toxic *Microcystis* by increasing mortality, decreasing growth and offspring fecundity, and delayed

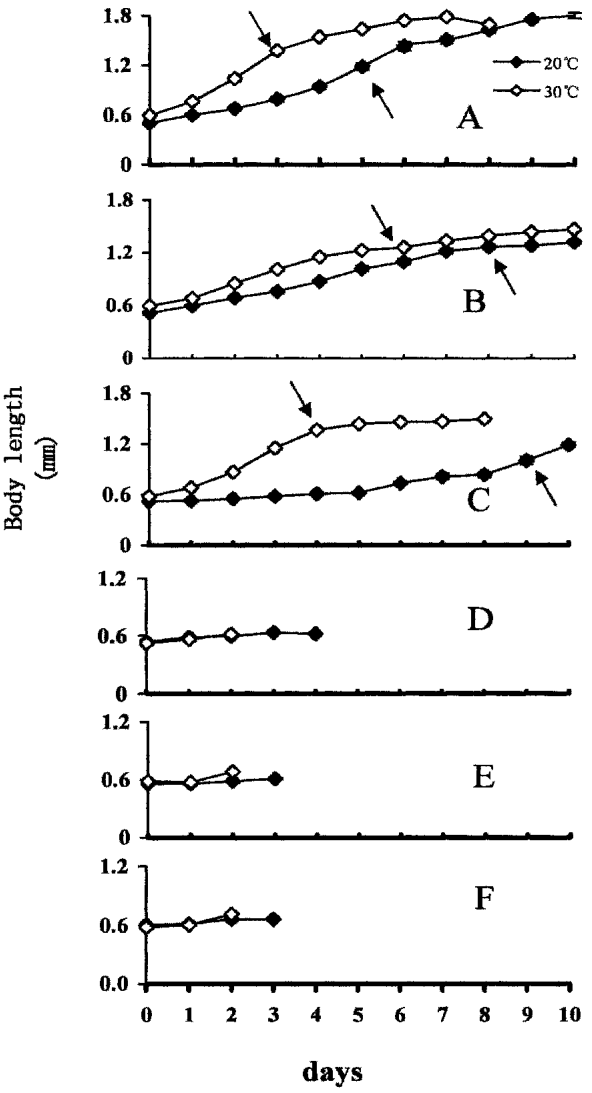

Figure 1.Daily changes in body length of *D. pulex* in different treatments (The arrows show the day for the appearance of first brood) A: 10^5cells/ml *S. obliquus*; B. 10^4cells/ml *S. obliquus*; C: 90% *S. obliquus* and 10% *M. aeruginosa* PCC7820 (9×10^4 cells/ml *S. obliquus*+ 1×10^4 cells/ml *M. aeruginosa* PCC7820); D: 50% *S. obliquus* and 50% *M. aeruginosa* PCC7820 (5×10^4 cells/ml *S. obliquus*+ 5×10^4 cells/ml *M. aeruginosa* PCC7820); E: 10^4cells/ml *M. aeruginosa* PCC7820; F: 10^5cells/ml *M. aeruginosa* PCC7820.

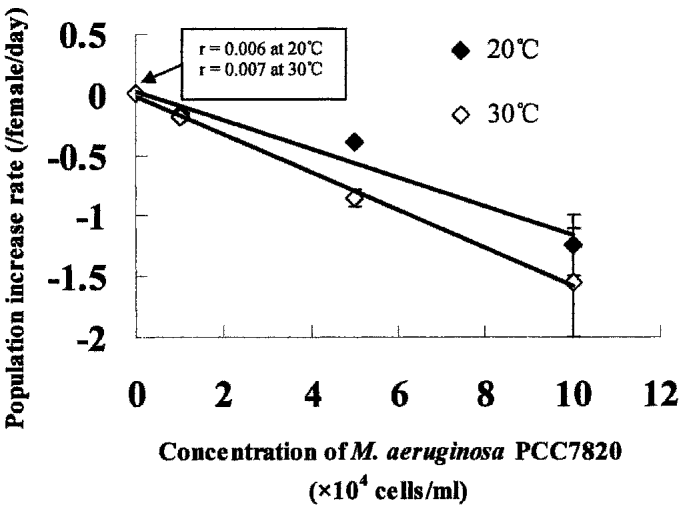

Figure 2. Relationship between concentration of *M. aeruginosa* PCC7820 and population increase rate of *D. pulex*. The sum of *S. obliquus* and *M. aeruginosa* PCC7820 in each treatment was 1×10^5 cells/ml.

maturation (Arnold 1971; Lampert 1987; Nizan et al. 1986; Reinikainen et al. 1994; Hietala et al. 1995). It is generally believed that the negative effects of *Microcystis* on *Daphnia* may be due to: (1) morphological features such as colonial size or filamentous appearance, (2) lack of essential fatty acids, (3) presence of microcystins and (4) feeding inhibition (Reinikainen et al. 1994; DeMott; M. Lürling 2003). In this study, *M. aeruginosa* PCC7820 was unicellular, and the animals fed with *M. aeruginosa* PCC7820 died faster than in starvation condition. Some studies have demonstrated that several *Microcystis* strains affect the feeding rate of daphnids, whatever the offered cyanobacteria are cells or colonies with the suitable size for food intake (Rohrlack et al. 1999).

In the combination treatments, analyses showed significant positive correlations between *S. obliquus* concentration (S% in the food) and population increase rate of *D. pulex* at both temperatures (at 20℃, $r^2 = 0.955$; at 30℃, $r^2 = 0.997$) (Fig.2). When fed with 10% *M. aeruginosa* and 90% *S. obliquus* (Fig. 1 C), *D. pulex* body length increased normally as Treatment B with only *S. obliquus* as food (Fig. 1 B) and the population increase rates were significantly higher than those fed with only *M. aeruginosa* (Fig.2). It indicates that the acute toxicity of *M. aeruginosa* PCC7820 can be reduced significantly by *S. obliquus*, and the more *S. obliquus* were added, the more the toxicity was reduced. Chen and Xie (2003) also got a similar result: *M. micrura* showed increased population rate with increased *S. obliquus* in the food.

The effects of temperature on the response of *D. pulex* population to the toxicity

M. aeruginosa PCC7820 were confirmed to exist. When temperature increased from 20℃ to 30℃, the mature time of *D. pulex* was shortened by at least two days under the same food conditions (see arrows in Fig.1). Fig.2 also shows the lower population increase rate at 30℃. It can be concluded that *D. pulex* were more sensitive to toxic *M. aeruginosa* PCC7820 at higher temperature (30℃), which is in accordance with earlier works by Hietala (1997) and Nandini (2000).

Our results conclusively revealed that the massive presence of the toxic *M. aeruginosa* strain PCC7820 was lethal to *D. pulex*, while the toxicity of the cyanobacterium was reduced significantly by the presence of edible green algae. The present results suggest that zooplankton community in a natural bloom may be affected not only by toxic cyanobacteria (toxin level and abundance of toxic algae) but also by the abundance of edible algae.

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