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Effects of Zooplankton Grazing on the Suppression of Harmful Algal Blooms by the Rotifer *Brachionus calyciflorus* in Freshwater Ecosystems

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To study the influence of the rotifer *Brachionus calyciflorus* on harmful algal bloom suppression, we focused on assessing the rotifer's abilities using several prey species : *Microcystis aeruginosa, Synechocystis* sp., *Chlorella vulgaris* and *Coelastrum* sp. of the warm-weather species and the cold-weather centric diatom *Stephanodiscus hantzchii*. Grazing effects and growth rates of rotifers *B. calyciflorus* were 94.5% and 1.29 d^{-1}, respectively, for *Synechocystis* sp., 87.4% and 0.60 d^{-1}, respectively, for *M. aeruginosa*, 95.2% and 0.65 d^{-1}, respectively, for *C. vulgaris*™, 78.6% and 0.45 d^{-1}, respectively, for *C. vulgaris* UTEX., 86.5% and 0.99 d^{-1}, respectively, for *Coelastrum* sp., and 82.6% and 0.40 d^{-1}, respectively, for *S. hantzchii*. Of these, although the growth of *Synechocystis* and *Coelastrum* was effectively suppressed by rotifer grazing, efficient suppression effects on *Stephanodiscus* blooms were unexpected. The present study revealed that reproduction of *B. calyciflorus* was greatly influenced by its food types in the initial stages and the efficiencies of bio-agents as sole food sources vary depending on the target algae and the agent.

Key words : rotifer *Brachinus calyciflorus*, grazing effect, harmful algae suppression

INTRODUCTION

Eutrophication in freshwater ecosystems is a worldwide problem resulting in extensive blooms of blue-green algae. These blooms have caused problems, such as foul odors, decreased aesthetic value, deterioration of water quality, and ecological damage (Sigee *et al*., 1999). To control nuisance algal blooms, direct applications of chemicals such as cupric-sulfate, smazine, dichromate, ozone and L-lysine have been conducted (Jeffries and Mills, 1990; Takamura *et al*., 2004). However, these applications have also caused damage to aquatic ecosystems by killing off beneficial organisms (Reyssac and Pletikosic, 1990). Thus, pan-ecological and environmental approaches have been sought for lake water conservation (Brabrand *et al*., 1983).

Predator-prey interactions are crucial processes of energy flow through food chains in ecosystems. In particular, grazing pressure by higher trophic level organisms appears to be a major contributor to the loss of phytoplankton blooms. High tro-

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Table1. Experiments information of rotifer *Brachionus calyciflorus* on six prey types as sole food sources.

Prey species	Source	Temp.	Medium	Initial prey conc (cells mL^{-1})
Synechocystis sp. PCC6803	Kyungpook National Univ.	28°C	BG-11	4.4×10^6
Microcystis aeruginosa	Inje Univ.	28°C	BG-11	4.4×10^6
Chlorella vulgaris™	Daesang Co., in Korea	28°C	PP	15×10^6
Chlorella vulgaris UTEX26	UTEX Culture Collection of Algae	28°C	PP	2.8×10^5
Coelastrum sp.	Isolation from Pal'dang Reservoir	28°C	BG-11	8.1×10^6
Stephanodiscus hantzschii	CCAP Culture Collection of Algae	15°C	DM	4.0×10^4

phic level predators such as zooplankton may experience not only food quantity constraints on growth, but also food quality constraints (Lürling, 2006). The grazing and digestion rates of zooplanktons also depend on algal cell and colony shape and size, wall architecture, absence or presence of spines, and bristles (Porter, 1973).

Rotifers are generally considered phagotrophs because of their considerable filtration activity. They are widely used as living food for fish and shellfish larvae culture due to their suitable size and nutritional value (Navarro, 1999; Nandini, 2000). Also, large brachionids rotifers, *Brachionus calyciflorus*, are particularly suitable test organisms for eco-toxicological studies because of their global distribution, rapid reproduction, short generation time, simplicity of culture and availability of resting eggs (Radix *et al*., 2002). More than three generations are produced within 72 h, which constitutes one of the shortest life cycles of any animal (Radix *et al*., 2002). Therefore, the rotifer may be expected to be a contributor to the loss of their algae bloom in freshwater ecosystem because they have considerable grazing impact on the biomass of small size phytoplankton. This study investigated the growth and grazing rates of rotifer *B. calyciflorus* on six different prey types, and discussed the extent of the rotifer's biological control on harmful algae bloom by top-down effects.

MATERIALS AND METHODS

1. Isolation and culture of the rotifer *Brachionus calyciflorus*

A rotifer *B. calyciflorus*, was isolated from the Pal'dang Reservoir (37° 31′620″N, 127° 16′853″E) in July 2002, in Korea. To isolate the healthy rotifer, 10 females were individually kept in a 20 mL test tube for 10 days using a micropipette under an inverted microscope (Stemi SV11, ZEISS, Germany). We established a monoclonal rotifer culture using a repetitive serial isolation process consisting of enrichment, dilution and single cell isolation steps. Rotifers were maintained by condensed *Chlorella*™ (Daesang Co., Korea) as prey. Culture conditions of the rotifer in the laboratory were performed at 28°C in CB media under a light intensity of 35∼40 μmol m^{-2} s^{-1} of a cool white fluorescent lamp with a 12 : 12 h light : dark cycle.

2. Cultures of six prey types

To understand the growth and grazing effects of the rotifer *Brachionus calyciflorus*, we used the following species as prey : two species of cyanophyceae : *Synechocystis* sp. PCC6803 and *Microcystis aeruginosa* MA001; three strains of chlorophyceae : *Chlorella vulgaris*™, *Chlorella vulgaris* UTEX26, and *Coelastrum* sp.; and one species of bacillariophyceae : *Stephanodiscus hantzchii* CCAP1079/4 (Table 1).

Synechocystis sp. and *M. aeruginosa* were obtained from Kyungpook National University and Inje University in Korea. *C. vulgaris*™ was received from Daesang Co., in Korea. *S. hantzchii* was obtained from the Culture Collection of Algae and Protozoa, USA. Also, *Chlorella vulgaris* UTEX was obtained from UTEX Culture Collection of Algae, USA. *Coelastrum* sp. were isolated from the Pal'dang Reservoir.

Of these, *Synechocystis* sp. and *M. aeruginosa* were cultured at 28°C in BG-11media. *Chlorella*™ and *C. vulgaris* UTEX were cultured at 28°C in PP media. *Coelastrum* sp. and *S. hantzchii* were grown at 28°C in BG-11 media and at 15°C in DM media, respectively. Preys were chosen from the exponential growth phase, and were monitored by an inverted microscope (Stemi SV11, ZEISS, Germany). All experiments were performed under a light intensity of 35∼40 μmol m^{-2} s^{-1} using a cool white fluorescent lamp with a 12 : 12 h light :

dark cycle.

3. Feeding experiments

To evaluate the food selectivity and quality of the prey fed to the rotifer *B. calyciflorus*, the rotifer was put into 500 mL conical flasks containing 300 mL of exponential-phase cultures of the following test-cultures of *Synechocystis* sp., *M. aeruginosa*, *C. vulgaris*^{TM}, *C. vulgaris* UTEX, *Coelastrum* sp., and *S. hantzchii* as sole food sources. The initial densities of these prey ranged from 4×10^4 cells mL^{-1} to 1.5×10^7 cells mL^{-1}, which are considered their bloom concentrations in the Pal'dang Reservoir. *B. calyciflorus* had been starved for 2 days to minimize possible residual growth resulting from ingestion of prey during batch culture. No rotifers were added for the control. The initial densities of rotifers were 2 inds. mL^{-1}. Triplicate cultures were incubated for 7 days at 15°C only for *S. hantzchii* and 28°C for the other prey under a light intensity of 35~40 μmol m^{-2} s^{-1} of a cool white fluorescent light with a 12 : 12 h light : dark cycle. To monitor changes in the concentration of prey algae and rotifers, each flask was gently swayed and 5 mL of culture suspension was collected every day. These samples were fixed with a Lugol solution (final concentration 2%). Rotifer monitoring was measured daily by a 1 mL Sedgwick-Rafter chamber under an inverted microscope. Prey organism counting was performed daily by a hemocytometer. Differences between the sole food treatment and control without food were tested using a one-way ANOVA.

The specific growth rates of rotifers were estimated by Eq. 1.

$$\mu=\ln N_t-\ln N_0/t_1-t_0 \qquad \text{(Eq. 1)}$$

where N_t and N_0 were rotifer population densities in ind. mL^{-1} at the initial time (t_0) and at 4 days (t_1). Grazing effects of the rotifers were calculated by Eq. 2.

$$\text{Grazing effects (\%)}=(1-T_t/C_t)\times100 \qquad \text{(Eq. 2)}$$

where T (treatment) and C (control) are the *B. calyciflorus* population densities with- and without and t is the inoculation time (4 days).

RESULTS

Rotifer *B. calyciflorus* showed active growth when feeding on cyanophyceae *Synechocystis* sp. (Fig. 1A). After being inoculated for one day, the prey population sharply decreased in the treatment and had a continuously low population density, implying that the rotifer had fed on most of the prey. Rotifer abundances gradually increased during the first 3 days, then they significantly increased between the 3rd and 5th day. In contrast, *Synechocystis* sp. density in the control was clearly higher than that in treatments (ANOVA, $F_{control/treatment}=9.235$, $P<0.001$). For the cyanophyceae, *M. aeruginosa*, the rotifer gradually increased on the final day, whereas *M. aeruginos* gradually decreased in the treatment and increased in the control within pass of culture times (Fig. 1B; ANOVA, $F_{control/treatment}=288.36$, $P<0.001$). The functional response of rotifer *B. calyciflorus* on *C. vulgaris*^{TM} (ANOVA, $F_{control/treatment}=1312.5$, $P<0.001$) and *C. vulgaris* UTEX (ANOVA, $F_{control/treatment}=78.41$, $P<0.001$) significantly differed even though they are from the same species (Figs. 2A, B; $P<0.001$). *C. vulgaris*^{TM} sharply decreased after the first day, and then remained at low densities. On the other hand, *C. vulgaris* UTEX gradually decreased in the final experiments. Chlorophyceae *Coelastrum* sp. also sharply decreased in the treatments, and then continuously remained at low densities (ANOVA, $F_{control/treatment}=1610.5$, $P<0.001$). Rotifer abundances increased after 3 days (Fig. 3A). These trends were similar to that of rotifer *B. calyciflorus* on cyanophyceae, *Synechocystis* sp. mentioned above. Finally, we did not observe significant rotifer proliferation when they were allowed to feed on diatom *S. hantzschii*. Reducing trends of *S. hantzschii* in the control and in the treatment were similar on the final day, although there was a significant difference between the control and the treatment (Fig. 3B; $F_{control/treatment}=463.7$, $P<0.001$).

Specific growth rates and grazing effects of rotifer *B. calyciflorus* feeding on several prey measured during the 4-day culture are shown in Fig. 4. Grazing effects and growth rates of rotifers were 94.5% and 1.29 d^{-1}, respectively, for *Synechocystis* sp., 87.4% and 0.60 d^{-1}, respectively, for *M. aeruginosa*, 95.2% and 0.65 d^{-1}, respectively, for *C. vulgaris*^{TM}, 78.6% and 0.45 d^{-1}, respectively, for *C. vulgaris* UTEX., 86.5% and 0.99 d^{-1}, respectively, for *Coelastrum* sp., and 82.6% and 0.40 d^{-1}, respectively, for *S. hantzchii*. Therefore, three strains, *Synechocystis* sp., *Coelastrum* sp., and *C. vulgaris*^{TM} triggered high growth of *B. calyciflorus*, while *M. aeruginosa*, *S. hantzschii* and *C.*

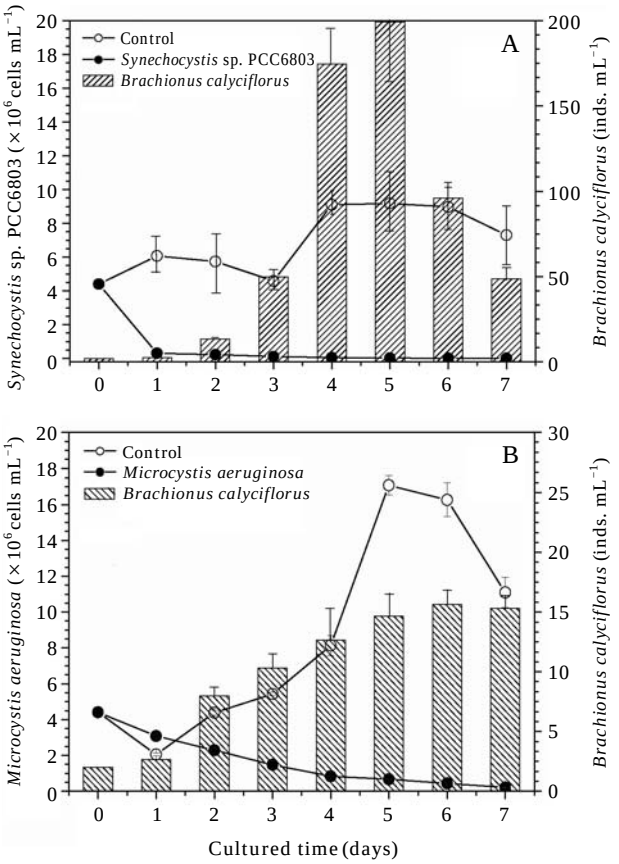

Fig. 1. Changes in *B. calyciflorus* while feeding on *Synechocystis* sp. PCC6803 (A) and *Microcystis aeruginosa* MA001(B) as sole food sources. Controls refer to treatments without food. Histograms with oblique lines are the biomass of *B. calyciflorus*. Data represent mean abundances of *B. calyciflorus* and prey species±standard error.

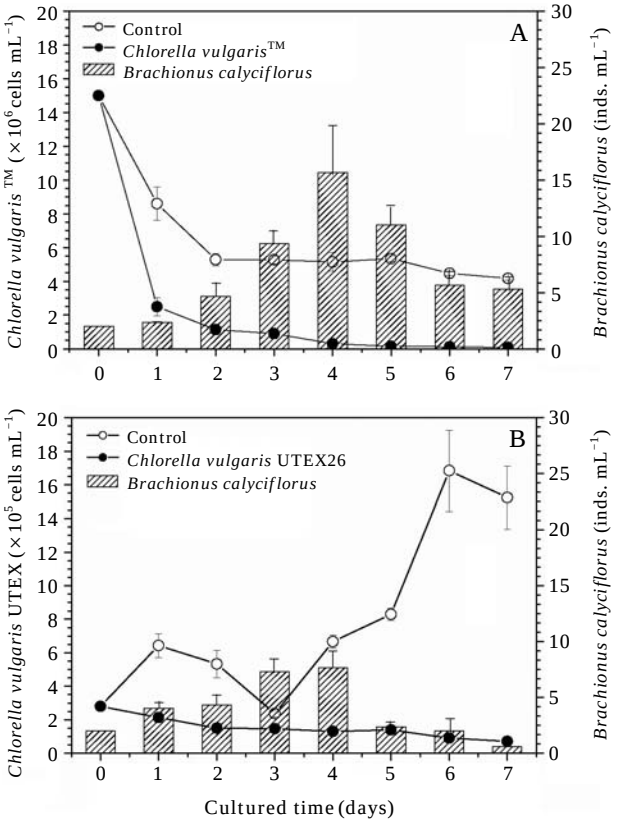

Fig. 2. Changes in *B. calyciflorus* while feeding on *Chlorella vulgaris*™ (A) and *Chlorella vulgaris* UTEX 26 (B) as sole food sources. Controls refer to treatments without food. Histograms with oblique lines are the biomass of *B. calyciflorus*. Data represent mean abundances of *B. calyciflorus* and prey species±standard error.

vulgaris UTEX induced relatively low *B. calyciflorus* growth effects.

DISCUSSION

The prey species of *Microcystis*, *Synechocystis*, *Chlorella* and *Coelastrum* are often found during warm seasons above 20°C, while *Stephanodiscus* is more common during cold seasons below 20°C in the Pal'dang Reservoir (Han *et al*., 2002; Hong *et al*., 2002). The reservoir provides drinking water and industrial water for a population of more than 20 million. These organisms are representative causative species of harmful algal blooms in the reservoir, and have caused serious problems such as the deterioration of water quality. To control the harmful algal blooms, previous studies have confirmed the abilities of some allelochemicals (Park *et al*., 2006), aquatic bacteria (Kang *et al*., 2005; Kim *et al*., 2007), grazing organisms such as heterotrophic nanoflagellates (Kim *et al*., 2006) and ciliates (Kim *et al*., 2007). However, algal bloom suppression by the grazing pressure of rotifers is not fully understood. This study investigated the functional response of rotifer *B. calyciflorus* on several prey to provide a better understanding of the biological control effects.

Genus *Brachionus* produced a significantly smaller number of eggs on nutrient limited food than on non-limited food, which has also been observed in *Daphnia* (Urabe and Sterner, 2001; Lürling, 2006). Reproduction of *B. calyciflorus* as

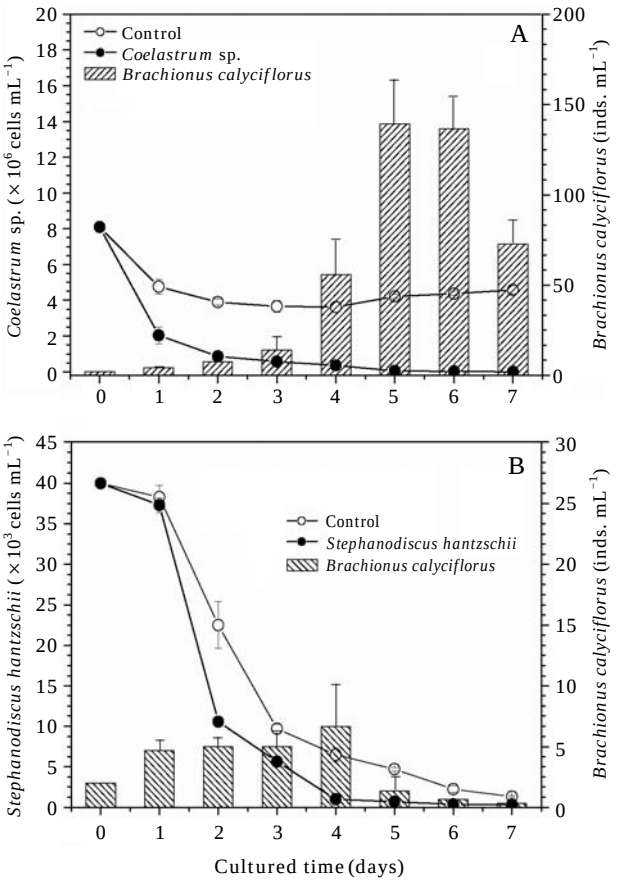

Fig. 3. Changes in *B. calyciflorus* while feeding on *Chlorella vulgaris*™ (A) and *Stephanodiscus hantzschii* CCAP 1079/4 (B) as sole food sources. Controls refer to treatments without food. Histograms with oblique lines are the biomass of *B. calyciflorus*. Data represent mean abundances of *B. calyciflorus* and prey species ± standard error.

suspension-feeders relies on algal prey (Mohr and Adrian, 2002). They are also able to regulate their food uptake by modification of their feeding behaviors with respect to prey density and type of prey (Navarro, 1999; Mohr and Adrian, 2002). According to Lürling (2006), egg production of the rotifer *B. calyciflorus* is influenced by the nutritional quality and quantity of food. In the present study, *B. calyciflorus* abundances gradually decreased after being inoculated for 3 ~ 4 days when fed on *C. vulgaris*™, *C. vulgaris* UTEX and *S. hantzschii* compared to other prey types such as *Synechocystis* sp., and *Coelastrum* sp.. Indeed, when feeding on *Synechocystis* sp. and *Coelastrum* sp., the rotifer induced the longest lasting egg holdings and the lowest mortality from the 5th to the 7th day. In the cases of *S. hantzschii* and *C. vulgaris* UTEX, the rotifer produced egg holdings for only a short period at the beginning of the culture, implying that the abundances of adults could not remain at high levels due to the loss of egg holding and production in the initial stages. Schröder (2005) also suggested that the population dynamics of the rotifer were likely affected by the initiation of sexual reproduction and the diapausing of resting eggs. Our findings revealed that the egg production of *B. calyciflorus* was greatly influenced by its food types in the initial stages, as mentioned by Mohr and Adrian (2002). Moreover, zooplankton should be able to gain at least some important nutrients by feeding on protozoans to maintain their metabolic demands (Porter *et al*., 1985; Sanders and Wickham, 1993).

Recently, zooplankton grazing has received attention as a tool for water quality management in relation to biomanipulation (Reynolds, 1994; Kobayashi *et al*., 1996). In lakes and reservoirs, *Brachionus* and *Daphnia* individuals are regarded as key zooplankton grazers in reducing the biomass of phytoplankton because they have a broader food-particle size range (Burns, 1968; Kobayashi *et al*., 1996) and show little selectivity in their type of food items (Kerfoot and Kirk, 1991; Mohr and Adrian, 2002). The fact that *B. calyciflorus* gained at least some nutritional benefit from feeding on *Synechocystis*, *Microcystis*, *C. vulgaris*™ and *Coelastrum* implies that these prey organisms have a supplemental effect to algal prey. However, feeding by these rotifer in the presence of algal prey *C. vulgaris* UTEX and *Stephanodiscus* seems to be inefficient for *B. calyciflorus*. Generally, zooplankton grazing depends on algal cell size and colony shape, and grazing rates increases exponentially with increasing body sizes of zooplankton. Rothhaupt (1990) reported that *B. calyciflorus* feed most efficiently on particles with an equivalent spherical diameter (ESD) around 10 μm. Based on the ESD of tested organisms, they was arranged the following; *Synechocystis* (2.35 ± 0.46 μm) < *Microcystis* (3.0 ~ 4.5 μm) < *Chlorella* (3.7 ± 0.52 μm) < *Coelastrum* (6 ~ 10 μm) < *Stephanodiscus* (12 μm) (Rothhaupt, 1990; Pagano, 2008). Indeed, the grazing effects and growth rates of rotifer *B. calyciflorus* were depended on the ESD of prey, except when feeding *Coelastrum* (Fig. 4). Therefore, prey selection of *B. calyciflorus* might be expected to reflect their food size and qualities, suggesting that the

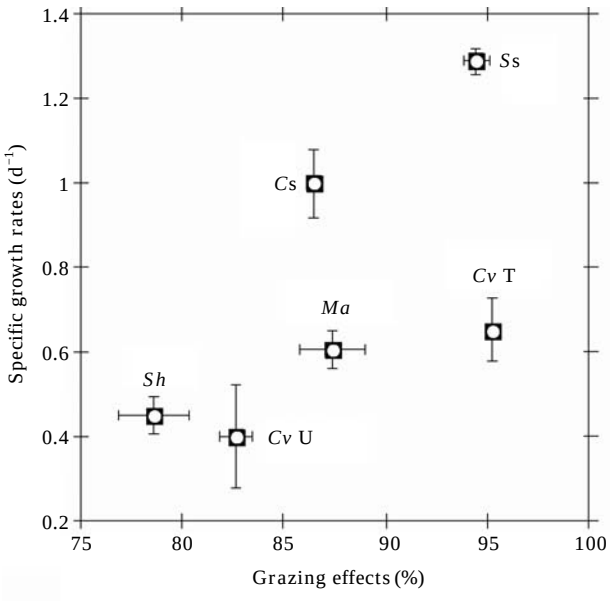

Fig. 4. Grazing effects and specific growth rates of *B. calyciflorus* while feeding on several algal prey. *Ss* : *Synechocystis* sp. PCC6803, *Ma* : *Microcystis aeruginosa*, *Cv* T : *C. vulgaris*^{TM}, *Cv* U : *C. vulgaris* UTEX26, *Cs* : *Coelastrum* sp., *Sh* : *Stephanodiscus hantzschii* CCAP 1079/4.

rotifer could be contributed to the control of population of small sizes algae in freshwater ecosystem.

In general, egg production and growth rate for most zooplankton species is influenced by the relationship between food and temperature (Ricci, 2001; Mohr and Adrian, 2002). *Brachionus* is in the pantropical genus with a capacity to successfully reproduce at temperatures greater than 25 ˚C (Ricci, 2001). Park *et al*. (2001) also reported dramatic growth rates of rotifer *B. calyciflorus* at high temperatures ranging from 28 to 32˚C. In fact *B. patulus* grows well even at 35˚C (Sarma and Rao, 1990). In contrast, the abundance of *B. calyciflorus* was maintained relatively high around 15˚C in Lake Müggelsee, Germany (Mohr and Adrian, 2002). The *S. hantzschii* bloom occurs annually in the Pal'tang Reservoir, mainly during the cold season (from late autumn to the following spring) when temperatures are below 20˚C (Han *et al*., 2002; Hong *et al*., 2002), while rotifer *B. calyciflorus* has a capacity to successfully reproduce at temperatures higher than 20˚C (Park *et al*., 2001). In the present study, the rotifer in treatment remained at a lower level of abundance, and grazing effects and growth rates of the rotifer also had low values compared to other prey experiments. These rotifer growths may be related to the temperature conditions, suggesting that *B. calyciflorus* can negatively affect the diatom *S. hantzschii*, which dominates in cold waters. According to Kang *et al*. (2005), *S. hantzschii* blooms were strongly suppressed by algicidal bacteria in the laboratory and in a mesocosm field study. Therefore, *S. hantzschii* bloom control maybe not be feasible via grazing pressure because *B. calyciflorus* does not have a strong capacity to successfully reproduce under low temperatures.

Genus *Microcystis* is a common harmful cyanobacteria which usually blooms in tropical freshwater ponds and lakes (Pearl, 1988). Their colonial structure and production of microtoxins are defensive functions against the grazing pressure of predators, such as zooplankton (De Bernardi and Giussani, 1990). Also, the growth status of zooplankton grazing on phytoplankton depends on different prey (Nandini, 2000). Our data show that the grazing effects and growth rates of rotifers *B. calyciflorus* were 87.4% and 0.60 d^{-1}, respectively, for *M. aeruginosa*. These were relatively lower levels than those of some prey. Ecologically, a bloom event of a phytoplankton species is a function not only of relative specific growth rates, but also of the mortality rate by grazing. *M. aeruginosa* population dynamics would also be affected by mesozooplankton grazing, and hence lower grazing pressures on a given organism may create an advantage over competitors (Fulton and Paerl, 1987; Nandini, 2000). In particular, the *M. aeruginosa* is believed to constitute a poor food source for zooplankton due to their colonial structure and production of microtoxins (De Bernardi and Giussani, 1990; Lürling, 2003). Therefore, although *M. aeruginosa* bloom suppression by the grazing pressure of a rotifer was partly accepted, more attention should be paid to the biological control effects because *M. aeruginosa* have a better advantage with regards to defensive functions against the grazing pressure of predators.

In conclusion, the rotifer *B. calyciflorus* had a good effect on controlling *Synechocystis* and *Coelastrum*, but was inefficient for the suppression of *Stephanodiscus*. The efficiencies of bio-agents as sole food sources in this study depended on the target algae. Further studies focusing on the relationship between co-applied bio-agents and changes of nutrients balance in aquatic ecosystems is

needed for controlling harmful algal blooms.

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