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Growth and grazing rates of rotifer, *(Brachinous calyciflorus)* on several algae

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ABSTRACT: To understand the dietary contribution of phytoplankton on the growth of the rotifer *Brachionus calyciflorus* feeding on four algal preys; *Microcystis aeruginosa*, two strains of *Chlorella vulgaris* and *Stephanodsicus hantzschii*, the growth rates, egg holdings and mortalities were measured. Two strains, *M. aeruginosa* and *C. vulgaris*™ induced a high growth of *B. calyciflorus*, while *S. hantzschii* and *C. vulgaris* UTEX26 induced a low growth. Rotifers strongly decreased the densities of *C. vulgaris* UTEX26 and *S. hantzschii* within 2-3 days, compared to those of *M. aeruginosa* and *C. vulgaris*™ in the same time period. Cyanobacterium *M. aeruginosa* induced the longest lasting egg holdings and the lowest mortality of *B. calyciflorus* within 7 days, while *S. hantzschii* produced the egg holdings for only a short period in the beginning of the culture with a high mortality. This study shows that a toxic cyanobacterium, *M. aeruginosa*, can be applied as a fruitful diet for the development of zooplankton population, like rotifer.

Key Words: rotifer, *Brachionus calyciflorus*, growth rates, algal preys, *Microcystis aeruginosa*

Introduction

Rotifers are considered to be phagotrophs, because of their considerable filtration activity, and are widely used as live food for larval fish and crustacean culture due to their size, nutritional value and behavior (Hoff and Snell, 1989). They are small animals, ranging from 100 to 2500 μm and are free-living herbivores and/or bacteriovores. Genus *Microcystis* is one of the most common cyanobacteria, which occur in tropical freshwater ponds and lakes (Pearl, 1988). It is generally known that their colonial structure and microtoxin productions are a defense function to the grazing pressure of predators, such as zooplankton (Lampert, 1981; Fulton and Paerl, 1987; De Bernardi and Giussani, 1990). Meanwhile, there were opposite opinions that the growth of the zooplankton population on cyanobacteria depends on the kinds of zooplankton and phytoplankton (Nandini, 2000). Also, large brachionids rotifer, *Brachinous calyciflorus* and *Euchlanis dilatata lucksiana* fed (Gulati *et al.*, 1993) and reproduced (Rothhaupt, 1991) on cyanobacterial diets, even on daphnia-toxic strains (Starkweather and Kellar, 1983).

In this study, we describe laboratory experiments with *Brachinous calyciflorus* and the cyanobacterium *Microcystis aeruginosa.* The results are compared with experiments in which other three algal preys were used. The aim was to investigate the reliable density and dietary contribution of four algal preys to the growth of the rotifer *B. calyciflorus* such as specific growth rate, egg holdings and daily mortality.

Materials and Methods

Preparation of algal preys

As prey, cyanobacterium *Microcystis aeruginosa* MA001 was cultured and maintained in BG-11 media at 28℃ , *Chlorella vulgaris* UTEX26 in P.P. media at 28℃ and *Stephanodiscus hantzchii* CCAP1079/4 in D.M. media at 15℃ , respectively. Light condition was 12:12 h light:dark cycle and 35 ~ 40 μE m^{-2} s^{-1}. Condensed freshwater *Chlorella*™ (*Chlorella* Ind. Co. Ltd., Japan) was also used.

Predator culture

Rotifer *B. calyciflorus* was obtained from the Faculty of Bioscience & Technology, Kangnung University, Korea. To isolate the healthiest rotifer colony, 30 females were individually cultured in a 20-ml test tube for 10 days with condensed *Chlorella*™ in the tap water.

Feeding experiments

The prey requirements of the rotifers were determined by using a different density of the four prey; *M. aeruginosa, C. vulgaris, S. hantzchii* and condensed *Chlorella*™ (Table 1). Each experiment was run in sterilized 10ml six-well plates. The initial density

of rotifers was 2 ind.ml^{-1} in each well (8 ml) after being starved at least 48h prior to the beginning of the experiments. The experiments were performed in triplicate under smooth shaking (40 rpm) in 12;12 h light:dark cycle at 35 ~ 40 μE m^{-2} s^{-1}. The feeding experiments were carried out with prey of algal exponential phases. To understand the growth of the rotifers after prey introduction to the well, 1ml subsamples were enumerated at 24-h intervals for 7days. Simultaneously, the density of the prey was

enumerated using a hemacytometer and a Sedgwick-Rafter chamber. The specific growth rate of the rotifers was calculated by the following equation.

$r = (\ln N_t - \ln N_0)/T$ .....(Eq. 1)

Where, T is culture days of the maximum number of rotifers and N_0 and N_t are the initial and maximum number of rotifers after t days, respectively (Rico-Martinez and Dodson, 1992).

The maximum growth rate of rotifers with the density of prey were analyzed by relating the resulting growth rates (μ) to the corresponding initial algal preys cell numbers N_0. These data were fit to a modified Michaelis -Menten (M-M) model,

Table 1. Initial concentration (cells ml^{-1}) for four species of algal preys and growth data for rotifer *Brachionus calyciflorus* fed three species of algal preys. Parameters are for numerical response from Eqs. (1) and (2) as presented in Fig. 3. μ_{max} (maximum growth rate day^{-1}), K_0 (the food concentration sustaining 0.5 μ_{max}), x` (threshold prey concentration).

Species	Density (x 10^4cells ml^{-1})	μ_{max}	Kt	x′
Microcystis aeruginosa	20, 60, 80, 100, 400, 500	0.54	0.27	5.15
Chlorella vulgaris	80, 200, 400, 600, 800, 1000	0.62	0.31	3.49
Stephanodiscus hantzschii	2, 4, 6, 8, 10, 50	0.52	0.26	2.12

which has been frequently used to describe numerical responses of protozoa (Montagnes 1996).

$\mu = [\mu_{max} \times (N_o - K_o)] \times [K_t + (N_o - K_o)]^{-1}$ .....(Eq. 2)

Where μ_{max} is the maximum growth rate, K_o is the x intercept or threshold concentration' (the food concentration where $\mu = 0$) and K_t is the 'half saturating concentration' (the food concentration at which $\mu = \mu_{max} / 2$). Curves were fit to the data using SigmaPlot 5.0 software (SPSS Inc., Chicago, IL).

Results and Discussion

The highest growth of rotifer *B. calyciflorus* was induced by two algal diets; *M. aeruginosa* and condensed *Chlorella*™ (Fig. 1). In particular, *M. aeruginosa* continuously induced growth for 7 days, while other three preys strongly inhibited growth after the 4th day. Our study indicates that reproduction of the rotifer population is divided into 4 groups; non-reproductive, laying 1 egg, laying 2 more eggs and death, respectively (Fig. 2). Animals fed *M. aeruginosa* showed the most varied stages and the lowest mortality in the study, while 3 other preys induced low growth and high mortality. Compared to the previous studies (Smith and Gilbert, 1995; Nandini, 2000), this positive contribution of *M. aeruginosa* to the growth of rotifers is beyond expectation. Although it is not fully understood, we surely suggest that cyanobacterium *M. aeruginosa* can be a favorable and effective food in

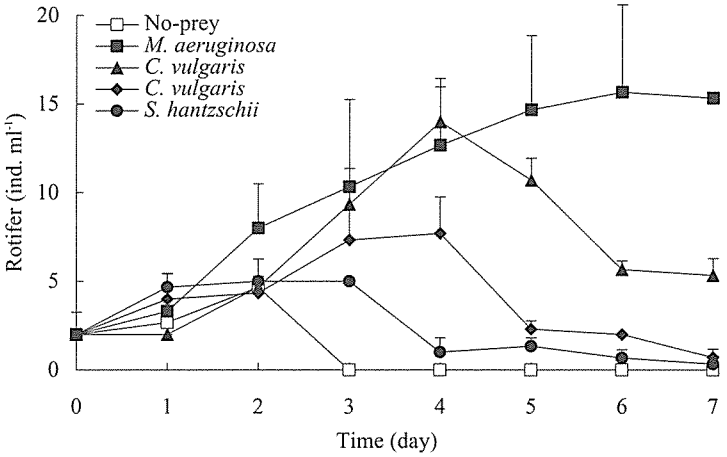

Fig. 1. Growth pattern of rotifer *Brachionus calyciflorus* fed several algal prey.

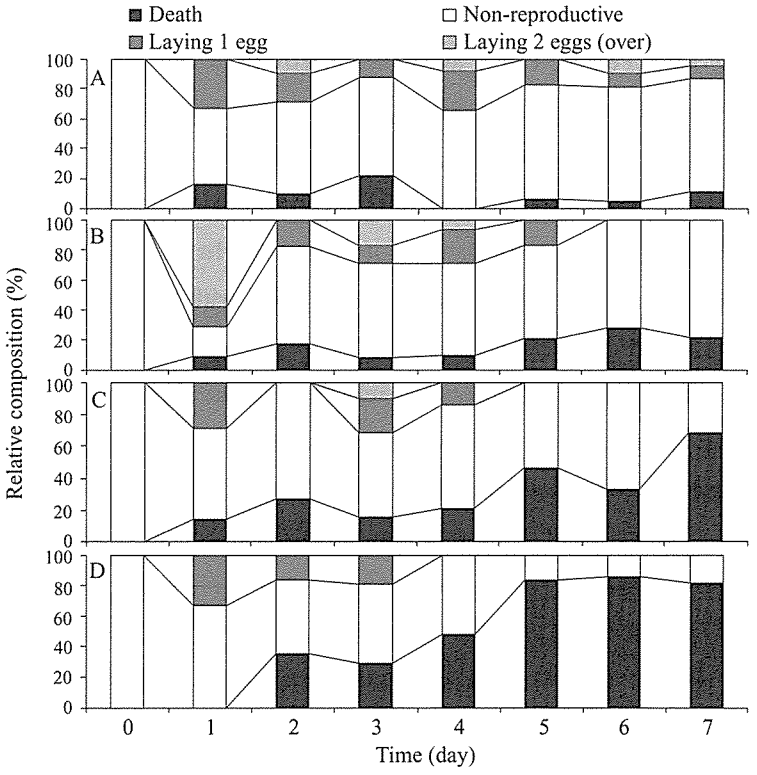

Fig. 2. Relative composition (%) of rotifer *Brachionus calyciflorus* at various reproductive stages; (A) *Microcystis aeruginosa*, (B) *Chlorella vulgaris*™, (C) *Chlorella vulgaris* and (D) *Stephanodsicus hantzschii.*

the development of rotifer populations. However, further study is needed for more generalizations on the feeding habitats and food preferences of rotifer *B. calyciflorus* in aquatic ecosystems.

Growth rates of animals according to density of each preys with *M. aeruginosa, C. vulgaris* and *S. hantzschii* showed $-0.38 \sim 0.63$ day^{-1}, $0.07 \sim 0.97$ day^{-1} and $-0.22 \sim 0.55$ day^{-1}, respectively (Table 1 and Fig. 3). Figure 3 shows that the M-M models of animals plotted using a growth rate (μ) of rotifer calculated in each density of preys. Of three algal preys, *C. vulgaris* induced the highest maximum growth rate (μ_{max}) of rotifers, as 0.6 day^{-1}, while *M. aeruginosa* and *S. hantzschii* are comparatively low, and similar each other, as 0.52 day^{-1}. The μ_{max} of rotifer, *B. calyciflorus* during the study is similar to the previous studies with $0.5 \sim 1.02$ day^{-1} (Erman, 1962; Halbach, 1972; Richard, 1985). These results showed that the μ_{max} of *B. calyciflorus* with *C. vulgaris* is less than that of *Cryptomonas* sp. (Richard, 1985), because of the nutritional value, such as polyunsaturated fatty acid (Petkov, 1993), and food selectivity of zooplankton (Nandini, 2000). Richard (1985) reported that the threshold (x′) food level, the $\mu_{max}/2$ food level, and the value of μ_{max} provide a simple theoretical framework for interpreting species distributions in nature. In this study, threshold (x′) for rotifer *B. calyciflorus* with *M. aeruginosa* is the highest value, 5.15 day^{-1}, while those with *C. vulgaris* and *S. hantzschii* relatively low, as 3.49 day^{-1} and 2.12 day^{-1}, respectively (Table 2). This result suggests that cyanobacterium *M. aeruginosa* often occurs in a nutrient-rich water environment, inducing a high threshold and high μ_{max}, the opposite of, *C. vulgaris* and *S. hantzschii* which are abundant in low nutrients and can lead to a low threshold and maximum growth rate of zooplankton (Richard, 1985)

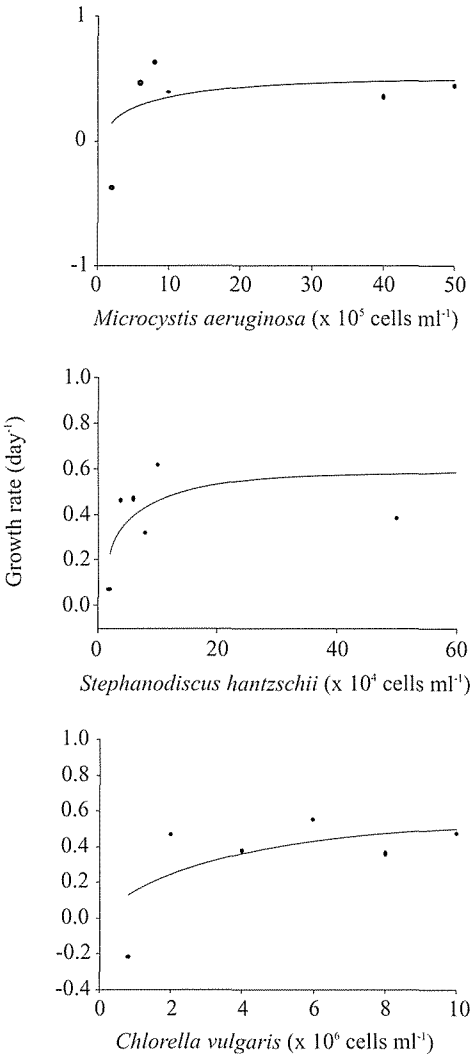

Fig. 3. Specific growth rate of rotifer *Brachionus calyciflorus* as a function of mean prey concentration when feed on (A) *Microcystis aeruginosa*, (B) *Chlorella vulgaris*™ and (C) *Stephanodsicus hantzschii*

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