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The effect of caloric restriction on the population density of freshwater rotifers (*Brachionus calyciflorus*)

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Abstract: The effects of caloric restriction (CR) on the population density of freshwater rotifers (*Brachionus calyciflorus*) were investigated in the present study. During experiments with different CRs, groups of rotifers were fed with *Chlorella* sp. at the same concentrations of 1.5×10^6 cells/mL at 12, 24, 36, 48, 60, and 72 h intervals for 20 days. Significant changes were observed both in the population density and the peak days in groups subjected to different CRs ($P < 0.01$). The highest peak in population density was obtained on the fifth day for rotifers fed in 12 h intervals (269 ± 40.12 individuals/mL, mean ± SD). The rate of population increase (r) varied from 0.608 ± 0.073 to 0.288 ± 0.053, depending on the feeding intervals. The results show that CR affected the population density of *B. calyciflorus*.

Key words: Rotifera, *Brachionus calyciflorus*, caloric restriction, population density

Kalorik sınırlamanın tatlı su rotiferi (*Brachionus calyciflorus*)'da populasyon yoğunluğu üzerine etkisi

Özet: Bu çalışmada, kalorik sınırlamanın (KS) tatlı su rotiferi (*Brachionus calyciflorus*)'nde populasyon yoğunluğu üzerine etkisi araştırılmıştır. KS deneyleri 12, 24, 36, 48, 60, ve 72 saat aralıkla 1 ml ($1,5 \times 10^6$ hücre/mL) *Chlorella* sp. ile beslenen rotifer gruplarından oluşturulmuştur. Uygulanan KS şartlarında gruplar arasında hem populasyon yoğunluğunda hem de pik günlerinde önemli değişikler gözlenmiştir ($P < 0,01$). En yüksek populasyon yoğunluğu 12 saat aralıkla beslenen grupta (269 ± 40,12 birey/mL) 5. günde elde edilmiştir. Gruplara ait populasyon artış oranları ise yemleme aralığına bağlı olarak 0,608 ± 0,073 ile 0,288 ± 0,053 arasında değişmiştir. Araştırma sonuçları KS'nin tatlı su rotiferi *B. calyciflorus* populasyon yoğunluğunu etkilediğini göstermektedir.

Anahtar sözcükler: Rotifera, *Brachionus calyciflorus*, kalorik sınırlama, populasyon yoğunluğu

Introduction

Rotifers are the most important live food organisms used for the rearing of fish larvae due to their size, nutritional value, and behavior (Lubzens et al., 1989, 2001; Arimoro, 2006). The freshwater rotifer *Brachionus calyciflorus* is a suitable organism and adequate food source for ornamental freshwater fish larvae such as dwarf gourami (*Colisa lalia*) and brown discus (Lima et al., 2003; Sales and Janssens, 2003). Shiri et al. (2003) reported that *B. calyciflorus* could

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be used efficiently as a starter food for burbot, *Lota lota*. However, little is known about the culture, nutrition, and population dynamics of freshwater rotifer, *B. calyciflorus* (Rajendiran and Subramanian, 2007). This information is important for inland freshwater aquaculturists (Arimoro and Ofojekwu, 2004). Although a number of techniques have been developed for the mass production of rotifers (Dhert et al., 2001; Hagiwara et al., 2001), problems concerning unexpected stagnant growth and the sudden collapse of the rotifer mass culture have not yet been solved (Yoshinaga et al., 2001). On the other hand, various studies have shown that one considerable factor influencing the rotifer culture is food supply and concentration (Edmondson, 1960; Lucía-Pavón et al., 2001; Sarma et al., 2001; Sarma and Nandini, 2001; Yoshinaga et al., 2001; Lucía-Pavón-Meza et al., 2005).

Caloric restriction (CR) as a form of food availability has been studied extensively by researchers in the field of aging (Verdone-Smith and Enesco, 1982; Masoro, 1992, 2000; Remmen et al., 2001; Weindruch et al., 2001; Masoro, 2003; Spindler, 2003; Fisher, 2004). It has also been investigated genetically (e.g. Lee et al., 1999; Kaneko et al., 2002; Koubova and Guarente, 2003; Yoshinaga et al., 2003b), physiologically (e.g. Kirk et al., 1999), biogerontologically (e.g. Kirk, 2001; Bourg, 2006), and ecologically (e.g. Kirk, 1997a; Ricci and Perletti, 2006; Weithoff, 2007).

However, studies on the effect of CR on the population density of rotifers are rare and have so far focused on demographic parameters such as reproduction, survival, and lifespan (Yamaza at al., 2002; Weithoff, 2007). The data obtained from studies on CR showed that CR suppressed fecundity and increased longevity in a variety of species, such as yeast, fish, rotifers, and rodents. For example, Yoshinaga et al. (2001) reported that *B. plicatilis* reproduction was suppressed under periodical starvation, but their lifespan was 2 times longer than those of nonstarved animals. As these changes in the life history parameters of the members of the population and food availability will result in alterations of the population density, it is very important to know how the organisms cope with the scarcity and abundance of food, not only at the individual but also at population levels. Thus, in this study, the effect of CR on population density was investigated in the freshwater rotifer, *B. calyciflorus*, in order to bring a different approach to feeding strategies with CR in rotifer cultures.

Materials and methods

Culture conditions

B. calyciflorus Pallas was obtained from Florida Aquatic Eco-Systems, Inc. (Apopka, FL) in the form of resting eggs. Rotifers were cultured in 5 glass tubes of 50 mL capacity, each containing 30 mL of hard water (EPA) (Hoff and Snell, 2001) as the medium. The rotifers were fed with the algae *Chlorella* sp. (3×10^6 cells/mL). A stereomicroscope was used for counting the rotifers.

Algal cultures consisted of a total of 3 glass beakers (1 L capacity) and were cultured semicontinously (Richmond, 1986) in a Bold 1NV medium (Starr and Zeikus, 1993). Algal cell density was estimated using a Neubauer hemocytometer under a trinocular microscope (Novex WF 10×).

Both algal and rotifer cultures were carried out at 27 ± 1 °C, pH 7.0-7.5, and under continuous fluorescent illumination (40 W 1000 lux). Experimental rotifer cultures were kept in total darkness to prevent algal growth, except during observations (Yoshinaga et al., 2000; Weithoff, 2007).

Experimental design

CR was applied by lengthening the intervals of feeding in rotifer groups and by feeding a monotype food (*Chlorella* sp.) containing the same amount of cells (1 mL of 1.5×10^6 cells/mL). Groups were fed by replenishing their medium with fresh algae at intervals of 12, 24, 36, 48, 60, and 72 h. The group that was fed every 12 h was designated as the control group.

The experimental design consisted of a total of 24 glass tubes of 10 mL capacity, each containing 5 mL of EPA. Four replicates were maintained in each group. Individuals of *B. calyciflorus* were inoculated into each of the glass tubes with an Eppendorf automatic pipette (1 µL) at an initial density of 8 individuals/mL (age <6 h).

Following inoculation, the living rotifers were counted every 12 h before feeding. After counting, rotifers were transferred, using an automatic pipette, to new tubes containing fresh medium with algal food. The experiment was terminated after 20 days, when *B. calyciflorus* began to decline.

The growth rates of rotifer population (r) were calculated from the exponential phase of the population using the following formula: $r = (\ln N_t - \ln N_0)/t$, where N_0 and N_t are the initial and final population densities, respectively; t is time in days. The r-value was calculated for each replicate separately, for different time intervals (3-14 days), and the mean of 6-7 values was derived (Krebs, 1985; Sarma et al., 2001; Sarma and Nandini, 2001).

Statistical Analysis

Differences between groups were analyzed with variance analysis of the repeated measurements tests and averages of the groups were tested using a multiple comparison test (Duncan's test) at the $\alpha = 0.05$ level. Raw data were subject to log-transformation prior to ANOVA. The results are presented as means ± SD. The analyses were performed using SPSS Version 13.0.

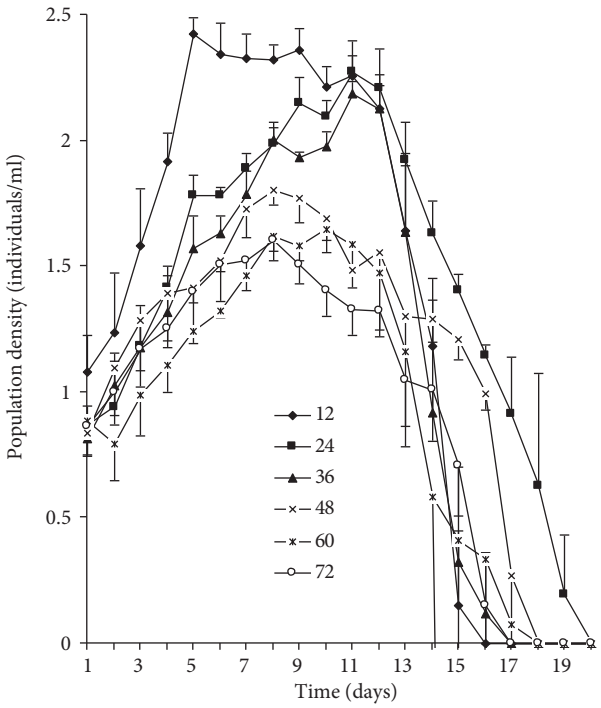

Figure. Time course of population density of *B. calyciflorus* under the CR conditions. Label numbers on graph shows the feeding intervals (h). Data are log-mean ± SD values based on 4 replicate recordings.

Results

The time course of the population peak density of *B. calyciflorus* under the CR conditions is shown in the Figure. CR had a significant effect on the peak population density of *B. calyciflorus* ($P < 0.01$, see Table). Similarly, the day at which the peak population abundance occurred was also significantly affected by CR ($P < 0.01$, see Table). It was observed that the population peak density decreased when the length of feeding intervals was increased in the rotifer groups. The highest peak in population density was seen on the fifth day in the groups fed at 12 h intervals (269 ± 40.12 individuals/mL, mean ± SD) (Table).

Table. Population peak density obtained under the CR conditions, peak days, and growth rates of population (r) means. Superscripts in a row with different letters represent significant differences ($P < 0.05$). Data are mean ± SD values based on 4 replicate recordings.

Feeding interval (hours)	Population peak density (ind./mL) (n = 24)	Peak day	r ± SD
12 (control)	269.0 ± 40.12	5	0.608 ± 0.073^{a}
24	193.0 ± 48.26	11	0.496 ± 0.056^{b}
36	154.5 ± 17.69	11	0.455 ± 0.049^{b}
48	64.25 ± 9.21	8	0.335 ± 0.075^{c}
60	45.25 ± 10.21	10	0.260 ± 0.051^{d}
72	40.75 ± 7.22	8	0.288 ± 0.053^{d}

The rate of population increase (r) varied from 0.608 ± 0.073 to 0.288 ± 0.053, depending on feeding intervals, while the highest rate of population growth (r) was recorded in the group fed at 12 h intervals (0.608 ± 0.073) (Table).

Discussion

Various studies have shown the effect and importance of food concentration on the population density of zooplankton, both from field collections and laboratory studies (Edmondson, 1960; Vanni, 1987; Rothhaupt, 1990; Dumont et al., 1995; Lucía-Pavón et al., 2001; Sarma et al., 2001; Sarma and Nandini, 2001; Yoshinaga et al., 2003a; Lucía-Pavón-Meza et al., 2005). However, studies on the effect of CR on population density are rare and have so far focused on demographic parameters such as reproduction, lifespan, and survival (Verdone-Smith and Enesco, 1982; Kirk, 2001; Yoshinaga et al., 2003a; Weithoff, 2007). This is because food limitation causes both a pressure on fecundity and an increase in mortality. These changes in the life history parameters of the members of the population and food availability will result in an alteration of the population density.

B. calyciflorus was taken into the culture by way of incubation of dormant eggs in this study, in order to have identical cyst quality (size, food history). Jensen and Verschoor (2004) reported that this type of culture is important as embryonic development depends strongly on egg size. The numerical values in many studies conducted on population density and demographic parameters of the rotifer species showed changes according to the culture conditions (algae type and density, temperature, etc.) (Sarma et al., 1999; Lucía-Pavón et al., 2001; Sarma and Nandini, 2002; Enríquez-García et al., 2003; Lucía-Pavón-Meza et al., 2005; Xi et al., 2005). Therefore, the results obtained from the present study were discussed by taking these factors into account.

Sarma (1991) noted that, among the brachionid rotifers, population densities exceeding 1000 individuals/mL have been reported for *B. calyciflorus* and *B. plicatilis*, and rarely for other species such as *B. patulus*. Such high densities are normally obtained by employing algal food concentrations in the range of 0.5×10^6 to 25×10^6 cells/mL of phytoplankton, depending on the algal type, cell diameter, etc. (Sarma and Nandini, 2001). However, for *Chlorella*, most researchers have normally used algal concentrations ranging from 0.1×10^6 to 10×10^6 cells/mL in *Brachionus* cultures to maximize the rate of population increase (Sarma and Rao, 1991; Sarma et al., 1999; Lucia-Pavón et al., 2001; Sarma et al., 2001; Xi et al., 2001). Sarma and Nandini (2001) reported that increasing mortalities were seen in low (0.25 and 0.5×10^6 cells/mL) and high (4.5×10^6 cells/mL) food concentrations. In the present study, the *Chlorella* concentration was adjusted to 1.5×10^6 cells/mL. This density was found to be over the incipient limiting level (~0.17×10^6 cells/mL) (Flores-Burgos et al., 2005) and was also common in the natural bodies of water in which the present rotifer species are commonly found (Sarma et al., 1999; Flores-Burgos et al., 2005; Nandini et al., 2005). Mohr and Adrian (2002) reported that the use of food densities above the incipient limiting level ensured optimal growth rates. Sarma and Nandini (2001) also showed that a 1×10^6 cells/mL food level had an intermediate algal density for a higher survivorship.

Rad (1992) reported that *B. calyciflorus* reached the highest population density on the eighth day of the experiment (101.17 ± 1.56 individuals/mL) when fed with algae (*Ankistrodesmus* spp.) at the concentration of 2-3 × 10^6 cells/mL. Lucía-Pavón et al. (2001) reported a population density for *B. calyciflorus* ranging from 55 ± 1 individuals/mL (under 0.5×10^6 cells/mL) to 471 ± 72 individuals/mL (under 4.5×10^6 cells/mL) with *Chlorella*. Sarma et al. (2001) noted that the highest density for *B. calyciflorus* and *B. patulus* fed with *Chlorella* at the concentration of 3×10^6 cells/mL was 103 ± 8 individuals/mL and 296 ± 20 individuals/mL, respectively. In the present study, the highest population peak-density was obtained from the group fed at 12 h intervals (269 ± 40.12 individuals/mL) on the fifth day of culture, and the population peak density was decreased when the length of feeding intervals were increased in rotifer groups.

The growth rates of rotifer population (r) observed here (0.608-0.288) are within the range recorded earlier for Brachionidae (0.14-0.61 for *B. patulus*: Sarma and Rao, 1991; 0.45-0.86 for *Anuraeopsis fissa*: Dumont et al., 1995; 0.28-0.40 for *Keratella cochlearis*:

Smith and Gilbert, 1995; 0.79-1.49 for *B. calyciflorus*: Sarma et al., 1996; 0.14-0.37 for *B. angularis*: Gama-Flores et al., 2004). Ooms-Wilms et al. (1998) demonstrated that an increase in food availability results in increased population density and higher growth rates (Sarma et al., 1999). Kirk (2001) reported that the r-value depended on food concentration and could also be affected by the culture conditions. Many studies have also shown that the growth rates of rotifer populations are limited in bodies of water depending on food availability (Stemberger and Gilbert, 1985, 1987; Kirk, 1997a, 1997b, 2001; Sarma et al., 2001; Sarma and Nandini, 2001). On the other hand, at high food concentrations, many species showed reduced growth rates. For example, Nandini and Sarma (2000) showed that *Chlorella* concentrations higher than 4.5×10^6 cells/mL actually caused decreased growth rates for *Moina macrocopa*. In our study, such an inverse relation between food concentration and growth rate was not observed because the food concentration was not so high (1.5×10^6 cells/mL).

High concentrations of food can be given in order to produce high numbers of rotifers in a short time, but this condition can cause deformation of the culture water or even the collapse of the entire culture in a short time (Hindioğlu and Serdar, 2001). Yoshinaga et al. (2001) also reported that a higher population growth may cause a deterioration of quality in the next generation, and consequently, the stability of the population will decrease. In the present study, although the higher population density was obtained by feedings with 12 h intervals, the collapsing of this group earlier (15th day) than the other groups can be explained with this reason.

The results of this investigation showed that caloric restriction plays an important role in the population density and growth rate of *B. calyciflorus*. As more severe restrictions (such as in feedings at 48, 60, and 72 h intervals) significantly diminished rotifer populations, milder levels of dietary limitation (such as feedings at 24 and 36 h intervals) may actually be more useful for rotifer cultures. In this situation, the negative conditions caused by excessive food (pollution, putrefaction, etc.) can also be minimized and the same culture tanks can be used over longer periods. This will save time and labor.

In conclusion, we can suggest that caloric restriction may be included as a strategy in the feeding regime of rotifer cultures and may provide valuable information for future aquaculture studies.

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