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Effect of three food types on the population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera: Brachionidae)

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Abstract: We compared the population growth of *B. calyciflorus* and *B. patulus* using the green alga *Chlorella vulgaris*, baker's yeast *Saccharomyces cerevisiae* or their mixture in equal proportions as food. Food was offered once every 24 h in two concentrations (low: 1×10^6 and high: 3×10^6 cells ml^{-1}) separately for each species. The experiments were terminated after 15 days. In general, at any food type or concentration, *B. patulus* reached a higher population density. A diet of *Chlorella* alone supported a higher population growth of both rotifer species than yeast alone. *B. calyciflorus* and *B. patulus* achieved highest population densities (103±8 ind. ml^{-1} and 296±20 ind. ml^{-1}, respectively) on a diet of *Chlorella* at 3×10^6 cells ml^{-1}. When cultured using the mixture of *Chlorella* and yeast, the maximal population densities of *B. calyciflorus* were lower than those grown on *Chlorella*. Under similar conditions, the maximal abundance values of *B. patulus* were comparable in both food types. Regardless of food type and density the rate of population increase per day (r) for *B. calyciflorus* varied from 0.13±0.03 to 0.63±0.04. These values for *B. patulus* ranged from 0.19±0.01 to 0.37±0.01. The results indicated that even though *Chlorella* was a superior food for the tested rotifers, yeast can be effectively used at low concentrations to supplement algal requirements in rotifer culture systems.

Key words: Population growth, alga, yeast, Rotifera.

Laboratory cultivation of brachionid rotifers has been successfully done using green algae. In order to supplement the algal quantity, Hirata and Mori (1967) introduced the use of bakers' yeast as food for the saline water species *Brachionus plicatilis*. Since then a number of investigators have used bakers' yeast as food for this species; rotifers grown in this way have also been nutritionally enriched (Fernandez-Reiriz and Labarta 1996, Lie *et al.* 1997). A vast majority of researchers used yeast only for *B. plicatilis* and *Brachionus rotundiformis*. In aquaculture, in addition to these two species, several other rotifer taxa such as *B. calyciflorus*, *B. rubens* and *B. patulus* are used as starter food (Rottmann *et al.* 1991, Mookerji and Rao 1994).

Several algal species have been used while testing the use of freshwater rotifer species. Since algal cultivation under controlled conditions is laborious, time consuming and expensive, alternative food types such as yeast, wastewater from food industry and livestock have been used for mass rotifer cultures (Klekot and Klimowicz 1981). However, controlled laboratory experiments using these food types for rotifers are necessary to compare the population growth with conventional algal diets. It is also not known whether different *Brachionus* species show different population growth rates when grown on yeast. At the same time, a comparative information about the growth of different rotifers grown on alga, yeast and their mixture separately has rarely been published (Guevara *et al.* 1996).

The aim of this study was to test the effect of different concentrations of green alga and yeast and their mixture on the population growth of two brachionid rotifer species commonly found in freshwater systems.

MATERIALS AND METHODS

We used clonal populations of each of the two rotifer species *Brachionus calyciflorus* and *Brachionus patulus* maintained at least for 3 months prior to testing. Both the rotifer species were mass-cultured (40 l glass aquaria) using the green algae *Chlorella vulgaris* as the exclusive food. Two weeks prior to experimentation, the test rotifer species were also offered baker's yeast *Saccharomyces cerevisiae* in addition to the algal diet. For maintaining mass rotifer cultures we used reconstituted hardwater (EPA) as medium (Anonymous 1985). This was also used as medium for rotifer growth experiments.

Chlorella was cultured using Bold-basal medium. Log phase algae were harvested, centrifuged and resuspended in EPA medium. Commercially available baker's yeast was freshly procured, resuspended in EPA medium and filtered using a 20 µm mesh to remove clumps. Algal and yeast cell density was estimated using haemocytometer. For each rotifer species we offered food in the following ways: 1. only alga, 2. only yeast, and 3. alga+yeast in equal density.

Each food type was offered in two densities viz. low ($1x10^6$) and high ($3x10^6$ cells ml^{-1}). For each food type and density, we maintained four replicates. Thus for population growth of *B. calyciflorus*, we maintained a total of 24 plastic jars (3 food types x 2 food concentrations x 4 replicates), each containing 20 ml of EPA medium with appropriate food density. The initial density of rotifers in each test jar was 5 ind. ml^{-1}. For the population growth of *B. patulus* also the above design was used. Experiments were conducted at 23±2°C.

Following inoculation of *B. calyciflorus* or *B. patulus*, at every 24 h interval, we counted the number of female rotifers alive under a stereomicroscope. For this we either counted the whole volume of the test vessel or two aliquot samples each of 1-5 ml depending the density of rotifers per container. After estimating the density, the individuals were transferred to fresh EPA medium containing appropriate food type. The transfer of rotifers to fresh medium was done either individually, when the densities were low, or using a 50 µm mesh during later stages of the study. Experiments were terminated after 15 days when most populations began to decline. Thoughout this study males were not encountered.

The rotifer population growth was obtained from a mean of 4-5 values during the exponential phase using the equation $r = (\ln N_t - \ln N_o)/t$, where, N_o = initial population density, N_t = density of population after time t (days) (Krebs, 1985).

RESULTS

The population growth curves of *B. calyciflorus* and *B. patulus* reared under three food types and two densities are shown in Figs. 1 and 2. The maximal population density of *B. patulus* influenced significantly ($p<0.01$) by food type, its concentration as well as their interaction but not for *B. calyciflorus* ($p>0.05$, ANOVA). In general, at any food type or concentration, *B. patulus* reached higher population density when compared to *B. calyciflorus*. In $1x10^6$ cells ml^{-1}, *B. calyciflorus* reached 77±12 ind. ml^{-1}; at the same food level *B. patulus* attained 109±26 ind. ml^{-1}. At $3x10^6$ cells ml^{-1} density, *B. calyciflorus* reached a peak density of 103±8 ind. ml^{-1}. Under comparable conditions, *B. patulus* reached much higher peak abundance 296±20 ind. ml^{-1}. When yeast was used as exclusive food, the maximal abundance values reached by *B. calyciflorus* were

62±19 and 57±25 ind. ml^{-1} under low ($1x10^6$ cells ml^{-1}) low and high ($3x10^6$ cells ml^{-1}) food concentrations, respectively. On the other hand, *B. patulus* showed peak population abundances of 97±17 and 50±6 ind. ml^{-1} in low and high concentrations of yeast. *B. calyciflorus* reached a peak abundance of 54±9 and 86±3 ind. ml^{-1} for low and high food densities when both these food types offered in equal concentrations. Comparable values of *B. patulus* were 251±12 and 259±32 ind. ml^{-1}.

The day of maximal population density was not significantly different for the food concentrations used for both *B. calyciflorus* and *B. patulus* ($p>0.05$). However, food type had a significant effect on this variable ($p<0.01$) for both the species. The highest rate of population growth (r) recorded for *B. calyciflorus* was 0.63±0.04 and the lowest r value (0.13±0.03) was observed for the same species when grown in high concentration of yeast. Regardless of food type and density the r values of *B. patulus* ranged from 0.19±0.01 to 0.37±0.01 (Fig. 3). The r values for *B. patulus* were significantly affected by food type, density and their interaction ($p<0.001$). For *B. calyciflorus* only the food type had a significant effect ($p<0.01$). An inverse relation occurred for both *B. calyciflorus* and *B. patulus* when the daily rate of population increase was plotted againt the population density of the same day (Figs. 4 and 5).

DISCUSSION

Studies concerning the population growth of *B. patulus* using baker's yeast have not been published so far. It is evident from the present study that yeast can be used for culturing *B. patulus* together with alga. The range of algal food densities chosen here were earlier used for growth studies of *B. calyciflorus* and *B. patulus*. As shown in many other studies (Halbach and Halbach-Keup 1974), an increase in *Chlorella* level from $1x10^6$ cells ml^{-1} to $3x10^6$ cells ml^{-1} resulted in an increase in the maximum population abundance of both *B. calyciflorus* and *B. patulus* (Figs. 1 and 2). However, when different food types were combined, the maximum peak density of *B. calyciflorus* was not statistically significant due to similar growth curves of rotifers fed yeast in low and high food densities. Sarma *et al.* (1996) have grown *B. calyciflorus* in a wide range of *Scenedesmus* ($0.5x10^6$ to $40.5x10^6$ cells ml^{-1}) and found no inhibitory effect of the algae, although the mean peak population abundances did not exactly correspond to the food levels offered. The present peak abundance values of *B. calciflorus* are comparable to those of Sarma *et al.* (1996) under similar food densties. Sarma and Rao (1987) used $1x10^6$ - $4x10^6$ cells ml^{-1} of *Chlorella* for growing *B. patulus* and reported peak abundance values of 110 - 325 ind. ml^{-1}. In the present study, we found peak abundance values ranged from 109±26 to 296±20 depending on the algal food level.

The rates of population increase (r) observed here for both *B. calyciflorus* and *B. patulus* are within the range recorded earlier for Brachionidae (Table 1). In general, *B. calyciflorus* has a higher population growth rate compared to *B. patulus*. This is also evident from Fig. 3. It is however, important to note that species with higher r values need not always be competitively superior to those with lower growth rates (Sarma *et al.* 1999).

The inverse relation between population density and per capita rate of increase as recorded for *B. calyciflurus* and *B. patulus* was earlier observed for other zooplankton (*Daphnia*: Kerfoot *et al.* 1985, *Anuraeopsis*: Dumont *et al.* 1995).

This study showed that *Chlorella vulgaris* is a superior food as compared to *Saccharomyces cerevisiae* for these rotifer species. However, when offered a mixture of alga and yeast at low food density, *B. calyciflorus* and *B. patulus* reached higher peak population abundances comparable to or higher than on a diet of alga alone. It was found that the freshwater rotifers *Brachionus calyciflorus* and *B. patulus* were able to grow well on a mixed diet of *Chlorella vulgaris* and baker's yeast at $1x10^6$ cells ml^{-1} density. Only yeast was not suited for both rotifer species under $1x10^6$ and $3x10^6$ cells ml^{-1} density. Thus, although a diet of yeast alone was not comparable to that of *Chlorella*, it can be effectively used at low concentrations to supplement algal requirements in rotifer culture systems.

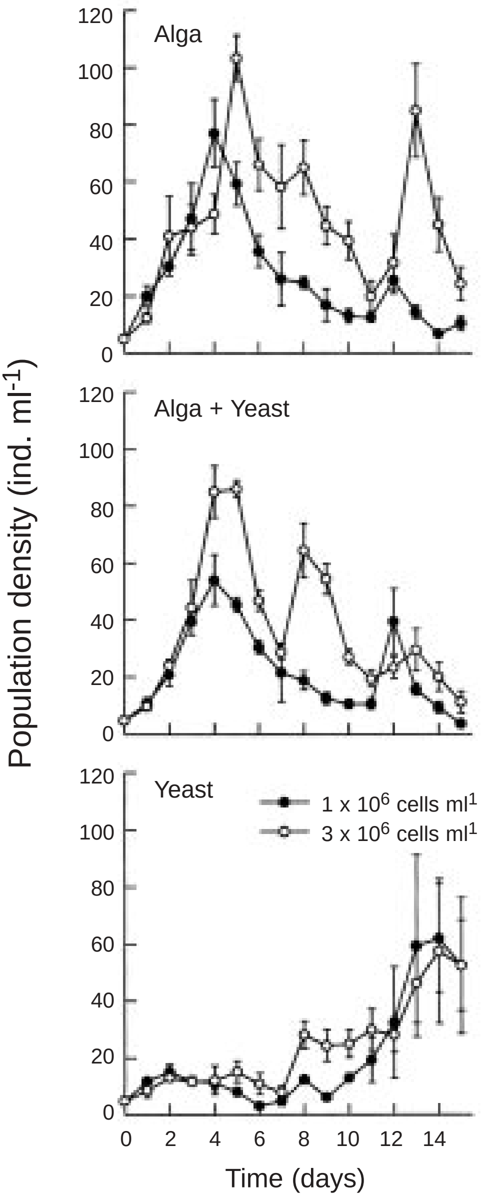

Fig. 1. Population growth curves of *B. calyciflorus* in relation to food type and density. Shown are the mean±SE values based on four replicate recordings. Other details as in Fig. 2.

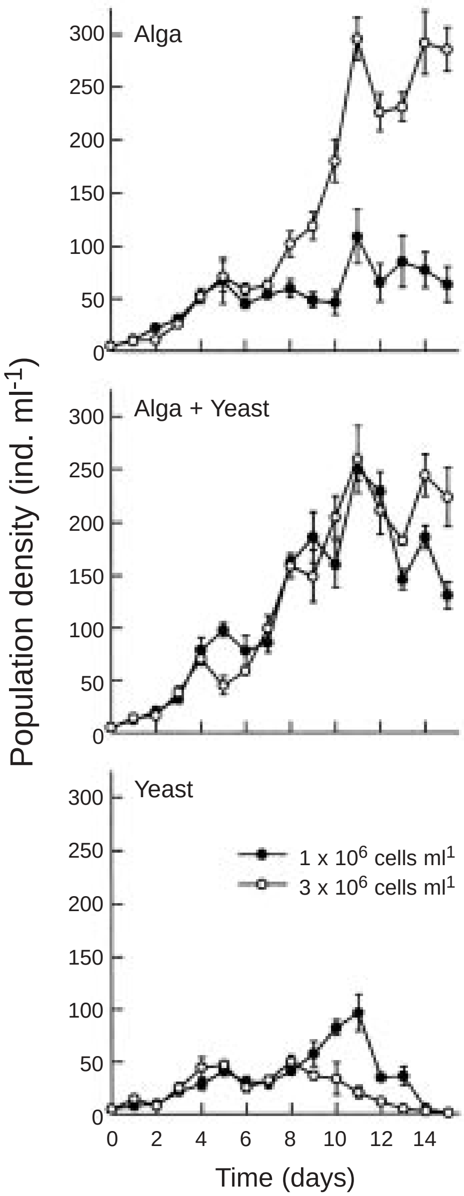

Fig. 2. Population growth curves of *Brachionus patulus* in relation to food type and density. Shown are the mean±SE values based on four replicate recordings.

TABLE 1

Rate of population increase (r) per day of selected rotifer species, family Brachionidae

Species	Experiment	Food type	Food level	r value	Reference
Anuraeopsis fissa	Population growth	*Scenedesmus obliquus*	0.5 - 8 x 10^6 cells ml^{-1}	0.45 - 0.86	Dumont *et al.* , 1995
	Population growth	*Scenedesmus acutus*	0.5 - 40.5 x 10^6 cells ml^{-1}	0.44 - 0.88	Sarma *et al.* , 1996
	Life table	-	-	0.10	Ooms-Wilms, 1997
Brachionus angularis	Population growth	*Stichococcus bacillaris*	-	0.58	Walz, 1993
B. calyciflorus	Life table	-	-	2.20	Wang and Li, 1997
	Population growth	*Scenedesmus acutus*	0.5 - 40.5 x 10^6 cells ml^{-1}	0.79 -1.49	Sarma *et al.* , 1996.
	Population growth	Various types of algae	-	0.80	Rothhaupt, 1990
B. patulus	Life table	*Chlorella*	1-4 x 10^6 cells ml^{-1}	0.14-0.61	Sarma and Rao, 1991
	Population growth	*Chlorella*	1-3 x 10^6 cells ml^{-1}	0.12-0.24	Sarma and Rao, 1990
B. plicatilis	Population growth	*Tetrathelmis tetrathele*	0.05 x 10^6 cells ml^{-1}	0.24 - 0.49	Okauchi and Fukusho, 1984
	Population growth	*Chlorella*	1.5 x 10^6 cells ml^{-1}	0.16-0.47	Okauchi and Fukusho, 1984
B. rubens	Population growth	Various types of algae	-	0.80	Rothhaupt, 1990
	Population growth	*Chlorella*	3 x 10^6 cells ml^{-1}	0.79	Iyer and Rao, 1993
B. urceolaris	Life table	-	-	1.32	Wang and Li, 1997
Keratella cochlearis	Population growth	*Cryptomonas erosa*	0.005-0.01 x 10^6 cells ml^{-1}	0.28-0.40	Smith and Gilbert, 1995
	Population growth	-	-	0.13	Ooms-Wilms, 1997
K. crassa	Population growth	*Cryptomonas erosa*	0.005-0.01 x 10^6 cells ml^{-1}	0.32	Smith and Gilbert, 1995
K. testudo	Life table	-	-	0.15 -0.39	Stemberger, 1988

The method of calculation of r also depends on the source of data and culture conditions. The negative r values as a result of stress (*e.g.* toxicant) are not included.

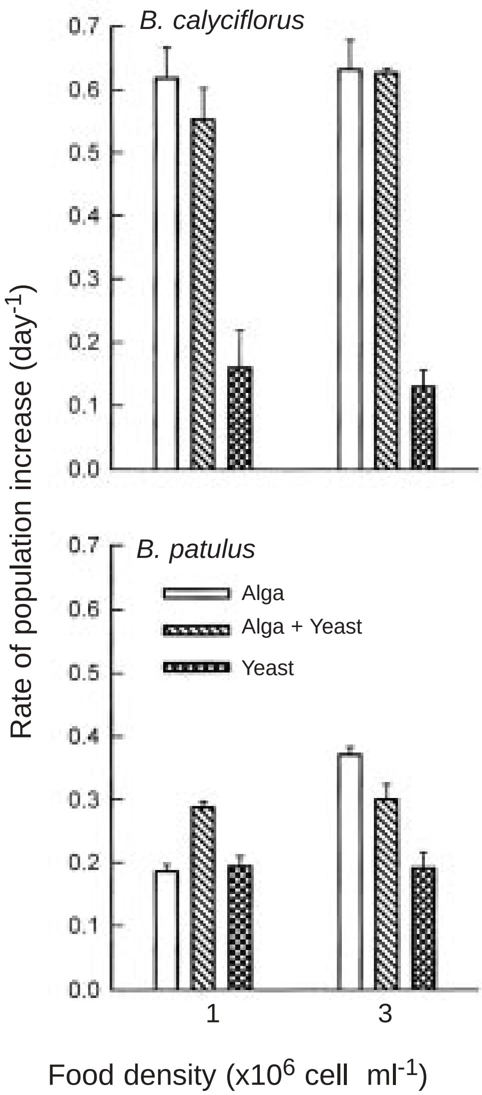

Fig. 3. Rate of population growth (r) per day for *B. calyciflorus* and *B. patulus* in relation to food type and density. Shown are the mean±SE values based on four replicate recordings.

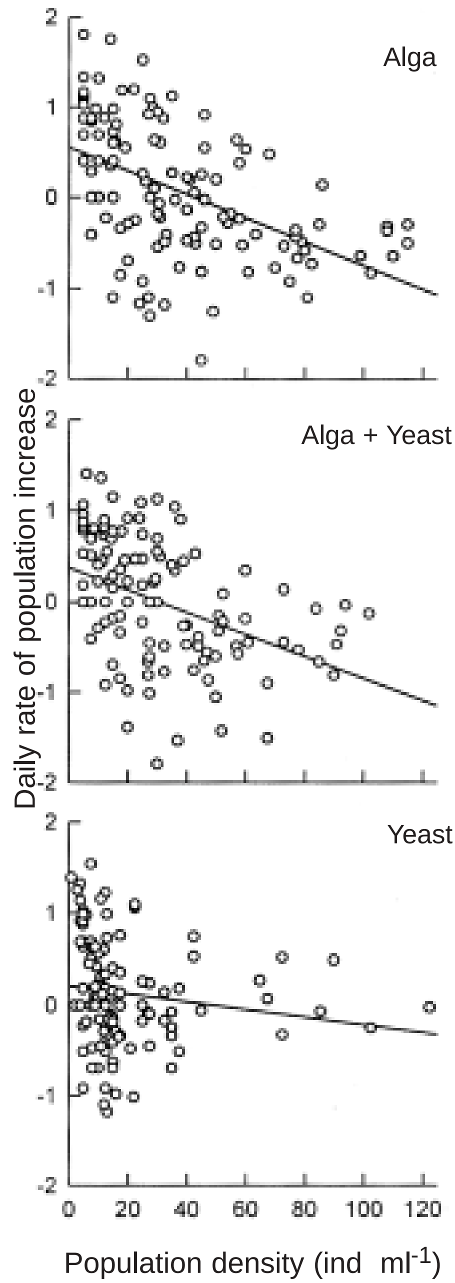

Fig. 4. Relation between population density and daily growth rate of *B. calyciflorus* in relation to food type and density. Shown are the mean±SE values based on four replicate recordings.

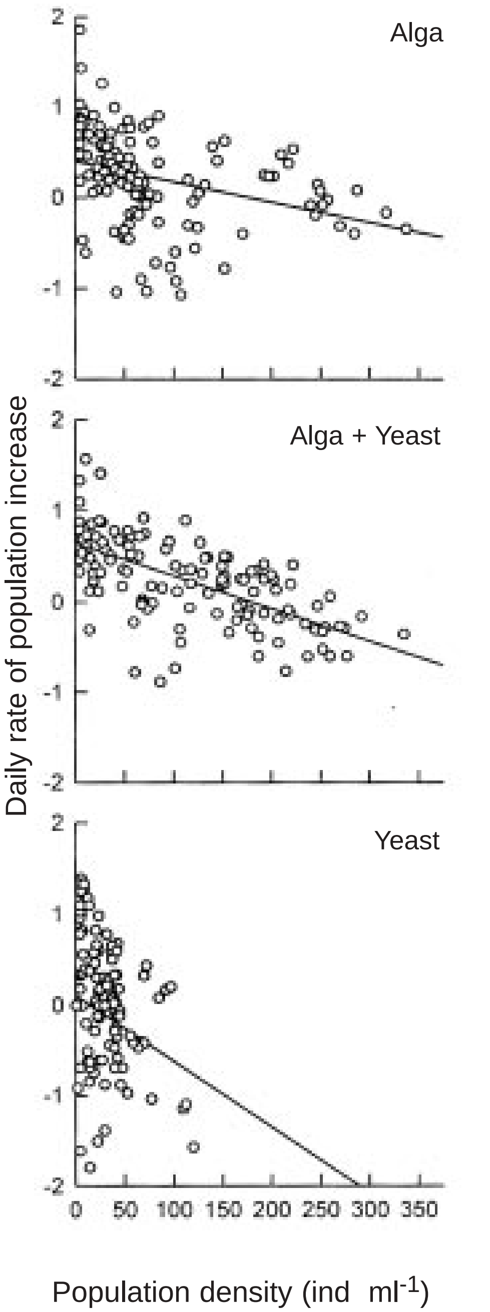

Fig. 5. Relation between population density and daily growth rate of *B. patulus* in relation to food type and density. Shown are the mean±SE values based on four replicate recordings.

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RESUMEN

Se comparó el crecimiento poblacional de dos especies planctónicas (*B. calyciflorus* y *B. patulus*) desarrolladas con el alga verde *Chlorella vulgaris,* la levadura de cerveza *Saccharomyces cerevisiae* y la mezcla de ambas dietas en proporciones iguales. *B. patulus* alcanzó las mayores densidades con cualquier tipo de alimento utilizado en comparación con *B. calyciflorus*. La dieta a base de *Chlorella vulgaris* sola promovió el mayor crecimiento poblacional en relación con la dieta de levadura sola. *B. calyciflorus* y *B. patulus* alcanzaron las mayores densidades de 103±8 ind. ml^{-1} y 296±20 ind. ml^{-1}, respectivamente, con la dieta de *Chlorella* en $3x10^6$ células ml^{-1}. En condiciones similares, los valores máximos de abundancia de *B. patulus* fueron semejantes para ambos tipos de alimento. La tasa de incremento poblacional por día (r) para *B. calyciflorus* vario de 0.13±0.03 a 0.63±0.04, sin importar el tipo y densidad de alimento. Los resultados indican que la dieta a base de *Chlorella* fue mejor para los rotíferos considerados, y que la levadura puede usarse de manera efectiva a concentraciones bajas para complementar los requerimientos algales del sistema de cultivo de rotíferos.

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