Rev. Biol. Trop. 49(3-4): 895-902, 2001
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Effect of different densities of live and dead *Chlorella vulgaris* on the population growth of rotifers *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera)

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Recibido 12-VI-2000. Corregido 31-I-2001. Aceptado 25-II-2001.

Abstract: In order to maintain rotifer populations during periods of low algal production, it is necessary to offer alternate diets, some of which include forms of preserved algae. The present work is based on the effect of live and dead *Chlorella vulgaris* on the population growth of *Brachionus calyciflorus* and *Brachionus patulus*. The experimental design consisted of three algal levels ($0.5x10^6$, $1.5x10^6$ and $4.5x10^6$ cells ml^{-1}) offered in three forms (living, frozen and heat-killed). The maximal population density values for *B. calyciflorus* ranged from 55±1 ind. ml^{-1} (at $0.5x10^6$ cells ml^{-1}) to 471±72 ind. ml^{-1} (at $4.5x10^6$ cells ml^{-1}) with live *Chlorella*, but was much lower (6±1 to 26±6 ind. ml^{-1}) with frozen or heat-killed alga under comparable food levels. However, the maximum population density of *B. patulus* under live or or heat-killed *Chlorella* was similar at comparable algal levels but when offered frozen algae it was four times less. The highest mean peak population density was 1 227±83 ind. ml^{-1} under $4.5x10^6$ cells ml^{-1}. The rate of population increase for *B. calyciflorus* varied from 0.50 to 0.79 using live *Chlorella*, but under comparable conditions, this range was lower (0.21 to 0.31) for *B. patulus*. Results have been discussed in light of possible application for aquaculture.

Key words: Rotifera, *Chlorella*, population growth, live-alga, dead-alga.

Rotifers of the genus *Brachionus* are widely used as live food in rearing larval fish, crabs, prawns, shrimps and molluscs under both marine and freshwater culture conditions (Sarma 1991). For mass production of *B. plicatilis* various types of food, both natural (*e.g.*, algae) and artificial diets (microencaspulated pellets) have been used. Hirata and Mori (1967) replaced green algae with baker's yeast for mass production of *B. plicatilis*. However, due to low nutritional value of normal yeast-fed rotifers, a number of enrichment procedures have to be followed (Hirayama and Funamoto 1983). Although some of these enrichment procedures are easily done, the use of microalgae in rotifer cultures has been largely practised (Pourriot 1991).

Since large-scale algal production is relatively cheap, both under field and laboratory conditions, various types of algae are routinely being produced for feeding planktonic rotifers (Groeneweg and Schluter 1981). However, some times algal cultures crash (Suminto and Hirayama 1997) which may, in turn, lead to problems of finding adequate food for rotifers. Therefore, stored algae could be used as substitute. On the other hand, at times there may be excessive production of microalga which

could be stored for future use (Millamena *et al*. 1990, Martínez-Jeronimo and Espinosa-Chavez 1994). The nutrional quality as well as the digestibility of the stored-microalga may vary considerably and consequently the growth responses of zooplankton feeding on them (Gatesoupe and Robin 1981, Dobberfuhl and Elser 1999). In this context, population level responses of rotifers to stored microalgae has not been well documented (Starkweather and Bogdan 1980, Lubzens *et al.* 1995).

The aim of this study was to compare the population growth of two freshwater rotifers, *B. calyciflorus* and *B. patulus* using *Chlorella vulgaris* in live, frozen and heat-killed forms under three food levels.

MATERIALS AND METHODS

The test rotifer species *B. calyciflorus* and *B. patulus* were isolated from lake Chapultepec (Mexico City) and the waterbody Presa Santa Elena (State of Mexico), respectively. Clonal populations of both these species were established using the single celled green algae *C. vulgaris*. For mass cultures as well as for experiments, we used *C. vulgaris*, raised on Bold's basal medium (Borowitzka and Borowitzka 1988). Algae in log phase of growth were harvested, centrifuged at 3 000 rpm for 5 min., rinsed with distilled water and resuspended in reconstituted moderately hardwater, (the EPA medium), which was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in one litre of distilled water (Anonymous 1985). The stock algal density was estimated using a haemocytometer. Test rotifers were offered *C. vulgaris* in three forms: live, cold-killed (freshly frozen) and heat-killed (with boiling water). For experiments, we used algae of no more than two days old, following harvest.

The experimental design for *B. calyciflorus* consisted a total of 27 transparent test jars (50 ml capacity) containing 50 ml EPA medium. We used three food levels namely, $0.5x10^6$, $1.5x10^6$ and $4.5x10^6$ cells ml^{-1} in EPA medium. For each food density and form, we used three jars per treatment. Into each of the test jars we inoculated individuals of *B. calyciflorus* at an initial density of 5 ind ml^{-1} using finely drawn Pasteur pipette. The test jars were maintained at 22±2 ºC, pH 7.2-7.5 and continous but diffused flourescent illumination (1 000 lux). Following inoculation the density of *B. calyciflorus* was estimated daily using total counts or 2-3 aliquots of 1 ml each. We counted the number live rotifers (females). Due to uncertainity of the time of death, dead individuals were not estimated. After estimating the density, rotifers were transferred using 50 mm mesh to new jars containing appropriate algal food density and form (*i.e.* live, frozen or heat-killed state). The experiment was discontinued after two weeks by which time *B. calyciflorus* in most replicates began to decline. Concurrently but separately, experiments were also conducted using the other brachionid rotifer *B. patulus*. For this species also, the experimental design and the test conditions were similar to those *B. calyciflorus*, except for the duration, which was 40 days.

Based on the data collected, we calculated the rate of population increase (r) using the exponential growth 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). The r was obtained from a mean of 4-5 values during the exponential phase of the population growth for each species.

RESULTS

Population growth curves of *B. calyciflorus* in relation to different *Chlorella* levels and forms are presented in Fig. 1. Regardless of form in which *Chlorella* was offered, *B. calyciflorus* showed increased population abundance with increasing food level. Within the three forms of *Chlorella*, live algae supported the best growth of *B. calyciflorus*. The maximal population density values for *B. calyciflorus* ranged from 55±1 ind. ml^{-1} (under $0.5x10^6$ cells ml^{-1}) to 471±72 ind. ml^{-1} (under

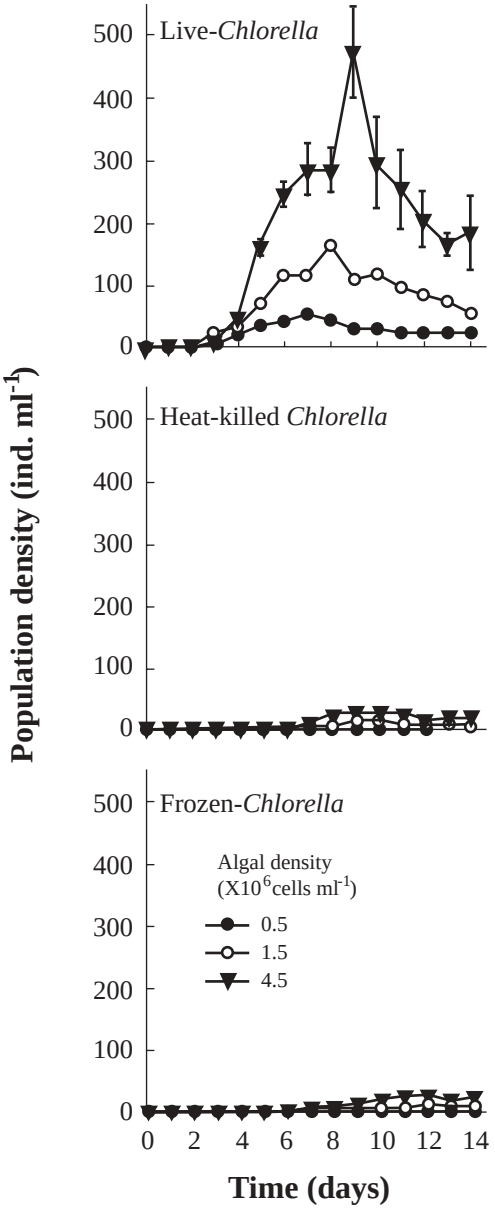

Fig. 1. Population growth curves of *B. calyciflorus* in relation to *Chlorella* density offered as live, heat-killed and frozen forms. Shown are the mean±SE values based on three replicate recordings.

4.5x10^6 cells ml^{-1}) with live *Chlorella*, but was much lower (6±1 to 26±6 ind. ml^{-1}) with frozen alga under comparable food levels (Fig. 2A). The maximum population density of rotifers was significantly affected by both the algal level and the form (Table 1, $p < 0.001$, ANOVA, Sokal and Rohlf 1985). The rate of population increase for *B. calyciflorus* varied from 0.50 to 0.79 on live algae, depending on the food level. However, the values were much lower (0.10 to 0.41) when heat-killed or frozen *Chlorella* was used as food (Fig. 3A). Thus, both food level and form as well as their interaction had a significant effect on the rate of population increase of *B. calyciflorus* (Table 1).

Brachionus patulus needed from 27 to 40 days to show a declining trend in the population densities (Fig. 4). The maximum population density of *B. patulus* under live or or heat-killed *Chlorella* was similar at comparable algal levels (Fig. 2B), with the trend of increasing population abundance with increasing availability of food. Thus the highest mean peak population density was 1 227±83 ind. ml^{-1} under 4.5x10^6 cells ml^{-1}. *Brachionus patulus* offered frozen algae increased only one-fourth of this. The rate of population increase varied from 0.21±0.003 to 0.31±0.002 under 0.5x10^6 cells ml^{-1} and 4.5x10^6 cells ml^{-1} of live *Chlorella*, respectively (Fig. 3B). Both form and level of *Chlorella*, as well as their interactions signficantly ($p < 0.05$, ANOVA, Table 1) influenced the mean maximal population density and rate of increase.

DISCUSSION

Various studies have shown the importance of food density on the population growth of zooplankton, both from field collections and laboratory studies (Edmondson 1960, Halbach and Halbach-Keup 1974). Rotifers being opportunistic species, respond more rapidly to changes in food levels (Nogrady *et al.* 1993). It is known that rotifers show an almost linear numerical increase with increasing food levels (Dumont *et al.* 1995). *Chlorella* is one the most widely used food for culturing planktonic rotifers (Pourriot and Rougier 1997). Studies on the use of preserved algae for zooplankton growth are rare because of a general idea that non-living algae do not support their growth

TABLE 1
Analysis of variance on maximal population density and the rate of population increase in B. calyciflorus *and* B. patulus *offered* C. vulgaris *in three forms at three densities*

Source of variation	DF	SS	MS	F
B. calyciflorus				
Max. population density				
Algal form (A)	2	271 055.03	135 527.52	75.56***
Algal density (B)	2	118 384.06	59 192.03	33.00***
Interaction (A x B)	4	164 411.72	41 102.93	22.92***
Error	18	32 286.69	1 793.70	-
Rate of population increase				
Algal form (A)	2	0.965	0.482	280.20***
Algal density (B)	2	0.305	0.152	88.56***
Interaction (A x B)	4	0.022	0.006	3.19*
Error	18	0.031	0.002	-
B. patulus				
Max. population density				
Algal form (A)	2	314.740	157.370	132.78***
Algal density (B)	2	16.963	8.482	7.16**
Interaction (A x B)	4	24.814	6.203	5.23**
Error	18	21.334	1.185	
Rate of population increase				
Algal form (A)	2	0.026	0.013	234.00***
Algal density (B)	2	0.093	0.0465	837.00***
Interaction (A x B)	4	0.002	0.0005	9.00***
Error	18	0.001	5.556x10-5	

DF = degrees of freedom, SS = sum of square, MS = mean square, F = F ratio, levels of singificance *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$.

(Kumlu 1997, Baer and Goulden 1998). Our study showed that this is true for *B. calyciflorus* but not for *B. patulus*. For example, *B. calyciflorus* did not grow well on either heat-killed or frozen *Chlorella* while, *B. patulus* was able to utilize heat-killed (but not to the same extent on frozen) algae more effectively and reached densities comparable to those of live *Chlorella* at all tested levels.

Data available in literature on the use of various forms of algae such as frozen, dry powder and freeze-dried so far have concentrated on *B. plicatilis* and usually under a single food density (Yamasaki *et al*. 1989, Yúfera and Navarro 1995, Navarro 1999). Although these results form the basis, they cannot be extrapolated to other commonly co-occuring species of the same genus. Thus, the present study provides the possibility of comparing two rotifer species belonging to the same genus, not only under the same food levels, but also under identical test conditions. It is evident from the data on population growth that *B. calyciflorus* (Fig. 1) could reach peak abundances much earlier than *B. patulus* (Fig. 4). This was also reflected in the rates of population increase. Among the planktonic rotifers, *B. calyciflorus* is known to have the highest growth rates (Bennett and Boraas 1989). In the present study also, under comparable live *Chlorella* levels, *B. calyciflorus* had higher growth rates than those of *B. patulus* (Fig. 3). The mean maximal population density reached by a given rotifer species is dependent on its body-size. Generally, large rotifer species have lower numerical abundance per unit volume of

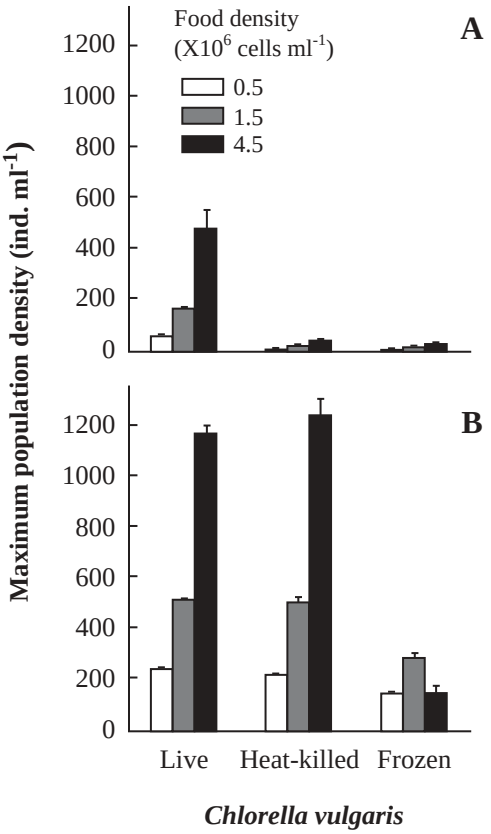

Fig. 2. Maximum population density (ind. ml^{-1}) of *B. calyciflorus* (A) and *B. patulus* (B) in relation to *Chlorella* density offered as live, heat-killed and frozen forms. Shown are the mean±SE values based on three replicate recordings.

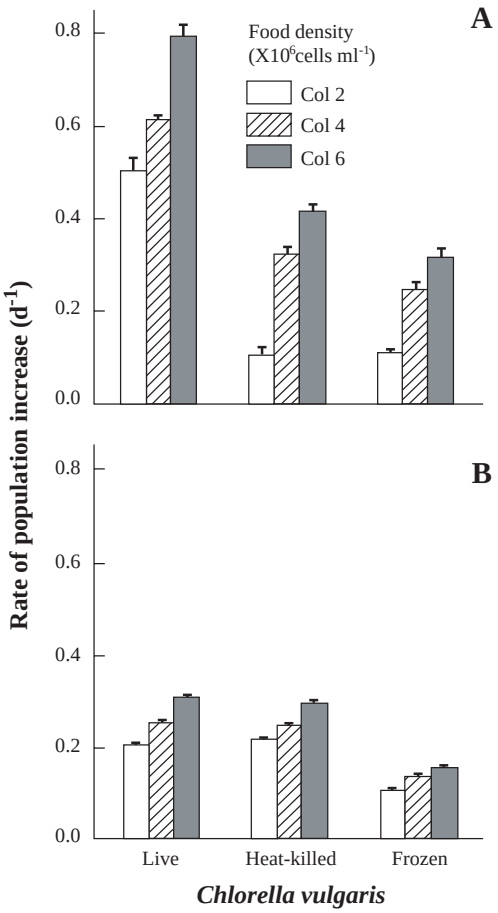

Fig. 3. Rate of population growth (r) per day for *B. calyciflorus* (A) and *B. patulus* (B) in relation to *Chlorella* density offered as live, heat-killed and frozen forms. Shown are the mean±SE values based on three replicate recordings.

medium compared to smaller species (Sarma *et al.* 1999). For example, the rotifer *Anuraeopsis fissa* with a body lenght of about 70 µm, could reach an abundance of > 9 500 ind ml^{-1} but under comparable food levels, a large species (*B. calyciflorus*, lenght about 255 µm) could reach only one-tenth of this density (Sarma *et al.* 1996). In the present study also, *B. patulus* being smaller than *B. calyciflorus* reached much higher abundances. The r values obtained in this study are comparable to those reported in a review of growth rates of selected rotifer species by Sarma *et al.* (2001).

The differential responses of *B. calyciflorus* and *B. patulus* to non-living *Chlorella* is probably related to their natural feeding habits. For example, *B. calyciflorus* is typically planktonic, while *B. patulus* is normally associated with vegetation, probably feeding on detritus and epiphytic algae (Ruttner-Kolisko 1972). This is reflected in the higher maximal population abundances of *B. patulus* fed on heat-killed *Chlorella* compared to *B. calyciflorus*. However, both the rotifer species could not utilize frozen algae effectively. This suggests that frozen *Chlorella* is probably difficult to digest and/or the tested rotifers might have the capacity to discreminate different forms of the same alga (DeMott, 1986). Lubzens *et al.* (1995) have also shown that the nutritonal quality of frozen alga is not significantly different from

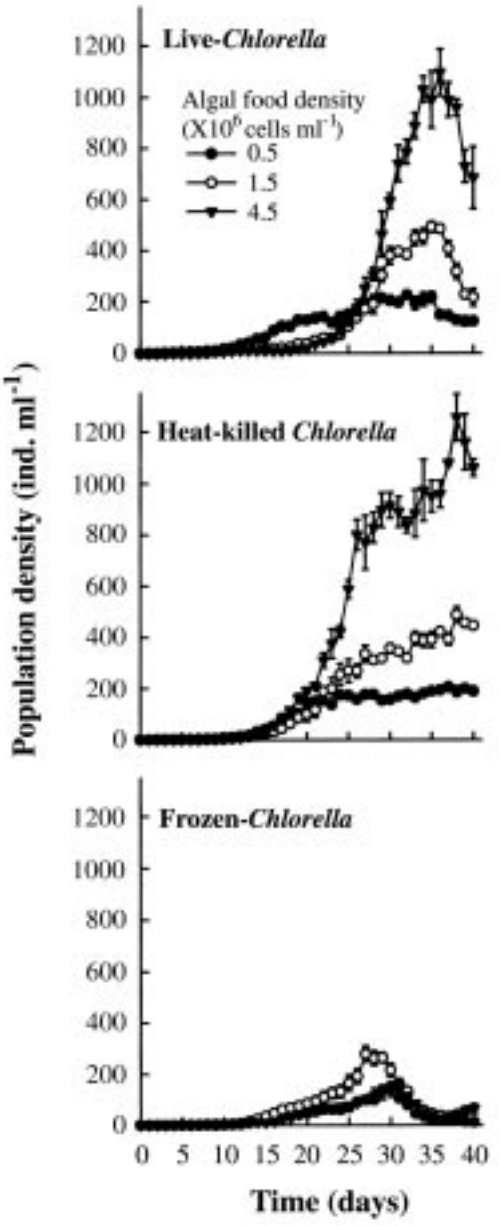

Fig. 4. Population growth curves of *B. patulus* in relation to *Chlorella* density offered as live, heat-killed and frozen forms. Shown are the mean±SE values based on three replicate recordings.

live alga. When heat-killed, algal digestibility probably would increase but the nutritional quality may decrease (Hohman *et al.* 1982, Brown 1995). However, the fact that *B. patulus* could grow well on the heat-killed *Chlorella* suggests that the response of brachionids could vary considerably based on their adaptations to the natural conditions they inhabit. This could also be due to the discriminatory capacity of rotifer species to live versus dead food particles (Starkweather and Bogdan 1980, DeMott 1986).

In conclusion, some implications of the present work are that although frozen *Chlorella* could not support growth of the test species, it could be used just for maintaining the high densities of rotifers, until live alga is available. Also, if the nutritional quality of rotifers fed on heat-killed algae is found comparable to those fed on live alga, then it could be possible to heat-kill and preserve algae for later use to maintain rotifer cultures.

ACKNOWLEDGMENTS

This investigation was supported by a Project from PAPIIT (IN223799). Additional support from a grant awarded for SNI members by ENEP-Iztacala to SSSS is gratefully acknowledged. ELP thanks CONACyT (Ref. 67991017) for a scholarship. SN thanks the National System of Investigators (SNI-20520).

RESUMEN

Para mantener poblaciones de rotíferos durante periodos con escasez de microalgas, es necesario ofrecer dietas alternativas, incluyendo algunas formas de microalgas preservadas. El presente trabajo analiza el efecto de *Chlorella vulgaris* viva y muerta sobre el crecimiento poblacional de *Brachionus calyciflorus* y *B. patulus.* El diseño experimental consistió en tres niveles de algas ($0.5x10^6$, $1.5x10^6$ y $4.5x10^6$ células ml^{-1}) ofrecidas en tres formas (viva, congelada y muerta con agua caliente). Las abundancias máximas de población de *B. calyciflorus* variaron desde 55±1 ind. ml^{-1} (en $0.5x10^6$ células ml^{-1}) a 471±72 ind. ml^{-1} (en $4.5x10^6$ células ml^{-1}) con *Chlorella* viva, mientras que, cuando se utilizó alga congelada o muerta con agua caliente, la abundancia fue mucho menor (6±1 a 26±6 ind. ml^{-1}). Sin embargo, la máxima densidad de población de *B. patulus* con *Chlorella* viva o muerta con calor fue similar bajo niveles de algas comparables, mientras que cuando se ofreció alga congelada, la abundancia fue cuatro veces menor. La abundancia máxima de *B. patulus* fue 1 227±83 ind. ml^{-1}

bajo $4.5x10^6$ células ml^{-1}. La tasa de crecimiento poblacional de *B. calyciflorus* varió desde 0.50 hasta 0.79 usando *Chlorella* viva, pero bajo las mismas condiciones, el rango es menor (0.21 a 0.31) para *B. patulus*. Los resultados se discuten para su posible aplicación en la acuacultura.

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