

## Combined effect of concentrations of algal food (*Chlorella vulgaris*) and salt (sodium chloride) on the population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera)

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**Abstract:** Salinity is an important variable influencing the density and diversity of rotifers. Studies on salt tolerance of rotifers have so far concentrated on euryhaline species while very little information is available on non-euryhaline taxa. In the present work, we have evaluated the combined effects of *Chlorella vulgaris* and sodium chloride on the population growth of two freshwater rotifers *B. calyciflorus* and *B. patulus*. A 24 hr acute tolerance test using NaCl revealed that *B. calyciflorus* was more resistant ( $LC_{50} = 3.75 \pm 0.04 \text{ g l}^{-1}$ ) than *B. patulus* ( $2.14 \pm 0.09 \text{ g l}^{-1}$ ). The maximal population density (mean  $\pm$  standard error) for *B. calyciflorus* in the control at  $4.5 \times 10^6 \text{ cells ml}^{-1}$  (algal level) was  $80 \pm 5 \text{ ind. ml}^{-1}$ , which was nearly a fifth of the one for *B. patulus* ( $397 \pm 7 \text{ ind. ml}^{-1}$ ) under comparable conditions. Data on population growth revealed that regardless of salt concentration, the density of *B. calyciflorus* increased with increasing food levels, while for *B. patulus*, this trend was evident only in the controls. Regardless of salt concentration and algal food level, the day of maximal population density was lower ( $4 \pm 0.5$  days) for *B. calyciflorus* than for *B. patulus* ( $11 \pm 1$  day). The highest rates of population increase ( $r$  values) for *B. calyciflorus* and *B. patulus* were  $0.429 \pm 0.012$  and  $0.367 \pm 0.004$ , respectively, recorded at  $4.5 \times 10^6 \text{ cells ml}^{-1}$  of *Chlorella* in the controls. The protective role of algae in reducing the effect of salt stress was more evident in *B. calyciflorus* than *B. patulus*.

**Key words:** Rotifera, Population growth, Salt stress, *Brachionus patulus*, *Brachionus calyciflorus*.

Rotifers, in natural waters, experience extremes of variations with respect to both abiotic (e.g. temperature, salinity) and biotic factors (e.g., food availability, predation) (Nogrady *et al.* 1993). Studies on the effect of food concentration and temperature on the population growth of rotifers have been well-documented (Edmondson 1965). Among abiotic factors salinity is an important variable strongly influencing the density and diversity of freshwater zooplankton (Green 1993, Zhao *et al.* 1996). Rotifers, generally being less halotolerant, are restricted to freshwaters

(Nogrady *et al.* 1993). Of the nearly 2000 taxa of rotifers, only about 150 species are considered to be marine (Koste 1978). Various mechanisms have been suggested to explain the poor survival of freshwater rotifers to salt stress. Some of them are a) failure of osmoregulation b) energetic costs associated with osmoregulation, c) reduced swimming rate, d) possible effect of salt on algal survival, e) low hatching success or even heavy mortality of neonates, even though some reproduction may have occurred in the population and f) biochemical changes (Epp and Lewis 1984,

Yamasaki and Hirata 1985, Frolov *et al.* 1991, Oeie and Olsen 1993).

Salinity tolerance studies on rotifers have concentrated on selected euryhaline species such as *Brachionus plicatilis* and *Brachionus rotundiformis*, because of their role as starter food in mariculture for larval stages of fish and crustaceans (Snell 1986, James and Abu-Rezeq 1990). A number of freshwater rotifers such as *Brachionus calyciflorus*, *B. rubens* and *B. patulus* are also used in freshwater aquaculture or even in rearing brackishwater crustaceans (Sarma 1991). It is therefore necessary to understand the extent to which freshwater rotifers of the genus *Brachionus* can survive under different levels of salt stress.

Anthropogenic causes such as agricultural activities and natural changes such as seasonal evaporation of freshwater bodies can contribute to increase the salinity in many aquatic ecosystems (Dash *et al.* 1995, Williams *et al.* 1998). Under these circumstances, it is not known whether changes in the abundances of certain rotifer species in natural waterbodies are result of diminution of food supply or increased salinity levels or both.

The aim of the present work is to study the combined effects of food levels and salt (sodium chloride) concentrations on the population growth of two freshwater rotifer species *Brachionus calyciflorus* and *Brachionus patulus* under laboratory conditions.

## MATERIALS AND METHODS

We used two laboratory-cultured rotifer species *Brachionus calyciflorus* Pallas and *Brachionus patulus* (Müller) (synonyms: *Platyias patulus* and *Platinius patulus*), originally isolated from lake Chapultepec (Mexico City) and the waterbody Presa Santa Elena (State of Mexico), respectively. Starting with a single parthenogenetic female from each species, clonal populations were established in large aquaria (40 l) using the single-celled green alga *Chlorella vulgaris* as exclusive food and reconstituted moderately hardwater as the

medium (EPA medium, Anonymous 1985). The EPA medium was prepared by dissolving 96 mg NaHCO<sub>3</sub>, 60 mg CaSO<sub>4</sub>, 60 mg MgSO<sub>4</sub> and 4 mg KCl in one litre of distilled water. Rotifers in mass cultures were fed daily with *Chlorella* (centrifuged and resuspended in EPA medium) at a density of 1 X10<sup>6</sup> to 2 X10<sup>6</sup> cells ml and the medium was changed every alternate day. Normally the mass cultures of both the rotifers had an average density of 40 to 50 ind. ml<sup>-1</sup>; at higher densities they were harvested.

For regular feeding in rotifers mass culture tanks as well as in experiments, we used *Chlorella vulgaris*, cultured on Bold's basal medium (Borowitzka and Borowitzka 1988). Algae in log phase of their growth were harvested, centrifuged at 3 000 rpm for 5 minutes, rinsed with distilled water and resuspended in EPA medium. The stock algal density was estimated using a haemocytometer. From this stock we prepared three densities, viz. 0.5 X10<sup>6</sup>, 1.5 X10<sup>6</sup> and 4.5 X10<sup>6</sup> cells ml<sup>-1</sup> using EPA medium. Standard grade (99.9% purity) sodium chloride was used in the experiments. A stock solution (nominal concentration) of 10 g l<sup>-1</sup> was prepared using distilled water. From this stock solution, we prepared seven salinity levels (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g l<sup>-1</sup>) using EPA medium. While preparing various combinations of algal-sodium chloride mixtures, we considered the effect of mutual dilution and adjustments were made accordingly.

In order to detect the range of salt concentration that would permit population growth of rotifers for a reasonable period of time, it was necessary to conduct acute tolerance tests for a 24 h period. For both species, we used neonates obtained from hatching parthenogenetic eggs. For both, *B. calyciflorus* and *B. patulus*, we used 6 concentrations of sodium chloride (0, 1.0, 2.0, 3.0, 4.0 and 5.0 g l<sup>-1</sup>). For each concentration we maintained 3 replicates, each one with 50 individuals. In order to keep rotifers active, we added *Chlorella* at a density of 0.5 X10<sup>6</sup> cells ml<sup>-1</sup>. After 24 h of inoculation, we counted the number of rotifers alive in each replicate. The

data were used to derive median lethal concentration (LC50) using probit method (Finney 1971).

Based on the results of the acute tolerance tests, we chose five salt concentrations (0, 0.5, 1.0, 2.0 and 3.0 g l<sup>-1</sup>) for *B. calyciflorus* and four (0, 0.5, 1.0, 2.0 g l<sup>-1</sup>) for *B. patulus*. For *B. calyciflorus*, the experimental design consisted of a total of 45 (= 5 salt concentrations X 3 algal levels X 3 replicates) transparent test jars (50 ml capacity) containing 50 ml of EPA medium with one of the selected combinations of *Chlorella*-sodium chloride mixture. Into each of these test jars, we introduced a mixed population (young and non-egg bearing adults) of *B. calyciflorus* at an initial density of 5 ind. ml<sup>-1</sup> using finely drawn Pasteur pipette under a stereomicroscope at a magnification of 30 X. The test jars were maintained at 25 ± 2°C, pH 7.2 to 7.5 under continuous but diffused fluorescent illumination. Following inoculation, the density of live individuals of *B. calyciflorus* was estimated daily using total counts or 2-3 aliquots of 1 ml each. Males were rarely encountered. Following the counting, rotifers were transferred (using 50 µm mesh) to new jars containing appropriate salt concentration and algal food density. We discontinued the experiment after 14 days, the time when *B. calyciflorus* began to decline in most replicates.

The experimental design for *B. patulus* was similar to that used for *B. calyciflorus*. In this case however, we continued the experiments for 20 days after then we observed a declining trend in all test jars.

Based on the data collected, we calculated the rate of population increase (r) using the exponential growth equation:  $r = (\ln N_t - \ln N_0)/t$ , where,  $N_0$  = initial population density,

$N_t$  = density of population after time t (days) (Krebs 1985). The r was obtained, as far as possible, from a mean of 4-5 values during the exponential phase of the population growth for each rotifer species.

## RESULTS

Data on the median lethal concentration of sodium chloride for *B. calyciflorus* and *B. patulus* are presented in Table 1. *B. calyciflorus* was more resistant to salt concentrations than *B. patulus*. The differences in the LC50 values for both the species were statistically significant ( $p < 0.001$ , F-test, Table 2). Population growth curves of *B. calyciflorus* and *B. patulus* in relation to different concentrations of sodium chloride and algal food levels are presented in figures 1 and 2, respectively. Regardless of salt concentration, *B. calyciflorus* showed increased population density with increasing food levels, while for *B. patulus*, this trend was evident only in the controls. The maximal population density (mean ± standard error) for *B. calyciflorus* in the control at 4.5 X 10<sup>6</sup> cells ml<sup>-1</sup> algal level was 80 ± 5 ind. ml<sup>-1</sup>, which was nearly one-fifth of the one for *B. patulus* (397 ± 7 ind. ml<sup>-1</sup>) under comparable conditions (Fig. 3). *B. calyciflorus* reached the maximal population density in a shorter time (4 ± 0.5 days) than *B. patulus* (11 ± 1 days) regardless of salt concentration and algal food level.

The rate of population increase per day (r) (mean ± standard error) for *B. calyciflorus* varied from 0.013 ± 0.001 (at 0.5 X 10<sup>6</sup> cells ml<sup>-1</sup> of food level and 2.0 g l<sup>-1</sup> of sodium chloride) to 0.429 ± 0.028 (at 4.5 X 10<sup>6</sup> cells ml<sup>-1</sup> of food level in the control). The highest r value

TABLE 1

Data on the median lethal concentration of sodium chloride (nominal concentration, g l<sup>-1</sup>) for rotifers at 24h. Low *Chlorella* density (0.5 X 10<sup>6</sup> cells ml<sup>-1</sup>) was used in order to avoid starvation-caused mortalities.

Values represent mean ± standard error. Values were derived using probit analysis

Species	LC50, 24h (g l <sup>-1</sup> of NaCl)
<i>Brachionus calyciflorus</i>	3.75 ± 0.04
<i>B. patulus</i>	2.14 ± 0.09

TABLE 2

Analysis of variance performed on selected population variables of *B. calyciflorus* and *B. patulus* offered *Chlorella vulgaris* under different concentrations of sodium chloride. The levels of significance are \*\*\* =  $p < 0.001$ , \*\* =  $p < 0.01$ , \* =  $p < 0.05$ ; ns =  $p > 0.05$ , non-significant

Source of variation	DF	SS	MS	F
<i>Comparison of LC50</i>				
Between rotifer species	1	3.877	3.88	234.40***
Error	4	0.066	0.02	
<i>B. calyciflorus</i>				
Max. population density				
Salt concentration (A)	4	1 372.055	343.01	14.46***
Algal density (B)	2	16 060.746	8 030.37	362.82***
Interaction (A X B)	8	8 887.977	1 111.00	40.16***
Error	30	664.000	22.13	
Day of max. population density				
Salt concentration (A)	3	5.861	1.95	4.14*
Algal density (B)	2	25.722	12.86	27.24***
Interaction (A X B)	6	3.389	0.56	1.20ns
Error	24	11.333	0.47	
Rate of population increase				
Salt concentration (A)	3	0.010	0.003	2.35ns
Algal density (B)	2	0.004	0.002	1.41ns
Interaction (A X B)	6	0.664	0.110	78.12***
Error	24	0.034	0.001	
<i>B. patulus</i>				
Max. population density				
Salt concentration (A)	2	56 834.000	28 417.00	113.76***
Algal density (B)	2	49 725.250	24 862.63	99.53***
Interaction (A X B)	4	46 286.375	11 571.59	41.18***
Error	18	4 496.500	249.81	
Day of max. population density				
Salt concentration (A)	3	1 206.555	402.19	245.40***
Algal density (B)	2	20.055	10.03	6.12**
Interaction (A X B)	6	27.945	4.66	2.84***
Error	24	39.333	1.64	
Rate of population increase				
Salt concentration (A)	2	0.038	0.019	42.75***
Algal density (B)	2	0.014	0.007	15.75***
Interaction (A X B)	4	0.010	0.0025	5.62**
Error	18	0.008	0.0004	
Comparison of day of max. abundance: <i>B. calyciflorus</i> vs <i>B. patulus</i>				
Between species	1	806.681	806.681	42.13***
Error	70	1 340.194	19.15	

( $0.367 \pm 0.004$ ) for *B. patulus* was recorded at  $4.5 \times 10^6$  cells  $\text{ml}^{-1}$  of food level in the control and the lowest was ( $0.209 \pm 0.003$ ) observed at  $0.5 \times 10^6$  cells  $\text{ml}^{-1}$  of *Chlorella* at a salt concentration of  $1.0 \text{ g l}^{-1}$  (Fig. 4).

Maximal population density, day of peak population density and the rate of population increase were all significantly affected by the concentrations of sodium chloride, food level as well their interaction ( $p < 0.05$ , ANOVA) for

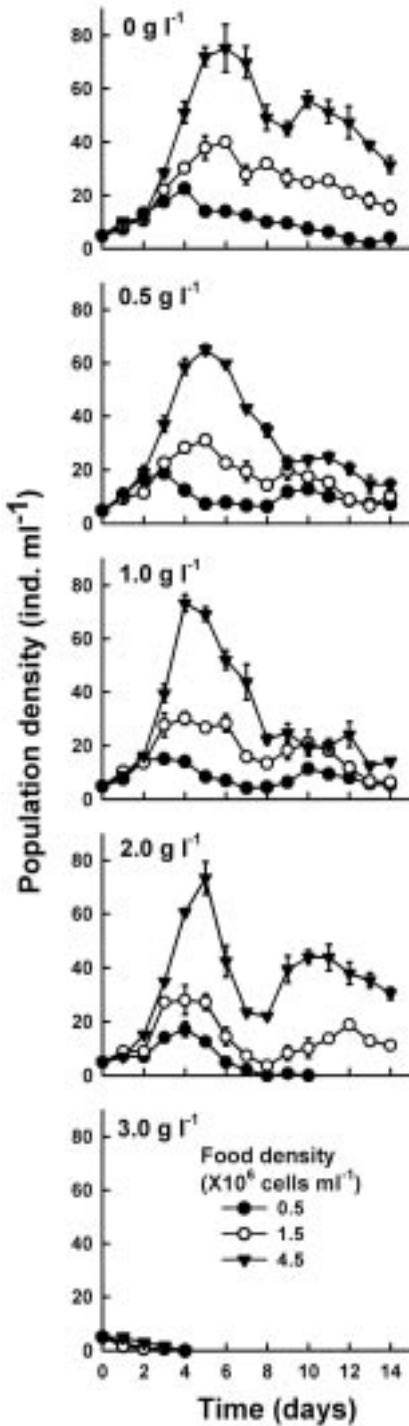


Fig. 1. Population growth of *Brachionus calyciflorus* in relation to different concentrations of sodium chloride ( $\text{g l}^{-1}$ ) under three *Chlorella* levels. Data represent the mean  $\pm$  standard error based on three replicate recordings.

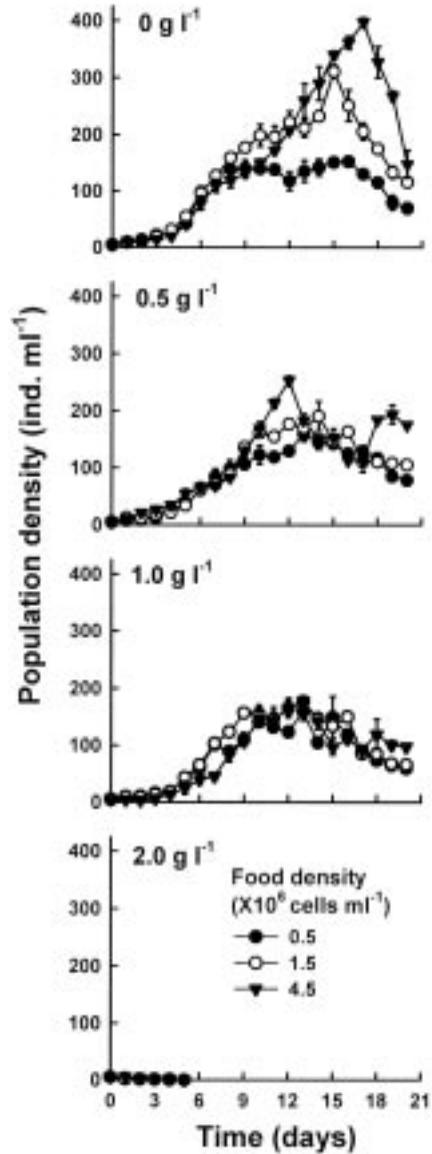


Fig. 2. Population growth of *Brachionus patulus* in relation to different concentrations of sodium chloride ( $\text{g l}^{-1}$ ) under three *Chlorella* levels. Data represent the mean  $\pm$  standard error based on three replicate recordings.

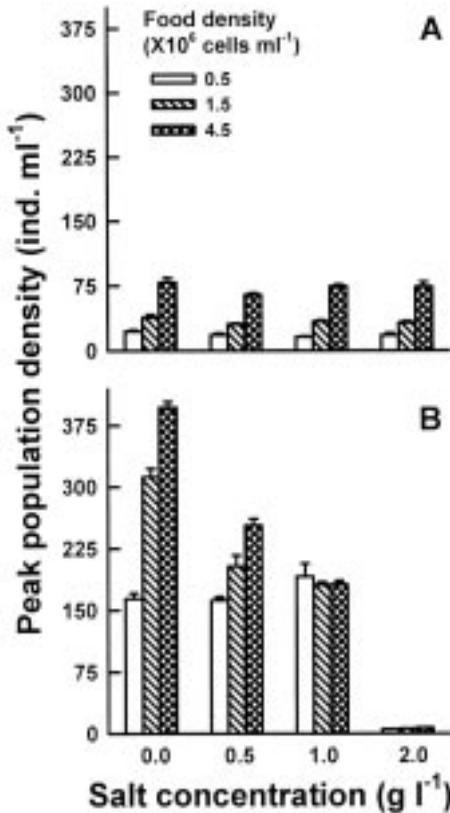


Fig. 3. Peak population densities (ind. ml<sup>-1</sup>) of *B. calyciflorus* (A) and *B. patulus* (B) in relation to different concentrations of sodium chloride (g l<sup>-1</sup>) under three *Chlorella* levels. Data represent the mean  $\pm$  standard error based on three replicate recordings.

*B. patulus*. However, for *B. calyciflorus* the interaction between salt concentration and algal food level on the day of maximal population abundance was not significant ( $p > 0.05$ ). Similarly, the rate of population increase for *B. calyciflorus* was not significantly affected by salt concentration or algal food density (Table 2).

## DISCUSSION

It is evident that both the rotifer species were negatively affected by salt concentrations of 4 g l<sup>-1</sup> as demonstrated in the acute tolerance tests, and 2 g l<sup>-1</sup> when exposed chronically. In general, our results agree with field observa-

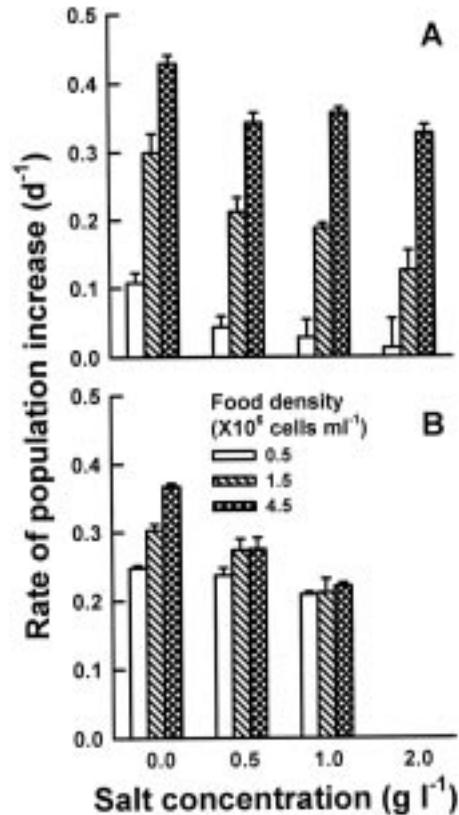


Fig. 4. Rates of population increase (d<sup>-1</sup>) of *B. calyciflorus* (A) and *B. patulus* (B) in relation to different concentrations of sodium chloride (g l<sup>-1</sup>) under three *Chlorella* levels. Data represent the mean  $\pm$  standard error based on three replicate recordings.

tions, where most members of the family Brachionidae are nearly absent when the salinity exceeds 2 g l<sup>-1</sup> (Greenwald and Hurlbert 1993). Relatively higher salt tolerance ( $> 5$  g l<sup>-1</sup>) of *B. calyciflorus* in some laboratory studies using rotifers obtained from hatching resting eggs (Snell *et al.* 1991) is probably due to strain differences or origin of the test population. Generally, neonates hatched from resting eggs are more resistant than those from parthenogenetic eggs (Sarma 2000). In our case, the rotifer population was obtained from parthenogenetic eggs.

Regardless of salt concentration and food levels, the growth curves obtained in the present study are typical for planktonic rotifers

(Walz 1995). An increase in density with increasing algal food level has been well-documented for several rotifer genera of Brachionidae e.g., *Anuraeopsis* (Dumont *et al.* 1995), *Brachionus* (Halbach and Halbach-Keup 1974), *Keratella* (Walz 1983) and *Notholca* (May 1980). In the present study, *B. calyciflorus* and *B. patulus* have also increased in population abundance with increasing availability of *Chlorella*. Nevertheless, the magnitude of rotifer increase was influenced by the concentration of sodium chloride (Figs. 1-2).

For single species studies, maximal population density, day of maximal abundance and the rate of population increase are important variables which are sensitive to changes in the medium (Sarma *et al.* 1998). Usually an increase in the available food, results in an increase in the population growth rate (Dumont *et al.* 1995). This is evident in the present work (Fig. 3). However, some factors can modify this relation such as temperature, salinity or both (Oltra and Todoli 1997). Effect of temperature and salinity on the life history variables of *B. plicatilis* has been reviewed by Miracle and Serra (1989). According to them, the intensity of salt stress has a greater influence on the fecundity of rotifers when compared to temperature variation. However, these conclusions are based on the euryhaline *B. plicatilis*, which inhabits freshwater environments and can survive even in hypersaline conditions (Walker 1981). In the present work, both rotifer species showed nearly a similar response to salinity, i.e. up to a certain level of sodium chloride (2 g l<sup>-1</sup> in case of *B. calyciflorus* and 1 g l<sup>-1</sup> for *B. patulus*); although they continue to grow but beyond this, they suddenly crashed in all the replicates.

Information published so far does not support the hypothesis that death of the algae under the range of salt concentrations used in this study could have contributed to the low population growth of the species tested (He *et al.* 1993). Reduced swimming speed of rotifers as a result of stress could probably cause reduced food intake (Lee and Macko 1981, Korstad *et al.* 1995). This is probably respon-

sible for low population growth of *B. calyciflorus* and *B. patulus* under 3 g l<sup>-1</sup> and 2 g l<sup>-1</sup> of sodium chloride, respectively. The protective role of algae in reducing the effect of salt stress was more evident in *B. calyciflorus*. Thus, if the sodium chloride had a uniform effect up to 2 g l<sup>-1</sup>, this would have caused more or less similar density of *B. calyciflorus* either at 0.5 X10<sup>6</sup> or 4.5 X10<sup>6</sup> cells ml<sup>-1</sup> of *Chlorella*. Nevertheless, under salt concentrations up to 2.0 g l<sup>-1</sup>, *B. calyciflorus* showed population growth rates dependent on food level. Conversely, due to relatively low tolerance to salt stress, *B. patulus* showed no food level-dependent population growth at 1.0 g l<sup>-1</sup>. The absence of population growth at 3.0 g l<sup>-1</sup> for *B. calyciflorus* and 2.0 g l<sup>-1</sup> for *B. patulus* even under 4.5 X10<sup>6</sup> cells ml<sup>-1</sup> of *Chlorella* suggested that food density could protect rotifers from the salt stress up to a certain limit, beyond which the effect of salinity is independent of food supply. In conclusion, our data show that both *B. calyciflorus* and *B. patulus* have a narrow range of tolerance to salt concentration and that the algal food had a protective role in reducing the effect of sodium chloride but only up to a concentration of 2.0 g l<sup>-1</sup>.

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#### RESUMEN

La salinidad es una variable importante que tiene influencia sobre la densidad y la diversidad de los rotíferos. Los estudios de rotíferos sobre tolerancia a la sal que se tienen hasta ahora se han concentrado en especies eurihalinas, sin embargo, hay muy poca información sobre taxas no eurihalinas. En el presente trabajo, se evaluaron los efectos combinados de las concentraciones de *Chlorella vulgaris* y cloruro de sodio sobre el crecimiento poblacional de dos rotíferos de agua dulce, *B. calyciflorus* y *B. patulus*. Una prueba de toxicidad aguda de 24 h utilizando cloruro de sodio reveló que *B. calyciflorus* fue más

resistente ( $CL_{50} = 3.75 \pm 0.04 \text{ g l}^{-1}$ ) que *B. patulus* ( $2.14 \pm 0.09 \text{ g l}^{-1}$ ). La máxima densidad de población (media  $\pm$  error estándar) de *B. calyciflorus* en el lote control, utilizando una concentración  $4.5 \times 10^6$  células  $\text{ml}^{-1}$  de alga fue de  $80 \pm 5 \text{ ind. ml}^{-1}$ , casi una quinta parte de *B. patulus* ( $397 \pm 7 \text{ ind. ml}^{-1}$ ) sobre condiciones comparables. Datos sobre el crecimiento poblacional revelaron que cualquier concentración de sal carece de efecto, la densidad de *B. calyciflorus* se incrementa cuando aumentan los niveles de alimento. Sin embargo para *B. patulus* esta tendencia fue evidente únicamente en los controles. Independientemente de las concentraciones de sal y los niveles de alimento, el día de abundancia máxima fue menor para *B. calyciflorus* ( $4 \pm 0.5$  días) que para *B. patulus* ( $11 \pm 1$  días). Los valores de la máxima tasa de crecimiento poblacional ( $r$ ) fueron para *B. calyciflorus* y *B. patulus* de  $0.429 \pm 0.012$  y  $0.367 \pm 0.004$ , respectivamente en lotes control con  $4.5 \times 10^6$  células  $\text{ml}^{-1}$  de *Chlorella*. El papel de protección de alga para reducir el efecto del estrés de la sal fue más evidente en *B. calyciflorus* que en *B. patulus*.

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