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Effect of salinity on competition between the rotifers *Brachionus rotundiformis* Tschugunoff and *Hexarthra jenkinae* (De Beauchamp) (Rotifera)

S.S.S. Sarma[1], Bertin Elguea-Sánchez[2] & S. Nandini[3]
[1]*Laboratory of Aquatic Zoology (UMF), Division of Research and Postgraduate Studies, National Autonomous University of Mexico, Campus Iztacala, Av. de los Barrios s/n, Los Reyes, AP 314, CP 54090, Tlalnepantla, State of Mexico, Mexico*
Fax: +52 5 623 1256 & +52 5 390 2918. E-mail: sarma@servidor.unam.mx
[2]*Colegio de Ciencias y Humanidades, Plantel No. 2, Naucalpan, Calzada de los Remedios S/N, Col. Bosque de los Remedios, C.P. 53030, Municipio de Naucalpan de Juárez Estado de México, Mexico*
[3]*CyMA Project-Aquatic Ecology, Division of Research and Postgraduate Studies, National Autonomous University of Mexico, Campus Iztacala, Av. de los Barrios s/n Los Reyes, AP 314, CP 54090, Tlalnepantla, State of Mexico, Mexico*
E-mail: nandini@servidor.unam.mx

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Abstract

We studied the effect of different concentrations (0, 3, 6, 9 and 12 g l^{-1}) of sodium chloride at one food level of *Chlorella* (1×10^6 cells ml^{-1}) on competition between the rotifers *B. rotundiformis* and *H. jenkinae*, both of which were isolated from a saline lake. The population growth experiments were conducted for 3 weeks. Both the rotifer species did not survive beyond one week at a salinity of 0 g l^{-1}. Regardless of salt concentration and the presence of a competitor, *H. jenkinae* reached higher densities than *B. rotundiformis*. When grown alone, both *B. rotundiformis* and *H. jenkinae* showed optimal peak population densities at the salinity of 6 and 9 g l^{-1}. Since biomass wise, *B. rotundiformis* was larger than *H. jenkinae*, it showed a lower numerical abundance. Thus, the maximum peak population densities of *B. rotundiformis* and *H. jenkinae* recorded in this study were 107±3 and 203±28 ind. ml^{-1}. The maximal rates of population increase for *B. rotundiformis* and *H, jenkinae* when grown alone were 0.264±0.003 and 0.274±0.004, respectively. Our results also indicated that *B. rotundiformis* and *H. jenkinae* coexisted better at a salinity of 6 and 9 g l^{-1} of sodium chloride while a salinity of 3 g l^{-1} favoured *Hexarthra* over *B. rotundiformis*. At 12 g l^{-1}, both the rotifer species grown alone or together showed lower growth rates compared to those at lower salinity levels. Except 0 g l^{-1}, in all other salinity treatments, *H. jenkinae* was a superior competitor to *B. rotundiformis*.

Introduction

Rotifera are among the few phyla, that show a higher diversity of species in freshwater as compared to marine ecosystems. Only a few species of the genera *Brachionus*, *Synchaeta* and *Hexarthra* are found in inland saline lakes (Koste, 1978). It is known that the diversity of rotifers in saline waterbodies is strongly controlled by the physical and chemical constituents of water. Of these, salinity is generally the single determining factor (Green, 1993). Rotifers in saline lakes suffer from biotic interactions of competition and predation, similar to the zooplankton in freshwater bodies (Lampert & Sommer, 1997). Competition among freshwater rotifers has been documented using species such as *Anuraeopsis fissa*, *Brachionus calyci-*

florus, *B. rubens* and *B. patulus* (Rothhaupt, 1988; Sarma et al., 1996, 1999). Although some attempts to detect the salt tolerance capacity of freshwater taxa have been made (Peredo-Alvarez et al., 2002), the effect of natural levels of salinity on the competition between co-occurring taxa of rotifers from saline waterbodies is relatively unknown (Bosque et al., 2001).

Among members of the genus *Brachionus*, *B. plicatilis* has received more attention, particularly as a first diet for fish larvae in mariculture as compared to *B. rotundiformis* (Lubzens et al., 1997), although both coexist simultaneously in many waterbodies (Sarma et al., 2000). Members of *Hexarthra* are largely adapted to freshwater conditions but *H. jenkinae* and *H. fennica* are capable of tolerating various levels of salinity (Ruttner-Kolisko, 1974). In the lake Alchichica (a natural saline waterbody in the State of Puebla, Mexico), both *B. rotundiformis* and *H. jenkinae* are frequently found. Since the food spectrum of *Hexarthra* and *Brachionus* overlaps (Nandini & Rao, 1998), competition for limited resources is probable. Also, since the biomass of *Hexarthra* is generally lower than *Brachionus* (Walz et al., 1995), in the absence of competition, the former may occur in higher numerical abundance than the latter at a given food density. However, competition between them can alter their relative abundances. It has been documented that the availability of food in terms of algal chlorophyll a in this lake is rather low throughout the year (Lugo-Vásquez, 2000). The seasonal changes in the concentration of dissolved salts can further shift the competitive outcome of rotifers (DeMott, 1989).

In this context, the aim of the present work was to study the competitive interactions between *B. rotundiformis* and *H. jenkinae* under different levels of salinity.

Materials and methods

The test rotifer species *B. rotundiformis* and *H. jenkinae* were isolated from Lake Alchichica in the State of Puebla, Mexico. Clonal cultures of these species were established using the single celled green alga *Chlorella vulgaris* as the exclusive food. *C. vulgaris* was cultured in 2l transparent bottles using Bold's basal medium (Borowitzka & Borowitzka, 1988). *B. rotundiformis* and *H. jenkinae*, were maintained in EPA medium (Anon, 1985) which was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in 1 l of distilled water. In order to maintain the salinity level close to the range observed in the field, we added NaCl 5 g l^{-1} to the EPA medium. Mass cultures (40 l volumes) were maintained at this salinity for both the rotifer species. For mass cultures or for the experiments we used resuspended *Chlorella*, which was obtained by centrifugation at 3000 rpm for 10 min. Mass cultures as well as experiments were conducted at 25 ± 2 °C, under fluorescent but diffused continuous illumination.

The experiments were conducted in 25 ml transparent jars containing 20 ml medium with specified *Chlorella* levels and sodium chloride concentrations. Based on a preliminary test, we selected 5 concentrations of salinity viz. 0, 3, 6, 9 and 12 g l^{-1} at one level of *Chlorella* (1×10^6 cells ml^{-1}) for both *B. rotundiformis* and *H. jenkinae*. The initial inoculation density of each rotifer species alone or in combination was 1 ind. ml^{-1}. For each treatment, we used 4 replicates. Thus into each test jar containing EPA medium at a known salt concentration and food level, we introduced exactly 20 individuals of either *B. rotundiformis* or *H. jenkinae* (or 10 individuals each in jars under competition) using a finely drawn Pasteur pipette under a stereomicroscope at 30×. Following introduction of the test rotifers, every day we counted the total number of living rotifers (initially whole count and later two aliquots of 1 ml each) from each test jar and transferred to new containers with appropriate salinity-algal food combinations. The growth experiments were terminated after 3 weeks by which time most replicates showed a declining trend. From the data collected, we calculated the rate of population increase (r) using the following equation (Krebs, 1985):

$$r = (\ln N_t - \ln N_0)/t,$$

where N_0 and N_t are the initial and final population densities respectively, and t is time in days. Varying data points along the growth curve were used to calculate the mean per replicate. In general, 4–6 data points were taken during the exponential phase of population growth following Dumont et al. (1995). Data on the maximum population abundance and rate of population growth were analysed using 2-way ANOVA (Sokal & Rohlf, 1993).

Results and discussion

Population growth curves of *B. rotundiformis* and *H. jenkinae* alone and in competition with each other in

Table 1. Statistical evaluation using ANOVA on the peak population density and the rate of population increase alone and in the presence of competition of *B. rotundiformis* and *H. jenkinae* under different salinity levels. DF = degrees of freedom, SS= sum of squares, MS = mean square, F = F-ratio. Levels of significant: *** = $p < 0.001$, ** = $p < 0.01$; ns = non-significant ($p > 0.05$)

Source	DF	SS	MS	F
		B. rotundiformis		
Peak population density				
Competition (A)	1	28565.96	28566.00	248.04***
Salinity (B)	3	096.83	98.94	5.69**
A × B interaction	3	174.34	91.45	3.19ns
Error	16	842.66	115.17	
Rate of population increase				
Competition (A)	1	0.153	0.153	612.00***
Salinity (B)	3	0.04	0.0133	53.33***
A × B interaction	3	0.008	0.0027	10.67***
Error	16	0.004	0.00025	
		H. jenkinae		
Peak population density				
Competition (A)	1	27202.69	27202.69	39.47***
Salinity (B)	3	13138.34	4379.45	5.96**
A × B interaction	3	4721.66	1573.89	2.14ns
Error	16	11026.66	689.17	
Rate of population increase				
Competition (A)	1	0.039	0.039	312.00***
Salinity (B)	3	0.005	0.00167	13.33***
A × B interaction	3	0.003	0.001	8.00**
Error	16	0.002	0.000125	

relation to different salt concentrations at 1×10^6 cells ml^{-1} of food density are presented in Figures 1 and 2, respectively. Both the rotifer species did not survive beyond one week at a salinity of 0 g l^{-1}. Regardless of the salt concentration and the presence of a competitor, *H. jenkinae* reached higher densities than *B. rotundiformis*. When grown alone, both *B. rotundiformis* and *H. jenkinae* showed optimal peak population densities at 6 g l^{-1}. The maximal population densities of *B. rotundiformis* and *H. jenkinae* were 107±3 and 203±28 ind. ml^{-1}, respectively in controls at the optimal salt concentration (Fig. 3). The maximal rates of population increase for *B. rotundiformis* and *H, jenkinae* when grown alone were 0.264±0.003 and 0.274±0.004, respectively (Fig. 4). The presence of competition negatively affected both the rotifer species. However, *B. rotundiformis* was more strongly affected by the presence of *H. jenkinae* than vice versa. The peak population density and the rate of population increase of *B. rotundiformis* and *H. jenkinae* were significantly affected by salinity levels and competition but the interaction of these factors was not significant (p<0.05, ANOVA) (Table 1).

While much information is available on the ecology and culturing of *B. rotundiformis* (Fielder et al., 2000), very little is known on these aspects of *H. jenkinae*. Morphological observations including the structure of trophi (with 9 uncal teeth) of *H. jenkinae* tally with the descriptions available in literature (Ruttner-Kolisko, 1974). *B. rotundiformis* is known to tolerate a wide range of salinity levels up to 40 g l^{-1} (Koste, 1978). In our study, this strain is failed to grow at a salinity of 0 g l^{-1}. *H. jenkinae* differs from *H. fennica* in their natural habitats. *H. jenkinae* occurs in inland saline lakes with a high concentration of carbonates, while *H. fennica* occurs in salt

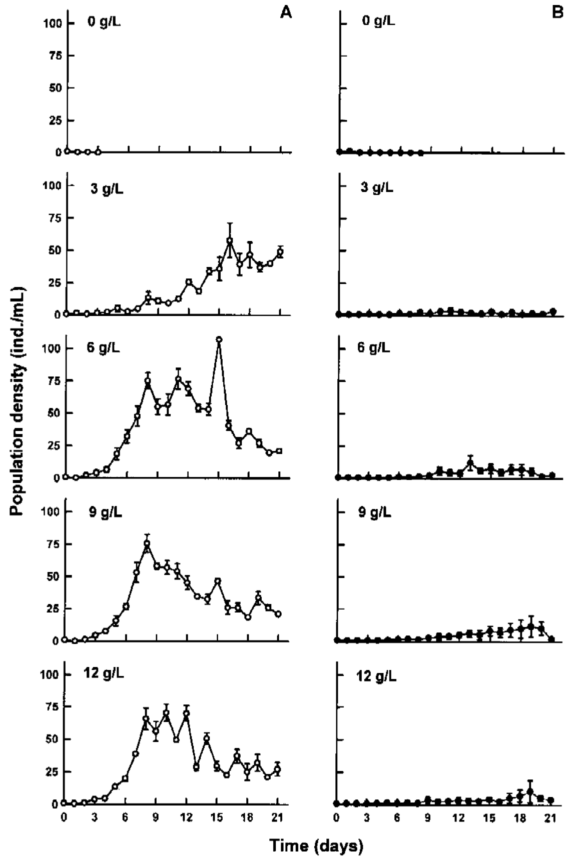

Figure 1. Population growth of *B. rotundiformis*, (grown alone; column A, or in the presence of *H. jenkinae*: column B) in relation to different concentrations of sodium chloride. Shown are mean ± standard error based on four replicates.

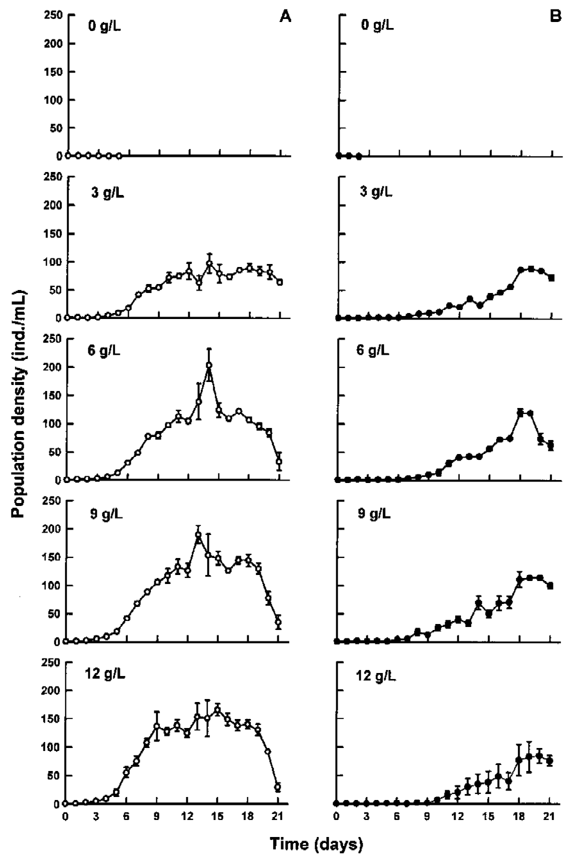

Figure 2. Population growth of *H. jenkinae*, (grown alone; column A, or in the presence of *B. rotundiformis*: column B) in relation to different concentrations of sodium chloride. Shown are mean ± standard error based on four replicates.

waters with high levels of chlorides (Ruttner-Kolisko, 1974). The origin of *H. jenkinae* in our study is also from an inland saline lake rich in carbonates and bicarbonates (Lugo-Vásquez, 2000), thus supporting Ruttner-Kolisko's (1974) field observations. However, the fact that this species could tolerate salinity in the form of chlorides must be considered while analysing zooplankton samples from brackish waterbodies because both *H. fennica* and *H. jenkinae* may simultaneously occur if the competitive exclusion does not take place.

The culture of brachionid rotifers under laboratory conditions using green alga such as *Chlorella* and *Scenedesmus* has been well documented since the mid 1960s (Pourriot, 1965). However, the long-term culture of any species of *Hexarthra* is rare (Iyer & Rao, 1996; Kak. & Rao, 1998). Studies on competitive interactions among planktonic rotifers have mostly been documented using brachionids (Sarma et al., 1999). In the planktonic samples of saline waters, *Hexarthra* and *Brachionus* are very common (Brain et al., 1995). In the Lake Alchichica too, we found both *H. jenkinae* and *B. rotundiformis* simultaneously. Compared to *Brachionus*, *Hexarthra* have rapid escaping movements and thus avoid predation (Sarma, 1993) but cannot escape totally from competition for limited food. Therefore, in the presence of *B. rotundiformis* the peak population density and the rate of population increase of *H. jenkinae* were significantly reduced. *B. rotundiformis* was also negatively affected at the same time. In general the peak population abundance and growth rates observed for the two species tested here are within the range recorded for many rotifer taxa (0.1–2.0) (Sarma et al., 2001).

The fact that *H. jenkinae* could reach higher abundances than *B. rotundiformis* in controls can be interpreted in terms of relative biomass. Numerical abundances for many zooplanktonic taxa are closely related

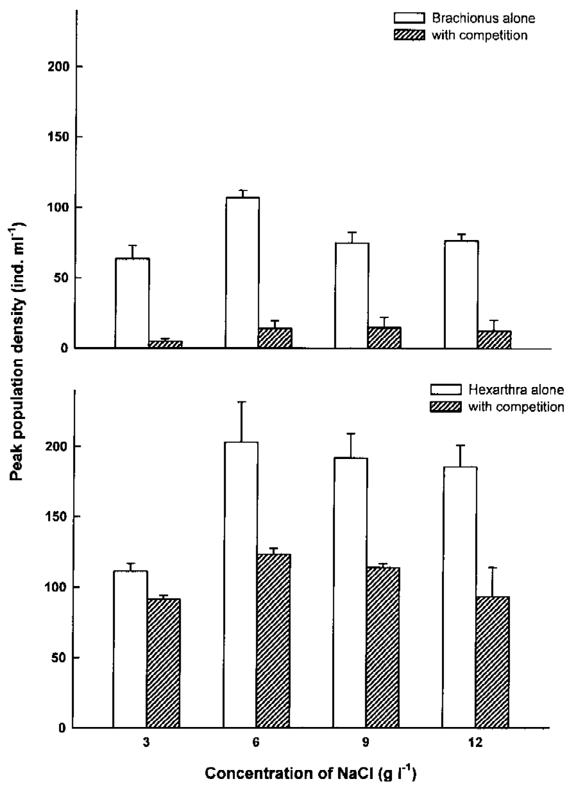

Figure 3. Peak population densities of *B. rotundiformis* and *H. jenkinae* (grown alone or in the presence of competition) in relation to different concentrations of sodium chloride. Shown are mean ± standard error based on four replicates. Data at the salinity of 0 g l^{-1} were not shown since the two rotifer species did not grow.

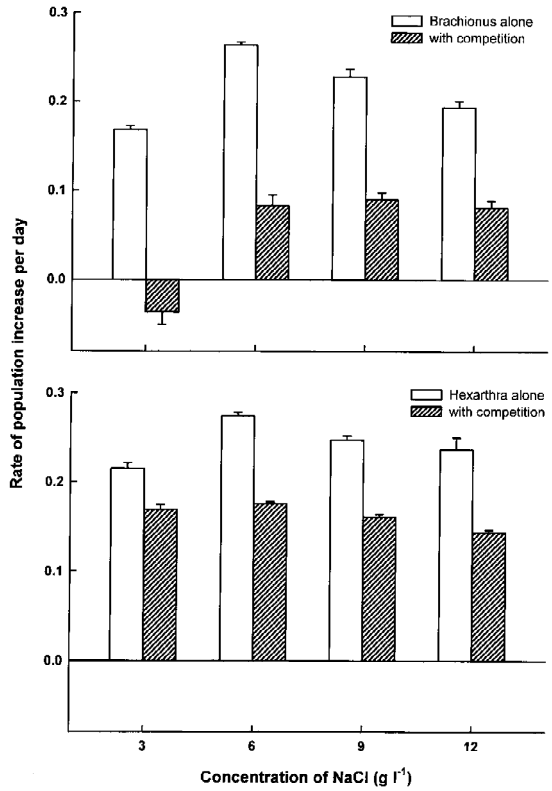

Figure 4. Data on the rate of population increase of *B. rotundiformis* and *H. jenkinae* (grown alone or in the presence of competition) in relation to different concentrations of sodium chloride. Shown are mean ± standard error based on four replicates.

to the biomass (Downing & Rigler, 1984). *B. rotundiformis* has a higher biomass than *Hexarthra* (Walz et al., 1995) and hence could occur in lower numerical abundance. Also, when both these species continue to grow under limited algal supply, *Hexarthra* dominated over *Brachionus* possibly due to its lower threshold food requirements (Lampert & Sommer, 1997). It is, however, probable that more than one factor, particularly food availability and initial inoculation density, could influence the outcome of competition (Sarma et al., 1996).

The influence of salinity on the outcome of competition between rotifers is also of considerable interest in saline water-bodies. Strong seasonal variations in salinity could favour certain species during certain times of the year (Hutchinson, 1967). From the range of salt concentration used here, both the tested rotifer species showed optimal growth at 6 and 9 g l^{-1}. These values are close to the annual mean of water salinity in the lake Alchichica (Lugo-Vásquez, 2000). At 0 g l^{-1} none of the rotifer species survived. It is quite possible that these species were well adapted to salinity higher than 3 g l^{-1}, since salinity levels lower than this were never observed in this lake (Lugo-Vásquez, 2000). Our results showed that a better coexistence of *B. rotundiformis* and *H. jenkinae* between 6 and 9 g l^{-1} of sodium chloride while a salinity of 3 g l^{-1} favoured *Hexarthra* over *B. rotundiformis*. At 12 g l^{-1}, both the rotifer species grown alone or under competition showed lower growth rates compared to those at lower salinity levels.

In conclusion, our results indicated that *H. jenkinae* is a superior competitor to *B. rotundiformis* at salinity levels of 3–12 g l^{-1} of sodium chloride and with green alga as food. *H. jenkinae* showed higher numerical abundance than *B. rotundiformis* when fed *Chlorella vulgaris* at 1×10^6 cells ml^{-1}. The paucity of planktonic rotifer species in saline lakes may be also due to intense competition leading to extremely low densities which cannot be easily detected.

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