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Research Paper

Population Growth of Six Iranian *Brachionus* Rotifer Strains in Response to Salinity and Food Type

key words: rotifer, *Brachionus plicatilis*, *Brachionus urceolaris*, algae

Abstract

Intrinsic rates of population increase (r) were evaluated as a measure of population dynamics of four strains of *Brachionus plicatilis* and two strains of *B. urceolaris* from Iran in response to different salinities and feeding algae. Each rotifer strain was cultured at four salinities: 5, 20, 25 and 30‰ and fed with two microalgal species: *Chlorella vulgaris* and *Nannochloropsis oculata*. Salinity of 5‰ was critical for all the examined strains, at which r was at minimum and was different from the other salinities ($P < 0.05$). For *B. plicatilis* strains, the maximum r was observed in those fed on *Chlorella* at salinities of 10 and 30‰ (64 ± 0.01 day^{-1}). While, in *B. urceolaris*, maximum r was for *Nannochloropsis* fed rotifers at salinity of 20‰ (0.69 ± 0.01 day^{-1}). Maximum final population density (FD) was obtained for a strain of *B. urceolaris* fed on *Nannochloropsis* at 20‰ (329.3 ± 10.9 ind.mL^{-1}). FD was relatively lower in *B. plicatilis* strains among all examined salinities. ANOVA showed the significant effect of salinity and rotifer strain, and algae × rotifer strain on both r and FD, and salinity × rotifer × algae on FD ($P < 0.05$).

1. Introduction

Rotifers are considered as ideal model organisms for various kinds of biological laboratory studies including population dynamics (Yoshinaga *et al*., 1999; Sarma, 2006; Snell and Desrosiers, 2008). They have also become a valuable and, in many cases, indispensable food organism for first feeding of a large variety of cultured marine finfish and crustacean larvae (Douillet, 2000).

Success in rotifer culture seems, to a great extent, to be dependent on providing adequate information on the life history characteristics and environmental preferences of each biotype. An increasing number of studies have been carried out on the effects of salinity, temperature and the type and concentration of food on the demographic traits and population growth of rotifers (Cabrera *et al.*, 1993; Oltra and Todoli, 1997; Fielder *et al.*, 2000; Sarma and Nandini, 2002; Athibai and Sanoamuang, 2008). These studies were accomplished using several rotifer species (Xi *et al.*, 2001; Sarma and Nandini, 2001; Suchar and Chigbu, 2006; Wullur *et al.*, 2009). However, the most intensely investigated rotifers have been

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the species and strains of the genus *Brachionus* (HAGIWARA and LEE, 1991; XI *et al*., 2001; SARMA *et al*., 2001; SARMA and NANDINI, 2001, 2002; KOSTOPOULOU and VADSTEIN, 2007; ATHIBAI and SANOAMUANG, 2008). *Brachionus* is a major contributor of biological studies on zooplankton, with 56 species described so far (FONTANETO *et al*., 2008). This genus has received greater importance in laboratory studies, because of its higher diversity, well-established culture methods, and its role as live food in aquaculture and as bioassay organism in ecotoxicological studies (SARMA and NANDINI, 2002). One of the recent concerns on the biology of rotifers is the diverse characteristics of *B. plicatilis* species complex and its strains originated from different geographic regions (GOMEZ *et al*., 2002; SUATONI *et al*., 2006). Exploring the life history characteristics and population dynamics of distinct members of *Brachionus* rotifers can help in accumulation of knowledge on their discriminative criteria from other related forms, their role in nature, their feasibility for biological studies, and the usage in aquaculture purposes.

In this study, population growth of six native strains of *Brachionus* rotifers from Iran in response to different salinities and food types were studied. This was assessed by the intrinsic rate of population increase and final population density of the rotifers.

2. Materials and Methods

2.1. Rotifer Strains

Six rotifer strains belonging to two species, *B. plicatilis* and *B. urceolaris*, were used in this experiment. Four strains of *B. plicatilis* were sampled from scattered localities in northwest Iran, and named according to the local name of their sampling sites. Two *B. urceolaris* strains were sampled from South Iran. For the identification of *B. plicatilis* strains the prevalent taxonomy of *B. plicatilis* s. l. by CIROS-PÉREZ *et al*., (2001) was followed. Characteristics of sampling sites and the experimental rotifers are shown in Table 1.

2.2. Stock Cultures

Laboratory culture of the rotifers was provided to obtain stocks of the strains; rotifers from each strain were first cultured in 6-well Plexiglas tissue-culture microplates containing their natural waters. The animals were then acclimated to the laboratory culture conditions, *i.e.,* to a water salinity of 15‰, temperature of 25 °C, pH of 7–7.5 and feeding on *Chlorella vulgaris*, and consequently, transferred to Erlenmeyer flasks to provide stock cultures for the experiments.

2.3. Experimental Design

The analyzed variables included two food types: *Chlorella vulgaris* (*Chl*) and *Nannochloropsis oculata* (*Nanno*), four salinity levels: 5, 10, 20 and 30‰, four rotifer strains of *B. plicatilis* and two strains of *B. urceolaris*. The algae were provided from our laboratory batch cultures using Walne's medium (WALNE, 1974) containing B_1 and B_{12} vitamins at salinity of 30‰ and pH 8. The experiment was conducted in 6-well Plexiglas tissue-culture microplates, with each well containing a volume of 10 mL of culture medium. Required salinities were made by diluting sterile saline water with distilled water. The microplates were kept at a constant temperature of 25 ± 2 °C under diffused and continuous fluorescent illumination (1000 lux). All treatments were initiated by inoculating an equal number of 10 amictic egg-bearing female rotifers (see LUBZENS and ZMORA (2003) for a description of amictic egg) in each well with three replicates. Cultures were kept for 10 days and renewed every two days. In each renewal time rotifers were transferred to new wells by pipetting under a stereomicroscope and the fresh medium was added. During the course of study, the rotifers were fed on one of the aforementioned algae keeping a daily concentration of 1.5×10^6 algal cell mL^{-1} of the medium.

Table 1. Rotifer strains used in this study, the characteristics of their sampling sites and their lorica length.

strain	sampling site	system type	province	position	ambient salinity (‰)	rotifer L. L. (μm)
Zbl	Zanbil	natural pond	West Azarbaijan	N 37°44′59″ E 45°14′44″	10–19	190 ± 14.1
Qo	Qoobi	wetland	West Azarbaijan	N 36°57′23″ E 45°53′11″	9.5–21	210 ± 11.9
Sht	Shatloo	seasonal pond	West Azarbaijan	N 39°36′15″ E 44°42′39″	4–19.5	192 ± 9
Segu	Seyrangul	wetland	West Azarbaijan	N 36°50′17″ E 45°34′10″	10–34	234 ± 13.6
Ti	Tiab	lagoon	Hormozgan	N 27°06′36″ E 56°49′48″	39	173 ± 7.7
Ba	Bandar Abbas	artificial pond	Hormozgan	N 27°14′06″ E 56°15′32″	37	178 ± 11.3

Zbl, Qo, Sht and Segu strains of *B. plicatilis*; Ti and Ba strains of *B. urceolaris*. Salinity ranges denote minimum and maximum values based on the seasonal measurements in the years 2007–2008.
L. L. Lorica length

On the eleventh day, by the observation of the cultures under a stereomicroscope with an overall magnification range from 25X to 40X, final population density (FD) (ind. mL^{-1}) were estimated by taking three random samples of 200 μl from each well and counting their rotifers. Intrinsic rate of population increase (r) was calculated as $r = \ln N_t - \ln N_0/t$, where $\ln N_0$ = natural logarithm of initial population density and $\ln N_t$ = natural logarithm of population density after time t (t = 10 days).

2.4. Data Analysis

Normal distribution and homogeneity of variances were assessed primarily using Kolmogorov-Smirnov test. For exploring significant differences between each two groups of the parameters, Chi-square test was employed for FD (non-continuous data) and t-test was used for r (continuous data). A three-way analysis of variance (ANOVA) was applied to evaluate the independent as well as interactive effects of salinity, algae and rotifer strain on the measured parameters. Significance level for all analyses was set at $P < 0.05$. All analyses were done using SPSS 14.0.

3. Results

Except for Ti strain, none of the examined rotifers could tolerate the salinity of 5‰ for the whole period of the experiment and their population declined to zero after 2–6 days at this salinity (data not shown). Thus, with the exception of Ti, r was null in all strains at 5‰ followed by a notable increase at 10‰. With slight differences, three of four *B. plicatilis* strains had similar trends for their r values at different salinities. However, two conspecies strains, Ti and Ba, had different r values at different salinities (Fig. 1). In most strains, r was fairly constant at 10‰ and the higher salinities. The highest r value belonged to *Nanno* fed group of Ba strain at 20‰ (0.69 ± 0.01 day^{-1}), followed by *Nanno* fed rotifers of Ti strain at 10‰ (0.68 ± 0.0 day^{-1}). In an intra-specific comparison, r was significantly different between *Chl* fed groups of Ti and Ba at all salinities except 5‰, and between *Nanno* fed groups of the strains at all salinities except 30‰. Among the strains of *B. plicatilis*, r was significantly

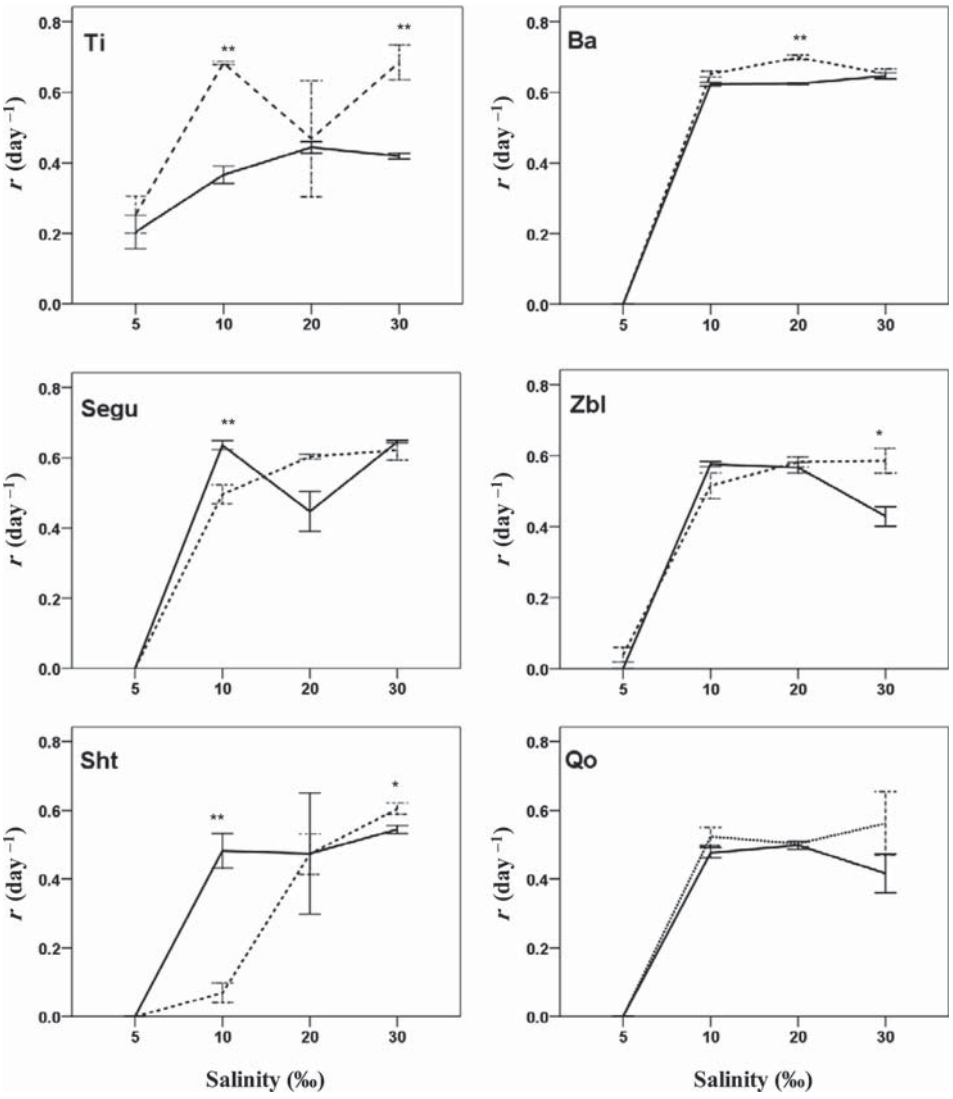

Figure 1. Mean ± standard error of the population growth rate (*r*) in two rotifer strains of *B. urceolaris*, Ti and Ba, and four strains of *B. plicatilis*, Segu, Zbl, Sht and Qo, fed with two algal species at different salinities. Dotted and solid lines are for the rotifers fed *Nannochloropsis oculata* and *Chlorella vulgaris*, respectively. At each salinity, significant differences in *r* values between rotifers fed on the two algae are shown by * ($P < 0.05$) and ** ($P < 0.01$).

different at salinities of 10 and 30‰ for *Chl* fed groups and at 10 and 20‰ for *Nanno* fed groups ($P < 0.05$) (Table 2).

With the exception of Ti strain, all the strains had null final population density (FD) at 5‰. In both feeding groups, FD increased at 10‰. However, the trend was different for the groups in some strains (Fig. 2). In *B. urceolaris* strains, *Nanno* fed animals had higher FD at all salinities tested, and the highest FD was recorded for *Nanno* fed Ba at

Table 2. *P*-values obtained from comparisons of population growth rate (*r*) and final population density (FD) within each rotifer species at different salinities and food types. Chl: *Chlorella vulgaris*; Nanno: *Nannochloropsis oculata.*

algae	salinity (‰)	rotifer	FD	*r*
Chl	5	*B. urceolaris*	0.058	0.05
		B. plicatilis	–	–
	10	*B. urceolaris*	0	0.001
		B. plicatilis	0.002	0.009
	20	*B. urceolaris*	0	0
		B. plicatilis	0.002	0.82
	30	*B. urceolaris*	0	0
		B. plicatilis	0	0.003
Nanno	5	*B. urceolaris*	0.002	0.009
		B. plicatilis	0.06	0.069
	10	*B. urceolaris*	0.033	0.035
		B. plicatilis	0.004	0
	20	*B. urceolaris*	0.003	0.297
		B. plicatilis	0.015	0.048
	30	*B. urceolaris*	0.446	0.566
		B. plicatilis	0.975	0.866

20‰ (329.3 ± 10.9 ind. mL^{-1}). The strains of *B. plicatilis* had apparently lower FD than *B. urceolaris* strains at the examined treatments. The highest FD was for *Chl* fed rotifers of Segu strain at 30‰ (252 ± 4.9 ind. mL^{-1}). There were significant differences between the FD values of Ti and Ba after feeding *Chl* at salinities of 10, 20 and 30‰ ($P < 0.05$). Whereas, when they were fed by *Nanno*, their FD differed significantly at 5, 10 and 20‰ ($P < 0.05$). The FD values of four *B. plicatilis* strains fed by *Chl* were different at all examined salinities, while, when they were fed by *Nanno*, their FD was significantly different only at 10 and 20‰ ($P < 0.05$). The *r* and FD were significantly different between *Nanno* and *Chl* fed groups of Ti, Segu and Sht strains at 10‰ ($P < 0.01$) (Figs. 1 and 2).

ANOVA indicated that rotifer strain and salinity significantly affected both *r* and FD values. In contrast, effects of salinity × rotifer on the parameters were not significant. Effects of algal type and its interaction with rotifer strain on *r* were uncertain, but their influence on the average FD was clear. Salinity × algae had rather weak and insignificant effects on FD and *r* values, respectively. The combined effects of salinity × rotifer × algae on *r* were not apparent but the effects on FD were significant (Table 3).

4. Discussion

The intrinsic rate of population increase (*r*), also called population growth rate (Sarma *et al.,* 2001; Yin and Zhao, 2008) and specific growth rate (Hotos, 2003), is a comprehensive parameter including age-specific survival and fecundity, and reproductive interval. Therefore, it is not surprising that both food type and salinity have significant influences on this parameter (Xi *et al.*, 2005). This is a practical variable which has been estimated as an indicator of rotifer population dynamics in several life table and demographic studies (Sarma and Nandini, 2001; Bosque *et al.,* 2001; Sarma and Nandini, 2002; Yin and Zhao, 2008), as well as for evaluation of production in rotifer mass culture systems (Suantika *et al.,* 2000, 2003). Theilacker and McMaster (1971) used *r* value for predicting the

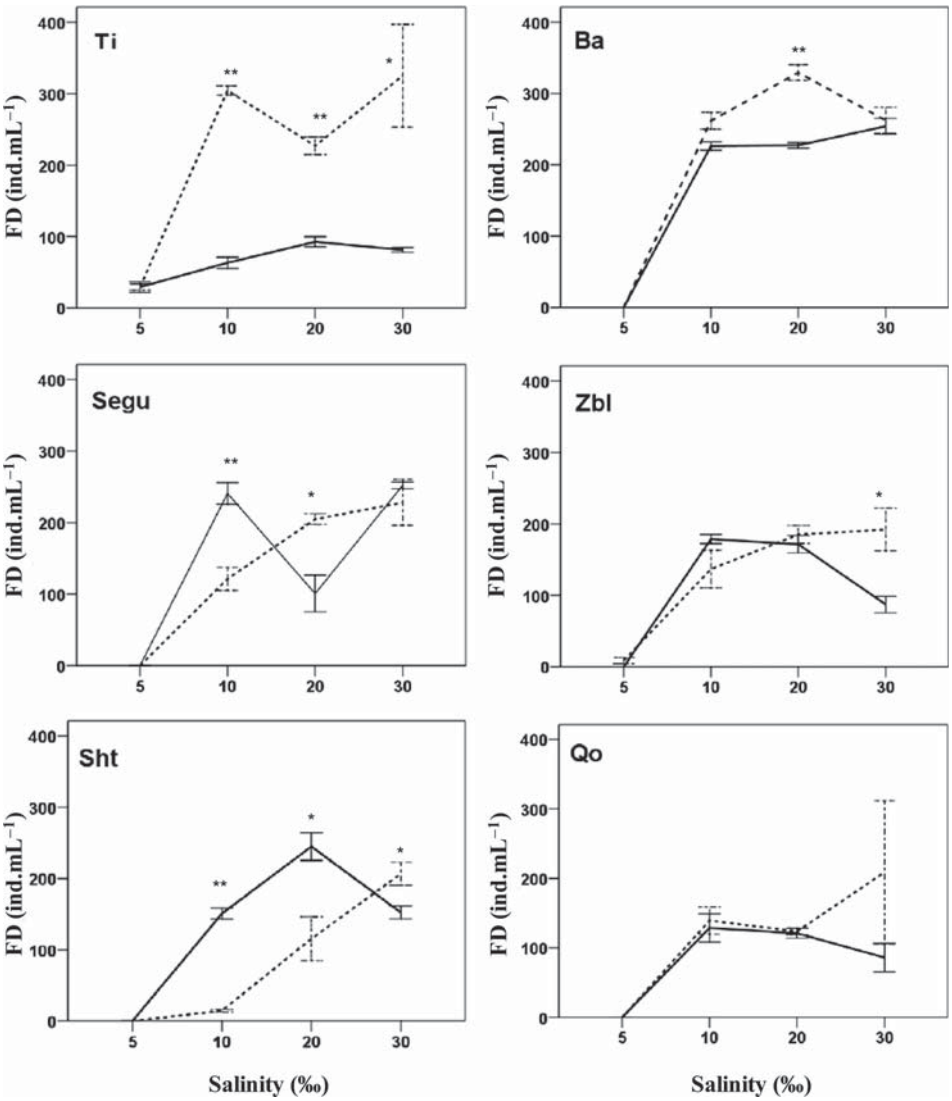

Figure 2. Mean ± standard error of the final population density (FD) of two rotifer strains of *B. urceolaris*, Ti and Ba, and four strains of *B. plicatilis*, Segu, Zbl, Sht and Qo, fed with two algal species at different salinities. Dotted and solid lines are for the rotifers fed *Nannochloropsis oculata* and *Chlorella vulgaris*, respectively. At each salinity, significant differences in FD values between rotifers fed on the two algae are shown by * ($P < 0.05$) and ** ($P < 0.01$).

doubling time of a rotifer population. In this study, r was determined as a central index of relative suitability of different cultural conditions for different rotifer strains from Iran.

It is important to know the food preferences and range of environmental factors tolerated by a species, in order to optimize culture condition for obtaining a maximum production of rotifers. Food is a major component of a rotifer culture and directly affects its reproduction and consequently, population growth rate (SRIVASTAVA *et al.*, 2006). Studies have

Table 3. Results of the three-way analysis of variance (ANOVA) for independent as well as combined effects of salinity, food type (algae) and rotifer strain on the population growth rate (r) and final population density (FD) of the examined rotifer strains.

source	FD		r	
	F	P-value	F	P-value
rotifer	23.600	0.000	14.273	0.000
salinity	76.313	0.000	142.888	0.000
algae	20.001	0.000	5.369	0.022
rotifer × salinity	1.585	0.196	0.833	0.478
rotifer × algae	21.633	0.000	5.588	0.020
salinity × algae	3.598	0.015	1.174	0.322
rotifer × salinity × algae	4.627	0.004	3.203	0.026

shown that the type of algae used as food affects rotifer production (Suchar and Chigbu, 2006). Successful rotifer cultures have been accomplished using a few number of species of microalgae as feed. The list of usable microalgal species has remained almost unaltered for many years. *C. vulgaris* and *N. oculata* are among the main algal foods commonly used in mass production of the *Brachionus* rotifers (Hotos, 2003). However, not many studies have focused on food selectivity by the rotifers (Hotos, 2002). Using different food types to grow rotifer were practiced by several researchers (Lie *et al.*, 1997; Douillet, 2000; Hotos, 2002; Matsumoto *et al.*, 2009). According to our results, effect of food type at different salinities on r and FD of the rotifers was strain-specific. Most examined rotifers showed a positive response to a shift from *Chlorella* – based food in our stock cultures to feeding with *Nannochloropsis* in the experiment. In general, using *Nanno* provided better conditions for the growth of all the strains. This might partly be because of the difference in cell size of the two algae. The two studied algae may commonly be used in rotifer culture systems interchangeably.

Salinity has been found to influence life time, the mode of reproduction and the growth rate of rotifers (Bosque *et al.*, 2001; Kostopoulou *et al.*, 2007). Salinity of 5‰ was critical for all strains, and as a general trend, all the strains tended to grow at higher salinities. The rotifer population decline at 5‰ seemed to be a result of both the lack of physiological adaptability and the induction of sexual reproduction which consequently could lead to the population deterioration. Although there were some differences in the salinity preferences of the rotifers of two feeding groups, *i.e.*, those fed either *Chl* or *Nanno*, ANOVA did not show strong effects of salinity × algae on the estimated parameters (Table 3). In some previous studies, combined effects of food × salinity on rotifer population growth were found to be significant (Oltra and Todoli, 1997; Yin and Zhao, 2008). Except for one strain, FD was higher in *Nanno* fed rotifers at higher salinities, and in most strains, it seems that the increase of salinity from 20 to 30‰ did not affect r and FD values. The highest FD for Ba and Ti strains at higher salinities is in accordance with their natural preference, as they were sampled from waters with high salinities ranged from 35 to 40‰. Some of the *B. plicatilis* strains had been sampled from the water bodies adjacent to the saline Urmia Lake. Under the influence of the lake and the alkaline lands of the region, these waters are able to reach salinities up to 20‰ in some seasons. This can explain the ability of these rotifers to grow at higher salinities. *B. plicatilis* is a common rotifer species in brackish waters, but shows strong tolerance to high salinity (Hagiwara *et al.*, 2001). Decrease in r and FD of Ti and Segu strains at 20‰ can be a result of physiological differences between the inoculated rotifers of different salinity treatments, or of an experimental artifact. Using more replicates

could increase the confidence of the results and remove the inconsistency. Notwithstanding, there is no data available on the salinity preference of *B. urceolaris* in culture conditions. This species showed considerable tolerance to wide range of salinity in our laboratory cultures. Based on our observations, it could also be concluded that *Brachionus* rotifers can better tolerate shifting to higher salinities than to lower ones, *i.e.*, 5‰. For a different genus of rotifers, it was found that salinity has a marked negative effect on the rotifer average lifespan (OLTRA and TODOLI, 1997) and fecundity of females and intrinsic growth rate (BOSQUE *et al.*, 2001).

The maximum r observed in this study ($r = 0.69 \pm 0.01$ day^{-1} for *Nanno* fed Ba at 20‰) was a quite high r value compared to the values obtained for different rotifer species in previous works (SUCHAR and CHIGBU, 2006). Most rotifers have r values less than or close to 1 day^{-1} (SARMA and NANDINI, 2002). A study by KOSTOPOULOU and VADSTEIN (2007) on two strains of *B. plicatilis*, using *N. oculata* resulted in a high r value (1.57 ± 0.07 day^{-1}), and the value had positive correlation with concentration of food offered to the rotifers. YIN and ZHAO (2008) examining six *B. plicatilis* strains fed on algal species with different equivalent spherical diameters (ESD), obtained r values less than 1 day^{-1}. They concluded that salinity had a notable effect on population increase, and that this effect was clone-specific. Feeding *B. plicatilis* with a higher cell density, *i.e.*, 4×10^6 cell mL^{-1}, of *N. oculata* resulted in r values between 0.62 and 0.82 day^{-1} (THEILACKER and MCMASTER, 1971). In the study of HOTOS (2003), feeding *B. plicatilis* by a higher density of *C. vulgaris* (2.5×10^6 cell mL^{-1}) at salinity of 30‰ led to r values ranged 0.43–0.61 day^{-1}. Using different concentrations of *C. vulgaris* resulted in relatively low r values (0.06–0.2 day^{-1}) in two *Brachionus* species (SARMA and NANDINI, 2002). For *B. urceolaris*, a high r value (1.32 day^{-1}) has been reported by SARMA *et al.* (2001). BOSQUE *et al.* (2001) provided a review of population growth rates of different rotifer species at different salinities.

Rotifer strain had significant influences on both the examined parameters ($P < 0.01$) (Table 3). This may be a result of different genetic background of these geographically distinct taxa. Our current molecular work can resolve the genetic relationships of these strains. BOSQUE *et al*. (2001) found that origin of the clones of *B. plicatilis* had an influence on their tolerance to salinity. HAGIWARA *et al*. (2001) suggested that resistance to environmental stress during mass culture varies among rotifer strains.

Population growth studies are particularly useful because rotifers may have strain-specific features in population growth potential. Such studies can provide invaluable information about the native and newly identified taxa. A significance of this experiment can be the introduction of *B. urceolaris* as a potential rotifer species for laboratory and mass cultures. It has a relatively small size (145 to 180 μm) and shows high growth rate in various cultural conditions. This potent species has, however, been addressed in only a few studies such as that of XI *et al.* (2001) and SARMA *et al.* (2001).

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