IJST (2012) A3: 245-250
Iranian Journal of Science & Technology

http://www.shirazu.ac.ir/en

Interactive effects of food and salinity on the reproductive and growth indices of two *Brachionus* rotifer strains from Iran

R. Malekzadeh-Viayeh

Artemia and Aquatic Animals Research Institute, Urmia University,
P.O. Box 165, Dr. Beheshti Street, Urmia, Iran
E-mail: malekzadeh@urmia.ac.ir

Abstract

Two Iranian strains of *Brachionus* rotifers were cultured under different food and salinity regimes. The rotifers were fed with five algal types (freshwater and marine *Chlorella vulgaris*, *Nannochloropsis occulata*, *Isochrysis galbana* and *Scenedesmus obliquus*) at three different salinities (5, 15 and 25 g/L) and their reproductive and growth parameters were assessed. The maximum number of ovigerous females (73 ± 7 ind/mL), population density (354 ± 3 ind/mL) and specific growth rate (0.75 ± 0.01 / day) were obtained for the rotifers fed with freshwater *Chlorella*. In Zbl strain, minimum growth rate (0.04 ± 0.05/ day) and population density (13 ± 4 ind/mL) were for the rotifers fed with *Isochrysis* at 25 g/L, while in Ba strain, population growth and density were null after feeding *Scenedesmus* at 5 g/L. The rotifer strains were differentially affected by salinity of medium, and Zbl and Ba strains had their maximum growth rates and population densities at 5 and 25 g/L, respectively. Analysis of variance showed significant effects of food type, salinity and their combined effects on all the estimated parameters, while rotifer strain and its interaction with food type and salinity significantly affected growth rate and population density of the rotifers ($P < 0.05$).

Keywords: Rotifer; *Brachionus*; reproduction; growth; salinity; food

1. Introduction

Rotifers of the genus *Brachionus* constitute an important group of organisms in aquatic ecosystems but also play a notable role in technological research for exploiting aquatic living resources [1]. They are also used worldwide, alone or in conjunction with other types of food, to rear early developmental stages of marine finfish and crustaceans [2]. *Brachionus* spp. are ideal as a first exogenous food source for aquaculture species due to their small size, slow swimming speed, ability to stay suspended in the water column and ease of culture [3]. The economic profitability of larval rearing in marine finfish hatcheries depends to a large extent on the continuous availability of high quality live food. In this respect, the demand for rotifers has gradually increased over the last few years [4]. On the other hand, the possibility of studying these organisms in the laboratory has promoted a significant rise in the quantity and variability of scientific papers on some species of *Brachionus* [1].

Food and salinity are among the most important factors that determine community typology in aquatic ecosystems [5]. They also influence the life history characteristics and population dynamics of rotifers [6, 7]. Food has an important role in maintaining rotifer culture stability [8]. Several food items including various algal species have been used to produce rotifer mass cultures and to improve their nutritional quality [9]. Effects of salinity on life span, reproduction and population growth of different rotifer species have been documented in previous studies [7, 10-12]. Rotifers can have optimal reproduction at any salinity within the range 4-35 g/L. However, they are generally cultured at salinities between 10 and 20 g/L [3].

Different rotifer strains originating from distinct geographic regions can have different environmental and food preferences. Thus, studying their growth performance under different conditions can provide valuable information about their biology and culture potentials. In this study the effects of different food types and medium salinity on growth and reproductive traits of two Iranian *Brachionus* rotifer strains are evaluated.

Received: 8 January 2011 / Accepted: 19 May 2012

2. Materials and methods

Two *B. plicatilis* rotifer strains used in this study were identified according to the descriptions provided by Ciros-Pérez et al. [13]. The rotifers were coded as Zbl and Ba based on the name of

their sampling sites, Zanbil (Urmia) and Bandar Abbas, located in northwest and south Iran, respectively. Geographical coordinates of the sampling sites and values of their main environmental variables are shown in Table 1.

Pure stock cultures of the rotifers were provided in 2 L glass beakers containing water with temperature of 25 ± 2°C, salinity of 15 g/L and pH of 7–8 under continuous illumination at~8000 candela. The rotifers were fed with marine *Chlorella vulgaris* at a density of 1×10^6 cell/mL.

The study was carried out using a completely randomized design in 6-well tissue culture microplates containing 5 mL culture medium. The examined treatments comprised feeding the rotifers with five algal types, freshwater and marine *Chlorella vulgaris*, *Nannochloropsis occulata*, *Isochrysis galbana* and *Scenedesmus obliquus*, at three salinities of 5, 15 and 25 g/L in triplicates. Except for freshwater *C. vulgaris*, all the algae were cultured at 25 g/L. In order to produce a sufficient amount of different algal species, algae were cultured separately in Walne medium [14] containing vitamins B1 and B12. Media of different salinities were made by diluting filtered and autoclaved saline water with distilled water. To avoid alteration of salinity of the media having lower salinities by addition of the food, the algae were concentrated by centrifugation and diluted to the assigned salinities by distilled water. Prior to the start of the experiment, subcultures from the stock rotifers were acclimated to either of the corresponding salinities and foods for two weeks. The experiment was started by transferring parthenogenetic-egg-bearing female rotifers to each well at a density of 10 rotifers/mL and ran for 12 days. The cultures were renewed every three days by precise pipetting of the animals into new plates followed by addition of fresh medium at the same salinity. The daily density of the algae offered to the rotifers was 1.5×10^6 cell/mL. At the end of the experiment, by taking three random samples of 200 µl from each well and counting their rotifers using a stereomicroscope (40X magnification) the number of ovigerous females bearing amictic eggs (OF) were counted and population density (PD) of the rotifers in each treatment was estimated as number of individuals per milliliter of the culture medium (ind/mL). Specific growth rate (SGR) was calculated as SGR= ln Nt–lnN0/t, where lnN0=natural logarithm of initial population density and lnNt = natural logarithm of population density after time t (t=12 days). The *r* was calculated for each replicate separately.

Data were analyzed using SPSS 17.0. A Kolmogorov-Smirnov test was used to check the normal distribution and homogeneity of variances. The independent and combined effects of salinity, food and rotifer strain on the estimated parameters were evaluated using a factorial design for a univariate ANOVA.

3. Results

Estimated values of the parameters (mean ± SE) for the studied rotifers are shown in Table 2. Maximum OF (73 ± 7 ind/mL) was observed in Zbl strain fed with freshwater *Chlorella* at 5 g/mL. However, there was no significant difference in the number of ovigerous females of Zbl strain between salinities of 5 and 25 g/L ($P > 0.05$). In Zbl strain, minimum OF (1 ± 0 ind/mL) was obtained in the rotifers fed with *Isochrysis* at 25 g/L, while in Ba strain OF was null in the rotifers fed with *Scenedesmus* at 5 g/mL. Salinity did not produce differences in the number of ovigerous females of the Zbl strain fed with marine *Chlorella*, *Nannochloropsis* and *Isochrysis* and of the Ba strain fed with *Scenedesmus*.

Minimum and maximum SGR was zero and 0.75 ± 0.01 /day for Ba fed with *Scenedesmus* at 5 g/L and fed with freshwater *Chlorella* at 25 g/L, respectively. The SGR increased up to 0.77 / day in Ba fed with freshwater *Chlorella* at 25 g/L.

Minimum and maximum PD were zero and 354 ± 3 ind/mL for Ba strain fed with *Scenedesmus* at 5 g/L and with freshwater *Chlorella* at 25 g/L, respectively. The PD reached 473 ind/mL in Ba strain fed with freshwater *Chlorella* at 25 g/L. Significant differences among different food and salinity treatments of each rotifer strain ($P < 0.05$) are shown by different alphabetic letters in Table 2.

Univariate ANOVA revealed significant influence of food type, salinity and their combined effects on all the estimated parameters, while rotifer strain and its interaction with food type and salinity significantly affected growth rate and population density of the rotifers ($P < 0.05$) (Table 3).

4. Discussion

Quantification of changes in the population numbers of zooplankton offered different diets is one of the common approaches to evaluate the diet effect on the zooplankton growth [15]. Food type can affect growth rate of rotifers [16]. In this study, influence of algae type on reproduction and growth of the rotifer strains was notable and the results attested to the positive response of the rotifers to feeding by freshwater *Chlorella*. However, Suchar and Chigbu [2] suggested that no alga species seems suitable for culturing all rotifer species, as the feeding habits of rotifers are diverse and the structure and size of their corona, mastax, and mouth determine the limits of the food they can ingest. Besides, results from study on one or two strains cannot be generalized for others because members of the same genus rarely show similar

characteristics even if cultured under similar algal type [17].

Table 1. Geographical position of the rotifers sampling sites and values of environmental parameters

Rotifer strain	Sampling site	Coordinates	Temperature (ºC)	Salinity (g/L)	pH	DO (mg/L)
Zbl	Urmia	N 37°44′59″ E 45°14′44″	12–26	10–19	8.2–8.5	2.18–11.9
Ba	Bandar Abbas	N 27°06′36″ E 56°49′48″	24–35	40–42	8-8.2	3– 5

Data ranges denote minimum and maximum values based on periodic measurements in the years 2007–2008; DO= dissolved oxygen

Table 2. Mean± standard error (SE) of the estimated parameters of two *B. plicatilis* rotifer strains fed different algae at different salinities

Algae	Salinity (g/L)	OF (ind/mL)	SGR (/day)	PD (ind/mL)
		Zbl strain		
Chlorella -fw	5	73 ± 7^{a}	0.7 ± 0.03^{a}	332 ± 46^{a}
	15	33 ± 5^{b}	0.5 ± 0.04^{c}	104 ± 21^{c}
	25	65 ± 8^{a}	0.6 ± 0.03^{b}	194 ± 32^{b}
Chlorella -m	5	3 ± 1^{ef}	0.20 ± 0.05^{f}	29 ± 7^{f}
	15	3 ± 1^{ef}	0.16 ± 0.02^{g}	23 ± 2^{g}
	25	3 ± 2^{ef}	0.25 ± 0.02^{ef}	36 ± 4^{de}
Nannochloropsis	5	10 ± 1^{d}	0.22 ± 0.02^{f}	31 ± 3^{ef}
	15	10 ± 3^{d}	0.22 ± 0.05^{f}	32 ± 3^{ef}
	25	13 ± 1^{c}	0.27 ± 0.01^{e}	38 ± 2^{d}
Isochrysis	5	2 ± 0.3^{f}	0.07 ± 0.05^{g}	15 ± 4^{h}
	15	3 ± 1^{ef}	0.10 ± 0.02^{f}	17 ± 2^{h}
	25	1 ± 0^{f}	0.04 ± 0.05^{g}	13 ± 4^{h}
Scenedesmus	5	15 ± 1^{c}	0.49 ± 0.01^{d}	119 ± 5^{b}
	15	2 ± 0^{f}	0.47 ± 0.01^{d}	105 ± 7^{c}
	25	5 ± 2^{e}	0.49 ± 0.02^{d}	119 ± 13^{b}
		Ba strain		
Chlorella -fw	5	19 ± 2^{b}	0.42 ± 0.02^{d}	54 ± 7^{ef}
	15	12 ± 1^{c}	0.7 ± 0.01^{a}	325 ± 11^{a}
	25	58 ± 3^{a}	0.75 ± 0.01^{a}	354 ± 3^{a}
Chlorella- m	5	2 ± 0.6^{ef}	0.38 ± 0.02^{ed}	68 ± 7^{e}
	15	8 ± 2^{d}	0.55 ± 0.02^{b}	160 ± 15^{b}
	25	2 ± 1^{ef}	0.51 ± 0.02^{c}	132 ± 10^{c}
Nannochloropsis	5	20 ± 1^{b}	0.34 ± 0.01^{e}	54 ± 3^{ef}
	15	8 ± 4^{d}	0.17 ± 0.08^{g}	24 ± 12^{h}
	25	15 ± 2^{c}	0.25 ± 0.01^{f}	35 ± 3^{g}
Isochrysis	5	4 ± 2^{e}	0.32 ± 0.06^{e}	55 ± 16^{ef}
	15	1 ± 0.3^{f}	0.32 ± 0.05^{e}	52 ± 6^{f}
	25	1 ± 0^{f}	0.17 ± 0.04^{g}	24 ± 5^{h}
Scenedesmus	5	0^{f}	0^{h}	0^{i}
	15	2 ± 1^{ef}	0.35 ± 0.05^{e}	61 ± 1^{ef}
	25	2 ± 0^{ef}	0.46 ± 0.02^{d}	99 ± 1^{d}

For each rotifer strain, data having different alphabetic letters are statistically different ($P<0.05$) OF= ovigerous females; PD= population density of the rotifers; SGR=specific growth rate; m=marine; fw= freshwater

Table 3. Results of univariate ANOVA for the independent and combined effects of rotifer strain, food type, algae, and salinity on the estimated parameters

Source	Parameter	*df*	*F*	*P*
algae	OF	3	64.006	0.000
	SGR	3	70.668	0.000
	PD	3	44.062	0.000
salinity	OF	2	4.734	0.013
	SGR	2	8.901	0.001
	PD	2	4.445	0.017
rotifer strain	OF	1	0.135	0.715
	SGR	1	22.879	0.000
	PD	1	23.347	0.000
algae × salinity	OF	6	5.340	0.000
	SGR	6	10.522	0.000
	PD	6	11.180	0.000
algae × rotifer	OF	3	10.999	0.000
	SGR	3	91.978	0.000
	PD	3	60.884	0.000
salinity × rotifer	OF	2	0.459	0.635
	SGR	2	9.901	0.000
	PD	2	5.104	0.010
algae × salinity × rotifer	OF	6	8.219	0.000
	SGR	6	11.471	0.000
	PD	6	10.083	0.000

OF= ovigerous females; PD= population density of the rotifers; SGR=specific growth rate

Number of eggs produced by a female rotifer is one of the indices of rotifer conditions [11]. Similarly, the ratio of ovigerous females to the total number of the rotifers can be applied for characterization of the conditions of a culture medium. In suitable conditions, reproduction rate and consequently, number of egg-bearing females increases. In both the studied rotifer strains maximum number of ovigerous females were observed in those fed with freshwater *Chlorella*, but at different salinities. This suggests that strains with different environmental preferences could favor similar food items.

Salinity has variable effects on productivity of different rotifer strains [3]. Zbl and Ba strains had their maximum densities at 5 and 25 g/L, respectively. In general, *B. plicatilis* strains are known to prefer lower salinities and their optimum reproduction occurs below 35 g/L [6]. Oltra and Todoli [10] reported that although the euryhaline *B. plicatilis* is able to tolerate a wide range of salinities, it grows more quickly in moderate salinities from 10 to 20 g/L. Kostopoulou et al. [12] found that at the lower salinities, the number of rotifers having parthenogenetic eggs increased substantially. Increase in salinity decreases reproduction rate and shortens life span of rotifers [7, 10]. Increased reproductive and growth rates of Ba strain at higher salinity can be attributed to its long-term adaptation to higher salinities of its natural habitat. Water salinity in such habitat may rise up to 45 g/L in summer. Further, in laboratory cultures, this rotifer strain showed higher growth rates at higher salinities.

Specific growth rate is a comprehensive parameter which can provide valuable insight into the suitability of the ambient conditions for the rotifers [18]. This is yet another important variable derived from the population growth studies [17]. For both rotifer strains, the maximum growth rates were obtained after feeding freshwater *Chlorella*. The rates were maximal for Zbl and Ba strains at 5 and 25 g/L, respectively. The growth rate values obtained in this study are comparable with those estimated for the other rotifer species [2] and indicate the suitability of these newly introduced strains to be grown in laboratory and mass producing plants. Most rotifers have specific growth rates less than or close to 1/day [17]. Bosque et al. [7] reviewed population growth rates of different rotifer species obtained at different salinities. A range of growth rates from 0.06 to 1.57/ day have been estimated for rotifer species fed with different microalgal types and densities [17, 19-23].

In the studies of the effects of algae on rotifer growth, in addition to the algal species, influence of the parameters including algal cell concentration

[18], cell size of the algae and the filtration rate of the rotifers [1, 21] have been considered. Hotos [24] studied the effects of algal cell volume on the rotifer feeding rate and concluded that rotifer grazing on the algae were a function of algal size. The species of algae used in this study have almost similar sizes, thus, their size could not be determinative for their preference. The acceptability and the efficiency of different algal species for rotifers may be influenced by some other factors such as age and starvation, which are not adequately evaluated [21].

Various morphotypes of *Brachionus* rotifers have been explored worldwide [25, 26]. These morphotypes can show different life characteristics in different environmental conditions [7]. For instance, Miracle and Serra [5] proposed that the salinity at which rotifers have their maximum growth is related to their genotype. Thus, characterizing endemic rotifer biotypes such as those investigated in this study can be of great importance to our understanding of their biological roles and cultural potentials.

Acknowledgment

This study was funded by Artemia and Aquatic Animals Research Institute of Urmia University and was performed as part of a research project (No. 005/A/86).

References

[1] Yúfera, M. (2001). Studies on *Brachionus* (Rotifera): an example of interaction between fundamental and applied research. *Hydrobiologia*, *446/447,* 383-392.

[2] Suchar, V. A. & Chigbu, P. (2006). The effects of algae species and densities on the population growth of the marine rotifer, *Colurella dicentra*. *Experimental Marine Biology and Ecology, 337*, 96-102.

[3] Fielder, D. S., Purser, J. & Battaglene, S. C. (2000). Effect of rapid changes in temperature and salinity on availability of the rotifers *Brachionus rotundiformis* and *Brachionus plicatilis*. *Aquaculture, 189*, 85-99.

[4] Suantika, G., Dhert, P., Sweetman, E., O'Brien, E. & Sorgeloos, P. (2003). Technical and economical feasibility of a rotifer recirculation system. *Aquaculture*, *227,* 173-189.

[5] Miracle, M. & Serra, M. (1989). Salinity and temperature influence in rotifer life history characteristics. *Hydrobiologia*, *186/187,* 81-102.

[6] Shiri Harzevili, A. R., Duffel, H. V., Defoort, T., Dhert, P. H., Sorgeloos, P. & Swings, J. (1997). The influence of a selected bacterial strain *Vibrio anguillarum* TR 27 on the growth rate of rotifer in different culture conditions. *Aquaculture International, 5,* 183-188.

[7] Bosque, T., Hernańdez, R., Pérez, R., Todolì, R. & Oltra, R. (2001). Effects of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. *Journal of Experimental Marine Biology and Ecology*, *258,* 55-64.

[8] de Arauju, A. B. & Hagiwara, A. (2001). Application of enzyme activity test for the diagnosis of rotifer mass culture. *Bulletin of Faculty of Fisheries of Nagasaki University, 82,* 85-91.

[9] Srivastava, A., Hamre, K., Stoss, J., Chakrabarti, R. & Tonheim, S. K. (2006). Protein content and amino acid composition of the live feed rotifer (*Brachionus plicatilis*): With emphasis on the water soluble fraction. *Aquaculture, 254,* 534-543.

[10] Oltra, R. & Todoli, R. (1997). Effects of temperature, salinity and food level on the life history traits of the marine rotifer *Synchaeta cecilia valenrina,* n. subsp. *Journal of Plankton Research, 19*(*6*), 93-702.

[11] Hagiwara, A., Gallardo, W. G., Assavaaree, M., Kotani T. & de Araujo, A. B. (2001). Live food production in Japan: recent progress and future aspects. *Aquaculture, 200,* 111-127.

[12] Kostopoulou, V., Miliou, H., Krontira, Y. & Verriopoulos, G. (2007). Mixis in rotifers of the lineage 'Nevada', belonging to the *Brachionus plicatilis* species complex, under different feeding regimes. *Aquaculture Research, 38,* 1093-1105.

[13] Ciros-Pérez, J., Gómez, A. & Serra, M. (2001). On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. *Journal of Plankton Research, 23,* 1311-1328.

[14] Walne, P. R. (1974). *Culture of Bivalve Molluscs*. 50 Years Experience at Conway. Fishing News (Books), *UK*: *West Byfleet, Surrey*,173 pp.

[15] Peñta-Aguado, F., Nandini, S. & Sarma, S. S. S. (2005). Differences in population growth of rotifers and cladocerans raised on algal diets supplemented with yeast. *Limnologica, 35,* 298-303.

[16] Douillet, P. A. (2000). Bacterial additives that consistently enhance rotifer growth under synxenic culture conditions: 1. Evaluation of commercial products and pure isolates. *Aquaculture, 182,* 241-248.

[17] Sarma, S. S. S. & Nandini, S. (2002). Comparative Life Table Demography and Population Growth of *Brachionus macracanthus* DADAY, 1905 and *Platyias quadricornis* Ehrenberg, 1832 (Rotifera, Brachionidae) in Relation to Algal (*Chlorella vulgaris*) Food Density. *Acta Hydrochimica Hydrobiologica, 30,* 128-140.

[18] Xi, Y. L., Hagiwara, A. & Sakakura, Y. (2005). Combined Effects of Food Level and Temperature on Life Table Demography of *Moina macrocopa* STRAUS (Cladocera). *International Review of Hydrobiology, 90,* 546-554.

[19] Theilacker, G. H. & McMaster, M. F. (1971). Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as food for larval anchovies. *Marine Biology, 10,* 183-188.

[20] Sarma, S. S. S., Jurado, P. S. L. & Nandini, S. (2001). Effect of three food types on the population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera: Brachionidae). *Revista de Biologia Tropical, 49,* 77-84.

[21] Hotos, G. N. (2003). Growth, filtration and ingestion rate of the rotifer *Brachionus plicatilis* fed with large (*Asteromonas gracilis*) and small (*Chlorella* sp.)

Celled algal species. *Aquaculture Research, 34,* 793-802.
[22] Kostopoulou, V**.** & Vadstein, O. (2007). Growth performance of the rotifers *Brachionus plicatilis*, B. 'Nevada'and B.'Cayman' under different food concentrations. *Aquaculture, 273,* 449-458.
[23] Yin, X. W. & Zhao, W. (2008). Studies on life history characteristics of *Brachionus plicatilis* O. F. Müller (Rotifera) in relation to temperature, salinity and food algae. *Aquatic Ecology, 42,* 165-176.
[24] Hotos, G. N. (2002). Selectivity of the rotifer *Brachionus plicatilis* fed mixtures of algal species with various cell volumes and cell densities. *Aquaculture, 33,* 949-574.
[25] Gómez, A., Serra, M., Carvalho, G. R. & Lunt, D. H. (2002). Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution, 56,* 1431-1444.
[26] Hagiwara, A., Suga, K., Akazawa, A., Kotani, T. & Sakakura, Y. (2007). Development of rotifer strains with useful traits for rearing fish larvae. *Aquaculture, 268,* 44-52.