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Studies on life history characteristics of *Brachionus plicatilis* O. F. Müller (Rotifera) in relation to temperature, salinity and food algae

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Abstract Effects of temperature (18, 24, and 30°C), salinity (5–40 ppt, five intervals) and algal foods (*Synechococcus* sp., *Chlorella pyrenoidosa*, *Isochrysis zhanjiangensis*, *Dunaliella salina* and *Tetraselmis cordiformis*) on the life table demography of six geographical *Brachionus plicatilis* sensu stricto clones, which had been identified according to the prevalent taxonomy and biometric analysis of *B. plicatilis* sensu lato, were studied. The results showed that temperature, salinity and temperature × salinity significantly influenced the life history parameters. Genotype (clone) had no effect on the population growth rate but did influence the net reproductive rate, generation time and lifespan. All rotifer clones showed the expected increase in population growth rate with increasing temperature. *B. plicatilis* s. s. attained a higher population growth rate at low–medium salinities (5–20 ppt) than at high salinities (25–40 ppt). The equivalent spherical diameter (ESD) of food algae, salinity and ESD × salinity had significant effects on the life history parameters. In this case, genotype had no effect on population growth rate, net reproductive rate and generation time but did influence lifespan. The population growth rate of *B. plicatilis* s. s. evaluated against particle retention spectrum of algae at two salinities resulted in bell-shaped curves. *Dunaliella salina* with an ESD = 7.7 μm was considered to be the best food for *B. plicatilis* s. s. while *Synechococcus* appeared to be an inadequate food algae.

Keywords *Brachionus plicatilis* sensu stricto · Food algae · Population growth rate · Salinity · Temperature

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Introduction

The euryhaline rotifer *Brachionus plicatilis* is cosmopolitan, cyclically parthenogenetic organism that appears regularly and typically in salt lakes and coastal lagoons. Due to it short generation time and because it is easily cultured in the laboratory and in commercial fishery facilities, *B. plicatilis* is widely used in aquaculture as live food for larval fish, shrimp and crabs (Lubzens et al. 2001) and has been the subject of much basic research in ecology, evolution and environmental physiology (Epp and Winston 1978; Miracle and Serra 1989; Gómez et al. 2002; Lowe et al. 2005).

The reproduction of *B. plicatilis* depends on temperature, salinity, food quantity and quality and is genotype (clone) specific (Snell et al. 1983; Lubzens et al. 1985, 1989; Snell 1986; Korstad et al. 1989; Miracle and Serra 1989; Vadstein et al. 1993; Hansen et al. 1997; Hotos 2002). Temperatures

supporting the highest population growth rate vary not only between species (Rumengan and Hirayama 1990) but also between geographical strains (Lubzens et al. 1989). The effect of salinity on rates of reproduction within a given temperature range depends on genotype (Miracle and Serra 1989). For *B. plicatilis*, the optimal temperature and salinity for asexual reproduction is 20–25°C and 10–25 ppt, respectively (see Lubzens et al. 2001). The population growth rate (r) of *B. plicatilis* is also influenced by the quantity (cell concentration) and quality (particle size and nutrition content) of the food (reviewed in Lubzens 1987; Korstad et al. 1989; Vadstein et al. 1993; Hansen et al. 1997; Hotos 2002). The optimal food for *B. plicatilis* is supposed to be *Tetraselmis* (around 8 μm ESD) at a concentration of 0.2 mg C l^{-1} (Hansen et al. 1997; Ciros-Pérez et al. 2001b).

When studying organisms, biologists usually use the species designation as labels to associate ecology, physiology, behavioral aspects, among other features. Thus, any description and identification of a species that is unclear will result in deficient databases and defective information retrieval (Knowlton 1993).

Although there is a considerable body of data on the life history variables in *B. plicatilis* in relation to temperature, salinity and food algae, the status of *B. plicatilis* sensu stricto collected from field water bodies and used in various studies is probably undecided and questionable due to morphological similarity, sympatric ecological distribution and coexistence of sibling species in *B. plicatilis* sensu lato (Sudzuki 1987; Gómez and Snell 1996; Ciros-Pérez et al. 2001a; Ortells et al. 2003) as well as inconsistent taxonomic criterion. This situation can be misleading in studies aimed at understanding the ecological phenomena or lead to overestimations of the scope of niche involved in rotifer populations. To this end, we have designed a factorial approach to study the effects of temperature, salinity and algae diet on the life history characteristics of *B. plicatilis* s. s. recognized according to prevalent taxonomy of species in *B. plicatilis* s. l. with the aim of improving laboratory data bases and obtaining information for use in improving the raising of this rotifer species in aquaculture. We evaluate the effects of both temperature and food combined with salinity and their interactions on the life table demography of *B. plicatilis* s.s. In order to test whether different geographic clones yield different patterns with respect to these factors, we used six geographically different clones belonging to six water bodies located in different parts of China that greatly vary in both abiotic and biotic conditions.

Materials and methods

All six *B. plicatilis* s. s. clones used in this work were collected from salt and brackish ponds, lakes and lagoons in the areas of China during 2001–2003. The geographical locations and physical features of the sampling sites are shown in Table 1. One individual of each pond, lake or lagoon was isolated and allowed to reproduce parthenogenetically to obtain a culture of genetically identical individuals. Since their collection, the rotifers have been continuously cultured in seawater, which is supplied to the laboratory from a nearby fjord by means of a direct pipeline and subsequently sterilized by filtering through a 0.45-μm filter and diluted with distilled water to the desired concentration, under constant illumination (approximately 50 μEin m^{-2} s^{-1}) at 24 ± 0.5°C and 30 ppt. The culture medium was renewed weekly. The rotifers were incubated in a diurnal growth chamber and fed daily with the green alga *Chlorella pyrenoidosa* as the exclusive food at a density of 2×10^6 cells ml^{-1}.

The identification of *B. plicatilis* s. s. was based on the prevalent taxonomy of *B. plicatilis* s. l. (Ciros-Pérez et al. 2001a) and the biometrical analysis described below. Morphometric values were compared using rotifers of identical age. Additionally, three *B. rotundiformis* clones and one clone of *B. plicatilis* Yamamoto's type (see Sudzuki 1987), isolated from one of our study ponds (Dalian), were used for comparison. For each rotifer clone, thirty 48-h-old individuals were fixed with 5% (final concentration) formaldehyde and measured at a magnification of 600×. The morphometric characters measured in this study are shown in Yin and Zhao (2005).

Experiment 1: temperature and salinity

To test the combined effect of temperature and salinity on life table demography of *B. plicatilis* s. s., Experiment 1 was designed as follow. Prior to the experiments, the rotifers were acclimated for 2 weeks to the corresponding experimental combinations of

Table 1 Sampling sites location and the temperature and salinity of habitats at the time of the first collection of *Brachionus plicatilis* sensu stricto

Clone	Location (China)	Sampling sites	Temperature (°C)	Salinity (ppt)
DL	Dalian	38°53′ N; 121°12′ E	27.5	4.0
LN	Panjin	40°27′ N; 121°56′ E	21.5	14.5
TJ	Tianjin	37°15′ N; 116°35′ E	19.0	34.0
SD	Dongying	35°42′ N; 117°44′ E	28.0	12.0
NM	Sanggendalai	39°48′ N; 105°38′ E	9.5	14.0
XZ	Qiangbei	32°09′ N; 74°19′ E	7.5	21.0

temperatures (18, 24 and 30°C) and salinities (5, 10, 15, 20, 25, 30, 35 and 40 ppt), giving a total of 144 (= three temperatures × eight salinities × six clones) beakers (50-ml capacity) containing 30 ml medium. The rotifers were fed daily with *C. pyrenoidosa* at a density of 2×10^6 cells ml^{-1}, and the culture medium was renewed every 3 days. Because no individuals of the LN, TJ and NM clones survived at the 18°C−40 ppt combination during the acclimation period, these three treatments were eliminated from the experimental designs.

Following acclimation, 12 newly hatched rotifers (age: <6 h) were randomly caught from each beaker and separately piped into 96-well culture plates, with each well containing 0.2 ml culture medium with 2×10^6 cells ml^{-1} *C. pyrenoidosa*, for treatment incubation. The experimental designs consisted of a total 141 [= (three temperatures × eight salinities × six clones) − the three eliminated combinations] culture plates. Experimental individuals were transferred to fresh medium daily, and each rotifer was monitored until death. In addition to survival, the number of neonates were recorded and used to calculate the life history parameters.

Life table demography, such as net reproduction rate (R_0), generation time (T) and population growth rate (r), were calculated following the formulae described in Krebs (1994). The mean and variance of population growth rates were based on 500 Jackknife samples of individual survivorship and fecundity schedules (Meyer et al. 1986).

Experiment 2: food algae and salinity

In order to establish the combined effect of food algae and salinity on life history characteristics of *B. plicatilis* s. s., Experiment 2 was designed as follows. The concentration of each food algae (Table 2) contained in the acclimation and incubation treatments was maintained at 0.128 mg C ml^{-1} (equivalent to 2×10^6 cells ml^{-1} *C. pyrenoidosa*). The particle linear dimensions of the algal cells used in the experiments were measured under a microscope at a magnification of 600× (n = 50) and, based on Hansen et al. (1997), equivalent spherical diameter (ESD) = $(vol/0.523)^{0.33}$ was worked out. We selected two levels of salinity (15 and 30 ppt) as the other experimental factor. The temperature of the incubations was 24°C.

Six *B. plicatilis* s. s. clones were acclimated for 2 weeks to the experimental combinations of algal foods (Table 2) and salinities (15 and 30 ppt), giving a total of 60 (= five algae × two salinities × six clones) beakers (50-ml capacity) containing 30 ml medium. Approximately 1 ml culture medium was taken from the beakers each day to determine the density of food

Table 2 Food algae used in the experiments, and volume (μm^3), equivalent spherical diameter (ESD; μm) and shape. The algal species were cultured at room temperature (20 ± 5°C), under natural illumination and in Conway media (Lourenço et al. 1997) (resembling seawater; diluted to 30 ppt)

Food algae	Volume	ESD	Shape
Synechococcus sp.	1.57	1.4	Bacilliform
Chlorella pyrenoidosa	13.90	3.0	Spherical
Isochrysis zhanjiangensis	90.90	5.5	Spherical
Dunaliella salina	249.75	7.7	Oval
Tetraselmis cordiformis	1297.55	13.2	Cordiform

algae. The food density in the medium was then regulated to 0.128 mg C ml^{-1}. The culture medium was renewed every 3 days. During the acclimation period, no individuals of LN and XZ clones survived at the *Synechococcus*–30 ppt combination. These two treatments were therefore removed from the experimental designs.

Following acclimation, 12 newly hatched rotifers (age: <6 h) were randomly selected and separately placed into 96-well culture plates, with each well containing 0.2 ml culture medium with the required food algae and salinity. The experimental design consisted of a total of 58 [= (five algae × two salinities × six clones) − two eliminated combination] culture plates. Each day, the surviving females together with attached eggs were transferred to fresh medium as described above, and the number of surviving females and neonates was counted. The experiment ended when all individuals died. The methods used to obtain the life table parameters are as described in Experiment 1.

Results

Biometric analysis resulted in a single cluster for the experimental clones that was consistent with the taxon *B. plicatilis* s. s. as a unique, single species (Fig. 1), in accordance with the prevalent taxonomy of the *B. plicatilis* s. l. Consequently, the status of *B. plicatilis* s. s. in our experiments was verified.

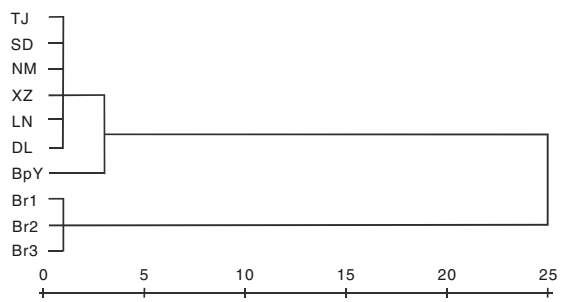

Fig. 1 Branch tree of *Brachionus plicatilis* s. s., *B. rotundiformis* and *B. plicatilis* Yamamoto's type based on biometric characters obtained using the between-groups linkage cluster method and squared Euclidean distance interval measurement. *BpY*,Yamamoto's type clone; *Br1*, *B. rotundiformis* clone 1; *Br2*, *B. rotundiformis* clone 2; *Br3*, *B. rotundiformis* clone 3

Temperature and salinity

The effects of temperature and salinity were clearly associated with the population growth rate (*r*; Fig. 2). All rotifer clones showed the expected increase in *r* with increasing temperature. All clones at 18°C started with an *r* around 0.4–0.5, which then decayed. At 24°C, all clones had an *r* ranging from 0.6 to 0.7 at the lowest values of salinity (5 ppt); this increased slightly at 10 or 15 ppt and then declined with increasing salinity. The variability of the clones cultured at low and high salinity (5 and 40 ppt) and 30°C was higher, but all clones showed a peak *r* again at 10 or 15 ppt, which then decreased as the salinity rose. According to the ANOVA tests, temperature and salinity were the main factors influencing the variability of *r*, and the genotype (clone) produced no significant effect (Table 3). The interaction of temperature × salinity and temperature × clone had a significant influence on *r*, whereas, salinity × clone showed no significant effect (Table 3).

A similar ANOVA were calculated for net reproductive rate (R_0), generation time (T) and lifespan (e). The results showed that R_0, T and e are not only temperature and salinity dependent but also genotype dependent and, moreover, temperature × salinity and temperature × clone were also significant (Table 3). Salinity × clone also had no influence on R_0, T and e.

The effects of temperature and salinity on the age-specific survivorship (l_x) and age-specific fecundity (m_x) curves of the DL clone are shown in Fig. 3 (other rotifer clones showed a similar distribution pattern with l_x, and m_x in relation to temperature and salinity). The lifespan varied with temperature and salinity and tended to shorten when the temperature increased. At identical levels of salinity, l_x curves of 18 and 24°C and 18 and 30°C were significantly different (Mann–Whitney *U*-tests, $P < 0.05$), but there were no discernible differences in the l_x curves of 24 and 30°C (Mann–Whitney *U*-tests, $P > 0.05$). Regardless of the salinity, higher survivorship was obtained at 18°C. The m_x curves were affected by temperature and modulated by salinity. At any one level of salinity, the maximum value of fecundity increased and the time to first neonate decreased with increasing temperature. The production of neonates at any one temperature was higher at low salinity than at high salinity.

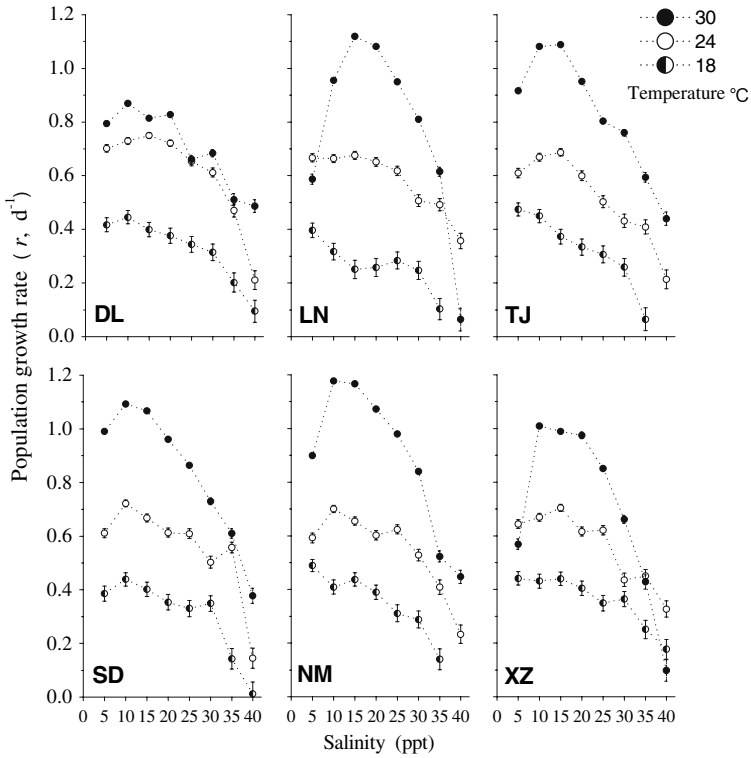

Fig. 2 Effects of temperature and salinity on population growth rate of six *B. plicatilis* s. s. clones. *Error bars* that are not apparent are smaller than the *symbols*

Food algae and salinity

The population growth rate (r) of *B. plicatilis* s. s. evaluated against the particle retention spectrum (ESD) at two salinities resulted in bell-shaped curves (Fig. 4). Almost all rotifer clones at the two salinities had the highest r with *D. salina* (ESD = 7.7 μm) as food algae. *Synechococcus* (ESD = 1.4 μm) was assessed to be the most inadequate food algae in that it resulted in the lowest population growth rate.

ANOVA tests revealed that ESD and salinity had significant effects on the population growth rate (r), net reproductive rate (R_0) and generation time (T), but that the genotype (clone) had no influence on these (Table 4). ESD × salinity and ESD × clone had significant effects on r, R_0 and T. Lifespan was significantly influenced by ESD, clone and their interaction. In contrast, salinity × clone had no influence on them.

The effects of food algae and salinity on the age-specific survivorship (l_x) and age-specific fecundity (m_x) curves of DL clone are shown in Fig. 5 (other rotifer clones showed a similar distribution pattern with l_x and m_x in relation to food algae and salinity). Lifespan varied with food algae and salinity, ranging from 12 to 18 days at 15 ppt, and from 13 to 24 days at 30 ppt. The maximum life span was obtained with *I. Zhanjiangensis*, and the minimum life span was recorded with *Synechococcus* as food. The m_x curves showed a more or less normal distribution pattern, but much lower production was observed when the rotifers were fed with *Synechococcus*.

Discussion

The results of this study reveal that temperature and salinity have a large effect on the population growth rate of *B. plicatilis* s. s. The direct effect of salinity on the population growth rate was genotype independent and, therefore, no geographical differences were found among the clones of our study, which is an interesting observation. Algae particle size and salinity have a significant effect on population growth rate, which is also genotype-independent. According to our results, *B. plicatilis* s. s. grows and reproduces better under conditions of 5–10 ppt at 18°C and 10–15 ppt at 24 or 30°C, with *D. salina* as the sole food alga.

Table 3 Results of analysis of variance (two-way ANOVA) performed for different variables obtained in the Experiment 1

Source	SS	*df*	MS	*F*	*P*
Population growth rate (r)					
Temperature	5.11	2	2.55	453.58	<0.001
Salinity	3.01	7	0.43	76.31	<0.001
Clone	0.05	5	0.01	1.76	0.13
Temperature × salinity	0.47	14	0.03	5.90	<0.001
Temperature × clone	0.30	10	0.03	5.26	<0.001
Salinity × clone	0.14	35	0.004	0.72	0.86
Error	0.38	67	0.006		
Total	54.43	141			
Net reproductive rate (R_0)					
Temperature	40.14	2	20.07	4.10	0.02
Salinity	1,210.81	7	172.97	35.36	<0.001
Clone	330.81	5	66.16	13.53	<0.001
Temperature × salinity	294.21	14	21.02	4.30	<0.001
Temperature × clone	397.41	10	39.74	8.12	<0.001
Salinity × clone	199.15	35	5.69	1.16	0.29
Error	327.75	67	4.89		
Total	22,355.87	141			
Generation time (T)					
Temperature	693.13	2	346.57	680.14	<0.001
Salinity	85.67	7	12.24	24.02	<0.001
Clone	20.54	5	4.11	8.06	<0.001
Temperature × salinity	39.25	14	2.80	5.50	<0.001
Temperature × clone	26.22	10	2.62	5.15	<0.001
Salinity × clone	16.85	35	0.48	0.95	0.56
Error	34.14	67	0.51		
Total	7,131.79	141			
Lifespan (e)					
Temperature	1,318.69	2	659.34	215.91	<0.001
Salinity	81.98	7	11.71	3.84	0.001
Clone	126.12	5	25.22	8.26	<0.001
Temperature × salinity	135.96	14	9.71	3.18	0.001
Temperature × clone	174.63	10	17.46	5.72	<0.001
Salinity × clone	102.74	35	2.94	0.96	0.54
Error	204.60	67	3.05		
Total	23,304.01	141			

SS, Sum of squares; *df*, degrees of freedom; MS, mean square; *F*, *F*-ratio; *P*, probability of *F* exceeded

Temperature, salinity and food particle size are considered to be the main factors influencing the life history characteristics in rotifers. By regulating the embryonic development time, fecundity, survivorship and time of reproduction (Herzig 1983; Lubzens et al. 1985; Snell 1986; Galkovskaja 1987; Korstad et al. 1989; Hirayama and Rumengan 1993), temperature, salinity and food algae will affect the reproductive rates of rotifers, and the responses to temperature, salinity and food algae depend on genotype (Lubzens et al. 1989; Miracle and Serra 1989). The results of the present investigation verify the assumptions that

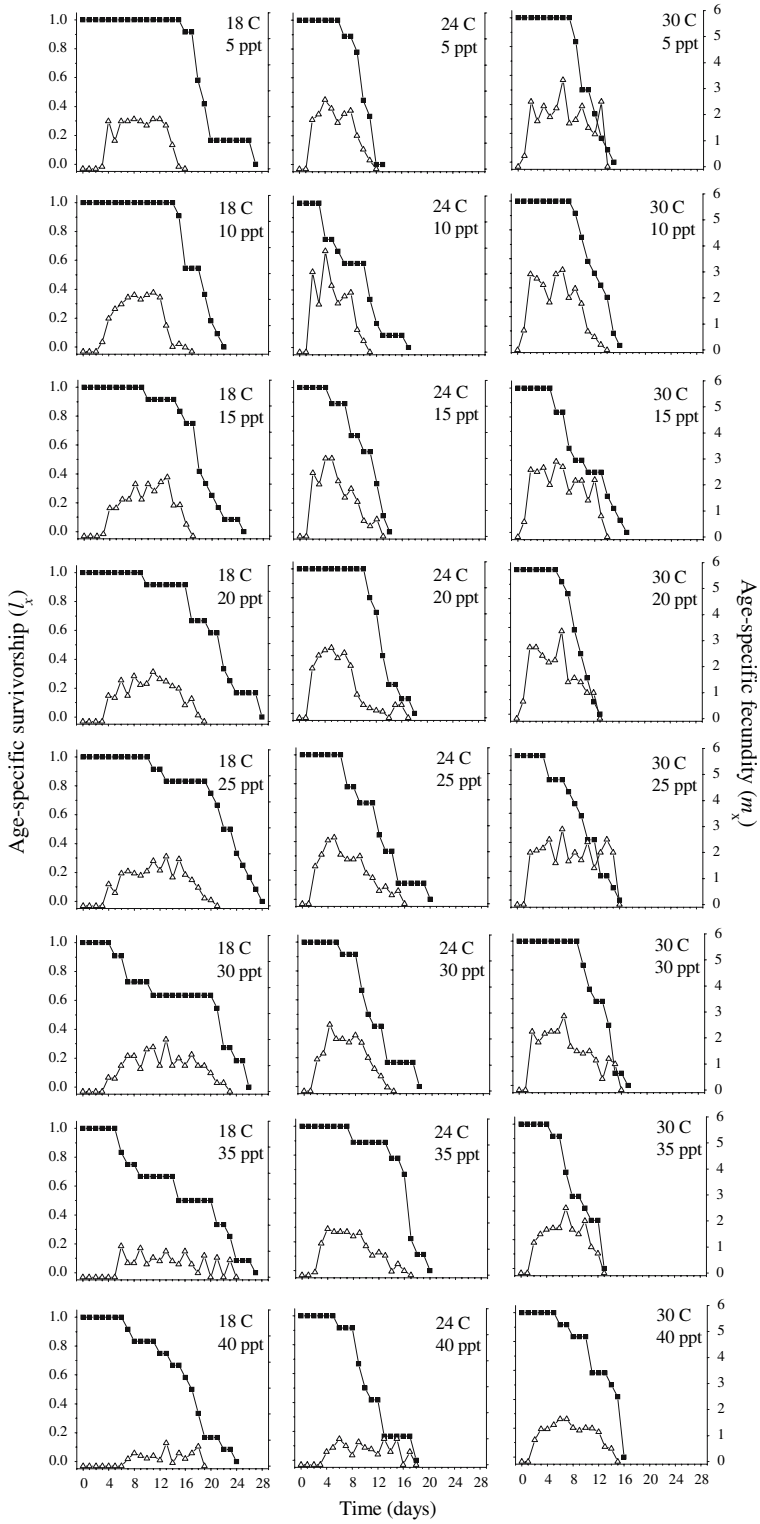

Fig. 3 Age-specific survival (*filled squares*) and age-specific fecundity (*open triangles*) for *B. plicatilis* s. s. of the Dalian clone at three temperatures and eight salinities. *C* °C

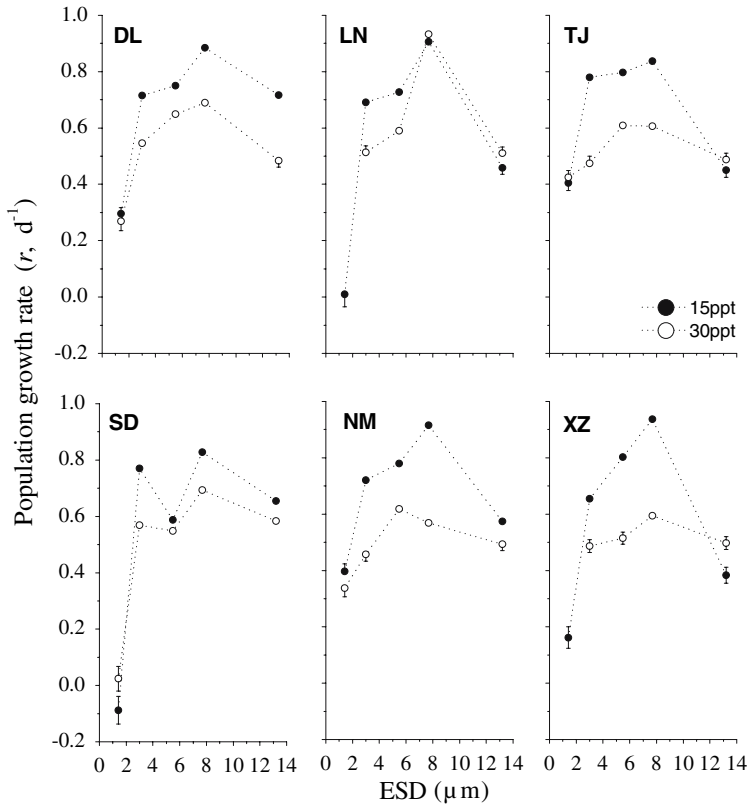

Fig. 4 Relationship between population growth rate and food particle size in six *B. plicatilis* s. s. clones. *Error bars* that are not apparent are smaller than the *symbols*

the temperature, salinity and algae particle size are the main factors influencing the reproduction rates. However, our results are inconsistent with the conventional opinion that the response patterns of ecotypes will match their habitats of origin in that we observed that genotype had no effect on the rates of reproduction (Tables 3, 4).

Compared to the rotifer clones used in Lubzens et al. (1989) and Miracle and Serra (1989), the status of *B. plicatilis* s. s. clones used in our study are better defined, which guarantees the dependability of our data. Although the biotic and abiotic features of the water bodies from which the six geographically separated *B. plicatilis* s. s. clones were first isolated vary greatly (unpublished data and Table 1), identical response patterns to the changes of temperature, salinity and algae particle size were observed with all *B. plicatilis* s. s. clones (Figs. 2, 4). This strongly suggests that it is the value of the reproduction rate – not the pattern – which varies due to characteristics of the original habitats of the genotype. The change patterns in relation to environmental factors within a rotifer species may be genotype-independent, as suggested by Rothhaupt (1990a) and Hirayama and Rumengan (1993).

Rotifers are supposed to have the ability of osmotic regulation, and they can regulate the salt concentration of the pseudocoelomic fluid through the movement of flame cells (Pontin 1966). Although *B. plicatilis* s. l. has long been considered to be an osmo-conformer (Bayly 1972; Epp and Winston 1977), a recent study by Lowe et al. (2005) confirms that *B. plicatilis* s. l. is an osmo-regulator and that the decreases in growth rate and egg production are a direct cost of osmo-regulation under conditions of high salinity. Therefore, the energy allocated to reproduction is lower at high salinities than at low and medium salinities which, in turn, results in the reduced reproduction rate of *B. plicatilis* s. s. at high salinities observed in our experiments (Fig. 2).

The particle retention spectrum for *Brachionus* ranges from 0.3 to 22.0 μm ESD (Rothhaupt 1990a, b; Vadstein et al. 1993; Hansen et al. 1997; Hotos 2002). The optimal particlefor *B. plicatilis* s. l. is supposed to be approximately 8 μm ESD, and grazing efficiency decreases as the size of the food algae

Table 4 Results of the analysis of variance (two-way ANOVA) performed for different variables obtained in Experiment 2

Source	SS	*df*	MS	*F*	*P*
Population growth rate (r)					
ESD	1.62	4	0.41	78.65	<0.001
Salinity	0.20	1	0.20	38.01	<0.001
Clone	0.07	5	0.01	2.55	0.07
ESD × salinity	0.14	4	0.04	6.90	0.002
ESD × clone	0.35	20	0.02	3.35	0.006
Salinity × clone	0.05	5	0.01	1.97	0.13
Error	0.09	18	0.01		
Total	22.15	58			
Net reproductive rate (R_0)					
ESD	1,222.63	4	305.66	56.03	<0.001
Salinity	29.37	1	29.37	5.38	0.03
Clone	41.10	5	8.22	1.51	0.24
ESD × salinity	102.24	4	25.56	4.69	0.009
ESD × clone	395.42	20	19.77	3.62	0.004
Salinity × clone	7.06	5	1.41	0.26	0.93
Error	98.19	18	5.46		
Total	11,531.33	58			
Generation time (T)					
ESD	11.76	4	2.94	4.35	0.01
Salinity	12.11	1	12.11	17.91	0.001
Clone	2.40	5	0.48	0.71	0.62
ESD × salinity	7.71	4	1.93	2.85	0.05
ESD × clone	34.11	20	1.71	2.52	0.03
Salinity × clone	1.37	5	0.27	0.40	0.84
Error	12.17	18	0.68		
Total	1,848.12	58			
Lifespan (e)					
ESD	236.48	4	59.12	21.60	<0.001
Salinity	3.956	1	3.96	1.45	0.25
Clone	80.78	5	16.16	5.90	0.002
ESD × salinity	33.15	4	8.29	3.03	0.045
ESD × clone	240.57	20	12.03	4.40	0.001
Salinity × clone	32.94	5	6.59	2.41	0.08
Error	49.26	18	2.74		
Total	9,075.90	58			

SS, Sum of squares; *df*, degrees of freedom; MS, mean square; *F*, *F*-ratio; *P*, probability of *F* exceeded

deviates from this value (Hansen et al. 1997). In the present study, almost all *B. plicatilis* s. s. clones attained the highest population growth rate (r) with *D. salina* (ESD = 7.7 µm) as food at two salinities (Fig. 4), and the r plotted against particle retention spectrum resulted in bell-shaped curves that resembled the curve embodying the relationship between grazing efficiency or relative growth rate and particle size (Rothhaupt 1990b; Hansen et al. 1997).

In addition to particle size, the size-frequency distribution of algae in culture vessels is another feature of food quality (Korstad et al. 1989). Our

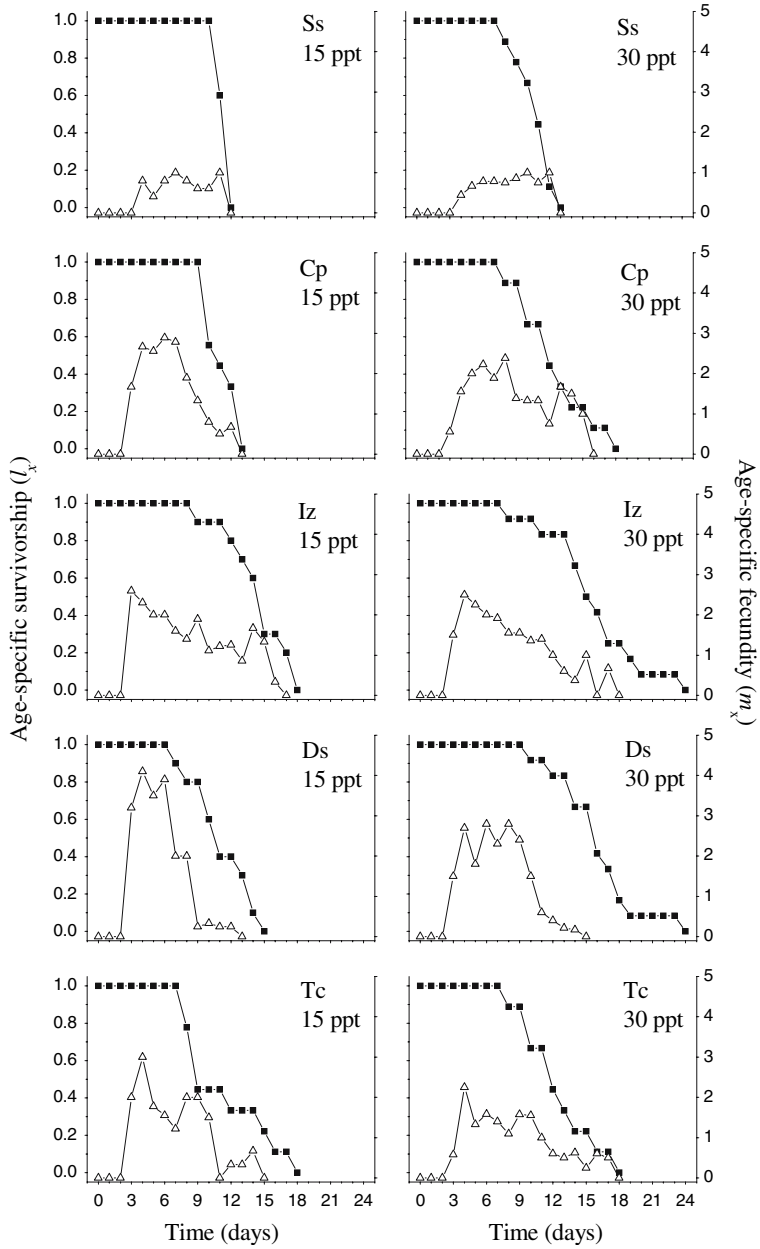

Fig. 5 Age-specific survival (*filled square*) and age-specific fecundity (*open triangle*) for *B. plicatilis* s. s. of the Dalian clone with all food algae at two salinities. *Ss*, *Synechococcus*; *Cp*, *C. pyrenoidosa*; *Iz*, *I. zhanjiangensis*; *Ds*, *D. salina*; *Tc*, *T. cordiformis*

experiments show that *D. salina* and *I. zhanjiangensis* are better food alga for *B. plicatilis* s. s. than *T. cordiformis* (Fig. 4). *Dunaliella* and *Isochrysis* are flagellated algae and can distribute evenly in culture vessels. *Tetraselmis* cells are also motile; however, they prefer to attach to the surface of the culture medium and the wall of culture plate wells (see Korstad et al. 1989). Moreover, the cell density of *Tetraselmis* used in the experiments is lower (2.2×10^4 cells ml^{-1}) than that of *Dunaliella* (11.1×10^4 cells ml^{-1}) and *Isochrysis* (28.6×10^4 cells ml^{-1}). All of these factors decrease the availability of *Tetraselmis* cells to rotifers, which accounts for the lower reproductive rates of *B. plicatilis* s. s. with *Tetraselmis* as food.

We observed that the population growth rate of *B. plicatilis* s. s. fed with blue-green algae *Synechococcus* was much lower than that of *B. plicatilis* fed

with other algae (Fig. 4). However, other field and laboratory studies have found that *B. plicatilis* s. l. attains a high growth rate with *Synechococcus* as food (Ito 1955; Hirayama et al. 1979). The use of blue-green algae for feeding rotifers is questionable, both in terms of food quality and applicability (Dumont 1977). It is believed that blue-green algae can jam the feeding mechanism of filter animals (Edmondson 1945). The cell density of *Synechococcus* used in the present study was 1.8×10^7 cells ml^{-1}. Such a high density of *Synechococcus* cells may clog the filtering organ of rotifers and ultimately results in a low population growth rate.

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