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Limnologia 38 (2008) 56–62

LIMNOLOGICA

www.elsevier.de/limno

Demographic parameters and mixis of three *Brachionus angularis* Gosse (Rotatoria) strains fed on different algae

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Received 9 April 2007; received in revised form 29 July 2007; accepted 17 August 2007

Abstract

Rotifers are one of the smallest metazoans, served as perfect material for evolution theories and excellent food resources to larva in aquaculture. Population dynamics of rotifers help to optimize culture conditions, and to understand selection of asexual and sexual reproduction types. We described population dynamics of three *Brachionus angularis* strains using the life table method, with three types of food. The following demographic parameters were analyzed: intrinsic rate of increase (r), net reproductive rate (R_0), generation time (T), average lifespan (L), and the total percentage of mictic females in the offspring. The results showed that there were significant effects of strain on the parameters except for R_0 . However, the food type significantly affected r , R_0 , T , and the total percentage of mictic females in the offspring. Demographic parameters differed among strains, showing the possible adaptation to local niches. In comparison to *Chlorella pyrenoidosa*, *Scenedesmus obliquus* served more nutrition to enlarge population size, and more mixis was induced in rotifer populations fed on it.

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Keywords: Rotifer; *Chlorella pyrenoidosa*; *Scenedesmus obliquus*; Sex reproduction; Population dynamics

Introduction

Rotifers are all relatively small, short-lived and fast-reproducing metazoans. The life cycle of monogonont rotifers involves both asexual and sexual reproduction. Those small metazoans are a perfect material in the evolution field; and recent studies on the reproduction types have aroused discussion on the topic of ancient evolution of sex (Birky, Wolf, Maughan, Herbertson, & Henry, 2005; Judson & Normark, 1996; Martens, Rossetti, & Horne, 2003). Population dynamics and

environmental factors both affect sexual reproduction of rotifers (Stelzer & Snell, 2006).

Life table seems to be a common tool to describe population dynamics. It has been widely used to investigate the demographic characteristics of populations in rotifers (Rao & Sarma, 1986; Sarma, Nandini, & Flores, 2001). Previous studies have shown that some factors are most likely to influence the rotifer life table parameters, such as food quality (Korstad, Olsen, & Vadstein, 1989; Snell & Hawkinson, 1983; Xi & Huang, 1999), food quantity (Roberston & Salt, 1981; Sarma & Nandini, 2001; Xi & Huang, 1999), temperature (Awaïss & Kestemont, 1992; Snell, 1986; Xi & Huang, 2000a), and salinity of culture medium (Snell, 1986).

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Rotifers are also excellent food resources to larval fish and crustaceans. In initiating mass cultivation of rotifers, their reproductive rates at the available environmental conditions should be carefully considered (Lubzens, Tandler, & Minkoff, 1989). Except for the population growth, the body size of rotifers is another factor that influences its application in aquaculture, for larva of different species and ages need corresponding amounts of food. Strain is an important endogenous factor affecting the population growth and body size of rotifers (Hu & Xi, 2006; Snell & Carrillo, 1984; Xi, Liu, & Jin, 2002; Yufera, 1982). The culturing of different rotifer strains offered a better solution to obtain different rotifer sizes (Snell & Carrillo, 1984).

Selecting appropriate rotifer strains helps to increase efficiency in culture. Population dynamics of rotifer helps to optimize culture conditions, and to understand selection of asexual and sexual reproduction types. In the present study, we described the difference of the life table variables among different strains of *Brachionus angularis* fed on three types of algal food, using the life table method. We also discussed the population growth and body size of different rotifer strains. We tried to demonstrate the effect of strain on the demographic parameters, and to find the relationship between mixis and food conditions.

Materials and methods

B. angularis is a common freshwater species. Its largest population density can reach 2000 ind. mL⁻¹ in laboratory culture, with green alga, *Scenedesmus obliquus*, as food at 25 °C. Its body size is in the range of 2.0 × 10⁶–4.5 × 10⁶ μm³ (lorica length ranged from 110 to 150 μm), which is available for larval fish and crustacean culture.

Three strains of *B. angularis* were used in this study, collected, respectively, from Guangzhou, Qingdao, and Wuhu City (GZ, QD, and WH strain). We obtained rotifer strains by hatching resting eggs in the sediments of a pond in the above three cities. All the strains were clonally cultured from one amictic female for more than 1 year at 25 ± 1 °C, on a 16 h:8 h light:dark photoperiod at 130 lux provided by fluorescent light from an illumination incubator, using Gilbert's (1963) medium and green algae *Chlorella pyrenoidosa* as a source of food. Before the experiment commenced, the three strains of rotifers were cultured in 15 mL tubes containing 10 mL rotifer medium with 3.6 × 10⁶ cells mL⁻¹ of *S. obliquus*, 9.0 × 10⁶ cells mL⁻¹ of *C. pyrenoidosa* (the mean cell volume of these algae was 500 and 200 μm³, respectively; Zhang & Huang, 1991), or 1:1 volume mixture (1.8 × 10⁶ cells mL⁻¹ of *S.*

obliquus, together with 4.5 × 10⁶ cells mL⁻¹ of *C. pyrenoidosa*), for more than 1 week. During the period, the rotifer populations were kept in log-phase growth. Algae were grown in semi-continuous culture with HB-4 medium and refreshed daily at 40% (Xi, Liu, & Jin, 2002). Algae in exponential growth was centrifuged and suspended in the rotifer medium. Algae concentration was determined by counting algae using a microscope, and diluted to the desired experimental food concentration.

Life table experiments were conducted in 5 mL glass beakers containing 3 mL rotifer medium with corresponding algal food and density mentioned as above. Twenty neonates (0–2 h age) of each strain were introduced into each beaker. Three replicates were taken for every cohort (3 replicates × 3 strains × 3 algae). All the experiments were accomplished at the same conditions as above. The rotifers were observed every 6 h under a dissecting microscope at 25 × magnification, and the numbers of the original test individuals, alive and new hatched ones, were recorded. The initial parental females were transferred to new beakers with the appropriate food type and density daily. The newly hatched neonates were cultured with corresponding algae for about 30 h, and then the total amount of the mictic females was counted. The experiment continued till every original test individual had died. Based on the data collected, the demographic parameters were calculated and analyzed following Krebs (1985) and Birch (1948):

l_x : age-specific survival rate, the proportion of surviving individuals at age x to original cohort.

m_x : age-specific fecundity rate, the mean number of female offsprings produced in a unit of time by a female aged x .

r : intrinsic rate of increase, the maximum potential for reproduction of an individual at a particular condition. r can be obtained through the equation $\sum_{x=0}^n e^{-rx} l_x m_x = 1$.

R_0 : net reproductive rate, the number of eggs produced per original individual in her life span, $R_0 = \sum_{x=0}^{\infty} l_x m_x$.

T : generation time, the duration from the birth of the parent to the birth of the offspring, $T = \ln(R_0)/r$.

L : average lifespan.

Two-way analysis of variance (ANOVA) was conducted to identify significant effects of algal type, rotifer strain, and interaction of algal type × rotifer strain on the life history parameters and the total percentage of mictic females in offspring, using generalized linear model in SPSS. The data of the total percentage of mictic females in offspring was arcsin-transformed. To determine the significant difference between strains and food types, multiple comparison was conducted with least significant difference (LSD) test.

Results

Demographic parameters of the three *B. angularis* strains fed on different algae

The age-specific survival rate (l_x) and the age-specific fecundity rate (m_x) of the three *B. angularis* strains fed on three types of algae were presented in Figs. 1 and 2, respectively. Survival rate of QD strain was lower almost on all days, and that of WH strain was obviously higher. Rotifers fed on *S. obliquus* had a higher survival rate than those fed on *C. pyrenoidosa*. The three strains of rotifers had similar fecundity rate. However, rotifers fed on *C. pyrenoidosa* shared much lower fecundity rate than those fed on the other two food types. Most of the rotifers finished reproduction within the initial 4 days.

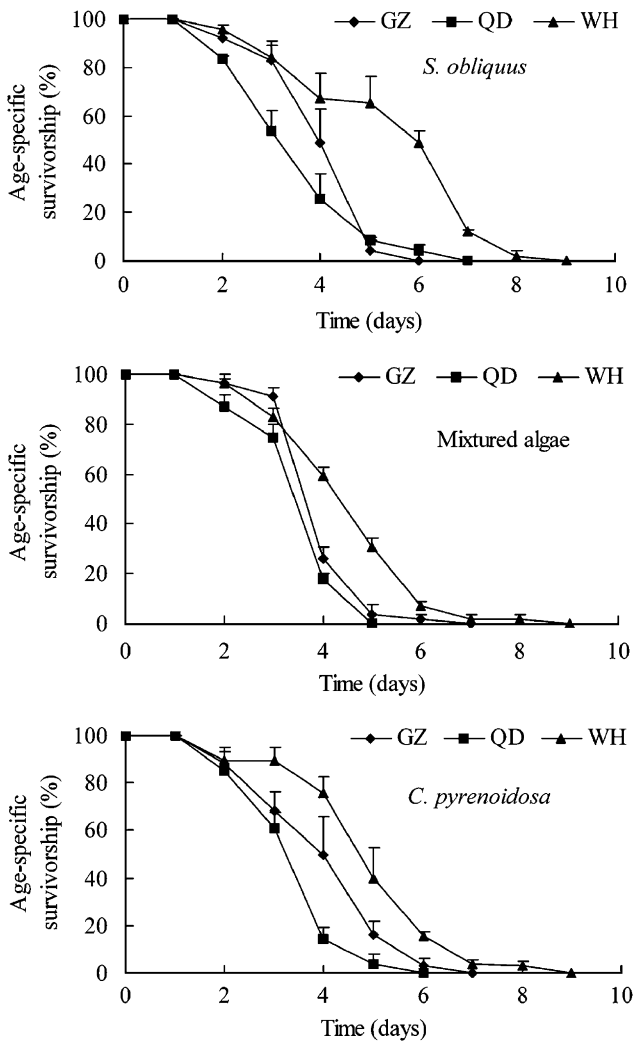


Fig. 1. Age-specific survival rate (l_x) of the three *Brachions angularis* strains fed on different algae. Bars represent standard errors.

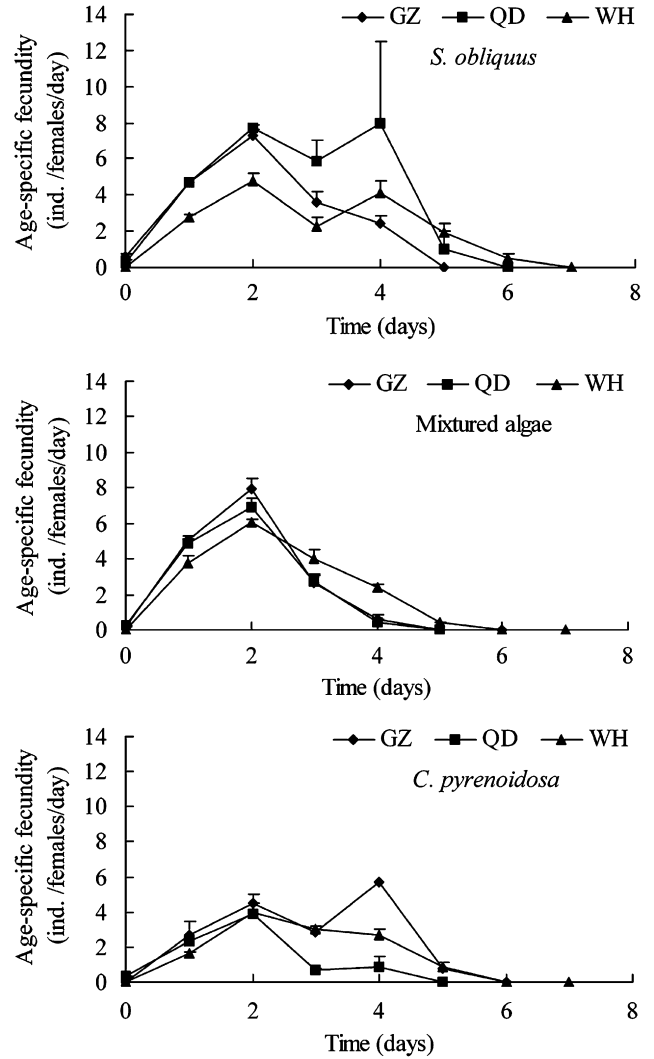


Fig. 2. Age-specific fecundity rate (m_x) of the three *Brachionus angularis* strains fed on different algae. Bars represent standard errors.

Demographic parameters of the three *B. angularis* strains fed on different algae are shown in Table 1. There were significant effects of strain on intrinsic rate of increase (r), generation time (T), and average lifespan (L), but there was no significant effect on the net reproductive rate (R_0) (Table 2). Multiple comparison test demonstrated that among the three strains, r of GZ strain was larger than that of WH strain. But WH strain had longer T and L . On the other hand, the type of algal food had a significant effect on the above parameters except for L . Rotifers fed on *S. obliquus* had larger r than those fed on the other two types of food. The interaction of strain and food type only significantly affected T in all the life history variables of *B. angularis* (Table 2).

Table 1. Demographic parameters of the three *Brachionus angularis* strains fed on different algae

	r (h^{-1})				R_0 (ind.)		
	GZ ^{a*}	QD ^{ab}	WH ^b		GZ	QD	WH
<i>S. obliquus</i> ^{1*}	0.1153 (0.0201)	0.0870 (0.0074)	0.0585 (0.0010)	<i>S. obliquus</i> ¹	16.3519 (1.4993)	15.6401 (0.2891)	13.3833 (0.8502)
Mixed algae ¹	0.0828 (0.0023)	0.0853 (0.0020)	0.0675 (0.0014)	Mixed algae ¹	15.5109 (1.4509)	13.3349 (1.4776)	14.4939 (0.7419)
<i>C. pyrenoidosa</i> ²	0.0566 (0.0014)	0.0609 (0.0155)	0.0471 (0.0018)	<i>C. pyrenoidosa</i> ²	12.4651 (3.4564)	6.4986 (1.9759)	10.2346 (0.8830)
	T (h)				L (h)		
	GZ ^b	QD ^b	WH ^a		GZ ^b	QD ^c	WH ^a
<i>S. obliquus</i> ²	25.4206 (3.7211)	32.0716 (2.8265)	44.3419 (1.4675)	<i>S. obliquus</i>	102.2400 (3.3460)	90.5000 (4.4763)	138.1224 (6.3193)
Mixed algae ²	33.0937 (2.0578)	30.1950 (0.7323)	39.5666 (0.0937)	Mixed algae	100.6316 (2.5013)	91.5918 (2.9972)	115.1186 (3.6173)
<i>C. pyrenoidosa</i> ¹	43.3017 (3.9501)	30.1630 (2.2408)	49.2906 (1.9837)	<i>C. pyrenoidosa</i>	99.3600 (4.2161)	87.5000 (3.4633)	124.7059 (4.8965)

*Numbers in parentheses are standard errors. The same letter/number in the upper right corner of the name of algal food/rotifer strain suggests that there are no significant differences of the parameter between the three rotifer strains/three types of algal foods.

Table 2. Effects of algae type and strain on the demographic parameters of *Brachionus angularis*

Parameters	Source	df	SS	MS	F	P -value
r	Algae type	2	0.0050	0.0025	10.4540	0.0010*
	Strain	2	0.0036	0.0018	7.5140	0.0042*
	Algae type \times strain	4	0.0021	0.0005	2.2246	0.1070
R_0	Algae type	2	155.2714	77.6357	9.4972	0.0015*
	Strain	2	41.3325	20.6663	2.5281	0.1077
	Algae type \times strain	4	34.7233	8.6808	1.0619	0.4038
T	Algae type	2	278.2497	139.1248	7.8265	0.0036*
	Strain	2	911.7176	455.8588	25.6445	5.36×10^{-6} *
	Algae type \times strain	4	353.5788	88.3947	4.9727	0.0071*
L	Algae type	2	4619.5490	2309.7745	2.0422	0.1309
	Strain	2	1.01×10^5	5.06×10^4	54.9546	4.06×10^{-22} *
Percentage of Mictic offspring	Algae type	2	0.0397	0.0198	118.5616	4.33×10^{-11} *
	Strain	2	0.0061	0.0030	18.1891	4.77×10^{-5} *
	Algae type \times strain	4	0.0054	0.0013	8.0278	0.0007*

Two-way ANOVA. * $P < 0.01$.

Percentage of mictic females in the offspring of the three *B. angularis* strains fed on different algae

The total percentage of mictic females in the offspring of the three *B. angularis* strains fed on three types of algae is presented in Fig. 3. Both strain and food type significantly affected the percentage (Table 2). However,

when fed on *S. obliquus*, no significant difference was observed in the percentage among the three *B. angularis* strains ($P > 0.05$). When fed on mixed food, WH strain shared the smallest percentage. When fed on *C. pyrenoidosa*, only GZ strain had mictic females. Rotifers fed on *S. obliquus* had a significantly higher percentage than those fed on *C. pyrenoidosa*.

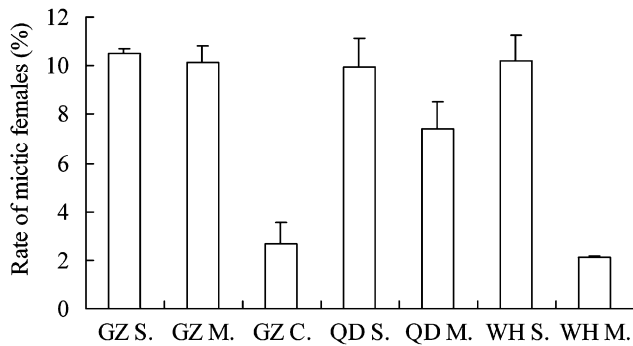


Fig. 3. The total percentage of mictic females of offspring in the culture of the three *Brachionus angularis* strains fed on different algae. Bars represent standard errors. (S. = *Scenedesmus obliquus*; M. = mixed algae; C. = *Chlorella pyrenoidosa*, GZ, QD, WH = rotifer strains. No mictic females had been obtained in QD and WH rotifer strains fed on *C. pyrenoidosa*).

Discussion

Differences among different rotifer strains

Previous studies have demonstrated that strain is one of the main factors causing the deviation of population growth and body size. Results have shown that rotifer strain significantly affected the population growth rate of *B. plicatilis* and *B. calyciflorus* (Snell & Carrillo, 1984; Xi, Liu, & Jin, 2002). The body size among different rotifer strains has also been confirmed to be significantly different in *B. plicatilis* (Snell & Carrillo, 1984; Yufera, 1982) and *B. calyciflorus* strains (Xi et al., 2002). In the present study, we found that the intrinsic rate of increase, generation time, and average lifespan of the three rotifer strains were all significantly different in the same culture condition. The body size of the three *B. angularis* strains had also been demonstrated to be significantly different (Hu & Xi, 2006). In a word, rotifers of different strains shared different demographic parameters, even cultured in the same condition.

However, there was no significant difference of R_0 among the three strains in our study, indicating that the mean amount of offspring produced by each amictic mother of the three strains was similar. Pourriot (1986) pointed out that the rate at which planktonic rotifers multiply during the parthenogenetic phase was more affected by the time that the embryo needs to develop and the early period of life (post-embryonic development) than by the net reproduction rate. GZ strain shares shorter periods of embryonic development and post-embryonic development than WH strain (Hu, Xi, & Geng, 2003), which should perhaps be an important

reason for the higher intrinsic rate of increase of the former.

Is there any genetic difference among rotifer strains? Gomez & Serra (1995) reported significant differences of allozyme markers among three sympatric strains of *B. plicatilis* and found there was no gene flow between those sympatric strains because of the different ecological niches (Gomez & Serra, 1995; Gomez, Carona, & Serra, 1997). Birky et al. (2005) demonstrated difference in the mitochondrial gene sequence of obligatory asexual bdelloid rotifers, and found that the bdelloid rotifers have undergone substantial speciation in the absence of sexual reproduction. Asexual reproduction existed in bdelloid individuals for millions of year, and they share no gene flow within the population. However, in monogonont rotifers involving both asexual and sexual reproduction, different strains of rotifers also show significantly different genetic distance (Xiang, Xi, & Hu, 2006). All those studies confirmed that differences of the rotifer body size and the life history variables are primarily genetically determined.

Mixis of rotifer population

Reproduction condition and population growth significantly affected rotifer sex reproduction. Recently studies showed that in the rotifer *Brachionus*, sexual reproduction (mixis) was induced by a chemical signal produced by the rotifers that accumulated during population growth. The chemical signal could induce mixis in each other, even in distant species in *B. plicatilis* complex (Snell et al., 2006; Stelzer & Snell, 2006). Our results found rotifers that fed on *S. obliquus* had more offsprings with sex reproduction. But when fed on inferior foods (esp. *C. pyrenoidosa*), the percentage of mictic females in the offspring decreased even to zero. Maybe, appropriate food served enough nutrition to rotifers, which enlarged population sizes rapidly, and then more mixis was induced. However, when food was inferior, the population size was smaller, and less mixis was formed. The factors inducing rotifer sex reproduction affirmatively exists in population structure.

The studies on the condition of mictic female formation and the difference in percentage of mictic females in the offspring of different rotifer strains are important for the mass production of rotifer resting eggs (Xi & Huang, 1999). In the present study, similar mixis rate in the offspring among the three *B. angularis* strains fed on *S. obliquus* showed that it might be worthless to select one of the three strains for promoting the mass production of *B. angularis* resting eggs when fed on optimum food. Considering the use of *S. obliquus* could increase the formation of mictic females, *S. obliquus* might be more suitable for the mass production of

resting eggs, which conformed the results obtained by Xi & Huang (2000b).

Effect of different algal food on the rotifer population growth

Many studies showed that the food type could affect the reproductive rates of *B. plicatilis* (Heerkloß & Hlawa, 1995; James & Abu-Rezeq, 1988; Korstad et al., 1989; Snell & Hawkinson, 1983), *B. calyciflorus* (Rothhaupt, 1990; Xi, Huang, Jin, & Liu, 2001; Xi et al., 2002), *B. urceolaris* (Xi & Huang, 1999), and *B. quadridentatus* (Heerkloß & Hlawa, 1995).

Xi et al. (2001, 2002) found that *C. pyrenoidosa* was a better food than *S. obliquus* for the culture of *B. urceolaris* and *B. calyciflorus*. In the present study, the rotifers fed on *S. obliquus* had higher population growth, which proved that *S. obliquus* tended to be a better algal food for *B. angularis*. According to Heerkloß and Hlawa (1995), the mechanism for accepting food particles involves the cilia of the pseudotrochus, the buccal funnel, the mastax jaw, and the feeding behaviors as well. Pourriot (1977) pointed out that rotifers select predominantly small cells with a diameter of about 20 µm or less. The average diameter and length of the spindle-shaped alga *S. obliquus* used in the present study were 5.36 and 9.41 µm, respectively, and the average diameter of the spherical alga *C. pyrenoidosa* was 4.25 µm. Both of them were in the available feeding scale of *B. angularis* and *B. calyciflorus*. Different species of rotifer might have formed adaptation to different food types.

Acknowledgments

This work was supported by the Natural Science Foundation PR China (No. 30270221), the Excellent Youth Foundation of Anhui Province, PR China (No.04043050), and the Special Project Grants of Anhui Normal University (2005zx19).

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