206

Hydrobiologia **255/256**: 139–143, 1993.
J. J. Gilbert, E. Lubzens & M. R. Miracle (eds), Rotifer Symposium VI.

Asexual and sexual reproduction in the rotifer *Brachionus plicatilis* cultured at different salinities

M. Pozuelo[1] & L. M. Lubián[2]
[1] *Departamento de Fisiología y Biología Animal, Laboratorio de Psicobiología. Universidad de Sevilla, Avda. S. Francisco Javier s/n. 41005 Sevilla, Spain;* [2] *Instituto de Ciencias Marinas de Andalucía (CSIC), Polígono Río San Pedro s/n. 11008 Puerto Real (Cádiz), Spain*

Key words: reproduction, sexuality, salinity, growth rates, rotifers, *Brachionus plicatilis*

Abstract

Two strains of the rotifer *Brachionus plicatilis* (L-type) differing in the levels of mictic female and male production, were grown in batch cultures with the alga *Nannochloropsis gaditana* as food, at two low (2.5 and 10‰), and two high (40 and 50‰) salinities. While both the low (strain S-1) and the high (strain S-3) sexual reproducing strains developed similar growth cycles at 2.5 and 10‰, the population growth response at 40 and 50‰ showed that; 1) in strain S-1, mixis can be suppressed in conditions that still allow asexual reproduction, and 2) in strain S-3 mictic female and male production are possible at nearly zero asexual population growth rates. In strain S-3, a double log linear relationship between the densities of males and females was found. These results show that mixis can occur over a wide ranges of female population density, and support the hypothesis that sexual reproduction is a strain dependent component of the general reproductive response.

Introduction

The rotifer *Brachionus plicatilis* reproduces both sexually and asexually as other monogonont rotifers. Two types of females can be distinguished; the amictic which through ameiotic parthenogenesis produces diploid eggs that hatch into mictic or amictic females, and the mictic female whose oocytes undergo meiosis and if unfertilized hatch parthenogenetically into haploid males (Birky & Gilbert, 1971; Gilbert, 1974, 1977). It is considered that the first step in the shift from asexual to sexual reproduction is the production of the mictic female, this event has been found to be controlled in *Asplachna* by tocopherol (Gilbert, 1980) and by photoperiod in *Notommata* (Pourriot & Clément, 1981). In *Brachionus* a clear-cut relationship of mictic female production and population density was demonstrated by Gilbert (1963) in *B. calyciflorus*. In *B. plicatilis*, Lubzens *et al.* (1980) reported sexual reproduction to occur at salinities lower than that of the full strength sea water, and related later (Lubzens *et al.*, 1985) mictic female production to asexual population growth rates that were salinity dependent. Algal quality effects on mixis in *B. plicatilis* were communicated by Ben-Amotz & Fishler (1982) and Lubzens & Minkoff (1988); the latter authors concluded that mixis is genetically determined and environmentally modulated. Snell (1986) found sexual reproduction to be more severely restricted than asexual reproduction by environmental extremes of food, temperature and salinity, and concluded that sexual reproduction must not be only a phenomenon of induction, but a repressible event. Later, Snell & Boyer (1988) also determined the threshold values for induction or repression of sexual reproduction by factors such as food levels, ammonia and population density. Furthermore, they found amictic population growth rates to act as a trigger of mictic female production, and concluded that mixis is a component of general reproduction, its environmental regulation depending on induction and repression.

In the present work, we investigated the *B. plicatilis* population growth response with its sexual and asexual components at low and high salinity levels.

Material and methods

Two strains of the L type of *B. plicatilis* O. F. Müller isolated from saltmarshes near Cádiz (South of Spain) were used. The strain S-1 was isolated in February, 1974 and maintained since then in the Instituto de Ciencias Marinas de Andalucía (CSIC) in Cádiz. The strain S-3 was isolated in November, 1990. They differ widely in the levels of mictic female and male production, S-3 showing consistently higher levels than S-1.

The alga used as food was the euryhaline eustigmatophycean *Nannochloropsis gaditana* Lubián (B-3 strain, Lubián & Yúfera, 1989). Natural seawater (35–37‰ salinity) was collected near the shore at Cádiz, filtered through 1 μm glass fiber filter, and used to prepare the experimental culture salinities of 2.5, 10, 40, and 50‰ by either diluting with deionized water or by concentrating through evaporation. Enriched culture medium (f/2) was prepared according to Guillard & Rhyter (1962). Algae and rotifers were maintained at 25 °C and continuous illumination using daylight fluorescent lamps (2500 lux). Rotifers were previously acclimated at each experimental salinity for at least two weeks prior to experiments.

Population growth experiments were initiated by inoculating rotifers (initial density 1–5 rotifers ml^{-1}) into algal cultures in the log phase of growth at non limiting cell densities ($18–22 \times 10^6$ cells ml^{-1}). Cultures were performed in 500 ml glass flasks (three replicates), stirred by aeration and the rotifers were allowed to grow without any renewal of the medium or of the food resource, until they consumed it and completed a growth cycle. Salinity was periodically monitored with a Shibuya S-10 refractometer and maintained constant by adding small amounts of deionized water. Rotifer densities was determined by daily counts, under a stereomicroscope of a 5 or 1 ml (depending on density) live sample from each of the three replicates.The number of females without eggs, females with amictic eggs, females with haploid mictic eggs, and males was recorded separately in each sample.

According to Snell (1986), the exponential growth rate of the female population measures the asexual growth rate, and that of the males the sexual growth rate. Thus, the asexual growth rate of rotifers was calculated as the slope of the semi-log plot of female density (total number of females) versus time during the exponential phase, and the sexual growth rate was estimated from a similar calculation for the males.

Results

The general pattern of population growth of *B. plicatilis* S-1 and S-3 strains at the low (2.5 and 10‰) and high (40 and 50‰) salinities is shown in Fig. 1. The pattern at the lower salinities is essentially the same as that reported by Ito (1960). It develops as expected in a food-limited system, with a rapid exponential growth followed by a sharp decrease in numbers caused by starvation resulting from the depletion of the food resource. The growth cycle includes mictic and amictic reproduction, with the levels of the former being higher in the S-3 strain. However, strain S-1 showing lower levels of sexual reproduction, grew faster and had a shorter growth cycle (Fig. 1; Tables 1 and 2).

In the cultures at the higher salinities (40 and 50‰) the pattern of population growth shows low or zero growth rates with a prolonged stationary phase. This phase may a last for twenty or more

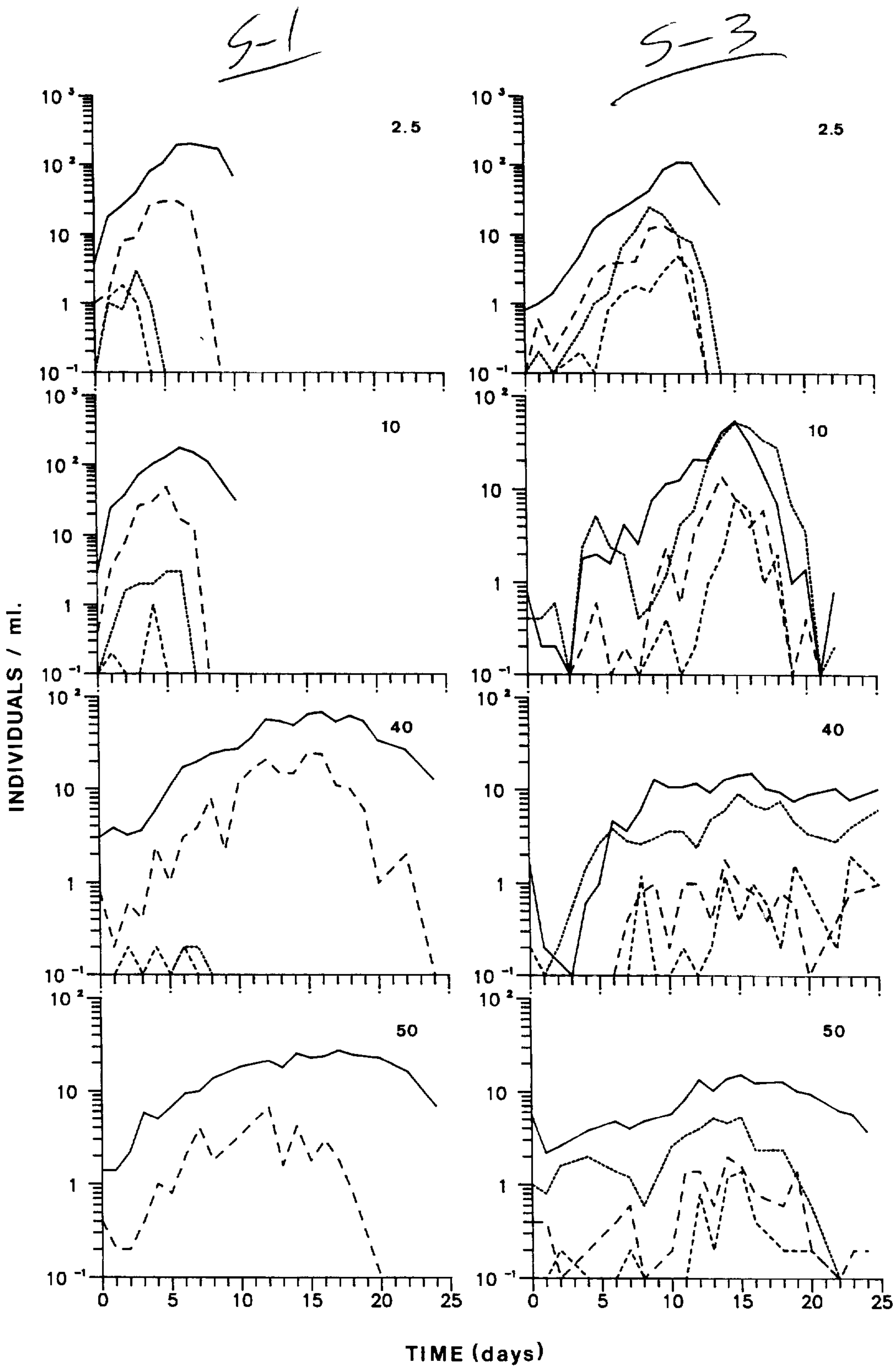

Fig. 1. Population growth cycles of *Brachionus plicatilis* in batch cultures at different salinities showing the total number of females (solid lines), females with amictic eggs (broken lines, long), females with mictic eggs (broken lines, short), and males (dotted lines). The salinities (‰) are shown on the top right of each graph. The results for strain S-1 are shown by the left hand graphs and those corresponding to the S-3 strain, on the right side. Each graph corresponds to one of three replicates.

Table 1. Growth rates for sexual and asexual reproduction in S-1 strain at different salinities.

Salinity (‰)	K (d^{-1})	SE (d^{-1})	K' (d^{-1})	SE
2.5	0.456	0.012	0.251	0.041
10	0.491	0.005	0.336	0.004
40	0.294	0.010	–	
50	0.000	0.006	NM	

K: growth rate for female population.
K': growth rate for male population.
SE: standard errors.
Labels are: negative value; NM: no males were produced.

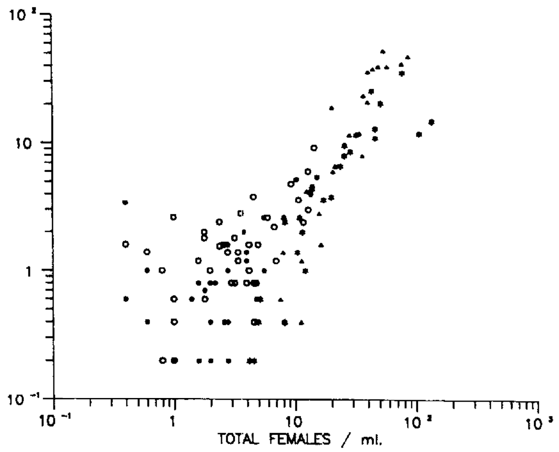

Fig. 2. The pattern of male production in *Brachionus plicatilis* (strain S-3) described by the relationship between population density of males and that of females. Points are pooled data of three replicates for each of the four salinities tested. Stars: 2.5‰, Triangles: 10‰, Open circles: 40‰, Closed circles: 50‰.

days; in some of the replicates it lasted for more than one month. Furthermore, an important difference appears between the two strains at high levels of salinity. Whereas in the S-1 strain, female and male production is depressed at 40‰ and completely inhibited at 50‰, the S-3 strain is able to produce lower, but still substantial levels of mictic females and males over many days (Fig. 1; Tables 1 and 2).

The relationship between the density of males to that of females (Pozuelo, 1977) can describe the sexual reproduction response. This is shown in Fig. 2, for the high male producing S-3 strain, at the four tested salinities, showing a double-log linear relationship.

Discussion

The observed variations in asexual reproductive rates at the four salinities tested in the course of the present study are in general agreement with those previously reported in the literature for *B. plicatilis*, with faster rates at low or intermediate salinities, showing optimum values at salinities ranging between 2 and 20‰ (Ito, 1960; Pascual & Yufera, 1983; Lubzens *et al.*, 1985; Ruttner-Kolisko, 1972). Our results, with the highest reproductive rates at 10‰ are similar to that reported by Lubzens *et al.* (1985). However, our figures are consistently lower possibly due to the different conditions of culture. In our experiments we used batch cultures in 500 ml flasks, stirred by air bubbling, while those reported, were maintained in 50 ml flasks on a rotating shaker and had the media renewed periodically.

Table 2. Growth rates for sexual and asexual reproduction in S-3 strain at different salinities.

Salinity (‰)	K	SE	K'	SE
2.5	0.416	0.057	0.580	0.100
10	0.325	0.342	0.682	0.075
40	0.189	0.049	0.116	0.030
50	0.000	0.081	0.000	0.048

Symbols as in Table 1.

At high salinities the population growth curve shows a stationary phase resulting from a equilibrium between the algae and the rotifers, until the food resource is finally depleted, ending the rotifer growth cycle. Clearly, the equilibrium depends on both the lower rates of food consumption and reproduction. It is known that food intake is depressed at high salinities (Hirayama & Ogawa, 1972; Yamasaki & Hirata, 1985). The reproductive rates depend on birth and death rates, and the decrease caused by the high salinities has been reported as due to a decrease in fertility and survival, the effect of salinity on *r* being dependent on genotype (Miracle & Serra, 1989). While the appearance of the stationary phase at the high salinities is a common feature to both strains (S-1 and S-3), they differ in the production of mictic females and males. This was severely depressed (40‰) or completely suppressed (50‰) in strain S-1, but was maintained in strain S-3, and developed into a stationary pattern similar to that of the asexual reproduction, showing first that mictic female and male production are possible at nearly zero asexual population growth rates (strain S-3), and secondly that mixis can be suppressed in conditions of high salinity that still allow asexual reproduction to occur (S-1 strain). High salinity appears as a factor that depresses both the sexual and asexual components of reproduction, leading to the disappearance of sexuality first in strains of low mictic level. The males to females density relationship shows that mixis, if expressed, follows a quantitative pattern similar to that of asexual reproduction. A possible explanation for this is that mixis and asexual reproduction may have in common some causal mechanism.

Acknowledgements

We acknowledge editorial comments by Dr E. Lubzens that improved the manuscript.

References

Ben-Amotz, A. & R. Fishler, 1982. Induction of sexual reproduction and resting egg production in *Brachionus plicatilis* by a diet of salt-grown *Nannochloris oculata*. Mar. Biol. 67: 289–294.

Birky, C. W., Jr & J. J. Gilbert, 1971. Parthenogenesis in rotifers: The control of sexual and asexual reproduction. Am. Zool. 11: 245–266.

Gilbert, J. J., 1963. Mictic female production in the rotifer *Brachionus calyciflorus*. J. exp. Zool. 153: 113–123.

Gilbert, J. J., 1974. Dormancy in rotifers. Trans. am. micros. Soc. 93: 490–513.

Gilbert, J. J., 1977. Mictic female production in monogonont rotifers. Arch. Hydrobiol. Beih. 8: 142–155.

Gilbert, J. J., 1980. Female polymorphism and sexual reproduction in the rotifer *Asplachna*. Evolution of their relationship and control by dietary tocopherol. Am. Nat. 16: 409–431.

Guillard, R. R. L. & J. H. Rhyter, 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8: 229–239.

Hirayama, K. & S. Ogawa, 1972. Fundamental studies on physiology of rotifer for its mass culture. I. Filter feeding of rotifer. Bull. Jap. Soc. Sci. Fish. 38: 1207–1214.

Ito, T., 1960. On the culture of mixohaline rotifer *Brachionus plicatilis* O. F. Müller in the sea water. Rep. Fac. Fish. Pref. Univ. Mie. 3: 708–740.

Lubián, L. M. & M. Yúfera, 1989. Colección de cepas de microalgas del Instituto de Ciencias Marinas de Andalucía (CSIC). In Yúfera, M. (ed.) Acuicultura Intermareal. Inst. Cien. Mar. Andalucía, Cádiz: 69–78.

Lubzens, E. & G. Minkoff, 1988. Influence of the age of the algae fed to rotifers (*Brachionus plicatilis* O. F. Müller) on the expression of mixis in their progenies. Oecologia (Berlin) 75: 430–435.

Lubzens, E., R. Fishler & V. Berdugo-White, 1980. Induction of sexual reproduction and resting egg production in *Brachionus plicatilis* reared in the sea water. In H. J. Dumont & J. Green (eds), Rotatoria. Developments in Hydrobiology I. Dr W. Junk Publishers, The Hague: 55–88. Reprinted from Hydrobiologia 73.

Lubzens, E., G. Minkoff & S. Marom, 1985. Salinity dependence of sexual and asexual reproduction in the rotifer *Brachionus plicatilis*. Mar. Biol. 85: 122–126.

Miracle, M. R. & M. Serra, 1989. Salinity and temperature influence in rotifer life history characteristics. In C. Ricci, T. W. Snell & C. E. King (eds), Rotifer Symposium V. Developments in Hydrobiology 52. Kluwer Academic Publishers, Dordrecht: 81–102. Reprinted from Hydrobiologia 186/187.

Pascual, E. & M. Yúfera, 1983. Crecimiento en cultivo de una cepa de *Brachionus plicatilis* O. F. Müller en función de la temperatura y la salinidad. Inv. Pesq. 47: 151–159.

Pourriot, R. & P. Clément, 1981. Action des facteurs externes sur la reproduction et le cycle reproducteur des rotifères. Acta Oecol. gen. 2: 131–155.

Pozuelo, M., 1977. Male production in seawater cultured *Brachionus plicatilis*. Arch. Hydrobiol. Beih. 8: 172–173.

Ruttner-Kolisko, A., 1972. Der einfluss von temperatur und salzgehalt des mediums auf stoffwechsel und vermehrungsintensitatt von *Brachionus plicatilis* (Rotatoria). Dt. Zool. Ges. 65: 89–95.

Snell, T. W., 1986, Effect of temperature, salinity and food level on sexual and asexual reproduction in *Brachionus plicatilis* (Rotifera). Mar. Biol. 92: 157–162.

Snell, T. W. & E. M. Boyer, 1988. Thresholds for mictic female production in the rotifer *Brachionus plicatilis* (Müller). J. exp. mar. Biol. Ecol. 124: 73–85.

Yamasaki, S. & H. Hirata, 1985. Effects of salinity in food conversion rate of rotifer *Brachionus plicatilis*. Mem. Fac. Fish. Kagoshima Univ. 34: 37–43.