Effect of temperature, salinity and food level on sexual and asexual reproduction in *Brachionus plicatilis* (Rotifera)

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Abstract

The reproductive response of sexual and asexual female *Brachionus plicatilis* (Muller) was examined over temperatures ranging from 20° to 40 °C, salinities from 5 to 40‰ S, and food levels from 0.25 to 20 μg *Chlorella vulgaris* dry-weight per ml. Reduced food levels, as well as temperature and salinity extremes, reduced reproduction of both sexual and asexual females, but did so differentially. Reproduction by sexual females was reduced to a greater extent at environmental extremes than asexual females. The broad, flat reproductive response curve of asexual females extended beyond the limits of the narrower, more sharply peaked curve of sexual females. Thus zones of exclusively asexual reproduction exist at environmental extremes where sexual reproduction is physiologically restricted. These results are corroborated by a comparison of the lifetime fecundity of individual sexual and asexual females over a 20 °C temperature range. No differences in lifetime fecundity occurred between sexual and asexual females at 18° and 28 °C. At 38 °C, however, asexual female fecundity reached its highest level, while sexual female fecundity declined 15%. The appearance of sexual females in rotifer populations is the result of both inducible and repressible factors.

Introduction

Monogonont rotifers are cyclical parthenogens capable of either sexual or asexual reproduction. Internal and external factors have been implicated in the switch from asexual to sexual reproduction. Genotype, diet, population density, and photoperiod are important in freshwater species (see Gilbert, 1977, 1980; Pourriot and Clement, 1981; Pourriot and Snell, 1983 for reviews). Temperature (Hino and Hirano, 1984) and salinity (Lubzens *et al.*, 1980, 1985; Ben-Amotz and Fishler, 1982) play an important regulatory role in the marine species *Brachionus plicatilis*.

The reproductive physiology of asexual (amictic) and sexual (mictic) females is substantially different. Amictic and mictic females differ in size (Gilbert, 1975; Gilbert and Litton, 1978) and in the types of eggs that they produce (Gilbert, 1983). Their mechanism for oogenesis differs, with amictic females producing diploid and mictic females haploid eggs (Gilbert, 1983). Egg shell composition, yolk contents and lifetime fecundity are also markedly different between amictic and mictic females (Gilbert, 1983).

As a result of these differences in reproductive physiology, amictic and mictic females may be expected to respond quite differently to extreme environments. As environmental conditions deviate from optimal, differences between these females are likely to be accentuated. The hypothesis examined in this paper is that amictic and mictic females have different reproductive requirements and their responses to environmental extremes are not identical. I have investigated this hypothesis by comparing the reproductive responses of *Brachionus plicatilis* amictic and mictic females over a wide range of temperatures, salinities and food levels.

Materials and methods

Brachionus plicatilis (Muller), McK8 strain, was originally collected in 1978 from McKay Bay in Tampa, Florida (Snell and Hawkinson, 1983). This bay was sampled monthly for two years and *B. plicatilis* was found in the plankton only from July through September. Temperatures ranged from 27° to 31 °C and salinities 18 to 23‰ S during this period. Since its collection, this strain has been continuously in culture in F medium (Guillard, 1983) made with artificial seawater (Instant Ocean from Aquarium Systems, Mentor, Ohio) at 20‰ S, 25 °C, constant illumination of 4 300 lux, on a diet of *Chlorella vulgaris*.

Prior to each population growth experiment, rotifer stock cultures were acclimated to the experimental tem-

peratures or salinities for at least 10 d. After acclimation, rotifers were transferred to fresh medium without food for 48 h before initiating the experiments. This starvation treatment caused the population to switch to almost exclusively asexual reproduction as the population began to decline. As a result of this treatment, each experiment began with only asexual females and we followed the appearance of mixis through a 10- to 15-d population growth cycle. The appearance of mixis was followed by counting the number of males and females present. Since unfertilized mictic females produce only males, male numbers are correlated (0.64 ± 0.03) with the number of mictic females present. The number of males in a population is determined by the rate of production of mictic females, the fecundity of each individual female, and female and male survival. It is possible to separate the first two factors experimentally.

Population growth experiments were initiated by transferring ten adult asexual females into 25 ml of fresh medium in a 50-ml flask containing *Chlorella vulgaris*. Each treatment contained three replicates on a 24-h light photoperiod at 4 300 lux. Unless otherwise indicated, the experimental conditions were 25 °C, 20‰ S, and a food level of 10 μg C. *vulgaris* ml^{-1}. Temperature was varied in two experiments, the first ranging from 20° to 30 °C, and the second 32° to 40 °C. Salinities from 5 to 40‰ S were investigated and, in a separate experiment, C. *vulgaris* food levels from 0.25 to 20 μg dry wt per ml were explored.

The medium was changed each day and the numbers of males and females in the population were recorded. From these daily census counts, the rate of change in male and female numbers each day was calculated as $r = \ln N_t - \ln N_{t-1}$, where: N_t = number of individuals (male or female) on Day t and N_{t-1} = number of individuals in the population on the preceding day. Because of the rotifer life cycle, the rate of female population growth estimates the amount of asexual reproduction occurring in a population. In this application, r is a conventional measure of the rate of parthenogenetic reproduction and mortality among females. Male population growth rate, in contrast, estimates the level of sexual reproduction in a population and is determined by the rate of mictic female production, mictic female survival and fecundity and male mortality. The use of r in this case is unusual because the factors determining the number of male and female rotifers are very different. However, r simply estimates the growth rate of male and female populations and this application seems appropriate. The mean r of male and female populations was calculated separately for the log phase of the population growth cycle. Log phase is defined by the population growth curves in various environments. The intervals used to compare the mean rates of male and female production are identified on sample population growth curves.

To initiate the individual female fecundity experiment, a population of *Brachionus plicatilis,* McK8 strain, was fed with excess *Chlorella vulgaris* and *Tetraselmis tetrathele* from log-phase cultures and maintained at 26 °C, 20‰ S

and constant light of 4 300 lux. These growth conditions promoted the development of mixis. When the culture reached exponential growth, several ovigerous females were collected. Neonate females were placed individually into wells of a Falcon #3047 multiwell plate containing 1 ml of medium with 10 μg C. *vulgaris* cells per ml. Forty-eight neonates were tested in each of three temperature treatments: 18°, 28°, and 38 °C. The offspring of every female were counted and removed at 24-h intervals in the 18° and 28° treatments and at 12-h intervals in the 38° treatment. Because this experiment was of short duration and low population density, the medium was not renewed. Algal growth occurred during the experiment so that addition of food beyond the initial inoculation was not required. Consequently, the fecundity estimates presented are for conditions where food was not limiting.

Results

Examples of population growth curves in a variety of environments are presented in Fig. 1. The intervals used to calculate the mean log-phase growth rate (r) of male and female populations are indicated with arrows on each female curve. Temperature, salinity and food level all caused marked shifts in the population growth curves. At 30 °C, for example, log growth began immediately, but at 20 °C, the initiation of log growth was delayed until Day 3. The beginning of log growth was also delayed at 40 °C, and at both 20° and 40 °C, population size during the experimental period reached only 3% of that attained at 30 °C. The effects of salinity on the population growth curves were generally less severe than temperature, but a marked delay of log growth was recorded at 40‰ S. Food level effects were also substantial; as starvation became more intense log phase was delayed, and in the 0.25-μg ml^{-1} treatment log growth never developed.

The effect of temperature, salinity and food level on the reproduction of sexual and asexual females is given in Figs. 2, 3, and 4. In each case, the reproductive response of asexual females is broad and flat, indicating that they are not sensitive to variation in these factors. In contrast, the reproductive response of sexual females is peaked, indicating that sexual reproduction is sensitive to variation in these factors.

In Fig. 5, the lifetime fecundity (total offspring production) of individual sexual and asexual females cultured in isolation is compared over a twenty degree temperature range. At 18° and 28 °C, no significant differences between female and male production were recorded. At 38°, asexual reproduction reached its highest level, while male production declined 15%. The Q_{10}'s for sexual females are 1.23 and 0.85 for the 18° to 28° and 28° to 38 °C intervals, respectively. Asexual female Q_{10}'s are 1.29 and 1.15 for the same temperature intervals. This experiment has been repeated with a different strain of *Brachionus plicatilis* (RUS, see Snell and Hawkinson, 1983) and a similar
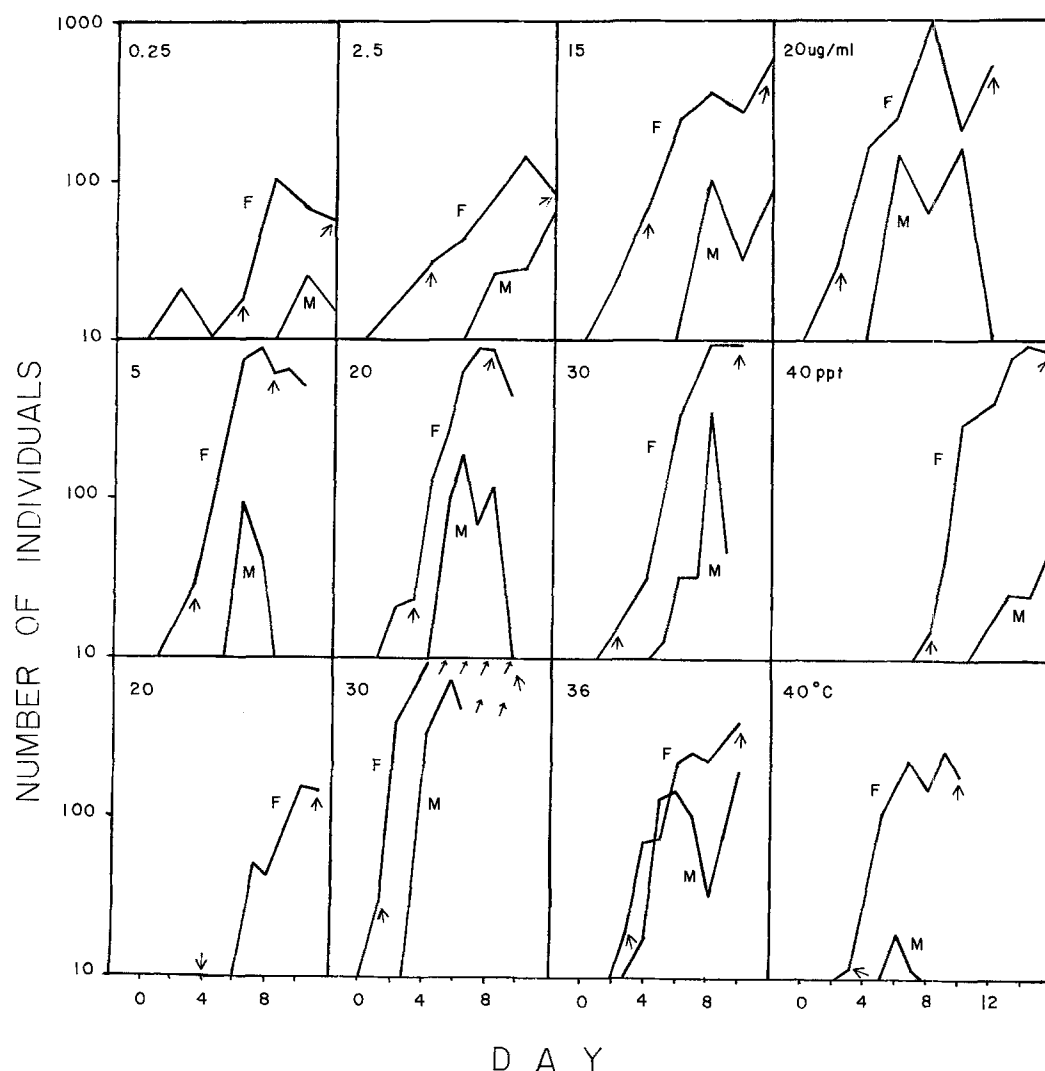
Fig. 1. *Brachionus plicatilis.* Sample population growth curves from a variety of environments. The top row is the food level treatment in μg *Chlorella vulgaris* ml^{-1} (25 °C, 20‰ S), middle row is salinity in parts per thousand (25 °C, 10 μg *C. vulgaris* ml^{-1}), and bottom row is temperature in °C (20‰ S, 10 μg *C. vulgaris* ml^{-1}). Only one of three replicates is plotted for each treatment. Female growth curves are labeled F and male curves M. Arrows on the female growth curves indicate periods of log growth; the additional arrows at the top of the 30 °C treatment indicate values off the scale
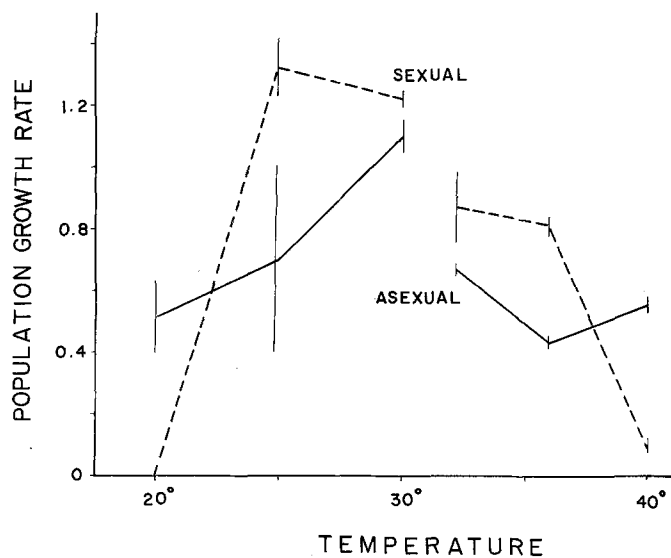

Fig. 2. *Brachionus plicatilis.* The reproductive response of sexual and asexual females to temperature. Mean $r\pm$ standard errors of three replicate populations is plotted for each temperature
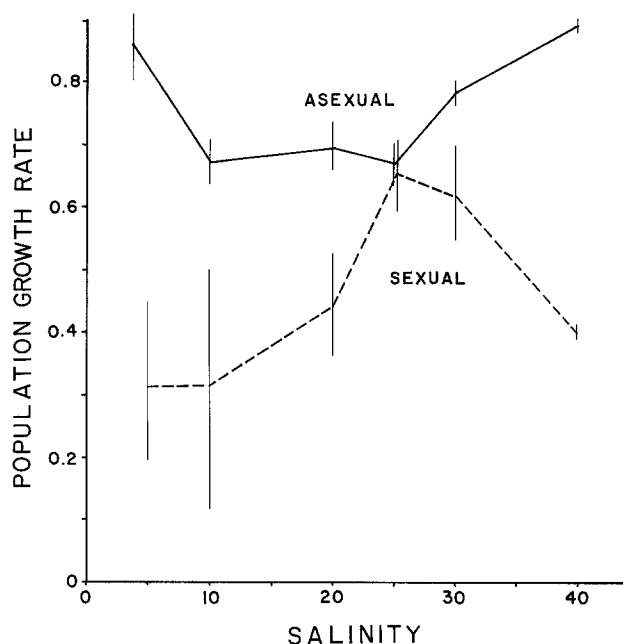
Table 1. *Brachionus plicatilis.* A comparison of mean lifespans of amictic and mictic females at 18°, 28° and 38 °C

Temp	Female type	Mean lifespan	SE	n	t	P
18 °C	amictic	9.87	0.52	38	1.11	0.28
	mictic	8.67	0.94	9		
28 °C	amictic	6.39	0.44	33	0.95	0.35
	mictic	5.92	0.23	12		
38 °C	amictic	3.33	0.17	30	1.21	0.24
	mictic	3.00	0.21	17		

Fig. 3. *Brachionus plicatilis.* The reproductive response of sexual and asexual females to salinity in ppt. Mean r ± standard errors of three replicate populations is plotted for each salinity
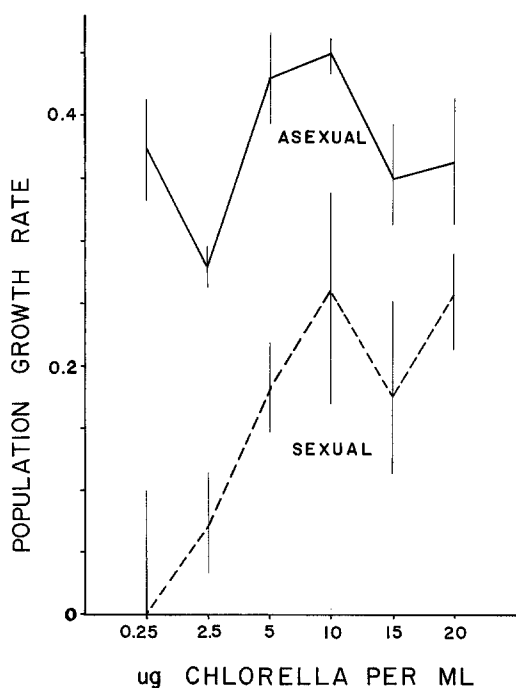

Fig. 4. *Brachionus plicatilis.* The reproductive response of sexual and asexual females to reduced food levels. Mean r ± standard errors of three replicate populations is plotted for each food level

Fig. 5. *Brachionus plicatilis.* Comparison of individual sexual and asexual female fecundity at different temperatures. Mean ± standard error lifetime fecundity (number of offspring) of sexual and asexual females is plotted for each of three temperature treatments

Fig. 6. *Brachionus plicatilis.* Generalized model of the reproductive response curves of sexual and asexual females. The environmental gradient refers to temperature, salinity, or food level. Reproductive rate is measured as r. The diagonal lines at the extremes of the reproductive response curves represent zones of exclusively asexual reproduction

reproductive pattern for sexual and asexual females was observed: no differences in their responses at 18° and 28 °C, but a substantial decline in male production at 38 °C while asexual reproduction increased. No significant differences in lifespan between sexual and asexual females were found at any temperature tested (Table 1).
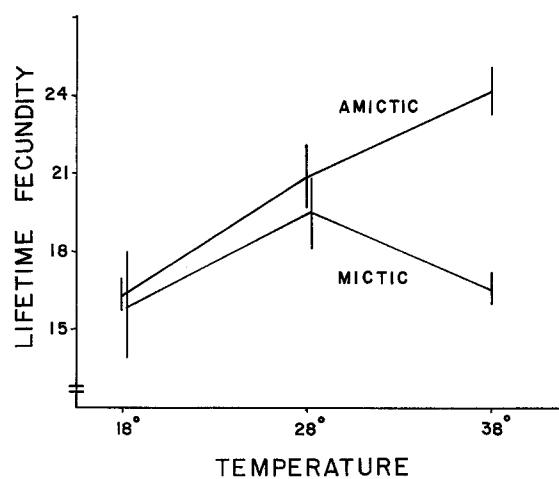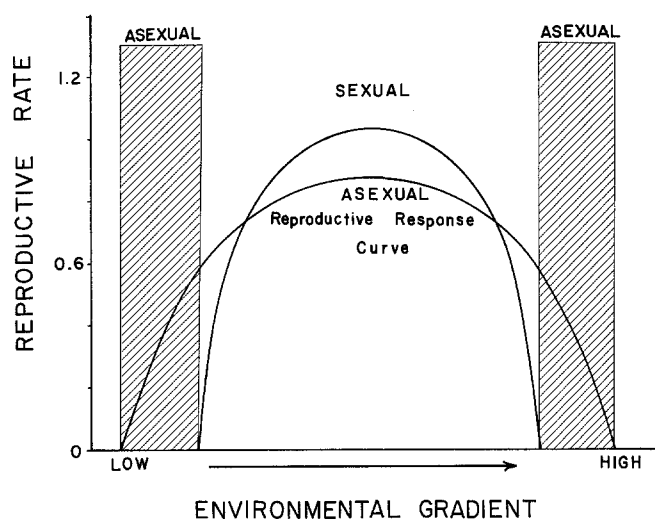
Discussion

The rate of appearance of males indicates the level of sexual reproduction occurring in rotifer populations. In the cyclically parthenogenetic life cycle of rotifers, asexual reproduction occurs by simple ameiotic parthenogenesis, but sexual reproduction is complex (Birky and Gilbert, 1971; King and Snell, 1977). It begins with mictic female production, followed by male production, and then the appearance of fertilized females bearing resting eggs. This entire process requires 2 to 3 generations for completion. The production of mictic daughters represents an irrevocable attempt at sexual reproduction by a female. If these

mictic daughters are not fertilized, their haploid eggs develop directly into males. If these males do not fertilize other mictic females, their mictic mothers effectively die without leaving offspring. In this life cycle, the number of males can be used to estimate the number of females attempting sexual reproduction. These females may not successfully complete sexual reproduction and produce resting eggs, but they nonetheless have the capacity to do so. A more direct measure of the level of sex occurring in rotifer populations is the number of females bearing resting eggs. Unfortunately, none of these females appeared in my experimental populations, so it was not possible to use this criterion as a measure of sex.

The first hint of the physiological diversity of mictic and amictic females was provided by Luntz (1926). He showed that amicitic *Testudinella (Pterodina) elliptica* females are able to withstand osmotic pressures and pHs that caused reduced survival among mictic females, but the reproductive effects of these factors were not measured. King (1970) found that amictic, unfertilized mictic and fertilized mictic female *Euchlanis dilatata* respond differently in net reproduction to temperature and recorded Q_{10}'s of 2.8, 3.6, and 2.4, respectively. King's results demonstrated that net reproduction of unfertilized mictic females increased most when temperature was raised from 19° to 27 °C. However, since the experiments were conducted at moderate temperatures, they reveal nothing about the reproductive response of mictic and amictic females to extreme environments. In a bioenergetic analysis of *Brachionus rubens,* Pilarska (1977 a, b, c) was unable to find differences between mictic and amictic females in the rate of food consumption, O_2 consumption, assimilation efficiency and production. However, all of her measurements were also made in moderate environments. Her measures of production further assumed that amictic, unfertilized mictic and resting eggs all have the same energy content. Resting eggs have greater lipid reserves (Wurdak *et al.,* 1978; Gilbert, 1983), so it is likely that Pilarska overestimated amictic and unfertilized mictic female production.

The data presented in this paper support the notion that sexual reproduction has more constraints than asexual reproduction. Sexual reproduction in rotifers is restricted by population density (Gilbert, 1980; Snell and Garman, in press) and by bioenergetic factors (Gilbert, 1980). The physiological requirements of reproduction, as modified by temperature, salinity and food levels, are broader for asexual than for sexual females. These constraints are evidenced by reduced levels of male production at environmental extremes. In the population growth experiments, the reduced rate of male production could be explained by lower production of mictic females, lower fecundity of mictic females, or greatly decreased male or mictic female survival. The decreased male production cannot be explained by decreased mictic female survival since the mean lifespans clearly show that there is no differential effect of temperature on amictic and mictic female survival. Decreased male survival, however, remains

a possible explanation for the observed reduction in male numbers at environmental extremes. Returning to the first two explanations, the role of lowered mictic female production vs mictic female fecundity in reducing male production can be contrasted. The absence of differences in mictic and amictic female fecundity at 20 °C suggests that the reduced level of male production at this temperature is primarily due to a lower rate of mictic female production. One is led to this conclusion because mictic females, if present, would experience reduced, but positive fecundity. In contrast, at high temperatures, mictic female fecundity is reduced while that of amictic females reaches its highest level. Therefore, reduced mictic female fecundity makes a large contribution to the lower level of male production at 38 °C.

Beauchamp (1935) found that relatively less sexual reproduction occurred in the freshwater, predatory rotifer *Asplanchna girodi* at extreme temperatures or low food levels, but no quantitative comparisons were made. Hino and Hirano (1984) found that the rate of mictic female production in their strain of *Brachionus plicatilis* is greatly reduced at higher temperatures. At 15 °C they recorded mictic female production of 28%, which was significantly higher than the 4% mictic rate of 30 °C. This reduction of mixis at higher temperatures is consistent with my results, but the McK8 strain that I used is subtropical in origin and therefore likely to have a higher temperature tolerance than Hino and Hirano's strain. Unfortunately, Hino and Hirano (1984) did not investigate the effect of temperatures lower than 15 °C on mictic reproduction, nor did they present data on how amictic reproduction responded to their experimental environments. Lubzens *et al.* (1985) found that mictic female production is reduced at salinity extremes. A lower percentage of mictic females was produced at 2‰ S and no mixis occurred above 36‰ S, although asexual reproduction continued at a substantial rate. Lubzens *et al.* (1985) is the only study that investigated a range of conditions which included environmental extremes and that made direct comparisons of amictic and mictic reproduction.

The clear implication is that reduced sexual reproduction is a "general" response to physiological stress of several types. The cited works have investigated one or two factors in isolation and have not interpreted the environmental effects as one, general reproductive response. Furthermore, this hypothesis predicts that the production of sexual females and their fecundity will be similarly diminished by extremes of other factors such as pH, dissolved oxygen or ammonia concentrations. A generalized model of the reproductive response curves of sexual and asexual females is presented in Fig. 6. The reproductive rate of asexual and sexual females is hypothesized to be affected differentially by extreme temperatures, salinities, and low food levels. Asexual females are capable of reproduction over a broader range of environments than sexual females. A consequence of the broader amictic curve is that zones of exclusively asexual reproduction exist at environmental extremes. In this

region, male production ceases, presumably because of the failure to produce sexual females and/or reduced sexual female fecundity. This model suggests that sexual reproduction in rotifers does not occur when environmental conditions deteriorate as suggested by Williams (1975, p 24). Rather, the greater physiological constraints on sexual reproduction require sex to occur in moderate environments where sexual female production and fecundity are not repressed.

The appearance of mictic females in rotifer populations has consistently been interpreted as an induction. Particular stimuli such as photoperiod in *Notommata* sp. (Pourriot and Clement, 1981), alpha-tocopherol in *Asplanchna* sp. (Gilbert, 1980), and population density in *Brachionus plicatilis* (Gilbert, 1977; Pourriot and Clement, 1981; Pourriot and Snell, 1983) have been identified as mictic inducers, although the mechanisms of action are still not understood. The data presented here suggest that the mictic response in *B. plicatilis* might best be viewed as a release from repression. At environmental extremes of temperature, salinity, or food level, mictic reproduction is differentially repressed compared to amictic reproduction. As the environment moderates, mictic females begin to appear. I believe that this appearance of mictic females when an environment becomes more moderate is best viewed as a release from repression rather than an induction as has been suggested by Lubzens *et al.* (1980, 1985). If this hypothesis is correct, our view of the mictic response in rotifers needs to be modified to incorporate both inducible and repressible modes of life cycle regulation.

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