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**The Effect of Temperature on Reproductive Characteristics of an
Asexually Reproducing Rotifer (Class Bdelloidea)**

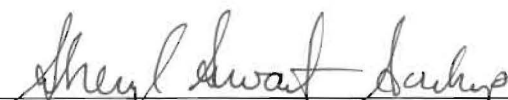
A Senior Research Honors Paper Presented
by

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April 24, 1997

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ABSTRACT

Most organisms exhibit sexual reproduction. Rotifers of the class Bdelloidea, however, seem to be a notable exception to this pattern. No male bdelloid individuals have ever been observed, and females apparently reproduce entirely through parthenogenesis. Sexual reproduction occurs in rotifers of the class Monogononta, and in many cases it is induced by environmental cues (e.g. temperature, diet). In this study, *Philodina* sp. was examined to determine if variations in temperature could induce a sexual cycle in bdelloid rotifers. Sibling individuals (clones) were raised with equal amounts of food at 20°C and 30°C. Newly hatched offspring produced by these individuals were counted and removed at approximately 12 hour intervals until the parent died. Individuals exhibiting unusual characteristics were isolated as possible males and raised for closer examination. No males were positively identified. However, temperature greatly affected reproduction rates. At 30°C individuals had a significantly greater rate of reproductive ($Q_{10} = 2.34$) and produced more offspring than those at 20°C. Furthermore, age at the start of reproduction was significantly earlier at 30°C than at 20°C. Unexpectedly, presumed genetically identical clones of *Philodina* sp. showed significant variation in lifespan when raised in equivalent environmental conditions

Key Words: *Philodina*, Rotifera, Bdelloidea, asexual reproduction

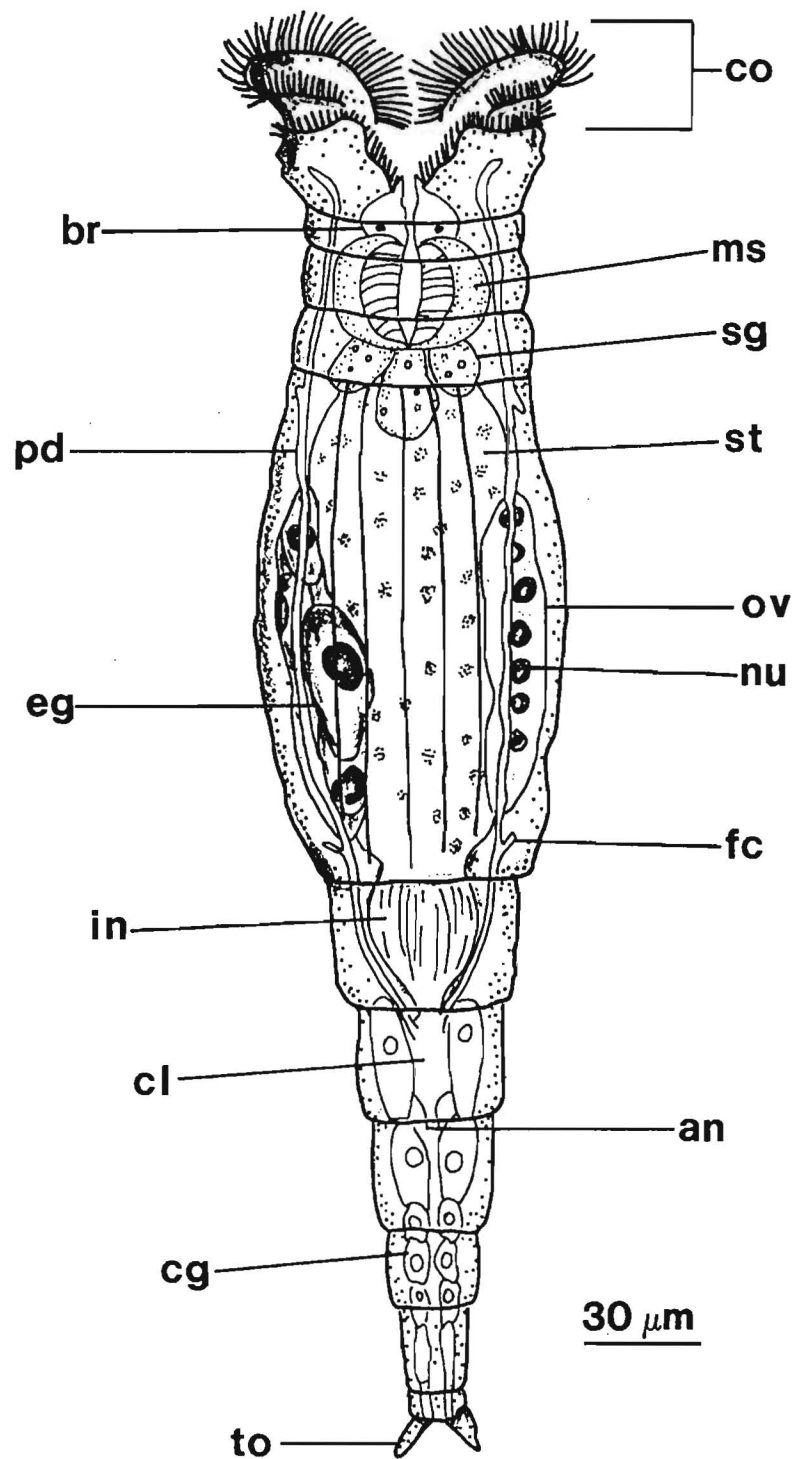
INTRODUCTION

Rotifers in the class Bdelloidea range in size from 0.05 to 1.0 mm and are common among freshwater microscopic fauna. Terrestrial species are found in low, moist vegetation such as mosses, lichens, and liverworts. As semisessile inhabitants of benthic habitats, they typically move about with an inch-worm motion that is created by alternately attaching to and releasing from the substratum with adhesive glands located on their toes and on an anterior dorsal projection called the rostrum (Fig. 1). Bdelloids can also swim by a ciliary water current produced by two trochal discs that form the corona. The corona, a defining characteristic of rotifers, is also used by bdelloids to generate a feeding current which sweeps food particles to the buccal field and into the mouth.

Bdelloids are digonants, meaning that they possess two gonads. They are eutelic organisms; no cell division occurs in the postembryonic period (Clément and Wurdak, 1991). Eggs are formed in paired ovaries (vitellaria) which contain the cytoplasmic elements and all the nuclei of potential egg cells. During embryonic development, germinal nuclei undergo two mitotic divisions to produce one diploid egg and two polar bodies (Hsu, 1956).

The most striking characteristic of bdelloid rotifers is that they appear to be the only major group of animals that does not exhibit sexual reproduction (Maynard Smith, 1978). Male bdelloids have never been observed. Females reproduce asexually via parthenogenesis, and since parthenogenetically produced offspring gain all of their

Figure 1. A typical bdelloid rotifer. An = anus, br = brain (with eyespots), cg = cement gland, cl = cloaca, co = corona, eg = egg, fc = flame cell, in = intestine, ms = mastax, nu = nucleus, ov = ovary, pd = protonephridial duct, sg = salivary gland, st = stomach, to = toe. The ovary is a vitellarium which contains the nuclei and cytoplasmic elements of future egg cells. (Modified from Wallace and Snell, 1991)



genetic material from a single parent, they are assumed to be genetically identical to the parent.

Other rotifers can reproduce sexually (Class Monogononta). In this group, two female types are present (Fig. 2): amictic (asexual), and mictic (sexual). Amictic females produce diploid eggs that develop without fertilization into amictic females (Starkweather, 1987). Periodically, however, amictic females of some species will produce eggs which give rise to mictic females (also diploid). The frequency at which the mictic cycle occurs is often controlled by environmental stimuli such as changes in diet or temperature (e.g. Lubzens et al., 1980; Gilbert and Thompson, 1968), and these environmental cues differ among species. The mictic eggs that mictic females produce are the haploid products of meiosis and unless fertilized, they will develop into a haploid male. In some species, amphoteric females exist (Fig. 2) that can produce both mictic and amictic eggs. When mictic eggs are fertilized they become resting eggs, which are highly resistant to adverse conditions (e.g. dessication, extreme cold or heat). Resting eggs give rise to amictic females. Males are generally smaller and shorter lived than females (Starkweather, 1987). They usually lack a gut and have a reduced corona.

A complete break from the near universal reproductive strategy of sexual reproduction is apparently evident in bdelloid rotifers. A central theme of evolutionary theory is the need for genetic variability (assumed to be generated through sexual reproduction). If indeed bdelloid rotifers have only asexual reproduction, the current view of sexual reproduction's role in maintaining genetic variability comes into question. However, complete asexuality in bdelloid rotifers has not yet been satisfactorially

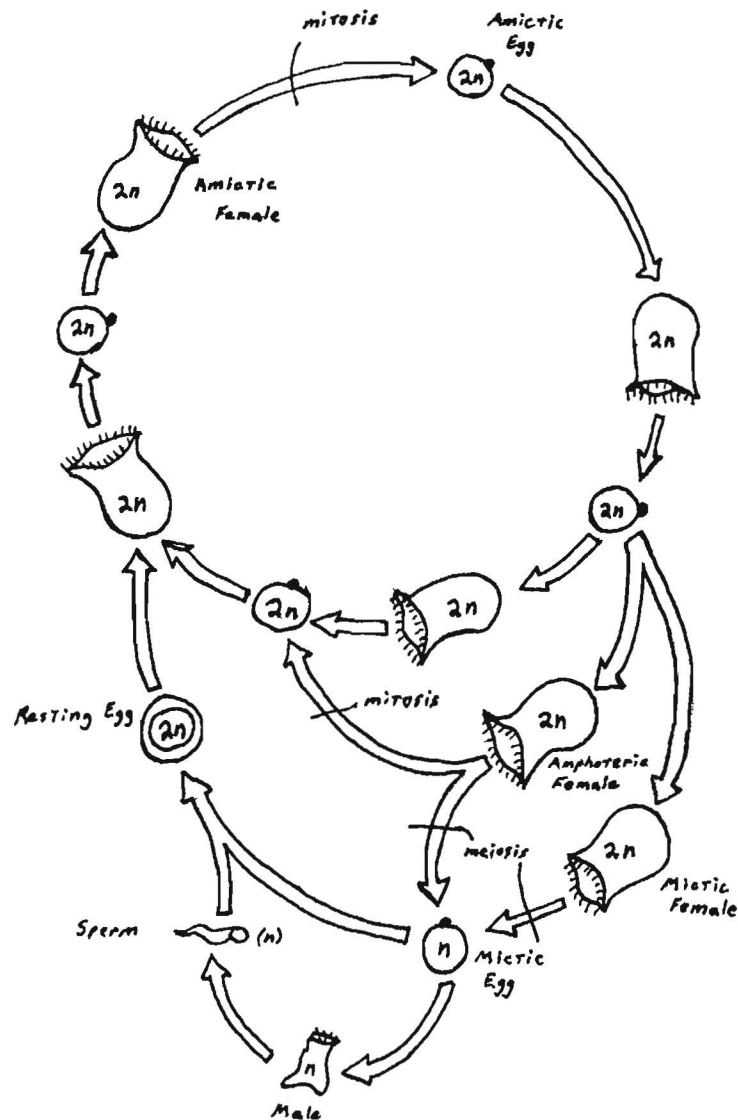


Figure 2: Generalized life cycle of monogonont rotifers. The asexual cycle involves a diploid amictic female producing a diploid egg which directly develops into another female. In response to environmental stimuli, amictic females produce eggs which develop into mictic (sexual) females. The eggs produced by mictic females are haploid and they either develop into haploid males or they are fertilized and become resting eggs. (Modified from King and Snell, 1977)

determined. Though they have been well studied, little work has been done attempting to induce sexual reproduction. This project examined the reproductive cycle of a representative bdelloid rotifer, *Philodina* sp., to determine the ability of temperature to stimulate sexual reproduction. Also, the effect of temperature on the reproductive rate, the number of offspring produced, and the length of the prereproductive period, reproductive period, and lifespan was evaluated.

METHODS

Two experiments were run in which parent (P) individuals of *Philodina* sp. were raised in individual, covered wells with equal food amounts (1×10^5 cells/ml, measured with a haemocytometer) at two temperatures (20°C and 30°C). This food concentration was in excess of the daily clearance rate of these rotifers, as was evident by the observation of uneaten food particles in wells 24 hours after feeding. The diet consisted primarily of active baker's yeast (Fleischman's) mixed in filtered pond water (filtered to 0.4 μ m). However, on alternating days a 1:1 mixture of yeast and the unicellular alga *Chlamydomonas reinhardtii* was provided. Pond water was obtained from Angler's Lake in Bloomington, IL. Animals were raised in 1 ml of food and water and this mixture was replaced daily. Individuals of *Philodina* sp. were obtained from Carolina Biological Supply. When newly hatched offspring were observed in a P individual's well, the parent was transferred to an empty well. Offspring which exhibited unusual characteristics (e.g. deformed corona, slow growth) were isolated as possible males and raised for further study. If they produced eggs they were considered to be females.

Mean values for the rate of reproduction, the number of offspring produced, and the length of the prereproductive period, reproductive period, and lifespan of animals reared at 20°C and 30°C were compared using a t-test. To ascertain which type of t-test to use (i.e. assuming equal or unequal variances), an F-test was performed to determine if variances were equal between data sets. Prereproductive period was measured from the hatching time of the P individual to the hatching time of the first offspring produced. Reproductive period was measured from the first to the last offspring produced by the P individual. Rate of reproduction was calculated using a least-square regression analysis of the number of progeny produced during the reproductive time.

Experiment 1:

Ten P individuals (not siblings) which were all hatched within 48 hours of each other were raised at each temperature. Once a day, newly hatched offspring (F1 individuals) were isolated and raised until they were observed to produce a third generation (F2). Due to the rapid increase in rotifer numbers, this experiment soon became too time consuming to continue and was not completed. Recovered data were limited.

Experiment 2:

Five P sibling individuals (clones) were raised at each temperature. These individuals were produced by a single offspring of a 20°C P individual from Experiment 1, and all hatched within 24 hours of each other. Twice a day, at approximately twelve hour intervals, wells were examined for new F1 individuals. These individuals were counted and discarded.

RESULTS

No males were positively identified. Individuals which were isolated as potential males exhibited unusual characteristics such as delayed reproduction (i.e. the prereproductive period was greater for the animal than it was for its siblings by 2 or more days), maternal delayed reproduction (i.e. the animal was produced after a pause of 3 or more days in the mother's offspring production), and morphological variations (e.g. small corona, hooked posterior end). All but two individuals which were isolated as possible males produced offspring. Of the two animals which did not reproduce, one disintegrated upon death and the other fully contracted when it was fixed in formaldehyde. In both cases, further examination of the animal was impossible.

Animals from the 20°C treatment (Expt. 1) were excluded from further analysis because the experiment was terminated before sufficient offspring were produced. More offspring were produced by the 30°C animals in Experiment 1, so data for rate of reproduction and prereproductive period were able to be obtained. However, the experiment ended before the 30°C individuals stopped reproducing, so lifespan, reproductive period, and total number of offspring could not be recorded.

There was no significant difference between the mean prereproductive periods ($t = 0.23$, $df = 13$, $P = 0.82$) and the mean reproductive rates ($t = 0.42$, $df = 9$, $P = 0.68$) of the two 30°C experiments (Table 1, Figs. 3, 4).

Temperature significantly influenced the reproductive biology of *Philodina* sp. The mean prereproductive period of the 30°C (Expt. 1) treatment was significantly longer ($t = 9.7$, $df = 12.8$, $P = 4.8 \times 10^{-7}$) than that of the 20°C (Expt. 2) treatment. Also, the mean

Table 1. Average values (mean \pm standard deviation) for all measured variables. Negative signs (-) indicate that insufficient data were collected to record a value.

	Expt. 1, 30°C (n = 10)	Expt. 2, 30°C (n = 5)	Expt. 2, 20°C (n = 5)
Prereproductive period (hrs)	45.6 \pm 21.0	36 \pm 12	120 \pm 8.5
Reproductive period (hrs)	-	305.2 \pm 28.2	360 \pm 156.2
Lifespan (hrs)	-	434.4 \pm 111.7	523.2 \pm 226.2
Rate of reproduction (#/hr)	0.19 \pm 0.092	0.18 \pm 0.028	0.076 \pm 0.017
Number of offspring	-	54.6 \pm 6.1	29.4 \pm 16.0

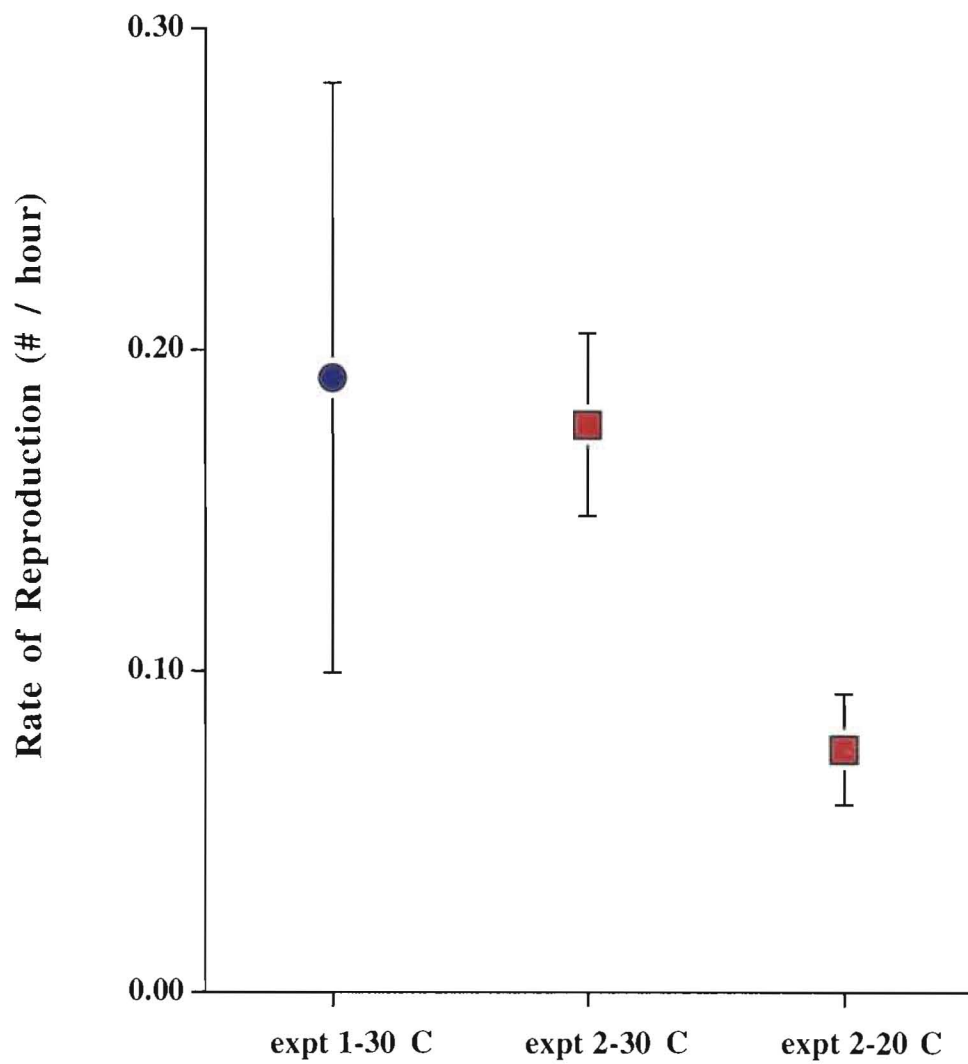


Figure 3. Rates of reproduction (mean \pm 1 standard deviation) for animals in each experiment. The rate of reproduction is calculated from the first to the last offspring produced.

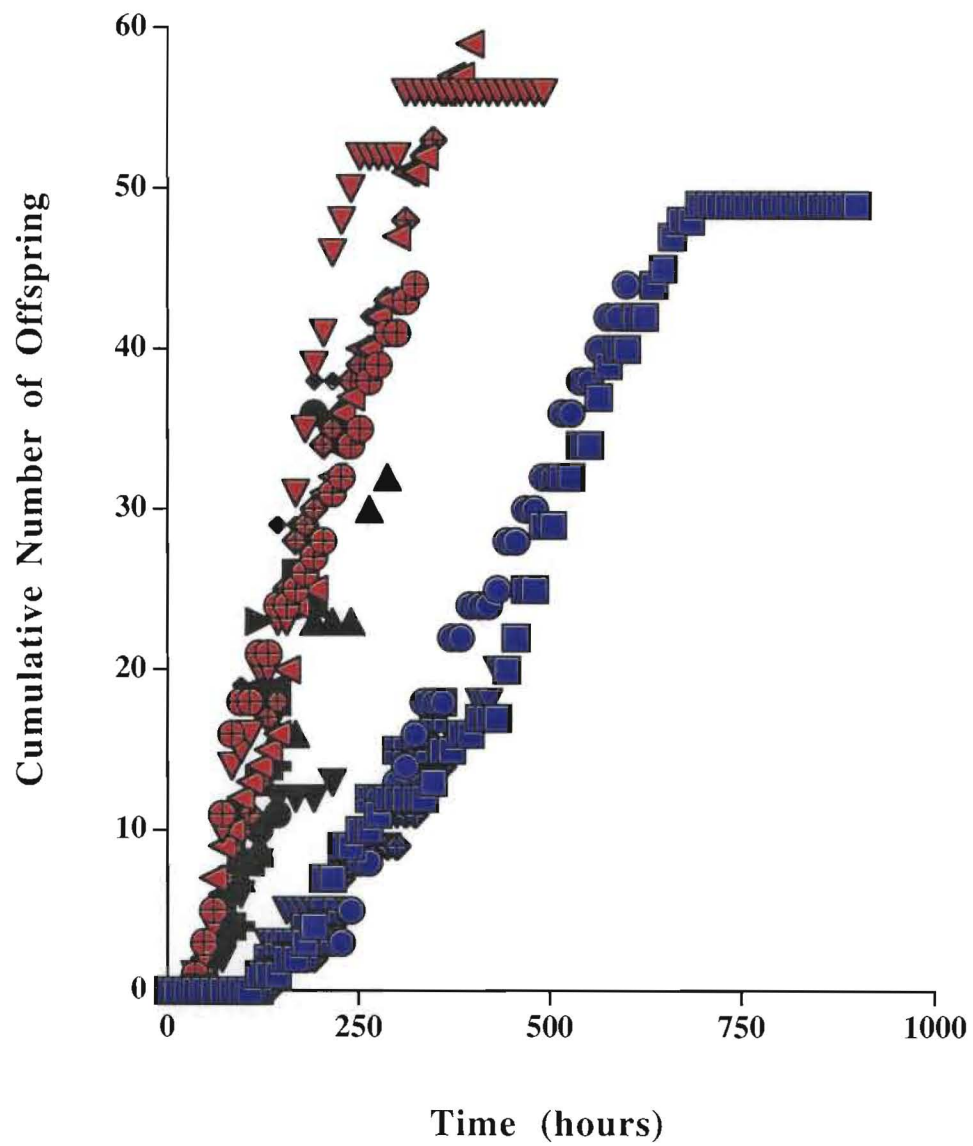


Figure 4. Total number of offspring produced by each animal. red symbols = Expt. 2 (30°C), blue symbols = Expt. 2 (20°C), black symbols = Expt. 1 (30°C). Each symbol represents a different individual.

reproductive rate of the 30°C animals in Experiment 1 was significantly greater ($t = 3.5$, $df = 8$, $P = 0.0086$) than the mean rate of the 20°C (Expt. 2) animals.

Within Experiment 2, the mean prereproductive period of the 20°C individuals was significantly longer ($t = 12.8$, $df = 8$, $P = 1.3 \times 10^{-6}$) than that of the 30°C individuals; the mean total offspring produced at 30°C was significantly greater ($t = 4.2$, $df = 5.6$, $P = 0.01$) than at 20°C; and the mean reproductive rate was significantly greater for animals at 30°C than for those at 20°C ($t = 6.8$, $df = 8$, $P = 0.00014$). Average lifespan did not differ significantly ($t = 0.79$, $df = 6$, $P = 0.46$) between the 20°C and 30°C treatments of Experiment 2 (Table 1, Fig. 5).

DISCUSSION

The fact that no male *Philodina* sp. were observed in this study does not convincingly demonstrate that males do not exist. Temperature is only one environmental variable, and even in this study it was tested only partially. Before temperature can be ruled out as a cue to initiate sexual reproduction, a wider range of temperatures (e.g. 10°C to 50°C) must be studied. If the results of a more comprehensive temperature study are negative, there remains the possibility that a different environmental variable such as diet or population density is responsible for triggering sexual reproduction in bdelloid rotifers.

Temperature had a strong and significant effect on the length of the prereproductive period, the rate of offspring production, and the total number of offspring produced (Figs. 3, 4). The effect of a 10°C increase in temperature on a rate is called the

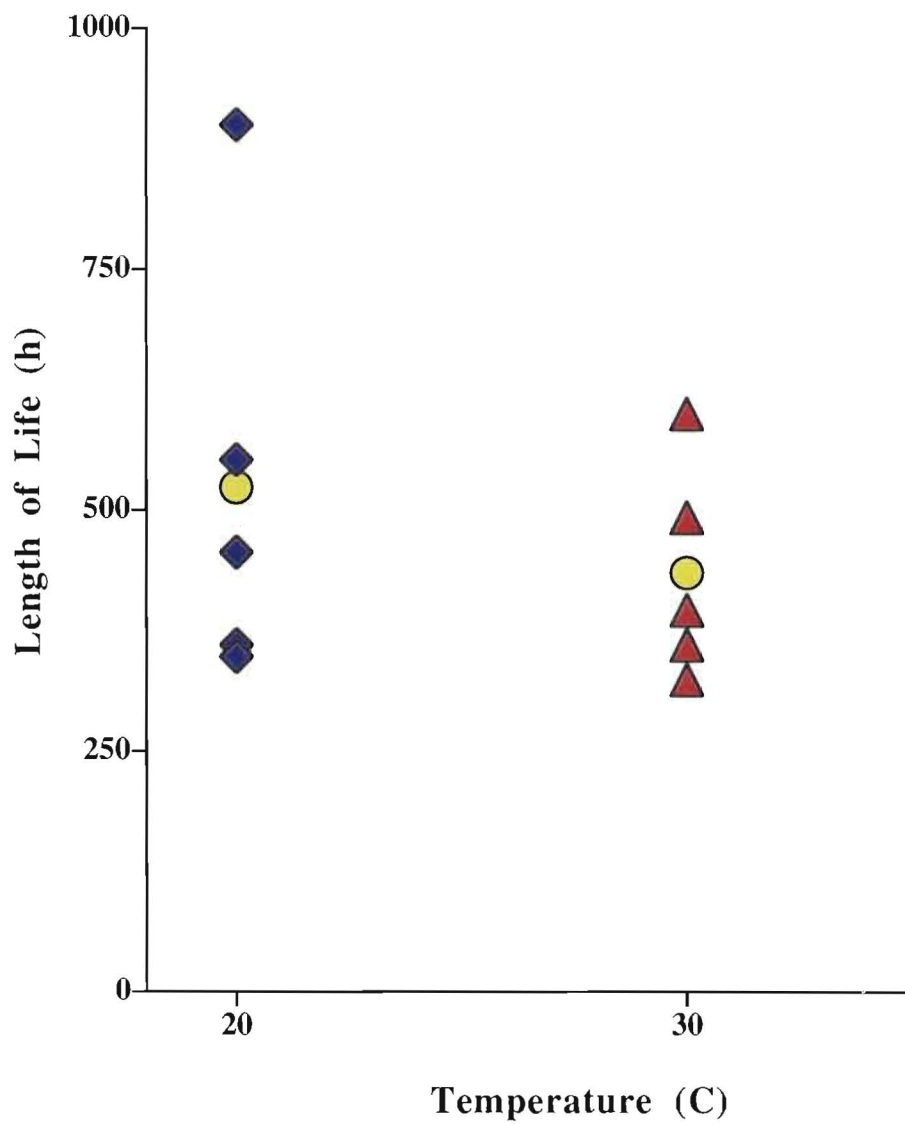


Figure 5. Range of lifespans for individuals at each temperature for Expt. 2. yellow circles = mean value.

Q_{10} (Schmidt-Nielsen, 1983). Q_{10} is typically used to describe metabolic rate changes, but in this case it was applied to the increase in rate of reproduction observed between 20°C and 30°C in Experiment 2. Since it is likely that the observed increase in reproductive rate is due to an increase in metabolic activity, it is reasonable to apply a Q_{10} calculation to rate of reproduction. The Q_{10} value for this rate change was 2.34, meaning that an increase of 10°C would increase reproductive output by a factor of 2.34. This value is similar to those calculated by Epp and Lewis (1980) for metabolic rate changes in a monogonont rotifer, *Brachionus plicatilis*, over broad temperature ranges (e.g. $Q_{10} = 1.9$ for 18°C to 30°C, and $Q_{10} = 2.4$ for 15°C to 32°C). Despite the increased reproductive activity at higher temperatures, the mean lifespans of individuals at each temperature were not significantly different, implying that bdelloid rotifers have a predetermined lifespan. This result is surprising and is likely due to the high variation in the lifespans of individuals found within each temperature treatment (Fig. 5).

Variation in the lifespan of individuals within temperature treatments is presently paradoxical. Bdelloid rotifers are thought to be completely asexual. Offspring are parthenogenetically produced clones and, therefore, are assumed to be genetically identical. However, results presented here indicate a large degree of variation in the lifespans of siblings. If each offspring from a single parent possesses the same genetic material, one implication is that some non-genetic, possibly environmental, factor caused the observed lifespan variation (Starkweather, 1987). However, within each temperature in these experiments, attempts were made to hold all known variables constant, thus reducing environmental influence as a source of variation.

Environmental variables that could not be completely controlled included variations in airflow within the incubators and time spent searching each well during offspring counts. Although each plate was covered, evaporation from cultures did occur. Since airflow was uneven inside the incubators, some wells may have evaporated more quickly than others, resulting in different food and ion concentrations in the culture media. However, it is unlikely that this would significantly influence rotifer lifespan because cultures were refreshed every day. This reduced build-up of potentially harmful ions. In addition, effects due to variations in food concentration should have been minimal because animals were fed an excess of food each day.

It is more difficult to dismiss differences in treatment of parents during offspring counts as an environmental cause of lifespan variation. All animals for each temperature were removed from and returned to their appropriate incubators as a group, so all were subjected to the same period of time outside of their experimental temperatures. However, some plates were searched longer than others, subjecting those animals to the microscope's intense light source for a longer period of time. Also, it was necessary to use force when transferring some individuals from well to well. A focused stream of water was used to remove animals which had adhered to the well wall. Although care was taken not to injure the animals, it is possible that some sustained damage and suffered a decrease in lifespan as a result. However, the behavior of animals which were treated in this way did not appear to differ from other individuals, and there was no apparent relationship between early mortality and forceful removal.

An alternative explanation for variation in lifespan is given by Lansing (1947) who determined that variation in survivorship and reproduction rates among sibling rotifers is related to maternal age. Offspring generated by young females have a greater reproductive success and a longer lifespan than offspring produced by older females. This effect is cumulative, and over successive generations in a lineage derived from only old females, a noticeable decrease in lifespan and reproduction will occur. The reverse is true for a lineage derived from young females. Lansing proposed that at the cessation of growth, a cytoplasmic "aging factor" accumulates in the female and this is inherited by offspring. In a review of rotifer aging studies, King and Miracle (1980) mention that the Lansing effect is found in bdelloids. However, they dismiss further consideration of bdelloids because "the complete lack of sexual reproduction in this group renders them inappropriate for most types of genetic manipulation."

The results of this study are not explained by the Lansing effect. The variation in lifespan found in Experiment 2 was among sibling individuals that had all hatched within 24 hours of each other, and it is doubtful that the difference in maternal age was great enough to produce an effect as extreme as those observed here (range of lifespans: 20°C, 348 - 900+ hrs; 30°C, 324 - 600 hrs, Fig. 5). These data suggest another source of variation.

It is possible, though highly speculative, that variation in lifespan in bdelloid rotifers results from mechanisms which allow or produce extremely high mutation rates. If such mutations occurred frequently in the egg nuclei of a female's vitellarium, they could potentially lead to variation among siblings. Deleterious mutations would be

selected against, but positive ones would be fixed in the population quickly by the high fecundity of rotifers. Assuming these mutations were specifically associated with genes controlling longevity, rate of reproduction and other characteristics would not be affected. This would explain the low degree of variation found in the reproduction rates of *Philodina* sp. However, there is no clear explanation for why a high mutation rate would be limited to genes which controlled longevity, and this possibility is suggested here only as an explanation for the lack of variation observed in other reproductive characteristics. To test the possibility of variation among siblings due to mutation, it will be necessary to compare the genomes of siblings and parents.

A final point merits discussion. The mean lifespans of animals from the 20°C and 30°C treatments in Experiment 2 were not significantly different (Fig. 5, Table 2). This implies a pre-established lifespan for bdelloid rotifers that is not dependent upon environmental variables. If this is the case, however, our results indicate that individuals raised at low temperatures will not reach their full reproductive potential. The 20°C individuals from this experiment produced half the number of offspring that the 30°C individuals produced (Table 1). Since rotifers are eutelic, it is assumed that the total number of potential egg cells that they can produce is established during embryonic development. No new egg nuclei can be produced in the post-embryonic stage, so the greater number of offspring observed in the 30°C treatment can not be explained by an increase in egg nuclei production after maturity is reached. The 20°C individuals produced fewer offspring because they died before they finished their reproductive cycles. Premature mortality due to a pre-set lifespan would be disadvantageous if it did

not permit an animal to reach its full reproductive potential. Natural selection should favor a lifespan that permits the entire reproductive cycle to be carried out, thus ensuring a maximal reproductive output.

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