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# Experimental Studies on the Effects of Certain Environmental Factors on Fecundity and Longevity of the Rotifer

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# Experimental Studies on the Effects of Certain Environmental Factors on Fecundity and Longevity of the Rotifer[1]

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*Abstract. Philodina megalotrocha* were grown in isolation culture in Knop's solution or variations thereof, buffered at pH 8.4, at a constant temperature of 30° C. Temperature toleration ranged from 5° C. to 35°C. Upper and lower limits of pH tolerance were pH 10.2 and pH 4.0. Longevity in low salt concentrations (0.02 percent) was increased over that of high concentrations; egg production was not altered except at high concentrations (0.06 percent), at which point it was very low. The effects were tested, one at a time, of high and low concentrations of calcium, magnesium, and potassium. In low calcium solutions longevity was increased, but when magnesium was used as a variable and calcium and potassium held constant, the pattern for calcium did not occur. The results with potassium were similar to those obtained with calcium. Also, potassium, under the conditions of this experiment, appeared to affect longevity. In low potassium concentrations fertility was approximately doubled.

It is a well known fact that animal populations in nature exhibit a seasonal rhythmicity that is predictable within reasonable limits. Further, a complex of environmental factors is generally known to affect directly or indirectly these seasonal fluctuations.

Concerning the Phylum Rotifera, relatively little is known of the causative agents active in the marked seasonal population fluctuations shown by this group. The first experimental work carried on with rotifers was stimulated by the peculiar patterns of reproduction exhibited by these animals. Shull (1911) and Whitney (1914) using *Hydatina senta* demonstrated environmental changes which affected the ratio of parthenogenetic to sexual forms. They showed that any marked or sudden change in the environment (food, temperature, oxygen, or ionic) could cause males to be produced and hence winter or resting eggs formed.

After the early impetus of the problem of male production began to wane, others took up the work of determining the effect of variations of the culture medium upon the life history of rotifers (Noyes, 1922; Dal Bianco, 1924; Finesinger, 1926; Jennings and Lynch, 1928; Behrens, 1933; and Lansing, 1942).

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It was the purpose of this investigation to obtain, in the laboratory under controlled conditions, further information concerning the effects of certain environmental factors upon the rotifer.

## Materials and Methods

The rotifer *Philodina megalotrocha* was used throughout the investigation. The rotifers were kept in stock cultures of the ordinary wheat-grain infusion type.

The isolation technique was employed in all of the experiments. Individual organisms were cultured in one drop of medium in concavities of depression slides, which were placed on glass supports over water in a closed moist chamber. Throughout the experiments the test animals were observed at twenty-four hour intervals. At this time records were taken on general condition, number of eggs laid, and time of death. In making the observations, both a binocular dissecting microscope and a compound microscope were employed. Also at the time of observation, the rotifers were transferred by means of a micropipette to fresh drops of culture fluid on clean depression slides.

In order to start an experimental series, eggs known to be of similar age were hatched and the resulting animals used as test organisms. In this way it was possible to get experimental animals that were of approximately the same age, an important factor in an investigation of this type. For each experiment twenty rotifers formed a sample. After preliminary trials it was determined that this number was sufficient to give valid results.

A well defined medium was selected so that variations could easily be carried out and accurately measured. With this in mind, a 0.04 percent Knop's solution (buffered at pH 8.4) was employed as a control medium. This solution contained three salts—calcium nitrate, potassium nitrate, and magnesium sulphate. The culture solution was prepared as follows: A 1 percent stock solution was made by adding 10 ml. of 10 percent calcium nitrate to 7.5 ml. of 5 percent potassium nitrate and 7.5 ml. of 5 percent magnesium sulphate. Then a 0.04 percent solution was prepared by adding 4 ml. of the 1 percent stock solution to 96 ml. of distilled water. The pH was properly adjusted, and 5 ml. of the correct buffer solution was added for every 100 ml. of culture fluid. The buffer solutions were prepared after the system devised by Mathews (1931). All pH values were repeatedly checked with a Beckman glass electrode pH meter.

The animals grown in 0.04 percent Knop's solution or variations thereof were able to develop and to lay eggs which were viable. The longevity and fecundity, to be sure, were lessened somewhat in

comparison with experiments on well fed individuals. However, it was felt that this technique allowed for a greater degree of control of the components of the medium. Other workers have employed this technique with the rotifer (Dal Bianco, 1924; Finesinger, 1926).

## Discussion and Results

### Temperature

Although temperature is not the only limiting environmental factor, it does trigger and regulate many biological reactions. Edmondson (1946) summarized this, ". . . temperature may be imagined as a sort of master control which determines the basic rates at which organisms can metabolize".

For this series of experiments the standard stock solution of 0.04 percent Knop's solution was prepared and buffered at pH 8.4. Temperatures ranging from 5° C. to 35° C. were tested, and the results are given in Table 1. It was found that the optimum temperature for this species in this medium was 25° C. At this point the greatest longevity and fecundity resulted. The animals fared about as well at 30° C., and this was the temperature that was selected as a constant for the remainder of the investigation. Temperatures above 35° C. or below 5° C. were incompatible with life for this rotifer.

**Table 1**

The Effects of Temperature on Longevity and Fecundity of *P. Megalotrocha;* Animals in 0.04 Percent Knop's Solution, Buffered at pH 8.4.

| Temperature | Number in sample | Number of eggs | | Length of life (days) | |
|---|---|---|---|---|---|
| | | Total | Average | Maximum | Average |
| 5°C. | 20 | 1 | 0.05 | 0 | 0 |
| 10°C. | 20 | 8 | 0.4 | 5 | 2.1 |
| 20°C. | 20 | 22 | 1.2 | 7 | 3.7 |
| 25°C. | 20 | 39 | 2.0 | 10 | 5.5 |
| 30°C. | 20 | 28 | 1.4 | 8 | 4.8 |
| 35°C. | 20 | 2 | 0.1 | 5 | 1.8 |

### Hydrogen Ion Concentration

The value of hydrogen ion concentration as a limiting factor for the rotifer has long been a controversial issue. The idea of acid and alkaline limited species is well known to anyone working extensively with the group. Actually the number of rotifer species tolerant to both types of environment is greater than both of the groups restricted to either acid or alkaline habitats.

In all of the experiments standard stock media of 0.04 percent Knop's solution were prepared as described earlier; solutions ranging from pH 4.0 to pH 11.2 were tested for their physiological effects. As can be seen in Table 2, at pH 4.0 the rotifers did not live through

the first twenty-four hour period; however, at pH 5.0 the animals were able to survive and to lay eggs so that the first complete set of data was obtained at this pH value. From pH 5 to pH 8.4, the length of life and fecundity were increased. Levels of pH above this were detrimental, and longevity and fecundity were progressively lessened thereafter until pH 11.2 was reached. At this value there were no survivors at the end of the first twenty hours (Figure 1).

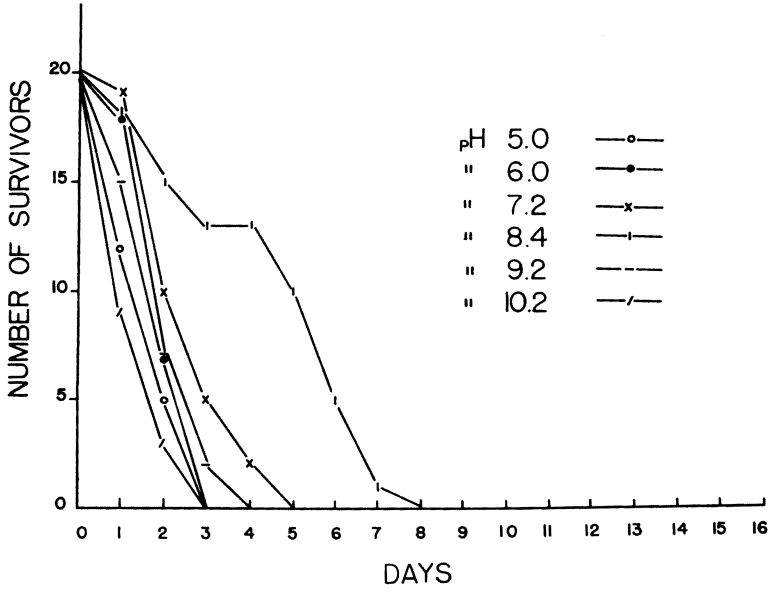


Figure 1. The effect of varying pH on the duration of life of *Philodina megalotrocha*.

When the data are reviewed, it can readily be seen that during immaturity and adulthood the curves are quite similar. It is in the senile phase that the pH of 8.4 allows for a greater longevity. Lansing (1942) noted this phenomenon, although he obtained a steady increase in longevity until pH 9.6 was reached. He suggested that perhaps the high alkalinity offset the increased acidity that is characteristic of ageing cells, thus increasing their longevity. It is also known that sodium hydroxide increases permeability of the cell. Since higher amounts of this compound are present in the buffer for high pH values, it is possible that the increased permeability could cause greater longevity. Above pH 8.4, however, the concentration of some one of the ions probably becomes toxic, and a decrease in longevity results.

The animals cultured in media buffered at pH 6.0 and 7.2 have a relatively short period of senility, whereas those at pH 8.4 have a much longer senile period. At pH 7.2 only 2 animals survived to

the fourth day, and these were dead on the fifth day. The pH of 8.4 seemed to be the optimum figure for *P. megalotrocha,* since 13 animals were alive on the fourth day, 10 on the fifth, 5 on the sixth, 1 on the seventh, and the last one was dead on the eighth day. On the other hand, only 2 animals were alive on the third day at pH 9.2 and these died by the fourth day. At pH 10.2, none survived to the third day.

Fecundity did not vary so markedly as length of life through the pH range studied. The optimum egg production was at pH 8.4 with a total of 28 eggs laid—an average of 1.4 per animal. At pH 7.2, 14 eggs were laid, with an average of 0.7 per individual. At pH 9.2, 9 eggs were laid, and the average per indivdual was 0.4 eggs (Table 2). Thus it can be seen that pH 8.4 is optimum, under the conditions of this experiment, for both fecundity and longevity of *P. megalotrocha.*

Table 2
The Effect of pH on Length of Life and Fecundity of *P. Megalotrocha;* Animals in 0.04 Percent Knop's Solution, at a Constant Temperature of 30° C.

| pH | Number in sample | Number of eggs | | Length of life (days) | |
|---|---|---|---|---|---|
| | | Total | Average | Maximum | Average |
| 5.0 | 20 | 3 | 0.2 | 3 | 1.8 |
| 6.0 | 20 | 7 | 0.4 | 3 | 2.3 |
| 7.2 | 20 | 14 | 0.7 | 5 | 2.8 |
| 8.4 | 20 | 28 | 1.4 | 8 | 4.8 |
| 10.2 | 20 | 9 | 0.4 | 4 | 2.2 |
| 9.2 | 20 | 2 | 0.1 | 3 | 1 6 |

## Total Salt Concentration

In order to get an idea of the tolerance of *P. megalotrocha* to varying salt concentrations, a series of experiments was devised using Knop's solution of 0.02 percent, 0.04 percent, and 0.06 percent. Preliminary experiments showed that the animals could exist in 0.01 percent Knop's solution, but could not survive in concentrations above 0.06 percent (Table 3).

Table 3
Effects of Variation of the Total Salt Concentration on Longevity and Fecundity of the Rotifer. Culture Medium Buffered at pH 8.4 and at a Constant Temperature of 30° C.

| Salt concentration | Number in sample | Number of eggs | | Length of life (days) | |
|---|---|---|---|---|---|
| | | Total | Average | Maximum | Average |
| Low salt 0.02 percent | 20 | 32 | 1.6 | 10 | 5.9 |
| Medium salt 0.04 percent (control) | 20 | 28 | 1.4 | 8 | 4.8 |
| High salt 0.06 percent | 20 | 8 | 0.4 | 6 | 3.2 |

Animals grown in the 0.02 percent solution (buffered at pH 8.4) exhibited a longer life span than the controls (0.04 percent at pH 8.4), and showed a still greater increase in longevity over the group in the 0.06 percent solution. The life span of the rotifers grown in the 0.02 percent solution reached nine days, with 5 alive on the seventh, 3 on the eighth, and 1 on the ninth day. In the control, only one individual was alive on the seventh day, and all were dead on the eighth day. The longevity in the high salt concentration of 0.06 percent was greatly reduced when compared with the other two samples (Figure 2).

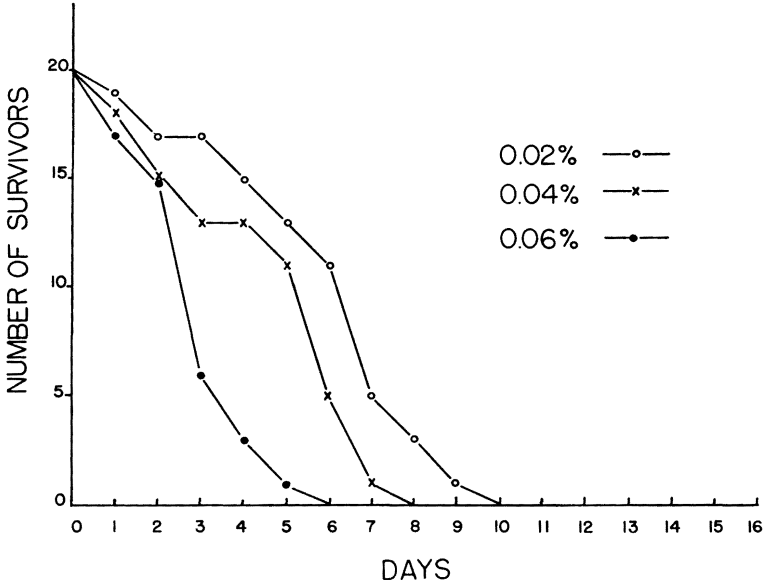


Figure 2. Survival of *Philodina megalotrocha* in high, medium, and low total salt concentrations.

The reproductive rates at these salt concentrations were about equal for those in the 0.02 percent and 0.04 percent solutions. A total of 32 eggs was laid, averaging 1.6 per individual, in the low (0.02 percent) salt concentration; and 28 eggs, averaging 1.4 per animal, were produced in the 0.04 percent solution. Egg deposition in the 0.06 percent solution fell off to a total of 8 for all individuals (Figure 4). It appears that total salt concentrations much above 0.04 percent are detrimental to both fecundity and longevity of *P. megalotrocha*.

## Variation of Salt Balance

In order to test variations of the salts used in this investigation, three experimental series were devised. First, using calcium as a variant, two media were prepared—one with high calcium content

with respect to magnesium and potassium, and the other with low calcium content with respect to magnesium and potassium. Second, using magnesium as the variant, similar media to those for calcium were prepared. Third, the same procedure was utilized to prepare media with high and low concentrations of potassium. In all cases, the media were so prepared that the total salt concentration was not altered and always remained at 0.04 percent. A control was run in the normal 0.04 percent Knop's solution for comparison.

Calcium. Lansing (1942), working with cells of rotifers, toads, and planarians, showed that calcium increased in ageing cells and that this increase was more marked after the cessation of growth. He suggested that the increased calcium in the cell formed insoluble compounds that decreased the permeability of the cell membrane. Since any breakdown in permeability would destroy the normal cellular function, the cell "aged" or went through a process of breakdown leading to the death of the cell.

Stock solutions were prepared as follows: 5 ml. of 10 percent calcium nitrate, 10 ml. of 5 percent potassium nitrate, and 10 ml. of 5 percent magnesium sulphate were combined to give a 1.0 percent solution. Then 4 ml. of this stock solution were added to 96 ml. of distilled water to produce a 0.04 percent salt solution of low calcium content. Similarly, a high calcium-low potassium and magnesium solution was prepared by adding 15 ml. of 10 percent calcium nitrate to 5 ml. of 5 percent potassium nitrate and 5 ml. of 5 percent magnesium sulphate. From this stock solution a 0.04 percent high calcium solution was made in the same manner as above. Lastly, a control was set up of the normal 0.04 percent Knop's solution. All of the solutions were buffered at pH 8.4.

It can be seen (Table 4 and Figure 3) that animals cultured in the low calcium-high potassium and magnesium medium exhibited a greater longevity than those grown in the other concentrations. All of the animals in the high calcium and the control media were dead on the seventh and eighth days, respectively. In the low calcium-high potassium and magnesium cultures 7 were alive on the eighth day, 5 on the ninth, 4 on the tenth, and the last animal was not dead until the fifteenth day. This is a marked increase in longevity.

Egg production under the conditions of this experiment did not vary appreciably from that of the animals kept in the control and in the low calcium media. However, the high calcium medium prevented the "normal" egg deposition.

Lansing (1942), working with *Rotifer vulgaris,* obtained similar results on longevity; but he noted that the animals in the high calcium medium produced slightly more eggs than the rotifers in the other two groups, which is in contrast to the findings of the present

work. Lansing's theory of accumulation of calcium as an agent in the ageing process of cells is substantiated in the present work. The survival curves show that the increase in life duration was actually an increase in length of the senile phase. This is in accordance with the theory that calcium begins to accumulate rapidly after the cessation of growth (Lansing 1942, 1948).

**Table 4**

The Effect of High, Medium, and Low Concentrations of Calcium, Magnesium, and Potassium, With Respect to the Other Ions of the Medium, on Longevity and Fecundity of the Rotifer. Medium at pH 8.4 and a Constant Temperature of 30° C.

| Salt concentration | Number in sample | Number of eggs | | Length of life (days) | |
|---|---|---|---|---|---|
| | | Total | Average | Maximum | Average |
| Low calcium | 20 | 35 | 1.8 | 15 | 7.6 |
| Medium calcium | 20 | 28 | 1.4 | 8 | 4.8 |
| High calcium | 20 | 16 | 0.8 | 7 | 4.3 |
| Low magnesium | 20 | 12 | 0.6 | 14 | 4.9 |
| Medium magnesium | 20 | 28 | 1.4 | 8 | 4.8 |
| High magnesium | 20 | 20 | 1.0 | 12 | 5.0 |
| Low potassium | 20 | 62 | 3.1 | 10 | 5.6 |
| Medium potassium | 20 | 28 | 1.4 | 8 | 4.8 |
| High potassium | 20 | 14 | 0.7 | 16 | 8.9 |

Magnesium. In order to ascertain the individual role of magnesium in relation to calcium and potassium, solutions of high and low magnesium content were prepared in a similar manner to that already described for calcium. A low magnesium medium was made by adding 4 ml. of 5 percent magnesium sulphate to 9 ml. of 5 percent potassium nitrate and 12 ml. of 10 percent calcium nitrate. Four ml. of this stock were added to 96 ml. of distilled water to produce a 0.04 percent total salt concentration. In the same manner 11.5 ml. of 5 percent magnesium sulphate was added to 5.5 ml. of 5 percent potassium nitrate and 8 ml. of 10 percent calcium nitrate. This was then diluted to give a 0.04 percent total salt concentration.

As can be seen in Table 4, the results in this series of experiments are not conclusive. In both the high and low magnesium solutions the test animals lived four to six days longer than those in the controls. The animals in the low magnesium culture dropped rapidly in number through the first three or four days. They then leveled off until on the ninth day 4 individuals were still alive; 3 remained on the tenth, 2 through the eleventh, twelfth, and thirteenth days, and all were extinct on the fourteenth day. The animals in the high magnesium solution exhibited a more normal curve, with the last survivor extinct on the twelfth day.

The record of egg production shows that there is very little difference between the number of eggs laid per day by the animals in the high and low magnesium concentrations. The production of eggs in the control sample was somewhat higher than the other two, suggesting that this concentration of magnesium in relation to calcium and potassium is optimal for egg production for this species.

If we accept the theory that calcium in high concentrations is detrimental to long life, as shown in the previous experiment, then we would expect the pattern of longevity to follow the concentration of calcium in any consequent experiment. Thus with magnesium, when the magnesium concentration was low, calcium concentration was high and vice versa. We would expect the survival curve to follow the calcium ion concentration, exhibiting a shortened life span in the low magnesium-high calcium and potassium solution and a lengthened life span in the high magnesium-low calcium and potassium medium. This was not the case, however, since the low magnesium-high potassium and calcium medium supported the longest life span.

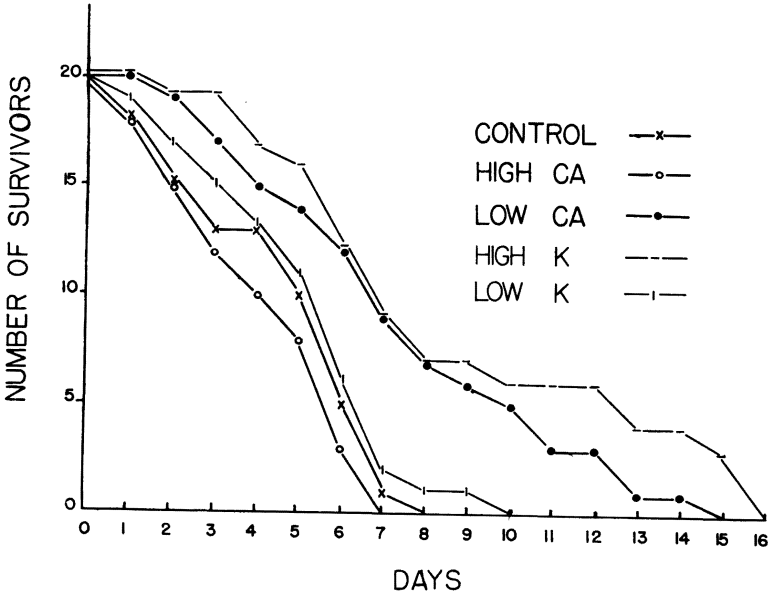


Figure 3. Effect of variation of calcium and potassium concentration on duration of life of *Philodina megalotrocha*.

Potassium. The usual stock solutions of high and low concentrations were prepared for this experiment. To provide a medium of low potassium content, 4 ml. of 5 percent potassium nitrate were added to 12 ml. of 10 percent calcium nitrate and 9 ml. of 5 percent magnesium sulphate. This was then diluted with distilled water to

give a total salt concentration of 0.04 percent. The high potassium solution was prepared by adding 11.5 ml. of 5 percent potassium nitrate to 8 ml. of 10 percent calcium nitrate and 5.5 ml. of 5 percent magnesium sulphate. This was then diluted to give the usual 0.04 percent total salt concentration. Three series of experiments were run, including the control. The data obtained for the series are given in Table 4.

The survival curve shows that the animals grown in the high potassium solution exhibited a much greater longevity than either the control or low potassium animals (Figure 3). The latter two had similar curves with the last survivor extinct on the eighth and tenth days, respectively. On the tenth, eleventh, and twelfth days, six animals in the high potassium solution were still alive; five remained on the thirteenth and fourteenth days, and three survived to the fifteenth day. All animals in this group were dead on the sixteenth day. Thus, contrary to the results obtained with magnesium, the survival of the animals seemed to follow the pattern set by the calcium ion as shown in previous experiments.

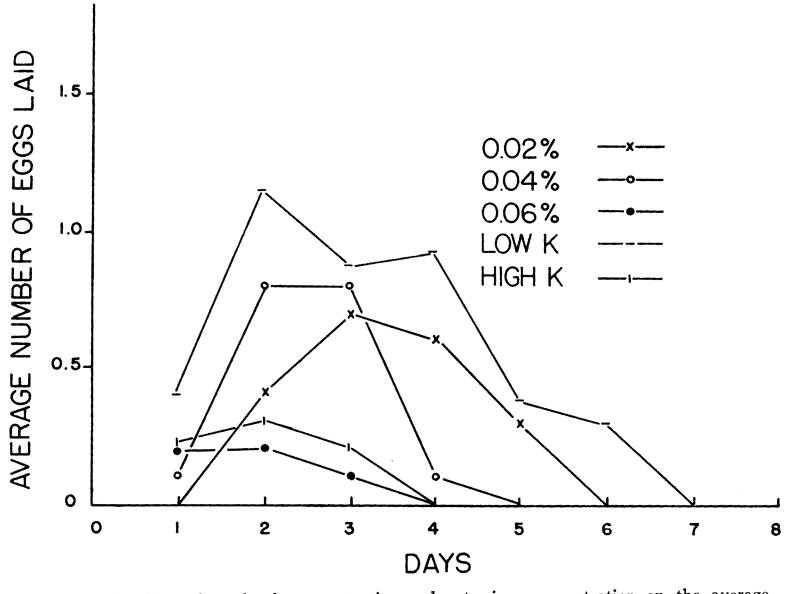


Figure 4. The effect of total salt concentration and potassium concentration on the average number of eggs laid per day by *Philodina megalotrocha*.

The effects of low potassium concentration upon fecundity of *P. megalotrocha* are very striking (Figure 4). The total number of eggs produced was 62, with an average of 3.1 per animal. This, when compared to the control with a production of 28 eggs and an average of 1.4 per animal, gives evidence that low concentrations of

potassium favor fecundity in this rotifer. The egg deposition in the high potassium solution was lower than the control; the animals in this medium produced 14 eggs, averaging 0.7 per animal.

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