Life history and ecology of Daphnia pulex ssp. pulicoides Woltereck 1932
by Blaine W LeSuer

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Botany
Montana State University

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A detailed study was made on the life history, natality, growth,, and mortality of Daphnia pulex ssp. pulicoides Woltereck 1932. In addition, a grazing study was carried out at temperatures of 5°, 10°, 15°, 20°, and 25° C. and at instar levels one through ten. Grazing data is presented in tabular form and summarized with a graph. Temperature effect on grazing rates was noted. Respiration studies were carried out at temperatures of 10°, 15°, and 20° C. at instar levels one through ten. A Q10 was calculated for oxygen consumption and also for carbon dioxide production. The Q10 was between the temperature levels of 10° and 20° C. A disucssion and a review of literature is presented. Part V includes a short summary.

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Any errors or omissions in this thesis are the sole responsibility of the author.

ABSTRACT

A detailed study was made on the life history, natality, growth, and mortality of Daphnia pulex ssp. pulicoides Woltereck 1932. In addition, a grazing study was carried out at temperatures of 5°, 10°, 15°, 20°, and 25° C. and at instar levels one through ten. Grazing data is presented in tabular form and summarized with a graph. Temperature effect on grazing rates was noted. Respiration studies were carried out at temperatures of 10°, 15°, and 20° C. at instar levels one through ten. A Q_{10} was calculated for oxygen consumption and also for carbon dioxide production. The Q_{10} was between the temperature levels of 10° and 20° C. A disucssion and a review of literature is presented. Part V includes a short summary.

PART I

INTRODUCTION

The purpose of this study was to obtain data on the biology of Daphnia pulex ssp. pulicoides Woltereck, 1932 which could be applied to the calculation of secondary productivity in Canyon Ferry Reservoir. This subspecies is listed as Daphnia schodleri Sars, 1862 in Brook's monograph (1958) and is the major zooplankter in Canyon Ferry Reservoir, an artificial impoundment located on the Missouri River near Helena, Montana (Wright, 1958).

Although it was not the primary purpose of this study to calculate secondary productivity, certain factors concerning the biology of the secondary producer must be known before an accurate measurement of secondary productivity can be obtained. These factors include rates of growth, reproduction, mortality, grazing, and respiration. In order to arrive at a better knowledge of these factors, three studies were carried out -- a life history study, a grazing study and respiration studies (oxygen consumption and carbon dioxide production).

Financial assistance was obtained from National Science Foundation Research Grant No. 3063. Laboratory facilities were made available by the Botany and Bacteriology Department, Montana State College, Bozeman, Montana.

PART II

METHODS

Life History Study

Animals collected from Canyon Ferry Reservoir were brought to the laboratory. One female was placed in a two liter flask containing filtered lake water and a generous supply of Ankistrodesmus for food. This female was watched closely for the releasing of young first-instar animals. When the young were released, they were immediately obtained, measured and placed in a 200 ml. flask containing filtered lake water and a generous amount of Ankistrodesmus cells to insure an abundant supply of food. Thirty-six flasks, each containing one first-instar D. pulex, were set up. A lighted controlled-temperature cabinet maintained at 16° C. was used to keep the experimental flasks at a constant temperature.

Daily, following the commencement of the experiment, each animal was picked out, anesthetized with six drops of chlorobutanol administered with a pipette and measured. After the beginning of the reproductive phase, the eggs or embryos carried by each mother were counted. When a mother released young they were removed from the experimental flask and counted. Cast carapaces, increases in lenght, and the number of young released were used as criteria for the determination of the passing of the animals from one instar to the next. Additional cells of Ankistrodesmus were added from time to time in order to keep the experimental animals in a well-fed condition.

To verify the reliability of the data obtained from the original 36 animals, an additional series of 12 animals was run after the first series had been completed. Thus, the life histories of 48 D. pulex were followed day by day from birth to death.

Grazing Study

The experimental animals were conditioned in filtered pond water for 24 hours prior to the commencement of each experiment. Various numbers of animals of a given instar were placed in a flask containing 100 ml. of filtered pond water. Log phase Chlamydomonas cells which were grown in liquid Modified Bristol's Solution (Bold, 1949) were centrifuged from the culture media to remove any toxic material which may have been produced by the algae. These cells were resuspended in the pond water contained in the experimental flasks. An attempt was made to obtain data on grazing rates of instars one through ten at temperature levels of 5°, 10°, 15°, 20°, and 25° C.

The cell concentration does not influence the filtering rates of zooplankton to any great extent It is for this reason that cell concentrations were not considered as important as long as they were above the level of 0.15 million cells per milliliter (Ryther, 1954).

One milliliter aliquots were withdrawn at the start and at the finish of each experiment and placed in a Sedgewick-Rafter Counting Chamber. Fifty fields were counted using a Whipple micrometer disc as the boundaries of the field. The differences in cell concentrations were then applied to Gauld's equations (1951);

$$\frac{C_t}{C_o} = e^{-nk} \qquad (1)$$

where C_o is the initial cell concentration, C_t the cell concentration at time (t), n the number of hours, and k the exponential function, and

$$F = Vk \qquad (2)$$

F is the filtering rate and V the volume of water per animal.

Experiments were limited to one to two hours to reduce the error brought about by the algae settling out (Ryther, 1954). The animals were counted and measured at the termination of each experiment. They were then placed in a weighed crucible, oven-dried at 100° C. for 16 hours and tared.

Respiration Studies

Oxygen Consumption Determinations

Oxygen uptake was measured by means of the polarometric method of Petering and Daniels (1938). A Fischer Elecdropode was used. The dropping mercury electorde was calibrated for dissolved oxygen concentrations by measuring the difference between galvanometer deflections at -0.1 volt and -1.0 volt. Oxygen concentration in the sample was determined by the Winkler method. Several calibrations were made at various oxygen concentrations. Oxygen concentrations were plotted against the corresponding galvanometer deflection differences and a regression line fitted to the points.

It was sometimes necessary to add a supporting electrolyte to the water sample. Potassium chloride (0.1 N) was used as the electrolyte when needed.

The animals used in the experiments were conditioned in filtered pond water. Each group of animals to be used in a given experiment was kept for 24 hours in pond water held at a temperature corresponding to that at which the experiment would be run. Twenty-five animals of approximately the same instar were picked from the conditioning water and placed in 125 ml. steam sterilized glass-stoppered bottles containing fresh filtered pond water. A control bottle lacking animals was set up to correct for microorganism respiration. Observations of oxygen uptake and carbon dioxide production were obtained at the beginning and again after 24 hours, at which time the experiments were terminated.

The animals were recovered at the end of each experiment and their lengths were measured with a microscope containing an ocular micrometer disc. They were then placed in a weighed crucible, oven-dried for 16 hours at 100° C., and weighed. Experiments were run at temperatures of 20°, 15°, and 10° at instar levels one through ten.

Carbon Dioxide Production Determinations

Carbon dioxide addition to water during animals respiration was measured by a modification of the method employed by Verduin (1956a). A Beckman model GS pH meter was employed in order to give a higher degree of accuracy. The Beckman GS pH meter has an expanded scale of 1,000 units which encompasses a pH range of three pH units. The expanded scale is calibrated in terms of millivolts which need not be converted to the pH scale in this case. Since one milliliter of 0.010 N NaOH is equivalent to 10 micromoles of carbon dioxide, the number of

micromoles of CO_2 equivalent to a one unit change on the expanded scale can be calculated.

For example, if the expanded scale reading changed from 470 to 500 MV due to animal respiration and the reading became 460 MV after the addition of one milliliter of 0.010 N NaOH, then 500 minus 460 or 40 units is equivalent to 10 micromoles CO_2 per liter. Hence one unit equals $\frac{10 \text{ micromoles}}{40}$ or 0.25 micromoles CO_2 per unit. Animal respiration caused a change of 30 units (500 - 470); therefore, 30 times 0.25 micromoles CO_2 per unit equals 7.50 micromoles CO_2 per length of time covered by the experiment.

PART III

RESULTS

Life History Study

Instar-Weight Relationship

Figure 1 presents the instar-weight relationship by instar. The weights were obtained by selecting animals of each instar group and placing a known number on a tared cover slip. These animals were then oven-dried at 100° C. for 16 hours, cooled in a desiccator and weighed.

The instar weight relationship obtained in this expierment was similar to that found by Richman (1958) with Daphnia pulex var. pulicaria Forbes and Edmondson (1955) with Daphnia pulex var. tenebrosa Sars.

Growth Per Instar

Figure 2 shows the increase in length in millimeters per instar. The slope of the line is negative; that is, the younger animals showed a greater rate of growth per instar than did the older animals.

Duration of Each Instar

The average time in days for passage of one instar to the next is shown in Figure 3. Here again, the age of the animal influenced the rate of change of this process. Instars one through five passed through each succeeding instar rather rapidly. Passage from instar one to instar two took only 1.39 days, while passage from instar four to instar five

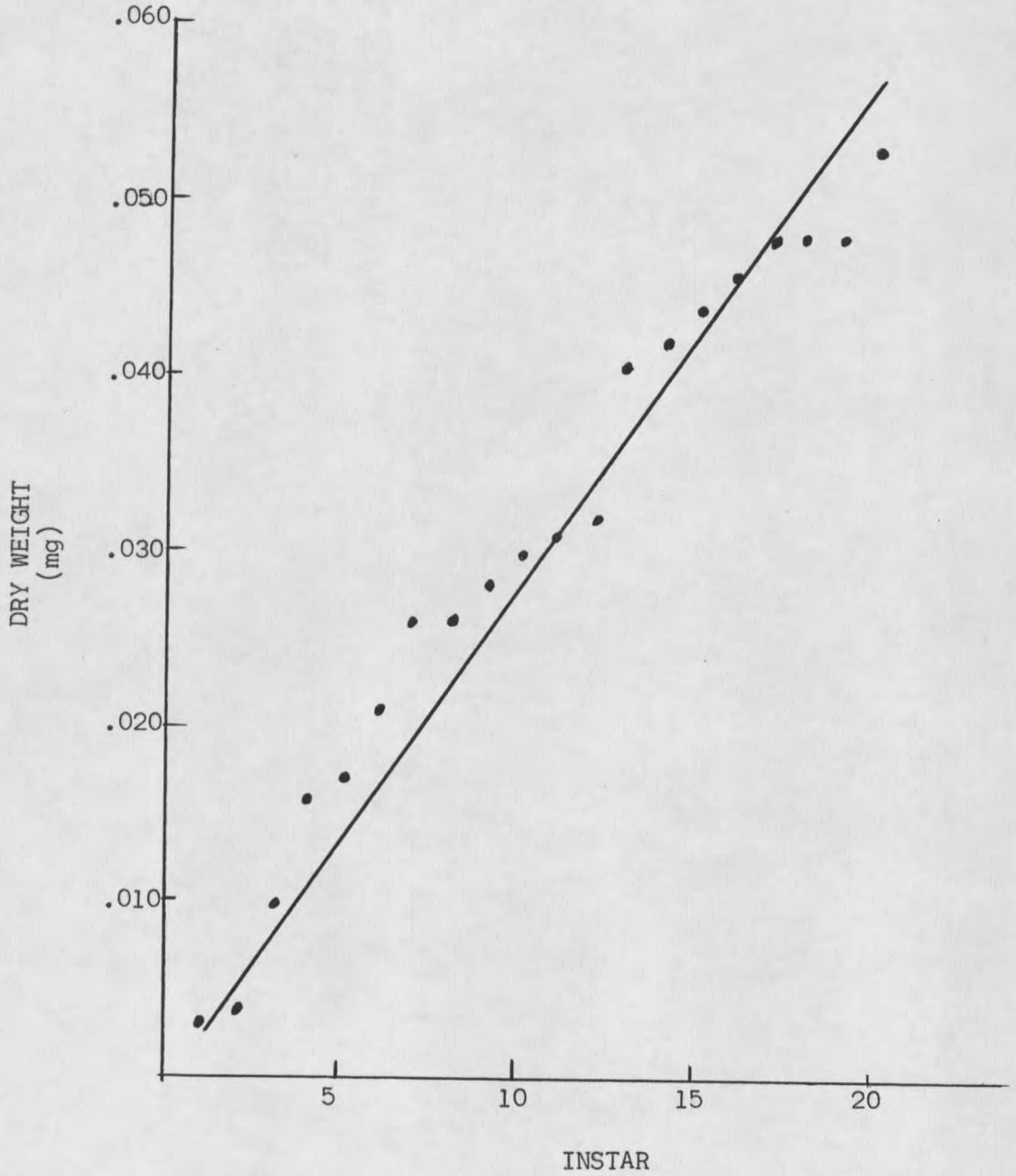


Figure 1. Instar-weight Relationship.

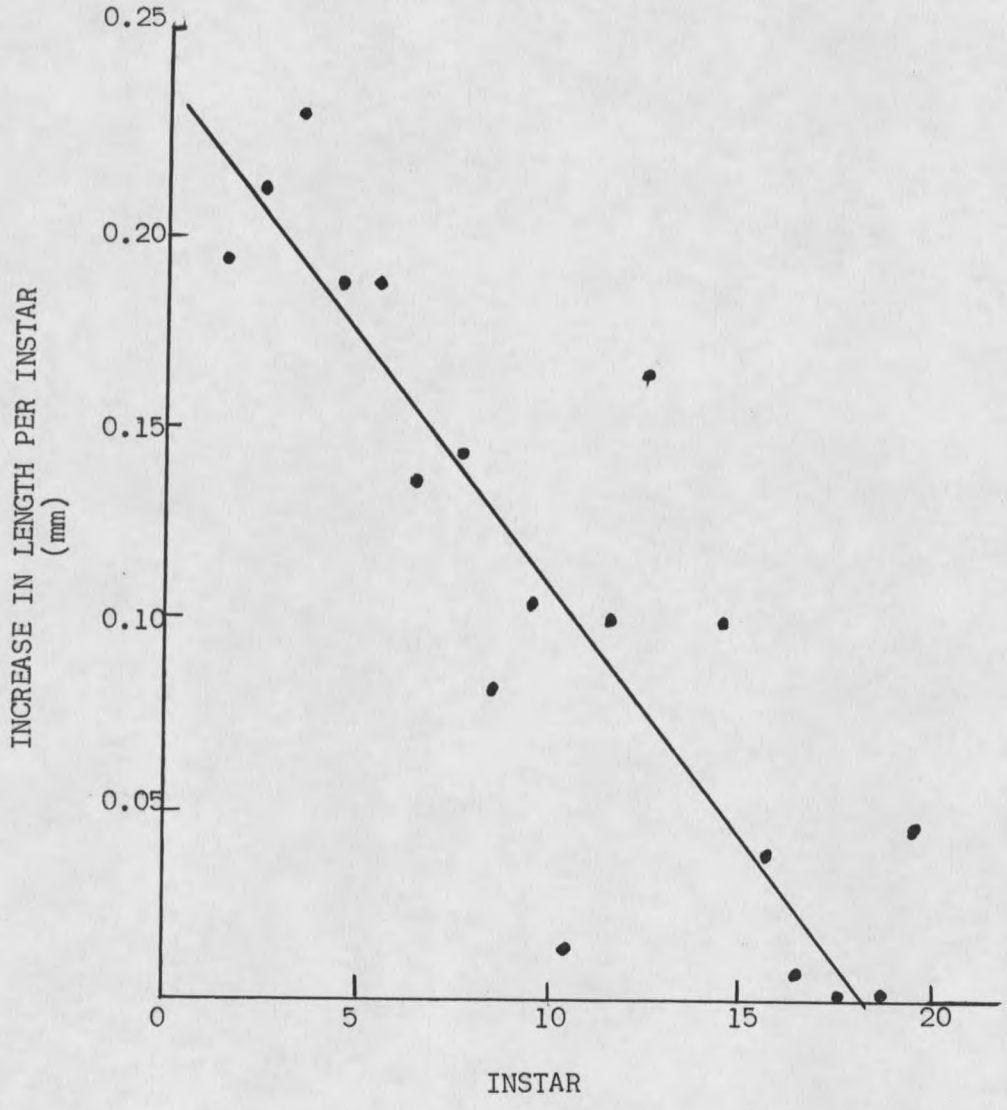


Figure 2. Average Increase in Length of Each Instar.

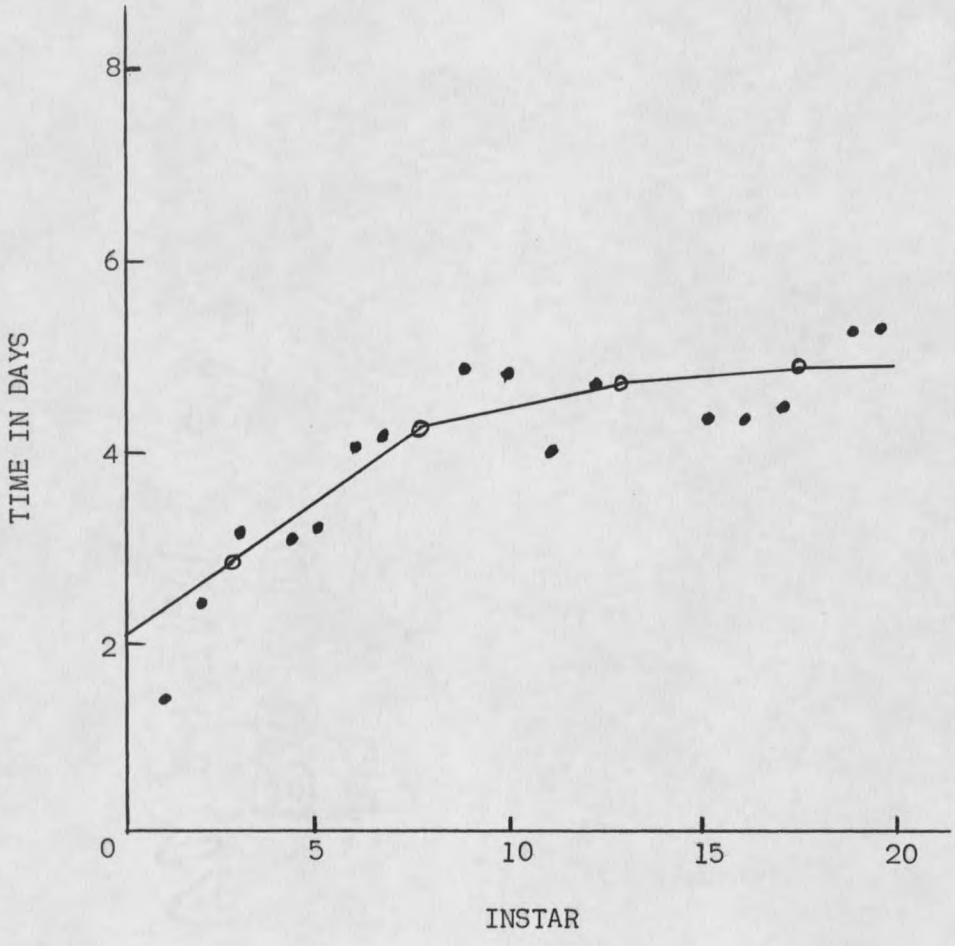


Figure 3. Average Time in Days Required for Passage from One Instar to the Next.

took 3.00 days. The duration of each instar increased until passage from instar 19 to instar 20 took 5.25 days.

Reproduction

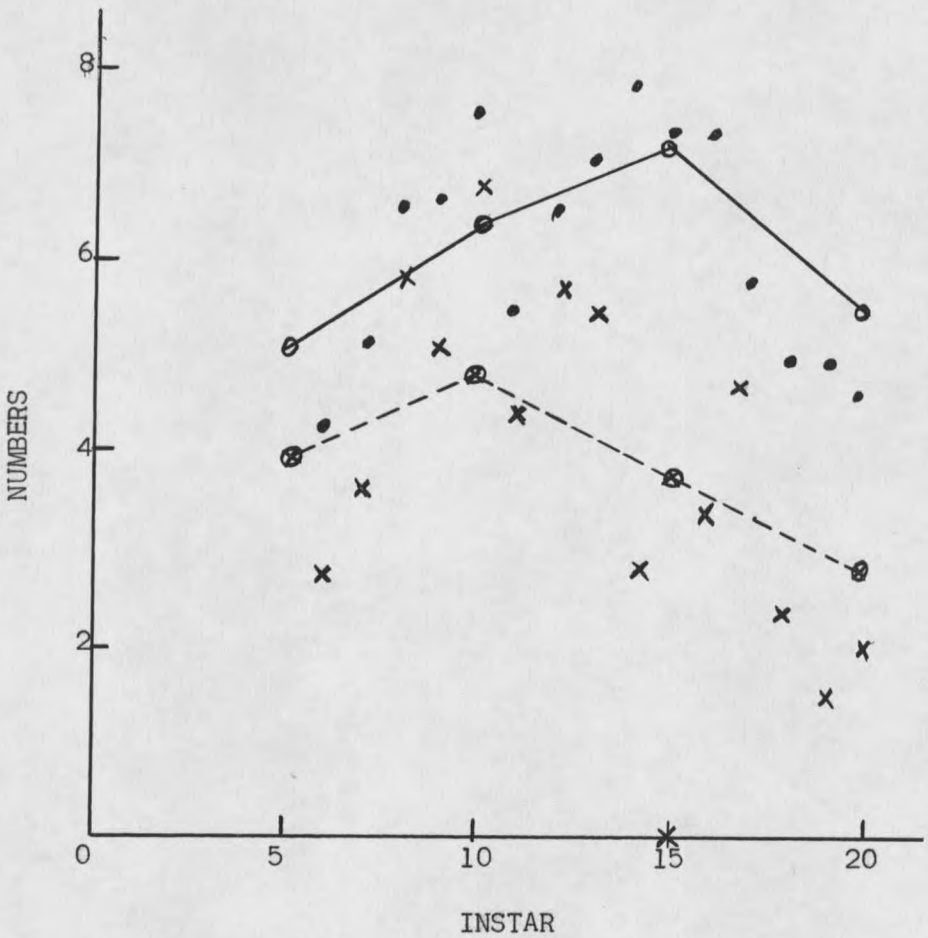
Reproduction generally began at the fifth instar level and occasionally the sixth. When reproduction began, the duration of each instar was markedly increased over that of the younger, nonreproductive instars. In life history studies of Daphnia longispina (Wood and Banta, 1933 and Ingle, 1933) and Daphnia magna (Anderson and Jenkins, 1942), the experimental animals were also found to be primiparous at the fifth instar and less frequently at the sixth instar.

Figure 4 shows the average number of eggs formed and the average number of young released in each instar. Previous papers on life history studies of Daphnia have assumed that all eggs formed per instar were viable. This was not found to be the case with Daphnia pulex ssp. pulicoides in this study.

The highest percentage of viable eggs was 77 percent produced during the fifth instar. The lowest was 51 percent produced during the fifteenth instar. The over-all percentage of viability was 61.

Eggs that were formed but not viable took on a watery appearance, whereas viable eggs developed embryos. The nonviable eggs shrank in size and finally disappeared from the brood chamber by the time the young were to be released.

In some cases, the entire brood of eggs was attacked and destroyed by a fungal mycelium. This fungus did not appear to affect the mother as



•_____• Eggs Formed
x- - -x Young Released

Figure 4. Average Number of Eggs Formed and Average Number of Young Released Per Reproductive Instar.

she would produce a brood comparable to other specimens during the following instar. The fungus was not studied in this investigation.

Mortality Rate

Figure 5 gives the number of survivors per instar. The highest mortality rates were encountered in the nonreproductive instars. Beginning with the reproductive instars, the curve tends to level off. The older the population became, the lower was the mortality rate of the population.

In Figure 5, data on 26 instars are included. Beyond the twentieth instar there were too few individuals to satisfactorily represent the population.

Table I gives pertinent data on instars one through 26. Seventy-six days elapsed between instars one and 20, and 73 days between instars 20 and 26, or a total of 149 days from birth to death.

Ingle (1933) using Daphnia longispina found 46.75 days to be the maximum longevity of his experimental animals. Anderson and Jenkins (1942) found D. magna, primiparous in the sixth instar, to have a life span of 53.54 days involving passage of 22 instars. Anderson and Zupancic (1933) reported Daphnia pulex as reaching the twentieth instar at which time the experiment was concluded. Ingle and Wood (1937) using D. longispina found the maximum longevity of their animals to be 51.19 days; this occurred among animals starved until the fifteenth instar. Their animals attained instar 23.

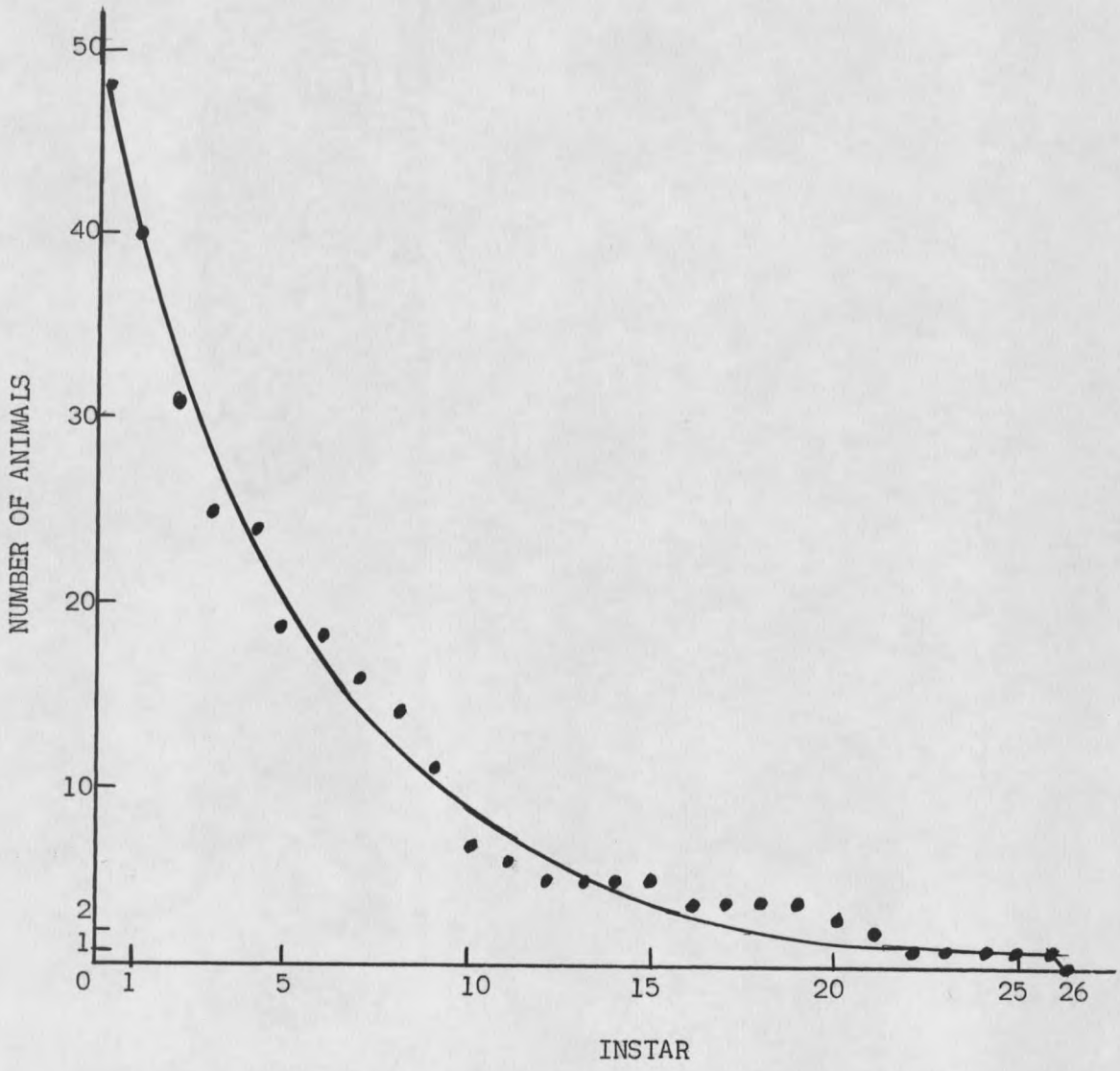


Figure 5. Survivors Per Instar.

TABLE I. DATA ON INSTARS ONE THROUGH TWENTY-SIX.

Instar	No. Animals	Average Length (mm)	Average Increase (mm)	Average Dry Weight (mg)	Average No. Eggs	Average No. Young	Average Time (days)
1	48	.6603	---	.003	---	---	---
2	40	.8579	.1976	.004	---	---	1.39
3	31	1.0703	.2124	.010	---	---	2.32
4	25	1.3033	.2330	.016	---	---	3.10
5	24	1.4826	.1793	.017	5.08	3.92	3.00
6	19	1.6615	.1789	.021	4.33	2.77	3.30
7	18	1.7977	.1362	.026	5.11	3.65	4.05
8	16	1.9414	.1437	.026	6.60	5.87	4.13
9	14	2.0247	.0833	.028	6.71	5.07	4.07
10	11	2.1287	.1040	.030	7.63	6.81	4.91
11	8	2.1488	.0158	.031	5.50	4.40	4.70
12	6	2.2489	.1001	.032	6.60	5.75	4.00
13	5	2.4142	.1653	.041	7.14	5.57	4.50
14	5	2.3889	.2053	.042	8.60	2.80	4.60
15	5	2.4901	.1012	.044	7.40	0.00	4.80
16	5	2.5280	.0379	.046	7.40	3.40	4.20
17	4	2.5359	.0079	.048	5.80	4.60	4.20
18	4	2.5359	.0000	.048	5.00	2.25	4.25
19	4	2.5359	.0000	.048	5.00	1.50	5.25
20	4	2.5833	.0474	.053	4.75	2.00	5.25
21	3	2.5833	.0000	.053	5.00	2.80	6.00
22	2	2.5916	.0083	.055	5.00	3.00	6.21
23	1	2.6544	.0628	.058	8.00	6.00	9.00
24	1	2.7492	.0948	.060	10.00	6.00	10.00
25	1	2.8440	.0948	.065	6.00	6.00	14.00
26	1	2.9072	.0628	.068	6.00	6.00	27.00

Grazing Study

Tables II, III, IV, V and VI give the results of the experiments conducted on grazing rates for each instar at each temperature level.