UW-Madison.
MET Publication No.53.11.R1.

No. 10

REPORT TO THE UNIVERSITY OF WISCONSIN

LAKE INVESTIGATIONS COMMITTEE

The Schwerdtfeger Library
1225 W. Dayton Street
Madison, WI 53706

LABORATORY EXPERIMENTS ON DAPHNIA

by

Robert A. Ragotzkie
(formerly Zoology Department)

Department of Meteorology

November 1953

ERRATA

Due to an oversight, the report on feeding experiments with Daphnia, presented to the Lakes and Streams Committee by Dr. Ragotzkie, was not sufficiently well labelled as a piece of research done in the Department of Zoology. It was distributed to the Committee by the Department of Meteorology only because Dr. Ragotzkie was associated with this department at the time of presentation of the report. Dr. Ragotzkie and the Department of Meteorology wish to emphasize that the research was done entirely in the Department of Zoology.

Please attach this sheet to your copy of Report No. 10.

8553338

The Schwerdtfeger Library
1225 W. Dayton Street
Madison, WI 53706

LABORATORY FEEDING EXPERIMENTS ON DAPHNIA

I. Introduction

Grazing by zooplankton represents the primary means of converting the phytoplankton crop into animal protoplasm. The subject has received widespread attention by investigators, but its complexity and inherent difficulty has made progress exceedingly slow. There are two main points of view from which to examine the problem. (1) In the sea, where the phytoplankton is composed largely of diatoms, the question usually arises - "Is there sufficient food available for the zooplankton population to flourish?" (2) On the other hand, in freshwater lakes, where the composition of the phytoplankton is highly variable, the question is often raised - "To what extent does the zooplankton control the phytoplankton population?" This last question is especially pertinent in cases where blue-green algae cause undesirable blooms. These two questions are not fundamentally different as they seek roughly the same information for two distinct situations. However, they do illustrate two of the principal ways of looking at the problem.

II. Literature

Work on the grazing of zooplankton was at first directed toward the qualitative aspects of the problem. Pütter (1909) proposed the theory that particulate material cannot possibly satisfy the metabolic requirements of zooplankton and that dissolved organic matter must therefore serve as an important food source. Lebour (1922) and

Marshall (1923) made studies on the gut contents of Calanus and found a variety of phytoplankton is consumed by the animals. Later on work by Krogh (1931), Gellis and Clarke (1935) and Clarke and Gellis (1935) showed that in the cases of Daphnia magna and Calanus finmarchicus dissolved material is not an important food source.

Investigators next turned toward the quantitative aspects of grazing. Lucas (1936) measured the rate of diatom consumption by estuarine zooplankton. At Woods Hole, Fuller and Clarke (1936) used carmine particles to determine the filtering rate of Calanus and obtained the mean value of 5.61 ml. water per day at 12° C. Respiration rates of Calanus as measured by Marshall, Nicholls, and Orr (1935) showed that Calanus would have to filter 72 ml. of water per day in order to subsist in Vineyard Sound. In 1937, Fuller, using Nitzschia closterium as food obtained filtering rates per day of 0.35 ml. at 3° C., 2.83 ml. at 8° C., and 1.09 ml. at 13° C. Harvey (1937) found that the filtering rate varied with the size of the food organism. His values ranged from 1.2 to 240 ml. per day for different diatoms.

More recently Riley, Stommel, and Bumpus (1949) in their report, "Quantitative ecology of the plankton of the western North Atlantic" made some feeding rate measurements while at sea. They obtained a value of 9 ml./day/mg. wet weight of zooplankton for Atlantic slope water and 34-52 ml./day/mg. wet wt. for the Sargasso Sea. By a separate method which is indirect and highly involved they calculate that the

actual rate in the sea varies from 80 to 110 ml./day/mg. wet weight. Gauld (1951) made a thorough study of feeding rates of marine copepods using Chlamydomonas as the food organism. His results varied from 4.3 to 71 ml./day depending on the copepod used.

III. Grazing Rates of Daphnia on Chlorella

A. Methods and Materials

1. Daphnia Cultures

Although Daphnia are known to be easily raised in the laboratory, to obtain a constant supply of healthy, vigorous animals requires care and experience. For this reason it seems worthwhile to describe the methods used. The animals are raised in filtered lake water in large battery jars. They are fed exclusively on Chlorella pyrenoidosa (Pringscheim). Cultures of two species of Daphnia, D. longispina O. F. Müller and D. pulex de Geer, are maintained. The first colony of each is started by a single female obtained from Lake Mendota, thus eliminating genetic differences in the laboratory colonies. Reproduction by parthenogenesis soon establishes a vigorous colony. The density of the population follows the usual pattern observed in pure cultures of practically all organisms. After an initial delay the numbers begin to increase geometrically. At a certain level "crowding effects" begin to appear and the numbers level off. Then begins a period of population fluctuation punctuated by occasional sharp decreases from which recovery is slow. In Daphnia cultures these depressions, as they are called, frequently

culminate in the complete destruction of the colony. Depressions occur in spite of regular, heavy feeding and are accompanied by a heavy growth of bacteria. In order to eliminate this difficulty new colonies are started by transfers from the old one during the period of most rapid increase. This period can only be determined by regular observation of the population. No fixed rules can be given; it is mostly a question of experience and regular observation. Timing is important, for if the transfer is made after the peak is reached, the new culture will exhibit an extra long delay or die out altogether. By always transferring before the peak, the depression is in effect postponed indefinitely. Thus healthy, vigorous animals are always available.

Feeding is done on a demand basis; that is, whenever the water appears clear, Chlorella is added. This is usually every 2 or 3 days in the beginning and every day after the population begins to increase rapidly. Also larger amounts are added as the population increases. Although no special study was made, it may be noted that production of winter eggs usually accompanies periods of starvation in a fairly crowded colony.

2. Chlorella cultures

A constant supply of fresh Chlorella is required to maintain the Daphnia colonies. This small green alga is cultured in one-liter Erlenmeyer flasks in the following nutrient solution:

Compound	Mg.
KH_2PO_4	2.450
K_2HPO_4	1.010
$Mg\ SO_4$	2.400
KNO_3	2.525
$FeSO_4 \cdot 7H_2O$	0.0015
A - Z solution (trace elements)	1.0 ml.
Distilled water	1000. ml.

Sterilization of the medium is unnecessary due to its low pH which prevents excessive growth of most bacteria. After innoculation, the flasks are aerated with compressed air. By vigorous aeration a sufficiently large supply of CO_2 is obtained from atmospheric air to permit rapid growth of the alga. The light source is an ordinary 100 watt bulb. A heavy green "soup" is produced in a week or less under these conditions. The resulting Chlorella suspension is allowed to settle out and the spent nutrient solution decanted. The cells are then resuspended in tap water. This suspension is refrigerated and used within a week. Prolonged storage of the cells is undesirable since "old" Chlorella tends to inhibit reproduction and increase mortality in laboratory Daphnia cultures.

3. Feeding-rate experiments

The feeding rates of D. longispina and D. pulex are measured by placing the animals in a suspension of Chlorella and determining the rate of decrease of the cells. Chlorella is suspended in filtered lake

lake water (no. 42 fine precipitate filter paper) and a dilute suspension in amounts varying from 50 to 200 ml. placed in 250 ml. Erlenmeyer flasks. Either 5 or 10 Daphnia are allowed to feed for periods of 2 to 24 hours. Most of the tests were made using 10 Daphnia in 100 ml. of suspension over a 4 hour period. Temperature control is achieved by immersing the flasks in a "home-made" constant temperature water bath.

The concentration of Chlorella is determined by counting samples of the suspension in a Levy-Hausser haemacytometer; the mean of 10 counts is taken as the concentration. Since the largest unit of the haemacytometer contains 10^{-4} ml., the number of cells per unit $\times 10^{4}$ is the number of cells per ml. of suspension.

Three flasks are used in each experiment. Two receive Daphnia while one is kept as a control. Before introducing the Daphnia the algal-concentration is determined for each flask and the results of the 3 flasks averaged. The rates of consumption of the Chlorella by the Daphnia are measured by cell counts made at 1 or 2 hour intervals except in the 20 and 24 hour experiments. Since it recuires about 15 minutes to make the 10 counts for a single flask, the starting times for the 3 flasks are staggered so that counts can be made for feeding periods of the same lengt for all flasks.

Chlorella, being a non-motile green alga, tends to settle out gradually. In the 20 and 24 hour experiments this sedimentation was observed in the control flasks, and t e concentrations in the feeding-flasks are corrected in proportion to their observed concentrations.

Subsequently the difficulty was eliminated by two measures. First by conducting shorter experiments sedimentation is materially reduced. Second, gentle swirling of the flasks every hour or so reduces the settling effect to practically zero. Flasks are always swirled immediately before counting.

Direct observations of the movement of material through the gut of Daphnia longispina were made by placing Daphnia fed on Chlorella in a suspension of carmine particles in a watch glass. The elapsed time from the first appearance of carmine on the appendages until it passes out the anus is taken as the time of passage. The times of passage in 8 Daphnia varied from 3 minutes 15 seconds to 10 minutes with a mean time of 7 minutes. The eliminated Chlorella and carmine is in the form of mucous bound clumps. In the feeding flasks these clumps settle out rapidly. Vigorous shaking tends to break them up and artificially increase the algal concentrations, while gentle swirling resuspends only those cells that are not clumped.

In a few experiments the concentrations in the control flasks decreased in an irregular manner in spite of all precautions. Data from these experiments were discarded.

B. Results

When Chlorella-concentrations are plotted against time in a typical feeding experiment, a parabolic curve results (Figs. 29 and 30). However, when logarithms of the concentrations are plotted against time,

a straight line results (Figs. ~~29~~ 1 and ~~30~~ 2). This sort of relationship suggests a constant filtering rate rather than a constant rate of food consumption. If one takes a filter-feeding mechanism as a working hypothesis, the filtering rate can be calculated from the experimental data by the following method:

Let $C_t = C_o e^{-kt}$

where C_o = Initial concentration of Chlorella cells

C_t = Concentration of cells at time t

$-k$ = Filtering constant

t = Time (seconds)

Differentiating logarithmically with respect to time

$$\ln C = \ln C_o - kt \ln e$$

$$\frac{d \ln C_t}{d t} = \frac{d \ln C_o}{d t} - \frac{d\, kt \ln e}{d t}$$

$$\frac{d \ln C_t}{d t} = -k$$

k can now be obtained by

$$k = \ln C_1 - \ln C_2$$

Since k is a constant with the units 1/sec., the actual filtering rate (F_s) as ml./Daphnia/~~day~~ sec. is given by

$$F_s = vk$$

where v = ml./Daphnia

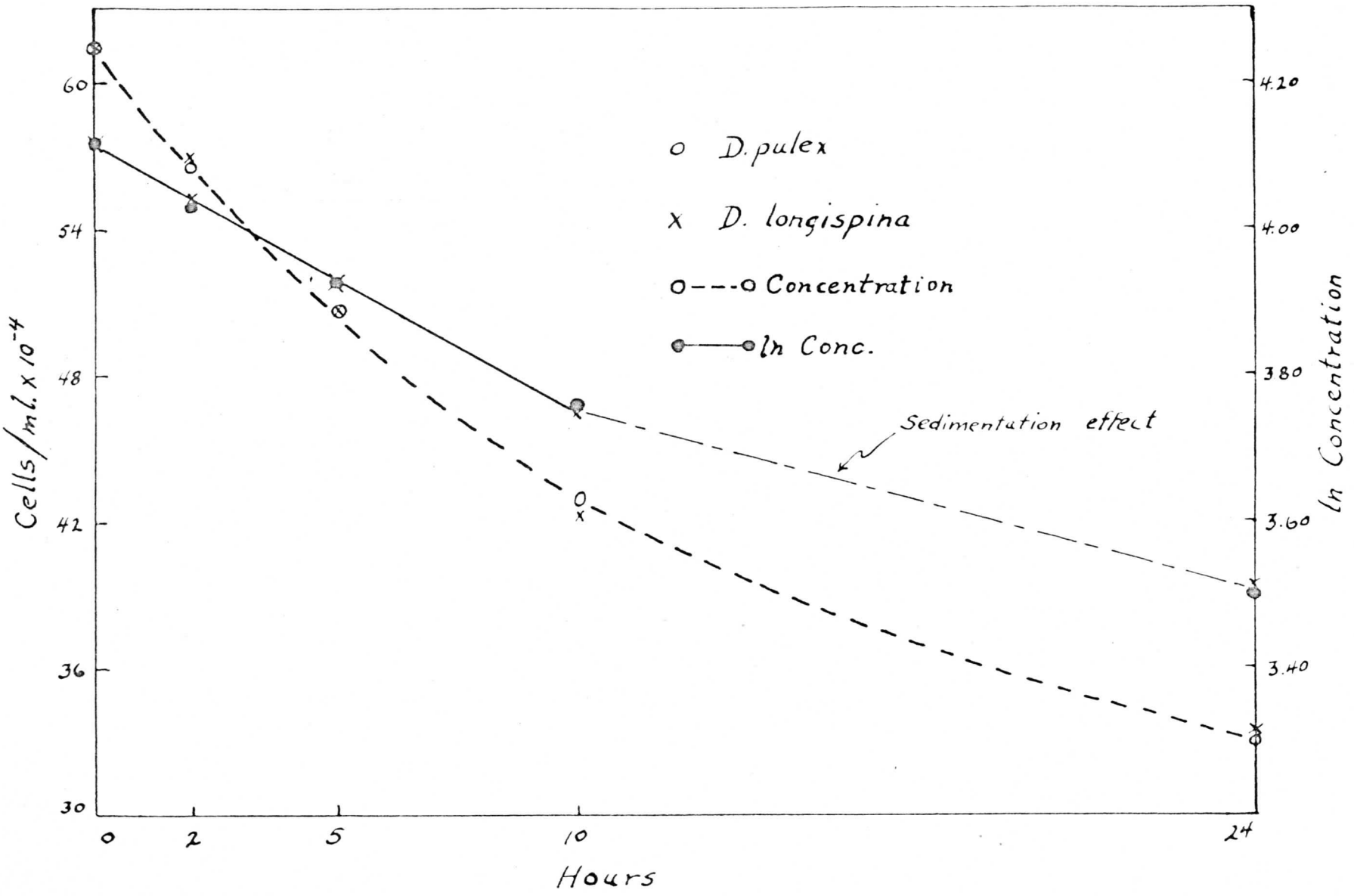

Fig. 1 Results of a typical 24-hour feeding experiment

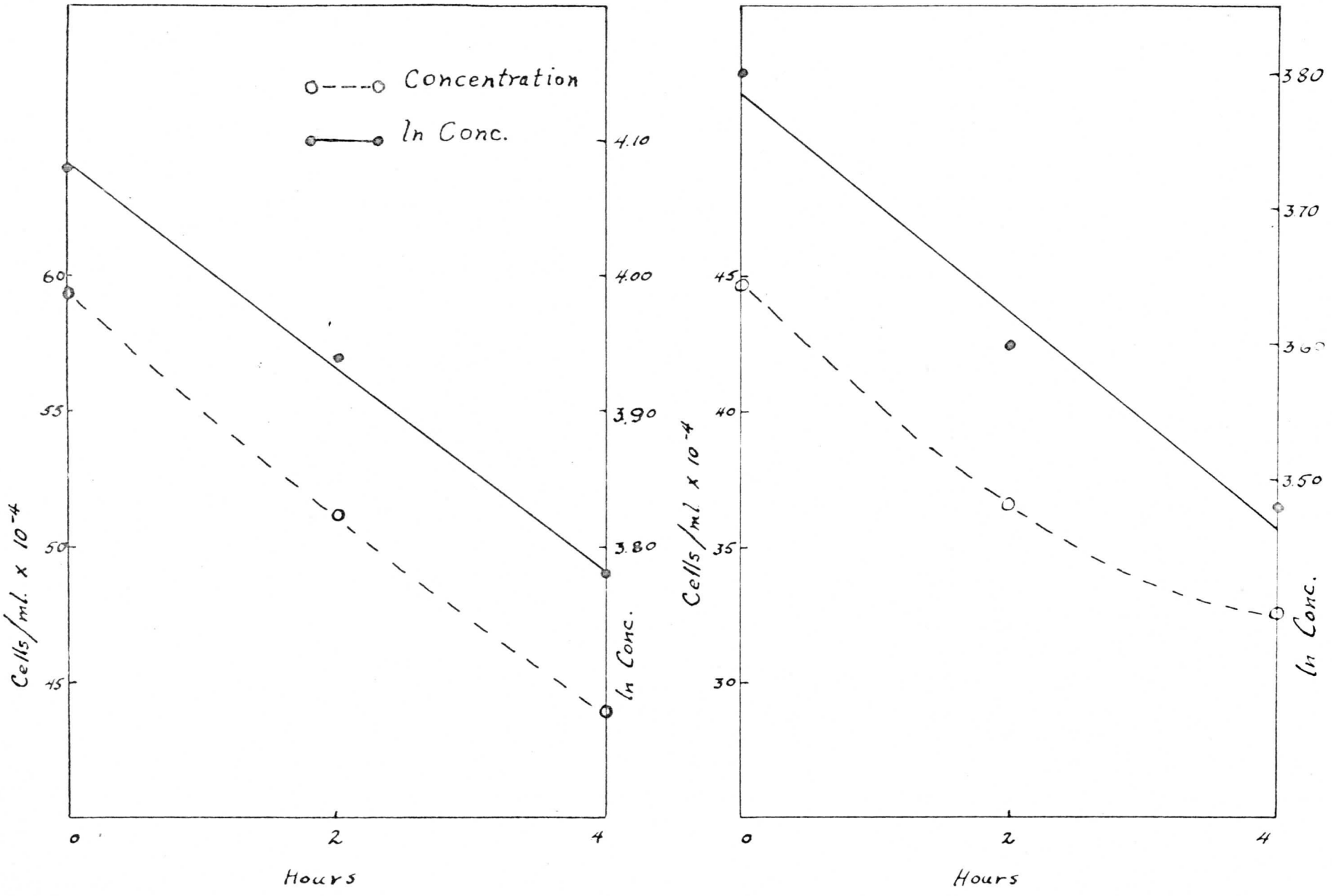

Fig. 2 Results of two typical 4-hour feeding experiments

In this manner all the results of each experiment can be expressed as a filtering rate. Actually, since there are more than two points for each experiment and since these points do not fall in a straight line, an estimate of the slope (k) has to be made. This is obtained by the method of least squares with time as the independent variable and ln concentration as the dependent variable. Table 1 gives a summary of the results of all the experiments. Filtering rates are expressed as ml./Daphnia/day.

Sixteen of the 18 experiments were done with D. longispina and 2 with D. pulex. Experiments 13 and 14 are identical except for the species of Daphnia; nearly identical filtering rates are obtained for both species. No systematic study of inter-specific differences or similarities was carried out, mainly because variations within a single species (D. longispina) are so large that study of these was given priority. As shown in Table 1, with the exception of one filtering rate of 48 ml./day and two rates of less than 10 ml./day, all the values fall in a range of 9 to 27 ml./day. Attempts to relate these rates with temperature and food concentration (to be discussed below) met with little success. Although the range of values represents considerable relative variation, the absolute range is small and may be unimportant in field applications of the data.

Table 1

Filtration Rates of *Daphnia longispina* When Fed on on *Chlorella pyrenoidosa*

Exper. No.	Temp. °C.	Duration of exper. Hours	Vol./ *Daphnia* ml.	Initial Conc. Cells/ml. x 10^{-4}	Filtration Rate ml./day
1	10	4	10	59.4	12.04
2	10	4	10	53.8	17.68
3	10	4	10	74.3	6.62
4	10	4	10	34.5	22.17
5	10	4	10	31.6	10.96
6	10	4	10	33.0	23.67
7	10	5	10	20.7	10.77
8	15	4	10	59.1	10.57
9	15	4	10	49.7	22.67
10	15	4	10	44.7	18.85
11	17	5	15	82.3	4.52
12*	22	20	10	85.0	9.38
13	22	24	20	61.5	11.88
14	22	24	20	61.5	12.05
15	25	2	10	35.0	27.29
16	25	2.5	10	28.0	25.75
17	25	2	10	33.6	19.77
18	25	1	10	31.6	48.52

Daphnia pulex ecperiments.

C. Discussion ~~Methods and Materials~~

Certainly the most striking feature of the results is their variability. Several factors possibly influencing Daphnia's filtering rate must be considered. Temperature is immediately suspect in this respect. Fuller's work on Calanus (1937) demonstrates the existence of an optimum temperature for maximum filtering rates. Riley et al (1949) assume that grazing rates vary with temperature in the same manner as respiration rates, and they adjust their grazing-rate data to the desired temperatures on this basis.

The experiments on the rate of feeding of Daphnia reported here were done at 10, 15, 17, 22, and 25° C. However when the mean filtering rate at each temperature is plotted against temperature, no optimum or relation between the two variables is apparent. The correlation coefficient between temperature and filtering rate is +0.07 which is not significantly different from zero. If the filtering rate is assumed to be related to temperature by the Van't Hoff principle, then there should be a higher correlation between the log of the filtration rate and the temperature. However, the points are so widely scattered that there is no suspicion of this nor is there any hint of the existence of an optimum temperature. Absence of a temperature effect is most surprising. It could be that other variables affecting the filtering rates tend to mask an expected temperature effect, but further analysis of the other known factors does not suggest this.

Precise control of the age and size of the experimental animals was not practical, but by using 10 individuals per flask and 2 flasks per experiment, an average filtering rate is obtained which minimizes size differences. Furthermore results from replicate flasks of any one experiment give results that agree very closely.
Yet 2 experiments done within a 3-day period under identical physical conditions yielded variable results.

The filter-feeding hypothesis is favored by the individual feeding experiments as there is no indication that the rate of depletion of Chlorella cannot be accurately described by the exponential equation

$$C_t = C_o e^{-kt}$$

On the same basis it follows that filtering rates obtained should be independent of the food concentration. To test this the filtering rates were plotted against algal-concentrations (Fig. 3). The result is suggestive of a relationship between the two quantities, but the correlation coefficient between F and C_o is only +0.107. Application of the "t" test shows that +0.107 is not significantly different from zero at the .10 level (calculated t = 0.43, tabular t = 1.7 for N = 18). Statistically there is no reason to reject the hypothesis that the filtering rate is unrelated to the food concentration. Despite this negative evidence, an element of doubt still persists. Further experiments are necessary to substantiate the fact or fiction of the food concentration effect. If future evidence should support the existence of a relationship with

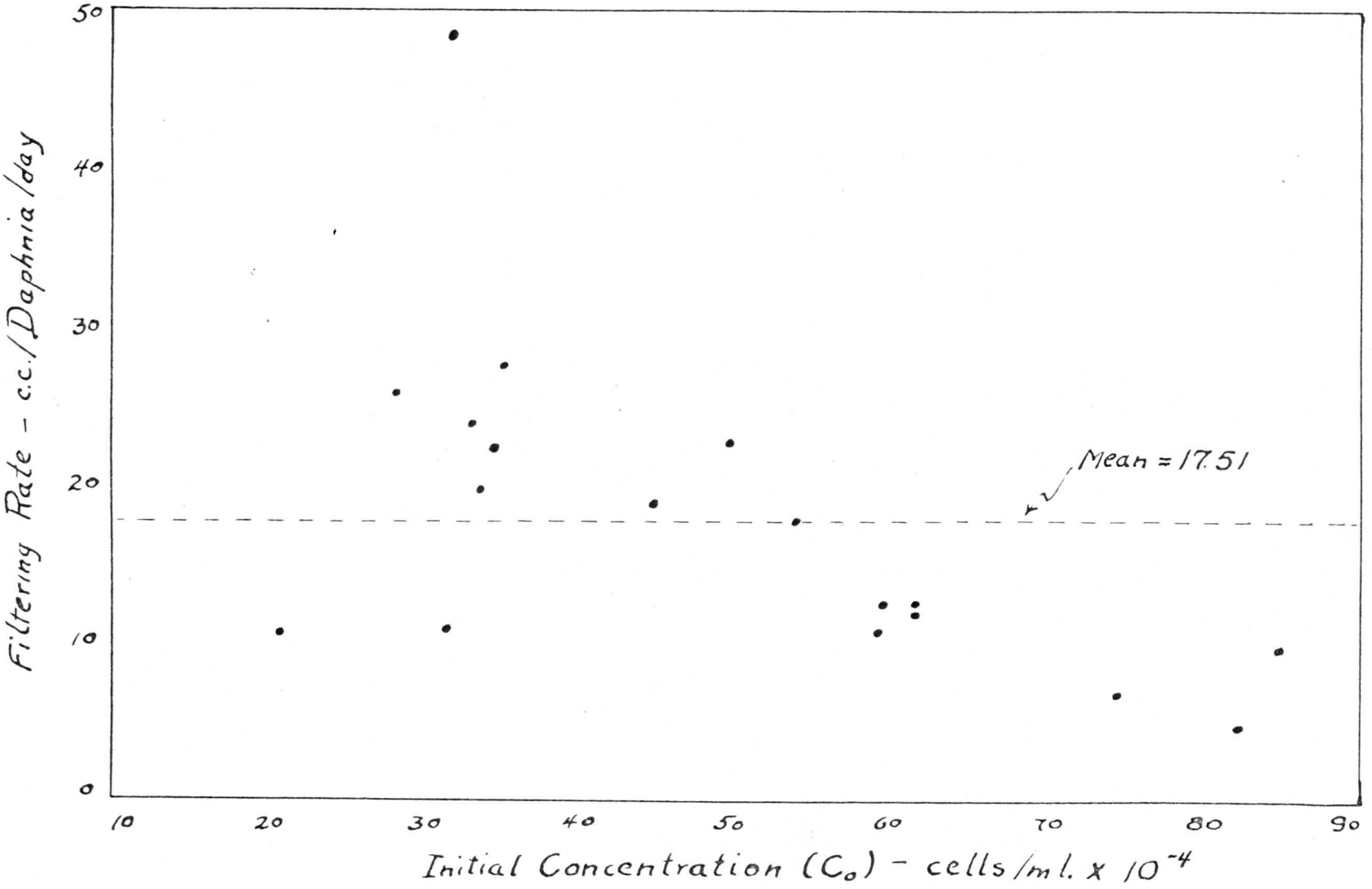

Fig. 3 Filtering rates of Daphnia vs. initial concentration of Chlorella cells

the algal-concentration, an explanation compatible with the filter-feeding hypothesis will not be simple. It may be that the filtering rates reported here were measured under such high food (algae) concentrations that some other factor besides the filtering mechanism began to operate as a limit of the rate at which cells were removed from suspension. Peristaltic rates or the size of the mouth opening are possible limiting factors. Finer techniques which permit accurate rate measurements at food concentrations ten or a hundred times less than those used here are desirable to investigate this possibility. In any event on the basis of the data given here the filter-feeding hypothesis is not seriously challenged.

IV. The Grazing Problem

"To what extent does the zooplankton control the phytoplankton population?" It has been shown that the feeding rate of Daphnia can be conveniently expressed as a filtering rate. By making certain assumptions, the expression for filtering rate can be used to derive an elementary solution of the grazing problem.

Assume the following conditions:

1. The phytoplankton population consists of a suspension of single-celled algae.
2. The filtering rates exhibited by Daphnia under laboratory conditions also hold for conditions in the lake.

3. The Daphnia population is not changing. This is essentially true in the summer months when algae "blooms" occur.

Starting with the filtering rate equation (Ch. VI)

$$C_t = C_o e^{-kt}$$

and substituting

$$k = F/v - FC_z$$

where F = filtering rate

v = volume per Daphnia

$C_z = 1/v$ = Daphnia concentration

we obtain

$$C_t = C_o e^{-FC_z t}.$$

This equation describes the grazing effect of Daphnia in terms of the filtering rate and the Daphnia concentration.

Assuming simple division as the mode of reproduction, the phytoplankton population may be described by

$$C_t = C_o 2^{t/T}$$

where C_t = algae concentration at time t

C_o = initial algae concentration

t = days

T = days per division

It is clear that grazing tends to decrease the phytoplankton population while growth of the algae tends to increase it. Ignoring other factors, such as mortality rate of the phytoplankton population, a perfect balance

of grazing by Daphnia and growth of the algae will result in a phytoplankton population which is neither increasing nor decreasing.

To examine the conditions for a static phytoplankton population the expressions for grazing and algae growth are equated

$$C_o e^{-FC_z t} = C_o 2^{t/T}.$$

Solving for generation time, T, we obtain

$$T = \frac{\log_e 2}{-FC_z}$$

The average filtering rate for all the laboratory feeding experiments is 17.5 ml./Daphnia/day. Using this value, the following table is constructed to show the generation times of algae which will be exactly balanced by various concentrations of Daphnia. Note that the balance between growth of the algae and grazing by Daphnia. Note that the balance between growth of the algae and grazing by Daphnia is entirely independent of the algae concentration.

Table 2

C_z - Daphnia/liter	T - generation time in days
1	39.6
5	7.9
10	4.0
15	2.6
20	2.0
25	1.6
50	0.79
100	0.40

Practically nothing is known about the generation times for algae under natural conditions. At 25° C. and under favorable light and nutrient conditions, Microcystis sp. cultures have demonstrated a generation time of 2 to 3 days (Gerloff, personal communication, 1953). If this is taken as an upper limit, it is apparent from the table that 15 to 20 Daphnia per liter could effectively balance the growth potential of the algae. Daphnia concentrations of this order are very common on Lake Mendota during the summer months. Yet heavy "blooms" of blue-green algae occur every year. This contradiction is good evidence that the blue-green algae of the lake are not being removed by Daphnia according to the grazing equation.

V. Blue-green Algae as Food for Daphnia.

To what degree Daphnia can utilize blue-green algae as food is not known. In the theoretical development of the previous section it is shown that Daphnia do not remove blue-green algae from Lake Mendota in accordance with the grazing rates obtained in the laboratory. As a more direct approach several laboratory experiments were conducted to determine the ability of Daphnia to live and reproduce when fed pure cultures of various species of blue-green algae.

A. Methods and Materials

Pure cultures of the following genera of blue-green algae were obtained from Profs. Gerlaff and Fitzgerald of the Botany Dept.: Microcystis, Anabaena, Gloeotrichia, Aphanizomenon, Lyngbya. The physical

characteristics of the suspensions were as follows:

Microcystis	suspension of single cells
Anabaena	separated filaments, 20-40 cells each
Gloeotrichia	diffuse colonies and many single cells in suspension
Aphanizomenon	large masses of filaments, broken up with glass beads before using.
Lyngbya	large mass of filaments, teased apart with needles before using

Five feeding experiments were performed. In the first two experiments Daphnia longispina was used as the test animal. In order to eliminate miscellaneous particulate food material, lake water was filtered through cotton and then through No. 2 filter paper. One hundred ml. of water was added to 200 ml. screw-top sample jars, and one adult Daphnia was placed in each jar. The jars were then divided into groups of five. Each group received a different alga as a source of food. One ml. of the algal suspension was adopted as a "standard feeding" except in the case of Lyngbya where a small mass of filaments was used. One group of test jars received Chlorella and one group received no food. The Daphnia were observed each day, and the young counted when they appeared. The experiments were continued until most of the animals died and until all reproduction had ceased.

Daphnia pulex was used in the last three experiments. In these

tests Microcystis was compared to Chlorella as a food source for the animals. Ten or twelve Daphnia were added to 1500 ml. of cotton-filtered water in battery jars, and larger amounts of food were added. After eight days the number of Daphnia in each battery jar was recorded.

B. Results

The results of the first two experiments are given below. The values given are mean values of each group of five jars except in the number of Daphnia reproducing. As Chlorella is a good source of food for Daphnia, the group receiving this alga may be considered as the control group.

Table 3

Effect of Feeding Blue-green Algae to D. longispina

Food Source	Days to death		Number of Daphnia reproducing		Tot. No. Produced per jar	
Exper. No.	1	2	1	2	1	2
Chlorella (controls)	17.6*	15.0	5	4	4.4	15.6
No food (Starved)	14.0	6.6	2	1	1.6	0.4
Aphanizomenon	14.8	13.0	4	5	2.8	3.4
Gloeotrichia	17.0	13.0	5	5	3.8	5.4
Lynghya	16.6	9.8	2	1	0.4	0.2
Microcystis	6.0	10.2	1	2	0.2	0.4
Anabaena	--	13.7	-	2	-	1.2

*All values except number of Daphnia reproducing are means of five jars.

Experiments 3 and 4 were designed to compare Microcystis with Chlorella as a food source for Daphnia pulex.

Table 4

Microcystis vs. Chlorella as Food for D. Pulex

	Number of Daphnia after 8 days	
Food source	Exper. 3*	Exper. 4
Chlorella	325	47
Chlorella	301	41
Microcystis	19	0
Microcystis	1	2

*Initial number of Daphnia: Exper. 3 - 12
Exper. 4 - 10

In the next experiment (No. 5) Microcystis and Chlorella were compared separately and then used mixed to determine whether starvation was operating in experiments 3 and 4.

Table 5

Effect of Feeding a Mixture of Chlorella and Microcystis to D. Pulex*

Food Source	Number of Daphnia after 8 days
20 ml. Chlorella	417
70 ml. Microcystis	164
10 ml. Chlorella + 35 ml. Microcystis	158
10 ml. Chlorella + 35 ml. Microcystis	131

*Initial number of Daphnia - 10.

C. Discussion

As expected the results of these feeding experiments are highly variable. Even under apparently identical conditions no two populations of Daphnia behave exactly alike. This may be attributed to differences in vigor of the parent individuals, differences in egg number in the first brood, slight variations in time between molts and probably other unknown factors. In spite of all these uncontrollable factors the results of feeding experiments 1 and 2 show tendencies of the animals to grow better on Chlorella, Aphanizomenon, and Gloeotrichia than on Lyngbya, Microcystis, and Anebaena. This is shown most clearly by the number of Daphnia reproducing and the total number produced in each jar. As mentioned previously, the clumps of Lyngbya resisted attempts to break them up into a suspension of individual filaments. This may well have rendered the alga less available to Daphnia. Anabaena was only used in one experiment and so results were not checked by repitition. Microcystis produced very poor growth in both experiments. Furthermore it was in a suspension of single cells and would most likely be readily available to Daphnia.

The last three experiments in which Microcystis was compared to Chlorella as a source of food further verified this result. In all cases the Daphnia populations showed less growth when fed Microcystis. When Microcystis and Chlorella were mixed (Exper. 5), the numbers of Daphnia produced were practically the same as when Microcystis was fed in a pure form. This would seem to rule out starvation as the limiting

factor. Rather it would seem that Microcystis has an inhibitory effect on colonies of Daphnia.

In all tests fresh living algae cultures were used as the food supply. What effect dying or decomposing blue-green algae would have is not known. However in order to control a blue-green alga population in a lake, Daphnia would necessarily have to attack the living, reproducing cells. Any detrimental effect that blue-green algae have on Daphnia will automatically be reflected in a lessened ability of the Daphnia to control a blue-green "bloom" by grazing.

REFERENCES

1. Clarke, G. L. and S. S. Gellis. 1935. The nutrition of Copepods in relation to the food-cycle of the sea. Biol. Bull., 68(2):231-246.

2. Fuller, John L. and George L. Clarke. 1936. Further experiments on the feeding of Calanus finmarchicus. Biol. Bull., 72(2): 308-~~316~~ 320.

3. Fuller, John L. 1937. Feeding rate of Calanus finmarchicus in relation to environmental conditions. Biol. Bull., 72(2): 233-246

4. Cauld, D. T. 1951. The grazing rate of planktonic copepods. J. Mar. Biol. Ass. U. K., 29: 695-706.

5. Cellis, S. S. and G. L. Clarke. 1935. Organic matter in dissolved and in colloidal form as food for Daphnia magna. Physiol. Zool., 8(2): 127-137.

6. Gerloff, G. C. 1953. Personal communication.

7. Harvey, H. W. 1937. Notes on selective feeding by Calanus. J. Mar. biol. Ass. U. K., 22: 97-100.

8. Hasler, A. D. 1935. The physiology of digestion of plankton crustacea. I. Some digestive enzymes of Daphnia. Biol. Bull. 68: 207-214.

9. Krogh, A. 1931. Dissolved substance as food for acuatic organisms. Biol. Rev., 6: 412-442.

10. Lebour, M. V, 1922. The food of plankton organisms. J. Mar. biol. Ass. U. K., 12: 644-677.

11. Lucas, C. E. 1936. On certain inter-relations between phytoplankton and zooplankton under experimental conditions. J. Cons. int. Explor. Mer, 11:343-362.

12. Marshall, Sheina. 1923. The food of Calanus finmarchicus during 1923. J. Mar. biol. Ass. U. K. 13: 473-479.

13. Marshall, S. M., A. G. Nicholls and A. P. Orr, 1935. On the biology of Calanus finmarchicus. Part VI. Oxygen consumption in relation to environmental conditions. J. Mar. biol. Ass. U. K., 20: 1-27.

14. Pütter, A. 1909. Die Ernährung der Wassertiere und der Stoffhousgekalt der Gewasser. Gustav Fischer. Jena.

15. Riley, G. A., H. Stommel, and D. F. Bumpus. 1949. Quantitative ecology of the plankton of the western North Atlantic. Bull. Bingham. oceanogr. Coll., 12: 3, 169 pp.