STUDIES ON THE CULTURE OF WATER FLEAS, MOINA MACROCOPA STRAUS, IN ARTIFICIAL CULTURE MEDIUM

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By

Shiro MURACHI and Takeo IMAI

Department of Fisheries, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Introduction

Many works have been reported on the culture of water fleas, which are not only a favourite food organism for fresh water fish frys, but also convenient materials for various biological experiments.

In most of the culture methods hitherto reported, natural organic substances were used as fertilizers, such as a mixture of garden soil and horse manure (Banta) (1), a mixture of garden soil and cotton seed meal (Chipman) (2), yeast (Bond) (3), soy bean meal, butter milk and sheep manure (Embody and Sadler) (4), wheat bran (Shluchter) (5), lettuce leaves (Hyman) (6), clover-extract (Matudaira) (7) and hay infusion (Imai and Sato) (8).

In these culture media the bacteria grow in the course of organic decomposition and serve as the principal foods for water fleas (Banta) (1). Later on Banta (9) recognized the food value of the unicellular algae for *Daphnia magna* and Hasler (10) and Pratt (11) bred *Daphnia magna* on unicellular algae, *Coccomyxa simplex* and *Chlorella pyrenoidosa*. Terao (10) and Matudaira (7) used *Scenedesmus* in the culture of *Moina macrocopa* and *Daphnia pulex* respectively.

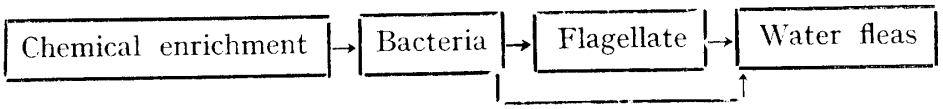
In the fertilized culture media, the end products of organic decomposition such as ammonia, carbon-dioxide and other inorganic substances are likely to promote the growth of unicellular algae in the medium and favour the growth of water fleas. Indeed, Matudaira (7) demonstrated the beneficial effect of organic enrichment of clover-extract on the growth of *Scenedesmus* and accordingly on the propagation of water fleas.

Recently, Imai and Sato (8) recognized the value of non-colored naked

flagellate as food for the water fleas. They proved that *Moina macrocopa* grew more efficiently when they were fed with the flagellate than when they were fed with bacteria or *Scenedesmus*. Subsequently, Imai and Hatanaka (13) in their study on the cultural requirements of marine non-colored naked flagellate, *Monas sp.*, demonstrated that the flagellate can be cultured in Miquel's sea water enriched with glucose in place of hay infusion.

The present research was planned to find out means to grow water fleas in the media with the composition of known chemical substances and to open the gate for quantitative analysis of water flea production.

In the experiment, glucose was added as an organic fertilizer to synthesized media of inorganic salts, and *Moina* were reared on flagellate and bacteria grown in the media. The organic transformation in the culture system can be expressed schematically as follows,



Material and Methods

The water flea, *Moina macrocopa*, of non-sexual cycle was used in the experiment. First of all, young from a clutch, within 24 hours after hatching, were washed in a 200 cc of sterilized culture medium five times, each for a few minutes, in order to remove as many micro-organisms as possible from the body surface.

The non-colored naked flagellate was isolated from a culture pond of water fleas in a carp nursery and its strain was cultured in a Bristol's solution enriched with 200 ppm of glucose. The flagellate was a non-colored naked form with a size of 6 to 14μ in length and 4 to 10μ in width. They had two flagella with unequal length. We could not identify the specific name but it was considered to be a *Monas* type. It was ascertained with the flagellate, vital-stained by neutral-red, that *Moina* ingested an abundance of the organisms.

A solution composed of 9 parts of saturated phenol, 1 part of formalin and 10 parts of distilled water was used to fix the flagellate for counting. A few drops of the fixative were used for 10 cc of the medium. For the fixation of bacteria, 5-10 % of Lugol-eosin solution (Imai and Hatanaka) (13) was used. The density of micro-organisms was measured by the help of Thoma's haemocytometer under the microscope.

Erlenmeyer's flask of 500 cc, filled with 400 cc of culture medium, was used for the experiment of flagellate culture. For the culture of water fleas, a square glass vessel of 11×24×7 cm was used with 1,000 cc of culture medium. A small milk bottle of 180 cc capacity containing 100 cc of culture medium was used as

the means for counting the number of young produced by single mother water flea. The vessels and solutions were sterilized before they were used. All the cultures were carried at the constant temperature of 25°C in darkness.

Experiments

1. *Growth of flagellate and Moina in synthesized culture solution.*

It was confirmed, in the preliminary experiments, that the slightly modified Bristol's solution was satisfactory as a culture medium for the flagellate. Its composition was as follows :

$MgSO_4 \cdot 7H_2O$	150 mg
$CaCl_2 \cdot 2H_2O$	50 mg
NaCl	50 mg
KBr	2 mg
KI	1 mg
$FeCl_3 \cdot 6H_2O$	10 mg
Dist. Water	1,000 cc

(pH was adjusted to 7.2 with $NaHCO_3$ after the preparation.)

In order to find the optimum concentration of salts for flagellate growth, various dilutions of Bristol's solution were prepared and were enriched with 50 ppm of KNO_3, 6 ppm of $Na_2HPO_4 \cdot 12H_2O$ and 100 ppm of glucose (P : N : C ≒ 1 : 13 : 77) and a few drops of the stock culture of flagellate were inoculated. Peak densities of the flagellates grown in various dilutions are shown in Table 1.

Table 1. The growth of non-colored naked flagellate in the various dilutions of modified Bristol's solution enriched.

Culture No.	1	2	3	4	5	6
Dilutions	× 100	× 50	× 10	× 5	× 2	× 1
Maximum density of non-colored flagellate per cc	675,000	875,000	1,150,000	950,000	910,000	955,000
Period of incubation in days.	3	3	3	3	3	3

Thus, the highest density was obtained in the solution diluted ten times with distilled water, indicating the optimum condition for flagellate growth. The growth of bacteria and flagellates in the 10 : 1 dilution is detailed in Figure 1. Daily changes of pH and phosphor content in solution are also shown in the figure.

There occurred a rapid growth of bacteria and then followed a growth of flagellate. The flagellate reached a peak density on the 5th day of culture and then declined rapidly. Phosphor had been consumed rapidly by the 4th day of culture : 0.442 ppm of it was gone. After the 5th day phosphor began to

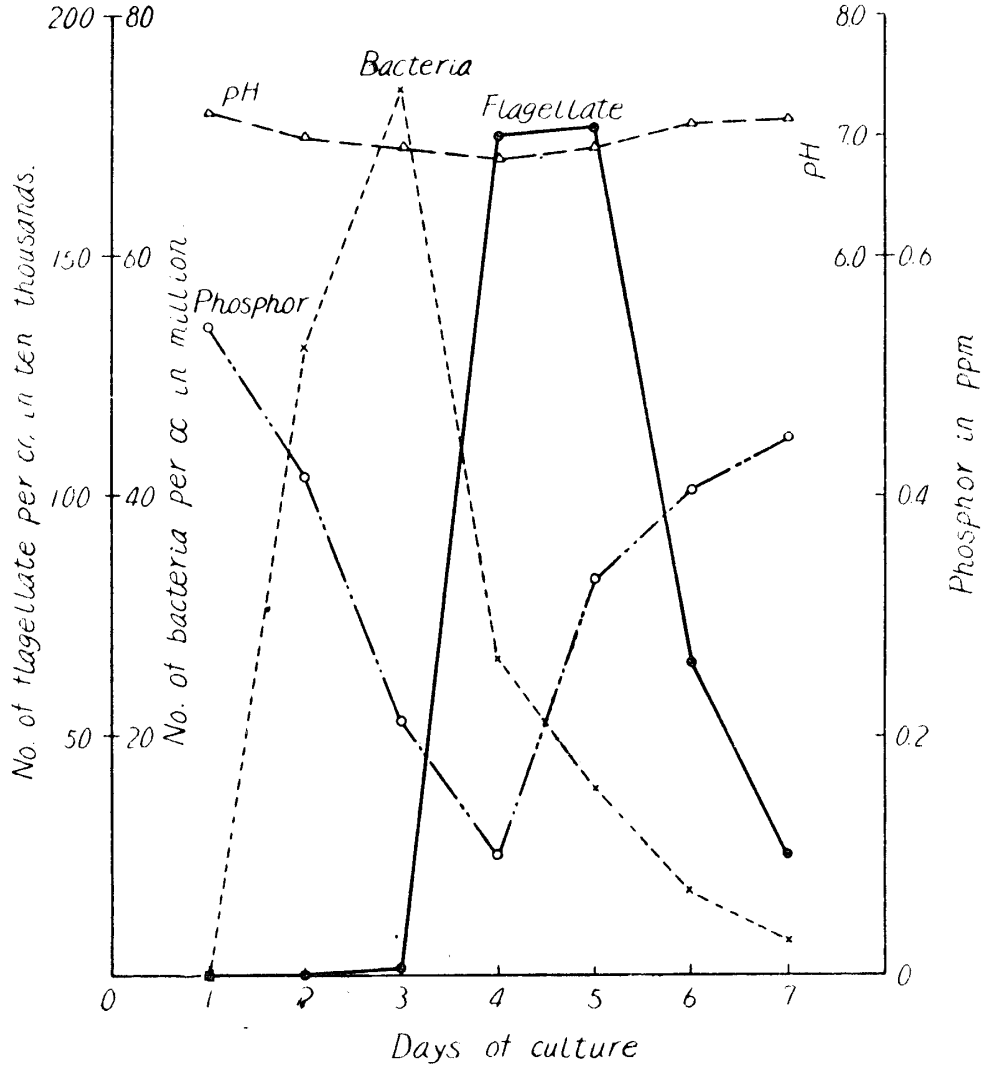


Fig. 1. Growth of non-colored naked flagellates in the ten times dilution of modified Bristol's solution enriched.

recover and on the 7th day 78 per cent of the consumed phosphor recovered. From the maximum consumption of phosphor and the amount of glucose added, P : C ratio of 1 : 90 was obtained. pH value remained inside a narrow range between 7.2 and 6.8 during the course of culture.

In the following experiments, it was proved that the same dilution of Bristol's solution as in flagellate culture was also good for the culture of the water fleas, *Moina macrocopa*. 100 cc of culture media of various dilutions were enriched with 50 ppm of KNO_3, 6 ppm of $Na_2HPO_4 \cdot 12H_2O$ and 5 ppm of glucose in milk bottles. A few drops of stock culture of flagellate were inoculated and 24 hours later, one young *Moina* was put into each bottle and total number of youngs produced in each bottle was counted. The results are summarized in Table 2.

Table 2 Production of *Moina* youngs in various dilutions of modified Bristol's solution enriched.

Culture No.	1	2	3	4	5	6
Dilutions	× 100	× 50	× 10	× 5	× 2	× 1
Maximum density of non-colored flagellate per cc	170,000	165,000	205,000	185,000	210,000	200,000
Total number of youngs produced by a mother in average.	3.7 (0–8)	4.7 (2–9)	24.7 (22–29)	24.3 (18–30)	25.0 (21–28)	23.0 (16–33)

The table tells that *Moina* produced many youngs in the media of concentrations above 1/10 of Bristol's solution. While in the solutions with higher dilution, the growth was disturbed and only a few youngs were produced.

From these experiments it was concluded that the culture medium of one part of modified Bristol's solution and 9 parts of distilled water was good for both flagellate and *Moina*. Therefore this dilution enriched with 50 ppm of KNO_3 and 6 ppm of $Na_2HPO_4 \cdot 12H_2O$ was used thoroughly in the experiments followed. The composition is shown in Table 3. It should be mentioned that the contents of phosphate and nitrate in the solution are sufficient for 100 ppm of glucose enrichment.

Table 3. The composition of basic culture solution for *Moina macrocopa*.

$MgSO_4 \cdot 7H_2O$	15 mg	
$CaCl_2 \cdot 2H_2O$	5 mg	
NaCl	5 mg	
KBr	0.2 mg	
KI	0.1 mg	
$FeCl_3 \cdot 6H_2O$	1.0 mg	
KNO_3	50 mg	P : N ≒ 1 : 13
$Na_2HPO_4 \cdot 12H_2O$	6 mg	
Dist. water	1,000 cc	

2. *Growth of flagellate and Moina in culture media repeatedly enriched with glucose.*

As shown in the above experiments, the period of growth cycle of flagellate on single enrichment of glucose was very short. Therefore, in order to obtain a dense culture of *Moina* it was necessary to sustain the density of flagellate at a certain level by adding glucose repeatedly at intervals. In the following experiments, the mode of growth of flagellate in the culture media repeatedly enriched with glucose was studied.

One group of culture (I) was enriched with 5 ppm of glucose at 3-day intervals, while the other group (II) was enriched with 2.5 ppm on the 1st and 4th day, 5 ppm on the 7th, 10 ppm on 10th, and 20 ppm on the 13th day. The results

are shown in Figure 2.

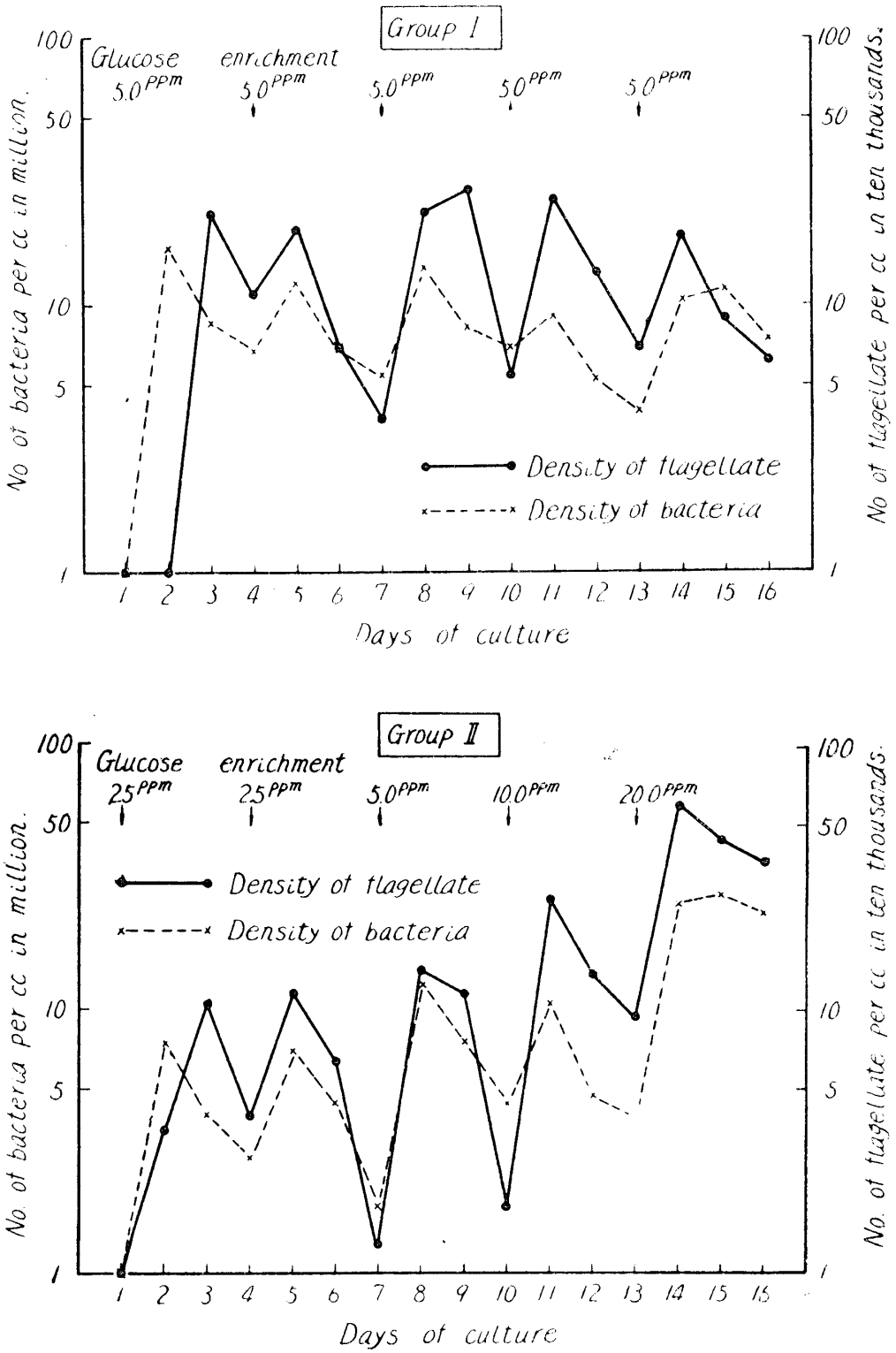


Fig. 2. Growth of non-colored naked flagellates in the basic culture media enriched repeatedly with glucose.

In Group I, the population of flagellates showed an oscillation with peak levels at a little above 200,000 per cc, while in Group II, the first two enrichments resulted in a density of 100,000 per cc and as the amount of enrichment increased the density peaks became higher and finally reached over 500,000 per cc.

During the experiments, pH of the medium remained inside the range of 7.0–7.2 and the amount of phosphor in the solution never decreased below 0.26 ppm.

Then the growth of *Moina* population in repeatedly fertilized culture medium was studied in the following series of experiments. Series A comprises the cases where the same amount of glucose was added at 3 days' intervals. The amount of glucose was, 10 ppm (A-I), 5 ppm (A-II), 2.5 ppm (A-III) and 0 ppm (A-IV) at a time. Three *Moina* youngs were put in the culture medium 24 hrs after the inoculation of flagellate. The results of experiments are shown in Fig. 3.

Almost no growth of *Moina* occurred in the culture A-I where 10 ppm of glucose was added at 3 days' interval. It indicated that the fertilization was excessive and the culture medium turned unfavorable to *Moina*.

On the other hand, in culture A-II and A-III where 5 and 2.5 ppm of glucose was added respectively at a time, *Moina* grew well and reached peak densities of 1,570 and 1,500 per 1,000 cc on 12th and 13th day of culture respectively. Control culture (A-IV) showed a peak density of only 140 young.

It was noticed both in cultures A-II and A-III that in the cyclic oscillation of flagellate population, the peak level gradually declined as the enrichment was repeated. This fact suggested that the enrichment had to be increased gradually as the culture proceeded. Such method of enrichment was tried in the next series of experiments (B). The amount of glucose at each enrichment and the results of culture are shown in Fig. 4.

In culture B-I, *Moina* density of 1,800 per 1,000 cc was obtained showing a slight improvement over group A. While in culture B-II, the flagellate was sustained at a high level and *Moina* density of a liltte over 3,000 per 1,000 cc was obtained. This was, so far, the highest density obtained in the culture media enriched with glucose. In control culture (B-III) the peak density was 70.

It may be worthwhile to compare the efficiency of glucose enrichment in producing *Moina* in these series of experiments. The efficiency can be expressed by the number of *Moina* youngs produced per 1 mg of organic carbon enriched. It will be calculated by the formula,

$$Y = (M - N) / C$$

where Y is the number of *Moina* produced per 1 mg of organic carbon enriched ; M, maximum number of *Moina* obtained in the culture ; N, number of *Moina* in the control culture ; C, the amount of organic carbon in mg enriched until

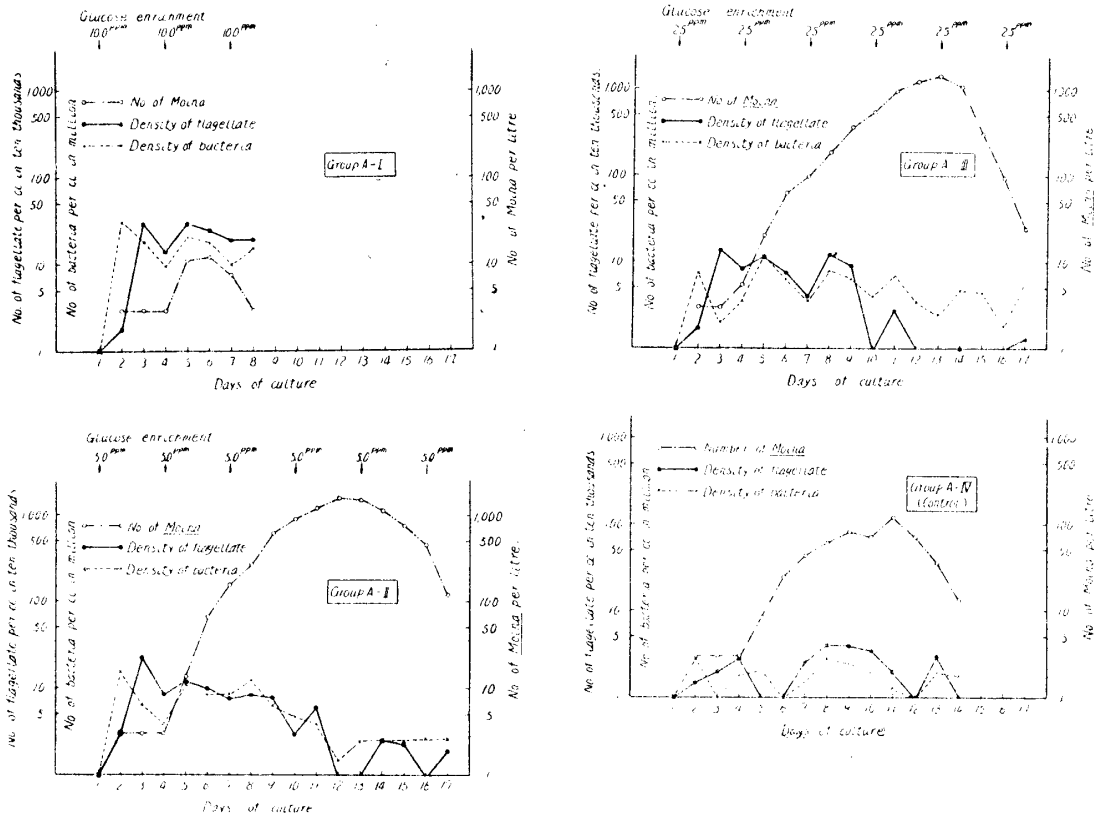


Fig. 3. Growth of *Moina* in the basic culture media enriched repeatedly with glucose. Series A.

the *Moina* population of each group reached its peak. Y values thus attained for each culture are shown in Table 4.

Table 4. Efficiency of glucose enrichment in the production of *Moina*, in various cultures.

Culture	A–II.	A–III.	B–I.	B–II.
Total amount of glucose added	20 ppm	10 ppm	20 ppm	80 ppm
Peak density of *Moina*	1,570	1,500	1,800	3,000
Y value	181.6	346.8	220.3	94.6

The efficiency was the highest in culture A-III where 2.5 ppm of glucose was added at 3-day intervals. When the amount of glucose was doubled (culture A-II) it dropped to 50 %. The efficiency was improved slightly in culture B-I where the enrichment was increased gradually as the culture proceeded. In the culture where the highest population of 3,000 per 1,000 cc was obtained, the efficiency was the lowest. Four to eight times of glucose was used to double the peak density.

Conclusions and Discussions

It was proved in this study that water fleas, *Moina macrocopa*, can be cultured

on flagellate and bacterial diet in a synthesized culture media of known chemicals with glucose as an organic enrichment. A diluted Bristol's solution with a slight

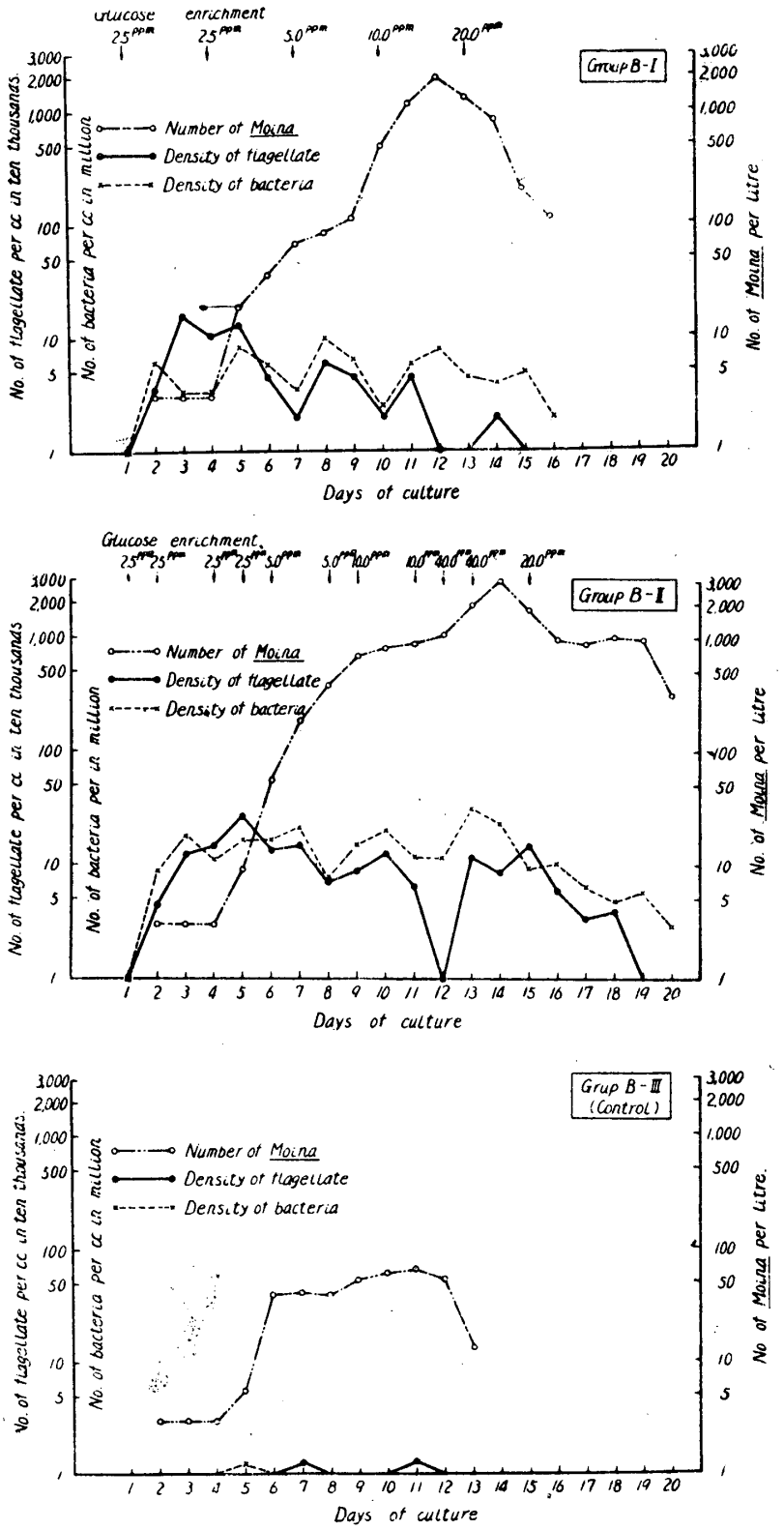


Fig. 4. Growth of *Moina* in the basic culture media enriched repeatedly with glucose. Series B.

modification which has a composition shown in Table 3 was found to be satisfactory as a basic culture media for both flagellate and *Moina*.

For flagellate culture, glucose could be added up to 100 ppm at a time. While in *Moina* culture 10 ppm of glucose at a time was the upper limit. In enriched culture medium flagellates grew rapidly and soon after the peak density was reached they declined sharply. Therefore, in order to obtain a high density of *Moina* population, it was necessary to give glucose enrichment repeatedly and sustain the flagellate population at high level.

The highest density of *Moina* obtained in the series of experiments was a little over 3 per cc of culture media. Matudaira (7) recorded the highest density of 0.6 *Daphnia pulex* per cc on *Scenedesmus obliquus* without renewing the medium during the culture. Hoshi (14) obtained the density of 1.8 of *Simocephalus vetulus* per cc on *Scenedesmus* also without renewing the culture medium. Record of Pratt (11) was 4.8 and 2.5 *Daphnia magna* per cc at 18°C & 25°C on *Chlorella pyrenoidosa* and Terao (12) obtained 30 *Moina macrocopa* per cc of media on *Scenedesmus*. But these high densities were obtained in the water renewed frequently. Therefore their figures can not be compared with ours.

On the other hand, we counted only 3 to 5 individuals per cc even in the well-fertilized culture ponds of water fleas in the carp nursery. Then the density of 3 *Moina* per cc in our laboratory experiment seems to be by no means a low one. But we hope that further improvement of the culture method will give a much higher density of water fleas in a synthesized medium.

References

1) Banta, A. M. (1921). Science, NS, **53**. pp. 557-558.
2) Chipman Jr. W. A. (1934). Science, NS, **79**. pp. 59-60.
3) Bond, R. M. (1934). Science, NS, **79**. p. 60.
4) Embody, G. C. & W. O. Sadler. (1934). Quoted by Galtsoff, P. S. et al. (1937). Culture methods for invertebrate animals. I Ed. p. 216, Comstock publishing, New York.
5) Schluchter, A. W. (1937). Quoted by Galtsoff et al. (1937). p. 215.
6) Hyman, L. H.(1937). Quoted by Galtsoff et al.(1937). p. 216
7) Matudaira, T. (1943). Bull Jap. Sci. Fish., **12**, 1. pp. 1–17 (in Japanese).
8) Imai, T. and R. Sato. (1949). Bull. Inst. Agric. Res. Tohoku Univ., **1**, 2. pp. 99-104. (in Japanese)
9) Banta, A. M.(1937). Quoted by Galtsoff et al. (1937). p. 207.
10) Hasler, A. D. (1937). Ibid. p. 214.
11) Pratt, D. M. (1943). Biol. Bull., **85**, 2. pp. 116-140.
12) Terao, A. & T. Tanaka. (1928). Proc. Imp. Acad. (Tokyo), **4**, 9. pp. 550-552.
13) Imai, T. And M. Hatanaka. (1950). Sci. Rep. Tohoku Univ. (Biol), **18**, 3. pp. 304-315.
14) Hoshi, T. (1949). Sci. Rep. Tohoku Univ. (Biol), **18**, 2. pp. 153-158.