

Studies on the Winter Eggs of the Water Flea, *Moina macrocopa* STRAUS.

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(Plates 1-2; Tables 1-3, Text-figs. 1-16)

(I) INTRODUCTION

The family Daphnidae contains many of the most important water fleas found in the fish culture ponds in Japan. Among others *Moina macrocopa* occupies the first place, followed by *Daphnia pulex*. In Japan the artificial propagation of *Moina macrocopa* has a great importance, because it is the most desirable and nutritious natural food for newly hatched fry in the carp-culture farm. It has been also used as a good material for ecological study of the Cladocera in general.

Many works have been reported on the biology of the Cladocera, but most of them are related to their parthenogenetic or asexual reproduction and little was added to the knowledge on their sexual reproduction and sexual eggs produced in the ponds. Therefore it is much desirable that a survey be made on the degree of relationship existing between the stock of winter eggs (sexual eggs) and the population growth.

Sexual reproduction of *Moina macrocopa* takes place at mid-May in the carp-culture ponds of the Kanto district and its winter egg production is also seen during last ten days of that month. *Moina* disappears in the ponds from June to April of the following year and it spends as long as about eleven months in the form of the winter eggs at the bottom of the ponds. This phenomenon is called "Sleeping" of the winter eggs.

Hence, the present studies have been carried out on the winter eggs of *Moina macrocopa* to make clear the mechanism of the sleeping.

Before going further, the author is happy to express his thanks to Professor emeritus, Dr. I. AMEMIYA and Prof. Dr. Y. OSHIMA for their guidance during the progress of this work. Further he wishes to express his thanks to Prof. Dr. Y. MATSUE and Ass. Prof. Dr. S. EGUSA for their valuable suggestions on many matters. And lastly acknowledgement is also made to Mr. T. ONBE for technical assistance in these experiments.

(II) MATERIAL AND METHODS

Present study was carried out by using the ponds of the Gunma Prefectural Fisheries Experimental Station in the Kanto district during these ten years from

1949 to 1959. But material was collected not only from the above mentioned ponds but also from those distributed in many prefectures, Tokyo, Shizuoka, Okayama, Hiroshima and Yamaguchi.

Experiments were made by using many winter eggs obtained from both the ponds and the small aquaria in the laboratory, because the ponds were not uniform in their character and not quite suitable for exact study.

For the purpose of analyzing the population of *Moina* in the ponds, samples were collected at the eight stations situated to the marginal region of the pond by a horizontal towing of a small plankton net with its mouth 10 cm. in diameter for the distance of 2 meters once a day for ten days.

In order to examine the distribution of the winter eggs in the pond bottom silt they were collected at 23 stations by using a cylinder mud sampler (10 cm. in diameter) and then counted.

The points which were observed with particular attention were as follows:

1) Growth form of population 2) Type of winter egg formation 3) Distribution of eggs in the pond 4) Non existence of pseudo-sexual egg 5) Mechanism of sleeping 6) A parasitic fungus, *Lagenidium* sp. 7) Technique of long time preservation of eggs.

(III) OBSERVATIONS

1. *Moina macrocopa* in the fish culture pond.

The accordance between the peak of the population growth of *Moina* and the hatching time of fish is one of the most important problems in the fish culture, but both of them are not always stable as we know that they are under the influence of climate and other environmental factors. In the Kanto district spawning of carp takes place usually early in May when the water temperature is about 20°C and the hatching is expected a week later. Accordingly the pond must be prepared for *Moina* culture at mid or last ten days of April. For this purpose some preparation are usually taken practically, that is, exposure, lime-sprinkling and fertilization of the pond bottom.

It is well known that the Cladocera have two different generations and there is the alternation of generations, such as, acyclic, monocyclic, dicyclic and polycyclic. But our informations on the conditions and environmental factors controlling the cycle of generations of the Cladocera are not sufficient. Is *Moina* monocyclic or polycyclic in the ponds? The answer is not so easy, but as is above mentioned, it may safely be said that *Moina* in the pond of Gunma Prefecture is monocyclic, because as is shown in Figure 1 (A) it appears in the pond mostly only in May and it keeps on to early June only occasionally.

2. Form of population growth of *Moina* in the pond.

It is well known that quantitative and qualitative investigations are necessary to know the course of population growth. For this purpose the following factors relating to population-increase or -decrease were taken up and their fluctuations were

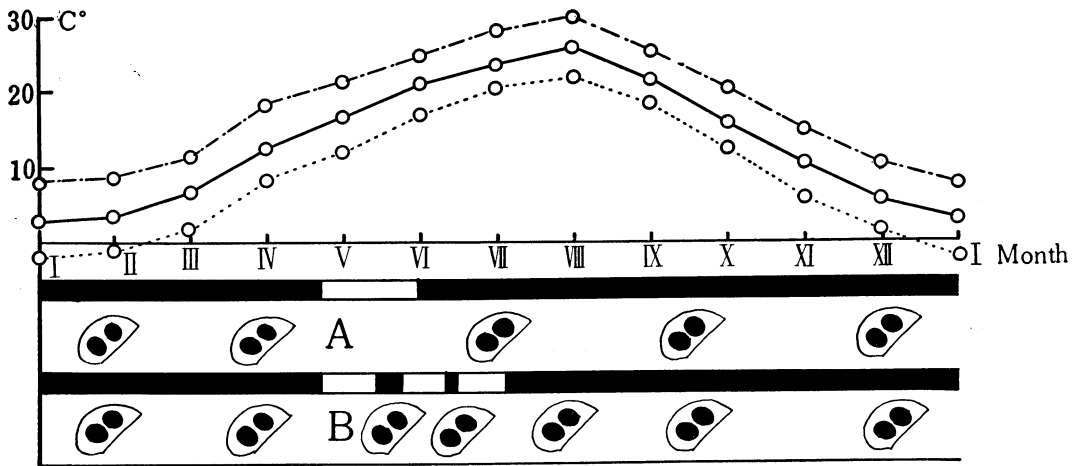


Fig. 1. *Moina macrocopa* STRAUS in the carp-culture ponds of the KANTO DISTRICT.

A : Monocyclic.

B : Polycyclic.

White parts mean the propagation of *Moina*.

Black parts mean the disappearance of *Moina* from the ponds, but many Winter Eggs are found at the bottom of the ponds.

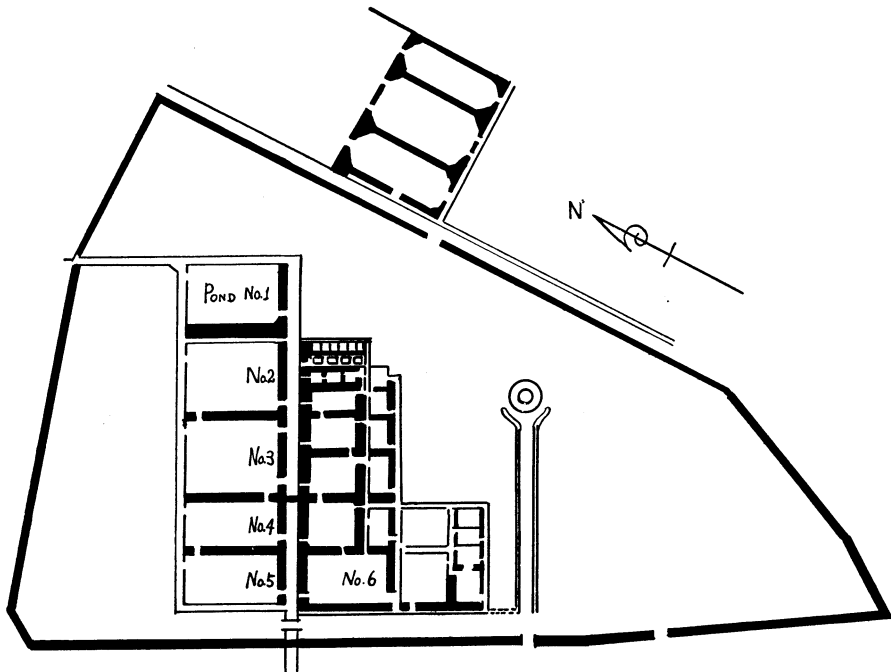


Fig. 2. A plane figure of Gunma Prefectural Fisheries Experimental Station

Pond No. 1	890m ²	No. 2	990m ²	No. 3	990m ²	No. 4	630m ²
No. 5	730m ²	No. 6	580m ²				

observed, i.e. body size composition, sex ratio, fecundity of parthenogenetic females and the ratio of sexual females (ephippial female) to parthenogenetic ones.

This investigation was carried out during the period from May 16th to 27th of

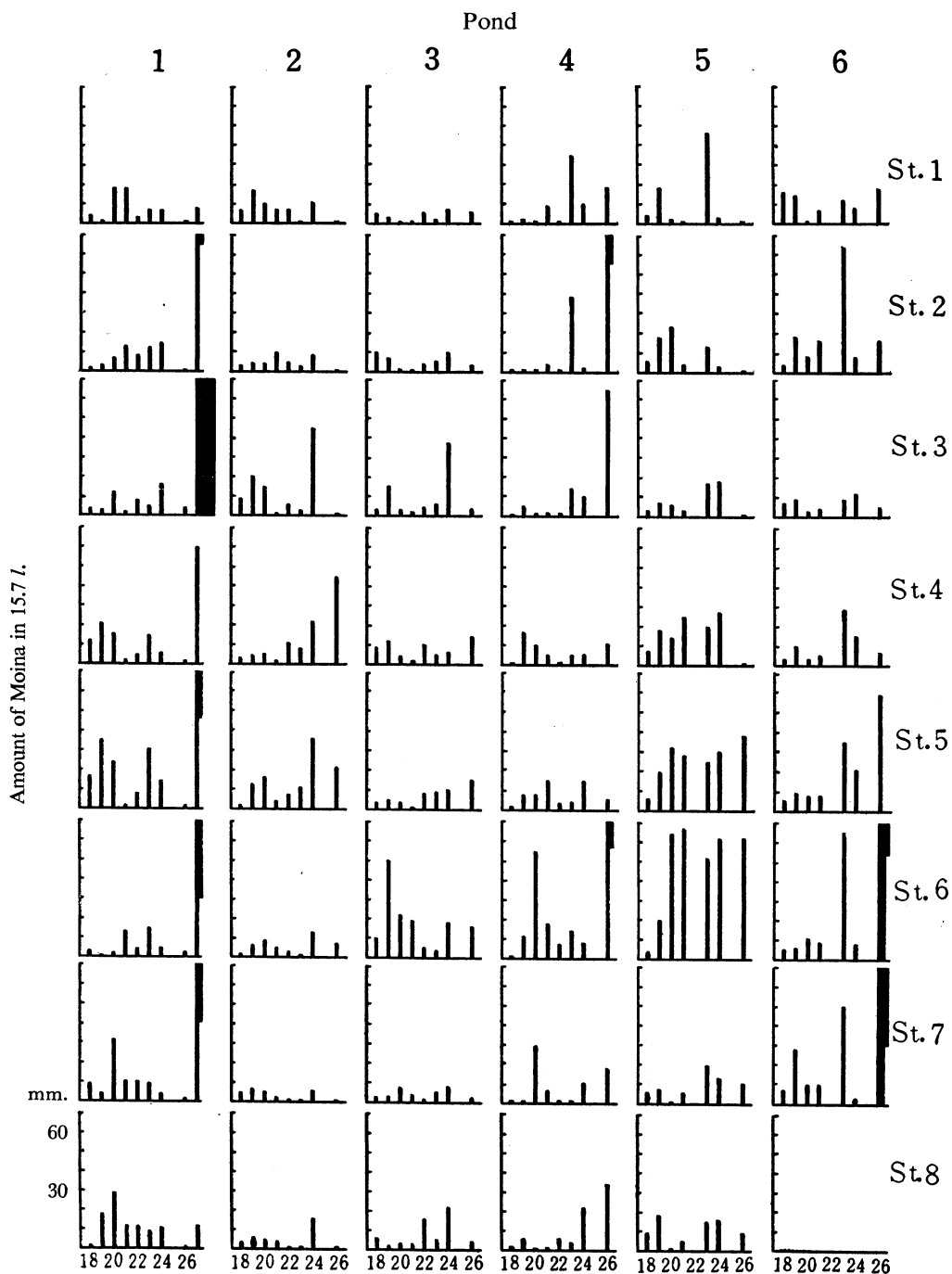


Fig. 3. Quantitative analysis of the population of Moina in the ponds.

Table 1. Average value of the amount of Moina distributed at eight stations of the six ponds of Gunma Prefecture.

Pond No. Station	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
	mm.					
1	7.4	7.6	3.8	9.5	10.7	10.9
2	7.9	5.0	5.9	16.6	9.7	19.0
3	6.8	13.8	9.1	12.3	7.9	7.0
4	9.6	12.8	7.6	6.9	16.0	10.1
5	16.6	13.9	6.9	7.6	25.6	20.9
6	5.6	5.8	16.9	24.8	47.0	37.1
7	10.0	3.9	4.3	8.4	8.9	41.0
8	11.9	4.6	7.6	10.7	10.4	—

These values mean the amount of Moina in 15.7 l. The value of 1 mm means 78.5 mm³ in volume (=0.08 gr.)

1956, when a remarkable fluctuation of population growth was observed. Data were shown in Table 1 and Fig. 3. Samples were obtained by the above mentioned method and they were filtrated through a small wire gauze of quantitative filter (10 mm. in diameter, 100 mm. in length). In Fig. 3 population size is represented in millimeter, in this case 1 mm. corresponds to 78.5 cubic millimeter in volume, and its value means the amount of Moina in 15.7 litre. Fig. 3 shows the pattern of the distribution of Moina at the eight stations in the six ponds. Granted that these figures are representing the population size, it will be able to discuss the differences of the character of distribution of Moina in the ponds.

Following results were obtained:

(1) Alarming changes in abundance have been observed in some ponds.

For instance, in the pond No. 1 all stations except St. 1 and St. 8 showed a remarkable increase on 27th, while on 26th we could not find any Moina at all stations, but the next day they appeared again in great abundance. Notwithstanding the sudden disappearance of the Cladocera have been observed by many investigators, the fluctuations between the 26th and 27th in the pond, No. 1 must be thought as a remarkable change in abundance. 350 mm. of St. 3 was the maximum value gained during this investigation and it may be safely said that it is nearly the maximum density of natural population of Moina. These phenomena were observed partially in other ponds, for example, Pond No. 2, No. 4 and No. 6 showed the same fluctuations on 24th and on 26th respectively.

(2) Evidence of the independence of the stations.

Regarding this problem the pattern of the Pond No. 5 indicated some interesting figures at St. 5 and St. 6, where the fluctuations were rather small and the population had been maintained at high level during ten days. This was proved by the data of 26th when other stations showed low value but the St. 5 and St. 6 showed the same high value of the day before.

The distribution of Moina in the ponds that were estimated to have the same condition of location, as a whole, did not indicate the same pattern. Accordingly

it is rather difficult to find a universally applicable model of the distribution of *Moina* in the ponds.

(3) Standard population size was estimated.

As above mentioned the distribution of *Moina* shows different pattern in the six ponds, but standard population size was estimated from Table 1 where average value of the samples collected at eight stations of six ponds during ten days were recorded.

It shows that the value of 20 m.m. obtained by the quantitative wire-gauze sampler indicates a dependable population size demanded by fish farmer in this country.

(4) Size composition, ratio of ehippial female to parthenogenetic female and the fecundity of parthenogenetic females.

Fluctuation of size composition of *Moina* in the ponds must be clarified, because number of individuals do not indicate the true population size of *Moina*. Number of individuals is the generally adopted expression of population density, nevertheless it does not indicate the true amount of *Moina* in the pond, because in the cladoceran crustacea the population is constituted of individuals of various size, larva and mother. For instance, in this expression as they are similarly counted there is no difference between the mothers which have many larvae in the brood chamber and the larvae just hatched. And the population density expressed in number of individuals indicates occasionally decuple value, in spite of the same volume.

Sample was obtained from eight stations of each pond once a day, and it was divided into four size-classes, A, B, C and D by using filters of different meshes.

Four size-classes of body length are as follows:

A: >1.2 mm., B: 1.0–1.2 mm., C: 0.8–1.0 mm., D: <0.8 mm.

After their volume was measured through above mentioned quantitative filter, their percentages in volume were calculated.

In order to make clear the ratio of the number of ehippial females (sexual female) to parthenogenetic ones they were examined microscopically. As the results it was known that A, B, C and D were respectively constituted of parthenogenetic and ehippial mother females, ehippial females and large size males, immature small males and females, and the smallest male and female larvae.

As the population-increase or -decrease factors, the fecundity of the parthenogenetic females must be examined. Then the number of larvae of summer eggs in the brood chamber of mother female were counted under the binocular microscope. Obtained data were shown in Fig. 4.

Several noteworthy results were obtained as follows:

(i) Three types of fluctuations of size-composition were observed in the population of *Moina* in the pond.

In the first type the difference in volume between the groupe of A plus B and the group of C plus D is large and it shows the steady growth. Consequently the population is constituted only of ehippial mother group. Pond No. 1, No. 2, No. 5

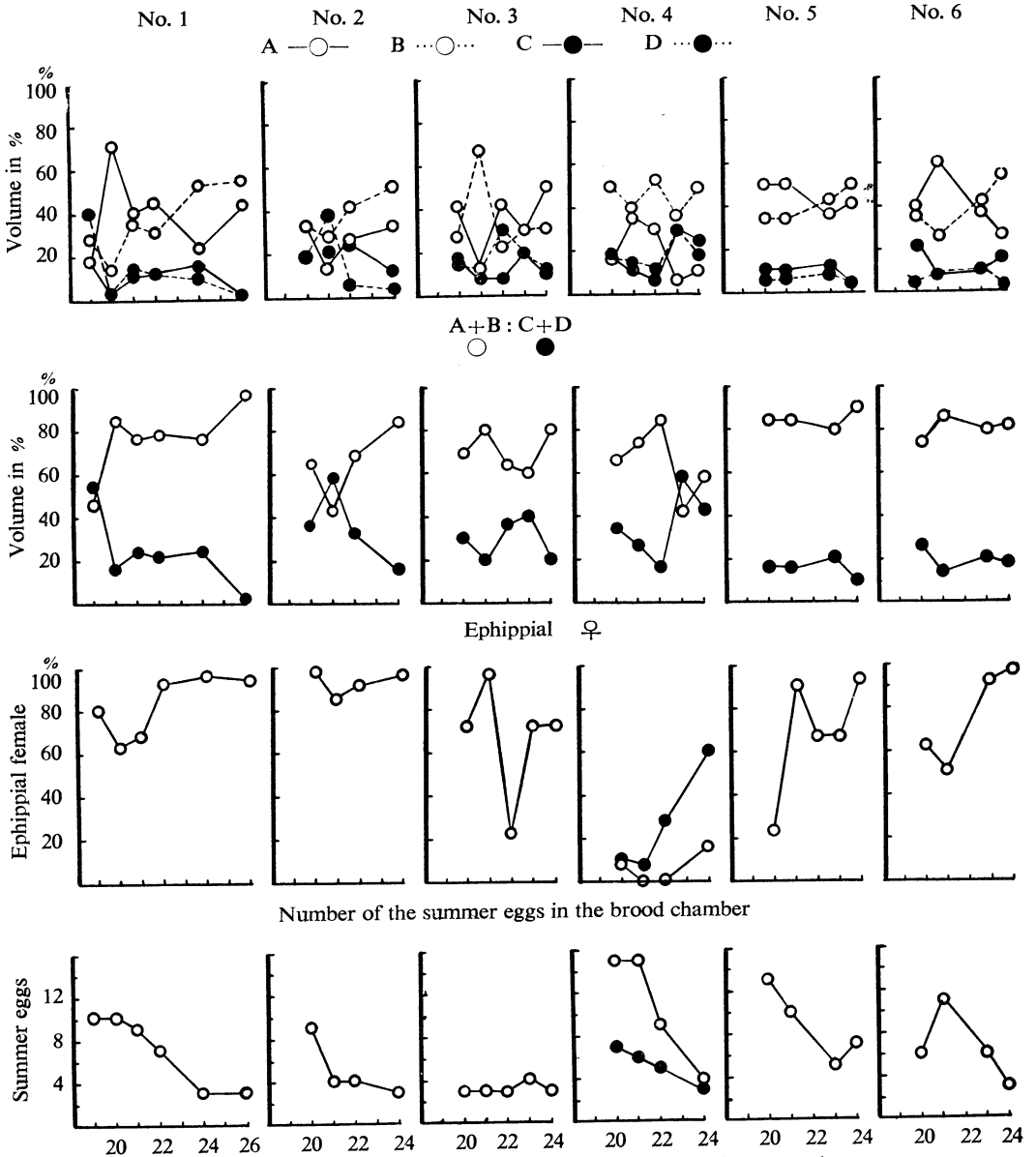


Fig. 4. Qualitative analysis of the population of Moina in the ponds.

and No. 6 belong to this type.

In the second type the difference between two groups is not so large as in the first type, and the fluctuations are wavy. In this type if it goes typically the population of Moina is always constituted of two groups of moderate amount. Pond No. 3 seems to belong to this type from the view of its size composition.

The third type differs essentially from the above mentioned two types. In this case, the population is constituted of two different species of Cladocera. In this investigation the population of cladocera of the pond No. 4 was constituted of both

Moina macrocopa and *Moina weismanni*, and the latter showed its peak of abundance later than the former.

(ii) In the fish ponds parthenogenetic generation of *Moina* does not last for a long time, because the fecundity of parthenogenetic females in all ponds decrease in a short time. For instance, *Moina* in the Pond No. 4 showed its best condition on the 21st, namely summer eggs in their brood chamber were fifteen, but three days after they dropped to four, and the percentage of the ephippial females to the parthenogenetic ones increased.

(5) An examination on the problem of additional fertilizer.

Additional fertilizing is widely used for practical method to increase the *Moina* in the ponds, whenever the population decrease takes place. Regarding this problem little is known, but if it is effective really, this treatment means to supply the deficiency of food of *Moina*.

Ammonium sulphate and boiled wheat flour have been used by many fish farmers. But as mentioned above, the changing of the generations from parthenogenetic to bisexual, is the chief cause for the population decrease of *Moina*, and when it occurs, the increase of the percentage of ephippial females may invariably be observed. Accordingly, experiments were intended to make clear the possibility of changing of the ephippial females to parthenogenetic ones by the treatment of additional fertilizing.

A great number of ephippial females of *Moina* were collected from the aquaria in the laboratory, and their gonads were examined microscopically. Their gonads are not uniform in shape and stages of the development of winter egg were observed as is described in Fig. 5. The direct effect of the additional fertilizing was examined in the following method. Ephippial females were put individually into a vessel fertilized with organic fertilizer. In this experiment dry chick dung was used.

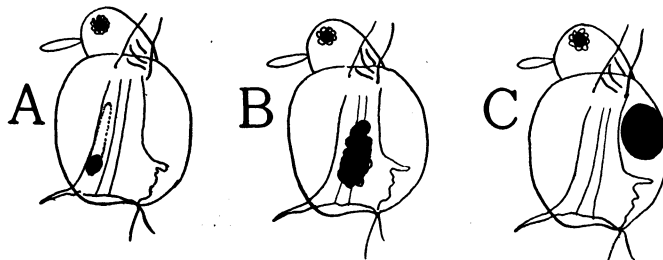


Fig. 5. Three stages of winter egg.

A : The origin of the winter egg. It is situated to the left side of the intestine.

B : Middle stage of the growing winter egg. It is on the intestine.

C : Winter egg in the brood chamber, ephippium is already completed.

As is shown in Fig. 6 the effect of the additional fertilizer is related to the stage of the maturation process of the sexual egg. For example, when the eggs developed in the length over the value of thirty percent of body length, the ephippial mother could not be changed to parthenogenetic ones. She continues the formation of sexual

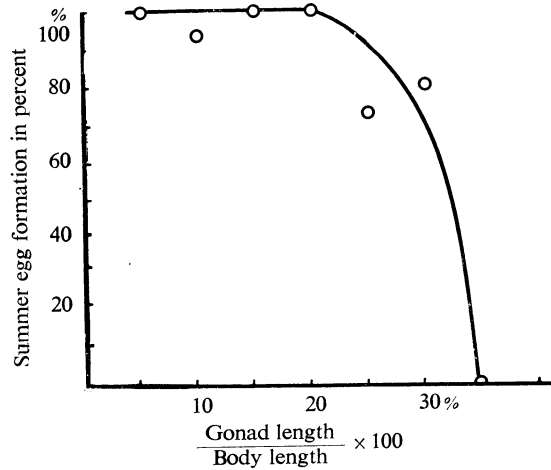


Fig. 6. Effect of the additional fertilizer to the winter egg forming Moina.

egg and completes it in the ephippium, even if it was put in the well fertilized solution. On the contrary, opposite result is expected when the gonad length is less than thirty percent of its body length.

3. Observations on the stock of the winter eggs of Moina in the pond, with a special reference to its decreasing.

It was already mentioned that the winter egg of Moina has a long sleeping at the bottom of the pond until the next favorable season comes. At Gunma Prefecture in the Kanto district the sleeping period is about eleven months from June to April of the following year. It is doubtful whether the pond bottom is a suitable place to keep the eggs for a long time, because it is ordinarily covered with organic silt in some depth and it bears many inadequate conditions to keep the eggs.

We have little informations with the amount of winter eggs in the ponds and their decrease in a year. In order to clarify these problems the following points were observed.

- (1) Types of winter egg formation.

A great many samples were collected, not only from many fish ponds but also from small outdoor and indoor aquaria.

Morphological study of these samples were performed. Besides these morphological study, observation with the procedure of winter egg formation of Moina in nature and in the laboratory were done. And it may be given as a conclusion that there are two types of winter egg formation, the one is ordinarily seen in fish ponds and the other is observed usually in a small aquaria in the laboratory. They have not only the different size of the produced egg but also the different frequency of production. In fish ponds the same individual produces repeatedly many winter eggs, but in a small vessel of the laboratory winter egg formation takes place only one time in its generation. These two types are explained in Fig. 7.

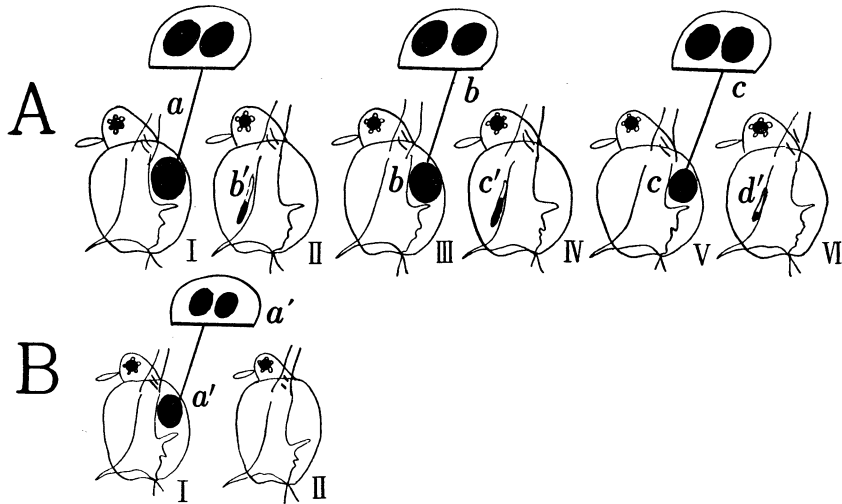


Fig. 7. Explanation of two types of winter egg formation in *Moina*.

A : This type is found commonly in the carp-culture ponds. The same mother produces repeatedly many winter eggs.

In II, IV, VI she shows the origin of the winter egg just after she produced a, b and c.

B : This type is observed in a small vessel in the laboratory. Winter egg formation is seen once in her generation.

Winter eggs produced in A type are larger than that in B.

(2) Non-existence of pseudosexual egg in *Moina*.

It was reported already that the pseudosexual eggs exist in some species of *Daphnidae*. And it is well known that there is a remarkable difference between the pseudosexual egg and the true sexual egg, in spite of their same shapes, namely, the former has a diploid and the latter has a haploid chromosome. Following experiments were performed to prove the non-existence of pseudosexual egg in *Moina*.

25 ephippial females with well developed gonad were put in a small vessel, and vigorous males were poured into this vessel. Females used are all 1200–1300 μ in length and their gonad length are also over 30% of their body length. Males are 800–900 μ in length. Following five combinations are prepared.

$$\text{♀} : \text{♂} \quad 25 : 0, \quad 25 : 5, \quad 25 : 10, \quad 25 : 15, \quad 25 : 25$$

Obtained results were shown in Fig. 8.

True winter eggs are not produced in any case in the vessel which is free from males. Instead of true winter eggs there exist many empty ephippia. Accordingly, winter eggs of *Moina* are always fertilized eggs.

(3) Distribution of the winter eggs in the ponds.

The amount of the first larvae in the propagation of *Moina* in the pond is decided by the amount of the winter eggs produced at late May of the previous year and its survival rate during eleven months, but our knowledge regarding the distribution and the amount of the winter eggs of *Moina* in the ponds are too me-

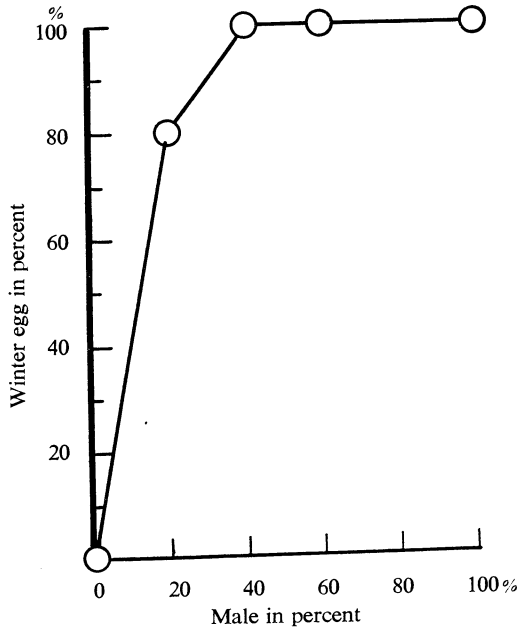


Fig. 8. Relation between sex ratio and the produced sexual eggs (%).

Females that are free from males do not produce the true sexual eggs. They produce only empty ephippia. This experiment shows non-existence of pseudo-sexual eggs in Moina.

agre up to now.

Three ponds in two prefectures far from each other were selected, two of them are located in Gunma and one is in Yamaguchi. Investigation was performed December 1956 and March 1957. Survival rate was calculated from the difference between the estimated early stock and the real stock at present. Obtained details were recorded in Table 2 and Fig. 9.

Results obtained from this investigation are as follows:

(i) The distributions of the winter eggs of Moina in three ponds are not uniform in their pattern, and it was difficult to find a standard pattern even if in two neighbouring ponds of Gunma Prefecture.

(ii) The amount of last survivor is estimated as 4,000–50,000/m² and decuple variation existed between these three ponds.

(iii) Survival rate ranged between 2–8 percent.

(iv) Distribution of bottom silt is related to the distribution of winter eggs in the Pond No. 4 of Gunma Prefecture.

(4) Survival curve for the winter eggs of Moina in the pond.

The factors related to the survival rate of the winter eggs will be divided into two categories, namely biological and physicochemical. Data in Fig. 9 and Table 2 indicate mean value of last survivor, but the details in the course of decreasing were unknown. Hence, in 1959 an attempt was made to make clear the decreasing

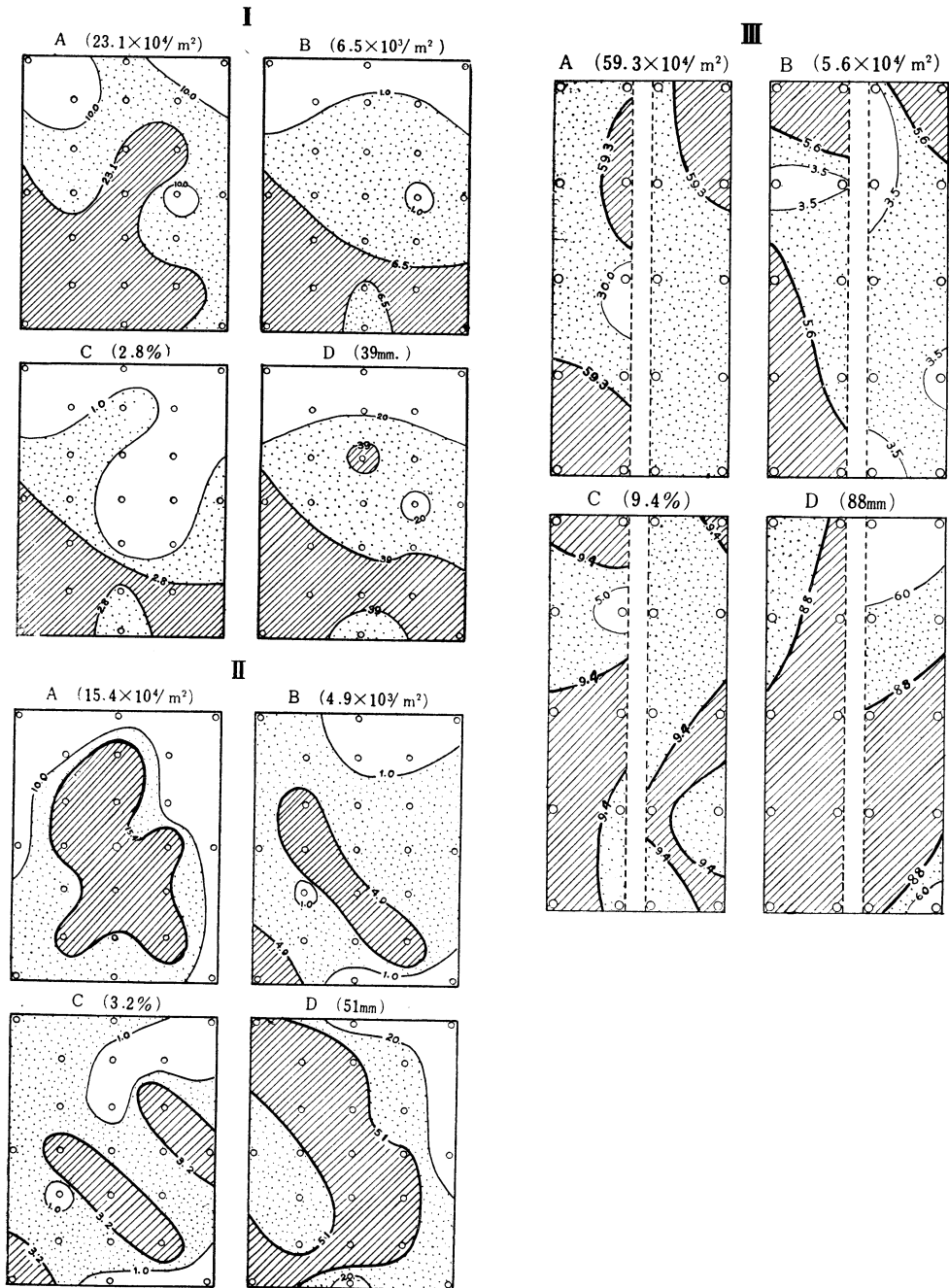


Fig. 9. Maps showing the variation in the distribution of winter eggs and mud.

I, II and III: ponds Nos. 3 and 4 in Gunma and the pond in Yamaguchi respectively.

A) estimated total number of winter eggs produced ($/m^2$), B) number of winter eggs at the present investigation ($/m^2$), C) survival rate of winter eggs ($B/A \times 100$) (%), D) mud depth (mm.). Average values for each item are given in brackets. Areas showing higher values than average are crosslined, and open circles represent the sampling stations.

Table 2. Number of winter eggs obtained in various ponds.

Pond	Number of sampling stations	Estimated number of winter eggs produced /m ²		Number of winter eggs in the present investigation /m ²		Survival rate of winter eggs %		Mud depth mm.	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Gunma, No. 3	23	26,900— 838,500	231,300	0— 48,000	6,500 (4,900)	0— 8.5	2.8(2.1)	0— 96	39
“ , No. 4	23	13,300— 410,400	154,300	0— 35,000	4,900 (3,600)	0—12.1	3.2(2.4)	0—120	51
Yamaguchi	20	260,600—1,210,500	593,300	28,100—146,900	56,000(47,000)	3.7—18.3	9.4(7.9)	50—120	88
Note Gunma No. 5	14	0—209,500	52,300	0—9,900	1,400(1,000)	0—6.3	2.3(1.9)	15—90	30

Figures in brackets are the corrected values determined after examining the hatchability of the eggs. Hatchabilities were 75% in Gunma samples and 84% in Yamaguchi ones.

process of the eggs in the ponds. Collections of samples were performed in the ponds of Gunma, Tokyo and Hiroshima Prefecture. Survival curve was shown in Fig. 10.

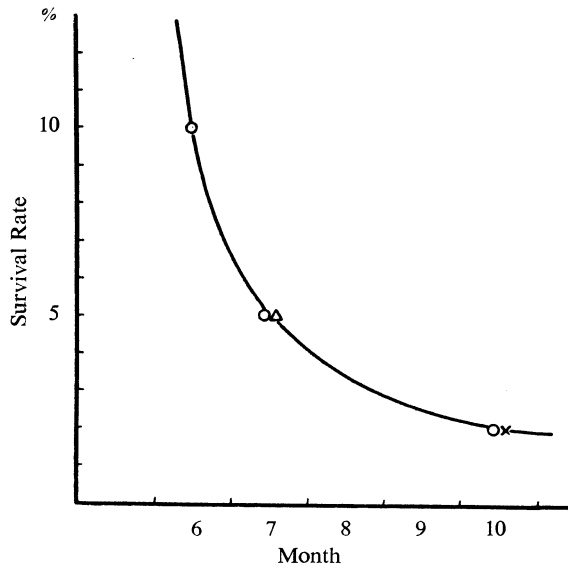


Fig. 10. Survivorship curve for the Winter Eggs of *Moina* in the carp-culture ponds.

○ Gunma △ Hiroshima × Tokyo

In Fig. 10, it is clear that the curve drops sharply in a month just after the winter eggs were produced in the ponds, then it goes gently sloping and after late October it seems to continue in the same level, because the value of late October of 1959 agrees with the value of 1956. Conceivable factors related to the survival of the winter eggs are as follows.

- i) Percentage of fertilization
As mentioned above, copulation or fertilization is the first requisite factor to produce the winter eggs.
- ii) Percentage of imperfect ephippia.
When the winter eggs are not closed perfectly in the ephippia, they fall out easily and only empty ephippia are found there.
- iii) Bacteria attack will be the most innocuous factor.
- iv) Fungus parasite
Investigations on this problem are explained in the next chapter.
- (5) Sleeping of the winter eggs of *Moina* in the ponds.

Sleeping is the most characteristic and noteworthy phenomenon in the ecological and physiological studies with the Cladocera, because the keys to solve many problems with the Cladocera are expected to exist in the explanations of sleeping of the winter eggs. For example, the cycle of alternation of their generations and the survival rate of the winter eggs are deeply related to this phenomenon.

Sleeping of the winter eggs is generally accepted as a kind of the delayed germination, and if it is true, the sleeping control factors will be inherent in the winter eggs themselves, not in the environmental conditions, and the differences of the number of cycles of alternation of generations will be caused by these inherent differences. For instance, if Moina shows three types of cycle, namely, monocyclic, dicyclic and polycyclic, these differences indicate the existence of three different winter eggs of Moina each having different sleeping times.

From the point of this view, the monocyclic aspect of Moina in the carp-culture ponds of Gunma Prefecture could be explained as follows: The eggs produced in late May are not mature enough to begin their development immediately and their maturation proceeds slowly with the elapsed time until their hatching takes place on early May of the following year.

But there is another opinion with this sleeping of the winter eggs of the Cladocera, this is, an explanation based on environmental factors of the places where the winter eggs are preserved for a long time.

Apparently there is a discrepancy in interpretation between these two opinions. One is chiefly based on the biological inherent natures of the winter eggs themselves, on the other hand, the other gives priority to the environmental factors.

Many experiments were performed in the laboratory with the hatching of the winter eggs of Moina collected from many carp-culture ponds in every season round a year, but their results were always denying the hypothesis that the sleeping of the winter eggs of Moina is a kind of delayed germination, because in all cases of these experiments their hatching rate always indicated a fairly high value, even if the material was collected on late May just after they were produced.

Winter eggs of Moina that were obtained in aquaria of the laboratory under various conditions were also tested, but they showed always the same results as in nature.

On the contrary, following fact was experimentally proved that when the winter eggs of Moina were buried into the deposit of the Moina culture tank, the hatching of them did not take place, even if other conditions were favorable to their development. Bottom silt of the carp-culture ponds and its filtrate are also similarly effective in inhibiting their development.

Furthermore, when those controlled winter eggs were transferred into tap water their hatching rate showed the same high value as those not controlled by the above mentioned substances, so it is right to interpret the sleeping of the winter eggs of Moina at least as a phenomenon to be that their development is inhibited by environmental factors.

Both in Moina culture tank and in carp-culture ponds, a great amount of organic matter are used as a fertilizer and the deposit or bottom silt are more or less polluted with their decomposition added by the disassimilation of organisms. Rarely, they show always a positive ammoniac reaction to the Nessler reagent.

It is well known that ammonia and ammonium compounds have a toxic action to aquatic organisms, and as above mentioned the sleeping of the winter eggs of

Moina is a kind of compelled inhibition of the development, then the following experiments were done to make clear the relations between them.

Fifty winter eggs of Moina were sealed into ampoules (15 cc in volume) which have different concentrations of ammonia and ammonium compounds solution. These ampoules were kept at 25°C, and they were tested at intervals of a week.

Development control power of ammonia and ammonium salts solutions were decided by the percentages of the number of the deformed winter eggs during preservation, not by the hatching rate, because as the first prerequisite the size immutability is demanded for the sleeping of the winter eggs.

And it is also well known that pH value is very important, but in these experiments the solutions NH_4Cl , NH_4CO_3 and $(\text{NH}_4)_2\text{SO}_4$, were not regulated, accordingly their pH values show less than 7.

Experiments with solutions of ammonium salts showed the following results that all winter eggs kept therein were deformed, even if their concentrations of ammonium ion are more than 100 p.p.m.(N). So it will be said that the sleeping of the winter eggs of Moina can not be attained solely by application of ammonium ion. On the contrary, experiments in ammonia water show some interesting results. As showed in Fig. 11 ammonia water over 10 p.p.m.(N) apparently showed to have control power to the development of the eggs, and according with the decrease of the amount of ammonia their control power dropped. Here, it was also experimentally proved that high pH value, for example, pH 9-10 of the H_3BO_3 , KCl and NaOH buffer solutions could not inhibit the development of the eggs, and as above mentioned the amount of not ionized ammonia are effective more than ten times of ionized ammonia. So it is conceivable that free ammonia plays an important role for the sleeping of the winter eggs of Moina.

But such amount of free ammonia of 10 p.p.m. of ammonia water could not be expected in nature, even if the amount of ammonium ion indicates the same value. Furthermore, it is unforgettable that the survival rate of the winter eggs of Moina in the carp-culture ponds are usually only 2-3 percent and the sleeping phenomenon is rather imperfect at the bottom of the ponds. Besides ammonia and ammonium ion as the sleeping control environmental factors the following ones are expected, namely water temperature and the amount of the dissolved oxygen. For example, low value of these factors inhibit the development of the eggs, but on the contrary, both high temperature and plenty amount of dissolved oxygen activate the development.

In Fig. 11 the long time preservation of the eggs in 10 p.p.m. ammonia water indicates the decrease of hatchability, and so it is desirable to use weak ammonia water to preserve the winter eggs for a long time.

From this point of view, bottom silt of the ponds were again tested, as a practical preserving method of the eggs, because if it is alkaline, the organic polluted mud always contains more or less ammonia and the consumption of the dissolved oxygen is always expected there.

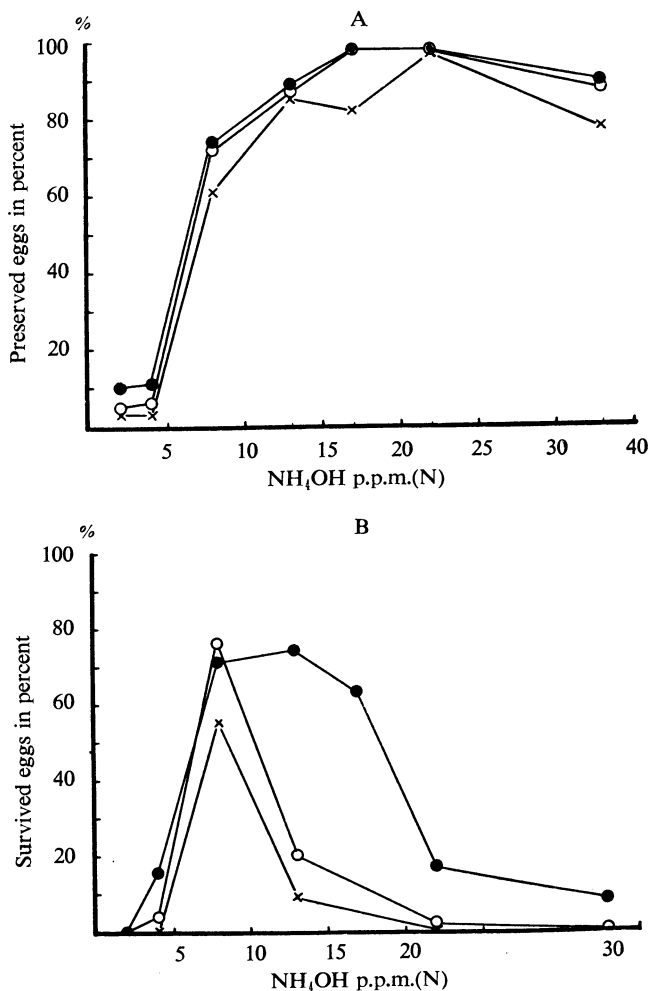


Fig. 11.

A : Relation between the concentration of ammonia and its effect to prevent the development of the Winter Eggs.

B : Relation between the survival rate and the concentration of ammonia.

- Preserved in 10 days
- " 20 "
- ×— " 30 "

One example of many experiments is shown in Fig. 12.

For the long time preservation of the winter eggs of Moina, the amount of organic matter of the bottom silt is important, because if it is in excess, the putrefaction usually takes place, and the death of eggs happens.

Practically, satisfactory results are always obtained when the winter eggs are preserved in the bottom silt of the ponds, but here it is necessary that the mud must be filtrated with tap water several times. For instance in Fig. 13 the survival rate and hatchability of the eggs preserved in this method is shown.

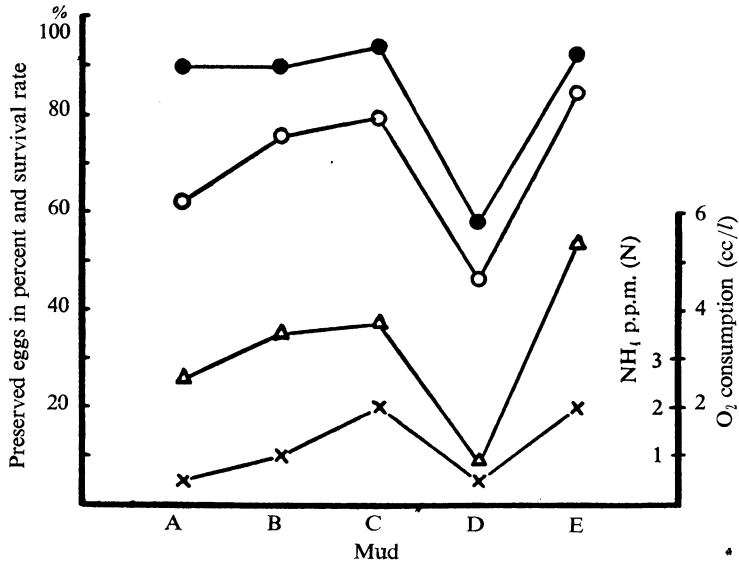


Fig. 12. Relation between the sleeping of the Winter Eggs and the bottom silt of the carp-culture ponds. (January 1958)

—●— Preservation rate —○— Survival rate —△— O₂
 —x— NH₄ p.p.m. (N)

Table 3 Effect of the bottom silt to the sleeping of the Winter eggs of Moina

Mud	Pond	Date	O ₂ consumption cc/l 9°C 24 hrs.	NH ₄ (N)	Preservation rate %	Survival rate %
A	Gunma No. 5	1956. 5	2.64	1>	89.5	62.3
B	"	1956. 12	3.47	1	89.6	75.5
C	Shizuoka Eel-culture Pond	1958. 1	3.76	2	94.0	79.4
D	Yamaguchi Carp-culture Pond	1957. 2	0.88	1>	57.7	46.3
E	Laboratory	1957. 10	5.39	2	92.2	84.3

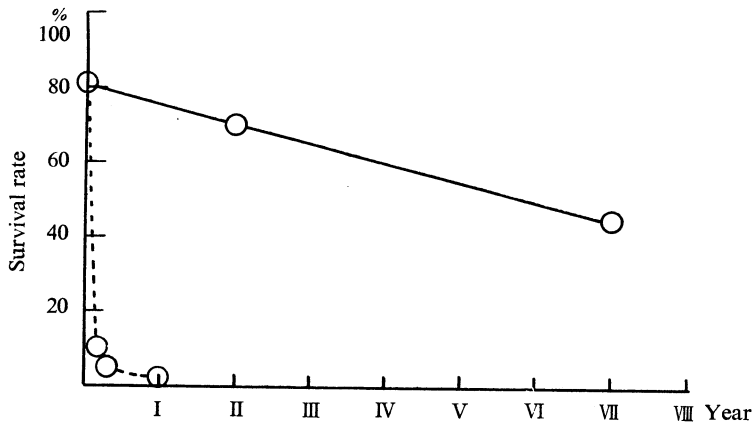


Fig. 13. Comparison of the survival rate of the artificially preserved Winter Eggs and that in nature.
 Eggs in nature — Artificially preserved Eggs

Abnormal eggs of Moina

It is already mentioned that in Moina pseudosexual eggs do not exist, but the existence of abnormal eggs is one of the most characteristic and noteworthy phenomena.

Specimens used in this experiments are always microscopically examined with their size and shape, but it is impossible to know whether they are normal or abnormal by the morphological examination only, because occasionally their hatching rate shows very small value in spite of their normal shape. These abnormal winter eggs are only distinguished by the difference of their viscosity from the normal ones, the former is less than the latter.

Existence of these abnormal eggs is one of the most important causes of the seemingly unreasonable results of the increase of the hatchability of the eggs at the later season. If the number of these abnormal eggs is much at earlier season than at later season, and they decay to empty ephippia with the elapsed time, the hatchability of the tested winter eggs indicate high value. So the fluctuation of hatchability must be necessarily examined with the reference of constitution of the eggs.

Other preservation methods of the winter eggs were also examined, but good

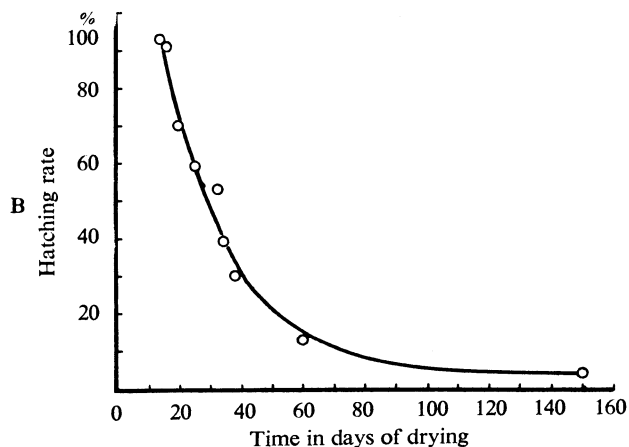
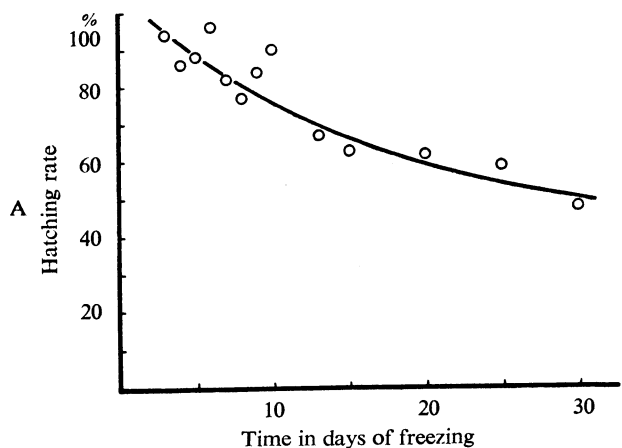


Fig. 14. Preservation of the Winter Eggs of Moina by freezing and drying.

results were not obtained than in the case of the methods of above mentioned bottom silt. In Fig. 14, the results of the two methods, freezing and drying are shown.

4. A parasitic fungus, *Lagenidium* sp.

In early June, 1959, fungus parasited winter eggs were obtained in carp-culture ponds of Gunma Prefecture. This was the first collection during this ten years observation with the winter eggs of *Moina* in many carp-culture ponds in Japan. These specimens were observed by microscope in the Laboratory of the University of Tokyo, and its asexual reproduction was partially clarified as follows. Under aerobic conditions the parasite fungus shows interesting figures as described in Fig. 15 and Plate 1 and 2. This fungus was identified to belong to *Lagenidium* sp. by the kind aid of Mrs. SUNAYAMA of Tōhō University.

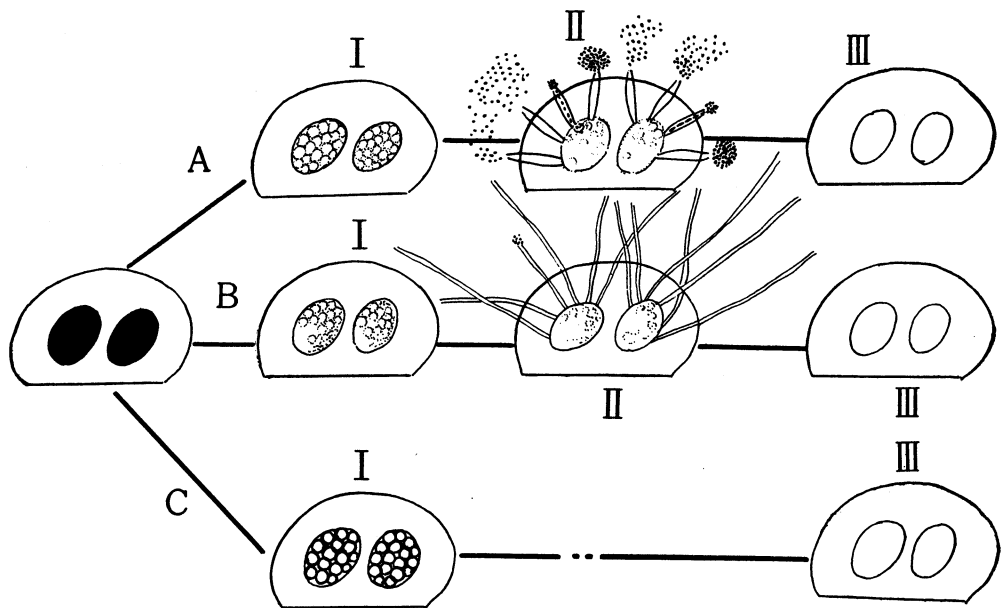


Fig. 15. Explanation of the asexual reproduction of a *Lagenidium* sp.

Three types were observed.

A I shows the deformed Winter Eggs infected by the zoospores of a *Lagenidium* sp.

A II shows many discharge tubes and liberation of the zoospores. These zoospores attack again other healthy Winter Eggs.

A III When zoospores liberated out, there remains a empty ephippium.

B I is like to A I.

B II Many long and narrow discharge tubes are formed, but zoospore can not be found.

When the zoospores are liberated, they are very few.

B III B II changes to a empty ephippium.

C is different from A and B. It makes resting spores with thick membrane. These spores do not change to zoospores in a short period.

Under the favorable water temperature, 20–30°C, many infection experiments were performed and the cycle of their asexual reproduction were observed. This fungus is holocarpic and when the zoospores entered into the healthy winter eggs

of Moina, the formation of discharge tubes were observed, then many zoospores come out through the tube in a single file, and encysted just outside of the mouth of this discharge tube. The length of tubes was 100–700 μ , sometimes over 1500 μ , and its width was 10–25 μ .

Soon after they became encysted, they formed a spherical aggregation, (80–120 μ in diameter), containing 20–200 zoospores. These spherical aggregation grew rapidly. After an active rotating movement, the zoospores swam out into the water almost as the same time, not separately. Zoospores are bean shaped (10–20 μ in length) and biciliate.

These escaped spores successively attack the healthy winter eggs and there remain many empty ephippia.

Concerning the distribution of this fungus little is known, but it is supposed that it will take wide distribution in the carp-culture ponds in Japan, because they were found in the ponds of Hiroshima Prefecture of the Chugoku district in July 1959.

The infection of this fungus can be apparently observed in nature when they form discharge tubes in the winter eggs of Moina, but at the earlier stage it is rather difficult to distinguish the parasite eggs from normal one.

Percentage of the infected eggs observed in nature shows less than 10%, but in laboratory it shows more than 50%. Here, the bottom silt again plays an interesting role, namely, the fungus in the winter eggs is preserved in it for a long time, and it develops when the eggs were set under aerobic condition.

It was experimentally proved that when one infected winter eggs is mixed in the healthy ones, their hatchability remarkably decreases, occasionally no hatched larvae were observed, even if 99% of the winter eggs are healthy ones.

Infection experiments by this fungus to the winter eggs of Moina have an

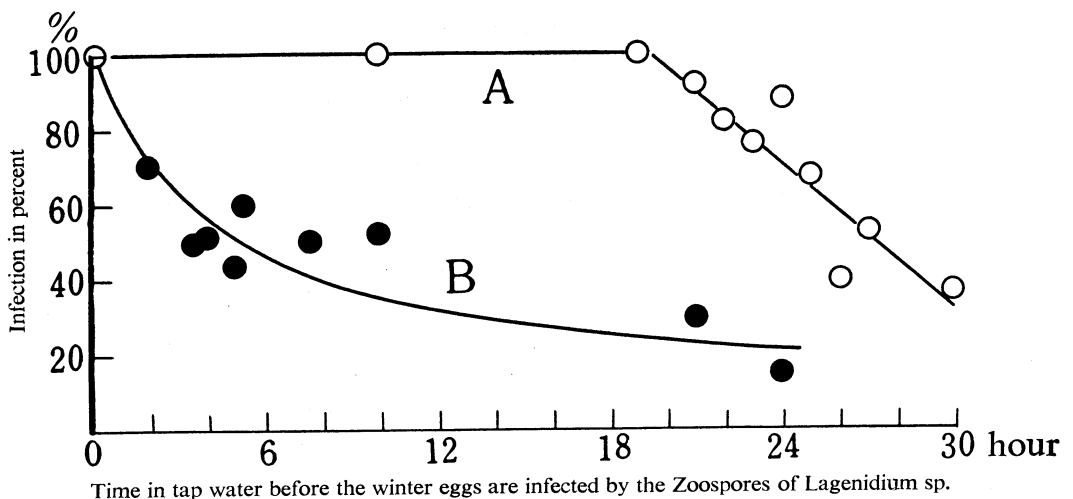


Fig. 16. Relation between the stage of the development of the winter eggs and their infection rate.

The development of A goes slowly than B. Using these infection experiments the activity of the winter eggs can be tested in a short time.

unexpected availability to know the hatchability of the eggs in a short time. In order to know the hatching rate of the winter eggs in tap water several days are needed but the cycle of the asexual reproduction of this fungus is observed in thirty hours.

It was experimentally known that the infection rate of this fungus to the winter eggs is related to the activity of the eggs. For instance as shown in Fig. 16, the infection rate shows different figures in different materials and in different developmental stages of the same material.

Winter eggs that have different exposure times in tap water were infected artificially with this fungus zoospores. In sample A the infection rate shows the same value of 100% till 19 hours exposure, but at 20 hours it begins to drop almost in rectilinear form till 28% at 30 hours exposure. Accordingly by the test of this infection experiment, the winter eggs of sample B is more active than those of sample A, in other words, from the point of view of quality, the sample B is better than sample A.

(IV) DISCUSSION

In almost all fish farms in Japan organic substances are used as fertilizer in Moina culture, such as cattle manure, barnyard manure and soy lees, and they furnish plenty of foods for Moina, containing bacteria, protozoa and unicellular phytoplankton.

Among these foods for Moina, the bacterial growth will serve as the principal ones. Concerning the culture methods of water fleas many works have been reported, for instance, BANTA, CHIPMAN, PRATT, IMAI and SATO, MATSUDAIRA, MURACHI, FUJITA and many other investigators reported on this problem with special references to the food organisms.

Population growth and its fluctuations of water fleas have been taken as one of the most interesting problems in ecological and physiological studies of zooplankton in fresh water, and the works with these problems have been reported by many investigators, for example, PRATT, TERAOKA, FRANK and SUGINOME. But our knowledge with the growth form of population of Moina in large carp-culture ponds are meagre, notwithstanding the history of propagation of this water flea goes back to old times in this country.

In this investigation efforts have been made to obtain the standard feature for the propagation of Moina, and special attentions have been directed to the qualitative and quantitative fluctuations of population. In the culture of water fleas not only in laboratory but also in nature, the maintenance of the parthenogenetic reproduction during long time is the most ideal and desirable form.

As long as the theoretical population growth of Moina holds good, a great amount of it is expected in the ponds. For instance, if this water flea matures by four days from its hatching and she produces ten larvae every day and ten times in the generation, the following tremendous increase of the number of individuals is theoretically expected.

DAY	1	5	10	15	20	25	30
Number of Individuals	1	11	360	12. 900	478. 000	19. 020. 000	767. 170. 000

But in this calculation the following conditions are necessary:

- 1) Population is constituted with females;
- 2) productivity of mother shows always high level: 10 summer eggs are produced in her brood chamber at one time;
- 3) winter egg formation does not take place;
- 4) difference between the amount of young and mother is rather small.

But the analysis of the population growth form of Moina in the carp-culture ponds indicates that those conditions cannot be expected to exist in any way in the ponds. And so it is necessary to introduce a new technical treatment to solve these problems. From this point of view the amount of the winter eggs in the ponds must be carefully examined, because it is directly related to the amount of the first population of Moina. Furthermore, it will have more important meaning with the consideration of the fact that the first larvae hatched from the winter eggs produce summer eggs during their generation and they do not produce winter eggs.

Amount of the survived winter eggs during a year shows about ten times difference in the investigated ponds, from 5×10^4 to $4 \times 10^3/m^2$, and this difference may be significant when parthenogenetic reproduction does not continue for a long time.

Additional fertilizer and the seeding of winter eggs will be available techniques. As a practical method to preserve winter eggs as "seeds", the bottom silt in the ponds is useful. Caution must be paid to the parasitic fungus and its infected winter eggs, but details are remained in further investigation.

(V) SUMMARY

1) *Moina Macrocopa* STRAUS appears in the carp-culture ponds in May, but disappears during following eleven months, from June to April of the following year remaining dormant in the form of winter eggs kept in the bottom silt.

2) Population growth of Moina in the ponds was investigated, and it is classified into three types. The first is characteristic by the sharp decline of the parthenogenetic reproduction which then shifts to the bisexual phase. In the second type the growth shows a fluctuation taking a wave shape, and in this type considerable population growth is expected. The third differs fundamentally from those above mentioned. In this case two distinct species of Moina come into being showing their growth peaks at different times.

3) Community of Moina even in the neighboring ponds shows different patterns, and there its great fluctuations are met with occasionally.

4) The amount of the winter eggs of Moina was from 6×10^5 to $5 \times 10^4/m^2$ on the bottom of the pond, and their survival rate were estimated as 2-3%.

5) 'Sleeping' of the winter eggs of Moina is explained as that it is a kind of

inhibition of development enforced by environmental factors, and is not a delayed germination attributable to their own inherent character.

6) Ammonia is undoubtedly effectively related with the sleeping, but the best method to preserve sleeping eggs for a long time is the use of bottom silt.

7) Two types of winter egg formation exist in nature, but in the carp-culture ponds the type of successive formation of the winter eggs by the same mother is common, and this is the reason why there exists such a great amount of winter eggs in the ponds.

8) Non existence of the pseudosexual eggs was experimentally proved.

9) A sexual reproduction of a parasitic fungus, *Lagenidium* sp., was proved clearly by the infection experiments of its zoospores to the healthy winter eggs of *Moina*. This fungus is available to know the activity of the egg development.

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EXPLANATION OF PLATES 1-2.

Successive liberation of zoospores of the six different discharge tubes of *Lagenidium* sp. at 25°C.

Fig. 1, 2, 3, 4. Process of the forming of the spherical aggregation of zoospores and their liberation of the discharge tube a.

Fig. 5, 6, 7, 8, 9, 10. Process of the encystment, and the liberation of the newly formed discharge tube b. Enlargement of the spherical aggregation of zoospores is observed.

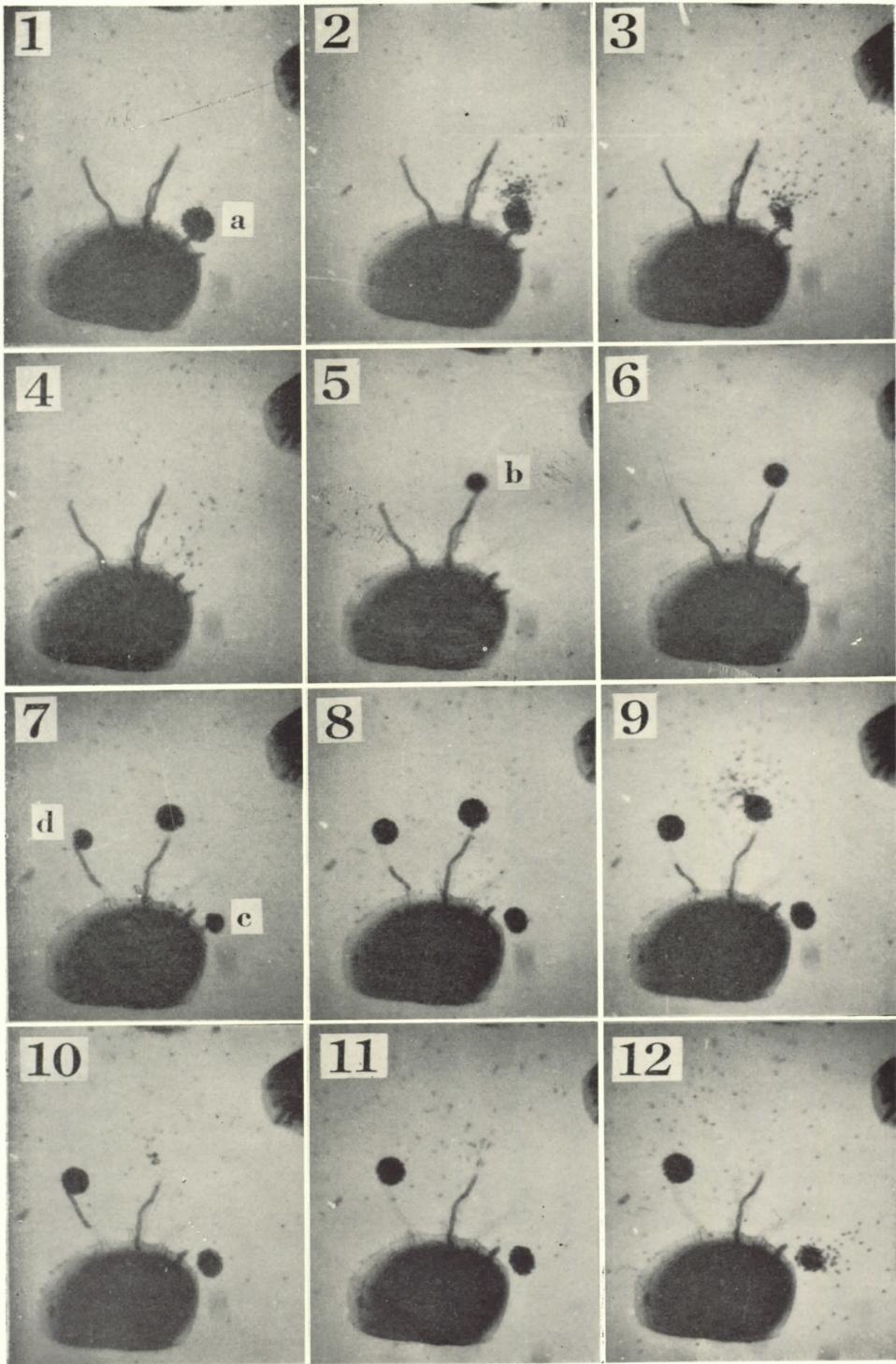
Fig. 7, 8, 9, 10, 11, 12, 13. The same process of the third tube c.

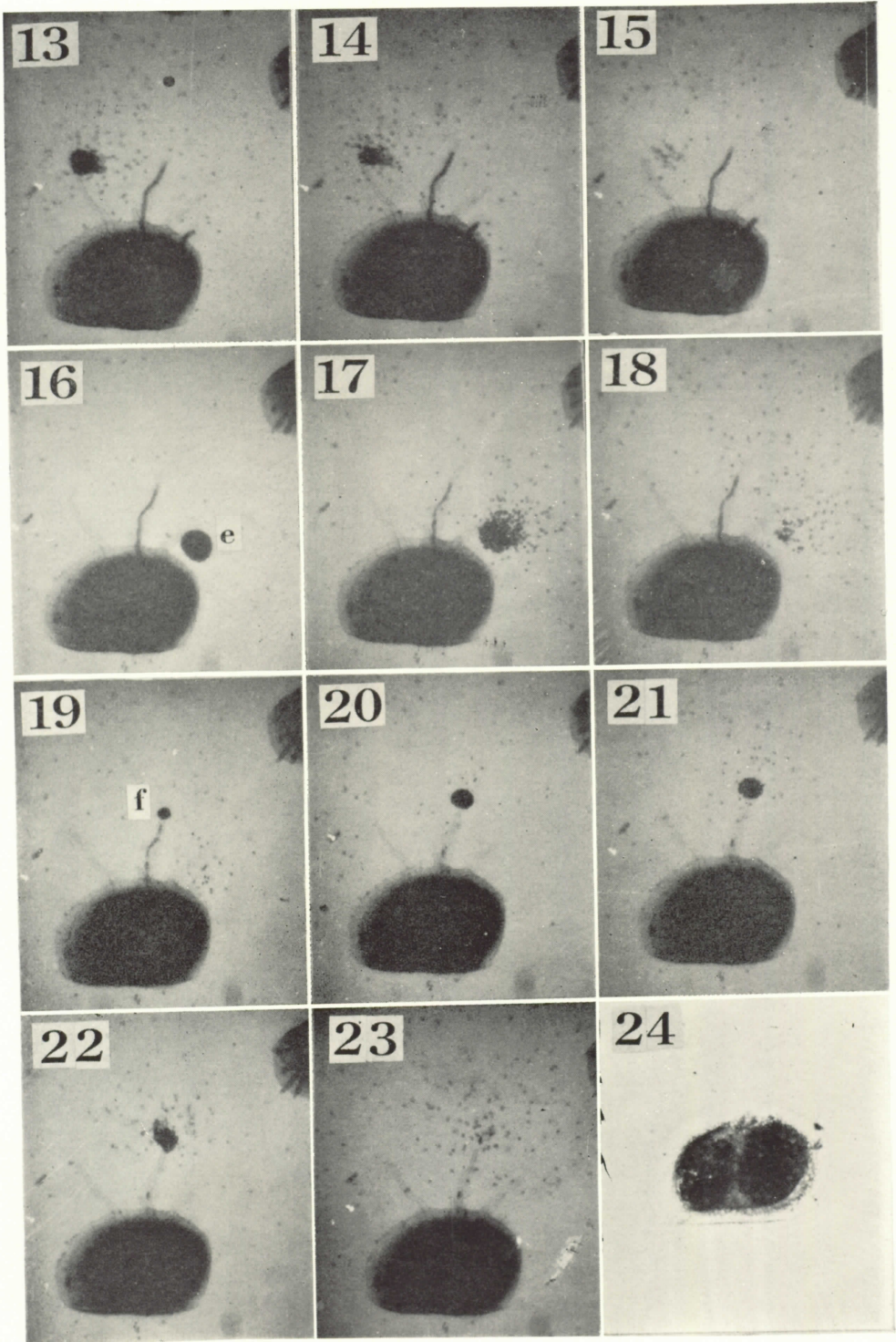
Fig. 7, 8, 9, 10, 11, 12, 13, 14, 15. Ditto process of the fourth discharge tube d. In Fig. 9, 10 indicate the escape spore and the empty tube.

Fig. 16, 17, 18. The same process in the fifth discharge tube e. Liberation of zoospores takes place all at once.

Fig. 19-23. The same process in the sixth discharge tube f.

Fig. 24. Showing the winter eggs packed with resting spores with thick membrane.





MURAKAMI : Winter Eggs of *Moina*

