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Factors influencing the start of development in Daphnia pulex winter eggs.

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I. INTRODUCTION

The winter eggs of Daphnia pulex, after passing safely through the winter, develop and hatch in the spring in numerous ditches and pools and subsequently lay summer eggs several times, multiplying by themselves, while some males emerging among them with the changes in environment produce fertile eggs, which are universally known as winter eggs (lasting eggs). These eggs, however, do not always pass safely through the winter. Given a suitable environment in the autumn, they hatch out in a very short time, again producing fertile eggs (winter eggs). Occasionally, at this period of varied generations, a multitude of Daphnia pulex may be seen in quite a small pool of water. So far, however, very little investigation has been done in connection with the factors governing the development of each separate generation of Daphnia pulex.

As regards artificial methods of treatment for initiating development in winter eggs, the drying process, temperature treatment or sunlight method are said to be the most effective. There are also reports by Banta (1921), Galtsoff (1937), Obreshkove (1940) and Matsudaira (1943), on the morphological changes during development and the breeding of living creatures; not one of these, however, gives a clear explanation of the factors influencing the start of development in winter eggs. The writers attempted to investigate these factors, by giving the eggs various artificial stimuli, through the spring and autumn season of 1950. The methods used for the experiments varied from centrifugal force, immersion in water, needle prick stimulation, drying process and sunlight radiation to chemical stimulation with a variety of substances. These experiments, though numerous, have not yet produced conclusive results. In the case of the temperature treatment, however, a number of interesting results have been obtained which are reported below.

II. Material and Methods.

Daphnia pulex winter eggs collected from pools in the Institute's compound, were used as material. The hard protective shells of the eggs were first removed with a needle and then the eggs placed in a glass container for the experiments. The eggs were subjected to

(1) sudden changes of temperatures ($2^\circ - 60^\circ$ C);

(2) cold conditions by putting them in the refrigerator ($2^\circ - 5^\circ$ C);

(3) maintenance at different temperatures ($9^\circ - 35^\circ$ C) within the scale suitable for living creatures, and

(4) treatment with alternating temperatures.

The apparatus used for the experiment consisted of a thermostatically controlled water tank and a refrigerator. All the eggs that began to develop were identified within 2 or 3 days after treatment. The water temperature at the pools from which the eggs had been collected was constantly taken during the experiments for comparative reference in assessing the results of the experiments.

III. Results of the experiments.

A) Sudden changes of temperature: the eggs collected from the pool (water temperature about 25°C) were put in the water in the glass container, in groups of 40 and were immersed in water at 40°C., 50°C., and 60°C., for 1, 2, 3, 4 and 5 seconds respectively and then released into an indoor water tank (at approx. 25°C) and examined. Although the experiments were repeated several times, the results were almost negative. Only a few among those treated for 2 or 3 seconds at 50° C showed any signs of development and no significant differences were recognised compared with the control group (untreated eggs). Experiments were made with groups of 40-120 eggs, the treatment being of the same type as in the previous case, with the sole difference of immersion in 5 stages at 11.6°C ± 1.5°C, 21° C ± 0.8°C, 25°C ± 0.2° C, 28°C ± 0.2° C, 35°C ± 0.2° C for 3 to 4 days; the results showed no development in any of the contrasting groups. The latter experiment was carried out in the beginning of May (water temperature about 17°C) and the eggs especially selected for this experiment were restricted entirely to those produced in the spring of the year.

B) Low temperature treatment: eggs collected in the middle of June (water temperature about 19°C) were placed in the regrigerator at 2°C-5°C for 5 - 15 days and subsequently in the thermostatic water tank at 23°C. This experiment was repeated 5 times, but the result only showed a few cases of initial development and no significant difference was observed on comparison with untreated eggs. No greater success was achieved with the treatments given in reverse order, i.e. 20°- 25°C followed by cold.

C) Alternate low and high temperature treatment: the eggs were treated alternately at low ($2^{\circ}C - 5^{\circ}C$) and high temperatures ($23^{\circ}C$) respectively for 1-15 days. For comparative purposes others were kept at $2^{\circ}C - 5^{\circ}C$ and 23° C respectively throughout the whole period of the experiments. These experiments were repeated several times. As a result although the eggs remained undeveloped at both the low and high temperatures, some degree of development was observed in the case of alternate treatment. Table I shows the best results. In this Table, the number of developed eggs, indicates those identified after the second alternate low and high temperature treatment. No change was noticed, however, in those returned, after treatment, to the outside pools.

Table 1. Experiment with low and high temperatures repeated every 48 hours.

No. of experiments	No. of eggs used.	Undeveloped.	No. developed.	Development ratio %
I	36	34	2	5.6
II	41	33	8	19.5
III	40	40	0	0

D) Experiment at optimum temperature for development after Low temperature treatment:

As approximately 10% development was observed in the previous experiments, especially among eggs that received treatment at various temperatures after low temperature treatment and including those accidentally developed through some defects in the arrangement during the low temperature treatment of B, it appears possible that temperature has some effect upon the development of the eggs. It is furthermore now known from the results that there is an optimum temperature for this development and, that this optimum temperature is in the region of 12° C. Accordingly experiments were carried out on the basis of this hypothesis; i.e., the eggs treated at low temperatures (2°-5°C) for 5-7 days were kept at 9° - 14° C for 1 - 4 days. The results were remarkably good. Table 2 shows the result of the experiment when the eggs were placed at the optimum temperature (9° - 14° C) after 5 days of low temperature treatment. In the case of those receiving the low temperature treatment for 6 - 7 days, the result proved to be almost the same as in Table 2. The number of developed eggs shown in the Table was based on observations up to the second day after treatment and no change was noticed for several days afterwards.

Table 2.

Days of low temp. treatment	Days of optimum temp. treatment	Number of eggs treated	Number of developed eggs	Development ratio (%)
5	1	17	14	82.4
5	2	19	19	100
5	3	19	19	100
5	4	20	20	100

Low temp. treatment only .... Development ratio 0%

High temp. treatment only (25° -27° C) ... Dev. ratio 0%

E) <u>Relative comparison of low optimum and high temperature treatments:</u>

As the eggs used in the previous experiments were almost all produced naturally in the pools and collected from them, it may be reasonably assumed that some might well have been produced this spring and had therefore, not passed a winter, or some might have been produced last year and have passed a winter but were unable to develop in the spring. Would it have been possible to separate these and, if so would it have made any difference to the results when they were treated at various temperatures? To settle such queries some experiments were made towards the middle and end of September with eggs placed at the optimum temperature after the low temperature treatment for 4 - 6 days and with others placed at the optimum, high and low temperatures only. The results are shown in Table 3. An experiment, apart from these, to apply the optimum temperature only was made at the end of July, when treatment for 1 - 4 days at 9° -14°C was tried. All produced similar good results.

TABLE 3.

Method of treatment	Number of eggs treated	Number of eggs develo--ped	Development ratio (%)
Low temp. 4 days optimum temp.	20	20	100
Low temp. 6 days optimum temp.	18	18	100
Optimum temp.	58	58	100
High temp. (25°-27°C)	20	0	0
Low temp.	18	0	0

Developed eggs observed on the 3rd day of the optimum temperature treatment.

No change in undeveloped eggs for several weeks afterwards.

IV. Observations.

For the artificial incubation of the winter eggs of Daphnia pulex, the drying process is reported by many experts as giving the best results. In the case of insect eggs, sunlight radiation is also said to promote development. There is furthermore a method based on the state of inertia; taking those and other artificial self-procreative processes applicable to many other forms of animal life into consideration, the writers attempted many and various mechanical, chemical and physical methods of stimulation, but all in vain as was stated before. It is nevertheless felt that there are many aspects which require further research. On the point of temperature, however, some conclusive results have been obtained, as reported previously. That an optimum temperature is necessary in order to stimulate development and that this optimum temperature is in the region of 12°C is in agreement with the natural development conditions of the winter eggs. In experiment A, the eggs were places at temperatures in the 11°-60°C range, but scarcely any showed signs of development. This shows that as a direct stimulus for the development of the eggs, a simple application of temperature variation has no effect on the eggs. It also supports the fact that, even if the eggs from water at 25° - 27°C in July, August and September are placed at a temperature as low as 2° - 5°C, this does not necessarily constitute a stimulus for their development. Again, it is scarcely probable that a mere process of refrigerating - wintering under natural conditions - would promote development or that direct transfer of wintered eggs into a high temperature such as the summer water temperatures of 23°-27°C would have such an effect. In considering the results of the alternative treatment of low and high temperatures in experiment C, although it is true that climatic conditions in the spring and autumn in the natural world change constantly and water temperatures go up and down in these seasons, such variations in temperature are not thought to be in the direct cause for the development of winter eggs.

On the other hand, the experimental results showed a small number of undeveloped eggs to have been stimulated by one cause or another during the experiments. Such development may have been stimulated by the optimum temperature conditions for development (stated below) which were by chance created during the alternate treatment. In the case of experiment D, the two stage treatment, first at low temperature and then at optimum temperature of about 12°C, is the direct and important factor for the development of winter eggs. Furthermore, in this experiment, when the eggs were treated at low temperature for 5 days and remained at the optimum temperature for over 2 days, the results showed a great increase in the number of developed eggs, almost 100%. Exactly the same tendency was found in the case of low temperature treatment for 6 or 7 days in the experiments conducted in similar manner, so that a little over one day may be considered as the shortest period for the duration of the optimum temperature treatment. Moreover, there was an instance in one experiment when only a small number of eggs developed when the period of the optimum temperature treatment was less than one day. For the duration of the optimum temperature treatment, therefore, over one day is considered to be necessary, but a minimum two day period is probably the best. On this question of the basic time limits for low temperature and optimum temperature treatment, further investigation requires to be made. In experiment E, no difference in development was observed between eggs that received the two stage treatment of low and optimum temperatures and those receiving the optimum temperature treatment only. In each case, the development was 100%. As similar experiments since July produced exactly the same results, the low temperature might be considered unnecessary. But it cannot be definitely discarded as unnecessary, in view of the case in experiment A (early May) in which eggs treated at 11.6° - 1.5° C temperatures did not develop at all. The difference between the two cases was probably influenced by the season of the experiments. That is to say, the A experiment was in early May and the eggs were all winter eggs, definitely produced that spring in the experimental room and consequently these winter eggs, immediately after production, may have required a period of dormancy. The existence of such a dormant period has often been discussed and the optimum temperature treatment after the dormant period may probably cause the initiation of development. If so, can the low temperature treatment be substituted for the dormant period? To settle these and other points, experiments are in progress and reports will be made at a later date.

V. Summary.

1) Naturally produced winter eggs of Daphnia pulex are placed in water in a glass container, after removal of the protective shells, and treated at varying temperatures. The following are the results obtained.

2) No development is observed in the winter eggs after temperature variation treatment only.

3) No development after low temperature (2° - 5° C) treatment only.

4) 100% development is obtained with eggs treated at optimum temperature (about 12° C) for more than two days after low temperature treatment.

5) Eggs develop with optimum temperature treatment only after the lapse of a definite period of time.

6) Whether the low temperature treatment can be substituted for the lapse of a definite period) remains to be settled by further research.

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Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.