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ZOOLOGY

Apparent Effects of Refrigeration on the Rate of Embryonic Development of Mayfly (Hexagenia limbata) Eggs

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Introduction: Hunt (1953) states that temperature is an important factor influencing the rate of embryonic development of *Hexagenia* (mayfly) eggs. Pasvogel (1962) has reported that 46 days is the time limit *Hexagenia* eggs can remain dormant at temperatures slightly above freezing. It was the object of this investigation to continue this line of research and to determine the effects of temperature on the embryonic development of *Hexagenia limbata* eggs.

Methods: All mayflies collected and used for this laboratory experiment were collected on August 7, 1962 at Winona, Minnesota, near the Mississippi River, from beneath a light. The imagoes were transported back to the laboratory, without separating the males from the females. Thirty to forty imagoes were placed in a liter beaker of water. This stimulated the females to release their eggs, which drifted to the bottom of the beaker. The eggs were pipetted out of the beaker in 0.5 cc. portions, and put in polyethylene bags (10.1 x 22.9 cm.) filled with 250 cc. of distilled water. Numbered cards were stapled to the tops of the bags for taking notes concerning each individual bag. Twenty of the filled bags were refrigerated at 12°C and another twenty bags were refrigerated at 7°C. Periodically (about every three days) one bag of eggs was removed from each refrigerator and allowed to incubate at room temperature (approximately 22°C.). The time it took the eggs to begin hatching was recorded and correlated to the time of refrigeration.

Results: The eggs refrigerated at 12°C. responded to the cooler temperature in an expected manner. That is, the longer the period of refrigeration, the shorter was the period of incubation when exposed to room temperature (Fig. 1). This group of eggs hatched in the refrigerator after 127 days. One could say, therefore, that the incubation period of *H. limbata* eggs at 12°C is 127 days, compared to 12 days at 22°C (Fig. 1).

Eggs refrigerated at 7°C. responded to the cooler temperature in an unsuspected, disorganized manner. These eggs never took less than the normal 12 days of incubation to hatch. Inspection of data (Fig. 1) shows that the period of incubation (after refrigeration at 7°C.)

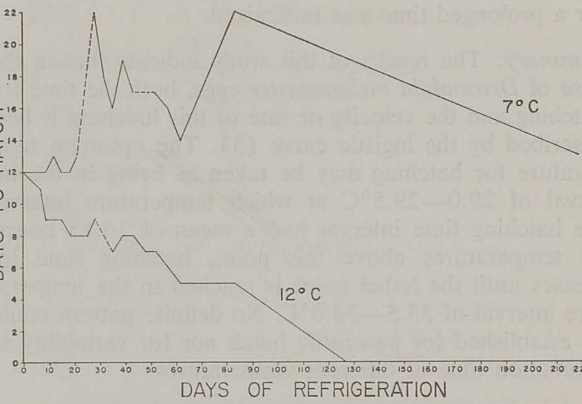

FIGURE 1. Graph, observed days to hatch vs. number of days of refrigeration at 12°C. and 7°C.

was usually much longer than normal and that there was no predictable trend established as to the period of incubation. These eggs failed to hatch in the refrigerator even though they were kept there for over 200 days.

Eggs were also exposed to temperatures above 22°C. (normal) to see if this might not shorten the incubation period. The results of this test were affirmative. Eggs kept at 32°C. took only 10 days of incubation to hatch. This also established that there is an inverse relationship between temperature and time of incubation.

Discussion: The summation of the heat energy required to complete a given stage in the life history of an animal is often expressed in degree days (Allee et-al, 1955). This quantity of heat is the same whether it takes place in one or several steps. A degree day represents one degree of mean temperature above the threshold temperature lasting for one day. Before degree days can be used as a measure, however, the threshold temperature must be established. The threshold temperature of an organism is that temperature below which the process being observed does not occur (Richards, 1959). The threshold temperature for hatching of *H. limbata* eggs was calculated to be 11.3 ± 0.7°C. The following equation was used to determine the hatching threshold:

Degrees above threshold x number of days = number of degree days

if; A = days of refrigeration
T = threshold temperature
t_1 = refrigeration temperature
B = days to hatch
t_2 = room temperature

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D = degree days

$(\,'\,)$ = second case

$$\text{then; } (A(t_1\text{-}T)) + (B(t_2\text{-}T)) = D$$

$$\text{and; } (A'(t'_1\text{-}T)) + (B(t'_2\text{-}T)) = D$$

$$\text{therefore; } (A(t_1\text{-}T)) + (B(t_2\text{-}T)) = (A'(t'_1\text{-}T)) + (B'(t'_2\text{-}T))$$

$$\text{or: } T = \frac{A't'_1 + B't'_2 - At_1 - Bt_2}{A' + B' - A - B}$$

The degree days required in one observation is equal to that required in another, because of this there are many different combinations of two that can be used in the derived algebraic equation for determining threshold temperature. Ten of the possible combinations were selected randomly and the threshold temperature for each determined. The mean was then calculated and used as the threshold temperature.

Once the threshold temperature was determined, the number of degree days required for hatching of *H. limbata* eggs was calculated to be 121 ± 8.6.

Inspection of Figure 1 shows that the eggs refrigerated at 12°C. responded to the cooler than normal incubation temperature in a manner which closely approximates that calculated in Figure 2. It seems evident embryonic development was taking place at 12°C. but at a much retarded rate.

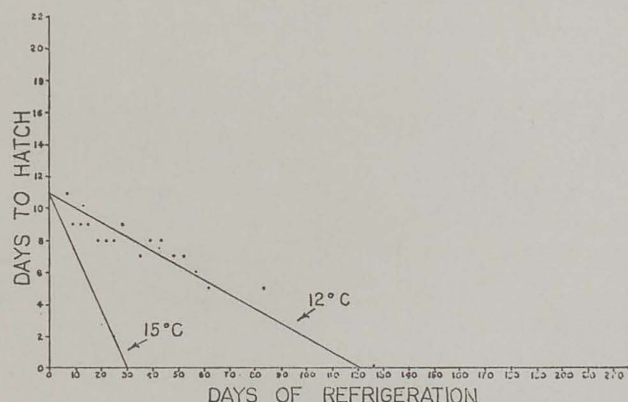

FIGURE 2. Graph, Calculated days to hatch vs. number of days of refrigeration at 12°C. and 15°C. (points represent observed).

The effects of exposing organisms to temperatures below the threshold and then returning them to higher temperatures are not uniform (Allee *Et-Al*, 1955). This can be observed by following the erratic rate of development of those eggs refrigerated at 7°C. (Fig. 1). The discrepancies between the observed rate of development and the predicted rate may be interpreted as follows: Some processes slow down more than others causing disorganization. Disorganization increases with time and if the disorganization has gone too far, the process cannot be completed or completion will follow only at a retarded rate. The fact that it always took as long or longer than 12 days of incubation to hatch at room temperature, shows that no embryonic development was taking place at 7°C. and that the eggs were below the threshold temperature for hatching (ie. they were not accomplishing the total number of degree days needed for hatching).

It was noted throughout this investigation that the percentage of hatch and the vitality of the nymphs after hatching was progressively less as refrigeration time increased. A suggested reason for this is that more and more energy is required to complete development the longer an organism is exposed to near threshold temperatures (Richards, 1959). Thus those eggs refrigerated for longer periods would be required to use more energy than those not refrigerated as long—resulting in a loss of nymphal vitality and a higher frequency of death. The eggs refrigerated at 7°C. always had a lower percentage of hatch and nymphal vitality than the eggs refrigerated at 12°C.

Conclusion: A definite relationship exists between the rate of embryonic development of *H. limbata* eggs and temperature. The cooler the temperature (to a critical point) the slower the rate of embryonic development and vice-versa. The lowest temperature at which these eggs can be incubated successfully is 11.3 ± 0.7°C.–the effective threshold temperature. Below this temperature embryonic development does not occur, disorganization takes place, and the percentage of successful hatches decreases.

The summation of energy (degree days) required for successful incubation of *H. limbata* eggs is 121 ± 8.6 degree days.

In knowing the threshold temperature and the required degree days for incubation for *H. limbata* eggs, it is possible to predict in advance the incubation periods at different temperatures (Fig. 2).

LITERATURE CITED

ALLEE, W. W., A. E. EMERSON, O. PARK, T. PARK, AND K. P. SCHMIDT. 1955. *Principles of Animal Ecology*. Philadelphia, Saunders.

RICHARDS, V. A. G. 1959. Studies on Temperature Thresholds in Insect Development. *Biol. Zbl.* 78:308-314.

PASVOGEL, C. H. 1962. Factors Affecting the Viability of *Hexagenia bilineata* (Say) and *Hexagenia limbata* (Serville) Mayfly Eggs. Graduate Research Paper. Winona State College Library. Winona, Minnesota.

HUNT, P. H. 1953. *The life History and Economic Importance of a Burrowing Mayfly,* Hexagenia limbata *in Southern Michigan Lakes*. Michigan Conservation Department. Bul. Inst. Fish. Res. 4.