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## MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

No. 90

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Atlantic Biological Station

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# Some Observations on the Effect of Changes of Temperature on Respiratory Rhythm in Flounders.

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## 1. Introduction.

Crozier has pointed out that the method of analysing the effect of temperature on the velocity of a reaction by calculating the temperature coefficient, $Q_{10}$, which is the ratio of velocities for intervals of ten degrees, leads to an erroneous result, as this quantity is not constant, but varies for different intervals, decreasing as the temperature becomes higher. In addition to this, it does not adequately indicate the abrupt changes which occur in many biological temperature relations at certain critical temperatures.

Arrhenius (1889, 1915) first introduced the principle of calculating the effect of temperature on physiological processes by the empirical formula for "irreversible" chemical reactions

$$K_1 = K_0 e^{\frac{\mu}{2}\left(\frac{T_1 - T_0}{T_0 T_1}\right)}$$

where $T_0$ and $T_1$ are two temperatures in degrees absolute, and $K_1$ and

$K_0$ the velocities of the reaction at temperatures $T_1$ and $T_0$ respectively, and $\mu$ a constant, denoting the energy of activation. The more rapidly the velocity increases with increase of temperature, the larger $\mu$ will be. If $T_1-T_0$ represents a small interval of temperature, $T_0T_1$ will not change very much in the interval. The equation can then be written

$$K_1 = K_0 e^{\frac{\mu}{2}(T_1 - T_0)}$$

or $$\log K_1 - \log K_0 = \frac{\mu}{2}(T_1 - T_0)$$

log. K is thus approximately a linear function of the temperature, and $\mu$ can be calculated graphically. Following Crozier, $\mu$ will be designated in this communication as the <u>temperature characteristic</u> in distinction to $Q_{10}$, the temperature coefficient.

The value $\mu$ for any given process is presumably dependent on $\mu$ of the slowest reaction of the complex of chemical reactions involved in any vital activity (Blackman and Putter). Thus the slowest reaction is the controlling reaction. Crozier hopes, through an analysis of the values of $\mu$ for various vital activities, to identify processes governed by "the same species of active molecule". Where a change in the value of $\mu$ occurs as the temperature changes, it is interpreted as a shift at that temperature from one controlling reaction to another. These changes occur normally in many reactions, and Crozier and Stier have induced them in the respiratory rhythm of the abdomen of grasshoppers by experimental mutilation.

It is not unreasonable to postulate the thesis that the same physiological action in members of the same class of organisms is governed by the same series of reactions, and hence by the same controlling reaction. If Crozier's method is of value in the identification of definite types of reactions, $\mu$ for the respiratory rhythm of the flounder might be expected to correspond to that for the goldfish (Crozier, 1925). Experiments carried out on two species of flounders at the Biological Station at St. Andrews, New Brunswick, have so far failed to show this correspondence, the thermal increment varying not only with different individuals subjected to the same experimental conditions, but with the same individual subjected to the same experimental temperatures in the same way on different days. The respiratory rhythm also showed variation in the value of $\mu$ when the experimental sequence of temperature was altered.

## 2. Methods.

The flounders were kept in a tank of running sea water for a week before the beginning of the experiments. No food was supplied other than the microscopic fauna accidentally introduced through the supply tap. After a day there was a deposit of fine organic detritus on the floor of the aquarium, on which the fish appeared to feed.

Individuals required for observations were isolated in a deep petri dish and kept in running water for twenty minutes to eliminate disturbances inevitable in transferring the fish from the large tank. Muscular movements, even when confined to the fins, produce irregularities while in progress and for a short time after their cessation. All counts of the rate of beating of the operculum, which was used as the indicator of the respiratory rhythm, were made while the flounders were motionless.

The vessel containing the fish was put into a larger glass vessel of water, and this again into a still larger granite pan of water. To the last the heating or cooling was directly applied. The temperature was raised by applying a flame to the granite pan, and lowered by using ice or freezing mixtures in this same pan. The method, though crude, changed the temperature of the vessel containing the experimental animals gradually and evenly, and the largest variation during any count was 0.5° C., the usual fluctuation being within 0.25°C.

The rate of change of temperature was 1° in 5 minutes. The animals were kept at the experimental temperature five minutes before making the counts, to ensure equilibrium of the body temperature of the fish with the surrounding water. The individuals varied from 6.5 to 8.5 cms. in length, with a maximum thickness of 0.75 cm., the flattened shape of the flounder giving an advantageous relation of the area which was exposed to the medium, with the volume of body which required to be brought into temperature equilibrium with the water surrounding it. An additional five minutes was required for making the counts of the frequency (number of beats per second) of the opercular movements. The figures used in calculation are the average time for four counts of 100 beats each. This made a total of twenty minutes between each observation at intervals of two degrees, twenty-five for intervals of three degrees, etc.

No oxygen other than that dissolved in the water was supplied to the Pseudopleuronectes used in the preliminary experiments. In the later experiments on Liopsetta air was bubbled through the water in the petri dish to keep it saturated with oxygen at all temperatures.

3. Experiments on Pseudopleuronectes americanus.

The respiratory rate of flounders living at a temperature of 13° in the tank was observed at three degree intervals between limiting temperatures of 6° and 20°, with a count of the frequency of the opercular movement at the normal temperature of 13° between each reading at an abnormal temperature. The same animals were subjected to the same experimental procedure on several successive days, but gave in no case a constant thermal characteristic. A typical experiment was:

| Day. | $\mu$ |
|---|---|
| 1st. | 12,500 |
| 2nd. | no increment |
| 3rd. | 9,200 |
| 4th. | 11,500. |

In two experiments, one tabulated above, the temperature characteristic in 1 day's experiment was nil. This was a temporary condition in the respiratory rhythm of the fish concerned, as recovery took place on succeeding days. Omitting these two abnormalities, there was still a wide variation in the value of $\mu$, the extreme values obtained being 9,000 and 12,700, and the average of all the values 11,600.

The values at 13° were not constant in any experiment, probably because of insufficient time for recovery from the abnormal temperature preceding. Gray (1923) found that the effect of temperature on the amplitude of the beat of the gill cilia of *Mytilus* was instantly reversible up to a certain point, but after that temperature varying lengths of time were required for the beat to regain its normal state.

A second series of experiments, carried out over a wider range and without intervening temperatures of 13° between each alternate reading, showed a phenomenon common to all the series. Crozier has noted the abrupt change which occurs in some reactions at about 16°, in the value of $\mu$. In this series, readings were taken at 3° intervals on a descending temperature gradient from 13.5° to 1°, and continuously on the same animals up the scale again from 1° to 20°; the temperatures were thus normal to low to normal to high. A change in the value of $\mu$ at from 13.5°-16° was very definite in all experiments, the animals showing no increase in frequency of respiratory movements at temperatures higher than this (Fig.2). In addition to this change occurred another which has repeated itself in all the experiments: <u>$\mu$ for the descending scale of temperature was less than for the ascending scale for the same animals</u>. This same phenomenon will be noted in experiments carried out in a different way, on the flounder *Liopsetta putnami*.

A typical experiment of the above type (<u>Pseudopleuronectes</u>) is figured below: figure 1 shows descending temperatures, $\mu$ = 11,000, and figure 2 ascending temperatures for the same fish in the same continuous experiment, $\mu$ = 15,000 below 13.5°, and nil above that temperature.

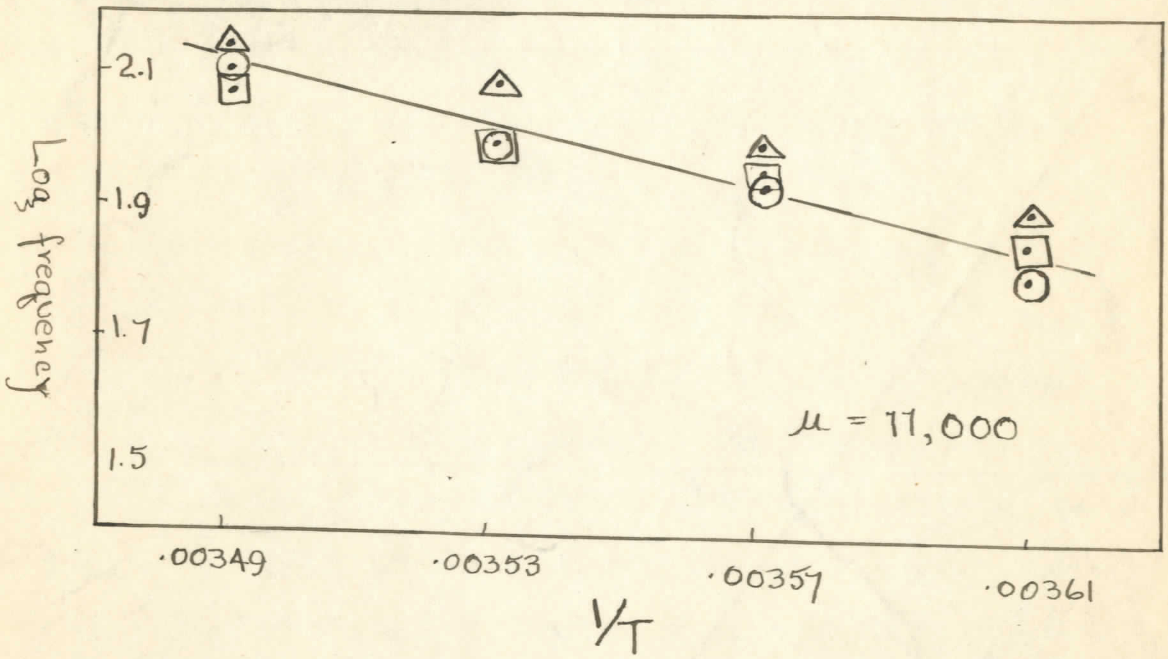


FIG. I. Pseudopleuronectes americanus. μ for the respiratory rhythm plotted between 13.5 and 4°, descending temperatures.

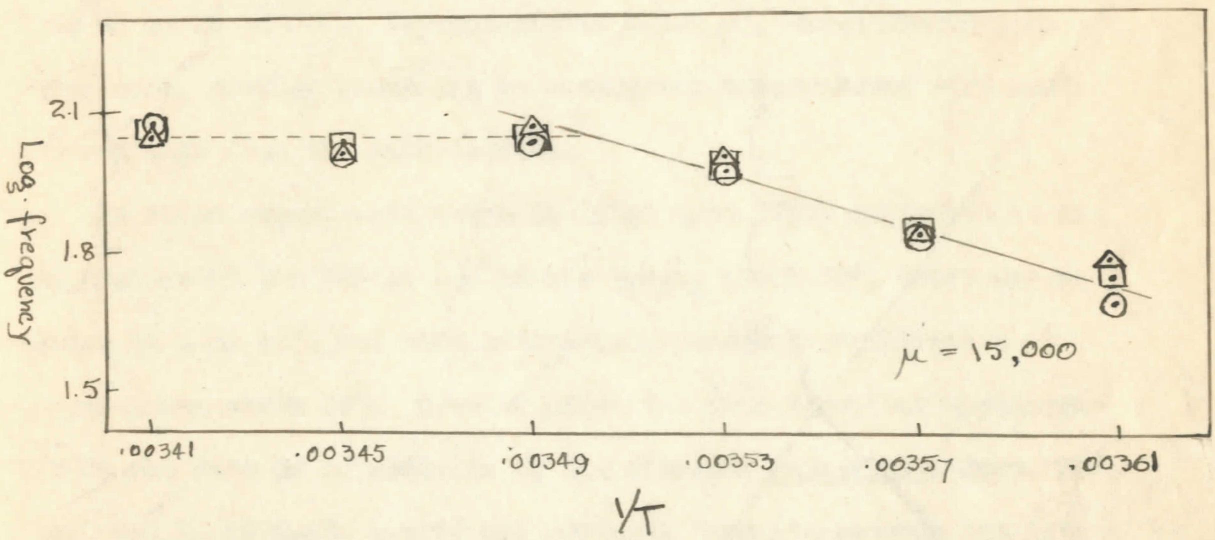


FIG. 2. Pseudopleuronectes americanus. Graph of the same individuals as in FIG. 1, plotted in ascending temperatures from 4 to 20°, showing the change in the value of μ at 13.5°.

### 4. Experiments on Liopsetta putnami.

The procedure in this series was to put the experimental animal directly from its normal living temperature of 13° into one or the other temperature at the extreme of the range, and then raise or lower the temperature as described till the other extreme was reached. Readings were taken at 2° intervals. The range of temperature was from 0° to 20 or 22°. Irregularities below 4°, which occurred in every case, whether ascending or descending temperatures were used, are excluded from the calculations.

In those experiments where the fish were first subjected to the temperature at the hotter end of the range, about 20°, there was no change in $\mu$ at 16°, but some individuals showed irregularities at temperatures above 19°. Crozier noted the same behaviour when working on the rate of progression of the diplopod Parajulus, (Jour. Gen. Phys. vol.7, 1924-25, p.129) and excluded from his records the individuals which failed to show increase in rate of progression after 15°. He does not state the proportion of these aberrants present. In my experiments, fully a third of the fish failed to exhibit frequencies greater than those obtained at 19°; the frequencies of these are shown on the graph with those of the more regularly increasing individuals (Fig. 3, ○, △, □)

The values of $\mu$ obtained for various experimental animals varied from 7,400 to 8,000, which indicates a fairly constant response of the respiratory rhythm to temperature changes.

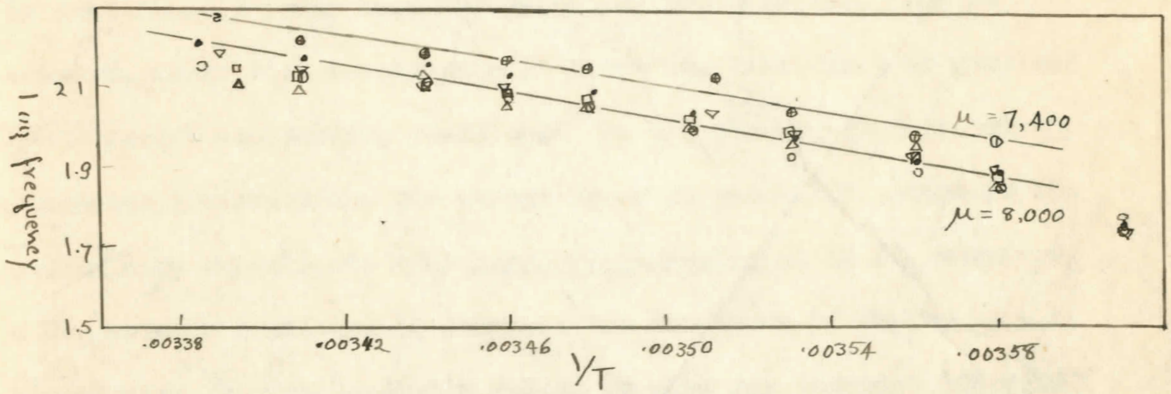


FIG. 3. Liopsetta putnami. Six individuals in descending temperatures from 22° to 6°.

Individuals started at the cold end of the experimental range did not give a constant value for $\mu$, and moreover did not yield any satisfactory degree of correspondence between the behaviour of individuals subjected to the same conditions of varying temperatures. $\mu$ varied from 9,000 to 15,500 (Figs. 4 and 5). This wide range of variation reaches from Crozier's critical $\mu$ of 11,500 to nearly the higher critical $\mu$ of 16,500, which repeat themselves in many biological activities, the latter being that of the respiratory rhythm of the goldfish. In no experiment carried out during the present investigation was a higher value than 15,500 obtained for $\mu$ which is considerably lower than the value for the goldfish. Yet it seems unlikely that respiration in these two fish would be governed by different controlling reactions. In all the experiments using ascending temperatures, the abrupt break at about 16°, noted in the preliminary experiments with Pseudopleuronectes at 13.5°, occurred. A few animals continued to increase the frequency of rhythm at a slower rate, but the majority failed to show any increase after 16 or 17°.

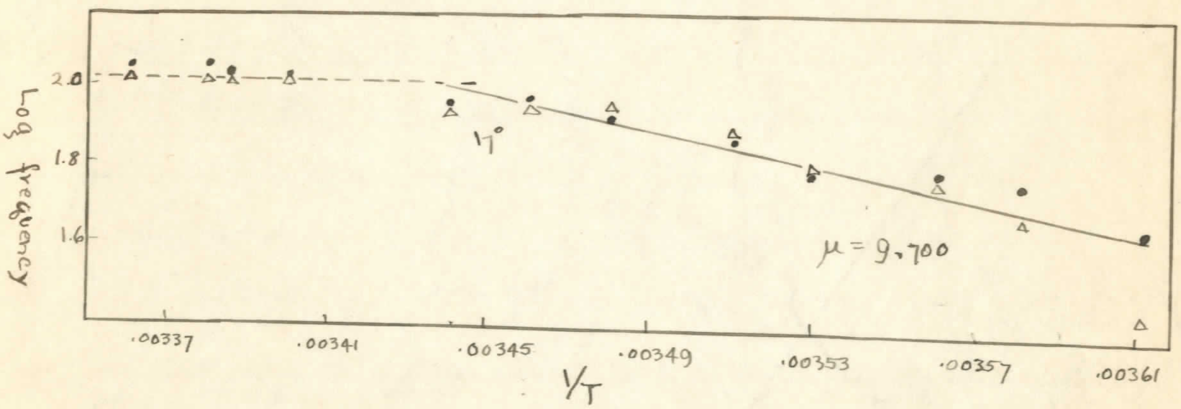


FIG. 4. Liopsetta putnami. Ascending temperatures from 4 to 25°, showing the change in $\mu$ at 17°.

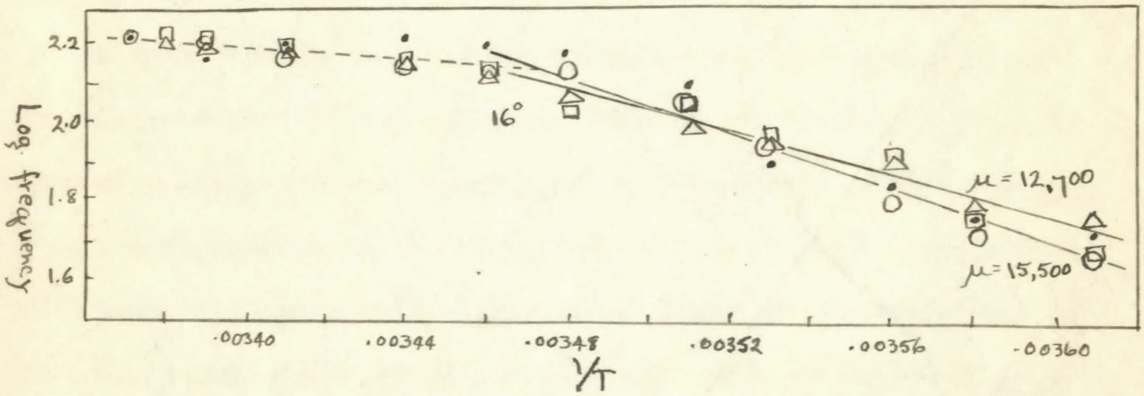


FIG. 5. Liopsetta putnami. Ascending temperatures from 5 to 23°, with a change in the value at 16°. The extreme values of μ are plotted.

## 5. Summary.

Experiments carried out on flounders with the object of determining $\mu$ for the respiratory rhythm did not give the expected value of 16,500, determined by Crozier for the same physiological action in the gold-fish.

Varying values were obtained when the same fish was subjected to the same experimental conditions on different days. There does not seem to be any adequate reason to suppose that a different set of reactions, with a different controlling reaction, is involved.

A difference is obtained in the value of $\mu$ for ascending and descending series of temperatures. This is true whether the same fish or different fish are used, and is independent of the previous experimental history of the fish. The values of $\mu$ for various fish of the same species show a satisfactory degree of correspondence for the experiments conducted on a descending scale of temperature, but not for those on an ascending scale, and the values for the ascending scale are always greater than for the descending scale.

| Species. | Descending. | Ascending. |
|---|---|---|
| Pseudopleuronectes | 11,000 | 15,000. |
| Liopsetta | 7,400-8,000 | 9,700 - 15,500. |

In the ascending scale of temperature, there is always an abrupt change in the value of $\mu$ at from 13.5°-17°, some individuals failing to increase the frequency of the respiratory rhythm after that tem-

perature. This change does not occur in the descending scale, but some individuals show irregularity above 19°.

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