THE EFFECT OF TEMPERATURE ON THE FINAL INSTAR NYMPHS OF THREE SPECIES OF AUSTRALIAN EPHEMEROPTERA.

By JANET E. HARKER.

(*New England University College, Armidale, N.S.W., Australia.*)

FROM work done on incipient lethal temperatures of fish (Richet, 1885; Plateau, 1872; Mayer, 1914; Huntsman and Sparks, 1923) there appears to be a relationship between the incipient lethal temperature of a species and the type of habitat in which it occurs. The range of temperatures which the animal can tolerate may also appear to be larger in those which come from environments with a very variable temperature range.

There is, however, a marked degree of ambiguity in the precise meaning which previous writers have implied by the phrases " Incipient Lethal Temperature " and " Thermal Resistance," so that confusion has arisen by indiscriminate use of these two terms. To avoid further confusion in this paper these two terms are intended to describe the following ideas. The incipient lethal temperature is the temperature above which an organism can no longer live for an indefinite period of time; the thermal resistance is the measure of the ability of an organism to withstand a temperature above the incipient lethal temperature. It will therefore be seen that Whitney's (1939) use of the term " temperature resistance " corresponds with my use of incipient lethal temperature.

Further work done on acclimatization (Loeb and Wasteneys, 1912; Jacobs, 1919; Hathaway, 1927; Fry Hart and Walker, 1946) substantiates this thesis, so that it would be expected that animals which have been living in high-temperature environments would have a higher incipient lethal temperature than those living in relatively low temperature environments.

It has been shown by Whitney (1939) that six species of mayfly nymphs from slow or still water in Great Britain have a higher incipient lethal temperature than those from swift waters. These results suggest at least two questions: (1) Are nymphs from different habitats irreversibly acclimatized? In other words, if mayfly nymphs are taken from habitats with different mean temperatures and acclimatized to a uniform temperature, will the incipient lethal temperature of the nymphs from each habitat show a relationship to the temperatures of the original habitat? (2) Are the incipient lethal temperatures of the penultimate instar nymphs correlated with the ability to emerge as a subimago at various temperatures?

In an attempt to elucidate this problem the following experiments were performed.

METHOD.

Three sets of experiments were performed using two species of mayfly from different habitats, and a third which was found in both these habitats. The species used were *Atalophlebia albiterminata* Tillyard from Dumaresque Creek, Armidale, N.S.W., in a region of rapid flow, *Caenis scotti* Tillyard taken from a small silted pond in the grounds of New England University College, Armidale (about a quarter of a mile distant from the stream), and *Leptophlebia crassa* Harker which was taken from both habitats. The temperature of the stream varied during the period of collection from 20–24° C., and that of the pond from 25°–32° C. Prior to each experiment each sample was kept at 18 ± 2° C. for a week.

A sample of twenty-five nymphs in the penultimate instar (as far as could be judged from an examination of the wing buds) was chosen for each experiment. The sample was placed in a constant temperature water bath (± 1° C.) heated by means of a luminous heating element, which was partially shaded by a tin cylinder. A control experiment using a similar sample of nymphs was placed beside the water bath in approximately the same light intensity, but at a temperature of 18 ± 2° C. This control served as a check both on the possible effect of the light, and on the changes in the rate of emergence with the passage of time. The animals were placed in a beaker containing water which was aerated by having air bubbled through it at intervals. This was partially immersed in the water bath at an initial temperature of 18° C., which was then gradually altered to that required for the experiments. Each experiment was run for a period of seven days. All dead nymphs or emerged subimagines were removed daily.

The difficulty of keeping a constant temperature below 18° C. made it, at the time, impossible to study the effect of low temperatures, but it was found that nymphs kept at 3° C. all died within a few hours. It should be noted that this only occurs in the later stages of the nymphs; earlier instars can tolerate low temperatures.

Results.

From Table I it can be concluded that the percentage of deaths of the nymphs from the higher temperature habitat (the pond) was lower than those from the low temperature habitat (the stream). That is the ratio of percentage of deaths of *Atalophlebia albiterminata* and *Caenis scotti* was 29 : 11 at 30° C. and 40° C., and in the case of *Leptophlebia crassa* at these temperatures the ratio of stream nymph deaths to pond nymph deaths was 13 : 6.

Table III contains a list of the mean temperatures of emergence of the subimagines, and it will be observed from this table that the values for the nymphs from the low temperature habitats are lower than those from the high temperature habitats. *Leptophlebia crassa* has a mean temperature of emergence in the ratio of stream to pond forms 23° C. : 26° C., *A. albiterminata* (stream) to *C. scotti* (pond) 24° C. : 27° C.

Table I.—*The Percentage Number of Dead Nymphs and Emerged Subimagines at* 18°, 25°, 30°, 40° C.

Species	Stream. *Atalophlebia albiterminata.*				Pond. *Caenis scotti.*				Stream. *Leptophlebia crassa.*				Pond. *Leptophlebia crassa.*			
	° C. 18.	° C. 25.	° C. 30.	° C. 40.	° C. 18.	° C. 25.	° C. 30.	° C. 40.	° C. 18.	° C. 25.	° C. 30.	° C. 40.	° C. 18.	° C. 25.	° C. 30.	° C. 40.
Percentage of dead nymphs after seven days	7	7	21	95	7	7	10	36	2	1	30	100	3	2	15	45
Percentage of subimagines which emerged in seven days	8	82	0	0	10	63	80	0	50	72	22	0	61	60	95	20

TABLE II.—*The Percentage Number of Dead Nymphs and Emerged Subimagines During Same Period in Control Experiment at* 18° C.

Species	Stream. *Atalophlebia albiterminata.*				Pond. *Caenis scotti.*				Stream. *Leptophlebia crassa.*				Pond. *Leptophlebia crassa.*			
Percentage of dead nymphs after seven days	7	6	7	7	7	7	5	6	1	3	1	2	1	2	2	1
Percentage of subimagines which emerged after seven days	15	12	22	10	20	25	27	26	50	65	58	63	65	54	60	61

TABLE III.—*The Mean Death and Emergence Temperatures of Pond- and Stream-dwelling Nymphs.*

Species.	Habitat.	Range of temperature of habitat.	Mean death temperature.	Mean emergence temperature.
Atalophlebia albiterminata Tillyard	Stream	20–24° C.	36° C.	24° C.
Caenis scotti Tillyard	Pond	25–32° C.	28° C +	27° C.
Leptophlebia crassa Harker	Stream	20–24° C.	33° C.	23° C.
,, ,, ,,	Pond	25–32° C.	36° C. +	26° C.

The maximum emergence of subimagines occurs in all the species tested at temperatures close to those of the habitat from which they came, stream insects at 25° C. and pond insects at 30° C.

DISCUSSION.

From these results it appears that the mayfly nymphs used in these experiments from the stream have a lower incipient lethal temperature than those from the pond. The nymphs from the pond also had a lower mean temperature of emergence.

The application of these results to conditions in the field has been mentioned for the breeding aspect, but the death point temperatures are not directly applicable to field conditions because any thermal deaths in nature are more likely to take place under changing temperature conditions.

The nymphs of these two populations of *Leptophlebia crassa* have supposedly both come from the same original population, as the pond and the stream are the only two places in which these nymphs are found in an area of ten square miles. If this is so, then there appears to have been a thermal acclimatization of the two populations to their environments resulting in a difference in thermal tolerance and favourable breeding temperatures. Imagines have been observed flying at distances from both the pond and the stream equivalent to the distance between these two areas, so that there is no complete geographical barrier to interbreeding. From the result obtained, however, it is suggested that the dates of emergence would differ for the two populations, which would cause an effective barrier to interbreeding.

Whether the period spent at 18° C. before the experiments were begun has altered the temperature tolerance range of the two populations of *Leptophlebia crassa* cannot be determined as no experiments were conducted omitting the initial period of acclimatization at 18° C. But it is significant that this acclimatization period has not brought the two populations back to a similar thermal tolerance range. Previous workers have recorded results of acclimatization when the animals concerned have been kept at the acclimatization temperatures for very short periods of time. The results of these experiments give a marked indication that the time factor is of extreme importance in acclimatization.

Summary.

1. The incipient lethal temperature has been determined for the penultimate instar nymphs of *Atalophlebia albiterminata* Tillyard from a stream, *Caenis scotti* Tillyard from a pond, and *Leptophlebia crassa* Harker from the pond and stream.

2. The final instar nymphs from still water have a higher incipient lethal temperature than those from swift water. The subimagines from still water emerged at a higher temperature than nymphs from running water.

3. The maximum emergence of the subimagines occurs at a temperature close to the mean temperature of the habitat.

4. A barrier to interbreeding is associated with the different emergence temperatures of the two populations of *Leptophlebia crassa*.

References.

Duodoroff, P., 1938, Reactions of marine fishes to temperature gradients. *Biol. Bull. Wood's Hole* **75** : 494–509.

Fry, F. E. J., Hart, J. S., and Walker, K. F., 1946, Lethal temperature relations for a sample of young speckled trout (*Salvelinus fontinalis*). *Univ. Toronto Stud.*, *Biol. Series* 54, *Publ. Ont. Fish. Res. Lab.* **66** : 9–35.

Harker, J. E., 1950, Australian Ephemeroptera. Part I. Taxonomy of New South Wales species, and evaluation of taxonomic characters. *Proc. Linn. Soc. N.S.W.* **75** : 1–34.

Hathaway, E. S., 1927 (= 1929), Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. *Bull. U.S. Bur. Fish.* **43** : 169–192.

Huntsman, A. G., and Sparks, M. I., 1924, Limiting factors for marine animals. 3. Relative resistance to high temperature. *Contr. Canad. Biol.* (n.s.) **2** : 102–113.

Jacobs, M. H., 1919, Acclimatization as a factor affecting the upper thermal death points of organisms. *J. exp. Zool.* **27** : 427–442.

Loeb, J., and Wasteneys, H., 1912, On the adaptation of fish (*Fundulus*) to higher temperatures. *Ibid.* **12** : 543–557.

Mayer, A. G., 1914, The effects of temperature upon tropical marine animals. *Pap. Tortugas Lab.* **6** : 1–24.

Plateau, F., 1872, Recherches physico-chimiques sur les articules aquatiques. 2me partie. Résistance à l'asphyxie par submersion, action du froid, action de la chaleur, température maximum. *Bull. Acad. Belg. Cl. Sci.* (2) **34** : 274–321.

Richet, C., 1885, De quelques températures élevées auxquelles peuvent vivre des animaux marins. *Arch. Zool. exp. gén.* **3** : vi–viii.

Whitney, R. J., 1939, The thermal resistance of mayfly nymphs from ponds and streams. *J. exp. Biol.* **16** : 374–385.