UPPER LETHAL TEMPERATURE RELATIONS OF PARAGNETINA MEDIA WALKER (PLECOPTERA: PERLIDAE)

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ABSTRACT

UPPER LETHAL TEMPERATURE RELATIONS OF
PARAGNETINA MEDIA WALKER
(PLECOPTERA: PERLIDAE)

By

Dennis R. Heiman

Continuous water flow and static test apparatus were employed to evaluate the influence of acclimation temperature, size and sex, season, life stage, current velocity, and testing method upon the upper-lethal-temperature of the nymphal stage of the stonefly, *Paragnetina media* Walker.

Lethal temperature was found to be raised by acclimation to higher temperatures throughout the thermal range examined. The rate of gain of heat resistance was approximately 5 C per day. Downard acclimation to colder temperatures was found to be a much slower process.

Body size had no significant effect on upper-lethal-temperature during the summer and autumn test periods. Larger nymphs were significantly less tolerant than smaller nymphs during winter and spring. This heterogeneous response to high temperature was attributed to differences in male and female nymphs and not to the effects of body size.

Nymphs tested in static waters were significantly less tolerant than in comparable tests under flowing water conditions. An increase in current velocity significantly increased heat resistance at all lethal temperatures examined.

Upper lethal temperatures showed seasonal differences, independent of acclimation. Stonefly nymphs were least tolerant during the spring, when temperature resistance was influenced by sex, molting condition, and proximity to emergence.

Experiments with early instar nymphs indicate that the period immediately following egg hatching is the most temperature sensitive life history stage.

The distribution of head capsule measurements indicates P. media requires two years to complete its nymphal growth. Maximum growth period is June to September.

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Dennis R. Heiman

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

INTRODUCTION

Temperature is an intrinsic ecological factor determining to a great extent, the abundance, the duration of the life cycle and distribution of many aquatic organisms.

Few cases have been recorded of heat death in fishes or macroinvertebrates in the natural environment. Nevertheless, information on their upper thermal tolerance becomes increasingly important with the advent of more and larger electric-power-generating plants, particularly those with atomic reactors. Present estimates indicate that about one-sixth of all the water in the nation wide annual runoff, some 1,200 billion gallons per day, will be needed by 1980 to cool the country's steam electric stations. Thus, some 200 billion gallons of cooling water will be needed daily to meet projected needs (Sport Fishing Institute Bulletin, September, 1968).

The present study was conducted to learn more of the upper lethal temperature relations among aquatic insects. The stonefly, Paragnetina media Walker was selected as the test organism.

Hart (1947) divided the quantitative study of the lethal effect of temperature on organisms into three phases: (1) establishing in the laboratory a method of

assessment that permits prediction of lethal effects in the field; (2) application of this method to the description of the species; and (3) relating this laboratory description to the distribution and success of the species in nature.

The use of P. media in a study of thermal tolerance is justified in that the temperature of the streams and rivers in which it occurs is subject to modification by alterations in the surrounding land, such as tree removal or by the installation of power generating plants along its banks which discharge large amounts of heated effluent.

In a discussion of water temperature criteria to protect aquatic life, Mihursky and Kennedy (1967) stated:

> It is perhaps superfluous to indicate temperature research is necessary on many more species. To focus on this need is the fact that no single species has been subjected to multivariate studies for all life history stages. Also, of the almost 1,900 fish species listed in the American Fisheries Society's list of fishes from the United States and Canada (1960) less than 5% have been specifically examined for their response to temperature (even less for macroinvertebrates)--quite a small figure upon which to base water temperature requirements for the entire United States.

LIFE HISTORY AND ECOLOGY

Concurrent with conducting a laboratory evaluation of environmental parameters it is essential to conduct field studies on the general biology and life-history patterns of the selected test organism. Laboratory survival data can have ecological significance only by interpreting in light of information on environmental conditions.

There have been few detailed life-history studies of species of Plecoptera in North America. Notable exceptions include Holdsworth (1941), Smith (1913) and Wu (1923).

Distribution

Paragnetina media is common and widely distributed throughout the state of Michigan. Recorded distribution includes nearly every county with suitable habitat. *P. media* is surpassed only by *Perlesta placida* Hagen in the number of streams in Minnesota from which the species has been collected (Hardin and Mickel, 1952). Field studies in Wisconsin further indicate that *P. media* is an important component of the northern stonefly fauna and

that northwestern Illinois probably represents the extreme southern edge of its distribution in the central states (Frison, 1935).

Collection

During the months from October 1967 to September 1968 detailed observations and growth estimates were recorded for P. media in several streams close to the laboratory, in particular Dumont Creek.

P. media nymphs are commonly found associated with riffle areas having a rubble streambed. The nymphs were taken primarily under the rock substrate, although they were also found among logs, sticks, leaf packets, and a variable assortment of stream debris. The numphs were taken most frequently in the fast-flowing riffle areas but they also inhabit the quiet sections of the stream.

The only other Plecoptera collected from Dumont Creek were Isoperla signata and Taeniopteryx sp. Other associated fauna includes: Stenonema sp. (Heptageniidae); Baetis sp. (Baetidae); Hydropsyche sp. (Hydropsychidae); Gomphus sp. (Gomphidae) and the amphipod Hyalella azteca Saussure.

Stonefly collections were generally made two or three times per month, with the frequency of sampling and number taken being dependent on the need for test organisms in the laboratory. The sample methods were not quantitative, but care was taken to collect all

the nymphs from each hand screen sample in an attempt to obtain nymphs representing all available size categories.

Development

Figures 1 and 2 show seasonal variation in size distribution. The distribution of head-capsule measurements indicates that P. media requires two years to complete its nymphal growth (Figure 2).

The stoneflies exhibited maximum growth during June, July, August and September. By the end of October the year-class II nymphs have nearly reached maximum growth as indicated by head capsule width. The unexpected size distribution recorded for October reflects the small sample taken. Year-class I nymphs were completely overlooked during October, November, and December, and the numbers of very small stoneflies are not adequately represented in the growth histograms. Very small nymphs were collected in January, however, and their rate of development was recorded for the remainder of their life cycle (Figure 2).

Growth as indicated by head capsule width was minimal during the winter and spring months for the year-class II nymphs. This was not the case with total body weight, however. Figure 3 shows the relationship between head capsule width and dry body weight for each season. Little difference was noted in the summer and autumn data, but the apparent alteration in the slope of the

Figure 1.--Seasonal variation in size distribution of *Paragnetina media* nymphs. Ordinate refers to the percentage composition of specimens in each size class for each month. Sample sizes: January, 202; February, 276; March, 135; April, 240; May, 281; June, 246; July, 271; August, 205; September, 118; October, 85; November, 102; December, 183.

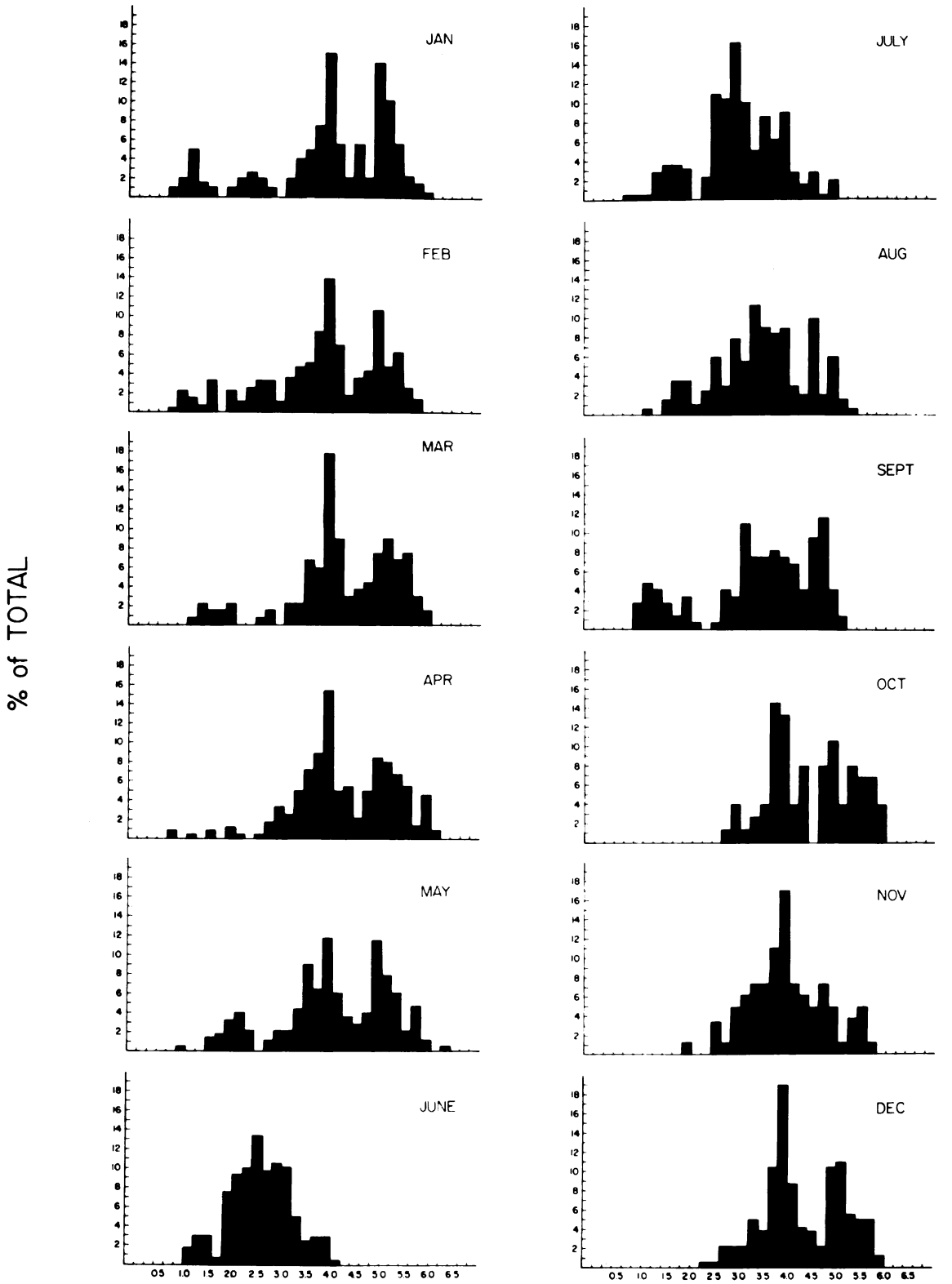

JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
% of TOTAL
HEAD CAPSULE WIDTH (mm)

Figure 2.--Numbers of *Paragnetina media* nymphs collected each month. Dashed line connects the mean head-capsule width for each month.

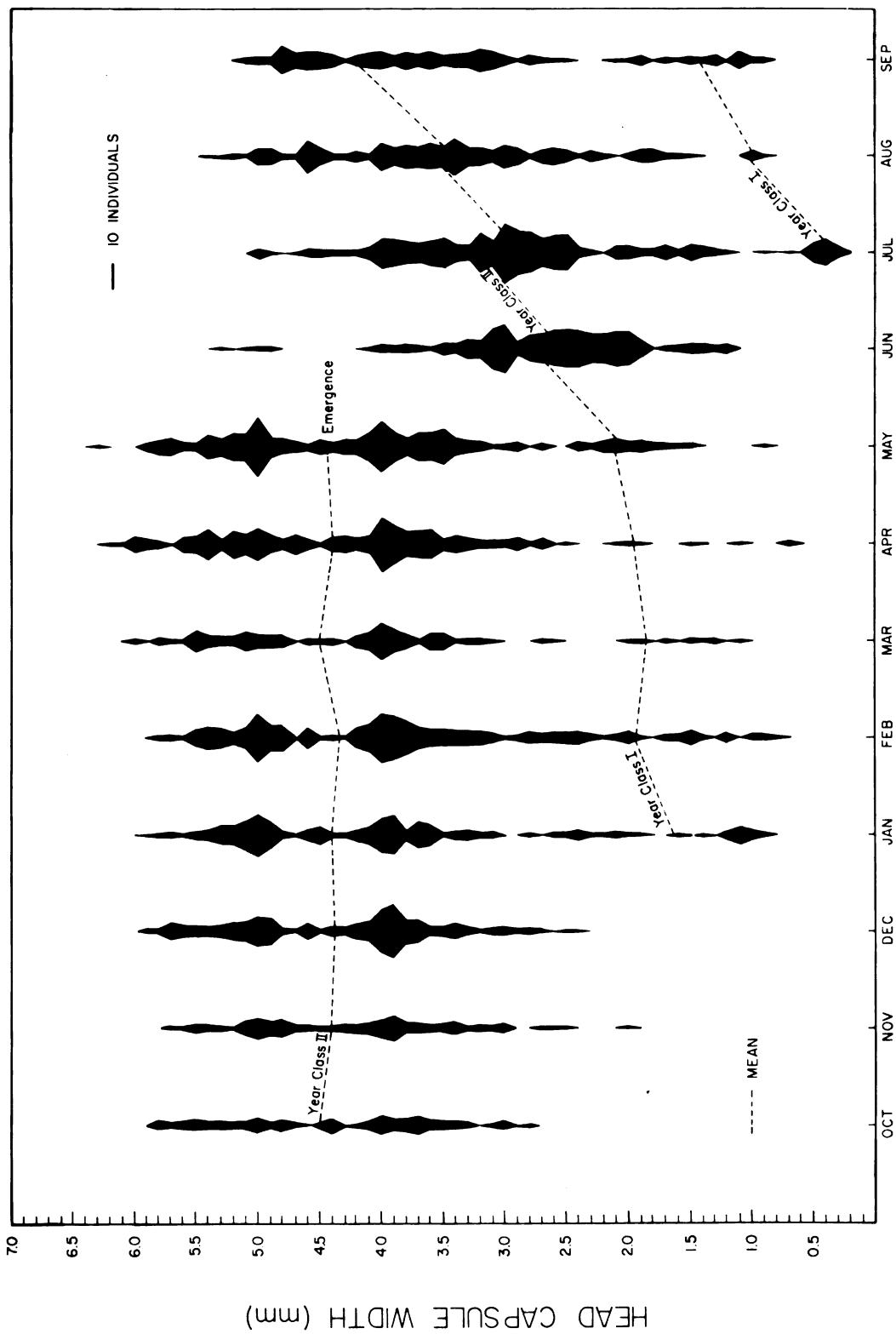
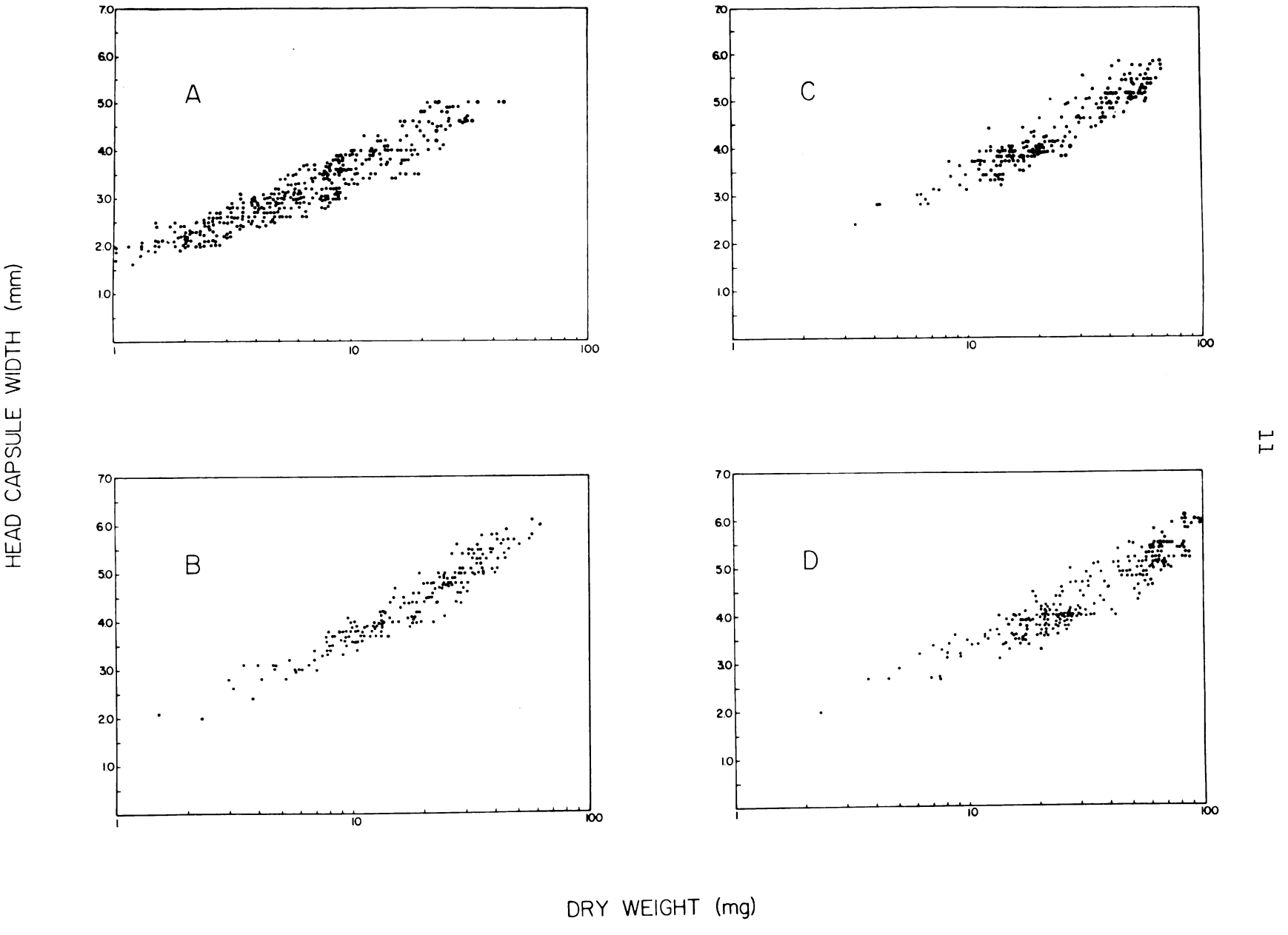

HEAD CAPSULE WIDTH (mm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
10 INDIVIDUALS
MEAN
Emergence
Year Class II
Year Class I
Year Class II
Year Class I
1967
1968

Figure 3.--Relation between head-capsule width (mm) and dry body weight for *P. media* nymphs during summer (A), autumn (B), winter (C), and spring (D).


HEAD CAPSULE WIDTH (mm)
A
B
C
D
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1
10
100
DRY WEIGHT (mg)

regression (Figure 3) during the winter and spring months is the result of an increase in total body weight. Head-capsule width measurements and personal observations on various body proportions during this period showed little or no increase. An increase in body weight is particularly evident during the spring, just prior to emergence, which may account in part for the noticeable distention of the nymphal body at that time. A large percentage of this weight increase can undoubtedly be attributed to the development of reproductive tissue in both male and female nymphs. No external morphological alterations were apparent during this period of developing reproductive organs in the nymph.

A bimodal population in the year-class II nymphs in December to May became evident in the course of plotting size distributions. Examination of emerging adults confirmed the suspicion that these two populations were the result of distinct size differences between male and female nymphs. Head-capsule widths for males and females were respectively 3.0-4.5 and 4.6-6.0 mm. Similar findings have been reported for the stonefly Phasganophora capitata Pictet (Alward, personal communication, 1968) and the mayfly Epeorus pliuralis Banks (Minshall, 1967).

Molting

As with all arthropods, growth in stoneflies is accompanied by molting. Occasional molts were observed

in nymphs collected from June to December. The cessation of growth in body dimensions during the mid-winter months resulted in an extensive intermolt period. The exo-skeleton became very hard with an attendant unicolorous dark shading. Accompanying the warming trend in water temperatures, most of the year-class II nymphs began to molt near the end of March and throughout April. Considerable change in the general pattern and texture of the exo-skeleton resulted from this molt. The nymphal bodies, following molting, were very soft and brightly colored with contrasting light and dark patterns.

Emergence

Emerging adults were observed during the last week of May in both 1967 and 1968. In the laboratory, adults emerged several days earlier when the nymphs were maintained at 30 C. Males emerged before the females, for only adult males were collected during the week following the first emergence. Frison (1935) states that the adults of P. media are diurnal and congregate on vegetation near rivers in which the nymphs live. Mating takes place during the day. The adults emerge from nymphs which leave the water at night.

Male and female stoneflies mated readily in glass rearing cages. A large egg mass formed at the genital opening of the female within one hour of copulation. The extruded egg mass (estimated at 200 to 500 eggs)

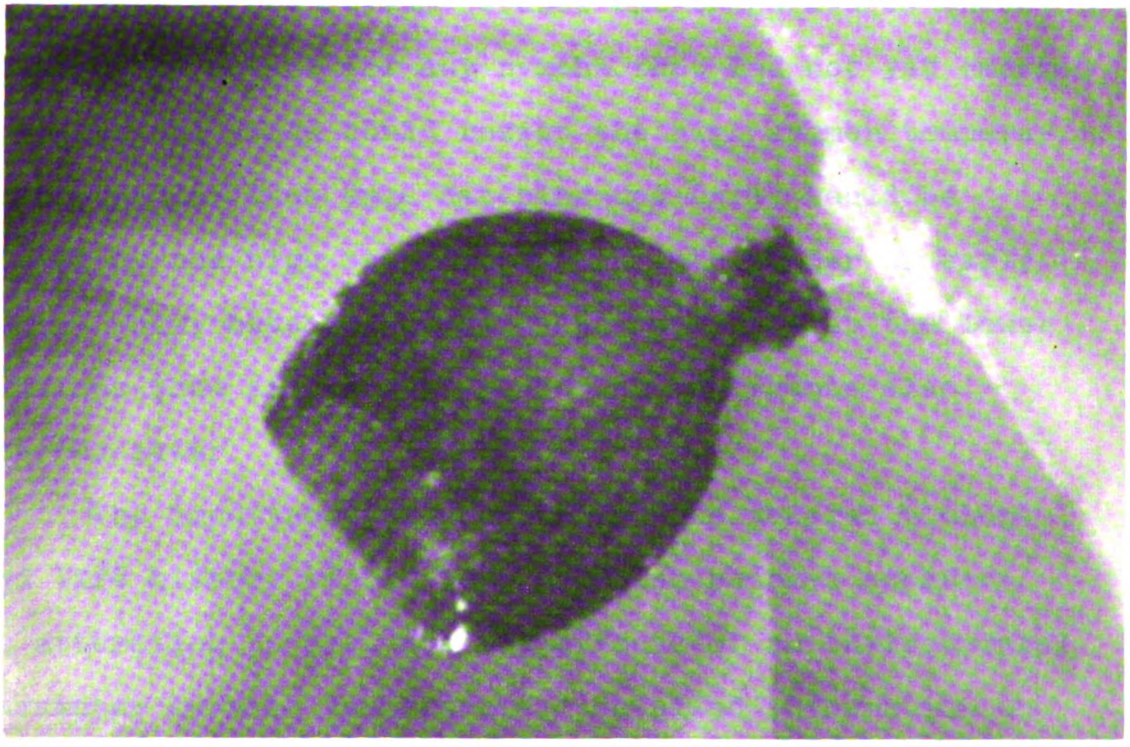
was removed from the female and placed in a shallow dish containing small stones and well-aerated stream water. Eggs collected before they had become too dry affixed readily to the first solid object they touched--by means of a sticky gelatinous base and several fine strands supporting the egg (Figure 4).

The first hatching occurred after a 30 day incubation period at room temperature (24-26 C). P. media eggs contain a circular aperture near the top, creating an opening from which the nymphs escape as from a "flip-top box." Figure 4 shows a nymph of P. media within 5 days of hatching. These tiny animals, though very active at this time, were completely helpless when dislodged from the substrate. Numerous cast skins were observed, indicating that the nymphs underwent intensive molting soon after hatching. Little growth was observed in laboratory reared-nymphs, and most died after several weeks.

Attempts failed to locate either eggs or newly hatched nymphs in the field. Therefore, the year-class I animals plotted for July (Figure 2) were those reared in the laboratory. Year-class I nymphs were collected in August, and their developmental rate can be followed in the histograms.

The variable size range of small nymphs taken in the field collections indicates an extended hatching period, or delayed nymphal growth after hatching, or

Figure 4.--*Paragnetina media* egg and newly hatched nymph. Egg size, 0.4-0.5 mm. Nymph size, 0.35 mm head-capsule width.



both. The emergence period extending over several weeks suggests that hatching may also occur over an extended period.

It appears that temperature is one of the factors influencing the initiation of stonefly hatching as well as triggering emergence. Brink (1949) pointed out that the emergence of many species of Plecoptera occurred progressively later in colder zones in Sweden. The main reason he gave was the slower growth in colder waters, which necessitates a longer larval life. Ide (1935), in studying the distribution of several species of mayflies in a stream, found that emergence started earlier and ended later near the source of the stream than at the lower levels. He postulated that the emergence period ended earlier downstream because the temperatures there soon became lethal for any nymphs that had not yet emerged. Only the egg stage could resist the high summer temperatures; nymphs from eggs hatching too early would be killed by the high temperatures in late summer or early fall. Thus, hatching usually started later at the lower stations than at the source, so that emergence also started later.

The importance of considering the general biology and life history of the test organism becomes apparent in attempting to evaluate the effects of an environmental parameter such as temperature. Detailed information on

the life history of P. media enabled this investigator to differentiate between male and female nymphal populations, define periods of maximum growth, and delimit temperature sensitive physiological states such as pre-molt, pre-emergence, and egg hatching.

METHODS AND MATERIALS

Collection and Initial Handling of Stocks

The test organism used was collected from Dumont Creek (T2N R13W S6) near Allegan, Michigan, a shallow stream, averaging less than 0.5 meter in depth, and characterized by swift water flowing over a loosely cemented rock and cobble bed. The stream receives a considerable quantity of allochthonous material from surrounding farm and woodland drainage. During the late summer months, dense growths of Cladophora glomerata become attached to much of the bottom substrate.

Most of the nymphs were collected with a hand screen, while others were collected by hand picking of rocks. The animals were transported to the laboratory in a 10-liter polyethylene pail.

Air and water temperatures were recorded in the field with a mercury thermometer at the time of each collection.

Acclimation Tanks

In the laboratory, the stoneflies were separated into one liter polyethylene bottles and supplied with

rock and bark substrates. Approximately 100 animals were placed in each flask. Sufficient dissolved oxygen was provided each bottle by means of air stones and compressed air. The bottles so provisioned were placed in constant temperature water baths. The baths were maintained at 10, 20 and 30 C (± 0.5 C) by electrical water heaters and a portable cooling unit. The acclimation temperatures were chosen for their close approximation to the normal thermal range of the test organism.

Test animals retained in the laboratory longer than four weeks were discarded. Although test animals were not fed during acclimation or experimental evaluation, nymphs held for three weeks showed no significant loss of heat tolerance. A 10 to 20 per cent mortality due to cannabilism was indicated by a decrease in the number of test organisms during acclimation and the presence of partial body segments.

Both acclimation and testing were conducted in Augusta Creek (T1S R9W S21) water transported from the stream to the laboratory in five-gallon polyethylene containers. P. media is relatively abundant in this stream. A chemical analysis of Augusta Creek water is given in Table 1.

Thermal Tolerance Apparatus

Initially, the technique used in determining upper thermal limits was static water bioassay. P. media

TABLE 1.--Chemical analysis of Augusta Creek water sampled during August, 1968.[1]

pH	8.2
Total alkalinity	4.6 meq/l*
CO_3 Alk.	0.0 meq/l
HCO_3 Alk.	4.46 meq/l
Boron	0.0 meq/l
Chloride	0.15 meq/l
Sulfate	0.45 meq/l
Nitrate	0.06 meq/l
Sodium	0.11 meq/l
Calcium	2.80 meq/l
Magnesium	1.83 meq/l
Potassium	0.04 meq/l

[1]Provided in part by the Department of Water Science and Engineering, Chemical Laboratory, University of California, Davis.

*Milliequivalent

occurs in streams of considerable current velocity, however, and it was therefore conjectured that water flow had a significant effect on its thermal resistance. Consequently, both static and flowing bioassays were employed and the results compared.

Six laboratory streams were constructed for use in obtaining upper lethal temperature information in a flowing water system. Wooden troughs, 120 x 10 x 9.5 cm,

treated with onert epoxy paint, were provided with a substrate of gravel and rock (approximately 8 cm in diameter). A centrifugal pump transported test water from a catch reservior (ten-gallon aquarium) to the upper end of the artificial stream, producing a continuous and uniform system of water recycling (Figure 5).

Wire screen (#20 mesh) was provided at each end of the trough to stabilize the substrate and prevent the animals from migrating or drifting from the test area.

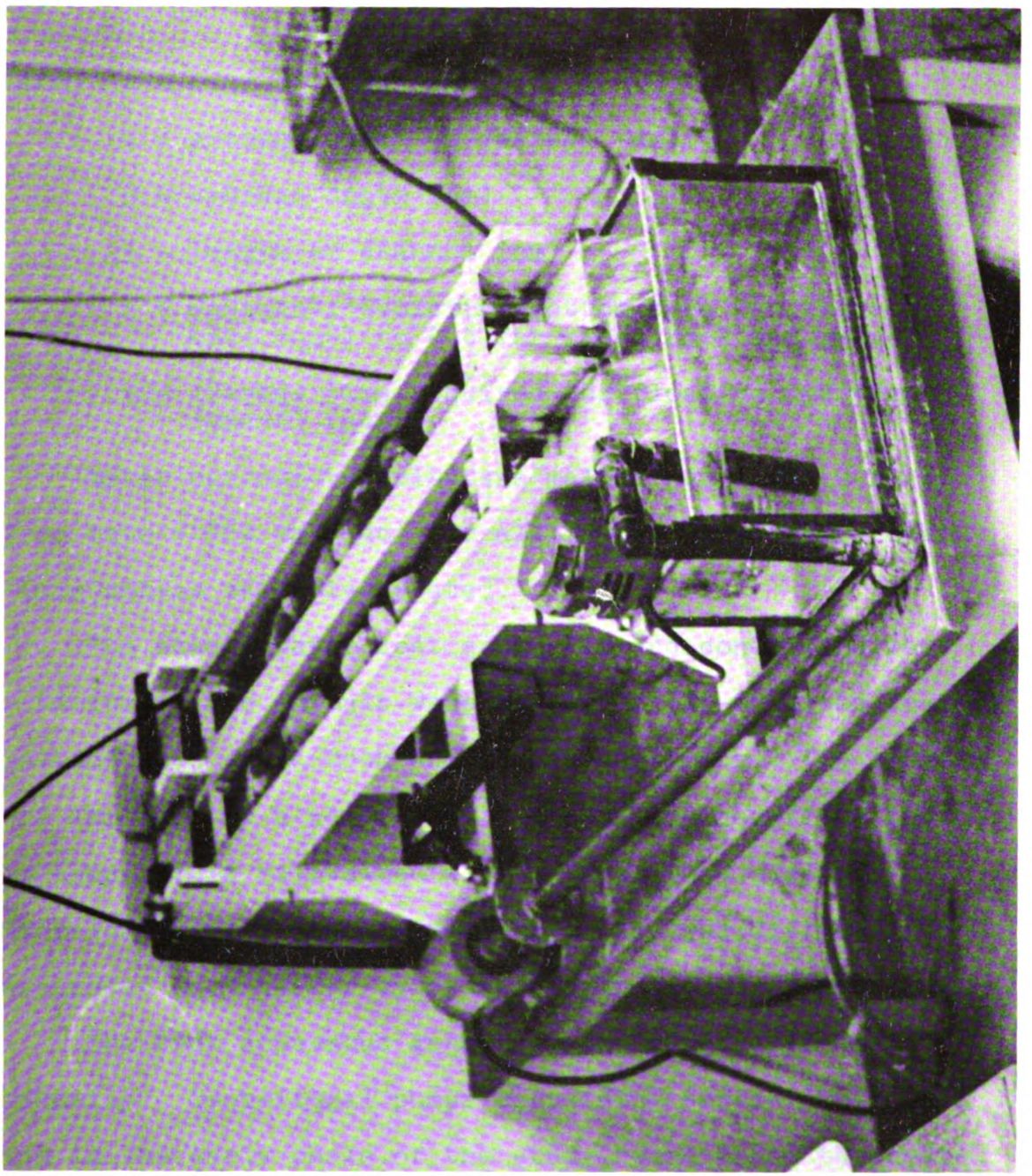
Constant temperature was maintained within the test apparatus with an immersion heater and bimetal or mercury thermoregulator coupled to a temperature controller incorporating a sensitive relay, power relay, and a control-circuit transformer. With this arrangement, bath temperature was controlled within $\pm$ 0.1 C.

Aeration was accomplished during recycling by agitation of the water. No attempt was made to control lighting.

Flow rate was regulated with an adjustable ball-valve in the output line of the centrifugal pump.

Surface velocity of water flow in the test apparatus was determined with a cork float 1.5 cm in diameter. Mean current velocity was approximated by taking the average of five trials (time elapsed for the float to traverse the stream bed) and multiplying by a correction factor of 1.33 for water depth (Welch, 1948).

Figure 5.--Test apparatus employed in determining upper lethal temperature of *P. media* nymphs under a constant water flow.



Rate of water flow was calculated from Embody's formula:

$$r = \frac{wdal}{t}$$

where: r = rate of flow (cm^3/sec)

w = average width of channel (cm)

d = average depth (cm)

l = length of channel (cm)

a = constant, depicting bottom type (in this case equal to 0.8 for loose rock and gravel)

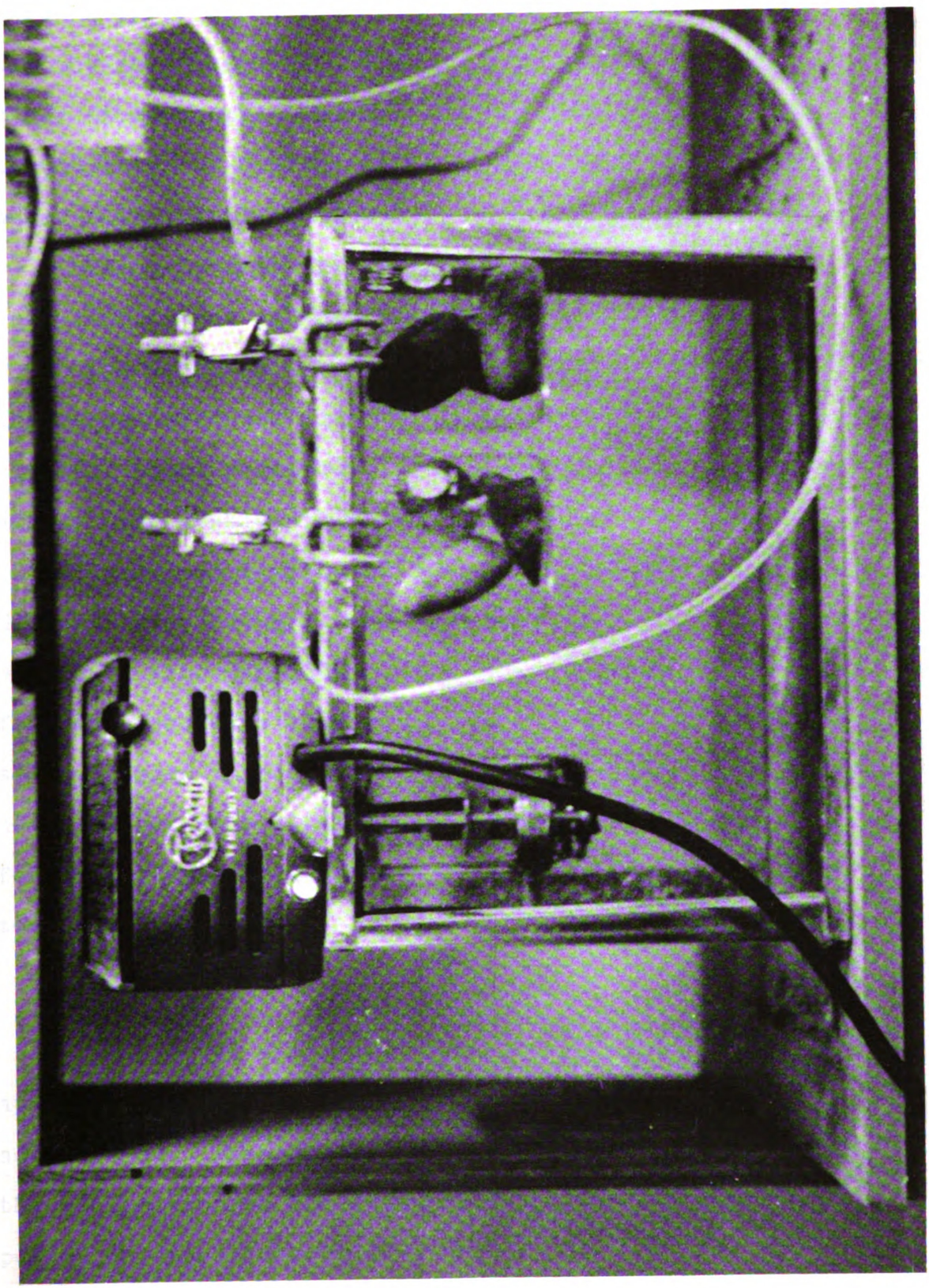
t = average time required for float to traverse channel (sec)

Water current velocity was maintained at 31.9 cm/sec (flow rate = 792 cm^3/sec) for all lethal-temperature evaluations unless otherwise stated.

The static water test apparatus and conditions were as follows: the animals were placed in 600-ml glass beakers provided with small rock and gravel substrate. The beakers were immersed in a water bath (five-gallon aquarium) and the temperature maintained within $\pm$ 0.1 C by electric immersion water heaters. Compressed air was diffused throughout each beaker by means of air stones (Figure 6).

The test apparatus generally performed quite satisfactorily. Centrifugal pumps containing bronze impellers, used in the early phase of the study, were found to create toxic conditions for the test organism. They were replaced with pumps having a stainless steel shaft and

Figure 6.--Test apparatus employed in determining upper lethal temperature of *Paragnetina media* nymphs in static conditions.



impeller. In several control runs made thereafter for periods up to three weeks, the nymphs remained healthy and active throughout. Florke et al. (1960), in a study of metabolism and heat resistance, found that an accumulation of 0.02 mg Cu per liter from a coil in the water bath greatly accelerated mortality in the gudgeon.

These examples illustrate the importance of any toxic substances in the water during bioassays.

The electronic relay unit coupled to a mercury thermoregulator was found to be more dependable than the bi-metal thermoregulator for maintaining constant temperatures over considerable periods of time.

Dissolved oxygen was determined frequently by the Micro-Winkler Method, Azide Modification, using 0.001 N $Na_2S_2O_3$ with replicate samples in both the static and flowing bioassay evaluations. The concentration of dissolved oxygen in both sets of experiments was maintained near 100 per cent saturation. Oxygen stress was therefore assumed to be nonexistent in effect on upper lethal-temperature levels.

Test Procedure

Acclimated stoneflies were introduced into test apparatus maintained at the same temperature used for acclimation. Then the water temperature was raised from the acclimation to the test level during a tempering period of approximately 30 minutes. This procedure

reduced shock or "heat rigor" that resulted if the temperature change was too rapid. Heat rigor was particularly evident in tests under static conditions. The 30-minute tempering period was initially assumed not to alter the acclimation temperature to any appreciable extent. This assumption was later confirmed in experiments testing the rate of gain and loss of heat resistance. This method presumably approximates natural conditions more closely and yields a better estimate of actual lethal temperature than does introducing the test organism abruptly from acclimation temperature to test temperature. This method should also be superior to the gradually raising of temperature until death ensues, a method which ignores the time factor in resistance.

The organisms were inspected at frequent intervals, the time of inspection being dictated by experimental conditions. At each inspection, dead animals were removed and the time lapse recorded. The criterion of death was cessation of all movement and failure to respond to mechanical stimulation. This was determined to be a good approximation of death when numerous test animals deemed "dead" developed no sign of recovery when placed in a well aerated water chamber for 24 hours at room temperature.

Numerous measurements were made on dead animals removed during the test period for assessment of size classes. Such measurements included head capsule width

(taken directly posterior to the compound eye), pronotum length and total body length (anterior margin of the head to the apex of the abdomen).

Oven dry weights were determined by placing the test organisms in a drying oven at 104 C for 48 hours, desicating for two hours and weighing to the nearest 0.1 mg.

Graphic Analysis and Statistical Evaluation of the Data

The measure of resistance was time to 50 per cent mortality at a given constant temperature. Results were analyzed by the methods of Litchfield (1949). In this procedure, resistance times are determined by plotting accumulative percentage dead at the end of successive observation intervals against time of exposure. A line is fitted by inspection to the points, and the LT50 (lethal time for 50 per cent of the test population) is read from the graph. The variability of a test population is shown by the slope of the line. It is denoted numerically by the slope function, S, and is defined as $1/2(LT_{84}/LT_{50}+LT_{50}/LT_{16})$; the greater the value of the slope function, the more variable the response of the test population.

The number of animals and the slope function are used in conjunction with a series of nomographs to obtain 95 per cent confidence limits for the LT50. These confidence limits are calculated by multiplying and dividing the estimate of the parameter (in this case the LT50) by

a factor, f. This procedure corresponds to adding the standard error to and subtracting it from the logarithm of the parameter. In other words, the factor, f, is equal to the antilogarithm of the standard error (Litchfield, 1949). The equations are solved to obtain f by means of a nomograph.

This method also allows for the comparison of two curves to determine whether they deviate significantly from parallelism. If the two curves are parallel within experimental error, they can then be compared for significant difference.

Considered to represent indefinite survival was 10,000 minutes (approximately 7 days). In no instance in this study did 50 per cent mortality occur when tests were extended beyond this point, occasionally for as long as three weeks. This appears to be a fairly accurate estimate of the level of resistance to the direct lethal effects of high temperatures, later mortality would be considered the result of a complex of factors.

The relation between test temperature and LT50 was plotted on semilogarithmic grid paper, and the points were fitted with a straight line by inspection. The point at which this line intercepted 10,000 minutes marked the incipient lethal level (ILL)--the level of the environmental identity (in this case temperature) beyond which the organism can no longer live for an indefinite

period of time (Fry, 1947). Thermal resistance is the measure of the ability of an organism to withstand a temperature above the ILL.

Respiration

The effect of temperature on oxygen consumption of P. media nymphs was evaluated with a Gilson Medical Electronics refrigerated Differential Respirometer, Model GR 14. The procedures for oxygen consumption evaluations and calculations followed those set forth by Umbreit et al. (1959) and the Gilson operating instruction manual (Gilson Medical Electronics, Middleton, Wisconsin).

RESULTS

Field Observations

Figure 7 shows the mean monthly water temperature of Dumont Creek for the periods of October to December (1967) and January to September (1968). Summer water temperatures for Dumont Creek averaged 20 to 25 C, with a maximum of 28 C recorded on August 20, 1968. As expected, spring and autumn included periods of rapidly changing water temperature. Winter temperatures were quite stable, generally averaging between 0.5 and 2.0 C. An extensive covering of anchor ice was observed on the stream bed in January, 1968. No serious effects on the population of *P. media* were apparent, for large numbers were collected immediately following release of the ice.

Relation Between Lethal and Acclimation Temperatures

Figure 8 shows the relation between 48-hour upper lethal temperature and acclimation temperature. It is evident that lethal temperature was raised by acclimation to higher temperatures throughout the thermal range examined.

Figure 7.--Mean monthly water temperature recorded for Dumont Creek in 1967-68.

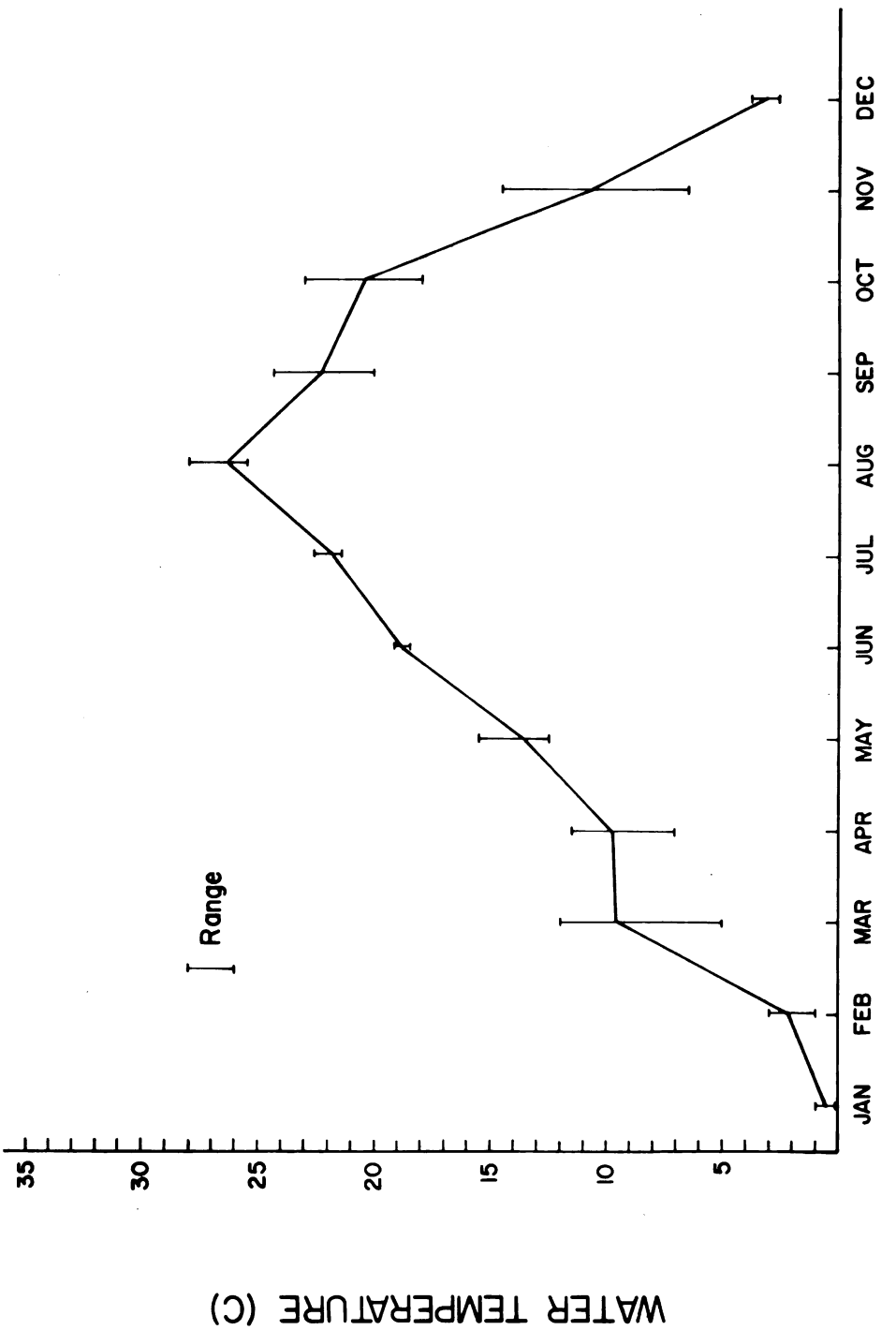

WATER TEMPERATURE (C)
35
30
25
20
15
10
5
Range
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
MONTHS

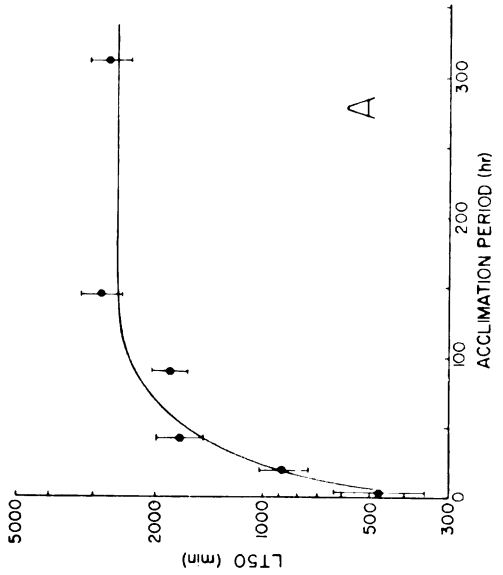
Figure 8.--Relation between 48-hour upper lethal temperature and acclimation temperature in P. media nymphs during winter, spring, and summer.

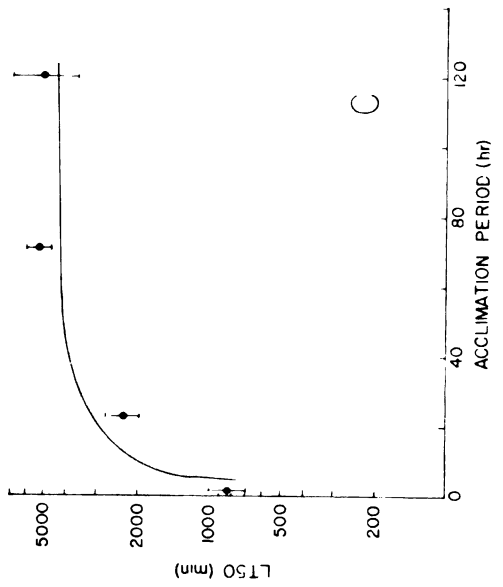
The rate of increased temperature tolerance with a rise in acclimation temperature was greatest in spring, and minimal in summer. This may reflect difficulty in attaining downward acclimation. The diagonal line on the right side of the panel is a construction line marking the locus of points where acclimation and lethal temperatures are the same. The point of intersection of this line, indicating the level at which lethal temperature can no longer be raised by acclimation, corresponds with Fry's (1947) ultimate upper incipient lethal temperature (35.3 C).

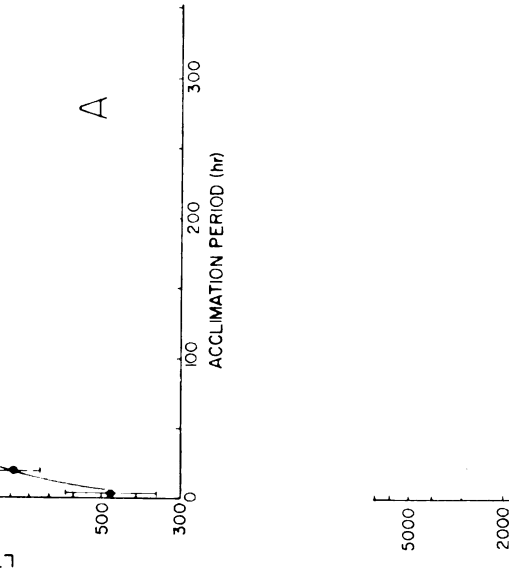
Rates of Gain and Loss of Heat Resistance

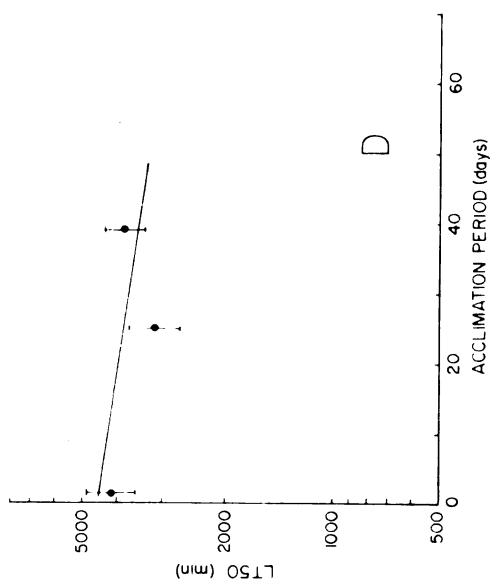
Experiments were conducted on four separate dates to determine the rates of gain and loss of heat resistance. Nymphs were collected from the stream during the period of near minimal water temperatures and slowly acclimated to 10 C. They were then immediately introduced to the 30 C acclimation bath. Groups of 8 to 10 were selected for determination of LT50's at various intervals thereafter, and tests continued until no change occurred in LT50. The selected test temperature was 35 C. From previous results, it was known that this temperature would result in rapid mortality of P. media nymphs acclimated at 10 C, whereas nymphs acclimated to 30 C could tolerate this temperature for approximately 48 hours. The results are presented in Figure 9.

Figure 9.--Rates of gain and loss of heat resistance in P. media nymphs. Temperature shift: (A) 10-30 C; (B) 10-20 C; (C) 20-30 C; (D) 30-10 C. Ninety-five per cent confidence limits for each LT50 are indicated by a vertical line.


A
LT50 (min)
5000
2000
1000
500
300
0
100
200
300
ACCLIMATION PERIOD (hr)


C
LT50 (min)
5000
2000
1000
500
200
0
40
80
120
ACCLIMATION PERIOD (hr)


B
LT50 (min)
5000
2000
1000
500
200
0
40
80
120
ACCLIMATION PERIOD (hr)


D
LT50 (min)
5000
2000
1000
500
0
20
40
60
ACCLIMATION PERIOD (days)

Rate of Gain

The acclimation temperature of P. media was raised from 10 to 30 C in approximately 120 hours (Figure 9A). The maximum level of resistance was maintained for 300 hours, when this series of experiments was terminated. In Figure 9B, acclimation was raised from 10 to 20 C in 45 hours, an apparent rate of 5 degrees per day, whereas in series 9C the rise was from 20 to 30 C in slightly less than 40 hours, an increase of 5.8 degrees per day.

The rate of gain of heat resistance for P. media was found to be approximately 5 C per day.

Decrease in resistance with prolonged acclimation did not significantly alter the LT50.

Rate of Loss

Figure 9D shows the rate of loss of heat resistance when animals acclimated to 30 C were introduced to the 10 C bath. Resistance decreased only slightly during 39 days of holding. LT50's for tests at 1 and 39 days were not significantly different at the 0.05 level of significance. Exact rates were not determined, but it is concluded that downward acclimation is much slower than upward acclimation.

Effects of Certain Factors on Thermal Resistance

Size and Sex

The factors of size and sex are inseparable in the present study with stonefly nymphs as it was impossible to

differentiate between male and female P. media on the basis of morphological characteristics. In the process of recording growth increments, however, it became evident that two distinct and separate populations were discernible (see Life History and Ecology section, Figure 2). By sexing adults it was concluded that the bimodal population was in fact a result of sex differences with the larger nymphs being females and the smaller being males. Sex could thus be determined fairly accurately on the basis of nymphal size class.

As mentioned in the Methods section, survival time was recorded for each animal along with measurement of body size. During the summer, autumn, and early winter periods, inspection of test results of individual experiments, and analysis of the correlation coefficient from regressions of body size and order of death (Snedecor and Cochran, 1967), failed to show any significant effect of size on resistance time. In these and all subsequent lethal temperature experiments, care was taken to select an equal number of representatives from all size classes so that if any such effect was operating it would not alter mortality distributions seriously.

Near the end of the winter test period, time-per cent curves began to indicate that size was affecting thermal resistance, though inconsistently. For this reason, a number of experiments were conducted in which

nymphs of two distinct sizes (and consequently different sexes) were tested separately and concurrently at the same temperature and with the same thermal histories.

The results are presented in Figures 10 and 11, and their statistical comparison is given in Table 2.

The hypothesis tested was:

(a) H_o: $b_m = b_f$

(b) H_1: $b_m \neq b_f$

where b_m = time-per cent curve for males, and b_f = time-per cent curve for females.

With 10 C acclimation (Figure 11A) male stoneflies showed significantly greater tolerance than females at test temperatures of 32-34 C but not at 35-37 C. All the nymphs in Figure 11A had recently molted, except those at 32 C. This could account for the unexpected loss of heat tolerance at 32 C. In Figure 11B, males showed significantly greater tolerance than females at 34 C but not at 35 C. The time-per cent curves for 33 C were not parallel and could not be compared statistically, though a trend was evident toward greater tolerance by males at the lower test temperatures.

The tests at 20 C acclimation were not compared statistically because of the "split probit" response exhibited in the time-per cent curves (Figure 10). The results show similar trends, the males being more tolerant at the lower test temperatures. The test at

Figure 10.--Comparison of time-per cent mortality curves of male and female P. media nymphs at 10, 20, and 30 C acclimation temperatures. Lines are labeled with test temperature and date.

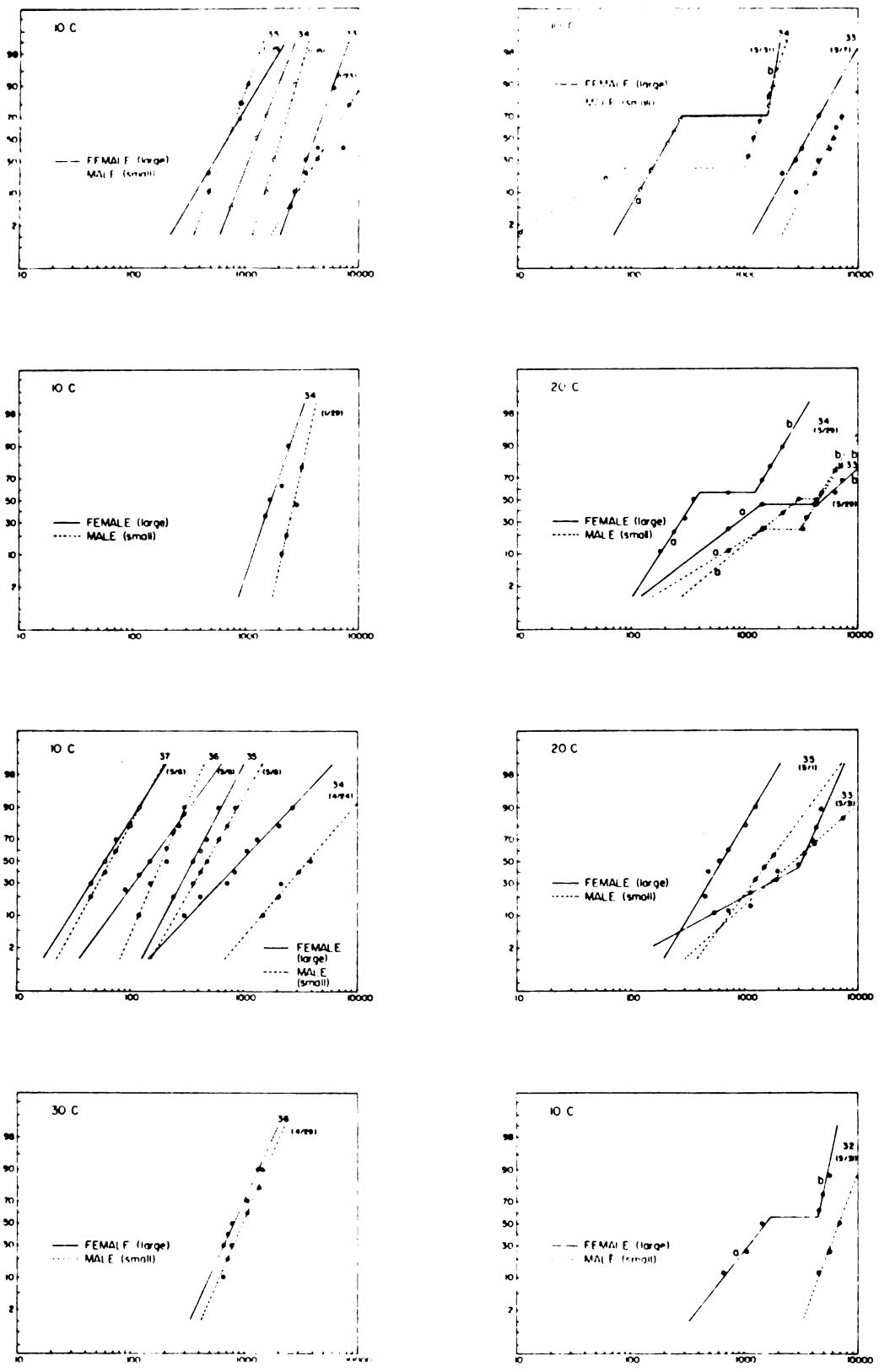

10 C
FEMALE (large)
MALE (small)
20 C
30 C
MORTALITY (%)
EXPOSURE (min)

Figure 11.--Comparison of times to 50 per cent mortality of male and female P. media nymphs. Acclimation temperatures 10 C (A and B) and 20 C (C). Test dates, (A) 4/24/68-5/31/68; (B) 1/15/68-1/29/68; (C) 5/1/68-5/31/68.

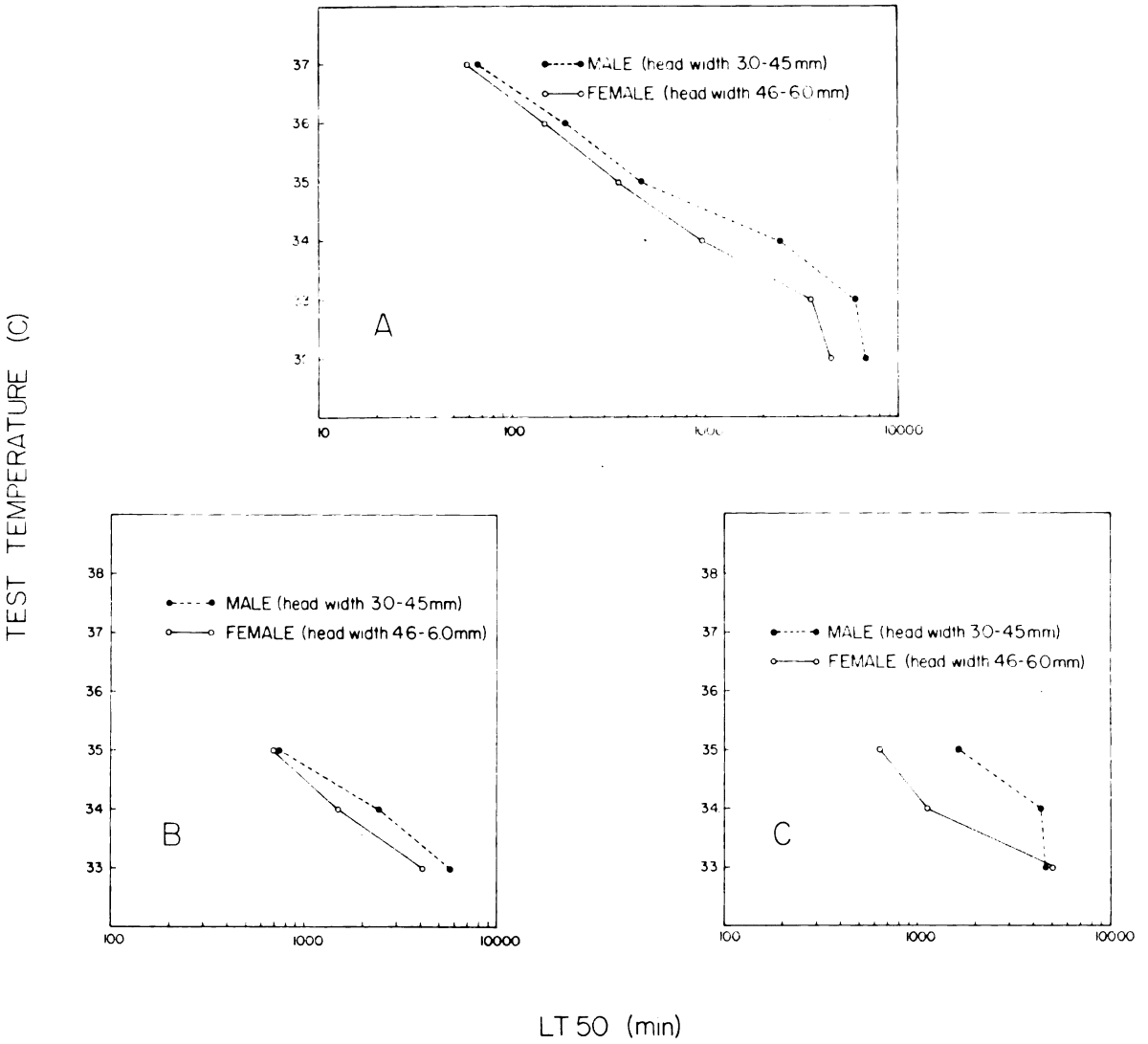

TEST TEMPERATURE (C)
MALE (head width 30-45mm)
FEMALE (head width 46-60mm)
A
B
C
LT 50 (min)

TABLE 2.--Statistical comparison of time-per cent curves from upper-lethal-temperature experiments using male and female Paragnetina media nymphs[1] (see appendix for analysis of time-per cent curves).

Date	Test temp (C)	SR (S_1/S_2)	f_{SR} (fs_1/fs_2)	H_o: b_m parallel to b_f	RR ($ET50_1/ET50_2$)	f_{RR} ($fet50_1/fet50_2$)	H_o: $b_m = b_f$*
5/6	37	1.06	1.36	accepted*	1.14	1.15	accepted
5/6	36	1.29	1.37	accepted	1.27	1.54	accepted
5/6	35	1.04	1.34	accepted	1.31	1.50	accepted
4/24	34	1.09	1.83	accepted	3.94	2.07	rejected
5/7	33	1.04	1.52	accepted	1.68	1.64	rejected
5/31	32	1.16	1.3	accepted	1.55	1.35	rejected
1/15	35	1.23	1.3	accepted	1.00	1.42	accepted
1/15	34	1.08	1.06	accepted	1.61	1.28	rejected
1/29	34	1.15	1.05	accepted	1.65	1.24	rejected
1/29	33	1.22	1.31	rejected			

[1]Litchfield (1949).

*Level of acceptance 0.05.

33 C in Figure 11C was complicated by the close proximity to emergence.

At high test temperatures the ensuing death processes are apparently so rapid that the expression of separate male and female tolerance levels is suppressed.

Since no significant differences in resistance of different size groups were found in the summer and autumn, the differences found during winter and spring are probably attributable to physiological properties associated with sex, and not with the size of the organism.

Static vs. Flowing Conditions

As stated earlier, the static bioassay method was replaced as the test procedure by a flowing bioassay apparatus. Static tests were conducted for comparative purposes during the summer and winter months, and the results are shown in Figures 12 and 13 (detailed graphic analysis of the time-per cent curves is given in the Appendix). Figure 14 compares static bioassay results with results in flowing water.

The animals were considerably more resistant to temperature effects in the flowing system than in the static. Comparison of concurrent tests by the method of Litchfield (1949) confirmed this. The differences were significant at the 0.05 level in all tests compared. The trend was similar for estimated ILL's (Table 3).

Figure 12.--Time-per cent mortiality curves for P. media nymphs tested in static conditions at 10, 20, and 30 C acclimation temperatures. Lines are labeled with test temperatures (C). The 95 per cent confidence limits for each time to 50 per cent mortality are indicated by a horizontal line. Bracketed point indicates zero or 100 per cent mortality. (A, B) December; (C, D, E) January; (F, G, H) February. Based on groups of animals with mean weights from 15.09 to 40.11 mg.

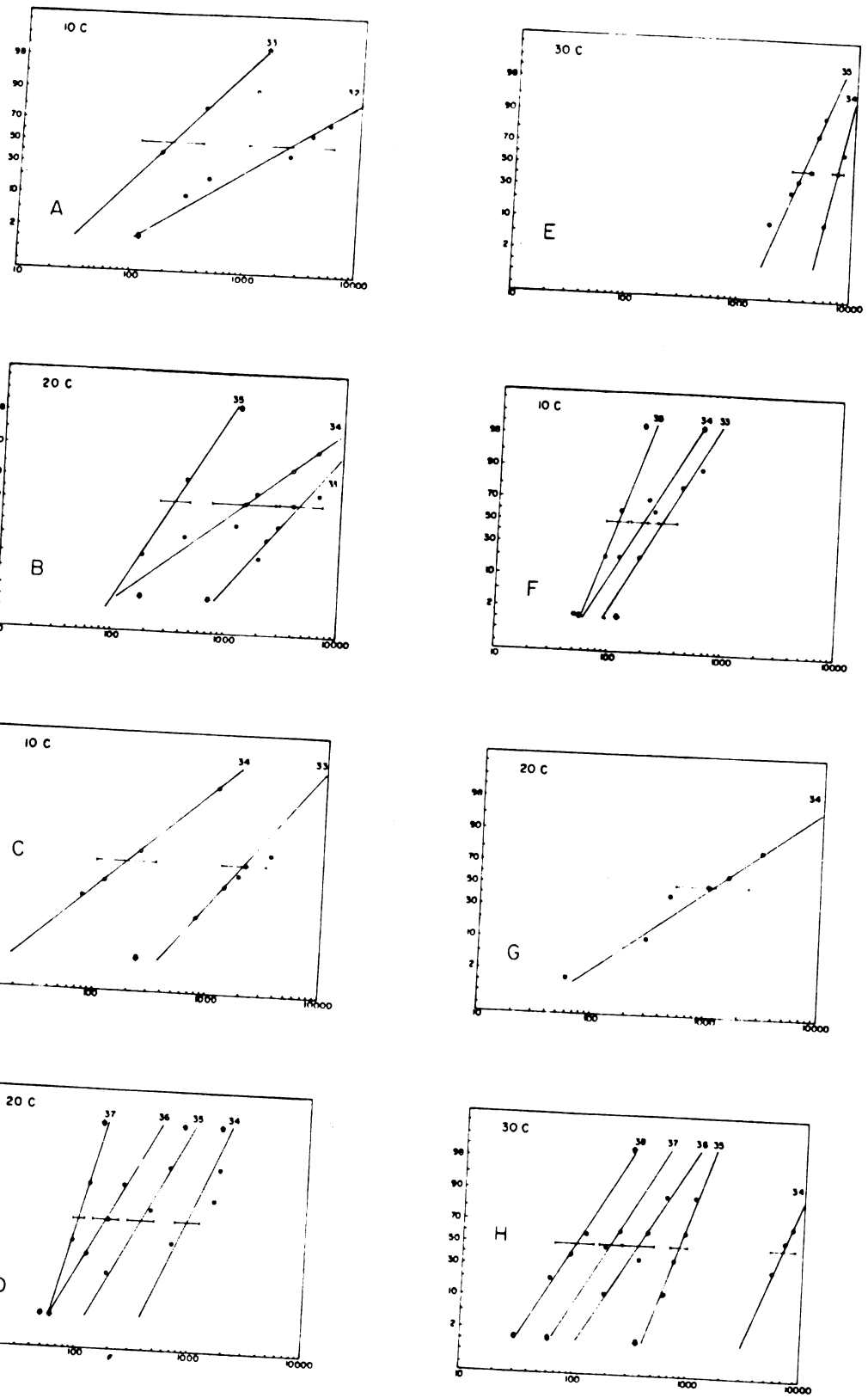

10 C
A
20 C
B
10 C
C
20 C
D
30 C
E
10 C
F
20 C
G
30 C
H
MORTALITY (%)
EXPOSURE (min)

Figure 12.--Time-per cent mortiality curves for P. media nymphs tested in static conditions at 10, 20, and 30 C acclimation temperatures. Lines are labeled with test temperatures (C). The 95 per cent confidence limits for each time to 50 per cent mortality are indicated by a horizontal line. Bracketed point indicates zero or 100 per cent mortality. (A, B) December; (C, D, E) January; (F, G, H) February. Based on groups of animals with mean weights from 15.09 to 40.11 mg.

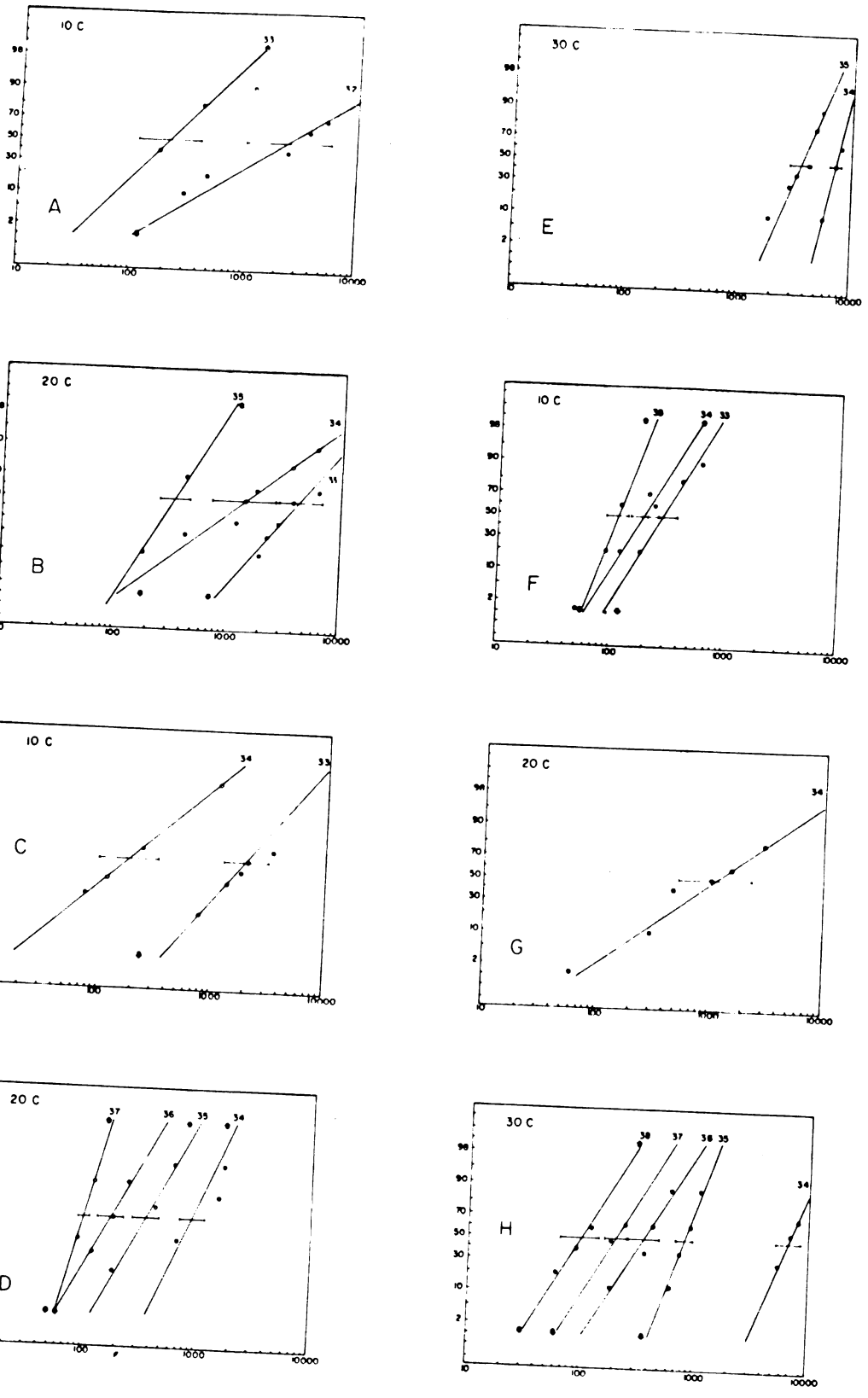

10 C
A
30 C
E
20 C
B
10 C
F
10 C
C
20 C
G
20 C
D
30 C
H
MORTALITY (%)
EXPOSURE (min)

Figure 13.--Time-per cent mortality curves for P. media nymphs tested in static conditions at 10, 20 and 30 C acclimation temperatures. Lines are labeled with test temperatures (C). The 95 per cent confidence limits for each time to 50 per cent mortality are indicated by a horizontal line. Bracketed point indicates zero or 100 per cent mortality. (A) June; (B, C) July; (D, E, F) August; (G, H) October. Based on groups of animals with mean weights from 3.31 to 26.87 mg.

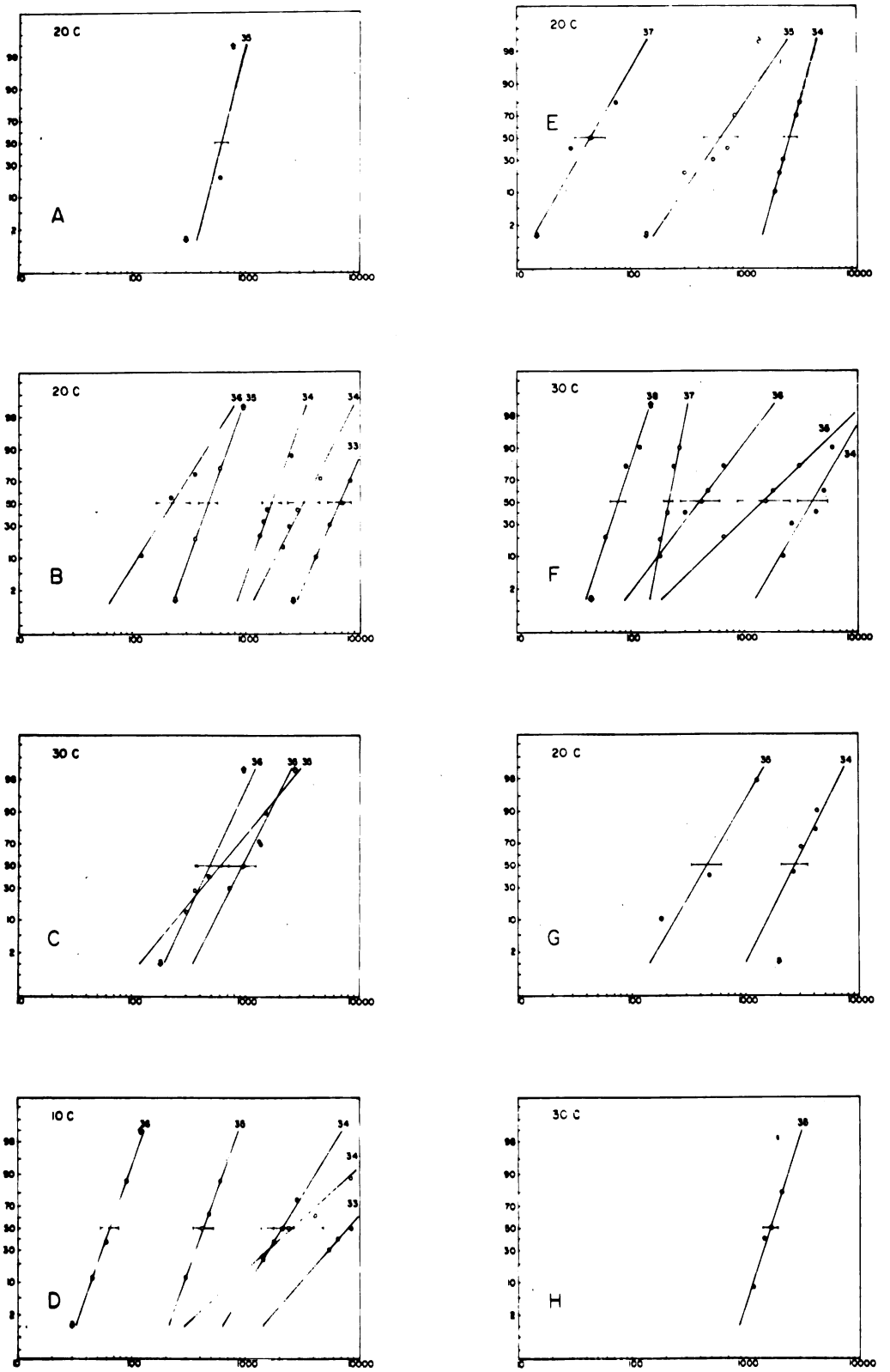
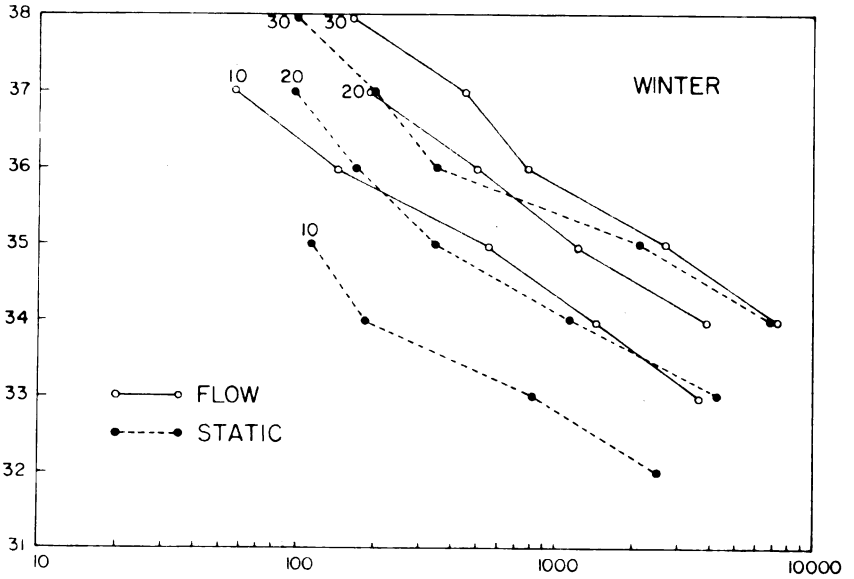
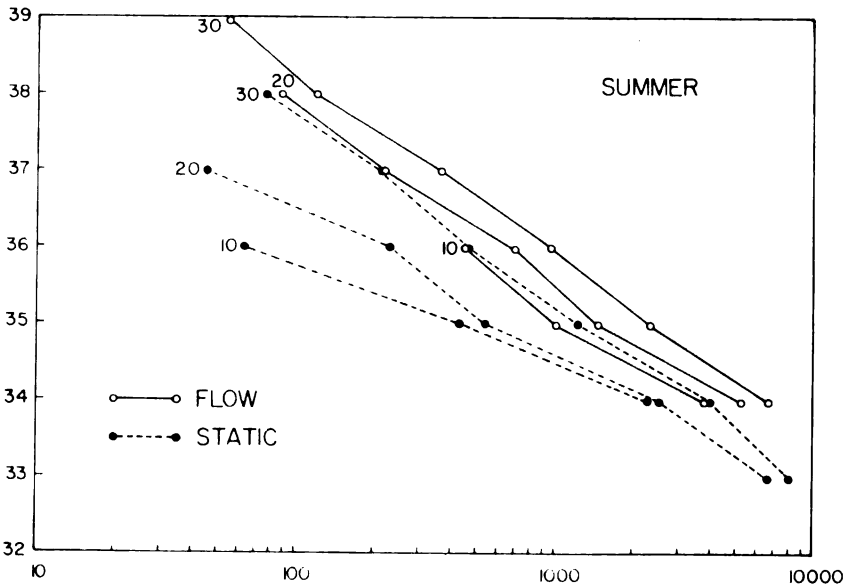

20 C
A
20 C
E
20 C
B
30 C
F
30 C
C
20 C
G
10 C
D
30 C
H
MORTALITY (%)
EXPOSURE (mn)

Figure 14.--Comparison of times to 50 per cent mortality for P. media nymphs tested in static and flowing conditions. Lines are labeled with acclimation temperatures.





TEST TEMPERATURE (C)

LT 50 (min)

TABLE 3.--Comparison of incipient lethal levels for upper-lethal-temperature experiments conducted on *Paragnetina media* nymphs in static and flowing conditions.

Accl temp (C)	ILL (C)			
	Winter		Summer	
	Static	Flow	Static	Flow
10	30.6	32.2	33.0	33.0
20	32.1	32.8	32.7	33.3
30	33.5	33.6	32.8	33.7

The animals appeared less able to adapt to higher temperatures when tested in static conditions. The nymphs would often succumb to heat rigor during the rise from acclimation temperature to test temperature. Even when death did not ensue immediately, the nymphs were frequently unable to regain normal muscular coordination. This resulted in excessive test variability as indicated by the rather large value of S in some of the statistical curves.

Under flowing-water conditions the nymphs retained normal mobility until near death.

These results indicate a significant difference in thermal resistance between the static and flowing-water experiments. Consequently, further tests were conducted with the same apparatus but regulating the rate of flow.

Effect of Flow

Figure 15 presents the results of lethal temperature experiments at current velocities of 5.2 cm/sec (flow rate = 171 cm^3/sec) and 40.0 cm/sec (flow rate = 1312 cm^3/sec). Table 4 gives the graphic analysis and statistical comparison. The results indicate that thermal tolerance was significantly greater for the higher current velocity at all test temperatures.

Lethal High Temperatures

Upper-lethal-temperature experiments were conducted at acclimation temperatures of 10, 20, and 30 C for all seasons from October, 1967, to September, 1968. Time-per cent curves for these experiments are presented in Figures 16-18 with detailed graphic analysis of the time-per cent curves given in the appendix. Replicate tests were conducted when the test results appeared questionable. LT50's obtained for similar seasons and under the same conditions were combined whenever possible.

Winter

Figure 19 presents the relation between test temperature and median resistance time for December, January, and February. The LT50's for this period are aligned along a straight line which passes within their 95 per cent confidence limits. This is partially a result of the uniform effect of acclimation temperature

Figure 15.--Comparison of times to 50 per cent mortality of P. media nymphs tested under conditions of high and low current velocity. Acclimation temperature at 20 C. The 95 per cent confidence limits for each LT50 are indicated by a horizontal line.

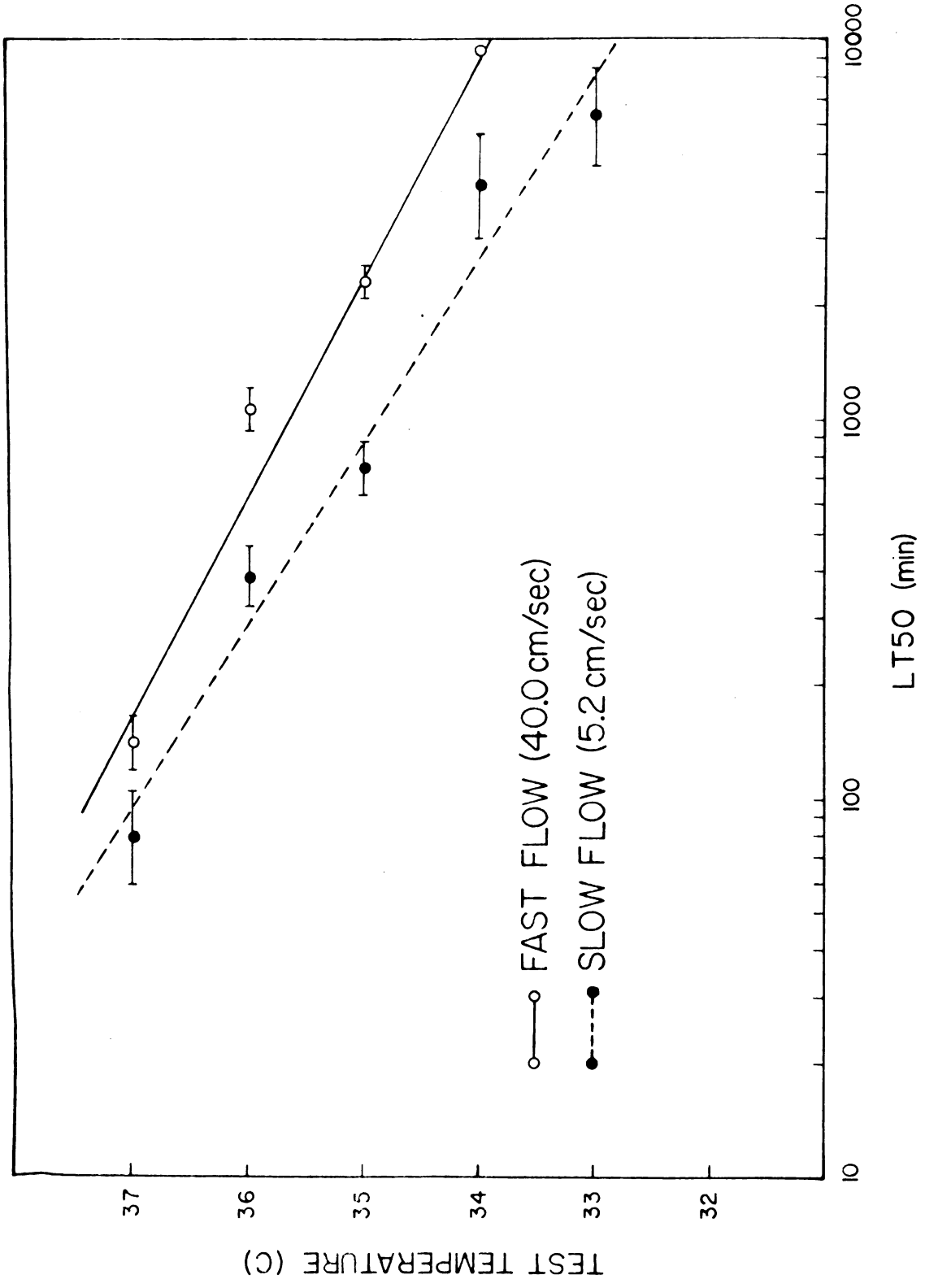

TEST TEMPERATURE (C)
37
36
35
34
33
32
10
100
1000
10000
LT50 (min)
FAST FLOW (40.0 cm/sec)
SLOW FLOW (5.2 cm/sec)

TABLE 4.--Analysis and statistical comparison of time-per cent curves from upper-lethal-temperature experiments with Paragnetina media nymphs conducted at high current velocity (40 cm/sec) and low current velocity (5.2 cm/sec). 20 C acclimation temperature.[1]

Current velocity (cm/sec)	Test temp (C)	LT50 and 95 per cent conf limits (min)	S	Ho: $b_H = b_L$*
40.0	37	140 (119-165)	1.29	rejected
5.2		79 (59-106)	1.61	
40.0	36	1090 (952-1249)	1.21	rejected
5.2		385 (318-466)	1.365	
40.0	35	2340 (2108-2597)	1.16	rejected
5.2		750 (636-885)	1.31	
40.0	34	9500 (5337-16910)	2.13	rejected
5.2		4200 (3000-5880)	1.715	
40.0	33	**		
5.2		6400 (4706-9344)	1.83	

[1]Litchfield (1949). *Level of acceptance 0.05.

**Did not get 50 per cent mortality in 10,000 minutes.

Figure 16.--Time-per cent mortality curves for P. media nymphs tested in flowing conditions at 10, 20, and 30 C acclimation temperatures. Lines are labeled with test temperatures (C). The 95 per cent confidence limits for each time to 50 per cent mortality are indicated by a horizontal line. Bracketed point indicates zero and/or 100 per cent mortality. Based on groups of animals with mean weights from 14.65 to 41.20 mg. (A, B, C) December; (D, E) January; (F, G, H) February.

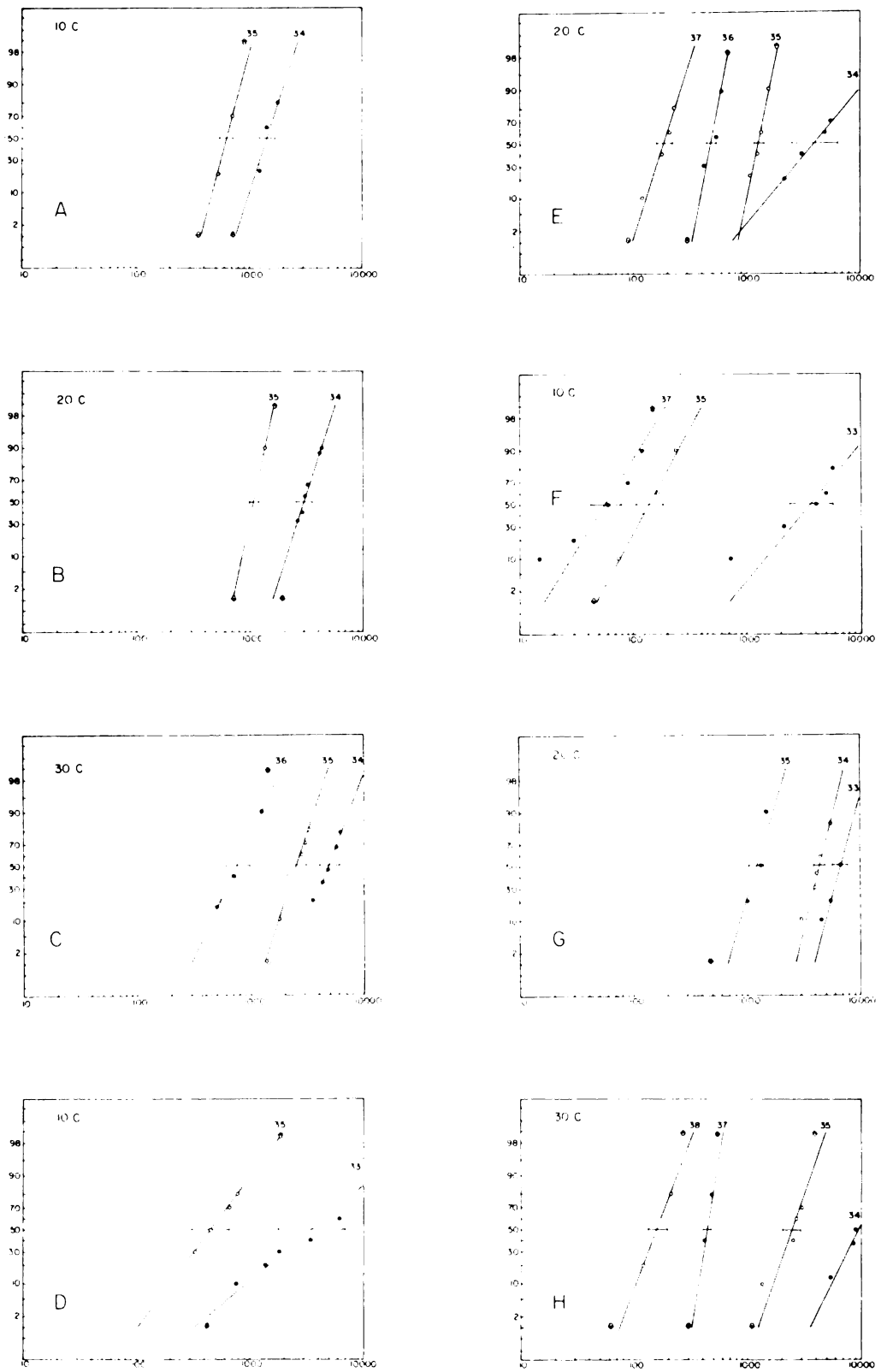

10 C
A
20 C
E
20 C
B
10 C
F
30 C
C
G
10 C
D
30 C
H
MORTALITY (%)
EXPOSURE (min)

Figure 16.--Time-per cent mortality curves for P. media nymphs tested in flowing conditions at 10, 20, and 30 C acclimation temperatures. Lines are labeled with test temperatures (C). The 95 per cent confidence limits for each time to 50 per cent mortality are indicated by a horizontal line. Bracketed point indicates zero and/or 100 per cent mortality. Based on groups of animals with mean weights from 14.65 to 41.20 mg. (A, B, C) December; (D, E) January; (F, G, H) February.

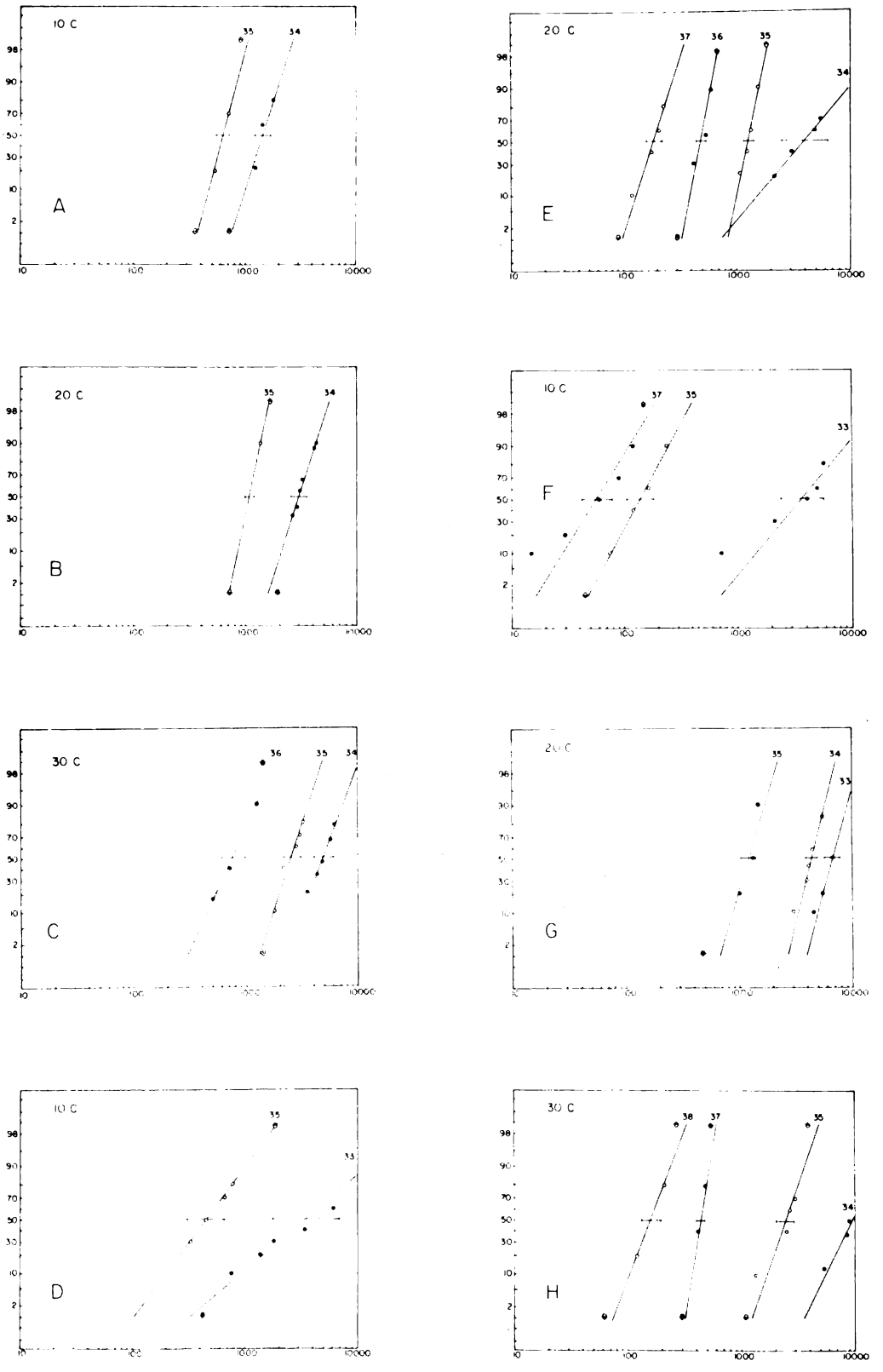

10 C
A
20 C
E
20 C
B
10 C
F
30 C
C
G
10 C
D
30 C
H
MORTALITY (%)
EXPOSURE (min)

Figure 17.--Time-per cent mortality curves for P. media nymphs tested in flowing conditions at 10, 20, and 30 C acclimation temperatures. Lines are labeled with test temperatures (C). The 95 per cent confidence limits for each time to 50 per cent mortality are indicated by a horizontal line. Bracketed point indicates zero and/or 100 per cent mortality. Based on groups of animals with mean weights from 2.68 to 63.36 mg. (A, B) March; (C, D) April; (E, F, G) May; (H) June.

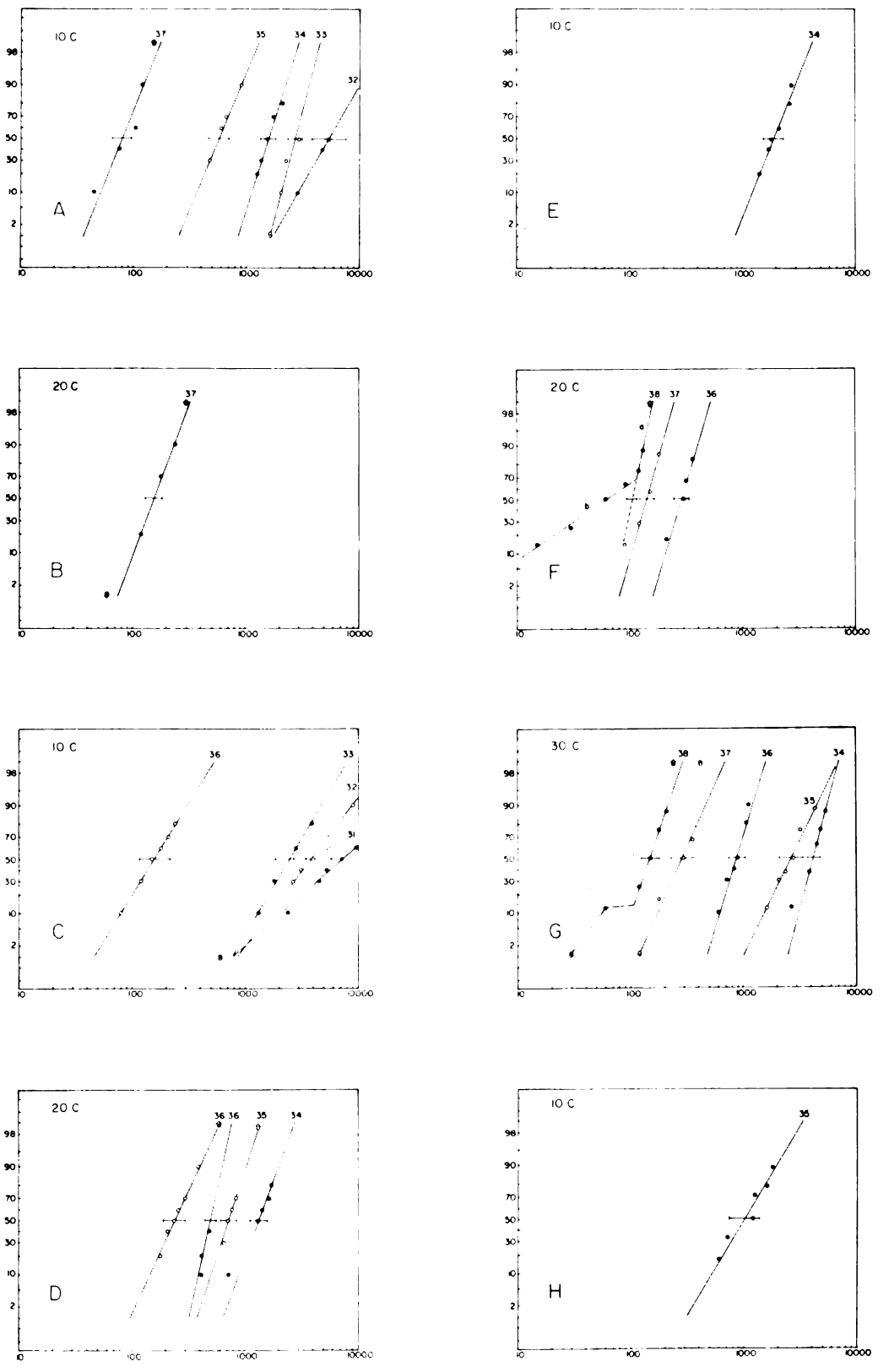

10 C
A
37
35
34
33
32
10 C
E
34
20 C
B
37
20 C
F
38
37
36
10 C
C
36
33
32
31
30 C
G
38
37
36
34
35
20 C
D
36
36
35
34
10 C
H
35
MORTALITY (%)
EXPOSURE (min)

Figure 18. Time-per cent mortality curves for P. media nymphs tested in flowing conditions at 10, 20, and 30 C acclimation temperatures. Lines are labeled with test temperatures (C). The 95 per cent confidence limits for each time to 50 per cent mortality are indicated by a horizontal line. Bracketed point indicates zero and/or 100 per cent mortality. Based on groups of animals with mean weights from 2.77 to 20.23 mg. (A, B) June; (C, D) July; (E, F) August; (G, H) October.

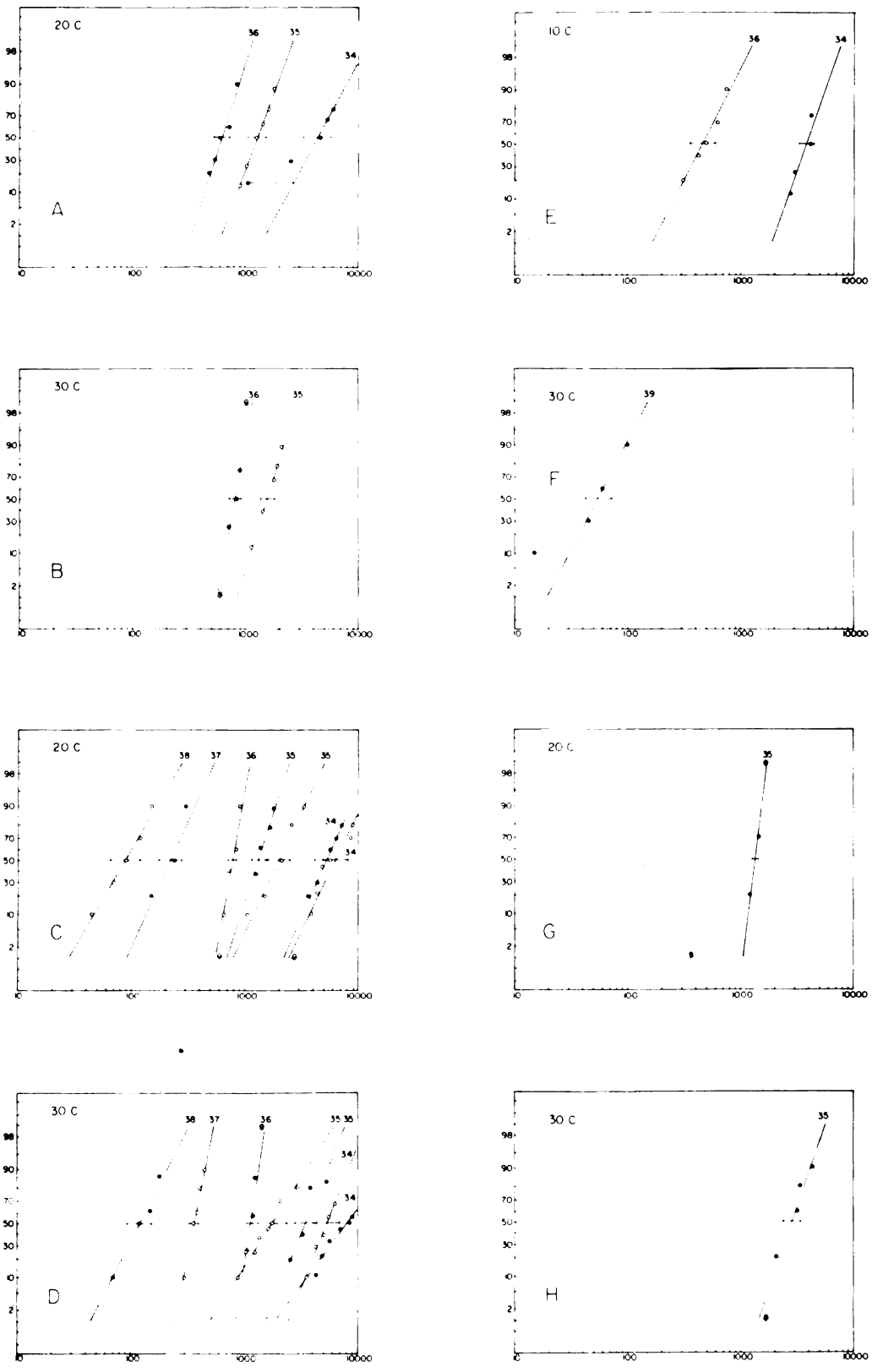
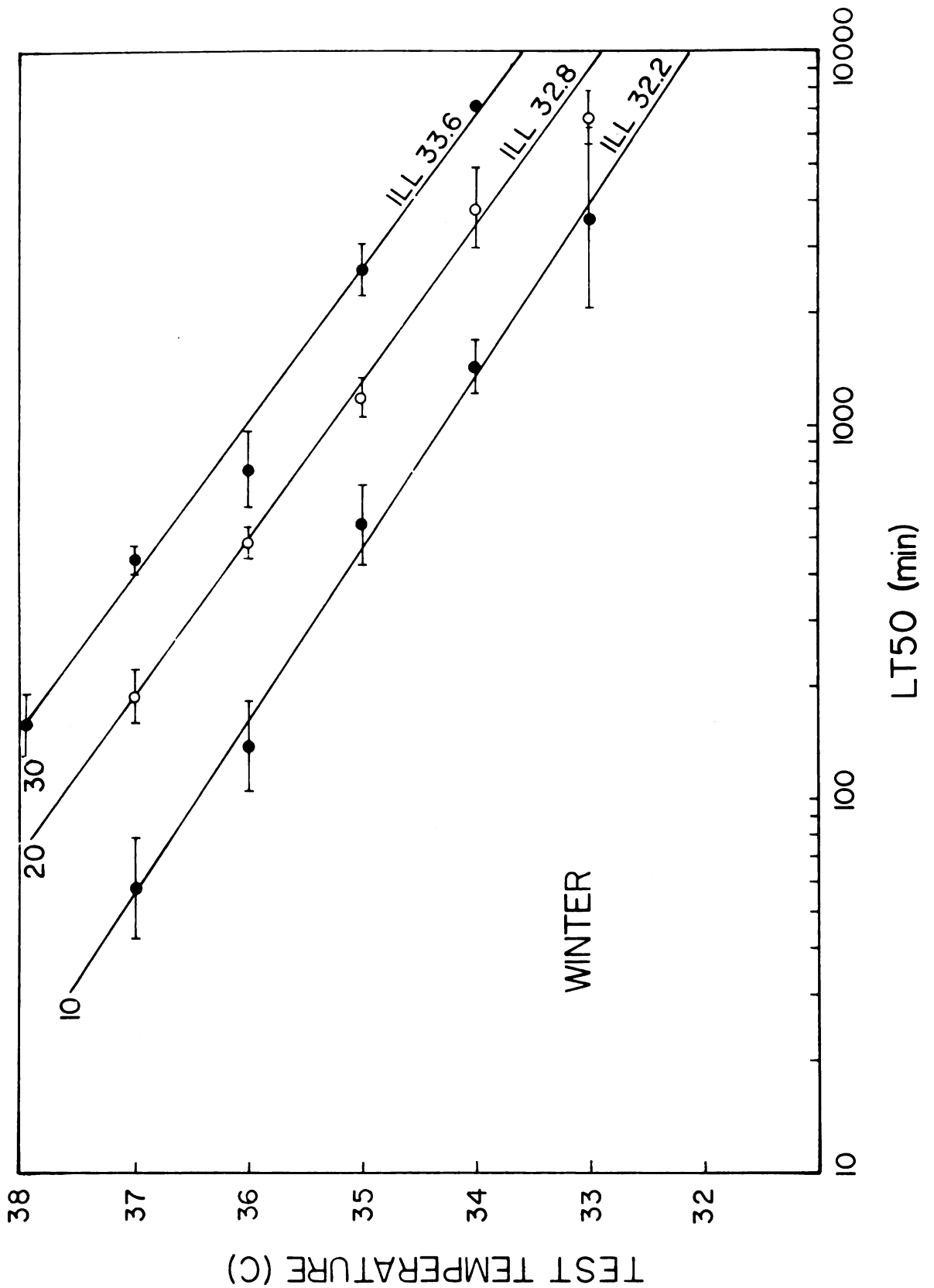

20 C
A
36
35
34
10 C
E
36
34
30 C
B
36
35
30 C
F
39
20 C
C
38
37
36
35
35
34
34
20 C
G
35
30 C
D
38
37
36
35
35
34
34
30 C
H
35
MORTALITY (%)
EXPOSURE (min)

Figure 19.--Times to 50 per cent mortality of P. media nymphs during winter for 10, 20, and 30 C acclimation temperatures. The 95 per cent confidence limits of each LT50 are indicated by a horizontal line. Based on groups of animals with mean weights from 14.65 to 41.20 mg.


ILL 33.6
ILL 32.8
ILL 32.2
30
20
10
WINTER
38
37
36
35
34
33
32
10
100
1000
10000
LT50 (min)
TEST TEMPERATURE (C)

on thermal resistance, with the actual influence of acclimation on lethal temperature being most accurately manifested during this period.

The minimum temperature found to result in 50 per cent mortality during the winter test period was 33 C following 10 C acclimation. The ILL was estimated for each acclimation temperature, and the maximum temperature at which 50 per cent of the animals could survive indefinitely was found to be 32.2 C (Fig. 19). The relation between lethal temperature and acclimation temperature (Fig. 8) indicates that this incipient lethal level would be slightly less if the animals were acclimated to the actual environmental temperature, which ranged from 1 to 4 C during this period.

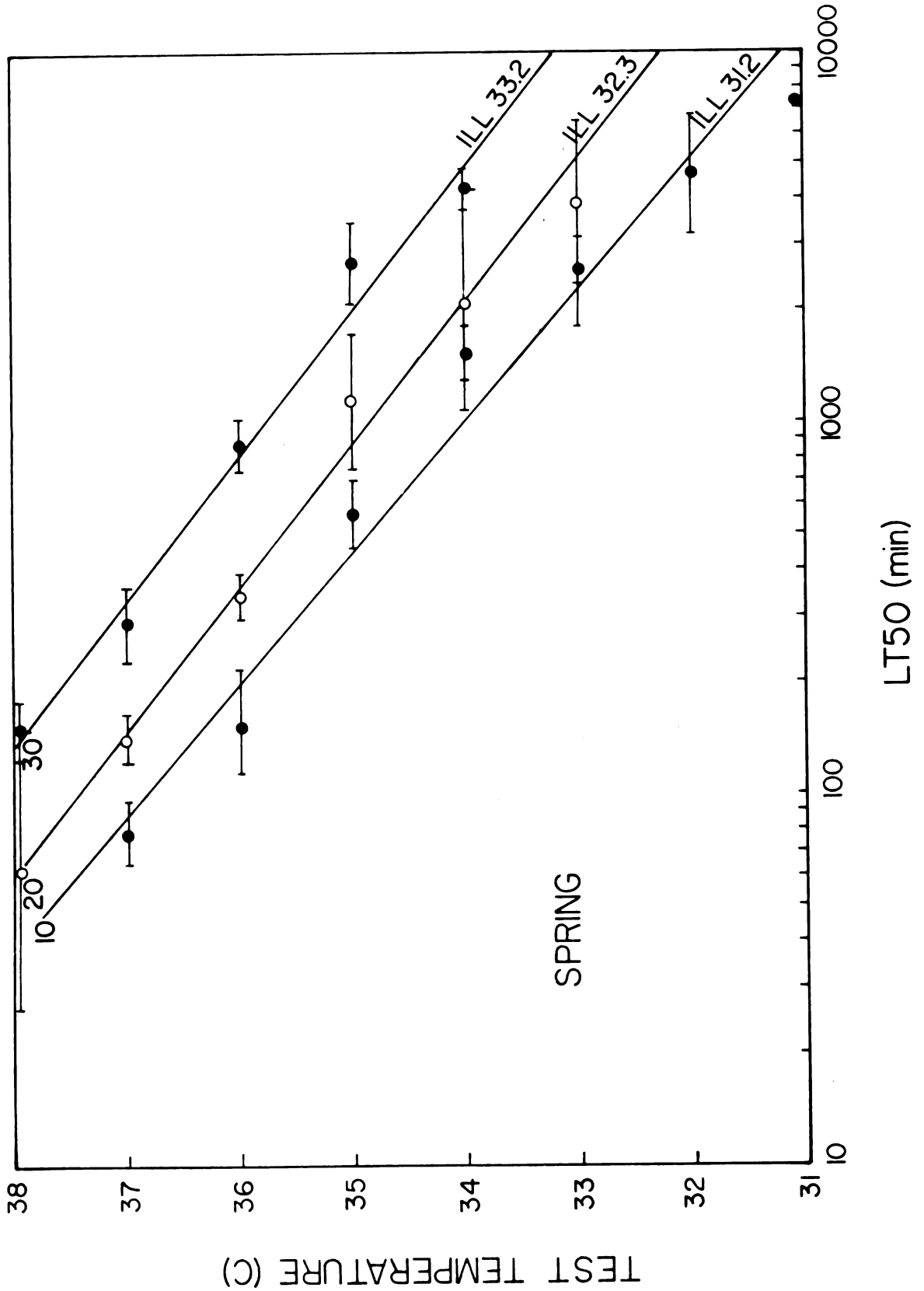
Spring

Response to abnormally high temperature was found to be more variable in the spring months (March, April, May). Figure 20 presents the results of upper-lethal-temperature experiments conducted in the spring.

Three separate and distinct physiological states could be assigned to the nymphs during the spring period:

1) Prior to Spring Molt (March 1-April 14). Ecdysis is important in the physiology of stoneflies because of the rapid growth which accompanies molting. Cast skins were observed occasionally in the field and in the acclimation baths throughout most of the year.

Figure 20.--Times to 50 per cent mortality of P. media nymphs during spring for 10, 20, and 30 C acclimation temperatures. The 95 per cent confidence limits of each LT50 are indicated by a horizontal line. Based on groups of animals with mean weights from 6.15 to 63.36 mg.


TEST TEMPERATURE (C)
38
37
36
35
34
33
32
31
LT50 (min)
10
100
1000
10000
ILL 33.2
ILL 32.3
ILL 31.2
30
20
10
SPRING

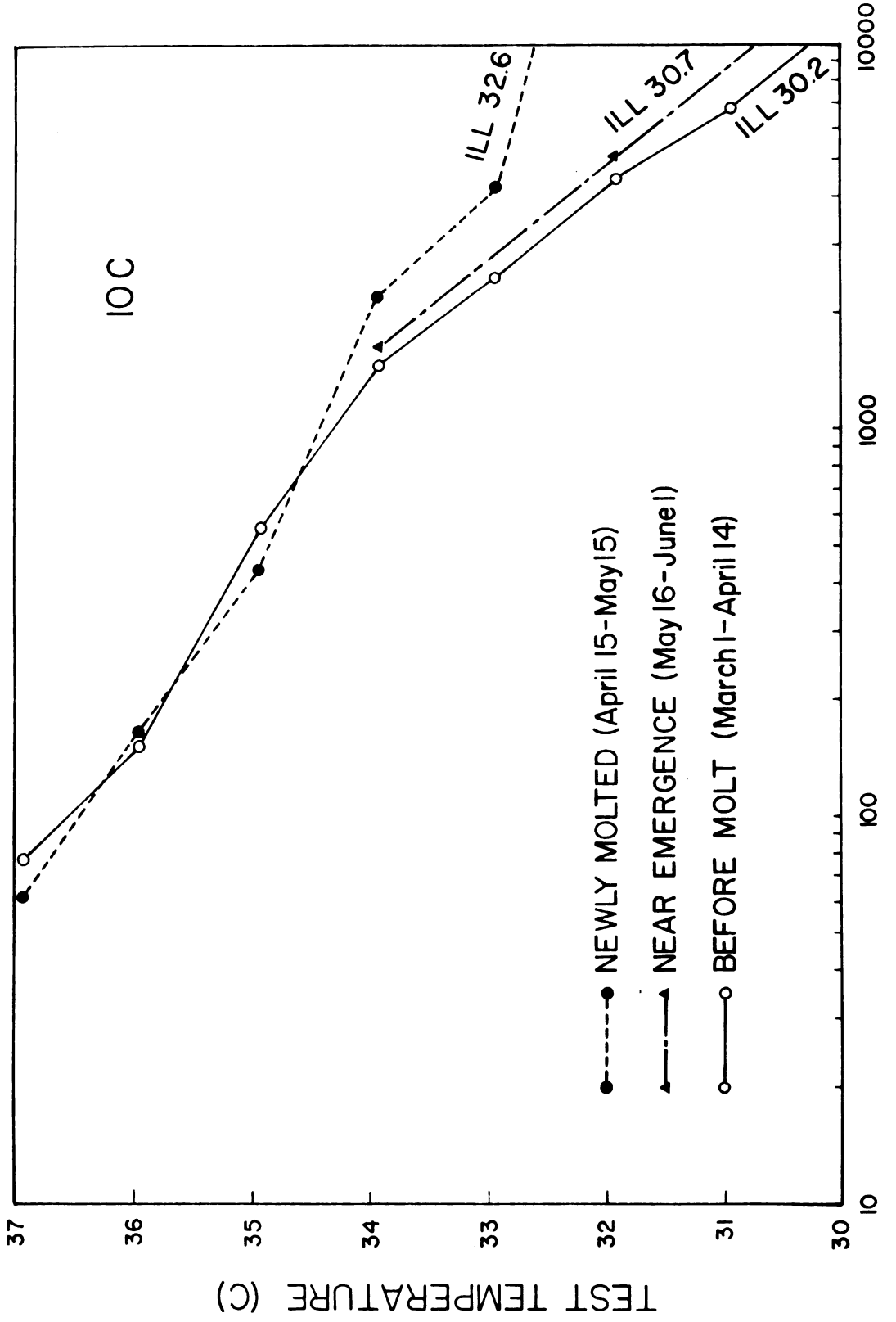
During the winter and early spring months very little growth was evident (see Life History and Ecology section, Fig. 2) and consequently an extensive intermolt period occurred at this time. As this intermolt period approached termination the nymphal exoskeleton became very hard and darkened. It was at this time that the nymphs reached maximum vulnerability to high temperatures.

On April 14, nymphs acclimated at 10 C and tested at 31 C showed an LT50 of 7,200 minutes. This was the minimum temperature found to result in 50 per cent mortality. The trend of thermal resistance for test temperatures 31-34 C in Figure 21 gives an estimated ILL of 30.2 C. In Figure 20 a straight line fitted by inspection to all the test temperatures for the spring period (31-37 C) at 10 C acclimation gives a somewhat higher estimation of the ILL because of increased resistance in tests conducted earlier in the spring. Heat resistance decreased continually until the time at which the spring molt takes place.

Attempts to acclimate the nymphs at 30 C resulted in a high percentage of mortality at this time. Results from other tests indicate that, in static conditions of acclimation, the ILL was below 30 C.

Nymphs in the pre-molt condition exhibited considerable body swelling prior to and accompanying the death from high temperature. This phenomenon gave rise to the suspicion that the osmoregulatory process was breaking down. This hypothesis is discussed in a later section.

Figure 21.--Times to 50 per cent mortality of P. media in three contrasting physiological states. Acclimation temperature at 10 C.


10C
ILL 32.6
ILL 30.7
ILL 30.2
NEWLY MOLTED (April 15-May 15)
NEAR EMERGENCE (May 16-June 1)
BEFORE MOLT (March 1-April 14)
TEST TEMPERATURE (C)
LT50 (min)
37
36
35
34
33
32
31
30
10
100
1000
10000

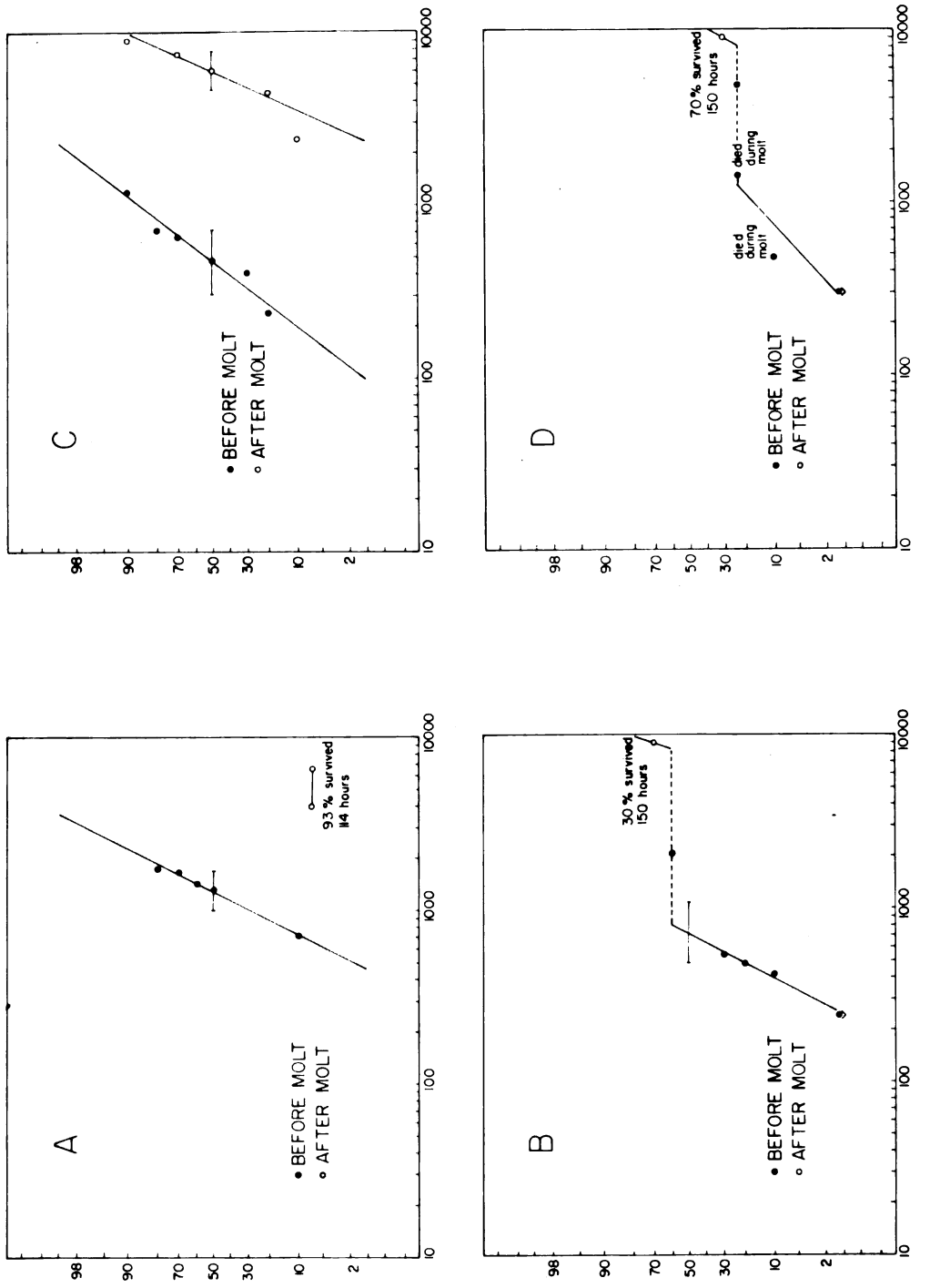
2) After 1st Spring Molt (April 15-May 15). Rising water temperature was accompanied by extensive molting throughout April. The newly molted nymphs exhibited a significant increase in tolerance to high temperature. Figure 22 compares the effects of high temperature on nymphs in two contrasting physiological states. These data indicate that the nymphs were significantly more resistant after molting. Further evidence is shown in Figure 21, where the ILL was estimated at 32.6 C, compared with 30.2 C for nymphs in the pre-molt condition.

Abnormal body swelling was not observed in newly molted nymphs dead from exposure to increased water temperature.

3) Prior to Emergence (May 16-June 1). Near the middle of May the nymphal body becomes slightly swollen and the wing pads become very dark. This period is accompanied by a decrease in thermal resistance (Fig. 21). Response to high temperature is extremely variable at this time, often resulting in a "split probit" in mortality distribution (Fig. 10). This response is indicated by a horizontal section in the regression. Such a split is generally interpreted as two mechanisms of death, both in response to high temperature in the present study.

Temperature resistance at this time appears to be governed directly by proximity to emergence, with

Figure 22.--Effect of molting on cumulative per cent mortality of P. media nymphs. (A, B, D) test temperature 34 C, acclimation temperature 20 C; (C) test temperature 33 C, acclimation temperature 10 C. Test dates 4/10-4/14. Nymphs tested either separately in two concurrent trials (A and C) or coincidently in the same trial (B and D).


A
B
C
D
• BEFORE MOLT
◦ AFTER MOLT
93 % survived 114 hours
30 % survived 150 hours
70% survived 150 hours
died during molt
MORTALITY (%)
EXPOSURE (min)

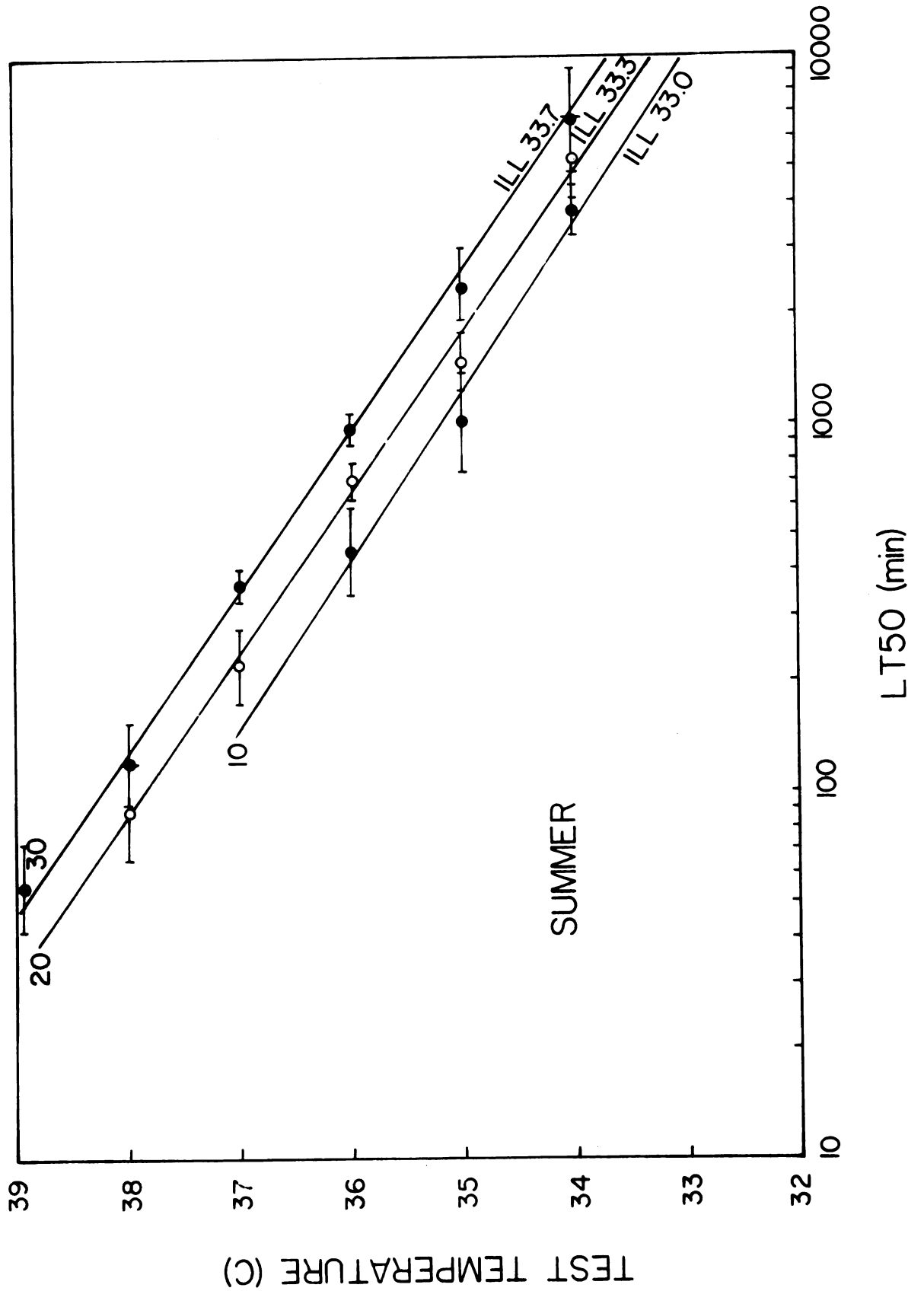
nymphs very near to emergence being less tolerant. Stonefly nymphs subjected to water temperatures up to 30 C successfully completed their life cycle whereas temperatures in excess of 30 C resulted in inability to emerge from the nymphal stage. Nymphs are apparently more tolerant during emergence than in the pre-molt condition (Fig. 21).

Summer

The months of June, July, and August are characterized by increased water temperature and rapid stonefly growth. It is during this period that the nymphs acquire their greatest level of heat resistance (Fig. 23). Few tests were conducted in the autumn months, but the results were similar to those in the summer. This might be accounted for by the very slow rate of downward acclimation of stonefly nymphs and the fact that environmental water temperatures were decreasing at this time. For this reason results for summer and autumn are combined.

Table II (Appendix) compares the ILL for each acclimation temperature during winter, spring and summer. No attempt was made to compare individual time-per cent curves, since the differences in tolerance are quite clear-cut and computation of the probability of their significance would be superfluous. A comparison of the ILL's does indicate that the trends are real and indicates a temperature tolerance that is maximum in summer, minimum in spring, and intermediate in winter.

Figure 23.--Times to 50 per cent mortality of P. media nymphs during summer for 10, 20, and 30 C acclimation temperatures. The 95 per cent confidence limits of each LT50 are indicated by a horizontal line. Based on groups of animals with mean weights from 2.68 to 20.23 mg.


TEST TEMPERATURE (C)
LT50 (min)
SUMMER
ILL 33.7
ILL 33.3
ILL 33.0
30
20
10
39
38
37
36
35
34
33
32
10
100
1000
10000

Resistance of Eggs and Newly Hatched Nymphs

On June 1, 1968, flasks containing P. media eggs fertilized and deposited in the laboratory were placed at 1, 10, 24, 27, and 30 C. This was done in an effort to determine the effect of temperature on egg hatching rate and survival. This experiment was rather unsuccessful since the 10 and 27 C eggs were destroyed by Saprolegnia sp. and the 1 C eggs by dessiccation. On July 1, 1968 a large number of nymphs were hatched at 24 C. Eggs at 30 C hatched on July 2 but nymphs died in the next several days.

Laboratory reared P. media nymphs were tested within 1 to 3 weeks of hatching to assess the effects of high temperature on the survival of young stoneflies.

Static lethal temperature experiments were conducted in 100-ml glass beakers. The animals were extremely difficult to transfer from the rearing bath to the test bath because of their very small size. Often the nymphs were unable to grasp the small stones provided in the test bath, and therefore floated helplessly to the surface where they were caught, unable to break the surface film and return to the substrate. Despite considerable difficulty in the testing procedure, the data obtained were sufficient to give some insight on the thermal resistance of very young stonefly nymphs.

Time-per cent curves were plotted, and LT50's estimated, for test temperatures 30 to 34 C. Because of the uncertainty and variability of the results, interpretations must be considered tentative, and no detailed graphic analysis was attempted. The results of the tests (Fig. 24) indicate that nymphs are more susceptible to high temperature when newly hatched than in the later stages of their life cycle. Figure 24 gives an estimated ILL of approximately 29 C. If the trend is real, some degree of tolerance is apparently lost immediately after hatching for eggs hatched at the 30 C rearing temperature.

Respiration

Figure 25 and Table 5 present the effects of slowly rising water temperature on the respiratory rate of P. media nymphs. The data represent the mean rates of 9 animals from the size class 6.16-11.86 mg dry body weight. Rates were ascertained over a 20-hour period of increasing water temperature: one-hour at each temperature with one-hour intervals for adaptation to the next-highest level.

P. media nymphs appear to remain metabolically active up to the lethal death point. A rather sharp increase in QO_2 is observed at 33 C, which might possibly indicate an "overshoot" in oxygen consumption resulting from stress from the approaching incipient lethal level.

Figure 24.--Times to 50 per cent mortality of newly hatched P. media nymphs acclimated to room temperature (approximately 24 C).

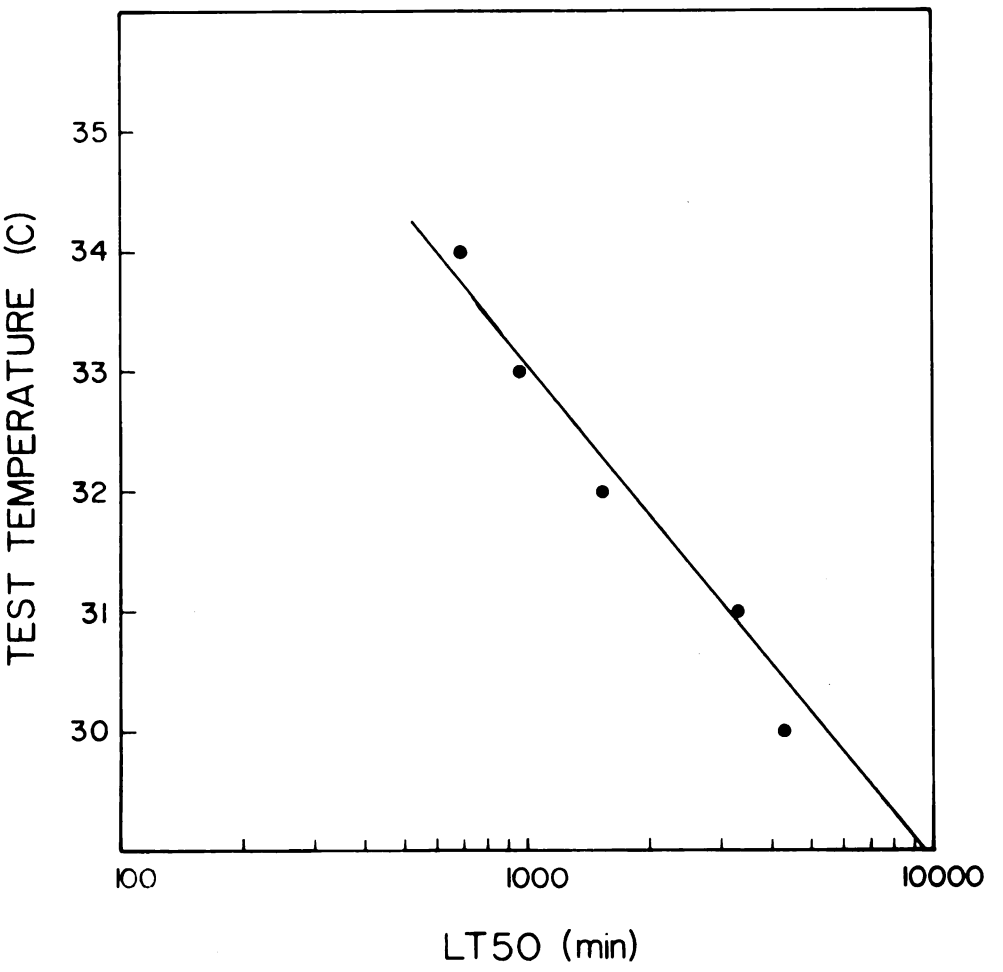

TEST TEMPERATURE (C)
35
34
33
32
31
30
100
1000
10000
LT50 (min)

Figure 25.--Mean respiratory rates of nine Paragnetina media nymphs tested in conditions of slowly rising water temperatures. Vertical lines indicate ± one standard error. Weight range 6.16-11.86 mg.

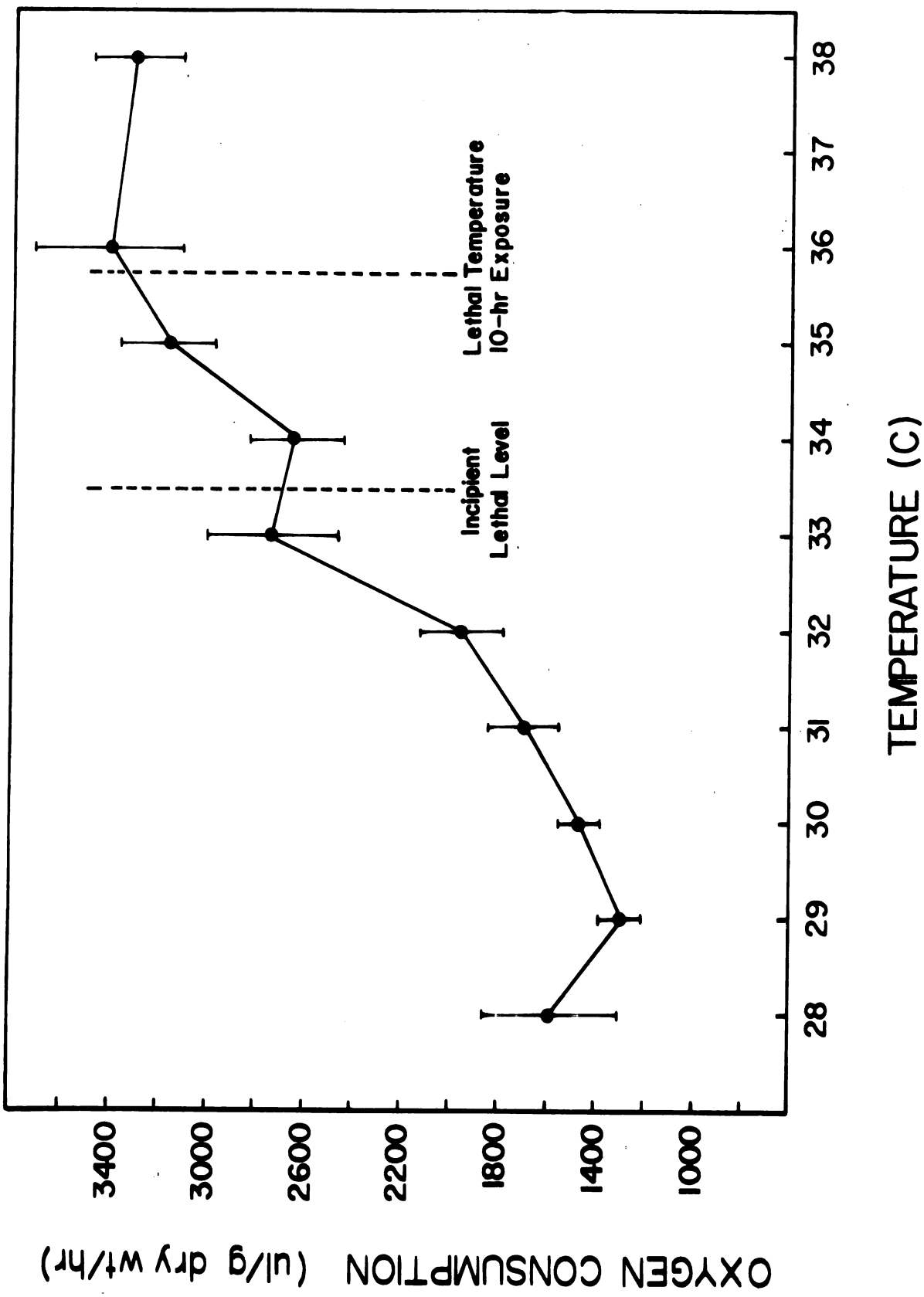

OXYGEN CONSUMPTION (ul/g dry wt/hr)
3400
3000
2600
2200
1800
1400
1000
28
29
30
31
32
33
34
35
36
37
38
TEMPERATURE (C)
Incipient Lethal Level
Lethal Temperature 10-hr Exposure

TABLE 5.--Details of experiments showing the effect of temperature on oxygen consumption (µl/g dry wt/hr) of Paragnetina media nymphs.

Temperature (C)	n	Oxygen consumption		
		$\bar{x}$	Range	$S_{\bar{x}}$
28	9	1588.0	3476.0-726.6	308.0
29	9	1293.7	1969.8-1033.3	102.1
30	9	1465.7	2061.4-1183.2	98.6
31	9	1690.1	2519.5-1183.2	150.1
32	9	1963.4	3093.0-1508.6	178.0
33	9	2725.6	4558.1-2061.5	280.4
34	8	2625.1	3951.2-2054.6	213.1
35	8	3168.3	4473.1-2606.5	209.7
36	8	3403.6	4971.6-2120.7	320.0
38	8	3300.0	4328.9-2725.0	180.1

It is also apparent that at the thermal death point the respiratory rate had not decreased, which demonstrates that "heat death" does not imply that all the constituent tissues are necessarily dead.

DISCUSSION AND CONCLUSIONS

Relation Between Lethal and Acclimation Temperatures

The limits of an animal's tolerance zone are not "fixed" but depend to some extent upon the condition of the animal (Andrewartha and Birch, 1954). Temperature effects are greatly modified by the past thermal history of an individual; hence, acclimation state should be qualified.

Acclimation, in regard to temperature adaptation, refers to the animal's compensation for a persistent change in environmental temperature, usually in the laboratory (Prosser, 1962).

The mechanisms involved in acclimation have been reviewed and discussed by several workers (Bullock, 1955; Fry, 1957; Prosser, 1962). Generally, as acclimation temperature increases, lethal temperatures progressively increase. Examples of this phenomenon are numerous, and only a few selected examples are presented here.

Fry (1942) reported that in the goldfish, Carassius auratus L., a 3 C increase in acclimation temperature was accompanied by a rise in lethal temperature of about 1 C.

A considerably lesser rate of change was obtained for yearling speckled trout, Salvelinus fontinalis Mitchill, which required a 7 C change in acclimation temperature to produce a 1 C change in lethal temperature (Fry et al., 1956). When acclimation temperature was raised from 10 to 20 C the 24-hour lethal temperature was increased by 1.9 C in Gammarus fasciatus Say, 1.3 C in Asellus intermedius Forbes, 0.5 C in Gammarus pseudolimnaeus Bousfield, and apparently not at all in Hyalella azteca Saussure (Sprague, 1963). Mcleese (1956) showed that the lobster, Homarus americanus Milne-Edwards, tested in water containing dissolved oxygen of 6.4 mg/l, raised its lethal temperature 3 C when the acclimation temperature was increased from 10 to 20 C. The lethal temperature determined for the crayfish, Orconnectes rusticus (Girard), was increased approximately 1 C for each 10 C increase in acclimation temperature (Spoor, 1955). Lethal temperature in the giant scallop, Placopecten magellanicus (Gmelin), increased 1 C with a 5 C rise in acclimation temperature (Dickie, 1958).

An exception to the general rule is presented by Matutani (1961). He found that the copepod, Tigriopus japonicus Mori, had a greater heat resistance when acclimated at 5 C than at 10 C. This inconsistency was ascribed to the inactivation of aerobic metabolism by cold, the effects of which remain long after the temperature is raised.

It has been shown, primarily in fish (Fry et al., 1942; Brett, 1944), that there is an upper limit to the temperatures at which acclimation takes place, and there is a corresponding limit on the temperature tolerance which can be induced by acclimation. This phenomenon was not demonstrated in P. media since acclimation was not attempted beyond 30 C. Information on other species, however, indicates that, for P. media, the straight line extending to the point where acclimation temperature equals lethal temperature (Fig. 8) would actually "plateau" if acclimation temperatures were above 30 C.

Because acclimation temperature was increased more readily than decreased, the period of December, January, and February is most representative of the effect of acclimation in P. media. An increase of 0.75 C in lethal temperature for each 10 C rise in acclimation temperature is somewhat moderate when compared with values for other species. Typically, over most of their biokinetic ranges fish will show an increase of about 1 C in upper lethal temperature for each 3 C change in acclimation temperature (Gilson, 1955).

Rates of Gain and Loss of Heat Resistance

In evaluating rate of acclimation to changing temperature, the thermal history of a species under consideration must be established, then the resistance to either a high or a low temperature can be followed by moving either up

or down the temperature scale. Difficulty in comparing rates of acclimation among different species results from the fact that no common factor has been established, either in the method of approach or in the range and level of temperature used. The method employed in this investigation, used originally by Loeb and Wasteneys (1912), is essentially the tracing of average mortality at a given high temperature until mortality ceases.

It has been shown that organisms tend to acclimate upward to high temperatures faster than they acclimate downward to lowered temperatures. This is important from an ecological point of view because it means that animals will not be acclimated as quickly to decreasing temperatures in autumn as to increasing temperatures in spring. It also means that acclimation to daily changes in temperature will proceed at different rates, adjusting more rapidly to temperature increases. In consequence, the animals will be acclimated to a higher level than the mean daily temperature.

A. intermedius increased its acclimation by 4 C per day whereas H. azteca required 2 days to acquire a new level of heat resistance after being moved from a 14 C acclimation bath to a 20 C bath (Sprague, 1963). The acclimation of male G. fasciatus was increased by 5 C in one day, whereas females took twice as long (2.5 C per day) in adjusting from 15 to 20 C (Sprague, 1963).

Downward acclimation was not determined precisely in these animals. Dickie (1958) indicated that the giant scallop acclimates upward by 1.7 C per day but may take up to 3 months to lose this acclimation. Spoor (1955) found that the heat resistance of O. rusticus increased quite rapidly, one day or less when temperature was raised from 4 to 24 C, but that the heat tolerance was lost much more slowly. The American lobster requires at least 24 days to acclimate from 14.5 to 23 C (McLeese, 1956). Considerable study has been devoted to the acclimation rates of fish (Fry, 1942; Sumner and Doudoroff, 1938). In general, upward acclimation is rapid in the region of 20 C and above, with a change of 8 to 10 C usually taking one to three days, depending on the species (Brett, 1946). Loss of this increased tolerance appears to be slow in fish, with a change of 10 C requiring 20 days or more (Doudoroff, 1942; Brett, 1944).

The acclimation to temperature increase in P. media of 5 C per day is similar to rates indicated for other aquatic invertebrates. Acclimation appears to be slower in macroinvertebrates than for the species of fish tested and the effect on lethal temperature more moderate.

Heinicke and Houston (1965) summarize as follows:

> It would seem likely that a basic feature of the acclimatory process is the re-establishment of dynamic equilibria among processes whose rates are differentially influenced by temperature variations. Thus, a measure of acclimation rate may be based upon the time required for the organism to reach a new stationary state following a change in state-influencing environmental conditions.

The ability to become acclimated to changing temperatures is important for aquatic species living in lakes and rivers that undergo extremes in temperature. It has been shown, though infrequently, that fish in such situations can be killed from the direct influence of high temperature (Huntsman, 1946). Without adaptation, the death rate would doubtless be higher and more frequent, greatly reducing the populations.

Effects of Size and Sex

Much information has been compiled relating body size to order of death at high temperatures, and the reported responses show considerable diversity.

In testing G. fasciatus and A. intermedius, Sprague (1963) found as a general tendency that the larger animals had shorter resistance times. With H. aztica that was acclimated at 20 C and tested at 35, 36, and 37 C, no difference in resistance was found between small individuals (0.6 to 1.1 mg) and medium-sized organisms (4 mg). Sprague recalculated the data of Bovee (1949) for H. azteca and found the results similar to his own findings. In two species of mayflies tested by Whitney (1939) the smaller individuals were more resistant to high temperature.

Among the decapod crustacea, Spoor (1955) found no difference in temperature resistance between crayfish 17 and 82 mm long. Roberts (1957) concluded that the

variable Q_{10} rate in the crab, Pachygrapsus crassipes Randall, at the higher temperature range of 16 to 23.5 C was the result of increased sensitivity of larger animals to thermal stress at temperatures approximating the environmental maximum. McLeese (1956) failed to find a relation between size and lethal temperature in the American lobster, H. americanus.

Moving up the phylogenetic scale, Huntsman and Sparks (1924) noted that in the winter flounder, Pseudopleuronectes americans (Walbaum), susceptibility to high temperature increased with size. Hart (1952) found a size effect in only three of fourteen species of freshwater fish studied. Temperature resistance decreased inversely with size in two, and directly with size in the third. A lack of correlation between the size of individual specimens and order of death at high temperatures has been found by many investigators: Sumner and Doudoroff (1938) with the marine goby, Gillechthys mirabilis; Doudoroff (1942), with the greenfish (Girella nigricans (Ayres); Fry et al. (1946), with the speckled trout, S. fontinalis; Timit (1963), with several species of the Adriatic littoral; and Alabaster (1967), with the salmon, Salmo salar L., and the sea trout, S. trutta L.

Tests in summer and autumn indicate no significant effect of size on the heat resistance of P. media nymphs.

Less information has been compiled on the effects of sex differences on temperature resistance.

Sprague (1963) found no difference in four species of crustaceans in resistance between nonbreeding females and those carrying eggs or having large oostegites. Bovee (1949), Spoor (1955) and Bowler (1963) found that sex did not effect the heat tolerance of the amphipod, H. azteca, the crayfish, O. rusticus, or the crayfish, Astacus pallipes.

Gibson (1954) studied the variable response between sexes in the guppy, Lebistes reticulus. At 37 C the males succumbed first in both unselected and inbred stocks, but at lower temperatures this particular response is lost. Hoar (1955) found that male goldfish were the more resistant than females in both winter and summer. Since the sex differences appeared in both winter and summer, resistance was apparently independent of the degree of sexual maturity.

Male and female P. media nymphs differed in response to high temperature at the lower test levels. This response indicates seasonal variation and is closely aligned with the physiological state of the animal. Females approaching sexual maturity were more susceptible both prior to and immediately following ecdysis. Mature female nymphs exhibited body dilation more frequently than males.

Lethal Temperature as Affected by Test Media and Rate of Flow

The use of the bioassay has been stimulated by the complexity of interactions in the natural environment and the need to isolate and determine the response of organisms to a variety of environmental parameters. The methods most commonly employed are continuous flow and the static bioassay.

In comparing results from both methods, in this study it is apparent that, to avoid possible effects on the general level of toxicity, the method that more closely approximates the natural habitat of the test organism should be employed--in this instance, continuous flow.

Considerable work has been done in the past relating the thermal resistance of animals to the types of habitat in which they are found (Huntsman and Sparks, 1924-25; Hathaway, 1927; Whitney, 1939; Walshe, 1947). The general principle which has emerged is that the thermal resistance of a species of animal is related to past environmental conditions. Hence animals from shallow, warm water ponds have been shown to possess greater heat resistance than similar species from cold stream environments. The standard test apparatus for determining heat resistance has included a static constant temperature bath, regardless of the habitat of the test organism.

Both the immediate and long term responses of P. media nymphs to high temperature differed between static and flowing water conditions. Most notably, thermal shock was intensified in the static-water tests. Thermal shock results from an abrupt change in thermal environment (Mihursky and Kennedy, 1967).

Tyler (1966) reported thermal shock in temperature evaluations with the minnow, Chrosomus eos Cope, and consequently divided the mortality distribution into first and second "lethal effects." He attributed initial mortality (first effect) to thermal shock. He found that the first effect did not always occur in winter test and was not evident at all in summer tests. P. media showed an inconsistency in the first effect.

During several static bioassay experiments a vigorous "pumping" behavior was exhibited by many stoneflies in the early phases of the test. Knight and Gaufin (1963) described in detail these rhythmic body undulations in the stonefly nymph, Acroneuria pacifica Banks, when the concentration of dissolved oxygen was reduced. They interpreted this behavior as an attempt to destroy an oxygen gradient developing around the nymphal body and gills. P. media nymphs also manifested vigorous pumping behavior, however, in a dissolved oxygen concentration of near 100 per cent saturation and extremely high temperatures. This behavior also can thus be interpreted as a response to general conditions of stress.

P. media increased its thermal resistance at a higher rate of current velocity. The results of tests conducted in static and slow water current are strikingly similar and lend support to the speculation that the differences encountered in the static and flowing water tests were an effect of water current, not of the different test apparatus. The fact that resistance was greater in the static tests than with low current velocity might be explained by the compressed air turbulence in the static bath, resulting in a greater water movement than that in the very slow flowing water test.

Whitney (1939) tested the thermal resistance of six species of mayfly nymphs and found that nymphs from slow or still waters had a greater resistance to high temperatures than comparable nymphs from swift waters. It is interesting to speculate whether results would have been altered if the tests had been conducted with flowing water. Walshe (1948) obtained similar results with seven species of chironomid larvae from static and flowing waters. The same question of testing method and water current would apply in her study.

Lethal High Temperatures

Seasonal Variations

There are numerous reports of seasonal changes in lethal levels or physiological rates in aquatic poikilotherms (Bullock, 1955; Fry, 1958; Hoar, 1955).

In work with animals possessing one- or two-year life cycles, such as P. media, seasonal variability and changes in life-history stage must be evaluated.

It has been shown that P. media nymphs, independent of acclimation, attain a slightly higher heat resistance in summer than in winter.

Brett (1944) found a substantial increase in the heat resistance of the bullhead, Ameiurus nebulosus (LeSueur), in summer. These experiments were, however, more nearly a determination of the effect of acclimation temperature on lethal temperature, for the fish were tested directly from their environmental temperature, with no attempt to alter their thermal history through acclimation. Hoar (1955) compared winter- and summer-tested goldfish that had been maintained at a constant temperature and fed the same diet. He found that summer fish were more resistant to heat stress and suggested that photoperiodically controlled changes in endocrine physiology were responsible for changes in resistance. Sprague (1963) tested four freshwater crustaceans and found that when the effect of weight was considered, laboratory acclimation eliminated seasonal variation in resistance. Dickie (1958) reported a reversal phenomenon in scallops. Scallops taken from cold water in winter and spring tolerated higher temperatures than scallops fished during the warmer summer and autumn months. He

gave no explanation for this reversal, but work by Matutani (1961), discussed previously, might help explain the seemingly contradictory results.

Comparison of spring, summer, and winter evaluations is difficult because of the complex effects of physiological state. Spring nymphs of P. media were less tolerant than summer and winter nymphs, and this level of tolerance was affected further by sex, condition of molting, and proximity to emergence.

References relating molting condition to temperature resistance are rare. Spoor (1955) concluded that stage of molt cycle had no effect on the heat tolerance of crayfish, O. rusticus, despite the physiological changes associated with molting and the stress thought to be placed on the animal during this process. McLeese (1963) found that molting lobsters were less resistant than hard-shelled lobsters to high temperature and low levels of salinity and oxygen.

Temperature susceptibility of P. media in the premolt condition was characterized by noticeable body dilation, particularly in the abdominal region, proceeding death of the organism.

It would be appropriate at this time to discuss present knowledge--or lack--on the mechanisms involved in thermal death. Bowler (1963) summarizes as follows:

> A variety of suggestions have been made as to the cause of heat death. The classical theory that protein coagulation was the main agency responsible seems unlikely at the lethal temperatures 31 to 37 C, although at much higher temperatures it may be the responsible factor. Mayer (1941) points out that most marine animals die if exposed to temperatures of 46 C though the most readily coagulated protein does not congeal below 56 C. Sizer (1943) believed that the deactivation of enzymes was an important factor, however, Rahn and Schroeder (1941) have shown from work on bacteria that when 99.9% of the bacteria are dead the enzymes catalase and succinic dehydrogenase are still 50% active. An old theory supported by Mayer was that the oxygen supply becomes inadequate at high temperatures. Fraenkel and Herford (1940) have shown that this is not the case in insects. Cellular lipoids change their state at elevated temperatures; however, Fraenkel and Hopf (1940) report that two species of blowfly with the same kind of fats have different thermal death points and conclude that these physical properties cannot be the cause of heat death. It can be stated that the agencies responsible for heat death in a species are probably complex and not the same at different lethal temperatures.

In the same paper, Bowler later showed that the death of the crayfish, A. pallipes, at lethal temperatures was not caused by lock of oxygen. Doudoroff (1945) and Brett (1952) suggested the possibility of osmoregulatory malfunction in cold death. Heinicke and Houston (1964) further advanced this theory in a series of experiments describing some of the osmoregulatory correlates of heat death in the goldfish. A decrease in plasma chloride concentration and cellular phase volume, and an increase in tissue water content and extracellular phase volume, they concluded, indicate that heat shock is associated with an impairment of the osmoregulatory capabilities.

Wikgren (1953; from Heinicke and Houston) showed that the water permeability of the lamprey, Petromyzon fluviatilis, varies directly with temperature. An increase in surface permeability after exposure to heat would, of course, lead to increased endosmosis and a consequent increase in tissue water content.

These findings lend considerable weight to the supposition that heat death in P. media is caused, at least in part, by malfunction of the osmoregulatory process and an increase in tissue water content, which, in impermeable pre-molt nymphs, results in considerable body dilation.

Knight and Gaufin (1964) noted this apparent inability to regulate body-fluid volume when the plecoptera nymph Pteronarcys californica, was subjected to decreasing concentrations of dissolved oxygen. Nymphs relieved of excess body fluid by means of a hypodermic syringe assumed the normal form and were quite healthy when placed in oxygen-saturated water. Stoneflies not thus relieved usually died within a short time in oxygen-saturated water.

It is recognized that thermal deaths in a species may not always arise from a single cause.

In experiments with Pacific salmon, Brett (1952) indicated three causes of death from low temperature: (1) a very rapid agent, usually effective within 60

minutes of exposure, and probably a disturbance of the central nervous system; (2) related to osmotic balance; and (3) of unknown origin, delayed in time of onset.

In P. media, rapid death typified by "heat rigor" is probably associated with disruption of the central nervous system, for body-coordinating mechanisms are affected in this type of death.

The decrease in temperature resistance prior to emergence is undoubtedly a result, in part, of additional physiological stress placed on the organism at this time. Osmoregulatory disturbances are apparently placing an additional strain on stoneflies.

Harker (1950) found that the incipient lethal temperature of final instar mayfly nymphs is lower in those taken from a stream than in those taken from a pond. She did not compare early and final instars, however, for differences in heat resistance. She further indicated that, in reference to breeding conditions, death-point temperatures are not directly applicable to field conditions, because thermal deaths in nature are more likely to take place under conditions of changing temperature. The importance of this aspect was demonstrated in P. media nymphs when animals close to emergence and acclimated to 20 C were moved to a 30 C acclimation bath. A high mortality and large numbers of partially deformed adults resulted, whereas nymphs

retained at 30 C throughout the acclimation period emerged with little difficulty.

Age and Development

Numerous investigators have shown that temperature effects depend on the life-history stage of the organism. In most species of fish, the survival range is narrower for eggs, especially during the hatching process, than in any other life-history stage (Mihursky and Kennedy, 1967). After egg deposition, developmental rates are also temperature-dependent. Many fish are unable to complete their life cycles unless the temperatures at spawning and hatching are as much as 15 C below median tolerance limits (Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission, 1956).

Brett (1952) demonstrated three zones of temperature tolerance for young sockeye salmon. The inhibiting level for spawning ranged from 12 to 14 C, while the limits for activity and growth, which he termed the "loading" level, ranged from 16 to 18 C. In comparison, the lethal level for 50 per cent of the individuals was approximately 23 C.

The direct lethal effects of high temperature to P. media are probably most critical during egg hatching and early development. In addition, it appears that some level of temperature tolerance is temporarily lost soon after hatching.

Barr (1951) studied the thermal death times of the aquatic stages of Anopheline mosquitos and found the eggs to be more resistant than either the larval or pupal stage. MacFie (1920) (from Barr, 1951) found the same to be true of Ades aegypti.

Temperature has been demonstrated, in part, to have a profound effect on the rates of embryonic, postembryonic and nymphal growth of P. media.

Bovee (1951) found that the postembryonic developmental rate of the aquatic sowbug, H. azteca, at 26 to 28 C was nearly double that at 20 to 22 C.

As previously stated, one of the original objectives in this study was to determine lethal temperatures of stoneflies taken throughout the year to discover any variations in temperature tolerance and to elucidate high-temperature effects on natural survival.

The only upper-lethal-temperature which has any real significance on natural survival under normal conditions is the minimum seasonal upper lethal. Because of rapid acclimation to increased temperature and the remarkably slow loss of high-temperature acclimation, the animals tend to become acclimated to the highest temperature they have recently encountered in the environment. Therefore, the concept of 10 C acclimation of stoneflies in the summer condition is ecologically meaningless.

By comparing the mean water temperatures recorded over a 12-month period from Dumont Creek (Fig. 7) with the incipient lethal-temperature values for each season at 10, 20, and 30 C acclimation temperatures, one can obtain a prediction of the lethal effects of temperature in the field. In terms of natural survival, it seems apparent that the direct effect of upper-lethal-temperature is not a limiting factor for P. media. Most investigators working in the aquatic environment have reported similar results.

These experiments, like others before, indicate that death is of questionable ecological value as an end point criterion. That is not to say, however, that high temperature does not have limiting effects on geographical distribution and successful existence in a lake or stream.

Certainly, any change in temperature in the aquatic habitat will affect the animals living in it, even though the change is within the tolerable range. If temperatures are held, by environmental alteration, at 5 C above the level to which existing organisms have become adapted, the reproductive cycles, growth rates, and other phases of the life history might be affected so as to alter the entire population. Water temperature has extremely critical effects on the final stages of nymphal development, emergence patterns and survival after hatching. If heat-producing industries affect waters to the point

of allowing little seasonal variation in temperature, organisms which require the eggs to be chilled, for example, might not be able to maintain themselves, or emergence peaks might occur too early in the spring, exposing adults to lethal cold air.

Temperature resistance was found in this study to be affected by a number of variables: season, size and sex, previous thermal history, life stage, testing method, and rate of water flow. Examples of factors modifying thermal resistance in other studies include level of dissolved oxygen (Alabaster, 1962), photoperiod (Hoar and Robertson, 1958), diet (Hoar and Cottle, 1952), and water hardness (Craigie, 1963). In view of the number of variables to be taken into account, the biological implications of a temperature change are extensive. Obviously, there is need for additional research.

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LITERATURE CITED

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APPENDIX

TABLE I.--Forty-eight-hour upper lethal temperatures of Paragnetina media nymphs recorded in winter, spring and summer in relation to acclimation temperature.

Accl Temp (C)	48-hour Upper Lethal Temperature		
	Winter	Spring	Summer
10	33.4	32.8	34.2
20	34.1	33.6	34.6
30	34.9	34.6	35.0
Increase in lethal temperature per 10° C increase in Accl Temp.	0.75	0.90	0.40

TABLE II.--Comparison of incipient lethal levels for upper lethal temperature experiments conducted on Paragnetina media nymphs in winter, spring and summer.

Accl Temp (C)	ILL (C)		
	Winter	Spring	Summer
10	32.2	31.2	33.0
20	32.8	32.3	33.3
30	33.6	33.2	33.7

TABLE III.--Characteristics and response of Paragnetina media nymphs used to show rate of gain of heat resistance.

Test Conditions				Test Organisms				Response			
Date	Accl. Range (°C)	Accl. Time (Hr)	Exp. Temp. (°C)	No.	Weight Mean (mg)	Weight Range (mg)	Notes	No.	ET50 (min)	95% Confidence Limits	S (fct50)(fs)
2/29/68	10-30	0	35	10	31.60	11.50-62.18		10	470	348- 635	1.63 (1.35)(1.24)
3/1/68		20		8	27.22	19.00-53.60		8	880	739-1047	1.32 (1.19)(1.13)
3/2/68		44		10	25.54	7.23-70.31		10	1700	1440-2006	1.30 (1.18)(1.125)
3/4/68		90		7	18.24	9.14-28.56		7	1810	1616-2027	1.18 (1.12)(1.076)
3/6/68		140		7	12.25	4.22-17.01		7	2850	2478-3277	1.26 (1.15)(1.11)
3/13/68		312		10	15.89	8.67-25.93		10	2680	2330-3082	1.25 (1.15)(1.105)
4/22/68	10-20	0	35	10	42.98	6.65-79.18		10	240	186- 309	1.51 (1.29)(1.20)
4/23/68		26 1/2		10	32.52	15.81-88.78		7	530	376- 747	1.705 (1.91)(1.34)
4/24/68		46		7	14.00	7.93-21.80		6	960	774-1190	1.425 (1.24)(1.18)
4/25/68		69 1/2		10	21.15	9.89-50.85		10	1000	833-1200	1.35 (1.20)(1.14)
4/26/68	20-30	0	35	5	22.22	10.58-46.59		5	840	712- 991	1.31 (1.18)(1.125)
4/27/68		23		6	23.40	7.70-30.88		6	2300	1917-2760	1.35 (1.20)(1.14)
4/29/68		71		8	45.54	34.74-58.39		8	5300	4609-6095	1.25 (1.15)(1.105)
5/1/68		120		10	23.04	9.64-34.01		10	5000	3620-6900	1.67 (1.38)(1.26)

TABLE IV.--Characteristics and response of Paragnetina media nymphs used to show rate of loss of heat resistance.

Test Conditions				Test Organisms				Response			
Date	Accl. Range (°C)	Accl. Time (Hr)	Exp. Temp. (°C)	No.	Weight Mean (mg)	Weight Range (mg)	Notes	No.	LT50 (min)	95% Confidence Limits	S (fct50)(fs)
7/15/68	30-10	1	34	8	8.71	1.62-12.53		7	4150	3532-4876	1.30 (1.175)(1.125)
8/5/68		25		8	7.53	10.05-2.10		5	3100	2678-3709	1.32 (1.19)(1.13)
8/29/68		39		8	10.33	15.01-4.87		8	3800	3332-4393	1.25 (1.15)(1.105)

TABLE V.--Characteristics and response of Paragnetina media nymphs separated into distance size groups and tested in concurrent upper lethal temperature experiments (mean current velocity 32 cm/sec).

Test Conditions				Test Organisms				Response			
Date	Accl. Temp. (°C)	Accl. Period (days)	Exp. Temp. (°C)	No.	Weight Mean (mg)	Weight Range (mg)	Notes	No.	ET50 (min)	95% Confidence Limits	S (fet50)(fs)
1/23/68	10	8	35	10	40.14	35.21-55.59	Females	10	705	518- 959	1.655 (1.36)(1.27)
				10	15.26	7.32-24.79	Males	10	706	588- 847	1.35 (1.20)(1.11)
1/23/68	10	8	34	10	53.58	34.77-75.[illegible]	Females	10	1300	1057-1599	1.38 (1.23)(1.18)
				10	16.29	9.66-24.[illegible]	Males	10	2000	1736-2445	1.28 (1.17)(1.10)
1/23/68	10	8	33	10	48.68	37.[illegible]-58.17	Females Bodies Distented	10	4100	3388-4961	1.36 (1.21)(1.14)
				10	16.70	11.[illegible]-27.3[illegible]	Males No noticeable body distentions	8	5750	4160-7939	1.66 (1.37)(1.31)
5/31/68	10	15	32	8	68.35	51.34-79.06	Females Most emergence Bodies distented "Split probit"	8(a)	1570	872-2826	1.965 (1.80)(1.55)
								(b)	4400	3777-5126	1.185 (1.165)(1.12)
				8	31.98	19.79-53.44	Males	6	6800	5231-8840	1.37 (1.30)(1.27)
5/7/68	10	6	33	9	71.58	50.21-93.72	Females	9	3580	2387-5370	1.625 (1.50)(1.44)
				10	28.72	18.29-35.26	Males	9	6000	4511-7980	1.565 (1.33)(1.24)
5/31/68	10	15	34	9	83.33	42.17-100.30	Females	9(a)	220	154- 315	1.635 (1.43)(1.30)
								(b)	could not compute		
				6	37.00	16.17-56.69	Males	5(a)	could not compute		
								(b)	1220	984-1513	1.345 (1.24)(1.20)
				7	1.76	0.11-2.78		7	1350	1174-1553	1.18 (1.15)(1.14)
4/24/68	10	5	34	10	71.51	44.08-98.15	Females	9	940	570-1551	2.235 (1.65)(1.46)

				10	20.48	8.37-28.20	Males	5	3700	2176-6290	2.05 (1.70)(1.61)
5/6/68	10	5	35	10	77.84	52.44-101.39	Females	10	360	273- 475	1.56 (1.32)(1.22)
				10	24.33	13.95-55.80	Males	10	470	348- 635	1.63 (1.35)(1.25)
5/6/68	10	5	36	8	69.88	37.06-106.62	Females	8	150	103- 219	1.87 (1.46)(1.32)
				10	22.50	7.84-48.02	Males	10	190	152- 238	1.445 (1.25)(1.18)
5/6/68	10	5	37	10	75.73	47.14-103.81	Females	10	59	43- 81	1.69 (1.38)(1.26)
				10	23.96	14.71-32.35	Males	10	67	50- 89	1.60 (1.33)(1.23)
5/31/68	20	4	33	9	63.03	55.33-71.84	Females	8	3200	2370-4320	1.46 (1.35)(1.30)
				7	17.08	14.62-19.71	Males	6	2800	1573-4984	2.46 (1.78)(1.58)
5/29/68	20	2	33	9	54.36	37.84-71.06	Females	6(a)	3100	1240-7750	3.165 (2.50)(2.40)
								(b)	4600	3134-6604	1.72 (1.44)(1.34)
				9	19.68	11.01-43.98	Males	7(a)	1600	640-4000	3.01 (2.50)(2.21)
								(b)	5000	2404-10400	2.50 (2.08)(2.00)
5/29/68	20	2	34	9	47.12	26.31-66.65	Females	7(a)	370	231- 592	1.745 (1.60)(1.52)
								(b)	1110	716-1720	1.67 (1.55)(1.48)
				8	23.88	11.72-35.92	Males	6(a)	3000	1345-6690	2.76 (2.23)(2.15)
								(b)	4400	2839-6820	1.67 (1.55)(1.48)
5/16/68	20	15	35	10	64.75	57.52-72.60	Females	9	640	467- 877	1.66 (1.37)(1.27)
				9	22.40	6.48-40.80	Males	5	1650	1025-2657	1.88 (1.61)(1.55)
4/29.68	30	17	36	10	47.83	31.24-67.68	Females	10	810	638-1029	1.475 (1.27)(1.18)
				10	15.43	10.30-23.03	Males	10	970	776-1212	1.435 (1.25)(1.175)

TABLE VI.--Characteristics and response of Paragnetina media used in upper lethal temperature experiments conducted in static water conditions.

Test Conditions				Test Organisms				Response			
Date	Accl. Temp. (°C)	Accl. Period (days)	Exp. Temp. (°C)	No.	Weight Mean (mg)	Weight Range (mg)	Notes	No.	ET50 (min)	95% Confidence Limits	S (fet50)(fs)
12/26/68	10	7	32	10	19.88	6.32-39.60		7	2500	1042-6000	3.38 (2.40)(2.10)
12/26/68	10	7	33	10	22.82	3.30-47.40		10	220	116- 418	2.29 (1.90)(1.60)
12/28/68	20	9	33	10	33.17	4.20-67.80		6	4300	2654-6966	2.04 (1.62)(1.55)
12/30/68	20	11	34	10	29.67	9.94-60.00		9	1440	720-2880	3.30 (2.00)(1.70)
12/30/68	20	11	35	10	25.46	10.15-30.38		10	340	246- 469	1.68 (1.38)(1.26)
1/8/68	10	20	33	10	25.42	10.35-53.65		10	1980	1253-3128	2.04 (1.58)(1.39)
1/16/68	10	7	34	10	32.36	6.84-57.17	"pumping" behavior	10	190	973- 333	2.685 (1.85)(1.57)
1/4/68	20	16	34	10	34.97	11.89-74.69		10	830	693-1117	1.46 (1.27)(1.175)
1/5/68	20	17	35	10	15.99	11.82-30.26		10	350	263- 465	1.58 (1.33)(1.23)
1/19/68	20	10	36	10	37.15	16.06-59.70		10	170	128- 226	1.575 (1.33)(1.225)
1/21/68	20	12	37	10	31.88	13.84-56.82	vigorous "pumping"	10	99	876-111.9	1.24 (1.13)(1.105)
1/4/68	30	16	34	10	18.99	6.09-39.75		5	7300	6348-8395	1.20 (1.15)(1.14)
1/5/68	30	17	35	10	15.99	11.82-30.26		10	3500	2845-4456	1.385 (1.23)(1.16)
2/8/68	10	4	33	10	38.76	15.52-61.74	muscle tremors	10	280	207- 378	1.635 (1.35)(1.25)
2/18/68	10	9	34	10	40.11	10.71-66.95	body twisted, muscle tremors	10	190	140- 258	1.65 (1.35)(1.25)
2/23/68	10	13	35	5	22.21	26.56-14.99		5	114	87.7-148	1.35 (1.30)(1.22)
2/22/68	20	13	34	10	30.57	11.15-56.64		8	1120	521-2408	3.30 (2.15)(1.75)
3/3/68	30	10	34	8	16.62	9.35-42.32		8	6600	5116-8514	1.41 (1.28)(0.24)
3/5/68	30	10	36	8	27.15	11.35-42.73		8	350	255- 479	1.66 (1.37)(1.26)
2/26/68	30	17	37	8	21.79	11.24-36.86		8	200	141- 284	1.65 (1.42)(1.28)
2/24/68	30	15	38	5	25.64	13.06-57.90		5	100	63.7- 157	1.66 (1.57)(1.39)
6/29/67	20	7	35	10	6.25	18.89-1.09		10	610	531- 701	1.24 (1.149)(1.10)

7/13/68	20	7	36	9	5.84	9.46-2.01	nymphs irritable "pumping" activity	9	230	161- 328	1.73 (1.43)(1.30)
7/12/67	20	6	35	10	11.88	23.82-6.37		9	470	382- 578	1.37 (1.23)(1.16)
7/3/67	20	11	34	9	3.31	8.49-1.46		8	1700	1393-2074	1.355 (1.22)(1.155)
7/6/67	20	14	34	7	4.77	9.45-1.79		5	3250	2305-4583	1.58 (1.41)(1.33)
7/30/68	20	20	33	10	8.98	15.41-3.45		7	6700	5276-8509	1.45 (1.27)(1.23)
7/13/67	30	7	36	10	7.61	16.25-2.74		10	480	375- 614	1.49 (1.28)(1.195)
7/10/67	30	18	35	7	5.74	11.99-2.45		7	600	361- 996	2.00 (1.66)(1.45)
7/21/67	30	10	35	10	7.57	14.03-2.52		9	940	712-1241	1.54 (1.32)(1.23)
8/20/68	10	40	36	8	6.95	12.66-3.56		8	64	52.5-78.1	1.345 (1.22)(1.16)
8/22/68	10	39	35	8	8.96	19.95-4.42		7	430	347- 533	1.36 (1.24)(1.17)
8/21/68	10	41	34	8	9.65	20.91-2.89		6	2100	1772-2489	1.685 (1.185)(1.13)
8/21/67	10	11	34	9	6.98	21.65-1.94		7	2600	1376-4914	2.57 (1.89)(1.58)
8/21/68	20	21	37	10	7.69	15.87-3.21		10	44.80	32.9-60.9	1.65 (1.36)(1.25)
8/15/67	20	8	35	10	14.48	30.00-6.73		10	630	438- 907	1.815 (1.44)(1.30)
8/15/67	20	8	34	10	13.97	31.34-2.31		8	2600	2232-3029	1.275 (1.165)(1.13)
8/21/68	30	21	38	10	6.79	13.19-2.33		10	77	65.3-90.8	1.325 (1.18)(1.13)
8/6/68	30	7	37	10	10.30	26.79-4.71		10	213	192- 236	1.18 (1.11)(1.076)
8/22/68	30	22	36	10	12.01	22.34-3.31		10	405	270- 607	1.93 (1.50)(1.31)
8/18/67	30	6	35	10	9.50	28.76-2.88		10	1490	861-2578	2.45 (1.73)(1.50)
8/5/68	30	7	34	10	9.96	18.73-4.53		10	4000	2920-5480	1.66 (1.37)(1.26)
8/21/67	30	11	33	10	6.44	17.21-1.44		5	8000	4511-14000	2.11 (1.75)(1.64)
10/26/67	20	15	35	10	17.68	34.10-8.78		10	450	328- 616	1.67 (1.26)(1.37)
10/28/67	20	17	34	10	26.87	41.00-5.25		7	2700	2045-3564	1.54 (1.28)(1.32)
10/26/67	30	15	35	10	26.42	62.11-9.60		10	1640	1390-1935	1.31 (1.13)(1.18)

TABLE VII.--Characteristics and response of Paragnetina media used in upper lethal temperature experiments at mean current velocity of 32 cm/sec.

Test Conditions				Test Organisms				Response			
Date	Accl. Temp. (°C)	Accl. Period (days)	Exp. Temp. (°C)	No.	Weight Mean (mg)	Weight Range (mg)	Notes	No.	ET50 (min)	95% Confidence Limits	S (fet50)(fs)
12/11/67	10	10	34	10	26.78	37.60-12.22		10	1450	1224-1718	1.32 (1.185)(1.13)
12/28/67	10	9	35	10	24.74	35.09-11.40		10	640	557- 736	1.25 (1.15)(1.11)
12/6/67	20	9	34	10	23.40	33.66-17.44		9	3050	2563-3629	1.32 (1.19)(1.14)
12/28/67	20	9	35	10	28.10	43.26-15.13		10	1090	982-1209	1.19 (1.11)(1.082)
12/28/67	30	9	34	10	30.01	55.25-17.02		10	5000	4065-6150	1.39 (1.23)(1.18)
12/11/67	30	13	35	10	31.20	53.68-13.10		10	2600	2166-3120	1.33 (1.20)(1.14)
12/26/67	30	15	36	10	14.66	28.66-4.31		10	770	602- 986	1.48 (1.28)(1.19)
1/8/68	10	20	33	10	31.69	32.92-11.84		10	3500	1750-7000	2.715 (2.00)(1.85)
1/16/68	10	7	35	10	39.71	48.06-15.62		10	450	302- 670	1.90 (1.49)(1.33)
1/19/68	20	10	34	10	41.20	58.06-11.66		7	4000	2500-6400	2.075 (1.60)(1.50)
1/5/68	20	17	35	10	35.23	51.13-17.23		10	1290	1157-1438	1.185 (1.11)(1.082)
1/19/68	20	10	36	10	30.56	36.07-12.16		10	490	443- 541	1.17 (1.105)(1.075)
1/21/68	20	12	37	10	34.12	41.78 13.58		10	190	160- 226	1.315 (1.19)(1.13)
2/8/68	10	4	33	10	33.00	65.21-21.19	bodies distented prior to death		3700	2387-5735	2.01 (1.55)(1.43)

2/16/68	10	7	36	10	40.29	55.59-18.06		10	140	105-186	1.58 (1.33)(1.23)
2/13/68	20	9	33	10	29.21	53.36-17.64	5 nymphs alive at 150 hr.	5	6700	5630-7973	1.255 (1.19)(1.17)
2/13/68	20	9	34	7	17.82	36.25-13.41		7	4400	3894-4972	1.225 (1.13)(1.095)
2/22/68	20	13	35	10	36.75	49.60-11.86	bodies distented	10	1220	1043-1427	1.29 (1.17)(1.12)
2/22/68	30	13	35	10	33.14	66.17-23.00		10	2480	2084-2951	1.335 (1.19)(1.14)
2/18/68	30	14	37	10	25.07	33.64-10.93		10	440	406- 477	1.14 (1.085)(1.06)
2/18/68	30	17	38	10	21.83	40.60-13.10		10	160	131-195	1.385 (1.22)(1.135)
2/24/68	30	15	34	10	27.80	45.61-13.05		5	9050		
3/29/68	10	21	32	10	22.91	52.77-25.64	bodies distented prior to death. One nymph died during ecdysis	5	5400	3750-7776	1.62 (1.44)(1.39)
3/29/68	10	21	33	10	59.76	73.15-25.70	bodies distented	5	2700	2308-3159	1.235 (1.17)(1.155)
3/28/68	10	22	34	10	38.52	54.01-17.55	one nymph with distented body	10	1570	1330-1853	1.31 (1.18)(1.125)
3/28/68	10	22	35	10	42.37	78.33-25.45		10	570	460-707	1.41 (1.24)(1.168)
3/31/68	10	25	37	10	42.59	55.33-19.42		10	79	64.2-97	1.40 (1.23)(1.16)
3/31/68	20	24	37	10	46.78	59.66-19.38		10	155	129-186	1.35 (1.20)(1.14)
4/14/68	10	9	31	10	50.56	75.01-18.38	nymphs had not molted. One nymph molted successfully. Bodies distented.	6	7200	3789-13680	2.57 (1.90)(1.77)
4/14/68	10	9	32	10	46.27	82.27-15.29	Bodies distented. Nymphs had not molted.	9	3900	2617-5811	1.90 (1.49)(1.35)

TABLE VII.--continued.

	Test Conditions			Test Organisms					Response		
Date	Accl. Temp. (°C)	Accl. Period (days)	Exp. Temp. (°C)	No.	Weight Mean (mg)	Weight Range (mg)	Notes	No.	ET50 (min)	95% Confidence Limits	S (fet50)(fs)
4/4/68	10	28	33	10	34.25	84.65-11.82	Had not molted	8	2500	1812-3450	1.67 (1.38)(1.29)
4/2/68	10	27	36	10	37.75	64.31-21.87	Had not molted	10	158	114- 218	1.68 (1.38)(1.26)
4/2/68	20	5	36	5	41.27	67.21-2.16	Had not molted	5	500	446- 560	1.195 (1.12)(1.085)
4/10/68	20	5	36	10	41.39	77.11-19.40	Bodies distended. Had not molted	10	240	190- 304	1.47 (1.265)(1.185)
4/10/68	20	5	34	10	[illegible]	[illegible]	Had not molted	10	1350	1116-1634	1.375 (1.21)(1.15)
4/10/68	20	5	35	10	[illegible]	[illegible]	Bodies distended. Had not molted	10	750	610- 950	1.32 (1.18)(1.13)
5/27/68	10	12	34	10	[illegible]	[illegible]		10	[illegible]	1667-2350	1.405 (1.225)(1.168)
5/30/68	20	3	40	8	[illegible]	[illegible]-14.77	Very near emergence. "Split profile."	7	62	26- 140	3.455 (2.40)(1.75)
5/30/68	20	3	37	7	31.41	[illegible]		7	140	125- 165	1.28 (1.175)(1.15)
5/16/68	20	15	36	6	[illegible]	71.12-[illegible]		6	[illegible]	[illegible]- 335	1.30 (1.17)(1.12)
5/31/68	30	5	34	9	[illegible]	15.14-[illegible]		9	[illegible]	4.7-3745	1.20 (1.135)(1.095)
5/31/68	30	5	35	8	[illegible]	[illegible]-1.5		8	2750	[illegible]-3443	1.51 (1.29)(1.20)
5/16/68	30	15	36	10	[illegible]	[illegible]		10	870	740-1022	1.295 (1.175)(1.125)
5/29/68	30	5	37	6	19.22	35.18-11.25		6	870	740-1022	1.295 (1.175)(1.125)
5/31/68	30	8	38	8	25.28	57.90-13.34	Wing pads very dark	8	150	124- 182	1.335 (1.21)(1.175)
6/27/68	10	13	35	10	2.77	4.35-1.75		10	1010	737-1384	1.67 (1.37)(1.26)
6/25/68	20	11	36	10	5.26	8.43-2.14		10	620	525- 732	1.31 (1.18)(1.13)
6/24/68	20	10	35	10	3.52	7.16-1.71		10	1280	1049-1562	1.37 (1.22)(1.15)

6/19/68	20	5	34	10	6.52	16.28- 2.41	One nymph died in emergence	10	4350	3295-5742	1.555 (1.32)(1.22)
6/25/68	30	11	35	5	2.68	3.14- 2.58		5	808	703- 929	1.165 (1.15)(1.11)
6/24/68	30	10	35	10	4.19	17.24- 0.10		10	1580	1362-1833	1.29 (1.16)(1.125)
7/18/68	20	12	35	10	3.96	8.10- 1.09		10	1950	1512-2516	1.505 (1.29)(1.20)
7/24/68	20	13	34	10	6.92	13.09- 1.78		9	5200	4127-6552	1.43 (1.26)(1.18)
7/15/68	20	10	38	10	6.67	18.44- 1.33		10	88	64.7-120	1.65 (1.36)(1.25)
7/15/68	20	10	37	10	4.04	9.05- 1.10		10	220	143- 279	1.47 (1.27)(1.185)
7/15/68	20	10	36	10	6.81	16.05- 1.37		10	780	716- 850	1.17 (1.09)(1.065)
7/8/68	20	12	35	5	5.03	13.16- 2.87		5	1300	1119-1558	1.31 (1.18)(1.125)
7/2/68	20	6	34	7	3.81	9.85- 1.36		6	6300	4884-8127	1.505 (1.29)(1.23)
7/15/68	30	5	38	10	3.89	18.44- 3.45		10	120	92.3-156	1.54 (1.30)(1.21)
7/15/68	30	5	37	10	5.66	8.76- 3.49		10	365	329- 405	1.18 (1.11)(1.077)
7/15/68	30	6	36	10	5.63	22.73- 3.99		10	1130	1041-1226	1.14 (1.085)(1.060)
7/8/68	30	10	35	5	5.49	7.96- 1.68		5	1800	1314-2466	1.74 (1.37)(1.32)
7/2/68	30	6	34	7	4.84	10.61- 1.08		5	8100	5328-12312	1.88 (1.52)(1.44)
7/18/68	30	8	35	7	4.97	11.41- 3.54		6	3500	2777-4410	1.46 (1.26)(1.85)
7/15/68	30	6	34	10	6.60	16.38- 1.34		10	5400	4355-6696	1.42 (1.24)(1.17)
8/22/68	10	21	36	10	5.90	13.91- 1.54		10	450	338- 598	1.59 (1.33)(1.19)
8/19/68	10	39	34	8	5.76	13.66- 3.83		6	3800	3234-4465	1.35 (1.175)(1.18)
8/21/68	30	21	39	10	4.01	10.69- 1.09		10	55	41.7-72.6	1.56 (1.32)(1.22)
10/26/67	20	10	35	10	20.22	33.05- 5.19		10	1350	1256-1444	1.12 (1.025)(1.053)
10/11/67	30	8	35	10	15.13	29.24- 4.72		10	2850	2375-3420	1.35 (1.15)(1.20)

TABLE VIII.--Details of upper lethal temperature experiments with Paragnetina media nymphs in winter (December, January, February). Values in parentheses following test temperature indicate number of replicates.

Accl. Temp. (C)	Test Temp (C)	LT50 and 95% Confidence Limits (min)
10	37(1)	58 (42-80)
10	36(1)	140 (105-186)
10	35(2)	545 (425-703)
10	34(1)	1450 (1224-1718)
10	33(2)	3600 (2069-6368)
10	32(1)	*
20	37(1)	190 (160-226)
20	36(1)	490 (443-541)
20	35(3)	1200 (1061-1358)
20	34(3)	3817 (2986-5000)
20	33(1)	6700 (5630-7973)
20	32(1)	*
30	38(1)	160 (131-195)
30	37(1)	440 (406-477)
30	36(1)	770 (602-986)
30	35(3)	2660 (2297-3103)
30	34(2)	7250 (5738-9471)
30	33(2)	*

*50 per cent mortality did not occur in 10,000 minutes.

TABLE IX.--Details of upper lethal temperature experiments with *Paragnetina media* nymphs in spring (March, April, May). Values in parentheses following test temperature indicate number of replicates.

Accl Temp (C)	Test Temp (C)	LT50 and 95% Confidence Limits (min)
10	37(1)	79 (64.2-97)
10	36(1)	158 (114-218)
10	35(1)	570 (460-707)
10	34(1)	1570 (1330-1853)
10	33(2)	2600 (1812-3450)
10	32(2)	4650 (3184-6794)
10	31(1)	7200 (3789-13680)
20	38(1)	62 (26-149)
20	37(1)	140 (120-165)
20	36(3)	341 (293-400)
20	35(2)	1145 (746-1782)
20	34(2)	2055 (1031-4205)
20	33(4)	3900 (2385-6582)
20	32(1)	*
30	38(1)	150 (124-182)
30	37(1)	290 (228-368)
30	36(1)	870 (740-1022)
30	35(1)	2700 (2093-3483)
30	34(1)	4280 (3755-4879)
30	33(1)	*

*50 per cent mortality did not occur in 10,000 minutes.

TABLE X.--Details of upper lethal temperature experiments with Paragnetina media nymphs in summer (June, July, August). Values in parentheses following test temperature indicates number of replicates.

Accl Temp (C)	Test Temp (C)	LT50 and 95% Confidence Limits (min)
10	36(1)	450 (338-589)
10	35(1)	1010 (737-1384)
10	34(1)	3800 (3234-4465)
10	33(1)	*
20	38(1)	88 (64.7-120)
20	37(1)	220 (173-279)
20	36(2)	700 (621-791)
20	35(4)	1475 (1234-1770)
20	34(3)	5283 (4102-6807)
20	33(2)	*
30	39(1)	55 (41.7-72.6)
30	38(1)	120 (92-156)
30	37(1)	365 (329-405)
30	36(2)	969 (872-1077)
30	35(4)	2345 (1957-3032)
30	34(2)	6750 (4842-9504)
30	33(2)	*

*50 per cent mortality did not occur in 10,000 minutes.