SELECTED TEMPERATURE AND RESISTANCE IN AQUATIC DIPTERA

EFFECTS OF AGE AND THERMAL ACCLIMATION ON THE SELECTED TEMPERATURE AND THERMAL RESISTANCE OF CULICID AND SIMULIID LARVAE (DIPTERA)

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SCOPE AND CONTENTS:

Black-fly larvae, and mosquito larvae and pupae, are shown to select certain temperature ranges when given a choice in a linear gradient. Larval age has little effect on the temperature selected, but pupae are tolerant of higher temperatures than larvae. The significance of this observation is discussed. The temperature selected is affected by the previous thermal history of the insect, although acclimation to the ambient temperature may be rapid. The final selected temperature of fourth-instar Aedes aegypti larvae was determined.

Rearing temperature has a profound effect on the thermal resistance of Aedes aegypti larvae exposed to high temperatures.

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INTRODUCTION

Acclimation can be considered as one of the levels at which poikilothermic animals compensate for changes in their environment, and is usually applied to such compensatory changes as occur in the laboratory where the animals are maintained under controlled conditions. Compensation for the more complex situation occurring in nature is usually termed acclimatization (Prosser, 1961; Hoar, 1966). That animals vary in the extent of their temperature tolerances, and thus in their capacity for acclimation, is seen in the wide use of the terms stenothermal and eurythermal. Besides affecting thermal tolerance, acclimation may have an effect on an animal's behaviour. When placed in a temperature gradient animals often aggregate within certain temperature limits. Entomologists have termed this temperature range, or part of it, the temperature preference (Fulton, 1928; Nicholson, 1934; Gunn, 1935; Campbell, 1937; Gunn and Walshe, 1942; Falconer, 1945; Henson, 1957; Zacharuk, 1962; Chapman, 1965), the preferred temperature (Wigglesworth, 1953; Nielsen and Nielsen, 1959; Atkins, 1966), the thermal or temperature preferendum (Uvarov, 1931; Deal, 1941; Wilkes, 1942; Belehradek, 1957; King and Riley, 1960), and the thermopreferendum or range of temperature indifference (Hafez, 1953) of the species being tested. Gunn and Cosway (1938) used the term preferred but suggested that ecritic temperature was less anthropomorphic. Those studying vertebrates have restricted themselves to the terms selected or preferred temperature (Fry, 1947; Brett, 1952; Pitt et al., 1956; Garside and Tait,

1958; Ogilvie and Anderson, 1965; Hoar, 1966; Licht et al., 1966). Fry (1958) used the term thermal indifference.

These terms have been used with little uniformity by entomologists. Fulton (1928) gave, as the theoretical optimum, and Henson (1957) as the temperature preference, the temperature (°C) most frequented by the test insects. Davies (1949) gave the selected temperature as the range of two Centigrade degrees, Omardeen (1957) as the range of four Centigrade degrees, Chapman (1955) as the range of five Centigrade degrees, and King and Riley (1960) as the range of four Fahrenheit degrees in which the mode, of a frequency distribution, occurred. Deal (1941) determined the temperature preferendum of 23 insect species, presented the results as graphs, and chose the temperature range with the greatest frequency of individuals as the preferendum for that particular species. But he gave no indication of the criteria used to determine the limits of the ranges. Nieschulz (1933, 1935) gave the mean as the preferred temperature of muscid flies. Nielsen and Nielsen (1959) defined the preferred temperature as the average of the temperatures at which the insects came to rest under the conditions of the experiments. Chapman (1965) regarded the preferred temperature zone as the range where a negative thermokinesis became dominant, for Schistocerca this range was about 10 Centigrade degrees. Gunn and Walshe (1942) gave the temperature preference of Ptinus simply as the temperature (24 C) around which most of the insects aggregated. Other workers have given the range in which a certain percentage of the test insects occurred as the selected temperature. Thus, Gunn (1935) gave the range in which 100%, Bodenheimer and Schenkin (1928), and Bodenheimer and Klein (1930) the range in which

50 - 80% of their observations occurred. Campbell (1937) regarded 'the preferendum' as the concentrated interval in which 50% or more of the insects were found. Wilkes (1942) gave the range of 50 - 80% and Zacharuk (1962) the interquartile range as the selected temperature. Brett (1952), Pitt et al., (1956), and Garside and Tait (1958) gave the mean ± standard deviation, and the mode in their results, indicating that the latter is the statistic that best describes the preferred temperature since it is the most frequently occupied position and thus is in fact the definition of preferred temperature. Ogilvie and Anderson (1965) regarded the selected temperature as the range, one Centigrade degree, corresponding to the mode of the frequency distribution plot. Licht et al., (1966) defined the mean preferred temperature as the average temperature selected by representatives of a species when provided with a wide choice of temperatures under standard conditions.

The first workers to show that selected temperature was associated with the previous temperature experiences of insects were Herter (1923) with Formica and Bodenheimer and Schenkin (1928) with Tribolium. Campbell (1937) showed that acclimation affected the temperature selected by wireworms, although individuals did not alter their selected temperature until subjected to a higher or lower temperature for at least one month. The selected temperature of the pierid, Ascia monuste (Linnaeus), is correlated with the seasonal changes in temperature (Nielsen and Nielsen, 1959). Zacharuk (1962) suggested that complex interactions of physical and chemical factors in the soil influence the behaviour of Ctenicera larvae and that temperature was a most significant one, although there was no consistent

simple relationship between acclimation and selected temperatures. Atkins (1966) showed that after several days acclimation to 2 - 4 C, bark beetles selected a lower temperature than controls kept at 23 C. Some workers have recorded negative results, i.e. selected temperature was independent of the previous thermal history. Bodenheimer and Klein (1930) concluded that the selected temperature of ants remained the same in different months in spite of the great differences in the environmental temperature. Hafez (1953) concluded that blowfly larvae did not alter their selected temperature when exposed to a higher or a lower temperature. Chapman (1955) stated that the temperature selected by Locusta nymphs could be raised after an acclimation period of only three hours. But in a later paper (1965) he repudiated these results, explaining them as poor experimental technique, and concluded that the selected temperature did not vary following preconditioning at different temperatures. The negative results obtained by Deal (1941) and Falconer (1945) with wireworms may not be valid since their maximum acclimation period was only 12 days. Deal (1941) reported that the selected temperature of Tribolium, Lasioderma, and Calandra was lower when the insects were kept at a high temperature for one month than when kept at a lower one; Tribolium selected 25 - 30 C when kept at 20 C, but when kept at 27 C this decreased to 14 C. Most poikilothermic vertebrates show a positive relationship, selected temperature increases with an increase in acclimation temperature (Doudoroff, 1938; Fry, 1947; Brett, 1952; Pitt et al., 1956). There is a point where the selected temperature does not alter with an increase in the acclimation temperature. The point where the selected is the same as the acclimation temperature has been termed

the final preferendum (Fry, 1947). This temperature has been determined for many fish species but apparently never for an insect. More than one final preferendum has never been determined for any species. Usually the selected temperature will be higher than the acclimation at temperatures below the final preferendum and lower than the acclimation at temperatures above the final preferendum, thus there is only one point where the curve relating selected to acclimation temperature intersects a 45 degree line through the origin. The effect is that animals in a temperature gradient will ultimately, if no other factors are limiting, aggregate at the final preferendum. The time this takes will depend upon the rate at which the species acclimates.

The majority of the temperature selection work with insects has been with terrestrial forms and results have had to be interpreted with respect to the influence of relative humidity. Similarly, studies with some aquatic insects cannot be separated from oxygen requirements. Since mosquito larvae and pupae are air breathers their selected temperature can be measured directly. Only two workers have made any laboratory study on the temperature selected by the immature stages of mosquitoes. Ivanova (1940) studied fourth-instar larvae of Anopheles maculipennis Meigen. Omardeen (1957) determined the selected temperature of every instar, except the first, of Aedes aegypti (Linnaeus). Neither of these workers studied the effect of thermal acclimation on the selected temperature.

Much of the work on thermal resistance has been with insects reared or acclimated to one temperature and no account has been given

of the effects of acclimation to other temperatures (Wright, 1927; Walshe, 1948; Farid, 1949; Barr, 1952; Harker, 1952; Lal, 1953; Pielou and Glasser, 1954; Bar-Zeev, 1957). Mellanby in a series of papers (1939, 1940, 1954, 1960) showed that acclimation can alter an insect's thermal resistance. The thermal resistance of Aedes aegypti larvae increased by 2 C, from 42 C to 44 C, after 24 hr acclimation to a higher temperature, from 30 C to 37 C (Mellanby, 1954). Similar results have been obtained for larvae of Calliphora and Phormia (Fraenkel and Hopf, 1940), for Dahlbominus (Baldwin, 1954), for Neodiprion larvae (Baldwin and House, 1954), for a cockroach, Blatta, (Colhoun, 1954), for Tribolium (Edwards, 1958), and for the larvae of Agria (Pseudosarcophaga) (House et al., 1958). The entire thermal history was shown to influence the thermal resistance of Drosophila. Imagines reared and subsequently kept at 25 C had a greater resistance to high temperatures than those reared at 15 C and acclimated to 25 C for five days. The acclimation which occurred in individuals reared at 25 C was long lasting, since flies subsequently kept at 15 C for one week had a longer survival time than those reared and kept at 15 C. In contrast, the acclimation during adult life was of short duration. Flies reared at 15 C, kept at 25 C for four days and then at 15 C for two days, survived only slightly longer than flies reared and kept at 15 C and shorter than flies reared at 15 C and kept at 25 C. This suggested that two types of acclimation occurred, long lasting developmental acclimation during pre-adult life, and a more transitory one in the adult, physiological acclimation (Maynard-Smith, 1956, 1957). Such an hypothesis receives support from Harker (1950). Working with the same species of mayfly nymph, Leptophlebia, collected

from a pond and a nearby cooler stream, she found that those from the pond had a higher thermal resistance than those from the stream even after both had been kept at the same temperature for one week prior to testing. She suggested that the nymphs were irreversibly acclimated to the temperature conditions of their original habitat. Other workers have found that closely related species from lentic and lotic habitats have different thermal resistances for the same acclimation temperature. Species from lotic habitats usually have a lower thermal resistance than species from lentic habitats, suggesting that the insect is adapted to the temperature conditions of its environment (Whitney, 1939; Walshe, 1948; Harker, 1950).

Acclimation, with regard to thermal resistance, may be a rapid process, a slow process, or non-existent. In Dahlbominus, acclimation to a higher temperature begins immediately upon exposure and reaches its maximum within three hours (Baldwin and Riordan, 1956). Larvae of Anopheles quadrimaculatus (Say) also acclimate rapidly, 40 min acclimation increased the thermal resistance almost fivefold (from six to 28 min) (Love and Whelchel, 1957). Mellanby (1954) suggested that insects are fully acclimated within 20 hr, longer acclimation producing no increase in thermal resistance. However, Whitney (1939) found no significant difference between the thermal resistances of mayfly nymphs, Baetis, kept at 10 C and those kept at 15 C for 40 hr. The thermal resistance of Agriotes larvae kept at 16 C was not significantly different from larvae kept at 6 C (Falconer, 1945). With extensive acclimation there may even be a reversal of the normal relationship. Tribolium maintained at 18 C for several months showed a higher

resistance to 40 C than controls kept at 30 C and 38 C, also those kept at 38 C had a lower resistance than those kept at 30 C (Edwards, 1958). Immobilization by cold, chill-coma, can affect the ability of an insect to acclimate. Larvae of Aedes aegypti reared at 30 C did not survive a 17 hr exposure to 0.5 C, but when similar larvae were exposed to 20 C for 24 hr before testing 93% survived. However, larvae placed in water at 10 C went into a chill-coma and only 2% survived the 17 hr exposure to 0.5 C (Mellanby, 1960). In contrast, Falconer (1945) found that Agriotes larvae were able to acclimate when in a state of chill-coma below 0 C.

The experimental techniques and the subsequent analysis of thermal resistance data for fish has been thoroughly documented and standardized by Fry and his co-workers at Toronto (see Brett, 1952). Thermal resistance data for insects has been obtained by different experimental methods and thus its comparison is made difficult. The techniques used include :

1/ The simplest, and probably least satisfactory, method is to gradually increase the water temperature until the desired test temperature is reached, keep the water at this temperature for the test period, and then allow it to cool to the original acclimation temperature (Lal, 1953; Love and Whelchel, 1957).

2/ A variation of the first method is to simulate the actual temperature conditions of the natural habitat by having a daily rhythm of fluctuating temperatures (Love and Whelchel, 1957; Davies and Smith, 1958). Such a method seems suitable only for species inhabiting lotic waters where temperature variations within habitats are probably slight.

The temperature of lentic waters often varies, depending on shade, depth, etc., and many insects are capable of selecting temperature zones (see Haufe, 1957).

3/ After gradual heating to the desired test temperature the water is held at this temperature and the time to death of each insect is recorded (Harker, 1950).

The other three methods involve exposing the experimental insects to the lethal temperature with as short a time lag as possible.

4/ Expose a number of samples to a series of temperatures for one time period and determine the temperature at which death, of a predetermined number, occurs. This temperature has been termed the 'thermal death point' (Beattie, 1928; Mellanby, 1960), 'thermal index' (Whitney, 1939), 'thermal resistance' (Walshe, 1948), and the '50% lethal temperature' (Farid, 1949).

5/ Expose the insects to one temperature and remove samples at definite time intervals over a period where the shortest exposure causes about 10% mortality and the longest 90% mortality. In this way the time to a given mortality, usually 50%, can be determined. This is the thermal death time or effective dose for a given temperature (Barr, 1952; Baldwin, 1954; Baldwin and House, 1954; Pielou and Glasser, 1954; Baldwin and Riordan, 1956; Bar-Zeev, 1957; Love and Whelchel, 1957; Edwards, 1958; House et al., 1958).

6/ Expose the insects to the test temperature and record the time to death of each individual (Baldwin, 1954; Maynard-Smith, 1956).

The methods involving the gradual heating of the test insects (1, 2, 3) are difficult to analyse because of the acclimation effect

which may occur during the initial heating period. Data obtained from methods 4 and 5 can be analysed by the technique applicable to dosage-mortality experiments (Bliss, 1935), and from method 6 by time-mortality (Bliss, 1937). The main difficulty with time-mortality experiments is deciding when a test animal dies. A criterion often used is the lack of response to mechanical stimulation. Insects often show a delayed effect of high temperatures. Baldwin (1954) found that three hours after the end of a one hour exposure to 43 C the number of immobile, apparently dead, insects was greater than at five hours. The insects actually dead increased from seven to 30 hr and then levelled off. Consequently, he did not record mortality until 48 hr after the end of each test. The method of time-mortality is an ideal one for long term experiments when the animals are exposed for many days and has been used for Dahlbominus at less extreme lethal temperatures (Baldwin, 1954) and for Drosophila (Maynard-Smith, 1956).

Criteria for the survival of mosquito larvae have varied from the ability to move 18 hr after the beginning of the test (Farid, 1949; Barr, 1952) to pupation and emergence of the imagines (Mellanby, 1960). Wright (1927) determined survival ten days after his tests, whereas Lal (1953), Bar-Zeev (1957), and Love and Whelchel (1957) used movement 24 hr after testing as their criteria for survival. Any experiment in which mortality is determined some time after the end of the exposure cannot be analysed by the method of time-mortality.

The term 'incipient lethal temperature' (Fry et al., 1946), simplified to 'lethal temperature' by Brett (1952), is a most useful one

and is probably the shortest summary of the thermal resistance of an animal. It can be regarded as the temperature at which survival of 50% of the population is indefinite, i.e. death is due to some factor other than temperature. For any animal species at a given acclimation temperature there is one upper and one lower incipient lethal temperature for any given set of environmental conditions. The temperature range between the lower and upper incipient lethal temperatures can be regarded as a zone of tolerance, a zone compatible with the continued existence of the organism. All other temperatures cause more than 50% mortality and fall within a zone of resistance. The time an animal can survive in the zone of resistance, apart from other factors, depends upon the intensity of the lethal factor.

The factors , besides acclimation, affecting the thermal resistance of insects are :

1/ Developmental stage. Whenever tested, thermal resistance has been shown to vary from one instar to the next (Thomson, 1940; Farid, 1949; Barr, 1952; Lal, 1953; Bar-Zeev, 1957; Love and Whelchel, 1957). Usually with mosquitoes the first-instar is the most resistant, and the fourth-instar the most susceptible to heat.

2/ Age. The age within an instar can affect the thermal resistance. Mature eggs of Aedes aegypti and Culex pipiens are more resistant to high temperatures than recently laid ones (Davis, 1932; Farid, 1949). Differences were found in all stages of Anopheles quadrimaculatus between those which were only a few hours old, in the given instar, and those within a few hours of moulting to the next. The

younger larvae were more resistant than the older ones (Barr, 1952). Young Aedes aegypti pupae were more resistant to low and high lethal temperatures than were old pupae (Bar-Zeev, 1957). Baldwin's (1954) initial experiments using imagines of unknown ages, varying from recently emerged to four days old, gave such variable results that for each future experiment he used imagines whose maximum age difference was only one hour.

3/ Sex. Tests on different Anophelines suggested that male pupae were more susceptible to high temperatures than female pupae (Barr, 1952). No other workers have suggested that sex may affect the thermal resistance of aquatic insects, but it has been shown to have an effect on mature Drosophila (Maynard-Smith, 1956) and Tribolium (Edwards, 1958).

4/ Diet. Starvation has been shown to decrease the thermal resistance of a number of adult insects (Mellanby, 1934). House et al. (1958) showed that the actual diet an insect is given can affect its thermal resistance. The time of exposure to 45 C required to cause 50% mortality of Agria (Pseudosarcophaga) was 184 min for larvae reared on a mixture containing unsaturated fatty acids, whereas for larvae reared on a diet containing a high proportion of saturated fatty acids it increased to 218 min.

5/ Crowding. Anopheles larvae reared under crowded conditions had an increased resistance to high temperatures (Barr, 1952).

Only a few species of mosquitoes have been studied with regard to the thermal resistance of the aquatic stages. Aedes species have been investigated by MacFie (1920), Wright (1927), Davis (1932), Mellanby (1940, 1960), and Bar-Zeev (1957, 1958); Anopheles species by Wright (1927),

De Meillon (1934), Muirhead-Thomson (1940), Pal (1943), Barr (1952), Lal (1953), and Love and Whelchel (1957); Culex species by Wright (1927), Karamchandani (1935), and Farid (1949). The only experiments on the effects of acclimation were those of Muirhead-Thomson (1940), Love and Whelchel (1957), and Mellanby (1954, 1960).

The present study was conducted to determine the effects of age and thermal acclimation on the selected temperature of blackfly larvae, on the selected temperature of mosquito larvae and pupae, and on the thermal resistance of Aedes aegypti larvae. An attempt was made to determine the incipient upper lethal temperature of Aedes larvae from one acclimation temperature.

MATERIALS AND METHODS

Selected Temperature Apparatus

This apparatus consisted of a trough (24 inches long, 3/16 inch wide, 7/16 inch deep) cut out of a copper bar (30 inches long by 3/4 inch square) so that three inches of solid copper remained at each end of the trough. The trough was painted white, to render the insects more visible, and then coated with a clear plastic. To test for any toxic substances in the trough from either the paint or the copper, insects (Aedes aegypti) were reared in it at frequent intervals. No undue mortality was recorded.

Six holes, 3/16 inch diameter, were drilled 9/16 inch into one side of the bar just 1/32 inch beneath the trough bottom. These holes were to house thermistor probes and were spaced at 2, 6, 10, 14, 18, and 22 inches from one end of the trough. The copper bar was encased in a piece of styrofoam plastic except for the three inch lengths at each end, one of which was cooled, either by packing in ice and salt or by a stream of cold water constantly pouring over it, whilst the other end was inserted into a box (10 x 6 x 6 inches) and heated by a 100 watt bulb controlled by a Powerstat.

The temperature measuring equipment consisted of a YSI model 44TD Tele-Thermometer and six interchangeable stainless steel thermistor probes (YSI - 403) which were plugged into the holes in the copper bar. Temperature readings were taken by turning the selector switch of the Tele-Thermometer to the desired probe.

A mirror, 24 x 6 inches, was supported six inches above the trough at an angle of 45 degrees. By uniform illumination of the trough and darkening of the laboratory it was possible to observe the insects, through the mirror, with a minimum of disturbance to them. As an extra precaution a board was placed between the trough and the observer.

The top surface of the wall of the trough and the adjacent styrofoam were marked off into 1/4 inch sections to facilitate the recording of the insect's positions.

Since the thermistor probes were not in contact with the water, the temperature readings obtained from them had to be corrected to obtain the water temperatures. In an ambient temperature of 23 C and a relative humidity of 50% the temperature of the water in the trough varied from that of the copper only at the ends of the gradient. A nomogram was constructed, with copper temperatures as the abscissa and water temperatures as the ordinate, and from it the actual water temperatures during the experiments were obtained.

In all the experiments with simuliids the thermistor probes were placed in the water in the trough. This had the advantage that the water temperature could be read directly, essential for flowing water, but the disadvantage that each probe could act as a barrier to larval movements. However, in the experiments the probes did not appear to affect larval behaviour.

Lethal Temperature Apparatus

The lethal temperature bath was an enamel tray (16 inches long, 10 inches wide, and 2 inches deep) supported in a well insulated 12

gallon water bath in which the water was heated by a 125 watt low-lag knife heater and the temperature controlled by a micro-set, differential range type, thermoregulator (sensitivity ± 0.005 F) working through an electronic relay control box (mere-to-mere Precision). The water was circulated by means of an electric pump.

A sheet of styrofoam plastic closed the top of the lethal temperature tray. Six holes, three and one half inches in diameter, were cut out of the styrofoam to house the larval containers which were one-pint plastic food containers, three and one half inches deep by three and one half inches in diameter. The bottoms of these containers were cut out and nylon screening substituted.

Species Used

Experiments were conducted using the immature stages of four simuliid species, Prosimulium magnum Dyar and Shannon, Cnephia mutata (Malloch), Simulium venustum Say, and Simulium vittatum Zetterstedt; and two mosquito species, Culiseta inornata (Williston) and Aedes aegypti (Linnaeus).

The simuliid larvae were collected from local streams and kept in the laboratory in artificial streams (Wood and Davies, 1966) for periods up to one month before being used.

Culiseta inornata was reared according to the method of McLintock (1952, 1964) at a temperature of 20 C (20.5 ± 0.5 C). Eggs were floated on distilled water and after three days the hatched larvae were transferred to the rearing medium. Rearing proved to be difficult with high mortality in the first-larval and pupal stages. After the

experiments with C. inornata were concluded, a different rearing technique for this species was published (Hanec and Brust, 1967). In the present study a light photoperiod of 18 hr was used. The light in the rearing incubator was set to switch on at 0600 hr. When used for an experiment, the larvae and pupae had had a minimum of six hours light conditioning.

Aedes aegypti larvae were reared in enamel trays (16 inches long, 10 inches wide, and 2 inches deep) in an incubator which maintained the water temperature within ± 0.5 C. Larvae were reared at 16, 20, 25, 30, 31.5, and 35 C, and with a light photoperiod of 12 hr. When used for an experiment they had had a minimum of six hours light conditioning. By limiting the number of larvae to 300 per tray and feeding rabbit chow and brewers' yeast twice daily, uniform growth and development was achieved. Fourth-instar larvae were used exclusively throughout the experiments.

All species were used for the selected temperature studies, but only Aedes aegypti could be reared in sufficient quantity to permit lethal temperature studies to be conducted.

Experimental Method-

1/ Selected Temperature Studies

The technique used involved establishing a temperature gradient in the trough, distributing the insects by means of a pipette along its whole length and allowing from five to ten minutes for the gradient to adjust. Observations were then made at definite time intervals, which differed for the different species.

The position of every insect was recorded to the nearest 1/4 inch, actively swimming mosquito larvae were not recorded until they stopped. Positions were plotted on a graph with trough divisions as the abscissa and time, in minutes, as the ordinate. At the end of the experimental period, isotherms were plotted on the same graph as the insects' positions with temperature as the abscissa (e.g. Fig. 9). On occasions where insects were exactly on an isotherm, they were equally distributed to either side of that isotherm. The total number of insects observed between each isotherm were summed and plotted as an histogram (e.g. Fig. 10) from which the modal temperature was obtained by inspection. A cumulative frequency curve was constructed (e.g. Fig. 10), and from it the median temperature and the interquartile range were obtained.

With Cnephia mutata, larval positions and trough temperatures were recorded every five minutes for 90 min, with Simulium venustum every five minutes for 70 min. Data were recorded with Prosimulium magnum every 10 min for 100 min and with Simulium vittatum every 30 min for 270 min. Insects' positions and trough temperatures in the Culiseta inornata experiments were recorded every three minutes for 90 min. and for the Aedes aegypti experiments every two minutes for 60 min.

Two types of temperature gradient were used in the experiments. Control experiments, the water at a uniform temperature, showed that the simuliid larvae and all the instars of Culiseta inornata were relatively inactive. Test insects formed minor aggregations in various parts of the trough and rarely moved from them. To stimulate locomotory activity in these species a shifting temperature gradient was used. This was achieved by varying the output of the heater by means of the Powerstat.

Control experiments with Aedes aegypti larvae from all the rearing temperatures showed them to be active. The larvae consistently formed aggregations at both ends of the trough, but individuals continually moved along its length. Since larval positions at any uniform temperature were predictable, and the larvae were active, a static temperature gradient was used in all the Aedes aegypti experiments. Examination of the Culiseta inornata distribution plots suggested that the shifting temperature gradient may have caused a biased result. Therefore, three experiments using a static temperature gradient were conducted and the results compared with those obtained from the shifting gradient.

During the experiments involving a shifting temperature gradient the actual gradient and its rate of shifting was different for every experiment. In the Culiseta experiments the rate of temperature change at any given point was never greater than 1 C in a three minute period. With a static gradient it was possible to keep it the same for every experiment. With Aedes larvae cold stupor set in at temperatures below 12 C regardless of the rearing temperature. To prevent an aggregation of larvae at the cold end of the trough, the lowest temperature in the gradient had to be kept above 12 C.

With two simuliid species, P. magnum and S. vittatum, flowing water was used in the trough. One end of the trough was slightly raised to cause the water to flow and a stream of cold water was introduced at this end. The other end was heated by steam. Standing water was used for the other two species, C. mutata and S. venustum.

In an attempt to keep the temperature gradient in the trough linear, the first two and last two inches of the trough were not used for any of the mosquito experiments. Mesh barriers were placed at divisions '2' and '22' (two and twenty-two inches from the cold end of the trough). The trough was re-calibrated to make the mesh barriers at positions '0' and '20'.

Two other factors, besides acclimation temperature, were found to affect larval behaviour in the mosquito studies. These were :

1/ Light.

When insects were taken from the dark incubator and placed in the experimental trough they continually moved up and down the gradient and no precise temperature selection was demonstrated. However, when they were exposed to light (a ten watt bulb 12 inches above the rearing trays) a few hours before an experiment, activity during the experiment was reduced and a precise temperature selection was seen. Thus before every experiment, insects were given a minimum of six hours 'light conditioning'.

2/ Food.

During the first hour of any experiment with Aedes, the larvae formed a compact aggregation. But after about 90 min this aggregation ceased and the larvae dispersed throughout the trough. When food, yeast, was placed in the trough, the larvae formed an aggregation around it, even when such food was placed in a temperature that was previously stimulating them into locomotory activity. Since feeding reactions appeared to overrule temperature selection it was decided not to feed the larvae during an experiment, although they had excess food

available immediately before, and restrict the experimental time to 60 min.

2/ Lethal Temperature Studies with *Aedes aegypti* Larvae

Experiments were conducted using fourth-instar larvae reared at 20, 25, 30, and 31.5 C. An attempt was made to use larvae of the same physiological age. Larvae to be used for experimentation were collected from the rearing trays at the first appearance of pupae in these trays. For larvae reared at 20 C pupation began at approximately 210 hr after hatching, whilst for larvae reared at 30 C it began 94 hr after hatching. All the larvae used for experimentation, except those reared at 25 C and 111 hr old, were regarded as "old" fourth-instars since they were within 12 hr of pupation. The thermal resistance of the 111 hr old larvae ("young" fourth-instars) was compared with that of the "old" larvae (132 hr old) reared at 25 C to determine the effect of instar age on thermal resistance. The experimental technique consisted of separating seven batches of 50 larvae, of the same age (± 1 hr) and from the same rearing temperature, and collecting each batch in a large pipette. A container was removed from the lethal tray and the larvae were transferred to it, the container was then replaced. A total of six batches of larvae were introduced into the lethal tray at one minute intervals. This method ensured that the temperature of the lethal tray remained constant (temperature drop $<$ 0.2 C) during the transfer of the larvae. The seventh batch of larvae were placed in a tray at 30 C and acted as a control. Each batch of larvae, except the control, was exposed to the

test temperature for a different time period, measured with a stop-watch, and then placed in a tray at 30 C. Due to the delayed lethal effect of high temperatures, mortality could not be recorded until several hours after the exposure. Larvae continued to die up to 48 hr after the exposure; little mortality occurred after 48 hr. Thus, final mortality was not recorded until 48 hr after the start of the exposure to the high high temperature. An insect was recorded as dead if it showed no movement when stimulated with a glass rod.

Analysis of Results

The analysis of the results obtained in selected temperature studies presents a major difficulty. One problem is what statistic, or statistics, should be used to describe the selected temperature? Gunn (in Fraenkel and Gunn, 1961 p. 202) suggested that the selected temperature should not be regarded as a single point, but as a zone. The problem then, of course, is, what are the limits of this zone? He suggested that when describing selected temperature a histogram is always necessary, and that, if it resembles a normal distribution, the mean ± standard deviation is a proper measure of the extent of the selected range. Nielsen and Nielsen (1959) were critical of gratuitously assuming the view that selected temperature determinations fit a normal distribution pattern. They could find no way of making their data fit a normal curve. Chapman (1965) discussed this problem and concluded that the only suitable method of determining selected temperature was by the inspection of the results plotted as a histogram. In the present study the results of the experiments have been presented as tables and

histograms. It was felt that the behaviour patterns seen in these studies were best expressed as histograms. Tables have been included to record the actual numbers of insects observed in each temperature range during the experiments. The terms " selected temperature range " and " modal selected temperature " have been used to mean the interquartile range and the mode of the final frequency distribution plot respectively.

The lethal temperature studies were treated as standard dose-effect experiments (Bliss, 1935). The effect, per cent mortality, was plotted against dosage, exposure time in minutes, on probability x logarithmic paper. The methods used for the analysis of the data were those of Litchfield and Wilcoxon (1949) and are shown in the Appendix. The ED_{50}, effective dose to kill just 50% of the sample, was used as the measure of larval resistance to high temperatures.

RESULTS

Selected Temperature Studies using Simuliids

These experiments were of a preliminary nature and only four were carried through to completion.

1/ Cnephia mutata - standing water.

Sixth-instar larvae, collected at 3.5 C and acclimated in the laboratory for 20 days at 7 C, were used for the experiments.

In the initial experiment a static temperature gradient of 5-40 C was used. When placed in the trough two types of behaviour were observed. All larvae immediately attached either to the sides or the bottom of the trough, or to the thermistor probes. Most remained stationary with an occasional opening and closing of their head fans, but a few proceeded to crawl actively, by looping, either up or down the temperature gradient. These active larvae rarely changed their direction unless obstructed by a thermistor probe or the end of the trough. It was expected that the larvae would stop and turn back when encountering temperatures of about 30 C (avoiding reaction, klino-kinesis; Fraenkel and Gunn, 1961 p. 211). However, this was not so, they continued moving, often at an increased rate, until they reached the end of the trough where they died within seconds. This experiment was not carried through to completion.

The other experiment with 34 larvae lasted 90 min. It can be divided into three parts of 30 min.

a) 0 - 30 minutes

During this period the temperature gradient remained almost constant and static at 0-12 C. Many larvae had moved up the gradient and came to rest at the 'hot end' (Table 1; Fig. 1a). This suggested that they were selecting a temperature above 10 C (Table 1; Fig. 2a).

b) 30 - 60 minutes

Immediately after the larval positions were recorded at time 30 min the heat was increased and the temperature gradient began to shift, so that at time 60 min it ranged from 0-18 C. During this period the temperature change at any given point averaged about 3 C. Results show that the larval positions changed little (Table 1; Fig. 1b) and that the large observed frequency at 10-12 C seen during the first 30 min of the experiment was drastically reduced (Table 1; Fig. 2b).

c) 60 - 90 minutes

During this period the gradient was continually increasing until at time 90 min it ranged from 2-27 C. Results again show that there was little positional change by the larvae (Table 1; Fig. 1c), and now the selected temperature appeared to be around 20 C (Table 1; Fig. 2c).

2/ Simulium venustum - standing water

Twenty-four third-instar larvae, collected at 16 C and acclimated in the laboratory at 17 C for two days were used in an experiment which lasted 70 min.

It is convenient to divide this experiment into two parts of 35 min.

a) 0 - 35 minutes

During this period the temperature ranged from 3-20 C. The most

obvious behaviour was a movement of larvae away from the hot end of the trough and an aggregation about the centre (Table II; Fig. 3a). The larvae appeared to be selecting a temperature of about 13 C (Table II; Fig. 4a).

b) 35 - 70 minutes

During this period the temperature range continued to increase, the hot end of the trough reaching 26 C. The movement of the larvae away from the higher temperatures was continued, so that now the aggregation was well into the coldest half of the trough (Table II; Fig. 3b). Once again the selected temperature was around 13 C, with a minor aggregation at 7 C (Table II; Fig. 4b).

The final results obtained (Figs. 4, 5) were :

Median temperature..... 11.8 C

Modal selected temperature..... 13 C

Selected temperature range..... 8 - 14 C

3/ Prosimulium magnum - flowing water

Larvae were collected at 3 C and acclimated in the laboratory for eight days at 7 C.

Fifty fifth-instar larvae were introduced at the head of the experimental stream, position 24, and were immediately carried downstream. They attached between positions 24 and 9, in a temperature range of 8-25 C. During the next 30 min the gradient had decreased to 8-22 C and two larvae had moved downstream. During the next 20 min the steam was shut off and the trough temperature fell to a uniform 8 C. A total of three larvae had moved downstream to the mesh barrier at position 1. For the next 30 min, 50-80 min from the start of the experiment, the steam

was on and the gradient in the entire trough had reached 8-38 C at 80 min (8-25 C from position 24-9). The only change in the larval positions was that one more larva had moved downstream. The four larvae now at the mesh barrier were dead. The heat was turned off once again,,the temperature gradient disappeared, no larva moved and the experiment was terminated after 100 min (from the start of the experiment).

4/ Simulium vittatum - flowing water

Young larvae were collected at 3.5 C and kept in the laboratory for one month at 7.5 C. Forty-six of these, now in the sixth-instar,were evenly spaced in the trough and allowed to attach. The temperature gradient was 5-12 C. The trough was inclined and water flowed into it. After 120 min little temperature change had occurred and no larva had moved its position. During the following 150 min the temperature gradient was shifting continuously. There was no significant change in larval positions (Fig. 6).

Selected Temperature Studies using Culiseta inornata

Distribution in a Trough at Uniform Temperature. Larvae reared at 20 C

The results from three experiments with first-instar larvae in the trough with the water a uniform 20 C are given in Table III and Fig. 7. This type of behaviour, insects forming minor aggregations throughout the trough length, was typical of every instar.

Distribution in a Shifting Temperature Gradient - Primary Acclimation

1/ First-instar larvae reared at 20 C

Three experiments each with 20 larvae, and three with 10 larvae were conducted. The larvae were 24 ± 3 hr old (after hatching).

Table IV shows the results obtained from each experiment, and the cumulative total of larvae selecting the highest indicated temperature or less. The histogram (Fig. 8) was constructed from the pooled results of the six experiments, and the frequency curve (Fig. 8) from the cumulative total.

The final results obtained were :

Median temperature..... 21.2 C

Modal selected temperature..... 21 C

Selected temperature range..... 18.2 - 24.0 C

2/ Second-instar larvae reared at 20 C

Four experiments with 20 larvae each, and one with 10 larvae were conducted. The instar age was 21 ± 3 hr.

In one experiment the larvae showed an 'ideal' behaviour pattern, and thus it is recorded in detail. Twenty larvae were spaced equally in the trough, the temperature gradient being 14-28 C. The larvae showed a very precise temperature selection, remaining within the zone of 20-26 C throughout the experiment (Fig. 9). In the other four experiments the behaviour pattern was similar but the larvae were dispersed over a greater temperature range.

Table V and Fig. 10 show the results obtained, which were :

Median temperature..... 22.1 C

Modal selected temperature..... 23 C

Selected temperature range..... 20.1 - 23.7 C

3/ Third-instar larvae reared at 20 C

Five experiments were conducted, one with 10 larvae and four with 20 larvae. Instar age was 24 ± 3 hr.

Table VI and Fig. 11 show the results obtained, which were :

Median temperature..... 21.6 C

Modal selected temperature..... 23 C

Selected temperature range..... 19.0 - 23.8 C

4/ Fourth-instar larvae reared at 20 C

Five experiments were conducted, one with 10 larvae and four with 20 larvae. Instar age was 24 ± 3 hr.

Table VII and Fig. 12 show the results obtained, which were :

Median temperature..... 21.5 C

Modal selected temperature..... 21 C

Selected temperature range..... 19.0 - 23.7 C

5/ Male pupae reared at 20 C

Four experiments were conducted, three with 20 pupae each and one with 10, with insects having an instar age of 36 ± 12 hr.

In control experiments with the water at a uniform 20 C all pupae were inactive. After the initial activity caused by placing them in the trough they were never seen to move spontaneously. When distributed in a temperature gradient, pupae came to rest at temperatures below 32 C. The work sheet presented (Fig. 13) is typical of pupal behaviour. In this experiment they maintained a position on the coolest side of the 30 C isotherm as the temperature increased. However, as this isotherm moved back, from time 45 min onwards, the pupae did not move with it. The work sheets from the other three experiments were basically the same as the one figured; the high temperature which initiated locomotory activity was sharply defined, the low temperature was less obvious. The thermal indifference zone, using initiation of locomotory

activity as the criterion, of the pupae in each of the four experiments is recorded below :

Experiment	Thermal indifference zone
#1	18 - 30 C (Fig. 13)
#2	18 - 32 C
#3	18 - 28 C
#4	14 - 32 C

One experiment was conducted with 10 male pupae 18 ± 3 hr old. These were active and the greatest frequency was observed between the 18 C and 20 C isotherms (Table VIII; Fig. 14).

Distribution in a Shifting Temperature Gradient - Secondary Acclimation

Third-instar larvae secondarily acclimated to 5 C

In the first experiment, 10 larvae reared at 20 C and of instar age 20 ± 3 hr were kept at 5 C for 70 hr before being placed in the experimental trough. Table IX and Fig. 15 show the result, which was :

Modal selected temperature..... 17 C

In the other experiment, 10 larvae reared at 20 C (20 ± 3 hr old), were kept at 5 C for 96 hr before being placed in the trough. The experiment was planned to last 90 min, but towards the end of this period the larvae appeared to be selecting a higher temperature than that selected at the beginning of the experiment. Thus the experiment was continued for a further 45 min. Table X and Fig. 16 show the results, which were :

	0 - 90 min	90 - 135 min
Median temperature	12.8 C	16.0 C
Modal selected temperature	13 C	17 C

	0 - 90 min	90 - 135 min
Selected temperature range	9.4 - 16.2 C	14.3 - 17.8 C

Distribution in a Static Temperature Gradient - Primary Acclimation

Second-instar larvae reared at 20 C

The results from the three experiments, each with 20 second-instar larvae 21 ± 3 hr old, are given in Table XI and Fig. 17.

Median temperature..... 19.0 C

Modal selected temperature..... 19 C

Selected temperature range..... 16.2 - 21.5 C

The results from all the temperature selection experiments with Culiseta inornata are shown in Fig. 18.

Selected Temperature Studies using Aedes aegypti

Larvae were reared at 16, 20, 25, 30, 31.5, and 35 C. For each rearing temperature five experiments, each with 20 fourth-instar larvae, were conducted. Thus the final results are based on 3000 observations.

Control experiments, the trough temperature uniform and the same as the rearing temperature, were conducted with larvae from each rearing temperature. The one experiment cited, Table XII and Fig. 19, is typical of the result obtained from every control experiment. The larvae consistently aggregated at both ends of the trough.

Distribution in a Static Temperature Gradient - Primary Acclimation

1/ Larvae reared at 16 C

Larval age varied between 14 and 16 days after hatching. Table XIII shows the result of each experiment and the cumulative total of

larvae selecting the highest indicated temperature or less. The histogram and frequency distribution curve (Fig. 20) were constructed from the pooled results of the five experiments and the cumulative total respectively. The final results obtained were :

Median temperature..... 23.7 C

Modal selected temperature..... 22.0 C

Selected temperature range..... 21.2 - 26.6 C

2/ Larvae reared at 20 C

The larvae were 10-11 days old. Table XIV and Fig. 21 show the results, which were :

Median temperature..... 24.5 C

Modal selected temperature..... 24.5 C

Selected temperature range..... 21.6 - 27.8 C

3/ Larvae reared at 25 C

Larval age was 114 ± 4 hr. Table XV and Fig. 22 show the results, which were :

Median temperature..... 26.9 C

Modal selected temperature..... 27.5 C

Selected temperature range..... 23.7 - 29.5 C

4/ Larvae reared at 30 C

Larval age was 90 ± 6 hr. Table XVI and Fig. 23 show the results, which were :

Median temperature..... 29.3 C

Modal selected temperature..... 30.5 C

Selected temperature range..... 26.9 - 31.0 C

5/ Larvae reared at 31.5 C

Larval age was 90 ± 5 hr. Table XVII and Fig. 24 show the results, which were :

Median temperature..... 31.1 C

Modal selected temperature..... 31.5 C

Selected temperature range..... 29.3 - 32.5 C

6/ Larvae reared at 35 C

Larval age was 72 ± 3 hr. Table XVIII and Fig. 25 show the results, which were :

Median temperature..... 31.5 C

Modal selected temperature..... 32.5 C

Selected temperature range..... 29.8 - 33.0 C

Distribution in a Static Temperature Gradient - Secondary Acclimation

Experiments were conducted to determine the rate at which larvae acclimate.

1/ Larvae reared at 20 C

In one experiment 20 larvae were secondarily acclimated at 31.5 C for 12 hr, and in another for 24 hr, before being placed in the temperature gradient. The results obtained are shown in Table XIX and Fig. 28, and were :

Secondary acclimation period	Modal selected temperature
12 hr	29.5 C
24 hr	31.5 C

2/ Larvae reared at 25 C

A total of four batches of 20 larvae each were secondarily acclimated at 31.5 C; one each for 2, 6, 12, and 24 hr. Table XX and

Figs. 29, 30 show the results, which were :

Secondary acclimation period	Modal selected temperature
2 hr	29.5 C
6 hr	31.5 C
12 hr	31.5 C
24 hr	30.5 C

3/ Larvae reared at 35 C

Two experiments were conducted with larvae secondarily acclimated for 24 hr at 16 C. Since the behaviour of the larvae was similar in each experiment the results were pooled. Initially the larvae formed a loose aggregation at the cold end of the trough, but as the experiment progressed they moved towards the hot end. The result of this behaviour pattern can be seen in Table XXI and Fig. 31 where the observations have been divided into ten-minute periods. The results obtained were :

Period	Modal selected temperature
0-10 min	17.5 C
10-20 min	24.5 C
20-30 min	23.5 C
30-40 min	26.5 C
40-50 min	26.5 C and 28.5 C
50-60 min	26.5 C

Although the mode was higher for the 10-20 min period than for the 20-30 one, the histograms (Fig. 31 a-f) suggest that the larvae, as a whole, were selecting a higher temperature during the 20-30 min period. The significance of this warmward shift was tested statistically. The

results from the two time periods were arranged in a contingency table in which the distribution of larvae up to the 25 C interval was compared with the distribution beyond this range. The probability that the calculated value of Chi-squared (20.6) would occur by chance alone was less than 0.01. Thus significantly more larvae selected above 25 C during the 20-30 min period than during the 10-20 min period.

During the 40-50 min period larval distribution was bi-modal at 26.5 C and 28.5 C, whilst it was uni-modal at 26.5 C during the 50-60 min period. The results from these two time periods were compared, by means of a contingency table and the Chi-squared test, to determine whether there was a coldward shift of larvae during the 50-60 min period. The distribution of larvae up to the 27 C interval was compared with the distribution beyond this range. The Chi-squared value obtained (2.56) was not significant (probability >0.05). Thus, there was no significant difference between the number of larvae selecting above 27 C during the two time periods, and, therefore, no significant coldward shift of larvae during the 50-60 min period.

The results of all the experiments with Aedes aegypti are shown in Fig. 32.

Lethal Temperature Studies with *Aedes aegypti* Larvae

Delayed mortality

The result of the set of experiments cited, larvae reared at 30 C and exposed to 41 C (Table XXII; Fig. 33), is typical of the delayed mortality effect seen throughout the experiments. Mortality continued up to 48 hr after the exposure.

Larvae exposed to 41 C

1/ Larvae reared at 20 ± 0.5 C

Larval age was 210 ± 6 hr (after hatching). The results of the eight experiments conducted, each with 50 larvae per dose, are shown in Table XXIII. The calculation of the regression line (Fig. 34) is shown in Table XXIV, and further analysis of the data in Table XXV. The final result was :

ED_{50} (95% confidence limits) = 46 (38-57) minutes

2/ Larvae reared at 25 ± 0.5 C

a) Larvae 132 ± 3 hr old

The results of the six experiments conducted, five with 50 larvae per dose and one with 25, are shown in Table XXVI. The calculation of the regression line (Figs. 34, 35) is shown in Table XXVII, and further analysis of the data in Table XXVIII. The final result was :

ED_{50} (95% confidence limits) = 63 (51-76) minutes

b) Larvae 111 ± 3 hr old

The results of the five experiments conducted, each with 50 larvae per dose, are shown in

Table XXIX. The calculation of the regression line (Fig. 35) is shown in Table XXX, and further analysis of the data in Table XXXI. The final result was :

ED_{50} (95% confidence limits) = 77 (62-94) minutes

3/ Larvae reared at 30 ± 0.5 C

Larval age was 94 ± 3 hr. The results of the five experiments conducted, each with 50 larvae per dose, are shown in Table XXXII. The calculation of the regression line (Fig. 34) is shown in Table XXXIII, and further analysis of the data in Table XXXIV. The final result was :

ED_{50} (95% confidence limits) = 100 (81-124) minutes

4/ Larvae reared at 31.5 ± 0.5 C

Larval age was 90 ± 3 hr. The results of the nine experiments conducted, each with 50 larvae per dose, are shown in Table XXXV. The calculation of the regression line (Figs. 34, 36) is shown in Table XXXVI, and further analysis of the data in Table XXXVII. The final result was :

ED_{50} (95% confidence limits) = 109 (100-119) minutes

The effect of the rearing temperature on the subsequent larval resistance to 41 C was compared by means of the "Potency Ratio" test (Litchfield and Wilcoxon, 1949). This test, using the ED_{50}'s and their factors, the slope function and their factors, showed no significant difference (19/20 probability) in resistance between the larvae reared at 30 C and those reared at 31.5 C, nor between the two age classes of larvae reared at 25 C. However, there was a significant difference in

resistance between larvae reared at 20 C and those reared at 25 C (132 hr old), between those reared at 25 C (132 hr old) and those reared at 30 C, and, of course, between those reared at 20 C and those reared at 31.5 C. The results were :

Rearing temperatures (°C)	Resistance ratio (95% confidence limits)
25 (132 hr) : 20	1.4 (1.0-1.8)
30 : 25 (132 hr)	1.6 (1.2-2.1)
31.5 : 20	2.3 (1.8-2.9)

Two other sets of experiments were conducted, each experiment with 50 larvae per dose, in the attempt to determine the upper incipient lethal temperature of fourth-instar larvae reared at 31.5 ± 0.5 C and 90 ± 3 hr old.

1/ Larvae exposed to 40 C

The results of the six experiments conducted are shown in Table XXXVIII. The calculation of the regression line (Fig. 36) is shown in Table XXXIX, and further analysis of the data in Table XL. The final result was :

ED_{50} (95% confidence limits) = 254 (222-290) minutes

2/ Larvae exposed to 43 C

The results of the six experiments conducted are shown in Table XLI. The calculation of the regression line (Fig. 36) is shown in Table XLII, and further analysis of the data in Table XLIII. The final result was :

ED_{50} (95% confidence limits) = 14.8 (11.2-19.5) minutes

The potency ratio test was used to compare the effects of the three exposure temperatures on the larvae reared at 31.5 ± 0.5 C. The results were :

Exposure temperatures (°C)	Potency ratio (95% confidence limits)
41 : 40	2.3 (2.0-2.7)
43 : 41	7.4 (5.5-9.9)
43 : 40	17.2 (12.6-23.3)

DISCUSSION

Selected Temperature

The present study was to determine the effects of age and thermal acclimation on the selected temperature, and as such, at least for the mosquito species, it was successful. Any extension of these results into the mechanism of temperature selection would be speculative. Nevertheless, the behaviour involved appeared to be of the ortho-kinetic type; the insects aggregated within certain temperature limits because they were initially more active outside these limits. Such a behavioural effect is probably due to a change of temperature (Fraenkel and Gunn, 1961 p. 211).

Simuliid larvae from a lotic habitat

Although simuliid larvae are adapted for attachment to the substratum, they are capable of active locomotion upstream and downstream. Also, larvae are known to detach from the substrate when conditions are unfavourable and to drift passively downstream, usually on a silken thread. Such unfavourable conditions have been recorded as silt accumulating around the larvae (Wu, 1931), excessive sunlight (Davies, 1949), and an alteration in the current velocity (Wu, 1931; Phillipson, 1956). No evidence was found in the literature to suggest that a change in water temperature would initiate larval detachment, although no one appears to have looked specifically for this factor. The present study, with flowing water, indicates that temperature per se or a change in temperature will not initiate detachment, and, therefore, black-fly

larvae cannot be considered to exhibit any temperature selection. Thus it is suggested that if temperature x time accumulations should exceed the tolerance limit for survival, the larvae will be killed *in situ*. Such a hypothesis is comparable with the results of a study on the effects of insecticides on *Simulium* larvae (Jamnback and Frempong-Boadu, 1966). They found that in flowing water, larvae killed by high doses of DDT remained *in situ* in apparently normal feeding positions. Low doses of DDT were thought to cause contraction of the anal disc, thus loosening the hooklets holding the larva to the substrate (Field, 1961), but this reaction seems not to be elicited by high temperatures. Another possibility is that the temperatures reached during the present experiments were still within the larva's tolerance zone for survival, and therefore, did not initiate detachment. However, this is unlikely since Davies and Smith (1958) showed that the ED_{50} of *Prosimulium* larvae (acclimated to 4-5 C) exposed to 22 C was between one and two days, and in the present study the temperature reached 38 C in the experiment with *Prosimulium magnum* and 31 C in the one with *Simulium vittatum*. Only one record was seen in the literature of locomotion not being initiated by temperatures that would possibly prove to be fatal within a short period of time. Grossman (1929) found that locomotory activity in boll weevils was not initiated by high temperatures below 55 C.

The results obtained with simuliid larvae in standing water are conflicting. Locomotory activity was greater than in the experiments with flowing water. Possible explanations are that the larvae were stimulated into activity by the lack of a water current, or that in flowing water the current was inhibiting locomotion. The next

consideration is the direction in which they moved. The Cnephia mutata larvae moved up the temperature gradient and aggregated at the end of the trough. Since these larvae could not be made to move back down the trough it is assumed that they were showing no precise temperature selection. However, the fact that they did aggregate at the "hot" end of the trough suggested that they were capable of some temperature selection, if only by moving away from colder water. The experiment with Simulium venustum showed the opposite effect. The larvae continually avoided high temperatures and aggregated around 13 C. Although the cold stupor temperature of larvae acclimated to 17 C was not determined, it seems possible that the minor aggregation of larvae at 7 C was due to this metabolic effect of temperature. Since C. mutata larvae are usually found in cooler water than S. venustum, the differences in behaviour seen between them may be more related to the fact that the former were mature larvae and near pupation whilst the latter were third-instars and still actively feeding, and less related to a species difference. Hafez (1950) found that feeding larvae of blow-flies selected a temperature range of 8-20 C but that fully fed larvae were not stimulated into locomotory activity by high temperatures less than 33 C.

Culiseta inornata larvae of different instars

The results of the experiments with C. inornata suggested that there was no change in the selected temperature throughout larval development. Although the modal selected temperatures did differ, these differences cannot be regarded as significant since they were never greater than the range into which the larvae were grouped. Omardeen (1957) studied Aedes aegypti and found that although the selected

temperature did not vary significantly between instars, the immature stages became progressively more selective throughout development with pupae the most selective stage. Applying this argument to the present study it is seen that the second-instar larvae were the most selective with 31% of the observations being within ± 1 C of the mode, third-instar larvae the next with 23%, fourth-instars with 21%, and first-instar larvae the least selective with 19%. Pupae of C. inornata with an instar age of less than 21 hr can be regarded as having the same selected temperature as the larvae. Pupae with an instar age greater than 24 hr had a much wider temperature tolerance, being indifferent, from the point of view of locomotory activity, to temperatures between 18 C and 32 C. This disagrees with the behaviour of Ae. aegypti pupae (Omardeen, 1957). Pupae of this species were intolerant of temperatures outside the range 28-32 C, and during the experiments as many as 80% of the pupae aggregated within this range. The fact that C. inornata larvae selected temperatures lower than 24 C whereas maturing pupae did not respond to high temperatures lower than 32 C, may be of adaptive significance. Recent studies on the thermal resistance of C. inornata have shown that the deleterious effects of high temperatures are cumulative through the instars (Hanec and Brust, 1967). None of their larvae lived to attain the fourth-instar when removed from 21 C to 29 C during the second-instar. However, when the larvae were reared at 21 C to the fourth-instar and then transferred to 29 C, 72% emerged as healthy adults. I suggest that at temperatures up to 32 C development is so rapid that temperature x time combinations can never accumulate to cause significant mortality.

Few workers have studied the effect of age on the selected temperature. The work of Omardeen (1957) has been mentioned. Deal (1941) studied stored-product pests, mostly beetles, and found that the immature stages had the same selected temperature as the adults, Other results are contradictory. Bodenheimer (1929) found that there was a marked increase in the selected temperature with each instar of Schistocerca gregaria, ranging from 27-28 C for the first-instar nymphs to 37-38 C for the fifth-instar. Chapman (1965) working with the same species could find no change in the selected temperature between different instars. The selected temperature range was wide with a peak at 40 C. Chapman (1965) also studied the possibility that the selected temperature may change within an instar. He was non-commital but suggested that there was an apparent decrease in the selected temperature during the latter part of each instar.

The present study, with C. inornata, on the effect of secondary acclimation to a temperature lower than that of the rearing temperature, showed that the selected temperature decreased and that the extent of the decrease was dependent on the length of the exposure to the secondary acclimation. However, these secondarily acclimated larvae appeared to be continually acclimating and altering their selected temperature. The same phenomenon was observed with Ae. aegypti larvae and will be further discussed later.

When determined in a shifting temperature gradient, the selected temperature range of second-instar C. inornata larvae was higher than the range when determined in a static temperature gradient. This discrepancy is difficult to explain satisfactorily, but it is thought

that the technique used for the determination was a significant factor. The reasoning behind this statement is best explained by reference to the experiment with male pupae (Fig. 13). Initially the insects were dispersed between the 20 C and 28 C isotherms. As the temperature increased, they moved down the gradient in response to the 30 C isotherm. Because of this, an aggregation of pupae formed within the temperature range 26-30 C when the gradient was increasing, 44% of the observations being within this range. When the heat source was removed and the gradient decreased, the insects did not move until the 18 C isotherm reached them. Thus, during the latter half of the experiment only 9% of the observations were between 26-30 C and the selected temperature now appeared to be 20-22 C. This effect, the insects apparently selecting a higher temperature during the period when the gradient was increasing and a lower one when it was decreasing is also seen, although it is less obvious, in the experiment with second-instar larvae (Fig. 9). During the initial heating period, 50% of the larvae appeared to be selecting 22-24 C, but during the cooling period that followed, only 25% selected 22-24 C with the majority, 72%, selecting 20-22 C.

Although the actual selected temperature of C. inornata was undetermined, it is considered that the results of the experiments in a shifting temperature gradient are comparable and that the developmental stage has little effect on the selected temperature.

Acclimation in fourth-instar Aedes aegypti larvae

The results of the experiments with fourth-instar Ae. aegypti larvae showed that the selected temperature varied with the rearing temperature. An increase in the rearing temperature resulted in an

increase in the selected temperature (Fig. 26). From these data the final selected temperature, the point where the selected temperature was the same as the acclimation temperature, was determined as being 31.5 C. Except at the lowest rearing temperature, the extent of the selected temperature range decreased with increasing rearing temperatures up to the final selected temperature. This relationship has been expressed as the percentage of larvae selecting the mode (± 0.5 C) at each rearing temperature (Fig. 27). As the rearing temperature increased, up to the final selected temperature, the larvae became more selective. Omardeen (1957) also determined the selected temperature of fourth-instar Ae. aegypti larvae. He made no mention of the rearing temperature, but since his control experiments were conducted at 23.5 C it is assumed that this was the rearing temperature. For a test time of 45 min, his larvae selected 30 C. This mode is higher than that obtained in the present study, which, by interpolation from Fig. 26, would be about 26.5 C.

No reports were found in the literature on the effect of thermal acclimation on the temperature selected by aquatic insects. However, a few results are available for terrestrial forms. Formica, when acclimated to 4 C selected 23 C, and when acclimated to 28 C selected 33 C (Herter, 1923). This is a 10 C increase in selected temperature for a 24 C increase in acclimation. In the present study there was a 10.5 C increase in selected temperature for a 19 C rise in acclimation temperature. Campbell (1937) recorded a 15 F (65-80 F) increase in the temperature selected by wireworms between early spring and early fall. These insects were collected locally from fields (southern California) but no mention was made of the soil temperature. Nielsen and Nielsen

(1959) recorded a relationship between the selected temperature and the ambient temperature in a pierid butterfly. In January-February when the mean air temperature was 19 C, adults of Ascia monuste selected 22 C, whilst in September (air = 28 C) they selected 27 C.

The results of the present study, with Ae. aegypti, are strikingly similar to those obtained by Pitt et al. (1956) for the common carp, Cyprinus carpio. They acclimated their fish to 15, 20, 25, 30, and 35 C, and the modal selected temperatures obtained were 25, 27, 31, 31, and 32 C, respectively. The final selected temperature was 32 C. This suggests that the physiological and behavioural responses involved in thermal acclimation and temperature selection may be universal for poikilothermic animals.

The experiments conducted to determine the rate of acclimation to a temperature higher than that of the rearing temperature showed that the larvae were fully acclimated within a few hours. Acclimation to a temperature lower than that of the rearing temperature occurred within 24 hr. Neither in the experiments involving secondary acclimation to a temperature higher than that of the rearing temperature, nor in the experiments with larvae primarily acclimated to the rearing temperature, was there any indication that further acclimation was occurring during the experimental period (1 hr). However, in both C. inornata and Ae. aegypti, when the secondary acclimation temperature was lower than the rearing temperature the subsequent selected temperature was transitory, suggesting that the selected temperature was acting as an acclimation temperature even during periods as short as 10 min. Not only did the tolerance for higher temperatures increase, but the tolerance for low

temperatures decreased. These results are comparable with those obtained for the Douglas-fir beetle, Dendroctonus pseudotsugae Hopkins, (Atkins, 1966). Newly emerged beetles, apparently reared at 23 C, were given a choice between 11 C and 22 C; they selected the latter. After several days at 2-4 C the beetles were re-exposed to 23 C for two hours. When placed in the choice chamber they selected 11 C, but after a four hour exposure to 23 C they selected 22 C.

Thermal Resistance

The thermal resistance of fourth-instar Aedes aegypti larvae was determined for a number of acclimation and exposure temperatures. With higher acclimation temperatures there was increased thermal resistance. At an exposure temperature of 41 C, larvae reared at 25 C were between 1.0 and 1.8 (19/20 probability) times more resistant than larvae reared at 20 C, and larvae reared at 30 C were between 1.2 and 2.1 times more resistant than larvae reared at 25 C. These results agree with those of Mellanby (1954), that Ae. aegypti larvae respond to an increase in acclimation temperature by an increase in thermal resistance. Only two other mosquito species appear to have been studied with respect to thermal acclimation. Muirhead-Thomson (1940) found no difference between the thermal death times of larvae of Anopheles minimus Theob. kept at 30 C and similar larvae secondarily acclimated at 35 C for 20 hr. However, when fourth-instar An. quadrimaculatus Say, reared at 85 F, were exposed to 107 F, the ED_{50} was recorded as six minutes, but similar larvae, gradually acclimated to this temperature over a period of 40 min, showed

only 30% mortality even after a 28 min exposure (Love and Whelchel, 1957). The literature on the effects of acclimation on the thermal resistance of other aquatic insects is sparse. Whitney (1939) appears to be the only worker to have attempted to alter the thermal resistance of an aquatic insect (other than a mosquito) by acclimation to another temperature. He acclimated nymphs of a mayfly, Baetis, to a higher temperature for 40 hr but found no significant difference between their thermal resistance and controls from the original temperature. Walshe (1948) found that chironomid larvae from a pond at 20 C had a higher thermal resistance than those from a stream at 15 C. However, it is difficult to say whether this difference was a species difference or was a result of the thermal acclimation. The most thorough work on acclimation and thermal resistance, for terrestrial insects, was that of Baldwin (1952). Studying a parasitic insect, Dahlbominus fuscipennis (Zett.), he recorded that a 12 C increase in the rearing temperature (17 to 29 C) resulted in an increase in the ED_{50} from 290 to 450 min at an exposure temperature of 41 C (Baldwin, 1954). In the present study an increase in the rearing temperature of 11.5 C (from 20 to 31.5 C) resulted in an increase in the ED_{50} from 46 to 109 min. Thermal acclimation also affected the thermal resistance of Drosophila (Maynard-Smith, 1958). For fruit flies reared at 15 C, the mean survival time, at 33.5 C, was 44 min, whereas for insects reared at 25 C it was 112 min.

Although the effects of acclimation were not considered, Bar-Zeev (1957) determined the ED_{50} of fourth-instar Ae. aegypti larvae, apparently reared at 28 C. At an exposure temperature of 41 C, the ED_{50}

was recorded as 205 min, which, compared to the present study, was extremely high (larvae reared at 30 C having an ED_{50} of only 100 min). This difference may, in part, be due to two factors, both of which Bar-Zeev did not mention in her paper. These factors are instar age and rearing conditions (i.e. crowding), the effects of which have been previously discussed in this thesis. Another possibility is that there may be genetically different "thermal strains" of Aedes aegypti.

In the present study, instar age was found to affect larval resistance to 41 C, younger larvae appeared to be more resistant than older larvae, although the difference in the ED_{50}'s was not significant ($P > 0.05$).

Upper incipient lethal temperature

An attempt was made to determine the upper incipient lethal temperature of fourth-instar Ae. aegypti larvae reared at 31.5 C. For fish, the relationship between the ED_{50}'s at different exposure temperatures has been shown to fall on a straight line when time is converted to its logarithm. The temperature at which a break in such a semi-logarithmic plot occurs is the point where mortality due to temperature as a primary cause has ceased. This temperature is the incipient lethal temperature for that acclimation (Fry et al., 1946). For Ae. aegypti larvae reared at 31.5 C, an exposure of 41 C was 2.0 to 2.7 (19/20 probability) times more lethal than an exposure to 40 C, whilst 43 C was 5.5 to 9.9 times more lethal than 41 C, and 43 C was 12.6 to 23.3 times more lethal than 40 C. This relationship between exposure temperatures and ED_{50}'s is shown in Fig. 37. Thermal resistance varies

within and between instars and thus can only be determined for relatively extreme temperatures so as to keep the exposure time as short as possible. In the present study, the ED_{50} for a test temperature of 39 C can be predicted at about 600-700 min, whilst at 38 C the ED_{50} approaches 2000 min. During an exposure time of 10 hr, the rate of dying would change by an unknown amount and possibly some larvae would pupate during the exposure. Thus it was felt that the upper incipient lethal temperature of fourth-instar Ae. aegypti larvae reared at 31.5 C could not be determined.

SUMMARY

Certain black-fly and mosquito larvae were shown to have some control over their body temperature by selecting a temperature range, often quite narrow, when given a choice of temperatures in a linear gradient.

Temperature did not initiate locomotion in Prosimulium magnum and Simulium vittatum larvae, when such larvae were in flowing water. Standing water appeared to initiate locomotion in Cnephia mutata and Simulium venustum larvae, but the direction of larval movement appeared to be influenced by temperature. There was some evidence to suggest that mature larvae were tolerant of higher temperatures than young larvae.

Developmental stage had little effect on the modal selected temperature of Culiseta inornata larvae, although second-instar larvae were the most selective stage. Mature pupae were tolerant of temperatures up to 32 C, whilst larvae and young pupae were intolerant of temperatures above 24 C. Such a difference in tolerance may be of adaptive significance.

The temperature selected by C. inornata and Ae. aegypti larvae was dependent on the immediate past thermal history of the insect. Acclimation to a temperature other than that of the rearing temperature, secondary acclimation, was rapid, occurring in less than two hours in Ae. aegypti larvae.

Culiseta inornata larvae appeared to have a higher selected temperature when subjected to a shifting temperature gradient, than when

placed in a static gradient.

Both the rearing and the exposure temperatures had a profound effect on the thermal resistance of fourth-instar Ae. aegypti larvae exposed to high temperatures. The higher the rearing temperature, the greater the larval resistance to 41 C. An 11.5 C increase in rearing temperature (from 20 to 31.5 C) resulting in a 2.3-fold increase in resistance (from 46 to 109 min). The lower the exposure temperature, the greater the resistance of larvae reared at 31.5 C. A 1 C drop in exposure temperature (from 41 to 40 C) resulting in a 2.3-fold increase in resistance (from 109 to 254 min), whilst a 3 C decrease (from 43 to 40 C) resulted in a 17.2-fold increase (from 14.8 to 254 min) in thermal resistance.

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TABLES

TABLE I

Distribution of sixth-instar Chephia mutata larvae, acclimated in the laboratory to 7°C, in a shifting temperature gradient and standing water.

Trough divisions inches	Observed frequencies 0 - 30 min	30 - 60 min	60 - 90 min
0 - 2	0	0	13
2 - 4	7	11	10
4 - 6	22	13	7
6 - 8	22	28	22
8 - 10	28	19	20
10 - 12	22	20	17
12 - 14	14	13	7
14 - 16	20	23	28
16 - 18	20	25	7
18 - 20	49	52	73
Temperature °C			
0 - 2	2	3	5
2 - 4	13	7	9
4 - 6	18	14	4
6 - 8	42	28	8
8 - 10	43	31	12
10 - 12	86	41	21
12 - 14		38	21
14 - 16		30	12
16 - 18		12	15
18 - 20			23
20 - 22			36
22 - 24			25
24 - 26			2
26 - 28			11

Temperature gradient in trough from 0 - 30 min : 0 - 12°C
30 - 60 min : 0 - 18°C
60 - 90 min : 0 - 27°C

TABLE II

Distribution of third-instar *Simulium vernustum* larvae, acclimated in the laboratory to 17°C, in a shifting temperature gradient and standing water.

Trough divisions inches	Observed frequencies 0 - 35 min	35 - 70 min
0 - 2	16	18
2 - 4	16	29
4 - 6	9	7
6 - 8	19	18
8 - 10	15	23
10 - 12	20	26
12 - 14	18	16
14 - 16	11	8
16 - 18	14	15
18 - 20	9	1
20 - 22	8	0
22 - 24	13	7

Temperature °C			Cumulative total of larvae selecting highest indicated temperature or less
2 - 4	5		5
4 - 6	20	13	38
6 - 8	19	28	85
8 - 10	20	18	123
10 - 12	29	18	170
12 - 14	40	36	246
14 - 16	23	20	289
16 - 18	8	10	307
18 - 20	4	12	323
20 - 22		6	329
22 - 24		4	333
24 - 26		3	336

Temperature gradient in trough from 0 - 35 min : 3 - 20°C
35 - 70 min : 4 - 26°C

TABLE III

Distribution of first-instar Culiseta inornata larvae reared at 20°C. Control : water temperature 20°C.

Trough divisions inches	Observed frequencies Expt. #1	Expt. #2	Expt. #3
0 - 2	22	12	13
2 - 4	8	46	57
4 - 6	13	89	27
6 - 8	43	49	50
8 - 10	41	20	24
10 - 12	5	13	7
12 - 14	14	15	52
14 - 16	19	46	8
16 - 18	48	6	2
18 - 20	87	4	60

TABLE IV

Distribution of first-instar Culiseta inornata larvae, reared at 20°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	#6	Cumulative total
2- 4	*	*	*	*	*	*	
4- 6	*	*	*	*	2	*	2
6- 8	12	1	2	3	12	0	32
8-10	15	10	11	6	10	2	86
10-12	7	10	33	18	11	1	166
12-14	9	14	50	8	12	17	276
14-16	10	15	61	6	22	21	411
16-18	40	32	69	13	28	45	638
18-20	70	62	102	16	77	76	1041
20-22	100	86	147	56	61	65	1556
22-24	110	130	91	71	36	53	2047
24-26	120	126	32	61	20	14	2420
26-28	70	81	2	31	3	6	2613
28-30	31	27	0	4	1	0	2676
30-32	6	6	0	5	0	0	2693
32-34	0	0	*	2	1	0	2696
34-36	0	0	*	*	1	*	2697
36-38	*	*	*	*	3	*	2700
38-40	*	*	*	*	*	*	
Mode :	25°C	23°C	21°C	23°C	19°C	19°C	

*These temperature ranges not present in the trough during the experiment.

TABLE V

Distribution of second-instar Culiseta inornata larvae, reared at 20°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative total
2- 4	*	*	*	*	*	
4- 6	*	*	1	*	*	1
6- 8	*	0	9	3	*	13
8-10	*	3	5	2	*	23
10-12	*	2	19	2	12	57
12-14	0	16	39	0	16	128
14-16	0	15	33	2	17	195
16-18	0	36	26	22	19	298
18-20	11	140	79	35	26	589
20-22	176	219	163	95	74	1314
22-24	267	134	135	207	82	2139
24-26	129	30	83	170	44	2595
26-28	17	3	7	58	13	2693
28-30	0	2	1	3	0	2699
30-32	0	0	0	1	0	2700
32-34	0	*	0	0	0	
34-36	0	*	*	*	0	
36-38	*	*	*	*	0	
38-40	*	*	*	*	*	
Mode :	23°C	21°C	21°C	23°C	23°C	

*These temperature ranges not present in the trough during the experiment.

TABLE VI

Distribution of third-instar Culiseta inornata larvae, reared at 20°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative total
44- 6	*	*	*	*	*	
6- 8	0	0	0	*	*	
8-10	1	2	*	3	1	7
10-12	2	1	*	3	4	17
12-14	7	7	2	7	4	44
14-16	28	24	17	16	2	131
16-18	87	98	31	30	26	403
18-20	105	126	85	74	41	834
20-22	104	133	124	159	104	1458
22-24	119	99	179	166	100	2121
24-26	95	57	82	93	16	2464
26-28	43	42	57	44	2	2652
28-30	6	11	21	5	0	2695
30-32	3	0	2	0	0	2700
32-34	0	0	0	0	0	
34-36	0	0	0	0	0	
36-38	*	*	*	*	*	
Mode :	23°C	21°C	23°C	23°C	21°C	

*These temperature ranges not present in the trough during the experiment.

TABLE VII

Distribution of fourth-instar Culisota inornata larvae, reared at 20°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative total
4- 6	*	*	*	*	*	
6- 8	10	*	*	*	*	10
8-10	7	4	*	0	0	27
10-12	19	5	1	1	5	58
12-14	15	5	3	9	4	93
14-16	23	14	7	37	58	233
16-18	44	47	23	40	58	445
18-20	81	99	43	159	85	912
20-22	52	148	114	159	109	1494
22-24	31	125	195	94	115	2054
24-26	11	86	136	72	93	2452
26-28	4	49	48	24	40	2617
28-30	2	17	23	5	23	2687
30-32	1	1	3	0	3	2695
32-34	0	0	4	0	1	2700
34-36	*	*	*	*	*	
Mode :	19°C	21°C	23°C	20°C	23°C	

*These temperature ranges not present in the trough during the experiment.

TABLE VIII

Distribution of male Culiseta inornata pupae, 18 hrs. old and reared at 20°C, in a temperature gradient.

Temperature °C	Observed frequency
2 - 4	*
4 - 6	5
6 - 8	16
8 - 10	11
10 - 12	9
12 - 14	12
14 - 16	35
16 - 18	47
18 - 20	62
20 - 22	41
22 - 24	26
24 - 26	32
26 - 28	2
28 - 30	2
30 - 32	0
32 - 34	0
34 - 36	0
36 - 38	*

Mode : 19°C

*These temperature ranges not present in the trough during the experiment.

TABLE IV

Distribution of third-instar Culiseta inornata larvae, reared at 20°C and acclimated at 5°C for 70 hrs., in a temperature gradient.

Temperature °C	Observed frequency
4 - 6	*
6 - 8	1
8 - 10	12
10 - 12	24
12 - 14	32
14 - 16	48
16 - 18	55
18 - 20	46
20 - 22	19
22 - 24	45
24 - 26	15
26 - 28	3
28 - 40	0
40 +	*

Mode : 17°C

*These temperature ranges not present in the trough during the experiment.

TABLE X

Distribution of third-instar Culiseta inornata larvae, reared at 20°C and acclimated to 5°C for 96 hrs., in a temperature gradient.

Temp. °C	Observed frequencies		Cumulative totals	
	0 - 90 min	90 - 135 min	0 - 90 min	90 - 135 min
0- 2	2	0	2	
2- 4	10	0	12	
4- 6	15	0	27	
6- 8	21	2	48	2
8-10	39	0	87	2
10-12	40	4	127	6
12-14	54	24	181	30
14-16	41	38	222	68
16-18	40	46	262	114
18-20	25	25	287	139
20-22	8	6	295	145
22-24	4	0	299	145
24-26	0	4	299	149
26-28	1	1	300	150
28-30	*	*		
Mode :	13°C	17°C		

*This temperature range not present in the trough during the experiment.

0 - 90 min..... 30 observations
90 - 135 min..... 15 observations

TABLE XI

Distribution of second-instar Culiseta inornata larvae, reared at 30°C, in a static temperature gradient.

Temperature °C	Expt. #1	#2	#3	Cumulative total
2 - 4	*	*	*	
4 - 6	4	14	9	27
6 - 8	5	20	38	90
8 - 10	15	32	21	158
10 - 12	16	25	19	218
12 - 14	44	31	28	321
14 - 16	52	37	48	458
16 - 18	105	46	137	746
18 - 20	153	74	98	1071
20 - 22	103	103	90	1367
22 - 24	55	100	59	1581
24 - 26	31	60	33	1705
26 - 28	17	58	14	1794
28 - 30	*	*	6	1800
30 +	*	*	*	
Mode :	19°C	21°C	17°C	

*These temperature ranges not present in the trough during the experiments.

TABLE XII

Distribution of fourth-instar Aedes aegypti larvae in the trough at a uniform water temperature.
Control : larvae reared at 30°C, trough temperature 30°C

Trough divisions inches	Observed frequency
0 - 2	129
2 - 4	59
4 - 6	31
6 - 8	29
8 - 10	21
10 - 12	24
12 - 14	21
14 - 16	59
16 - 18	54
18 - 20	173

TABLE XIII

Distribution of fourth-instar Aedes aegypti larvae, reared at 16°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative total
11-12	*	*	*	*	*	
12-13	2	5	1	1	2	11
13-14	1	1	0	1	1	15
14-15	0	2	1	1	2	21
15-16	4	8	0	2	4	39
16-17	4	35	2	4	15	99
17-18	6	46	5	13	18	187
18-19	14	48	15	19	42	325
19-20	23	34	6	34	52	474
20-21	30	38	23	77	80	722
21-22	61	40	54	73	63	1013
22-23	63	35	58	82	53	1304
23-24	50	63	62	44	52	1575
24-25	44	84	61	60	38	1862
25-26	46	49	72	52	59	2140
26-27	47	34	38	35	34	2328
27-28	41	28	57	41	20	2515
28-29	55	26	25	34	20	2685
29-30	48	8	31	14	31	2817
30-31	52	9	36	12	11	2937
31-32	3	6	25	0	3	2974
32-33	1	1	15	1	0	2992
33-34	2	0	3	0	0	2997
34-35	2	0	0	0	0	2999
35-36	1	0	0	0	0	3000
36 +	*	*	*	*	*	

*These temperature ranges not present in the trough during the expts.

TABLE XIV

Distribution of fourth-instar Aedes aegypti larvae, reared at 20°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative total
11-12	*	*	*	*	*	
12-13	2	4	1	5	3	15
13-14	3	1	1	2	4	26
14-15	1	3	0	4	13	47
15-16	5	17	1	8	14	92
16-17	9	3	2	7	18	131
17-18	9	16	14	8	22	200
18-19	15	21	18	22	31	307
19-20	26	16	30	46	28	453
20-21	24	42	25	57	35	636
21-22	58	35	26	39	33	827
22-23	65	59	46	57	40	1094
23-24	81	62	41	39	37	1354
24-25	75	53	58	47	54	1641
25-26	69	49	37	34	23	1853
26-27	57	39	64	32	33	2078
27-28	26	47	34	53	48	2286
28-29	31	50	64	36	28	2495
29-30	13	24	45	27	32	2636
30-31	15	16	42	33	28	2770
31-32	9	21	21	13	30	2864
32-33	3	13	19	19	25	2943
33-34	4	7	10	10	12	2986
34-35	0	2	1	2	6	2997
35-36	0	0	0	0	3	3000
36 +	*	*	*	*	*	

*These temperature ranges not present in the trough during the expts.

TABLE IV

Distribution of fourth-instar Aedes aegypti larvae, reared at 25°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative total
13-14	*	*	*	*	*	
14-15	11	19	*	21	1	52
15-16	15	10	*	1	2	80
16-17	5	6	17	3	2	113
17-18	9	10	28	2	4	166
18-19	14	16	21	1	10	228
19-20	12	16	16	5	8	285
20-21	12	18	16	4	19	354
21-22	26	32	35	5	23	475
22-23	41	44	32	14	27	633
23-24	50	39	26	30	37	815
24-25	48	37	37	34	37	1008
25-26	44	41	53	57	56	1264
26-27	61	51	44	57	59	1536
27-28	63	47	40	85	66	1837
28-29	47	40	27	87	74	2112
29-30	30	43	23	85	72	2365
30-31	25	24	42	50	46	2552
31-32	36	32	40	31	31	2722
32-33	20	37	22	20	16	2837
33-34	22	24	22	5	5	2915
34-35	9	14	31	3	5	2977
35-36	*	*	17	*	*	2994
36-37	*	*	6	*	*	3000
37 +	*	*	*	*	*	

*These temperature ranges not present in the trough during the expts.

TABLE XVI

Distribution of fourth-instar Aedes aegypti larvae, reared at 30°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative total
11-12	*	*	*	*	*	
12-13	3	7	7	4	7	28
13-14	2	4	14	0	1	49
14-15	0	4	5	3	4	65
15-16	1	1	3	0	1	71
16-17	5	6	8	1	4	95
17-18	3	6	4	0	0	108
18-19	3	8	6	0	0	125
19-20	0	6	6	0	3	140
20-21	8	14	13	2	1	178
21-22	3	18	19	1	0	219
22-23	0	8	42	4	2	275
23-24	8	27	51	1	2	364
24-25	10	32	46	2	9	463
25-26	13	50	40	7	20	593
26-27	22	69	39	14	24	761
27-28	42	73	59	59	27	1021
28-29	44	116	76	114	54	1425
29-30	59	78	56	91	114	1823
30-31	91	15	62	144	123	2258
31-32	123	19	24	74	94	2592
32-33	112	34	12	45	47	2842
33-34	29	3	3	10	38	2925
34-35	12	2	4	14	17	2974
35-36	7	0	1	10	8	3000
36 +	*	*	*	*	*	

*These temperature ranges not present in the trough during the expts.

TABLE XVII

Distribution of fourth-instar Aedes aegypti larvae, reared at 31.5°C, in a temperature gradient.

Temp. °C	Observed frequencies Expt. #1	#2	#3	#4	#5	Cumulative Total
13-14	*	*	*	*	*	
14-15	*	19	*	2	*	21
15-16	5	6	1	0	*	33
16-17	0	8	0	2	0	43
17-18	1	3	0	1	0	48
18-19	0	1	0	0	0	49
19-20	0	1	0	0	1	51
20-21	4	1	3	0	0	59
21-22	3	5	4	0	0	71
22-23	14	4	5	3	0	97
23-24	1	6	3	3	2	112
24-25	9	8	12	6	5	152
25-26	18	20	13	6	1	210
26-27	40	22	13	11	5	301
27-28	50	28	26	28	15	448
28-29	37	61	38	39	40	663
29-30	53	63	78	59	100	1016
30-31	61	110	88	99	89	1463
31-32	83	104	113	125	165	2053
32-33	61	78	83	99	92	2466
33-34	75	37	66	60	60	2764
34-35	62	7	34	36	25	2928
35-36	21	6	16	14	0	2985
36-37	2	1	3	6	0	2997
37-38	0	1	0	1	0	2999
38-39	*	*	0	0	*	2999
39-40	*	*	1	*	*	3000

*These temperature ranges not present in the trough during the expts.

TABLE XVIII

Distribution of fourth-instar *Aedes aegypti* larvae, reared at 35°C, in a temperature gradient.

Temp. °C	Expt. #1	Observed frequencies #2	#3	#4	#5	Cumulative total
11-12	*	*	*	*	*	
12-13	*	7	*	*	*	7
13-14	8	4	1	*	*	20
14-15	3	1	2	*	2	28
15-16	1	1	1	1	1	33
16-17	0	0	1	1	1	36
17-18	1	1	0	1	1	40
18-19	2	1	1	2	3	49
19-20	0	3	2	0	1	55
20-21	1	5	2	1	4	68
21-22	1	1	1	1	3	75
22-23	0	1	4	0	1	81
23-24	2	6	4	1	3	97
24-25	1	9	7	4	6	124
25-26	9	10	7	11	7	168
26-27	12	28	5	6	13	232
27-28	15	37	14	18	18	334
28-29	16	34	41	20	18	463
29-30	34	84	99	71	53	804
30-31	58	112	100	70	117	1261
31-32	111	81	99	88	87	1727
32-33	94	88	87	115	133	2244
33-34	137	52	76	119	71	2699
34-35	47	18	25	59	40	2888
35-36	30	4	12	6	15	2955
36-37	17	12	9	1	2	2996
37-38	*	*	*	4	*	3000

* These temperature ranges not present in the trough during the expts.

TABLE XIX

Distribution of fourth-instar Aedes aegypti larvae, reared at 20°C and acclimated at 31.5°C, in a temperature gradient.

Temperature °C	Observed frequencies	
	12 hrs acclimation	24 hrs acclimation
14 - 15	*	*
15 - 16	*	2
16 - 17	*	5
17 - 18	*	2
18 - 19	*	1
19 - 20	*	2
20 - 21	*	6
21 - 22	*	6
22 - 23	*	8
23 - 24	57	8
24 - 25	26	10
25 - 26	33	14
26 - 27	40	12
27 - 28	28	32
28 - 29	74	43
29 - 30	114	89
30 - 31	93	126
31 - 32	76	148
32 - 33	31	69
33 - 34	18	15
34 - 35	8	1
35 - 36	1	1
36 - 37	1	0
37 - 38	*	*

*These temperature ranges not present in the trough during the experiments.

TABLE XX

Distribution of fourth-instar *Aedes aegypti* larvae, reared at 25°C and acclimated at 31.5°C, in a temperature gradient

Temp. °C	Acclimated :	Observed frequencies 2 hrs	6 hrs	12 hrs	24 hrs
12-13		*	*	*	*
13-14		*	*	*	61
14-15		*	*	*	6
15-16		*	*	*	2
16-17		*	*	*	0
17-18		*	*	*	1
18-19		2	16	*	2
19-20		2	1	*	1
20-21		4	0	*	1
21-22		7	1	*	1
22-23		9	1	*	2
23-24		19	14	30	6
24-25		30	3	53	4
25-26		33	2	57	12
26-27		44	0	41	21
27-28		49	1	52	22
28-29		54	5	58	46
29-30		77	67	66	72
30-31		62	143	54	154
31-32		72	241	70	100
32-33		70	85	41	50
33-34		37	13	23	24
34-35		16	7	3	12
35-36		13	0	2	*
36-37		*	*	*	*

*These temperature ranges not present in the trough during the expts.

TABLE XXI

Distribution of fourth-instar Aedes aegypti larvae, reared at 35°C and acclimated at 16°C for 24 hours, in a temperature gradient.

Temp. °C	Min :	Observed frequencies 0-10	10-20	20-30	30-40	40-50	50-60
12-13		*	*	*	*	*	*
13-14		2	1	3	3	0	3
14-15		2	0	0	0	1	0
15-16		2	0	1	0	0	0
16-17		16	2	1	1	1	1
17-18		32	4	6	1	0	3
18-19		19	12	2	1	1	1
19-20		7	12	1	1	3	2
20-21		10	21	8	7	3	6
21-22		26	18	23	10	10	6
22-23		18	17	18	14	8	3
23-24		17	33	33	17	19	7
24-25		21	39	21	25	17	13
25-26		14	13	22	25	15	21
26-27		11	11	24	39	25	31
27-28		0	6	13	14	23	19
28-29		1	2	6	16	21	27
29-30		0	2	9	15	25	21
30-31		0	0	3	4	15	17
31-32		0	1	2	4	8	8
32-33		0	4	2	1	3	4
33-34		0	2	2	2	2	5
34-35		2	0	0	0	0	1
35-36		0	0	0	0	0	0
36-37		0	0	0	0	0	1
37 +		*	*	*	*	*	*

*These temperature ranges not present in the trough during the expt.

TABLE XXII

Mortality of fourth-instar Aedes aegypti larvae reared at 30°C and exposed to 41°C.

Dose (min)	Total mortality - % Hours after start of test 11	24	51	72	96
Control	2	2	4	6	6
60	6	26	30	32	34
90	2	32	42	42	42
120	8	42	54	56	58
150	12	62	68	74	74
180	16	60	74	74	74
210	30	64	86	86	88

Larvae kept at 30°C after test period

TABLE XXIII

Mortality of fourth-instar Aedes aegypti larvae reared at 20°C and exposed to 41°C.

Dose (min)	Number dead							
	Expt. #1	#2	#3	#4	#5	#6	#7	#8
Control	3	0	1	0	0	2	0	0
30	14	26	16	13				
40	15	26	28	17	24	26	8	
50	21	38	38	24	32	27	10	
60	24	40	31	37	38	24	24	25
70		42	40	28	50	37	32	
80				28	50	41	25	38

In each experiment, 50 larvae were exposed at each dose.

TABLE XXIV

Calculation of the regression line for the mortality of fourth-instar *Aedes aegypti* larvae reared at 20°C and exposed to 41°C.

X	w	w^2	Y	Z	wZ
30	1.477	2.18	34.5	-0.40	-0.591
40	1.602	2.56	41.1	-0.22	-0.352
50	1.699	2.88	54.3	0.11	0.187
60	1.778	3.15	60.8	0.27	0.480
70	1.845	3.40	76.3	0.72	1.328
80	1.903	3.61	72.8	0.60	1.142
N=6	Σw=10.304	Σw^2=17.78		ΣZ=1.08	ΣwZ=2.194

m = 3.42

k = -5.69

TABLE XXV

Analysis of data from the experiments with fourth-instar Aedes aegypti larvae reared at 20°C and exposed to 41°C

Dose (min)	Dead/ Exposed	% Mortality Observed	Expected	O-E	(Chi)^2*
30	69/200	34.5	26.5	8.0	0.0310
40	144/350	41.1	41.5	0.4	0.0000
50	190/350	54.3	54.3	0.0	0.0000
60	243/400	60.8	65.0	4.2	0.0075
70	229/300	76.3	73.0	3.3	0.0055
80	182/250	72.8	79.0	6.2	0.0230
					Σ(Chi)^2 = 0.0670

*From Litchfield and Wilcoxon (1949) (nomograph #1)

ED_{84} = 91.5 minutes

ED_{50} = 46.4 "

ED_{16} = 23.5 "

S = 1.97

A = 1.67

R = 2.67

fED_{50} = 1.2

fS = 1.6

TABLE XXVI

Mortality of fourth-instar Aedes aegypti larvae reared at 25°C, 132 hr old, and exposed to 41°C.

Dose (min)	Number dead Expt. #1	#2	#3	#4	#5	#6*
Control	0	0	0	0	0	3
30	12	5	13			
45	15	10	11	5	11	7
60	19	20	23	9	24	13
75	38	32	44	21	32	17
90	38	42	38	28	43	21
105	45	45	45	33	41	22

*In experiment #6, 25 larvae were exposed to each dose. In the other experiments #1 to #5, 50 larvae were exposed to each dose.

TABLE XXVII

Calculation of the regression line for the mortality of fourth-instar *Aedes aegypti* larvae reared at 25°C, 132 hr old, and exposed to 41°C.

X	w	w^2	Y	Z	wZ
30	1.477	2.18	15.5	-1.02	-1.507
45	1.653	2.73	21.5	-0.79	-1.306
60	1.778	3.15	39.3	-0.27	-0.480
75	1.875	3.50	66.9	0.43	0.806
90	1.954	3.82	76.4	0.72	1.407
105	2.021	4.08	84.0	0.99	2.001
N=6	Σw=10.758	Σw^2=19.46		ΣZ=0.06	ΣwZ=0.921

m = 4.97

k = -8.92

TABLE XXVIII

Analysis of data from the experiments with fourth-instar Aedes aegypti larvae reared at 25°C, 132 hr old, and exposed to 41°C

Dose (min)	Dead/ Exposed	% Mortality Observed	Expected	O-E	$(Chi)^2$
30	31/200	15.5	6.0	9.5	0.1600
45	59/275	21.5	24.1	2.6	0.0035
60	108/275	39.3	46.5	7.2	0.0200
75	184/275	66.9	65.0	1.9	0.0016
90	210/275	76.4	78.1	1.7	0.0018
105	231/275	84.0	86.5	2.5	0.0050
					$\Sigma(Chi)^2 = 0.1919$

ED_{84} = 99.5 minutes

ED_{50} = 62.5 "

ED_{16} = 39.2 "

S = 1.59

A = 1.22

R = 3.5

fED_{50} = 1.2

fS = 1.3

TABLE XXIX

Mortality of fourth-instar *Aedes aegypti* larvae reared at 25°C, 111 hr old, and exposed to 41°C.

Dose (min)	Number dead Expt. #1	#2	#3	#4	#5
Control	0	0	0	0	0
45	3	0	10	12	19
60	5	11	12	15	22
75	15	14	17	22	22
90	21	27	37	36	47
105	41	34	42	43	42
120	47	41	43	45	47

In each experiment, 50 larvae were exposed at each dose

TABLE XXX

Calculation of the regression line for the mortality of fourth-instar *Aedes aegypti* larvae reared at 25°C, 111 hr old, and exposed to 41°C.

X	w	w^2	Y	Z	wZ
45	1.653	2.73	17.6	-0.93	-1.535
60	1.778	3.15	26.0	-0.64	-1.138
75	1.875	3.50	36.0	-0.36	-0.675
90	1.954	3.82	67.2	0.45	0.879
105	2.021	4.08	80.8	0.87	1.757
120	2.079	4.31	89.2	1.25	2.599
N=6	Σw=11.360	Σw^2=21.59		ΣZ=0.64	ΣwZ=1.387

m = 7.35

k = -13.84

TABLE XXXI

Analysis of data from the experiments with fourth-instar *Aedes aegypti* larvae reared at 25°C, 111 hr old, and exposed to 41°C.

Dose (min)	Dead/ Exposed	% Mortality Observed	Expected	O-E	$(Chi)^2$
45	44/250	17.6	4.5	13.1	0.4000
60	65/250	26.0	22.0	4.0	0.0085
75	90/250	36.0	47.0	11.0	0.0450
90	168/250	67.2	70.0	2.8	0.0030
105	202/250	80.8	84.0	3.2	0.0085
120	223/250	89.2	92.6	3.4	0.0120
					$\Sigma (Chi)^2 = 0.4770$

ED_{84} = 105.0 minutes

ED_{50} = 76.5 "

ED_{16} = 56.0 "

S = 1.37

A = 1.12

R = 2.67

fED_{50} = 1.2

fS = 1.3

TABLE XXXII

Mortality of fourth-instar *Aedes aegypti* larvae reared at 30°C and exposed to 41°C.

Dose (min)	Number dead Expt. #1	#2	#3	#4	#5
Control	0	2	0	0	1
60	8	15	16	0	16
90	18	21	21	21	
120	30	27	42	41	33
150	36	34	41	43	33
180	36	37	47	47	45
210		43	50	50	49

In each experiment, 50 larvae were exposed at each dose.

TABLE XXXIII

Calculation of the regression line for the mortality of fourth-instar *Aedes aegypti* larvae reared at 30°C and exposed to 41°C.

X	w	w^2	Y	Z	wZ
60	1.778	3.15	22.0	-0.77	-1.369
90	1.954	3.82	40.5	-0.24	-0.469
120	2.079	4.32	69.2	0.50	1.040
150	2.176	4.73	74.8	0.66	1.436
180	2.255	5.08	84.8	1.03	2.323
210	2.322	5.38	96.0	1.75	4.060
N=6	Σw=12.564	Σw^2=26.48		ΣZ=2.93	ΣwZ=7.021

m = 5.31

k = -10.63

TABLE XXXIV

Analysis of data from the experiments with fourth-instar Aedes aegypti larvae reared at 30°C and exposed to 41°C.

Dose (min)	Dead/ Exposed	% Mortality Observed	Expected	O-E	$(Chi)^2$
60	55/250	22.0	12.2	9.8	0.0850
90	81/200	40.5	40.5	0.0	0.0000
120	173/250	69.2	65.9	3.3	0.0045
150	187/250	74.8	83.8	9.0	0.0550
180	212/250	84.8	90.8	6.0	0.0400
210	192/200	96.0	95.4	0.6	0.0000
					$\Sigma(Chi)^2 = 0.1845$

ED_{84} = 156 minutes

ED_{50} = 100 "

ED_{16} = 65 "

S = 1.55

A = 1.19

R = 3.5

fED_{50} = 1.2

fS = 1.3

TABLE XXXV

Mortality of fourth-instar Aedes aegypti larvae reared at 31.5°C and exposed to 41°C.

Dose	Number dead								
(Min)	Expt. #1	#2	#3	#4	#5	#6	#7	#8	#9
Control	1	0	0	0	0	0	0	1	0
30	1	0	2						
60	0	4	5	5	10	1	3	6	
90	12	5	6	22	28	9	19	13	19
105			35	18	26	10	28	25	21
120		20		38	46	23	25	22	23
135				35	44	24	37	26	34
150		29	26	43	42	34	37	30	42
180		38	36						44

In each experiment, 50 larvae were exposed at each dose.

TABLE XXXVI

Calculation of the regression line for the mortality of fourth-instar *Aedes aegypti* larvae reared at 31.5°C and exposed to 41°C.

X	w	w^2	Y	Z	wZ
30	1.477	2.18	2.0	-2.06	-3.043
60	1.778	3.15	8.5	-1.39	-2.471
90	1.954	3.82	29.6	-0.54	-0.955
105	2.021	4.08	46.6	-0.09	-0.182
120	2.079	4.30	56.3	0.16	0.333
135	2.130	4.54	66.7	0.44	0.937
150	2.176	4.72	70.8	0.55	1.197
180	2.255	5.08	78.7	0.30	1.804
N=8	Σw=15.870	Σw^2=31.87		ΣZ= -2.13	ΣwZ= -2.380

m = 4.76

k = -9.71

TABLE XXXVII

Analysis of data from the experiments with fourth-instar *Aedes aegypti* larvae reared at 31.5°C and exposed to 41°C.

Dose (min)	Dead/ Exposed	% Mortality Observed	Expected	O-E	$(Chi)^2$
30	3/150	2.0	0.4	1.6	0.0600
60	34/400	8.5	11.0	2.5	0.0067
90	133/450	29.6	34.5	4.9	0.0100
105	163/350	46.6	46.6	0.0	0.0000
120	197/350	56.3	57.5	1.2	0.0000
135	200/300	66.7	66.0	0.7	0.0000
150	283/400	70.8	74.0	3.2	0.0050
180	118/150	78.7	84.4	5.7	0.0230
					$\Sigma(Chi)^2 = 0.1047$

ED_{84} = 178 minutes

ED_{50} = 109 "

ED_{16} = 67 "

S = 1.63

A = 1.16

R = 6.0

fED_{50} = 1.1

fS = 1.1

TABLE XXXVIII

Mortality of fourth-instar *Aedes aegypti* larvae reared at 31.5°C and exposed to 40°C.

Dose (min)	Number dead					
	Expt. #1	#2	#3	#4	#5	#6
Control	1	0	0	1	0	0
120	4	4	7	3	2	2
180	5	8	9	12	3	9
240	17	20	27	15	21	24
300	32	40	31	33	31	34
360	42	43	37	40	45	48
540	48	46	48	48	50	47

In each experiment, 50 larvae were exposed at each dose.

TABLE XXXIX

Calculation of the regression line for the mortality of fourth-instar *Aedes aegypti* larvae reared at 31.5°C and exposed to 40°C.

X	w	w^2	Y	Z	wZ
120	2.079	4.31	7.3	-1.46	-3.035
180	2.255	5.08	15.3	-1.03	-2.323
240	2.380	5.66	41.3	-0.32	-0.525
300	2.477	6.13	67.0	0.44	1.090
360	2.556	6.53	85.0	1.04	2.658
540	2.732	7.46	95.7	1.70	4.644
N=6	Σw=14.479	Σw^2=35.17		ΣZ=0.47	ΣwZ=2.510

n = 6.11

k = -14.67

TABLE XL

Analysis of data from the experiments with fourth-instar *Aedes aegypti* larvae reared at 31.5°C and exposed to 40°C.

Dose (min)	Dead/ Exposed	Observed	Expected	O-E	$(Chi)^2$
120	22/300	7.3	2.4	4.9	0.1000
180	46/300	15.3	18.0	2.7	0.0050
240	124/300	41.3	44.5	3.2	0.0040
300	201/300	67.0	67.5	0.5	0.0000
360	255/300	85.0	82.8	2.2	0.0040
540	287/300	95.7	97.9	2.2	0.0270
					———
					$\Sigma(Chi)^2 = 0.1400$

ED_{84} = 367 minutes

ED_{50} = 254 "

ED_{16} = 175 "

S = 1.45

A = 1.11

R = 4.5

fED_{50} = 1.1

fS = 1.1

TABLE XLI

Mortality of fourth-instar Aedes aegypti larvae reared at 31.5°C and exposed to 43°C.

Dose (min)	Number dead					
	Expt. #1	#2	#3	#4	#5	#6
Control	2	0	0	0	1	0
5	5	3	4	2	5	
10	17	9	7	7	7	11
15	18	17	11	17	16	
20	45	45	45	26	35	29
25	47	44	40	31	46	
30	48	48	46	39	43	40

In each experiment, 50 larvae were exposed at each dose.

TABLE XLII

Calculation of the regression line for the mortality of fourth-instar *Aedes aegypti* larvae reared at 31.5°C and exposed to 43°C.

X	w	w^2	Y	Z	wZ
5	0.699	0.49	7.6	-1.44	-1.006
10	1.000	1.00	19.3	-0.87	-0.870
15	1.176	1.38	31.6	-0.47	-0.553
20	1.301	1.69	75.0	0.67	0.872
25	1.398	1.95	83.2	0.96	1.342
30	1.477	2.18	88.0	1.17	1.728
n=6	Σw=7.051	Σw^2=8.69		ΣZ=0.02	ΣwZ=1.513

m = 3.66

k = -4.29

TABLE XLIII

Analysis of data from the experiments with fourth-instar *Aedes aegypti* larvae reared at 31.5°C and exposed to 43°C.

Dose (min)	Dead/ Exposed	% Mortality Observed	Expected	O-E	$(Chi)^2$
5	19/250	7.6	4.2	3.4	0.0270
10	58/300	19.3	26.5	7.2	0.0260
15	79/250	31.6	51.0	19.4	0.1400
20	225/300	75.0	68.1	6.9	0.0220
25	208/250	83.2	79.0	4.2	0.0100
30	264/300	88.0	87.0	1.0	0.0010
					$\Sigma(Chi)^2$ = 0.2260

ED_{84} = 27.7 minutes

ED_{50} = 14.8 "

ED_{16} = 7.9 "

S = 1.87

A = 1.27

R = 6.0

fED_{50} = 1.3

fS = 1.4

FIGURES

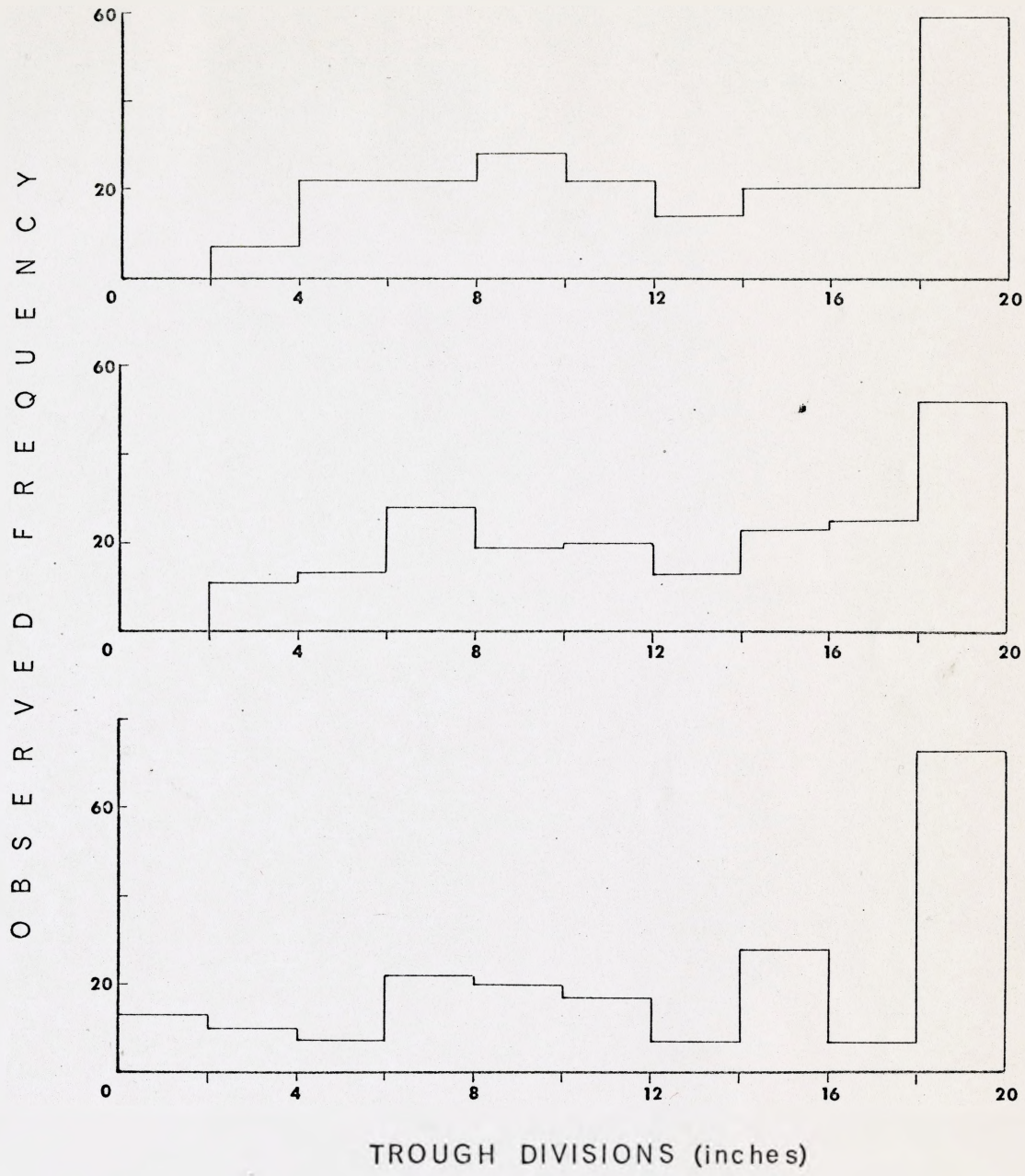


Fig. 1 Distribution of sixth-instar Cnephia mutata larvae, acclimated in the laboratory to 7°C, in a shifting temperature gradient.

a... 0 - 30 minutes, temperature gradient 0°- 12°C

b... 30 - 60 " " " 0°- 18°C

c... 60 - 90 " " " 0°- 27°C

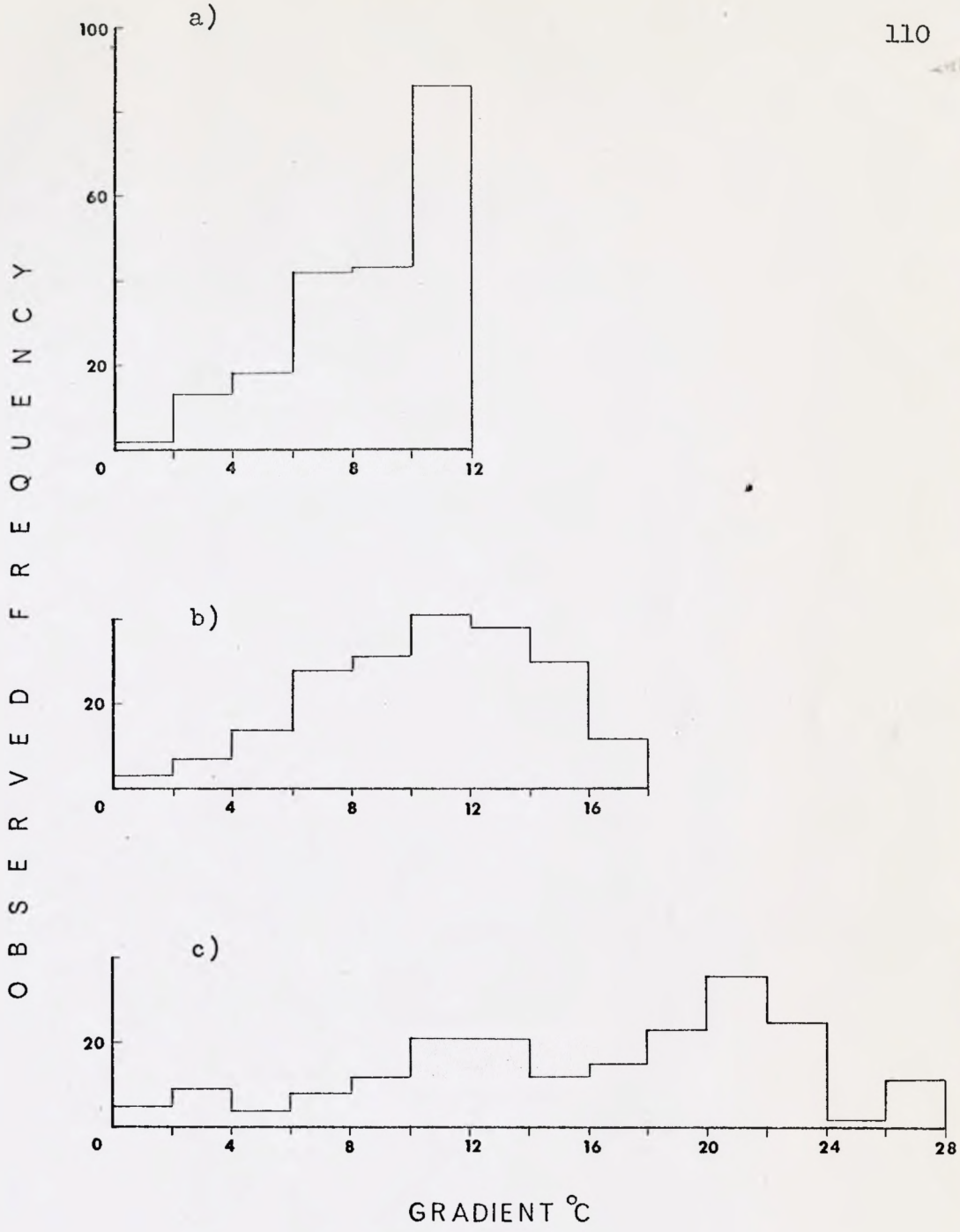


Fig. 2 Distribution of sixth-instar Cnephia mutata larvae, acclimated in the laboratory to 7°C, in a shifting temperature gradient.

a... 0 - 30 minutes

b...30 - 60 minutes

c...60 - 90 minutes

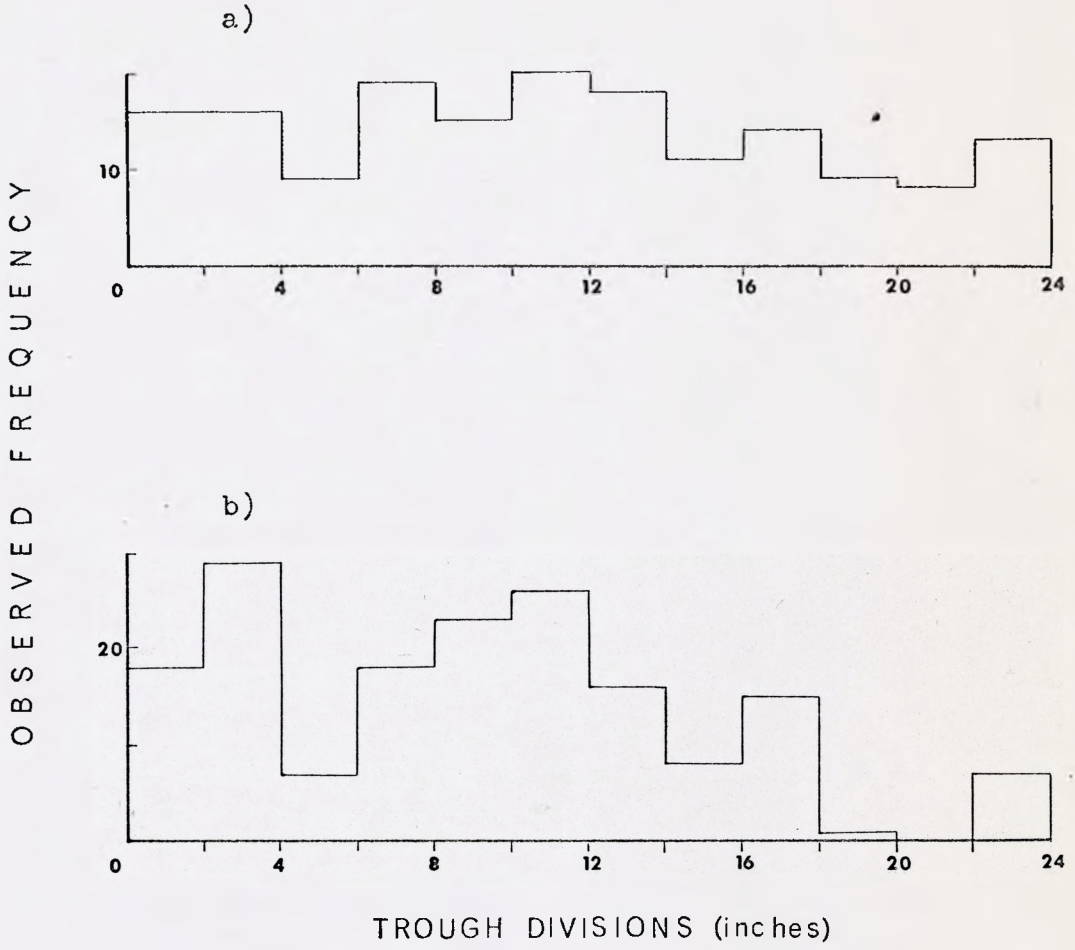


Fig. 3 Distribution of third-instar Simulium venustum larvae, acclimated in the laboratory to 17°C, in a shifting temperature gradient.

a... 0 - 35 minutes, temperature gradient 3°- 20°C

b... 35 - 70 " " " 4°- 26°C

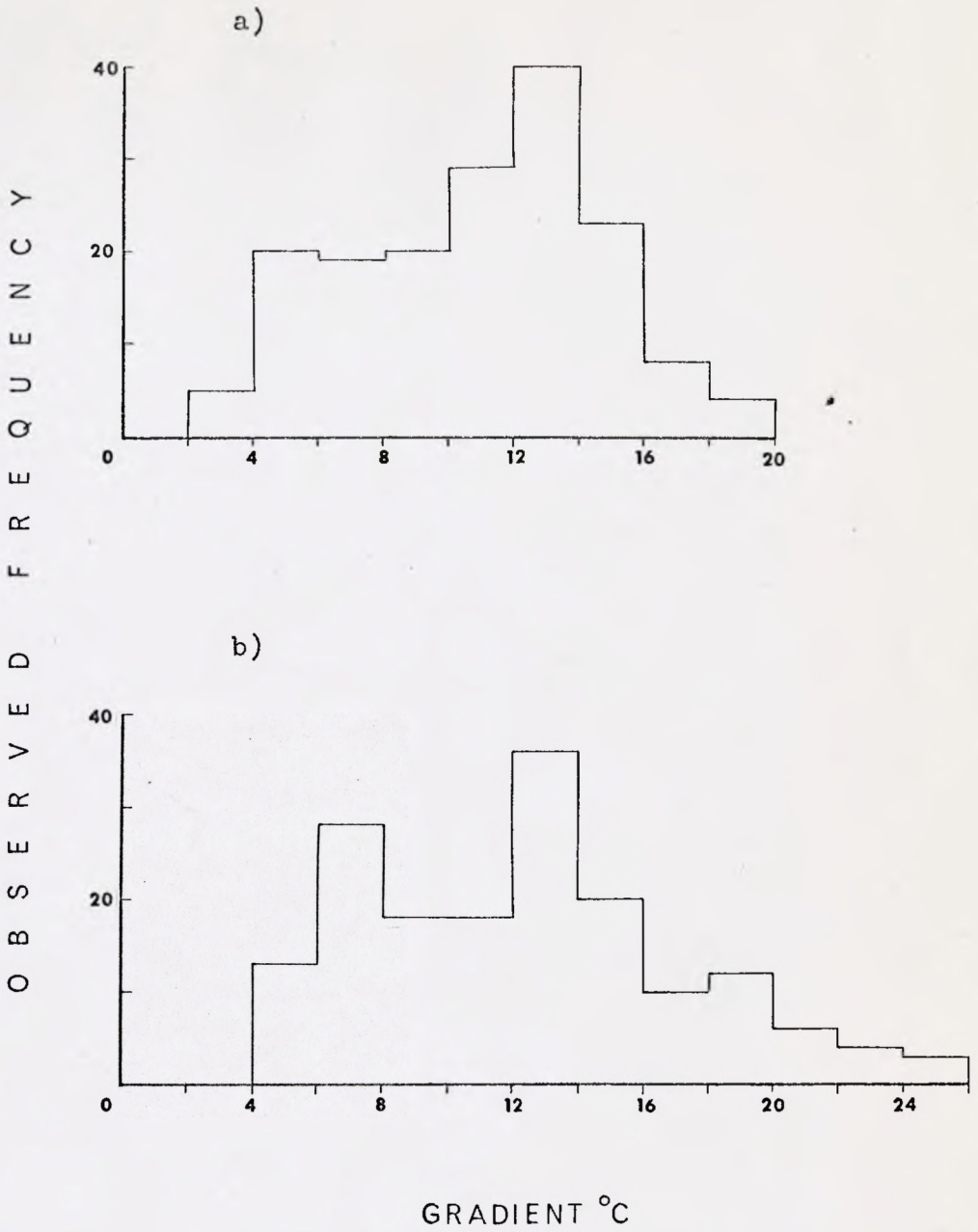


Fig. 4 Distribution of third-instar Simulium venustum larvae, acclimated in the laboratory to 17°C, in a shifting temperature gradient.
a... 0 - 35 minutes
b... 35 - 70 minutes

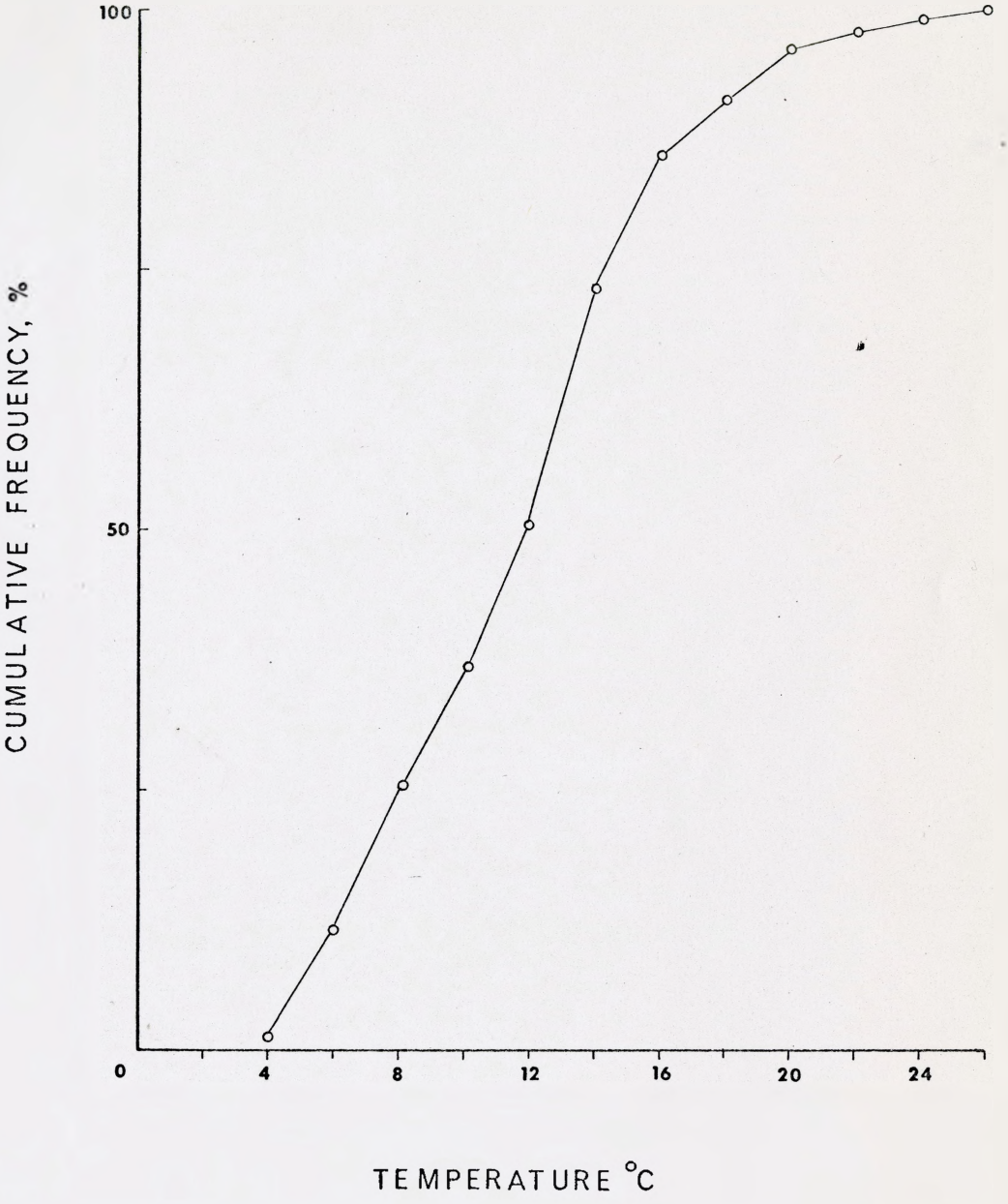


Fig. 5 Cumulative frequency distribution of third-instar Simulium venustum larvae, acclimated in the laboratory to 17°C, in a shifting temperature gradient.

Fig. 6 Distribution of sixth-instar Simulium vittatum larvae, acclimated in the laboratory at 7.5°C, in a shifting temperature gradient.
Flowing water.
Each dot represents one larva.

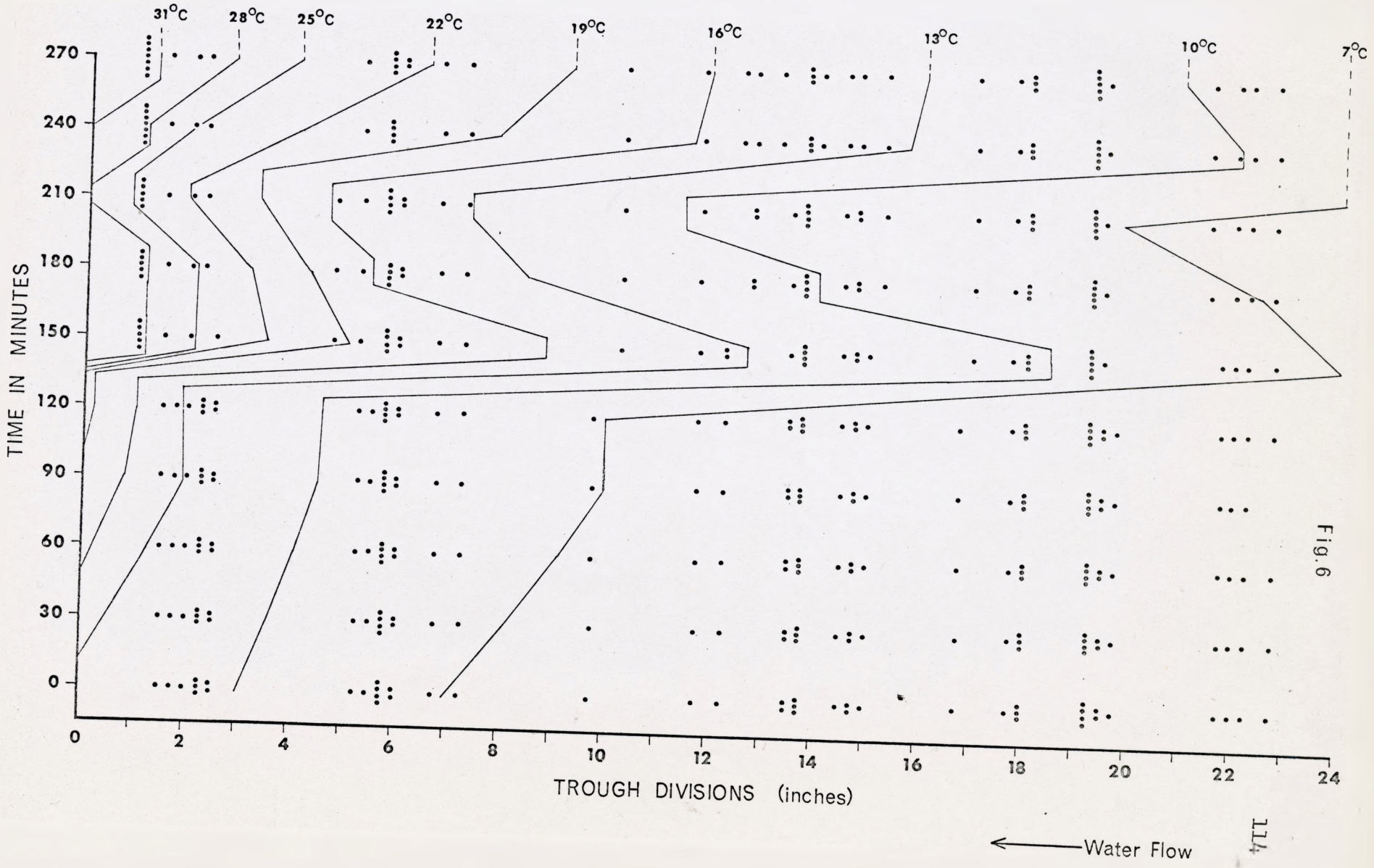

31°C
28°C
25°C
22°C
19°C
16°C
13°C
10°C
7°C
TIME IN MINUTES
270
240
210
180
150
120
90
60
30
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TROUGH DIVISIONS (inches)
Water Flow

Fig.6

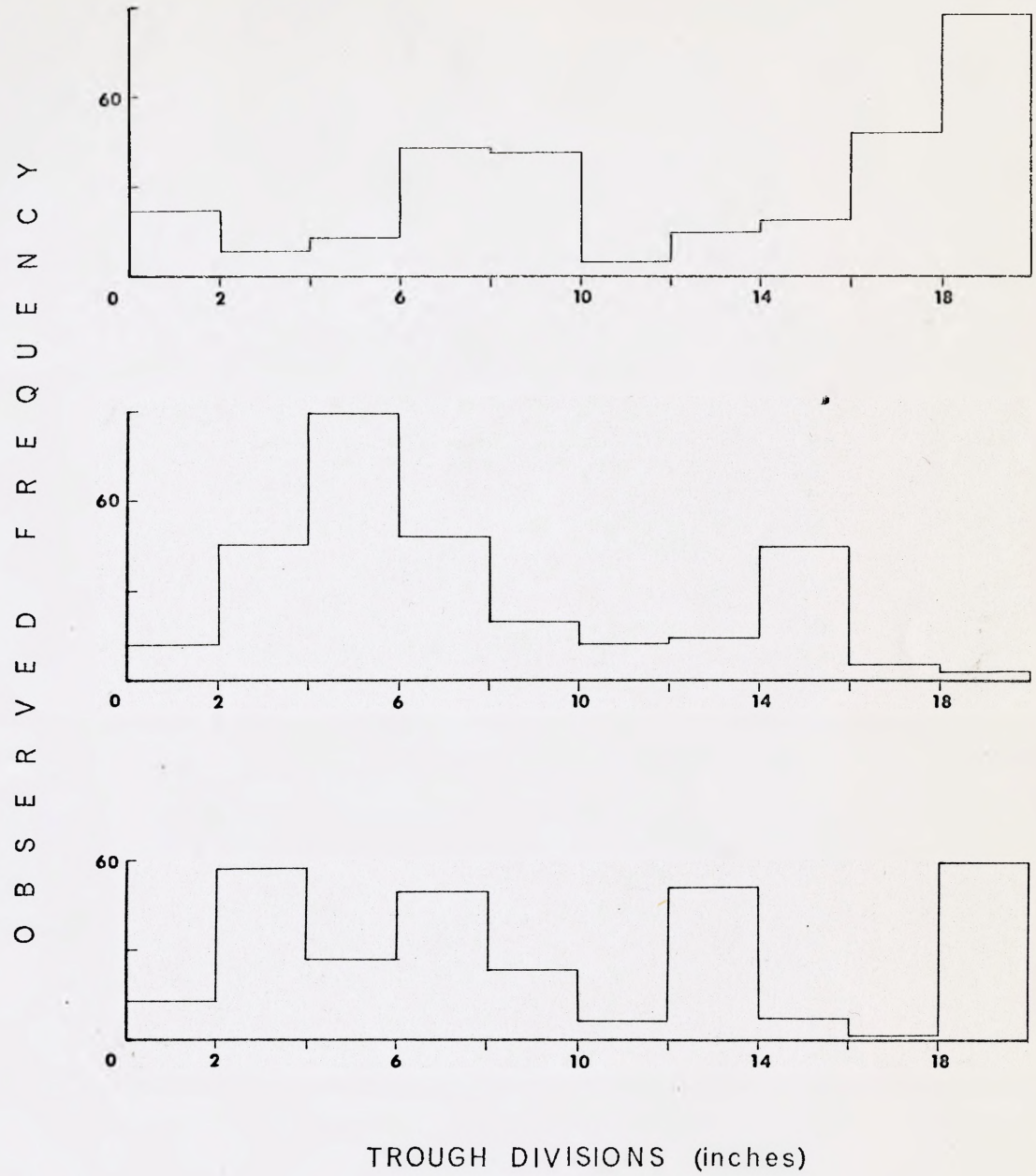


Fig. 7 Distribution of first-instar Culiseta inornata larvae reared at 20°C.
Control : water temperature 20°C

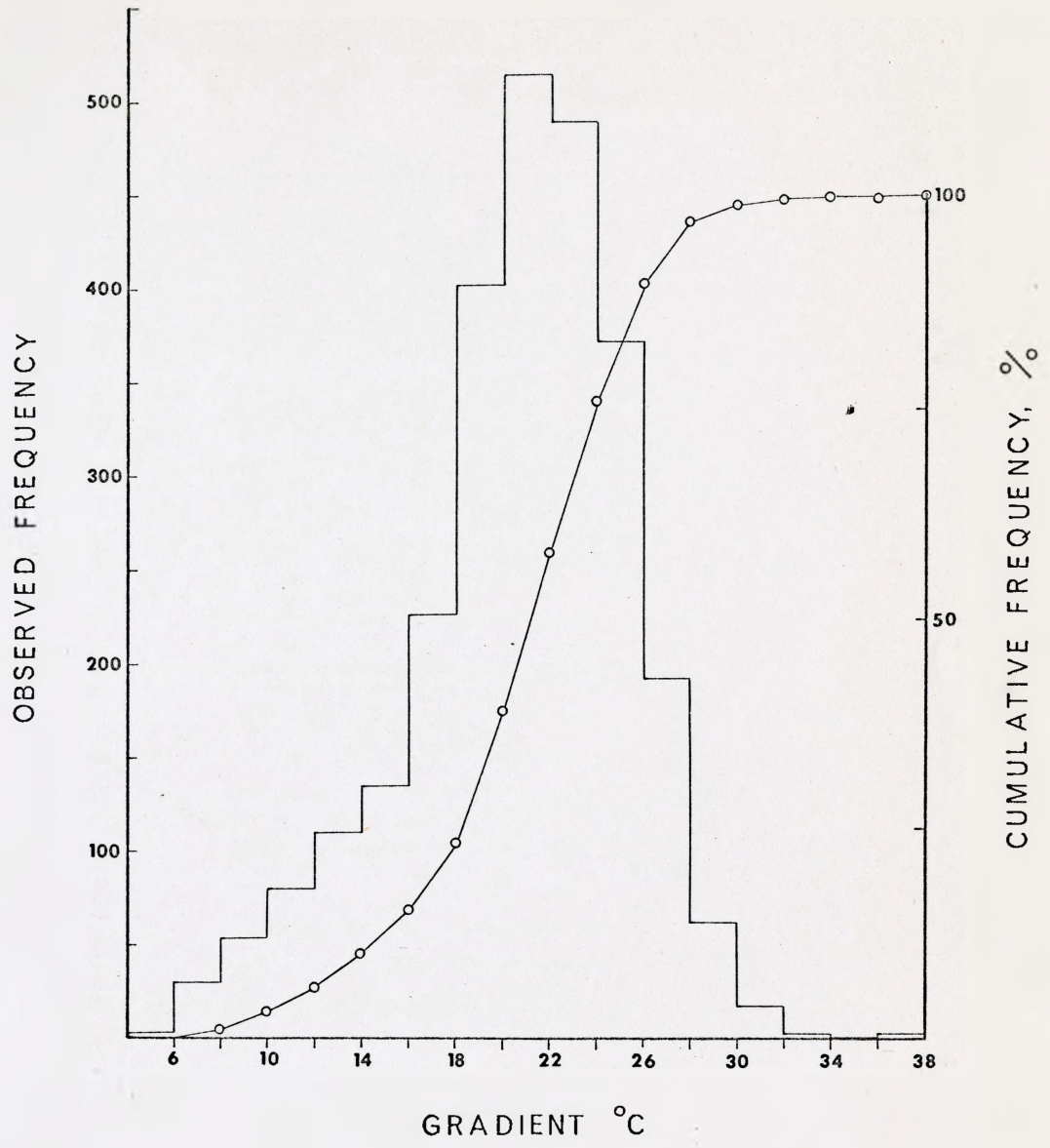
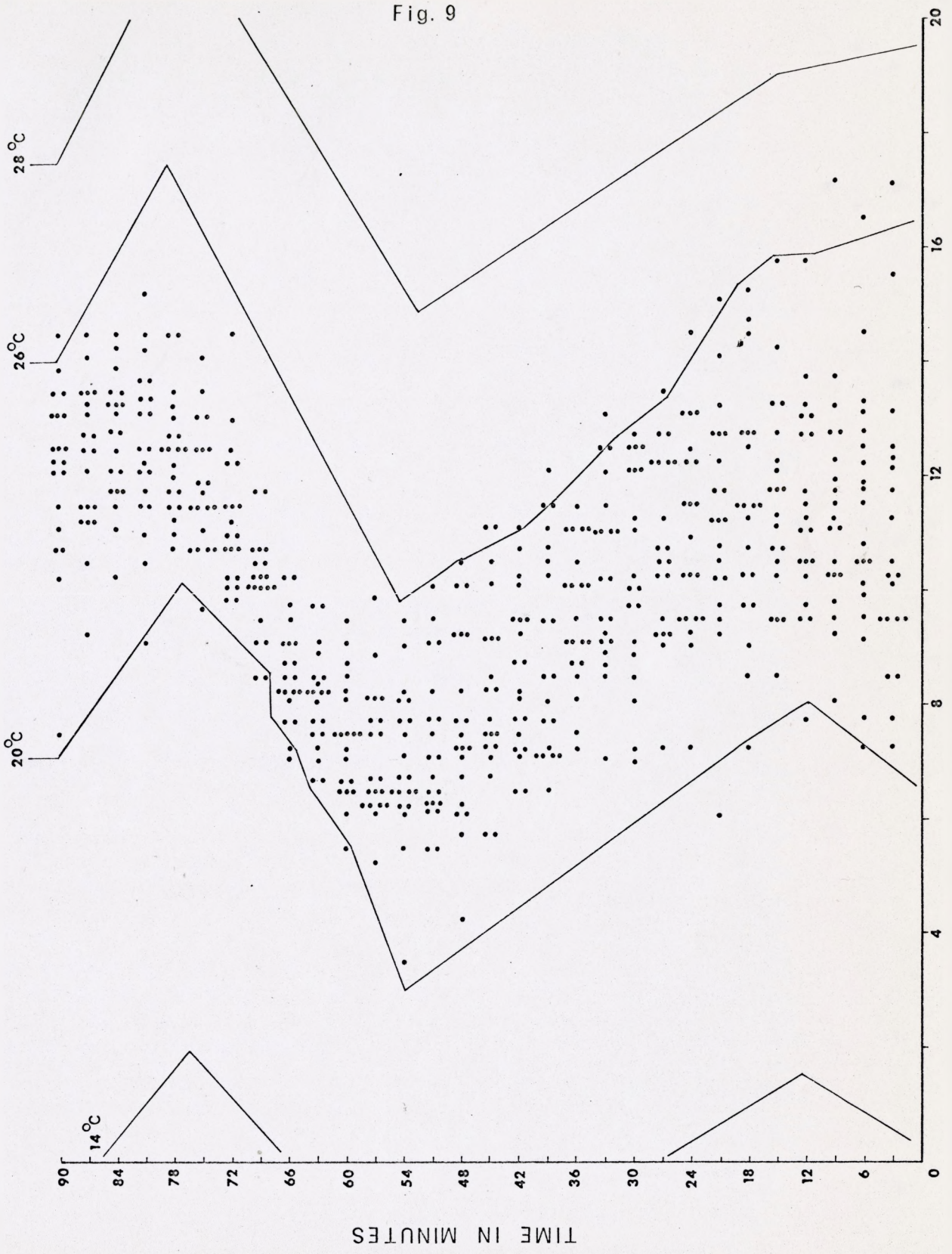


Fig. 8 Distribution of first-instar Culiseta inornata larvae, reared at 20°C, in a temperature gradient.
Total observations : 2700

Fig. 9 Distribution of second-instar Culiseta inornata larvae, reared at 20°C, in a shifting temperature gradient.

Each dot represents one larva.

Fig. 9


28°C
26°C
20°C
14°C
90
84
78
72
66
60
54
48
42
36
30
24
18
12
6
0
TIME IN MINUTES
20
16
12
8
4
0
TROUGH DIVISIONS (inches)

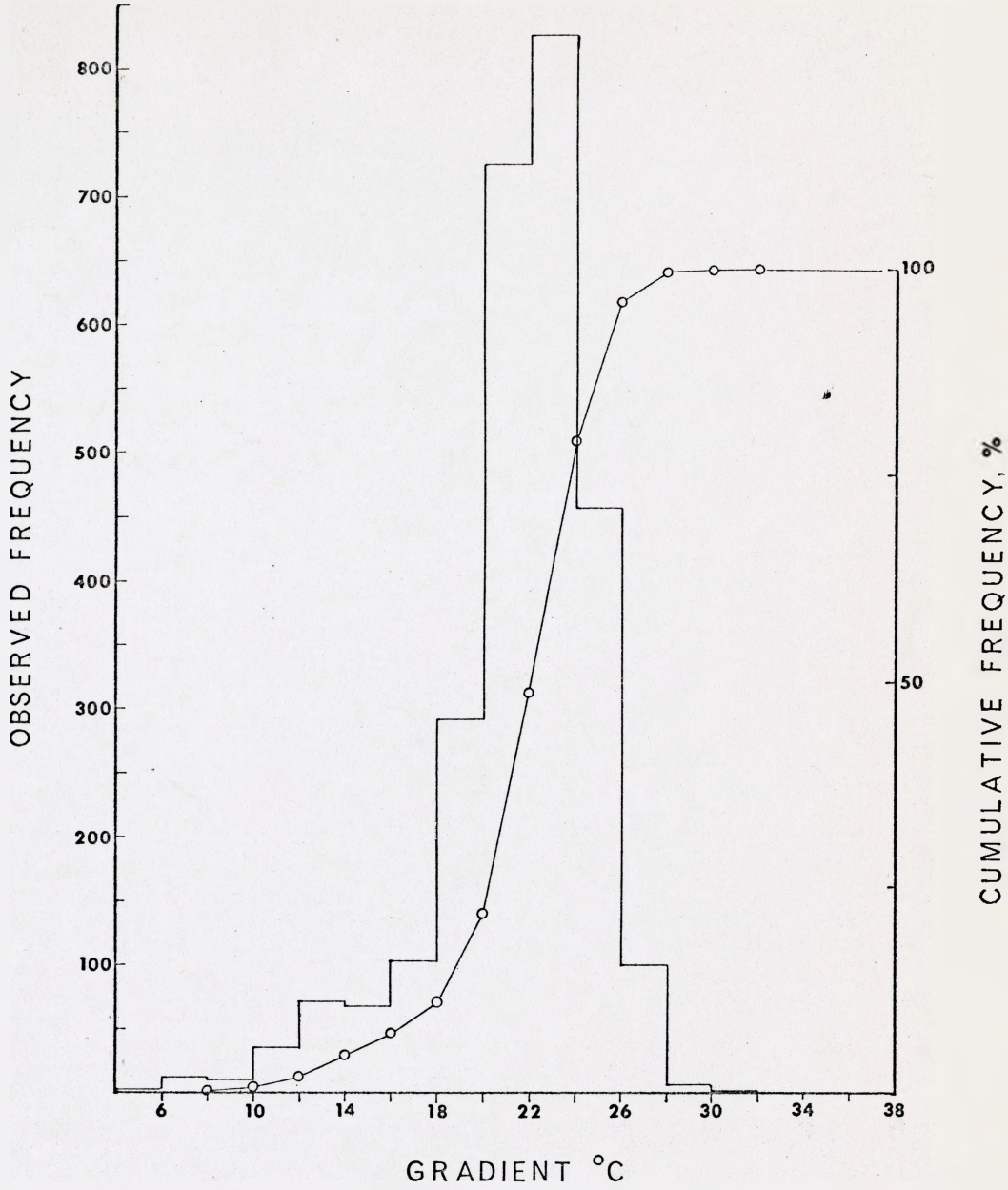


Fig. 10 Distribution of second-instar Culiseta inornata larvae, reared at 20°C, in a temperature gradient.
Total observations : 2700

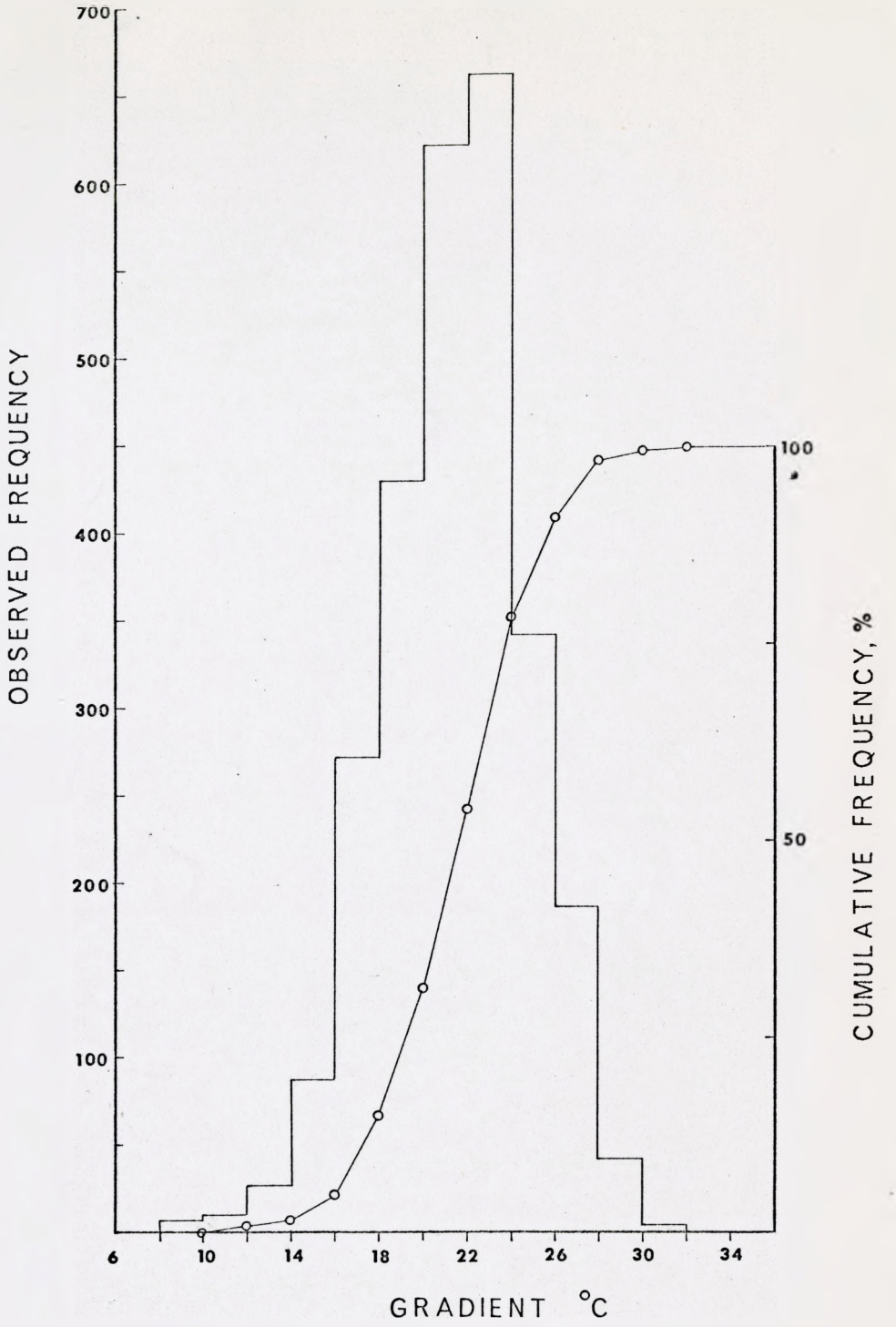


Fig. 11 Distribution of third-instar Culiseta inornata larvae, reared at 20°C, in a temperature gradient.
Total observations : 2700

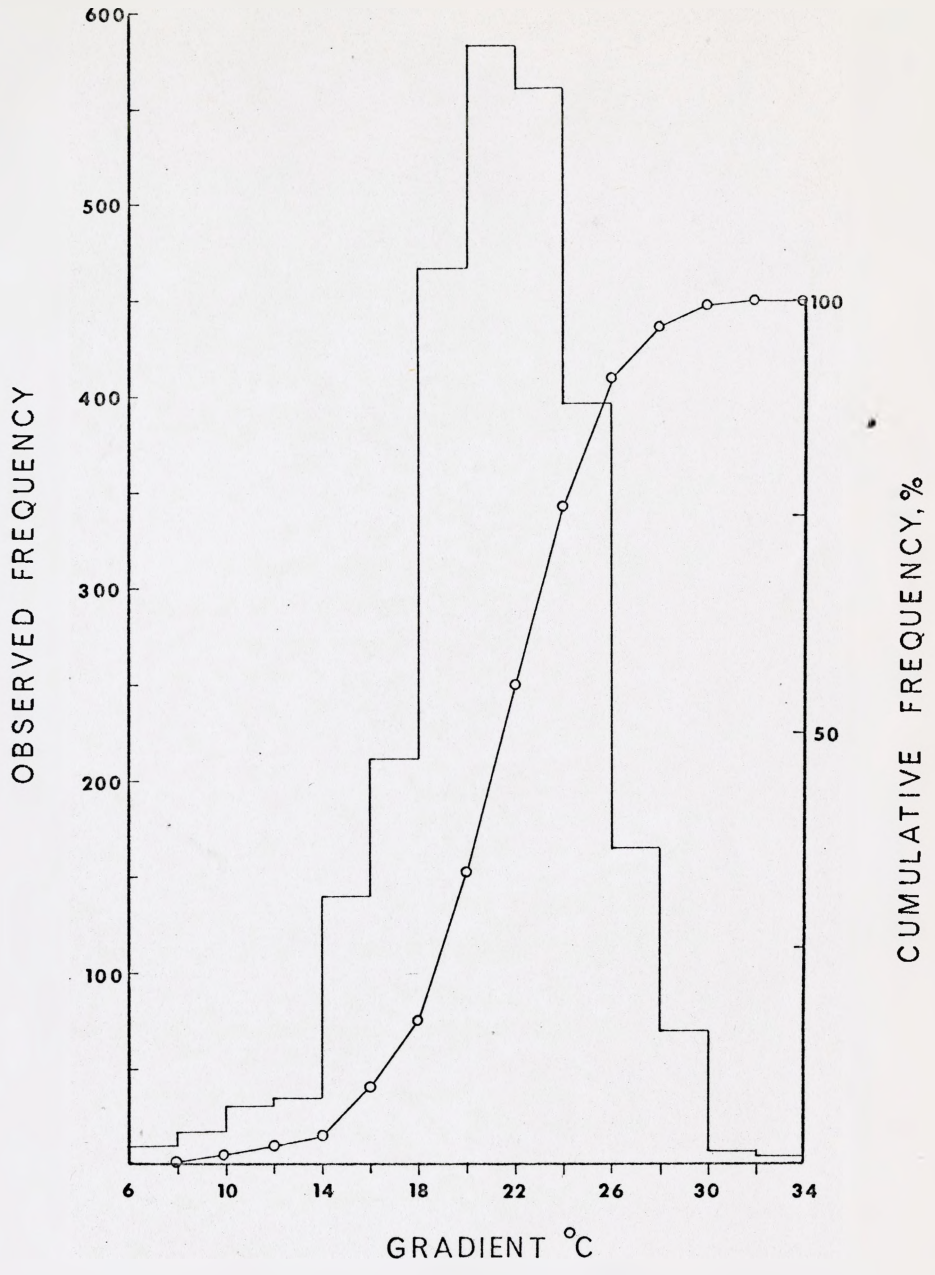
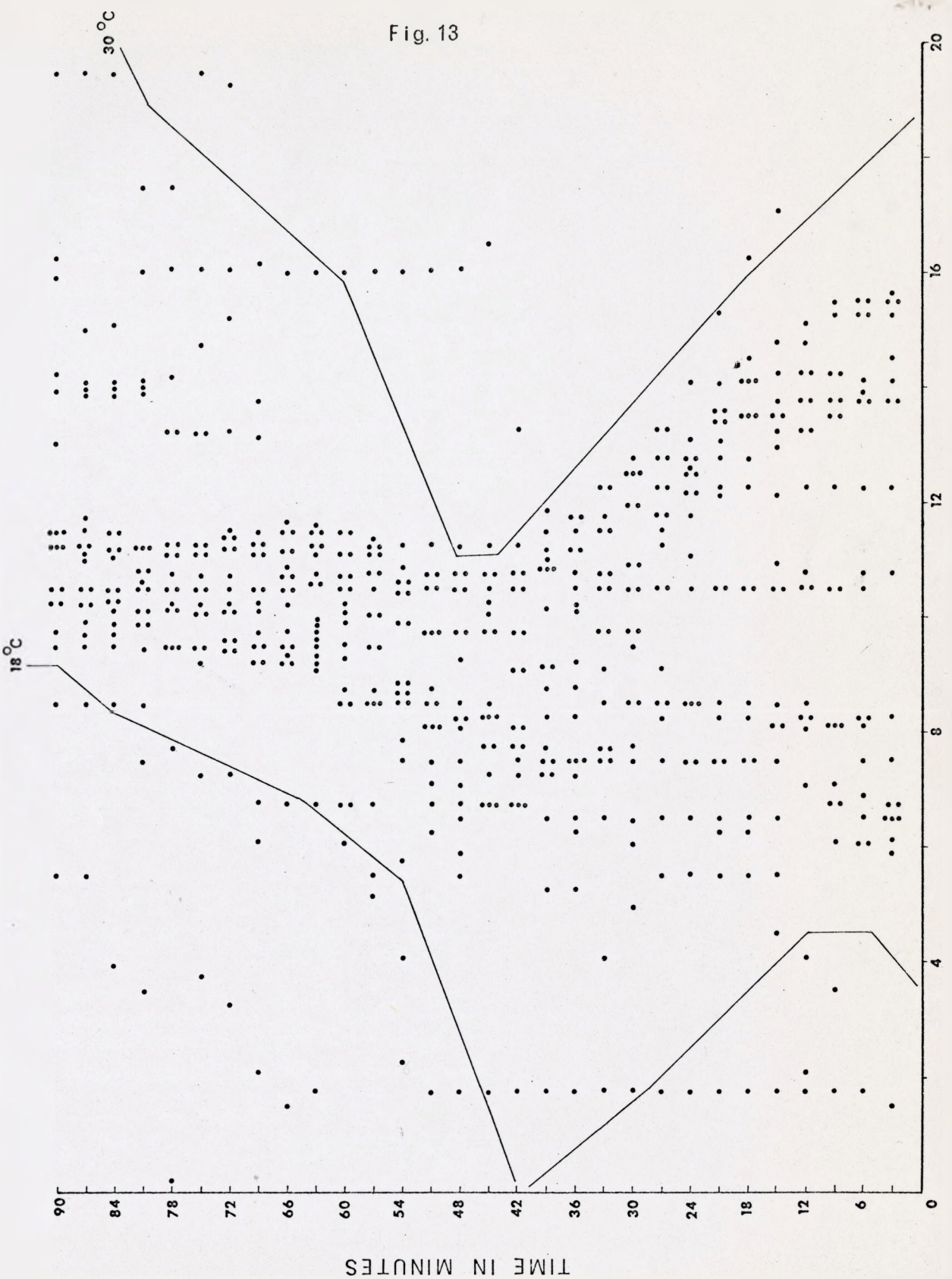


Fig. 12 Distribution of fourth-instar Culiseta inornata larvae, reared at 20°C, in a temperature gradient.
Total observations : 2700

facing p 121

Fig. 13 Distribution of male Culiseta inornata pupae, reared at 20°C and with an instar age of 36 ± 12 hr, in a shifting temperature gradient.
Each dot represents one pupa.

Fig. 13


30 °C
18 °C
TIME IN MINUTES
90
84
78
72
66
60
54
48
42
36
30
24
18
12
6
0
TROUGH DIVISIONS (inches)
20
16
12
8
4

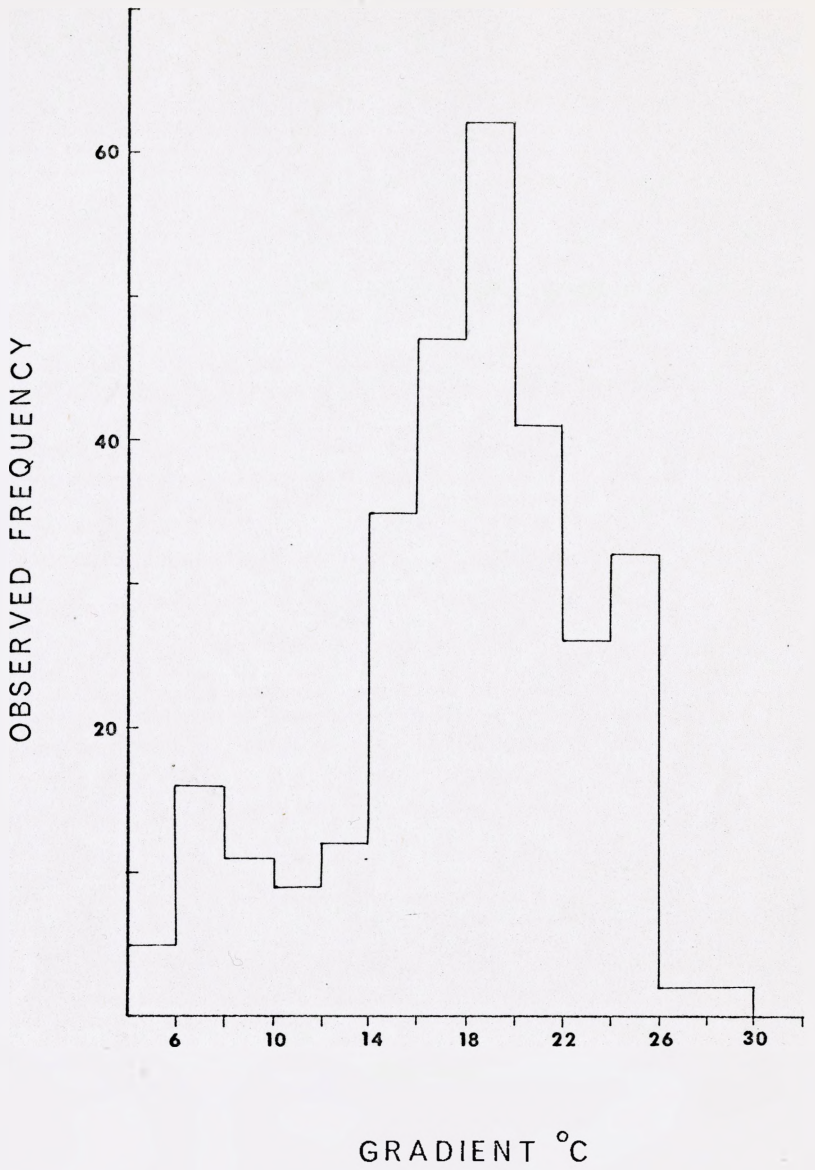


Fig. 14 Distribution of male Culiseta inornata pupae, reared at 20°C and with an instar age of less than 24 hours, in a temperature gradient.

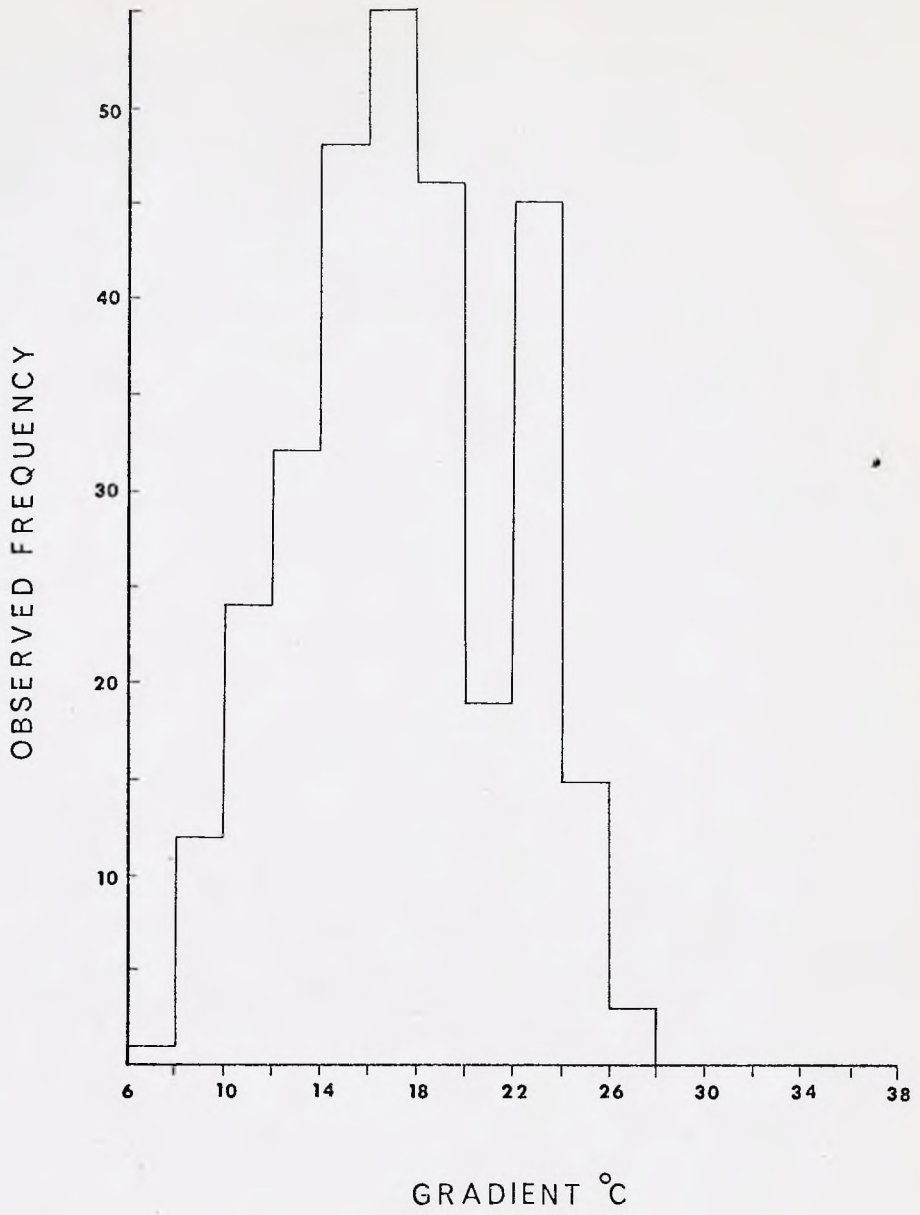


Fig. 15 Distribution of third-instar Culiseta inornata larvae, reared at 20°C and secondarily acclimated to 5°C for 70 hours, in a temperature gradient.

Fig. 16 Distribution of third-instar Culiseta inornata larvae, reared at 20°C and secondarily acclimated to 5°C for 70 hours, in a temperature gradient.

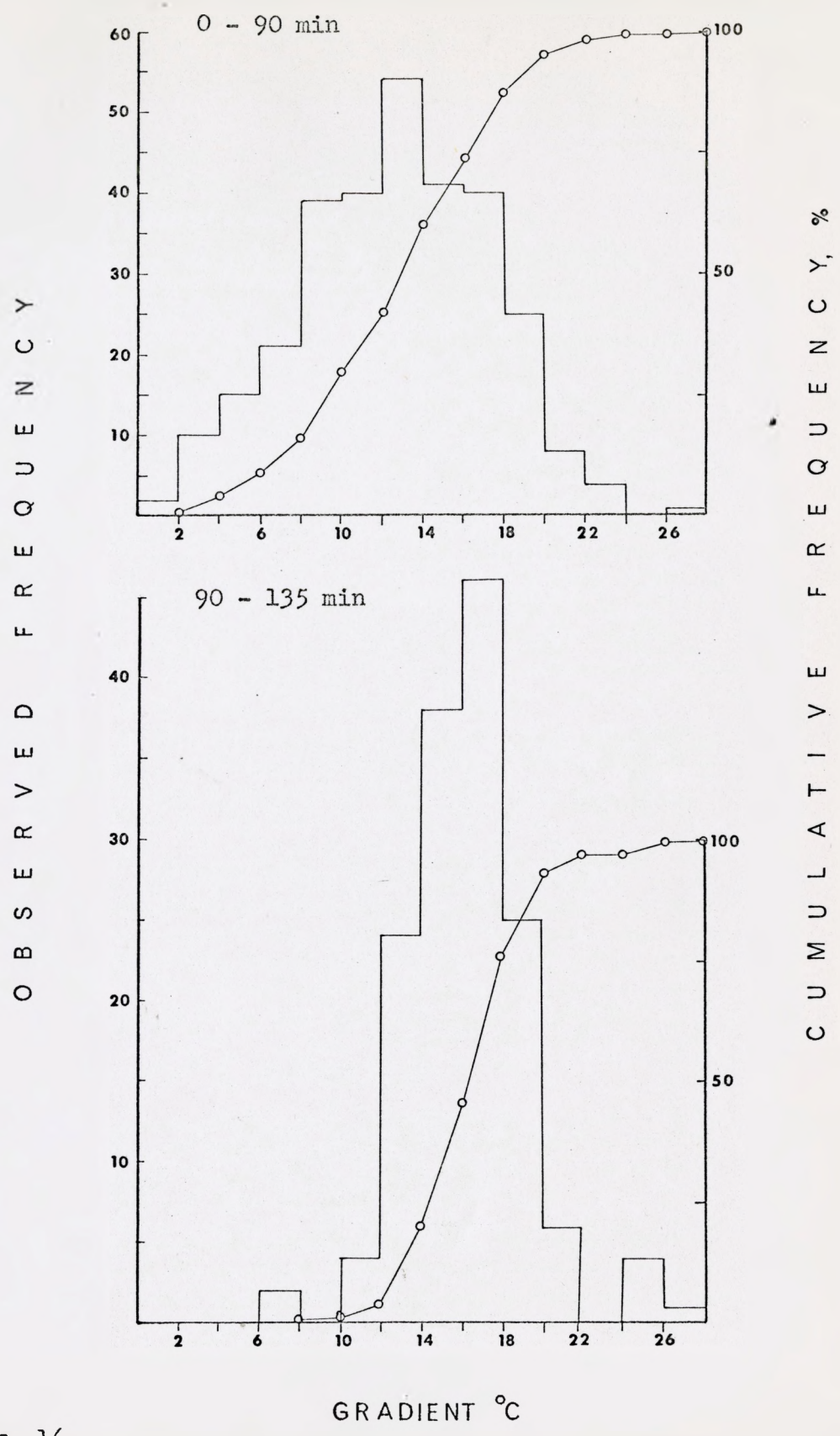

0 - 90 min
90 - 135 min
OBSERVED FREQUENCY
CUMULATIVE FREQUENCY, %
GRADIENT °C
60
50
40
30
20
10
100
50
2
6
10
14
18
22
26

Fig. 16

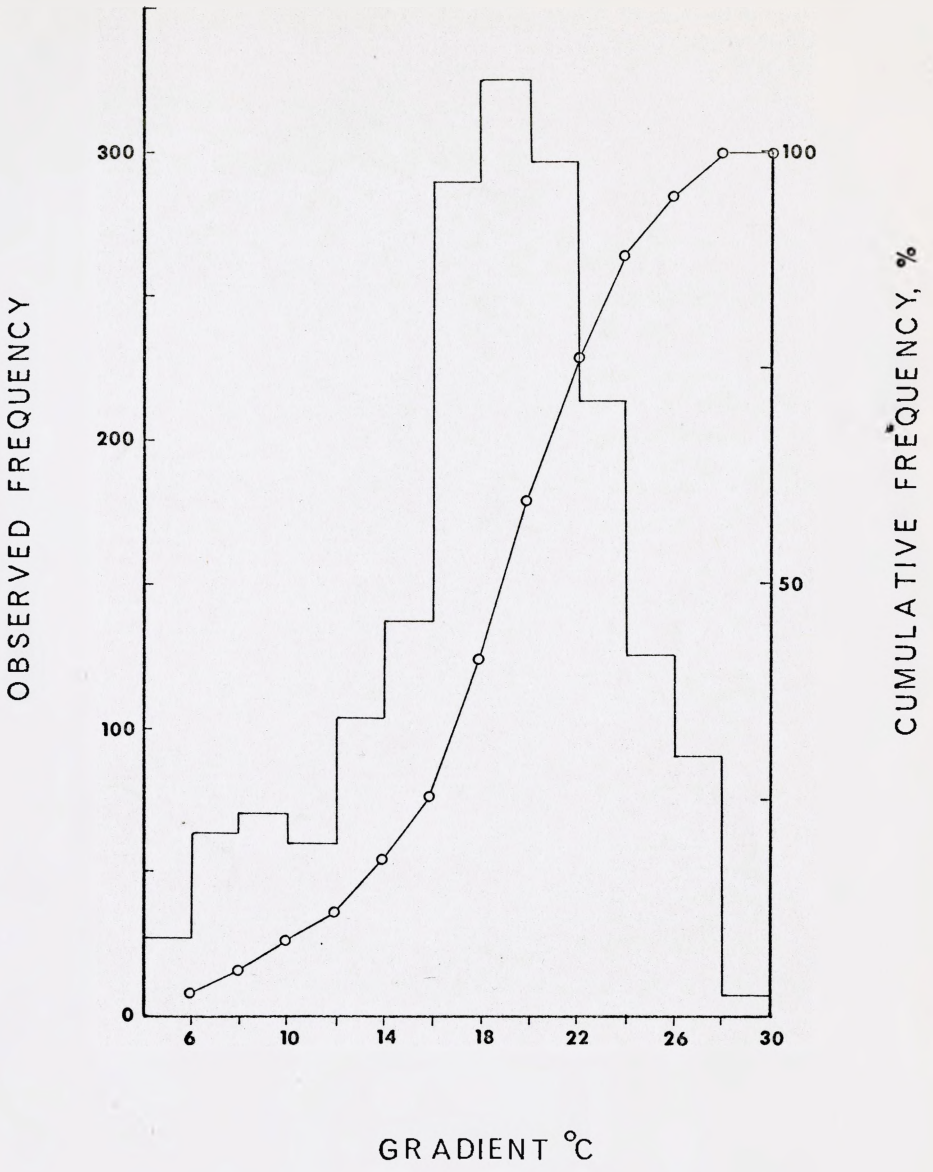


Fig. 17 Distribution of second-instar Culiseta inornata larvae, reared at 20°C, in a static temperature gradient.

Fig. 18 Results of the selected temperature studies with Culiseta inornata larvae.

Primary acclimation, larvae reared at 20°C

a) Shifting temperature gradient

+ Median temperature

⊙ Modal selected temperature

o Mode of a single experiment

_____ Selected temperature range

b) Static temperature gradient

----- Selected temperature range

⊘ Modal selected temperature

⊘ Mode of a single experiment

X Median temperature

Secondary acclimation, larvae reared at 20°C and acclimated at 5°C for 96 hours (third-instar larvae)

Selected temperature range :

0 - 90 min – – – – – 90 - 135 min ------

Modal selected temperature :

0 - 90 min ⊙ 90 - 135 min ⊙

Median temperature :

0 - 90 min ⊕ 90 - 135 min ⊕

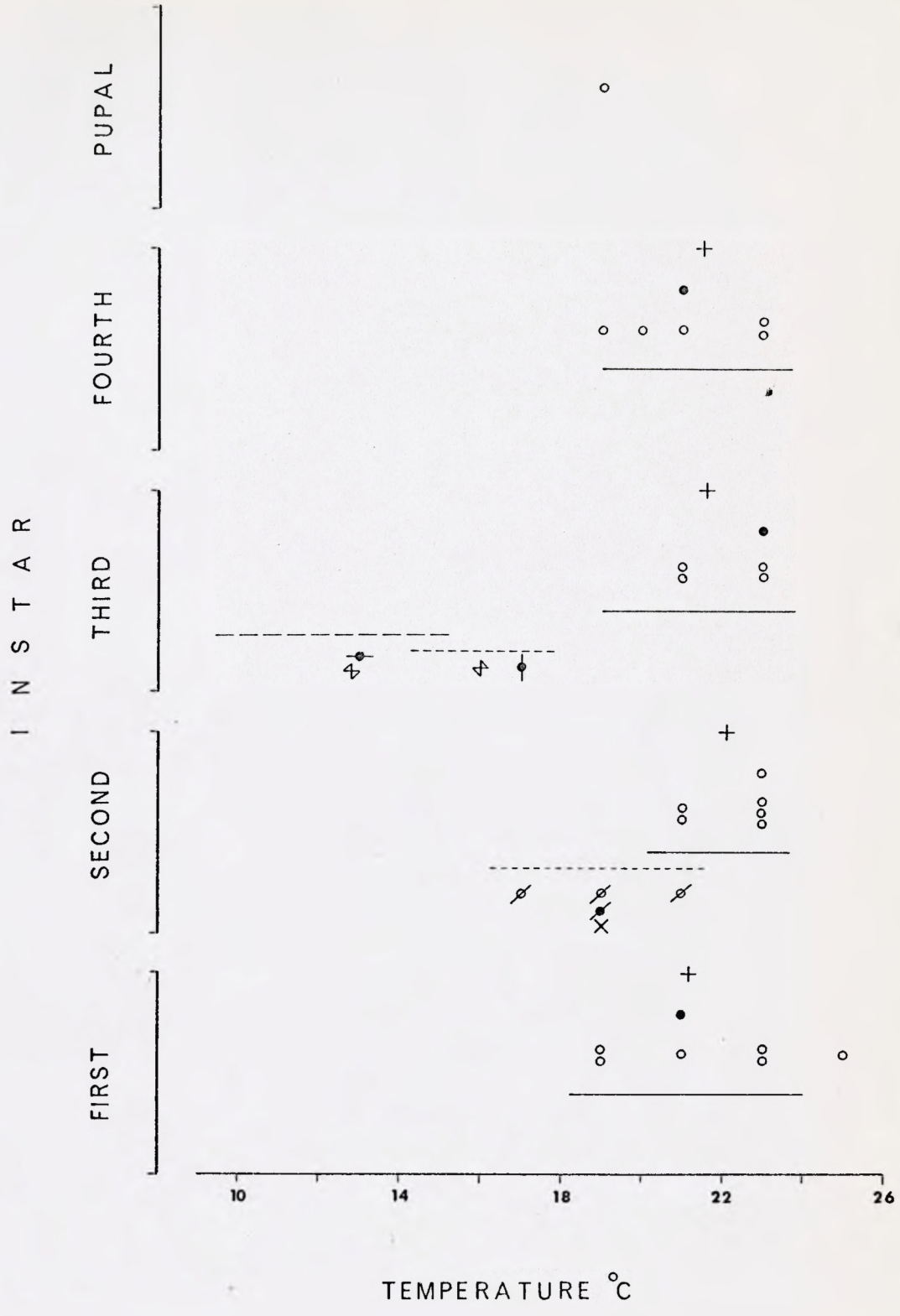

PUPAL
FOURTH
THIRD
SECOND
FIRST
INSTAR
10
14
18
22
26
TEMPERATURE °C

Fig. 18

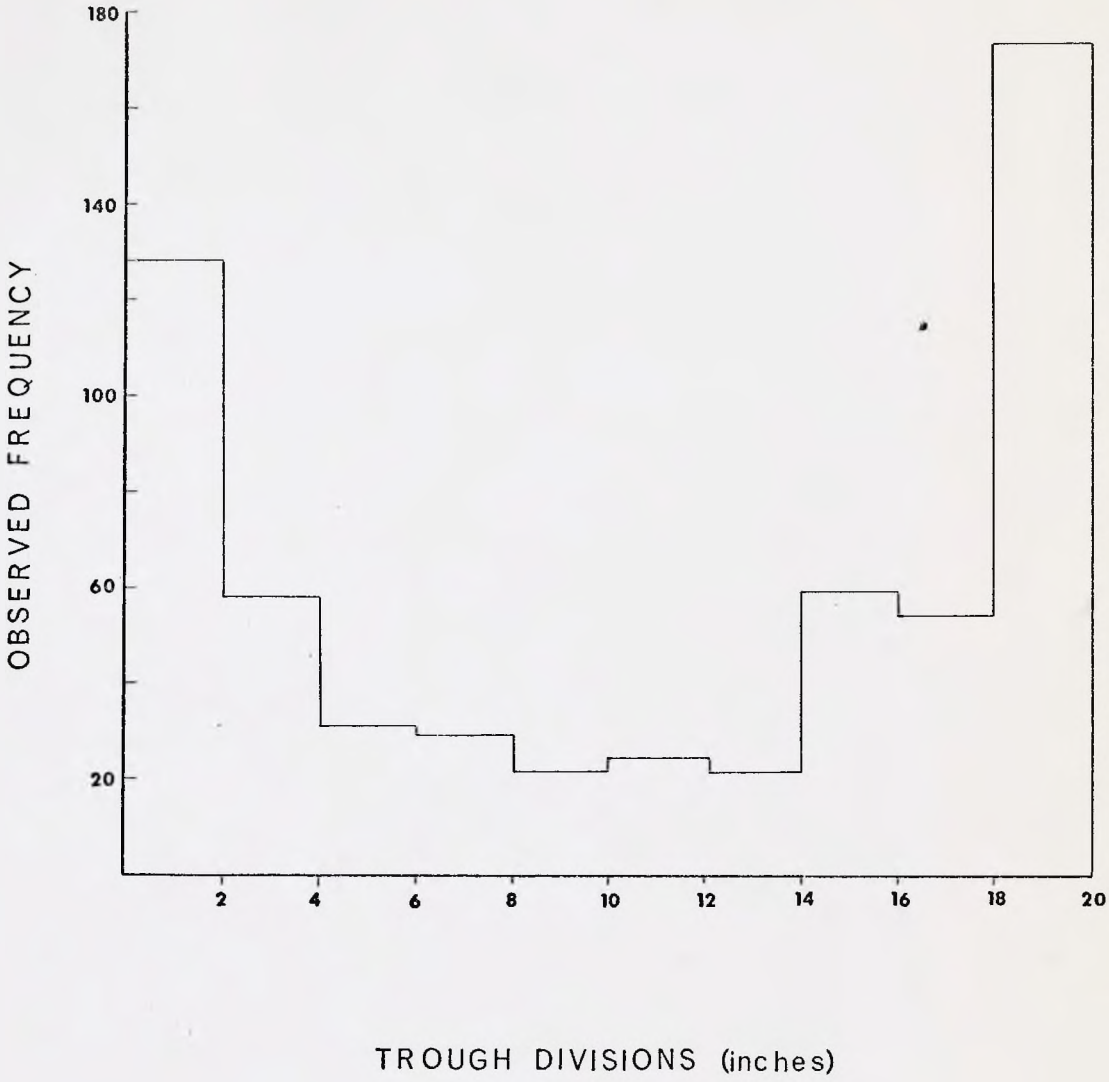


Fig. 19 Distribution of fourth-instar Aedes aegypti larvae, reared at 30°C, in the temperature gradient trough. Control : water temperature a uniform 30°C.

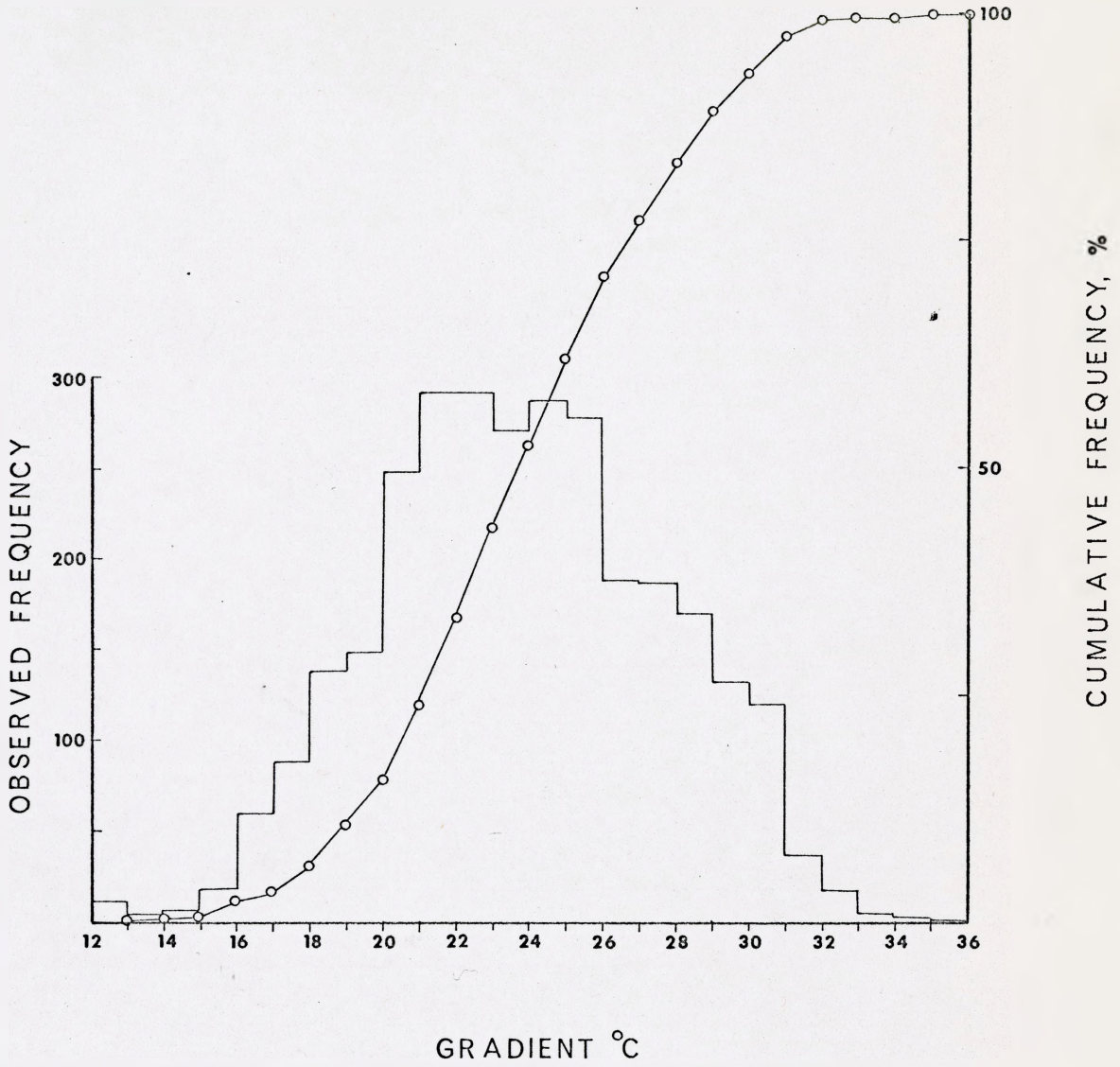


Fig. 20 Distribution of fourth-instar Aedes aegypti larvae, reared at 16°C, in a temperature gradient.

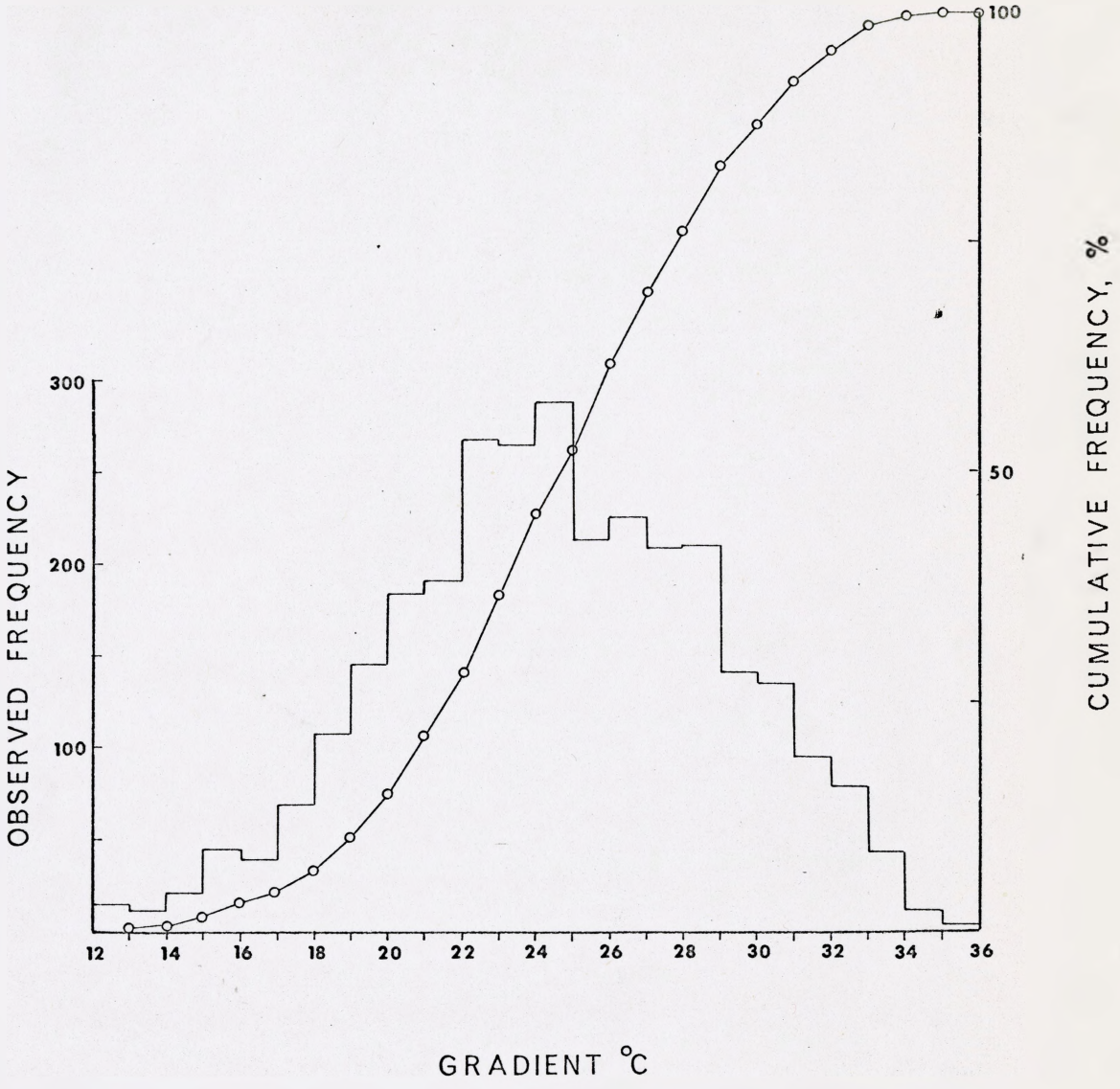


Fig. 21 Distribution of fourth-instar Aedes aegypti larvae, reared at 20°C, in a temperature gradient.

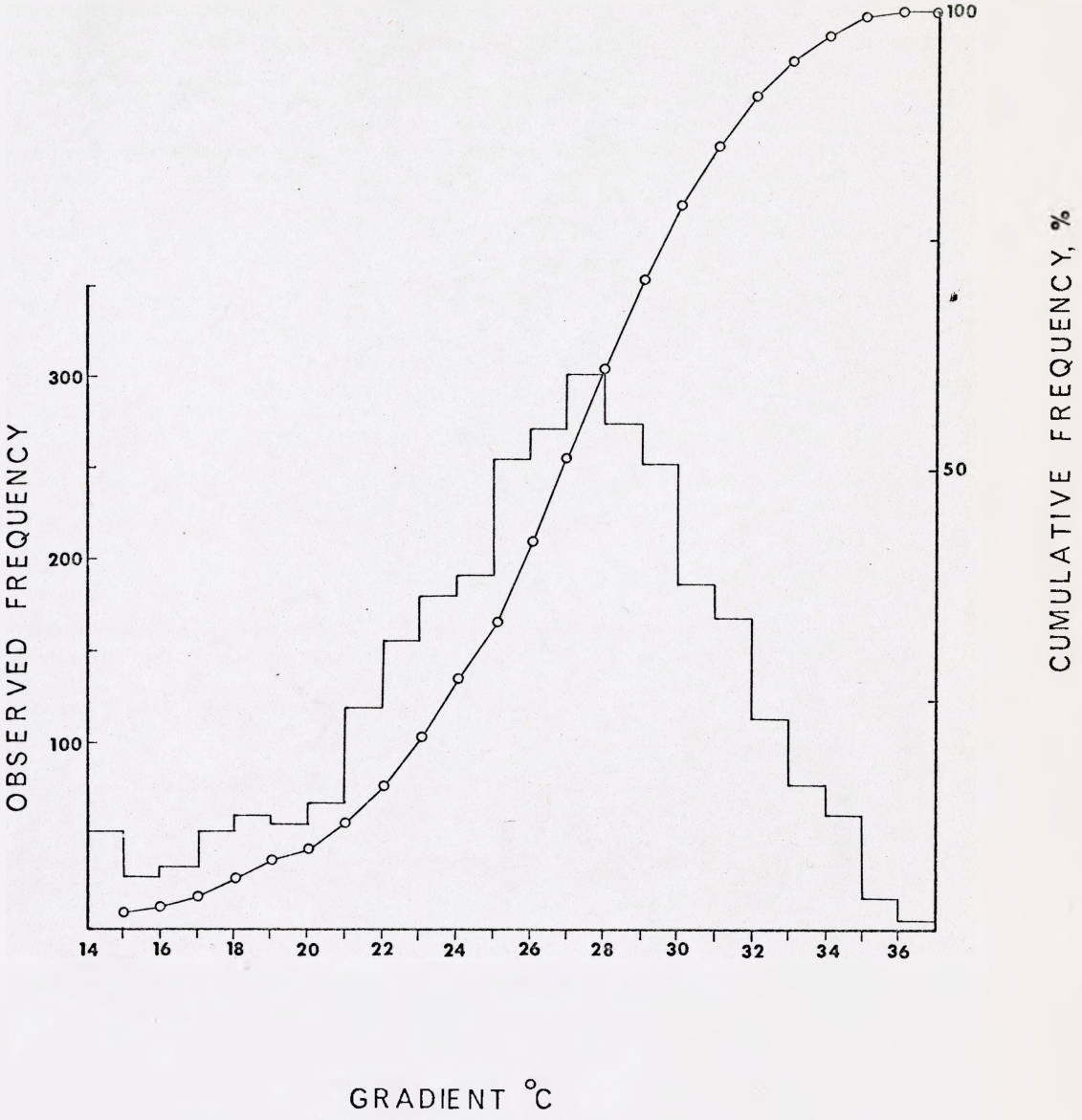


Fig. 22 Distribution of fourth-instar Aedes aegypti larvae, reared at 25°C, in a temperature gradient.

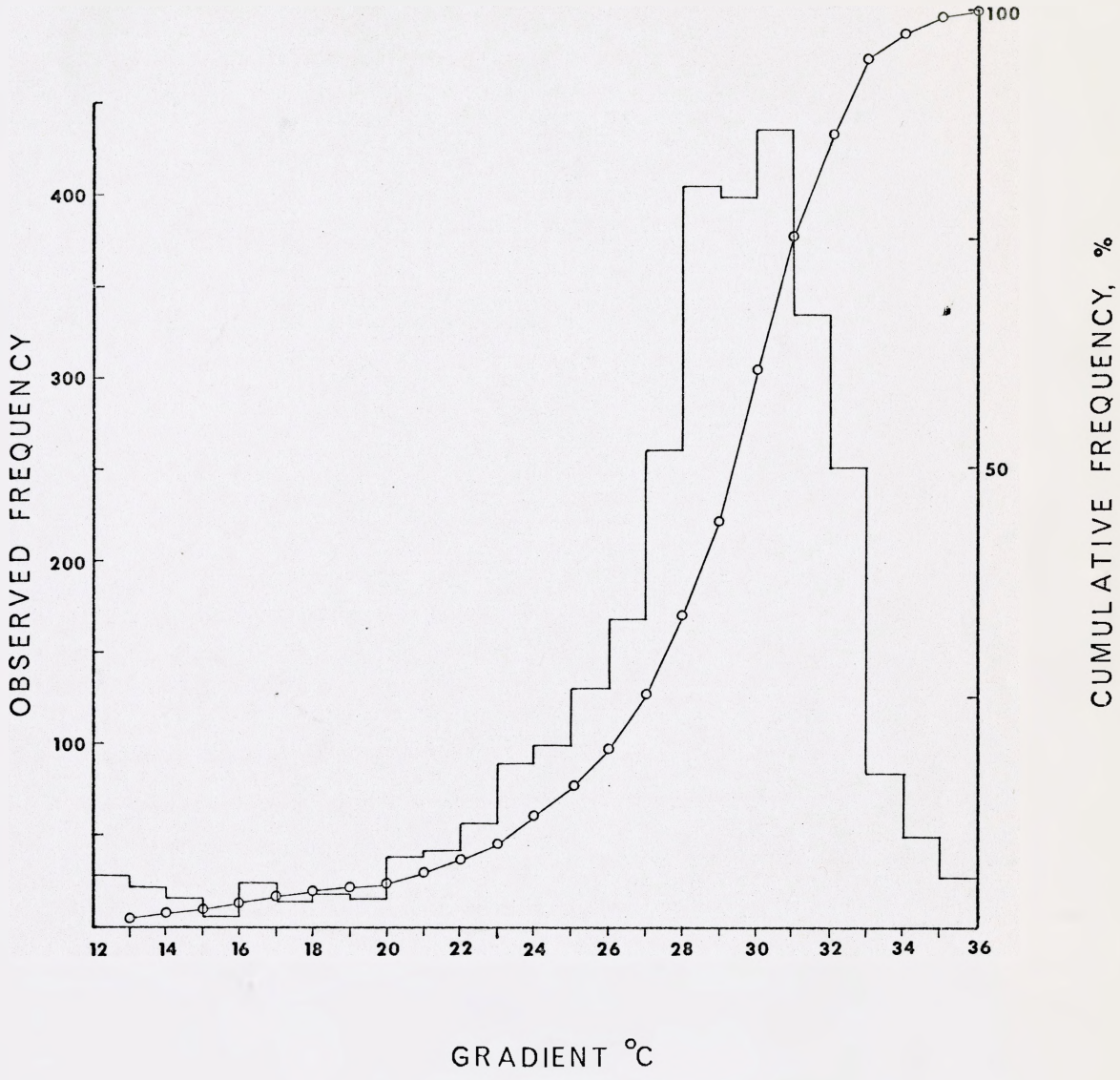


Fig. 23 Distribution of fourth-instar Aedes aegypti larvae, reared at 30°C, in a temperature gradient.

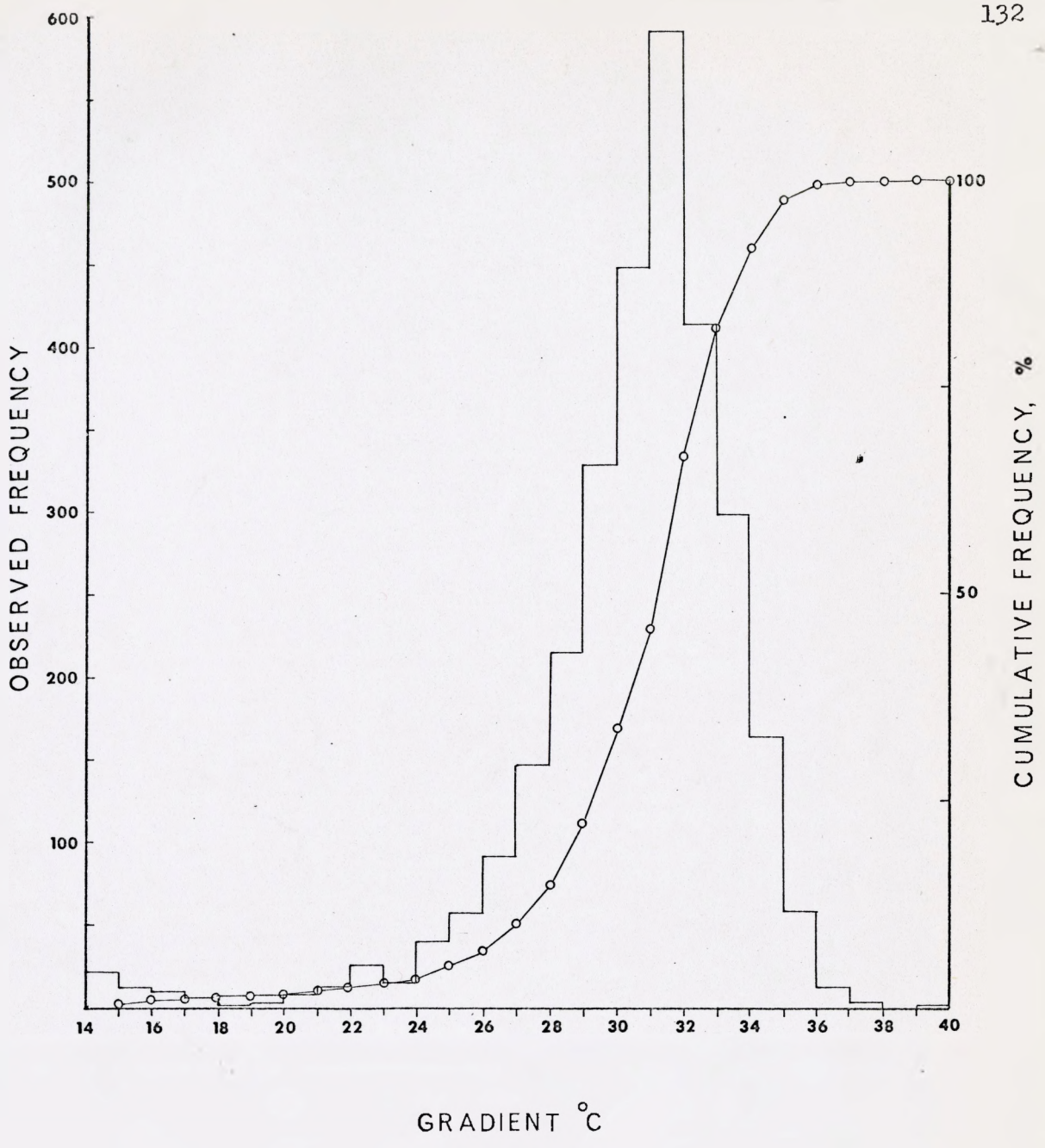


Fig. 24 Distribution of fourth-instar Aedes aegypti larvae, reared at 31.5°C, in a temperature gradient.

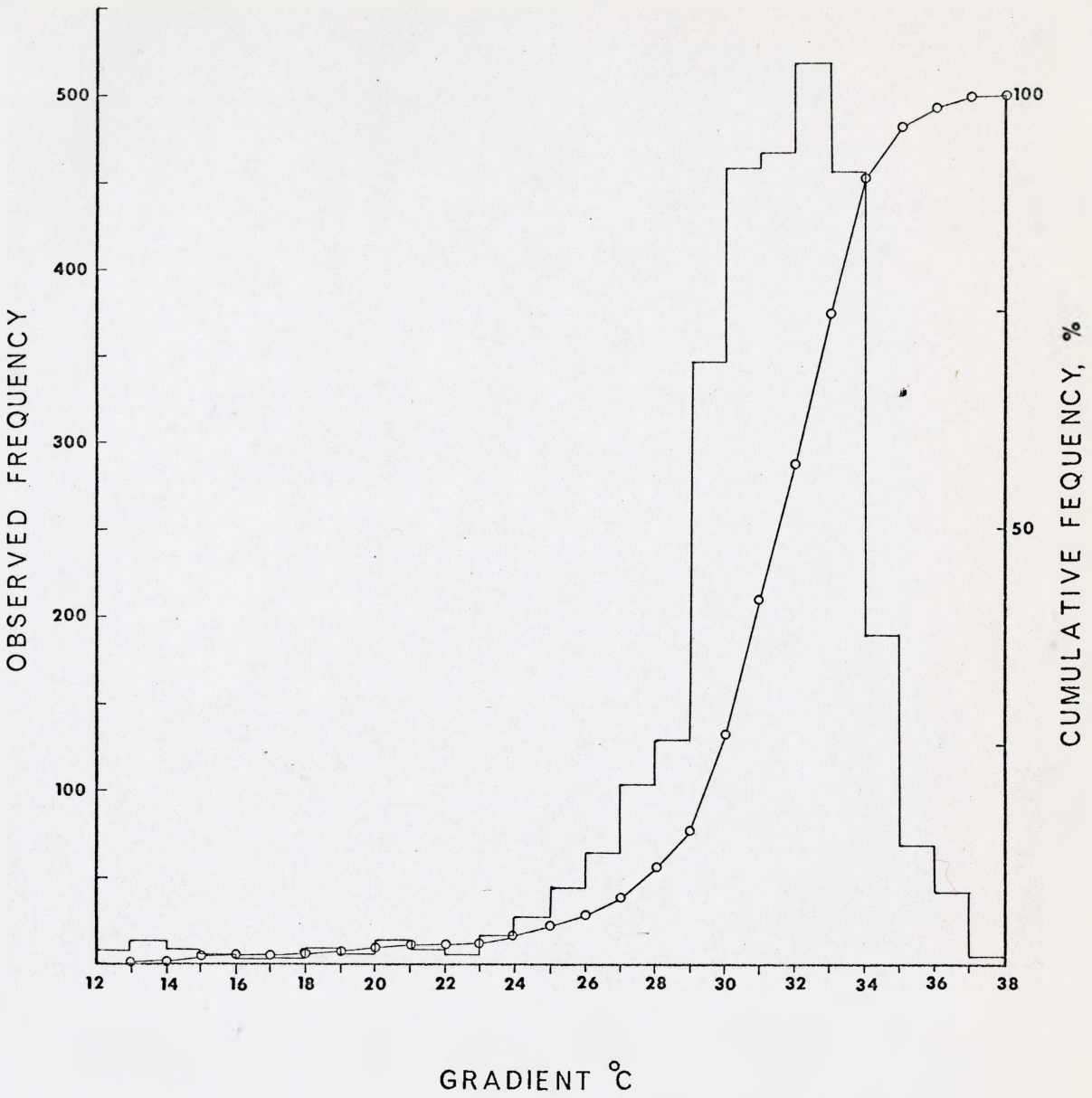


Fig. 25 Distribution of fourth-instar Aedes aegypti larvae, reared at 35°C, in a temperature gradient.

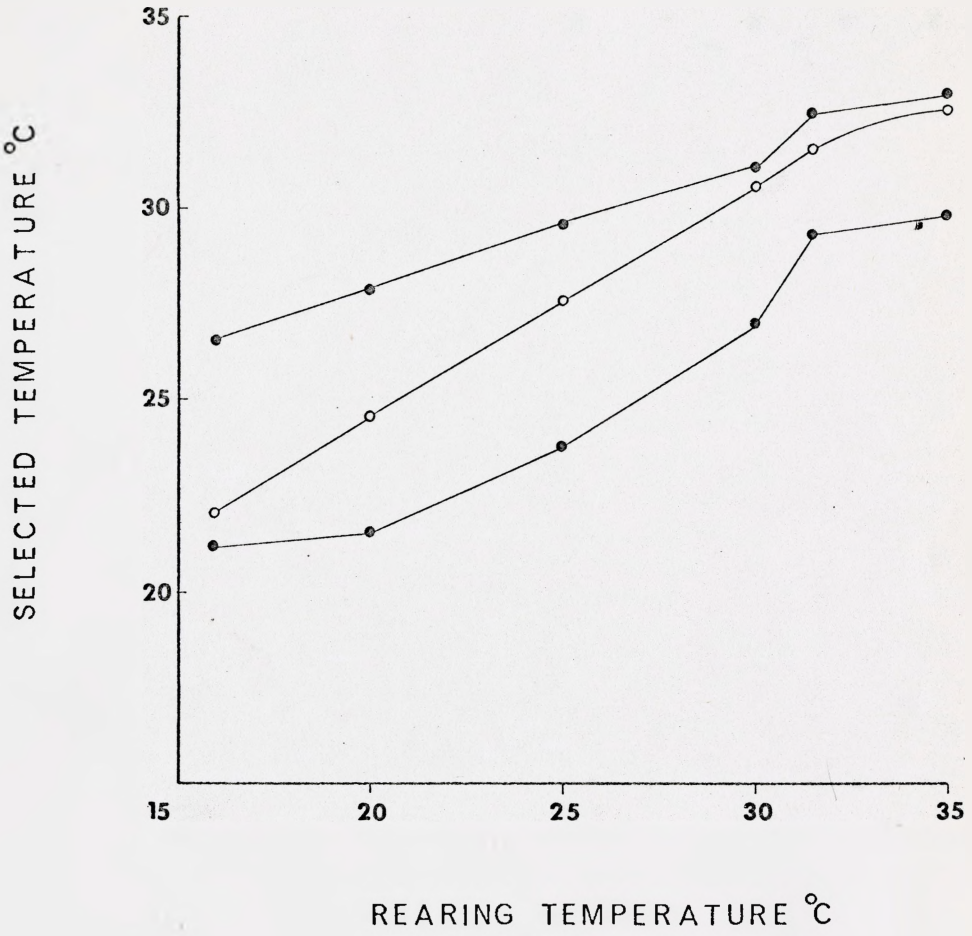


Fig. 26 Relation between the selected temperatures and the rearing temperatures of fourth-instar Aedes aegypti larvae.

o Modal selected temperature

Selected temperature range

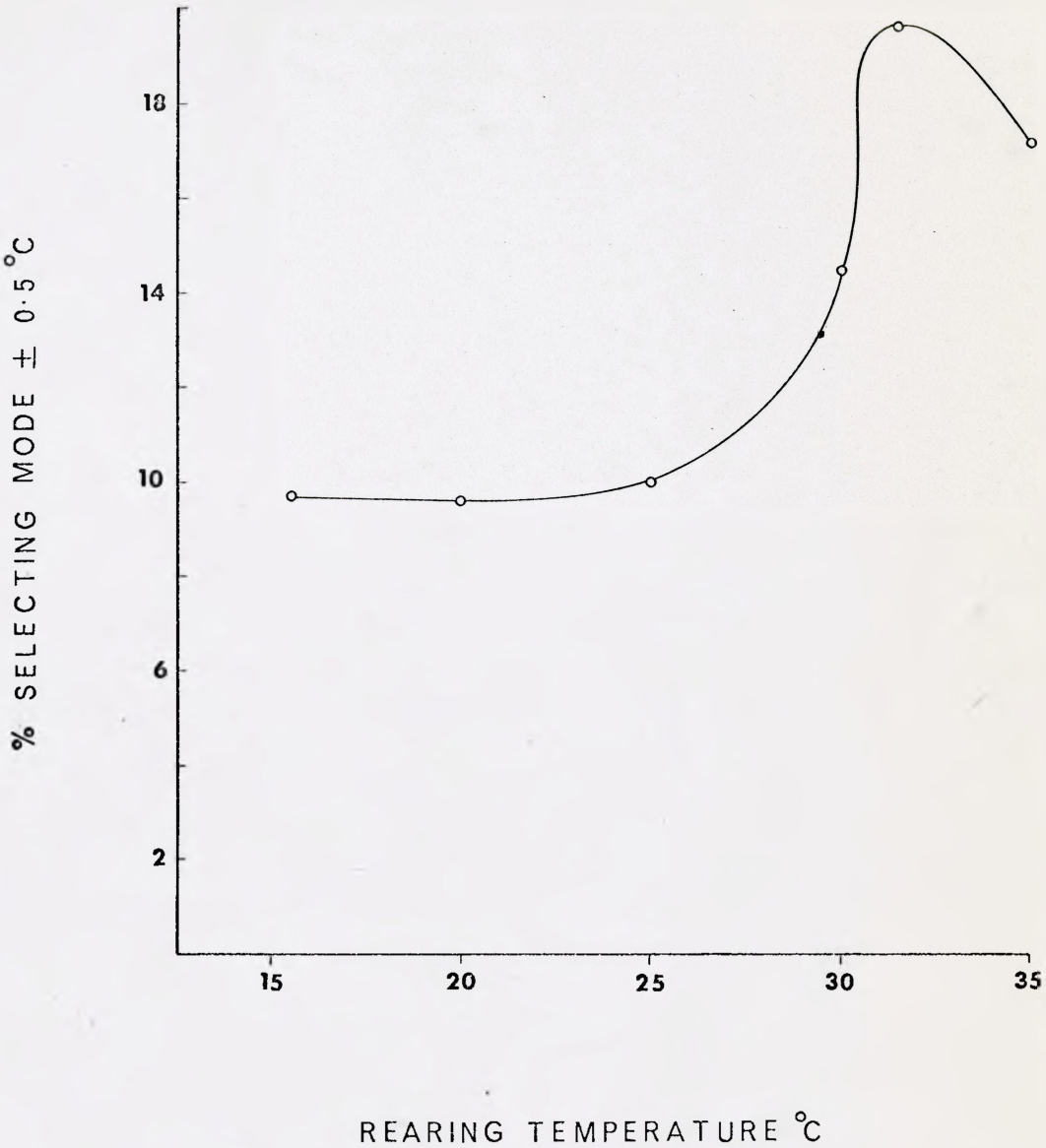


Fig. 27 Relation between the rearing temperature and the percentage of fourth-instar Aedes aegypti larvae selecting the mode ± 0.5°C.

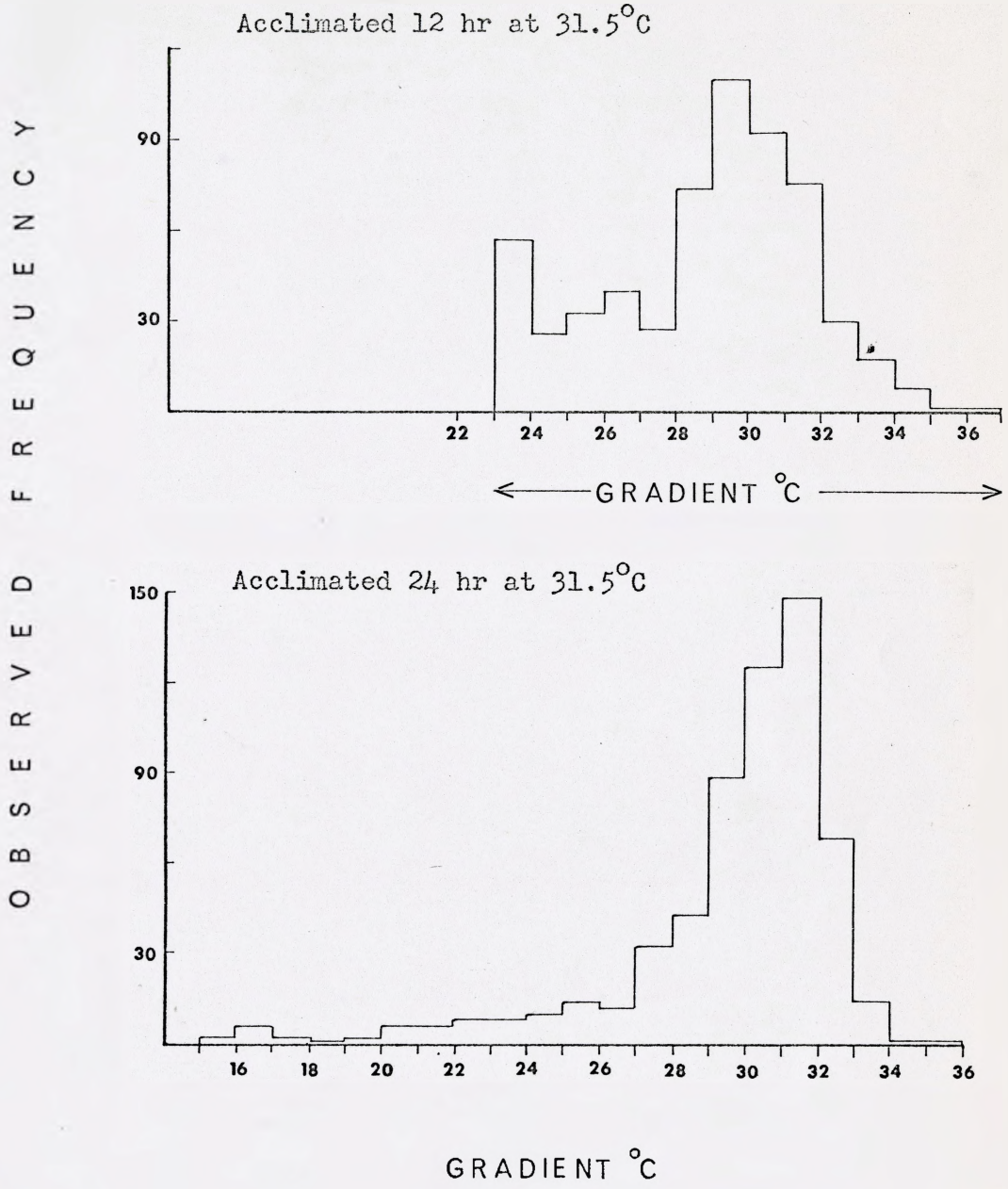


Fig. 28 Distribution of fourth-instar Aedes aegypti larvae, reared at 20°C and secondarily acclimated at 31.5°C, in a temperature gradient.

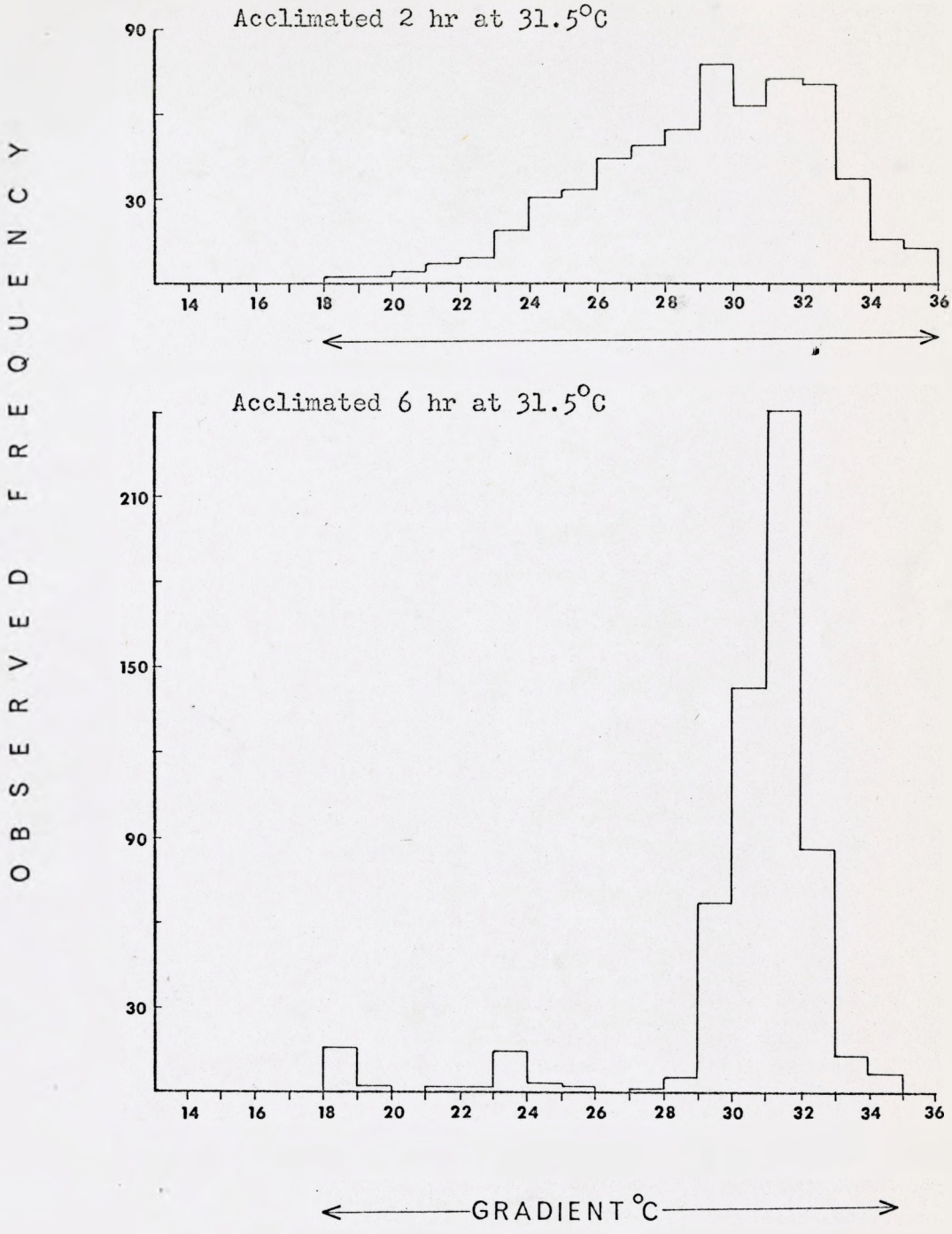


Fig. 29 Distribution of fourth-instar Aedes aegypti larvae, reared at 25°C and secondarily acclimated at 31.5°C, in a temperature gradient.

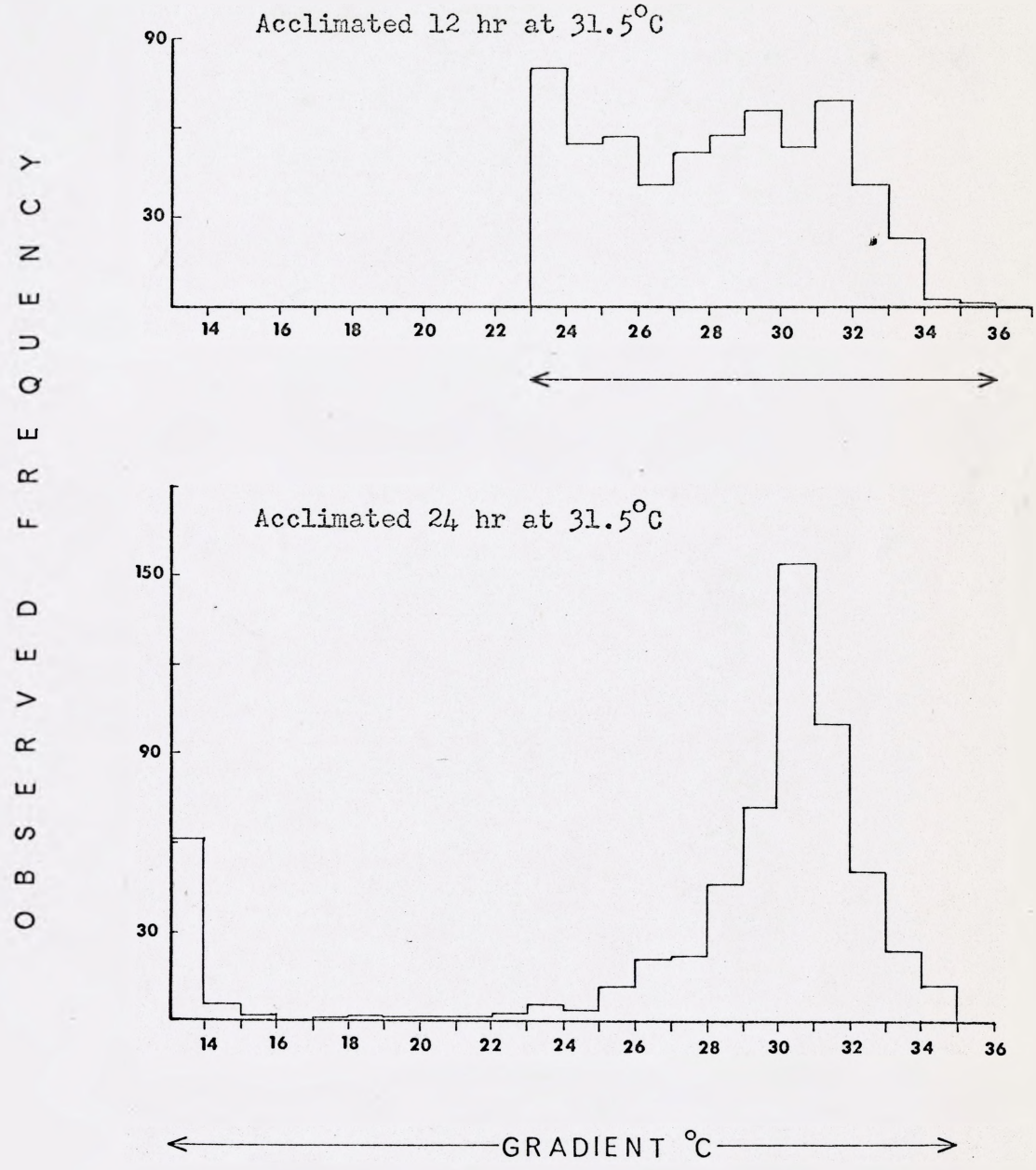


Fig. 30 Distribution of fourth-instar Aedes aegypti larvae, reared at 25°C and secondarily acclimated at 31.5°C, in a temperature gradient.

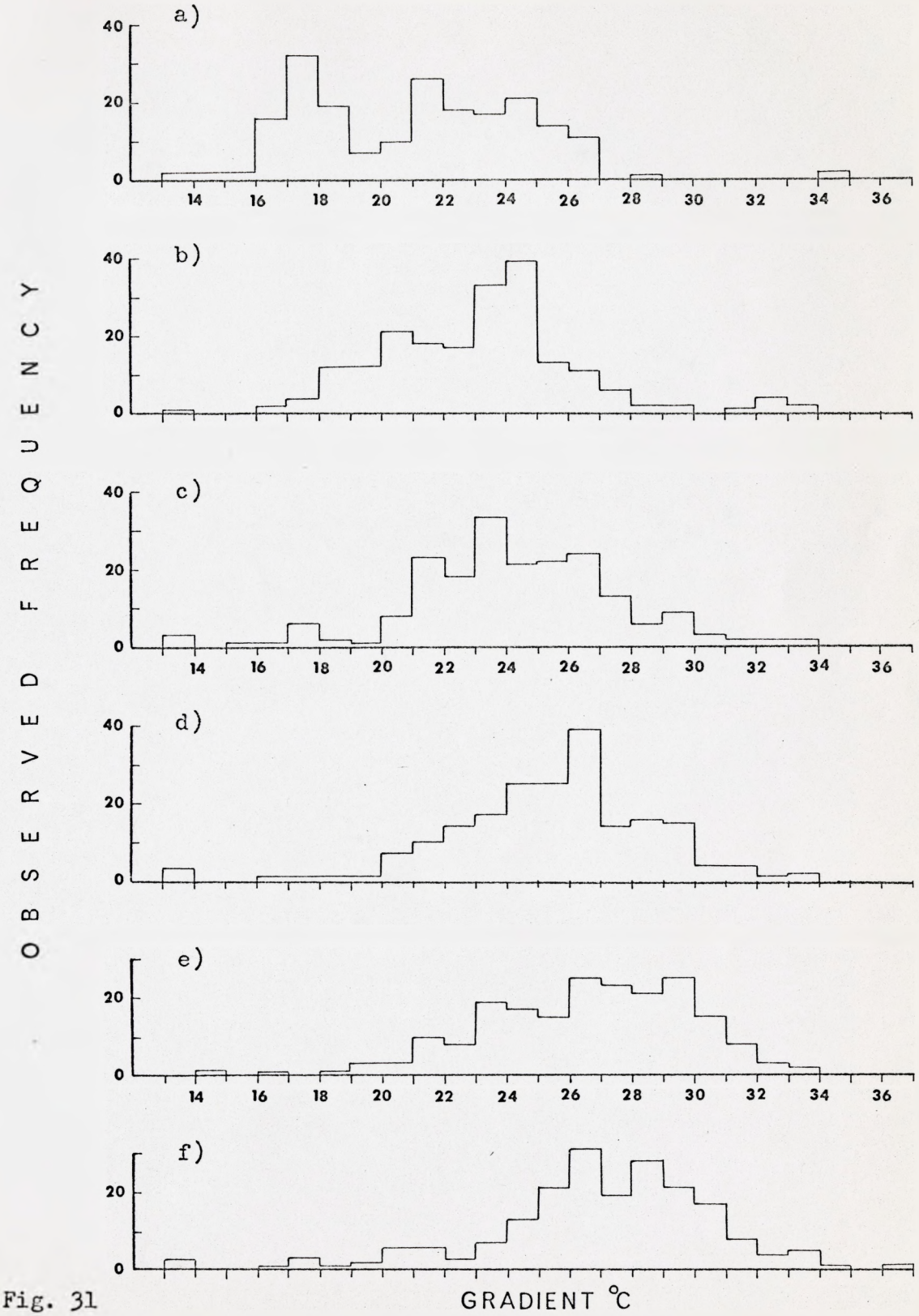

a)
b)
c)
d)
e)
f)
40
20
0
14
16
18
20
22
24
26
28
30
32
34
36
OBSERVED FREQUENCY
GRADIENT °C

Fig. 31

facing p. 13

Fig. 31 Distribution of fourth-instar Aedes aegypti larvae, reared at 35°C and secondarily acclimated at 16°C for 24 hours, in a temperature gradient.

a) 0 - 10 minutes

b) 10 - 20 "

c) 20 - 30 "

d) 30 - 40 "

e) 40 - 50 "

f) 50 - 60 "

Fig. 32 Results of all the selected temperature studies with fourth-instar Aedes aegypti larvae.

Primary acclimation

+ Median temperature

● Modal selected temperature

o Mode of a single experiment

o o Modes of experiments where histograms were bi-modal

——— Selected temperature range

Secondary acclimation

⌀ acclimated 2 hr at 31.5°C

⌀ " 6 hr at 31.5°C

ϕ " 12 hr at 31.5°C

⊖ " 24 hr at 31.5°C (larvae reared at 25°C)

⊖ " 24 hr at 16°C (larvae reared at 35°)

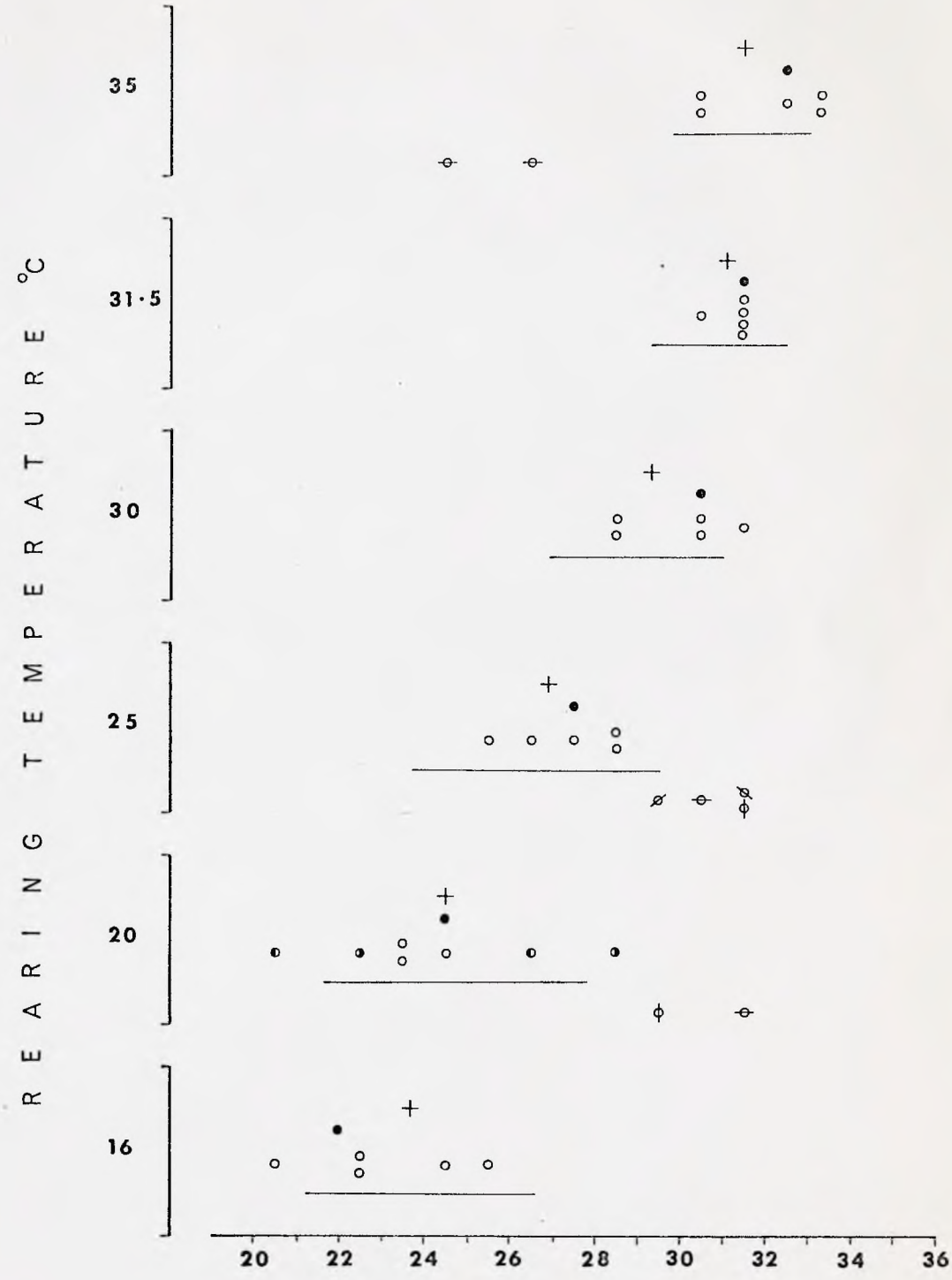

REARING TEMPERATURE °C
35
31·5
30
25
20
16
20
22
24
26
28
30
32
34
36
SELECTED TEMPERATURE °C

Fig. 32

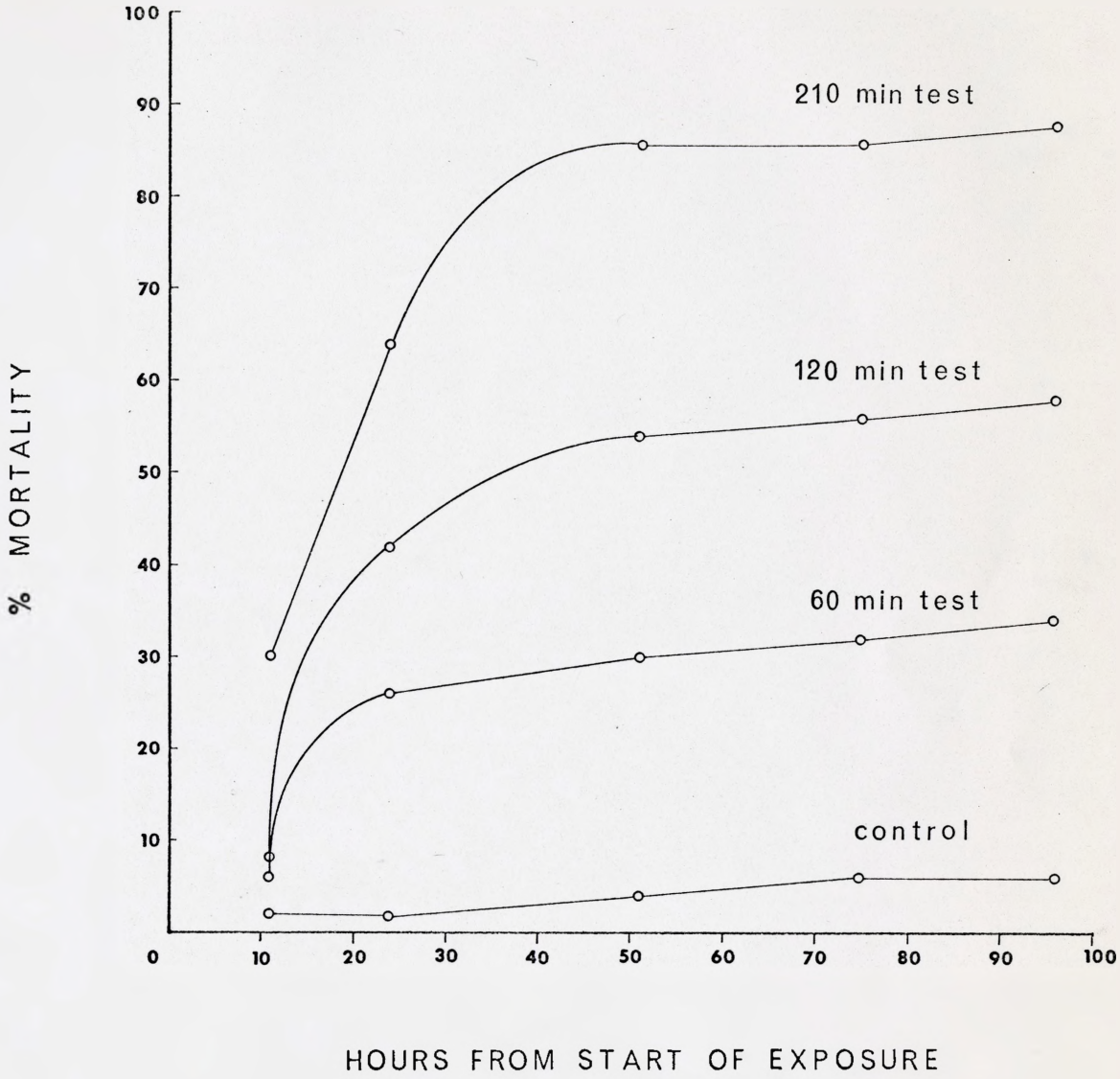


Fig. 33 Mortality of fourth-instar Aedes aegypti larvae, reared at 30 ± 0.5°C, exposed to 41 ± 0.1°C. Larvae kept at 30°C after the exposure.

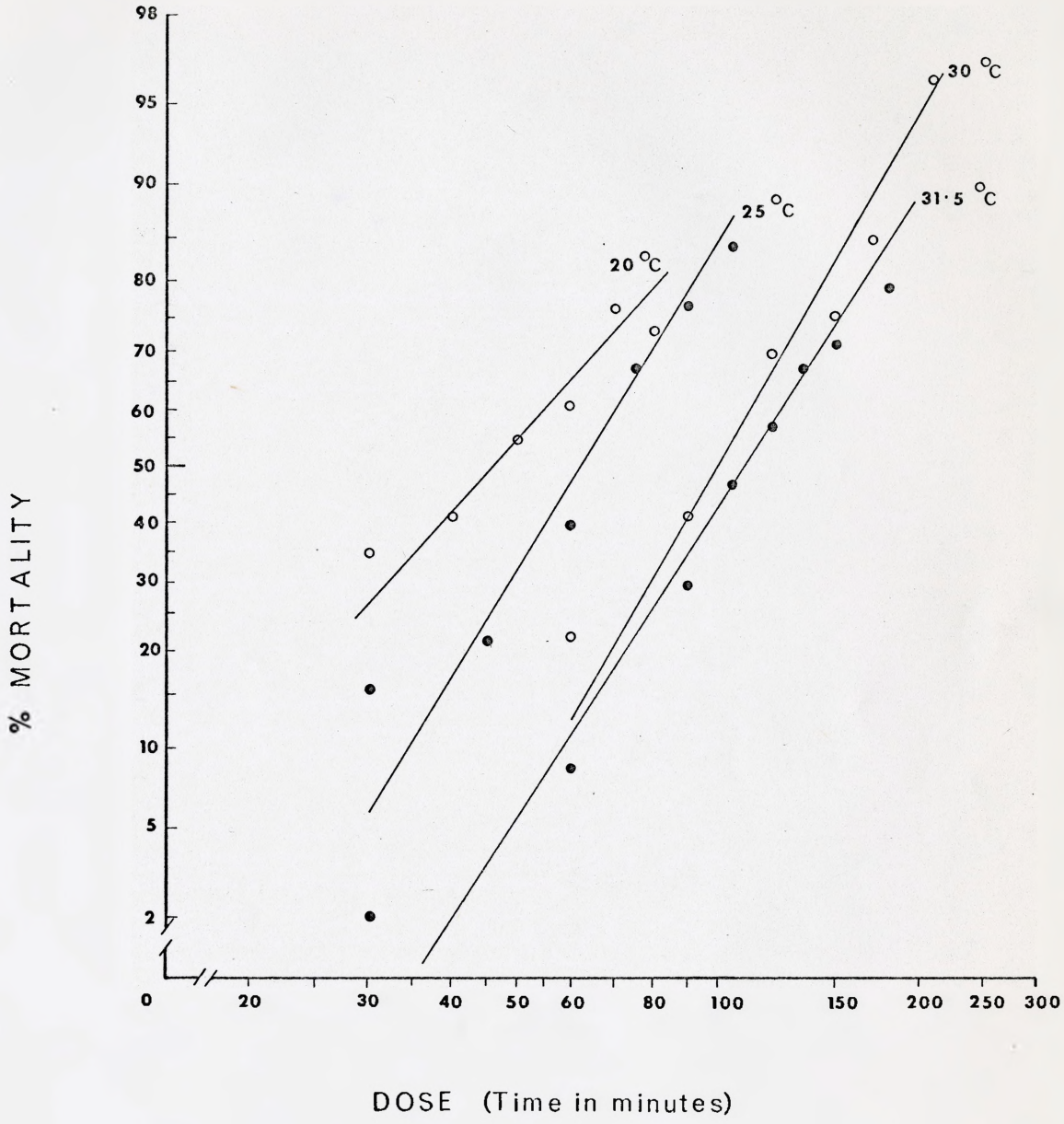


Fig. 34 Regression lines showing the mortality of fourth-instar Aedes aegypti larvae exposed to 41 ± 0.1°C. Rearing temperatures are indicated.

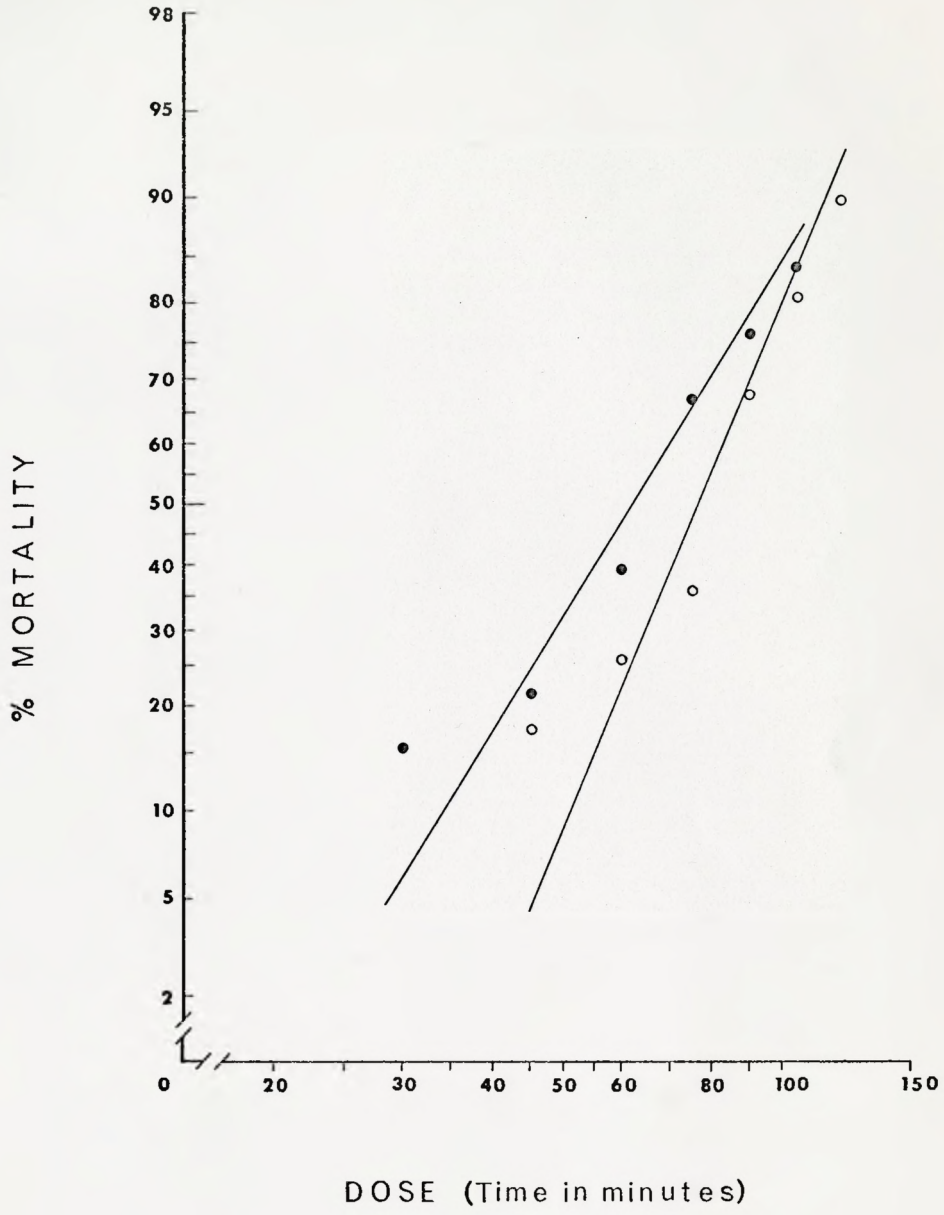


Fig. 35 Regression lines showing the mortality of fourth-instar Aedes aegypti larvae, reared at 25 ± 0.5°C, exposed to 41 ± 0.1°C.

- ∘ 'young' larvae, 30 hours from pupation
- • 'old' larvae, 12 hours from pupation

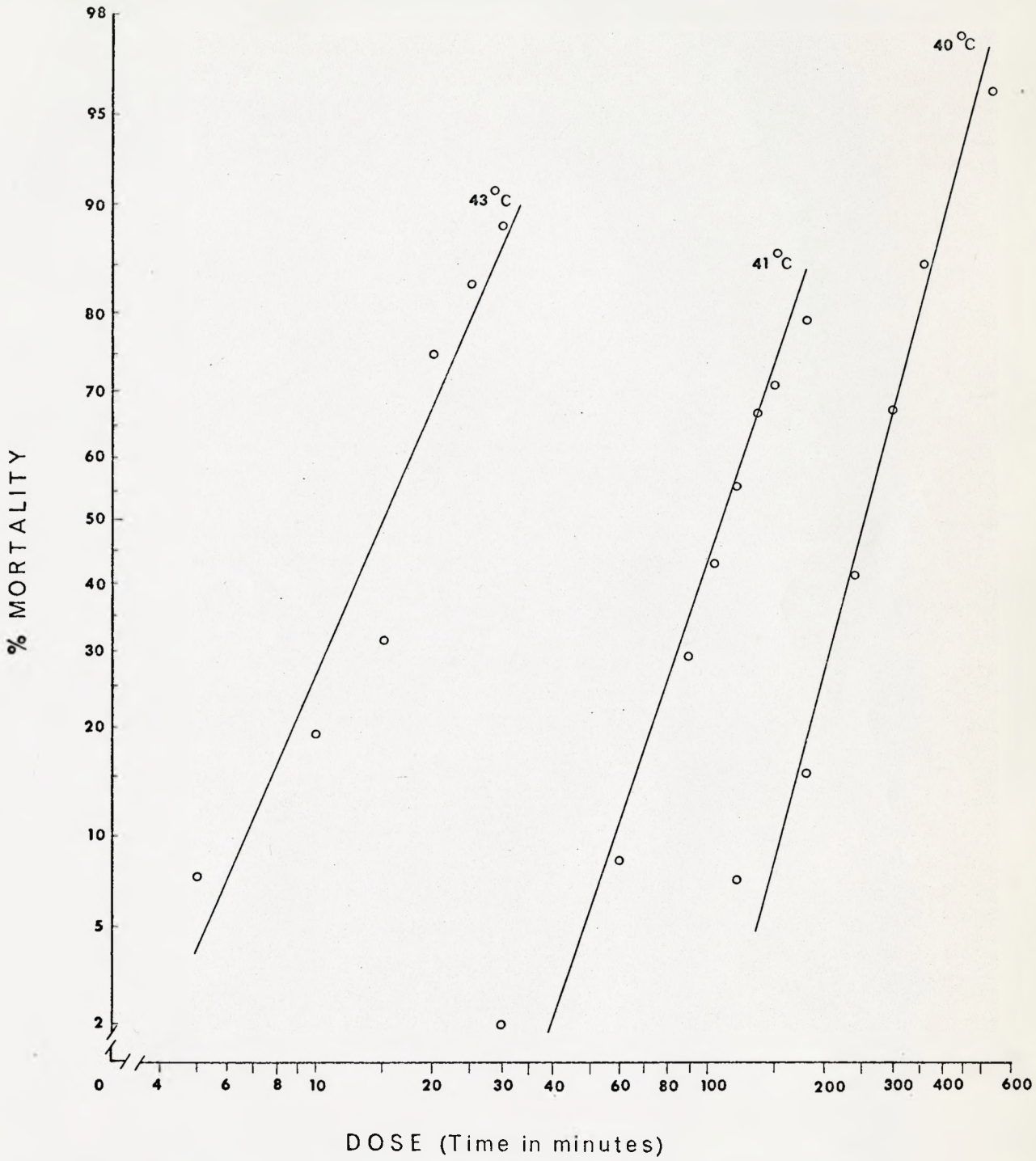


Fig. 36 Regression lines showing the mortality of fourth-instar *Aedes aegypti* larvae reared at 31.5°C. Exposure temperatures are indicated.

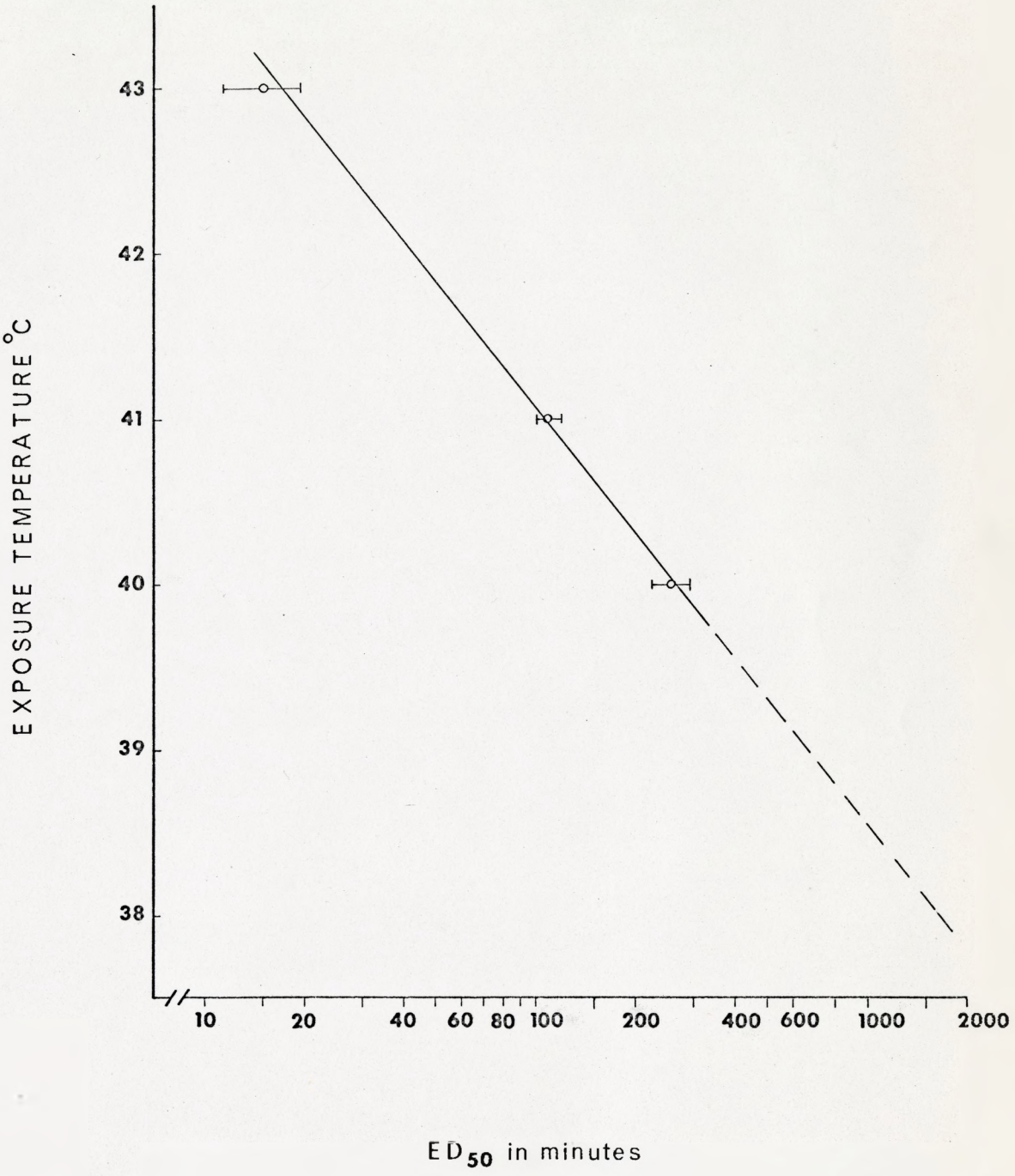


Fig. 37 Median resistance times to high temperatures among fourth-instar Aedes aegypti larvae reared at 31.5°C.

⊢○⊣ ED_{50} and its 95% confidence limits

APPENDIX

1/ Formula used for the calculation of the regression lines in the thermal resistance experiments with Aedes aegypti larvae :

$$Z = mw + k$$

where,

$$m = \frac{N \Sigma wZ - \Sigma w \Sigma Z}{N \Sigma w^2 - (\Sigma w)^2}$$

$$k = \frac{\Sigma wZ \, \Sigma w - \Sigma w^2 \Sigma Z}{(\Sigma w)^2 - N \Sigma w^2}$$

and,

Z = normal deviate of the Y value

w = log. X

N = no. of doses

X = dose (time in minutes)

Y = effect (% mortality)

2/ Analysis of the data from the thermal resistance experiments with Aedes aegypti larvae.

e.g. : Experiment with larvae reared at 20°C and exposed to 41°C.

Total no. of larvae exposed = 1850

Number of doses, N = 6

Larvae per dose = 1850/6 = 308.3

$(Chi)^2$ of regression line = 308.3 x 0.0670 = 44.56

Degrees of freedom, n = N - 2 = 4

$(Chi)^2$ for n = 4, = 9.49 (P = 0.05)

Since the $(Chi)^2$ of the regression line is greater than 9.49, the line is a poor fit, the data are significantly heterogeneous. The value of t, for n = 4, is noted :

t = 2.78

ED_{84} = 91.5 minutes
ED_{50} = 46.4 "
ED_{16} = 23.5 "

} obtained from the regression line by inspection

Slope function, $S = \frac{ED_{84}/ED_{50} + ED_{50}/ED_{16}}{2} = \frac{1.97 + 1.97}{2} = 1.97$

The total number of larvae at those doses whose expected effects were between 16 and 84%, N' = 1850

Dosage range, R = largest dose/smallest dose, = 80/30 = 2.67

A = 1.67 (from Litchfield and Wilcoxon, 1949; nomograph no. 3)

Calculation of factors; including correction for heterogoneity.

Factor for ED_{50}, $fED_{50} = S^{\text{exponent}}$

$\text{exponent} = 1.4t\sqrt{(Chi)^2 / nN'}$

$= 1.4 \times 2.78\sqrt{44.5 / 4 \times 1850}$

$= 0.302$

$fED_{50} = 1.97^{0.3}$

$= 1.225$ (from Litchfield and Wilcoxon, 1949; nomograph no. 2)

Confidence limits of the ED_{50}

$ED_{50} \times fED_{50}$ = upper limit for 19/20 probability

ED_{50} / fED_{50} = lower limit for 19/20 probability

$46.4 \times 1.225 = 56.8$ minutes

$46.4 / 1.225 = 37.8$ minutes

Factor for S, $fS = A^{\text{exponent}}$

$\text{exponent} = \left[5.1t\,(N-1)\sqrt{(Chi)^2 / nN'}\right]/N$

$= 5.1 \times 2.78 \times 5\sqrt{0.00602/6}$

$= 0.916$

$fS = 1.67^{0.916}$

$= 1.6$ (from Litchfield and Wilcoxon, 1949; nomograph no. 2)

3/ "Potency Ratio" test.

Determination of the thermal resistance ratios for the experiments with Aedes aegypti larvae.

e.g. : Comparison between larvae reared at 20°C and those reared at 25°C (132 hr old); exposed to 41°C.

a) Test for parallelism

Slope function ratio, S.R. = S_1 / S_2 , where S_1 is the larger value :

S.R. = 1.97 / 1.59

= 1.24

Using fS_1 and fS_2, fS.R. = 1.7 (from Litchfield and Wilcoxon, 1949; nomograph no. 4)

Since the S.R. value is less than its factor, the regression lines may be considered parallel within experimental error, and thus the resistance ratio may be computed.

b) Resistance ratio

Resistance ratio, R.R. = ED_{50_1} / ED_{50_2}, where ED_{50_1} is the larger value :

R.R. = 62.5 / 46.4

= 1.35

Using fED_{50_1} and fED_{50_2}, fR.R. = 1.34 (from Litchfield and Wilcoxon, 1949; nomograph no. 4)

Since the R.R. value exceeds the value of its factor, the two sets of larvae differed significantly in their resistance to 41°C.

Confidence limits of the resistance ratio is given by :

R.R. x fR.R. = upper limit for 19/20 probability

R.R. / fR.R. = lower limit for 19/20 probability

1.35 x 1.34 = 1.81

1.35 / 1.34 = 1.00