THE EFFECTS OF WATER TEMPERATURE ON OVIPOSITION AND OTHER ASPECTS OF THE LIFE HISTORY OF *AEDES AEGYPTI* (L.) AND *CULEX PIPIENS* L.

by

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The effects of water temperature on oviposition and other aspects of the life history of Aedes aegypti (L.) and Culex pipiens (L.)

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ABSTRACT

This study investigates effects of water temperature of the breeding site on various aspects of the life history of *Aedes aegypti* (L.) and *Culex pipiens* L. in the laboratory.

The effects of water temperature on duration of development, survival to adult, and size of imago were used as indicators of the range of water temperatures favourable for *Ae. aegypti* and *C. pipiens*. The effect of temperature of rearing on the fecundity of *Ae. aegypti* adults was determined. These parameters were then compared to the number of eggs laid by each species in water at various temperatures.

The "optimum" for *Ae. aegypti* was 25 C and for *C. pipiens* was 20 C.

Both species showed reduced oviposition at high and low temperatures when no other option was available. Oviposition of *Ae. aegypti* in water at high temperatures was influenced by the temperature at which they were reared and by air temperature.

When given a choice, *Ae. aegypti* oviposited more in water between 20 and 30 C, whereas the range for *C. pipiens* was between 20 and 25 C. Within these ranges both species had the shortest time of development, and survival, size of imago, and fecundity (*Ae. aegypti* only) were greatest.

The possibility of manipulating temperature in a mosquito control programme is discussed in the light of the results of this work.

ACKNOWLEDGMENTS

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GLOSSARY OF SPECIAL ABBREVIATIONS

CI	Confidence interval.
ET50	Time in days for 50% of the males or 50% of the females to emerge. Based on those surviving to adult.
n	Sample size.
SD	Standard deviation.
SE	Standard error.
s.l.	Sensu lato, in the broad sense, as when a species name is used in the sense of including subspecies.

INTRODUCTION

Larvae of different species of mosquito are found in specific habitats. It was once thought that mosquitoes scattered their eggs indiscriminately and that only certain species were able to survive in a given habitat. Field studies have failed to support this as the larvae found in a given breeding site correspond to the eggs found there (Bates, 1940). It seems more likely that the restriction of a given species to a certain type of larval habitat is due to selective oviposition by the adult female (Wallis 1954).

Various factors have been found to influence site selection. There are two groups of these (Buxton & Hopkins 1927, cited by Wallis 1954): extrinsic factors such as light and darkness, rain, wind, landing place; and intrinsic factors relating to the oviposition site, such as water vapour, temperature, ammonia, alcohol, salinity, pH, dissolved oxygen, microorganisms, aquatic vegetation (Angerilli 1977), soil moisture (Russo 1977) and other chemical factors such as pheromones (Osgood 1971).

The effect of temperature of the water at the breeding site on site selection has not been intensively studied under controlled conditions.

Hecht (1930) studied oviposition and temperature in four species but water temperatures were not well controlled and variations between 1 and 7 C for any particular site were common. Ambient air temperatures during the experiments were not stated. He concluded that females will select a specific range of temperatures for oviposition when given a choice.

Sharma and Sen (1921, cited by Hecht 1930) showed (table I) that different species had different patterns of selection.

Thomson (1940), studying *Anopheles minimus* Theobald, concluded that temperature differences between still and running water at night were so small

TABLE I

Oviposition responses of various species of mosquitoes to water of various temperatures.*1

	number of eggs or egg masses laid in water		
	Hot	Normal	Cold*2
Culex pipiens fatigans (Wiedman) [=*C. fatigans*]*3	31	14	2
Culex vishnui Theobald*4	19	2	2
Culex gelidus Theobald*4	3	0	0
Culex sp. (yellow)	10	1	0
Aedes albopictus (Skuse) [=*Stegomyia albopicta*]*3	1381	415	393
Aedes aegypti L. [=*Stegomyia sugens*]*2	33	60	0
Armigeres obturbans (Walker)*4	50	0	0
Anopheles subpictus Grassi [= *A. rossi*]*3	108	393	0
Culex concolor Edwards*4	2	0	2

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*1 Table from Sharma and Sen (1921) cited from Hecht (1930).

*2 Hot was 5-7 C above normal, normal was 23-35 C, and cold was 2-3 C below normal.

*3 Updated names from Stone *et al*. (1959) and Gillett (1971).

*4 The names of authors were not given in the original tables and were added to this one using the above references.

and irregular that water temperature could play no part in site selection of the gravid female. In laboratory tests he found that the ovipositing female usually avoided temperatures higher than those of breeding places at night.

Surtees (1967) found that when Aedes aegypti (L.) was allowed to lay in a range of water temperatures from 26 to 44 C nearly 80% of the eggs were laid below 30 C and only 0.2% at 39 and 44 C.

According to Christophers (1960) survival at extreme water temperatures may be studied in two ways: lethal limits for short exposures may be determined; or the temperature limits within which the species can exist, undergo growth and complete development may be ascertained.

Lethal limits over short exposures vary greatly with the conditions of the test. The effects of high or low extreme temperatures can be modified by a period of acclimatization at less extreme temperatures

(Mellanby 1960). Mellanby uses the term acclimatization for this process. Other authorities (Prosser and Brown 1950) would call it acclimation. Different instars may vary in their response to temperature. Mature eggs of Ae. aegypti were the instar most tolerant of high temperatures, and young pupae were more tolerant to temperature extremes than older pupae in a study by Bar-Zeev (1957).

Exposure for short periods does not indicate the suitablity of a particular temperature for growth and development. A partial measure of this would be the percentage survival, from larva to imago at a particular temperature.

Optimum survival temperature has been defined as the temperature giving the highest percentage of adult survival within the shortest developmental period (Blunck 1924, cited by Brust 1967). Bates (1949) suggested that the temperature scale for any given species of mosquito could be considered to include a lower fatal limit, a zone of unfavourable low temperatures, a zone of favourable temperatures, a zone of unfavourable high temperatures, and an upper fatal limit (thermal death point). These definitions were designed to avoid the word optimum. The "true optimum" temperature for a species would be almost impossible to determine as our definition and the measurements we make to determine the "optimum" are often inadequate, so the word must be used with care and the criteria used to define the optimum stated clearly.

The influence of temperature on growth has been investigated in many species of insects, and gain in weight has been shown to vary inversely with rearing temperature by many workers (van den Heuvel 1963).

Martini (1923, cited by Christophers 1960) noted that mosquito larvae subjected to warm temperatures produced smaller adults than those produced at cooler temperatures. This effect may be the direct result of temperature altering metabolic processes and the endocrine system or it may be due to a change in food supply, e.g. temperature may affect the growth of microorganisms that serve as food (Roberts and Hsi 1977). This effect is commonly seen in nature in species of *Anopheles* (Christophers 1960), and also in *Ae. aegypti* as specimens bred at lower temperatures are larger than those bred at higher temperatures (Christophers 1960; Costello 1974). Kurihara (1963) found that wing lengths of adult *C. pipiens* s.l. were largest in those bred at low temperatures and became smaller with increased temperature.

There are many ways to measure size. Weight can be used as long as the condition of the insect is clearly stated. Weight can vary due to feeding, gravid condition, age and water uptake (Christophers 1960). A linear measure is useful to describe size. Christophers (1960) found a correlation between weight and wing length. Wet or fresh weights and wing lengths give a satisfactoy index of size for comparisons between species and temperatures.

Although size decreases with increasing temperature, speed of development increases in many species of mosquito (Christophers 1960; Kurihara 1963; Brust 1967). The rate of postembryonic growth follows an S-shaped curve. Below a developmental threshold no growth occurs, above the threshold the rate of growth increases with increasing temperature, reaching a maximum at the so called "optimum temperature" above which it declines (Clements 1963). This "optimum" is defined as the temperature at which development is the fastest but does not take mortality into account as Blunck's (1924) definition does.

Colless and Chellapah (1960) found that a direct relationship between egg production and body weight could be demonstrated when Ae. aegypti larvae were starved. The larger the adult the greater the egg production. Extreme temperatures may also affect egg production as a large range in the sizes of adults is produced by rearing at various constant temperatures.

Two species of mosquito were used in this study: Ae. aegypti and C. pipiens. Ae. aegypti is found throughout most of the tropical and subtropical regions of the world (Carpenter and LaCasse 1955). Eggs are deposited in artificial containers, either just above the water level or on the surface of the water. Larvae have been found in the rot cavities of trees and in rock pools in the United States and Africa (Carpenter and LaCasse 1955) but are rarely found far from human habitations. A quiescent period may be passed in the egg stage however, breeding is usually continuous throughout the year in warmer climates.

C. pipiens is a cosmopolitan speces, which, with its subspecies and varieties, is found almost all over the world wherever there is land, except in the far north and south (Gillett 1955). The variety used in this study was collected in a temperate region so its response to temperature should differ from *Ae. aegypti*. *C. pipiens* lays its eggs in rafts at the surface of the water. Larvae are found in foul water in rain barrels, tubs, catch basins, faulty cesspools, ditches and similar habitats (Carpenter and LaCasse 1955). Adult females pass the winter hibernating in warm places which afford protection from the cold.

The purpose of this study was to investigate the effects of water temperature on various aspects of the life cycle of *Ae. aegypti* and *C. pipiens*. The aspects studied were:

A) The effects of water temperature upon survival and development time of the immature instars and size of the imago.

B) The effects of temperature of rearing upon the fecundity of the adults produced.

C) The effects of water temperature on oviposition site selection.

In the summary and conclusions section the oviposition behaviour of the two species in response to water temperature was compared with other aspects of the life cycle which were studied.

GENERAL METHODS AND MATERIALS

A) Rearing Techniques

1) Aedes aegypti

Eggs were obtained as needed from a colony that has been maintained at Simon Fraser University since 1966. The air temperatures in the rearing room of the main colony were held to a minimum of 20 C and upper extremes in the summer may reach 30 to 32 C.

The eggs were hatched at room temperature (20 ± 2 C) and the larvae reared in 2000 ml of distilled water in white enamel trays 40 by 25 by 4.5 cm deep. Larval density varied from 0.3 to 0.6 larvae per cm3. Finely-ground TetraMin (made by Tetra-Werke, Melle) was provided daily as food and dried brewers yeast was provided 4 days after hatching. Yeast provided nutrients which are not provided in the Tetramin. Quantities of food followed Gerberg's (1970) schedule (table II,

TABLE II

Regular and excess (125% of regular) feeding schedules for *Aedes aegypti* and *Culex pipiens* larvae reared at various constant temperatures. Food was ground TetraMin.

Aedes aegypti

Regular		Excess	
Day 0*	0.2 mg per larva	Day 0*	0.25 mg per larva
Day 1	0.2	Day 1	0.25
Day 2	0.3	Day 2	0.38
Day 3	0.4	Day 3	0.5
Day 4 on	0.6	Day 4 on	0.75
On Day 3 and every 7 days after this--.0045 mg per larva of brewers yeast.		On Day 3 and every 7 days after this-.006 mg per larva of brewers yeast.	

Culex pipiens

Regular		Excess	
Day 0*	0.15 mg per larva	Day 0*	0.19 mg per larva
Day 1	0.25	Day 1	0.31
Day 2	0.25	Day 2	0.31
Day 3	0.3	Day 3	0.38
Day 4	0.3	Day 4	0.38
Day 5	0.4	Day 5	0.5
Day 6	0.5	Day 6	0.63
Day 7 on	0.7	Day 7 on	0.88
On Day 3 and every 7 days after this--.0045 mg per larva of dried bakers yeast.		On Day 3 and every 7 days after this--.006 mg per larva of dried bakers yeast.	

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*Day of hatching

regular). TetraMin was used rather than ground dog food in all rearing because the latter caused formation of scum on the surface of the water in the rearing trays.

Pupae were sexed by size and placed in separate cages so that the approximate number of males and females available for experimentation was known.

Adult emergence and maintenance cages were 18 by 12 by 16 cm high. The front had a clear sliding Perspex door with a central hole fitted with a #3 rubber stopper. The back was nylon mesh. The bottom, top and other two sides (16 by 12 cm) were wood.

Adults were fed by soaking absorbent cotton rolls in a 10 percent sucrose solution. Relative humidity was maintained at 60 to 65 percent by partially immersing a paper towel wick in a beaker of water. Costello (1974) used this method to maintain adults and found they could be kept alive and in apparent good health for up to six weeks. Adults were removed with an aspirator when needed. Photoperiod followed the light regime of the normal work day and that provided naturally from the windows.

2) *Culex pipiens*

A colony was started in September, 1975, by obtaining egg rafts from a colony maintained at Simon Fraser University since July, 1974. The colony was started from larvae collected in Richmond, B.C. (Angerilli 1977).

Mosquitoes were reared continuously as described by McLintock (1960) in two cages. All stages were kept in each cage, 50 by 50 by 90 cm high, with a wooden frame supporting a sliding, clear, Plexiglas front having a 23 cm diameter hole in the center fitted with a sleeve. The cage had a wooden bottom, and sides and top of nylon mesh. The cage was large enough to provide space for swarming.

Larvae and pupae developed in white enamel trays, 40 by 25 by 4.5 cm deep, placed in the bottom of the cages. A water depth of 3.5 cm was maintained. Air was slowly bubbled through the water to prevent build-up of scum. 400 mg of ground TetraMin and 50 mg of ground Fleishmann's dry active baker's yeast were added three times a week.

A restrained shaved guinea pig was left in the cage overnight once a week for blood meals. A 10 percent sucrose solution in a 250 ml Erlenmeyer flask stoppered with 3 cotton wicks was always available as a source of food.

The two cages were maintained at room temperature and illuminated by two 40-watt bulbs for 16 hours each day. The rest of the room was lit by fluorescent lights which were on for 10 to 12 hours of the above 16 hours. Windows also provided some natural light. This light regime appeared satisfactory as fertile egg rafts were produced.

When adult _C. pipiens_ were needed for experiments, egg rafts were removed from the main colonies and larvae reared following the same procedure as for _Ae. aegypti_ except for a different feeding schedule (table II, regular) taken from Gerberg, Hopkins, and Gentry (1969).

B) Statistical Analyses

Except when stated otherwise, mean values are reported in the text plus or minus one standard error (SE) with the number of cases, n, in brackets, when n varies for the means reported. In the figures, mean values are plotted plus or minus one standard deviation (SD).

Analyses of variance were used to determine the significance of the effect of treatments and the interactions between them. Student-Newman-Keuls multiple range tests were used to determine which means were significantly different (Sokal & Rohlf 1969; Nie _et al_. 1975).

Regression analyses were used when effects appeared to be linearly related to treatments. Significance of the fit of the line was tested using an F-test. Slopes of lines were tested to determine if they were significantly different from zero by calculating 95 percent confidence limits and seeing if zero was included (Goldstein 1964). Slopes of two lines were compared using modified T-tests (Zar 1974).

Tests were considered significant when $p \leq 0.05$ and highly significant when $p \leq 0.01$. The null hypothesis can be accepted when $p \geq 0.50$, in analysis of variance. It is important to remember that to accept a null hypothesis is not "proof" that it is true. It is only a statement that there is not sufficient evidence to conclude that it is false (Zar 1974).

The only transformation used was an arcsin square root transformation which is used to normalize data that are percentages or proportions (Sokal and Rohlf

1969). Means from transformed data were retransformed to the original scale before being reported in the text. Confidence intervals (CI), also retransformed, are reported on a table or graph as the transformations used were nonlinear (Sokal & Rohlf 1969). These confidence limits are asymmetrical, so transforming SE or SD to the original scale would be misleading (Sokal & Rohlf 1969).

PART I

EFFECTS OF DIFFERENT CONSTANT TEMPERATURES OF REARING OF *AEDES AEGYPTI* AND *CULEX PIPIENS* ON THE SIZE OF ADULT, SURVIVAL TO IMAGO, AND RATE OF DEVELOPMENT.

This study was conducted to determine the suitability of various constant water temperatures for survival of *Ae. aegypti* and *C. pipiens*. Survival from first instar larva to imago was measured. Development time and size of the imago, as measured by weights and wing lengths, was also recorded for each mosquito as a measure of the other effects that rearing temperatures have on the life cycle of each species of mosquito.

Methods and Materials

On the first day of an experiment eggs were hatched at room temperature and the larvae separated into groups of 50. Four replicates were done for each species at each temperature.

The larvae were placed in 200 ml of distilled water at room temperature in a glass finger bowl, 10 cm in diameter and 4 cm deep. The bowls were then put into incubators set at 10 different temperatures ranging from 10 to 40 C. The water level was maintained by the addition of water each day.

The number of living larvae was estimated each day and the appropriate amount of food added following the schedules described earlier (table II, excess). Food was supplied in excess to eliminate it as a limiting factor. Yeast was added on Day 3 and every 7 days thereafter until pupation was complete.

On Day 6 and every 7 days thereafter the number of living larvae was accurately determined as they were transferred singly with a medicine dropper to fresh water at the appropriate temperature. This also helped prevent mortality from the build up of bacterial scum on the surface of the water.

When pupation began, each bowl was placed in a small cage and put back into the appropriate incubator. Adults were removed daily with an aspirator and placed in vials, 4.5 cm by 1.5 cm in diameter, stoppered with cotton plugs. The adults were preserved by freezing and were sexed, counted, and had their weights and wing lengths measured at a convenient time.

Survival was recorded as a proportion of those reaching the adult stage. Data were transformed using an arcsin square root transformation before being compared across temperatures for each species. A oneway analysis of variance and Student-Newman-Keuls test were used.

Weights were recorded in milligrams and wing lengths in millimeters. Wing lengths were measured from the tip, excluding the fringe, to the bend in the trailing edge of the distal end of the alula (van den Heuvel 1963). Size of females and males was analyzed separately as the male is generally smaller than the female (Christophers 1960). For each species and sex an analysis of variance was done and a regression analysis applied.

The time for 50% of the males and 50% of the females to emerge was calculated in each replicate for each sex separately by interpolation from graphs of cumulative emergence. This is referred to as an ET50.

Results

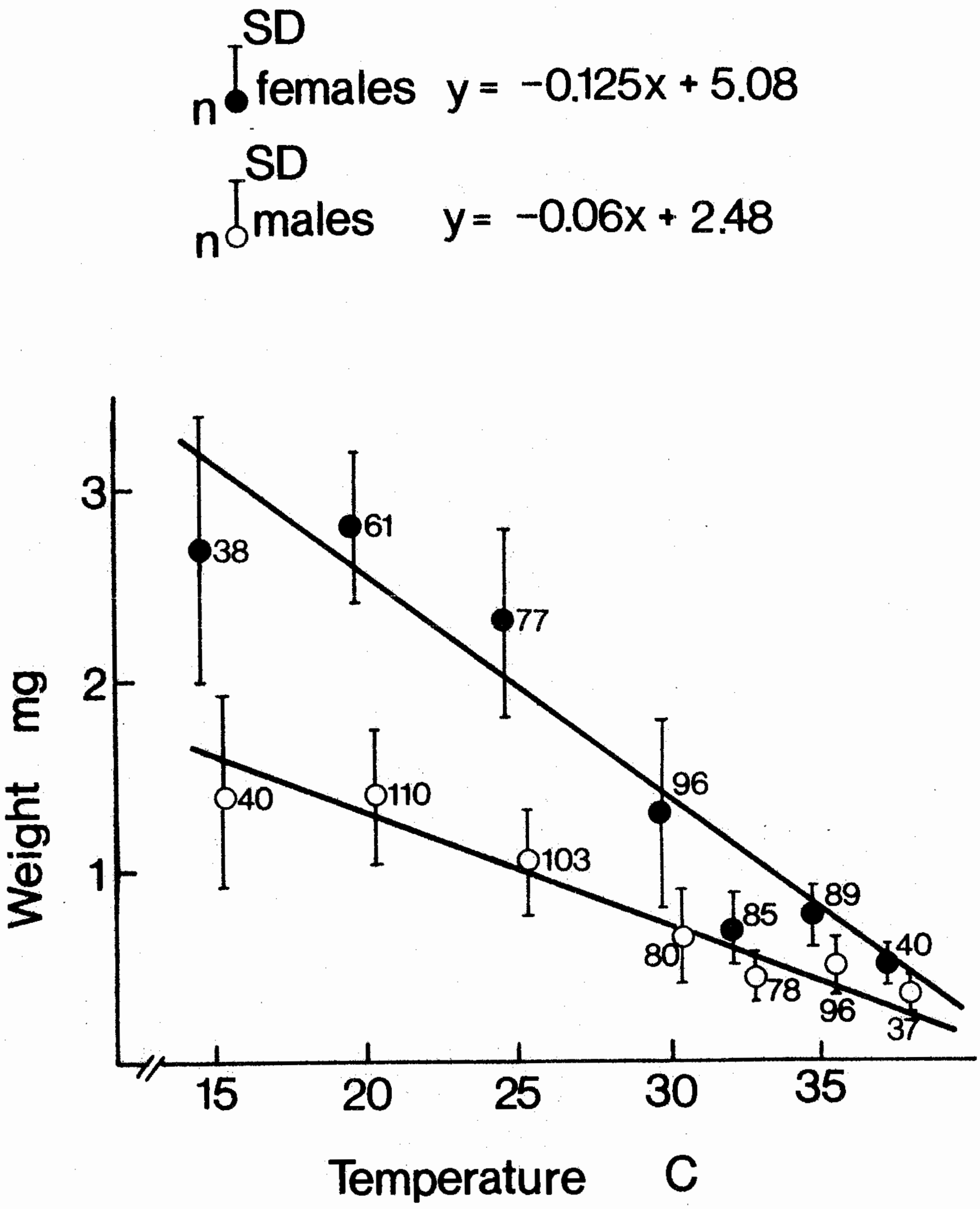
A) Size

The size of adults, as measured by weights and wing lengths, decreased with increased larval rearing temperature for both species of mosquito.

Temperature and sex influenced both weight and wing lengths for the two species studied (analysis of variance $p \leq 0.01$). Measurements of males and females of both species were analyzed using linear regression and the same tests of significance were used for all four sets of data.

The regression line for weights of Ae. aegypti females had the equation $y = -0.125x + 5.08$ (fig. 1) while the one for males had the equation $y = -0.06x + 2.48$ (fig. 1) different (T-test).

Fig. 1 Mean weights ± one SD of adult *Ae. aegypti* reared at different temperatures. The number of mosquitoes measured (n) is along side each point. Regression lines have been plotted, and the equations are on the graph.


SD
n ● females y = −0.125x + 5.08
SD
n ○ males y = −0.06x + 2.48
38
61
77
96
85
89
40
40
110
103
80
78
96
37
3
2
1
Weight mg
15
20
25
30
35
Temperature C

The regression line for wing lengths of Ae. aegypti females had the equation $y = -0.06x + 5.09$ (fig. 2) whereas the one for males had the equation $y = -0.04x + 3.84$ (fig. 2). Both relationships were significant. The slopes were significantly different from zero and the slopes for females and males were significantly different.

The regression line for weights of C. pipiens females had the equation $y = -0.018x + 1.55$ (fig. 3) whereas the one for males had the equation $y = -0.016x + 1.01$ (fig. 3). Both relationships were significant. The slopes were significantly different from zero and the slopes for males and females were not significantly different.

The regression line for wing lengths of C. pipiens females had the equation $y = - 0.045x + 5.62$ (fig. 4) while the one for males had the equation $y = -0.046x + 4.8$ (fig. 4). Both relationships were significant. The slopes were significantly different from zero and

Fig. 2 Mean wing lengths ± one SD of adult *Ae. aegypti* reared at different temperatures. The number of mosquitoes measured (n) is along side each point. Regression lines have been plotted, and the equations are on the graph.

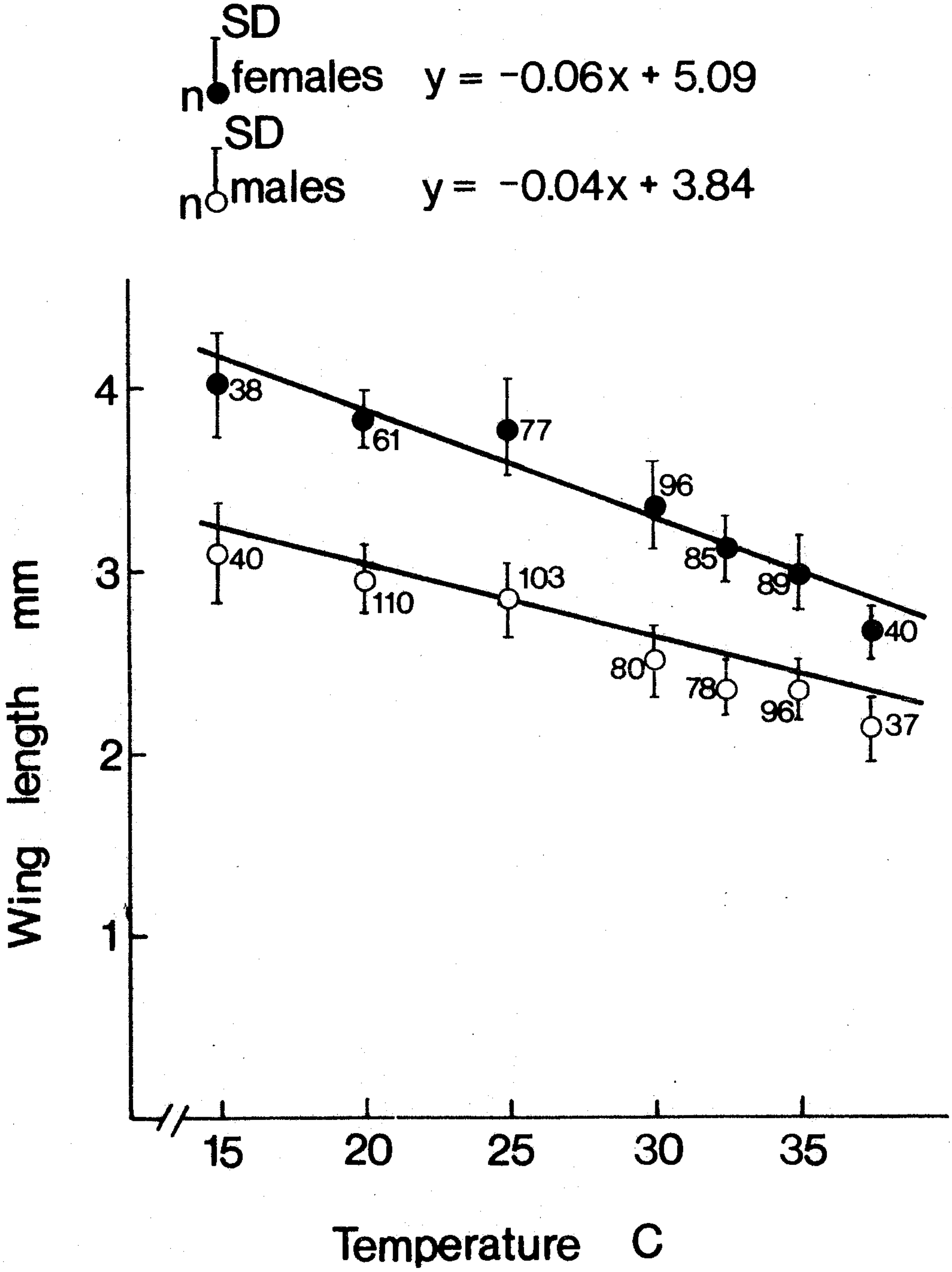

SD
n females y = −0.06x + 5.09
SD
n males y = −0.04x + 3.84
Wing length mm
4
3
2
1
38
61
77
96
85
89
40
40
110
103
80
78
96
37
15
20
25
30
35
Temperature C

Fig. 3 Mean weights ± one SD of adult *C. pipiens* reared at different temperatures. The number of mosquitoes measured (n) is along side each point. Regression lines have been plotted, and the equations are on the graph.

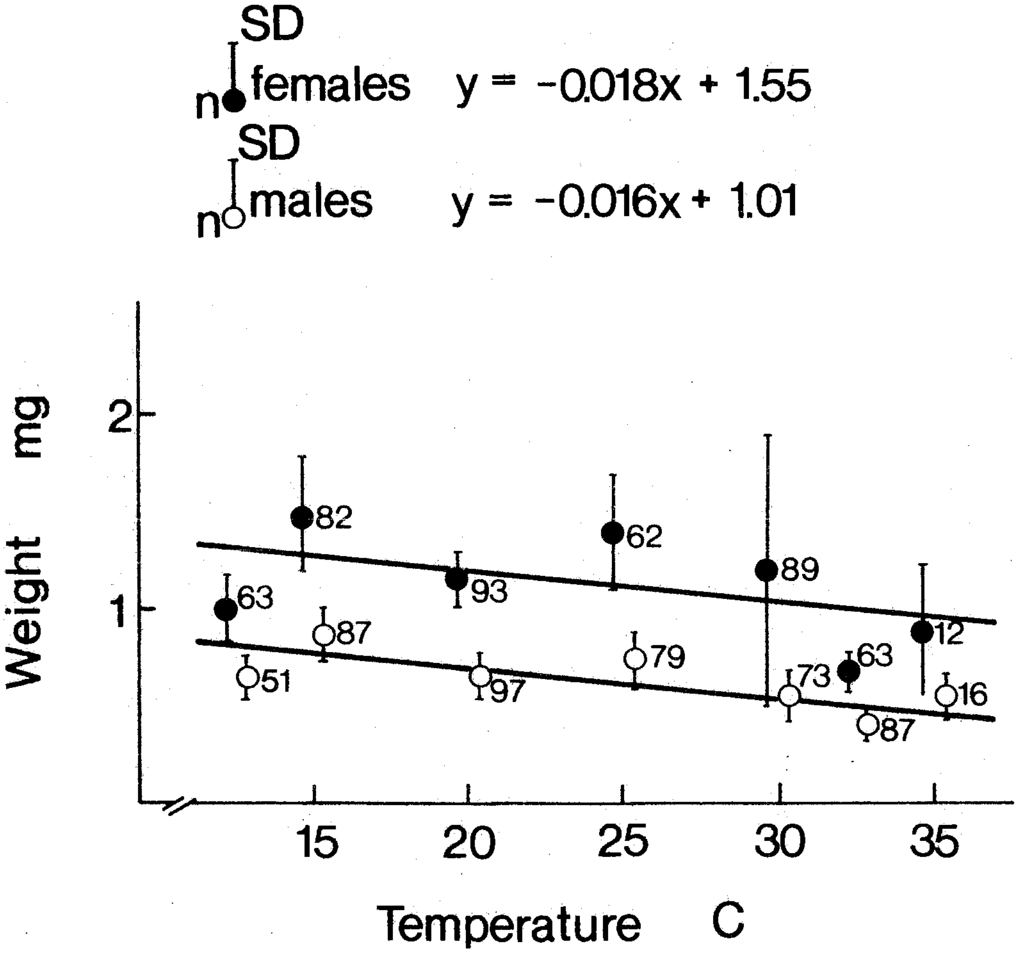
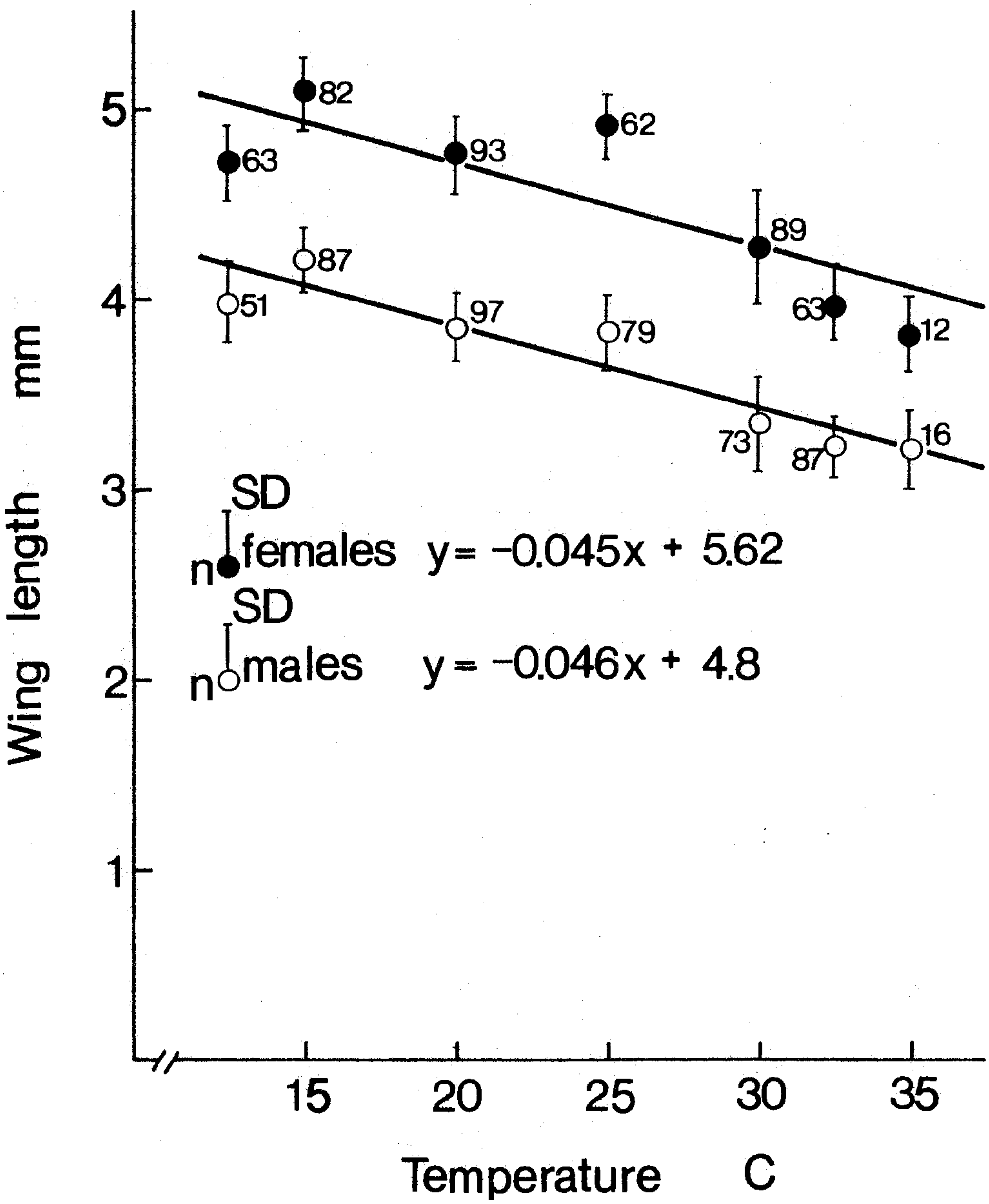

SD
n ● females y = -0.018x + 1.55
SD
n ○ males y = -0.016x + 1.01
Weight mg
2
1
82
62
89
63
93
12
87
79
63
73
51
97
16
87
15
20
25
30
35
Temperature C

Fig. 4 Mean wing lengths ± one SD of adult *C. pipiens* reared at different temperatures. The number of mosquitoes measured (n) is along side each point. Regression lines have been plotted, and the equations are on the graph.


Wing length mm
5
4
3
2
1
15
20
25
30
35
Temperature C
82
63
93
62
89
63
12
87
51
97
79
73
87
16
SD
n females y= −0.045x + 5.62
SD
n males y= −0.046x + 4.8

the slopes for females and males were not significantly different.

B) Survival

Both species survived to the adult stage over a wide range of temperatures. *Ae. aegypti* survived at temperatures ranging from 15 to 37.5 C (fig. 5) while *C. pipiens* survived at temperatures ranging from 10 to 35 C (fig. 6).

The survival of *Ae. aegypti* was depressed at 15 and 37.5 C with a mean proportion of .41 (fig 5) surviving at each temperature (see appendix A for raw data). Between 20 and 35 C survival was high with the mean proportion surviving ranging from .85 to .96 (fig. 5). Over this range of temperatures the differences in survival were not significant (Student-Newman-Keuls).

The survival of *C. pipiens* was depressed at 10 and 35 C with mean proportions of 0.007 and .14 surviving respectively (fig. 6, see appendix B for raw data).

Fig. 5 Survival to imago of Aedes aegypti at various constant temperatures. Means (n=4) and 95% CI were retransformed from an arcsin square root transformation. Means followed by the same letter are not significantly different (Student-Newman-Keuls).

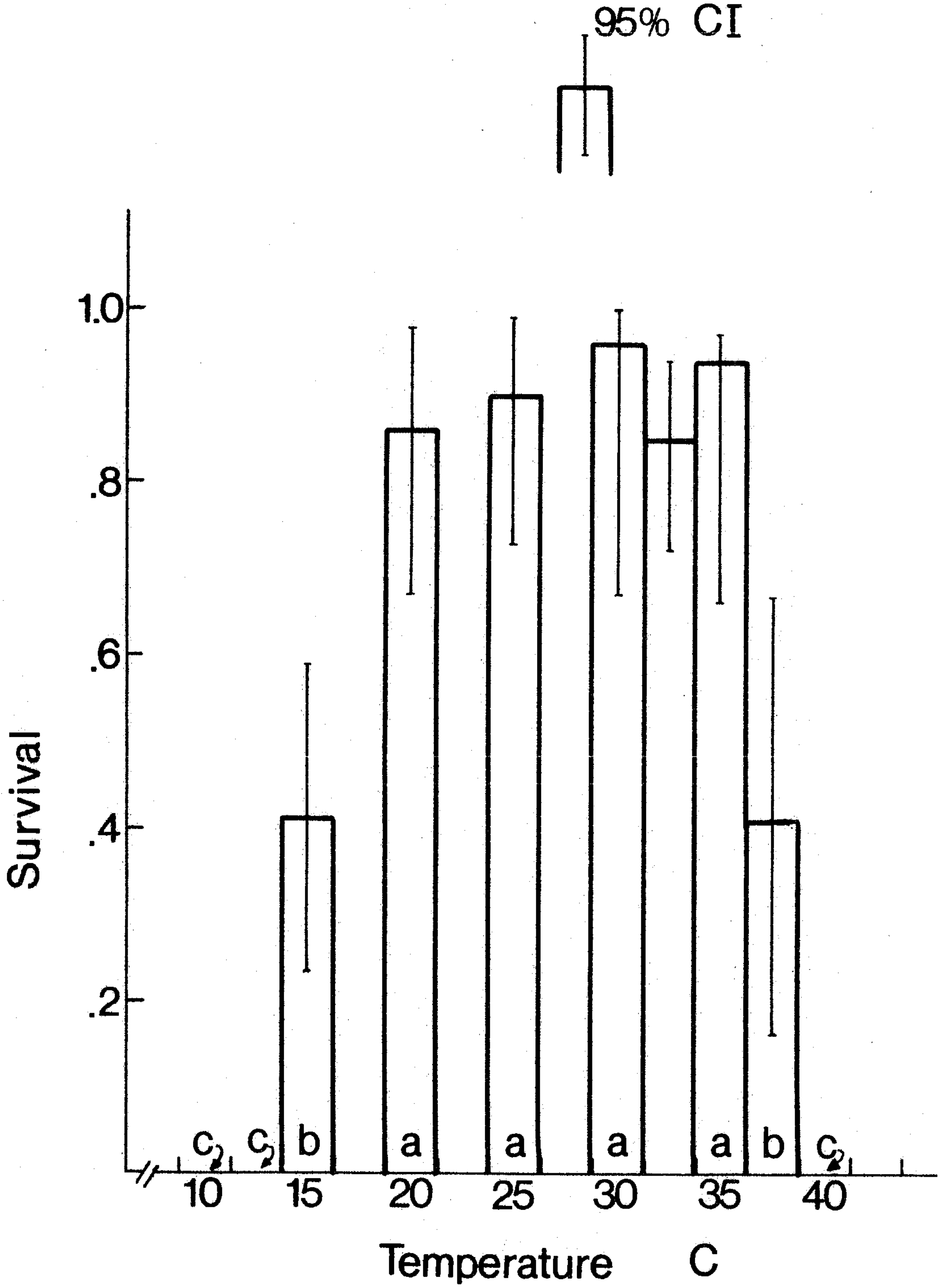
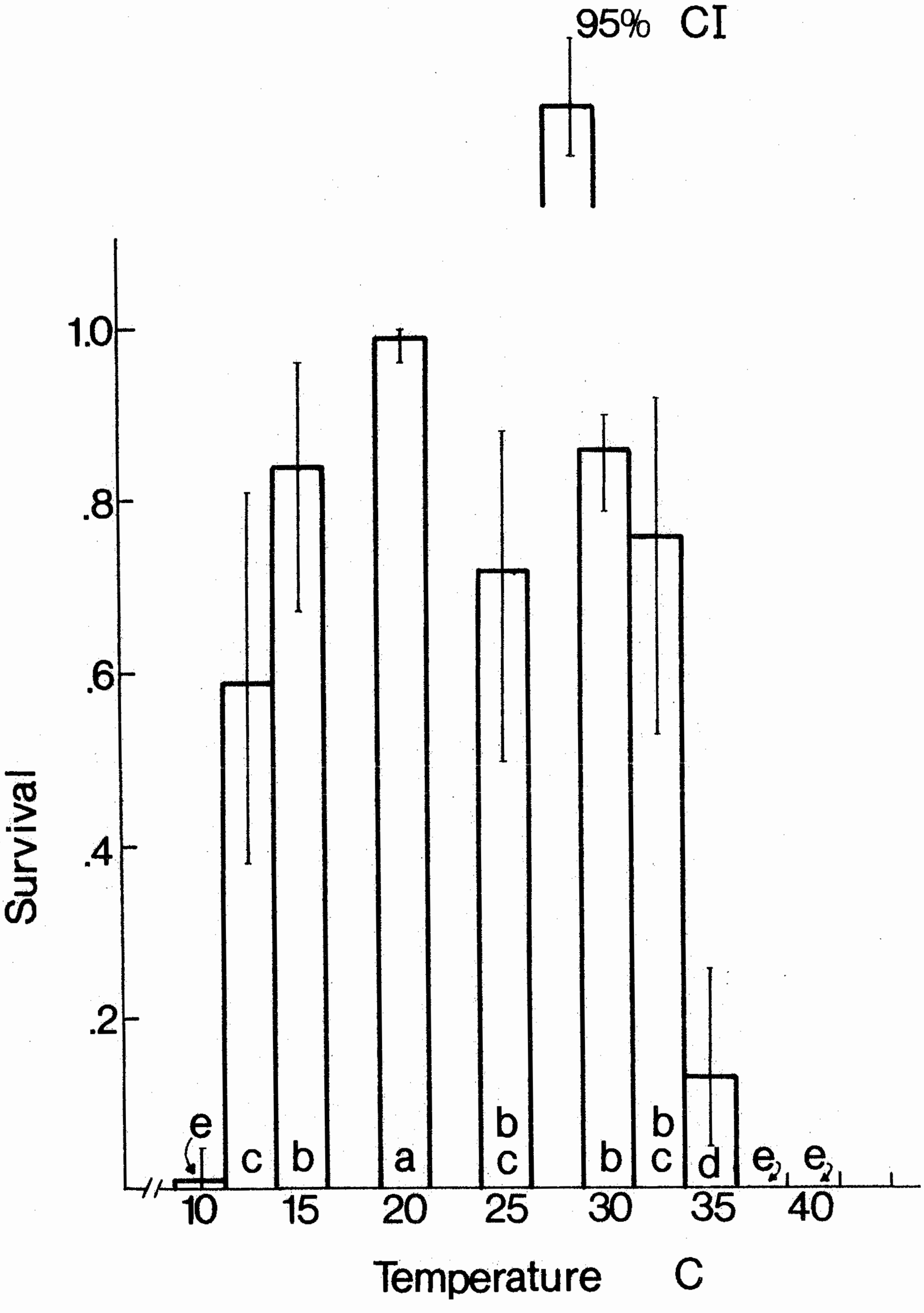

95% CI
1.0
.8
.6
.4
.2
Survival
c
c
b
a
a
a
a
b
c
10
15
20
25
30
35
40
Temperature C

Fig. 6 Survival to imago of _Culex pipiens_ at various constant temperatures. Means (n=4) and 95% CI were retransformed from an arcsin square root transformation. Means followed by the same letter are not significantly different (Student-Newman-Keuls).


95% CI
Survival
1.0
.8
.6
.4
.2
e
c
b
a
b
c
b
b
c
d
e
e
10
15
20
25
30
35
40
Temperature C

The mean proportion surviving at 12.5, 15, 25, 30 and 32.5 C ranged from .59 to .86. Maximum survival was observed at 20 C where an average of .99 survived. Survival at 20 C was significantly different from survival at all other temperatures (Student-Newman-Keuls).

C) Development Time

The time taken for 50% emergence (ET50) for males or females decreased with increasing larval rearing temperatures for both species of mosquito (tables III & IV). Each ET50 was calculated from those surviving, out of 50, to adult in each of four replicates.

The mean ET50 for *Ae. aegypti* females at 15 C was 28.0 ± 0.52 days and at 37.5 C was 6.1 ± 0.11 days. Males took 27.7 ± 0.75 days at 15 C and 5.9 ± 0.17 days at 37.5 C.

The mean ET50 for *C. pipiens* females at 12.5 C was 39.6 ± 0.54 days and at 35 C was 8.4 ± 0.42 days.

TABLE III

Mean ET50, as calculated from those surviving out of 50, of male and female _Aedes aegypti_ reared at various constant temperatures (n=4).

Females

Temperature	Mean*	Standard Error
15	28.0 a	0.52
20	14.9 b	0.19
25	10.5 c	0.08
30	7.7 d	0.18
32.5	6.8 e	0.04
35	7.1 de	0.07
37.5	6.1 f	0.11

Males

Temperature	Mean	Standard Error
15	27.7 g	0.75
20	13.9 h	0.18
25	9.8 i	0.12
30	6.8 j	0.08
32.5	6.6 j	0.17
35	6.3 j	0.10
37.5	5.9 j	0.17

---

*Means followed by the same letter are not significantly different (Student-Newman-Keuls, $p \leq 0.05$). Test was done on males and females separately.

TABLE IV

Mean ET50, as calculated from those surviving out of 50 of male and female *Culex pipiens* reared at various constant temperatures (n=4).

Females

Temperature	Mean*	Standard Error
12.5	39.6 a	0.54
15	23.5 b	0.24
20	13.6 c	0.20
25	11.8 d	0.30
30	10.4 e	0.03
32.5	10.0 e	0.07
35	8.4 f	0.42

Males

Temperature	Mean	Standard Error
12.5	37.5 g	0.96
15	22.2 h	0.15
20	11.9 i	0.13
25	10.6 j	0.07
30	8.0 k	0.14
32.5	8.2 k	0.25
35	7.1 k	0.35

---

*Means followed by the same letter are not significantly different (Student-Newman-Keuls, $p \leq 0.05$). Test was done on males and females separately.

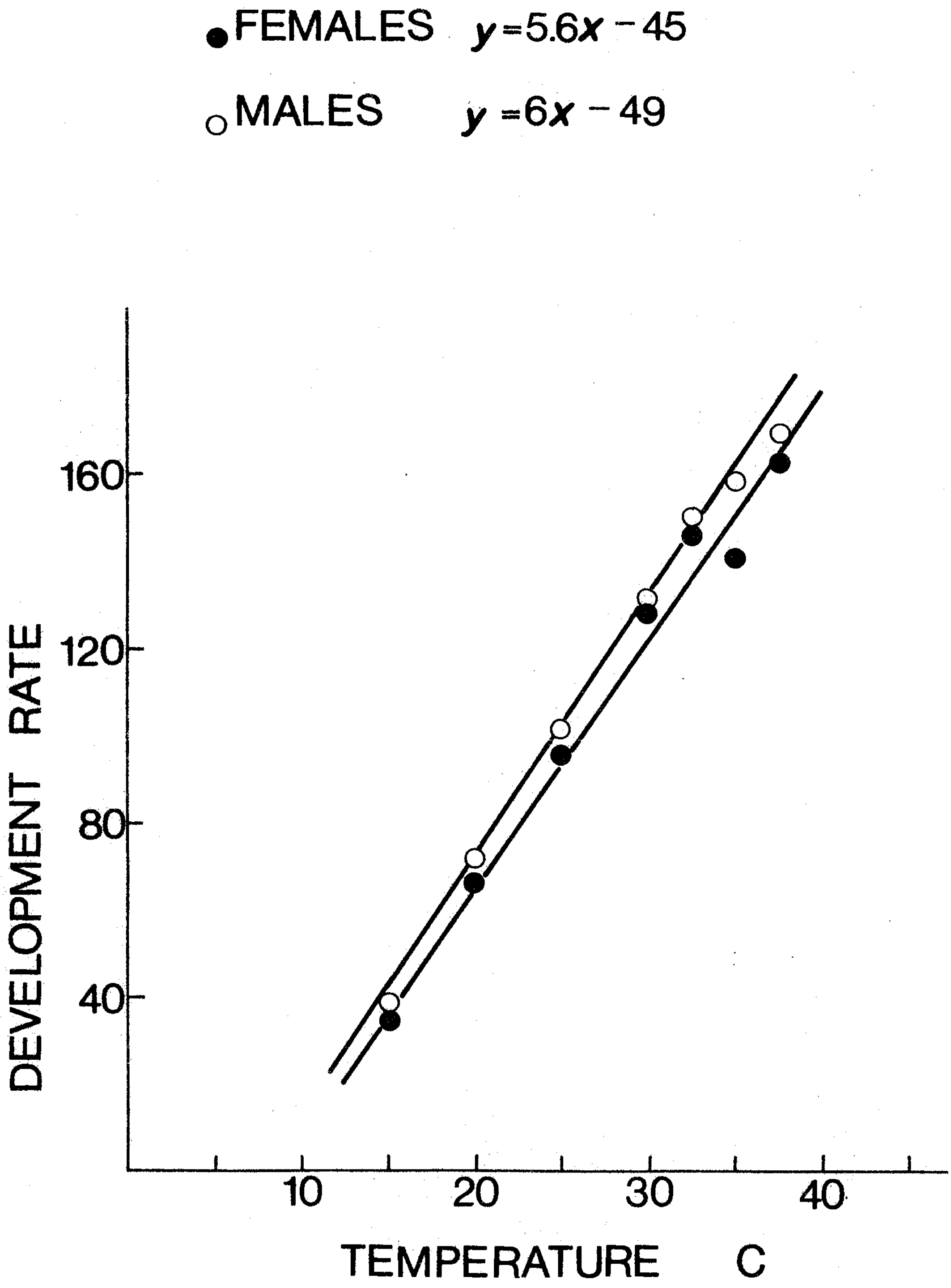
Males took 37.5 ± 0.96 days at 12.5 C and 7.1 ± 0.35 days at 35 C.

The relationship of development time to the sex and to the temperature of rearing were both highly significant (analysis of variance), for the two species studied. Males generally emerged before females and development time decreased with increasing rearing temperature.

The data was transformed to rates of development, 1000/time, as this has been found to have an almost linear relationship with rearing temperature (Bar-Zeev 1958; Clements 1963).

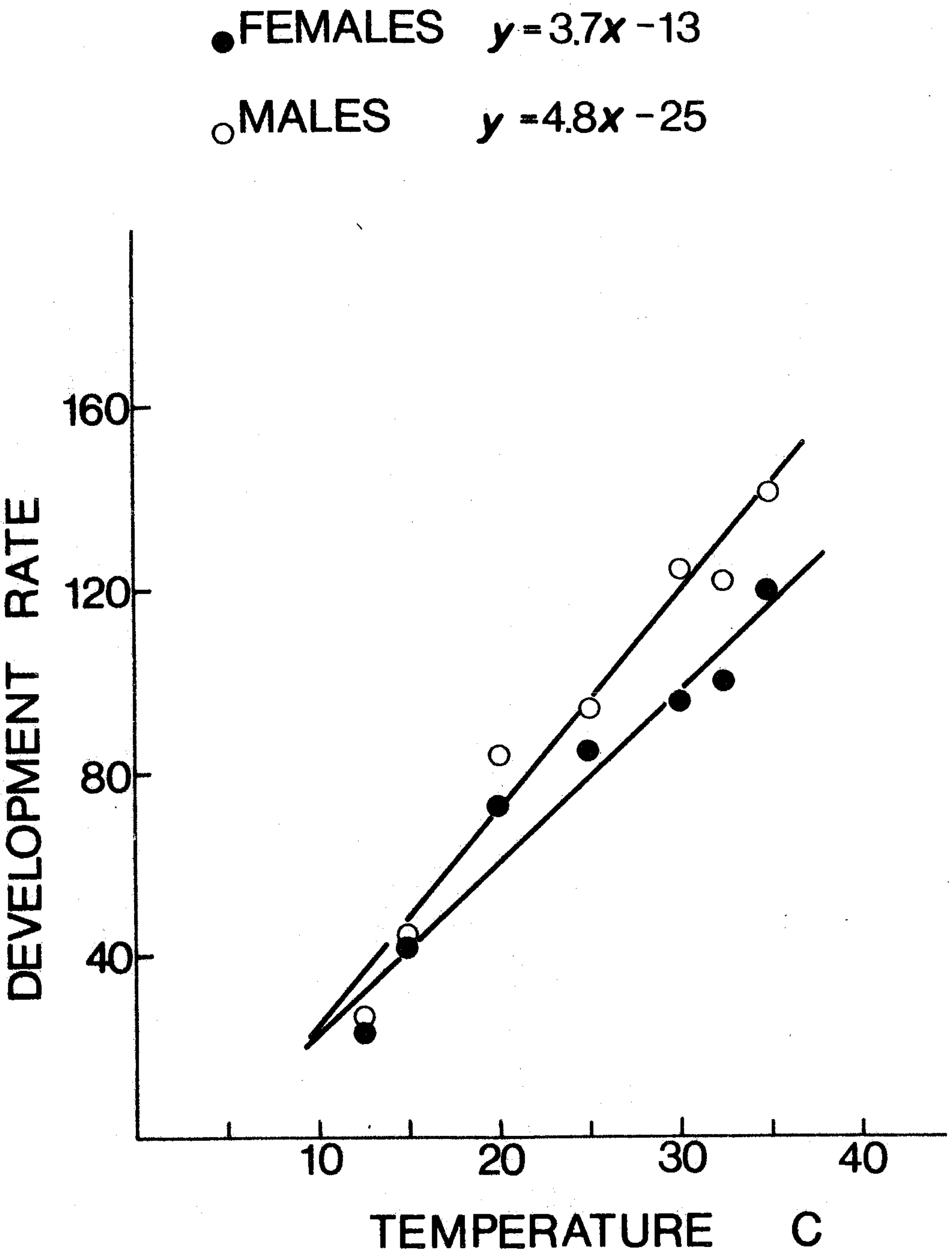
The regression line for _Ae_. _aegypti_ females had the equation $y = 5.6x - 45$ (fig. 7) while the one for males had the equation $y = 6.0x - 49$ (fig. 7). Both relationships between rate of development and temperature were significant (F-test). The slopes were significantly different from zero. The slopes for female and male development rates of _Ae_. _aegypti_ were not significantly different from each other (T-test).

Fig. 7 Relationship between temperature and mean (n=4) rate of development, 1000 divided by ET50 (based on those surviving to adult) of *Aedes aegypti*. Regression lines for males and females are plotted separately.


● FEMALES $y = 5.6x - 45$
○ MALES $y = 6x - 49$
DEVELOPMENT RATE
160
120
80
40
10
20
30
40
TEMPERATURE C

The regression line for C. pipiens females had the equation $y = 3.7x - 13$ (fig. 8) whereas the one for males had the equation $y = 4.8x - 25$ (fig. 8). Both relationships between rate of development and temperature were significant (F-test). The slopes were significantly different from zero. The slopes for female and male development rates were significantly different from each other (T-test).

Fig. 8 Relationship between temperature and mean (n=4) rate of development, 1000 divided by ET50 (based on those surviving to adult) of *Culex pipiens*. Regression lines for males and females are plotted separately.


● FEMALES $y = 3.7x - 13$
○ MALES $y = 4.8x - 25$
DEVELOPMENT RATE
160
120
80
40
10
20
30
40
TEMPERATURE C

Discussion

The size of the imago decreased with increasing rearing temperatures, as expected, in both species of mosquito. The two species differed in the way in which male and female size was influenced by changes in rearing temperature.

The size of male and female *C. pipiens* changed by the same amount in response to increasing rearing temperature, i.e., the slopes of the regression lines (figs. 3 & 4) are not significantly different, indicating similar responses by males and females.

The size of *Ae. aegypti* males and females was affected differently by increased rearing temperatures. There was a greater change in the size of females than in males, i.e., the slopes of the regression lines (figs. 1 & 2) are significantly different. Both weights and wing lengths change more in females as rearing temperatures are increased. The size of females decreased at a faster rate with increasing rearing temperature than did the size of males.

This difference in male and female responses accentuates the need to distinguish between the sexes when measuring physiological parameters.

Hosoi (1954) with *Culex pipiens* var. *pallens* (Coq.), Mer (1937, cited by Christophers 1960) with *Anopheles sacharovi* Faur. and van den Heuvel (1963) with *Ae. aegypti* noted that at extreme temperatures wing length changed disproportionately in comparison to other measures of size. At high temperatures wing lengths were shorter than expected and at low temperatures they were longer than expected. This is not a large effect, and therefore as long as it is acknowledged, wing length can still be used as an index of size. Christophers (1960) stated that wing length is the simplest and most reliable measure of size as it is not liable to vary with the condition of the insect and is roughly correlated with weight.

Fay (1964) found the average weight of newly emerged Ae. aegypti males to be 1.18 to 1.4 mg and females to be 2.4 to 2.9 mg which is the range of sizes observed at 25 C (fig. 1) in the present study. Rearing temperatures were not stated in Fay's paper. Data on C. pipiens size is lacking in the literature, and is probably variable depending on the subspecies.

High temperatures produce smaller mosquitoes but whether or not these mosquitoes are less able to survive or reproduce cannnot be determined from this parameter alone. Number surviving to adult is an appropriate measure of the suitability of any particular rearing temperature for species continuance.

The survival of Ae. aegypti (fig. 5) was high between 20 and 35 C. Bar-Zeev (1958) determined the lowest temperature at which development to adult occurred to be 14 C and the highest to be 36 C. Some adults were obtained at 15 and 37.5 C in this study. Headlee (1940; 1941; 1942) gives temperature limits for species continuance as 60 to 94 F, which is approximately 16 to 34 C. For the colony studied here the lower limit would be higher than that stated by Headlee.

The survival of C. pipiens (fig. 6) was high between 15 and 32.5 C, but was highest at 20 C. Main (1966) found that temperatures of 38 and 46 C were fatal to eggs and larvae. Kurihara (1963) recorded rates of pupation (table V) and found pupation was high between 15 and 32 C but was the highest at 18 C.

For both species there is a wide range of temperatures at which survival is high. As might be expected from the temperature zones in which the species are found, Ae. aegypti survives at higher temperatures while C. pipiens survives at lower temperatures.

As expected, the rates of development decreased with increased temperatures of rearing in both species of mosquito. For practical purposes, rates of development increased linearly with temperature, as Clements (1963) also pointed out.

TABLE V

Rates of pupation of _Culex_ _pipiens_ s.l. reared at various constant temperatures.*1

Temperature C	Percent Pupation
10	0
15	81.3
18	92.6
20	71.3
25	78.0
28	78.0
32	68.3
38	0

---

*1 From Kurihara (1963).

The two species differ in the way in which male and female rates of development were influenced by changes in rearing temperatures. The rates of development of the two sexes of *Ae. aegypti* changed by the same amount in response to increased temperatures of rearing, whereas in the case of *C. pipiens*, increased rearing temperatures have a greater effect on male developmental rates than on that of females. This is opposite to the effect of rearing temperature on the size of imago in these two species. *Ae. aegypti* females showed a greater decrease in size than males with increasing rearing temperature whereas *C. pipiens* male and female size changed at the same rate.

Ae. aegypti shows faster developmental rates than *C. pipiens* at higher temperatures indicating that *Ae. aegypti* is better suited to higher temperatures.

On the basis of survival and developmental times established in this work (tables III & IV), the optimum survival temperatures for *Ae. aegypti* are between 30 and 35 C., because within this range survival is still high and development is relatively rapid. The optimum, using the above paramaters, is more difficult to determine for C. pipiens because survival is highest at 20 C but development is still relatively slow.

The lethal low temperature for Ae. aegypti, in terms of survival to adult, is between 12.5 and 15 C, whereas for C. pipiens it is about 10 C. The lethal high temperature for Ae. aegypti is between 37.5 and 40 C whereas for C. pipiens it is between 35 and 37.5 C.

A wide range of temperatures is suitable for survival and development but higher temperatures produce progressively smaller adults that may be less strong and less capable of reproduction than those reared at lower temperatures. This is investigated in the following section.

PART II

EFFECTS OF TEMPERATURE OF REARING UPON FECUNDITY OF *AEDES AEGYPTI*.

This study was conducted to determine if the rearing temperature of the immature instars of *Ae. aegypti* will have any effect on the fecundity of the adults produced. This is a measurement of the suitability of the rearing temperature for the species.

Methods and Materials

Eggs were hatched at room temperature (20±2 C). The larvae were then placed in enamel trays, 25 by 20 by 4.5 cm deep with 750 ml of distilled water. One hundred larvae were placed in each of three incubators maintained at 20, 25 and 30 C while 200 larvae were placed at both 15 and 35 C as fewer were expected to survive to the adult stage at these temperatures. Food was provided following the schedule outlined in table II (Excess).

Adults were removed as they emerged and placed in a cage 15 by 15 by 45 cm high, having a 15 by 45 cm front of clear Plexiglas with a central hole fitted with a plastic sleeve, and the remaining sides, top and bottom of nylon mesh. Seven to ten days after the majority of the adults had emerged, a restrained shaved guinea pig was provided and females were allowed to feed to repletion. One blood-fed female was placed in each of 20 individual cages 10 by 6 by 10 cm high with the front (10 by 10 cm) of clear Plexiglas, the opposite side of nylon mesh and the rest of the walls, top and bottom of wood. A 20 ml beaker lined with a 3 by 12 cm piece of paper toweling and containing 15 ml of distilled water was provided for oviposition.

Before blood feeding, the females had opportunity to mate with males reared at the same temperature. To check on the mating ability of the males reared at extreme temperatures, ten females were placed with males reared at the same temperature and ten females were placed with males reared at room temperature.

Gillett (1955) showed that in one strain of Ae. aegypti mating was a necessary stimulus for full development of eggs and oviposition. If males were unable to mate, a decrease in the number of females actually laying could be observed. The cages were then placed in a cupboard at room temperature and after 7 days the wing length of each female measured and the number of eggs counted.

Females were dissected and examined for retained eggs and these were then added to the total egg count for that female before the analysis was done. Mosquitoes which had not developed any eggs were not included in the analysis, following the procedure of Colless & Chellapah (1960).

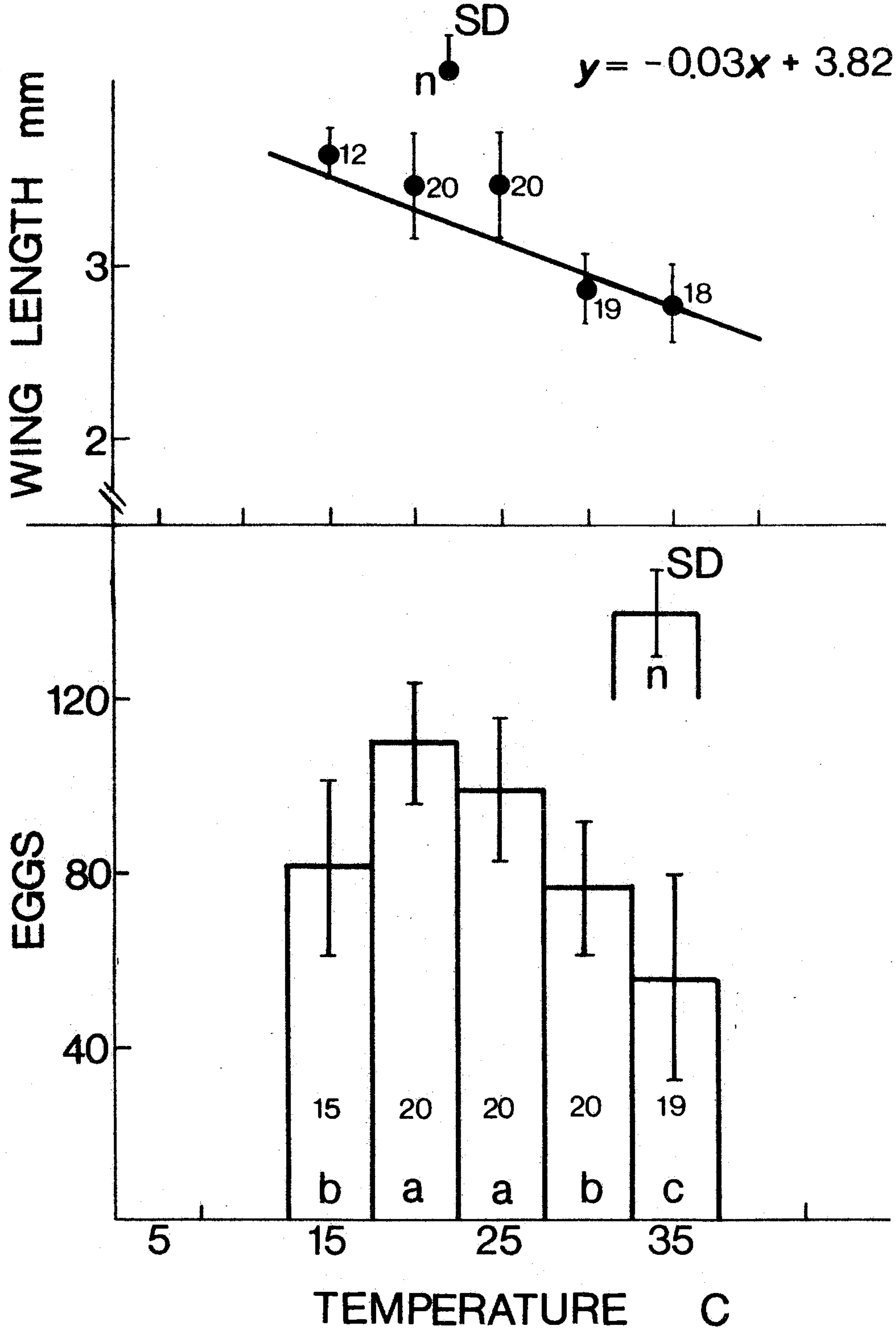
Results

The number of eggs laid by Ae. aegypti, reared in the larval stage at various constant temperatures, varied with the rearing temperature (fig. 9, see appendix C for raw data). The largest number of eggs was laid by females reared at 20 and 25 C, which averaged 109.8 ± 3.23 (n=20) and 98.6 ± 4.79 (n=20) respectively. Fewer eggs were laid by females reared at 15 and 30 C, an average of 82.2 ± 4.89 (n=15) and 78.0 ± 3.59 (n=20) eggs respectively. The smallest number was laid by females reared at 35 C, an average of 56.5 ± 5.38 (n=19) eggs (fig. 9).

As expected, size, as measured by wing lengths, decreased with increasing temperatures (fig. 9).

Highly significant differences were found between the number of eggs produced by females reared at different constant temperatures (analysis of variance), and the temperature of rearing of the male present

Fig. 9 Mean number of eggs laid ± SD and mean wing lengths ± SD of *Aedes aegypti* reared at various constant temperatures. Sample size varied for the different temperatures and is indicated on the graph. Mean eggs followed by the same letter are not significantly different (Student-Newman-Keuls). A regression line has been plotted for wing lengths.


SD
n
y= -0.03x + 3.82
WING LENGTH mm
3
2
12
20
20
19
18
SD
n
EGGS
120
80
40
15
20
20
20
19
b
a
a
b
c
5
15
25
35
TEMPERATURE C

during oviposition appeared to have a significant effect on the numbers (analysis of variance). Females with males reared at room temperature during oviposition appear to produce fewer eggs than females with males reared at the same temperatures (table VI) although no significant differences were found using a Student-Newman-Keuls test. The majority of the females laid some eggs so egg-laying was seldom completely inhibited.

Four mosquitoes died without developing eggs and two lived to the end of the test but did not develop any eggs. All the mosquitoes that died before laying were reared at 15 C. Six mosquitoes reared at either 15 or 35 C had retained eggs, 3 of them due to premature death.

TABLE VI

Mean number of eggs ± SD laid by Aedes aegypti reared at various constant temperatures. All had opportunity to mate before blood feeding with males reared at the same temperature but for oviposition females were isolated either with males reared at the same temperature or at room temperature. Means followed by the same letter are not significantly different (Student-Newman-Keuls).

Rearing Temperature C	Females caged with Males reared at same temperature			Females caged with Males reared at room temperature (20 C)		
15	85.3±6.8	n=8	bc	78.7±7.4	n=7	cd
20	112.3±3.2	n=10	a	107.3±5.7	n=10	ab
25	106.7±4.9	n=10	ab	90.5±7.6	n=10	abc
30	83.2±4.9	n=10	c	72.6±4.9	n=10	cd
35	57.0±5.0	n=10	d	56.0±10.3	n=10	d

Discussion

The fecundity experiments were done only with Ae. aegypti because C. pipiens would not mate and oviposit when isolated in small (less than 1 cubic foot) cages (Gerberg 1970).

Many authors have found a marked upward trend in egg production correlated with larger blood meals in a number of species of mosquito (Woke et al. 1956; Roy 1936; Colless & Chellapah 1960; Edman & Lynn 1975).

Christophers (1960) found no suggestion of "any close relation between the number of eggs and the size of the female" when studying Ae. aegypti and Woke et al. (1956) found that the number of eggs increased with body weight. Although this increase suggested a linear relationship, a statistically significant trend was not established. Colless and Chellapah (1960) observed that body weight affected fecundity in Ae. aegypti and concluded that they obtained these results, contrary to those of the above authors, because of rearing techniques used during the larval stages. The other workers had experimented with adults reared from well-fed larvae, whereas Colless and Chellapah deliberately starved *Ae. aegypti* larvae to obtain smaller adults.

In the present work the mosquitoes were not weighed before or after blood feeding and were not disturbed during feeding. It has been observed that mosquitoes would feed to repletion when not interrupted (Roy 1936), therefore in the present work it can be assumed that complete blood meals were taken by the test animals.

MacDonald (1956) suggested that small adults would ingest a smaller amount of blood and so could be expected to lay fewer eggs.

When comparing results of this work with those of others it is important to remember that there may be differences in the strains of mosquitoes used and that methods for tabulating and analyzing the data may differ. Colless and Chellapah (1960) distinguished between those that develop eggs whether they lay them or not (layers) and those that do not (nonlayers). In their test, 12 out of 124 mosquitoes had retained eggs, 9 of them owing to premature death. Nonlayers were not considered in the analysis. This procedure was adopted in recording data in this study.

Ae. aegypti isolated in tubes rarely showed retained eggs after oviposition had occurred: Christophers (1960) found that less than 2% of the mosquitoes examined showed one or two retained eggs in the common oviduct or the ovary. In the present study up to 64 retained eggs were observed which indicates that large numbers of eggs may be retained if the mosquito is not hardy and dies before laying or does not lay for some other reason, e.g. they may not have been inseminated.

A decrease in fecundity was observed in females reared at 20 to 35 C (fig. 9) and this correlates with a decrease in size as measured by wing lengths.

Mosquitoes reared at 15 C were bigger generally than those mosquitoes reared at 20 C but they were observed to produce fewer eggs. This effect may be the result of a general lack of vitality as mentioned above. Edman and Lynn (1975) found that more sugar-fed Culex nigripalpus Theobald females developed eggs from partial blood meals than did individuals with depleted energy reserves. MacDonald (1956) suggested that when weak adults were given their first blood meal, either of two results can be expected: the ovaries will not develop past stage 2, which is the stage reached without a blood meal (Christophers 1960), or the number of eggs formed will be less than 50. As the rearing conditions are improved, so a larger proportion of adults will form eggs after one blood-meal and the number of eggs will increase. This may be what has occurred with the mosquitoes reared at 15 C. The low temperature may have produced weakened adults. It was difficult to get females reared at 15 C to ingest blood from human or guinea pig. Hatchett (1946, cited by Fay 1964) observed that Ae. aegypti adults from larvae that required over 30 days for growth seldom feed on blood.

Perhaps the females reared at 15 C did not or could not obtain a large enough blood meal to develop a full complement of eggs even though they were allowed to feed to repletion.

The most favourable rearing temperatures for maximum fecundity of Ae. aegypti appears to be in the range of 20 to 25 C as decreased egg laying was observed at higher and lower temperatures.

When high temperatures were applied to developing larvae of certain species of mosquitoes maleness was suppressed and femaleness remained unchanged (Anderson & Horsfall 1963). Anderson and Horsfall call this anomalous development. The extent of male suppression depends upon how extreme the rearing temperatures are. This could have occurred during rearing at high temperatures (35 C) and may have masked the potential fecundity of the females. The results obtained from the controls (male reared at room temperature present during oviposition) failed to indicate that anomalous development occurred even though the F value was significant. Actually the controls laid fewer eggs (table VI). If anomalous development had occurred, more females with all their eggs retained would have been expected at 35 C in the experimental group (male reared at same temperature present during oviposition). None of those females reared at 35 C that developed any eggs retained all of them.

It does not appear that anomalous development was induced--perhaps the critical temperature was not reached or *Ae. aegypti* is not susceptible. Another explanation for these results could be that the strain used in these experiments did not require mating to stimulate egg development and oviposition.

PART III

OVIPOSITION OF MOSQUITOES IN WATER AT VARIOUS TEMPERATURES.

The selection of an oviposition site may be influenced by many factors as discussed in the Introduction. This study was conducted to determine if site selection can be modified by the temperature of the water present. A second purpose was to determine if _Ae_. _aegypti_ and _C_. _pipiens_ show any patterns of selection when given a choice between sites with water at different temperatures. The results from this section will be compared with the results obtained in parts I and II in the summary and conclusions section.

Methods and Materials

A) General

Two oviposition sites were available at opposite ends of each of two plywood experimental cages, 182 by

61 by 122 cm high. The Plexiglas front of each cage was covered, as external stimuli, such as light intensity, influenced site selection by the ovipositing female.

At each site there was a bowl, 17 cm in diameter and 8.5 cm deep, with 1000 ml of distilled water, a heater with thermostat, a thermometer, and a temperature probe leading to a chart recorder. The bowl was placed on a cold water circulating chamber which provided cooling. The water temperature within 1.5 cm of the surface could be regulated plus or minus 1 C with this system.

B) *Aedes aegypti*

The experimental cage was illuminated by two 40-watt bulbs, one over each oviposition site, from 6 a.m. to 10 p.m. Three- to five-week old females were exposed to a restrained shaved guinea pig in a glass aquarium 32 by 62 by 30 cm high with a cardboard top having an 18 cm hole fitted with a plastic sleeve.

Males and females were introduced to this cage one day before the guinea pig was made available for blood feeding.

After one day, 30 blood-fed females and 30 males were introduced to the experimental cage. A piece of paper toweling, 24 by 8.5 cm, lined the bowl and provided a surface for oviposition. The papers were replaced daily between 8 and 9 a.m. and the eggs counted. Water level in the bowls was kept constant by adding water at this time. The experiment was terminated when oviposition ceased. Each experiment lasted about one week.

A control series was run in which the two sites were kept at the same water temperature in order to check whether or not each site was equally attractive, and to determine if the same number of eggs would be laid at both sites at the various temperatures used. Three replicates were done for each temperature, ranging from 15 to 45 C in 5 C increments.

A series was done in which the two sites differed in their water temperatures by 5 and 10 C over a range between 15 and 45 C. At least two replicates were done for each temperature comparison. Both of the above sets of experiments were run at air temperatures between 19 and 21 C.

A small number of experiments were carried out with high air temperatures, 28 to 32 C. Two lots of mosquitoes were used for this experiment: one group was reared at 19 to 21 C and a second group was reared at 28 to 32 C. Adults were maintained at the high temperature until they were required for experiment. Two replicates with water temperatures set at each of 40 and 45 C at both sites were done with mosquitoes reared at room temperature. Two replicates with water temperatures as above were done with mosquitoes reared at high temperatures. Two replicates of each of the following water temperature pairs were also done with mosquitoes reared at high temperatures: 30 with 40 C and 40 with 45 C.

A test with mosquitoes reared at high temperatures but with air temperatures during oviposition set between 19 and 21 C was also done. One test with water temperatures of 40 C at both sites was done with these mosquitoes.

Data from experiments with both sites at the same water temperature were analyzed using analysis of variance to determine if site and temperature had any effect on the number of eggs laid.

The data from experiments with the two sites at different constant temperatures was analyzed by comparing the number of eggs at the higher temperature with 50% of the total eggs laid at both sites to see if it was less than or greater than expected. One thousand was added to the difference between these two to give an index of response. Control data were treated in the same way but site 2 was arbritarily chosen to be examined as both sites were at the same temperature. These indices were analyzed using a oneway analysis of variance and a Student-Newman-Keuls test, to see if the variation in the indices in the different temperature comparisons were different from the variation observed in the experiments with both sites at the same constant temperatures.

The results of experiments where the effects of air temperature during oviposition and temperature of rearing of the larvae and pupae on the adult's oviposition were analyzed as above, but selected temperature comparisons from the other experiments were used for comparison.

C) *Culex pipiens*

The experimental cage was illuminated by two 60-watt bulbs from 8 a.m. to 8 p.m. and one central 15-watt bulb from 6 a.m. to 10 p.m. This gave two hours of low intensity light at each end of the photophase.

Two to three hundred of each sex were introduced to the experimental cage. At each end of the cage there was a 100 ml Erylenmeyer flask containing a 10% sucrose solution and stoppered with two cotton wicks. After one week a restrained, shaved guinea pig was made available for blood feeding on four consecutive nights.

Egg rafts were collected over the next ten days. Each raft was counted as an oviposition event. Eggs in each raft were not counted as this has been found to be independent of the suitability of the characteristics of the water at the breeding site (Wallis 1954).

A control series similar to that of the Ae. aegypti experiments was done with temperatures ranging from 15 to 35 C in 5 C increments. Only one experiment was done at each temperature to determine if females would lay at that specific temperature, given no other choice, and to determine if the sites were equally attractive. It could not be established whether the same number of rafts, from a certain number of blood-fed females, would be laid at the various temperatures because the exact number of blood-fed females was not known. The size of the experimental cage and the activity of the mosquitoes prevented an accurate count of blood-fed females. No blood-fed females laid egg rafts when they were handled so that an exact number of blood-fed *C. pipiens* could not be introduced to the experimental cage, as was done for *Ae. aegypti*.

A series was done in which the two sites differed by 5 and by 10 C over a range between 15 and 30 C. Two replicates were run for each temperature comparison. All experiments were conducted at air temperatures between 19 and 21 C.

This series was analyzed in the same way as the *Ae. aegypti* experiments except that the data was changed to proportions before it was analyzed and thus an arcsin square root trnasformation was done before the data was analyzed. Proportions of total rafts laid were used because the number of blood-fed females could not be controlled. Also, for this reason, the results from experiments with both sites at the same constant temperatures were not analyzed for temperature effects.

Results

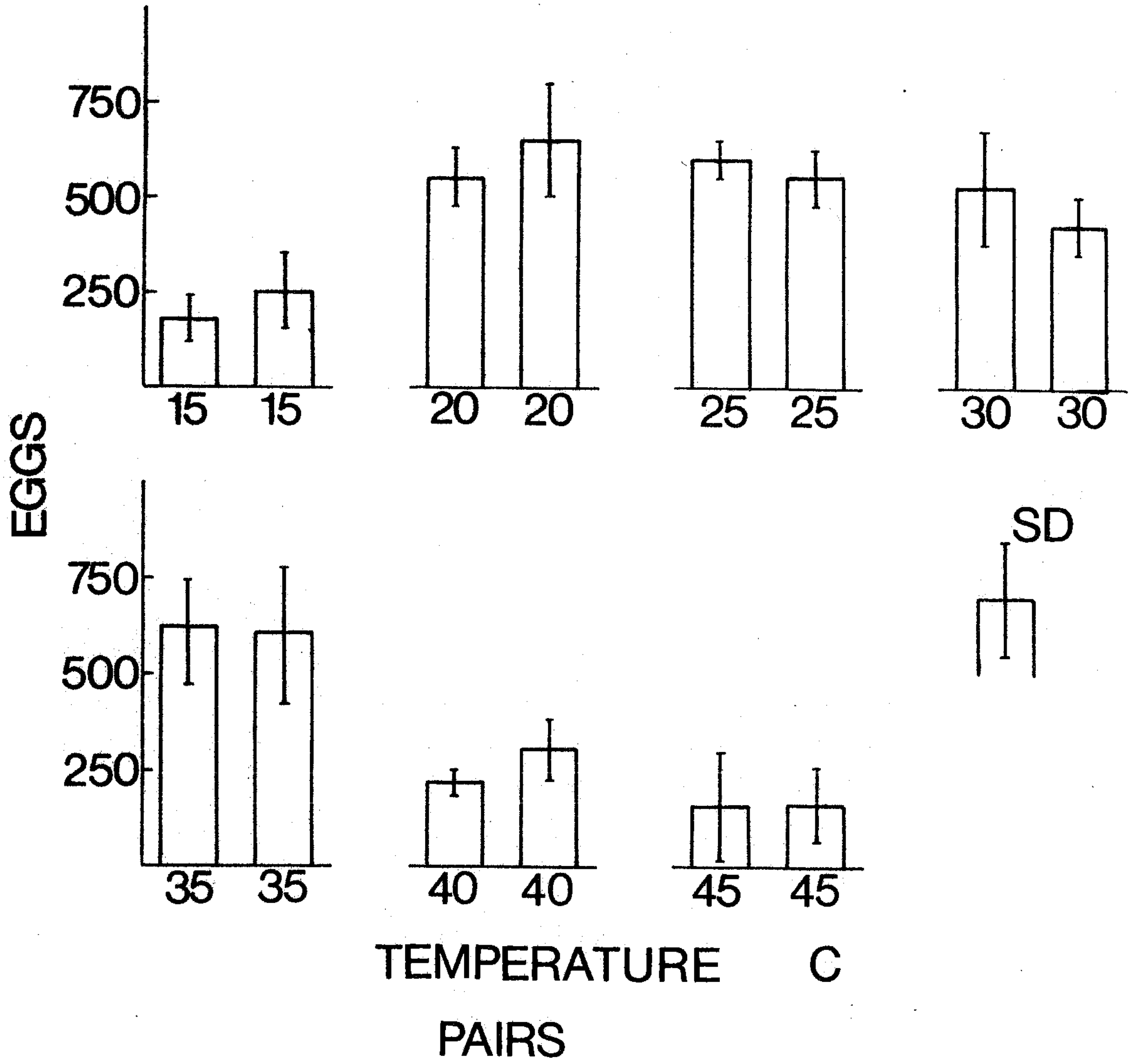
A) *Aedes aegypti*

1) Two oviposition sites at the same water temperatures.

The control series indicated that *Ae. aegypti* will oviposit over a wide range of water temperatures. Oviposition occurred at temperatures ranging from 15 to 45 C (fig. 10).

A two way analysis of variance (see appendix D for raw data) showed a highly significant effect of temperature on the number of eggs laid but no effect due to position of the oviposition site. The null hypothesis that there was no effect caused by position was true ($p \geq 0.05$) and this is shown clearly in fig. 10. The effect of temperature was investigated by plotting the data disregarding site, i.e., tabulating the total number of eggs laid (fig. 11) in any one

Fig. 10 Mean (n=3) number of eggs laid ± SD by *Aedes aegypti* at each of two oviposition sites with both sites at the same constant water temperatures.


EGGS
750
500
250
15
15
20
20
25
25
30
30
SD
750
500
250
35
35
40
40
45
45
TEMPERATURE C
PAIRS

experiment. A Student-Newman-Keuls test indicated that more eggs were laid at 20 , 25, 30, and 35 C (Group I) water than at 15, 40, and 45 C (Group II), Between 951 ± 131 (n=3) and 1258 ± 181 (n=3) were laid at group I temperatures while between 322 ± 132 (n=3) and 523 ± 59 (n=3) eggs were laid at group II temperatures (fig. 11).

2) Two oviposition sites at contrasting water temperatures.

Three significantly different (Student-Newman-Keuls) response groups were found in the experiments when the mosquitoes had a choice between sites at two different water temperatures (fig. 12, see appendix E for raw data) and the indices (defined in methods) were analyzed. In fig. 12 the differences in number of eggs in temperature pairs that were labeled I and III are all significantly different from each other. Those with hatched bars designate temperatures which have been shown to be unfavourable for oviposition even when there was no option (fig. 11).

Fig. 11 Mean (n=3) of the total number of eggs laid ± SD by Aedes aegypti at the two sites combined at various constant water temperatures. Means followed by the same letter are not significantly different. (Student-Newman-Keuls).

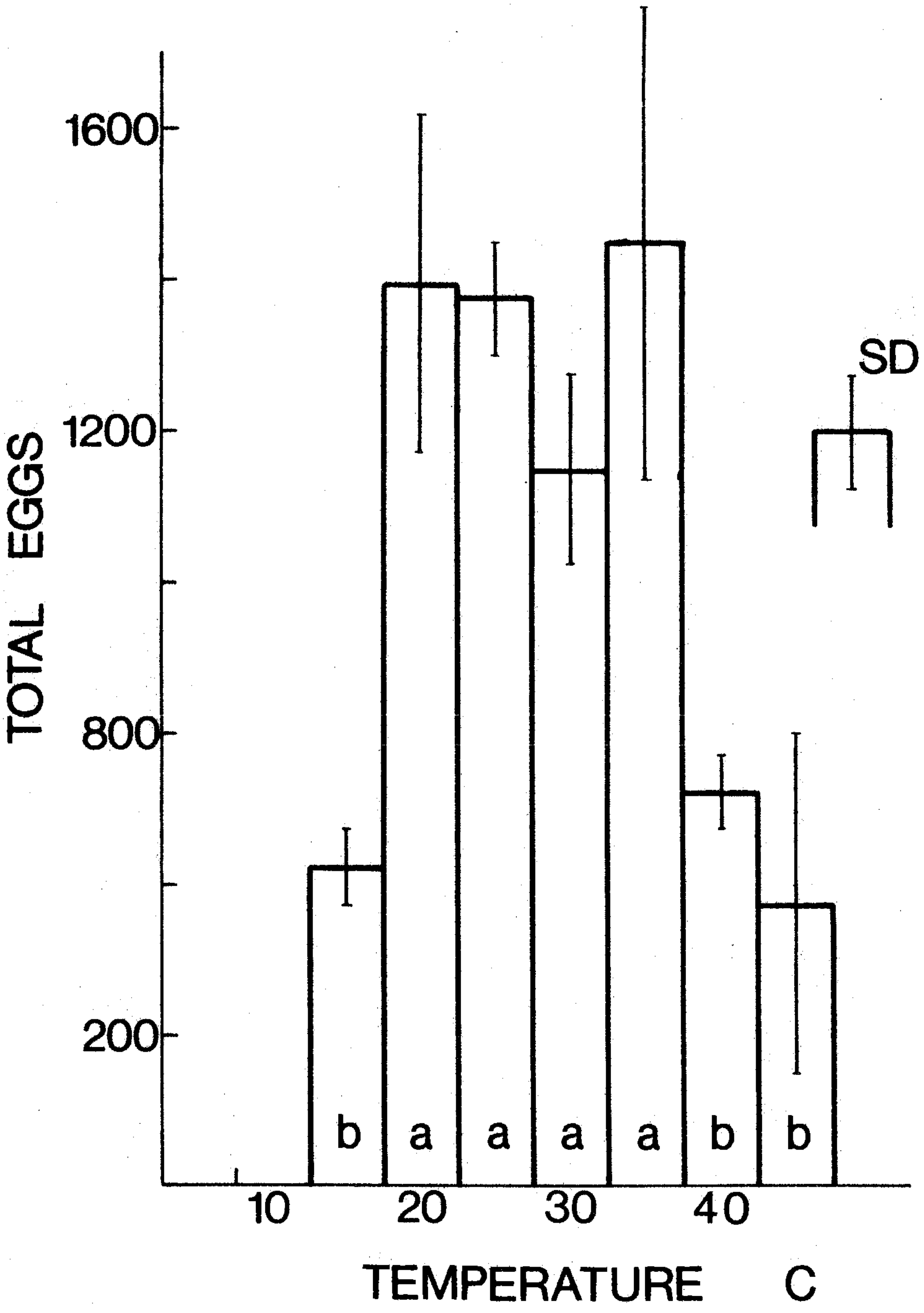
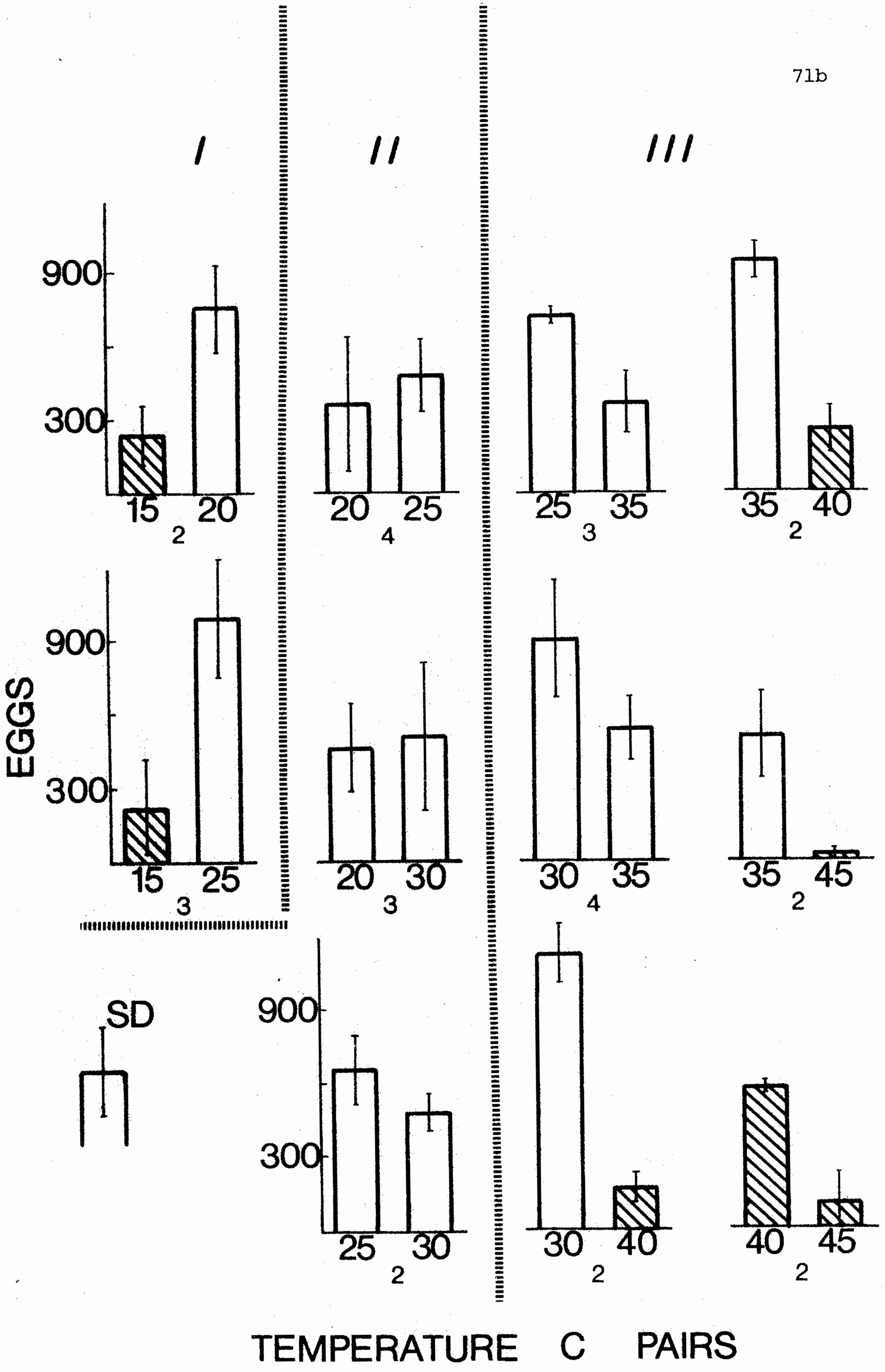

TOTAL EGGS
1600
1200
800
200
SD
b
a
a
a
a
b
b
10
20
30
40
TEMPERATURE C

Fig. 12 Mean number of eggs laid by Aedes aegypti ± 1 SD at each oviposition site, given a choice between various constant water temperatures. Number of replicates varies for the different temperature pairs and is indicated under the temperatures for each experiment on the graph. Analysis indicated three different (Student-Newman-Keuls) response groups: I; where more eggs are laid at the higher temperature, II; where there is no difference between the number of eggs at the two sites, and III where more eggs are laid at the lower temperature. Hatched bars designate temperatures at which the total number of eggs laid was reduced in other experiments where no option but to oviposit at these temperatures was available.


I
II
III
900
300
15
20
2
20
25
4
25
35
3
35
40
2
EGGS
900
300
15
25
3
20
30
3
30
35
4
35
45
2
SD
900
300
25
30
2
30
40
2
40
45
2
TEMPERATURE C PAIRS

When the lower temperature was 15 C (fig. 12, I) the higher temperature in the pair had more eggs. The comparisons in this group were 15 with 20 C and 15 with 25 C. In the first comparison 246 ± 88 (n=2) were laid at 15 while 751 ± 138 (n=2) eggs were laid at 20 C. In the second comparison a larger difference was observed: 212 ± 114 (n=3) eggs at 15 C and 995 ± 128 (n=3) eggs at 25 C.

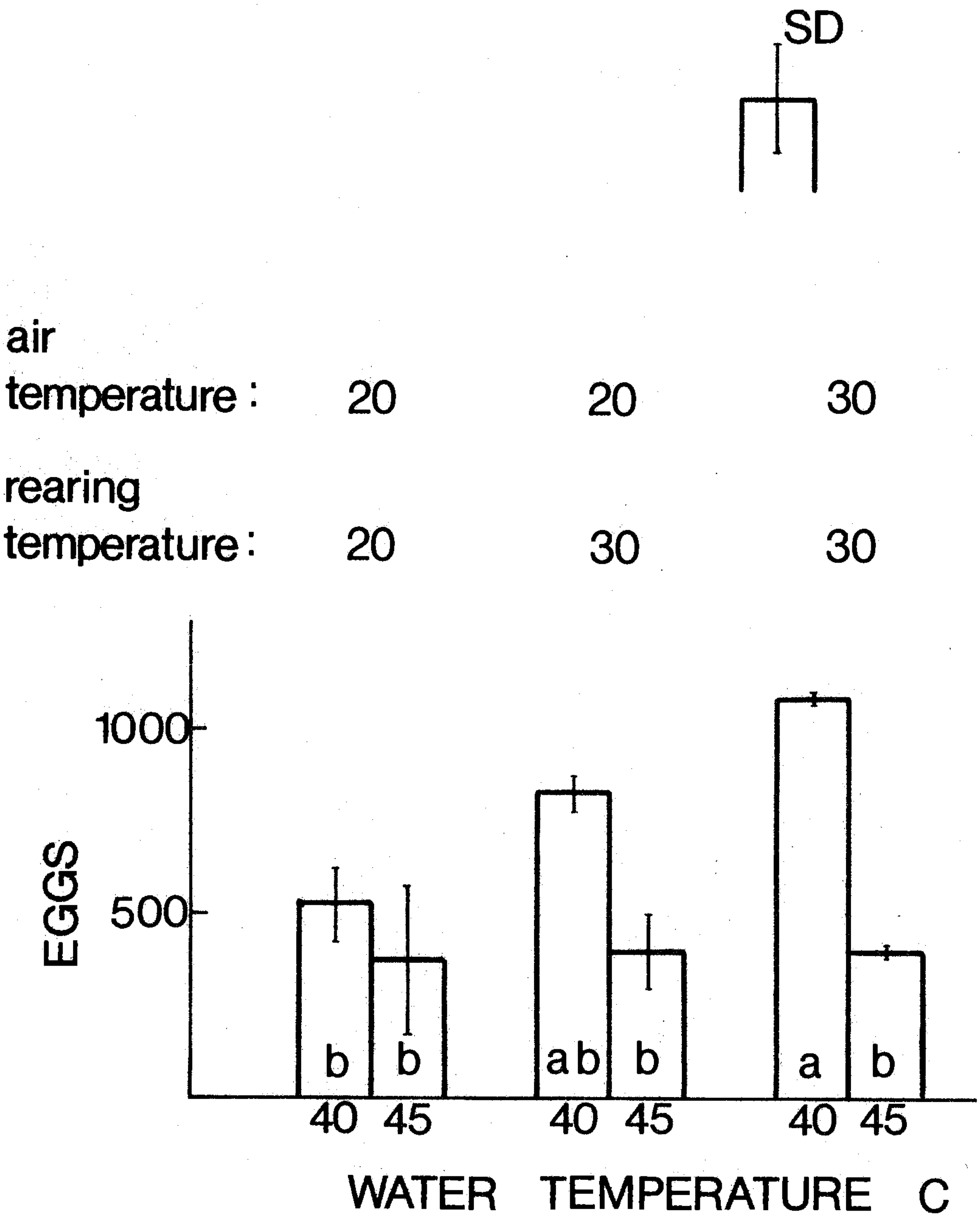
With temperatures between 20 and 30 C (fig. 12, II) the number of eggs laid at the two sites showed differences no greater than those found in the experiments with both sites at the same water temperatures. The comparisons in this group were 20 with 25 C, 20 with 30 C, and 25 with 30 C. The actual number of eggs laid were as follows: 381 ± 136 (n=4) eggs at 20 C and 479 ± 77 (n=4) eggs at 25 C; 463 ± 101 (n=3) eggs at 20 and 521 ± 163 (n=3) eggs at 30 C and 654 ± 104 (n=2) eggs at 25 C and 476 ± 61 (n=2) eggs at 30 C.

When the higher temperature was 35 C or more (fig. 12, III) fewer eggs were laid at the high temperatures. The comparisons in this group were 25 with 35 C, 30 with 35 C, 30 with 40 C, 35 with 40 C, 35 with 45 C, and 40 with 45 C. The actual number of eggs laid were as follows: 720 ± 16 (n=3) eggs at 25 C and 358 ± 65 (n=3) eggs at 35 C; 905 ± 128 (n=4) eggs at 30 C and 531 ± 69 (n=4) eggs at 35 C; 1130 ± 98 (n=2) eggs at 30 C and 165 ± 56 (n=2) eggs at 40 C; 933 ± 53 (n=2) eggs at 35 C and 252 ± 70 (n=2) eggs at 40 C; 515 ± 132 (n=2) at 35 and 16 ± 16 (n=2) and 570 ± 10 (n=2) eggs at 40 C and 100 ± 81 (n=2) eggs at 45 C.

3) The effect of temperature of rearing and air temperature upon the number of eggs laid at high water temperatures.

Both the rearing temperature of larvae and pupae and the air temperature during oviposition influenced the total number of eggs laid at high water temperatures (fig. 13, see appendix F for raw data). A large number of eggs, 1077 ± 2.5 (n=2) were laid at 40

Fig. 13 Mean (n=2) ± SD of the total number of eggs laid by _Aedes aegypti_ at the two sites combined when the larvae were reared at various water temperatures and air temperatures were held at various constant levels during oviposition. Means followed by the same letter are not significantly different (Student-Newman-Keuls).


SD
air temperature : 20 20 30
rearing temperature : 20 30 30
EGGS
1000
500
b
b
ab
b
a
b
40 45 40 45 40 45
WATER TEMPERATURE C

C by mosquitoes reared at high temperatures and allowed to oviposit at high air temperatures. These mosquitoes showed a depressed number of eggs, 380 ± 8 (n=2) at 45 C.

Fewer eggs, 810 ± 40 (n=2), were laid at 40 C by the mosquitoes reared at room temperature and allowed to oviposit at high air temperatures. These mosquitoes also showed a depressed number of eggs, 394 ± 82 (n=2) at 45 C.

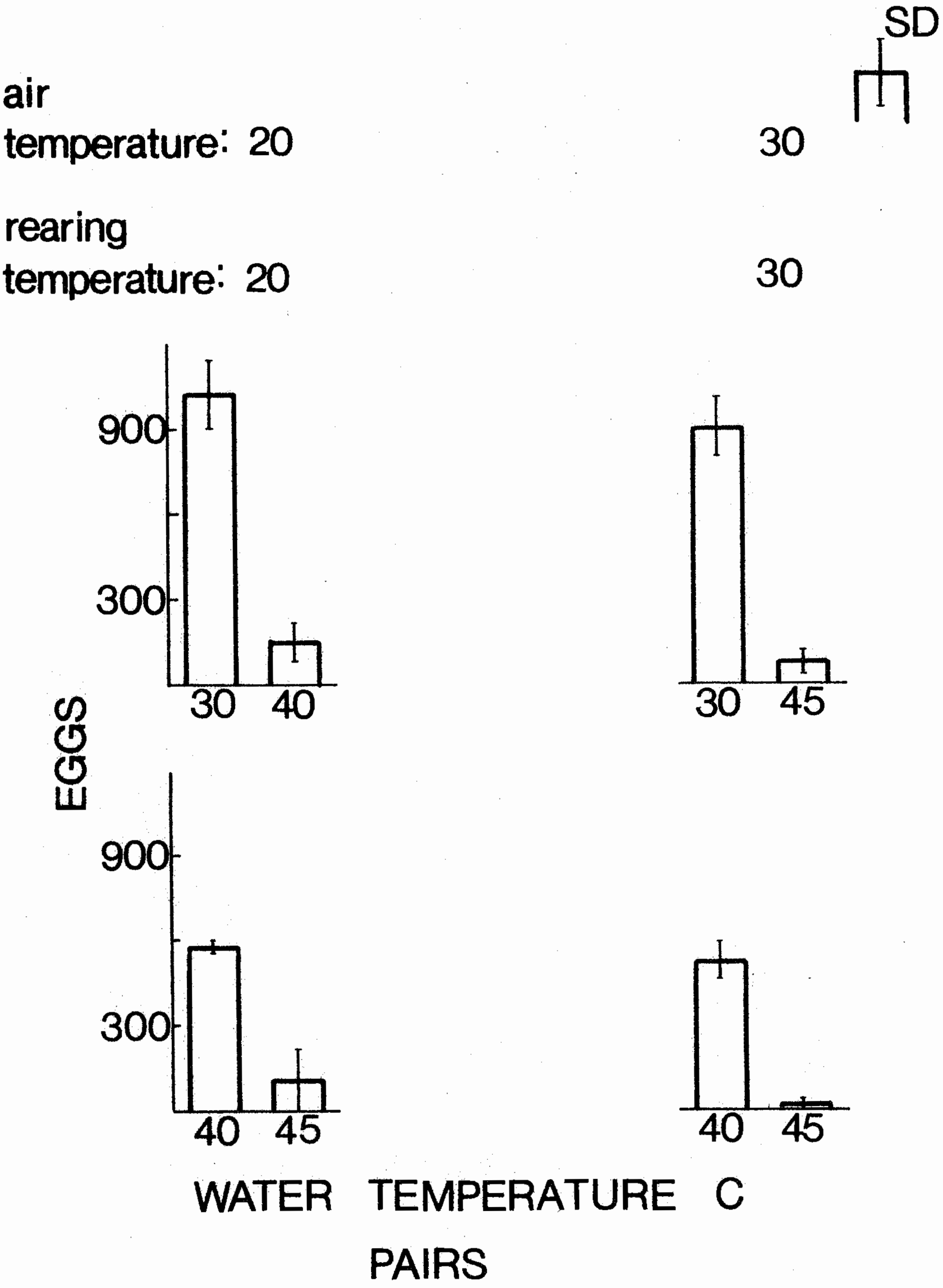
Only one trial was done with mosquitoes reared at high temperatures and allowed to oviposit at air temperatures of 20 C. A total of 1061 eggs were obtained at water temperatures of 40 C when the females were reared at high temperatures and allowed to oviposit at room temperature.

The number of eggs from mosquitoes reared and allowed to lay at room temperature was depressed (figs. 11 & 13) at both 40 and 45 C. 523 ± 59 (n=3) and 322 ± 132 (n=3) eggs respectively were laid by these mosquitoes at the above two temperatures.

A significant effect of rearing and air temperature was found on the number of eggs laid by *Ae. aegypti* (analysis of variance). The number of eggs laid at 40 C by the mosquitoes reared at high temperatures and allowed to oviposit at high air temperatures was significantly different from the number laid by the mosquitoes reared and allowed to oviposit at room temperature (Student-Newman-Keuls). The number laid by the mosquitoes reared at room temperature and allowed to lay at high air temperatures was an intermediate value between the above two (fig. 13).

The response of mosquitoes reared at high temperatures and allowed to oviposit at high air temperatures to a choice between 30 with 40 C and 40 with 45 C was similar (fig. 14, see appendix F for raw data) to that found in the original experiments (see part 1 of this section) with mosquitoes reared and allowed to oviposit at room temperature. In both cases more eggs were laid at the lower temperature. The

Fig. 14 Mean (n=2) ± SD of the number of eggs laid at two sites by *Aedes aegypti* at two sites when given a choice between water temperatures. Two rearing temperatures and air temperatures when oviposition was occurring were used.


SD
air
temperature: 20
30
rearing
temperature: 20
30
900
300
30
40
30
45
EGGS
900
300
40
45
40
45
WATER TEMPERATURE C
PAIRS

actual number of eggs laid by the mosquitoes reared and allowed to oviposit at high air temperatures were: 906 ± 74 (n=2) eggs at 30 C and 75 ± 15 (n=2) eggs at 40 C; and 519 ± 44 (n=2) eggs at 40 C and 1 ± 1 (n=2) eggs at 45 C.

B) *Culex pipiens*

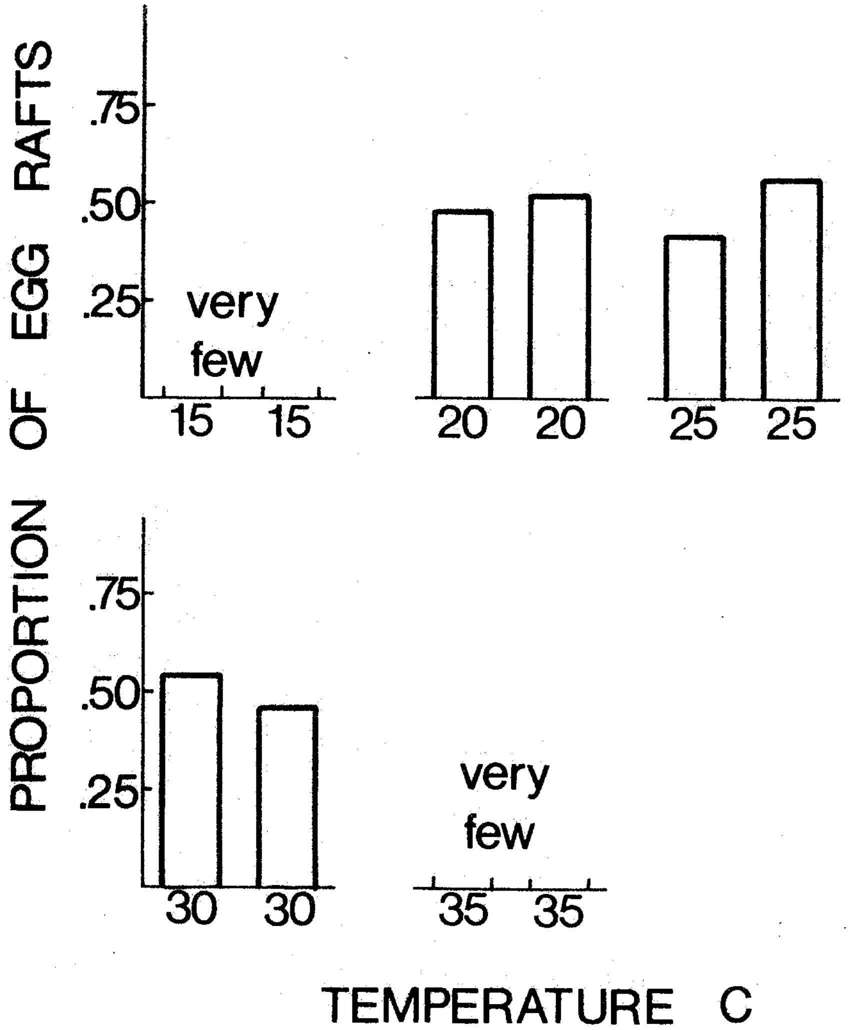
1) Two oviposition sites at the same water temperatures.

The position of the oviposition site had no effects on the proportion of egg rafts laid (fig. 15, see appendix G for raw data) at various constant water temperatures (analysis of variance). Fifteen and 35 C were not included in this analysis because only 4 and 10 egg rafts respectively were laid in those experiments.

2) Two oviposition sites at contrasting water temperatures.

Three significantly different

Fig. 15 Proportion of total number of egg rafts laid by Culex pipiens at two sites at various constant water temperatures.

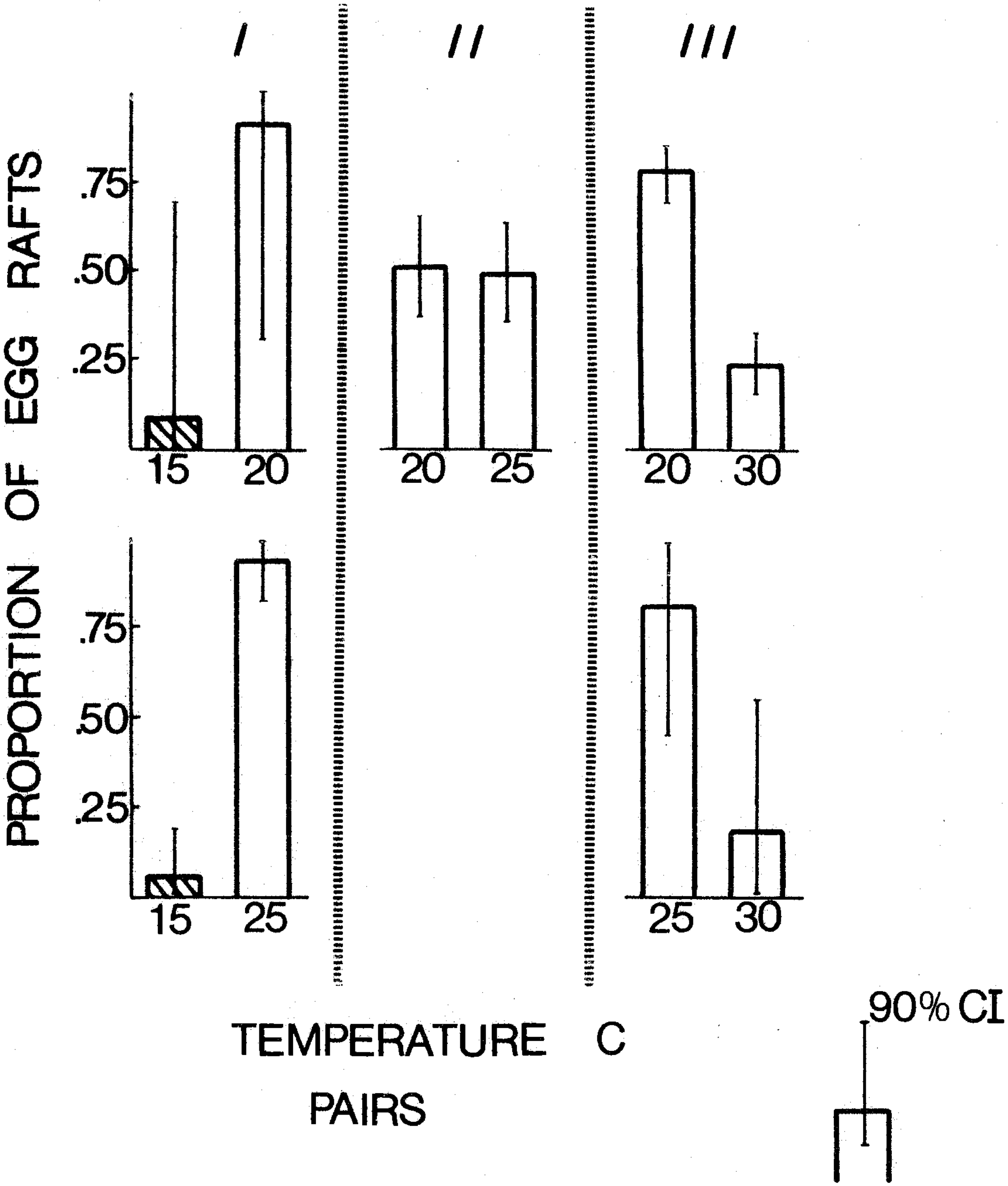

PROPORTION OF EGG RAFTS
.75
.50
.25
very few
15
15
20
20
25
25
.75
.50
.25
30
30
very few
35
35
TEMPERATURE C

(Student-Newman-Keuls) response groups, similar to those found with Ae. aegypti, were found in the experiments when the mosquitoes had a choice between sites at different water temperatures (fig. 16, see appendix G for raw data). In fig. 16 the differences in the proportion of egg rafts laid in temperature pairs labeled I and III are all significantly different from each other. Those with hatched bars designate temperatures which have been shown to be unfavourable for oviposition even when there was no option (fig. 15).

When the lower temperature was 15 C the higher temperature had more egg rafts. The comparisons in this group (fig. 16, I) were 15 with 20 C and 15 with 25 C. The retransformed mean proportion of total egg rafts laid at 15 C was 0.08 and 0.92 at 20 C while in the other comparison 15 C had 0.06 and 35 C had 0.94.

With temperatures between 20 and 25 C (fig. 16, II) the proportion of egg rafts laid showed differences no greater than those found in the experiments with

Fig. 16 Mean proportion (n=2), with 90% CI, of the total egg rafts laid by *Culex pipiens* at two sites when given a choice between water temperatures. Means and CI were retransformed from an arcsin square root transformation. Analysis indicates three different (Student-Newman-Keuls) response groups: I; where more rafts are laid at the higher temperature, II; where equal numbers are laid at the two sites and III; where more rafts are laid at the lower temperature. Hatched bars designate temperatures at which the laying of egg rafts was reduced in other experiments where no option but to oviposit at these temperatures was available.


I
II
III
PROPORTION OF EGG RAFTS
.75
.50
.25
15
20
20
25
20
30
.75
.50
.25
15
25
25
30
TEMPERATURE C
PAIRS
90% CI

both sites at the same water temperatures. Only the 20 with 25 C comparison fits in this group. The proportion observed were 0.51 at 20 C and 0.49 at 25 C.

When the high temperature was 30 C (fig. 16, III) fewer eggs were laid at the high temperature. The comparisons in this group were 25 with 30 C and 20 with 30 C. The proportion observed were 0.82 at 25 C and 0.18 at 30 C and 0.77 at 20 C and 0.28 at 30 C.

Discussion

A) Aedes aegypti

The total number of eggs laid was depressed at 15, 40 and 45 C in the control series. Theoretically 3000 eggs could be expected from the 30 females if 100 eggs were laid by each (Christopher 1960, also see PART II) and if none of them died before or during oviposition. Approximately one third of the maximum number of eggs (as suggested by Christophers) were laid in the oviposition experiments with both sites at the same water temperatures at temperatures shown to be favourable for oviposition (Group I: 20, 25 & 30 C; see fig. 11).

Low and high temperatures (Group II: 15, 40 & 45 C; see fig. 11) caused a depression in egg laying. Perhaps the females "prefer" not to lay at these temperatures and retain their eggs. There was no source of food in the experimental cage, so they probably died without laying.

These results will have to be considered when the results of the experiments with the two sites at contrasting water temperatures are examined. It can be predicted that group I temperatures will be favoured over group II temperatures when they are presented together. These temperatures are marked with hatched bars on fig. 12.

Fewer eggs were laid at group II temperatures when a group I temperature was the alternative (fig. 12). The response shown when 15 C was the lower temperature falls into this category (fig. 12, I) as do the 30 with 40 C and 40 with 45 C (fig. 12, III) experiments. In the first case some eggs were laid at 15 C but most were laid at the higher water temperatures. In the second case fewer eggs are laid at the higher water temperatures.

Temperataures between 20 and 30 C (fig. 12, II) were equally favourable. Within this range of water temperatures the females do not appear to discriminate between changes in water temperature, as indicated by the numbers of eggs laid.

In the 25 with 35 C and the 30 with 35 C (fig. 12, III) experiments the mosquitoes showed bias towards the lower temperature. All of these temperatures were favourable for oviposition in the experiments with both sites at the same water temperatures. Forty and 45 C were unfavourable for oviposition but given a choice of only these temperatures more eggs were laid at 40 C (fig. 12, III). This provides evidence of discrimination between water temperatures that seemed equally favourable or unfavourable in those experiments with both sites at the same water temperatures.

These results correspond with those of Hecht (1930). He found that females favoured a middle range of temperatures between 26 and 28 C, and 23 and 28 C (table VII, A & C). His results become less clear when the temperature ranges almost overlapped (table VII, B) although 21-25 C had 50% of the total number of eggs laid. Hecht's results presented here are the total

TABLE VII

Results from Hecht (1930). Effect of water temperature on oviposition of *Aedes aegypti*.

A	Temperature range	26-28 C	18-22 C	11-15 C
	total eggs	631	270	322
	percent	51%	22%	27%
B	Temperature range	30-34 C	27-29 C	21-25 C
	total eggs	711	886	1522
	percent	22%	28%	50%
C	Temperature range	34-38 C	23-28 C	12-13 C
	total eggs	158	1058	450
	percent	10%	63%	27%

number of eggs laid in a series of experiments. Surtees (1967) also found that temperatures below 30 C were favoured for oviposition by Ae. aegypti.

The results presented here and those of other authors agree well considering the different methods, and the possibility that different strains of mosquitoes were used.

The rearing of larvae and pupae at high temperatures, and keeping the air temperature high when the resultant adults were ovipositing, appeared to change the mosquitoes's response to 40 C. It was no longer an unfavourable temperature for oviposition as these mosquitoes laid more eggs than those both reared and allowed to lay at room temperature. Varying these factors may change how the adult mosquito perceives the various water temperatures.

Rearing the larvae and pupae at 20 and then moving them to air temperatures of 30 C for ovipostion also appeared to make 40 C a more favourable temperature but not enough to distinguish it statistically from a favourable or an unfavourable one. A single experiment with mosquitoes that had been reared at 30 C and allowed to lay at air temperatures of 20 C showed high oviposition at 40 C when no choice was available, in comparison with those which were reared and allowed to oviposit at room temperature.

It seems that rearing temperature is more critical in determining what response an ovipositing mosquito will give to a particular water temperature than air temperature during oviposition although the latter also modified the behaviour of the mosquitoes. More experiments are needed before definite conclusions on this phenomenon can be reached.

When given a choice between 30 with 40 C and 40 with 45 C the mosquitoes reared and allowed to oviposit at high air temperatures still showed the same "preference" as those reared and allowed to oviposit at room temprature (fig. 14). In the 40 with 45 C experiment with mosquitoes both reared at and allowed to oviposit at high air temperatues it appears that 45 C is still having an unfavourable effect on the mosquitoes, as the total number of eggs laid is depressed. This also occurs in the 35 with 45 C comparison with mosquitoes both reared and ovipositing at room temperature (fig. 12, III). It appears that 45 C had an irreversible effect on some of the mosquitoes and they could not or would not oviposit when a lower water temperature was also available.

B) Culex pipiens

Experimentation with *C. pipiens* was more diffecult than with *Ae. aegypti*, and a less extensive study was done with them. Very few egg rafts were obtained at 15 and 35 C. Wallis (1954) stated that *Culex* mosquitoes will not oviposit in water that has a salt concentration unacceptable to them. Bellamy and Corbet (1974) found resorption of eggs in *Culex tarsalis* Coq. It is possible that because 15 and 35 C are unfavourable temperatures for oviposition, only a few laid rafts and the others resorbed their eggs or died without laying.

These results must be taken into account when the results of the experiments with the two sites at contrasting water temperatures are examined. The results in the set where 15 C is the lower temperature (fig. 16, I) can be predicted from those of the control series. Some egg rafts were laid at 15 C but most were laid at the higher water temperatures. Temperatures between 20 and 25 C (fig. 16, II) were equally favourable for oviposition. Within this range of water temperatures the females do not appear to discriminate between differences in water temperature, as indicated by the number of eggs rafts laid.

In the 20 with 30 C and 25 with 30 C experiments (fig. 16, III) the mosquitoes showed bias towards the lower temperature. None of these temperatures was unfavourable for oviposition in experiments where the two sites were maintained at the same constant temperatures, so it appears that the mosquitoes were discriminating between water temperatures that appeared equally favourable in the experiments when both sites were at the same water temperatures.

Hecht (1930) obtained the following number of egg rafts with C. pipiens at sites maintained at the indicated temperatures; 2 at 15 to 17 C, 14 at 20 to 29 C and 12 at 30 to 34 C. He also found an avoidance of temperatures around 15 C but he obtained a large number of egg rafts above 30 C which is contrary to what was found in this study. His water temperatures were not well controlled, as temperatures could vary by 7 C and he only reported the results of one experiment with C. pipiens. He might have obtained different results under different experimental conditions.

C) General Discussion

There appear to be two types of discrimination between water temperatures in both species of mosquito. One level involves temperatures which are avoided even when no other option is available. In this case the majority of the mosquitoes do not oviposit. The other level involves water temperatures at which eggs will be laid if there is no other option; however if given a choice these temperatures will be avoided.

How the mosquitoes discriminate between water temperatures is not known. Warm and cold receptors which respond to air temperature have been found in the antennae of *Ae. aegypti* (Davis and Sokolove 1974). The activity of the cold receptor was highest at 26 C and activity for the warm receptor was highest at 28.5 C. Davis and Sokolove suggested that these receptors may be important in host-seeking behaviour, but it is possible that they could also respond to air streams emanating from a body of water. The temperatures of maximum activity for these receptors fall within the range of temperatures found to be the most favourable for oviposition.

Another type of antennal sensilla responded to water vapour with an increase in the action potential rate (Kellogg 1970) and was capable of detecting a sudden increase of two percent in the relative humidity. Kellogg postulated that these sensilla, as well as some responding to carbon dioxide, could be involved in host-finding; these receptors or similar ones could also be active in water finding.

Dwarakanath *et al.* (1974) found that after blood feeding, *Culex fatigans* females responded to the wet side in an alternative chamber for testing response to relative humidity. Before blood feeding they responded to the dry side. Kennedy (1942) found that, in more or less still air, *C. pipiens* females were not attracted to water either from above or from the side from more than 10 cm away. Still air does not normally exist in nature. The water vapour, which normally rises as it is lighter than air, emanating from a body of water would not take the form of a series of gradients radiating out in all directions. It would take the form of a more gentle gradient in only one direction according to the direction of local air movement (Kennedy 1942). The joint effects of activation by a moist air current and orientation of flight along that current very probably play an important part in water-finding in the field.

In the cages used in the laboratory it is likely that the mosquitoes found the oviposition sites by random movement, rather than orientation along a moist air current, but in some cases (e.g. Group II temperatures with *Ae. aegypti*) found them unsuitable for oviposition.

Another factor which has been shown to influence mosquito oviposition and that would be influenced by water temperature is the presence of microbes, and associated chemical cues, particularly those from bacteria (Roberts and Hsi 1977). Distilled water was used in the oviposition experiments but the sites were not sterile. The range of temperatues which are favourable for oviposition may permit the greatest microbial growth. Investigation of this phenomenon could possibly explain the different responses of the mosquitoes to the oviposition sites if they are not responding to water temperature.

For both species, a favourable range of water temperatures for oviposition can be defined. *Ae. aegypti* favours temperatures between 20 and 30 C while

C. pipiens is more restricted and favours temperatures between 20 and 25 C.

It has been suggested that artificial pools could be used to determine presence and abundance of disease-carrying mosquitoes (Belton 1967; Smith & Jones 1972). It has been found that oviposition surveys offer an economic, rapid and sensitive method for determining the presence of Ae. aegypti adults in the field (Fay & Eliason 1966).

The knowledge of this favourable range of water temperatures could be used to increase the chance of oviposition in artificial pools. There appears to be some potential for using temperature in an integrated control progam: possibly attractive oviposition sites could be created for surveying.

Many volatile chemicals have been found which are attractive to specific species of mosquito, e.g. Mulla et al. (1970). They suggest that, by using these volatiles, oviposition may be intensified in a portion or in portions of the breeding area where the eggs or larvae could be eliminated by other means. These volatiles and other factors which favour oviposition could easily be used in combination with the favourable range of water temperatures, as determined for the specific species involved in surveying and possibly as control measures.

SUMMARY AND CONCLUSIONS

This final section is devoted to the synthesis of the results of the previous parts of this thesis. The overall temperature responses of the two species will be considered as well as the potential for using controlled temperature pools in a mosquito control program. Many points have been discussed in the individual sections so only the conclusions will be considered here.

A wide range of temperatures permited survival to adult in both species of mosquito. The survival of *Ae. aegypti* was high between 20 and 35 C while the survival of *C. pipiens* was high between 15 and 32.5 C. As expected, *Ae. aegypti* survives at higher temperatures than *C. pipiens* and *C. pipiens* survives at lower temperatures than *Ae. aegypti*.

Adult size, as measured by fresh weight and wing length, decreased linearly with increasing temperature of rearing; and rate of development increased linearly with increasing temperature of rearing for both species. The two species differed in the way in which males and females were influenced by rearing temperature. There was a greater change in the size of the females than in males with *Ae. aegypti* whereas *C. pipiens* males and females responded similarly. The change in rate of development due to temperature was different from the change in size observed for males and females in the two species. The rates of development of the two sexes of *Ae. aegypti* changed by the same amount in response to increased temperatures of rearing whereas developmental rates of male *C. pipiens* changed more than that of females.

On the basis of survival, rates of development, size of imago and fecundity (for *Ae. aegypti* only), the "optimum" temperature for *Ae. aegypti* was 25 C, whereas for *C. pipiens* it was 20 C. A rating system was used to calculate these optima and the calculations are shown in table VIII. The above parameters were given ratings at each temperature depending upon trends indicated in the analysis of the data. Products of

TABLE VIII

Determination of an "optimum" temperature for *Ae. aegypti* and *C. pipiens* by taking the product of ratings asigned to the listed parameters. The largest product indicates the most favourable temperature. This is an arbitrary rating system and the products can not be compared between species.

		Ratings of			Products of	
Temperature	Suvival	Rate development	Size	Fecundity	1x2x3	1x2x3x4
	(1)	(2)	(3)	(4)		
		Ae. aegypti				
15	1	1	5	2	5	10
20	2	2	4	3	16	48
25	2	3	3	3	18	54
30	2	4	2	2	16	32
32.5	2	4.5	1.5	-	13.5	--
35	2	5	1	1	10	10
37.5	1	5.5	0.5	-	2.8	--
		C. pipiens				
12.5	2	0.5	5.5	-	5.5	
15	3	1	5	-	15	
20	4	2	4	-	32	
25	2.5	3	3	-	21.5	
30	3	4	2	-	24	
32.5	2.5	4.5	1.5	-	16.8	
35	1	5	1	-	5	

these ratings were calculated and the temperature with the largest is called the "optimum".

Although a range of temperatures is favourable for development of these two species, it is likely that the "optima" determined by the above criteria is within the true "optima" range for that species.

Fecundity of _Ae_. _aegypti_ females was affected by temperature of rearing. A decrease in fecundity was observed in females reared from 20 to 35 C which was correlated with a decrease in size. Mosquitoes reared at 15 C laid fewer eggs than those reared at 20 C. This was attributed to a lack of hardiness as size was not an apparent factor.

The rearing temperatures used in the fecundity experiments did not appear to have any debilitating effects on the males, although no direct tests were done on the males. No anomalous development appeared to occur, as the majority of the females developed and laid eggs, so in all probability the males were capable of mating.

Oviposition of mosquitoes can be modified by the temperature of the water. At extremely high and low water temperatures both species of mosquitoe showed reduced oviposition when no other option was available. *Ae. aegypti* found 15, 40, and 45 C unfavourable for oviposition and few egg rafts were laid by *C. pipiens* at 15 and 35 C. The response of *Ae. aegypti* to high temperatures, specifically 40 C, is modified by the temperature at which the mosquitoes were reared and the air temperature at which oviposition occurred. More eggs were laid at 40 C when the mosquitoes had been reared at high water temperatures or when the air temperature was high during oviposition. Rearing temperature appeared more critical than air temperature in the modification of oviposition.

When given a choice between water temperatures for oviposition, *Ae. aegypti* favoured water temperatures between 20 and 30 C. whereas *C. pipiens* favoured water temperatures between 20 and 25 C. These temperature ranges overlap with the rearing temperatures that were "optimum" for each species as defined by the criteria measured in this study. Both species favour water temperatures for oviposition in a smaller range of temperatures than that in which they could survive. When survival, development time, size, and fecundity were considered the females chose water temperatures which would give them a large population increase in a short time.

Both species oviposited at temperatures unfavourable for survival when no other option was available. An attractive oviposition site could be created for surveying, particlarly early in the season when the range of temperatures most favourable for oviposition might not be present in the field.

APPENDIX A

Survival of *Aedes aegypti* (L.) from larva to adult at various constant temperatures--raw data.

Temperature C	Number surviving out of 50			
10	0	0	0	0
12.5	0	0	0	0
15	28	19	20	14
20	45	42	35	48
25	38	47	48	47
30	50	43	40	50
32.5	44	41	37	46
35	45	48	46	48
37.5	14	28	28	12
40	0	0	0	0

APPENDIX B

Survival of *Culex pipiens* L. from larva to adult at various constant temperature--raw data.

Temperature	Number surviving out of 50			
10	0	0	1	2
12.5	30	39	20	28
15	42	46	44	35
20	50	45	50	50
25	35	44	33	31
30	42	41	43	45
32.5	38	29	38	45
35	8	4	5	12
37.5	0	0	0	0
40	0	0	0	0

APPENDIX C

Eggs oviposited by <u>Aedes</u> <u>aegypti</u> (L.) reared at various constant temperatures. Zeros (no eggs laid) and missing values (wing lengths) are designated by --. (An * indicates that the female died before the end of the test. When retained eggs were found they are reported in brackets after the number of eggs laid.)

Temperature C

<u>Rearing Temperature Male Present</u>

Eggs Laid

15	20	25	30	35
--*	116	87	94	45(35)
109	120	125	107	62
72	123	109	68	70
101	121	96	82	23
95	127	103	108	64
50	100	137	85	49
75	103	102	67	55
97	107	118	67	42
83	101	96	82	64
--*	105	94	73	61

Wing Lengths of females which laid the above eggs

15	20	25	30	35
--	3.5	3.2	2.8	3.1
3.9	3.6	3.3	2.7	2.4
3.6	3.6	3.3	2.7	3.4
3.9	3.6	3.3	3.0	2.9
3.8	3.9	3.3	3.1	2.7
3.4	3.5	3.3	3.0	2.9
3.6	3.2	3.3	3.0	2.7
3.6	3.6	3.3	2.7	2.6
--	3.4	3.2	2.9	2.7
--	3.6	3.1	3.1	2.8

APPENDIX C continued

Room Temperature Male Present During Oviposition

Eggs Laid

86	116	93	91	83
78	119	94	66	45
17(64)	117	48	78	67
111	108	113	91	121
--*	68	82	77	54
51(20)	93	53	77	1(35)
1(44)	109	88	72	41
--*	132	117	68	33(7)
--	96	113	36	17
79	118	104	70	--

Wing Lengths of females which laid the above eggs

3.8	3.7	3.3	3.1	2.8
--	3.6	3.2	3.0	2.7
3.6	3.7	3.6	2.7	2.8
3.6	3.6	3.2	2.7	3.0
--	3.4	3.1	2.6	--
3.4	3.3	3.4	2.9	2.6
3.6	3.6	3.1	2.7	2.4
--	3.6	4.0	2.8	2.8
--	3.5	3.4	2.7	2.7
--	3.5	3.2	--	--

APPENDIX D

Number of eggs laid by *Aedes aegypti* (L.) females at two sites maintained at the same constant temperatures

Temperature C

15		20		25		30	
125	365	453	485	649	615	352	344
166	213	608	643	538	617	673	458
242	159	576	805	626	476	552	475

Temperature C

35		40		45	
750	677	250	382	1	94
687	764	189	244	201	119
481	416	220	287	275	277

APPENDIX E

Number of eggs laid by *Aedes aegypti* (L.) at two sites maintained at various constant water temperatures.

Temperature Pairs C

15	20	20	25	25	30	30	35
158	613	163	690	758	537	1237	650
334	889	190	354	551	415	642	365
		420	379			789	471
		751	493			951	639

Temperature Pairs C

35	40	40	45	15	25	20	30
880	182	580	19	29	744	517	489
987	322	560	181	184	1082	605	827
				422	1160	267	248

Temperature Pairs C

25	35	30	40	35	45
706	256	1228	221	648	31
703	339	1033	109	383	0
752	479				

APPENDIX F

Number of eggs laid by *Aedes aegypti* (L.) reared and allowed to oviposit at various air temperatures at two sites maintained at various constant water temperatures

Rearing temperature 30 C Air Temperature 30 C

Water Temperature Pairs C

40	40	45	45	30	40	40	45
672	407	167	205	980	60	563	2
598	476	181	207	833	90	475	0

Rearing temperature 20 C Air Temperature 30 C

Water Temperature Pairs C

40	40	45	45
295	483	176	299
495	365	154	158

Rearing temperature 30 C Air Temperature 20 C

Water Temperature Pairs

40	40
597	564

APPENDIX G

Number of egg rafts laid by *Culex pipiens* L. at two sites maintained at various constant water temperatures.

Water Temperature Pairs C

15	15	20	20	25	25	30	30
3	1	22	24	29	39	20	17

Water Temperature Pairs C

35	35	15	20	20	25	25	30
		1	32	17	15	33	10
		5	29	16	17	32	5

Water Temperature Pairs C

15	25	20	30
4	49	19	6
1	30	33	9

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