ECOLOGICAL STUDIES ON CONTAINER-BREEDING MOSQUITOES

Aedes geniculatus (OLIVIER) AND Aedes aegypti (L.)

by

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ABSTRACT

Several aspects of the ecology of container-breeding mosquitoes Aedes geniculatus (Olivier) and Aedes aegypti (L.) are described.

The work aims to provide a better understanding of the ecology of container-habitat mosquitoes through the evaluation of some of the factors which affect the population dynamics of mosquitoes breeding in small, transient bodies of water such as tree-holes and artificial containers.

Studies under field conditions of Aedes geniculatus, the most common tree-hole mosquito in England, are described. Larval populations were regularly monitored in 32 tree-holes in beech trees at Silwood Park during 1975 and 1976. Numbers of larvae were estimated, and oviposition in the field was also investigated in both years.

Laboratory experiments are described which were designed to examine larval and pupal development and survival of Aedes geniculatus. Adult populations were established in the laboratory to investigate reproductive behaviour, oviposition and egg diapause.

The effects of population density on the development of mosquito larvae in small artificial container-habitats were analyzed using laboratory populations of Aedes aegypti. Intraspecific factors, such as competition for food and space, and interactions between different stages of development were investigated. The strength of the effects of these intraspecific factors was measured in terms of numbers surviving to adult and of the quality of the individuals produced.

"IT IS NEARLY TRUE TO SAY THAT IF

IT HOLDS WATER MOSQUITOES WILL

BREED IN IT SOMEWHERE OR OTHER"

P.F. Mattingly, 1969

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SECTION 1

MOSQUITO-BREEDING IN CONTAINER-HABITATS

1.1   INTRODUCTION

Aedes geniculatus (Olivier) and Aedes aegypti (L.) (Diptera :
Culicidae) are both container-breeding mosquitoes: Aedes geniculatus
is a woodland species found in the Paleartic region restricted to
breeding in tree-holes. Aedes aegypti is a tropical species widely
adapted to many different kinds of container-habitats, especially
to tree-holes in tropical forests, but more important, has become
associated with small artificial containers in urban areas.

These two species were used as the subjects of the study on
aspects of the ecology of container-breeder.

A general review of mosquito breeding in container-habitats, especially in tree-holes is presented in this section. The second section deals with the characteristics of the tree-hole habitat in the study area and the occurrence of mosquito larvae in these tree-holes, while section 3 is concerned with oviposition and larval population dynamics of _Aedes geniculatus_ in the field. Section 4 deals with laboratory experiments on the bionomics of _Aedes geniculatus_; development, adult size, reproductive characteristics and egg diapause. Section 5 presents an analysis of the effects of larval density on the development and survival of pre-imaginal stages of _Aedes aegypti_: intraspecific factors and interactions between different pre-imaginal stages in small artificial containers are investigated.

1.2   THE CONTAINER HABITATS

Container-habitat is a term proposed by Shannon (1931) to cover habitats such as tree-holes, water containing plants, water in fallen leaves, bamboos, etc., all usually depend on rain and are subject to great variations of water level.

At least 40% of the Culicini, the whole of Sabethini and Toxorhynchitini and a negligible proportion of Anophelini breed in container habitats (Mattingly, 1969). He also pointed out that some authorities considered that the Culicidae originated as container-breeders.

Some classifications of the larval habitats of mosquitoes have been proposed by several authors, for example Boyd (1930), Shannon (1931), Bates (1949), Hopkins (1932) and more recently Mattingly (1969, 1973), who classified the mosquito larval breeding places as follows:

A - Running-water habitats

B - Still-water habitats

    1 - Ground-water habitats

        (a) Permanent

        (b) Temporary

    2 - Subterranean habitats

        (a) Artificial

        (b) Natural

    3 - Container habitats

        (a) Tree-holes

        (b) Bamboos

        (c) Leaf habitats

        (d) Fruit and husks

        (e) Artificial containers

        (f) Miscellaneous

Container breeders are rarely found in ground pools (other than rock pools) and ground-pool species rarely breed in containers. However, Mattingly (1973) pointed out that even although most species of mosquitoes can be categorized reasonably sharply with respect to their breeding places, a certain degree of plasticity has to be allowed.

Many mosquito species are adapted to a wide range of different types of habitats, while others are very specific in their breeding site, for example Aedes geniculatus breeds only in tree-holes. However, other members of the genus Aedes are found in both groups.

Such species which live in small bodies of water which are subject to drastic fluctuations in level of water, have adaptations which allow them to survive periods of drought. For example their eggs can withstand desiccation for prolonged periods and have an inconsistent hatching response when resubmerged.

1.3 MOSQUITOES IN TREE-HOLES

Although tree-holes are a common site for the breeding of several mosquitoes they have been frequently considered as a source of minor importance. Nevertheless, Horsfall (1972) listing the common breeding sites for nearly 1300 species of mosquitoes showed that 25% (350 spp.) had been reported to be breeding in tree-holes, rot-holes or bamboo stumps. Of these 350 species 190 were species of the genus Aedes and 12 of Anopheles.

The output of mosquitoes from small water collections such as tree-holes and bamboo stumps was considered of great importance in tropical conditions (Nasir, 1952).

In temperate regions the most common natural container habitat are tree-holes and few mosquito species breed in them; however, in tropical forests the mosquito fauna of tree-holes is large and varied.

Peterson and Lambrecht (1976) in Nigeria found that 34.1% of wet

tree-holes of 17 different tree species were positive for larvae of

13 mosquito species.

"In the tropics it is mainly species of Aedes, certain types of

Culex, Haemagogus, Orthopodomyia, Toxorhynchites and Wyeomia that

occupy the tree-hole niche."

Considerable work has been carried out on tree-hole mosquitoes

in Africa, principally because Aedes aegypti and Aedes africanus breed

in tree-holes and are important agents in the transmission of yellow

fever. (Dalziel, 1920; Dunn, 1926, 1927a, 1928; Taylor, 1934;

Service, 1965; Lambrecht and Peterson, 1976; Peterson and Lambrecht,

1976; Vogel, 1971; Trpis et. al., 1970).

In America, Jenking and Carpenter (1946) studied the ecology

of the tree-hole breeding mosquitoes of Neartic North America, and

more recently, Smith and Trimble (1973) in Canada did a general study

of the biology of tree-holes of Ontario. The distribution and ecology

of tree-hole mosquitoes in Iowa was investigated by Lunt and Peters

(1976), and Bradshaw and Holzapfel (1975) analysed some aspects of the

development of tree-hole mosquitoes.

Peterson and Lambrecht (1976) referred to Aedes aegypti as one

of the most ubiquitous species in tree-holes. He found it the third

most abundant species in a survey of tree-holes carried out in Nigeria,

occurring in 3.5% of wet tree-holes. Previously, Taylor (1934) and

Service (1965) had reported it in 10% of tree-holes. Moreover, Hanney

(1960) found in the Kaduna area that of the total of Aedes aegypti

larvae sampled near villages 54% were in tree-holes.

1.4 TREE-HOLE MOSQUITOES IN BRITAIN

Thirty-two mosquito species (Gillet, 1971) actually occur in the United Kingdom, and Ireland, of which 27 are Culicines. In tree-holes the most common species is _Aedes geniculatus_ (Olivier); _Anopheles plumbeus_ Stephens and _Orthopodomyia pulchripalpis_ (Rondani) are also found, but rarely.

Marshall (1938) had reported 29 species in Britain with no less than 17 laying their eggs in a wet substrate as _Aedes geniculatus_. Chinery (1974) referred to about 30 species of mosquitoes in Britain, of which he named four species of Anophelines, and pointed out that "although _Anopheles maculipennis_ is quite common in Britain malaria is rare here."

Shute (1954) said that in England the indigenous malaria was apparently transmitted by the tree-hole mosquito _Anopheles plumbeus_. Gillet (1971) listed _Aedes geniculatus_ as capable of transmitting viruses and _Anopheles plumbeus_ as capable of transmitting malaria to man in warmer parts of the World.

Mattingly (1969) reported _Aedes geniculatus_ as common in all types of tree-holes in Southern England, while _Anopheles plumbeus_ and _Orthopodomyia pulchripalpis_ are infrequently found. Even when the three species occupy similar habitats _Aedes geniculatus_ is by far the most common. The distribution of those three species in woodlands is

related to the condition of the tree-holes rather than of the trees.

In Silwood Park, Ascot, six species of Culicines and two of Anophelines were found in a survey of adults carried out in 1970 by F. Benton (personal communication), namely Aedes cantans (Meigen), Ae. punctor (Kirby), Ae. geniculatus (Olivier), Ae. cinereus (Meigen), Culex pipiens Linnaeus, Culiseta (= Theobaldia) annulata (Schrank), Anopheles plumbeus Stephens and Anopheles claviger (Meigen), of these only Aedes geniculatus and Anopheles plumbeus are found in tree-holes. No record of the presence of Orthopodomyia pulchripalpis is known for the area.

Among the papers which give some details regarding the general bionomics of English tree-hole mosquitoes are: Beattie and Howland (1929), Macan and Tutin (1932), Keilin (1932), Tate (1932), MacGregor (1932), Marshall and Staley (1933) and later Kitching (1969a, 1969b, 1971) and Service (1971a, 1971b, 1974).

1.5 ZOOGEOGRAPHICAL DISTRIBUTION OF THE SPECIES STUDIED

Aedes geniculatus, Anopheles plumbeus and Orthopodomyia pulchripalpis, the only three species reported in England as tree-hole breeders, are all restricted to the Paleartic Region.

Aedes geniculatus, a representative of the subgenus Finlaya has been recorded from numerous localities in the southern part of England. No records exist for Wales, Scotland or Ireland. (Marshall, 1938).

Edwards (1921) pointed out that _Aedes geniculatus_ is distributed throughout Europe from France to Galicia, and from South Sweden to Macedonia, wherever there are deciduous trees, such as beech, sycamore and chestnut in sufficient numbers. It also occurs in Corsica and Asia Minor.

Anopheles plumbeus, a species of the subgenus Anopheles, is widely distributed in Britain, and numerous records of it exist from England, Wales, Scotland and Ireland.

Edwards (1921) stated that _Anopheles plumbeus_ is distributed throughout Europe wherever there are deciduous trees in which rot-holes can form. It is also represented in Asia, but not in North Africa.

Anopheles plumbeus and _Aedes geniculatus_ were reported by Gillett (1971) as species living only in the Paleartic Region (Europe, North Africa, Middle East and North Asia).

Aedes aegypti, a species of the subgenus Stegomyia, is ubiquitous in the tropics and subtropics, and its range extends into various temperate zones. The ancestral populations seem to occur in Africa, showing a forest habit while the successful spreading of the species all over the world appears wholly related to its adaptation to urban areas and colonization of artificial containers, such as tyres, tin cans, water jars, etc.

1.6 AIMS OF THIS STUDY

The aims of the work described in this thesis were, firstly to investigate some aspects of the biology and ecology of Aedes geniculatus, the commonest tree-hole breeder in Britain. Secondly, to examine some aspects of the intraspecific effects on larval populations of mosquitoes naturally breeding in container-habitats by using Aedes aegypti as the experimental model on the laboratory work, on account of its importance as a common breeder in container-habitats and its easy handling in laboratory conditions.

It was hoped that the study would have importance for a better understanding of the ecology of these mosquitoes breeding in such transient small bodies of water.

SECTION 2

THE TREE HOLE HABITAT

2.1 INTRODUCTION

Tree-holes in beech trees (Fagus sylvatica L.) are common and occur from the ground level to the canopy. Kitching (1969a) divided them in two main categories which he referred to as rot-holes and pans. Rot-holes arise after damage to the trees extend into the heartwood, with the bark lining incomplete, while pans arise when branches or buttress grow together and are lined completely with bark, however, Kitching (loc. cit.) did not find any differences in the composition of the fauna of these two types of tree-holes at Wytham Wood, Berkshire.

2.2 THE STUDY AREA

All the ecological field work was undertaken in the south-east

woodland of Silwood Park (Fig. 2.2.1), where _Fagus sylvatica_ (the beech tree) is the dominant tree species, with a canopy about 12 to 14 metres above ground level.

The larval habitats studied through regular sampling were 32 tree-holes on the oldest beech-trees. These tree-holes keep water for long periods of time after rain and all of them have layers of soil and leaf litter at the bottom. Other tree-holes were occasionally sampled and used for complementary studies.

2.2.1   The Tree-holes at Silwood Park

Many old beech-trees are present in the wood at the south-east corner of Silwood Park. In a survey carried out during September 1974 one hundred and two beech trees were found in the area, 32 with a total of 74 holes in the trunks. The tree-holes occurred mainly at ground level (at less than 1 m high), and were of the pans type between buttress roots; relatively few holes were found at canopy level.

All 74 beech tree-holes were labelled and their water levels observed weekly during September and October 1974. At the end of the survey it was found that only 58 tree-holes (in 27 trees) were continuously water-filled, 49 at ground level and nine at canopy level. The remaining holes either dried out soon after rain or did not hold water at all and were discarded for the purposes of study. The nine holes at canopy level were not considered for further studies because

Fig. 2.2.1   The Study Area at Silwood Park, Ascot.

EAST LODGE

SOUTH GRAVEL

DRIVE LAWNS

△Screen

Well

DRIVE
FIELD

JAPANESE
GARDEN

THE HEATH

SOUTH LODGE WOOD

CANNON
WOOD

HEATH

LODGE
FIELD

SOUTH LODGE

⑤ Beech-tree with water-filled holes (27)

Regularly sampled tree-holes (32, Set A)

Occasionally sampled tree-holes (17, Set B)

⊛ Beech-trees without water-filled holes (75)

⊡ Other trees

Bushes

Grasses

△ Meteorological site
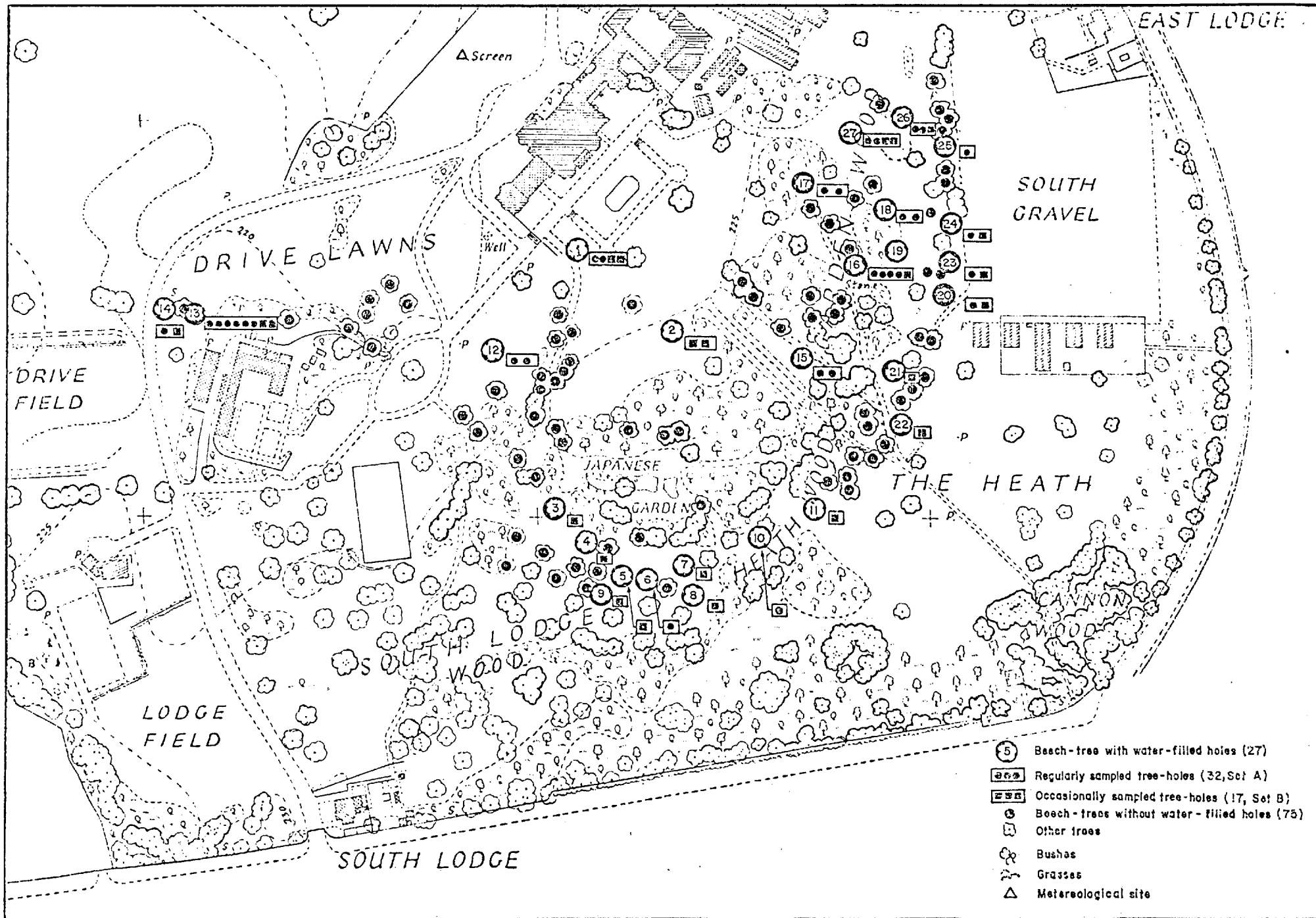
of their few number and inaccessibility.

2.3   THE SELECTED TREE-HOLES

The forty-nine tree-holes continuously water-filled at ground level were considered as appropriate breeding places for mosquito larvae and were classified into three categories on the basis of their size.

Measurements were made of the axes of the tree-holes at the maximum level of water and it was found that because of the elongate shape of the majority of the tree-holes a maximum axis could be clearly defined and used as a criterion of ranking. They were ranked into three categories: large, medium and small with maximum axes ranging from 30 to 50 cms, 16 to 28 cms and 7 to 14 cms respectively. As an additional measurement the maximum perpendicular to the maximum axis was considered and in general (70% of the tree-holes) it was 0.25 to 0.50 ($\bar{x}$ = 0.42) of the length of the respective maximum axis. Thus twelve tree-holes were ranked as large, twenty-six as medium and eleven as small (See Appendix 2. 3.1).

From those 49 measured tree-holes a group of thirty-two (Set A : 8 small, 16 medium and 8 large) were randomly selected to be used in a regular sampling programme. The 17 remaining ones (Set B : 4 small, 9 medium and 4 large) were reserved for other purposes (e.g. occasional sampling, as a source of material for laboratory studies, adults traps, etc.)

Measurements of the volume of the thirty-two tree-holes

selected for regular sampling (Set A) were carried out at the end of

the two years sampling programme (See Table 2.5.1). At the same time

the volumes of the tree-holes at different levels of water were

measured in order to know the actual volume of water present in each

of the tree-holes at the sampling dates, when it was only possible

to record the water levels. This was found to be necessary because

the shapes and depth of the holes varied considerably (Appendix 2.5.1).

2.4   CLIMATOLOGICAL FACTORS

Data on temperature and rainfall (Table 2.4.1 and 2.4.2) were

obtained from the Meteorological Site on the front lawn at Silwood Park.

2.4.1   Temperature

Monthly mean of temperatures of the daily mean ± 1°C from chart

reading, 4-hourly, starting at 02.00 hours in a 4-feet Stevenson

screen records. Mean monthly temperatures for the years 1974, 1975

and 1976 are shown in Table 2.4.1..

2.4.2   Rainfall

Monthly totals of rainfall of daily total ± 0.01 mm from a Dines

Tilting Syphon recorder checked by M.O. gauge. Table 2.4.2 shows

monthly rainfall values for 1974, 1975 and 1976.

TABLE 2.4.1   Temperature Data for Silwood Park, Ascot. 1974-1976

MONTH	1974			1975			1976		
	Mean temperature (^{o}C)			Mean temperature (^{o}C)			Mean temperature (^{o}C)		
	max.	min.	mean	max.	min.	mean	max.	min.	mean
January	8.77	3.44	6.24	9.57	4.27	7.27	8.40	1.95	5.22
February	8.60	2.19	5.64	7.50	1.00	4.67	7.50	1.90	4.57
March	9.54	1.77	5.43	9.48	1.79	4.63	8.85	1.10	4.88
April	13.68	2.99	8.05	12.70	4.25	8.39	13.29	2.52	8.04
May	16.09	5.32	10.96	14.48	5.54	9.93	18.13	6.77	12.43
June	19.59	8.44	14.24	21.03	8.13	15.13	24.43	11.11	17.69
July	19.76	10.98	15.62	23.09	11.85	17.73	24.54	12.20	18.63
August	20.27	9.77	15.01	24.98	12.54	18.74	24.42	9.99	17.33
September	16.48	7.97	11.79	18.37	8.78	13.37	18.52	8.66	12.94
October	10.44	4.12	6.96	14.13	5.44	9.78	13.83	7.17	10.50
November	9.69	4.08	7.25	9.20	0.82	5.29	9.04	2.63	5.69
December	10.44	5.60	8.29	6.94	0.93	5.07	4.74	-1.05	2.02

TABLE 2.4.2   Rainfall Data for Silwood Park, Ascot. 1974-1976

MONTH	Rainfall (mm)		
	1974.	1975	1976
January	75.40	81.90	18.60
February	78.70	28.20	27.30
March	42.60	91.50	15.30
April	16.30	53.00	7.10
May	24.80	60.80	13.10
June	75.60	11.90	11.20
July	38.30	37.70	24.90
August	72.90	0.30	13.80
September	145.60	118.90	132.70
October	67.00	15.10	118.90
November	147.30	62.10	91.30
December	41.70	29.60	34.80
ANNUAL TOTAL	826.20	698.10	509.00

The annual total precipitation in the area during the last three years, 1974, 1975 and 1976 were 826.20, 698.10 and 509.00 mm respectively. There were big differences in rainfall during the summer months (June, July and August) between 1974 and 1975-1976.

2.5 PHYSICAL FACTORS

The physical factors of the tree-hole environment considered in the present study were:

(a) position of the hole on the tree)
)

(b) surface area of the hole)   constants
)

(c) capacity of the hole)

(d) presence or absence of water in the hole)
)

(e) water level in the hole)   variables
)

(f) water volume in the hole)

The first two constants factors were estimated at the beginning of the study. The tree-holes considered (Set A and Set B) were 0 to 1 mt. above ground level; almost all the holes were of the pan-type between butress roots.

The measurements of maximum axis and maximum perpendicular (see Sec. 2.3) were used as indicators of surface area of the tree-holes for their ranking into small, medium and large size tree-holes.

2.5.1 Capacity of the Tree-holes

The volumetric capacity of the 32 tree-holes of Set A was measured at the end of the sampling programme (Table 2.5.1) and in general it did not change the ranking previously done according to measurements of the axes. Tree-holes Nos. 12a, 12b and 15a appear displaced in their size group, because of their inclined bottom surface they held less water than their surface areas indicated. Tree-hole No. 17a, with a large surface area was very shallow and held appreciably less water than the other holes in the large size group.

2.5.2 Presence or Absence of Water in the Tree-holes

The presence or absence of water and the levels of water in the tree-holes of Set A were observed on each sampling occasion. A summary of the presence of water in the tree-holes examined from December 1974 to December 1976 is shown in Table 2.5.2.

2.5.3 Volume of Water in the Tree-holes

Because the volume of water in the tree-holes studied could not be measured during the sampling period, only the levels of water were recorded for each sampling date. At the end of the sampling programme the tree-holes were completely emptied and small measured volumes of water were poured into each of them to reach the different levels of water recorded during sampling; there real volumes of free water were recorded and   were referenced to each sampling

TABLE 2.5.1   Capacity of the 32 Tree-holes (Set A) Studied

SMALL		MEDIUM		LARGE	
Hole number	Volume (cm^3)	Hole number	Volume (cm^3)	Hole number	Volume (cm^3)
10	400	1b	900	1c	1800
13f	400	6	1200	12a	800 *
14a	125	7b	1600	13c	2300
16c	400	12b	500 *	13e	1900
16d	250	13a	1200	13h	1700
24b	400	13g	750	16a	1700
26a	300	15a	300 *	17a	1200 *
27d	200	15b	900	18a	3200
		17b	600		
		18b	600		
		20a	1500		
		20b	700		
		23b	700		
		25	600		
		26b	600		
		27c	700		

*   See Section 2.5.1

TABLE 2.5.2   Summary of the Presence of Water in 32 Tree-holes (Set A)
Examined from December 1974 to December 1976

Sampling dates	No. tree-holes with water in 32 examined (Set A)	% with water	Sampling dates	No. tree-holes with water in 16 examined (Set A-1)**	% with water
17 Dec 74	20 *	100.0	11 Jun 76	16	100.0
15 Jan 75	32	100.0	21 Jun 76	16	100.0
13 Feb 75	31	97.9	29 Jun 76	16	100.0
14 Mar 75	32	100.0	7 Jul 76	16	100.0
3 Apr 75	31	95.8	15 Jul 76	16	100.0
8 May 75	19	64.5	30 Jul 76	16	100.0
22 May 75	29	89.6	9 Aug 76	16	100.0
5 Jun 75	27	84.4	24 Aug 76	16	100.0
19 Jun 75	14	43.8	6 Sep 76	16	100.0
3 Jul 75	10	31.3	6 Oct 76	16	100.0
10 Jul 75	31	97.9	10 Nov 76	16	100.0
17 Jul 75	31	97.9	13 Dec 76	16	100.0
31 Jul 75	18	75.0			
7 Aug 75	18	75.0	Sampling dates	No. tree-holes with water in 16 examined (Set A-2)	% with water
19 Aug 75	24	79.2			
27 Aug 75	16	54.2			
11 Sep 75	8	27.1			
26 Sep 75	32	100.0	11 Jun 76	2	12.5
9 Oct 75	31	95.8	21 Jun 76	3	18.8
29 Oct 75	24	75.0	29 Jun 76	2	12.5
19 Nov 75	24	75.0	7 Jul 76	1	6.3
18 Dec 75	27	84.4	15 Jul 76	0	0.0
23 Jan 76	19	59.4	30 Jul 76	3	18.8
19 Feb 76	26	81.3	9 Aug 76	0	0.0
18 Mar 76	26	81.3	24 Aug 76	0	0.0
8 Apr 76	9	12.5	6 Sep 76	2	12.5
6 May 76	4	12.5	6 Oct 76	16	100.0
20 May 76	2	6.3	10 Nov 76	16	100.0
2 Jun 76	2	6.3	13 Dec 76	7	43.8

*   only 20 tree-holes were examined in this occasion

**   16 tree-holes were artificially watered twice per week from the
     11th of June to September 1976

occasion (Appendix 2.5).

Tables 2.5.3 and 2.5.4 show the variations of volume of free

water in the 32 tree-holes sampled regularly.

During summer 1975 there was a long dry period that caused

volumes of water to decrease rapidly in some tree-holes, and even in

some of them, like 1b, 15a, 15b, 16c, 17a, 20b and 23b there was free

water for not more than five continuous sampling dates during that

period (Appendix 2.5.1)

During 1976, after a dry winter the situation was critical. By

the 18th of March, 81.3% (26) of the tree-holes held water, but on

the 8th of April only 12.5% (9 tree-holes) were holding water. During

May, with the increasing drought and the beginning of a very hot

summer most of the tree-holes became completely dry. For this reason

it was decided to divide the Set A into two sub-groups, one to be

artificially watered twice per week (Set A-1) and one to be kept in

natural conditions (Set A-2).

TABLE 2.5.3   Variations of Volumes of Free-water into the Tree-
Holes (Set A) Studied.   Dec. 74 to Dec. 75 (22
Sampling Occasions).

Hole number	Min. Value (cm^3)	Max. Value (cm^3)	Mean Value (cm^3)	No. times without free-water
10	0	400	252.27	11
13f	0	400	287.50	4
14a	0	125	54.41	5
16c *	0	400	250.00	10
16d	0	250	169.05	1
24b *	0	400	187.50	7
26a *	0	300	175.00	7
27d	75	200	112.50	0
1b	0	900	607.69	9
6 *	0	1200	846.43	7
7b	0	1600	1142.11	3
12b *	0	500	326.67	6
13a	200	1200	713.64	0
13g	0	750	478.57	1
15a *	0	300	190.91	10
15b	0	900	853.85	9
17b *	0	600	323.07	8
18b	0	600	325.00	1
20a *	0	1500	1252.94	4
20b	0	700	415.38	8
23b	0	700	436.36	11
25	0	600	355.00	1
26b	0	600	377.50	2
27c *	0	700	500.00	6
1c	0	1800	1168.18	0
12a *	0	800	631.58	2
13c	600	2300	1581.82	0
13e	0	1900	1276.47	5
13h	0	1700	1160.00	2
16a *	0	1700	988.89	3
17a *	0	1200	716.67	15
18a	0	3200	2447.62	1

* only 21 sampling occasions (not sampled on Dec. 1974)

TABLE 2.5.4   Variations of volume of free-water into the tree-holes

(Set A) studied. Jan. 76 to Dec. 76 (18 sampling

occasions

Hole number	Min. Value (cm^3)	Max. Value (cm^3)	Mean Value (cm^3)	No. times without free-water
10	0	400	31.94	16
13f *	0	400	101.39	7
14a	0	125	11.11	14
16c *	0	400	127.78	9
16d *	0	250	180.00	3
24b	0	400	52.78	12
26a	0	300	47.22	13
27d *	0	200	164.58	6
1b	0	900	133.33	15
6	0	1200	183.33	13
7b *	0	1600	725.00	6
12b *	0	500	161.11	10
13a	0	1200	241.67	12
13g	0	750	225.00	8
15a	0	300	16.67	16
15b	0	900	161.11	14
17b *	0	600	288.89	6
18b *	0	600	333.33	5
20a *	0	1500	833.33	6
20b	0	700	366.67	12
23b	0	700	55.56	16
25 *	0	600	227.78	4
26b *	0	600	280.56	5
27c *	0	700	419.44	5
1c *	0	1800	905.56	2
12a *	0	800	461.11	4
13c *	0	2300	1022.22	4
13e *	0	1900	944.44	6
13h	0	1700	200.00	14
16a	0	1700	111.11	13
17a	0	1200	66.67	15
18a	0	3200	728.00	12

*. Tree-holes watered artificially from 11th June to mid-September

2.6 THE TREE-HOLE MOSQUITOES STUDIED

In Britain only three mosquito species have been reported as tree-hole breeders, namely Aedes geniculatus (Olivier) Anopheles plumbeus Stephens and Orthopodomyia pulchripalpis (Rondani).

The most common species found in the tree-holes studies was Aedes geniculatus. It has been reported as associated with tree-holes in many localities throughout southern England. Also Anopheles plumbeus was found, but on few occasions and in very small numbers.

Fourth instar larvae and adults of Aedes geniculatus and Anopheles plumbeus were identified using the key of Coe, Freeman and Mattingly (1950). The identifications were later confirmed by Dr. Graham White of the British Museum, Nat. Hist. (personal communication).

2.6.1 Aedes geniculatus (Olivier)

The different stages have been described and illustrated in detail by Marshall (1938), a brief summary is given below:

The fourth instar larvae are easily separated from other British species by their very broad anal gills, comb-scales arranged in a single evenly-aligned row and the antennal hair is simple. The presence on the thorax and abdomen of numerous paired "stellate hairs" is another of the distinctive characters of the fourth instar larvae.

Aedes geniculatus, as the only British Aedes of the subgenus Finlaya could be easily recognised by the conspicuous white colour of the knee-joints and the lateral triangular patches on the abdominal tergites. Also the pleural region of the thorax has white patches and the mesonotum a median stripe and two broad lateral stripes of a yellowish colour. The wing scales are uniformly dark.

In the present study eggs were found deposited singly in the cracks of the tree-hole bark at different heights above the water level. Marshall (1938) stated that Aedes geniculatus lays its eggs exclusively in tree-holes. Also Wesenberg-Lund (1921), James (1923) and Seguy (1924) have reported the crevices in tree-holes as the normal oviposition site of the species.

Wesenberg-Lund (1919) observed larvae in holes of beech and oak especially those at the base of these trees. Later Beattie and Howland (1929) examining the feeding habits of the larvae found they were fairly indiscriminate feeders of tree-hole debris of all kinds.

As pointed out by Marshall (1938) different trees as beech, ash, elm, chestnut, hornbean, lime and sycamore are reported to provide breeding places for Aedes geniculatus, but Harold (1926) reported a case of larvae breeding in an open pool, and Stackelberg (1937) found larvae in ground pools among roots of hazel, oak, ash and beech in Russia. Also Callot and Ty (1944) reported large numbers of larvae in rock pools in a thick wood of beech and oak in France.

It seems that even the tree-hole bark is the specific ovi-
position site for this species, when <u>Aedes geniculatus</u> larvae are
found in ground pools it is a consequence of tree-holes being
flooded by rains, and larvae removed from the holes in the over-
flowing water.

In England, <u>Aedes geniculatus</u> larvae have been found associa-
ted with <u>Anopheles plumbeus</u>, and also with <u>Culiseta morsitans</u>
(Blacklock and Carter, 1920; Beattie and Howland, 1929; Macan, 1930;
Marshall, 1938; Kitching, 1969a,b).

A summary of the occurrence of larvae of <u>Aedes geniculatus</u> in
the 32 tree-holes examined from December 1974 to December 1976 is
shown in Table 2.6.1.

2.6.2   <u>Anopheles plumbeus</u> Stephens

During the present study <u>Anopheles plumbeus</u> was occasionally
associated with <u>Aedes geniculatus</u>. It was found only in a few holes
and in very small numbers.

A full description of the different stages is given by
Mashall (1938).

The fourth instar larvae may be easily separated from other
British Anophelines (<u>A</u>. <u>maculipennis</u>, <u>A</u>. <u>claviger</u> and <u>A</u>. <u>algeriensis</u>)
by their smooth antennal shaft, the single head hairs, particularly
the frontal and basal ones, and also by the pecten composed of 16

TABLE 2.6.1   Summary of the Occurrence of Larvae of <u>Aedes</u>

geniculatus, in Tree-holes Examined from December

1974 to December 1976 (41 Sampling Dates)

Date	No. of water-filled tree-holes, in 32 examined	% of tree-holes with A. geniculatus larvae	% of water-filled tree-holes with A. geniculatus larvae
17 December 1974	20 *	45.00	45.00
15 January 1975	32	18.75	18.75
13 February 1975	31	25.81	25.81
14 March 1975	32	53.12	53.13
9 April 1975	31	40.63	41.94
8 May 1975	19	40.63	68.42
22 May 1975	29	53.13	58.62
5 June 1975	27	37.50	44.44
19 June 1975	14	15.63	35.71
3 July 1975	10	6.25	20.00
10 July 1975	31	9.38	9.68
17 July 1975	31	31.25	32.26
31 July 1975	18	37.50	66.67
7 August 1975	18	28.13	50.00
14 August 1975	24	31.25	41.67
27 August 1975	16	37.50	75.00
11 September 1975	8	9.38	37.50
26 September 1975	32	21.88	21.88
9 October 1975	31	12.50	12.90
29 October 1975	24	15.63	20.88
19 November 1975	24	15.63	20..88
18 December 1975	27	12.50	14.81
23 January 1976	19	18.75	31.58
19 February 1976	26	18.75	23.01
18 March 1976	26	28.13	34.62
8 April 1976	9	18.75	66.67
6 May 1976	4	6.25	50.00
20 May 1976	2	0.00	0.00
2 June 1976	2	0.00	0.00

TABLE 2.6.1 (Continued)

Date	No. of water-filled tree-holes, in 32 examined	% of tree-holes with A. geniculatus larvae	% of water-filled tree-holes with A. geniculatus larvae
11 June 1976	2 **	0.00 **	0.00
21 June 1976	3	6.25	33.33
29 June 1976	2	0.00	0.00
7 July 1976	1	0.00	0.00
15 July 1976	0	0.00	0.00
30 July 1976	3	12.50	66.67
9 August 1976	0	0.00	0.00
24 August 1976	0	0.00	0.00
6 September 1976	2	0.00	0.00
6 October 1976	16	18.75	18.75
10 November 1976	16	6.25	6.25
13 December 1976	7	0.00	0.00

*   Only 20 tree-holes were examined in this occasion

**   From 11 June to 13 December 1976 only 16 holes were taken into account for this Table, the other 16 were artificially watered during this period

teeth more or less uniform in length.

In England larvae have been collected in every month of the year (MacGregor, 1921; Marshall, 1938; Gillet, 1971). Beattie and Howland (1929) reported the highest incidence of larvae from September to December. The species overwinter as larvae.

Blacklock and Carter (1920) examining 135 tree-holes in England found larvae only in sixteen of them.

From Russia, Horsfall (1971) has reported general references where rot-holes, abandoned wells, cisterns, domestic containers like vases and flower-pots are accounted as larval breeding sites for Anopheles plumbeus.

SECTION 3

3.1   INTRODUCTION

The relationship between mosquito species and the type of habitat where the larvae are found is principally determined by the choice of the oviposition site by the females and the ability of the larvae to successfully reach the adult stage there. Thus, water-filled tree-holes, as discrete and very restricted habitats required especially adapted groups of mosquito species.

In Britain, apart from the early works of Keilin (1927), Beattie and Howland (1929), Marshall and Staley (1929), Marshall (1930), Macan and Tutin (1932), MacGregor (1932) and Marshall (1938); and later Kitching (1969b) little is known of the preadult ecology of the mosquitoes breeding in water filled tree-holes.

As stated previously, <u>Aedes geniculatus</u> is restricted during its pre-adult life to water filled tree-holes and it is the most common tree-hole breeder in the Southern part of England.

A series of sampling programmes and experiments were done in order to study its oviposition pattern and fluctuations of larval populations in the field.

3.2　OVIPOSITION IN THE FIELD

3.2.1　Introduction

It was clearly pointed out by Service (1976a) that the ability to sample eggs and get population estimates in natural habitats is of paramount importance in ecological studies concerning population dynamics. In mosquitoes there is a close relation between the oviposition habits of the females and the requirements of the larva, therefore, the selection of sites for oviposition is of great importance in the ecology of species like the tree-hole breeding insects, which will spend their adult life in a completely different habitat; but in general little importance has been given to the study of oviposition habits of insects breeding in tree-holes.

Dunn (1926), in a study of the breeding habits of the yellow fever mosquito, _Aedes aegypti_ in West Africa, sampled dry tree-holes by removing the debris and scraping the inside of the holes. He demonstrates that the eggs of eight species of _Aedes_ found in those tree-holes were resistant to the drought of the dry season and remain viable until the beginning of the rains. Taylor (1934) also reported that the tree-hole breeding _Aedes_ of the Northern Provinces of Nigeria remain viable through a nine months rigorous dry season. The ability of _Aedes_ eggs to withstand desiccation also has been observed by other authors (Finlay, 1886; Theobald, 1901; Bacot, 1916; Coling, 1924; Buxton and Hopkings, 1927; Christophers, 1960).

The manner and timing of egg laying in several North-American

tree-hole mosquitoes were reported by Jenkins and Carpenter (1946), and the oviposition site selection by the tree-hole mosquito Aedes triseriatus Say was studied by Wilton (1968) in North-America. Wilton concluded that the most important influences on the selection of a site for oviposition are the colour of the container's wall, the optical density of the water and the presence of decaying organic matter. The preferences of Aedes triseriatus were for black walls, dark colour water and organic matter emitting methane gas.

The influence of the water container surface on oviposition of Aedes scutellaris scutellaris (Walker), Aedes aegypti (L.), Aedes albopictus (Skuse) and Aedes scutellaris katherinensis Woodhill was analysed by O'Gower (1955, 1957a, 1957b).

Only few methods have been developed for sampling mosquito eggs, however, Aedes geniculatus in England was sampled in a study of the seasonal oviposition of tree-hole insects in Wytham Woods Berkshire by Kitching (1969b) who, using black jars as ovitraps with and without beech bark chips, had very poor results during 1967 and 1968. His ovitraps worked properly for other tree-hole insects but not for Aedes geniculatus. However, Yates (1974) used, with very good results, sections of bamboo with an absorbent oviposition surface, which was dyed dark grey and had an embossed pattern.

The aim of this study was to get information about oviposition by Aedes geniculatus in the field, firstly by sampling the bark around the tree-holes and secondly by monitoring their oviposition over a period of time in small artificial containers placed near the tree-

holes under study.

3.2.2 Sampling the Bark of Tree-holes

3.2.2.1 Materials and methods

The bark sampler used was a cylindrical metallic corer, 2.5
cm in diameter and 8 cm long, with the edge of one end sharpened to
produce a cutting border. A block of hard wood was used to cover
the upper end of the corer when it was hammered through the tree
bark, until the corer had penetrated about 50 to 75 mm. The circular
sample of bark was then removed with the aid of a fine metallic
spatula.

Thirty-two samples of bark, each 4.91 cm^2 were taken on the
26th November 1974 from the 32 tree-holes (Set A) used later for the
studies of larval populations.

The samples were taken 0 to 5 cm above the water lines, which
at that time were almost at the maximum recorded levels.

Each circle of bark was placed in a labelled, shallow plastic
container with some tap water in it, and covered with a tight plastic
lid to be transported to the laboratory. Each sample was examined
under a binocular microscope, for eggs on the surface of the bark,
and especially in cracks and crevices.

The Aedes geniculatus eggs found were measured and compared with those laid by females in the laboratory populations.

3.2.2.2 Results

Aedes geniculatus eggs were found singly or in very small clusters. Besides Aedes geniculatus, few Collembola eggs were present on the bark samples studied. Acari and Collembola adults were found in some samples.

Of the thirty-two samples examined only five (15.63%) had Aedes geniculatus eggs (Table 3.2.1), with none from small holes. Although the medium sized holes accounted for only 50% of the holes examined, they contributed 88.89% of the eggs found.

TABLE 3.2.1   Aedes geniculatus Eggs in Bark Samples

Tree-hole size	No. of samples	No. of positives	% of pos. in the size	% of pos. in the total	No. of eggs	% of the total
Large	8	1	12.50	3.15	2	11.11
Medium	16	4	25.00	12.50	16	88.89
Small	8	0	0.00	0.00	0	0.00
TOTAL	32	5	-	15.63	18	100.00

Knowing that in general the bark samples were only a very small percentage of the moist band of 5 cm of bark above the water line and

considering the possible aggregate distribution of eggs in the bark

surface, the result of this sampling could only be considered as a

rough indication of the presence of Aedes geniculatus eggs in the

sides of the tree-holes sampled, but no comment can be done about

the abundance of Aedes geniculatus eggs in the tree-holes studied.

Because of the destructive nature of the sampling method, it

was not repeated, and artificial containers attached to the trees

were used as ovitraps during the two following summers.

3.2.3   Artificial Containers as Ovitraps

Three different experiments were set up to monitor the ovi-

position of Aedes geniculatus in the field. Small artificial con-

tainers were placed in woodland areas of Silwood Park, where tree-

holes were common. During the summers of 1974 and 1975 plastic

containers were used, while in 1976 beech-wood containers were used.

3.2.3.1   Containers used (Fig. 3.2.1)

Three different types of artificial containers were used in the

field experiments carried out during the summer months.

(a)   Type A : Circular transparent plastic boxes (10.5 cm top

diameter, 9.5 cm bottom diameter and 4.5 cm height), overflow holes

were made at 2.5 cm height and each containers was covered with half

of a plastic lid (to reduce evaporation). A filter paper band lined
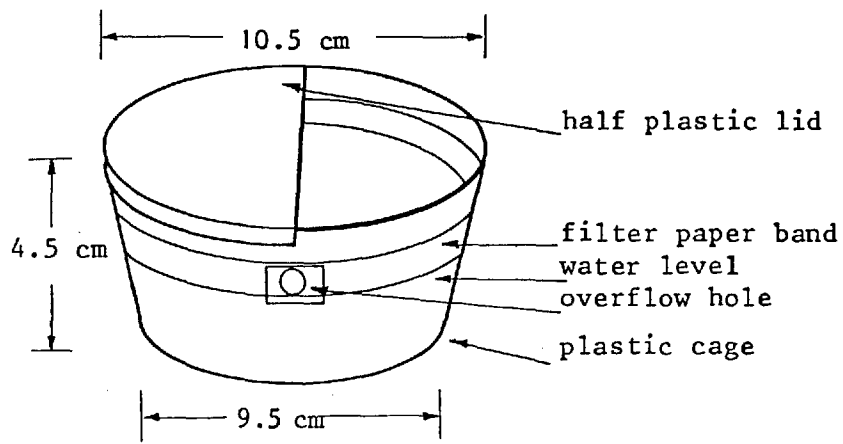
(a) Ovitrap type A
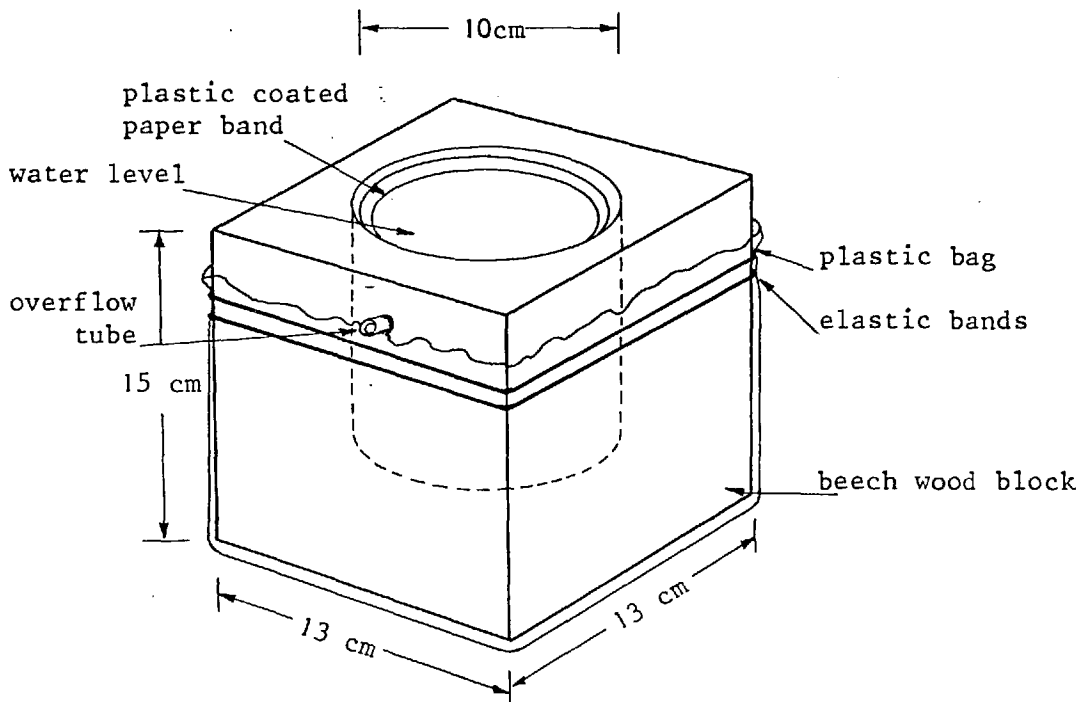
(b) Ovitrap type C

Fig. 3.2.1   Artificial containers used as ovitraps for Aedes

geniculatus

the sides of the containers, and 100 cm of water with some beech

leaves and small pieces of beech bark were provided in each container.

(b)   Type B : As type A but with the outsides of the containers

and half-lids painted black.

(c)   Type C : Cavities of 10 cm diameter and 10 cm deep were

cut in beech wood blocks of 13 x 15 x 13 cm.   In one side a 1 cm

diameter overflow hole was made in which a plastic tube with the

inner end covered with a terylene net was inserted.   The outside of

each container was dipped into molten paraffin wax to seal any cracks

and prevent them from splitting during their use in the field.   The

cracks in the inside were also filled with molten paraffin wax.

The side of the cavity was lined with a strong absorbent paper

band, which was plastic coated on the outside, and with a hole to

coincide with the overflow tube.

To further prevent loss of water, should a crack develop, the

blocks were placed in a plastic bag, up to the level of the overflow

and held in place with several elastic bands.

3.2.3.2   Experiment 1

During July and August 1974 two groups of 12 plastic containers

(Type A) were located in Heat Wood in the south-east corner of Silwood

Park and in Rookery Copse to the west.   The containers were attached to

small wooden platforms above stakes 0.75 mt high. The containers were water-filled and lined with filter paper, they were renewed weekly.

In the laboratory the bands of filter paper and fallen leaves from each ovitrap were examined for eggs under a binocular microscope.

No _Aedes geniculatus_ eggs were found during the two months period.

3.2.3.3   Experiment 2

During the summer of 1975 a series of 32 black plastic containers (Type B) were located near the tree-holes of Set A, and exposed for 14 weeks, from the 29th May until the 28th August. From the 5th June when the first pupae appeared in the tree-holes, the exposed filter paper bands were renewed weekly and examined for eggs in the laboratory. All the leaves that fell in the ovitraps were also examined.

The level of water in the ovitraps was checked every three or four days.

Throughout the study period two types of eggs were found:

(a)   _Dasyhelea_ spp: eggs horseshoe in shape, found in groups,

surrounded by gelatinous sheets of variable size; identified from description given by Kitching (1969).

(b) Aedes geniculatus: eggs ellipsoid in shape, found singly or in small clusters, dark black colour; identified by comparison with those laid by females of Aedes geniculatus in laboratory cultures and as described by Marshall (1938).

The only groups of Aedes geniculatus eggs were found the week ending the 24th July. Twenty eggs were in the ovitrap of the tree-hole No. 12a and 13 eggs in the ovitrap of tree-hole No. 27d. At the same time 4, 28, 18 and 3 groups of Dasyhelea eggs were found in the ovitraps from Nos. 20a, 6, 16a and 27d respectively. In later collections only Dasyhelea eggs were found, but were not counted.

No eggs of Aedes geniculatus were found on the leaves fallen into the ovitraps.

3.2.3.4   Experiment 3

During summer 1976 wooden containers (Type C) were used as ovitraps. Beuchkote ® absorbent paper was used instead of ordinary filter paper to line the containers.

Ten containers were placed near trees Nos. 1, 6, 7, 12, 13, 16, 20, 24, 26 and 27. They were exposed for 14 weeks, from the 20th May to the 26th August and the paper bands examined weekly from the 3rd June.

Even although the containers were bigger than those used in
the previous years, it was necessary to top up with water twice per
week because of the very dry conditions of 1976.

In the paper bands collected the week ending the 12th August,
a total of 40 eggs of _Aedes geniculatus_ were found, 16, 14 and 10 in
the ovitraps of trees Nos. 6, 7 and 13 respectively. Eggs of _Dasyhelea_
were less frequent than in the previous year.

3.2.4 Discussion

The small number of _Aedes geniculatus_ eggs found in the ovi-
traps is difficult to explain, the containers of the first two experi-
ments could be considered not suitable for oviposition of _Aedes_
geniculatus because of their white filter paper linings. However,
this was not true of the third experiment where the absorbent paper
and water in the wooden containers turned brown after 1 or 2 days of
exposure. In addition organic matter accumulated in the containers
during the 12 week period producing conditions considered to be suit-
able for tree-hole mosquitoes studied by Corbet (1963), O'Gower (1955,
1957a, 1957b) and Wilton (1968). It was thought that _Aedes geniculatus_
would respond to similar conditions, thus the choice of the wooden
containers as ovitraps.

Throughout the three experiments it was clear that the contain-
ers were not successful in attracting _Aedes geniculatus_ females to
oviposit in them, even during the droughts of 1975 and 1976 when there

were few wet tree-holes the ovitraps did not succeed. It seems that the natural population of adults in the area was small, and in addition females showed a high selectivity for oviposition sites.

The small number of Aedes geniculatus eggs found in the artificial containers used as ovitraps does not permit firm conclusions about their abundance or periodicity.

Kitching (1969b) reported only 25 eggs of Aedes geniculatus during an 18 month sampling with 16 jars in 1967-1968 at Wytham Wood, Berkshire; all the eggs were collected from black jars with bark chips provided as oviposition surface. Sixteen eggs were in such ovitraps placed at ground level and only nine in those placed in the tree canopy, but Yates (1974) sampling in Monks Wood, Huntingdon found over 20,000 eggs of Aedes geniculatus on the paper linings of 34 bamboo pots during 1972.

The big differences in number of eggs are difficult to explain other than by reason of very different abundances of adults.

3.3   LARVAL POPULATIONS

3.3.1   Introduction

Tree-holes are a very specialised habitat for the mosquito larvae, and not very much work has been done on the ecological research of these mosquito species, especially their populations. In Britain some early workers produced a small amount of information about the larval population of mosquitoes in tree-holes.

The larval incidence of Anopheles plumbeus in three rot-holes in beech-trees was investigated by Beattie and Howland (1929), and related to physico-chemical factors of the environment. Marshall and Staley (1929) reported the presence of Aedes geniculatus larvae in tree-holes at Hayling Island during April, May, August and November of 1928; pupae were found only during August. Marshall (1930), after a survey of five years, pointed out that in Southern England, Aedes geniculatus larvae were found from January to August and November to December, with adults from April to September.

More recently some information about Aedes geniculatus larvae has been presented by Kitching (1969b). Sampling every two months during two years in Wytham Woods, (Oxford), he reported low numbers (from 0.4 to 0.65 per 100 cc) of Aedes geniculatus larvae, with highest density in June and also high numbers in December and February.

The aim of this part of the project was to investigate seasonal changes of the larval population of mosquito breeding in tree-holes.

Over the total period of two years only two species of mosquito were found during the sampling programme, namely Aedes geniculatus and Anopheles plumbeus (see section 2.5), in the 32 tree-holes.

The main analysis of the seasonal fluctuations in larval population, refer only to Aedes geniculatus but some observations on Anopheles plumbeus are included here.

Larvae of Anopheles plumbeus were rare in the samples and throughout this study occurred only in 6 of the 32 tree-holes, namely 13a, 16a, 6, 25, 1c and 16d (Appendix 3.3.1). Their numbers were too few to indicate peaks and were in fact spread throughout the year even although it has been reported to breed mainly in tree-holes and over-winter in the larval stage.

Beattie and Howland (1929) found maximum numbers of larvae in tree-holes from September to December, and later Marshall (1938) reported that the first adults appear in April. Service (1969b) reported that biting populations during 1964 appeared in May and increased in numbers by August and September. He suggested that the species was bivoltine with a small proportion of adults emerging in May to June from over-wintering pre-adults, and a second generation in August to September.

However, Rettich (1971) referred to Anopheles plumbeus as probably having many generations per year in the Poděbrady area of Czechoslovakia.

3.3.2   Methods of Sampling

3.3.2.1   The sampler

The sampling apparatus used (Fig. 3.3.1) consisted of two parts: a liquid aspirator and an open ended graduate cylinder. The liquid aspirator was made of a large glass syringe, 17 cm long and with a volumetric capacity of 35 cm^3. It was operated by a plastic bulb at the upper end, where a plastic valve prevented the water passing into the bulb. At the narrowed and open end of the syringe a plastic extension tube was attached to make the aspirator the same length as the graduate cylinder. The liquid aspirator was used in conjunction with an open-ended graduate polythene cylinder, 21 cm long and 310 cm^3 of volume, which had a plastic foam ring glued to one end.

3.3.2.2   The sampling procedure

The sampler was employed as follows: firstly the graduated tube was placed vertically above the surface of the water in the tree-hole, and then was pressed down by hand until it reached the bottom of the tree-hole, the plastic foam ring helped to keep a seal with the bottom surface. The level of the water was then measured using the tube scale. Secondly, the liquid aspirator was inserted into the tube and the sample of water held there was drawn up into the syringe and transferred to a labelled polythene bag. The aspirator and tube were washed off into the bag with a small amount of distilled water.

(a) Liquid aspirator

plastic bulb

plastic valve

(b) Open-ended cylinder

glass syringe

21 cm

300

260

220

180

140

100

60

20

21 cm

plastic
extension

plastic foam ring
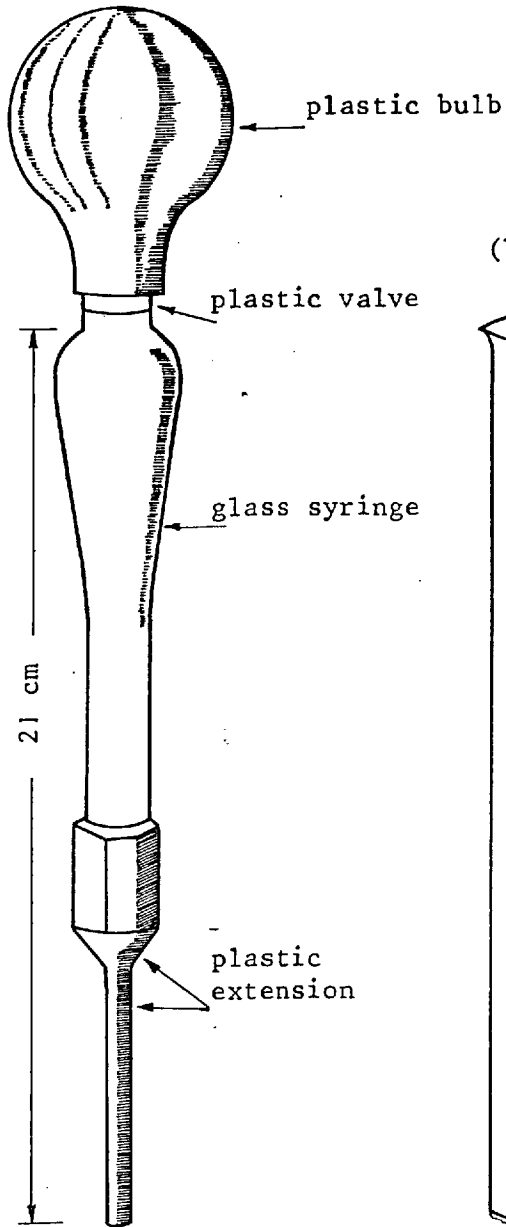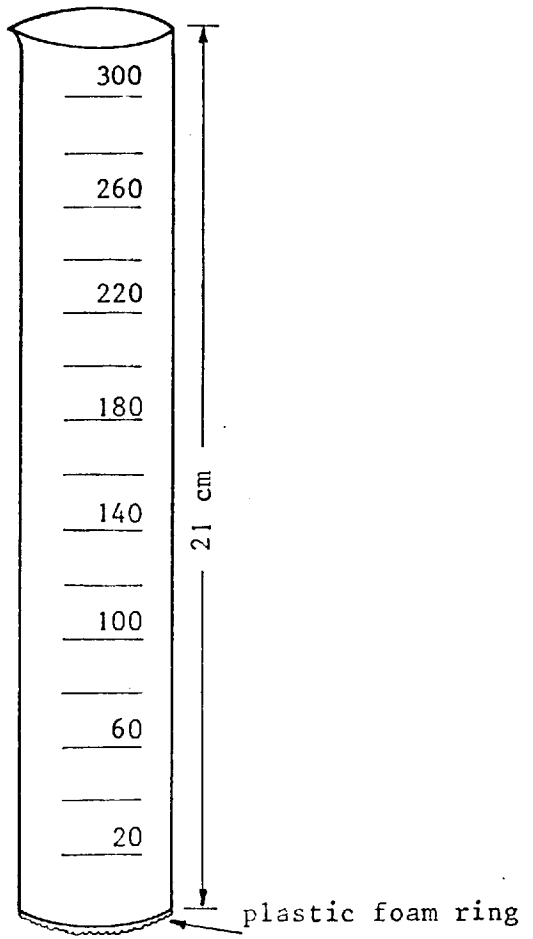
Fig. 3.3.1   The Sampler

3.3.2.3 Laboratory examination of samples

The samples were taken to the laboratory and kept at the same temperature as the tree-holes' water measured at the time of sampling.

In the laboratory the samples were removed from their bags to white plastic wide containers, where they were first examined by eye for large larvae and later under a low-power microscope. Animals found were counted and Aedes geniculatus larvae were measured and separated into instar by size (See Part 3.3.3). Following examination all the animals were returned to their respective bags and taken back to the tree-holes, usually the day after the sampling was done.

This sampling method was useful as a means of estimating the relative abundance of mosquito-larvae present in the tree-holes as it was not feasible to obtain the actual total densities. The size of sample taken was kept small because of the need to sample each hole repeatedly over a long period and the need to minimise disturbance.

3.3.3 The Separation of the Larval Instars

To separate morphologically the different larval instars of Aedes geniculatus in the samples taken during field studies, total body length of the live larva was used as an indicator of stage of development.

3.3.3.1 Methods

To relate the larval sizes found in the field with the actual larval instar, first instar larvae brought from the tree-holes into the laboratory, were placed in groups of 10 or 12 in larval cages (see Section 4.2) with standard culture medium of ten mg. of solid food mixture per larva, and 100 cm³ of water per container. Larvae were reared at 20°C in a constant temperature room and 16 hours light per day. They were inspected every 24 hours and the moults counted and taken out of the cages.

Measurements of 2nd, 3rd and 4th instar larvae were based on these laboratory reared larvae, and first instar size was measured on newly hatched larvae from the field; only those larvae with the egg-breaker still visible were used. As this character disappears when the first instar larva moults (Christophers, 1960) it was considered as a safe indicator to identify first instar larvae.

The live larvae were placed with a few drops of water in a small Petri dish with a millimetre graph paper fix to the under side. The total body length was measured from the mouth to the 8th abdominal segment with the aid of a binocular microscope.

No attempt was made to measure the width of the head or other parts of the larvae because the measurements had to be done in the same quick way as with larvae sampled in the field population studies in order to return them undamaged to their respective tree-holes.

3.3.3.2 Results

The results of measurements of the four larval instars are given in Figure 3.3.2.

The factors of increase of body length from instar to instar are 2.54, 1.56 and 1.48 respectively, with a mean value of 1.86. The high value of the first factor of increase seems to appear as a consequence of bias in the sample of larvae used (only newly hatched first instar larvae were measured).

The increased variability about the mean body length in the fourth instar as indicated by the bigger value of standard deviation is probably the cumulative effects of individual variation in size being larger in that instar in which the larvae spend more time and the expected consequence of geometric growth.

According to size the larvae clearly separated into four instars. The ranges did not overlap and were,

first instar	1.0 - 1.8 mm
second instar	3.0 - 4.0 mm
third instar	5.1 - 6.0 mm
fourth instar	7.4 - 9.0 mm

For practical convenience and having in mind the possible bias of first instar measurements their range was extended to 2.0 mm instead of 1.8 mm, for the ageing of field samples.
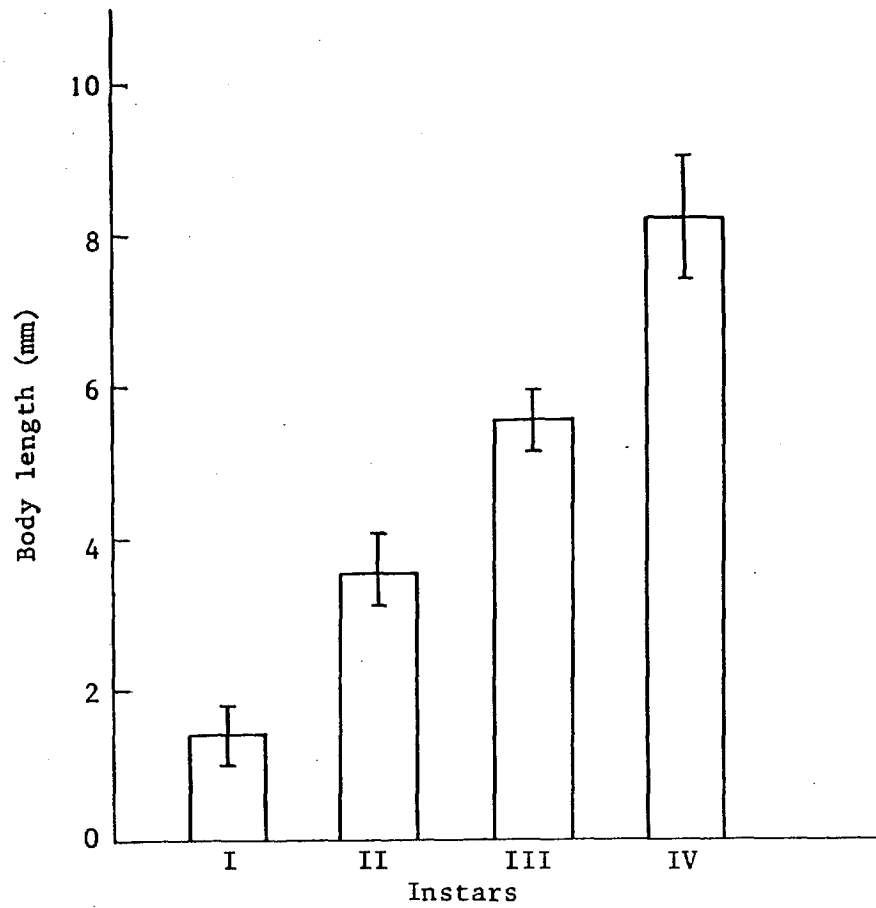
Fig. 3.3.2 Body length of Aedes geniculatus larvae; mean

and standard deviation based on 10 individuals

of each instar

3.3.4   Sampling Programme

The monthly sampling of the thirty-two selected tree-holes (Section 2.3) was designed to provide a series of figures for the seasonal fluctuations of the mosquito-larvae populations. More frequent samplings, weekly or fortnightly, were made during spring and summer in order to provide details of the pattern of development and composition by instar of the larval populations of Aedes geniculatus in the study area (Section 2.2.).

Samples were taken from December 1974 to December 1976, a total of 25 months. Samples of December 1974 were taken only in twenty holes; from January 1975 samples correspond to 32 tree-holes (Set A).

Because of the summer drought, during 1976 it was necessary to modify the design of the field sampling programme because almost none of the tree-holes held free water, and many became completely dry. From May 20 to June 11 almost the whole group of tree-holes was dry, (See Section 2.5.3).

Sixteen tree-holes (Set A-1): four small (13f, 16c, 16d, 27d), eight medium (7b, 12b, 17b, 18b, 20a, 25, 26b, 27c), and four large (1c, 12a, 13c, 13e) were artificially watered twice per week, from June 1976 until the end of the sampling programme in December 1976. The other sixteen tree-holes (Set A-2): four small (10, 14a, 24b, 26a), eight medium (1b, 6, 13a, 13g, 15a, 15b, 20b, 23b) and four large (13h, 16a, 17a and 18a) were kept in natural conditions for the remaining of the sampling occasions.

3.3.5 Composition by Instar of the Larval Populations of

Aedes geniculatus

In Britain the mosquito-eggs laid in tree-holes must cope with very fluctuating water levels and be able to hatch when the temperature of the water is sufficiently high that the larval development is favoured.

First instar larvae were more abundant in the samples from mid-March to mid-August (Appendix 3.3.2). However, some first instar larvae were present in some samples throughout almost the whole year. This may be caused by a marked differential hatch of the eggs from the previous year and due to different durations of diapause. These variations may also be related to the position of the eggs in the bark; eggs at a higher level will be submerged later than lower eggs. Only when heavy rain fills up the tree-holes at once will all the eggs become submerged at the same time, even so the hatch will be erratic, as is common in Aedes species (Marshall, 1938; Gillett, 1971).

It was observed that a high mortality probably occurred during the first instar. For example in the medium sized tree-holes 20a and 26b (Appendix 3.3.2) by 31 July 1975 there were 42 and 43 first instar larvae respectively in the samples while a week later (17 August) only 27 and 18 second instar larvae respectively were found, plus one third instar larvae in tree-hole 26b.

It seems that in a restricted habitat such as tree-holes, this early mortality may allow the remaining larvae to have a better opportunity to reach adult life.

70.

3.3.6   Seasonal Fluctuations of the Larval Populations

The number of larvae per sample were transformed to number of
larvae per tree-hole, relating the counts of larvae per sample
(Appendix 3.3.2) to the actual volume of free water in the tree-holes
at the time of sampling (Appendix 2.5.1).

When the estimated numbers of larvae per actual volume of water
in the tree-holes were examined (Appendices 3.3.3, 3.3.4 and 3.3.5) a
different pattern of larval populations was noted in different tree-
holes. Some tree-holes, mainly large and medium size, appear as
adequate breeding containers for the mosquito populations.

The others, mainly from small size group, were not able to main-
tain populations of larvae consistently over the whole period of study.

Following analysis of the 1975 field results the tree-holes were
grouped into four categories (Table 3.3.1) by taking into consideration
the presence of water and populations of mosquito-larva.

Tree-hole 14a was very small, while tree-holes 15a, 16c and 17a
were too shallow; the first two became dry from May and the second
from April. The absence of larvae in these tree-holes must have been
at least partly due to extremes of conditions and not even providing
the minimum conditions for building up a larval population, even if
females laid eggs in them.

TABLE 3.3.1   Different Types of Tree-hole/<u>Aedes geniculatus</u>

               <u>Larvae Relation During 1976</u>

(a)   Tree-holes without larvae

Small	Medium	Large
13f	15a	17a
14a	15b	
16c	27c	
24b		

(b)   Tree-holes only occasionally with larvae

Small	Medium
10	12b
26a	23b

(c)   Tree-holes water filled almost the whole year and regularly

populated by larvae

Small	Medium	Large
16d	7b	1c
27d	13a	12a
	13g	13c
	18b	13e
	20a	13h
	25	16a
	26b	18a

(d)   Tree-holes irregularly water-filled and irregularly populated

Medium
1b
6
17b
20b

From the estimated number of larvae per actual volume of water in the tree-holes (Appendices 3.3.3, 3.3.4 and 3.3.5) the mean number of larvae per hole was calculated for each tree-hole size category (small, medium and large) (Figs. 3.3.3, 3.3.4 and 3.3.5).

Figures 3.3.3, 3.3.4 and 3.3.5 show the seasonal fluctuation of larvae in each size category of tree-holes only until 11 of June 1976, when 16 of the tree-holes were first watered artificially. The results from June 1976 to Dec. 1976, in both groups, watered (Set A-1) and not watered (Set A-2) will be presented in section 3.3.8.

At the beginning of the sampling programme, in December 1974, an average of 1.2 larvae per tree-hole was present in the small-size tree-hole category, with more larvae in the medium-size category (13.9 larvae tree-hole) and none in large size tree-holes (29.1 larvae/tree-hole). The larvae were third and fourth instar (Appendix 3.3.2) and represented a small overwintering population remaining from the previous summer.

For most of the time numbers of larvae were consistently highest in the large tree-holes and lowest in the small size group.

3.3.6.1   Small size tree-holes

During 1975 the larval populations were always at low levels (Fig. 3.3.3), showing a moderate increase from mid-March to early May, with two later slight peaks in mid-June and mid-July. From September to December the level was near zero.
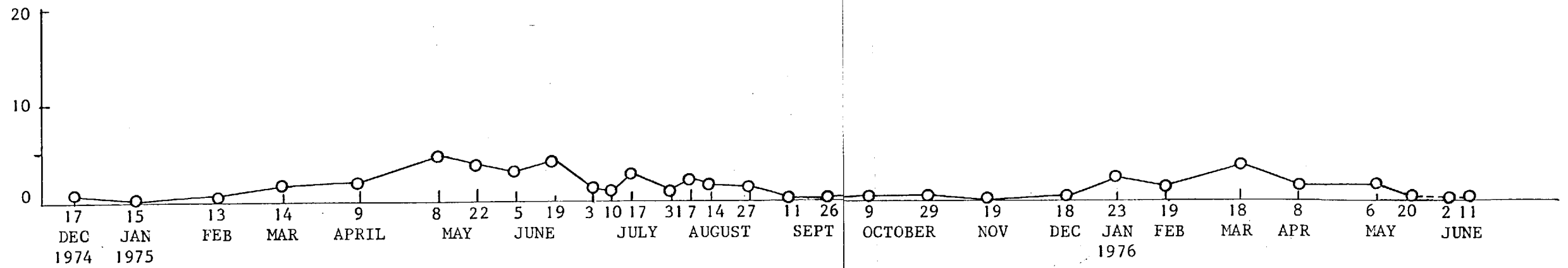
Fig. 3.3.3   Seasonal fluctuations of populations of _Aedes geniculatus_ larvae in small-size tree-holes
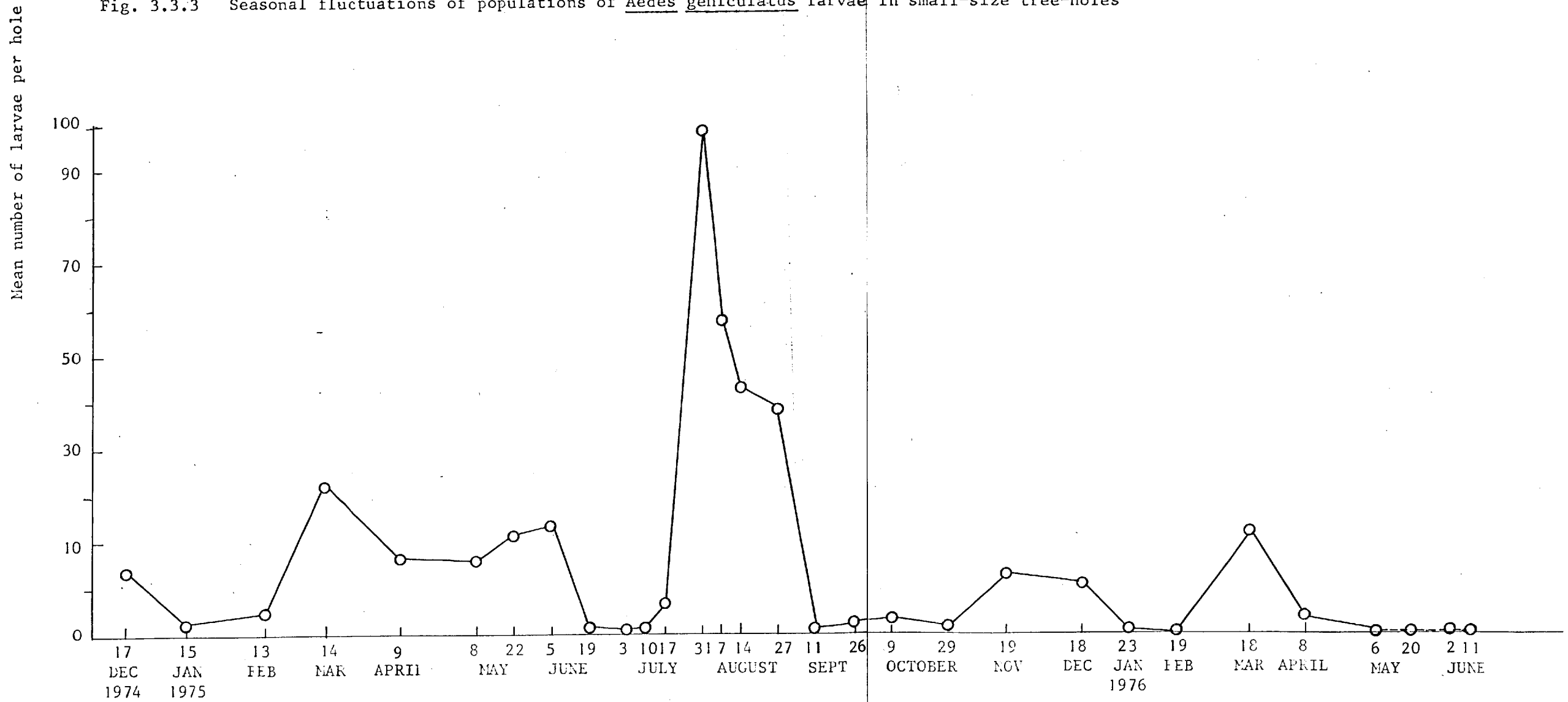
Fig. 3.3.4   Seasonal fluctuations of populations of _Aedes geniculatus_ larvae in medium-size tree-holes
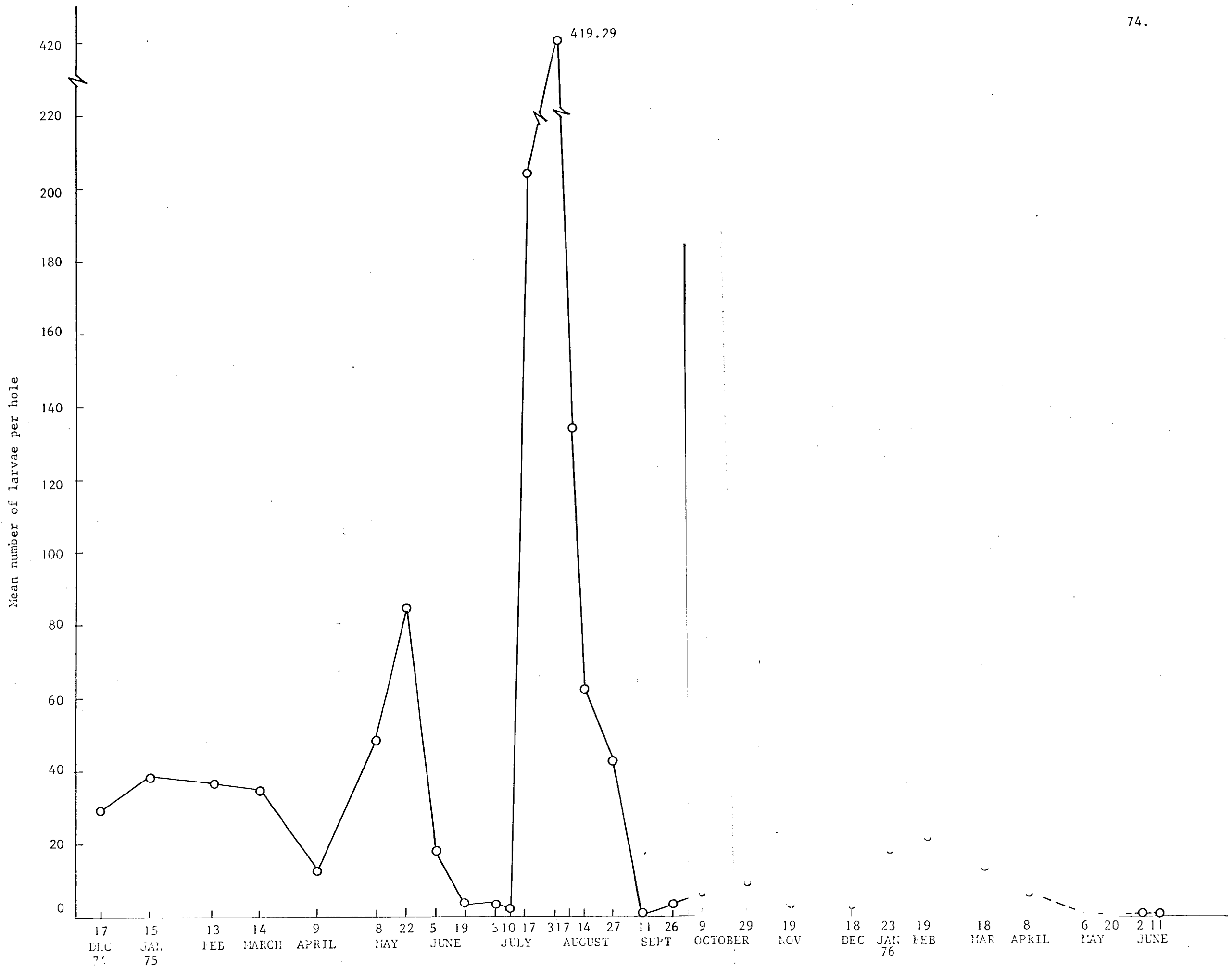
Fig. 3.3.5   Seasonal fluctuations of population of _Aedes geniculatus_ larvae in large-size tree-holes

From January 1976 the larval population began to build up again, with a peak in mid-March. Later, the small holes were seriously affected by the very dry season, and soon became dry leading to the complete loss of larval populations from May to June.

In June, two weeks after artificial watering had been started new larval populations began to build up in some small tree-holes. By 29th June 1976 (18 days after watering) 64 Aedes geniculatus larvae, (46 first and 18 second instar) were sampled in tree-hole 13f and two more (one first and one second instar) in tree-hole 16d.

3.3.6.2   Medium size tree-holes

The larval population in medium size tree-holes rose rapidly from February to a moderate peak in mid-March (Fig. 3.3.4) when temperatures were still very low (1.79 to 9.48°C, Table 2.4.1). Another increase of larval population occurred during May, with a high peak on the 5th June, followed by a drastic decline of the population during June until mid-July.

A third peak the highest in the year, occurred at the end of July and the populations were at high levels until the end of August, when a drastic fall of numbers occurred.

The three peaks of larval population coincided with the sampling occasions when very high proportions of the sampled larvae were in the first instar of development.

The first peak in mid-March was determined by the emergence of larvae on the previous days before the sampling, because 52.3% (see Appendix 3.3.2) of the larvae in the samples from medium sized tree-holes were first instar and 36.4% were second instar.

When the second peak occurred on the 22nd of May, 57.6% of the larvae were first instar and 21.2% were second.

Later, at the end of July, when the highest peak occurred 70.6% of the larvae were first instar while 27.9% were in the second instar.

From September the larval population was at very low levels, with a slight increase during November and December. The first peak of 1976 also occurred in March (90.5% first instar larvae), as in the previous year, but it was smaller probably due to the lack of normal rainfall (15.30 mm in March 1976 compared with 91.50 mm in March 1975, Table 2.4.2).

By April the larval population had declined severely because the tree-holes were drying out during the long drought (March to August): during May and June sampling was not possible because all the tree-holes were dry.

3.3.6.3   Large size tree-holes

During 1975 the estimated larval population in large tree-holes (Fig. 3.3.5) showed two very high peaks. The first on the 5th June,

when the first instar larvae accounted for 41.4% of the samples, and second instar for 34.5%.

Numbers declined during June, when the rainfall was only 11.90 mm (Table 2.4.2) and levels of water in the tree-holes were very low.

In July when rainfall increased to 37.70 mm populations rose rapidly to very high levels; a second peak, the highest of all was reached by the end of July. At that time first instar larvae accounted for 54.2% of the sample and second instar for 41.5%.

Numbers remained high until the end of August, but were drastically decreased by the beginning of September.

From September to December, the level of the populations was very low, and were mostly overwintering third and fourth instar larvae.

Despite the very little rainfall a small peak occurred on the 19th of February 1976, but the populations then declined until the beginning of April: again no sampling was then possible because the tree-holes were dry.

3.3.7   Pupal Populations

No pupae occurred in the samples before June (Table 3.3.2), and they were found mainly from June to August.

However, some pupae were collected during September and October

TABLE 3.3.2   Aedes geniculatus pupae found during the sampling
programme 1974-1976

Date	Tree-hole No.	No. of pupae per sample
a) Small size tree-holes		
5 June 1975	27d	1 ♂
19 June 1975	26a	1 ♂
31 July 1975	26a	1 ♀
7 August 1975	26a	1 ♀
14 August 1975	16d	1 ♂
27 August 1975	27d	1 ♀
9 August 1976	13f*	1 ♂
b) Medium size tree-holes		
5 June 1975	1b	4 (2♀, 2♂)
19 June 1975	13a	2 ♂
3 July 1975	7b	1 ♀
3 July 1975	13a	1 ♀
10 July 1975	13a	1 ♀
14 August 1975	7b	1 ♂
9 October 1975	26b	1 ♀
6 September 1976	25*	1 ♀
c) Large size tree-holes		
19 June 1975	1c	1 ♀
31 July 1975	12a	1 ♀
7 July 1976	13e*	1 ♂
15 July 1976	13e*	1 ♂
6 October 1976	13c*	1 ♀

* Tree-holes watered artificially after June 1976

of both years. The numbers of pupae sampled during 1976 was very small (4) because from May to June almost all of the 32 tree-holes were dry. After June, 16 of the tree-holes were dry during the whole summer. While the other sixteen were watered artificially from June to December and the populations were able to achieve some measure of recovery.

Adults in the field were found only during July and August; few adults were observed resting near the tree-holes.

Attempts were made during the summer of 1976 to catch adults in traps attached to six tree-holes (from Set B); only in two traps (tree-holes 3 and 1d) were adults caught (1 female and 2 males).

3.3.8   Larval populations in tree-holes watered artificially during
        the drought of Summer 1976

During the drought of 1976, when all the 32 regularly sampled tree-holes (Set A) had been dry from early May to early June, the set was divided at random in two sets, one for each size category as follows:

Set A-1	Set A-2	Set A-1	Set A-2	Set A-1	Set A-2
Small size group		Medium size group		Large size group	
13f	10	7b	1b	1c	13h
16c	14a	12b	6	12a	16a
16d	24b	17b	13a	13c	17a
27d	26a	18b	13g	13e	18a
		20a	15a		
		25	15b		
		26b	20b		
		27c	23b		

Set A-1 was watered artificially twice per week from 11th June to mid-December, in order to keep the water level in these holes at the maximum.

Tree-holes of Set A-2 were not watered, however, there was so little rain from June to August (Table 2.4.2) that these holes continued dry until mid-September. In mid-September all tree-holes studied (Set A-1 and Set A-2) were again filled by rain and remained water-filled until December 1976 when the sampling programme was ended.

After the tree-holes of Set A-1 were watered, they were checked twice weekly for larvae. It was not until 18 days after watering that first instar larvae appeared in the samples.

By the 29th June 1976, larvae were found in nine of the sixteen watered tree-holes (Appendix 3.3.6). 57.6% were first instar and 48.4% second instar.

.The number of larvae per actual maximum volume in each of the 16 tree-holes was calculated (Appendix 3.3.7) and also the mean number of larvae per hole for each tree-hole size category. (Table 3.3.3)

Peak larval populations occurred on the 29th June (Table 3.3.3) and on the 30th July in small and medium-size tree-holes respectively. Small tree-holes had smaller peak at the end of July.

The populations level reached in these occasions were much higher than during 1975. From August to December the larval populations returned

TABLE 3.3.3   Mean estimated number of larvae of <u>Aedes geniculatus</u>

per actual maximum volume of water in 16 tree-holes

watered artificially

Date of sampling	Mean number per size category tree-holes		
	Small size	Medium size	Large size
29 Jun. 1976	107.9	81.7	245.8
7 Jul. 1976	73.3	0.0	468.1
15 Jul. 1976	15.0	0.0	555.8
30 Jul. 1976	31.0	172.6	292.2
9 Aug. 1976	5.0	16.0	40.0
24 Aug. 1976	1.3	2.0	54.6
6 Sep. 1976	3.3	10.0	120.9
6 Oct. 1976	0.0	0.0	13.3
10 Nov. 1976	2.2	5.8	0.0
15 Dec. 1976	0.6	4.0	0.0

to similar low level as in 1975, and the larvae in the samples were mostly fourth instar (Appendix 3.3.6).

Medium size tree-holes had one peak at the end of June, but with the highest at the end of July, both mainly accounted for by the presence of first and second instar larvae. From August to the end of the year the population decreased to low levels, with fourth instar larvae the predominating age group.

It seems that because during this period of artificial watering the small and medium tree-holes were kept at their maximum level of water, almost all the eggs laid the previous year, and remaining un-hatched after the low level hatch during Spring (Figs. 3.3.3 and 3.3.4) were able to hatch during the first month of artificial water-ing. Hence the very high larval populations in some of the tree-holes (Appendix 3.3.6) compared with the previous year.

Large size tree-holes produced large populations of larvae from the end of June to the end of July, with a peak by mid-July. A moder-ate larval population was produced during August and September, while no larvae at all were sampled in large tree-holes during November and December, probably because remaining overwintering larvae were in a very low number or the few larvae hatched in early September completed development or die before winter.

The tree-holes from Set A-2 were dry throughout the whole period from June to mid-September, from there on with the restarting rain all of them were water filled by the October sampling. On that occasion tree-holes 1b, 13g and 16a contained 7, 1 and 2 first and second instar

Aedes geniculatus larvae respectively. Later in November only 2
fourth instar larvae were found in tree-hole 13a, but no larva in
December sample.

The scarcity of larvae in these rain filled tree-holes could
be explained by the low temperatures not favouring hatching. Even
so larger numbers might have been expected and the full reasons for
the differences are unknown.

3.3.9 Discussion

During the period of this work 1974 to 1976, Aedes geniculatus
larvae occurred in variable, but in general moderate, densities in the
beech woods of Silwood Park. Thirty-two beech tree-holes were monitored
throughout the period and some additional tree-holes were sampled
occasionally.

Large differences in the size of larval populations were found
in the three groups (small, medium, large) of tree-holes studied.

The large size tree-holes had the highest peak population and
in general the level of population was always higher than in medium and
small size tree-holes.

Factors, such as attraction of the tree-holes to the ovipositing
females, nutritional quality and quantity of the organic matter in the
tree-hole water, permanency of the water in the holes, related to the
size of the tree-hole, could have accounted for the differences.

Because large water-filled tree-holes are more permanent
habitats, the larval populations in them are more protected against
drastic changes in the level of water. It seems also that large
holes have more organic matter in them making this group of tree-
holes more adequate for the establishment of larval populations of
Aedes geniculatus and possibly are more attractive to ovipositing
females.

In all the size-groups of tree-holes the first instar larvae
begin to appear by mid-March, and to increase by May, but the
highest larval populations were noted by the end of July of 1975.
This was probably because the increase in rainfall during that month
which accompanied by summer temperatures, allowed eggs to hatch which
were not submerged during June when there was little rain.

Between September and the next spring the larval populations
were low and only a few third and fourth instar larvae remained in the
tree-holes; hatching does not occur during these winter months.

Although the study was carried out in two very dry years, it
seems that the observed levels of populations of Aedes geniculatus in
tree-holes are typical for this region of England. Kitching (1969b)
also reported very low levels of population for Aedes geniculatus
larvae in Wytham Woods, Oxford. Service (1969b) in 1964 caught a
total of 41 Aedes geniculatus adults at bait catches from June to
August in Brownsea. During 1965 and 1966, dry years, the adults
caught were only 2 and 1 respectively, during the same period of time
as in 1964.

The usually low numbers of larvae of Aedes geniculatus recorded in the field suggest that limiting factors, such as poor food supply and certainly the drought, kept the population at a low level during the period of study.

Kitching (1969b) considered the water-filled tree-holes as semipermanent habitats, which potentially, can persist for the whole life of the mature tree.

However, because they rely on water from rainfall, prolonged dry periods can cause drastic effects on the development of the mosquito larval population.

High larval mortality was noticed, mainly during the first instar. After high counts of first instar larvae the number of second instar the following week were markedly small.

Pupae, collected mainly from June to August, were always in very small numbers, thus the adult abundance in the area would be very low.

SECTION 4

LABORATORY STUDIES OF Aedes geniculatus

4.1   INTRODUCTION

In the mosquito literature there is only a small number of
references dealing with the bionomics of Aedes geniculatus.

Most of the papers report general accounts of the life history,
biology, seasonal incidence and geographical distribution;   among
them are: Marshall and Staⱡley (1929), Beattie and Howland (1929),
Macan and Tutin (1932), Keilin (1932), Marshall (1938), Clastrier
(1955), Kitching (1969 and 1971), Rettich (1971), Horsfall (1972)
and Service (1974).

Some other papers have been published which deal with more
specific aspects of the biology of this species. MacGregor (1932)

presented data of oviposition and hatching. Oviposition in the field was studied by Kitching (1969band 1971), feeding behaviour and host preferences were investigated by Service (1971a), while flight periodicity and vertical distribution was investigated by Service (1971b).

This section describes a general study of the bionomics of the different developmental stages of <u>Aedes geniculatus</u>. Reproductive aspects such as preoviposition period and number of eggs per batch are also described.

The series of experiments were done under constant temperature and light regime conditions. Small containers and low densities were used for larval rearing in all the experiments with the aim to relate the laboratory information with the natural situation of mosquito breeding in small volumes of water such as in the tree-holes in the field.

4.2   MATERIALS AND METHODS

4.2.1   Experimental Populations

The experimental populations came from larvae (mainly first instar) collected from beech tree-holes (Set B) naturally rain-filled at Silwood Park woodlands.

4.2.2   The Rooms

The experiments were carried out in two controlled environment rooms at constant temperatures of 10 and 20°C and with 16 hours light per day provided by flourescent tubes.

For comparison with conditions closer to those occurring in the field observations were made in the laboratory with variable temperature, and daylight through glass windows.

4.2.3   Larval Cages (Fig. 4.2.1)

Circular transparent plastic boxes (10.5 cm top diameter, 9.5 cm bottom diameter, and 4.5 cm height) were used as standard larval rearing containers. The lids had a 3 cm diameter hole in the centre, covered with terylene net to provide ventilation. 100 cm^3 of tap water were used in each container providing a water surface of 78.50 cm^2.

4.2.4 Larval Culture Medium

A mixture of 6 mg powdered dry yeast (Yestamin) and 4 mg dog biscuits (Spillers) per larva was used in 100 cm^3 of tap water, as the nutritive medium for densities between 10 and 16 larvae per container. The dog biscuits were ground in a fine mill. The grist was then sieved through a fine nylon mesh, so that the finest powder was separated and used with the yeast.

The solutions were prepared in litre quantities and incubated at 25°C for 24 hours, and then were transferred to the temperature at which the larvae were to be reared.

4.2.5 Pupal-cages (Fig. 4.2.2)

The containers used were circular transparent plastic cups (4 cm top diameter, 3 cm bottom diameter and 3.5 cm high) covered with a piece of terylene net held in position by two elastic bands. 10 cm^3 of tap water were used in each container in which only one pupa was placed.

4.2.6 Adult-cages

Two types of cages were used (A and B); the first for stock cultures and the second one for isolated females.

(a) Type A (Fig. 4.2.3) : Rectangular shaped cages of 18 x

Fig. 4.2.1 Larval cage

- 10.5 cm
- terylene net
- circular hole
- plastic lid
- 100 cm³ culture medium
- plastic cage
- 4.5cm
- 9.5 cm

Fig. 4.2.2 Pupal cage

- 4 cm
- terylene net
- elastic bands
- water
- plastic cage
- 3.5 cm
- 3cm

Fig. 4.2.3 Adult cage Type A

- 18 cm
- 20 cm
- transparent perspex
- terylene sleeve
- 28 cm
- sliding floor
- terylene net
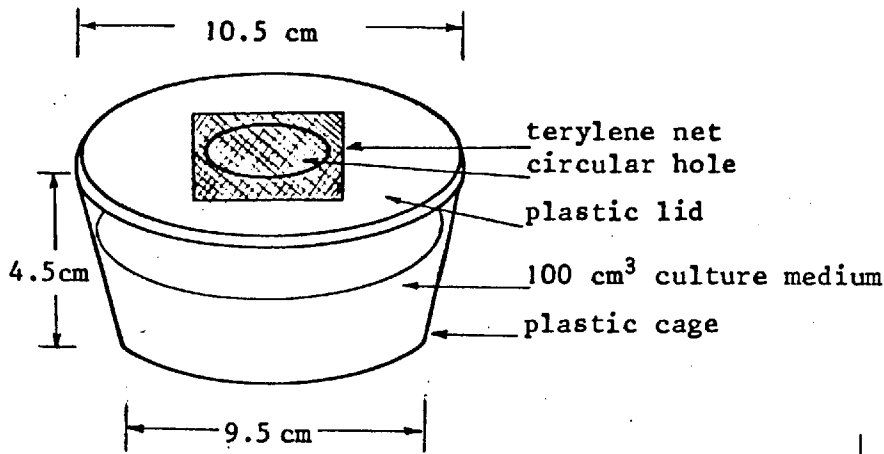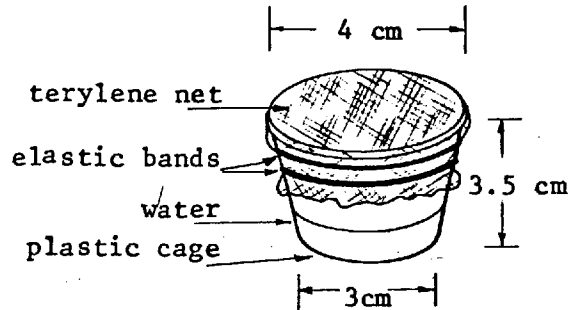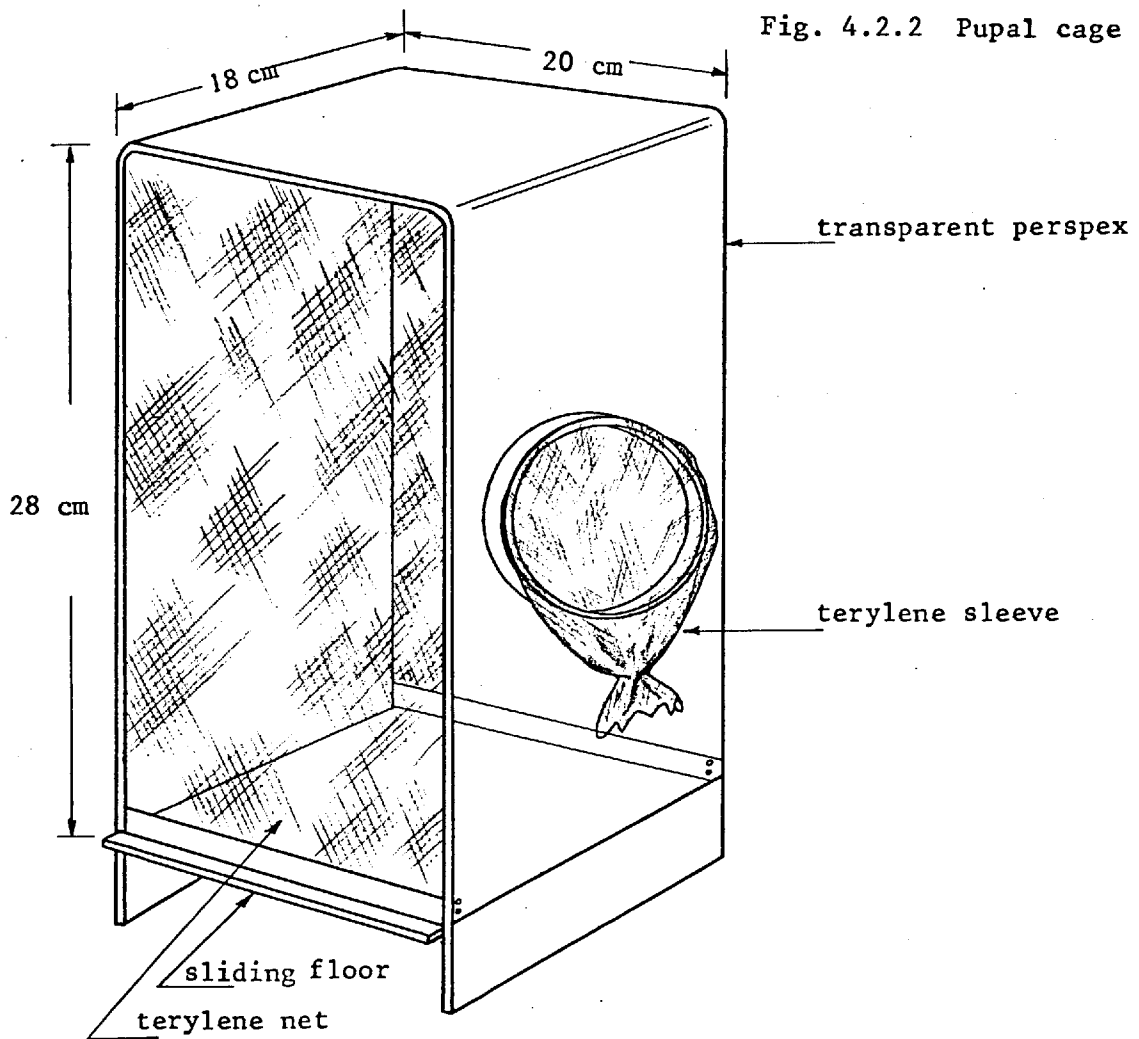
28 x 20 cm, with floor, top, front and back sides of transparent
perspex, and the two lateral sides of terylene net. A circular open-
ing (12 cm diameter) in the front held a terylene sleeve. Each cage
was provided with a feeding container and an oviposition container
(to be described later).

(b)   Type B (Fig. 4.2.4) : Individual adult cages were used
for isolated females after blood feeding. Each cage consisted of a
white paper cup (170 cm capacity) with thé top covered with a piece
of terylene net, held in position by two elastic bands. A circular
opening was made at the centre of the bottom, where a small ovi-
position container was held.

A cotton dental roll moistened with 10% sugar solution was
placed on the net and changed every 24 hours. The whole unit was
supported in a plastic cup (6 cm top diameter) to provide stability.

4.2.7   Adults Sugar Feeding Units (Fig. 4.2.5)

The containers used were glass tubes (30 cm^3 capacity) with 20
cm^3 of 10% sugar solution and a filter paper cylinder projecting 5 cm
out of the tubes. A piece of cotton wool was inserted into the top
of the cylinders to close the openings, so preventing mosquitoes
falling into the sugar solution. The tubes were inserted in thick
cork rings for stability.

Each adult-cage type A was provided with one of these units,

terylene net

elastic bands

paper cup

oviposition cage

plastic cup

Fig. 4.2.4 Adult cage Type B

cotton wool

sugar solution

filter paper cylinder

ring of cork
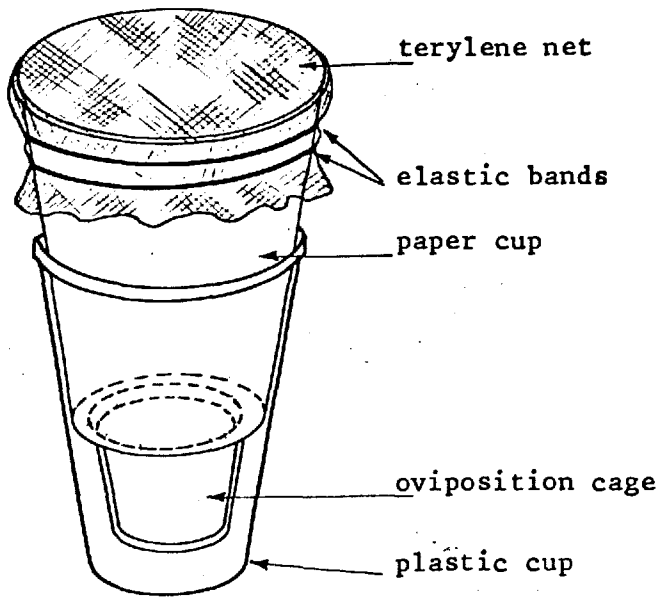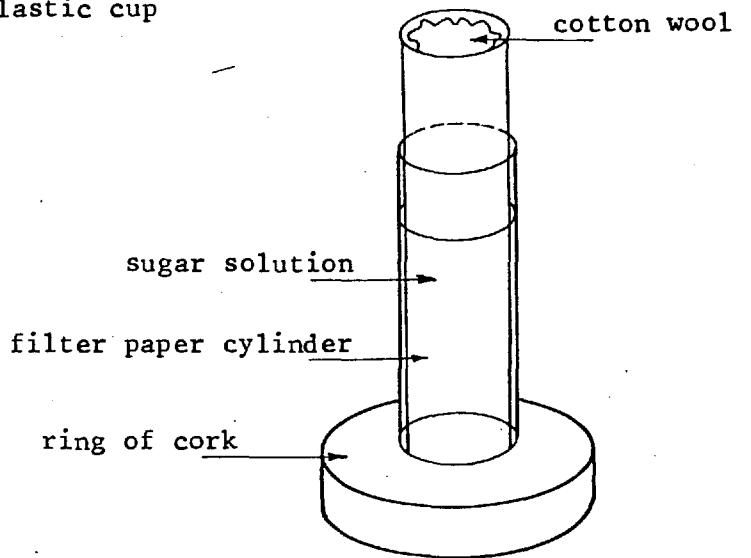
Fig. 4.2.5 Sugar feeding unit

filter paper band

water

plastic cage
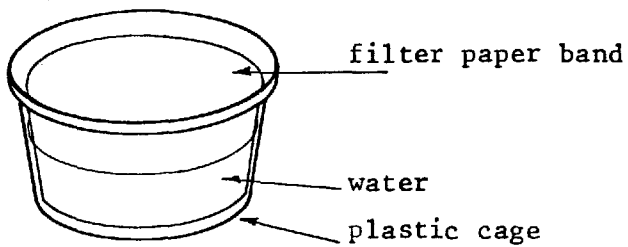
Fig. 4.2.6 Oviposition cage

and as a routine, sugar solutions and filter paper were changed for new ones every three or four days.

4.2.8 Blood-feeding of Females

For routine blood-feeding of cultures in the laboratory an anaesthetized rabbit was used, but on many occasions the females were fed on the author's hands.

For feeding on the rabbit : first, the sugar feeding unit and oviposition cage were removed from the adult cage type A which was then turned onto one side, so the rabbit could be laid on the terylene net of the cage, allowing access of the mosquitoes to the ventral rabbit skin. The rabbit was kept in that position for 30 minutes.

For feeding on the author's hands : the left hand was placed into the adult cage type A for 30 minutes, the sleeve of the cage was kept tight around the wrist. If the females were reluctant to feed, isolated individuals were placed in transparent plastic tubes (3 cm length and 2 cm diameter) with both ends covered with terylene net, the tubes were then attached to the left fore arm with elastic bands for 30 minutes.

4.2.9 Oviposition Cages (Fig. 4.2.6)

The containers used were circular plastic cages with filter

paper bands lining the inner-side. For the adult-cages type A, the oviposition-cages were 70 cm^3 capacity, with 40 cm^3 of tap water, and for the adult-cages type B, they were 35 cm^3 capacity with 20 cm^3 of tap water.

4.2.10 Humid Chambers

Circular transparent plastic containers the same size as used for larval cages (Sec. 4.2.2) were provided with a thick layer of damp cotton wool at the bottom. The cotton wool was covered with two fitting filter papers upon which the filter paper bands with eggs from the oviposition cages were placed 24 hours after the eggs being laid. A tight fitting plastic lid was used to ensure humid conditions in the chambers.

4.2.11 Methods of Rearing

Several attempts were made to maintain a breeding culture of Aedes geniculatus in the laboratory. In every case only one generation was achieved because it proved impossible to break the diapause of the eggs. Thus insects were available for experimentation only when larvae were brought in from the field (Set B of tree-holes) and reared in the laboratory.

Of the several rearing methods tried the most successful was the following:

Larvae brought from the field were kept in a 20°C constant temperature room, with 16 light hours per 24-hours day. Each culture was set up with 10 to 16 larvae placed in a larval cage (Sec. 4.2.2) with 100 cm³ of tap water and 10 mg solid food per larva (Sec. 4.2.4).

Pupae were removed from the larval cages every 48 hours, tentatively sexed, counted and placed into individual pupal cages (Sec. 4.2.5) with 10 cm³ of tap water; these were kept in the same room as the larvae and adults.

The adults were placed in the adult-cages type A (Sec. 4.2.6) the first day after emerging and were provided with sugar feeding units and oviposition cages.

Twice weekly an anaesthetized rabbit was offered to the females for a blood meal and the filter papers from the feeding units (Sec. 4.2.7), and the oviposition cages (Sec. 4.2.9) were replaced with new ones, and both containers refilled.

The eggs oviposited in the filter paper were removed from the cages and kept in the humid chambers (Sec. 4.2.10) for two weeks before any treatment was applied with the intention of breaking the eggs' diapause.

4.3   THE EGGS

The eggs of a number of English species of _Aedes_ were described by James (1922, 1923) and Marshall (1938).

The eggs of _Aedes geniculatus_ are elongate oval and about a millimetre in length with the anterior end thicker and more tapered than the posterior end. When first deposited they are pale grey coloured, but after a few hours they become black.

In general the eggs were laid at the water level on the fold of the filter paper lining the walls of the oviposition cages; only on a few occasions were eggs found on the surface of the water or at the bottom of the container, this probably happens when the female is disturbed while egg-laying.

Usually the eggs were deposited in short lines or small clusters.

4.3.1   Egg Size

The length and the greatest width of 20 eggs taken at random from the stock cultures were measured and the results are given in Table 4.3.1.

TABLE 4.3.1   Size of Aedes geniculatus Eggs

No. of eggs measured	Length (mm.)			Width (mm.)		
	Mean	S.D.	Range	Mean	S.D.	Range
20	1.04	0.02	1.00 - 1.07	0.34	0.01	0.32 - 0.35

4.3.2   Egg Diapause

In univoltine species diapause is usually an essential stage in individual development, but in multivoltines it is facultative, appearing only in certain generations under the influence of specific external conditions (Danilévskii, 1965).

Some Aedes species may have multiple generations (multivoltines) per year, others apparently have two, one in the spring and a second in the autumn, and others have only one per year (univoltines).

Facultative diapause of the eggs as known in multivoltine Aedes species usually appears when the eggs are subjected to unfavourable environmental conditions, but eggs will hatch at any time of the year if conditions are favourable.

Other Aedes species have an obligatory diapause and their eggs will hatch under favourable conditions only when diapause is broken.

Attempts by several authors to induce the eggs of Aedes geniculatus to hatch have produced erratic results. MacGregor (1932) reported hatching of eggs laid in the laboratory by females caught in

the field in August and fed on human or avian blood. However, no
larvae resulted from eggs laid by females which had emerged in the
laboratory and confined with males in large insect cages.

Horsfall (1972) discussed the results from several authors,
and pointed out that hatching in natural sites after a September rain
was reported by Rouband and Colas-Belcour (1938). When debris from a
tree-hole were submerged a second and a third time in the laboratory at $20^{\circ}C$
in October and December egg hatched. However, Rouband (1929)
reported that a group of 10 eggs submerged for two months in tap
water failed to hatch but when transferred 3 weeks later to a pepsin
solution larvae hatched within 48 hours.

Rouband and Colas-Belcour (1945) reported erratic results in
the laboratory. A 60% egg hatch was obtained when 15 eggs on a
piece of bark were dried for 3 months, then frozen at $0^{\circ}C$ for 4 months
and then placed in water from a tree-hole. In water at $25^{\circ}C$ the usual
percentage hatch when the above treatment was used was between 6 to 38%.

4.3.2.1 Experiments

In the present study some experiments were done to determine
whether egg diapause was obligatory or facultative.

The eggs collected in the ovitraps (Section 3.2.3) were exposed
to different regimes of temperatures. Thus, 6 groups of eggs were
held on the wet filter paper bands from the ovitraps in humid chambers
(Section 4.2.10) that were kept at $20^{\circ}C$ and $5^{\circ}C$ (Table 4.3.2).

TABLE 4.3.2   Treatments of <u>Aedes geniculatus</u> Eggs Collected in

the Field Ovitraps

Date of collection	Ovitrap number	Treatment number	No. of eggs treated	Weeks at 20°C	Weeks at 5°C
24 July 1975	12a	1	10	4	0
24 July 1975	12a	2	10	4	4
24 July 1975	27d	3*	13	12	12
12 August 1976	6	4	16	6	0
12 August 1976	7	5*	14	9	9
12 August 1976	13	6*	10	12	12

* Attacked by fungus

The filter paper bands with eggs of treatments 3, 5 and 6 were
attacked by fungus while in the humid chambers.

At the end of the treatments the filter papers with the eggs
were moved to larval cages with 100 cm³ of tree-hole water kept at
20°C and for a period of three weeks each container was checked every
day.

4.3.2.2   Results

No hatching was observed even after the eggs were soaked for
three weeks at 20°C so that none of the different temperature regimes
broke the egg diapause.

Similar treatments were performed with samples of <u>Aedes</u> <u>geniculatus</u> eggs collected from laboratory cultures during August and September 1976; in these cases also none of the eggs hatched.

When these eggs were examined after treatment a high percentage of them were observed to have collapsed. When samples of the eggs not collapsed were bleached with Diaphanol^R they were found to be infertile, with the yolk rounded up in a globular mass. <u>Aedes</u> <u>aegypti</u> eggs from laboratory cultures were treated at the same time in the same way and they showed fully developed larvae with thoracic and abdominal segments clearly shown, and 3 dark spots (ocellis and egg-breaker) at the anterior pole.

This indicates that probably the laboratory mating of <u>Aedes</u> <u>geniculatus</u> were not successful.

4.4   LARVAL AND PUPAL DEVELOPMENT

A brief description of the fourth instar larvae has been given in Section 2.6 and measurements of the different larval instars were already reported in Section 3.3.3.

Detailed studies of biological parameters were done with two groups of newly hatched first instar larvae brought from the field on two different occasions during July 1976.

4.4.1   Experiments

Forty-eight larvae were reared at 20°C in three replicate cultures of 16 larvae each and 33 larvae at 10°C in three replicate cultures of eleven larvae each. The cultures were provided with 10 mg standard solid food per larvae, and 100 cm³ tap water, all were kept in a constant regime of 16 hours light per day.

Each larval cage was checked every 24 hours. Dead larvae were removed from each cage and counted, also pupae were removed, sexed and placed into individual pupal cages, which were also checked every day until the adults emerged.

In the laboratory observations were made of newly hatched first instar larvae brought from the field during June and July 1976 when the mean temperatures were 17.6 and 18.6°C. The larvae were reared in standard culture medium at 20 and 15°C. It was observed that the ranges of times for these larvae to moult to second instar were 12 to 36 hours

at 20°C and 24 to 36 hours at 15°C. Therefore, it was estimated that the first instar larvae, which showed characters of newly hatched larvae and were used in the next experiments, were about one day old when the experiments began. Thus the hatching time was estimated as 24 hours earlier than the setting up of the cultures in the laboratory, and was taken into account for the records of developmental times, pupation and adult emergence.

4.4.2   Developmental Time of Larvae and Pupae

The developmental time of any stage was calculated as the time required for 50% of the individuals to moult into the next stage. At 20°C the developmental time ± S.D. for larvae was 15.5 ± 1.6 days for females with a range of 12 to 18 days, and 12.9 ± 2.4 days for males with a range of 11 to 16 days. For pupae the developmental time was 3.7 ± 0.3 days for females and 3.8 ± 0.4 for males, both in a range of three to four days, even although larval period was shorter for males.

At 10°C the developmental time for larvae was 29.0 ± 0.0 days for females, with a range of larval life of 26 to 31 days, and 24.0 ± 2.0 days for males, with a range of 22 to 29 days. For pupae the developmental times were 10.7 ± 0.6 days for females and 9.7 ± 0.6 days for males, both in a range of pupal life of 9 to 12 days.

Table 4.4.1 shows the comparative results of developmental times at 10 and 20°C.

Table 4.4.1   Larval and Pupal Developmental Times of _Aedes_
geniculatus Reared at 10 or 20°C

	10°C		20°C	
	Males	Females	Males	Females
Larval mean developmental time ± S.D. (days)	24.0 ± 2.0	29.0 ± 0.0	12.9 ± 2.4	15.5 ± 1.6
Range of larval life (days)	22 - 29	26 - 31	11 - 16	12 - 18
Number of pupae	14	16	22	18
Pupal mean developmental time ± S.D. (days)	9.7 ± 0.6	10.7 ± 0.6	3.8 ± 0.4	3.7 ± 0.3
Range of pupal life (days)	9 - 12	9 - 12	3 - 4	3 - 4
Number of adults	7	15	20	18

Pupae brought from the field and kept at low temperature (5°C) became dormant but they resumed activity as soon as they were at room temperature (August), mortality was not observed in these pupae, and they produced normal adults after three or four days.

4.4.3   Larval and Pupal Survival

At 20°C only 79.17% of the newly hatched first instar larvae reached the adult stage. The mean larval survival was 83.33% and the mean pupal survival was 95.54%. (Table 4.4.2)

Table 4.4.2   Larval and Pupal Survival of _Aedes geniculatus_ Reared

at 20°C

Replicate	Number of 1st instar larvae	% larval survival (No. of pupae)	% pupal survival (No. of adults)	% overall survival
A	16	87.50 (14)	92.86 (13)	81.25
B	16	100.00 (16)	93.75 (15)	93.75
C	16	62.50 (10)	100.00 (10)	62.50
Mean (%) ± S.D.		83.33 ± 19.09	95.54 ± 3.89	79.17 ± 15.75

There was no mortality of female pupae, and 90.91% of male pupae survived to the adult stage; only two pupae died, one as a young pupa and one during the process of casting the pupal skin.

At 10°C the overall percentage of survival to adult stage was only 66.67%. The mean larval survival was 90.91% and the mean pupal survival was 70.71%. (Table 4.4.3)

Table 4.4.3   Larval and Pupal Survival of _Aedes geniculatus_

Reared at 10°C

Replicate	Number of 1st instar larvae	% larval survival (No. of pupae)	% pupal survival (No. of adults)	% overall survival
A1	11	100.00 (11)	72.73 (8)	72.73
B1	11	81.82 (9)	66.67 (6)	54.55
C1	11	90.91 (10)	72.73 (8)	72.73
Mean ± S.D.		90.91 ± 9.09	70.71 ± 3.50	66.67 ± 10.50

4.4.4   Pupation

Pupation began 11 days after the eggs hatched and lasted for 8 days at 20°C (Fig. 4.4.1). 76.19% of the males pupated during the first two days with female pupation beginning a day later and was most numerous (47.37%) during the fourth and fifth days.

At 10°C pupation began 22 days after the eggs hatched and lasted for 10 days; females began to pupate 4 days later than males (Fig. 4.4.2).
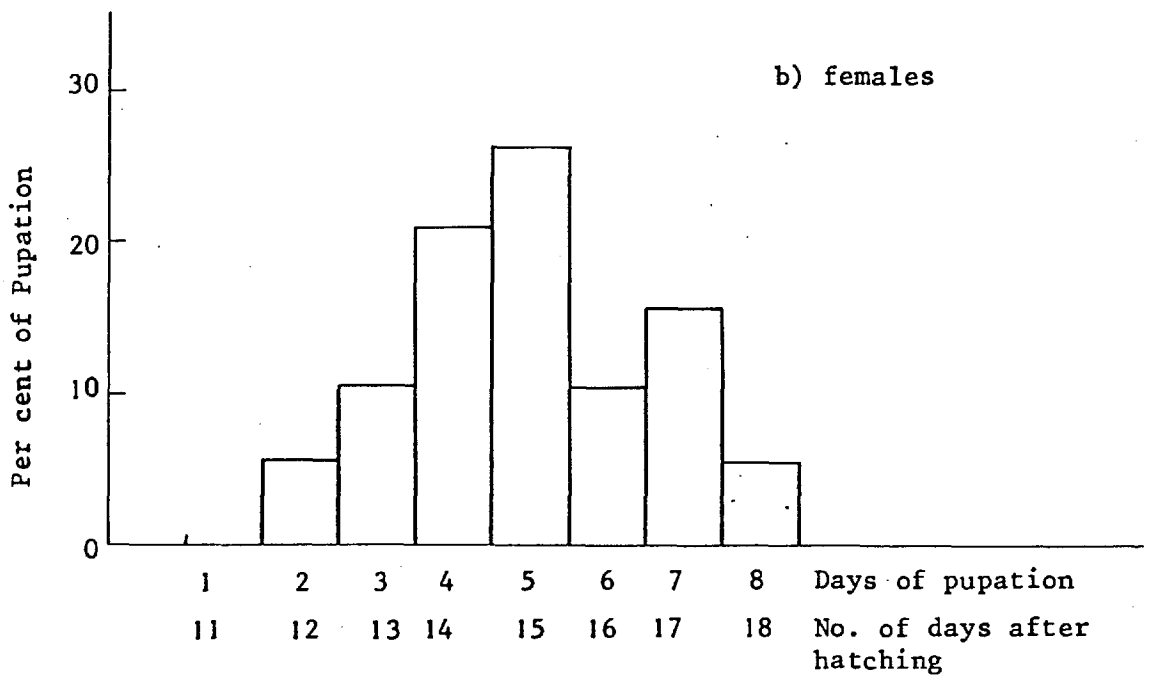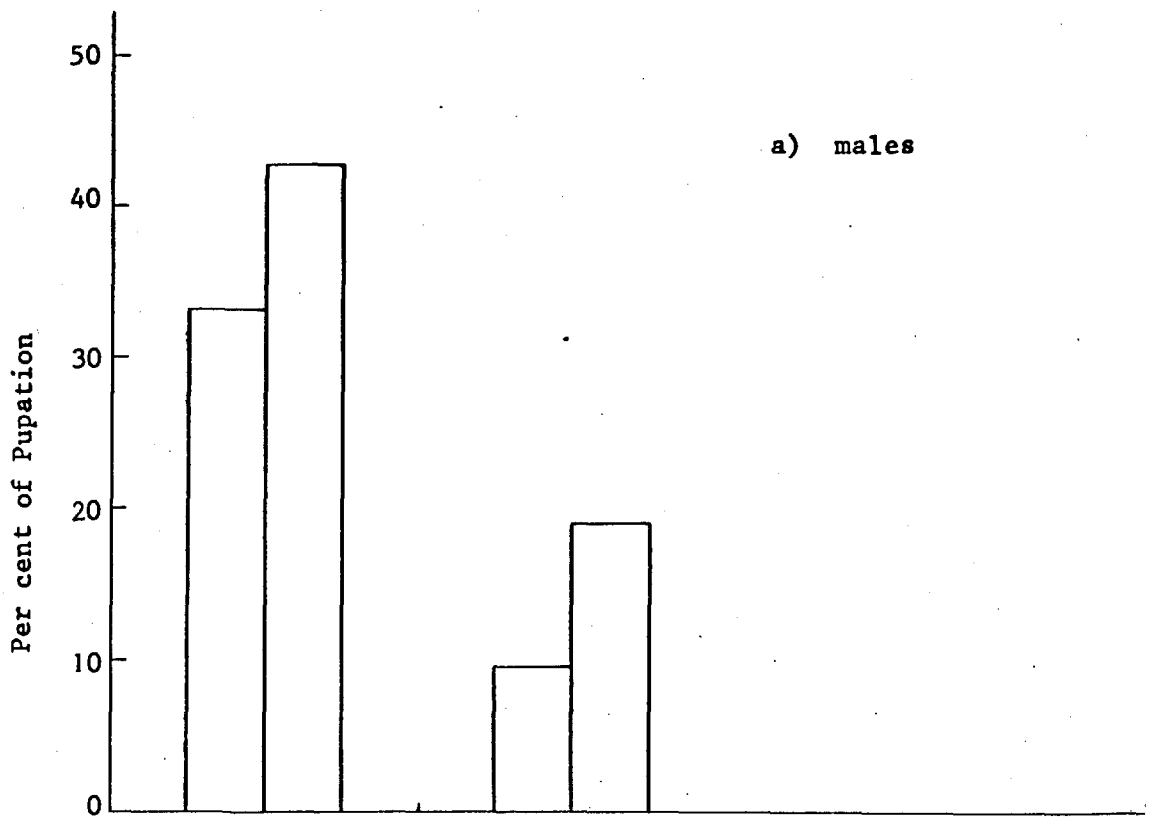
Fig. 4.4.1   Pattern of Pupation of <u>Aedes geniculatus</u> reared at 20°C
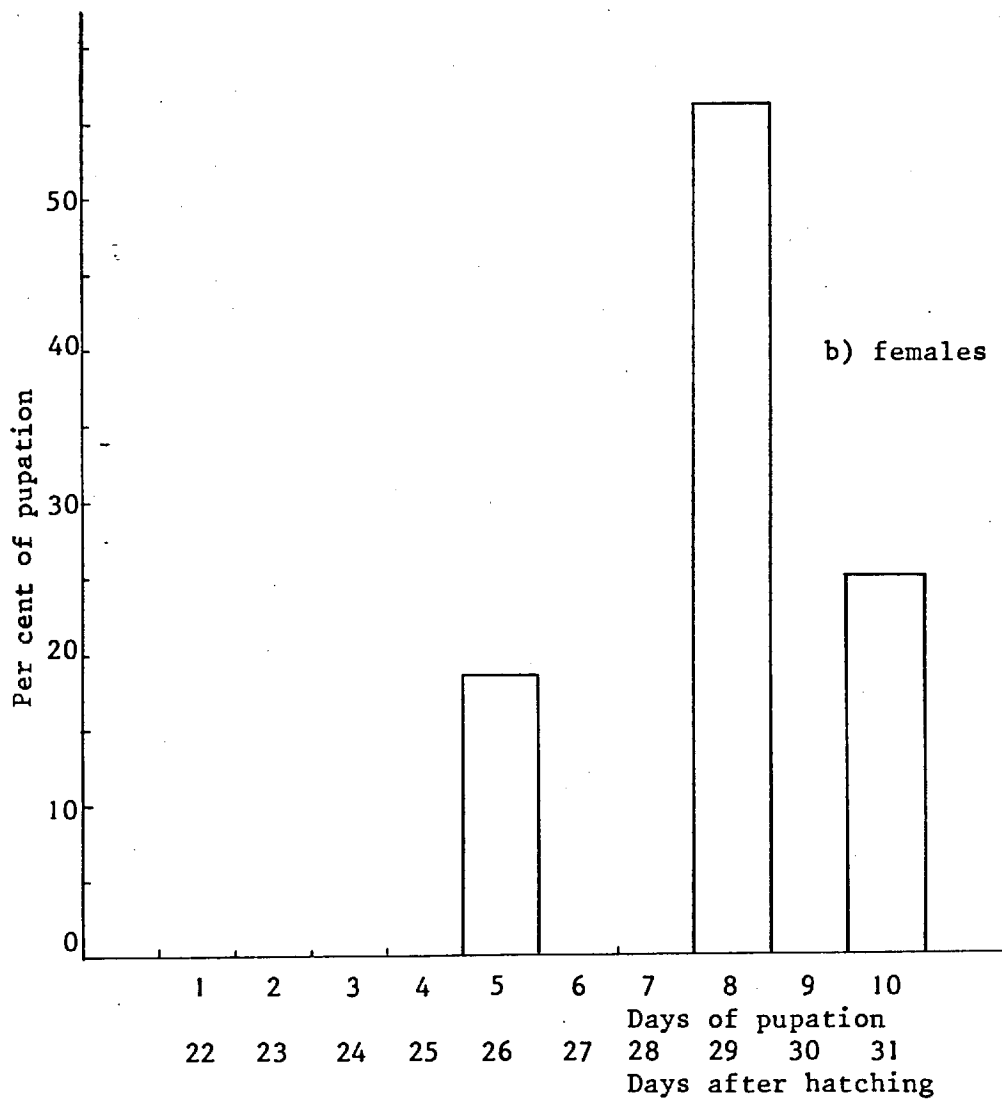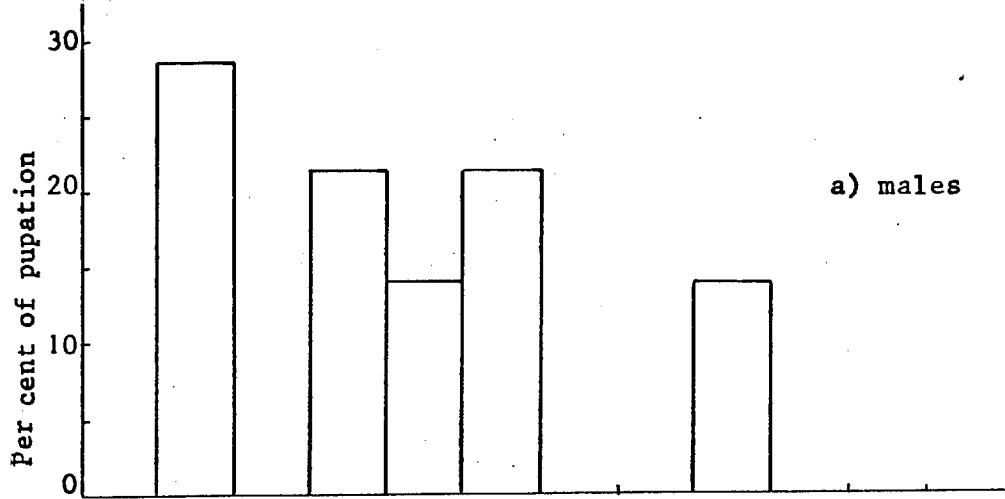
Fig. 4.4.2   Pattern of pupation of <u>Aedes</u> <u>geniculatus</u> reared

at 10°C

4.4.5 Emergence

Emergence of the adults began on the fifteenth day after the
eggs hatched and lasted for 8 days at 20°C. Seventy per cent of the males
emerged during the first two days (Fig. 4.4.3) while females started
to emerge two days after the males and it was a further two days before
a peak of female emergence (50%) occurred.

At 10°C the emergence began 31 days after the eggs hatched and
lasted for thirteen days. Females began to emerge 6 days later than
males (Fig. 4.4.4).
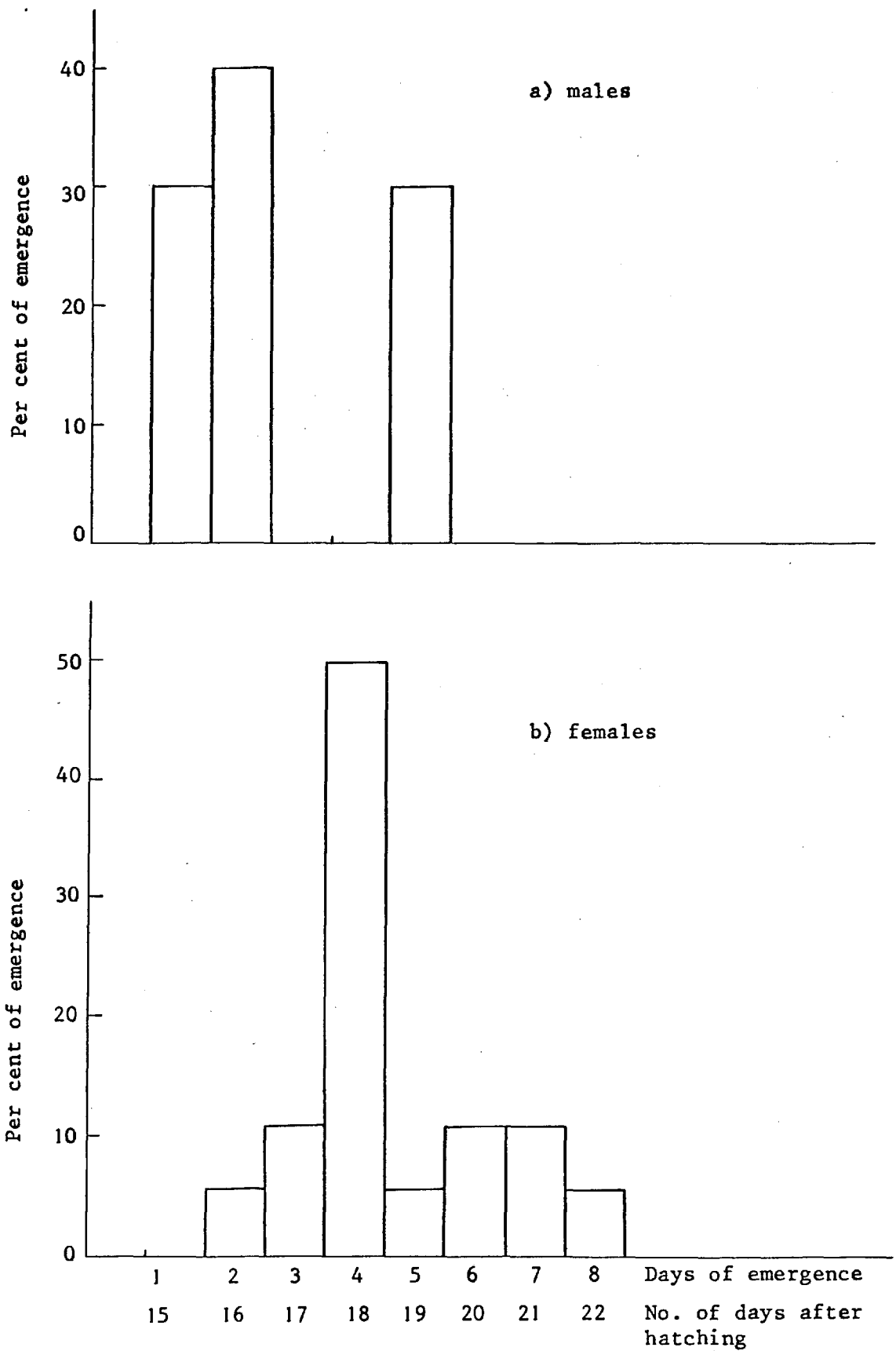
Fig. 4.4.3   Pattern of Emergence of Aedes geniculatus reared at 20°C
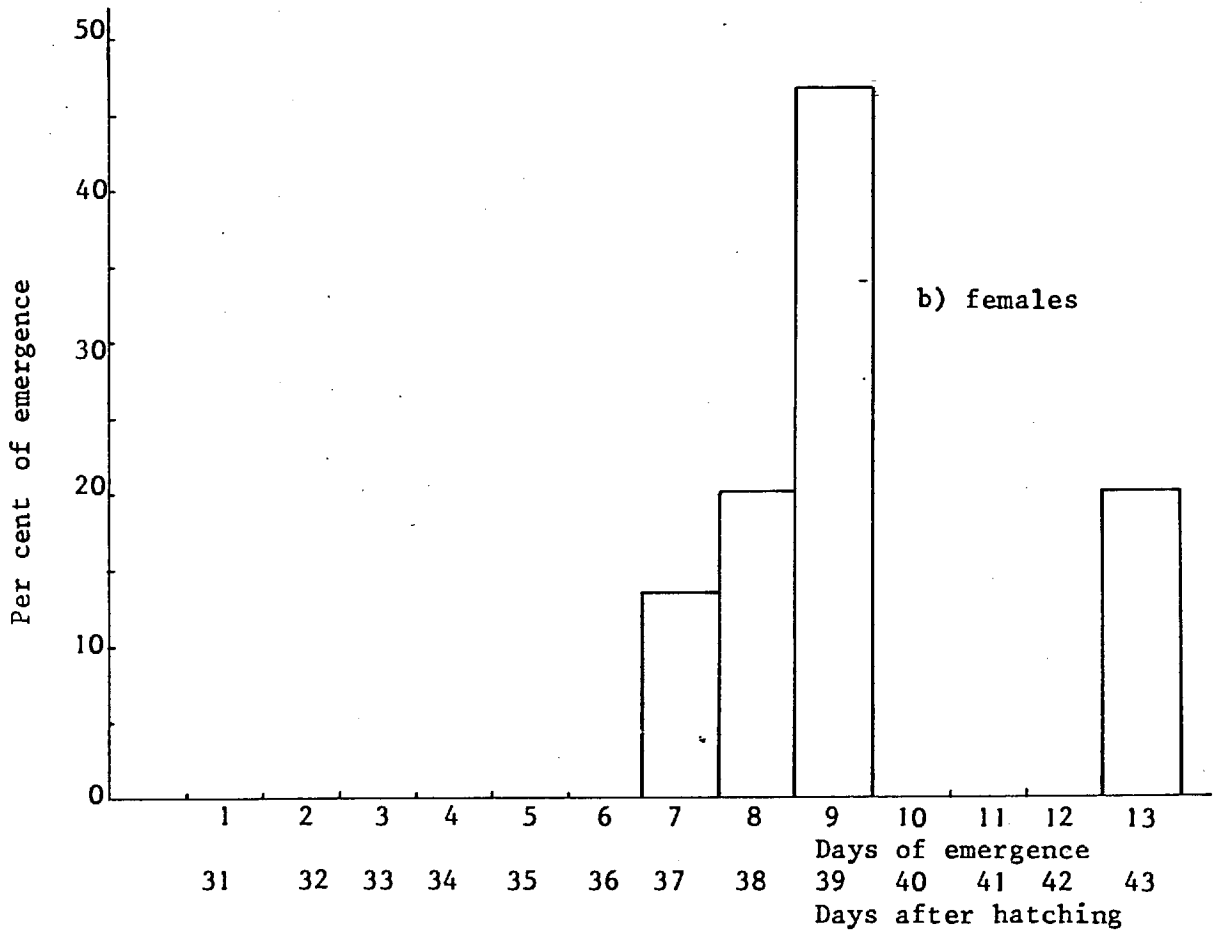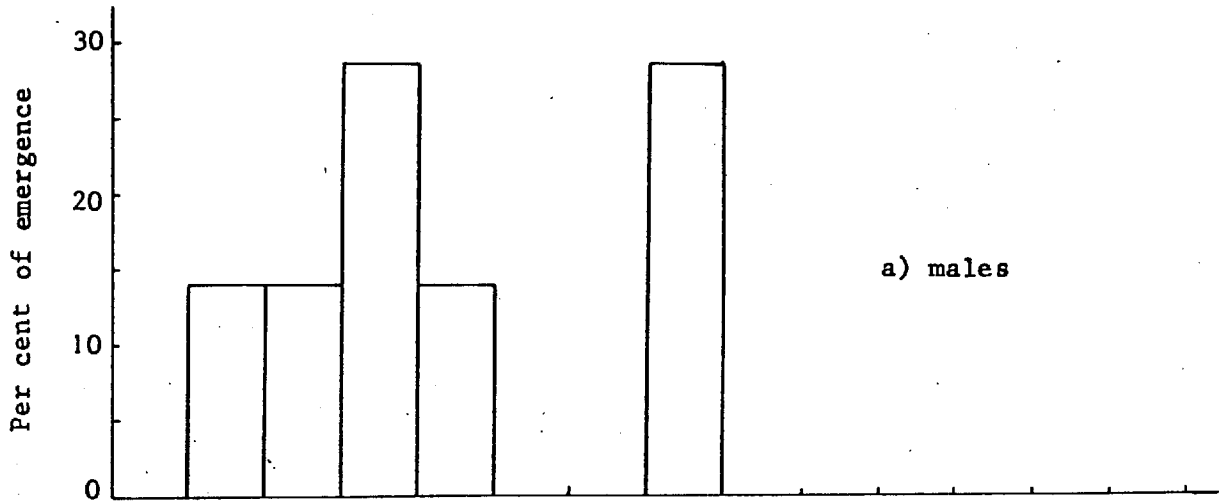
Fig. 4.4.4   Pattern of emergence of <u>Aedes geniculatus</u> reared at 10°C

4.5 ADULT BODY SIZE

The fresh body weight of recently emerged adults and the wing

length were chosen as indicators of body size of _Aedes geniculatus_.

It was considered that under controlled conditions of rearing these

characters would be reliable morphometric parameters to estimate

body size.

It was pointed out by Christophers (1960) that the weight of

adult mosquitoes was the most desirable index of size, but it can only

be used when exact information as to age, condition of feeding,

egg laying, etc. are available. Therefore, it was considered most

satisfactory to use a linear measurement that bears a simple and close

correlation with weight under given conditions. Christophers (_loc. cit._)

considered that wing length was a convenient measurement to fill that

role.

That breeding temperatures play an important role in the deter-

mination of wing length was clearly shown by van den Heuvel (1963).

He found that for mosquitoes reared at only one temperature the ratio

of body dry weight to wing length can be considered constant. The

wings were smaller at high rearing temperatures and larger at low ones,

in relation to body dry weight. Wing length varied more or less

linearly with temperature but thorax length and body weight varied

together in a similar non-linear manner. Also Mer (1937) found dispro-

portionately long wing lengths on mosquitoes bred at low temperatures.

Hosoi (1954) said that _Culex pipiens pallens_ (Coq.) wings, when

compared with body dry weight, were disproportionately short at high breeding temperatures and disproportionately long at low breeding temperatures.

4.5.1 Experiments

Forty pupae (19 females and 21 males) were individually isolated in pupal cages; these pupae were from three replicate cultures of 16 larvae each, reared at 20°C, sixteen hours of light daily, in a nutritive medium of 10 mg. of solid food per larva and 100 cm^3 of tap water. The resulting adults were kept isolated, without food and were anaesthetized, killed and weighed within 24 to 36 hours of emergence.

. Each specimen was individually weighed on a 10 mg. torsion balance, sensivity 0.01 mg. After weighing, the specimens were stored in previously labelled vials provided with glicerinate 70% alcohol, until the measurements of wing lengths were done.

Under a microscope the right wing of each mosquito was removed, and measured with the aid of a $^\times$10 micrometer eye piece and a x2 objective. The measurements were done from the tip, excluding the fringe, to the bend in the trailing edge at the distal end of the alula (Fig. 4.5.1) as indicated by van den Heuvel (1963).

4.5.2 Body Weight

The mean fresh body weight of females was 3.417 $\pm$ 0.474 mg. and
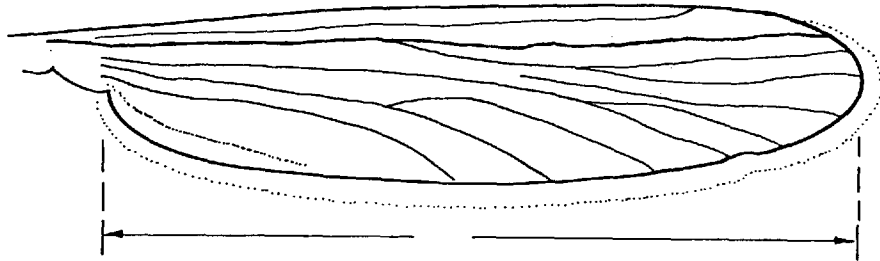
Fig. 4.5.1   Limits used in measurements of Wing

Length of _Aedes geniculatus_

was 1.713 ± 0.190 mg for males (Tables 4.5.1 and 4.5.2). Thus, the fresh body weight of the female was about twice that of the male, with a ratio of female/male mean weight of 1.83, 2.06 and 1.91 in the three groups studied.

4.5.3   Wing Length

The mean length of the right female wing was 5.16 ± 0.20 mm and for the male was 4.01 ± 0.12 mm (Tables 4.5.3 and 4.5.4).

4.5.4   Relationship Between Wing Length and Adult Weight

The cubed wing length in each sex was tested for correlation with fresh weight of one day old adults and the regression of fresh weight on wing length was calculated (Fig. 4.5.2 and 4.5.3).

The correlation coefficients between weight and the cubed wing length were significant ($r_{♀♀} = 0.752$, $r_{♂♂} = 0.569$; $P<0.01$) so it was possible to predict weight by the regression equations:

$$Y_{♀♀} = -0.190 + 0.026 X_{♀♀}$$

and

$$Y_{♂♂} = 0.130 + 0.024 X_{♂♂}$$

where $Y_{♀♀}$ and $Y_{♂♂}$ are the female and male fresh weights respectively and $X_{♀♀}$ and $X_{♂♂}$ are the female and male cubed length of the right wing.

TABLE 4.5.1   The Body Weight of Female Adults of _Aedes_
geniculatus Reared at 20°C

Replicate	Body Weight (mgr.)	Mean (mgr.)	Standard Deviation
A	3.76		
	3.18		
	3.10		
	3.39		
	2.60		
	3.24	3.206	0.371
B	3.28		
	4.38		
	3.54		
	3.18		
	4.20		
	3.78		
	3.24		
	3.98		
	3.52	3.677	0.434
C	3.26		
	3.30		
	2.58	3.046	0.404
TOTAL		3.417	0.474

TABLE 4.5.2   The Body Weight of Male Adults of *Aedes*
geniculatus Reared at 20°C

Replicate	Body Weight (mgr.)	Mean (mgr.)	Standard Deviation
A	1.84		
	1.82		
	1.72		
	1.56		
	1.62		
	1.95		
	1.74	1.750	0.133
B	1.86		
	2.10		
	1.76		
	1.76		
	1.78		
	1.46	1.786	0.205
C	1.46		
	1.24		
	1.74		
	1.74		
	1.72		
	1.68	1.596	0.204
TOTAL		1.713	0.190

TABLE 4.5.3   Length of the Right Wing of Female Adults of

Aedes geniculatus Reared at 20°C

Replicate	Wing Length (mm.)	Mean (mm.) ± S.D.
A	5.18	
	5.10	
	5.05	
	5.25	
	4.86	5.075
	5.00	± 0..137
B	5.23	
	5.30	
	5.33	
	5.12	
	5.48	
	5.38	
	5.16	
	5.39	5.293
	5.25	± 0.115
C	5.10	
	5.18	4.963
	4.61	± 0.308
TOTAL		5.165 ± 0.204

TABLE 4.5.4   Length of the Right Wing of Male Adults of

Aedes geniculatus Reared at 20°C

Replicate	Wing Length (mm.)	Mean (mm.) ± S.D.
A	4.16	
	4.12	
	3.96	
	3.98	
	3.72	
	4.20	4.027
	4.08	± 0.658
B	4.14	
	4.18	
	4.06	
	4.04	
	4.10	4.070
	3.90	± 0.977
C	3.92	
	3.86	
	4.03	-
	4.00	
	3.91	3.936
	3.90	± 0.064
TOTAL		4.013 ± 0.125
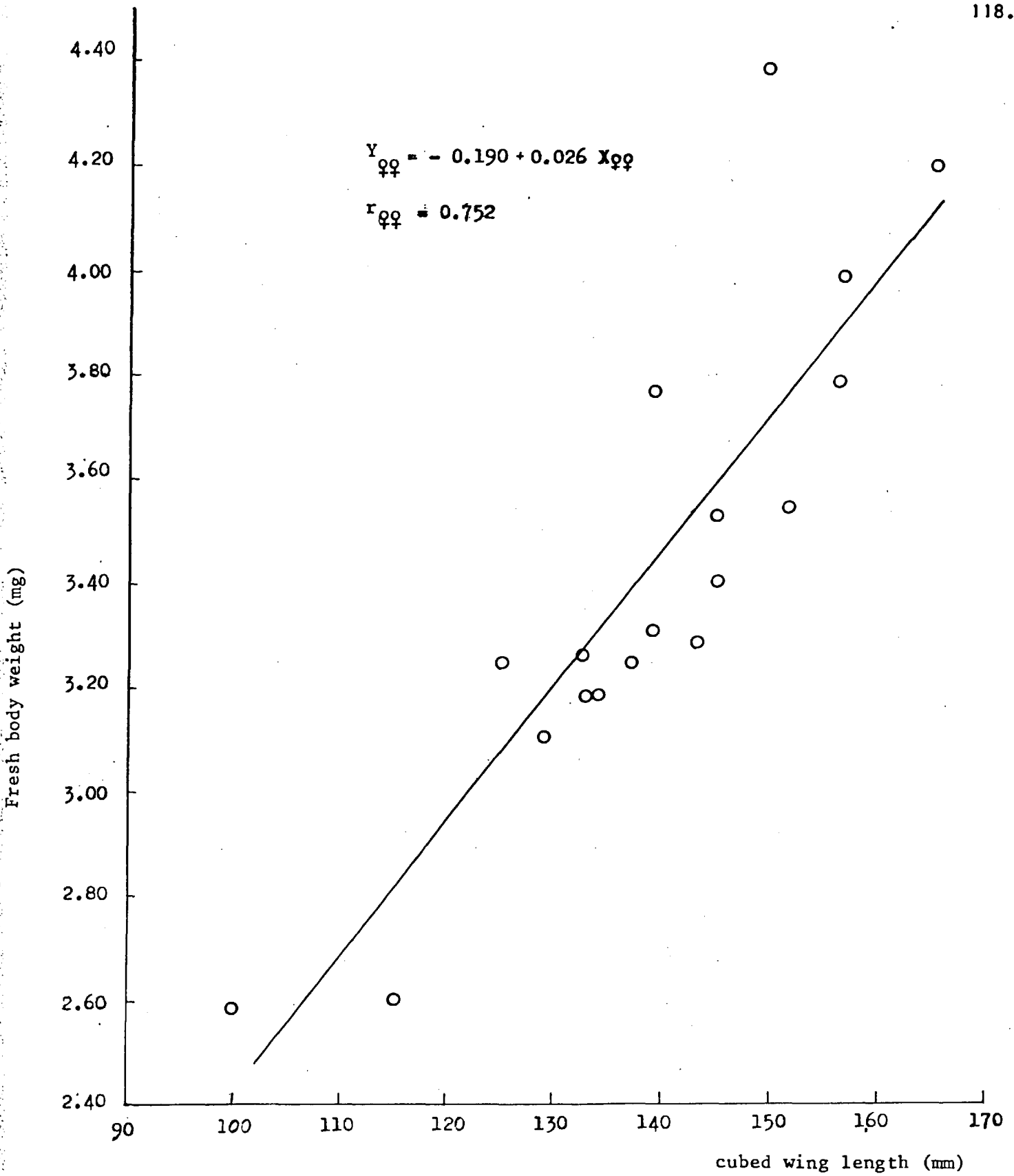
Fig. 4.5.2   Relationship between cubed wing length and weight of <u>Aedes</u>

<u>geniculatus</u> females reared at 20°C
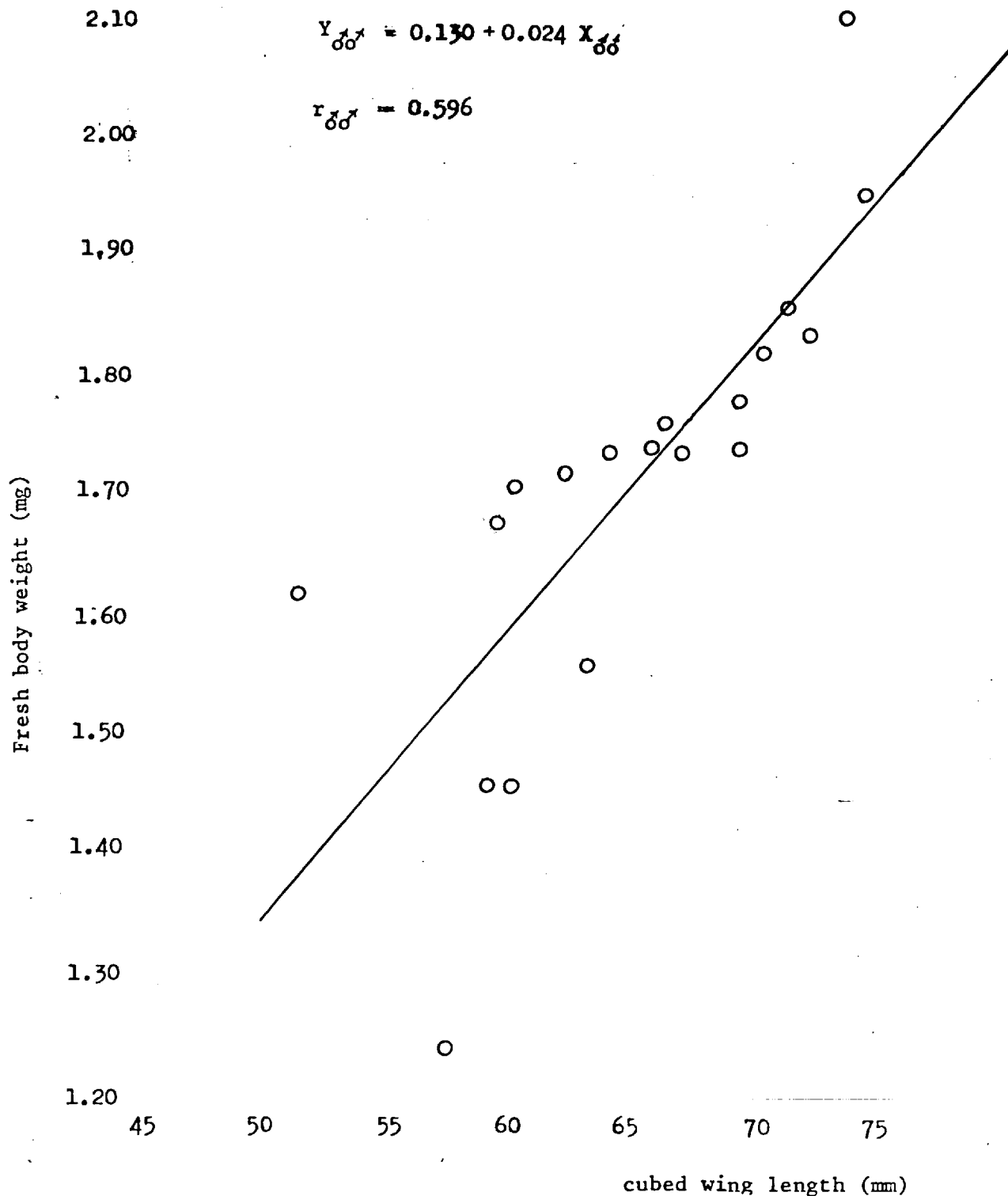
Fig. 4.5.3   Relationship between cubed wing length and weight of <u>Aedes</u>

<u>geniculatus</u> males reared at 20°C

The standard errors of b were 0.0005 for females and 0.0010 for males which gave 95% confidence limits for $b_{\varphi\varphi}$ of 0.0027 to 0.0049 and for $b_{\delta\delta}$ of 0.0022 to 0.0063.

The close correlation between fresh adult weight and wing length allows prediction of the weight of <u>Aedes</u> <u>geniculatus</u> adults from wing length which is easier to obtain than fresh weight.

4.6   OBSERVATIONS ON REPRODUCTION

(a)   Studies on preoviposition period

(b)   Studies on oviposition

4.6.1   Introduction

Many examples of research of oviposition in a wide range of insects are found in the literature, mainly because of their fundamental importance in ecological studies.

Marshall (1938) reported numerous data about oviposition of some British mosquitoes. Detailed information was presented by Christophers (1960) on the copulation and oviposition behaviour of Aedes aegypti.

More recently Gillet (1971) summarised information on mating, blood-feeding and egg-laying of several mosquito species.

Experiments were carried out to measure the duration of preoviposition period (i.e. period between first blood meal and laying of the first egg batch) and the number of eggs per batch produced by Aedes geniculatus.

4.6.2   Experiments

In all the experiments carried out, as soon as females emerged

in the 20°C room they were grouped with males in adult-cages type A

(Fig. 4.2.3) for periods of one, two or seven days when they were

offered blood meals for 30 minutes, either from an anaesthetized

rabbit or from the authors' arms (see Section 4.2.7). The engorged

females were individually isolated in adult-cages type B (Fig. 4.2.4)

immediately after their first blood meal, and were checked every day.

The oviposition containers were changed when the females oviposited,

or once a week when the cotton dental rolls were changed. The number

of eggs per batch was counted and each batch kept in a humid chamber

for later studies.

4.6.3   Results

Table 4.6.1 shows a summary of the nine experiments performed.

TABLE 4.6.1   Experiments on Oviposition of Aedes geniculatus

Experiment Number	Number of ♀♀	Age when fed (days)	Bloodmeal	Number of ♀♀ alive 1 week after blood feeding	Number of ♀♀ that oviposited
1	9	7	Rabbit	6	1
2	9	7	Rabbit	7	0
3	9	2	Human	9	0
4	7	2	Rabbit	5	0
5	6	2	Rabbit	6	6
6	5	2	Human	5	5
7	3	7	Human	3	3
8	6	2	Rabbit	6	2
9	4	2	Human	3	0
TOTAL	58	-	-	50	17

4.6.3.1   Preoviposition period

Of the 58 females blood fed and individually isolated, 8
(13.79%) died during the first week of the experiments, most being
drowned in the oviposition containers, 50 (86.21%) survived through-
out the oviposition period. Of these 50, 17 (34.0%) laid eggs while
33 (66.0%) lived between three and four weeks and died without lay-
ing any eggs.

The minimum duration of the preoviposition period observed
was 7 days in 23.53% of the females and the maximum was 23 days in
47.06% of them.

Of 34 females blood-fed 2 days after emergence and alive 1
week after feeding, 13 (38.24%) laid eggs, but of the 16 fed at
seven days from emergence only 4 (25%) laid eggs.

In total 17 females fed on human blood and of these 8 (47.06%)
oviposited, but only 9 (30%) of the 30 fed on rabbit oviposited.

Several attempts were made to feed the females during the first
24 hours, but in no case was it achieved. However, it was clear that
they were able to blood-suck from the second day after emergence,
when 13 (76.47%) of the ovipositing females had their first blood
meal.

The 17 isolated ovipositing females came from both groups of
those fed on the second and seventh day after emergence (Table 4.6.2).

Females which were fed on the seventh day had a shorter pre-oviposition period than those fed on the second day.

TABLE 4.6.2   Reproductive Characteristics of _Aedes geniculatus_

Females

Female Number	Female age when fed (days)	Preoviposition period (days)	Number of eggs in first batch	First reproductive age (α) in days
1.1	7	7	51	14
5.1	2	23	82	25
5.2	2	23	76	25
5.3	2	23	73	25
5.4	2	22	52	24
5.5	2	22	49	24
5.6	2	22	56	24
6.1	2	23	82	25
6.2	2	23	84	25
6.3	2	23	68	25
6.4	2	23	76	25
6.5	2	23	80	25
7.1	7	7	77	14
7.2	7	7	57	14
7.3	7	7	56	14
8.1	2	10	66	12
8.3	2	20	13	22
Mean	–	18.12	64.59	21.29
Standard Deviation	–	7.07	17.98	5.19

4.6.3.2 Oviposition

Eggs were deposited on the walls of the containers above the water level, mainly in the folds of the filter paper and only occasionally some of the eggs of the batches were found on the surface of the water.

The mean number of eggs produced by the seventeen females was 64.49 (Table 4.6.2). Sixteen of the females produced only one batch of eggs even although they were offered further blood meals. Only one female (No. 8.1) laid a second batch (17 eggs), 31 days after the first one.

The frequency distribution of number of eggs per batch (Fig. 4.6.1) illustrates the variation in size found in first oviposition of females fed during the first week (2 or 7 days) after emergence.

It was not easy to blood-feed Aedes geniculatus females in the laboratory. When feeding the colonies only a small portion of the females flew directly to the skin, punctured it at once and had a whole blood meal. On many occasions there was no feeding on the rabbit or my hand after 30 minutes exposure and sometimes a few fed only after several minutes of walking on the skin. Engorging with blood usually took about two minutes to complete.
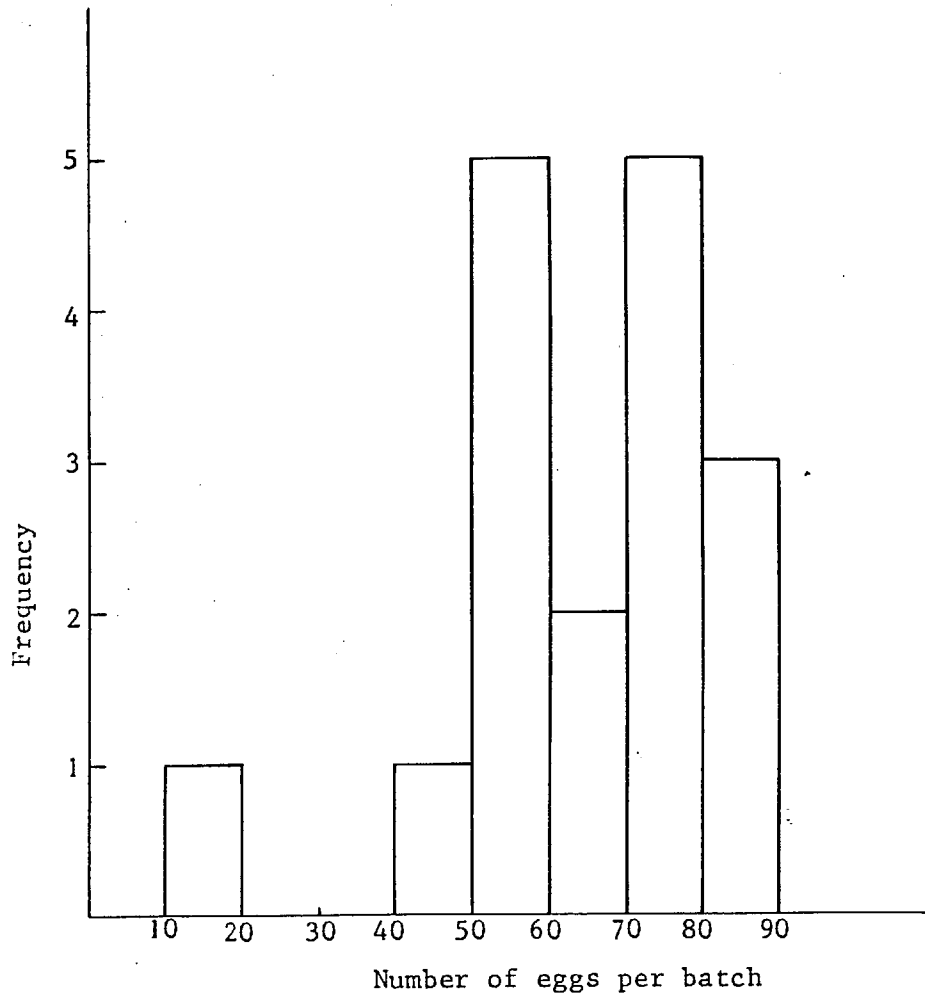
Fig. 4.6.1 The frequency distribution of eggs per batch

(first oviposition) in Aedes geniculatus

4.7   SECTION GENERAL DISCUSSION

Laboratory studies on some aspects of the biology of Aedes
geniculatus have been described.

In laboratory conditions Aedes geniculatus laid its eggs above
the water level, mainly fixed to the folds of the filter paper pro-
vided as the oviposition substrate;   similarly in the field eggs are
laid in cracks and crevices of the bark of the tree-holes.   In general
Aedes species lay their eggs above the water level (Wesemberg-Lund,
1921;   Dunn, 1926;   Shute, 1930;   Nieschulz, Bedford and du Toit, 1934).

The only published information on size of the eggs of Aedes
geniculatus was given by MacGregor (1932) who quoted the average
length as 0.654 mm and average width as 0.215 mm.   Clearly those eggs
were smaller than the eggs obtained in the laboratory in the present
study where length and width were 1.04 ± 0.02 mm and 0.34 ± 0.01 mm
respectively.

None of the eggs brought from the field hatched under the
different treatments applied, even although in the field eggs take
only two weeks to hatch after being submerged.   This was confirmed
during the 1976 drought, when several tree holes were filled with
water after being dry for more than three weeks and first instar
larvae appeared in the holes two weeks after adding the water.

Although females reared in the laboratory readily produce eggs
after a human blood meal they do not lay fertile eggs.   It seems that
the cageing and laboratory conditions are not conductive to successful

mating and so it was impossible to rear <u>Aedes geniculatus</u> continuously. It has already been reported by MacGregor (1932) that even after seven days fertilization did not occur when some females were caged with males.

<u>Aedes geniculatus</u> has been reported as overwintering in the egg stage, but authors disagree about the number of generations per year; it is reported to be univoltine by some and multivoltine by others. For example Lang (1920) reported that eggs laid in autumn, hatch next spring. He suggested that because of the dates on which larvae of various sizes were observed and because of their slow rate of growth that there was probably normally only one brood in the year. One or two generations per year were reported by Wesemberg-Lund (1921). MacGregor's (1932) opinion was that there are at least two generations annually, one appearing in mid-summer from the individuals which have emerged in the spring, and the other occurring in early to late autumn from the members of the summer generation. Finally, Rettich (1971) from Czechoslovakia reported that multivoltinism is shown by <u>Aedes geniculatus</u>.

The field data seem to confirm Lang's (<u>loc</u>. <u>cit</u>.) conclusion. In no case was diapause broken in the laboratory, and field sampling showed a clear period around mid-March when large proportions of first instar larvae occurred in the larval populations of the tree-holes. These points strongly suggest that eggs in the field enter an obligatory diapause that is not broken until next spring, around mid-March, when the first instar begin to appear in the tree-holes (Section 3.4).

Egg diapause is known in several subgenera of Aedes, namely
Aedes, Ochlerotatus, Finlaya and Stegomyia. Although egg diapause is
widespread in the genus Aedes the actual hatching characteristics vary
very much from species to species.

European species such as Aedes cinereus, Ae. caspius, Ae.
annulipes and Ae. cantans are known to spend the winter months in the
egg stage. Service (1977) suggests that diapause in Aedes cantans is
initiated by a reduction in temperature and/or daylight lengths, but
the factors which terminated diapause were not determined because he
could not induce eggs to hatch from October to December.

No reports are known about developmental times of larvae and
pupae of Aedes geniculatus; our results show a clear difference of
developmental times at 20 and $10^{\circ}C$. At $10^{\circ}C$ development was so slow
that the mean time taken for first instar larvae to reach the adult
stage was twice the time needed at $20^{\circ}C$ (39.7 against 19.2 days for
females, and 33.7 against 16.7 days for males).

More extreme temperature effects were reported by Service (1977)
for another English mosquito, Aedes cantans. At $20^{\circ}C$ the mean develop-
mental time from first instar to pupa was 26.4 days and at $10^{\circ}C$ it was
65.4 days.

It is well known for mosquitoes that preadult development is
shorter for males than for females; this difference was demonstrated
clearly at both $10^{\circ}C$ and $20^{\circ}C$ in the present study. At $20^{\circ}C$ the differ-
ence was 2.5 days, while it was 6 days at $10^{\circ}C$. The differences occur
principally during larval development, 29.0 days (females) against 24.0

days (males) at 10°C and 15.5 days (females) against 12.9 days (males) at 20°C.

At any temperature the developmental times estimated in the laboratory probably are shorter than in field conditions principally because adequate food is supplied in the laboratory cultures. Southwood et. al. (1972) reported that for _Aedes aegypti_ the durations of the various stages of development were shorter in the laboratory than in natural conditions.

The data gathered from this study show that _Aedes geniculatus_ has a high larval survival rate in laboratory conditions, 83.33% at 20°C and 90.91% at 10°C, when an adequate food supply was provided. Most of the larval mortality observed occurred during the first days of larval life.

When total larval and pupal mortality were considered together there was a clear difference between the two temperatures tested. Thus 79.17% survival was observed at 20°C, and 66.17% at 10°C. This difference was mainly attributable to the fact that survival of the pupal stage at 20°C was very high (95.54%) while only 70.71% at 10°C.

The duration of the pupal stage was greater (10 days) for the cultures at 10°C than for those kept at 20°C (8 days). Similarly the emergence of adults occupied less time (8 days) at 20°C than at 10°C (13 days).

Finally, we may conclude that the laboratory results indicate that the potential for increase of _Aedes geniculatus_ populations

between the range of temperatures studied is very high. However, it is difficult and dangerous to extrapolate from the laboratory into the field directly particularly since natural populations are subject to a plethora of often unknown limiting factors.

Adult body weight and wing length were used as indicators of body size of Aedes geniculatus reared at 20°C.

The significant correlation (P < 0.01) found between fresh body weight and cubed wing length of adult females and males indicated that wing length is a satisfactory index of size. The regression equations established allow the prediction of adult weight of Aedes geniculatus adults from measurements of wing length, which are easier to obtain than fresh body weights.

The minimum preoviposition time estimated agrees with the information from Marshall (1938) who reported 7 days as the shortest time from blood feeding to egg-laying for Aedes geniculatus, and between 4 and 9 days for other British Aedes.

The mean duration of the preoviposition period was rather long as compared with many other Culicidae. Preoviposition period of Aedes aegypti was reported by Christophers (1960) as 70 hours in grouped females and 80 to 90 hours in single females in tubes at 23°C. This time was about doubled at 18°C for females fed on the fifth to seventh day from emergence. In contrast Wallis and Lang (1956) had reported preoviposition periods of 7 days (168 hours) at 23-25°C in 55% of 128 virgin females and 89% of mated females of Aedes aegypti.

Grouped <u>Culex</u> <u>pipiens</u> <u>fatigans</u> at $25 \pm 1^{\circ}C$ begin to oviposit 5 days (120 hours) after the first blood meal when fed six days after emergence (Gómez <u>et al.</u>, 1977).

Our laboratory females reared from wild larvae showed a higher reproductive potential, laying an average of 64.5 eggs in the first batch, 4.5 times more than reported by MacGregor (1932). His <u>Aedes geniculatus</u> females reared from wild larvae laid only 14 eggs per female 4 days after feeding while wild caught females fed on human or avian blood laid an average of 46 eggs per female 4 days after feeding.

These data suggest that MacGregor laboratory-reared larvae were exposed to some stress such as, lack of space or food.

SECTION 5

EFFECTS OF LARVAL DENSITY ON MOSQUITO-DEVELOPMENT

IN SMALL CONTAINER-HABITATS

5.1   INTRODUCTION

Animals in general are affected in different ways by their density, mainly in the amount of food and space available for each individual.

Each species is affected in a particular way and what could be a disastrous density for one species might be the optimum for others.

There are two types of effects on density, namely effects on the

same species, or intra-specific effects and those on other species
or inter-specific, notably parasites, predators and also competitors.

The intraspecific effects of density can be divided into
what is generally regarded as a series of changes in the biology and
the behaviour of the individuals, but without any major change in
their morphology or physiology, and what is more easily recognisable
the "group effect", since it also includes changes in morphology and
physiology, such as those which occur in Lepidoptera (Chauvin, 1967).
Uvarov (1961) emphasised the fact that the studies on density effects
insects have frequently been concentrated on changes of colouration
and morphology of individuals, while important physiological and
behavioural effects have not been studied.

Among the more relevant papers on the effects of density on
insects are those by Barbosa and Peters (1970, 1977). They review
papers dealing with manifestations of overcrowding in different
- species, and the influence of population density on size, fecundity
and developmental rate of insects in culture.

Some other papers have been published which deal in more detail
with different Diptera species, for example Quraishi (1965) on
swarming, mating and density of _Anopheles mysorensis_ Sweet; Cohen
(1968) on developmental time of _Drosophila melanogaster_ Meigen on
chemically defined diets. Krishnan et al. (1969) studied the effects
of crowding on larvae of _Culex fatigans_ Wied in the laboratory, while
Ikeshoji and Mulla (1970, 1974a, 1974b) examined the chemical factors
produced by overcrowded mosquito larvae.

The influence of density on fecundity in insects has been studied by Watt (1960) and more specifically in mosquitoes by Bar-Zeev (1957). The effect of crowding during larval stages on growth, development and flight was examined in some mosquitoes my Nayar and Sauerman (1968, 1970, 1973).

More specific studies on Aedes aegypti were made by Greenough et al. (1971) on crowding of larvae in reduced experimental universes, also by Moore and Fisher (1969) on the effects of density on growth, mortality, fecundity and production of growth retardant. Later Moore and Whitacre (1972) described the production of larval growth retardant at various densities, and also the effect of larval overcrowding on larval and pupal respiration was investigated by Barbosa and Peters (1972, 1973).

Morphological changes in adult insects resulting from variations in the larval rearing conditions of the larvae is particularly well known in some groups of insects. The effects of varying larval density and food supply on pre-imaginal development, adult weight and length of wing of Aedes aegypti were examined in this study.

Although it had been intended that similar studies would be made of Aedes geniculatus, the difficulties encountered in laboratory rearing (Section 4. 4) made this impossible.

Thus, it was necessary to use only Aedes aegypti in all the experiments on account of its readiness to mate in captivity, quick embryonic development, lack of obligatory diapause, feasibility of storing embrionated eggs and the high percentage hatch of previously conditioned groups of eggs.

5.2   MATERIALS AND METHODS

5.2.1   The Stock Culture

Aedes aegypti eggs from laboratory cultures kept for several years at Silwood Park, Imperial College (J. Lenaham, personal communication) were used to establish the stock cultures.

Pupal cages, adult cages, sugar feeding units, blood feeding of females, oviposition cages and humid chambers were the same as already described in the Sections 4.2.5 to 4.2.10.

Continuous stock cultures were established in the laboratory. They were set up with groups of 100 first instar larvae placed in plastic boxes of 22 x 11.5 x 8 cm., with three, 3 cm. diameter circular terylene-covered, ventilation holes in the lid and provided with 1000 cm^3 of tap water and 10 mg solid food (Section 4.2.4) for larva.

Pupae were removed from the cultures every 48 hours and placed in plastic cups in adult cages each of which had a sugar feeding unit and an oviposition cage.

At least once per week after emergence each culture was offered a blood meal from an anaesthetized rabbit.

The eggs oviposited in the stock culture were collected every day and kept in humid chambers for another 24 hours to allow embrionation

before they were allowed to dry and then were stored dry at 20°C
until needed. These methods ensured a high percentage hatch when
the eggs were submerged later.

5.2.2 Experimental Populations

When larvae were needed for experiments the dry paper bands with
eggs were placed in humid chambers with several layers of wet filter
paper for 24 hours and then submerged in water with a little amount of
yeast powder.

First instar larvae collected 3 hours later after submersion
of the eggs were used to set up the larval cohorts for the different
experiments.

The nutritive medium used for the larval experimental cultures
was prepared as already explained in Section 4.2.4. The amount of
feed per larva and the larval cages used varied from experiment to
experiment and these details are given for individual experiments.

Pupae were removed from the larval cages every 24 hours, sexed,
counted and placed into pupal cages until the adults emerged.

5.2.3 The Rooms

All the stock cultures were kept in a controlled environment
room at constant temperature of 25°C and with 16 : 8 hours light : dark
cycle; the experimental populations were also kept in the same room.

Relative humidity was not controlled but it was always high because
of transpiration of many plants kept in the room.

5.2.4   The Measurements of the Adults

Adults were kept isolated, without food and were anaesthetized
and killed within 24 to 36 hours of emergence.

Each specimen was weighed and its right wing length was measured
in the same way as already described for _Aedes geniculatus_ (Section
4.5.1).

5.3 LARVAL AND PUPAL DEVELOPMENT OF Aedes aegypti AT DIFFERENT LARVAL DENSITIES

Larvae were reared in isolation or in groups of 2, 4, 8, 16, 32, 64, 80 and 100 larvae in standard larval cages (Section 4.2.3).

Each nutritive medium was prepared with 100 cm³ of tap water.

5 mg. solid food, per larvae, and was incubated for 24 hours at 25°C to

allow bacterial growth.

Newly-hatched first instar larvae (maximum 3 hours old) were assigned to the different densities. The cages were allocated random positions in trays placed in the 25°C room.

The cultures were examined every day and pupae were transferred to pupal cages until emergence when the sexual proportions were checked, and adult measurements were done.

5.3.1 Larval and Pupal Developmental Times

As previously done in section 4.4.2 the developmental time was calculated as the time required for 50% of the individuals to change into the next stage.

Larval mean developmental times for densities 8, 16 and 32 were the same, (Table 5.3.1) but slightly longer for density 64. At density 4 less than 50% of larvae reached the pupal stage and thus developmental time was not calculated. At densities 1 and 2 no larval development was

TABLE 5.3.1   Larval developmental times of _Aedes_ _aegypti_ (25°C) at different densities

	Density per container				
	4	8	16	32	64
Larval mean developmental time ± S.D. (days)	*	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.5 ± 0.6
Range of larval life (days)	6 - 14	5 - 7	5 - 11	5 - 10	5 - 9
Number of pupae	8	57	112	242	224
Initial number of larvae	32	64	128	256	256

* Less of 50% larvae changed to pupae

completed in any case, even although larvae survived for periods

ranging from 2 to 28 days and 2 to 26 days respectively; not one of

these larvae reached the four instar

The range of larval life was also similar in densities 8 to 64

larvae per container, but in density 4, where only 8 larvae moulted

to pupae, they took from 6 to 14 days to complete larval growth.

The peak of pupation in densities 8 to 64 occurred on day 6 of

larval life, when there was a massive pupation.

In cultures of 80 and 100 larvae per container no larval develop-

ment was completed. The large amount of food necessary in each con-

tainer caused the formation of a film on the surface of the rearing

medium restricting larval respiration. In these highly concentrated

media nearly all the larvae by the fourth day after setting up the

cultures.

Pupal mean developmental times were slightly shorter in densities

4, 8 and 16 than in densities 32 and 64 (Table 5.3.2). Although the

ranges of pupal life were greater at the lower densities, the peak of

emergence was on day five of pupal life, when a massive emergence

occurred.

5.3.2   Larval and Pupal Mortality

Larval mortality was 100% in densities 1, 2, 80 and 100 and very

high (75%) at density four (Table 5.3.3). For densities 8, 16, 32 and

TABLE 5.3.2 Pupal developmental times of <u>Aedes</u> <u>aegypti</u> from larvae reared at different densities (25°C)

	Density per container				
	4	8	16	32	64
Pupal mean developmental time ± S.D. (days)	5.0 ± 0.9	5.0 ± 0.5	5.0 ± 0.0	6.0 ± 0.0	5.5 ± 1.0
Range of pupal life (days)	4 – 8	3 – 7	4 – 6	3 – 6	3 – 6
Number of adults	6	56	108	237	221
Initial number of pupae	8	57	112	242	224

TABLE 5.3.3   Larval mortality of <u>Aedes</u> <u>aegypti</u> reared at various

densities (25°C)

Density	Initial Number of larvae	Number of dead larvae	% larval mortality
1	30	30	100.0
2	32	32	100.0
4	32	24	75.0
8	64	7	10.9
16	128	16	12.5
32	256	14	5.5
64	256	32	12.5
80	320	320	100.0
100	400	400	100.0

64 the larval mortality was in a low range, that is between 5.5 and 12.5%.

Mortality during the pupal stage was very low (1.34 to 3.57%) at densities eight to 64 (Table 5.3.4), but it was considerably higher (25%) for larvae reared four per container.

As it seems that very small amounts of solid food (5 mg to 20 mg) in 100 cm^3 of water is not enough to promote suitable bacterial growth in the medium to allow adequate larval growth, an additional experiment was carried out with isolated larvae. In this case 20 solitary larvae were provided with 50 mg of solid food per container and another 20 with 100 mg per container.

Table 5.3.5 shows the results from this experiment compared with those from isolated larvae of the previous experiment.

With 50 mg of food in each container there was no mortality at all, while with 100 mg only 10% of the larvae died.

When these mortalities were compared with the mortalities previously found at densities 1, 2 and 4 (Table 5.3.3), it seems clear, that the reason for the high mortalities was related to the low level of bacteria growing and/or that the amount of solid food provided a very weak medium such that the larvae have some difficulty in finding solid particles.

TABLE 5.3.4   Pupal mortality of <u>Aedes</u> <u>aegypti</u> from larvae

reared at various densities (25°C)

Density	Initial Number of pupae	Number of dead pupae	% pupal mortality
4	8	2	25.0
8	57	1	1.75
16	112	4	3.57
32	242	5	2.07
64	224	3	1.34

TABLE 5.3.5   Mortality of isolated <u>Aedes</u> <u>aegypti</u> larvae reared

with different amounts of food per larva (25°C)

Initial number of larvae	Amount of solid food/ larva	% larval mortality
30 *	5	100
20	50	0
20	100	10

*   data from Table 5.3.3

5.3.3 Sex Ratio at Adult Emergence

In densities 16, 32 and 64, where larval mortality was low, the proportion of males was slightly higher than females (Table 5.3.6). Only at density 8 was the proportion of males smaller than females, even though it is usual that in Aedes aegypti the proportion of males is usually higher than females (Christophers, 1960). At density 4 it seems that the high larval mortality (75%) affected female larvae more than males.

TABLE 5.3.6   Sex ratio of Aedes aegypti at emergence from larvae reared at different densities (25°C)

Density	Initial number of larvae	Number of adult females	Number of adult males	Female/male ratio
4	32	1	5	1 : 5.0
8	64	35	21	1 : 0.6
16	128	50	58	1 : 1.2
32	256	97	140	1 : 1.4
64	256	109	112	1 : 1.0

In an additional experiment at density 20, where a greater number of larvae (760) was reared in the same way as these in Table 5.3.6, the proportion of female/male adults was 1 : 1.4.

5.3.4 Adult Weight as a Measure of Larval Growth

Adult weight at emergence was used as a measure of growth of
Aedes aegypti larvae reared at densities of 4, 8, 16, 32 and 64 larvae
per container.

The heaviest individuals were those reared eight per container
(Table 5.3.7).

Males from densities 4, 32 and 64 were significantly lighter
(t test P < 0.05) than males from density 8, but there was no signifi-
cant difference between males from density 16 and 8.

Females from densities 16 and 64 were not significantly different
from those reared at density 8, while females from density 32 were
significantly lighter than females from density 8.

With 5 mg of food per larvae in 100 cm^3 of water, it appears
that greatest larval growth could be obtained at densities of 8 to 16
larvae per container.

The few adults produced at density 4 were clearly much lighter
than the rest of the adults obtained from densities 8 to 64.

This seems to confirm the previous observation that cultures
of 1, 2 and 4 larvae per container, conditions were not suitable for
larval growth.

Cultures of 80 and 100 larvae per container were too polluted
to allow larval growth.

TABLE 5.3.7   Adult weight of <u>Aedes aegypti</u> reared at different

larval densities (25°C)

Density	Initial number of larvae	No. of emergent adults		Adult weight ± S.D.(mg)	
		Females	Males	Females	Males
4	32	1	5	0.620 (1)*	0.70 ± 0.10 (5)
8	64	35	21	1.87 ± 0.28 (20)	1.06 ± 0.15 (20)
16	128	50	58	1.79 ± 0.15 (40)	1.00 ± 0.13 (40)
32	256	97	140	1.72 ± 0.20 (40)	0.97 ± 0.16 (40)
64	256	109	112	1.79 ± 0.26 (40)	0.97 ± 0.11 (40)

*   Number of individuals measured in parenthesis

5.4   INTERACTIONS BETWEEN LARVAE OF DIFFERENT AGES

To investigate possible competition effects between larvae of different ages, newly hatched larvae were introduced into already established cultures.

One, two or four first instar larvae were introduced into cultures of 16 larvae either 3 or 5 days old initially provided with 80 mg solid food in 100 ml of water. All the cultures were set up in standard larval cages and were allocated at random to trays in the 25°C room. Care was taken that all the cultures used had 16 alive larvae developing normally on the day of the introduction of first instar larvae.

Sixteen, eight and five replicates were used respectively in the experiments with 1, 2 and 4 introductions.

The effects of 3 and 5 days old cultures on introduced larvae are summarised in Tables 5.4.1 to 5.4.6.

5.4.1   Effects on Mortality

Comparing the preadult mortality (Table 5.4.1) of the introduced first instar larvae in cultures 3 days old (25, 12.5 and 40% for densities 1, 2 and 4 respectively) with the preadult mortality (15.63%) in 16-larvae cultures (section 5.3.2), it seems clear that only at a density of 4 first instar larvae was there a big difference in percentage mortality.

TABLE 5.4.1   Aedes aegypti preadult mortality of late-introduced

first instar larvae (1, 2 or 4) in cultures of 16

3-days old larvae (25°C)

Number of late-introduced larvae	Number of replicates	Preadult mortality (%)
1	16	25.0
2	8	12.5
4	5	40.0

When the first instar larvae where introduced into 5-days old

cultures (Table 5.4.2) the increase in mortality was remarkable for

densities 2 and 4 (43.8 and 65.0% respectively).

TABLE 5.4.2   Aedes aegypti preadult mortality of late introduced

first instar larvae (1, 2 or 4) in cultures of 16

5-days old larvae (25°C)

Number of late-introduced larvae	Number of replicates	Preadult mortality (%)
1	16	25.0
2	8	43.8
4	5	65.0

It seems that in 3-days old cultures the mortality of the late-

introduced first instar larvae was not affected very much by the pres-

ence of the established 16 3-days old larvae.

In addition, since mortality was not significant it seems that the medium had not been depleted nor altered detrimentally for the one or two late-introduced larvae treatments. In cultures where 4 first instar larvae were introduced the preadult mortality of these late-introduced larvae was high (40.0%), much more than the 7.42 and 15.65% preadult mortality in the previous cultures of 32 and 16 larvae respectively.

The effects on the late-introduced larvae seem clearer when the first instar larvae were introduced into 5-days old cultures (Table 5.4.2). Preadult mortalities of 25.0, 43.8 and 65.0% were found for densities of 1, 2 and 4 late-introduced first instar larvae.

5.4.2 Effect on Sex Ratio

In all the cases studied in both experiments (Tables 5.4.3 and 5.4.4) the sex ratio of emerging adults of the introduced larvae was highly biased to males as compared with the range of 1 : 1.2 to 1 : 1.4 found in previous cultures of 16 and 32 larvae per container respectively (Table 5.3.6). In both experiments the proportion of males increased with the density of the late-introduced larvae in the initial culture.

It seems that the stress upon the late-introduced larvae is much stronger for the female larvae than for males. Female larvae usually needs to reach a greater body size than males before being ready to pupate and thus it might be expected that the effect of restricted food would be greater on females than on males larvae.

TABLE 5.4.3   Aedes aegypti sex ratio at adult emergence of late-

introduced first instar larvae (1, 2 or 4) reared

in cultures of 16 3-days old larvae (25°C)

Number of late- introduced larvae	Number of replicates	Number of adult females	Number of adult males	Female/ male ratio
1	16	3	9	1 : 3.0
2	8	3	11	1 : 3.7
4	5	2	10	1 : 5.0

TABLE 5.4.4   Aedes aegypti sex ratio at adult emergence of late-

introduced first instar larvae (1, 2 or 4) reared in

cultures of 16 5-days old larvae (25°C)

Number of late- introduced larvae	Number of replicates	Number of adult females	Number of adult males	Female/ male ratio
1	16	4	8	1 : 2.0
2	8	2	7	1 : 3.5
4	5	1	6	1 : 6.0

5.4.3 Effects on Adult Body Size

In the 3-days and 5-days old cultures late-introduced first instar larvae produced significantly lighter females (t test $P < 0.01$) (Tables 5.4.5 and 5.4.6) than those produced from cultures of 16 larvae per container (1.79 ± 0.15 mg, Table 5.3.7) in the previous experiment. Males, with the exception of those from the one late-introduced larva treatment, were also significantly lighter than those produced in cultures of 16 larvae per container (1.00 ± 0.13 mg, Table 5.3.7).

TABLE 5.4.5   Aedes aegypti adult weight and right wing length from late-introduced first instar larvae (1, 2 or 4) reared in cultures of 16 3-days old larvae (25°C)

Number of replicates	Number of late-introduced larvae	Adult weight ± S.D. (mg)		Wing length ± S.D. (mm)	
		Females	Males	Females	Males
16	1	1.53 ± 0.09 (3)*	0.98 ± 0.09 (9)	3.60 ± 0.05 (3)	2.92 ± 0.10 (9)
8	2	1.30 ± 0.11 (3)	0.84 ± 0.09 (11)	3.48 ± 0.23 (3)	2.83 ± 0.18 (11)
5	4	0.73 ± 0.03 (2)	0.61 ± 0.08 (10)	2.86 ± 0.05 (2)	2.55 ± 0.14 (10)

* Number of individuals measured in parenthesis

TABLE 5.4.6   Aedes aegypti adult weight and right wing length from

late-introduced first instar larvae (1, 2 or 4) reared

in cultures of 16 5-days old larvae (25°C)

Number of replicates	Number of late-introduced larvae	Adult weight ± S.D. (mg)		Wing length ± S.D. (mm)	
		Females	Males	Females	Males
16	1	1.52 ± 0.28 (3)*	0.96 ± 0.19 (8)	3.52 ± 0.32 (4)	2.83 ± 0.24 (8)
8	2	0.93 ± 0.19 (2)	0.65 ± 0.29 (7)	3.15 ± 0.18 (2)	2.65 ± 0.24 (7)
5	4	0.79 (1)	0.58 ± 0.11 (6)	2.86 (1)	2.45 ± 0.13 (6)

*   Number of individuals measured in parenthesis

In both females and males there was a decrease in weight when

density of the late-introduced first instar larvae was increased.

All the female and male adults produced from the late-introduced

larvae were significantly lighter (t test $P < 0.05$) when introduced at

density four compared with two and one.

Finally, the weight of female and male adults from the late-

introduced larvae was compared with the weight of adults from larvae

reared 32 per container (1.72 ± 0.20 mg for females and 0.97 ± 0.16 mg

for males, Table 5.3.7).

The adult females from late-introduced larvae were in all cases significantly lighter (t test $P < 0.05$) than the females from density 32. The males, with the exception of those from one late-introduced larvae, were also significantly lighter than the males from density 32 of the previous experiment.

This indicates clearly that increase of densities to 17, 18 and 20 after 3 days of rearing cannot solely be responsible for the decrease in weight observed in adults from late-introduced larvae in old cultures.

Similar effects as with adult weight were found when wing lengths (Tables 5.4.5 and 5.4.6) were compared.

Wings of adults from density four were significantly shorter (t test $P < 0.01$) when compared with adults from density two and one, which were not significantly different.

When first instar larvae were introduced into cultures of 16 5-days old larvae (Table 5.4.6), adult weights and wing lengths parallel the trends of the 3-days old introductions.

There were no significant weight differences between adult males compared between 3 and 5-days introductions.

Only in the case of the two late-introduced larval treatment were adult female weights significantly different when 3 and 5-days introduction were compared.

No significant differences in wing length were found between the two experiments.

It seems that because the larval mortalities in the second experiment were very high (25.0, 43.8 and 65.0%, Table 5.4.2) the surviving larvae could reach almost the same level of growth as those at lower densities; the small differences in the means were not statistically significant.

5.5　EFFECT OF PREVIOUSLY USED MEDIUM ON LARVAL DEVELOPMENT

In view of earlier results this more comprehensive experiment was designed to determine the effect of previously used culture medium and different densities of larvae on pupation, emergence and adult weight of _Aedes aegypti._

Three different densities, 5, 20 and 40 were used and 4 different conditions of rearing medium were considered.

Initially a set of 120 standard larval cages (Section 4.2) were provided with 80 mg of solid food and 100^3cm of water each, and were incubated at 25°C for 24 hours.

Sixty of the containers (Group I) were kept without any modification for six days more and the other 60 containers (Group II) received 16 newly-hatched first instar larvae each 24 hours after setting up the media. All the containers were positioned at random in trays in the 25°C room.

Larvae in containers of Group II were allowed to develop for 6 days (pupation began the fifth day), then all larvae and pupae were removed from the containers and thus providing 60 cages with pre-used rearing medium (Group II) and a group of 60 cages of fresh medium (Group I); both groups were of the same age.

Twenty cultures of each group were allocated to each of the different densities (5, 20, 40) of newly-hatched first instar larvae.

Ten cultures of each density for both types of initial medium (fresh and pre-used) received 5 mg of solid food per larva.

These twelve treatments, each with 10 replicates were kept in the 25°C room.

All the cultures were checked and rotated every day, pupae were removed from the larval cages daily and transferred to pupal cages until emergence. The numbers of pupae and adults were recorded, and also samples of adult females and males from each treatment were killed and weighed within 24 to 36 hours of emergence.

5.5.1 Effect on pupation and emergence

In cultures of 20 and 40 larvae per container in pre-used medium without additional solid food the larval growth was inadequate to produce pupae (Table 5.5.1). Even at density 5 with the same previous conditions the larval growth was so poor that there was only 12% pupation and only 8% of the larvae reached the adult stage (Table 5.5.2).

Under similar densities in the fresh rearing medium there was a clear decreasing percentage of pupation and emergence with increased, namely 92 ± 10.3% at density 5 to 13.5 ± 8.8% at density 40.

In the treatments where 5 mg of addition food per larva were added to both fresh and pre-used medium high percentages of pupation and emergence were reached at density 5 and 20 of fresh medium and density

TABLE 5.5.1   Rate of pupation of <u>Aedes</u> <u>aegypti</u> reared at different densities (5, 20, 40) and 4 different conditions of the rearing medium (25°C) (10 replicates per treatment)

Initial condition of the rearing medium	Additional mg of food per larva at new densities	Pupation (%) ± S.D.		
		Density 5	Density 20	Density 40
Medium with 80 mg solid food in 100 cm^3 of water, kept for 7 days at 25°C (Group I, 60 containers)	0.0 (Group I-0)	94.0 ± 9.6	39.5 ± 20.2	14.6 ± 9.0
	5.0 (Group I-5)	100.0 ± 0.0	91.0 ± 6.6	74.2 ± 33.6
As previous medium, but 16 larvae were reared in each container from day 2 to day 6. (Group II, 60 containers)	0.0 (Group II-0)	12.0 ± 10.3	0.0	0.0
	5.0 (Group II-5)	100.0 ± 0.0	80.0 ± 11.8	30.8 ± 23.6

TABLE 5.5.2   Rate of emergence of <u>Aedes aegypti</u> reared at different densities (5, 20 and 40) and 4 different conditions of the rearing medium (25°C)   (10 replicates per treatment)

Initial condition of the rearing medium	Additional mg of food per larva at new densities	Adult emergence (%) ± S.D.		
		Density 5	Density 20	Density 40
Medium with 80 mg solid food in 100 cm of water, kept for 7 days at 25°C (Group I, 60 containers)	0.0 (Group I-0)	92.0 ± 10.3	37.0 ± 18.9	13.5 ± 8.8
	5.0 (Group I-5)	98.0 ± 6.3	91.0 ± 6.6	73.8 ± 34.0
As previous medium but 16 larvae were reared in each container from day 2 to day 6. (Group II, 60 containers)	0.0 (Group II-0)	8.0 ± 10.3	0.0	0.0
	5.0 (Group II-5)	96.0 ± 8.4	77.5 ± 14.8	30.3 ± 22.7

5 of pre-used medium; the percentages in the other treatments.

An analysis of variance of the factors considered was obtained for the percentage of pupation and emergence (Appendix 5.5.1 and 5.5.2). For the analysis all the values of % of pupation and emergence for each replicate were transformed to arc sin to normalise the data (Sokal and Rohlf, 1969).

The three factors considered, initial condition of the medium, posterior addition of food and density, were all significant (0.1% level) for percentages of pupation and emergence.

The interaction between pre-use of the medium and subsequent addition of food was significant (0.1% level) for both percentage of pupation and percentage of emergence. However, the interaction between effect of density and additional amount of food was only significant (5% level) for percentage of pupation but not for percentage of emergence. The interaction between pre-use of the medium and density was not significant neither for pupation nor for emergence.

The relationship between adult emergence (%) and larval density in the different treatments is shown in Fig. 5.5.1. By the lack of parallelism between the lines it can be inferred that these interactions might be significant as shown by the analysis of variance.

5.5.2 Effects on weight of adults

Because of the zero percentage emergence observed for some treatments
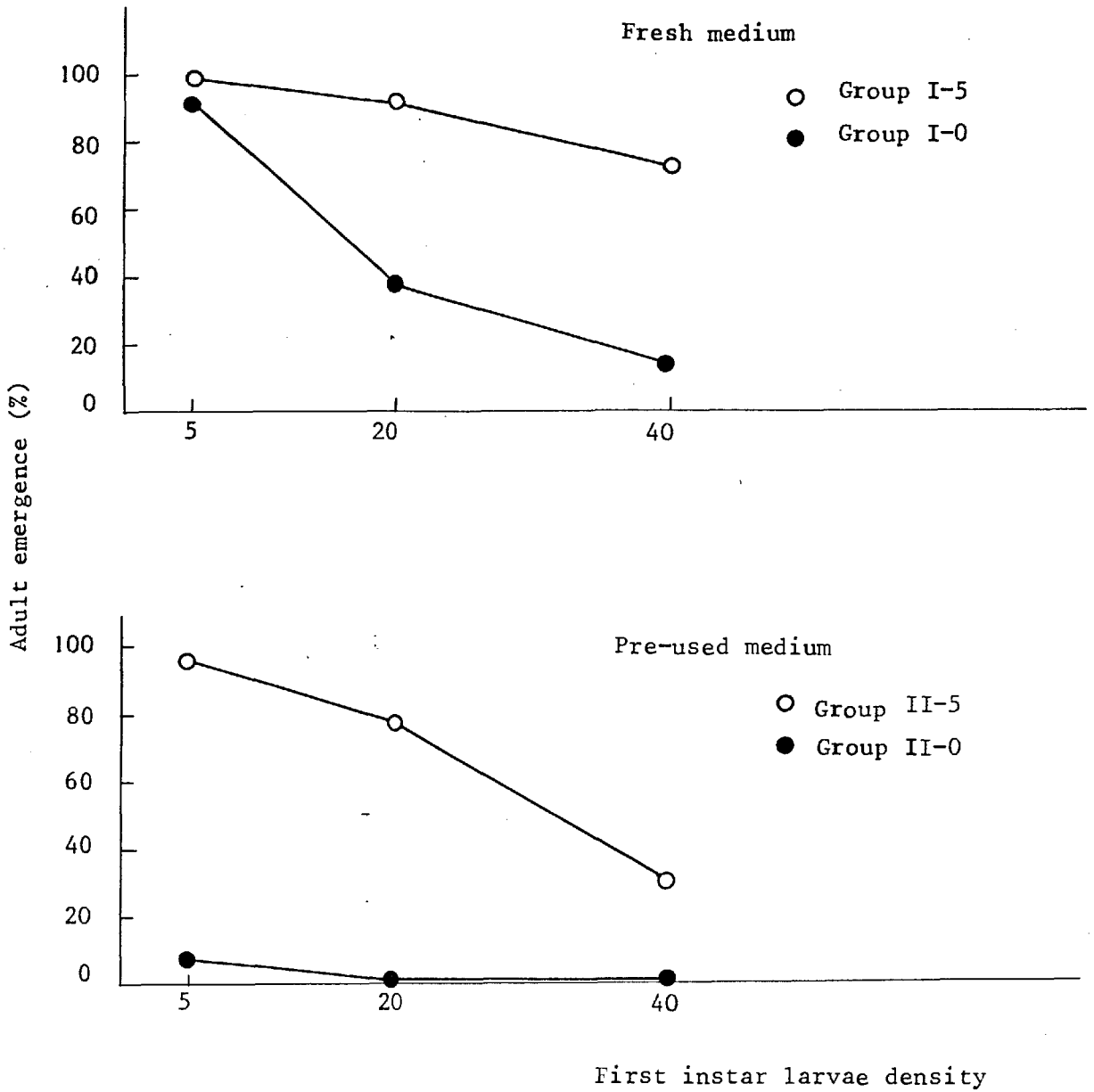
Fig. 5.5.1   Adult emergence (%) of _Aedes_ _agypti_ in relation to
the larval density and condition of medium

(Table 5.5.2) it was not possible to record adult weights in those cases. Thus a full analysis of variance was not attempted but specific comparison were made using a "t" test.

The adult weight from Group II-5 (Table 5.5.3) were compared with these from similar densities from Group I-5.

For both female and male adults the weight from Group II-5 at densities 5 and 20 were significantly smaller ("t" test $P < 0.01$ and $P < 0.05$ respectively). At density 40 neither females nor males were significantly different.

The only information about weights of Group II-0 was for males from density 5, where only 8% of larvae developed into adults (Table 5.5.2), and all were males. Only four were weighed, since the other 4 all died within 24 hours of emergence.

In Group I it was found that weights, both in females and males were not significantly different between Groups I-0 and I-5 at density 5, but there was a highly significant difference for densities 20 and 40 ($P < 0.001$).

For both females and males in Groups I-0 and I-5 there was a decrease of weight when density was increased.

The weight of females from density 5 was significantly greater ($P < 0.01$) than weights from densities 20 and 40 which were, however, not significantly different.

TABLE 5.5.3   Adult weight of _Aedes_ _aegypti_ reared at different densities and condition of medium (25°C)

Initial condition of the rearing medium	Additional food per larva of new densities (mg)	Female adult weight ± S.D. (mg)			Male adult weight ± S.D. (mg)		
		Density 5	Density 20	Density 40	Density 5	Density 20	Density 40
Medium with 80 mg solid food in 100 cm³ of water, kept for 7 days at 25°C (Group I, 60 containers	0.0 (Group I-0)	2.12 ± 0.46 (10)	0.67 ± 0.08 (9)	0.61 ± 0.07 (5)	1.28 ± 0.24 (10)	0.52 ± 0.06 (10)	0.41 ± 0.07 (10)
	5.0 (Group I-5)	2.26 ± 0.24 (10)	1.94 ± 0.23 (10)	1.76 ± 0.37 (10)	1.15 ± 0.10 (10)	1.06 ± 0.11 (10)	0.89 ± 0.09 (10)
As previous medium but 16 larvae were reared in each container from day 2 to day 6. (Group II, 60 containers)	0.0 (Group II-0)	**	**	**	0.47 ± 0.10 (4)	**	**
	5.0 (Group II-5)	1.76 ± 0.33 (10)	1.73 ± 0.35 (10)	1.89 ± 0.19 (10)	0.99 ± 0.13 (10)	0.95 ± 0.07 (10)	0.95 ± 0.09 (10)

*   Number of individuals measured in parenthesis

** No individuals emerged in these treatments

However, for males mean weights were not significantly different when compared between densities 5 and 20, but they were significantly different ($P < 0.01$) between densities 20 and 40.

In Group II-5, both females and males weight differences between densities were insignificantly different.

5.6 SECTION GENERAL DISCUSSION

Studies of the influences of larval densities upon the biology of species are of importance for a better understanding of the dynamic of the animal populations. Grasse (1946) and also Chauvin (1952) pointed out that the grouping of individuals, even in small numbers, may have a great influence upon the biology of the species considered. It has been pointed out by Lang (1953) that the response to density of a given species is related to its mode of life in nature.

Usually the effects of high larval density are accompanied by a shortage of food, and it was advised by Wada (1965) to separate the effect itself from that of starvation, since the two could be quite different processes. In Shannon and Putnam's (1934) experiments the amount of food per container was constant and the density increased, thus the larvae in high densities were affected not only by density, but also by shortage of food.

The effects of larval food on the fecundity of several species of Diptera have been studied by several authors (MacKerras, 1933; Mathis, 1938; Bodenheimer, 1955). Mathis (loc. cit.) found that larvae of _Aedes aegypti_ fed on an abundant diet laid more eggs than larvae fed on a poor diet.

According to Bates (1949) food is rarely a limiting factor for mosquito development, however, Bickley (1972) stated that larvae in small containers at times may be affected by food shortages. Ikeshoji (1965) showed that at the same amount of food per larva adult mosquito size and fecundity decreased when density increased.

In mosquitoes high larval densities produce high larval mortalities, increase in the duration of larval and pupal stages and decrease of size and weight of pupae and adults (Shannon and Putnam, 1934; Terzian and Stahler, 1949; Bar-Zee, 1957; Wata, 1965; Barbosa et al., 1972).

The effects of density on the biology of mosquitoes have been interpreted in several different ways.

Bar-Zee (1957) pointed out that delayed development of the larvae under crowded conditions is due to lack of food. Provided that the water was renewed to prevent development of a yeast film on the surface, he found metabolites had no effect on development or mortality.

Klomp (1964) interpreted additional effects of high density, such as prolongation of the larval and pupal stages, as mutual inter-ference and a consequence of intense physical contact under crowded conditions.

More recently the presence of chemical factors have been detected in cultures of Culex pipieus quinquefasciatus Say and Aedes aegypti (L.) where densities were above 5-7 larvae per ml of medium (Ikeshohi and Mulla, 1970), cultures less crowded showed no evidence of such factors. The overcrowding chemical factors are highly toxic to younger larvae and retard growth and development.

Overcrowding factors are not considered as a limiting factor in our experiments because in no case was the density near the overcrowded level of 5 to 7 larva/ml.

In our experiments (Section 5.3) larval and pupal developmental

times were not significantly different for cultures of 8, 16 and 32

larvae per container with the same amount of food per larva. However,

larvae at low densities (4, 2 and 1 larvae/container) with the same

amount of food per larva were not able to grow normally, although they

survived for very long periods (3 to 4 times more than cultures of 8,

16 and 32 larvae/container).

This response could be valuable in nature where larvae were

short of food, and where they could live for long periods with minimal

amounts of food; the population could be partially recovered as soon

as a good supply of food became available.

In the laboratory it was noted that larvae kept on a suboptimal

diet apparently developed in a normal way, as soon as they were trans-

ferred to a richer diet and produced adults of a normal weight.

The effects on mortality are seen mainly during larval develop-

ment where extreme values were found; one hundred percent mortality

at very low densities (1 and 2 larvae per container) and also at high

densities (80 and 100 larvae per container). In the cases of high

densities the mortality was produced mainly in the first 2 or 3 days

of larval life. It looks as if the factors affecting the cultures at

high densities were mainly mechanical; the large amount of food in

the containers produced a film on the water surface that did not allow

the normal process of respiration of the larvae. Similar results were

found by Bar-Zee (1957), who pointed out that larvae of _Aedes aegypti_

reared at low food (yeast) concentrations, develop slowly, and when

excess food is present, the mortality of the larvae is high as a

result of the development of a film of yeast on the water surface.

At densities, 16, 32 and 64, where low larval mortality occurred,

the proportion of adult males was slightly higher than females, which

is usual for Aedes aegypti. However, when larval mortality was high,

as in density 4, the females were more affected than males, and as a

consequence a very male-biased sex ratio was obtained.

The weights of female and male adult were very low at low

density (4 larvae/container), but although the higher weights were

each at densities 8 and 16, females at high densities (32 and 64 larvae/

container) were not significantly different from females at density 16.

In the case of males from high densities (32 and 64 larvae/container)

they were significantly lighter than males from density 8.

From the results in section 5.4 it seems that food was the

requisite in short supply and the increasing intensity of competition

among larvae when food is limited in quantity or quality, results in

increase of percentage of mortality and reduction of the adult weight

and wing length. In all cases the proprotion of males was higher than

normal. This very biased sex ratio could indicate that the competitive

ability varies between sexes. This could possibly operate if females

need a higher minimal larval weight to pupate than the males.

From experiments in section 5.5 it was noted that there were

marked increases in mortality of groups of larvae introduced into

established cultures when density and time of introduction was increased.

Obviously the established larvae consume more of and/or cause more

damage to the culture medium as time elapses and the late-introduced

larvae are subjected to more stress.

Again, in this series of experiments, the stress seems to be

greater upon the female larvae, because the adult sex ratio is biased

towards males.

Both females and males developing from late-introduced larvae

were in general significantly lighter than those produced from standard

cultures.

In all cases it was found that late-introduced larvae produced

lighter adults as density increased; the same trend was apparent in

wing length.

The experiments of section 5.5 showed that larval growth in

pre-used medium, without additional food provided after removing the

first established larvae, was inadequate to produce a normal larval

growth. Even at low densities larval mortality was very high. However,

when additional food was added high percentages of pupation and emergence

were reached at the lower densities (5 and 20 larvae/container) but

not at the higher density (40 larvae/container).

These results indicate that in the first situation there was an

inadequate food supply to support normal larval growth, and only low

densities of late-introduced larvae could have some success. When food

was added, however, the cultures behaved almost in the same way as

cultures in fresh medium except that in general, although of an inferior condition, they were smaller than those produced from standard optimal conditions. As density increased from 5 to 40 the emergence of adults decreased in a remarkable way, as shown in Table 5.5.2.

The lack of evidence of toxic effects would indicate that chemical overcrowding factors (Ikeshoji and Mulla, 1970) were not present.

Summing up, the small containers used in the experiments represent simplified conditions of artificial and natural containers to be found in nature. Several factors affect larval populations and larval development.

Clear density effects are produced within a certain range of larval densities when the same quantity of food is provided for each larva. These effects are detected in rate of development, preadult mortality and adult size characteristics.

At high densities large mortalities of larvae were noted in the first days of life. These were caused basically by the large concentrations of food in relatively small amounts of water, where the younger larvae could not cope with the formation of the bacterial film on the surface.

At very low densities, when the same amount of food was offered per larva in standard containers, other effects not attributable directly to density were detected. These included high larval mortalities and delayed larval development probably caused by starvation and possibly

associated with difficulties of the larvae in finding solid particles

of food in a very weak medium, despite there being the same amount of

food, in absolute terms, per larva as at successful mid-range densities.

SECTION 6

GENERAL DISCUSSION

The main part of the study has involved several different approaches designed to obtain a picture of the ecology of the pre-adult stages of Aedes geniculatus, a mosquito restricted to breeding in tree-holes, as a British representative of container-breeding mosquitoes. Aedes geniculatus is also the most common tree-hole breeder in Britain and, even although its populations are never very high at Silwood Park, was the most suitable for the study. Additional experiments were done in the laboratory with Aedes aegypti, also a container-breeder but more plastic in its selection of breeding places and thus occurs in several different types of containers.

The behaviour of Ae. aegypti in the laboratory and its responses

to varying rearing densities were to be compared with Ae. geniculatus

under similar laboratory conditions. However, the laboratory culture

of Aedes geniculatus could not be maintained and therefore, it was

impossible to obtain sufficient material, and of controlled quality,

to make the comparisons by experiments.

Detailed discussions of results are given within each section.

What are considered to be the more important points and characteristics

of the two species are now discussed briefly.

Tree-holes contain a very simple composition of animal

association which is probably the result of the nature of the habitat

which has extremes of environmental conditions. Those animals which

successfully colonize such habitats usually possess some special

characteristics of behaviour or physiology which allow them to over-

come short term, and in some cases even long term stress conditions.

Such responses would seem particularly advantageous to species

which colonize such transient bodies of water.

Breeding places like small amounts of water in tree-holes, leaf

axils and small artificial containers are liable to dry out very

quickly. Mosquito species like Ae. aegypti and Ae. geniculatus which

breed in such situations have some special characteristics which

enable them to overcome the hazard associated with such sites.

For example, Ae. geniculatus and Ae. aegypti lay their eggs dry

on the wall of containers having water in them and above the water level.

After rain if the eggs become flooded, eggs hatch and the first
instar larvae are released into the water. Provided that the eggs
are always laid above an existing water level then subsequent flooding
must guarantee a greater amount of water at hatching than was previously
in the hole.

During the period of study large fluctuations in the water
content of the tree-holes were observed and thus there were associated
large fluctuations in the character of the environment in which larvae
of Ae. geniculatus were developing. Other factors vary too but can all
be classified into general types, namely seasonal, for example temper-
ature, irregular, for example drought, and from site to site, for
example size and position of the hole and supply of food.

Accordingly numbers of larvae fluctuated widely from hole to
hole, but with clear seasonal trends, but generally reacted rapidly to
changes in environmental conditions. Thus, following dry periods, and
when temperatures allowed, populations soon increased through the
appearance of newly hatched first instar larvae in the tree-holes.

An important factor associated with the habit of laying eggs
dry is that the embryos can develop normally and the eggs remain viable
even although in an almost dry condition. The eggs can remain in this
state for long periods, perhaps for years, and hatch when flooding
occurs, and without apparent harm to the larvae. The eggs of mosquito
species which oviposit on the water surface usually hatch in less than
one week.

It seems that the eggs of Ae. geniculatus in Britain, unlike
the eggs of Ae. aegypti, have to go through a period of obligatory
diapause development before hatching. The conditions which break
this diapause are not known and attempts to do so by using various
durations of cold treatment were unsuccessful. This and the problem
of getting adults to mate successfully to produce fertile eggs were
the main reasons why a laboratory culture of Aedes geniculatus could
not be maintained.

Hatching is often inconsistent and erratic after flooding so
that all the eggs do not hatch at one time leaving a reservoir of un-
hatched eggs. From the evidence following the experiment where tree-
holes were watered artificially it seems that there is a considerable
delay before the first larvae hatch, 18 days. This suggests that at
summer temperatures the eggs need this period to mature following
flooding. By contrast eggs of Aedes aegypti often begin to hatch
within 48 hours following flooding. The length of the delay will have
important consequences for the re-establishment of larval populations.

The extended period over which hatching occurs after flooding
is apparent in the data from the watered tree-holes.

Howard, Dyar and Knab (1915) pointed out that only a proportion
of the mosquito eggs laid on wet surfaces hatch each time the breeding
places are submerged.

Atkin and Bacot (1917) found that hatching of Aedes aegypti
immersed after incubation and drying is generally erratic, a few eggs

may hatch after minutes of immersion while others may not hatch after days or even months under water.

Marshall (1938) reported that Aedes cantans eggs submerged may appear in instalments over a period of three months.

In some mosquito species it seems that selection has favoured prolonged diapause, in other shorter, while in other a high variation of response could be found, even within a single batch of eggs (Gillet, 1955). Gillet stated the opinion that variation in the hatching response of Aedes eggs to uniform stimulation has a genetic basis.

Pupae begin to appear mid-June with adult probably emerging from the end of June. Oviposition probably continued from June and throughout the summer but eggs were noted in ovitraps placed near the tree-holes only during July and August.

At Silwood Park Aedes geniculatus appears to have only a single generation per year and overwinters in the egg stage.

Some fourth instar larvae pass the winter in the tree-holes, probably producing the first adults of the following year.

Water temperatures in the tree-holes are usually low in the spring, ranging from 4.6 to 12.4°C during the period March to May (Table 2.4.1). Larval development in the laboratory at 10°C took an average of 29 days for females and 24 days for males (Table 4.4.1); development in the field would be slower.

During the summer months mean daily temperatures were between 14.2 and 18.7°C (Table 2.4.1) which would allow faster development; at 20°C the development times for female and male larvae in the laboratory were 15.5 and 12.9 days respectively. Since the laboratory conditions included adequate food for larvae then it might be expected that actual developmental times in the field would be greater. Even so, developmental times were short enough to allow more than one generation per year and the obligatory diapause must be the only factor which limits Ae. geniculatus to one generation per year.

In the tree-holes, larval mortality was most noticeable during the early instars, particularly in the first instar. This mortality probably was due to a poor supply of food in the tree-hole water. De Meillon et al. (1967) pointed out that lack of food during the first hours of larval life cause very high levels of mortality in the population, and reported 92% larval mortality of Culex pipieus fatigans hatching in water devoid of food for 12 hours.

The container experiments with Ae. aegypti also highlighted the susceptibility of first and second instars to stress. It was these young larvae which suffered most under crowded conditions or when food, although adequate in total, was scarce by being spaced out in the water.

It was interesting that conditioning factors could not be implicated as playing a part in the survival of successive colonies of larvae and that the only carry-over effect was depletion of food reserves.

Unfortunately the planned comparison of the effects of varying density on _Ae. aegypti_ and _Ae. geniculatus_ was not possible. It would be undoubtedly informative if such comparisons could be to elucidate the physiology as well as the dynamics of intraspecific competition particularly where small bodies of transient water are concerned and where, because through the mechanism of flooding of the eggs, flushes of larvae and therefore often high densities occur.

SUMMARY

1. Field and laboratory studies were undertaken from 1974 to 1976
    on the ecology of Aedes geniculatus and Aedes aegypti.

2. Water filled tree-holes are the most common natural containers-
    habitat for mosquitoes breeding in the temperate zone. Tree-
    holes in beech trees in a section of the woods of Imperial
    College at Silwood Park, Ascot were chosen for the study.

3. Two species of mosquito Aedes geniculatus and Anopheles plumbeus
    were found in the tree-holes studied. In no case was
    Orthopodomyia pulchripalpis found during this study.

4. Aedes geniculatus eggs are laid above the water level, in cracks
    and crevices of the bark lining the tree-holes.

5. Eggs of Aedes geniculatus were 1.04 mm in length and 0.34 mm
    width.

6. Ovipositing females of Aedes geniculatus show a high selectivity
    for ovipositing in tree-holes.

7. In the field, at summer temperatures Aedes geniculatus eggs
    take a minimum of 14 days to hatch after being submerged in water.

8. The larvae of Aedes geniculatus are present in the tree-holes at
    all times of the year, but show marked seasonal fluctuations.

SUMMARY (Continued)

9. The highest larval populations during 1975 in all sizes of
   tree-holes were noted at the end of July.
   In watered artificially tree-holes during 1976 the highest
   populations were noted in mid-July in large size tree-holes.

10. From September to February the larval populations are in very
    low numbers in all the size-groups of tree-holes and are usually
    composed entirely of fourth instar larvae.

11. The large size tree-holes had the highest peak population of
    Aedes geniculatus and in general the level of population was
    always higher than in medium and small size tree-holes.

12. Aedes geniculatus appear to have only a single generation
    per year, and pass the winter in the egg stage or as quiescent
    third or fourth instar larvae.

13. High larval mortality occurred, mainly during the first instar.

14. Limiting factors, such as inadequate supply of food, and
    certainly drought conditions keep populations of Aedes geniculatus
    at moderate levels.

15. Because very few pupae were sampled during the whole period of
    study it can be inferred that adults were not very abundant in
    the area.

SUMMARY (Continued)

16. In no case was diapause broken in the laboratory. In the field termination of diapause probably occurs by mid-March when the first peak of first instar larvae appear in the tree-holes.

17. Females reared in the laboratory did not lay fertile eggs.

18. Temperature has a drastic effect on the larval and pupal development of Aedes geniculatus; at $10^{\circ}C$, the pre-adult development was twice as long as at $20^{\circ}C$.

19. Pre-adult developmental time for Aedes geniculatus in the laboratory was shorter for males than for females. There was a difference of 2.5 days and 6 days at 20° and $10^{\circ}C$ respectively, between the male and female developmental times.

20. Aedes geniculatus has a high larval survival rate in laboratory conditions, both at 10° and $20^{\circ}C$, when an adequate food supply is provided.

21. Adult body weight and wing length were used as indicators of body size. In Aedes geniculatus a significant correlation was found between both characters.

22. Adult females reared from wild larvae laid an average of 64.5 eggs in the first batch.

23. The preoviposition time after first blood meal of Aedes geniculatus was a minimum of 7 days.

SUMMARY (Continued)

24. Clear density effects, detected in rate of development, pre-adult mortality and adult size were observed within a range of larval densities of Aedes aegypti.

25. Very delayed larval development and high mortalities were observed at very low densities of Aedes aegypti, probably caused by starvation.

26. Aedes aegypti adults from low density cultures (4 larvae/ container) were of very low weight.

27. Small containers, representing simplified conditions of artificial and natural containers found in nature, were used for the study of density effects in larval cultures of Aedes aegypti.

28. Drastic mortalities of larvae were observed during the first days of larval life of Aedes aegypti reared at high densities, basically caused by large concentrations of food in the containers.

29. At mid-densities (16 to 64 larva per container) the pre-adult mortality of Aedes aegypti was low and the sex ratio normal.

30. Intraspecific competition for food among larvae of Aedes aegypti resulted in an increase of pre-adult mortality, male biased sex ratio and reduction of adult size.

SUMMARY (Continued)

31. Marked increases in mortality of groups of larvae introduced into established cultures were observed when density was increased and time of introduction was delayed.

32. Female and male _Aedes aetypti_ adults developing from late-introduced larvae were in general lighter than those produced from standard cultures.

33. When food was added to medium already used, the new larvae reared in them behave almost in the same way as cultures in fresh medium, except that adults produced were smaller than those from standard optimal conditions.

34. The lack of evidence of toxic effects would indicate that chemical overcrowding factors were not present.

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* Original not seen.

APPENDIX 2.3.1 <u>Ranking of 49 Tree-holes in Three Categories</u>

<u>of Size (Large, Medium and Small) on the Basis</u>

<u>of Their Maximum Axes</u>

(a)   Large tree-holes (maximum axis between 30 and 50 cm)

Tree-hole number	Maximum axis (cm)	Maximum perpendicular (cm)	Max. axis/ Max. perp. ratio
1a	30	15	0.50
1c	30	14	0.47
12a	30	7	0.23
13c	33	10	0.30
13d	43	14	0.33
13e	34	10	0.29
13h	50	12	0.24
15b	30	10	0.33
16a	42	12	0.29
17a	38	18	0.47
18a	40	18	0.45
27a	32	7	0.22
			$\bar{X}_1 = 0.34$

APPENDIX 2.3.1 (Continued)

(b)   Medium tree-holes (maximum axis between 16 and 28 cm)

Tree-hole number	Maximum axis (cm)	Maximum perpendicular (cm)	Max. axis/ Max. perp. ratio
1b	24	8	0.33
1d	23	10	0.43
2a	20	10	0.50
3	21	9	0.43
6	20	12	0.60
7b	24	14	0.58
8	19	8	0.42
12b	21	5	0.24
13a	28	8	0.44
13b	24	16	0.67
13g	21	8	0.38
13i	28	8	0.29
15a	21	10	0.48
16b	25	12	0.48
17c	20	5	0.25
18b	16	11	0.69
20a	25	11	0.44
20b	22	7	0.32
23b	17	11	0.65
25	20	4	0.24
26a	28	4	0.14
26b	18	12	0.67
27b	17	6	0.35
27c	26	11	0.42
27d	16	4	0.25
27e	19	8	0.42

$$\bar{X}_m = 0.43$$

APPENDIX 2.3.1 (Continued)

(c)   Small tree-holes (maximum axis between 7 and 14 cm)

Tree-hole number	Maximum axis (cm)	Maximum perpendicular (cm)	Max. axis/ Max. perp. ratio
2b	10	2	0.20
10	10	8	0.80
13f	16	5	0.31
14a	15	4	0.27
14b	7	4	0.57
16c	11	5	0.45
16d	7	5	0.71
17b	12	8	0.67
23a	14	6	0.43
24a	14	4	0.29
24b	13	8	0.62
			$\bar{X}_s$ = 0.48

APPENDIX 2.5.1 Free-water Volume (mm.) into Tree-holes at each

Sampling Occasion

SMALL HOLES

Date	10	13f	14a	16c	16d	24b	26a	27d
17 Dec 1974	250	275	75	–	125	–	–	175
15 Jan 1975	250	350	75	350	150	250	300	200
13 Feb 1975	350	375	50	400	125	300	300	150
14 Mar 1975	200	300	125	400	250	150	200	175
9 Apr 1975	175	375	100	350	250	100	200	125
8 May 1975	NFW*	225	25	NFW	250	150	NFW	100
22 May 1975	150	275	25	NFW	250	50	100	125
5 Jun 1975	NFW	225	50	NFW	250	100	NFW	100
19 Jun 1975	NFW	NFW	NFW	NFW	150	NFW	100	75
3 Jul 1975	NFW	NFW	Dry**	Dry	NFW	Dry	NFW	75
10 Jul 1975	250	250	25	100	125	150	100	100
17 Jul 1975	250	300	50	100	125	275	100	125
31 Jul 1975	NFW	150	NFW	NFW	100	NFW	100	75
7 Aug 1975	NFW	200	50	NFW	125	NFW	250	50
14 Aug 1975	150	275	50	NFW	75	125	100	75
27 Aug 1975	NFW	NFW	NFW	NFW	75	100	100	75
11 Sep 1975	Dry	NFW	Dry	Dry	75	NFW	NFW	75
26 Sep 1975	400	400	50	250	250	275	250	100
9 Oct 1975	350	325	25	200	250	400	250	75
29 Oct 1975	NFW	225	50	200	175	NFW	NFW	125
19 Nov 1975	NFW	275	50	200	175	NFW	NFW	125
18 Dec 1975	NFW	375	50	200	200	200	NFW	175
23 Jan 1976	Dry	50	Dry	NFW	125	100	NFW	100
19 Feb 1976	NFW	NFW	50	400	175	200	100	200
18 Mar 1976	NFW	NFW	50	400	175	200	150	175
8 Apr 1976	Dry	Dry	NFW	NFW	100	NFW	NFW	NFW
6 Mar 1976	Dry	Dry	Dry	Dry	50	Dry	Dry	Dry
20 May 1976	Dry	Dry	Dry	Dry	NFW	Dry	Dry	Dry
2 Jun 1976	Dry	Dry	Dry	Dry	NFW	Dry	Dry	Dry
11 Jun 1976	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry

APPENDIX 2.5.1 (Continued)

MEDIUM HOLES

Date	1b	6	7b	12b	13a	13g	15a	15b
17 Dec 1974	700	–	1550	–	800	400	–	900
15 Jan 1975	600	1000	1550	500	1100	600	300	900
13 Feb 1975	900	1200	1550	500	1200	750	250	NFW
14 Mar 1975	850	1000	1600	300	1200	600	200	800
9 Apr 1975	500	900	1500	500	1200	600	200	800
8 May 1975	350	900	1300	NFW	800	350	NFW	NFW
22 May 1975	600	1000	1500	300	1100	500	NFW	300
5 Jun 1975	500	500	500	300	1000	400	NFW	400
19 Jun 1975	NFW	NFW	500	200	400	100	NFW	NFW
3 Jul 1975	NFW	NFW	300	100	300	NFW	Dry	Dry
10 Jul 1975	NFW	1000	1300	500	300	600	200	900
17 Jul 1975	NFW	1200	1400	500	700	600	100	800
31 Jul 1975	Dry	400	NFW	200	200	100	NFW	NFW
7 Aug 1975	Dry	NFW	300	250	200	200	NFW	Dry
14 Aug 1975	NFW	NFW	1000	250	400	400	NFW	NFW
27 Aug 1975	Dry	Dry	NFW	NFW	400	400	NFW	NFW
11 Sep 1975	Dry	NFW	NFW	NFW	150	300	NFW	Dry
26 Sep 1975	600	1200	1550	300	1200	700	200	900
9 Oct 1975	600	300	1400	200	1000	650	200	300
29 Oct 1985	350	NFW	600	NFW	600	600	200	400
19 Nov 1975	550	300	800	NFW	700	600	150	500
18 Dec 1975	800	950	1500	NFW	750	600	100	600
23 Jan 1976	NFW	300	800	NFW	600	600	NFW	NFW
19 Feb 1976	NFW	800	1400	NFW	700	600	NFW	800
18 Mar 1976	NFW	700	1300	NFW	700	600	NFW	800
8 Apr 1976	NFW	NFW	400	NFW	150	500	Dry	Dry
6 May 1976	Dry	Dry	Dry	NFW	Dry	350	Dry	Dry
20 May 1976	Dry	Dry	Dry	Dry	Dry	300	Dry	Dry
2 Jun 1976	Dry	Dry	Dry	Dry	Dry	200	Dry	Dry
11 Jun 1976	Dry	Dry	Dry	Dry	Dry	100	Dry	Dry

APPENDIX 2.5.1   MEDIUM HOLES (Continued)

Date	17b	18b	20a	20b	23b	25	26b	27c
17 Dec 1974	–	500	–	–	300	400	450	–
15 Jan 1975	500	600	1500	500	300	400	500	700
13 Feb 1975	600	700	1500	700	700	400	600	700
14 Mar 1975	600	500	1500	500	500	300	450	700
9 Apr 1975	400	500	1400	500	400	400	450	700
8 May 1975	NFW	400	NFW	NFW	NFW	350	300	400
22 May 1975	300	200	1400	300	250	300	250	400
5 Jun 1975	300	100	1300	200	400	300	300	300
19 Jun 1975	NFW	100	NFW	NFW	NFW	300	NFW	NFW
3 Jul 1975	Dry	100	Dry	Dry	Dry	50	NFW	NFW
10 Jul 1975	200	300	1500	500	600	300	450	500
17 Jul 1975	100	300	1500	350	400	450	400	450
31 Jul 1975	NFW	300	800	NFW	NFW	600	250	NFW
7 Aug 1975	NFW	300	700	NFW	Dry	200	600	NFW
14 Aug 1975	100	300	1300	NFW	NFW	300	300	350
27 Aug 1975	NFW	200	800	NFW	Dry	350	300	NFW
11 Sep 1975	Dry	NFW	NFW	NFW	Dry	NFW	250	NFW
26 Sep 1975	300	400	1500	600	700	450	600	500
9 Oct 1975	300	400	1400	350	250	450	400	500
29 Oct 1975	NFW	300	800	200	NFW	350	150	400
19 Nov 1975	200	300	1100	300	NFW	400	250	400
18 Dec 1975	300	200	1300	400	NFW	450	300	400
23 Jan 1976	NFW	300	NFW	300	Dry	200	200	300
19 Feb 1976	600	300	1200	400	NFW	300	300	450
18 Mar 1976	600	200	1100	400	NFW	300	300	400
8 Apr 1976	Dry	NFW	NFW	NFW	Dry	100	NFW	NFW
6 May 1976	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
20 May 1976	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
2 Jun 1976	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
11 Jun 1976	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry

APPENDIX 2.5.1 (Continued)

LARGE HOLES

Date	1c	12a	13c	13e	13h	16a	17a	18a
17 Dec 1974	1500	1	1800	1600	1500	-	-	2600
15 Jan 1975	1700	700	2300	1700	1400	1600	1000	2700
13 Feb 1975	1800	800	2300	1700	1800	1000	500	3200
14 Mar 1975	1700	800	2200	1700	1800	1700	500	3200
9 Apr 1975	1700	700	2200	1600	1700	1400	NFW	3200
8 May 1975	1100	700	2100	1200	700	600	NFW	3200
22 May 1975	1100	600	1500	1200	1400	1000	NFW	3100
5 Jun 1975	1100	600	2100	900	1400	1400	NFW	2700
19 Jun 1975	500	NFW	600	NFW	400	1000	Dry	1900
3 Jul 1975	500	NFW	600	Dry	NFW	300	Dry	1600
10 Jul 1975	1700	700	1500	900	600	1000	500	2100
17 Jul 1975	1400	700	2100	1400	1400	1000	1200	1900
31 Jul 1975	500	300	1100	700	400	NFW	Dry	1000
7 Aug 1975	200	400	900	NFW	400	NFW	Dry	1900
14 Aug 1975	800	500	1800	700	1000	400	Dry	1600
27 Aug 1975	800	600	1300	NFW	1800	1000	Dry	1600
11 Sep 1975	300	300	600	NFW	NFW	NFW	Dry	NFW
26 Sep 1975	1700	900	1300	1900	1700	1000	600	3100
9 Oct 1975	1400	600	1900	1500	1200	1000	Dry	2700
29 Oct 1975	1100	600	1300	900	700	800	Dry	2700
19 Nov 1975	1400	700	1500	900	900	800	NFW	2700
18 Dec 1975	1700	800	1800	1200	1000	800	NFW	2700
23 Jan 1976	800	600	1100	1000	NFW	300	Dry	2600
19 Feb 1976	1800	900	1500	1000	1400	400	300	2300
18 Mar 1976	600	500	600	NFW	NFW	NFW	NFW	1200
8 Apr 1976	600	500	600	NFW	NFW	NFW	NFW	1200
6 May 1976	800	Dry	NFW	Dry	Dry	Dry	Dry	NFW
20 May 1076	300	NFW	Dry	Dry	Dry	Dry	Dry	NFW
2 Jun 1976	NFW	NFW	Dry	Dry	Dry	Dry	Dry	NFW
11 Jun 1976	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry

* No free water, but wet hole   ** Dry hole

APPENDIX 3.3.1   Anopheles plumbeus Larvae Found During the Sampling

Programme 1974-1976.

Date	No. of Anopheles plumbeus per sample	Tree-hole No.	Tree-hole Size
17 Dec. 1974	2	13a	M
15 Jan. 1975	1	13a	M
15 Jan. 1975	1	16a	L
13 Feb. 1975	1	6	M
13 Feb. 1975	1	16a	L
14 Mar. 1975	1	6	M
8 May 1975	2	13a	M
9 Jun. 1975	1	13a	M
31 Jul. 1975	1	13c	L
7 Aug. 1975	1	13c	L
14 Aug. 1975	1	25	M
27 Aug. 1975	4	13a	M
26 Sep. 1975	1	1c	L
29 Oct. 1975	1	25	M
18 Dec. 1975	1	25	M
23 Jan. 1976	4	13a	M
18 Mar. 1976	1	1c	L
10 Nov. 1976	1	16d*	S

*   Tree-hole artificially watered after June 1976.

APPENDIX 3.3.2   Larval Populations of <u>Aedes geniculatus</u> by Instar (Four Instars)

SMALL SIZE TREE-HOLES

Date	10	13f	14a	16c	16d	24b	26a	27d
17 Dec 1974	(0)	(0)	(0)	*	0-0-3-0	*	*	(0)
15 Jan 1975	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
13 Feb 1975	(0)	(0)	(0)	(0(	5-0-0-0	(0)	(0)	(0)
14 Mar 1975	(0)	(0)	(0)	(0)	0-1-0-0	(0)	(0)	7-0-0-0
9 Apr 1975	(0)	(0)	(0)	0-1-0-0	(0)	(0)	0-2-0-0	2-1-0-0
8 May 1975	NFW	(0)	(0)	(0)	0-14-2-4	(0)	NFW	5-1-1-3
22 May 1975	1-0-0-0	(0)	(0)	NFW	6-2-2-0	(0)	NFW	0-7-1-2
3 Jun 1975	NFW	(0)	(0)	NFW	10-2-7-0	(0)	NFW	1-2-1-5
19 Jun 1975	NFW	NFW	NFW	NFW	0-5-5-6	NFW	(0)	0-2-4-2
3 Jul 1975	NFW	NFW	DRY	DRY	NFW	DRY	NFW	0-3-1-2
10 Jul 1975	(0)	(0)	(0)	(0)	(0)	(0)	(0)	0-5-0-1
17 Jul 1975	(0)	(0)	(0)	(0)	0-7-0-0	(0)	0-2-3-2	(0)
31 Jul 1975	NFW	(0)	NFW	NFW	2-1-0-0	NFW	0-2-1-0	(0)
7 Aug 1975	NFW	(0)	(0)	NFW	0-0-6-0	NFW	0-2-0-0	(0)
14 Aug 1975	(0)	(0)	(0)	NFW	1-1-2-4	(0)	(0)	(0)
17 Aug 1975	NFW	NFW	NFW	NFW	0-0-1-4	(0)	0-1-0-0	(0)
11 Sep 1975	DRY	NFW	DRY	DRY	0-0-1-0	NFW	NFW	(0)
26 Sep 1975	(0)	(0)	(0)	(0)	0-1-0-1	(0)	(0)	(0)
9 Oct 1975	(0)	(0)	(0)	(0)	0-1-1-1	(0)	(0)	(0)
29 Oct 1975	NFW	(0)	(0)	(0)	0-1-1-2	NFW	NFW	(0)
11 Nov 1975	NFW	(0)	(0)	(0)	(0)	NFW	NFW	(0)
18 Dec 1975	NFW	(0)	(0)	(0)	(0)	(0)	NFW	0-1-0-1
23 Jan 1976	DRY	(0)	NFW	NFW	0-0-1-5	(0)	NFW	0-2-3-4
19 Feb 1976	NFW	NFW	(0)	(0)	0-0-1-3	(0)	(0)	0-1-1-3
18 Mar 1976	NFW	NFW	(0)	(0)	12-0-0-4	(0)	(0)	3-2-1-0
8 Apr 1976	DRY	DRY	NFW	NFW	3-5-1-0	NFW	NFW	NFW
6 May 1976	DRY	DRY	DRY	DRY	1-3-4-0	DRY	DRY	DRY
20 May 1976	DRY	DRY	DRY	DRY	NFW	DRY	DRY	DRY
2 Jun 1976	DRY	DRY	DRY	DRY	NFW	DRY	DRY	DRY
11 Jun 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY

Continued .....

APPENDIX 3.3.2 (Continued)   MEDIUM SIZE TREE-HOLES

Date	1b	6	7b	12b	13a	13g	15a	15b
17 Dec 1974	0-0-0-2	*	(0)	*	(0)	(0)	*	(0)
15 Jan 1975	0-0-0-1	0-1-0-0	(0)	(0)	(0)	(0)	(0)	(0)
13 Feb 1975	(0)	0-1-0-0	(0)	(0)	(0)	1-0-0-0	(0)	NFW
19 Mar 1975	0-2-0-2	3-0-0-0	4-5-0-0	0-1-0-0	4-5-1-0	0-0-1-0	(0)	(0)
9 Apr 1975	0-1-0-1	6-4-0-0	0-0-1-0	(0)	(0)	(0)	(0)	(0)
8 May 1975	0-7-1-1	1-1-0-0	3-0-0-0	NFW	0-2-4-0	(0)	NFW	NFW
22 May 1975	1-1-0-0	2-0-0-0	2-0-0-0	(0)	2-0-0-0	1-0-0-0	NFW	(0)
5 Jun 1975	1-2-0-0	0-10-2-2	(0)	(0)	0-0-2-0	(0)	NFW	(0)
19 Jun 1975	NFW	NFW	(0)	(0)	0-0-3-0	(0)	NFW	NFW
3 Jul 1975	NFW	NFW	(0)	(0)	0-0-0-2	NFW	DRY	DRY
10 Jul 1975	NFW	1-0-0-0	(0)	(0)	(0)	(0)	(0)	(0)
17 Jul 1975	NFW	0-1-4-0	(0)	(0)	(0)	(0)	(0)	(0)
31 Jul 1975	DRY	(0)	NFW	(0)	(0)	1-31-0-0	NFW	NFW
7 Aug 1975	DRY	NFW	(0)	(0)	(0)	0-7-4-0	NFW	DRY
14 Aug 1975	NFW	NFW	(0)	(0)	(0)	0-3-1-0	NFW	NFW
27 Aug 1975	DRY	DRY	NFW	NFW	0-8-9-0	0-0-2-0	NFW	NFW
11 Sep 1975	DRY	NFW	NFW	NFW	0-0-2-0	0-1-0-0	NFW	DRY
26 Sep 1975	(0)	(0)	(0)	0-2-1-0	(0)	(0)	(0)	(0)
9 Oct 1975	(0)	(0)	(0)	(0)	(0)	(0)	0-1-0-0	(0)
29 Oct 1975	(0)	NFW	(0)	NFW	0-1-0-1	(0)	(0)	(0)
19 Nov 1975	(0)	(0)	0-3-6-0	NFW	0-0-0-1	(0)	(0)	(0)
18 Dec 1975	(0)	(0)	0-0-0-7	NFW	(0)	(0)	(0)	(0)
23 Jan 1976	NFW	(0)	(0)	NFW	(0)	(0)	NFW	NFW
19 Feb 1976	NFW	(0)	(0)	NFW	(0)	(0)	NFW	(0)
18 Mar 1976	NFW	1-1-0-0	1-1-0-0	NFW	2-6-0-0	(0)	NFW	(0)
8 Apr 1976	NFW	NFW	1-0-0-0	NFW	0-0-5-1	(0)	DRY	DRY
6 May 1976	DRY	DRY	DRY	NFW	DRY	(0)	DRY	DRY
20 May 1976	DRY	DRY	DRY	DRY	DRY	(0)	DRY	DRY
2 Jun 1976	DRY	DRY	DRY	DRY	DRY	(0)	DRY	DRY
11 Jun 1976	DRY	DRY	DRY	DRY	DRY	(0)	DRY	DRY

APPENDIX 3.3.2 (Continued)   MEDIUM SIZE TREE-HOLES

Date	17b	18b	20a	20b	23b	25	26b	27c
17 Dec 1974	-	0-0-0-1	0-0-1-0	-	0-0-1-0	0-0-2-3	0-0-0-2	-
15 Jan 1975	(0)	(0)	(0)	(0)	0-1-0-0	(0)	(0)	(0)
13 Feb 1975	1-2-0-0	0-2-1-0	(0)	(0)	(0)	(0)	(0)	(0)
14 Mar 1975	2-1-0-0	2-2-0-0	(0)	3-0-0-0	(0)	2-0-0-0	3-0-1-0	(0)
9 Apr 1975	(0)	0-0-2-0	(0)	0-0-1-0	(0)	0-2-2-0	0-1-0-0	(0)
8 May 1975	NFW	2-0-1-0	NFW	NFW	NFW	1-1-0-0	0-0-1-0	(0)
22 May 1975	0-5-0-0	0-1-1-3	(0)	8-0-1-1	(0)	0-0-0-1	3-0-0-0	(0)
5 Jun 1975	1-3-0-0	0-1-0-0	(0)	0-1-0-0	(0)	0-1-0-4	(0)	(0)
19 Jun 1975	NFW	(0)	NFW	NFW	NFW	(0)	NFW	NFW
3 Jul 1975	DRY	(0)	DRY	DRY	DRY	(0)	NFW	NFW
10 Jul 1975	(0)	(0)	(0)	1-0-0-0	(0)	(0)	(0)	(0)
17 Jul 1975	1-0-0-0	(0)	(0)	1-1-0-0	(0)	(0)	(0)	(0)
31 Jul 1975	NFW	3-0-0-0	43-0-0-0	NFW	NFW	0-0-2-0	43-4-0-0	NFW
7 Aug 1975	NFW	(0)	0-27-0-0	NFW	DRY	0-4-0-0	0-18-1-0	NFW
19 Aug 1975	(0)	(0)	2-19-4-0	NFW	NFW	0-10-3-0	0-7-0-0	(0)
27 Aug 1975	NFW	0-10-1-0	0-6-8-0	NFW	DRY	0-4-0-0	0-6-6-0	NFW
11 Sep 1975	DRY	NFW	NFW	NFW	DRY	NFW	(0)	NFW
26 Sep 1975	2-1-0-0	(0)	(0)	0-0-1-0	(0)	(0)	(0)	(0)
9 Oct 1975	0-1-0-0	(0)	(0)	(0)	(0)	(0)	(0)	(0)
29 Oct 1975	NFW	(0)	(0)	(0)	NFW	(0)	(0)	(0)
19 Nov 1975	0-0-2-2	(0)	(0)	(0)	NFW	(0)	(0)	(0)
18 Dec 1975	0-1-0-2	(0)	(0)	(0)	NFW	(0)	(0)	(0)
23 Jan 1976	NFW	(0)	NFW	(0)	DRY	0-0-2-0	(0)	(0)
19 Feb 1976	(0)	(0)	(0)	(0)	NFW	0-0-1-0	(0)	(0)
18 Mar 1976	4-0-0-0	2-0-0-0	(0)	(0)	NFW	13-2-0-0	(0)	(0)
8 Apr 1976	DRY	NFW	NFW	NFW	DRY	2-2-0-0	NFW	NFW
6 May 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
20 May 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
2 Jun 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
11 Jun 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY

APPENDIX 3.3.2 (Continued)   LARGE SIZE TREE-HOLES

Date	1c	12a	13c	13e	13h	16a	17a	18a
17 Dec 1974	0-0-1-0	*	(0)	(0)	(0)	*	*	0-0-4-0
15 Jan 1975	(0)	0-0-1-0	(0)	(0)	(0)	0-3-1-0	(0)	0-2-0-4
13 Feb 1975	0-2-0-1	1-1-0-0	(0)	(0)	(0)	3-4-1-0	(0)	(0)
14 Mar 1975	0-0-0-1	0-2-0-0	0-1-0-0	(0)	(0)	7-0-0-0	(0)	(0)
9 Apr 1975	(0)	2-0-2-0	(0)	1-0-0-0	(0)	0-1-0-0	NFW	(0)
8 May 1975	0-1-0-0	0-0-0-1	(0)	0-1-0-2	(0)	9-6-1-0	NFW	(0)
22 May 1975	1-0-0-0	0-1-2-1	(0)	(0)	(0)	10-9-4-0	NFW	1-0-0-0
5 Jun 1975	(0)	(0)	(0)	0-0-0-2	0-0-0-1	0-2-0-0	NFW	(0)
19 Jun 1975	0-0-0-0	NFW	(0)	NFW	(0)	0-0-0-1	DRY	(0)
3 Jul 1975	(0)	NFW	(0)	DRY	NFW	0-0-2-0	DRY	(0)
10 Jul 1975	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
17 Jul 1975	0-2-2-0	0-1-0-0	4-63-0-0	(0)	(0)	0-0-1-0	(0)	0-3-0-0
31 Jul 1975	11-20-0-2	0-11-0-0	42-21-0-0	12-21-0-0	12-7-0-0	NFW	DRY	0-0-4-0
7 Aug 1975	0-7-1-0	(0)	1-33-12-0	NFW	4-7-0-0	NFW	DRY	(0)
14 Aug 1975	0-0-1-0	(0)	0-3-4-0	1-2-0-0	0-0-3-0	(0)	DRY	2-0-1-2
27 Aug 1975	1-0-0-0	(0)	1-0-1-0	NFW	2-5-3-0	0-1-0-0	DRY	(0)
11 Sep 1975	(0)	(0)	(0)	NFW	NFW	NFW	DRY	NFW
26 Sep 1975	0-1-0-0	(0)	0-1-0-0	(0)	(0)	(0)	(0)	(0)
9 Oct 1975	0-0-1-1	(0)	(0)	(0)	(0)	(0)	DRY	(0)
29 Oct 1975	0-0-1-0	(0)	0-0-1-0	0-0-1-0	(0)	0-0-1-0	DRY	(0)
19 Nov 1975	(0)	0-0-1-0	0-0-1-0	0-0-2-0	(0)	(0)	NFW	(0)
18 Dec 1975	(0)	0-0-0-1	(0)	(0)	(0)	(0)	NFW	(0)
23 Jan 1976	1-0-0-0	(0)	0-0-2-0	0-1-1-1	NFW	(0)	NFW	(0)
19 Feb 1976	1-0-0-0	(0)	0-0-1-0	0-2-1-1	(0)	(0)	(0)	(0)
18 Mar 1976	1-0-0-0	(0)	(0)	NFW	(0)	(0)	(0)	3-1-0-0
2 Apr 1976	(0)	0-0-0-1	(0)	NFW	NFW	NFW	NFW	1-0-0-0
6 May 1976	(0)	DRY	NFW	DRY	DRY	DRY	DRY	NFW
20 May 1976	(0)	NFW	DRY	DRY	DRY	DRY	DRY	NFW
2 Jun 1976	NFW	DRY	DRY	DRY	DRY	DRY	DRY	DRY
11 Jun 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY

(0) No larvae in the sample   NFW No free water, but wet hole   * Not sampled   DRY Dry hole

APPENDIX 3.3.3   Estimated Numbers of Larvae of <u>Aedes geniculatus</u>

<u>per Actual Volume of Water in 8 Small Size Tree-holes</u>

Date	10	13f	14a	16c	16d	24b	26a	27d
17 Dec 1974	(0)	(0)	(0)	*	6.8	*	*	(0)
15 Jan 1975	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
13 Feb 1975	(0)	(0)	(0)	(0)	12.5	(0)	(0)	(0)
14 Mar 1975	(0)	(0)	(0)	(0)	2.5	(0)	(0)	24.5
9 Apr 1975	(0)	(0)	(0)	8.8	(0)	(0)	13.3	9.4
8 May 1975	NFW	(0)	(0)	NFW	50.0	(0)	NFW	28.6
22 May 1975	7.5	(0)	(0)	NFW	25.0	(0)	NFW	31.3
5 Jun 1975	NFW	(0)	(0)	NFW	47.5	(0)	NFW	3.3
19 Jun 1975	NFW	NFW	NFW	NFW	40.0	NFW	(0)	30.0
3 Jul 1975	NFW	NFW	DRY	DRY	NFW	DRY	NFW	22.5
10 Jul 1975	(0)	(0)	(0)	(0)	(0)	(0)	(0)	20.0
17 Jul 1975	(0)	(0)	(0)	(0)	17.5	(0)	35.0	(0)
31 Jul 1975	NFW	(0)	NFW	NFW	3.3	NFW	15.0	(0)
7 Aug 1975	NFW	(0)	(0)	NFW	15.0	NFW	21.4	(0)
14 Aug 1975	(0)	(0)	(0)	(0)	30.0	(0)	(0)	(0)
27 Aug 1975	NFW	NFW	NFW	NFW	18.8	(0)	5.0	(0)
11 Sep 1975	DRY	NFW	DRY	DRY	3.8	NFW	NFW	(0)
26 Sep 1975	(0)	(0)	(0)	(0)	5.0	(0)	(0)	(0)
9 Oct 1975	(0)	(0)	(0)	(0)	7.5	(0)	(0)	(0)
29 Oct 1975	NFW	(0)	(0)	(0)	9.3	NFW	NFW	(0)
19 Nov 1975	NFW	(0)	(0)	(0)	(0)	NFW	NFW	(0)
18 Dec 1975	NFW	(0)	(0)	(0)	(0)	(0)	NFW	7.0
23 Jan 1976	DRY	(0)	DRY	NFW	13.6	(0)	NFW	25.7
19 Feb 1976	NFW	NFW	(0)	(0)	9.3	(0)	(0)	17.5
18 Mar 1976	NFW	NFW	(0)	(0)	37.3	(0)	(0)	21.0
8 Apr 1976	DRY	DRY	NFW	NFW	25.7	NFW	NFW	NFW
6 May 1976	DRY	DRY	DRY	DRY	26.7	DRY	DRY	DRY
20 May 1976	DRY	DRY	DRY	DRY	NFW	DRY	DRY	DRY
2 Jun 1976	DRY	DRY	DRY	DRY	NFW	DRY	DRY	DRY
11 Jun 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY

(0) No larva in the sample   * Not sampled in this occasion

NFW No free water, but wet hole   DRY Dry hole

APPENDIX 3.3.4 Estimated Numbers of Larvae of Aedes geniculatus

per actual Volume of Water in 16 Medium Size Tree-holes

Date	1b	6	7b	12b	13a	13g	15a	15b
17 Dec 1974	25.5	*	(0)	*	(0)	(0)	*	(0)
15 Jan 1975	12.0	14.3	(0)	(0)	(0)	(0)	(0)	(0)
13 Feb 1975	(0)	15.0	(0)	(0)	(0)	6.8	(0)	NFW
14 Mar 1975	52.3	42.9	144.0	10.0	109.1	6.7	(0)	(0)
9 Apr 1975	25.0	150.0	21.4	(0)	(0)	(0)	(0)	(0)
8 May 1975	105.0	30.0	7.2	NFW	60.0	(0)	NFW	NFW
22 May 1975	24.0	28.6	42.9	(0)	22.0	6.3	NFW	(0)
5 Jun 1975	37.5	233.3	(0)	(0)	22.2	(0)	NFW	(0)
19 Jun 1975	NFW	NFW	(0)	(0)	24.0	(0)	NFW	NFW
3 Jul 1975	NFW	NFW	(0)	(0)	15.0	NFW	DRY	DRY
10 Jul 1975	NFW	14.3	(0)	(0)	(0)	(0)	(0)	(0)
17 Jul 1975	NFW	75.0	(0)	(0)	(0)	(0)	(0)	(0)
31 Jul 1975	DRY	(0)	NFW	(0)	(0)	106.7	NFW	NFW
7 Aug 1975	NFW	NFW	(0)	(0)	(0)	55.0	NFW	DRY
14 Aug 1975	NFW	NFW	(0)	(0)	(0)	24.6	NFW	NFW
27 Aug 1975	DRY	DRY	NFW	(0)	136.0	11.4	NFW	NFW
11 Sep 1975	DRY	NFW	NFW	NFW	12.0	6.0	NFW	DRY
26 Sep 1975	(0)	(0)	(0)	NFW	(0)	(0)	(0)	(0)
9 Oct 1975	(0)	(0)	(0)	30.0	(0)	(0)	6.7	(0)
29 Oct 1975	(0)	NFW	(0)	(0)	18.5	(0)	(0)	(0)
19 Nov 1975	(0)	(0)	160.0	NFW	10.0	(0)	(0)	(0)
18 Dec 1975	(0)	(0)	150.0	NFW	(0)	(0)	(0)	(0)
23 Jan 1976	NFW	(0)	(0)	NFW	(0)	(0)	NFW	NFW
19 Feb 1976	NFW	(0)	(0)	NFW	(0)	(0)	NFW	(0)
18 Mar 1976	NFW	28.0	43.3	NFW	80.0	(0)	NFW	(0)
8 Apr 1976	NFW	NFW	16.0	NFW	36.0	(0)	DRY	DRY
6 May 1976	DRY	DRY	DRY	NFW	DRY	(0)	DRY	DRY
20 May 1976	DRY	DRY	DRY	DRY	DRY	(0)	DRY	DRY
2 Jun 1976	DRY	DRY	DRY	DRY	DRY	(0)	DRY	DRY
11 Jun 1976	DRY	DRY	DRY	DRY	DRY	(0)	DRY	DRY

Continued .....

APPENDIX 3.3.4 (Continued)

Date	17b	18b	20a	20b	23b	25	26b	27c
17 Dec 1974	*	8.3	21.4	*	12.0	50.0	22.5	*
15 Jan 1975	(0)	(0)	(0)	(0)	12.0	(0)	(0)	(0)
13 Feb 1975	30.0	30.0	(0)	(0)	(0)	(0)	(0)	(0)
14 Mar 1975	30.0	33.3	(0)	30.0	(0)	20.0	45.0	(0)
9 Apr 1975	(0)	16.7	(0)	10.0	(0)	40.0	11.3	(0)
8 May 1975	NFW	24.0	NFW	NFW	NFW	20.0	10.0	(0)
22 May 1975	50.0	33.0	(0)	100.0	(0)	10.0	30.0	(0)
5 Jun 1975	40.0	5.0	(0)	10.0	(0)	20.0	(0)	(0)
19 Jun 1975	NFW	(0)	NFW	NFW	NFW	(0)	NFW	NFW
3 Jul 1975	DRY	(0)	DRY	DRY	DRY	(0)	NFW	NFW
10 Jul 1975	(0)	(0)	(0)	10.0	(0)	(0)	(0)	(0)
17 Jul 1975	10.0	(0)	(0)	17.5	(0)	(0)	(0)	(0)
31 Jul 1975	NFW	25.7	1120.0	NFW	NFW	20.0	470.0	NFW
7 Aug 1975	NFW	(0)	756.0	NFW	DRY	32.0	228.0	NFW
14 Aug 1975	(0)	(0)	700.0	NFW	NFW	88.0	84.0	(0)
27 Aug 1975	NFW	88.0	3733.3	NFW	DRY	40.0	120.0	NFW
11 Sep 1975	DRY	NFW	NFW	NFW	DRY	NFW	(0)	NFW
26 Sep 1975	30.0	(0)	(0)	10.9	(0)	(0)	(0)	(0)
9 Oct 1975	10.0	(0)	(0)	(0)	(0)	(0)	(0)	(0)
29 Oct 1975	NFW	(0)	(0)	(0)	NFW	(0)	(0)	(0)
19 Nov 1975	40.0	(0)	(0)	(0)	NFW	(0)	(0)	(0)
18 Dec 1975	30.0	(0)	(0)	(0)	NFW	(0)	(0)	(0)
23 Jan 1976	NFW	(0)	NFW	(0)	DRY	16.0	(0)	(0)
19 Feb 1976	(0)	(0)	(0)	(0)	NFW	10.0	(0)	(0)
18 Mar 1976	36.9	13.3	(0)	(0)	NFW	150.0	(0)	(0)
8 Apr 1976	DRY	NFW	NFW	NFW	DRY	DRY	NFW	NFW
6 May 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
20 May 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
2 Jul 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY
11 Jul 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY

(0) No Larvae in the sample   * Not sampled

NFW No free water, but wet hole   DRY Dry hole

APPENDIX 3.3.5   Estimated Numbers of Larvae of Aedes geniculatus per

Actual Volume of Water in 8 Large Size Tree-holes

Date	1c	12a	13c	13e	13h	16a	17a	18a
17 Dec 1974	23.08	*	(0)	(0)	(0)	*	*	122.4
15 Jan 1975	(0)	11.7	(0)	(0)	(0)	116.4	(0)	180.0
13 Feb 1975	67.50	22.9	(0)	(0)	(0)	200.0	(0)	(0)
14 Mar 1975	24.3	24.6	20.95	(0)	(0)	198.3	(0)	(0)
9 Apr 1975	(0)	46.7	(0)	26.7	(0)	28.0	NFW	(0)
8 May 1975	22.00	11.7	(0)	30.00	(0)	320.0	NFW	(0)
22 May 1975	22.00	48.0	(0)	(0)	(0)	575.0	NFW	31.0
5 Jun 1975	(0)	(0)	(0)	60.01	28.0	56.0	NFW	(0)
19 Jun 1975	(0)	NFW	(0)	NFW	(0)	25.0	DRY	(0)
3 Jul 1975	(0)	NFW	(0)	DRY	NFW	30.0	DRY	(0)
10 Jul 1975	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
17 Jul 1975	93.3	11.7	1407.0	(0)	(0)	25.0	(0)	95.0
31 Jul 1975	550.00	(0)	1155.0	1155.0	380.0	NFW	DRY	114.3
7 Aug 1975	106.7	(0)	888.0	NFW	220.0	NFW	DRY	(0)
14 Aug 1975	20.00	(0)	140.0	105.0	75.0	(0)	DRY	160.0
27 Aug 1975	20.00	(0)	37.1	NFW	257.1	25.0	DRY	(0)
11 Sep 1975	(0)	(0)	(0)	NFW	NFW	NFW	DRY	NFW
26 Sep 1975	24.3	(0)	(0)	(0)	(0)	(0)	(0)	(0)
9 Oct 1975	46.7	(0)	(0)	(0)	(0)	(0)	DRY	(0)
29 Oct 1975	28.0	(0)	(0)	30.0	(0)	22.9	DRY	(0)
19 Nov 1975	(0)	11.7	11.7	60.0	(0)	(0)	NFW	(0)
18 Dec 1975	(0)	12.3	12.3	(0)	(0)	(0)	NFW	(0)
23 Jan 1976	20.0	(0)	36.7	86.7	NFW	(0)	NFW	(0)
19 Feb 1976	24.0	(0)	18.8	133.3	(0)	(0)	(0)	(0)
18 Mar 1976	17.4	(0)	(0)	NFW	(0)	(0)	(0)	90.0
8 Apr 1976	(0)	12.5	(0)	NFW	NFW	NFW	NFW	30.0
6 May 1976	(0)	DRY	NFW	DRY	DRY	DRY	DRY	NFW
20 May 1976	(0)	NFW	DRY	DRY	DRY	DRY	DRY	NFW
2 Jun 1976	NFW	DRY	DRY	DRY	DRY	DRY	DRY	DRY
11 Jun 1976	DRY	DRY	DRY	DRY	DRY	DRY	DRY	DRY

(0) No larvae in the sample   * Not sampled

NFW No free water, but wet hole   DRY Dry hole

APPENDIX 3.3.6   Larval populations of _Aedes geniculatus_, by instar, per sample of 16 tree-holes watered artificially

Date	Small size tree-hole No.				Medium size tree-hole No.								Large size tree-hole No.			
	13f	16c	16d	27d	7b	12b	17b	18b	20a	25	26b	27c	1c	12a	13c	13e
29 Jun 76	46-18-0-0	(0)	1-1-0-0	(0)	6-7-0-0	(0)	(0)	2-0-0-0	(0)	0-24-0-0	(0)	10-3-0-0	0-3-0-0	(0)	1-16-0-0	16-5-0-0
7 Jul 76	3-37-4-0	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	1-0-0-4	(0)	9-2-6-0	23-12-31-0
15 Jul 76	5-4-0-0	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	17-0-0-0	(0)	54̶-7-0-0	13-2-0-5
30 Jul 76	0-15-3-0	(0)	0-0-2-0	(0)	0-43-40-0	(0)	0-3-0-0	(0)	(0)	0-1-0-0	0-1-0-0	(0)	6-3-0-0	(0)	51-0-0-0	0-0-3-0
9 Aug 76	0-2-1-0	(0)	(0)	(0)	0-6-2-0	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	0-2-4-0
24 Aug 76	(0)	(0)	1-1-0-0	(0)	0-0-0-1	(0)	(0)	(0)	(0)	(0)	(0)	(0)	1-0-0-0	(0)	0-0-0-3	0-5-0-0
6 Sep 76	1-1-0-0	(0)	(0)	(0)	0-3-2-0	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	0-3-1-0	13-2-0-0
6 Oct 76	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	0-0-0-2
10 Nov 76	(0)	(0)	0-0-0-2	0-1-0-0	(0)	(0)	(0)	(0)	(0)	0-0-2-0	0-0-0-2	(0)	(0)	(0)	(0)	(0)
15 Dec 76	(0)	(0)	0-0-0-1	(0)	0-0-0-2	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)

APPENDIX 3.3.7   Estimated number of larvae of *Aedes* *geniculatus* per actual maximum volume of water in 16 tree-holes watered artificially

Date	Small size tree-hole No.				Medium size tree-hole No.								Large size tree-hole No.			
	13f	16c	16d	27d	7b	12b	17b	18b	20a	25	26b	27c	1c	12a	13c	13e
29 Jun 1976	426.7	(0)	5.0	(0)	208	(0)	(0)	20	(0)	240	(0)	185.7	67.5	(0)	355.5	560.0
7 Jul 1976	293.3	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	112.5	(0)	355.5	1760.0
15 Jul 1976	60.0	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	382.5	(0)	1275.5	553.3
30 Jul 1976	120.0	(0)	5.0	(0)	1328	(0)	30	(0)	(0)	10	13	(0)	22.5	(0)	1066.4	80.0
9 Aug 1976	20.0	(0)	(0)	(0)	128	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	160.0
24 Aug 1976	(0)	(0)	5.0	(0)	16	(0)	(0)	(0)	(0)	(0)	(0)	(0)	22.5	(0)	62.7	133.3
6 Sep 1976	13.3	(0)	(0)	(0)	80	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	83.6	400.0
6 Oct 1976	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	53.0
10 Nov 1976	(0)	(0)	5.0	3.6	(0)	(0)	(0)	(0)	(0)	25	26	(0)	(0)	(0)	(0)	(0)
15 Dec 1976	(0)	(0)	2.5	(0)	32	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)

APPENDIX 5.5.1   Analysis of Variance of % of Pupation of

Aedes aegypti

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F. ratio	Significance
Pre-use (P)	1	6.723	6.723	162.06	* * *
Density (D)	2	10.083	5.041	121.52	* * *
Additional Food (F)	1	16.782	16.782	404.48	* * *
P x D	2	0.174	0.087	2.11	ns
P x F	1	1.933	1.933	46.61	* * *
D x F	2	0.305	0.152	3.68	*
P x D x F	2	2.089	1.044	25.18	* * *
Within replicates	108	4.481	0.041		
TOTAL	119	42.570			

*   Significant at 5% level

* * * Significant at 0.1% level

ns   non significant

APPENDIX 5.5.2   Analysis of Variance of % of Emergence of

Aedes aegypti

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F. ratio	Significance
Pre-use (P)	1	7.103	7.103	149.39	* * *
Density (D)	2	8.323	4.161	87.52	* * *
Additional Food (F)	1	16.813	16.813	353.60	* * *
P x D	2	0.287	0.143	3.03	ns
P x F	1	1.756	1.756	36.95	* * *
D x F	2	0.284	0.142	2.99	ns
P x D x F	2	2.169	1.084	22.82	* * *
Within replicates	108	5.135	0.047		
TOTAL	119	41.870			

* * *   Significant at 0.1% level

ns   non significant

POPULATION ANALYSIS OF *CULEX PIPIENS FATIGANS* WIED. (DIPTERA: CULICIDAE) UNDER LABORATORY CONDITIONS

By Cela Gómez[1], Jorge E. Rabinovich[2] and C. E. Machado-Allison[1]

Abstract: A laboratory experiment using 4 cohorts of *Culex pipiens fatigans* was set up to obtain age-specific mortality and fecundity information, and to derive statistical estimates of some population parameters. Figures for developmental times, survival, reproduction and sex ratio are provided. Life expectancy is calculated for both males and females. The following population parameters were estimated: intrinsic rate of natural increase (r = 0.01), instantaneous birth and death rates, net replacement rate ($R_o' = 161.4$), finite rate of increase ($\lambda = 1.17$), generation time (T = 44.7 days), reproductive value and stable age distribution. The colonizing features of *Cx. p. fatigans* are compared with the ones of *Aedes aegypti* as the result of applying 2 colonization models, and it is concluded that although *Ae. aegypti* is a better colonizer, *Cx. p. fatigans* is an excellent r-strategist that can be classified as an opportunistic species.

· *Culex pipiens fatigans* is an important vector of various pathogenic agents. Its role as a vector of diseases produced by viruses, protozoans and helminths, its wide distribution throughout the tropical and subtropical urban areas, and its great capacity to adapt to a wide variety of ecological conditions are well known (San Martín-Barbieri et

al. 1953, Suárez & Bergold 1968, Hammon & Reeves 1943, Collins 1963, Mattingly 1962, de Meillon et al. 1967a, Jordan 1962, Desowitz & Chellapah 1962, Laven 1967).

Little research on the biology of tropical *Cx. p. fatigans* has been done, and that mainly in Brazil, Puerto Rico and French Guayana. In Venezuela, Scorza (1972) recently studied the bionomics of a strain from the Valley of Caracas; he shows that this strain has become adapted to a diversity of environmental conditions and human habitats, and both ·larvae and adults have developed resistance to insecticides. These mosquitoes feed on a variety of vertebrates, and the larvae are able to develop in water that is toxic for other freshwater animals.

An understanding of the population dynamics of a vector, so necessary for optimal control strategy, requires considerable quantitative data, such as life table statistics, which have been largely lacking for this species. The present study is an attempt to fill this need.

MATERIALS AND METHODS

The experimental population came from a *Cx. p.*

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fatigans strain collected from the drains around the School of Biology, Colinas de Bello Monte, Caracas, by C. Machado & A. Fergusson, 3 August 1971 (strain Bello Monte, I.Z.T.).

Egg rafts laid the night before were put in 250-cc plastic cups with tap water until hatching occurred. Soon after hatching, 100 larvae were transferred with a dropper to each of 6 plastic jars containing 1 liter of tap water, providing a water surface of 530 cm². To each jar, 1 g of powdered liver was added. The jars with larvae were kept in a controlled temperature chamber at 26 ± 2 °C until pupation.

Pupae from each jar were separated by sex and were put in groups of 20 in 75-cc plastic cups with approximately 60 cc of tap water; each cup was kept inside a 0.5-liter cylindrical cardboard box covered with a nylon net. From the time of pupation, individuals were kept under laboratory environmental conditions (25 ± 1 °C and 73.5 ± 10% RH).

Each cohort was prepared with 50 adults (25 females and 25 males) taken at random after anesthetizing all the recently emerged adults (9–48 hr old).

Individuals of each cohort were kept in a 4.73-liter cylindrical cardboard breeding box covered with a nylon net and a flannel side sleeve. Each box had a 50-cc cup of tap water that was changed every 48 hr. The adults were fed with slices of cooked apple that were placed on the net and changed every 72 hr.

Every 4th night, beginning from the day the cohorts were organized, a blood meal was offered to the females by introducing into each box a young chick immobilized within a nylon-net bag; the chick remained in the box from 1900 hr until 0800 hr next day.

Development time of each pre-adult stage was determined by checks at intervals specified in the Results; in this way the durations of the embryonic, larval, and pupal stages and time ranges of pupation and adult emergence were recorded.

Each adult breeding box was checked every 48 hr to record mortality and the number of eggs laid. Dead adults were removed from each box and counted; the water cup was replaced, the egg rafts were removed, and the number of rafts and eggs per raft were counted.

The mortality and fecundity values at fixed intervals were used to compile life tables (Deevey 1947) for each cohort, and to estimate such population parameters as generation time (T), net reproductive rate (R_o), intrinsic rate of natural increase (r), birth rate (B) and, instantaneous birth and death rates (b and d) (Birch 1948). Similarly, the

stable age distribution and the reproductive value (v_x) (Fisher 1930) were also calculated.

On 4 occasions during the experiment, groups of eggs were taken at random from each cohort (3786 eggs total). These eggs were allowed to hatch and larvae were reared to the adult stage as explained above. This allowed the calculation of hatching, pupation and emergence percentages, sex ratio and percentage of pre-adult death (embryonic, larval and pupal mortality).

The life tables and the population parameters were estimated with a computer program (PARPO) elaborated by one of the authors (JER) and carried out on an IBM 1130 computer at IVIC.

RESULTS

Developmental time

Values are given in TABLE 1 for the mean time of development of each pre-adult stage and the mean time of the pupation and emergence processes.

Average embryonic development time was 37 hr. The majority of the larvae were obtained during the 1st hour of hatching; after 2 hr no more hatched. With 600 recently hatched larvae (6 replicates of 100 larvae each) larval development was checked. The mean (±1 S.D.) development time was 164.74 ± 9.26 hr (approximately 7 days), with a range of 262 hr.

Pupation began 146 hr after hatching and lasted for 116 hr; 52.5% of the larvae pupated within 18 hr after the first pupation. Cultures were checked every 2 hr during the first 34 hr after pupation began, at which time 91% of the larvae had pupated

TABLE 1. Pre-adult development time (in hours) and survival (%) in *Culex pipiens fatigans* at 26 ± 2 °C.

	MEAN ± S.D.
Developmental time* (hr)	
Egg development	37
Larval development	164.74 ± 9.26
Pupal development	48.75 ± 4.98
Mean pupation time**	17.41 ± 7.49
Mean emergence time (♀♀)**	16.84 ± 6.33
Mean emergence time (♂♂)**	17.82 ± 9.45
Total pre-adult development (♀♀)	253.83 ± 5.44
Total pre-adult development (♂♂)	247.15 ± 11.27
Survival*** (%)	
Egg (% hatching)	82.08 ± 6.97
Larvae (% pupating)	58.20 ± 10.33
Pupae (% emerging)	52.58 ± 7.09
Relative egg mortality	17.92 ± 6.97
Relative larval mortality	23.88 ± 7.93
Relative pupal mortality	5.62 ± 6.13
Total pre-adult mortality	47.42 ± 7.10

*From 6 replicates of 100 first-stage larvae each.

**Mean of times of pupation or emergence relative to the total period of the respective process.
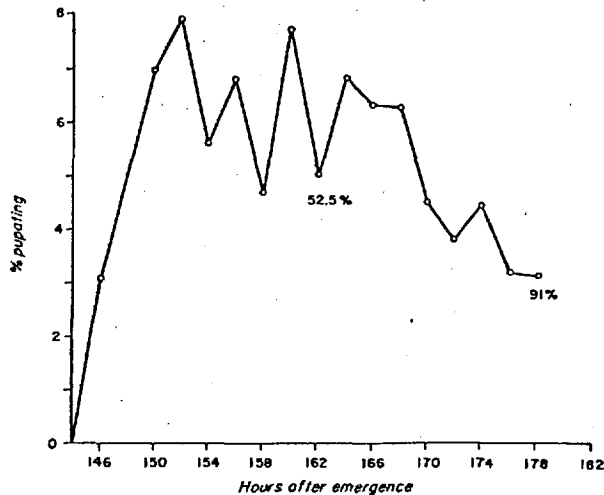
***From a total of 3786 eggs.

FIG. 1. Pupation in *Culex pipiens fatigans*: percentage of individuals pupating after the start of the process as a function of time.

TABLE 2. Reproductive characteristics of *Culex pipiens fatigans* ♀♀.

	Mean ± S.D.
First reproductive age (days) (α)	22.0 ± 2.0
Age of maximum reproductive effort (days) (γ)	86.0 ± 2.6
Total reproductive period (days)	.55.0 ± 14.1
Last reproductive age (days) (ω)	91.5 ± 6.4
Total number of eggs/replicate	12,115.5 ± 2127.1
Total number of eggs/♀	507.4 ± 45.1
Total number of eggs/oviposition	236.6 ± 21.7

(FIG. 1). The remaining 9% pupated during the following 82 hr. Mean pupation time over the period when pupation occurred was 17.41 ± 7.49 hr. Mean pupal development time was 48.75 ± 4.98 hr.

Mean time of emergence of females over the period of their emergence was 16.84 (±6.33) hr and this mean was slightly higher for males (17.82 ± 9.45 hr); the latter began emerging 190 hr after hatching while the former did so 194 hr after hatching. The process of emergence lasted 48 hr.

The overall pre-adult development time was 253.83 ± 5.44 hr for females and 247.15 ± 11.27 hr for males.

Mortality

Pre-adult mortality is expressed as relative mortality of each stage (number of dead individuals in each stage over the total number of dead individuals in the pre-adult stage × 100). Mean relative mortality of the larval and egg stages (23.88 and 17.92%, respectively) is markedly larger than that of the pupal stage (5.62%). Overall pre-adult mortality was 47.42%, i.e., only 52.58% of the original eggs reached the adult stage.

Reproduction

TABLE 2 shows values of the first (α), the last (ω) and the maximum reproductive effort (γ) (22, 91.5 and 86 days, respectively), and also the total number of eggs per cohort, the total number of eggs per female, and the number of eggs laid per oviposition (12, 115.5, 507.4 and 236.6, respectively).

FIG. 2-5 show the fecundity curves (m_x) for each replicate (number of eggs per female per 2-day

interval). The age-specific fecundity pattern of these curves is very irregular. However, there is one outstanding feature: the maximum reproductive effort (γ) takes place towards the end of reproductive life.

Life tables

TABLE 3 represents a "horizontal" life table (Southwood 1971) of the female population, obtained from the 4 cohorts. It is a compressed life table showing only the basic columns with the pivotal age (x), the survival at age x (l_x), and the life expectancy (e_x) at age x; the intermediate columns L_x and T_x necessary to compute the latter are not shown.

Adult survival

Adult mortality, checked at 48-hr intervals, resulted in a mean longevity of 60.25 days for females and of 42.65 days for males. Average maximum longevity was 102.5 days for the former and 63 days for the latter. These results are shown in TABLE 4, together with the percentage of adult females in the progeny.

An idea of age-specific female mortality can also be obtained from FIG. 2-5, which show the survival curves for females on the basis of the specific mortality values of each age (l_x) expressed as the prob-
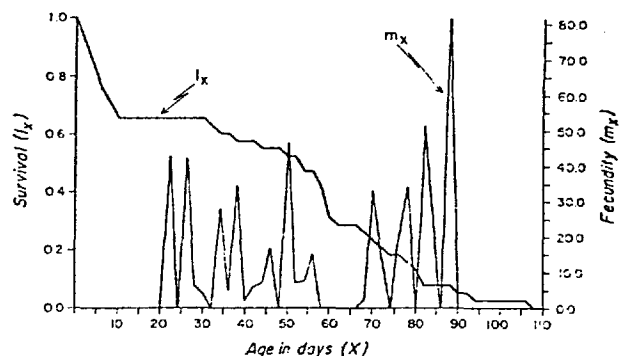

FIG. 2. Survivorship and fecundity curves of *Cx. p. fatigans* ♀♀. The survivorship curve shows the probability of arriving alive at a certain age x; the fecundity curve shows the number of ♀ eggs laid per ♀ every 2 days. Replicate No. 1.

TABLE 3. Average and 1 standard deviation of the survival (l_x) and life expectancy (e_x in 2-day units) columns of the life table of *Culex pipiens fatigans* ♀♀ beginning at oviposition. Data obtained from 4 replicates.

Age	l_x		e_x	
x	Mean ± S.D.		Mean ± S.D.	
1	1.000 ± 0.000		19.64 ± 2.12	
3	0.929 ± 0.049		20.14 ± 2.55	
5	0.848 ± 0.041		21.00 ± 2.67	
7	0.773 ± 0.046		22.01 ± 2.98	
9	0.704 ± 0.049		23.10 ± 3.04	
11	0.648 ± 0.043		24.03 ± 3.06	
13	0.611 ± 0.055		24.45 ± 2.76	
15	0.600 ± 0.048		23.84 ± 2.17	
17	0.600 ± 0.048		22.84 ± 2.17	
19	0.600 ± 0.048		21.84 ± 2.17	
21	0.600 ± 0.048		20.84 ± 2.17	
23	0.600 ± 0.048		19.84 ± 2.17	
25	0.600 ± 0.048		18.84 ± 2.17	
27	0.593 ± 0.060		18.07 ± 2.01	
29	0.581 ± 0.061		17.38 ± 1.57	
31	0.539 ± 0.086		17.78 ± 1.65	
33	0.533 ± 0.075		16.95 ± 1.57	
35	0.513 ± 0.079		16.59 ± 0.94	
37	0.513 ± 0.079		15.59 ± 0.94	
39	0.506 ± 0.069		14.76 ± 0.94	
41	0.506 ± 0.069		13.76 ± 0.94	
43	0.499 ± 0.079		12.97 ± 0.79	
45	0.487 ± 0.075		12.27 ± 0.63	
47	0.481 ± 0.080		11.42 ± 0.49	
49	0.475 ± 0.087		10.58 ± 0.52	
51	0.469 ± 0.079		9.71 ± 0.59	
53	0.469 ± 0.079		8.71 ± 0.59	
55	0.432 ± 0.037		8.39 ± 1.29	
57	0.400 ± 0.074		8.03 ± 0.56	
59	0.375 ± 0.058		7.52 ± 0.81	
61	0.337 ± 0.030		7.38 ± 1.82	
63	0.298 ± 0.047		7.24 ± 1.61	
65	0.243 ± 0.043		7.69 ± 1.36	
67	0.226 ± 0.044		7.35 ± 2.07	
69	0.201 ± 0.048		7.14 ± 1.57	
71	0.170 ± 0.055		7.47 ± 1.71	
73	0.163 ± 0.045		6.67 ± 1.64	
75	0.151 ± 0.031		6.16 ± 2.05	
77	0.125 ± 0.048		6.36 ± 1.89	
79	0.112 ± 0.048		6.06 ± 1.20	
81	0.105 ± 0.040		5.30 ± 1.05	
83	0.092 ± 0.037		5.06 ± 1.75	
85	0.081 ± 0.044		4.88 ± 1.69	
87	0.057 ± 0.025		6.33 ± 3.89	
89	0.050 ± 0.029		5.58 ± 3.49	
91	0.044 ± 0.023		5.04 ± 3.36	
93	0.037 ± 0.032		4.17 ± 3.14	
95	0.030 ± 0.030		4.29 ± 3.07	
97	0.024 ± 0.019		4.00 ± 2.68	
99	0.024 ± 0.019		3.25 ± 2.18	
101	0.024 ± 0.019		2.50 ± 1.68	
103	0.024 ± 0.019		1.75 ± 1.19	
105	0.024 ± 0.019		1.00 ± 0.71	
107	0.018 ± 0.012		0.38 ± 0.25	
109	0.000 ± 0.000		0.00 ± 0.00	

ability of arriving alive at age x. The 4 replicates show a similar pattern: stability until the first reproductive age (z) is passed, and afterwards a more or less gradual reduction in survival during

TABLE 4. Survival characteristics of *Culex pipiens fatigans*.

	Mean ± S.D.
Sex ratio (% ♀♀)	54.22 ± 2.52
♀ longevity (days)	60.25 ± 4.74
♂ longevity (days)	42.65 ± 1.60
Maximum ♀ longevity (days)	102.50 ± 9.00
Maximum ♂ longevity (days)	63.00 ± 2.82
Life expectancy at the 1st adult age (♀♀) (days)	24.03 ± 3.06
Life expectancy at the 1st adult age (♂♂) (days)	15.30 ± 1.46

approximately 100 days, until the oldest female dies.

FIG. 6 shows the average adult age-specific survival for both males and females. The male curve falls steeply after the 12th day of adult life.

Adult life expectancy

Life expectancy values (FIG. 7) of adult females decrease consistently with age in all replicates. TABLE 3 shows the average values with 1 standard deviation.

Immediately after emergence as adults, the females have an average life expectancy of 48 days, dropping down to 41.7 days on the 10th day of adult life.

TABLE 5 shows the estimated life expectancy values for males only, including their pre-adult risks; the average e_x at time of emergence of adults is 31.71 days, and only 23.57 days at the 10th day of adult life.

Population parameters

All population parameters here analyzed are derived from the survival and fecundity age-specific tables obtained from the cohort analysis. Since they are derived under experimental (supposedly near ideal) conditions, they must be interpreted as an estimation of their *maximum*, that is, an expression of a genetic feature of the species, and not an ecological characteristic. Their detailed description as well as the formulae used for their calculation can be found in some of the original works on these parameters (Birch 1948, Lotka 1945,
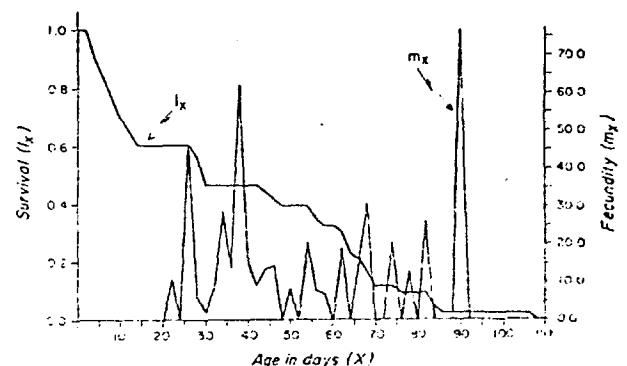

FIG. 3. Survivorship and fecundity curves of *Cx. p. fatigans* ♀♀. Replicate No. 2. Symbols as in FIG. 2.

Fisher 1930) or in a recent summary of their application to a triatomine species (Rabinovich 1972a).

TABLE 6 presents estimates of some of the more important population parameters; the standard deviations show a very low dispersion around the mean, most of them having a coefficient of variation below 10%.

DISCUSSION

The average embryonic development time of 37 hr is longer than the 27.11 $\pm$ 0.57 hr obtained at 28.1 $\pm$ 0.7 °C reported by de Meillon et al. (1967b) and longer than the 28 to 32 hr (at 29.4 °C) noted by Shriver & Bickley (1964) in a colony of *Cx. p. fatigans* from Malaya. The latter paper reported that the optimal temperature for embryonic development is from 23.9 to 29.4 °C; the authors noted that under these conditions, with strictly controlled temperature, 70% of the eggs hatch within approximately 30 hr.

Kitzmiller & Micks (1954) claimed that a tem-

TABLE 5. Life expectancy in *Culex pipiens fatigans* ♂♂ from time of oviposition (all units in 2 days).

| AGE x	Mean $\pm$ S.D.
1	27.39 $\pm$ 3.24
3	27.94 $\pm$ 3.18
5	28.62 $\pm$ 3.05
7	29.69 $\pm$ 2.86
9	30.25 $\pm$ 2.60
11	31.71 $\pm$ 1.94
13	30.57 $\pm$ 1.46
15	28.57 $\pm$ 1.46
17	26.57 $\pm$ 1.46
19	25.57 $\pm$ 1.54
21	23.57 $\pm$ 1.54
23	21.57 $\pm$ 1.54
25	19.78 $\pm$ 1.52
27	17.78 $\pm$ 1.52
29	17.61 $\pm$ 2.68
31	16.72 $\pm$ 3.39
33	15.07 $\pm$ 3.25
35	13.62 $\pm$ 2.57
37	13.01 $\pm$ 3.37
39	12.19 $\pm$ 4.04
41	12.89 $\pm$ 3.83
43	12.58 $\pm$ 2.75
45	10.93 $\pm$ 2.24
47	9.97 $\pm$ 1.58
49	7.97 $\pm$ 1.58
51	6.68 $\pm$ 1.10
53	5.15 $\pm$ 0.69
55	5.50 $\pm$ 1.69
57	4.29 $\pm$ 1.65
59	3.25 $\pm$ 0.99
61	1.91 $\pm$ 1.42
63	1.50 $\pm$ 2.38
65	0.75 $\pm$ 1.49
67	0.25 $\pm$ 0.50
69	0.00 $\pm$ 0.00
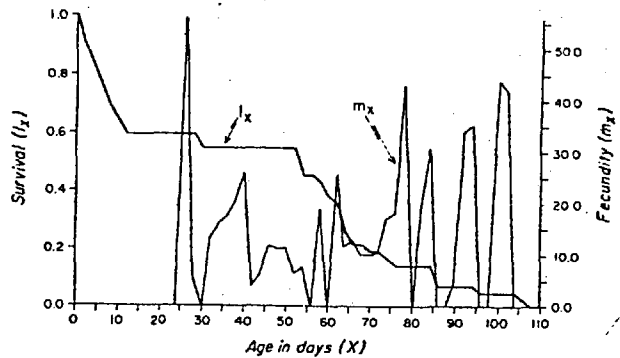
FIG. 4. Survivorship and fecundity curves of *Cx. p. fatigans* ♀♀. Replicate No. 3. Symbols as in FIG. 2.

perature of about 29 °C appears to be the optimum for the development of *Culex pipiens*. Our average time for larval development, 164.74 $\pm$ 9.26 hr at 26 °C, is greater than the average values (135 $\pm$ 4.4 hr for females and 118.4 $\pm$ 2.4 hr for males) at 28.6 °C cited by de Meillon et al. (1967b). Scorza (1972) reported developmental times of 175.7 hr for males and 205.8 hr for females, at 24.33 $\pm$ 0.43 °C. Kurihara (1963) reported variations in the time of larval development at different temperatures ranging from 20.4 days at 15 °C to 5.4 days at 28 °C. In addition to temperature, diet and culture density are also important; Klomp (1964) reported that development becomes slower as the intraspecific competition increases.

Nayar (1968) demonstrated that the development of *Cx. nigripalpus* is accelerated by increased temperature and slowed down by high salinity and high culture density. Nayar & Sauerman (1970) reported that pupation begins at 116 hr after hatching and lasts for 84 hr at 27 °C; in this study it began 146 hr after hatching, and lasted for 116 hr, although it should be noted that 91% of the pupae were formed within the first 34 hr. It should also be kept in mind that genetic differences in the strains employed by different investigators may have influenced results.

The average time of pupal life (48.75 hr) is close to the 43 hr for males and 45 hr for females reported

TABLE 6. Population parameters of *Culex pipiens fatigans* (all time units in days).

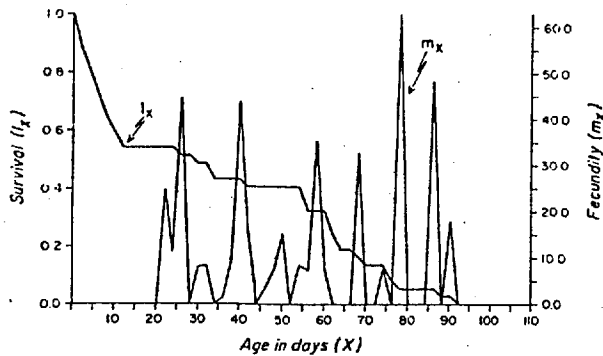|	Mean $\pm$ S.D.
Intrinsic rate of natural increase (r)	0.0103 $\pm$ 0.0103
Instantaneous birth rate (b)	0.4855 $\pm$ 0.0407
Instantaneous death rate (d)	0.3314 $\pm$ 0.0323
Net replacement rate (R$_0$)	161.3600 $\pm$ 30.43
Finite rate of increase (λ)	1.167 $\pm$ 0.121
Generation time (T)	44.68 $\pm$ 3.41
Total reproductive value (V)	1763.0 $\pm$ 355.5
Reproductive value of fecund ages	1233.00 $\pm$ 323.6

FIG. 5. Survivorship and fecundity curves of *Cx. p. fatigans* ♀♀. Replicate No. 4. Symbols as in FIG. 2.

by Nayar & Sauerman (1970). De Meillon et al. (1967b) obtained values of 34.16 hr for females and 32.95 hr for males.

Southwood et al. (1972), studying the population dynamics of *Aedes aegypti*, noted that the various stages of development in the laboratory are shorter than in nature, probably because of better food supply in the laboratory.

Pre-adult mortality of 47.42% occurs mainly in the pre-pupal stages; pupal mortality is very low.

Our data show that nearly 18% of the eggs failed to hatch; de Meillon et al. (1967b) reported only 8% (1.5% embryonic death plus 6.5% nonembryonated eggs). In our study, with 18% mortality, if there is a similar proportion of embryonic mortality, there would be 3.36% dying and 14.56% nonembryonated eggs. Shriver & Bickley (1964) reported a hatch of scarcely 70% of the total eggs. Of course, these estimates obtained under controlled conditions do not reflect the situation of natural populations exposed to a variety of factors inducing mortality.

In our study, 70.91% of the larvae pupated. De Meillon et al. (1967b) obtained 64% of pupae on the 6th day after eclosion; these authors emphasized that lack of food during the first hours of larval life may have a dramatic effect, reporting that in a group of larvae hatching in water devoid of food and maintained thus for 12 hr, only 8% of pupae were obtained 6 days later.

The estimated percentage of female adults was 54.22%. Qutubuddin (1952), in an experimental population based on 40 ovipositions, found 44.22% adult females; he emphasized that, in the majority of the hatchings from a single egg mass, the male/female ratio was close to 1. Only in a small fraction of egg masses was there a high preponderance of males or females. Scorza (1972) concluded that the sex ratio was very close to 1 in the population
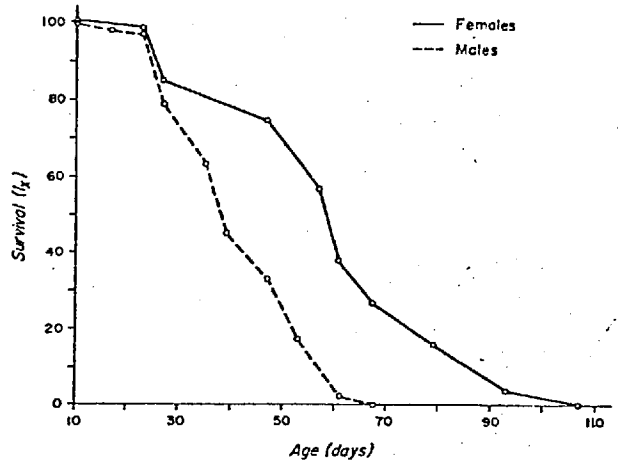

FIG. 6. Survivorship curves of ♂♂ and ♀♀ of *Cx. p. fatigans* in their adult stage.

of *Cx. p. fatigans* of Caracas.

Our results show that females survive much longer than males, the former having an average life span of 60.25 days, as against an average of 42.65 days for the latter. Even after 10 days of adult life, the females have a life expectancy greater than the males have at the moment of emergence. Wattal et al. (1961) reported an average adult life of 34 days in females fed only sucrose. Scorza (1972), who terminated his experiments at 30 days, reported that less than 50% of the females died within this period.

The adult survival curves we obtained are similar to those reported by Machado-Allison (1971) and Crovello & Hacker (1972) for strains of *Aedes aegypti*.

The lesser longevity of males appears to be a biological characteristic of the species; females are fertilized soon after emergence and 1 copulation by a single male is enough for all subsequent oviposi-
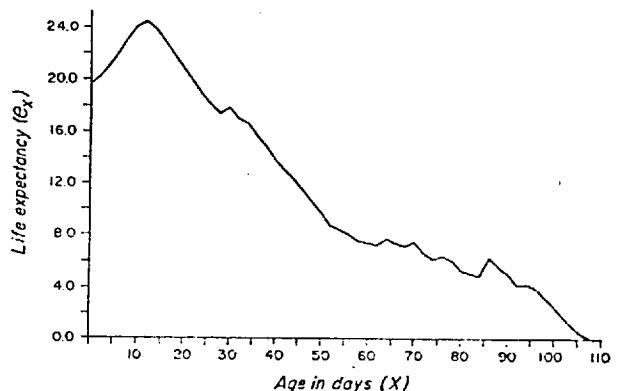

FIG. 7. Life expectancy of *Cx. p. fatigans* ♀♀ as a function of age after oviposition. Life expectancy increases until pre-adult risks of death have been overcome.
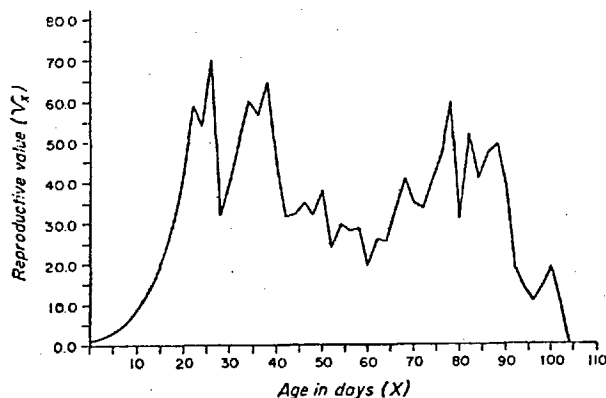
FIG. 8. Reproductive value of *Cx. p. fatigans* ♀♀ as a function of age.

tions. Once the males have completed their reproductive function, it appears that they are no longer necessary in the population. Comparison of FIG. 2–5 with FIG. 6 supports this conclusion; the drastic fall in male survival coincides with the start of the female oviposition period.

Roy & Majumdar (1939) observed that in a Calcutta strain of *Cx. p. fatigans*, after 48 hr of confinement in cages 20 × 20 × 15 cm, 100% of the females were fertilized, while in a larger container the percentage was smaller. Scorza (1972) reported that, with 50 males and 50 females confined for 48 hr in containers 8 cm in diam. and 10 cm in height (500 ml in volume), 96% of egg rafts laid contained fertile eggs.

The irregularity of the fertility pattern can be seen in all our experimental cohorts; it is noteworthy that in 2 cases (R_1 and R_2) the greatest reproductive effort (γ) was found at or close to the final reproductive age (ω). In a single case (R_3), γ took place 18 days before ω and in the last replicate (R_4), it happened 4 days before ω. In brief, the greatest reproductive effort was made towards the close of the reproductive span, which extended nearly to the end of life of the females. As shown in TABLE 2, females began oviposition at a mean age of 22 days (11 days after emergence and 5 days after the 1st blood meal). Actually, due to the fact that the reproductive behavior of females was not checked individually but followed for a cohort of females, the 1st age of reproduction (α) is rather an estimate of the age of the most precocious female of the cohort. Oviposition continued with very high variability up to an average age of 91.5 ± 6.4 days. The 1st blood meal was offered 2 days after emergence, but apparently only 3 out of 95 females fed. This early lack of appetite for blood was studied in detail by Scorza (1972), who found that in this species,

only 20% of virgin females engorged when offered blood at 2.6, 3 and 6 days after emergence. This percentage was doubled when females were offered a blood meal 7 days after emergence. A similar phenomenon was reported for *Aedes aegypti* by Armstrong & West (1965).

Blakeslee et al. (1962), working with a laboratory colony of a *Cx. erythrothorax* strain from California (at 23.9°C and 60–75% RH), reported that the 1st blood meal was taken 3–7 days after emergence, with oviposition beginning 5–12 days after the blood meal.

The average oviposition was of 236.62 ± 21.68 eggs, slightly higher than the average of 228 eggs reported by Scorza (1972). Somewhat lower values were given by Krishnamurti & Pal (1958) and by Wattal & Kalra (1963) in an Indian strain (204 and 167 eggs, respectively). De Meillon et al. (1967b) reported an average of 188 eggs per oviposition, at 28.1 ± 0.7°C and 90% RH.

The average total number of eggs per female was 507.41 ± 45.05. López (1972), working under similar conditions with *Aedes aegypti*, found an average of 644.48 ± 59.5 total eggs per female.

The adaptability of a population should be measured by several components, as emphasized by Machado-Allison (1971), Crovello & Hacker (1972) and Ayala (1970). They are in agreement that both r and R_0 are important components in measuring the adaptability of a population. Machado-Allison (1971) claims that the value of r is under genetic control; Wilson & Bossert (1971) also affirm that the parameter r in a particular environment is determined by the genetic composition, and is thus subject to evolutionary change.

The relatively high value of r (0.134 per day) obtained for *Cx. p. fatigans* is intermediate between

% of individuals in each age class
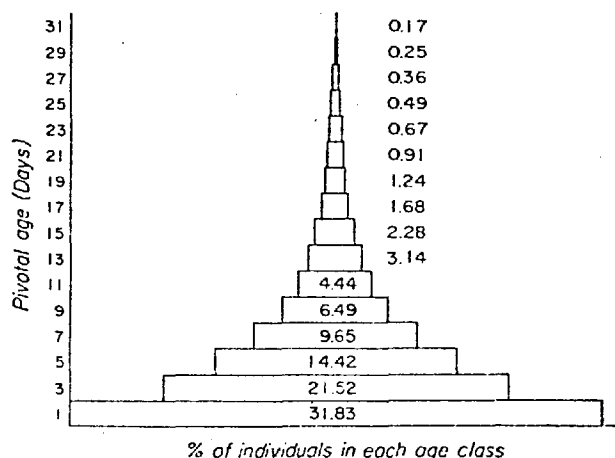
FIG. 9. Age pyramid corresponding to the Stable Age Distribution of a *Cx. p. fatigans* population.

the r values obtained by Rabinovich (1972a) for *Synthesiomyia nudiseta* (Diptera) and for *Cryptolestes ferrugineus* (Coleoptera), ranking 10th in a list of 25 species. López (1972), working under similar conditions, estimated an r value of 0.180 per day for *Ae. aegypti*, very close to the value of *Cx. p. fatigans*. Considering the similarity of the life cycles of the 2 species, it would seem that the pre-adult and adult mortality risks are very similar.

Crovello & Hacker (1972) found differences in r and R_0 between sylvatic and urban strains of *Ae. aegypti*; these values are significantly lower in the sylvatic strains, suggesting that urban conditions offer greater risks for the species.

The total reproductive value ($V_t = 1763$) is particularly high. Rabinovich (1972a) obtained a V_t of 530 for a population of *Triatoma infestans*. The reproductive value is fairly equally distributed among nearly all the adult ages (TABLE 7); however, there is a bimodal pattern with a set of major values corresponding to the ages between 21 and 39 days of reproductive life and a 2nd set of high values between the ages of 65 and 87 days (FIG. 8). The 2nd set may be considered of relatively little importance, since the stable age distribution of ages reveals that these values represent only 0.002% of the population.

Since the age-specific reproductive value v_x is a measure of the contribution of female individuals of age x to the succeeding generation, it is of the greatest importance in ecology, particularly in the study of predation and colonization (MacArthur & Wilson 1967, MacArthur 1960) and in control strategies (Rabinovich 1972b). Wilson & Bossert (1971) emphasized that individuals with a high v_x are the best colonizers, and that "prudent" predators do well to confine their attention to those prey individuals with the lowest reproductive value. In pest control, however, the preferred tactics should be those which operate most efficiently upon the ages with highest v_x. Rabinovich (1972b) emphasized the necessity of selecting those means of control which simultaneously reduce as much as possible the population density and the reproductive value of the surviving population.

From the evolutionary point of view, natural selection has a more significant action upon the ages of greatest reproductive values (Slobodkin 1966). Thus, Wilson & Bossert (1971) affirmed that "the genes that cause mortality in the individuals of high reproductive value tend to be removed from the population more rapidly than those which act upon other ages."

Analysis of the importance of each age in terms of its contribution to r shows that, although the

TABLE 7. Average and 1 standard deviation of the reproductive value (v_x) and the % of individuals of age x (after oviposition) in a Stable Age Distribution (S.A.D.).

Age x	v_x Mean ± S.D.	S.A.D. Mean ± S.D.
1	1.17 ± 0.01	31.831 ± 1.529
3	1.71 ± 0.11	21.520 ± 0.484
5	2.56 ± 0.19	14.417 ± 0.182
7	3.83 ± 0.39	9.650 ± 0.290
9	5.73 ± 0.67	6.493 ± 0.288
11	8.48 ± 1.11	4.442 ± 0.233
13	12.25 ± 1.59	3.136 ± 0.176
15	16.99 ± 2.15	2.284 ± 0.162
17	23.16 ± 3.35	1.680 ± 0.148
19	31.59 ± 5.16	1.236 ± 0.131
21	43.10 ± 7.89	0.910 ± 0.113
23	58.83 ± 11.96	0.670 ± 0.096
25	53.78 ± 7.21	0.490 ± 0.081
27	70.03 ± 10.92	0.355 ± 0.067
29	31.86 ± 9.30	0.249 ± 0.049
31	40.56 ± 13.19	0.175 ± 0.037
33	50.67 ± 20.47	0.126 ± 0.032
35	60.01 ± 23.61	0.091 ± 0.026
37	56.55 ± 18.51	0.067 ± 0.021
39	64.57 ± 17.82	0.049 ± 0.016
41	44.60 ± 15.97	0.036 ± 0.013
43	31.62 ± 2.07	0.026 ± 0.010
45	32.23 ± 8.78	0.019 ± 0.008
47	35.06 ± 8.94	0.014 ± 0.006
49	32.03 ± 8.48	0.010 ± 0.005
51	38.01 ± 15.56	0.007 ± 0.004
53	23.98 ± 2.70	0.005 ± 0.002
55	29.71 ± 6.63	0.004 ± 0.002
57	28.02 ± 9.31	0.002 ± 0.001
59	28.68 ± 18.53	0.002 ± 0.001
61	19.59 ± 10.93	0.001 ± 0.001
63	26.16 ± 17.45	0.001 ± 0.000
65	25.45 ± 9.80	0.000 ± 0.000
67	33.61 ± 9.68	0.000 ± 0.000
69	40.99 ± 1.41	0.000 ± 0.000
71	34.77 ± 22.09	0.000 ± 0.000
73	33.51 ± 15.02	0.000 ± 0.000
75	40.50 ± 16.57	0.000 ± 0.000
77	47.16 ± 21.49	0.000 ± 0.000
79	59.56 ± 21.08	0.000 ± 0.000
81	31.12 ± 10.39	0.000 ± 0.000
83	52.13 ± 31.48	0.000 ± 0.000
85	40.55 ± 19.90	0.000 ± 0.000
87	47.01 ± 10.33	0.000 ± 0.000
89	49.52 ± 28.23	0.000 ± 0.000
91	39.26 ± 36.19	0.000 ± 0.000
93	19.13 ± 38.26	0.000 ± 0.000
95	14.15 ± 28.29	0.000 ± 0.000
97	10.60 ± 21.21	0.000 ± 0.000
99	14.16 ± 28.32	0.000 ± 0.000
101	18.91 ± 37.81	0.000 ± 0.000
103	10.50 ± 21.00	0.000 ± 0.000
105	0.00 ± 0.00	0.000 ± 0.000
107	0.00 ± 0.00	0.000 ± 0.000
109	0.00 ± 0.00	0.000 ± 0.000

female reproductive period extends from 21 to 101 days (more than 90% of its adult life), the value of r is determined mainly (92%) by oviposition between 21 and 35 days of age, the first 16 days of the repro-

TABLE 8. Colonizing features of *Culex pipiens fatigans* and *Aedes aegypti* after the MacArthur & Wilson (1967) model.

	C. p. fatigans	*Ae. aegypti**
Intrinsic rate of natural increase (r)	0.1541	0.1800
Ratio r/b	0.3174	0.3667
Extinction time of 1 propagule when K = 1 (days)	3.02	3.21
Extinction time of 1 propagule when K = 10 (days)	132.8	194.1
Extinction time of 1 propagule when K = 10 (generations)	2.97	3.77
Dispersability** (λ) (in days)	0.6206	0.51937
Dispersability (λ) (in generations)	27.7	26.7
Extinction time increase when K goes from 10 to 20 (in %)	17.6	39.4

*Data for the calculations were obtained from the results with the optimal density of 25 larvae/liter given by López (1972).

**From the model by Richter-Dyn & Goel (1972); see text for discussion.

ductive period. However, only 38% of the total eggs are laid during these 16 days (by females aged 11 to 25 days of adult life). The remaining 62% on the eggs contribute only 8% of the value of r.

Information on the age distribution of a population is of great importance in any control program, especially when there are high percentages of certain age groups in the population, as is the case with *Cx. p. fatigans*. The stable age distribution estimated here indicates that about 50% of the population is in the egg stage, and that about 90% of the population is included between the egg stage and the beginning of adult life. Of the remaining 10% of the population comprised of adults, 9.1% have ages between 11 and 27 days, while adults of ages between 28 and 109 days comprise only 0.09% of the population (TABLE 7).

This result is in accordance with that of Odum (1971), who emphasized that a rapidly expanding population will have a large proportion of juveniles while a stable population has a more uniform distribution. However, it should be remembered that the stable age distribution is seldom achieved under natural conditions.

Wilson & Bossert (1971) considered as "r strategists" those species which are well adapted to short lives in new and unforseen environments. Such populations show early reproduction, high capacity of dispersion, and a high intrinsic rate of natural increase. These species perform an enormous reproductive effort to insure the survival of at least a small fraction of their progeny, and through these, the survival of the species.

CONCLUSIONS

The results of this analysis indicate that *Cx. p. fatigans* is a species with the following genetically determined population characteristics: (a) a short generation time, (b) a high population growth rate, (c) a type III survival curve (Deevey 1947), and (d)

a very irregular reproductive pattern.

These features, when coupled with the dispersal behavior of the species (high dispersal), and characteristics of its habitat (unstable and unpredictable), lead to the picture of a typical r-strategist (MacArthur & Wilson 1967, Pianka 1970).

The r/b ratio, considered by MacArthur & Wilson (1967) to be one of the most important indicators of the colonizing ability of a species, is 0.3174. This is an extremely low value, which indicates that *Cx. p. fatigans* is a very poor colonizer by this criterion. If we apply MacArthur & Wilson's model of colonization to this species, we obtain the results shown in TABLE 8, presented side by side with the same information for *Ae. aegypti*.

The extinction time of a population derived from the arrival of a propagule ("minimal number of individuals capable of reproducing—ordinarily a gravid female, or an unmated female plus a male"), when the carrying capacity of the environment (K) equals 1, is only 3.02 days for *Cx. p. fatigans*, slightly lower than the extinction time of *Ae. aegypti* (3.21 days). However, the same comparison, when K = 10, shows that the extinction time is much higher for *Ae. aegypti* (194.1 days) than for *Cx. p. fatigans* (132.8 days). This means that when the environment can support no more than 10 individuals, *Ae. aegypti* must "recolonize" the habitat at least once every 194 days if it is to maintain a permanent population, while *Cx. p. fatigans* must do so much more frequently, once every 133 days. Expressing these extinction times in generation units shows that, while *Ae. aegypti* should recolonize the habitat every 4 generations, *Cx. p. fatigans* must do so every 3 generations.

TABLE 8 also shows that the differences in extinction times reflect the lesser ability of *Cx. p. fatigans* to make "good" use of new resources, as compared with *Ae. aegypti*. The percentage increase in extinction time of a population derived from 10

propagules, when K increases from 10 to 20 individuals, is only 17.6% for *Cx. p. fatigans* compared with a 39.4% increase for *Ae. aegypti*.

In spite of not being a good colonizer, *Cx. p. fatigans* is an excellent r-strategist. This condition reflects its adaptation to an extremely unpredictable environment. An improved model of colonization proposed by Richter-Dyn & Goel (1972) allows the calculation of the minimum rate of immigration such that the population does not become extinct $(\tilde{\lambda})$; this rate has a value of 0.6206 propagules per day (or 27.7 propagules per generation. Thus, having $\tilde{\lambda} > 0.1$, $r/b < 0.5$, and $b/\mu < 2$, *Cx. p. fatigans* fits the characterization of an opportunistic species as proposed by Rabinovich (1974).

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