Studies on the overwintering of mosquitoes at Durham

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STUDIES ON THE OVERWINTERING

OF MOSQUITOES AT DURHAM

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CONTENTS

Page

TABLES AND FIGURES

			Page
Figure 1	:	Siphon of Theobaldia morsitans	89
Figure 2	:	Siphon of Theobaldia annulata	89
Figure 3	:	Siphon of Aedes punctor	89
Figure 4	:	Antennae of Anopheles claviger	89
Figure 5	:	Device for outdoor breeding	89
Figure 6	:	Map of Durham	90
Figure 7	:	Houghall I	91
Figure 8	:	Houghall III	91
Figure 9-a	:	Frequency curves of the siphonal index in Theobaldia morsitans 3rd instar	92
Figure 9-b	:	Siphonal index in the 4th instar	92
Figure 9-c	:	Siphon length	93
Figure 9-d	:	Siphon width (bimodal type)	94
Figure 9-e	:	Siphon width (unimodal type)	95
Figure 10	:	Relative abundance of instars	96
Figure 11-a	:	Association and succession at Houghall III	97
Figure 11-b	:	Association and succession at Houghall I	97
Figure 12	:	Anopheles claviger, 4th instar in January and March	98
Figure 13	:	Effects of density on mortality	99
Figure 14	:	Effects of density on mortality (curves)	100

INTRODUCTION

The present work deals with the overwintering of four common Mosquito species around Durham, namely *Theobaldia annulata* Schrank, *Theobaldia morsitans* Theobald, *Aedes punctor* Kirby, *Anopheles claviger* Meigen. These species have been studied in many parts of the world. In the British Isles they were studied mostly in the South of England, and notably in Hampshire (Hayling Island) (cf. Marshall 1938). But so far no study on these species has been done in the North of England and our aim in the present work was to see whether their overwintering stages, rule of development etc. would conform with the published information.

The studies were carried out in four ways :

a) by periodic sampling to determine the stages present at various times of the winter.

b) by breeding of larvae in the temperature of the laboratory (which is comparable to summer temperature) in order to see if there is quiescence or diapause.

c) by breeding of larvae in outdoor conditions where they could be observed and compared with field sampling.

d) by working out the possible effects of density on mortality and moulting rate. Various densities of larvae per 20cc were used. This experiment was intended to check whether larvae kept in the laboratory might not be kept at too high a density to give information relevant to field data.

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Part I. METHOD

A. SAMPLING

Regular sampling was done every 3-4 weeks. In most cases sampling every month was sufficient. Indeed, in most instances there was no, or little, change in the population during such an interval.

A plankton net was used, of 27.5 cm diameter, 38 cm deep, and with a handle of 150 cm. An effort was made each time to cover the different microhabitats of the pond studied. This precaution was particularly important for Houghall I (see Part II) where well-individualized microhabitats were found.

Special care was also taken in handling the larvae so that the individuals collected were in the best possible condition. Dead leaves were carefully avoided in sweeping so as not to exert too much pressure on larvae while they were in the net. After each sweep the contents of the net were poured into a white dish and the larvae picked up one by one with a pipette and put into the jar. At their arrival in the laboratory the larvae to be bred were left for 15-24 hours outside to settle down. Many moultings occurred outdoors during these hours following the catch. (cf. also Part IV, i-iii).

On each sampling day four groups of larvae were taken : one group for measurements, another for identification, the third for indoor, and the fourth for outdoor breeding.

These groups were sampled separately so that each might be reasonably considered as reflecting the pattern of the total population. The number per sample varied between 30 and 150, according to the yield available. Sometimes it was necessary to reduce the number in order to avoid depletion in small populations such as Brasside I, II, III (see Part II).

The case of samples from Brasside requires special explanation. Brasside I is mostly an Anopheles habitat. There is a high proportion (an average of 80%) per sample of Anopheline larvae and a regularly small sample of Culicine larvae (about 20% on average). As for Brasside II, the reduced size of the station (2 m x 1 m) and the scarcity of larvae limited even more than in Brasside I the available number per sample.

As an average, for each sample, there are :

20 to 100 larvae for measurements,
5 to 50 for indoor breeding,
5 to 50 for outdoor breeding.

B. MEASUREMENTS

Slide preparations were used for all measurements in spite of many disadvantages, i.e. the time spent on preparing the slides, the unchangeable position of the larvae, the loss of many taxonomically important hairs, the distortion of the abdomen etc. However, for the purpose of these studies the advantages of using slides were greater. The first advantage was that slides allow very accurate measurements of small organs; the second was the possibility of referring back to any larva at any time for further observations or measurements. It was, in fact, in the long run, time saving and easier to do numerous and delicate measurements on materials in slides rather than in fluids.

The larvae were dehydrated in successive baths of alcohol 70%, 95%, absolute alcohol, benzene. They were mounted in a moderately thick Canada balsam. The slides, labelled and oriented, were left to dry for a few days. The average number per slide was 20 for 4th and 3rd instar larvae, 35-40 for 2nd and 1st instar Culicine or Anopheline larvae.

The measurements were taken with a microscope. The magnification used was 40x so that one division of the eye-piece micrometer actually used corresponded to 1/40 mm on the larva studied.

B-1. <u>Measurements on Culicine larvae</u> (Figures 1, 2, 3)

The width of the head has frequently been used either to differentiate one species from another, or an instar within a species. (Sen & Das Gupta, 1958). This dimension is one means of identifying species quickly when 50 or more larvae are grouped on a slide. But it is difficult to take a satisfactory measurement of such a convex surface. More frequently used in these studies was the siphonal index obtained by dividing the length of the siphon by its width. This was preferred for the following reasons :

- the length of the siphon, at least for the three last larval instars, can be measured very accurately. Indeed, the basis as well as the apex of this organ is clearly marked (Figure 1, 1-4), and it is rigid and straight. The precision can be to the nearest 1/40 mm.
- the siphon is almost cylindrical (except for Aedes punctor) and is little altered in its shape and dimensions by the dehydration processes.
- the siphonal index, although situated within a narrow range of values, shows some variations which make possible some elementary analyses of the population under consideration.

The length of the body - thorax and abdomen - was also used to work out the importance of growth.

B-2. Measurements on Anopheline larvae (Figure 4)

The antenna was an excellent organ to measure, and its length is a reliable criterion to separate instars. It is straight and easily measurable at any position of the larva.

C. INDOOR BREEDING

200 cc jars were used, filled with pond water to 2 cm below the top, then covered with nylon material held in place by a rubber band. The larvae of each habitat were given pond water from the same habitat. The jars were put against a glass window in the laboratory, in a room not used by night, and therefore where the normal photo period of the season was rarely disturbed. Twice a week each jar was checked and the water temperature taken. The dead larvae and the exuviae were removed and preserved for eventual observation. Aquatic plants, such as filamentous algae, Lemna, were put in some jars. This was found to be rather detrimental to the larvae and, fairly regularly, larvae living with Lemna showed a higher mortality than contemporary larvae in the jars containing only water. Olenev (1950) reports that some substances diffusing from living plants killed insects and ticks. Sailer & Lienk (1954) noted

also that the presence of certain plants inhibits the development of mosquito larvae. There is probably some similarity between the death in the larval population that we studied and the facts reported by Olenev, Sailer & Lienk.

D. OUTDOOR BREEDING

A special device (Figure 5) was designed to meet the need for keeping under close control a sample taken from the field and living in as natural conditions as possible. For this purpose we used large sinks buried in the laboratory garden in a moderately shaded position and filled with pond water. The larvae were kept in plastic cups of 195 cc through which tiny holes allowed the water to move freely both ways. The plastic cups were covered with nylon bound with a rubber band. In order to follow the water level the cups were placed in holes made in a piece of cork 35 cm x 25 cm. The whole system was floating at the water surface. A regular check was made twice a week. Each time dead larvae and exuviae were removed and kept for further studies. The temperature at the surface of the water was read every day on a maximum-minimum thermometer, and twice a week at 10 cm below the surface.

E. IDENTIFICATION

The identification of species was made through keys given by Marshall (1938) for larvae and adults, and by P.F. Mattingly (1950) for adults. A rough identification was done by using particular characteristics of antennae and siphon for Culicines, and of the head (hairs and patches) for Anophelines. Furthermore, precise identification was made by Dr. L. Davies, and later on larvae were determined a posteriori by identification of the resulting adult, or vice versa.

The separation of instars was made by multiple cross-examination :

(1) - using characteristics reported by Marshall (1938). This was rather laborious because of the difficulty of observing the various hairs and because of individual variations.

(2) - by seriation according to the size and the siphonal length and index, a certain number of discontinuous series was obtained for each sample. Then using either the first instar (obvious with the egg breaker), or the 4th instar (obvious in moulting to a pupa) as reference points, it was possible to determine for each series the species and the instar.

Definite identification was made only after pupation and emergence, and in using results from breeding in the laboratory.

Table I shows the synopsis of characteristics used to separate species and instars.

Species	T.annulata	T.morsitans	Aedes punctor	Anopheles claviger
Instars	I II III IV	I II III IV	I II III IV	I II III IV
Morph. charact.	Siphonal tuft nearly basal	Siphonal tuft basal	Siphonal tuft intermediate	Head patches
Siph. Length	14 27 43 68	. 36 57 83	. 24 34 47	. . . .
Antenna Length	. . . .	. . . .	. . . .	. . 9 13

Table I : Characteristics used to separate species and instars in a sample. The population is known to contain only the four species. The unit used is that on the eyepiece micrometer (1 = 1/40 mm).

F. INTERPRETATION

1) Comparison of two samples

Our basic assumption is that each sample reflects the pattern of the whole population. It is therefore necessary, in order to study the successive states of the population, to find ways to compare samples of different sizes. To meet this need we used prefferably percentages instead of actual numbers in our interpretation of the numerical data. However, the exact numbers can be found in the tables accompanying the text.

2) Significance test

In a few instances t-test and d-test are used to estimate the validity of a conclusion.

Part II. HABITATS SAMPLED (Figures 6,7,8)

Two groups of habitats were sampled. The first was at Houghall, 1 mile S.E. of the laboratory, and the second around Brasside, 3 miles N.N.E. As some of them had small surfaces and small populations they had to be sampled sparingly. (Brasside II, for example).

A. THE HOUGHALL STATIONS (Figure 6)

1. Houghall I (Figure 7). This station is a permanent pond situated at about 200 metres from a farm. It was first studied on December 3rd, 1966. The pond is in a deep hollow. It is elongate in shape, with one end deeper than the other. From December 1966 to March 1967 *Cladocera* and Mosquito larvae were the main components of this pond; the *Cladocera* mostly at the deep end and the mosquito larvae at the shallow end and particularly between o-45 cm of water depth (Figure 7). This pond is partly a temporarily flooded place where in some places the plant materials of last year were still found very little decayed.

A few microhabitats could be fairly well distinguished and for each sampling every microhabitat was taken into consideration.

a. Microhabitat A. (Figure 7,A). The water was clear. The bottom was covered by some graminae species, still green all through the winter. The last three larval instars and pupae were found here.

b. Microhabitat B. (Figure 6,B). At this place semi-ligneous stems were still standing. The water surface was almost entirely covered with stem debris. B. was the favoured site of the 4th instar larvae and pupae.

c. Microhabitat C. (Figure 7,C). It had a muddy bottom on which lay a thick layer of dead leaves. At the edges were a few tufts of Deschampsia caespitosa L, partly covered by water. This was the favoured habitat for the first and second instar larvae. The rest of the habitat had two features : Shallow water (0-50 cm) and plant materials either on the surface or on the bottom. The majority of the third and fourth instar larvae were found there.

These microhabitats were distinct from one another not only by their physical aspect but also by their temperature and their lighting. A and C were better lit than B. Water was limpid in A and the bottom was visible, whereas in C a nearly continuous layer of floating dead stems prevented light from reaching the bottom. The temperature also varied from place to place. At the surface a difference of 1°C between two points two metres apart has been observed in January and at 10 cm below the surface a difference of 0.5°C. It is possible that these variations in light and temperature added their effect to those of physical and chemical properties to determine migrations (diurnal migrations and/or migrations linked with instars).

Relative abundance of species (Table II)

This year there was a clear dominance of *Theobaldia morsitans*. The next species in importance was *Theobaldia annulata* but it was far behind *Theobaldia morsitans*. *Anopheles claviger* was regularly found but only in one or two specimens out of an average of 90 or 100 larvae collected. By early March *Aedes punctor* appeared also at the station, increasing in importance in April.

2. Houghall II. The first study was made here on November 15th, 1966. This second habitat was a trough at the edge of a field near a group of trees. The trough was placed against a declivity and the grasses from the slope covered most of it. Unlike three others found in the vicinity this trough was almost completely protected against wind.

Along with Mosquito larvae, an abundant population of Cladocera was found and a thick layer of filamentous algae. Two species of mosquito were found in good number : Theobaldia annulata and Anopheles claviger. Theobaldia morsitans was found as a single specimen. The trough was accidentally dried up in December.

3. Houghall III - 1 (Figure 8). This habitat was a marshy basin, the source of a small stream. There were a few small pools and more or less open water in a thick vegetation of Phalaris arundinacea L. On the hillside there were a few hawthorn bushes. The greatest part of the basin was shaded by Salix trees.

In the Phalaris zone the bottom of the basin was covered with black mud rich in decayed plant materials. But above this mud the water was limpid. During the winter this zone was covered by slowly decaying stems and leaves of Phalaris arundinacea L. The larvae were mostly collected in this zone.

The distribution of the larvae was rather irregular. They were mostly found when the water depth was about 20-30 cm, when the water was limpid, and when vegetation was present but not very thick.

Most of the larvae were collected in an area of about 10 m^2 (Figure 8, 1). This area was situated in a pocket of the basin in which the slow movement of the water dies out against an embankment. It was also a moderately shaded part of the basin. There was probably constant though slow migration of larvae to this point. This would account for the rich concentration of larvae (about 3000 collected at this place through the winter), and also for the increasing proportion of *Aedes* larvae from February onwards. The latter hatched in Houghall III - 2 and migrated slowly from there to Houghall III - 1.

Houghall III - 1 was a type of habitat which, according to Seguy (1955) is highly favourable to Mosquito larvae. Indeed, this author reports that such a habitat with a moderately dense vegetation is more favourable to the development of Mosquito larvae than open water exposed to direct sunlight.

Relative abundance of the Species (Table II).

Theobaldia morsitans, *Theobaldia annulata*, *Anopheles claviger* were found regularly from December to June. From mid-February, *Aedes punctor* appeared and became numerically important.

4. Houghall III - 2. This was not a distinct habitat from Houghall III - 1. It was actually the upper part of the same basin, characterized by a series of small pools around a few thick tufts of *Deschampsia caespitosa*. The individuality of the habitat must be found in the presence of these tufts which seem to be used prefferably by female *Aedes* for laying their eggs. The Oviposition site preference of *Aedes* mosquitoes was studied by Beckel (1955). This author reports that when black and white, and rough and smooth surfaces were offered, the black was conclusively chosen over white, and rough over smooth.

Houghall III - 2 was a well-shaded place and *Deschampsia caespitosa* has very rough leaves, so the site seemed to be the type normally chosen by *Aedes*, according to Beckel's studies. This would account for the occurrence of first instar larvae under these tufts and nowhere else.

In May and June first instar larvae of *Theobaldia annulata* were also found at these sites. The latter species, with *Aedes punctor*, were the two species found in Houghall III - 2.

B. THE BRASSIDE STATIONS (Figure 6).

1. Brasside I. This habitat was a vast flooded field. Only the shallower parts along the border were inhabited by Mosquito larvae. These parts were actually grassy land covered by 0-50 cm of water. The water was limpid at these places and both green grass and dead stems of *Triticum* were found. Mortenson (1963) studied similar habitats in California, U.S.A., i.e. flooded grassy land, and concluded that they are particularly favourable to Mosquito production. He also cites McHugh (1958) who reports that emerging grass in shallow water is a highly productive habitat for *Aedes* and *Culex* mosquitoes.

The whole surface of this habitat was exposed to sunlight and wind. In January there was an almost continuous layer of ice of about 1 cm thick. The total exposure of the field sometimes made sampling difficult as the larvae, and more particularly the smaller ones, usually dive when the wind blows on the surface.

From December to March the decaying processes of plant materials were probably slow and there was no noticeable major change in the habitat. From March onwards a quicker decay took place so that the bottom was covered by soft, muddy material. This state of the habitat coincided with the development of a more abundant and more diversified community. Until the beginning of March the *Diptera* larvae were the main components with a few *Heteroptera* and *Ephemeroptera*.

Predators

From Spring the number of individuals and species increased, while the number of *Diptera* larvae decreased, presumably because of emergence and mortality and of predation. Indeed, predatory *Dityscidae* larvae were found in quantity with a catch of about half the number of Mosquito larvae per sample taken in the previous weeks.

Sailer & Lienk (1954) studied in some detail the effect of predation on Mosquito larvae and pupae. Without reaching any accurate assessment they point out that the widespread species in Alaska usually breed in habitats unfavourable to the predators, and that the permanent ponds, where the alleged predators abound, are usually poor in Mosquito larvae. Kuhlhorn (1961) reports that in Southern Germany the Mosquito larvae were destroyed by species of *Crustacea*, *Ephemeridae*, *Odonata*, *Heteroptera*, *Dityscidae*. He noted also that the presence of certain plants that spread over the surface of the water was a strongly limiting factor. Such a result can be compared with that of Olenev (1950) (cf. Part I, C). Around

Brasside there were many ponds covered with a thick superficial layer of Lemna and almost totally deprived of Mosquito larvae, though there were a great many other insect larvae. We would add to Kuhlorn's observation that water depth also plays a limiting role in the production of Mosquitoes.

Relative abundance of species (Table II).

Anopheles claviger was the dominant species. There was also a small but persistent proportion of Culicines.

2. Brasside II. This was first studied on January 14th, 1967. This site was unique in its type. It was a little pool, 2m x 1m, connected to a small stream and situated at the bottom of a narrow, deep ravine. The pool itself seemed to be supplied with water by a little spring. The water was limpid and there was a moderately abundant vegetation of Lemna. There was a small population of Mosquito larvae, mostly Theobaldia morsitans and rare Anopheles claviger larvae. The pool was shaded by thick vegetation of Hawthorn.

3. Brasside III. This was first studied on January 4th, 1967. The site studied was the grassy border of a permanent pond temporarily under water during the winter.

The larvae were found around tufts of *Carex* species and also in a lateral extension in a little well-shaded pool where the effects of wind on water were slackened. The Anopheles larvae were more abundant in this particular habitat.

The Culicine larvae were fewer than the Anophelines. They were exceptionally big and most of them were covered by colonies of *Epistylis* (Epistylidae, Ciliata).

C. CONCLUSION (Table III).

The two groups of habitats, Houghall and Brasside, are different from one another :

1. The habitats in Houghall are of a permanent type (except Houghall II). Their Mosquito larvae populations were mainly made up with Culicine, 68.6% of which are *Theobaldia morsitans*, and 16.5% of *Theobaldia annulata*.
2. The habitats in Brasside are of a more temporary type, i.e. fields flooded in winter, and there is a majority of *Anopheles claviger* (69.9%), and a minority of Culicine larvae.

It is possible that the Culicine species (*Theobaldia morsitans*, *T. annulata*, *Aedes punctor*) found in the fouled and permanent water of the Houghall sites a favourable breeding place, whereas *Anopheles claviger*, although capable of surviving in Houghall, breeds in better conditions when the sites are new and the water clean, as they were in Brasside.

TABLE II. (OVERLEAF). Synopsis of the relative abundance of the species in the habitats sampled.

Stations	Dates of Sampling	Theobaldia Annulata No.	%	Theobaldia Morsitans No.	%	Aedes Punctor No.	%	Anopheles Claviger No.	%	Monthly Total
Houghall I	Dec 3	10	15.8	51	80.2	0	0	2	3.1	63
	Jan 13	20	16.0	104	83.2	0	0	1	0.008	125
	Mar 13	11	10.6	87	84.4	4	3.8	1	0.009	103
	Apr 14	0	0	65	67.7	30	31.2	1	0.01	96
Houghall II	Nov 15	74	72.5	1	0.009	0	0	27	26.4	102
Houghall III-1	Dec 30	12	12.3	84	86.5	0	0	1	0.01	97
	Feb 18	2	1.3	79	53.0	67	44.9	1	0.006	149
	Mar 13	4	2.1	128	69.1	52	28.1	1	0.005	185
	Apr 14	0	0	35	35.3	63	63.6	1	0.01	99
Houghall III-2	Feb 18					69	100			69
	Mar 13					61	100			61
	Apr 14					158	100			158
	May 13	5	7.2			64	92.7			69
	June 10	249	79.5			64	20.7			313
TOTAL HOUGHALL		387	22.9	634	37.5	632	37.4	36	2.1	1689
Brasside I	Dec 17	4	3.3	19	15.9			96	80.6	119
	Jan 14			20	8.6			210	91.3	230
	Mar 18			18	15.6			97	84.3	115
	Apr 8			20	41.6			28	58.3	48
Brasside II	Dec 19			69	98.5			1	0.01	70
	Jan 14			40	90.9			4	9.0	44
Brasside III	Jan 14			27	25.7			78	74.2	105
	Feb 9			35	31.5			76	68.4	111
	Mar 11			17	15.5			92	84.4	109
	Apr 8			24	100					24
TOTAL BRASSIDE		4	0.004	289	29.6			682	69.9	975
GENERAL TOTAL		391	14.6	923	34.6	632	23.7	718	26.9	2664

TABLE II. Synopsis of the relative abundance of the species in the habitats sampled.

PART III THE OVERWINTERING SPECIES

Weather characteristics of 1966-1967 winter

The winter 1966-1967 has been a mild winter. From February to April the mean temperature for each month was higher than the average. It was also sunnier than usual, and from December to March the monthly total sunshine was regularly higher than normal.

The water temperature was read in many ways : at the surface, at 5 cm and at 10 cm below the surface. The readings from 5 cm below the surface are given on the table below along with information from Durham University Observatory.

	Temperature in °C			Sunshine in hours	
	Screen temperature		Water temp		
	Mean 1966-1967	Long-term average	Mean at -5cm	Mean 1966-1967	Long-term average
Nov	5.3	5.2	.	58.4	58.8
Dec	3.7	3.7	3.5	65.4	41.8
Jan	3.2	3.3	4.2	77.6	50.2
Feb	5.0	3.5	5.6	80.8	64.1
Mar	6.5	4.4	4.5	154.1	106.0
Apr	7.3	6.5	.	113.6	134.1
May	8.7	9.6	.	119.9	160.0

The four species studied : These were associate species, found together in all habitats sampled, though the dominant species varied with the habitat and the time of the year.

Theobaldia annulata Schrank

The literature available on this species deals mostly with taxonomy. Among others are the following works : Wesenberg-Lund (1921) in Denmark; Roukhadze (1926) in Russia; Natvig (1948) in Scandinavia. The species has been described or recorded probably under different names such as Culex annulatus (Wingate 1906), Culiseta morsitans (mostly by American and Canadian workers). In the British Isles the species has been studied by Theobald (1901), Harold (1926), Shute (1933), Marshall (1938). It is recorded as very common in Britain. Marshall states that there is no record of this species from Durham. However, as early as 1906, Wingate reported its occurrence there.

A. The conditions of occurrence at Durham.

Between November and April 137 larvae of this species were found out of a total of 1778 (7.7%). Most of them were from Houghall (97%) and a few from Brasside (2.9%). In Houghall the species amounted to 16.3% of the total collected in winter, and in Brasside 0.4%. A summer generation hatched in early June 1967 and adults were seen flying by June 15th.

Many authors report that this species breeds preferably in acid water, old trenches filled with leaves (Harold).

It has also been said that this species does not avoid impure water and that it can be found in foul water of the worst type (Harold). Marshall emphasizes the same tendency in saying that water contaminated by nitrogenous matter appears to provide an additional attraction, though the species does not show any particular preference as to breeding place.

The sites found in Durham were similar to those described by Harold and Marshall. In none of them, however, was Theobaldia annulata found to be prosperous. It was also in a small minority compared with either Theobaldia morsitans (Houghall) or Anopheles claviger (Brasside). It has been observed from Houghall and Brasside that when the water was heavily fouled and little permeable to light the larvae were dark coloured, dark brown, or dark green. Where the water was clean and permeable the larvae were lighter in colour.

B. The overwintering pattern of the larvae (Table IV).

(1) On November 15th, 74 larvae of this species were taken from a trough at Houghall Farm (Houghall II). 37 were of the first instar, 34 of the second, 2 of the third, and 1 of the fourth. The hatching probably took place in early November or at the end of October. Wesenberg-Lund reports that in Denmark the winter generation hatches in September - October. The situation at Durham was comparable to what has been observed by Wesenberg-Lund.

(2) In December, 33 specimens were collected from various places at Houghall and Brasside. Of these there were 1 second instar larva, 9 third instar, 15 fourth, and 8 pupae. No first instar was found. This may have been due to the small size of the sample. As a matter of fact the absence of first instar larvae has been noted constantly throughout the winter. On the other hand, the size of the sample from a given place was always about 70-100, and the number of *Theobaldia annulata* individuals was almost regularly 10% of the whole. There is some reason to think that no hatching had occurred since November, and that a slow and consistent development was in process in the field.

(3) In January the weather became generally cold, and in all habitats the water surface was covered with a layer of ice, 7 to 15 mm thick. Larvae were found either at places where there was no ice cover, or sometimes underneath it. The overall pattern of the population was still comparable to what it was in December ; 1/25 second instar, 8/25 third instar, 11/25 fourth instar, 5/25 pupae. The pupae were exclusively found at Houghall at microhabitat B.

(4) In February the temperature rose and an unusually high average of sunshine hours was found during this month. Only 9 specimens were found from sampling in Houghall and Brasside, 3 fourth instar, and 6 pupae. The absence of second and third instar in the samples seems to be connected with development. This tendency will be better outlined in the following month.

(5) In March nowhere were any larvae caught. Only 2 pupae were found in both groups of habitats. This was the last month of development of the winter generation. All the larvae hatched in October - November had moulted by the end of this month to the adult form.

C. The overwintering pattern of the adults.

A few adults (11) were caught in houses and cellars during the winter months, November to February. They usually came into the houses early in the evening, and the very good state of their scales suggests that most of them had just emerged when they came in.

Harold gives a very interesting detail about the males. He writes that they have a short span of life and that they provide useful information when found in winter about the date of emergence. Using this method, and taking into account climatic records and the results of breeding in the laboratory, one could place two favourable periods for adult emergence - November and February.

During the winter it is noteworthy that we found 11 adults flying, and 27 adults were obtained from the breedings. Out of these 38 adults, 11 were males and 27 females.

D. The Summer generation.

Wesenberg-Lund writes that in Denmark there is a summer generation of this species which is short-lived. In Durham we witnessed such a summer generation. On May 13th 2 first instar larvae were found along with a dense population of *Aedes punctor*. (Although similar, the larvae of the two species can easily be separated according to the siphonal tuft position).

On June 10th the situation was reversed and there was a dense population of *Theobaldia annulata* (88.9%) and a small minority of *Aedes punctor* (11.1%).

The development of this summer generation was very rapid. Hatched probably in the last week of May, there were on June 10th 2.8% first instar, 3.6% second instar, 49.7% third, 41.3% fourth, 2.4% pupae and many flying adults.

The adults caught in the fields in May were not as black as the specimens caught in houses in November. They were grey, even not very dark, and the "normally" white scales were yellowish. Lang (1920) also reports differences in colour and explains such differences as adaptations to particular climatic conditions. His example is taken from Mesopotamia where Theobaldia annulata has a form adapted to desert conditions, with white scales becoming yellow, and black scales becoming brown.

E. Conclusion and discussion

(1) The breeding site

The species in Durham has been found in at least five different types of breeding places. The water at Houghall II was very limpid and cool. Houghall I was moderately fouled, and the larvae and pupae were taken at microhabitat B where the bottom was covered by a thick mat of decaying leaves and the water surface covered by an almost continuous layer of herbaceous floating dried stems. As for Houghall III, some of the microhabitats found were filled with thick, bad-smelling water. It is clear from our obervations that the species can breed in various types of water, temporary or permanent. We would subscribe to Marshall's opinion that the species has no particular preference. We would add only that the breeding places found around Durham were all in the vicinity of a farm, or pastures and probably pools neighbouring the place where blood meals could be expected.

were the breeding places most likely to be used.

(2) The winter development

The development of *Theobaldia annulata* is of a particular type. It is not arrested like that of other species (the 4th instar *Theobaldia morsitans*, for example). It continues normally through the winter months. But the significant point to make is that it is very slow and very varied in length. While some individuals emerge in November, some others of the same generation do not emerge until February and March. And at the end, the time needed to complete the development of the winter generation is of the same length as it is for *Theobaldia morsitans* or *Anopheles claviger*. But as a consequence of the availability of adults since November and throughout the winter, there was in Durham this sudden eruption of a summer generation which is fast developing and compact in time. No other species had so early a second generation in the Durham area.

Marshall reports overwhelming evidence of the presence of all larval instars and pupae at Hayling Island in every month of the year.

He concludes that the female can lay eggs at any time when conditions happen to be favourable. At Hayling Island this species seems, therefore, to develop and reproduce continually, interrupted occasionally by harder climatic conditions which do not alter the fundamental pattern of the life cycle.

The situation is entirely different in Durham. There is only one winter generation. Hatching of eggs occurs early in winter and no other hatching takes place before June. All through the winter the age structure of the population and its evolutive changes show clearly that there is no more hatching in winter. Wesenberg-Lund (1921) reports that there are only two generations in Denmark, a winter and a summer one. The development of this species in Durham is more similar to that in Denmark than to that in Hayling Island. There are probably many comparable climatic and environmental factors between Denmark and Northern England. Indeed, the two regions are of the same latitude (around 55^{o}N.).

(3) The Biological range

For this species Durham appears to be in a biological range distinct from the range including Hayling Island.

The latter and Aldershot (Harold 1926) are probably the northern fringe of the continuous development range of this species. The northern part of the British Isles would be in the so-called "Denmark range" (Wesenberg-Lund) where the species tend to have well-developed breeding seasons and a well-defined number of generations.

Generation	Stations	T.annulata			T.morsitans			A.punctor			An.claviger			Total
		No.	$\vec{\%}$	%↓	No.	$\vec{\%}$	%↓	No.	$\vec{\%}$	%↓	No.	$\vec{\%}$	%↓	
Winter	Houghall	133	16.5	97.0	634	78.9	68.6	.	.	.	36	4.4	5.0	803
	Brasside	4	0.4	2.9	289	29.6	31.3	.	.	.	682	69.9	94.9	975
	Total	137			923						718			1778
Spring & Summer	Houghall	254	28.6	100	.	.	.	632	71.3	99.8	.	.	.	886
	Brasside	0	.	.	.	.	.	1	.	.	.	.	.	1
	Total	254						633						887
	Gen.Total	391			923			633			718			2665

Table III. Recapitulation of the catches from Houghall and Brasside for winter, spring and summer generations. Signs used : $\vec{\%}$ Relative importance of the species within an area for one generation;

%↓ Relative importance of the population of an area within a species for one generation.

Months	Larvae I	II	III	IV	P	Ad. flying ♂	♀	Emer. Lab. ♂	♀
November	37	34	2	1	.	2	5	.	.
December	.	1	9	15	8	.	1	1	14
January	.	1	8	11	5	.	1	.	1
February	.	.	.	3	6	1	1	1	2
March	.	.	.	.	2	.	.	6	2
April	.	.	.	.	.	.	.	.	.
May	2	.	.	.	3	.	.	.	.
June	7	9	124	103	6	Many			

TABLE IV. Recapitulation of catches of *Theobaldia annulata* in Houghall and Brasside (larvae and pupae) and in Durham City (adults) November 1966 - June 1967.

Theobaldia (Culicella) morsitans Theobald

A. Previous work on Theobaldia morsitans

Among the work done on the biology of this species are those of Wesenberg-Lund (1921) and Natvig (1948) in Denmark and Scandinavia, Carpenter & Lacasse (1955) in North America, Haufe (1952) in Canada. In the British Isles this species has been studied by Theobald (1901), Harold (1926), Shute (1929), Marshall (1938). This species is said to be common in Britain, but so far neither Wingate nor Marshall report its occurrence in Durham County.

B. Conditions of occurrence at Durham (Table IV, V, VI, Fig.9,10 I)

Theobaldia morsitans breeds in all the stations studied in Houghall and Brasside. It amounts to 52.9% of the larvae collected during the winter for population and development studies (923 out of 1742). Of this, 68.6% (634) were taken from Houghall and 31.3% (289) from Brasside. In Houghall the species amounts to 634 out of a total of 812 larvae studied, (78%), and in Brasside there were 289 out of a total of 993 larvae (31.0%). Theobaldia morsitans is undoubtedly the most widely distributed and the most abundant of the species overwintering in larval form at Durham. Houghall is a particularly favourable breeding area for this mosquito.

The reports on the breeding places do not assign a definite type for this species. Harold (1926) found larvae in "old trenches filled with leaves, in effluent from sewage plants, in grassy pools". Marshall states that the eggs are laid in hollow places to be flooded later in early winter. Our own observations happen to be comparable to these reports. Houghall III has the aspect of a grassy pool open in places and shaded in others. It has also some small pools filled with strongly fouled water, or filled with decaying leaves. Some parts of the site are to be flooded in early winter. The larvae of Theobaldia morsitans were found in all of these microhabitats. Houghall I is a pond filled with dead leaves and receiving washings from a rubbish tip probably for many years. Brasside II is also of this type but Brasside I is just a flooded field with clean and limpid water. It is not easy to distinguish from these various types of sites the best for the species. The most abundant population was found at Houghall III - 1, but this place was also a cul-de-sac where the slow movement of the water mass was stopped by an embankment, and the concentration of larvae owes much to this purely mechanical action.

The abundance of this species in Houghall could also be seen as a result of its particular habits. Marshall states that the eggs are laid in "dried up hollows or above the water level of partly-filled ones". They hatch in early winter when the water level rises. The sites in Houghall contain many such places, probably undisturbed for many years, in a basin where predation is almost completely absent and blood meals (if wanted) could easily be taken from the cattle, horses, sheep etc. of the farm. The population found in Houghall is probably a sedentary population reproducing in the same habitat for many years.

C. The overwintering pattern at Durham (Tables V & VI, Figures 9, 10, I).

(1) December 1966 : Dominance of the 3rd instar.

In all places sampled no first instar larvae were found. By that time of the year there was probably no hatching.

The 2nd instar larvae were found in many places, either in Houghall or in Brasside or in other sites sampled. In all cases they were very rare. However, no 2nd instar larvae were found in Brasside I and III. This absence may be due to the fact that at these places the larval population is more advanced in its development than in Houghall. The main feature of the larval population of *Theobaldia morsitans* in many stations is the numerical dominance of the 4th instar. It seems safe enough to consider this population more advanced in development.

(2) January 1967 : Dominance of the 3rd instar.

The main features remain as they were in December; no 1st instar larvae; the 2nd instar rare or absent; the 3rd instar dominant in Houghall and Brasside II; the 4th dominant in Brasside I and III.

(3) February 1967 : Dominance of the 4th instar.

The exceptional rise in temperature brought forth a resumption of development. Indeed three major changes occurred this month :

- the disappearance of the 2nd instar larvae in Houghall.
- the disappearance of the 3rd instar larvae in Brasside.
- the dominance of the 4th instar in Houghall and in Brasside.

(4) March 1967 : Dominance of the 4th instar.

This month was also an exceptional one. It was reported by Durham University Observatory as the warmest March since 1961, with a mean temperature of $6^{o}5C$ for a long-term average of $4^{o}4C$. It was also the sunniest March since 1953 with a total sunshine of 154.1 hours for an average of 106.0 hours.

The resumption of development started in February went on, leading to fewer and fewer 3rd instar larvae and a clear and firmly established dominance of 4th instar.

The frequency curves of the siphonal indices are of a bimodal type (Figure 9a-2). This is probably attributable to the resumption of growth. Such a growth may indeed modify the ratio between the length and width of the siphon, and also render the instar groups heterogenous. Among individuals of the same instar a fraction was at this stage for three months or even more, another had just moulted, and a third ranged from the youngest to the oldest group.

The comparison of two frequency curves (Figure 9a) of the same population and the same instar in December and in February shows that the population was more homogenous in December than in February (Figure 9a-1, 9b-1) and March (Figure 9b-3).

(5) April 1967 : Pupation and emergence.

In all habitats there was a complete disappearance of the 3rd instar larvae, and a certain proportion of pupae was taken from the field. The frequency curves showed a homogenous population. On the whole, there was probably some physical growth within an instar, and the increase in the mean siphonal index may be an indication of such a growth.

D. Significance tests on the development of T. morsitans

The conclusions on life-cycle are based upon the assumption that the relative importance of instars at any time reflects the state of development. When the dominance swings, for instance, from the 3rd to the 4th instar larvae, the majority of the larval population moulted from the third to the fourth instar. The significance of the ratio 3rd instar/4th instar has therefore to be tested for the validity of the conclusions. We used a 't-test' to evaluate this significance.

1. December - January : third instar dominance (data from Houghall)

Dates	No. 3rd inst.	No. 4th inst.	Difference
3.12.66(I)	34	14	20
30.12.66(III)	59	24	35
1. 1.67(I)	83	13	70

$\bar{x} = 41.666 \qquad s = 25.661 \qquad n = 3 \qquad \mu = 0$

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}} = \frac{41.666}{14.7} = 2.812$$

with 2 degrees of freedom

$P > 0.10$

2. February - April : 4th instar dominance (data from Houghall)

Dates	No.3rd inst.	No.4th inst.	Diff.
18.2.67 (III)	37	91	54
13.3.67 (I)	3	84	81
13.3.67 (III)	19	67	48
14.4.67 (I)	0	88	88
14.4.67 (III)	0	77	77

$\bar{x} = 69.600$ $s = 31.050$ $n = 5$

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}} = \frac{69.600 - 0.000}{13.886} = 5.01$$

no. degrees of freedom = 4

$0.01 < P < 0.02$

The 'd-test' has also been used to estimate the significance of the difference between the mean siphonal index in December and the mean siphonal index in April within the 4th instar population of Brasside I.

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$ with $\bar{x}_1 = 6.33$, $s_1 = 0.52$, $\bar{x}_2 = 5.93$, $s_2 = 0.42$

$$\frac{s_1^2}{n_1} = \frac{0.2704}{17} = 0.01590 \qquad \frac{s_2^2}{n_2} = \frac{0.1764}{10} = 0.01764$$

$$\bar{x}_1 - \bar{x}_2 = 0.40$$

$$d = \frac{0.40}{\sqrt{0.03354}} = \frac{0.40}{0.18} = 2.22 \qquad 0.02 < P < 0.05$$

d being larger than 1.96, there is some probability that the difference between the mean siphonal indices is significant. It reflects some physical growth taking place between December and April. This growth affecting the body length is probably of a disharmonic type (Teissier 1928). As far as the siphon is concerned, the length grows proportionally more than the width.

E. Conclusions and discussion.

(1) The second, third and fourth instar larvae were found in the field in various proportions from December to March, the second instar being rare since December

and absent since February.

(2) The second and third instar larvae are slow-developing instars. They moult normally to the next stage after some time and the moulting takes place in winter. Wesenberg-Lund (1921), Shute (1933), Marshall (1938), report that the fourth instar is reached early in winter before the period of frost, and the species overwinters in the fourth instar. In the Durham area the fourth instar is reached in November. But the third instar is dominant in number until February, and the second instar is still found until December. If there is swing from a third instar dominant stage to a fourth instar dominant one, and also if there is reduction followed by the disappearance of the second instar, and then the third, there are slow development and moulting occurring in the natural conditions during the winter as far as the second and third instars are concerned.

(3) The development of the fourth instar is different from that of the two previous ones. The larvae of this stage do not pupate either in the field or in the laboratory (mean temperature 18°C). It is in diapause. For instance, in Houghall fourth instar larvae were found at the proportion of 27.4% on December 3rd, but the first pupae were collected on April 14th. Between

these two dates pupation is unlikely to occur in the natural conditions.

(4) There is indication of some physical growth within an instar, between ecdysis. By growth we mean all changes in organ shapes and dimensions. Such changes may affect either the form of the body or its mass, or both. We found examples of physical changes affecting the siphon of Theobaldia morsitans (Figure 9, a,b,c,d,e).

i. If the siphonal indices are plotted against the frequency per cent, two types of curves may be obtained. The first type is unimodal (Figure 9a-1, 9b-1 and b-2). These curves picture the situation in a population when there is little or no growth. The third instar in December (9a-1) and the fourth in December and February (9b-1, 9b-2) are of this type. In these populations the frequency curves of both the length and the width of the siphon are also unimodal (Figure 9c-1, c-2, e-1, e-2).

ii. When there is resumption of growth the frequency curves become bimodal (Figure 9a-2, b-3). The third instar in February and the fourth instar in March fall in this case. The frequency curves of both the width and the length of the siphon cease to be unimodal and become both bimodal (Figure 9c-3, c-4, d-1, d-2). The bimodality of the frequency curves of the siphonal index is therefore due to the bimodality of both frequency curves of the length and the width of the siphon.

From these observations it seems that there is growth or at least readjustment of organ dimensions - even the sclerotized ones - after moulting. Both the length of the siphon and its width increase towards an ideal average for the instar. Clearly such a growth affects only a part of the population, and both the lower and the upper limits of the length and the width are not altered.

It is found that the changes causing bimodality in the frequency curves are not strictly contemporary in the third and fourth instars. It is actually earlier in the third. This fact leads us to consider that the physical growth affects mostly the newly moulted larvae. Indeed, the lower peak of the bimodal curves for indices is situated in the regions of the siphonal indices of the previous instar (Figure 9a & b).

Physical growth occurring between ecdyses has been reported by many authors. Clements (1963) stated that among Mosquito larvae the abdomen and the thorax increase continuously throughout larval stages but not the head or the siphon. We have, however, found examples of growth affecting the siphon as it has been shown. Such a growth is of the disharmonic type described by Teissier (1928) and more recently by Chanussot (1963).

There seems to be two different aspects of development : the development towards the achievement of the life cycle and the physical growth. The latter is not stopped by diapause in the fourth instar larvae of the winter generation.

(4) We have observed after Wesenberg-Lund, Harold, Marshall, Bates and others, that the larvae of this species can stay a long time under water. Among larvae bred in the laboratory it was a common occurrence. In some of the Houghall III microhabitats the water surface was covered with a layer of floating unidentified substances in such a way that the surface of the water was insulated from the contact of atmospheric oxygen. Many larvae were taken underneath these layers. Marshall and Wesenberg-Lund stated that the larvae remain submerged throughout the winter. We would suggest rather that such a possibility exists, but it is not the normal way of overwintering in the species. Most of our specimens were taken in sweeping the surface of the water, and we have observed larvae coming to the surface from time to time. Bates (1949)

gives an explanation for this ability of the larvae to stay underwater. There is a low consumption of oxygen consequent upon a low metabolism due to the low temperature. In these conditions cuticular respiration is sufficient. However, it should be noted that some other factors may be involved since other species living in the same conditions do not react in the same way.

Aedes (Ochlerotatus) punctor Kirby

The biology of Aedes punctor has been studied by many authors in various parts of the world. Among the works dealing with the larvae and the biology are those of Natvig (1948) in Scandinavia, Jachowski & Schultz (1948) in Alaska, Dyar (1920, 1928), Knight (1951), Carpenter & Lacasse (1955) in North America, and Haufe (1952), Haufe & Burgess (1956) in Canada. In the British Isles the species has been studied by Harold (1926) mostly in the South of England, Shute (1933) and Marshall (1938). In the North-east of England the species has been recorded in Westmorland, but so far there is no record from County Durham.

A. Conditions of occurrence at Durham (Tables II, III, VII).

Aedes punctor was found breeding at a very particular and limited spot in Houghall III-2, one generation from February to June 1967, and another in August. A single specimen was taken in Brasside. From February to the middle of May Aedes _punctor_ was the only species to be found at Houghall III-2. But in early June a great number of _Theobaldia annulata_ hatched and outnumbered the _Aedes_ population.

The site has been described elsewhere (cf. Part II A-4). Harold reports some observations about the breeding places of this species. It has been found in acid water (4.4 to 7.5) associated with _Theobaldia morsitans_. Natvig (1948) described somewhat similar breeding sites. Shute (1933) reports that this species breeds in waters shaded under canopy. These characteristics of the breeding places were found in Houghall. The site is swampy, shady, and the water acid (pH 5.5 - 6.5). Marshall noted also that acid waters are frequently found in the breeding places of this species.

Thus in Houghall III the species was strictly confined to a well-delimited area and was not found anywhere else. Such a case could be the result either of an excessive habit of being sedentary, or an indication of a recent immigration. But it may also indicate a narrow preference as for the breeding site. The information from the literature seems to suggest that this species has indeed a narrow preference for its breeding sites and that the site at Houghall III-2 meets such a preference particularly well.

A recent study by Beckel (1955) shed some light on the site-choosing behaviour of female _Aedes_ (cf. Part II, A-4). According to this author the females of _Aedes_ species choose regularly to lay eggs on dark and rough surfaces. Precisely these two conditions were found in the site at Houghall III-2.: the canopy of Salix and the tufts of Deschampsia caespitosa providing shade, and the leaves and dead leaves of the same graminae providing rough surfaces.

B. Hatching

Hatching occurred between January 20th and February 18th 1967. In Durham this year, this period coincided with a general rise of temperature. The mean temperature of February was 5.0°C for an average of 3.5°C. This rise in temperature may be one of the causes of this hatching. If so, this hatching would be an occasional one, an accidental event. However, Jachowski & Schultz (1948) state that in Alaska a temperature of 2.4°C is sufficient to cause hatching. Haufe & Burgess (1956) write that at 3.3°C the development of this species is rapid. The problem is to know whether the species responds to temperature changes in the same way in Durham (Lat. $54\frac{3}{4}$°N), Umiat, Alaska (Lat. $68\frac{1}{2}$°N), and Churchill, Manitoba (Lat. 59°N).

Marshall writes that the eggs are normally deposited in places to be flooded later on, and they hatch when submerged. At Durham the general conditions reported by the authors, namely rise in temperature and in water level, were fulfilled by the beginning of February. Probably the quantitative values of the increases are not the determining factors. More effective may be the somewhat catalytic effect of their very occurrence.

C. Development Pattern (Table VII, Figure 10 -II, Figure 11).

(1) February 1967 (Table VII).

On February 18th we found a large population with a dominant second instar (60.8%). The first instar was still important (34.7%). As for the third instar, they were in a small proportion (4.3%). The previous visit to the site was on January 20th, and the hatching occurred, presumably, by the beginning of February.

(2) March 1967 (Table VII).

The second instar was still the dominant stage (68.8%), but three important changes occurred : the presence of 4th instar larvae, the high proportion of third instar (22.9%), and the extreme scarcity of 1st instar larvae. The larvae were migrating downstream and many were found mixed with Theobaldia morsitans in Houghall III-1.

(3) April 1967 (Table VII).

A few first instar larvae were still found (5.3%). This was not another generation since the general pattern of the population was not altered. There was a swing to a third instar dominance (34.1%), followed very closely by the fourth instar (32.9%), and a good proportion of pupae (14.9%).

(4) May 1967 (Table VII)

The development speeded up since the last sampling and the dominance changed from the third to the fourth instar, and then to the pupal stage (45.1%) on May 13th. Some adults were already flying, but there were still some second instar larvae (3.2%). The fourth instar was important (38.7%), and the third instar reduced from 34.1% to 12.9%.

D. Associated Species (Figure 1a).

Though there are many microhabitats in Houghall III, (for example, Houghall III-1 with *Theobaldia morsitans*, *Theobaldia annulata*, *Anopheles claviger*, and Houghall III-2 with *Aedes punctor* and the summer generation of *Theobaldia annulata*), the continuity provided by the water brought much unity in the habitat. Thus all of the species found there could be considered as associates.

As the habitat of *Aedes punctor* is situated "upstream", and that of *Theobaldia morsitans* "downstream", the first invades the domain of the second, but the reverse does not happen. The two species can breed in the same conditions, and the difference in location for the first instar is due, presumably, more to the females' oviposition habit than to some particular larval requirements. Moreover, through the literature many authors report the association of *Aedes punctor* with *Theobaldia morsitans*, *Theobaldia annulata* and *Anopheles claviger*.

Harold (1926) reports that in the habitat he studied the *Aedes punctor* brood followed rather regularly that of *Theobaldia morsitans*. This was also observed in Durham. The succession was as follows :

I	(*Theobaldia morsitans*	(November - May)
	(*Theobaldia annulata* I	(November - April)
	(*Anopheles claviger*	(November - April)
II	*Aedes punctor*	(February - June)
III	*Theobaldia annulata* II	(May - June)
IV	*Aedes punctor*	(August)

E. Discussion and Conclusion.

1. From the literature and from our own observations in Durham *Aedes punctor* has some consistent specific habits for its breeding requirements. There are variations, but the ecology of the species remains little changed from $50^{O}N$ to $68\frac{1}{2}^{O}N$.

2. Harold suggested that there is only one brood of *Aedes punctor* per year, and he found confirmation of this opinion in Dyar's work on the same species in North America. At Durham there are probably two generations per year, one in the period February - June, and another found at the second instar in August, (but we have some doubt about our identification of this second generation). Marshall gives some larval records (1938, p.163) in which, for

instance, at Wittley, Surrey, in 1931, the dates of occurrence of Aedes punctor are comparable to the dates observed at Durham in February to June 1967.

Anopheles claviger Meigen

There is a vast literature about this species. Among the works on biology, and dealing particularly with the problem of overwintering, are those of Wesenberg-Lund (1921) in Denmark, Roukhadze (1926) and Vinogradova (1963) in Russia. In the British Isles the species has been studied, among others, by Lang (1920), Harold (1926), Shute (1933), and Marshall (1938). In the Mediterranean zone there are studies by Sautet (1933) in Corsica, Garrett-Jones (1951) in the Middle East. Wingate (1906) mentions the occurrence of this species at Durham under the name of Anopheles bifurcatus, though Marshall (1938) states that no record of this species from Durham was known.

A. The conditions of occurrence in Durham (Tables II, III).

Anopheles claviger was found in all the habitats studied, but in all, except at Houghall II, Brasside I, Brasside III, only one or two larvae per sample were found. 718 larvae out of 1778 studied during the winter belonged to this species (39.1%). Most of this total came from Brasside (94.9%). Only a small proportion (5.0%) came from Houghall.

The sites where this species was breeding in the Durham area can be put into two major groups. The first group including Houghall II, Brasside I and Brasside III could be termed "more or less permanent waters", or "growth of reeds at the margins of ponds", as Marshall described with precision the type of breeding place for this species. The second group including Houghall I, Houghall III, and other minor sites are of the type described by Harold as "foul waters", "marshy area", "channel receiving washing from a country road covered with cow-droppings". The species has been seen breeding "in pure, cool water frequently renewed". (Feytaud & Gendre, 1919, quoted by Horsfall 1955), or in old sewage runnels (Harold 1926). The species obviously has a wide range of adaptation as to breeding places.

B. Field observations

(1) The relative importance of the instars (Tables II, X).

Our first sampling was on November 15th 1966 at Houghall farm in a trough. 27 larvae were taken, 20 of the third instar, and 7 of the fourth. The trough was unfortunately dried up in December.

The rich station of Brasside I was found and first studied on December 17th 1966. The number of larvae of different sampling is shown in Table II. It will be noted :

(a) that in Brasside I as in Houghall II there were the two instars, 3rd and 4th. The proportion of larvae of the two instars encountered was variable.

(b) that all through the winter the percentage of 4th instar in the samples was persistently higher than that of the 3rd.

(c) that in all samples the percentage of 3rd instar decreased from December to March, whereas that of the 4th increased during the same time.

In Brasside III the first sample was taken on January 14th 1967. The larval population there appeared to be younger than that of Brasside I : 88.4% third instar, and 11.5% fourth instar larvae. Although the percentages changed in March, the third instar was still the predominant stage. This site was virtually destroyed by the beginning of April.

(2) Sizes (Table IX).

In handling the larvae we had the feeling that the variations in the size were more than individual variations of a normally homogenous population. To test this, two samples were chosen for closer study. We picked the January sample as typical of a mid-winter population, the March sample as representing the same population in Spring. The measurements and calculations give for the 4th instar a mean body length of 5.1 mm ± 0.6 in January, and 5.8 mm ± 0.8 in March. (See Table IX).

TABLE IX

	3rd instar			4th instar		
Date	Sample size	Length in mm Antennae	Body	Sample size	Length in mm Antennae	Body
14.1.67	90	0.224 ± 0.016	3.5 ± 0.3	120	0.335 ± 0.025	5.1 ± 0.6
11.3.67	12	0.222 ± 0.011	4.24 ± 0.3	84	0.339 ± 0.021	5.8 ± 0.8

If the body length is plotted against its frequency (expressed in percentage to facilitate comparison) curves can be shown as in Figure 12. Such figures and curves evidence the occurrence of a physical growth affecting the body length while affecting the antennae very little.

C. Significance Tests

(1) The relative importance of the third and fourth instar has been tested with a 't-test'.

TABLE X

Dates	No. of 3rd instar	No. of 4th instar	Difference
17.12.66	31	65	34
14. 1.67	90	120	30
6. 2.67	22	54	32
11. 3.67	13	84	71
8. 4.67	0	28	28
TOTAL	156	351	195

$\sum x^2 = 8905$ $\sum x = 195$ $\bar{x} = 39.000$ $(\sum x)^2 = 38025.$

$s^2 = 122.5$ $s = 11.067$

$$\frac{s}{\sqrt{n}} = \frac{11.067}{2.236} = 4.949$$

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}} = \frac{39.000}{4.949} = 7.880,$$ with 4 degrees of freedom.

$0.001 < P < 0.002$

(2) The 'd-test' was also used to estimate the significance of the difference between the mean body length of 3rd instar larvae in December and in March.

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s^2_1}{n_1} + \frac{s^2_2}{n_2}}}$$

$x_1 = 4.25$ $n_1 = 12$ $s_1 = 0.3$

$x_2 = 3.50$ $n_2 = 90$ $s_2 = 0.3$

$x_1 - x_2 = 0.75$

$s_1^2 = 0.09$ $s_2^2 = 0.09$

$$\frac{s_1^2}{n_1} = \frac{0.09}{12} = 0.007 \qquad \frac{s_2^2}{n_2} = \frac{0.09}{90} = 0.001$$

$$\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2} = 0.007 + 0.001 = 0.008$$

$$\sqrt{0.008} = 0.282$$

$$d = \frac{0.750}{0.282} = \underline{2.659}$$

D. Pupation (Table VIII).

The date of first pupation was important to know and this had been watched in the outdoor breeding tanks as well as in the natural population. The first pupation occurred between April 3rd - 10th and emergence between April 23rd - 28th. Unfortunately by that time the population in Brasside was almost totally depleted by predators as has been reported elsewhere (cf. Part II A-4). It was quite clear that this considerable reduction was not a result of massive emergence, as it might appear. Indeed, only a few pupae were taken among larvae by the end of March, and by April 10th the population had decreased sharply. In the laboratory by the middle of June pupation of the winter generation was still in process, and out of 13 individuals there were 6 larvae, 3 pupae, and 4 adults newly emerged (13.6.67).

E. Development

Wesenberg-Lund, quoted by Bates, distinguishes an "Anopheles claviger type of cycle" in which there is hibernation in the larval stage. Theobaldia morsitans, for example, has this type of cycle. There are conflicting reports about which larval instars are actually overwintering. Lang (1920) and Harold (1926) state that the species overwinters in the 3rd larval instar. Marshall criticises a general belief that Anopheles claviger passes the winter in the 4th instar, and

and he produces overwhelming evidence to show that all three later instars were found throughout the year at one place. So he concludes, and he is supported by Bates (1949), that this species is merely in quiescence during the winter. It overwinters in any larval instar and resumes development as soon as conditions become favourable, particularly if there is a rise in temperature.

At Durham we failed to resume artificially the development of the larvae collected. This may have been for some particular reason such as food. Many workers report also either the difficulty or the impossibility of resuming the development of the overwintering larvae of this species. Roukhadze (1926) links the resumption of development with some particular breeding techniques, such as a very slow rise in temperature. Sautet (1933) states that the temperature is but one, and not the most important, factor among others in the resumption of growth. He observes that if some oxidising chemical (eau de Javel) is added to the water, growth will resume, and he concludes that under normal conditions the winter cessation of development is broken by an increased rate of dissolved oxygen due to an increased chlorophyl production of water plants.

The situation at Durham is closer to those reported by Andrewartha (1952) and Vinogradova (1963). From these reports it appears that the activity and development of this species is variable and depends, among other factors, on photoperiod. Vinogradova states that though the species reproduces continually in the extreme south of its range, in higher latitudes the 4th instar is in diapause irrespective of temperature. The general pattern given by Wesenberg-Lund is also similar to that in Durham and different from the situation recorded by Marshall, probably from Hayling Island. We found, with Vinogradova, and probably Wesenberg-Lund also suggested it, that the 4th instar was in diapause. The 3rd instar was also in a form of quiescence which was very advanced and not very different from the diapause of the 4th instar. Some slow development seemed to occur in this instar. In any case the overwintering pattern found in Durham was very different from that reported by Marshall, and was nearer to the type reported by Wesenberg-Lund and said to be comparable to that of Theobaldia morsitans. As was the case for the latter species, Durham belongs, presumably, to a biological zone different from Hayling Island, as far as Anopheles claviger is concerned.

F. Laboratory Studies

(1) Mortality (Table VIII).

In the group kept inside the laboratory, mortality was very high from the second month following the collection. So far, almost all larvae died. Only 3 pupae were obtained out of 84 larvae reared. They gave adults which died when half pulled out from the pupal skins. Outdoors the mortality was much lower. Two groups of larvae were reared. The first, collected on January 14th 1967, reached May with 84% survivors. The second, collected on February 6th, reached May with 96%. This group was mainly used as a control group for field populations.

(2) Variations in Size (Table IX).

Among the species studied at Durham, *Anopheles claviger* was the only one to have such a wide range of individual variation. As a matter of fact, 3rd and 4th instar larvae individuals formed a continuous series as far as body length was concerned, ranging from 3.0 mm for the smallest 3rd instar larva found, to 6.5 mm for the largest 4th instar, with an overlap for the largest 3rd instar of 4.5 m and the smallest 4th instar of 4.1 mm. The occurrence of a wide range of variation among insects is a well-known phenomenon.

Teisser (1928) found in another group of insects even greater fluctuation within contemporary individuals of the same species of *Dixippus morosus*. In one population of this species the length varied from 15 mm to 85 mm in a continuous manner. He reported also that he could accentuate these individual variations by means of food changes.

However, in the population of Brasside I there was also another fact. The 4th instar larvae in January were 5.1 mm $\pm$ 0.6 long (head excluded), and the same instar in March were 5.8 mm $\pm$ 0.8. The frequency curves shown in Figure 12 are clearly distinct. Such data suggests that some physical growth took place between January and March. We have already reported Clements' statement according to which the thorax and abdomen of Mosquito larvae "grow continuously throughout larval life, showing no sudden change at ecdysis". We think also that there is physical growth among the overwintering larvae of *Anopheles claviger*. It is noteworthy that such growth takes place precisely among larvae found to be in diapause. Moreover, the fact has always been implicitly admitted by authors who stated that overwintering individuals were usually larger than short developing individuals because of the length of time spent in larval form.

G. Some peculiarities

(1) Partial moulting

Some odd cases of partial moulting were observed. The most frequent was the moulting limited to the head alone. This happened mostly with larvae collected in February and kept indoors. The bottom of one of the jars contained grain-like bodies which happened to be cephalic exuvia. At that time some larvae had transparent heads and normally dark brown bodies.

In other instances the posterior end of the abdomen, particularly the eighth segment, moulted and became transparent, whereas the rest of the larva did not show any change.

In some individuals of *Aedes punctor* we also noted partial moulting in which the body cast the old skin, but not the head or the siphon. For *Aedes* individuals this seems to be connected with the traumatic moulting due to transportation.

(2) Intermediate individuals

Some rare individuals (28/704) had either a 4th or 3rd instar head on a body intermediate in length (4.5 mm) between a 3rd instar body (mean 3.5 m) and a 4th (mean 5.1). They were mostly partially moulted individuals.

Partial moulting and intermediate individuals were found only among 3rd instar larvae and it may be an indication that the diapause affecting the 3rd instar larvae is different from the diapause of the 4th.

(3) Morphological variations

Some larvae had a white stripe on the dorsum of the abdomen. Marshall (1938) reported similar peculiarities among Anopheles maculipennis. The adults emerged from these larvae were identified as Anopheles claviger. The white line was also found on the resulting pupae. After observation it appears that a wide range of intermediate types exists between the brown type and the type with a mediodorsal white line. These intermediate types show white spots more or less packed in the median zone of the abdominal dorsum.

PART IV

Observations and laboratory study on moulting and mortality

A. MOULTING

1. Observations

Among the first three instars of _Theobaldia morsitans_ and in all instars of _Theobaldia annulata_ moulting normally occurred in the field between November and April. There are two periods when the rate of moulting is probably high : in the fortnight between the 17th and 28th of February and in the week between the 8th and 15th of April.

It has been observed that disturbance due to transportation causes moulting among larvae brought from the field.

2. Laboratory study : the effect of density on moulting

This study has been carried out with an experimental population mainly of _Theobaldia morsitans_ larvae in the fourth and third instars, reared in a room when temperature varied between 12°C and 18°C. Glass tubes of 20 cc were used, and there were eleven densities : one larva per jar, two larvae per jar, ... ten per jar, and fifteen per jar. For each density there were ten replicates.

The experiment started on 13th February and was stopped on April 15th. Between these two dates a regular census was taken twice a week : the dead larvae were removed and replaced by live ones to maintain the correct density; the exuvia were also removed and studied apart. The water was regularly replenished at each census time.

Results (Table XIII, Figure 15a)

The figures on Table XIII (grouped per week) show that moulting is somewhat independent of density and occurs almost necessarily at a certain time of the development. In some instances, however, stress conditions or disturbance cause moulting. The experiment shows the time of moulting more than the effect of density on it. Two periods can be seen at the reading of this table : the first between February 17th and 28th, and the second between March 8th and 15th.

If we take the most complete series of figures, that of the 8th week (March 8th - 15th), and if the percentage of moulting is plotted against density, the points are situated in such a way that the ideal curve may be a straight line - a decreasing linear function. (We admit that the high percentage of moulting for density 1 is an accident).

There is likely inverse linear function between density and moulting rate. But such correlation is strongly upset by the fact that moulting is due to happen at a certain time of the larval development. It can be delayed but not prevented from occurring.

Density	No. in each series	Weeks I-II		Weeks III-IV		Weeks V-VI		Weeks VII-VIII	
		No.	%	No.	%	No.	%	No.	%
1	10	.	.	.	.	.	.	2	20
2	20	.	.	1	5.0	1	5.0	7	35
3	30	.	.	.	.	9	30.0	14	46.6
4	40	.	.	3	7.5	12	30.0	20	50.0
5	50	2	4.0	2	4.0	20	40.0	29	58.0
6	60	2	3.3	14	23.3	34	56.6	37	61.6
7	70	1	.	14	20.0	43	61.0	35	50.0
8	80	.	.	20	25.0	49	61.2	42	52.0
9	90	.	.	21	23.0	51	56.0	57	52.2
10	100	.	.	36	36.0	64	64.0	53	53.0
15	150	.	.	77	51.3	110	73.3	90	60.0

TABLE XII. Variation of mortality with density.

Density	No. in each series	1st wk	2nd wk	3rd w	4th w	5th w	6th w	7th w	8th w
		17.2	21.2	4.3	11.3	18.3	31.3	8.4	15.4
1	10	.	.	.	.	.	2	1	8
2	20	.	.	.	.	.	1	.	8
3	30	.	.	.	1	.	.	.	5
4	40	2	1	.	1	2	.	.	13
5	50	.	.	.	.	1	.	.	15
6	60	2	4	.	.	.	.	.	12
7	70	1	5	.	1	.	.	.	6
8	80	1	5	.	1	.	.	.	14
9	90	4	3	.	1	1	1	.	9
10	100	4	9	.	1	.	1	.	12
15	150	6	22	.	1	2	1	.	19

TABLE XIII. Variation of moulting rate with density.

B. MORTALITY (Table XII, Figure 13, 14, 15)

1. Observations

Mortality was very high among larvae kept in the laboratory, particularly when the temperature in the jars exceeded 16°C. For the larvae reared outside mortality was much lower (cf. Table VIII & XI) and was mostly caused by post-moulting death. Almost regularly, death followed moulting from January to March, and particularly among newly moulted 4th instar of Theobaldia morsitans in March.

2. Particular laboratory study : The effect of density (Figure 13, Table XII).

The death rate was studied with the same lot of larvae and the same experiment as for moulting rate. There was the same number of densities (eleven) and the same routine check and census for the same length of time.

Results

The larvae used being mostly overwintering larvae (Theobaldia morsitans), their reaction was different before and after the reactivation of development in Spring. The effect is seemingly related to the state of the medium, the duration of the experiment, and the physiological state of the experimental population. In the following, the results are grouped by periods of two weeks.

a) Weeks I & II (Table XII, Figures 13,14,15)

The number of larvae that died is negligible (5/600). The medium is still new and the effect of crowding did not manifest yet.

b) Weeks III & IV (Table XII, Figures 13,14a)

The number of dead larvae is in sharp contrast with the previous period, particularly in higher densities. The ideal curve (Figure 14a) is likely to be a straight line, expressing a linear relation between density and mortality.

c) Weeks V & VI (Table XII, Figures 13,14b)

Relatively more larvae than in the previous census died. The ideal curve seems not to be a straight line. It is bent in the region of high densities.

d) Weeks VII & VIII (Table XII, Figures 13,15b)

There are fewer larvae that died than during the previous two-week period. The frequency curve (Figure 14b) is obviously split in two unconnected parts : the first six densities in one part, and the rest in another. There are two straight lines of different slopes corresponding to the two groups.

Discussion

The experiment is misleading because the actual results obtained are not consequent upon density only.

Many other factors interfere to cause the death of the larvae and the effects of these factors are not measured.

The first difficulty is that the population used is inevitably heterogenous; the reaction to stress is variable : some larvae had been under stress conditions for many days, some others had just been brought in. Their reaction will be necessarily different. Such a problem is particularly apparent on Figure 15b. The jars of higher densities happened to be replenished with newly collected larvae, and the mortality drops clearly.

The second difficulty is the discrepancy between the time needed by the effects of density to manifest and the time during which the larvae stay in that stage before moulting to pupae. The first may be longer than the second, particularly in Spring.

However, these reserves being expressed, the data collected suggests that density and mortality are in a linear function to one another, and the discrepancies are due to the strongly limiting effects of the conditions stated above.

C. Abnormal pupae

Between the middle of February and the end of March very small pupae were seen in two species : Anopheles claviger and Aedes punctor. The Anopheles pupae died regularly a few days after pupation and the first adult was obtained only on April 1st.

Among the small pupae of <u>Aedes punctor</u> we came across a very unusual case; one larva pupated at the 3rd instar (at least the exuvia is identical to other exuvia cast by larvae moulting from the 3rd to the 4th instar).

D. <u>Emergence</u>

Indoors <u>Theobaldia annulata</u> emerged in November, December, and January, but the conditions in the laboratory were so different from natural conditions that these results are of limited interest. One point should be made, however : when adults emerged in number in the laboratory there were many adults of the same species caught in many parts of Durham City.

In 1967, pupation in the field for species other than <u>Theobaldia annulata</u> took place in the last week of April, followed probably a few days later by emergence.

CONCLUSION

1. Breeding sites

All four species studied were found associated in all the habitats sampled in spite of the obvious difference of these sites. It is therefore likely that they can breed in many types of waters, and this conclusion is widely supported by the literature.

However, for each species there was a particularly favourable place : Houghall I and III for Theobaldia morsitans, Houghall II for Theobaldia annulata, Brasside I for Anopheles claviger, and Houghall III-2 for Aedes punctor.

For the particular case of Durham, all the habitats rich in Mosquito larvae fall within a short distance from a farm or a meadow. Most of them were sheltered from wind action : in hollow and under canopy like in Houghall, behind a curtain of hedge on an elevated road, (Brasside I and II). In all the places studied, farms or meadows are always found within half a mile distance.

It has been noted also that in the habitats found to be rich in larvae there was an almost total absence of freshwater invertebrates - except Cladocera spp. - This was true for all sites investigated. When, for instance, Cladocera species were abundant (such as in Houghall I), the Mosquito larvae migrate to other microhabitats of the pond. (This is also due to opposite tropism, Cladocera being attracted by light, and Mosquito larvae avoiding it). There is some link between the absence of animal population in Houghall (due to a heavily fouled water), and in Brasside (recently flooded place) and the abundance of Mosquito larvae at these places. In old ponds rich in fauna and vegetation in the same area, the Mosquito larvae were extremely scarce, if not totally absent.

From these studies we would think that the most favourable conditions for Mosquitoes in these areas are : - still, shallow, sheltered waters, temporary or permanent. - absence or scarcity of aquatic animals and floating vegetation. - proximity of farms and meadow.

We have met cases of overwintering which are cases of ecological isolation.

<u>2. Diapause, quiescence, and slow development</u>

In all the species studied there was a winter pattern of development. This pattern varies not only with species but also with individuals. But if all cases studied are put together, it appears that there is continuity and smooth transition from the slow development (<u>Theobaldia annulata</u>) and diapause (<u>Anopheles claviger</u> and <u>Theobaldia morsitans</u> 4th instars). We have not met a clear case of quiescence. The only observation along that line may be earlier completion of cycle among larvae reared at 16°C : two or three weeks ahead of the field populations.

A remarkable fact about the various winter patterns of development is that at the end the time needed to complete the cycle will be almost the same for a slow development like that of *Theobaldia annulata*, and for a mixed (slow and diapautic) like that of *Theobaldia morsitans* and *Anopheles claviger*.

Another point to be made is that diapause stops the development towards the completion of the cycle, but leaves the possibility of some physical growth, regressive or progressive. The increase in size, reported by some authors, has been implicitly recognized by many others who report that long-developing individuals are larger than short-developing ones.

We noted also that the winter pattern of development is correlated with geographical location. Species such as *Anopheles claviger* which reproduces continuously in the south parts of its range tend to have its breeding season more and more restricted to a narrow part of the year. In such species the extension and intensity of diapause are inversely proportional to the length of the possible breeding season.

3. Succession (Figure 11a, 11b, Table XIV).

In Houghall there was a succession of species from November 1966 to August 1967. The species found were the four already mentioned species, and in August *Culex pipiens* and probably a few species of Aedes. Two species are univoltine, *Theobaldia morsitans* and *Anopheles claviger*. *Theobaldia annulata* has two peaks, a massive hatching in November, and a second in late May. Aedes punctor has a second generation hatching in the last week of July or the first days of August.

SUMMARY

1. Methods

Regular sampling, measurements, laboratory breeding, were the main procedures used. Regular sampling was used to outline the general pattern of the population of Mosquito larvae through its development. Measurements were made in order to separate species and instar. They were used also to evidence growth or variations or abberations. Laboratory breedings were used as control experiment : the outdoor breeding as control for the field populations : the indoor breeding as control for the outdoor one.

2. Habitats

They were of two types. The habitats in Houghall were mostly permanent ponds or swamps with fouled water and permanent vegetation. The Culicine larvae were prosperous in these habitats. In Brasside there were mostly temporarily flooded places, with limpid water and grassy bottom. The Anophelines were more abundant than the Culicines.

3. The overwintering species

Theobaldia annulata had one generation in winter and another in summer. The development was not arrested but considerably lengthened and variable with individuals. Emergence took place from November to March.

Theobaldia morsitans. The second, third, and fourth instars were found together until February. The fourth instar,

unlike the three first ones, is in diapause. Pupation took place in April and adults emerged in May.

Aedes punctor. Hatching occurred in February and that generation completed the cycle in June. A second generation was found at the second instar dominant stage in August.

Anopheles claviger. This species was found in the 3rd and 4th instars, the fourth being in diapause, and the third progressing very slowly to the fourth (it is in fact in a looser form of diapause). Pupation took place in early April and emergence by the end of the same month.

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Stations	Dates of Sampling	Sample size	INSTARS Instars	No.	%	Siphon Length in mm	Siphonal Index
HOUGHALL I	Dec. 3rd	51	I	0	0	-	-
			II	3	5.8	0.900 ± 0.0	4.93
			III	34	66.6	1.425 ± 0.06	4.82 ± 0.32
			IV	14	27.4	2.125 ± 0.09	5.60 ± 0.38
	Jan. 13th	104	I	0	0	-	-
			II	8	7.6	0.900 ± 0.09	4.51 ± 0.30
			III	83	79.8	1.425 ± 0.05	4.86 ± 0.51
			IV	13	12.5	2.100 ± 0.05	5.77 ± 0.22
	Mar. 13th	87	I	0	0	-	-
			II	0	0	-	-
			III	3	3.4	1.400 ± 0.02	4.79
			IV	84	96.5	2.114 ± 0.08	5.84 ± 0.60
	Apr. 14th	100	I	0	0	-	-
			II	0	0	-	-
			III	0	0	-	-
			IV	88	88	2.136 ± 0.09	6.08 ± 0.50
			Pupae	12	12	-	-
HOUGHALL II	Dec. 30th	84	I	0	0	-	-
			II	1	1.1	0.900	3.60
			III	59	70.2	1.425 ± 0.06	4.70 ± 0.75
			IV	24	28.5	2.075 ± 0.07	5.22 ± 0.51
	Feb. 18th	128	I	0	0	-	-
			II	0	0	-	-
			III	37	28.9	1.400 ± 0.05	4.83 ± 0.70
			IV	91	71.0	2.116 ± 0.11	5.79 ± 0.52
	Mar. 13th	86	I	0	0	-	-
			II	0	0	-	-
			III	19	22.0	1.426 ± 0.05	4.49 ± 0.63
			IV	67	78.9	2.108 ± 0.10	5.54 ± 0.77
	Apr. 14th	100	I	0	0	-	-
			II	0	0	-	-
			III	0	0	-	-
			IV	77	77	2.223 ± 0.09	6.33 ± 0.52
			Pupae	23	23	-	-

TABLE V. *Theobaldia morsitans* : larvae from Houghall

December 1966 - April 1967

Stations	Dates of Sampling	Sample size	Instars	No.	%	Siphon Length in mm	Siphonal Index
BRASSIDE I	Dec. 17th	19	I	0	0	-	-
			II	0	0	-	-
			III	9	47.3	1.425 ± 0.09	4.92 ± 0.31
			IV	10	52.6	2.125 ± 0.10	5.93 ± 0.42
	Jan. 14th	20	I	0	0	-	-
			II	0	0	-	-
			III	7	35	1.450 ± 0.05	5.27 ± 0.30
			IV	13	65	2.250 ± 0.12	6.11 ± 0.32
	Mar. 11th	18	I	0	0	-	-
			II	0	0	-	-
			III	0	0	-	-
			IV	18	100	2.304 ± 0.07	6.35 ± 0.28
	Apr. 8th	20	I	0	0	-	-
			II	0	0	-	-
			III	0	0	-	-
			IV	17	85	2.223 ± 0.08	6.33 ± 0.52
			Pupae	3	15	-	-
BRASSIDE II	Dec. 19th	69	I	0	0	-	-
			II	2	2.8	0.900	4.86
			III	40	57.9	1.375 ± 0.07	4.99 ± 0.51
			IV	27	39.1	2.025 ± 0.07	6.05 ± 0.53
	Jan. 14th	40	I	0	0	-	-
			II	2	5	0.850	4.78
			III	28	70	1.325 ± 0.05	4.44 ± 0.68
			IV	10	25	2.075 ± 0.07	6.17 ± 0.73
BRASSIDE III	Feb. 9th	35	I	0	0	-	-
			II	0	0	-	-
			III	0	0	-	-
			IV	35	100	2.225 ± 0.10	6.20 ± 0.46
	Apr. 8th	24	I	0	0	-	
			II	0	0	-	
			III	0	0	-	
			IV	24	100	2.237	6.33 ± 0.52

TABLE VI. Theobaldia morsitans : larvae from Brasside

December 1966 - April 1967

Stations	Dates of Sampling	Sample size	INSTARS Instars	No.	%	Siphon Length in mm	Siphonal Index
HOUGHALL I	Apr. 1st	31	I	0	0	-	-
			II	2	6.4	0.700	2.62
			III	16	51.6	0.804 ± 0.050	3.00 ± 0.20
			IV	10	32.2	1.217 ± 0.035	3.65 ± 0.43
			Pupae	3	9.6	-	-
HOUGHALL III-1	Apr. 10th	71	I	0	0	-	-
			II	0	0	-	-
			III	26	35.6	0.808 ± 0.066	3.39 ± 0.47
			IV	37	50.6	1.202 ± 0.055	3.56 ± 0.38
			Pupae	10	13.6	-	-
HOUGHALL III-2	Feb. 18th	69	I	24	34.7	...	...
			II	42	60.8	0.604 ± 0.025	2.60 ± 0.44
			III	3	4.3	1.000	3.03
			IV	0	-		
			Pupae	0	-		
	Mar. 13th	61	I	0	0	-	-
			II	42	68.8	0.622 ± 0.035	2.95 ± 0.33
			III	14	22.9	0.858 ± 0.055	3.08 ± 0.21
			IV	5	8.1	1.055 ± 0.061	...
			Pupae	0	0	-	-
	Apr. 14th	167	I	9	5.3	...	...
			II	21	12.5	0.601 ± 0.035	2.90 ± 0.29
			III	57	34.1	0.853 ± 0.055	2.96 ± 0.32
			IV	55	32.9	1.175 ± 0.061	3.38 ± 0.46
			Pupae	25	14.9	-	-
	May 13th	31	I	0	0	-	-
			II	1	3.2	-	-
			III	4	12.9	0.866 ± 0.082	3.02 ± 0.45
			IV	12	38.7	1.205 ± 0.082	3.10 ± 0.47
			Pupae	14	45.1		
	June 10th	31	I	0	0	-	-
			II	0	0	-	-
			III	0	0	-	-
			IV	9	29.0	1.265 ± 0.066	3.16 ± 0.38
			Pupae	22	70.9	-	-

TABLE VII. *Aedes punctor* : larvae from Houghall

February - June 1967

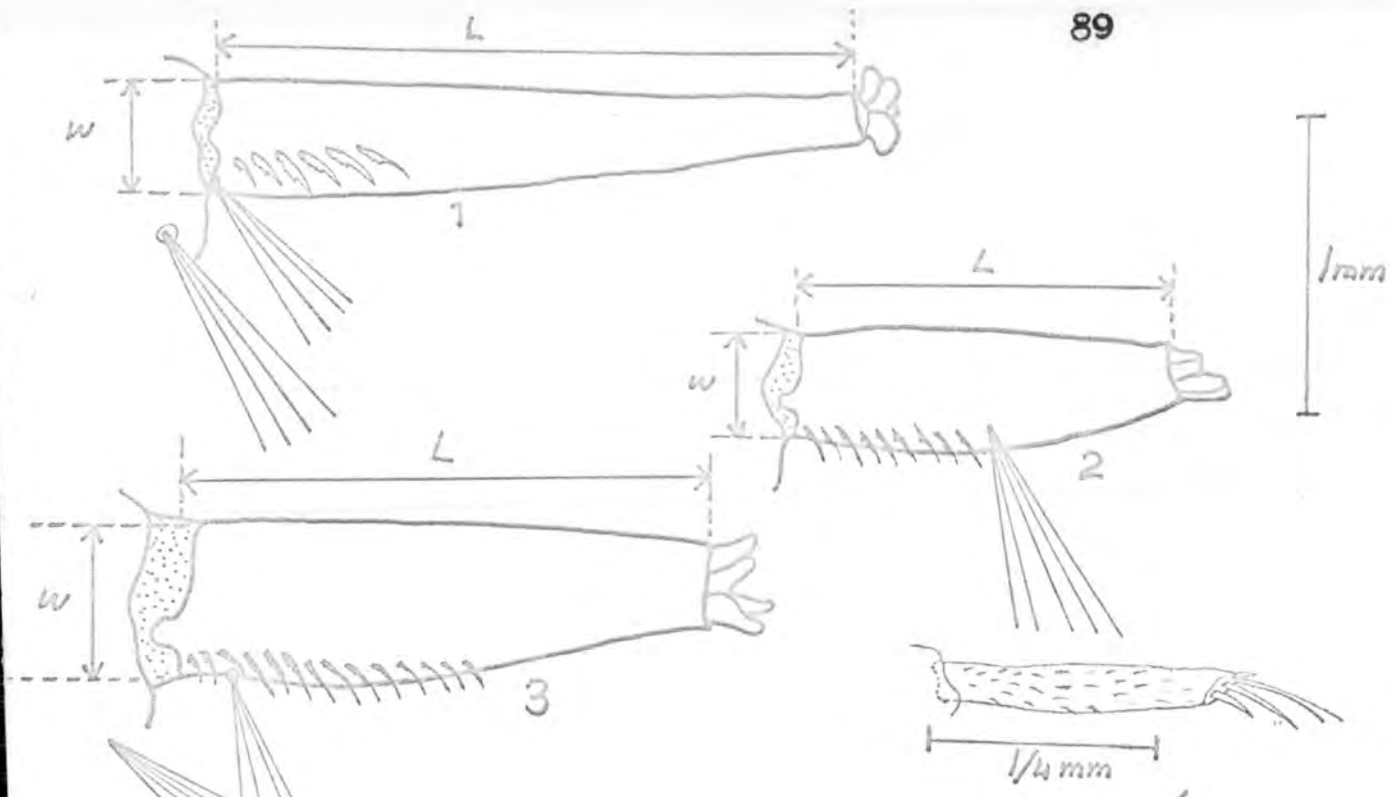
MONTHS	No. at start		Moult -ing	Pup.	Emer.	Dead	No. at end
	Pup.	Lar.					
November	0	10	4	0	0	9	1
December	6	60	16	8	17	11	38
January	0	38	3	1	0	22	16
February	0	90	74	6	3	50	37
March	4	83	9	9	13	54	25

TABLE XI. Indoor breeding from November to March (Mixed population of Culicine larvae)

Stations	SPECIES			Larval instars								Pupae	
				I		II		III		IV			
	Names	No.	% in sample	No.	% sp.	No.	% sp.	No.	% sp.	No.	% sp.	No.	% sp.
HOUGHALL I	C.pipiens	124	81.5	2	1.6	32	25.8	42	33.8	43	34.6	5	4.0
	T.annulata	19	12.5	..	...	5	26.3	8	42.1	6	31.5	.	...
	A.punctor	9	5.9	..	...	7	77.7	2	22.2	..	...	.	...
HOUGHALL III-2	A.punctor	77	100	4	5.1	67	85.8	7	8.9	..	...	.	...
HOUGHALL III-1	A.punctor	66	100	8	12.1	38	57.5	20	30.3	..	...	.	...

TABLE XIV. Results of the census made on August 16th

at HOUGHALL



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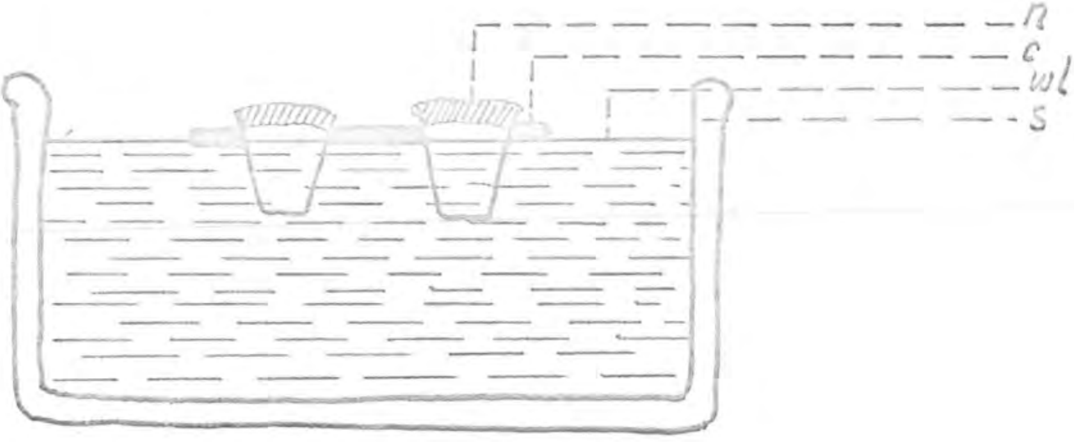

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Fig 1, 2, 3 Siphons of Theobaldia morsitans (1), Theobaldia annulata (3) and Aedes punctor (2) - Fig. 4 Antenna of Anopheles claviger. Fig 5 : Device for outdoor breeding
c : cork ; L : Length ; n : nylon cover ; s : sink ; w : width
wl : water level .

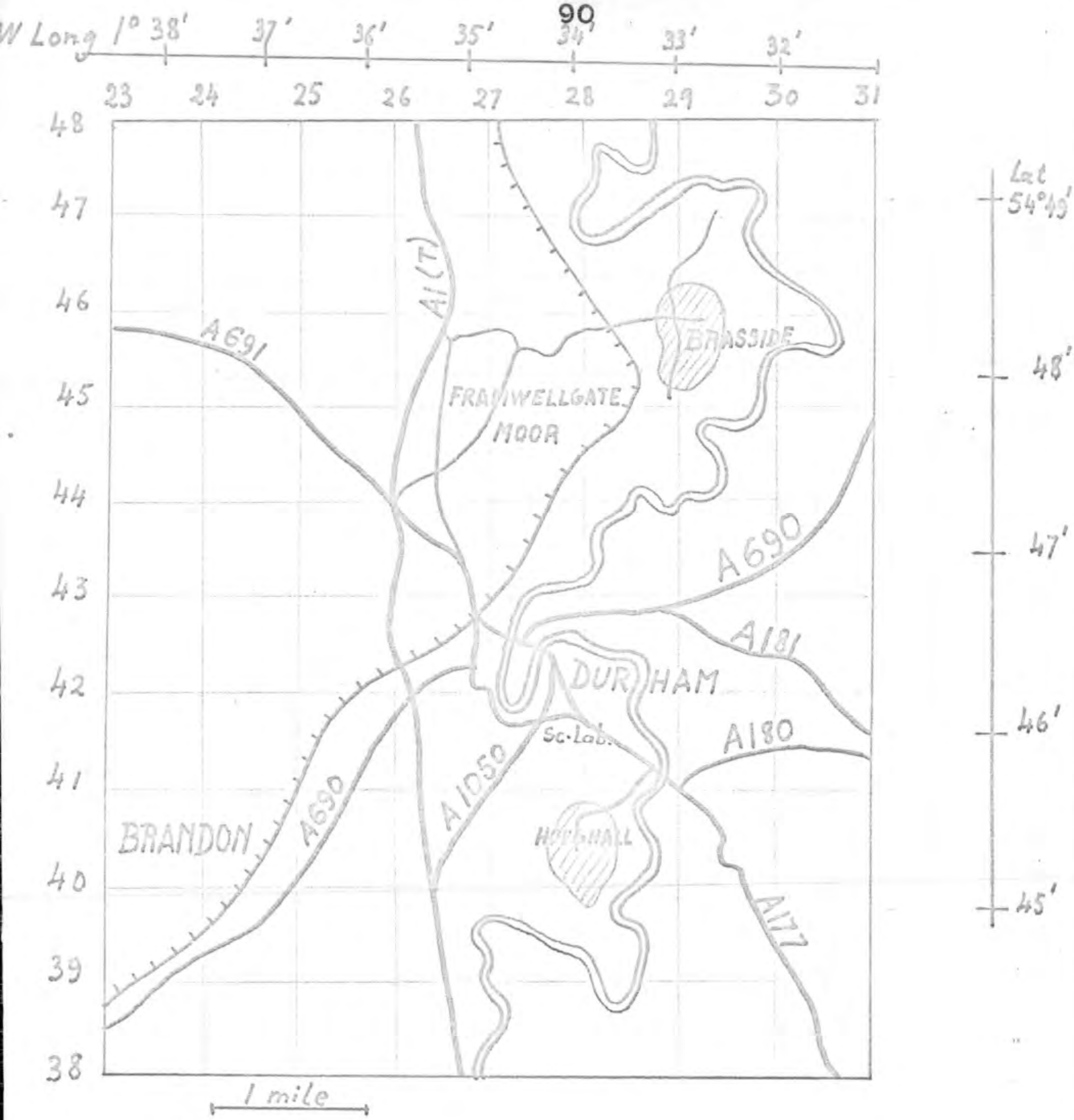


Fig 6 . Map of Durham showing the two areas studied : Houghall and Brasside (from Ordnance Survey Maps Sheet 85)

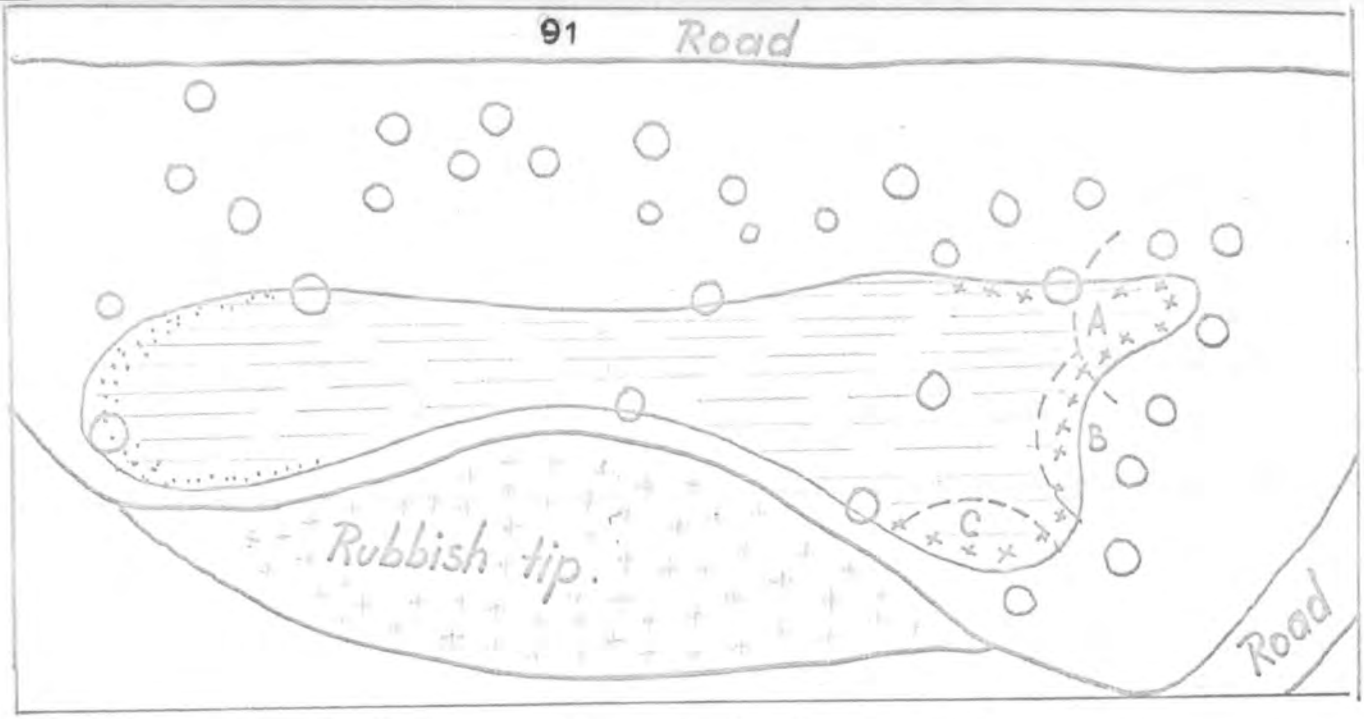


Fig. 7 Houghall I. [dotted box] Cladocera zone

[xx box] Mosquitoe zone ○ : Trees.

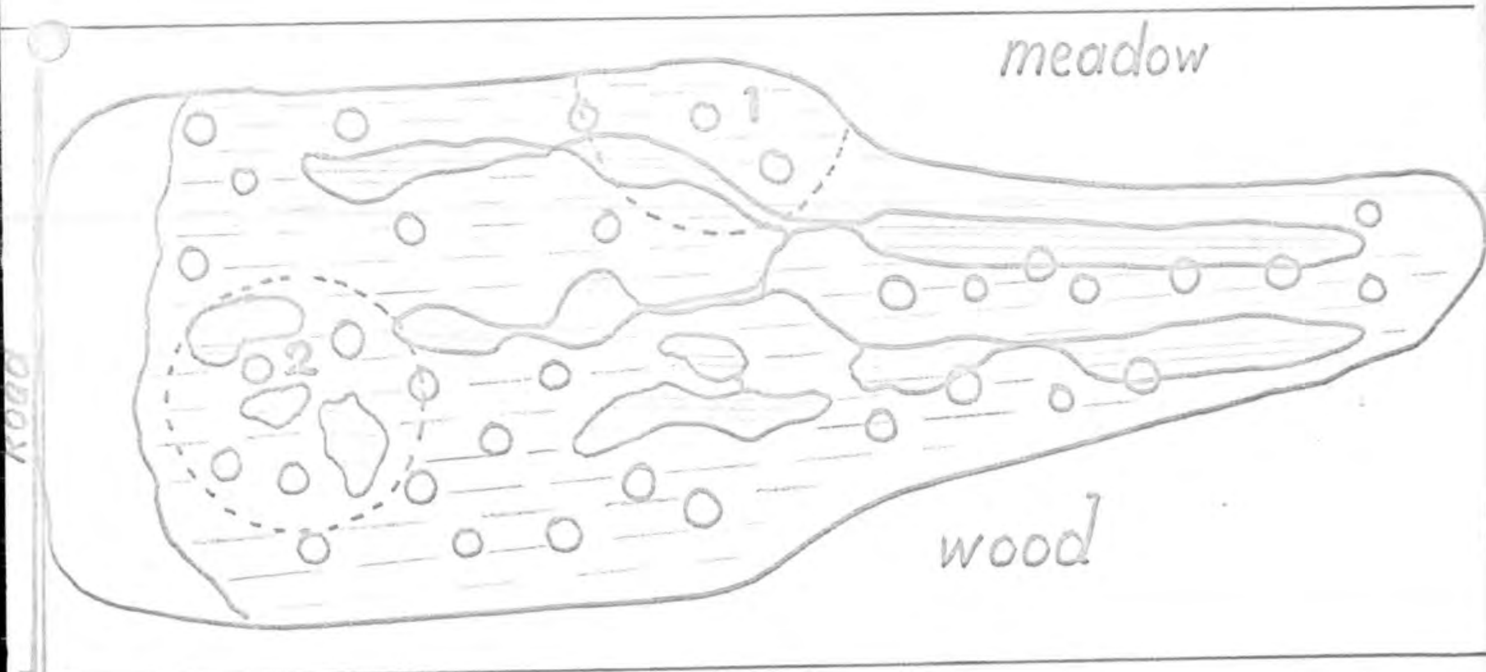


Fig. 8 Houghall III. [box] open water

○ : Trees

1 : Houghall III-1 2 : Houghall III-2

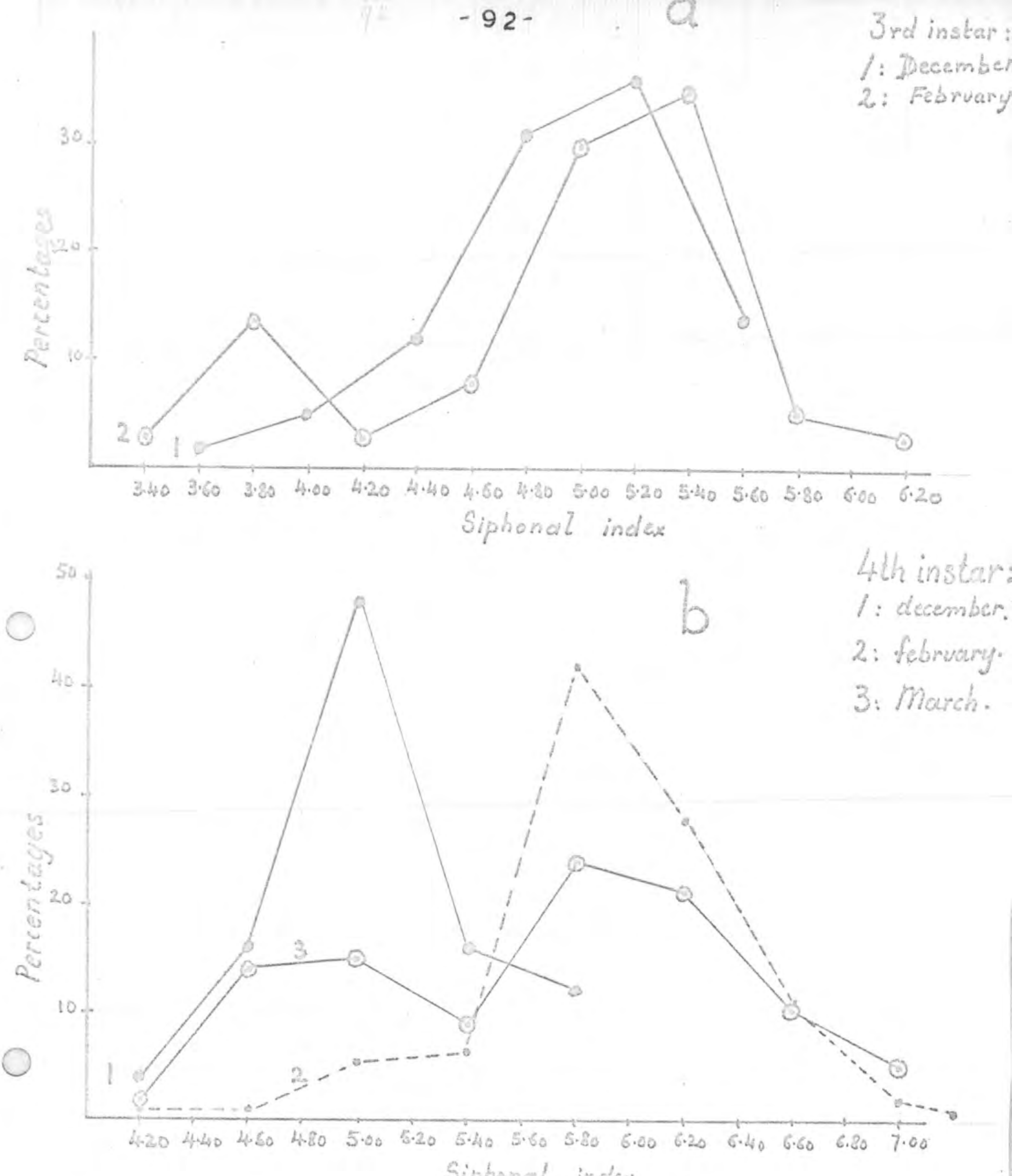


Fig 9a and 9-b. Unimodal and bimodal frequency curves a-1 and a-2 : 3rd instar ; b1, b2, b3 : 4th instar.

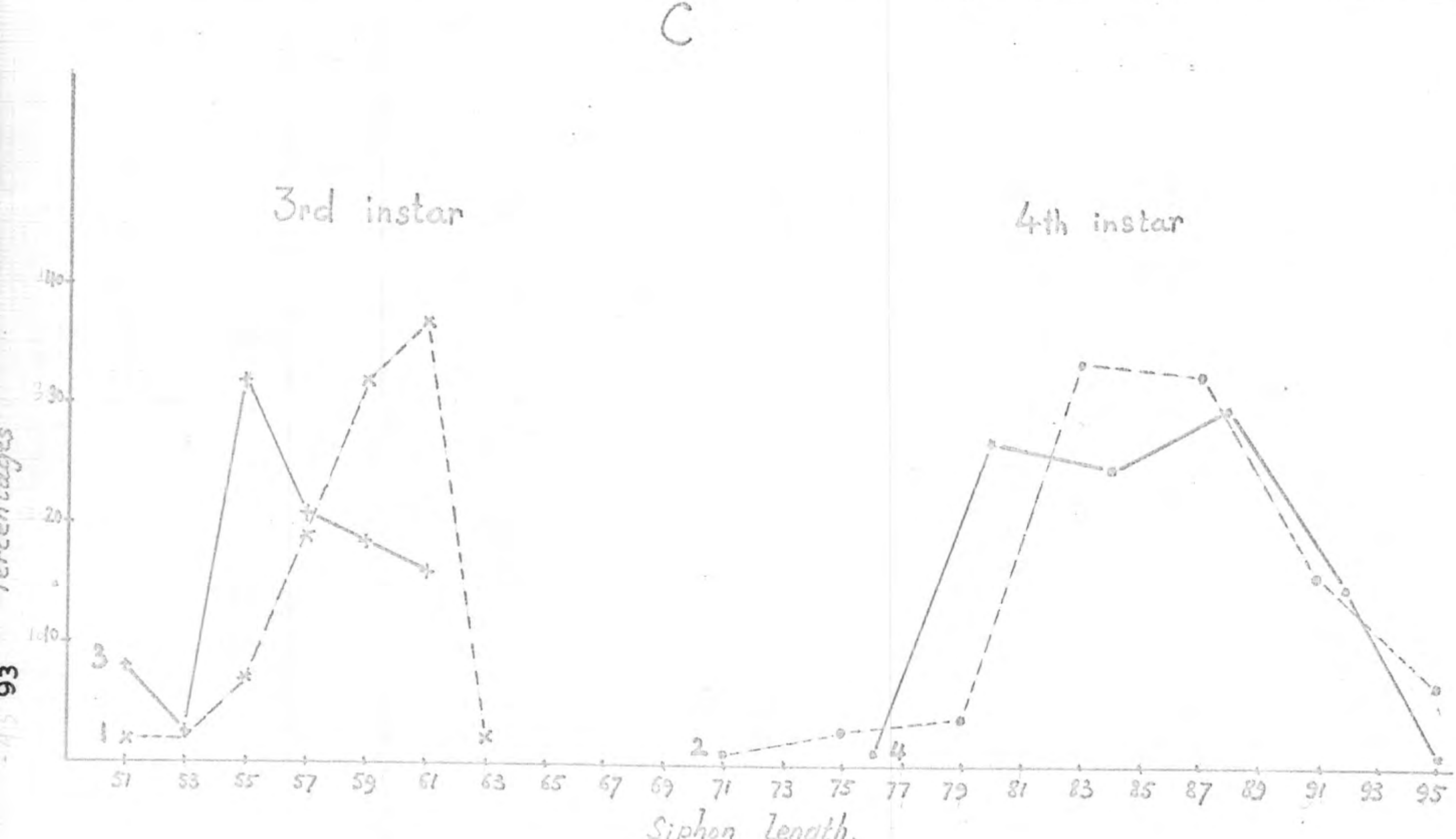


Fig. 9c. Siphon length. 1: 3rd instar in december; 2: 4th instar in February
3: 3rd instar in February; 4: 4th instar in March

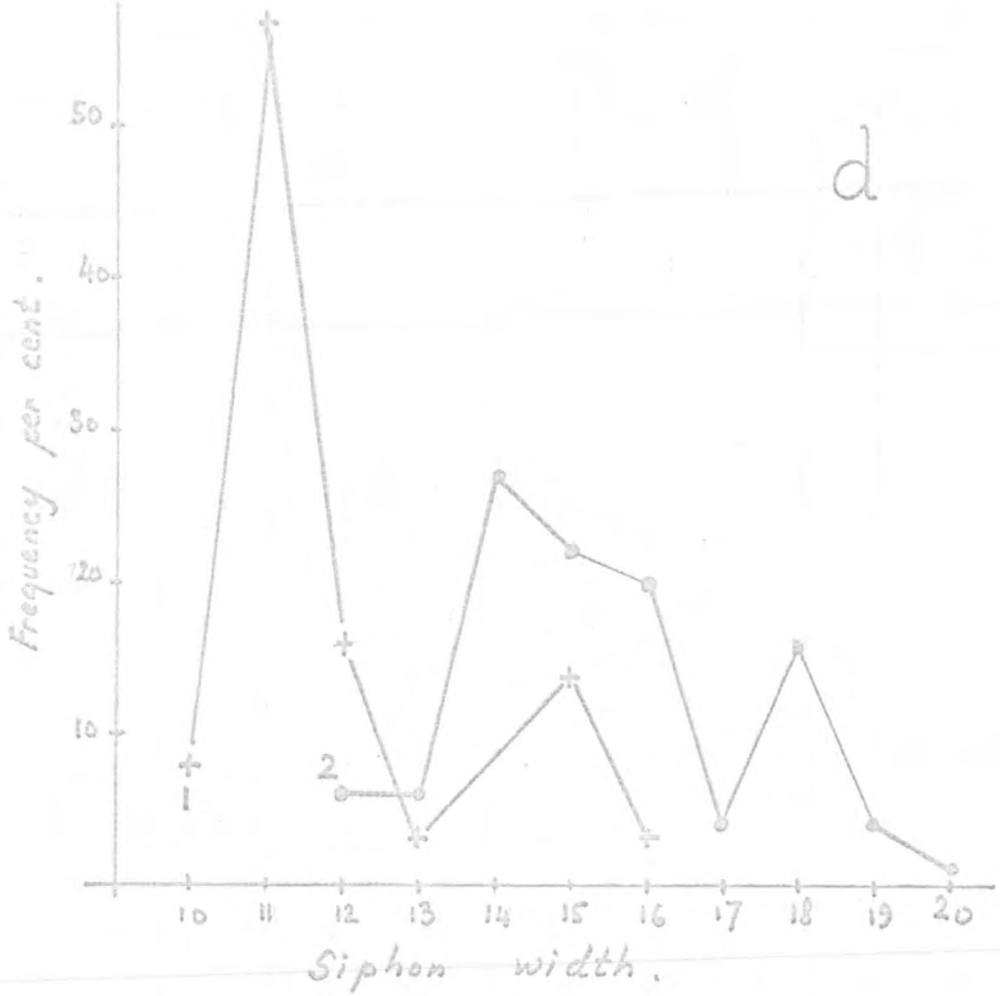


Fig. 9 d. Siphon width in a 3rd instar population in February (1) and 4th instar in March (2). *Theobaldia morsitans* from Houghall.

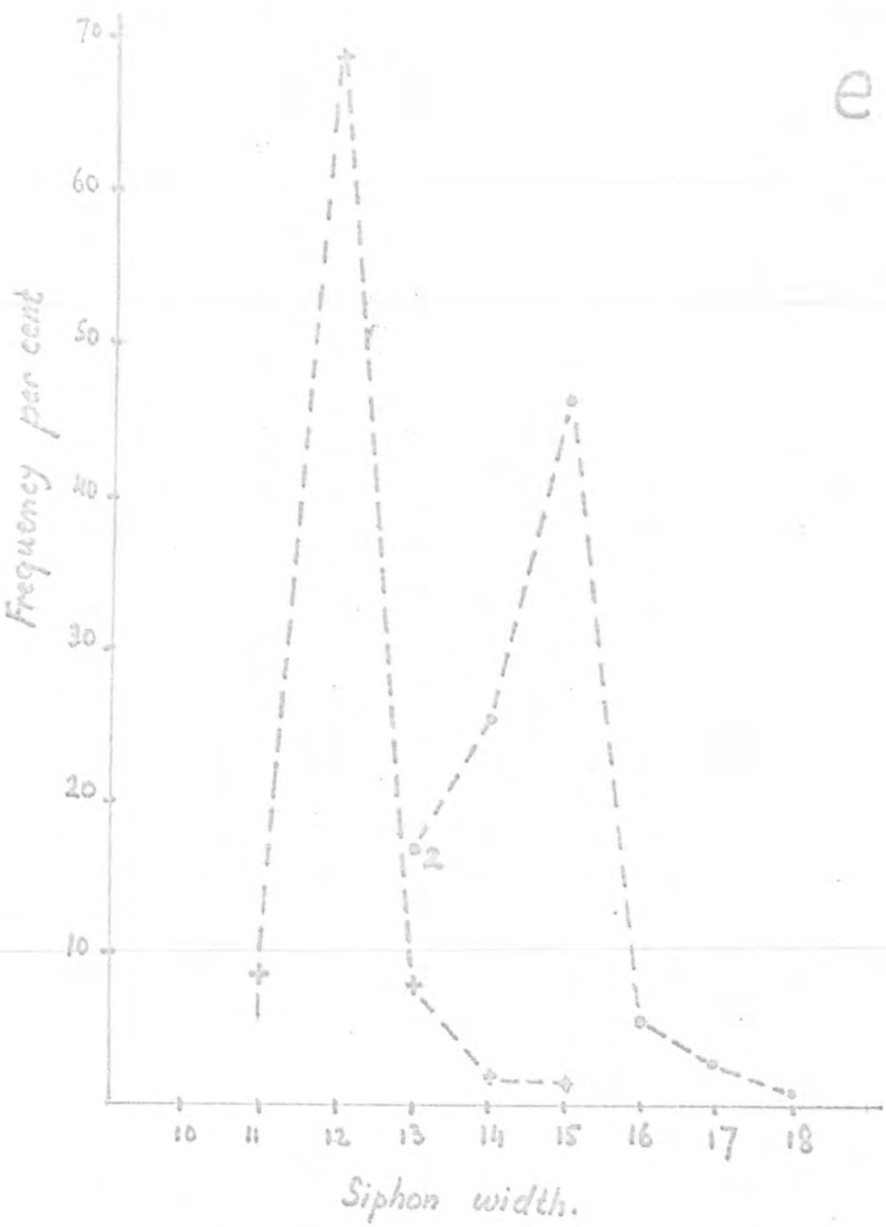


Fig. 9e. Siphon width of a 3rd instar populati[on] (1) in december, and a 4th instar population (2) in February (Th. morsitans from Houghall.)

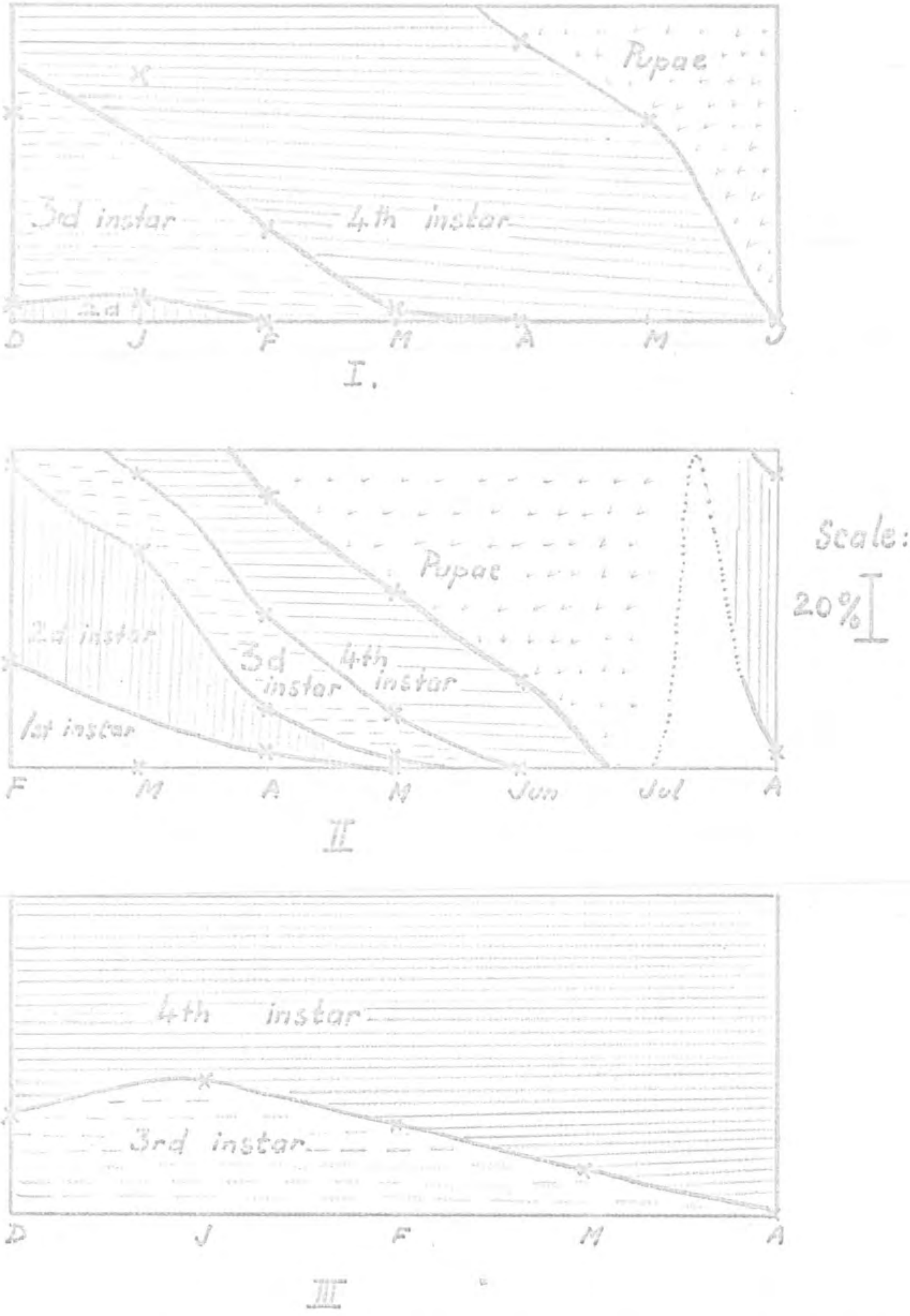


Fig. 10 Relative abundance (in percentages) of larval instars and pupae : I : Theobaldia marsitans from December to June at Houghall III ; II : Aëdes punctor from February to August ; III : Anopheles claviger from December to April in Brasside.

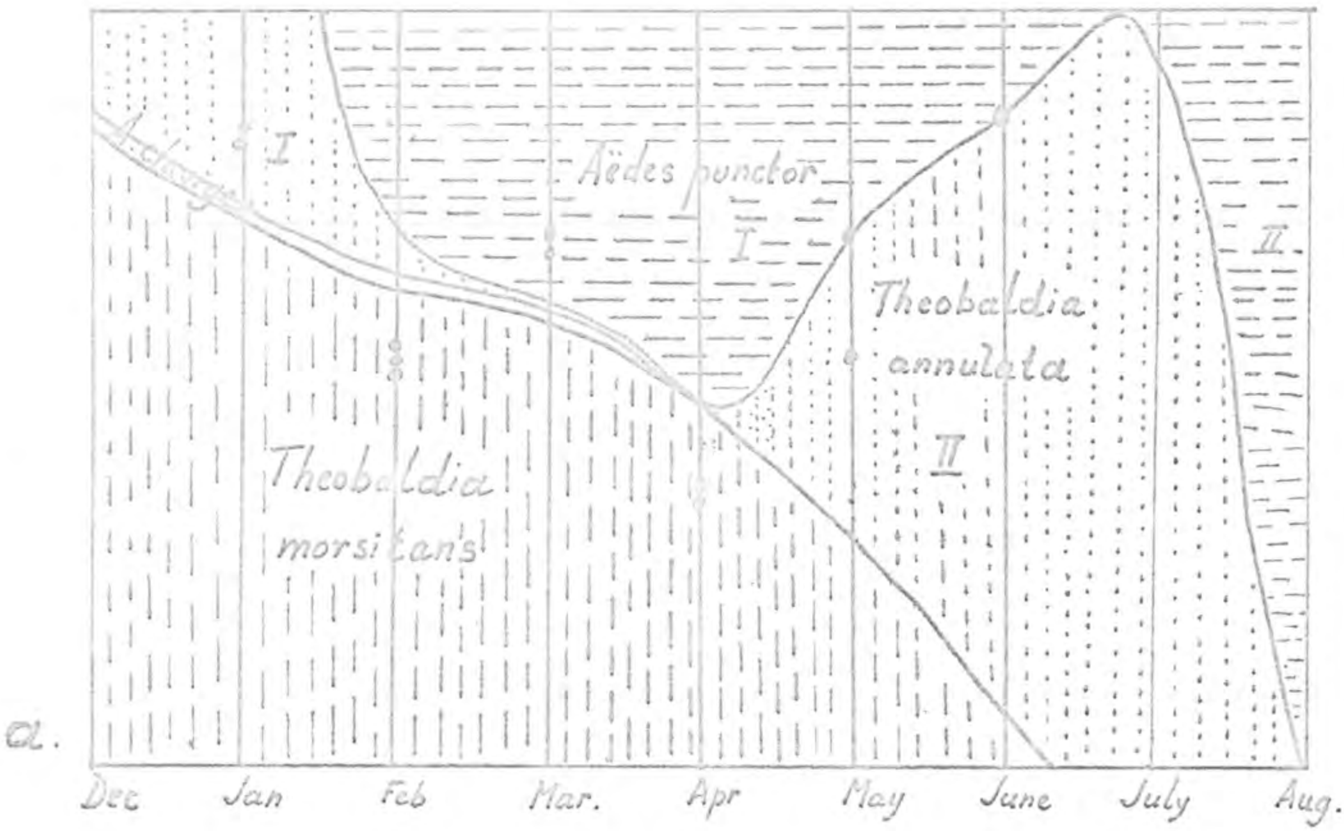


Fig II a. Association and succession of species at Houghall III from December, 1966 to August 1967.

Fig. II-b Association and succession at Houghall I from November to August. 1. A. claviger ; 2 T. morsitans ; 3 : T. annulata ; 4 : Aedes punctor ; 5 : C. pipiens

MONTHS	NUMBER AT START	MOULTED	PUPATED	EMERGED	DIED	NUMBER AT END
November	IN. 5 OUT. ·	0 ·	0 ·	0 ·	2 ·	3 ·
December	IN. 23 OUT. ·	0 ·	0 ·	0 ·	0 ·	23 ·
January	IN. 23 OUT. 36	0 0	0 0	0 0	17 5	6 31
February	IN. 82 OUT. 107	4 0	2 0	0 0	10 4	72 103
March	IN. 72 OUT. 103	0 0	1 0	0 0	55 3	18 100
April	IN. 17 OUT. 100	0 15	0 11	1 5	17 0	0 89 L.+6P.

Table VIII. *Anopheles claviger*: Results from laboratory breeding.

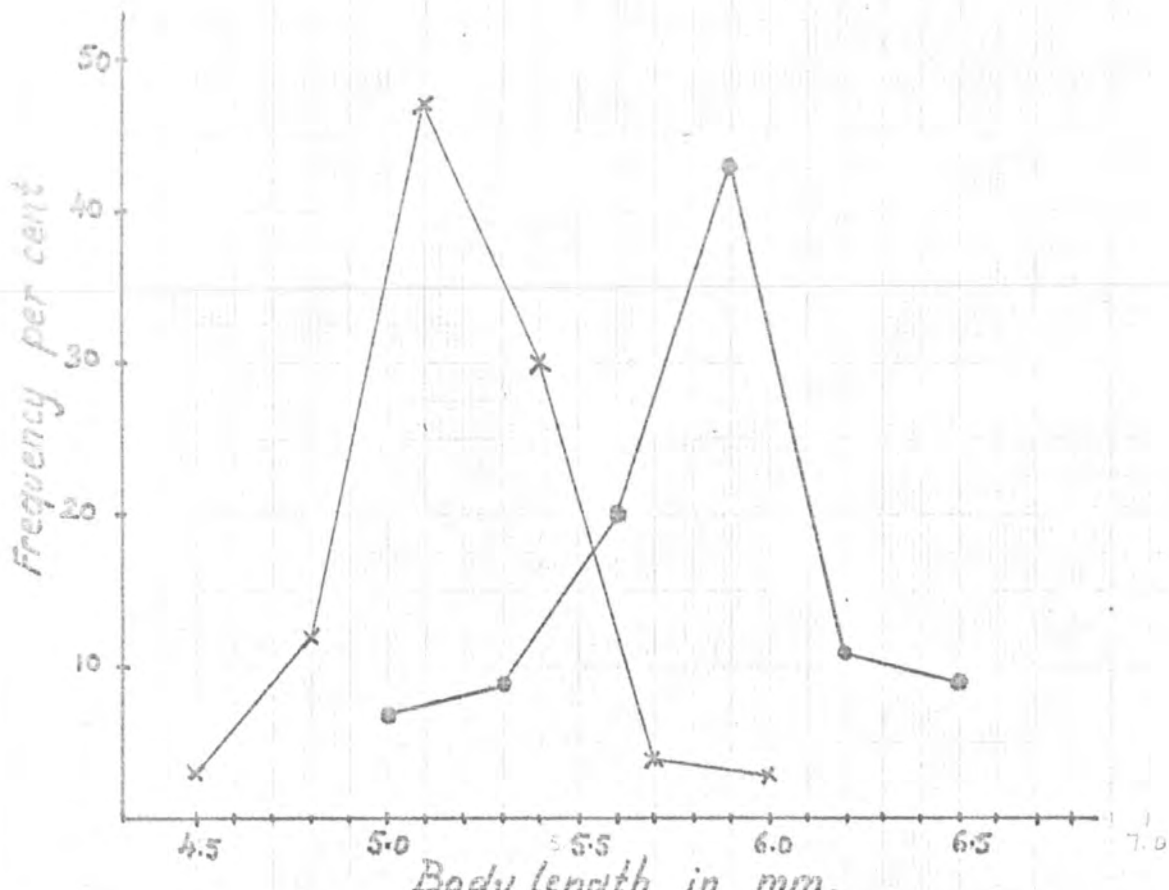


Fig 12. *Anopheles claviger*, 4th instar in January (x) and March (•)

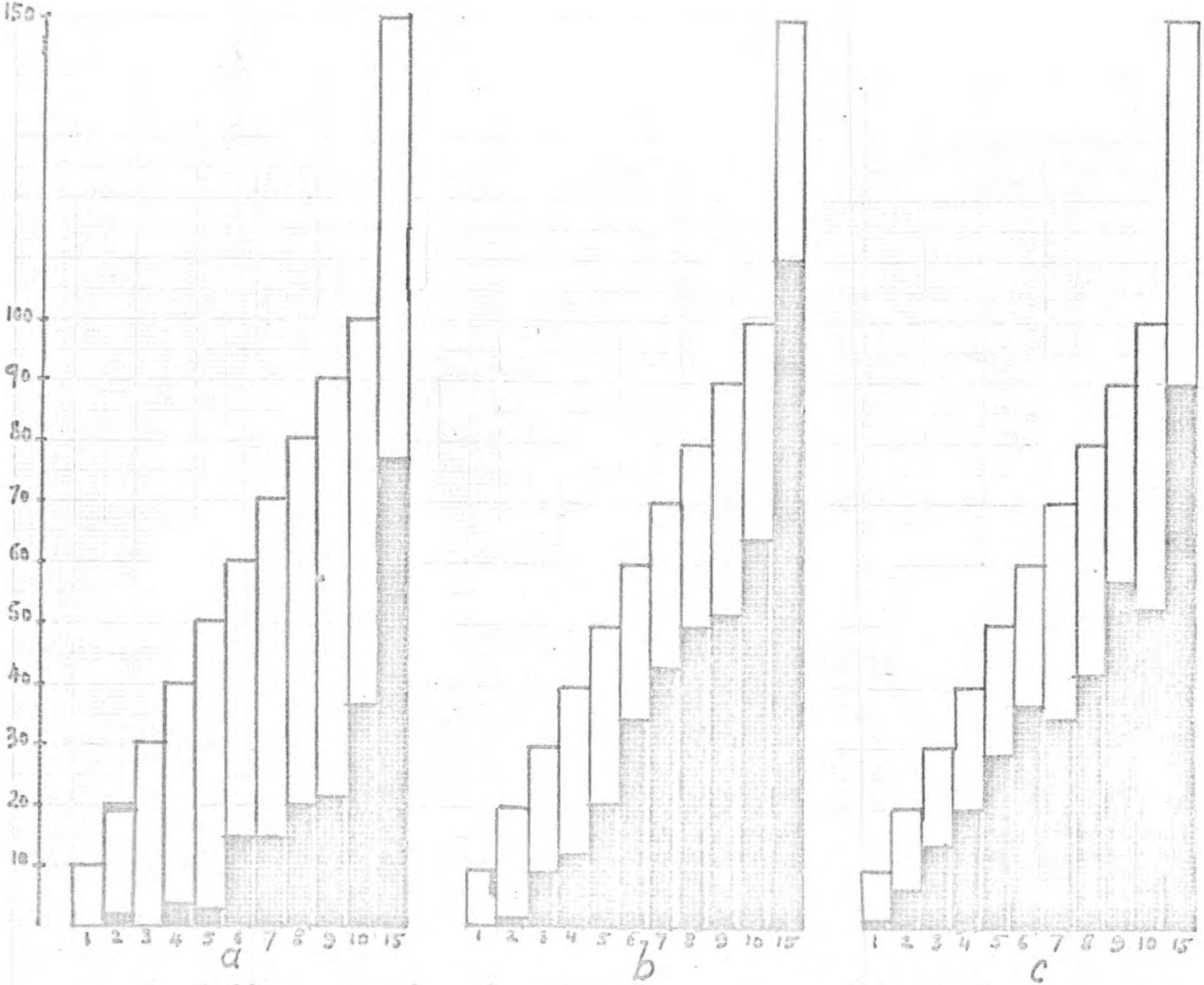
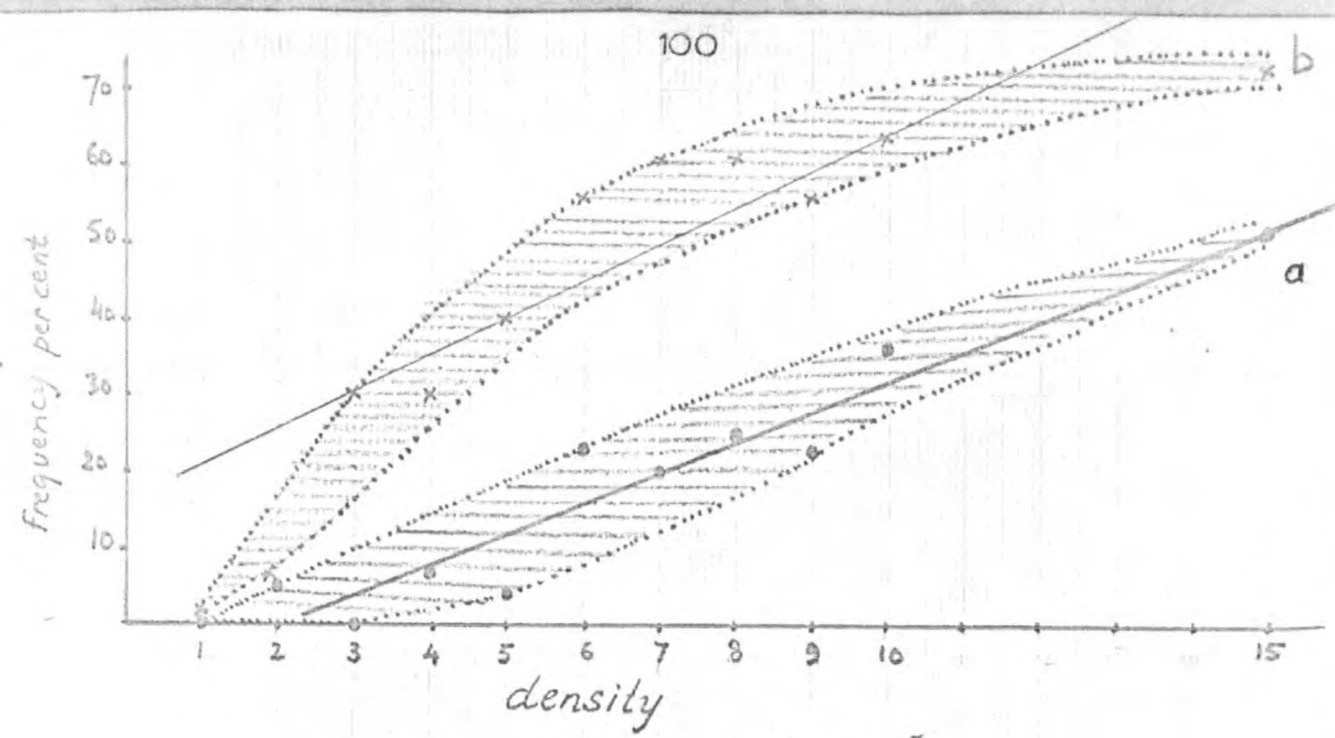
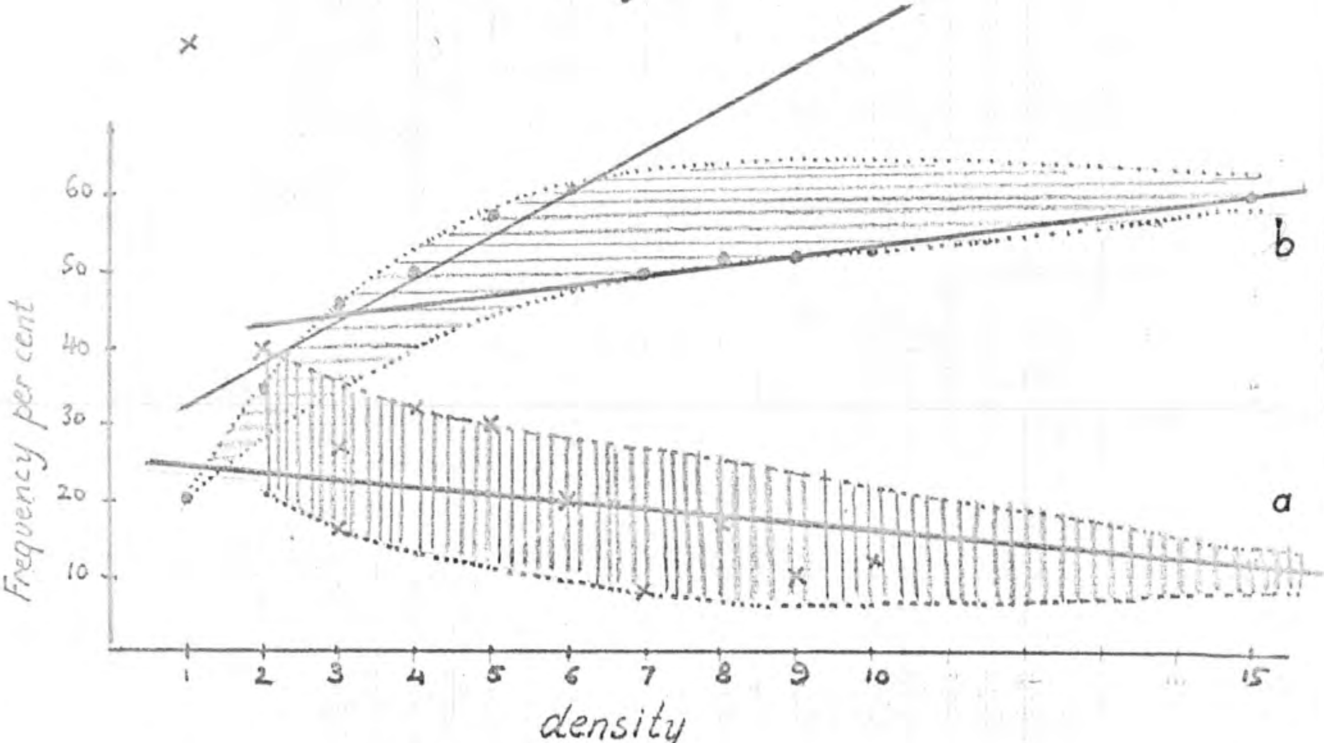


Fig. 13. Effects of density on mortality. In black: Number of larvae that died; in white: survivors. – a: end of the 4th week b: end of the 6th week – c: end of the 8th week.





Figs 14 (above) and 15 (below). Effect of density on mortality (14 a, b, 15 b) and on moulting rate (15 a).