THE ECOLOGY OF *AEDES CANTANS* (MEIGEN) AND BIOLOGY OF *CULEX PIPIENS* L. IN HIBERNATION SITES IN NORTHERN ENGLAND

by

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ABSTRACT

The biology and ecology of Aedes cantans was studied for three years in a small wood in north-west England. In addition laboratory studies were undertaken on egg batch size and duration of the gonotrophic cycle under different environmental conditions. Comparative larval sampling showed that quadrats gave more accurate samples of the different aquatic age-classes than a ladle or aquatic net. Aquatic light traps employing either a betalight or chemical light were evaluated and the latter proved to be of considerable value in trapping larvae. Various adhesives were used successfully in the laboratory to trap Ae. cantans larvae, but they were of little use in larval habitats.

The population dynamics of the immature stages of Ae. cantans were studied and survivorship curves and life-tables constructed. In 1980 mortality was highest amongst the later larval instars whereas in 1981 it was highest amongst earlier instars. The precipitin test identified dytiscid larvae, adult aquatic beetles, Mochlonyx culiciformis, Chaoborus crystallinus and sticklebacks as the more important predators, but they could not account for the estimated large pre-adult mortalities (79.7 - 95.1%) occurring each year. Other factors, including desiccation of parts of the larval habitats, deoxygenation and possibly larval competition for space, probably caused considerable mortality. Another serological method, the ELISA test, was evaluated for predator detection but was inferior to the precipitin test.

Adult behaviour and seasonal activities were studied by human-bait catches and by sweep-netting vegetation. Blood-feeding was delayed until about three weeks after emergence; maximum biting occurred in June and July. The principal hosts were cattle. Females took sugar meals both before and after blood-feeding. A few adults of Ae. cantans were parasitized by hydrachnellid and erythraeid mites. Monks Wood, CDC and chemical light traps were inefficient at sampling either newly emerged adults or older ones resting amongst vegetation. A carbon dioxide-baited trap also failed to attract adult mosquitoes.

The seasonal build-up and decline in populations of hibernating female Culex pipiens was studied over 3 years in man-made shelters in West Kirby, near Liverpool. It was considered that in one shelter the large reduction in the C. pipiens population occurring each November - December might be due to mosquitoes leaving it, but the reasons for such an exodus were not discovered. However, an exit trap fitted to another shelter caught very few mosquitoes during this period, although the population was decreasing. Spiders were present in both shelters but although they were considered to be predators their populations were too small to account for the large reduction in numbers of C. pipiens observed during hibernation.

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INTRODUCTION TO ECOLOGICAL STUDIES ON MOSQUITOES

Aedes cantans (Meigen) is a Palearctic mosquito with a distribution extending from Britain across Europe to China. It is essentially a woodland species and the larval habitats are, often temporary, collection of shaded water in woods and forests. Ae. cantans is a one-generation species (univoltine) and eggs laid by the females during the summer are oviposited among damp leaf litters of shaded woodland pools (Marshall, 1938, Covell and Shute, 1962 and Service 1977a). The conditions favourable for the hatching of submerged eggs arise in January or February with the result that 1st-instar larvae of Ae. cantans appear in large numbers; pupae are formed in late April or early May (Service 1977a). Service (1977a) and Lakhani and Service (1974) studied the mortalities of the Ae. cantans immature stages and using survivorship curves and life tables, found that mortality was greatest in the younger instars. Coelomomyces fungi, aniridescent virus, mermithid nematodes caused some larval deaths. Precipitin tests identified the most important predators of the pre-adults as larval Dytiscidae (Coleoptera) and adult flies as predators of emerging adults. The objectives of the present study were to evaluate different sampling techniques for sampling the immature stages of Ae. cantans and other mosquito species at Ness Woods on the Wirral peninsular. Also, to study the population dynamics of the immature stages of Ae. cantans and identify its principal causes of mortality. In addition to using the precipitin test to identify predators, the value of its Enzyme-linked immunosorbent assay (ELISA) method as a serological tool for identification of predators was evaluated.

Adult mosquitoes probably pass more time resting in natural shelters in the vegetation than in flight (Service, 1976), so sweep-netting vegetation was undertaken to obtain samples of males, and unfed, blood-fed, half-gravid and gravid females. The collection of blood-fed adults is useful because engorged females can be examined serologically to determine their natural host preferences. Besides collecting adults with a sweep-net, other sampling methods, namely human-bait collections, light traps catches and dry ice collections were tried. Regular human-bait catches, allowed the seasonal incidence of the adult population to be monitored.

The present account describes the ecological studies extending over three years (1979-1981) on the immature stages and adults of Ae. cantans at Ness Woods.

In Britain Culex pipiens hibernates during the winter in cool damp enclosed situations such as brick shelters and dark cellars. When the warm days of spring arrive usually in April, adults become active and leave their hibernation shelters to lay their eggs (Marshall, 1938, Covell and Shute, 1962, Service, 1969a). Females of Culiseta annulata Schrank also hibernate in the winter, but in only a "partial" state of hibernation. During spells of mild weather females leave their hibernation sites and feed on man or other suitable hosts at any time during the winter (Marshall, 1938, Covell and Shute, 1962). The last sections of this thesis present the result of studies on the build-up and decline in hibernating populations of C. pipiens and Cs.annulata in shelters at Leas School, West Kirby on the Wirral conducted, from December 1978 to June 1981.

DESCRIPTION OF WORKING AREA

The ecological work was undertaken in Ness Woods, a private woodland of some 6ha (Fig. 1) at about 33km (by road) south-west of Liverpool in the north-west of England. (3° 1.5 'W, and 53° 17 'N)

Fig. 2 shows the sketch map of Ness Woods and the surrounding areas. Adjacent to the northern part of Ness Woods is a road leading to a village called Ness about 1½ km west of NessWoods. Across the road is a small patch of woodland and to the north-east is the University of Liverpool's Faculty of Veterinary Science Animal Husbandary Farm. Extending from the north-east of Ness Woods towards the north-west is a large area of grazing fields for cattle, sheep and horses. To the east is a grazing field for cattle (Fig. 3) and a poultry farm, and to the west of Ness Woods is an agricultural farm for growing barley and potatoes.

Canopy vegetation

Dominant trees in the woodland were Sycamore, Acer pseudoplatanus L, and Oak, Quercus petraea (Mattuschka) Liebl., except around a few ponds and along the south-edge where Silver Birch, Betula pendula Roth., is the commonest tree (Figs 4 and 5). Other trees include a very few Horse Chestnuts, Aesculus hippocastanum L., scattered Elms, Ulmus procera Salisb., a few Ash, Fraxinus excelsior L., even fewer Elder, Sambucus nigra L, and one or two Aspen, Populus tremula L.

Scrub vegetation

Scrub vegetation composed mainly of younger Sycamore, Acer pseudoplatanus L. and Hawthorn , Crataegus monogyna Jacq., with a very few scattered Holly bushes, Ilex aquifolium L.

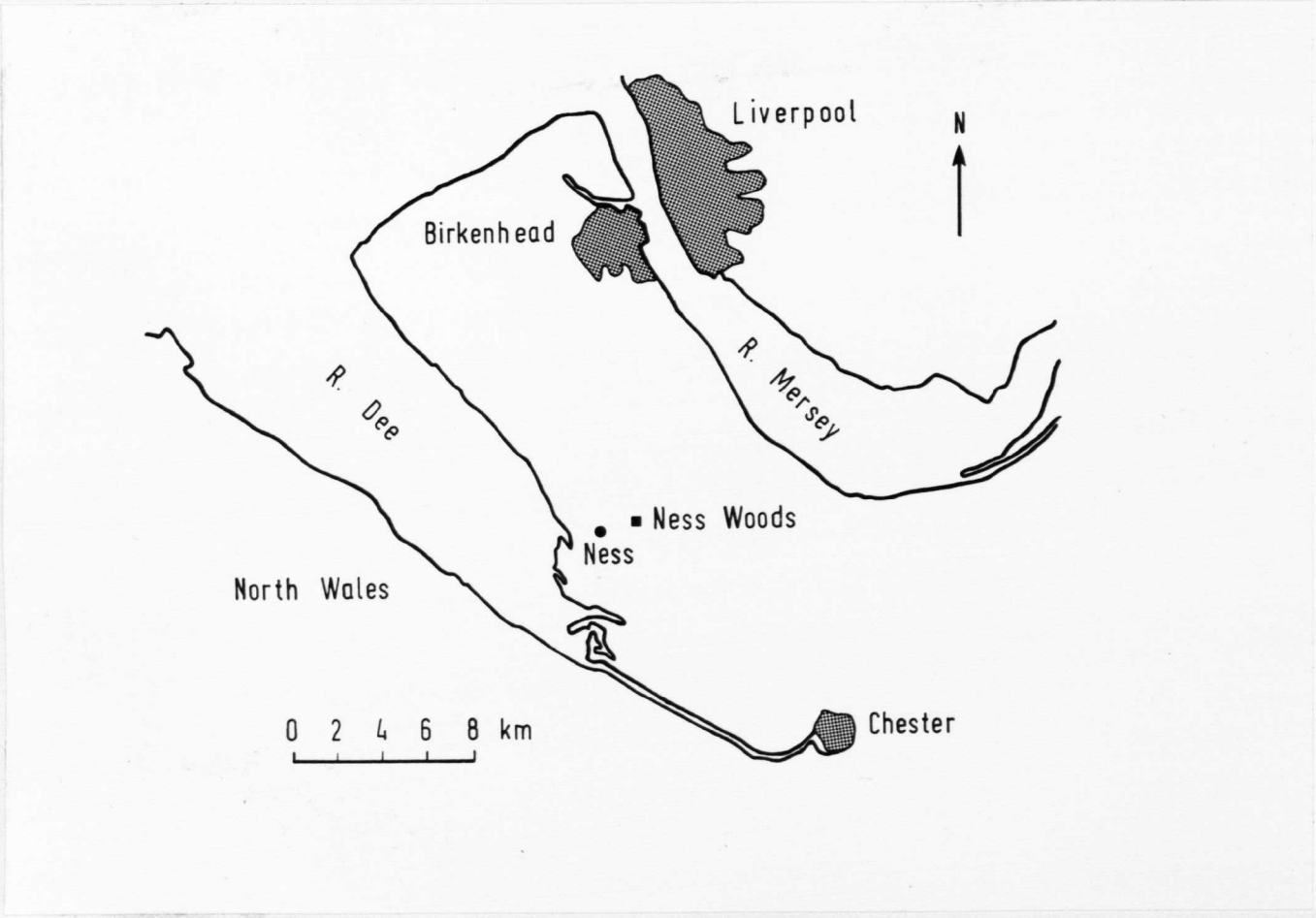

Fig. 1: Map of Wirral Peninsular and the location of Ness Woods (taken from map of North Wales produced and published by "Geographia" Ltd.)

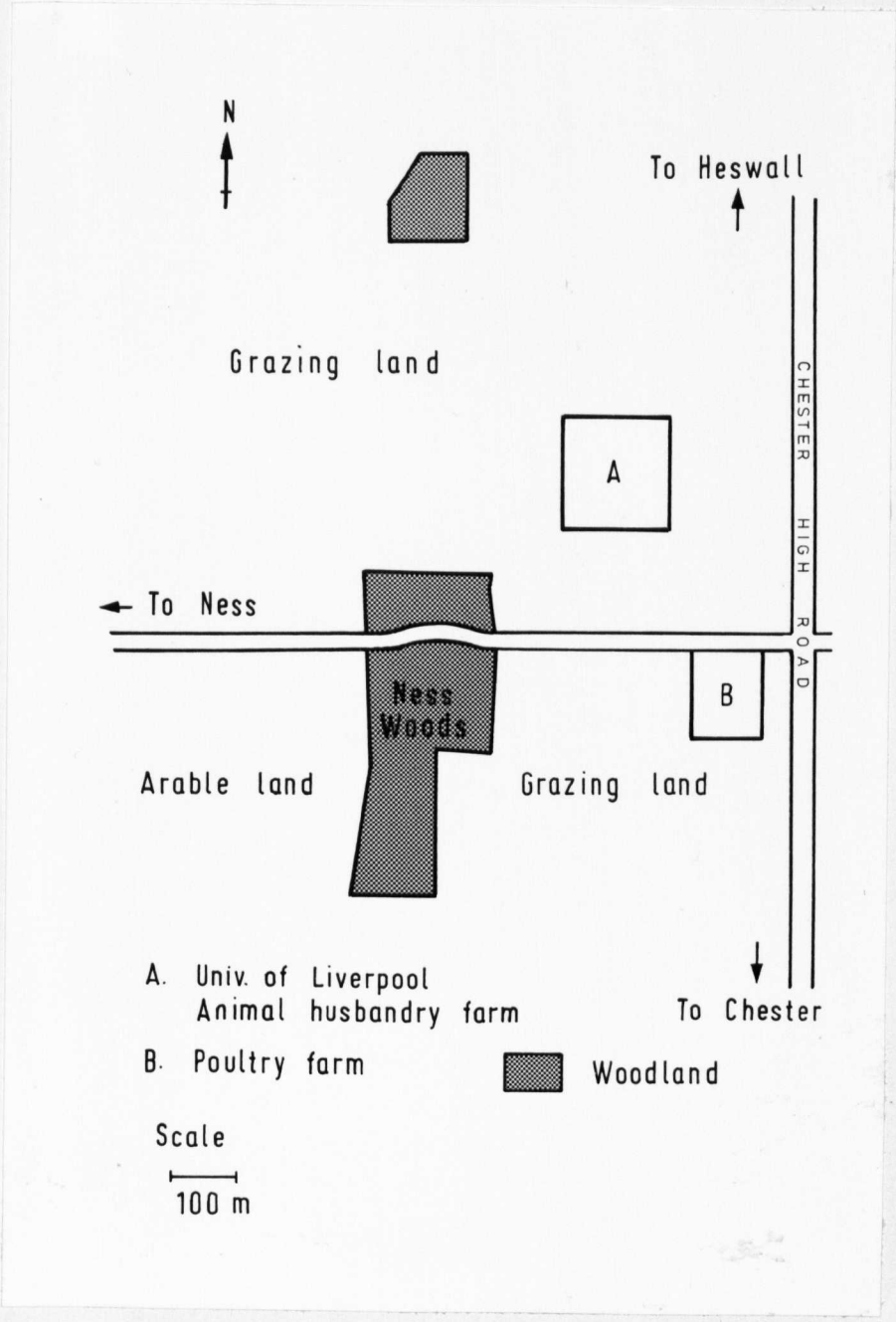

Fig. 2: Sketch map of Ness Woods and the surrounding areas.

Fig. 3: A field for cattle adjacent to Ness Woods.

Fig. 4: The ground vegetation and trees at Ness Woods during the summer.

Fig. 5: Typical area of trees at Ness Woods during the summer.

Ground vegetation

Ground vegetation comprised mainly mixtures and almost pure stands of Bracken, Pteridium aquilinum (L.) Kuhn, and Bramble, Rubus fruticosus L. (Figs 4 and 5), but on the southern border there were more grasses of the species Anthoxanthum odoratum L, Dactylis glomerata L and Milium effusum L. Ground vegetation was about 60 cm in the summer months (Fig. 4) . The common woodland plants were Bluebells, Scilla nonscripta (L.) Hoffmg. & Link, and Red Campion, Lychnis dioica L. Other plants include Rosebay Willow Herbs, Epilobium angustifolium L. Ladies Bedstraw, Galium verum L., Fox glove, Digitalis purpurea L. and Honeysuckle Lonicera periclymenum L.

Ponds and ditch

There were 12 ponds in the study area and one of them was subdivided into four separate ponds. On the eastern side of the woodland, running north to south was a ditch. Most of the ponds were filled with water throughout the year but a few which were shallow dried out in the summer but became filled with water each winter and spring and provided larval habitats for Ae. cantans, and most of the ecological studies on immature stages of Ae. cantans were studied in these shallow and waterlogged ponds (Figs 6 and 7).

Mammals were sometimes seen in the woodland namely rabbits, squirrels and mice. Occasionally ducks were seen in some of the ponds and birds were often seen in the canopy.

Ae. cantans was the commonest mosquito breeding in the ponds and ditch at Ness Woods. Smaller numbers of Ae. punctor (Kirby). Ae. rusticus (Rossi) and very low numbers of Culiseta annulata also occured in some ponds. However, in ponds of the adjacent woodland across the road the population of Ae. punctor was

Fig. 6: A typical habitat of Ae. cantans in Ness Woods during the winter.

Fig. 7: A typical habitat of Ae. cantans in Ness Woods during the winter.

reasonably high. Arboreal species found in Ness Woods include Aedes geniculatus Olivier and Anopheles plumbeus Stephens, but their population were never large.

Weather

Climatological data relevant to Ness Woods are given in Table 1 and rainfall plotted in Fig. 8. Although there is considerable variation in the amount of precipitation in the same months during different years, there is not much difference in annual rainfall.

April, 1980, the driest month ever recorded at this station since 1969, and also the sunniest April ever recorded, temperature was above average. From 30th March, 1980 to 18th May, 1980, rainfall was virtually nil (or trace). The highest daily rainfall during that period was only 0.5 mm.

Table 1 : Rainfall and Temperature Data Recorded at Ness Gardens (about 1.5 km west of Ness Woods)

Months	Total rainfall (mm) year 1978	1979	1980	1981	Mean monthly rainfall (mm) May 1969–June 1981	Mean maximum temp (°C) year 1978	1979	1980	1981	Mean minimum temp (°C) year 1978	1979	1980	1981
January	83.0	54.0	60.4	60.5	61.6	6.2	3.4	5.2	8.1	1.2	-2.5	-0.3	2.1
February	48.0	30.9	98.5	69.3	50.7	5.4	4.0	8.6	6.2	0.1	-1.2	2.7	0.2
March	42.3	68.2	93.6	102.5	58.6	10.4	7.7	7.6	11.0	3.4	1.4	1.9	5.1
April	38.7	78.1	2.1	34.6	47.9	10.4	10.7	12.5	12.3	2.9	4.2	5.4	3.0
May	40.0	91.7	41.5	61.5	60.4	16.0	13.2	16.8	15.4	7.4	5.3	6.4	7.6
June	91.0	18.4	88.1	47.3	55.4	16.7	17.7	17.1	16.4	9.9	9.8	10.1	9.1
July	78.0	21.4	49.1	29.2	50.3	17.7	19.8	18.7	18.3	11.0	12.0	11.2	11.9
August	98.3	52.0	62.7	-	58.6	18.1	18.9	19.6	-	11.9	11.0	12.4	-
September	59.9	19.7	39.2	-	63.1	17.6	17.4	18.5	-	10.8	9.6	11.1	-
October	23.4	64.3	88.0	-	55.4	15.2	14.7	12.1	-	8.9	7.8	6.0	-
November	59.7	81.5	61.7	-	85.5	11.7	10.6	9.6	-	5.4	4.1	4.4	-
December	116.4	137.3	44.2	-	62.9	6.4	8.5	9.3	-	0.6	3.3	3.1	-
Total	778.7	717.5	729.1	-	710.4								

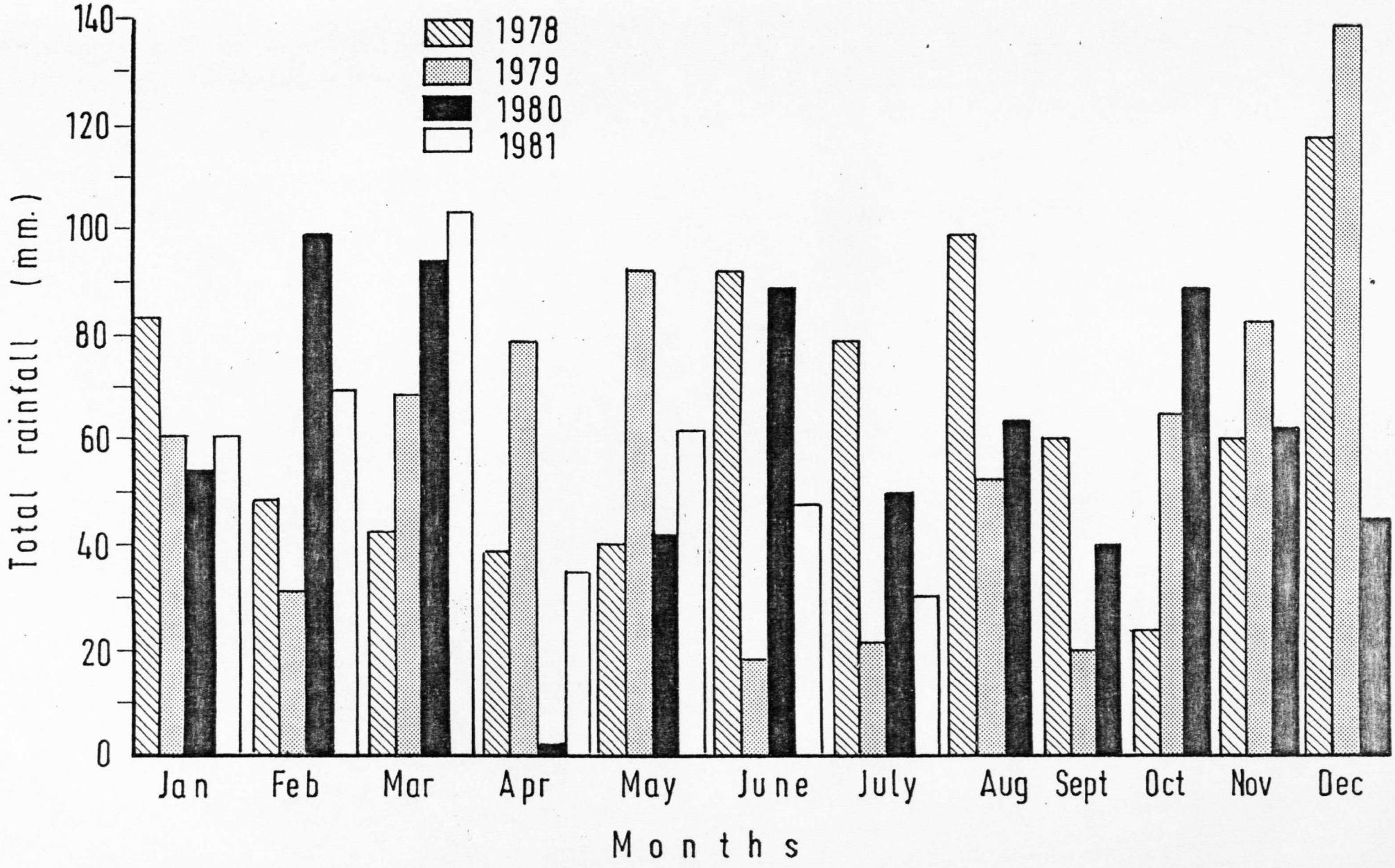

Fig. 8 : Total Rainfall (mm.) recorded at Ness Gardens, south Wirral January 1978 - July 1981.

LABORATORY STUDIES ON OVIPOSITION

The objective of this experiment was to find the number of eggs laid by Ae. cantans fed in the laboratory on man and by gravid females caught from the field. Also, to determine the duration from blood-feeding to becoming gravid, and from blood-feeding to egg laying under different environmental conditions.

MATERIALS AND METHODS

Unfed female Ae. cantans were caught from the field by human bait catches and were placed in paper-lined plastic tubes (diameter 4cm x length 13.5 cm.) as supplied by with the WHO insecticide susceptibility test kit for adult mosquitoes. These mosquitoes were then fed on the author. After feeding, up to 5 fully engorged mosquitoes were transferred to each WHO test kit lined with white paper. A piece of cotton wool soaked with tap water was placed on the top of each tube which was then left either in the insectary (28.9°C, R.H. 87%) or in a tea chest in the courtyard outside the School and thus under field conditions. Each mosquito that reached the gravid stage was transferred to a 7.5 x 2.5cm glass vial lined with white paper and with wet cotton wool at the bottom for oviposition. These oviposition tubes were kept in the insectary or in the tea chest.

Each gravid female Ae. cantans caught in the field during the summer (June to August) was also placed in vials which were then maintained in the tea chest.

The interval (in days) from blood-feeding to becoming gravid, and the interval up until egg-laying were recorded. The number of eggs laid by each mosquito from the above experiments was counted, and moreover each female was dissected after oviposition and the number of retained eggs recorded.

RESULTS AND DISCUSSION

Female Ae. cantans which were fed on human blood and kept in the insectary at a temperature of 28.9°C. (Table 2) laid a mean of 66.2 ± 1.8 eggs,with a range of between 25-90. The time taken to digest the blood-meal and become fully gravid was 3-4 days, afterwhich, in the laboratory at least, another 1-3 days elapsed before oviposition. When the above experiment was conducted outdoors in the courtyard in the summer (June to August), the duration from blood-feeding to becoming gravid was 6-8 days, again an additional 1-3 days occured before the eggs were laid (Table 3). The mean number of eggs laid was 69.3 ± 2.4 eggs, ranged between 42- 100. The combined percentage of females having retained eggs for both experiments (Tables 2 & 3) was 15.4%, the actual number of eggs ranging between 10- 67 eggs.

Gravid females Ae. cantans caught amongst vegetation at Ness Woods were kept in the courtyard and laid their eggs 2-9 days later (Table 4). These females laid a mean of 71.8 ± 3.2 eggs ranging between 46-101, and percentage of females having retained eggs was 27.3%, the number of eggs retained ranged between 1-43 eggs. There was little difference between the mean number of eggs laid by females fed in the laboratory on human blood and those laid by gravid females caught from the field. In the field cattle were found to be the main host of Ae. cantans as determined by precipitin tests from mosquito blood smears (Table 55). According to Service (1968a) it is unlikely that any British mosquitoes feed predominantly on man, therefore the size of egg batches derived from feeding adults on man may be expected to differ to some extent from those matured from wild-caught gravid females. This difference, however is unlikely to be great if feeding occurs predominantly on mammals, but larger differences would be expected if birds or reptiles were important hosts. Service (1968a) found there was a positive correlation between the size of the blood-meal and the

Table 2: Results of fecundity of *Ae. cantans* which were fed with human blood and kept in the insectary (temp. 28.9° C and R.H. 87%)

Mosquito No.	Time (in days) from bloodfed to gravid	Time (in days) of egg- laying after blood-feeding	No of eggs laid	No of retained eggs	Total No. of eggs.
1	4	5	25	0	25
2	4	5	13	67	80
3	4	5	59	0	59
4	4	6	60	10	70
5	4	5	84	0	84
6	4	6	27	39	66
7	4	5	80	0	80
8	4	5	67	0	67
9	4	5	76	0	76
10	4	5	57	0	57
11	4	5	52	4	56
12	4	5	83	0	83
13	4	7	52	0	52
14	4	6	69	0	69
15	4	6	44	0	44
16	4	6	58	0	58
17	4	5	62	0	62
18	3	4	65	0	65
19	3	4	54	0	54
20	3	4	59	0	59
21	3	5	79	0	79
22	4	5	70	0	70
23	4	5	76	0	76
24	3	5	62	0	62
25	3	5	77	0	77
26	4	5	35	26	61
27	4	7	59	28	87
28	3	5	90	0	90
29	3	6	70	0	70
30	3	5	81	0	81
31	3	4	62	0	62
32	3	5	61	0	61
33	3	4	56	0	56
34	3	4	64	0	64
35	3	4	83	0	83
36	4	5	81	0	81
37	4	6	50	0	50
38	4	5	63	0	63
39	4	5	41	0	41
40	3	4	68	0	68

Continued .....

Table 2: (Continued)

Mosquito No.	Time (in days) from bloodfed to gravid	Time(in days) of egg-laying after blood-feeding	No of eggs laid	No of retained eggs	Total No. of eggs
41	3	4	75	0	75
42	3	4	62	0	62
43	3	5	61	0	61
44	3	4	56	0	56
45	3	5	64	0	64
46	3	4	83	0	83
47	4	6	50	0	50
48	3	4	68	0	68
49	3	4	75	0	75

Total =3242

$\bar{x}$ = 66.2 $\pm$ 1.8

Table 3: Results of fecundity of Ae. cantans which were fed with human blood and kept in the teachest in the courtyard (June - August)

Mosquito No.	Time (in days) from bloodfed to gravid	Time (in days) of egg-laying after blood feeding	No of eggs laid	No of Retained eggs	Total No of eggs
1	6	10	48	0	48
2	6	10	100	0	100
3	6	11	38	47	85
4	6	9	20	65	85
5	7	10	74	0	74
6	7	10	52	0	52
7	8	9	44	27	71
8	8	10	48	0	48
9	8	10	29	24	53
10	8	10	46	0	46
11	7	11	52	0	52
12	7	9	46	0	46
13	8	10	92	0	92
14	7	10	64	0	64
15	6	8	57	0	57
16	6	8	90	0	90
17	7	8	59	0	59
18	7	8	73	0	73
19	7	8	64	0	64
20	6	9	90	0	90
21	6	8	42	0	42
22	6	8	76	0	76
23	6	8	79	0	79
24	6	8	70	0	70
25	7	9	87	0	87
26	6	8	37	25	62
27	7	10	53	19	72
28	7	10	51	0	51
29	6	7	92	0	92
30	6	8	57	0	57
31	6	8	90	0	90
32	7	8	59	0	59
33	7	8	73	0	73
34	7	8	64	0	64
35	6	9	90	0	90
36	6	8	76	0	76
37	6	9	79	0	79
38	6	8	70	0	70
39	7	9	87	0	87
40	6	8	37	25	62
41	6	9	53	19	72
42	7	10	51	0	51

Total = 2910

% of females having retained eggs = $\frac{14}{91} \times 100 = 15.4\%$

$\bar{x} = 69.3 \pm 2.4$

Range of retained eggs = 10 - 67 eggs

Table 4: Results of fecundity of gravid *Ae. cantans* caught from the field and kept in a teachest in the courtyard (June to August)

Mosquito No.	Time (in days) of egg laying after being caught from the field	No of eggs laid	No of Retained eggs	Total No of eggs
1	6	79	0	79
2	6	84	0	84
3	2	64	15	79
4	6	70	0	70
5	8	72	0	72
6	7	66	0	66
7	9	75	0	75
8	9	54	0	54
9	8	46	0	46
10	6	54	0	54
11	7	101	0	101
12	7	54	0	54
13	6	98	0	98
14	2	78	0	78
15	6	43	43	86
16	4	58	0	58
17	4	66	0	66
18	4	82	0	82
19	5	68	1	69
20	5	39	10	49
21	6	60	30	90
22	6	53	17	70

Total = 1580

$\bar{x}$ = 71.8 ± 3.2

% of females having retained eggs = $\frac{6}{22} \times 100 = 27.3\%$

Range of retained eggs = 1 - 43 eggs

number of eggs laid by Ae. cinereus Meig. and Ae. punctor, but not in Ae. detritus Haliday, Ae. cantans and Mansonia richiardii Ficalbi. The mean number of eggs laid by Ae. cantans fed on human blood was 52.3 ± 6.8, ranging between 23-78. This result is smaller than obtained in the present studies. Later Service (1977a) studied the ecology of Ae. cantans at Monks Wood, southern England and found that the numbers of eggs laid by Ae. cantans that fed on man was 31.3 ± 1.8 and those fed on rabbits 32.5 ± 0.8. Again, these results are much smaller than obtained in the present studies. Service (1977a) concluded that there was a positive correlation between wing length and the number of eggs produced. The smaller the wing length the smaller the egg batches, because the number of eggs laid by Ae. cantans is related to the size of the adult females. Service (1977a) also found that the time required for blood digestion for Ae. cantans diminished with increasing temperatures from fourteen days at 8 °C to only 58 hours at 35° C. He found that from June to August when maximum numbers of Ae. cantans are feeding in the field, blood is digested within about 7 days. Results of the present studies indicate that blood digestion in the courtyard in summer (June to August) was 6 - 8 days.

Several factors may affect the size of the egg batch in any species. The number of eggs laid shows a positive correlation with the size of the female Ae. aegypti (Colless and Chellapah, 1960) . According to Roy (1936),there exists a definite quantitative relationship between the weight of the blood-meal and the number of eggs produced by Ae. aegypti., and he believed that the amount of blood in its stomach exercises a greater influence than the mosquitoe's size. Woke (1937) compared the effects of the blood of man and a canary on egg-production of Culex pipiens. He concluded that eggs produced by the mosquitoes fed on canary blood averaged over twice as many eggs per mass or per milligram of blood ingested as those produced by the mosquitoes fed on the blood of man. C.pipiens is known to be ornithophilic.

From results of the present experiments it is concluded that gravid Ae. cantans females caught from the field laid a mean of 71.8 ± 3.2 eggs per batch ranging between 46- 101. The difference in numbers of eggs laid by Ae. cantans when fed on man and eggs laid by gravid female caught from the field was not significant. Blood digestion under 'field conditions ' in the courtyard took between 6-8 days, afterwhich the time of oviposition was delayed by a further 1- 3 days, However, when Ae. cantans females were kept in the insectary (28.9° C, R.H. 87%) blood was digested in 3- 4 days, this probably representing the fastest possible time for maturation of the eggs.

SAMPLING THE LARVAL POPULATION OF MOSQUITOES

INTRODUCTION

Collections of mosquito immature stages are usually made to determine the presence or absence of various species in different habitats, to monitor population changes associated with seasonal abundance or control measures, and sometimes to estimate the size of the absolute population in a habitat from number of larvae per dip or number enclosed in each quadrat.

Service (1976) summarised the methods used in sampling larval population of mosquitoes. The collecting techniques include dippers (ladle), nets, static quadrats, floating quadrats, larval collecting trays and light traps. The dipper is the most common tool for collecting mosquito larvae and pupae that occur in large and small collections of ground water, in rock pools and a variety of large container-type habitat. A ladle with a diameter of 9.5 cm and capacity 100ml was used for sampling Ae. cantans in a ditch for estimating population size and larval mortalities (Lakhani and Service, 1974, Service 1977a). A similar sampling technique was used for studying the mortalities of Anopheles gambiae Giles complex in Kenya (Service 1977b) and Nigeria and Kenya (Service 1971a). The ladle is most suitable for small collections of water such as puddles, ditches, hoofprints and experimental work involving small plots, but because of the small surface area sampled it is not particularly suitable for larger breeding places. An aquatic net is often better for sampling larger areas of water in a relatively short time. A disadvantage of the net is that it cannot easily be used when dense vegetation occurs in breeding places (WHO, 1973). Dippers and nets in general have the limitation of being difficult and even inaccurate to use when dense stands of rigid emergent plants occur, or where large amounts of debris or algae lie on the water surface.

According to Knight (1964) 'quadrats' used for collecting larval population of mosquitoes can be either mobile (nets or dippers) or they can be static (e.g. open ended cylinders) The latter being thrust down into the bottom of the habitat to enclose a volume of water, from which larvae can be removed and counted. Static quadrats can be used to estimate absolute densities by relating the surface area of water enclosed by the quadrat to the surface area of the entire breeding area. An advantage of static quadrats over dipping is that more opportunity exists to collect larvae that were frightened from the surface by the approach of the collector. Cambournac(1939) used a rectangular metal chamber open at both ends and long enough to rest on the bottom, but with the top above the water surface enclosing a surface area of 0.1 square metre for assessing anopheline larval populations in ricefields in Portugal. By thrusting this down at selected places in a ricefield and collecting the enclosed larvae as they surfaced, a satisfactory estimation of larvae per unit area could be obtained. But the limiting feature of the quadrat technique is the amount of tedious labour required to use them.

Roberts and Scanlon (1979) compared two sampling methods, namely the area sampler and the standard pint dipper. Approximately equal proportions of 2nd- and 3rd - instar larvae were collected by the two sampling methods, but significantly smaller proportions of pupae and 4th-instar larvae were collected with the dip method. The dipper is most effective at collecting larvae at or near the water surface. The area sampler should be equally effective for larvae at all depths and should realistically reflect the true numbers of larvae in the sampled area of water.

In the U.S.A. Christensen and Washino (1978) compared three immature mosquito samplers, namely the standard aquatic net, a 50 square inch plastic tub and the standard one-pint dipper. When Culex tarsalis Coquillett and Anopheles freeborni Aitken were sampled, the net had the highest mean number of larvae per sample, followed by the tub, and then the dipper. However, the tub was found to be the most precise device because the variation about its sample mean was the least of the three methods. In the U.S.A. Nagamine et al. (1979) compared the efficiency of three sampling devices namely the tub, dipper and area sampler. They concluded that dipper should be used if the objective is to collect numerous immature mosquitoes regardless of the amount of effort and time involved. However if the main concern is collecting the most mosquitoes with a minimum of effort and or time, then the tub should be utilised.

Submerged light traps have occasionally being used to sample aquatic insects. Hungerford et al. (1955) used a torch in a cylindrical trap for trapping a variety of invertebrates including chironomids, Chaoborus larvae, a few mosquito larvae, and tadpoles. They concluded that a subaquatic light trap may be of value in the study of many aquatic life problems. Carlson (1971) constructed an underwater light trap using a fluorescent tube as the light source which was powered by a 12-volt motorcycle battery. The trap was made from a polyethylene bucket and polythene funnel fixed into the opening at the bottom of the bucket with epoxy resin glue to form a funnel entrance. The spout of the funnel was cut off giving a $1\frac{1}{2}$ in - diameter opening. Fish were prevented from entering the funnel by placing a $\frac{1}{4}$in gauze mesh over the funnel entrance. A tight fitting plastic lid painted black to reduce its attraction to terrestrial insects was fixed to the top of the bucket. a 9-in, 6-W 'Actinic 5'(BL) bulb fluorescent tube mounted on a strip of wood was screwed to the underside of the lid.

The trap was lowered into the water and fixed to an upright support leaving about the upper quarter above the water level. Among the insects which the blacklight trap collected were Chironomidae and Chaoborinae larvae.

Ervin and Haines (1972) used a two lamptype of trap utilizing a brown plastic bottle with the bottom cut out. A plastic funnel of appropriate size was connected to the bottom of the bottle. The cap of the bottle had an amber lamp cemented into a hole drilled through it. Another lamp was placed about 3cm from the mouth of the funnel and attached to the funnel by means of three fine chains. The trap was suspended vertically in the water by means of the wire attached to this lamp. The lamps were wired separately so that they could be used independantly. In operation, the lamp over the funnel was turned on for a given length of time, after which it was turned off and the bottom lamp turned on. This cycle was repeated several times. Organisms that were attracted to the top lamp were drawn into the bottle by the bottom lamp; the funnel prevents organisms in the bottle from leaving during successive cycles of operation. Using this trap, they collected some Culicidae besides numerous Crustaceans. Bertram et al. (1970) used betalights for trapping mosquito larvae. The trap consisted of a biscuit tin with the light on the inside of the lid and an opening in the base through which larvae could pass towards the light. The low intensity luminescence of the betalight was derived from the action of tritium gas on a phosphor coating lining the glass tube of the light unit. Preliminary trials in Sarawak with betalights gave catches of 15- 95 Culex larvae, including Culex tritaeniorhynchus Giles, from ditches, compared with 0-2 larvae in control traps without a light. Betalights gave a 6 fold increase over control catches of Culex mimulus Edw. in forest pools.

OBJECTIVES OF THE SAMPLING PROGRAMME

The objectives of the present study were threefold. Firstly to compare the different proportions of larval instars and pupae of mosquitoes sampled by a ladle, a quadrat and a D-shaped aquatic net, so as to select the best method to study the population dynamics of the immature stages of Aedes cantans (see pages 46 - 75). Secondly, to evaluate two types of aquatic light traps (betalights and chemical lights) and thirdly, to evaluate the possibility of using various glues to trap mosquito larvae.

COMPARATIVE LARVAL SAMPLING

MATERIALS AND METHODS

The main objective of comparative larval sampling was to compare the different proportion of larval instars and pupae sampled by each of the following methods:

(i) a soup ladle (i.e. dipper) having a capacity of about 130ml and a diameter of 9.5 cm. (Fig. 9).

(ii) a metal quadrat which in fact was a metal wastepaper basket with the bottom removed having an internal diameter of 28 cm at the top, 23cm at the bottom end, and a length of 28cm (Fig. 17).

(iii) a D-shaped aquatic net, made of nylon mesh (Griffin, code YRF-700-G Net Beg, Scrim L05-049/075) fastened by popstuds over a D-shaped metal frame to which a 60cm wooden handle was attached (Fig. 10)

Comparative larval sampling at pond A

Pond A was a very large pond of area about 2000 square metres. From 7th March 1979 to 5th July 1979, samples were taken at random by tossing a coin along the perimeter of the pond, each at a distance of 1 metre apart. Every two samples by a ladle was

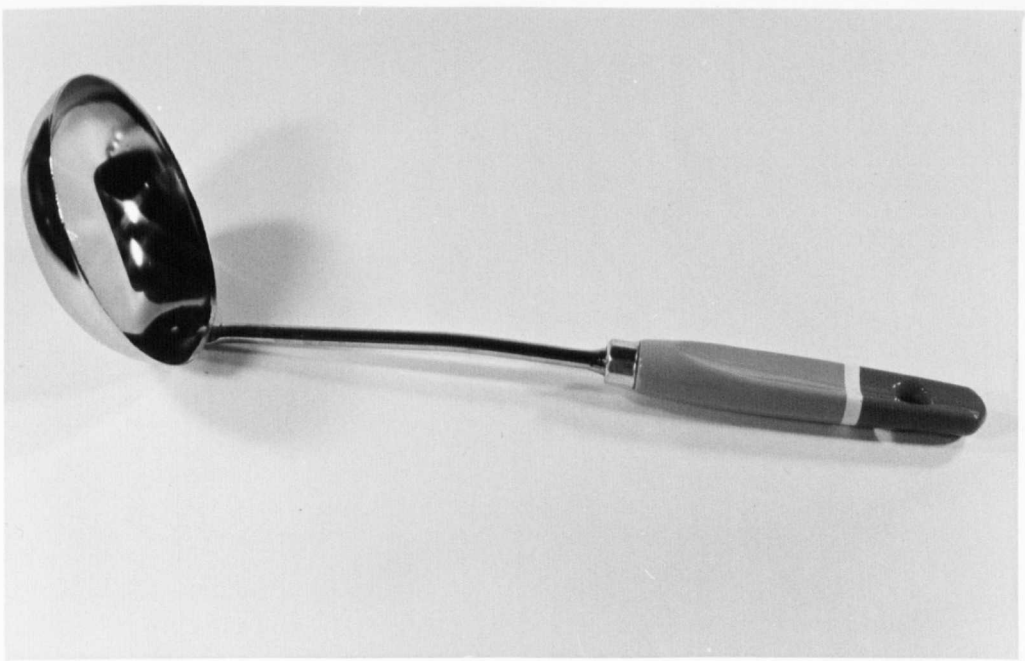

Fig. 9: A ladle (i.e. dipper).

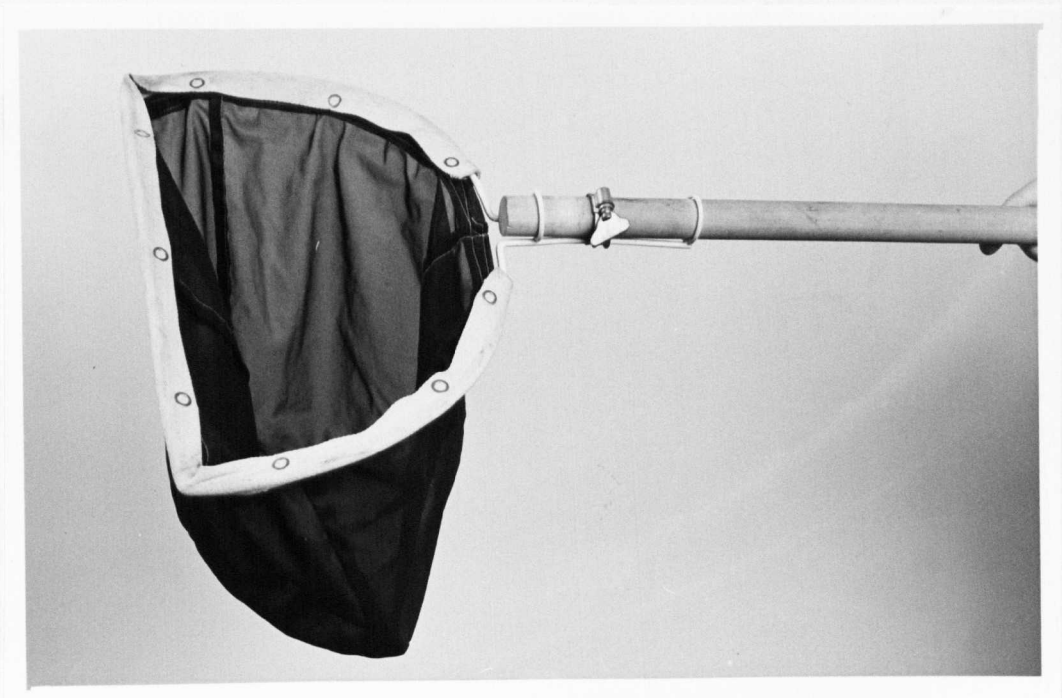

Fig. 10: A D-shaped aquatic net.

followed by one sample by a quadrat and one sample by the D-net. A total of 20 samples by the ladle, 10 samples by the quadrat and 10 samples by the D-net were taken each week.

Sampling using a ladle

The ladle was gently lowered until it reached the bottom of the pond and water allowed to flow into it, then it was drawn out of the pond. The contents were then tipped into a white plastic tray and larvae were transferred with a pipette to a 75 x 25mm vial containing 70% alcohol as a preservative. Pupae in each sample were placed in a different vial with pond water, taken to the laboratory and adults allowed to emerge. Numbers of larvae were recorded according to species and larval instars, and the number and species of each pupa was determined by identification of the emerging adult.

Sampling using a quadrat

The metal quadrat was pushed into the ground and all enclosed larvae and pupae removed by means of a white plastic tray and then pipetted into vials, larvae being preserved and pupae kept alive. Numbers of larvae and emergent adults (from pupae) were identified in the laboratory as in previous procedure. To ensure as far as possible that all larvae and pupae were removed from each quadrat, dipping with the white plastic tray in each quadrat was stopped only after the absence of larvae or pupae for five continuous dips.

Sampling using a D-net

The D-net was pushed into the pond until it reached the bottom, then drawn out and its contents quickly tipped into a white plastic tray containing pond water. The net was carefully flushed through with further pond water to remove any stranded larvae or pupae. Floating debris or leaf litter were vigorously shaken in the plastic

tray to detach larvae or pupae before being discarded. Larvae and pupae were collected, preserved or reared and identified as before.

Comparative larval sampling at pond B

Pond B was a small pond of about 30 square metres. From 24th February, 1981 to 3rd June 1981, samples were taken as described for pond A. Due to the small size of the pond there were fewer samples, but the total samples taken each week covered almost the whole perimeter of the pond. Every 5 samples by the ladle was followed by one sample with the quadrat and one sample by the D-net. A total of 10-20 samples by the ladle, 2-4 samples by the quadrat and 2-4 samples by the D-net were taken each week. The sampling techniques and the numbers of each larval instars and pupae were recorded as before in pond A.

RESULTS AND DISCUSSION

Pond A

Two mosquito species namely Ae. cantans and Culiseta annulata were found in Pond A. However, the presence of Culiseta annulata in Pond A was observed only during the later part of the sampling period (from early June, 1979) and its population was low. Consequently the results of the comparative larval sampling are based entirely on sampling the predominantly Ae. cantans population. The occurence of various larval instars and pupae of Ae. cantans was seasonal. Results of comparative larval sampling at pond A in 1979 showed that sampling with a quadrat was the more reliable method of determining the proportion of the various larval instars and pupae than sampling with a ladle or D-net. Appendix 1 and Figures 11 a-e show that sampling with a ladle when several larval instars were present often gave unrealistically low proportions of earlier instars compared to the late instars, even when the

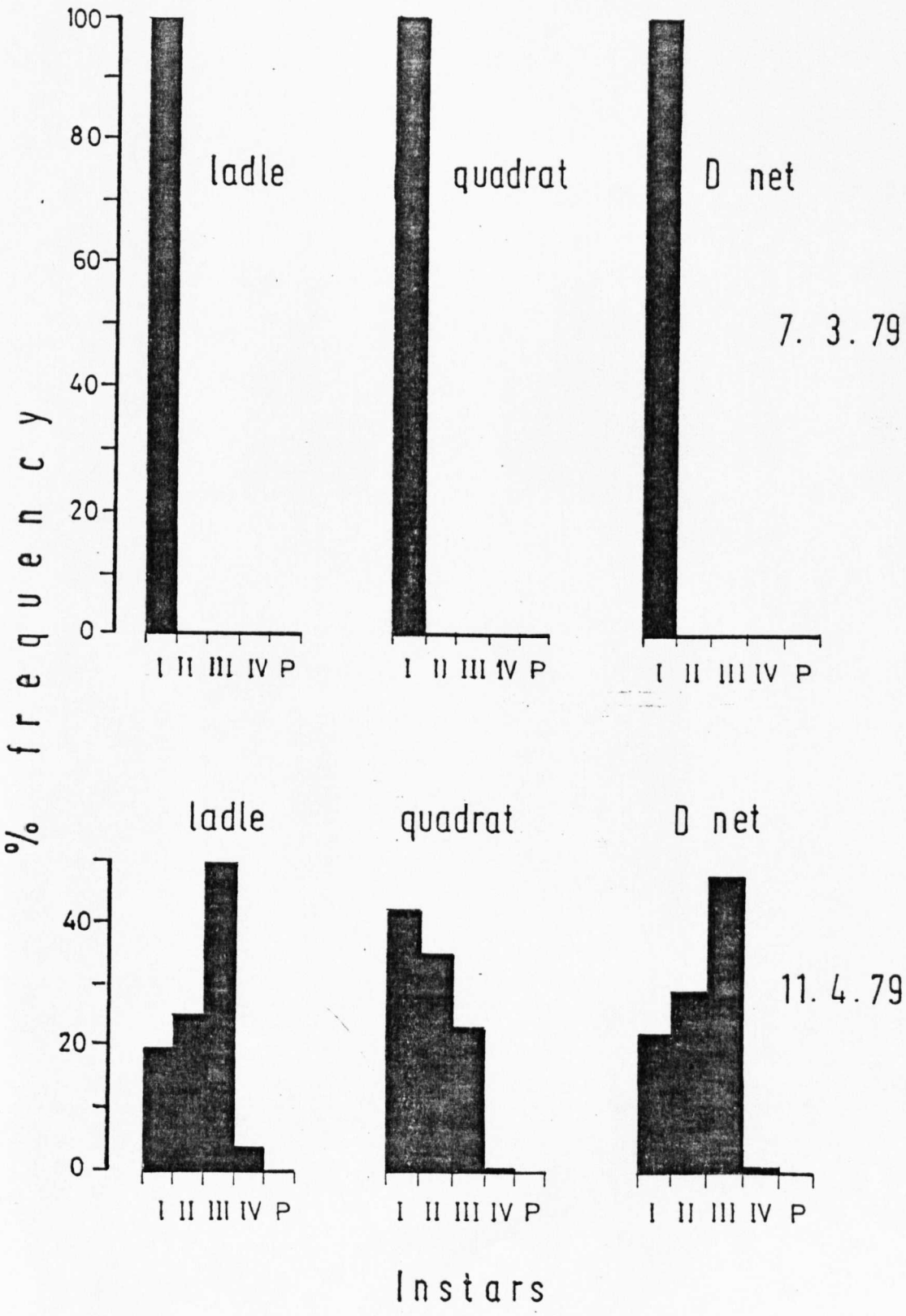

Fig 11a : % Frequency of Ae. cantans immature stages sampled by different methods at pond A Ness Woods in 1979.

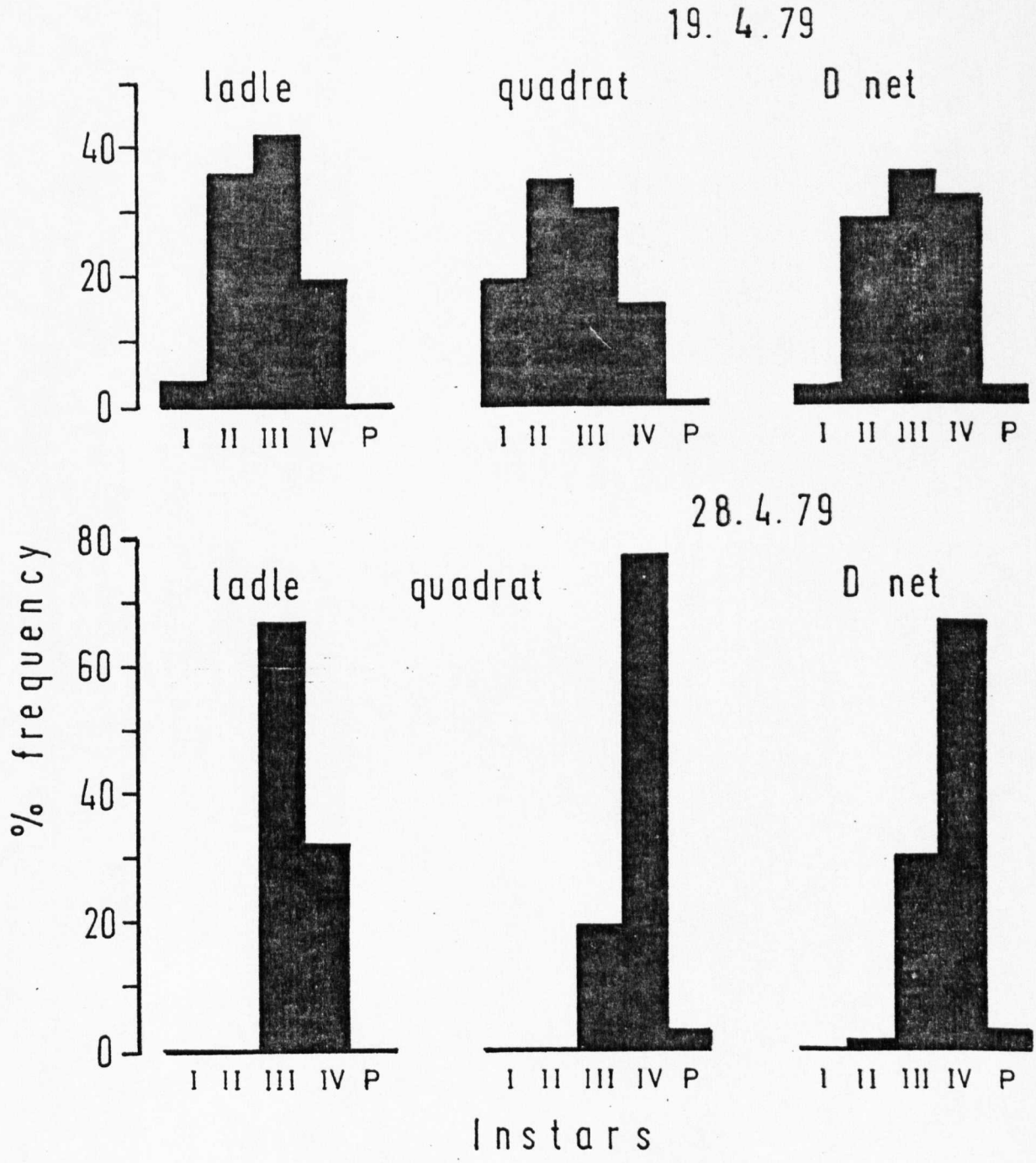

Fig. 11b: % Frequency of Ae. cantans immature stages sampled by different methods at pond A, Ness Woods in 1979.

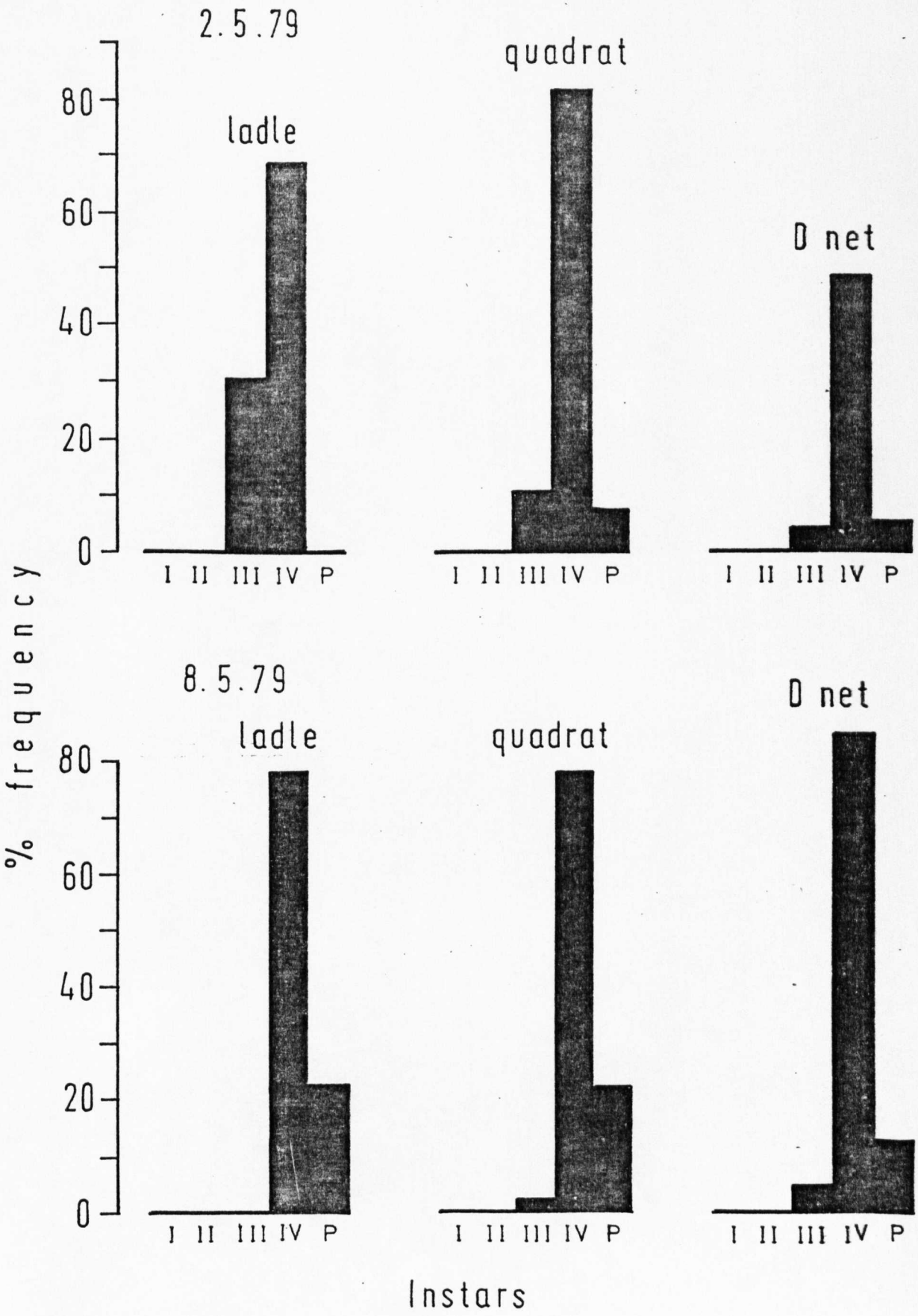

Fig. 11c: % Frequency of Ae. cantans immature stages sampled by different methods in pond A, Ness Woods in 1979.

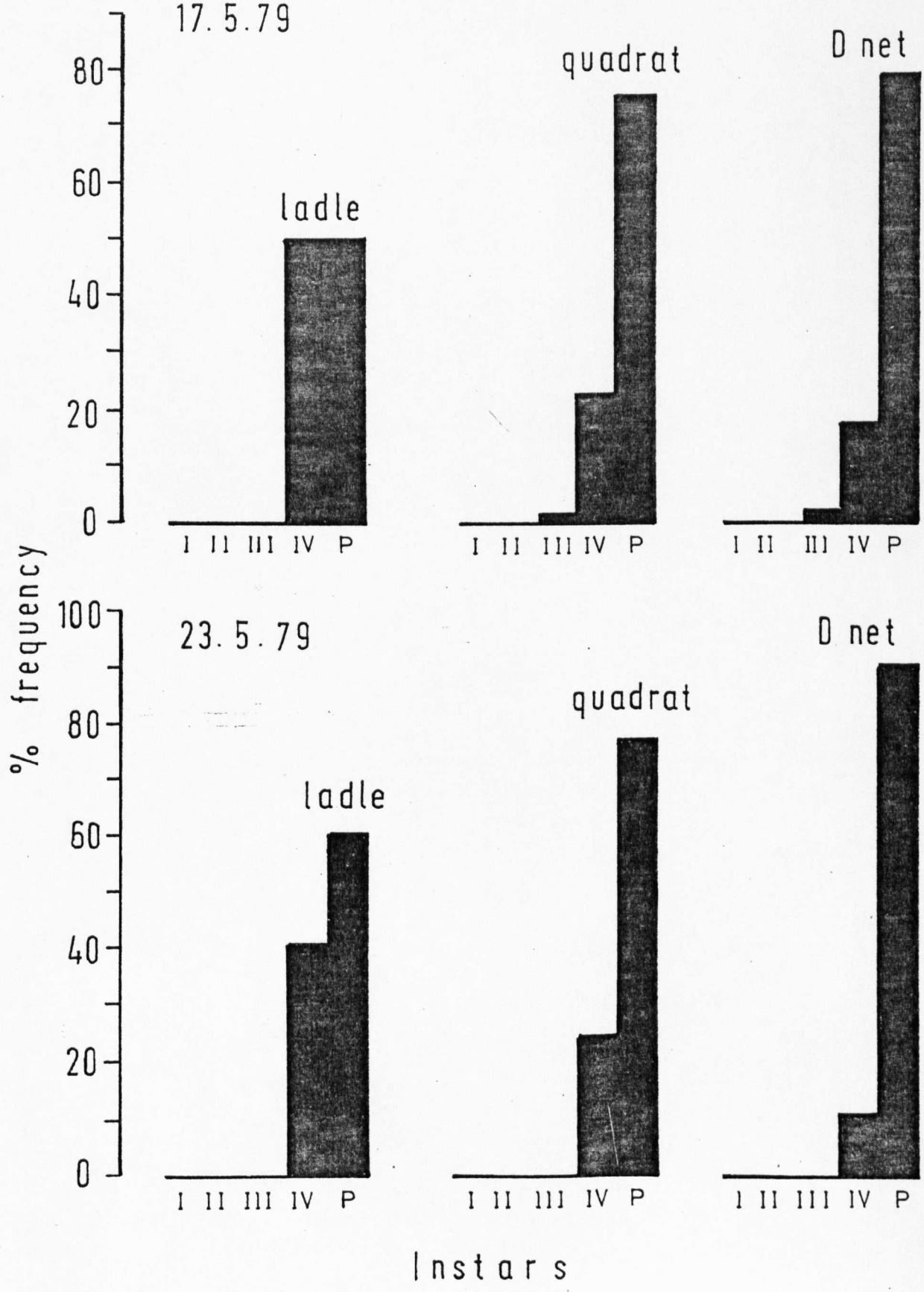

Fig. 11d: % Frequency of Ae. cantans immature stages sampled by different methods at pond A, Ness Woods in 1979.

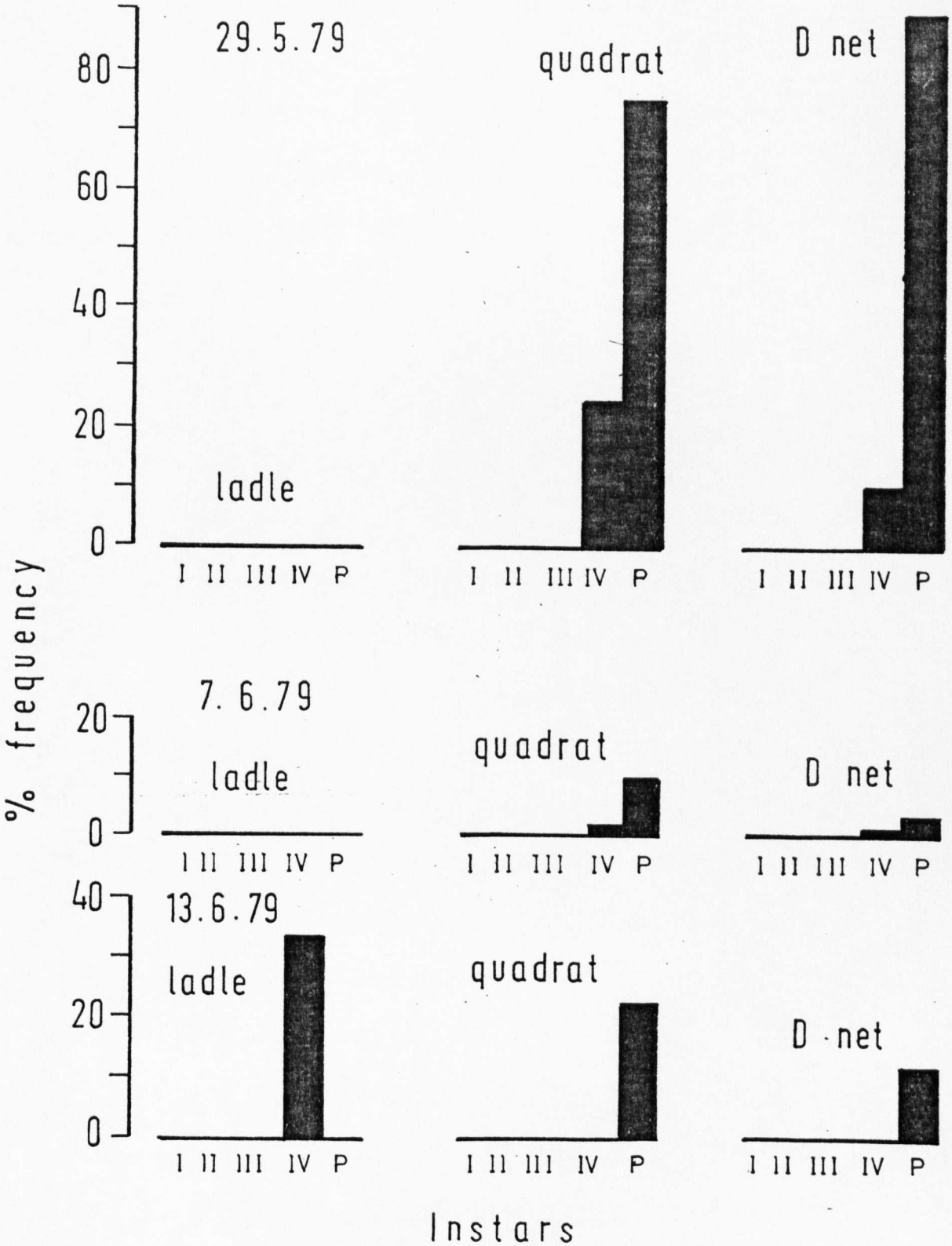

Fig. 11e: % Frequency of Ae. cantans immature stages sampled by different methods at pond A, Ness Woods in 1979.

population of the earlier instars was larger as determined by the other sampling methods, especially the quadrat. For example, sampling with a ladle onthe 11th April 1979 (Appendix 1 Fig 11a) the proportion of 3rd-instar Ae. cantans was the highest (50.4%) followed by the 2nd-instar (25.2%), 1st-instar (19.8%) and the 4th-instar (4.6%) larvae. On thecontrary, sampling using a quadrat on the same day, the 1st-instar larval population had the highest proportion (41.3%) followed by 2nd-instar (34.5%) , 3rd -instar (22.8%) and 4th-instar (1.5%) larvae. Sampling using a D-net had shown the population of 3rd-instar (47.3%) was the highest followed by 2nd-instar (29.0%), 1st-instar (22.3%) and 4th-instar (1.3%) larvae. Again, sampling on 19th April 1979 (Appendix 1, Fig. 11b), the ladle indicated that 3rd-instar (41.3%) larvae were the most numerous followed by 2nd-instar (35.6%),4th-instar (19.2%) and 1st-instar (3.8%) larvae. But the quadrat sample showed that 2nd -instar (34.8%) were the most prevalent followed by 3rd-instar (30.1%), 1st-instar (19.1%) and 4th -instar (15.9%) larvae and pupae(0.1%). Sampling with a D-net showed that 3rd-instar (35.2%) larvae was the highest proportion sampled followed by 4th-instar (32.0%), 2nd-instar (28.4%), 1st-instar (2.3%) larvae and pupae (2.1%).

The main difference between the results was that, the ladle and to a lesser extend the D-net, sampled mostly the later instars compared to the earlier instars. In contrast the quadrat always sampled the earlier instars more than the later instars during the time where the population of the earlier instars was high. According to Service (1976), dipping usually catches larvae and pupae at the water surface. Since both in the presence or absence of alarm reactions, different species and also different instars of the same species may remain at the water surface for varying periods, it follows that dipping may frequently be biased for a particular species or instar.

Nielsen and Nielsen (1953) observed that 1st-instar, and to a lesser extent 2nd-instar, larvae of Aedes taeniorhynchus (Wiedemann) came up to the water surface much less frequently than older larvae. Differences between submersion times of the different immature stages will probably result in sampling bias when the age-structure of the population is derived from dipping. Hagstrum (1971) investigated the reliability of dipping in sampling all larval instars of Culex tarsalis Coquillett by comparing a quadrat made of an aluminium frame (20 x 25cm and 25cm high) open at both ends and a pint dipper of surface area 95 cm^2. He concluded that the quadrat was more efficient in collecting 1st-instar larvae than dipping. On the contrary Chubachi (1976) used a dipper (8.4cm in diameter and 1.8 cm in depth) for sampling larvae and pupae of Culex tritaeniorhynchus summorosus Dyar and Anopheles hyrcanus sinensis Wied. in ricefields and an artificial container in Japan. He found that the efficiencies for sampling larvae and pupae of C.t. summorosus in the artificial container and the ricefields were identical. In the case of An. h. sinensis, the same finding was reported from the artificial container but not in the ricefields because the younger instars of An.h. sinensis were concentrated around the riceplants.

It is thought that because earlier instars of Ae. cantans prefer to remain submerged for a longer time than later instars, they were sampled less by the ladle and D-net. This can be explained as follows, when the ladle was slowly drawn upwards from the bottom of the pond towards the surface, the disturbance created might have caused more of the earlier than later instars to escape from the ladle, thus causing sampling bias against the earlier instars. With the D-net it is more difficult to see why the younger instars appeared to be under-sampled. In contrast sampling with the quadrat enabled almost all larvae of all instars trapped within

the quadrat to be removed. Therefore, there was a better chance of recovering the earlier instars from each sample than with the ladle or D-net. Thus, although the results of sampling with the ladle and D-net show more similarities in the proportion in the various instars than obtained with the quadrat, I believe the quadrat method reflected better the actual instar populations present in the pond. However, the disadvantage of using the quadrat is the time spent on dipping from each quadrat. Because of the seasonal nature of Ae. cantans and great decline in its numbers, sampling with the ladle in late May onwards became even more unreliable. But sampling with the quadrat was more appropriate with low density populations encountered at this time of the year.

Pond B

Results of comparative larval sampling at pond B in 1981 showed that there were mixed populations of three mosquito species, namely Ae. cantans, Ae. punctor and a very small population of Ae. rusticus (Figs 12 a-f). Because of the very small population of Ae. rusticus (a total of 17 1st-, 32 2nd-, 16 3rd- and 13 4th-instar larvae were collected), very few larvae were caught in a ladle (6.4%) and D-net (25.6%), by far the most were caught in the quadrat (67.9%). Results of sampling with the ladle on 10th March 1981 (Appendix 3, Fig. 12b) showed that the proportion of 1st-instar (24%) Ae. cantans caught was lower than the proportion of 2nd-instar (58.7%) larvae. Similarly, the D-net caught a lower proportion of 1st-instar (36.8%) than the 2nd-instar (48.3%) larvae of Ae. cantans on the same day. On 17th March 1981, however, the proportion of 1st-instar (33.5%) Ae. cantans larvae caught by the ladle was higher than the proportion of 2nd-instar (23.6%) larvae. Similarly, the D-net caught a higher proportion of 1st-instar (31.7%) than the 2nd-instar (22.4%) on this day. In contrast, when sampling with

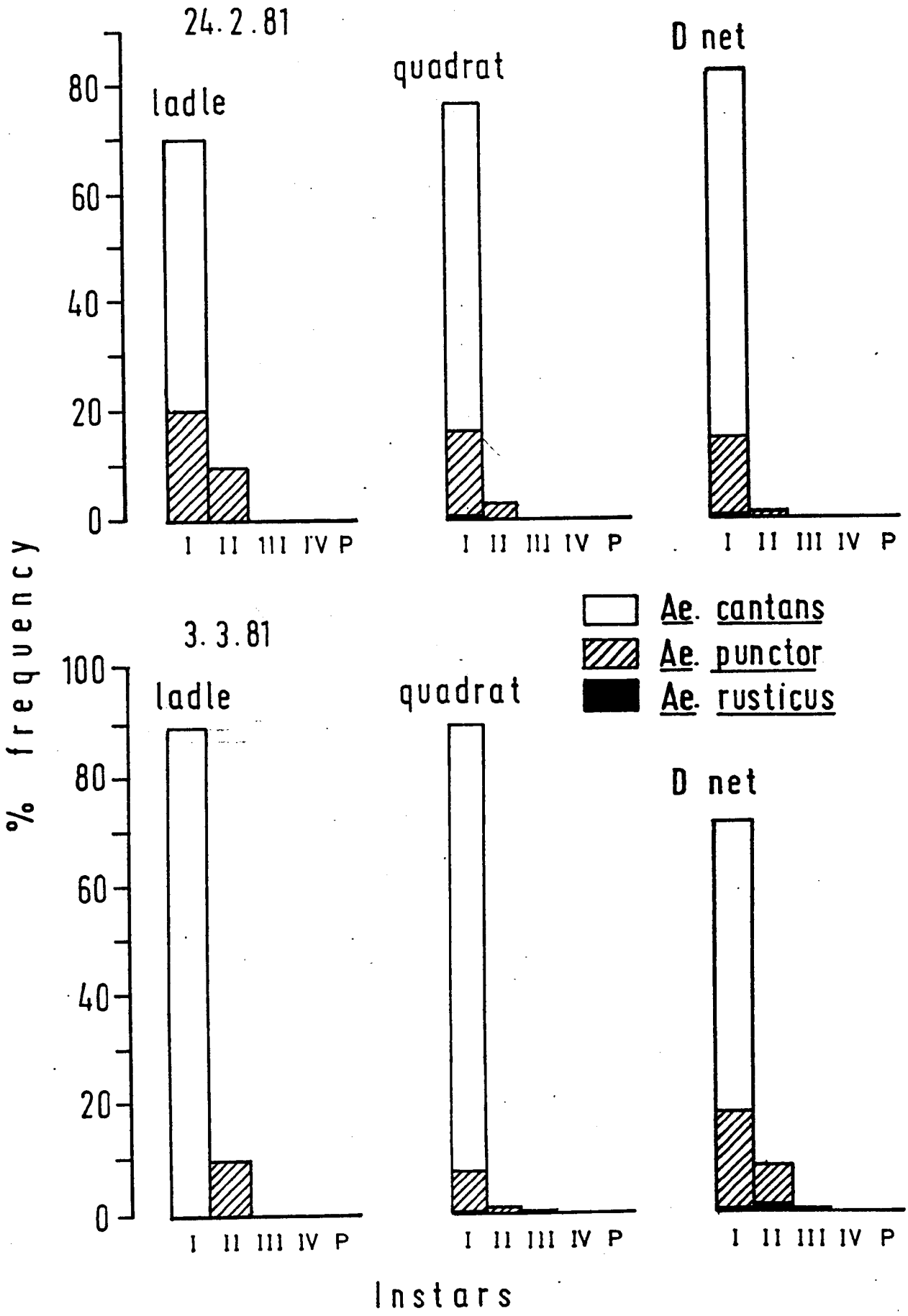

Fig. 12a: % Frequency of Ae. cantans, Ae. punctor, Ae. rusticus immature stages sampled by different methods at pond B, Ness Woods in 1981.

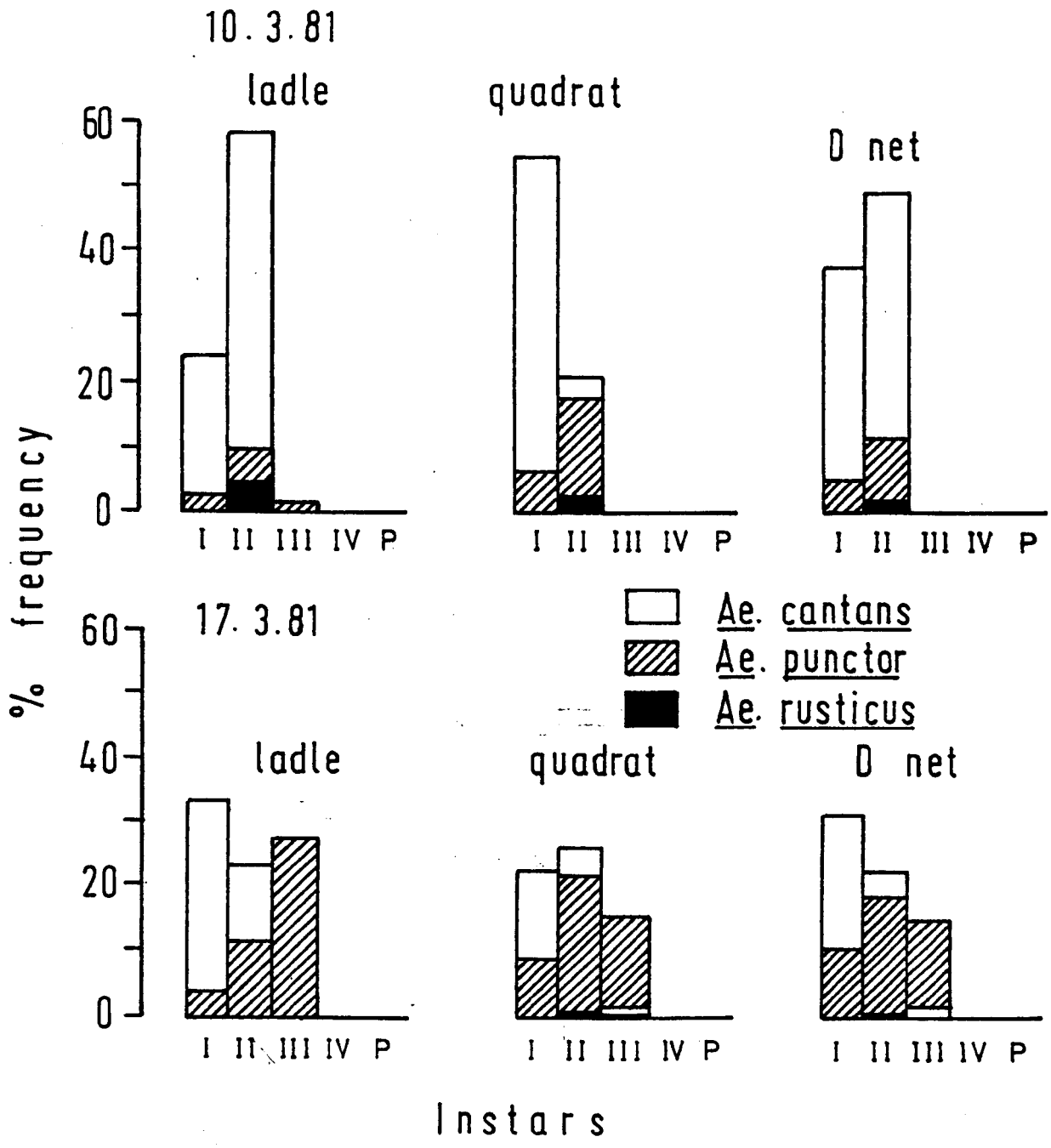

Fig. 12b: % Frequency of Ae. cantans, Ae. punctor and Ae. rusticus immature stages sampled by different methods at pond B, Ness Woods in 1981.

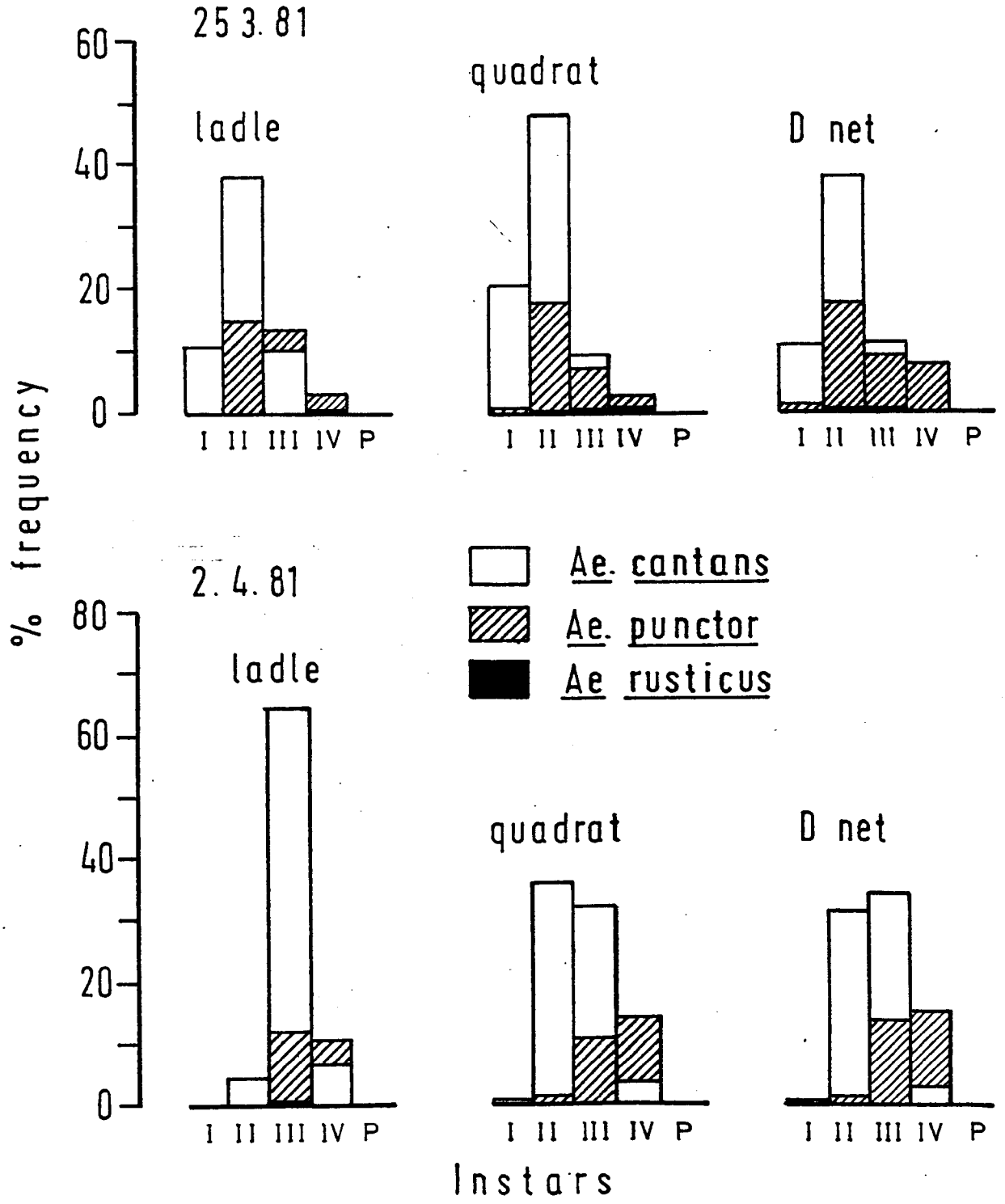

Fig. 12c: % Frequency of Ae. cantans, Ae. punctor and Ae. rusticus immature stages sampled by different methods at pond B, Ness Woods in 1981.

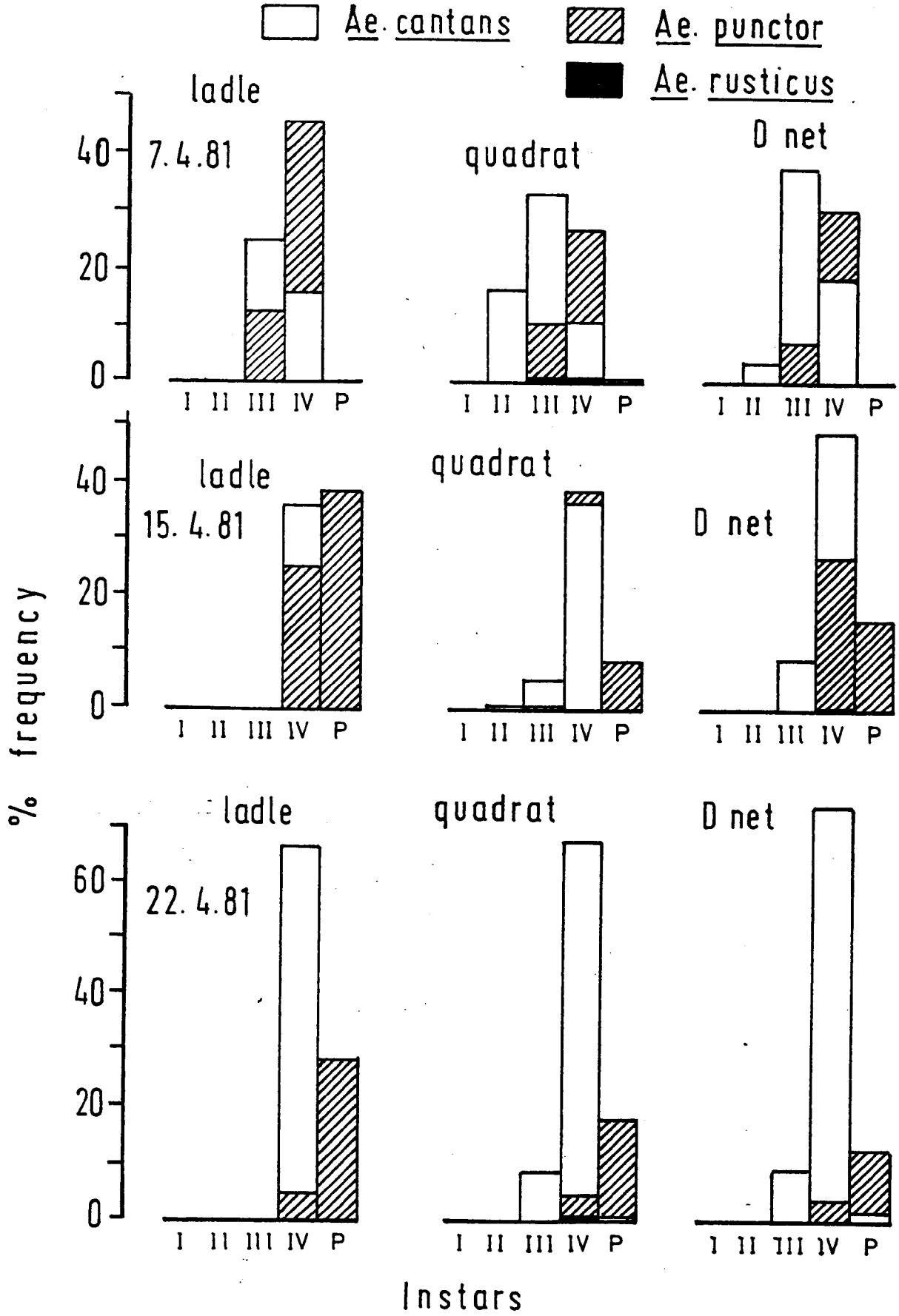

Fig. 12d: % Frequency of Ae. cantans, Ae. punctor and Ae. rusticus immature stages sampled by different methods at pond B, Ness Woods in 1981.

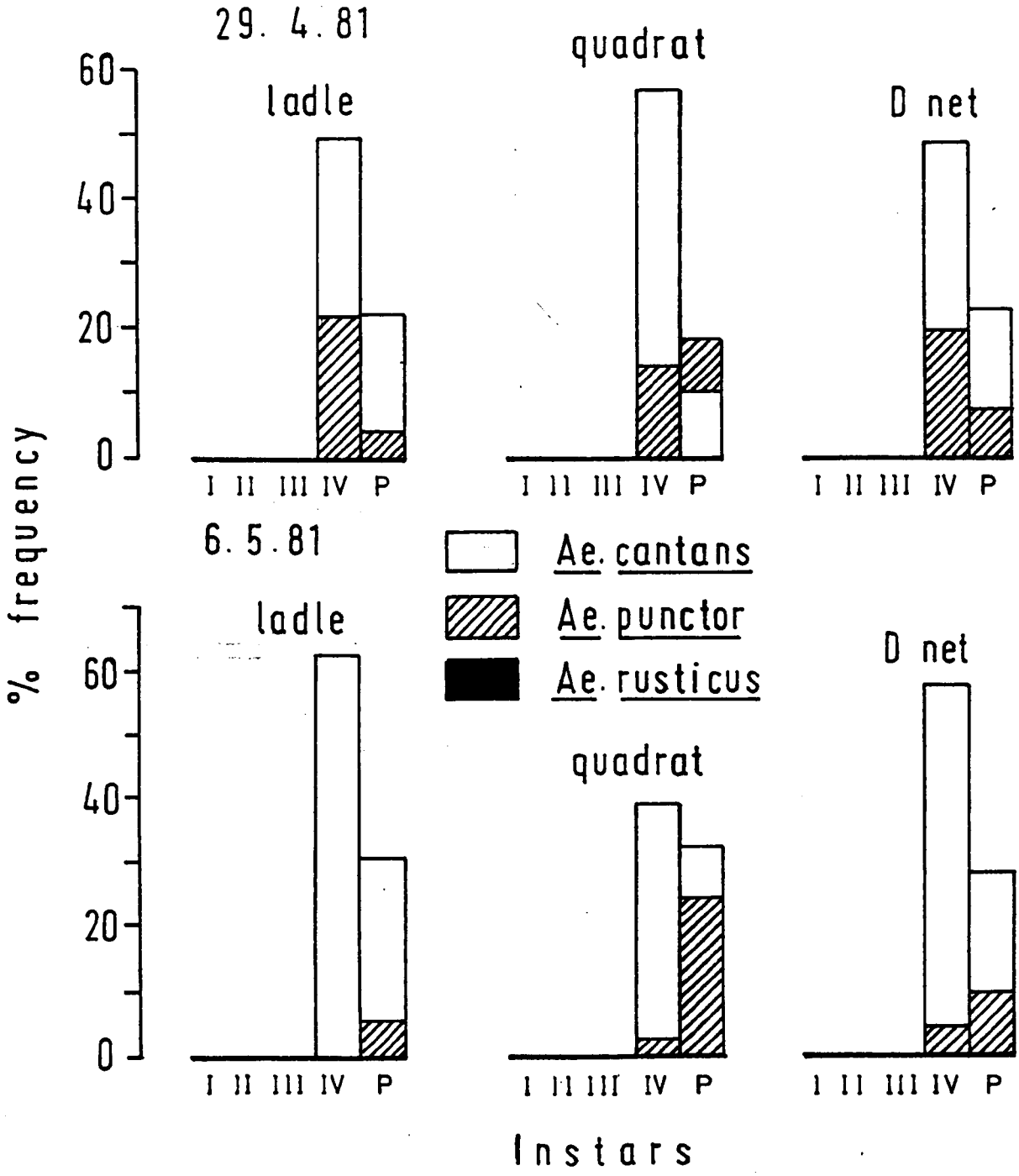

Fig. 12e: % Frequency of Ae. cantans, Ae. punctor and Ae. rusticus immature stages sampled by different methods at pond B, Ness Woods in 1981.

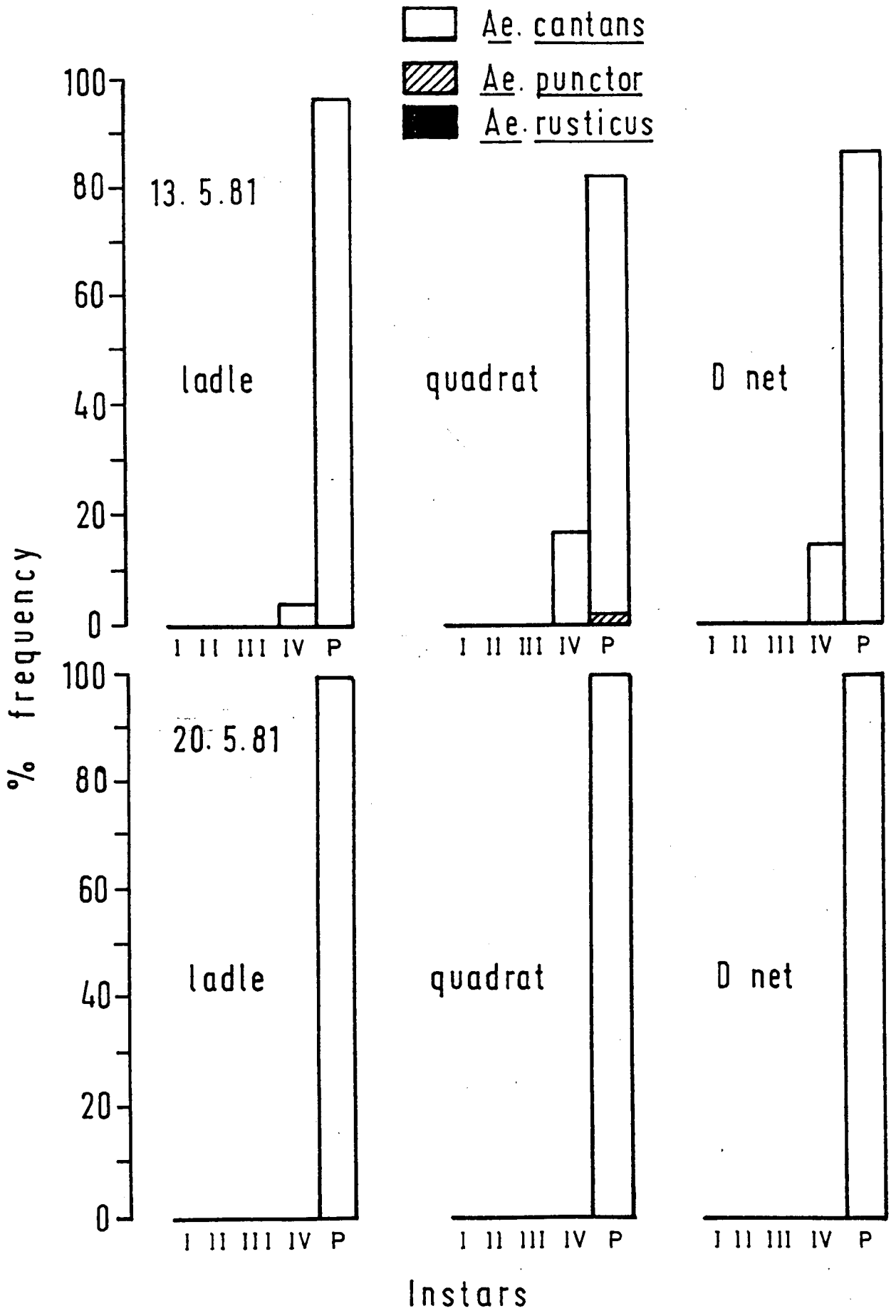

Fig. 12f: % Frequency of Ae. cantans, Ae. punctor and Ae. rusticus immature stages sampled by different methods at pond B, Ness Woods in 1981.

the quadrat on 10th March 1981, the proportion of 1st-instar (54.6%) larvae of Ae. cantans sampled was higher than the 2nd-instar (20.5%) larvae, and sampling a week later on 17th March 1981, the proportion of 1st-instar (23%) larvae was lower than the 2nd-instar (26.6%). Results from sampling on 2nd April 1981 (Appendix 3, Fig 12c) with the ladle showed that the proportion of 3rd-instar (64.8%) Ae. cantans was higher than the 4th- (6.6%) and 2nd-(4.4%) instar larvae. With the D-net, the proportion of 3rd-instar (34.5%) was greater than 2nd (31.6%) and 4th- (13%) instar larvae. However, on the same day using the quadrat the proportion of 2nd-instar (37%) was the highest, followed by 3rd-(32.6%) and 4th-(3.9%) instar larvae. So, results of sampling withthe quadrat are apparently more reliable because the proportions of the various instars sampled coincide with the seasonal abundance of each instar at a specific time during the larval breeding season.

These results show that sampling with a quadrat produced more representative results coinciding with the seasonal trend for Ae. cantans population in the ponds, compared to sampling with a ladle and a D-net.

Appendix 3 and Figure 12c show that using a D-net on 25th March 1981 some 1st-instar Ae. punctor were collected, but, no 1st-instar larvae were caught when a ladle was used. Again on 2nd April 1981 the ladle did not collect any 2nd-instar Ae. punctor whereas a small number were caught with both the quadrat and D-net. Therefore, as with Ae. cantans the ladle seemed to have shown sampling bias against earlier instars of Ae. punctor.

Samples on 22nd April 1981 at pond B showed that the proportion of pupae of Ae. punctor taken by all three methods was higher than 4th-instar larvae, but sampling a week later (on 29th April) the ladle and D-net sampled a higher

proportion of Ae. punctor 4th-instar larvae than pupae. However, I consider the quadrat produced the most reliable results, showing the pupae were more numerous than 4th-instar larvae on both sampling date. Service (1976) sampled Ae. rusticus and Ae. punctor immature stages in a ditch in England by taking each week 100 dips with a ladle and also by collecting all larvae and pupae enclosed within 12 open-ended cylinders. He concluded from the proportions of the age-classes enclosed by the cylinders, that both 4th-instar larvae and pupae of Ae. rusticus and Ae. punctor were seriously underestimated by dipping. He also thought that it was possible that the cylinders failed to give completely representative samples of the different instars, although they likely gave a more accurate representation of the instar proportions. Downing (1977) in the U.S.A. found that in New Jersey woodland pools; the dipper was efficient only at collecting Ae. canadensis (Theobald) larvae from the shallow parts of the pools. Its performance decreased rapidly as pool depth increased more than 7 inches, resulting in an underestimate of total larval density and a bias of collecting more late instar larvae. Knight (1964) considered that a desirable advantage of static quadrat collecting devices over dipping was that more opportunity existed to collect larvae that were frightened from the surface by the approach of the collector.

Appendices 2 and 4 show that sampling with all three methods the sample variance is greater, usually much greater, than the sample mean of all larval instars and pupae of Ae. cantans. This indicates that all age-classes had a highly contagious distribution. The same is also true for Ae. punctor, but it is difficult to deduce anything about the aggregation of Ae. rusticus because of its very low population. Service (1977a) also found that Ae. cantans regardless of instar tended to aggregate.

To conclude, among the three methods of sampling mosquito immature stages in ponds at Ness Woods, the quadrat proved more reliable in giving a more accurate representation of the different instar proportions in the ponds than the two other methods. The ladle showed considerable sampling bias especially in undersampling its earlier instars. Similar, but not so severe, bias occured when sampling was with the D-net, again its catch of the earlier instars was smaller than it should be.

SAMPLING THE MOSQUITO IMMATURE STAGES BY AQUATIC LIGHT TRAPS

MATERIALS AND METHODS

Betalight discs

Betalights are sealed glass capsules internally coated with a phosphor and filled with tritium gas. The tritium, an isotope of hydrogen, emits low energy beta particles (electrons) which strike the phosphor causing it to emit the light characteristic of the phosphor used. Betalights are perfectly safe with no external radiation hazards. The brightness is determined by the amount of tritium used. The useful life of a betalight is generally between 10 and 20 years when the light source will appear to the eye about half as bright as at the time of manufacture.

The betalight used in this experiment was an 'F' type disc (code F08/G/900) obtained from Saunders-Roe Developments Ltd., Millington Road, Hayes, Middlesex, England. The brightness of these betalight discs was 900 microlamberts.

Cyalume lightstick

These were manufactured by American Cyanamid Company, Organic Chemicals Division, Bound Brook, New Jersey, U.S.A. The lightstick was 152mm long and 19mm in diameter. When the plastic tube was bent, an inner glass ampoule was broken, releasing 1.5ml of colourless energy-producing solution that mixed with 6ml of a greenish fluorescer solution in the outer plastic tube. The strong chemical reaction produced, based on the principle of peroxyoxalate chemiluminescence, a greenish light. But measurement of spectral emission shows it to be rich inthe red end of the spectrum (M.W. Service pers. comm., 1981) Fig. 13.

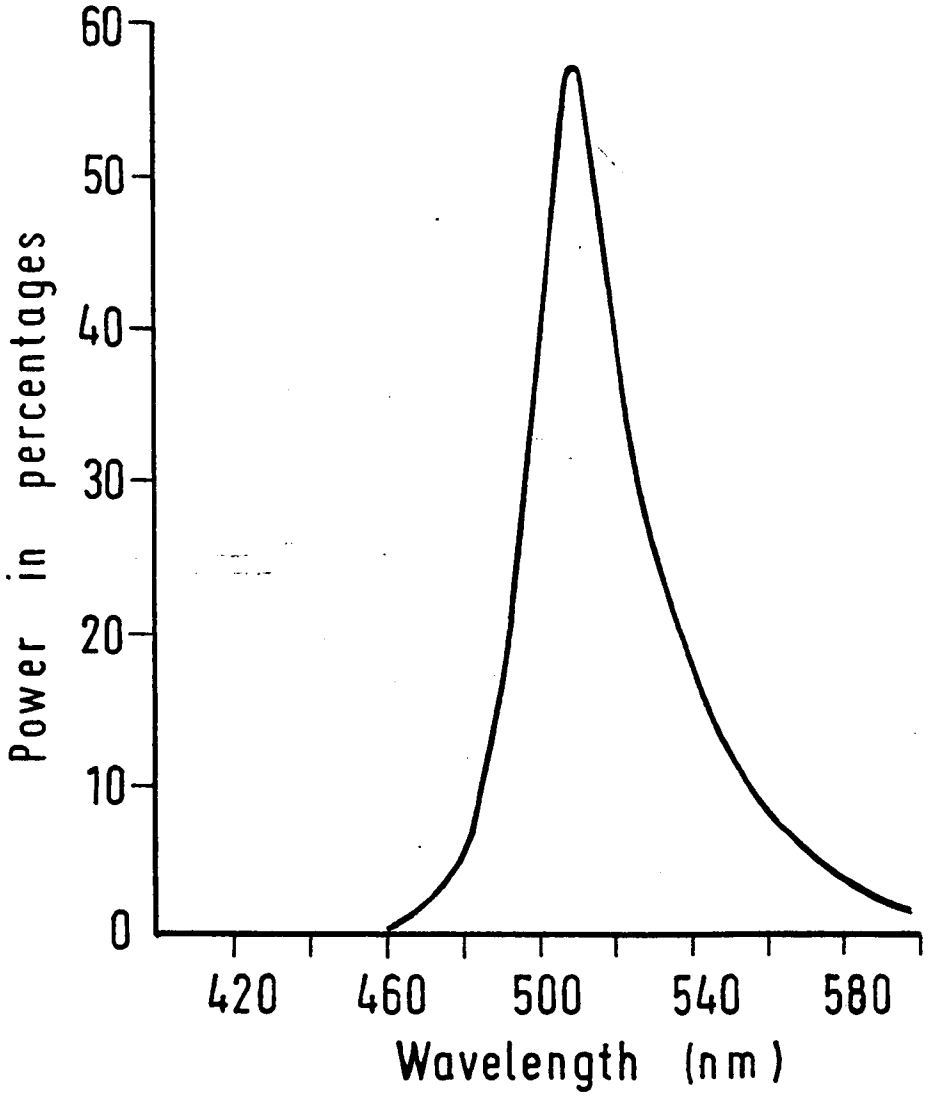

Fig. 13: Spectral emission of "Cyalume" lightstick obtained from Dr. M. W. Service (personal comm.)

Description of the aquatic light traps

Each aquatic light trap consisted a 36-cm length of polyvinylchloride tubing (Fig. 14) having an internal diameter of 14.5cm. The inner surface of each trap was painted white and a strip of "touch and close velcro fastener" glued by 'Evostik' around the inner surface of each end. This 'velcro' helped in retaining the transparent plastic cones fitted into the open ends of the trap.

For a betalight aquatic light trap, the bottom was cut from two glass vials (75 x 25mm), and a betalight disc inserted into each vial and held in position by a spring as shown in Figure 14. The two vials were then stuck together by waterproof insulating tape and tied to the middle of the fibre glass cylinder by wire.

For a chemical light trap, the Cyalume lightstick was bent to break the glass ampoule and the lightstick shaken so that the two solutions reacted chemically producing a greenish light. The whole lightstick was fixed vertically in the middle part of the tube.

Trapping immature stages of Ae. cantans in a pond at Ness Woods

In April and May 1979 and February to May 1980, during the larval breeding season, the above aquatic light traps were submerged along the perimeter of a large pond known to contain a large population of Ae. cantans. Selection of trap location was by tossing a coin along the perimeter of the pond, each at a distance of about 10 metres apart. For the control, a similar trap but without any light source was used. After 24hours of exposure, each trap was removed quickly and the water in each poured into a white plastic tray. Larvae were transfered from the tray into a glass vial with 70% alcohol added as a preservative.

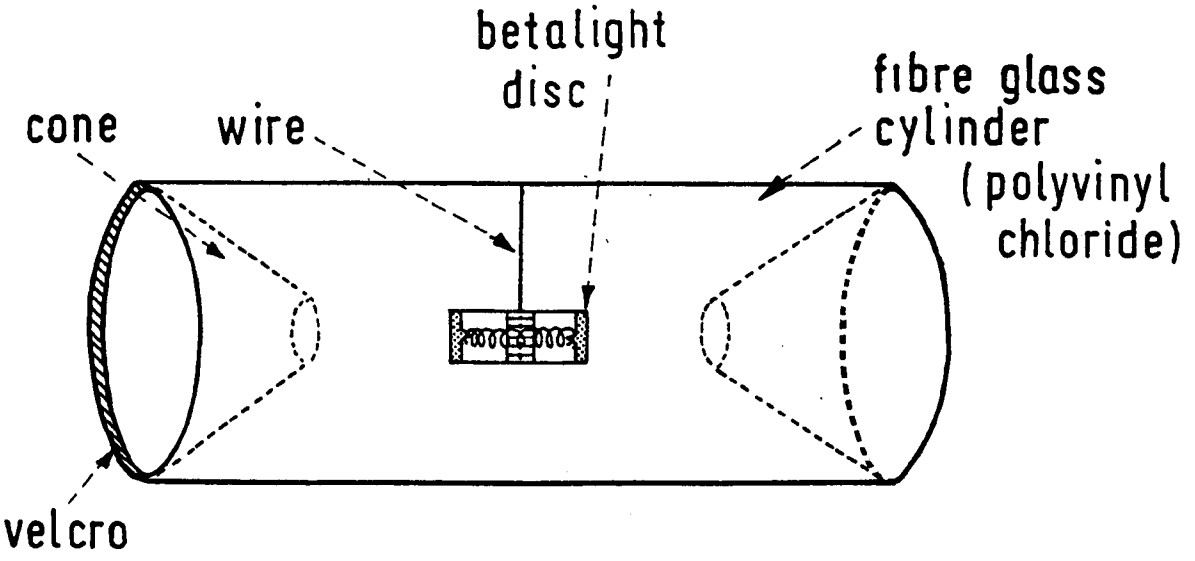

Diagram of an aquatic light trap

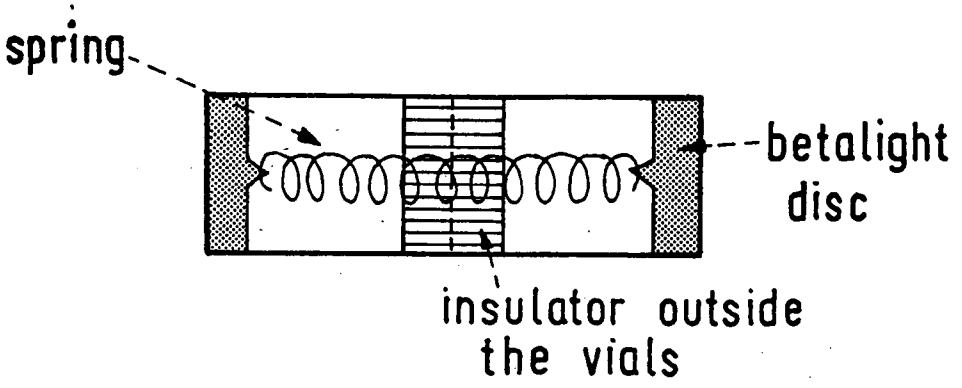

Diagram of a pair of betalight discs attached to the ends of two vials

Fig. 14: Diagrams of an aquatic light trap and betalight discs.

Any larvae or pupae attached in the interior part of the traps and cones were collected by forceps. Any pupae within the tray were transferred to a separate vial containing pond water. In the laboratory pupae from each trap were put aside for adult emergence. Experiments were repeated on different days and results analysed by the Kolmogorov - Smirnov two-sample test.

Trapping immature stages of mosquitoes in a ditch at Ness Woods

From February to May 1980 aquatic light traps were also evaluated in a ditch as Ness Woods. The traps were submerged in the middle of a ditch, the location of the traps being selected by tossing a coin along the ditch, each at a distance of about 10 metres apart.

RESULTS

The pond 1979 and 1980

In experiments in the pond at Ness Woods during April to May 1979 the chemical light trap caught between 41- 527 Ae. cantans immature stages in 24 hours, whereas the betalight trap caught only 1-30 immature stages of Ae. cantans. In the controls (i.e. traps without any light) only 1-8 Ae. cantans were trapped (Table 5). Results of Kolmogorov-Smirnov two-sample test shows that the percentage of Ae. cantans trapped by chemical light trap was significantly greater ($K6$, $P < 0.01$) thanby the betalight trap.The percentages of Ae. cantans trapped by chemical light trap ($K6$, $P < 0.01$) and betalight traps ($K5$, $P < 0.05$) were significantly greater than in the controls. Both chemical and betalight traps sampled the age-classes disproportionately. Results of previous experiment in sampling the same pond with a quadrat during the same period between 11th April 1979 to 17th May 1979 showed that the percentages of age-classes were 21.2% for the 1st-, 22.3% for the 2nd-, 19.9% for the 3rd-, 28.4% for the 4th-instar larvae

Table 5: Results of sampling Ae. cantans immature stages with aquatic light traps in a pond at Ness Woods in 1979

(sampling from 11-4-79 to 18-5-79)

Methods of Trapping

Date	Control							Betalight trap							Chemical light trap						
	1st - instar	2nd- instar	3rd- instar	4th- instar	Pupae	Total	%	1st - instar	2nd- instar	3rd- instar	4th instar	Pupae	Total	%	1st- instar	2nd- instar	3rd- instar	4th- instar	Pupae	Total	%
11.4.79	1	0	0	0	0	1	0.3	1	11	3	0	0	15	5.1	1	30	235	13	0	279	94.6
22.4.79	0	0	0	1	0	1	1.0	0	0	0	11	1	12	12.5	0	0	44	39	0	83	86.5
28.4.79	0	0	0	1	0	1	1.1	0	0	12	18	0	30	33.7	0	0	16	42	0	58	65.2
2.5.79	0	0	1	6	1	8	1.5	0	0	2	4	0	6	1.1	0	0	0	527	0	527	97.4
8.5.79	0	0	0	7	0	7	12.5	0	0	0	0	2	2	3.6	0	0	0	37	10	47	83.9
18.5.79	0	0	0	0	0	0	0.0	0	0	0	0	1	1	2.4	0	0	0	24	17	41	97.6
Total	1	0	1	15	1	18	1.6	1	11	17	33	4	66	5.9	1	30	295	682	27	1035	92.5
% in instar	5.6	0	5.6	83.3	5.6			1.5	16.7	25.8	50.0	6.1			0.1	2.9	28.5	65.9	2.6		

and 8.1% for the pupae. Table 5 shows that the chemical light trap sampled higher proportions of 3rd-instar (28.5%) and 4th-instar (65.9%) larvae of Ae. cantans and lower proportions of 1st-instar (0.1%) and 2nd-instar (2.9%) larvae, and also pupae (2.6%) than wereobtained by quadrat sampling. Similarly the betalight trap sampled higher proportions of 3rd-instar (25.8%) and 4th-instar (50%) larvae and lower proportions of 1st-instar (1.5%) and 2nd-instar (16.7%) and again pupae (6.1%) in comparison to those sampledby the quadrat.

Results of sampling the same pond in 1980 (Table 6), between 12th February 1980 and 27th February 1980, showed that the chemical light trap caught between 30-317 1st-and 2nd-instar Ae. cantans in 24 hour exposures, compared to 0-23 for the betalight trap, and 0-29 for the control experiments.

The percentage of the earlier immature stages (1st-and 2nd-instar larvae) of Ae. cantans trapped by the chemical light trap (K6, $P < 0.01$) was significantly greater than both betalight trap and control catches. The numbers caught by the betalight trap and the control trap did not differ significantly, whereas in 1979 the betalight trap catch was greater than in the control. The reason is that in 1979 late instars (3rd and 4th) were more common and formedthe bigger component of both the betalight trap (75.8%) and chemical light trap (94.4%) collections. This clearly shows that the betalight was more attractive towards the later instars of Ae. cantans larvae.

Between 16th April 1980 to 1st May 1980 in the same pond (Table 7) chemical light traps caught between 10-55 Ae. cantans immature stages in 24 hour exposures while the betalight trap caught between 7- 40 Ae. cantans. In the control experiments, no larvae or pupae of Ae. cantans were caught. Statistical analysis showed that the percentage of Ae. cantans trapped by

Table 6: Results of sampling Ae. cantans immature stages with aquatic light traps in a pond at Ness Woods in 1980

(sampling from 12.2.80 to 27. 2. 80)

Date	Methods of trapping											
	Control				Betalight trap				Chemical light trap			
	1st-instar	2nd-instar	Total	%	1st-instar	2nd-instar	Total	%	1st-instar	2nd-instar	Total	%
12.2.80	29	0	29	20.6	9	0	9	6.4	103	0	103	73.0
13.2.80	2	0	2	2.8	2	0	2	2.8	68	0	68	94.4
19.2.80	0	0	0	0	0	0	0	0	40	0	40	100.0
21.2.80	4	0	4	9.3	9	0	9	20.9	30	0	30	69.8
26.2.80	0	1	1	2.3	2	0	2	4.5	40	1	41	93.2
27.2.80	4	1	5	1.4	14	9	23	6.7	223	94	317	91.9
Total	39	2	41	6.0	36	9	45	6.6	504	95	599	87.4

Table 7: Results of sampling Ae. cantans immature stages with aquatic light traps in a pond at Ness Woods in 1980

(sampling from 16.4.80 to 1.5.80)

Date	Methods of Trapping																					
	Control							Betalight trap							Chemical light trap							
	1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%	1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%	1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%
16.4.80	0	0	0	0	0	0	0	0	2	22	15	0	39	43.3	0	18	24	9	0	51	56.7
17.4.80	0	0	0	0	0	0	0	0	0	10	1	0	11	29.7	0	0	20	6	0	26	70.3
22.4.80	0	0	0	0	0	0	0	0	0	0	8	0	8	27.6	0	0	0	20	1	21	72.4
24.4.80	0	0	0	0	0	0	0	0	0	2	8	0	10	15.4	0	0	10	43	2	55	84.6
30.4.80	0	0	0	0	0	0	0	0	0	0	0	7	7	41.2	0	0	0	10	0	10	58.8
1.5.80	0	0	0	0	0	0	0	0	0	0	20	20	40	44.4	0	0	0	35	15	50	55.6
Total	0	0	0	0	0	0	0	0	2	34	52	27	125	37.0	0	18	54	123	18	213	63.0
% instar									1.6	27.2	41.6	21.6			0	8.5	25.4	57.7	8.5		

the chemical light trap was significantly greater (K6, $P < 0.01$) than caught by the betalight trap. During this sampling period most of the immature stages of Ae. cantans were in late immature stages (3rd and 4th). Therefore, it again indicates that betalights are much more attractive to late instar larvae than the earlier stages, whereas chemical lights are better at trapping both early stages as well as late larval instars (Tables 5- 7).

The ditch 1980

The ditch had a mixed population, of largely Ae. cantans and a small population of Ae. rusticus and Ae. punctor. Between 13th February 1980 and 27th February 1980, the chemical light trap caught between 30-265 Ae. cantans immature stages (1st- and 2nd-instar larvae) and between 0-430 Ae. rusticus immature stages (2nd- , 3rd-, and 4th-instar larvae) in 24 hour exposures (Table 8). The highest number of 430 larvae of Ae. rusticus trapped comprised of 390 3rd-instar larvae and 40 4th-instar larvae. With the betalight trap the number of Ae. cantans earlier immature stages trapped was between 0-77 and Ae. rusticus between 5-39 larvae. In the control experiments, the number of Ae. cantans earlier immature stages trapped was only 1-4 larvae and Ae. rusticus between 0-2 larvae. Statistical analysis showed that the percentage of mosquitoes trapped by chemical light trap was significantly greater (K6, $P < 0.01$) than by the betalight trap. The percentage of mosquitoes trapped by the betalight was not significantly greater than by the controls, because of the predominance of Ae. cantans earlier instars during the sampling period which are not sampled favourably by betalight traps. Unfortunately, no earlier instars of Ae. punctor were trapped by any of the aquatic light traps. This could possibly be due to the very low population of its earlier instars occuring in the ditch during the sampling period. Although

Table 8: Results of sampling immature stages of mosquitoes with aquatic light traps in a ditch at Ness Woods in 1980

(sampling from 13.2.80 to 27.2.80)

Date	Species	Methods of trapping: Control 1st-instar	Control 2nd-instar	Control 3rd-instar	Control 4th-instar	Control Total	Control %	Betalight trap 1st-instar	Betalight trap 2nd-instar	Betalight trap 3rd-instar	Betalight trap 4th-instar	Betalight trap Total	Betalight trap %	Chemical light trap 1st-instar	Chemical light trap 2nd-instar	Chemical light trap 3rd-instar	Chemical light trap 4th-instar	Chemical light trap Total	Chemical light trap %
13.2.80	Ae. cantans	4	0	0	0	4	6.6	9	0	0	0	9	23.0	30	0	0	0	30	70.5
	Ae. rusticus	0	0	0	0	0		5	0	0	0	5		0	9	4	0	13	
14.2.80	Ae. cantans	4	0	0	0	4	7.8	0	0	0	0	0	7.8	65	0	0	0	65	84.4
	Ae. rusticus	0	0	2	0	2		0	0	6	0	6		0	0	0	0	0	
19.2.80	Ae. cantans	1	0	0	0	1	1.0	77	0	0	0	77	43.6	47	0	0	0	47	55.4
	Ae. rusticus	0	0	1	0	1		0	1	10	0	11		0	26	39	0	65	
21.2.80	Ae. cantans	1	0	0	0	1	0.7	3	0	0	0	3	8.9	265	0	0	0	265	90.5
	Ae. rusticus	0	0	1	0	1		0	2	7	15	24		0	3	3	4	10	
26.2.80	Ae. cantans	1	0	0	0	1	0.2	44	9	0	0	53	16.6	0	31	0	0	31	83.2
	Ae. rusticus	0	0	0	0	0		0	0	35	4	39		0	0	390	40	430	
27.2.80	Ae. cantans	2	0	0	0	2	1.2	28	35	0	0	63	40.0	40	30	0	0	70	58.8
	Ae. rusticus	0	0	0	0	0		0	0	5	0	5		0	0	25	5	30	
Total	Ae. cantans	13	0	0	0	13	1.2	161	44	0	0	205	21.6	447	61	0	0	508	77.2
% instar		100	0	0	0	-		78.5	21.5	0	0			88.0	12.0	0	0		
Total	Ae. rusticus	0	0	4	0	4		5	3	63	19	90		0	38	461	49	548	
% instar		0	0	100	0	-		5.6	3.3	70.0	21.1			0	6.9	84.1	8.9		

the Ae. rusticus population was also small during this sampling period (13th February 1980 to 27th February 1980), nevertheless a large proportion were in the late instars (3rd and 4th) (Table 8) and were attracted to the light traps.

Between 16th April 1980 and 1st May 1980 the chemical light trap caught between 37-1675 Ae. cantans immature stages from the same ditch within 24 hours (Table 9). The number of Ae. punctor caught was between 0-52 and the number of Ae. rusticus trapped was between 0-21. The highest number of mosquitoes caught after 24 hours exposure by the chemical light trap was 1675 Ae. cantans, 52 Ae. punctor and 21 Ae. rusticus giving a total of 1748 mosquitoes! The betalight trap caught between 3-135 Ae. cantans, 0-6 Ae. punctor and 0-2 Ae. rusticus after 24hour exposures. Control experiments caught between 0-28 Ae. cantans, 0-4 Ae. punctor and 0-1 Ae. rusticus. The percentage of mosquitoes trapped by the chemical light trap was significantly greater (K6, $P < 0.01$) than caught in either the betalight and control traps. Although the percentage of mosquitoes trapped by betalight trap was not statistically greater (Kd = 4, but at $\alpha = 0.05$ Kd = 5), than in the controls, the numbers caught were always larger, indicating that the betalight traps were infact more attractive than controls, to at least the late immature stages of mosquitoes.

DISCUSSION

Besides trapping mosquito immature stages, the chemical light trap, and to a lesser extent the betalight trap, caught a few adult beetles of the following species, - Hydroporus palustris (L.), Agabus bipustulatus (L.), and Acilius sulcatus (L.), also a few chironomids, Chaoborus crystallinus Degeer, Mochlonyx culiciformis Degeer and the stickleback Pygosteus pungitius (L.).

Table 9: Results of sampling immature stages of mosquitoes with aquatic light traps in a ditch at Ness Woods in 1980

(sampling from 16.4.80 to 1.5.80)

Date	Species	Methods of trapping: Control 1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%	Betalight trap 1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%	Chemical light trap 1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%
16.4.80	Ae. cantans	0	0	3	0	0	3	0.4	0	0	19	2	0	21	1.6	0	210	1220	245	0	1675	98.0
	Ae. punctor	0	0	3	0	0	4		0	0	0	6	0	6		0	0	0	52	0	52	
	Ae. rusticus	0	0	0	1	0	1		0	0	0	2	0	2		0	0	0	21	0	21	
17.4.80	Ae. cantans	0	0	1	1	0	2	1.2	0	5	23	66	0	94	42.1	0	20	85	25	0	130	56.6
	Ae. punctor	0	0	0	0	0	0		0	0	0	6	0	6		0	0	0	5	0	5	
	Ae. rusticus	0	0	0	1	0	1		0	0	0	2	0	2		0	0	0	2	0	2	
22.4.80	Ae. cantans	0	0	0	0	0	0	0	0	0	0	3	0	3	3.4	0	4	27	42	0	73	96.6
	Ae. punctor	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	4	4	8	
	Ae. rusticus	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	2	2	4	
23.4.80	Ae. cantans	0	0	7	21	0	28	75	0	0	35	100	0	135	36.2	0	0	59	139	2	200	56.3
	Ae. punctor	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	4	2	6	
	Ae. rusticus	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	3	1	4	
24.4.80	Ae. cantans	0	0	2	5	0	7	5.9	0	0	7	40	2	49	45.8	0	0	10	44	3	57	48.3
	Ae. punctor	0	0	0	0	0	0		0	0	0	0	5	5		0	0	0	0	0	0	
	Ae. rusticus	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	0	0	0	

Continued .....

Table 9: (Continued)

Date	Species	Methods of Trapping: Control 1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%	Betalight Trap 1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%	Chemical Light Trap 1st-instar	2nd-instar	3rd-instar	4th-instar	Pupae	Total	%
30.4.80	Ae. cantans	0	0	0	0	2	2	1.6	0	0	0	10	15	25	19.4	0	0	5	40	54	99	79.1
	Ae. punctor	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	0	1	1	
	Ae. rusticus	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	2	0	2	
1.5.80	Ae. cantans	0	0	0	3	4	7	12.5	0	0	0	4	8	12	21.4	0	0	2	15	20	37	66.1
	Ae. punctor	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	0	0	0	
	Ae. rusticus	0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	0	0	0	
Total	Ae. cantans	0	0	13	30	6	49	1.9	0	5	84	225	25	339	12.9	0	234	1408	550	79	2271	85.2
% instar		0	0	26.5	61.2	12.2			0	1.5	24.8	66.4	7.4			0	10.3	62.0	24.2	3.5		
Total	Ae. punctor	0	0	3	0	0	3		0	0	0	12	5	17		0	0	0	65	7	72	
% instar		0	0	100.0	0	0			0	0	0	70.6	29.4			0	0	0	90.3	9.7		
Total	Ae. rusticus	0	0	0	2	0	2		0	0	0	4	0	4		0	0	0	30	3	33	
% instar		0	0	0	100.0	0			0	0	0	100.0	0			0	0	0	90.9	9.1		

Katabazi (1981) in laboratory trials used 'Cyalume' chemical lightsticks in a very small version of the cylindrical trap used in the field. He placed them in an aquarium containing known numbers of mosquito larvae. He trapped 26-47% of the available Culex quinquefasciatus Say larvae and 22-28% of Ae. aegypti (L.) larvae in the aquarium. Anopheles stephensi Liston showed the greatest attraction towards the light and 49-82% of its larvae were trapped. In the control experiments only 1- 10% of the larvae entered the trap. In other experiments the cylindrical trap was placed vertically in the water and the only entrance faced upwards. The catch of C. quinquefasciatus and An. stephensi increased, ranging from 75-91% and 67-95% respectively of those available for capture. In contrast the catches for Ae. aegypti remained low, 12-20%. Service (personal comm., 1981) using a chemical light trap placed in a blocked off concrete drainage ditch in Legon, Ghana, caught 1631 Aedes vittatus (Bigot)? 621 Chironomus transvalensis Kieffer larvae and 32 nymphs of Libellulidae (Odonata).

CONCLUSION

These field trials in the pond and ditch at Ness Woods in 1979 and 1980 have most clearly shown that an aquatic light trap employing a chemical light source can trap large, sometimes very large, numbers of all instars of Ae. cantans. They also caught large numbers of late instar larvae of Ae. rusticus, some late instar larvae of Ae. punctor, but did not appear so effective in trapping the early stages. These results and the fact that a similar light trap in Ghana caught a very large number of Ae. vittatus clearly show the potential value of such traps in sampling mosquito populations.

Although more larvae of Ae. cantans, Ae. rusticus and Ae. punctor were caught in the traps containing the radioactive betalight than in control traps, this trap which produces a much smaller light output was very much less efficient than the chemical light trap. Moreover, it was not effective in sampling the early larval instars.

Further trials are merited to compare the efficiency of the chemical light trap directly with other sampling methods.

LARVAL SAMPLING WITH STICKY TRAPS

In discussions with L. Ryan (personal comm., 1979) it was learnt that there were a number of adhesives that remained sticky even when wetted. It was quickly realised that if adhesives were to remain sticky under water they might be able to be used to sample mosquito larvae. It was considered that if such glues were spread onto suitable surfaces and placed at the bottom of larval habitats then they might trap mosquito larvae as they descended to feed on the bottom, or if placed floating on the water surface they might trap larvae surfacing to breathe. With these prospects in mind and the idea that aquatic sticky traps might sample all age-classes without bias led to the initiation of, firstly, laboratory and, secondly, field trials with what appeared to be three of the most promising sticky compounds, namely Hyvis 200, Hyvis 2000, and a Rat Varnish. Hyvis 200 and Hyvis 2000 are clear and colourless polybutene compounds, and samples were kindly donated by the manufacturers, British Petroleum Chemical Ltd., Bo'ness, Graingemouth, Sterlingshire FK3 9XH, England. The Rat varnish is clear yellow in colour and is basically a lithographers varnish; it was purchased from Rodent Control Ltd., 70-78 Queens Road, Reading, Berkshire, England.

MATERIALS AND METHODS

Laboratory experiments in 1980

Experiment 1

Laboratory experiment using Hyvis 200, Hyvis 2000 and Rat Varnish for trapping 1st-instar larvae of Ae. cantans in pond water.

Submerged method

Each glue was smeared onto one surface of a 15 x 15cm piece of transparent plastic using a glass rod. The coated plastic sheet was then placed at the bottom, sticky side facing upwards, of a plastic tray (26 x 18 x 7cm) containing 1200ml of Ness Woods pond water. 25 1st-instar larvae of Ae. cantans were placed in each tray.

Floating method

A similar square plastic sheet coated on the underside with each glue had polystyrene floats attached and was floated on the surface of 1200 ml pond water contained in a plastic tray. 25 1st-instar larvae of Ae. cantans collected from the field were placed in each tray.

For the controls both floating and submerged methods were conducted without any glue. After 24 hours of exposure, the number and percentage of 1st-instar larvae of Ae. cantans trapped were recorded as well as any mortality in control experiment. Water temperature was recorded with a thermometer. The experiment was then repeated.

Experiment II

Laboratory experiment using Hyvis 200, Hyvis 2000 and Rat Varnish for trapping 4th-instar larvae of Ae. cantans in pond water.

This experiment using 4th-instar larvae was conducted with the same procedures as experiment 1 above.

Experiment III

Laboratory experiment using Hyvis 200, Hyvis 2000 and Rat Varnish for trapping 4th-instar larvae of Ae. aegypti in tap water.

The procedures of experiment I were repeated.

Experiment IV

Laboratory experiment using Hyvis 200, Hyvis 2000 and Rat Varnish for trapping 4th-instar larvae of Ae. aegypti in pond water.

The procedures of experiment I were repeated.

Experiment V

Laboratory experiment using Hyvis 200, Hyvis 2000 and Rat Varnish for trapping a mixed population of 4th-instar larvae of Ae. aegypti and 4th-instar larvae of Ae. cantans in pond water.

The procedures of experiment I were repeated.

using a mixture of 20 4th-instar larvae of Ae. aegypti and 20 4th-instar larvae of Ae. cantans in each tray.

Experiment VI

Laboratory experiment using Hyvis 200, Hyvis 2000 and Rat Varnish for trapping a mixed population of 4th-instar larvae of Ae. aegypti and 4th-instar larvae of Ae. cantans in pond water in incubator at 32° C.

The procedures of experiment I were repeated using a mixture of 20 4th-instar larvae of Ae. aegypti and 20 4th-instar larvae of Ae. cantans in each tray. All the trays were placed in an incubator at 32 °C and results recorded after 24 hours of exposure.

Experiment VII

Laboratory experiment using Hyvis 200, and Rat Varnish for trapping 4th-instar Ae. aegypti in tap and pond water in the cold room (temperature 5-6.5 °C)

Because of the poor results shown by Hyvis 2000 in previous experiments, only Hyvis 200 and Rat Varnish were tested in this experiment, and only the submerged method of trapping was used. Each glue was smeared on two squares of plastic sheets. One of them was submerged in a plastic tray containing 1200ml of pond water and the other submerged in a tray containing 1200ml of tap water. The trays were placed in a cold room and after about 1 hour for the water to reach the environmental temperature (5-6.5° C) , 40 4th-instar Ae. aegypti were placed in each tray (including control experiment). 24 hours later the number and percentage of Ae. aegypti trapped in each tray was recorded and temperature of water noted.

Laboratory experiments in 1981

Because of the poor results shown previously by Hyvis 2000, only Hyvis 200 and Rat Varnish were used in these experiments.

Experiment VIII

Laboratory experiment using Hyvis 200 and Rat Varnish for trapping 1st-instar larvae of Ae. cantans in tap water.

The floating and submerged methods of trapping 1st-instar Ae. cantans were conducted according to experiment I of 1980.

Experiment IX

Laboratory experiment using Hyvis 200 and Rat Varnish for trapping 1st-instar larvae of Ae. cantans in pond water.

The floating and submerged methods of trapping 1st-instar Ae. cantans were conducted according to experiment I of 1980.

Experiment X

Laboratory experiment using Hyvis 200 and Rat Varnish for trapping a mixed population of 3rd and 4th-instar larvae of Ae. cantans in pond water.

The floating and submerged methods of trapping a mixture of late instar larvae (3rd and 4th-instar) of Ae. cantans were conducted in the laboratory using 25 larvae in each tray. The number and percentage of larvae trapped to each method of trapping for each glue was recorded.

Experiment XI

Laboratory experiment using Hyvis 200 and Rat Varnish for trapping a mixed population of 3rd and 4th - instar larvae of Ae. cantans in tap water.

The procedures of experiment X were repeated in tap water.

Field experiments in 1980

Experiment XII

The same methods as used in the laboratory were used to float (Fig. 15) and submerge sticky traps in a pond and ditch at Ness Woods inwhich mosquito immature stages were abundant. Each pair of sticky traps (floating and submerged) representing each of the three glues namely Hyvis 200, Hyvis 2000 and Rat Varnish were placed along the perimeter of a pond or in the middle of a ditch. The location of each pair of sticky traps was chosen at random by tossing a coin, each at a distance of 10 metres apart. After 24 hours the mosquitoes sticking to the plastic sheets were carefully removed by forceps and placed in a glass vial containing 70% alcohol as a preservative. In the laboratory the catch was recorded according to the larval instars and species.

Field experiments in 1981

Experiment XIII

Field experiment using Hyvis 200 and Rat Varnish for trapping 1st-instar Ae. cantans larvae in plastic trays containing tap water at Ness Woods.

Fig. 15: A floating sticky trap in a ditch. The adhesive is coated on the undersurface of the transparent plastic sheet which is then floated on the water by a piece of polystyrene.

The floating and submerged methods of trapping 25 1st-instar larvae of Ae. cantans in plastic trays each containing 1200 ml of tap water was conducted at Ness Woods. The trays were then placed on the ground amongst vegetation of the wood. After 24 hours the number and percentage of larvae trapped to each method of trapping for each glue was recorded.

Experiment XIV

Field experiment using Hyvis 200 and Rat Varnish for trapping 1st-instar Ae. cantans larvae in plastic trays containing pond water at Ness Woods.

The procedures of experiment XIII were repeated in pond water.

Experiment XV

Field experiment using Hyvis 200 and Rat Varnish for trapping a mixed population of 3rd and 4th - instar larvae of Ae. cantans in pond water.

The procedures of experiment XIII were repeated.

Experiment XVI

Field experiment using Hyvis 200 and Rat Varnish for trapping a mixed population of 3rd and 4th-instar larvae of Ae. cantans in tap water.

The procedures of experiment XIII were repeated.

Experiment XVII

Field experiment at Ness Woods using Hyvis 200 and Rat Varnish for trapping mosquito immature stages in a pond and a ditch.

The floating and submerged methods of trapping mosquito immature stages were repeated following the method of experiment XII in 1980. The temperature of the ditch and pond was also recorded using maximum and minimum thermometers.

RESULTS AND DISCUSSION

Laboratory experiments (I - VII)

In 1980, three glues namely Hyvis 200, Hyvis 2000 and Rat Varnish were evaluated against 1st-instar Ae. cantans in pond water in the laboratory (Expt. 1a-1c). The percentage of larvae trapped by each glue was very low, never beyond 20% with Hyvis 200, up to 8% with Hyvis 2000 and up to 16% with Rat Varnish (Table 10) When the glues were tested against 4th-instar larvae of Ae. cantans in pond water in the laboratory (Expt. IIa-IIc), Hyvis 200 managed to trap up to 32%, Hyvis 2000 up to 4% and Rat Varnish up to 36% (Table 11).

In laboratory trials using 4th-instar Ae. aegypti in tap water (Expt. IIIa-IIIe), Hyvis 200 in the floating position trapped up to 65% of Ae. aegypti larvae and up to 95% when submerged. Hyvis 2000 in the floating position trapped up to 22.5% and in submerged position up to 40% of the 4th-instar larvae of Ae. aegypti. Rat Varnish in the floating position caught up to 70% and in submerged position up to 97.5% of the 4th-instar Ae. aegypti larvae (Table 12). Generally, all the glues trapped significantly more larvae ($P < 0.05$ to $P < 0.005$) when placed at the bottom of the containers (i.e. submerged position) than when placed on the water surface (i.e. the floating position). Among the three glues being compared, Rat Varnish was the most efficient in trapping Ae. aegypti 4th-instar larvae in tap water ($P < 0.05$, $P < 0.001$).

When the three glues were tried against Ae. aegypti 4th-instar larvae in pond water (Expt. IV), Hyvis 200 in the floating position trapped up to 25% and in the submerged position up to 60% of the larvae (Table 13). Hyvis 2000 in the floating position trapped up to 25% and in the submerged position up to 10% of 4th-instar Ae. aegypti larvae. Rat Varnish in the floating position in pond water

Table 10: Results of laboratory experiments using Hyvis 200, Hyvis 2000, and Rat Varnish for trapping 1st instar *Ae. cantans* in plastic trays containing pond water in 1980. 25 larvae were used in each experiment with each glue

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
19.2.80	1a	Hyvis 200	Floating	2	8%	17.5
			Submerged	4	16%	17.5
		Hyvis 2000	Floating	0	0%	17.5
			Submerged	2	8%	17.5
		Rat varnish	Floating	3	12%	17.5
			Submerged	2	8%	17.5
		Control	Floating	no mortality		17.5
			Submerged	no mortality		17.5
21.2.80	1b	Hyvis 200	Floating	4	16%	17.5
			Submerged	5	20%	17.5
		Hyvis 2000	Floating	0	0%	17.5
			Submerged	0	0%	17.5
		Rat varnish	Floating	1	4%	17.5
			Submerged	0	0%	17.5
		Control	Floating	no mortality		17.5
			Submerged	no mortality		17.5
26.2.80	1c	Hyvis 200	Floating	3	12%	18.0
			Submerged	2	8%	18.0
		Hyvis 2000	Floating	0	0%	18.0
			Submerged	0	0%	18.0
		Rat varnish	Floating	4	16%	18.0
			Submerged	0	0%	18.0
		Control	Floating	no mortality		18.0
			Submerged	no mortality		18.0

Table 11: Results of laboratory experiments using Hyvis 200, Hyvis 2000, and Rat Varnish for trapping 4th-instar *Ae. cantans* in plastic trays containing pond water in 1980. 25 larvae were used in each experiment with each glue.

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp. of water °C
30.4.80	IIa	Hyvis 200	Floating	1	4%	20
			Submerged	6	24%	20
		Hyvis 2000	Floating	0	0%	20
			Submerged	0	0%	20
		Rat Varnish	Floating	0	0%	20
			Submerged	4	16%	20
		Control	Floating	no mortality		20
			Submerged	no mortality		20
5.5.80	IIb	Hyvis 200	Floating	1	4%	20
			Submerged	7	28%	20
		Hyvis 2000	Floating	0	0%	20
			Submerged	1	4%	20
		Rat Varnish	Floating	2	8%	20
			Submerged	9	36%	20
		Control	Floating	no mortality		20
			Submerged	no mortality		20
6.5.80	IIc	Hyvis 200	Floating	2	8%	21
			Submerged	8	32%	21
		Hyvis 2000	Floating	0	0	21
			Submerged	0	0	21
		Rat Varnish	Floating	2	8%	21
			Submerged	8	32%	21
		Control	Floating	no mortality		21
			Submerged	no mortality		21

Table 12: Results of laboratory experiments using Hyvis 200, Hyvis 2000, and Rat Varnish for trapping 4thinstar Ae. aegypti in plastic trays containing tap water in 1980. 40 larvae were used in each experiment with each glue

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
23.6.80	IIIa	Hyvis 200	Floating	4	10%	24
			Submerged	32	80%	24
		Hyvis 2000	Floating	1	2.5%	24
			Submerged	11	27.5%	24
		Rat Varnish	Floating	10	25%	24
			Submerged	39	97.5%	24
		Control	Floating	no mortality		24
			Submerged	no mortality		24
28.6.80	IIIb	Hyvis 200	Floating	22	55%	24
			Submerged	38	95%	24
		Hyvis 2000	Floating	0	0%	24
			Submerged	12	30	24
		Rat Varnish	Floating	13	32.5%	24
			Submerged	39	97.5%	24
		Control	Floating	no mortality		24
			Submerged	no mortaltiy		24
6.7.80	IIIc	Hyvis 200	Floating	26	65%	20
			Submerged	36	90%	20
		Hyvis 2000	Floating	9	22.5%	20
			Submerged	9	22.5%	20
		Rat Varnish	Floating	28	70%	20
			Submerged	38	95%	20
		Control	Floating	no mortality		
			Submerged	no mortality		
8.7.80	IIId	Hyvis 200	Floating	6	15%	20
			Submerged	18	45%	20
		Hyvis 2000	Floating	2	5%	20
			Submerged	16	40%	20
		Rat Varnish	Floating	5	12.5%	20
			Submerged	30	75%	20
		Control	Floating	no mortality		20
			Submerged	no mortality		20
15.7.80	IIIe	Hyvis 200	Floating	21	52.5%	20
			Submerged	33	82.5%	20
		Hyvis 2000	Floating	5	12.5%	20
			Submerged	5	12.5%	20
		Rat Varnish	Floating	28	70%	20
			Submerged	38	95%	20
		Control	Floating	no mortality		20
			Submerged	no mortality		

Table 13: Results of laboratory experiments using Hyvis 200, Hyvis 2000, and Rat Varnish for trapping 4th -instar Ae. aegypti in plastic trays containing pond water in 1980. 40 larvae were used in each experiment with each glue

Date	Expt. No.	Glues	Method of trapping	No. of larvae trapped	% of larvae trapped	Temp of water °C
29.6.80	IVa	Hyvis 200	Floating	0	0%	21
			Submerged	5	12.5%	21
		Hyvis 2000	Floating	0	0%	21
			Submerged	4	10%	21
		Rat Varnish	Floating	9	22.5%	21
			Submerged	5	12.5%	21
		Control	Floating	no mortality		21
			Submerged	no mortality		21
2.7.80	IVb	Hyvis 200	Floating	10	25%	26
			Submerged	24	60%	26
		Hyvis 2000	Floating	1	2.5%	26
			Submerged	3	7.5%	26
		Rat Varnish	Floating	13	32.5%	26
			Submerged	23	57.5%	26
		Control	Floating	no mortality		26
			Submerged	no mortality		26
4.7.80	IVc	Hyvis 200	Floating	8	20%	19.5
			Submerged	20	50%	19.5
		Hyvis 2000	Floating	1	2.5%	19.5
			Submerged	2	5%	19.5
		Rat Varnish	Floating	26	65%	19.5
			Submerged	33	82.5%	19.5
		Control	Floating	no mortality		19.5
			Submerged	no mortality		19.5
19.7.80	IVd	Hyvis 200	Floating	8	20%	19.5
			Submerged	20	50%	19.5
		Hyvis 2000	Floating	10	25%	19.5
			Submerged	2	5%	19.5
		Rat Varnish	Floating	26	65%	19.5
			Submerged	33	82.5%	19.5
		Control	Floating	no mortality		19.5
			Submerged	no mortality		19.5

trapped up to 65% and in the submerged position up to 82.5% of the 4th-instar (Table 13). So, the pond water seemed to have an effect in reducing the percentages of Ae. aegypti 4th-instar larvae caught by these glues. Results of statistical analysis have shown that Hyvis 200 is significantly much better than Hyvis 2000 in trapping Ae. aegypti 4th-instar larvae ($P < 0.025$) and Rat Varnish is significantly better than Hyvis 200 ($P < 0.05$). However, in contrast to experiments in tap water Rat Varnish in pond water is not significantly better than Hyvis 200 ($P > 0.05$).

When 4th-instar Ae. aegypti and 4th-instar larvae of Ae. cantans were mixed in pond water in laboratory trials (Expt. V), again the results obtained showed that Hyvis 200 caught only up to 15% of Ae. aegypti and 45% of Ae. cantans 4th-instar larvae. No larvae were trapped by Hyvis 2000, but the Rat Varnish managed to catch as many as 40% of Ae. aegypti and 30% of Ae. cantans 4th-instar larvae in the pond water (Table 14). Again, low percentages of larvae of both species were trapped in pond water by Hyvis 200 and Rat Varnish, while Hyvis 2000 failed to trap any larvae.

To find out whether temperature could play an important role, the sticky traps were placed in trays in an incubator at 32° C. (Expt. VI) and in a cold room (temperature 5-6.5° C) (Expt. VII). When these sticky traps were tested with a mixed population of 4th-instar larvae of Ae. cantans and Ae. aegypti in pond water at 32° C, results showed that Hyvis 200 managed to trap only 15% of Ae. aegypti, and 15% of Ae. cantans (Table 15). No larvae were trapped by Hyvis 2000. Rat Varnish managed to trap up to 15% of Ae. aegypti and 15% of Ae. cantans 4th-instar larvae (Table 15). So, generally a lower percentage of mosquito larvae were trapped at 32° C than those at room temperature (18 - 26° C).

Table 14: Results of laboratory experiments using Hyvis 200, Hyvis 2000, and Rat Varnish for trapping a mixed population of 4th-instar Ae. cantans and 4th -instar Ae. aegypti in plastic trays containing pond water in 1980.

In each experiment with each glue 20 Ae. aegypti and 20 Ae cantans larvae were used.

Date	Expt. No	Glues	Method of trapping.	No of Ae. aegypti trapped	% of Ae. aegypti trapped	No. of Ae. cantans trapped	% of Ae. cantans trapped
20.4.80	Va	Hyvis 200	Floating	0	0%	1	5%
			Submerged	3	15%	9	45%
		Hyvis 2000	Floating	0	0%	0	0%
			Submerged	0	0%	0	0%
		Rat Varnish	Floating	0	0%	1	5%
			Submerged	7	35%	5	25%
		Control	Floating	no mortality		no mortality	
			Submerged	no mortality		no mortality	
	Temp of pond water = 18 °C						
22.4.80	Vb	Hyvis 200	Floating	0	0%	0	0%
			Submerged	3	15%	3	15%
		Hyvis 2000	Floating	0	0%	0	0%
			Submerged	0	0%	0	0%
		Rat Varnish	Floating	0	0%	1	5%
			Submerged	3	15%	0	0%
		Control	Floating	no mortality		no mortality	
			Submerged	no mortality		no mortality	
	Temp of pond water = 19°C						
23.4.80	Vc	Hyvis 200	Floating	0	0%	1	5%
			Submerged	3	15%	3	15%
		Hyvis 2000	Floating	0	0%	0	0%
			Submerged	0	0%	0	0%
		Rat Varnish	Floating	1	5%	1	5%
			Submerged	8	40%	6	30%
		Control	Floating	no mortality		no mortality	
			Submerged	no mortality		no mortality	
	Temp of pond water = 20°C						

Table 15: Results of laboratory experiments using Hyvis 200, Hyvis 2000, and Rat Varnish for trapping a mixed population of 4th -instar Ae. cantans and 4th -instar Ae. aegypti in plastic trays containing pond water in an incubator at 32°C in 1980. In each experiment with each glue 20 Ae. aegypti and 20 Ae. cantans larvae were used.

Date	Expt. No	Glues	Method of trapping	No of Ae. aegypti trapped	% of Ae. aegypti trapped	No. of Ae. cantans trapped	% of Ae. cantans trapped
24.4.80	VIa	Hyvis 200	Floating	0	0%	0	0%
			Submerged	0	0%	1	5%
		Hyvis 2000	Floating	0	0%	0	0%
			Submerged	0	0%	0	0%
		Rat Varnish	Floating	0	0%	0	0%
			Submerged	3	15%	0	0%
		Control	Floating	no mortality		no mortality	
			Submerged	no mortality		no mortality	
25.4.80	VIb	Hyvis 200	Floating	0	0%	0	0%
			Submerged	2	10%	2	10%
		Hyvis 2000	Floating	0	0%	0	0%
			Submerged	0	0%	0	0%
		Rat Varnish	Floating	0	0%	0	0%
			Submerged	0	0%	3	15%
		Control	Floating	no mortality		no mortality	
			Submerged	no mortality		no mortality	
28.4.80	VIc	Hyvis 200	Floating	0	0%	1	5%
			Submerged	3	15%	3	15%
		Hyvis 2000	Floating	0	0%	0	0%
			Submerged	0	0%	0	0%
		Rat Varnish	Floating	0	0%	1	5%
			Submerged	2	10%	3	15%
		Control	Floating	no mortality		no mortality	
			Submerged	no mortality		no mortality	

Continued .....

Table 15: (Continued)

Date	Expt No.	Glues	Method of trapping	No of Ae. aegypti trapped	% of Ae. aegypti trapped	No. of Ae. cantans trapped	% of Ae. cantans trapped
29.4.80	VId	Hyvis 200	Floating	0	0%	0	0%
			Submerged	0	0%	1	5%
		Hyvis 2000	Floating	0	0%	0	0%
			Submerged	0	0%	0	0%
		Rat Varnish	Floating	0	0%	0	0%
			Submerged	2	10%	2	10%
		Control	Floating	no mortality		no mortality	
			Submerged	no mortality		no mortality.	

An experiment (number VII) was then conducted between 5-6.5°C with submerged sticky panels in tap water. Hyvis 200 caught up to 80% of 4th-instar Ae. aegypti compared to 60% in pond water (Table 16). Rat Varnish trapped as much as 85% of 4th-instar Ae. aegypti in tap water, but only 60% in pond water. So, even at low temperature as in the cold room, both Hyvis 200 and Rat Varnish trapped higher percentages of 4th-instar Ae. aegypti larvae in tap water than in pond water. This suggests that pond water due possibly to its turbidity somehow effected the tackiness of the glues, possibly because they become coated with a very fine surface deposit. Surprisingly the adhesives caught more larvae when smeared onto submerged panels placed in pond water at low temperatures (5-6.5° C) than when placed in much higher temperature (32° C). The possible reason for this difference could be due to changes of viscosity of the glues at higher temperatures. Both Hyvis 200 and Rat Varnish in the submerged positions at laboratory temperatures (18 - 26°C) also caught more larvae than when submerged at 32°C.

Statistical analysis with paired t-tests show that Hyvis 200 submerged in tap water (5-6.5°C) is significantly much better in trapping larvae than Hyvis 200 submerged in pond water ($P < 0.005$). Rat Varnish panels submerged in tap water (5-6.5° C) caught significantly more larvae than when the panels were placed in pond water ($P < 0.025$).

Laboratory experiments (VIII-XI)

In 1981, Hyvis 200 and Rat Varnish sticky traps were again tested on 1st-instar larvae of Ae. cantans in the laboratory using tap water (Expt. VIII). In a floating position Hyvis 200 sticky traps caught as many as 60% of the larvae available over 24 hour exposures, and as high as 80% of the larvae when the sticky panels were submerged (Table 17). Using pond water in the laboratory, Hyvis 200 in the floating

Table 16: Results of laboratory experiments (in cold room) using Hyvis 200 and Rat Varnish for trapping Ae. aegypti 4th -instar larvae in plastic trays containing pond or tap water in 1980. 40 larvae were used in each experiment with each glue.

Date	Expt No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
26.7.80	VIIa	Hyvis 200	Submerged in tap water	32	80%	5.0
			Submerged in pond water	24	60%	5.0
		Rat Varnish	Submerged in tap water	28	70%	5.0
			Submerged in pond water	4	10%	5.0
		Control	Submerged in tap water	no mortality		
			Submerged in pond water	no mortality		
4.8.80	VIIb	Hyvis 200	Submerged in tap water	27	67.5%	6.5
			Submerged in pond water	14	35%	6.5
		Rat Varnish	Submerged in tap water	34	85%	6.5
			Submerged in pond water	11	27.5%	6.5
		Control	Submerged in tap water	1 larva died		6.5
			Submerged in pond water	1 larva died		
8.8.80	VIIc	Hyvis 200	Submerged in tap water	19	47.5%	6.5
			Submerged in pond water	14	35%	6.5
		Rat Varnish	Submerged in tap water	31	77.5%	6.5
			Submerged in pond water	24	60%	6.5
		Control	Submerged in tap water	1 larva died		
			Submerged in pond water	1 larva died		
16.8.81	VIId	Hyvis 200	Submerged in tap water	24	60%	6.5
			Submerged in pond water	16	40%	6.5

Continued .....

Table 16: (Continued)

Date	Expt No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
		Rat Varnish	Submerged in tap water	22	55%	6.5
			Submerged in pond water	10	25%	6.5
		Control	Submerged in tap water	no mortality		
			Submerged in pond water	no mortality		

Table 17: Results of laboratory experiments using Hyvis 200, and Rat Varnish for trapping 1st instars *Ae. cantans* in plastic trays containing tap water in 1981

25 larvae were used in each experiment with each glue.

Date	Expt No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
27.2.81	VIIIa	Hyvis 200	Floating	2	8%	18.5
			Submerged	10	40%	18.5
		Rat Varnish	Floating	22	88%	18.5
			Submerged	21	84%	18.5
		Control	Floating	no mortality		
			Submerged	no mortality		
4.3.81	VIIIb	Hyvis 200	Floating	2	8%	17.0
			Submerged	18	72%	17.0
		Rat Varnish	Floating	22	88%	17.0
			Submerged	23	92%	17.0
		Control	Floating	no mortality		
			Submerged	no mortality		
6.3.81	VIIIc	Hyvis 200	Floating	6	24%	20.0
			Submerged	18	72%	20.0
		Rat Varnish	Floating	20	80%	20.0
			Submerged	23	92%	20.0
		Control	Floating	no mortality		
			Submerged	no mortality		
9.3.81	VIIId	Hyvis 200	Floating	15	60%	18.0
			Submerged	18	72%	18.0
		Rat Varnish	Floating	21	84%	18.0
			Submerged	21	84%	18.0
		Control	Floating	no mortality		
			Submerged	no mortality		
12.3.81	VIIIe	Hyvis 200	Floating	10	40%	21.0
			Submerged	19	76%	21.0
		Rat Varnish	Floating	15	60%	21.0
			Submerged	22	88%	21.0
		Control	Floating	no mortality		
			Submerged	no mortality		
17.3.81	VIIIf	Hyvis 200	Floating	8	32%	20.0
			Submerged	20	80%	20.0
		Rat Varnish	Floating	10	40%	20.0
			Submerged	22	88%	20.0
		Control	Floating	no mortality		
			Submerged	no mortality		

position caught only up to 8%, and in the submerged position up to 24% of 1st-instar larvae of Ae. cantans (Expt. IX) (Table 18). When Rat Varnish was used in a floating position in tap water up to 88% of the larvae were caught and up to 92% when in the submerged position. In marked contrast when Rat Varnish sticky panels were tested in pond water in a floating position only up to 12%, and in the submerged position only up to 24% , of 1st-instar Ae. cantans larvae were trapped. These results with both Hyvis 200 and Rat Varnish clearly demonstrate that catches are very low in pond water compared to tap water, possibly due to fine particles masking the nature of the sticky surface of the adhesives.

When Hyvis 200 was tested against a mixed population of 3rd and 4th-instar larvae of Ae. cantans in pond water in the laboratory (Expt. X) , only up to 68% of the late immature stages were trapped in both the floating and submerged positions (Table 19). But when a similar experiment was conducted in tap water (Expt. XI) (Table 20), Hyvis 200 in a floating position trapped up to 84% of the larvae and as much as 96% in a submerged position. Using Rat Varnish in pond water in the laboratory up to 56% of the larvae were trapped on floating panels, and 88% on submerged panels (Table 19). However, when the experiment was conducted in tap water, Rat Varnish in either a floating or submerged position sometimes caught all the 3rd-and 4th-instar Ae. cantans larvae available during the exposure period. So, when experiments using either adhesive were conducted in the laboratory in tap water a much higher percentage of the larvae were caught than when the sticky traps were placed in pond water.

Table18: Results of laboratory experiments using Hyvis 200 and Rat Varnish for trapping 1st - instars Ae. cantans in plastic trays containing pond water in 1981. 25 larvae were used in each experiment with each glue

Date	Expt No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
25.2.81	IXa	Hyvis 200	Floating	0	0%	17.5
			Submerged	6	24%	17.5
		Rat Varnish	Floating	1	4%	17.5
			Submerged	0	0%	17.5
		Control	Floating	no mortality		
			Submerged	no mortality		
2.3.81	IXb	Hyvis 200	Floating	1	4%	18.0
			Submerged	3	12%	18.0
		Rat Varnish	Floating	0	0%	18.0
			Submerged	4	16%	18.0
		Control	Floating	no mortality		
			Submerged	no mortality		
5.3.81	IVc	Hyvis 200	Floating	0	0%	18.0
			Submerged	3	12%	18.0
		Rat Varnish	Floating	0	0%	18.0
			Submerged	6	24%	18.0
		Control	Floating	no mortality		
			Submerged	no mortality		
11.3.81	IXd	Hyvis 200	Floating	0	0%	18.0
			Submerged	0	3%	18.0
		Rat Varnish	Floating	0	0%	18.0
			Submerged	2	8%	18.0
		Control	Floating	no mortality		
			Submerged	no mortality		
19.3.81	IXe	Hyvis 200	Floating	1	4%	18.0
			Submerged	2	8%	18.0
		Rat Varnish	Floating	0	0%	18.0
			Submerged	1	4%	18.0
		Control	Floating	no mortality		
			Submerged	no mortality		
25.3.81	IXf	Hyvis 200	Floating	2	8%	21.0
			Submerged	4	16%	21.0
		Rat Varnish	Floating	3	12%	21.0
			Submerged	4	16%	21.0
		Control	Floating	no mortality		
			Submerged	no mortality		

Table 19: Results of laboratory experiments using Hyvis 200 and Rat Varnish for trapping 3rd and 4th -instars Ae. cantans in plastic trays containing pond water in 1981. 25 larvae were used in each experiment with each glue

Date	Expt No	Glues	Method of trapping	% of larvae trapped	Temp of water °C
7.4.81	Xa	Hyvis 200	Floating	16%	20
			Submerged	20%	20
		Rat Varnish	Floating	56%	20
			Submerged	88%	20
		Control	Floating	no mortality	
			Submerged	no mortality	
13.4.81	Xb	Hyvis 200	Floating	44%	25
			Submerged	68%	25
		Rat Varnish	Floating	52%	25
			Submerged	76%	25
		Control	Floating	no mortality	
			Submerged	no mortality	
14.4.81	Xc	Hyvis 200	Floating	44%	25
			Submerged	68%	25
		Rat Varnish	Floating	48%	25
			Submerged	76%	25
		Control	Floating	no mortality	
			Submerged	no mortality	
16.4.81	Xd	Hyvis 200	Floating	12%	25
			Submerged	48%	25
		Rat Varnish	Floating	32%	25
			Submerged	48%	25
		Control	Floating	no mortality	
			Submerged	no mortality	
21.4.81	Xe	Hyvis 200	Floating	60%	18
			Submerged	20%	18
		Rat Varnish	Floating	56%	18
			Submerged	88%	18
		Control	Floating	no mortality	
			Submerged	no mortality	
29.4.81	Xf	Hyvis 200	Floating	68%	19
			Submerged	64%	19
		Rat Varnish	Floating	56%	19
			Submerged	84%	19
		Control	Floating	no mortality	
			Submerged	no mortality	

Table 20: Results of laboratory experiments using Hyvis 200 and Rat Varnish for trapping 3rd and 4th instars Ae. cantans in plastic trays containing tap water in 1981. 25 larvae were used in each experiment with each glue.

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
6.4.81	XIa	Hyvis 200	Floating	20	80%	21
			Submerged	24	96%	21
		Rat Varnish	Floating	15	60%	21
			Submerged	24	96%	21
		Control	Floating	no mortality		
			Submerged	no mortality		
8.4.81	XIb	Hyvis 200	Floating	21	84%	22
			Submerged	22	88%	22
		Rat Varnish	Floating	25	100%	22
			Submerged	25	100%	22
		Control	Floating	no mortality		
			Submerged	no mortality		
11.4.81	XIc	Hyvis 200	Floating	17	68%	21
			Submerged	14	56%	21
		Rat Varnish	Floating	21	84%	21
			Submerged	23	92%	21
		Control	Floating	no mortality		
			Submerged	no mortality		
15.4.81	XId	Hyvis 200	Floating	11	44%	24
			Submerged	16	64%	24
		Rat Varnish	Floating	21	84%	24
			Submerged	19	76%	24
		Control	Floating	no mortality		
			Submerged	no mortality		
20.4.81	XIe	Hyvis 200	Floating	15	60%	25
			Submerged	22	88%	25
		Rat Varnish	Floating	24	96%	25
			Submerged	25	100%	25
		Control	Floating	no mortality		
			Submerged	no mortality		
27.4.81	XIf	Hyvis 200	Floating	10	40%	17
			Submerged	17	68%	17
		Rat Varnish	Floating	22	88%	17
			Submerged	16	64%	17
		Control	Floating	no mortality		
			Submerged	no mortality		

Field experiments (XII-XVII)

Results of field experiments conducted in a pond at Ness Woods in 1980 (Expt. XII) (Table 21) showed that from a total of 13 trials Hyvis 200 in the floating position caught only 9 1st-instar larvae of Ae. cantans, and in the submerged positions again only 9 1st-instar larvae, and 2 4th-instar larvae. Hyvis 2000 in a floating or submerged position did not trap any larvae. Rat Varnish in a floating position trapped only 1 1st- and 1 3rd-instar larvae of Ae. cantans, and in the submerged positions, 3 1st- and 3 4th-instar larvae.

From 13 trials in a ditch at Ness Woods in 1980 (Table 21) Hyvis 200 sticky traps in a floating position caught only 2 1st- instar Ae. cantans, and in a submerged position 1 2nd- and 6 4th-instar larvae. No larvae were trapped by Hyvis 2000. Rat Varnish sticky traps in a floating position did not trap any larvae and in the submerged positions only 1 3rd- and 6 4th-instar larvae. These disappointing results showed that for some reason the sticky traps were not working well in the field despite their promising potential shown by laboratory trials.

When Hyvis 200 was tested against 1st-instar Ae. cantans larvae in tap water in plastic trays placed within Ness Woods (Expt. XIII) , only up to 12% of the 1st-instar larvae were trapped. Repeating these field trials with Rat Varnish showed that up to 36% of 1st-instar larvae of Ae. cantans were trapped (Table 22). In a similar experiment conducted in pond water Hyvis 200 caught up to only 4%, and Rat Varnish up to only 8% of 1st-instar Ae. cantans (Table 23). These results showed that besides the nature of the pond water causing glues to be less tacky, the low temperature in the field (2-14 °C seasonal temperature) also seemed to cause a reduction in catching efficiency, even in tap water. But in laboratory trials with Hyvis 200 and Rat Varnish (Expts. VIII & IX) considerably higher catches were

Table 21: Results of field experiments using Hyvis 200, Hyvis 2000, and Rat Varnish for trapping Ae. cantans immature stages in a pond and a ditch at Ness Woods in 1980

Dates of trapping	Expt No.	Location	Glues	Method of trapping	Total larvae trapped
13 February 1980 to 1 May 1980	XII (13 trials)	pond	Hyvis 200	Floating	9 1st-instar Ae. cantans
				Submerged	9 1st-instar Ae. cantans 2, 4th-instar Ae. cantans
			Hyvis 2000	Floating	0
				Submerged	0
			Rat Varnish	Floating	1 1st-instar Ae. cantans
					1, 3rd-instar Ae. cantans
				Submerged	3, 1st -instar Ae. cantans
					3, 4th-instar Ae. cantans
13 February 1980 to 1 May 1980	XII (13 trials)	ditch	Hyvis 200	Floating	2, 1st-instar Ae. cantans
				Submerged	1, 2nd-instar Ae. cantans
					6, 4th-instar Ae. cantans
			Hyvis 2000	Floating	0
				Submerged	0
			Rat Varnish	Floating	0
				Submerged	1, 3rd-instar Ae. cantans
					6, 4th-instar Ae. cantans

Table 22: Results of field experiments using Hyvis 200 and Rat Varnish for trapping 1st-instars Ae. cantans in plastic trays containing tap water at Ness Woods in 1981. 25 larvae were used in each experiment with each glue.

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
27.2.81	XIIIa	Hyvis 200	Floating	0	0%	2
			Submerged	0	0%	2
		Rat Varnish	Floating	0	0%	2
			Submerged	0	0%	2
		Control	Floating	no mortality		
			Submerged	no mortality		
5.3.81	XIIIb	Hyvis 200	Floating	0	0%	3
			Submerged	0	0%	3
		Rat Varnish	Floating	0	0%	3
			Submerged	1	4%	3
		Control	Floating	no mortality		
			Submerged	no mortality		
6.3.81	XIIIc	Hyvis 200	Floating	0	0%	8
			Submerged	0	0%	8
		Rat Varnish	Floating	0	0%	8
			Submerged	0	0%	8
		Control	Floating	no mortality		
			Submerged	no mortality		
12.3.81	XIIId	Hyvis 200	Floating	3	12%	14
			Submerged	1	4%	14
		Rat Varnish	Floating	3	12%	14
			Submerged	9	36%	14
		Control	Floating	no mortality		
			Submerged	no mortality		
18.3.81	XIIIe	Hyvis 200	Floating	0	0%	8
			Submerged	1	4%	8
		Rat Varnish	Floating	0	0%	8
			Submerged	2	8%	8
		Control	Floating	no mortality		
			Submerged	no mortality		
24.3.81	XIIIf	Hyvis 200	Floating	0	0%	11
			Submerged	1	4%	11
		Rat Varnish	Floating	0	0%	11
			Submerged	2	8%	11
		Control	Floating	no mortality		
			Submerged	no mortality		

Table 23: Results of field experiments using Hyvis 200 and Rat Varnish for trapping 1st-instars Ae. cantans in plastic trays containing pond water at Ness Woods in 1981. 25 larvae were used in each experiment with each glue

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
25.2.81	XIVa	Hyvis 200	Floating	0	0%	2
			Submerged	0	0%	2
		Rat Varnish	Floating	0	0%	2
			Submerged	0	0%	2
		Control	Floating	no mortality		
			Submerged	no mortality		
26.2.81	XIVb	Hyvis 200	Floating	0	0%	2
			Submerged	0	0%	2
		Rat Varnish	Floating	0	0%	2
			Submerged	0	0%	2
		Control	Floating	no mortality		
			Submerged	no mortality		
3.3.81	XIVc	Hyvis 200	Floating	0	0%	2
			Submerged	0	0%	2
		Rat Varnish	Floating	0	0%	2
			Submerged	0	0%	2
		Control	Floating	no mortality		
			Submerged	no mortality		
4.3.81	XIVd	Hyvis 200	Floating	0	0%	3
			Submerged	0	0%	3
		Rat Varnish	Floating	0	0%	3
			Submerged	0	0%	3
		Control	Floating	no mortality		
			Submerged	no mortality		
10.3.81	XIVe	Hyvis 200	Floating	0	0%	12
			Submerged	0	0%	12
		Rat Varnish	Floating	0	0%	12
			Submerged	0	0%	12
		Control	Floating	no mortality		
			Submerged	no mortality		
11.3.81	XIVf	Hyvis 200	Floating	0	0%	12
			Submerged	0	0%	12
		Rat Varnish	Floating	0	0%	12
			Submerged	0	0%	12
		Control	Floating	no mortality		
			Submerged	no mortality		
17.3.81	XIVg	Hyvis 200	Floating	0	0%	12
			Submerged	1	4%	12
		Rat Varnish	Floating	0	0%	12
			Submerged	2	8%	12
		Control	Floating	no mortality		
			Submerged	no mortality		

obtained in both tap and pond water (Tables 17 & 18). The reason for this could be due to the higher temperature in the laboratory (17-21° C). In the field, when Hyvis 200 was tested with a mixed population of 3rd and 4th-instar larvae of Ae. cantans in pond water in plastic trays (Expt. XV) (Table 24), up to only 8% of these larvae were trapped by Hyvis 200 in a floating position, and up to 24% in a submerged position. Using Rat Varnish up to 32% were caught on floating sticky traps while with submerged traps up to 48% of the Ae. cantans larvae were caught. In tap water in the field, Hyvis 200 in a floating position caught up to 96% and in a submerged position up to 84% of the late instars Ae. cantans. Rat Varnish in a floating position trapped up to 88% of the 3rd and 4th-instar larvae, and up to 96% when submerged (Expt. XVI) (Table 25). The water temperature during this period was between 10-16°C. Rat Varnish caught significantly more larvae of 3rd and 4th-instar Ae. cantans than Hyvis 200 in pond water ($P < 0.05$) and tap water ($P < 0.025$). A higher percentage of 3rd and 4th-instar larvae were caught by both glues when the experiment was conducted in tap water than in pond water. Even for temperatures between 10-16°C encountered in the field a high percentage of late immature stages of Ae. cantans were caught by both glues.

Finally, the sticky traps were placed in the pond in 1981 (Expt. XVII), and from a total of 27 trap catches, Hyvis 200 in a floating position caught just a single 4th-instar Ae. cantans, and in a submerged position, 10 4th-instar Ae. cantans. Rat Varnish in a floating position caught 3, 4th-instar Ae. cantans and in a submerged position 3, 1st-, 2 2nd-, 13 3rd- and 129 4th-instar Ae. cantans (Table 26). There were also 27 trap catches in the ditch at Ness Woods (Expt. XVII). In a floating position Hyvis 200 did not trap any mosquito larvae and in a submerged position only a single 4th-instar larva of Ae. cantans. Using Rat Varnish in a

Table 24: Results of field experiments using Hyvis 200 and Rat Varnish for trapping 3rd and 4th-instars *Ae. cantans* in plastic trays containing pond water at Ness Woods in 1981. 25 larvae were used in each experiment with each glue

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
7.4.81	XVa	Hyvis 200	Floating	0	0%	12
			Submerged	0	0%	12
		Rat Varnish	Floating	2	8%	12
			Submerged	10	40%	12
		Control	Floating	no mortality		
			Submerged	no mortality		
9.4.81	XVb	Hyvis 200	Floating	2	8%	15
			Submerged	2	8%	15
		Rat Varnish	Floating	1	4%	15
			Submerged	1	4%	15
		Control	Floating	no mortality		
			Submerged	no mortality		
14.4.81	XVc	Hyvis 200	Floating	1	4%	15
			Submerged	6	24%	15
		Rat Varnish	Floating	8	32%	15
			Submerged	12	48%	15
		Control	Floating	no mortality		
			Submerged	no mortality		
15.4.81	XVd	Hyvis 200	Floating	1	4%	13
			Submerged	2	8%	13
		Rat Varnish	Floating	2	8%	13
			Submerged	5	20%	13
		Control	Floating	no mortality		
			Submerged	no mortality		
23.4.81	XVe	Hyvis 200	Floating	0	0%	7
			Submerged	3	12%	7
		Rat Varnish	Floating	1	4%	7
			Submerged	5	20%	7
		Control	Floating	no mortality		
			Submerged	no mortality		
30.4.81	XVf	Hyvis 200	Floating	0	0%	10
			Submerged	2	8%	10
		Rat Varnish	Floating	6	24%	10
			Submerged	5	20%	10
		Control	Floating	no mortality		
			Submerged	no mortality		

Table 25: Results of field experiments using Hyvis 200 and Rat Varnish for trapping 3rd and 4th-instars Ae. cantans in plastic trays containing tap water at Ness Woods in 1981. 25 larvae were used in each experiment with each glue

Date	Expt. No.	Glues	Method of trapping	No of larvae trapped	% of larvae trapped	Temp of water °C
8.4.81	XVIa	Hyvis 200	Floating	7	28%	11.5
			Submerged	6	24%	11.5
		Rat Varnish	Floating	9	36%	11.5
			Submerged	6	24%	11.5
		Control	Floating	no mortality		
			Submerged	no mortality		
16.4.81	XVIb	Hyvis 200	Floating	21	84%	16
			Submerged	19	76%	16
		Rat Varnish	Floating	22	88%	16
			Submerged	24	96%	16
		Control	Floating	no mortality		
			Submerged	no mortality		
21.4.81	XVIc	Hyvis 200	Floating	22	88%	15
			Submerged	21	84%	15
		Rat Varnish	Floating	20	80%	15
			Submerged	21	84%	15
		Control	Floating	no mortality		
			Submerged	no mortality		
22.4.81	XVId	Hyvis 200	Floating	10	40%	10
			Submerged	11	44%	10
		Rat Varnish	Floating	17	68%	10
			Submerged	17	68%	10
		Control	Floating	no mortality		
			Submerged	no mortality		
28.4.81	XVIe	Hyvis 200	Floating	24	96%	10
			Submerged	19	76%	10
		Rat Varnish	Floating	19	76%	10
			Submerged	23	92%	10
		Control	Floating	no mortality		
			Submerged	no mortality		
29.4.81	XVIf	Hyvis 200	Floating	7	28%	10
			Submerged	15	60%	10
		Rat Varnish	Floating	8	32%	10
			Submerged	17	68%	10
		Control	Floating	no mortality		
			Submerged	no mortality		

Table 26: Results of field experiments using Hyvis 200 and Rat Varnish for trapping *Ae. cantans* immature stages in a pond and a ditch at Ness Woods in 1981.

Dates of trapping	Expt.	Location	Glues	Method of trapping	Total larvae trapped
19February 1981 to 30 April 1981	XVII (27 trials)	Pond	Hyvis 200	Floating	1,4th-instar *Ae. cantans*
				Submerged	10,4th-instar *Ae. cantans*
			Rat Varnish	Floating	3, 4th-instar *Ae. cantans*
				Submerged	3, 1st-instar *Ae. cantans*
					2, 2nd-instar *Ae. cantans*
					13, 3rd-instar *Ae. cantans*
					129, 4th-instar *Ae. cantans*
19 February 1981 to 30 April 1981	XVII (27 trials)	Ditch	Hyvis 200	Floating	0
				Submerged	1, 4th-instar *Ae. cantans*
			Rat Varnish	Floating	1, 3rd-instar *Ae. cantans*:
					3, 4th-instar *Ae. cantans*
				Submerged	14, 4th-instar *Ae. cantans*

floating position 1 3rd - and 3 4th-instar Ae. cantans were caught while in a submerged position 14 4th-instar larvae were trapped (Table 26).

Results of Mr. B. Katabazi's experiments

In laboratory trials using aquaria Katabazi (1981) found that Rat Varnish was superior than Hyvis 2000; the former trapped up to 90% of the Ae. aegypti larvae present whereas Hyvis 200 trapped less than 30%. He found that Rat Varnish was best for trapping Ae. aegypti whether the sticky traps were floating or submerged. However, Rat Varnish was not very successful in trapping larvae of Anopheles stephensi Liston, only 4-6% of mixed instars were caught when the coated panels were placed at the surface, and 15-16% when they were on the bottom of the aquarium. When panels were coated with Rat Varnish and placed at the bottom of the aquarium filled with pond water and mixed instars of Ae. aegypti, Katabazi (1981) found that 78-80% of the available larvae were caught.

When, however , the trials were conducted outside the laboratory in the colder conditions prevailing at that time only up to 21% of the added larvae were caught. Here again is more evidence that low temperature reduces the catch.

CONCLUSION

Three glues namely, Hyvis 200, Hyvis 2000 and Rat Varnish were initially evaluated against Ae. aegypti larvae in the laboratory and against Ae. cantans larvae in both the laboratory and field. Hyvis 2000 performed poorly in trapping larvae of both species and was therefore not used in further experiments. With tap water in laboratory trials Hyvis 200 trapped as many as 80% of the available 1st-instar larvae of Ae. cantans, while the Rat Varnish trapped up to 92%.

But, when pond water was substituted both Hyvis 200 and Rat Varnish trapped up to only 24% of the larvae. So, it seems that pond water, possibly due to suspension of fine particles on its sticky surfaces reduced the tackiness of the glues. When Hyvis 200 was evaluated in the field with 1st-instar larvae of Ae. cantans in tap water up to only 12% were caught, and under the same conditions the Rat Varnish trapped up to 36% of the added larvae. When this experiment was repeated with pond water, Hyvis 200 trapped up to only 4% and Rat Varnish up to 8% of the larvae. Consequently, it seems that the combination of water turbidity and low temperatures encountered in the field (ranging between 2-14 °C) caused a great reduction in size of the catches. Finally, when both glues were tested on numerous occasions, in a ditch and pond of Ness Woods few larvae of Ae. cantans were trapped.

When the glues were tested against a mixed population of 3rd- and 4th-instar Ae. cantans in the laboratory in tap water, Hyvis 200 managed to trap up to 96% and the Rat Varnish up to 100% of the larvae. Using pond water in the laboratory, Hyvis 200 caught up to 68% and Rat Varnish up to 88% of the larvae. In tap water in plastic trays placed in Ness Woods both Hyvis 200 and Rat Varnish caught as many as 96% of the larvae when temperatures were 10-16° C. Similar experiments using pond water showed that Hyvis 200 caught a maximum of only 24% , and Rat Varnish 48% of the added larvae. Hence, for late larval stages of Ae. cantans the pond water itself caused large reduction in the percentage of the larvae trapped by each glue. Low temperatures (10-16 °C) seemed to have no effect on the efficiency of the glues in trapping 3rd- and 4th-instar larvae in tap water, but adversely affected their efficiency when used in pond water. It seems that the small particles present in pond water settled on the sticky surfaces and then reduced

the tackiness of the glues. However, in the laboratory, it was high temperature (32° C) more than cold temperature (5-6.5° C) that seemed to drastically reduced the catch of Ae. aegypti larvae.

When the glues were tested with 4th-instar Ae. aegypti larvae in the laboratory at room temperature, Hyvis 200 caught up to 95% in tap water, and up to 60% in pond water while the Rat Varnish caught up to 97.5% in tap water and 82.5% in pond water. In the laboratory at temperature of 5-6.5 °C, Hyvis 200 caught as many as 80% of the larvae in tap water and 60% in pond water. Rat Varnish caught a maximum of 85% of the larvae in tap water and 60% in pond water. It seems that the turbidity of pond water is the main factor reducing the catch of larvae by both glues, and that low temperatures (5 - 6.5°C) cause a smaller reduction in numbers caught.

Comparing the two glues, Rat Varnish seemed to be the better one and it is worth trying in the tropics.

These long series of comparative trial in the laboratory and field have not unequivocally answered all the question as to why the glues do not work very well in the field, although water turbidity and to a less extent low water temperature, are adverse factors.

Nevertheless, these experiments demonstrate clearly that the most promising adhesive tested is the Rat Varnish. This adhesive is consequently worth testing in the field in different types of water, and in places where field temperatures are higher such as in the tropics. For example, submerged sticky traps could be placed at the bottom of water containers used in villages and towns to collect and store domestic water to evaluate their effectiveness in trapping Ae. aegypti and other larvae.

POPULATION DYNAMICS OF THE IMMATURE STAGES OF MOSQUITOES

INTRODUCTION

Life-table analysis has recently been used in mosquito ecology to study the population dynamics of the immature stages of mosquitoes to obtain mortality estimates of the pre-adults (Service, 1971a, 1973a,b, 1977a,b; Lakhani and Service, 1974; Southwood, 1972; Onyeka, 1980). The methods used depended on estimating the durations of the various larval instars and pupae, experimentally, or by direct observations, and an accurate assessment of the proportions of the various age-classes. The survivorship picture was obtained using a graphical approach based on the construction of the stage-specific age-distributions of the pre-adults. A smooth curve was then drawn through the age-distribution histograms to produce an approximation to the survivorship curve. From this curve appropriate data, such as numbers entering each age-class could be obtained and thus mortalities could be computed to give a life-table.

The idea of using the precipitin test as a serological tool to determine predators of mosquito larvae was first tested by Brooke and Proske (1946). This work revealed that this technique could be utilised satisfactory but their observations were not followed up. Hall et al.(1953) demonstrated the effectiveness of the precipitin test to study a reduviid predator (Zelus exsanguis (Stahl)) of the forest tent caterpiller (Malacosoma disstria Hbn.), and several other workers used the test to detect predators on various agricultural insects. It was Service (1973a,c, 1977a,b) who used the technique to study extensively the predators of Ae. cantans and the Anopheles gambiae complex (Giles) and predators of the

Simulium damnosum complex (Service and Lyle 1975; Service and Elouard, 1980). Service (1973c, 1977a) identified the predators of Ae. cantans using the precipitin test based on the reaction of Ae. cantans proteins in the gut of a predator with the blood serum of a rabbit which had been immunized with cell-free extracts of the prey (i.e. Ae. cantans). According to Service (1973c) an advantage of serological techniques in predator-prey studies is that the intensity of predation occuring in the field under natural conditions is measured. Such a critical and objective assessment is difficult to obtain from either direct observations of predation in the field, or from laboratory experiments. Another advantage is that, once gut smears have been made, precipitin tests need not be performed until many months later. However, the precipitin test is not without limitations. It is not always possible to produce specific antisera, although this is not a serious disadvantage if the species with which they cross react do not coexist to any great extent with the species under study.

The enzyme linked immunosorbent assay "ELISA" developed in recent years represent a significant addition to existing serological tools. Encouraging preliminary results obtained through its application to a number of parasitic diseases such as malaria, trichinosis, schistosomiasis, trypanosomiasis during the last two years indicate the need for further investigations and trials so as to get a more complete evaluation of this technique (WHO, 1976). Theakston et al. (1977) successfully used ELISA for detecting and assaying snake venom and venom antibodies. Voller and Bidwell (1976) using an indirect enzyme immunoassay method and alkaline phosphatase conjugated antiglobulins showed satisfactory results for detection of antibody to measles and cytomegalovirus. Voller et al.(1976a) found that the "double antibody sandwich method" could be used for detecting plant viruses.

However, the ELISA test also showed cross reactions in some cases, as found by McLaren et al.(1978) when using ELISA for testing Schistosoma mansoni infections in Sudan. They found that many cross reactions occured in infections with other human (and animal) schistosomes, although to a lesser extent with other helminths. Rotmans and Mooij (1980) in studying separation and comparative immunoassays with antigen from adult Schistosoma mansoni also found cross reactions occured with other helminth infections, mainly with high molecular weight antigens.

The objective of this study was firstly to measure the mortalities of the different immature stages of Ae. cantans at Ness Woods, and secondly to find the factors causing the observed mortalities and thus responsible for regulating the population of Ae. cantans. The precipitin test was used to detect predators of Ae. cantans immature stages and the possibility of using ELISA test as a new serological tool for detecting predators was investigated.

MATERIALS AND METHODS

Determination of instar durations

In late November 1979 and late November 1980, 20 egg batches of Ae. cantans were each immersed in 200ml of pond water in a plastic cup and placed in teachests in the School's courtyard. Numbers of eggs hatching and larvae moulting to successive instars were recorded over the following seven months. When the eggs hatched, the first instar larvae were transferred to plastic cups, each containing 200ml of pond water and crushed leaf litters as nutrient. A maximum of 10 larvae were placed in each plastic cup. Whenever the larvae moulted to the successive instars they were recorded and transferred to different plastic cups with pond water and crushed leaf litters. When the fourth instar larvae had pupated , the plastic cups containing pupae were covered with nylon netting

for adult emergence. Pond water as well as crushed leaf litters were renewed every two weeks to ensure that as far as possible larvae were in water chemically or biologically almost similar to that in the larval habitats.

The development time of each instar for a particular month is the time taken in days for 50% to change into the next instar. In order to get the weighted average instar durations used in mortality estimates as used by Onyeka (1980) were calculated as the sum over the breeding season thus:-

$$\frac{\text{No. of larvae in instar } i \text{ sampled per month}}{\text{Total no. of larvae in instar } i \text{ sampled in the season}} \times \frac{\text{development time of}}{\text{instar } i \text{ per month}}$$

Sampling a pond with a quadrat

The population dynamics of the immature stages of Ae. cantans was studied in a small pond of area about 28 square metres (Fig 16). This pond was part of a larger pond but was blocked off by a bank of earth in order to prevent mosquito immature stages and predators from leaving and entering during the breeding season. Ae. cantans formed 100% of the population in 1980 and 99.7% in 1981. In early December, 1979 and 1980 a D-shape aquatic net was used to sample mosquito larvae once or twice a week to make sure that the beginning of hatching of Ae. cantans was not missed. An aquatic net sampled a larger volume of water than other methods and was therefore appropriate for detecting the beginning of egg hatching. When the first hatching was observed, larval sampling was done using a metal quadrat which in fact was a metal waste paper basket with the bottom removed (internal diameter 28cm at the top, 23cm at the bottom end and length of 28cm). Along the perimeter of the pond, 5-10 samples were taken once a week, each at a distance of 1 metre from each other and chosen at random by tossing a coin. The metal

Fig. 16: A small pond in which the population dynamics of Ae. cantans immature stages was studied.

quadrat was pushed into the ground and larvae or pupae were removed by means of a white plastic tray and the numbers of each larval instar and pupae counted and placed in another white plastic tray. To ensure as far as possible that all larvae and pupae were removed from the quadrat, dipping with the white plastic tray stopped only after the absence of larvae or pupae for five continuous dips. After counting, all larvae and pupae were then returned to the respective quadrat.

In 1981 besides recording the number of Ae. cantans immature stages in each quadrat, the number of different species of predators were also recorded to estimate the population of predators.

Sampling a pond with permanent quadrats

In early February 1980 when first instar larvae of Ae. cantans were seen in the field, 5 metal quadrats of the type described above were pushed into the ground along a perimeter of a large pond known to have large number of Ae. cantans larvae (Fig. 17). The metal quadrats were left permanently in position until the following year. Once a week, larvae and pupae were removed by means of a white plastic tray and the numbers of larvae in each instar and pupae counted, afterwhich they were returned as described in the previous experiment. But in late February 1980, due to the increase in water level of the pond, it was feared that the top of the existing quadrat would become submerged in the water. To prevent this a metal extension was fitted into the top of each of the 5 quadrats. A plastic sponge foam lining around the top ensured a water-tight part in case the water level rise (Fig.18).

From April 1980 onwards, the 5 permanent quadrats were covered with nylon netting for adult emergence and numbers of adults that emerged were recorded. In addition, the netting also prevented new ovipositions in the quadrat during the summer and early autumn of 1980. In the following year the number of immature

Fig. 17: A permanent quadrat in a pond.

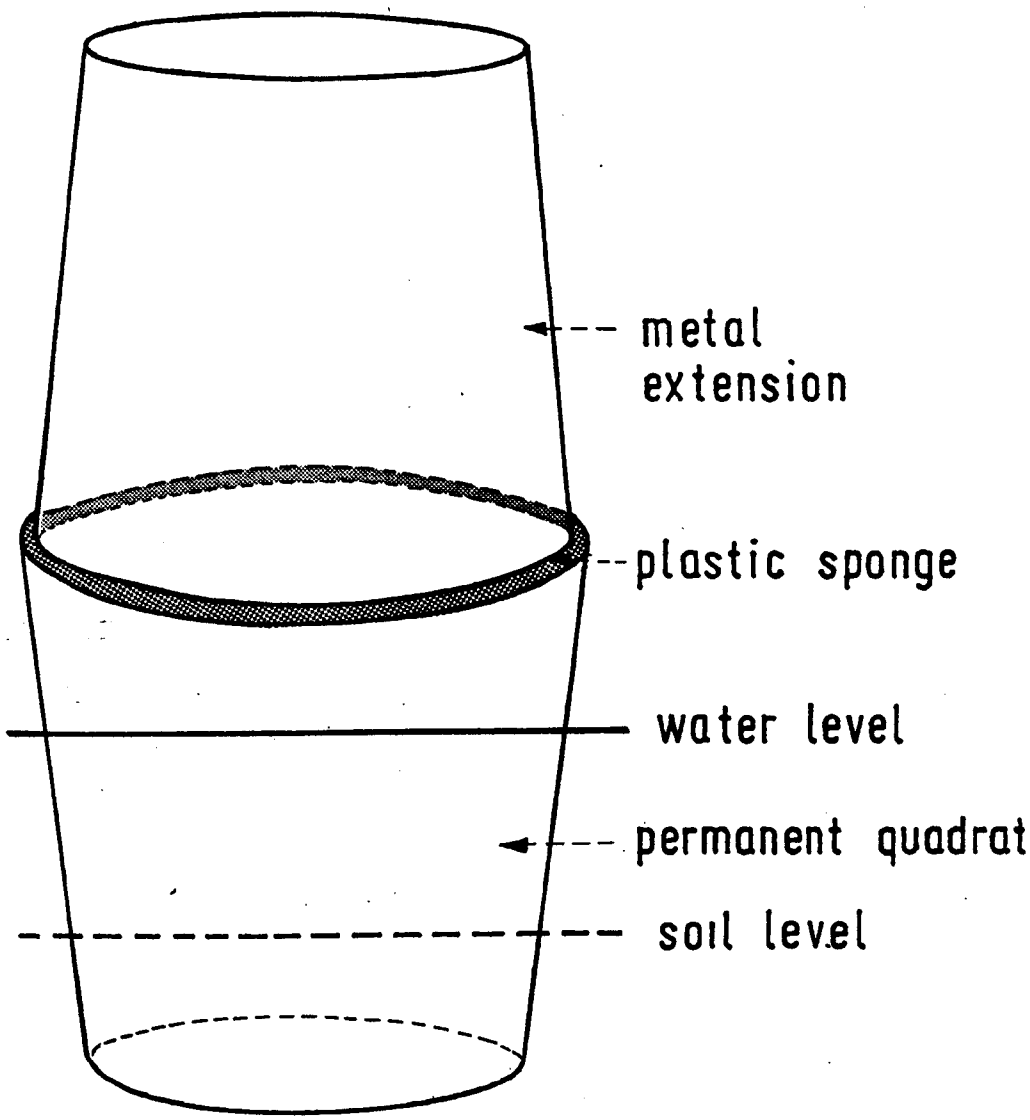

Fig. 18: Diagram of a metal extension fitted on top of a permanent quadrat

stages and adults were counted as before in order to find out whether there were any eggs that had not hatched in 1980 but had hatched in 1981.

Sampling a pond with cylindrical permanent quadrats

In December, 1980, 5 cylindrical aluminium quadrats (internal diameter 32cm and height 51 cm) were pushed into the ground along the perimeter of the same pond as in the previous experiment (Fig. 19). As before, the number of larvae in each instar and pupae were recorded once a week and returned to the respective quadrat after counting. When pupae were seen, each cylindrical permanent quadrat was covered with nylon netting and adults that emerged each week were removed and recorded.

Estimation of instar mortalities of Ae. cantans

Model and procedures

The procedures of estimating instar mortalities of Ae. cantans were done according to Service (1973a, 1977a,b; Lakhani and Service, 1974), but with the modification of Onyeka (1980) of using weighted average instar durations instead of instar duration for the season. Basically the approach was to use the total numbers of each instar collected each year divided by the appropriate weighted average instar duration in days (Tables 27 and 28) to give the age distribution of the pre-adult stages of mosquitoes. These values were plotted against age in days of the immature stages and the resultant histogram produced a graph of stage-specific-age distribution. A smooth curve drawn through the graph gives the age-specific- age distribution. This graph mimics the survivorship curve assuming that the population was in a steady state during the sampling period. The numbers of the immature stages of mosquitoes surviving to each age in days were read off from the curve to give the numbers of larvae surviving to age x (n_x column of Table 29).

Fig. 19: A cylindrical aluminium quadrat showing mosquito netting fitted on top to prevent emerging adults from escaping and oviposition within the enclosed area.

Table 27: Results of weighted average instar duration of Ae. cantans for the permanent quadrats in 1980 at Ness Woods

Quadrat	Instar	Weighted average instar duration in days
1	I	14.9
	II	15.3
	III	18.2
	IV	12.5
	Pupae	8.1
2	I	14.5
	II	14.9
	III	15.6
	IV	9.2
	Pupae	7.2
3	I	14.5
	II	13.6
	III	19.3
	IV	10.9
	Pupae	7.9
4	I	14.9
	II	15.6
	III	21.0
	IV	11.9
	Pupae	7.3
5	I	14.8
	II	14.4
	III	14.1
	IV	9.5
	Pupae	7.5

Table 28: Results of weighted average instar duration of Ae. cantans for pond 1 in 1980 and 1981 at Ness Woods

Year	Instar	Weighted average instar duration in days
1980	I	14.9
	II	15.7
	III	21.1
	IV	13.1
	Pupae	10.2
1981	I	8.5
	II	11.7
	III	13.5
	IV	18.6
	Pupae	10.2

Life-tables for pre-adult mosquitoes were then constructed starting with a convenient 1000 individuals (l_x column, Table 29). From estimates of the probability of a larva of age x dying before reaching age $x + 1$ gives the d_x column (Table 29), and P_x is the probability that a larva of age x survives to age $x + 1$, and finally e_x is the expectation of life for larvae of age x.

A simpler method than the construction of a life-table is the calculation of instar mortalities. For this it must be assumed that the relative mortality rate during each instar is constant. The steps used in calculating the relative proportions dying daily in each instar are outlined in Table 30.

Larval mortalities in pond 1

In 1980 first instar larvae were first seen on 6th February and the last pupae were seen on 21st May, thus the period covered by the immature stages was 106 days. With a mean of 5 samples (quadrats) per week, 6 823 1st-, 6 651 2nd-, 7 938 3rd-, 1 023.5 4th-instar and 335.5 pupae were recorded (Appendix 5). Dividing the total number of each instar by the appropriate weighted average instar durations (Table 28) gives the age structure of the immature population and allows plotting of the survivorship curve (Fig 20.). After the survivorship curve has been plotted for Ae. cantans, a time-specific life table can be constructed (Table 29). The two most informative statistics are given in the last 2 columns, namely q_x which is the probability of a larva of age x dying before reaching age $x + 1$ and e_x which is the mean expectation of further life of these larvae that have already attained age x. Table 30 is a more simplified approach of analysis of the survivorship curve and shows the relative proportions dying in each instar, and clearly shows that most mortality occured amongst the 4th-instar larvae and pupae, there being little mortality in the earlier larval instars.

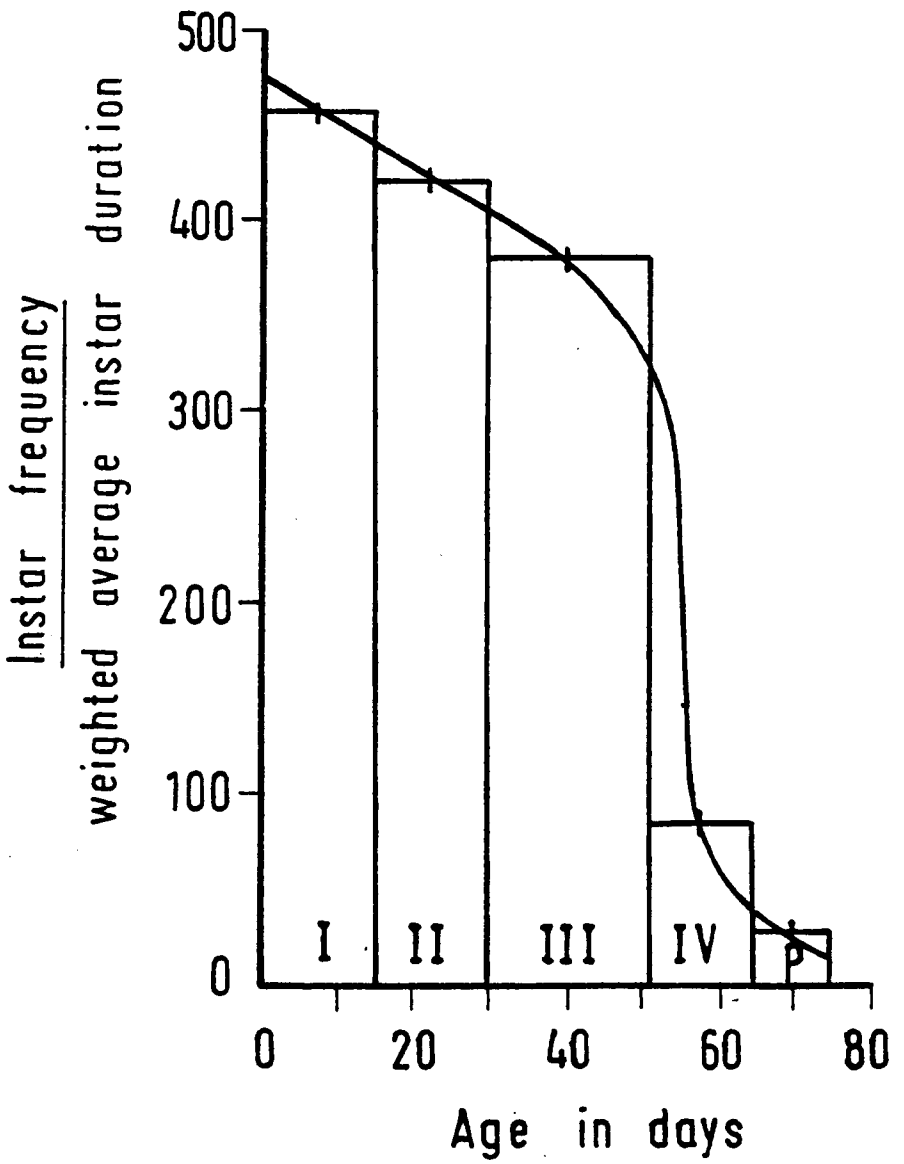

Fig. 20: Age distribution and survivorship curve of the immature stages of Ae. cantans in 1980 at pond 1, Ness Woods.

Table 29: Life-table for Ae. cantans pre-adults at pond 1, Ness Woods in 1980

x	n_x	l_x	d_x	p_x	q_x	e_x
0	474	1000	4	0.996	0.004	49.063
1	472	996	9	0.991	0.009	48.258
2	468	987	4	0.996	0.004	47.694
3	466	983	4	0.996	0.004	46.886
4	464	979	4	0.996	0.004	46.075
5	462	975	5	0995	0.005	45.262
6	460	970	4	0.996	0.004	44.493
7	458	966	4	0.996	0004	43.675
8	456	962	6	0.994	0.006	42.854
9	453	956	5	0.995	0.005	42.120
10	451	951	4	0.996	0.004	41.339
11	449	947	6	0.994	0.006	40.512
12	446	941	4	0.996	0004	39.767
13	444	937	5	0.995	0.005	38.934
14	442	932	4	0.996	0.004	38.141
15	440	928	4	0.996	0.004	37.303
16	438	924	6	0.994	0006	36.462
17	435	918	4	0.996	0.004	35.697
18	433	914	5	0.995	0.005	34.851
19	431	909	6	0993	0.007	34.040
20	428	903	4	0.996	0.004	33.263
21	426	899	4	0.996	0.004	32.409
22	424	895	7	0.992	0.008	31.551
23	421	888	4	0.995	0.005	30.796
24	419	884	4	0.995	0.005	29.933
25	417	880	7	0992	0.008	29.067
26	414	873	6	0.993	0.007	28.296
27	411	867	2	0.998	0.002	27.488
28	410	865	4	0.995	0.005	26.551
29	408	861	4	0.995	0.005	25.672
30	406	857	5	0.994	0.006	24.789
31	404	852	8	0.991	0.009	23.932
32	400	844	4	0.995	0.005	23.154
33	398	840	5	0.994	0.006	22.262
34	396	835	6	0.993	0.007	21.392
35	393	829	6	0.993	0.007	20.543
36	390	823	7	0.991	0.009	19.689
37	387	816	6	0.993	0.007	18.854
38	384	810	6	0.993	0007	17.990
39	381	804	4	0995	0005	17.121
40	379	800	7	0991	0.009	16.204

Continued .....

Table 29 : (Continued)

x	n_x	l_x	d_x	p_x	q_x	e_x
41	376	793	10	0.987	0.013	15.342
42	371	783	9	0.989	0.011	14.532
43	367	774	12	0.984	0.016	13.695
44	361	762	11	0.986	0.014	12.903
45	356	751	13	0.983	0.017	12.085
46	350	738	14	0.981	0.019	11.289
47	343	724	15	0.979	0.021	10.496
48	336	709	17	0.976	0.024	9.707
49	328	692	21	0.970	0.030	8.934
50	318	671	21	0.969	0.031	8.197
51	308	650	26	0.960	0.040	7.446
52	296	624	33	0.947	0.053	6.736
53	280	591	45	0.924	0.076	6.084
54	259	546	82	0.850	0.150	5.544
55	220	464	84	0.819	0.181	5.435
56	180	380	110	0.711	0.289	5.526
57	128	270	101	0.626	0.374	6.574
58	80	169	23	0.864	0.136	9.204
59	69	146	13	0.911	0.089	9.575
60	63	133	13	0.902	0.098	9.462
61	57	120	6	0.950	0.050	9.433
62	54	114	9	0.921	0.079	8.904
63	50	105	6	0943	0.057	8.624
64	47	99	6	0939	0061	8.116
65	44	93	4	0.957	0.043	7.608
66	42	89	5	0944	0.056	6.927
67	40	84	6	0.929	0.071	6.310
68	37	78	4	0.949	0.051	5.756
69	35	74	6	0.919	0.081	5.041
70	32	68	5	0.926	0.074	4.441
71	30	63	4	0.937	0.063	3.754
72	28	59	6	0.898	0.102	2.975
73	25	53	4	0.925	0.075	2.255
74	23	49	5	0.898	0.102	1.398
74	21	44				0.500

Table 30: Instar mortalities of *Ae. cantans* at pond 1 in 1980 at Ness Woods

Instars	Age in days at beginning of instar ($t_i - 1$)	No entering instar ($S_{ti} - 1$)	Deaths in instar (D_i)	Relative proportion dying in instar $\left(\frac{D_i}{S_{ti} - 1}\right)$	Population dying daily in instars $1-\left(\frac{S_{ti}}{S_{ti} - 1}\right)^{1/d^*}$
I	0	474	34	0.0717	0.0050
II	14.9	440	36	0.0818	0.0054
III	30.6	404	102	0.2525	0.0137
IV	51.7	302	258	0.8543	0.1367
Pupa	64.8	44	23	0.5227	0.0699
Adult	75.0	21			

d^* = weighted average instar duration

In 1981, first instar larvae were first found on the 10th February and the last pupae were seen on 3rd June, the period covered being 114 days. With a mean of 5 samples (quadrats) per week, 1 188 1st-, 939 2nd-, 547 3rd-, 603 4th-instar and 240 pupae were recorded (Appendix 6). A survivorship curve was plotted (Fig. 21) and life table. constructed (Table 31). Table 32, a simplified approach of analysis of survivorship curve shows that relative proportion dying in instar and pupulation dying daily in instars were highest for the 2nd-instar larvae, followed by 1st-instar larvae, the pupae, 3rd- and 4th- instar in that order. The marked difference in the mortality pattern for the two years was possibly due to the fact that in 1980 very little rainfall from late March to mid May (Table 1). The highest daily rainfall during that period was only 0.5mm. In addition, April 1980 was the driest month recorded at Ness Gardens since 1969, and also the sunniest April ever recorded, temperatures were above average. Consequently the water level of the pond receded due to the combined effects of the above factors. The water tended to become deoxygenated and smelt sulphurous and this may have caused the large mortalities especially of the late instar larvae. On the contrary in 1981 more rainfall occured in spring compared to the previous year (Table 1) and mortality occured predominantly among the first two instars, followed by pupae; there was relatively little mortality of the 3rd and 4th-instar larvae. Table 33 shows that in pond 1, for the year 1980 there was 95.1% pre-adult (1st-instar $\widehat{\text{pupae}}$) mortality whereas in 1981, this mortality was only 79.7%. However, the population of immature stages was greater in 1980 than 1981. (Appendices 5 and 6).

Table 34 shows that the total number of Ae. cantans immature stages and predators collected between 10th February 1981 to 3rd June, 1981 at pond 1 was 197.6 : 1. Therefore the predators were greatly outnumbered by the Ae. cantans

immature stages. However, this may not be a very accurate figure as there was probably some sampling bias in collecting the mosquitoes and predators, but nevertheless convincingly shows that there was a much smaller population of predators than Ae. cantans.

Larval mortalities in permanent quadrats 1-5

Due to the difficulty and sampling bias that often occured in sampling mosquito populations in large habitats such as ponds, ricefields and swamps, the purpose of the present experiment is to concentrate sampling in limited areas within permanent quadrats to study the population dynamics of Ae. cantans.

By using the same procedure applied to pond 1, realistic age distribution and survivorship curves were obtained for permanent quadrats 1 and 5 (Figs. 22and 26) and life-tables constructed (Tables 35 and 37) (but difficulties arose in plotting the survivorship curves and constructing life-tables for permanent quadrats numbers 2,3 and 4). In 1980, for permanent quadrat 1, a total of 617 1st-, 324 2nd-, 35 3rd-, 23 4th-instar larvae, 15 pupae and 8 adults Ae. cantans were recorded (Appendix 7). Figure 22 shows that mortality predominantly occured amongst the 1st, 2nd and 3rd-instar larvae and far less mortality amongst 4th-instar and pupae. Similarly, the estimated mortalities (Table 36) show that the relative proportion dying in the instars and the proportion dying daily in the instars were highest in 3rd instar followed by 2nd-, 1st-, 4th- instar and pupae. So, mortality occured largely among the early immature stages as was recorded in pond 1 in 1981. However, in 1980, mortality of Ae. cantans immature stages in pond 1 was predominant among the late instars. The mortality of 1st instar to adult emergence was 98.7% and mortality of pre-adults (1st-instar to pupae) was 97.6% (Table 39). This result was almost similar to that of pond 1 . As already indicated results from permanent quadrat numbers 2,3 and 4, as shown in Figures 23-25 are clearly non-sensible. The difficulty

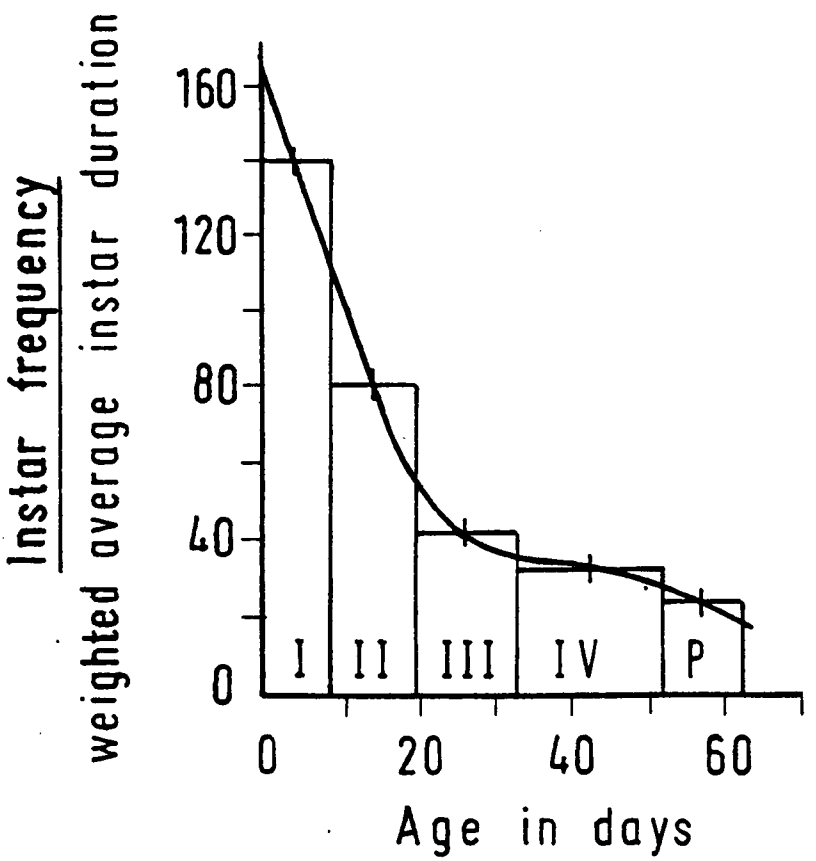

Fig. 21: Age distribution and survivorship curve of the immature stages of Ae. cantans in 1981 at pond 1, Ness Woods.

Table 31: Life-table for Ae. cantans pre-adults at pond 1, Ness Woods in 1981

x	n_x	l_x	d_x	p_x	q_x	e_x
0	164	1000	30	0970	0.030	21.635
1	159	970	37	0.962	0.038	21.289
2	153	933	37	0.960	0.040	21.113
3	147	896	36	0.960	0.040	20.964
4	141	860	36	0.960	0.040	20.821
5	135	823	37	0.964	0.036	20.735
6	130	793	30	0.953	0.047	20.500
7	124	756	37	0.952	0.048	20.479
8	118	720	36	0.949	0.041	20.478
9	112	683	37	0.991	0.009	20.560
10	111	677	6	0.901	0.099	19.738
11	100	610	67	0.910	0.090	20.851
12	91	555	55	0.968	0.032	21.868
13	88	537	18	0.931	0.069	21.584
14	82	500	37	0.926	0.074	22.144
15	76	463	37	0.948	0.052	22.874
16	72	439	24	0.945	0.055	23.097
17	68	415	24	0.925	0.075	23.404
18	63	384	31	0.969	0.031	24.253
19	61	372	12	0.935	0.065	24.019
20	57	348	24	0.945	0.055	24.641
21	54	329	19	0.945	0.055	25.035
22	51	311	18	0.961	0.039	25.455
23	49	299	19	0.936	0.064	25.457
24	46	280	12	0.957	0.043	26.150
25	44	268	12	0.955	0.045	26.299
26	42	256	12	0.953	0.047	26.508
27	40	244	6	0.975	0.035	26.787
28	39	238	6	0.975	0.035	26.450
29	38	232	6	0.974	0.026	26.121
30	37	226	3	0.987	0.013	25.801
31	36.5	223	3	0.987	0.013	25.141
32	36	220	7	0.968	0.032	24.477
33	35	213	3	0.986	0.014	24.265
34	34.5	210	3	0.986	0.014	23.605
35	34	207	3	0.986	0.014	22.940
36	33.5	204	3	0.985	0.015	22.270
37	33	201	3	0.985	0.015	21.595
38	32.5	198	0	1.000	0.000	20.914
39	32.5	198	0	1.000	0.000	19.914
40	32.5	198	3	0.985	0.015	18.914

Continued .....

Table 31 (Continued)

x	n_x	l_x	d_x	p_x	q_x	e_x
41	32	195	0	1.000	0.000	18.197
42	32	195	0	1.000	0.000	17.197
43	32	195	3	0.985	0.015	16.197
44	31.5	192	0	1.000	0.000	15.443
45	31.5	192	3	0.984	0.016	14.443
46	31	189	3	0.984	0.016	13.664
47	30.5	186	3	0.984	0.016	12.876
48	30	183	3	0.984	0.016	12.079
49	29.5	180	3	0.983	0017	11.272
50	29	177	3	0.983	0.017	10.455
51	28.5	174	3	0.983	0.017	9.626
52	28	171	6	0.965	0.035	8.769
53	27	165	6	0.964	0.036	8.070
54	26	159	7	0.956	0044	7.355
55	25	152	3	0.980	0.020	6.671
56	24.5	149	6	0.960	0040	5.795
57	23.5	143	3	0.979	0.021	5.017
58	23	140	12	0.914	0.086	4.114
59	22	134	6	0.957	0.043	3.276
60	21	128	6	0.977	0.023	2.406
61	20.5	125	3	0.952	0.048	1.452
62	19.5	119				0.500

Table 32: Instar mortalities of Ae. cantans at pond 1 in 1981 at Ness Woods

Instars	Age in days at beginning of instar ($t_i - 1$)	No entering instar ($S_{ti} - 1$)	Deaths in instar (D_i)	Relative proportion dying in instar $\left(\frac{D_i}{S_{ti} - 1}\right)$	Population dying daily in instars $1 - \left(\frac{S_{ti}}{S_{ti} - 1}\right)^{1/d}$ *
I	0	164	49	0.2988	0.0409
II	8.5	115	58	0.5043	0.0582
III	20.2	57	22	0.3860	0.0355
IV	33.7	35	7	0.2000	0.0119
Pupa	52.3	28	9	0.3214	0.0373
Adult	62.5	19			

d* = weighted average instar duration

Table 33: The mortality of Ae. cantans pre- adult in pond 1 in 1980 and 1981 at Ness Woods

Year	Total no of first instars sampled	Total no of pupae sampled	Mortality of pre-adult (1st-instar - pupae)
1980	6823	335.5	95.1%
1981	1188	241.0	79.7%

Table 34: Total number of Ae. cantans immature stages and predators sampled between 10.2.81 to 3.6.81 at pond 1, Ness Woods

	Ae. cantans					Predators				
No of samples with a Quadrat	I	II	III	IV	Pupae	Dytiscidae larvae (unidentified)	Agabus bipustulatus adult	Colymbetes fuscus adult	Hydroporus palustris adult	Mochlonyx culiciformis larvae
155	1899	1062	630	1067	480	9	3	1	1	12

Total no of Ae. cantans immature stages = 5138

Total no of Predators = 26

Ratio of Ae. cantans immature stages : Predators = 197.6 : 1

is due to the presence in the samples of larger numbers of late instars than earlier ones. Clearly there cannot be more late instar larvae than earlier ones, so some type of sampling error has been introduced. Because of this problem the construction of life-tables, and estimation of instar mortalities are impossible for permanent quadrats 2,3 and 4. Nevertheless, Table 39 shows that for permanent quadrat 2, the mortality of 1st-instar to adult emergence was 91.4% and mortality of pre-adults (1st-instar to pupae) was 72.8%. For permanent quadrat 3, the mortality of 1st-instar to adult emergence was 88.2% and mortality of pre-adults (1st-instar to pupae) was 74.0%. For permanent quadrat 4, the mortality of 1st-instar to adult emergence was 96.5% and the mortality of pre-adults (1st-instar to pupae) was 84.5%. Hence, the overall mortality for Ae. cantans 1st-instar to adult emergence was in the range of 88.2% to 98.7% and the pre-adult mortality (1st-instar to pupae) was in the range of 72.8% to 97.6%. Results which are not dissimilar to those obtained from the pond and permanent quadrats 1 and 5.

During the summer and autumn of 1980, permanent quadrats 1-5 were still covered with nylon netting to prevent new oviposition and in January 1981 until June 1981, weekly sampling was done with the same procedure as before. The objective of this experiment was to determine the numbers of unhatched eggs in 1980 that hatched in 1981. In permanent quadrat 1, a total of 15 1st-, 6 2nd-, 6 3rd-, 8 4th-instar larvae, 6 pupae and 3 adults were recorded (Appendix 12). However, in permanent quadrats 2 and 3, no larvae or pupae were observed. In the permanent quadrat 4, a total of 10 1st-, 8 2nd-, 8 3rd-, 9 4th-instar, 6 pupae and 5 adults were recorded. In the permanent quadrat 5, a total of 12 1st-, 8 2nd-, 8 3rd-, 9 4th- instar, 7 pupae and 2 adults were recorded. Hence a few eggs that were unhatched in 1980 were hatched in 1981.

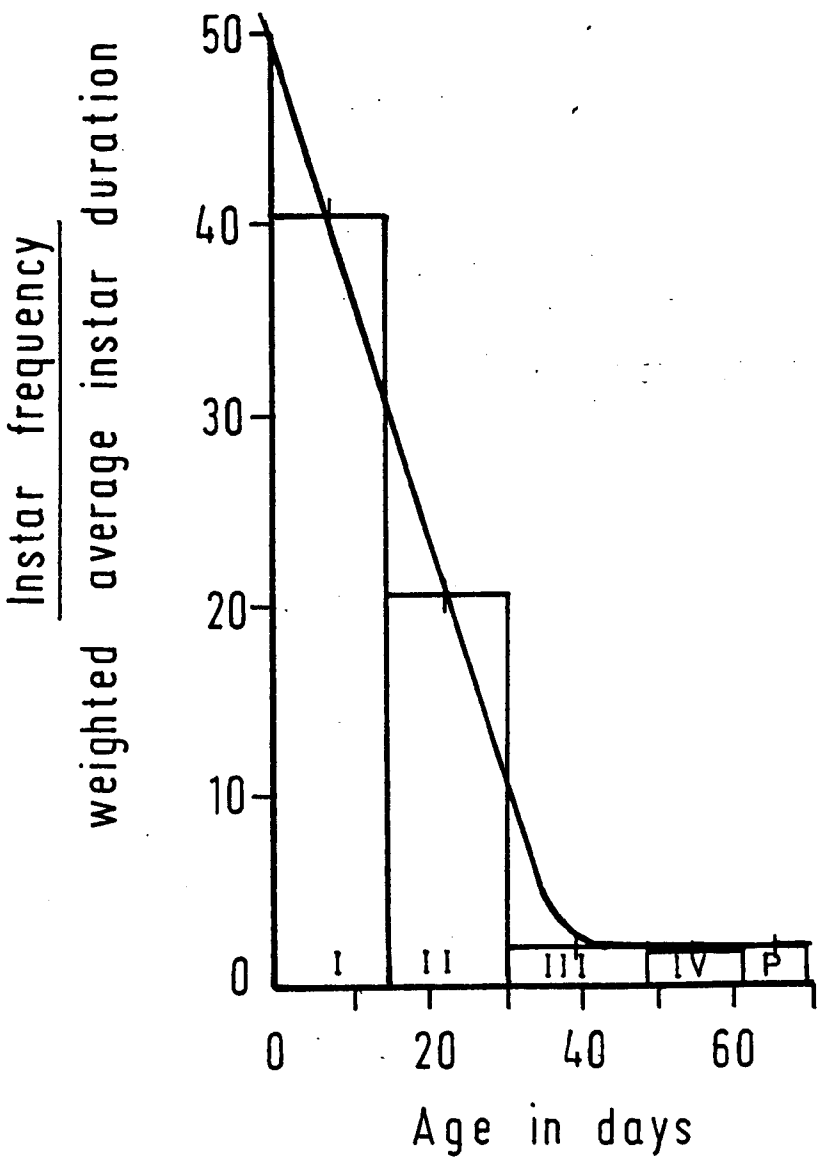

Fig. 22: Age distribution and survivorship curve of the immature stages of Ae. cantans in 1980 at permanent quadrat 1, Ness Woods.

Table 35: Life- table for Ae. cantans pre-adults at permanent quadrat 1, Ness Woods in 1980

x	n_x	l_x	d_x	p_x	q_x	e_x
0	50	1000	20	0980	0020	20.178
1	49	980	28	0.971	0.029	19.580
2	47.6	952	28	0971	0.029	19.141
3	46.2	924	24	0.974	0.026	18.706
4	45	900	28	0.969	0.031	18.191
5	43.6	872	28	0.968	0.032	17.760
6	42.2	844	28	0.967	0.033	17.332
7	40.8	816	24	0.971	0.029	16.909
8	39.6	792	28	0.965	0.035	16.407
9	38.2	764	24	0.969	0.031	15.990
10	37	740	28	0.962	0.038	15.492
11	35.6	712	28	0.961	0.039	15.081
12	34.2	684	24	0.965	0.035	14.678
13	33	660	28	0.958	0.042	14.194
14	31.6	632	24	0.962	0.038	13.801
15	30.4	608	28	0.954	0.046	13.326
16	29	580	28	0.952	0.048	12.945
17	27.6	552	28	0.949	0.051	12.576
18	26.2	524	28	0.947	0.053	12.221
19	24.8	496	24	0.952	0.048	11.883
20	23.6	472	24	0.949	0.051	11.462
21	22.4	448	28	0.938	0.062	11.049
22	21	420	24	0.943	0.057	10.752
23	19.8	396	28	0.929	0.071	10.374
24	18.4	368	24	0.935	0.065	10.125
25	17.2	344	24	0.930	0.070	9.797
26	16	320	28	0.913	0.087	9.494
27	14.6	292	24	0.918	0.082	8.538
28	13.4	268	28	0.896	0.104	9.149
29	12	240	28	0.883	0.117	9.158
30	10.6	212	20	0.906	0.094	9.302
31	9.6	192	28	0.854	0.146	9.219
32	8.2	164	24	0.854	0.146	9.707
33	7	140	24	0.829	0.171	10.286
34	5.8	116	28	0.759	0.241	11.310
35	4.4	88	16	0.818	0.182	13.750
36	3.6	72	16	0.778	0.222	15.694
37	2.8	56	8	0.857	0.143	19.036
38	2.4	48	12	0.750	0.250	21.125
39	1.8	36	0	1.000	0.000	27.000
40	1.8	36	0	1.000	0000	26.000

Continued .....

Table 35 : (Continued)

x	n_x	l_x	d_x	p_x	q_x	e_x
41	1.8	36	0	1.000	0.000	25.000
42	1.8	36	0	1.000	0.000	24.000
43	1.8	36	0	1.000	0.000	23.000
44	1.8	36	0	1.000	0.000	22.000
45	1.8	36	0	1.000	0.000	21.000
46	1.8	36	0	1.000	0.000	20.000
47	1.8	36	0	1.000	0.000	19.000
48	1.8	36	0.	1.000	0.000	18.000
49	1.8	36	0	1.000	0.000	17.000
50	1.8	36	0.	1.000	0.000	16.000
51	1.8	36	0	1.000	0.000	15.000
52	1.8	36	0	1.000	0.000	14.000
53	1.8	36	0	1.000	0.000	13.000
54	1.8	36	0	1.000	0.000	12.000
55	1.8	36	0	1.000	0.000	11.000
56	1.8	36	0	1.000	0.000	10.000
57	1.8	36	0	1.000	0.000	9.000
58	1.8	36	0	1.000	0.000	8.000
59	1.8	36	0	1.000	0.000	7.000
60	1.8	36	0	1.000	0.000	6.000
61	1.8	36	0	1.000	0.000	5.000
62	1.8	36	0	1.000	0.000	4.000
63	1.8	36	0	1.000	0.000	3.000
64	1.8	36	0	1.000	0.000	2.000
65	1.8	36	0	1.000	0.000	1.000

Table 36: Instar mortalities of *Ae. cantans* at permanent quadrat 1 in 1980 at Ness Woods

Instar	Age in days at begining of instar ($t_i - 1$)	No entering instar ($S_{ti} - 1$)	Deaths in instar (D_i)	Relative proportion dying in instar $\left(\frac{D_i}{S_{ti} - 1}\right)$	Proportion dying daily in instars $1 - \left(\frac{S_{ti}}{S_{ti} - 1}\right)^{1/d^*}$
I	0	50	19.5	0.3900	0.0326
II	14.9	30.5	19.7	0.6459	0.0656
III	30.2	10.8	8.8	0.8148	0.0884
IV	48.4	2.0	0.2	0.1000	0.0084
Pupa	60.9	1.8	0	0	0.0000
Adult	69.0	1.8			

d^* = weighted average instar duration

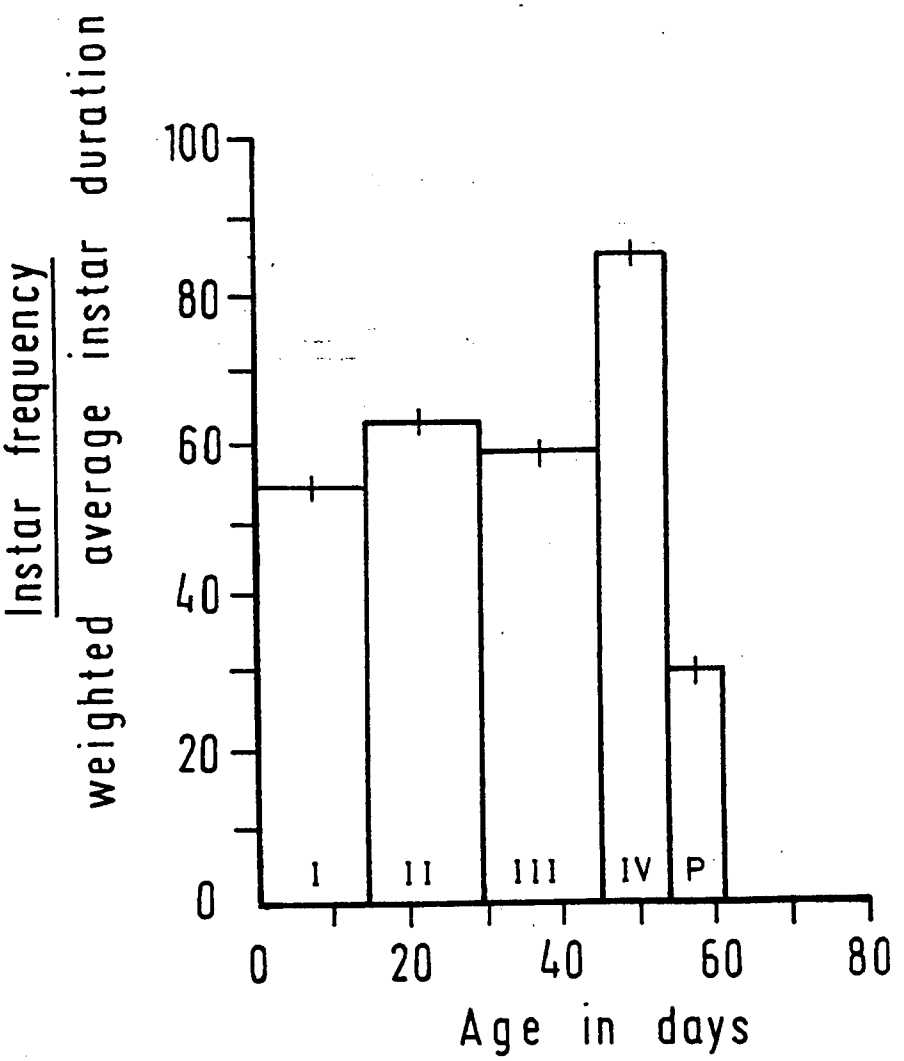

Fig. 23: Age distribution and survivorship curve of the immature stages of Ae. cantans in 1980 at permanent quadrat 2, Ness Woods.

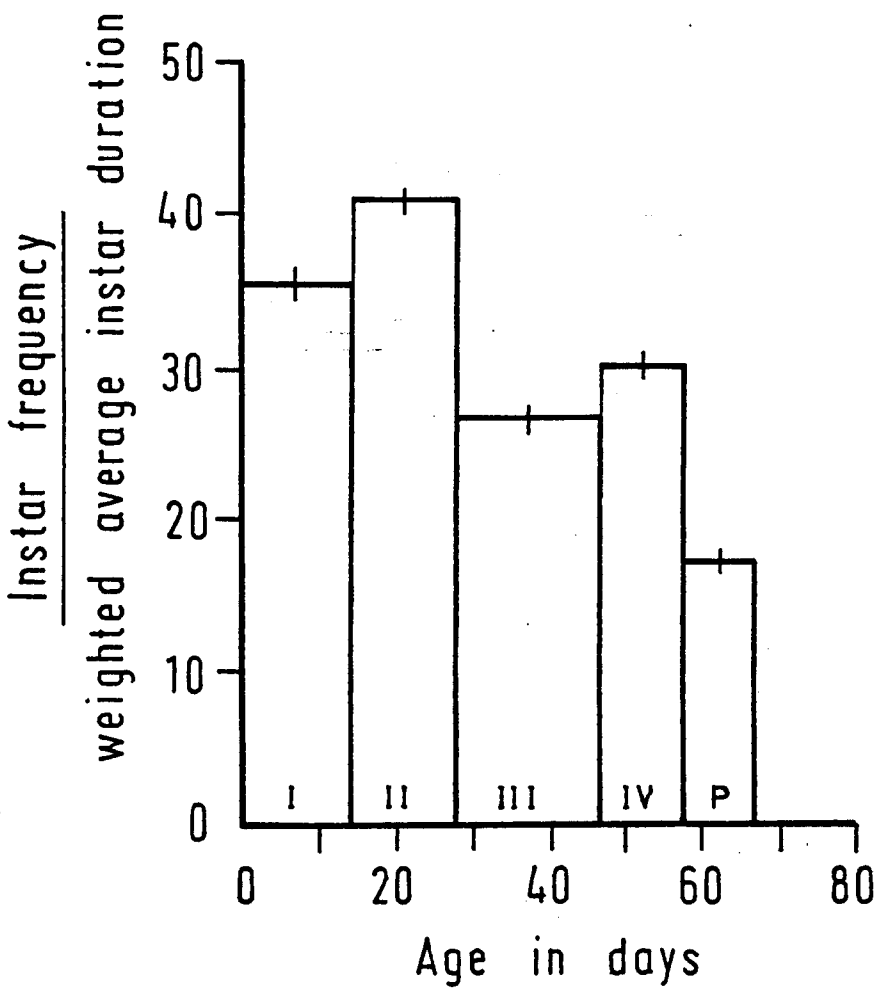

Fig. 24: Age distribution and survivorship curve of the immature stages of Ae. cantans in 1980 at permanent quadrat 3, Ness Woods.

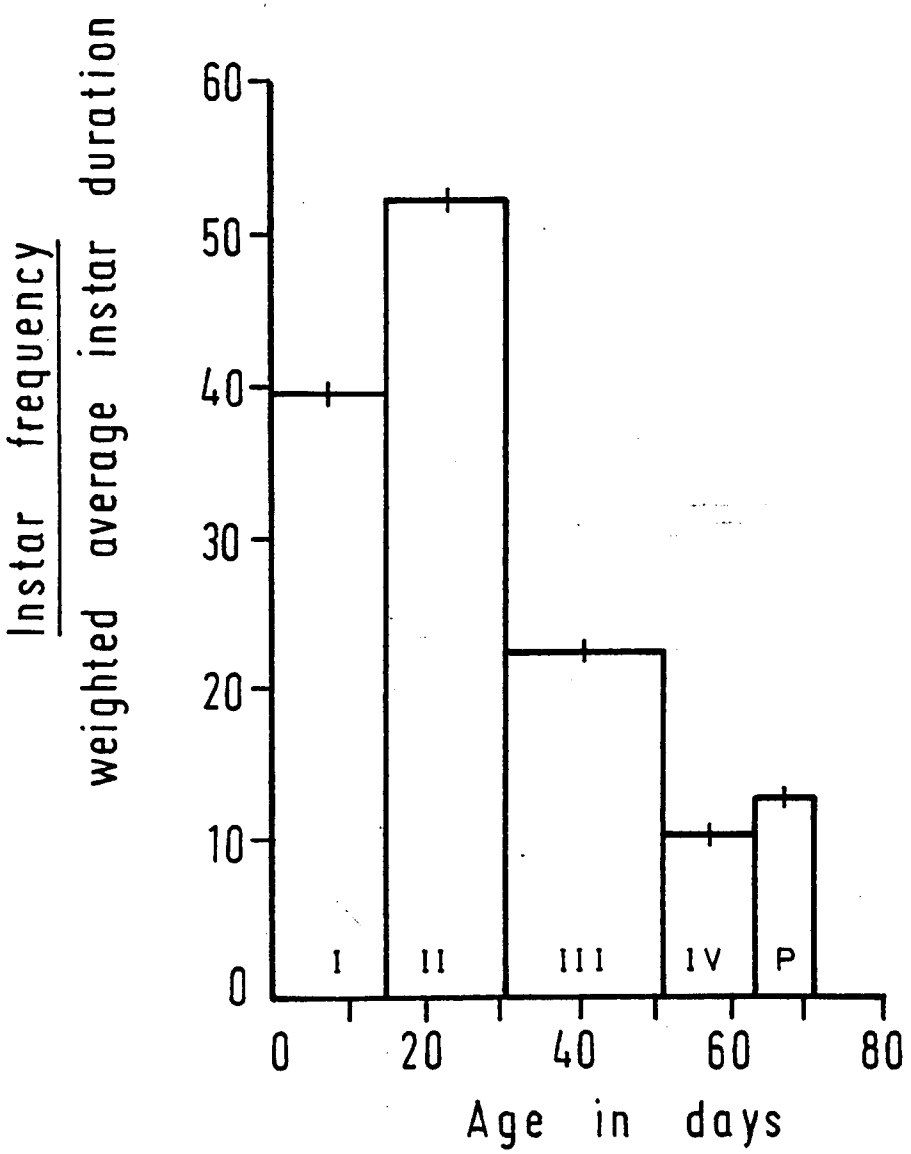

Fig. 25: Age distribution and survivorship curve of the immature stages of Ae. cantans in 1980 at permanent quadrat 4, Ness Woods.

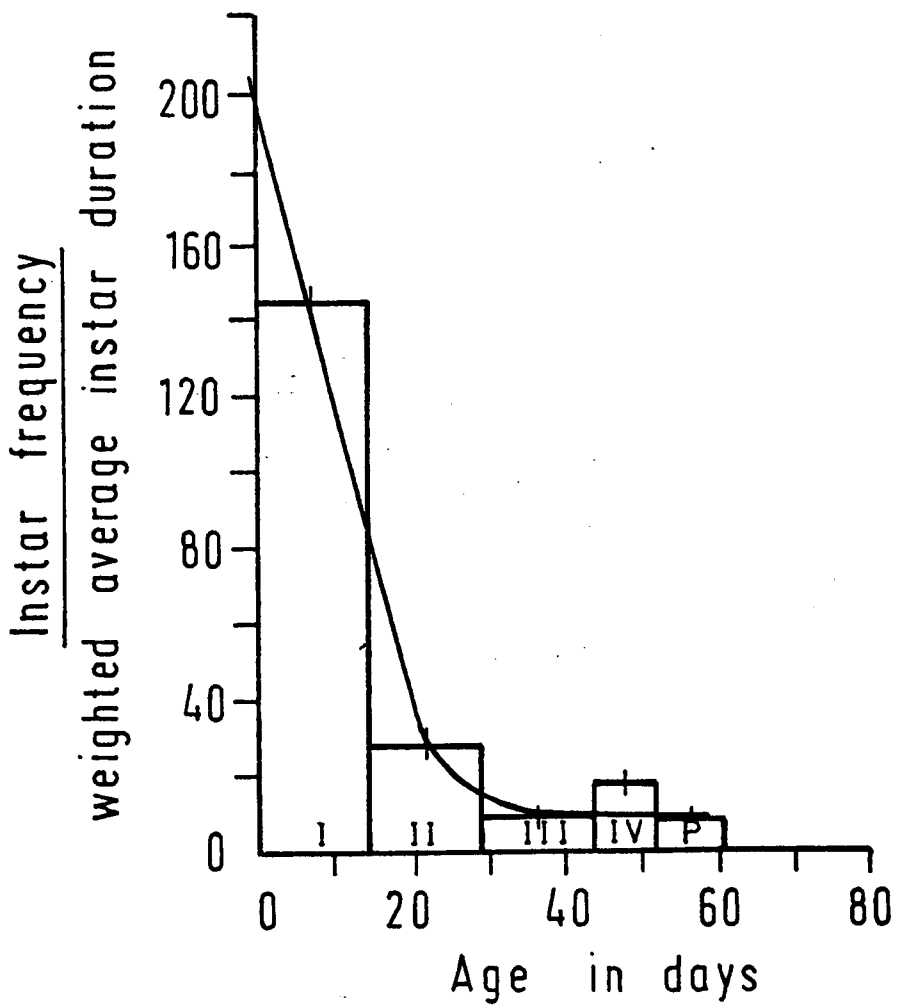

Fig. 26: Age distribution and survivorship curve of the immature stages of Ae. cantans in 1980 at permanent quadrat 5, Ness Woods.

Table 37: Life-table for Ae. cantans pre-adults at permanent quadrat 5, Ness Woods in 1980

x	n_x	l_x	d_x	p_x	q_x	e_x
0	204	1000	34	0966	0034	14.594
1	197	966	43	0955	0.045	14.090
2	188	923	41	0.956	0.044	13.723
3	180	882	39	0.956	0.044	13.338
4	172	843	44	0.948	0.052	12.932
5	163	799	34	0.957	0.043	12.616
6	156	765	15	0.980	0.020	12.155
7	153	750	64	0.915	0.085	11.388
8	140	686	39	0.943	0.057	11.404
9	132	647	39	0.940	0.060	11.061
10	124	608	39	0.936	0.064	10.738
11	116	569	40	0.930	0.070	10.440
12	108	529	39	0.926	0.074	10.192
13	100	490	39	0.920	0.080	9.963
14	92	451	44	0.902	0.098	9.782
15	83	407	39	0904	0096	9.785
16	75	368	35	0905	0095	9.769
17	68	333	39	0.883	0.117	9.743
18	60	294	39	0.867	0.133	9.969
19	52	255	39	0.847	0.153	10.418
20	44	216	40	0.815	0.185	11.208
21	36	176	34	0.807	0.193	12.642
22	29	142	19	0.866	0.134	14.549
23	25	123	10	0.919	0.081	15.720
24	23	113	15	0.867	0.133	16.066
25	20	98	0	0.898	0.102	17.449
26	18	88	5	0.943	0.057	18.375
27	17	83	8	0.904	0.096	18.452
28	15.4	75	6	0.920	0.080	19.367
29	14	69	5	0.928	0.072	20.007
30	13	64	5	0.922	0.078	20.531
31	12	59	5	0.915	0.085	21.229
32	11	54	3	0.944	0.056	22.148
33	10.5	51	2	0.961	0.039	22.422
34	10	49	2	0.959	0.041	22.316
35	9.5	47	3	0.936	0.064	22.245
36	9	44	0	1.000	0.000	22.727
37	9	44	0	1.000	0.000	21.727
38	9	44	0	1.000	0.000	20.727
39	9	44	0	1.000	0.000	19.500
40	9	44	1	0977	0.023	18.727

Continued .....

Table 37 :(continued)

x	n_x	l_x	d_x	p_x	q_x	e_x
41	8.8	43	0	1.000	0000	18.151
42	8.7	43	1	0.977	0.023	17.151
43	8.6	42	0	1.000	0.000	16.548
44	8.5	42	1	0.976	0.024	15.548
45	8.4	41	0	1.000	0.000	14.915
46	8.4	41	0	1.000	0.000	13.915
47	8.3	41	1	0.976	0.024	12.915
48	8.2	40	0	1.000	0.000	12.225
49	8.1	40	1	0.975	0.025	11.225
50	8.0	39	0	1.000	0.000	10.500
51	8.0	39	0	1.000	0.000	9.500
52	8.0	39	0	1.000	0.000	8.500
53	8.0	39	0	1.000	0.000	7.500
54	8.0	39	0	1.000	0000	6.500
55	8.0	39	0	1.000	0.000	5.500
56	8.0	39	0	1.000	0.000	4.500
57	8.0	39	0	1.000	0.000	3.500
58	8.0	39	0	1.000	0.000	2.500
59	8.0	39	0	1.000	0.000	1.500
60	7.9	39	0	1.000	0000	0.500

Table 38: Instar mortalities of *Ae. cantans* at permanent quadrat 5 in 1980 at Ness Woods

Instar	Age in days at begining of instar (t_i -1)	No entering instar (S_{ti} -1)	Deaths in instar (D_i)	Relative proportion dying in instar $\left(\frac{D_i}{S_{ti} - 1}\right)$	Proportion dying daily in instars $1 - \left(\frac{S_{ti}}{S_{ti} - 1}\right)^{1/d^*}$
I	0	204	118	0.5784	0.0567
II	14.8	86	72	0.8372	0.1184
III	29.2	14	5	0.3571	0.0308
IV	43.3	9	1	0.1111	0.0123
Pupa	52.8	8	1	0.1250	0.0176
Adult	60.3	7			

d^* = weighted average instar duration

Table 39: The mortality of Ae. cantans in permanent quadrats in 1980 at Ness Woods

Permanent quadrats	Total no. of first instars	Total no of pupae	Total no of adults emerging	Mortality of pre-adults (1st-instars→pupae)	Mortality of 1st instar →adult emergence
1	617	15	8	97.6%	98.7%
2	791	215	68	72.8%	91.4%
3	515	134	61	74.0%	88.2%
4	592	92	21	84.5%	96.5%
5	2134	59	28	97.2%	98.7%

Larval mortalities in permanent cylindrical quadrats

In December, 1980, 5 tall cylindrical aluminium quadrats were pushed into the ground along the perimeter of a pond known to have had large numbers of Ae. cantans immature stages in the Spring of 1980. The objective of this experiment was to study the population dynamics of the same habitat which I had already studied from February to June, 1980, (using metal quadrats made out of waste paper baskets with the bottoms removed).

Appendix 13 showed that in the permanent cylindrical quadrat 1, only 4 1st-instar, 4 2nd-, 4 3rd-, 4 4th-instar larvae, 3 pupae and 3 adults Ae. cantans were recorded in 1981. There was no immature stages and adults in permanent cylindrical quadrat 2. In permanent cylindrical quadrat 3, only 6 1st-instar, 5 2nd-, 3 3rd-, 3 4th-instar larvae, 3 pupae and 3 adults Ae. cantans were recorded. In permanent cylindrical quadrat 4, only 8 1st-instar, 5 2nd-, and 3 3rd-instar larvae Ae. cantans were recorded. No immature stages and adults of Ae. cantans was seen in permanent cylindrical quadrat 5. Due to the very low numbers of Ae. cantans immature stages recorded in the above permanent cylindrical quadrats, it is impossible to draw age distribution and survivorship curves and construct life tables. These results showed that very much fewer immature stages of Ae. cantans were recorded in this pond (Appendix 13) in 1981 compared to the larger numbers recorded from permanent quadrats (Appendices 7-11) in 1980 in the same habitat. This clearly shows that for some reason there was a large reduction in population of Ae. cantans immature stages in 1981 as compared to the population in 1980.

Causes of Mortality

Predators : methods of serological detection

Precipitin test

The techniques of preparation of antigen, production of antiserum and the precipitin test were done according to Service (1973c) with some modifications.

Preparation of antigen

Large numbers of 4th-instar larvae of Ae. cantans were collected from various ponds at Ness Woods. Larvae were kept in the laboratory for about 24hours in clean tap water to empty their guts. Larvae, and the few pupae formed during the holding period, were then dried on filter paper, weighed and ground in normal saline (0.85%) in a small tissue grinder at the rate of 2 gm of Ae. cantans per 10ml normal saline. The extract was placed in 15ml centrifuge tubes and left for about 20 hours at 4° C before centrifuging at 700 g, for 15 minutes. The extracted was then filtered through a sterile Seitz E.K. filter pad and 0.01% of sodium azide added as preservative. Quantities of 2.5 ml of sterile antigen (mainly proteins) were placed in sterile small glass bottles and kept at -20°C.

Production of antiserum

The proteins of the antigen were precipitated with 0.1 ml of 0.4% potassium alum and the pH adjusted to 6.8 by sterile 4% HCl or sterile 4% NaOH. The suspension was then injected into the hind leg muscle of 2 New Zealand white rabbits weighing 4.5 - 5 Kg. Both rabbits received 4 injections. The rabbits were test bled from the marginal ear vein 10 days after second, third and fourth injections. The blood was centrifuged and serum was obtained. The strength of the antiserum was measured by a precipitin test against 2-fold serial dilutions of the sterile antigen. All tests were made by underlying the antigen with antiserum in 2-2.5mm bore glass tubes. After 2 hours the tubes were examined with top

illumination against a dark background and any precipitin ring formed at the interface of antiserum and antigen recorded (Fig. 27). The final titer of the antisera, that is the lowest dilution of Ae. cantans or original antigen giving a positive reaction was the same for both rabbits. A precipitin reaction occured at 1/200 dilution with homologous antigen 10 days after the second inoculation, 10 days after the third injection a precipitin reaction occured at 1/800 with the homologous antigen, 10 days after the fourth injection a reaction occured at a dilution 1/4000. The titer dropped to 1/2000, 21 days after 4th injection. The titer against a single 4th-instar larva of Ae. cantans was 1/1000 at both 10 and 21 days after 4th-injection.

About 60-80 mls of blood was taken from each rabbit 10 days after 4th-injection. The blood was centrifuged, and the serum sterilized through a sterile Seitz E.K. filter pad and 0.01% sodium azide added as a preservative. Quantities of 2.5ml sterile antiserum were placed in sterile small glass bottles and kept at -20°C.

Preparation of predator smears

Guts were extracted from larger suspected predators(e.g. most Coleoptera, fish such as Sticklebacks) and smeared onto as small an area as possible of filter paper while whole individuals of small arthropods were crushed directly on to the paper. These labelled smears were placed in a desiccator over phosphorus pentoxide for rapid drying to reduce deterioration. Since predator meals were only detectable up to about 18 - 24 hours (Service, 1973c), I frequently made the smears in the field.

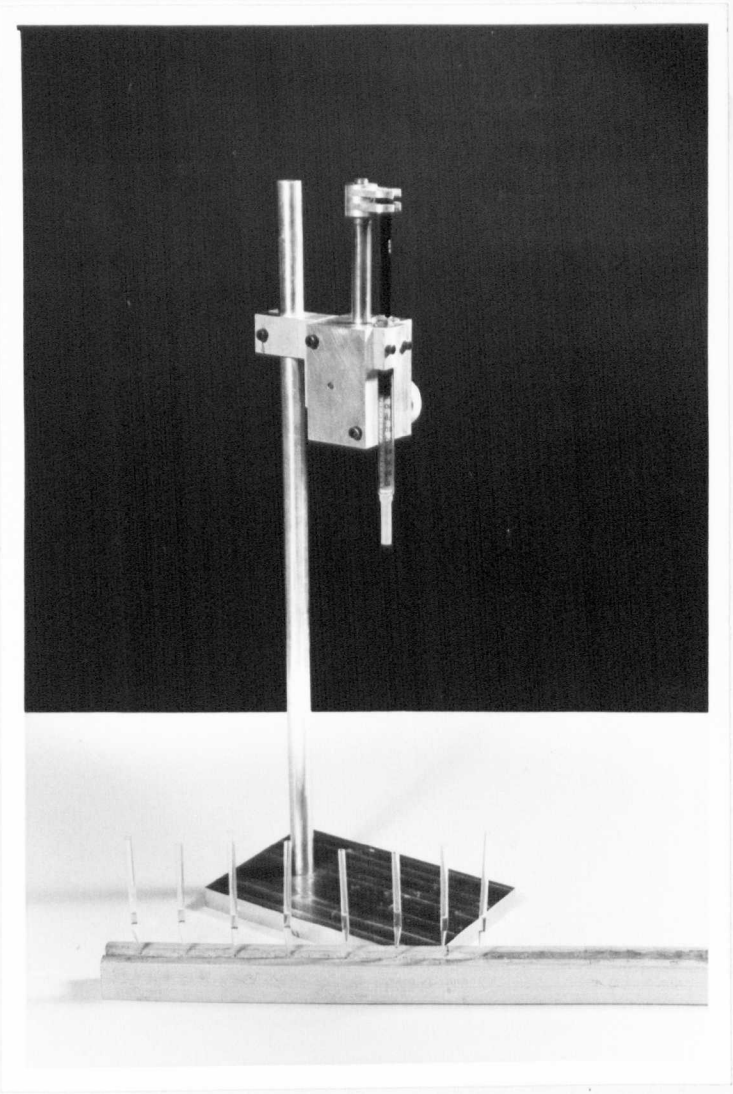

Fig. 27: The equipment used in the precipitin test.

The precipitin test

Smears were cut from the filter paper, placed in 0.5 ml centrifuge tubes and soaked according to the size of the smears, in either 0.1, 0.2 or 0.3ml normal saline for 20 hours at room temperature. The soaked smears were then centrifuged for 15 minutes at 500G. A small quantity about 0.02ml of the supernatant fluid was drawn up into a small capillary tube followed by about an equal volume of antiserum. Care was taken not to cause mixing. Air was then drawn up so that the 2 fluids came into the straight part of the tube, the end of which was pushed into a tray of plasticine (Fig. 27). The tube was left at room temperature for 2 hours and then examined. The presence of a distinct precipitin ring at the interface of the 2 fluids indicated that Ae. cantans was included in the predator's meal. It was essential that both antiserum and saline extract of the predator's gut were perfectly clear. The tubes were wiped clean of finger prints so that faint reactions were not missed. After testing the tubes were discarded. The small centrifuge tubes in which the smears were soaked were cleaned and boiled in distilled water before they were used.

Collection of predators

Suspected predators were collected from ponds in Ness Woods known to have large population of Ae. cantans. In 1979, collection of suspected predators began in early March and continued until about the middle of June, and in 1980 and 1981 collections were made from early February to the middle of June. This being the period when the Ae. cantans population is in the larval and pupal state. Suspected predators were collected using an aquatic sweepnet and the contents were poured into a white plastic tray and small predators collected and smeared onto filter paper. Large predators were dissected and the gut contents were smeared

on the filter papers. These labelled smears were placed in a desiccator over phosphorus pentoxide for rapid drying to reduce deterioration.

The Enzyme linked immunosorbent ... assay (ELISA) test

Plates: Disposable polystyrene plates (Linbro E.I.A. microtitration Plate) with 96 flat bottom wells, well capacity 0.35 ml approximately. (Fig. 28).

Antigen: Suspected predators were collected from ponds in Ness Woods, smeared onto filter paper. These labelled smears were placed in desiccator over phosphorus pentoxide. Besides suspected predators, fourth instar larvae of Ae. cantans, Ae. rusticus and Ae. punctor were also collected from a ditch in Ness Woods and smeared on filter paper.

IgG - obtaining IgG from rabbit anti-cantans serum

IgG was obtained by the method of Voller et al.(1976a). 1 ml of antiserum and 1 ml of 36% sodium sulphate were pipetted into a glass beaker and the solution stirred at room temperature for 30 minutes. It was then centrifuged at 4000rpm for 20 minutes.

The supernatant was discarded and sediment was suspended in 2ml 18% sodium sulphate. After centrifuging the sediment was washed with 18% sodium sulphate and centrifuged. The sediment was dissolved in 1ml PBS (Phosphate buffer saline) pH 7.4 and dialysed overnight at 4 °C against PBS pH 7.4 in a glass bottle on a magnetic stirrer. The following day the IgG was kept in a small vial and frozen at -20 °C.

Conjugate:- Preparation of conjugate

The principle was based on Voller et al.(1976a,b) and modified by R.D. G. Theakston (1980, pers.comm.)

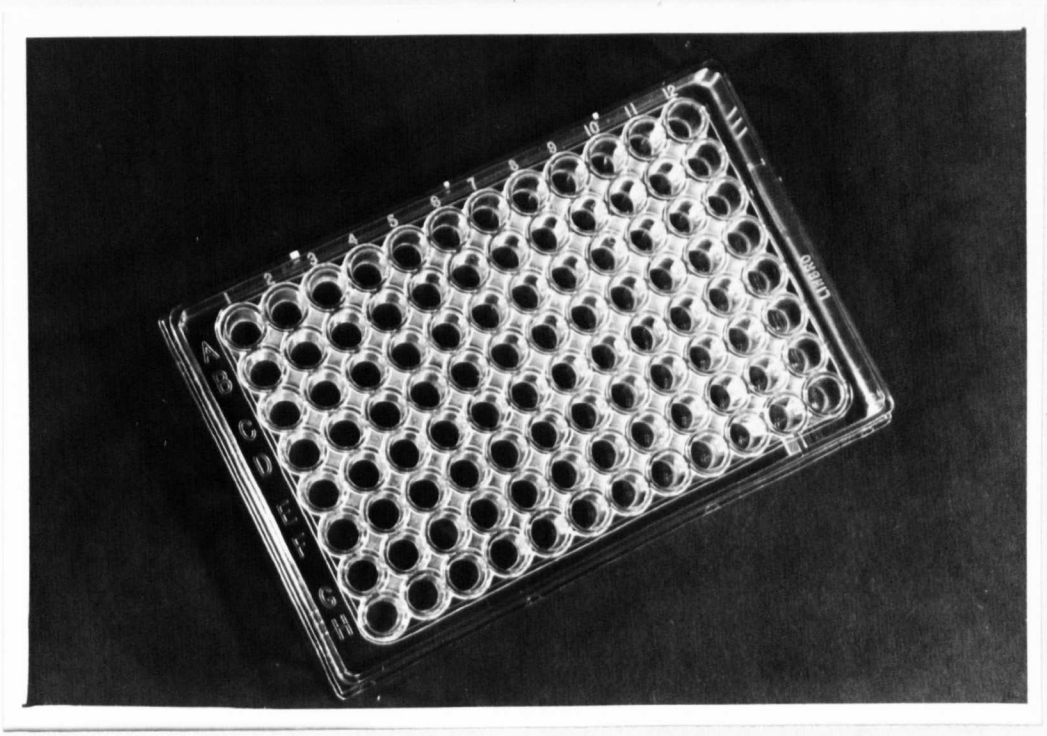

Fig. 28: The ELISA plate.

Day 1 - obtaining IgG from serum.

Same procedure as obtaining IgG from rabbit anti-cantans serum.

Day 2

The contents of the dialysis tube were placed in a small glass test-tube and 1/25 dilution was prepared (100µl IgG + 2400µl PBS). The optical density was read on Gilford Spectrophotometer. at 280 nm and 260nm using PBS alone as blank.

Using the following Warburg and Christian's (in Dawson et al., 1969) formula:-

Protein (mg/ml) = Extinction at 280nm x Factor x 1 x 25 .

0.5mg protein IgG was removed and made up to 1.0ml with PBS pH 7.4. 0.32ml alkaline phosphatase (5mg/ml) was centrifuged and supernatant discarded. PBS/IgG mixture was added to the alkaline phosphatase, mixed and dialysed overnight against PBS in a glass bottle with stirrer at 4° C.

Day 3

4.2% glutaraldehyde solution PBS was freshly prepared (0.1ml 25% glutaraldehyde + 0.5ml PBS). The contents of the dialysis tubing was placed in a glass tube and 10µl of 4.2% glutaraldehyde was added, mixed and left at room temperature for 3 hours. As before, it was dialysed overnight at 4°C against PBS.

Day 4

Dialysis was done against 0.05M Tris-HClpH 8.0 (6g/liter distilled H_2O) during the day. Tris was renewed and dialysing was continued overnight.

Day 5

The content of the dialysing tubing was placed into a stoppered tube and 1mg bovine serum albumin, 200µg NaN_3 were added and was stored at 4° C.

The double antibody sandwich method of microelisa for detection of antigen

The principle of detection of antigen by the double-antibody method was described by Voller et al.(1976a,b,c,) . Using this method immunoglobulin containing specific antibody was used to sensitize the carrier surface. The solution containing the antigen was then incubated with the sensitized surface and the excess solution was washed away. A conjugate consisting of enzyme-labelled specific antibody was then added and this became attached to the antigen already "captured" by the sensitized surface. After incubation, the excess conjugate was washed off and the amount attached was measured by the rate at which it degraded the added substrate.

Chequer-board titrations were made to determine the best concentration of antigen, IgG and conjugate that produced a colour change and a high optical density read on spectrophotometer. It was found that for the antigen the concentration was 1:2 , IgG 1:16 and conjugate 1:25 dilutions respectively.

The test procedure was based on Voller et al,(1976a,b,c).

1. Each well in the plate was coated for 5 hours at 37 °C with 100 µl of 1 : 16 dilution of IgG in coating buffer.
2. The plate was washed 3 times with washing buffer.
3. Supernatant from soaked smears (smears were cut from filter paper placed in 0.5ml centrifuge tubes and soaked according to the size of the smears ranging from 0.1ml to 0.3ml normal saline for 20 hours at room temperature and centrifuged) were diluted to 1:2 with incubation buffer. 100 µl from each test sample was placed in each well. As reference samples antigens of 4th-instar larvae of Ae. cantans, Ae. punctor and Ae. rusticus were used (obtained by soaking each smear in 0.1 ml normal saline for 20 hours at room temperature and centrifuged). The supernatants were incubated at 4° C overnight.

4. The plate was washed 3 times with washing buffer.

5. 100 µl of 1:25 dilution of conjugate diluted in incubation buffer was added to each well and incubated at 37°C for 3 hours.

6. The plate was washed 3 times with washing buffer.

7. 100 µl substrate was added to each well.

(substrate = 40mg capsule of p-Nitrophenyl Phosphate Disodium dissolved in 40mls of diethanolamine buffer pH 9.8).

8. The reaction was allowed to continue for 2½ hours and using a Titertek Muttiskan Spectrophotometer standardized at 405nm, the optical density for each well was read.

Reagents used

1. Coating buffer pH 9.6

1.59 g Na_2CO_3
2.93 g $NaHCO_3$
0.2 g NaN_3
Dissolve in 1000mls distilled H_2O.

2. Incubation buffer

900mls PBS
0.45 ml Tween 20
0.18g NaN_3

3. Phosphate Buffered Saline pH 9.4 (PBS)

8g NaCl
0.2g KH_2PO_4
0.2 g KCl
1000ml distilled H_2O.

4. Diethanolamine buffer pH 9.8

9.7ml diethanolamine
80ml distilled H_2O
10.1mg $MgCl_2 \cdot H_2O$
Adjust to pH 9.8 with 1N HCl
Dilute to 100ml with distilled H_2O
Add 0.02gm NaN_3 as preservative

RESULTS OF THE PRECIPITIN TEST

A total of 359 gut smears from possible predators were tested using the precipitin test for the presence of larvae and pupae of Ae. cantans. Species of predators in which positive reactions occured are included in Table 40.

Larvae of Chaoborus crystallinus Degeer were common in Ness Woods and 57.0% of gut smears reacted with the anti-cantans serum (Table 40). From field observations they were also seen to feed on larvae of Ae. cantans and in laboratory trials each Chaoborus crystallinus larva consumed 2-4 first instar or 1 fourth instar Ae. cantans (partially or entirely) in 24 hours. They were undoubtedly the most important larval predators in Ness Woods. However, they were greatly outnumbered by Ae. cantans larvae and pupae, therefore they were unlikely to cause a very great reduction in the population of Ae. cantans. Mochlonyx culiciformis Degeer larva could also consumed 1 fourth instar larva of Ae. cantans partially or entirely in 24 hours.

Five species of aquatic beetles occured in the ponds of Ness Woods, but they were not as common as Chaoborus crystallinus and Mochlonyx culiciformis. Results of precipitin test showed that 72.7% of Acilius sulcatus (L.) adults showed positive reaction with anti-cantans serum; 37.2% of Hydroporus palustris (L.) adults, 38.9% of Colymbetes fuscus (L.) adults, 53.8% of Agabus bipustulatus (L.) adults and 100% of Dytiscus circumcinctus Ahrens adult. In the laboratory, Colymbetes fuscus adult could consumed 1 whole larva of 4th-instar Ae. cantans in 24 hours. Hydroporus palustris adult could consumed partially 1-2 larvae of 4th-instar Ae. cantans in 24 hours. Although these adult beetles were predators of Ae. cantans larvae and pupae, their numbers were too small to cause much reduction in populations of Ae. cantans immature stages in the field. The larval dytiscids showed 52.8%

Table 40: List of predators that showed positive reactions with anti-cantans sera using the precipitin test

Species		Predators of larvae and pupae No. tested	No. +ve	% +ve
Coleoptera				
	Dytiscidae			
1.	Dytiscid larvae (unidentified)	36	19	52.8
2.	Acilius sulcatus (L.) adult	11	8	72.7
3.	Colymbetes fuscus (L.) adult	18	7	38.9
4.	Agabus bipustulatus (L.) adult	13	7	53.8
5.	Dytiscus circumcinctus Ahrens adult	2	2	100.0
	Hydrophilidae			
6.	Hydroporus palustris (L.) adult	43	16	37.2
	Corixidae			
7.	Hesperocorixa sahlbergi (Fieb.) adult	29	0	0
	Trichoptera			
8.	Trichostegia minor (Curtis) larvae	15	0	0
	Diptera			
9.	Chaoborus crystallinus Degeer larvae	100	57	57.0
10.	Mochlonyx culiciformis Degeer larvae	75	13	17.3
	Gasterosteidae			
11.	Pygosteus pungitius (L.)	11	9	81.8
	Amphibia			
12.	Tadpoles	6	0	0

postive reaction to anti-cantans serum. Dytiscid larvae were seen in the field killing and biting Ae. cantans larvae. This happens normally in March and April when dytiscid larvae were seen capturing 3rd and 4th-instar Ae. cantans by the thorax or abdomen. In the laboratory each dytiscid larva early instar could consume 1-2 4th-instar Ae. cantans larvae in 24 hours, and later instar larvae could kill as many as 17-19 4th-instar larvae of Ae. cantans in 24 hours. Although these larvae were voracious predators, their numbers were not great and they were therefore not considered as important as Chaoborus or Mochlonyx at reducing the numbers of Ae. cantans.

The stickleback Pygosteus pungitius (L.) had shown 81.8% postive reaction to anti-cantans serum but their numbers were much too few to be a dominant predator causing reduction in population of Ae. cantans. Moreover, they were present in only a few of the ponds containing Ae. cantans larvae. Other species were tested for predation namely the Trichopteran Trichostegia minor (Curtis), the corixid Hesperocorixa sahlbergi (Fieb.) and tadpoles but they showed negative results to anti-cantans serum. Therefore they were not predators of Ae. cantans in Ness Woods.

RESULTS OF THE ELISA TEST

Some degree of cross-reactions occured between Ae. cantans and other mosquito species namely Ae. rusticus and Ae. punctor. However, using the spectrophotometer, the difference in optical density of the reaction from various species could be read and compared. Table 41 indicated that Ae. cantans antigen generally has a higher optical density than the two other species in this reaction. The mean optical density for Ae. cantans for different experiments were 0.261 and 0.293 respectively giving the overall mean optical density of 0.277.

The mean optical density for Ae. rusticus for both experiments were 0.069 and 0.074 respectively giving the overall mean optical density of 0.072. The mean optical density for Ae. punctor for both experiments were 0.020 and 0.034 respectively, giving an overall mean optical density of 0.027. Although some degree of variations in optical density for each individual and species occured, which is thought to be due to differences in size of the fourth instar larvae of the different species, this method could nevertheless differentiate between these species. To standardize positive reaction the optical density of 0.2 was taken as the predetermined value, since the mean optical density for Ae. cantans was 0.277.

Table 42 shows that the lowest optical density for Ae. cantans reference sera was 0.176 and the highest was 0.752. Results showed that the lowest optical density for Ae. cantans was generally higher than those shown by negative reference sera (except for one case of Ae. rusticus indicating an optical density of 0.446 but this could be false positive).

Results of ELISA test for identification of predators (Table 43) indicated that Chaoborus crystallinus larvae showed 25% positive reaction to anti-cantans serum, Mochlonyx culiciformis larvae showed 10.7% positive reaction, Pygosteus pungitius showed 8.7% positive reaction and dytiscid larvae showed 10.0% positive reaction. The dytiscid adults did not show positive reaction. No positive reactions were showed by the adults Hydroporus palustris, Trichopteran Trichostegia minor and Corixidae Hesperocorixa sahlbergi. The single tadpole showed a negative reaction but in the laboratory a tadpole when hungry could eat a small number of fourth-instar Ae. cantans larvae. Tadpoles were rare in the ponds at Ness Woods, and in fact were absent from many of them. It is known that young tadpoles are herbivorous, only in later life do they become predaceous.

Table 41: The optical density of positive and negative reference antigens read from Spectrophotometer in ELISA test

Date	Antigens	Wells (of Plate)	Optical Density
6.1.81	Ae. cantans	No. 1	0.319
		No.2	0.260
		No.3	0216
		No.4	0.208
		No.5	0.300
			$\bar{x}$ = 0.261
	Ae. rusticus	No. 1	0.318
		No.2	0.001
		No.3	0.002
		No.4	0.010
		No.5	0.012
			$\bar{x}$ =0.069
	Ae. punctor	No.1	0.107
		No.2	0.023
		No.3	0.035
		No.4	0.008
		No.5	0.012
			$\bar{x}$ = 0.020
13.1.81	Ae. cantans	No.1	0.339
		No.2	0.286
		No.3	0.314
		No.4	0.251
		No.5	0.275
			$\bar{x}$ = 0.293
	Ae. rusticus	No.1	0.000
		No.2	0.000
		No.3	0.082
		No.4	0.000
		No.5	0.286
			$\bar{x}$ = 0.074
	Ae. punctor	No.1	0.159
		No.2	0.000
		No.3	0.000
		No.4	0.003
		No.5	0.010
			$\bar{x}$ = 0.034

The overall mean optical density for

(i) Ae. cantans = 0,277
(ii) Ae. rusticus = 0.072
(iii) Ae. punctor = 0.027

Table 42: The optical density of positive and negative reference antigens as control in ELISA test for detection of predators of Ae. cantans

Date	Antigens	Optical Density		
20.1.81	Ae. cantans	a)	0.176	
		b)	0.204	
	Ae. rusticus	a)	0.000	
		b)	0.141	
	Ae. punctor	a)	0.122	
		b)	0.106	
27.1.81	Ae. cantans	a)	0.381	
		b)	0.221	
	Ae. rusticus	a)	0.116	
		b)	0.446	(false positive)
	Ae. punctor	a)	0.157	
		b)	0.135	
3.2.81	Ae. cantans	a)	0.752	
		b)	0.317	
	Ae. rusticus	a)	0.158	
		b)	0.169	
	Ae. punctor	a)	0.133	
		b)	0.140	

Table 43: List of predators that showed positive reactions with anti-cantans sera using ELISA test

Species		Predators of larvae and pupae		
		No. tested	No. +ve	% +ve
Coleoptera				
	Dytiscidae			
1.	Dytiscid larvae (unidentified)	40	4	10.0
2.	Acilius sulcatus (L.) adult	1	0	0
3.	Colymbetes fuscus (L.) adult	14	0	0
4.	Agabus bipustulatus (L.) adult	3	0	0
5.	Dytiscus circumcinctus Ahrens adult	0	0	0
	Hydrophilidae			
6.	Hydroporus palustris (L.) adult	6	0	0
	Corixidae			
7.	Hesperocorixa sahlbergi (Fieb.) adult	8	0	0
	Trichoptera			
8.	Trichostegia minor (Curtis) larvae	6	0	0
	Diptera			
9.	Chaoborus crystallinus Degeer larvae	4	1	25.0
10.	Mochlonyx culiciformis Degeer larvae	28	3	10.7
	Gasterosteidae			
11.	Pygosteus pungitius (L.)	23	2	8.7
	Amphibia			
12	Tadpole	1	0	0

Comparison of precipitin test and ELISA test

According to Voller et al. (1974) the ELISA test appears to be eminently suitable for seroepidemiological programmes. Although it is certainly an elegant and useful serological method it has the disadvantage of being very time consuming. Voller et al. (1976b) suggested that it is always best to use the most highly titred antisera that are available, because this allows the conjugate to be used in a more dilute form and so requires less enzyme which is the most expensive reagent in these tests. In using the ELISA test for identifying predation of Ae. cantans at Ness Woods, the titers used for IgG was 1:16; antigen 1:2 and conjugate 1:25 for giving a yellow colour after adding the substrate. These titers were considered low therefore more enzyme must be used in each experiment. Hence a disadvantage is that the test is not economical. Another problem encountered is using the ELISA test is that some of the predators' guts produced absorbance values less than the standard positive reference sera (optical density of 0.2), yet higher than negative reference sera, or PBS and were considered negative reactions. But, they could indicate true predation although the amount of antigen available in each gut was low, because either most of it had been destroyed by degeneration or few Ae. cantans pre-adults were consumed. Consequently the ELISA test produced an optical density of less than the standardized 0.2 when read from spectrophotometer. Comparison of the numbers of positive reaction and predators identified in Tables 40 and 43 clearly showed that in all cases, except for Mochlonyx culiciformis larvae, predator rates detected by the ELISA method are much lower than indicated by the Precipitin Test. However, the ELISA test has the advantage of differentiating cross-reactions between mosquito species, by reading the optical density from the spectrophotometer.

The precipitin test according to Service (1973c) has the disadvantage that it is not always possible to produce specific antisera although this is not a serious disadvantage if the species with which they cross-react do not co-exist to any great extent with the species under study. The precipitin test has the advantages that less time and much simpler equipment is needed to perform the test compared to the time consuming and sophisticated ELISA test. Another advantage of the Precipitin test is that less amount of antigen or antisera needed to perform test compared to the ELISA test.

In conclusion although the sophisticated ELISA technique might at first sight appear to have more potential than the simpler precipitin test, its usefulness is severely limited due to the available amount of prey - proteins (e.g. Ae. cantans tissues) in its predator's guts.

DISCUSSION

Using the age distributions and survivorship curves, Lakhani and Service (1974) and Service (1977a) estimated the life-table parameters for Ae. cantans at Monks Wood in southern England. They found that in all years from 1969 to 1971 the shape of the survivorship curves was similar and mortality was most intense in the younger stages, and that the few individual that survived to advanced stages had a relatively high expectation of survival. Investigations on the mortalities of Anopheles gambiae complex in Nigeria and Kenya by Service (1971a, 1973a) showed that mortality was usually greatest in the fourth-instar larvae. The large mortality of the immature stages of An. gambiae that occured could have been caused by a variety of factors including adverse climate conditions, limited food supply, competition, parasites and pathogens but predations was probably the most important limiting factor. But in his studies in Kenya on An.gambiae in small

and more or less temporary pools (Service, 1977b), mortality was heaviest towards the end of the aquatic phase of development, whereas in the ricefields there were substantial deaths in the younger instars, resulting in a more constant mortality rate. There were much smaller aquatic predator populations in the pools and ponds than in the ricefields and it seems reasonable to believe that in these temporary habitats predators were not so important in regulating the numbers of An.gambiae. Reisen and Siddiqui (1979) studied the larval survivorship and development of Culex tritaeniorhynchus Giles in Pakistan and found that the mortality of early instars was low during the pre-monsoon and monsoon season, but increased markedly during the post-monsoon period. Predation was high during the post-monsoon compared to monsoon season. Bown and Bang (1980) studied the ecology of Aedes simpsoni (Theobald) in southeastern Nigeria and found that the highest mortality rates in cocoyam axils occured amongst the 3rd- and 4th-instar larvae. There is probably a density- dependant phenomenon associated with the reduction of water volume in the axils as they become looser and begin to dry out at this time of year. Predators also had a contribution to Ae. simpsoni mortality, indeed several species of Coleoptera and Diptera were frequently seen in the axils.

Results of this study at Ness Woods, revealed that for Pond 1 in 1980, mortality largely occured among the late instars but in 1981 mortality was higher among the earlier- instars of Ae. cantans. A possible reason for the change in survivorship pattern was that at the beginning of late March 1980 until mid-May 1980 little rainfall occured during this period (Table 1), in addition it was the driest and sunniest April ever recorded at Ness Gardens (about 1.5km west of Ness Woods) since 1969. The mosquitoes in the pond at this time were mainly late instars, therefore due to the decrease in water volume and level in the pond, as a result

of the combination of the above meteorological factors, the mortality of the late instars Ae. cantans increased subsequently. According to Service (1978) the most important weather variable affecting the survival of larvae and pupae and the productivity of adult mosquitoes is undoubtedly rainfall. Drought that occurs during periods of normal aquatic development can cause high pre-adult mortality and greatly diminish the emerging adult population. Larsen (1978) in his studies on the mortality of the immature stages of Aedes spp. in Danish forest pools found that mortality caused by predation was of minor importance because the most important predators, the dytiscid larvae only occured very late in the spring compared to Aedes larvae and pupae. The drying up of the pools seem to have been the most important known mortality factor. Service (1973c) also found that dessication of larval habitats probably causes the greatest mortality of Ae. cantans immature stages at Monks Wood, south of England. In Japan Mogi et al.(1980) found that death of Culex tritaeniorhynchus due to drying out occured frequently in experimental floating cages in 1974 but not in 1973. This difference resulted from the difference in precipitation between the two years.

In 1980, the mortality of Ae. cantans immature stages in permanent quadrats 1 and 5 were higher among earlier than later instars (Tables 36 and 38, Figs 22 and 26). On the contrary in pond 1 in the same year, mortality was higher among the later instars (Table 30 and Fig. 20). It is thought that the main reason for high mortality of early instars in permanent quadrats 1 and 5 was due to density-dependant factors. For example, it is suggested that the restricted space and volume of pond water in these quadrats were insufficient to support the population of Ae. cantans immature stages confined to the quadrats, consequently higher mortality occured among the early instars. However, permanent quadrats 2-4 contained larger volumes of

water because of their position in the deeper parts of the pond, therefore they had enough water to support the population of Ae. cantans earlier instars during February and March. Subsequently, as the water level started receding due to little rainfall at the end of March onwards large mortalities of later instars occured. These experiments using quadrats to measure natural larval mortalities demonstrate the difficulties of trying to isolate part of a natural much larger population.

Because of the well-known difficulties of sampling all age-classes of mosquitoes due to sampling bias, it was thought reasonable to try to enclose a small natural population within quadrats in order to be able to more or less count the total population of the various instars. Clearly, the approach did not work very well, and more meaningful results on the population dynamics of Ae. cantans were obtained from sampling the much larger and unrestricted pond population. The fact that the age-distribution plotted in Figures 23-25 for permanent quadrats 2-4, show increases in numbers of older instars compared to the younger ones, an impossible solution, shows that this technique has in fact not overcome the sampling problem, but introduced them.

Service (1973c, 1977a) studied the predators of Ae. cantans at Monks Wood and other localities in southern England using the precipitin test as the serological tool. He found that Trichostegia minor (Curtis) and Glyphotaelius pellucidus (Retzius) were common and a small percentage of gut smears (1.3 - 1.5%) reacted with anti-cantans serum. He considered that these caddis fly larvae were not predators but scavengers. Trichostegia minor were found in Ness Woods but showed negative result on predation to Ae. cantans. Moth Iversen (1971) in his studies of Ae. communis at Danish woodland pools failed to detect any predation on Ae. communis larvae by Trichostegia minor.

Mochlonyx culiciformis larvae were common in Ness Woods, and showed a 17.3% positive reaction against anti-cantans serum using the precipitin test and 10.7% with ELISA. Moth Iversen (1971) studied the ecology of Ae. communis Deg. in a Danish beechwood and found that Mochlonyx culiciformis mainly fed on Copepods but they could take Aedes larvae.

According to Freeman (Coe et al., 1950), the larvae of the British genera of Chaoborinae are carnivorous, feeding on mosquito larvae, small Crustacea, etc. In Ness Woods, Chaoborus crystallinus larvae were common and using the precipitin test 57% of the gut smears reacted with anti-cantans serum, with ELISA test only 25% showed positive reactions. Although Chaoborus crystallinus larvae were predators they were considered unlikely to cause a great population reduction of Ae. cantans in the ponds of Ness Woods because they were largely outnumbered by Ae. cantans immature stages in habitats where Ae. cantans were seen.

Service (1973c, 1977a) found that the dytiscid Agabus bipustulatus had preyed upon Ae. cantans in Monks Wood. Some 37-47% of the gut smears from dytiscid larvae were positive and they were undoubtedly the most important larval predators in Monks Wood and other localities in southern England. Moth Iversen (1971) found that the dytiscid , Colymbetes fuscus, larvae were the main cause of mortality of Ae. communis in the pool.

Baldwin et al (1955) studied the predators of Ae. stimulans (Wlk.) and Ae. trichurus (Dyar.) in a mosquito pool in a woodland swamps in Ontario, Canada, and found that dytiscids were important predators. James (1966) tagged mosquito larvae with radioactive P^{32} that were released in woodland pools to identify their predators. He found that the leading predators were Dytiscidae. Larsen (1978) in his studies of Aedes spp. inhabiting some Danish forest pools concluded that mortality

caused by predation must have been of minor importance because the most important predators, the dytiscid larvae only occured very late in the spring compared to Aedes larvae and pupae. In this studies at Ness Woods, although dytiscid larvae were the most voracious predators, late-instar larvae eating a large number of larva per day, their population was small. Consequently the mortality caused by their predation was not the main factor for causing the large reduction in population of Ae. cantans (Tables 29-39) at Ness Woods. The Stickleback, Pygosteus pungitius, was a predator in Ness Woods, but due to its very small numbers, and absence in many ponds, it seems unlikely to have caused much reduction in the population. Similarly there were few tadpoles, and they are considered unimportant predators. Quantitative studies of the number of predators and Ae. cantans immature stages sampled by quadrats in pond 1 in 1981 (Table 34) showed that the ratio of Ae. cantans immature stages : Predators during the larval period was 197.6: 1. Therefore predators were greatly outnumbered by Ae. cantans immature stages. So, although predators undoubtedly caused mortality of Ae. cantans immature stages they were not the main ecological factor that causes the great population reduction recorded in Ness Woods.

Service (1973c, 1977a) in his studies of predators of Ae. cantans at Monks Wood, found that predaceous flies such as Hilara interstincta (Fallen), H. lugubris (Zetterstedt) and Rhomphomyia crassirostris (Fallen) fed on emergent adults of Ae. cantans but, in my studies at Ness Woods, predaceous flies were not seen. Similarly, various pathogens and parasites can cause mortality to Ae. cantans larvae (Service, 1977a). A mosquito iridescent virus which turned fourth-instar larvae an iridescent green and killed them prior to pupation infected a very small proportion of Ae. cantans larvae in Monks Wood and other localities in

southern England (Service, 1977a; Tinsley et al., 1971). Karpenko and Buchatskii (1978) also found that Ae. cantans larvae were infected with a mosquito iridescent virus and suggested that it could be transmitted transovarially due to the presence of this virus in the primordial gonads. Service (1977a) also found that pathogens such as Coelomomyces psorophorae infected the larvae of Ae. cantans and that larvae usually die prior to pupation. Parasitic nematodes were also seen to infect larvae of Ae. cantans in southern England. However, despite regular searches in the ponds at Ness Woods, neither pathogens nor parasites were seen to infect immature stages of any Ae. cantans. Southwood et al., (1972) studied the life-budget of Ae. aegypti (L.) in Thailand and postulated that food might be the factor in regulating the numbers of Ae. aegypti breeding in village pots. But in Ness Woods, Ae. cantans larvae feed on a variety of microscopic organisms and also browse on leaf litters which were abundant. Although nutritional requirement are unknown, food seems unlikely to be the limiting factor. However, competition for space could be responsible for the large population loss that occured yearly. In Ness Woods the Ae. cantans larvae were normally seen aggregated in large numbers and particularly the earlier instars were mostly found at the water's edge and competition for space could arise. Ikeshoji and Mulla (1970a) were the first to show that chemicals could be produced when larvae became overcrowded and they were able to characterise these so-called overcrowding factors biologically and entomologically. They showed that these chemical factors, produced by overcrowded 3rd stage larvae of Culex pipiens quinquefasciatus Say, were toxic to the younger larvae of this species and also to the larvae of Ae. aegypti, An. albimanus Wiedemann and Culex tarsalis Coquillett. Subsequently Ikeshoji and Mulla (1970b) studied the toxic and growth retarding components of larval by-product obtained under

highly overcrowded conditions, the physiological effects of these larval factors and their bacteriostatic effects were established. According to Ikeshoji (1977), self-limiting ecomones which are substances produced by certain species circulate within their ecosystem, to limit their own population growth. Such self-limiting ecomones include overcrowding factors, antagonistic excretory chemicals and bioactive substances causing morphogenic aberration and even migration. The theory being that mosquito larvae regulate their own population density by means of toxic ecomones (Ikeshoji, 1978) produced during overcrowding conditions. The actual mosquito larvae and /or their associated microflora secrete 7-methyloctadecane and 8- methylnonadecane which retards the growth of young instar larvae. These hydrocarbons are then degraded by Pseudomonas spp. in the breeding water to methyl-branched fatty acids which inflict high mortality at their ecdysis. Similarly, Ae. cantans could produce these toxic substances and cause the large observed mortality of the early instars.

To conclude, although predators found in the larval habitats at Ness Woods caused some mortality of Ae. cantans immature stages they were not the main factor responsible for the large reductions in their populations. The main factor causing large reductions in population of Ae. cantans in 1980 was the drying up of parts of the pond, due to little rainfall together with the occurence of the sunniest and warmest April recorded since 1969. Also, unknown factors possibly competition for space, and overcrowded conditions were responsible for causing large mortalities of the population.

FIELD STUDIES ON ADULT BEHAVIOUR AND ACTIVITIES

INTRODUCTION

According to Service (1977c), methodology is of paramount importance in sampling insect populations. Many methods have been devised during the last few decades for sampling adult mosquitoes but too frequently with little thought as to what the samples represent. The types of samples and sampling procedures usually differ according to whether mosquitoes are collected for virus isolations, for studying resting habits, host preferences, dispersal, life-span, degree of mosquito-man contact, faunas or the effect of control measures, or for estimating relative or absolute population size.

Human bait catches are made for a variety of reasons, including the estimation of biting rates and infection rates, the assessment of the effectiveness of control operations and the monitoring of temporal changes in relative population size. In studying the seasonal changes in population size the attraction of man as bait must not change over the sampling period. Such a change could result from fluctuations in either the population size or availability of attractive hosts or from physiological or behavioural changes in the mosquitoes (Service, 1976). According to Service (1969a, 1971b, 1977c) in England species which were essentially crepuscular and nocturnal were caught in large numbers during the day whenever bait catches were performed in sheltered sites where unfed females were resting amongst vegetation.

Standardized catches during the middle of the day have therefore proved sufficient for measuring variations in seasonal biting rates and differences between species numbers and composition in different localities. According to Service (1976) adult mosquitoes probably pass more time resting in natural, or man-made, shelters than in flight, and collections of such resting populations provide more representative samples of the population as a whole than do most other sampling methods. In addition to unfed females, males and both blood-fed and gravid females are caught. The collection of blood-engorged adults is useful for studying natural host preferences. Another advantage is that the age-structure will be more representative than that caught at bait or in traps.

Light traps have been widely used in U.S.A. and Japan whereas in many tropical countries they have been little used, although there has been renewed interest in certain parts of Africa. Light traps may be very useful in catching large numbers of certain mosquito species and in measuring the relative changes in abundance of these species in time and space, but as an ecological tool they are of strictly limited value because they sample different species unequally (Service, 1976).

The objectives of the present study were to obtain information on adult behaviour and activities by employing different sampling techniques, namely human-bait catches, sweep-netting vegetation, light traps catches, dry ice collections and marking the adults with 'Dayglo' fluorescent dusts.

MATERIALS AND METHODS

Human-bait catches

The seasonal incidence of Ae. cantans was derived from human-bait catches. The procedure of conducting a bait catch was done according to Service (1976). In April of each year, 1979-1981, a time when pupae were first recorded in the ponds at Ness Woods 1-2 human-bait catches a week were performed until the end of each October. A permanent site in Ness Woods was chosen for the catches which were undertaken from 11.00 - 12.00 hr GMT. The author sat at the selected site on the ground with legs outstretched and allowed hungry unfed mosquitoes to alight on his clothing or exposed skin. A raincoat was worn with a hood pulled over the head. Mosquitoes being unable to bite the back of the author because of the close weave of the raincoat were forced to the front where, after settling they were caught by carefully placing a test-tube (125 x 18mm) over them. Each test-tube could accommodate some 4-5 mosquitoes separated by cotton wool plugs. In the laboratory the mosquitoes were counted according to species and dissected for determining the parity by ovarian tracheation (Detinova, 1962). However, in 1981, bait collections had to be performed about 50 metres from the original site because the owner of the wood had cut down many trees and cleared much undergrowth from around the previous catches site.

Sweep-netting vegetation

Adult populations of mosquitoes were monitored by sweep-netting vegetation. Adults resting amongst ground vegetation were collected using a sweep-net consisting of a strong white calico bag fastened by pop studs over a D-shaped metal frame, to which a 60cm wooden handle was attached. From April to October 1979-1981,

adults were collected by sweep-netting vegetation during the day once or twice per week. Samples of mosquitoes were taken from different types of vegetation and compared; and in addition adults resting on trees were collected by an aspirator (= pooter). Gut smears of bloodfed adults were sent to Imperial College Field Station, Silwood Park, Ascot to determine, by the precipitin test, the hosts they had fed upon. Unfed adults were dissected to determine their parity, the presence or absence of sperms in spermathecae, and the presence of sugar solution in the crops. In 1981, the presence of sugar in the crops was determined by the anthrone test (Van Handel, 1972; Young et al. 1980) with slight modification as described below.

Anthrone test

Mosquitoes were crushed individually with a glass rod in a well of an ELISA plate. To avoid contamination the glass rod was washed with tap water after crushing each mosquito. Four drops (with a pipette) of a 0.1% ($^{w}/_{v}$) solution of anthrone in 72% ($^{v}/_{v}$) of sulphuric acid was added to each well and the plate was kept at room temperature. The reagent turns green or blue depending on the amount of sugar present; if there is no colour change after about an hour the test should be considered negative.

Collections with light traps and Trinidad no. 10 baited with dry ice

Three different types of light traps were used to sample the adult populations of mosquitoes, namely the Monks Wood trap, a chemical light trap and a CDC light trap.

Monks Wood light trap

The trap was developed by Service (1970) and later modified by Ross and Service (1979). Light was provided by a 23-cm 6 W fluorescent tube mounted vertically between three white plastic baffles (Fig. 29). The fluorescent tube could be operated continuously or be repeatedly flashed on and off at different rates. An aluminium

75-mm diameter fan blade was mounted on the spindle of a small 12-V DC Maxon motor. Power was supplied by a 12-V car battery. The collecting bag had a slit-like opening along about a third of its circumference in the middle, the edges of the opening were covered with Velcro (a touch- and - close fastening) allowing the opening to be easily closed.

Chemical light trap

Four rechargeable nickel-cadmium 1.25V dry-cell batteries were placed in series in a 4-cell battery holder enclosed in an aluminium box (5 x 10 x 15cm). These batteries powered a 6-V DC motor and small fan mounted in a clear plastic cylinder (135mm long, 105mm diameter). The light source was a 152-mm long and 19-mm diameter "Cyalume" lightstick tied to a mesh screen fixed to the bottom of the plastic cylinder below the fan (Fig. 30). When this flexible plastic tube was bent, an inner glass ampoule was broken releasing 15ml of colourless energy-producing solution that mixed with 6 ml of a greenish fluorescer solution in the outer plastic tube. The strong chemical reaction produced a greenish light. Mosquitoes attracted to the light were sucked up into a nylon mesh cage placed above the cylinder and from which the trap was suspended.

CDC miniature light trap

For trapping emergent adults in May 1980, the CDC miniature light trap developed by Sudia and Chamberlain (1962) was used. A 4-V torch bulb was mounted directly above the motor at the top of the trap body, the trap operated from 6-V supplied from four 1.25 -V torch batteries (Fig. 31).

In experiments designed to catch mosquitoes resting amongst vegetation, that is a trap not placed over the larval habitats, the CDC miniature light trap differed slightly. The cylindrical part consisted of a 14.8-mm long piece of brown plastic tubing and a 6-V, 0.25- A torch bulb was used.

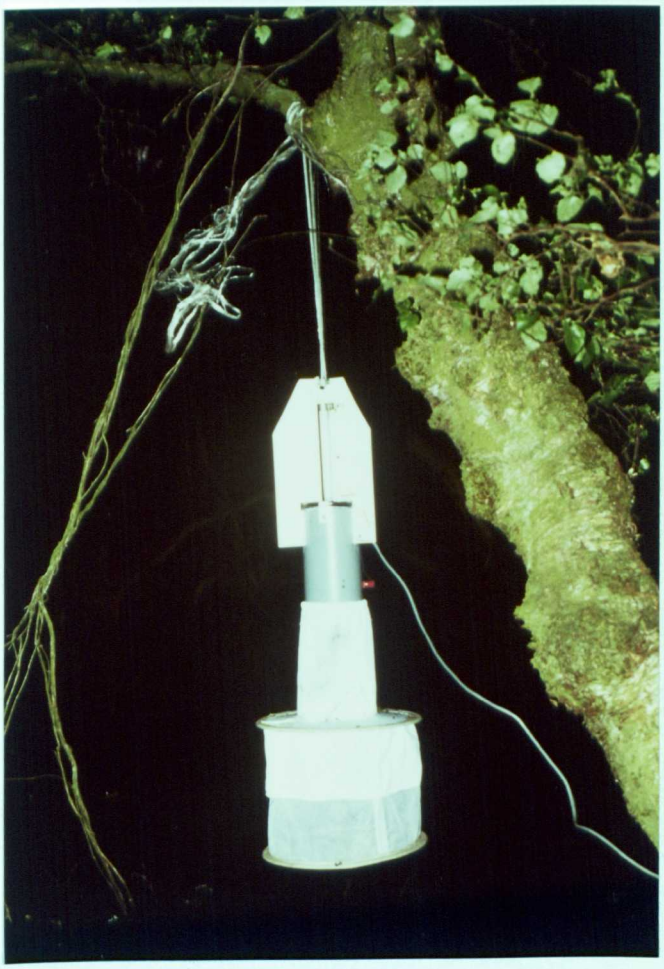

Fig. 29: A Monks Wood light trap.

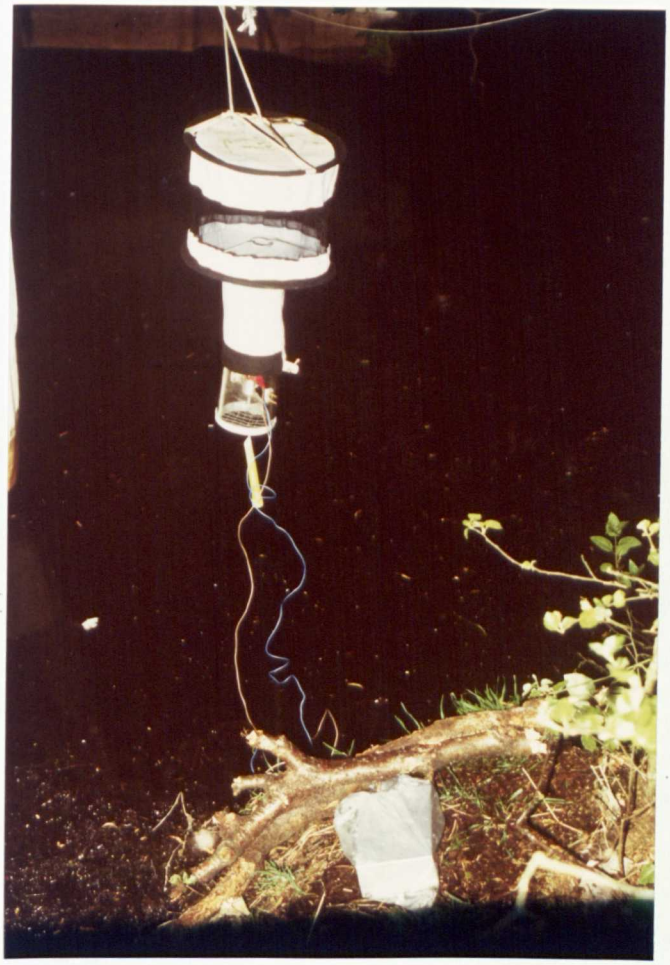

Fig. 30: A chemical light trap suspended on a pond for trapping emerging adults. The aluminium box containing batteries is lying on the ground.

Fig. 31: A CDC miniature light trap in the foreground for trapping emerging adults and a mosquito bednet in the background, both sited over a pond.

Operation of light traps

Between 12-24th May 1980 a Monks Wood light trap, chemical light trap and a CDC light trap were suspended from branches of trees or from string securing mosquito nets in position (Figs. 29-31), so as to be about 10cm above water surface of the pond or ditch.

From 13th June - 29th September 1979 a Monks Wood light trap and a chemical light trap were suspended 1-1.5 metre above the ground amongst scrub vegetation at Ness Woods. In the following year, a Monks Wood light trap, chemical light trap and a CDC miniature light trap were again suspended amongst the scrub vegetation. To avoid sampling bias their positions were changed on different catch nights.

In all collections the lights operated from 2030 - 1000 hr GMT.

Trinidad no. 10 mosquito trap baited with dry ice

Between 6th May - 23rd June 1981, 1kg of dry ice was placed in a Trinidad no. 10 trap which was suspended about 1-1.5 metres above the ground amongst the scrub vegetation from 20.30 to 1000 hr GMT. The dry ice, which consisted of 8 x 3 cm pellets made in the laboratory from a gas cylinder, were wrapped in polystyrene and placed in 5 plastic containers (10 x 12 cm). These open- topped containers were placed on a wooden support placed in each V-section of the trap (Fig. 32). On the following morning any trapped mosquitoes were collected with an aspirator. The location of the trap was changed on different catch night to avoid sampling bias due to position.

Occasionally a Monks Wood light trap, chemical light trap or a CDC light trap (with or without dry ice) were also operated for comparison of catches.

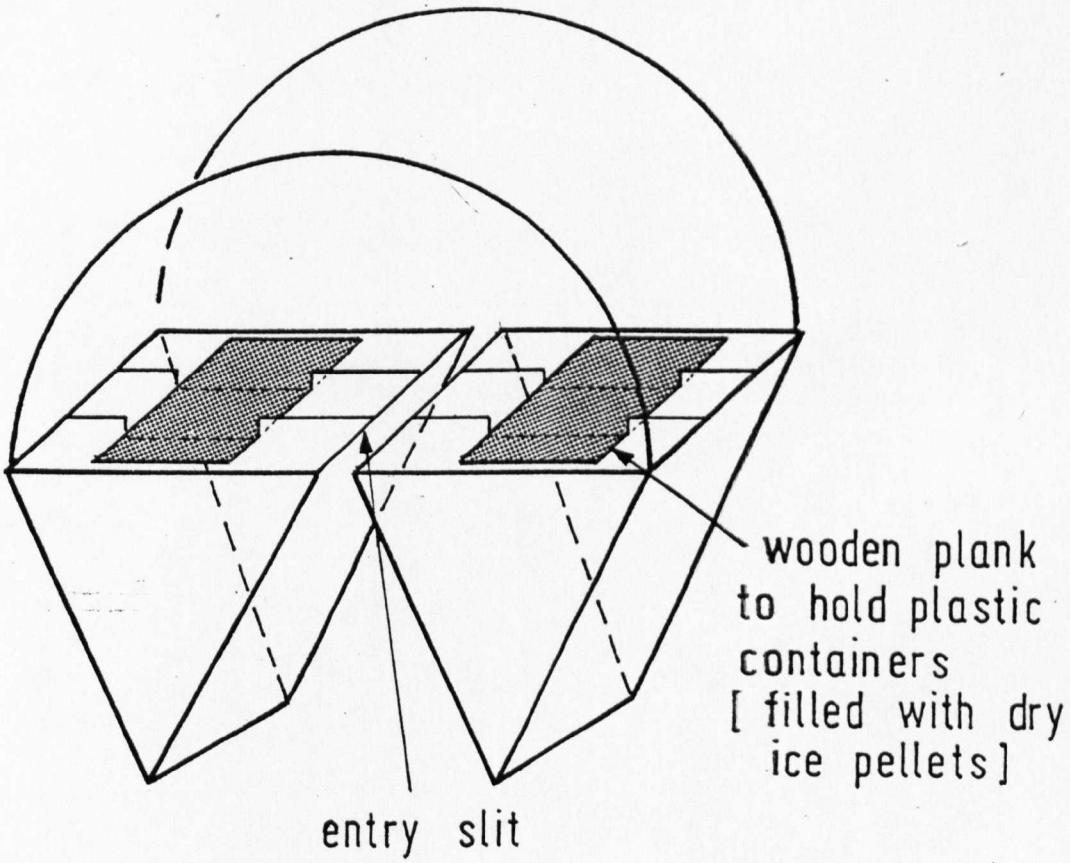

Fig. 32: Diagram of a Trinidad no. 10 trap with mosquito gauze removed.

Marking emergent Ae. cantans adults and release and recapture studies

Between 5th - 18th May 1981, newly emerged Ae. cantans adults were trapped by means of 4 bednets placed over two ponds (Fig. 33). Every 2-3 days during this period, both male and female Ae. cantans caught within the nets were marked with one of the following 'Dayglo' fluorescent dusts - Arch chrome A, Saturn yellow A, Horizon blue A, Signal green A, Blaze A and Corona magenta A. Each colour denotes one particular day of marking the mosquitoes. Marking was done by aspirating the mosquitoes into a transparent plastic bag containing a few milligrams of each fluorescent dust. The bag was gently shaken and rotated. Mosquitoes were then allowed to escape but those unable to fly out of the bag were discarded. The objectives of this experiment were to determine

i) the interval between emergence and parity,

ii) the period between emergence and blood-feeding as determined by bait collection and sweep-netting and

iii) the longevity of both male and female Ae. cantans.

RESULTS

Biting populations

Seasonal incidence of Ae. cantans

The seasonal incidence of Ae. cantans was derived from human-bait catches undertaken between April to October in 1979-1981 and performed between 11.00 - 12.00 hr GMT at a permanent collecting site. However in 1981 the permanent site for bait catches was abandoned and another site about 50 metres away was chosen (see page 78). For comparison of catches in different months and years the total numbers of mosquitoes caught each month were divided by the

Fig. 33: A mosquito bednet placed over a pond for trapping emergent adult Ae. cantans.

number of 1-hour catches to give mean monthly values. Table 44 and Fig. 34 show that in 1979 the highest mean monthly population of Ae. cantans occured in June with a mean of 79 mosquitoes per hour at bait, although the highest 1-hr catch of 187 Ae. cantans was recorded on 10th July. In 1980, maximum biting was again in June (34.8), and the highest single 1 hr-bait catch was on 3rd June 1980 with 93 Ae. cantans. In 1981 the highest mean monthly catch was a month later in July (25.4), but the maximum catch was on 10th June 1981 with 60 Ae. cantans caught. The lowest population of Ae. cantans was in September for all three years, and the adults died off during this month. Table 46 shows that the first capture of female Ae. cantans at bait was on 6th June in 1979, 19th May in 1980 and 1st June in 1981. The last capture was on 19th September in 1979, 9th September in 1980 and 7th September in 1981. Thus, the duration of biting was 106 days in 1979, 114 days in 1980 and 99 days in 1981.

During these bait catches (except for the bait catch on 6th June 1979) the numbers arriving at bait were segregated into 5-minute intervals (Table 45). Figure 35 clearly shows that in all years the greatest proportion of Ae. cantans were caught in the first 5 minutes, and that approximately half (46.0 - 52.8%) of the total hour's catch was obtained during the first 15 minutes. Similar responses have been observed before (Service, 1971b). The explanation is that the high initial catch is due to the arrival at bait of hungry females resting in the immediate vicinity of the bait; later catches consist mainly of adults arriving from further away.

Population size.

The total numbers of Ae. cantans caught in 1979 (June - September) in 31 bait catches was 1297, while in 1980 (May - September) 306 Ae. cantans were caught in 43 catches, and in 1981 (June - September) a total of 411 Ae. cantans

Table 44: Mean monthly numbers of females caught per 1-hr human bait catches, 1979 - 1981. (Total caught in parentheses)

Species	Months and Years																				
	April 1979	May 1979	June 1979	July 1979	Aug. 1979	Sept. 1979	Oct. 1979	April 1980	May 1980	June 1980	July 1980	Aug. 1980	Sept. 1980	Oct. 1980	April 1981	May 1981	June 1981	July 1981	Aug. 1981	Sept. 1981	Oct. 1981
Ae. cantans	0	0	79.0 (474)	60.2 (482)	32.4 (324)	2.4 (17)	0	0	3.7 (33)	34.8 (209)	4.9 (54)	1.3 (9)	0.1 (1)	0	0	0	21.9 (175)	25.4 (203)	5.3 (32)	0.1 (1)	0
Ae. punctor	0	3.6 (32)	1.1 (7)	0.6 (5)	0	0	0	0	0.7 (6)	0.2 (1)	0	0	0	0	0	0.1 (1)	0 8 (6)	0	0	0	0
Ae. detritus	0	0	0	0	0	22.4 (157)	0.8 (5)	0	0	0	0	0.1 (1)	0.2 (3)	0.8 (4)	0	0	0.3 (2)	0	0	0	0
Ae. geniculatus	0	0	0	0.5 (4)	0.2 (2)	0	0	0	0	0.2 (1)	0.1 (1)	0	0	0	0	0	0	0	0	0.1 (1)	0
Anopheles plumbeus	0	0	0	0	0	0.1 (1)	0	0	0	0	0	0.1 (1)	0	0	0	0	0	0	0	0.1 (1)	0
Culiseta annulata	0	0	0	0	0	0	0.2 (1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0

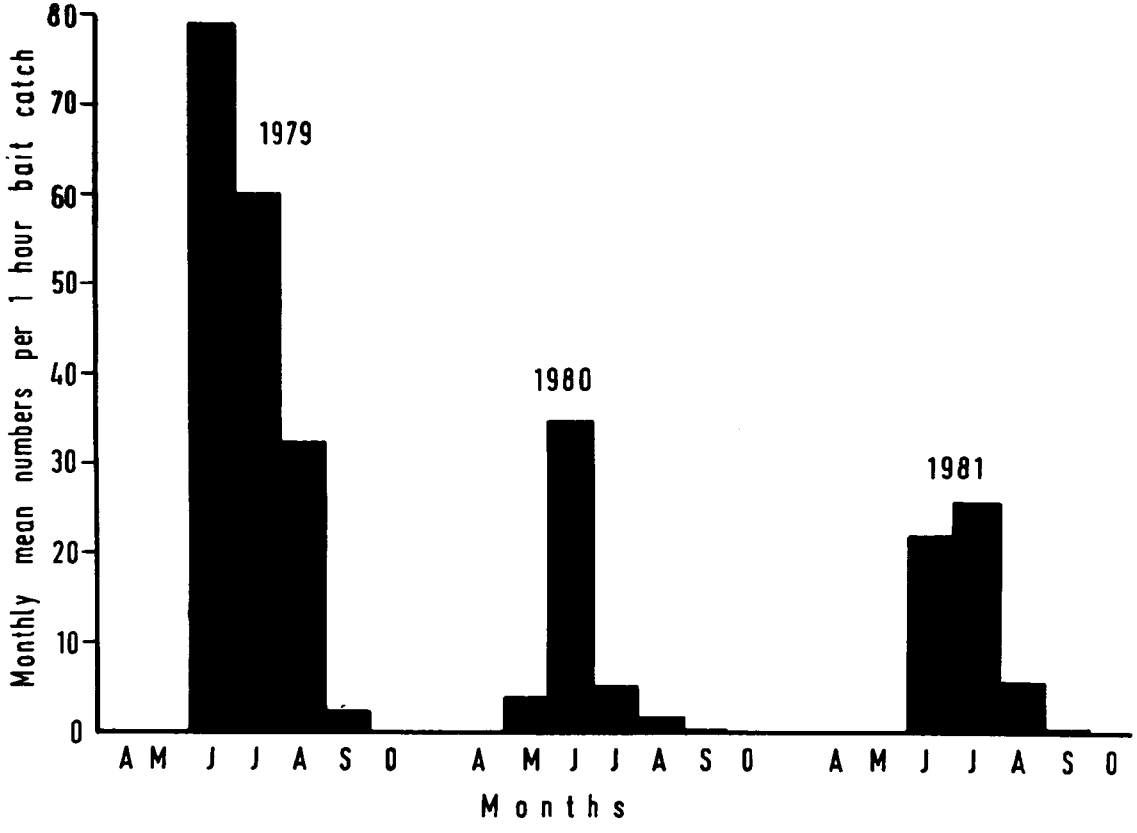

Fig. 34: Seasonal incidence of female Ae. cantans showing monthly mean numbers of females caught by human-bait catches.

Table 45: Results of human-bait catches at intervals of 5 - minutes from 11.00 - 12.00 GMT in 1979-1981 at Ness Woods.

Total Ae. cantans caught (percentages in parentheses)

Year	0 - 5 min.	5- 10 min.	10-15 min.	15-20 min.	20-25 min.	25-30 min.	30-35 min.	35-40 min.	40-45 min.	45-50 min.	50-55 min.	55-60 min.
1979	262 (23.0)	138 (12.1)	124 (10.9)	79 (6.9)	83 (7.3)	82 (7.2)	78 (6.8)	72 (6.3)	58 (5.1)	53 (4.6)	53 (4.6)	59 (5.2)
1980	95 (31.0)	40 (13.1)	23 (7.5)	23 (7.5)	26 (8.5)	28 (9.2)	17 (5.6)	17 (5.6)	10 (3.3)	10 (3.3)	9 (2.9)	8 (2.6)
1981	102 (24.8)	64 (15.6)	51 (12.4)	32 (7.8)	29 (7.1)	21 (5.1)	18 (4.4)	23 (5.6)	19 (4.6)	20 (4.9)	18 (4.4)	14 (3.4)

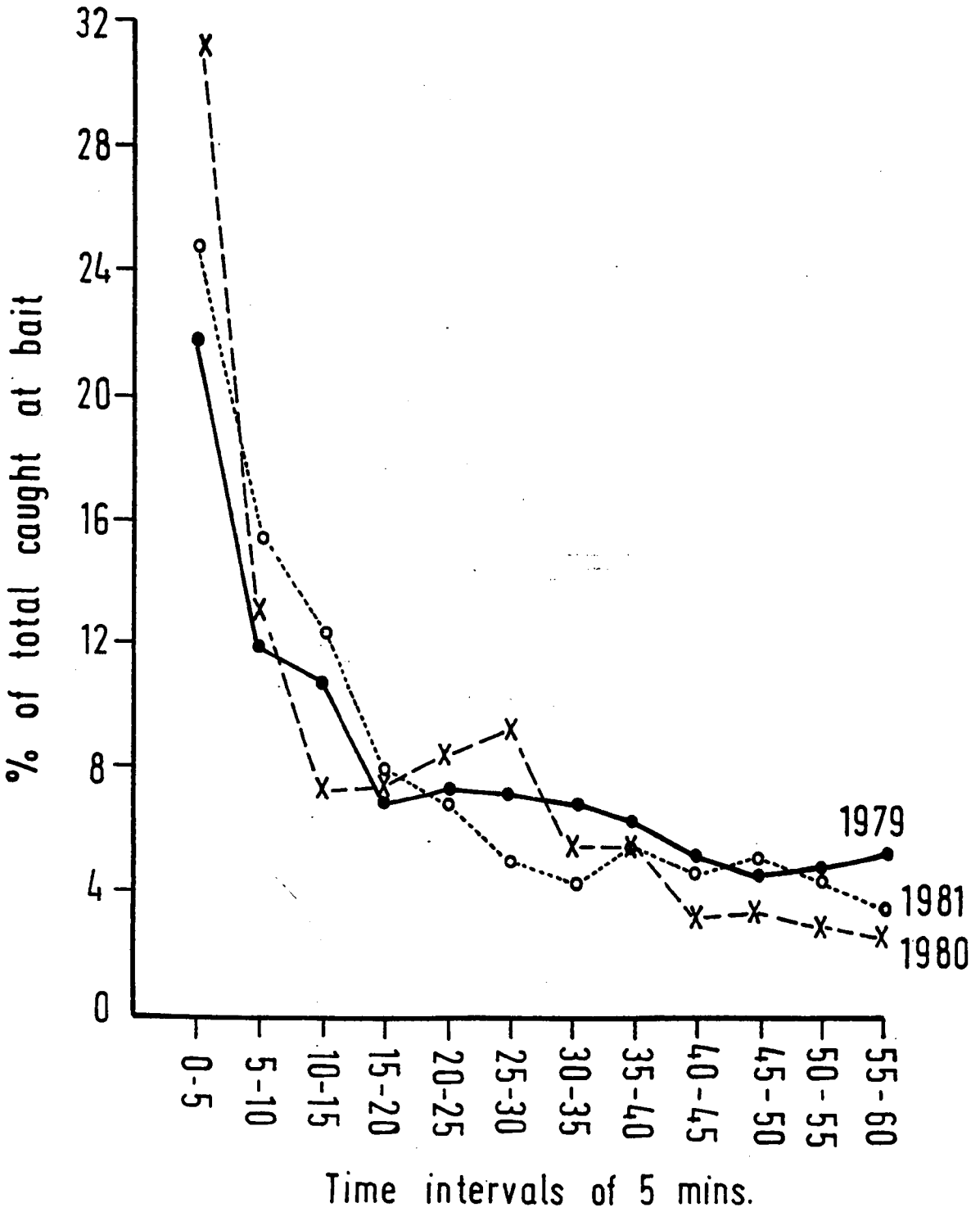

Fig. 35: The percentage of total Ae. cantans caught at human-bait at intervals of 5 minutes from 11.00 - 12.00 GMT in 1979 - 1981 at Ness Woods.

Table 46: Dates of capture of first and last females of Ae. cantans each year at human bait and sweep-netting vegetation, together with dates of capture at bait and sweep-netting vegetation of first parous females.

Human-bait catches

Year	First capture	Last capture	First parous at bait	Duration of biting (days)	Interval between first biting and first capture of parous at bait (days)
1979	6.6.79	19.9.79	22.6.79	106	16
1980	19.5.80	9.9.80	12.6.80	114	24
1981	1.6.81	7.9.81	18.6.81	99	17

Sweep-netting vegetation

Year	First capture	Last capture	First parous at sweep-netting vegetation	Interval between first capture by sweep-netting vegetation and first capture of parous by sweep-netting vegetation (days)
1979	14.5.79	13.9.79	22.6.79	39
1980	6.5.80	2.9.80	12.6.80	37
1981	11.5.81	15.9.81	18.6.81	38

were caught in 32 bait collections. The population of biting adults was clearly considerably greater in 1979 than in the later years. These variations in population size were expected from larval collections, and were also recorded in adults caught by sweep-netting vegetation.

A few other mosquito species with low numbers were also caught. Ae. detritus, breeds only in saline waters, and most surprising adults suddenly appeared at bait in September and October 1979, when 157 and 5 females were collected respectively. The following year 8 females were caught from August - October, and then 2 adults were caught in June 1981, but none later in the year. The nearest breeding place is on the coast about 3 km away. A few Ae. punctor were caught biting, 44 in 1979 and 7 in both 1980 and 1981. This species is breeding in relatively small numbers in Ness Woods but is more common in the small wood to the north, about 250 metres away. Ae. geniculatus is a tree-hole breeding species and only 6 adults were caught at bait in 1979, 2 in 1980 and 1 in 1981. An. plumbeus, is another tree-hole species at Ness Woods, only 1 adult was caught at bait in each year. Larvae of Cs. annulata occur in ditch and ponds and a single female was caught at bait in 1979.

Sugar feeding

No male mosquitoes were caught at bait. Females consisted largely of unfed individuals, but two half-gravid and 1 gravid Ae. detritus were caught at bait in 1979, another half-gravid Ae. detritus in 1980 and 1 gravid Ae. cantans was caught in 1981.

In 1981, some Ae. cantans caught at bait were subjected to the anthrone test to determine the presence of sugar in their crops. From a total of 304 Ae. cantans tested, 68 (22.4%) nulliparous and 69 (22.7%) parous Ae. cantans gave a positive reaction, indicating that sugar-meals in nature are taken both before and after blood-feeding.

Parity rates

Table 47 shows the monthly parity rate of unfed female Ae. cantans caught at human-bait. In June (1979-1981) the monthly parity rate of Ae. cantans was between 10.2 - 33.1%, in July between 68.8 - 96.2%, in August between 84.4 - 100% while in September a 100% monthly parity rate was recorded. In 1979 the last nulliparous Ae. cantans, having spermatozoa in the spermathecae, was caught on 28th August, in 1980 it was caught on 14th July and in 1981 on 12th August. In 1981, when 4 bednets (Fig. 33) were placed over 2 ponds for trapping emerging adults, the last day on which female Ae. cantans emerged was 18th June. Thus, the difference in time between the last adult emergence and the last nulliparous adult caught at bait in 1981 was 55 days. This indicates that some female Ae. cantans can remain nulliparous without blood-feeding for as long as 55 days. Bed nets were not used in the earlier years, but the last pupae were seen on 23rd June 1979 and 11th June 1980. If 10 days are allowed for pupal duration then the last adults probably emerged on 3rd July 1979 and 21st June 1980. Comparing these dates with the last appearance of nullipars at bait in these two years indicates that they were 56 and 23 days old.

The first parous Ae. cantans was found at bait in 1979 on 22nd June, in 1980 on 12th June and in 1981 on 18th June. The intervals between first biting and first capture of parous at bait were between 16-24 days (Table 46), and the period between first adult emergence and parity was 37-39 days.

Because of the low numbers of other species caught at bait catches it is difficult to see the seasonal trend in parity of these species. But for Ae. detritus it is worthwhile recording that in September and October 1979 when 157 and 5 females respectively suddenly appeared at bait, 55.7% of the 149 dissected were

Table 47: Monthly parity rate of unfed Ae. cantans caught from human-bait catches at Ness Woods

Month and Year	No. caught	No dissected	Nulliparous	Parous	% Parous
May 1979	0	0	0	0	0
May 1980	33	33	33	0	0
May 1981	0	0	0	0	0
June 1979	474	447	299	148	33.1
June 1980	209	207	183	24	13.1
June 1981	175	127	114	13	10.2
July 1979	482	454	62	392	86.3
July 1980	54	53	2	51	96.2
July 1981	203	189	59	130	68.8
August 1979	324	323	6	317	98.1
August 1980	9	9	0	9	100.0
August 1981	32	32	5	27	84.4
September 1979	17	13	0	13	100.0
September 1980	1	1	0	1	100.0
September 1981	1 (but escaped)	0	0	0	0.0

nulliparous. This shows that newly emerged Ae. detritus as well as older females dispersed inland from the coast for about 3km in search of blood-meals. As this is entirely a salt-marsh species, then for any benefit for the species they would have to fly back again to the coast to lay eggs.

Ectoparasites

Two species of mites were seen to be attached to the neck and thorax of Ae. cantans, their numbers ranging from 1- 5 per mosquito. They were Thyas barbigera and a larval erythraeid which appears to belong to the genus Leptus. Table 48 shows that in 1980, a single parous female Ae. cantans caught at bait was parasitized by a mite whereas in 1981, 24 female Ae. cantans caught biting were parasitized by mites. Nine of these were nulliparous and 15 parous. The incidence of parasitism was very low, 0.3% in 1980 and 5.8% in 1981.

Comparison of sweep-netting various types of vegetation

In the summer of 1979, collections of mosquitoes by sweep-netting were made in different types of ground vegetation, namely brambles, ferns, and grasses in Ness Woods; in addition pootering with an aspirator collected adults resting on tree trunks up to a height of 2 metres. A total of 565 sweeps over 12 sampling days were made from each type of vegetation and during the same time there collections were made from 565 trees.

Table 49 shows that sweep-netting brambles collected 289 female Ae. cantans comprising 83 unfed, 31 bloodfed, 78 half-gravid and 97 gravid individuals. In addition 186 male Ae. cantans were collected, thus giving a total of 475 Ae. cantans adults. Sweep-netting ferns yielded 227 female Ae. cantans, comprising 91 unfed, 15 bloodfed, 55 half-gravid and 66 gravid adults. Males totalled 157 and the total catch of Ae. cantans was 384. When grasses were

Table 48 : Results showing the percentage of Ae. cantans (caught by sweep-netting vegetation and human-bait catches) parasitized by mites and also the parity of the parasitized unfed mosquitoes

Year	Method of collection	No of ♀ Ae. cantans caught	No of ♀ Ae. cantans parasitized by mites	parity	% of female Ae. cantans parasitized	No of ♂ Ae. cantans caught	No of ♂ Ae. cantans parasitized by mites	% of ♂ Ae. cantans parasitized
1980	Sweep-netting vegetation	598	2(1 unfed 1 gravid)	1 parous	0.3%	347	0	0%
1981	Sweep-netting vegetation	369	5(3 unfed 1 bloodfed 1 half-gravid)	3 nulliparous	1.4%	175	1	0.6%
1980	Human-bait catches	306	1 (unfed)	1 parous	0.3%	-	-	-
1981	Human-bait catches	411	24 (all unfed)	9 nulliparous 15 parous	5.8%	-	-	-

Table 49: Numbers and percentages in parentheses of Ae. cantans caught resting on 565 tree trunks and from 565 sweep-net collection amongst different ground vegetation over 12 days in 1979 at Ness Woods

	Sweep-netting brambles	Sweep-netting ferns	Sweep-netting grasses	Aspirating from tree trunks
Unfed	83 (28.7)	91 (40.1)	169 (50.0)	29 (85.3)
Bloodfed	31 (10.7)	15 (6.6)	32 (9.5)	1 (2.9)
Half-gravid	78 (27.0)	55 (24.2)	72 (21.3)	2 (5.9)
Gravid	97 (33.6)	66 (29.1)	65 (19.2)	2 (5.9)
Total females	289	227	338	34
Total males	186	157	265	9
Total Ae. cantans	475	384	603	43

sweep-netted 338 female Ae. cantans were collected, comprising 169 unfed, 32 bloodfed, 72 half-gravid and 65 gravid individuals, and 265 males; thus giving a total of 603 Ae. cantans adults.

Only 34 female and 9 male Ae. cantans were collected from searching 565 tree trunks, most of the females were unfed individuals. Clearly tree trunks are not a favoured resting site of Ae. cantans, and it appears the species prefers more sheltered positions provided by dense vegetation.

Results of statistical analysis using t-tests have shown that there is no significant difference between the total numbers of Ae. cantans sampled among the three different types of ground vegetation. However, there were significantly more Ae. cantans resting on brambles than trees ($P < 0.02$), on ferns than trees ($P < 0.01$) and on grasses than trees ($P < 0.01$).

Seasonal incidence of Ae. cantans caught by sweep-netting vegetation

The first male Ae. cantans captured by sweep-netting vegetation in 1979 was on 14th May and the last capture was on 2nd August. In 1980, the first capture was on 6th May and the last on 15th July, in 1981, these dates were 29th April and 1st July respectively. Table 46 shows that the first female captured by sweep-netting vegetation in 1979 was on 14th May and last female caught was on 13th September. In 1980, the first capture was on 6th May and last on 2nd September, and in 1981 the first and last captures were on 11th May and 15th September respectively. Comparison of these dates indicate that both sexes appeared more or less together amongst vegetation, but males of Ae. cantans were last collected 6-10 weeks before the last females. It seems that males longevity is considerably less than that of the females.

Tables 50-52 show the seasonal variation in numbers of mosquitoes resting amongst the vegetation at Ness Woods during 1979 - 1981; based on the mean monthly numbers caught per 100 sweeps. In 1979 the highest populations of unfed Ae. cantans was recorded in May and June (22.70 and 22.29 Ae. cantans per 100 sweeps respectively). The highest numbers of bloodfed, half-gravid and gravid female Ae. cantans were recorded in June with 5.90 bloodfed, 16.48 half-gravid and 14.00 gravid females per 100 sweeps (Table 50). In 1980, the highest populations of unfed Ae. cantans was recorded in May and June with 13.40 and 10.50 mosquitoes per 100 sweeps respectively (Table 51). The highest number of bloodfed female Ae. cantans was recorded in June with 1.67 bloodfed. The highest numbers of half-gravid (1.67, 2.44) and also gravid (4.08, 4.78) females were both recorded in June and July. In 1981, the highest populations of unfed, bloodfed, half-gravid and gravid female Ae. cantans were recorded in June with 12.58 unfed, 1.42 bloodfed, 1.50 half-gravid and 2.50 gravid female Ae. cantans per 100 sweeps (Table 52). Comparing the population of all physiological stages of female Ae. cantans sampled by sweep-netting vegetation throughout the three years (1979-1981) shows that the highest population was in 1979 followed by 1980 and the lowest was in 1981. This same decrease in population size was obtained by human bait catches (See pages 83-84). Similarly the largest numbers of male Ae. cantans were collected in 1979 with a peak of 44.95 mosquitoes per 100 sweeps in June, followed by 1980 with a peak of 15.41 mosquitoes in May, but only 8.83 male Ae. cantans per 100 sweeps were caught in June 1981. In May, at the beginning of adult emergence in 1979 males comprised 61.4% of the total catch of Ae. cantans giving a male : female sex ratio of 1 : 0.6, and in June males formed 43.4% of the total catch giving a male : female sex ratio of 1 : 1.3. Combining the catches in May and June the male : female

Table 50: Mean monthly numbers of mosquitoes caught per 100 sweeps of the vegetation at Ness Woods in 1979.

Total numbers caught in parentheses

Months	Total no. of sweeps	Species	Females: Unfed	Bloodfed	Half-gravid	Gravid/ fatty tissues*	Total females	Males	Total
April	150	Ae. cantans	0	0	0	0	(0)	0	(0)
		Ae. punctor	0	0	0	0	(0)	0	(0)
May	600	Ae. cantans	22.70 (136)	0	0	0	(136)	36.00 (216)	(352)
		Ae. punctor	0.33 (2)	0	0	0	(2)	1 (6)	(8)
June	1050	Ae. cantans	22.29 (234)	5.90 (62)	16.48 (173)	14.00 (147)	(616)	44.95 (472)	(1088)
		Ae. punctor	0.10 (1)	0.10 (1)	0.10 (1)	0	(3)	0	(3)
July	1350	Ae. cantans	9.41 (127)	2.15 (29)	5.04 (68)	10.07 (136)	(360)	3.04 (41)	(401)
		Ae. punctor	0. 15 (2)	0.07 (1)	0.07 (1)	0.15 (2)	(6)	0	(6)
		Cs. annulata	0	0	0.07 (1)	0.15 (2)	(3)	0	(3)

Continued .....

Table 50: (continued)

Months	Total no of sweeps	Species	Females: Unfed	Bloodfed	Half-gravid	Gravid/ fatty tissues*	Total females	Males	Total
August	1950	Ae. cantans	1.69 (33)	0.15 (3)	0.62 (12)	1.08 (21)	(69)	0.27 (5)	(74)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Cs. annulata	0.31 (6)	0	0	0.10 (2)	(8)	0.92 (18)	(26)
September	1050	Ae. cantans	0.10 (1)	0	0.48 (5)	0.29 (3)	(9)	0	(9)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0.67 (7)	0.10 (1)	6.86 (72)	4.67 (49)	(129)	5.14 (54)	(183)
		C. pipiens	0.19 (2)	0	0	0.38* (4)	(6)	1.14 (12)	(18)
		Cs. annulata	1.33 (14)	0	0.10 (1)	0.20 (2)	(17)	1.43 (75)	(92)
October	450	Ae. cantans	0	0	0	0	(0)	0	(0)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0	0	0	0.22 (1)	(1)	0	(1)
		C. pipiens	0	0	0	0.22* (1)	(1)	0	(1)
		Cs. annulata	0	0	0	1.11* (5)	(5)	3	(8)

Table 51: Mean monthly numbers of mosquitoes caught per 100 sweeps of the vegetation at Ness Woods in 1980.

Total numbers caught in parentheses

Months	Total no. of sweeps	Species	Females					Males	Total
			Unfed	Bloodfed	Half-gravid	Gravid/ Fatty tissues*	Total females		
April	150	Ae. cantans	0	0	0	0	(0)	0	(0)
		Ae. punctor	0	0	0	0	(0)	0	(0)
May	1350	Ae. cantans	13.40 (181)	0.22 (3)	0.07 (1)	0	(185)	15.41 (208)	(393)
		Ae. punctor	0.37 (5)	0	0.15 (2)	0	(7)	0.67 (9)	(16)
June	1200	Ae. cantans	10.50 (126)	1.67 (20)	1.67 (20)	4.08 (49)	(215)	9.92 (119)	(334)
		Ae. punctor	0	0	0	0	0	0.17 (2)	(2)
July	1800	Ae. cantans	2.39 (43)	0.83 (15)	2.44 (44)	4.78 (86)	(188)	1.11 (20)	(208)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Cs. annulata	0.06 (1)	0	0	0	(1)	0.11 (2)	(3)

Continued .....

Table 51: (Continued)

Months	Total no. of sweeps	Species	Females					Males	Total
			Unfed	Bloodfed	Half-gravid	Gravid/ Fatty tissues	Total females		
August	750	Ae. cantans	0	0.13 (1)	0.67 (5)	0.27 (2)	(8)	0	(8)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0.13 (1)	0	0	0	(1)	0	(1)
		C. pipiens	0.40 (3)	0	0	0.80* (6)	(9)	0.27 (2)	(11)
		Cs. annulata	0	0	0	0	(0)	0.53 (4)	(4)
September	1500	Ae. cantans	0.07 (1)	0	0.07 (1)	0	(2)	0	(2)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0	0	0.07 (1)	0	(1)	0	(1)
		C. pipiens	0.07 (1)	0	0	0.20* (3)	(4)	0.27 (4)	(8)
		Cs. annulata	0	0	0	0	(0)	0.33 (5)	(5)
October	750	Ae. cantans	0	0	0	0	(0)	0	(0)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0	0	0.27 (2)	0	(2)	0	(2)
		C. pipiens	0	0	0	0	(0)	0.13 (1)	(1)
		Cs. annulata	0	0	0	0	(0)	0.67 (5)	(5)

Table 52: Mean monthly numbers of mosquitoes caught per 100 sweeps of the vegetation at Ness Woods in 1981

Total numbers caught in parentheses

Months	Total no of sweeps	Species	Females					Males	Total
			Unfed	Bloodfed	Half-gravid	Gravid/ Fatty tissues*	Total females		
April	600	Ae. cantans	0	0	0	0	(0)	0.17 (1)	(1)
		Ae. punctor	0	0	0	0	(0)	0	(0)
May	1650	Ae. cantans	4.00 (66)	0	0	0	(66)	4.00 (66)	(132)
		Ae. punctor	0.67 (11)	0.06 (1)	0.06 (1)	0	(13)	0	(13)
June	1200	Ae. cantans	12.58 (151)	1.42 (17)	1.50 (18)	2.50 (30)	(216)	8.83 (106)	(322)
		Ae. punctor	0.17 (2)	0	0	0.08 (1)	(3)	0	(3)
		Ae. detritus	0.08 (1)	0	0	0	(1)	0	(1)
July	1350	Ae. cantans	3.41 (46)	0.30 (4)	0.44 (6)	1.41 (19)	(75)	0.07 (1)	(76)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0	0	0	0	(0)	0	(0)
August	1050	Ae. cantans	0.67 (7)	0	0.10 (1)	0.29 (3)	(11)	0	(11)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0	0	0	0	(0)	0	(0)
		C. pipiens	0.29 (3)	0	0	0	(3)	0.29 (3)	(6)

Continued .....

Table 52: (Continued)

Months	Total no of sweeps	Species	Females				Total females	Males	Total
			Unfed	Bloodfed	Half-gravid	Gravid/ Fatty tissues*			
		Cs. annulata	0	0	0	0	(0)	0.67 (7)	(7)
September	1500	Ae. cantans	0	0	0	0.07 (1)	(1)	0.07 (1)	(2)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0	0	0	0	(0)	0	(0)
		C. pipiens	0	0	0	0	(0)	0.07 (1)	(1)
		Cs. annulata	0	0.07 (1)	0.07 (1)	0.07* (1)	(3)	0.67 (10)	(13)
October	600	Ae. cantans	0	0	0	0	(0)	0	(0)
		Ae. punctor	0	0	0	0	(0)	0	(0)
		Ae. detritus	0	0	0	0	(0)	0	(0)
		C. pipiens	0	0	0	0	(0)	0	(0)
		Cs. annulata	0	0	0	0	(0)	0.17 (1)	(1)

sex ratio is 1 : 1.1, which indicates about an equal production of both sexes, but with males being more common in May than June. In July and August, males comprised only 10.2% and 6.8% of the total Ae. cantans respectively, indicating heavier mortality than in the females. In May of 1980, males comprised 52.9% of the total catch of Ae. cantans giving a male : female sex ratio of 1 : 0.9, in June males formed 35.6% of the catch giving a male : female sex ratio of 1 : 1.8. After combining the catches in May and June the male : female sex ratio is 1: 1.2. In July, males comprised only 9.6% of the total Ae. cantans, again indicating heavier mortality than in the females. No males were recorded from August onwards. In May 1981, males comprised 50.0% of the total catch of Ae. cantans giving a male : female sex ratio of 1: 1, in June males formed 32.9% of the total catch of Ae. cantans giving a male : female sex ratio of 1: 2. Combining the catches in May and June the male : female sex ratio is 1: 1.6 suggesting that in this year the number of emerging females was greater than the males, but as the sex ratio was equal in May it seems that the number of emerging males and females was again about equal, and that the reduced catch of males in June, reflected more male mortality than an unequal sex ratio. In July, males comprised only 1.3% of the total Ae. cantans, again as in the previous two years indicating heavier mortality than in the females.

Besides Ae. cantans which was the predominant species at Ness Woods, small populations of Ae. punctor, Ae. detritus, C. pipiens and Cs. annulata were also caught by sweep-netting vegetation. Ae. detritus, a salt-marsh mosquito was suddenly found biting at Ness Woods in September and October 1979. In the same months both males (54) and females (130) were found resting amongst vegetation.

In the following year, however, only 4 females were collected from August - October, and only a single female was caught in the woods in June of 1981. C. pipiens is an ornithophilic mosquito and it would not be expected to appear at bait, and none has been found breeding within the woods, but 2 unfed females, 5 females with well developed fat reserves, and 12 males were caught by sweep-netting during September - October, 1979. During August - October 1980, 4 unfed females and 9 with fat reserves plus 7 males were caught and finally in 1981 3 unfed females , 4 males from August - September. Adults are known to begin entering hibernation sites during these months (see page 107) and presumably those caught in Ness Woods represented adults seeking suitable hibernation sites.

Small numbers of Ae. punctor and Cs. annulata were caught in all years, they were also occasionally seen as larval stages in some of the ponds at Ness Woods.

Physiological condition of resting adults

Sugar feeding

Table 53 shows that in May of 1979- 1981, the presence of a liquid, taken as indicative of sugar feeding, in the crop of female Ae. cantans caught by sweep-netting vegetation was very high, ranging between 94.1 - 100%. This percentage of crops filled with liquid decreased in June, July and August. In 1981 the fluid in the crop was subjected to the anthrone test and this confirmed that the fluid was indeed sugary. This shows that in May, a period when females are usually not seeking blood-meals, female Ae. cantans feeds on sugar. In June with the onset of blood-feeding the incidence of sugar feeding decreased, although as many as 39.1-57.1% still fed on sugar as late as August in both 1979 and 1981.

Table 53: Sugar (liquid) content of the crop of unfed female Ae. cantans caught by sweep-netting vegetation

Year	Months	No caught	No dissected or tested*	No with liquid	% with liquid
1979	April	0	0	0	0
	May	136	136	128	94.1
	June	234	191	118	61.8
	July	127	76	37	48.7
	August	33	23	9	39.1
	September	1	0	0	0
1980	April	0	0	0	0
	May	181	181	176	97.2
	June	126	126	77	61.1
	July	43	31	16	51.6
	August	0	0	0	0
	September	1	0	0	0
1981*	April	0	0	0	0
	May	66	53	53	100.0
	June	151	102	56	54.9
	July	46	43	27	62.8
	August	7	7	4	57.1
	September	0	0	0	0

* In 1981, the test for sugar was conducted using the Anthrone test.

Parity rates

Table 46 shows that the first parous Ae. cantans was seen on 22nd June, 12th June and 18th June in the years 1979-1981. The same dates were obtained for the first parous adults caught at bait. The interval between first capture by sweep-netting vegetation and first capture of parous by sweep-netting vegetation was 39 days in 1979, 37 days in 1980 and 38 days in 1981. The parity rate of unfed female Ae. cantans in June ranged between 9.0 - 32.0%, in July between 79.1 - 93.0% and in August and September a 100%. Thus the parity rates of Ae. cantans caught by sweep-netting vegetation were more or less similar to those caught by bait catches (Tables 47 and 54).

Ectoparasites

Table 48 shows that in 1980, 2 female Ae. cantans (1 parous unfed and 1 gravid) caught by sweep-netting vegetation were parasitized by mites. In 1981, 5 female Ae. cantans (3nulliparous unfed, 1 bloodfed and 1 half-gravid) and 1 male were parasitized by mites. Therefore it is concluded that mites parasitize both nulliparous and parous Ae. cantans. As with adults caught at bait the percentage parasitized is very small.

Host preferences

Filter paper smears of the stomach contents made from blood-fed mosquitoes caught by sweep-netting were identified by precipitin tests by Dr. P.F.L. Boreham of the Imperial College of Science and Technology at Ascot. The results of these serological tests on adults collected in 1979 are pooled (Table 55).

Table 54: Monthly parity rate of unfed Ae. cantans caught by sweep-netting vegetation at Ness Woods

Month and Year	No caught	No dissected	Nulliparous	Parous	% Parous
April 1979	0	0	0	0	0
April 1980	0	0	0	0	0
April 1981	0	0	0	0	0
May 1979	136	136	136	0	0
May 1980	181	175	175	0	0
May 1981	66	53	53	0	0
June 1979	234	219	149	70	32.0
June 1980	126	126	97	29	23.0
June 1981	151	111	101	10	9.0
July 1979	127	125	22	103	82.4
July 1980	43	43	3	40	93.0
July 1981	46	43	9	34	79.1
August 1979	33	33	0	33	100.0
August 1980	0	0	0	0	0.0
August 1981	7	7	0	7	100.0
September 1979	1	1	0	1	100.0
September 1980	1	1	0	1	100.0
September 1981	0	0	0	0	0.0
October 1979	0	0	0	0	0.0
October 1980	0	0	0	0	0.0
October 1981	0	0	0	0	0.0

Table 55: Numbers, with percentages (in parentheses) of feeds in 1979 on various hosts

Species	Man	Bovid	Sheep/Goat	Rabbit	Horse	Unidentified mammal	Unidentified bovid	Negative	Total
Ae. cantans	2 (1.0)	161 (81.3)	7 (3.5)	2 (1.0)	1 (0.5)	4 (2.0)	12 (6.1)	9 (4.5)	198
Ae. punctor	0	4 (57.1)	2 (28.6)	0	0	0	1 (14.3)	0	7
Ae. detritus	0	14 (77.8)	1 (5.6)	0	0	0	1 (5.6)	2 (11.1)	18
Cs. annulata	0	0	1 (100)	0	0	0	0	0	1

Negative results indicate that the smears were of poor quality, probably the blood proteins were too far digested and the blood could not be identified. Unidentified mammalia feeds refer to poor quality smears that could not be identified further. The term bovid indicates that feeds were from cattle and not sheep or goats and unidentified bovids that only weak precipitin reactions were obtained and that sheep and goats cannot be excluded as hosts.

Table 55 shows that bovid (cattle) was the principal host of Ae. cantans, Ae. punctor and Ae. detritus. From 198 smears of Ae. cantans tested 2 smears (1%) were positive for feeding on man, and were probably derived from the author while sweep-netting the vegetation. 161 (81.3%) of these gut smears had bovid blood, showing that cattle were the principal hosts. A few smears were positive for sheep/goat and horse, all these animals including cattle are kept in fields adjacent to Ness Woods. Rabbits were present in the woods, but were not common and only 2 feeds were from this animal. In fact very few smears could have been from any animals living within the wood, because of the paucity of small mammals in the wood. Clearly Ae. cantans at Ness Woods feeds predominantly on domestic animals kept in neighbouring fields.

Very few blood engorged females of other species were collected and tested, but again the results indicate that cattle were the preferred hosts.

Light traps collections of emerging adults

Between 12th - 24th May 1980 a Monks Wood light trap, chemical light trap and a CDC light trap were suspended about 10cm above the water surface of ponds or a ditch known to have large numbers of mosquito larvae. In 4 night catches with a Monks Wood trap, no mosquito was caught; from 4 night catches with a chemical light trap placed both above a pond and a ditch, only a single unfed Cs. annulata

was caught; finally 4 catches with a CDC light trap over a pond gave only a single male Ae. cantans. These results show that despite the emergence at this time of year of large numbers of Ae. cantans, they are not attracted to light traps.

Light traps collections of mosquitoes resting amongst vegetation

Between 13th June - 29th September 1979, a Monks Wood light trap and a chemical light trap were suspended 1- 1.5 metre above ground level amongst vegetation. From a total of 14 catches with a Monks Wood light trap 9 unfed female Ae. cantans; 1 unfed, 2 half-gravid and 4 gravid female Ae. detritus; 14 unfed female and 5 male C. pipiens; 19 unfed female and 46 male Cs. annulata were caught. Although there were large population of Ae. cantans both resting amongst vegetation and biting, the Monks Wood trap was inefficient in sampling them. The 7 male Ae. detritus were caught in September, a time when they suddenly appeared in the wood at bait and at rest in vegetation. Similarly the 19 C.pipiens were caught at a time when the species is seeking hibernation sites (September - October), and when adults were also caught for the first time resting amongst vegetation of Ness Woods. Although Cs. annulata is much less common in Ness Woods than Ae. cantans, more (65) were caught, suggesting that the Monks Wood light trap is rather more attractive to this species than to the others.

In 1979 a chemical light trap managed to catch only 5 unfed female Ae. cantans and 2 gravid Cs. annulata during the same period.

Between 3rd July - 2nd October 1980, in addition to the Monks Wood and chemical light traps, a CDC light trap was also used for sampling adults resting amongst vegetation. Even fewer mosquitoes were caught than in 1979. The Monks Wood trap caught 1 unfed female and 1 male Ae. cantans, 1 unfed female Ae. detritus

and 3 male C. pipiens from a total of 13 night catches. The chemical light trap caught just 2 gravid female Cs. annulata from 13 night catches, and the CDC light trap caught in 13 nights 1 male Ae. detritus; 1 unfed and 1 female C. pipiens with fat reserves, 2 male C. pipiens and 1 gravid female Cs. annulata.

From 6th May - 23rd June 1981, 3 night collections with a Monks Wood trap yielded only 2 unfed female C. pipiens, 3 night catches with a chemical trap gave no mosquitoes while 3 similar catches with the CDC trap resulted in trapping 2 unfed female C. pipiens. Finally, a CDC trap supplemented with 400gm of dry ice on one night caught 1 unfed female Ae. cantans and 1 unfed female An. plumbeus.

From these results, Ae. cantans seems not to be attracted to light traps. However, a few mosquitoes of other species such as C. pipiens and Ae. detritus which were not breeding in this woodland were caught by the light traps, and these traps may be more effective in catching these species, and as mentioned above Cs. annulata.

Carbon dioxide trap

In 1981, a Trinidad no. 10 trap containing 1kg of dry ice was evaluated on 5 nights at Ness Woods from 6th May - 23rd June 1981. Only 1 unfed female Ae. cantans was caught by the trap. Dry ice, even when combined with a light (see above) does not seem suitable for sampling Ae. cantans or the other mosquitoes present in Ness Woods.

Results of marking, release and recapture of emergent Ae. cantans

Between 5th May - 18th May 1981, a total of 1409 Ae. cantans comprising 665 females and 744 males were collected as emerging adults in the bed nets and marked with 'Dayglo' fluorescent dusts. Only 1 marked parous female was re-caught by sweep-netting vegetation, and that 58 days after marking and release (Table 56), giving a recapture rate of 0.07%. Unfortunately little information can be obtained from this experiment because of the extremely low recapture rate.

Table 56: Number of *Ae. cantans* marked at Ness Woods in 1981.

Date	Type of fluorescent dust	No of ♂ marked	No of ♀ marked	Total
5.5.81	Arc chrome A	30	5	35
8.5.81	Saturn yellow A	130	30	160
11.5.81	Horizon blue A	140	116	256
13.5.81	Signal green A	199	230	429
15.5.81	Blaze A	124	169	293
18.5.81	Corona magenta A	121	115	236
		Total = 744	Total = 665	Total = 1,409

DISCUSSION ON ADULT STUDIES

Service(1977a) studied the ecology of Ae. cantans in southern England during 1967 - 1972. He found that the monthly mean number of Ae. cantans caught at bait was highest in July throughout his six years of study. Females were not caught at human-bait until about 20 days after emergence had begun. By marking newly emerged females and recapturing them in human-bait catches, Service (1977a) confirmed that marked females were never caught biting until 21- 23 days after their emergence. Hence, there was definitely an interval of about three weeks between adult emergence and blood-feeding. During the first 3 or 4 weeks at bait all females were still nulliparous. The reason for this consistent delay in blood-feeding was not discovered. The fact that nullipars were found amongst females that were up to eight weeks old suggested that Ae. cantans may experience difficulty in getting a blood-meal.

The present studies at Ness Woods show that maximum biting of Ae. cantans in 1979 was in June, although the peak population was reached on 10th July with 187 Ae. cantans. In 1980, most intense biting was recorded in June with the highest single 1-hr bait catch of 93 Ae. cantans on 3rd June. In 1981, the highest population of Ae. cantans occurred in July, but the maximum number in a single 1-hr bait catch was still in June, with 60 Ae. cantans caught on 10th June. Each September all remaining adults of Ae. cantans die off.

The interval between the first capture of Ae. cantans from sweep-netting vegetation and its capture at bait in 1979 was 24 days, 14 days in 1980 and 22 days in 1981. This indicates that Ae. cantans began searching for the first blood-meal 2-3 weeks after emergence - assuming that the day mosquitoes were first found amongst vegetation is near their day of emergence. The reason for this

delay in feeding is not known, but has been observed before in this species (Service, 1977a) and also in other species (Service, 1969a). This behaviour is in marked contrast to that of most tropical species which generally feed within a day or so after emergence. The interval between first biting and first capture of parous at bait varied from 16-24 days, Service (1977a) in southern England found this interval was 27-32 days. In 1981, the difference in time between the last adult emergence and the last nulliparous adult (with spermatozoa in spermathecae) caught by bait catches was 55 days, indicating that some Ae. cantans could remain without blood-feeding for a long time. The anthrone test for sugar, on Ae. cantans caught at bait showed that parous as well as nulliparous females were positive, indicating that non-bloodmeals were taken both before and after bloodfeeding.

The largest population of Ae. cantans, as indicated by bait catches and sweep-netting, occured in 1979 with decreasing population size in 1980 and 1981. A few other mosquito species were also caught biting and from sweep-netting vegetation, but their populations were very small compared with that of Ae. cantans. However, an interesting finding was the sudden appearance in 1979 of Ae. detritus, a salt-marsh mosquito, at bait, in sweep-net and light trap samples. According to Covell and Shute (1962) Ae. detritus adults have been found occasionally some 8 - 11 km inland. Service (1980) considered that mosquito species which breed in and inhabit exposed habitats are more likely to be hindered in finding hosts and oviposition sites because of windy weather inhibiting their flight, than sylvan species, and also that such species will more likely be passively transported by wind to other areas. It seems possible that the Ae. detritus caught in Ness Woods were part of a larger population being swept inland by on-shore winds, and the individuals caught at Ness Woods represented those whose flight was blocked

by the wood, with the result that they settled in this small woodland. It is also conceivable that more than just a passive transportation was involved, and that during September 1979 and to a lesser extent in August 1980 and June 1981 Ae. detritus had changed their behaviour such as possibly flying higher from the ground to enable them to be dispersed. September is the time of the year when active breeding is decreasing and such behaviour, if it does occur, would enable the species to disperse to other areas for egg-laying and colonization. Whatever the reason for this appearance at Ness Woods it seems most likely that, irrespective of whether they obtained blood meals, they represent a loss to the population, because it seems most unlikely that they will fly back again to lay their eggs in a coastal habitat.

From the end of August to October C. pipiens and Cs. annulata females sampled by sweep-netting vegetation were found to have fatty reserves. C. pipiens does not breed in Ness Woods, its nearest larval habitats are probably 300 metres away, consisting of domestic and farm receptacles. It is only caught resting amongst vegetation in the wood at a time when adults stop feeding on birds and are seeking hibernation sites. There is evidence to suggest that their flight levels change during this time (Service 1971c). It seems that the adults caught in the wood represent adults, whose flight behaviour and orientation had changed. It is also interesting to record that a few were collected in light traps at this time of the year, whereas at other times they were absent.

Sweep-netting different types of ground vegetation namely brambles, ferns and grasses failed to reveal any significant preference of Ae. cantans for these types of vegetation. However, in Dorset, Service (1971b) found that very few mosquitoes rested amongst grassland or exposed heathland vegetation despite

the fact that in one area the principal larval habitats of Ae. punctor, one of the commoner mosquitoes, was heathland pools. At a wooded site in Dorset, although large numbers of mosquitoes rested in the wood, they were not evenly distributed amongst the vegetation. For example, none was found resting in the dense shelter provided by the growths of bracken (Pteris aquilinum (L.)), although substantial numbers were collected from adjacent grassy vegetation (Molinea caerulea (L.) Moench, Agrostis setacea (Curt.)). There was a large number of of holly bushes (Ilex aquifolium L.) in this wooded site in Dorset and surprisingly large numbers of mosquitoes were collected from them, but none was collected from the foliage of young birch (Betula pubescens Ehrh.) saplings. The small clump of Erica cinerea L. and Erica tetralix L. provided the most attractive resting site for mosquitoes in the copse.

The majority of mites parasitizing mosquitoes are aquatic (Hydrachnellae) and belong to 2 families - Thyasidae and Arrenuridae .Thyas barbigera, Thyasides sphagnorum and Euthyas truncata were reported for the first time as mosquito parasites by Mullen (1975) and according to him 4 terrestrial mite families also parasitize mosquitoes - Erythraeidae, Johnstonianidae, Trombellidae and Trombidiidae. Service (1969a) recorded parasitic water mites on some British mosquitoes. The highest incidence of 3.6% was recorded on Ae. cantans which he caught at Arne in Dorset where 1-9 mites were attached to the neck and thorax of 24 of the 718 specimens examined. Another two had two mites attached on the ventral surface of the abdomen. Nine of approximately 750 Cs. annulata examined had 1-5 mites on the thorax and three out of some 6,000 Ae. punctor had 1-2 mites on the thorax. Of 92 An. claviger examined, one had a mite on the ventral abdominal surface. Surprisingly, two of the 6,500 adults of the salt-marsh species

of Ae. detritus had two and three mites, respectively, on the thorax. He found that all parasitized adults were nulliparous. Gillett (1957) proposed that the presence of parasitic mites (hydrachnids) on female mosquitoes might indicate that the latter were nulliparous. This is because of the mites' life-history. Immature mites attach to the mosquito host as it emergence from the water and then drop off when females first return to the water to oviposit. The validity of this means of age-grading mosquitoes was supported by Corbet (1970), but Graham (1969) found that the presence of mites was not necessarily correlated with a particular physiological age of the mosquito host. According to McCrae (1976) there are limitations to using the presence of mites to age-grade female mosquitoes. For example, careful preliminary studies are required in each new area or with each new host species, to evaluate the usefulness of the method, not all nulliparous (or pars) are infested with mites and frequently overall infestation rates are very low, and finally the incidence of mite infestation may vary with time and from place to place. The method therefore cannot be used to determine the physiological age of all mosquitoes, not by itself to compare samples of mosquitoes collected on different dates and from different areas. But , when infestation rates are high then McCrae (1976) concluded that the method may nevertheless have the following advantages:

a) It may be a very rapid accessory method of recognising host age-grades, with or without calculable error.

b) It may indicate short-term (e.g. circadian) fluctuations of age structure within a host population, although it should first be ascertained whether or not the mites have their own short-term patterns of incidence.

c) It may allow living wild-caught nullipars to be recognized without killing them, so that infection-free material is available for laboratory studies. Partially-grown mites may be especially accurate in this respect.

d) It might, after careful study of the duration of each mite growth class and other variables, provide a means of determining the age structure of male populations for comparison with female population structure.

e) In a few cases, the relative incidence of mite growth classes may possibly be used as a supplementary means of assessing daily host mortalities, even though analysis would be extremely complex.

At Ness Woods, two species of mites were found to be parasitic on Ae. cantans adults, namely Thyas barbigera and a larval erythraeid mite which appears to belong to the genus Leptus (G.R. Mullen, 1981 personal comm.) Unfed Ae. cantans infested with mites, caught atbait and sweep-netting vegetation when dissected were found to be parous as well as nulliparous. Therefore, the presence of mites was not an indicator of nulliparity among Ae. cantans at Ness Woods.

The precipitin test has been widely used as the basic serological tool for blood-meal identification (Edman and Downe, 1964, Tempelis, 1975, Kay et al. 1979). Service (1971d) found that cattle often constituted the principal host (48.5-73.4%; 76.9%) of mosquitoes such as Ae. cantans, Ae. cinereus Meigen, Ae. detritus and Ae. punctor collected from several sites in Dorset, Huntingdon and Kent. However, 69.4% of the 36 individuals of Ae. cantans caught from a wood where there were no nearby cattle had fed on rabbits which were common in the area. The present studies indicate that cattle was the principal hosts of Ae. cantans (81.3%), Ae. punctor (57.1%) and Ae. detritus (77.8%). Besides feeding on

cattle, Ae. cantans also fed on sheep, rabbit, horse, unidentified mammal and man. Therefore Ae. cantans had a broad spectrum of hosts. Besides feeding on cattle Ae. punctor and Ae. detritus in Ness Woods, and a single Cs. annulata tested had also fed on sheep. Now, from personal observations and information from local farmers it seems that at the time of the study there were few rabbits or other suitable mammals in Ness Woods or in the wooded area across the road to the north, although a considerable number of rabbits did exist to the south of Ness Woods. So it seems that in this situation the large numbers of Ae. cantans produced in the woods have to leave at night to feed on domestic farm animals and return to the woods afterwards to seek shelter amongst vegetation while digesting their blood meals. As there are only a limited number of cattle near Ness Woods and it seems that mosquitoes must be biting them in very large numbers possibly to the detrement of the cattle.

Chandler and Highton (1975) evaluated a modified CDC light -traps for sampling emerging adult population of mosquitoes from ricefields. Thirty-five species of mosquitoes totalling 10,782 adults were caught in their CDC light traps during the 27-week rice cultivation cycle at Ahero, Kenya. Only 0.8% were bloodfed and 5.1% gravid indicating that the traps were in fact sampling newly emerged adults. However, when at Ness Woods a CDC light trap was suspended over a pond (known to have a large population of Ae. cantans and a few mosquitoes of other species) for 4 nights, only a single male Ae. cantans was caught. Furthermore, only a single female Cs. annulata was caught when a chemical light trap was placed over larval habitats containing a few larvae of other species and many Ae. cantans larvae, and no mosquito were caught when a Monks Wood light trap was sited over a pond. Light traps are clearly of no use

in sampling emerging population of Ae. cantans. In the Poole area, Dorset a Monks Wood light trap with a white light tube operated for 10 nights caught 65 female and 22 male Culiseta morsitans (Theo.), 3 female and 2 male Culiseta litorea (Shute), 1 female and 14 male Cs. annulata, 8 female Cs. subochrea (Edw.) 37 female and 52 male C. pipiens, 3 female and 12 male Culex torrentium Mart., 16 female Ae. detritus, 49 female and 9 male Mansonia richiardii (Fic.) (Service, 1970). When a similar trap was evaluated for 10 nights in the Huntingdon area 3 female Cs. annulata, 15 female and 11 male C. pipiens, 4 female and 4 male Ae. cantans and 1 female Ae. detritus were caught (Service, 1970). All females caught were unfed and some of the Culex species had well developed fat reserves. Because of this limited success with the Monks Wood trap it was thought worthwhile testing it in Ness Woods. However, when placed amongst vegetation only 10 unfed female, 1 male Ae. cantans, 2 unfed, 2 half-gravid and 4 gravid female Ae. detritus, 17 unfed female and 5 male C. pipiens, 19 unfed female and 46 male Cs. annulata were caught from 27 night collections. These disappointing results show that the Monks Wood light trap was inefficient at trapping Ae. cantans population, especially when compared with the large numbers of Ae. cantans present in the wood. Similarly, Service (1970) caught very few (8) Ae. cantans in Monks Wood, Huntingdon, despite the presence of large populations biting and breeding in the wood. Nevertheless studies at Ness Woods indicate that adults of Ae. detritus, C. pipiens and Cs. annulata are more attracted to light traps, although their numbers were never very large.

A CDC light trap was likewise unsuccessful in sampling Ae. cantans in Ness Woods, and from 13 trap nights only 1 male Ae. detritus, 1 unfed and 1 female C. pipiens with fat reserves and 1 gravid female Cs. annulata were caught.

In North America, C. pipiens is regularly caught in CDC traps and recently Wilton (1981) studied the responses of C. pipiens from northern U.S.A. and C. quinquefasciatus from the southern U.S.A. to CDC light traps. In these laboratory experiments he found that C. pipiens had the greatest attraction and C. quinquefasciatus the least to the light traps. Hybrids, resulting from crosses between these two sibling species, were attracted more than C. quinquefasciatus but less than C. pipiens.

Finally, because of the recent success achieved by Service and Highton (1980) with a chemical light trap in Ghana and Kenya in catching several mosquito species, they were tried in Ness Woods. But from a total of 27 catches only 5 unfed female Ae. cantans and 2 gravid female Cs. annulata were caught. These chemical light traps were also evaluated by Service at Malham Tarn, Yorkshire, at a time when large biting population of Culicoides were present, but very few were caught in the trap, more were collected in Monks Wood traps, especially when a UV tube was used. But in South America a chemical lightstick surrounded by a clear transparent sheet of plastic coated with castor oil has proved exceptionally successful in catching Lutzomyia species. More were caught of several species than were collected from light traps employing a torch bulb (R. Lane 1981 personal communication to M.W. Service).

Trinidad no. 10 traps containing about 1 kg of dry ice were found to be more effective in Dorset than the more generally used cylindrical carbon dioxide trap (Service 1969b), and 9 mosquito species were caught. The maximum catch occured at night and consisted of 92 Ae. detritus, 8 An. plumbeus, 3 An. claviger Meigen and 2 Mansonia richiardii Ficalbi. However, when a similar trap was evaluated at Ness Woods on 5 nights, only a single unfed female Ae. cantans was caught, but at the time of this sampling the mosquito population was not very large.

Fluorescent dusts have been used successfully in several studies on the dispersal, behaviour and survival of mosquitoes (Brust, 1980, Nelson and Milby, 1980), yet only one marked Ae. cantans was recaught by sweep-netting at Ness Woods. This individual was caught 58 days after marking and release, and although nothing can really be deduced from this unsuccessful experiment, at least it confirms that Ae. cantans is long-lived. The reasons for the very low recapture rate (0.07%) are not known, conceivably handling and marking the mosquitoes reduced their survival rates, or maybe stimulated them to disperse out of the wood. Although these 'Dayglo' powder have been used by several workers, and there was no reason to suspect that marked adults lost their powders, a recent experiment by Lillie et al.(1981) showed that 'Dayglo' powders may not be retained, at least on Culicoides variipennis (Coquillett), as well as generally believed.

STUDIES ON HIBERNATING POPULATIONS OF CULEX PIPIENS AND CULISETA ANNULATA

INTRODUCTION

Culex pipiens passes the winter months as fertilised female adults. In winter the females remain inert in cool, damp enclosed situations nourished by the fat body which develops in their abdomen at the approach of autumn (Marshall, 1938). In April hibernating females leave their winter quarters and after taking avian blood-meals deposit their eggs rafts in collections of water (Marshall, 1938).

Culiseta annulata is unusual in that it passes the winter in either the adult or larval form. The females frequently hibernate either in cellars or attics of houses or in buildings having domestic animals such as cattle and horses (Marshall, 1938). Cs. annulata is a "partial" hibernator whereby the females during spells of mild weather obtain bloodmeals from domestic animals and birds as well as man (Marshall, 1938; Covell and Shute, 1962, Kuhlhorn, 1974).

Service (1969a) studied a population of C. pipiens hibernating in old abandoned brick shelters on Brownsea Island, Dorset and found that adults entered the shelter as early as 19th August in 1964 and 3rd August in 1965. By December, however, there was a sharp reduction and after this month very few adults occured. In the same shelters Cs.annulata were first found on 9th and 19th October in 1964 and 1965 respectively, later than C. pipiens. A population peak was reached in the second half of November.

Onyeka (1980) studied hibernating populations of C. pipiens and Cs.annulata in underground cellars of a house at Sunninghill, near Ascot and found the build-up of C. pipiens population reached its peak in late October or early November each year.

In December onwards there was a gradual reduction in the number of mosquitoes until late April. Hibernating mosquitoes were last seen on the 10th, 12th, and 15th May, in 1978, 1979 and 1980 respectively.

A small population of Cs.annulata was found in the same sites between October and May and their population fluctuated without defined peak. (Onyeka, 1980).

The objective of the present study was to observe the build-up and decline in populations of C. pipiens and Cs.annulata in shelters at Leas School, West Kirby on the Wirral, about 22km south-west of Liverpool.

MATERIALS AND METHODS

The study was conducted in an underground bombproof shelter (Shelter 1) at Leas School, West Kirby. The shelter was made of concrete, 9.35metres long, 2.24 metres wide and 2.08 metres high. There were two entrances to the shelter, the first entrance consisted of a 63.5-cm square opening on its roof. The second entrance was a door covered with wire netting and measured 78.7 cm by 190.4cm.

Sixteen sections each from both sides of the wall and roof were marked as sampling sites (Fig.36). At a regular interval of about 10-12 days hibernating mosquitoes resting on each section were counted and recorded from December 1978 to June 1981.

In shelter 2, which was similar in structure and dimension to shelter 1 and adjacent to it, the main door was locked and all holes and crevices covered with plastic foams glued to the wall to prevent mosquitoes inside the shelter from escaping. The main door was covered with a dark red curtain on the inside to darken the shelter. The second entrance, an opening in the roof was covered with a wooden board 78.7 cm square with a circular hole cut from its centre. On top of the board a

Fig. 36: The sections on both sides of the wall and roof of shelter no. 1 where C. pipiens and Cs. annulata rest during hibernation.

Fig. 37: An exit trap placed on top of shelter no. 2 for trapping mosquitoes in order to measure their exodus.

mosquito cage of size 35.5 x 35.5 x 45.7 cm was attached as an exit trap for the mosquitoes inside the shelter (Fig.37).

Twice a week from 7th November, 1980 until the end of June, 1981, the number of C. pipiens, Cs. annulata and spiders were recorded from all walls and ceiling of the shelter, and also of mosquitoes trapped in the exit trap.

The objective for blocking both entrances of shelter no. 2 was to prevent possible predators such as bats and birds from entering at night and leaving again, to prevent mosquitoes entering or leaving so that the mortality in the shelter of the mosquitoes, and exodus as measured by the exit trap, could be determined.

RESULTS

Culex pipiens populations

On 13th December 1978 when counting was first started, the population of C. pipiens recorded in shelter 1 was 73 (Appendix 14). Thereafter the population declined until it reached a zero population on 22nd May. 1979. In 1979, the first adults of C. pipiens were seen in hibernation on 14th July when 5 mosquitoes were observed. Thereafter there was a build-up in the population of C. pipiens reaching its first peak on 14th September. 1979 with 2888 C. pipiens (Fig. 38). After reaching its first peak the population dropped to 1588 mosquitoes on 25th September 1979, but then the population increased again to a second and even higher peak of 2913 on 9th November. 1979. After reaching its maximum population on 9th November the population declined again until only 1 C. pipiens was found on 29th June 1980 (Appendix 14). In 1980 the first C. pipiens in the same shelter was seen on 9th July 1980 with 2 mosquitoes, reaching a single peak population of 2080 mosquitoes on 23rd October (Fig 38). The population in 1980

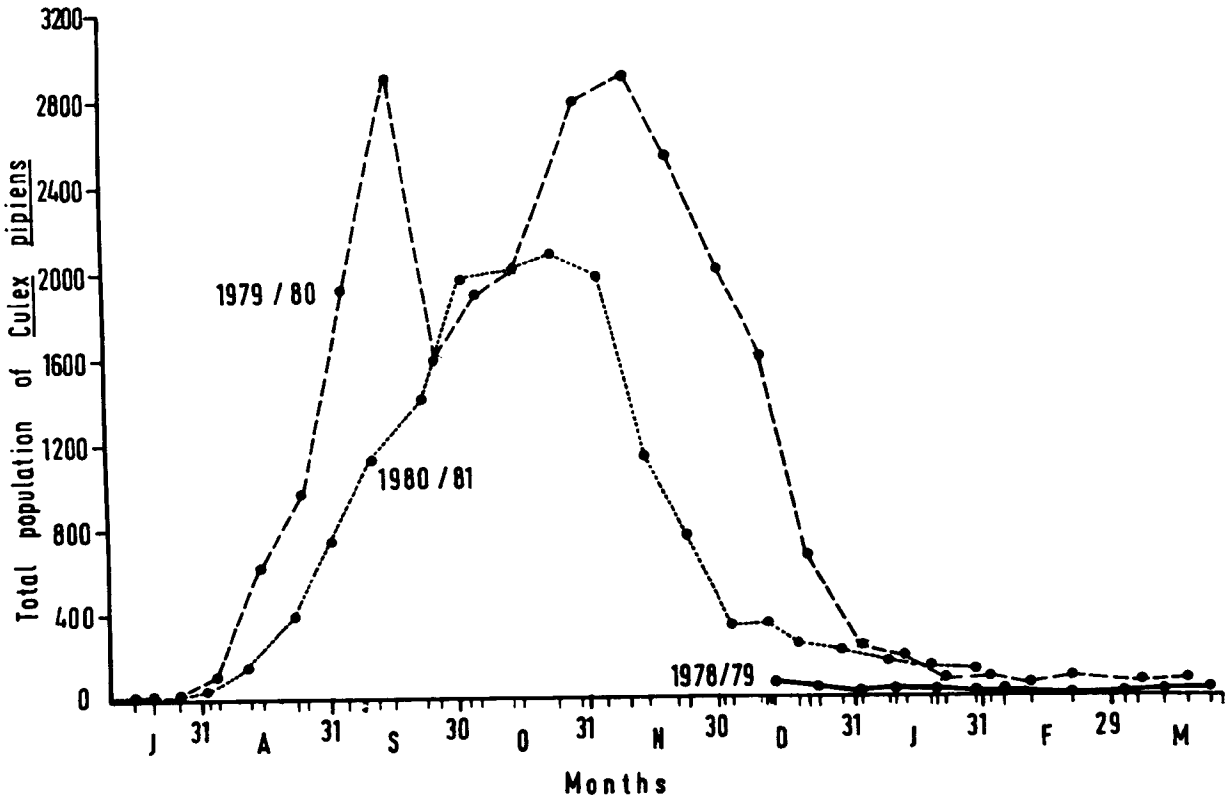

Fig. 38: Population of hibernating ♀ Culex pipiens in shelter 1, West Kirby, Wirral.

was smaller than recorded in 1979. Figure 39 shows that in 1979 there was a sharp increase in population of hibernating C. pipiens from 1.8% on 4th August 1979 to 99.1% on 14th September 1979, then the population declined to 54.5% on 25th September but increased again to a maximum of 100% on 9th November 1979. The reduction in population after reaching its first peak on 14th September 1979 was thought to be due to mosquitoes leaving the shelter but the reason for leaving during this time of the year was not known. In 1980 the population of C. pipiens increased sharply and more or less steadily from 2nd August 1980 with 1.7% to 2nd October 1980 with 94.9%, then a slower build-up to a maximum of 100% on 23rd October (Fig. 39).

The rapid reduction in the hibernating populations of C. pipiens began in 1979/80 on 19th November 1979 with 13.1% reduction of its maximum population of 2913 females to 2nd January 1980 with 92.2% reduction from maximum population (Fig. 40), gradually reaching to 99.9% reduction on 29th June 1980. For the year 1980/1981 the population decline began on 3rd November 1980 with 5.2% reduction to 84.0% reduction on 3rd December 1980 with further reduction occured gradually eversince until reaching to 99.9 % reduction on 29th May 1981.

Fungi are known to cause considerable mortality to hibernating C. pipiens (Service,1969a). He found that two fungi of the species Cephalosporium possibly C. coccorum and Entomophthora sp. nr conglomerata caused considerable mortality to C. pipiens, while other species such as Penicillium, Mucor and Sporotrichum species were saprophytic. However, in this shelter in West Kirby no fungal infection was seen. This was due to the fact that the shelter was dry and not suitable for fungal growths.

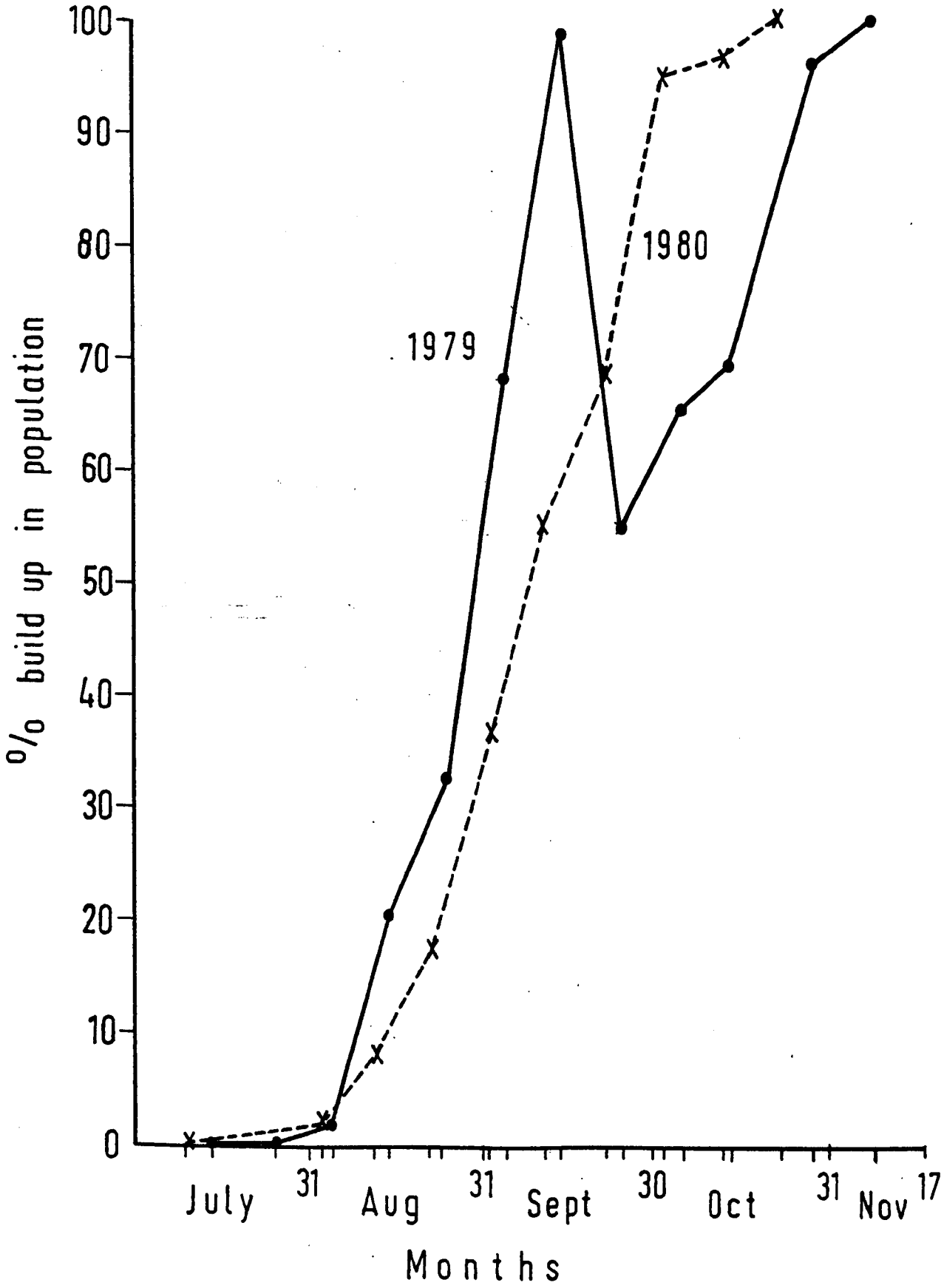

Fig. 39: % of Build-up in population of ♀ Culex pipiens in shelter 1, West Kirby, Wirral.

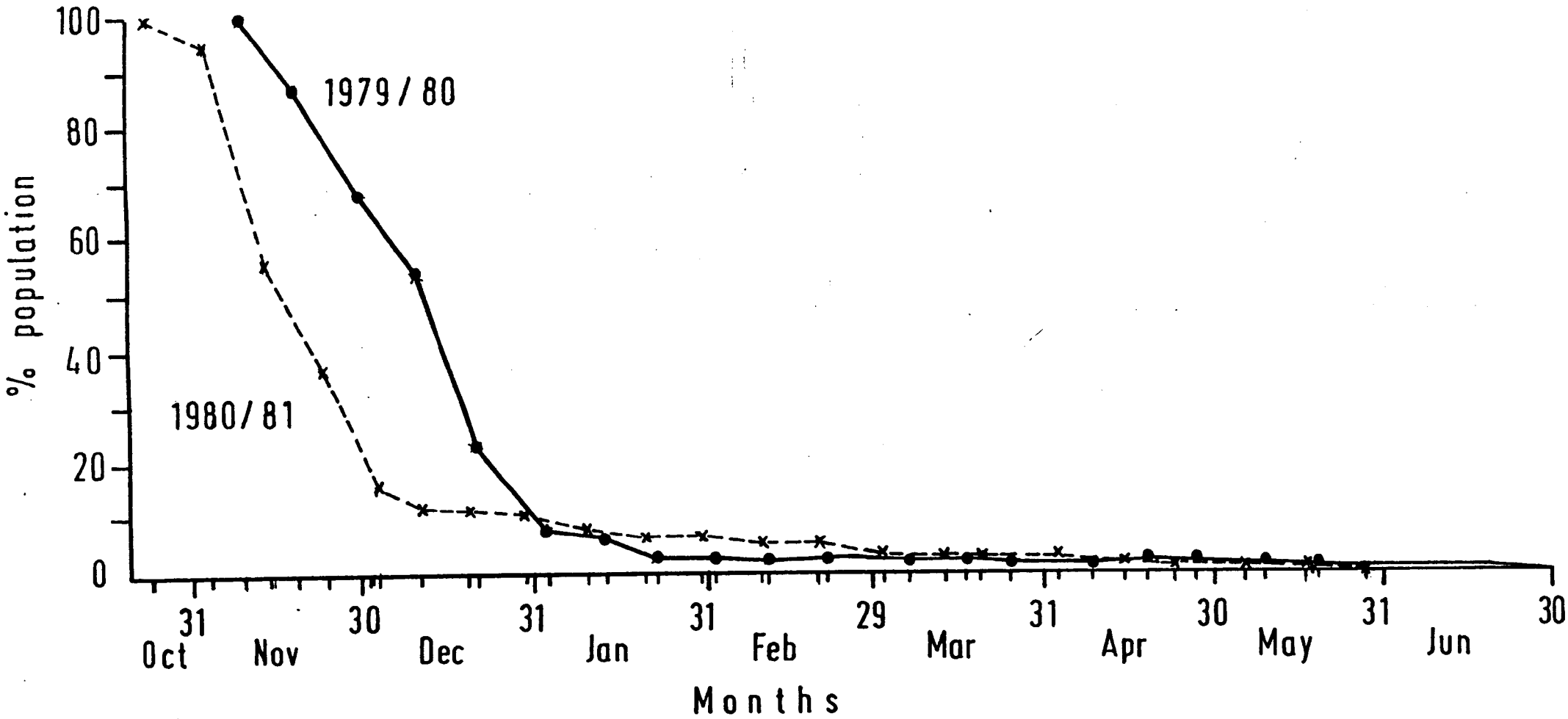

Fig. 40: Reduction of ♀ Culex pipiens population in shelter 1, West Kirby, Wirral.

Spiders were also observed to be feeding on hibernating C. pipiens (Service, 1969a). A few spiders of the species Lepthyphantes leprosus (Ohlert) were seen in the present shelter. Although they were not seen to be feeding directly on the mosquitoes they were considered likely to be predators on Culex pipiens. P. Merrett (personal communication, 1980) considered that they are likely to be predators of Culex pipiens. But because of the few spiders that existed in the shelter they were not thought to be the dominant factor in reducing the population.

The shelter was divided into equal sized sections, 16 on each side, labelled 1A to 16A and 1B to 16B (Fig. 41). The distribution of the C. pipiens was highest in both years on section A 15, then A9 and A1 in that order (Figs.42 &43). Berg and Lang (1948) studied hibernating mosquitoes in Massachusetts, U.S.A. during the winter of 1944-45 and found that the darker corners of the resting stations appeared to be most attractive to resting mosquitoes. Service (1969a) in his observation of hibernating mosquitoes on Brownsea Island found that the grouping of mosquitoes in a vertical shaft was most probably in response to a light gradient. Onyeka (1980) observed that throughout hibernation period, mosquitoes tended to concentrate more to the areas towards the entrances. Because section A15 was located near the main entrance and an area marked x on Fig. 41 caused it to be darkened, therefore it was a favourable place for resting C. pipiens. Section A1 was also near an entrance, but because of the smaller size of this entrance and because of its position in the roof exposed directly to sunlight, relatively few mosquitoes preferred to rest on section A1. For both years the population of C. pipiens was higher on section A15 than B16 (Figs.42-45). The distribution of C. pipiens on B sections was in both years highest on the middle section (Figs.44&45).

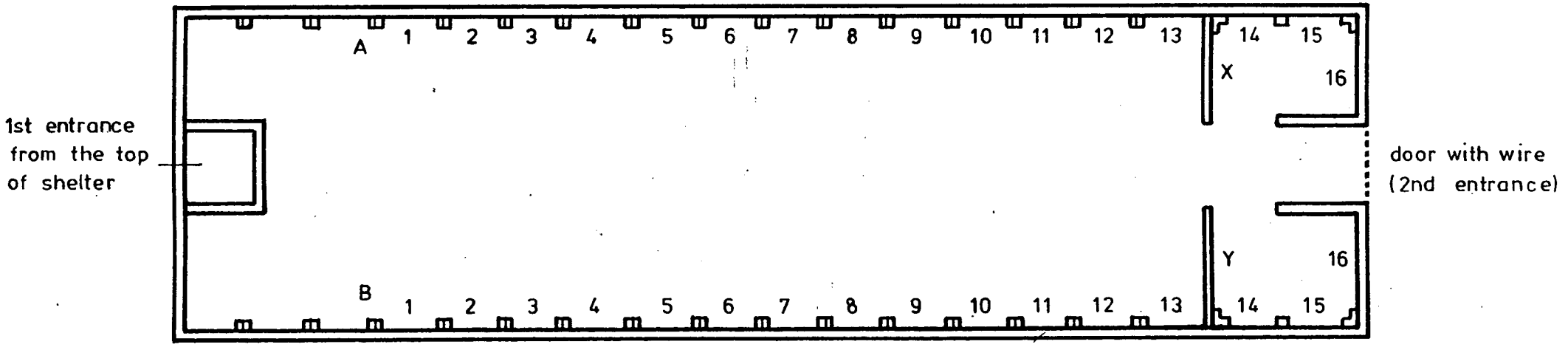

Fig. 41: Sketch map of shelter 1, West Kirby, Wirral.

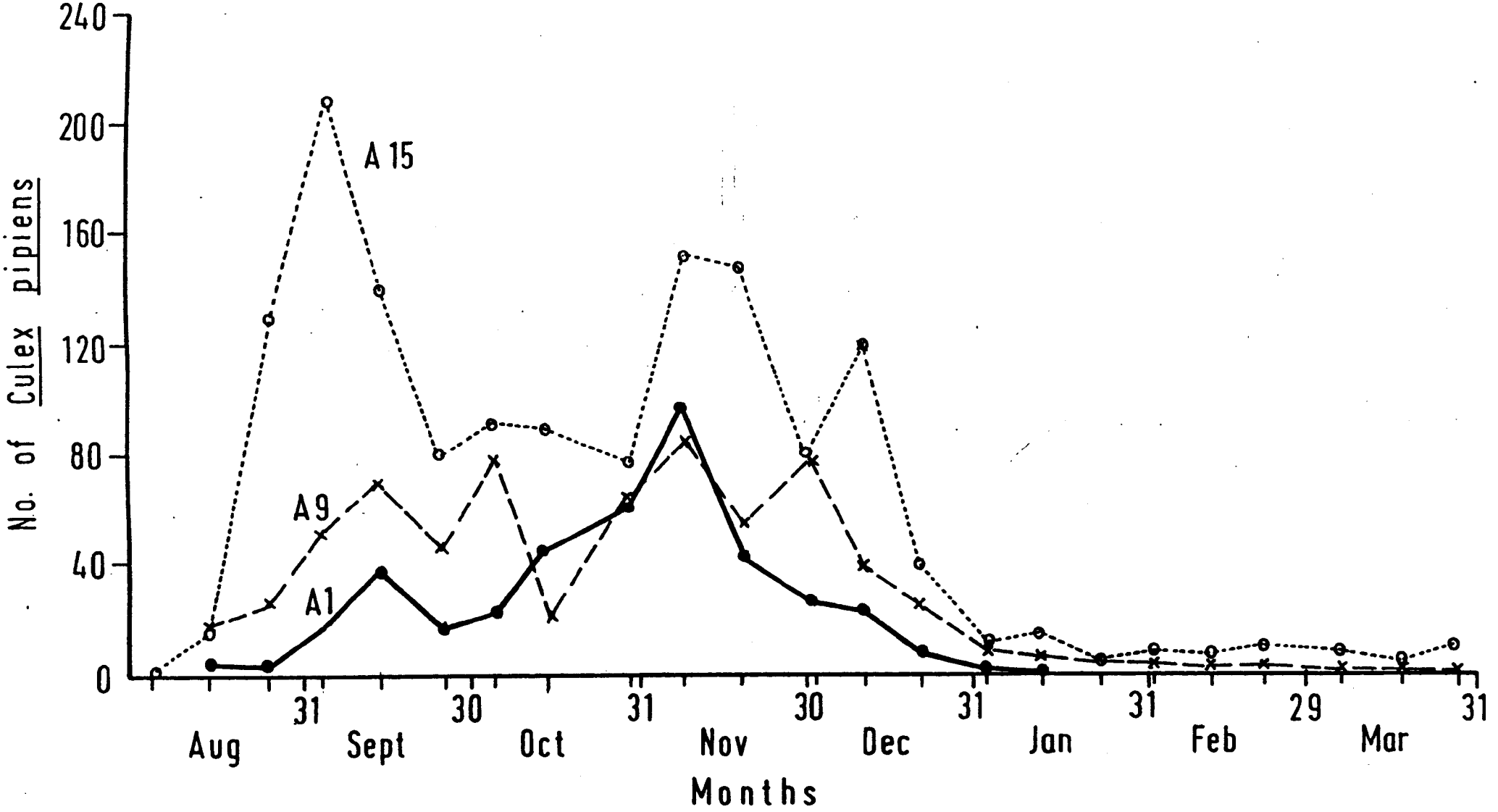

Fig. 42: Distribution of ♀ Culex pipiens population on various sections in the year 1979 / 1980.

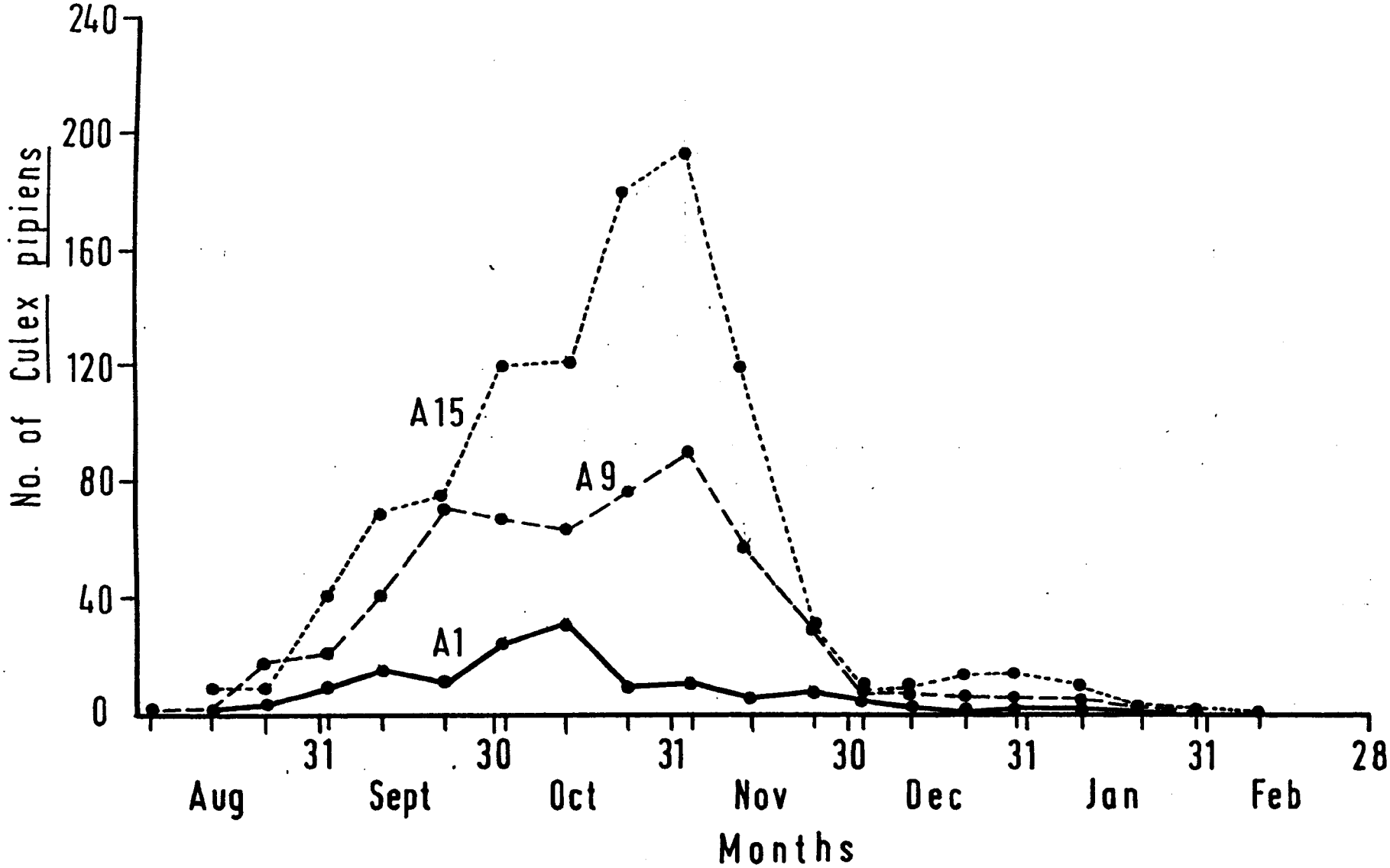

Fig. 43: Distribution of ♀ Culex pipiens population on various sections in shelter 1 in the year 1980/1981.

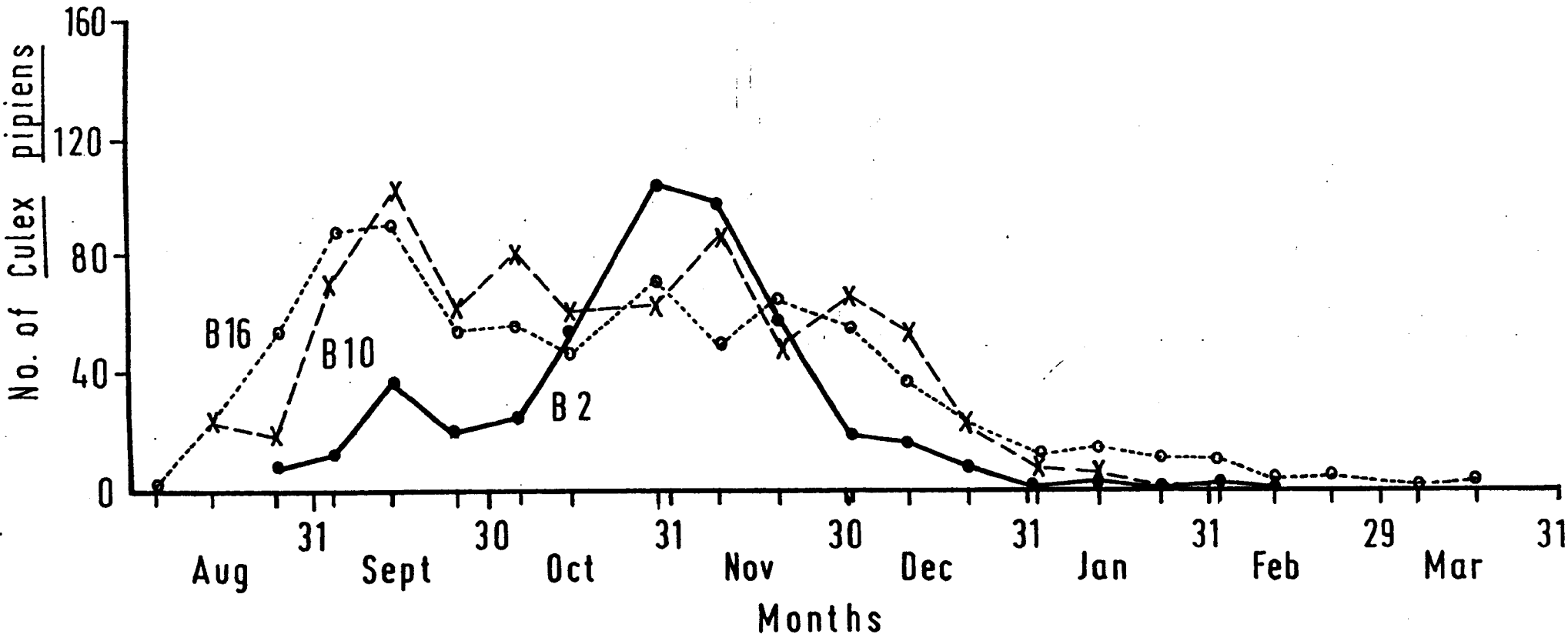

Fig. 44: Distribution of ♀ Culex pipiens population on various sections in the year 1979/1980.

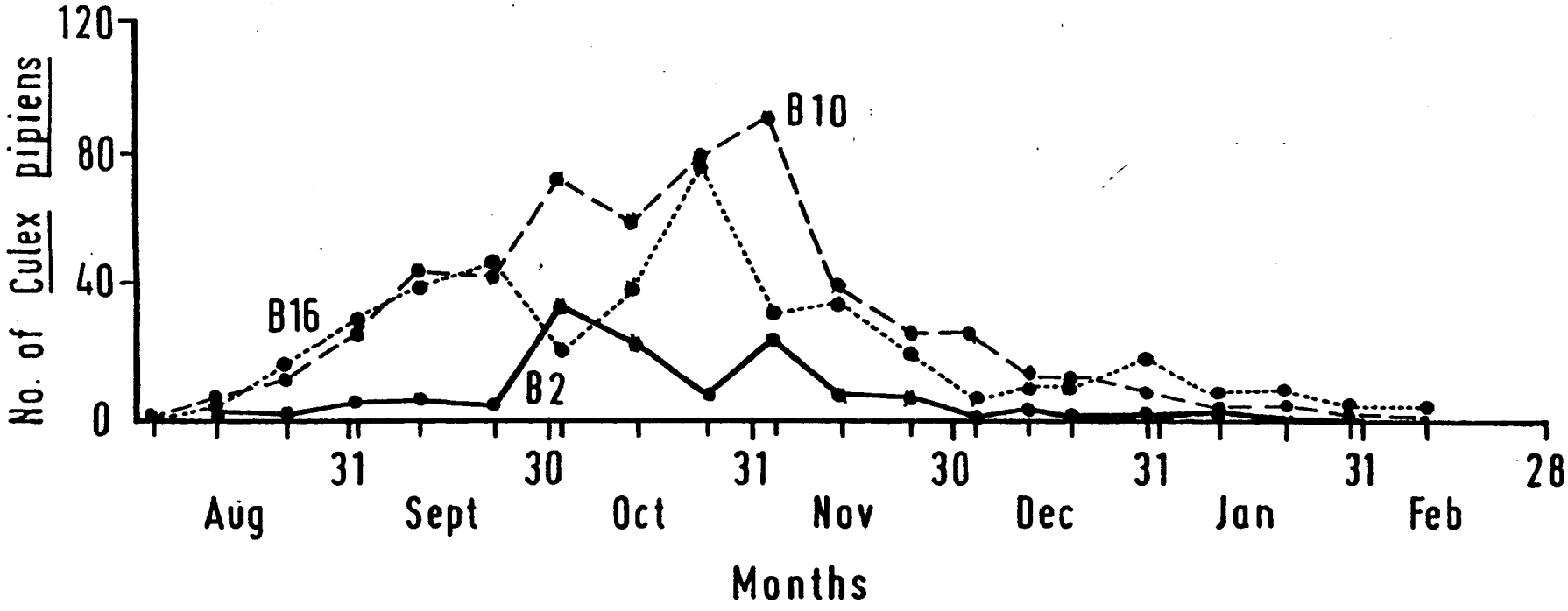

Fig. 45: Distribution of ♀ Culex pipiens population on various sections in shelter 1, in the year 1980/1981.

This was possibly because of the lay out of the shelter, which would let breezes blow towards the middle of the A sections, and this might have caused the mosquitoes to disperse towards the middle of B sections. Whatever the reasons the mosquitoes were not regularly distributed between the two walls or along each wall, in other words they had a highly aggregated distribution which remained similar in all years.

In 1980, in shelter no. 2 the main entrance, the doorway, was locked and an exit trap (Fig.37) was placed on the second entrance, the opening in the roof, so that the only escape route for the mosquitoes was via exit trap. The population loss observed was presumably due to mortality of the mosquitoes in the shelter. The population reduction was only 5.4% by 24th November 1980, 19.7% by 29th December 1980 and 21.8% by 29th January 1981. (Table 58). In marked contrast in shelter no. 1 whereby neither entrances was covered there was a population reduction of 63.7% by 24th November 1980, 89.7% by 29th December 1980 and 93.9% by 29th January 1981 (Table 57). This large reduction in the population in shelter no. 1 could have been due to an exodus of adults, although this was not indicated by the exit trap fitted to shelter no. 2 or to mortality. Conceivably predators entered the shelter and caused this reduction, predators being prevented from such entry in shelter no. 2. It is thought that due to the few predators (e.g. spiders) seen in shelter no. 1 and no sign of fungal infections among mosquitoes, mortality due to predators or other causes played a minor role in population reduction and exodus seemed to be the main factor of this large reduction in population. On the contrary in shelter no. 2 there was no exodus because there was no escape route except the exit trap for the mosquitoes and a reduction in population throughout the hibernation period was thought to be due

Table 57 : Decline in population of ♀ C. pipiens in shelter 1, West Kirby, Wirral (1980-81)

Date	Population of ♀ C. pipiens	% reduction from maximum population
23.10.80	2080	-
3.11.80	1971	5.2%
13.11.80	1160	44.2%
24.11.80	755	63.7%
3.12.80	333	84.0%
11.12.80	339	83.7%
18.12.80	244	88.3%
29.12.80	214	89.7%
9.1.81	164	92.1%
19.1.81	125	94.0%
29.1.81	126	93.9%
9.2.81	104	95.0%
19.2.81	103	95.0%
2.3.81	41	98.0%
12.3.81	52	97.5%
23.3.81	55	97.4%
2.4.81	63	97.0%
13.4.81	37	98.2%
23.4.81	29	98.6%
5.5.81	26	98.8%
14.5.81	4	99.8%
22.5.81	3	99.9%
29.5.81	1	99.9%
8.6.81	1	99.9%
18.6.81	0	100.0%

Table 58 : Decline in population of ♀ C. pipiens in shelter 2, West Kirby, Wirral (1980-81)

Date	Population of ♀ C. pipiens	% reduction from maximum population
7.11.80	2234	-
14.11.80	2231	0.1%
17.11.80	2186	2.1%
21.11.80	2136	4.4%
24.11.80	2113	5.4%
1.12.80	2074	7.2%
3.12.80	2058	7.9%
8.12.80	2036	8.9%
11.12.80	1971	11.8%
15.12.80	2105	5.8%
18.12.80	2006	10.2%
22.12.80	1994	10.7%
25.12.80	1953	12.6%
29.12.80	1795	19.7%
1.1.81	1857	16.9%
5.1.81	1893	15.3%
9.1.81	1723	22.9%
12.1.81	1833	17.9%
15.1.81	1804	19.2%
19.1.81	1744	21.9%
22.1.81	1687	24.5%
26.1.81	1655	25.9%
29.1.81	1747	21.8%
2.2.81	1679	24.8%
5.2.81	1690	24.4%
9.2.81	1508	32.5%
12.2.81	1546	30.8%

Continued .....

Table 58 : (Continued)

Date	Population of ♀ C. pipiens	% reduction from maximum population
17.2.81	1556	30.3%
19.2.81	1536	31.2%
23.2.81	1462	34.6%
25.2.81	1413	36.8%
2.3.81	1326	40.6%
5.3.81	1571	29.7%
9.3.81	1323	40.8%
12.3.81	1534	31.3%
16.3.81	1500	32.9%
19.3.81	1499	32.9%
23.3.81	1372	38.6%
26.3.81	1329	40.5%
31.3.81	1418	36.5%
2.4.81	1314	41.2%
6.4.81	1298	41.9%
9.4.81	1325	40.7%
13.4.81	1033	53.8%
16.4.81	839	62.4%
20.4.81	668	70.1%
23.4.81	733	67.2%
27.4.81	652	70.8%
30.4.81	612	72.6%
5.5.81	321	85.6%
7.5.81	269	88.0%
11.5.81	110	95.1%
14.5.81	48	97.9%
18.5.81	30	98.7%
22.5.81	16	99.3%
26.5.81	9	99.6%

Continued .....

Table 58 : (Continued)

Date	Population of ♀ C. pipiens	% reduction from maximum population
29.5.81	7	99.7%
1.6.81	1	99.9%
4.6.81	0	100%
8.6.81	0	100%
11.6.81	2	99.9%
15.6.81	1	99.9%
18.6.81	0	100%

to predation by spiders or by other causes.

Table 60 shows that between November and third week of March only very few C. pipiens were caught occasionally in the exit trap. But from the beginning of the last week of March until the first week of June (except on one occasion, 27th April 1981) C. pipiens were seen in the exit trap. A total of 1128 ♀ C. pipiens were collected in the exit trap from the original population of 2234 in the shelter or 50.5%. The total female Cs.annulata collected in the exit trap was 6 or again 50.0% of the original population.

Table 61 shows that 1104 of the original 2234 ♀ C. pipiens in the shelter was estimated to be dead (49.4%). Between November 1980 and March 1981 the estimated monthly mortality of ♀ C. pipiens was in the range of 118-244 mosquitoes. For the months of April and May a total of 207 C. pipiens were estimated to be dead. Mortality is thought to be caused by spiders and other causes such as depletion of fat reserves. As suggested by Buxton (1935) and Buffington and Zar (1968) fat reserves would be depleted by the end of hibernation period particularly in March and April. So, the additional stress of resuming active metabolism maybe another factor contributing to mortality (Buffington, 1972). It was difficult to find any mosquitoes that were dead and dropped on the floor. Similar problems were also encountered by Buffington (1972) in his studies of C. pipiens hibernation population in Illinois, U.S.A.

Table 62 shows that over the 3 years, male C. pipiens found in shelter 1 represented only 0.3% of the total C. pipiens in the shelter. Male C. pipiens were seen in the shelter from August to October in 1979 and from September to October in 1980. The highest percentage of male C. pipiens seen was 1.3% on 5th October 1979 and 1.6% on 1st September 1980.

C. torrentium was first described in Britain in 1951 (Mattingly, 1951). Its biology is very similar to that of C. pipiens and the immature stages occur in the same habitat as those of C. pipiens and are morphologically indistinguishable. It is assumed that, as with C. pipiens, adult females overwinter as hibernating adults, but so far none has been found in man-made or any other shelters. Therefore occasionally a few samples of mosquitoes were collected from the shelter and brought and examined in the laboratory to determine whether or not C. torrentium were present in the shelter. Identification of C. torrentium was based on the presence of scale insertions to determine whether or not pre-alar scales had been present. Males were readily identified on the form of the terminalia (Service 1968b) However, no C. torrentium was discovered hibernating in the shelter throughout the study period.

Culiseta annulata populations

Figure 46 shows that throughout the 3 years study period the population of Cs.annulata was always smaller than the previous year. The largest number of Cs.annulata observed was on the 13th December 1978 when 45 adults occured. Then a sharp reduction in population occured until a zero population was recorded on 22nd April 1979. Adults of Cs.annulata were first seen in the shelter again on 5th July 1979 reaching a peak population of 34 mosquitoes on 9th November 1979. On the 2nd January 1980 the population had been reduced to zero but after that date there was a fluctuation in the population of Cs.annulata (Fig. 46). In 1980/81 the population was much lower than the previous two years and fluctuating.

No male Cs.annulata were seen throughout the study period in shelter 1. However, in shelter 2, 2 male Cs.annulata were seen in the exit trap on

Table 59 : Number of hibernating C. pipiens, Cs.annulata and spiders recorded in shelter 2, West Kirby, Wirral (1980-81)

Date	No of ♀ C. pipiens in shelter	No of ♀ Cs. annulata in shelter	Total no of spiders in shelter
7.11.80	2234	12	4
14.11.80	2231	10	21
17.11.80	2186	10	23
21.11.80	2136	10	24
24.11.80	2113	10	32
1.12.80	2074	10	19
3.12.80	2058	9	26
8.12.80	2036	9	29
11.12.80	1971	6	29
15.12.80	2105	9	27
18.12.80	2006	5	16
22.12.80	1994	9	17
25.12.80	1953	8	15
29.12.80	1795	7	19
1.1.81	1857	7	17
5.1.81	1893	6	17
9.1.81	1723	3	11
12.1.81	1833	3	13
15.1.81	1804	5	14
19.1.81	1744	4	10
22.1.81	1687	4	11
26.1.81	1655	4	9
29.1.81	1747	4	9
2.2.81	1679	5	9
5.2.81	1690	5	7
9.2.81	1508	3	8
12.2.81	1546	3	11
17.2.81	1556	2	9

Continued .....

Table 59 : (Continued)

Date	No of ♀C. pipiens in shelter	No of ♀Cs.annulata in shelter	Total no of spiders in shelter
19.2.81	1536	2	8
23.2.81	1462	2	5
25.2.81	1413	5	5
2.3.81	1326	1	6
5.3.81	1571	2	9
9.3.81	1323	0	4
12.3.81	1534	1	5
16.3.81	1500	1	7
19.3.81	1499	1	4
23.3.81	1372	1	2
26.3.81	1329	0	5
31.3.81	1418	0	6
2.4.81	1314	0	5
6.4.81	1298	0	7
9.4.81	1225	0	4
12.4.81	1033	0	3
16.4.81	839	0	3
20.4.81	668	0	1
23.4.81	733	0	1
27.4.81	652	0	0
30.4.81	612	0	1
5.5.81	321	0	1
7.5.81	269	0	0
11.5.81	110	0	0
14.5.81	48	0	1
18.5.81	30	0	0
22.5.81	16	0	0
26.5.81	9	0	0

Continued .....

Table 59: (Continued)

Date	No of ♀ C. pipiens in shelter	No of ♀ Cs.annulata in shelter	Total no of spiders in shelter
29.5.81	7	0	1
1.6.81	1	0	2
4.6.81	0	0	1
8.6.81	0	0	0
11.6.81	2	0	1
15.6.81	1	0	0
18.6.81	0	0	0

Table 60: Total number of ♀ C. pipiens and ♀ Cs.annulata caught at different times in exit trap fitted on shelter 2, West Kirby, Wirral (1980-1981)

Date	No of ♀ C. pipiens	No of ♀ Cs. annulata
7.11.80	1	0
14.11.80	0	0
17.11.80	3	0
21.11.80	0	0
24.11.80	2	0
1.12.80	0	0
3.12.80	0	0
8.12.80	0	0
11.12.80	0	0
15.12.80	0	0
18.12.80	0	1
22.12.80	0	0
25.12.80	0	0
29.12.80	0	0
1.1.81	1	0
5.1.81	0	0
9.1.81	0	0
12.1.81	0	1
15.1.81	0	0
19.1.81	0	0
22.1.81	1	0
26.1.81	0	0
29.1.81	1	0
2.2.81	1	1
5.2.81	0	0
9.2.81	0	0
12.2.81	0	0
17.2.81	0	0
19.2.81	0	0
23.2.81	0	0
25.2.81	0	0

Continued .....

Table 60 : (Continued)

Date	No of ♀ C. pipiens	No of ♀ Cs. annulata
2.3.81	0	0
5.3.81	0	0
9.3.81	2	1
12.3.81	2	0
16.3.81	0	1
19.3.81	0	0
23.3.81	0	1
26.3.81	2	0
31.3.81	7	0
2.4.81	25	0
6.4.81	26	0
9.4.81	19	0
12.4.81	134	0
16.4.81	123	0
20.4.81	95	0
23.4.81	43	0
27.4.81	0	0
30.4.81	69	0
5.5.81	77	0
7.5.81	69	0
11.5.81	308	0
14.5.81	48	0
18.5.81	35	0
22.5.81	15	0
26.5.81	12	0
29.5.81	2	0
1.6.81	2	0
4.6.81	3	0
8.6.81	0	0

Continued .....

Table 60 : (Continued)

Date	No of ♀ C. pipiens	No of ♀ Cs. annulata
11.6.81	0	0
15.6.81	0	0
18.6.81	0	0
Total	1128	6
Percentage of the original population in shelter	50.5%	50.0%

Table 61 : Estimated monthly mortality of ♀ C. pipiens in shelter 2, West Kirby, Wirral 1980-1981

Month & Year	Highest no of ♀ C. pipiens		Total no of ♀ C. pipiens in exit trap		Estimated mortality of ♀ C. pipiens.	Highest no of spiders
November, 1980	2234	160	6		154	32
December, 1980	2074	181	0		181	29
January, 1981	1893	203	3		200	17
February, 1981	1690	119	1		118	11
March, 1981	1571	257	13		244	9
April, 1981	1314		534	1100	207	7
May, 1981	321		566			1
June, 1981	2		5		-	2
			Total = 1128		Total = 1104 = 49.4% of population	

Table 62 : Population of ♂ Culex pipiens in shelter 1, West Kirby, Wirral

Date	No of ♂ C. pipiens	Percentage in total population of C. pipiens
14.8.79	2	0.3
25.8.79	1	0.1
4.9.79	3	0.2
14.9.79	11	0.4
25.9.79	5	0.3
5.10.79	25	1.3
14.10.79	15	0.7
29.10.79	26	0.9
1.9.80	12	1.6
11.9.80	5	0.4
22.9.80	5	0.4
2.10.80	10	0.5
13.10.80	8	0.4
23.10.80	6	0.3

Percentage of ♂ C. pipiens for the 3 years $= \frac{134}{41372} \times 100\% = 0.3\%$

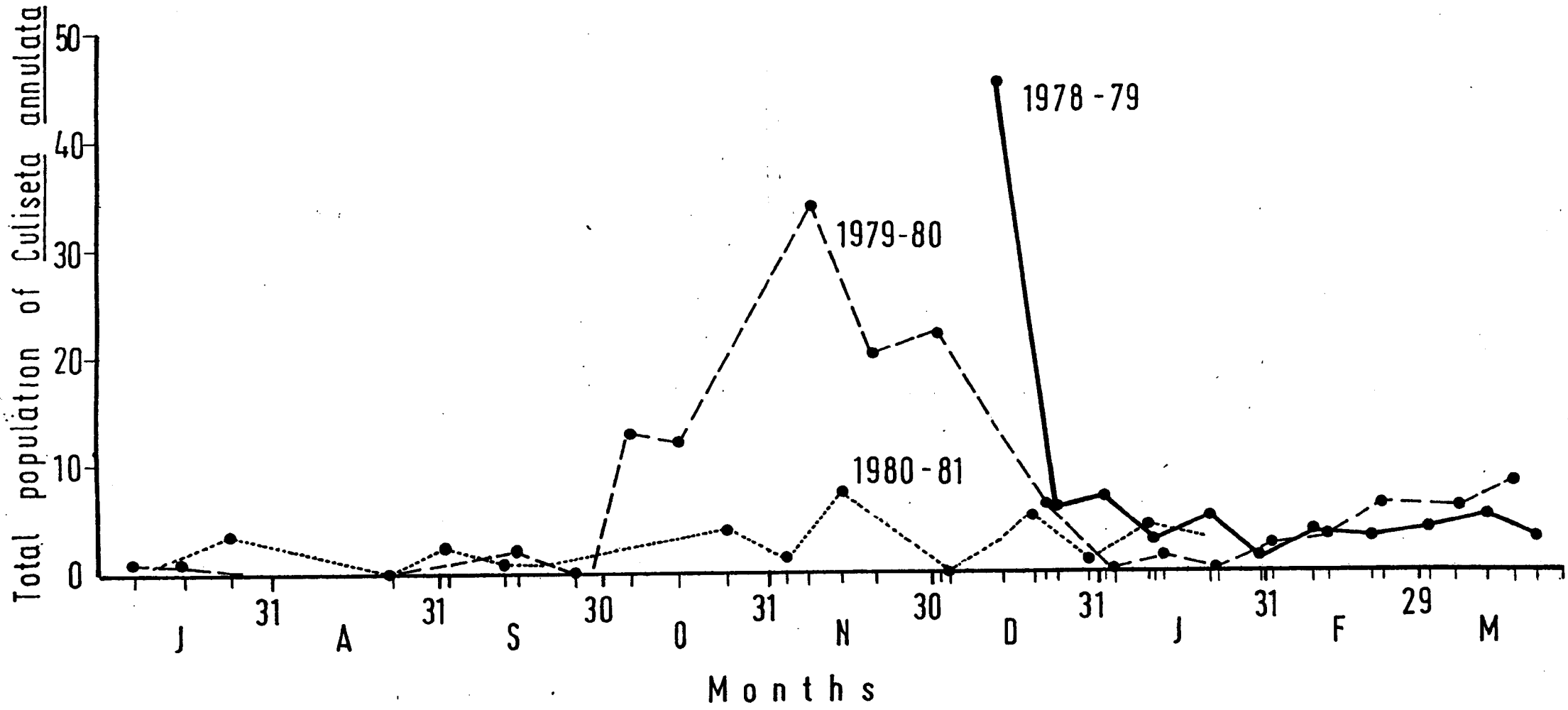

Fig. 46: Population of hibernating ♀ Culiseta annulata in shelter 1, West Kirby, Wirral.

7th November 1980. Adults were not infected with fungi in either shelters, and no blood engorged adults were seen. In autumn and winter the females Cs.annulata have fat bodies.

DISCUSSION ON HIBERNATING C. PIPIENS AND CS ANNULATA

This study in West Kirby (latitude 53° 22N) indicated that C. pipiens began hibernating in July. Similar studies conducted by Service (unpublished data) in Monks Wood, (latitude 52° 20N) Huntingdonshire showed that hibernating C. pipiens entered shelters on 15th August and 11th August in 1968 and 1969 respectively. A similar study on Brownsea Island (latitude 50° 43N) (Service, 1969a) found that C. pipiens began hibernating as early as 19th August in 1964 and 3rd August in 1965. Onyeka (1980) studied the hibernating population of C. pipiens in a house at Sunninghill (latitude 51° 25N), near Ascot and found that in 1978 and 1979 hibernating C. pipiens first entered the sites on the 8th and 16th August respectively. This difference in time of first hibernation of C. pipiens between the present studies in the north-west of England and the south of England conducted by Service (1969a, unpublished data for 1968-1970) and Onyeka (1980) might be due to variations in daylength. As observed by Oda and Kuhlow (1974) in their studies of hibernating C. pipiens in Hamburg (latitude 53° 33N), Germany, adult diapause began in July and ended in March and was induced by changing daylength rather than temperature. Buffington (1972), however, suggested that time of the first frost is a contributing factor in initiating hibernation of C. pipiens in Massachusetts, U.S.A.

The peak population for the years 1979 and 1980 were 9th November and 23rd October respectively. Service (1969a) observed the peak populations of C. pipiens on Brownsea Island, were in late October or early November. His studies in Monks Wood, Huntingdonshire showed the peak population of C. pipiens occured on 25th October and 27th October in 1969 and 1970 respectively (Fig 47) Onyeka (1980) also observed the peak population of C. pipiens in late October or early November in his studies at Sunninghill. Therefore, not much difference on time of peak population for hibernating C. pipiens occured in north west and south of England. C. pipiens had totally disappeared from the shelter by 22nd May 1979 but in 1980 C. pipiens were seen throughout the year with a minimum of one C. pipiens seen on 29th June 1980. But C. pipiens seen in the summer months were not in hibernation but were gonoactive females using the shelter as a resting site. In Monks Wood, Huntingdonshire , Service (unpublished data) found that C. pipiens had disappeared in May or early June (Figs 47 and 48).

There was a large reduction in population of C. pipiens in November and December of 1980 and 1981 respectively. This sort of situation was also seen by Service (1969a).

Spiders of the species Meta segmentata, Meta merianae, Tegenaria silvestris, Tegeneria atrica and Amaurobius sp. were observed feeding on hibernating adults of C. pipiens (Service, 1969a). He also discovered that at least two fungi namely Cephalosporium sp. possibly C. coccorum and Entomophthora sp. nr. conglomerata caused considerable mortality. Dead mosquitoes became infected with saprophytic Penicillium, Mucor and Sporotrichum species. But in both shelters in West Kirby, fungi were totally absence due to their dryness. Spiders of the species Lepthyphantes leprosus were the only suspected predators seen to exist in the shelters.

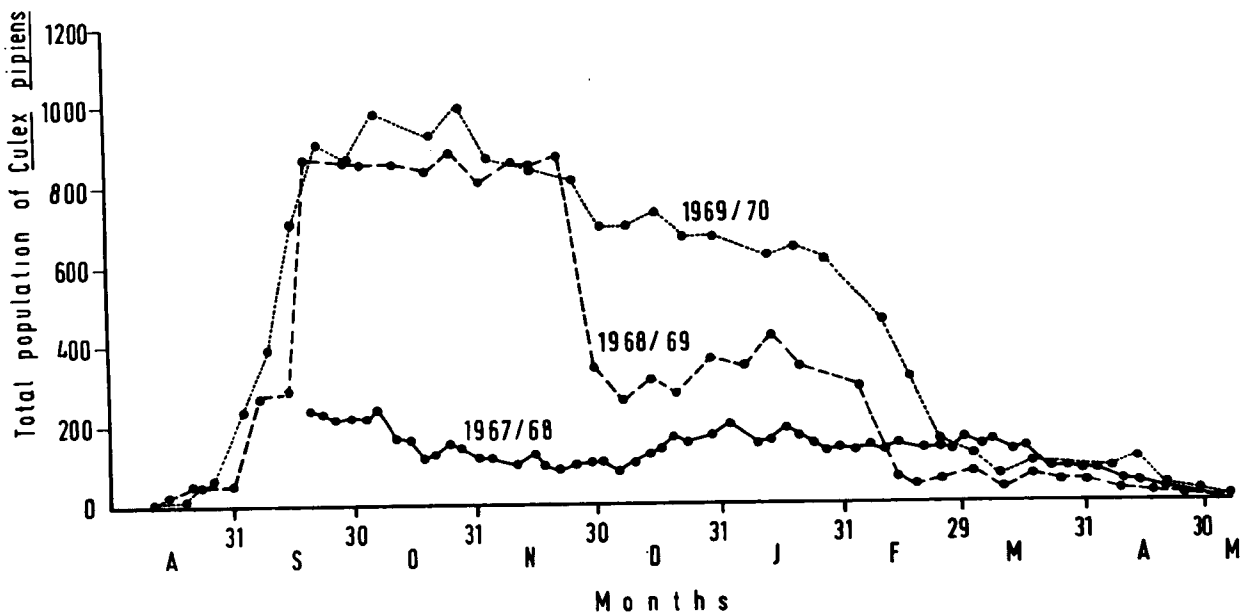

Fig. 47: Population of hibernating ♀ Culex pipiens at Monks Wood, Huntingdonshire (unpublished results of M.W. Service).

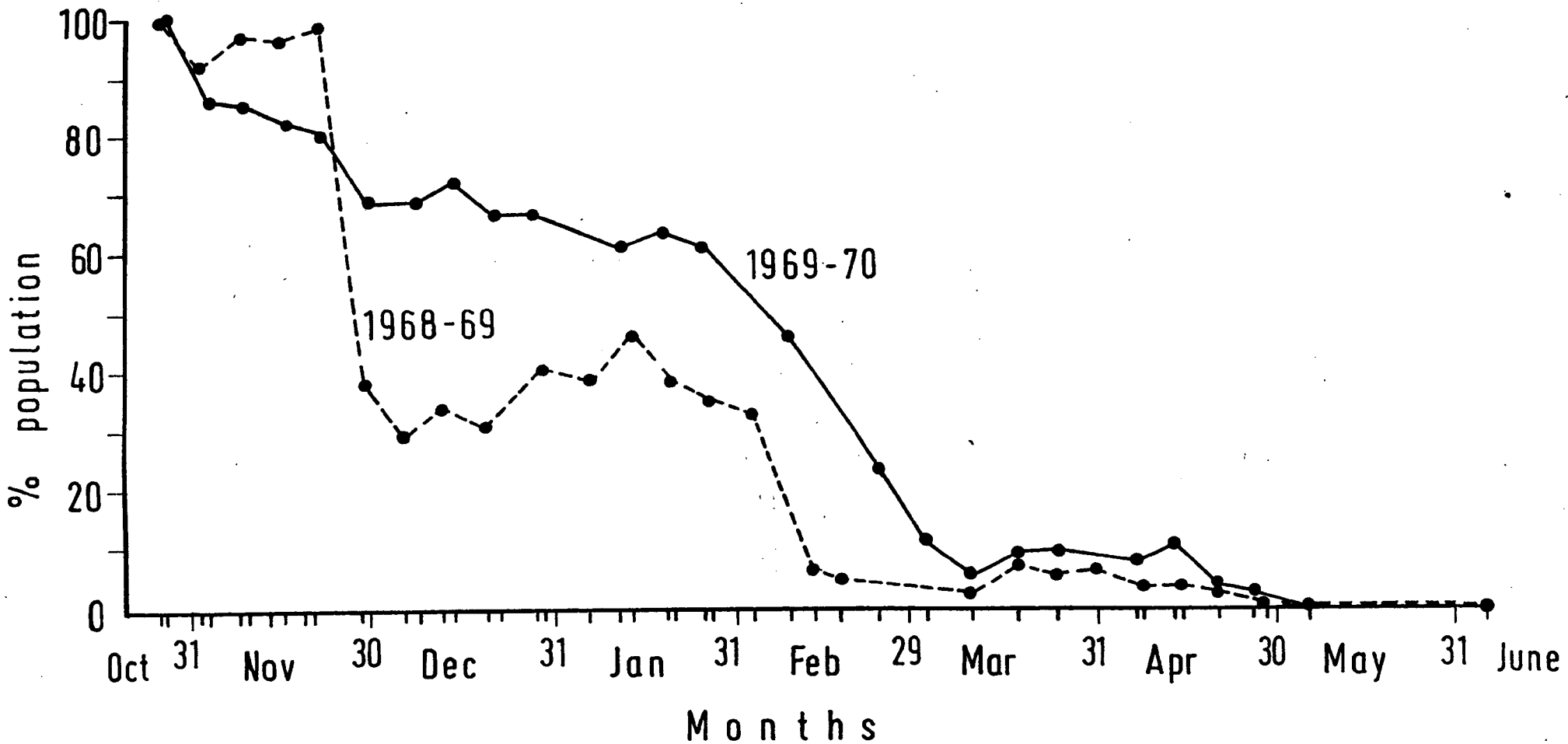

Fig. 48: Reduction of ♀ Culex pipiens population in a shelter, Monks Wood, Huntingdonshire (unpublished data of M.W. Service).

P. Merrett (personal communication, 1980) thought that Lepthyphantes leprosus was likely to be a predator of C. pipiens in such a habitat.

However, results obtained during 1980-1981 from shelter no. 2, to which an exit trap was built and the door and any crevices covered, indicated that light played a significant role in the exodus of C. pipiens from the shelters. In this shelter when exodus was prevented, only 6 C. pipiens were found in the exit trap in November and none was seen in December 1980, 3 C. pipiens in January, and 1 C. pipiens in February respectively (Table 61).

Table 58 shows that a reduction in population of C. pipiens was 5.4% by 24th November 1980, 19.7% by 29th December 1980, 21.8% by 29th January 1981 and 36.8% by 25th February 1981. This loss is presently accounted for by mortality in the shelter and was likely caused by spiders and depletion of fat reserves. As mentioned by Buffington (1972) additional stress of resuming active metabolism could be a factor contributing to mortality. Spiders were seen in the shelter and the population of spiders was as high as 32 (Tables 59 & 61). In contrast, in shelter no. 1, where both entrances were uncovered, Table 57 shows that there was a reduction of 63.7% of C. pipiens by 24th November 1980, 89.7% by 29th December 1980, 93.9% by 29th January 1981 and 95% by 19th February 1981. It seems therefore that the main factor contributing to the population loss recorded in shelter no. 1 was due to them leaving the shelter in the winter months. Similar observation was also seen by Service (1969a) whereby the decrease in C. pipiens population was mainly due to exodus of hibernating adults although spiders and fungi caused substantial reduction in the population. The breeding period for C. pipiens is between April and October and the reason for C. pipiens leaving the shelter in winter is unknown. However, C. pipiens that left the

shelter during the mid-winter could possibly be hibernating in other hibernation sites. Alternatively, and more likely, they died after leaving the shelter.

Males accounted for only 0.3% of the C. pipiens population in the shelter throughout the study period. The latest times male C. pipiens were seen, were 29th October in 1979 and 23rd October in 1980. Onyeka (1980) also found very few male C. pipiens in hibernation and the latest time male C. pipiens were seen was 22nd October in 1979. Clearly males do not survive the winter; they have no well developed fat bodies.

Female Cs.annulata go into semi-hibernation during cold weather and they feed on man, birds and other mammals in winter and summer (Marshall 1938, Kuhlhorn, 1974). Because of this behaviour even in the winter months there was a fluctuation in the population of adults Cs.annulata in the shelter no. 1. This could be due to adults occasionally emerge from hibernation to take bloodmeals (Service, 1969a). However, no bloodfed Cs.annulata were seen in the shelter. Male Cs.annulata were not found in shelter no. 1 but 2 male Cs.annulata were seen in the exit trap of shelter no. 2 on 7th November, 1980. Service (1969a) also found male Cs.annulata in shelter in Brownsea during October to February. Adults in both shelters were not seen to be infected with fungi and Service (1969a) also did not find adults Cs.annulata infected with fungi in Brownsea although C. pipiens in the same hibernation site were infected.

GENERAL DISCUSSION

Ae. cantans life strategy and population dynamics

The season's first adults of Ae. cantans emerge in late April or early May, feed on naturally occuring sugary secretions and rest amongst ground vegetation. No preferences for resting in different types of vegetation were found in Ness Woods. Then, about 2-3 weeks after emergence females commence blood-feeding. They fly out of the woods at night to feed on farm animals, mainly cattle, then return to the woods for shelter. Blood digestion takes about 6-8 days, afterwhich there is a delay of 1-3 days before the mature eggs are laid. Ae. cantans females lay about 72 eggs at each oviposition. Egg laying starts in late June and continues to September, but no eggs hatch during this period. This behaviour is similar to that observed by Service (1977a) in Monks Wood, Huntingdonshire; he found that Ae. cantans did not take blood-meals until they are about three weeks old, and that blood digestion took about 7 days. In Ness Woods all adults died off by the end of September, and the population is only in the egg state from October to early February when the eggs start to hatch.

During February to May the population is in larval state. Most mortality of an Ae. cantans population studied in a pond during 1980 occured amongst the later instars, but in 1981 it was highest among the earlier instars. A possible reason for this change in survivorship pattern was that there was very little rainfall from the beginning of late March 1980 until to mid-May 1980; in fact that April was the driest and sunniest month ever recorded in the area. Now, during this period of little rainfall, most of the Ae. cantans immature stages were in their late instars, and the decrease in volume of water in the pond seemed to cause high

larval mortalities. In this, and other years, predators also caused some mortality but they were not the main factor causing the large reductions in the population of Ae. cantans. For example, dytiscid larvae were the most voracious predators but their populations were too small to account for the estimated large reductions in the Ae. cantans population. Other predators included Chaoborus crystallinus and Mochlonyx culiciformis but again they were considered unlikely to cause much reduction of Ae. cantans because their numbers were far fewer than Ae. cantans. It is concluded that the main factor causing large reductions in population of Ae. cantans in 1980 was the drying up of parts of the pond. In addition it is considered that a number of unknown factors, possibly competition for space and overcrowded conditions, were responsible for most larval mortality.

The purpose of constructing a life-table is to summarise the survival and mortality rates of a population, but several problems are associated with construction of survivorship curves and life-tables for the immature stages of mosquitoes. Firstly, there is the problem of overlapping generations and prolonged or continual recruitment. Thus at any fixed point of time the population is composed of individuals of mixed age-classes. It is essential that all stages are sampled equally, otherwise the shape of the survivorship curve reflects not only survival rates but the catchability of the different age-classes. Therefore, sampling technique is important in order to get a representative proportion of the different instars in the population. For this reason the quadrat was utilised, in the studies at Ness Woods, because it was considered to give less biased samples than either dipping with a ladle or using an aquatic net. Service (1977b) also mentioned that before a survivorship curve can be drawn the instar durations must be determined, because the number of mosquitoes caught in each age-class must be "corrected" by dividing

by these durations. A problem here is that accurate measurements of instar durations are often difficult in natural habitats, especially when there are fluctuating environmental conditions such as temperature, larval density and food supply. Onyeka (1980) tried to overcome some of these difficulties by using weighted average instar durations. This is appropriate since it takes into account the differences in the numbers of pre-adult stages of mosquitoes sampled each month.

Despite the various problems encountered in building life-tables they are still worth trying to construct because they can provide comparative information of mortalities under different environmental condition and in different habitats. Although the quadrat method proved the best sampling method for estimating instar mortalities, there is still need for improving the technique, for example it might be possible to pump out the water enclosed by the quadrat. This would save considerable time and thus allow more quadrat samples to be taken, and might also be more efficient at removing the larvae and pupae. Instar durations should, wherever possible, be calculated by sampling the larval habitat, rather than based on laboratory studies. It would be worth trying to find out to what extent factors such as competition for space and overcrowding have on the survival rate of the immature population of Ae. cantans in Ness Woods, and also elsewhere.

Serological methods have the great advantages of measuring the intensity of predation in the field under natural conditions. But a disadvantage is that it is not always possible to produce specific antisera, although this is not a serious problem if the species to which the antiserum cross reacts do not coexist to any great extent with species under study. According to Service (1973 c), although absorbed serum is generally more specific, it loses some sensitivity; thus absorbed and unabsorbed antisera each have their merits. In Ness Woods Ae. cantans is by

far the predominant species and therefore serological identifications of predators using the stronger unabsorbed antisera is the most useful.

The ELISA test is very time consuming and requires highly titred antisera, because this allows the conjugate to be used in a more dilute form and so requires less enzyme which is expensive. In the present studies the titres used for IgG, antigen and conjugate are rather low, therefore more enzyme must be used and this makes the test costly, whereas in contrast the precipitin test is economical and simple. Another difficulty is that the gut contents (antigen) of the predators had low protein values, either because proteins in the meal of Ae. cantans had been destroyed by digestion or few prey were consumed. Consequently, the optical density of the yellow reaction colour resulting from the degradation of the enzyme substrate (its rate of degradation depends on the amount of enzyme-labelled antibody present and that in turn depends on the amount of antigen in the test sample) measured by Titertek Muttiskan Spectrophotometer could be lower than the standardized value. Therefore, the reading was recorded as negative, although actually prey had been consumed. In theory the ELISA test has the advantage of being more specific. Thus it should be able to differentiate better any cross-reactions between mosquito species, and therefore it should have potential when two or more mosquito species occur together. However, in practice, a comparison of the two tests, ELISA and precipitin, showed the latter to be more useful for identifying predation.

Evaluation of sampling methods

Of the three methods used to sample mosquito immature stages at Ness Woods, the quadrat proved more reliable than the ladle or aquatic net. Sampling with the quadrat enabled almost all immature stages trapped within the quadrat to be removed,

thus there was a better chance of recovering the earlier instars than when samples were taken with a ladle or aquatic net. According to WHO (1973),a ladle is suitable for small collections of water such as puddles, hoofprints and other small habitats, but unsuitable for larger breeding places. WHO (1973) considered an aquatic net more suitable for sampling larger bodies of water, especially as this method could cover a large surface in a relatively short time, but a disadvantage is that it cannot easily be used when dense vegetation is present. Knight (1964) concluded that dippers were inaccurate when dense stands of rigid emergent plants occur or where large amounts of debris or algae lie on the water surface, however, a quadrat is not subject so much to this limitation.

Nielsen and Nielsen (1953) found that earlier instars of Ae. taeniorhynchus less frequently rise to the water surface than later instars, and Hagstrum (1971) concluded a quadrat was more efficient in collecting 1st-instar larvae than dipping. Wada and Mogi (1974) found that the efficiency of a dipper differed considerably for the different age-classes of Culex tritaeniorhynchus summorosus and also for the 4th-instar larvae of this species and C.p. pallens. They concluded that there was a lower dipping efficiency for young stage larvae than for old stage larvae. Moreover, dipping efficiency for C. p. pallens was much lower than for C. t. summorosus suggesting the different behaviour of the two species in their ricefields. Now, there are likely differences in the submersion time of the different instars of Ae. cantans, and consequently dipping is likely to introduce bias. Although the quadrat seems likely to be more efficient in sampling the different age-classes of mosquitoes in the ponds at Ness Woods, the chief disadvantage is that it is time consuming. But the technique could be improved. For example, Ikemoto (1976) when using a square steel quadrat (25 x 25 x25cm) sucked out the larvae from each quadrat into a bottle with a small suction pump, and then poured

the contents into a larval concentrator. This considerably reduced the time spent in sampling from each quadrat.

Of the two aquatic light traps, namely betalights and chemical lights, that were evaluated, the latter clearly have potential for sampling mosquito populations. The chemical light trap is better presumably because it has a brighter light which is clearly seen even in daylight. In Ghana (M.W. Service personal comm., 1981), an M.Sc student, Mr. R. Esena,at the University of Ghana, caught as many as 18,500 mosquito larvae (presumably Culex thalassius Theobald) in a chemical light trap within 12 hours of exposure in a salt water pond. This clearly shows that chemical light traps can be very efficient in trapping larvae and deserves much more detailed evaluation.

Various glues, namely Rat Varnish, Hyvis 200 and Hyvis 2000, were tested as a mean of trapping mosquito larvae, and Rat Varnish seemed to be the better one. In the laboratory, Rat Varnish caught up to 92% of the 1st-instar and 100% of 3rd- 4th instar larvae of Ae. cantans larvae placed in tap water but only up to 24% and 88% of these larvae when they were in pond water. However, in the field, in tap water in plastic trays, Rat Varnish caught up to 36% of the 1st-instar and 96% of the 3rd-4th instar Ae. cantans larvae, but in pond water up to only 8% and 48% of the larvae were caught. Consequently, it seems that the combination of water turbidity and low temperatures (2- 14° C) caused a great reduction in size of the catches. Finally when the Rat Varnish was tested in a pond and ditch, very few Ae. cantans larvae were caught, thus showing its inefficiency in trapping mosquito larvae in the field at Ness Woods.

Because of the successful experiments in the laboratory at room temperature (17-26° C), field trials in the tropics where temperatures are higher should be worthy of evaluation. In fact, Mr. R. Esena working in Ghana caught over 24 hours as many as 1,755 larvae and 1,420 pupae (presumably Culex thalassius) on a plastic sheet (30 x 16cm) coated with Rat Varnish, placed in a salt water pond (M.W. Service personal comm., 1981). More evaluation of sticky traps are needed for sampling the immature stages of mosquitoes.

For adult sampling, bait collections are still the best for measuring biting population; light traps and a carbon dioxide trap were found to be of no use in sampling Ae. cantans adults at Ness Woods. Often great difficulties are encountered in finding adults mosquitoes resting in vegetation, but not so in Ness Woods, probably because there is a large emerging population concentrated within a relatively small wood. So, sweep-netting was very successfully employed to collect segments of the population not orientated to bloodfeeding.

Hibernation of Culex pipiens

A few hibernating adults of C. pipiens entered their hibernation shelters, as early as July, but the sharp increase in numbers did not began until early August, reaching a peak population in late October or early November. Thereafter there was a large reduction in population size, most loss occuring in November and December. This pattern of build-up and decrease has been observed by other workers (Service, 1969a, Onyeka, 1980). It seemed that this large reduction in numbers might be due to C. pipiens leaving the shelter although the reasons for this, if it happened, were not understood.

To try to demonstrate such an exodus an exit trap was fitted in November 1981 to another shelter. Although the population in this shelter declined greatly very

few adults were caught in the trap. However the presence of comparatively large numbers of mosquitoes in this exit trap during April and May, when hibernation is terminated and adults leave their shelters, clearly shows that the trap is capable of catching C. pipiens leaving the shelter. It seems therefore that the population loss is caused by some other factor, such as mortality within the shelter. Although spiders were present and were considered to be predators, their numbers were too small to have caused such large reductions in hibernating C. pipiens. So, the reason for the population loss is still unknown.

Further research is needed to find out whether mosquitoes are dying in the shelter. This could be determined by placing some mosquitoes inside a cage free of predators and counting the numbers of dead mosquitoes weekly. It might also be useful to determine the maximum numbers of mosquitoes that can be eaten by spiders, such as by placing a known number of spiders in small cages containing mosquitoes and recording their reduction in numbers each week.

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Appendix 1 Results of comparative larval sampling at Pond A, Ness Woods in 1979

Date of sampling	Method of sampling	No of samples	Total no. and % of Ae. cantans immature stages caught												Total no and % of Culiseta annulata, immature stages caught.									
			I	%	II	%	III	%	IV	%	Pupae	%	I	%	I	%	II	%	III	%	IV	%	Pupae	%
7.3.79	Ladle	20	298	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	259	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	519	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11.4.79	Ladle	20	26	19.8	33	25.2	66	50.4	6	4.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	560	41.3	468	34.5	309	22.8	20	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	50	22.3	65	29.0	106	47.3	3	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19.4.79	Ladle	20	4	3.8	37	35.6	43	41.3	20	19.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	155	19.1	283	34.8	245	30.1	129	15.9	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	11	2.3	135	28.4	167	35.2	152	32.0	10	2.1	-	-	-	-	-	-	-	-	-	-	-	-
28.4.79	Ladle	20	-	-	-	-	27	67.5	13	32.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	-	-	-	-	75	19.5	299	77.9	10	2.6	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	-	-	4	1.2	95	29.5	214	66.5	9	2.8	-	-	-	-	-	-	-	-	-	-	-	-
2.5.79	Ladle	20	-	-	-	-	8	30.8	18	69.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	-	-	-	-	34	10.6	261	81.6	25	7.8	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	-	-	-	-	15	4.7	155	48.4	17	5.3	-	-	-	-	-	-	-	-	-	-	-	-
8.5.79	Ladle	20	-	-	-	-	-	-	14	77.8	4	22.2	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	-	-	-	-	3	1.2	190	77.2	53	21.5	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	-	-	-	-	5	4.1	102	83.6	15	12.3	-	-	-	-	-	-	-	-	-	-	-	-
17.5.79	Ladle	20	-	-	-	-	-	-	6	50.0	6	50.0	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	-	-	-	-	4	1.6	56	22.9	185	75.5	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	-	-	-	-	3	2.8	19	17.8	85	79.4	-	-	-	-	-	-	-	-	-	-	-	-

Continued .....

Appendix 1 Results of comparative larval sampling at Pond A, Ness Woods in 1979 (Continued)

Date of sampling	Method of sampling	No of samples	Total no. and % of *Ae. cantans* immature stages caught												Total no and % of *Culiseta annulata*, immature stages caught									
			I	%	II	%	III	%	IV	%	Pupae	%	I	%	II	%	III	%	IV	%	Pupae	%		
23.5.79	Ladle	20	-	-	-	-	-	-	2	40	3	60.0	-	-	-	-	-	-	-	-	-	-		
	Quadrat	10	-	-	-	-	-	-	25	23.8	80	76.2	-	-	-	-	-	-	-	-	-	-		
	D-net	10	-	-	-	-	-	-	5	10.4	43	89.6	-	-	-	-	-	-	-	-	-	-		
29.5.79	Ladle	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Quadrat	10	-	-	-	-	-	-	8	24.2	25	75.8	-	-	-	-	-	-	-	-	-	-		
	D-net	10	-	-	-	-	-	-	1	11.1	8	88.9	-	-	-	-	-	-	-	-	-	-		
7.6.79	Ladle	20	-	-	-	-	-	-	-	-	-	-	-	-	2	100	-	-	-	-	-	-		
	Quadrat	10	-	-	-	-	-	-	1	2.0	5	10.0	-	-	15	30	26	52.0	3	6.0	-	-		
	D-net	10	-	-	-	-	-	-	1	1.6	2	3.3	-	-	14	23	40	65.6	3	4.9	1	-		
13.6.79	Ladle	20	-	-	-	-	1	33.3	-	-	-	-	-	-	-	-	-	-	2	66.7	-	-		
	Quadrat	10	-	-	-	-	-	-	-	-	4	22.2	-	-	-	-	-	-	14	77.8	-	-		
	D-net	10	-	-	-	-	-	-	-	-	5	12.8	-	-	-	-	6	15.4	27	69.2	1	2.6		
23.6.79	Ladle	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Quadrat	10	-	-	-	-	-	-	1	3.4	-	-	-	-	-	-	1	3.4	5	17.2	22	75.9		
	D-net	10	-	-	-	-	-	-	-	-	1	6.3	-	-	-	-	4	25.0	5	31.3	6	37.5		
28.6.79	Ladle	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	Quadrat	10	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	4	66.7	2	33.3		
	D-net	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	85.7	1	14.3		
5.7.79	Ladle	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	33.3	2	66.7		
	Quadrat	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	42.9	4	57.1	-	-		
	D-net	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	16.7	3	50.0	2	33.3		

Appendix 2 : Results of comparative larval sampling at Pond A, at Ness Woods in 1979

			Ae. cantans														
				Instar I			Instar II			Instar III			Instar IV			Pupae		
Date of sampling	Method of sampling	No of samples	Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance
7.3.79	Ladle	20	298	14.9	356.5	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	10	259	25.9	499.9	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	10	519	51.9	1571.5	-	-	-	-	-	-	-	-	-	-	-	-
11.4.79	Ladle	20	26	1.3	11.2	33	1.65	20.6	66	3.3	4.7	6	0.3	0.6	-	-	-
	Quadrat	10	560	56.0	2319.0	468	46.8	1497.2	309	30.9	359.3	20	2.0	3.4	-	-	-
	D-net	10	50	5.0	48.8	65	6.5	57.1	106	10.6	49.4	3	0.3	0.4	-	-	-
19.4.79	Ladle	20	4	0.2	0.5	37	1.85	26.8	43	2.15	1.7	20	1.0	1.3	-	-	-
	Quadrat	10	155	15.5	140.1	283	28.3	449.8	245	24.5	76.7	129	12.9	50.5	1	0.1	0.1
	D- net	10	11	1.1	7.1	135	13.5	1040.5	167	16.7	444.0	152	15.2	220.4	10	1.0	3.2
28.4.79	Ladle	20	0	0	0	0	0	0	27	1.35	1.7	13	0.65	0.9	-	-	-
	Quadrat	10	0	0	0	0	0	0	75	7.5	12.1	299	29.9	897.1	10	1.0	1.2
	D-net	10	0	0	0	4	0.4	0.2	95	9.5	55.1	214	21.4	226.6	9	0.9	0.9
2.5.79	Ladle	20	0	0	0	0	0	0	8	0.4	0.5	18	0.9	0.3	0	0	0
	Quadrat	10	0	0	0	0	0	0	34	3.4	9.8	261	26.1	513.6	25	2.5	29.1
	D-net	10	0	0	0	0	0	0	15	1.5	1.7	155	15.5	304.3	17	1.7	14.8
8.5.79	Ladle	20	0	0	0	0	0	0	0	0	0	14	0.7	0.4	4	0.2	0.2
	Quadrat	10	0	0	0	0	0	0	3	0.3	0.2	190	19.0	422.2	53	5.3	62.8
	D-net	10	0	0	0	0	0	0	5	0.5	0.5	102	10.2	53.6	15	1.5	8.5
17.5.79	Ladle	20	0	0	0	0	0	0	0	0	0	6	0.3	0.2	6	0.3	0.3
	Quadrat	10	0	0	0	0	0	0	4	0.4	0.8	56	5.6	40.6	185	18.5	304.5
	D-net	10	0	0	0	0	0	0	3	0.3	0.4	19	1.9	5.3	85	8.5	31.9

Continued .....

Appendix 2: Results of comparative larval sampling at Pond A, Ness Woods in 1979 (Continued)

Ae. cantans

Date of sampling	Method of sampling	No of samples	Instar I			Instar II			Instar III			Instar IV			Pupae		
			Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance
23.5.79	Ladle	20	0	0	0	0	0	0	0	0	0	2	0.1	0.1	3	0.15	0.13
	Quadrat	10	0	0	0	0	0	0	0	0	0	25	2.5	10.9	80	8.0	72.8
	D-net	10	0	0	0	0	0	0	0	0	0	5	0.5	1.5	43	4.3	49.4
29.5.79	Ladle	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	10	0	0	0	0	0	0	0	0	0	8	0.8	1.0	25	2.5	11.9
	D-net	10	0	0	0	0	0	0	0	0	0	1	0.1	0.1	8	0.8	0.4
7.6.79	Ladle	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	10	0	0	0	0	0	0	0	0	0	1	0.1	0.1	5	0.5	0.5
	D-net	10	0	0	0	0	0	0	0	0	0	1	0.1	0.1	2	0.2	0.2
13.6.79	Ladle	20	0	0	0	0	0	0	1	0.05	0.1	0	0	0	0	0	0
	Quadrat	10	0	0	0	0	0	0	0	0	0	0	0	0	4	0.4	0.2
	D-net	10	0	0	0	0	0	0	0	0	0	0	0	0	5	0.5	0.9
23.6.79	Ladle	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	10	0	0	0	0	0	0	0	0	0	1	0.1	0.1	0	0	0
	D-net	10	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1	0.1
28.6.79	Ladle	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D-net	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3 : Results of compartive larval sampling at pond B Ness Woods in 1981

Date of sampling	Method of sampling	No of samples	Total no and % of Ae. cantans immature stages caught										Total no and % of Ae. punctor immature stages caught									
			I	%	II	%	III	%	IV	%	Pupae	%	I	%	II	%	III	%	IV	%	Pupae	%
24.2.81	Ladle	10	35	70	-	-	-	-	-	-	-	-	10	20	5	10	-	-	-	-	-	-
	Quadrat	2	217	75.9	-	-	-	-	-	-	-	-	48	16.8	9	3.1	-	-	-	-	-	-
	D -net	2	153	81.8	-	-	-	-	-	-	-	-	28	15.0	4	2.1	-	-	-	-	-	-
3.3.81	Ladle	10	36	90.0	-	-	-	-	-	-	-	-	-	-	4	10.0	-	-	-	-	-	-
	Quadrat	2	519	90.3	-	-	-	-	-	-	-	-	43	7.5	6	1.0	-	-	-	-	-	-
	D-net	2	128	71.1	-	-	-	-	-	-	-	-	33	18.3	15	8.3	-	-	-	-	-	-
10.3.81	Ladle	10	18	24.0	44	58.7	-	-	-	-	-	-	2	2.7	7	9.3	1	1.3	-	-	-	-
	Quadrat	2	237	54.6	89	20.5	-	-	-	-	-	-	25	5.8	75	17.3	-	-	-	-	-	-
	D-net	2	202	36.8	265	48.3	-	-	-	-	-	-	21	3.8	55	10.0	-	-	-	-	-	-
17.3.81	Ladle	20	54	33.5	38	23.6	1	0.6	-	-	-	-	6	3.7	18	11.2	44	27.3	-	-	-	-
	Quadrat	4	167	23.0	193	26.6	13	1.8	-	-	-	-	66	9.1	164	22.6	114	15.7	-	-	-	-
	D-net	4	198	31.7	140	22.4	10	1.6	-	-	-	-	66	10.6	115	18.4	94	15.0	-	-	-	-
25.3.81	Ladle	15	16	10.7	57	38.0	15	10.0	-	-	-	-	-	-	22	14.7	20	13.3	4	2.7	-	-
	Quadrat	3	90	11.1	395	48.7	79	9.7	4	0.5	-	-	6	0.7	141	17.4	62	7.6	24	3.0	-	-
	D-net	3	54	11.4	183	38.6	53	11.2	2	0.4	-	-	6	1.3	85	17.9	45	9.5	42	8.9	-	-

Continued .....

Appendix 3 : Results of compartive larval sampling at pond B Ness Woods in 1981 (Continued)

Date of sampling	Method of sampling	No of samples	Total no and % of Ae. cantans immature stages caught										Total no and % of Ae. punctor immature stages caught									
			I	%	II	%	III	%	IV	%	Pupae	%	I	%	II	%	III	%	IV	%	Pupae	%
2.4.81	Ladle	15	-	-	4	4.4	59	64.8	6	6.6	-	-	-	-	-	-	11	12.1	10	11.0	-	-
	Quadrat	3	1	0.1	324	37.0	286	32.6	34	3.9	-	-	-	-	13	1.5	97	11.1	120	13.7	-	-
	D- net	3	1	0.2	139	31.6	152	34.5	13	3.0	-	-	1	0.2	7	1.6	59	13.4	68	15.5	-	-
7.4.81	Ladle	15	-	-	-	-	10	25.6	6	15.4	-	-	-	-	-	-	5	12.8	18	46.2	-	-
	Quadrat	3	-	-	104	16.4	212	33.3	69	10.8	-	-	-	-	-	-	69	10.8	174	27.4	3	0.5
	D-net	3	-	-	5	3.6	53	38.4	27	19.6	-	-	-	-	-	-	10	7.2	43	31.2	-	-
15.4.81	Ladle	15	-	-	-	-	-	-	13	36.1	-	-	-	-	-	-	-	-	9	25.0	14	38.9
	Quadrat	3	-	-	3	0.5	30	5.2	212	36.6	-	-	-	-	-	-	4	0.7	223	38.4	108	18.6
	D-net	3	-	-	-	-	20	8.3	117	48.3	-	-	-	-	-	-	-	-	65	26.9	38	15.7
22.4.81	Ladle	15	-	-	-	-	-	-	14	66.7	-	-	-	-	-	-	-	-	1	4.7	6	28.6
	Quadrat	3	-	-	-	-	32	8.7	249	68.0	2	0.5	-	-	-	-	-	-	15	4.1	66	18.0
	D-net	3	-	-	-	-	16	8,9	133	73.9	3	1.7	-	-	-	-	-	-	6	3.3	22	12.2
29.4.81	Ladle	15	-	-	-	-	-	-	11	50.0	5	22.7	-	-	-	-	-	-	5	22.7	1	4.5
	Quadrat	3	-	-	-	-	-	-	100	57.1	18	10.3	-	-	-	-	-	-	25	14.3	32	18.3
	D- net	3	-	-	-	-	-	-	25	49.0	12	23.5	-	-	-	-	-	-	10	19.6	4	7.8

Continued .....

Appendix 3 : Results of compartive larval sampling at pond B Ness Woods in 1981 (Continued)

Date of sampling	Method of sampling	No of samples	Total no and % of *Ae. cantans* immature stages caught										Total no and % of *Ae. punctor* immature stages caught									
			I	%	II	%	III	%	IV	%	Pupae	%	I	%	II	%	III	%	IV	%	Pupae	%
6.5.81	Ladle	15	-	-	-	-	-	-	12	63.1	6	31.6	-	-	-	-	-	-	-	-	1	5.3
	Quadrat	3	-	-	-	-	-	-	50	39.7	42	33.3	-	-	-	-	-	-	3	2.4	31	24.6
	D- net	3	-	-	-	-	-	-	43	58.1	21	28.4	-	-	-	-	-	-	3	4.1	7	9.5
13.5.81	Ladle	15	-	-	-	-	-	-	1	3.8	25	96.2	-	-	-	-	-	-	-	-	-	-
	Quadrat	3	-	-	-	-	-	-	31	16.3	156	82.1	-	-	-	-	-	-	-	-	3	1.6
	D-net	3	-	-	-	-	-	-	23	13.5	148	86.5	-	-	-	-	-	-	-	-	-	-
20.5.81	Ladle	15	-	-	-	-	-	-	-	-	15	100.0	-	-	-	-	-	-	-	-	-	-
	Quadrat	3	-	-	-	-	-	-	-	-	112	100.0	-	-	-	-	-	-	-	-	-	-
	D-net	3	-	-	-	-	-	-	-	-	30	100.0	-	-	-	-	-	-	-	-	-	-
27.5.81	Ladle	15	-	-	-	-	-	-	-	-	3	100.0	-	-	-	-	-	-	-	-	-	-
	Quadrat	3	-	-	-	-	-	-	-	-	9	100.0	-	-	-	-	-	-	-	-	-	-
	D-net	3	-	-	-	-	-	-	-	-	6	100.0	-	-	-	-	-	-	-	-	-	-
3.6.81	Ladle	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981

Ae. cantans

Date of sampling	Method of sampling	No of samples	Instar I Total	Instar I Mean	Instar I Variance	Instar II Total	Instar II Mean	Instar II Variance	Instar III Total	Instar III Mean	Instar III Variance	Instar IV Total	Instar IV Mean	Instar IV Variance	Pupae Total	Pupae Mean	Pupae Variance
24.2.81	Ladle	10	35	3.5	4.1	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	2	217	108.5	870.3	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	2	153	76.5	1806.3	-	-	-	-	-	-	-	-	-	-	-	-
3.3.81	Ladle	10	36	3.6	1.6	-	-	-	-	-	-	-	-	-	-	-	-
	Quadrat	2	519	259.5	2256.3	-	-	-	-	-	-	-	-	-	-	-	-
	D-net	2	128	64.0	256.0	-	-	-	-	-	-	-	-	-	-	-	-
10.3.81	Ladle	10	18	1.8	4.0	44	4.4	28.6	-	-	-	-	-	-	-	-	-
	Quadrat	2	237	118.5	462.3	89	44.5	156.3	-	-	-	-	-	-	-	-	-
	D-net	2	202	101.0	576.0	265	132.5	5550.3	-	-	-	-	-	-	-	-	-
17.3.81	Ladle	20	54	2.7	12.4	38	1.9	2.9	1	0.05	0.1	-	-	-	-	-	-
	Quadrat	4	167	41.75	566.19	193	48.25	423.19	13	3.25	2.19	-	-	-	-	-	-
	D-net	4	198	49.5	700.75	140	35.0	279.5	10	2.5	5.25	-	-	-	-	-	-
25.3.81	Ladle	15	16	1.07	0.6	57	3.8	24.96	15	1.0	1.2	-	-	-	-	-	-
	Quadrat	3	90	30.0	42.67	395	131.67	2534.22	79	26.33	310.22	4	1.33	0.22	-	-	-
	D- net	3	54	18.0	8.67	183	61.0	56.0	53	17.67	54.89	2	0.67	0.22	-	-	-
2.4.81	Ladle	15	0	0	0	4	0.27	033	59	3.93	17.66	6	0.4	0.77	-	-	-
	Quadrat	3	1	0.33	0.22	324	108.0	2420.67	286	95.33	3456.22	34	11.33	81.56	-	-	-
	D- net	3	1	0.33	0.22	139	46.33	491.56	152	50.67	1074.89	13	4.33	16.22	-	-	-

Continued .....

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981 (Continued)

Ae. cantans

Date of sampling	Method of sampling	No of samples	Instar I			Instar II			Instar III			Instar IV			Pupae		
			Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance	Total	Mean	Variance
7.4.81	Ladle	15	0	0	0	0	0	0	10	0.67	0.62	6	0.40	0.64	0	0	0
	Quadrat	3	0	0	0	104	34.67	1480.22	212	70.67	104.22	69	23.0	348.67	0	0	0
	D-net	3	0	0	0	5	1.67	5.56	53	17.67	88.22	27	9.0	16.67	0	0	0
15.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	13	0.87	0.78	0	0	0
	Quadrat	3	0	0	0	3	1.0	0.67	30	10.0	2.0	212	70.67	696.22	0	0	0
	D-net	3	0	0	0	0	0	0	20	6.67	4.22	117	39.0	60.67	0	0	0
22.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	14	0.93	1.80	0	0	0
	Quadrat	3	0	0	0	0	0	0	32	10.67	150.22	249	83.0	3200.67	2	0.67	0.89
	D-net	3	0	0	0	0	0	0	16	5.33	1.56	133	44.33	1342.89	3	1.0	2.0
29.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	11	0.73	0.60	5	0.33	0.36
	Quadrat	3	0	0	0	0	0	0	0	0	0	100	33.3	1136.22	18	6.0	32.67
	D-net	3	0	0	0	0	0	0	0	0	0	25	8.3	10.89	12	4.0	2.67
6.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	12	0.8	2.16	6	0.4	0.51
	Quadrat	3	0	0	0	0	0	0	0	0	0	50	16.7	91.56	42	14.0	18.0
	D-net	3	0	0	0	0	0	0	0	0	0	43	14.33	24.89	21	7.0	24.0

Continued .....

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981 (Continued)

Ae. cantans

Date of sampling	Method of sampling	No of samples	Instar I Total	Instar I Mean	Instar I Variance	Instar II Total	Instar II Mean	Instar II Variance	Instar III Total	Instar III Mean	Instar III Variance	Instar IV Total	Instar IV Mean	Instar IV Variance	Pupae Total	Pupae Mean	Pupae Variance
13.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	1	0.07	0.06	25	1.67	3.42
	Quadrat	3	0	0	0	0	0	0	0	0	0	31	10.33	0.22	156	52.0	304.67
	D-net	3	0	0	0	0	0	0	0	0	0	23	7.67	14.89	148	49.33	193.56
20.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	15	1.0	1.07
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	112	37.33	294.89
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	30	10.0	28.67
27.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	3	0.2	0.29
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	9	3.0	0.67
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	6	2.0	0.67
3.6.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Continued .....

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981 (Continued)

Ae. punctor

Date of sampling	Method of sampling	No of samples	Instar I Total	Instar I Mean	Instar I Variance	Instar II Total	Instar II Mean	Instar II Variance	Instar III Total	Instar III Mean	Instar III Variance	Instar IV Total	Instar IV Mean	Instar IV Variance	Pupae Total	Pupae Mean	Pupae Variance
24.2.81	Ladle	10	10	1.0	0.6	5	0.5	1.05	0	0	0	0	0	0	0	0	0
	Quadrat	2	48	24.0	225.0	9	4.5	6.25	0	0	0	0	0	0	0	0	0
	D -net	2	28	14.0	4.0	4	2.0	4.0	0	0	0	0	0	0	0	0	0
3.3.81	Ladle	10	0	0	0	4	0.4	0.24	0	0	0	0	0	0	0	0	0
	Quadrat	2	43	21.5	0.25	6	3.0	1.0	0	0	0	0	0	0	0	0	0
	D-net	2	33	16.5	110.25	15	7.5	56.25	0	0	0	0	0	0	0	0	0
10.3.81	Ladle	10	2	0.2	0.16	7	0.7	0.41	1	0.1	0.09	0	0	0	0	0	0
	Quadrat	2	25	12.5	30.25	75	37.5	0.25	0	0	0	0	0	0	0	0	0
	D-net	2	21	10.5	42.25	55	27.5	110.25	0	0	0	0	0	0	0	0	0
17.3.81	Ladle	20	6	0.3	0.81	18	0.9	1.39	44	2.2	7.16	0	0	0	0	0	0
	Quadrat	4	66	16.5	117.25	164	41.0	142.5	114	28.5	216.75	0	0	0	0	0	0
	D-net	4	66	16.5	114.25	115	28.75	258.69	94	23.5	52.25	0	0	0	0	0	0
25.3.81	Ladle	15	0	0	0	22	1.47	1.72	20	1.33	2.49	4	0.27	0.6	0	0	0
	Quadrat	3	6	2.0	2.67	141	47.0	8.67	62	20.67	110.89	24	8.0	8.0	0	0	0
	D-net	3	6	2.0	8.0	85	28.33	182.89	45	15.0	72.0	42	14.0	144.67	0	0	0

Continued .....

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981 (Continued)

Ae. punctor

Date of sampling	Method of sampling	No of samples	Instar I Total	Instar I Mean	Instar I Variance	Instar II Total	Instar II Mean	Instar II Variance	Instar III Total	Instar III Mean	Instar III Variance	Instar IV Total	Instar IV Mean	Instar IV Variance	Pupae Total	Pupae Mean	Pupae Variance
2.4.81	Ladle	15	0	0	0	0	0	0	11	0.73	1.00	10	0.67	0.49	0	0	0
	Quadrat	3	0	0	0	13	4.33	0.89	97	32.33	451.56	120	40.0	992.0	0	0	0
	D-net	3	1	0.33	0.22	7	2.33	6.89	59	19.67	128.22	68	22.67	464.22	0	0	0
7.4.81	Ladle	15	0	0	0	0	0	0	5	0.33	0.22	18	1.2	2.03	0	0	0
	Quadrat	3	0	0	0	0	0	0	69	23.0	28.67	174	58.0	1420.67	3	1	2
	D- net	3	0	0	0	0	0	0	10	3.33	6.22	43	14.33	10.89	0	0	0
15.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	9	0.6	0.51	14	0.93	1.80
	Quadrat	3	0	0	0	0	0	0	4	1.33	0.22	223	74.33	1300.22	108	36.0	130.67
	D-net	3	0	0	0	0	0	0	0	0	0	65	21.67	126.89	38	12.67	43.56
22.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	1	0.07	0.06	6	0.40	0.51
	Quadrat	3	0	0	0	0	0	0	0	0	0	15	5.0	0.67	66	22.0	12.67
	D-net	3	0	0	0	0	0	0	0	0	0	6	2.0	0.67	22	7.33	0.89
29.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	5	0.33	0.36	1	0.07	0.06
	Quadrat	3	0	0	0	0	0	0	0	0	0	25	8.33	38.89	32	10.67	38.22
	D- net	3	0	0	0	0	0	0	0	0	0	10	3.33	2.89	4	1.33	0.89

Continued .....

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981 (Continued)

Ae. punctor

Date of sampling	Method of sampling	No of samples	Instar I Total	Instar I Mean	Instar I Variance	Instar II Total	Instar II Mean	Instar II Variance	Instar III Total	Instar III Mean	Instar III Variance	Instar IV Total	Instar IV Mean	Instar IV Variance	Pupae Total	Pupae Mean	Pupae Variance
6.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	1	0.07	0.06
	Quadrat	3	0	0	0	0	0	0	0	0	0	3	1	0.67	31	10.33	1.56
	D-net	3	0	0	0	0	0	0	0	0	0	3	1	2.0	7	2.33	1.56
13.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	3	1.0	2.0
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27.5.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.6.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Continued .....

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981 (Continued)

Date of sampling	Method of sampling	No of samples	Ae. rusticus Instar I Total	Instar I Mean	Instar I Variance	Instar II Total	Instar II Mean	Instar II Variance	Instar III Total	Instar III Mean	Instar III Variance	Instar IV Total	Instar IV Mean	Instar IV Variance	Pupae Total	Pupae Mean	Pupae Variance
24.2.81	Ladle	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	2	8	4	1.0	3	1.5	2.25	1	0.5	0.25	0	0	0	0	0	0
	D-net	2	2	1	1.0	0	0	0	0	0	0	0	0	0	0	0	0
3.3.81	Ladle	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	2	6	3	0	0	0	0	1	0.5	0.25	0	0	0	0	0	0
	D-net	2	1	0.5	0.25	2	1	1	1	0.5	0.25	0	0	0	0	0	0
10.3.81	Ladle	10	0	0	0	3	0.3	0.41	0	0	0	0	0	0	0	0	0
	Quadrat	2	0	0	0	8	4.0	6.0	0	0	0	0	0	0	0	0	0
	D-net	2	0	0	0	6	3.0	4.0	0	0	0	0	0	0	0	0	0
17.3.81	Ladle	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	4	0	0	0	6	1.5	0.25	2	0.5	0.25	0	0	0	0	0	0
	D-net	4	0	0	0	2	0.5	0.25	0	0	0	0	0	0	0	0	0
25.3.81	Ladle	15	0	0	0	0	0	0	0	0	0	1	0.07	0.06	0	0	0
	Quadrat	3	0	0	0	1	0.33	0.22	5	1.67	0.22	4	1.33	0.89	0	0	0
	D-net	3	0	0	0	1	0.33	0.22	3	1.0	2.0	1	0.33	0.22	0	0	0

Continued .....

Appendix 4: Results of comparative larval sampling at pond B, Ness Woods, in 1981 (Continued)

Ae.rusticus

Date of sampling	Method of sampling	No of samples	Instar I Total	Instar I Mean	Instar I Variance	Instar II Total	Instar II Mean	Instar II Variance	Instar III Total	Instar III Mean	Instar III Variance	Instar IV Total	Instar IV Mean	Instar IV Variance	Pupae Total	Pupae Mean	Pupae Variance
2.4.81	Ladle	15	0	0	0	0	0	0	1	0.07	0.06	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	1	0.33	0.22	0	0	0	0	0	0
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	1	0.33	0.22	4	1.3	1.6	0	0	0
	D-net	3	0	0	0	0	0	0	1	0.33	0.22	4	1.3	1.6	0	0	0
15.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D-net	3	0	0	0	0	0	0	0	0	0	2	0.67	0.22	0	0	0
22.4.81	Ladle	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3	0	0	0	0	0	0	0	0	0	2	0.67	0.89	0	0	0
	D-net	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Between 29.4.8 to 3.6.81	Ladle	15 x 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quadrat	3 x 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D-net	3 x 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 5: Results of weekly sampling with a quadrat from pond 1, Ness Woods in 1980 with average of 5 samples per week

Date of sampling	Ae. cantans I	II	III	IV	Pupae
31.1.80	0				
6.2.80	713				
13.2.80	2506				
20.2.80	2301	143			
27.2.80	770	2266			
5.3.80	107	1102	1224		
12.3.80	95	1597	2598		
19.3.80	82	488	1451	18	
26.3.80	104	332	900	30	
2.4.80	63	284	729	22	
9.4.80	76	313	727	155	
16.4.80	6	126	297	460	
23.4.80	-	-	11	278	46
30.4.80	-	-	1	58	225
7.5.80	-	-	-	1	57
15.5.80	-	-	-	1	6
21.5.80	-	-	-	0.5	1.5
28.5.80	-	-	-	-	-
Total	6823	6651	7938	1023.5	335.5

Appendix 6: Results of weekly sampling with a quadrat from pond 1, Ness Woods in 1981 with average of 5 samples per week

	Ae. cantans				
Date of sampling	I	II	III	IV	Pupae
5.2.81	0				
10.2.81	1				
17.2.81	8				
23.2.81	3				
2.3.81	235	1			
9.3.81	283	5			
16.3.81	181	114	12		
24.3.81	412	272	28		
31.3.81	65	309	39	12	
6.4.81	-	235	397	127	
14.4.81	-	3	67	210	6
21.4.81	-	-	3	164	50
28.4.81	-	-	1	56	69
5.5.81	-	-	-	29	46
12.5.81	-	-	-	5	60
19.5.81	-	-	-	-	6
26.5.81	-	-	-	-	2
3.6.81	-	-	-	-	1
10.6.81	-	-	-	-	-
16.6.81	-	-	-	-	-
Total	1188	939	547	603	240

Appendix 7: Results of weekly sampling from permanent quadrat 1 at Ness Woods in 1980

Date of sampling	I	II	III	IV	Pupae	Adults
12.2.80	248					
19.2.80	289	8				
26.2.80	41	157				
4.3.80	16	87	6			
11.3.80	2	34	3			
18.3.80	3	18	5			
25.3.80	8	10	4	1		
1.4.80	7	6	4	1		
8.4.80	3	4	8	1		
15.4.80	-	-	4	7		
22.4.80	-	-	1	7		
29.4.80	-	-	-	4	4	
6.5.80	-	-	-	1	6	1 ♂
13.5.80	-	-	-	1	3	3 ♂
20.5.80	-	-	-	-	2	2 ♀
27.5.80	-	-	-	-	-	2 ♀
3.6.80	-	-	-	-	-	-
Total	617	324	35	23	15	8

Appendix 8: Results of weekly sampling from permanent quadrat 2 at Ness Woods in 1980

Date of sampling	I	II	III	IV	Ae. cantans Pupae	Adults
12.2.80	250					
19.2.80	365	35				
26.2.80	3	208				
4.3.80	3	148	60			
11.3.80	-	83	84			
18.3.80	20	79	71			
25.3.80	58	60	81	2		
1.4.80	31	77	83	16		
8.4.80	57	71	152	26		
15.4.80	4	97	159	44		
22.4.80	-	52	85	159	1	
29.4.80	-	27	66	156	23	
6.5.80	-	9	56	141	57	
13.5.80	-	-	29	142	28	11 ♂ 10 ♀
20.5.80	-	-	-	79	40	4 ♂ 6 ♀
27.5.80	-	-	-	16	30	2 ♂ 2 ♀
3.6.80	-	-	-	3	32	1 ♂ 5 ♀
11.6.80	-	-	-	-	4	12 ♂ 15 ♀
1.7.80	-	-	-	-	-	- -
Total	791	946	926	784	215	68

Appendix 9: Results of weekly sampling from permanent quadrat 3 at Ness Woods in 1980

	Ae. cantans					
Date of sampling	I	II	III	IV	Pupae	Adults
12.2.80	156					
19.2.80	275	18				
26.2.80	-	209				
4.3.80	24	145	46			
11.3.80	-	45	142			
18.3.80	2	43	53			
25.3.80	8	14	73	2		
1.4.80	24	14	49	26		
8.4.80	22	32	59	18		
15.4.80	4	28	46	52		
22.4.80	-	6	31	80		
29.4.80	-	2	12	71	29	
6.5.80	-	-	1	45	49	10 ♂
13.5.80	-	-	-	29	40	17 ♂ 4 ♀
20.5.80	-	-	-	4	10	8 ♂ 14 ♀
27.5.80	-	-	-	-	4	2 ♂ 3 ♀
3.6.80	-	-	-	-	2	3 ♀
11.6.80	-	-	-	-	-	- -
Total	515	556	512	327	134	61

Appendix 10: Results of weekly sampling from permanent quadrat 4 at Ness Woods in 1980

					Ae. cantans				
Date of sampling	I	II	III	IV	Pupae	Adults			
12.2.80	170								
19.2.80	387	21							
26.2.80	3	344							
4.3.80	4	248	52						
11.3.80	-	72	184						
18.3.80	9	55	93						
25.3.80	15	40	36						
1.4.80	2	30	32						
8.4.80	2	3	53	1					
15.4.80	-	4	18	33					
22.4.80	-	1	2	52					
29.4.80	-	-	2	24	29				
6.5.80	-	-	-	9	41	1	♂		
13.5.80	-	-	-	2	22	12	♂	2	♀
20.5.80	-	-	-	-	-	1	♂	2	♀
27.5.80	-	-	-	-	-	-		3	♀
3.6.80	-	-	-	-	-	-		-	
Total	592	818	472	121	92	21			

Appendix 11: Results of weekly sampling from permanent quadrat 5 at Ness Woods in 1980

	Ae. cantans					
Date of sampling	I	II	III	IV	Pupae	Adults
12.2.80	965					
19.2.80	861	39				
26.2.80	204	176				
4.3.80	16	62	12			
11.3.80	3	27	1			
18.3.80	15	18	2			
25.3.80	24	8	8			
1.4.80	22	15	15	6		
8.4.80	9	15	28	4		
15.4.80	15	17	25	14		
22.4.80	-	15	18	31	2	
29.4.80	-	8	10	38	5	
6.5.80	-	-	9	34	14	2 ♂
13.5.80	-	-	-	32	18	4 ♂ 3 ♀
20.5.80	-	-	-	4	17	2 ♂ 1 ♀
27.5.80	-	-	-	-	3	10 ♂ 6 ♀
3.6.80	-	-	-	-	-	- -
Total	2134	400	128	163	59	28

Appendix 12: Total number of immature stages and adults Ae. cantans in permanent quadrats (without new ovipositions in summer 1980) at Ness Woods in 1981.

	Ae. cantans								
Permanent Quadrats	I	II	III	IV	Pupae	Adults			
1	15	6	6	8	6	2	♂	1	♀
2	-	-	-	-	-	-		-	
3	-	-	-	-	-	-		-	
4	10	8	8	9	6	4	♂	1	♀
5	12	8	8	9	7	1	♂	1	♀

Appendix 13: Total number of immature stages and adults Ae. cantans in permanent cylindrical quadrats at Ness Woods in 1981

	Ae. cantans						
Permanent Cylindrical Quadrats	I	II	III	IV	Pupae	Adults	
1	4	4	4	4	3	2 ♂	1 ♀
2	-	-	-	-	-	-	-
3	6	5	3	3	3	2 ♂	1 ♀
4	8	5	3	-	-	-	-
5	-	-	-	-	-	-	-

Appendix 14 : *Population of ♀ C. pipiens and ♀ Cs.annulata in shelter 1, West Kirby, Wirral (1978-81)*

Date	Total no of ♀ C. pipiens	Total no. of ♀ Cs.annulata
13.12.78	73	45
23.12.78	47	6
1.1.79	37	8
10.1.79	39	3
20.1.79	34	5
30.1.79	25	1
9.2.79	20	3
19.2.79	6	3
2.3.79	6	4
12.3.79	23	5
22.3.79	19	3
1.4.79	23	3
11.4.79	25	2
22.4.79	15	0
2.5.79	5	0
12.5.79	1	0
22.5.79	0	0
1.6.79	0	0
12.6.79	0	0
26.6.79	0	0
5.7.79	0	1
14.7.79	5	1
25.7.79	11	0
4.8.79	53	0
14.8.79	587	0
25.8.79	945	0
4.9.79	1981	1
14.9.79	2888	2
25.9.79	1588	0

Continued .....

Appendix 14 : (Continued)

Date	No no of ♀ C. pipiens	Total no of ♀ Cs.annulata
5.10.79	1894	13
14.10.79	2008	12
29.10.79	2795	24
9.11.79	2913	34
19.11.79	2531	20
1.12.79	1996	22
11.12.79	1565	14
21.12.79	649	6
2.1.80	228	0
12.1.80	184	1
22.1.80	73	0
1.2.80	86	2
11.2.80	59	3
21.2.80	84	6
7.3.80	59	6
17.3.80	77	8
27.3.80	47	3
7.4.80	44	2
18.4.80	43	1
28.4.80	28	0
9.5.80	10	0
19.5.80	4	14
28.5.80	7	9
9.6.80	11	1
19.6.80	8	0
29.6.80	1	3
9.7.80	2	1
23.7.80	3	3
2.8.80	35	2
12.8.80	174	1

Continued .....

Appendix 14: (Continued)

Date	Total no of ♀ *C. pipiens*	Total no of ♀ *Cs. annulata*
22.8.80	377	0
1.9.80	742	2
11.9.80	1150	1
22.9.80	1404	1
2.10.80	1973	3
13.10.80	2007	3
23.10.80	2080	4
3.11.80	1971	1
13.11.80	1160	7
24.11.80	755	3
3.12.80	333	0
11.12.80	339	6
18.12.80	244	5
29.12.80	214	1
9.1.81	164	4
19.1.81	125	3
29.1.81	126	5
9.2.81	104	4
19.2.81	103	2
2.3.81	41	4
12.3.81	52	2
23.3.81	55	0
2.4.81	63	0
13.4.81	37	0
23.4.81	29	0
5.5.81	26	0
14.5.81	4	0
22.5.81	3	0
29.5.81	1	0
8.6.81	1	0
18.6.81	0	1