

STUDIES ON THE BIOLOGY AND ECOLOGY OF
CERATOPOGONID MIDGES IN SOUTHERN ENGLAND

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ABSTRACT

Aspects of the biology and ecology of Culicoides spp. in Southern England were studied at Silwood Park, Ascot, Berkshire from September 1968 to December 1970.

By applying the stratified random sampling technique on a delimited area of Culicoides breeding sites, the seasonal variations in the population levels and spatial distributions of the different immature stages in the soil were determined. Attempts at constructing the population budgets are presented for the period of study.

Observations were made on the differences in the landing-rates of adult Culicoides females, following the arrival of a human-bait at sites located at various distances from the breeding area. An account is also given of ecological investigations, including the seasonal as well as diurnal variations and biting habits, of the adults of four most abundant species at Silwood Park. The roles played by various weather factors on the total daily and diel flight activities, as obtained from suction trap catches, were determined.

Aerial dispersion of adult Culicoides was assessed by landing-rate and sticky trap methods (horizontal) and by suction traps (vertical). The observed differences in the dispersion of the sexes are discussed. Investigations on the feeding behaviour of Culicoides females in relation to host preferences and the attractiveness of carbon dioxide contained in exhalant breath, as one of the factors which induce landing, are presented and discussed.

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1. Introduction

This thesis is primarily an account of some ecological observations on Culicoides species in Southern England. Of the 14 species occurring at Silwood Park, only four are numerically abundant. These are C. impunctatus, C. obsoletus, C. pulicaris and C. pallidicornis.

Section 2. deals essentially with the seasonal and annual variations in the populations of Culicoides immature stages in a delimited area of the breeding sites at Cascade Marsh, Silwood Park, during the period from December 1968 to November 1970. An attempt has been made to assess quantitatively the mortality occurring at each developmental stage with a view of constructing a population budget for Culicoides. Estimates of spatial distribution within and between stages have been determined by various methods which acted as mutual checks on one another.

A considerable part of this study (Sections 3-7) is concerned with some aspects of the biology and ecology of the adult Culicoides. These include the biting-rates in relation to distance from the breeding area, diel and seasonal periodicities of flight and biting activities and vertical and horizontal dispersion from the breeding sites of the most abundant species. The role of weather factors on the flight activity of Culicoides are also considered.

Some aspects of Culicoides feeding behaviour such as host preference and the attractiveness of carbon dioxide which forms part of the constituents of expired breath, are investigated in Sections 8 and 9 respectively.

1.1 Economic Importance

The species of Culicoides are of importance both as disease vectors and as blood-sucking pests of man and domestic animals.

Culicoides and other Caratopogonids constitute a major pest problem because of the irritation and annoyance which their bites cause. They are known to disrupt tourist trade in Florida and the Bahamas and the Caribbean area. Three species, Culicoides furens (Poey), C. barbosai (Wirth and Blanton) and Leptoconops becquaerti (Kieffer) are particularly troublesome in these areas (Linley and Davies, 1971). Culicoides have been blamed for the slow progress in the development of some Southern States in U.S.A. (Dove, Hall and Hull, 1932) and the Highlands of Western Scotland (Edwards et al, 1939). Sherlock and Guitton (1964, 1965) established that the bites of Culicoides were responsible for the rapid increase in cases of dermatitis between 1958 and 1961 in Salvador, Brazil, following a cessation in the control of Aedes aegypti, inadequate disposal of garbage and increase in the amount of open drainage as the city grew. An allergic condition in horses, Queensland Itch, has also been attributed to their bites (Riek, 1954).

Culicoides transmit pathogenic organisms to domestic animals but not to man. C. austeni is a vector of the non-pathogenic human filariasis, Acanthocheilonema perstans in West Africa (Sharp, 1927, 1928). Henrard and Peel (1949), Chardome and Peel (1949) and Duke (1952) incriminated C. grahami as a vector of filaria parasite, Dipetalonema streptocerca. Buckley (1934) observed the complete development of Mansonella ozzardi in females of C. furens. Steward (1933) established that C. obsoletus and C. nubeculosus are transmitters of Onchocerca reticulata which causes a condition known as fistulous withers in horses while Buckley (1938) indicated that O. gibsoni in cattle in Australia and Malaya, which can lead to the condemnation of carcasses, is transmitted by Culicoides species.

Perhaps the most important lethal organism transmitted by Culicoides is the blue-tongue virus of sheep. Du Toit (1944) in Ondestepoort, South Africa, recorded three positive infections of this virus in sheep and one positive infection in a horse after intravenous injections with emulsions of wild Culicoides. One sheep also developed blue-tongue infection after bites by Culicoides fed ten days earlier on an infected sheep. Foster et al (1963) successfully transmitted the virus with C. variipennis in the laboratory and Bowne and Jones (1966) observed the virus in the salivary glands of the same Culicoides species.

Fallis and Wood (1957) observed the development of a blood protozoan parasite, Haemoproteus nettionis in an unidentified Culicoides species and the sporogony of H. canachites in C. sphagnumensis was also observed by Fallis and Bennett (1960). Epidemiological studies further showed that heavy parasitaemia of these parasites in immature birds is usually preceded by the peak abundance of Culicoides (Bennet, 1960; Fallis and Bennett, 1960). In Japan and South-east Asia, Leucocytozoon caulleryi, an important poultry disease, is transmitted by C. arakawae (Akiba et al, 1959; Akiba, 1960).

1.2. Breeding Sites

Culicoides species have a very wide range of breeding habitats which are commonly wet. In Britain, Kettle and Lawson (1952) identified six distinct breeding habitats:-

1. Bogland (C. impunctatus, C. obsoletus).
2. Fresh Water Marsh (C. pallidicornis, C. cubitalis).
3. Swamps (C. odibilis, C. pulicaris, C. punctatus).
4. Mud (C. odibilis, C. stigma, C. nubeculosus).
5. Salt Marsh (C. halophilus, C. maritimus, C. salinarius).
6. Dung (C. chiopterus, C. dewulfi).

Indications that Culicoides have been adapted to utilizing some restricted ecological niches for breeding purposes have been reported from different parts of the world. These include rotting cocoa pods, Theobroma cacao (Williams, 1964, 1966), rotting banana and plantain stumps (William, 1964; Hopkins, 1952), decaying flowers of Balister, Heliconia elongata and Cachibou, Calathea lutea (Williams, 1964), decaying stalks and piles of mixed bedding and cow manure (Jones, 1961a), loose layers of beech leaves, Fagus gradifolia (Jamnback and Wirth, 1963), tree holes and old dug out African canoes (Cater, Ingram and Macfie, 1920), sandy beaches (Jamnback and Wall, 1968), areas of soil covered with Sphagnum, Polytrichum and Sphagnum mixed with carex species of grass (Nielsen, 1963), crab holes, Oesipleura cordata (Forratini et al, 1958) and Mangrove swamps (Williams, 1962).

1.3. Life Cycle of Culicoides in Britain

The adults of Culicoides species commence emergence in early spring, i.e., between late March and early April, and continue to late summer or September. The peak emergence varies between species and is dependent on the prevailing weather conditions.

The banana-shaped eggs (0.3 to 0.5 mm long), which are white when newly laid but quickly darken, are laid singly or in small groups on the mud surface of suitable breeding sites (Linley and Davies, 1971). Each egg has longitudinal rows of sucker-like processes.

The larva has a dull-white or translucent cylindrical segmented body with an oval brownish head. There are no appendages, movement being by wriggling. Respiration is cuticular and they feed on other small forms of life such as algae, protozoa and free-living nematodes which occur in the substrate (Busvine, 1966; Linley, 1968; 1969). Hill (1947), Kettle and Lawson (1952) and Linley (1969) have distinguished four instars in the larval stage. Culicoides over-winter in the larval form. Parker (1950) indicated that C. grisescens over-winters in the egg stage.

Pupation begins when the late instar larvae migrate from the muddy substrate to a drier part of the habitat (Busvine, 1966; Linley and Davies, 1971), or swim to the surface, if the soil is flooded, where they seek support to keep them in contact with atmospheric air (Bidle-mayer, 1957; Linley 1966a). The pupa has a rigid thorax and a slightly flexible abdomen which is capable of only limited movements. A pair of trumpets on the back of the thorax is adapted for breathing atmospheric air.

The small darkly coloured adult, with a wing-span of about 2-3 mm, emerges through a split on the dorsal aspect of the pupal thorax. The antennae are moderately long, hairy in females and bushy in males.

As in mosquitoes, the females have mouthparts adapted for piercing and sucking blood from vertebrate hosts. The males are said to feed on nectar of flowers.

Reported observations on the life-history of Culicoides are scanty and in most cases incomplete. Cater et al (1920) did not observe the larval duration of some West African species, but noted that their pupae lasted only 3-5 days. For C. oxystoma, Patel (1921) reported that the eggs hatched after 3-11 days and the larval and pupal durations were 14-140 days and 3-7 days respectively. Writing on C. canithorax and C. dovei, Dove et al (1932) pointed out that the larval development could last at least 6 months while the pupae only 4-7 days. In Britain, Jobling (1929) and Edwards (1939) suggested that C. vexans and C. obsoletus produced one and two generations a year respectively.

Only a few Culicoides species have been, to some extent, bred successfully in the laboratory. Mating in females of C. impunctatus and C. obsoletus (Hill, 1947) and C. nubeculosus (Megahed, 1956) is independent of blood meal although the latter is important for egg maturation. Male swarms (Downes, 1955) are also not entirely necessary for mating purposes as Megahed (1956) reported successful mating of C. nubeculosus in 3" x 1" glass tubes but not in bigger cages. Table 1 is a summary of the literature on laboratory breeding of some Culicoides species.

In the field, however, the development of immature stages is much slower than indicated by the laboratory breedings (Table 1).

The incubation periods of the eggs and the pupal durations are short but the larvae have a particularly long developmental period. In univoltine species, the larvae remain in the soil for as long as one year and Kettle (1962) suggested that in bivoltine species, overlapping generations of the overwintering larval populations are responsible.

Author and Date	Hill (1947)	Hill (1947)	Megabed (1956)	Jones (1960)
Species	<u>C.</u> <u>obsoletus</u>	<u>C.</u> <u>impunctatus</u>	<u>C.</u> <u>nubeculosus</u>	<u>C.</u> <u>veriipennis</u> <u>sonorensis</u>
No. of blood meals per Gonotrophic cycle.	5*	7*	1	1
Period between feed- ing and oviposition (Days)	17	14	3-4	3
Average no. of eggs laid per batch	75	50	135	-
Incubation period (Days)	3	14	3-6	2
Average larval duration. (Days)	105	150	-	21
Average pupal duration. (Days)	5	5	4-7	3
Breeding temperature (Controlled)	16-19°C	16-19°C	20±5°C	75-80°F
Percentage Relative Humidity	Very high	Very high	80-90	40-50

Table 1. A summary of Literature on laboratory breedings of some Culicoides species.

* Similar numbers of eggs are laid after one meal as after more than one.

1.4. Review of literature on topics studied

a) Ecology.

Culicoides, which are small blood-sucking midges, have a world wide distribution (Hill, 1947; Campbell and Pelham-Clinton, 1960; Arnaud and Wirth, 1965; Imms. 1970 Edition) but have not been reported from New Zealand (Macfie, 1932), Patagonia and Southern Chile (Ingram and Macfie, 1931). The genus was originally grouped with other non-biting flies in the family Chironomidae and latter placed in the Ceratopogonidae with the genera Lasiohelea and Leptoconops, which feed on warm-blooded animals, and Forcipomyia and Atrichopagon which depend on blood of other insects (Edwards et al, 1939).

Kettle (1962) recognised that 'the study of the bionomics and control of Culicoides and Leptoconops is still in its infancy' and 'much of the inadequacy in our knowledge of Culicoides arises from limitations imposed by techniques.' The need for behavioural and physiological studies is also emphasised but these have been hindered by the difficulties of rearing Culicoides spp. in the laboratory. Eight hundred Culicoides species are known (Jorgensen, 1969) and 41 of these occur in Britain. (Campbell and Pelham-Clinton, 1960). In Britain, only C. nubeculosus; Megahed, 1956) has been successfully reared while Hill (1947) and Parker (1950) were unsuccessful in keeping C. impunctatus and C. obsoletus beyond a single generation. However, in America, Jones (1957, 1960) and Linley (1968) have respectively colonized C. variipennis and C. furens.

Prior to the present studies, there has been no published attempts at constructing life-budgets for Culicoides spp. or of studies on the seasonal spatial distribution changes in the immature stages. Nielsen (1963), studied the microdistribution of Culicoides larvae by taking soil samples from adjoining sub-plots and found that they were not randomly distributed. Hill (1947) sampled along transects at intervals of 20-30 feet (6-9 m) when she determined the population levels of the

various immature stages. Kettle (1950), Kettle et al (1956) and Kettle and Parish (1957) located the breeding centre of C. impunctatus and assessed the effects of larvicidal treatments by random soil samples. Because of lack of detailed systematic studies on the immature stages, emergence traps have been used for identification of breeding sites and for estimations of species abundance (Nielsen, 1963; Dove et al, 1932). Kettle (1956) obtained a positive correlation between larval population in the soil and the amount of rainfall during the preceding period of adult activities.

In various aspects of study on Culicoides adult populations, different sampling methods have been used. These include sweep net* (Parker, 1949: Scotland; Nielsen, 1963: Denmark) dark cloth (Hill, 1947: Liverpool), emergence traps (Campbell & Pelham-Clinton, 1960: Scotland; Nielson, 1963: Denmark), biting rates* (Service, 1969a: Dorset, Southern England; Parker, 1949: Scotland; Kettle, 1957: Scotland; 1969: U.S.A.; Kettle and Linley, 1967a,b; 1969a,b: U.S.A.), Suction traps* (Reuben, 1963: Scotland), black cylindrical sticky traps* (Kettle, 1951 & 1960: Scotland), Light traps (Blanton et al, 1955: Panama; Jamnback & Watthews, 1963: U.S.A.; Service, 1969b: Dorset, Southern England; Khalaf, 1952: U.S.A.), baited traps (Snow et al, 1960; Minter, 1961: U.S.A.) and Vehicular collection (Jorgensen, 1969: U.S.A.). The techniques marked with an Asterisk have been used in the present studies.

In Britain, Cameron et al (1946) focussed attention on Culicoides problems in Scotland. Since then, work on the bionomics of the genus has been concentrated in that area and particular attention has been paid to C. impunctatus (Kettle, 1949, 1950, 1951, 1957 & 1960; Parker, 1949; Reuben, 1963) which Campbell and Pelham-Clinton (1960) described as the most troublesome human-biting species in highland Britain. Variations in the seasonal and diurnal flight as well as biting cycles of the species

have been studied by the above authors and Kettle (1951, 1960) determined its vertical and horizontal dispersion under woodland and moorland conditions. In Liverpool, Hill (1947) carried out an extensive study on Culicoides spp. occurring in Northern England and recently in Southern England, Service (1969a,b) reported on the biting habits and seasonal periodicities of the species in Dorset.

Downes (1955) observed the swarming flights and mating of Culicoides spp. and Kettle (1955) found the sex-ratios to be normal in 26 British species. The detailed description of the anatomy and morphology of Culicoides nubeculosus immature stages have been given by Lawson (1951). A Key for the identification of the early stages of British Culicoides and allied genera was provided by Kettle and Lawson (1952). Parker (1950) studied the effect of changes in the microenvironment on the egg stage of various Culicoides species.

Ecological investigations on Culicoides in England, particularly in the southern regions, are limited and it is hoped that this work will provide some additional basic information.

b) Attraction to hosts

Culicoides females are haematophagous, a blood meal being necessary for egg maturation (Reuben, 1963; Linley, 1966; Linley et al, 1970). There is little information about the feeding habits of Culicoides which feed on animals other than man. Various authors, including Fallis (1958), Bennet and Fallis (1960), Snow et al (1958), Wirth and Bottimer (1956) and Messersmith, (1965) have investigated host preference among Culicoides spp. which feed on birds and Jones (1959), Hair and Turner (1968), Bennet and Fallis (1960) and Braverman et al (in press) on mammalian and other vertebrate hosts.

Literature on the attraction of Culicoides females to hosts

is limited but the various factors inducing mosquitoes to land and feed on a host have been extensively studied by various workers in the laboratory. Dethier (1957) reviewed the responses of Haematophagous insects to physical and chemical stimuli associated with the host and Gatehouse (1969) pointed out that the degree of responsiveness is dependent on intrinsic as well as environmental factors.

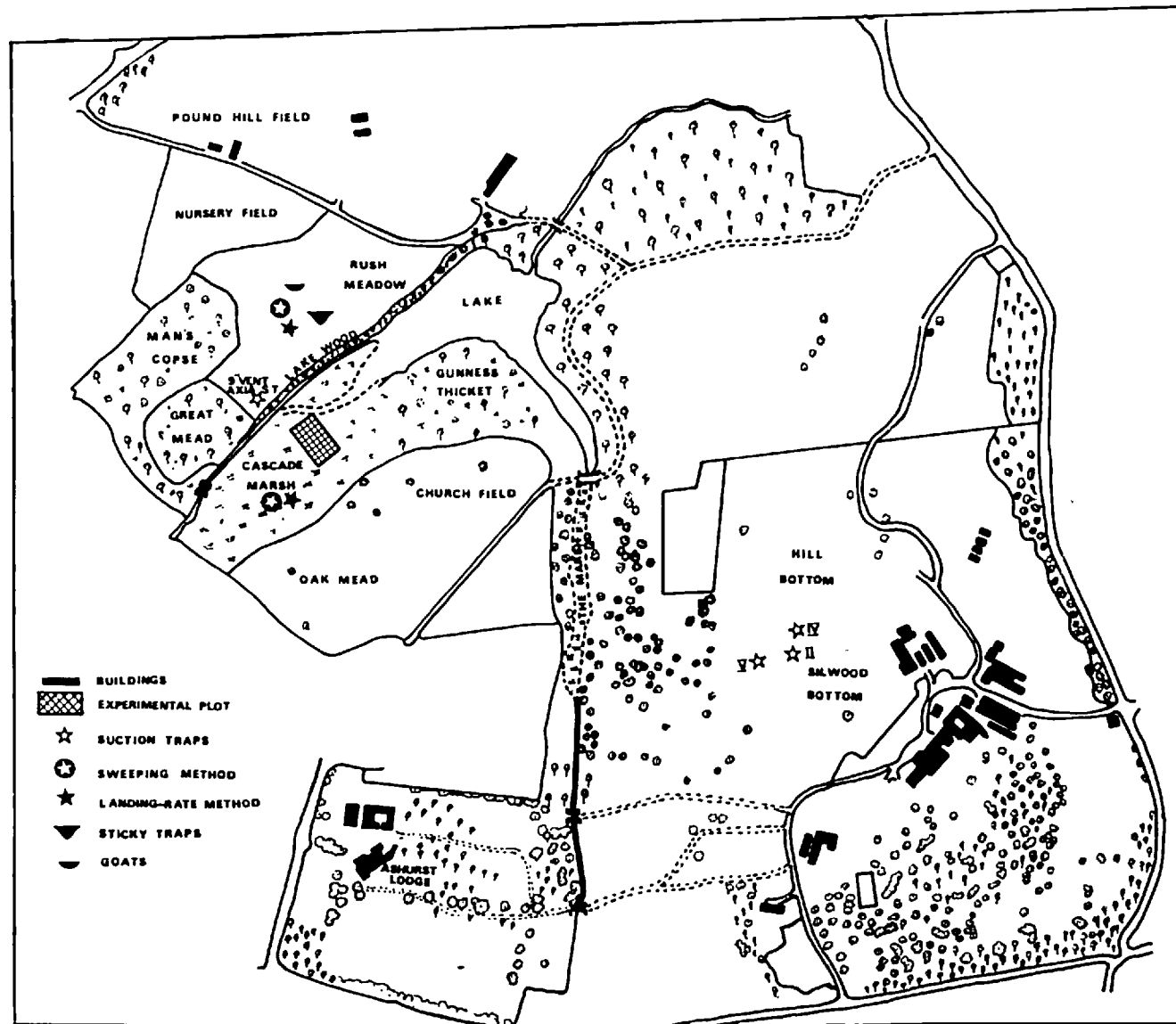
The sharp contrast of black and white strips on the revolving cylinder of Haufe-Burgess trap is attractive to mosquitoes (Haufe and Burgess, 1960). Similarly, Tabanidae respond to the suspended black or red sphere of the Manitoba horsefly trap (Bracken et al, 1962). Southwood (1966) quoted the detailed studies of Verheijen (1960) which showed that the effectiveness of a light trap in catching insects is dependent on the contrast between the illumination source and the darker surroundings.

Wood and Wright (1968) regard warmth and humidity as the main factors responsible for inducing Aedes aegypti to land on an inanimate host. Wright (1962) observed that activated A. aegypti did not alight either on moist cloths over unheated copper bars or dry cloths over heated bars. Both factors are necessary to induce a positive landing response (Khan et al, 1966; Khan and Maibach, 1966). Wood and Wright (1968) thought this response could be due primarily to the convection currents which warmth and moisture generate. Such convection currents rise from a mammal (Cummings and Wright, 1967; Lewis et al, 1969) and also contain volatile body secretions and exhalent breath.

Traps baited with blocks of solid carbon dioxide attract both sexes of Culicoides and Leptoconops (Nielsen, 1965; Whitsel and Schoeppner, 1965). In the laboratory, using cage olfactometers, Brown et al (1951), Willis and Roth (1952), Burgess (1959) and Kellog and Wright (1962) have

shown that carbon dioxide induces landing in A. aegypti. Gatehouse (1969) and Laarman (1955) have found human breath attractive to Stomoxys calcitrans and Anopheles I. atroparvus respectively. The latter species also responded to an airstream which was passed over a rabbit and then treated to remove carbon dioxide and moisture (Laarman, 1955). The odour of human apocrine sweat has been found to be attractive to A. aegypti (Parker, 1948; Brown et al, 1951; Rahm, 1957) and Laarman (1955) obtained a strong response from A. L. atroparvus to air bubbled through citrated rabbit blood.

FIG1 Map of Silwood Park.



1.5. The Study Areas

The studies on the adults and immature stages were done in the Cascade Marsh and Rush Meadow which are in the grounds of Imperial College Field Station, Silwood Park, Sunninghill, Ascot, Berkshire, Waloff (1968) has given a general description of Silwood Park.

Fig. 1. is a sketch map of the Field Station.

a) Cascade Marsh

The Cascade Marsh is limited in the north by the Silwood Lake and Lake Wood, in the south by Oak Mead and Church Field and in the east by the Guinness Thicket. The whole area is located at an altitude of about 160 feet (48.93 m). It is a woodland area consisting mainly of Alnus glutinosa (Common Alder), Salix spp., Betula pubescens (Birch), Quercus robur (Pendunculate Oak), Sambucus nigra (Elder) and Fagus spp. (Beech). The branches of these trees hang over to form a 40 feet (12.2 m) high canopy, producing a shade underneath. The undergrowth is comparatively low with isolated patches of young and smaller B. pubescens and fallen Salix spp.

Ranunculus repens (Creeping Buttercup), R. flammula (Lesser spearwort), Juncus articulatus (Jointed Rush), Typha latifolia (Bulrush) and Oenanthe fistulosa (Common Water Dropwort) are predominantly among the cover vegetation of the almost constantly wet, marshy ground. Locally, however, on firmer and sometimes isolated open grounds, patches of Chamaenerion angustifolium (Rose Bay), Carex spp., Polystichum lobatum (Hard Fern) and Dryopteris filix-mas (Male Fern) are found.

b) Rush Meadow

The Rush Meadow is apparently an undisturbed pasture area which was used previously for cattle grazing. A three feet (0.92 m) deep drainage system runs parallel to and about 25 yards (22.9 m) from Lake Wood. The Meadow is on a slope which rises gently northwards towards Pound Hill Field. A narrow, marshy, A. glutinosa and B. pubescens

woodland (Lake Wood) separates the Meadow from Silwood Lake in the south. Q. robur, Acer pseudoplatanus (Sycamore), Ilex aquifolium (Holly) and B. pubescens of the Great Mead and Marls Copse are to the west and the acid grassland area, which forms the Nursery Field, to the north.

The Rush Meadow has a floristic composition which is essentially graminaceous: Holcus mollis forming the predominant ground cover with Ranunculus bulbosa (Bulbous Buttercup), Stellaria alsine (Bog stitchwort) and Carex spp. in close association. Other gramineae and herbaceous flowering weeds which are common include Dactylis glomerata, Deschampsia cespitosa (Tufted Hair grass), Juncus effusus (Soft Rush), J. conglomeratus (Common Rush), Rumex acetosa (Common Sorrel), Lysimachia vulgaris (Yellow Loose Strife), Cirsium arvense (Creeping thistle), C. palustre (Marsh thistle) and Rubus fruticosus (Agg.). A dense patch of Pteridium aquilinum also occurs in the west end of the Meadow.

2. Survival in The Immature Stages

2.1. Experimental plot, material and method

A survey of possible breeding areas of Culicoides species at Silwood Park was made in the autumn^{of} 1968. Three positive identifications were obtained from which the experimental site was randomly selected. It consisted of a strip of land in the Cascade Marsh surrounded by a dense Alnus glutinosa woodland and covered by growths of Ranunculus flammula, R. repens, Juncus articulatus, Calthia patustris and Lysimachia vulgaris.

A plot, 24 by 18 feet (7.3 x 5.5 m), was marked out and divided into 12 sectors, each measuring 6 by 6 feet (1.8 x 1.8 m). Each sector was further divided into 36, 1 by 1 foot (0.3 x 0.3 m) sub-sampling units. The plot was situated near a drainage from the woodland into the nearby Silwood Lake and hence was marshy for nearly the whole year.

Stratified random sampling method (Healy, 1962) was adopted. Equal numbers of randomly selected sampling units were taken from each sector to ensure an unbiased coverage of the whole population of immature stages of Culicoides species in the plot. Thirty six or twenty four soil samples were taken monthly i.e., nine or six each week from December 1968 to November 1970.

The sampler used consisted of a metal frame, 2 mm thick, which was open both at the top and bottom, and with an internal measurement of 8" x 8" x 3" (0.2 m x 0.2 m x 0.08m). The bottom edges were ground to facilitate its insertion into the soil. During sampling, the sampler was positioned centrally in a sampling unit and then pushed into the soil until the top edges were level with the ground surface. Mud was then scooped out from the inside of the frame until its level was the same as that of the lower edges of the sampler. In firmer areas, however, it was more convenient to lift clear the sampler and its content of soil

with a spade, causing minimum disturbance to the surrounding units in the process. Each soil sample was put in a separate polythene bag and labelled. After each sampling, the units were filled with earth from areas not likely to be breeding habitats.

2.2. Extraction methods used

The larvae and pupae of Culicoides were extracted from the soil samples by two methods: sand extraction which involved an active response of the mobile stages to stimuli (Southwood, 1966) and soil washing which was purely a mechanical process. The latter method was used, not only for the extraction of the relatively immobile pupae, but also served as a check on the efficiency of the former. The sand extraction method was economical in terms of time and labour and the larvae were recovered undamaged.

2.2.a. The sand extractor

The simple sand extractor described by Bidlingmayer (1957) and used by Williams (1960a,b) was further improved. It consisted of two main parts: a bottom container with an internal volume equal to that of the sampler and a tin box which was positioned at the open end of the container by means of two plates. Both ends of the tin box were open. To allow for application of extra pressure during the positioning, two plate handles were provided at the top end of the box. A medium-mesh wire gauze sitting on supports attached to the inside walls of the box, 2 mm. from its bottom end, separated the natural substrate in the container from the sand trap medium in the top box. A slot, 2 m.m. wide, was provided on one side for a plate to be pushed in under the wire gauze to effect a complete separation of the substrates when necessary.

The procedure for the use of the apparatus illustrated in Fig. 2 was as follows. The container was completely filled with a soil sample and placed in a glass tank measuring 18" x 18" x 24" (0.5 m x 0.5 m x 0.6 m).

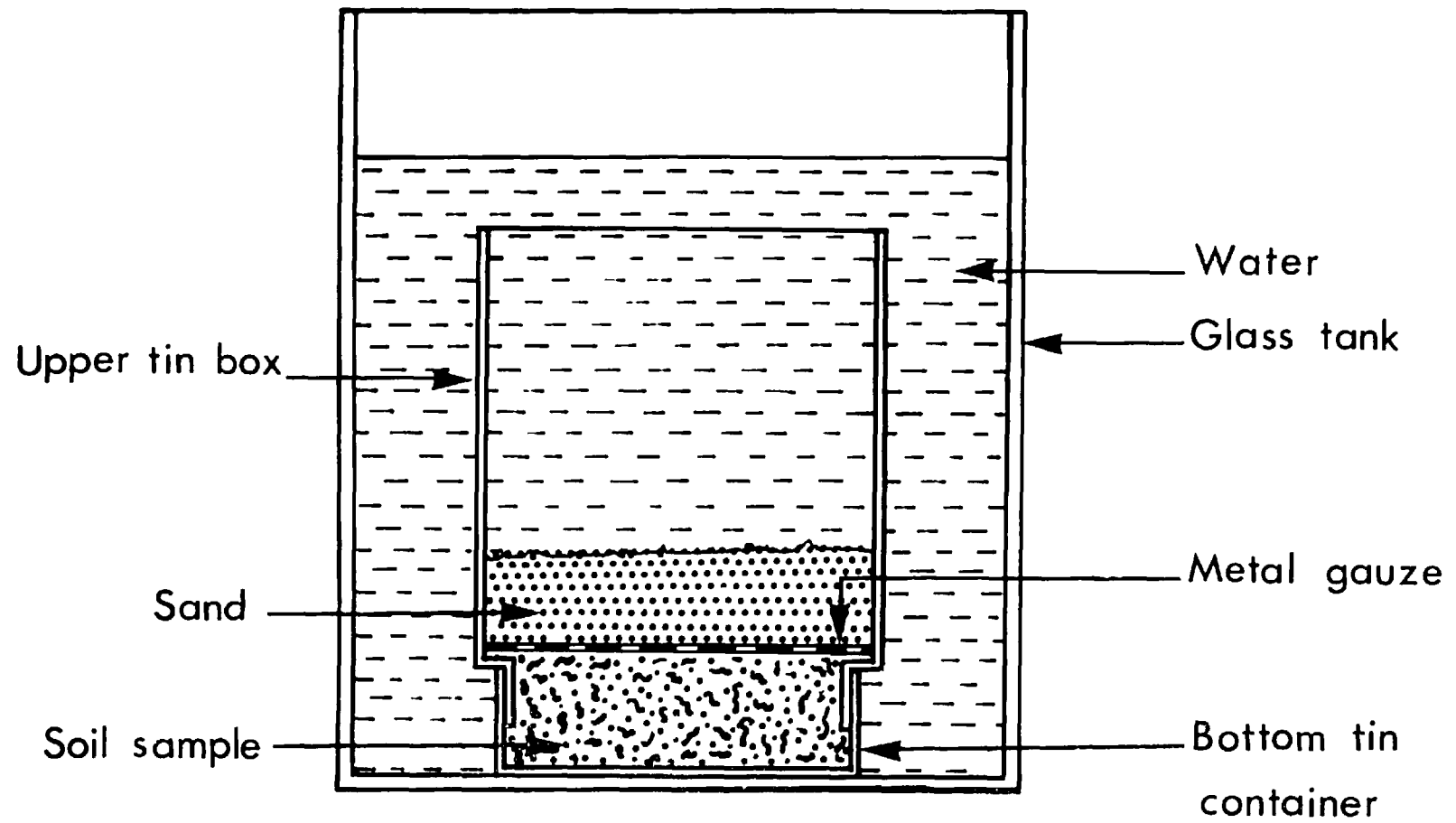


FIG. 2 Transverse section of sand extractor.

The wire gauze was positioned in the upper box and the latter fixed securely onto the container. Clean sand was then spread on the wire-gauze to a depth of about three inches. This was usually enough to produce contact between the two substrates through the gauze. The tank was then filled with water leaving the apparatus completely submerged. After 48 hours the water was drained through a three-inch diameter plastic collecting tube, with a rubber tubing. Since the apparatus was not water tight, some larvae escaped into the surrounding water in the glass tank. Thus, this operation recovered such larvae. The upper box was then carefully lifted off and its content of sand emptied into a plastic bowl. A clean separation of the two substrates was achieved in this way if the right quantity of sand was used. It was not necessary to use the dividing plate for this purpose. The sides of the tank and the tin box were washed into the bowl. Four such apparatus were used simultaneously.

Convenient portions of the sand were scooped into a plastic beaker and agitated with a strong jet of water. The water was immediately poured through the collecting tube leaving the large particles of sand behind. This process was repeated five times to ensure that all the animals had been extracted. When all the sand in the bowl had been similarly treated, the fine particles of sand and animals in the collecting tube were washed into a large beaker with saturated magnesium sulphate solution (S.G. 1.2). The content of the beaker was then agitated with a glass rod and the beaker slowly filled with more saturated MgSO_4 solution from a wash bottle. The sand particles settle to the bottom and the 'float' decanted into a piece of muslin cloth placed loosely on the wide end of a funnel but held in position with a rubber band. This was repeated as many times as necessary until the top and sides of the beaker were clean. The concentrated float was then washed free of MgSO_4 and transferred into a petri-dish containing 70% alcohol. This

left the larvae straight and relaxed and easy to pick out and count against a dark background under a binocular microscope.

2.2.b. Soil washing

The Salt and Hollick technique (1944) is an efficient method for extraction of soil arthropoda from soil samples, (Raw, 1962). Raw (1955) described a scaled-down version of the apparatus. Only an outline of the technique is given here as detailed illustrated descriptions can be found in recent books (Kevan, 1955; 1962; Kühnelt, 1961; Murphy, 1962; Southwood, 1966).

Briefly, the soil in the container after the previous extraction process, was washed through coarse and medium sieves for the removal of unwanted coarse particles. The filtered small particles were mixed with saturated MgSO_4 solution (S.G. 1.2). On settling, the soil particles sank and all the organic matter floated to the top. The Ladell tank was then tipped forwards allowing the 'float' to pass over the lip into a collecting tube. The concentrated 'float' was treated for examination as described earlier.

2.3. Emerging adults

The emergence of adult Culicoides was recorded in the plot by setting up a series of emergence traps. Each trap consisted of an inverted 8" square by 9" high (0.2 m x 0.2 m x 0.22 m) biscuit tin fitted at the top with a 3" x 1" specimen tube containing a celluloid trap funnel (Fig 3,4). Thirty six emergence traps were used in randomly selected sampling units.

In 1969, the traps were not moved monthly to other sub-sampling units. Consequently, only adults emerging from the immature stages which overwintered (1968-1969) were actually sampled. In 1970, however, a plan was devised to allow for some traps to be moved, on the first day of the month, to other randomly selected sub-sampling units. Thus, it

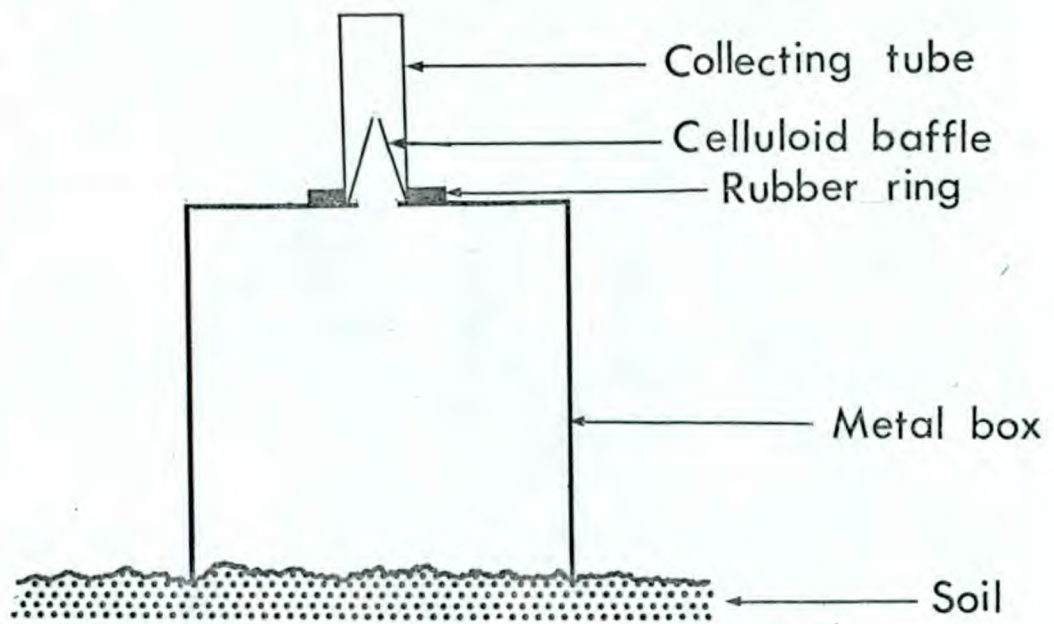


FIG.3 Emergence trap.



FIG.4 Experimental plot and emergence traps.

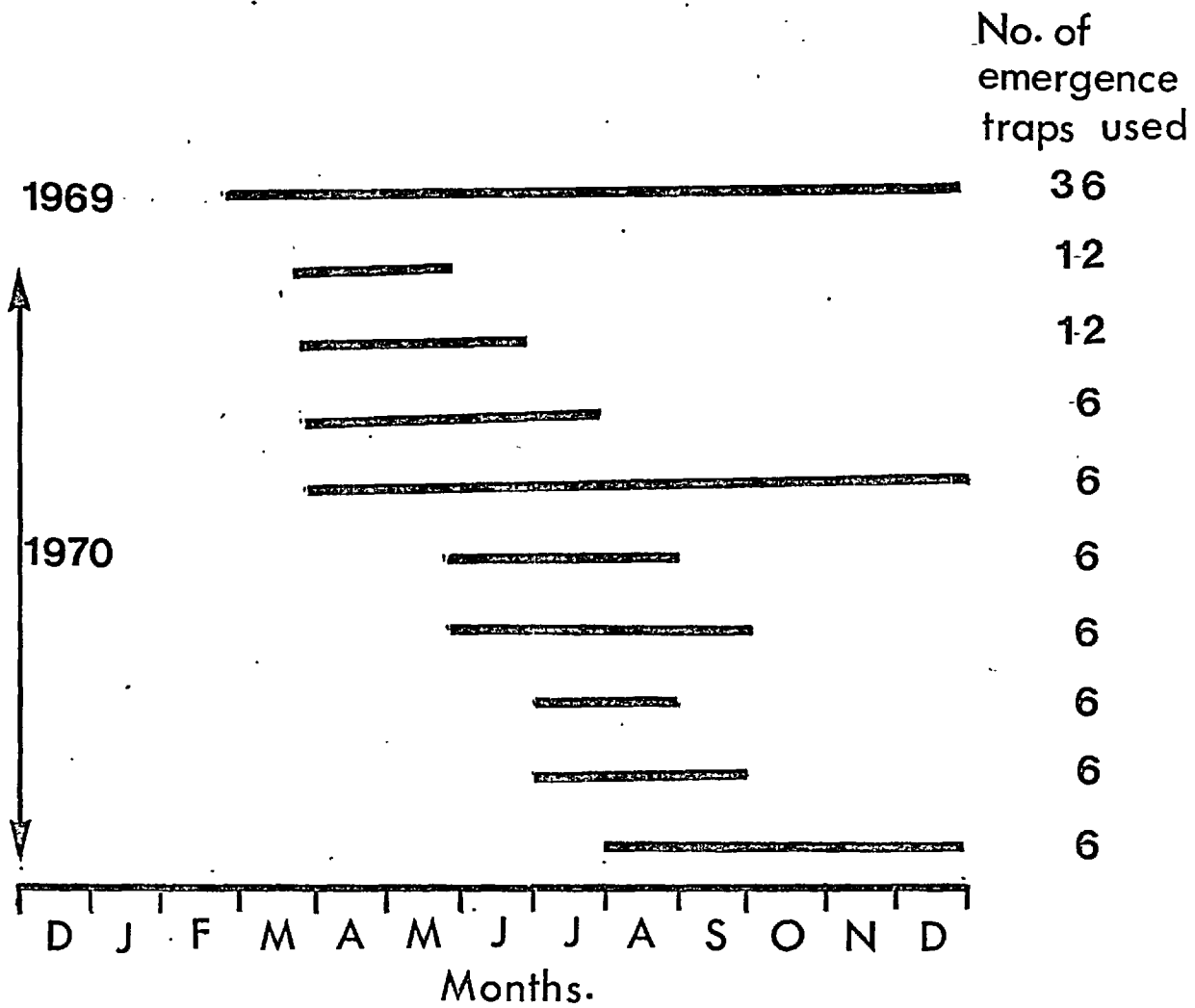


FIG.5 Operational chart for sampling adult emergence in the experimental plot.

was possible for the two peaks of the bimodal distribution of adult Culicoides emerging, to be sampled including the tails of the overlapping generations.

The operational chart is given in Fig. 5 and shows the distribution of the traps in relation to time.

2.4. Seasonal population fluctuations of the developing Culicoides Stages

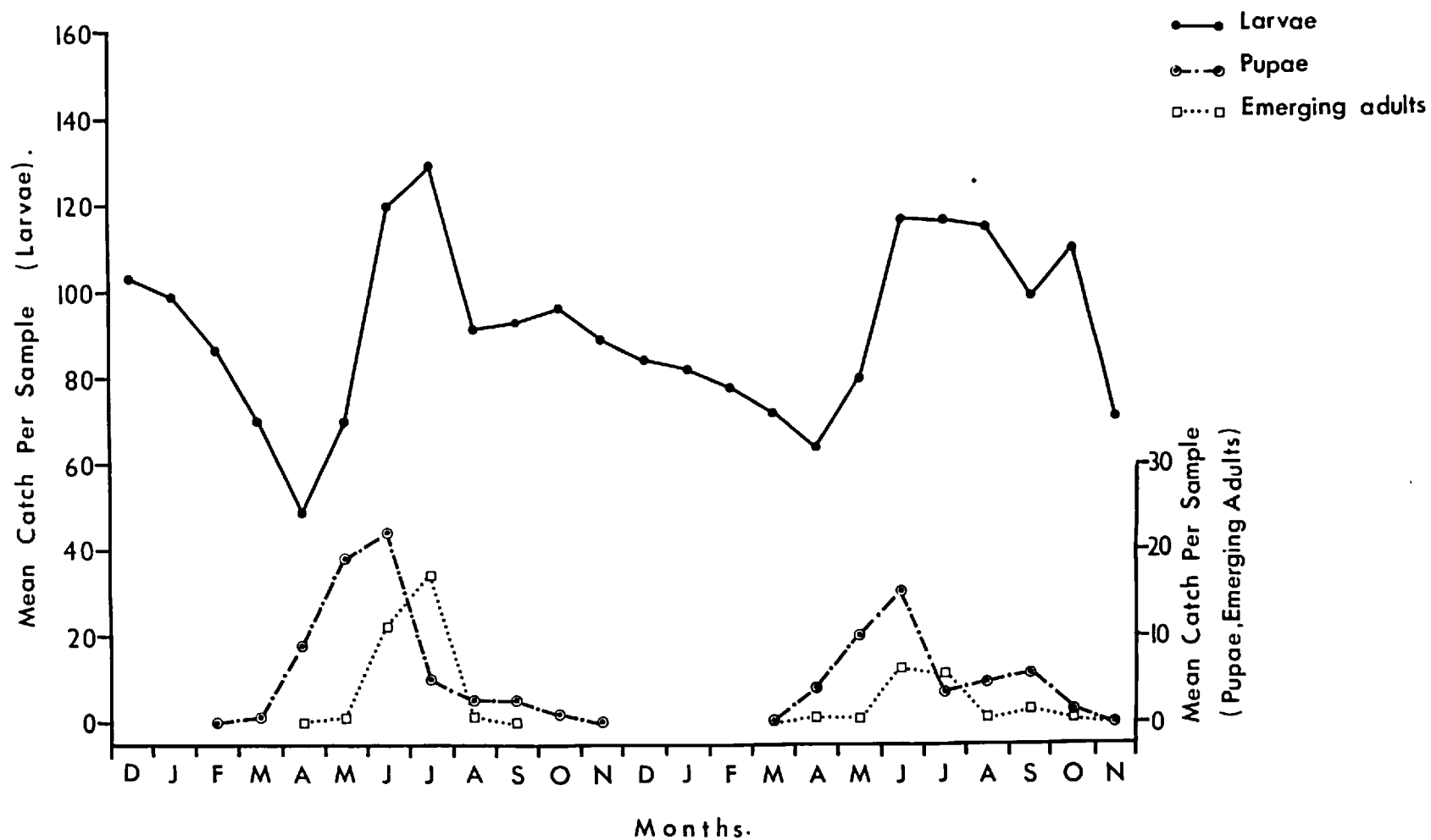
A graphical representation of the seasonal changes in the mean catches, between December 1968 and November 1970, of the different developmental stages of Culicoides is shown in Fig. 6.

The mean catches of Culicoides larvae decreased steadily during the winter months from 103 and 84 in December 1968 and 1969 to 49 and 64 in April 1969 and 1970 respectively. This was followed by a rapid increase, reaching a maximum of 129 in July 1969 and 117 in June 1970. In subsequent months, however, the mean catches fell progressively and by November were 88 (1969) and 71 (1970).

The first appearance of pupae in soil samples coincided with the apparent decrease in the larval population in March of both years. The last pupae were taken in October. The peak occurrence of pupae in the field was closely related to the peak emergence of adults in July. The latter was similarly associated with build up in the larval populations in May, June and July. A small recovery in September of the pupal populations could be a summer generation from which the second adult population emerged in 1970.

Catches in emergence traps were predominantly (82%) C. pallidicornis except in 1970 when the small second generation consisted of C. obsoletus only. Moreover, the population trend of the emerging adults was consistent with that obtained for C. pallidicornis by other methods described later (Section 4). No C. pulicaris were collected and comparatively few C. impunctatus and C. obsoletus appeared in the emergence traps.

FIG 6. Seasonal Population Fluctuations of Different Culicoides Stages.



It can thus be concluded that the experimental plot was unintentionally located in a basically C. pallidicornis breeding habitat.

Whereas only three Culicoides species were taken in the emergence traps in 1969 and 1970, the nine inch Vent Axia Suction trap operated at the Rush Meadow during the same period captured twelve species. This suggests that not only are the breeding sites of Culicoides localised, but individual species are capable of selecting a preferred breeding site from a wide range of potentially suitable habitats, although overlapping generally occurs.

2.5. The distribution of immature stages and emerging Adults of Culicoides in the field

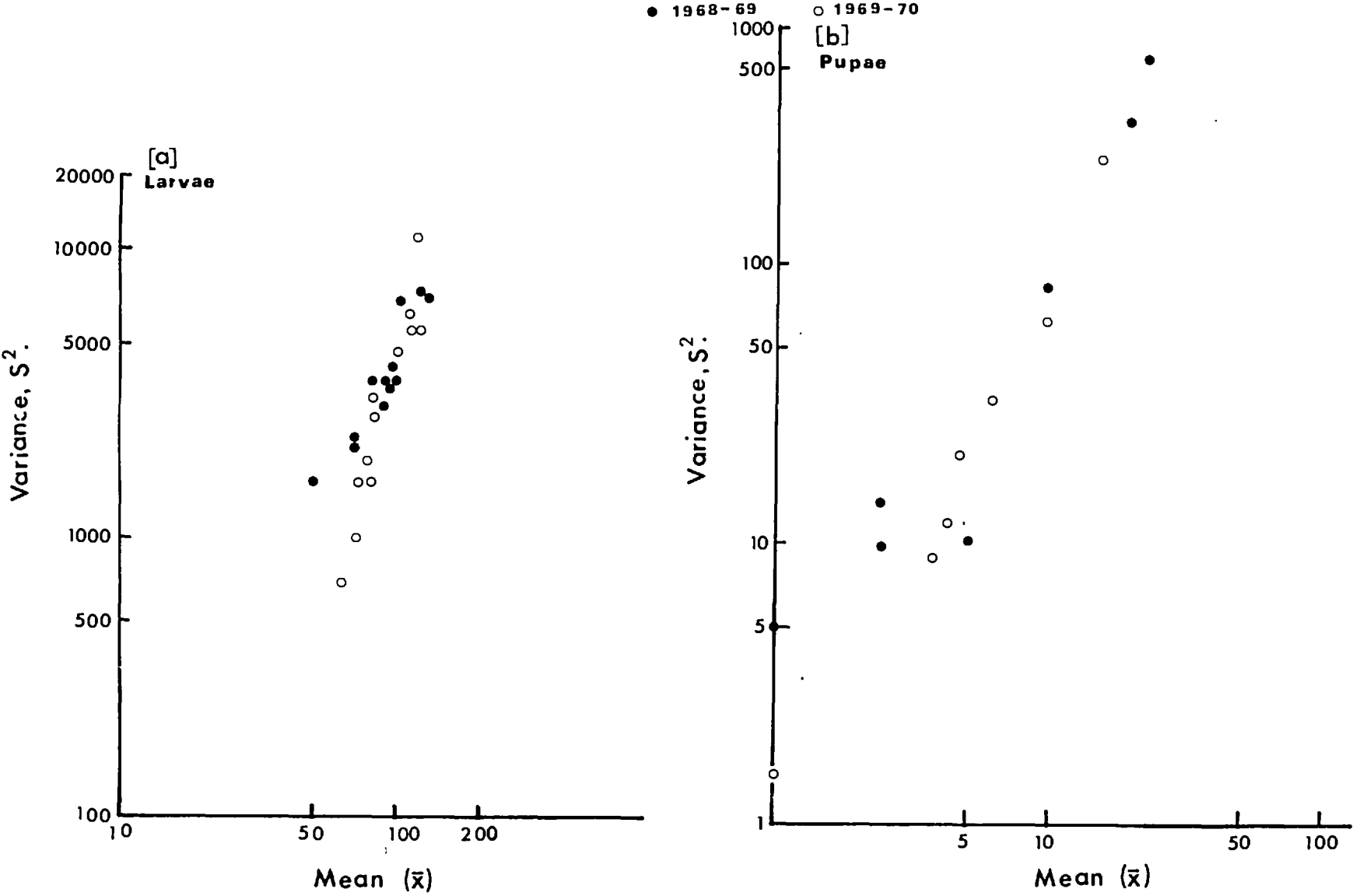
Southwood (1966) reviewed the techniques of application of the various statistical indices available for the description of spatial distribution or degrees of aggregation of insect species populations.

i. Variance : Mean Ratio

If the different developmental stages of Culicoides were randomly distributed in the field, the ratio of the sample variance to the mean, i.e. $\frac{S^2}{\bar{x}}$, should be equal to unity (Bliss and Owen, 1958; Fisher and Bliss, 1953). Thus, the ratio is a test for randomness in biological distributions. It indicates aggregation when greater than unity and under-dispersion when below unity (Hutchinson, 1962).

For each stage, monthly values of the variance : mean ratio was obtained. It was consistently and significantly greater than unity except when the population is low, i.e., at the beginning and end of both the pupal and the adult emergence periods. Due to the low samples taken in the latter cases, the ratios approximated to unity and were not significantly different from random distribution or Poisson series. Chi-squared test, i.e., $\chi^2 = (n-1) \frac{S^2}{\bar{x}}$, where n = number of observations, S^2 = sample variance and $\bar{x}$ = sample mean, was used. Figures 7a, b indicate the relationship between variance and mean for larvae and pupae counts.

FIG.7 Relationship between sample variance and mean for counts of immature Culicoides stages.



Thus, a general indication was given that the different Culicoides stages were to some extent aggregated in their spatial distributions.

ii. The Negative Binomial Distribution

Biological aggregations have been adequately expressed by the negative binomial (Bliss and Owen, 1958). The monthly data obtained for the different Culicoides stages were further tested by this method. The negative binomial is described by two parameters: the sample mean and the exponent K which is a measure of aggregation. K is not a constant for a species. Its value is variable and generally increases with the sample mean. It ranges from zero, maximum aggregation, to infinity when it describes randomness. Large values of K, however, usually indicate an approach to the latter condition. Roblés (1969) noted that since the negative binomial describes only aggregated distributions, the use of the K exponent as an estimate of aggregation should be limited to samples where the ratio of the variance on sample mean is greater than unity.

The values of the parameter K was obtained by the Bliss and Fisher (1953) maximum likelihood method for each stage sample. Chi-squared tests of the goodness-of-fit for the negative binomial distributions was calculated using Roblés (1969) 'TOPFIT' computer model. Each sample fitted the negative binomial distribution since the probability P, for the hypothesis of agreement between the observed and expected distributions is greater than the 0.05 (5% level (Bailey, 1959). When the populations were low the variance : mean ratio indicated an approach to randomness but a better fit was obtained with the negative binomial except in October 1970 when a negative value for the K exponent was obtained. In the latter case, the population of the emerging adults was very low, only four were taken in the emergence traps for the month. A summary of the chi-squared tests for the goodness-of-fit of the negative binomial and Poisson series distributions is presented in Table 2.

TABLE 2. CHI-SQUARED TESTS OF GOODNESS-OF-FIT FOR NEGATIVE BINOMIAL & POISSON SERIES DISTRIBUTIONS OF CULICOIDES DEVELOPING STAGES IN THE EXPERIMENTAL PLOT.

(CASCADE MARSH)

(CASCADE MARSH)

1968 - 69						1969 - 70						
1. LARVAE	NEGATIVE BINOMIAL			POISSON SERIES			NEGATIVE BINOMIAL			POISSON SERIES		
	χ^2	df.		χ^2	df.		χ^2	df.		χ^2	df.	
DEC.	2.07	4	0.80>P>0.70	$\bar{X}$ TOO LARGE FOR EXPONENTIATION			3.50	1	0.10>P>0.05	20.31	2	P>0.0001
JAN.	4.12	3	0.30>P>0.20	"	"	"	0.60	1	0.50>P>0.30	12.71	2	0.01>P>0.001
FEB.	1.50	4	0.90>P>0.80	58.55	4	P>0.001	22.03	18	0.30>P>0.20	10.49	2	0.01>P>0.001
MAR.	2.70	4	0.70>P>0.50	49.58	4	P>0.001	2.03	1	0.20>P>0.10	11.73	2	0.01>P>0.001
APR.	31.16*	27	0.30>P>0.20	693.92	28	P>0.001	21.61	20	0.50>P>0.30	15.49	2	P>0.001
MAY	7.64	4	0.20>P>0.10	39.32	4	P>0.001	0.99	1	0.50>P>0.30	13.83	2	P>0.001
JUNE	1.21	1	0.30>P>0.20	$\bar{X}$ TOO LARGE FOR EXPONENTIATION			20.55	21	0.50>P>0.30	$\bar{X}$ TOO LARGE FOR EXPONENTIATION		
JULY	4.83	1	0.05>P>0.02	"	"	"	0.83	1	0.50>P>0.30	"	"	"
AUG.	5.16	4	0.30>P>0.20	"	"	"	0.42	1	0.70>P>0.50	"	"	"
SEPT	6.29	4	0.20>P>0.10	"	"	"	0.24	1	0.70>P>0.50	"	"	"
OCT.	3.03	1	0.10>P>0.05	"	"	"	0.41	1	0.70>P>0.50	"	"	"
NOV.	21.66*	16	0.20>P>0.10	897.31	17	P>0.001	32.51	18	0.05>P>0.02	117.62	19	P>0.001
2. PUPAE												
MAR.	0.85	2	0.70>P>0.50	8.88	3	0.05>P>0.02						
APR.	9.13*	15	0.90>P>0.80	178.72	16	P>0.001	3.63	1	0.10>P>0.05	29.05	7	P>0.001
MAY	17.78*	20	0.70>P>0.50	395.49	21	P>0.001	1.09	1	0.30>P>0.20	12.25	2	0.01>P>0.001
JUNE	1.58	1	0.30>P>0.20	20.67	2	P>0.001	2.62	1	0.20>P>0.10	17.34	1	P>0.001
JULY	9.62	7	0.30>P>0.20	24.59	8	0.01>P>0.001	9.96*	5	0.10>P>0.05	14.79	1	P>0.001
AUG.	0.82	1	0.50>P>0.30	13.96	2	P>0.001	14.28	8	0.10>P>0.05	10.58	1	0.01>P>0.001
SEPT.	0.14	1	P = 0.70	10.76	2	0.01>P>0.001	8.77	9	0.50>P>0.30	9.58	1	0.01>P>0.001
OCT.	3.23	3	0.50>P>0.30	10.90	1	P>0.001	0.79	2	0.70>P>0.50	1.77	1	0.20>P>0.10
3. EMERGING ADULTS												
APR.							INSUFFICIENT df.			0.89	1	0.50>P>0.30
MAY	INSUFFICIENT df.			0.36	1	0.70>P>0.50	2.37	1	0.20>P>0.10	4.18	2	0.20>P>0.10
JUNE	27.48*	15	0.05>P>0.02	343.51*	16	P>0.001	6.95	2	0.05>P>0.02	10.66	3	0.02>P>0.01
JULY	14.03	18	0.80>P>0.70	670.01*	19	P>0.001	18.18	11	0.10>P>0.05	68.67	3	P>0.001
AUG.	INSUFFICIENT df.						INSUFFICIENT df.					
SEPT										3.00	3	0.50>P>0.30
OCT.										7.32	1	0.01>P>0.001

iii. Other Measures of Aggregation

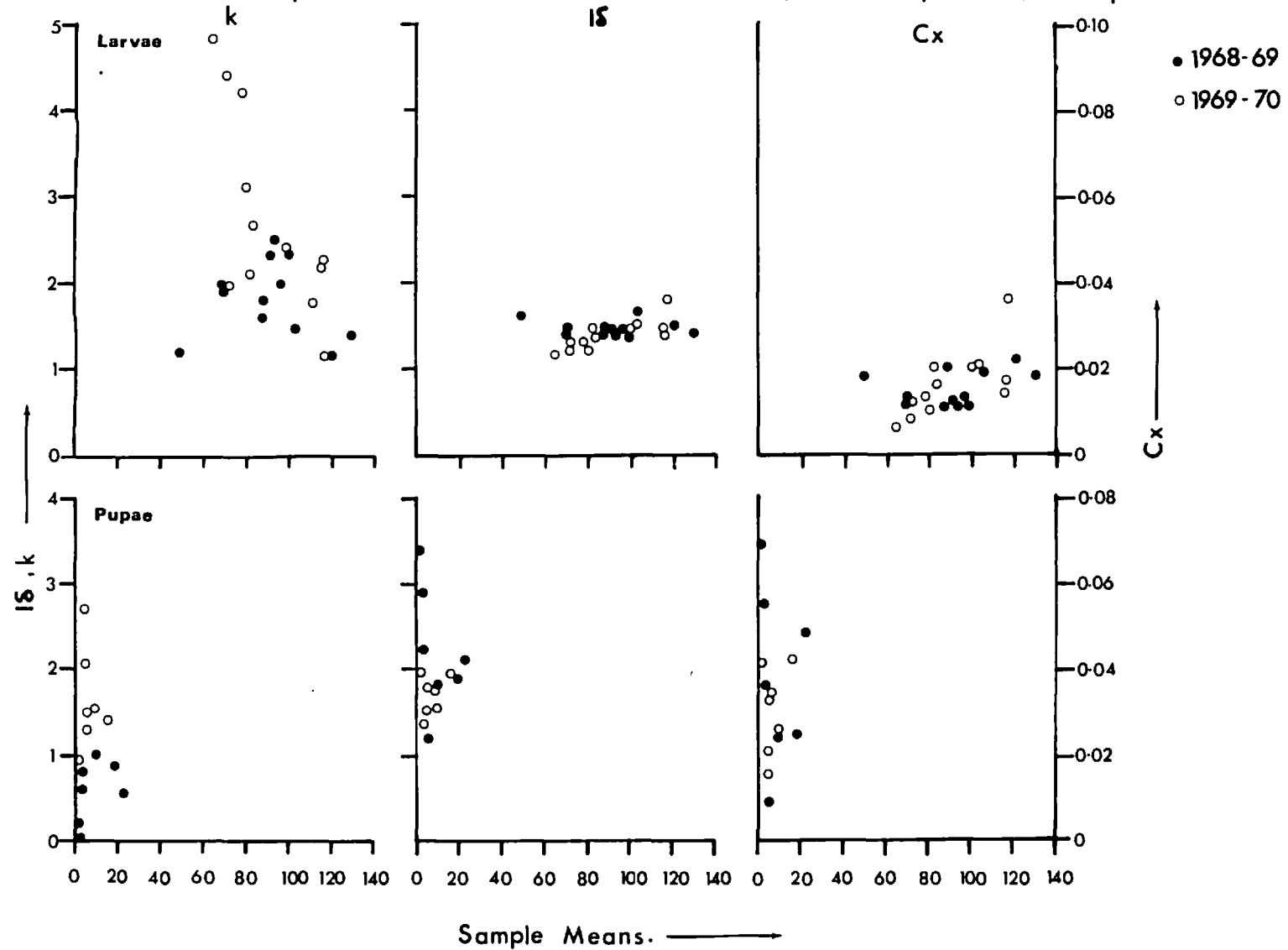
Green (1966) proposed the Cx coefficient as an index appropriate for measurements of aggregation. $Cx = \frac{S^2}{\bar{X}} - 1$, where $S^2 = \frac{\sum X^2 - \frac{(\sum X)^2}{N}}{N - 1}$ variance, $\bar{X}$ = sample mean and $\sum X - 1$ = total number of individuals sampled. Its value range from zero to one, that is, from randomness to maximum aggregation. The Cx coefficient is not influenced by variations in the size and number of samples or by the population size.

Marisita's (1959, 1962) Index of Dispersion, $I\delta$, is also a useful measure of aggregation. It also has the advantages of being relatively independent of the type of distribution, the number of samples or the mean size. $I\delta = \frac{N\sum X^2 - (\sum X)^2}{(\sum X)^2 - \sum X}$, where N = total number of samples, X = number of individuals in a sample and $\sum X$ = total number of individuals in all samples. When the distribution is random, $I\delta$ equals unity, when aggregated (that is, negative binomial) it is greater than unity and in regular or binomial distributions, it is less than unity.

Southwood (1966) suggested that since the dispersion of a population is a description of the condition of the population, changes in the latter should be considered along-side their dispersion pattern. Estimates of the population densities, sample variance and their corresponding dispersion indices (K, Cx and $I\delta$) are presented in Tables 3, 4 and 5. Excepting the exponent K, the indices are relatively unaffected by the sample mean (Fig 8).

Both the Cx and $I\delta$ indices were used for comparison with the large variations obtained in the values of K exponent. Figures 9a,b show the seasonal fluctuations of K, Cx and $I\delta$ indices of dispersion for the different developmental stages of Culicoides. The Cx and $I\delta$ values were closely similar in their descriptions of the changes taking place, in relation to time, in the aggregations. Both were comparatively

FIG.8 Relationship of Morisita's Index, $I\delta$, Green's Index, Cx , and the exponent k , to sample mean.



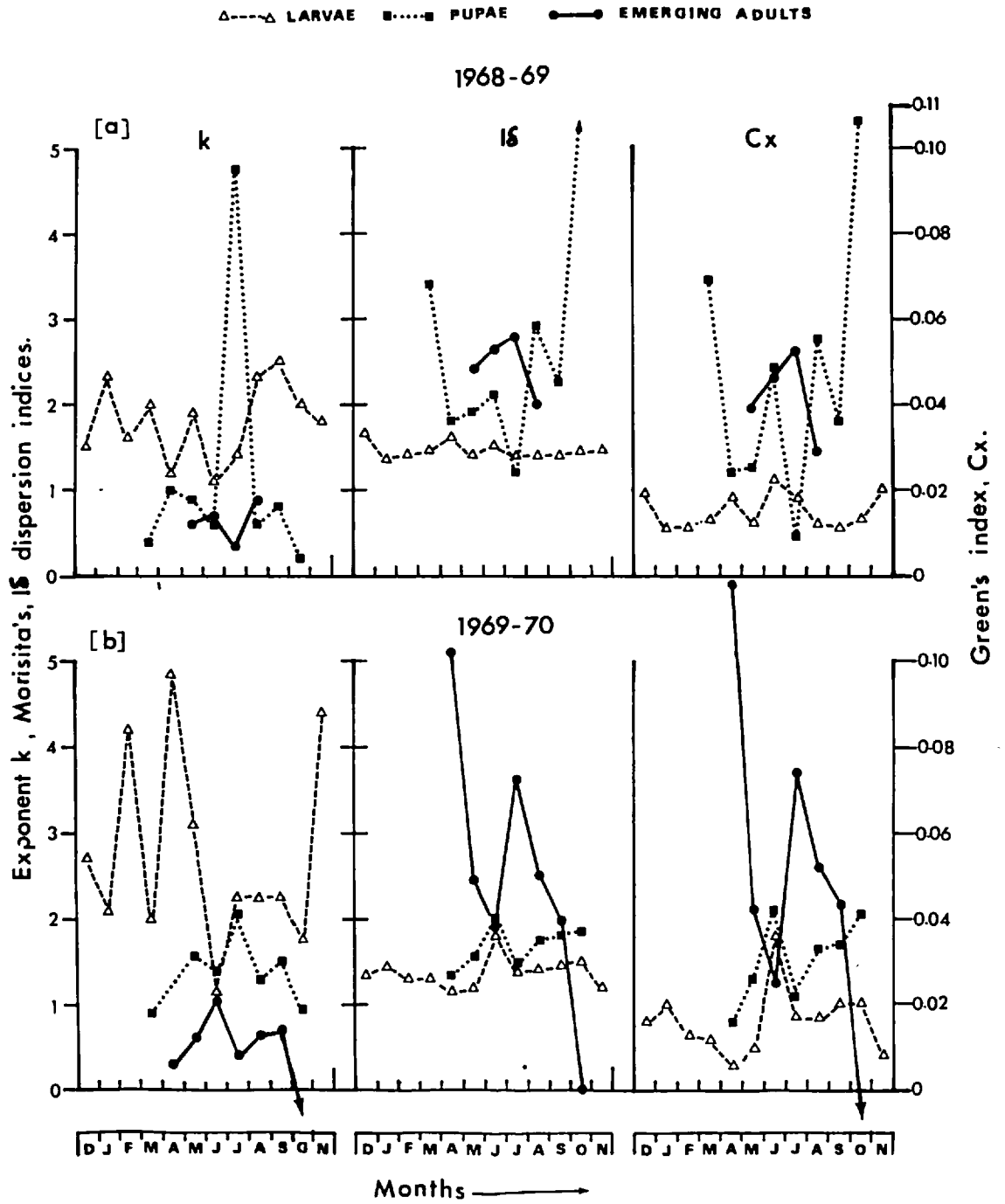


FIG.9 Seasonal changes in the spatial distribution of immature Culicoides stages.

Table 3. LARVAE: Estimates of population densities, sample variance and corresponding indices of dispersion.

K = negative binomial exponent, Cx = Green's (1966) Coefficient and IS = Morisita's (1959, 1962) index of dispersion

		Total No. of Indivi- duals	Mean $\pm$ Standard Error	Estimated Population In Plot	Estimated Population Per Square Metre	Sample Variance = S^2	Standard Deviation = S	$\frac{S^2}{\bar{x}}$	Green's (1966) Index = Cx	Morisita's (1959) Index IS	Negative Binomial K
<u>1968-69</u>	DEC.	3721	103.36 $\pm$ 14.16	100456	2503	7222.29	84.98	69.87	0.019	1.65	1.47
	JAN.	3454	98.69 $\pm$ 10.20	96242	2398	3742.28	61.17	37.92	0.011	1.36	2.33
	FEB.	3134	87.06 $\pm$ 9.32	84603	2108	3126.34	55.91	35.91	0.011	1.39	1.58
	MAR.	2536	70.44 $\pm$ 8.08	68469	1706	2346.14	48.45	33.30	0.013	1.49	1.97
	APR.	1752	48.67 $\pm$ 6.60	47278	1178	1566.17	39.57	32.18	0.018	1.62	1.19
	MAY.	2504	69.56 $\pm$ 7.70	67586	1684	2175.23	46.64	31.27	0.012	1.42	1.91
	JUN.	2884	120.17 $\pm$ 14.79	116790	2910	7875.54	88.74	65.54	0.022	1.51	1.14
	JULY	3090	128.75 $\pm$ 17.50	125098	3117	7350.20	85.73	57.09	0.018	1.42	1.38
	AUG.	3285	91.25 $\pm$ 9.67	88656	2209	3576.14	59.80	39.19	0.012	1.41	2.31
	SEPT	3355	93.19 $\pm$ 9.90	90542	2256	3527.42	59.39	37.85	0.011	1.38	2.50
	OCT.	3437	95.47 $\pm$ 9.90	92790	2312	4224.20	64.99	44.25	0.013	1.44	1.98
	NOV.	2099	87.46 $\pm$ 12.48	85004	2118	3741.48	61.17	42.78	0.020	1.46	1.82
<u>1969-70</u>	DEC.	2003	83.46 $\pm$ 10.60	81111	2021	2699.65	51.96	32.35	0.016	1.36	2.66
	JAN.	1955	81.46 $\pm$ 11.57	79144	1972	3216.00	56.71	39.48	0.020	1.45	2.12
	FEB.	1864	77.67 $\pm$ 8.94	75492	1881	1920.41	43.82	24.73	0.013	1.29	4.17
	MAR.	1737	72.38 $\pm$ 8.11	70355	1753	1580.33	39.75	21.84	0.012	1.28	1.96
	APR.	1528	63.46 $\pm$ 5.33	61186	1537	682.87	26.13	10.76	0.006	1.15	4.84
	MAY.	1925	80.21 $\pm$ 8.20	77940	1942	1614.78	40.18	20.13	0.010	1.23	3.13
	JUN.	2806	116.92 $\pm$ 22.21	117358	2923	11848.86	108.85	101.34	0.036	1.82	1.14
	JULY	2790	116.25 $\pm$ 15.32	116676	2906	5637.33	75.08	48.49	0.017	1.39	2.24
	AUG.	2756	114.83 $\pm$ 15.32	115270	2871	5634.58	75.06	49.07	0.017	1.40	2.27
	SEPT	2379	99.13 $\pm$ 14.05	96322	2400	4740.29	68.85	47.82	0.020	1.45	2.42
	OCT.	2658	110.75 $\pm$ 16.34	107640	2682	6410.89	80.07	57.89	0.021	1.49	1.76
	NOV.	1712	71.36 $\pm$ 6.59	69352	1728	1043.10	32.30	14.62	0.008	1.18	4.37

Table 4. PUPAE: Estimates of population densities, sample variance, and corresponding indices of dispersion.

K = negative binomial exponent, Cx = Green's Coefficient and IS = Morisita's (1959, 1962) index of dispersion

		Total No. of Indivi- duals	Mean $\pm$ Standard Error	Estimated Population in Plot	Estimated Population Per Square Metre	Sample Variance = S^2	Standard Deviation = S	$\frac{S^2}{\bar{x}}$	Green's (1966) Index = Cx	Morisita's (1959) Index IS	Negative Binomial K
1968-69	MAR.	15	0.42 $\pm$ 0.15	401	10	0.82	0.91	1.97	0.069	3.43	0.41
	APR.	340	9.44 $\pm$ 1.54	9191	229	84.83	9.21	8.98	0.024	1.82	1.03
	MAY.	669	18.58 $\pm$ 3.03	18060	450	330.71	18.19	17.80	0.025	1.88	0.87
	JUNE	518	21.58 $\pm$ 4.81	20990	523	556.17	23.58	25.77	0.048	2.10	0.58
	JULY	122	5.08 $\pm$ 0.66	4936	123	10.51	3.24	2.07	0.009	1.20	4.76
	AUG.	86	2.39 $\pm$ 0.62	2328	58	13.62	3.69	5.70	0.055	2.94	0.58
	SEPT	87	2.42 $\pm$ 0.52	2368	59	9.85	3.14	4.08	0.036	2.25	0.83
	OCT.	31	0.86 $\pm$ 0.38	843	21	5.09	2.26	5.92	0.163	6.74	0.22
1969-70	MAR.	5	0.21 $\pm$ 0.10	201	5	0.26	0.51	1.24			0.88
	APR.	90	3.75 $\pm$ 0.61	3652	91	8.98	3.00	2.39	0.016	1.36	2.69
	MAY	224	9.64 $\pm$ 1.62	9472	236	63.29	7.96	6.50	0.026	1.54	1.54
	JUNE	360	15.00 $\pm$ 3.16	15056	375	239.30	15.47	15.95	0.042	1.96	1.42
	JULY	101	4.21 $\pm$ 0.90	4216	105	12.87	3.59	3.06	0.021	1.47	2.05
	AUG.	110	4.58 $\pm$ 0.94	4455	111	21.04	4.59	4.59	0.033	1.76	1.28
	SEPT	138	5.75 $\pm$ 1.16	5580	139	32.54	5.70	5.66	0.034	1.78	1.50
	OCT.	21	0.88 $\pm$ 0.26	883	22	1.59	1.26	1.82	0.041	1.94	0.95

Table 5. EMERGENCE ADULTS. Estimates of population densities, sample variance, and corresponding indices of dispersion. K = negative binomial exponent, Cx = Green's (1966) Coefficient and IS = Morisita's (1959, 1962) index of dispersion.

	Total No. of Indivi- duals	Mean $\pm$ Standard Error	Estimated Population in Plot	Estimated Population per square metre	Sample Variance = S^2	Standard Deviation = S	$\frac{S^2}{\bar{x}}$	Green's (1966) Index = Cx	Morisita's (1959) Index = IS	Negative Binomial K
MAY	14	0.38 $\pm$ 0.13	361	9	0.59	0.77	1.51	0.039	2.37	0.59
JUNE	400	11.11 $\pm$ 2.45	11162	278	216.79	14.72	19.51	0.046	2.62	0.72
JULY	610	16.94 $\pm$ 3.91	17024	424	549.60	23.44	32.44	0.052	2.81	0.33
AUG.	9	0.25 $\pm$ 0.09	241	6	0.31	0.55	1.23	0.029	2.00	0.91
APR	7	0.19 $\pm$ 0.10	201	5	0.33	0.58	1.71	0.118	5.14	0.26
MAY	20	0.56 $\pm$ 0.17	562	14	1.00	1.00	1.79	0.042	2.46	0.71
JUNE	226	6.28 $\pm$ 1.07	6304	157	41.35	6.43	6.59	0.025	1.87	1.12
JULY	200	5.56 $\pm$ 1.56	5581	139	87.23	9.34	15.70	0.074	3.59	0.38
AUG	9	0.30 $\pm$ 0.11	321	8	0.42	0.65	1.41	0.052	2.50	0.65
SEP	27	1.13 $\pm$ 0.31	1124	28	2.38	1.54	2.11	0.043	1.98	0.69
OCT	4	0.33 $\pm$ 0.12	321	8	0.24	0.49	0.73	0.091	0.00	-1.22

more stable than the K exponent. However, all three indices were affected by low population densities.

In general, it would appear that developing Culicoides stages are aggregated in nature rather than randomly distributed. Nielsen (1963) obtained a similar result with C. impunctatus larvae.

Figures 9a,b indicate that aggregation becomes greater with decrease in population levels. Aggregations in the pupal and emerging adult populations were similar in 1969 since the dispersion indices values seem to be within the same range. However in 1970, the emerging adults were distinctly more aggregated than the pupae. In both years, both these stages showed greater aggregation than indicated for the larval populations.

Since the behaviour of the different immature Culicoides stages in the soil breeding medium was not specifically studied, a possible explanation for the differences in the degrees of aggregation between the stages can only be speculative. Gravid female Culicoides select relatively drier parts of the breeding habitat for oviposition (Kettle, 1962). The young larvae wriggle in the thin film of moisture available; thus effecting dispersion into the wetter areas of the soil where food and space for their wandering behaviour are available. It is difficult to assume a similarity in the dispersion of the eggs and larvae populations although it should be admitted that nearness of the oviposition sites to the substrates suitable for larval habitation may well have some influence. The fully grown larvae move sluggishly and pupate in the drier superficial areas of the habitat. Such areas were comparatively limited in the experimental plot, resulting in a more patchy distribution of the pupae. Thus the behaviour of fully grown larvae in selection of pupation sites reflects on the aggregation of the pupae. The pupae are relatively immobile and hence the emerging adults

could be similarly distributed. Differences in the aggregations of the pupae and emerging adults is a reflection on the mortality pattern within the pupal populations.

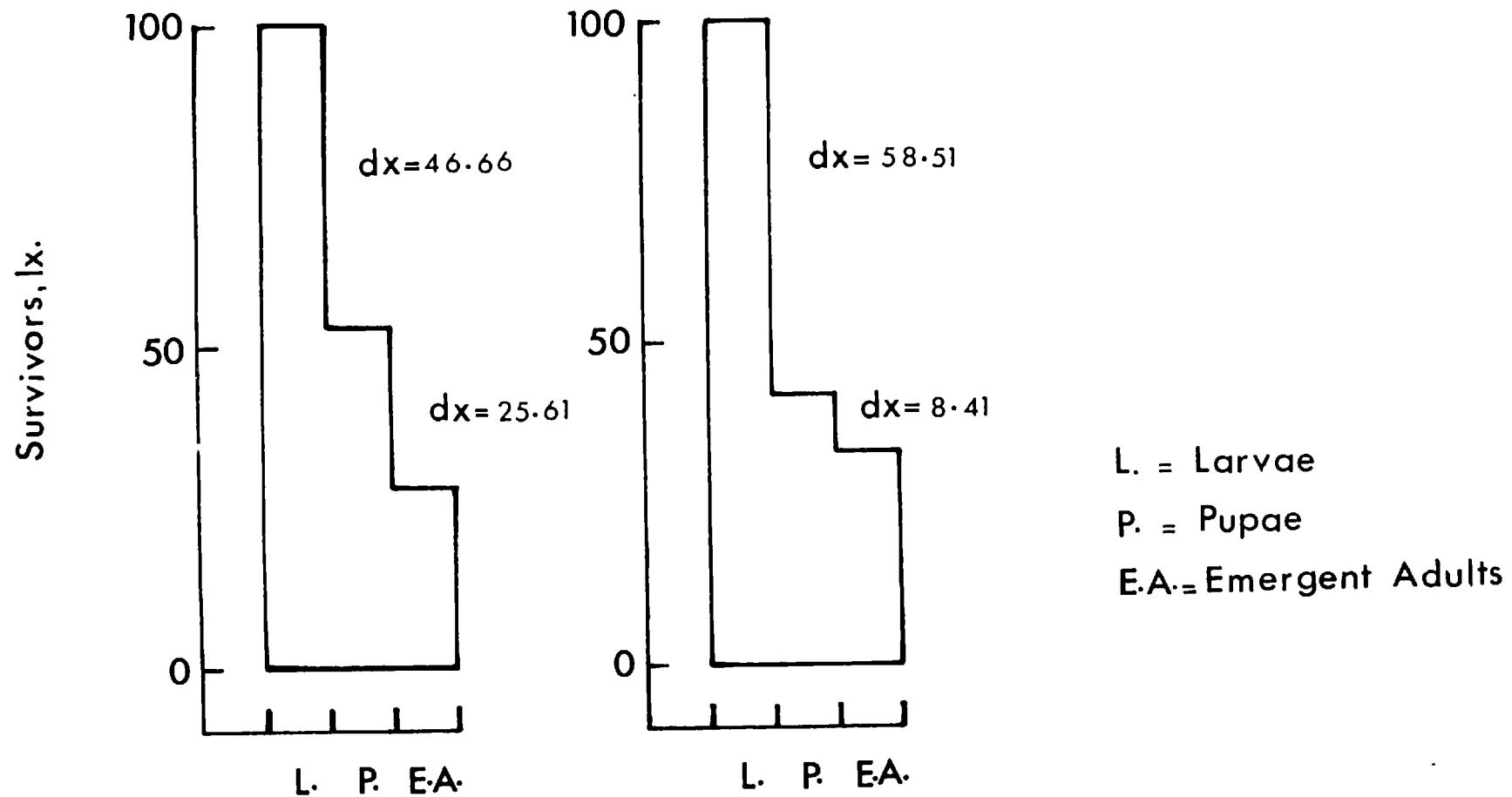
2.6. Estimation of Survival and Mortality in the immature stages

Culicoides larvae have long durations in the field and consequently different immature stages occur simultaneously between March and October. The numbers of the different stages are reduced by death and metamorphosis while being simultaneously increased by oviposition, hatching and subsequent metamorphosis. This condition makes the analysis of data collected from such populations difficult.

Various methods are available for estimating recruitment of individuals into a stage and mortality occurring within that stage (See Southwood, 1966). The reliability of these methods depends on the insect population under consideration. They were, however, found not to be suitable for the analysis of these results. A method similar to that previously described by Richards (1940) was adopted instead. Monthly estimates of the absolute population densities per square metre of the different successive development Culicoides stages in the study area were obtained separately.

No adult Culicoides, and hence no oviposition, occurred during the winter months, i.e. November to April of the following year. Also pupae were not taken in the soil samples from November until March. Thus, the estimated larval densities in December 1968 and 1969 can justifiably be taken as the initial populations from which subsequent Culicoides stages developed in the 1969 and 1970 seasons, respectively. Attempts were made, particularly in 1970, to identify and separate the summer generations of pupae and emergent adults, which occur from August to October, from those that actually developed from the overwintered

FIG.10 Trend in the survival and mortality of Culicoides immature stages.



larvae. In 1969 only adults from the overwintered larvae were taken since the emergent traps were not moved but in 1970 the two adult generations were sampled separately. The summer generation was very small.

The total number of pupae developed from the initial larvae population in December was obtained by summing the monthly pupae estimates per square metre from March to July, 1969 and March to September, 1970. Similarly, the total number of adults emerging was the sum of estimates from May to August, 1969 and from April to August, 1970.

The trend in the survival and mortality of the immature stages in both years are shown in Figure 10.

One hundred was arbitrarily taken as the initial larvae population size in December, 1968 and 1969. The survivors, lx , in each stage was found by subtracting the deaths, dx , which occurred in the previous stage from the survivors in that stage (Deevey, 1947; Southwood in Williams et al, 1969). If x and y are the population estimates of larvae and pupae respectively, the mortality, dx , in the larval stage is equal to $\frac{x - y}{x} \cdot 100$. By removing this value from 100, the number surviving to the pupal stage is then obtained. By similar calculations the emerging adult survivors are determined with x = estimate of pupae, y = estimate of emerging adults and 100 is replaced by the number of surviving pupae.

2.7. Population budget

An attempt at producing a population budget is presented in Tables 6a,b for 1968-1969 and 1969-1970 respectively. Admittedly, these budgets are incomplete since the egg stage was not sampled because of intense practical difficulties. The sex-ratio in the emergence trap catches was nearly 1:1. Assuming that the average number of eggs per batch is 50 /C. impunctatus; Hill, (1947); Service, (1969). Neither of these authors managed to obtain a second batch of eggs⁷, then the maximum potential total eggs per square meter of the experimental plot is estimated as 17,356 (i.e., $694/2 \times 50$, where 694 = total number of adults per m² in 1969). The estimated value for eggs is incorporated (in parenthesis. Table 6b) into the 1969-1970 population budget. These budgets are of the type proposed by Richards and Waloff (1961) for Phytodecta olivacea. It summarises the changes in the population size and mortality variations of the successive developmental stages of Culicoides in the two seasons.

The mortalities are expressed as percentages of the initial larval populations (and initial estimated egg population, in parenthesis, in the 1969-1970 budget) and also as percentages of the numbers actually entering that stage.

In relation to the initial larval populations, the greater mortalities apparently occurred in the larval stages in both years. Mortalities in the pupal stages were considerably lower than in the larval stage although the pupal mortality was higher in 1969 than in 1970. The accumulated mortalities in these two stages were 72.28% and 64.24% in the two seasons respectively. When the percentage mortalities within the stages were considered, however, it was found that mortalities in both larval and pupal stages were nearly equal in 1968-1969 while the larval mortality in 1969-1970 was about twice that occurring in the pupal stage.

Table 6. Population Budget

		1968 - 1969				
a)	Stage	No. entering stage per m ² .	No. dying within stage	% of the stage dying	Mortality of that stage as % of initial larvae No.	Accumulated mortality as % of larvae No.
	Larvae	2,503	1168	46.67	46.67	46.67
	Pupae	1,335	641	48.01	25.61	72.28
	Emerging Adults	694				
		1969 - 1970				
b)	Stage	No. entering stage per m ² .	No. dying within stage	% of the stage dying	Mortality of that stage as % of initial larvae or estimated egg No.	Accumulated mortality as % of larvae or egg No.
	Egg	(17,356)	(15,335)	(88.36)	(88.36)	(88.36)
	Larvae	2,021	1,190	58.88	58.88 (6.87)	58.88 (95.23)
	Pupae	831	169	20.34	8.36 (0.97)	67.24
	Emerging Adults	662				

The apparent mortality experienced in the egg stage is 88.36% and the accumulated mortality of the eggs and larvae accounts for 95.23% of the total mortality occurring. Dessication (Lingley and Davies, 1971; Parker, 1950), fungal and bacterial diseases (Jones, 1960) and even predation may possibly be responsible for such high mortality in the egg stage particularly as Downes (1950) found that C. nubeculosus females oviposit more readily in company than when alone, thus resulting in high local concentration of eggs.

3. LANDING RATE IN RELATION TO HOST ARRIVAL

3.1. Introduction and Method

The relative aerial densities of biting C. impunctatus (Kettle, 1957; Service, 1969) and C. sanguisuga (Jamnback and Watthews, 1963) have been previously estimated by landing rate counts. Species of Culicoides are known to respond differently to the attractiveness of hosts of the same or different species. This work was carried out firstly, to provide a basis for standardisation of the bait/collector method used for the determination of seasonal distributions of the various Culicoides species occurring at Silwood Park and secondly, to investigate any differences in the landing rates at three different sites located at different distances from the breeding areas.

Site I was in the breeding area of Cascade Marsh while Sites II and III were located in the Rush Meadow at distances of 20 (18.3 m) and 100 yards (91.7 m) respectively away from Lake Wood which also forms part of the breeding area.

The method employed was similar to that used by Jamnback and Watthews (1963). The bait sat on the ground and counted the number of Culicoides landing on his exposed legs (knees to ankles) for every consecutive two minutes during a one hour period - after his arrival at the site. The flies were removed with a pooter during counts. Twenty such counts were made on separate days at each of the three sites. On each occasion counts were limited to the hour of sunset.

3.2. Results

The landing rate data obtained in 1969 and 1970 were combined and expressed as percentages of the total catch for each catching period in each of the three separate sites (Figs 11-13). Comparison of catches between the sites is based on three criteria; the landing peak, the period during which maximum landing occurred (i.e. occasions when landing exceeded 5% of the total catch) and the 'waiting period' (i.e. the time between the arrival of the bait and the commencement of maximum landings). These showed a shift towards the right of the histograms in relation to time after the arrival of the bait. Linear relationships are obtained when values of the waiting periods and the times of peak landings are plotted against the distances of the sites from the breeding areas (Fig 14). The slopes of the regression lines indicate an increase of 72 seconds in the 'waiting-periods' and 60 seconds for the attainment of maximum landing for every 10 yards (9.2 m) away from the breeding area.

The first 50% of the total Culicoides taken at each site landed on the bait 12, 16 and 24 minutes after his arrival at Sites I, II and III respectively. Using chi-squared tests, there are no significant differences between Sites I and II and Sites II and III but the difference between Sites I and III is statistically significant ($P = 0.05$).

A possible explanation for this result is proposed. As in other blood-sucking insects (Gatehouse, 1969), Culicoides host-finding movements can be divided into two components: 1. Long range flights which bring the insects to the host's vicinity and 2. Short range orientation towards the host.

The former are random movements while the latter are directional, responding actively to visual and/or olfactory stimuli from a potential host.

Due to interactions between various environmental factors Culicoides are crepuscular in Southern England. C. austeni has been reported to feed at night (Hopkins, 1952) and Reuben (1963) caught large numbers of

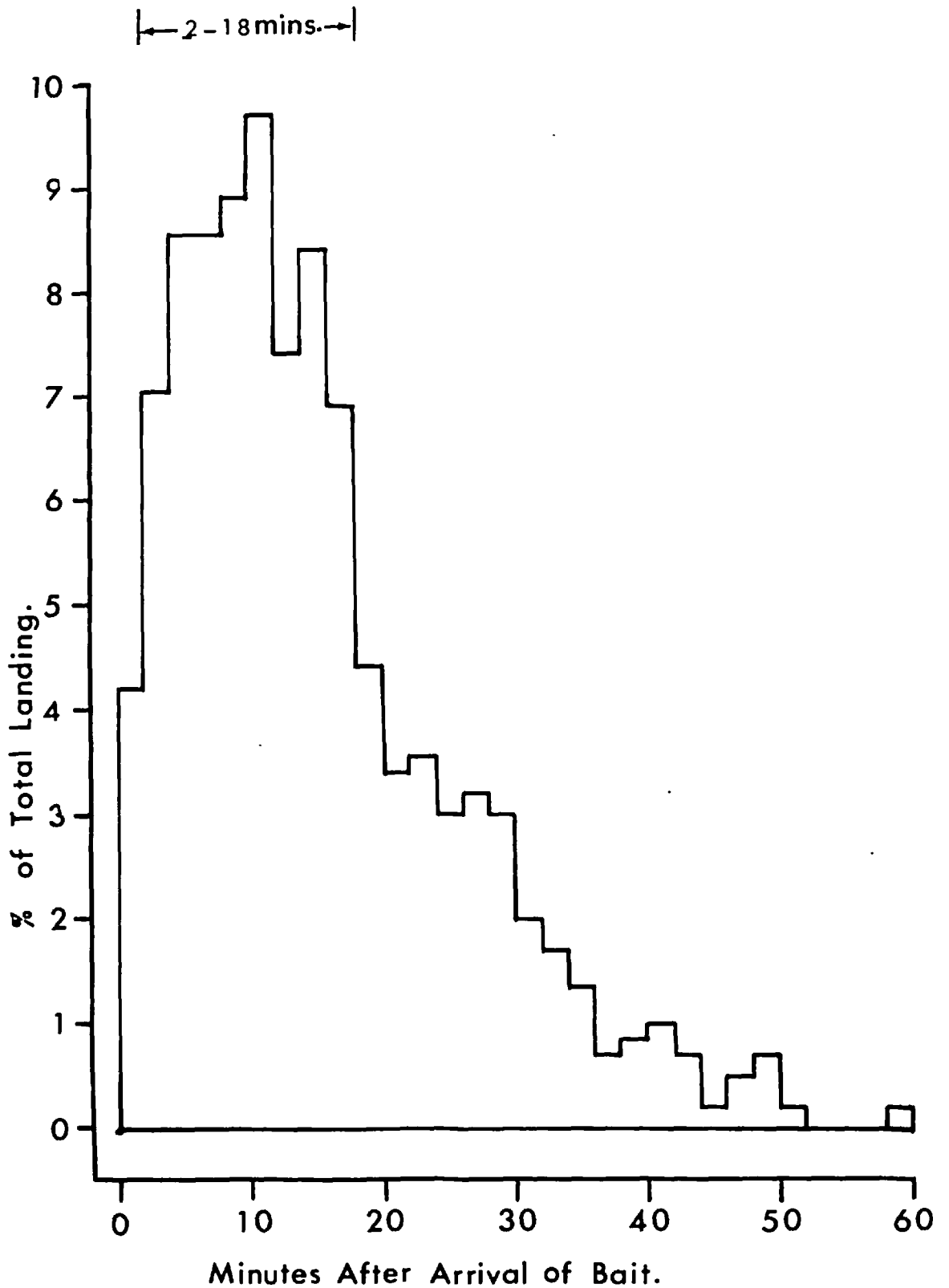


FIG.11. Landing Rates of Adult *Culicoides* at Site I.
[Breeding Site, Cascade Marsh]

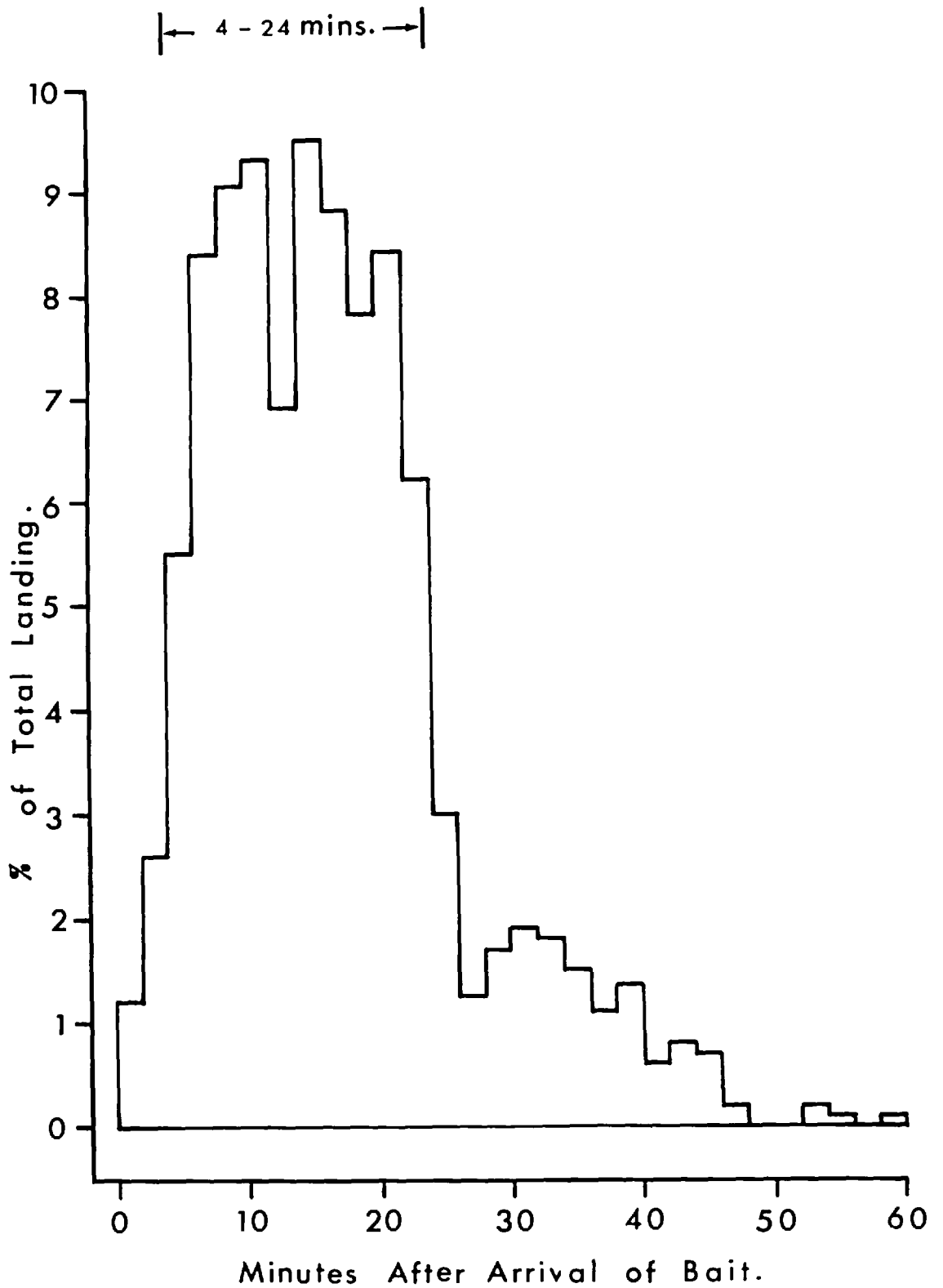


FIG.12. Landing Rates of Adult *Culicoides* at Site II
[20 yds. From Breeding Site, Rush Meadow]

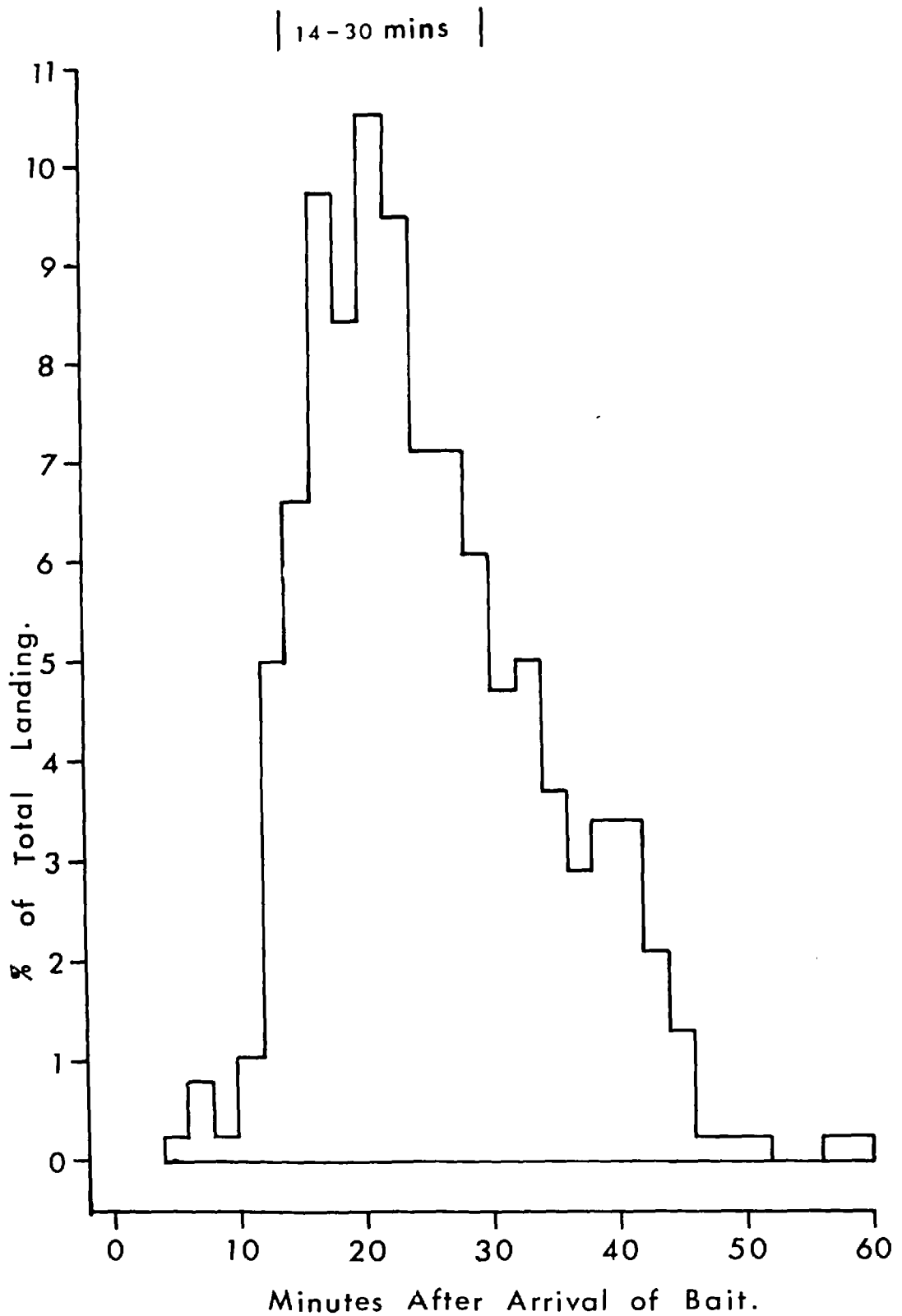


FIG.13. Landing Rates of Adult *Culicoides* at Site III
[100yds. From Breeding Area, Rush Meadow]

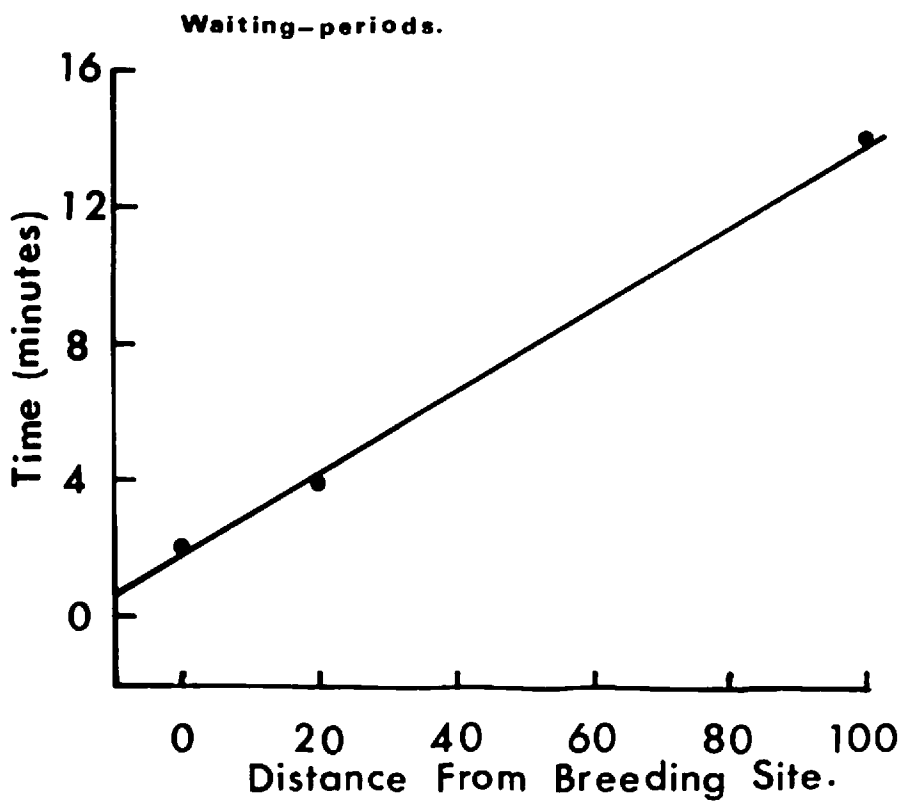
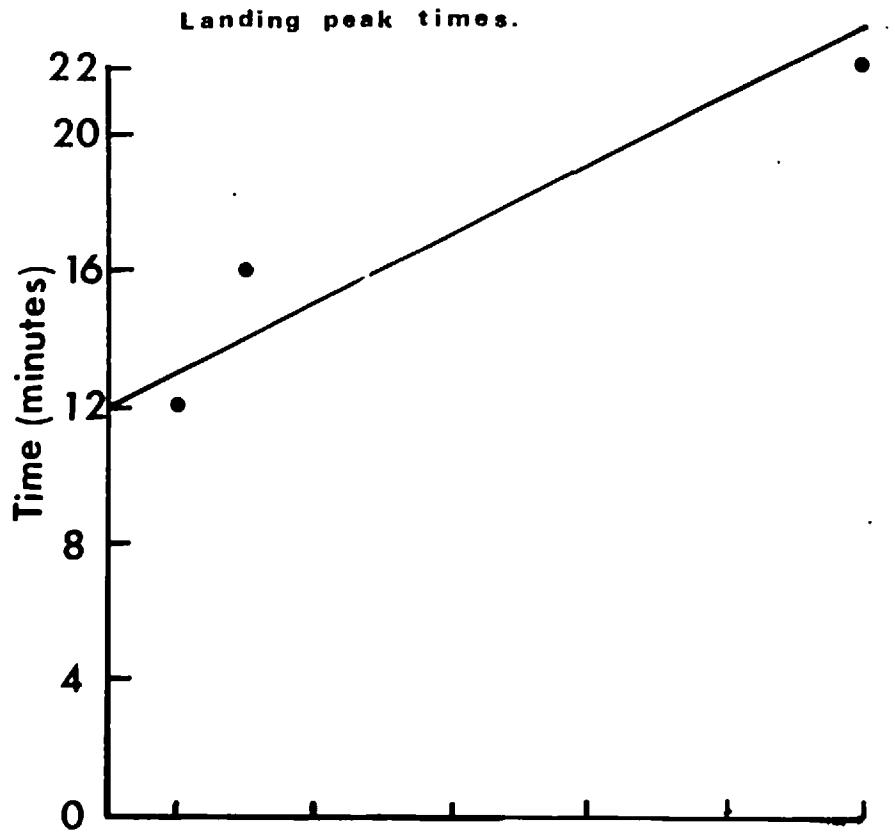


FIG.14 Relationship between 'waiting-period' and peak landing time to the distance of site from breeding area.

C. impunctatus at night in suction traps. It is therefore doubtful if visual response is as important as olfactory stimuli in host finding by Culicoides.

However, the body surface and the exhaled breath are the main source of olfactory stimuli (Gatehouse, 1969) and naturally are more concentrated in the microenvironment of the bait, diffusing in all directions in windless conditions or carried down wind. Due to turbulence in the environmental air-flow, concentration of vapours may not be evenly distributed. Thus, the early build up of the number of Culicoides landing on the host shortly after his arrival at Site I (in the breeding areas of the Cascade Marsh) and Site II (in the open Rush Meadow, 20 yards (18.3 m) from the Woodland), can possibly be both resting and flying Culicoides within the range of influence of the bait's stimuli. Consequently, random flight is terminated in favour of a directional approach towards the host and hungry resting flies are activated into similar responses. Lower catches after the peak landing probably comprised of randomly searching individuals encountering the olfactory stimuli by chance and orientating towards the bait. The time of arrival on the bait depends on when the stimuli are encountered and its distance from the bait. It should be noted, however, that peak landing was achieved two minutes later at Site II than in Site I. This suggests that Culicoides can make short range stimuli-controlled directional flights from its natural habitat into the adjacent Rush Meadow for feeding purposes.

In Site III (in the Rush Meadow, 100 yards (91.7 m) from the woodland), maximum landing was achieved 10 and 6 minutes later than in Site I and II respectively. The implication of this is that hungry Culicoides in their random searching flights for blood meals may fly into open surrounding areas. A chance encounter with the host's stimuli orientates them to him. All catches at this site may comprise only of individuals whose long range random flights were terminated and converted into short range

stimuli-controlled flights. Thus, the catches are lower and the time of peak landings depends on when the largest number of individuals enter the sphere of influence of the host's stimuli simultaneously.

Since the host-finding behaviour of Culicoides was not investigated, it may be generalised that the basic movements of a hungry female Culicoides is a random search on which a non-random directional approach is superimposed once within the vicinity of a host. Furthermore Jackson's (1933) 'feeding ground hypothesis', in which he proposed that Glossina morsitans and G. swynnertoni, when hungry fly from the 'breeding grounds' or 'home' in the dense bush into the 'feeding grounds' in open bush, may be applicable to Culicoides, with the Cascade Marsh as its natural breeding and resting habitat and the Rush Meadow the 'hunting ground' for hosts when not available in the former habitat. The movement of individuals between these two distinct regions, depends on their physiological state; the gorged flies returning to the breeding area within the woodland for egg maturation and oviposition as this facility is not available in the Meadow.

Specific investigations into this aspect of Culicoides behaviour are necessary.

4. Seasonal distribution of adults of various species

4.1 Methods of collection

For the purpose of comparison of results, a combination of three separate methods were used for the determination of the seasonal distributions of the Culicoides species.

4.1a Sweeping method

Collections of adult Culicoides by sweeping with a hand net (Parker, 1949) were made along two linear transects, each approximately 100 yards (91.7 m) long. Transect I ran through the woodland of Cascade Marsh, the major breeding area of Culicoides at Silwood Park. It was located South-west of Silwood Lake and 100 yards (91.7 m) South-east of Cascade Bridge. The soil was muddy throughout the whole year. Transect II was located 200 yards (183.4 m) north of Transect I on the Rush Meadow. It ran parallel to and about 100 yards (91.7 m) north of the southern limits of the Meadow with Lake Wood. The latter forms part of the breeding area.

Weekly collections at each transect consisted of 500 semi-circular strokes in the air, about 5 feet (1.5 m) above the ground, while the collector maintained an even pace from one end of the transect to the other and back. Only one collector was involved. Collections were alternated each week i.e. in one week, the operation was first carried out in transect I, in the next week, transect II. Sweeping was executed within the hour of sunset.

To avoid any losses, which may not be detected because of the minute size of the midges, the sweep net was not emptied immediately after each set of strokes. The thick cotton material with which the net was made, was quickly folded tightly over the straight edge of its D-shaped open end and held securely in position with four large paper clips to avoid any possible escapes. Two marked nets were used, one

for each transect. In the laboratory, the nets with their catches were put in a deep-freeze for two or three minutes after which the Culicoides were removed with a pooter.

4.1b Bait-collector method

This is the most widely used method for studies on adult Culicoides (Jamnback and Watthews, 1963; Service, 1969; Trapido, 1947; Sailer et al, 1956; Jenkins, 1948). Due to the bias introduced by the attractiveness of the bait, standardisation was necessary for any meaningful quantitative data. Weekly landing-rate counts were made on the same bait/collector throughout the 1969 and 1970 seasons in Cascade Marsh and Rush Meadow to avoid variations due to differences between individual potential hosts (Jamnback and Watthews, 1963; Kettle and Linley, 1960). Counts were limited to the hour of sunset when the activity of the midges was at its peak; thus reducing to a minimum the possible presence of other human hosts in the vicinity. The same sites were regularly used in both habitats. The sites, L.R.I and L.R. II were located in the middle of transects I and II and corresponding to sites I and III in the landing-rate experiments (Section 3) respectively.

Catches of adult Culicoides females landing on the outstretched and exposed legs of the sitting bait/collector were made with an aspirator. The catching periods were limited to 15 minutes at each site with two and 14 minutes 'waiting-periods' at sites L.R.I and L.R.II respectively. Catches were alternated as in the sweeping method (see 4.1a). The Culicoides caught were killed in the field with ethyl acetate vapour.

4.1c Suction trap

Reuben (1963) reported the use of nine inch Vent Axia suction trap with automatic hourly sorting device in her studies on C. impunctatus in Scotland. Bidlingmayer (1961) also used a 'powered-

aspirator' - a suction trap with a five-inch fan. Unlike other methods employed for collections of adult Culicoides, suction traps have the advantage of passively sampling the aerial populations independent of effects due to the presence of bait or any other attractants.

Catches obtained at 4 feet (1.2 m) above the ground by an 18 inch propeller trap type in 1968, 1969 and 1970, were examined. The trap is one of three suction traps which are operated continuously and simultaneously at different heights above the ground on the grass lawn of Silwood Bottom.

Johnson and Taylor (1955a) and Southwood (1966) have given illustrated detailed descriptions of the 18-inch propeller suction trap type. Briefly, it consists of an 18-inch propeller fan, mounted in a galvanized iron cylinder, which sucks in air into a filtering cone made of 32-mesh copper gauze. The insects are collected in a glass collecting jar containing 70% alcohol which is screwed into a socket at the base of the cone. The trap sucks in air at the rate of 163800 cu. ft. per hour.

The collecting tube is changed daily at 10.00 hours except during the week-ends. Catches on Tuesday and Thursday of each week were examined.

4.2. Comparison of Sampling Methods Used

The results obtained from Rush Meadow and Silwood Bottom by the three sampling methods are compared for 1969. Both habitats are similar open meadow lands, but Rush Meadow is much closer to the potential breeding sites.

Twelve (9 inch Vent-Axia suction trap, Rush Meadow) and eleven (18 inch propeller suction trap, Silwood Bottom) Culicoides species occurred in the suction trap catches while five and six species were collected by sweeping and bait/collector methods (Rush Meadow) respectively. Of these, only four species were common in all the catches by the different sampling methods and were sufficiently abundant for consideration. The 9 inch Vent-Axia suction trap caught 2.2 and 1.5 times and the 18 inch propeller type 10.3 and 6.8 times as many individuals as by sweeping and bait/collector methods respectively. The 18 inch propeller trap type caught 4.1 times as many individuals as the sum of catches by sweeping and bait/collector methods which was approximately equal to that obtained by the 9 inch Vent-Axia suction trap (Table 7). Table 8 shows the number of the various Culicoides species caught by the different sampling methods.

Sampling Method	No. of Sampling Occasions	Total Catch	Mean (log n+1) Catch Per Sampling Occasion $\pm$ 95% Confidence Limit	Standard Error	Standard Error As % of Mean
Suction Trap (Rush Meadow)	25	844	1.40 ± 0.14	0.0687	4.91
Suction Trap (Silwood Bottom)	56	3972	1.46 ± 0.17	0.08839	6.03
Sweeping	24	384	0.92 ± 0.24	0.1173	12.76
Bait/Collector	25	580	1.07 ± 0.27	0.1310	12.26

Table 7. Total catches of Adult Culicoides by the different Methods, with their confidence limits.

SPECIES	S U C T I O N T R A P				SWEEPING No. %		BAIT/COLLECTOR No. %	
	9 in. Vent Axia RUSH MEADOW No. %		18 in. Propeller Type SILWOOD BOTTOM No. %					
* <u>C. impunctatus</u>	165	18.9	113	5.7	152	39.5	303	52.2
* <u>C. obsoletus</u>	126	14.5	369	18.5	179	46.6	223	38.0
* <u>C. pulicaris</u>	297	34.1	1335	67.0	27	7.0	32	5.5
* <u>C. pallidicornis</u>	186	21.4	139	7.0	25	6.5	19	3.3
<u>C. chiopterus</u>	1		1		-		2	
<u>C. nubeculosus</u>	-		4		-		1	
<u>C. odibilis</u>	32		14		1		-	
<u>C. heliophilus</u>	11		9		-		-	
<u>C. albicans</u>	-		3		-		-	
<u>C. vexans</u>	18		4		-		-	
<u>C. stigma</u>	1		3		-		-	
<u>C. brunnicans</u>	7		-		-		-	
<u>C. parrotti</u>	1		-		-		-	
<u>C. fascipennis</u>	26		-		-		-	
Totals	871		1994		384		580	
No. of species	12		11		5		6	

Table 8. Numbers of various Culicoides species caught.

The confidence limits of the means ($\log n+1$) are similar (Table 7) but the values of the percentage errors of mean indicate that the suction trap catches are more reliable than the similar values obtained for the other two methods. This suggests that suction traps provide the best method for obtaining unbiased estimates of aerial adult Culicoides

densities. The increase in the percentage of the standard error of mean by the sweeping and bait/collector methods are about twice the value for suction trap catches. This is no doubt due to the presence, and hence the attractiveness, of the operator in both the former methods; introducing a bias which illicited a positive response from the hungry female populations.

No males were taken on the bait/collector and males constituted 9.4%, 38.1% and 1.3% of the total catches obtained by 9 inch Vent-Axia trap type at Rush Meadow, 18 inch propeller trap type at Silwood Bottom and Sweeping at Rush Meadow, respectively in 1969.

The proportions (%) of C. impunctatus and C. obsoletus caught by sweeping and bait/collector methods are significantly greater than those obtained by both suction trap catches. C. pulicaris was, however, more common in the latter, with the highest proportion occurring in the 18 inch propeller type at Silwood Bottom. The 9 inch Vent-Axia trap type at Rush Meadow captured a greater proportion of C. pallidicornis than the other three methods.

C. pulicaris although the most abundant species in the aerial fauna, as determined by suction trap samples, was relatively little attracted to the bait or, and probably consequently, little caught in the sweep net samples. In contrast, C. impunctatus and C. obsoletus indicate strong attraction to the bait and are hence probably human-biting species.

Samples by sweeping and bait/collector methods were taken at the hour of sunset which corresponds to the period of ^{peak} activity of Culicoides species at Silwood Park (Section 6). This, it is admitted, could be a contributory factor for these differences. Since similar catches are commonly used for assessments of daily abundance of the species (Parker, 1949; Hill, 1947; Service, 1969a), a comparison with standard 24 hour catches by suction traps is valid.

4.3 Seasonal distributions

In the tropics Culicoides species are present all through the year (Rees and Smith, 1958; Lingley and Davies, 1971), but in the temperate regions ~~usually~~ only one or two generations are usually produced annually. Bimodality in the distribution of some temperate species is probably due to two overwintering larval populations in the preceding year (Kettle, 1962). At Silwood Park, only four species, C. impunctatus, C. obsoletus, C. pulicaris and C. pallidicorvus, are numerically abundant to justify considerations of their seasonal fluctuations (Figs 15, 16, 17).

a) C. impunctatus

There has been conflicting field observations on the seasonal distributions of adult C. impunctatus. In Britain, the species has been reported as unimodal by Hill (1947) at Knowsley Park, Liverpool, Parker (1949) at Rosdhu Park, Loch Lomond, and Service (1969) at Brownsea Island, Dorset and as bimodal by Kettle (1950), at Bannachra and Western Bannachra, Loch Lomond, and Reuben (1963) at Lephinmore, Argyllshire. At Silwood Park, however, C. impunctatus was unimodal in 1969 but a bimodal distribution was detected in 1970. The latter was confirmed by the graphical inflection method on probability paper.

Except in the Cascade Marsh, 1969, when the first adult was taken in the third week of May, all collections by the various methods in the Marsh, Meadow and Silwood Bottom, indicated their first appearance in the last week of May. The population peak was reached in the first and second weeks of June in 1969 and 1970 while the second peak in 1970 occurred in the first week of July. The peaks in June 1969 and July 1970 were followed by decreases in the abundance of the species and complete disappearance by the first week of August.

Kettle (1950) suggested that the two peaks in the bimodal

FIG.15a. Seasonal Distribution of Adult Culicoides.

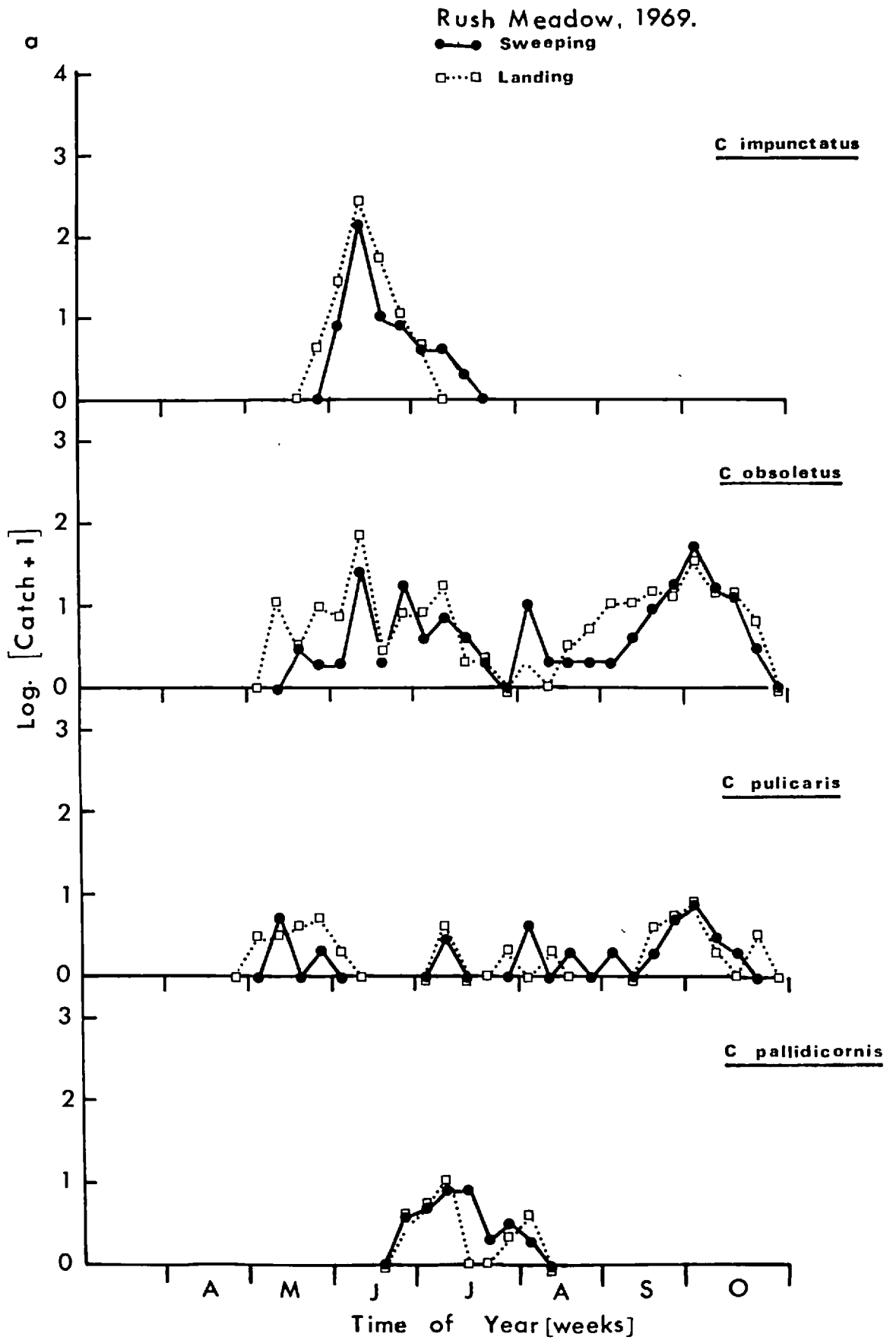


FIG15b. Seasonal Distribution of Adult Culicoides
Cascade Marsh, 1969.

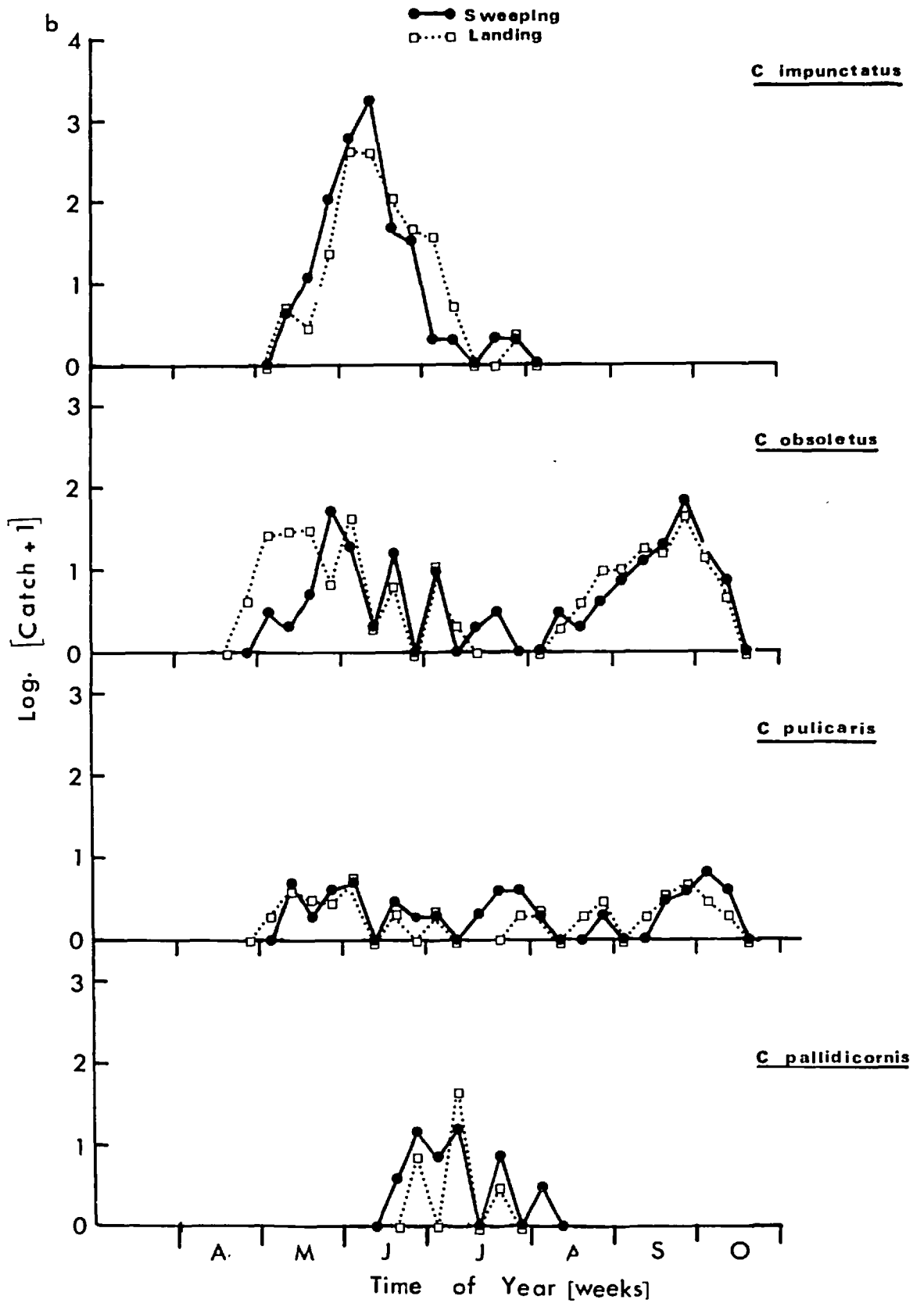


FIG.16a. Seasonal Distribution of Adult Culicoides.
Rush Meadow, 1970.

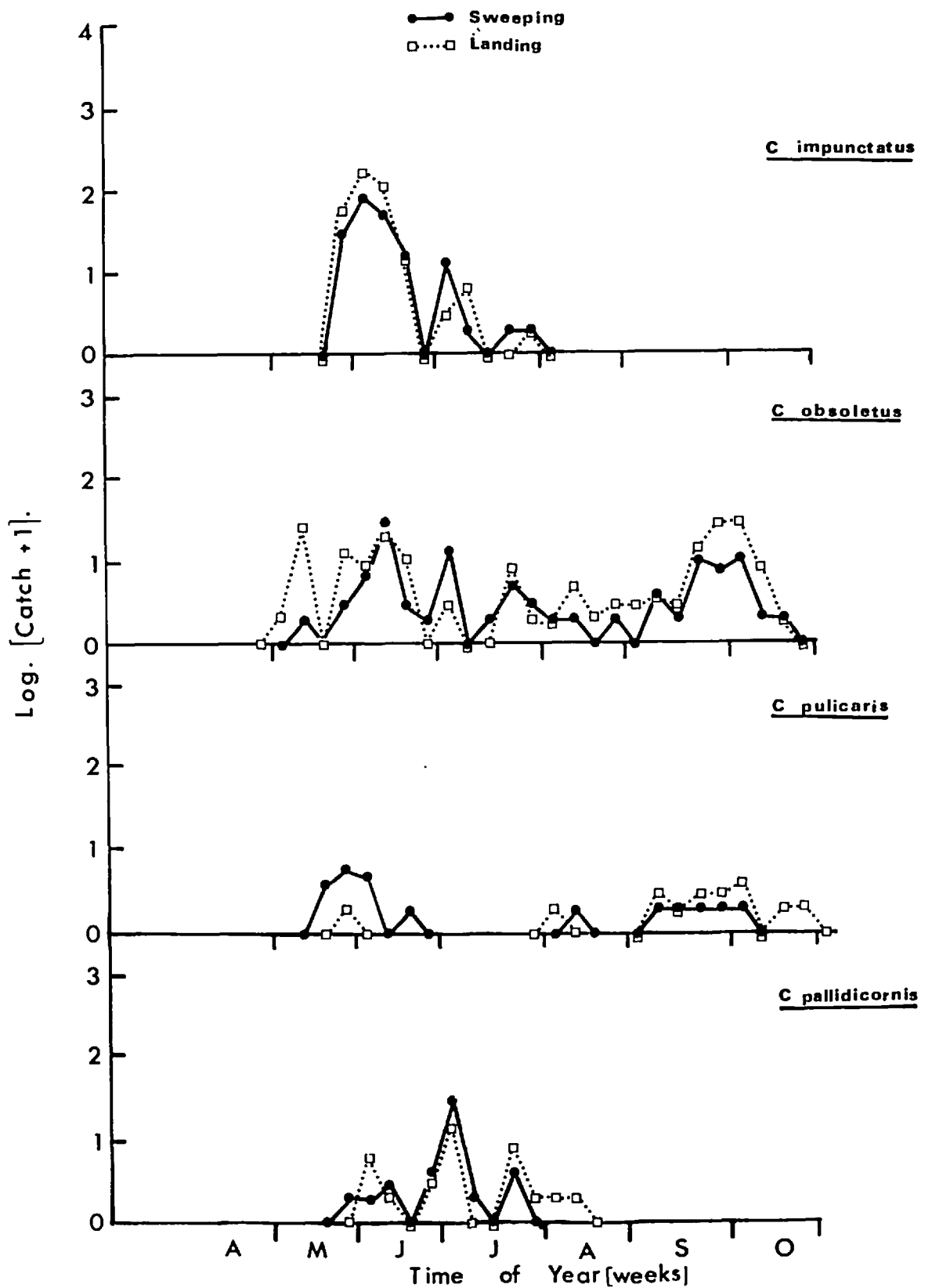
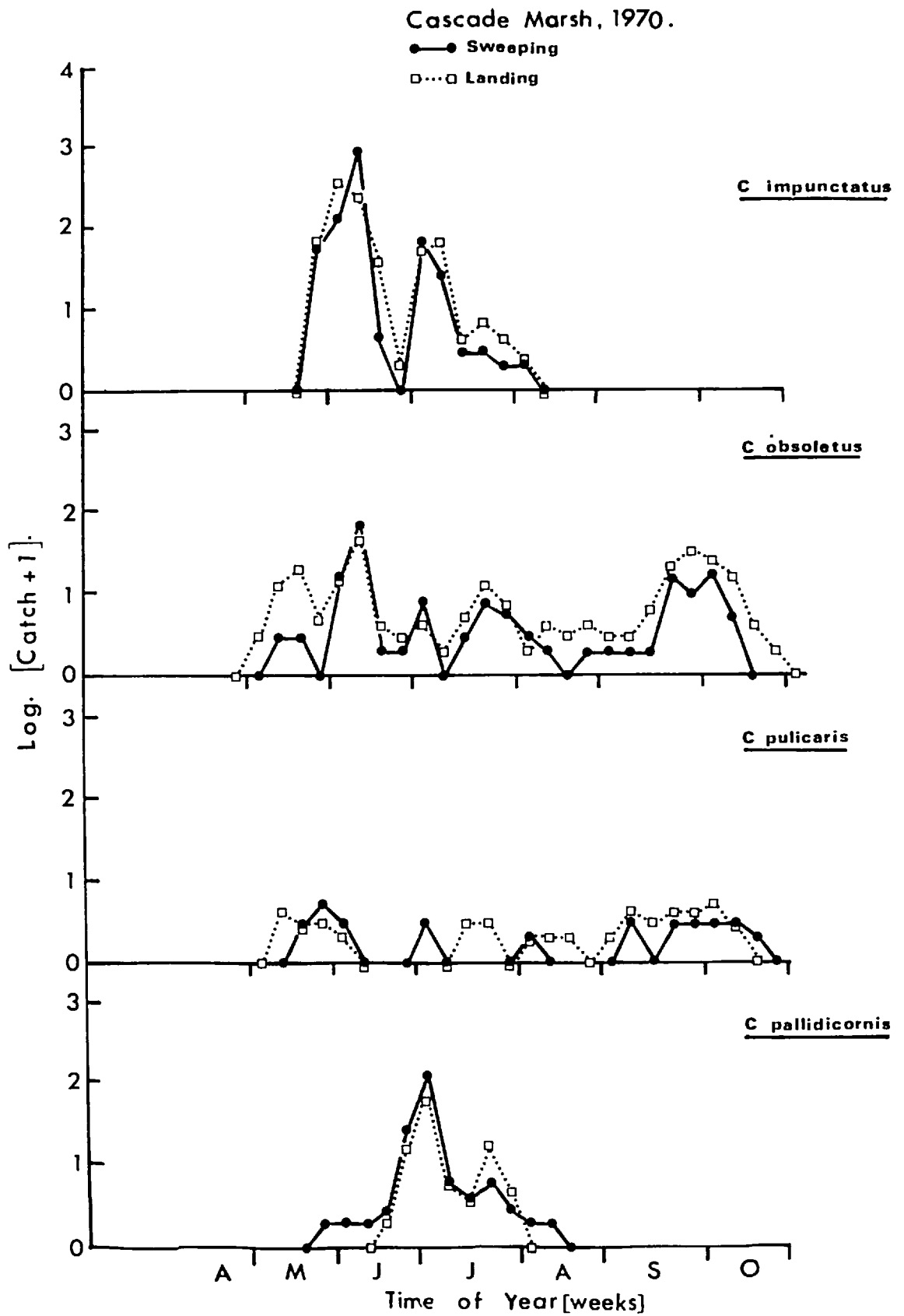


FIG.16b. Seasonal Distribution of Adult Culicoides.



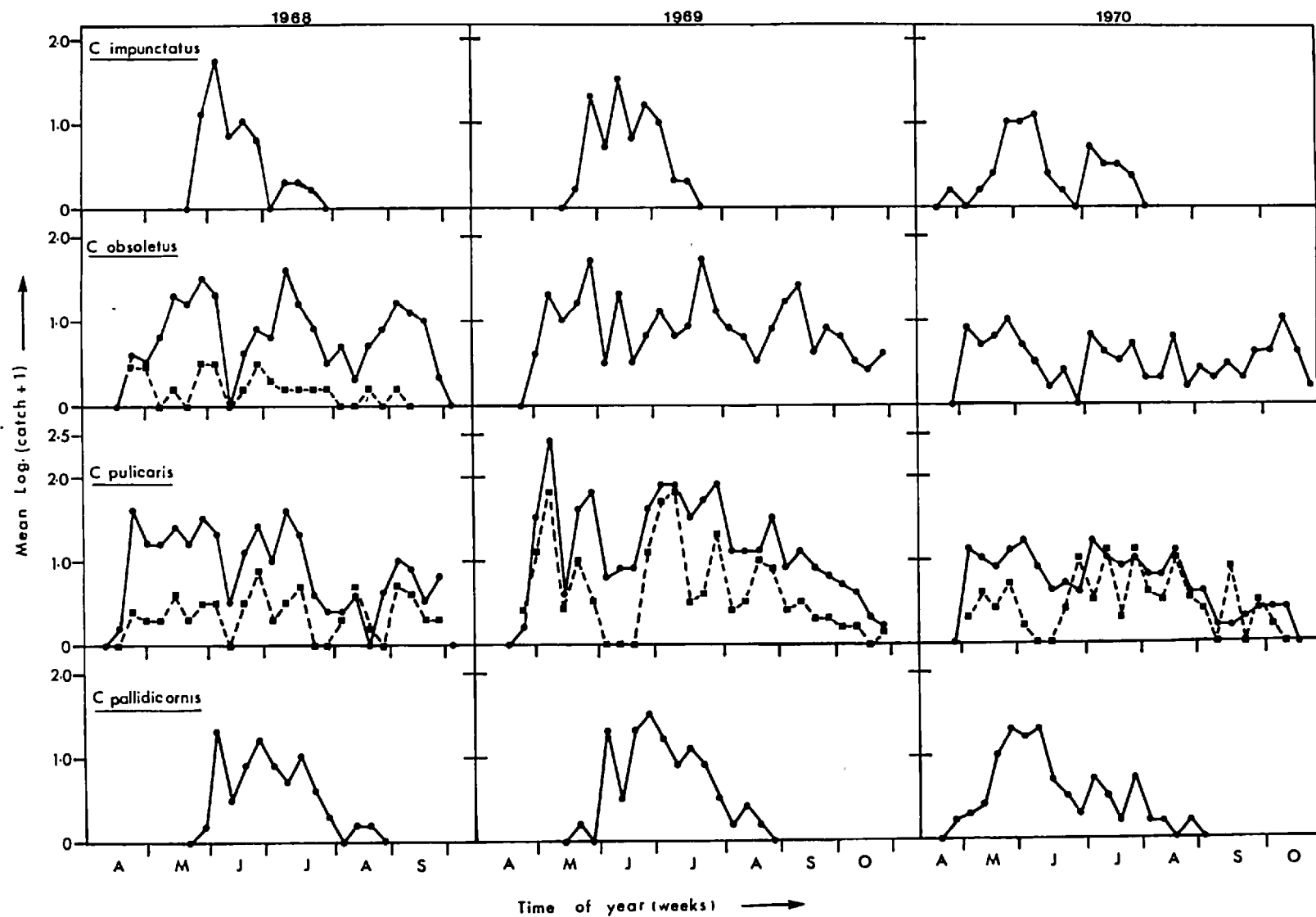


FIG-17 Seasonal distribution of various *Culicoides* species. Silwood Bottom; Suction trap.

distribution of C. impunctatus consist of different biological races. He based his hypothesis on the following features; 1. times of their seasonal appearance, 2. their sex-ratios and 3. their vertical and horizontal distributions. In view of the results in this investigation, where the distribution is unimodal in 1969 at the three sites and bimodal at the same sites in 1970, this hypothesis is doubtful or at least not applicable to the population of C. impunctatus at Silwood Park. The second peak may have been a summer population whose immature stages developed simultaneously with the overwintered stages under favourable climatic conditions. It should be pointed out that in such situations the two generations do overlap.

b) C. obsoletus

On the seasonal distribution of C. obsoletus, Parker (1949) did not obtain any definite peak abundance but Service (1969) observed that maximum biting occurred in May. Both authors, however, reported unimodality. Hill (1947) indicated that the species is bimodally distributed with peaks in June and late September.

At Silwood Park, C. obsoletus first appeared in catches in the last week of April (Suction trap, Silwood Bottom and on bait/collector, Cascade Marsh in 1969) or in the first week of May. In the Marsh, Meadow and at Silwood Bottom, the species occurred throughout the whole season and the last adults were taken in late October in 1969 and 1970. In catches obtained by sweeping and bait/collector methods at Rush Meadow and Cascade Marsh, the numerical fluctuations of the population levels of C. obsoletus indicated bimodality in its seasonal distribution. The peaks occurred during May and June, and September and October. In the suction trap catches three peaks are distinguishable - in the last week of May, third and fourth weeks of July and in September - in the seasons of 1968, 1969 and 1970. This appears to suggest that the species is possibly multivoltine.

c) C. pulicaris

Like C. obsoletus, C. pulicaris occurred throughout the whole season from May to October. Catches by sweeping and bait/collector methods at Rush Meadow and Cascade Marsh were very low. The seasonal fluctuation in population levels was insignificant at the two habitats and no peak was distinguishable. However, catches by the suction trap were numerically greater than by the other two methods - but no distinct peak was evident in 1968, 1969 and 1970 seasons.

d) C. pallidicornis

C. pallidicornis adults were first taken by sweeping and bait/collector methods at Cascade Marsh and Rush Meadow in the third and fourth weeks of June 1969 and in the last week of May in 1970. In both seasons peak abundance was reached in late June and early July and the last adults were taken in the first or second week of August. The suction trap catches of the species showed a similar trend although the last individuals were taken in September.

A combined effect of the overlapping distributions of the various Culicoides species at Silwood Park, results in the persistence of the biting adult populations throughout the whole year except during the winter months i.e., from the beginning of November to about the third week in April of the following year. Maximum biting are thus expected to occur in May and June and again in September and October, with the former being the bigger peak.

5. Adult Dispersal from Breeding Sites

5.1. Introduction

The dispersal of an insect population has been described as a process in which the individuals are displaced over an area, resulting in an increase in the mean distance between the individuals (Andrewartha and Birch, 1954; Kennedy, 1961; Southwood, 1962). Insect movement types have been classified by Southwood (1962) as migratory when a dispersive effect is produced and trivial when such movements are concerned with searching for food or mate in response to visual and olfactory stimuli. Johnson (1957) studied the empirical relation of insect population densities to height and concluded that densities diminished with height. Behavioural aspects which are associated with insect movement types have been discussed by Johnson (1960, 1969).

The major mode of locomotion of Culicoides, both inside and outside its natural habitat, is flight but during this investigation, most females have been observed to walk after landing on the bait's legs before probing. The importance of walking as a means of dispersion is not known. However, Gluchova (1958, quoted in Kettle, 1962) in distinguishing between active hunting flights of Culicoides females and activity induced by a host's arrival, noted that in the latter case, resting individuals can be stimulated to crawl up the host's legs.

Apart from dispersive flights which the insects actively initiate by launching themselves into the air (Andrewartha and Birch, 1954), some small winged insects such as aphids depend to a greater extent on air currents for their dispersal (Broadbent, 1948; Johnson, 1950; Kennedy, 1950a,b). The latter condition is often referred to as passive or wind-bourne dispersal.

5.2. Method of Study

Three methods were employed.

5.2a. Landing-rate at various distances from the breeding sites

Seven landing sites, each consisting of a small clearing in the ground vegetation, were linearly located at Rush Meadow, from the edge of Lake Wood towards Pound Hill Field in the north. The marshy surroundings of Cascade Marsh and Lake Wood constitute Culicoides breeding sites in this area. The landing sites were separated at 40 yards (36.7 m) intervals and the first 20 yards (18.3 m) from the edge of Lake Wood.

Twenty separate samples were taken at each experimental site at dawn. Culicoides landing on the exposed legs of the siting bait were removed with a pooter. A waiting period of two minutes was allowed at each site and catching was limited to a five minute period. All Culicoides still around the bait at the end of this period were removed with a sweep-net. This procedure was adopted to prevent or at least reduce to a minimum the movement of individuals from one site to another with the bait. An attempt was made to correct the bias introduced by the bait's behaviour by alternating the order of the catching sites between sampling occasions i.e., sites 1,2 --- and 7 in week 1, sites 2,3 ---- 7 and 1 in week 2 and so on. Catches at each site were retained in the marked collecting tubes for identification.

5.2b. Sticky traps

Kettle (1951, 1960) and Nielson (1963) have previously used cylindrical sticky traps, originally described by Broadbent et al (1948) for sampling alate aphids, in investigations of aerial Culicoides populations. At Rush Meadow in 1969, three rows (3,2,2) of catching stations, each consisting of three cylindrical sticky traps at heights of two feet (0.6 m), six feet (1.8 m) and ten feet (3.0 m), were

arranged parallel to and away from Lake Wood. The first row was 20 yards (18.3 m) from the edge of the latter while the second and third rows were 40 yards (36.7 m) and 80 yards (73.4 m) respectively from the first. Results obtained from this arrangement confirmed that the breeding sites in Cascade Marsh and Lake Wood were the source of dispersing adult Culicoides on the Meadow.

The linear arrangement of five stations in 1970 was similar to that of the landing sites (Section 5.2a.) and determined from positions of stations with maximum catches in 1969. The first and last stations were 20 yards (18.3 m) and 180 yards (165.1 m) respectively from Lake Wood with an interval of 40 yards (36.7 m) between them.

Each trap consisted of a black cylindrical fruit tin measuring 12" high by 6" diameter and covered by a sheet of cellulose acetate on which a tree banding grease was lightly smeared. Hill (1947) found that black colour is attractive to female Culicoides. The three cylinders of a catching station were separated by two feet (1.2 m) long garden nylon strings which were tied to light wire crossbars fixed across the open ends of each cylinder. The traps were then suspended vertically by means of a pulley fixed to the end of a metal bracket which was attached to the top of a 12 feet (3.6 m) wooden post. The centres of the cylinders were then 2 feet (0.6 m), 6 feet (1.8 m) and 10 feet (3.0 m) above ground level. To prevent the traps from being greatly displaced in the wind, the nylon string from over the pulley was anchored to an attachment at the side of the post and that from the lower end of the 0.6 m cylinder was tied to a peg placed directly under it.

Due to minute size of Culicoides, all small insects trapped were removed at weekly intervals with a knife and placed in jars containing trichloroethylene which freed them from the grease. However, on examination, a large proportion was found damaged. This might have occurred during the removal process. Since identification of Culicoides

species is based mainly on the characteristics of the wing patterns, the catches were separated into the different sexes only.

5.2c. Suction traps

The numbers of Culicoides captured on Tuesday and Thursday of each week during three seasons (1968-1970) by three suction traps, which sampled the air at three different heights above the ground, were examined. The suction traps were of two types - two 18 inch propeller type (II, IV) and a 40 feet type (V) with air delivery capacities of 163,800 cu. ft. per hour and 96,000 cu. ft. per hour respectively. Trap II sampled at 4 feet above the mown grass-lawn at Silwood Bottom. Trap IV was suspended on a tower at the boundary between Silwood Bottom and Hill Bottom and sampled at a height of 28 feet (8.5 m), 20 feet (6.1 m) north of Trap II. Trap V sampled at 40 feet (12.2 m) above the ground, 100 feet (30.6 m) west of Trap II. During the three seasons, all the three traps were operated continuously and simultaneously from early May to late September and the daily catches were removed at 10.00 hours, except at the week-ends. The catches were preserved in alcohol.

A brief description of the 18 inch propeller suction trap has been given (See Section 4.1c.). There is no published description of the 40 feet (12.2 m) trap type but Mr. R. A. French of the Rothamsted Experimental Station has provided the following information. The trap consists of a $1\frac{1}{2}$ horse power electric motor driving a 12 inch centrifugal fan; this fan draws air down a 10 inch diameter tube, the opening of which is 40 feet (12.2 m) above the ground. The insects contained in the sampled air are filtered out by a monel gauze net and collected in a bottle at the lower end of the net, the air being exhausted at ground level.

5.3. Vertical Dispersion

5.3a. Suction traps

Suction trap catches are convenient for aerial density estimates of various insect species because they sample a known volume of air in unit time. Johnson and Taylor (1955a,b) and Taylor (1951, 1962a) described the aerial density of an insect species as the number of individuals in a unit volume of air standardised at 10^6 cu. ft.

Wind speed, insect size and the fan performance affect the absolute efficiencies of suction traps (Taylor, 1955, 1962a). Wind speeds vary with height, becoming greater with increasing height above the ground. The traps have maximum efficiency in still air but this decreases as wind speed increases. Hence, the actual numbers of Culicoides species taken by the 18 inch propeller suction trap (II, IV) and the 40 feet (12.2 m) trap type V at the different heights, are not comparable.

Tables of conversion factors for the different trap sizes, which provide corrections for the effects of wind speed and insect size on the trap efficiency, have been given by Taylor (1962a). The sum of log. catch per hour for each day's sample and the appropriate correction factor (in logs) equals the log density per 10^6 cu. ft. of air. Conversion factors are, however, not available for the 40 feet (12.2 m) trap type but Dr. L. R. Taylor (private communication) Rothamsted Experimental Station, confirmed that the absolute efficiency of this trap type is nearly 100% in all relevant wind speeds. Since Culicoides are minute in size, it is assumed that the trap also captured them efficiently.

The aerial density of each Culicoides species at 4 feet (1.2 m) and 28 feet (8.5 m) above the ground were estimated as follows:-

- a). The log catch per hour of the conventional day of the species, i.e., 18.00 hours to 06.00 hours, was found.
- b). The average wind speed during this period at the different heights and the standard size of Culicoides (i.e. length x wing span) were

determined and used for obtaining the conversion factors from Taylor's tables.

c). The sum of the results of a) and b) then gave the log density of the species per 10^6 cu. ft. of air. Such daily estimates for a species at each height were summed to give a value for the log density.

The results obtained from hourly catches by a nine inch Vent Axia suction trap operated at Rush Meadow during 1969 and 1970, showed that maximum flight activity of each Culicoides species occurs from 18.00 hours to 06.00 hours, i.e., 12 hours, with intermittent flights at other times of the day. The average wind speed during this period at 28 feet (8.5 m) above the ground was calculated from hourly spot readings in 1968, 1969 and 1970 obtained from the Meteorological Office in Bracknell, six miles west of Silwood Park.

Windspeed measurements at two metres by Imperial College Meteorological Department at Pound Hill Field were used as approximately equal to that at 1.2 m above ground level.

For comparative purposes. the aerial densities of Culicoides species at 40 feet (12.2 m) were estimated as follows:-

$$\frac{\text{Catch} \times 10^6}{96000 \times 12} = \text{Density}/10^6 \text{ cu. ft. of air,}$$

Where 96,000 = Volume of air sampled per hour and 12 = number of hours of maximum flight.

$$\text{Thus, Catch} \times 0.864 = \text{Density}/10^6 \text{ cu. ft.}$$

$$\text{or Log. catch} + 1.9385 = \text{Log Density}/10^6 \text{ cu. ft.}$$

As for the other heights, the daily estimates were summed.

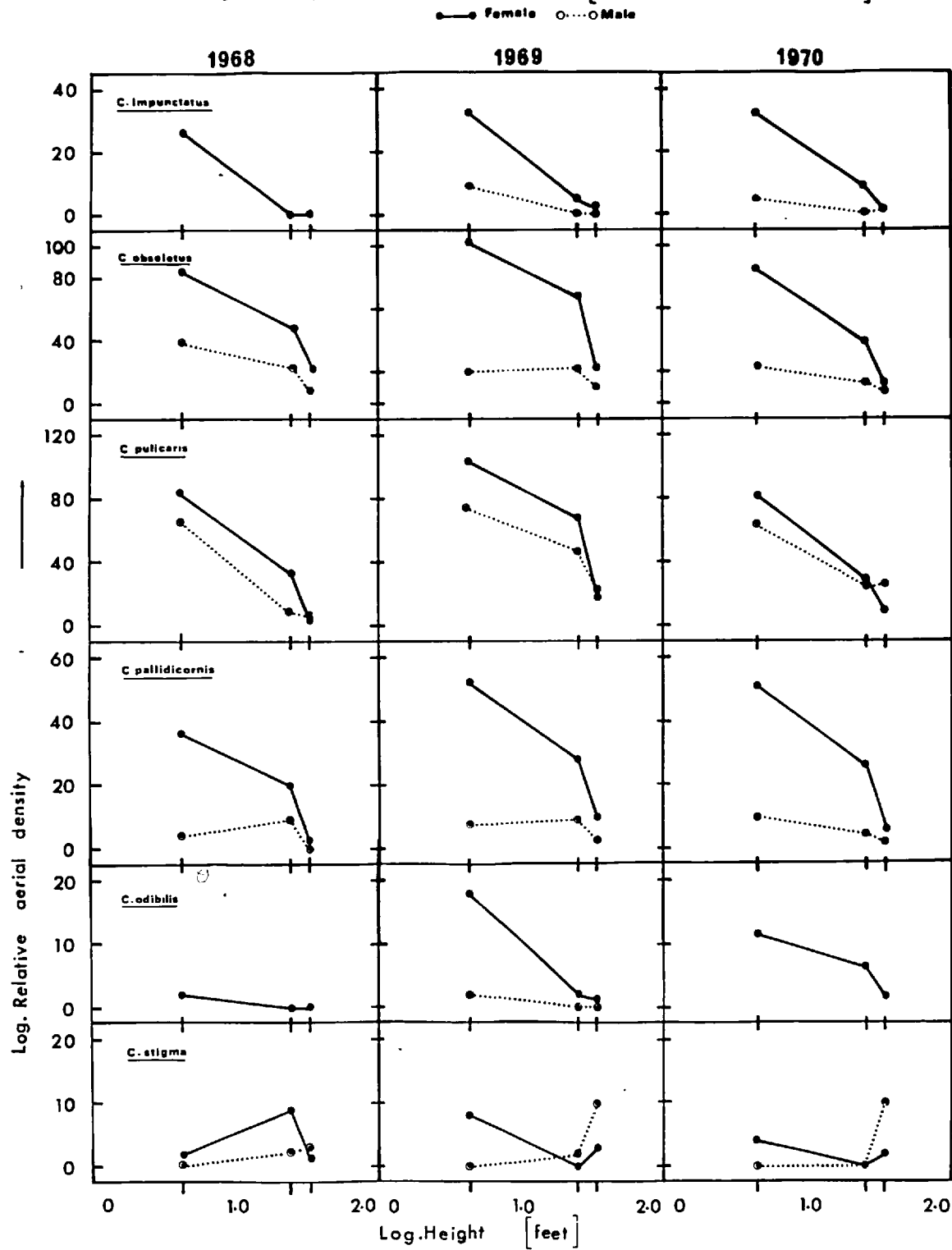
The actual uncorrected numbers of Culicoides taken by the traps at the different heights (Table, 9a) apparently indicate greater numbers at 40 feet (12.2 m) than at 28 feet (8.5 m) above the ground. This is due to the greater efficiency of the 40 feet (12.2 m) suction type trap whose air filtering cone has steeper sides than in the 18 inch propeller type.

A.	1968						1969						1970					
	TRAP II		TRAP IV		TRAP V		TRAP II		TRAP IV		TRAP V		TRAP II		TRAP IV		TRAP V	
	1.2 m		8.5 m		12.2 m		1.2 m		8.5 m		12.2 m		1.2 m		8.5 m		12.2 m	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
<u>C. impunctatus</u>	197	3	0	0	2	0	224	4	1	0	5	0	86	4	2	0	4	2
<u>C. obsoletus</u>	554	29	33	17	129	19	743	12	66	16	134	27	201	14	28	6	67	57
<u>C. pulicaris</u>	578	100	21	5	19	19	1933	736	75	35	113	148	344	176	20	16	41	177
<u>C. pallidicornis</u>	191	2	18	2	5	0	275	2	28	3	63	6	233	6	16	2	28	10
<u>C. odibilis</u>	2	0	4	1	1	0	23	1	1	0	5	0	7	0	3	0	7	0
<u>C. stigma</u>	2	0	0	0	8	16	4	0	0	1	13	51	2	0	0	0	8	56
B.																		
<u>C. impunctatus</u>	26.06	1.98	0	0	0.46	0	32.26	7.76	2.18	0	1.10	0	31.80	3.94	4.36	0	0.64	0.66
<u>C. obsoletus</u>	84.48	39.00	48.22	21.92	20.52	3.80	104.48	21.52	68.18	21.92	21.52	5.22	85.06	23.34	38.44	13.08	13.08	7.18
<u>C. pulicaris</u>	82.88	65.02	32.82	8.74	3.82	3.62	103.96	74.38	68.28	46.06	18.14	23.22	82.22	63.60	28.50	25.08	8.74	26.84
<u>C. pallidicornis</u>	37.08	1.96	19.78	4.36	1.10	.0	46.10	3.88	24.20	4.38	9.66	1.28	51.84	9.76	26.24	4.36	5.58	2.18
<u>C. odibilis</u>	1.96	.0	.0	.0	0.22	.0	17.70	1.94	2.18	.0	1.08	.0	11.66	.0	0.54	.0	1.54	.0
<u>C. stigma</u>	1.96	.0	8.72	2.18	1.06	2.82	7.76	.0	.0	2.18	2.62	9.90	3.88	.0	.0	.0	1.72	10.24

Table 9. A. Numbers of Culicoides species caught by Suction traps II and IV (18 inch propeller type) at 4 feet (1.2m) and 28 feet (8.5 m) above the ground respectively and by the 40 feet (12.2 m) trap type, V.

B. Logarithms of the calculated aerial density estimates at different heights.

FIG. 18 Height- Density profile of adult *Culicoides*. [Suction traps; Cascade Marsh]



In the latter, minute insects may be lost when they are trapped in or forced through the gauze mesh by the force of impaction as the trap sucks in air.

The corrected numbers, allowing for trap efficiency and expressed as log. aerial density per 10^6 cu. ft. of air for each species at the various heights are given in Table 9b and presented graphically in Figure 18.

C. impunctatus, C. obsoletus, C. pulicaris, C. pallidicornis, C. odibilis and C. stigma are considered. Excepting C. stigma where the numbers are small, the largest numbers of both sexes of each species flew at 4 feet (1.2 m) above the ground. C. stigma males were not caught at this level and very few C. impunctatus of both sexes were taken above this height. In all three seasons, 1968-1970, both sexes of each species showed a definite fall-off in numbers in relation to increasing height, except C. stigma where almost all the males occurred at 40 feet (12.2 m). The females of this species were also slightly more at 40 feet (12.2 m) than at 28 feet (8.5 m). It is probable that C. stigma does not breed or feed near the suction trap sites. In general, however, the densities of C. obsoletus and C. pulicaris females diminished more rapidly with increasing height than those of C. impunctatus, C. pallidicornis, C. odibilis and C. stigma but the rate of fall-off between 28 feet (8.5 m) and 40 feet (12.2 m) was more rapid in C. obsoletus, C. pulicaris and C. pallidicornis. Excepting C. pulicaris, the fall-off of male numbers with height above the ground was more gradual. Males of C. pulicaris and C. stigma were more numerous at 40 feet (12.2 m) than the females in 1969 and 1970, and in the latter species more males flew at this height than at 28 feet (8.5 m).

For confirmation of the above results, the density/height mean regressions for the four most abundant species were calculated. The

mean density estimates for all three seasons for males and females were obtained and treated separately. The mean (all years) values of the regression coefficients of log density/log height are given in the following table:-

	<u>Regression coefficients</u>	
	<u>Females</u>	<u>Males</u>
<u>C. impunctatus</u>	- 30.5316	- 4.6943
<u>C. obsoletus</u>	- 64.2589	- 18.5485
<u>C. pulicaris</u>	- 77.8904	- 52.3968
<u>C. pallidicornis</u>	- 35.1798	- 3.0205

From these values, it is evident that the rate of fall-off of females is steeper than that of the males of the various species. C. pulicaris females fell-off, in relation to height, more rapidly than those of C. obsoletus which in turn had a steeper slope than either C. impunctatus or C. pallidicornis females. Similarly, C. pulicaris and C. obsoletus males fell-off more steeply than those of the other two species. The differences were statistically not significant because of the small degree of freedom.

5.3b. Sticky traps

	Height above vegetation					
	6 inches (0.2m)		4.5 feet (1.4m)		8.5 feet (2.6m)	
	Females	Males	Females	Males	Females	Males
<u>1969</u>						
Total Catch	6	13	201	155	161	297
Log.Catch/ 10^6 cu. ft.	1.07	1.40	1.98	1.87	1.84	2.12
<u>1970</u>						
Total Catch	6	4	70	56	89	123
Log.Catch/ 10^6 cu. ft.	1.21	0.89	1.53	1.43	1.59	1.73

Table 10. Total numbers of Culicoides caught on Cylindrical sticky traps and corresponding estimated densities per 10^6 cu. ft. of air at the various heights.

The total catches of both Culicoides sexes on cylindrical sticky traps at heights of 0.2 m, 1.4 m and 2.6 m above the 1.5 feet (0.5 m) ground vegetation at Rush Meadow are given in Table 10.

The log density estimates at the respective heights are also given. The above estimates were obtained by using correction factors (Taylor, 1962b) which were calculated from Johnson's (1950) data in which he compared catches by suction trap, white cylindrical sticky traps and a tow net. By summing the log catch per hour and the appropriate correction factor, the log density per 10^6 cu. ft. of air is obtained. Taylor's table of conversion factors is suitable for insects up to $\frac{1}{4}$ inch long and corrects variations in the efficiency of the traps caused by changes in wind speed. Fig. 19 relates the log density estimates to the logarithm of the various heights.

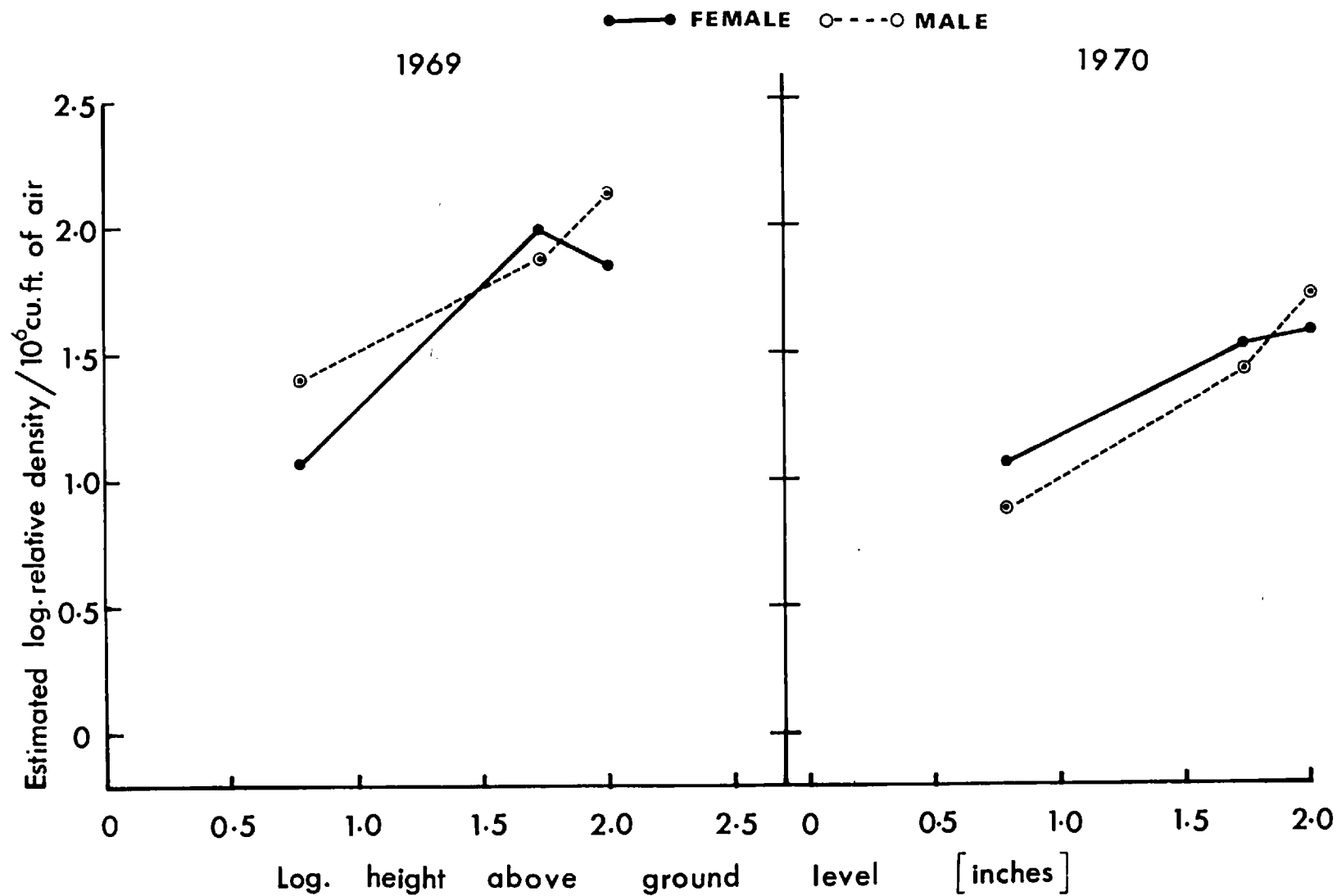


FIG.19 Height-Density profile of adult *Culicoides*. [Sticky traps; Rush Meadow]

As for the suction trap catches, the conventional flying day of adult Culicoides has been taken as 12 hours (i.e., 18.00 hours to 06.00 hours), giving a total of 588 and 420 sampling hours in 1969 and 1970 respectively. The wind speed data were obtained from measurements by Mrs. P. Reader and Imperial College Meteorological Department.

In both years, the estimated aerial densities of both sexes at 6 inches (0.2 m) above the vegetation were lower than at 4.5 feet (1.4 m) and 8.5 feet (2.6 m). Chi-squared tests, however, showed that the observed differences of both sexes between the various heights were statistically not significant; thus suggesting that flying Culicoides are nearly evenly distributed between 0.2 m and 2.6 m above the vegetation.

5.4. Horizontal Dispersion

Assuming that searches by flying Culicoides are not organized but random, except when actually responding to stimuli, it would be expected that the numbers caught at any of the landing sites or cylindrical sticky trap stations are directly dependent on the flight range of the species and hence on the proximity of that site or station to the breeding or resting sites.

The numbers of Culicoides females landing on the bait at the different sites and those of both sexes caught by the sticky traps at the different catching stations were subjected to logarithmic transformation (Williams, 1947) and summarized in Tables 11a,b:

Distance From Breeding Sites (yds)	<u>C. impunctatus</u>			<u>C. obsoletus</u>			<u>C. pallidicornis</u>		
	Arith. Total	Sum log. (n+1)	Mean log. (n+1)	Arith. Mean	Sum log. (n+1)	Mean log. (n+1)	Arith. mean	Sum log (n+1)	Mean log. (n+1)
20	387	25.82	1.29	151	18.33	0.92	73	13.20	0.66
60	363	25.28	1.26	141	17.83	0.82	81	13.65	0.68
100	190	19.23	0.96	107	15.55	0.78	54	10.92	0.55
140	241	20.09	1.10	88	14.45	0.72	21	5.82	0.29
180	223	21.48	1.07	66	12.32	0.62	13	3.54	0.18
220	146	18.17	0.91	56	11.31	0.57	8	2.40	0.12
260	73	12.32	0.62	45	10.00	0.50	5	1.50	0.08

Table 11a. Arithmetic totals, sum log (n+1) and mean log (n+1) of C. impunctatus, C. obsoletus and C. pallidicornis females in relation to distances from the breeding sites in Lake Wood and Cascade Marsh. Landing-rate Method, 1970

Distance From Breeding Sites (yds)	Females			Males		
	Arith. Total	Sum log. (n+1)	Mean log. (n+1)	Arith. Total	Sum log. (n+1)	Mean log. (n+1)
20	46	5.04	0.86	49	5.54	0.92
60	41	4.78	0.80	46	5.50	0.92
100	29	4.10	0.68	34	3.89	0.65
140	29	3.96	0.66	24	3.88	0.65
180	20	3.60	0.60	30	4.54	0.76

Table 11b. Arithmetic totals, sum log (n+1) and mean log (n+1) of female and male Culicoides in relation to distance from breeding sites in Lake Wood and Cascade Marsh. Cylindrical sticky trap method, 1970.

The transformed data of numbers landing were also used in the analysis of variance calculations to show if differences between the numbers taken at the different sites were significant. The variance was divided into three components; that due to differences in the distance of the sites from the breeding area, that due to differences between sampling occasions and the residual or experimental error. The analysis of variance for C. impunctatus, C. obsoletus and C. pallidicornis are presented in Tables 12a, b and c.

The number of C. pulicaris landing on the bait was very small for statistical analysis. The differences between the sites are highly significant ($P < 0.001$) for all the species considered separately. The difference between the sampling occasions was unexpectedly insignificant for C. impunctatus but significant for C. obsoletus ($P < 0.001$) and C. pallidicornis ($0.05 < P > 0.01$).

a). C. impunctatus

Source	df.	S.S.	M.S.	F.
Sampling occasions	19	0.6857	0.0361	1.5973
Sites	6	6.9792	1.1632	51.4690 ***
Residual	114	2.5720	0.0226	
Total	139	10.2369		

b). C. obsoletus

Source	df.	S.S.	M.S.	F.
Sampling occasions	19	1.1070	0.0583	7.2875
Sites	6	3.1693	0.5283	66.0375 ***
Residual	114	0.9123	0.0080	
Total	139	5.1886		

c). C. pallidicornis

Source	df.	S.S.	M.S.	F.
Sampling occasions	19	0.9202	0.0484	3.3151
Sites	6	8.2086	1.3681	93.7059 ***
Residual	114	1.6936	0.0146	
Total	139	10.8224		

Table 12. Analysis of variance

FIG.20 The effect of distance from breeding site on the fall-off
in numbers of flying Culicoides species

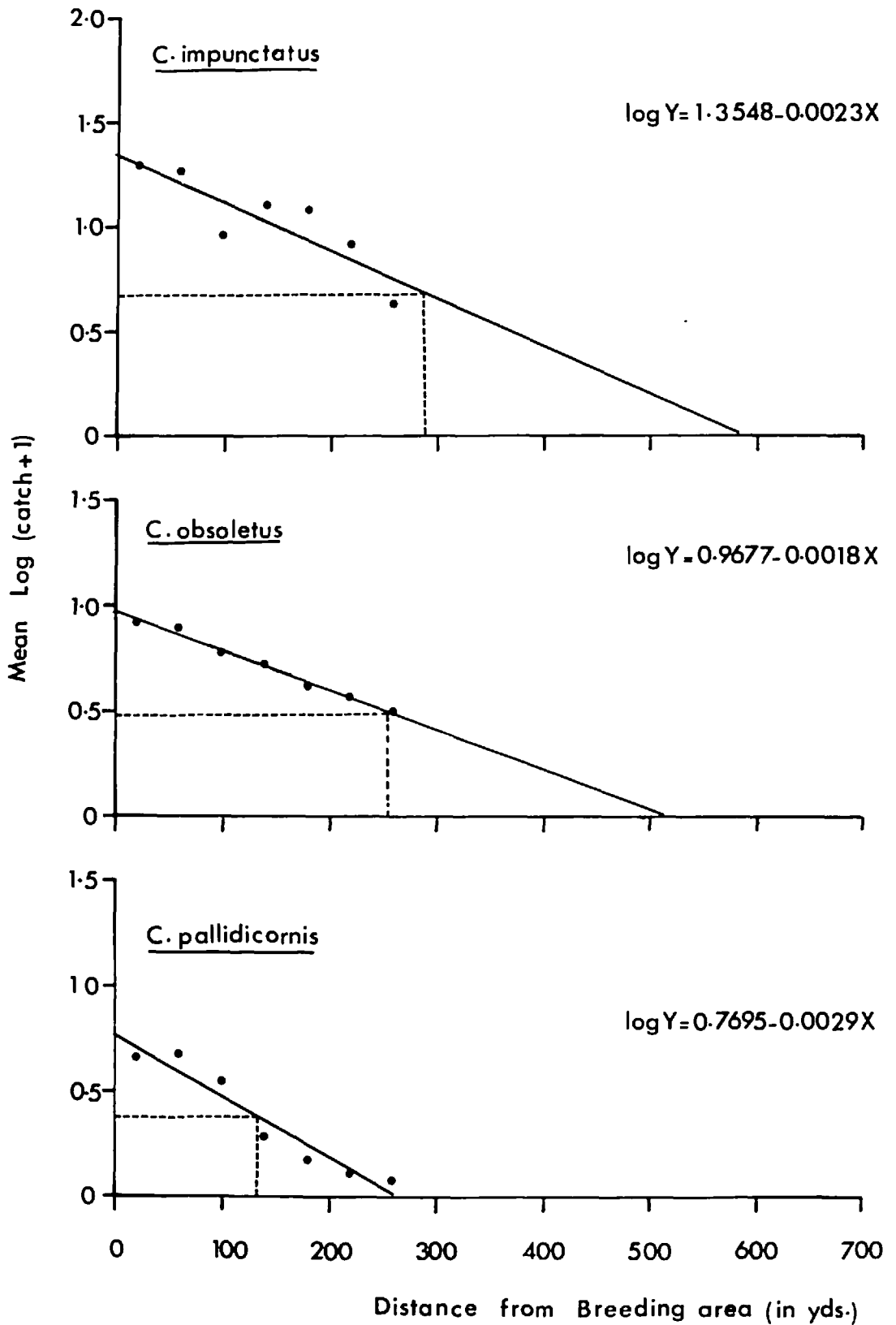
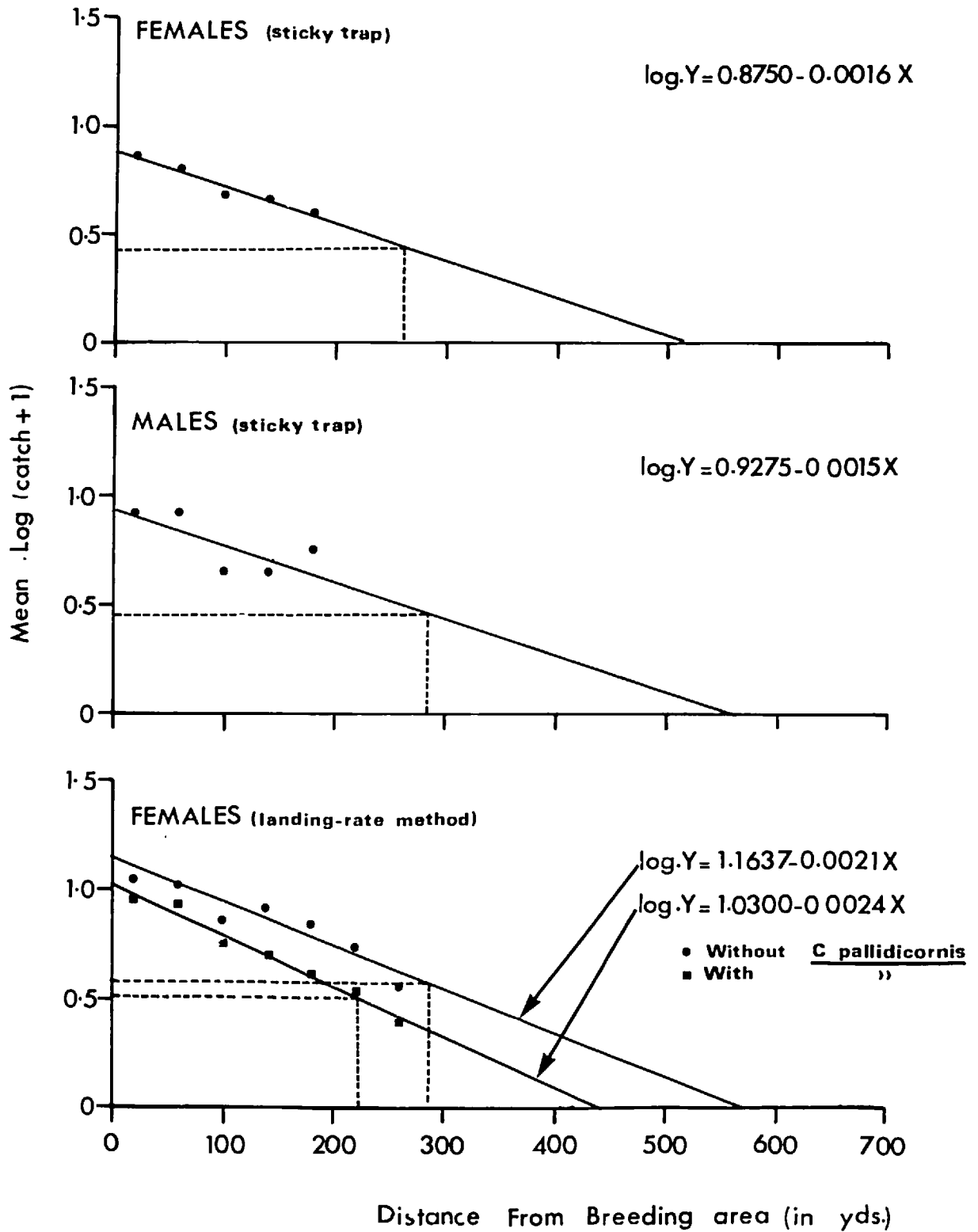


FIG.21 The effect of distance from breeding site on the fall-off
in numbers of flying Culicoides.



Both the landing-rate and sticky trap data were further tested by calculation of regression equations. Southwood (1966) listed five such formulae previously proposed by various authors for the description of fall-off in numbers of dispersing insect populations with distance from the release point or breeding sites. Kettle's (1951) expression of density-distance relationship of C. imunctatus was adopted because it gave the best fit.

$\log y = \log a + bx$; where $y = \text{Culicoides density at a distance } x$ from the breeding site, $a = \text{density at the breeding centre}$ and $b = \text{regression coefficient}$. Since the numbers of aerial Culicoides decrease with increase in distance from the dispersion source, the regression coefficient is always negative. Graphical representations of the effect of distance on the dispersion of females of three species (landing-rate method) and of males and females of all species (landing-rate and sticky trap methods) are given in Figures 20a, b and c, and 21a, b and c, respectively with their corresponding regression expressions.

As shown in Fig. 20, the distances at which the numbers of flying female C. impunctatus, C. obsoletus and C. pallidicornis fell to 50% of their original sizes at the breeding sites were 290 yards (266 m), 250 yards (229 m) and 130 yards (119 m) respectively, away from Lake Wood. The low value for C. pallidicornis is possibly due to the low catches. By extrapolating the regression lines, it appears that the above species are capable of flying distances of up to 590 yards (541 m), 525 yards (482 m) and 265 yards (243 m) respectively away from their breeding sites. By the sticky trap method, the aerial numbers of females and males were reduced to 50% of their original sizes at 260 yards (239 m) and 290 yards (266 m) respectively from Lake Wood and by extrapolating the regression lines, can possibly travel 520 yards (477 m) and 550 yards (505 m) respectively.

For comparison with results obtained by sticky traps (Fig. 21a), the landing-rate data for females were summed, with and without C. pallidicornis, and the regression coefficients calculated (Fig. 21c). The combined numbers of the three species decreased by 50% at 220 yards (202 m) from Lake Wood but with C. pallidicornis excluded, this value was 290 yards (266 m). The likely flight range for C. impunctatus and C. obsoletus combined was 560 yards (514 m) and almost similar to that obtained for all species by the sticky trap method. Thus, the estimates obtained for C. pallidicornis are considered unreliable, and since only three out of a total of twelve species, recorded from suction trap catches at Rush Meadow, landed in considerable numbers on the bait, the others are almost certainly non-human biters.

5.5. The different dispersal of sexes

Dispersion of both sexes of Culicoides from their breeding sites is vertical as well as horizontal.

Figure 18 shows a differential activity in both sexes in relation to height above ground level. The sex-ratios at 4 feet (1.2 m), 28 feet (8.5 m) and 40 feet (12.2 m) in 1968, 1969 and 1970 seasons are given in Table 13. The percentage of males in the aerial density at each height for the various species were calculated from the combined estimates of the three seasons (Table 14). Estimates of the aerial densities were used in all the calculations. Although the densities of both sexes tend to decrease with increasing height (Fig. 18) in accordance with Johnson's (1957) hypothesis, the ratio of males to females of the same species seem to increase with increasing height (Fig. 22).

C. stigma males were not taken at four feet (1.2 m) but the proportion of males increased very significantly with successive heights. The proportion of males in the aerial populations of C. impunctatus and C. pulicaris were similarly distributed with increasing height and showed the highest proportion of males at 40 feet (12.2 m). No C. impunctatus occurred at 28 feet (8.5 m) and the proportion of C. pulicaris males was lowest at this height but not significantly different from that at 4 feet (1.2 m). In C. obsoletus and C. pallidicornis the sex-ratios were relatively stable although males of the latter indicated slight increases with successive heights while C. obsoletus showed the highest proportion of males at 28 feet (8.5 m). Both species, however, showed no significant differences in their sex-ratios between the various heights. C. odibilis males did not occur in catches above 4 feet (1.2 m).

Despite variations between years, these results indicate that Culicoides are capable of flying at great heights with the proportion of males of the various species also increasing with height above ground

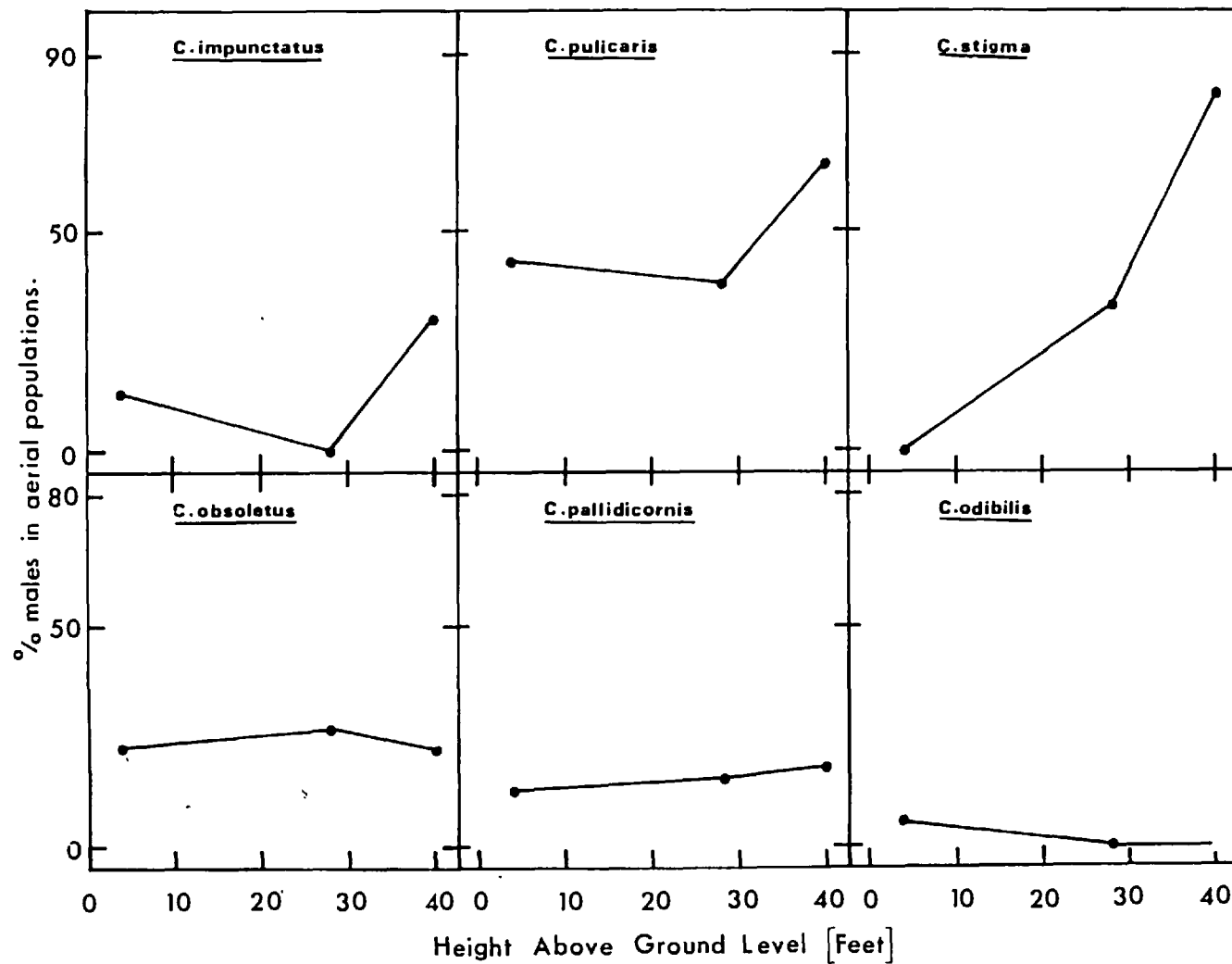
<u>Culicoides</u> spp.	1968			1969			1970		
	TRAP II 1.2 m	TRAP IV 8.5 m	TRAP V 12.2 m	TRAP II 1.2 m	TRAP IV 8.5 m	TRAP V 12.2 m	TRAP II 1.2 m	TRAP IV 8.5 m	TRAP V 12.2 m
	F : M	F : M	F : M	F : M	F : M	F : M	F : M	F : M	F : M
<u>C. impunctatus</u>	13.16:1	-	-	4.16:1	-	-	8.07:1	-	1:1
<u>C. obsoletus</u>	2.11:1	2.20:1	5.40:1	4.86:1	3.11:1	4.12:1	3.64:1	2.94:1	1.8:1
<u>C. pulicaris</u>	1.27:1	3.76:1	1.06:1	1.40:1	1.48:1	0.78:1	1.29:1	1.18:1	0.33:1
<u>C. pallidicornis</u>	19.38:1	4.56:1	--	11.88:1	5.53:1	7.55:1	5.31:1	6.02:1	2.56:1
<u>C. odibilis</u>	--	--	--	9.12:1	--	--	--	--	--
<u>C. stigma</u>	--	4.00:1	9.38:1	--	--	2.65:1	--	--	0.17:1

Table 13. Sex-ratio of Culicoides species caught by suction traps II, IV and V at 4 feet (1.2 m), 28 feet (8.5 m) and 40 feet (12.2 m) above the ground.

<u>Culicoides</u> species	TRAP II 1.2 m	TRAP IV 8.5 m	TRAP V 12.2 m
<u>C. impunctatus</u>	13.18	0	30.00
<u>C. obsoletus</u>	23.43	26.88	22.59
<u>C. pulicaris</u>	43.00	38.13	64.87
<u>C. pallidicornis</u>	10.30	15.72	17.47
<u>C. odibilis</u>	5.83	0	0
<u>C. stigma</u>	0	33.33	80.96

Table 14. Males of the various Culicoides species as percentages of the total aerial density of each species at the different heights.

FIG 22 Sex-ratio expressed as percentage of males in aerial density of Culicoides species at different heights. Data for 1968,1969,1970.



level. MacCreary (1941), using light-suction traps to sample aerial populations of various mosquito species near a salt marsh, found that 92% of Aedes vexans at 103 feet (32 m) above the ground were males compared with only 24% at 4-5 feet. Downes (1955) observed columns of $\frac{1}{2}$ -8 feet (0.2 m - 2.4 m) above the ground of Culicoides male swarms over markers which included dark mud patches, fresh wet cow dung or induced by laying a dark piece of cloth on the ground. Each swarm consisted of one species and he suggested that swarming functions as a means of bringing dispersed males and females into close proximity for mating purposes. Formation of swarms was at wind speeds below 2-3 m.p.h. It is doubtful if suction traps are suitable as markers and the average wind speeds at 8.5 m and 12.2 m are much higher than the optimum required for swarm formation. Thus it may be suggested that Culicoides individuals occurring in the aerial insect populations at these heights are probably migratory forms.

At lower heights, however, both male and female Culicoides are more evenly distributed (Fig. 19), as shown from sticky trap catches at Rush Meadow. The sex-ratios, expressed as percentages of males in the populations at the various heights are compared with those calculated from Kettle's (1951) data, obtained by sticky traps over morrland in Scotland, in the following table:-

	Height above ground		
	2 feet	6 feet	10 feet
	(0.6 m)	(1.8 m)	(3.0 m)
a. Rush Meadow, 1969	56.7	48.6	53.5
b. " " 1970	45.4	48.3	52.1
Kettle (1951) on <u>C. impunctatus</u>			
a. Wester Bannachra Moorland	49.2	47.3	53.7
b. Bannachra "	48.8	50.6	50.9

Both investigations show a general tendency for slightly higher proportion of males with increasing height. Reuben (1963), using suction traps captured slightly greater numbers of C. impunctatus females at 6 feet (1.8m) than at 3 feet (0.9 m) and significantly less at 6 inches (0.2 m) above ground level.

5.6. Discussion of results

In considering the dispersion of adult Culicoides, particular attention is paid to the females because they bite and suck blood and in certain cases transmit disease organisms. Their dispersal, therefore, has a significant bearing on the cost and planning of control programmes.

The determination of migrant and non-migrant females of an insect population has been related to the rate of development of the ovaries and flight apparatus; the flight muscles developing faster than the ovaries in the migrants and vice versa in non-migrants (Johnson, 1969). Southwood (1962) has also distinguished between migratory and trivial flight movements. Since no gorged females were netted or taken in suction traps both at Silwood Bottom and Rush Meadow, it can be assumed that individuals alighting on the bait are hungry and ready to bite. Reuben (1963) trapped large numbers of unfed and fertilized C. impunctatus females with immature ovaries. It can thus be said that aerial Culicoides populations are composed of both flitting and migrating individuals. The flitting individuals consist of females which have newly emerged or have oviposited and searching for another blood-meal or failed in previous attempts to obtain a blood-meal and have returned to search again. The males belonging to this group form swarms (Downes, 1955) which attract females for mating. Attempts to find what males feed on were unsuccessful.

The height-density profile of both sexes of Culicoides as determined from suction trap catches at 1.2 m, 8.5 m and 12.2 m above ground level at Silwood Bottom was consistent with Johnson's (1957) hypothesis, i.e., aerial numbers diminished with increasing height. Glick (1939) and Breeland & Smith (1962) have respectively captured Culicoides at heights of 13,000 feet (3975 m) and 90 feet (28.0 m), thus indicating that Culicoides can fly at great heights. Culicoides are minute and individuals flying at heights greater than 8.5 m may be

air-borne in strong air currents. This suggests they could have taken off at wind speeds stronger than the optimum required for normal flitting movements. Pratt et al (1949) observed that wind speeds above 3.5 m.p.h. inhibited flights of C. tristriatulus. Thus, individuals at these heights are probably true migrants and may include flitting individuals inadvertently caught in strong wind currents. The latter are more likely to fly nearer ground level where chances of encountering visual and/or olfactory stimuli from suitable hosts are greater.

Williams (1955) observed that certain Culicoides species prefer to fly at heights of 40 feet (12.2 m) and 25 feet (7.6 m) and in the present study, C. impunctatus and C. odibilis are comparatively scarce at 12.2 m while C. stigma males are more common at this height and did not occur at 1.2 m. Kettle (1951) suggested that in oak woodland, where the leaf canopy is about 20 feet (6.1 m) above the ground, Culicoides had a self-imposed ceiling of about 10 feet (3.05 m). There were no traps above 10 feet. Snow (1955) also described the upward movements of C. paraensis and C. spinosus on trunks and spreading into the leaf canopy during the day with a reverse movement in the evening. The above authors thus seem to indicate that, unlike open surroundings, the leaf canopy in woodland areas tend to restrict the height at which Culicoides fly. Both Bidlingmayer (1961) and Kettle (1951) thought that fewer males are taken by various sampling methods because they prefer to rest several feet above the ground. As observed here, this also implies that they prefer to fly at higher levels. The reason for this is not known but it is probably a significant factor in their feeding behaviour and may also be important in their dispersion.

The breeding areas of Culicoides at Silwood Park are at least 300 yards (274 m) from the suction traps operated at Silwood Bottom and

catches by trap II at 4 feet (1.2 m) give an indication on how far both sexes can fly. The catches may include flitting as well as dispersing individuals since the 'homing mechanism' of Culicoides, particularly at such distances from the breeding area, is not known. Estimates obtained by extrapolating the regressions of numbers alighting on a bait on distance from breeding sites, indicate that C. impunctatus and C. obsoletus have a probable flight-range of 590 yards (541 m) and 525 yards (482 m) respectively, the value for C. pallidicornis being unreliable because of low catches and the bait was apparently unattractive to C. pulicaris. Trap II also captured both sexes of all four species in substantial numbers. The indication is therefore given that Culicoides are capable of travelling considerable distances when flitting or randomly searching for hosts. The average distance flown by C. impunctatus under woodland conditions is 80 yards (73.4 m) (Kettle, 1951) but in open cattle grazing areas the species might travel up to $\frac{3}{4}$ of a mile or more depending on host availability (Kettle, 1960). Whitehead (1935; quoted in Johnson, 1969) also reported the annoyance caused to cattle by C. variipennis two miles from their breeding sites and Bidlingmayer (1961) netted C. furens females 1530 yards (1404 m) from their breeding sites. Hill (1947) thought that passive transport by wind was the only means by which Culicoides travel distances greater than 300 yards (274 m) but Breeland and Smith (1962) and Williams (1962) concluded that C. furens are carried by wind for distances of between 2-4 miles.

6. Diurnal Periodicity of Flight

6.1. Introduction

The daily biting cycle of C. impunctatus (Kettle, 1957; Service, 1969) and of C. obsoletus (Service, 1969) have been studied previously by landing-rate method. Reuben (1963) used a 9 inch Vent-Axia suction trap to sample flying populations of C. impunctatus throughout the day while Parker (1949) determined the diurnal distributions of various Culicoides species by a standard sweep net method. The above authors, excepting Service, worked in Scotland.

Three methods were used in this study; suction trap, landing-rate and sweep net. Suction trap catches are independent of the physiological responses of the insects and hence a more reliable estimator of population densities. The landing-rate method samples only hungry females and is therefore selective while the presence of the operator influences sweep net catches.

6.2. Methods of study

6.2a. Suction trap.

A 9 inch Vent Axia suction trap, with a built in mechanical device which segregates catches automatically into hourly samples (Johnson, 1950; Taylor, 1951), was used for collecting actively flying Culicoides species throughout 24 hour periods. It has an air delivery capacity of 16620 cu. ft. of air per hour. Detailed illustrated descriptions of the trap have been given by the above authors and by Southwood (1966). Gate jaws, which release a metal disc every hour to retain in the collecting tube insects caught in that hour, are operated by a time switch. The collecting tube, containing 24 one hour samples, was replaced at 14.00 hours daily. Insects contained in each compartment between two discs, which constitute an hour's catch, were killed by a 1% solution of pyrethrum in petrol ether previously applied on the muslin

attached to the under surface of each disc and allowed to dry.

The trap was operated from June to August, 1969 and from May to September, 1970. It was located in the south-western corner of Rush Meadow, 10 yards (9.2 m) from both Lake Wood and Great Mead Woodlands.

6.2b. Landing-rate and sweeping methods

In June and August, 1969, hourly collections from aerial Culicoides populations were made by landing-rate and sweep net methods in the Cascade Marsh. There were three sets of collections. Catching commenced at 12.00 hours on one day, continued through the night and ended at the same hour the next day.

The following procedure was adopted:-

Landing rate counts were made on the exposed legs of the sitting bait for 5 minutes, beginning at 10 minutes past each hour. Site L.R.1 was used. The bait then left the vicinity for 15 minutes, then returned to sweep the air at a height of approximately 5 feet (1.6 m) above the ground along Transect 1 (See 4.1a, b). Each sweeping occasion commenced at 30 minutes past each hour and consisted of 200 semi-circular strokes. The operator tried to maintain an even pace during this operation. Thus, both collecting techniques were used during each hour.

In the dark, however, landing-rate counts were made when the bait felt the pain of a biting insect. He then flashed his torch to check and collect if it was a midge. Sweeping operations were done normally as during the day but the net was emptied and the Culicoides counted in the laboratory.

6.3. Results and Discussion

Estimates of the hourly density per 10^6 cu. ft. of air for females of C. impunctatus, C. obsoletus, C. pulicaris and C. pallidicornis captured in sufficient numbers by the suction trap, were found as follows:-

The total monthly catch during each hour of the day was divided by the number of trapping days during that month and the value obtained was then multiplied by the anti-log of the corresponding conversion factor (Taylor, 1962a). The results for 1969 and 1970 seasons are presented in Figures 23 and 24. Each species showed a distinct single peak at dusk which is closely related to the sunset hour. The daily biting rhythm and flight activity of the above species, found respectively by landing-rate and sweeping methods at Cascade Marsh, were similar and bimodally distributed with peaks at dusk and in the morning, shortly after sunrise. (Fig. 25). The former peak was larger. Following the classification of distribution curves by Taylor and Lewis (1965), the peaks obtained by the three methods can be described as normally distributed, i.e., there is no evidence of the distributions skewing towards or away from maximum light. The indications are that the numbers of flying Culicoides increase and fall sharply or evenly during dusk. Intermittent flights, however, occur at different times of the day outside the period of peak activity (Figs. 23 and 24) but no females were taken on the bait from after midnight until at least an hour after sunrise although a few night-fliers were taken in sweep net catches during this period (Fig. 25). The sweep-net operator and the bait were one and the same person and his presence influenced the sweep-net catches. Both methods attracted only hungry females of the aerial population. Consequently, the similarity of the diurnal distributions and size of the catches by the two methods may be attributable to these factors. Only four males were taken in the sweep-net catches.

FIG.23 Diurnal flight periodicity of various Culicoides species. 2-in.Vent Axia suction trap.

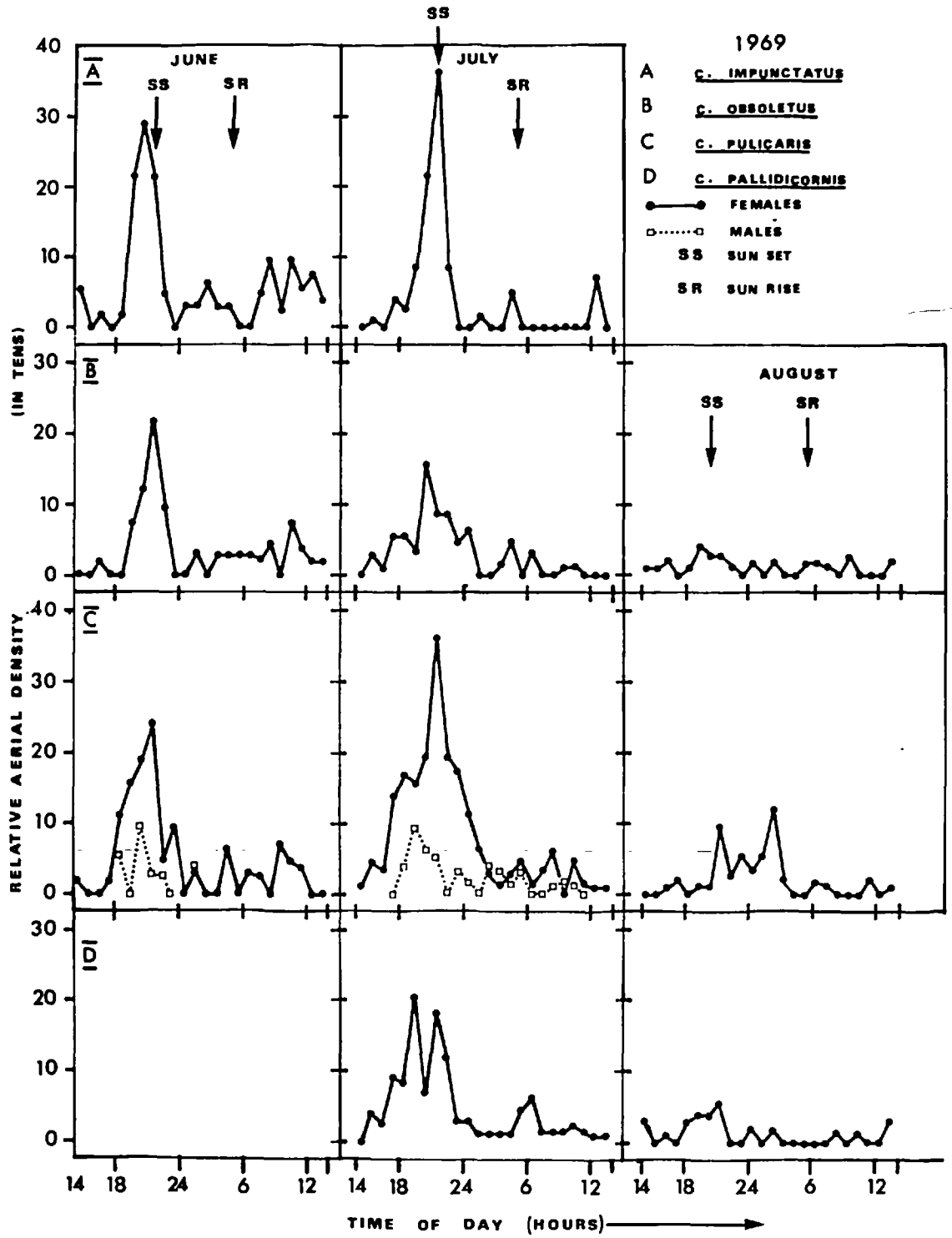
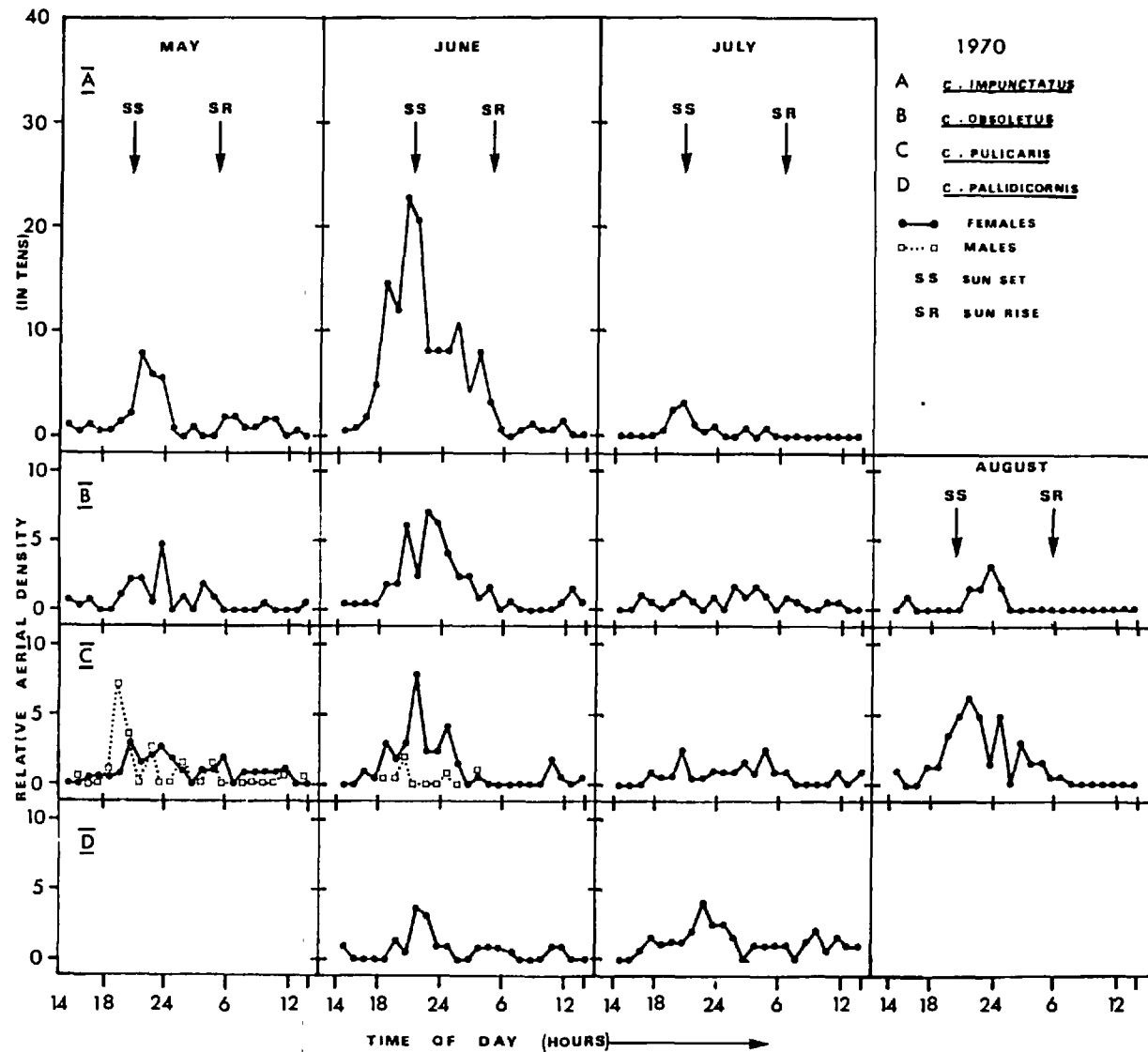


FIG. 24 Diurnal flight periodicity of various Culicoides species. 2in.Vent Axia Suction Trap.



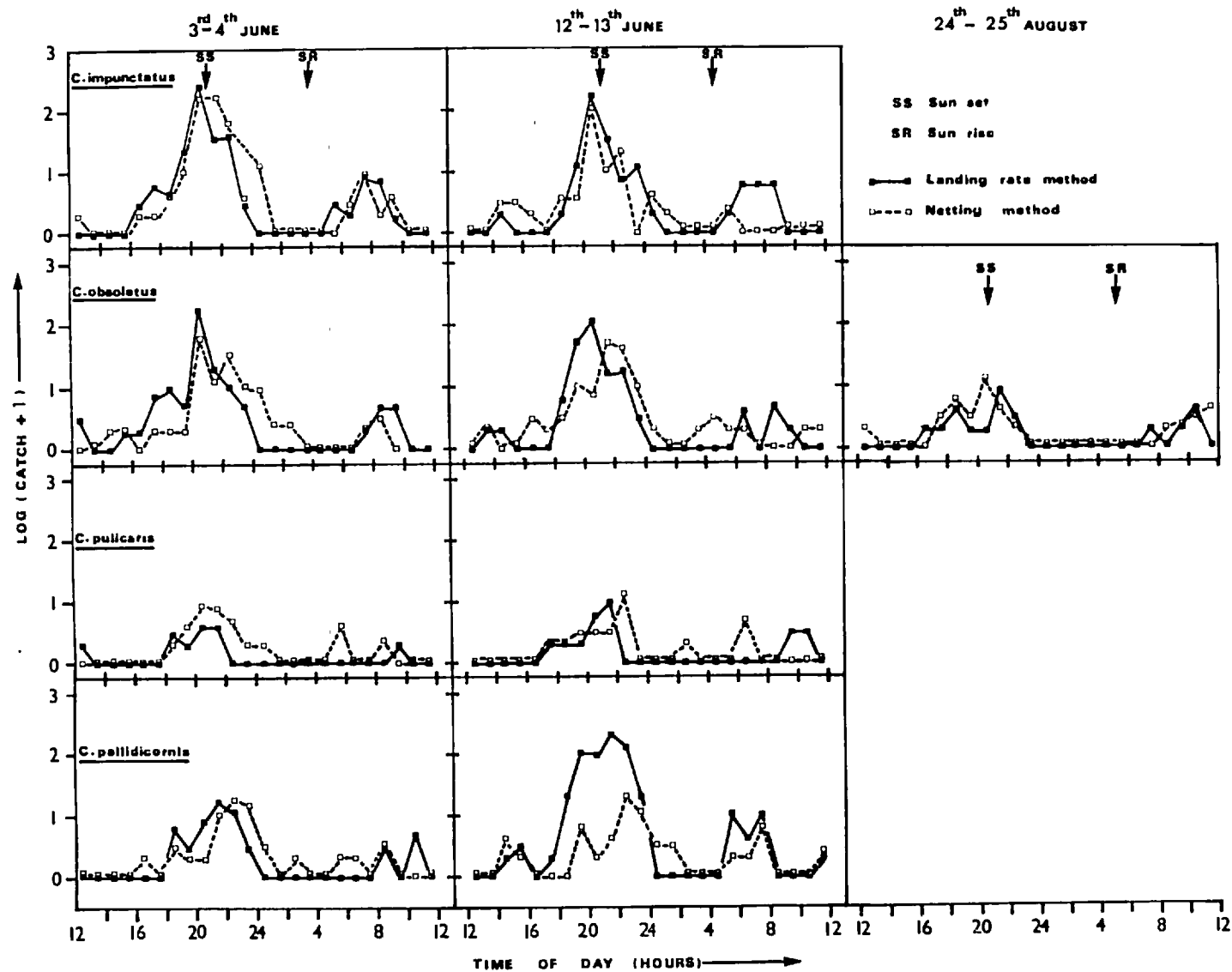


FIG.25 Diurnal periodicity of *Culicoides* species. Cascade Marsh; Landing rate and netting method. 1969

For this investigation, it was thought convenient to describe the duration of peak flight as the number of hours when the aerial numbers of a species exceeded the average per hour for that species that day. The peak flight durations are given in Table 15a and b.

		1969		1970	
a.	Months	No. of Hours		No. of Hours	
		Before Sunset	After Sunset	Before Sunset	After Sunset
<u>C. impunctatus</u>	May	-	-	2	3
	June	2	1	3	5
	July	2	2	3	3
	Aug.	-	-	-	-
<u>C. obsoletus</u>	May	-	-	2	3
	June	2	2	3	8
	July	4	4	-	-
	Aug.	2	3	0	4
<u>C. pulicaris</u>	May	-	-	5	8
	June	3	3	3	5
	July	4	4	0	0
	Aug.	0	6	3	9
<u>C. pallidicornis</u>	May	-	-	-	-
	June	4	2	2	4
	July	2	2	4	6
	Aug.	-	-	-	-

		3/4 June		12/13 June		24/25 Aug.	
b.		Before Sunset	After Sunset	Before Sunset	After Sunset	Before Sunset	After Sunset
<u>C. impunctatus:</u>	Landing	5	3	3	4	-	-
	Sweeping	3	4	3	3	-	-
<u>C. obsoletus:</u>	Landing	4	3	3	3	5	2
	Sweeping	1	6	3	3	4	2
<u>C. pulicaris:</u>	Landing	3	4	4	1	-	-
	Sweeping	3	1	4	2	-	-
<u>C. pallidicornis:</u>	Landing	3	3	3	3	-	-
	Sweeping	3	4	2	5	-	-

Table 15. The number of hours of peak activity of various Culicoides species taken by a. Suction trap and b. Landing-rate and sweeping methods. (- = no data).

Excepting C. obsoletus in May and August 1970 (suction trap), C. pallidicornis on 3rd - 4th and 12th - 13th June 1969 and C. pulicaris on 24th - 25th August 1969 (sweeping), when peak activity was about 2-3 hours after sunset, all catches (Figs 23, 24 and 25) indicate peak flight at sunset hour or an hour before or after. Parker (1949) observed earlier flights on darker than on lighter evenings, which suggests that the amount of cloud cover at this time might affect the duration of maximum flight. The mean duration of peak flight, as obtained from Table 15, are as follows:-

	Suction Trap		Landing Rate		Sweeping	
	Before Sunset	After Sunset	Before Sunset	After Sunset	Before Sunset	After Sunset
<u>C. impunctatus</u>	2.4	2.8	4.0	3.5	3.0	3.5
<u>C. obsoletus</u>	2.2	4.0	4.0	2.7	2.7	3.7
<u>C. pulicaris</u>	3.0	5.8	3.5	2.5	3.5	1.5
<u>C. pallidicornis</u>	3.0	3.5	3.0	3.0	2.5	4.5

Few males of all species were taken in the suction trap. C. pulicaris males were comparatively more common in catches in June and July 1969 and May and June 1970, (Figs 23 and 24). Like the females, the males were more active around sunset but the peak activity of the latter occurred an hour (June 1969, May and June 1970) or two hours (July 1969) before sunset, i.e. earlier than the peak activity of the females during the same periods. Parker (1949) obtained similar results for C. pallidicornis. Since the number of males taken in catches are generally low, it is difficult to know whether this difference in peak activity between the sexes also occurs in other species.

The daily distribution of flight activity of various British Culicoides species have been described by Hill (1947), Parker (1949),

Kettle (1957) and Reuben (1963) in Scotland, and Service (1969a) in Southern England. These authors obtained well defined cycles similar to the results of this study except Reuben (1963) in Scotland who found a generalised increase in the evenings with a peak just before midnight for C. impunctatus. There is no published record of the periods of oviposition and emergence of British Culicoides but in Jamaica, C. furens and Leptoconops bequaerti have been reported to emerge between 07.00 and 11.00 hours (Linley 1966a, 1968c). The explanation put forward by Nielsen and Haeger (1954) for evening peak activity in Aedes taeniorhynchus and by Taylor (1957) for morning peak activity in aphids, may be applicable to Culicoides. Endogenous factors control synchronization of pupation and temperature determines the duration of pupal development and the time required by the emerging flies to be ready for flight. This results in a build up of flight-mature flies against dusk flights when the thresholds of the various exogenic (climatic) factors are reached. Endogenous factors may also be involved in this process. These factors may also be responsible for morning peaks. It is of interest to notice that morning peaks were not obtained from suction trap catches but from catches where a man was involved, either as a bait or the operator of a sweep net. Culicoides which are active during this period consist possibly of females which failed to obtain a blood meal the previous evening or oviposited during the night.

During June - August 1969 and May - August 1970, a shift in the time of peak activity of the different Culicoides species seem related to the changing times of sunset (Figs 23 and 24). A precise measurement of this relationship was not made as the time-switch of the 9 inch Vent Axia suction trap was set to be operational at hourly intervals. However, Taylor and Lewis (1965) using a similar trap described the linear relationship of the two changes from catches of ten insect species

(including C. obsoletus) and indicated that the time during which most of the insects were flying changed by an interval approximately equal to the change in the time of sunset.

7. Weather

7.1. The effects of weather factors on the total daily catch by suction trap.

The numbers of C. impunctatus, C. obsoletus, C. pulicaris and C. pallidicornis captured by suction trap II at Silwood Bottom vary between days. These are dependent not only on the population size of each species at the source of dispersion but also on the numbers which are actually flying. Weather factors as well as endogenous factors influence the latter. The distance of the trap from the breeding sites of the insects is also a contributory factor as Culicoides become fewer, both vertically and horizontally, with increasing distance from the source.

Williams (1940) considered catch as being proportional to activity and population size, and obtained the following relation between catch and the various weather factors which affect activity independently.

$$\text{Log catch} = (\text{log. act. Temp.} + \text{log. act. Wind} + \dots) + \text{log. Population.}$$

The use of multiple regression analysis in determining the role of the different factors on insect activity has the advantage of distinguishing between population size and activity and also indicates the influence which each factor exerts on the size of the catch. It also has a predictive value. In the analysis two assumptions are made: firstly, that no one factor acts to limit the effect of any of the others, i.e. all the weather factors act together to produce an effect comparable to that obtained by summing the effects of each of them and secondly, that a linear relationship exists between catch size and each of the weather variables as the non-linear relationship of a factor may produce statistically insignificant results (Hughes, 1955).

Both sexes were combined and the species treated separately. The nine variable local weather factors used (Table 16) were obtained from

VARIABLES	SUM	MEAN	STANDARD DEVIATION
1. Mean daily radiation (milliwatt-hours/cm ²)	2490.5	23.4953	9.4670
2. Mean radiation 3 hours before sunset (")	1233.7	11.6387	57.9068
3. Total daily sunshine hours	682.2	6.4358	3.9589
4. Mean daily temperature (T°C)	1473.9	13.9047	3.7155
5. Mean temperature 3 hours before sunset (T°C)	1684.4	15.8906	3.9649
6. Mean daily relative humidity (%)	8807.5	83.0896	10.3852
7. Mean relative humidity 3 hrs before sunset (%)	8283.0	78.1415	12.8095
8. Total daily rainfall (mm)	160.9	1.5179	4.4651
9. Mean daily wind speed (m.p.h.)	619.3	5.8425	3.1222
10. Numbers caught: <u>C. impunctatus</u> 10C ₁	333.0	3.1415	9.0158
<u>C. obsoletus</u> 10C ₂	1368.0	12.9057	18.4295
<u>C. pulicaris</u> 10C ₃	3777.0	35.6321	64.7857
<u>C. pallidicornis</u> 10C ₄	662.0	6.2453	11.8232

Table 16. Sum, Mean and Standard deviations of the variables.

Number of observations = 106

1	2	3	4	5	6	7	8	9	
-0.0481	0.7675	0.0014	0.2700	0.9201	0.8633	0.1387	0.02797	-0.0986	10C ₁
0.3911	-0.0214	0.2700	0.3366	0.2206	0.0944	0.0789	0.0905	0.0144	10C ₂
0.4414	-0.0214	0.3366	0.9201	0.2261	0.8633	0.0789	0.0905	0.0144	10C ₃
-0.2386	-0.0017	-0.2617	0.2206	0.2261	0.8633	0.0789	0.0905	0.0144	10C ₄
-0.3314	-0.0895	-0.3600	-0.1752	0.0944	0.8633	0.0789	0.0905	0.0144	
-0.0776	-0.0307	-0.0407	0.1190	0.0376	0.0789	0.1387	0.0905	0.0144	
0.0501	0.1398	0.0958	0.1054	-0.0069	0.1456	0.0905	0.02797	-0.0986	
0.3479	-0.0400	0.0911	0.1727	0.1382	-0.2299	-0.1733	0.0144	-0.0986	
0.0749	-0.0661	-0.1716	0.1022	0.1058	-0.0218	0.0156	-0.0637	-0.2729	
0.0221	-0.0236	-0.0451	-0.0082	0.0011	-0.0713	-0.0347	-0.0353	-0.2109	
0.3624	-0.3974	0.0993	0.3523	0.2936	-0.1993	-0.1283	-0.0383	-0.2037	

Table 17. Multiple correlation coefficients of variables.

	<u>C.</u> <u>impunctatus</u>	<u>C.</u> <u>obsoletus</u>	<u>C.</u> <u>pulicaris</u>	<u>C.</u> <u>callidicorn</u>
1. Multiple Regression Coefficient, R	0.5064	0.4545	0.2375	0.6120
2. R square (R^2)	0.2565	0.2066	0.0564	0.3745
3. % of numbers caught accounted for by the variables	25.65	20.66	5.64	37.45
4. F ratio for analysis of variance df. $n_1 = 6$ $n_2 = 96$	3.6790 ($P > 0.001$)	2.7774 ($P > 0.01$)	0.6378 N.S.	6.3872 ($P > 0.001$)

Table 18. Comparison of multiple regression coefficients obtained for the various Culicoides species

	a	bX_1	bX_2	bX_3	bX_4	bX_5	bX_6	bX_7	bX_8	bX_9
<u>C. impunctatus</u>	9.5649	+0.5989	+0.0024	-0.9764	+0.6113	-0.3917	-0.2580	+0.0803	+0.0662	-0.2510
<u>C. obsoletus</u>	16.1270	+0.8381	-0.0013	-2.3820	+0.6368	-0.0708	-0.1574	+0.0865	-0.2489	-1.4718
<u>C. pallidicornis</u>	10.9922	+0.6698	+0.0072	-1.1429	+2.0446	-1.0327	-0.3538	+0.1228	-0.1642	-0.8861

Table 19. Multiple regressions of numbers of different Culicoides species trapped on various meteorological factors.

b = individual regression coefficients; X = variables and a = intercept constant.

measurements at the Field Station; but wind speed data, available only at 30 feet (9.2. m) were taken from records in the Meteorological office, Bracknell. Because the daily catches extended from 10.00 hours on one day to 10.00 hours on the next, the meteorological variables were considered for the same period. 1969 and 1970 data were combined and put through the College computer (IBM7090) for analysis by Dr. G. Murdie.

Details of the multiple regression analyses of the numbers of the various species caught on the nine weather variables are given in Tables 16 and 17 and the results are summarized in Table 18.

The F-tests for analyses of variance on the various multiple regression coefficients are highly significant except for C. pulicaris where R^2 equals 0.0564. Table 19 shows the individual regression coefficients on the different variables. The major weather factors which appear to influence the total daily catches of Culicoides are heat (radiation and temperature), sunshine and wind speed. Regression on the daily mean values of the first two are positive and on the last two are negative. The total daily rainfall does not seem to affect the size of catch, but the relative humidity three hours before sunset has a small positive influence. The inhibitory effects of strong wind may be due to exposed Culicoides increasing their hold on the vegetation as Hughes (1955) observed on Meromyza variegata or shelter among the lower vegetation, between one and two feet above ground (Bidlingmayer, 1961) to avoid being blown off. Thus, from the results in Table 19, it can be suggested that maximum total daily Culicoides catches by suction trap II are expected on warm, cloudy but windless days as these conditions seem ideal for flitting flights from the breeding sites towards Silwood Bottom.

The analyses do not account for 74.4% (C. impunctatus), 79.3% (C. obsoletus) and 62.6% (C. pallidicornis) of the variability in numbers of the species trapped and this, it is thought, underlines the importance of

endogenous factors which control Culicoides flight activity.

7.2. The thresholds of three major weather factors for flight of Culicoides

The cumulative effects of various weather factors during each hour on Culicoides activity are reflected in the total daily catch. The thresholds of these factors mark a change in the level of activity of an insect species and also determines the length of the period during which flight occurs. These thresholds are found by a method introduced by Taylor (1963). A catch of one or more individuals indicate activity and when there is no catch, activity is zero.

Culicoides have been shown to be crepuscular, with a high level of activity between dusk and dawn i.e. from 18.00 hours to 06.00 hours. The thresholds of three weather factors, radiation, temperature and humidity, were determined from 9 in. Vent-Axia suction trap hourly catches during this period. Data for all species were combined because catches of individual species were low. Tables 19, 20 and 21 show the data for radiation, temperature and relative humidity threshold distributions of Culicoides and these are plotted in Figures 26, 27 and 28.

Figure 26 shows that flight activity in relation to radiation falls into two distinct groups; higher activity corresponding to low radiation intensities from 0-0.8 milliwatt-hours/cm² and lower activity to higher radiation intensities from 0.9->6.1 milliwatt-hours/cm². The threshold for increased activity, therefore, is between 0.8 and 0.9 milliwatt-hours/cm², i.e. 0.85 milliwatt-hours/cm². Below this value of radiation intensity Culicoides flight activity is maximal but above it flight is inhibited.

Solar radiation is transmitted through the atmosphere to the earth's surface as a broad energy spectrum and consists of short waves (visible

FIG-26 The radiation threshold for Culicoides
(all species)

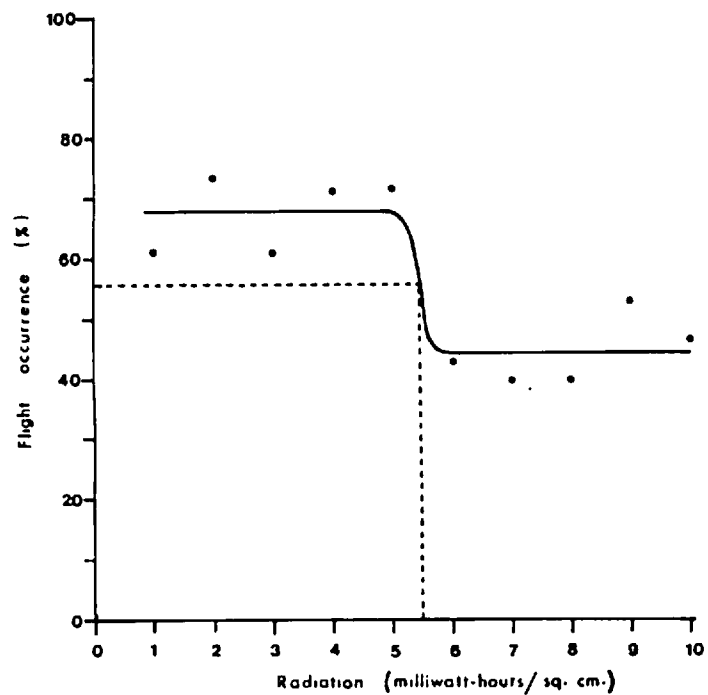


FIG-27 The upper and lower temperature thresholds
for Culicoides (all species)

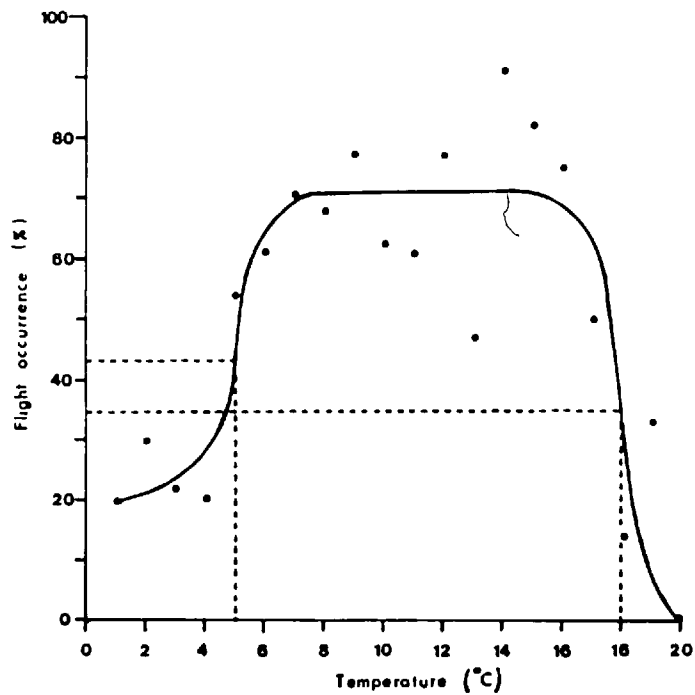
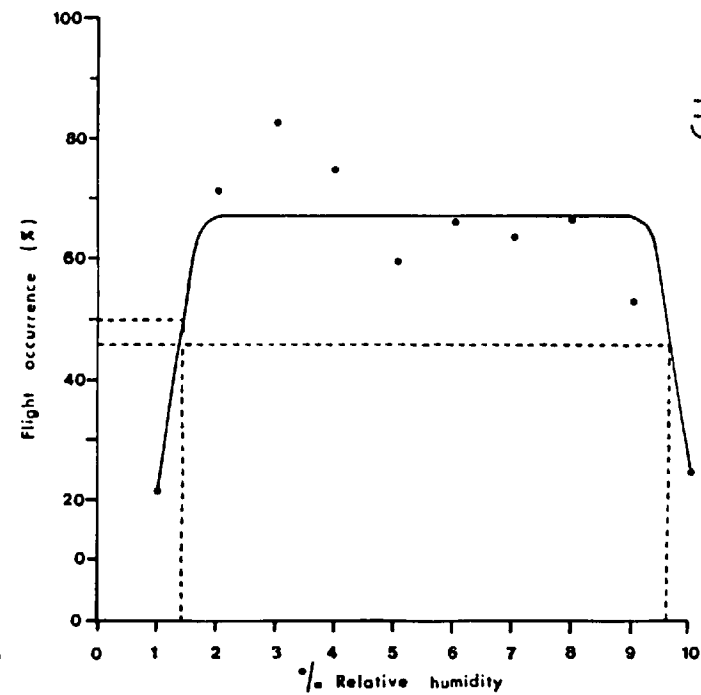


FIG-28 The upper and lower humidity thresholds for Culicoides
(all species)



Class No.	Class limits (milliwatts-hours per sq. cm.)	No. of occurrences	Frequency of catch	% Flight occurrence
1	0	204	125	61.3
2	0.1-0.2	34	25	73.5
3	0.3-0.4	18	11	61.1
4	0.5-0.6	7	5	71.4
5	0.7-0.8	7	5	71.4
6	0.9-1.0	7	3	42.9
7	1.1-2.0	15	6	40.0
8	2.1-4.0	30	12	40.0
9	4.1-6.0	19	10	52.6
10	>6.1	43	20	46.5
		384	222	

Table 20. Data for radiation threshold distribution

Class No.	Class limits (T°C)	No. of Occurrences	Frequency of catch	% Flight occurrence
1	6.1-7	5	1	20.0
2	7.1-8	10	3	30.0
3	8.1-9	23	5	21.7
4	9.1-10	15	3	20.0
5	10.1-11	28	15	53.6
6	11.1-12	28	17	60.7
7	12.1-13	34	24	70.6
8	13.1-14	50	34	68.0
9	14.1-15	40	31	77.5
10	15.1-16	32	20	62.5
11	16.1-17	31	19	61.3
12	17.1-18	22	17	77.3
13	18.1-19	15	7	46.7
14	19.1-20	11	10	90.9
15	20.1-21	11	9	81.8
16	21.1-22	4	3	75.0
17	22.1-23	4	2	50.0
18	23.1-24	7	1	14.3
19	24.1-25	3	1	33.3
20	>25.1	11	0	0
		384	222	

Table 21. Data for temperature threshold distribution

Class No.	Class limits (R.H.%)	No. of Occurrences	Frequency of catch	% Flight Occurrence
1	51-55	9	2	22.2
2	56-60	7	5	71.4
3	61-65	6	5	83.3
4	66-70	16	12	75.0
5	71-75	20	12	60.0
6	76-80	21	14	66.7
7	81-85	39	25	64.1
8	86-90	63	42	66.7
9	91-95	191	102	53.4
10	96-100	12	3	25.0
		384	222	

Table 22. Data for relative humidity threshold distribution

light and radiant heat) as distinct from long waves which are continually being absorbed and re-emitted by the earth's terrestrial atmosphere (Sutton, 1953; Brunt, 1948). Measurements of the former have been used above for determining the radiation threshold for adult Culicoides. Variable quantities, such as amount of cloud cover and particles in the atmosphere, result in fluctuations of radiation intensities between places and times. It is therefore difficult to relate radiation measurements to light intensities. However, from information obtained from the Meteorological Office, Bracknell, an approximate equivalent in terms of light intensity can be found by multiplying the former by a factor of 0.87. Thus, the radiation threshold value of 0.85 milliwatt-hours/cm² is related to a light intensity value of approximately 0.74 Kilolux-hours/cm². Under normal circumstances, both these estimates occur at dusk and dawn and possibly during very cloudy conditions. It is of interest to note that although higher radiation intensities have inhibitory effects on flight activity the latter is not entirely stopped.

Figure 27 shows that the maximum flight activity of Culicoides occur between a lower threshold of 11°C and an upper threshold of 24°C. This suggests that temperatures below 11°C are too cold for flight while those above 24°C are too hot and might possibly result in desiccation of the insects.

Figure 28 indicates that Culicoides generally prefer high relative humidities of between 56% and 95% for flight. No definite thresholds are distinguishable but the percentage flight occurrences at 51-56% (class 1) and 95-100% (class 10) are low. This seems to imply, therefore, that conditions in which the relative humidity is below 56% or about 100% are not ideal for flight, as the insects are exposed to desiccation in the former and air saturated with water vapour is possibly too wet.

It is noticeable that in the three figures referred to above, the points do not all lie on the respective curves. Although the different

weather factors, acting independently or by interactions between them and other physical factors, provide thresholds which allow intermittent flights at various hours of the day or mass flights at dusk and/or dawn, endogenously controlled behavioural responses appear to play an important role in the timing of flight activities. It is considered probable that the outburst of Culicoides activity at dusk is a momentary response to the approach and passing of the radiation threshold when the inhibitory effects of the solar beams are removed and the insects are released to execute their intrinsic flight behaviours. The duration of maximum flight after the initial outburst, is dependent on how long the ambient temperature remains between 11°C and 24°C and the relative humidity between 56% and 95%. Flight activity at dawn may also be explained in these terms. In the tropics where L. bequaerti is diurnal (Kettle and Linley, 1967b), the above explanation may be applicable in a reverse form. Thus, it can be further speculated that in Britain, and perhaps in some other parts of the world, weather factors do not initiate flights on their own but act as a release mechanism, allowing normal behavioural flights to commence and continue until the weather factors again become unfavourable.

It is, however, important to recognise that the role of wind may be a decisive factor in the activities of Culicoides. As obtained in this study with suction trap catches (see 7.1), Kettle (1957) and Reuben (1963) respectively found that the biting rate and suction trap catches of C. impunctatus were negatively related to wind speed. Travis (1949) observed that C. tristriatulus and C. yukonensis activities ceased at wind speeds above 3.5 m.p.h. and Kettle and Linley (1967b) found that although L. bequaerti remained active at 5 to 10 m.p.h., complete cessation occurred at 15 m.p.h. Kettle (1969b) also noted that C. furens and C. barbosai activities declined rapidly at wind speeds above 2 m.p.h.

and ceased totally at 6 m.p.h.

Parker (1949) and Reuben (1963) did not obtain a relationship between Culicoides activities and temperature. Kettle (1969b) suggested this could have been due to the small range of temperatures that occurred during observations. However, that there is a temperature range during which Culicoides fly unimpeded (Fig. 27) has also been shown by various authors such as Travis (1949) who found that below 55°F (13°C) the activities of C. tristriatulus and C. yukonensis declined rapidly and Minář (1962; quoted in Kettle, 1969b) obtained a range of 9-22°C (48-72°F) for maximum activity of C. pallidicornis and C. obsoletus. Kettle (1969b) also obtained a threshold of 68°F (20°C) for C. furens and C. barbosai in the tropics. A range of 11-24°C (52-75°F) for maximum flight of Culicoides of all species obtained in this study, compares favourably with the results above.

Parker (1949) reported earlier dusk activities on days with dull evenings and Kettle (1957) observed that in cloudy mornings the duration of dawn activity is longer. Reuben (1963) obtained a radiation threshold of 22.7 cal/cm²/hr. for C. impunctatus while Minář (1962, quoted in Kettle, 1969b) found light intensities of between 1,000 and 10,000 lux are preferred for activities by C. pallidicornis and C. obsoletus. Kettle (1969b) obtained negative regressions of numbers of C. furens and C. barbosai biting on log light intensities but found that the role of illumination ceased to be significant for C. barbosai, and played a minor role in the biting activity of C. furens, when other factors were included in the analysis. A solar radiation threshold of 0.85 milliwatt-hours/cm² was obtained for Culicoides (all species) in this study,

Although Reuben (1963) believes that humidity was a major factor which determines the timing of the peak activity of C. impunctatus, other workers such as Parker (1949) on various Culicoides species, Kettle and

Linley(1967b) on C. furens and C. barbosai and Minař (1962 in Kettle, 1969b) on C. pallidicornis and C. obsoletus showed that humidity and saturation deficit do not influence activity. Parker (1949) obtained maximum catches at relative humidities ranging from 60-95% which is comparable to a range of 56-95% obtained in this study.

8. Host Preference - Landing on Different Hosts

8.1. Introduction

Observations on the feeding behaviour of female Culicoides on wild hosts are impracticable in natural habitats. The use of man and other domestic animals as baits provide information on host preference which does not necessarily reflect their choice under natural conditions. The identification of Culicoides blood meals by precipitin tests have been reported for species in Canada (Downes, 1958), India (Das Gupta and Sen, 1958) and Israel (Braverman, Boreham and Calun, 1971. In press). Such reports only describe host range but do not indicate host preference.

Jones (1959) using caged animals found that C. variipennis sonorensis preferred cattle, sheep and rabbits and mice. Culicoides feeding on avian hosts have also been reported by Akiba (1960) and Messersmith (1965) in poultry.

Jones (1959) used caged mammals in the laboratory to determine host preferences of C. variipennis sonorensis. Similar experiments have been carried out by Bennett (1960) on 20 species of birds and Hair and Turner (1968) on 14 hosts including mammals, birds, reptiles and amphibians.

This section describes the investigation of the host preference of various Culicoides species for man and goats in the field.

8.2. Material and Method

The host preferences of various Culicoides species at Rush Meadow were determined by the relative attractiveness of a man (J.O.; black) and three tethered domestic goats which consisted of two white females (G.W.1 and G.W.2; the latter being the kid of the former) and a black female (G.B.).

In 1969, the numbers of the various Culicoides species alighting on man (J.O.) and G.W.1 were compared while in 1970, all four baits were used. On each catching occasion, between May and September, G.W.1, G.W.2 and G.B. were tethered to form an approximate triangle with the human-bait in the middle. The latter was also the collector and he wore dark clothes during these operations. Black hoods were pulled over the heads of the goats and this made them relatively motionless during the collection of Culicoides alighting to feed on their sides. Individuals alighting on the exposed legs of the human-bait were captured. Separate aspirators were used for collection from each bait.

The order in which collections were made from the baits was alternated between catching occasions. Four separate five minute catches were taken alternatively from each bait; giving a combined total of twenty minutes catch on each bait during each experimental occasion.

8.3. Result and Discussion

The following Culicoides species caught alighting on the baits were identified on examination of catches: C. impunctatus, C. obsoletus, C. pulicaris, C. pallidicornis, C. heliophilus, C. vexans, C. nubeculosus, C. chiopterus and C. odibilis. No C. nubeculosus was caught on any of the baits in 1969.

Since the human-bait and the catcher were the same person, any possible influence on the numbers landing on each animal-bait due to his proximity during catching, was corrected by subtracting the numbers of each species which landed on him from those caught on G.W.1., G.W.2. and G.B. on each experimental occasion. The relative attractiveness of each bait expressed as total numbers of various Culicoides species landing in 1969 and 1970 are summarized in Table 23 and Figure 29. The 1970 results do not include catches taken after 25th July when G.W.2. died as the result of an accident.

In 1969, eight Culicoides species were collected landing on G.W.1. as against six on the human-bait. Of the six species taken on both baits, significantly greater numbers occurred on G.W.1. This suggests that G.W.1. was comparatively more attractive to hungry female Culicoides than man. Since the human bait was black and the goat, G.W.1. white, the 1970 collections were intended to show whether preference for the latter in 1969 was a response to visual or olfactory stimuli. Of the four baits used in 1970, man (black), G.W.1. and G.W.2. (goats, white) and G.B. (goat, black), the black goat was significantly more attractive than the other baits to C. impunctatus, C. obsoletus, C. pulicaris, C. pallidicornis, C. heliophilus, C. chiopterus and C. odibilis. Although larger numbers of C. impunctatus, C. obsoletus, C. pulicaris, C. pallidicornis, C. heliophilus and C. vexans were taken from G.W.1. than from G.W.2. and more C. nubeculosus from G.W.2. than G.W.1., the

<u>Culicoides</u> spp.	Man (Black)		G.W.1		Goats G.W.2		G.B.	
	Total	%	Total	%	Total	%	Total	%
1. <u>C. impunctatus</u>	303	8.77	893	25.85	702	20.45	1552	44.9
	(234)	(24.07)	(738)	(75.93)				
2. <u>C. obsoletus</u>	191	10.36	359	19.48	270	14.65	1023	55.5
	(215)	(35.77)	(386)	(64.23)				
3. <u>C. pulicaris</u>	6	6.45	16	17.20	10	10.75	61	65.5
	(23)	(37.10)	(39)	(62.9)				
4. <u>C. pallidicornis</u>	10	2.58	98	25.26	59	15.21	221	56.9
	(33)	(18.23)	(148)	(81.77)				
5. <u>C. heliophilus</u>	1	6.67	3	20.00	2	13.33	9	60.0
	(0)	(0)	(13)	(100)				
6. <u>C. vexans</u>	1	5.00	8	40.00	6	30.00	5	25.0
	(1)	(5.56)	(17)	(94.44)				
7. <u>C. nubeculosus</u>	0	0	2	22.22	3	33.33	4	44.4
8. <u>C. chiopterus</u>	0	0	0	0	1	33.33	2	66.6
	(2)	(14.29)	(12)	(85.71)				
9. <u>C. odibilis</u>	0	0	0	0	1	16.67	5	83.6
	(0)	(0)	(3)	(100)				

Table 23. Total numbers and percentages of Culicoides species alighting on the various baits in 1970. 1969 results are in paranthesis.

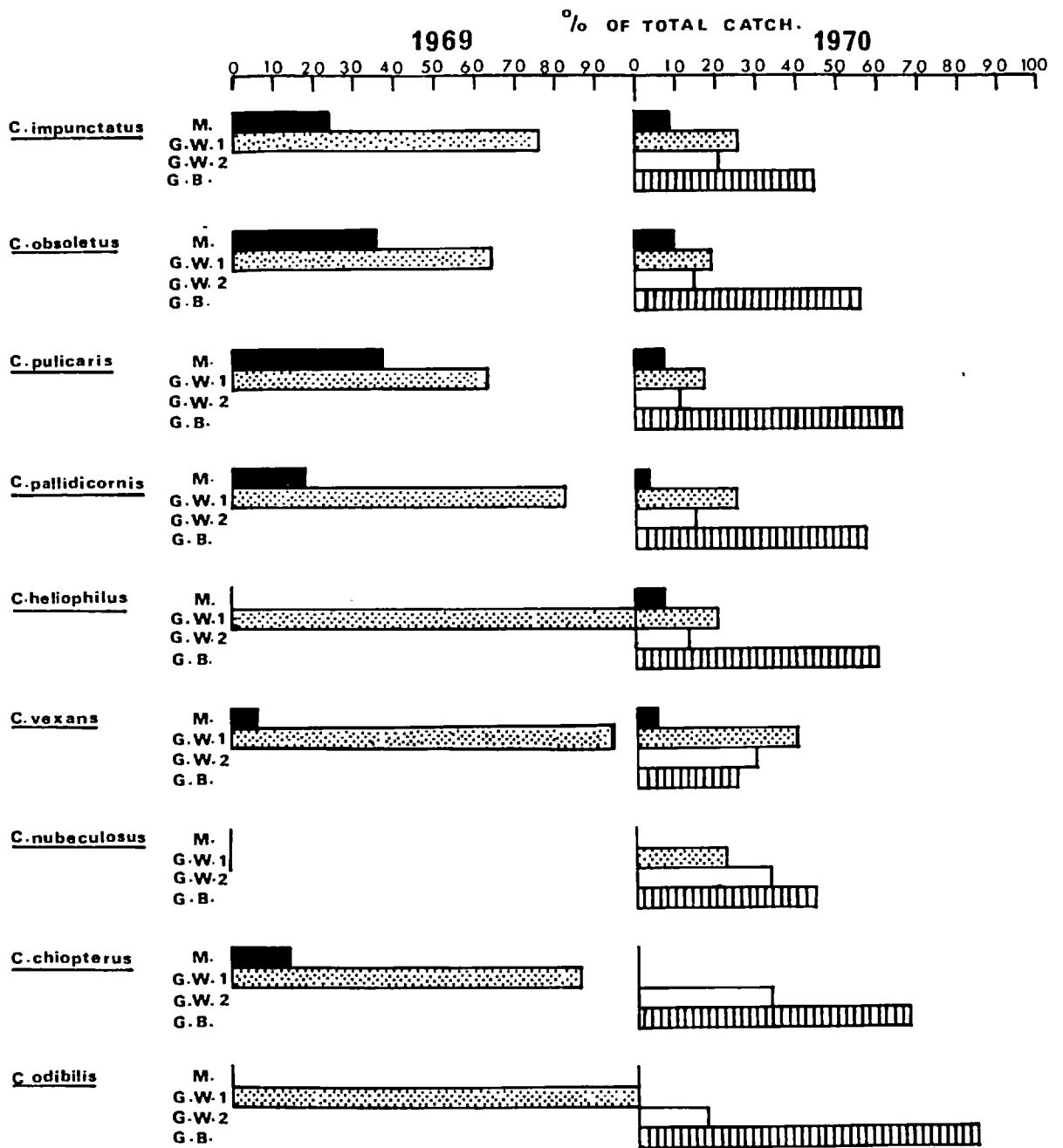


FIG 29 Host Preferences.

differences were not statistically significant. C. heliophilus and C. odibilis in 1969 and C. nubeculosus, C. chiopterus and C. odibilis in 1970, did not occur in catches taken on the human-bait. Also in 1970, C. chiopterus and C. odibilis were not taken from G.W.I. The human bait appeared to be the least preferred of the four baits.

These results seem to suggest that a bait's skin colour may not be a primary but possibly a secondary factor in the feeding behaviour of hungry Culicoides females. The quality of olfactory stimuli from individual potential hosts is therefore more important in selection of the preferred host. Such olfactory stimuli include carbon dioxide released in exhalent breath (4-5% in human breath) and in small quantities by diffusion through the skin (0.3-4.2 ml/100 sq. cm/hr in humans - Rothmans, 1955), volatile constituents of blood which diffuse through the lung epithelium and contained in exhalent breath and skin secretions containing many compounds which when decomposed by the bacterial flora of the skin, give rise to secondary compounds (Gatehouse, 1969). Haddow, (1942) showed that huts containing unwashed children attracted more Mansonia and Anopheles than those containing washed children. The hairy texture of the goat's skin may have acted in their favoured preference by landing Culicoides. Thus, Culicoides are capable of preferential selection of suitable potential hosts from between as well as within host species, as indicated by the results of this investigation. In this context, man appears to be an incidental host.

Bursell (unpublished observation quoted by J. Ford in Mulligan, 1970) noted that the mean fat content of Glossina caught when about to probe a human bait was very much less than that of flies caught when about to probe an ox; thus suggesting that man is not a suitable host and is fed upon under the compulsion of extreme hunger in the absence of suitable hosts. This may, to some extent, be true of Culicoides.

Bennett (1960) using caged avian hosts belonging to twenty different species, and exposed simultaneously, showed that Culicoides near piliferous fed mainly on common duck while C. sphagnumensis, C. stilobezzioides and C. crepuscularis preferred woodland birds. Jones (1959) found that C. variipennis sonorensis preferred red cattle, sheep and man in that order. Hair and Turner (1968) used fourteen hosts including mammals, birds and reptiles and amphibians and showed some Culicoides species did not prefer any particular host species while others fed exclusively on mammals or birds. Du Toit (1962; quoted by Braverman, Boreham and Calun, in press) stated that Culicoides species which suck blood from both cattle and sheep, usually showed a distinct preference for the former. The above authors also confirmed this observation when Dr. P.F.L. Boreham carried out precipitin tests on blood-containing female C. pallidipennis collected by New Jersey light traps on a farm holding sheep, goats, cattle, horses and donkeys in the grounds of the Veterinary Institute, Bet Dagan, Isreal. They found that the ratio of blood-meals containing goat/sheep to those containing cattle blood was 48 : 62. Leptoconops bequaerti (Kettle and Linley, 1967a) and C. furens (Kettle and Linley, 1969b) preferred to feed on the legs of human-baits when given the choice of arm or leg, while C. barbosai (Kettle and Linley, 1969a,b) preferred the arm during daylight but the preference shifted to the legs at night. All three species, common in Florida, Bahamas and Caribbean area, showed marked preference for certain individual human-baits.

In the present investigation, the order of preference, beginning with the most preferred bait, of the various Culicoides species, is: black goat (G.B.), white goat (G.W.1.), white goat (G.W.2.) and man (black). The preferential behaviour of feeding female Culicoides to certain baits/hosts is important from epidemiological view point as some Culicoides species are confirmed vectors of various disease organisms.

9. The Effect of the Removal of Carbon-dioxide Contained in Exhalent Breath on the Biting Rates of Culicoides

9.1. Introduction.

The importance of carbon dioxide contained in exhalent breath as a constituent of the olfactory stimuli affecting the attractiveness of a host to hungry female Culicoides was investigated. The apparatus used was similar to that of Snow et al (1968) who demonstrated the effect on the biting-rates of Aedes cantans when carbon dioxide in exhalent breath is removed by breathing through soda-lime.

9.2. Apparatus and Method

The different parts of the carbon dioxide absorption apparatus are shown in Fig. 30. Its two main components are a Ruben non-rebreathing valve and a soda-lime canister. The former consists of a T-shaped chamber with gas-inlet and gas-outlet channels and a third channel for direct connection to the subject. A movable part inside the chamber closes the outlet channel when the subject breaths in and closes the inlet channel when he breathes out. The canister was tightly packed with 'Calona' soda-lime as its efficiency in absorbing carbon dioxide diminishes appreciably after $\frac{1}{2}$ hour (W.F. Snow, personal communication). The different components of the apparatus are held together with rubber tubings and hose-clips.

When in use, the face mask was tightly fitted over the subject's nose and mouth and the whole apparatus was then supported by hand. The subject inhaled fresh air through the inlet channel but exhaled through the outlet channel into the canister containing soda-lime where carbon dioxide contained in the exhalent breath is removed and the rest released at the open end of the canister.

The attractiveness of the subject with and without the apparatus,

Ruben
non-rebreathing
valve

Canister



Mask

Rubber Tubings

U-shaped glass tubing

Fig. 30. Apparatus for the extraction of carbon dioxide from exhalent breath.

i.e. with and without carbon dioxide in the exhalent breath, was determined by taking counts of the numbers of Culicoides alighting on the subject's exposed legs and arms. Sixty three pairs of observation were made. Each count during each condition was for a period of 10 minutes with a five minute interval between counts. The alighting Culicoides were counted and squashed to prevent repeat visits.

9.3. Results and Discussion

The total number of Culicoides alighting on the subject:

without apparatus = 977. Mean 15.51 ± 4.87

with apparatus = 504. Mean 8.00 ± 2.83

Difference = 473 (i.e. 48.41 %).

t - test for significance of observed difference is approximately
P = 0.05.

The significant reduction in numbers of Culicoides landing on the subject when carbon dioxide is extracted from the exhalent breath underlines the importance of this factor as a constituent of the olfactory stimuli inducing landing. Snow et al (1968) using a similar apparatus in Sussex obtained 60% fewer Aedes cantans attacking a subject with the apparatus than those attacking a control subject breathing out normally. Gatehouse (1969), in his experiments in a wind tunnel type of flight chamber, tested the effects of exhalent breath and different concentrations of carbon dioxide in the numbers of Stomoxys calcitrans alighting on the test targets. He found that the numbers landing on the test target, through which exhalent breath was released into the flight chamber, was significantly larger than those on the control target. He concluded that the response to the test target was due solely to the carbon dioxide content of exhalent breath as neither relative humidity nor the rate of flow of airstream affected response.

Several workers including Van Thiel (1937), Brown, Sarkaria and Thompson (1951), Willis and Roth (1952) and Brown (1951) have studied, in olfactometer cages, the role played by carbon dioxide as an olfactory factor inducing Aedes aegypti to land or probe on a test target and concluded that it exerted an attractive influence. However, Burgess (1959), Kellogg and Wright (1962a) and Daykin, Kellogg and Wright (1965) thought carbon dioxide acted as an activator rather than as an attractant. Other olfactory factors include volatile constituents of blood which diffuse through lung epithelium and are exhaled or diffused through the skin (Gatehouse, 1969). Parker (1948) and Brown et al (1951) found that human apocrine sweat was attractive to Aedes aegypti but the latter authors noted that concentrated sweat was repellant. Rahm (1957) also reported that human sweat also enhanced their response to warm moist current although odours from human skin were more attractive. Laarman (1955) bubbled air through citrated rabbit blood and found this very attractive to Anopheles I. atroparvus. Burgess and Brown (1957), investigating the responses of Aedes Aegypti to beef blood, concluded that volatile substances in the blood plasma were attractive and that attraction of blood was due not only to evolved carbon dioxide. Clements (1963), however pointed out that none of the constituents forming body odour has yet been identified.

The response to individual factors such as body odour, caused by skin secretions (which include diffusing small quantities of carbon dioxide) or environmental factors i.e. heat and moisture, or the interaction of these factors may account for the high numbers (34% of total landings) of Culicoides alighting when carbon dioxide was extracted from exhalent breath of the subject. 66% of the total landings were counted when the subject was breathing normally. Thus, it may be suggested that variations in the attractiveness of potential hosts to

hungry Culicoides females is a reflection on the 'quantity' and 'quality' of their respective body odours. Carbon dioxide is relatively unimportant in this respect as hosts of the same species can be assumed to expire approximately equal volumes but may be important when hosts of different species and size are considered (Reeves, 1953).

10. General Discussion

The results described in this thesis have been discussed in the relevant sections. It is intended here to relate the conclusions drawn from this work to possible effective control measures. It is, however, not pretended that a study of only $2\frac{1}{2}$ years duration on Culicoides population is sufficient for the formulation of any definite ideas: Richards (1961) proposed a study period for insect ecology of at least 5 years.

Insect population changes are influenced by environmental factors, such as weather, habitat, food and the effects of inter- and intra-specific factors. Allee et al (1949) and Richards (1961) recognised that the range of distribution of animal species is determined by the effects of climatic factors and the abundance of a species is related to immigration, emmigration and mortality factors. Temperature appears to be an important factor on which the seasonal distribution of Culicoides species are dependent. In the tropics Culicoides are multivoltine with adult emergence occurring throughout the whole year (Linley and Davies, 1971) while in the temperate regions, as shown in the present studies and by Hill (1947), Parker (1949), Service (1969a,b) and Kettle (1950) in Britain, the seasonal distribution of the adults extends from late spring to late autumn. In the latter, the duration of flight activity of the individual species vary: at Silwood Park, C. obsoletus and C. pulicaris occur throughout the whole season, C. impunctatus from May to early August and C. pallidicornis from mid-June to early August (Figs 15, 16 and 17). Pupation and adult emergence commence in late spring (Fig 6) when the soil temperature is considerably higher than in the winter.

Temperature as a component of the environment also influences the rate of development of the immature stages. The survival of Culicoides

spp. from one season to another in Britain is ensured by the long durability of larval stages in the soil and their capability to withstand and survive the cold winter months. The effects of low temperature and other mortality factors, such as predation and parasitism, on the overwintering larval populations are reflected in the size of 'winter-disappearance': 52% in 1968-1969 and 24% in 1969-1970. Parker (1950) found that although most eggs of Scottish Culicoides hatch within a few days after laying, C. grisescens eggs laid in September did not hatch until February or later, indicating that this species overwinters in the egg stage. In Southern Louisiana, U.S.A., Khalaf (1966) captured small numbers of C. crepuscularis, C. haematopodus and C. hollensis in light traps during winter.

The breeding habitats of Culicoides are localized. This may be due to the selection by females of oviposition sites (Smith and Lowe, 1948; quoted in Kettle, 1962; Linley, 1968b) particularly as they oviposit readily in groups rather than alone (Downes, 1950) and the selection of drier parts of the habitat for pupation by mature larvae (Bidle, 1957; Linley, 1966a). The life history of Culicoides, from the egg stage to the pupal stage takes place in the soil and the immature stages have been shown not only to be aggregated in their spatial distributions but also that the degree of aggregation fluctuates between months in the same stage and differ between stages (Fig. 9.). The pattern of emergence of adult Culicoides in a delimited plot in Cascade Marsh showed greater aggregation than in the pupal population which in turn was more aggregated than in the larval population. Because of the behaviour of the ovipositing gravid females, it is probable that the eggs are laid in patches which may show greater aggregation than obtained for the larval population. Apart from the egg stage which was not sampled, the highest mortality in the life cycle occurs during the larval stage. (Fig. 10). The difference

in aggregation between the relatively immobile pupae and the emergence pattern of the adults reflects on the pattern of the efficiency of mortality factors acting on the former; they appear to be more effective on the peripheries of the pupal clumps. The mortality occurring in the egg stage may be much higher (Table 6b), since they are deposited in large numbers on the soil surface and are consequently vulnerable not only in adverse weather conditions but also to the various natural enemies. The different mortality factors operating on the different stages were not studied but Linley and Davies (1971) quoted Bacon (in press) as having reviewed about 33 parasites and 16 predators of sand flies.

The construction of life-budgets and their relevance to the planning of control measures have been little considered in insect vector studies. Muirhead-Thomson (1968) quoted a WHO (1967a) report in which such studies were advocated. While the construction of a life-budget "is vital to the description and understanding of the population dynamics of a species" (Southwood, 1966), the importance of the different mortality factors operating in the different immature stages can be recognised when used in conjunction with Varley and Gradwell's (1960) key factor analysis. An attempt has been made to construct a life-budget for Culicoides (Tables 6a, b). Although incomplete, the results do illustrate the special problems which require further investigations. Firstly, although Kettle and Lawson (1952) produced an identification Key for the immature stages of Culicoides in Scotland, there still is no means of identifying the 1st and 2nd instar larvae to species level and the identification characteristics of the 3rd and 4th overlap considerably between and within species. Secondly, the extraction of the eggs from the soil medium presents immense practical difficulties as they are easily confused with some tiny seeds. The sand extractor is economical in terms of labour but separates

only the mobile larval stages.

It is evident from the above discussions that this study on the dynamics of Culicoides immature stages is incomplete and should be regarded as preliminary. Practical difficulties have to be resolved and costs, in terms of labour, minimized. The value of such studies on the epidemiology of insect-bourne diseases should be fully appreciated as informations obtained can be valuable for prediction of vector outbreaks and the planning of control programmes.

The importance of Culicoides in Britain is associated with their intolerable biting habits when contact between man and the females of the human-biting species is close. Nash (1948) and Hutchinson (1953) have described the man-fly contact, in relation to Glossina, as 'personal' when a group of tsetse flies repeatedly feed on man because both rely on a common water-hole for survival during the hot dry season in Northern Nigeria and 'impersonal' when the association is casual with man not being the main host. The degree of man-Culicoides contact can be described as 'impersonal', as the occasional association between both occurs during a leisurely evening stroll or when working sites and tourists hotels are located in close proximity to Culicoides breeding habitats or within the flight range of the adult females. When, however, animal farms are sited within the flight range of the females, a permanent association is established and contact between the animals and Culicoides is close and 'personal'. The popular sampling techniques, such as exposure of animal and human baits, for measuring the abundance of Culicoides and other biting flies are dependent on responses illicited from the latter. Sweep-net catches are subject to similar bias because of the operator's presence. Estimates by these methods are relative and as Muirhead-Thomson (1968) pointed out such catches are influenced by the nature, number and relative attractiveness of the individual baits, duration of exposure, presence of

other attractive hosts in the neighbourhood, time of day in relation to the diel biting cycle and various weather factors. In this study, no gorged females were taken landing on the human-bait or in the sweep-net and the number of males taken in the latter were negligibly small. Consequently, these methods are selective, favouring hungry females of human-biting species and do not adequately reflect the species abundance. They are, however, useful for rapid assessments of the effectiveness of control measures (Trapido, 1947). Suction traps, which neither attract nor repel insects (Taylor, 1962a) and sample the air throughout the day, have been shown to be a more reliable sampling technique for Culicoides than the previous two (Table 7). It is important to point out, that in the use of human-or animal-baits for sampling purposes, the rate at which Culicoides land on the bait to feed is not evenly distributed during the first hour after his arrival at the site. Figs 11, 12 and 13 show that the attainment of maximum landing in relation to time after arrival varies between sites i.e. the further away the site is from the Culicoides breeding area, the longer it takes for maximum number of landings to be achieved (Fig. 14) and the fewer the number of flies landing. Also, man does not appear to be the main host when other preferred hosts are available. (Fig. 30).

In the costing and planning of control programmes for adult Culicoides it is necessary to understand fully the dispersal and seasonal as well as diel flight activity of the species occurring in that particular locality. Application of control measures such as fogging at the wrong time of the day or to areas likely to be quickly infiltrated by adults dispersing from untreated areas, may be ineffective. In Culicoides, the usual mode of dispersion is by flight. The present study shows that Culicoides of both sexes are dispersed in two dimensions: vertically and horizontally away from their breeding or resting sites. Individuals dispersing . . .

horizontally decrease in number with distance away from the source and probably consists of those females executing flitting flights in search of a blood meal. Considerable distances from the source of dispersion are covered during the process. It is not suggested that such movements are continuous; the search for a host is random and probably discontinuous as well. Both males and females have a similar flight-range (Fig 21a,b). Wind also plays a role in the transportation of individuals over long distances (Breeland and Smith, 1962; Williams, 1962). The height-density profile of both Culicoides sexes determined from suction trap catches at various heights (1.2, 9.1 & 12.2 m) show a decrease in aerial numbers with increasing heights above the ground (Fig 18) at Silwood Bottom, 300 yards (275 m) from the nearest breeding site. The sex-ratios at the different heights also show a greater proportion of males of the various species as height above the ground level increases and in two species - C. pulicaris and C. stigma - the aerial numbers of males are actually greater than those of females at 12.2 m. Considering the size of Culicoides it may be said that individuals at such heights are likely to be airbourne and probably true migrants but may also include flitting individuals which are caught inadvertently in strong wind currents. From the above discussions, it appears certain that dispersal must result in the death of a considerable number of the dispersing individuals; it is not known how effectively Culicoides are capable of 'homing' from long distances away from their resting or breeding habitats and such individuals as well as those which are airbourne may be 'stranded' in habitats not suitable for their survival. It may be reasonable to suggest that the time of day during which dispersion occurs coincides with the diel period of peak activity of the species. At Silwood Park, using a 9-in. Vent-Axia suction trap, the peak flight activity is associated with the sunset hour (Figs 23 & 24) but

the diel biting cycle is bimodally distributed with peaks at dusk and dawn, the former being the larger (Fig. 25). The morning peak could possibly have consisted of individual females that failed to obtain a blood-meal the previous evening, oviposited during the night or emerged earlier that morning and searching for their first blood meal.

It is convenient at this point to consider the effects of weather factors on the total daily and hourly Culicoides flight activities. Multiple regression analyses have shown that weather factors account for a small percentage of the variability in the total daily catches (Table 18 & 19). The major factors affecting the latter are radiation, temperature, wind speed and total daily sunshine-hours. Regressions on mean daily wind speed, mean temperature at dusk and total daily sunshine-hours are negative while those on mean daily temperature and mean daily radiation are positive. These suggest that maximum flight activity can be expected on warm, cloudy and windless days. The threshold distributions for radiation, temperature and relative humidity were also determined (Figs 26, 27 & 28). Maximum flight activity occurs at radiation intensities of below 0.8-0.9 milliwatt-hours/cm² but above this value activity is reduced. The lower and upper temperature thresholds are 11°C and 24°C respectively and the preferred range of relative humidity is 56% -95%. Since the points are scattered around the respective threshold curves, it is concluded that these weather factors do not initiate flight by themselves but act, independently or by interaction between them and other physical factors, as a release mechanism for the intrinsic flight behaviours of the insects.

Unlike mosquitoes, where there is a vast amount of literature, the influence which chemical and physical stimuli have on the feeding behaviour of Culicoides females has been relatively neglected. The

reasons for this could be the difficulties involved in rearing Culicoides spp. in the laboratory. The only aspect of this problem investigated in this study is the role which carbon dioxide, contained in the exhalent breath, plays in the attraction of females to a host. The results (Fig. 30) show that significantly fewer Culicoides landed on the bait from whose exhalent breath carbon dioxide is extracted than on the same bait breathing out normally. It is recognized that other factors, such as the warm, humid convection current in the micro-environment of a host, the constituents of body odour which are contained in the latter and to some extent visual stimuli, contribute to his attractiveness to hungry female Culicoides. Studies of this kind in the laboratory are necessary and important for the development of ideas concerning the formulation of attractants for possible control measures and repellants which can give some measure of protection to a subject.

In the light of the above discussions and conclusions, it appears that the planning of an adequate control measure should be based on the reduction of Culicoides at its source of dispersion. Since the adult population is directly related to the number actually surviving from the egg stage to the pupal stage and Culicoides in Britain, overwinter in the larval stage, a well planned combined attack on the latter by ecological as well as chemical control measures at the breeding sites may be effective. Not enough is known of the natural enemies for inclusion. Larvicidal treatments may be best applied in early winter and/or late winter-early spring. Heavy rainfall is required to carry the chemicals into the soil for immediate and effective control (Hill and Roberts, 1947). Full short term controls have been achieved for various species by the application of DDT and gamma-BHC to the soil breeding medium (Hill and Roberts, 1947; Kettle, Nash and Hopkins, 1956; Kettle and Parish, 1957; Kettle, Parish

and Parish, 1959). Culicoides breeding areas can be extensive and associated with lakes and river systems; making the use of large quantities of persistent larvicides for proper coverage of such areas not only economically unjustifiable but also may have disastrous consequences on other wild life. Rogers (1962), Clements and Rogers (1964) and Linley and Davies (1971) have recommended long term control measures for C. furens and C. barbosai, in Florida, U.S.A., and the Caribbean areas, in which the areas available for breeding are reduced by dyking and flooding of swampy surroundings, by drainage systems which make the soil too dry for larval habitation or by reclaiming such areas for developmental purposes. In view of the current opinion on the environment, it is considered that adequate drainage systems in the marshy areas will limit the potential breeding areas into small and manageable isolated pockets which can then be more easily treated with larvicides. Although the initial costs of such operations will be high, this approach may ultimately lead to a more permanent control or complete eradication of Culicoides from certain areas. Attempts at controlling adult Culicoides by barrier spraying with DDT on walls and vegetation (Kettle, 1949; Trapido, 1947) and by fogging (Bruce and Blakeslee, 1948; Travis, 1949) produced only temporary relief as Culicoides quickly infiltrated the treated areas soon afterwards. These results are not surprising since the long distances flown during flitting movements can render this approach to controlling the adults ineffective. Linley and Davies (1971) recommended the use of repellants such as deet, T.M.P.D. (2,2,4-trimethyl-1, 3-pentanedioil) or D.M.P. (dymethyl phthalate) against the Caribbean species. The repellants, when applied to exposed parts of the body, provide a measure of personal protection for a few hours.

SUMMARY.

1. Oviposition, pupation and adult emergence of Culicoides species at Silwood Park occur between April and September. The entire life-cycle, from eggs to pupae, takes place in marshy soil. The survival of the species is ensured by the over wintering larval stages which suffer considerable mortality or 'winter-disappearance' during the winter months.
2. The spatial distribution of the different populations of the immature stages fluctuates in relation to time. Higher aggregations correspond to decrease in the population densities. Estimates of population densities and the degrees of aggregation were obtained from data collected by stratified random samples taken from a delimited area of the breeding site. C. pallidicornis formed 82% of the emerging adult population, indicating that the experimental plot was probably a preferred breeding site of the species.
3. The degree of aggregation in the populations of Culicoides immature stages in the soil increases in the following order: larvae, pupae and emerging adults. Differences in the latter two suggest that mortality factors acted on the periphery of the relatively immobile pupal clumps. The eggs were not sampled because of intense practical difficulties.
4. The population budgets for 1968/69 and 1969/1970 are presented and show that larval populations experienced the highest mortalities. It is probable that the early instar larvae are more exposed to various natural mortality factors, particularly during dispersion from the egg batches which are deposited on the soil surface.
5. Fourteen Culicoides species occur at Silwood Park but only four are numerically abundant. These are C. impunctatus, C. obsoletus, C. pulicaris and C. pallidicornis. A comparison of samples obtained with a 9 in. VentAxia suction trap, an 18 in. propeller suction trap,

a sweep-net and a bait/collector show that the former two give more reliable estimates of species abundance as well as aerial densities.

C. impunctatus and C. obsoletus females are strongly attracted by a human-bait which is comparatively less attractive to C. pulicaris and C. pallidicornis.

6. Culicoides adults occur in the field throughout the flying season, i.e. from May to October. This is due to the differences in the peak occurrence of the various species:

	May	June	July	Aug.	Sept.	Oct.
<u>C. impunctatus</u>		✓	✓			
<u>C. obsoletus</u>	✓	✓			✓	✓
<u>C. pulicaris</u>						
<u>C. pallidicornis</u>			✓			

The adult population is comparatively low in August. No definite peaks were evident in the seasonal distribution of C. pulicaris.

7. Culicoides landing-rates following the arrival of a human-bait at three separate sites located at various distances from the breeding sites are compared. Both the times required for maximum landings to commence (waiting-period) and the occurrence of peak landing, increase with distance from the breeding sites.

8. The aerial numbers of both sexes decrease with increasing height above the ground. It is suggested that individuals caught above 8.5 m are probably airbourne and hence migratory forms. There is no significant difference between the horizontal flight range of both sexes. This has been estimated as between 500-600 yards (459-550 m).

9. The sex-ratios of the various species at 1.2, 8.5 m and 12.2 m above the ground, as determined from suction trap samples, show an increase in the proportion of males with increasing height above the ground except

C. pallicaris and C. stigma where the males are numerically more abundant than the females of the same species at 12.2 m.

10. The diel biting and flight activities of the four abundant Culicoides species, as determined by landing-rate and sweep-net sampling techniques, show a bimodal distribution with a larger peak at dusk and a smaller one shortly after dawn. Suction trap catches show a single peak of flight activity at dusk. A possible explanation for the difference is offered.

11. The mean daily wind speed, mean temperature at dusk and total sunshine hours are negatively correlated with the total daily catch of adult Culicoides by an 18 in. propeller suction trap. The mean daily temperature and mean daily radiation are positively correlated with the latter.

The preferred range of temperature and relative humidity for flight are found graphically to be 11°-24°C and 56-95% respectively. Flight activity is increased when the radiation intensity is lower than the estimated threshold value of 0.8-0.9 milliwatt-hours per sq. cm.

12. Host preference experiments at Rush Meadow show that the females of nine Culicoides species occurring prefer a black goat (G.B.) white goat (G.W.1), white goat (G.W.2) and man (black) in that order. This suggests that man is the least preferred host when other more suitable hosts are available. It is also likely that olfactory stimuli play a greater role in inducing landing than visual stimuli.

13. By extracting carbon dioxide contained in the exhalent breath of a human-bait, it was found that his attraction was significantly lower than when breathing out normally.

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APPENDIX



I. Cascade Marsh



II Rush Meadow



III 9-in. Vent Axia suction trap at Rush Meadow



IV Suction traps II & IV (18-in propeller type at 4 and 28 feet above the ground respectively) and suction trap V at 40 feet. Silwood Bottom.



V Sticky traps at Rush Meadow



G.W.2

G.B.

G.W.1.

VI Domestic goats used for host preference experiments.
Rush Meadow.