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Seasonal and diurnal flight activity patterns in some species of black-flies (diftera: simdliidae)

Roberts, D. M.

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SEASONAL AND DIURNAL FLIGHT ACTIVITY PATTERNS IN SOME

SPECIES OF BLACK-FLIES (DIPTERA: SIMULIIDAE)

by D. M. ROBERTS B.Sc.

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ABSTRACT

The flight activity of three species of Simuliidae was studied in the
Eden Valley, Cumberland. The flies were caught using a net mounted on a
vehicle, which was driven at 48 km/h along a circuit, consisting of four roads
parallel to the River Eden (at distances of 0.5, 1.5, 3.0 and 6.0 km from the
river), and the main road at right angles to the river, between the villages
of Langwathby and Melmerby.

Equipment in the back of the vehicle allowed the flow of insects from the
net to be divided into catches, each of which was caught over a distance of 1 km.
An anemometer on the mouth of the net allowed the insects to be calculated as a
concentration, so that the catches on the four roads could be directly compared.

The female flies were all dissected and thus divided into different
physiological groups. The main divisions were into nulliparous and parous
flies, each of which was sub-divided into blood-thirsty and blood-fed flies,
and into gravid and parasitised flies.

The diurnal rhythm of the three simuliid species was studied, noting the
differences between the different physiological groups. The effect of the
weather on these rhythms was examined.

By comparing the four parallel roads, the spatial distribution of the
three species was studied. The distribution, and thus powers of dispersal,
of the nulliparous and parous flies was compared, and the effect of the presence
of parasites on this ability to disperse was noted.

INDEX

I INTRODUCTION

1. General Introduction

In many tropical countries, Simuliidae are of medical importance due to the fact that they are vectors of the nematode parasite <u>Onchocerca volvulus</u> Leuckart. The parasite is transmitted to humans during bloodsucking by the simuliid and results in the disease onchocerciasis, in which there is fibrosis of the skin and often blindness. In West and Central Africa, the vector is principally <u>Simulium damnosum</u> Theobald (Blacklock 1927, Crosskey 1954, Duke 1973, Lewis 1953), while in Central and South America, the vectors are <u>S.metallicum</u> Bellardi, <u>S.callidum</u> Dyar and Shannon, and <u>S.ochraceum</u> Walker (Dalmat 1958, Lewis and deAldecoa 1962). In England, <u>S.ornatum</u> Meigen has been shown to transmit the parasite <u>Onchocerca gutturosa</u> Neumann (Steward 1937). A number of simuliids have been shown to be vectors of bird parasites. <u>S.rugglesi</u> Nicholson and Michel, for example, transmits <u>Leucocytozoon simondi</u> Mathis and Leger to ducks (Fallis et al 1956).

In the sub-arctic regions of Canada and Russia, several species of Simuliidae are of economic importance because they may occur in vast numbers, resulting in a severe nuisance to humans and in the death of livestock. For example, <u>S.venustum</u> Say, <u>Prosimulium fuscum</u> Syme and Davies, and <u>P.mixtum</u> S & D are major man-biting species (D. M. Davies 1956, L. Davies 1961, 1963), while <u>S.arcticum</u> Malloch has been responsible for many cattle deaths (Cameron 1922, Fredeen 1956, Peterson and Wolfe 1958).

In a region in which onchocerciasis occurs, the actual area infected is therefore largely limited by the flight range of the simuliid vector (though there may be other factors such as the movement of humans). The flight range (the maximum distance flown by the adult flies from their breeding grounds) is however less important than the spatial distribution (change in concentration of

the flies with distance from the breeding ground), since the concentration of the flies will affect the degree of transmission of the disease to humans.

In Canada, where the simuliids are important due to their vast numbers, the spatial distribution is similarly important. Thus Rempel and Arnason (1947) found that large numbers of cattle were killed up to 96 km from the breeding site by swarms of S.arcticum, small numbers of cattle were killed up to 160 km fro m the breeding site, but no cattle were killed within the first 48 km. Further examples of the flight range and spatial distribution of different species of Simuliidae are given at the beginning of chapter 4.

In the case of simuliids acting as vectors of disease, it is not the total flight range that is important, but only that of the parous flies (those flies which have already undergone one or more gonotrophic cycles and have therefore already had a previous blood meal), since only they can transmit the disease, having picked up the parasite during a previous blood meal. Thus L. Davies (1961) found that parous Prosimulium mixtum S & D dispersed less than the nulliparous flies, being concentrated around the breeding sites. Furthermore, since Lewis (1961, 1965) found that only 13 - 25% of parous S.damnosum contained O.volvulus microfilariae, the spatial distribution of the parasitised flies must also be considered. Dalmat and Gibson (1958), for example, found that simuliids infected with O.volvulus dispersed less than uninfected flies. Consequently, when studying the spatial distribution of the flies, the different physiological groups (nulliparous, parous, parasitised etc) must be considered.

Many species of simuliids have been shown to have a marked diurnal rhythm of flight activity (for example, Crosskey 1955, L. Davies 1957 a, Peterson and Wolfe 1958 - these and other examples are given in more detail at the beginning of chapter 3). In S.damnosum it has been shown that the parous have a peak of activity at a different time of the day (midday) to that of the nulliparous (afternoon) (Le Berre 1966, Duke 1968, Disney 1972). Consequently, the diurnal rhythm is also important, since a person will have a greater chance of being infected with onchocerciasis at midday rather than later in the afternoon. Furthermore, the diurnal rhythm must be taken into account when studying the spatial distribution, since it is not usually possible to sample at different sites simultaneously.

This thesis is therefore the result of research on the flight activity of three species of Simuliidae in the Eden Valley, Cumberland. It was designed to test the efficiency of a new type of vehicle-mounted trap (described in chapter 2), which allowed the actual concentration of the flies in the air to be measured.

The area in which the vehicle trap was tested was designed to be equivalent to the Simulium damnosum situation in Africa. Like S.damnosum, the two main species studied, S.reptans L and S.equinum L, only breed in large rivers, in this case the River Eden. Since breeding was restricted to one river, it was possible to study the spatial distribution by collecting the flies at different distances from the river.

The female flies caught were all dissected and divided into categories based on their physiological condition. These categories are described in detail in chapter 2. The spatial distribution and diurnal rhythm of the different categories could therefore be compared with one another.

It was therefore possible to see whether, for example, the presence of parasites had an effect on the spatial distribution, or whether there was a difference between the spatial distribution of the nulliparous and parous flies.

In this way, it was hoped that the research done in the Eden Valley could shed some light on some of the problems occurring in the equivalent S.dammosum situation in Africa.

2.   PREVIOUS RESEARCH TECHNIQUES

a)   Sampling methods

To study the dispersal or diurnal rhythm of flight activity of a species necessitates having a method of trapping the flies, in order to sample the population.   A wide range of trapping methods have been used, but these can be divided into a few basic types.   The primary division is into attractant traps, the most important of which are bait traps and light traps; and non-attractant traps, such as sticky traps (though whether these are actually non-attractant is highly debateable - but they are normally considered to be non-attractant and so have been included in this category), suction traps and vehicle-mounted net traps.

Each type of trap has its disadvantages, and have been used to different extents for studying different groups of insects.   In this brief survey, apart from the Simuliidae, the groups considered have been restricted to mosquitoes (Culicidae) and biting midges (Ceratopogonidae), since all three families are small blood-sucking insects, and so have similar problems to overcome and therefore probably have much in common.

(i)   Bait traps

The type of bait naturally depends on the food preference of the species being studied, but because of their medical importance, research has been largely concentrated on anthrophilic species, using human bait.   Research on Simulium damnosum in Africa, for example, has been largely based on the use of "fly-boys" - counting the number of flies landing on a human within a given period of time and expressing the results as the number of flies/fly-boy/hour (for example, Crosskey 1955, 1961; Duke 1968; Lewis 1960).   This had the disadvantage in that the different humans had markedly different powers of attraction to simuliids and so would result in different catches.   There is also the danger of the different "fly-boys" had different catching efficiencies. However, by using the same person in the same location, "fly-boys" have been extensively used for studying the diurnal and seasonal changes in the fly population (Crosskey 1955; Disney 1972; Duke 1968; Le Berre 1966).

But, because bait traps are attractant traps, they only sample a proportion of the population, which in this case is the blood-thirsty flies. Therefore little is known about the blood-fed and the gravid flies (those flies with fully mature eggs).   Because of its limitations, little research has been done on the spatial distribution using this method, so that studies of dispersal have been principally limited to that of the flight range (Crosskey 1958; Lewis 1953, 1957; Le Berre 1966).

In Canada, a similar collecting method has been extensively used involving netting flies attracted to a human bait within a given period of time, or using a standard number of sweeps of the net (D.M. Davies 1952; L. Davies 1963; Peterson 1959; Wolfe and Peterson 1960).

Other bait animals have been used, depending on the species of simuliid being studied. For example, L. Davies (1957a) trapped S.ornatum Meigen on cattle; S.euryadminiculum Davies has been trapped on the common loon (Gavia immer Brunswick) by Lowther and Wood (1964) and Fallis et al (1967); S.latipes Meigen and S.aureum Freis were caught on the ruffed grouse (Bonasa umbellus L) by Fallis and Bennett (1958).

Bait traps have therefore been the main method of studying the flight activity of adult simuliids, despite the difficulties of making the catches comparative, and despite the fact that only blood-thirsty flies can be studied.

In studies on mosquitoes (Culicidae), bait traps have been used to study the circadian rhythm of biting activity (Gillett 1957; Haddow and Ssenkubuge 1973; Snow 1955), but only in a few cases to study dispersal (Jenkins and Hassett 1950). Most of the studies of dispersal have used other methods, especially light traps and vehicle traps.

Bait traps have been extensively used to study dispersal by midges (Ceratopogonidae), though much of this research has been based on irregular observations of biting flies at different distances from the breeding sites (for example, Dorsey 1947; Linley and Davies 1972). But trapping has been used more systematically to study dispersal by Nicholas (1953) and Williams (1962). However, most of the systematic research on dispersal in midges has used non-attractant traps.

(ii) Light traps

Only a few studies of Simuliidae have involved the use of light traps (Williams and Davies 1957 ; Davies and Williams 1962), largely because they could only be used at night, while flight activity of Simuliidae is mainly diurnal.

In contrast, light traps have been extensively used to study mosquitoes because their flight activity is principally crepuscular or nocturnal. Light traps have the advantage over bait traps in that they do not only catch blood-thirsty flies, but they still have the disadvantage of being attractant and stationary traps. Light traps have been shown to be very sensitive to the local environment, since the size of the catch can be markedly altered by the amount of reflection from surrounding vegetation (Verheijen 1958). Being attractant traps, the catch is not representative of the actual flying fauna. Thus Bidlingmayer (1967) found that woodland species were repelled by the light and so were not found in the trap, while slow flying species were less likely to be caught than fast flying species. Because of these factors, it is difficult to equate the size of the light trap catch with the concentration of the flying population. However, this has not prevented their extensive use in the studies of mosquito dispersal (for example, Chant and Baldwin 1972; Clarke 1943; Dow 1971; Provost 1957).

Light traps have been little used for studying the flight activity of midges.

(iii) Sticky traps

Little use has been made of sticky traps to study simuliids or mosquitoes, though L. Davies (1957a) used them to catch ovipositing S.ornatum. But, they have been used to study the dispersal of midges (Bidlingmayer 1961; Kettle 1951, 1961).

(iv) Suction traps

Suction traps have the advantage over bait traps and light traps of being a non-attractant method of sampling. They will therefore catch all the flying insects and will not bias the catch. They have however been little used to study simuliids, but have been used for mosquitoes (Bidlingmayer 1967, 1971; Service 1971).

(v)   Vehicle-mounted net traps

These are non-attractant and so have the advantages of the suction traps.
They have the further advantage over all the previous traps in that they are
not stationary.   Stationary traps may be markedly affected by the local
environment, such as the type of vegetation surrounding the trap.   It is
therefore difficult to equate the catches from traps in different sites.
Vehicle traps, however, collect flies over a distance of usually many kilometres
and so reduce the effect of local differences in the vegetation etc.

Despite these advantages, vehicle traps have been little used to study
Simuliidae.   Though one was used by Davies and Peterson (1956), it was not
used for dispersal studies.   In contrast, vehicle traps have been extensively
used to study the dispersal of mosquitoes (Stage 1947; de Zulueta 1950;
Provost 1957; Bidlingmayer 1967), and of midges (Bidlingmayer 1967; Nelson
and Bellamy 1971; Dyce 1972).

b)   Experimental methods to study adult dispersal

The dispersal of flying insects has been studied by two main methods: a)
capture-recapture studies, recapturing marked insects at different distances
from the release point; b) collecting insects at different distances from the
nearest known breeding ground.

In capture-recapture studies, the insects were usually marked by either
a dye (usually an aniline compound sprayed over the adult) or by a radioactive
tracer (usually P^{32} fed to the larvae - examples are given at the beginning of
chapter 4).   However, when using flies dispersing from a point source, it is
difficult to study the spatial distribution since the flies radiate outwards.

Thus when collecting at some distance from the release point, there is less chance of recapturing a fly than there is when collecting close to the release point (if the 360° of possible flight directions from the release point are divided up into arcs, then the area within the arc increases with distance from the source). This problem has been compounded by the widespread use of stationary attractant traps, such as bait traps and light traps, to recapture the flies (Chant and Baldwin 1972; Clarke 1943; Dalmat 1952; Jenkins and Hassett 1950; Provost 1957). As mentioned earlier, experiments using stationary and attractant traps are difficult to analyse because of the large differences between individual traps, even when they are equidistant from the release point. Consequently, capture-recapture studies usually concentrate on the flight range and not the spatial distribution of the species.

Most studies of spatial distribution have therefore depended on catching flies at different distances from the nearest breeding area, so that there is no problem in having a point source of release. However, it does depend on all the breeding grounds of the species involved being known within the collecting area.

3. PRESENT RESEARCH

Because of its obvious advantages, a vehicle-mounted trap was used to sample the flying population. It was non-attractant and unlikely to be biased by local environmental conditions since flies were collected over a distance of one kilometre. It was also capable of filtering over 27,000 m^3 of air/h, and so was sensitive to changes in the flight activity. It was therefore a useful sampling method for studying the spatial distribution. However, it had two disadvantages, in that trapping was dictated by the existing road system, and in that it was not suitable for swarming insects since the numbers caught would then depend on how much of the swarm the net happened to go through.

Therefore, as male Simuliidae tend to fly in swarms, the research was concentrated on the females.

The two main species studied, <u>S.reptans</u> and <u>S.equinum</u>, only bred in the River Eden and so since they were dispersing from a known source, capture-recapture methods with all their problems of analysis were not necessary. The flies were simply caught at different distances from the river.

II.   THE COLLECTING EQUIPMENT; FIELD AND LABORATORY PROCEDURES

1.   Earlier vehicle mounted net traps

The earliest equipment, for research on mosquitoes, employed cone-shaped insect nets fixed to a vehicle (Chamberlin and Lawson 1945; Stage 1947; De Zulueta 1950; Provost 1952).   These had the danger of losing insects when the vehicle stopped (since the insects were only held in the net by the force of the airflow).   Later equipment consisted of a large pyramidal net mounted on the roof of a vehicle, having a mouth 0.6 m by 2.1 m and terminating in a small muslin bag.   This bag could be rapidly removed when the vehicle stopped, thus reducing insect loss (Provost 1957; Bidlingmayer 1966; Nelson and Bellamy 1971; Dyce 1972).   Though Dyce's experiments did include Simuliidae, the only specific research on Simuliidae using a vehicle trap was by Davies and Peterson (1956) who used a simple screen cage fixed on a jeep.

All the above equipment necessitated relatively low speeds (usually below 30 km/h), because of the problem of wind resistance by the net, and required long catches (6 km - Provost 1952; 5 km - Bidlingmayer 1966; 0.5 h - Dyce 1972) to reduce the inconvenience of repeatedly stopping the van and emptying the net catch.   For this present research, new equipment was designed which allowed the flow of insects from the net to be divided up into sequential catches controlled by the driver.   Thus repeated catches were made along roads divided into one kilometre stretches, without the vehicle having to stop.   Furthermore, since the volume of air was continually monitored, the catch could be calculated as an actual concentration (per 2000 m^3 of air), and therefore different 1 km stretches from different roads could be directly compared with one another.

PLATE 1. The van on Road 3. Note the scarp slope in the background (about 4 km), and the open vegetation beside the road.

PLATE 2. Road 3. Note the closeness of the trees to the road, providing the flies with more sheltered conditions.

This equipment was used in the Eden Valley collecting along a standard circuit, consisting of four roads roughly parallel to the River Eden and a connecting road roughly at right angles to these. The area and collecting methods are described in more detail later in the chapter.

2. Collecting equipment

Flies were caught by a pyramid - shaped net mounted on the roof rack of a 5 cwt Ford Escort van (Plate 1), which was driven at a standard speed of 48 km/h (30 mph). Though this speed was not exceeded, since the roads used were narrow country lanes (see Plate 2), there were bends where the speed was reduced below 48 km/h. However, these sections were short and would not affect the catch, since the speed was not reduced below 30 km/h. Insects filtered out of the air were funnelled into a pipe at the rear end of the net, which led via a hole cut in the roof into equipment in the back of the van. This equipment (Plate 3) allowed the flow of insects to be divided up into separate catches, by changing the collecting tube through which the air was flowing.

The net had a mouth 91.5 cm (3 ft) wide and 61 cm (2 ft) high, with its lower lip 182 cm above the ground. It tapered over its length of 137 cm to a diameter of only 10 cm. Since it was made from polyester netting with a mesh size of 13.3 meshes/cm and having 50% open area, the net allowed much of the air to escape without losing the flies, so that there was no appreciable build up of air pressure in front of it at speeds up to 48 km/h. The metal lip of the net was bolted to a frame on the roof rack (Plate 1), so that it could be taken down when travelling to and from the catching area. The net was kept as taut as possible by moving the roof rack forwards and by pulling the sides of the net apart using hooks attached to the supporting frame (visible in Plate 1).
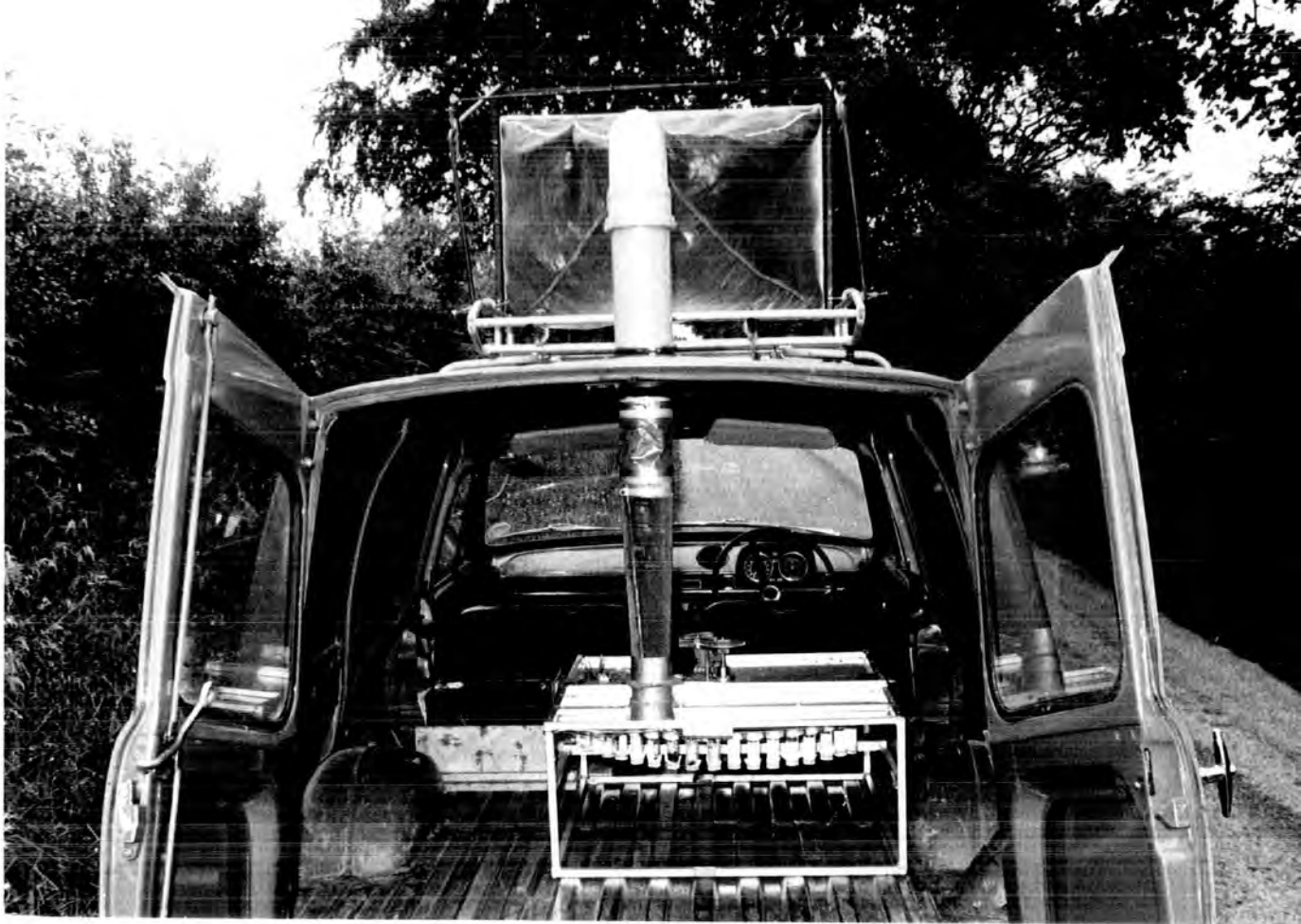
PLATE 3. The collecting equipment.

PLATE 4. Close-up of the tubes on the rotating perpex disc.
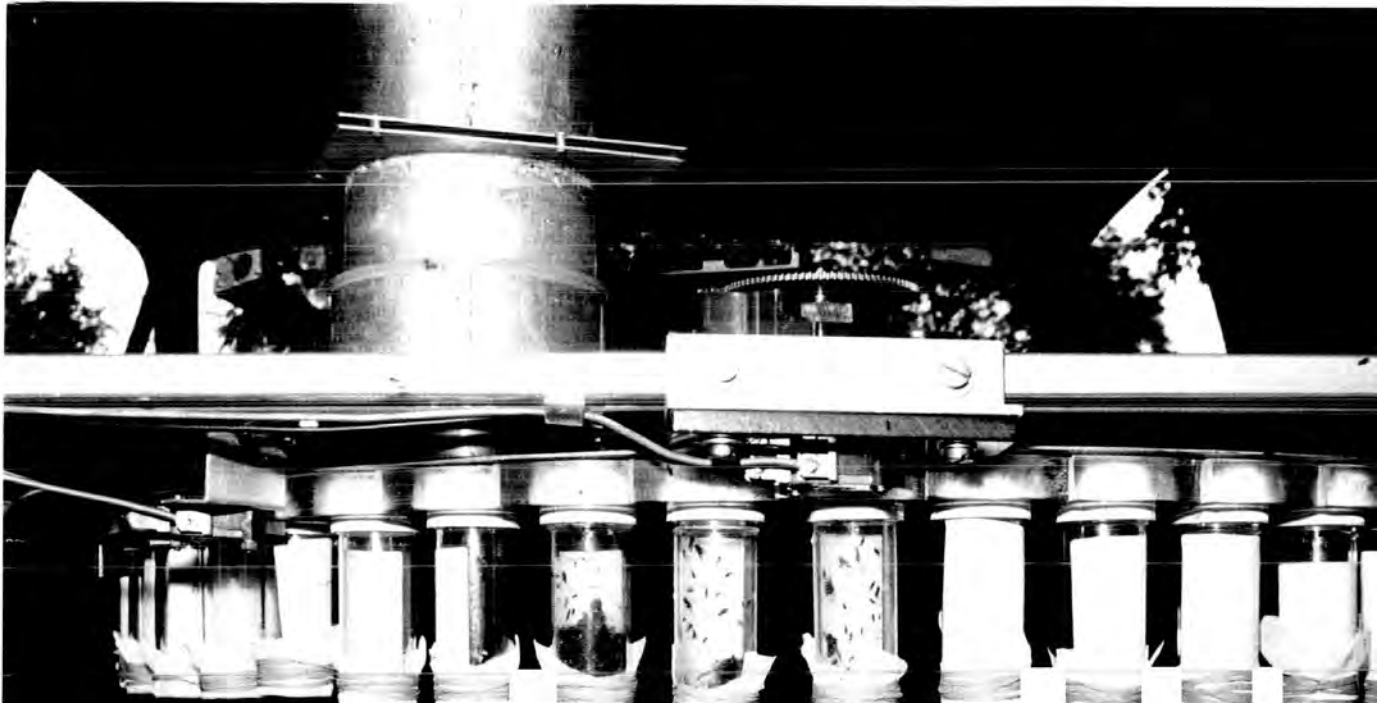
Otherwise the force of the airstream produced depressions in the net which trapped some insects, though they were mainly Chironomids and not Simuliids (more compact shape). The front edge of the net was raised 23 cm above the roof so as to largely avoid the slipstream of air passing over the windscreen, since this would have been at a greater velocity than the surrounding air.

In calm conditions, the volume of air sampled depended on the van speed and on the cross-sectional area of the net mouth, so that for every metre the van moved forwards, the net would filter $0.5581 \, m^3$ of air. However, when there was a wind blowing, it increased or decreased the volume of air depending on the wind speed and its direction relative to the direction of travel of the van. To measure this effect, a cup-counter anemometer (see Plate 1) reading to 0.1 km was mounted on the net mouth, so that it measured the actual volume of air entering the net. The anemometer could be read from inside the van using a pair of mirrors mounted inside a black perspex box, which was attached to the windscreen (see Plate 1).

The 10 cm diameter plastic tube from the net was connected to a collecting tube 2.6 cm in diameter by a 33 cm tapered gauze cone (Plate 3 and Fig 2). Tests using an anemometer fixed under the collecting tube to measure the air velocity travelling through it, showed that when the van was travelling at speeds up to 48 km/h, the velocity of the air passing through the tubes approximately equalled the van speed. But above 48 km/h, the velocity dropped relative to the van speed showing that an air pressure barrier had built up, probably in front of the net. At low van speeds (30 km/h), the air velocity was not sufficient to force large insects down into the tubes, so that they tended to collect on the sides of the gauze cylinder. The van was therefore kept as far as possible at 48 km/h, when these problems did not occur. A removable coarse-meshed large-insect excluder (Fig 2) with a mesh of 4.5 meshes/cm was inserted beneath the gauze cylinder to prevent large insects
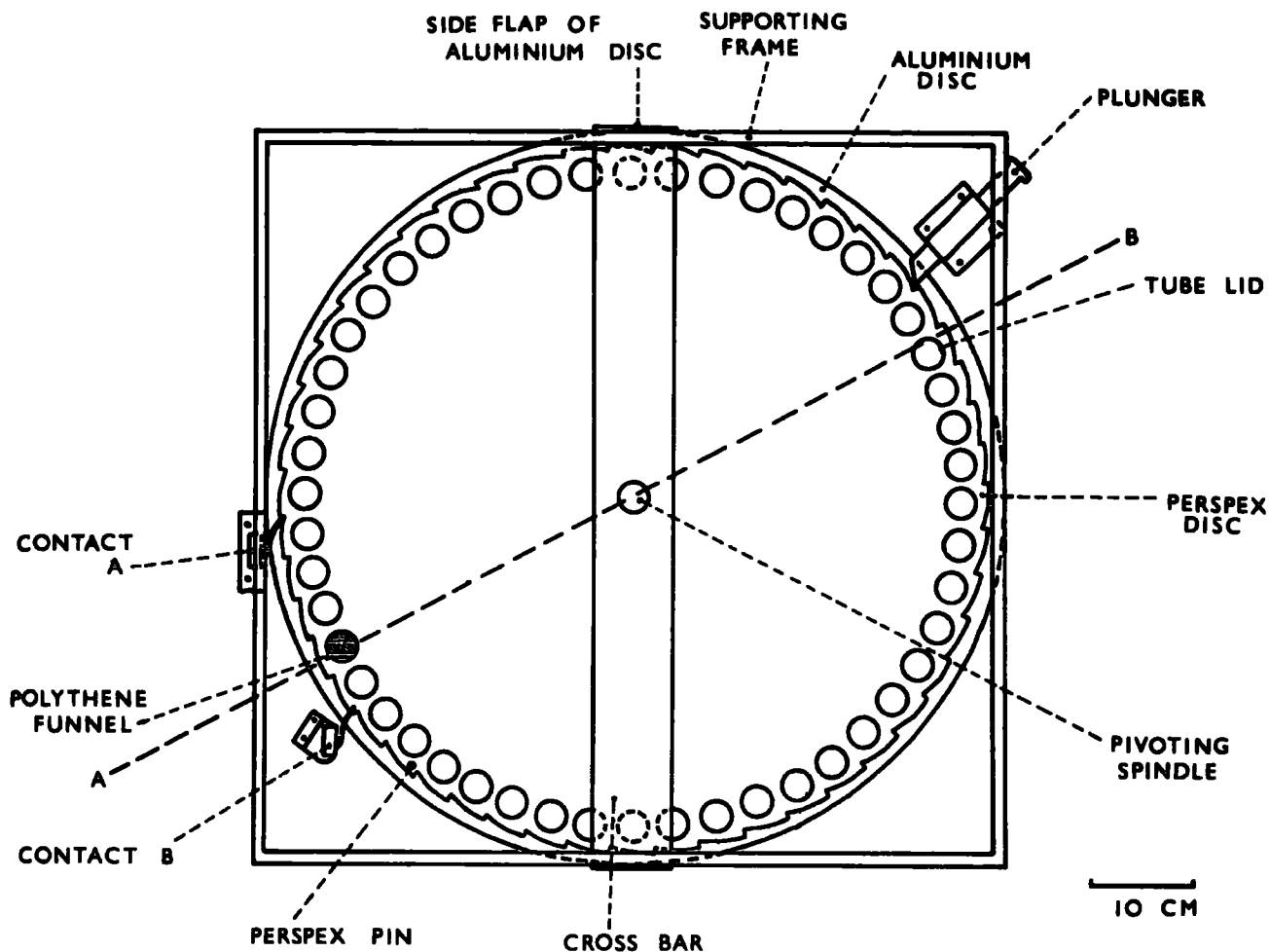
FIG. 1   Underside view of segregrating apparatus.

FIG. 2   Section along A-B (Fig.1) of the apparatus. [W = perspex
         washer; X = cross bar; Y = pivoting spindle ; Z = driving spindle
         of the perspex disc, geared to the   motor].
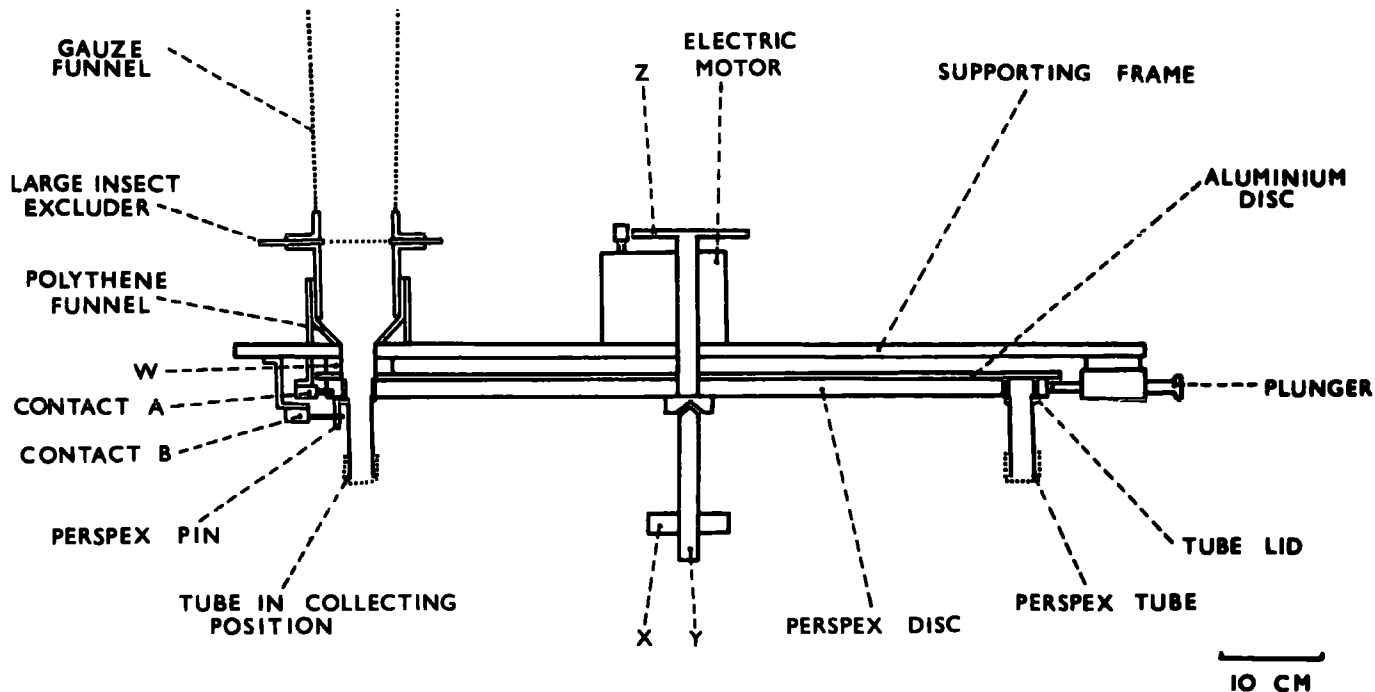
from crushing the Simuliidae when they were forced by the air velocity into
the small collecting tube. In practice, however, the simuliids were found
to be completely undamaged by the small number of large flies found in the
Eden Valley, so that the excluder was not found necessary.

The insects in the catch were filtered out in a 7.7 x 2.6 cm (3 x 1 in)
collecting tube, which had had its bottom sawn off and replaced by a fine
30 mesh/cm nylon gauze. This mesh was found necessary, since under the
pressure of the 48 km/h air flow, many simuliids became entangled in coarser
meshes and were therefore difficult to remove. Fifty of these tubes were
screwed into their lids, which after having had their centres removed, had
been embedded in the 1.3 cm thick perspex disc, to form a circle around the
edge (Fig. 1 and Plate 4). The first tube under the nylon funnel, through
which the air current was passing, collected the flies caught over one kilometre.
The disc was then rotated clockwise by a 12 v electric motor, connected to the
van's battery and to an auxiliary battery within the van, until the next tube
was under the funnel.

The electric motor was wired up through two alternative circuits, both
with switches. One of the circuits went through circuit A (Fig. 1 and visible
in Plate 4) which had a lever pressing against the side of the perspex disc.
When the circuit was at rest, the lever engaged into one of the fifty notches
cut into the circumference of the disc, so that the circuit in contact A was
broken. To start the rotation of the disc at the end of a kilometre stretch,
the driver pressed a switch on the dashboard of the van, closing the second of
the circuits and thus starting the motor. The switch was kept pressed in
until the disc had started to rotate, pushing in the lever of contact A and
thus closing the first circuit. The disc therefore continued to rotate until
the lever slipped into the next notch, breaking the circuit again. In this way,
rotation of the disc was started by the driver and stopped automatically when
the next tube was correctly aligned under the funnel.

Since contact A closed the circuit by having its lever pressed in, it was found to be very sensitive to jolting when travelling on rough roads. To prevent the disc thus being accidentally set off, a plunger (see Fig. 1) was mounted so that it engaged into a notch on the disc. The plunger was spring-loaded, the tension being adjusted until it was sufficient to prevent accidental movement of the disc, but not too great to prevent the motor rotating the disc.

To prevent the flies escaping from the tubes once they had rotated, the perspex disc was covered by a "floating" aluminium plate, which acted as a lid for all the tubes except the one under the funnel (the aluminium plate can be seen in Plate 4 covering the perspex disc). To record when all fifty tubes were full, a perspex pin (see Fig. 1) was mounted on the underside of the disc. After the 49th tube was full, the pin engaged with contact B, illuminating a light mounted on the dashboard and thus warning the driver that the last tube was being filled.

In the first season (1971), a cruder equipment was used (Plate 5) consisting of six jars mounted on a rotating disc, unlike the fifty tubes used in 1972 and 1973. Each jar was 10 x 16 cm and had its bottom replaced by polyester gauze. Because the jars were so bulky, they could not be stored in large numbers in the van. So as soon as all six jars were full, the catch in each one had to be killed with ethyl acetate and then be emptied into smaller tubes. This involved a considerable wastage of time during fieldwork, so that in 1972 and 1973, the equipment was modified to that described above.
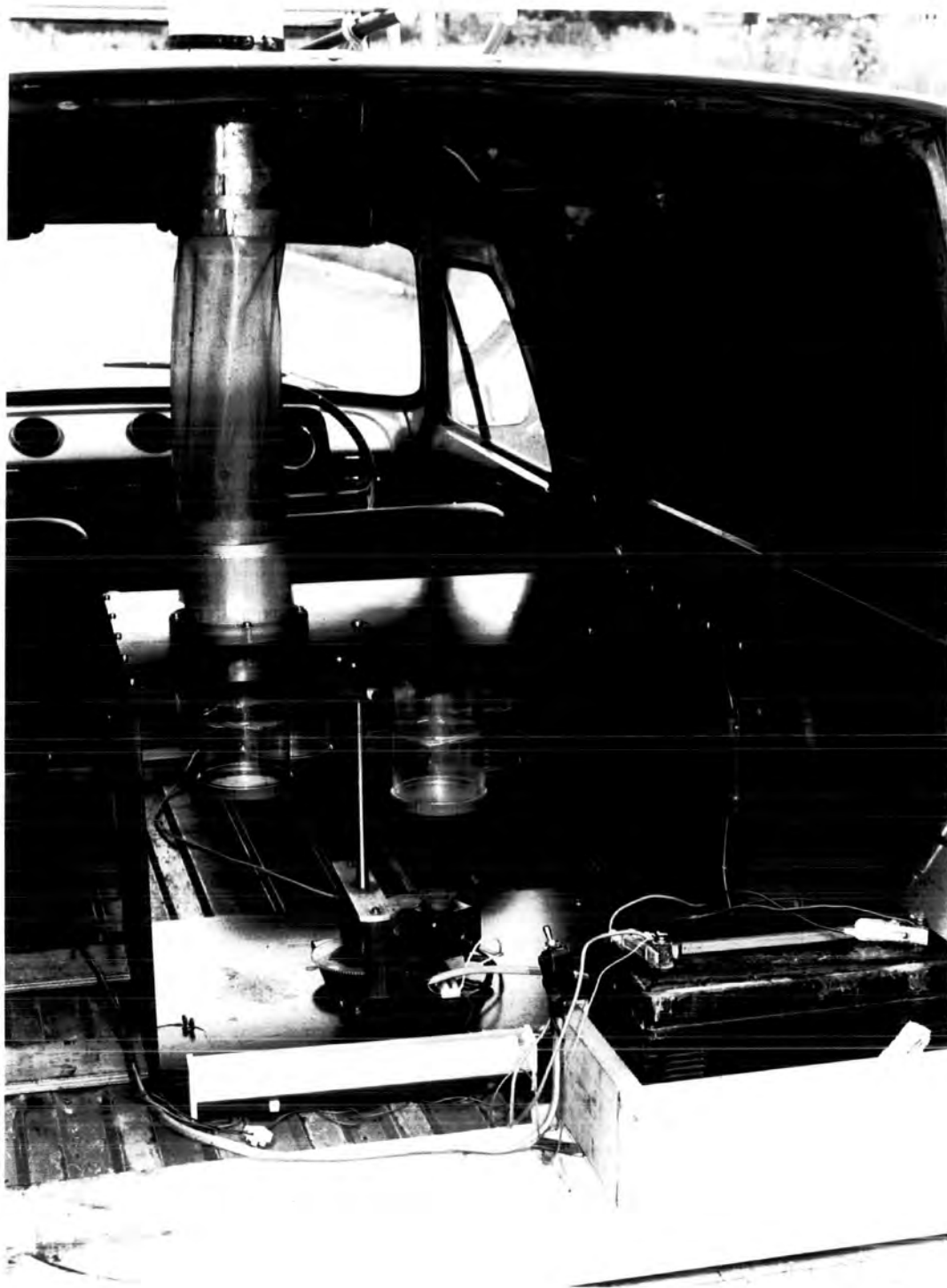
PLATE 5. The old collecting equipment (1971)

3. COLLECTING AREA

The van was used in the Eden Valley (Cumberland) in an area about 6 km north-east of Penrith between the villages of Langwathby, situated on the banks of the River Eden, and Melmerby (shown in Plate 6), at the foot of the scarp slope of the Pennines. The Eden Valley at this point was approximately 18 km wide, the south-east boundary of the valley being the Lake District and the north-east boundary being the Pennines. In the area of study, the valley was fairly flat (see Plate 6), but dissected by a small stream, Briggle Beck. The valley was a very intensive dairying region with large fields containing cattle, and to a lesser extent, sheep. There were also supporting crops such as oats, barley and hay. Consequently, the land was fairly open, but there were many small patches of woodland (Plate 6). Apart from the main road, the roads used were narrow lanes (Plate 2) and were usually bordered by low hedges (under 2 m), stone walls or fences (Plate 1). However, several stretches were lined with trees or woods, the distribution of these stretches being shown in Fig. 53.

The scarp slope of the Pennines formed a sharp boundary to the valley floor, rising over 350 m in less than 1.5 km (Plate 1). It therefore probably formed a barrier to insect flight activity, this effect being increased by the frequent adiabatic winds which blew downhill, generated by the steep slope.

The River Eden was the only large river in this area, apart from the River Eamont which flowed across the other side of the valley (see Fig. 4). The River Eden (Plate 7) was a wide, shallow, fast-flowing river, with frequent rapids along its length. It therefore provided ideal breeding conditions for the two main simuliid species, S.reptans L. and S.equinum L.

PLATE 6. Eden valley viewed from the scarp slope. Melmerby (at
the foot of the slope) can be seen in the foreground.
Lake District in the background.

PLATE 7. River Eden at Langwathby. A fast-flowing shallow river,
providing ideal breeding grounds for S.reptans and S.equinum.

4.   FIELD PROCEDURES

In 1971, using the large containers described earlier, catches were made over a considerable distance of road.   This was done partly to give a catch containing a reasonable number of flies necessary for statistical analysis, and partly to reduce the frequency with which the containers had to be emptied. This was necessary because much time was wasted during the fieldwork in having to kill the flies and transfer them to smaller tubes which could be stored in the van.

Catches were made on the four roads parallel to the River Eden.   These roads were at the following approximate distances from the river:-
Road 1 = 0.5 km; Road 2 = 1.5 km; Road 3 = 3.0 km; Road 4 = 6.0 km.
The catch on each road consisted of two round trips (that is 4x the road length).   On each road, the catch was therefore of the following length:-
Road 1 = 18.1 km; Road 2 = 12.0 km; Road 3 = 12.8 km; Road 4 = 16.0 km.
The catches were done in a circuit in the order:- Road 4, Road 3, Road 2 and Road 1.   This took 2.5 h, so that in the entire day only four circuits were completed, compared with nine circuits per day in the following years.

In 1972 and 1973, the van was driven along a circuit of 44 km, comprising the main road between the two villages of Langwathby and Melmerby (A686), and the four roads at right angles to this, roughly parallel to the river.   The circuit was divided up into approximately 1 km stretches (Fig. 3) using the van's tachometer, marking the divisions with strips of white cloth tied to the hedge or wall.   The driver therefore rotated the perspex disc at the end of each kilometre streth, to bring a fresh tube into the collecting position. Since the tachometer was not very accurate, in order to eliminate any differences in the length of the catches, the number of flies in each catch was calculated as a concentration (number of flies/2000 m^3 of the filtered air), using the readings obtained from the anemometer.
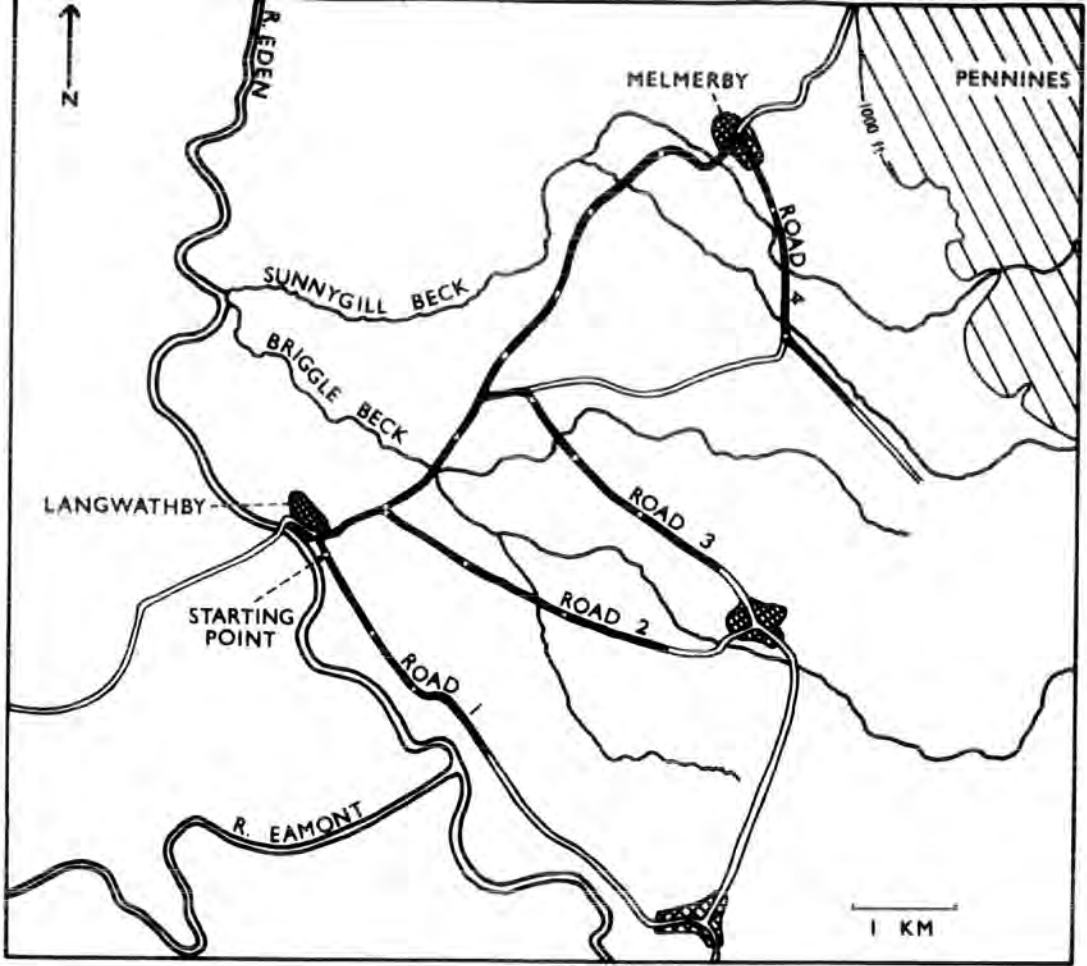
FIG. 3 Map of the Eden Valley, showing the collecting circuit in 1972-73. (roads marked in black).

FIG 4 Map showing collecting circuit in 1971 (roads marked in black), and larval collecting points (crosses)
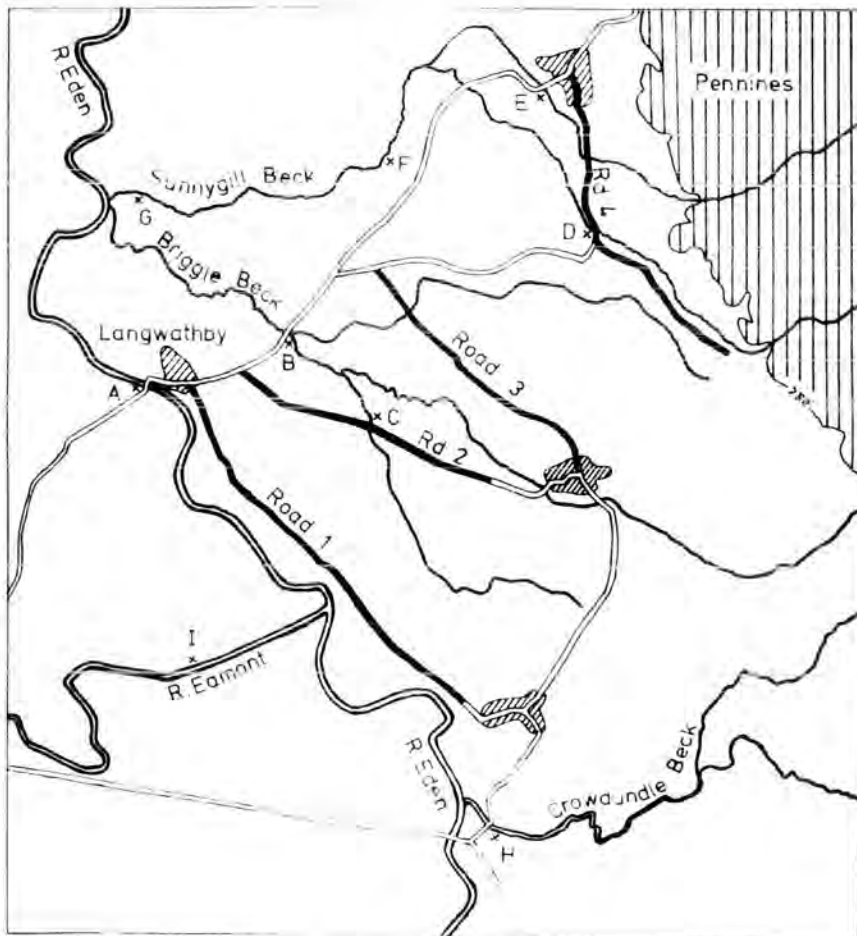
Each circuit started at Langwathby and was divided into two parts. The first part consisted of catches in the following order:- six catches along Road 1 (three up to the terminus opposite the River Eamont, then three on the return journey - see Fig. 3 where the kilometres are marked by white spots); seven catches along the main road from Langwathby to Melmerby; six catches on Road 4; then seven catches back along the main road to Langwathby. The first part thus consisted of 26 catches and took approximately 40 mins. The tubes containing the "kilometre catches" were unscrewed from the perspex disc, sealed with their plastic lids and placed in a labelled polythene bag. This, together with taking the temperature and wind velocity (see next section) took approximately ten minutes.

The second part of the circuit consisted of:- three catches on the main road, from Langwathby up to the start of Road 3; six catches along Road 3, returning to the main road; two catches on the main road to the start of Road 2; six catches along Road 2; and a final one catch along the main road back to Langwathby. The second part therefore consisted of 18 catches, taking approximately 30 mins. The tubes were then removed and placed in the polythene bag with those from the first part of the circuit. A fresh set of tubes were screwed into the disc, and temperature and wind speed measurements were taken. This took approximately 20 mins. Thus the entire circuit took approximately 1 h 40 mins, compared with 2 h 30 mins in 1971, and eight circuits were completed per day in 1972, compared with four in 1971. In 1973, the number of circuits was increased to nine by starting earlier.

The polythene bags containing the tubes of flies were stored in a large insulated polystyrene box (76 x 47 x 32 cm) and a layer of ice cubes placed on top to immobilise the insect catch (otherwise they all formed a sticky ball from which it was difficult to extract the simuliids). Deep frozen ice cubes (-20°C)

were found to be successful at keeping the flies frozen throughout the collecting period (from 06.00 h to 02.00 h = 20 h), whereas crushed ice melted within eight hours on a hot day, and ice blocks were found not to cool the flies sufficiently. The flies were thus kept near freezing point until they were brought back to the laboratory at the end of the day, when they were put into a deep-freeze to await dissection. However, it was found that if the flies were kept in the collecting tubes for more than a week, they dehydrated due to freeze-drying (since one end of the collecting tube was only covered by netting). If they were not going to be dissected immediately, they were therefore transferred into small sealed tubes.

5.   THE WEATHER

Weather conditions have been shown by previous workers to have a marked effect on the flight activity of simuliids, for example by Anderson and DeFoliart (1961), D.M. Davies (1952), L. Davies (1957a and 1963), Peterson and Wolfe (1958), Rubtzov (1939), and Wolfe and Peterson (1960).

Simuliids have a temperature threshold, below which flight activity is severely curtailed. Thus D.M. Davies (1952) and L. Davies (1963) found that flight activity of S.venustum Say in Ontario, Canada, was markedly reduced at temperatures below 12°C. Anderson and DeFoliart (1961) studying a number of simuliid species in Wisconsin, U.S.A., similarly found a threshold at 12°C. However, Wolfe and Peterson (1960) in Quebec, Canada, studying mainly S.venustum concluded that the temperature threshold was at 7°C.

High temperatures also reduce flight activity. D.M. Davies (1952) considered that this was due to the resulting reduction in humidity, and thus an increase in the rate of water loss by the flies. He found (D.M. Davies 1952) that activity in S.venustum decreased in temperatures above 27°C, while Wolfe and Peterson (1960) found a reduction at temperatures above 32°C. Rubtzov (1939) found that in a number of Russian species, activity was reduced at temperatures above 29°C.

Greater flight activity was found in cloud conditions compared with sunny conditions by Anderson and DeFoliart (1961), D.M. Davies (1952), Peterson and Wolfe (1958), Rubtzov (1939), and Wolfe and Peterson (1960). This was probably also due to the higher humidity found under cloudy conditions. High humidity (70 - 90%) was found by D.M. Davies to increase activity. Similarly, Rubtzov (1939) found activity greatest between humidities of 75 - 90%, and Peterson and Wolfe found it greatest between 70 - 95%. However rain was found by these authors to reduce activity, possibly because it is usually associated with lower temperatures.

A reduction in light intensity produces an increase in flight activity. Wolfe and Peterson (1960) found activity greatest at 15 ft-candles (163 lux) so that there was an evening peak of activity. However very low light intensity levels markedly reduced flight activity (D.M. Davies 1952), so that little activity was found at night. Peterson and Wolfe (1958) found that activity had virtually ceased by the time the light was reduced to 11 lux.

Wind also markedly inhibited flight activity. Thus Peterson and Wolfe (1958) and Wolfe and Peterson (1960) found activity reduced when the wind velocity was over 3 km/h; Anderson and DeFoliart (1961) and L. Davies (1957a) found activity reduced for winds over 8 km/h; but D.M. Davies (1952) only found a reduction at winds over 24 km/h. Wind possibly has an effect by increasing the rate of evaporation from the flies (D.M. Davies 1952).

Because the principal object of this research was to compare catches on different roads (and therefore taken at different times), it was important to have as near constant weather conditions as possible, so that the effect of the weather could be ignored when comparing the catches. Furthermore, since large catches were necessary for the statistical analysis, it was important

for the weather to be suitable.   No collections were therefore made on days
in which there was a wind over 5 km/h, or when there was rain, since these
factors were found to markedly reduce flight activity.   About half the days
were eliminated because even at midday the temperature hardly rose above 13°C,
so that fly concentrations remained extremely low.   As far as possible, catches
were made on days when it was either sunny all day or else cloudy all day,
since it was found that when the sun went behind a cloud, there was a dramatic
change in the flight activity patterns (see chapter 3).   It will be appreciated
that this did not leave many suitable days.

6.   WEATHER RECORDING

At the start of each half of the circuit (or in 1971, each full circuit)
the temperature was noted from a thermometer kept in the shade of a hedge.
The wind velocity was recorded using a small propellor-driven anemometer,
taking a reading over a two minute period at a height of 180 cm above the
ground (approximately level with the lower lip of the net).   The wind direction
was noted.   During each "kilometre catch", the presence or absence of sunshine
was noted, so that if the sun shone for 50% or more of the time the catch was
taken as being "sunny", if less than 50% it was "cloudy".   Since each catch
only lasted about 1.5 minutes, the result was a fairly continuous recording
of the amount of sunshine.   Similarly, the presence or absence of rain was
recorded.   In the evenings, light intensity readings were taken at ten minute
intervals using a selenium cell photometer fixed to a window of the van (see
Plate 2), and the time of sunset was noted.

7.   SPECIES PRESENT

The Simuliidae caught were almost entirely S.reptans L, S.ornatum Meig,
and S.equinum L.   The flies caught in the three seasons 1971 - 3 consisted of:-

65.6% S.reptans L (31,352 flies)

17.1% S.ornatum Meig. (8,198)

13.9% S.equinum L (6,648)

1.8% S.variegatum Meig. (846)

0.9% S.aureum group - probably mainly S.angustipes Edw. (417)

0.5% S.latipes group - probably mainly S.dunfellensé Davies (261)

0.2% S.monticola Fried. (88)

8.   DISSECTION

All the female flies were dissected. They were immersed in saline solution with a drop of detergent added to help wet the specimens, and the abdomen were opened by a median cut through the ventral sterna using microneedles (0.1 mm diameter tungsten steel pins). In each dissection, the following were noted:-

1.   The ovaries. These were used to determine the physiological age of the fly.

a)   The ovaries were divided into:-

i)   Nulliparous. These were flies which had not laid eggs before and so were "young" flies, still in their first gonotrophic cycle. The ova were tightly packed together; the trachea were coiled at their ends; the oviducts were usually narrow (though this was not found to be very reliable); and there were no corpora lutea.

ii)   Parous. These were flies which had already undergone a gonotrophic cycle and so were relatively "old" flies. The ova were loosely arranged (as a result of the ovary having enlarged and contracted during the previous gonotrophic cycle); the trachea tended to be unravelled at their ends; there were corpora lutea and often relict eggs present; and the oviduct was usually wide.

The parous features could not be reliably identified in gravid flies (which had fully mature eggs) because the corpora lutea had by that stage shrunk to an inconspicuous size, and the large eggs had caused the ovaries to expand, obscuring the other characters. Gravid flies were therefore kept in a separate category.

Detinova (1958) and L. Davies (1961), using the number of dilations of the intima of the ovarioles, subdivided the parous flies according to the number of gonotrophic cycles they had undergone. This was not done in the present work due to the small size of the ovaries and the large number of flies dissected (over 57,000 females of which the majority were parous).

b) The ova were subdivided into five stages of development (Plate 8), based on the classification of Wanson and Lebied (1948) and Ruhm (1970):-

Stage 1 - 50 to 90μ long, colourless lacking in yolk.

Stage 2 - 65 to 130μ long, some yellowish yolk present.

Stage 3 - 80 to 155μ long, ovum filled with yolk but still small.

Stage 4 - 145 to 245μ long, larger but still roughly spherical.

Stage 5 - 220 to 310μ long, fully mature, triangular in shape.

2. The blood meal. Blood in the mid-gut was divided into the following categories:-

a) Fresh - bright red, semi-liquid.

b) New - medium red, gelatinous.

c) Old - dark red and gelatinous, or black and solid.

The three main species of Simuliidae present were anautogenous, requiring a blood meal for the development of their ovaries. Consequently, all the flies with Stage 3 or Stage 4 eggs had blood present in the mid-gut.

PLATE 8   STAGES OF OOCYTE DEVELOPMENT IN S.REPTANS

× 160
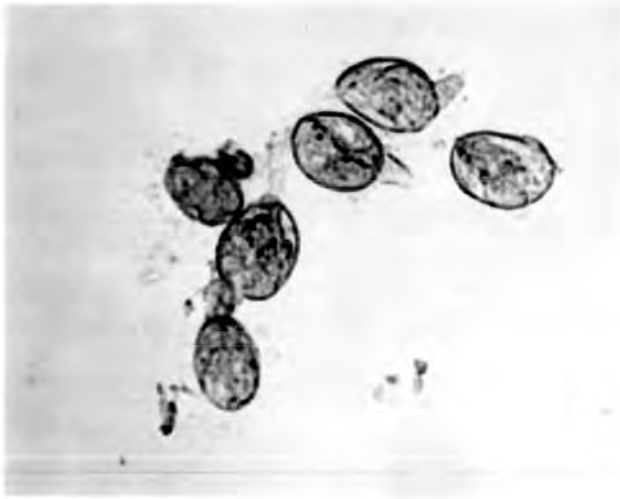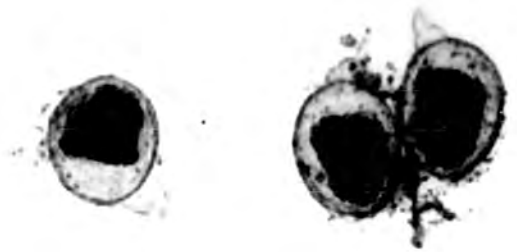
STAGE 1

STAGE 2

STAGE 3

STAGE 4

STAGE 5

91.8% of Stage 2 flies had a blood meal, while between 0.7 and 6.0% of the
Stage 1 flies had had a blood meal.   Therefore, the eggs will not develop
beyond Stage 2 without the fly having a blood meal.

3.   The crop.   The dilation of the crop was noted but not used in
the analysis since its volume was found to be affected by dehydration in the
deep-freeze.   Thus 50% of the freshly caught flies had large crops, compared
with less than 10% of the flies which had been kept for four months in the
deep-freeze.

4.   Parasites.   These were mermithids, micraosporidia and fungi.

9.   THE BREEDING SITES

Since this research was principally concerned with the spatial distribution
of the different simuliid species, it was necessary to know their breeding ground
and thus the direction and distance which the flies had travelled.   Consequently
samples of larvae and pupae were taken from nine samples in the area covered
(see map, Fig. 4), sampling all the streams present.

The sampling points were as follows:-

A.   River Eden at Langwathby (see Plate 7), national grid reference NY336565.
The river was about 25 m wide, very shallow (under normal conditions, most
of the river was less than two metres deep), with extensive rapids.   The
larvae and pupae were found on the River Crowfoot (Ranunculus fluitans Lamb.),
which can be seen in Plate 7.   This sample site was representative of most
of the River Eden.

B.   Briggle Beck, where crossed by the main road (grid reference NY341584).
The stream was two metres wide, but slow flowing with few rapids.   Pupae
were found on vegetation trapped in the rapids.

C. Williekeld Sike – a tributary of Briggle Beck, sampled where it was crossed by Road 2 (grid reference NY593330). The stream was 0.5 m wide with a gravel bottom. Vegetation trailing in the stream was sampled.

D. Sunnygill Beck, where crossed by Road 4 (grid reference NY618356). The stream was three metres wide, shallow with rapids over much of its length. The stony bottom was heavily encrusted with algae and some moss, with little other vegetation present.

E. Dale Beck, a small tributary of Sunnygill Beck, sampled in the village of Melmerby (grid reference NY 614372). The stream was 0.5 m wide, with very little flow of water.

F. Sunnygill Beck, opposite section 1 of the main road (grid reference NY614372). The stream was 0.5 m wide, with very little flow of water.

G. Sunnygill Beck at the village of Little Salkeld (grid reference NY565360). The stream was 2 – 3 m wide, with a rapid flow of water amongst rocks. Pupae were collected from trailing grass.

H. Crowdundle Beck, sampled at grid reference NY608282. The stream was four metres wide and very shallow, with rapids along its length over a stony bottom. Pupae were collected from trapped dead vegetation.

I. River Eamont, sampled at grid reference NY573304. The River Eamont was up to 25 m wide, but only had short stretches of rapids, most of the river being very deep and slow flowing. Pupae were collected from Ranunculus fluitans.

Samples taken on 19:vi:73 at the nine sites contained the following number of pupae:-

Species	A	B	C	Sample sites D	E	F	G	H	I
S.reptans	318	-	-	-	-	2	52	41	291
S.equinum	313	2	-	-	-	-	12	-	410
S.ornatum	54	31	2	6	-	8	6	-	16

The samples were the result of five minutes collecting at each site, and so were only roughly comparable. The River Eden sample (A) was in fact a gross underestimation compared with the other samples. This was due to the large clumps of Ranunculus fluitans which acted as a substrate for the larvae and pupae. Only small samples were taken for each clump, so that the five minutes search was therefore made inefficient for this collecting site.

The only major breeding sites for S.reptans were therefore at sites A, G, H and I. Site G was at the mouth of Sunnygill Beck and was remote from the collecting area (2.5 km from the nearest point on the main road). Since other sections of Sunnygill Beck (F, E and D) contained virtually no S.reptans, breeding was probably confined to a short stretch around G where the stream was faster flowing, and so would have little effect on the total adult population. It was possible that S.reptans may have bred over a short stretch at the mouth of Briggle Beck, though no samples were taken from this region. However, no breeding occurred within the collecting area (B and C), so that like Sunnygill Beck, it would have little effect on the adult population. Crowdundle Beck was probably also unimportant, being 3.5 km from the nearest part of the collecting circuit (shown in Fig. 3).

Therefore the only major breeding areas were the River Eden and the River Eamont. These not only had higher concentrations of pupae than the other streams, but because of their relatively vast width, and thus far greater breeding area, they would make the populations on the other breeding sites insignificant in comparison. However, as mentioned above, the River Eamont only had intermittent stretches of rapids separated by long stretches of deep calm water. It must therefore have had a much smaller overall population of larvae than did the River Eden, which had rapids all along its length. Furthermore, the River Eamont extended at right angles away from the collecting area. Virtually all of the adult S.reptans caught in the collecting area must therefore have come from the River Eden.

S.equinum had a similar breeding distribution, except that it did not occur in Crowdundle Beck. Therefore, like S.reptans, it was virtually restricted to breeding in the River Eden.

S.ornatum in contrast had a much smaller population in the River Eden. This can be shown by combining the number of pupae collected on the four days in which the River Eden was sampled in 1973 (25:v:73, 4:vi:73, 19:vi:73 and 3:vii:73) and thus reducing the effect of the seasonal rhythm of the different species:-

	Number of pupae	Percentage of total
S.reptans	1276	69
S.equinum	476	26
S.ornatum	92	5
S.variegatum	1	-

In 1973, <u>S.ornatum</u> therefore only constituted 5% of the simuliid population in the River Eden, whereas it constituted 23% of the adult flies caught in that year (compared with 68% <u>S.reptans</u> and 6% <u>S.equinum</u>). Many of the adult <u>S.ornatum</u> caught in the collecting area must have therefore come from Briggle Beck (sample B) and Sunnygill Beck (samples D, F and G).

Breeding in <u>S.reptans</u> and <u>S.equinum</u> was therefore largely restricted to the River Eden, while <u>S.ornatum</u> also bred in the smaller streams present.

10. UNITS AND USAGE OF TERMS

All concentrations in this thesis = number of flies/2000 m^3 of filtered air.

All means = arithmetic means, except for the diurnal rhythm where geometric means were used (see chapter 3).

All diurnal times were measured as British Summer Time (B.S.T.), which is one hour ahead of Greenwich Mean Time (G.M.T.).

The flight range has been taken to be the maximum distance at which adult flies have been found from their breeding ground.

The spatial distribution is the change in concentrations at different distances from the breeding ground.

All the tables and the graphs have been labelled as figures.

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III. DIURNAL RHYTHM OF FLIGHT ACTIVITY

1. INTRODUCTION

Many species of Simuliidae have been shown to have a definite diurnal rhythm of flight activity, though the rhythm breaks down under adverse weather conditions. This is because the activity is affected by the temperature, wind, humidity and light intensity, (see previous chaper).

Crosskey (1955) showed that S.damnosum had a diurnal biting rhythm consisting of a steady increase in activity in the morning, a peak at about 13.00 h, and a steady decrease throughout the afternoon. Le Berre (1966), Duke(1968) and Disney (1972) showed that this rhythm was the combined result of two separate rhythms, the nulliparous having an afternoon peak and the parous having a midday peak. L. Davies (1957b) similarly found in S.ornatum Meig. that there was a difference between the nulliparous and parous rhythms, the ratio of nulliparous : parous being highest in the early morning and late evening. He also found (Davies 1957a) that the rhythm consisted of a sharp evening peak with little other activity on a sunny day, while on a cloudy day, the rhythm broke down becoming irregular. Peterson and Wolfe (1958) working in E.Quebec (Canada) found that for a number of species (though predominantly S.venustum Say) there was a sharp dawn and evening peak. D.M. Davies (1952) working in Ontario (Canada), however, found no definite diurnal rhythm in S.venustum, but then his results were based on only a few days taken under very variable weather conditions, especially with high winds (up to 50 km/h).

All the above results were based on the use of bait trapping (in Africa, S.damnosum were collected when they landed on "fly-boys"; in Canada, flies hovering above humans were caught by sweep netting; and in England, L. Davies

caught S.ornatum which were feeding on cattle) and so caught only blood-thirsty flies. Some research has been done on ovipositing gravid females using hand-netting and sticky traps. For example, L. Davies (1957a) using sticky traps at oviposition sites found a post sunset peak for S.ornatum. Other research has been done by Davies and Peterson (1956); J.B. Davies (1962); Fredeen et al (1951); Peterson and Wolfe (1958); and Peterson (1959). But apart from oviposition, little else is known about the diurnal rhythm of flight activity for gravid flies. Nor is much known about the activity of blood-fed flies, or non blood-fed flies (other than those actively seeking a blood meal).

2. RESEARCH METHOD

Research was principally concentrated on the females of S.equinum L, S.ornatum Meig. and especially S.reptans L. The females of each species were divided into nulliparous (those which had not laid eggs before and so were "young"), parous (those which had laid eggs before and so were "old"), and gravid (those with fully mature eggs and so were too difficult to classify into the previous categories). Parasitised flies were excluded, being dealt with separately in chapter 6. The concentrations of each of these categories (expressed as number of flies/2000 m^3 of air) were compared at different times of the day along one of the roads. The data for 1971 could not be used because the old equipment (described in chapter 2) resulted in only four circuits being made each day at 2.5 h intervals. In 1972, using the new equipment eight circuits were made each day, while in 1973, by starting earlier, nine circuits were completed.

The concentrations were expressed as geometric means of the six "kilometre catches" made on each circuit along each road, (geometric means were used to reduce the effect of abnormally large or small catches).

FIG. 5 DIURNAL RHYTHM S. REPTANS 5:6:73 NULLIPAROUS
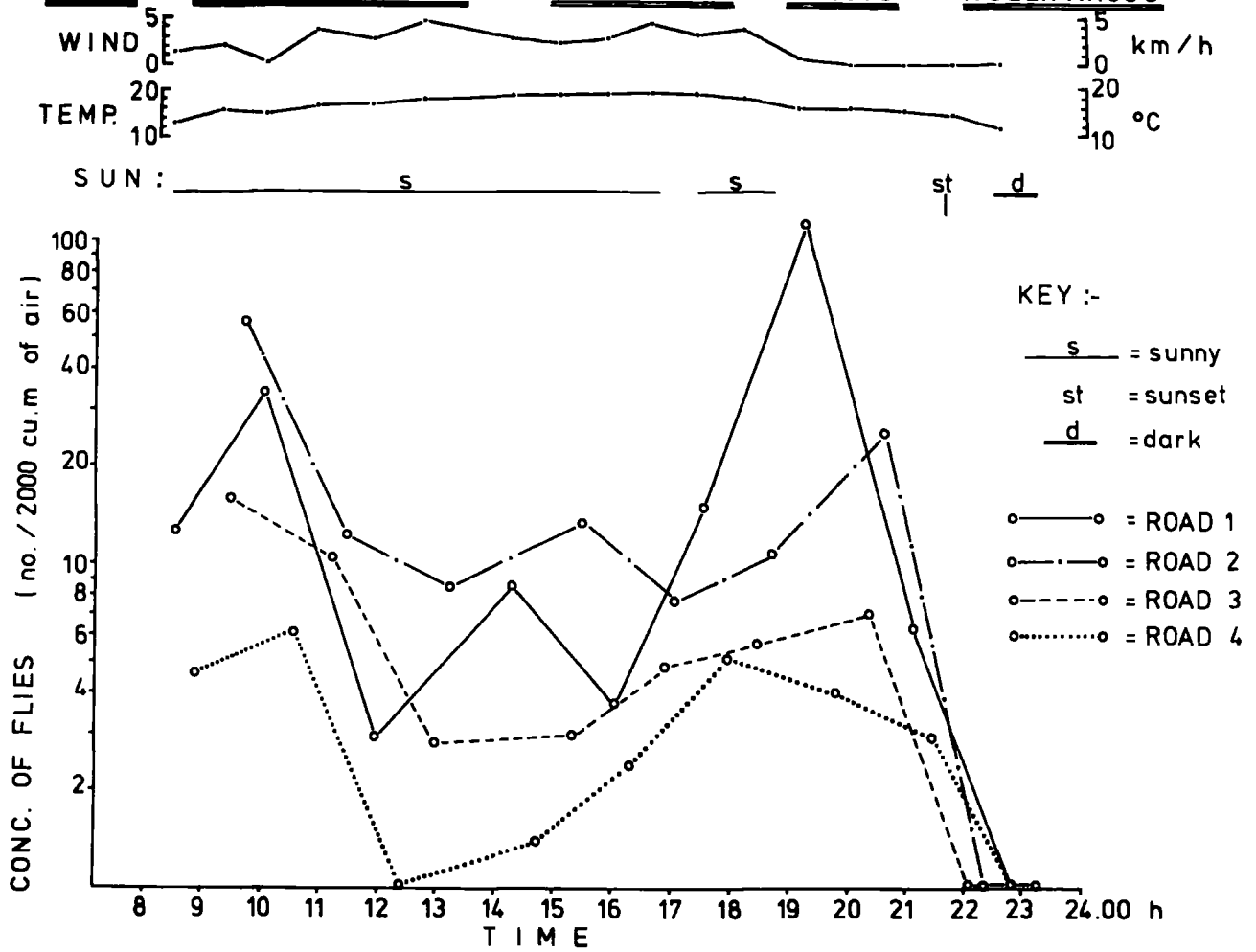
FIG. 6 DIURNAL RHYTHM S. REPTANS 27:6:73 PAROUS
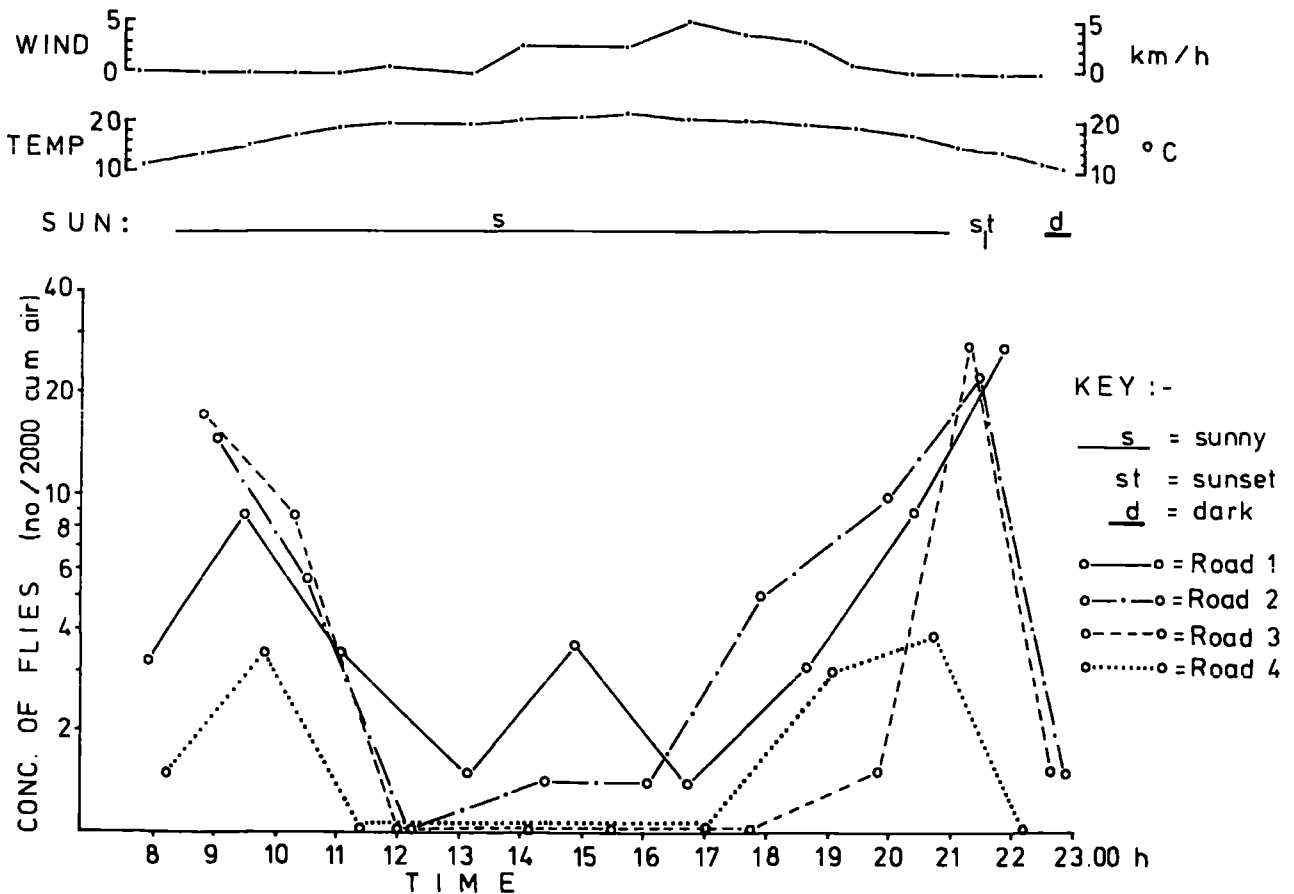
Only one road was used as an example for each day, because the four parallel roads were found to have the same diurnal rhythm (e.g. Fig. 6). However, it was found that the evening peak of activity was very brief, often less than an hour, whereas each circuit lasted 1 hr 40 min., so that on a particular road, it was possible for the peak to occur between circuits (e.g. Fig. 5, where the peak occurred while collecting along Road 1). The road on which the peak occurred was therefore used as the example for that day.

In 1971, a 24 h catch was made along a road running beside the River Tweed, starting at 12.30 h on one day and finishing at 13.00 h on the following day (Fig.20). This catch was almost entirely S.reptans. A 21 h catch was made in the Eden Valley on 12:vii:73 (Fig.19), starting at 03.45 h and finishing at 24.00 h.

3. DIURNAL RHYTHM OF S.REPTANS FEMALES

    a) Normal rhythm (sunny conditions)

The diurnal rhythm was most markedly seen on sunny days (Fig. 7-11 – N.B. in the graphs, presence of sunshine was recorded as a line above the graphs, breaks in the line = cloudy weather). There was a short peak of activity in the morning, followed by low concentrations in the afternoon. This difference was most marked on days with high temperatures (Figs. 7 and 8), and so was probably due to a reduction in humidity. In the evening, as the humidity rose again, there was a large peak of activity half an hour before sunset. This appeared to be synchronised by the rapid drop in light intensity rather than by the actual light intensity, since the peak occurred at light levels varying from 640 to 70 lux (measured by a selenium cell photometer fixed to the van window, see Plates 1 and 2).

FIG. 7   DIURNAL RHYTHM   S. REPTANS   18:7:72   ROAD 1
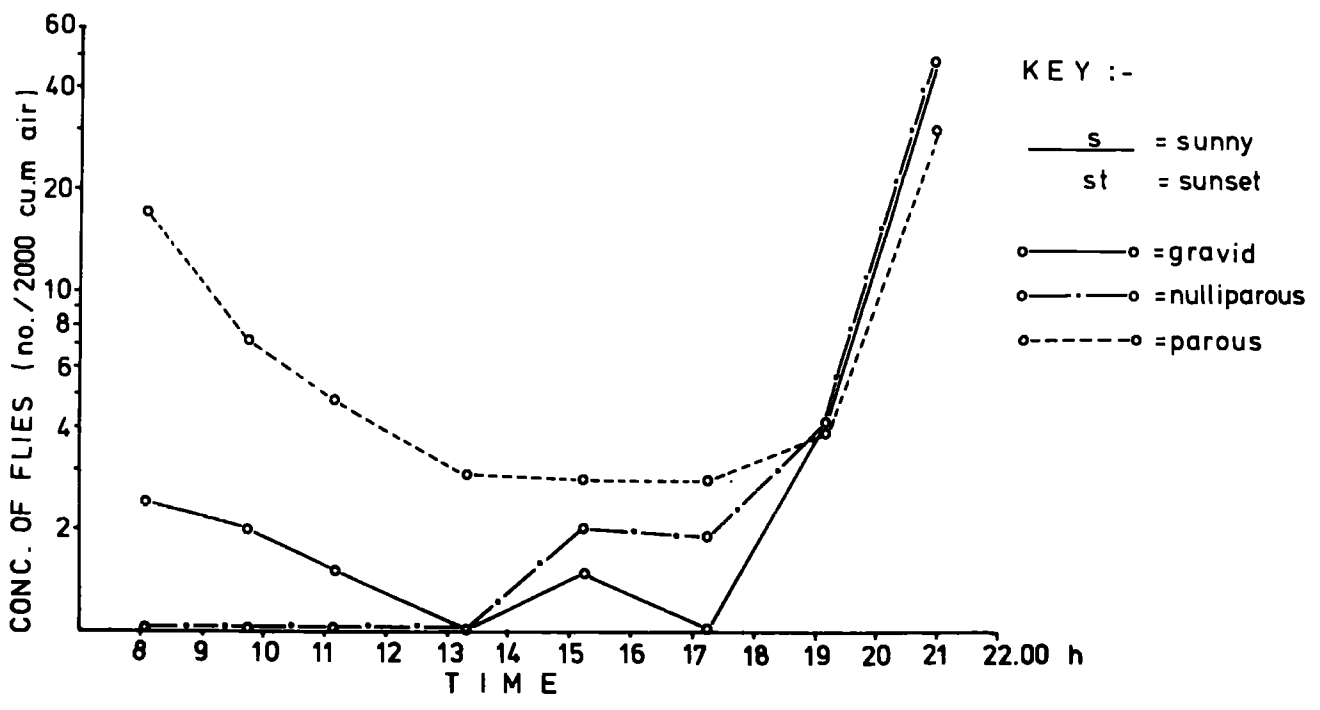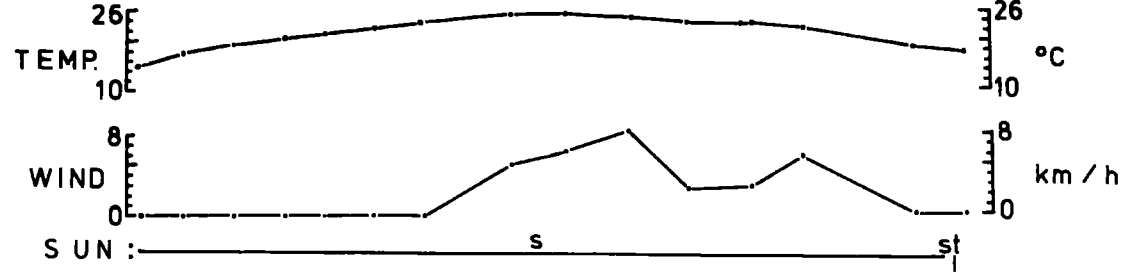
FIG. 8   DIURNAL RHYTHM   S. REPTANS   27:6:73   ROAD 2
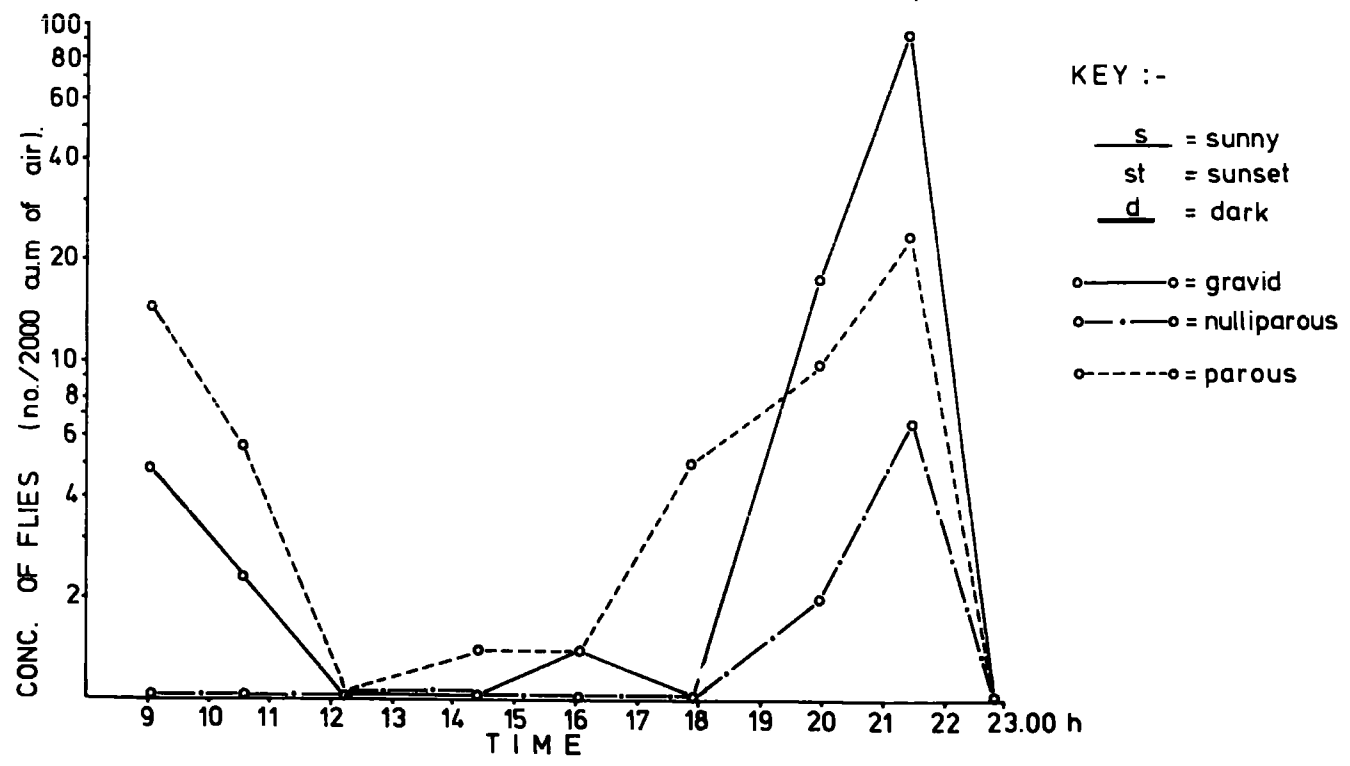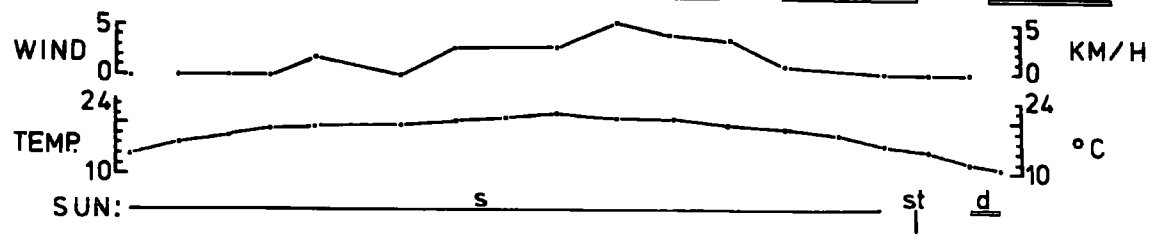
Flight activity decreased rapidly after sunset. This can be shown by plotting all the catches in the order in which they were taken during the evening. This has been shown for three sunny days in Figs. 28 - 30. Along the 'x' axis in each graph is plotted the catches, the break between Road 2 and Road 1 being the end of a circuit. These graphs, and Fig. 42, show that S.reptans had only a brief peak of activity, which in each case occurred before sunset though between different light intensity levels. Activity then fell off rapidly, and so had virtually ceased by half an hour after sunset. The 24 h catch in the Tweed Valley (Fig. 20) showed that activity was extremely low by dark (1 h after sunset) and remained low till dawn. In the nine circuits taken during the night (22.05 - 04.20 h) only eleven S.reptans females were caught, consisting of six gravid and five non blood-fed parous flies. These gave a mean concentration of only 0.3 flies/2000 m^3 of air, compared with a mean during daylight of 33.7 flies. The drop in activity at dusk was unlikely to be due to a lowering in the temperature (see below), since for example in Fig. 20, even during the middle of the night the temperature did not drop below $13^{\circ}C$, which was above the temperature threshold. The activity must therefore be controlled by the change in light intensity.

b) Effect of cloud on the rhythm

On 5:vi:73 (Fig.10), the evening peak was brought forwards to over two hours before sunset, instead of the normal half an hour. This was due to the sky clouding over, which causes a rapid reduction in the light intensity and a drop in the temperature (thus reducing the rate of evaporation). Figs. 11 and 12 show similar examples of the flight activity increasing when the sky clouded over. Figs. 13 and 14 show an extreme example where activity was principally limited to the cloudy period, despite being in the middle of the afternoon, decreasing again when the sunshine returned. Fig. 14 shows

FIG. 9   DIURNAL RHYTHM   S. REPTANS   26:5:71   ROAD 1
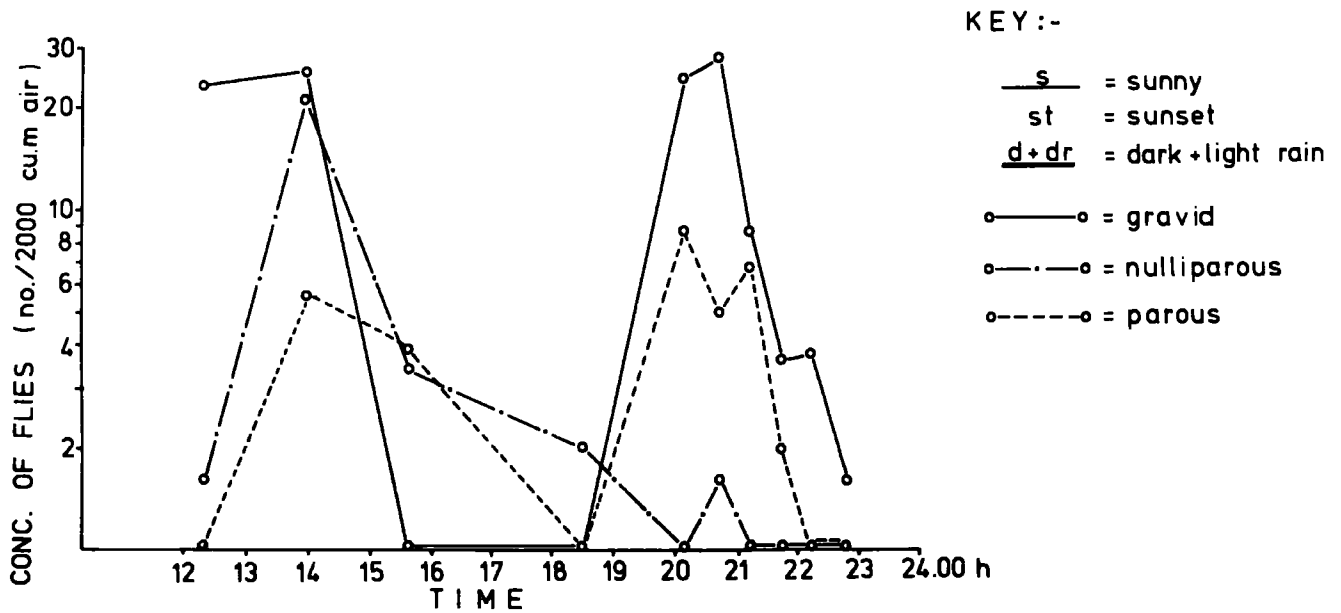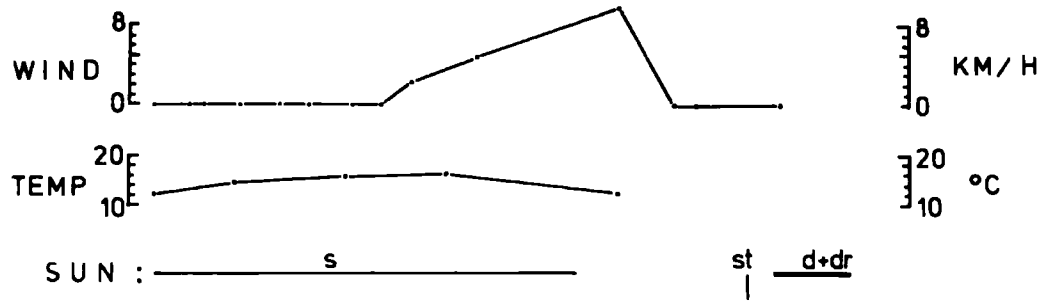
FIG. 10   DIURNAL RHYTHM   S. REPTANS   5:6:73   ROAD 1
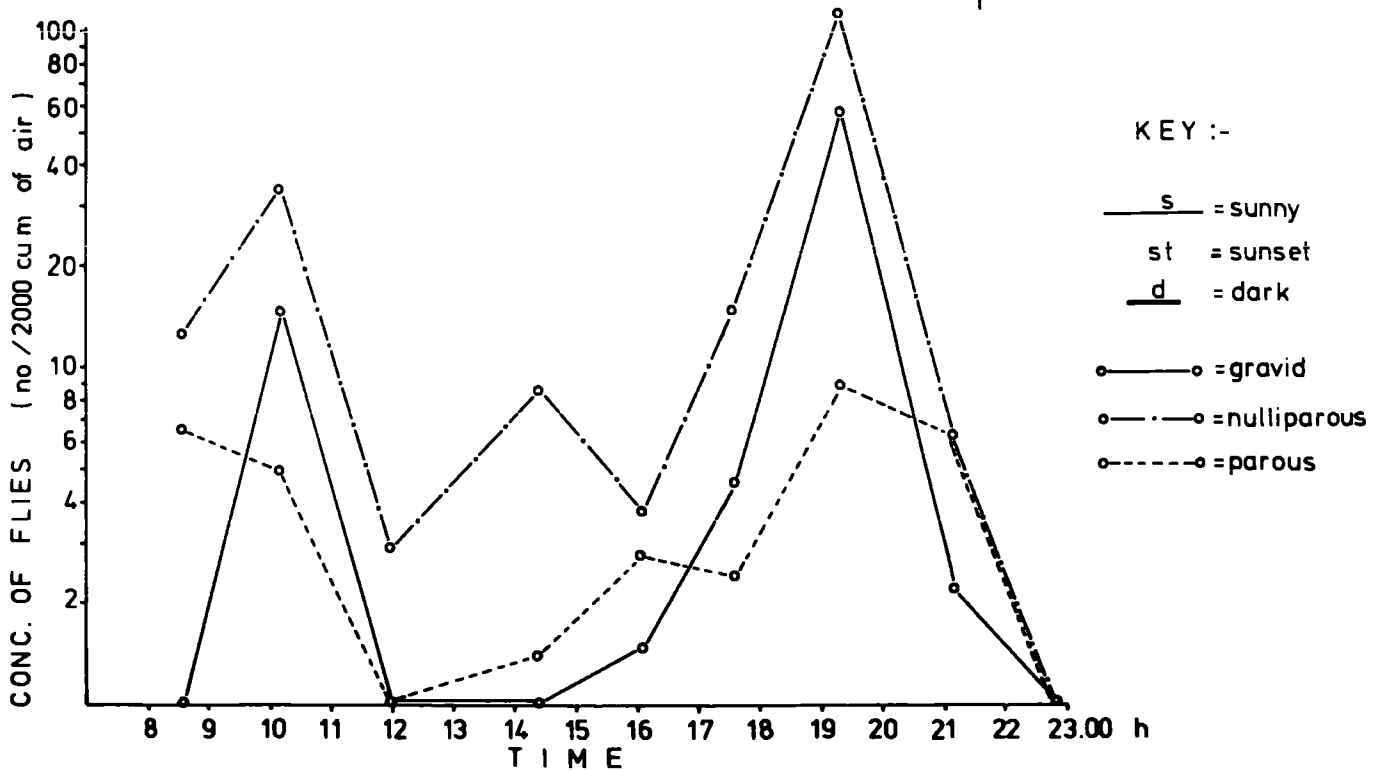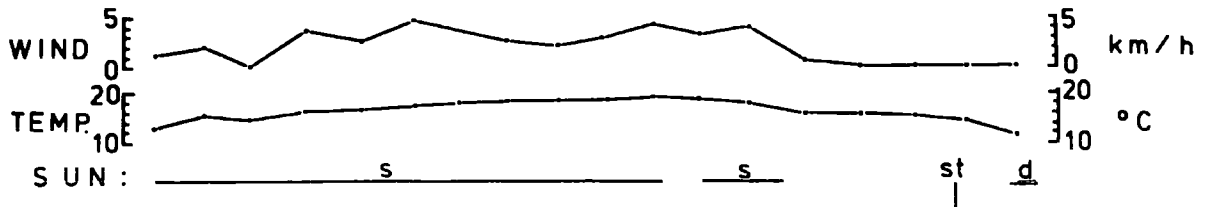
FIG.11 DIURNAL RHYTHM S. REPTANS 21:6:73 ROAD 2
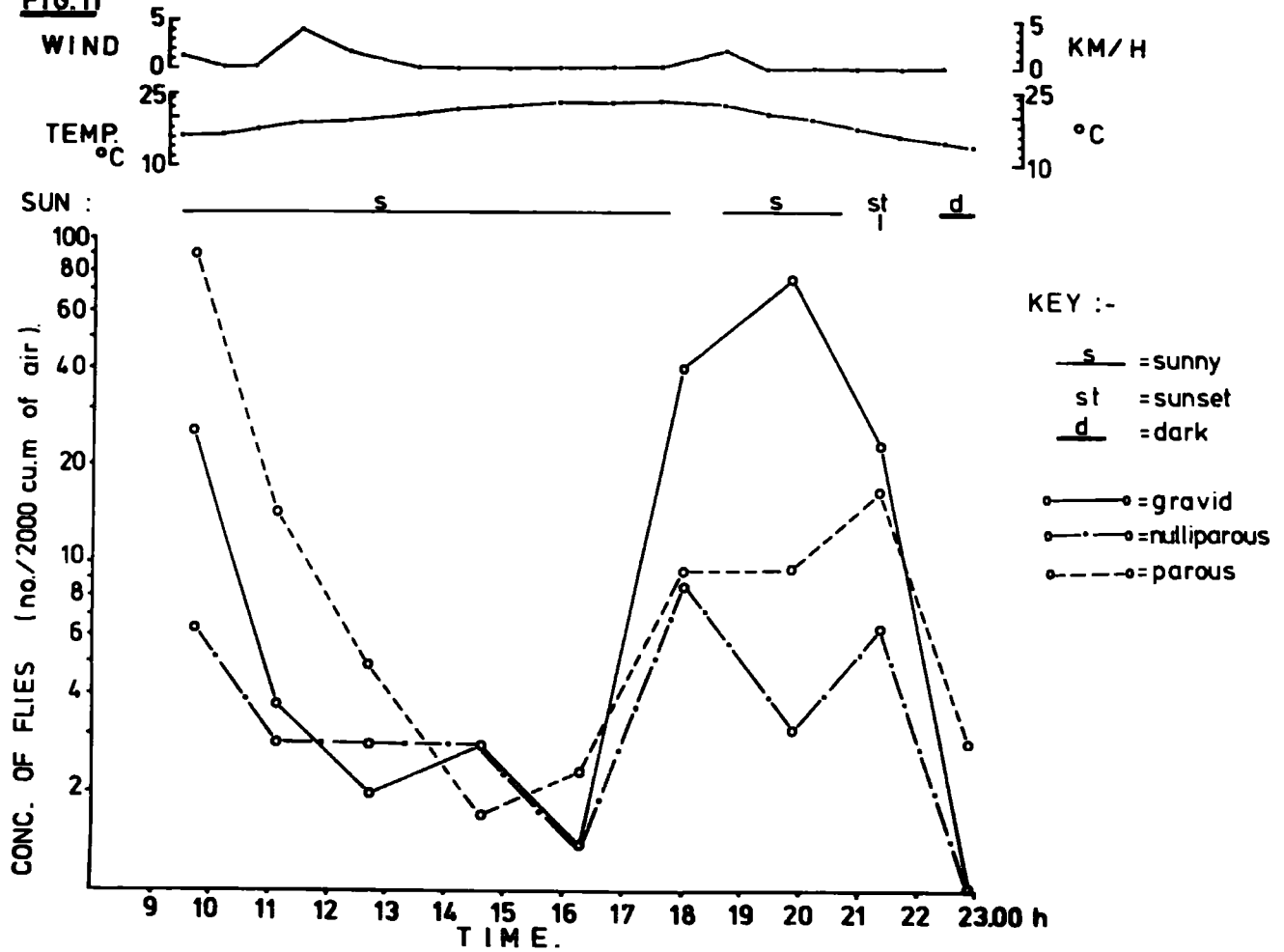
FIG. 12 DIURNAL RHYTHM S. REPTANS 14:6:72 ROAD 2
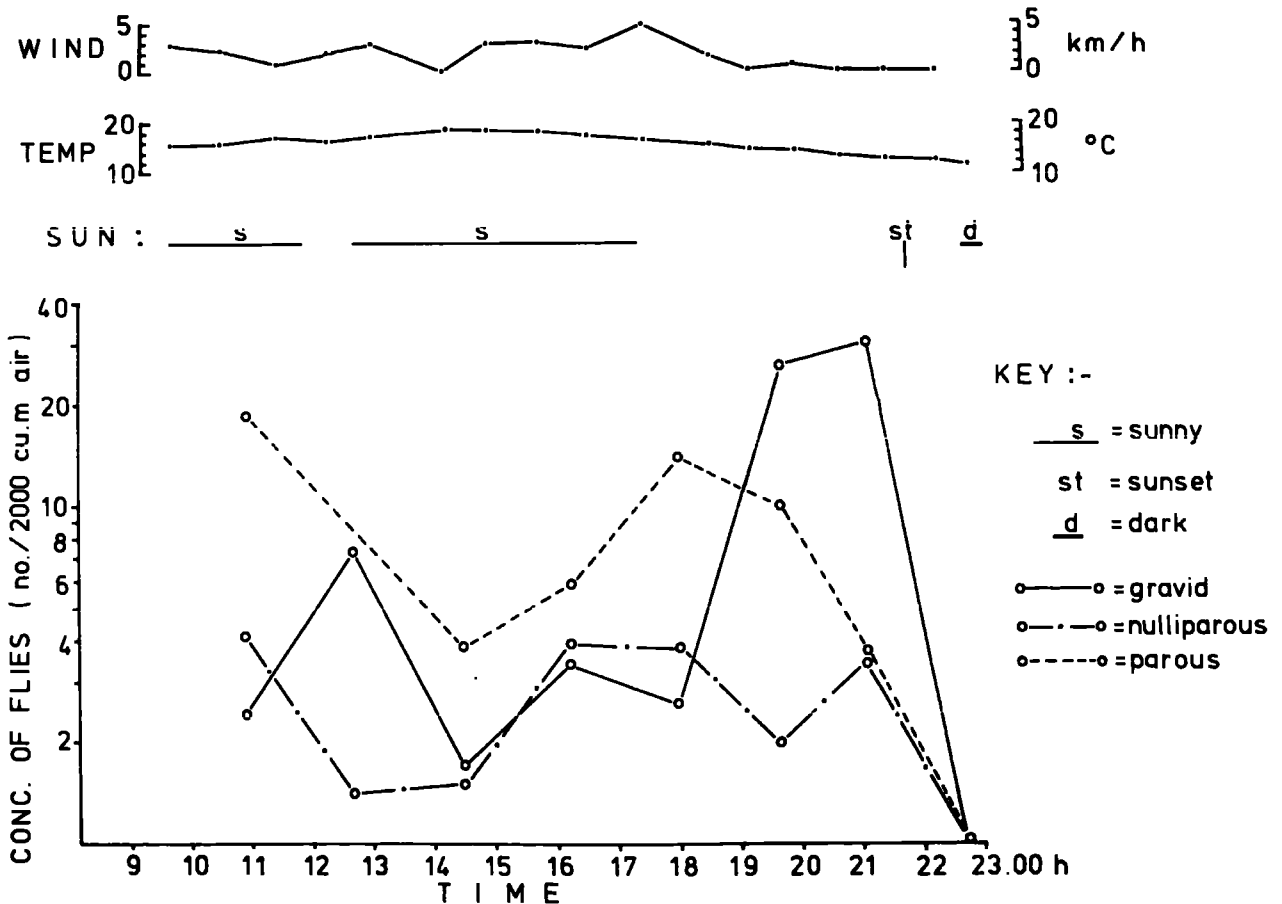
FIG. 13 DIURNAL RHYTHM S. REPTANS 31:5:73 ROAD 1
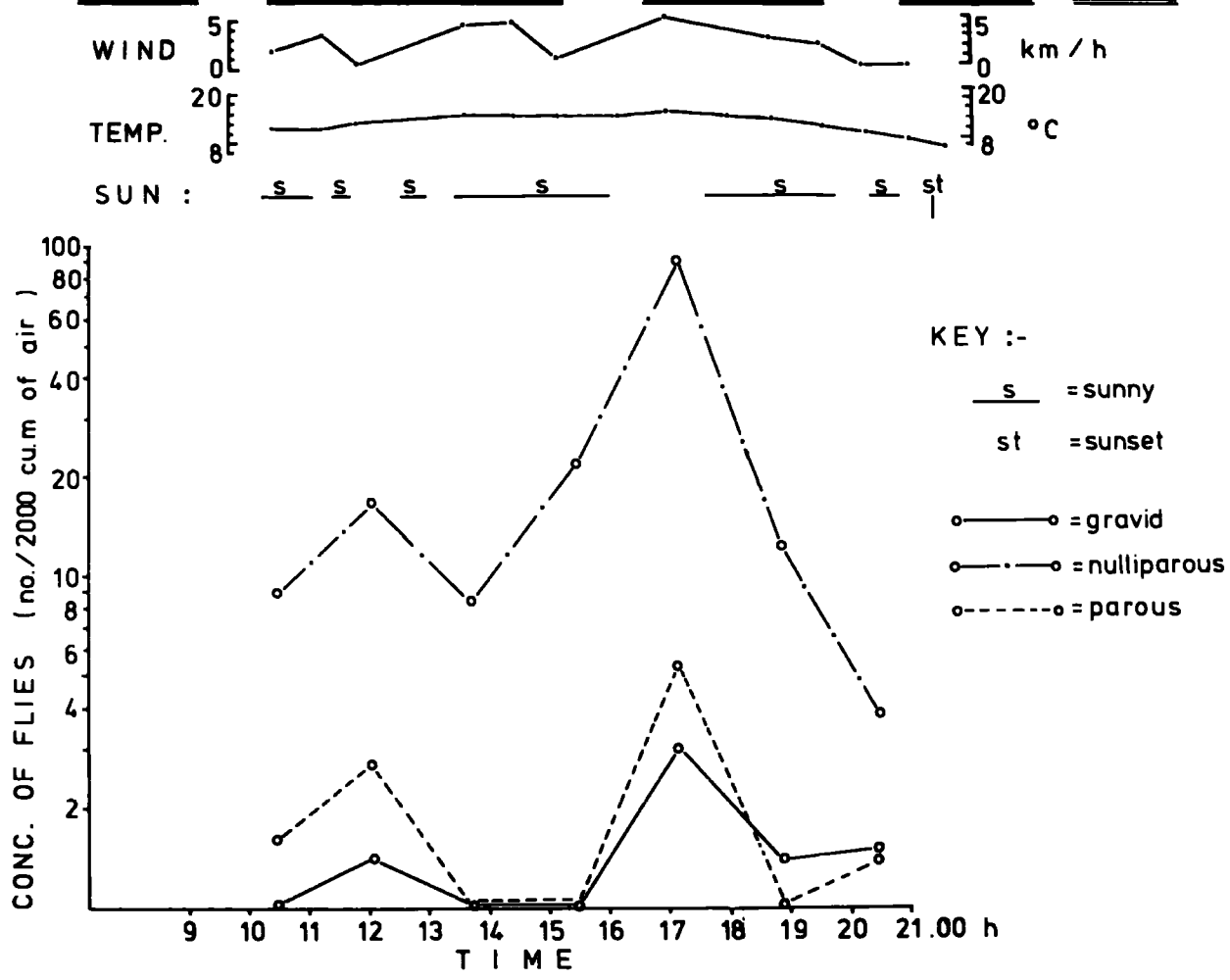
FIG. 14 DIURNAL RHYTHM S. REPTANS 31:5:73 NULLIPAROUS
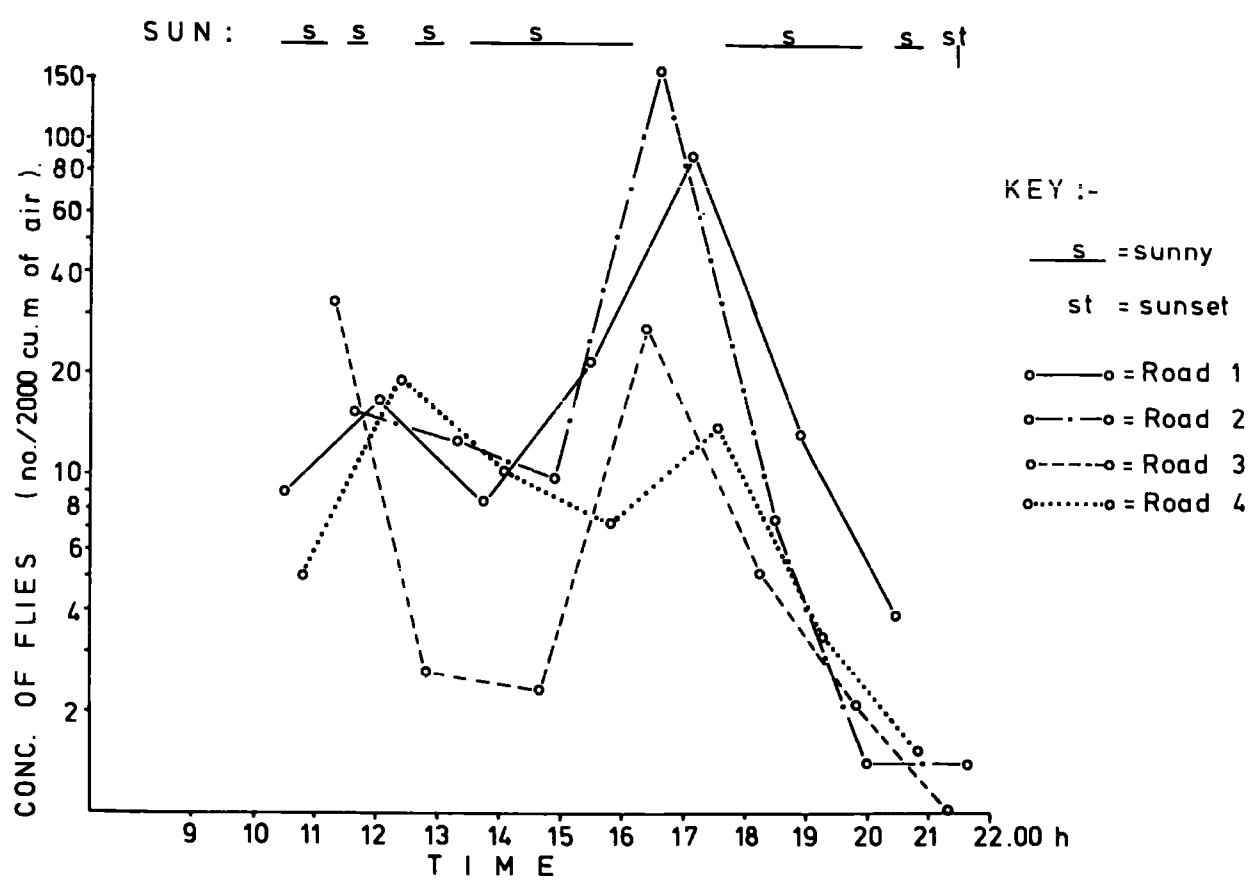
that this occurred on all four roads. Figs. 15 - 17 show two further examples. In Fig. 16, there were peaks of activity on Roads 1 and 4, but not on Roads 2 and 3, because no catches were taken on these roads during the cloudy period.

Figs. 18 - 20 show examples of completely cloudy days. Cloudy weather, with lower temperatures and higher humidities, produced a much less pronounced diurnal rhythm with higher concentrations throughout the morning and afternoon, though there was still a reduction in activity before the evening peak. Figs. 19 and 20 also show the presence of a brief dawn peak. Like the sunset peak, this was probably caused by a rapid change in the light intensity.

c) Effect of temperature

The reduction in flight activity in mid-afternoon (shown in most of the graphs, Figs. 7 - 19) was probably due to the humidity being lowest at this time of the day. When temperatures were high (over $20^{\circ}C$ e.g. Fig. 7 and 8), there were low concentrations throughout most of the day. High temperatures therefore probably reduce flight activity by increasing the rate of evaporation and lowering the relative humidity. In Figs. 7 and 8, there was only a brief morning peak of activity while temperatures were still low, and an evening peak which occurred after the temperatures had dropped again. In contrast to this, Fig. 9 shows a sunny day (26:v:71) in which the temperature remained low because it was early in the season, so that there was a prolonged morning peak extending into the early afternoon.

Low temperatures had a threshold at approximately $12^{\circ}C$, below which flight activity was severely inhibited. Thus the timing of the morning peak was dependent on the rate at which the temperature rose. This was clearly shown on 14:vi:73 (Fig. 21) when the temperature did not rise above the threshold until after collecting had started. This was also shown to a lesser extent on 27:vi:73. Thus on 14:vi:73, the concentration of flies on Road 1

FIG. 15 DIURNAL RHYTHM S. REPTANS 27:7:72 ROAD 1

KEY :-

s = sunny
st = sunset
o——o = gravid
o—·—o = nulliparous
o----o = parous
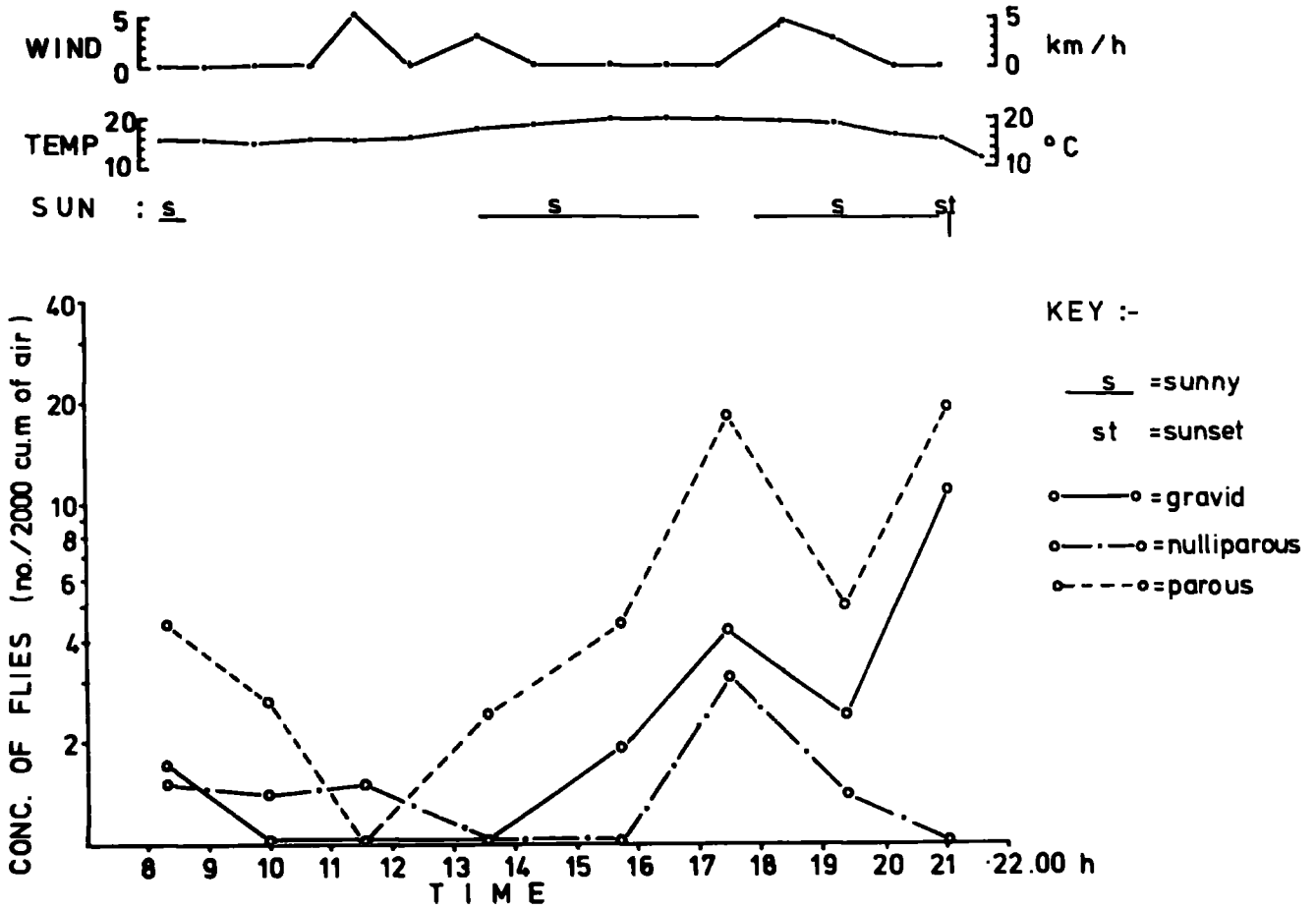
FIG. 16 DIURNAL RHYTHM S. REPTANS 27:7:72 PAROUS

KEY :-

s = sunny
st = sunset
o——o = Road 1
o—·—o = Road 2
o----o = Road 3
o·····o = Road 4
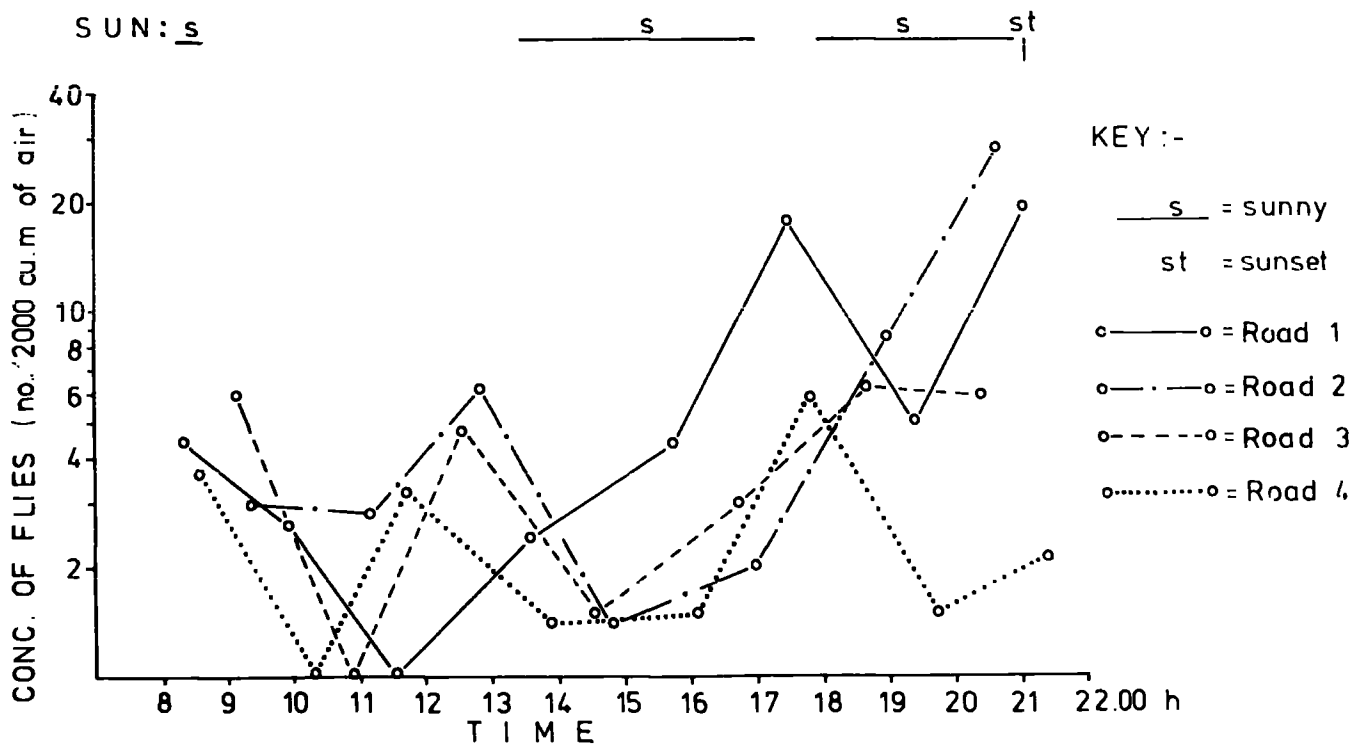
FIG. 17 DIURNAL RHYTHM S. REPTANS 4:7:73 ROAD 2
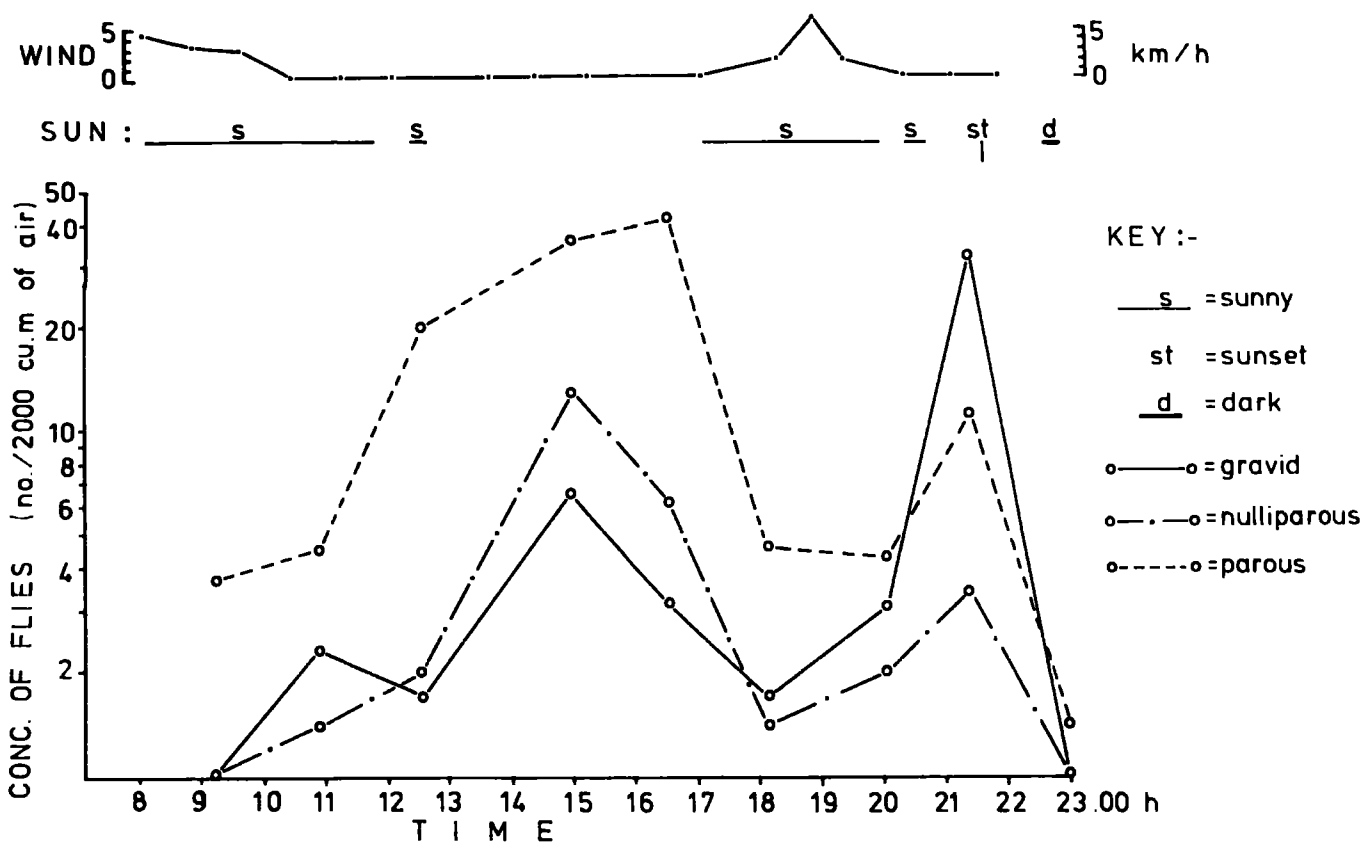
FIG 18 DIURNAL RHYTHM S. REPTANS 14:6:73 ROAD 2
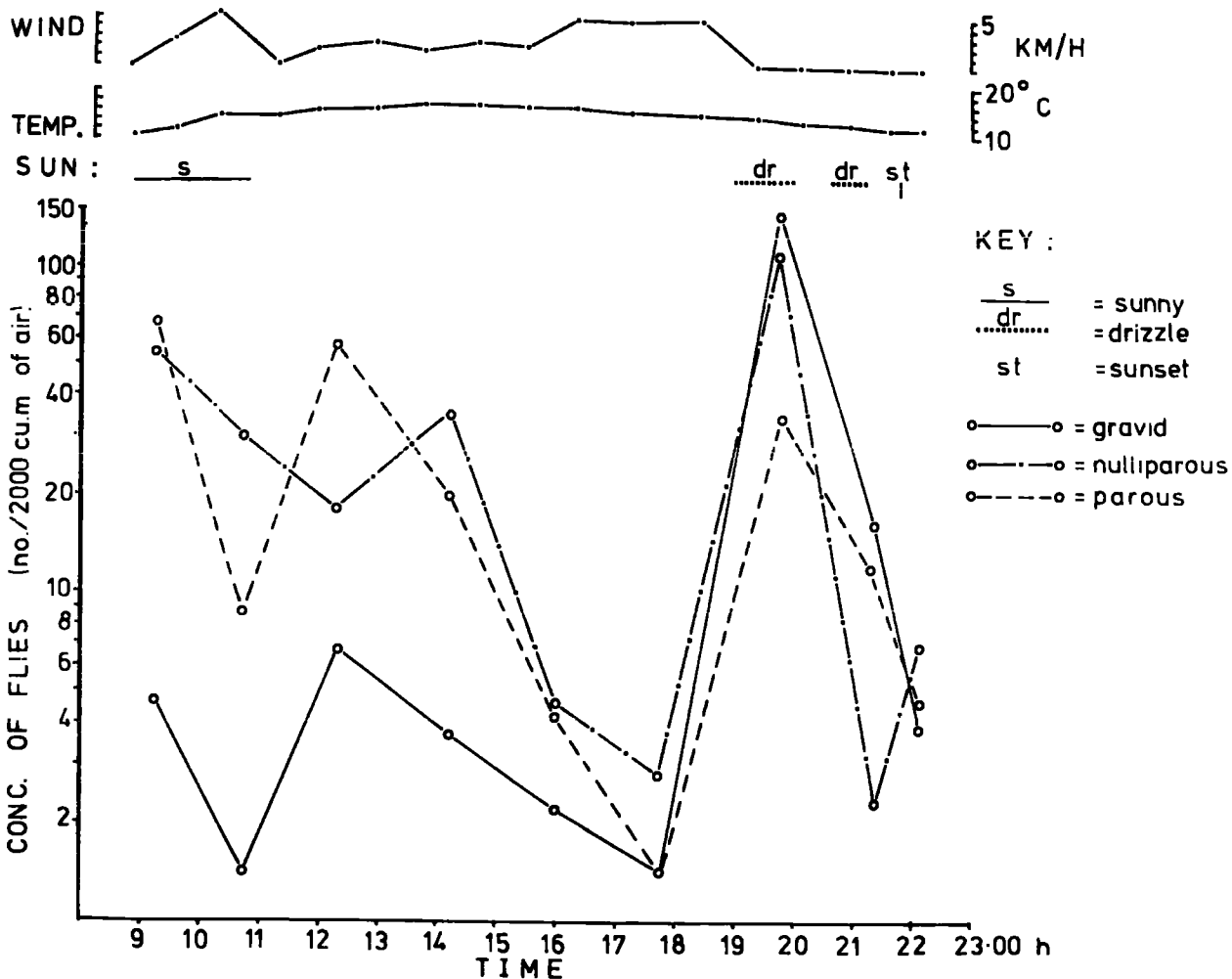
FIG. 19   DIURNAL RHYTHM   S. REPTANS   12:7:73   ROAD 2

WIND 5/0 km/h

TEMP 20/10 °C

SUN : SR   R ST   D

KEY :-

SR = sunrise
R ..... = rain
ST = sunset
D ▬ = dark
———o = gravid
—·—o = nulliparous
----o = parous

CONC. OF FLIES (no./2000 cu. m of air)

80 60 40 20 10 8 6 4 2

TIME

4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24.00 h
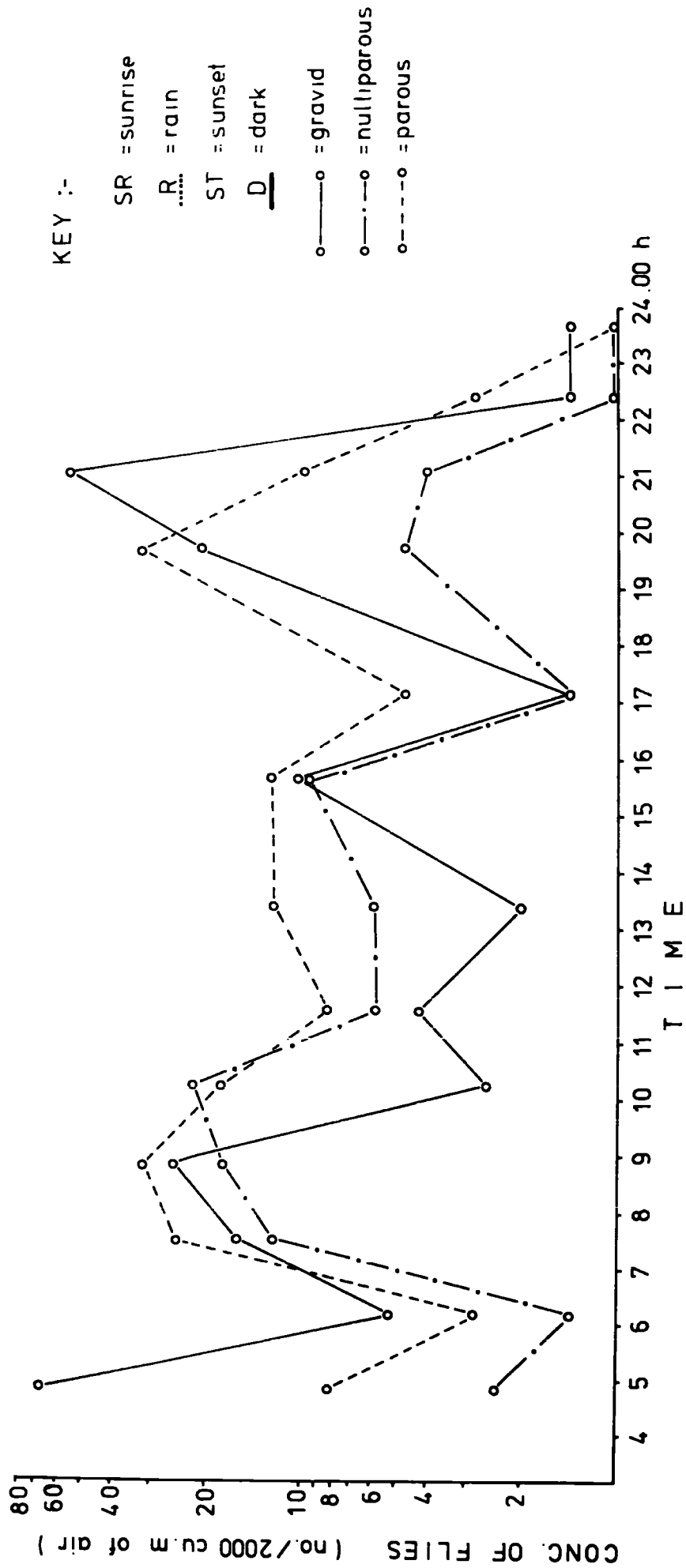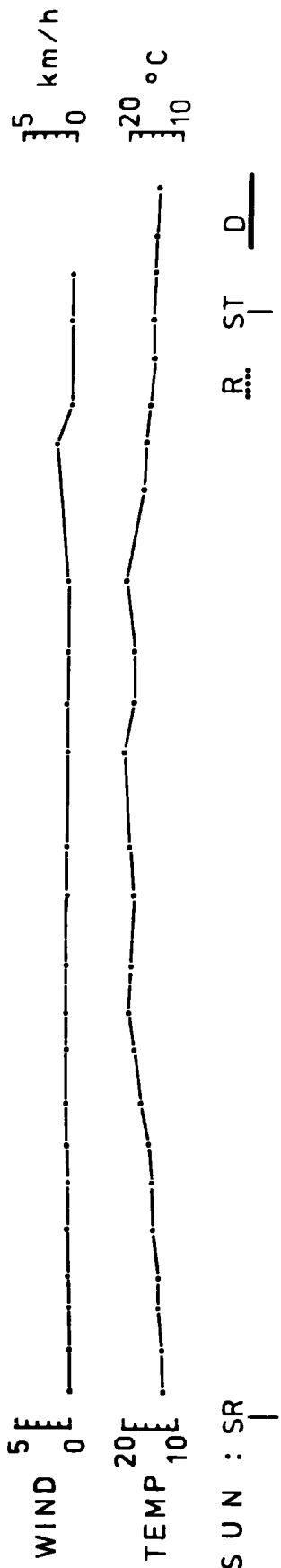
FIG. 20   DIURNAL RHYTHM   S. REPTANS (TWEED VALLEY)   27 - 28 : 7 : 71

WIND $5 \atop 0$ km/h

TEMP. $20 \atop 10$ °C

SUN:

KEY:-

st = sunset
d = dark
sr = sunrise

o = gravid
o = nulliparous
o = parous

CONC. OF FLIES (no./2000 cu. m of air)

27 th JULY   28 th JULY

13.00 h
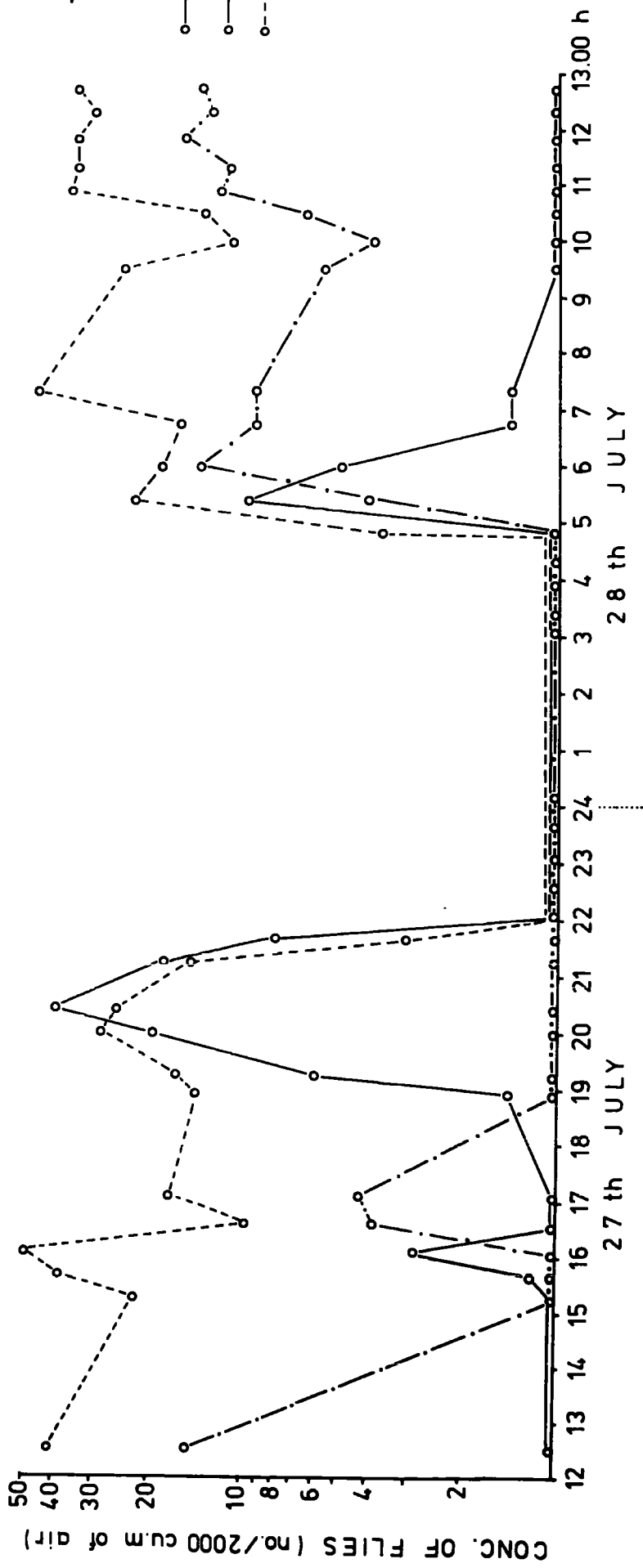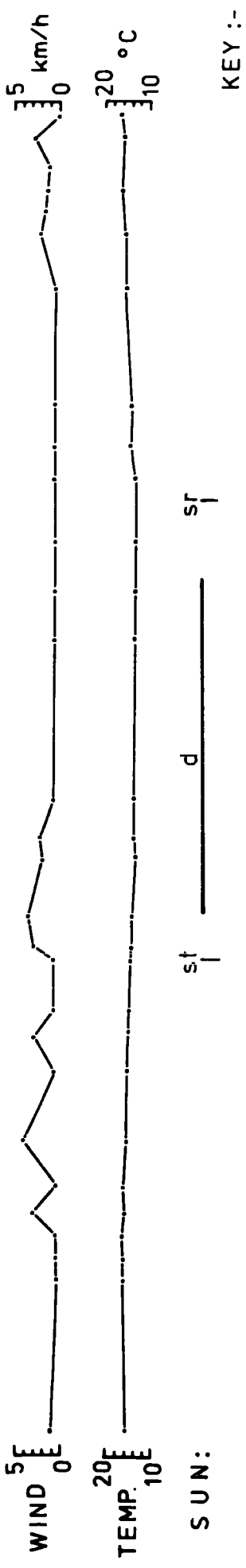
FIG. 21   EFFECT OF TEMPERATURE ON THE MORNING PEAK IN S.REPTANS

			14:6:72		27:6:73	
			Conc.	Temp.	Conc.	Temp
Circuit 1	Road	1	2·9	7·4	4·6	11·6
	Road	4	6·7		1·5	
	Road	3	13·0	10·5	20·0	13·8
	Road	2	128·9		21·0	
Circuit 2	Road	1	39·2	11·9	8·7	15·8
	Road	4	32·8		3·4	
	Road	3	12·9	14·4	10·0	17·6
	Road	2	46·1		9·3	
Circuit 3	Road	1	17·0	14·1	7·0	18·9

FIG. 22   S.REPTANS   PERCENTAGE NULLIPAROUS FOR DIFFERENT CIRCUITS DURING THE DAY

Date	1	2	3	4	5	6	7	8	9	10	11	12
31:5:72				91·0	89·4	100·0	95·6	64·4	100·0	100·0		
14:6:72				18·3	15·6	28·3	39·8	21·5	16·8	48·6	–	
26:6:72				4·2	24·8	26·9	7·3	50·0	23·0	28·7	–	
18:7:72				20·4	–	53·3	41·2	33·3	0·0	32·6	33·1	
27:7:72				0·0	33·3	34·4	50·0	69·2	19·1	6·4	–	
5:6:73			95·7	79·6	76·4	100·0	42·9	73·0	87·2	0·0	–	
14:6:73			45·2	77·5	24·0	64·4	52·3	33·3	77·6	16·6	59·7	
21:6:73			6·6	15·5	32·6	62·2	37·8	47·5	24·4	27·8	0·0	
27:6:73			0·0	0·0	–	0·0	0·0	0·0	16·9	21·9	–	
4:7:73			0·0	23·7	9·1	26·3	12·9	23·3	31·8	23·3	0·0	
12:7:73	29·6	33·3	31·2	36·1	55·2	41·1	32·6	43·4	23·0	12·9	29·0	0·0

sunset (21·10–21·45)

circuit 1 = 3·45 ; circuit 3 ≙ 9·15 h ; circuit 11 ≙ 23·00 h.

in the first circuit was extremely low due to the low temperature, while in the second circuit the concentrations on Road 1 had risen by a factor of ten, before starting to fall again in the third circuit. Since on a sunny day in the latter part of the season (July and end of June), the temperature rose rapidly during the morning due to the strong sunlight, there was only

a brief morning peak between the time when the temperature crossed the lower threshold for flight activity and the time when the temperature lowered the humidity sufficiently to reduce flight activity. This was shown on 27:vi:73 (Fig. 6) and 18:vii:72 (Fig. 7). In contrast to this, at the beginning of the season (Fig. 9) the sunlight was not strong enough to raise the temperature above $17^{\circ}C$, so that there was a prolonged morning peak extending into the early afternoon.

Low temperatures may also curtail flight activity in the evening. On 31:v:73 (Fig. 13 and 14) there was no evening peak because the temperature fell below $12^{\circ}C$ two hours before sunset (the peak normally being half an hour before sunset).

d) **Physiological condition of the flies**

i) Diurnal rhythm of nulliparous and parous females.

Research on S.damnosum (Le Berre 1966; Duke 1968; Disney 1972) showed that the nulliparous flies had an afternoon peak, and the parous flies a midday peak. L. Davies (1957b) found that in S.ornatum, the proportion of parous flies was highest in the early morning and late evening.

The nulliparous and parous S.reptans were plotted as a percentage for each circuit during the day. The gravid and parasitised flies were ignored, since it was not easy to tell whether the gravids were nulliparous or parous (the definitive characters being obscured by the developed eggs) and also

because both gravid and parasitised flies probably had different diurnal rhythms (the gravid flies are shown in the next section and the parasitised flies in chapter 6). The percentage nulliparous for the circuits on Road 2 are shown in Fig. 22, in which the circuits have been arranged with respect to sunset (circuits did not start at the same time each day). However, it should be remembered that during most of the day the concentrations were usually very low and so the percentages were more unreliable.

There does not appear to be any consistent distinction between the diurnal rhythms of the nulliparous and parous flies. Thus on the two hot days (Figs. 7 and 8), nulliparous were caught virtually only during the evening peak, whereas on three days (Figs. 9, 19 and 20) there was little nulliparous activity in the evening, despite large parous peaks.

ii)   Diurnal rhythm of gravid flies.

Gravid flies were females with fully mature eggs.   As mentioned in the above section, they were not divided into nulliparous and parous due to the difficulty in doing so and due to the probability of their having a different diurnal rhythm.

The activity of gravid flies was almost entirely confined to the morning and evening peaks (Figs. 7 - 20), so that the percentage of flies that were gravid was higher during the evening peak than during the rest of the day. This is shown in Fig. 23. The low concentration of gravid flies during the rest of the day was not due to the population of gravids being very small, since during the evening peak, the concentration of gravid flies was normally greater than that of the nulliparous or parous flies (e.g. Figs. 8, 11, 17 and 20). It is possible, therefore, that the gravid flies were more sensitive to dehydration than the non-gravid flies and so were only active when the

FIG. 23   S.REPTANS   PERCENTAGE GRAVID IN EVENING PEAK

Date	a	b	d	significance
14:6:72	42.1	82.3	5.68	0.0001
18:7:72	36.7	42.6	0.95	n.s.
5:6:73	24.2	30.3	1.96	0.05
14:6:73	19.2	42.8	11.50	0.0001
21:6:73	50.7	65.1	3.00	0.01
27:6:73	67.5	75.2	2.32	0.02
4:7:73	24.1	72.7	7.69	0.0001
12:7:73	47.0	78.9	6.25	0.0001

a = percentage gravid during whole day

b = percentage gravid during evening peak

d = standard deviation

FIG. 24   DIURNAL RHYTHM   S.REPTANS   BLOOD-FED FEMALES

o———o = nulliparous blood-fed on 5:6:73 (compare fig. 10)

o—·—o = nulliparous blood-fed on 18:7:72 (compare fig. 7)

o-----o = parous blood-fed on 27:6:73 (compare fig. 8)

o··········o = parous blood-fed on 21:6:73 (compare fig. 11)
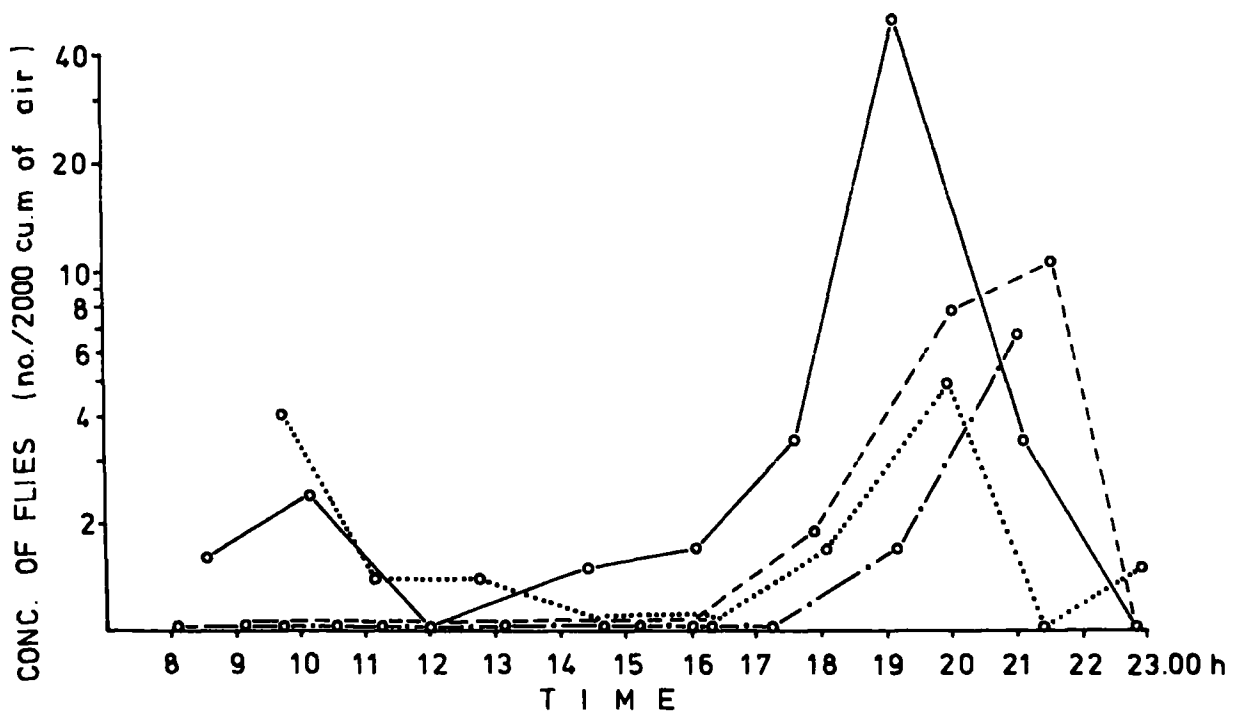
humidity was highest (early morning and late evening). The two 24 h catches (Figs. 19 and 20) show that there were also high concentrations during the dawn peak.

In most species of Simuliidae, oviposition occurs in the evening (Davies and Peterson 1956; L. Davies 1957a; J.B. Davies 1962; Fredeen et al 1951; Peterson and Wolfe 1958; and Peterson 1959). However, this could not be the reason for the observed evening peak of activity of the gravid flies, since the peak occurred on all four roads and even Road 1 was up to 1 km from the River Eden (which was the only major oviposition site - see chapter 2).

iii) Blood-fed flies.

The blood-fed flies were those which contained a blood meal in the mid-gut. Because the concentrations were very low, the blood-fed flies could not be sub-divided into the three categories of fresh, new, and old, which were described in chapter 2. Even when treated as a composite category, there were too few blood-fed flies on many of the days to study the diurnal rhythm. Only four days could therefore be used (Fig. 24). When compared with the corresponding graphs for the non blood-fed flies (Figs. 7, 8, 10 and 11), it will be seen that both the parous and nulliparous blood-fed flies have a rhythm similar to the gravid flies, with activity mainly restricted to an evening peak. Like the gravid flies, they are therefore probably sensitive to dehydration when there is a low humidity.

4. DIURNAL RHYTHM OF S.ORNATUM FEMALES

For each circuit on Road 2, the geometric mean concentration of female S.ornatum is shown in Fig. 25d. Three days at the beginning of the season had very low concentrations and have therefore not been included. The circuits have been arranged in Fig. 25 with respect to sunset, the final circuit each day

a) S.EQUINUM (ROAD 1)

Circuits (see below)

Date	1	2	3	4	5	6	7	8	9
26:6:72		62.3	22.9	20.3	19.1	27.9	23.7	11.6	1.7
18:7:72		8.3	14.4	27.9	43.4	57.0	50.5	45.5	73.9
27:7:72		5.2	6.0	5.2	13.9	34.4	41.7	25.1	18.9
14:6:73	-	13.3	5.2	10.7	5.8	2.9	4.8	24.0	3.6
21:6:73	7.5	20.9	20.7	6.0	10.6	7.8	10.2	15.0	1.4
27:6:73	-	3.1	2.9	7.3	3.4	5.8	2.8	10.4	7.1
4:7:73	1.5	6.5	9.0	11.8	13.0	4.4	10.7	5.5	2.8

(st above column 9)

circuit 1 ≏ 8.00 h ; circuit 9 ≏ 21.50 h   st=sunset (21.10 -21.45 h)

b) S.REPTANS (ROAD 1)

(st above column 9)

Date	1	2	3	4	5	6	7	8	9
26:6:72		61.5	56.0	32.4	15.3	13.0	7.3	24.1	13.7
18:7:72		19.4	10.5	6.3	2.9	6.3	6.2	13.7	132.6
27:7:72		7.6	4.0	1.5	2.4	6.3	25.7	8.8	34.9
14:6:73	2.9	39.2	17.0	21.9	10.5	8.8	14.4	99.3	46.2
21:6:73	43.1	28.4	8.3	8.2	11.0	10.3	19.8	40.2	57.6
27:6:73	4.6	8.7	5.0	1.5	3.6	1.4	4.6	34.6	44.6
4:7:73	3.0	7.0	9.3	18.4	22.7	7.0	8.0	11.5	46.0

c) S.REPTANS (ROAD 2)

Date	1	2	3	4	5	6	7	8	9	10	11	12
26:6:72				128.2	95.4	8.9	50.9	2.8	14.8	44.7	-	
18:7:72				23.2	-	4.4	4.8	7.6	12.9	60.7	28.6	
27:7:72				4.5	5.2	12.1	5.2	9.3	17.9	39.9		
14:6:73			128.2	46.1	112.7	63.2	23.6	9.8	306.0	40.5	18.5	
21:6:73			122.4	22.2	11.0	8.7	7.5	63.8	101.9	77.7	2.8	
27:6:73			21.0	9.3	-	1.4	5.2	5.0	31.9	131.2	-	
4:7:73			3.7	8.2	23.6	57.2	53.6	7.7	11.8	48.4	1.4	
12:7:73	78.5	9.4	53.4	79.4	45.6	21.2	23.4	33.7	9.9	58.6	72.0	4.2

(st above/below column 11)

circuit 1= 3.45 h; 3 ≏ 9.15 h ; 11 ≏ 23.00 h.

d) S.ORNATUM (ROAD 2)

Date	1	2	3	4	5	6	7	8	9	10	11	12
26:6:72				6.1	6.9	-	7.1	1.4	5.8	27.2	-	
18:7.72				6.6	-	1.4	1.4	1.5	20.6	124.7	83.6	
27.7.72				2.5	-	2.8	1.4	9.1	6.4	98.6		
14.6:73			4.8	1.4	8.9	4.6	3.7	-	23.5	18.0	4.8	
21:6:73			6.6	-	1.4	1.7	3.4	10.0	38.6	20.9	-	
27:6:73			7.5	1.4	-	2.9	-	1.8	29.9	58.6	2.8	
4:7:73			-	2.8	3.7	20.5	24.4	3.6	18.3	153.6	1.4	
12:7:73	50.3	2.6	3.2	2.8	-	3.0	4.6	5.2	1.4	14.4	89.2	3.9

sunset (below column 11)

being made approximately one hour after sunset (except on 12:vii:73). The
blank spaces in the table are due to the circuits starting at different times
for the two years (in 1972 eight circuits were completed per day, while in
1973 this was increased to nine by starting earlier), while dashes mean that
no flies were caught in the catch.

Fig. 25 shows that activity was mainly restricted to an evening peak,
though the catch on 12:vii:73 shows that there was a large dawn peak. The
last two circuits before sunset (circuits 9 and 10 in Fig. 25) were calculated
as a percentage of the rest of the day - the final circuit of each day was
ignored because they were taken under variable light conditions (thus 18:vii:72
and 12:vii:73 have large concentrations because they were taken while it was
still light, whereas 21:vi:73 was taken half an hour after dark). The
percentages are shown in Fig. 26 and vary from 63.4 to 96.5%, with a mean
of 79.5%. If there was no diurnal rhythm, so that all the circuits had equal
activity, the percentage would be expected to be 22% (two circuits out of nine).
S.ornatum therefore had a very pronounced evening peak of activity. In
comparison, only 48.6% of the S.reptans on Road 2 were caught in the two
circuits before sunset (Fig. 26). S.reptans therefore had greater activity
in the rest of the day (due to a morning peak, which was absent in S.ornatum).
This is further shown in Fig. 27, where for each circuit S.ornatum was expressed
as a proportion of S.reptans (taking S.reptans = 100). The figures in brackets
in the table are where only S.reptans were caught. Since this would give a
proportion equal to infinity, a proportion was estimated using S.equinum = 1
instead of nought. Fig. 27 therefore shows that the proportion of S.ornatum
to S.reptans increased markedly during the evening, so that S.ornatum had a
much more pronounced evening peak.

FIG.27 **DIURNAL RHYTHM.** **S.EQUINUM AS A PROPORTION OF S.REPTANS (WHERE S.REPTANS =100) ON ROAD 1**

Circuits

Date	1	2	3	4	5	6	7	8	9
26:6:72		101	41	63	125	215	325	48	12
18:7:72		43	137	443	1497	905	815	331	56
27:7:72		68	150	347	579	546	162	285	54
14:6:73	(3)	34	31	49	55	33	33	24	8
21:6:73	17	74	249	33	47	76	52	37	6
27 6 73	(2)	36	58	487	94	414	61	30	16
4.7:73	50	72	97	64	57	63	134	48	6

sunset

S.ORNATUM AS A PROPORTION OF S.REPTANS (WHERE S.REPTANS =100) ON ROAD 2

Date	1	2	3	4	5	6	7	8	9	10	11	12
26:6.72				5	7	(1)	14	50	39	61	-	
18:7:72				28	-	32	29	20	160	205	292	
27:7:72				56	(2)	23	27	98	36	247		
14:6:73			4	3	8	7	16	(1)	8	44	24	
21:6:73			5	(1)	13	19	45	16	38	27	(4)	
27:6:73			36	15	-	207	(2)	36	94	46	280	
4:7:73			(3)	34	16	36	46	47	155	317	100	
12:7:73	64	28	6	4	(1)	14	20	15	14	25	124	93

sunset

Brackets = only S.reptans caught, so proportion estimated as a reciprocal.

FIG.26 **RELATIVE IMPORTANCE OF THE EVENING PEAK IN THE DIURNAL RHYTHM OF THE THREE SPECIES**

Date	(a)S.orn	(b) S.rept	d	signif.	(e) S.rept	(f)S.equin	d	signif.
26:6:72	66.7	15.9	9.96	0.0001	17.9	7.4	2.23	0.03
18:7:72	96.5	73.9	6.53	0.0001	73.7	37.2	5.69	0.0001
27:7:72	90.5	70.2	4.54	0.0001	47.8	29.3	1.79	n.s.
14:6:73	75.6	43.0	6.05	0.0001	55.7	36.4	2.12	0.04
21:6:73	74.2	41.9	6.46	0.0001	43.0	15.7	3.41	0.001
27:6:73	91.5	87.0	1.59	n.s.	74.1	40.9	2.41	0.02
4:7:73	77.4	23.4	14.40	0.0001	43.3	12.1	2.92	0.01
12:7:73	63.4	33.1	8.76	0.000				
Mean	79.5	48.6			50.8	28.6		

(a) S.orn = evening catches (circuits 9 +10) as a percentage of the total — S.ornatum on Road 2

(b) S.rept. = ,, ,, ,, ,, ,, ,, —S.reptans on Rd 2

(e) S.rept = ,, ,, (circuits 8 +9) ,, ,, —S.reptans on Rd 1

(f) S.equin = ,, ,, ,, ,, ,, ,, —S.equinum on Rd 1

d = standard deviation

Whereas for S.ornatum the evening peak formed a consistently high percentage of the total daily catch (Fig. 26), in S.reptans the percentage varied markedly from day to day (from 15.8 to 87.0%), depending on the weather conditions. Thus on the two hot,dry days (18:vii:72 and 27:vi:73) flight activity was restricted to the evening, which therefore formed 73.8% and 87.0% respectively of the total catch. These percentages, however, are still lower than those for S.ornatum. The percentages for the two species in Fig. 26 were compared using the formula:-

$$d = \frac{k_1 - k_2}{\sqrt{k(1-k)(\frac{1}{n_1} + \frac{1}{n_2})}}$$

where $k_1 = \frac{a_1}{n_1}$; $k_2 = \frac{a_2}{n_2}$; $k = \frac{a_1 + a_2}{n_1 + n_2}$

d = standard deviation

a_1 = number of S.ornatum caught during the evening peak

n_1 = number of S.ornatum caught throughout the day

a_2 = number of S.reptans caught during the evening peak

n_2 = number of S.reptans caught throughout the day

(Formula from Bailey N.T.J. 1959 Statistical methods in biology. pg. 38 - English University Press, London.)

There was a highly significant difference between S.ornatum and S.reptans on all the days except 27:vi:73 (which as mentioned above had an exceptional peak of S.reptans).

Since the flight activity of S.ornatum was largely restricted to the evening, it is possible that the peak of activity occurred later than in S.reptans. On a sunny day, the sunset peak for S.reptans occurred between

thirty minutes before sunset, up to sunset. This can be deduced by comparing the diurnal rhythm for the four parallel roads (e.g. Fig. 5). Since the peak was brief, it only occurred on one road, the rhythms on the other roads having a trunchated appearance. This can be better shown by plotting the catches in the order in which they were taken during the circuits. An example is shown in Fig. 42 (for 5:vi:73) in which the evening peak circuit is plotted with part of the preceding and following circuits. The graph shows that there was a sharp evening peak occurring between 35 and 20 minutes before sunset.

Though this showed the diurnal rhythm, super-imposed on this was a pronounced spatial distribution effect (see next chapter). Thus, the increase in concentration on Road 2 of the evening peak catch in Fig. 42 compared with Road 4 was a result of the spatial distribution and not the diurnal rhythm. Therefore, when comparing the sunset peaks of S.ornatum and S.reptans by this method, it must be remembered that the two species have different spatial distributions, S.reptans being highest on Road 2 and Road 1, while S.ornatum was highest on Road 4 and Road 2.

A further complication is that in S.reptans the timing of the evening peak was probably due to the rate of change of the light intensity rather than due to the actual light intensity (see earlier). Thus, when there was cloud present, the peak of activity was synchronised by clouding over of the sun, rather than by sunset. The sunset peaks of S.ornatum and S.reptans were therefore compared on four days (Figs. 28 - 31), three of which were sunny with sharp evening peaks for S.reptans, while the fourth (4:vii:73) was cloudy in the evening.

FIG. 28   SUNSET PEAK   S.ORNATUM + S.REPTANS   27:7:72

KEY :-

— = S.ORNATUM

····· = S.REPTANS

st = sunset

Light intensity (lux):- 970 520 580 510 400 330 240 200 120 70 (st) 50 24 9

Conc. of flies (no./2700 cum. of air) 70 60 50 40 30 20 10

CATCH SEQUENCE

Rd 1   Main rd   Rd 4   Main rd   Rd 3   Rd 2   Rd 1   Main rd   Rd 4   Main rd
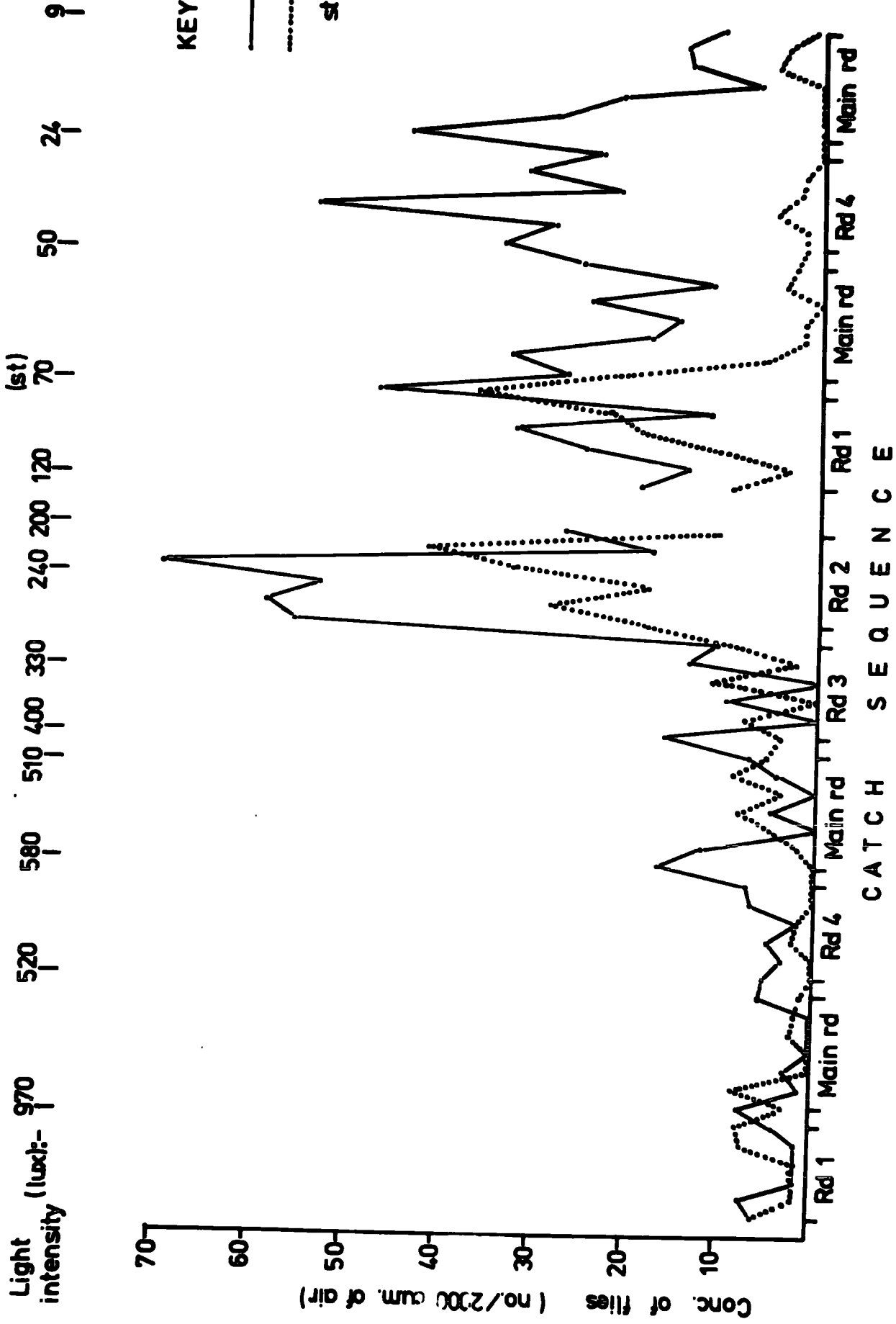
FIG. 2.9 SUNSET PEAK S.ORNATUM + S.REPTANS 18:7:72
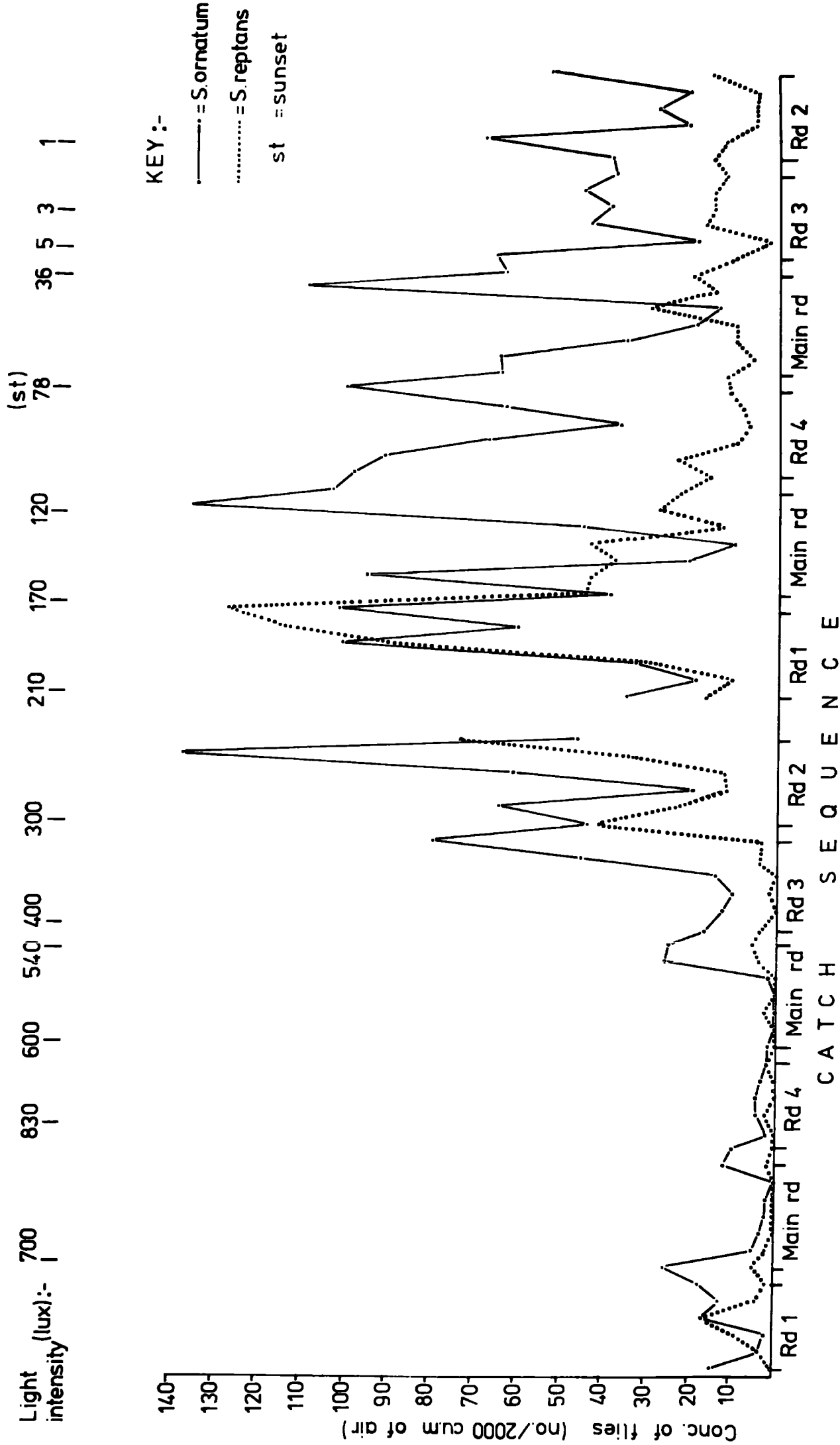
FIG. 30   SUNSET   PEAK   S.REPTANS + S.ORNATUM   27.6.73

KEY:-

— = S.ornatum

···· = S.reptans

ST = sunset

FIG. 31   SUNSET PEAK   S.REPTANS + S.ORNATUM

Light intensity (lux):-   950   770   510   280   200   110   92   64   44   20   8   4   Dark

4:7:7 3   (st)

KEY:-
.‑. = S.ORNATUM
.... = S.REPTANS

st = sunset

Conc. of flies (no./2000 cu.m of air)

150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

Main rd   Rd 3   Rd 2   Rd 3   Rd 1   Main rd   Rd 4   Main rd   Rd 3   Rd 2   Main rd   Rd 1   Main rd   Rd 4   Rd 3

CATCH SEQUENCE
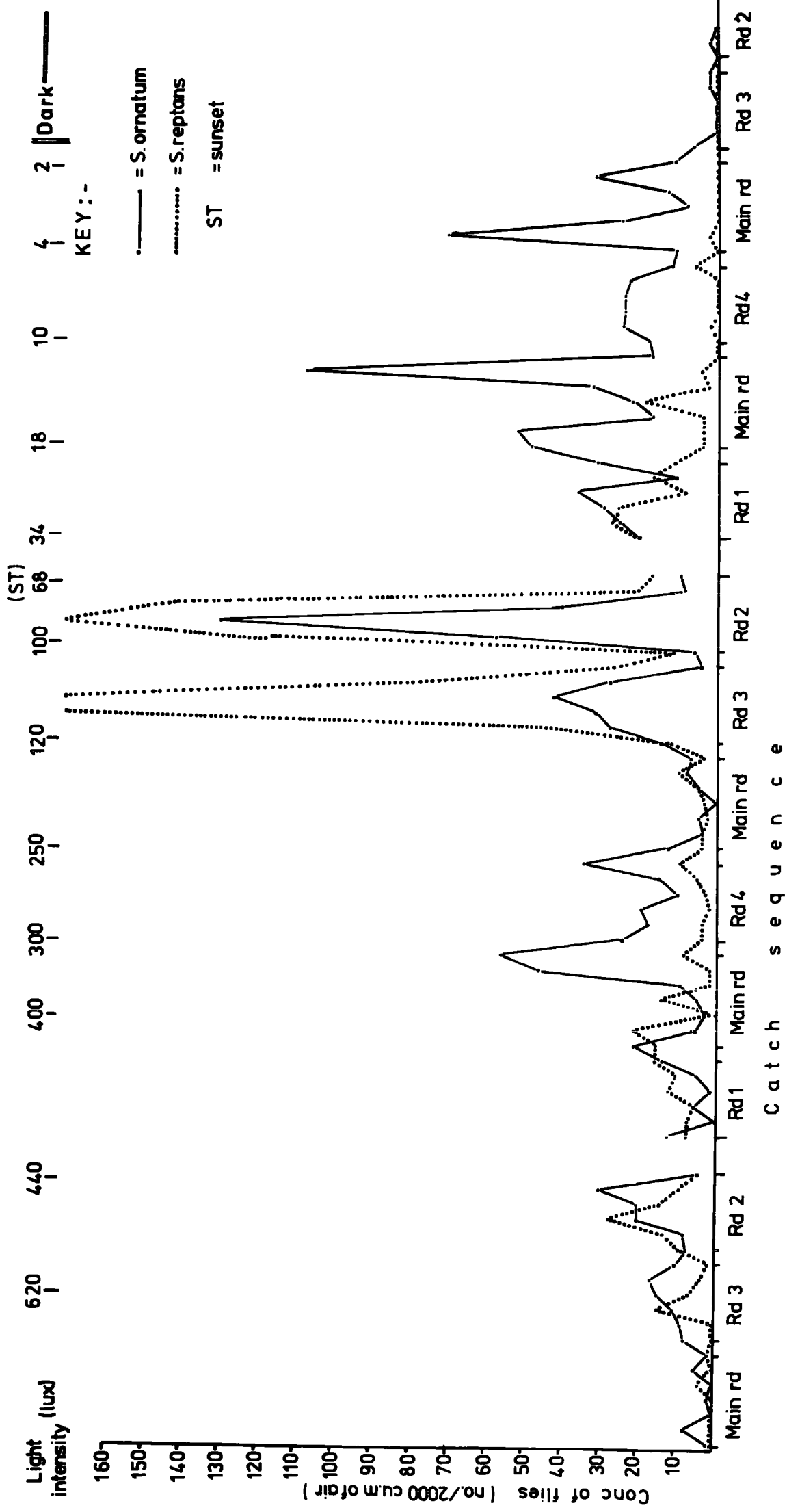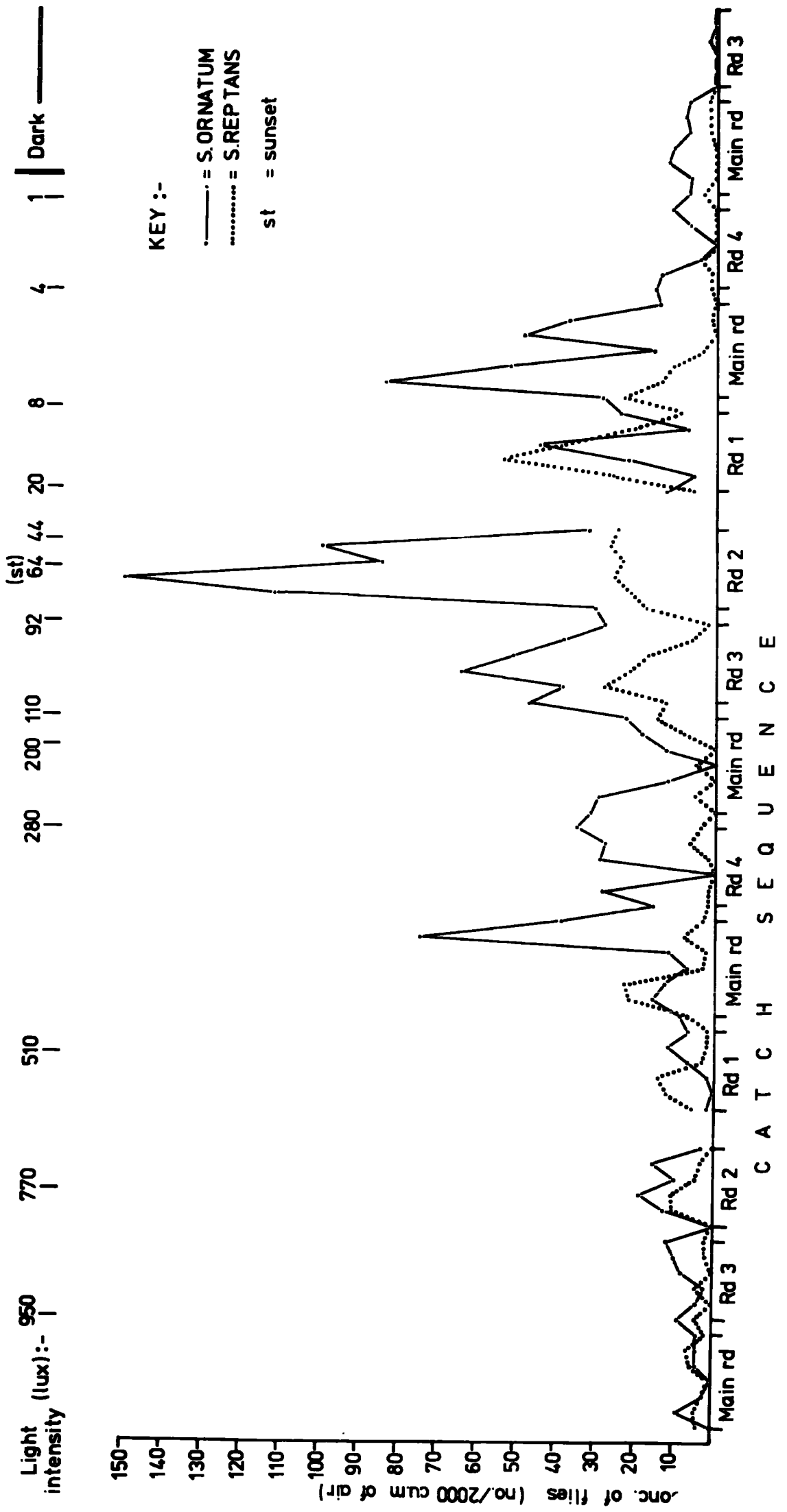
On the three sunny days, S.reptans had a short evening peak before sunset, though this occurred between varying light intensity values (measured in lux, by a small selenium cell photometer attached to a window of the van). On two of the days (27:vii:72 and 27:vi:73), activity of female S.reptans had virtually ceased by half an hour after sunset (20 lux on 27:vii:72 and 10 lux on 27:vi:73), while on the third day activity remained after the peak up to dark (one hour after sunset). On the cloudy day (4:vii:73), there was no sharp peak of activity, but once again activity had virtually ceased by half an hour after sunset. On each of the days, S.ornatum had a much longer peak of activity, starting at between 300 and 400 lux (which on two of the days - Fig. 30 and 31 - was half an hour before S.reptans) and finishing at dark. On the cloudy day (4:vii:73) activity was greatest at sunset, but on the other days activity remained relatively constant throughout the peak (taking into account the effect of the spatial distribution). The peak of activity in S.ornatum therefore continued up to dark (one hour after sunset), while in S.reptans it finished at sunset, with little activity occurring by half an hour after sunset. This agrees with L. Davies (1957a) who found that on sunny days S.ornatum had a peak of biting activity in the hour between sunset and dark.

The diurnal rhythm of the nulliparous, parous and gravid S.ornatum are shown in Figs. 33 - 38, though on several of the days the concentration of nulliparous was too low to include them. As in S.reptans, the activity of gravid flies was more restricted to the evening peak than were the non gravid flies. This is shown in Fig. 32, in which the percentage of female flies which were gravid in the last circuit before sunset (circuit 10 in Fig. 25d) are compared with the percentage gravid for the whole day.

FIG. 32 S.ORNATUM PERCENTAGE GRAVID IN EVENING PEAK ROAD 2

Date	a	b	d	significance
18 : 7 : 72	49.1	56.0	1.40	n.s.
27 : 7 : 72	52.0	56.1	0.78	n.s.
14 : 6 : 73	14.4	37.5	2.72	0.01
21 : 6 : 73	52.6	54.8	0.22	n.s.
27 : 6 : 73	56.1	59.2	0.54	n.s.
4 : 7 : 73	71.3	82.1	2.89	0.01
12 : 7 : 73	73.6	88.9	3.61	0.001

a = percentage gravid during whole day

b = percentage gravid during evening peak

d = standard deviation

As for S.reptans, the significance of the difference between the
percentage gravid in the evening peak (column b in Fig. 32) and the percentage
gravid for the whole day (column a) was tested using the formula:-

$$d = \frac{k_1 - k_2}{\sqrt{k(1-k)\ (k_1 + k_2)}}$$

where $k_1 = \dfrac{a_1}{n_1};$ $\quad k_2 = \dfrac{a_2}{n_2};$ $\quad k = \dfrac{a_1 + a_2}{n_1 + n_2}$

d   =   standard deviation

a_1 =   number of gravid caught during evening peak

n_1 =   total females caught during evening peak

a_2 =   number of gravid caught during whole day

n_2 =   total females caught during whole day

(from Bailey 1959)

Because the non gravid flies were also largely confined to the evening peak,
the difference between the two percentages was much smaller than in S.reptans
(Fig. 23).

On 4:vii:73, S.ornatum showed an increase in flight activity due to a
drop in the light intensity when the sun clouded over in the afternoon (Fig. 37).
However, this increase was small compared with that in S.reptans (Fig. 17),
since the parous flies, for example, had an afternoon peak only one third that
of the evening peak, whereas the parous S.reptans had an afternoon peak three
times that of the evening.   S.ornatum must therefore be much less sensitive
to changes in the light intensity.   On a completely cloudy day (12:vii:73),
S.reptans had high concentrations throughout the day with only a small evening
peak (Fig. 19).   S.ornatum, however, had the same rhythm as on a sunny day,
with a sharp evening peak and low concentrations during the rest of the day
(Fig. 38).

FIG. 33   DIURNAL RHYTHM   S.ORNATUM ON ROAD 2   18:7:72

KEY :-

s   = sunny
st   = sunset
o—·—o = nulliparous
o— — —o = parous
o———o = gravid
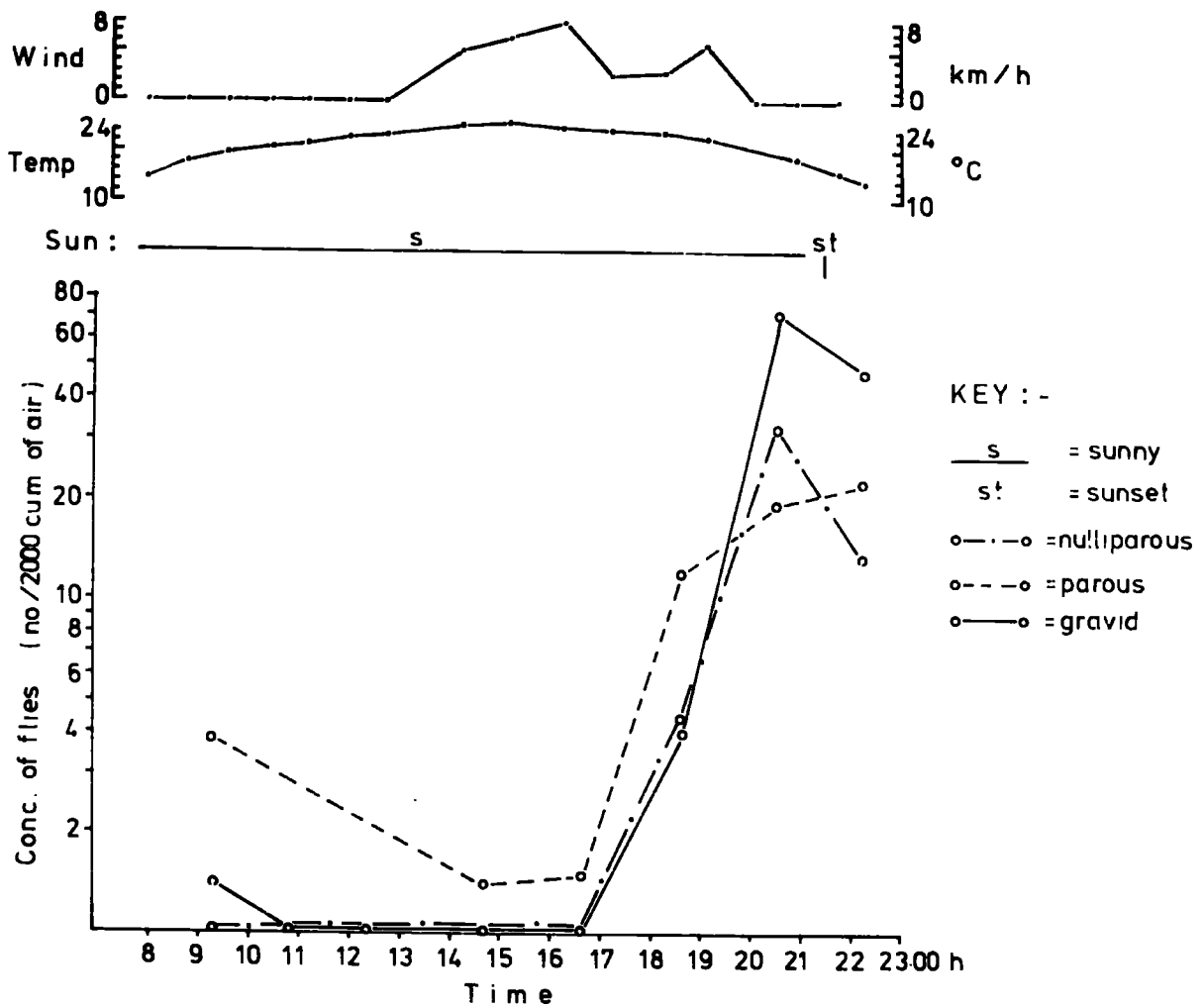
FIG. 34   DIURNAL RHYTHM   S.ORNATUM ON ROAD 2   27:7:72

KEY :-

s   = sunny
st   = sunset
o—·—o = nulliparous
o— — —o = parous
o———o = gravid
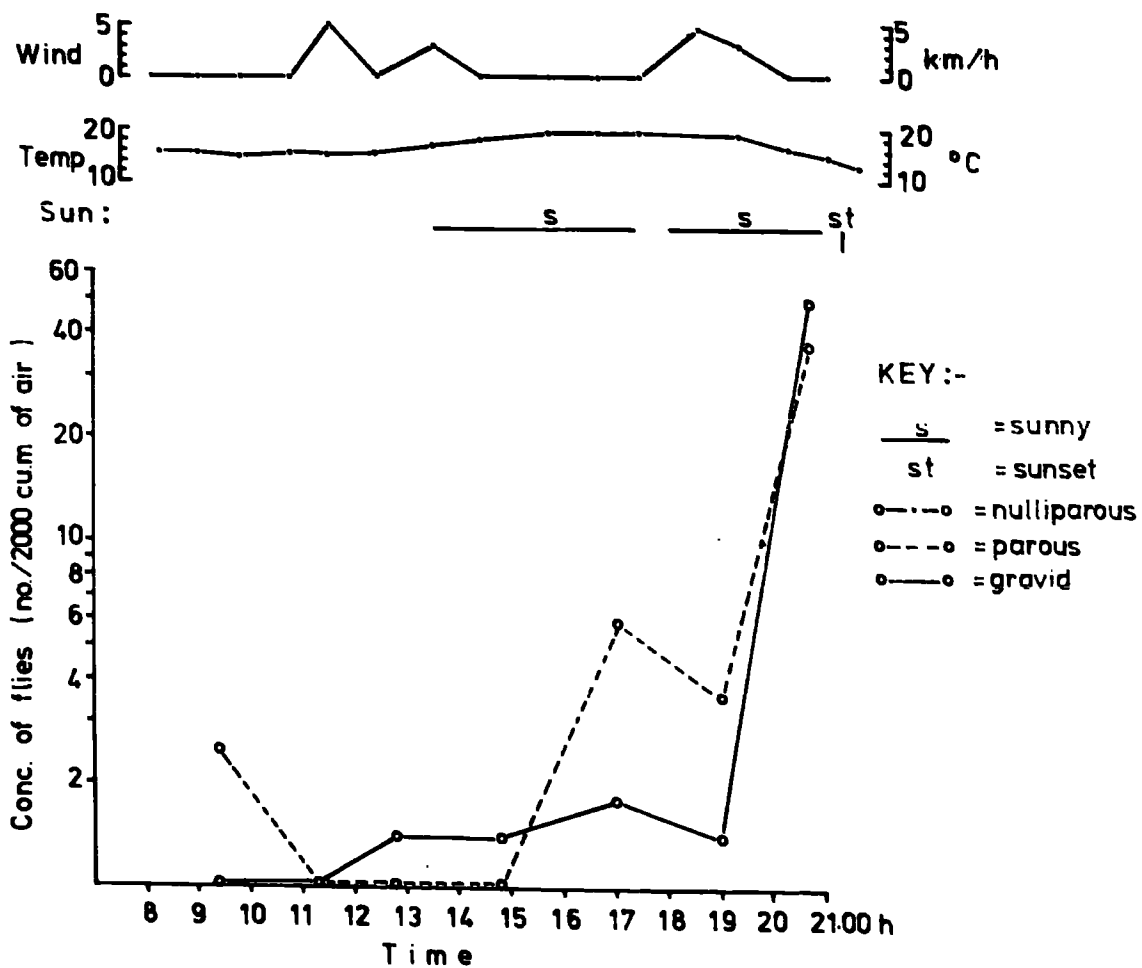
FIG. 35   DIURNAL RHYTHM   S.ORNATUM ON ROAD 2   21:6:73

KEY :-

s   = sunny
st = sunset
d   = dark
o—·—o = nulliparous
o— —o = parous
o———o = gravid
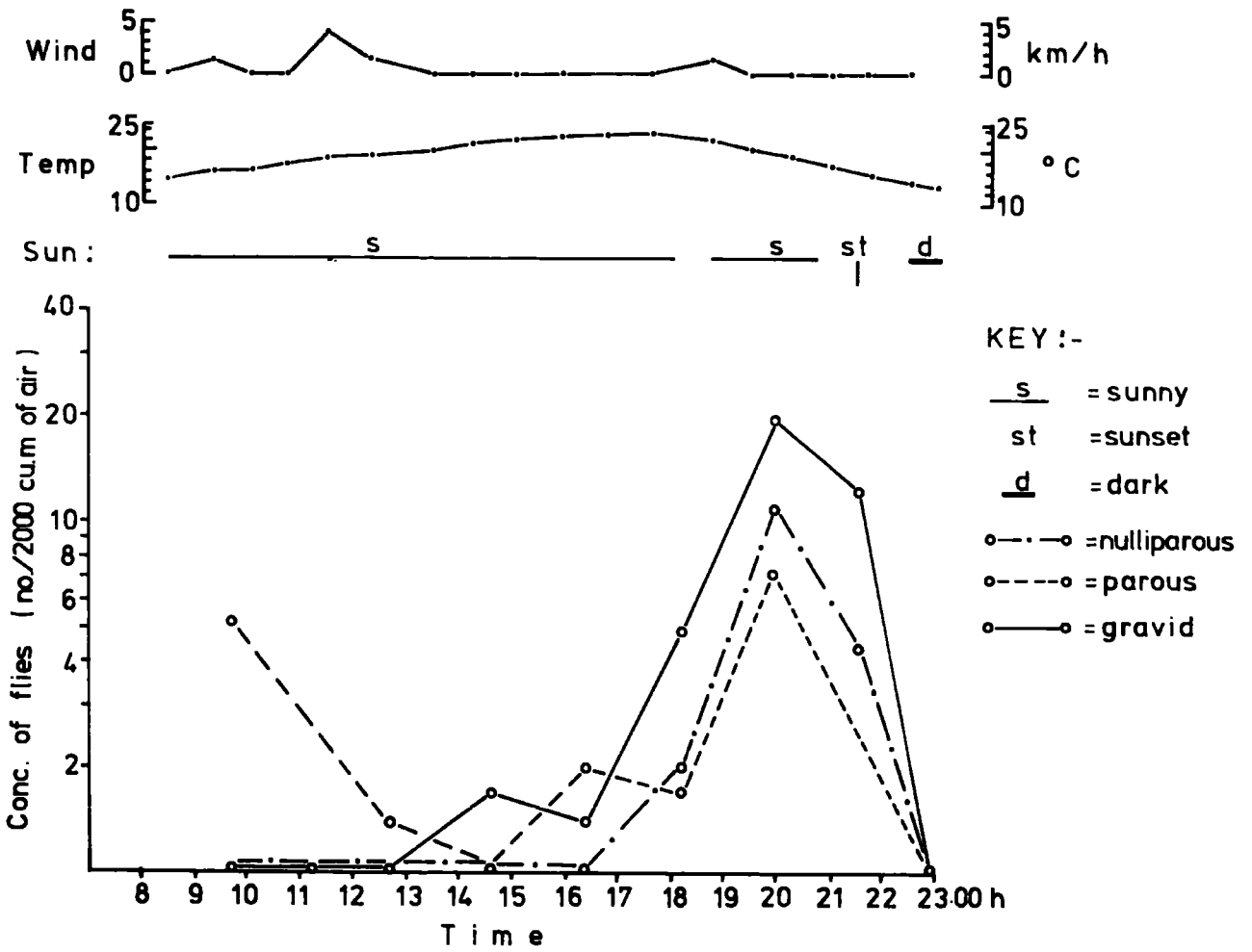
FIG. 36   DIURNAL RHYTHM   S.ORNATUM ON ROAD 2   27:6:73

KEY :-

s   = sun
st = sunset
d   = dark
o———o = gravid
o— —o = parous
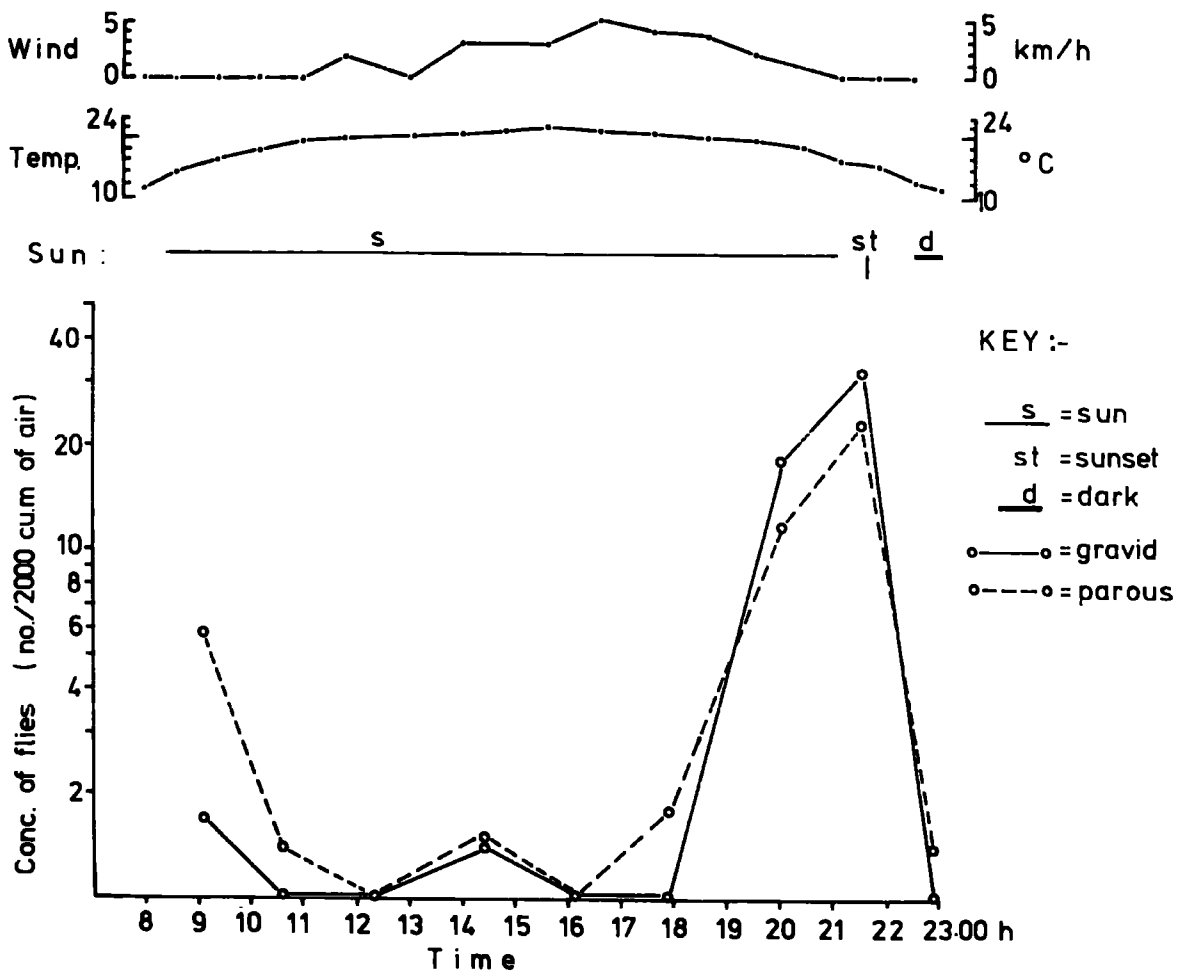
FIG. 37   DIURNAL RHYTHM   S.ORNATUM ON ROAD 2   4:7:73

KEY :-

$\underline{s}$ = sun
st = sunset
$\underline{d}$ = dark
o——o = gravid
o----o = parous
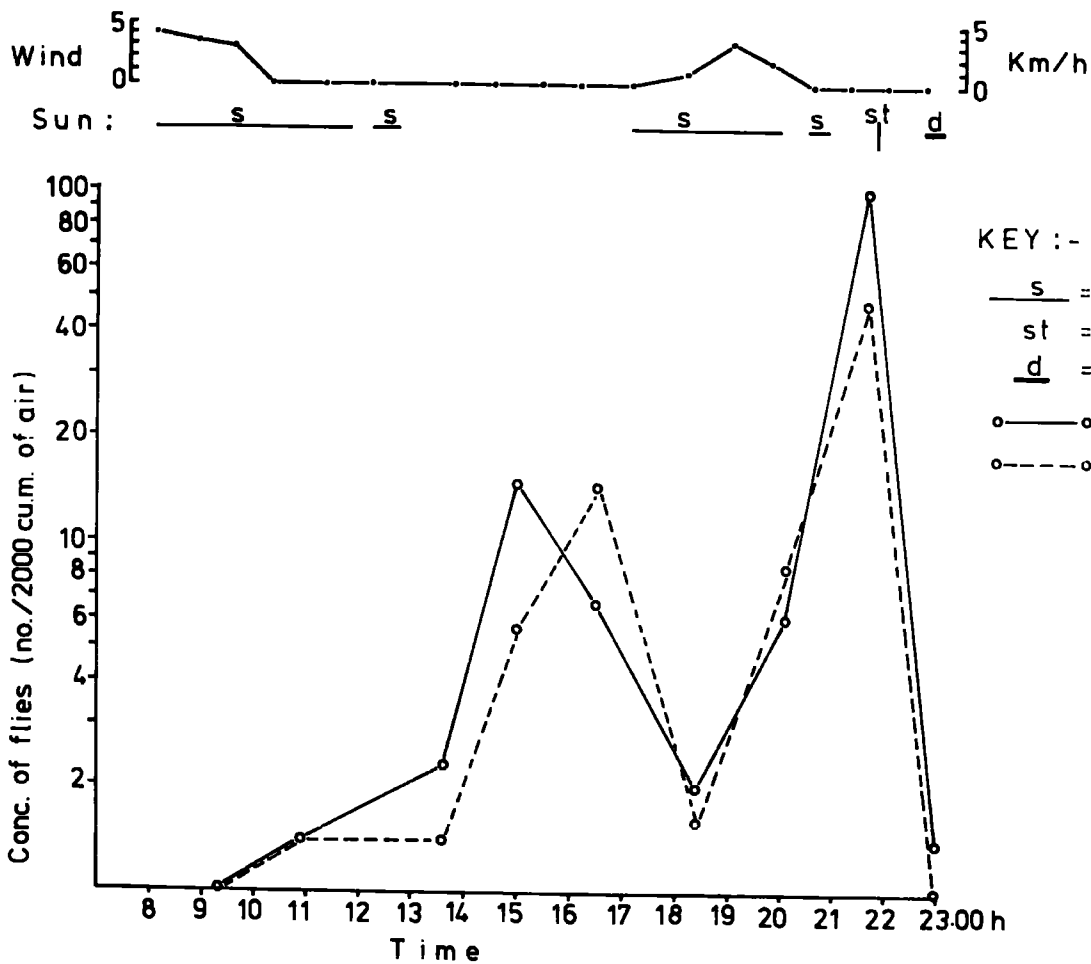
FIG.38   DIURNAL RHYTHM   S.ORNATUM ON ROAD 2   12:7:73

KEY :-

sr = sunrise
..r.. = rain
st = sunset
$\underline{d}$ = dark
o——o = gravid
o----o = parous
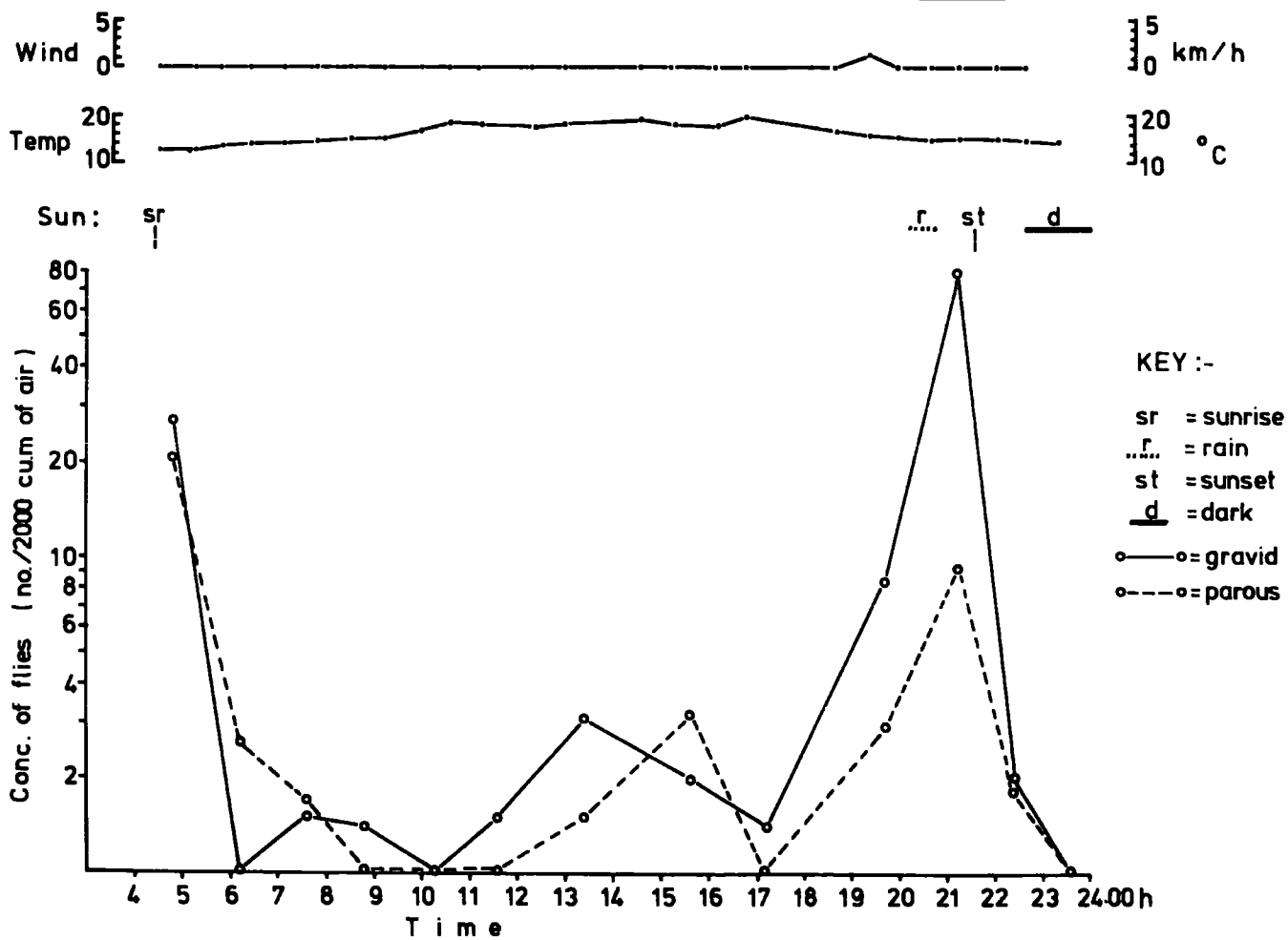
Therefore, whereas S.reptans appeared to react to light intensity changes, rather than to the absolute light levels, S.ornatum appeared to only have a marked increase in activity when the light intensity fell below a threshold. This threshold (possibly at 400 lux - judging from the graphs, Figs. 28 - 31) would appear to be lower than the reduced light resulting from clouding over of the sun. Clouds do not usually lower the light intensity below 600 lux and are normally over 800 lux. Therefore, unlike S.reptans, clouding over was not sufficient to cause a peak in flight activity.

5. DIURNAL RHYTHM OF S.EQUINUM FEMALES

Unlike S.reptans and S.ornatum, S.equinum did not consistently have an evening peak (Fig. 25a). Its diurnal rhythm could not be directly compared with that of S.ornatum, since S.equinum was largely confined to Road 1, whereas S.ornatum had low concentrations on this road. However, it could be compared with S.reptans (Fig. 26). Whereas in S.reptans, the evening peak (that is the last two circuits) made up between 17.9 to 74.1% (with a mean of 50.8%) of the total daily catch, in S.equinum, the last two circuits varied from 7.4 to 40.9% (mean of 28.6%). The relatively low significance of the difference between the percentages of the two species is only due to the small sample involved (due to the lack of S.equinum). S.equinum therefore had a significantly lower activity in the evening.

When expressed as a proportion of S.reptans (Fig. 27), S.equinum formed a much lower proportion in the last circuit before sunset (circuit 8) compared with the earlier circuits, while the final circuit (which on Road 1 was only shortly after sunset) had a very marked reduction in the proportion. This corresponded with the actual concentrations (Fig. 25a) in which apart from the two days, 18:vi:72 and 27:vii:72, activity had virtually ceased by the circuit just after sunset (circuit 9). In contrast, S.reptans had high concentrations during the final circuit, with on most of the days, the evening peak occurring at this time.

In the first circuit of each day in 1973 (circuit 1 in Figs. 25a and 27), which was taken at approximately 08.00 h, the concentration of S.equinum was extremely low compared with S.reptans. In fact, on two of the days no S.equinum were caught on this circuit. Flight activity of S.equinum therefore not only ceased earlier in the evening, but also started later in the morning. It is possible that this was due to S.equinum having a higher temperature threshold below which activity was curtailed (that in S.reptans was at approximately 12°C).

The diurnal rhythm of S.equinum appeared much less consistent than in the other two species, though this was based on fewer days and much lower concentrations, and so was less reliable. Unlike the other two species, there did not appear to be a reduction of activity in the afternoon, while on several of the days (for example, all three days in 1972) there was actually an afternoon peak of activity.

Because of the low concentrations of S.equinum and because of the high percentage that were parasitised (for example, 74% on 26:vi:72 - see chapter 6), it was only possible to plot the diurnal rhythm of nulliparous, parous and gravid flies on two days (Figs. 39 and 40), which may therefore not be typical rhythms. However, 18:vii:72 (Fig. 39) was of special interest, since it was sunny, hot (up to 26°C) and dry. The nulliparous had an afternoon peak of activity at 15.10 h, while the parous and gravids also had high concentrations at this time, though having a larger evening peak. This was in marked contrast to S.reptans (Fig. 7) and S.ornatum (Fig. 33) which had virtually no activity throughout the afternoon due to the low humidity. 27:vii:72 (Fig. 40) was of interest, since it had sunny and cloudy periods. Both S.reptans (Figs. 15 and 16) and to a lesser extent S.ornatum (Fig. 34) had activity largely confined to the cloudy periods, when the rate of evaporative water loss was lowest.

FIG. 39   DIURNAL RHYTHM   S.EQUINUM ON ROAD 1   18:7:72
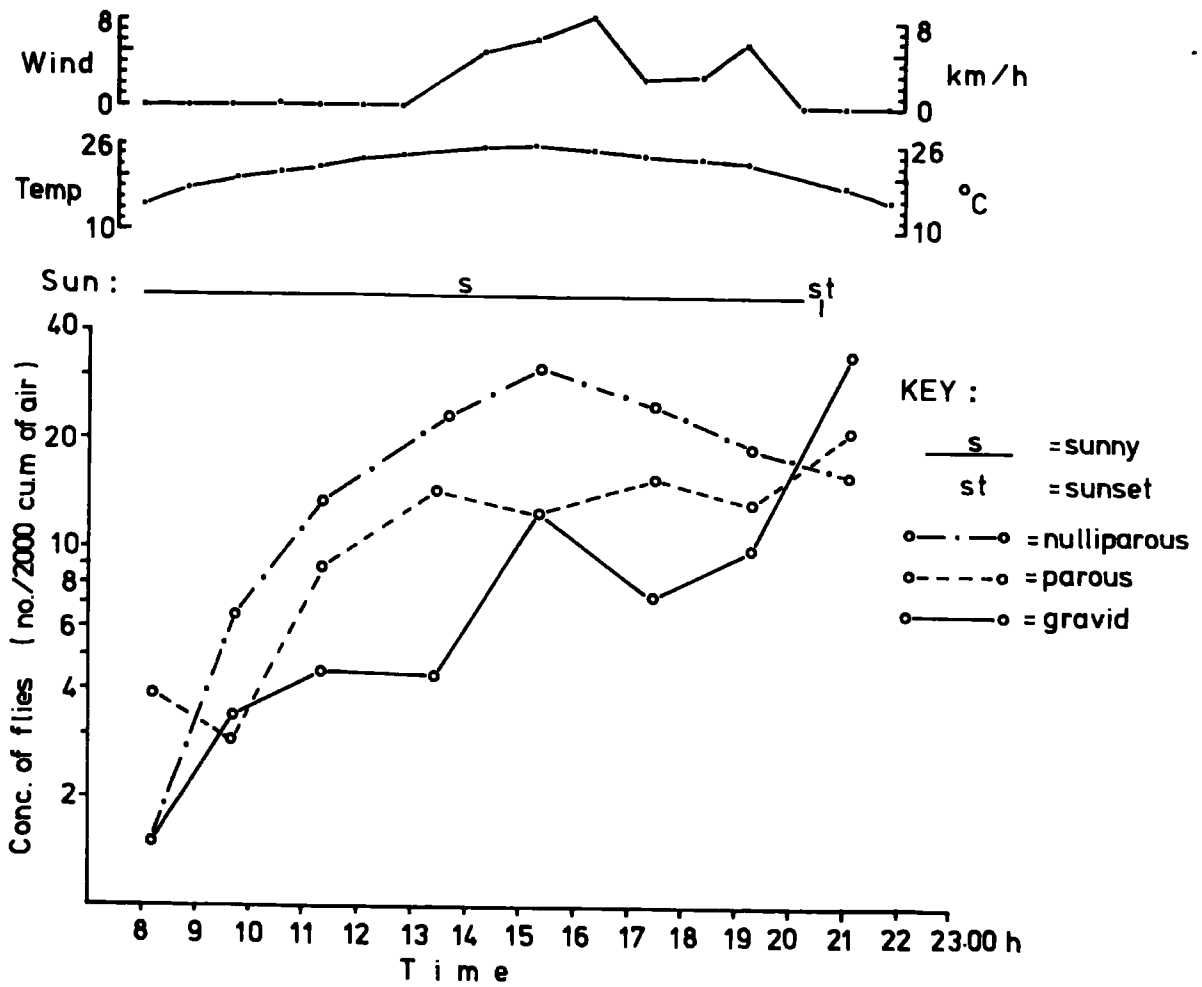
FIG. 40   DIURNAL RHYTHM   S.EQUINUM ON ROAD 1   27:7:72
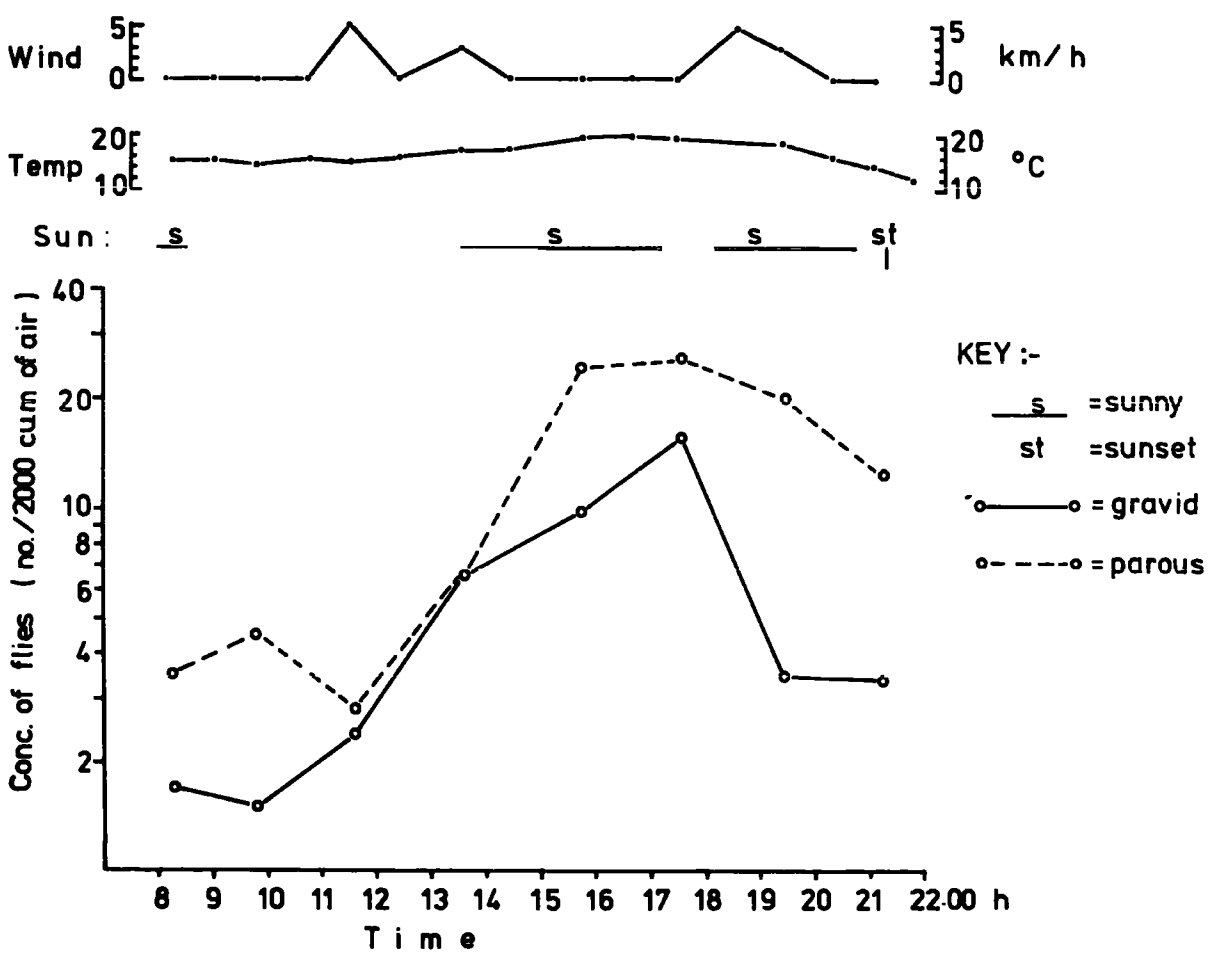
However, S.equinum again had an afternoon peak, independent of the sun: cloud changes. In the case of the parous flies, the concentration was the same at 15.40 h (during sunshine) as at 17.25 h (during cloud).

Though it is not possible to tell whether an afternoon peak of activity was normal in S.equinum (and several of the days, for example 14:vi:73 and 21:vi:73 in Fig. 25a did not have one), it was less affected by periods of dehydration than the other two species. Thus the concentrations were not lower during sunny periods than in cloudy periods, and there was an afternoon peak of activity on 18:vii:72, despite the sunny and dry conditions. 18:vi:72 also had the highest total daily catch of S.equinum for all the twelve days studied, while in contrast it had the second lowest catch of S.reptans, due to the harsh conditions.

IV.   SPATIAL DISTRIBUTION OF THE SIMULIIDAE

1.   INTRODUCTION

Studies on dispersal in insects can give two types of information:-

a)   The flight range.   This is the maximum distance which a species can disperse from its breeding site, and therefore defines the area which a population will occupy.

b)   The spatial distribution.   This is the change in the concentration of the flies with distance from the breeding site, and so shows how the population is distributed within the flight range.

Much of the studies of dispersal depend on recapturing marked insects. The insects were marked by either a dye (usually an aniline compound sprayed over the adult) or by radioactive tracers (usually radioactive phosphorus - P^{32} - fed to the larvae).   Dispersal has also been studied by calculating the distance to the nearest known breeding site.

a)   Studies of Simuliidae

Most of the research on dispersal in Simuliidae has only been concerned with the flight range.   Lewis (1953 and 1957) found that the flight range of S.damnosum Theobald was 10 - 20 km; however, Le Berre (1966) considered the range to be 41 km; while Gibbins (1937) considered the range to be 72 km. S.griseicolle Becker was found by Lewis (1953 and 1957) to have a flight range of 80 km; while Crosskey (1958) considered the range to be 24 km.   Peterson and Wolfe (1958) found that both P.hirtipes Freis and S.venustum Say could fly over 8 km; however, Hocking and Richards (1952) found that by eliminating larvae from a large area using insecticide that P.hirtipes only had a flight range of 3 km, while S.venustum could fly 9.5 km.   Fredeen (1956) states that S.venustum could fly 16 km and that S.luggeri N & M flew up to 42 km (Fredeen 1953).

Using aniline dyes for capture - recapture studies, Dalmat (1950) released 19,580 marked adults and collecting by "fly-boys" at twenty stations recaptured 21. From this, he found that S.ochraceum Walker has a flight range of 11.8 km. In a second experiment (Dalmat 1952), he released 52,685 and collecting at 33 stations, caught 31. From this, he concluded that S.metallicum Bellardi has a flight range of 15.5 km. Marking larvae with P^{32}, Bennett (1963) found that S.rugglesi N & M disperses over 10 km.

Since the flight activity of most Simuliidae was adversely affected by winds over 5 km/h (see chapter 2), the flight range of most species is probably normally not much affected by the wind. However, some species regularly fly in strong winds and so are carried great distances. For example, S.arcticum Malloch has been caught in swarms up to 220 km downwind of the breeding sites, during periods of high winds (Peterson and Wolfe 1958).

Simuliidae have therefore been shown to have flight ranges of many kilometres. However, how far it is possible for a few individuals of a species to disperse is of less importance than how far the majority of the flies travel, thus the spatial distribution is important. In S.arcticum, for example, Rempel and Arnason (1947) found that though some flies flew up to 160 km, most occurred at 96 km from the breeding site, and that there were very few flies within the first 48 km of the river. Bennett and Fallis (1971), studying the distribution of S.euryadminiculum Davies at different distances from the edge of a lake (the flies were attracted to lakes since they feed on the common loon - Gavia immer Brunswick), marked 94,000 flies with P^{32}. He found that though some were caught at 75 m inland, most were caught over the lake in the morning and 8 m inland in sedge meadows during the afternoon and evening. He also found that they dispersed up to 8 km from the point of release (mainly following the lake edge and rivers) but most were found within 2.4 km.

L. Davies (1961) found that there was a difference in the spatial

distributions of nulliparous and parous flies of Prosimulium mixtum S & D,

the nulliparous flies being more evenly distributed, while the parous flies

were more restricted to the river and so dispersed less.   Downes et al (1962)

similarly found that in S.venustum, the parous flies stayed closer to the rivers.

Much research has also been done on dispersal in other insects, especially

mosquitoes and midges.

b)   Studies of Culicidae

Chant and Baldwin (1972) marked 250,000 larvae of Aedes communis DeGeer

using P^{32}.   Recapturing using a 'T' shaped distribution of light traps, he

caught 31 adults.   However, over half were caught in the outer traps, 1.5 km

from the release point.   He therefore concluded that the adults dispersed

widely and that most of them moved outside the trapping area.

Jenkins and Hassett (1950) also studied A.communis.   They marked 4 million

larvae with P^{32} and estimated that 3 million emerged.   Flies were collected

at different distances up to 8 km by netting around human bait, resulting in

the recapture of 141 adults.   The flight range was only 1.6 km (in marked

contrast to Chant and Baldwin) and 90% of them were caught within 100 m of

the release point - though this was biased by the distribution of the collecting

points since almost half were within 200 m of the release point.

Shemanchuck et al (1955) marked 415,000 Aedes flavascens Muller with

P^{32}.   By bait netting, 82 adults were collected at distances up to 10.6 km,

though most were found around the breeding site (80% were caught within 200 m).

De Zulueta (1950) studying various mosquitoes on the llanos in Colombia using a vehicle trap and bait traps found an even distribution of the flies due to the closeness of the breeding sites. The flies were thus capable of dispersing at least 1.8 km.

Gillies (1961) marked 132,000 larvae of _Anopheles gambiae_ Giles with P^{32} and recaptured 1,019 adults. However, he found that the dispersion was not random but heavily correlated with the distribution of human settlements. He found that 78% were caught within 1.5 km, 18% up to 3 km and 4% over 3 km (with a maximum of 3.6 km).

Provost (1957) marked 1,400,000 larvae of _Aedes taeniorhynchus_ Weidermann with P^{32}. Using 64 light traps, 8 bait traps, 2 vehicle traps and 1 rotor trap, collecting at distances up to 64 km, he recaptured 1,550 adults. He found that the flies had a definite migration on the first night, so that by the second night, they had travelled up to 14 km. On the following nights, they had short appetential flights while looking for a blood meal and travelled up to a maximum distance of 40 km from the release point.

Stage et al (1937) caught _Aedes vexans_ Mg. and _Aedes aldrichi_ D & K marked by dyes at up to 48 km from the release point, with large numbers being found at 13 km.

Clarke (1943) marked mosquitoes with aniline dyes and after only one day recaptured _Aedes vexans_ at up to 22 km, _Culex pipiens_ L up to 14.2 km and _Anopheles punctipennis_ Say up to 16.8 km from the release point.

Dow (1971) studying the dispersal of _Culex nigropalpus_ Theobald marked with P^{32}, found by using light traps that the concentration of young flies decreased with distance from the breeding ground at only half the rate of that of the old flies. Thus the young flies had a much greater dispersion and were caught up to 4.8 km from the point of release.

The research on dispersal in mosquitoes has therefore produced very variable results. Many of the species appear to have a much poorer power of dispersal than the simuliid species studied, with most of the flies concentrated in less than 0.5 km of the breeding site. However, others such as _Aedes taeniorhynchus_ and _Aedes vexans_ disperse over great distances.

c)   Studies of Ceratopogonidae

Kettle (1951) found that the number of _Culicoides impunctata_ Goetghebuer in woodland decreased by a tenth for every 65 m, so that at 200 m, the numbers had fallen to one thousandth of the original. However, when collecting on moorland (Kettle 1961), he found no change in the concentrations over a distance of 1.2 km, the only changes being due to the presence or absence of livestock hosts.

Nicholas (1953) found that when _Culicoides grahamaii_ was collected by "fly boys" at different distances from the breeding ground, the numbers caught fell by one tenth over a distance of only 370 m.

Linley and Davies (1971) found that _Culicoides barbosai_ Wirth and Blanton mainly stayed within 400 m of the breeding ground, but may be carried up to 1.5 km by the wind. The numbers of _Leptoconops becquaerti_ Keiffer decreased by one third over the first 200 m, with very few flies dispersing more than 1 km, though a few were caught up to 8.8 km downwind. _Culicoides furens_ Poey however dispersed much wider than the other two species, being more readily carried by the wind up to 5 - 6 km.

Williams (1962) also found _Culicoides furens_ up to 6.4 km from the nearest breeding site. He also found (Williams 1951) that _Culicoides tristriatulus_ Hoffman dispersed up to 8 km from the nearest breeding site.

Dorsey (1947) only found _Culicoides peleliouensis_ Tookunga up to 3.2 km.

Midges being poorer fliers than mosquitoes or black-flies are probably therefore more dependent on the wind for dispersion over distances of more than a few kilometres. Thus most of the flies probably only fly a few kilometres, while small numbers of the flies are carried out of the breeding area by the wind.

d)   Basis of present research

In flies with poor powers of flight such as midges, large flight ranges were probably due to small numbers of flies blown by the wind. These are important in finding new breeding sites but otherwise have little importance, since unless the wind changes, they are unlikely to be able to return to the breeding ground. What is important therefore, is not the flight range but the spatial distribution - the area over which high concentrations occur. In more powerful fliers, such as Simuliidae, it could be expected that the spatial distribution would cover a more extensive area (many of the midge species studied in the above research only had high concentrations for less than a kilometre from the breeding sites).

In the present research, the spatial distribution of two species of Simuliidae were studied over a distance of six kilometres, between the breeding grounds on the River Eden and the scarp slope of the Pennines. Each species was divided into nulliparous and parous, and into blood-thirsty, blood-fed and gravid, to see what effect these different stages in the gonotrophic cycle affected the overall distribution. Parasitised flies were also studied to see whether the presence of parasites had an effect.

It was in fact not possible to study the flight range due to the presence of the scarp slope (see Fig. 4), which limited collecting activity. However, the concentrations on Road 4 (6 km from the River Eden) were often high. Thus for the evening peak of 14:vi:73, the concentration of female S.reptans was 125.8 flies/2000 m^3 of air, so that the flight range for this species must have been much greater than the 6 km.

2. RESEARCH METHOD

Since the spatial distribution is the change in concentration of flies with distance from the breeding ground, it was studied by comparing catches taken at different distances from the River Eden. This, however, is only possible when the River Eden was the only breeding ground, so that the source of origin would be known for any adult flies caught, and thus the distance and direction from which they had come. Only S.reptans and S.equinum could therefore be studied in this way, since S.ornatum also bred in smaller streams and so the source of the adult flies caught would not be known.

In order to study the spatial distribution of the flies, data was used from two sources:-

a) The parallel roads. These were parallel to the River Eden (Fig. 3) at approximate distances of 0.5 km (Road 1); 1.5 km (Road 2); 3.0 km (Road 3); and 6.0 km (Road 4). The catches on the four roads were therefore compared for each circuit. This had the disadvantage in that the peaks of activity during the diurnal rhythms (see chapter 3) were often short and so occasionally only occurred on one road (since each circuit lasted 1½ hours). An example is shown in Fig. 42 in which the evening peak only occurred on Road 1 and one direction of the main road.

b)   The main road.   This ran at right angles to the river and was divided
into seven sections (see Fig. 3) between Langwathby and Melmerby (sections D-J).
The parallel roads were roughly equivalent in distance from the river to the
following main road sections:-

Parallel road		Main road section
Road 1	=	D
Road 2	=	E + F
Road 3	=	F + G
Road 4	=	J

The main road had the advantage over the parallel roads in that there was only
thirty minutes between the first and last catch in each circuit and so was less
affected by the diurnal rhythm.   But it had the disadvantage in that the
concentrations were lower than on the comparable parallel roads and there
were only two catches for each section (one in either direction), compared
with six on each parallel road (three in either direction).   The main road
catches were therefore less reliable in estimating the changes in concentration.

Comparison of the concentrations on the parallel roads or main road
sections was difficult due to the very large fluctuations resulting from the
diurnal rhythm and changes in the weather (see Chapter 3).   As explained in
Chapter 2, changes in the level of flight activity and thus concentration of
the catches, due to a change in the weather during a circuit, were eliminated
as far as possible by only studying those days in which the weather conditions
remained constant throughout the day.   The effect of the diurnal rhythm was
the reason for not analysing the 1971 catches.   This was because the old
equipment (see Chapter 2) resulted in each circuit lasting $2\frac{1}{2}$ h, which was
sufficiently long for a change in the diurnal rhythm to have occurred between
one road and another.   The spatial distribution results for 1971 were unreliable,
having a diurnal rhythm superimposed on them.   In 1972 and 1973, the circuits
were reduced to $1\frac{1}{2}$ h which largely eliminated this.

The only times of the day when the concentrations were high enough to safely compare the four roads of a circuit was during the morning and evening peaks of activity. Unfortunately, it was not possible to study the spatial distribution at any one moment, since it took 100 minutes to complete a circuit. Consequently, since the concentrations were changing rapidly due to the diurnal rhythm, it was not really possible to study the spatial distribution of individual circuits.

On the twelve days studied, a total of 103 circuits were made. Combining the results of these circuits to study the overall spatial distribution was difficult due to the enormous changes in the concentrations between different circuits. For example, during a circuit in the afternoon of a hot day (12.45 - 14.50 h on 18:vii:72) the concentrations (flies/2000 m^3 of air) of female S.reptans were only:-

Road 1 = 1.8; Road 2 = 3.6; Road 3 = 0; and Road 4 = 0;

whereas during a circuit in the evening peak (18.25 - 20.00 h) of 14:vi:73, the female S.reptans concentrations were:-

Road 1 = 110.1; Road 2 = 539.1; Road 3 = 126.1; and Road 4 = 125.8.

These differences between the circuits were due to the diurnal rhythm (see Chapter 3), the seasonal rhythm (see Chapter 5) and changes from day to day (Chapter 5).

The data from the main and parallel roads was therefore analysed in two ways:-
a) An arithmetic mean concentration was found for the whole day, on each road. All the concentrations were expressed as the number of flies/2000 m^3 of filtered air. The actual mean concentration for each day will have little meaning, since it will vary depending on the diurnal rhythm and the weather. Thus circuits made after sunset will contain very few flies because of the diurnal rhythm (see Chapter 3) and so would lower the overall mean concentration

for the day. Similarly, cloudy days (such as 14:vi:73 - see Fig. 41) had higher mean concentrations than did sunny days (such as 27:vi:73), because on cloudy days activity occurred throughout the day, while on sunny days activity was restricted to the morning and evening, so that catches taken during the afternoon lowered the daily mean.

The mean concentration therefore depended not only on the actual concentration of flies, but also on the timing of the circuits with respect to diurnal rhythm and the weather. However, all four roads within a circuit would be affected to the same degree (except during the evening peak - see below), so that the relative differences between the roads would remain constant even if the mean concentrations were very variable from circuit to circuit. Since it was only the relative differences of the four roads that was important and not the absolute size of the mean concentrations, a mean for all the twelve days studied was found on each road. Taking a mean of a mean helped to reduce the effect of unusually large catches resulting from the evening peak of activity.

Using mean concentrations can result in a bias to one of the roads as a result of the diurnal rhythm. When there was a sharp evening peak, it was often so brief (less than twenty minutes) that it only occurred on one road. This one catch was often so large that it swamped all the rest of the days catches, so that when calculated as a daily mean, the highest mean occurred on whichever road had the evening peak catch. This occurred on the following days:-

Date	Road on which peak occurred
14:vi:72	Main Road
18:vii:72	Road 1 + Main road
5:vi:73	Road 1 + Main road (see Figs. 5 + 42)
14:vi:73	Road 2
27:vi:73	Road 2 + 3

b)   The roads were compared in pairs.   The number of circuits in which one road had higher concentrations than a second was compared with the number of circuits in which the second was higher than the first.   The significance of the difference between the two totals was then tested using a chi-square test. This had the advantage of making all the circuits of equal importance.   It therefore reduced the effect of diurnal peaks of activity and of seasonal changes in the overall concentrations, and so was more reliable than using mean concentrations.

In summary, therefore, the parallel road results were much more reliable than the main road results because they had higher concentrations and were based upon three times as many catches per circuit.   The main road results have however been included for comparison.

The calculations using the mean concentrations were likely to be biased because many of the days had such a pronounced diurnal rhythm that most of the activity occurred within a short period of time (and thus on only one or two of the roads), swamping all the rest of the catches in that day.   Because a mean has been used, the absolute mean concentrations had little meaning, since because of the diurnal rhythm, the mean depended on the times at which the catches were taken.   However, since all four roads would be affected by the same amount, the means can still be used for comparing them.   Chi-square

FIG. 41   SPATIAL DISTRIBUTION OF S.REPTANS FEMALES
(TOTAL CATCH)

DATE	Parallel Roads				Main Road						
	Rd 1	2	3	4	D	E	F	G	H	I	J
31:5:72	24.1	32.3	10.2	9.6	23.4	17.5	15.8	7.6	5.8	14.1	3.0
14:6:72	18.2	23.0	8.9	7.1	22.5	17.5	19.9	12.9	10.5	13.6	6.8
26:6:72	27.9	43.2	21.9	15.0	25.1	30.3	23.2	18.0	7.9	13.2	9.2
18:7:72	26.8	20.3	10.4	6.4	17.5	10.4	11.2	9.0	3.7	7.1	6.0
27:7:72	8.0	13.2	7.1	3.3	7.8	9.6	5.1	2.6	1.8	2.0	2.2
31:8:72	11.2	16.5	5.6	6.6	8.8	13.2	19.6	9.4	3.0	8.2	4.8
5:6:73	43.1	30.4	10.5	7.2	34.3	30.8	12.5	4.4	8.8	12.2	4.2
14:6:73	28.9	83.1	21.4	18.8	42.8	50.1	17.8	6.4	4.7	18.7	15.0
21:6:73	25.1	46.1	16.3	12.5	19.6	23.2	10.4	9.4	8.1	12.3	11.8
27:6:73	12.1	22.7	16.1	2.9	4.9	6.7	2.2	4.4	2.1	1.9	2.2
4:7:73	14.8	27.0	10.8	6.2	9.5	13.9	11.1	8.0	5.2	7.4	7.3
12:7:73	25.3	54.4	29.5	12.7	16.6	13.0	10.5	10.8	6.9	7.4	7.4
MEAN	23.2	35.4	14.2	9.2	19.4	19.7	13.3	8.6	5.9	9.8	6.7

D=Langwathby ;   J= Melmerby

Mean Concentration   (no. / 2000 cu.m of air)

FIG. 42

CONC. OF FLIES (no 2000 cu m of air)

SUNSET PEAK

S. REPTANS 5 : 6 : 73

KEY : -

o———o = gravid

o— — -o = nulliparous
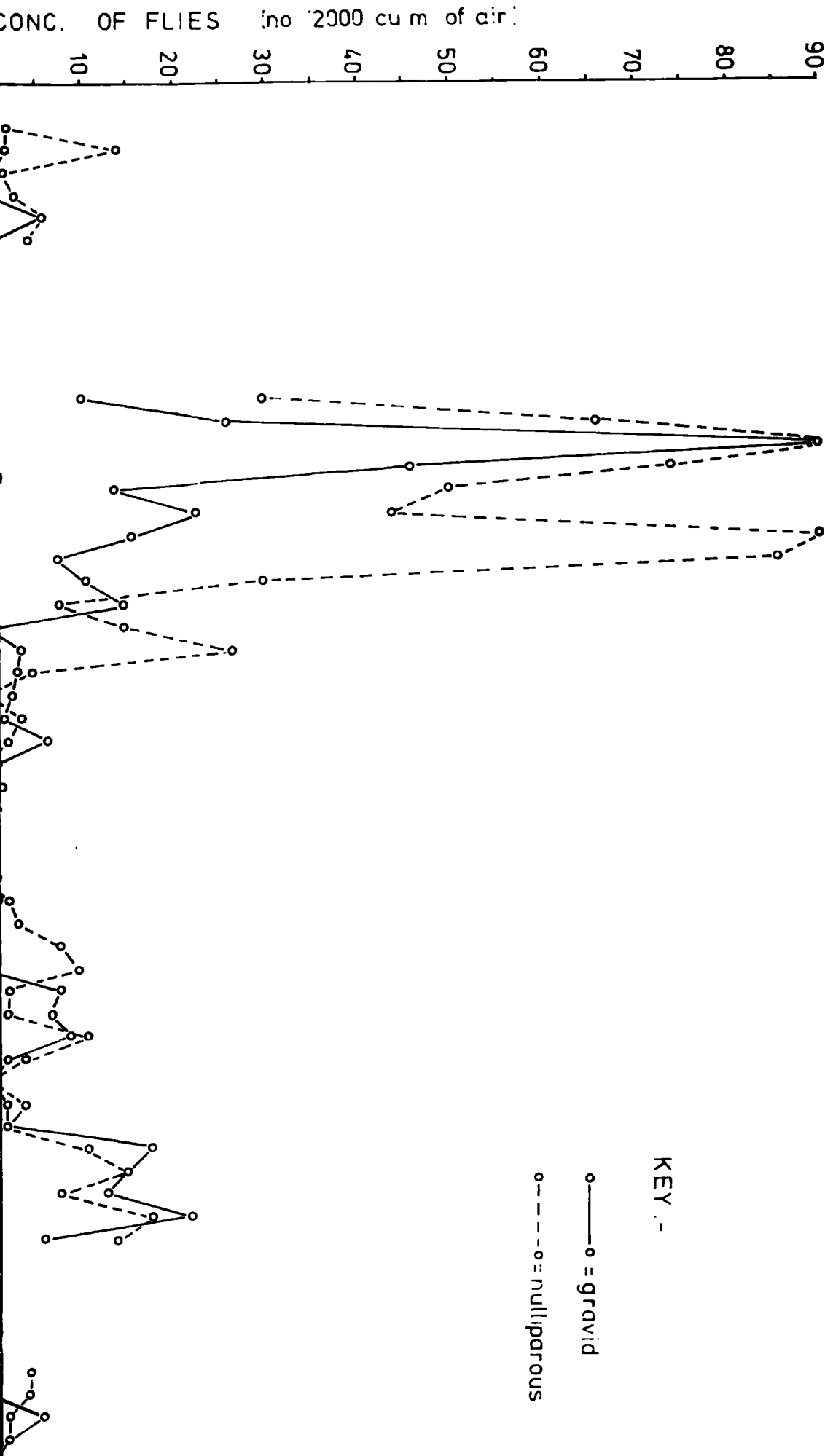
analysis was therefore much more reliable because it made all the circuits

of equal importance, preventing the diurnal rhythm from having an undue effect.

The mean concentrations have therefore only been included for comparison.

3. SPATIAL DISTRIBUTION OF S.REPTANS

   a) Male and female flies

   The mean concentration of female flies on the parallel roads are shown in

Fig. 41.   The concentrations were highest on Road 2, and decreased in the order

Road 2, Road 1, Road 3 and Road 4.   The main road (also shown in Fig. 41)

differed from the parallel roads in that there was no difference between the

means for the first two sections D and E (equivalent to Roads 1 and 2).   It

also differed in having a small increase on section 1.   This was probably due

to the presence of woods beside the road (see section 3e).

   Since this research was principally concerned with the female flies, the

males were only investigated on four days.   The mean concentrations on the

parallel roads for the males and females, on the same days were:-

	Males	Females	
Road 1	9.1	11.4	conc. = no./2000 m^3 air
Road 2	17.1	22.2	
Road 3	5.0	6.3	
Road 4	4.7	3.1	

To make the males and females directly comparable with each other, the

concentrations on each road were expressed as a percentage of the total male

and females:-

	Male	Female	Signif.of the diff.
Road 1	25.4	26.5	n.s.
Road 2	47.6	51.6	n.s.
Road 3	13.9	14.7	n.s.
Road 4	13.1	7.2	p=0.01

The significance of the difference between the males and the females was found for each road using the formula:-

$$d = \frac{k_1 - k_2}{\sqrt{K(1-K)(\frac{1}{n_1} + \frac{1}{n_2})}}$$

$$\text{where} \quad k_1 = \frac{a_1}{n_1}; \quad k_2 = \frac{a_2}{n_2}; \quad K = \frac{a_1 + a_2}{n_1 + n_2}$$

d = standard deviation

a_1 = number of males caught on one road

n_1 = total males caught on all four roads

a_2 = number of females caught on the one road

n_2 = total females caught on all four roads

(from Bailey 1959)

Therefore on the days compared, the only significant difference between the spatial distribution of males and of females was on Road 4, though it is possible that this was due to the low concentrations involved.

The female flies, as explained in Chapter 2, were divided into nulliparous (young flies, still in their first gonotrophic cycle) and parous (old flies which have completed at least one cycle). The gravid flies (with fully mature eggs) were put into a third category, because of the difficulty of dividing them into nulliparous and parous. Parasitised flies were not included in these categories and are considered separately in Chapter 6.

FIG. 43   MEAN CONCENTRATIONS OF S.REPTANS FEMALES
ON THE PARALLEL ROADS

DATE	No. of circuits	Nulliparous				Parous				Gravid			
		Rd 1	2	3	4	Rd 1	2	3	4	Rd 1	2	3	4
31:5:72	7	22 6	29.3	10.3	8.6	1.6	1.5	0.2	0.3	1.0	2.5	0 2	0.0
14:6:72	8	2.0	2.5	0.7	1.2	7.4	7.9	4 0	3.1	4.9	9.2	2.3	2.0
26 6:72	8	2 9	3.5	1.5	1.2	15.3	21.2	9.7	7.1	3.1	6.9	4.5	2.4
18 7:72	7	8.2	3.2	1.7	0.9	9.2	8.8	5.0	2.3	8.2	7 1	1.6	2.2
27 7:72	7	1.1	2.1	1.2	0.6	5.2	7.4	3.4	2.0	1.5	1.9	1.5	0 8
31 8.72	5	-	-	-	-	11.2	16.5	5.6	6.6	9 8	10 8	1 4	1.2
5:6:73	9	21.7	14 8	5 5	2.9	3.7	2.1	1.3	0.5	8 9	4 6	1.8	2.2
14:6:73	9	7 1	30.2	10.3	7.2	8.3	22.9	4.7	6.6	4.2	20.3	3 9	1 3
21 6 73	9	2.9	3.8	1.2	1.6	12.6	16.7	5.9	3.6	6 7	22.2	6.7	5.7
27 6:73	9	0.7	1.0	0.7	0.0	6.8	6.8	6.2	1.5	3 9	13.4	7.4	1.2
4 :7:73	9	0.7	3.3	1.0	0.8	10.9	14.2	6.2	4.1	2.8	5.9	3 2	1.0
12:7:73	13	3 0	6.6	3.5	2.5	9.1	12.8	7 1	3.1	4.2	16.1	8.4	2.6
MEAN CONC.		6.1	8.4	3.1	2.3	8.4	11.6	4.9	3.4	4.9	10.1	3.6	1.9

Conc. = no. of flies/ 2000 cu.m of air

FIG. 44   MEAN CONCENTRATIONS ON THE PARALLEL ROADS

S. REPTANS FEMALES

KEY:-

o———o = gravid

o—·—o = nulliparous
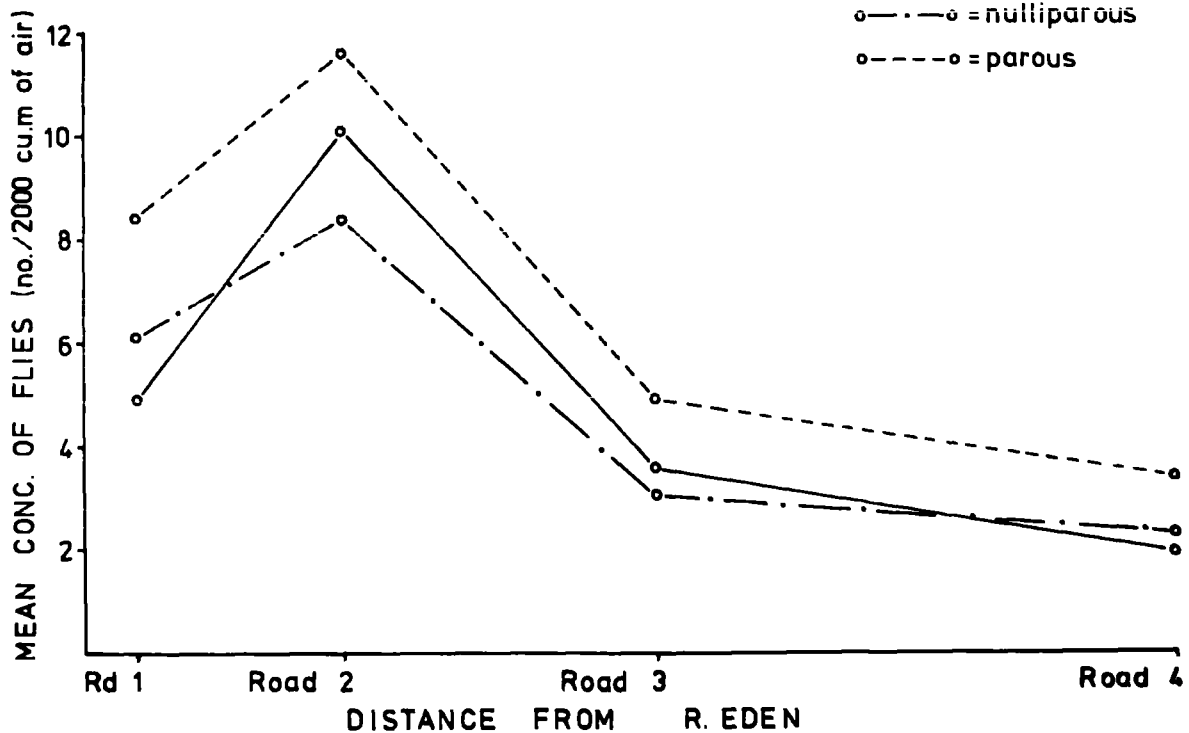
o————o = parous

FIG. 45   **MEAN CONCENTRATIONS OF S.REPTANS FEMALES ON THE MAIN ROAD**

Date	Nulliparous							Parous							Gravid						
	D	E	F	G	H	I	J	D	E	F	G	H	I	J	D	E	F	G	H	I	J
31:5:72	21.4	12.6	12.2	5.9	4.2	12.6	2.6	-	1.9	0.2	0.7	-	-	-	0.2	0.5	0.7	0.2	-	-	-
14:6:72	1.8	0.4	0.7	-	0.3	0.5	0.6	12.1	2.2	4.2	2.7	2.4	1.0	1.1	4.7	10.9	12.7	8.5	6.2	8.9	2.7
26:6:72	1.1	2.3	1.1	0.6	0.6	0.8	0.8	10.4	12.3	9.3	8.9	0.9	3.4	4.0	4.5	4.4	5.7	3.4	3.4	2.5	0.8
18:7:72	2.0	1.9	1.6	2.8	0.3	1.0	0.8	8.0	4.7	5.2	3.1	0.9	3.1	2.8	7.2	5.1	4.1	2.8	2.5	2.9	2.1
27:7:72	0.9	0.9	1.1	0.4	-	-	0.2	4.0	3.5	2.6	1.1	1.5	1.5	1.3	2.2	5.1	0.6	1.1	-	0.5	0.2
31:8:72	-	-	-	-	-	-	-	5.6	6.0	9.4	4.0	1.6	4.6	2.4	2.8	6.4	9.0	5.4	1.4	2.8	2.0
5:6:73	20.6	17.3	7.3	1.9	3.6	4.6	0.9	2.4	1.7	0.6	0.4	0.6	0.2	0.6	34.3	30.8	12.5	4.4	8.8	12.2	4.2
14:6:73	15.3	14.0	4.5	1.8	2.5	8.7	6.0	10.7	10.3	3.7	0.9	0.6	3.9	4.3	3.8	14.1	4.1	2.1	0.8	3.6	1.7
21:6:73	1.8	2.5	1.0	0.2	0.8	0.7	1.2	8.0	8.4	2.6	2.8	1.3	2.2	3.4	9.2	10.2	5.7	5.8	5.1	9.2	6.6
27:6:73	0.8	-	-	0.2	-	-	0.4	2.4	2.7	1.8	2.1	1.0	1.1	0.4	4.9	6.7	2.2	4.4	2.1	1.9	2.2
4:7:73	0.5	1.1	0.4	0.6	0.3	1.0	0.4	7.1	8.4	6.6	5.9	4.1	5.2	5.5	1.9	3.3	3.6	1.2	0.8	1.2	1.0
12:7:73	2.7	1.1	1.2	0.8	0.4	1.6	1.0	8.3	6.3	4.6	3.4	1.8	2.2	2.3	5.1	6.7	4.3	6.4	4.5	2.9	4.0
Mean Conc.	5.7	4.5	2.6	1.3	1.1	2.0	1.2	6.6	5.7	4.2	3.0	1.4	2.4	2.3	4.1	6.2	4.6	3.3	2.3	3.3	2.0

Conc. = no. of flies/2000 cu.m of air

FIG. 46   **MEAN CONCENTRATIONS OF S.REPTANS FEMALES ON THE MAIN ROAD**

KEY :-
o———o = gravid
o—·—o = nulliparous
o———o = parous
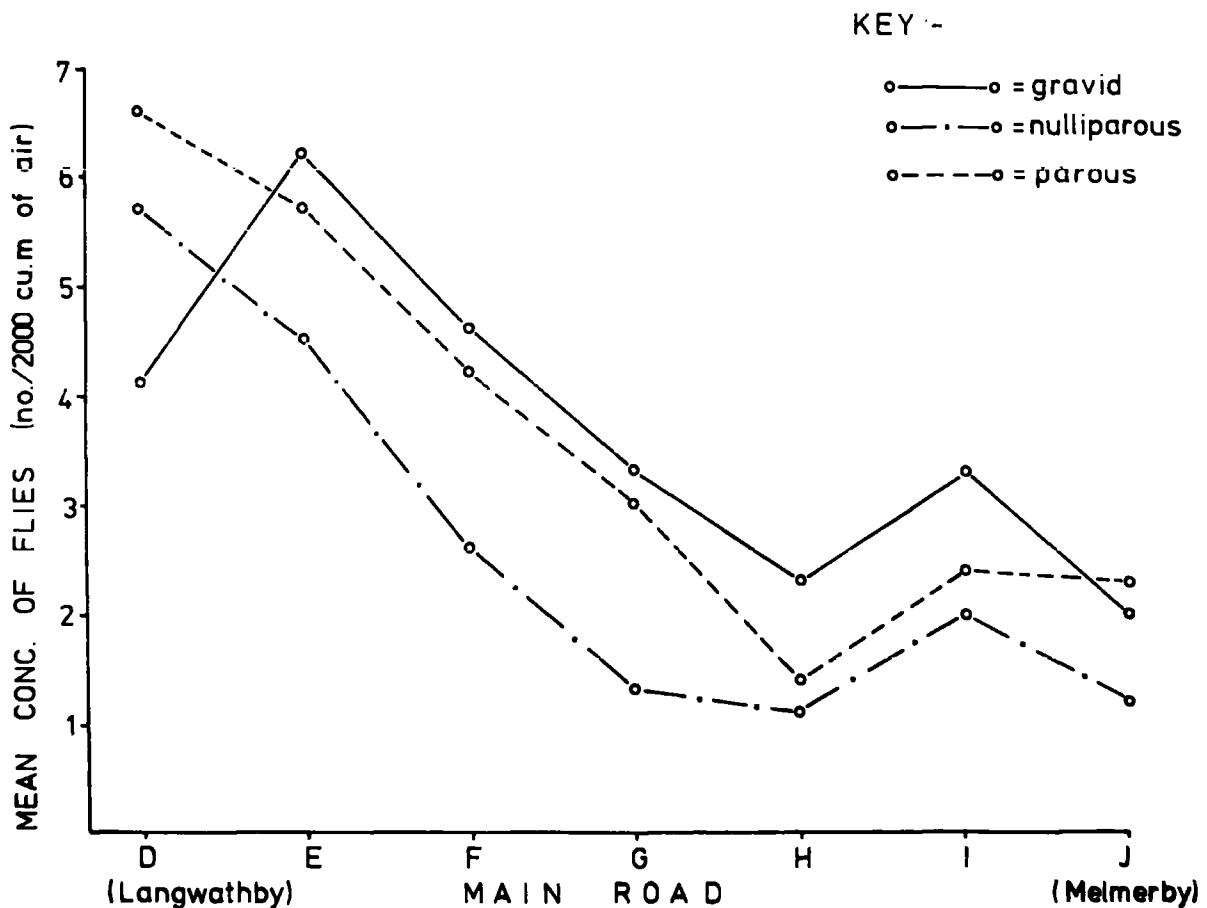
b)   Distribution of nulliparous and parous females

The nulliparous and parous concentrations are shown in Fig. 43 and 44 for the parallel roads, and Figs. 45 and 46 for the main road.

On the parallel roads, the concentrations of both nulliparous and parous flies decreased in the order Road 2, Road 1, Road 3 and Road 4.   Though Road 1 was closer to the River Eden (see Fig. 3), it had lower concentrations than Road 2 on every day, other than 18:vii:72 and 5:vi:73.   These two days were mentioned above as being biased to Road 1, by having large evening peaks on this road.

When the four roads were compared in pairs (Fig. 47), counting the number of circuits in which the concentrations on one road were higher than on the other, and vice-versa, then testing the significance of the difference of the two totals using chi-square, the situation was slightly different.   The nulliparous flies had significantly higher concentrations on Road 2 than on Road 1, whereas the parous had no significant difference between the two (in fact Road 1 was slightly higher).   Since the chi-square figures show the relative difference between the road pairs, they were plotted in a graph form (Fig. 49).   The parous flies showed a rapid decrease in concentrations with distance from the river, while the nulliparous had a marked increase up to Road 2 followed by a decrease to Road 3.   The relatively small difference between Road 1 and Road 4 for the nulliparous flies, compared with the very large difference for the parous, showed that the nulliparous were much more evenly distributed throughout the valley, whereas the concentrations of parous fell off much more markedly away from the river.

FIG. 47

FIG. 47 — CHI-SQUARE FOR S.REPTANS FEMALES ON THE PARALLEL ROADS

ROADS	Nulliparous			Parous			Gravid		
	Total	X^2	signif.	Total	X^2	signif.	Total	X^2	signif.
1 + 2	25 } 55	11.3	p=.001	50 } 47	0.1	n.s.	30 } 60	10.0	.01
1 + 3	47 } 24	7.5	p=.01	73 } 18	33.2	.001	51 } 30	5.4	.02
3 + 4	35 } 27	1.0	n.s.	55 } 28	8.8	.01	45 } 23	7.1	.01

FIG. 48 — CHI-SQUARE FOR S.REPTANS FEMALES ON THE MAIN ROAD

Section	Nulliparous			Parous			Gravid		
	Total	X^2	signif.	Total	X^2	signif.	Total	X^2	signif.
D + E	31 } 18	3.4	n.s.	42 } 33	1.2	n.s.	24 } 38	3.2	n.s.
E + F	30 } 15	5.0	p=.05	43 } 31	1.9	n.s.	35 } 20	4.1	.05
F + G	27 } 15	3.4	n.s.	43 } 16	12.4	.001	32 } 18	3.9	.05
G + H	16 } 15	0.0	n.s.	40 } 14	12.5	.001	27 } 15	3.4	n.s.
H + I	9 } 25	5.4	.02	18 } 40	8.3	.01	16 } 29	3.8	n.s.
I + J	25 } 13	3.8	.05	29 } 26	0	n.s.	29 } 22	1.0	n.s.
H + J	14 } 17	0	n.s.	17 } 40	9.3	.01	15 } 29	4.5	.05
G + J	20 } 21	0	n.s.	32 } 25	0.9	n.s.	29 } 26	0	n.s.

FIG. 49   SPATIAL DISTRIBUTION OF S.REPTANS FEMALES
AS SHOWN BY CHI-SQUARE

o——o = gravid
o—·—o = nulliparous
o————o = parous

χ^2

Rd 1   Road 2   Road 3   Road 4

Distance   from   R.Eden
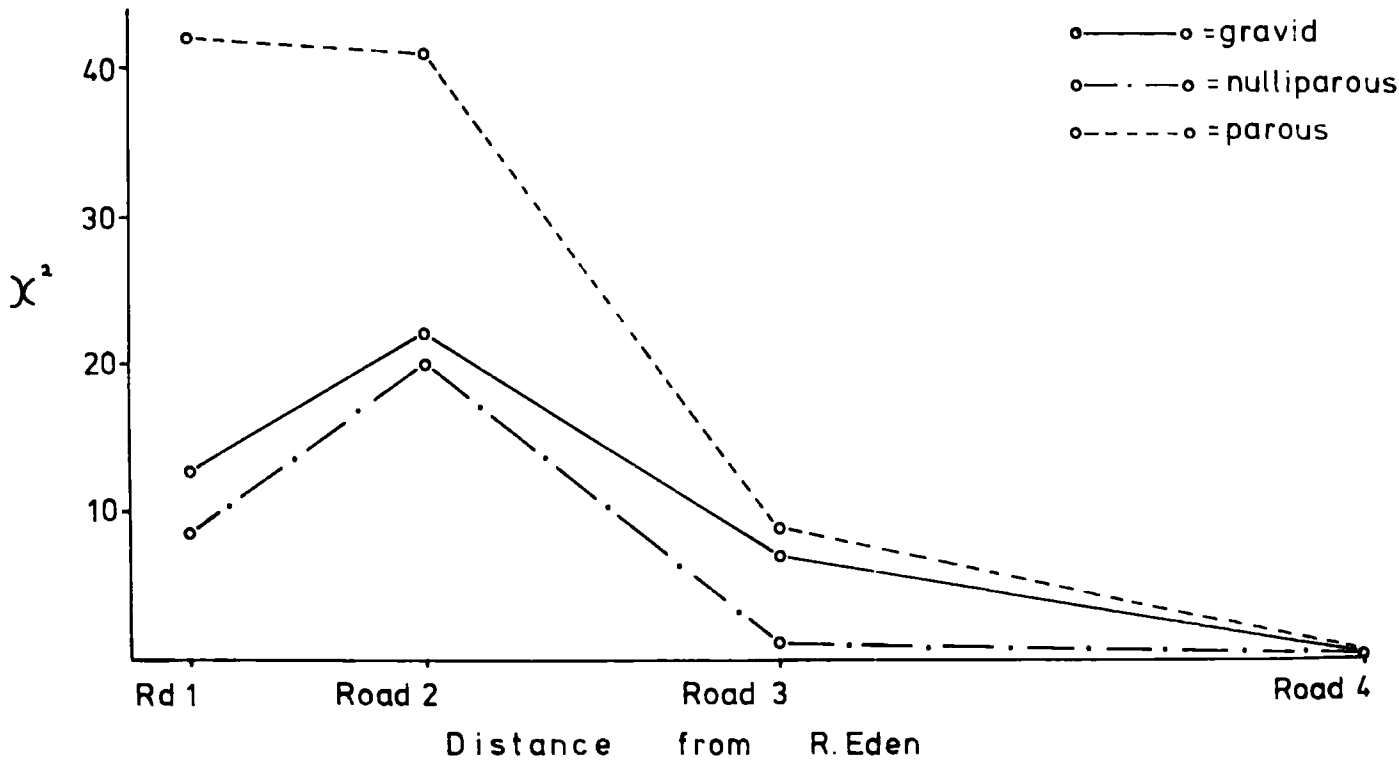
FIG. 50   CHI-SQUARE FOR S.REPTANS FEMALES ON MAIN ROAD

o——o = gravid
o—·—o = nulliparous
o————o = parous

χ^2

D   E   F   G   H   I   J

Distance from R.Eden
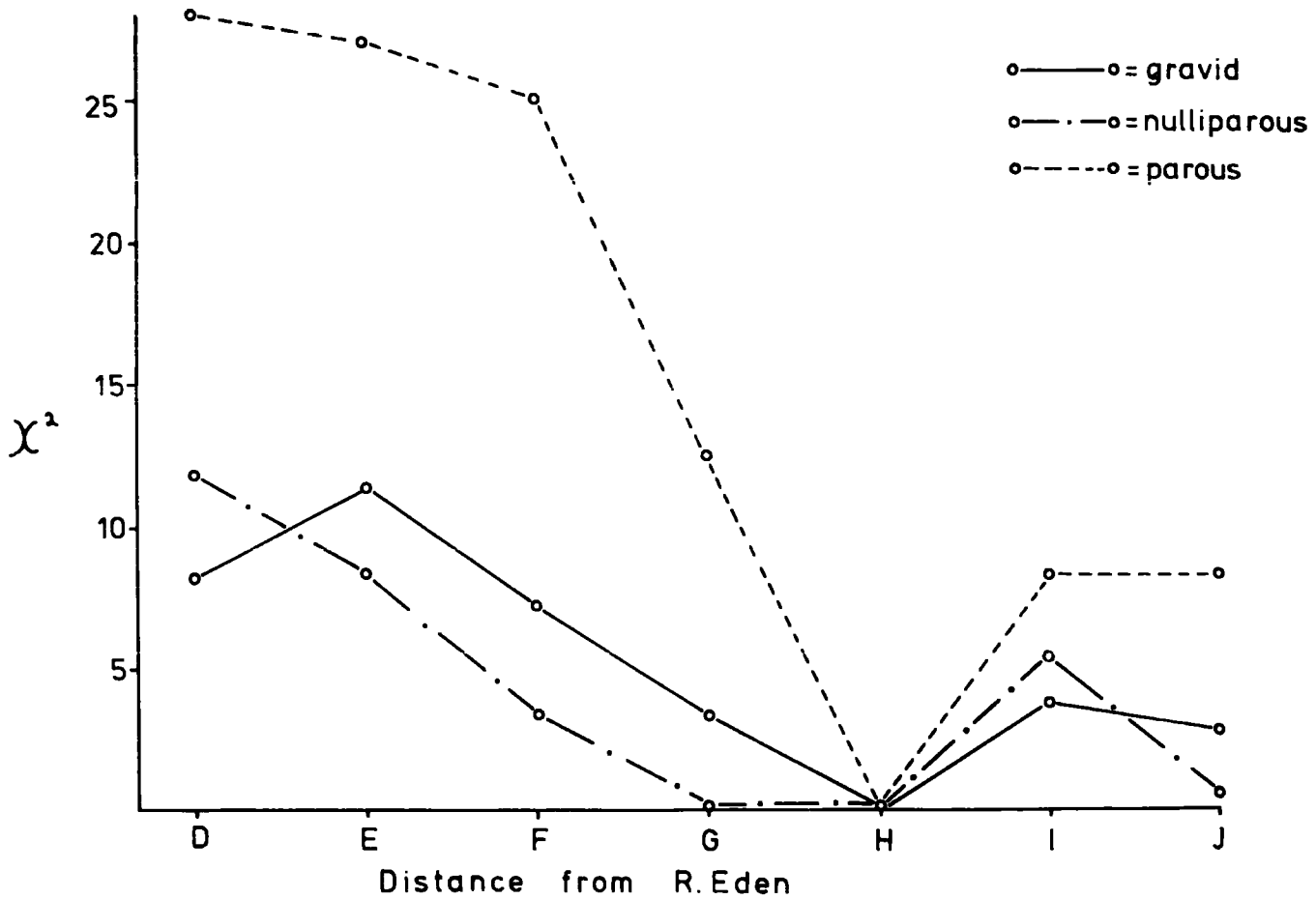
On the main road, concentrations of both nulliparous and parous flies decreased with distance from the river between sections D and H, but then increased again in section I (Figs. 45 and 46). When the sections were compared in pairs using chi-square (Figs. 48 and 50), there was no significant difference between D and E (unlike the parallel roads, where there was a marked difference between Roads 1 and 2 for the nulliparous flies). But as on the parallel roads, the difference between D and J was much greater for the parous than the nulliparous flies (shown in Fig. 50). Thus, the nulliparous flies were more evenly distributed, while the parous flies had a marked decrease in concentrations with distance from the river. For both nulliparous and parous, there was no significant difference between G and J, despite the considerable distance involved. This was similar to the situation on the parallel roads where there was only a small difference between Roads 3 and 4. Though this difference was significant for the parous ($X^2 = 8.8$), it was small compared with the difference between Roads 2 and 3 ($X^2 = 33.2$). Thus beyond the first three kilometres from the river, there was a drop in the rate at which the concentrations of nulliparous and parous flies declined with distance from the river.

The main road sections were less reliable than the parallel roads, because the concentrations were generally lower with many of the catches consisting of only one or two flies (and so chi-square was less reliable). The sections were also only over a distance of one kilometre and therefore more likely to be biased by factors such as the presence of trees beside the road. The sections were originally measured using the van's tachometer and so were inaccurate, the actual lengths being:-

$$D = 0.97 \text{ km} \qquad G = 1.05 \text{ km}$$

$$E = 0.92 \text{ km} \qquad H = 0.74 \text{ km}$$

$$F = 0.97 \text{ km} \qquad I = 0.91 \text{ km}$$

$$J = 1.03 \text{ km}$$

Section H was therefore considerably shorter than the others, and so with the very low concentrations involved, a large proportion of the catches contained no flies. For moderate catches, calculating the flies as a concentration would adjust for the length of the section, but this obviously has no effect when there were no flies caught. Consequently, the very low concentrations on section H were probably partly an artefact.

The parous flies included all those which had undergone one or more gonotrophic cycles. No attempt was made to subdivide this group using the number of dilations of the ovarioles (which gives the number of gonotrophic cycles undergone) as has been done by Detinova (1958) and L. Davies (1961), because of the large number of flies dissected and the small size of the ovaries. However, since a proportion of the parous flies contained relict eggs, which have been left over from previous gonotrophic cycles, it would be expected that the proportion would increase as the number of gonotrophic cycles increased. Furthermore, flies from later gonotrophic cycles would be more likely to contain more than one relict egg. The relict eggs had the following frequency distribution:-

No. of relict eggs/fly	No. of flies	No. of relict eggs/fly	No. of flies
1	257	7	13
2	78	8	13
3	38	9	6
4	13	10	5
5	17	11-15	23
6	16	16+	26

Percentage of flies with relict eggs, containing more than 1 = 49.1%

Percentage of flies with relict eggs, containing more than 3 = 26.1%

The parous flies were therefore arbitrarily aged using the following divisions:-

 i)   Parous flies without relict eggs

 ii) Parous with only one relict egg

 iii) Up to three relict eggs

 iv) Over three relict eggs

These divisions are shown in Fig. 51 for the four parallel roads. The latter three divisions were also expressed as a percentage of the parous on each road, so that the spatial distribution of the divisions could be compared. The significance of the differences between the percentages was tested using the formula:-

$$d = \frac{k_1 - k_2}{\sqrt{K(1-K)(\frac{1}{n_1} + \frac{1}{n_2})}}$$

where $k_1 = \dfrac{a_1}{n_1}$; $k_2 = \dfrac{a_2}{n_2}$; $K = \dfrac{a_1 + a_2}{n_1 + n_2}$

d = standard deviation

a_1 = number of flies in one of the age categories

n_1 = total number of parous flies

a_2 = number of flies in a second age category

n_2 = total number of parous flies

(from Bailey 1959)

FIG. 51   SPATIAL DISTRIBUTION OF   S.REPTANS   FEMALES   WITH RELICT EGGS

(a) = number of flies with more than 1 relict egg
(b) = ...   more than 3 relict eggs
R.E. = relict egg

Road	Total P	Total R.E.	% R.E.	1 (a)	% of P	3 (b)	% of P
1	1330	168	12.6	77	5.8	47	3.5
2	2059	207	10.1	103	5.0	50	2.4
3	719	78	10.8	42	5.8	20	2.8
4	449	52	11.6	27	6.0	15	3.3

Date	Rd	Total P	Total R.E.	%R.E.	1 (a)	3 (b)
14:6:72	1	100	11	11.0	5	3
	2	98	10	10.2	4	1
	3	42	5	11.9	2	0
	4	29	2	6.9	1	0
26.6.72	1	167	26	15.6	9	5
	2	267	17	6.4	7	0
	3	117	12	10.3	4	0
	4	87	8	9.2	3	1
18 7.72	1	109	7	6.4	3	3
	2	100	13	13.0	5	1
	3	52	8	15.4	3	3
	4	20	3	15.0	2	1
27:7:72	1	82	11	13.4	7	4
	2	84	8	9.5	5	4
	3	35	4	11.4	1	1
	4	16	1	6.3	1	0
31:8:72	1	91	10	11.0	6	5
	2	125	10	8.0	4	0
	3	34	4	11.8	2	1
	4	36	1	2.8	0	0

Date	Rd	Total P	Total R.E.	%R.E.	1 (a)	3 (b)
5:6:73	1	47	11	23.4	3	2
	2	21	6	28.6	1	1
	3	14	5	12.2	4	0
	4	6	4	66.7	3	3
14:6:73	1	117	24	20.4	12	4
	2	521	58	11.1	25	10
	3	59	7	11.9	5	2
	4	101	18	17.8	9	6
21:6:73	1	198	29	14.6	12	8
	2	261	32	12.3	14	5
	3	81	10	12.3	4	1
	4	48	8	16.7	5	3
27:6:73	1	88	13	14.8	7	5
	2	108	17	15.7	11	9
	3	85	10	11.8	8	7
	4	11	1	9.1	0	0
4:7:73	1	149	13	8.7	5	3
	2	197	14	7.1	9	6
	3	82	4	4.9	3	2
	4	44	3	6.8	2	1
12:7:73	1	182	13	7.1	8	4
	2	277	22	7.9	18	12
	3	118	9	7.6	6	3
	4	51	3	5.9	1	0

There was no significant difference between the roads for any of the categories. Since each category therefore formed the same percentage of the parous flies on all four roads, they must each have had the same spatial distribution. So if these categories were a true reflection of the age of the flies, the older parous flies must have had the same powers and pattern of dispersal as the younger parous flies.

In summary, the nulliparous and parous flies had markedly different patterns of spatial distribution, as shown by the chi-square results. The decrease in concentrations between Road 1 and Road 4 (Fig. 49) were four times as great for the parous as the nulliparous. On the main road (Fig. 50), the difference was smaller, but if sections I and J were ignored since they were biased by the presence of trees (see section e of this chapter), the decrease in concentrations between D and H was still twice as great for the parous. The mean concentrations (Figs. 44 and 46) do not show this difference, but were unreliable due to reasons given earlier. The mean concentrations of both nulliparous and parous were higher on Road 2 than on Road 1, but this was due to the presence of trees (see section e).

Since the chi-square results were comparative, they could not be used to estimate the absolute decrease in concentrations between Road 1 and Road 4. However using the mean concentrations (and remembering that they were not too reliable), the concentrations decreased over the six kilometres between Road 1 and Road 4 by only a third, so that S.reptans had considerable powers of dispersal. This was emphasised by the fact that during the evening peaks, when there was a maximum of flight activity, the concentrations on Road 4 were often remarkably high. For example, on 14:vi:73 the evening catch had a concentration of 125.8 female S.reptans/2000 m^3 of air.

c)   <u>Distribution of gravid flies</u>

Like the nulliparous and parous, the gravid flies had their highest mean concentrations on Road 2 (Figs. 43 and 44).   Comparison of the parallel roads in pairs using chi-square (Figs. 47 and 49) showed a significantly higher number of flies on Road 2 compared with Road 1.   Since the gravids consisted of both nulliparous and parous flies (it being too difficult to separate the two when the ovaries were mature), they would be expected to have an intermediate distribution.   On the twelve days considered, the nulliparous to parous ratio was approximately 2:3.   The gravids would therefore be expected to have chi-square values of Road 2/Road 1 = 4; Road 1/Road 3 = 23; Road 3/Road 4 = 6. Therefore Road 2 had markedly higher concentrations than would be expected, while Road 1 had much lower concentrations.

On the main road, the gravids differed from the non-gravids in having higher concentrations on section E than on section D.   This was also shown by the chi-square figures, though the difference was not significant, and therefore agreed with the parallel road results.   Apart from section D, the gravids were similar to the nulliparous and more evenly distributed than the parous (as they were on the parallel roads).

d)   <u>Distribution of blood-fed flies</u>

The concentration of flies containing a blood meal in the midgut was often very low:-

Date	Percentage Blood-fed	Date	Percentage Blood-fed
31:v:72	3.5	5:vi:73	10.8
14:vi:72	3.2	14:vi:73	1.9
26:vi:72	3.7	21:vi:73	5.6
18:vii:72	10.1	27:vi:73	8.0
27:vii:72	5.1	4:vii:73	12.3
31:viii:72	21.1	12:vii:73	6.0

so that the actual number of flies caught was used (Fig. 52) rather than the concentration. The fresh blood-fed flies (where the blood was bright red and liquid, and probably less than one day old) were analysed separately, as well as being included in the total. The roads were expressed as a percentage for each category, so that the spatial distribution of each category could be compared using the formula:-

$$d = \frac{k_1 - k_2}{\sqrt{K(1-K)(\frac{1}{n_1} + \frac{1}{n_2})}}$$

where $\quad k_1 = \frac{a_1}{n_1}; \quad k_2 = \frac{a_2}{n_2}; \quad K = \frac{a_1 + a_2}{n_1 + n_2}$

d = standard deviation

a_1 = number of fresh/total blood-fed caught on one road

n_1 = total fresh/total blood-fed caught on all four roads

a_2 = number of non blood-fed flies caught on one road

n_2 = total non blood-fed caught on all four roads

For the parous flies, there was no difference in the spatial distribution of the three categories (Fig. 52). But for the nulliparous flies, though the total blood flies had the same distribution as the non blood-fed flies, the fresh blood-fed flies were significantly different on Roads 1 and 2. This however was probably an artefact due to the data of 5:vi:73. This contained 54% of all the nulliparous fresh blood-fed flies, and as mentioned previously, was biased to Road 1 by having a large evening peak on this road. Therefore when this day's catch was removed, there was no significant difference between the spatial distribution of the fresh blood-fed and the non blood-fed flies (Fig. 52).

FIG.52 DISTRIBUTION OF BLOOD-FED FLIES (EXPRESSED AS PERCENTAGES)

	Nulliparous				Parous			
	Rd 1	2	3	4	Rd 1	2	3	4
Fresh blood-fed	44.1	26.5	23.5	5.9	29.6	36.5	23.9	10.1
Total blood-fed	36.6	37.1	17.4	8.9	29.4	43.0	18.2	9.4
Non blood-fed	30.7	42.2	15.6	11.6	29.7	41.0	17.3	12.0
Significance:-								
Fresh/Non	.03	.01	n.s.	n.s	n.s.	n.s.	n.s.	n.s.
Total/Non	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
With 5:6:73 removed :-								
Fresh	34.0	36.2	19.2	10.6				
Total	28.1	40.8	19.1	12.0				
Fresh/Non	n.s.	n.s.	n.s.	n.s.				
Total/Non	n.s.	n.s.	n.s.	n.s.				

BLOOD-FED S.REPTANS ON THE PARALLEL ROADS

Date	Total Blood-fed								Fresh Blood-fed							
	Nulliparous				Parous				Nulliparous				Parous			
	Rd 1	2	3	4	Rd 1	2	3	4	Rd 1	2	3	4	Rd 1	2	3	4
31:5:72	17	26	10	7	-	-	-	-	5	3	1	1	-	-	-	-
14:6:72	3	7	-	2	8	11	5	4	-	-	-	-	2	1	1	-
26:6:72	2	3	4	2	26	31	11	4	1	1	-	-	3	3	-	-
18:7:72	12	14	6	2	15	30	12	4	5	8	2	1	4	12	5	3
27:7:72	-	-	1	1	4	17	2	2	-	-	1	-	2	7	2	1
31:8:72	-	-	-	-	34	79	28	23	-	-	-	-	7	15	9	6
5:6:73	85	53	25	7	3	2	-	-	29	10	15	1	-	-	-	-
14:6:73	10	19	16	3	2	8	4	1	1	-	3	2	1	1	1	-
21:6:73	17	25	5	10	18	20	8	2	9	3	2	-	9	4	1	-
27:6:73	-	-	-	-	21	42	30	4	-	-	-	-	5	3	9	-
4:7:73	2	1	-	-	54	49	10	13	2	-	-	-	9	8	4	2
12:7:73	12	14	8	5	32	28	14	12	5	2	-	1	5	3	6	4
Total	160	162	75	39	217	317	134	69	45	26	24	6	47	58	38	16

The fresh blood was probably less than one day old, so that the flies containing it had probably taken their blood meal near where they were caught. Since the distribution of fresh blood-fed flies was not significantly different from that of the non blood-fed flies, the presence of hosts for blood meals does not appear to affect the fly distribution. The main source of blood meals for S.reptans was cattle (L. Davies et al 1962). The Eden Valley is an intensive dairying region, with large numbers of cattle dispersed throughout the valley.

e)   Effect of the roadside vegetation on the catches

The distribution of flies on the parallel and main roads (Figs. 44, 46, 49 and 50) would be expected to show a steady decrease in the concentrations with distance from their breeding ground, the River Eden. There were however two major anomalies:-

i)   The mean concentrations of nulliparous flies was greater on Road 2 than on Road 1 (Fig. 44), so that the concentrations were greater at 1.5 km from the river than they were at 0.5 km. However, on the main road, there was no such increase in concentrations away from the river.

ii)   On the main road, the concentration of flies was greater in the last two sections I and J than in the previous two sections G and H. On the parallel roads, however, Road 4 (equivalent to I and J) had, as would be expected, lower concentrations than Road 3 (equivalent to G).

These anomalies in the fly concentrations were probably due to local environmental effects, the most important of which was probably the roadside vegetation.

The roadside vegetation was classified into three groups:-

i)   Woods - forming a solid barrier to cross-winds and giving dense shade.

ii)   Tall hedges or row of trees over three metres tall (that is, above

       the height of the net) and within one metre of the road - these

       would form a less dense, and thus less effective barrier.

iii)   Exposed - only a low hedge or wall below the level of the net, and

       so providing no shelter.

The distribution of these groups are shown in the map (Fig. 53).

The only major stretches of woods were on sections I and J of the main

road, and sections S and T of Road 2.   The stretch marked on section L of

Road 4 was in fact mainly in a valley below the level of the road.   The wood

on section B of Road 1 was only about 30 m wide and set back from the road by

about 5m.   Rows of trees or tall hedges were found on sections L and M of

Road 4, section O of Road 3 and section C of Road 1.

Since the three sections on each parallel road were approximately

equidistant from the River Eden, they would be expected to have equal

concentrations if the vegetation had no effect on the catch.   Therefore on

each road, the three sections were compared with one another in pairs.   The

number of circuits in which one road had higher concentrations than a second

was counted, and vice-versa, then the totals were compared by chi-square.

The results are shown in Fig. 54; part (a) being for the total female

catch, while in part (b) the total is separated into the three groups:

nulliparous, parous and gravid.   For the total catch, there was no significant

difference between any of the sections on Road 3 or on Road 4.   The presence

of a row of trees beside the road does not therefore appear to affect the

distribution of the flies.   Section O, for example, did not have a significantly

higher catch than P or Q, despite the presence of a row of trees on either side

of the road (see Plate 2).   Similarly, though section K on Road 4 had a lower

catch than L or M, the difference was not significant.

FIG. 53

MAP OF ROADSIDE VEGETATION

R.Eden

Road 4

Road 3

Road 2

Road 1

≠=≠ = km sections

▥ = wood

······ = tall hedge
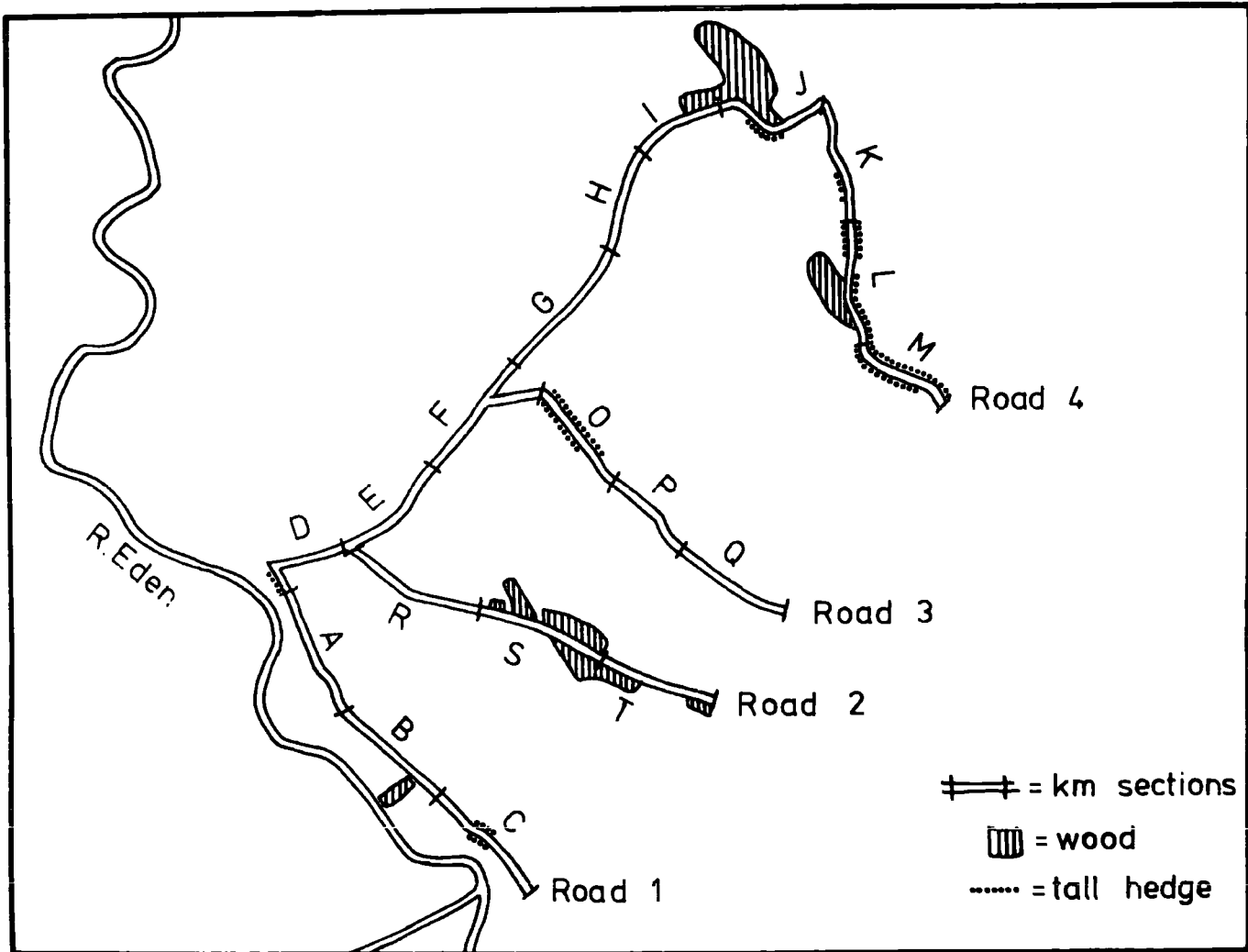
FIG. 54

COMPARISON OF THE CATCHES SHOWING THE EFFECT OF VEGETATION ON S.REPTANS

a) TOTAL CATCH

	Sections	Totals	X^2	Signif.
Road 1	A / B	47 / 49	0.04	n.s.
	B / C	30 / 70	8.00	0.001
	A / C	31 / 67	13.22	0.001
Road 2	R / S	15 / 69	34.71	0.001
	S / T	58 / 35	5.69	0.02
	R / T	18 / 64	25.81	0.001
Road 3	O / P	46 / 36	1.22	n.s.
	P / Q	37 / 48	1.42	n.s.
	O / Q	46 / 38	0.76	n.s.
Road 4	K / L	36 / 50	2.28	n.s.
	L / M	38 / 45	0.60	n.s.
	K / M	34 / 49	2.72	n.s.

b) PHYSIOLOGICAL GROUPS

Section	Nulliparous			Parous			Gravid		
	Totals	X^2	Signif.	Totals	X^2	Signif.	Totals	X^2	Signif.
A / B	30/32	0.06	n.s	44/39	0.30	n.s	31/34	0.14	n.s.
B / C	23/35	2.48	n.s.	26/63	15.38	0.001	25/45	5.72	0.02
A / C	27/33	0.60	n.s.	28/62	12.84	0.001	21/48	10.56	0.01
R / S	20/53	14.91	0.001	21/59	18.05	0.001	16/52	19.06	0.001
S / T	41/33	0.86	n.s.	51/35	2.98	n.s.	43/38	0.31	n.s.
R / T	23/40	4.59	0.05	26/65	29.40	0.001	21/53	13.84	0.001
O / P	33/15	6.76	0.01	36/31	0.38	n.s.	27/24	0.18	n.s.
P / Q	16/26	2.38	n.s.	38/35	0.12	n.s.	19/29	2.08	n.s.
O / Q	26/22	1.34	n.s.	41/30	1.70	n.s.	26/29	0.16	n.s.
K / L	15/29	4.46	0.05	30/39	1.18	n.s.	27/19	1.14	n.s
L / M	24/25	0.02	n.s.	31/34	0.14	n.s.	22/23	0.02	n.s.
K / M	17/32	4.60	0.05	29/37	0.96	n.s.	28/23	2.25	n.s.

In contrast, the presence of woodland had a marked effect on the catches of Road 2. Of the three sections on Road 2, section R was completely exposed with no woodland or tall hedges present; section S was wooded on most of the north side and half of the south side of the road; while section T was only wooded on half the south side of the road. The chi-square results (Fig. 54) show that the exposed section R had significantly lower catches than the two wooded sections, and that the heavily wooded section S had higher catches than the partly wooded section T. On Road 1, section C had significantly higher catches than sections A and B. The reason for this however is not clear, since it would be surprising if the small section of trees had such a marked effect (considering that the much greater stretch of trees on section O of Road 3 did not give a significant increase.

The presence of woods beside the road could be responsible for an increase in the concentration of flies by affecting a number of different factors:-

a)   Producing shade. This has two related effects:-

   i)   Increases the humidity. The shade provided by the trees would have a lower temperature, and thus rate of evaporation, than sunny areas. There would also be an increase in the humidity due to transpiration. Since the marked diurnal rhythm of Simuliidae (see Chapter 3) was probably an adaptation so the greatest activity occurred when humidity was highest, it is probable that the flies would prefer to fly in shade rather than in sunlight. Thus the concentration of flies would be greatest where there were woods beside the road.

   ii)   Reduces the light intensity. Whereas the presence of high humidities may increase the concentration of flies occurring in that region, a reduction in the light intensity probably acts as a stimulus to flight activity (see Chapter 3). The method of collecting meant that

the results depended not only on the actual concentration of flies
present, but also on the level of flight activity. Thus if the flight
activity was increased, increasing the number of flies in flight at
any one moment, then the number of flies caught would increase, even
though the actual number of flies present remained constant.

b)   Acting as a wind break.   Simuliid activity was markedly affected by the
wind strength.   Consequently, when there were cross-winds, presence of trees
would provide sheltered calm conditions.

c)   Barrier to flight activity.   Tall vegetation might act as a barrier to
flight activity, causing flies to accumulate on their boundaries.   However,
the woods were fairly open, while the most effective barrier would be a tall
hedge, such as occurred on section O of Road 3.   But this has been shown not
to have greater concentrations than the open sections P and Q.   The woods
could not therefore have been acting as a barrier.

d)   Marker for visual orientation.   It is possible that the flies preferred
to orientate by a marker, such as a line of trees, when flying.   They would
therefore fly along the edge of the wood.   Thus Giglioli (1965) found that
the mosquito, Anopheles gambiae Theobald flew alongside the edge of a bush-
clearing interface, while Nash (1969) found that tsetse flies follow game
trails through the grass.   However, as in the above section, tall hedges or
trees did not increase the catch, even though they would form a more prominent
visual object than the presence of woods.

The woods therefore probably affect the catch either by acting as a
source of shade or as a wind break.

T. Lewis (1969) considered that hedges acted as wind breaks. He found that a range of different species of flying insects collected on the leeward side of a hedge, and that the proportion of insect sheltering there increased as the wind velocity increased. However, this was only based on one hedge and ignored the effect of sunlight/shade differences. He also assumed that the flies were passively blown by the wind, whereas Simuliidae are strong robust flyers and there is no evidence that the species involved are blown downwind (though other species such as S.arcticum Malloch - Peterson and Wolfe 1958, are known to travel great distances downwind).

Bidlingmayer (1971) did not find any difference between the leeward and windward side of a hedge when studying mosquitoes, except under very strong wind conditions. Since he was working at night, there would be little humidity changes involved. He therefore considered that the very large catches caught beside the hedges were due either to the hedge acting as a barrier which the flies flew around rather than over, or that they visually orientated to the hedge so that there was a flight path parallel to the hedge, or that they reacted to the lower light intensity levels. In the same paper, he considered that the reason why woodland mosquitoes remained in the wood and did not enter the adjacent swamp was due to the lower light intensities in the wood.

As far as the net catches were concerned, hedges must have had little effect on the humidity or light intensity, since they were not tall enough to cast a shadow across the net (which was between two and three metres above the ground). The hedges would similarly have had little effect as a wind-break, and if anything appeared to channel the wind into the road. The woods on Road 2, however, were not only tall (over ten metres), but also overhung the road so that catches in both directions along the road were in the shade.

Unfortunately, it was not possible to assess the relative importance of the effects of increasing the humidity and of as acting as a wind break due to the lack of data. For example, there were only twelve circuits which were sunny and calm (calm has arbitrarily been taken as less than 1 km/h, since it is not known at what velocity the wind starts to have an effect on the fly), and only two circuits which were not sunny and had south-west cross-winds. South-west winds were therefore normally associated with sunny conditions, so that the effect of cross-winds could not be assessed independently of the effect of sunshine. No catches were made on days with north-east cross-winds, partly because of the associated low temperatures and thus low catches, and partly because the scarp slope of the Pennines greatly increased the velocity of the wind by an adiabatic process. North-east winds were therefore associated with strong cold winds and thus low fly concentrations.

In an attempt to evaluate the relative effects of the woods acting as a wind-break and as a shade, the catches on Road 2 were analysed by a multivariate analysis. Since by far the most important variables were the diurnal and seasonal rhythms, and the effect of the weather, these were eliminated by using percentages. In each circuit, six catches were made on Road 2 (three sections collected in either direction). The concentration of female flies in each catch was therefore expressed as a percentage of the total for the six in that circuit.

There were therefore only six variables left to be analysed:-

i)   The percentage of the female flies in the catch.

ii) The three sections, where:-

R = 0   (exposed section)

T = 1   (partly wooded)

S = 2   (mainly wooded)

iii) The catch direction, where:-

catch going south = 0

catch going north = 1

iv) The sun, where:-

absence of sun during the catch = 0

presence = 1

v) Wind presence, where:-

absence of wind = 0

presence = 1

vi) Wind direction, where:-

length winds (north or south) = 0

cross winds (east or west) = 1

This was then analysed by a stepwise regression using the M.T.S. computer program BMDO2R. The statistics involved are explained in Dixon (1968). The results for the six variables were:-

Variable	DF	Coefficient	SE
sections	2	6.25649	0.63981
wind presence	1	0.46197	1.13355
catch direction	1	0.29409	1.04483

where; DF = degrees of freedom = no. of possibilities (e.g. 3 sections)-1

SE = standard error

the coefficients for sunshine and wind direction were too small to be computed.

The significance of the above results can be tested:-

$$\frac{\text{coefficient}}{\text{S.E.}} = t$$

thus:-

variable	t	signif.
sections	9.779	p = 0.02
wind presence	0.408	n.s.
catch direction	0.282	n.s.

Therefore, the only variable which had a significant effect on the catch was the three sections. The principal difference between the three sections was the presence or absence of woodland, but since none of the sections were totally wooded (see Fig. 53) and since the presence of woodland was unlikely to be the only difference between the sections which affected the catch, it was not possible to calculate the percentage of the catch which was due to the presence of the woods (if it was possible, the mean concentrations on Road 2 could have been adjusted to eliminate the effect of the woods).

The multi-variate analysis was not therefore able to evaluate whether the effect of woodland was due to the trees acting as a wind break, or by producing shade, due to the lack of data.

As the presence of woods had such a marked effect on the distribution of flies along the road, their presence would have biased the spatial distribution results. The only woods occurring on the parallel roads were on section S and T of Road 2. Therefore, the fact that higher concentrations were found on Road 2 than on Road 1 (Fig. 44) was probably due to the presence of woods and not a result of the spatial distribution.

Unfortunately, as mentioned above, it was not possible to calculate the effect of the woods from the multi-variate analysis, though it indicated that the effect was probably very marked. If, however, for the sake of the calculation, it was assumed that the presence of the woodland was the only difference between the sections, and that section S was totally wooded and section T half wooded, then the effect of the woodland could be calculated:-

Coefficient of the woodland (assumed=section) = 6.25

Degree of freedom = 2

The percentage due to the woodland on section S = coeff. x DF

$$= 12.5\%$$

However, this was based on a mean percentage of flies = 16.667%
(to convert the data into percentages, each catch had been calculated as a
percentage of the total six catches in the circuit - see earlier, thus each
catch = a sixth of the total = 0.16667).

Therefore, the percentage of the catch on section S due to the woodland

$$= \frac{12.5 \times 100}{16.667} = 75.0\%$$

As section T has been assumed to be half wooded, the percentage of the catch
due to the woodland on section T = 37.5%.

Since section R is assumed to be non-wooded, section T to be half-wooded
and section S to be fully-wooded, then a mean catch for the whole of Road 2
would be equivalent to a half-wooded section. Thus the mean concentration
of Road 2 would be 37.5% too high due to the presence of woods. The mean
concentration (Fig. 41) = 35.4 flies/2000 m of air.

Therefore, the concentration due to the presence of woods = $35.4 \times \frac{37.5}{100}$

$$= 13.3\%$$

Therefore, without the woods, the conc. = 35.4 - 13.3 ·   = 24.1

This is very close to the mean conc. of Road 1 (Fig. 41)   = 23.2

Remembering that this was based on the assumption that all the differences
between the sections were entirely due to the presence or absence of woodland,
and that section S was totally wooded (it was in fact only about 75% wooded -
see Fig. 53) so that this calculated effect of the woodland is an exaggeration,
then the concentrations on Road 2 were at least as great as those on Road 1,
and probably larger.

The effect of the woodland can be estimated by a second method. Since section R was unaffected by woods, if it was assumed to be typical for its distance away from the river of 1.5 km (and thus unaffected by other local habitat effects), then it can be compared with sections A and B of Road 1 using chi-square (section C was ignored because its catches were unexplainably large - see Fig. 54). The number of circuits in which section R was higher than section A, and the number in which A was higher than R was compared using chi-square. This was then repeated for sections R and B:-

	Totals section A	R	X	Signif.
Nulliparous	20	28	1.34	n.s.
Parous	42	33	1.32	n.s.
Gravid	28	35	0.78	n.s.
	section B	R		
Nulliparous	27	31	0.28	n.s.
Parous	43	41	0.04	n.s.
Gravid	27	36	0.64	n.s.

Therefore for all three categories of nulliparous, parous and gravid, there was no significant difference between section R (Road 2) and sections A and B (Road 1). This agrees with the multi-variate analysis results, but is in marked contrast to Figs. 47 and 49, where Road 2 (taking the sum of all three sections R, S and T) was significantly higher for the nulliparous and gravids, and slightly though non-significantly lower for the parous, compared with Road 1.

In the comparison of the catches on the two sections R (exposed) and S (wooded) not only was there a significant difference between the two total catches (Fig. 54a) but also when the three physiological groups were considered (Fig. 54b). Since the chi-square values were similar, the nulliparous, parous

and gravid would seem to be affected by the presence of woods to equal extents, and should therefore be biased on Road 2 compared with Road 1 to equal extents. Consequently, if the three sections A, B and R were therefore genuinely representative of their distances from the river (sections A and B being 0.5 km and section R being 1.5 km from the River Eden), and not biased by other local habitat effects, then the nulliparous, parous and gravid would appear to remain at relatively constant concentrations up to 1.5 km from the river.

Since only Road 2 was biased by the presence of woods increasing the catch, the previous conclusions still hold, in that the drop in concentrations between Roads 1 and 4 (see Fig. 49) was four times as great for the parous as for the nulliparous, which therefore had greater powers of dispersal. With the increase in concentrations on Road 2 accounted for, the parallel roads showed the same pattern of spatial distribution as did the main road (Fig. 50). On the main road, there was no significant difference between the first two sections (Fig. 48), that is up to 1.5 km from the river, but thereafter, the concentrations decreased steadily away from the river. As on the parallel roads, the parous had a much greater decrease in concentrations than did the nulliparous (Fig. 48). An anomaly, however, was an increase in concentrations on sections I and J. But, like Road 2, these two sections were wooded (see map - Fig. 53), which would account for this increase in fly concentrations.

4. SPATIAL DISTRIBUTION OF S.EQUINUM FEMALES

The concentrations of S.equinum were much lower than those of S.reptans. This was aggravated by the high level of parasitism (see Chapter 6), so that the concentrations of nulliparous, parous and gravid were very low and so much less reliable. This was especially true for the main road, where not only were the concentrations lower than on the parallel roads, but they were also only based on two catches per circuit for each section, compared with six catches

per circuit for each parallel road. Consequently, the main road sections were too unreliable to be analysed.

The mean concentrations on the parallel roads are shown in Figs. 55 and 56. Compared with S.reptans (Figs. 43 and 44), there was a very rapid exponential decrease in concentrations with distance from the river. This is clearly shown if the overall mean concentrations for the total female flies is considered:-

	Road 1	2	3	4
Conc. (no./2000 m^3)	16.6	10.0	4.6	2.4

The nulliparous, parous and gravid flies showed similar exponential decreases, so that there was no difference between the dispersal of these three groups.

The increase in concentrations of S.reptans on Road 2 compared with Road 1 has been shown to be due to the effect of the woods on Road 2. However, the lack of an increase on Road 2 for S.equinum cannot just be due to this species being unaffected by the presence of woods, because there is a much greater drop in the concentrations on Road 3 and Road 4 than there was in S.reptans. Thus, when the concentration on Road 1 was expressed as a proportion of that on Road 3 and Road 4 for the two species (taking the proportion on Road 1 as 100):-

	S.equinum Rd1 Rd3	S.reptans Rd1 Rd3	S.equinum Rd1 Rd4	S.reptans Rd1 Rd4
Nulliparous	100 : 22	100 : 51	100 : 6	100 : 38
Parous	100 : 21	100 : 58	100 : 10	100 : 40
Gravid	100 : 22	100 : 73	100 : 13	100 : 39
Total	100 : 28	100 : 61	100 : 14	100 : 40

(Total includes parasites)

FIG. 55   MEAN CONCENTRATIONS OF S.EQUINUM FEMALES

	Nulliparous				Parous				Gravid			
	Rd. 1	2	3	4	Rd. 1	2	3	4	Rd. 1	2	3	4
26:6:72	3.0	1.3	1.6	0.5	1.8	1.8	1.3	0.6	0.6	0.6	0.2	0.2
18:7:72	17.0	11.1	3.1	0.9	11.5	8.1	4.1	1.0	9.7	2.4	1.7	0.8
27:7:72	0.6	0.9	0.2	0.2	12.0	5.4	2.7	0.9	5.6	4.2	1.7	0.8
31:8:72	-	-	-	-	14.1	5.6	0.9	1.7	4.9	2.3	0.3	0.6
14:6:73	2.1	3.2	0.6	-	0.8	1.1	0.2	-	-	0.2	-	0.2
21:6:73	2.1	0.5	0.2	0.2	2.4	0.7	0.4	0.2	1.5	0.3	0.7	-
27:6:73	0.3	-	0.2	-	2.5	1.3	0.2	0.5	1.0	0.5	0.5	0.2
4:7:73	0.5	-	-	-	3.8	1.8	0.4	0.2	2.6	1.2	0.4	0.2
Mean	3.2	2.1	0.7	0.2	6.1	3.2	1.3	0.6	3.2	1.5	0.7	0.4

Conc. of flies / 2000 cu.m of air

FIG. 56   MEAN CONCENTRATION OF S.EQUINUM FEMALES ON THE
PARALLEL ROADS

KEY :-

o———o = gravid
o—.—o = nulliparous
o————o = parous
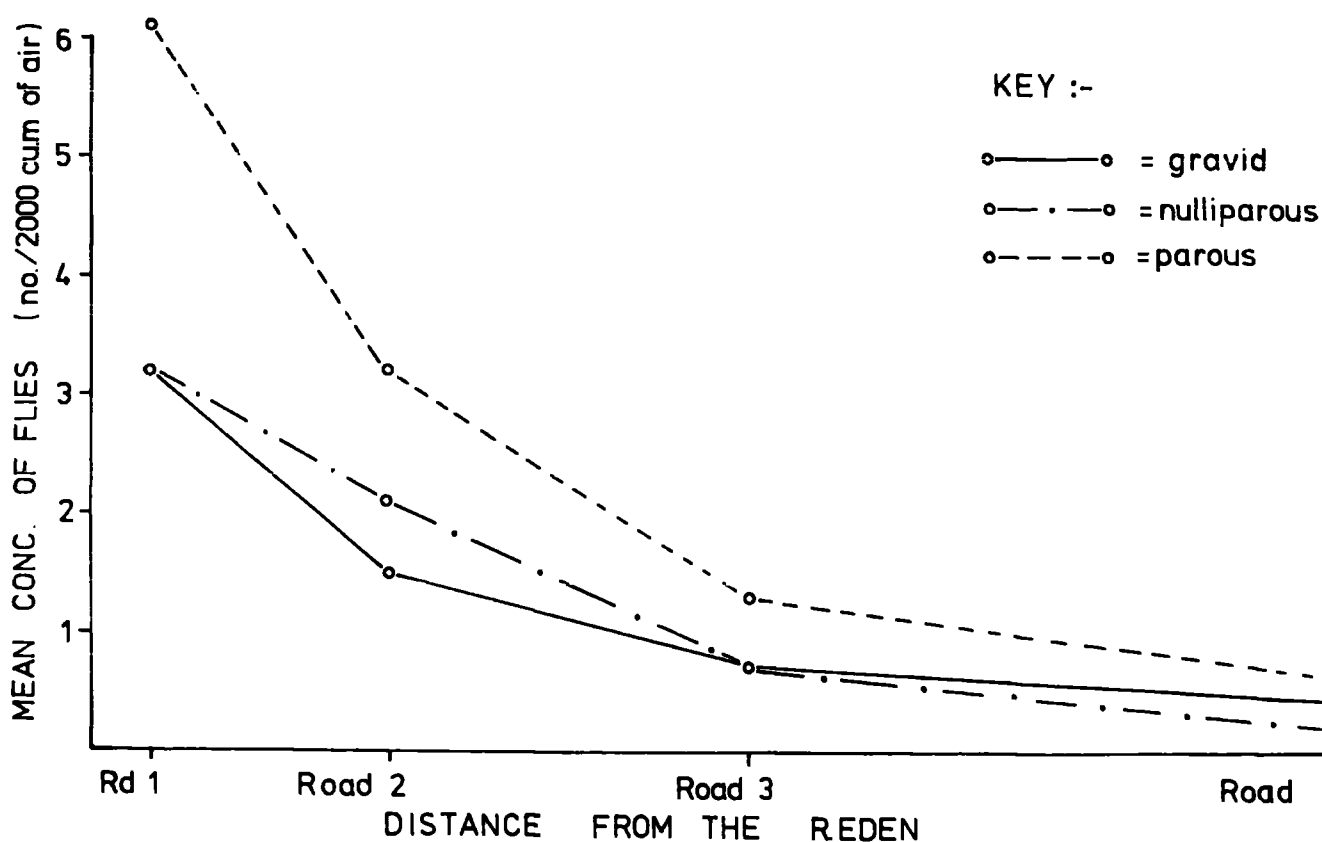
The smaller difference between the three roads for the total S.equinum females compared with the three physiological groups was due to the remarkable distribution of the parasitised flies (see Chapter 6) which made up a large proportion of the total catch.   Between Road 1 (at 0.5 km from the River Eden) to Road 3 (3.0 km from the river), the mean concentration of parous S.equinum decreased to 0.21 that of Road 1, while the parous S.reptans only decreased to 0.58 of Road 1.   Between Road 1 and Road 4 (6.0 km) the mean concentration of parous S.equinum decreased to 0.1 of that on Road 1, while S.reptans only decreased to 0.4 of that on Road 1.   Thus between Road 1 and Road 3, the concentrations of parous S.equinum decreased more than twice as much as that of S.reptans.   Between Road 1 and Road 4, the decrease in concentration was four times as great as that in S.reptans.   The greater difference between Road 1 and Road 4 for the two species was because S.reptans had only a small drop in concentration between Roads 3 and 4 (Fig. 44) while in S.equinum the concentrations continued to drop rapidly with distance from the river (Fig. 56).

The four parallel roads were compared in pairs using chi-square.   However, because of the low concentrations, a large proportion of the circuits had no catch, this being especially true on Roads 3 and 4 due to the marked spatial distribution.   Consequently, the chi-square figures were based on much fewer circuits than in S.reptans and so were less reliable.   The results are shown in Figs. 57 and 58.   When compared with S.reptans (Figs. 47 and 49), S.equinum had a much greater difference between Roads 1 and 4 for the nulliparous and gravids.   In S.reptans, the parous flies had a marked drop in concentrations with distance from the river, while the nulliparous and gravid flies were much more evenly distributed, and so dispersed more widely.   In S.equinum, all three groups showed a rapid drop in concentrations with distance from the river, though as in S.reptans, the parous flies showed the most marked reduction and so dispersed less than the other two groups.

FIG. 57 SPATIAL DISTRIBUTION OF THE S.EQUINUM FEMALES ON
THE PARALLEL ROADS AS SHOWN BY CHI-SQUARE

Roads	Nulliparous			Parous			Gravid		
	Total	X^2	signif.	Total	X^2	signif.	Total	X^2	signif.
1 + 2	27 } 8	10.3	p=0.01	42 } 16	11.7	p=0.001	33 } 11	11.0	p=0.001
2 + 3	22 } 5	10.7	0.01	40 } 7	23.2	0.001	26 } 10	7.1	0.01
3 + 4	14 } 4	5.6	0.02	22 } 9	5.5	0.02	18 } 6	6.0	0.02

FIG. 58   SPATIAL DISTRIBUTION OF S.EQUINUM FEMALES AS SHOWN BY
CHI -SQUARE

o———o = gravid
o—·—o = nulliparous
o- - - -o = parous

χ^2

Rd 1   Road 2   Road 3   Road 4

Distance from R.Eden
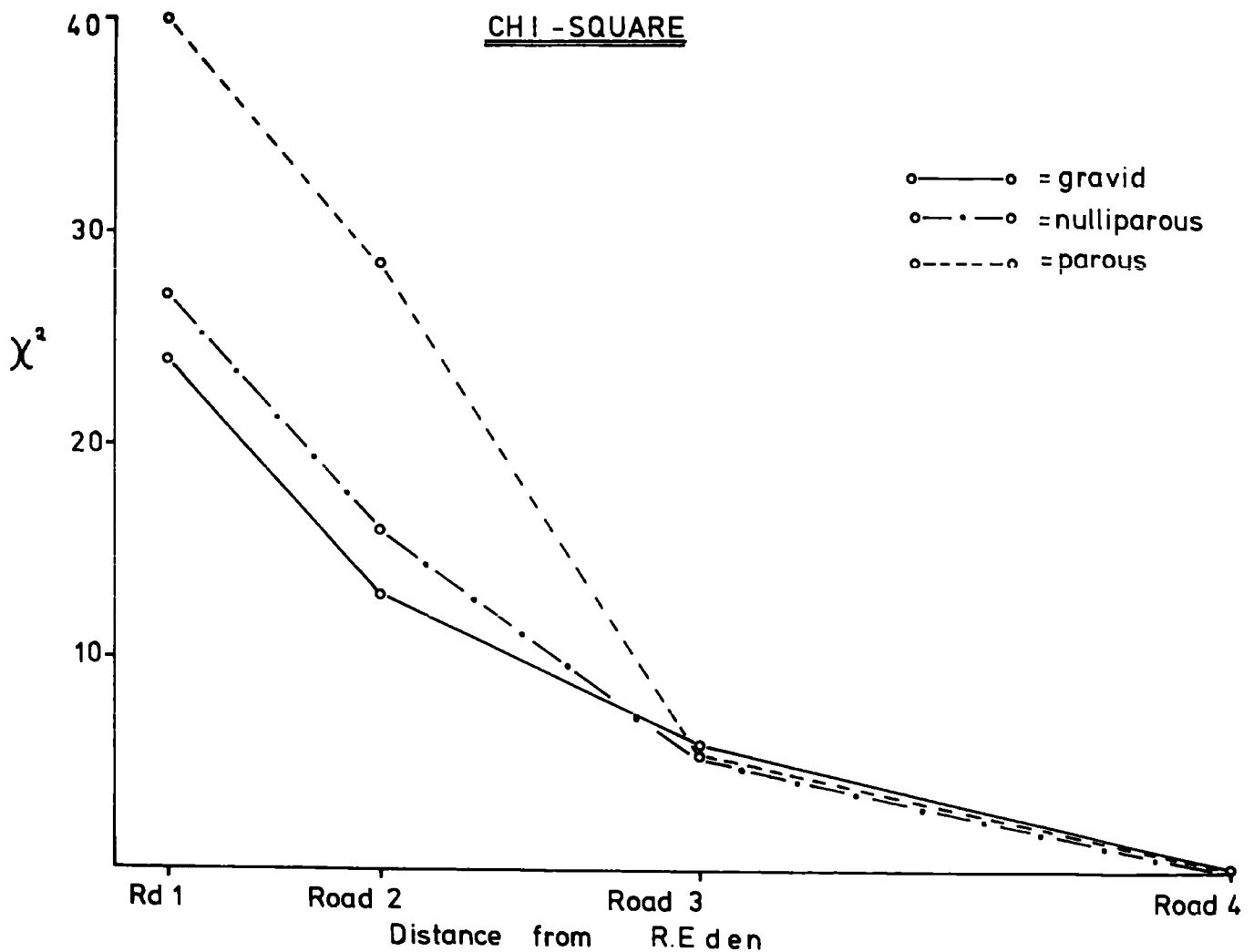
Since in S.reptans, the very high concentrations on Road 2 were largely due to the presence of trees, it is probable that the low concentrations of S.equinum on Road 2 were partly due to this species being unaffected by changes in the vegetation. To test this, the three sections on Road 2 were compared with each other using chi-square:-

	Totals	X	Signif.
R / S	23 / 29	0.692	n.s.
S / T	34 / 15	7.361	p = 0.01
R / T	34 / 15	7.361	p = 0.01

The reason for section T being significantly lower than the other two sections is not known, but section S, which was heavily wooded, did not have significantly higher concentrations than section R, which was exposed. This was in marked contrast to S.reptans (Fig. 54a) in which section S was significantly higher than T (p = 0.02) and T was significantly higher than R· (p = 0.001).

Therefore, whereas S.reptans preferred shaded conditions for flight activity, probably because it was sensitive to dehydration, S.equinum showed no such preference. This is correlated with its diurnal rhythm (see Chapter 3) in which activity was greatest during the middle of the afternoon, when humidity would be at its lowest and the rate of water loss at a maximum. S.equinum also differed from S.reptans in having much poorer powers of dispersal, the rate of decline of the concentrations with distance from the river being four times as great as that in S.reptans.

5.   SPATIAL DISTRIBUTION OF S.ORNATUM

Since S.ornatum bred in Sunnygill Beck and Briggle Beck, as well as in the River Eden (see Chapter 2), it was not really possible to study its powers of dispersal, since the direction and distance flown by the flies caught would

not be known.   However, studying its spatial distribution will show to
some extent the relative importance of the breeding sites.

The mean concentrations of the females are shown for the parallel roads
in Fig. 59 and the main road in Figs. 60 and 61.   Many of the days had
concentrations which were too low to be included and on the remaining days
the concentration of nulliparous flies was extremely low and therefore
unreliable.   However, on the parallel roads, the highest concentrations of
the parous and gravid flies were on Road 2 and the lowest were on Road 1 (Fig. 59).
On the main road, the concentrations of the parous flies were highest on
sections D, E, I and J.   The gravid flies had a similar distribution, but
were especially high on section I (Fig. 61).

Since the concentration of female flies would be expected to be highest
around the breeding sites, it is of interest that the two tributaries of
Sunnygill Beck cross the main road in sections I and J (see map - Fig. 4),
and Briggle Beck crosses through section E.   Section D is of course nearest
the River Eden.

It was not possible to compare the number of flies produced by the
different breeding sites, but the River Eden would be expected to be by far
the most important due to its great width (see Plate 7), whereas both the
becks were less than three metres wide at the point where they crossed the
main road.   It is therefore surprising that the concentrations on Road 1
were so low and that the concentrations on section D were not larger.

Considering the great variations in concentrations (Fig. 61) over
relatively short distances (for example, section G had a mean gravid
concentration of 2.3 flies/2000 m^3 of air, while section I - separated by
a distance of 1 km - had a mean of 13.4), compared with the much smaller

FIG. 59   MEAN CONCENTRATIONS OF S.ORNATUM FEMALES ON

THE PARALLEL ROADS

Date	Nulliparous				Parous				Gravid			
	Rd 1	2	3	4	Rd 1	2	3	4	Rd 1	2	3	4
26:6:72	–	1.2	–	0.4	1.3	4.0	2.2	1.9	0.2	0.5	0.9	0.9
18:7:72	6.8	6.2	4.8	2.5	5.4	7.6	4.4	7.9	8.6	15.5	9.4	10.6
27:7:72	0.2	0.5	0.4	0.3	3.7	6.1	3.0	5.0	0.8	8.2	1.8	6.0
21:6:73	0.7	1.9	2.1	0.3	2.5	3.1	1.5	1.3	2.5	4.4	2.4	2.5
27:6:73	0.7	0.2	0.9	0.4	3.2	5.1	2.5	3.6	4.0	5.9	5.3	6.8
4:7:73	0.9	0.7	0.6	0.2	4.5	9.0	3.8	4.9	4.6	14.9	8.5	4.1
12:7:73	0.5	0.4	0.5	1.2	2.2	3.8	2.8	4.1	2.3	10.5	5.2	2.9
Mean	1.4	1.6	1.3	0.8	3.3	5.5	2.9	4.1	3.3	8.6	4.8	4.8

Concentration = number of flies / 2000 cu.m of air

FIG. 60 Mean concentration of S.ornatum females on the main road

Date	Parous							Gravid						
	D	E	F	G	H	I	J	D	E	F	G	H	I	J
18:7:72	9.8	7.5	4.4	0.4	1.2	5.7	7.6	9.4	18.4	4.3	1.9	5.9	18.6	12.8
27:7:72	4.5	3.7	1.6	1.1	0.3	2.1	6.3	4.3	3.9	2.5	2.6	5.9	3.4	4.0
21:6:73	2.4	1.4	1.0	0.4	0.6	2.1	2.0	1.8	5.1	1.0	0.6	1.4	10.1	4.6
27:6:73	6.2	3.7	2.5	1.6	1.9	7.8	3.4	4.4	7.8	3.4	2.9	7.0	20.3	7.4
4:7:73	3.9	7.0	5.0	3.6	3.0	5.6	4.9	4.7	11.3	8.9	3.6	10.4	14.6	10.8
Mean	5.8	5.0	2.9	1.4	1.4	4.7	4.8	4.9	7.3	4.0	2.3	6.1	13.4	7.9

Conc. = no. of flies / 2000 cu.m of air

Nulliparous are not included due to the very low concentrations.

FIG. 61   SPATIAL DISTRIBUTION OF S.ORNATUM FEMALES ON THE MAIN ROAD
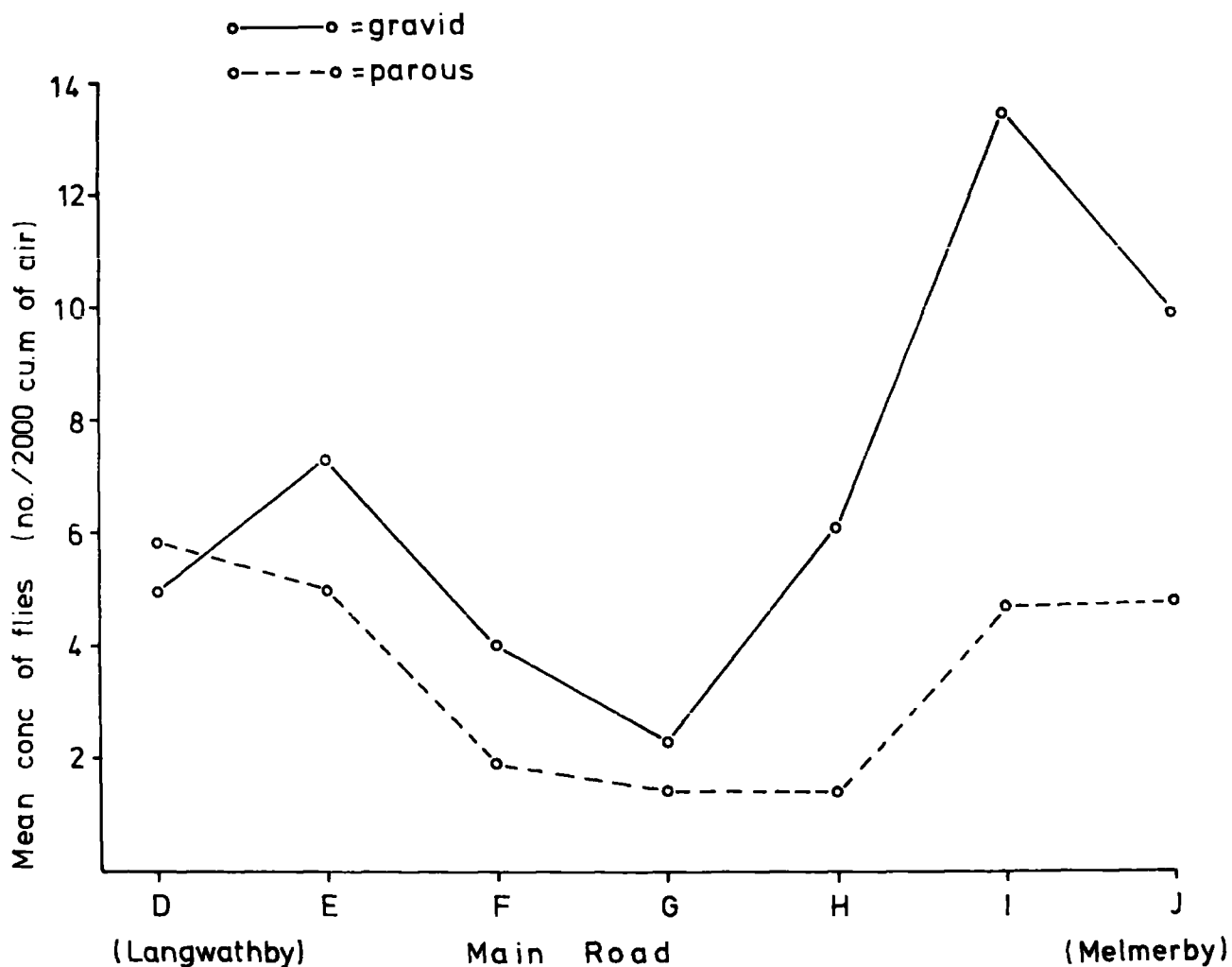
difference in S.reptans (Fig. 46), it is possible that S.ornatum had poor

powers of dispersal, so that the concentrations decreased rapidly away from

the breeding sites. It was of interest in this respect that the highest

concentrations occurred on the main road and not the parallel roads (which

were 3 km long), so that the high concentrations were probably very localised

around the breeding sites.

V.   DAILY, SEASONAL AND ANNUAL CHANGES IN FLIGHT ACTIVITY

The concentration of flies caught not only changed during the day due to the diurnal rhythm (Chapter 3), but also changed from day to day, due to daily changes and due to the seasonal rhythm.   Since the vehicle-mounted trap only caught flying insects, changes in the concentration of flies on one particular road could be due to two factors:-

i)   Changes in the level of flight activity.

ii)   Changes in the size of the population present.

The diurnal rhythm was therefore due to activity changes, whereas the seasonal rhythm was due to population changes.   Daily changes, which were irregular changes from day to day within the overall change due to the seasonal rhythm, were due to a combination of activity changes (principally due to changes in the weather from day to day) and to population changes of the different physiological groups.

1.   DAILY CHANGES IN ACTIVITY

The mean concentration of female flies fluctuated markedly from day to day as a result of changes in the weather conditions.   Thus on a warm ($14 - 18^{\circ}C$) cloudy, calm, humid day such as 14:vi:73, the mean concentration of S.reptans females was 32.2 flies/2000 m^3 of air, where as on a cold (below $14^{\circ}C$) windy day (normal weather conditions), the mean concentration was less than 3 flies/2000 m^3 of air.   These changes due to the weather were considered in Chapter 3, and so will not be considered further.   When the effect of the weather was eliminated by expressing the different physiological groups as a percentage of the total (S.reptans, Fig. 62), there were still marked changes in the percentages from day to day.   This was due to the population changes and will be considered in more detail for the S.reptans blood-fed females.

FIG. 62 **CHANGES IN THE PERCENTAGES OF THE DIFFERENT PHYSIOLOGICAL GROUPS FOR S.REPTANS**

	% blood-fed	% fresh blood-fed	% gravid	% para- sitised	% blood- thirsty
31:5:72	4·3	(0·8)	4·1	7·4	84·2
14:6:72	4·5	(0·4)	48·5	16·9	30·1
26:6:72	4·7	(0·7)	23·6	24·6	47·1
18:7:72	15·0	(5·1)	27·7	2·7	54·6
27:7:72	8·0	(3·0)	28·5	5·6	57·9
31.8:72	28·7	(5·5)	39·3	4·6	27·4
5:6:73	16·2	(4·7)	42·0	22·8	19·0
14:6:73	2·3	(0·3)	23·9	6·2	67·6
21:6:73	7·8	(2·0)	49·1	5·4	37·7
27:6:73	10·2	(2·0)	54·9	7·1	27·8
4:7:73	17·7	(3·4)	23·2	3·1	56·0
12:7:73	7·1	(1·5)	41·4	4·0	47·5
Mean	10·5	(2·5)	33·8	9·3	46·4

[Fresh blood-fed are included in total blood-fed]

a)   Blood-fed S.reptans females

The overall mean percentage of flies containing a blood meal in the midgut (Fig. 62) was remarkably low, being only 10.5% of the total flies compared with 33.8% for the gravid flies and 46.6% for the blood-thirsty flies. But since the truck trap only caught flying insects, the low percentage could be due to two factors:-

i)   Duration of the blood-fed stages.   If the flies stayed in a blood-thirsty condition (ovary stage I) longer than in a blood-fed condition (ovary stages I - IV), then there would be less chance of catching the blood-fed flies. Lewis (1953) found that in S.damnosum Theo. maturation of the eggs from the time of blood feeding took 2 - 3 days.   However, this was in flies kept at a constant 25°C.   Le Berre (1966) found that the rate of egg development was dependent on the temperature, so that at 23°C the eggs matured in 90 h, while at 27°C they took 70 h.   These temperatures were far higher than the mean temperatures in the Eden Valley.   L. Davies (1957b) found that development in S.ornatum took 4 - 5 days.   S.reptans was therefore probably similar.

S.damnosum starts searching for a blood meal on the day after emergence (Disney 1970), and most flies find a blood meal within 2 - 3 days, since Le Berre (1966) found that if insecticide was added to an isolated breeding site to stop further emergence, then the nullipars disappeared from the biting population within this time.   S.reptans was probably similar since it bites mainly cattle (L. Davies et al 1962), which were abundant throughout the Eden Valley (an intensive dairying region).   It is therefore unlikely that the flies remained in a blood-thirsty condition as long as in a blood-fed condition, so that the low percentage of blood-fed flies could not be due to this factor.

ii) Activity. Since the trapping method only caught flying flies, if the blood-fed flies were less active there would be less chance of catching them. Unfortunately, there is very little data on resting flies due to the difficulty of finding them. Disney (1969) found that out of 1,135 resting flies caught amongst riverside vegetation in the savannah region of the Federal Cameroons Republic, 92 (8.1%) were blood-fed, which is still a low percentage.

There has been some research on the resting sites of other biting insects, but these do not usually consider the proportion of blood-fed flies. However, De Zulueta (1950) found that of the culicid mosquitoes resting amongst the grass of the Colombian llanos, 11% were blood-fed. Bidlingmayer and Edman (1967), studying the mosquito <u>Psorophora confinnis</u> Lynch Arribalzaga, found that using a vehicle trap only 12% were blood-fed, but when catching resting flies using a mechanical aspirator 53% were blood-fed. Similar results were found for other mosquito species. Their results therefore showed that blood-fed flies were far less active than blood-thirsty flies. Their experiments were actually done to disprove the popular assumption that blood-fed flies do not fly because they have no need to. The authors showed that in a further experiment (Edmans and Bidlingmayer 1969) that blood-fed mosquitoes were capable of flying at least 1.5 km on the same night as having the blood meal. They considered that the reason for flight by blood-fed flies was to reach shelter amongst trees before daylight (the flies were caught in the middle of a large marsh).

In the blood-fed simuliids caught in the Eden Valley, however, all four stages of egg development were caught in equal numbers (whereas Bidlingmayer caught mainly fresh blood-fed flies):-

	ovary stage 1	stage 2	stage 3	stage 4
% of blood-fed	22.3	24.4	24.2	29.1

(N.B. the duration of each stage was unknown and so may not be comparable with each other.)

The flies with old blood meals therefore appeared to fly as much as the flies with fresh blood meals (which could be expected to be seeking shelter), so that the reason for flight is not obvious. However, though all the blood-fed flies had a similar level of activity (assuming that the stages were of equal duration), the low percentage of the total catch which they formed was probably due to them being much less active than the blood-thirsty flies.

Though the mean percentage of blood-fed flies was low, it varied considerably from day to day. This was probably due to a combination of the gonotrophic cycle and the effect of the weather. The gonotrophic cycle was as follows:- the blood-thirsty flies mate and dispersed away from the river (after emergence if nulliparous, or after oviposition if parous), then searched for a blood meal; the blood-fed flies had a reduced flight activity while the oocytes mature; when mature, the gravid flies return to the river for oviposition. There were two stages in the cycle, blood feeding and oviposition, which were heavily dependent on the weather. Both activities were at a maximum during warm, calm, humid evenings (Davies and Peterson 1956, L. Davies 1957a, Fredeen et al 1951).

On the majority of days, when the weather was poor, there would thus tend to be a build up in the concentrations of gravid and blood-thirsty flies. When suitable weather arrived, there would be a marked drop in the percentage of gravids and an increase in the percentage of blood-fed flies (the blood-thirsty group gaining flies from gravids which oviposited and losing flies to the blood-fed group). Thus the gonotrophic cycle would produce changes in the population which were controlled by previous weather conditions. Therefore on a particular day, the size of the population of blood-fed flies would be due to the previous weather conditions (and due to the seasonal rhythm - see later), while the level of activity would be due to the present weather. Since the

concentration of the flies caught was a combination of the two factors of population size and activity level, then the daily changes would be marked and difficult to analyse.

The daily changes in the percentage of blood-fed flies are shown in Fig. 62 and varied from 2.3% on 14:vi:73 to 28.7% on 31:viii:72. The fresh blood-fed flies showed similarly large fluctuations. It was difficult to correlate the results with the weather on the date of the catch. For example, the highest percentage of blood-fed flies was caught on 31:viii:72, which was cloudy and humid, while the second highest percentage of fresh blood-fed flies was on 18:vii:72, which was very hot and dry. Since the weather conditions were only measured on the days on which flies were collected and not on previous days, it was not possible to analyse these daily fluctuations to show the effect of the weather on the gonotrophic cycle and the level of activity.

b)   Gravid S.reptans

The percentage of gravid flies are shown in Fig. 62. The percentages were much higher than for the blood-fed flies (mean of 33.8% compared with 10.5%). Since it is unlikely that the duration of the gravid stage (stage V) was longer than that of the blood-fed stages (stages I - IV), the higher percentages show that the flies were more active. This would be expected since they have to migrate back to the river to lay their eggs. Like the blood-fed flies, the mean daily percentages varied markedly from 4.1% on 31:v:72 to 45.9% on 27:vi:73.

2.   SEASONAL CHANGES IN ACTIVITY

The adult flies only occur during the summer. Since for this research the main species of interest was S.reptans, collecting commenced when the flies started to emerge in large numbers at the end of May in 1972, and the beginning of June in 1973. Collecting was stopped in the middle of July due

to the large number of flies caught by then, so that it was not known when the adult season ended. However, five circuits were made on 31:viii:72, which showed that moderate concentrations of flies were still present (see Fig. 41).

The average age of the adult flies in the flying population will have a seasonal change, since it is affected by the rate of emergence of adults from the pupae. The change in the age of the flies can be shown by dividing them into nulliparous (young) and parous (old) flies. Unfortunately, this change in the age of the flies cannot easily be shown by comparing the mean concentrations or the total number of flies caught, since these are heavily dependent on the weather conditions. The nulliparous and parous flies were therefore expressed as a percentage of the total flies, thus eliminating daily changes due to the weather.

a)   S.reptans

The number of nulliparous and parous flies caught on each of the parallel roads were expressed as a percentage for each day (see Fig. 63). The percentage of nulliparous was very highm at the beginning of the season, being 95.2% on 31:v:72 and 85.5% on 5:vi:73. Since there were large numbers of flies caught on these days (1,698 on 31:v:72 and 1,886 on 5:vi:73), there must have been a mass emergence over a relatively short period. Otherwise, since the gonotrophic cycle only lasted about a week, by the time the concentrations had built up to high numbers there would have been a much higher percentage of parous (only 4.8% on 31:v:72). During June, there was a steady decline in the percentage of nulliparous (down to 14.6% on 26:vi:72 and 10.1% on 27:vi:73) as more flies became parous and the rate of emergence fell off. However in the middle of July, there was a marked increase in the percentage of nulliparous (35.6% on 18:vii:72 and 32.7% on 12:vii:73), showing that there had been a second peak of emergence.

FIG. 63 SEASONAL CHANGE IN PERCENTAGE OF NULLIPAROUS / PAROUS OF S.REPTANS

	Nulliparous					Parous				
	Rd 1	2	3	4	Total	Rd 1	2	3	4	Total
31:5:72	93.4	95.1	97.2	96.6	95.2	6.6	4.9	3.8	3.4	4.8
14:6:72	21.3	24.0	14.9	28.9	22.2	78.7	76.0	85.1	72.1	77.8
26:6:72	16.9	14.2	13.4	14.5	14.6	84.1	85.8	86.6	85.5	85.4
18:7:72	47.1	26.7	25.4	29.1	35.6	52.9	73.3	74.6	71.9	64.4
27:7:72	17.5	21.1	26.1	23.1	21.7	82.5	77.9	73.9	76.9	78.3
31.8:72	–	–	–	–	–	100.0	100.0	100.0	100.0	100.0
5:6:73	85.4	87.6	80.9	85.3	85.5	14.6	13.4	19.1	24.7	14.5
14:6:73	46.1	56.9	68.7	52.2	56.3	53.9	43.1	31.3	47.8	43.7
21:6:73	18.2	18.5	16.9	30.8	19.7	81.8	81.5	83.1	69.2	80.3
27:6:73	9.3	12.8	10.1	0	10.1	90.7	87.2	89.9	100.0	89.9
4:7:73	6.0	18.9	13.9	16.3	14.1	94.0	81.1	86.1	83.7	85.9
12:7:73	24.8	34.0	32.0	44.6	32.7	75.2	66.0	67.0	55.4	67.3

FIG. 64 SEASONAL CHANGE IN PERCENTAGE OF NULLIPAROUS / PAROUS

a) S.EQUINUM

1972	Nulliparous	Parous	1973	Nulliparous	Parous
31:5:72	33.9	66.1	5:6:73	100.0	–
14:6:72	100.0	–	14:6:73	92.5	7.5
26:6:72	94.1	5.9	21:6:73	77.1	23.9
18:7:72	61.7	38.3	27:6:73	24.6	65.4
27:7:72	6.0	94.0	4:7:73	10.9	89.1
31:8:72	–	100.0	12:7:73	4.4	95.6

b) S.ORNATUM

1973	Nulliparous	Parous	1973	Nulliparous	Parous
31:5:72	18.8	81.2	5:6:73	31.5	68.5
14:6:72	24.7	75.3	14:6:73	54.2	45.8
26:6:72	30.0	70.0	21:6:73	43.6	56.4
18:7:72	45.3	54.7	27:6:73	16.5	83.5
27:7:72	8.3	91.7	4:7:73	11.2	88.8
31:8:72	12.7	87.3	12:7:73	8.7	91.3

This seasonal rhythm was mirrored by that of the flies parasitised by mermithids (see Chapter 7). Since the mermithids only occurred in nulliparous flies, they were virtually only caught at the beginning of the season and in the middle of July (see Fig. 69a).

S.reptans therefore had a mass emergence at the end of May and beginning of June, and a second smaller emergence in the middle of July.

b)   S.equinum

The seasonal change in the percentages of nulliparous and parous S.equinum is shown in Fig. 64. Emergence on a large scale started later than in S.reptans. Thus the total female catch (see Fig. 65) for 31:v:72 contained only 19 S.equinum(compared with 1,698 S.reptans), while 5:vi:73 had 7 S.equinum (compared with 1,886 S.reptans). The percentage of parous increased steadily throughout the season, this being especially shown in 1973, so that the emergence of nulliparous flies gradually decreased over a period of a month. S.equinum did not therefore have a short emergence period as occurred in S.reptans, and there was no second emergence later in the season (or at least not during the period in which collecting occurred).

c)   S.ornatum

In both 1972 and 1973, the nulliparous remained low throughout the collecting period (see Fig. 64). The nulliparous varied between 54.2% and 8.3% of the catch. It is possible therefore that S.ornatum had an extended emergence period. Thus the number of flies caught (see Fig. 65) increased steadily throughout June and July (highest catches in 1972 were on 18:vii:72 and in 1973 on 4:vii:73; S.reptans had highest catches on 26:vi:72 and 14:vi:73). This agrees with L. Davies (1957a) who found that there was a continuous emergence of S.ornatum from June to October.

FIG. 65   SEASONAL CHANGE IN THE TOTAL DAILY CATCH

Date	S.reptans	S.equinum	S.ornatum
31:5:72	1,698	19	58
14:6:72	1,303	95	40
26:6:72	2,251	1,405	252
18:7:72	1,010	1,285	1,928
27:7:72	625	634	774
5:6:73	1,886	7	87
14:6:73	3,409	450	257
21:6:73	1,888	297	533
27:6:73	1,218	98	943
4:7:73	1,050	158	1,307
12:7:73	2,189	30	825

FIG. 66   ANNUAL CHANGES IN THE SIMULIID CATCH

Species	1971		1972		1973	
	No.	%	No.	%	No.	%
S. reptans	12,047	80.4	7,665	48.1	11,640	68.2
S. equinum	1,840	12.2	3,768	23.6	1,040	6.1
S. ornatum	758	5.0	3,488	21.9	3,952	23.2
S. latipes group	41	0.3	159	1.0	61	0.4
S. aureum group	175	1.2	248	1.5	169	1.0
S. variegatum	116	0.8	552	3.5	178	1.0
S. monticola	10	0.1	61	0.4	17	0.1

3. ANNUAL CHANGES

The number of flies of each species are shown for the three seasons in Fig. 66. The actual numbers of flies caught are obviously not directly comparable for the three years, since the totals depend on the number of days on which collections were made, and on the weather for these days. It is therefore not possible to see whether the overall number of adult simuliids changed from year to year. However, there were marked annual changes in the relative abundance of the three species. Thus in 1971, S.reptans was the only important species present (80.4% of the total catch), with S.equinum second at 12.2%; in 1972, S.reptans dropped to only 48.1% of the catch while both S.equinum markedly increased their proportion (23.6% and 21.9% respectively); in 1973, S.reptans was once more the major species (68.2%), while S.equinum had dropped to only 6.1% of the catch and S.ornatum remained constant. The changes in the populations therefore occurred independently for the different species.

VI.   INTERNAL PARASITES

1.   INTRODUCTION

Internal parasites consist of mermithids (Nematoda), microsporidia (Protozoa), and fungi.   There were also ectoparasitic mites.

The parasites infected the Simuliidae during their aquatic larval stage in the River Eden.   Both mermithids (Phelps and DeFoliart 1964) and microsporidia (Strickland 1913) infect the larvae by being ingested, then boring through the wall of the digestive tract into the haemocoel.   Many authors have described very high levels of larval parasitism.   Strickland (1913) found up to 25% of the larvae infected with mermithids and up to 80% with microsporidia, and considered that virtually all these would die due to inhibition of pupation.   D.M. Davies (1956) found 10% of the larvae infected with microsporidia, and also considered that these would result in the death of the larvae.   Phelps and DeFoliart (1964) found up to 80% infected by mermithids, but discovered that a proportion of these were able to pupate and form adults. What determines whether the infected larvae are able to pupate is not known.

In the cases where the larva dies, the parasites penetrate the body wall and so were released back into the river as adult mermithids or microsporidian spores.   Since both mermithids and microsporidians have poor powers of locomotion, they drift downstream in the current.   As the infected simuliid larvae also migrate downstream, if the parasites only infected the larvae their whole population would gradually move downstream (Phelps and DeFoliart 1964). It is therefore necessary for a proportion of the parasite population to infect the adult flies.

The parasites usually developed in the adult fly at the expense of its fat body, and thus of the ovaries. As the parasites develop, the fly behaved as if it was gravid, migrating upstream and depositing the parasites back into the river. Thus, D. M. Davies (1958) found that when collecting ovipositing Prosimulium fuscum S & D and P.mixtum S & D, 15 - 60% of them were infected by mermithids. Phelps and DeFoliart (1964) also caught mermithid parasitised flies amongst ovipositing S.vittatum Zett. Infecting of the adult flies would therefore help to maintain the parasite population in the river.

2.   PARASITES PRESENT IN THE ADULT SIMULIIDS

It was impossible to identify the actual species of the parasites, since the mermithids can only be identified in their adult stage (which would have necessitated keeping the flies alive until the mermithids emerged), while the microsporidia are only described for the stages existing in the simuliid larvae (in the adult, they occur as spores). The mermithids were therefore considered as one group, while the microsporidia and fungi were divided into five groups (A to E - Plate 9).

Types A, B and E were microsporidia. Type A was oval in shape and 22 - 30µ long; Type E was also oval and 22 - 30µ long; Type B was spherical and increased in size from 15µ in the freshly emerged flies up to 50µ in older flies. The older Type B were surrounded by a thick transparent layer (see Plate 9).

Type C and D were fungi. Type D were tetrahedron-shaped spores, 16µ across, and like the microsporidians Type A and E occurred in vast numbers swelling up the abdomen of the infected fly. Type C were hyphae, which were up to 500µ long and only occurred in small numbers per fly.

PLATE 9   SIMULIID PARASITES

Type A   x 160   Type B   x 160   Type C   x 160
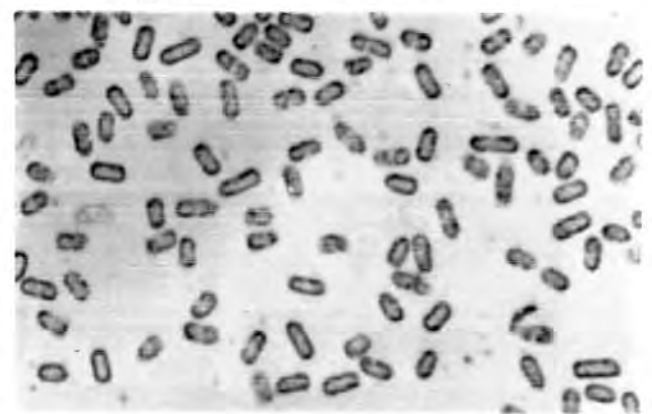

Type D   x 250   Type E   x250

Mermithids

Type A only occurred in the fly's ovarioles and invariably resulted in the autolysis of the fly's oocytes. The other parasites occurred in the haemocoel. Type B usually resulted in the development of the oocytes being arrested. However, after the parasites had been 'laid', the oocytes appeared to develop normally. Thus, later in the season, many of the flies with developing oocytes also contained a small number of Type B parasites. Furthermore, the parasites were all in the large condition (50µ) with a thick transparent layer, and so had been present for some time. This was especially conspicuous in 1972, when in the early part of the season, 74% of S.equinum were parasitised by Type B, so that later in the season most of the flies still contained small numbers of the parasites. These flies were abnormal in that the stage 1 ovaries had many of the characteristics of a parous condition, such as the minute mis-shapen oocytes (due to the ovarioles being squashed by the mass of the parasites), but naturally no corpora lutea were present. Thus the flies must have previously been heavily parasitised. The oocytes then develop normally, so that genuinely parous flies were found with small numbers of Type B still present. Therefore, unlike Type A which destroyed the ovaries, Type B only delayed the development of the oocytes.

In all the cases of infection by the fungus Type C observed, the number of parasites per fly was relatively small. This resulted in only a proportion of the oocytes being retarded, while the rest developed to maturity.

Mermithids invariably resulted in a complete arresting of the development of the oocytes. 19.4% of the parasitised flies contained decomposing mermithids, so that even in adult flies a large proportion of the mermithids do not survive.

3.   DISTRIBUTION AMONGST SIMULIID SPECIES, AND SEASONAL CHANGES

From the three seasons 1971-1973, the parasitised flies caught are shown
in Fig. 67.   The two most abundant parasites were the mermithids (though these
were remarkably absent for S.equinum), and the microsporidian Type B.   S.equinum
had a markedly higher overall percentage of parasitised flies, despite the lack
of mermithids, due to Type B which occurred in almost one quarter of the flies.
No fungal parasites were found in S.reptans.   S.ornatum contained all the
types of parasites, but only in low numbers.   There was therefore a marked
difference in the frequency with which the different parasites occurred in
the three main species of Simuliidae.

Therewere marked annual changes in the relative abundance of some of the
different parasites (Fig. 68).   In S.reptans and S.equinum, Type B infected
low numbers of flies in 1971, but larger numbers in the following two years
(in S.equinum, there was an increase from 1.6% infected up to 29.4%).   Type A
decreased each year from 1971 to 1973 for both S.equinum and S.ornatum.   The
mermithids showed a similar decrease in S.ornatum but remained constant each
year in S.reptans.   Apart from the mermithids, therefore, the parasites showed
the same annual population changes for all three simuliid species.

Since the parasites infect the simuliids during the larval stage and were
returned to the water by the adult fly, they only infected nulliparous and not
parous flies.   Because of this, they had a marked seasonal rhythm (see Chapter 5).
The mermithids (see S.reptans Fig. 69) only occurred in large numbers at the
beginning of the season (31:v:72 and 5:vi:73), and in smaller numbers around
the middle of July (27:vii:72 and 12:vii:73).   This corresponded to the timing
of the two peaks of nulliparous concentrations, which resulted from the two
peaks of emergence (see Chapter 5).

FIG. 67   DISTRIBUTION OF PARASITES AMONGST SIMULIID SPECIES

	Mermithid		Type A		Type E		Type B		Type C		Type D		Total ♀ flies	% parasitised
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
S.reptans	641	2.0	30	0.1	2	–	1603	5.1	–	–	–	–	31,352	6.7
S. equinum	1	–	99	1.5	–	–	1624	24.4	3	–	–	–	6,648	25.9
S.ornatum	90	1.1	8	0.1	9	0.1	131	1.6	68	0.8	7	0.1	8,198	3.8
S.variegatum	–	–	–	–	1	0.1	1	0.1	5	0.6	1	0.1	846	1.0
S.aureum gp.	–	–	1	0.2	–	–	1	0.2	2	0.5	–	–	417	1.0
S.latipes gp.	–	–	–	–	–	–	2	0.8	2	0.8	–	–	261	1.5
S.monticola	2	2.3	–	–	–	–	–	–	–	–	–	–	88	2.3

FIG. 68   ANNUAL CHANGES IN PARASITE ABUNDANCE

S.REPTANS

	1971		1972		1973	
	No.	%	No.	%	No.	%
Mermithids	242	2.0	172	2.2	227	2.0
Type A	10	0.1	8	0.1	12	0.1
Type B	57	0.5	825	10.8	721	6.2
Type E	1	–	1	–	–	–
Total	310	2.6	1006	13.1	960	8.2

Total flies :-
1971 = 12,047
1972 = 7,665
1973 = 11,640

S.EQUINUM

	1971		1972		1973	
	No.	%	No.	%	No.	%
Mermithids	–	–	1	–	–	–
Type A	80	4.3	19	0.5	–	–
Type B	30	1.6	1109	29.4	485	46.6
Type C	1	–	2	0.1	–	–
Total	111	6.0	1131	30.0	485	46.6

Total flies –
1971 = 1,840
1972 = 3,768
1973 = 1,040

S.ORNATUM

	1971		1972		1973	
	No.	%	No.	%	No.	%
Mermithids	76	10.0	10	0.3	4	0.1
Type A	4	0.5	4	0.1	–	–
Type B	7	0.9	57	1.6	67	1.7
Type C	5	0.7	28	0.8	35	0.9
Type D	–	–	2	–	5	0.1
Type E	–	–	2	–	7	0.2
Total	92	12.1	103	3.0	118	3.0

Total flies –
1971 = 758
1972 = 3,488
1973 = 3,952

FIG. 69   PARASITES OF S.REPTANS

a) MERMITHIDS   SPATIAL DISTRIBUTION AND SEASONAL CHANGES

Date	Parallel Roads Rd 1	2	3	4	Main Road D	E	F	G	H	I	J	Total	% of flies
31:5:72	18	40	5	11	9	8	9	3	5	2	1	111	6.5%
14:6:72	3	6	4	2	3	1	1	1	1	–	–	22	1.7%
26:6:72	–	2	–	–	–	1	–	1	–	–	–	4	0.2 %
18:7:72	–	1	–	1	–	–	–	–	–	–	–	2	0.2 %
27:7:72	5	11	4	–	1	–	4	–	1	–	2	28	4.5 %
31:8:72	2	2	1	–	–	–	–	–	–	–	–	5	0.6 %
5.6:73	34	55	8	3	9	12	7	4	4	3	2	141	7.5 %
14:6:73	1	1	1	1	1	–	1	–	–	–	1	7	0.2 %
21:6:73	–	1	–	–	–	–	–	–	–	–	–	1	
27:6:73	1	–	–	–	–	–	–	–	–	–	–	1	0.1 %
4:7:73	–	5	2	–	–	1	1	–	–	–	–	9	0.9 %
12:7:73	13	21	11	4	2	5	3	2	1	4	2	68	3.1 %
Total	77	145	36	22	25	28	26	11	12	9	8		
% of flies	27.8	52.4	13.0	7.9	21.0	23.5	21.8	9.2	10.2	7.6	6.7		

b) MICROSPORIDIA TYPE B

Date	Parallel Roads Rd 1	2	3	4	Main Road D	E	F	G	H	I	J	Total	% of flies
31:5:72	4	6	2	1	1	–	–	–	–	–	1	15	0.9 %
14:6:72	47	42	18	10	15	15	10	6	8	18	9	198	15.2 %
26:6:72	85	157	93	40	26	46	32	22	9	24	15	549	24.4 %
18:7:72	9	3	8	1	1	2	–	1	–	–	–	25	2.5 %
27:7:72	–	4	1	–	1	–	–	–	–	–	1	7	1.1 %
31:8:72	15	8	1	1	1	1	2	–	–	1	1	31	4.0 %
5:6:73	95	79	18	17	15	22	14	8	6	10	5	289	15.3 %
14:6:73	32	97	14	15	5	18	11	3	3	1	4	203	6.0 %
21:6:73	16	40	20	8	1	6	3	4	1	1	2	102	5.4 %
27:6:73	12	31	34	3	–	2	–	1	1	1	–	85	7.0 %
4:7:73	6	3	2	4	–	3	1	–	1	1	2	23	2.2 %
12:7:73	4	7	6	1	–	–	–	–	–	1	–	19	0.9 %
Total	325	477	217	101	66	115	73	45	29	58	40		
% of flies	29.9	42.6	19.4	9.0	15.5	27.0	17.1	10.6	6.8	13.6	9.4		

c) MICROSPORIDIA TYPE A

18:7:72	3
31:8:72	5
14:6:73	1
12:7:73	11

Microsporidia Type A, which hardly occurred in 1973, only occurred in the 1972 season after 18:vii:72 for both S.reptans (Fig. 69) and S.equinum (Fig. 70). This was the date on which the second peak of emergence occurred, so that Type A appeared to only parasitise this later generation of flies.   Type B occurred throughout the season, but in 1972, the highest numbers were caught on 14:vi:72 and especially 26:vi:72, for both S.reptans and S.equinum (Fig. 71); while in 1973, there was a peak on 5:vi:73 for S.reptans and 14:vi:73 for S.equinum. Therefore apart from in S.reptans in 1973, there was a marked lack of the parasite at the beginning of the simuliid season.

During the season, there was thus a succession of parasites.   The mermithids occurred at the beginning of the season (end of May) and reappearing in the middle of the season, when the second emergence occurred (middle of July); Microsporidia Type B occurred throughout June; Microsporidia Type A only occurred in the second emergence (middle of July).

Strickland (1913), working on the larval stages of Simuliidae, found that since microsporidia infected the larvae by being ingested, they infected all the simuliid species present in the river.   However, they could only infect very young larvae, that is those in which the peritrophic membrane had not yet lined the entire mesenteron.   The larvae were therefore only sensitive to infection for a short period.   As the different microsporidian species only have infective spores for a short period, they would infect the different simuliid species to different extents.   This is because the eggs of the different simuliid species hatch at different times, so that their sensitive periods would overlap the microsporidian's infective period to different extents.

FIG. 70   PARASITES   OF   S.EQUINUM

a) TYPE   B

| | Parallel | | Roads | | Main | | Road | | | | | | |
DATE	Rd 1	2	3	4	D	E	F	G	H	I	J	Total	% of flies
31:5:72	-	-	-	-	-	-	-	-	-	-	-	-	-
14:6:72	2	2	5	7	1	3	1	1	2	8	2	34	35.8
26:6:72	204	170	113	93	80	85	84	47	22	75	62	1035	73.7
18:7:72	7	6	7	1	1	-	1	1	1	-	-	25	1.9
27:7:72	2	2	1	-	-	1	-	-	1	-	-	7	1.1
31:8:72	1	2	-	-	1	1	1	-	1	1	-	8	2.4
5.6:73	1	-	-	-	-	1	-	-	-	-	-	2	28.6
14:6:73	72	132	24	18	29	17	2	1	-	5	6	282	62.7
21:6:73	59	15	14	4	9	21	11	6	3	3	1	146	49.2
27:6:73	6	3	6	-	-	1	1	1	1	-	-	19	19.4
4:7:73	3	4	-	-	-	-	-	-	-	1	1	9	5.7
12:7:73	-	-	1	-	-	-	-	-	-	-	-	1	3.3
Total	357	336	170	123	121	130	101	57	31	93	72		

b) TYPE A

18:7:72	14
27:7:72	4
31:8:72	1

FIG. 71   SEASONAL RHYTHM OF PARASITES

o———o = Mermithid in S.reptans
o------o = Type B   in S.reptans
o—.—o = Type B in S.equinum
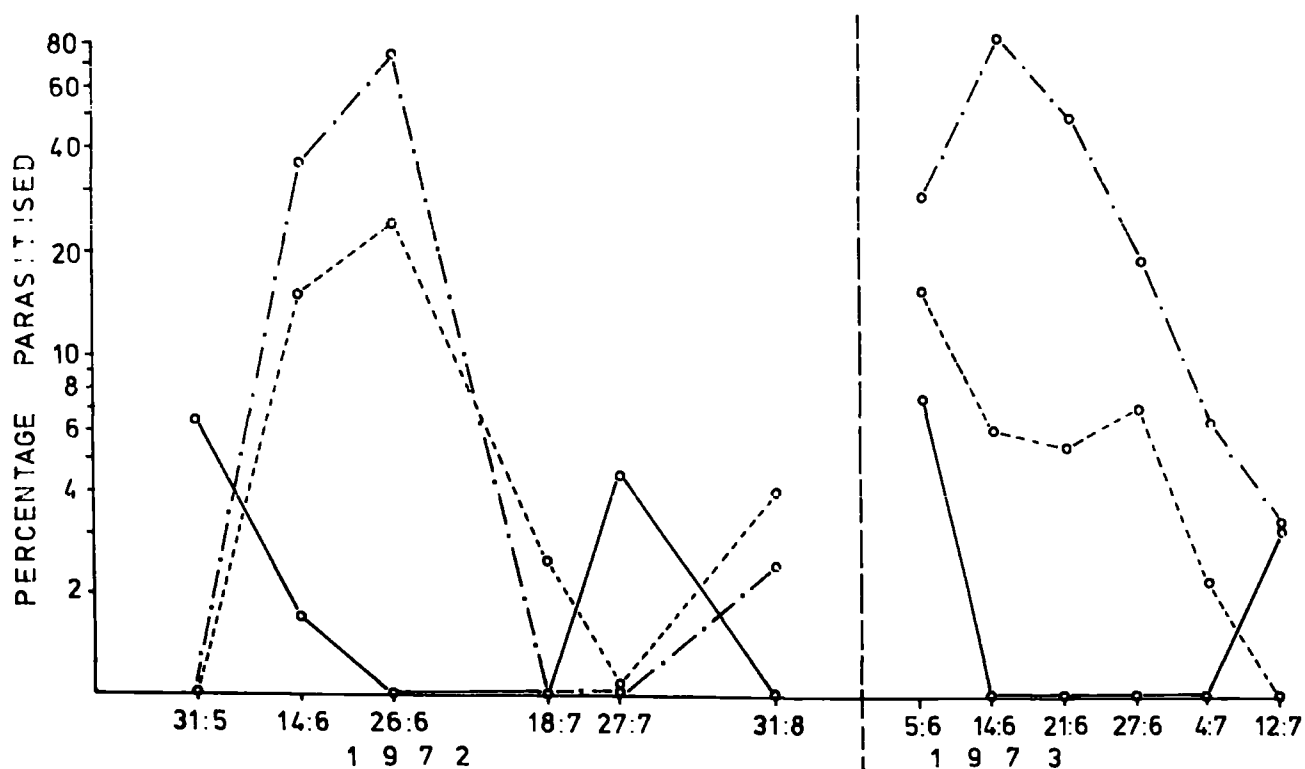
Ezenwa (1974) found a close correlation between the time of hatching of the different microsporidia and mermithid species, and that of the simuliid species, so that each parasite species tended to be principally restricted to one species of simuliid, but would also affect the other simuliid species depending on how much their time of hatching overlapped that of the main vector species. This specificity was shown by Phelps and DeFoliart (1964) who found up to 91% of S.vittatum Zett. were infected with mermithids, whereas in the same stream, no S.tuberosum Lundstrom were infected. This corresponds with the present research in which 641 S.reptans were infected with mermithids, 90 S.ornatum were infected, but only one S.equinum contained a mermithid. It would also explain the succession of parasites during the season, which would therefore occur due to different hatching times of the parasite species.

However, since this research was only done on the simuliid adults and not the larvae, it was possible that the differential rate of parasitism in the three main species of Simuliidae was due to differential mortality at pupation. Strickland (1913) found that virtually all larvae infected with mermithids died at pupation, while D.M. Davies (1956) similarly concluded that virtually all larvae infected with microsporidia at pupation. Thus in the species they studied, larval parasitism resulted in a high mortality. Therefore it is possible, for example, that the low rate of parasitism by mermithids in adult S.equinum was due to a very high rate of mortality in the infected larvae compared with the other two species, while the high percentage of Microsporidia Type B present in the adults could be due to S.equinum larvae being resistant and so having little mortality at pupation.

4. SPATIAL DISTRIBUTION

Dalmat and Gibson (1952) allowed simuliids, mainly S.ochraceum but also S.metallicum and S.callidum to feed om persons infected with Onchoceroa volvulus. The flies were then stained with an aniline dye and released. They were recaptured by bait trapping at different distances from the release point. Out of the 40,474 simuliids released, three infected flies were recaptured at distances upto 4.6 km. Since uninfected flies were caught upto 15 km from the release site, they concluded (Dalmat and Gibson 1958) that the presence of the parasite amongst the thoracic flight muscles limited the dispersal of the fly. However, data based on three flies is obviously not very reliable.

Crosskey (1954b) found that the percentage of humans infected with Onchocerca microfilariae remained within 40-48 % upto 12 km from the River Galma. Even at 20 km from the river, the percentage infected was over 16 % , which implied that the infected flies dispersed widely.

Since the infective larvae of O.volvulus were relatively small (0.65 mm – Duke 1974), they might be expected to have less effect on the flight activity than the mermithids found in the Eden Valley. These were often over 10 mm long, and not only occupied the thorax but also invaded the head (see Plate 9).

a) S.reptans

The numbers of parasitised flies caught on the main road and parallel roads are shown in Fig. 69. For the parallel roads, the mermithids and microsporidia Type B were calculated as a percentage for each road, so that the distributions could be compared:-

	Road 1	Road 2	Road 3	Road 4
Mermithids	27.8	52.4	13.0	7.8
Type B	29.0	42.6	19.4	9.0
Total nulliparous	30.6	42.2	15.6	11.6

The distributions of the parasitised flies were compared with that of the nulliparous flies (since most of the parasitised flies were nulliparous) and the significance tested using the formula:-

$$d = \frac{k_1 - k_2}{\sqrt{K(1-K)(\frac{1}{n_1} + \frac{1}{n_2})}}$$

$$\text{where} \quad k_1 = \frac{a_1}{n_1}; \quad k_2 = \frac{a_2}{n_2}; \quad K = \frac{a_1 + a_2}{n_1 + n_2}$$

d = standard deviation

a_1 = number of mermithids/Type B caught on one road

n_1 = total number of mermithids/Type B caught on all four roads

a_2 = number of nulliparous caught on one road

n_2 = total number of nulliparous caught on all four roads

(from Bailey 1959)

The only significant difference was on Road 2, where the mermithids were significantly higher ($p=0.001$) than the total females. Both mermithids and Type B had lower percentages on Road 4 than the non-parasitised flies, though the difference was not significant. However, if the combined catches on Roads 3 and 4 were considered, the mermithids, but not Type B, were significantly lower than the non-parasitised ($p = 0.03$).

On the main road, though the numbers involved were admittedly small, and so less reliable, there were the following distributions:-

	D	E	F	G	H	I	J
Mermithids	21.0	23.5	21.8	9.2	10.2	7.6	6.7
Total B	15.4	27.0	17.0	10.6	6.8	13.6	9.4
Total nulliparous	31.0	24.5	14.1	7.1	6.0	10.9	6.4

In section D, the mermithids were significantly lower than the total nulliparous females (p = 0.03), while Type B had a highly significant difference (p = 0.0001). In section F, the mermithids were significantly higher than the total nulliparous flies (p = 0.03). This was therefore similar to the parallel roads where there was a significant difference on Road 2.

The parasitised flies therefore had their highest concentrations on sections E and F, whereas the non-parasitised flies (see Fig. 46) other than the gravids were highest on section D. Apart from this, on the main road there was little significant difference between the spatial distribution of parasitised and non-parasitised flies. As the results for the parallel roads were more reliable (due to higher concentrations and a larger number of samples), the presence of mermithids probably reduces the flight range of the flies, though over the distances studied (6 km) this effect was not very marked. Microsporidia do not appear to have any such effect.

b)   S.equinum

The mean concentration of parasitised flies was found for each parallel road and expressed as a percentage of the total females:-

	Road 1	Road 2	Road 3	Road 4
Mean conc. of parasitised	4.1	3.2	1.9	1.2
Mean for total females	16.6	10.0	4.6	2.4
Percentage parasitised	24.7	32.0	41.3	50.0

The percentage of flies that were parasitised therefore increased with distance from the River Eden, so that whereas on Road 1 (0.5 km from the river) only one quarter were parasitised, on Road 4 (6 km from the river) half the female flies were parasitised. However, it should be noted that on Road 4 the concentrations were extremely low and so not too reliable.

The presence of parasites (almost entirely Microsporidia Type B) did not therefore reduce the powers of dispersal of the flies, but seemed to actually increase it.

VII. DISCUSSION

1. USES OF THE VEHICLE TRAP

A vehicle-mounted net was successfully used in sampling the flying simuliid population for the purpose of studying the patterns of flight activity and the spatial distribution of the flies. The vehicle trap had the advantage of being efficient, in that it was capable of detecting small changes in the flying population (down to 2 flies/2000 m^3 of air), even though the concentrations of flying simuliids was generally very low (the overall mean concentrations of S.reptans on Road 2 was 35.4 flies/2000 m^3 of air). The efficiency was due to the large volume of air sampled. Thus when driven at 48 km/h, the net sampled 27,000 m^3 of air/h. Large suction and rotor traps are capable of sampling similar volumes of air. Bidlingmayer (1971), for example, used a suction trap capable of filtering 22,300 m^3 of air/h, while Chamberlin and Lawson (1945) used a rotor trap which filtered 23,040 m^3 of air/h. However, because they are stationary, the efficiency of these traps was much less than would appear from the amount of air filtered. Even a large suction trap can only suck in insects over a distance of a few metres, so that stationary traps are liable to constantly resample the same air. Consequently, the rate at which the flies were sampled was totally dependent on the speed at which the flies move into the area (which even when moving downwind is likely to be less than 10 km/h). In contrast, the vehicle trap actively sought the flies, so that the rate of trapping was mainly dependent on the vehicle speed of 48 km/h (though also affected by the wind speed).

A second advantage of the vehicle trap was that it was non-attractant. Consequently, the catch could be calculated as a concentration of flies per unit volume of air, and so could be directly compared with catches taken in

different areas. Other non-attractant sampling methods, such as suction traps,
can also theoretically give the catch as a concentration, but this would be
dependent on there being no recycling of the filtered air.

Being non-attractant, the net caught all the different physiological
groups of simuliids. Previous research was largely restricted to information
about the activity of blood-thirsty females caught by bait-trapping, and
ovipositing gravid flies caught by sweep netting and sticky traps. There was
therefore little known about the blood-fed flies, or the gravid flies away from
the breeding grounds. Nor, by using different methods, was it possible to
compare the relative abundance of the gravid and blood-thirsty flies. The
vehicle trap, on the other hand, can be used to directly compare the different
physiological groups by expressing them as concentrations.

Since the vehicle trap was non-attractant, it could be used for studying
the circadian rhythm by collecting throughout the day and night. Attractant
traps are usually only effective over part of this period. For example, light
traps only work at night. Since the vehicle was driven at 48 km/h, there was
no evidence that the use of headlamps at night affected the catch, the flies
having insufficient time to react to the light beam before being caught. This
was similarly concluded by de Zulueta (1950).

A third advantage of the vehicle trap was that it was not stationary, and
so was less affected by the surrounding habitat. This is an important factor
when studying the spatial distribution. However, though the catches did not
seem to be affected by the presence of a row of trees or a tall hedge beside
the road, there was a marked increase in the concentrations of S.reptans females
whenever the road was bordered by a wood. Fortunately, woods only occurred on
a few sections of the circuit (Fig. 53).
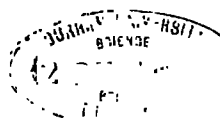
The vehicle trap did have a number of disadvantages in its use. When mounted on a van, it required an existing road network. When studying dispersal, roads at right angles to the breeding ground had the disadvantage that the distance from the breeding ground changed constantly during the catch. Thus catches could only be made over short distances, with a resulting small catch which would be difficult to analyse. Roads parallel to the breeding ground allow catches to be made over much greater distances, but parallel road systems are not common.

A second disadvantage of the vehicle trap was that, since the catch was calculated as a mean concentration for each section, the trapping method was not suitable for insects which form local aggregations such as swarms (but then nor is any other trapping method). Thus, in the case of male Simuliidae, most swarms would be very considerably larger than the average catch of only 3 - 4 males/kilometre section. Consequently, the size of the catch would depend principally on what proportion of the swarm the net passed through, rather than other factors such as the spatial distribution.

A third disadvantage of the vehicle trap was that catches could only be made at a fixed height. On the present equipment, the net caught simuliids flying at a height of 2 - 3 metres above the ground. However, many of the mosquito species have been shown to have a pronounced vertical distribution (Happold 1965; Service 1971), and some of the species have been shown to have a diurnal rhythm of vertical movement (Snow 1955, 1957). Thus when collecting at a fixed height, the catching efficiency for different physiological groups may alter during the day. Furthermore, some of the section of the population may be completely missed. For example, Provost (1957) found that migrating Aedes taeniorhynchus were not caught at all in vehicle traps, whereas on subsequent nights, the mosquitoes taking appetential flights were caught in large numbers. He therefore concluded that mosquitoes migrated at a great height.

Despite their obvious advantages, vehicle traps have been largely ignored in the studies of Simuliidae. In contrast, vehicle traps have been used for a long time to study the flight activity of mosquitoes (Chamberlin and Lawson 1945; Stage 1947; de Zulueta 1950; Provost 1952, 1957; Bidlingmayer 1966; Nelson and Bellamy 1971; Dyce 1972). However, these earlier traps did not have a mechanical method of dividing the flow of insects into separate catches, and so necessitated catches over much greater distances. The present equipment divided the insects into 1 km catches, so that each catch was taken over a period of about 1.5 minutes. The equipment was therefore sensitive to rapid changes in the flying population and so could be used, for example, to study the effect of the weather on flight activity. With its sensitivity and short collecting time, it is also useful for studying the spatial distribution of simuliids, since it allowed a circuit of catches at different distances from the breeding grounds to be made within a short period of time, thus reducing problems from the diurnal rhythm.

The vehicle trap would be especially useful in Africa where studies have been largely limited to using "fly-boys", with all the attendant problems of analysis. In the savannah regions, the lack of roads could in some localities be overcome by mounting the equipment on a landrover or similar four-wheel drive vehicle. Thus de Zulueta (1950) studied the dispersal of mosquitoes on the llanos of Colombia using a net mounted on a small truck (lorry).

2.   DIURNAL ACTIVITY WITH RELATION TO THE WEATHER

The study of the diurnal rhythms of the simuliids in the Eden Valley showed a marked difference between the three species. S.reptans had a diurnal rhythm consisting of a morning and evening peak. There was probably also a dawn peak, but this was only studied on two days (Figs. 19 and 20). The diurnal rhythm broke down on cloudy days, and seemed to be partly controlled

by rapid changes in the light intensity. Thus when the sun clouded over, flight activity markedly increased. The dawn and evening peaks were thus probably due to rapid changes in light intensities. The evening peak occurred between a wide range of light intensity values (640 - 70 lux) and so was not due to a threshold at an absolute value, but was due probably to the rate of change of the intensities.

Low light intensities inhibited activity, there being little activity of S.reptans females after sunset (about 80 lux).

S.ornatum reacted in a similar way to changes in the light intensities, but activity was principally restricted to the evening, there being little activity in the morning or afternoon (Figs. 33 - 38). Thus when, for example, the sun clouded over in the afternoon (Fig. 37), there was an increase in activity but it was much less than in S.reptans. S.ornatum therefore appeared to have an upper light intensity threshold above which activity was inhibited. Though S.ornatum was sensitive to high light intensities, activity was inhibited later in the evening that in S.reptans. Activity continued almost up to darkness, one hour after sunset (Figs. 28 - 31), so that S.ornatum must have a much lower threshold, below which activity was inhibited, than does S.reptans.

The diurnal rhythm of S.equinum was not very clear from the few days available. However, there was often no evening peak (Fig. 25), and flight activity was reduced much earlier than in S.ornatum and S.reptans. Furthermore, activity started later in the morning so that concentrations in the first circuit (08.00 - 09.45 h) were usually extremely low. The lack of activity in the evening could conceivably be due to the flies having a much higher light intensity threshold, so that activity ceased earlier than in the other

species.   However, this could not be the reason in the morning, since the

light intensity was over 1000 lux many hours before 08.00 h.   There must

therefore be other limiting factors involved, the most important of which

was probably the temperature.

S.reptans appeared to have a lower temperature threshold for flight at

approximately 12°C.   The twelve days studied were especially chosen as being

warm and so do not show the effect of the threshold to a great degree.   On

the majority of the days which have not been included, the temperatures did

not rise above 12°C until after midday.   There were thus very low concentrations

(less than 2 flies/2000 m³ air).   Even on the days sampled, the first few

catches of the first circuit (08.00 h) often had low concentrations because

the temperature was still below 12°C (see Fig. 21).   Because of this, it is

debateable as to whether a dawn peak is 'normal', since though peaks occurred

on the two days sampled (Figs. 19 and 20), these days were unusual in having

temperatures over 12°C at dawn.   A temperature threshold at 12°C, is similar

to that found in many of the Canadian species.   Thus S.venustum in Ontario

was found to have a threshold at 12°C (D.M. Davies 1952, L. Davies 1963),

though in Eastern Quebec where temperatures are cooler, the threshold was at

7°C (Wolfe and Peterson 1960).   In Wisconsin, U.S.A., Anderson and DeFoliart

(1961) found a number of simuliids had a flight threshold at 12°C.

In the evening the threshold may also have a limiting effect, and on a

cool evening may eliminate the peak (Fig. 13).   However, the lack of activity

at night was probably primarily due to the light intensity threshold rather

than the temperature, since even when the temperature stayed above 14°C (Fig. 20),

there was little activity.   Previous research on other species using light

traps and suction traps has often revealed a high level of night activity.

Thus C.B. Williams (1965) using a suction trap caught 1,238 S.latipes Meigen

in October 1956, an average of 40/flies/night.   Whereas in the present research,

vehicle trap catches over a seven hour period of a warm night (Fig. 20) only caught 13 flies, despite what would seem to be optimum conditions. This somewhat surprising result therefore needs, for research, to find the factors affecting night activity.

While low temperatures were inhibitory, high temperatures also seemed to reduce activity in S.reptans. There did not appear to be a definite threshold, but temperatures over 20°C were accompanied by very low concentrations (Figs. 7 and 8). Since the temperatures were highest in the afternoon (on a sunny day, temperatures were highest around 15.00 h), the concentrations were lowest around this period. On days early in the season, such as the end of May and beginning of June, the sun was not strong enough above 18°C (Fig. 9) so that the concentrations were only low for a few hours in the afternoon. However, later in the season when the sun was much stronger, the temperature rose rapidly on the morning of a sunny day so that the concentrations were low from mid-morning up to sunset (Figs. 7 and 8).

The diurnal rhythm in S.reptans was therefore largely the result of two factors:- the temperature and the light intensity. The rhythm could however be broken down by the presence of clouds or winds over 5 km/h. The question therefore arises as to how these factors affect the flight activity.

The lower temperature threshold was the point below which flight activity was curtailed because the temperature was too low for the correct functioning of the metabolic processes involved in flight, such as ATPase activity (Johnson 1969). Very high temperatures may similarly curtail activity by degrading protein. However, in the Eden Valley temperatures never went above 26°C on the days sampled, so that this cannot have been the reason for the high temperatures reducing activity. The effect on the simuliids was therefore

probably due to dehydration (D.M. Davies 1952). As the temperature rises, the relative humidity would decrease so that there will be a corresponding increase in the rate of water loss by the simuliids.

When there were sunny and cloudy periods during a day, there was a marked increase in the flight activity of S.reptans females during the cloudy periods. This preference for cloudy conditions was probably also due to the rate of water loss under these conditions. Sunshine has the dual effect of raising the air temperature and thus lowering the relative humidity, and of increasing the rate of evaporation due to direct insolation. Under these circumstances, it was not surprising that far higher concentrations of S.reptans were caught on section S of Road 2 which was shaded by woodlands, rather than on section R which was exposed.

The deleterious effect of winds on flight activity has been considered to be due to an increase in the rate of water loss (D.M. Davies 1952). In the case of S.reptans, winds over 5 km/h appeared to reduce flight activity. This is similar to results found in Canadian research. Peterson and Wolfe (1958) and Wolfe and Peterson (1960) found that winds over 3 km/h affected activity, while Anderson and DeFoliart(1961), and L. Davies (1957a) found that winds over 8 km/h reduced activity. A few species such as S.arcticum are known to fly in relatively strong winds (Fredeen et al 1951), but since they fly downwind, the relative windspeed will be low.

Since S.reptans appears therefore to be adversely affected by dehydration, the increase in flight activity when there were rapid drops in the light intensity is probably a mechanism to ensure that flight activity was greatest when the humidity was highest (early morning and late evening), so that water loss would be at a minimum. In S.reptans, the highest concentrations and therefore greatest activity occurred during the evening peak, which was usually

between an hour and half an hour before sunset. Except on sunny days when the
temperature rose rapidly, there was also a large morning peak. The timing of
this depended on when the temperature rose above the threshold, and on when
the humidity dropped too low, but was often between 08.00 and 13.00 h. In
S.ornatum, there was usually no morning peak so that most of the flight
activity was restricted to the evening peak, which extended much later than
in S.reptans. Activity was therefore concentrated in the period of highest
humidities, so that S.ornatum would appear to be more sensitive to dehydration
than S.reptans.

S.equinum, on the other hand, had a mid-afternoon peak of activity even
on the two hot days, 18:vii:72 (Fig. 7) and 27:vi:73 (Fig. 8), and so must
have been res$_{\wedge}$tant to dehydration. This is further shown by the fact that the
wooded section S of Road 2 did not have higher catches than the exposed section
R. Thus unlike S.reptans, the concentrations of S.equinum were not increased
by the presence of woods, which would have provided sheltered conditions and
higher humidities.

The female flies were subdivided into nulliparous (those flies still on
their first gonotrophic cycle and thus "young"), parous (those which had
undergone more than one cycle and so were "old"), and gravid (those with
fully mature ovaries). The first two groups were subdivided into blood-
thirsty, blood-fed and parasitised. The gravid flies were not divided into
nulliparous and parous because of the difficulty in doing so.

In S.reptans, there appeared to be no significant difference in the flight
activity rhythm between the nulliparous and parous flies. This may possibly
have been due to the small number of days involved in the analysis, since a
difference has been found by previous research on other species. In S.damnosum,
for example, the nulliparous have an afternoon peak and the parous a midday peak
(Le Berre 1966; Duke 1968; Disney 1972). In S.ornatum, the parous form a higher
percentage of the catch in the early morning and late evening (L. Davies 1957a).

The graphs of nulliparous and parous S.reptans (Figs. 7 - 20) consisted of both blood-thirsty and blood-fed flies. But the blood-fed flies only formed a mean of 10.5% of the total, so that the graphs were effectively of the blood-thirsty flies and show the typical pattern of diurnal rhythm described above.

Both nulliparous and parous blood-fed flies S.reptans (Fig. 24) had a rhythm consisting almost entirely of an evening peak. The gravid flies (Figs. 7 - 20) were similarly largely restricted to the evening. Because the vehicle trap only sampled flying simuliids, the low concentrations could be due to two factors: firstly, if only a low proportion of the adult population was in flight at any one moment (in other words, there was a low level of flight activity); secondly, if the adult population in that particular category was small.

The size of the total adult population was controlled by the seasonal rhythm - the rate of emergence of new adults from the pupae and the death rate. But when divided into the physiological groups, the proportion of flies in each group was dependent on the duration of that stage. The gonotrophic cycle was divided into five ovarian stages (see Chapter 2), but for the analysis the flies have been divided into three physiological groups: the blood-thirsty flies (ovarian stage I), the blood-fed (stages I - IV), and the gravid flies (stage V). If therefore the flies were gravid for a shorter period than blood-thirsty, then there would be a smaller gravid population. Because the vehicle trap was not efficient at sampling very low concentrations, then if the population was small,only the peaks of activity would be recorded.

However, during the evening peak the gravid flies often formed a much higher percentage of the total female catch than did the blood-thirsty flies (see Fig. 23). Consequently, the gravid population must have been large,

so that the diurnal rhythm, with low concentrations during the morning and afternoon, must have been a result of changes in the level of flight activity. Since the blood-thirsty flies lasted for four ovarian stages, it is also unlikely that they had a small population, so that their diurnal rhythm was similarly due to a lack of activity during the rest of the day.

Since activity of both gravid and blood-fed S.reptans was restricted to the evening peak, it is possible that both the gravid and blood-fed flies were less resistant to dehydration than the blood-thirsty flies. Most of the blood-thirsty flies had their crops distended with nectar. Though nectar feeding is normally considered to be a method of ontaining sugars to provide energy for flight activity (Nayar and Van Handel 1971), it also consists of a large supply of water and so may allow the fly to cope with greater water loss. The crop contents of blood-fed flies tended to be small, but most of the gravid flies had large crops, so that a difference in the ability to withstand dehydration may not have been the reason for the difference in the diurnal rhythms.

It was possible that the short period of flight activity each day by the blood-fed and gravid flies was simply because they had less motivation for flying. Blood-thirsty flies must find a blood meal and so will have a strong motivation for flight. The reason for flight by blood-fed simuliids is not clear. Edman and Bidlingmayer (1969) found that most of the blood-fed mosquitoes caught in a marsh contained fresh blood meals. They therefore considered that the reason for flight was to find a resting site on wooded islands in the marsh. However in the present research, though little is known about the resting sites of Simuliidae, it is unlikely that they had very far to fly. Furthermore, not only were simuliids with fresh blood meals found (which could be expected to be searching for a resting site), but also flies with all the other stages of blood digestion, so that the reason for flight is not known. However, it is probable that they had less motivation for flying than the blood-thirsty flies, so that flight was restricted to the most ideal conditions occurring at sunset.

3.   SPATIAL DISTRIBUTION

The three species of Simuliidae in the Eden Valley showed marked difference in their spatial distributions.

In S.reptans, the females had high concentrations for the first two kilometres away from the river.   It was difficult to compare the concentrations on Roads 2 and 1, because of the presence of woods beside two of the sections of Road 2, which greatly increased the catch.   However, if the effect of the woods was eliminated using multivariate analysis, or if only the non-wooded section R was used, then the concentrations on Road 2 (1.5 km from the river) were actually slightly higher than on Road 1 (0.5 km from the river).   Even on Road 3 (3 km from the river), the concentrations had only fallen to 0.61 of that of Road 1, and on Road 4 (at 6 km) it had only fallen to 0.4 of that on Road 1. Consequently, the S.reptans females would seem to have a vigorous dispersal away from the river, so that the concentrations increased with distance from the river up to approximately 1.5 km, then gradually decreased again, but still remaining high at 6 km from the river.

In contrast, the S.equinum females had an exponential decrease in concentrations away from the river, so that by Road 4 the concentrations had fallen to 0.14 that of Road 1.   Thus, the concentrations of S.equinum females decreased over a distance of 6 km by over three times as much as did the S.reptans.   Furthermore, whereas the concentrations of S.reptans were highest on Road 2 away from the river, the concentrations of S.equinum were only high on Road 1 close to the river.   S.equinum would therefore appear to have comparatively poor powers of dispersal.

Yet S.reptans was probably active for a shorter period each day, since activity was largely concentrated in the short morning and evening peaks, whereas S.equinum was active for most of the morning and afternoon. The poorer dispersal by S.equinum was therefore unlikely to be due to the flies having a shorter period of activity, and thus less time to disperse than S.reptans.

The spatial distribution of S.ornatum could not easily be studied, since it not only bred in the River Eden but also in the smaller streams. There were therefore a number of breeding sites within the collecting area which were only a few kilometres apart. But, considering the rapid changes in the concentrations on the main road, especially the sharp drop in concentrations between the breeding site of Sunnygill Beck at section I and between section G, only 2 km away (Fig. 61), it is probable that like S.equinum, S.ornatum only had poor powers of dispersal. However, if this was so, it was surprising that the concentrations were not higher near the River Eden (Road 1 and section D), since due to its relatively vast area, the Eden must have been quantitatively the main breeding ground. The highest concentrations on the main road were found where the road was crossed by the two streams, Briggle Beck (section E) and Sunnygill Beck (sections I and J). Pupal samples showed that S.ornatum bred in these two streams.

The male S.reptans appeared to have a similar spatial distribution to the females. However, this was only based on a few days and so was not very reliable. But the results do show that the males were capable of dispersing widely, up to at least 6 km.

The female flies were divided into nulliparous and parous. In S.reptans, the decrease in concentrations between Road 1 and Road 4 was four times as great for the parous as the nulliparous flies, (Fig. 49). The concentrations shown in Fig. 49 were increased by the presence of woods on Road 2, but if the effect

of the woods was eliminated, then the parous still showed a rapid decrease in the concentration away from the river, while in contrast the concentration of nulliparous was higher on Road 2 (at 1.5 km from the river) than on Road 1 (0.5 km). The nulliparous flies therefore dispersed much more widely than the parous flies. Despite this, there were still moderate concentrations of parous flies on Road 4, at 6 km from the river.

In S.equinum, there appeared to be less difference in the dispersal of the nulliparous and parous flies. Both had rapid exponential decreases in concentrations with distance from the river, and thus very low concentrations on Roads 3 and 4. There was, however, still some evidence that the nulliparous flies dispersed further than the parous flies.

There has been little previous research on the spatial distribution of simuliids, due to the limitations of the sampling methods. L. Davies (1961), using netting around human bait to catch P.mixtum, found that the parous blood-thirsty flies were largely restricted around the breeding sites, whereas the nulliparous flies dispersed much more widely. Downes et al (1962) found similar results for S.venustum. Both these studies therefore found a similar situation to that of S.reptans. Rempel and Arnason (1947), studying cattle deaths by S.arcticum, found few deaths within the first 48 km of the breeding site, large numbers at 96 km and moderate numbers up to 160 km. However, S.arcticum differed from the simuliid species in the present research in that dispersal was wind-borne. The authors therefore concluded that most of the population was removed by the wind and deposited tens of kilometres away.

Extensive research has been done on the dispersal of mosquitoes (Culicidae) and midges (Ceratopogonidae). A number of the mosquito species and many of the midges have been shown to have a very rapid decrease in concentrations

with distance from the breeding site, so that most of the population stays within one or two kilometres (Jenkins and Hassett 1950; Linley and Davies 1971; Nicholas 1953; Shemanchuck et al 1955).

In contrast to this situation, a number of mosquito species have been shown to have a dispersal which has similarities with that of S.reptans. Stage 1937, marking mosquitoes with dyes found large numbers of Aedes vexans and Ae. aldrichi up to 13 km from the breeding site. Clarke (1943) similarly used aniline dyes. He found that on the first night after emergence Ae. vexans was capable of travelling up to 22 km, Culex pipiens dispersed up to 14.2 km and Anopheles punctipennis dispersed 16.8 km. He therefore concluded that these species had a definite migration on the night of emergence.

It is necessary at this point to define what is meant by 'migration'. The description generally accepted by entomologists studying the flight patterns of mosquitoes has been summarised by Provost (1952, 1953). He considers that the difference between 'migrating' and 'non-migrating' insects is purely due to the type of behaviour patterns they exhibit. Thus flight behaviour can be divided into two types: 'appetential' (or 'appetitive') in which the insect flies in order to satisfy an internal 'drive' which results from a physiological need, such as to find a mate or blood meal, and thus exhibits searching behaviour until this 'drive' is consummated; 'migratory' or non-appetential behaviour in which the internal drive is purely to fly, so that flight activity and flight direction are unaffected by the presence of food, etc.

Provost (1957) studying Aedes taeniorhynchus found that they were capable of travelling up to 14 km on the first night. He found that the migrating flies were not attracted to light (35 flies were caught in a suction-light trap situated 30 m from the point at which 1,400,000 mosquitoes emerged).

He was also unable to catch any in a vehicle trap or in bait traps.   He
therefore concluded that they migrated at a height greater than that of the
vehicle trap (and therefore greater than normal), and that they probably flew
throughout the night, ignoring normal stimuli such as the presence of a blood
meal.   On the following night they reverted to the 'normal' behaviour of
making short appetential flights and so were caught in all the traps.

Dow (1971) studying Culex nigropalpus did not divide his adult females
into nulliparous and parous, but only into the roughly equivalent 'young'
and 'old' flies.   He found that the concentration of 'old' flies decreased
twice as fast as the 'young' flies, when sampled at different distances from
the breeding ground.   The young flies dispersed much further (4.8 km).   He
therefore concluded that the young flies migrated on emergence dispersing over
many kilometres.   After returning to the breeding ground to oviposit, they
did not migrate again, but instead made short appetential flights, and so
dispersed less.

Like C.nigropalpus, the S.reptans nulliparous flies dispersed widely,
while the parous flies had much poorer powers of dispersal.   It is possible,
therefore, that the nulliparous S.reptans migrated after emergence.   S.reptans
did not show the same range of dispersal as did some of the mosquito species,
such as Ae.vexans and Ae.taeniorhynchus.   However, the mosquitoes migrated
throughout the night, while S.reptans in contrast had a pronounced diurnal
rhythm with only short periods of activity.   It is therefore probable that
the 'migration' in S.reptans lasted perhaps only an hour at sunset.   The
flies would thus only have time to travel a few kilometres away from the river.

S.equinum had much poorer powers of dispersal than S.reptans, with the concentrations decreasing very rapidly with distance from the breeding grounds of the River Eden.   This was true for both the nulliparous and parous flies. The S.equinum must therefore either not have a migration, the dispersal being due to appetitive flights, or else the migration must be very brief and short.·

In S.reptans, the spatial distribution of the fresh blood-fed and total blood-fed flies were considered.   When each of these were divided into nulliparous and parous, it was found that there was no significant difference between the spatial distribution of the fresh blood-fed flies and the respective total nulliparous or parous flies.   Since the fresh blood-fed flies had probably fed within the last 12h or less, this showed that the distribution of hosts (probably mainly cattle - L. Davies et al 1962) did not affect the distribution of the flies.   This is not really surprising since the Eden Valley is an intensive dairying region, with large herds of cattle dispersed throughout the collecting area.   The total nulliparous and parous blood-fed flies similarly had the same spatial distribution as the respective nulliparous or parous flies.

The spatial distribution of the gravid flies was more surprising, since compared with the non-gravid flies, they had a greater proportion of their population on Road 2.   Yet since the gravid flies must return to the River Eden to lay their eggs, it would be expected that the highest concentrations would be on Road 1 nearest the river.   The reason for this distribution is not known, but since the diurnal rhythm indicates that they were more sensitive to dehydration than the blood-thirsty nulliparous and parous flies, it is possible that the presence of shade from the woods on Road 2 had a greater attraction for the gravids than the other two groups.   However, this would not explain why the concentrations were also low on section D of the main road, where there were no woods to affect the concentrations.

The gravid flies were widely distributed, with even moderate concentrations on Road 4. If the gravid flies started to return in the direction of the river as soon as their eggs matured, it would be expected that compared with the non-gravid flies, there would be a more marked increase in the concentrations towards the river. This did not occur. If the gravid flies did not move at all, it would be expected that their spatial distribution would be inter-mediate between that of the nulliparous and parous flies, since it is a composition of these two categories. Its spatial distribution should therefore be dependent on the nulliparous:parous ratio of the total flies in the twelve days collected. However, when this was calculated, the gravid flies were in fact more widely dispersed than they should have been. It would therefore appear that the gravid flies do not 'know' in which direction the river lies, and simply move randomly until they either find the river by chance or else die.

If the flies depend on chance to find the river, then having a migratory flight and so widening the area covered by the population will on the one hand be dangerous to the continued survival of the population by reducing the chance of the flies returning to the breeding site, but on the other hand would increase the chance of finding new breeding sites.

4.   OCCURRENCE OF PARASITES, AND THEIR EFFECT ON FLIGHT ACTIVITY

Some simuliid parasites are of medical importance because they can infect livestock, or in the case of Onchocerca volvulus, infect humans. These parasites spend part of their life cycle in the adult simuliid, and the other part in their second host, being transferred during blood-sucking by the simuliid. Being medically important, many of these parasites, especially O.volvulus have been extensively studied (Blacklock 1927; Duke 1968; Lewis 1953).

Other parasites infect only the simuliids and so have been less studied. These parasites are mainly microsporidia and mermithids. They infect the simuliids during their aquatic larval stage, and for some reason have only really been studied in this stage, possibly because it has often been thought that the parasites are largely restricted to the larvae. Strickland (1913) stated that infection by microsporidia or mermithids invariably resulted in the death of the larva at pupation. Twinn (1939) considered that all cases of parasitism by mermithids resulted in the death of the larvae, and D.M. Davies (1956) similarly considered that microsporidia killed the larvae.

However, in the present research large numbers of parasitised adult simuliids were found. The incidence of parasitism in the adults had a marked seasonal rhythm. Since the parasites were almost entirely confined to the nulliparous flies, this rhythm was partly controlled by the simuliid seasonal rhythm. In S.reptans, there was a main peak of emergence around the end of May/beginning of June, and a second smaller emergence in the middle of July. Consequently, the proportion of nulliparous flies markedly increased at these periods (Fig. 63).

The occurrence of mermithids in the adult population was closely correlated with these two emergences (Fig. 71), but the other main parasite present, Mircosporidia Type B, was highest in the middle of June, though occurring over much of the season (Fig. 71). The period of occurrence of Type B in S.equinum was the same as in S.reptans.

There was a marked difference in the percentage parasitised for the three simuliid species. S.ornatum had very little parasitism (only 3.8% of the total catch); S.reptans also had a low level of parasitism (7.2%); S.equinum on the other hand had a mean of 25.9% infected, with a peak on one day (26:vi:72) of 73.7% infected. This was almost entirely due to the microsporidian Type B.

Since the mermithids and microsporidians are only able to infest the
similiid larvae during a short period in the first larval instar before the
peritrophic membrane lines the entire mesenteron (Strickland 1913; Phelps and
DeFoliart 1964), it is possible that the difference in the rate of infection
between the three similiid species depended on the level of synchronisation
between the period when the parasites were infective and the period when the
larvae were susceptible. However, since the larvae in the River Eden were not
studied, it was also possible that the difference between the species was due
to the rate of mortality at pupation, since this has been shown to be high by
previous researchers (Strickland 1913; Twinn 1939; D.M. Davies 1956). It is
conceivable, therefore, that the large percentage of S.equinum adults parasitised
by microsporidian Type B was a result of S.equinum being resistant to the
parasite, whereas most of the infected S.reptans and S.ornatum died at pupation.

Since many parasites were found amongst the adult population, the question
arises as to what is the advantage to the parasite of invading the adult. The
normally accepted life cycle of mermithids and microsporidians (Strickland 1913;
Phelps and DeFoliart 1964) is that the infective juvenile mermithids or micro-
sporidian spore enters the first instar similiid larvae via its gut. The
parasite matures until just before larval pupation, when it leaves the similiid
larva and so kills it. The microsporidians leave the larva as spores which
become infective. The mermithids, after leaving the larva, become adult, mate
and produce eggs. These then hatch to produce infective juveniles. The
cycle is thus repeated, with a free living stage in the water and a parasitic
stage in the similiid larva.

However, if this was the entire cycle, the parasite population would
steadily move downstream, because the similiid larvae move downstream, and
the free living parasites are not strong enough to move upstream against the current.

The simuliid population is maintained by the adults moving upstream to lay
their eggs.   It is therefore necessary for a proportion of the parasites to
infect the adult simuliids, so that they too can maintain their populations.
Parasitised flies have been noted amongst ovipositing gravid flies (D.M. Davies
1958; Phelps and DeFoliart 1964) and so appear to 'oviposit' the parasites back
into the stream.   This is correlated with the fact that though parasites were
almost entirely confined to nulliparous flies, a few parous flies contained
small numbers of mature Type B spores, which therefore appear to have been
left over when the rest were laid.   Some parous flies contained mermithids,
but these were either dead and decaying or else very small, as if they had
failed to mature.

In medically important parasites such as O.volvulus, it is necessary to
know what effect they have on the flight activity of the simuliid.   Dalmat
and Gibson (1952) considered that the O.volvulus infective larvae reduced the
dispersal of the flies.   However, this was only based on three flies and so
was very unreliable.   Crosskey (1954b) considered that the presence of O.volvulus
had little effect on the dispersal.   He found that the percentage of humans
infected remained between 40 - 48% for up to 12 km from the breeding site.

The infective larvae of O.volvulus were only 0.65 mm long (Duke 1974),
whereas in the present research the mermithids were often over 10.0 mm long,
and therefore should have a much more marked effect on dispersal.   However,
a comparison of the spatial distributions of S.reptans parasitised by mermithids
and of non-parasitised nullipars showed only a small relative reduction in the
concentrations on Roads 3 and 4 for the parasitised flies.   Microsporidia had
no effect on the spatial distribution (they probably had no more effect on the
fly than gravid eggs).   Mermithids did therefore appear to reduce the dispersal
of the simuliid, but the effect was not very great.

Though the O.volvulus larvae were much smaller than the mermithids, it was possible that they had a greater effect on the simuliid's powers of dispersal, since they occurred within the flight muscles, whereas the mermithids occurred within the haemocoel. But, it was in fact surprising what the flies were able to put up with, and yet still be able to fly (which they must have been, to have been caught in the vehicle trap). Some of the simuliids contained up to four mermithids, which not only occupied the abdomen, but also the thorax between the flight muscles, and even projecting into the head (Plate 9).

One of the limitations of the present research was that several mutually exclusive fields of study were covered. In the future, therefore, it would be desirable to study the circadian rhythm in more detail, especially the effect of the weather on the flight activity. In the present research, the study of the spatial distribution had priority, and so the study of the circadian rhythm was limited by having to collect on four roads. Furthermore, as the maximum catch possible was desirable to study the dispersal, the effect of the weather was deliberately eliminated as far as possible. In the study of dispersal, it would be desirable to try collecting at different heights and to study in more detail the effect of woods on the catch size. If the weather had been more suitable, it would have been useful to study the spatial distribution every day for a period of a fortnight, so that the changes could be seen in more detail.

In the onchocerciasis regions of Africa, there is plenty of scope for research using a vehicle trap, providing that the right field conditions can be found. Because of its advantages as a non-attractant method, catching flies at all stages, it must provide important desired information on the adults of Simulium.

SUMMARY

1.   The flight activity of three species of Simuliidae was studied in the Eden Valley.

2.   The flying population was sampled using a net mounted on the roof rack of vehicle, which was driven at 48 km/h along a circuit of roads.   Equipment in the back of the vehicle divided the flow of insects from the net into a series of catches.   This was controlled by the driver, so that each catch contained the insects collected over a distance of 1 km.   Using an anemometer mounted in the mouth of the net, the actual concentration of the flies could be calculated.

3.   The vehicle was driven along a circuit of four roads parallel to the River Eden, and along the main road at right angles to this, between the villages of Langwathby and Melmerby.   Each circuit took approximately 1 h 40 min, so that eight circuits were made per day in 1972, and nine circuits in 1973 (by starting earlier).

4.   The weather conditions were recorded.   Temperature was measured by a thermometer kept in the shade of a hedge.   Wind speed and wind direction were noted using a hand-held anemometer.   The presence of sunny or cloudy conditions were noted.   In the evening, the light intensity was recorded using a selenium cell photometer.

5.   Research was concentrated on the female flies.   All the female flies were dissected.   On the basis of this, the females were divided into nulliparous and parous, and the development of the oocytes was divided into five stages.   The presence, size and age of blood-meals in the mid-gut was noted.   The presence of parasites was noted.

6.   All the streams in the area were sampled for simuliid larvae and pupae in order to survey the breeding sites.   S.reptans and S.equinum were found to be largely restricted to the River Eden, though small numbers were also found in the lower reaches of the other streams.   S.ornatum was much less restricted to the River Eden, and was found in moderate quantities in the other streams.

7.   The diurnal rhythm of activity was studied for the three species, by comparing the catches on one of the roads for all the circuits during the day.   On two days, 24 h catches were made, but only a very low level of flight activity was found during the period of darkness.

a)   S.reptans

    i)   On sunny days, the flies had a morning peak of activity, little activity in the afternoon and a large evening peak, often about half an hour before sunset.   On the two days in which it was sampled, there was also a dawn peak of activity.   On cloudy days, the rhythm was much less pronounced.

    ii) The dawn and evening peaks seemed to be synchronised by a rapid change in the light intensity, rather than the actual intensity, which varied between 640 and 70 lux.   Similarly, a clouding over of the sky on a sunny day produced a sharp increase in activity.   Little activity occurred after half an hour after sunset.   This seemed to be due to the low light intensities rather than due to other factors.

    iii) There was a temperature threshold at approximately $12^{\circ}C$, below which activity was curtailed.   This controlled the timing of the beginning of the morning peak.   On one of the days (31:v:73), low temperatures also eliminated the morning peak.

iv)   High temperatures (over 20°C) reduced activity.   This is believed to be due to the resulting low humidities.   Presence of sunshine and of winds over 5 km/h also reduced activity, probably for the same reason.   The low activity in the afternoon was probably because activity was lowest at this time.   Hot (over 20°C) sunny days resulted in a reduced morning peak because of the rapid rise in temperatures.

v)   There was no evidence of a difference between the diurnal rhythms of the nulliparous and parous flies, but this may have been due to the small number of days examined (twelve) and the variable weather conditions.

vi)   Both gravid and blood-fed flies had activity largely reduced to an evening peak.   They were therefore possibly more sensitive to dehydration than the blood-thirsty flies, which showed the typical rhythm described above.

b)   S.ornatum

Activity was much more restricted to the evening than for S.reptans. S.ornatum was therefore possibly more sensitive to dehydration.   In the evening, activity continued up to dark (one hour after sunset), which was later than in S.reptans.   The lack of activity during most of the day was possibly controlled by an upper light intensity threshold, above which activity was reduced. Clouding over of the sky on a sunny day produced an increase in activity, but it was much smaller than in S.reptans.   On cloudy days, there was still an evening peak of activity.

c)   S.equinum

The diurnal rhythm could not be studied in detail, because of the small number of days with reasonably high concentrations.   However, activity started later in the day than in S.reptans and on most days had virtually ceased by sunset.

It was possible that this was due to the species having a higher temperature threshold than in S.reptans. The diurnal rhythm appeared irregular on the few days sampled, but on the hot day 18:vii:72, when the temperatures went above $26^{o}C$, there was an afternoon peak of activity, so that S.equinum appeared to be resistant to dehydration. Similarly, clouding over of the sky on sunny days did not reduce the activity.

8. The spatial distribution of S.reptans and S.equinum was studied by comparing the catches on the four parallel roads, and by comparing the seven sections along the main road, which was at right angles to the River Eden. The four parallel roads were at the following distances from the River Eden:-
Road 1 = 0.5 km; Road 2 = 1.5 km; Road 3 = 3.0 km; Road 4 = 6.0 km.

a) S.reptans

i) No difference was found between the spatial distribution of males and females, though they were only compared on a few days. However, this does show that the males dispersed as much as the females, with moderate concentrations even on Road 4.

ii) The female flies had their highest concentrations on Road 2 rather than on Road 1, as might be expected. This however was largely due to the presence of woods on two sections of Road 2. The presence of woods beside the road thus markedly increased the catch, whereas the presence of a row of trees or tall hedge seemed to have no such effect. The presence of woods may have an effect by either providing shade with associated high humidities, or by acting as a wind break. However, on the few days examined, it was not possible to distinguish between the two effects. The effect of the winds could be eliminated by multi-variate analysis.

iii) When taking into account the effect of the woods on Road 2, the parous flies had their highest concentrations on Road 1, with a marked reduction in the concentrations with distance from the river. The nulliparous flies had little difference between the concentrations on Roads 1 and 2, and in fact the concentrations were probably slightly higher on Road 2, even when taking into account the effect of the woods. The concentrations then decreased with distance from the river, but were still moderately high even on Road 4. The decrease in numbers between Roads 1 and 4 was four times as great for the parous as for the nulliparous flies. The nulliparous flies therefore dispersed widely, while the parous flies had much poorer powers of dispersal.

iv) Considering that the gravid flies were made up of nulliparous and parous flies, they were much more widely dispersed than would be expected. It is probable that they do not 'know' their way back to the breeding grounds of the River Eden, and so continue to disperse outwards.

v) The blood-fed flies, when divided into nulliparous and parous, had the same spatial distribution as the respective non blood-fed nulliparous and parous flies. The presence of hosts (probably cattle) does not therefore affect their distribution.

b) S.equinum

i) The concentrations of both nulliparous and parous flies decreased rapidly with distance from the river. The drop in concentrations between Roads 1 and 4 was over three times as great as for S.reptans, so that S.equinum has much poorer powers of dispersal. However, as in S.reptans, the nulliparous appeared to disperse more widely than the parous.

ii)   Corresponding with its apparent lack of sensitivity to dehydration, as shown by the diurnal rhythm, the S.equinum catches were not increased by the presence of woods on Road 2.

c)   S.ornatum

Though it was not possible to study the spatial distribution in detail, due to the fact that S.ornatum bred in the smaller streams within the collecting area as well as in the River Eden, the concentrations appeared to decrease rapidly with distance from the nearest breeding site, so that the flies had poor powers of dispersal.

9.   Since the adults only occur in the summer, there was a seasonal change in the population size.

a)   S.reptans

There was a mass emergence of nulliparous flies at the end of May and beginning of June.   There was a second smaller emergence in the middle of July. There was a corresponding change in the population of nulliparous and parous flies.

b)   S.equinum

Emergence started later than in S.reptans (middle of June) and was of a more extended duration, so that the proportion of nulliparous decreased steadily during the season.

c)   S.ornatum

The proportion of nulliparous flies was generally low throughout the season, so that the emergence would appear to have started some time before the collecting began at the end of May.   The emergence probably continued at a relatively steady rate.

10.   Apart from the seasonal change in the overall population numbers, there were marked daily changes in the concentrations of the different physiological groups.   This was principally due to changes in the weather, and due to the gonotrophic cycle.

11.   The proportion of blood-fed flies was generally low (mean of 10.5%). This was probably due to them being relatively inactive, rather than being due to the blood-fed stage having only a short duration.   However, the concentration of blood-fed flies was surprisingly high, considering the popular assumption that blood-fed flies are largely inactive.   Furthermore, not only were flies with fresh blood meals caught (which could be expected to be seeking shelter), but also all the other stages of blood digestion were caught, and so must have been active.

Gravid flies formed a much higher proportion of the total flies (33.8%). This was probably due to their being more active than the blood-fed flies.

12.   Internal parasites consisted of mermithids, microsporidia, and to a lesser extent, fungi.

a)   There was a marked difference in the distribution of the different types of parasites amongst the three simuliid species.   In 1972, for example, 29% of S.equinum contained microsporidian Type B, whereas only 11% of S.reptans and 2% of S.ornatum contained this parasite.   There was also a marked change from year to year.   This seemed to be principally due to a change in the parasite population, since it was relatively constant for all three simuliids.

b)   The parasites had a marked seasonal change of occurrence in the adults. The mermithids were restricted to the nulliparous flies, and so fluctuated with the pattern of simuliid emergence, occurring at the beginning of June and again during the middle of July.   Microsporidian Type A, on the other hand,

only infected the nulliparous flies of the second emergence, and so occurred from the middle of July onwards. Microsporidian Type B, though largely restricted to the nulliparous flies occurred during most of the season, but had a peak around the middle of June.

c)   The presence of mermithids reduced the dispersal of the S.reptans nulliparous flies, though the amount of reduction was not very great, so that moderate concentrations of parasitised flies still occurred on Road 4. Microsporidia did not appear to significantly affect the dispersal.

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