

Ecological studies on *Culicoides impunctatus*
(Diptera: Ceratopogonidae)
with reference to its control in the
Highlands of Scotland

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Thesis presented for the degree of
Doctor of Philosophy
University of Edinburgh
1986



Abstract

Biting midges (*Culicoides* spp. – Diptera: Ceratopogonidae) were studied by Loch Awe in Argyllshire, Scotland. In this holiday area, *Culicoides impunctatus*, the 'Highland midge', presents a real threat to tourism. Most of the conclusions relate to this species.

The diel flight periodicity of *Culicoides impunctatus* was found to be dependent on trapping technique and location of capture. Using suction-trap catches, typical crepuscular activity was observed in a forest. The 'rest phase' during the daytime was considered to be dictated by circadian rhythms. A similarly-positioned human bait, however, not only recorded dusk and dawn peaks, but also indicated that a potential blood-source could induce daytime attack activity in previously resting adults. By contrast, simultaneous flight-periodicity determinations by both a human bait and a suction trap in a nearby exposed field were similar in form to that observed using a suction trap in the forest. The less favourable environmental conditions and the paucity of resting sites in the field were considered to explain the discrepancy between the two human-bait catches.

An attempt was made to model the flight activity of *Culicoides impunctatus* using a 'least squares' regression analysis. The necessity to allow for the possible effect of circadian rhythms was emphasized. The model, in consequence, not only incorporated elements to describe (theoretically) the influence of both the more conspicuous weather factors and any change in the population density, but also analysed the relationship to 'time of day'; an indirect way of modelling rhythmically-determined activity. Furthermore, data taken from within the putative rest phase of the diel activity cycle were omitted. Although the results seemed plausible – for instance, flight activity was found to be negatively correlated with wind speed and strongly related to time of day – little confidence was placed in the parameter estimates. Regression techniques, it was concluded, are ill-suited to describe the complexities of flight activity. Entomologists were strongly urged to recognise the limitations of statistics and to interpret their data accordingly.

A time-dependent Poisson process was used to investigate whether midges (almost exclusively *Culicoides impunctatus* and *C. obsoletus* grp) attack a human in a random manner. The results indicated that the landing distribution was clumped with respect to time. The adaptive significance of this behaviour is difficult to explain.

The problem that midges pose to the tourist industry of the Highlands and Islands was considered at length in relation to insect control methods currently in use worldwide. Although the situation for the paying tourist could be improved, the actual elimination of the pest species, it was felt, would be neither economically sound nor environmentally desirable. Ultimately it was concluded that the severity of a particular highland area's midge problem is very subjective, strongly determined by sociological considerations.

Declaration

This thesis has been composed by myself, and the work described in it is my own unless explicitly acknowledged.

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Acknowledgments

To begin with, I would like to thank the Natural Environment Research Council (N.E.R.C.) who, in association with the Forestry Commission, provided the C.A.S.E. studentship for this work.

I was fortunate in having two excellent supervisors, Philip Ashmole and John Stoakley. During my time in Edinburgh their support and tolerance has been unwavering. I am profoundly grateful to them both. Although always willing to discuss my work, they gave me the freedom to pursue my own ideas and to learn for myself about scientific research, with its pleasures, demands and pitfalls. I have certainly learnt from, and have been *severely* humbled by, my mistakes. In addition, I would like to thank Duncan Campbell, Head of The Environment Branch of the Forestry Commission. His understanding of financial matters was very important to me.

I am extremely thankful to Chris Theobald, David Williams and Margaret Shotter of Edinburgh University's Statistics Department. Not only did they answer the frequent salvos of questions that I fired at them (putting up with my wild statisticulations), but they also entertained the possibility – which is admittedly remote – that a zoologist might actually find statistics interesting.

I am pleased and proud to number amongst my friends, John Linley and John Boorman: ceratopogonists of repute, but more importantly fine people. Both have cast an educated eye over this work at various times; their advice is invaluable to any apprentice entomologist. Thank you gentlemen. Thanks also to the people who have been cajoled into reading chapters of this offering: Joss Bartlett, Tony Collins, Arthur Ewing, George Hendry, Jules Johnson, Simon Ryder and David Saunders. The work has benefited immensely from their comments. I remain, however, fully responsible for its failings – venial or otherwise.

My thanks are extended to the adults of Dalavich, Argyllshire, for tolerating a Sassenach in their midst; to their children for not vandalising my equipment; and to the holiday-makers for treating me as a benign English eccentric, indeed a novelty; and for offering me tea and other forms of sustenance during the all-night vigils. With reference to the eight 24-hour catches performed in this work, I have to thank Ken Thomson. In addition to enduring partial exsanguination for 'the cause', he learnt to cope with and help me during my all-too-frequent bouts of manic 'hectorberliosis'. As a field partner he was second to none. Equally important to me during my field seasons, people who I am now pleased to have as friends, were Bill Dye, the Rees family and Rob and Audrey Lightfoot. Bill in particular, from day one of the field study, gave me unending help and friendship. This will *always* be remembered.

Next, to my friends in Zoology who were spared the torment of reading this work: Viv Brown, Nigel Clark, Andy Evans, Kevin Fowler, Heather Gates, Geoff Harper, Joanne Innes, Paul Ward and Pat and Philip Whitfield. The strong sense of camaraderie that my fellow students created made the Zoology Department an enjoyable as well as an academically stimulating place to work.

My non-zoological friends were no less special to me. Of all the people, many of them in the Humanities, who have shaped my development during my time at this university, Mark Drummond, Annie Findlay, Bruce Grant, Norman Ingram, Timothy Killingback, Mark Kingwell, Ferencz Liszt, Nancy Lorimer, Marina Marrelli, (the mellifluous, the avuncular, the euphonic) Charlie McNair & his jazzband, Terry Patten, Philip Siaguru, Robert 'Isaac' Stern, Sophie Thornton and Christian Weber were particularly close and deserve special mention. Not only did they culture an alarming number of dormant elements in my character, but each, in his or her own special way, made my 'Edinburgh years' the happiest and most fulfilling of my life so far.

Finally, I would like to thank both my parents, John and Enid Marsh, and my Grandfather, Percy Vernon Wiltshire. I lack the vocabulary to express fully my appreciation for the support and understanding that they have given me throughout the ridiculous amount of time that this study has taken. Herein lies my only non-entomological regret: not that I strayed outside the period considered by the Government to be reasonable for such a study (though I'm certainly not proud of it), but that my dear Grandmother, Ellen Mary Wiltshire, didn't live to see its completion.

General introduction

1.1. Conception of the study

Culicoides impunctatus Goetghebuer (1920, Diptera: Ceratopogonidae), dubbed the "Highland midge" by Cameron (1947), is inimical to many people's enjoyment of a holiday in the Highlands and Islands of Scotland. Indeed, it is reckoned that this species of biting midge presents a major problem to the tourist industry during the summer months. The loss of revenue has stimulated many studies aimed at finding suitable control methods (Cameron, 1946, 1947, 1948; Cameron *et al.*, 1946; Kettle, 1949, 1950, 1951a, 1951b, 1955, 1956, 1957, 1960, 1961; Kettle & Lawson, 1952; Kettle & Parish, 1957; Kettle *et al.*, 1956, 1959; MacGregor *et al.*, 1948; Reuben, 1959, 1963; see Chapter 6). In spite, however, of the considerable input – in terms of both finance and expertise – the 'midge problem' remains as acute as ever.

The Forestry Commission owns and runs a number of holiday complexes in the western Highlands. Naturally concerned about the loss of income from tourism due to midges, the Commission sought advice on their control by means of a C.A.S.E. Studentship (in association with the Natural Environment Research Council). The proposed study was, however, not restricted to control *per se*. It was recognised that to control a pest its ecology must be fully understood: traditional ecological studies therefore comprise a considerable proportion of this thesis.

1.2. The genus *Culicoides*: a brief introduction

Of the 60 genera that comprise the family Ceratopogonidae (Order Diptera: Sub-order Nematocera), only four (*Culicoides*, *Austroconops*, *Leptoconops* & *Forcipomyia*) feed on warm-blooded animals (Kettle, 1984). (The females take blood; Ceratopogonid males feed solely on nectar. The remaining Ceratopogonid females either predate other insects or parasitise them, or they may feed on plant sap (Edwards *et al.*, 1939; Kettle, 1984).) The genus *Culicoides*, the largest of these (almost 1000 species, approximately 25% of the family's total), has a worldwide distribution (Hill, 1947; Kettle, 1984; Service, 1980); being found from "the tropics to the tundra, and from sea level to 4,200 m in Tibet" (Kettle, 1984).

Haematophagous *Culicoides* spp. are referred to by various popular names: 'midges' in Scotland; 'punkies' and 'no-see-ums' in most of the United States and Canada; 'sandflies' in the Atlantic and Gulf coasts of the United States and in the West Indies; 'moose-flies' in Alaska; 'jejenes' in Latin America ('maruins' in Brazil) and 'no-nos' in Polynesia (Blanton & Wirth, 1979; Edwards *et al.*, 1939; Hill, 1947). In entomological circles the name 'biting midge' is usually preferred – at least for initial clarification – thereby distinguishing them from other small (wing length usually < 2 mm) flies commonly also called midges which, however, don't feed on blood (e.g. the Chironomidae or dancing midges).

In Scotland, as in many areas, *Culicoides* spp. are simply a pest (Chapter 6); vexatious to humans and their livestock but posing no significant threat to health. (In the Highlands, their biting activities may reduce milk yields (Cameron, 1946).) At lower latitudes, however, certain species are vectors of both human and animal pathogens.

Mansonella perstans (Manson) (formerly *Dipetalonema* (*Acanthocheilonema*) *perstans*), a non-periodic filarial parasite, is transmitted in

Africa by *Culicoides milnei* Austen, *C. grahamii* Austen and *C. ornatipennis* Carter, Ingram & Macfie (Blanton & Wirth, 1979; Hill, 1947; Kettle, 1984; Service, 1980). This nematode was introduced into the Americas (e.g. Mexico, Colombia, Guyana, French Guiana & Surinam) and the Caribbean with the slave trade. The New World vectors have yet to be identified. Previously regarded as non-pathogenic to humans, *Mansonella perstans* is now known to cause eosinophilia, pruritis, transient swelling, liver and joint pain, fever and fatigue (Linley *et al.*, 1983). There is, however, a considerable individual variation in response to infection.

Culicoides grahamii and *C. milnei* are also vectors of the mild skin pathogen, *Mansonella streptocerca* (Macfie & Corson) (previously *Dipetalonema (Acanthocheilonema) streptocerca*), in the rain forest zones of West and Central Africa (e.g. Zaire, Cameroun & Ghana). *Mansonella ozzardi* Manson, a filariid of the Caribbean, Central and South America, can be transmitted by *Culicoides* vectors; for example, *C. furens* (Poey), *C. phlebotomus* (Williston), *C. pifanoi* Ortiz and *C. paraensis* (Goeldi). Symptoms include severe articular pain and varying degrees of eosinophilia. Filarial counts are typically higher in males and tend also to increase with age (Linley *et al.*, 1983).

Oropouche, a Simbu group virus (family Bunyaviridae) which is strongly pathogenic to man, has been found in the Amazon basin of Brazil, and in Trinidad, Haiti and Colombia (Blanton & Wirth, 1979; Kettle, 1984; Linley *et al.*, 1983). An infection with Oropouche may lead to acute headache, myalgia, arthralgia, dizziness and photophobia; with meningitis being a potential complication. *Culicoides paraensis*, a species which breeds profusely in peridomestic accumulations of cacao pods and rotting banana stems, is suspected of being the principal vector.

Other viral diseases of man and/or animals of which *Culicoides* species are potential vectors include: Rift Valley Fever, Congo and Dugbe viruses,

various encephalitides (e.g. eastern equine encephalitis & Venezuelan equine encephalitis), bovine ephemeral fever, epizootic haemorrhagic disease of deer, African horse sickness virus and bluetongue virus (Blanton & Wirth, 1979; Kettle, 1984; Linley *et al*, 1983; Onyiah, 1971; Wild, 1983). Bluetongue virus (B.T.V., family Reoviridae), present in all the world's zoogeographic areas, is pathogenic principally to sheep; symptoms in cattle tend to be milder or non-existent. In sheep an infection with B.T.V. may lead to foot lesions, enteritis, tongue ulcerations, abortion and a reduction in both the quality and quantity of the fleece (Kettle, 1984). Midges belonging to the subgenus *Avaritia* are considered to be major agents of transmission.

Ceratopogonid midges are also vectors – of greater or lesser significance – of pathogenic protozoa. As an illustration, *Leucocytozoon caulleryi* Mathis & Leger parasitises chickens in South-east Asia and Africa, causing lethargy, loss of appetite, diarrhoea, convulsions and anaemia. The principal transmitter of this protozoan is *Culicoides arakawae* (Arakawa). Similarly, *Culicoides adersi* Ingram & Macfie has been implicated as a vector of *Hepaticystis kochi* (Laveran), a plasmodium-like parasite of *Cercopithecus* monkeys in East Africa (Blanton & Wirth, 1979; Kettle, 1984).

1.3. Life history of *Culicoides impunctatus*

1.3.1. Egg stage

Working near Liverpool, Hill (1947) found (indirectly using larval studies) that *Culicoides impunctatus* generally laid its eggs in an area where the following plant species occurred: *Hydrocotyle vulgaris* L., *Rhododendron ponticum* L., *Juncus articulatus* L., *Carex goodenowii* Gay., *Pteridium aquilinum* L., *Polytrichum commune* L. and *Gymnocola inflata* Dum. In general, she

concluded that this species favours a "peaty soil with an acid reaction". Kettle (1961) performed a similar study in Scotland; finding high concentrations of larvae in typical acid bogland, where mosses, such as *Sphagnum* spp. and *Polytrichum* spp., grew in profusion.

The oviposition process under laboratory conditions was described by Hill (1947): "The eggs would emerge like a string of sausages and were usually laid asymmetrically, though sometimes in echelon and sometimes in a double row, the eggs of one row alternating with those of the other, like turned-in footprints". The eggs of this species are typically cigar shaped, approximately 490 μ in length and a dark greyish-brown. The chorion bears ansulae - sucker-like structures (Hill, 1947) - which may act as a plastron.

Clutch size varies according to the reproductive status of the female. Boorman and Goddard (1970) recorded a mean clutch size of 43.5 ± 10.3 (range 18-62) for parous midges that had been blood-fed in the laboratory; for non-blood-fed nullipars (see below) collected from tree trunks, a mean egg number of 79.6 ± 14.0 (range 56-114) was obtained. Hill (1947), working with parous individuals, recorded a mean clutch size of 50 (approx., range 30-65). These eggs hatched after seven to 20 days depending on the temperature (16-19°C). Under mean field temperatures of approximately 15°C, hatching was recorded by Service (1969c) to occur 7-9 days after oviposition.

1.3.2. Larval stage

Ceratopogonid larvae are apodous and vermiform. 11 segments comprise the body, and the head capsule is heavily sclerotised. The larvae possess two pairs of retractile anal papillae. It is unlikely that these have a respiratory function (midge larvae are supposed to respire cutaneously); more probably they serve to absorb salts from the surrounding medium (Kettle, 1984).

Little is known about larval ecology. Hill (1947) found over 90% of all larvae (excluding the first instars, < 1 mm in length) within one inch of the surface. This finding was corroborated by Kettle and Lawson (1952). In their study, 91%, 85% and 74% respectively of second, third and fourth stage larvae occurred at a depth of up to an inch. The larval stages of *Culicoides impunctatus* – in common with the larvae of most other Ceratopogonids (Kettle, 1984) – are probably opportunistic scavengers. Hill (1947) suspected that they may on occasions show cannibalism.

Culicoides impunctatus undergoes four larval stages; overwintering as a fourth instar (mean length 5 mm, Hill, 1947; Kettle, 1984). Prior to pupation, the fourth stage larva develops copious fat-bodies in the abdomen.

1.3.3. Pupal stage

The pupal stage of *Culicoides impunctatus* has been little studied. The pupae (length approx. 2.1 mm, Hill, 1947) are “culicid in appearance” (Kettle, 1984), bearing two respiratory trumpets (propneustic) on the cephalothorax. Caudal spines aid in locomotion. Hill (1947) found that pupation usually occurred in the drier margins of the larval habitat offered in the laboratory. The pupal stage in her study lasted for about five days; Kettle (1950), however, records its duration as from one to two weeks.

1.3.4. Adult stage

Boorman and Goddard (1970), working in southern England, collected seven *Culicoides impunctatus* (Group 1, see below) from tree trunks (*Quercus* L., *Alnus* Miller & *Betula* L.) at the very beginning of its flight season (16.5.69). All these midges had ovaries in early Stage II of development (see Linley,

1965a); none had spermatozoa in their spermathecae; and their fat-bodies - "two large refractile masses of white tissue lying alongside the gut" - were fully replete. (Stage II is the 'resting stage' in ovarian development (Linley, 1965a).) By comparison, resting midges (Group 2) taken after the 16th of May (up until the 27th of June) showed various states of ovarian development: Stage I 31%; Stage II 30%; Stage III 13%; Stage IV 10% and Stage V 15%. They observed that "The fat-bodies of all midges at stages IV and V were much depleted: all other midges except ... [10 in Stage I] had large full fat-bodies"; and further that "None of the midges dissected contained visible traces of vertebrate blood in either the mid-gut or hind-gut". (Resting *Culicoides impunctatus* taken after May were found to have been inseminated.) The third group analysed was composed of individuals taken by a human bait. All of 39 host-seeking *Culicoides impunctatus* had ovaries in either Stage I (69%) or Stage IIA (31%) of development. The fat bodies of these insects were fully depleted; 'blood hungry' individuals were invariably inseminated.

Although this is in part surmise, Boorman and Goddard's results may be explainable as follows: after emerging, females of *Culicoides impunctatus* rest on the surrounding vegetation. These teneral midges are likely to comprise Group 1 (see above). Upon achieving the necessary physiological state, they probably enter nearby mating swarms (Downes, 1950) composed almost entirely of males. (*Culicoides impunctatus* is protandrous (Boorman & Goddard, 1970; Hill, 1947; Kettle, 1984; pers. obs.).) The inseminated nullipars then seek local, sheltered and moist resting sites, where the first batch of eggs are developed using the fat-bodies as the requisite source of protein and fat, etc. With the exception of the 10 insects with depleted fat-bodies whose ovaries were in Stage I, Group 2 was probably composed of such individuals. Only after the first oviposition, do the females, all with spent fat-bodies, typically engage in crepuscular/nocturnal host-seeking forays into surrounding areas

(Group 3 females). During the daytime, blood-hungry midges rest in the shelter of vegetation. It is likely that the remainder of Group 2 (see above) was composed of these parous individuals.

Culicoides impunctatus is thus an autogenous species, able to develop the first batch of eggs without a blood meal using the fat-bodies acquired as a larva (Boorman & Goddard, 1970). Autogeny explains the existence of large midge populations in the Highlands and Islands where, one must presume, warm-blooded hosts are few and far between (Boorman & Goddard, 1970). (In this study, only a minute percentage of the *Culicoides impunctatus* caught in the suction traps had blood in the abdomen.) Moreover, the fact that nulliparous individuals are not compelled to find a blood meal and, therefore, probably mature and lay their eggs very close to the site from which they emerged, gives a partial explanation for the composition of the suction-trap catches taken at Dalavich. The aerial population of *Culicoides impunctatus* was composed almost entirely (> 99.5%) of parous – presumably host-seeking – females (cf. Reuben, 1959, 1963). The lack of males in these catches probably indicates that mating is restricted to the immediate vicinity of the breeding areas.

(In my study, 35 suction-trap caught females (Group 3, see above) were dissected in insect saline under a stereomicroscope. All had heavily-depleted fat-bodies and possessed the characteristic burgundy abdominal pigmentation which signifies parity in many *Culicoides* spp. (Boorman & Goddard, 1970; Dyce, 1969). Of the 35 midges, 32 were considered to have ovaries in Stage I of development (eight at Stage N & 24 at Mer's Stage I); the remainder were assigned to Stage II (two at early Stage IIA & one at late Stage IIA). Follicular relics (see Linley, 1965b), another indicator of parity, were occasionally identified with certainty. Considerable difficulty was experienced in dissecting out these dilatations: they were very delicate and many undoubtedly broke

during the dissection procedure. All the *Culicoides impunctatus* examined had spermatozoa in their spermathecae.)

Little is known about the resting sites selected by British *Culicoides* spp. (Service, 1973). As noted above, Boorman and Goddard (1970) found (mostly nulliparous) *Culicoides impunctatus* resting on tree trunks in a copse. Females, unlike males, hid in "small cracks and crevices" in the bark. Few midges were observed less than three feet from the ground; the majority resting between five and nine feet. By inference from daytime human-bait catches (refer to Chapters 3 & 5), my own observations indicate that *Culicoides impunctatus* will rest - often in considerable numbers - wherever the vegetation affords moist, sheltered resting sites. Areas with a thick carpet of moss shaded by conifers seemed to be particularly favoured.

In Scotland, adult *Culicoides impunctatus* first start emerging around the beginning of May (Kettle, 1950; Reuben, 1959, 1963; pers. obs.). Although at lower latitudes in Britain the seasonal distribution seems (in general) to be unimodal (Hill, 1947; Onyiah, 1971; Service, 1969a), two peaks of adult activity during the summer are common in Scotland (Kettle, 1950; Reuben, 1959, 1963; pers. obs.). This bimodality does not indicate that *Culicoides impunctatus* is bivoltine (Kettle, 1950). (Kettle discounted this possibility by monitoring the larval population from February to August. No rapidly-growing larvae were found after the first adult peak.) Instead, a bimodal seasonal distribution suggested to Kettle that, two biological - and, in his work, morphologically-inseparable - races of this species may occur. To explain Hill's (1947) results, he cautiously offered the suggestion that a single (third) race may exist at Liverpool. Adult populations of *Culicoides impunctatus* usually die out around the end of September, coinciding with the first frosts.

1.4. Aspects of *Culicoides* ecology and control investigated

More exhaustive introductions to the different topics covered in this thesis are reserved for the relevant chapters. This section serves merely as a brief overview of the work; primarily a guide to its structure.

An initial perusal of the published literature indicated that although the diel flight periodicity of British *Culicoides* spp. has been studied in fair detail (Hill, 1947; Kettle, 1957; Lewis & Taylor, 1964; Onyiah, 1971; Parker, 1949; Reuben, 1959, 1963; Service, 1969b, 1971b), the specific question of the relationship between flight activity pattern and both microclimate and method of capture has not been fully addressed. Chapter 3 attempts to answer this (at least in part); describing a study in which the diel flight activity of (principally) *Culicoides impunctatus* was monitored simultaneously in a field and in a forest, using both a suction trap and a human bait in each habitat. Of particular personal interest was the role of circadian rhythms (Saunders, 1977, 1982) in governing any field periodicity; though it was understood at the outset that the results could never offer incontrovertible evidence for the existence of one (or two) circadian oscillator(s).

In Chapter 4, an attempt is made to 'model' the flight activity of *Culicoides impunctatus* using a multiple linear regression analysis. The field of chronobiology, being relatively new (David Saunders, pers. comm.), seems in general not to have impinged fully on work of a similar nature. It was felt that a causal link between insect-catch size and microclimate can only be postulated – and then only very tentatively – when activity determined by circadian rhythms has been allowed for. Otherwise, a correlation – even though statistically robust – may be merely a reflection of the natural co-variation of both flight intensity and weather variable with time (see Section 4.1). Naturally, I concede that it will be impossible to separate accurately the

exogenous components (i.e. those related to weather) of flight activity from that dictated by circadian rhythms. Nevertheless, assuming – implicitly through one's analysis – that flight activity is purely exogenously determined will invariably lead to correlations with little or no causal meaning. The model developed in this chapter attempts to avoid this problem of "nonsense correlations" (Kendall & Stuart, 1967).

The short-term temporal attack patterns of *Culicoides impunctatus* and *C. obsoletus* grp are considered in Chapter 5. This study was prompted by the preliminary field observation that *Culicoides impunctatus* appears to land on a human bait in a clumped (i.e. a non-random) manner with respect to time. A time-dependent Poisson process (a distant relation of the standard Poisson distribution) was used to test the veracity of this observation.

Chapter 6, an essay on 'Midges and the tourist industry of the Highlands and Islands', discusses this complex subject in detail. (This work will be written up from a more practical angle for the Forestry Commission to fulfill the obligation of a C.A.S.E. student.) The chapter considers the likely efficacy of a broad range of insect control (i.e. elimination) techniques. Methods of reducing man-midge contact are then discussed; and, in association with this, some practical work on the reduction of midge activity in limited areas is described. This offered possibilities for future research of value.

Chapter 2

Materials and methods

2.1. The study area

The fieldwork for this thesis was conducted in and around the village of Dalavich, situated on the western shore of Loch Awe in Argyllshire (Ordnance Survey NS 969127, Figures 2-1 & 2-2). Until recently, Dalavich was almost exclusively a logging community serving the Inverliever forest, an area of some 13,000 hectares afforested after 1908 by His Majesty's Office of Woods. (This government department later became the Forestry Commission.) In 1978, however, Dalavich was chosen – owing to its facilities, scenic beauty and recreational potential (Duncan Campbell, pers. comm.) – as the site for a proposed Forestry Commission holiday complex. 40 chalets comprise this development which was first opened in May 1981 to offer self-catering holidays. In addition to a diverse and interesting biota, the area boasts many miles of thoughtfully-planned forest walks; making it a prime holiday destination for naturalists and walkers alike.

The breeding sites of *Culicoides impunctatus* in the vicinity of Dalavich were not mapped. Casual observation indicated, however, that suitable areas of *Sphagnum* bog (Section 1.3.1) were both local and extensive; many having been planted with commercial softwoods, such as Norway spruce (*Picea abies* (L.) Karsten) and Sitka spruce (*P. sitchensis* (Bong.) Carriere).



Figure 2-1. The village of Dalavich (far right) by Loch Awe, Argyllshire

Figure 2-2.

Map of the study area

(Scale 1:13,500 approx.)

Reproduced with the kind permission of
Ordnance Survey

Key: A = Field Site (Chapter 3)

B = Forest Site (Chapter 3)

C = Human-bait catches (Chapter 5)



2.2.2. Air temperature

Air temperature was obtained from the 'dry-bulb' thermistor of a miniature aspirated psychrometer unit (Delta-T Devices Ltd, psychrometer unit Type WVU). The thermistor was linked via a linearising bridge circuit to the input of the logging device; the input amplification was set to accommodate readings from 0–30°C.

2.2.3. Relative humidity

The unit used to determine air temperature also measures the air's 'wet-bulb temperature' using another thermistor, covered by a wick wetted with distilled water. From the 'wet-bulb depression' (i.e. dry-bulb temperature – wet-bulb temperature) values of relative humidity were calculated (Monteith, 1980).

2.2.4. Wind: speed and direction

Owing to a midge's low flight speed (approx. 60 cm s⁻¹, pers. obs.), an anemometer with a low starting threshold had to be used. Initially three thermistor-based thermoanemometers were built (Unwin, 1980). Their output was, however, found to be partly dependent on ambient temperature and they were consequently discarded. On reflection, it is doubtful whether they would have been sufficiently robust to survive the rigours of fieldwork. Moreover, under field conditions, water may have condensed on the thermistors leading to artificially-high wind speed readings (Reuben, 1959).

Porton A100E (Vector Instruments) rotating-cup anemometers were finally selected. When fitted with a Type R60 staggered six-cup polystyrene rotor, the

transducer has a threshold response of 0.15 m s^{-1} and a low distance constant (one metre $\pm$ 5%). (The distance constant is a measure of the run of wind required to produce a 63% transducer response to a step-change in wind speed (Woodward & Sheehy, 1983).) Considering the relative infrequency of recording (once per minute), a transducer with a higher distance constant – less responsive to eddies and gusts – would have been preferable. Starting thresholds and distance constants are, however, positively linked (Woodward & Sheehy, 1983); in the present study, sensitivity to the lower wind speeds was considered to be more important. Each anemometer was calibrated separately in a wind tunnel using a pitot static tube as a reference (Unwin, 1980).

Wind direction in the Field Site (consult Chapter 3) was determined using a Porton potentiometric weather cock-style wind vane (Vector Instruments, Type 200). This had a response threshold of 0.6 m s^{-1} .

2.2.5. Light

Light levels (330–700 nm, peak response 575 nm) were measured with Vactec® selenium photo-voltaic cells (Norbain Electro-optics Ltd, R75EB; John Grace, pers. comm.). Calibration in micro-einsteins $\text{m}^{-2} \text{ s}^{-1}$, over the full range of natural light intensities encountered in Scotland, was performed using a quantum sensor (Macam Photometrics Ltd, Macam Quantum Sensor® SD101/Q) as a standard.

Unwin (1980) noted the importance of measuring the same component of the electromagnetic spectrum as is perceptible to the organism under study. No one has studied the spectral sensitivity of any *Culicoides* sp. (Wild, 1983). In general, however, most insects – especially nocturnal and crepuscular species – show a strong ultra violet (U.V.) response; and a relative insensitivity to the deeper shades of red (Clements, 1963; Goldsmith & Bernard, 1974;

Romoser, 1973; Wigglesworth, 1972; Wild, 1983). The near U.V. is relatively well represented in the output of selenium cells. Other typically-used quantum sensors, such as silicon and cadmium sulphide, are proportionately more responsive to the 'red end' of the electromagnetic spectrum. Theoretically, therefore, the system should have measured (approximately) those wavelengths to which midges are sensitive.

The logging system available had only 240 input levels. In consequence, measuring the normal diel range of light intensities (zero to approximately 1400 micro-einsteins $\text{m}^{-2} \text{s}^{-1}$) proved problematical. The light-monitoring system usually indicated 'complete darkness' between 22.30 and 03.30 B.S.T. (refer to Figure 3-6): some light levels of undoubted biological significance were therefore not recorded. I concede that this is a major limitation to the study. The possibility of using two sensors – one for diurnal and one for nocturnal irradiance levels – was considered but was found to be unfeasible.

2.3. Suction traps

Suction traps are "the most efficient and least biased non-attractant traps available for sampling aerial populations of insects" (Service, 1976). They have been used widely in the study of *Culicoides* spp. (Koch & Axtell, 1979; Lewis & Taylor, 1964; Onyiah, 1971; Reuben, 1959, 1963; Service, 1971b, 1974; Walker, 1976, 1977; Wild, 1983).

The design of trap adopted for this work was first used in southern England to study first-instar larvae of the felted beech scale, *Cryptococcus fagisuga* Ldgr. (Wainhouse, 1980; Figures 2-4 & 2-5); although it was slightly modified to suit this particular application. An inverted Airmax® fan (A.K. Fans Ltd, Type PR-Y4393) drew air into the trap at approximately $300 \text{ m}^3 \text{ hour}^{-1}$. The fan motors (31 W) were powered by 120-ampere hour 12-volt batteries

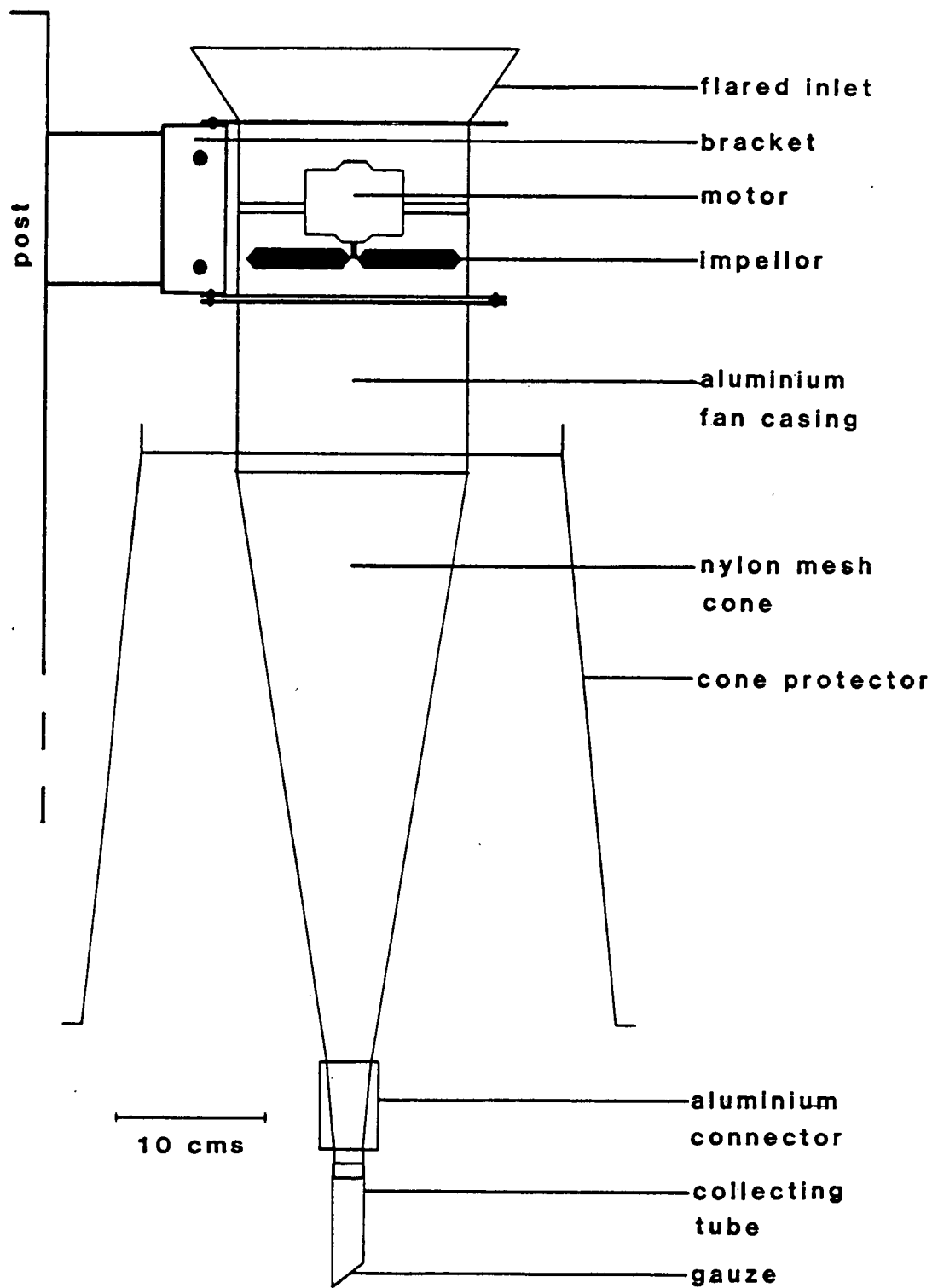


Figure 2-4. Cross-sectional view of a modified 'Wainhouse' (1980) suction trap



Figure 2-5. The suction trap in the Forest Site

(Lucas Batteries Ltd, Type 355 ATVP 17T8): a trap could be operated for 24 hours without a significant long-term fluctuation in its air intake. The traps were calibrated using the "concentric rings technique" (Johnson, 1950a); and were always run on the battery with which they had been calibrated. Collecting receptacles were changed manually.

To lessen the effect of cross-winds on sampling efficiency, the collecting cone (mesh size 300 μ ; Henry Simon Ltd, Special Products) was protected by a plastic skirt (Stewarts Plastics Ltd, 538 (Handy bin); Johnson & Taylor, 1955; Service, 1976, 1977; Southwood, 1978; Taylor, 1955, 1962). In addition, the log-efficiency equation devised by Taylor (1962) was used to compensate for the reduction in sampling rate caused by cross-winds of varying strengths passing over the fan intake.

Normally it is assumed that suction traps neither attract nor repel flying insects (Johnson, 1950b; Taylor, 1962). It is certainly possible, however, that in this study midges could have been attracted not only to the frequency – actual or harmonic – of the fan motor but also to its warmth (Service, 1976).

2.4. Human-bait catches

Human baits – the author (P.M.M.) and another Ph.D. student (Ken Thomson, R.C.K.T.) – were used to determine the flight periodicity of insects actively seeking a host (Chapter 3). Midges that landed on a target area (21 cm X 5 cm) drawn on the left forearm (Figure 2-6) were aspirated off. At night the target zone was illuminated using a red headlamp (Petzl, Frontale Zoom®, Service, 1976); the assumption being that these crepuscular/nocturnal Nematocera are blind to these wavelengths (refer to Section 2.2.5). Clothes, rubber gloves and a modified bee-keeping hat (Steele & Brodie Ltd, M14100 bee hat) with a veil of nylon gauze protected the rest of the body from attack.



Figure 2-6. The human bait (P.M.M.) catching in the Forest Site

Variation in individual attractiveness to anthropophilic Diptera is well known (Kettle, 1969a, 1969b; Kettle & Linley, 1967a, 1967b, 1968, 1969; Service, 1976, 1977). In addition, a single person's attractive capacity – which is species specific – may vary over comparatively short time periods (Service, 1976). This last – though of most import in the present study – cannot be compensated for. (To reduce the 'noise' in the data as far as possible, both catchers wore the same clothes for all the trials.)

The human baits were not 'standardised' (Kettle & Linley, 1967a, 1968, 1969; Service, 1976, 1977); for one's interest lies in the proportion of attack experienced during each half-hourly period of the 24 hours and not in the absolute number of midges caught. Indeed, little of concrete value could be gained from such data. In apparently homogeneous environments, populations of insects are known to vary spatially both in size and in species composition – a patchiness which itself varies with time (Kettle & Linley, 1967a, 1968, 1969; Service, 1971a, 1971b, 1976; Wild, 1983).

A single human bait (the author) was used to study the temporal landing distribution of *Culicoides* spp. on a host (Chapter 5). Numbered sticky labels replaced the 'pooter' as the method of capture.

2.5. Preserving and sorting

All catches were preserved for later identification in a mixture of formalin (5% approx.), saline (0.8% approx.) and detergent (0.2% approx., Birley & Boorman, 1982; John Boorman, pers. comm.). The detergent acts as both a wetting and cleansing agent (Walker & Boreham, 1976).

In addition to *Culicoides* spp., the suction trap caught a broad array of other aerial invertebrates; especially during the daytime. Before the catch could be counted and identified, it was spread out in a small dissecting tray

and the larger insects (e.g. moths and cyclorrhaphan flies) were removed with forceps.

2.6. Sub-sampling and identification

The human-bait catches were always counted in their entirety. This was also true for the midges taken by the suction trap in 1983; though it proved extremely laborious. (A 24-welled tissue-culture tray (Costar®) enabled the catch to be divided into manageable units which facilitated counting.) After a certain amount of experimentation, therefore, a sub-sampling system was devised. Low catches were still fully counted. Larger catches (> 100 approx.), in the preserving medium, were spread evenly by eye between eight wells of the tissue-culture tray. If a randomly-selected well had fewer than 20 midges, then the insects in all eight wells were counted. The contents of four wells (all randomly chosen) were considered (and appropriately multiplied) if there were 21–40 midges; a count of above 40 insects in the first well meant that only 25% of the catch (i.e. two wells) was counted.

Species were identified using the key to dry-mounted specimens produced by Campbell and Pelham-Clinton (1960); together with the account of Edwards *et al.* (1939) and the kind assistance of John Boorman. The following page carries a list of species of midge actually identified. It is, however, conceded that this is (very probably) far from exhaustive for the area; as will become apparent, attention in this thesis was focussed on *Culicoides impunctatus* owing to its dominance. No attempt was made to separate females of the *Culicoides obsoletus* grp. The four species that comprise this group are separable only in the male (Birley & Boorman, 1982).

Species of *Culicoides* identified at Dalavich

Culicoides cubitalis Edwards

Culicoides delta Edwards

Culicoides grisescens Edwards

Culicoides heliophilus Edwards

Culicoides impunctatus

Culicoides nubeculosus (Meigen)

Culicoides obsoletus grp

(*Culicoides chiopterus* (Meigen))

(*Culicoides dewulfi* Goetghebuer)

(*Culicoides obsoletus* (Meigen))

(*Culicoides scoticus* Downes & Kettle)

Culicoides pallidicornis Kieffer

Culicoides pictipennis (Staeger)

Culicoides pulicaris (L.)

Culicoides punctatus (Meigen)

Chapter 3

Flight periodicity

in relation to microclimate and method of capture

3.1. Introduction

There is no dearth of published literature on the diel flight activity of biting midges. Flight periodicity in British *Culicoides* spp. has been studied previously using suction traps (Lewis & Taylor, 1964; Onyiah, 1971; Reuben, 1959, 1963; Service, 1971b), human-bait catches (Kettle, 1957; Onyiah, 1971; Service, 1969b), sweep-net catches (Kettle, 1957; Onyiah, 1971; Parker, 1949), and traps which utilise the attractive capacity of a black cloth (Hill, 1947). The observed periodicities – even for a single species taken by an identical trapping method – have proved to be extremely variable.

This chapter describes a comparison study in which the effect on flight periodicity of both trapping technique (suction trap and human bait) and habitat (field and forest) were examined. The results raised some important questions hitherto only rarely addressed in similar work. Why do flight activity patterns vary with trapping technique and location of capture? Why is crepuscularity fairly typical of the genus? Are the individuals caught during periods of low activity physiologically distinct from those taken at times of peak flight? The answers offered are on occasions necessarily speculative.

3.2. Control of flight periodicity

It is now widely accepted that field periodicities of (for example) flight are not determined purely by the prevailing weather conditions (i.e. exogenously). The overwhelming majority have proved to be "a 'mixture' of endogenous and exogenous components" (Saunders, 1982).

Circadian rhythms, reproductive and nutritional status, and previous experience comprise the endogenous components of activity (Corbet, 1966a); of these, rhythms usually have the greatest input into any overt field periodicity. Saunders (pers. comm.) defines circadian rhythms as, "Physiological oscillations that have free-running (unentrained) periods close to that of the solar day. Their periodicity is temperature compensated and entrainable to an exact 24-hour period by a variety of environmental cycles, principally light".

These genetically-determined biological chronometers provide a broad framework for diel activity (Aschoff, 1966; Brady, 1974; Brady & Crump, 1978; Cloudsley-Thompson, 1960; Corbet, 1960, 1966a, 1966b; Saunders, 1977, 1982; Taylor, 1969). This is modified to a greater or lesser extent by other endogenous and exogenous factors. Environmental conditions, in addition to having a *direct* effect on rhythmically-determined activity, also entrain the rhythm to the period of the solar day.

The relative importance of rhythmic, other endogenous (Section 3.7.3) and exogenous factors (Chapter 4) in governing field periodicity varies inter-specifically (Corbet, 1966a, 1966b; Saunders, 1977, 1982). Homoiotherms and poikilotherms vary in their dependence on 'pre-set' activity patterns (Brady & Crump, 1978); there is also a considerable variation with latitude (Corbet, 1966a, 1966b; Saunders, 1982). Troglobites (obligate cavernicoles, e.g. the beetle *Laemostenus navarricus*) are, in general, arrhythmic (Saunders, 1982).

Brady and Crump (1978) felt that under normal field conditions approximately 80% of the biting activity of *Glossina morsitans morsitans* Westw. is under endogenous control; around 20% is, in their estimation, due to the effects of temperature. (They did not differentiate between rhythms *sensu stricto* and other endogenous components, see Section 3.7.3.) Conversely, although most atypically, activity is thought to be under almost pure exogenous control in two species of British millipede (*Blaniulus guttulatus* (Bosc) & *Oxidus gracilis* (C.L. Koch), Cloudsley-Thompson, 1960), and in many high-latitude insects (Corbet, 1966a, 1966b). Corbet (1966b) believed that in insects living north of the arctic circle (e.g. *Aedes impiger* (Walker) & *Ae. nigripes* (Zetterstedt)), the only truly significant non-exogenous components of behaviour are likely to arise from, for example, 'feeding drives' and age effects (Section 3.7.3). At lower latitudes, however, endogenous rhythms seem to be ubiquitous and are "every bit as much a part of the organism as its morphological organisation" (Saunders, 1977, 1982).

Consider the black salt marsh mosquito, *Aedes taeniorhynchus* (Wied.). In a light:dark (LD) regime of 12:12 (i.e. 12 hours of light alternating with 12 hours of darkness), this mosquito shows the typical bimodal 'bigeminus' pattern (Aschoff, 1966); with peaks of flight activity occurring at both 'light-off' and 'light-on' (Nayar & Sauerman, 1971). This periodicity is seen both in groups of adults and in isolated individuals. The 'dusk' peak (duration approximately one hour) is the larger. Activity in the scotophase is usually at a fairly high level, though variable; in the photophase there is practically no activity. In constant darkness (DD), however, in the absence of perceptible (to humans) time-cues which could dictate the overt periodicity, this bimodal activity *persists* (though in a slightly modified form) and 'free-runs' revealing both its endogenous component and period (τ). (τ for *Aedes taeniorhynchus* = 23.5 hours.)

The persistence of an activity pattern similar in form to the normal field periodicity under supposedly constant conditions is certainly not peculiar to *Aedes taeniorhynchus* (Aschoff, 1966; Jones *et al.*, 1967; Saunders, 1977, 1982). (Nielsen and Nielsen (1962) and Tychsen and Fletcher (1971), however, note examples in which the activity periodicity observed under normal field conditions bears almost no relation to that displayed in constant darkness in the laboratory.) Indeed, referring to crepuscular species, Aschoff (1966), the 'Father' of the 'Rhythmic School', concluded that "the basic two-peak pattern of locomotor activity is a persistent property of the circadian oscillating system". Moreover, it seems that a 'resting' phase during the subjective day of such a periodicity is fairly universal (Aschoff, 1966; Jones *et al.*, 1967; Nayar & Sauerman, 1971; Saunders, 1977, 1982).

In view of these results, it would seem acceptable for dark-active species to divide the 24-hour period into two: the 'rest phase', those hours when the population is rhythmically 'programmed' to rest, and the 'flight phase'. Within the flight phase, the segment of the daily cycle when flight activity is initiated or permitted by rhythms, 'endogenous levels' of intensity would be expected to exist (Robert Lewis, pers. comm.; David Saunders, pers. comm.). These concepts, the flight phase, the rest phase and the endogenous levels of activity, should be borne in mind in subsequent discussions. It must be noted, however, that although this terminology is useful for explanatory and analytical purposes, the limits assigned to, or the values calculated for, these concepts are necessarily rough.

Diel changes in light levels (or sometimes temperature) act as 'zeitgebers' (Aschoff, 1966) which entrain the rhythm. Thus, the inbuilt oscillator adopts (by discrete adjustments of phase angle known as transients) the precise 24-hour periodicity of the entrainer and, concurrently, assumes a characteristic phase angle to it. The magnitude and direction of the phase-shift depends on the

time, with respect to the circadian oscillator, at which the zeitgeber acts. Such phase-response curves are species-specific; varying quantitatively but not qualitatively (Saunders, 1977, 1982).

The value of τ is of great ecological significance. If an organism 'chooses' to be active at (say) dusk, a value of τ greater than 24 hours will be selected for. The precise phase-delay (as dictated by the phase-response curve) necessary to bring the periodicity into synchrony with the zeitgeber is generated when light falls late in the subjective day. Conversely, dawn-active organisms require a τ of less than 24 hours.

The presence of a single oscillator governing a crepuscular flight periodicity cannot explain the apparent entrainment of the activity peaks to seasonal changes in daylength. For example, Lewis and Taylor (1964) found that the interval between the dusk and dawn activity maxima of crepuscular species became greater with increase in nightlength. Onyiah (1971) and Parker (1949) obtained similar results; Reuben (1959, 1963), however, found no such change. To explain these results, it may be necessary to postulate the existence of separate 'dawn' and 'dusk' oscillators, each of which will have a characteristic phase angle to the zeitgeber (Nayar & Sauerman, 1971; Robert Lewis, pers. comm.; David Saunders, pers. comm.). Alternatively, only one peak may be governed by an oscillator; the other maximum being an exogenous effect of rapid illumination changes (Saunders, 1982).

Incidentally, a double-oscillator system could explain why, in crepuscular species, the dusk peak almost invariably occurs at a lower light intensity than the dawn activity maximum (Robert Lewis, pers. comm.; David Saunders, pers. comm.). This asymmetry – possibly an adaptation to avoid the lower temperatures typically encountered at dawn – has been observed by, amongst others, Akey and Barnard (1983), Barnard and Jones (1980), Hill (1947), Onyiah (1971) and Service (1969b, 1971b). Corbet (1965), however, thinking in purely

exogenous terms, offered the explanation that a lag-phase may exist between the 'release' of an individual (by a critical light intensity) and the onset of the observed behaviour. This lag-phase was considered to be a period of physiological preparation and/or the time taken for a recently 'activated' insect to reach (say) a human bait.

Although light is usually the principal zeitgeber (the "master factor", Cloudsley-Thompson, 1960), temperature may also entrain the activity periodicity of some species. Nelson and Bellamy (1971), working in California on *Culicoides variipennis* (Coquillett), compared five "summer" evenings (22nd June - 25th August) with five "fall" evenings (12th October - 16th November). They found that, "peaks of flight activity began later in the summer, relative to sunset, than in the fall"; and concluded that, "phases of activity probably are entrained by temperature [which is markedly lower in the fall] ... as well as by light". Akey and Barnard (1983) and Barnard and Jones (1980), working on the same species in northeastern Colorado, obtained similar results.

A seasonal fluctuation in activity peak distribution - whether wholly or partly governed by light - is of importance to the attempt at 'modelling' flight activity (Chapter 4). Consequently, all the trials of this experiment were conducted between the 2nd of July and the 2nd of August to try and keep any 'zeitgeber noise' to a minimum.

Thus, in the planning stage of this experiment it was presumed that flight activity in midges has a rhythmic element. The results of this thesis cannot prove this premise to be correct; only studies under truly constant conditions (see above) could offer such incontrovertible evidence. Corbet (1966a) felt, however, that, "The pervasiveness of rhythmicity imposes obligations on behaviourists and physiologists to allow for it when experiments are being designed or interpreted". This belief was echoed by Nelson and Bellamy (1971): "It cannot be concluded from field observations that the periodic activity of *C.*

variipennis has an endogenous basis, but the possibility should be recognised". Certainly *Culicoides* spp. would be highly exceptional if their activity were to be purely exogenously determined (Philip Corbet, pers. comm.; David Saunders, pers. comm.).

The 'model', presented in Chapter 4, represents an attempt to disentangle any rhythmic component to flight activity from the effects of weather. Quite obviously though, in view of the complexity of the problem relative to the limitations of such a study, the results remain equivocal.

3.3. How are rhythms adaptive?

The adaptive advantage conferred by rhythms varies depending on an organism's environment and ecology. Saunders (1977, 1982) considers that rhythms are of survival value because they permit "temporal organisation". Thus activities (either physiological or behavioural) can be performed "at the right time of day" (Saunders, 1977); for example, when competition (either intra- or inter-specific) is reduced, when a food source is regularly available, when predators can be avoided, or when environmental conditions are most likely to be favourable. Rhythmicity can also ensure mating synchronisation and, between sibling species, prevent hybridisation (e.g. in *Dacus tryoni* (Frogg.) (Diptera: Tephritidae), Tychsen & Fletcher, 1971).

A predictable exogenous factor could ensure temporal organisation. What, therefore, are the selective advantages of rhythmicity? First, weather conditions are not always predictable. An organism with a significant degree of rhythmic control to its activity may still, however, be active in otherwise favourable conditions even though the time cue is occasionally absent (Corbet, 1966a; Saunders, 1977, 1982). Second, the possessor of an internal 'clock' will be able to anticipate (i.e. physiologically prepare for) its normal period of

activity. Indeed, a regularity of behavioural and physiological activities – independent of the vagaries of a rapidly changing environment – might be a prerequisite for longevity (Saunders, 1977, 1982).

Corbet (1966a, 1966b) felt that rhythms only offer an advantage to organisms living in temperate and tropical areas. He argues that, at higher latitudes, where the weather is generally more severe and has a less pronounced diel cycle, organisms become opportunistic, living a marginal and usually short-lived existence in comparatively simple, less-diverse ecosystems. Not only, therefore, in his view is the need for temporal niche-diversification less acute, but advantage can be gained if an organism – not under strict endogenous control – can capitalise on favourable conditions whenever they prevail.

3.4. Methods

3.4.1. Experimental sites

a) The Field Site

The Field Site (Figures 3-1 & 3-2), a wild flower meadow, was exposed to the full range of environmental conditions prevailing during the experiment. In this poor acid grassland the following plant species predominated: creeping soft-grass (*Holcus mollis* L.), Yorkshire fog (*H. lanatus* L.), creeping (red) fescue (*Festuca rubra rubra* L.), common bent (brown top) (*Agrostis tenuis* Sibth.), heath grass (*Sieglingia decumbens* Bernh.), white clover (*Trifolium repens* L.), red clover (*T. pratense* L.), meadow buttercup (*Ranunculus acris* L.), creeping buttercup (*R. repens* L.), ribwort (*Plantago lanceolata* L.), and sheep's-bit (*Jasione montana* L.). (All species were identified, with the help of Dr Geoff

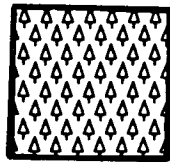
Figure 3-1.

Map of the experimental site

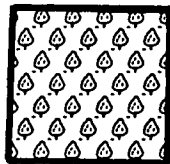
Key: H = human bait

 S = suction trap

 W = weather station



= softwoods (principally)



= hardwoods

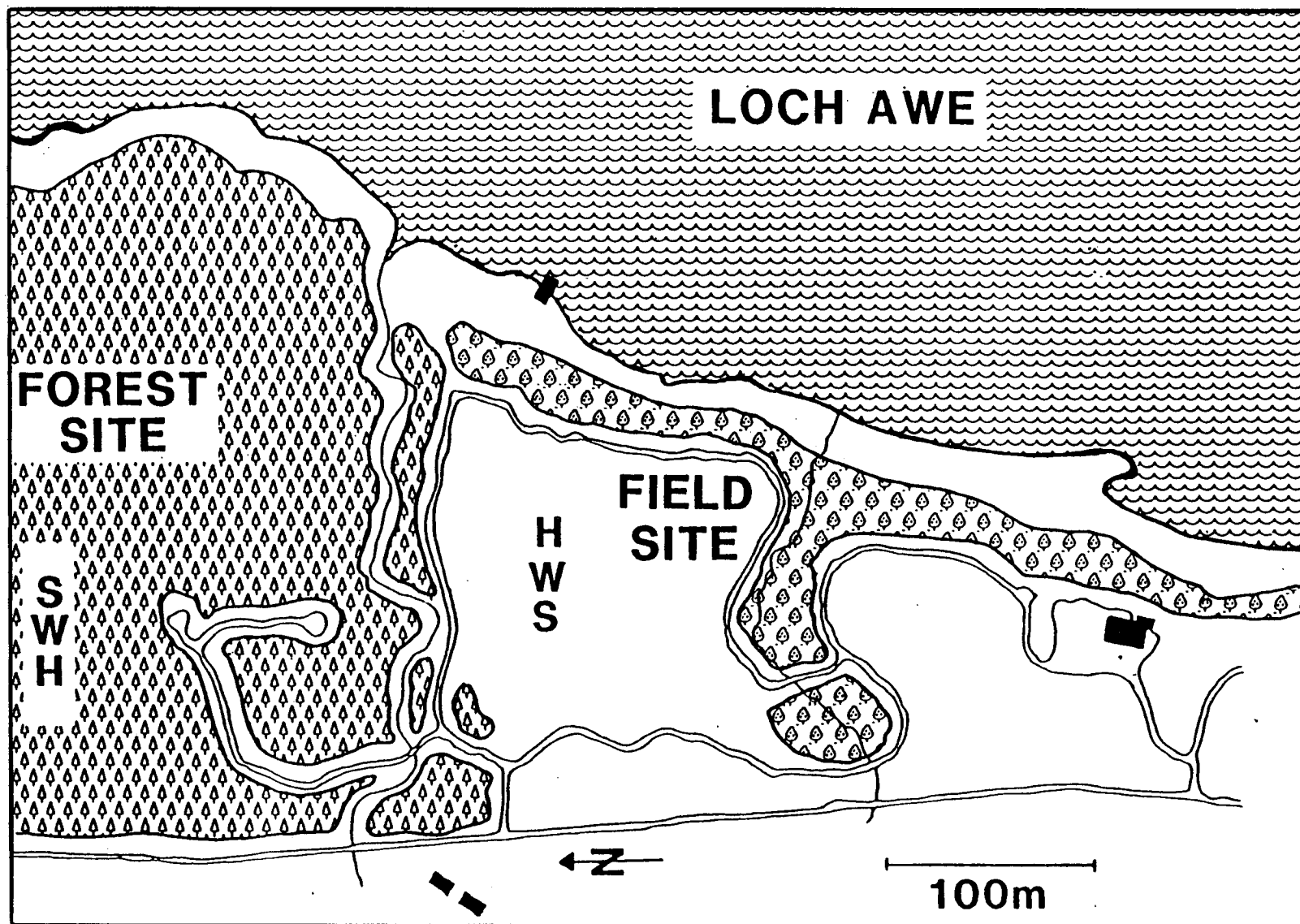




Figure 3-2. The Field Site, showing the human bait, the micrometeorological equipment and the suction trap.

Harper, using Clapham, Tutin and Warburg's *Excursion Flora of the British Isles* (1968).)

To reduce the effect of progressive trampling on wind speed at the experimental height, a rectangle of grass (approx. 50 m X 15 m), in which to perform the catching and environmental monitoring, was cut to a height of 10 cm or less. (Wind speed increases with the logarithm of height above the 'roughness length' of the meadow. Although this is related to the shape and density of the vegetation, it is largely dependent on its height. The roughness length is defined as, "the height at which the neutral wind profile extrapolates to zero wind speed" (Oke, 1978).) Only a relatively small area was cut, however, for this sward was home to the greater butterfly orchid (*Platanthera chlorantha* (Cust.) Reichenb.) and other plants of increasing rarity. I chose to lessen the study's environmental impact.

b) The Forest Site

The Forest Site (Figure 3-1) offered a more heterogeneous microclimate than the Field Site. The canopy was composed mainly of Norway spruce, Sitka spruce and Scots pine (*Pinus sylvestris* L.). An occasional mature oak (*Quercus robur* L.), together with a few mountain ash (*Sorbus aucuparia* L.), hairy birch (*Betula pubescens* Ehrh.) and spruce saplings, also grew in the experimental area. Isolated bushes (*Rhododendron* L. sp.) comprised the shrub layer. The herb layer was dominated by bracken (*Pteridium aquilinum*) in unevenly distributed clumps, frequent tussocks of purple moor-grass (*Molinia caerulea* (L.) Moench) and mosses (e.g. *Polytrichum* & *Sphagnum* spp.) in abundance.

3.4.2. Experimental design

24-hour flight patterns were monitored simultaneously in the Field and Forest Sites using both a suction trap and a human bait in each habitat. The experiment started at 10.00 B.S.T. The suction traps were first changed at 10.30 and then at subsequent half-hourly intervals. Immediately after attending to the suction trap in his catching site (i.e. either turning it on or changing it), each human bait caught the midges alighting on his left forearm for five minutes (Section 2.4). P.M.M. worked in the forest. (Concurrent with this experiment, R.C.K.T. studied the diel flight activity of *Haematopodus pluvialis* (Diptera: Tabanidae) in the Field Site using attractive-panel traps.) The catches were then labelled and stored. In both sites environmental conditions (air temperature, relative humidity, wind speed and light) were automatically recorded every minute and later averaged to give mean half-hourly values which corresponded to the insect catches (refer to Section 2.2). Wind direction was only recorded in the Field Site.

The suction traps ran continuously for the duration of each trial and thus could be expected to give a reasonable account of flight periodicity. By contrast, the five-minute human-bait catches are unlikely to be fully representative of the mean activity for the half-hourly periods. In view of this, it would have been preferable to perform them in the middle and not at the beginning of each half-hourly interval. The duration and timing of the human-bait catches were necessitated by the length of the trials.

All the transducers, the arm of the host and the inlet of the suction trap were positioned at approximately one metre from the ground to avoid 'noise' associated with variation in activity and microclimate with respect to height (Monteith, 1980; Service, 1973; Woodward & Sheehy, 1983). Moreover, to prevent the human bait modifying the flight periodicity monitored by the

suction trap, the human bait and the suction trap in each site were separated by approximately 40 metres (see Chapter 5).

Eight trials of the '24-hour experiment' were performed: four in 1983 (July 11th–12th, 17th–18th, 24th–25th, and 27th–28th) and four in 1984 (July 2nd–3rd, 17th–18th, 25th–26th, and August 1st–2nd).

3.5. Expressing flight periodicity: measures of central tendency

Williams (1937) discussed the merits of using a geometric (logarithmic) as opposed to an arithmetic mean to express central tendency. The 'Williams' mean' (see Haddow, 1960), a geometric mean that accommodates the presence of zeros in the data, has been widely used and is now accepted by many as the 'correct' method of averaging replicate flight periodicities (Barnard & Jones, 1980; Haddow, 1954, 1960; Kettle, 1969a; Kettle & Linley, 1967a, 1967b; Nathan, 1981; Service, 1969b; Williams, 1937). The Williams' mean is sometimes expressed as a percentage which can permit the comparison of flight patterns taken using different trapping methods or from areas with different population densities (Corbet & Smith, 1974; Haddow, 1954).

The number of adult insects in a specific locality can, however, fluctuate rapidly and with large amplitude. A pure geometric mean will not compensate for this and the result will be weighted in favour of the larger catches. Certainly in the present study the change in actual population density between the trials is likely to be significant – especially as this experiment spanned two field seasons.

Adopting an arithmetic mean based on the percentage of the total catch of each trial obtained in each time period obviates the problem of a population change (Blau & Stinner, 1983; Brady & Crump, 1978; Service, 1971b). When a trial's catch is low, however, even catching a single insect is likely to have a

significant but unwarranted effect on the mean (Haddow, 1954). Catches taken on days unfavourable for flight will thus be weighted for. (Subjecting the percentages to the angular transformation (Sokal & Rohlf, 1969) would reduce the effect of an atypically large 'catch' on the overall mean.) The use or rejection of a measure based on percentages ultimately depends on whether the trials – however severely affected by adverse weather conditions – are considered to be equally important in the final flight periodicity representation. There seems to be no perfect solution. Flight periodicities presented in this work were calculated using a Williams' mean expressed as a percentage of the total diel 'activity'.

3.6. Results

3.6.1. Environmental conditions

The eight trials encompassed a broad range of environmental conditions. This variability was necessary for the meaningful execution of the multiple linear regression analysis (Chapter 4). The accuracy of weather forecasting by the Glasgow Meteorological Office made it possible to 'choose' the conditions under which to perform a trial. Unfortunately, however, the recording system would malfunction if contacted by free water. This meant that the data logger – though enclosed and waterproof whilst running – could only be set up in dry conditions. Consequently, trials could be carried out on periods predicted to finish with dull, rainy conditions, but not on periods starting in this way. Not only did this have a depressing effect on the dawn activity peak, but also could have meant that flight continued later into the morning than would be expected on average (see Parker, 1949).

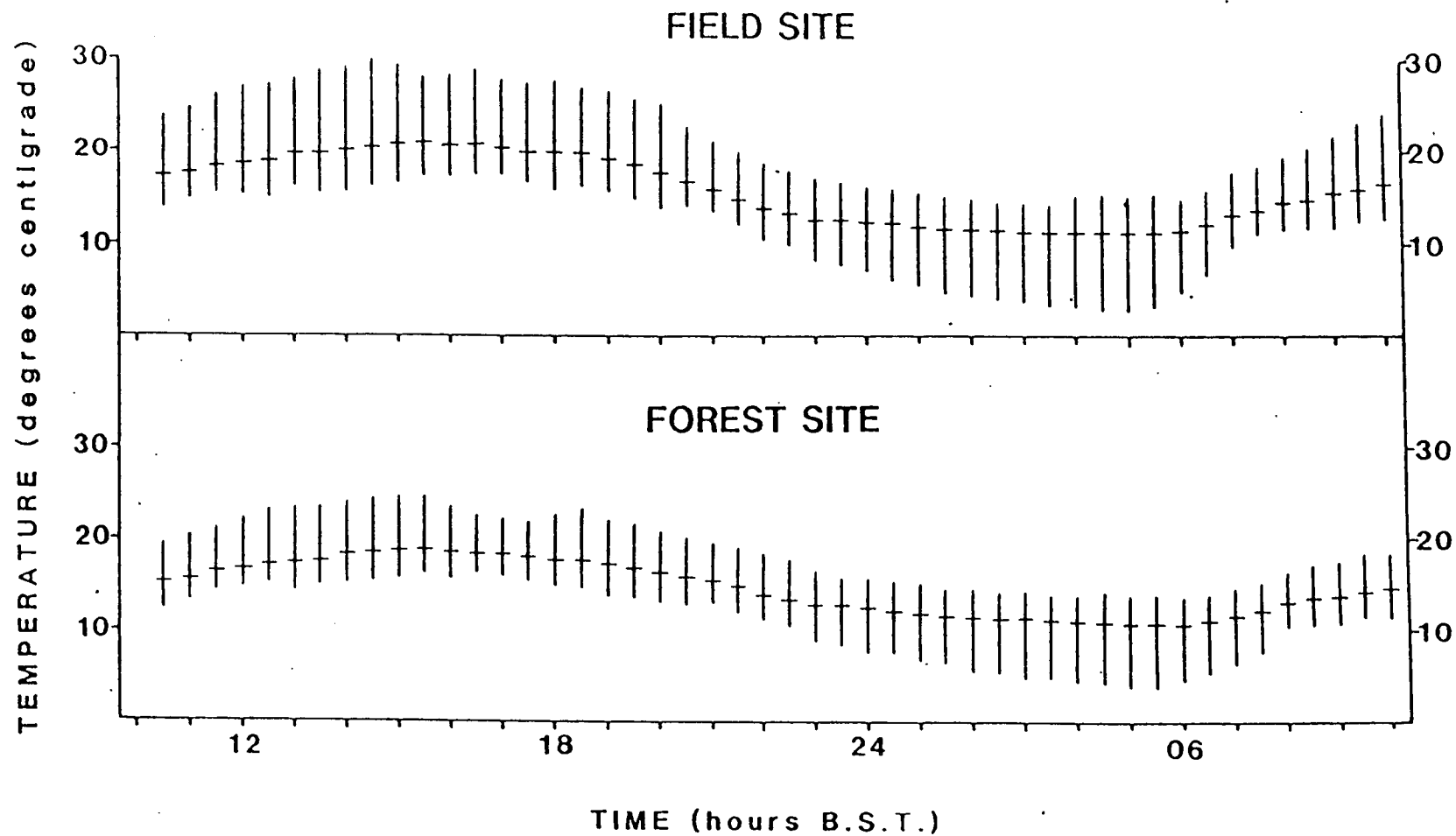


Figure 3-3. Diel air temperature cycles in the Field and Forest Sites:
mean and range.

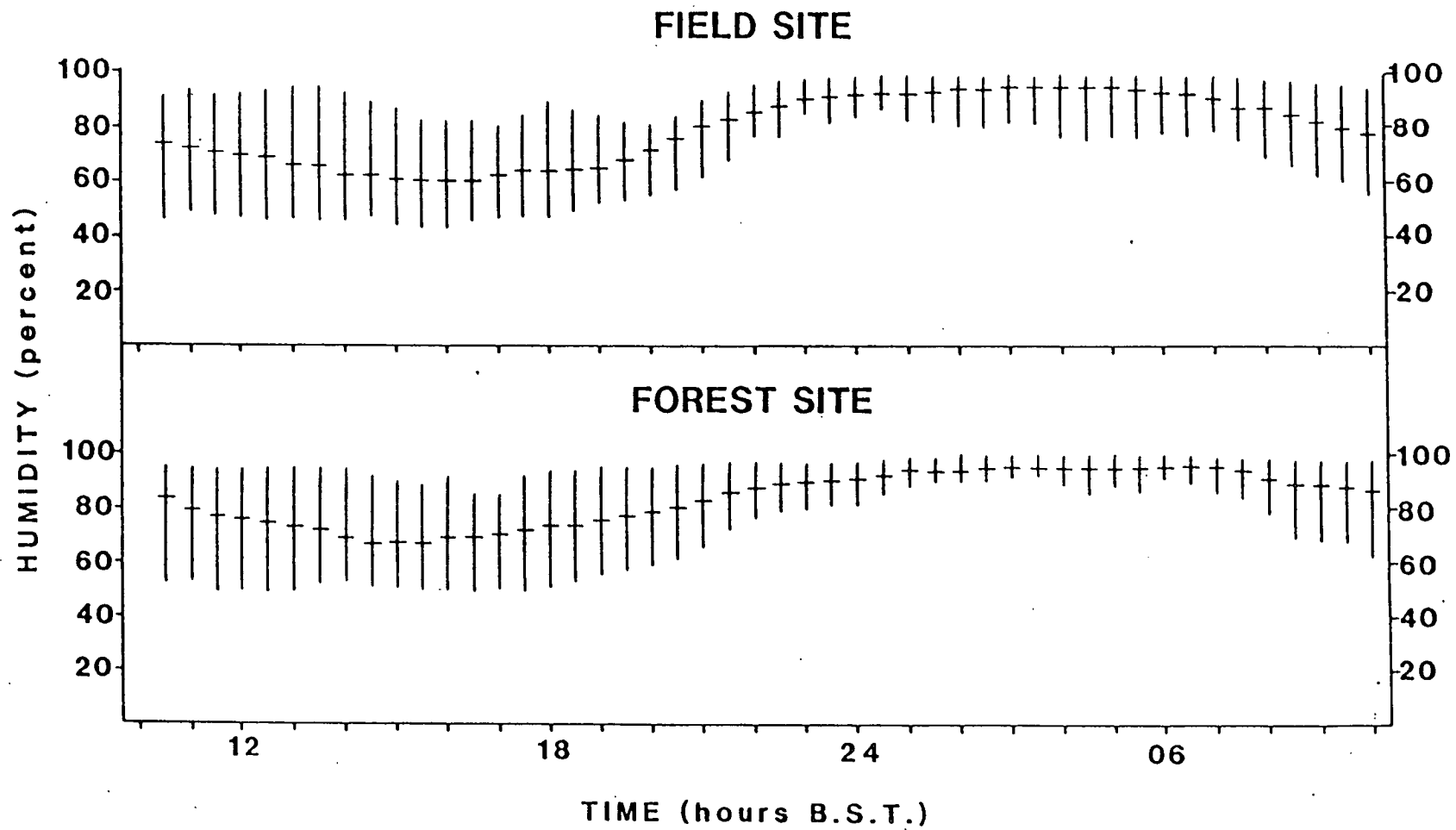


Figure 3-4. Diel humidity cycles in the Field and Forest Sites:
mean and range.

a) Air temperatures (Fig. 3-3)

The air temperatures on the experimental days for any specific half-hourly period were normally different in both mean and range in the two experimental sites. Both cycles were, however, typically sinusoidal (Monteith, 1980); 'peaking' in mid-afternoon and reaching their lowest point around dawn. (I concede that it would have been more correct to have plotted all the graphs in this chapter using the mid-point of the half-hourly intervals and not the end. For example, the mean temperature reading for the period, 10.00-10.30, should have been plotted at 10.15 and not at 10.30. This error was rectified in Appendix 1.) The Forest Site experienced a narrower range of temperatures, being generally cooler by day and warmer at night (between 20.30 and 02.30 B.S.T.). The vegetation cover maintains a more consistent microclimate; reducing daytime short-wave input and hindering night-time long-wave loss. In an extremely dense forest, the long-wave radiation emitted by the trees themselves would help maintain a warm, equable environment (Monteith, 1980; Oke, 1978).

b) Relative humidities (Fig. 3-4)

Humidity fluctuated in anti-phase with air temperature ($r = -0.645$, $P < 0.1\%$); reaching its highest levels during the night. (The correlation coefficients given in this chapter were calculated from the eight sets of the 48 half-hourly (averaged) environmental readings taken from the Field Site, using 'Minitab', Ryan *et al.*, 1976.) Mean values in both sites, were almost always above 90% between 22.30 and 07.00; though during the afternoon in the Field Site humidities of less than 50% were common. The canopy of the Forest Site retards moisture loss through evapo-transpiration. Consequently, in the forest the mean humidities were higher and their range was smaller.

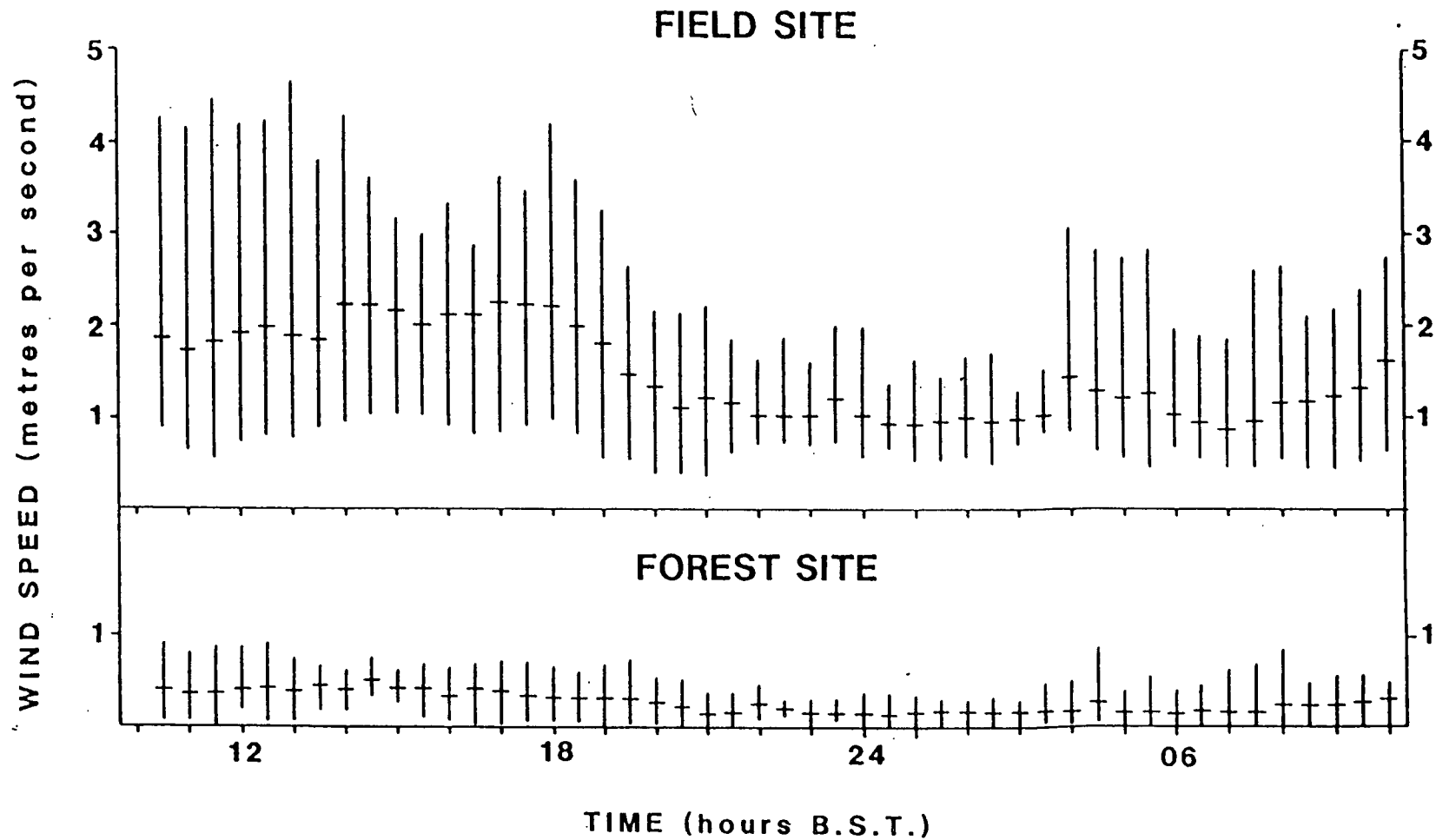
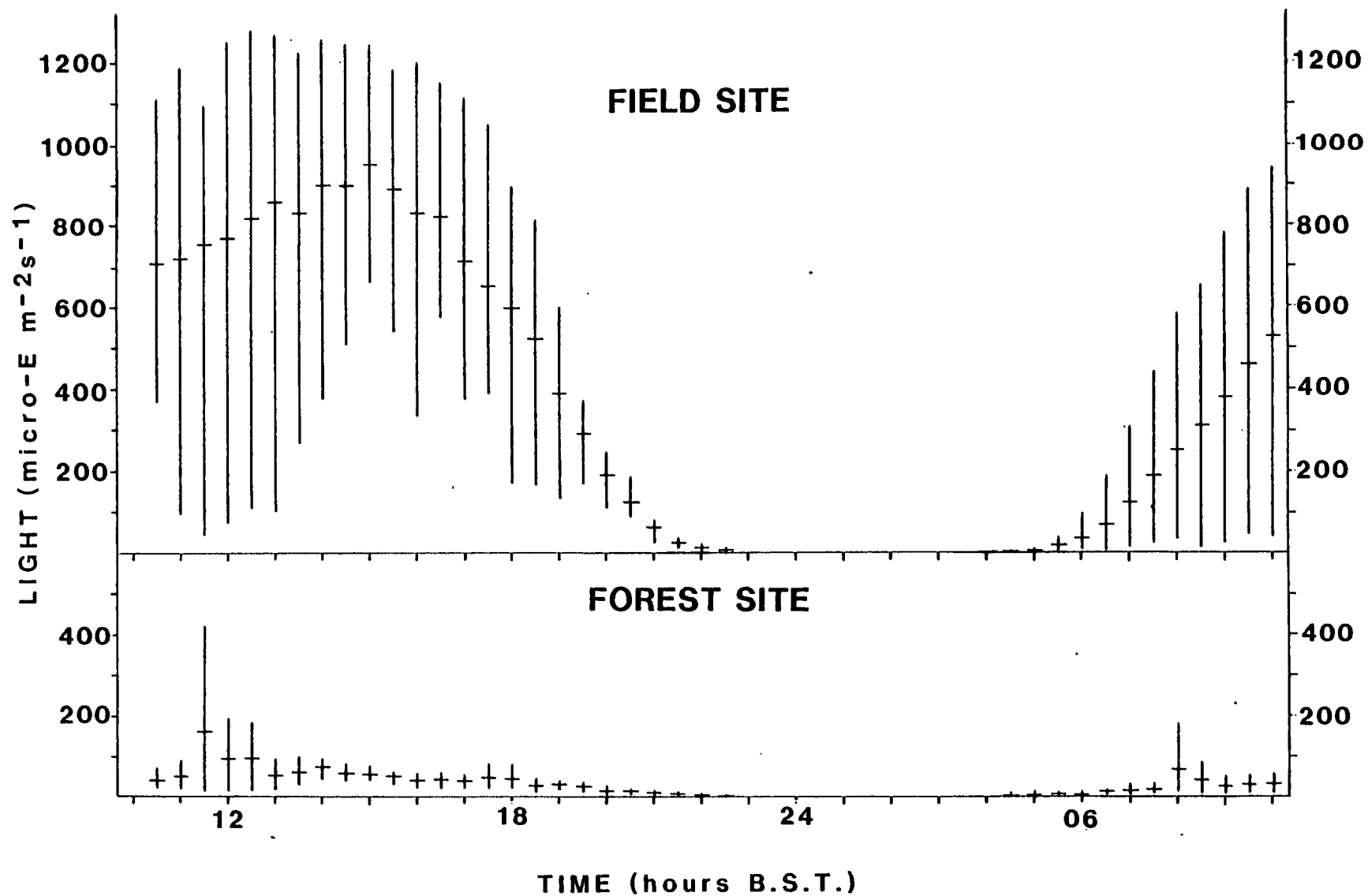


Figure 3-5. Diel wind speed cycles in the Field and Forest Sites:
mean and range.



c) Wind speeds (Fig. 3-5)

Mean wind speeds in the Forest Site never exceeded 1 m s^{-1} . By comparison, daytime half-hourly wind speeds in the Field Site of over 4 m s^{-1} were occasionally recorded. The higher daytime temperatures give rise to a convective wind system ($r = 0.265$, $P < 0.1\%$). Advective winds were also common over Loch Awe (Monteith, 1980; Oke, 1978; Woodward & Sheehy, 1983); this persistent 'sea breeze' (normally of over 0.5 m s^{-1}) made it impossible to record the cycle of flight activity in complete calm (see Figure 4-3).

d) Light levels (Fig. 3-6)

The foliage of the forest canopy reflects, transmits and absorbs the short-wave solar input. As a result, the radiation incident on the forest floor will be qualitatively and quantitatively different from that experienced in the meadow (Monteith, 1980; Oke, 1978; Woodward & Sheehy, 1983). Indeed, a reduction of the above-canopy radiation of 80% or more is common at ground level in a coniferous forest (Oke, 1978). A similar attenuation was noted in this study.

'Expected' solar input (taken from, for example, the Air or Nautical Almanacs) was lower at dawn than at dusk for two reasons: firstly, the mountains on the eastern side of the experimental site (the Ben Cruachan Massif) were significantly higher than those on the west. Secondly, morning fog and mist, of considerable depth, was common over the loch.

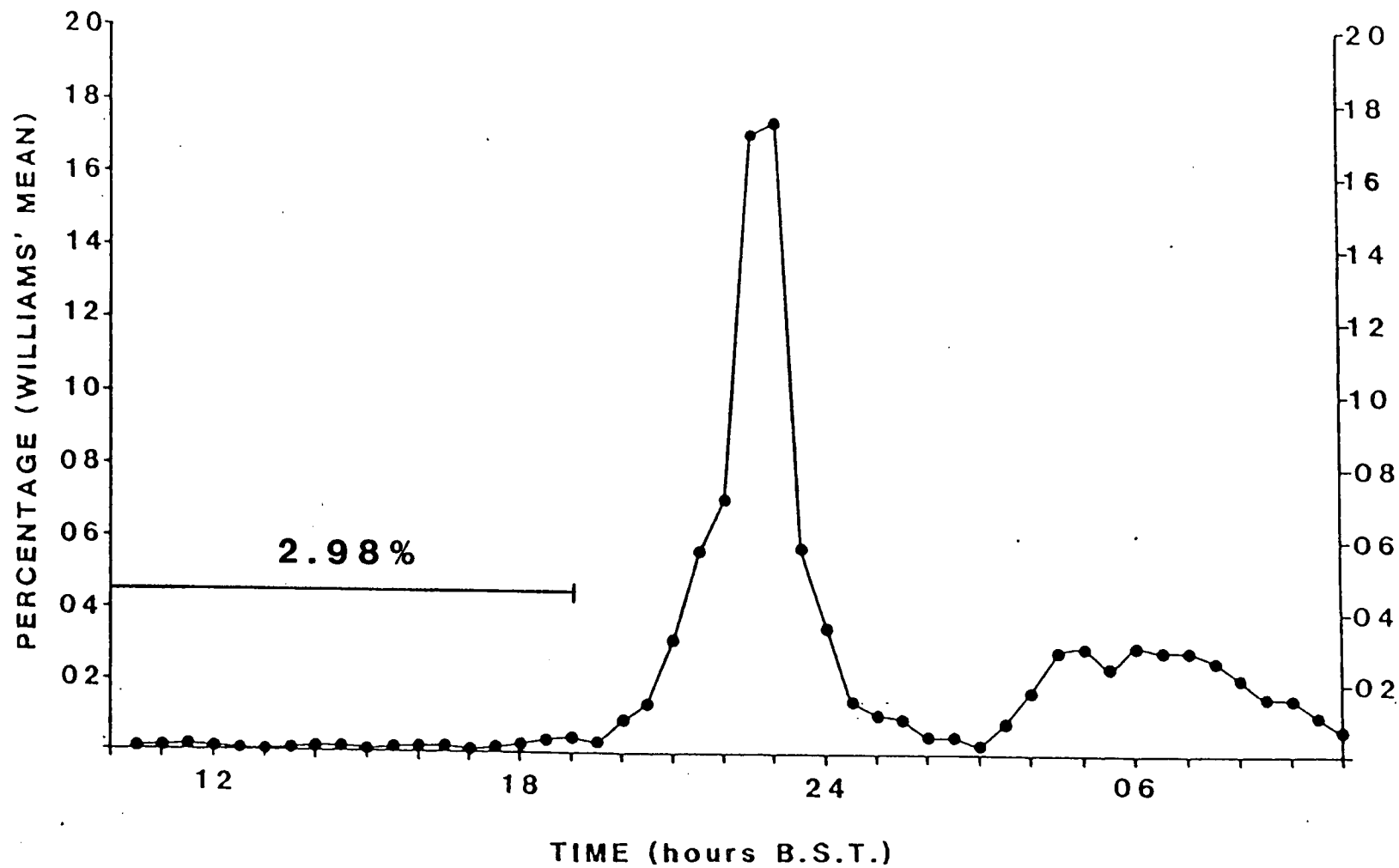


Figure 3-7. Flight periodicity of female *Culicoides impunctatus*:
Forest Site and suction trap.

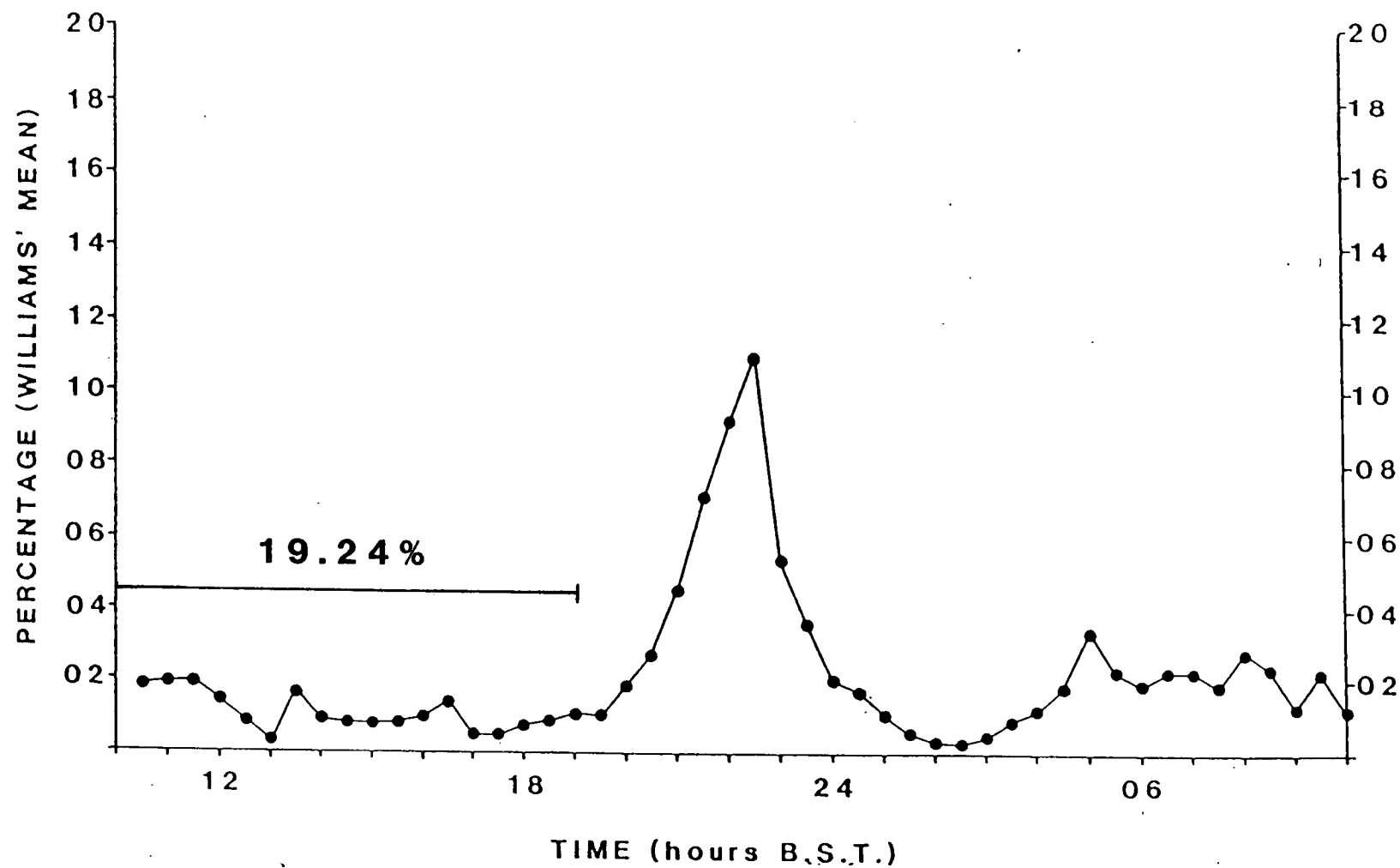


Figure 3-8. Flight periodicity of female *Culicoides impunctatus*:
Forest Site and human bait.

3.6.2. Flight periodicities of *Culicoides impunctatus*

a) Forest Site – suction trap (Fig. 3-7)

In the forest *Culicoides impunctatus* caught by a suction trap showed a bimodal (crepuscular) periodicity. The dusk activity, which peaked around 22.00–23.00, was considerably larger and more sharply defined than the dawn 'peak'. Little night activity occurred in between. The catch during the 'daylight hours', defined as the period 10.00 to 19.00, accounted for only 2.98% of the total daily activity.

b) Forest Site – human bait (Fig. 3-8)

In contrast to the result obtained using a non-attractive trapping technique, the 'daylight hours' catch by a human bait (P.M.M.) in the same locality was significant, though variable; comprising 19.24% of the total diel activity. Apart from this apparently induced daytime flight (discussed in Section 3.7.1), the periodicity also showed bimodality. The two maxima (22.00–22.30 & 04.30–05.00) were again markedly unequal and of different definition.

c) Field Site – suction trap (Fig. 3-9)

The flight pattern observed with a suction trap located in the field was closely similar in form to the suction-trap catch in the forest. The dusk activity maxima were of similar magnitude; furthermore, in the field the 'daylight hours' catch also accounted for a relatively minute proportion of the total diel activity (0.58%). At dusk, however, significant activity started one

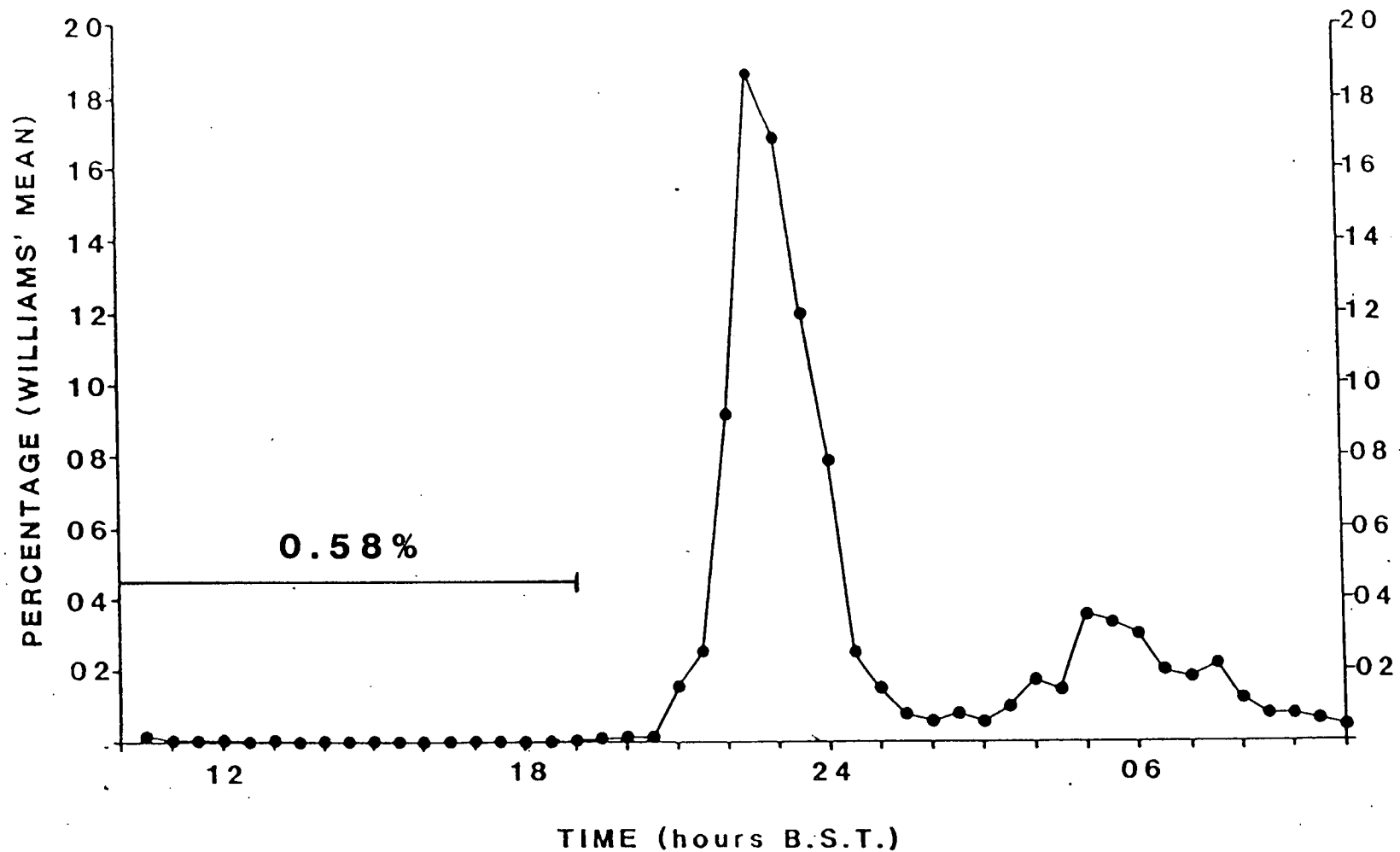


Figure 3-9. Flight periodicity of female *Culicoides impunctatus*:
Field Site and suction trap.

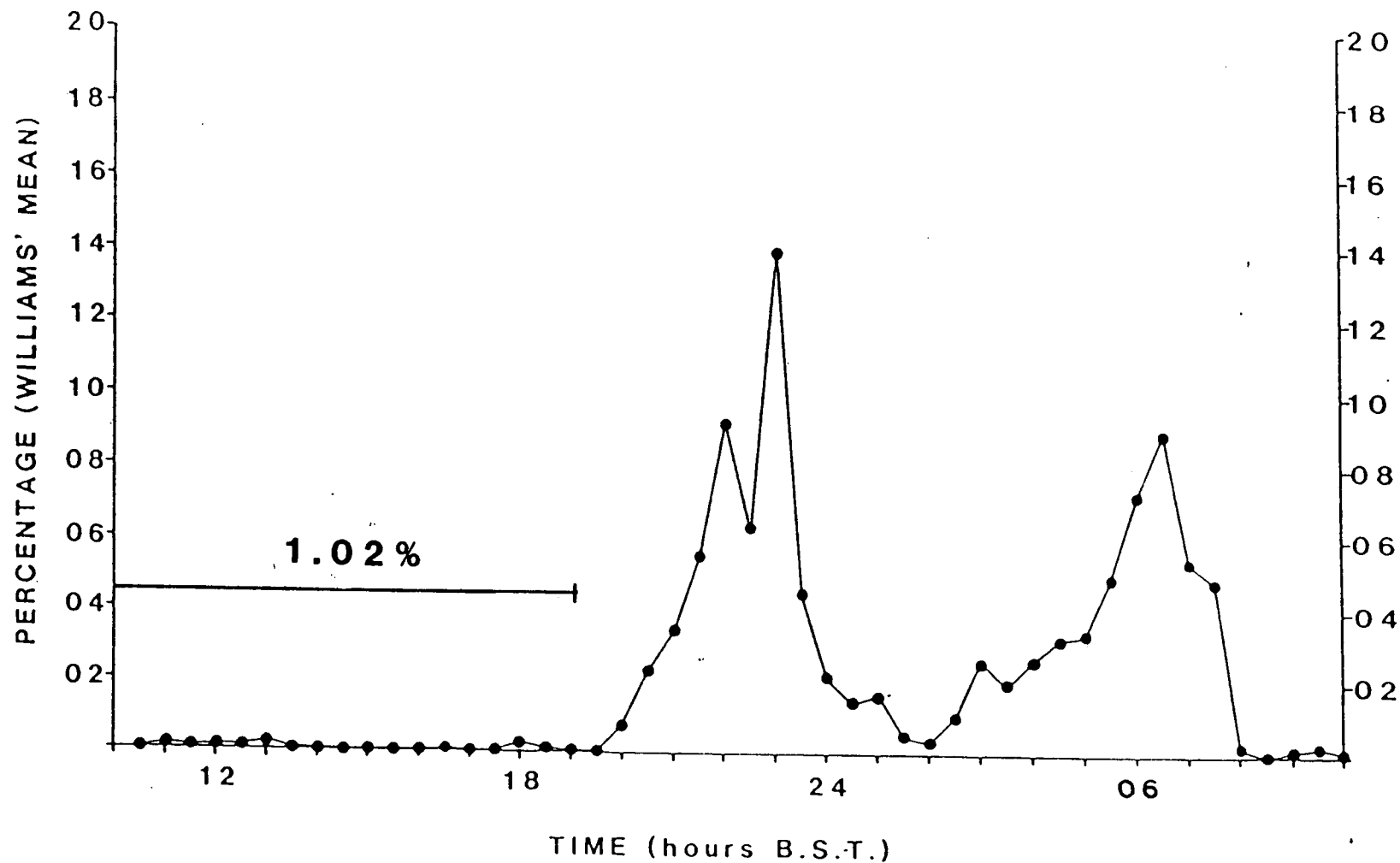


Figure 3-10. Flight periodicity of female *Culicoides impunctatus*:
Field Site and human bait.

hour later in the Field Site; additionally, flight intensity in the field was lower and more variable after dawn.

A considerable variation in flight pattern was noted between the trials (see Appendix 1). On occasions, the dawn peak was the larger of the two maxima (Trials 3 & 6); in some other trials, this activity was much reduced or absent (Trials 5, 7 & 8). Trials 1 and 3 were peculiar in that significant flight activity was recorded throughout the scotophase. Sometimes sharp peaks of flight activity – more characteristic of a purely exogenous response – were noted (Trials 4 & 5). This could indicate that light has an excitatory effect (Haddow, 1956; Saunders, 1977, 1982). As discussed in Section 3.2, it is believed that the variability in flight periodicity noted is attributable to the effect of exogenous factors acting on an endogenous framework for activity.

d) Field Site – human bait (Fig. 3-10)

The diel flight pattern determined by a human bait (R.C.K.T.) in the field – although still bimodal – was fairly irregular in form. The five-minute catching periods, in combination with the more unstable conditions experienced in this exposed area, probably account for the variation observed. In contrast to the human-bait catch in the forest, only 1.02% of the total diel activity was experienced during the 'daylight hours'.

3.6.3. The 'daylight hours' catches: a statistical comparison

A Wilcoxon matched-pairs signed-ranks test (Siegel, 1956; SPSS Inc., 1983) was used to test the null hypothesis that the mean percentage of the total catch taken during the 'daylight hours' did not vary with method and location of capture. The human-bait catch of the 'daylight hours' in the forest

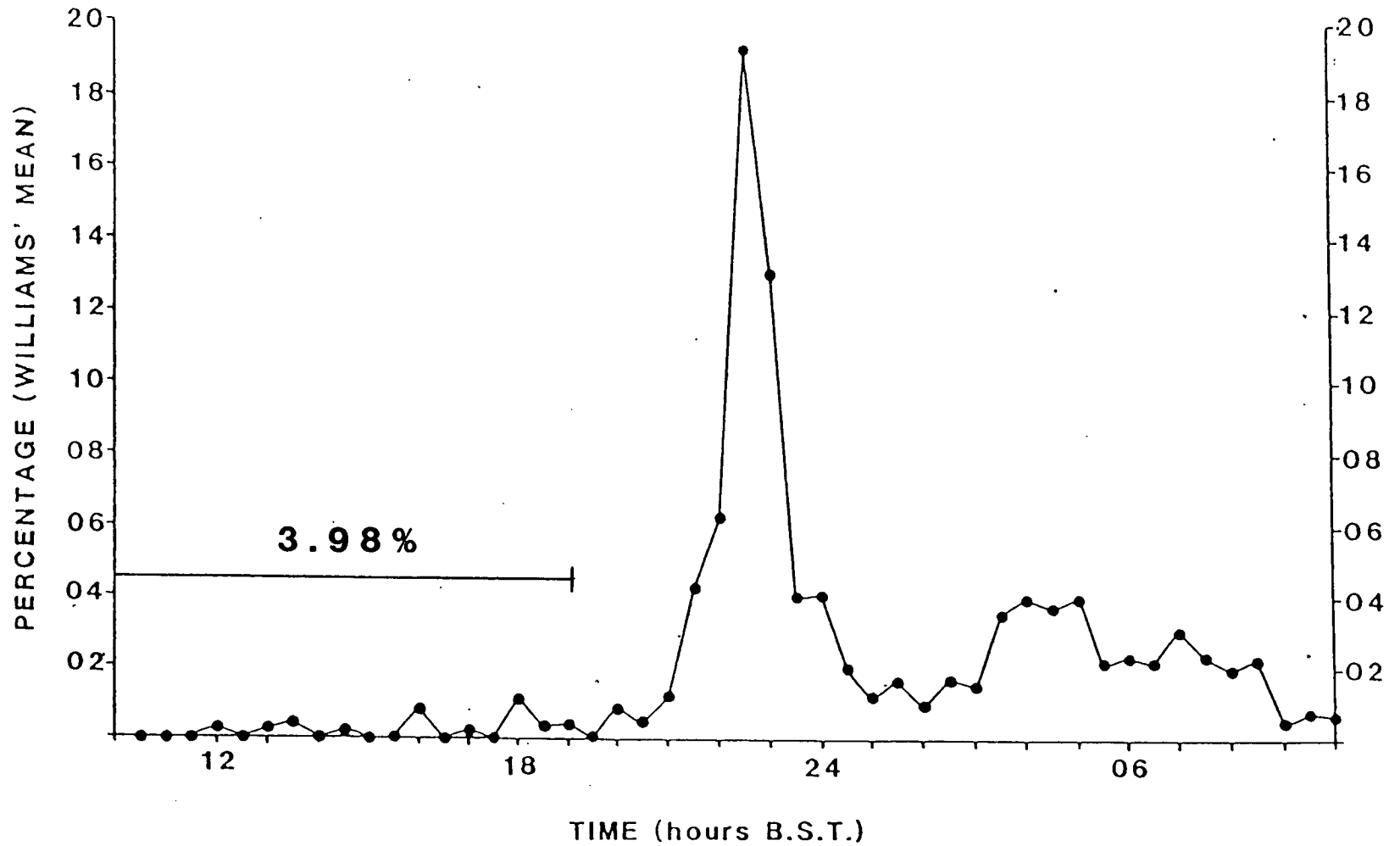


Figure 3-11. Flight periodicity of female *Culicoides obsoletus* grp:
Forest Site and suction trap.

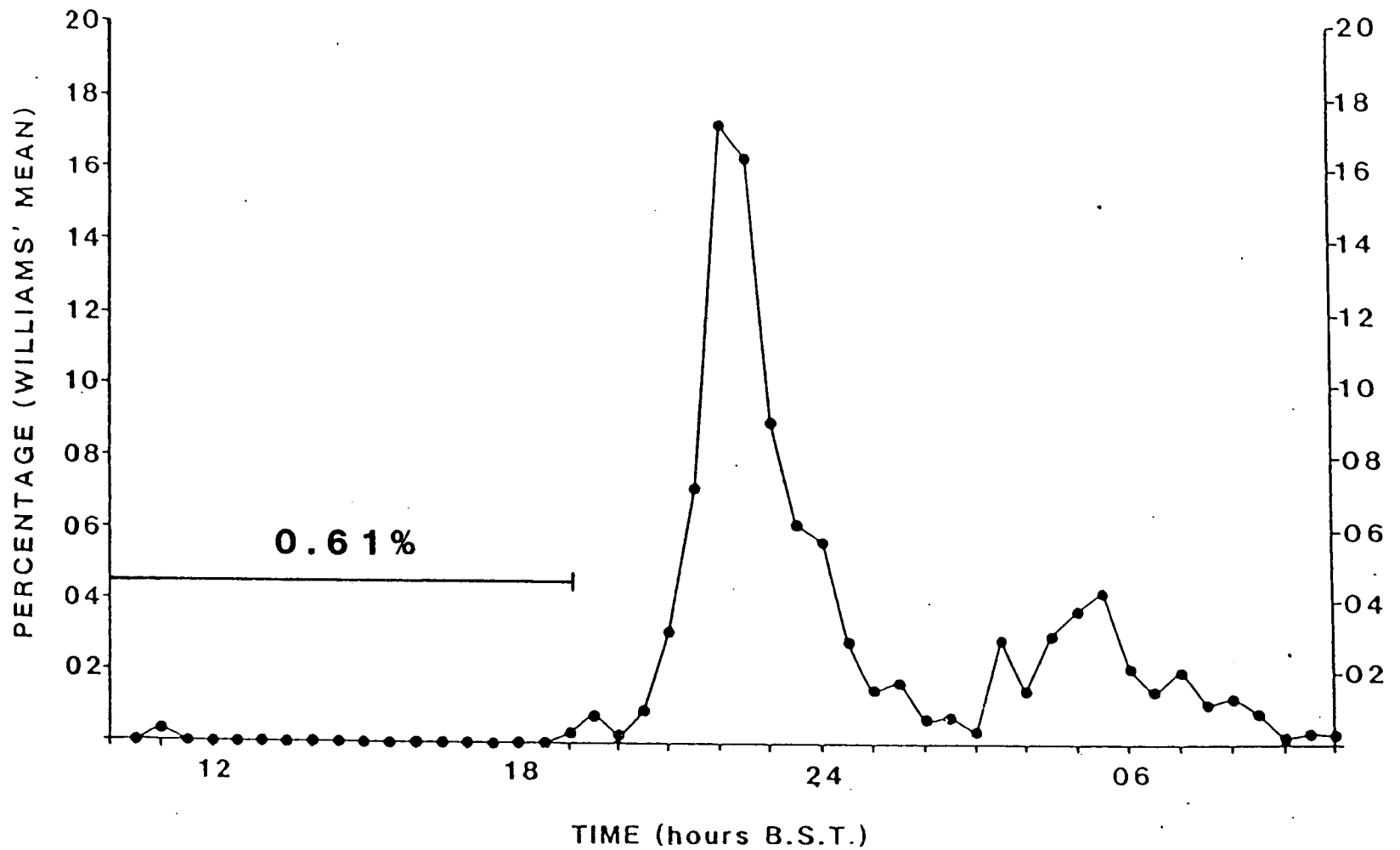


Figure 3-12. Flight periodicity of female *Culicoides obsoletus* grp:
Field Site and suction trap.

was significantly larger ($P < 0.01\%$: one-tailed) than that of the suction trap in the same locality; whose catch in turn was greater than that of both the suction trap ($P < 0.02\%$: two-tailed) and the human bait ($P < 0.4\%$: two-tailed) in the Field Site. The two Field Site catches were inseparable statistically ($P > 30\%$: two-tailed).

3.6.4. Flight periodicities of other species

Suction-trap caught *Culicoides obsoletus* grp females also showed crepuscularity; once again the peaks were markedly unequal (see Figures 3-11 & 3-12). In common with the suction-trap catches of *Culicoides impunctatus*, little 'daylight hours' activity was noted: 3.98% in the forest; 0.61% in the field. (Very few ($< 0.5\%$) *Culicoides obsoletus* grp females were caught by the human baits.) All the other species listed in Chapter 2, with the exception of *Culicoides heliophilus*, appeared to be crepuscular/nocturnal in habit. (They were caught too infrequently, however, for reliable flight periodicities to be plotted (Philip Ashmole, pers. comm.).) *Culicoides heliophilus* – as its name suggests – was observed to fly in full sunlight at all hours of the daytime (see Edwards *et al.*, 1939).

3.7. Discussion

All the flight periodicities determined in this study show a bimodality (associated with dusk and dawn) to a greater or lesser extent. Indeed, it seems as though crepuscularity may be fairly typical of the genus (see 3.7.2).

Service (1969b), using four continuous human-bait catches between 17.00 and 10.00 (G.M.T.), found *Culicoides impunctatus* and *C. obsoletus* to be crepuscular. Considerable activity was associated with sunset; no insects were

caught biting between 23.00 and 04.00; and, after sunrise, a smaller dawn peak (06.30) was noted. In a later study, using suction traps within the shelter of vegetation (Service, 1971b), flight activity in unfed females of the *Culicoides obsoletus* grp peaked at both 20.00–21.00 and 04.00–05.00 (G.M.T.). Few insects were caught at other times. Crepuscularity has also been observed by Hill (1947) in *Culicoides impunctatus* using landing rates on a black cloth; in Onyiah's (1971) human-bait and sweep-net collections of *C. impunctatus*, *C. obsoletus*, *C. pulicaris* and *C. pallidicornis*; and in Parker's (1949) sweep-net catches of female *C. impunctatus*, *C. pallidicornis* and *C. obsoletus*, and on *C. pulicaris* males.

This periodicity of flight, however, has not always been observed in British midges (see also Section 3.6.4). For example, flight periodicities of doubtful bimodality were recorded by Hill (1947) in her black-cloth landing-rate studies on *Culicoides obsoletus* and *C. chiopterus*; in Kettle's (1957) single exploratory 24-hour human-bait catch of *C. impunctatus*; in the work of Lewis and Taylor (1964) on *C. obsoletus*; by Onyiah (1971) for suction-trap caught males and females of *C. impunctatus*, *C. obsoletus*, *C. pulicaris* and *C. pallidicornis*; and by Service (1971b), using a suction trap, for *C. punctatus*, *C. pulicaris* and *C. pictipennis*. In these examples, the dusk peak was always present and pronounced. The dawn peak, in contrast, was either very poorly defined or absent. In general, little activity of significance was recorded during the daytime. The loss or reduction of the dawn peak may be explainable, in part, by microclimatic effects: I do not consider these periodicities too dissimilar to those measured at Dalavich. Contrastingly, the work of Reuben (1959, 1963) demands comment.

Reuben worked on *Culicoides impunctatus* in an exposed, fairly dry, field situation using a suction trap. Although she recorded a flight increase at dusk (which stabilised at a fairly high level during the night), highly significant flight

activity also persisted throughout the daytime. (A similar periodicity was noted occasionally by Onyiah (1971) for suction-trap caught *Culicoides impunctatus*, *C. obsoletus*, *C. pulicaris* and *C. pallidicornis* in a lush meadow situation.) In view of the results obtained in my study, I find this flight pattern particularly difficult to explain. It is, however, just possible that in Reuben's work, those insects that were caught during the daytime had been induced to feed by the presence of livestock (refer to Section 3.7.1); her trapping site "was fenced off with wire-netting and barbed wire to exclude animals". (Onyiah makes no mention of animals being present in his trapping site.)

3.7.1. Why do flight activity patterns vary with trapping technique and location of capture?

The number of insects caught in a suction trap at any specific instant during the 24-hour period will normally depend on the time of day, the density and 'blood avidity' (Section 3.7.3) of the population, and the microclimate (Chapter 4). One extra factor affects the catch by a human bait (during the daytime): the number of resting sites in the 'immediate vicinity' (see below).

Midges and some other haematophagous insects seem to be "opportunistic" (Service, 1973). Consequently, the presence of a host is able to modify their normal activity pattern and induce previously resting individuals to attempt to feed. The induction of activity obviously depends on whether 'hungry' insects can perceive a blood-source. The immediate vicinity (i.e. the attraction distance) is not only species-specific but also depends on the prevailing microclimate (Chapter 5). I have no data for *Culicoides* spp. For mosquitoes, Service (1971a) found that during the day under woodland conditions, *Aedes cantans* (Meigen) could be attracted to a stationary human from up to 7-10 metres. It would seem reasonable to suggest, therefore, that

the 19.24% of the total diel activity recorded by the human bait in the Forest Site during the 'daylight hours', represents induced activity in those individuals that were resting in the vegetation close to the bait. That this flight was stimulated is evidenced by the low catch (2.98%) of the suction trap in the same habitat. (The experiment described in Chapter 5 relied on the fact that a human bait could induce significant attack activity during the daytime in moist, sheltered forest conditions.)

This type of induced-feeding activity has also been reported by Gluchova (1958) - who termed it "lurking" or "waylaying attack" - for *Culicoides grisescens* and *C. pulicaris*; from the laboratory actograph studies of Harker (1956) on *Periplaneta americana* L.; by Nathan (1981) for *Culicoides phlebotomus* (Williston); and in the work of Jamnback and Watthews (1963) on *C. sanguisuga* (Coquillett). Induced activity is typically local and short in duration; ceasing almost immediately after the host's departure. By contrast, "active, hunting flight" (Gluchova, 1958), which in the majority of *Culicoides* species is limited to the crepuscular and nocturnal periods, is prolonged and, as noted above, is likely to be primarily rhythmic in origin.

Service (1971a) observed: "There appeared to be little movement of daytime resting populations [of mosquitoes] unless a suitable host entered the area ...". In this study in southern England on (principally) *Aedes cantans*, he found that in a "sheltered" site with thick undergrowth, significant numbers of mosquitoes could be caught between 11.00 and 12.00 (B.S.T.). A similar catch at the same time of day in an exposed field, however, yielded no insects. He concluded that, "It is clear that during the day unfed *Aedes cantans*, ..., rest in heavily-shaded vegetation". Although such an interpretation is very likely correct for this mosquito, it is certainly possible that midges do rest in normal field situations (though probably in smaller numbers), but that the daytime extremes of microclimate experienced therein usually inhibit flight. In the

present study, therefore, whilst cutting the vegetation in the Field Site (refer to 3.4.1.a) ensured approximate homogeneity of microclimate, potential resting sites could have been destroyed from which biting individuals might have emerged during favourable daytime conditions had they been stimulated. Surely dense long grass could constitute "heavily-shaded vegetation" for an insect the size of a midge. Indeed, this must be the case – at least in some moorland areas – for midges can still be very abundant in areas devoid of a significant shrub or tree-layer within the likely flight range (Section 6.8).

The disparity between the 'daytime catches' of the two human baits used in this study (19.24% relative to 1.02%) may therefore be attributable to differences in both the number of resting sites in, and the microclimate of, the two habitats. These factors could also account for the higher percentage of the 24-hour catch taken during the daytime by the forest-located suction trap (2.98%) as compared with its counterpart in the field (0.58%). Although it would seem that *Culicoides impunctatus* is not really 'programmed' to fly during the daytime, resting individuals might make short avoidance flights if the vegetation on which they are resting becomes insulated (Service, 1971a, 1973). Additionally, the human changing the suction trap could have induced some activity subsequently monitored by the suction trap.

If there is really a paucity or absence of resting sites in the entire Field Site, one would expect a diel migration from and to the forest at dusk and dawn as predicted by Gluchova (1958) and Service (1971a). Substantial activity started one hour later in the meadow (compare Figures 3-7 & 3-9). Thus a possible egress may start only when exogenous factors in the field become permissible for controlled flight. (An attempt to monitor any diel population movement using two-dimensional oil-covered perspex 'interception traps' failed owing to insufficient numbers of midges being present at the end of the 1984 season.)

3.7.2. Why is crepuscularity fairly typical of the genus?

It has been observed that crepuscularity is very common in the genus *Culicoides* (Kettle, 1984; Onyiah, 1971; Reye, 1964; Wild, 1983). A precise explanation for this is difficult to give. It seems, however, reasonable to suggest that an organism might evolve a crepuscular (or indeed a nocturnal) existence to avoid the more extreme environmental conditions that generally prevail during the daytime.

At night, when temperature inversions are to be expected, wind speeds are usually lower and, in consequence, will be more favourable for controlled flight (Lewis & Taylor, 1964; Figure 3-5). (Lewis and Taylor (1964) showed convincingly that small migratory insects utilise daytime convective-instability to facilitate their dispersal.) Additionally, the higher illuminances, frequent temperature extremes and lower humidities typical of the daytime could affect activity indirectly. Owing to its high surface area/volume ratio, the likelihood of desiccation (and perhaps temperature excess) for a small active insect will always be relatively high (Oke, 1978; Willmer, 1982).

Although daytime environmental conditions may be comparatively unfavourable to flight activity of *Culicoides* spp., some species, for example *C. heliophilus* (Section 3.6.4), are day-flyers. (It could be noteworthy that this species flies in the early part of the season (Edwards *et al.*, 1939; pers. obs.), thus avoiding the heat of mid-summer.) Similarly, Nathan (1981) found *Culicoides phlebotomus* to be diurnal. It seems, therefore, that a diurnal existence is probably not denied to midges through their inability to evolve, for instance, the necessary flight capacity or the required resistance to desiccation. Indeed, Lewis and Taylor (1964) believe that "Day flight is likely ... to be primitive, and the evolutionary trend in the Nematocera may well be from small, dark, day-flyers to larger, paler, night-flyers". (*Culicoides heliophilus* is heavily

pigmented.) In contradiction, Cloudsley-Thompson (1960) theorised that crepuscularity/nocturnality is a primitive trait. Although a consideration of the phylogenetic origin of the Ceratopogonidae is clearly beyond this work, considering their size and feeding habits (see below), the members of this family would seem far more suited to exploit a crepuscular/nocturnal niche.

Crepuscular and/or nocturnal activity could also be related to food availability and location. Lewis and Taylor (1964) separated their catch according to adult-feeding habit and mean flight time. Species which are heavily dependent on olfactory means for food location (e.g. feeders on decaying organic matter) showed nocturnality and/or crepuscularity. Although not explicitly mentioned, haematophagous insects would fall into this category. At night the air is generally still and moist. Although these conditions are not as conducive to host-odour dissemination as those experienced during the daytime, a strongly-defined scent gradient near a potential blood-source is probably required for accurate 'homing in' (refer to Chapter 5).

The tentative explanations offered above do not explain crepuscularity as opposed to nocturnality. Such a periodicity could be a compromise between night-time optimal environmental conditions and a midge's optical limitations (Cloudsley-Thompson, 1960; Lewis & Taylor, 1964; Nelson & Bellamy, 1971). Visual acuity declines rapidly after twilight. For example, although *Drosophila melanogaster* (another crepuscular species) has full visual acuity at illumination levels less than those at sunset, this falls to approximately 35% in full zenith moonlight and the insect is almost 'blind' at lower light intensities (Hecht & Wald, 1934, from Lewis & Taylor, 1964). This could explain why flight activity on moonlit nights is generally higher than that during new or crescent moon phases (Barnard & Jones, 1980; Nelson & Bellamy, 1971). By contrast, the work of Akey and Barnard (1983) indicates that an increase in nocturnal illumination does not necessarily augment trap catches of ceratopogonids; the response of

Culicoides variipennis was found to depend on reproductive condition.

The arguments mentioned in Section 3.3 regarding genetic isolation and mating synchronization are difficult to apply – at least to *Culicoides impunctatus*. Not only are the activity maxima relatively broad but, more importantly, one is dealing almost entirely with parous individuals all of which already have a supply of sperm (refer to Section 1.3.4).

It is possible that the time of evening maximum flight coincides with the onset of roosting in birds (Barnard & Jones, 1980; Corbet, 1960; Service, 1973; Philip Corbet, pers. comm.). Additionally, rodents and rabbits emerge around dusk; Service (1973) felt, however, that their high level of activity may severely hamper blood-feeding attempts.

3.7.3. Are the individuals caught during periods of low activity physiologically distinct from those taken at times of peak flight?

Field populations of female *Culicoides impunctatus* can be separated into 'non-flying' and 'flying' groups. The first seems to be composed of all nullipars, together with blood-fed pars that are maturing eggs. 'Hungry' pars typically comprise the aerial population (Section 1.3.4).

The 'non-flying' individuals seem to lack a 'feeding drive'. Brady (1975) showed that blood feeding has a depressing effect on the activity of *Glossina morsitans morsitans* (Machado). Armstrong (1968) obtained similar results for *Anopheles quadrimaculatus* (Say). Klowden and Lea (1979a, in an elegant paper) have demonstrated that in *Aedes aegypti* (L.) at least, the ovaries produce a "host-seeking inhibitory factor" during oogenesis. A comparable haemolymph-borne substance (possibly ecdysone, Jones & Gubbins, 1978) may be present in *Culicoides* spp.

"Behaviour is essentially a probabilistic phenomenon" (Brady, 1975). For 'non-flying' midges, the probability of capture by either a human bait or a suction trap is effectively zero. For 'flying' individuals this likelihood can be possibly related to their 'feeding drive' or blood avidity. The qualitative form of this drive in an individual is likely to be bimodal and dictated by rhythms. The quantitative level, in part rhythmically determined, will also be related (for example) to the hunger experienced by an insect and its age (Bailey & Meifert, 1973; Blau & Stinner, 1983; Brady, 1970, 1975; Corbet, 1960; Haufe, 1967; Jones & Gubbins, 1978).

(British *Culicoides*, unlike some other Nematocera, are assumed not to migrate. Kettle and Linley (1967b) believe that the ceratopogonid, *Leptoconops becquaerti*, could have a dispersal phase. Such migrants would be expected to have no perceptible feeding drive (Provost, 1953).)

Brady (1975), for instance, estimated that in male tsetse flies that have been starved for five days, the feeding drive – as displayed by spontaneous activity and visual responsiveness – is approximately 50 times as great as that of recently blood-fed individuals. The drive increases geometrically for five days after a blood meal; after which, a "pre-moribund" decline sets in. He states: "Evidently the depletion of food reserves affects the behaviour of the fly". In his opinion, the fly possibly obtains feedback about its nutritional status from "monitoring its weight [relative to that at eclosion] or abdominal volume".

If midges whose nutritional reserves are low show a greater than normal flight 'motivation', then such individuals, in addition to being generally more active in favourable weather, might be taken relatively more frequently during sub-optimal conditions. This feeding drive could induce them to start flying earlier and to continue later than their satiated conspecifics. These, in contrast, might concentrate the majority of their flight on the more favourable times of the 24-hour cycle – which could generally be at dawn and dusk (see Section

3.7.2). The multiple linear regression analysis (performed in Chapter 4) relies on a broad variation in response to identical environmental conditions.

As noted above, variation in physiological motivation, and consequently an insect's response to weather factors, does not necessarily have to be related to hunger. Consider the work of Goodenough and Snow (1979), for example. They reckoned that for the screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), "a gravid female needing an ovipositional site would probably fly sooner (and thus perhaps at either lower or higher temperatures) than a physiologically young [less motivated] female with no apparent need to find a host or food".

The findings of Nielsen and Nielsen (1962) are also of relevance here. Working on swarming in *Culex pipiens fatigans* Wied., they proposed the idea of an "urge to swarm". Using artificial sunrises, it was observed that if the 'twilight' period was short (11 minutes), and, in consequence, the mosquitoes had insufficient time to fully dissipate their swarming 'energy', then activity would persist at abnormally high illuminances (1.9 as opposed to 1.6 log lux). They summed up: "It seems that the urge to swarm is probably so strong that swarming continues into the period with light intensities that are normally inhibitory". The magnitude of such an urge should be highly correlated with a mosquito's physiological state.

Finally, whether or not an individual is caught under desiccating conditions could conceivably be related to its state of hydration. To explain: fully-hydrated individuals may be able to fly for a short period in dry conditions without too much physiological stress. An insect whose body fluids are more concentrated might, by contrast, have to remain inactive; being unable to survive further desiccation.

'Modelling' flight activity of *Culicoides impunctatus*

4.1. Introduction

"Even trained statisticians often fail to appreciate the extent to which statistics are vitiated by the unrecorded assumptions of their interpreters ... It is easy to prove [statistically] that the wearing of tall hats and the carrying of umbrellas enlarges the chest, prolongs life, and confers comparative immunity from disease ... A university degree, a daily bath, the owning of thirty pairs of trousers, a knowledge of Wagner's music, a pew in church, anything, in short, that implies more means and better nurture ... can be statistically palmed off as a magic-spell conferring all sorts of privileges ... The mathematician whose correlations would fill a Newton with admiration, may, in collecting and accepting data and drawing conclusions from them, fall into quite crude errors by just such popular oversights as I have been describing" (Shaw, 1911).

Although written many years ago, Shaw's words are only too relevant today. Observed covariation, however close, does not necessarily imply causation. The most common cause of such 'nonsense correlations' is the concomitant fluctuation of two variables with time (Aschoff, 1966; Huff, 1981; Kendall & Stuart, 1967).

All environmental variables, of which light is the most obvious example, show a pronounced and fairly predictable diel fluctuation (Monteith, 1980; Oke, 1978; Woodward & Sheehy, 1983; Figures 3-3, 3-4, 3-5 & 3-6). Insect activity too – if one accepts that activity in insects almost invariably has a strong rhythmic component (see Section 3.2) – can vary dramatically with time of day independent of any external influence. Thus, environmental ecologists should be wary when interpreting apparent relationships between weather conditions

and insect catch, if time – the variable with which both may be strongly correlated – has been ignored.

I believe that the failure to accommodate rhythmicity in most previous analyses of a similar nature has led to activity – or the lack of it – principally dictated by rhythms being attributed incorrectly to the prevailing environmental conditions. Typically only “correlative rather than causative” (Lewis & Hurst, 1967) relationships have been established.

To exemplify the point, consider the suction-trap catches taken in this study from the Field Site during the ‘daylight hours’ (see Section 3.6.2.c & Figure 3–9). Why were they almost always zero? One explanation – apparently favoured by the majority of environmental entomologists – is that the higher temperatures (for example) generally experienced during this period are inhibitory to flight. Certainly high temperatures can prevent flight in many of the smaller Diptera (Haufe, 1967; Taylor, 1963; Willmer, 1982). They can, however, no longer be regarded as inhibitory *per se*, if *Culicoides impunctatus* avoids these temperature extremes by being programmed rhythmically to be inactive when they are usually encountered (refer to Sections 3.6.2 & 3.7.1). Hence, temperature may have been “relegated to the category of ultimate (i.e., evolutionary) rather than proximate causes for the pattern observed under field conditions [at these times]” (Aschoff, 1966). In consequence, the apparent relationship between flight activity and temperature during the daytime could be merely a reflection of their mutual covariation with time.

(This is an extreme situation; one pertaining to the portion of the 24-hour period not included in the flight phase (refer to Sections 3.2 & 3.7.1). Within the flight phase, however, different endogenous levels of activity are likely to occur. Their possible existence must be recognised before suggestions are proffered about the direct effect of weather on insect activity.)

Aschoff (1966) noted the tendency amongst ecologists working in this field to draw unjustified cause and effect conclusions from corresponding activity and environmental data.

"Both in dealing with the two peaks and with the trough or quiescent stage in daily activity patterns [of species that have rhythmically-determined bimodal activity periodicities] many authors try to correlate the temporal pattern with specific stimuli in the environment. The peaks, for example, have been called dusk and dawn effects, or the trough has been related to the highest daily value of temperature, or the lowest value of humidity, shortly after noon. Authors frequently imply that these are cause-and-effect relations."

Aschoff's observations and subsequent words of caution have gone almost unheeded: the overwhelming majority of environmental entomologists have persisted with 'non-rhythmic' approaches. Not until the rhythmic element to a species' activity under field conditions is identified and accounted for, will it be theoretically possible to establish a proximate causal link between activity and microclimate. In reality, even if this could be achieved, it should be understood that our methods of analysis are far too crude to offer a reliable understanding of this exceedingly complex subject (see Section 4.2).

This chapter describes a new approach to the problem of 'modelling' flight activity in the field. One which, by the use of factors (see Section 4.3.2), attempts to analyse indirectly any variation in the data possibly associated with rhythms within the typical *flight* period of the species. (By only analysing data taken from the flight phase (Sections 3.2 & 3.7.1), the likelihood of obtaining non-sensical correlations should have been considerably reduced.) It is essential to understand, however, that although I consider this approach an improvement in some respects on previous analyses, I am sensitive to its limitations (see below).

4.2. Is a regression analysis applicable to the problem?

Many authors have tried to produce regression 'models' of insect catches in relation to weather conditions for predictive or standardisation purposes (Alverson & Noblet, 1977; Berlyn, 1978; Burnett & Hays, 1974; Gerhardt & Axtell, 1972; Goodenough & Snow, 1979; Kettle, 1969b; Kettle & Linley, 1967b; Koch & Axtell, 1979; Morton *et al.*, 1981; Onyiah, 1971; Read & Adames, 1980; Reuben, 1959, 1963; Vogt *et al.*, 1983, 1985; Wild, 1983; Williams, 1962). The technique has not been universally accepted. Taylor (1963, 1967) argued vigorously against trying to fit regressions – either recti- or curvilinear – to what, in his view for mono-specific populations, are simple threshold (binary) responses to environmental change.

I find the conclusions in Taylor's papers convincing only for the existence of upper and lower environmental thresholds for flight; that is (for example), temperatures above and below which flight is physically impossible. His method of analysis does not show, as is stated, that between these thresholds "both ability to fly, and willingness, are independent" of exogenous factors. This error of interpretation results from his use of "percentage flight occurrence"; an analytical method which ensures that "threshold" (*sensu* Taylor) definition becomes clearer with an increase in abundance of the species under question. Additionally, it prevents comparison between the different levels of flight activity within the permissive range – differences which he attributes entirely to "chance or population [fluctuation]".

(To evaluate 'percentage flight occurrence', catches are classified as positive (one or more insects) or zero. The number of positive catches within, for example, each temperature interval is then expressed as a percentage of the sum of the positive and zero catches for that interval. This 'percentage flight occurrence' is plotted against the corresponding temperature bracket.)

Onyiah (1971) applied Taylor's binary approach to suction-trap catches of *Culicoides* spp. At temperatures 6.1–7.0°C, 20% 'flight occurrence' was noted; this peaked at 90.9% in the temperature bracket 19.1–20.0°C. The results show no evidence for an abrupt temperature threshold and, even if the analysis had been applicable to the problem, do not support the rejection of a regression analysis.

Taylor does concede that "the threshold is not exactly the same for different individuals nor for different occasions and appears as a frequency distribution in a population. The chance of A [activity] being 1 [referring to his binary activity theory] on any particular occasion will increase as temperature increases through the threshold region and a probability distribution will appear, with repeated samples from a population in the field, corresponding to the threshold distribution for individuals within the population".

Taylor would seem not to be referring to actual ability to fly, for which there must surely be relatively little intra-specific variation. Any frequency distribution of thresholds for flight within a population is more probably related to variation in individual flight motivation and state of hydration; variation which, as discussed in Section 3.7.3, might lead to significant differences in response to identical weather conditions.

To conclude: providing one accepts the possibility that at some environmental extremes, insects – however physiologically motivated – will be physically incapable of flight, then I believe that there are strong grounds for generally preferring a regression analysis to the binary approach for data taken from the activity phase of a species (refer to Section 4.4.2). There are, however, undoubtedly good reasons for adopting a threshold type of analysis where a species' activity pattern is almost totally reliant on a critical light intensity. (See the work of Tychsen and Fletcher (1971) on *Dacus tryoni*, Section 4.3.4.d.)

It is vital to realise, however, that a multiple linear regression (M.L.R.) analysis may still be incapable of describing accurately variation in aerial density with respect to population change, weather and time of day. Such an analysis reduces an extremely complicated biological phenomenon to a simple 'skeleton' of rectilinear and curvilinear equations: the impotence of normal statistical procedures for data of this type became progressively more apparent. Regrettably, I cannot offer a preferable alternative. The word 'model' is, in consequence, used only very loosely in this account. It follows that the limitations of the mathematical analysis, and indeed those of the field study, must be reflected in the conclusions drawn from the data and the extent to which they should be 'milked' for supposedly *bona fide* relationships. Only rectilinear and quadratic (curvilinear) responses to the predictor variables were therefore considered; it was hoped that the analysis could separate out the main effects with a certain degree of reliability. These data, indeed those of this nature typically collected by entomologists, do not merit an analysis of y 's response to the numerous possible interaction terms between the x -variables and the factors (Chris Theobald, pers. comm.; David Williams, pers. comm.); some of which, by the stochastic effect of natural variation, are bound to be significant anyhow (Wild, 1983).

(The y -variable, the number of insects, is obviously the response (or dependent) variable. Environmental conditions constitute the x -variables, commonly known as the predictor, explanatory or independent variables (Section 4.3.4). Factors, which have mutually exclusive levels, were used to describe mathematically the effects due to the trial date (Section 4.3.2), the time of day (Section 4.3.3), the occurrence of rain (Section 4.3.4.e) and the wind direction (Section 4.3.4.f).)

4.3. Factors governing the size of suction-trap catches

Wild (1983) considered that the instantaneous catch in a non-attractive trap would be predicted by the following equation:

$$C_i = k_t \times D \times A_c \times [E(a) \times E(b) \times \dots \times E(z)]$$

C_i = instantaneous catch at some given time.

k_t = trap constant.

D = adult population density.

A_c = activity expected due to the circadian activity rhythm
under conditions ideally favourable for activity.

E(a) = influence of the environmental variable, a, on the
expression of the free circadian activity. This predictor variable can
be either real or derived (e.g. a higher order or interaction term).

This equation provides a theoretical starting point from which to consider the elements of the multiple linear regression approach adopted in this study.

Even though a term for the adult population density is included, the model above cannot, in isolation, give even a rough instantaneous prediction of **D** (Morton *et al.*, 1981; Vogt *et al.*, 1983). For this one would require a knowledge of (for example) the vertical distribution of the species under different environmental conditions, the area from which the catch was drawn, and the proportion of the adult population that were 'flying' at the time of capture (see Section 3.7.3).

4.3.1. The trap constant

k_t , a proportion constant, is a measure of trapping efficiency. In theory this should approach unity for the suction traps used in this study (see Section 2.3); the model (Section 4.6), however, requires only that the efficiency of trapping remains constant throughout the collection periods.

4.3.2. The adult population density (TRIAL)

The adult population density – one of the major determinants of the size of insect catches – would be expected to have varied dramatically during the data collection for this work (Section 1.3.4). Any analysis of this sort must, therefore, either analyse or obviate any variation in the data associated with a change in the number of insects available for capture.

The eight trials of the experiment described in Chapter 3, from which the data were taken (see 4.4.1), were factorised for the analysis (Wild, 1983). Catches taken from the same trial were assigned the same factor level: 1 corresponds to Trial 1, 2 to Trial 2 and so on (see Appendix 2). The analysis compared the number of insects caught in each of the Trials 2–8 with the catch taken in Trial 1 (the reference level), after theoretically allowing for the effects of weather and the other factors in the equation. (To clarify this, the trials were compared as follows: 1 & 2, 1 & 3, 1 & 4, etc. See Table 4–5.) The significance of each b , the estimate of the partial regression coefficient (β) for the change in catch between the factor levels, indicates the significance of the comparison; the sign of each b indicates the relative magnitude of the catch of the trial under question as compared with that of the reference trial (Trial 1). (McCullagh and Nelder (1983) demonstrate the use of factors in their example, "A study of ship damage", Section 6.3.2, p. 136. In addition, the reader is

referred to "Fisher's data on tuberculin response", included as a worked example in the GLIM manual (Baker & Nelder, 1978).)

Berlyn (1978) analysed seasonal population fluctuation in *Hydrotaea irritans* (Fallen) (Diptera: Muscidae) by describing the density change with a curvilinear (negative quadratic) function. Other environmental entomologists have negated population change through the use of 'first differences' (Dow & Gerrish, 1970; Reuben, 1963; Williams, 1962); analysis of covariance (ANCOVAR, Kettle, 1969b; Kettle & Linley, 1967b); and deviations from a "selected standard" (Kettle & Linley, 1967b; Reuben, 1959, 1963). Finally, some have performed experiments over, or have analysed experiments from, very short time periods to minimise population fluctuation (Kettle, 1969b; Kettle & Linley, 1967a, 1967b; Reuben, 1959, 1963).

(The analysis of 'first differences', as generally used in entomology, involves seeking a relationship between successive day-to-day differences in insect catch and the corresponding day-to-day differences in some environmental variable.)

The inclusion of **TRIAL** in the model (Section 4.6) therefore represents an attempt to separate "population" from "activity" effects on catch size (Taylor, 1967). The variation in the data attributable to this factor is determined by two elements which remain confounded within the parameter estimates: an inter-trial change not only in the actual number of adult insects but also in the proportion of 'flying' and 'non-flying' imagines within the population (refer to Section 3.7.3). It is hard to see, however, why the ratio of these two activity groups should change dramatically over the main flying season.

4.3.3. Rhythms (TIME)

Rhythms (A_c in Wild's model), considered at length in Chapter 3, are likely to play a major role in flight periodicity. As noted above, few ceratopogonists have acknowledged their possible existence in flight activity models. Those that have – with the exception of Kettle (1969b) – have relied on simple linear relationships to describe what, for the majority of biting midges, is probably a complex bimodal function with respect to time related to their crepuscularity (refer to Section 3.7.2).

In this study, the sums of squares (Mead & Curnow, 1983) associated with the time of trapping – an indirect and admittedly rough way of analysing any activity that may be governed by a rhythm – were incorporated in the model in the form of factors (see 4.3.2). Factorising the separate hourly intervals of the flight phase (Sections 3.2, 3.7.1 & 4.4.2) obviated the need to describe mathematically – through some complicated two peaked function – an innate crepuscularity that rhythms possibly dictate. (Plainly it would be incorrect to fit a multitude of bimodal mathematical functions to data of this type with the aim of finding the function of 'best fit'.)

4.3.4. Environmental variables and factors

"Weather affects insects as individuals (independently of the population density) and so acts as a *density independent* or *catastrophic factor*" (Varley *et al.*, 1980). This work endeavours to analyse the effect of air temperature, relative humidity (as one of the many possible measures of atmospheric moisture), wind speed and direction, light and rain on flight activity of *Culicoides impunctatus*. Other authors have, in addition, considered the importance of cloud cover (Alverson & Noblet, 1977; Burnett & Hays, 1974;

Kettle, 1957; Nathan, 1981; Nelson & Bellamy, 1971; Wild, 1983); cloud type (Alverson & Noblet, 1977); and atmospheric pressure (Alverson & Noblet, 1977; Bailey & Meifert, 1973; Blau & Stinner, 1983; Burnett & Hays, 1974; Koch & Axtell, 1979; Taylor, 1967). The failure to include these exogenous variables does not mean that I consider them to be unimportant. To the contrary, they were not included either due to a lack of equipment or because it would have been impossible in this study to take readings of sufficient accuracy to make the exercise worthwhile.

a) Air temperature

Many researchers have sought a relationship between the flight activity of various midge species and ambient temperature (Table 4-1). The majority have restricted their analysis to examining a rectilinear response; some of which have proved significant. Others, for example Wild (1983), have found, through considering a possible quadratic relationship, an optimum temperature for flight, above and below which flight activity is reduced until the thresholds *sensu stricto* are reached (see Section 4.4.3). Similarly, Kettle (1969b) combined three regression responses over different temperature ranges and concluded, "it would appear that below 70°F the biting rate of *C. furens* was positively related to temperature, between 70 and 75°F it was largely independent of temperature, and above 75°F negatively correlated". Indeed, a similar activity response to this environmental variable would seem to be fairly common amongst the Diptera. For example, an optimum temperature for flight has been found by Berlyn (1978) for *Hydrotaea irritans*; Dale and Axtell (1975) for *Tabanus nigrovittatus* Maquart (Diptera: Tabanidae); Gerhardt and Axtell (1972) for *Hippelates pusio* Loew (Diptera: Chloropidae); Goodenough and Snow (1979) for *Cochliomyia hominivorax* (Diptera: Calliphoridae); and Williams and Osman

(1960) for "the Diptera" caught near Cairo, Egypt.

Table 4-1

Some published work:The effect of air temperature on the activity of certain Ceratopogonids

(Significance levels: * = 5%, * * = 1%, * * * = 0.1%.

+ = positive response, - = negative response, n/s = not significant.

L.T. = Lower Threshold, U.T. = Upper Threshold.)

<u>Reference</u>	<u>Analysis</u>	<u>Units and/ or (interval)</u>	<u>Species</u>	<u>Response</u>
Barnard & Jones, 1980	field obs.	°C (various)	<i>C. variipennis</i> both sexes	curvilinear L.T. 7 U.T. 35
Jamnback & Watthews, 1963	field obs.	°F (2 min)	<i>C. sanguisuga</i> (Coquillett) females	L.T. 49-54
Kettle & Linley, 1967b	M.L.R. & ANCOVAR	(15 min)	<i>L. bequaerti</i> females	+
Koch & Axtell, 1979	M.L.R.	minimum T average T maximum T (hours)	<i>C. hollensis</i> (Melander & Brues) both sexes	* * * * * * * * direction not given
Nelson & Bellamy, 1971	field obs.	°C (15 min- 2 hours)	<i>C. variipennis</i> both sexes	curvilinear L.T. 10 U.T. 32
Onyiah, 1971	M.L.R.	mean daily temperature (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	+ + +

Onyiah, 1971	M.L.R.	mean temperature 3 hours before sunset (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	- - -
Read & Adames, 1980	field obs.	°C (hours)	<i>C. diabolicus</i> Hoffman females	curvilinear L.T. 18 U.T. 32
Reuben, 1959, 1963	M.L.R.	(hours)	<i>C. impunctatus</i> females	n/s
Reuben, 1963	M.L.R. on 1st diff's	minimum T previous day maximum T previous day (24 hours)	<i>C. impunctatus</i> females	n/s n/s
Walker, 1977	partial correlation	(hours)	<i>C. pallidipennis</i> C, I + M both sexes <i>C. schultzei</i> grp (End.) females	+ * +
Wild, 1983	M.L.R.	(various)	"courting" <i>C. austropalpalis</i> gravid female <i>C. austropalpalis</i>	-ve quadratic * * * -ve quadratic * * *

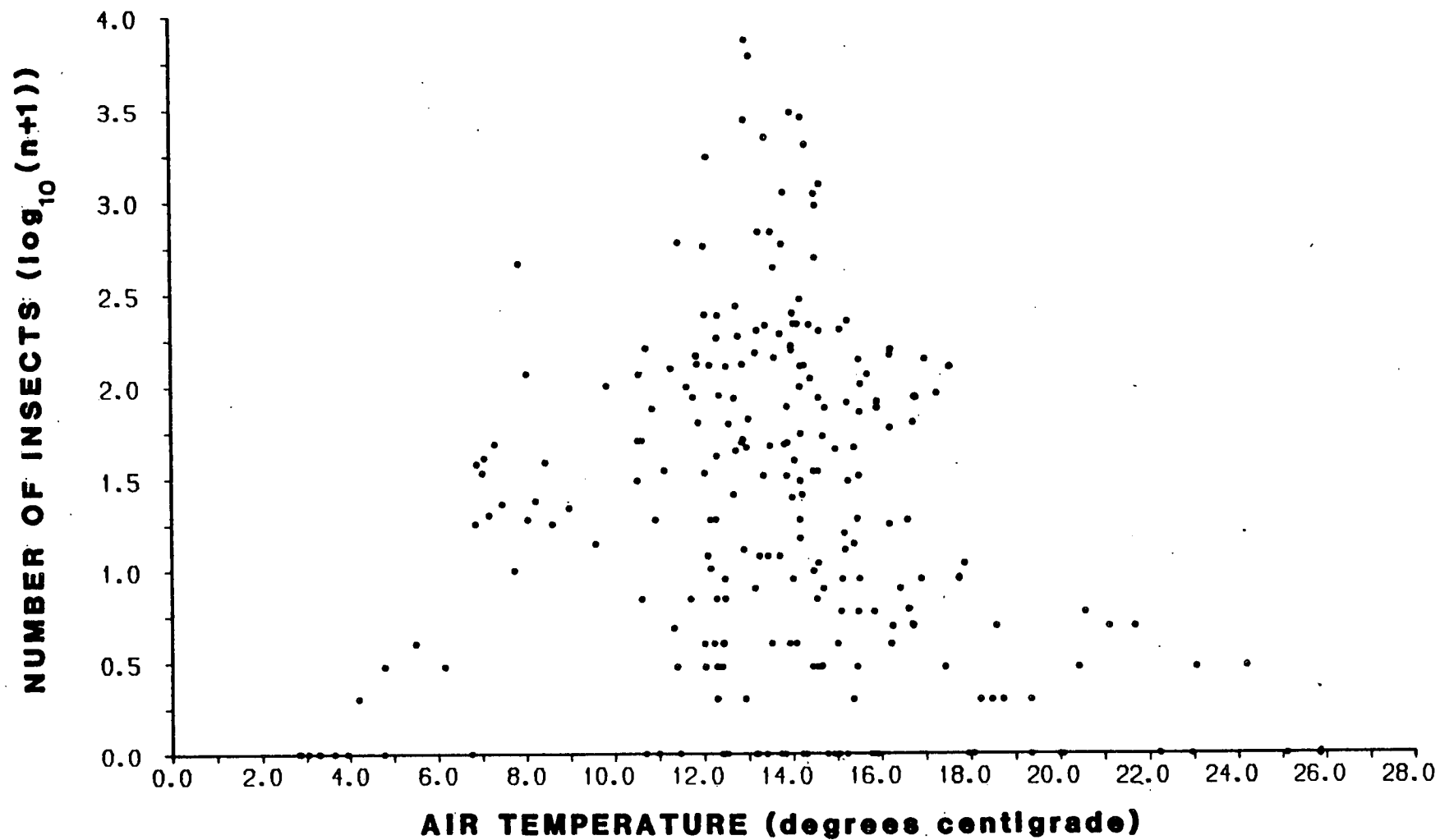


Figure 4-1. Scatter diagram: air temperature and number of insects

In my study, a scatter diagram of the mean temperature for a trapping period relative to the corresponding actual catch ($\log_{10}(n+1)$, Figure 4-1) appears to show that an optimum temperature (13°C approx.) for flight exists. (I used only the data points taken from within the flight phase, defined as the period 19.00 to 10.00 for the purpose of the analysis; see Sections 3.2, 3.7.1 and 4.4.2.) This simple interpretation may be wrong, however. Consider, for example, the catches taken at temperatures in excess of (say) 18°C. Do these higher temperatures really reduce flight activity, or are these catches low simply because such temperatures were only encountered at the beginning and at the end of the flight phase; when rhythmically-determined activity in the population had just started or was about to finish (refer to Sections 3.2 & 3.7.1)? Likewise, temperatures around the apparent optimum may have coincided with endogenous peaks of activity.

Naturally this is a major over-simplification of the field situation; each point is the product of the combined effect of many predictor variables and factors. It does, however, re-emphasise the point (made in the Introduction) that correlations between predictor and response variables may not be necessarily causative. Theoretically, the analysis should be able to isolate the effect of temperature – curvilinear, rectilinear or insignificant – from the other components of the model. A significant negative quadratic response (i.e. a significant negative parameter estimate for the higher order variable, **TEMPERATURE²**) may therefore indicate that *Culicoides impunctatus* has a real temperature preference, above and below which flight intensity is reduced.

The covariation of both the response and the predictor variables with time is not the only problem clouding the interpretation of this scatter diagram. Obviously, any change in population density between the trials will confuse the situation; so too will the non-orthogonality (i.e. inter-correlation) of the **x**-variables. Air temperature in this study (Section 3.6.1.a) was correlated with

light, wind ($r = 0.265$, $P < 0.1\%$) and humidity ($r = -0.645$, $P < 0.1\%$). Consequently, its effect will be difficult to determine both visually and mathematically. (When two variables are strongly correlated the analysis cannot be expected to apportion reliably variance between them (Mead & Curnow, 1983; Morton *et al.*, 1981; Vogt *et al.*, 1983; Walker, 1977; Wetherill, 1981; Williams, 1962).)

b) Relative humidity

The relationship of flight activity to relative humidity (R.H.) was studied in this analysis. Some dipterists involved in comparable work have, in contrast, adopted different measures of atmospheric moisture content and desiccating ability: vapour pressure (Haufe, 1967; Read *et al.*, 1978); vapour pressure deficit (V.P.D., Blau & Stinner, 1983; Dale & Axtell, 1975; Gerhardt & Axtell, 1972; Kettle & Linley, 1967b); dew point (Read & Adames, 1980; Wild, 1983); and evaporation rate (Burnett & Hays, 1974; Jamnback & Watthews, 1963). All are dependent on temperature. Evaporation rate – a composite measure, also determined by wind speed, the moisture content of the air and solar input – might be expected to be one of the principal variables governing flight activity of these minute Diptera (Jamnback & Watthews, 1963; Oke, 1978; Willmer, 1982; see 3.7.2). An atmometer compatible with the recording system available (Section 2.2) could not be found for use in this study.

Reuben (1963) observed that "catches of more than ten midges [*Culicoides impunctatus*] in an hour were only obtained when the relative humidity was above 60 per cent., and catches of over 100 per hour between 75 per cent. and 97 per cent. Very low catches were obtained at 100 per cent. relative humidity". Wild (1983) considered that there could be an optimum humidity range for flight which "might be very wide in some species". Onyiah (1971)

thought along similar lines, recording (for one of his analyses) 56–95% relative humidity as the favourable range.

Table 4-2

Some published work:The effect of atmospheric moisture on the activity of certain Ceratopogonids

(Significance levels: * = 5%, * * = 1%, * * * = 0.1%.

+ = positive response, - = negative response, n/s = not significant.

L.T. = Lower Threshold, U.T. = Upper Threshold.)

<u>Reference</u>	<u>Analysis</u>	<u>Units and/ or (interval)</u>	<u>Species</u>	<u>Response</u>
Jamnback & Watthews, 1963	field obs.	evaporation ml 2 min ⁻¹ (2 min)	<i>C. sanguisuga</i> females	- L.T. 1.8
Kettle & Linley, 1967b	M.L.R. & ANCOVAR	V.P.D. (15 min)	<i>L. becquaerti</i> females	n/s
Nelson & Bellamy, 1971	field obs.	R.H. % (15 min- 2 hours)	<i>C. variipennis</i> both sexes	L.T. 25
Onyiah, 1971	M.L.R.	mean daily R.H. (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	- - -
Onyiah, 1971	M.L.R.	R.H. 3 hours before sunset (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	+ + +
Read <i>et al</i> , 1978	field obs.	vap. press. millibars (hours)	<i>C. diabolicus</i> females	curvilinear U.T. 33.3

Read & Adames, 1980	field obs.	dew point °C (hours)	<i>C. diabolicus</i> females	curvilinear L.T. 18 U.T. 30
Reuben, 1959, 1963	M.L.R.	R.H. (hours)	<i>C. impunctatus</i> females	6ft n/s 3ft - *
Reuben, 1963	M.L.R. on 1st diff's	R.H. (24 hours)	<i>C. impunctatus</i> females	n/s
Walker, 1977	partial correlation	R.H. (hours)	<i>C. pallidipennis</i> <i>C. schultzei</i> grp both sexes	+ * * * + * - * *
Wild, 1983	M.L.R.	dew point (various)	"courting" <i>C. austropalpalis</i> gravid female <i>C. austropalpalis</i>	-ve quadratic * * * -ve quadratic * * *

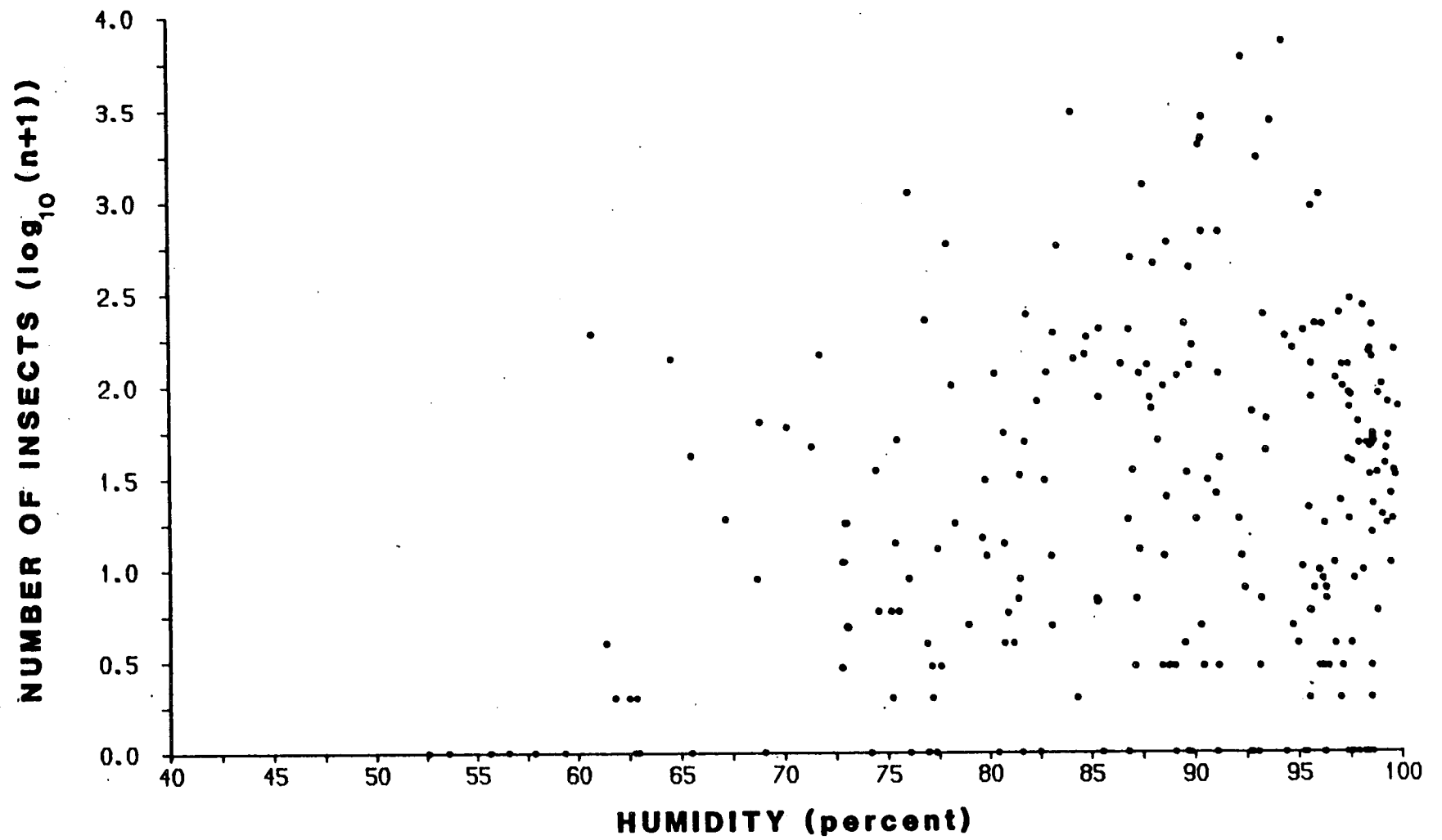


Figure 4-2. Scatter diagram: humidity and number of insects

Figure 4-2, a scatter diagram of catch size and the corresponding mean relative humidity for each half-hourly period, shows that in this study significant flight activity was recorded over a broad range of saturations (approx. 60-100%). Perusal of the distribution, however, indicates that catch size seems to increase with humidity up to a point (90-95%) after which an increase in saturation results in a diminished catch. (This accords well with Reuben's (1963) observation on the same species; see above.) The interpretation of this plot may, however, be confused by the aforementioned problems (Section 4.3.4.a).

c) Wind speed

Parker (1949) noted, with reference to his sweep-net catches in Scotland of *Culicoides impunctatus*, *C. pallidicornis*, *C. obsoletus* and *C. pulicaris*, that "Wind had a very pronounced effect; a sudden increase in its strength always resulted in an equally sudden diminution in the number of flying midges captured. Collections made during strong winds never yielded any adults". As is evident from Table 4-3, catch size is in general negatively correlated with wind speed. In fact, authors working on crepuscular/nocturnal midges have observed that even moderate breezes usually dramatically reduce flight activity. The flight periodicity of these species, as explained in Section 3.7.2, enables them to avoid higher wind speeds which typically prevail during the daytime (Figure 3-5); consequently, a strong flight capacity may not have been evolved or maintained.

By contrast, diurnally active species are necessarily stronger flyers. For example, Nathan (1981) found that *Culicoides phlebotomus* still flew in breezes in excess of 10-12 km hour⁻¹. Likewise, Kettle and Linley (1967a, 1967b), working in Jamaica, experienced considerable biting activity from the day-flying

ceratopogonid, *Leptoconops bequaerti*, in winds of up to 12.6 mph.

If midges are occasionally carried 'against their will' by winds in excess of their flight speed (as is believed by many authors, Kettle & Linley, 1967b; Koch & Axtell, 1979; Wild, 1983), or even at lower wind speeds sometimes utilise weak air currents for downwind locomotion, then the accuracy of any modelling exercise will be reduced. Indeed, trap catches should be correlated in part with wind speed, for insects will be carried into the zone of capture at a greater rate. Koch and Axtell (1979) noted that in light breezes ($< 3.2 \text{ km hour}^{-1}$) more *Culicoides furens* were caught in a wooded area than in a nearby "exposed" marsh. With an increase in wind strength ($3.2\text{--}5.0 \text{ km hour}^{-1}$), however, the situation was reversed. This result was attributed to "more wind-borne *Culicoides* being carried over the suction trap [located in the marsh,] helplessly".

Table 4-3

Some published work:The effect of wind on the activity of certain Ceratopogonids

(Significance levels: * = 5%, * * = 1%, * * * = 0.1%.

- = negative response, n/s = not significant. U.T. = Upper Threshold.)

<u>Reference</u>	<u>Analysis</u>	<u>Units and/ or (interval)</u>	<u>Species</u>	<u>Response</u>
Jamnback & Watthews, 1963	field obs.	mph (2 min)	<i>C. sanguisuga</i> females	- U.T. 1.5
Kettle, 1957	field obs.	(hours)	<i>C. impunctatus</i> females	-
Kettle & Linley, 1967b	M.L.R. & ANCOVAR	mph (15 min)	<i>L. bequaerti</i> females	- U.T. 15
Koch & Axtell, 1979	M.L.R.	(hours)	<i>C. furens</i> females males	* * * direction not given
Onyiah, 1971	M.L.R.	mean daily wind speed (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	- - -
Reuben, 1959	correlation	miles of wind (24 hours)	<i>C. impunctatus</i> females	- * *

Reuben, 1963	M.L.R. on 1st diff's	miles of wind (24 hours)	<i>C. impunctatus</i> females	- * * *
Travis, 1949	field obs.	mph	<i>Culicoides</i> spp.	U.T. 3.5
Walker, 1977	partial correlation analysis	m s^{-1} (hours)	<i>C. pallidipennis</i> <i>C. schultzei</i> grp sex not given	n/s U.T. 3 n/s U.T. 3
Wild, 1983	M.L.R.	(various)	"courting" <i>C. austropalpalis</i> gravid female <i>C. austropalpalis</i>	- * * * - * * *

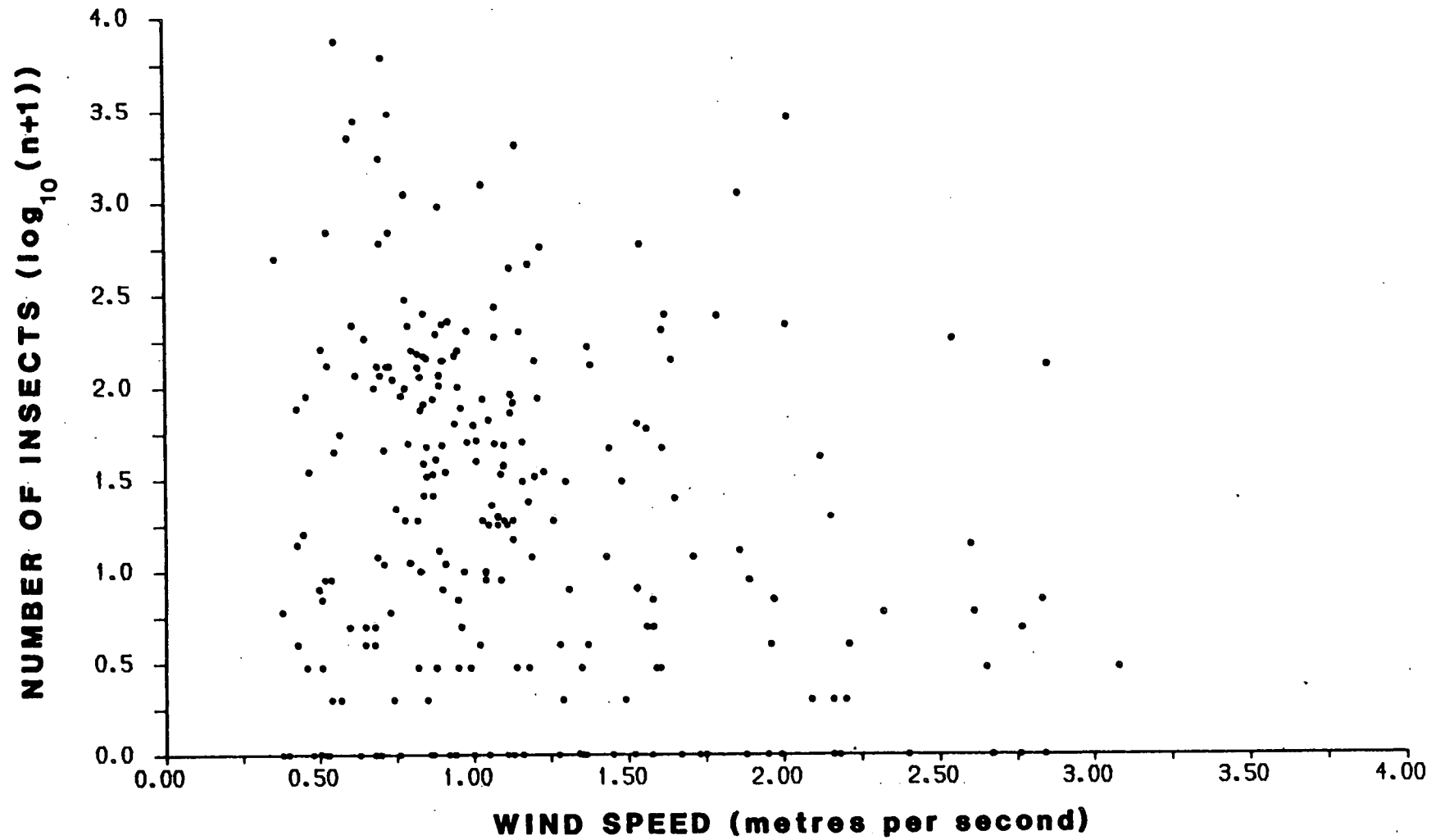


Figure 4-3. Scatter diagram: wind speed and number of insects

The scatter diagram of insect catch in relation to this environmental variable, Figure 4-3, indicates that a decrease in catch size was usually associated with an increase in wind speed; the interpretation of the plot is clouded by the problems noted in Section 4.3.4.a. The relationship, if truly inverse (i.e. if the parameter estimate for y 's response to the rectilinear term for wind speed proves to be negative), would seem far from robust. Considerable activity was noted in winds in excess of 2 m s^{-1} on a few occasions. This is significantly higher than their flight speed (approx. 60 cm s^{-1} , pers. obs.) and consequently supports the belief (see above) that midges may be dispersed passively (and probably 'involuntarily' unless migrants exist, Section 3.7.3) in strong air currents.

d) Light

"light [cycles] ... can entrain an endogenous oscillation by effecting phase-shifts which correct the endogenous period (τ) [of the activity pattern] to that of the environment [i.e. 24 hours]. In a large number of organisms, if not all, light has additional effects which are not to be confused with entrainment because they modify the level or occurrence of activity *directly*" (Saunders, 1977).

The extent to which light affects a field periodicity varies inter-specifically (Saunders, 1977, 1982). Changes in illumination at dusk and dawn may serve only to 'sharpen up' the activity peaks of crepuscular species which have an underlying rhythmically-determined bimodal periodicity (David Saunders, pers. comm.). Specific light intensities, or more probably rapid changes in illuminance, could have an excitatory effect on the activity of *Culicoides impunctatus* during the flight phase (Section 3.6.2.c & Appendix 1). Light may also exert a "masking" effect (Saunders, 1977); negative masking effects cause light to inhibit activity. These may only slightly modify an overt field activity

pattern. For instance, high light intensities might delay the dusk peak and curtail the dawn peak of activity in crepuscular species (David Saunders, pers. comm.). Alternatively, the natural light regime may almost totally override any rhythmic component to a periodicity. To illustrate, consider the work of Tychsen and Fletcher (1971) on *Dacus tryoni*, the Queensland fruit fly. They showed convincingly that although the endogenous phase (of the circadian oscillator) for "readiness to mate" permits mating for at least 14 hours, field light intensities concentrate this activity to 30 minutes around dusk. An illuminance of 0.8 lux ft^{-2} was found to be optimal for sexual activity. This example shows the futility of trying to model the behaviour of certain species using regression techniques; here Taylor's binary approach (Section 4.2) would be far more appropriate. The field periodicity of *Culicoides impunctatus* is obviously not as reliant on a specific and narrow band of illuminances as is that of *Dacus tryoni*. This midge not only flew under a broad range of light intensities (Figure 4-4), but also showed a similar flight periodicity under dramatically different light regimes (i.e. in the Field and Forest Sites, refer to Figures 3-6, 3-7 & 3-9). Even so, the role of illuminance in governing flight intensity is bound to be difficult to represent mathematically (see below).

Table 4-4

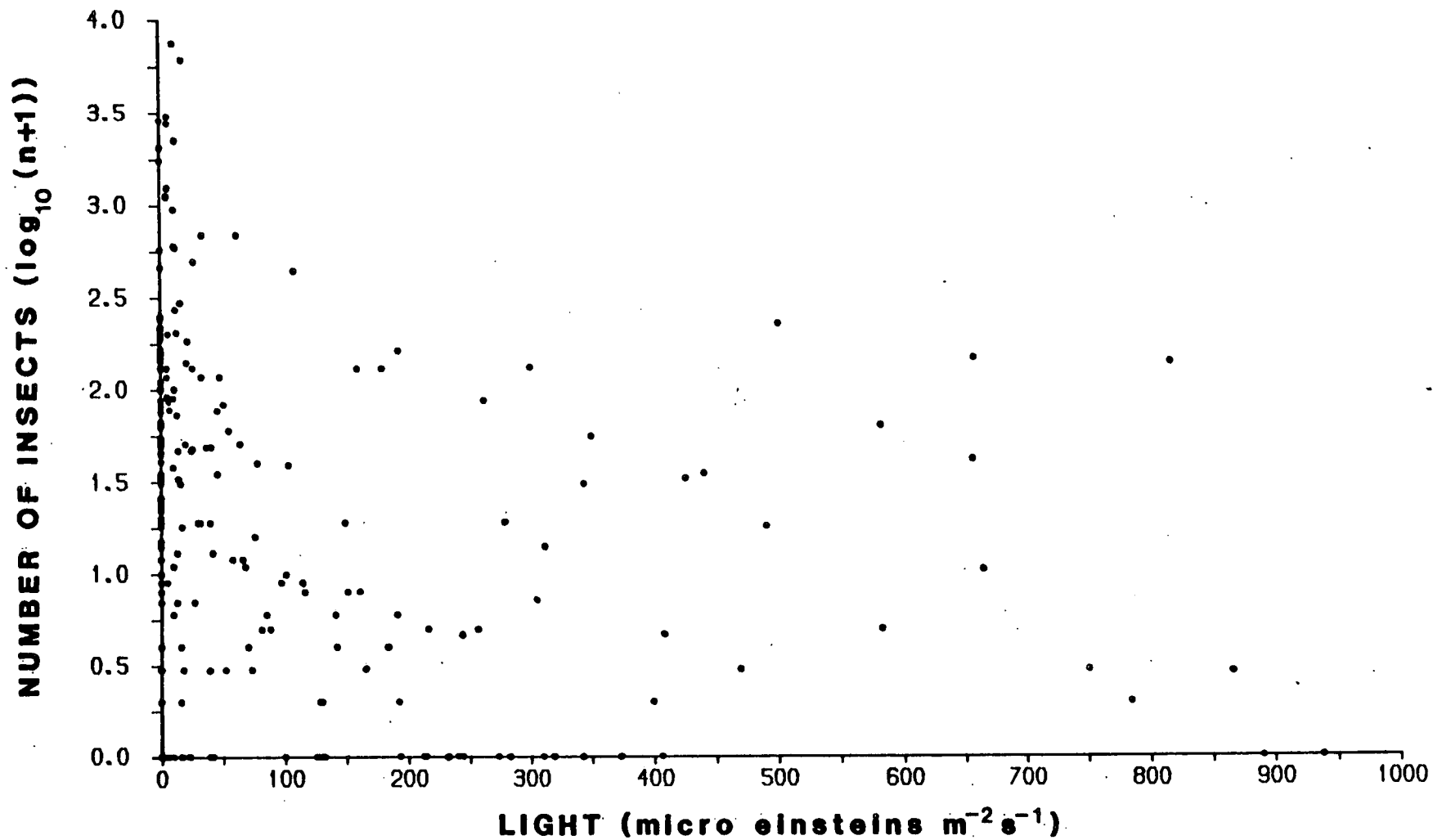
Some published work:The effect of light on the activity of certain Ceratopogonids

(Significance levels: * * = 1%. + = positive response, - = negative response.

U.T. = Upper Threshold.)

<u>Reference</u>	<u>Analysis</u>	<u>Units and/ or (interval)</u>	<u>Species</u>	<u>Response</u>
Kettle & Linley, 1967b	M.L.R. & ANCOVAR	(15 min)	<i>L. becquaerti</i> females	+
Onyiah, 1971	M.L.R.	mean daily radiation (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	+ + -
Onyiah, 1971	M.L.R.	radiation 3 hours before sunset (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	+ - +
Onyiah, 1971	M.L.R.	total daily sunshine hours (24 hours)	<i>C. impunctatus</i> <i>C. obsoletus</i> grp <i>C. pallidicornis</i> both sexes	- - -
Reuben, 1959, 1963	ANOVAR	cal cm ⁻² hour ⁻¹ (hours)	<i>C. impunctatus</i> females	U.T. 22.7

Wild, 1983	M.L.R.	(various)	"courting"	- * *
			<i>C. austropalpalis</i>	
			gravid female	- * *
			<i>C. austropalpalis</i>	



The actual half-hourly catches of insects ($\log_{10}(n+1)$) and the corresponding mean light intensities are plotted in Figure 4-4. A high percentage of the data was taken in 'complete darkness' (Section 2.2.5). The failure to monitor nocturnal illuminances must cast doubt on the parameter estimates obtained for y 's response to different light levels. It should be noted, however, that even if all light intensities of biological significance had been monitored, it is still highly unlikely that a simple rectilinear or quadratic function could describe the relationship adequately – especially considering the possible masking and excitatory effects of light.

e) Rain

The effect of rain on midge flight activity has, in comparison to other weather variables, been little studied. Trap catches of *Culicoides marksii* Lee & Reye, *C. austropalpalis* and *C. brevitarsis* Kieffer were found to be unaffected by rainfall (Wild, 1983). Onyiah (1971), likewise, failed to find a correlation between total daily rainfall and daily catch of *Culicoides impunctatus*, *C. obsoletus* and *C. pallidicornis*.

A few observations – all anecdotal – suggest that drizzle may stimulate activity in *Culicoides impunctatus*. I have certainly experienced considerable activity around noon in fine rain. It is not clear, however, whether my presence induced the attack or whether the drizzly conditions had actually initiated flight in previously resting individuals (Section 3.7.1).

Owing to the size of the data set and the relative infrequency of precipitation during the eight trials, only a very rough estimate of rain's effect on flight activity could be made. The data were thus divided into those half-hourly periods in which rain – however strong – of longer than approximately ten minutes duration was or was not recorded. Catches taken 'in

the rain' were assigned the factor level 1 (see Appendix 2).

f) Wind direction (WDIR)

Wind direction would be expected to be significant in the model if, as considered previously (Section 4.3.4.c), midges are occasionally carried passively in air streams. Winds blowing from areas with an abundance of resting sites might, therefore, be insect-laden; whereas, winds blowing over a loch or an arable area, for example, might be relatively insect-free..

Wild (1983) found that catches of *Culicoides brevitarsis* showed almost no relationship to wind direction; this species bred in widely scattered substrates in his study site. By contrast, collections of *Culicoides austropalpalis* were found to be dependent on the direction of the prevailing wind. This, Wild believes, indicates that *Culicoides austropalpalis* had a single discrete breeding site located to one side of his trapping device.

In this study, an estimate of the effect of wind direction was obtained by factorising (see 4.3.2) the catches according to whether, at the time of collection, the average wind direction was from the land (factor level 1) or from the loch (factor level 2). This division was necessarily rough (refer to Figure 3-1).

4.4. Selecting the data for the analysis

4.4.1. Source of the data

The data used in the M.L.R. analysis (listed in Appendix 2) were taken from the eight trials of the flight periodicity experiment described in Chapter 3. In each 24-hour trial, half-hourly insect catches were taken by two trapping

techniques (suction trap & human bait) from each of two study areas (the Field & Forest Sites). Of the four sets of diel flight activity data obtained, only the catches taken by the suction trap from the Field Site were modelled. Why? Firstly, suction-trap catches, as explained in Section 3.4.2, are likely to be more representative of a population's mean activity for any half-hourly period. Secondly, the suction traps caught significantly more insects than the human baits. In consequence, parameter estimates calculated from the suction trap data should be comparatively less adversely affected by the addition of 1 to the 'raw' catches (refer to Section 4.5, 'Transforming the response variable'). Thirdly and finally, the broader range of environmental conditions encountered in the Field Site (Figures 3-3, 3-4, 3-5 & 3-6) was necessary to demonstrate clear responses (if they exist) between the *x* and *y* variables, thereby reducing the ambiguity of the results (Kettle & Linley, 1967b; Snedecor & Cochran, 1980).

4.4.2. The 'daylight hours' catches: their exclusion

Only the data collected between the hours of 19.00 and 10.00 (termed the flight phase, refer to Sections 3.2 & 3.7.1) of the eight trials of the flight periodicity experiment were used in the M.L.R. analysis. Thus, I am assuming that *Culicoides impunctatus* is programmed rhythmically to be inactive during the 'daylight hours'; consequently, it makes sense to exclude this period from the *activity* model (Section 4.1). (It should be remembered that the definition of the flight phase is not claimed to be precise.) Is this a warranted assumption? Laboratory studies, of the type described in Section 3.2, would most probably have answered this question. Although I lack these data, I consider that the results obtained in the flight periodicity experiment (refer to Sections 3.6.2 & 3.6.3 and the subsequent discussion, 3.7.1) do, to a great extent, support the exclusion of the 'daylight hours' catches from the analysis.

Comparison of the two Forest Site catches, one by a suction trap and one by a human bait, indicates that midges would remain fairly inactive between 10.00 and 19.00 unless stimulated by the presence of a host. The human bait was considered to have induced flight in opportunistic resting individuals. Consequently, it seems that although environmental conditions did not actually prevent flight activity in the Forest Site during the 'daylight hours', the overwhelming majority of this period may not be included in the flight phase of the species. The 'daylight hours' can therefore probably be regarded as approximately synonymous with the rest phase of a circadian periodicity.

4.4.3. Thresholds

Although the crepuscularity/nocturnality typical of the genus has probably evolved partly in order to avoid limiting weather conditions (Section 3.7.2), environmental thresholds for flight may still be exceeded on occasions during the normal flight period. For example, owing to an exceptionally clear night sky in Trial 5, nocturnal mean temperatures of below 6°C were recorded between 00.30 and 06.00; the lowest being 2.81°C (04.30–05.00). At these temperatures midges would be physically incapable of flight (Trevor Lewis, pers. comm.; Sir Richard Southwood, pers. comm.); the inclusion of these data points would therefore tend to reduce the validity of the model.

(In Trial 5, significant activity was recorded around 7°C. (40 *Culicoides impunctatus* were caught at a mean temperature of 7.03°C, 23.30–24.00. See also Trial 7, 03.30–06.00.) Insects in Trial 5 were still, however, being caught below 6°C, down to a mean temperature of 4.19°C (consecutive half-hourly counts of 3, 2 & 1). These very low counts could represent actual flight activity. Alternatively, they might have resulted from insects – actually sucked in during the preceeding activity maximum – finally reaching the collecting

receptacle. Although the gauze was agitated each time the trap was changed, it is likely that a few midges were trapped in the mesh subsequently to be dislodged during the collection for a later time interval.)

Should these points beyond this predicted lower temperature threshold be removed? (No laboratory-determined data for the lower temperature threshold for flight of *Culicoides* spp. are available.) Although the case for omitting them is strong, they were left in the data set. To remove them would have meant that other selective exclusion arguments could then have been legitimately raised for the insects' response to the other exogenous factors included in this model. For example, the wind speed threshold for flight was probably exceeded relatively more frequently during the crepuscular and nocturnal periods than the temperature threshold.

4.5. Transforming the response variable

A logarithmic transformation of the response (y) variable was necessary on two accounts. To begin with, it seems more sensible biologically for the model to be multiplicative as opposed to additive in form (Mead & Curnow, 1983; Vogt *et al.*, 1983; Williams, 1937; Chris Theobald, pers. comm.; David Williams, pers. comm.). To illustrate: consider two catches, 100 (C_1) and 1000 (C_2), taken at temperature T_1 . The model adopted assumes that if C_1 increases to 200 in response to a favourable increase in temperature (T_2), then C_2 at T_2 will be 2000 (i.e. will have increased in proportion to its original level) and not 1100 (which would represent a simple additive increase). In addition, the logarithmic transformation should stabilise the variance; that is, render the variance relatively independent of the mean. This is a prerequisite of any regression analysis (Mead & Curnow, 1983).

Thus the usual transformation for data of this type, $Y' = \log (Y+1)$ was used; where Y equals the actual catch of insects for any half-hourly period.

4.6. The statistical model

$$\begin{aligned}
 Y' = & \beta_0 + \beta_1(\text{TEMPERATURE}) + \beta_2(\text{HUMIDITY}) + \beta_3(\text{WIND SPEED}) + \beta_4(\text{LIGHT}) \\
 & + \beta_5(\text{TEMPERATURE}^2) \dots\dots \\
 & + \text{CONSTANT FOR TRIAL} \\
 & + \text{CONSTANT FOR TIME OF DAY} \\
 & + \text{CONSTANT FOR THE EFFECT OF RAIN} \\
 & + \text{CONSTANT FOR THE EFFECT OF WIND DIRECTION} \\
 & + \text{ERROR TERM}
 \end{aligned}$$

It is important to realise that although the factors enter the model as constants, the value of each constant depends on, for example, the time of day when the catch was taken or whether or not it was raining at the time of collection (refer to Table 4-5).

The data were analysed using the Royal Statistical Society's regression analysis package, GLIM (Generalised Linear Interactive Modelling, Baker & Nelder, 1978; Richardson, 1982). The 'best regression equation' (i.e. an equation including only the influential x -variables and factors) was determined principally by using a mixture of 'backward' and 'stepwise' regression techniques (Draper & Smith, 1966; Wetherill, 1981). The only obvious constraint regarding variable deletion was that the quadratic term of a variable could not exist in the model without the associated rectilinear function (Chris Theobald, pers. comm.; David Williams, pers. comm.); the rectilinear term is required to scale the higher order

variable. A blind reliance should, however, never be placed on an 'automatic' selection technique: Draper and Smith (1966) emphasise the need to incorporate into a model an understanding of the phenomenon in question (see below).

4.7. Results

The results of the multiple linear regression analysis are listed in Table 4-5. One can seldom find an absolute 'best' regression equation for problems of this nature: frequently, the final model depends on the selection technique adopted. Additionally, the regression equation obtained using an accepted selection routine may yield results that are counter-intuitive. In such situations, one's knowledge of a species should override statistical considerations (David Williams, pers. comm.). For example, the quadratic function of light was found to be marginally superior (in a statistical sense) to its rectilinear term. The inclusion of **LIGHT²**, however, made the effects attributable to **TIME** incredible biologically (see below); **LIGHT²** was, in consequence, removed from the equation.

Table 4-5

Multiple linear regression analysis: results

(Significance levels: * = 5%, * * = 1%, * * * = 0.1%, n/s = not significant.)

<u>Factor or x-variable</u>	<u>Parameter estimate</u>	<u>Standard error</u>	<u>Significance</u>
TRIAL			* * *
Trial 1	reference	level	
Trial 2	- 1.59	0.56	* *
Trial 3	0.30	0.43	n/s
Trial 4	- 0.93	0.51	n/s
Trial 5	- 1.58	0.75	*
Trial 6	1.31	0.47	* *
Trial 7	0.57	0.56	n/s
Trial 8	0.29	0.48	n/s
TIME			* * *
19.00-20.00	reference	level	
20.00-21.00	0.40	0.54	n/s
21.00-22.00	1.99	0.59	* * *
22.00-23.00	3.02	0.62	* * *
23.00-24.00	2.49	0.63	* * *
24.00-01.00	0.73	0.64	n/s
01.00-02.00	- 0.12	0.65	n/s
02.00-03.00	- 0.12	0.65	n/s
03.00-04.00	0.81	0.66	n/s
04.00-05.00	1.39	0.66	*
05.00-06.00	1.53	0.65	*
06.00-07.00	0.44	0.62	n/s
07.00-08.00	0.26	0.59	n/s
08.00-09.00	- 0.35	0.59	n/s
09.00-10.00	- 0.42	0.62	n/s
TEMPERATURE	0.67	0.13	not applicable
TEMPERATURE² (X 100)	- 3.02	0.45	* * *
WIND SPEED	- 1.03	0.21	* * *
LIGHT (X 100)	0.27	0.10	* *

 $R^2 = 59.56\%$, F (overall) = 12.76 * * *

4.8. Discussion

The model accounts for almost 60% of the variation in the data. It should, however, be remembered that the total sum of squares were calculated on a logarithmic scale (i.e. $\log_e$). Consequently, the discrepancy between the actual catches and the predicted values may be considerable.

An analysis of the residuals (i.e. $\log_e$ observed values - $\log_e$ fitted values) indicated a tendency for high catches to be underestimated and for low catches to be overestimated. Although the problem was identified, it seemed imprudent - considering the large uncertainty in the data - to modify the linear functions adopted, in an attempt to obtain a more accurate equation.

Rectilinear and quadratic terms were investigated to see whether - in the most approximate sense - log catch size either varied in a constant manner, or (for example) increased and then decreased, with respect to a change in some environmental variable over the range of values observed in the study. A cubic function for (say) temperature (i.e. **TEMPERATURE³**) could have improved the 'fit' of the model. Even if the inclusion of this particular higher order term had reduced the residual deviance, its presence in the model might not represent an improvement in biological terms. One could be simply "fitting noise" in the data: sums of squares of no actual significance to *Culicoides impunctatus* (David Williams, pers. comm.). It would, therefore, be incorrect to state with any certainty that one higher order function of any variable is preferable biologically to another. Similarly, as noted in Section 4.2, the data are too 'noisy' and the statistical approach is too weak to justify the analysis of the myriad of interaction terms between the x-variables and factors. Although some of these may be important as regards the flight activity of this insect, the analysis cannot be expected to separate them with any reliability.

4.8.1. TRIAL

The activity equation predicted that the actual density of *Culicoides impunctatus* in the trapping area was greatest in Trial 6; followed, in descending order, by Trials 7, 3, 8, 1, 4, 5 and 2 (Table 4-5). The population density estimate for Trial 6 was significantly greater ($P < 1\%$) than that for Trial 1, the aliased parameter; whereas, the number of insects available for capture in Trials 5 and 2 was predicted to be substantially lower than the reference level ($P < 5\%$ & $P < 1\%$, respectively).

The overall effect of **TRIAL** was calculated to be very strong ($P < 0.1\%$). If, however, the **x**-variables and other factors failed to describe their respective components of the model accurately (which I consider is highly likely), then sums of squares – more properly assigned to other elements – may have been taken up incorrectly by this factor. This is an unavoidable problem where there are strong correlations between the constituents of a model (Section 4.3.4.a): the relative magnitude of the parameter estimates must, therefore, be interpreted with caution.

4.8.2. TIME

Activity – independent of the weather and population effects *accommodated by the model* – was predicted to have peaked at both 22.00–23.00 and 05.00–06.00 (B.S.T., refer to Table 4-5). The parameter estimates (calculated on a natural log scale) for the dusk and dawn peaks were markedly unequal and seemed to represent the field situation quite well (refer to Figure 3-9).

Do these results offer any evidence for the presence of an underlying circadian rhythm controlling activity? Although I have strong *a priori* reasons

for suspecting that flight activity in *Culicoides impunctatus* does have a rhythmic component (Chapter 3), it would, I feel, be naive to suggest that the estimates of the statistical model support my belief. The result could simply indicate that the equation devised was incapable of reliably portraying the true field situation. To reiterate: in a model that fits the data poorly, a factor such as **TIME** – so strongly correlated with the x-variables – would be expected to take up sums of squares more rightly associated with the prevailing weather conditions.

The limitations of statistics for problems of this type became progressively clearer to me: my initial aim of separating the effects of weather and rhythms has not been possible. Nevertheless, the incorporation of **TIME** into the equation, in combination with the exclusion of the data taken from the 'daylight hours' (the rest phase), should, I believe, have partly avoided the problem of 'nonsense correlations' discussed in the Introduction.

4.8.3. Temperature

A negative quadratic term for temperature (**TEMPERATURE²**) was found to be highly significant in the model ($P < 0.1\%$). Flight activity was calculated to have peaked at 11.16°C; the decrease in catch size associated with a departure from this optimum was assumed – by necessity – to be symmetrical (i.e. the same, irrespective of direction).

Reference both to Figure 4-1 and to the review of the literature (Section 4.3.4.a) indicates that this is a credible result. Moreover, it is a physiologically reasonable finding. Willmer (1982) reasoned that animal species will have an optimum temperature for activity "because physiological and biochemical mechanisms may be subverted at either extreme of the temperature range. Not only does the rate of each enzymic reaction depend upon temperature, but

the rates of reaction of other larger-scale processes (muscle activity, heart-beat, etc.) have different Q₁₀'s so that changes of temperature can disturb the balance between otherwise *complementary* phenomena". (The italics are mine.) In Section 3.7.3, it was argued that those insects which were caught under sub-optimal conditions could have possessed an atypically strong flight motivation. Such individuals may, therefore, be 'prepared' to tolerate a greater physiological stress. This, it will be noted, is largely conjecture.

4.8.4. Relative humidity

Both HUMIDITY and HUMIDITY² were observed to make an insignificant contribution to the explanatory power of the model. Within the range of humidities encountered in this study during the flight phase, it is certainly possible that this variable has little or no effect on flight intensity. If true, such a finding would support the feelings of Wild (1983, Section 4.3.4.b). Alternatively, the result could be an artefact of the experimental and statistical limitations.

4.8.5. Wind speed

The parameter estimate for WIND SPEED (i.e. the rectilinear term) was found to be negative ($P < 0.1\%$). In view of the discussion presented in Section 4.3.4.c, I feel that confidence can be placed in the sign of the response. Whether or not the magnitude of this parameter estimate is reasonable, however, is a matter of debate. The model predicted that flight would have ceased at approximately 8.7 m s^{-1} . This would naturally be a ridiculous result if midges only remained airborne in breezes lower than their flight speed. Figure 4-3 and Section 4.3.4.c indicate, however, that this is not the case. Even

so, I would have expected a greater parameter estimate for this variable; indicating a more rapid reduction in flight activity with increasing wind speed.

4.8.6. Light

The problems of modelling flight activity with respect to light were raised in Section 4.3.4.d. Indeed, I believe that the task is almost impossible. As noted above, the inclusion of **LIGHT²** was found to be preferable statistically to the rectilinear form of this variable. Incorporating the quadratic term, however, corrupted the parameter estimates related to **TIME**. More specifically, whilst still indicating a bimodal flight periodicity based around dusk and dawn (supposedly independent of weather and other factor effects), a model which included **LIGHT²** predicted that 'rhythmically-determined' flight intensity was *substantially* higher at the beginning of the flight phase (19.00–20.00) than during the period 24.00–03.00. Although it would be almost impossible to fully substantiate, I believe that this result stems from the inadequacies of both the analysis and the field work – especially the errors involved in measuring this variable (refer to Section 2.2.5). The term **LIGHT²** was, therefore, excluded. This move is definitely vulnerable to criticism; intuition and biological considerations took precedence.

4.8.7. RAIN and WIND DIRECTION

Both **RAIN** and **WIND DIRECTION** were found to exert almost no control over the magnitude of midge catches. It is unlikely that these findings represent reality. In fact, had a larger data set been collected, I think it probable that both factors would have been shown to have a possibly small though significant effect on catch size. In retrospect, I regret not having

amassed a greater number of data points on which to perform the analysis.

4.9. Conclusion

The work described in this chapter represents an attempt to produce a simple yet plausible 'rhythmic' model of the flight activity of *Culicoides impunctatus*. The initial aims of the study have not, in my opinion, been fully realised. During the formulation and fitting of the activity equation, it became increasingly apparent that standard regression techniques – though preferable to other analytical tools – are ill-suited for describing the intricacies of flight behaviour in the field (Section 4.2). All the results have, therefore, been interpreted with the required degree of caution.

The method of 'least squares', the 'traditional' approach to entomological modelling, was used. Recently, however, Generalised Linear Models (G.L.M.'s, McCullagh & Nelder, 1983) have been applied to problems of this type (Morton *et al.*, 1981; Vogt *et al.*, 1983, 1985). A log-linear model, one of many varieties of G.L.M., was investigated in this work. Regrettably, stable parameter estimates could not be obtained using GLIM; in consequence, the method of least squares was resorted to.

(A log-linear model assumes that the logarithm of the expectation of making a catch of a given size is a linear function of the predictor variables and factors. This contrasts with the approach adopted in this study, in which the expectation of obtaining a logarithmically-transformed catch of a certain magnitude is modelled. For high insect counts, the results obtained using a log-linear model should be only marginally superior (Vogt *et al.*, 1983; David Williams, pers. comm.). When catches are consistently low, however, the discrepancy between the results increases; the failings of a least squares approach are augmented further by the necessity to transform the response

variable (Section 4.5).)

In spite of its greater statistical elegance, a log-linear system would have still greatly over-simplified the complexities of flight activity. At best, a regression system can only be expected to demonstrate which of the predictor variables and factors are most influential in the actual control of activity (Section 4.2). The results are "suggestive" and not "predictive" (Wetherill, 1981). All too frequently, overly-precise predictions are made in the literature based on parameter estimates obtained from ill-fitting models. This represents an abuse of statistics and is to the detriment of the field. Entomologists – indeed biologists in general – must learn to use statistics with care.

Temporal attack patterns of *Culicoides impunctatus* and *C. obsoletus* grp

5.1. Introduction

The temporal landing pattern of mosquitoes on a human, Service (1973) observed, is "not usually regular, but shows an aggregated or patchy type of distribution. That is, catches are characterised by intervals of several seconds, or minutes, with few or no mosquitoes arriving, followed by the sudden arrival of several adults almost simultaneously". Pilot human-bait catches performed in this study indicated that a similar landing distribution may obtain to midges (almost exclusively *Culicoides impunctatus* & *C. obsoletus* grp females). Are these attack patterns, however, truly non-random? In other words, does the landing distribution differ significantly from that predicted by a *Poisson process*; the model by which it should be most closely approximated if each event (i.e. arrival) had occurred independently (Cox & Lewis, 1966; Cox & Snell, 1981; Chris Theobald, pers. comm.)? It should be remembered that in a Poisson process one would expect a relative preponderance of short inter-arrival times. This could conceivably be mistaken for a clumped landing distribution with respect to time.

A simple field experiment was devised to test whether the *Culicoides* midges present in the study area attacked the upper forearm of a human bait in a completely random manner. The investigation was combined with, and in a field situation inextricably linked to, an analysis of the longer-term temporal landing distribution: the variation in attack rate over periods of one hour.

Many authors have noted that the attack intensity of Nematoceros blood-sucking Diptera typically reaches a maximum between 10 and 20 minutes after the arrival of a host at a site in which hungry insects are *resting*. After this peak, a significant decline in biting rate with time has usually been recorded. For instance, Service (1971a), using daytime one-hour human-bait catches, found that peak numbers of the crepuscular/nocturnal mosquito, *Aedes cantans*, were obtained 15–20 minutes after he entered an area of dense shaded woodland. (The consecutive mean five-minute catches ($n = 111$), calculated from the percentage of each trial's total catch obtained in the separate five-minute periods, were as follows: 8.8%, 10.9%, 14.0%, 16.5%, 9.5%, 7.8%, 6.4%, 5.8%, 5.7%, 5.8%, 4.7% & 4.1%; 50% (approx.) of the catch was therefore taken in the first 20 minutes.) Jamnback and Watthews (1963), likewise, noted a long-term fluctuation in the landing rate of *Culicoides sanguisuga*. Maximum catches were obtained, on average, 10–12 minutes after the start of the human-bait catches; at the end of the 30-minute experimental periods, the mean attack intensity had fallen to less than half of its peak rate. Kettle (1957), Gluchova (1958) and Onyiah (1971) have recorded similar findings.

5.2. Materials and methods

5.2.1. Experimental procedure

The experiment was performed at Site C (see Figure 2-2) within the shelter of a spruce forest. Human-bait catches (refer to Section 2.4), of 60 minutes duration, were performed between the hours of 10.00 and 19.00 (B.S.T.); the putative rest phase of both *Culicoides impunctatus* and *C. obsoletus* grp females (consult Sections 3.2, 3.6.2, 3.6.3, 3.6.4 & 3.7.1). Only induced or opportunistic attack activity was therefore monitored.

For each 60-minute catching period (trial), I moved rapidly into a 'new' area of forest (carrying a chair and other necessary equipment), sat down and immediately started a stop-watch. (A site in the forest could not constitute a 'new' area, if a human-bait catch had been performed within 30 metres (approx.) of it during the same rest phase. See Section 5.4.) The arrival time of each insect that landed on the target area (21 cm X 5 cm, drawn on my left forearm) within the next 60 minutes was recorded. The arm was held immobile, fairly close to the body (Figure 2-6). Midges were not permitted to feed. Instead, an attempt was made to lift each insect gently off the target area with a numbered sticky label (approx. 3 cm X 1.5 cm). These were stored for later examination. A few insects avoided capture (in general, between 5% and 20% of an hour's total). The inevitable failure to catch all the midges which alighted restricted the study to considering the temporal landing distribution of all species together, not separately.

It can be appreciated that it was almost impossible – especially when subject to multiple attack – to note with precision the arrival time of each insect. Consequently, the following procedure was adopted: first, landing times were generally recorded to the nearest five-second point on the time scale (for example, 145, 150, 155 seconds, etc). (Very occasionally, times accurate as far as possible to the nearest second were taken. This inconsistency in the timing of the arrivals is, I believe, of no real significance to the investigation.) Secondly, when two insects were observed to arrive more or less simultaneously, or when another midge was found to have alighted after the stop-watch had been rapidly consulted to time a previous landing, a fairly arbitrary inter-arrival time of two seconds was used. Once again, this approximation should have introduced a relatively miniscule amount of error into the data.

Of far greater significance to the suitability of the experimental procedure to the phenomena under investigation, was the failure to consider those insects which landed outside the target area. If one assumes that midges frequently attack in unison, then it is certainly possible that, for example, a solitary insect alighting in the target zone could have been induced to attack by, or have stimulated attack in, another midge which landed elsewhere (e.g. on the underside of the arm). The strength of any 'mass attack' behaviour – if indeed it is shown by *Culicoides impunctatus* and *C. obsoletus* grp females – would, therefore, have been diluted by this unavoidable limitation to the field study.

Many trials had to be abandoned or discarded either because the attack intensity was too great to permit the accurate execution of the experiment, or because too few insects were caught for the data to be suitable for the analysis. A lower limit of 25 midges in an hourly period was used. For the inter-arrival time study, however, the cut-off point was later set at 40 insects hour⁻¹.

The work was conducted in the late August and early September of both 1983 and 1984. Population densities were low at these times; all attempts to collect data during June, July and early August failed owing to the inundation of the bait.

5.2.2. Statistical theory

Only the 22 hours which yielded 40 or more insects were used for the inter-arrival time analysis. A data file was created consisting of the time of landing (in seconds), of each of the 1388 midges (all species) which alighted, measured from the beginning of the respective hourly period. The landings were factorised according to the trial from which they came; that is, they were classified into 22 levels by the factor **HOUR** in precisely the same manner as

described in Section 4.3.2.

The preliminary calculations on the data (e.g. the computation of the inter-arrival times) and the majority of the final analysis were carried out using GLIM (Baker & Nelder, 1978; Richardson, 1982). Probability plots and other explorative graphic material were output through GENSTAT (Alvey *et al.*, 1980) to a graph plotter; necessary since large, detailed, informative plots are unattainable directly from GLIM.

In the Introduction, it was stated that a fully-random landing distribution with respect to time would conform most accurately to a Poisson process. To illustrate, consider a variable N_I , the number of arrivals within an arbitrary time period of length I (say 60 seconds). If, in this study, the temporal landing pattern were completely random, then N_I would have a Poisson distribution of mean λI . The constant λ , a measure of the mean landing rate over a substantial time period (e.g. 10 minutes), is termed the *probability rate of occurrence* of events (Cox & Lewis, 1966).

Symbolically, the Poisson distribution becomes:

$$\text{prob. } (N_I = k) = \frac{(\lambda I)^k e^{-\lambda I}}{k!} \quad (k = 0, 1, 2, \dots)$$

If one could safely assume that λ had remained constant throughout the experiment, then a test of randomness could be made by comparing the observed distribution of events over a set of time periods of length I , with that predicted by the equation above (χ^2 test, Mead & Curnow, 1983). (The reader is referred to Chapters 1, 2 (Sections 2.1 & 2.2) and 3 (Sections 3.1, 3.2 & 3.3) of Cox & Lewis', *The Statistical Analysis of Series of Events* (1966).)

By considering the distribution of arrivals over necessarily large time intervals, however, one is wasting a considerable amount of information in the data (Chris Theobald, pers. comm.). This can be avoided by analysing the inter-arrival times (T_i) which, if randomly generated, will show an *exponential* distribution (Cox & Lewis, 1966; Cox & Snell, 1981; Mead & Curnow, 1983). (The probability density function (p.d.f.) of T_i with respect to time t equals $\lambda e^{-\lambda t}$.) A test of randomness can then be made by comparing the pattern of the observed inter-arrival times with the corresponding exponential distribution dictated by the parameter λ (Cox & Lewis, 1966; see below).

The standard Poisson process assumes, however, that the overall rate of arrival, λ , remains constant within and between the trials of the experiment. This requirement is unlikely to have been satisfied in this work. First, λ is positively related to the absolute number of hungry insects that were prepared to attack in the zone of attraction (Section 5.4) around the human bait. In this, there is bound to have been a significant inter-trial variation. Why? To begin with, the experiment was conducted over two field seasons (see above). Additionally, even if the study had been completed within a comparatively short period of time, the resting population around the bait could not be assumed to have been constant in the different areas of the forest. Service (1971a), amongst others (Kettle & Linley, 1967a, 1968, 1969; Service, 1973, 1976; Wild, 1983), has noted that, in what appears to be a fairly uniform habitat, insects – in this case the mosquito, *Aedes cantans* – show a distinctly-patchy spatial distribution. Finally, the attack rate would have been affected by the weather conditions prevailing during a trial (Chapter 4). This last should have been comparatively insignificant: typically the trials were conducted in stable anticyclonic conditions.

Second, as noted in the Introduction, landing rates – and, in consequence, λ – would be expected to have varied over the course of each hour. This

leads to the idea of a *time-dependent Poisson process* (Cox & Lewis, 1966); that is, a Poisson process in which there is a temporal fluctuation in expectation. The arrivals, however, remain independent of one another. (Both Cox and Lewis (1966, 'Coal-mining disasters', Example 3.1, p. 42) and Cox and Snell (1981, 'Admissions to intensive care unit', Example A, pp. 53–57) deal with problems of a similar nature.)

To test for randomness, both sources of variation in λ must first be described mathematically. This is done by assuming a slowly-varying time-dependent rate function ($\lambda_h(t)$) for each trial. $\lambda_h(t)$, in this example, can be represented by one of two equations:

$$A) \lambda_h(t) = \exp (\alpha_h + \sum_{k=1}^r \beta_k t^k)$$

$$B) \lambda_h(t) = \exp (\alpha_h + \sum_{k=1}^r \beta_{hk} t^k)$$

h = trial number

k = a small integer (1 to r , describing the power of the rate function of time; see below)

Equation A includes both an element to describe the variation in insect number between the trials (the intercept, α), and a slowly-changing multiplicative time effect common to the trials. Equation B, a slight modification of this, differs in that it incorporates the possibility that the long-term landing rate may have varied significantly between the trials.

Fitting the factor **HOURL** in a GLIM model defines the value of the intercept for the trial under question. A log-link, gamma error structure and Scale of one were set. Such a combination of input directives corresponds to exponentially-distributed inter-arrival times with rate $\lambda_h(t)$ in hour h .

The choice between Equations A and B, and of the value of k , was made by 'exploring' the adequacy of various models; the reduction in deviance (twice the *maximum of the likelihood*) being related to χ^2 . If catches of one hour duration are typically characterised by an increase and then a decrease in landing rate (see Section 5.1 & Figure 5-1), then, for example, a quadratic ($k = 2$) or a cubic ($k = 3$) rate function may be able to describe the general trend in λ . This, as mentioned above, may either be specific to a single trial or a reasonable approximation for all of them.

Under the model of 'best fit', the inter-arrival times, T_i , should be – assuming that the model is correct – approximately exponentially distributed with expectation $1/\lambda_h(tm_i)$; where tm_i is the mid-point of the corresponding interval between arrivals. The GLIM package calculates fitted values $\hat{T}_i$, say, which are estimates of these expectations. To test the validity of the model, one examines the 'residuals', calculated as $T_i/\hat{T}_i$. If the temporal attack rate were random, then the residuals should also be approximately exponentially distributed, but now with unit expectation.

The assumption of independent exponentially-distributed inter-arrival times (i.e. of randomness) could then be tested using an *exponential probability plot*. For this, residuals, placed in ascending order, are plotted against the *expected order statistics* for a unit exponential distribution. A significant departure from linearity would indicate that midges tended to land in a non-random manner with respect to time.

It is, however, preferable to calculate $v_i = 1 - \exp(-T_i/\hat{T}_i)$, for which the expected distribution under the model is uniform (approx.) on the interval [0 to 1]. Deviations from uniformity (and, therefore, randomness) can then be inspected visually by first ordering the v_i and then plotting $v_i - u_i$ against u_i ; $u_i = i / (\text{number of observations} + 1)$. (This plot overcomes the disadvantage of the exponential probability plot, in which the information on the smaller

residuals tends to be compressed into the bottom left-hand corner of the graph.) The appropriateness of the model can then be tested further by analysing departure from uniformity using a modification of the one-sample Kolmogorov-Smirnov statistic, taking $C = \max_i |v_i - u_i|$ (Equation 291, p. 119, Pearson & Hartley, 1972).

I acknowledge the considerable assistance given to me by Chris Theobald, lecturer in statistics at the University of Edinburgh. Namely: introducing me to, and guiding me through, the Poisson process, and actually performing these particular calculations. It is correct to say that without his help, an analysis of such complexity could never have been tackled successfully.

5.3. Results

An examination of the sticky labels' contents indicated that the captured insects were almost entirely (> 98.0%) female *Culicoides impunctatus* and *C. obsoletus* grp. There was a substantial variation in the species composition of a catch between the trials. To illustrate: *Culicoides obsoletus* grp females comprised 95% of the actual catch of Trial 15; whereas, only *Culicoides impunctatus* females were caught during Trial 22.

5.3.1. Landing distribution of *Culicoides* spp. over periods of one hour

Figure 5-1 shows the mean percentage 'catch' (of all insects, whether actually captured or not) for each five-minute interval of the 30 'successful' one-hour trials performed. The biting activity, although low initially, peaked rapidly at between five and 15 minutes (12% approx.); this was followed by a gradual decline in the attack intensity. A mean of 41.80% of the total catch was taken in the first 20 minutes.

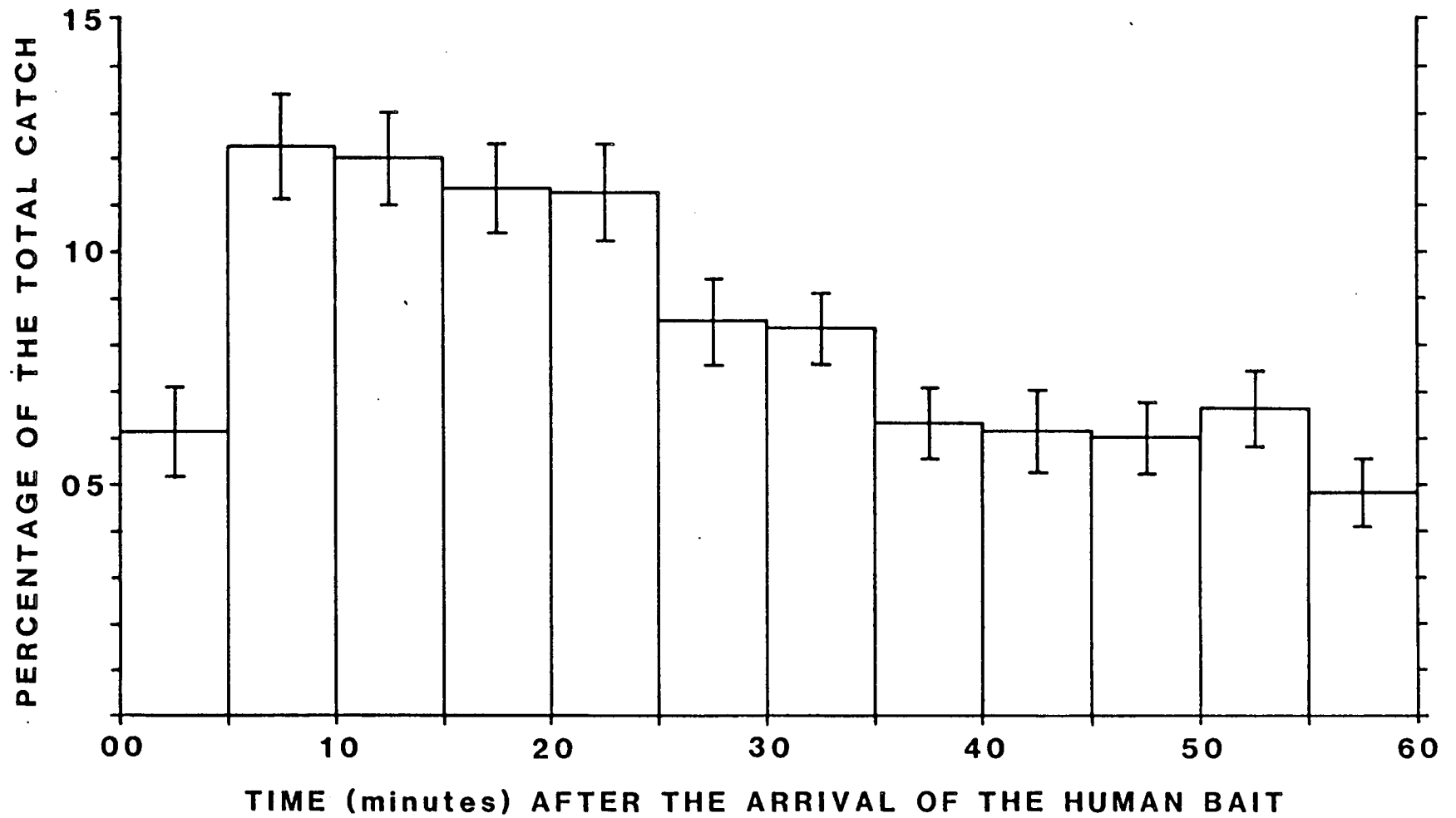


Figure 5-1. Landing distribution of *Culicoides* spp. over periods of one hour: mean and standard error

5.3.2. Inter-arrival times

The data were analysed for the two potential sources of variation in λ described in Section 5.2.2. First, fitting the factor **HOURLY** as a GLIM model indicated that there was a significant inter-trial variation in the density of hungry adult insects close to the bait ($P < 0.1\%$, $\chi^2 = 76$, on 21 degrees of freedom). **HOURLY** was, therefore, retained in the model.

The arrival times were then subject to a 'test for trend' (Cox & Lewis, 1966, p. 48, Equation 13). In essence, this analysis shows up any temporal trend in the landing rates by comparing the mean observed landing time with that which would be expected had λ been constant within each trial (i.e. 30 minutes). Deviations from expectation can be related to the z statistic (denoted by u in Cox & Lewis). The overall value (i.e. for the 22 trials used for this particular analysis) of z was -5.7 ($P < 0.1\%$); indicating that, in general, the mean arrival time was significantly less than 30 minutes. A time function was therefore required to describe this temporal fluctuation in expectation (refer to Section 5.2.2). (There was a substantial inter-trial variation. z in the individual trials ranged from -5.0 to 2.8 ; on occasions, therefore, the attack rate increased towards the end of the hourly period. Such variability is to be expected in data of this type: in addition to dealing with a biological system, one is working with relatively small numbers.)

Uniform probability plots of the raw data indicated that it would be sensible to exclude the first 15 minutes of the trials. Landing rates increased too rapidly and too erratically during this period for a reasonable estimate of $\lambda_h(t)$ to be made (Chris Theobald, pers. comm.). Working with the last 45 minutes, the first fit tried was a Type A model with a simple rectilinear time function (i.e. $k = 1$). This was followed by testing the adequacy of other similar models, in which the value of k was increased to five in increments of one.

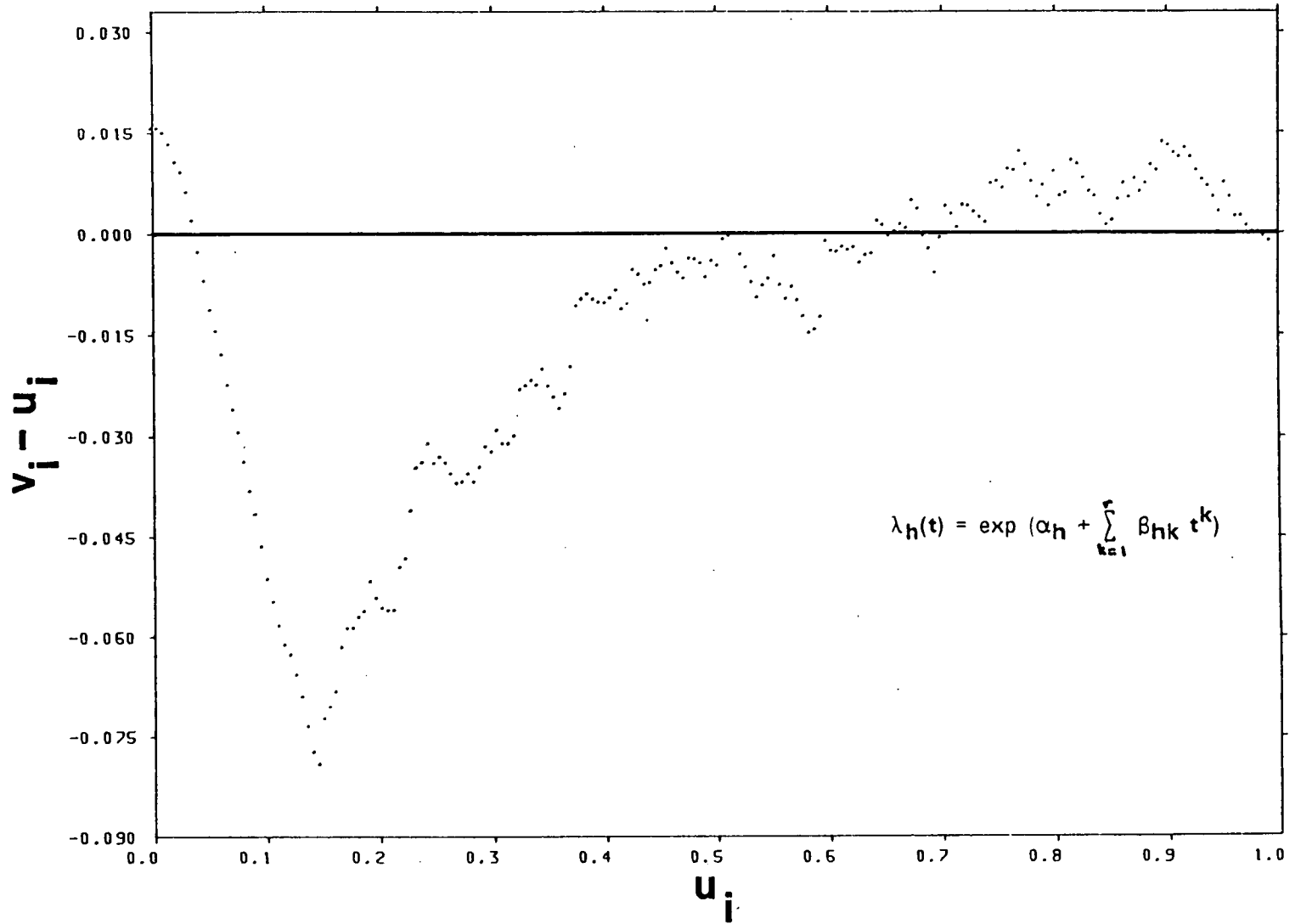


Figure 5-2. Deviations from uniformity plot (see text for details)

Type B models were assessed in a similar manner, except that k only took values 1 to 3. Models incorporating quartic and quintic terms were found to be prohibitively time-consuming – even using modern computing facilities. The best-fitting representation of $\lambda_h(t)$ was obtained from a Type B model with a cubic rate function. In reality, however, both the type of model and the value of k made little difference to the conclusions.

Figure 5-2, a *deviations from uniformity plot*, requires some explanation. (For clarity, only one out of every five points was plotted.) Its interpretation is as follows: after compensating for both sources of variation in λ , then had the landing distribution been random the points would have been fairly uniformly and closely distributed around the line drawn at $v_i - u_i = 0.00$ for all values of u_i . Instead, the results show that:

- a) The smallest v_i are too large ($u_i < 0.05$). This analytical artefact, of no real consequence to the results, was caused by taking the minimum inter-arrival time as two seconds.
- b) There is then a rapid switch, the v_i becoming much smaller than expectation ($u_i = 0.05$ to 0.65).
- c) Finally, these are balanced by values of v_i greater than expectation ($u_i = 0.65$ to 1.0).

In plain English: there is a super-abundance of very short inter-arrival times; a result that would be expected if midges tend to show a temporally-clumped landing pattern. As noted above, these are balanced by overly-long (relative to expectation) periods during which no insects alighted. The modified one-sample Kolmogorov-Smirnov statistic shows this deviation from uniformity to be statistically significant ($C = 0.078$ approx., $P < 2\%$).

5.4. Discussion

The mean landing distribution over the 60-minute trials (see Figure 5-1) shows that a definite lag time exists between the arrival of a host and the onset of peak attack in a previously resting hungry population (see Section 3.7.1). This is, I feel, to be expected. A human will emit kairomones. (Lincoln *et al.* (1982) define a kairomone as "An interspecific chemical messenger, the adaptive benefit of which falls on the recipient rather than the emitter". These include carbon dioxide, lactic acid, ammonia, amino acids (e.g. L-alanine, L-proline & L-arginine), diphenols (e.g. resorcinol & catechol), steroids, etc (Acree *et al.*, 1968; Clements, 1963; Gillies, 1980; Gillies & Wilkes, 1970; Muirhead-Thomson, 1982; Nelson, 1965; Vale, 1979). Gillies (1980) considers that carbon dioxide, emitted by a human at a rate of approximately 200 ml minute⁻¹, is the most important factor in host location.) These attractants will, under forest conditions, be disseminated relatively slowly into the surrounding environment; they will "activate" (Gillies, 1980) resting blood-hungry individuals, which will then have to orientate to and land on the host.

(Haematophagous insects are also known to respond – to a greater or lesser degree – to visual stimuli from a potential host; principally contrast and movement (Clements, 1963; Gillies, 1980). Midges reacting primarily to visual cues should, therefore, generally engage a host more rapidly than their congenics who, owing to their resting situation, will be activated only when the zone of (olfactory) stimulation reaches them. I offer no suggestion as to the importance of visual stimuli to the observed landing distribution.)

As a host's circle of influence extends, the landing rate will increase until the "immediate population" (Service, 1973, i.e. those insects that were resting within the maximum attraction distance) starts to become noticeably depleted. A consequent reduction in the landing rate follows (refer to Figure 5-1).

Jamnback and Watthews (1963) reason that this decrease in attack intensity "suggests that punkies [midges] are attracted to man from relatively short distances, otherwise the landing rate would remain fairly constant over a long period". As noted in Section 3.7.1, no data are available on the attraction distance of any midge species. Working on actively host-seeking mosquitoes, Gillies and Wilkes (1970) found that *Anopheles melas* Theo. and some other Anophelines (e.g. *A. ziemanni* Grunb., *A. squamosus* Theo. & *A. rufipes* (Gough)) responded both to a calf and to a human from approximately 15–20 metres in an open savannah environment. Of greater relevance to this study, however, is the work of Service (1971a) on *Aedes cantans*. Experimenting in an area which – one must assume – was as windless as Site C, he demonstrated that daytime resting populations of this mosquito were attracted to a stationary human bait from between seven and ten metres. (It is natural to expect that a host in an exposed situation will stimulate attack in blood-hungry insects at a greater range than in a calm environment (Clements, 1963). Kairomones will be dispersed more readily; any dilution in their concentration at the greater distances being partially offset by their linear concentration in the odour plume produced downwind of the source.) To give a good margin for error, trials performed in the same rest phase were always conducted at least 30 metres apart to avoid sampling a previously depleted population.

The results show that in this study midges (*Culicoides impunctatus* & *Culicoides obsoletus* grp) tended, on the whole, to land on a human bait in a clumped manner with respect to time. The mediation and adaptive significance of this phenomenon promise to be difficult to explain.

Ahmadi and McClelland (1985) theorised that if haematophagous insects attack "in waves", it "might plausibly reflect temporal changes in air currents or in the release of host kairomones, but it is also possible that feeding mosquitoes themselves ... demonstrate an assembly phenomenon". Support for

the latter idea was obtained from their study on the attack behaviour of *Aedes sierrensis* (Ludlow) under laboratory conditions. A guinea-pig which had females already feeding on it was found to be more attractive to "responder" mosquitoes than a similar animal not under attack. A comparable result was obtained by Alekseev *et al.* (1977) who coined the term "invitation effect". Their work on *Aedes communis* de Geer showed that a positive relationship existed between the landing rate of mosquitoes on a human arm and the number already feeding on it. (I am very grateful to Dr Geoff Harper for translating this paper.) In the choice-chamber apparatus used by Ahmadi and McClelland the 'responder' mosquitoes were unable to see the feeding mosquitoes, which led to the conclusion that "a chemical stimulus must be actively released by female *Ae. sierrensis* (and probably other mosquitoes) at, but not necessarily only at, the time of feeding". In support of this, Schlein *et al.* (1984) have demonstrated that females of the sandfly, *Phlebotomus papatasi* (Psychodidae), secrete a volatile assembly pheromone whilst taking a blood meal. The chemical originates from "large vacuolated cells" in the maxillary palps. It should be noted that any chemical released would be expected to 'say' more than "there is a host here"; surely a host capably advertises itself through the release of kairomones in large quantities.

It is very difficult to give a credible explanation for the adaptive significance of a temporally-clumped landing behaviour. It may be that the landing pattern in itself is of no adaptive value; this behaviour being merely an epiphenomenon of an 'invitation effect' in midges (see above). To illustrate: if a *flying* female midge were to emit an aggregation pheromone immediately on detecting a suitable patch of skin on which to feed, then we have a possible – though far from compelling – explanation for the temporal attack pattern demonstrated in this study. On alighting, the continued release of an aggregation pheromone would lead to the positive feedback mechanism

described by Alekseev *et al.* (1977). (If a pheromone is not involved, it is hard to imagine how else such a behaviour pattern might be mediated. A change in wing-beat frequency? Visual cues, etc? The pheromone idea seems most plausible, but is still doubtfully tenable.)

If a non-random landing distribution with respect to time is an inevitable consequence of the 'invitation effect', how do midges gain from feeding communally? The scant literature on the subject seems to indicate that such behaviour would be maladaptive as far as the individual is concerned. For instance, Edman *et al.* (1972) showed that if haematophagous insects feed singly, then the likelihood of their obtaining a full blood meal is maximised (cf. Waage & Nondo, 1982). Working with four species of cinconiiform birds as hosts, they found an inverse relationship between individual feeding success and the number of *Culex nigripalpus* attempting to feed. (Feeding success was measured both as the proportion actually obtaining a blood meal and the amount of blood taken.) The reduction in feeding success at the higher mosquito densities was attributed to intensified anti-mosquito activities by the test birds (see also Klowden & Lea, 1979b). Confronted with the "apparent inconsistencies in this phenomenon", Ahmadi & McClelland (1985) conclude, "While an advantage would clearly accrue to a population group, or even to a guild of bloodsuckers, the mechanism of selection remains unclear". Patently, there is a considerable amount of work yet to be done in this field.

Midges and the tourist industry of the Highlands and Islands

6.1. Introduction

Biting midges have long been recognised as a major hindrance to the full development of the Highlands and Islands' tourist industry (Cameron, 1946, 1947, 1948; Edwards *et al.*, 1939; Hendry, 1986a, 1986b; Kettle, 1949, 1960, 1961; Kettle & Parish, 1957; Kettle *et al.*, 1956, 1959; MacGregor *et al.*, 1948; Reuben, 1959, 1963). Their capacity to ruin an otherwise extremely pleasurable vacation should not be underestimated. Indeed, as Reye (1964) wryly observed (referring to the pest species of midge in Queensland, Australia, e.g. *Culicoides molestus* (Skuse) and *C. dycei* Lee & Reye), "The abrupt fall in morale and the strong desire to be elsewhere which they engender, are difficult to convey to those who have not experienced them". In short, midges are anathema to practically everyone; except, for example, to a handful of environmentalists who would prefer that humans were kept from despoiling these relatively untouched areas.

This chapter attempts to offer a good understanding of the midge problem, by reviewing some of the techniques of pest control currently in use world-wide in relation to the situation in the Highlands. Certainly some steps could be taken to ameliorate the situation. The improvement produced would, however, fall very far short of satisfying many people: people who either show acute symptoms after having been bitten, or who are unable to cope with the 'wildness' of the rural highland areas – of which midges are just a part.

6.2. Effect on the tourist

Tourists staying in and remaining near the centre of a major town (e.g. Oban) will usually suffer little vexation from midges. This essay relates primarily to the holiday-maker spending his or her vacation in the less-developed areas of the Highlands.

Midges in the rural environment can literally make life misery for the mentally-unprepared tourist. During the summer months, the presence of these insects – often in phenomenal numbers – may either actually prevent or seriously reduce the enjoyment of, for instance, outdoor meals and drinks, bathing and loch-side fishing. The situation is particularly acute at dusk and dawn, owing to the crepuscularity of the principal pest species (refer to Sections 3.6.2 & 3.6.4). People are not only at risk outdoors: midges will attempt to bite within human habitations, especially at night when attracted to lights (Dove *et al.*, 1932; Hill, 1947; Jamnback, 1961; Reye, 1964; pers. obs.). Of all the categories of tourist though, it is the camper who generally seems to be bitten worst. Tents are very 'permeable' to midges, few have adequate insect screens, and campsites tend to be located in areas most prone to midge activity (Section 6.11.3.a).

6.2.1. Physiological effects

Fortunately, midges in Scotland carry no organisms that are pathogenic to humans. In contrast, in the tropics they are vectors of the filariid nematodes, *Mansonella perstans*, *M. streptocerca* and *M. ozzardi*; together with a number of viral infections including Oropouche, Rift valley fever, Congo, Dugbe and various encephalitis viruses (see Section 1.2). The fact that midges are not vectors of human pathogens in the Highlands is, of course, significant; the

knowledge, however, holds little comfort for those individuals under attack.

The piercing of the human skin by a midge's proboscis is generally accompanied by a sharp stinging sensation; at the site of puncture there is usually a "tiny haemorrhagic point surrounded by a halo of redness" (MacGregor *et al.*, 1948). The overwhelming majority of individuals experience some after effects, although these vary in persistence and degree (Blanton & Wirth, 1979; Cameron, 1946; Edwards *et al.*, 1939; Hendry, 1986a, 1986b; MacGregor *et al.*, 1948; Romoser, 1973; W.H.O., 1982). Human females tend to show a higher degree of sensitivity than males (Edwards *et al.*, 1939; pers. obs.).

The normal mild skin reaction includes a temporary burning sensation and a slight localised swelling. More sensitive individuals may develop wheals, blisters, oedema of the face, neck and limbs, a blotchy appearance and an extreme tenderness of the skin. An unlucky few show a severe allergy to being bitten. This can lead to incapacitation; the individual may, on occasions, even require hospitalisation (Hocking, 1952; MacGregor *et al.*, 1948; pers. obs.).

Midge bites usually itch terribly and scratching (if bad enough) can result in both permanent scarring and secondary infection (Edwards *et al.*, 1939; Hocking, 1952; Linley & Davies, 1971; W.H.O., 1982). Owing to a midge's small size and the fact that few are usually permitted to feed to repletion, actual blood loss by a human is insignificant (Romoser, 1973).

6.2.2. Psychological effects

The psychological effects from being bitten range from a mild displeasure – the typical response of the local people who have grown used to midges and accept them as part of summer life – through intense anger to actual psychological disturbance. After having been bitten or even harassed by

midges, the majority of tourists become disaffected with the Highlands as a whole, hostile to the locals – on occasions almost blaming them for the problem – and intolerant of one another. By contrast, the retiring minority, people who know the Highlands and Islands and love them in spite of the insect problem, suffer in silence. For them, the beauty and solitude of these areas is sufficient recompense for the bites and inconvenience endured. This point – of fundamental import – will be expanded in the Conclusions (Section 6.12).

As noted above, midges can induce serious psychological disturbances. After a period of 'sensitisation' (i.e. a period of exposure to midges varying between individuals), even the presence of a few insects – whether real or imagined – can lead to various degrees of derangement. For example, an individual may try ever-more-vigorously to repel his or her attackers, descending rapidly "into a state bordering on dementia" (Hocking, 1952). Thankfully, such extreme entomophobia occurs only very infrequently.

6.3. Effect on the tourist industry

Midges are, I believe, responsible for a considerable reduction in the tourist potential of the Highlands and Islands. No quantitative data exists to substantiate this statement (Scottish Tourist Board, pers. comm.). Holiday-makers who have been 'badly' bitten (a very subjective evaluation) may leave the Highlands early, never to return; some have even been known to demand their money back. To quote Dove *et al.* (1932), "Tourists sometimes find that the best means of prevention [from attack] is obtained by moving to a locality where sandflies [midges] do not occur". More importantly, however, as far as the long-term well-being of the tourist industry is concerned, disgruntled, sometimes livid, holiday-makers might discourage other people

from taking a highland vacation (Linley & Davies, 1971; pers. obs.).

This midge problem is certainly not peculiar to the Highlands and Islands. In parts of the Caribbean and Florida, for instance, biting ceratopogonids are also held responsible for the slow and incomplete development of tourism (Apperson, 1975; Blanton & Wirth, 1979; Dove *et al.*, 1932; Edwards *et al.*, 1939; Linley & Davies, 1971; Reye, 1964; Reynolds & Vidot, 1978; Yaseen, 1974). Unfortunately, the situation is particularly serious in the Highlands. Not only is tourism one of the principal profitable industries, but also the main holiday season (late May to early September), which is short relative to that in the tropics, coincides only-too-well with the presence of midges as biting adults (refer to Sections 1.3.4 & 6.6).

6.4. History of midge control in Scotland

In the autumn of 1944, the Secretary of State for Scotland prompted a study (conducted in 1945) which had two main objectives: to determine a suitable formulation for a midge repellent and to investigate whether the actual elimination of biting midges would be practicable (Cameron, 1946; Cameron *et al.*, 1946). The suggested repellent, sponsored by the Scottish Tourist Board, was sold commercially. The report (composed of the two papers listed above) also recognised both the principal pest species and the need for a greater understanding of their ecology. This initial work was expanded, resulting in a second report to the Department of Health for Scotland (MacGregor *et al.*, 1948). In 1947, Cameron touched briefly on the use of combustible Gammexane smoke generators for localised midge control. A year later, he described the first study on the use of larvicides for ceratopogonid control in Scotland (Cameron, 1948; refer to Section 6.9.2).

Further midge control work was conducted under the aegis of Dr Kettle at Edinburgh University's Midge Control Unit (Kettle, 1949, 1950, 1951a, 1951b, 1955, 1956, 1957, 1960, 1961; Kettle & Lawson, 1952; Kettle & Parish, 1957; Kettle *et al.*, 1956, 1959; Reuben, 1959, 1963). Kettle and his co-workers concentrated on chemical methods of control, recognition of breeding sites and the relationship between flight range and control. None of their suggestions was ever adopted for general use in the Highlands. Since the closure of the unit, no formal academic work on midge control *per se* (apart from the present study) has been undertaken in Scotland.

6.5. Pest species in Scotland

Of the 41 species of *Culicoides* recognised by Campbell and Pelham-Clinton (1960) in Britain, only 35 occur in Scotland (Hendry, 1986b). The following Scottish species have been recorded as biting humans: *Culicoides achrayi* Kettle & Lawson, *C. chiopterus*, *C. cubitalis*, *C. delta*, *C. fascipennis* (Staeger), *C. halophilus* Kieffer, *C. heliophilus*, *C. impunctatus*, *C. lupicaris* Downes & Kettle, *C. nubeculosus*, *C. obsoletus*, *C. pallidicornis*, *C. pulicaris*, *C. punctatus*, *C. stigma* (Meigen) and *C. vexans* (Staeger) (Cameron *et al.*, 1946; Campbell & Pelham-Clinton, 1960; Edwards *et al.*, 1939; Hendry, 1986b; MacGregor *et al.*, 1948; pers. obs.).

Although *Culicoides halophilus* and *C. obsoletus* are reputed to have the most painful bites (Campbell & Pelham-Clinton, 1960; Edwards *et al.*, 1939), it is *C. impunctatus* – the “Highland midge” (Cameron, 1947) – that is most detrimental to the tourist trade. This species, as well as being widespread and abundant, also shows, in comparison to other Scottish midges, a marked preference for human blood. For example, Cameron (1946) noted that although *Culicoides impunctatus* comprised 45% of the midge population at his study

site near Dundee, it was responsible for almost 100% of the biting activity. In addition to *Culicoides impunctatus*, Cameron (1947) felt that possibly only three other species are regularly noxious to man: *C. obsoletus*, the "Urban" or "Garden midge"; *C. heliophilus*, the "Swamp midge"; and *C. pulicaris*, the "Farm midge".

6.6. Duration of the problem

Culicoides impunctatus starts to become vexatious towards the end of May. Even though its adult population undergoes a significant fluctuation over the summer months (refer to Section 1.3.4), petering-out in late August to early September, this species remains intolerably numerous – as far as most holiday-makers are concerned – for the duration of the main tourist season. Similarly, *Culicoides obsoletus* and *C. pulicaris* can be troublesome throughout the summer in many areas. *Culicoides heliophilus* is, by contrast, an early season flyer (Edwards *et al.*, 1939; pers. obs.); posing a problem in June and July.

6.7. What level of control is necessary?

Before considering the control options, it is important to get some idea of the level of reduction in midge density that would be required to eliminate the problem in the eyes of the average tourist. Linley and Davies (1971) reckoned that midges (or sandflies as they are generally termed in Florida and the Caribbean) are bearable to tourists if five or fewer bites are received per hour; they point out that "most people are willing to tolerate a higher level of annoyance where they live and work than in the place that they visit on vacation". This figure is, of course, exceedingly subjective. Moreover, it was

determined for tourists on the beaches of the Caribbean, the Bahamas and Florida – people with a different set of expectations to, and considerably more exposed skin than, the typical highland visitor.

Giglioli *et al.* (1980) used 'sandfly bats' to monitor adult midge density in the Cayman Islands, B.W.I. (Sandfly bats are simple midge traps, consisting of a piece of lightly-greased circular mosquito netting (diameter 7.5 cm) attached to a long wooden handle by a Bulldog® spring clip. The pest insects, attracted to the batter, adhere to the netting and are later washed off with kerosene (Giglioli *et al.*, 1980; Marco Giglioli, pers. comm.).) They thought that if fewer than five midges were caught in a five minute period, then "complete shirtless" comfort could be enjoyed. A catch of around 20 would, in their opinion, "remove tourists from the beaches"; and ca. 40 would "cause discomfort to entomologists".

Even though the estimates of these two sets of workers are not directly comparable, there would seem to be a large discrepancy between them. From my own observations, under good midge conditions, an attack rate of 5000 bites per hour is possible on an exposed upper forearm. This is admittedly an extreme situation. (The figure was obtained by extrapolation from five-minute insect counts. It could be argued that extrapolation is not legitimate here for factors such as competition for space would eventually limit the number of insects able to feed.) Even if the maximum attack rate possible on a normally-clad highland visitor were to be 1000 bites in an hourly period (an only-too-likely possibility on a calm summer's evening) and we consider Linley and Davies' estimate to be fairly accurate, the biting population would have to be reduced by at least 99.5% to render the area 'clean' in the tourists' eyes. This figure should be borne in mind when the control options are being reviewed.

One could object to having a simple cut-off point and argue in favour of trying to determine a continuous relationship between level of attack and loss of revenue (Linley & Davies, 1971; Geoff Harper, pers. comm.). These data would be of extreme value. The relationship could be used to justify the adoption or the rejection of ameliorative procedures. Collecting the information could prove prohibitively difficult.

6.8. Flight range and its bearing on midge control

Even if a near perfect level of control is required to keep the typical highland visitor content, the solution to the midge problem really hinges on the flight range of the pest species from their breeding area (Hendry, 1986b; Hocking, 1952; Kettle, 1951a, 1951b, 1960; Linley & Davies, 1971). If, for example, the mean flight distance were to be 50 metres, then the elimination of the breeding sites – though difficult – from only a comparatively small area around a tourist amenity would be required. This would be technically feasible and probably financially viable. Furthermore, the environmental degradation caused should be minimal.

Unfortunately, midges are able to disperse (either passively or actively) far from their breeding site. Kettle (1949) investigated the possibility of protecting holiday areas by the use of 'barrier spraying' with DDT. (This involves using a residual insecticide to treat a 'buffer zone' of vegetation around an area to be protected. Its efficacy relies on the insects having a very short flight range.) The use of 40 yard barriers was found to be totally ineffective; strongly suggesting that *Culicoides impunctatus* (principally) has a flight range much in excess of this distance.

Linley and Davies (1971, referring primarily to *Culicoides furens*) reckoned that if there are breeding sites within eight kilometres (five miles) of a tourist

area, then the holiday-maker is likely to experience annoyance. Kettle (1960) found that *Culicoides impunctatus*, aided by the prevailing winds and (in his view) the stimulatory effect of potential hosts, could travel in large numbers more than three quarters of a mile from its point of origin. This supports my own observations and means that virtually no tourist area in the Highlands and Islands – where breeding sites are both extensive and common – will enjoy total freedom from activity. Significantly reducing such massive breeding areas would undoubtedly prove impracticable; not only technically but also in terms of the direct (i.e. financial) and the indirect (e.g. environmental) costs of the control measures (Frank *et al.*, 1980; see below).

6.9. Chemical control

Chemical methods of insect control will be considered first. Although the control options in this chapter are dealt with individually, it is important to realise that most modern-day control programmes (integrated pest management schemes) are the product of an intelligent synthesis of many control techniques (Frank *et al.*, 1980; Service, 1983).

6.9.1. Adulticides

In laboratory tests, Service (1968) found that *Culicoides impunctatus* and *C. obsoletus* were extremely susceptible to Dieldrin and DDT. Dieldrin was found to be equally toxic to both species; *Culicoides impunctatus* succumbed more easily to an equivalent dose of DDT.

Kline *et al.* (1981) determined the adulticidal effect of four synthetic pyrethroids and of three organophosphorous compounds on field-collected *Culicoides mississippiensis* Hoffman. Using one-hour and 24-hour

post-treatment mortalities, NRDC-161 was found to be most effective, followed in order of descending toxicity by the other pyrethroids: Permethrin, Resmethrin and d-Phenothrin. The organophosphorous compounds proved less efficacious. Naled was more potent than Malathion; Fenthion was the least valuable adulticide. Apperson (1975) recommended Chlorpyrifos-methyl and Chlorpyrifos as general adulticides for ceratopogonid midges.

Demonstrating an insecticide's toxicity under laboratory conditions is far removed from proving its value as an adulticide in an insect's natural environment, where making contact with the target organism may prove extremely difficult (Kelson *et al.*, 1980; Knipling *et al.*, 1968; Reynolds & Vidot, 1978; Wall & Marganian, 1973). To destroy flying and resting adults, the "space spray applications" (W.H.O., 1982) used depend on the production of an insecticidal fog or aerosol (Service, 1983). (Ultra-low-volume (U.L.V.) techniques of insecticide application, which involve spraying only highly concentrated or undiluted (technical) insecticides, have recently facilitated the spraying operations.) The minute droplets of the toxicant either impact on the flying or resting individuals, or coat the vegetation with a biologically-active layer of insecticide (Kettle, 1949; Reynolds & Vidot, 1978; Romoser, 1973; W.H.O., 1982).

In the British West Indies, Giglioli and his co-workers (1980) found that U.L.V. field applications of Fenitrothion in aqueous solution at a dosage of 191.5 gm hectare⁻¹ A.I. (active ingredient), could give "near perfect control" of pestiferous ceratopogonids. Similarly, Haile *et al.* (1984) obtained 99% control (mostly of *Culicoides hollensis* and *C. melleus* (Coquillett)) using two U.L.V. applications of undiluted Dibrom 14 (85% formulation of Naled) at a rate of 73.1 ml hectare⁻¹. Only 24% control was achieved using 36.5 ml hectare⁻¹. This was attributed to the poor penetration of the insecticide into the vegetation.

Obviously adulticides can be effective at temporarily reducing adult midge populations. There is hardly any residual effect, however. A sprayed area would almost certainly be reinvaded as soon as the insecticidal mist had cleared (Apperson, 1975; Blanton & Wirth, 1979; Linley & Davies, 1971; Service, 1983; Smith *et al.*, 1959). (In contrast, Haile *et al.* (1984) reported that their control attempt was effective for three days; this work was, however, conducted on a small island where reinvasion would be minimal.) Another drawback is that the broadcast of insecticides could lead to the rapid development of resistant strains (Service, 1983); it would therefore be necessary to use different toxicants on a rotational basis (Marco Giglioli, pers. comm.).

Linley and Davies (1971) conclude that "in general, it is probably a waste of effort and materials to fog against sandflies [midges] as a routine measure". They do see a value for the use of adulticides in dealing with "emergency situations"; which, in their estimation, occur when the level of attack reaches "intolerable levels". In the Highlands, 'emergency situations' arise – in most people's opinion – practically every day.

To mount a successful adulticidal operation – assuming that it would be technically feasible – requires a high level of managerial efficiency and coordination, complex equipment, trained employees and a great deal of money (Linley & Davies, 1971; Smith *et al.*, 1959; W.H.O., 1982). This leads to the vital question, central to the whole issue of midge control in the Highlands and Islands: would ameliorative measures pay for themselves through increased tourism? As mentioned in Sections 6.3 and 6.7, no reliable information exists on the relationship between level of midge attack and loss of income from tourism. I consider that one could never recoup the considerable financial outlay required to undertake an efficient spraying programme. Indeed, I would doubt whether the effective implementation of the overwhelming majority of

control techniques would make financial sense.

The indirect costs too might be prohibitive – though impossible to quantify accurately. I believe that spraying an area would not only severely affect its biota, but would also render the site ‘unclean’ in the tourists’ eyes (Frank *et al.*, 1980; Giglioli *et al.*, 1979, 1980; Knipling *et al.*, 1968; Linley & Davies, 1971; Matthews, 1983; Provost, 1972a, 1972b, 1977; Reynolds & Vidot, 1978; Wigglesworth, 1976). Moreover, the holiday-makers would be legitimately concerned about the toxicant’s effect on their health. (For maximum efficiency, spraying would have to be done when the insects are most active, which is at dawn and dusk (refer to Sections 3.6.2 & 3.6.4). Clandestine spraying during the hours of darkness would produce few positive results.) These objections, together with the embarrassing coverage from the increasingly environmentally-conscious media, could discourage people from visiting the Highlands. Spraying might, therefore, deter more people from taking a highland holiday than the midges themselves do.

6.9.2. Larvicides

Cameron (1948) performed the first study on the efficacy of larvicides for midge control in Scotland; obtaining good control of *Culicoides pallidicornis* in test plots with DDT emulsions and Gammexane miscible oil (200 mg ft⁻²). Kettle and his research team (1956) investigated the possibility of using larvicides (p,p’DDT, γ BHC (HCH), Dieldrin, Chlordane & Malathion) for the control (both immediate and residual) of *Culicoides impunctatus*. The large variation in the mortality rate in this and in subsequent studies (Kettle & Parish, 1957; Kettle *et al.*, 1959) was attributed to differences in the water solubility of the preparations; in the total amount of rainfall between spraying and larval eclosion; and in the micro-topography of the land. All affect the degree of the

toxicant's penetration into the breeding medium. This is important since, as Kettle *et al.* (1956) point out, the larvae "are protected from insecticidal sprays by a layer of living moss, usually *Sphagnum* or *Polytrichum*, which may be six inches thick".

For immediate larval control, Kettle *et al.* (1956) recommended the use of 200 mg ft⁻² of either p,p'DDT (wetable powder or water-miscible concentrate) or γ BHC (HCH, wettable powder). The results were very variable. 200 mg ft⁻² is an extremely high dose which, apart from severely affecting the natural biota, was thought by Kettle (1949) to be of potential harm to grazing animals, particularly sheep. (Many midge breeding sites are used for grazing livestock.)

The residual control was, in general, more constant and lower dosages were found to be "effective". p,p'DDT, Dieldrin and Chlordane were equally or more potent three years after application. As an illustration, both 50 and 200 mg ft⁻² p,p'DDT (wetable powder) still exerted near absolute control after three years; whilst the efficacy of a dose of 50 mg ft⁻² of Chlordane (water-miscible concentrate) increased from 79% to 100% over the same period. Wall and Marganian (1973) achieved reasonable immediate control (51.6% - 80.4%) of *Culicoides furens* and *C. hollensis* by applying 2% Diazinon (0.2 pounds acre⁻¹) and 1% Dursban (0.05 & 0.2 pounds acre⁻¹) to the salt-marsh breeding sites.

Although the long-term control obtainable using larvicides in test plots can undoubtedly be impressive, it would be difficult to support their widespread use in the Highlands. Linley and Davies (1971) listed numerous objections which accord with my feelings. They noted that breeding sites are extensive and thus their treatment would require a substantial amount of expensive insecticides; adequate coverage would almost always be impossible (in the Highlands penetrating both the *Sphagnum* layer and, on occasions, the tree cover would be difficult or unfeasible); the environmental degradation would be

severe; and resistance would most probably develop. Indeed, they consider the whole approach to be "economically unsound and inadvisable".

To emphasize a point of crucial significance to all midge-control methods: if the average flight range of the pest species were to be less than 50 metres from their breeding site, one could perhaps condone the treatment with larvicides of the small buffer zone required to 'cleanse' an area. From the discussion on flight range (Section 6.8), it is obvious that to improve the situation noticeably as far as the tourist is concerned, all the breeding sites within a radius many kilometres from the area to be protected would have to be sprayed. The direct and indirect costs already considered render this an unviable proposition.

6.10. Biological control

The possibility of employing biological control techniques for the eradication of *Culicoides* spp. has been little studied. Without the inevitably massive injection of funds and expertise, it is unlikely that these rather complex modes of attack will play an important role in reducing midge populations (Bacon, 1970; Blanton & Wirth, 1979; Welch, 1962). This account cannot be exhaustive; it is a brief discussion of some of the more frequently used biological control techniques. Where possible references and ideas specific to midges are presented.

6.10.1. Predators, parasites and pathogenic microbes

Predators, parasites and pathogenic microbes suspected of being natural control agents of *Culicoides* spp. were recorded by Bacon (1970). The list included viruses, rickettsiae, bacteria, fungi, protozoa, nematoda, acarina,

annelida, insecta and vertebrata (pisces and reptilia). It is just possible that in the long term, some natural or – more probably – unnatural (i.e. introduced) control agent could effect a marked reduction in *Culicoides* populations in Scotland. At present, we know little about the “enemies” of Scottish midges (Hendry, 1986b). There is no quantitative, or indeed qualitative, evidence to indicate that a natural predator, parasite or pathogen causes a reasonable mortality that could be capitalised upon.

Nematodes of the family Mermithidae (*Mermis* & *Agamomermis*) are recognised as fatal parasites of the Ceratopogonidae (Callot, 1959; Chapman *et al.*, 1968; Hendry, 1986b; Sen & das Gupta, 1958; Smith, 1966; Smith & Perry, 1967; Whitsel, 1965). The parasitism of male imagines produces intersexes which are probably sterile (Hendry, 1986b). The head, antennae, mouthparts and wings become female in form, whilst the abdomen and terminalia remain masculine. Infected females are not perceptibly altered. The host, whether in the larval or in the adult stage, is killed when the nematode emerges. Sen and das Gupta (1958) reported an infection rate of less than 3% in *Culicoides alatus*; in general, the percentage parasitism would seem to be low (Chapman *et al.*, 1968; Whitsel, 1965). By contrast, under “favourable conditions”, between 30% and 90% of the adult *Culicoides haematopodus* Malloch, *C. stellifer* (Coquillett) and *C. crepuscularis* Malloch caught by Smith and Perry (1967) contained mermithids. They believed that the nematodes were effecting “a perceptible degree of natural control of *Culicoides* populations”. For mosquito control, *Romanomermis culicivorax* has been mass produced for large-scale evaluation in both temperate and tropical areas (Service, 1983; W.H.O., 1982).

The bacteria *Bacillus thuringiensis* (Serotype H-14) de Barjac and *B. sphaericus* Strain 1593, used as ‘microbial insecticides’, have proved efficacious in controlling larval mosquitoes (Service, 1983; W.H.O., 1982). Their specificity makes them environmentally safe. Indeed, these pathogens are so specific to

mosquitoes that, with a reasonable dose, no practical control of *Culicoides* spp. can be obtained (Kelson *et al.*, 1980; Lacey & Kline, 1983).

Chapman *et al.* (1968) recorded the fatal infection of at least two species of *Culicoides* by *Plistophora* spp. (Protozoa: Nosematidae). (Only one species of midge was identified: *Culicoides nanus* Root & Hoffman.) The same study revealed that larvae of *Culicoides arboricola* Root & Hoffman and *C. guttipennis* (Coquillett), collected from tree holes, were terminally infected with the Culicoides Iridescent Virus (Cu.I.V., Bacon, 1970; Yaseen, 1974).

6.10.2. Competitors

The use of competitors for insect control involves the displacement of a nuisance species by a more competitive ecological homologue that is not a pest (Romoser, 1973). For midge control a species of non-biting insect would be sought, whose larvae could successfully compete with those of the pestiferous midge(s). Yaseen (1974) investigated the possible seasonal displacement of *Culicoides insignis* Lutz and *C. furens* by the innocuous ceratopogonid, *Stilobezzia antennalis* (Coquillett). Displacement of the nuisance species did not occur.

6.10.3. Sterilization and genetic control

Sterile-insect control methods *sensu stricto* (van den Bosch & Messenger, 1974), so-called autocidal techniques, involve the inundative release of sterile males into a pest's natural habitat. These then mate with the wild, preferably monogamous, females. The procedure, if repeated over many generations, may lead to the substantial reduction or local extermination of the target insect population (van den Bosch & Messenger, 1974; Knipling *et al.*, 1968; Romoser,

1973; W.H.O., 1982).

Sterile males are produced either by irradiation or by chemosterilization. The method utilises a considerable number of individuals, of equal or greater sexual competitiveness than their wild counterparts. For instance, to eliminate two species of fruit fly (*Dacus cucurbitae* (Coquillett) and the Oriental fruit fly, *D. dorsalis* Hendel) on Rota, a small island near Guam, ten million individuals per week had to be released (van den Bosch & Messenger, 1974). The number needed to control *Culicoides impunctatus* in even a small area of the Highlands would certainly be markedly in excess of this figure. Furthermore, the financial costs would be too prohibitive, the areas requiring treatment too extensive, and the likelihood of reinvasion too great for it to be a practicable exercise.

In theory, chemosterilants (e.g. Thiotepa, Ismail *et al.*, 1981) could be applied to the breeding sites. Both males and females would be affected – an obvious advantage. Most of the available formulations are, however, mutagenic agents; their large scale broadcast would, therefore, be inadvisable (Knipling *et al.*, 1968; Romoser, 1973). The use of attractant chemosterilant-containing traps would avoid the environmental problem. Altering the genetic make-up of pests by artificial manipulation (e.g. hybrid sterility or cytoplasmic incompatibility) can make them less competitive, less fecund or sterile (van den Bosch & Messenger, 1974; Knipling *et al.*, 1968; Romoser, 1973; W.H.O., 1982). Obviously, there is a substantial amount of work to be done in the field. Who would fund it?

6.11. Environmental management

The W.H.O. Expert Committee on Vector Biology and Control offered the following definition of environmental management for the control of vector mosquitoes:

"The planning, organization, carrying out and monitoring of activities for the modification and/or manipulation of environmental factors or their interaction with man with a view to preventing or minimizing vector propagation and reducing man-vector-pathogen contact".

Although ceratopogonid midges in the Highlands and Islands are not vectors of human disease (Section 1.2), this definition appears useful and was subdivided into the following:

- a) Environmental modification: "A form of environmental management consisting in any physical transformation that is permanent or long-lasting of land, water and vegetation, aimed at preventing, eliminating or reducing the habitats of vectors without causing unduly adverse effects on the quality of the human environment."
- b) Environmental manipulation: "A form of environmental management consisting in any planned recurrent activity aimed at producing temporary conditions unfavourable to breeding of vectors in their habitats."
- c) Modification or manipulation of human habitation or behaviour: "A form of environmental management that reduces man-vector-pathogen contact."

6.11.1. Environmental modification

This section deals with the environmental modification methods that are most commonly used in mosquito and midge-control programmes. The principles behind each technique are discussed, examples of their implementation are given and specific objections to their use are forwarded. The efficacy of these methods has (to the best of my knowledge) never been evaluated for the control of *Culicoides impunctatus* in the Highlands. A case exists to perform some rigorous exploratory studies to rectify this. Even if,

however, small-scale field trials were to be encouraging, I believe that the necessarily extensive use of such techniques would be both too expensive and environmentally unsound.

a) Impounding breeding sites

Impounding breeding areas to destroy haematophagous insects involves encircling a breeding site with a dike capable of retaining a constant level of water (usually less than one metre) within the impounded area. The impounded section is flooded; thereby eliminating the breeding sites of those ceratopogonids whose larvae cannot survive in free-water environments (Blanton & Wirth, 1979; Linley & Davies, 1971; Rogers, 1962; Service, 1983; W.H.O., 1982). Control of pest *Culicoides* spp. typically approaches 100%. There may, however, be a certain amount of breeding around the dike's walls – of potential interest to the tourist – if they are not perpendicular to the water surface (Rogers, 1962).

Would impounding the breeding sites of *Culicoides impunctatus* be a viable exercise? Impounding operations really require that the breeding sites are located on flat ground; this species frequently breeds on slopes – many of which are fairly steep (pers. obs.). Furthermore, the direct costs would be excessive; varying dependent on a breeding site's size and location, etc. Additionally, income from both sheep grazing and from commercial forestry would be lost. Finally, land ownership problems would undoubtedly frustrate large-scale impoundment plans.

The indirect costs would be no less forbidding. The topography of the Highlands and their flora and fauna, that many people regard as quintessentially Scottish – the very appeal as a tourist destination – would be replaced by a patchwork of mostly shallow water-filled impoundments. Far from improving

the tourist potential of an area, flooding the large tracts of land necessary to significantly reduce midge-population levels could have the opposite effect. (In Florida, it has been argued that impounding an area increases its diversity and appeal (Provost, 1959, 1967).) In addition, one would experience considerable and (in my opinion) justifiable opposition from the environmental lobby. (The impoundment of large areas in the United States gave rise to the Wetlands Protection Acts (Frank *et al.*, 1980).) To sum up, I cannot envisage impounding playing a significant role in the control of *Culicoides impunctatus*.

b) Draining breeding sites

Lowering a breeding site's water table could make it too dry for midges to breed (Dove *et al.*, 1932; Hull & Shields, 1943; Rogers, 1962; Service, 1983; W.H.O., 1982). In theory, draining (both surface and sub-surface) could be accomplished by building a reticulum of drainage channels (open ditches, french drains, buried conduits and mole drains) in a breeding area, and/or by using pumps or vertical drainage techniques. In the Highlands, open ditches would have to be cleared periodically of *Sphagnum* to prevent new breeding sites forming.

Adequately draining a small area of *Sphagnum* bog might be impossible; especially where the land is flat and low lying. Knowledge of an area's topography, its soil's porosity and capillarity, etc, would be essential. The Highlands have an extremely high rainfall (Dalavich, Argyllshire has approximately 2.4 metres p.a., George Francey, pers. comm.). In addition, *Sphagnum* spp., the dominant plants of the larval habitat (Kettle, 1961; Section 1.3.1), have a high water-holding capacity. Hence it is likely that attempts to drain thoroughly even a small area would prove futile. Both Rogers (1962) and Hull and Shields (1943) found that their endeavours to drain a site by ditching

still allowed a significant amount of breeding of *Culicoides furens* (principally) to occur; especially in the ditches themselves. Finally, it is possible that if a breeding site of *Culicoides impunctatus* were drained, *C. obsoletus* could colonise the drier substrate (Hendry 1986b).

c) Land filling

Land filling is an expensive method of eliminating breeding sites (Linley & Davies, 1971; Rogers, 1962; W.H.O., 1982). Raising the soil's level above that of the water table by the use of land fill (e.g. rocks & earth) can make an area too dry for midges to breed. For example, Rogers (1962) obtained near-perfect control of *Culicoides furens* by filling the breeding sites to a level of 3.5 ft (average) above mean sea level with a mixture of sand, shells and clay. Apart from reducing midge populations, one obvious extra benefit is the improvement or formation of agricultural land. Even though this is an efficient control technique, its indispensably large-scale implementation would be unfeasible and generally unwelcome.

6.11.2. Environmental manipulation

Techniques of environmental manipulation, such as stream flushing and shade removal, are in widespread use in both the tropics and sub-tropics for pest and vector control (Romoser, 1973; W.H.O., 1982). These methods have been little used to eliminate *Culicoides* spp.

Davies (1969) found that one year after the shade of a breeding site had been removed by felling its mangroves, the larval population of *Culicoides barbosai* Wirth & Blanton was reduced by 90%. *Culicoides furens* and *C. insignis* showed no real preference for the degree of shade of their larval

habitat. *Culicoides impunctatus* breeds in acid bogs, whether they are shaded by trees or not (Kettle, 1961; pers. obs.).

6.11.3. Modification or manipulation of human habitation or behaviour

Actually eliminating midges on the scale necessary to reduce the midge problem to acceptable levels, would seem, for the reasons presented above, to be an impracticable proposition on the whole. The financial, technical and environmental objections are just too strong. The 'modification or manipulation of a human's habitation or behaviour' may, however, offer an accessible means of reducing the vexation experienced. This category of environmental management aims not at destroying midge populations, but at reducing the amount of man-midge contact. (Although it is not immediately apparent from the title, the W.H.O. Expert Committee (see above) intended methods of personal protection to be included under this heading.)

a) Location of tourist area

Even though midges are ubiquitous in the Highlands and Islands, the actual magnitude of an area's midge problem will depend on its location. This fact is typically overlooked by site planners, for whom aesthetic and leisure-facility considerations seem to be paramount. Linley and Davies (1971) considered the effect of ceratopogonids on the tourist industry of Florida, the Caribbean and the Bahamas. They note: "Though great imagination has almost invariably been shown in the provision of man-made facilities for the tourist, frequently considerably less skill has been demonstrated in entomological matters". They continue: "Unless an entomological evaluation is made, the owners of a magnificent new facility may soon find that they have a

monumental sandfly [midge] problem, which can be brought under control only after considerable difficulty and substantial expenditure". An entomological appraisal of a potential holiday area (e.g. mapping the breeding sites) must be undertaken. This cannot be stressed too strongly.

What criteria should be used to site a tourist area? First, holiday developments should (quite obviously) be located as far away as possible from breeding sites (Cameron, 1947; Kettle, 1951a, 1951b, 1960; Linley & Davies, 1971; W.H.O., 1982). Second, the smaller the actual area of these sites the better (Linley & Davies, 1971). Cameron (1947), using larval studies, estimated that an acre of good breeding site could support around nine million larvae. Clearly with such high larval densities, even a small breeding site close to a holiday area could severely impair its tourist potential. Third, tourist amenities should be situated – if possible – on land most frequently upwind of nearby breeding sites. Even though moderate breezes usually inhibit flight activity of *Culicoides impunctatus*, on occasions midges will be carried as part of the aerial plankton (Kettle, 1951a, 1951b, 1960; Linley & Davies, 1971; W.H.O., 1982; Sections 4.3.4.c & 4.3.4.f). It is obvious how a shift in wind direction could augment or reduce the insect problem (Linley & Davies, 1971). Fourth, an area's degree of exposure should be as high as possible. From a purely entomological perspective, sites on high ground or in non-wooded areas are preferable.

The work described in Chapter 3 is of relevance here. The form of the 24-hour flight cycles as determined by a human bait, proved to be very different when measured in a field and in a forest. In a forest, 19.24% of the total diel activity was recorded between the hours of 10.00 and 19.00 B.S.T. This contrasts strongly with the 1.02% of the diel activity experienced during the same period in the field (consult Sections 3.6.2.b, 3.6.2.d & 3.7.1 and Figures 3-8 & 3-10). This is a fairly extreme result. It should be noted that it would

be surprising to find a holiday habitation built in an area as sheltered as the Forest Site used in this work. Even so, if a tourist amenity, for example a chalet, is sheltered by trees and the surrounding vegetation affords resting sites, its occupants are likely to experience biting activity throughout the full 24-hour period. Tourists in a chalet sited in an exposed situation, however, would usually be free from attack during the daylight hours. At night both should be subject to similar levels of activity. There is unfortunately a certain amount of conflict here between what site planners and tourists usually consider preferable (i.e. sheltered areas that are tastefully and fairly heavily vegetated), and what is best in entomological terms.

b) Midge-proofing habitations

It should be apparent that little (see 6.11.3.c) at present can be done to prevent midges interfering with outdoor activities. One could, however, improve the situation for the tourists by ensuring that their habitation is relatively midge-free. This would be a comparatively inexpensive way of reducing the resentment felt by one's paying guests.

Buildings can be made relatively midge-proof by screening the doors and windows, and by closing other portholes of entry. In many countries – although hardly ever in Scotland – where insects are pests and/or vectors, mosquito screening is used (Mesh No.16 or No.18, Blanton & Wirth, 1979; Hocking, 1952; Linley & Davies, 1971; W.H.O., 1982; pers. obs.). Although midges can pass with ease through these meshes, treating the screens with insecticides (e.g. Dichlorvos, recommended by Linley & Davies, 1971) kills those insects which travel through them (Jamnback, 1961, 1963; Linley & Davies, 1971). Jamnback (1963) reported that a 6% solution of either Malathion or Bayer 39007 (Proxopur) weight/volume in ethanol (95%) or acetone, remained

effective for at least one month. The preparation used by Davies (1965, from Linley & Davies, 1971) of 7% Dichlorvos (weight/volume in 90% ethanol) also showed good persistence.

Meshes that physically prevent the ingress of midges (No. 32) cannot be recommended, for they reduce air-flow and light intensity (Dove *et al.*, 1932; Linley & Davies, 1971; W.H.O., 1982); if used, it should be in conjunction with an exhaust fan to maintain air movement (Linley & Davies, 1971). The use of air conditioning systems, provided that all points of entry are sealed, provides a preferable though expensive alternative to screening (Linley & Davies, 1971; pers. obs.). Air conditioning systems were installed and used to good effect in two chalets in Dalavich.

Appendix 3 describes a simple experiment aimed at determining whether artificially ventilating a restricted area of chalet balcony with an industrial fan could sufficiently reduce midge activity to allow humans to sit outside unmolested. The results were encouraging. Using a (vertical) wind speed of only one m s^{-1} , a human could enjoy practically midge-free conditions – even at times of peak activity. Although this breeze is welcome on warm evenings, convective and evaporative cooling can be a problem at other times. Additionally, many heavy-duty weather-resistant fans would be required to adequately ventilate even a moderately-sized area. Nevertheless, further field trials should be undertaken for this approach could offer an effective and long-term remedy to the midge problem in limited areas.

c) Personal protection

Exposed areas of skin, including the scalp, can be protected from attack by using commercial repellent formulations, such as Shoo®, Autan® or Jungle Formula®. Repellents can be applied in lotion, stick, cream or aerosol form

(Cameron, 1946, 1947; Grothaus *et al.*, 1976; Harlan *et al.*, 1983; Hocking, 1952, 1963; Linley & Davies, 1971; MacGregor *et al.*, 1948; Schreck & Kline, 1981; W.H.O., 1982). Protection time varies considerably; being dependent on the individual, the species of midge, the ambient conditions and the attack rate. DEET (N,N-diethyl-m-toluamide, Pfizer), developed for the American Armed Forces, was found by Davies (1967, from Linley & Davies, 1971) to be more efficacious than TMPD (2,2,4-trimethyl-1,3-pentanediol) and DMP (dimethyl phthalate) at repelling *Leptoconops becquaerti* (Diptera: Ceratopogonidae). All these preparations are mild irritants. There is a great deal of variation in individual tolerance to them, though care should always be taken to avoid contact with the eyes, nose and mouth. (In the absence of DEET or some other similar repellent, Schreck and Kline (1981) recommend the use of mineral-oil-containing substances; for example, Johnsons' Baby Oil®. These protect one from being bitten not because they repel adults, but because their oiliness traps insects trying to feed.)

To reduce the ephemerality of the protection and to avoid adverse skin reactions, preparations, principally of DEET, have been impregnated into special quarter-inch-mesh polyester and cotton jackets (Grothaus *et al.*, 1976; Harlan *et al.*, 1983; Graham White, pers. comm.), and also into head-veils, oversocks and gauntlets (Cameron, 1946, 1947; Hocking, 1952; MacGregor *et al.*, 1948). The commercially-available over-jackets were found by Grothaus *et al.* (1976) to offer protection against *Culicoides furens* for over seven weeks when treated with 0.25 gm of DEET per gram of netting material. When treated with 0.5 gm of repellent per gram of material, considerable wearer discomfort was noted. Harlan and his co-workers (1983) concluded that under conditions of high midge density, such treated jackets may not give adequate protection from attack even though they increase the long range repellency effect of the formulation relative to applying it directly onto the skin.

Further research into the causes of individual variation in attractiveness to haematophagous insects should be fruitful and ultimately lucrative (Myrtle Ashmole, pers. comm.). This could lead to the development of systemic repellents (Sherman, 1966); that is, repellents which when taken orally appear in the skin itself or in its secretions.

If midges get indoors, they can be repelled or eliminated by burning pyrethrum coils (e.g. Moskil®), by gradually vapourising a pyrethroid-containing tablet (Hocking, 1952; Graham White, pers. comm.), or by using any domestic insecticide in aerosol form. Electronic buzzers, which are commonly on sale in the highland areas, are ineffective at repelling mosquitoes (Lewis *et al.*, 1982; Graham White, pers. comm.). Although their precise effect on *Culicoides* species has not been evaluated, it is extremely unlikely that they will be of value because they (in theory) operate using the wing-beat frequency of a mosquito.

6.12. Conclusions

The tone of this chapter is not optimistic. Midges will probably always impinge on human life in the Highlands and Islands (Hendry, 1986a, 1986b). There are no panaceas. At present, although we have the expertise, the necessary financial resources would not be available to eliminate this pest. Even if they were, public opinion would not permit the widespread habitat destruction that would accompany the control techniques.

The situation would, however, undoubtedly be improved for *some* holiday-makers if the suggestions offered in this chapter were adopted (e.g. the installation of air-conditioning systems in chalets and the appropriate location of tourist amenities). Moreover, by actively 'introducing' the paying visitor to the midge, one could partly mitigate the problem that this insect presents to

the tourist industry. To illustrate: a very simple preliminary exercise was conducted at Dalavich, in which tourists were given a fact sheet on the midge as part of the introductory literature to the chalet site. This pamphlet explicitly acknowledged the problem; noting also that some exploratory control work was being undertaken. Additionally, by giving a brief and entertaining resume of midges, their life history and biting habits, it played a large role in reducing the entomophobia typical of visitors new to these rural areas.

Suggestions were also offered on behavioural modification. In common with the locals, some tourists learnt to organise their day to avoid peak midge activity; fewer lights were left on and windows were generally closed at night. Finally, the many positive aspects of the chalet site were emphasised. Any literature of this nature should impress on the holiday-maker that midges are an integral part of the Highlands – the ‘wildness’ that people from around the globe find so appealing. In essence, the tourist should be convinced that, as vexatious as midges undeniably are, they are “an essential feature of an environment which offers compensations in other directions” (Hocking, 1952). Whether or not a person enjoys a holiday in a midge-infested region, really depends on the mentality of the individual.

There are, of course, two types of tourist who are not predisposed to enjoy a holiday in the Highlands and Islands. The first, as noted in Sections 6.2.1 and 6.2.2, is hyper-sensitive to midge bites. The second is totally unwilling or, more probably, unable to make any concessions to his or her new environment. These people – almost invariably urbanites – are mentally ill-equipped to deal not only with the midges but also with the weather, the horseflies, the muscids, the meandering roads, and the lack of proximity to major conurbations, etc. For them the whole ‘highland experience’ is disagreeable. The ‘back to nature’ holiday that, from the comfort of their living room, seemed so idyllic, is in reality too much of a change from their normal

existence to be bearable. I find it depressing to think that, in addition to being completely unadaptable, some people are entirely oblivious to natural beauty.

Chapter 7

Concluding discussion

The work described in Chapters 3 and 4 frequently assumes that the diel flight activity of *Culicoides impunctatus* has a bimodal rhythmic component. Although, in view of both the published literature and the field data, this would seem a fair assumption, no incontrovertible evidence could be produced to support it. The statistical model only suggested that the flight periodicity of this midge is strongly determined by rhythms. (The parameter estimates for the separate hours of the flight phase included in the factor **TIME** seemed plausible. They predicted that 'rhythmically-determined' activity is bimodal and associated with dusk and dawn; the dusk peak being the larger.) As concluded in Chapter 4, however, this prediction could be seriously in error.

What pattern of diel flight activity would a single *Culicoides impunctatus* female show under constant darkness (DD) in the laboratory? (Although it would be easier to work with a population of midges (say 50), it should be remembered that if a bimodal activity pattern were to be determined for a population, it would not necessarily prove that each individual flies at both 'dusk' and 'dawn'. Indeed, Kettle and Linley (1967b) felt that, "In insects the periodicity is largely a character of the population. Where there are two peaks of activity it is likely that any particular individual will be involved in only one". This idea conflicts with the findings of Nayar and Sauerman (1971) and the belief of Aschoff (1966, refer to Section 3.2).) An actograph study, as noted previously, would be very difficult to perform on a solitary insect of this size (David Saunders, pers. comm.). Most comparable work has been conducted with larger insects (e.g. mosquitoes and tabanids) using flight chambers and microphones (Jones & Gubbins, 1978; Jones *et al.*, 1967, 1972; Nayar &

Sauerman, 1971). Even so, given the required electronic assistance, a similar, though necessarily more sensitive, system could probably have been developed for this study. In retrospect – especially considering the proportion of this thesis that is finally concerned with activity rhythms – such a laboratory study should have been attempted.

In Chapter 5, it was demonstrated that midges tend to land in a clumped manner with respect to time. Some means of communication may therefore exist between flying hungry females. (In the discussion, it was tentatively suggested that such behaviour could be mediated by an aggregation pheromone emitted by flying females just about to attack a host.) If attack signals do exist, are they species specific or common to the genus (or at least to those species of *Culicoides* within a geographical area)? This idea was researched by Chris Theobald, my statistics advisor, using data collected for this thesis. The mathematics are complex.

In brief: using information on the relative proportions of *Culicoides impunctatus* and *C. obsoletus* grp in each trial's catch, a statistic, combining data from all the trials, was developed to test for departures from the Hardy-Weinberg law (see Haldane, 1954 & Smith, 1970). This law relates to the segregation of a pair of autosomal genes within a population. Instead, however, of testing for the incidence of heterozygotes and homozygotes, the species comprising the pairs of midges which landed 'simultaneously' (i.e. within five seconds of each other) were analysed. To illustrate: assume that in a trial 80 midges were actually caught, of which 40 were *Culicoides impunctatus* and 40 were *C. obsoletus* grp females. If the pairs were generated randomly (i.e. if any signal to attack was not species specific), then the probability of a pair being composed of either two *Culicoides impunctatus* or two *Culicoides obsoletus* grp females should be around 25%. By subtraction for simplicity, a mixture of species would be expected in approximately half of

the cases. This is simple trinomial probability; combining the information from trials with different proportions of *Culicoides impunctatus* and testing for any departure from expectation, however, presented a fairly novel statistical problem.

The results indicated that although there was a slight preponderance of like pairs, the departure from expectation was not statistically significant ($P > 17\%$ approx.). Considering the numerous inherent inaccuracies in the experiment, this result should not be taken as definitive proof that, in this study, a female *Culicoides impunctatus* (say) was equally likely to land 'simultaneously' with a *C. obsoletus* grp female as with a conspecific.

Finally: it is obvious that this thesis offers little cause for hope to the Forestry Commission, its partial sponsor; indeed, to anyone who has a tourist concern in the Highlands and Islands. This, it should be said, is not an unexpected conclusion. (As indicated in Chapter 1, a report aimed more at the practitioner will be submitted to the Forestry Commission.)

For Chapter 6, a fairly exhaustive literature survey of the principal insect control techniques was conducted. Not one method of pest elimination was found that could be recommended for general use in the Highlands. It might of course be argued that the appraisal of the insect elimination methods was too subjective; prejudiced by the views of an environmentalist, obviously unwilling to condone any necessarily large-scale interference with the 'natural' ecosystem (even though almost entirely man made). 'Green' considerations aside, however, the financial and logistical objections are, I believe, still too compelling. (On reflection, preliminary costings for a small-scale spraying programme should have been made to substantiate fully some of the financial arguments. For these, Forestry Commission data on spraying for spruce pests are available.)

If money for midge control is available, then – as emphasized in Chapter 6 – it would be most profitably spent on reducing the amount of contact that the tourist has with midges (see Section 6.11.3). Midge-avoidance techniques, coupled with an imaginative programme to gently introduce the tourist to the midge (to dispel neophobia, Section 6.12), surely offer the only sound approach to reducing the resentment felt by the average holiday-maker – at least for the near future.

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Appendix 1

Flight activity patterns of female *Culicoides impunctatus*:

Field Site and suction trap

Trials 1 to 8

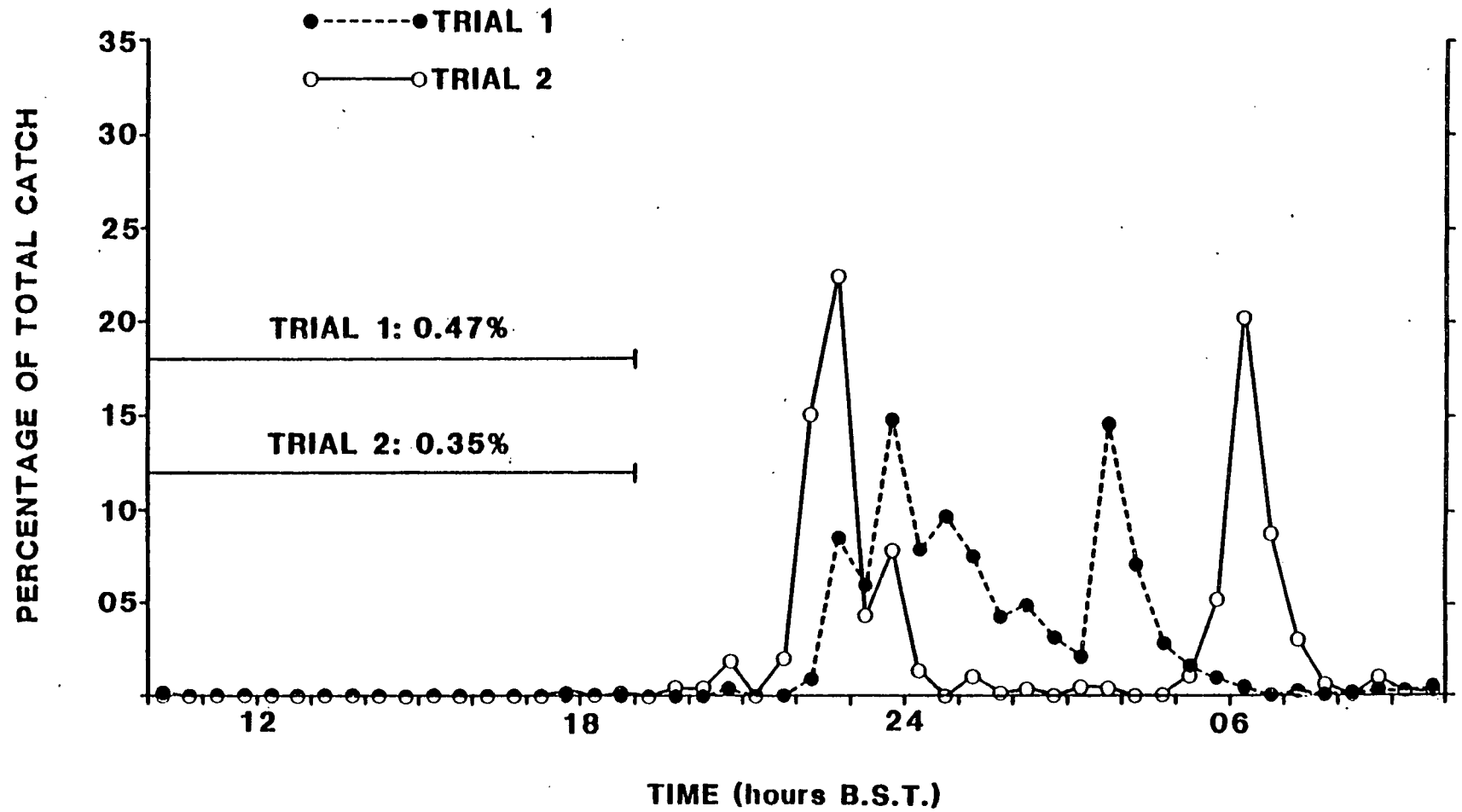


Figure A1-1. Flight activity patterns of female *Culicoides impunctatus*:
Field Site and suction trap.
Trials. 1 (11th-12th.7.83) and 2 (17th-18th.7.83).

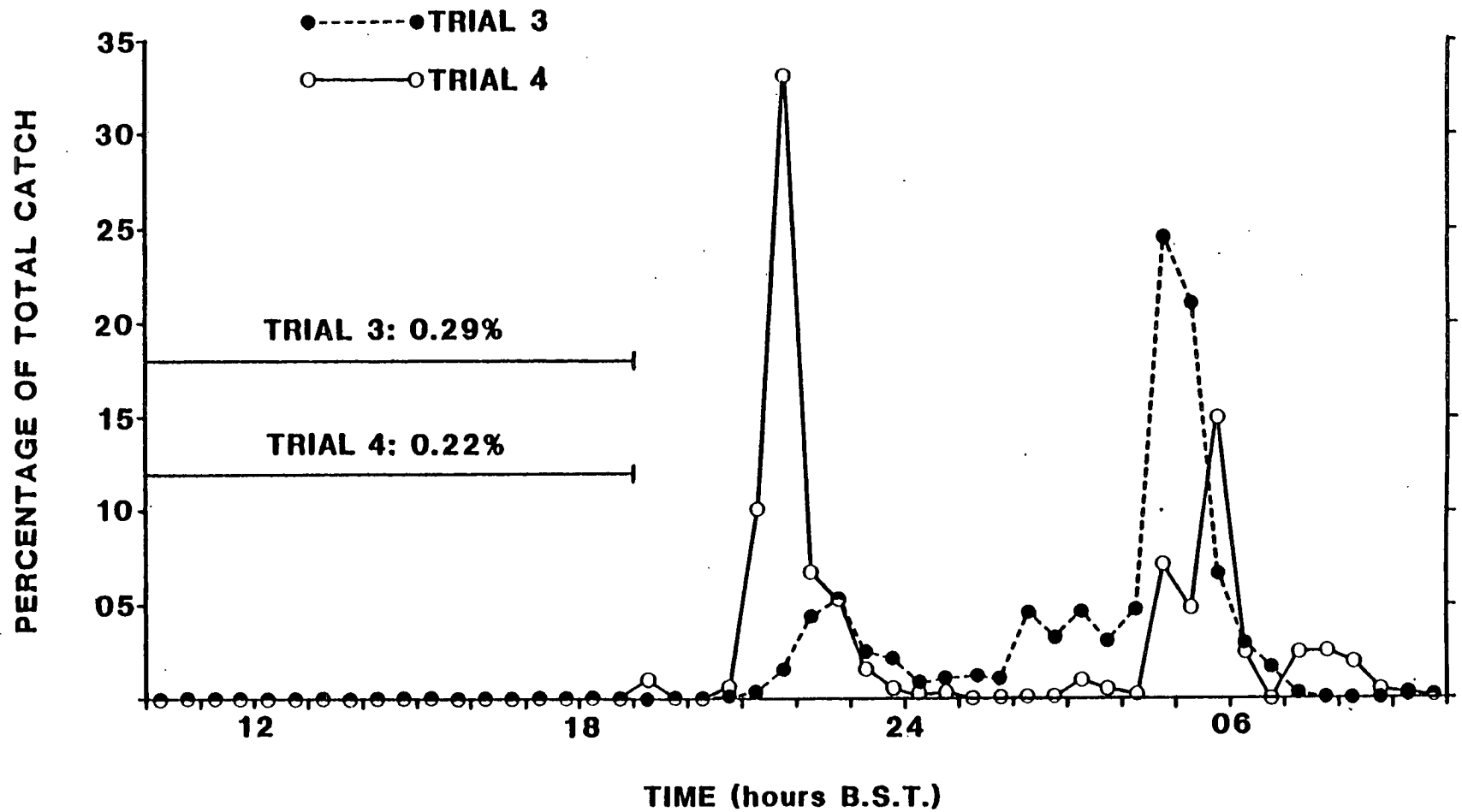


Figure A1-2. Flight activity patterns of female *Culicoides impunctatus*:
 Field Site and suction trap.
 Trials 3 (24th-25th.7.83) and 4 (27th-28th.7.83).

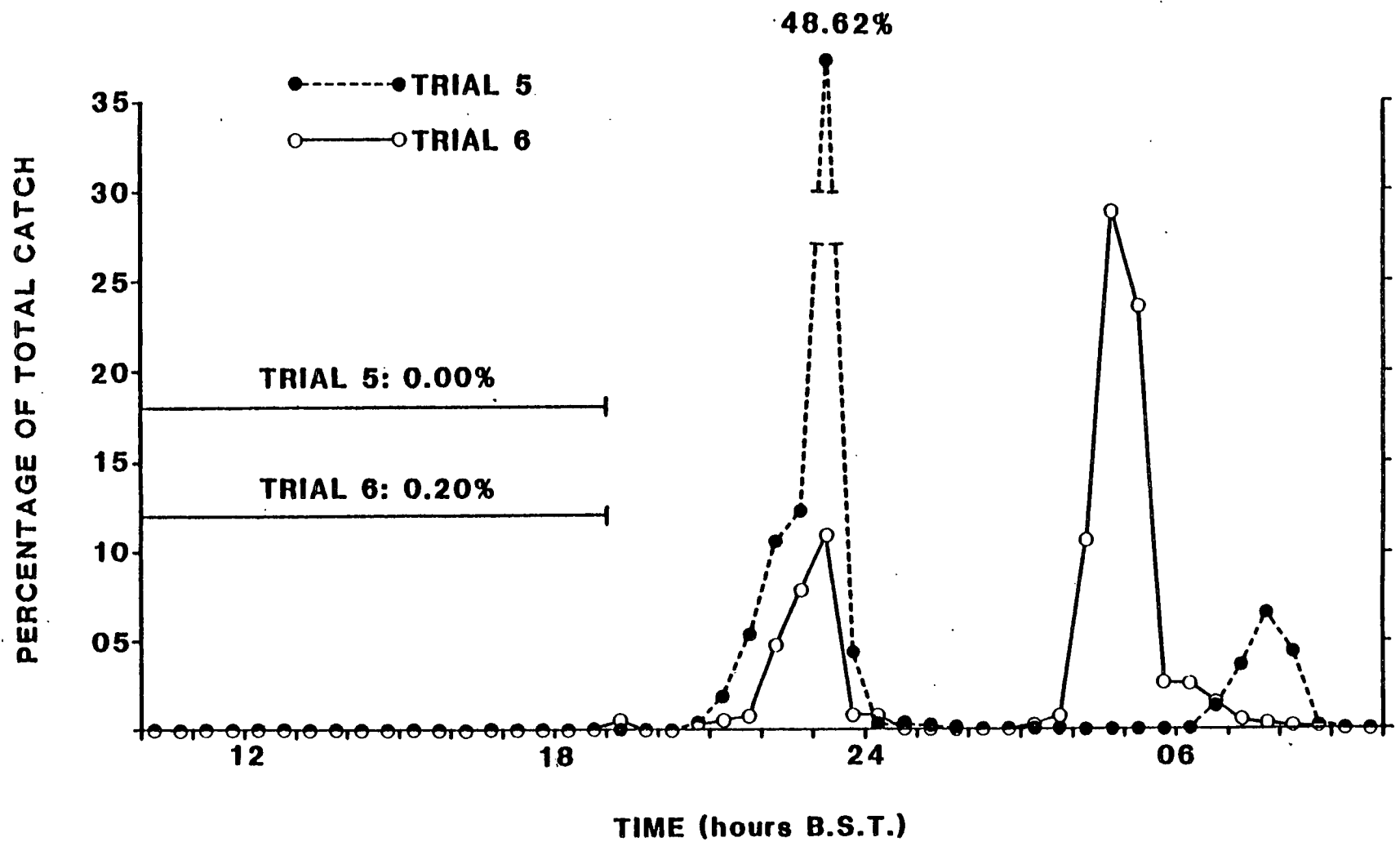


Figure A1-3. Flight activity patterns of female *Culicoides impunctatus*:
 Field Site and suction trap.
 Trials 5 (2nd-3rd.7.84) and 6 (17th-18th.7.84).

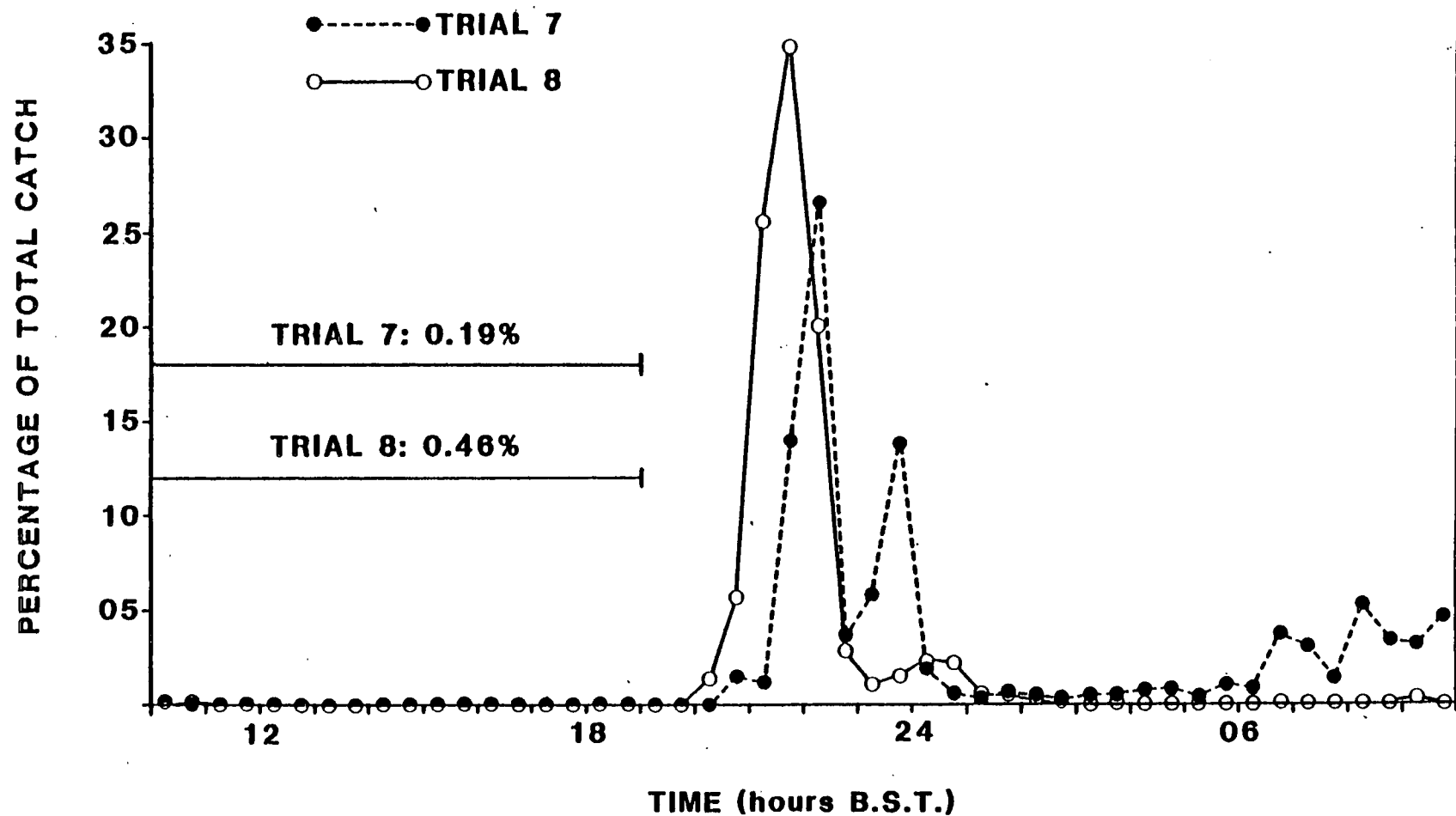


Figure A1-4. Flight activity patterns of female *Culicoides impunctatus*:
Field Site and suction trap.
Trials 7 (25th-26th.7.84) and 8 (1st-2nd.8.84).

Appendix 2

Multiple linear regression analysis: data

CIMP	TEMP	HUMID	WIND	LIGHT	WDIR	TRIAL	RAIN	TIME
0000	25.81	65.72	1.27	0368	1	1	2	01
0000	25.09	69.03	0.63	0241	1	1	2	01
0000	22.94	82.48	0.38	0125	1	1	2	02
0004	21.09	90.30	0.96	0081	1	1	2	02
0000	19.99	92.76	1.11	0039	1	1	2	03
0001	18.71	95.50	0.74	0016	1	1	2	03
0010	17.85	96.72	0.91	0010	1	1	2	04
0091	17.23	97.43	1.12	0005	1	1	2	04
0063	16.69	97.89	0.94	0000	1	1	2	05
0158	16.19	98.46	0.80	0000	1	1	1	05
0084	15.95	98.61	0.77	0000	1	1	1	06
0102	15.51	99.05	0.89	0000	1	1	1	06
0081	15.20	99.35	0.84	0000	1	1	1	07
0045	14.94	99.26	0.71	0000	1	1	1	07
0053	14.65	99.37	0.77	0000	1	1	1	08
0034	14.44	99.63	0.91	0000	1	1	1	08
0027	14.19	99.48	0.84	0000	1	1	2	09
0157	13.94	99.64	0.95	0000	1	1	2	09
0077	13.84	99.85	0.96	0007	1	1	2	10
0032	13.84	99.70	0.85	0014	1	1	2	10
0018	14.14	99.58	0.82	0030	1	1	2	11
0010	14.56	99.47	0.71	0068	1	1	2	11
0005	15.82	98.80	0.73	0141	1	1	2	12
0000	17.93	96.22	0.48	0214	2	1	2	12
0004	18.56	94.69	0.65	0261	2	1	2	13
0000	19.34	92.67	0.69	0406	1	1	2	13
0002	20.41	89.04	1.14	0469	2	1	2	14
0004	21.66	83.07	0.68	0582	2	1	2	14
0002	23.03	77.56	1.18	0751	2	1	2	15
0002	24.22	72.75	1.18	0865	2	1	2	15
0000	14.96	76.95	1.35	0343	1	2	2	01
0003	13.91	81.18	1.28	0184	1	2	2	01
0003	14.06	80.72	1.02	0183	1	2	2	02
0011	13.68	83.06	1.19	0066	1	2	2	02
0000	13.15	85.53	1.75	0023	1	2	2	03

CIMP	TEMP	HUMID	WIND	LIGHT	WDIR	TRIAL	RAIN	TIME
0012	12.87	87.35	0.89	0013	1	2	2	03
0086	12.65	87.84	1.03	0006	1	2	2	04
0128	12.47	89.76	0.82	0000	1	2	2	04
0025	12.64	91.05	0.87	0000	1	2	2	05
0044	12.69	93.42	0.55	0000	1	2	2	05
0008	12.45	96.15	1.04	0000	1	2	2	06
0000	12.51	95.25	0.52	0000	1	2	2	06
0006	12.46	96.31	0.51	0000	1	2	2	07
0001	12.27	98.51	0.57	0000	1	2	2	07
0002	12.28	98.51	0.46	0000	1	2	2	08
0000	12.43	98.18	0.70	0000	1	2	2	08
0003	12.21	97.53	1.37	0000	1	2	2	09
0002	11.38	96.40	3.08	0000	1	2	2	09
0000	10.98	95.36	2.84	0000	1	2	2	10
0000	10.69	94.38	2.76	0006	1	2	2	10
0006	10.60	93.18	2.83	0013	1	2	2	11
0030	10.50	90.64	1.48	0016	1	2	2	11
0116	10.53	91.15	0.62	0033	1	2	2	12
0050	10.60	88.23	0.98	0064	1	2	2	12
0018	10.90	86.79	1.26	0149	1	2	2	13
0004	11.31	85.34	1.57	0244	1	2	2	13
0000	11.45	81.59	1.35	0273	1	2	2	14
0006	11.70	78.92	1.97	0305	1	2	2	14
0001	12.28	77.16	1.49	0399	1	2	2	15
0004	12.54	73.01	2.76	0411	1	2	2	15
0000	22.30	63.16	0.51	0288	2	3	2	01
0005	20.55	74.53	0.38	0191	2	3	2	01
0001	19.33	84.31	0.54	0128	1	3	2	02
0002	17.41	90.42	0.82	0073	1	3	2	02
0018	16.57	90.09	1.10	0032	1	3	2	03
0072	15.49	92.76	1.12	0013	1	3	2	03
0200	14.57	95.24	1.15	0006	1	3	2	04
0249	13.96	96.97	0.84	0000	1	3	2	04
0110	14.37	96.79	0.74	0000	1	3	2	05
0099	14.13	97.13	0.78	0000	1	3	2	05

CIMP	TEMP	HUMID	WIND	LIGHT	WDIR	TRIAL	RAIN	TIME
0032	13.31	98.45	0.85	0000	1	3	2	06
0051	12.85	98.61	1.01	0000	1	3	2	06
0062	12.53	98.88	1.00	0000	1	3	2	07
0049	12.82	98.67	0.79	0000	1	3	2	07
0218	13.99	95.80	0.90	0000	1	3	2	08
0152	13.12	98.40	0.82	0000	1	3	2	08
0214	13.35	98.56	0.79	0000	1	3	2	09
0143	13.55	98.56	0.85	0000	1	3	2	09
0216	14.34	96.13	0.61	0000	1	3	2	10
1107	14.45	96.03	0.78	0005	1	3	2	10
0950	14.48	95.64	0.89	0011	1	3	2	11
0297	14.13	97.49	0.78	0016	1	3	1	11
0130	14.23	97.39	0.73	0026	1	3	1	12
0076	14.70	97.45	0.43	0046	1	3	1	12
0015	15.14	98.56	0.45	0076	1	3	1	13
0008	15.49	97.67	0.54	0098	1	3	1	13
0000	15.90	97.34	0.40	0132	2	3	1	14
0003	16.20	96.73	0.43	0142	2	3	1	14
0007	16.40	95.74	0.50	0151	1	3	1	15
0004	16.76	95.58	0.60	0255	2	3	1	15
0020	15.36	72.95	2.17	0274	1	4	2	01
0000	15.03	74.17	1.95	0212	1	4	2	01
0000	14.20	77.33	1.73	0128	1	4	2	02
0011	13.41	79.84	1.43	0058	1	4	2	02
0183	12.26	84.80	0.65	0022	1	4	2	03
0602	11.41	88.71	0.70	0011	1	4	2	03
0124	11.20	89.13	0.83	0005	1	4	2	04
0099	11.59	88.49	0.68	0000	1	4	2	04
0033	11.99	89.63	0.87	0000	1	4	2	05
0011	12.06	88.54	0.69	0000	1	4	2	05
0003	11.99	89.52	0.65	0000	1	4	2	06
0006	12.26	87.19	0.95	0000	1	4	2	06
0000	12.43	86.78	1.16	0000	1	4	2	07
0002	12.39	88.43	0.95	0000	1	4	2	07
0000	12.39	89.06	0.76	0000	1	4	1	08

CIMP	TEMP	HUMID	WIND	LIGHT	WDIR	TRIAL	RAIN	TIME
0002	12.27	91.13	0.88	0000	1	4	1	08
0018	12.24	92.13	0.78	0000	1	4	1	09
0009	12.09	95.15	0.83	0000	1	4	1	09
0002	12.01	97.09	0.99	0000	1	4	1	10
0130	12.10	97.09	0.69	0005	1	4	1	10
0089	12.31	97.53	0.46	0010	1	4	1	11
0272	12.69	98.13	1.07	0012	1	4	1	11
0046	12.93	98.45	1.44	0014	1	4	1	12
0000	13.19	98.56	1.13	0017	1	4	1	12
0047	13.46	98.56	0.85	0026	1	4	1	13
0048	13.78	97.94	0.90	0037	1	4	1	13
0039	14.01	97.38	1.01	0078	1	4	1	14
0009	14.45	95.98	0.97	0101	2	4	1	14
0007	14.67	96.30	0.90	0117	1	4	2	15
0002	14.68	95.85	1.57	0162	1	4	2	15
0000	15.83	52.53	2.67	0323	1	5	2	01
0000	15.76	53.58	2.18	0245	1	5	2	01
0000	14.76	56.55	2.16	0131	1	5	2	02
0003	13.50	61.38	2.21	0070	1	5	2	02
0018	12.12	67.14	1.13	0040	1	5	2	03
0050	10.51	75.42	1.15	0020	1	5	2	03
0100	09.81	78.10	0.95	0011	1	5	2	04
0116	08.00	87.33	0.89	0005	1	5	2	04
0461	07.83	88.05	1.18	0000	1	5	2	05
0040	07.03	91.21	0.88	0000	1	5	2	05
0002	06.13	93.12	0.88	0000	1	5	2	06
0003	05.48	94.95	0.68	0000	1	5	2	06
0002	04.78	96.18	0.51	0000	1	5	2	07
0001	04.19	96.98	0.85	0000	1	5	2	07
0000	03.93	97.54	0.87	0000	1	5	2	08
0000	03.63	97.60	1.05	0000	1	5	2	08
0000	03.23	98.34	0.86	0000	1	5	2	09
0000	03.03	98.33	0.92	0005	1	5	2	09
0000	02.85	98.33	0.94	0010	1	5	2	10
0000	02.81	97.88	1.00	0016	1	5	2	10

CIMP	TEMP	HUMID	WIND	LIGHT	WDIR	TRIAL	RAIN	TIME
0000	03.29	96.25	0.94	0042	1	5	2	11
0000	04.77	93.04	0.68	0100	1	5	2	11
0000	06.74	89.78	0.53	0193	1	5	2	12
0013	09.56	80.72	0.43	0311	2	5	2	12
0034	11.10	74.41	0.47	0441	2	5	2	13
0063	11.85	68.80	1.53	0581	1	5	2	13
0041	12.26	65.48	2.12	0655	1	5	2	14
0001	12.91	61.81	2.20	0784	1	5	2	14
0000	13.72	59.33	2.40	0890	1	5	2	15
0000	14.32	55.87	1.99	0940	1	5	2	15
0148	16.79	82.85	0.84	0169	2	6	2	01
0006	16.59	81.42	1.53	0161	1	6	2	01
0004	16.23	80.89	1.56	0088	1	6	2	02
0082	15.88	82.36	1.13	0051	1	6	2	02
0139	15.47	84.17	1.20	0021	1	6	2	03
0203	15.04	85.42	1.61	0013	1	6	2	03
1241	14.58	87.56	1.03	0006	1	6	2	04
2039	14.25	90.27	1.14	0000	1	6	2	04
2837	14.16	90.44	2.02	0000	1	6	2	05
0217	14.07	89.53	2.01	0000	1	6	2	05
0166	13.93	89.89	1.37	0000	2	6	2	06
0024	13.96	88.65	1.65	0000	1	6	2	06
0000	13.81	89.66	1.45	0000	1	6	2	07
0000	13.39	91.07	1.67	0000	1	6	2	07
0011	13.22	92.27	1.71	0000	1	6	2	08
0007	13.12	92.40	1.31	0000	1	6	2	08
0066	12.97	93.46	1.05	0000	1	6	2	09
0187	12.74	94.37	1.07	0000	2	6	2	09
2737	12.88	93.71	0.62	0006	2	6	2	10
7498	12.90	94.29	0.56	0012	1	6	2	10
6140	13.00	92.33	0.71	0018	2	6	2	11
0688	13.19	91.18	0.73	0034	2	6	2	11
0688	13.47	90.38	0.53	0062	2	6	2	12
0440	13.53	89.78	1.12	0108	2	6	2	12
0129	14.14	87.72	0.72	0159	2	6	2	13

CIMP	TEMP	HUMID	WIND	LIGHT	WDIR	TRIAL	RAIN	TIME
0086	14.55	85.38	0.87	0261	2	6	2	13
0030	15.22	82.71	1.16	0343	2	6	2	14
0032	15.47	81.48	1.20	0425	1	6	2	14
0017	16.16	78.26	1.11	0491	1	6	2	15
0011	17.83	72.87	0.76	0661	2	6	2	15
0000	20.03	57.77	1.59	0306	2	7	2	01
0001	18.46	62.50	2.16	0192	1	7	2	01
0001	18.20	62.84	1.29	0130	2	7	2	02
0059	16.17	70.10	1.56	0055	1	7	2	02
0046	15.36	71.30	1.61	0025	1	7	2	03
0590	13.72	77.88	1.54	0012	1	7	2	03
1123	13.76	75.99	1.86	0006	1	7	2	04
0147	11.80	84.71	0.94	0000	1	7	2	04
0242	12.28	81.83	1.79	0000	1	7	2	05
0575	11.97	83.37	1.22	0000	2	7	2	05
0075	10.83	87.92	0.83	0000	2	7	2	06
0021	08.96	95.50	0.75	0000	1	7	2	06
0017	08.58	96.25	1.08	0000	1	7	2	07
0023	08.20	97.02	1.18	0000	1	7	2	07
0018	08.02	97.44	1.03	0000	1	7	2	08
0009	07.72	98.12	1.04	0000	1	7	2	08
0022	07.44	98.62	1.06	0000	1	7	2	09
0019	07.14	99.07	1.08	0000	1	7	2	09
0033	06.99	98.82	1.09	0000	1	7	2	10
0037	06.86	99.21	1.10	0010	1	7	2	10
0017	06.83	99.28	1.05	0017	1	7	2	11
0048	07.27	98.30	1.10	0041	1	7	2	11
0038	08.42	97.58	0.84	0103	1	7	2	12
0161	10.69	94.71	0.51	0193	2	7	2	12
0131	12.83	86.46	0.53	0299	2	7	2	13
0055	14.15	80.68	0.57	0349	2	7	2	13
0226	15.21	76.79	0.92	0500	2	7	2	14
0147	16.17	71.70	0.84	0656	2	7	2	14
0140	16.96	64.53	1.64	0816	1	7	2	15
0206	17.44	60.36	2.50	0917	1	7	2	15

CIMP	TEMP	HUMID	WIND	LIGHT	WDIR	TRIAL	RAIN	TIME
0000	18.01	63.02	1.33	0232	2	8	2	01
0008	16.87	68.66	0.52	0114	1	8	2	01
0116	15.66	80.24	0.70	0048	2	8	2	02
0496	14.47	86.94	0.36	0027	1	8	2	02
2219	13.34	90.40	0.60	0012	2	8	2	03
3014	13.92	84.10	0.73	0006	2	8	2	03
1741	12.04	93.05	0.70	0000	1	8	2	04
0245	11.99	93.31	1.62	0000	1	8	2	04
0087	11.73	95.60	1.21	0000	1	8	2	05
0132	11.82	95.61	1.38	0000	1	8	2	05
0201	13.16	86.84	0.98	0000	1	8	2	06
0193	13.68	83.16	0.88	0000	1	8	2	06
0049	13.85	81.73	1.07	0000	2	8	2	07
0030	14.14	79.76	1.30	0000	2	8	2	07
0014	14.14	79.62	1.13	0000	2	8	2	08
0008	13.98	81.50	1.09	0000	2	8	2	08
0000	14.29	80.41	1.52	0000	2	8	2	09
0013	15.36	75.34	2.60	0000	2	8	2	09
0001	15.35	75.20	2.09	0000	2	8	2	10
0008	15.10	75.99	1.89	0005	2	8	2	10
0005	15.07	75.51	2.32	0010	2	8	2	11
0003	14.99	76.88	1.96	0016	2	8	2	11
0000	15.21	76.06	1.88	0022	2	8	1	12
0012	15.16	77.40	1.86	0042	2	8	1	12
0005	15.46	75.14	2.61	0085	2	8	1	13
0002	15.43	77.11	2.65	0052	2	8	1	13
0002	14.42	87.12	1.35	0018	2	8	1	14
0006	14.52	85.27	1.58	0027	2	8	1	14
0034	14.54	87.02	1.23	0046	2	8	1	15
0002	14.39	88.22	1.59	0042	2	8	1	15

Appendix 3

Artificial ventilation of a chalet balcony

A simple experiment was devised to test whether the artificial ventilation of a limited area of chalet balcony could reduce midge activity to a tolerable level. (As noted in Section 4.3.4.c, moderate breezes can significantly reduce midge flight activity.)

A 12-inch Vent-Axia® GX12 fan, controlled by a variac (Berco-Rotary-Regavolt®), was clamped to the chalet's gutter 110 cm above my arm (see Figure A3-1). A relationship was determined between the insect catch on a target zone (approx. 21 cm X 5 cm, drawn on my left forearm) and the wind speed over the target area ($0.0 - 1.5 \text{ m s}^{-1}$, in increments of 0.25 m s^{-1} ; see Figure A3-2). (The variac setting for each wind speed was determined using a Luftechnik® vane anemometer, Airflow Developments Ltd.) The experiment used catching periods of from two to five minutes at each wind speed; naturally, within any replicate, of which there were 20, the catching periods remained constant.

The mean catch at 1 m s^{-1} comprised 1.34% of the total over the range of wind speeds. Those insects that were caught at and above this wind speed were blown onto the target area; having, one assumes, been sucked into the fan intake. Fitting a gauze to the fan inlet could eliminate this problem.



Figure A3-1. Performing the 'artificial ventilation' experiment
(see text for details)

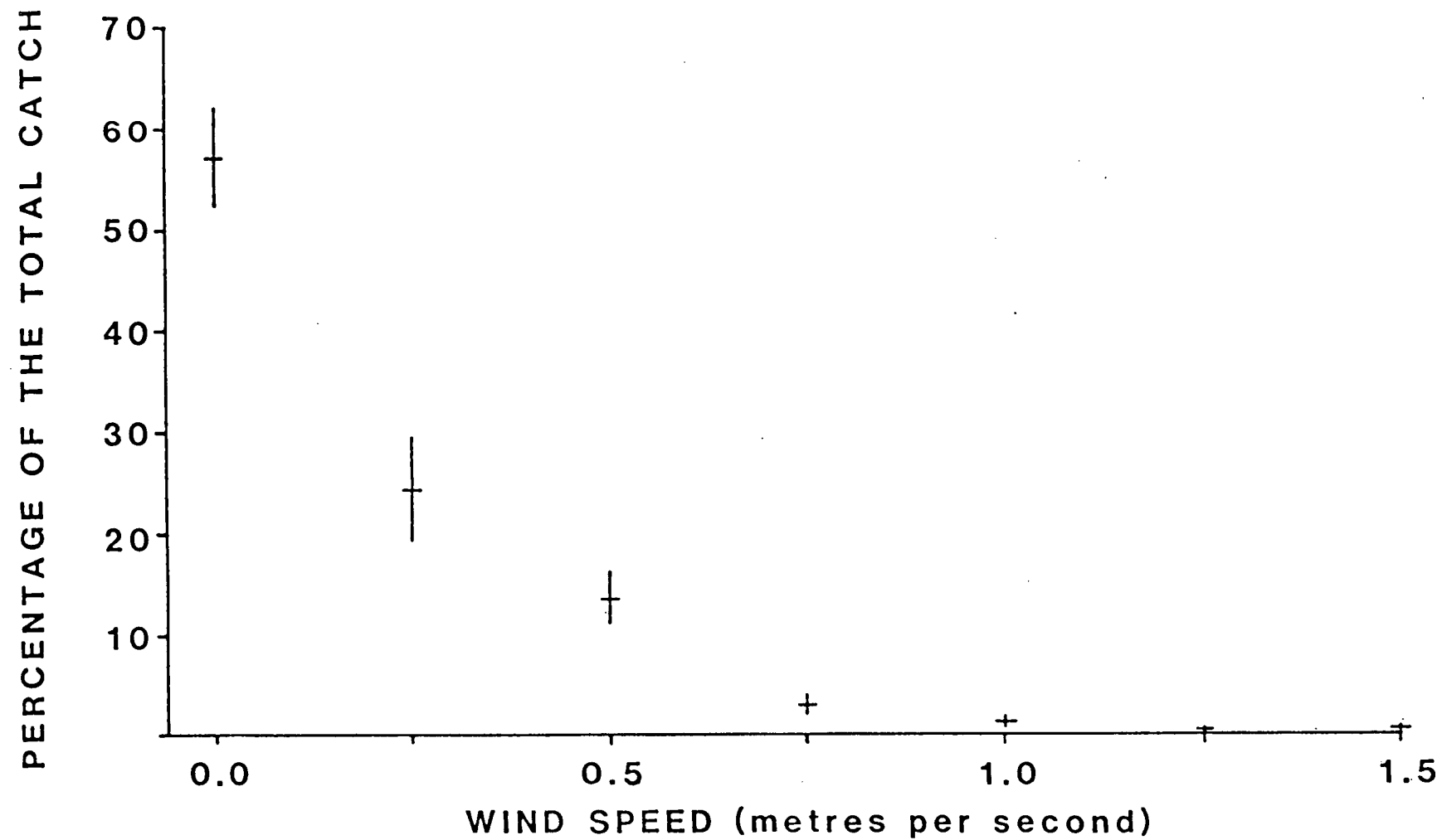


Figure A3-2. The effect of wind speed on catch size:
mean ($n = 20$) and standard error

Given the choice, I would grant this thesis its inalienable right to a place in the auto-da-fé.