POPULATION DYNAMICS AND ECOLOGY OF AEDES CANTANS (DIPTERA: CULICIDAE) IN ENGLAND

THESIS SUBMITTED IN ACCORDANCE WITH THE
REQUIREMENTS OF THE UNIVERSITY OF LIVERPOOL FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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CONTENTS

CHAPTER ONE - GENERAL INTRODUCTION

1.1 BRITISH MOSQUITOES

There are thirty-four species of mosquitoes found in Britain, five anophelines and twenty-nine culicines (Cranston et al., 1987).

1.2 HISTORY OF VECTOR-BORNE DISEASES IN THE UNITED KINGDOM

British mosquitoes are no longer of importance in human disease transmission, although some native species are disease vectors in other parts of the world. Malaria was endemic in some parts of Britain up to the beginning of the 20th Century and was especially common in south-east England and the Fens (Snow, 1987a). Anopheles atroparvus van Thiel transmitted the benign tertian form of malaria, Plasmodium vivax (Grassioc Felette), and this caused an acute fever known as ague. Drainage of marshlands and housing improvements separating people from livestock, led to the decline of malaria. During World War I, malaria transmission occurred in south-east England because large numbers of soldiers returned from the Middle East, some being infected with malarial parasites (Snow, 1987a).

The numbers of imported malaria cases has risen steeply from less than one hundred per year in the 1960's to approximately two thousand cases per year by the early 1980's as a result of increased foreign travel (Curtis and White, 1984). Most of these cases are P. vivax from Asia, and Plasmodium falciparum (Welsh) from Africa and also increasingly from Asia.

Yellow fever outbreaks occurred on ships arriving at British ports in the 19th Century. Occasionally Aedes aegypti (L.) maintained on board ship in water tanks and then infected by feeding on sailors with yellow fever, subsequently bit people in the surrounding ports and infected them. This, however, was a rare occurence (Snow, 1987a).

Various Aedes species, Culiseta morsitans (Theobald) and Culex pipiens L., all transmit arboviruses in Europe but not in Britain. British mosquitoes can transmit myxomatosis mechanically but their main nuisance remains that of being a biting pest (Service, 1971a).

Aedes cantans (Meigen) is the species that was investigated in this study. This species feeds on humans and a variety of other mammals (Service, 1971b). In Continental Europe it can transmit the arbovirus Tahyna while Sindbis virus has also been recorded in Ae. cantans in Sweden (Snow, 1991). Neither of these viruses has, however, been isolated in Britain. Snow (1991) considered that the Tayhna and Sindbis viruses are probably present but are, as yet, undetected in British Aedes including Ae. cantans. Tahyna virus gives symptoms close to influenza, and therefore clinically would be hard to detect, whilst Sindbis virus causes ochelba disease which causes pains in the muscles and joints, rash and moderate fever, and again may not be readily recognised by general medical practitioners as being the result of an arboviral infection.

1.3 AEDES CANTANS

1.3.1 DISTRIBUTION AND SEASONALITY

Aedes (Ochlerotatus) cantans is a univoltine mosquito which is particularly common in Britain, with a Palearctic distribution ranging from Britain and Ireland, through Europe, to China. It is a woodland species and lays its eggs in the damp leaf litter and mud of temporary pools which are often heavily shaded. Eggs are laid from May to September and remain unhatched until December to February. Once the eggs have hatched, larvae take several months to develop but usually pupate in early April and emerge as adults in April and May (Marshall, 1938).

Most work on this species has been concentrated in southern England; at Monk's Wood, Huntingdon and on Brownsea Island, Dorset (Service, 1968, 1971a, 1971c, 1971d, 1977a), and at Ness Woods on the Wirral Penninsula, Merseyside (Sulaiman, 1982).

1.3.2 EGGS

Service (1977a) conducted one of the few investigations on Ae. cantans eggs and looked briefly at diapause, distribution and sequential hatching. He attempted to break the diapause of the eggs by reducing the oxygen content of the water in which the eggs were placed. However, although this stimulates hatching in Aedes punctor (Kirby) (Fallis and Snow, 1983), it had only minor effect on Ae. cantans. He found that the eggs hatch sequentially, and that although some eggs hatch in mid-December, most hatch in mid-February.

1.3.3 LARVAE AND PUPAE

Somewhat surprisingly, very little work has been carried out on the larvae and pupae of this mosquito. Service (1977a), Lakhani and Service (1974) and Sulaiman (1982) investigated the mortality of Ae. cantans larvae and constructed survivorship curves and life-tables. Service (1977a) found that larval mortality was greatest in the early instar stages whilst Sulaiman (1982) obtained conflicting results, finding highest mortality in the early stages in one year and in later stages in the following year. One of the causes of larval mortality is predation. Larval predators include dytiscid larvae, adult aquatic beetles, Mochlonyx culiciformis (De Geer) and Chaoborus crystallinus

De Geer (Service 1973a). Coelomomyces fungi and mermithid nematodes also caused some larval deaths (Service, 1977a). Sulaiman (1982), working in Ness Woods, found that the major cause of larval mortality was the ponds drying up before the mosquitoes had time to emerge. In southern England, Service (1977a) estimated that there was 95% larval and pupal mortality.

The distribution of larvae is highly aggregated (Service, 1985) which can sometimes make standardised sampling difficult. Most larval sampling work in small temporary pools is done using a standard dipper. Younger instar Aedes tend to spend longer at the bottom of the pond than the larger ones because they rely more on cuticular respiration; they are therefore less likely to be sampled, at the surface with a dipper (Service, 1976).

1.3.4 ADULTS

Most work on Ae. cantans has been carried out on the adults. Emerging adults have been sampled using a variety of small and large cages (Service, 1977a) and more recently with bednets (Sulaiman, 1982) placed over ponds and ditches, whilst older adults are usually sampled by human-bait catches and sweep-netting vegetation (Service, 1976). Mark-release-recapture work has also been carried out on mosquitoes using both paint spots and fluorescent powders. Service (1977a), working at Monk's Wood, Huntingdon, found that newly emerged females fed on sugar secretions for up to three weeks and only then began to blood-feed. Maximum biting occured around sunset and the biting season extended from June to September, with peak biting in July. The ELISA test (Service et al., 1986) has been used to identify the hosts of Ae. cantans and other species. The average number of eggs laid per batch is 32 but there is a decrease in fecundity with decreasing adult female size (Service, 1977a). There is also a decrease in the number of eggs laid with each ovarian cycle. It takes approximately 15 days for the embryos to mature after which the eggs enter diapause.

1.4 OBJECTIVES OF THIS STUDY.

The main objectives of this project were to investigate the interactions between density-dependent and density-independent processes and their effects on the regulation of mosquito populations.

The importance of intra- and interspecific competition on larval stages was also investigated and attempts were made to relate these factors to the population dynamics of adult mosquitoes. This involved extensive measurement of certain larval and adult characters.

1.4.1 INTRA- AND INTERSPECIFIC COMPETITION

Each individual in a population affects and is affected by other individuals within the population. Competition has been described as the "manifestation of the struggle for existence in which two or more organisms of the same or of different species exert a disadvantageous influence upon each other because their more or less active demands exceed the immediate supply of their common resources" (Begon and Mortimer, 1983). The general idea is of harm being done to a number of animals striving for a resource which is in limited supply.

When studying populations one has to be able to measure the effect of competition and know its causes. This involves measuring the changes in the resource supply, the number of individuals competing, and also assessing the disadvantageous influences which may be reflected either in the number or proportion of the organisms, or as a decrease in growth rate, adult weight or reproductive capacity. Some of the resources that mosquitoes may find in limited supply include food and space for larvae, and hosts (food) and oviposition sites for adults.

Competition can be between individuals of the same species in which case it is termed intraspecific competition, or between individuals of different species, that is, interspecific competition.

There are several characteristics of intraspecific competition. Firstly, the ultimate effect of competition must be a decrease in the contribution of individuals to later generations, or a decrease from the potential contribution had there been no competitors. Secondly, the resource must be in limited supply, and in addition the competitors must be inherently equivalent. Finally the effect of competition on any one individual is greater the greater the number of competitors. The effects of intraspecific competition are therefore said to be density-dependent.

Whenever there is intraspecific competition, its effects, whether on survival, fecundity or both, are density-dependent. Not all density-dependent effects result from intraspecific competition but all density-dependent effects show a tendency to regulate population size. Regulation is described as the ability to decrease or increase the size of a population above or below the population's equilibrium, or its carrying capacity. Such regulation is extremely widespread, and may be universal. In this study intraspecific regulation in Ae. cantans larvae is examined in detail.

Natural populations are usually assemblages of species and in many habitats the neighbours of one individual may be a different species. One species may cause an increase or decrease in the fitness of another species or have no effect at all. There are many different types of relationships between different species of organisms including predator-prey, host-parasite, commensalism, amensalism and symbiosis. Interspecific competition is not investigated in great detail in this study, except in terms of predation and parasitism. Mosquito larvae are preyed upon by a variety of pond fauna including dytiscid larvae and adult aquatic beetles (Service, 1973a). Adults are sometimes parasitised by water mites (Mullen, 1974).

Density-independence and its effects were also investigated in the present study. Weather conditions were monitored to show the effect of temperature, windspeed and rainfall on larval and adult populations of Ae. cantans. Although weather conditions can have profound effect on the numbers of mosquitoes and also other organisms, the effects are termed density-independent because they have equal effect on large populations as well as individuals.

Considerable work has been carried out on the regulation of mosquito populations and the pertinent literature is discussed in the appropriate chapters.

Studies on mosquito larval ecology are usually complicated by their patchy distribution. Mosquito larvae tend to aggregate in more favourable environments, such as shallower water or warmer regions (Service, 1976). This aggregation means that there are often large differences between the numbers of larvae caught in different samples and so sampling intensity must be increased.

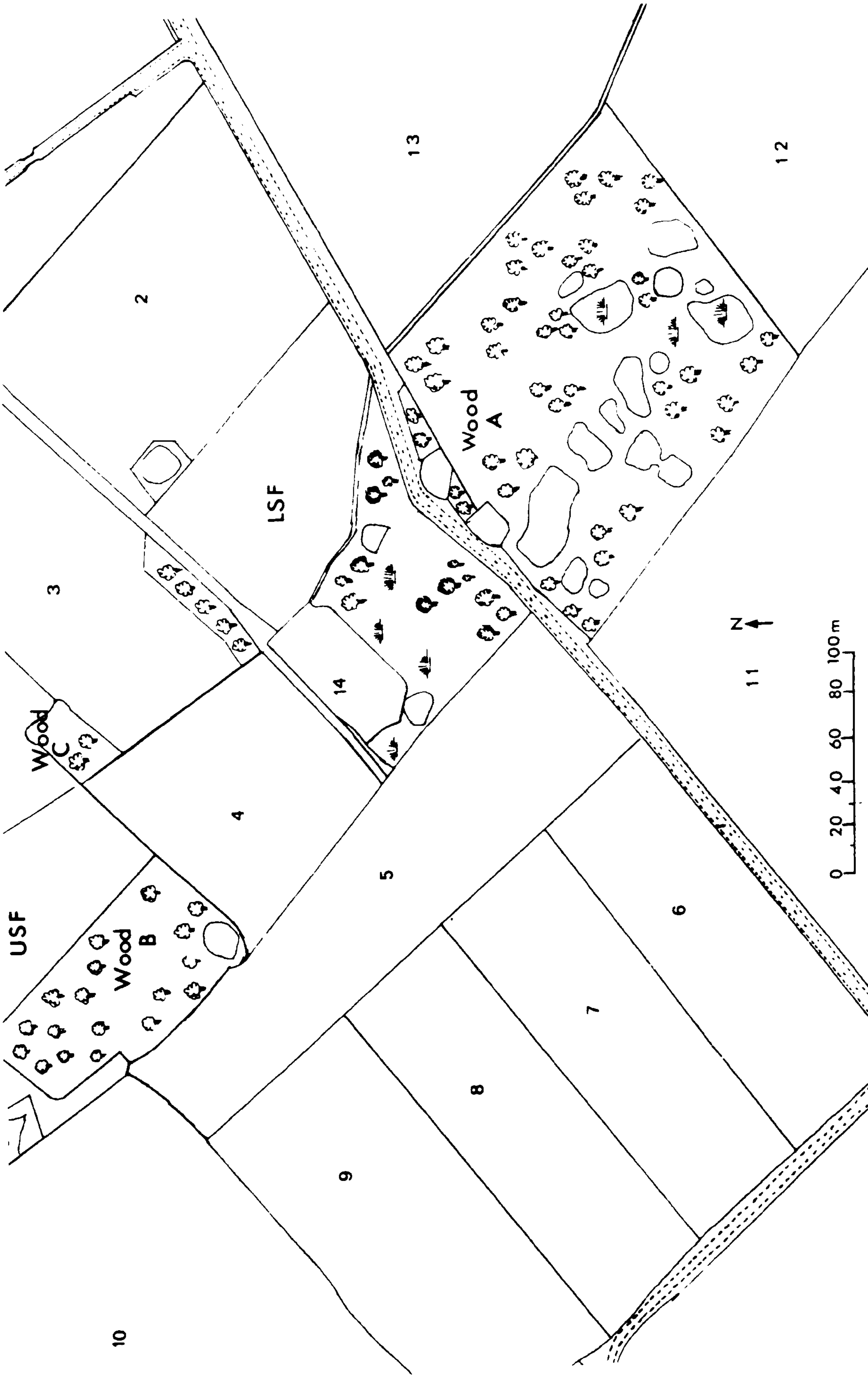
1.5 FIELD SITE

Fieldwork was carried out near to the village of Ness (3^0 1.5'W, 53^0 17'N) on the Wirral Peninsula, Merseyside (Fig.1.1).

The study area consists of three woods; Wood A, a private woodland known as Ness Woods, and Woods B and C, both located on the University of Liverpool's Faculty of Veterinary Science Animal Husbandry Farm. These woods are surrounded by fields used for grazing, mainly by sheep and cattle (Fig.1.2.).

Fig 1.1 : Map of the Wirral Peninsula and the location of the field site at Ness Woods.

Fig. 1.2: Map of the field site at Ness Woods, showing location of woods and the surrounding fields (1-14).



1.5.1 WOOD A (Fig.1.3)

1.5.1.1 FLORA.

Wood A, the main sampling site is approximately 6 hectares. It is dominated by three species of tree; sycamore (Acer pseudoplatanus L.) which makes up about 30% of the trees, and oak (Quercus petraea {Mattuschka} Liebl.) and silver birch (Betula pendula Roth) which each comprise approximately 20%.

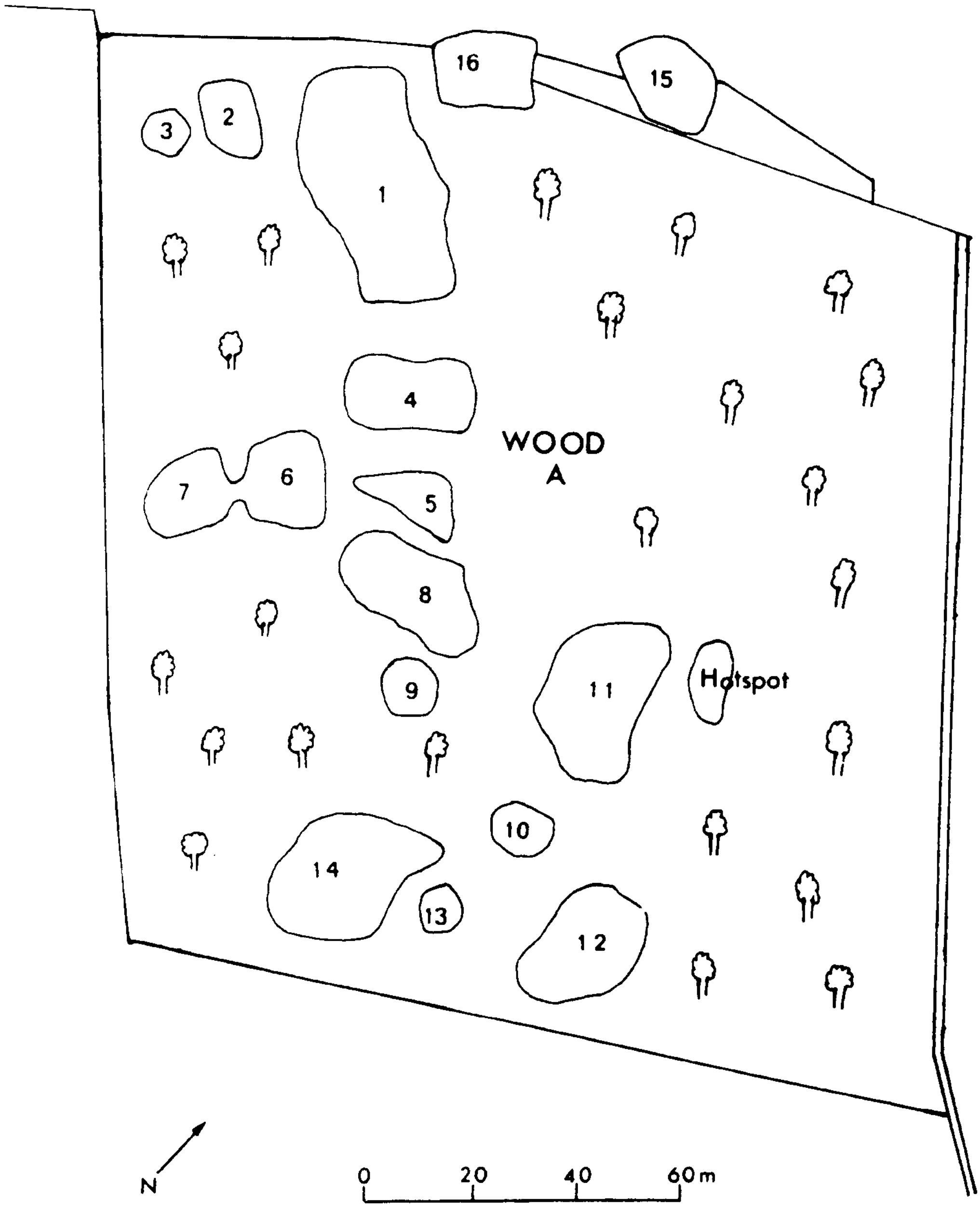
The remaining 30% is a mixture of the following species;

Elm (Ulmus procera Salisb).
Horse chestnut (Aesculus hippocastanum L.).
Ash (Fraxinus excelsior L.).
Elder (Sambucus nigra L.).
Rowan (Sorbus aucuparia L.)
Hawthorn (Crataegus monogyna Jacq.).
Crabapple (Malus sylvestris Miller).
Holly (Ilex aquafolium L.).

Ground vegetation is predominantly bramble (Rubus fruticosus L.) and bracken (Pteridium aquilinum {L.} Kuhn) and is especially thick during the summer, providing many resting sites for adult mosquitoes. There are some grassy areas, comprising predominantly scented vernal grass (Anthoxanthum odoratum L.). Other ground vegetation includes the following;

Pendulous sedge (Carex pendula Hudson).
Wood millet (Milium effusum L.).
Yellow oat grass (Trisetum flavescens {L.} Beauv.).
Woodland brome (Bromus ramosus Hudson).
Wood melick (Melica uniflora Retz).

Fig. 1.3: Map of Wood A, Ness Woods, showing location of ponds (1-16).



Bluebells (*Hyacinthoides non-scripta* {L.} Chouard ex Rothm).

Red campion (*Silene dioica* {L.} Clairv.).

Honeysuckle (*Lonicera periclymenum* L.).

Rose-bay willow herb (*Chamaenerion angustifolium* L.).

Stinging nettles (*Urtica dioica* L.).

Ladies bedstraw (*Galium verum* L.).

Foxglove (*Digitalis purpurea* L.).

These species were identified using Clapham *et al*. (1981).

1.5.1.2 FAUNA.

There is also a rich mammal fauna within the woods including; grey squirrels (*Sciurus carolinensis* Gmelin), foxes (*Vulpes vulpes* L.), badgers (*Meles meles* L.), moles (*Talpa europea* L.), woodmice (*Apodemus sylvaticus* L.), rats (*Rattus norvegicus* Erxleben) and voles (*Clethrionomys glareolus* Schreber).

Birds found within the wood include wrens (*Troglodytes troglodytes* L.), treecreepers (*Certhia familaris* L), nuthatches (*Sitta europaea* L), rooks (*Corvus fragilegus* L), wood pigeons (*Columba corone* L), kestrels (*Falco tinnunculus* L), little owls (*Athene noctua* Scopoli), barn owls (*Tyto alba* Scopoli), tawny owls (*Strix aluca* L), mallards (*Anas platyrhynchus* L), coots (*Fulica atra* L), greater spotted woodpeckers (*Dendrocopus major* L.) and robins (*Erithacus rubecula* L.).

1.5.1.3 PONDS.

Wood A contains 15 permanent ponds, 5 temporary ponds and 2 ditches. Most sampling work was carried out in two temporary ponds; Pond 9 and the 'Hotspot'.

Aedes cantans is the commonest mosquito found in Ness woods, comprising more than 99% of the adult mosquito population. *Aedes punctor*, *Aedes rusticus*

(Rossi), *Cs. morsitans*, *Culiseta annulata* (Shrank), *Aedes flavescens* (Muller), *Anopheles claviger* (Meigen), *Anopheles plumbeus* Stephens and *Culex pipiens* make up the remaining 1%.

Wood A is owned by Mr. G. Nichols, a conservationist farmer who ensures that there is little interference in the wood.

1.5.2 WOOD B (Fig. 1.2.)

Wood B is located at Leahurst Animal Husbandry Farm, and is approximately 4 hectares in size. It is a much more open wood consisting predominantly of oak (*Q. petraea*) and silver birch (*B. pendula*). Ground cover is sparser than in Wood A and consists of bracken, bramble and various grasses.

Mammals found in the wood include rabbits, hares, squirrels and rats. The bird fauna is similar to that found in Wood A.

There is one permanent pond, with no mosquito larvae, one semi-permanent pond, which was the only sampling site, and one dry ditch. This wood was used mainly for adult work.

1.5.3 WOOD C (Fig. 1.2.)

Wood C is a very small wood, less than 1 hectare, which is used for sweep-netting vegetation during the summer. The trees are predominantly birch, oak and holly and the groundcover consists of very dense brambles and some bracken. There is one permanent pond.

1.5.4 SURROUNDING FIELDS

The surrounding fields are grazed by cows and sheep, and one or two horses. The mammals use the fields on a rotational basis throughout the year.

1.6 WEATHER DATA

Both water and air temperatures were recorded during this investigation at the field site using a Squirrel Data Logger. Pond water temperatures were recorded just below the water surface using a probe shaded from sunlight. Air temperatures were measured using a probe suspended approximately 50 cm above the ground and shaded from sunlight. Weather data, including maximum and minimum daily temperatures recorded one metre above ground level using a screen and rainfall data were obtained from Ness Gardens, approximately 1.5 km west of Ness Woods. The relevance of using weather data collected at some distance from the field site is discussed in the relevant chapters. All three years were characterised by particularly dry, warm weather which presented several problems, especially for larval work.

CHAPTER TWO - LARVAL SAMPLING

2.1 INTRODUCTION

2.1.1 OBJECTIVES

The main objectives of the larval investigations were to examine density-dependent and density-independent regulation of Ae. cantans in its larval habitat. Special attention was paid to intraspecific competition in the field. Measurements of the larval siphon were taken to examine the effect of density and competition on size. The patterns of mortality were also assessed by drawing survivorship curves and constructing life-tables, and attention was paid to predation, parasitism, food, cannibalism and weather effects.

Larvae were sampled in all three years from ponds in Wood A (Fig. 1.3).

2.1.2 LARVAL BEHAVIOUR

It is generally more difficult to obtain unbiased samples of mosquito larvae and pupae than adults. One difficulty is that larvae and pupae are not randomly dispersed but have a highly aggregated distribution (Lakhani and Service, 1974; Service, 1985). Since most environments are not completely uniform, one would expect certain areas to be favoured at the expense of less attractive ones. In Canada, Hocking (1953) observed that larvae of Aedes communis (De Geer) aggregated around the edges of a pool, and Nielsen and Nielsen (1953) recorded the existence of 'balls' of Aedes taeniorhynchus (Wiedemann) 10-15 cm in diameter. Larval aggregation often results from differences in water temperature, availability of food and photonegative responses (Nayar and Sauerman, 1968).

Service (1985) examined Ae. cantans dispersion characteristics. He found that the sample variances were greater than the mean, indicating a highly aggregated larval distribution. However, the variance to mean ratio became smaller with successive instars, indicating that the degree of contagion decreased with age. Although the variance to mean ratio indicated that first and second instars were more highly aggregated than the later instars, the pattern of the distribution could not be consistently described by any mathematical models. In contrast, thirds and fourth instar larvae and pupae were well described by the negative binomial. Most biologically contagious distributions seem to fit this model.

Thomas (1950) (See Clements, 1963) found that regular repetition of changes in light intensity, such as shadows on the water surface caused a decrease in the numbers of larvae of Culex quinquefasciatus Say that dived to the bottom. The alarm response was density-dependent: the more larvae present, the more likely they were to submerge. Older larvae did not react by diving as often as younger ones. This is partly because younger instars have a larger surface area to volume ratio allowing them to obtain more oxygen by cuticular respiration of dissolved oxygen.

2.1.3 SAMPLING METHODS

Larval sampling may be carried out simply to determine the presence or absence of larvae, to monitor population changes associated with seasonal abundance or control, or alternatively to estimate the absolute population in a habitat from the number per dip or the number in a quadrat. Techniques for sampling larvae include the use of dippers (ladles), nets, static quadrats, floating quadrats, larval collecting trays and light-traps (Service, 1976). The dipper is the most commonly used tool for collecting mosquito larvae and pupae; however, it is most suited to smaller volumes of water such as puddles, ditches and hoofprints, whilst aquatic nets are better for larger bodies of water (Sulaiman, 1982). Static quadrats (area samplers) are thrust down into the bottom of a habitat to enclose a known volume of water from which larvae can be removed and counted. Static quadrats can be used to estimate absolute densities by relating the surface area enclosed by the quadrat to the surface area of the breeding site. An advantage over the dipper is that larvae frightened from the surface by the approach of the collector are still sampled. A disadvantage is that it is labour intensive.

Sulaiman (1982), working in Ness Woods, assessed the best method for collecting immatures, by comparing a soup ladle (capacity 130 ml, diameter 9.5 cm), a metal quadrat and a D-shaped aquatic net. He found that sampling with the quadrat was the more reliable method, since the ladle, and to a lesser extent the D-net, sampled predominantly later instars. According to Service (1976), dipping usually catches larvae and pupae at the water surface. Since different species and instars remain at the surface for different periods, dipping may frequently be biased in favour of a particular species or instar. Nielsen and Nielsen (1953) observed that first, and to a lesser extent, second instar larvae of Ae. taeniorhynchus surfaced much less frequently than older larvae. The differences in submersion times of different immature stages will result in sampling bias when the age-structure of the population is derived from dipping.

Many studies have investigated the efficiency of the ladle over other sampling methods, and several authors have found that dipping is less efficient at catching the earlier instars (Andis et al., 1983; Hagstrum, 1971; Roberts and Scanlon, 1979; Wada and Mogi, 1974), or larvae in deeper water (Downing, 1977). Knight (1964) considered that a desirable advantage of static quadrat collecting over dipping is that larvae frightened from the surface can be collected. Dipping may therefore be biased for particular species and instars.

In contrast, Chubachi (1976) found that there was no difference in the efficiency of a dipper in comparison to a static quadrat in sampling *Culex tritaeniorhynchus* Dyar and *Anopheles hyrcanus sinensis* Wiedemann in ricefields and an artificial container in Japan. Croset *et al*. (1976) considered that dipping gives estimates of population size similar to those obtained by mark-release-recapture and removal methods.

In this investigation, dipping was the most commonly used technique for sampling larvae, although static quadrats were used in the second and third years, and nets were used to survey larger ponds in the woods.

With regard to the estimation of larval mortalities, Service (1977*b*) stated that there are several problems associated with the construction of survivorship curves and life-tables for immature stages of mosquitoes. Problems include overlapping generations and prolonged or continued recruitment, so that at any fixed point in time the population is composed of individuals of mixed age classes. This was not a problem in my investigation because with the exception of the Hotspot in 1989, there was a single generation in the ponds as a result of low rainfall levels.

2.1.4 DENSITY-DEPENDENCE AND DENSITY-INDEPENDENCE

Density-dependent processes regulate population size about an equilibrium density whilst density-independent processes, although capable of inflicting large mortalities, cannot regulate a population (Service, 1985). Habitat desiccation may be the most significant mortality factor experienced by a mosquito population and may often lead to temporary extinctions, but it acts independently of larval numbers.

Intraspecific competition occurs when a number of animals of the same species utilise common resources whose supply is limited. This is often competition for food, refugia or warmer spots (Klomp, 1963). Although resource limitation may be density-dependent when it involves intraspecific competition, this is only likely to occur at high population densities, near the habitat's carrying capacity.

In species that live in very small water bodies, such as container breeders or tree-hole species, intraspecific competition has been proposed to account for larval mortality (Mori and Wada, 1978; Southwood et al., 1972) as it has in laboratory studies (Chubachi, 1979; Gilpin and McClelland, 1979; Reisen, 1975). Many medically important vectors colonize larger, less stable groundwater habitats which are less easy to study.

Mortality is density-independent at low densities, but is density-dependent at high densities. As soon as mortality becomes more density-dependent, the smallest individuals are increasingly and disproportionately prone to mortality. In insect populations, increasing population density during the larval stages leads to increasing mortality. Density-dependent mortality, and therefore intraspecific competition, becomes increasingly apparent with increasing density. Frequently, most mortality occurs in the youngest stages (Wall and Begon, 1986).

2.1.5 CAUSES OF LARVAL MORTALITY

2.1.5.1 PREDATION

Sulaiman (1982) examined predation in larval habitats in Ness Woods, identifying predators by the precipitin and ELISA tests. He found that the most important predator of mosquito larvae was C. crystallinus. Mochlonyx culiciformis, and adults of five species of aquatic beetle also showed positive results with the precipitin test: Acilinus sulcatus (L.) Hydroporus palustris (L.), Colymbetes fuscus (L.), Agabus bipustulatus (L.) and Dytiscus circumcinctus Ahrens. Sulaiman (1982) concluded that although these adult beetles were predators of Ae. cantans, they were present in numbers too small to cause a significant decrease in the size of the population. Larval dytiscids were positively identified as predators and they were seen in the field attacking and killing third and fourth instar Ae. cantans in March and April (Sulaiman, 1982).

Service (1973, 1977a) identified the predators of Ae. cantans larvae at Monks Wood and other localities in southern England using the precipitin test. He found that the most important predators of Ae. cantans were larval Dytiscidae, e.g. Agabus bipustulatus (L.), but concluded that predation caused little decrease in population size. Baldwin et al. (1955), Iversen (1971) and James (1966) also found that larval dytiscids were the most important predators of mosquito larvae; however, Larson (1978) concluded that larval mortality due to predation was low, since dytiscids, the most voracious predators, were only present in small numbers. In Monk's Wood, several different predator species consumed a relatively large number of larvae, but their populations were small, and consequently they were unlikely to cause any important decrease in the larval population (Service, 1973).

2.1.5.2 FOOD

The most common method of feeding employed by mosquito larvae is filter-feeding, but larvae also feed by gnawing on large items, by browsing on filamentous algae, fungi and bacteria which cover submerged surfaces, by ingesting the film of living and non-living matter found at the water surface, or by crushing and swallowing small crustacea and other plankton (Clements, 1963). A few species are predatory and feed on insect larvae up to their own size. Southwood et al. (1972) studied the life budget of Ae. aegypti in Thailand and postulated that food might be the factor regulating the numbers of Ae. aegypti in village pots. In contrast, in Ness Woods, Sulaiman (1982) concluded that Ae. cantans has an abundant food supply, feeding on a variety of microscopic organisms and also browsing on leaf litter. He felt that although the specific nutritional requirements were unknown, food seemed unlikely to be the limiting factor.

Service (1985) was not convinced that there is a food deficit for culicines that browse and gnaw at decaying leaves and other organic debris which is so abundant in many ground pools in temperate deciduous woodlands. Overcrowding leading to intraspecific competition for food may be a major mortality factor of container-breeding mosquitoes, but is probably less important in species colonising larger habitats, such as ponds and marshes.

2.1.5.3 CANNIBALISM

Cannibalism is defined as combined acts of killing and ingesting conspecifics. Begon (1982) stated that cannibalism is a process with considerable potential for regulating populations, and its frequency is underrated. Cannibalism is very often density-dependent and requires a minimum size-ratio to be exceeded before the larger animal eats the smaller. One would not expect cannibalism to occur in Aedes larvae since they are filter feeding detritivores. However, cannibalism has been recorded in a number of typical filter feeders. The disappearance of newly hatched larvae in the presence of older larvae must be attributed to cannibalism provided that there is no reduction in the number of older larvae and the time period is too short for larval decomposition.

Mogi (1978a) observed cannibalism in the typical filter feeders, Culex tritaeniorhynchus Giles and An. sinensis. Koenekoop and Livdahl (1986) investigated cannibalism in Aedes triseriatus (Say). Contrary to expectations, they found that the rate of cannibalism was not significantly affected by larval density and the availability of yeast decreased cannibalism but did not eliminate it. They reported that the vulnerability of first instar larvae decreased during the first 24 hours, partly because consumption time increased with increased size, but mainly because increased size led to increased agility. Cannibalism has also been recorded in Anopheles stephensi Liston (Reisen and Emory, 1976). Cannibalism is often overlooked, and widespread facultative cannibalism may be a strong factor in the evolution of egg hatching inhibition in the presence of larvae (Fox, 1975).

2.1.5.4. PARASITES AND PATHOGENS

A great variety of parasites and pathogens have been recorded infecting mosquito larvae, including Ae. cantans (Service, 1977a). Although Service found that Coeleomomyces fungi, an iridescent virus, mermithid nematodes and other infections led to some larval deaths, he concluded that pathogens and parasites contributed little to the overall mortality of Ae. cantans. No such infections have been found by me or Sulaiman (1982) in Ness Woods.

2.1.5.5 GROWTH RETARDANT FACTORS

Some studies have shown that larval crowding elicits the production of autotoxins or growth retardant factors (GRFS) in at least three mosquito species: Ae. aegypti (Moore and Fisher, 1969; Moore and Whitacre, 1972), An. stephensi (Reisen, 1975) and Cx. quinquefasciatus (Ikeshoji and Mulla, 1970). These GRFS retard larval development, increase mortality and lead to smaller adults. In addition to this, interference competition, exploitative interactions for available food supplies, may produce similar results nutritionally (Nayar, 1969), although Moore and Whitacre (1972) suggested that these effects may be indistinguishable. However, others have been unable to demonstrate the existence of GRFS in Ae. aegypti (Bar-Zeev, 1957; Dye, 1982; Gilpin and McClelland, 1979).

2.1.6 WEATHER

Weather can have an important influence on the mean density levels, but due to it acting in a density-independent manner cannot have any regulating influence (Klomp, 1963).

2.1.6.1 EFFECT OF WEATHER ON EGGS

The most important weather factor affecting egg survival and population size is precipitation or flooding (Service, 1978). Delayed flooding due to absence of rain will delay egg hatching of aedine species and may postpone the production of adult mosquitoes. Above-average precipitation will cause the opposite effect, a large number of viable aedine eggs will be immersed and if they are physiologically ready to hatch, large larval populations will result. If during times of high water level, aedine mosquitoes are ovipositing, many of the eggs will be in places that will not be flooded unless there is subsequent excessive rainfall (Service, 1978).

2.1.6.2 EFFECT OF WEATHER ON LARVAE AND PUPAE

RAINFALL

Rainfall is the most important weather variable affecting the survival of larvae and pupae and the productivity of adult mosquitoes. When drought occurs during periods of normal aquatic development, this can cause high pre-adult mortality and may greatly diminish the emerging adult population. Mosquitoes breeding in temporary small habitats, such as the ones in this investigation, are extremely susceptible to mortality caused by desiccation of larval habitats. Long-term spells of dry weather can result in small mosquito populations. For example, Shute (1930) found that in England the hot dry spring of 1929 postponed and delayed the emergence of aedine species. In the USA an _Anopheles_ _quadrimaculatus_ (Say) population was drastically reduced during 1953-1955 due to successive years of drought (Platt _et_ _al_., 1957).

Sulaiman (1982) found in 1980 in Ness Woods that larval mortality was at a maximum in late instars and this was due to the lack of rain. Similarly, Service (1973) found that desiccation of larval habitats caused the greatest mortality of Ae. cantans in Monk's Wood in southern England. It seems that catastrophic mortality occurs frequently, especially as a result of ponds drying up before development is completed (Service, 1968b; Yates, 1979).

The particularly dry weather encountered in the second two years of my study provided a good chance to study the effects of lack of rainfall on mosquito production from the ponds in Wood A. Rainfall data were recorded at Ness Gardens in all three years.

TEMPERATURE

Generally, the warmer the weather the more rapid the completion of the aquatic stages and the greater the number of adults and generations produced (Bar-Zeev, 1958).

Le Sueur and Sharp (1991) working on Anopheles merus Doenitz in South Africa found an inverse relationship between larval head capsule width and seasonal fluctuations in air and water temperatures. The mean head capsule width of all instars was significantly greater in winter than during the summer.

Water temperature was recorded during this study using a squirrel data logger.

2.1.7 GROWTH AND MEASUREMENTS

The heavily-sclerotised parts of the integument, the head capsule and the siphon, increase in size immediately after ecdysis but not between ecdyses. The thorax and abdomen, which are covered in a thin extensible cuticle, continue to grow throughout larval life, showing no sudden increase at ecdysis (Clements, 1963). The growth of the head capsule follows Dyar's rules in some species but not in others, whilst the soft parts do not grow at the same rate. The rate of growth increases with rising temperatures, but rapid growth is also dependent on adequate food supply, and in conditions of starvation the larval stage may last for several months, despite highly favourable weather.

In this investigation the siphon was used as a measure of larval size and also to distinguish the different instars. Identification of the instars can be crucial for certain studies on larval population dynamics, such as determining whether mortality is greater on younger or older instars (Service, 1976; 1977a). Accurate instar determination may also be required to calculate precise larvicide dosages for toxicological experiments, and frequently identification is based just on relative body size, not on actual instars.

2.2 MATERIALS AND METHODS

2.2.1 INITIAL SURVEY

An initial survey was made of all the ponds in Wood A using a combination of pond netting and dipping with white plastic trays. All ponds having mosquito larvae were recorded. A careful check was kept of ground depressions that might fill with water, because previous work in the woods suggested that it was such temporary pools which yielded large numbers of Ae. cantans larvae (Sulaiman, 1982). The main ponds selected for larval studies were the temporary Pond 9 and the Hotspot (Fig. 1.3).

2.2.2 TRANSECT SAMPLING

Wooden sticks were fixed across Pond 9 and the Hotspot in November 1988 to form linear transects, and these were subdivided into sampling points 20 cm apart. Once to twice weekly the ponds were sampled using a 120 ml capacity dipper.

At each point along the transect, ten dips were taken and the contents emptied into white trays, the number of larvae in each dip being recorded separately. Whenever possible, sampling was undertaken by me standing on dry land and reaching into the pond and trying not to cast shadows, so that the larvae were disturbed as little as possible. Some larvae were immediately placed in 70% alcohol and killed, whilst others were put in pond water and taken back alive to the laboratory. The depth of the pond was measured at each transect point. The water temperature was also measured at each point along the transect, just below the surface of the pond using a probe shaded from the sun.

2.2.3 CYLINDRICAL QUADRATS

In 1990, cylindrical samplers (quadrats) were placed in the ponds before larval sampling began, at set points along the transects. Use of static quadrats prevents larval immigration or emigration, and sampling is from a known volume, making density estimates easier. Ten dips were taken from each cylinder and the water depths and temperatures measured. Occasionally, the cylinders were emptied completely so that the exact number of larvae contained within them was known.

2.2.4 PREDATORS AND PARASITES

Throughout the sampling programme, all other pond fauna were noted and any possible predators removed. Once or twice monthly, a search was made for possible predators using pond nets, and some samples were taken from along the bottom of the ponds. This sampling was confined to days on which mosquito larvae were not collected so as to reduce disturbance of the pond.

2.2.5 IDENTIFICATION AND MOUNTING

Larvae were identified to species and instar according to Marshall (1938). It was occasionally difficult to distinguish between the first instars of *Ae. cantans* and *Ae. punctor*, but after examining first instars under a dissecting microscope and then allowing them to moult into second instars, all the original identifications proved correct.

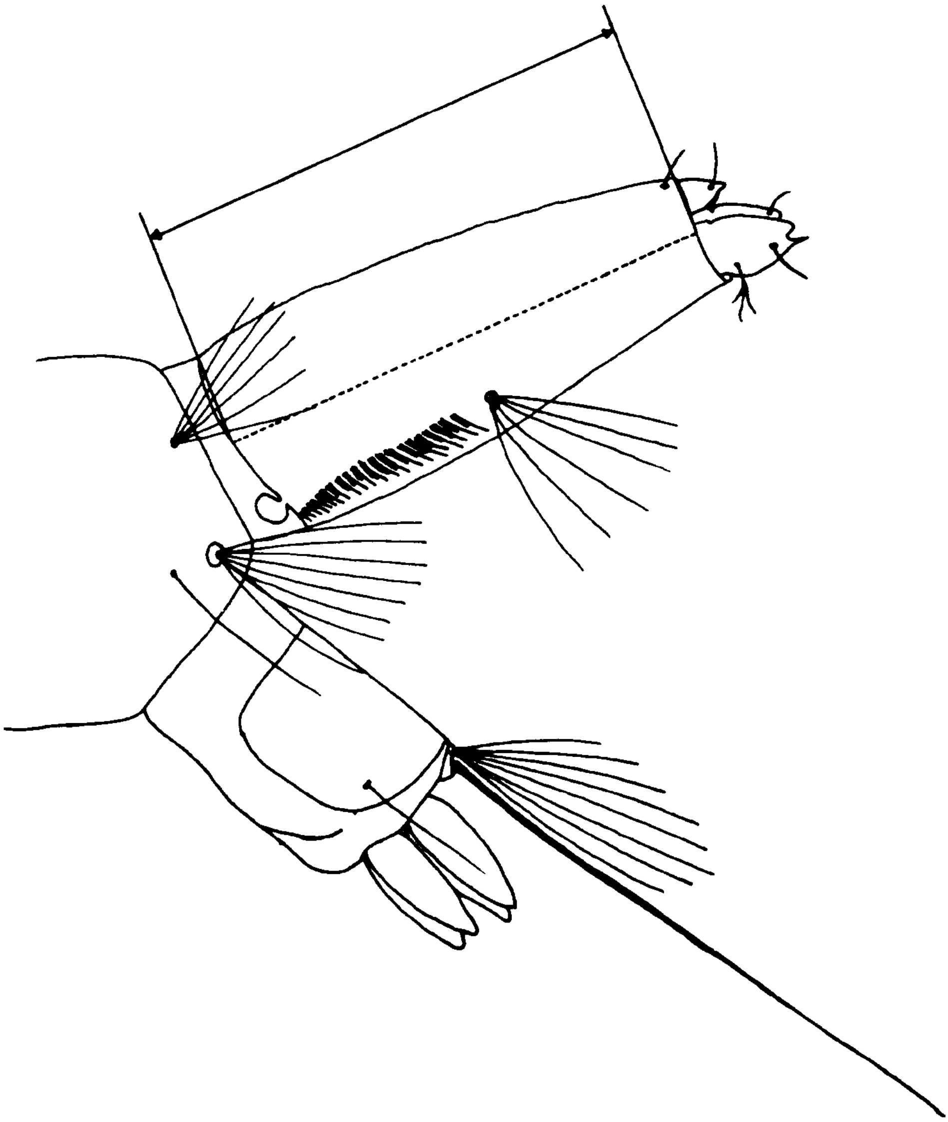
Larvae were mounted in polyvinyl-lactophenol (chitomount) on glass slides, covered with a coverslip and then the slides were allowed to dry for one week. The larvae were measured at a magnification of X100 using a *camera lucida* on a Vickers microscope.

2.2.6 MEASUREMENTS

The larval characters selected in the first year for measurement were siphon length, siphon width and head sclerite width. Figure 2.1a shows how the siphon length was measured. These are all regions which have a fixed size that does not vary with desiccation, or grow during an instar. These characters were measured on a sample of slides for a second time six months later and no significant difference was noted in the size of any of these three characters. During the second two years siphon length only was measured as this was found to represent a sufficient measure of larval size.

Mounting larvae on slides unfortunately prevents the use of dry weight to confirm the correlation between the character measured and the actual body weight, which is the most widely accepted measure of size.

Fig. 2.1a: Siphon of Ae. cantans showing the dimensions measured.



Some larvae were not killed but were placed in trays or plastic pots for observation of grazing behaviour and larval interactions, and used for density experiments.

2.2.6.1 CALIBRATION

All the larvae were measured using a camera lucida at a magnification of X100. The measurement data presented in this investigation are in units.

1 unit = 0.0057 mm

10 units = 0.057 mm

The microscope was calibrated each time it was used.

2.2.7 CANNIBALISM

First and second instar larvae were transported alive from the field in 3 X 1" tubes containing pond water but no leaf-litter. Once in the laboratory, it was noted that there were fewer larvae in the tubes than were initially introduced and in addition there was an absence of corpses. This suggested the possibility of larval cannibalism, and so cannibalism experiments were set up.

Small plastic pots were filled with 100 ml of tap water and were set up in the laboratory. Half of them had 50 g of leaf-litter added, the other half had no leaf-litter. Fifty first instar larvae were added to each pot, with some of the larvae being newly hatched and others being older. The numbers of larvae surviving after five days were recorded, as were the numbers of corpses.

The larvae in the pots were also observed to see whether cannibalism could actually be seen.

2.2.8 CAGE EXPERIMENTS

Cage experiments were carried out in 1990 and 1991 in Ponds 9 and 13, and the Hotspot. Aedes cantans eggs were obtained from laboratory oviposition experiments in 1989 and 1990 (Chapter 3), and were placed in tubes and kept out of doors for the winter. Samples of eggs were placed in water to see whether they would hatch at a time when hatching was observed in the field. Other eggs were used as a source of larvae for cage density experiments.

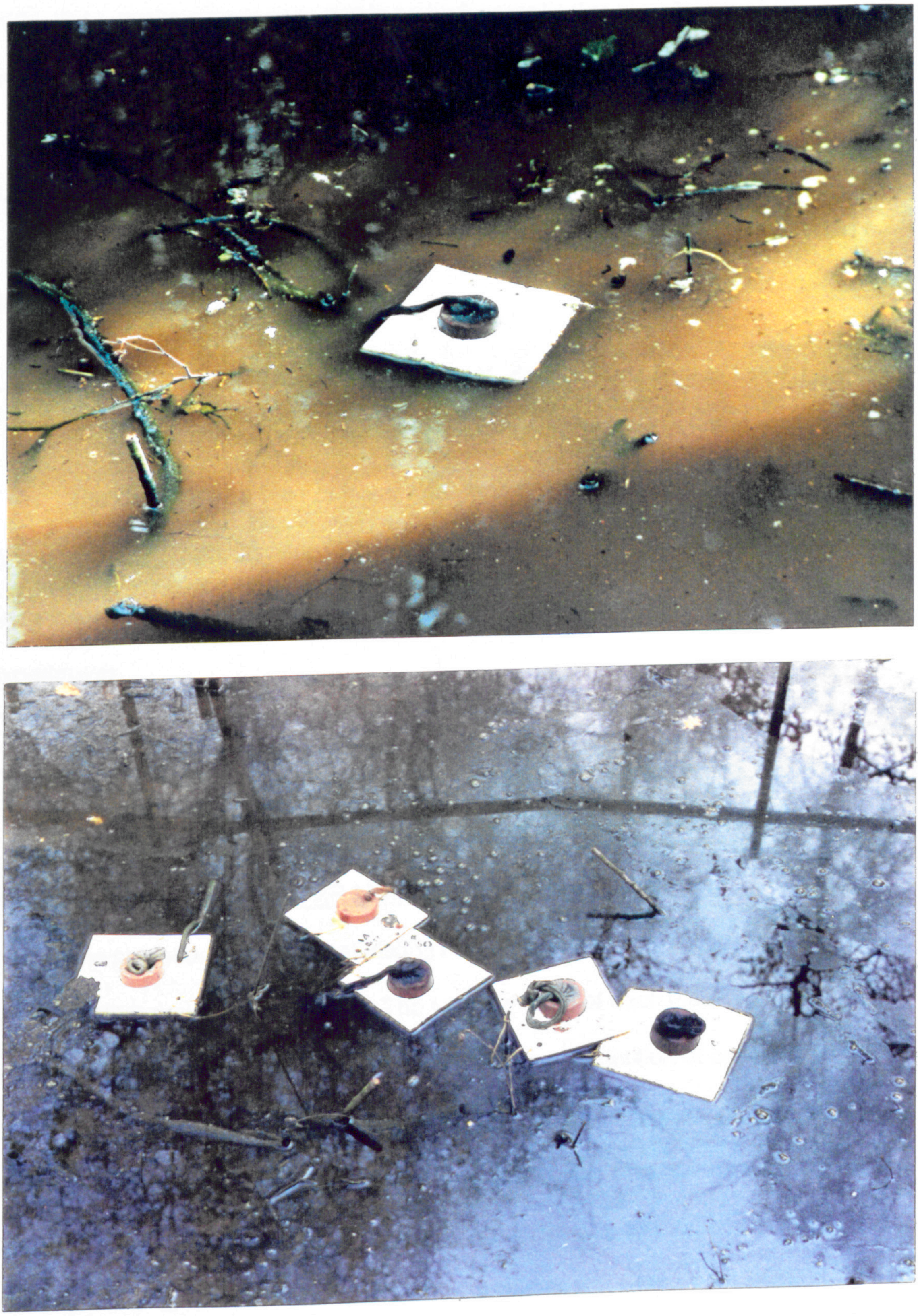
The cages comprised inverted 150 ml brown plastic plant pots with the bases cut out. The pots were covered with nylon mesh and were floated in Pond 13 using a polystyrene tile (Fig.2.1). The volume of water enclosed by the pots was 100 ml.

These cages allowed a known number of larvae to be maintained in them free of predators, but still allowing normal nutrient circulation, so that food resources were as natural as possible, that is, like the pond. The effect of different densities on larval development and survival could then be examined.

Eggs were soaked in water 24 hours before the cages were set up, and the numbers of hatched larvae counted and removed. Larvae obtained from eggs laid by different sized adults were mixed. Larvae were then sorted into densities of 10, 50, 100, and 250 and these were placed in the cages in Pond 9, the Hotspot and Pond 13. The positions of the cages were rotated regularly to reduce positional effects. In addition, temperature measurements were taken to ensure that all pots were placed in water of the same temperature. There were two or three replicates of each density. Larvae were killed after they had become second instars. The siphon lengths of all larvae were measured and first instar larval mortality was recorded.

In 1991, leaf-litter was added to half the cages to determine the effects of additional food on size and survival rates.

Fig. 2.1: Cage experiments in situ in Pond 13, Wood A.



In 1990, six replicates of larvae at a density of 100 per pot were placed into Pond 13 to examine the effect of density on survivorship from first instars through to pupae, and survivorship curves and life-tables were constructed from these data. In 1991, the experiment was repeated but with four replicates at densities of 10, 50, 100 and 200. Again, survivorship curves were drawn from these results.

2.2.9 DETERMINATION OF INSTAR DURATIONS

The duration of each instar was estimated according to the method used by Sulaiman (1982) -

$$\frac{\text{N of instar } i \text{ sampled per month}}{\text{Total } N \text{ of instar } i \text{ sampled in season}} \quad \text{X} \quad \begin{array}{c}\text{development time of instar } i\\ \text{per month}\end{array}$$

The development time of instar *i* per month is the time it takes for 50% of larvae placed in pots to develop to the next instar. The instar durations were calculated separately for each year, and each pond, and also for each density in the pot experiments.

2.2.10 ESTIMATION OF INSTAR MORTALITIES OF AE. CANTANS

The procedures used for estimating instar mortalities were those of Lakhani and Service (1974) and Service (1977a). The total numbers of each instar collected were divided by the average instar duration to give the age-distribution of pre-adult stages. These values were plotted against age in days of the immature stages and the resultant histogram was the stage-specific age distribution. The numbers of immature stages surviving to each age can be read off the curve to give the numbers of larvae surviving to age x. Life-tables for pre-adult mosquitoes were then constructed.

2.3 RESULTS

2.3.1 WEATHER CONDITIONS

Weather data were recorded at Ness Gardens, 1.5 km from the field site. All three years were characterised by very dry conditions, with low rainfall levels delaying larval hatching and leading to premature desiccation of the habitats.

Figure 2.2 shows the rainfall data.

2.3.2 POND SURVEY

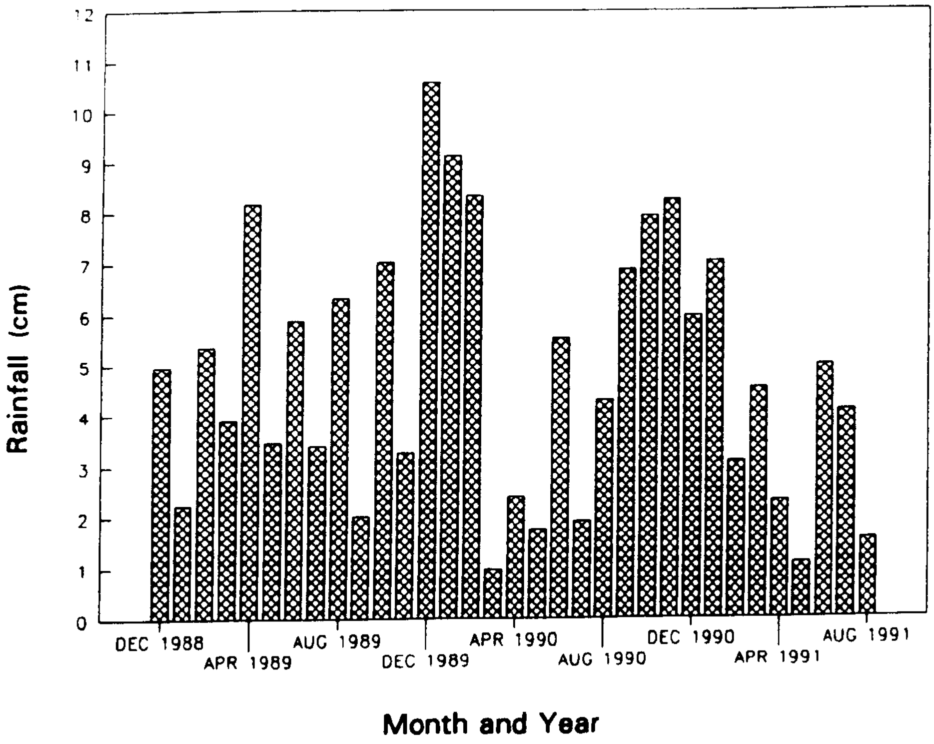
Wood A has 16 permanent ponds, five temporary ponds and a ditch (Fig. 1.3). All ponds were surveyed for mosquito larvae throughout the duration of the study using a pond net. Ten ponds in Wood A had mosquito larvae in them, namely numbers 6, 7, 8, 9, 10, 11, 12 and 13 and the Hotspot, along with the ditch. The species identified included Ae. cantans, Ae. punctor, Ae. rusticus, Cs. morsitans and Cs. annulata.

Pond 9 and the Hotspot were the two temporary ponds chosen for the majority of the larval work, although Ponds 12 and 13 were also studied, particularly during periods of adult emergence.

2.3.3 SEASONALITY

In 1988, the Hotspot was dry until 10 December, and first instar larvae were present from 15 December. The Hotspot began to dry up on 4 May, before emergence was completed and because of this, pupae were rescued and placed into buckets under the emergence nets (Chapter 3), and also water was pumped through a filter from the larger Pond 11 into the Hotspot in order to maintain the water volume until emergence was completed. This meant that the pond was maintained artificially for the last three weeks of May, so as to prevent a total failure of emergence.

Fig. 2.2: Rainfall data for the years 1989-1991 recorded at Ness Gardens weather station, Wirral.



In 1988 Pond 9 was dry until 15 December and larvae were present from 19 December. Pond 9 also began to dry up in early May 1989 and water was pumped and filtered from Pond 8 into Pond 9 to maintain some water in the pond until emergence could be completed.

In 1989, the Hotspot was dry until 17 December but the larvae did not hatch until 6 February 1990. It is interesting to note that Ae. cantans eggs kept immersed in water in jars in my garden hatched on 4 February. The Hotspot began to dry up in late April and again water was pumped from Pond 11 until emergence was completed. In the same year, Pond 9 was dry until 9 January 1990 and the larvae hatched on 6 February 1990. Pond 9 also began to dry up in late April and water was again pumped into it from Pond 8.

In 1991, the Hotspot was dry until 21 February and the larvae hatched on 25 February. Pond 9 was dry until 10 March 1991 and the larvae hatched on 12 March. Eggs maintained in pots in my garden hatched earlier than this, on 7 February, indicating that low rainfall levels delayed the onset of the larval season. The year 1991 was characterised by especially low rainfall levels (Fig. 2.2) and both Pond 9 and the Hotspot began to dry up before the end of March, that is before any third instars were present in the ponds. The ponds were completely dry by the beginning of April.

The total number of larvae and the different species in both ponds in all three years are shown in Table 2.1.

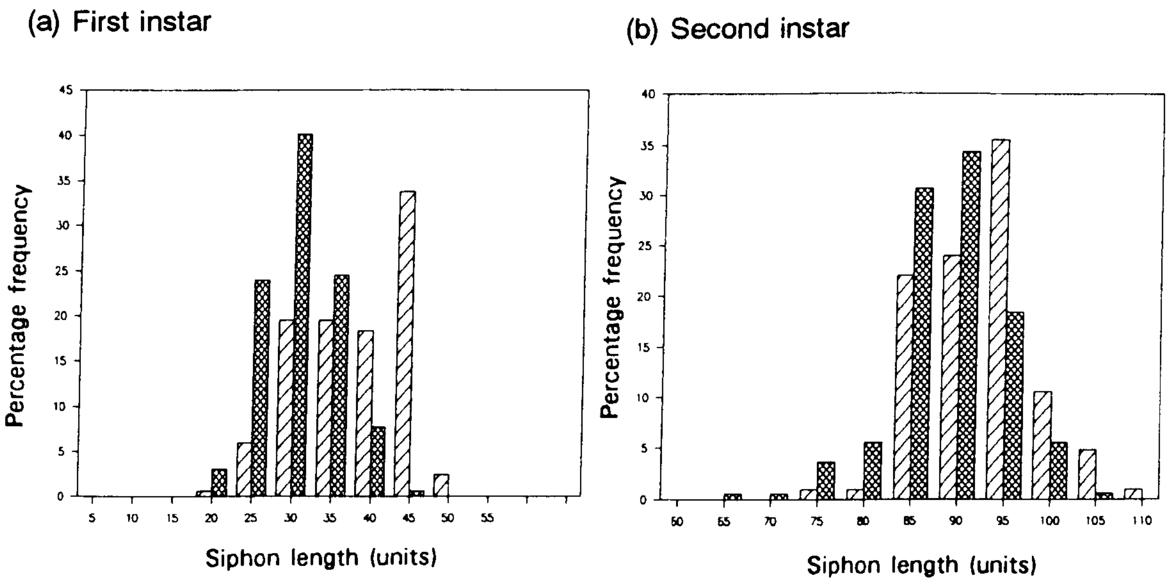
2.3.4 SIZE DISTRIBUTIONS OF LARVAE IN THE FIELD

Figure 2.3 shows the siphon length measurements of first, second, third and fourth instar Ae. cantans caught in the Hotspot and Pond 9 in 1989. Table 2.2 shows the statistical analysis of these results. With all instars, there was a significant difference in the siphon length of larvae between the two ponds in 1989, with Pond 9

Table 2.1: Numbers and species of mosquito larvae caught in Pond 9 and the Hotspot in 1989-1991.

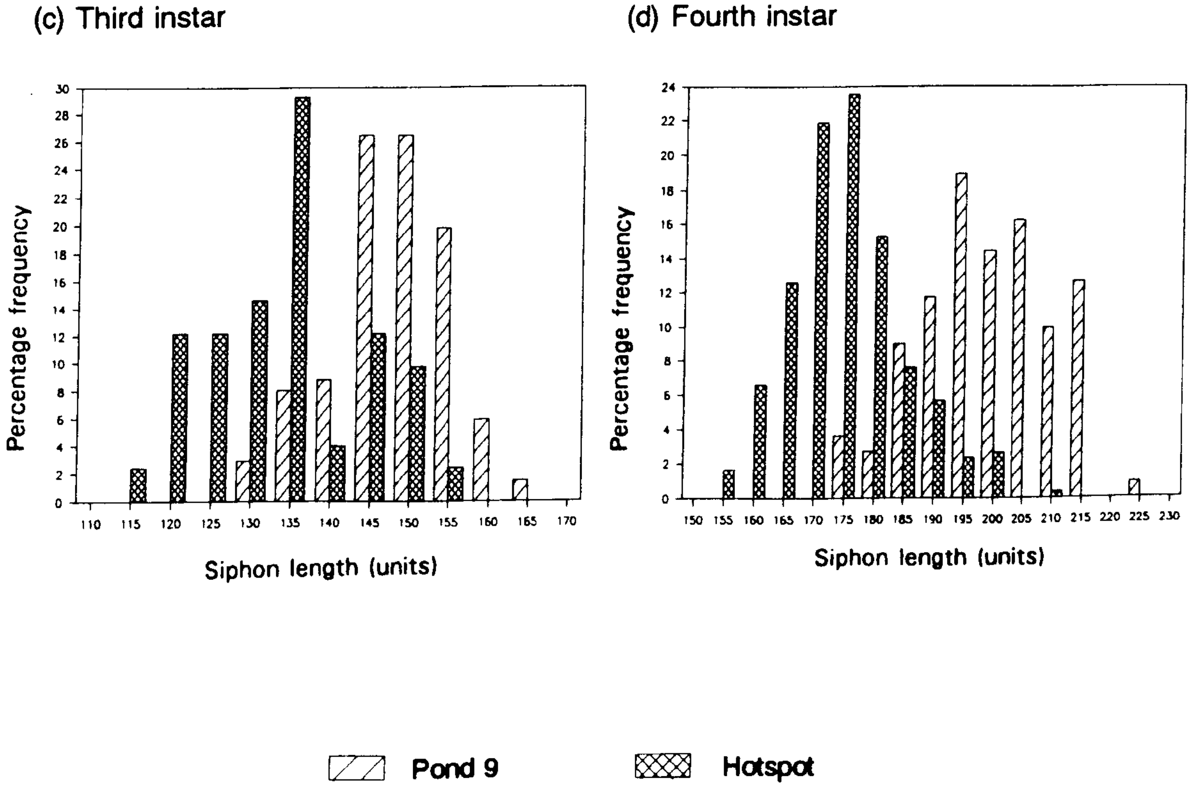
Year	Species	Pond 9		Hotspot	
		number	%	number	%
1989	*Ae. cantans*	3,430	96.87	13,973	98.35
	Ae. punctor	25	0.70	10	0.07
	Ae. rusticus	6	0.17	119	0.84
	Cs. morsitans	80	2.26	106	0.74
	Total	3,541	100	14,208	100
1990	*Ae. cantans*	341	97.99	873	99.66
	Ae. rusticus	7	2.01	3	0.34
	Total	348	100	876	100
1991	*Ae. cantans*	43	100	2,687	100

Fig. 2.3: Frequency distributions of siphon lengths for *Ae. cantans* larvae sampled from the Hotspot and Pond 9 in 1989.



(a) First instar

(b) Second instar



(c) Third instar

(d) Fourth instar

Table 2.2: Differences in siphon length measurements between larvae sampled from the Hotspot and Pond 9 in 1989.

Instar	Site	Number	Mean	SE	Probability
First	Hotspot	121	17.43	2.53	P<0.0001
	Pond 9	58	22.29	2.87	
Second	Hotspot	163	91.69	6.09	P<0.0001
	Pond 9	104	95.53	5.66	
Third	Hotspot	248	130.75	8.48	P<0.0001
	Pond 9	253	140.01	7.74	
Fourth	Hotspot	301	168.05	9.62	P<0.0001
	Pond 9	111	192.30	10.8	

Table 2.3: Differences in siphon width measurements between larvae sampled from the Hotspot and Pond 9 in 1989.

Instar	Site	Number	Mean	SE	Probability
First	Hotspot	121	17.43	2.53	P=0.0001
	Pond 9	58	22.29	2.87	
Second	Hotspot	119	41.80	5.68	P=0.0015
	Pond 9	103	44.22	5.54	
Third	Hotspot	235	64.38	6.48	P=0.0009
	Pond 9	251	66.41	6.88	
Fourth	Hotspot	302	75.95	7.53	P=0.0015
	Pond 9	111	78.82	8.21	

Statistical analysis = Two-sample T-tests.

larvae being significantly larger than those caught in the Hotspot. Similarly, the siphon width was significantly larger in Ae. cantans caught in Pond 9 than in the Hotspot (Table 2.3). There was no significant difference in the head sclerite width between the two ponds, and the data are not shown.

Figure 2.4 shows the siphon length frequency distributions of Ae. cantans caught in Pond 9 and the Hotspot in 1990. Table 2.4 shows the statistical analysis of this data. For all instars, there was no significant difference in the lengths of the siphons between the two ponds.

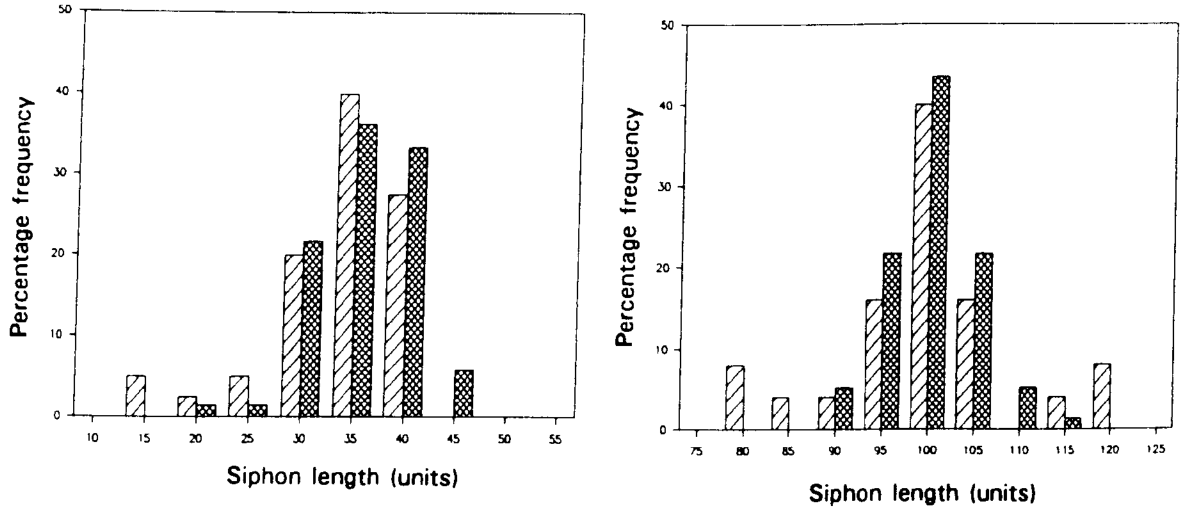
Since very few larvae were caught in 1991, and the two ponds dried up long before the larval season could be completed, the siphon lengths of larvae from these two habitats were not compared.

Larvae from both the Hotspot and Pond 9 in 1989 were significantly smaller in terms of siphon length than those sampled in 1990.

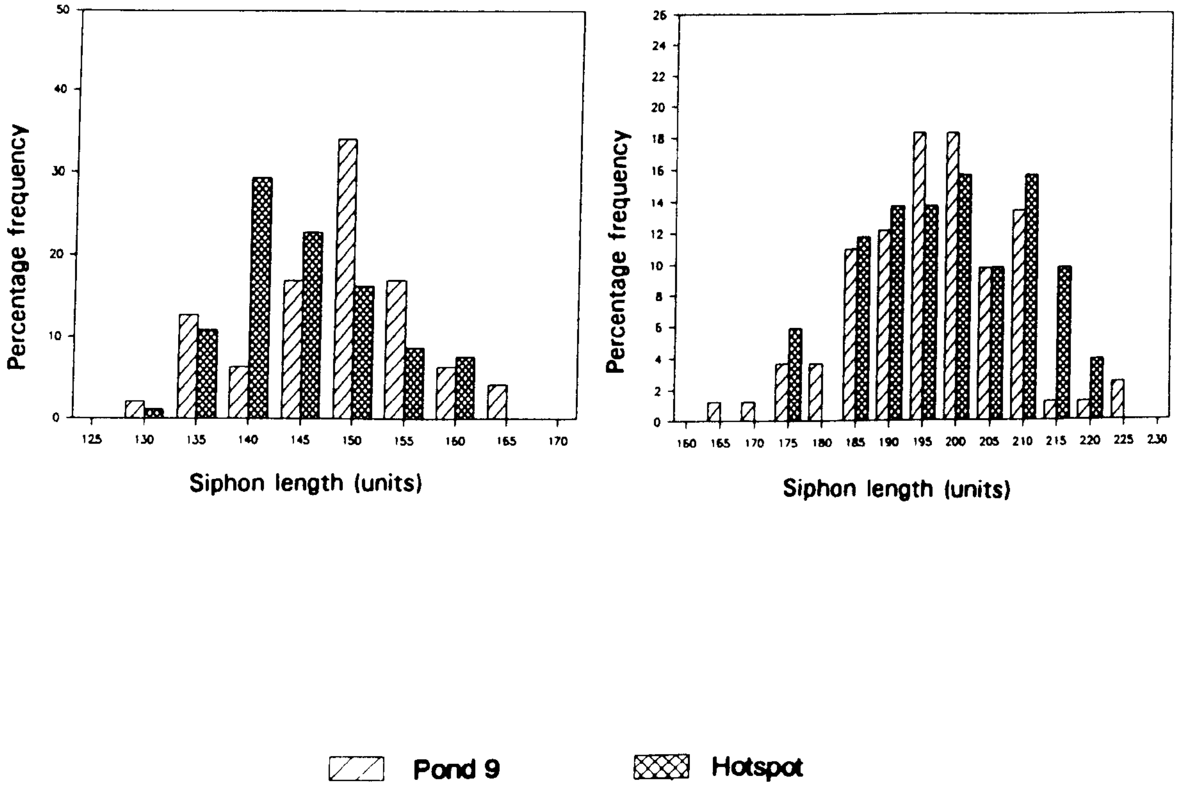
Figure 2.5 shows the regression lines of siphon length and siphon width of larvae sampled from the Hotspot and Pond 9 in 1989. All four instars can be clearly distinguished in Pond 9 (Fig 2.5a). In the Hotspot, third and fourth instars are not as clearly separated as in Pond 9, and it would be difficult to identify a few larvae as being either third or fourth instars (Fig 2.5b). One interesting aspect is that the size of the instars exhibit some degree of overlap. In 1989, the fourth instars in the Hotspot were the same size as third instars in Pond 9. Therefore, siphon measurements can only be used to distinguish between different instars from the same habitat in the same year.

In 1989, the distributions of siphon length for larvae from the Hotspot were skewed to largeness for all instars but not in Pond 9, and in 1990, the size distributions of siphon length from both the Hotspot and Pond 9 were not skewed.

Fig. 2.4: Frequency distributions of siphon lengths for Ae. cantans larvae sampled from the Hotspot and Pond 9 in 1990.


(a) First instar

(b) Second instar


(c) Third instar

(d) Fourth instar

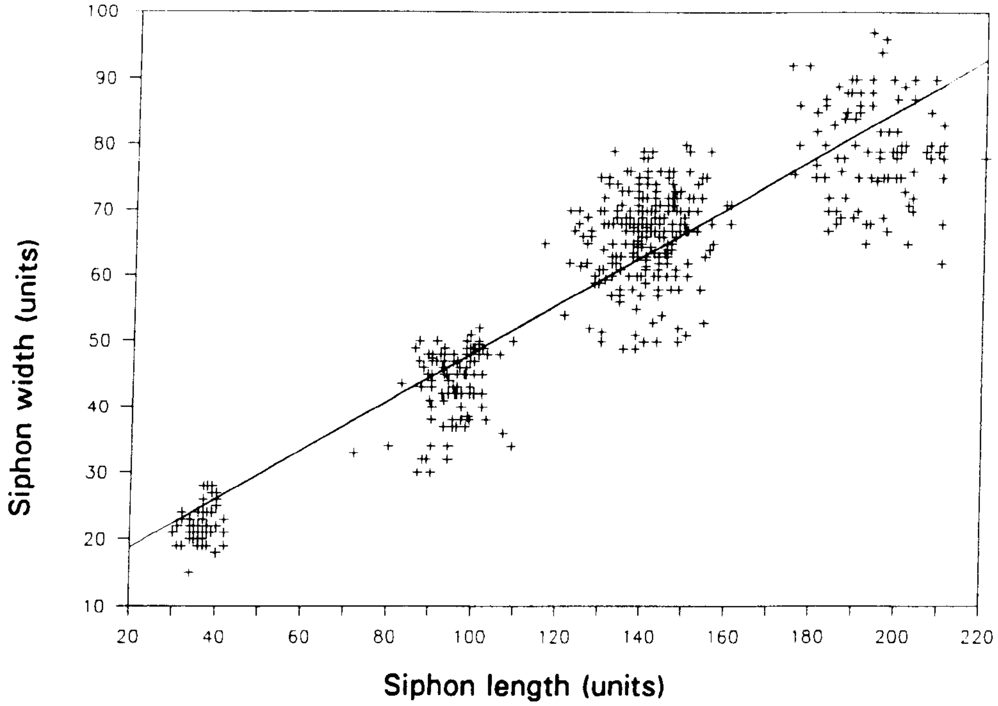
Table 2.4: Differences in siphon length measurements between larvae sampled from the Hotspot and Pond 9 in 1990.

Instar	Site	Number	Mean	SE	Probability
First	Hotspot	69	33.71	5.05	P=0.10
	Pond 9	40	31.70	6.66	
Second	Hotspot	78	97.72	4.88	P=0.69
	Pond 9	25	97.88	9.99	
Third	Hotspot	92	144.76	9.03	P=0.14
	Pond 9	47	146.94	7.75	
Fourth	Hotspot	82	193.90	13.6	P=0.08
	Pond 9	51	197.80	11.7	

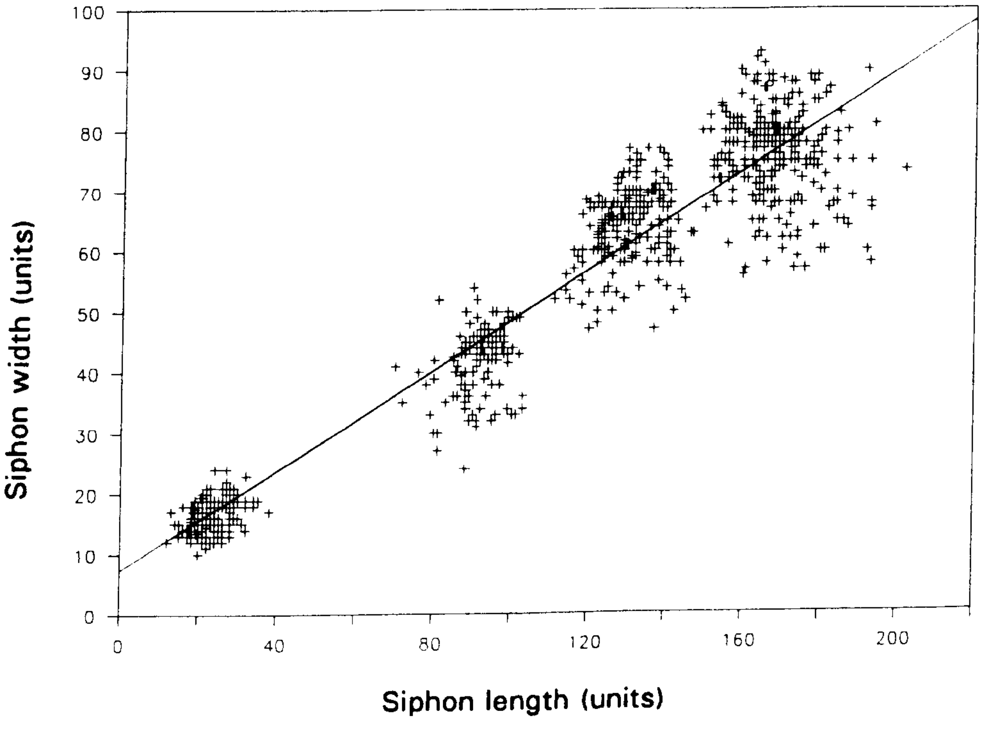
Statistical analysis = Two-sample T-tests.

Fig. 2.5: Linear regression of siphon length and siphon width of <u>Ae</u>. <u>cantans</u> larvae sampled from Pond 9 and the Hotspot,1989.

(a) Pond 9, 1989



(b) Hotspot, 1989



First instar siphon length measurements are unreliable because the siphon does not become fully sclerotised until late into the instar. Figure 2.6 illustrates the growth of the siphon from hatching until just before moulting to the second instar.

2.3.5 SIZE DISTRIBUTIONS FROM CAGE EXPERIMENTS

In the first cage experiment carried out in 1990, larvae were placed in cages at densities of 10, 50 and 100, and siphon lengths were measured once the larvae reached the second instar. Analysis of variance showed that larvae maintained at a density of 10 were significantly larger than those maintained at a density of 50, which in turn were significantly larger than those maintained at a density of 100 (Table 2.5). In the second cage experiment in 1990, larvae were maintained at densities of 10, 100 and 250 per cage. Analysis of variance again showed that the larvae maintained at a density of 10 were significantly larger than those maintained at densities of 100 and 250. Larvae maintained at a density of 250, however, were significantly larger than those at a density of 100. The original density of 250 caused such high initial mortality that the numbers in the cage were reduced to densities lower than in the cages that originally contained 100 larvae. The size distribution of the first cage experiment in 1990 is shown in Fig. 2.7.

In the first cage experiment in 1991, larvae were introduced at densities of 10, 50, 100 and 200, with four replicates at each density, two with additional leaf-litter added to the cages and two without. The addition of leaf-litter to the cages had no significant effect on the size of second instar larvae in terms of siphon length at densities of 10, 100 and 200, however, at a density of 50, larvae with leaf litter were significantly larger than those without leaf-litter in both replicates (Table 2.6a).

Larvae at a density of 10 with additional leaf-litter were significantly larger than those at higher densities with or without additional leaf-litter. Similarly, larvae at a

Fig. 2.6: Changes in the first instar siphon length from hatching.

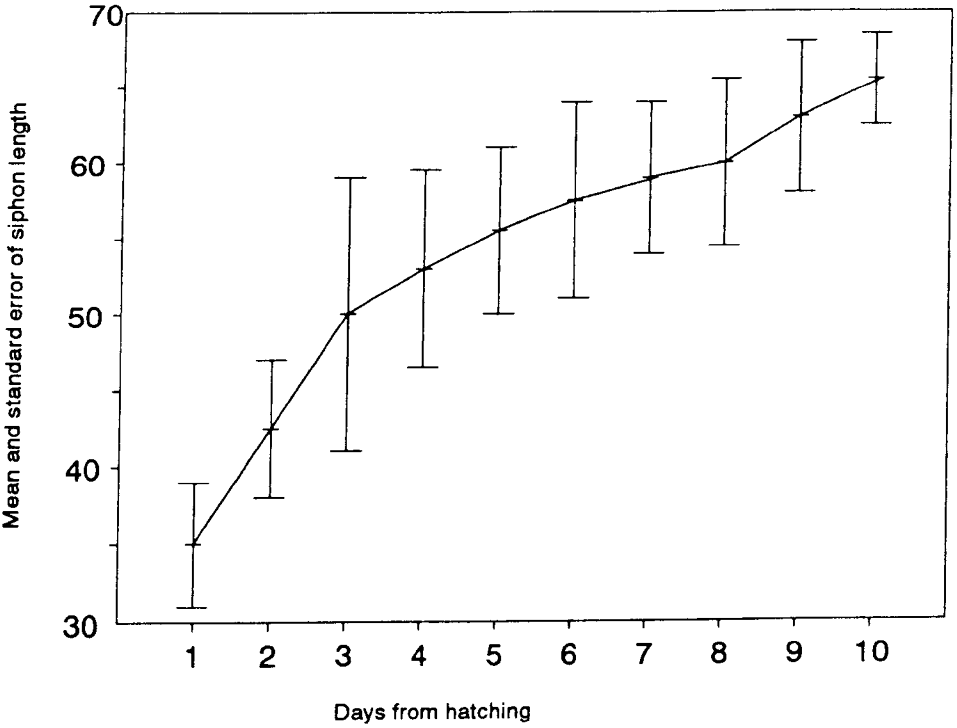


Table 2.5 Size differences in *Ae. cantans* second instar larvae raised in cages at different densities in 1990.

(a) Experiment 1. Analysis of variance

Source	DF	SS	MS	F	P
Factor	2	3984.0	1992.0	106.24	P<0.001
Error	97	1818.7	18.70		
Total	99	5802.8			

Density	n	mean	St. dev	Pooled St. deviation
10	10	90.40	8.53	4.330
50	36	76.53	4.29	
100	54	69.56	3.13	

(b) Experiment 2. Analysis of variance

Source	DF	SS	MS	F	P
Factor	2	2993.1	748.3	21.86	P<0.001
Error	104	3559.5	34.2		
Total	108	6552.6			

Density	n	mean	St. dev	Pooled St. deviation
10	10	92.20	5.05	5.569
100	55	77.61	5.50	
250	44	81.54	5.85	

Fig. 2.7: Frequency distribution of second instar *Ae. cantans* larval siphon lengths from cage experiments, 1990.

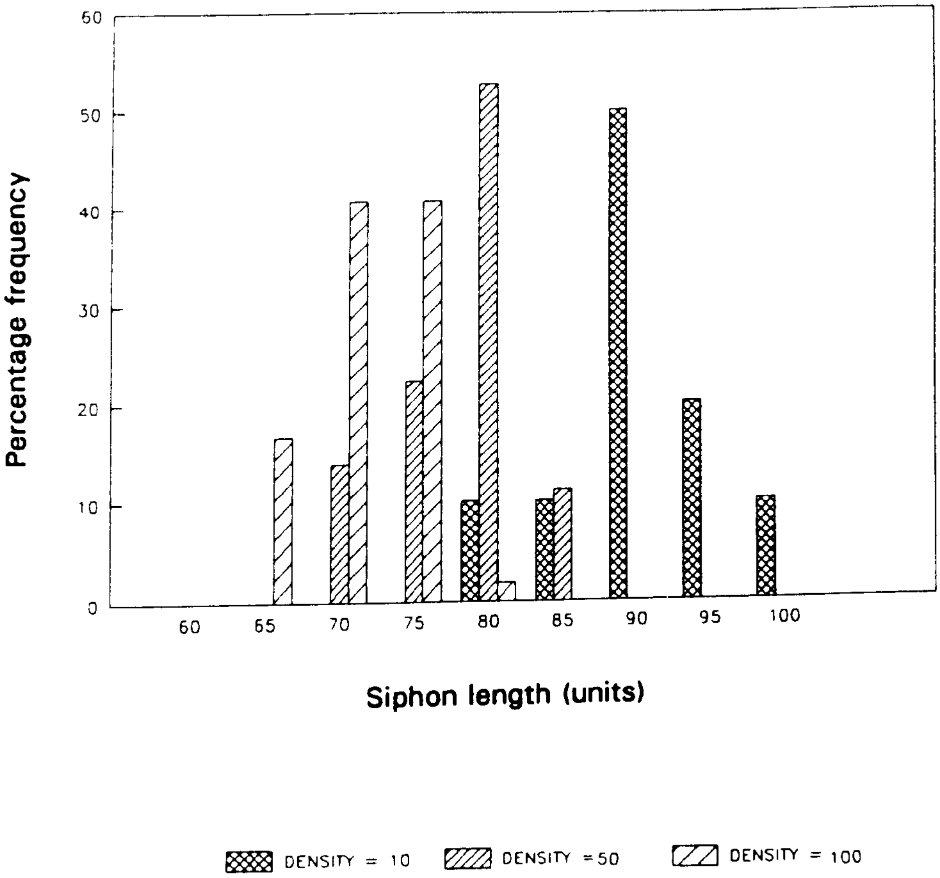


Table 2.6a: Differences in siphon length measurements of second instar Ae. cantans from cage experiments in 1991. Larvae were maintained at various densities with or without leaf litter

Larval Density	Mean	SE	Probability
10 with litter	99.25	2.50	P=0.11
10 no litter	96.00	3.37	
50 with litter	93.32	6.29	*P=0.0005
50 no litter	87.00	8.85	
100 with litter	83.00	6.84	P=0.16
100 no litter	82.1	5.40	
200 with litter	78.44	5.55	P=0.18
200 no litter	77.34	4.84	
10 with litter	95.60	11.90	P=0.5
10 no litter	91.56	3.05	
50 with litter	87.80	4.94	P<0.0001
50 no litter	82.03	3.44	
100 with litter	84.47	3.94	*P=0.46
100 no litter	83.64	6.95	
200 with litter	83.43	7.94	P=0.66
200 no litter	84.09	6.06	
10 with litter	99.10	1.60	P=0.25
10 no litter	97.00	2.24	
50 with litter	92.00	3.86	P<0.0001
50 no litter	86.00	3.43	
100 with litter	82.10	4.82	P=0.34
100 no litter	81.54	5.85	
200 with litter	77.64	5.50	P=0.65
200 no litter	75.32	4.80	

Note: All measurements are stated in terms of units, see text for translation.
Statistical analysis = Two sample T-test except Mann-Whitney analysis when starred (*).

density of 10 without leaf-litter were significantly larger than larvae at all higher densities, except for those maintained at a density of 50 with additional leaf-litter. All larvae maintained at a density of 50, with and without leaf-litter, were significantly larger than larvae maintained at the higher densities. At a density of 100, the larvae were significantly larger than those maintained at a density of 200 (Table 2.6b).

In the second cage experiment in 1991, there was again no significant difference between the larval siphon lengths in cages with or without leaf-litter at densities of 10, 100 and 200 but there was again a significant difference between those at a density of 50, larvae with additional leaf-litter being significantly larger. As before, larvae at a density of 10 with or without leaf-litter were significantly larger than those at all higher densities. Larvae maintained at a density of 50 with or without leaf-litter were significantly larger than larvae maintained at higher densities. There was no significant difference between the length of the larval siphons from larvae maintained at densities of 100 and 200, with or without leaf-litter (Table 2.6b).

In the third cage experiment in 1991, larvae at a density of 50 were again significantly larger when leaf-litter was added than those at a density of 50 without leaf-litter, but at densities of 10, 100 and 200 there was no significant difference. Larvae at a density of 10 were significantly larger than those at a density of 50 which in turn were significantly larger than those at a density of 100 which were in turn significantly larger than those at a density of 200 (Table 2.6b).

Figure 2.8 shows the mean and standard deviation of the siphon length measurements for the first cage experiment in 1991.

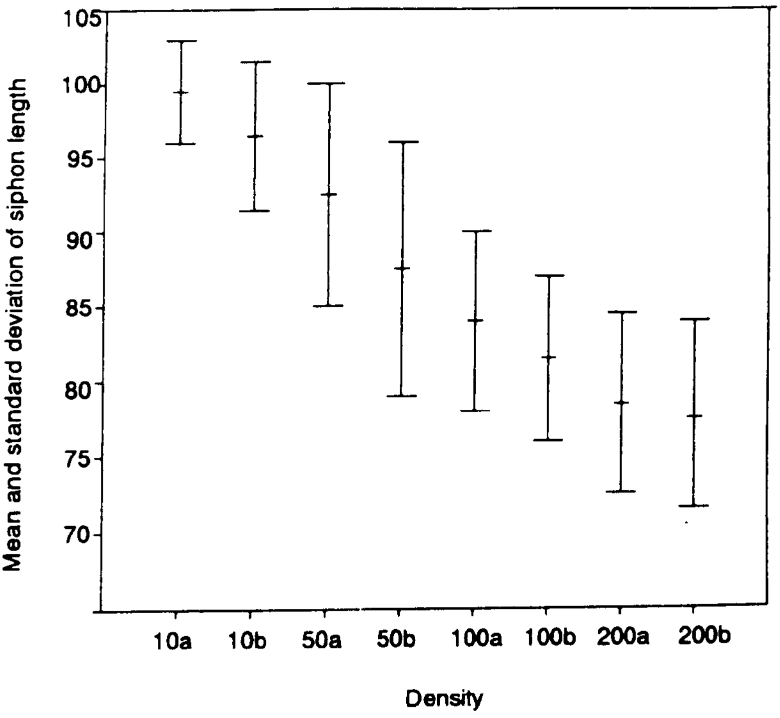
2.3.6 LARVAL DISTRIBUTION IN THE FIELD

The variance to mean ratios are shown in Table 2.7 for both the Hotspot and Pond 9 in 1989 and 1990.

Table 2.6b: Differences in siphon length measurements of second instar Ae. cantans from cage experiments in 1991. Comparisons for all pairwise combinations of densities, with or without leaf litter

Larval Density	Probability		
	Experiment 1	Experiment 2	Experiment 3
10+L v 10-L	P>0.05	P>0.05	P>0.05
10+L v 50-L	P<0.001	P<0.001	P<0.001
10+L v 50+L	P<0.001	P<0.001	P<0.001
10+L v 100-L	P<0.001	P<0.001	P<0.001
10+L v 100+L	P<0.001	P<0.001	P<0.001
10+L v 200-L	P<0.001	P<0.001	P<0.001
10+L v 200+L	P<0.001	P<0.001	P<0.001
10-L v 50-L	P<0.001	P<0.001	P<0.001
10-L v 50+L	P>0.05	P<0.001	P<0.001
10-L v 100-L	P<0.001	P<0.001	P<0.001
10-L v 100+L	P<0.001	P<0.001	P<0.001
10-L v 200-L	P<0.001	P<0.001	P<0.001
10-L v 200+L	P<0.001	P<0.001	P<0.001
50+L v 50-L	P<0.001	P<0.001	P<0.001
50+L v 100-L	P<0.001	P<0.001	P<0.001
50+L v 100+L	P<0.001	P<0.001	P<0.001
50+L v 200-L	P<0.001	P<0.001	P<0.001
50+L v 200+L	P<0.001	P<0.001	P<0.001
50-L v 100-L	P<0.001	P<0.001	P<0.001
50-L v 100+L	P<0.001	P<0.001	P<0.001
50-L v 200-L	P<0.001	P<0.001	P<0.001
50-L v 200+L	P<0.001	P<0.001	P<0.001
100+L v 100-L	P>0.05	P>0.05	P>0.05
100+L v 200-L	P<0.001	P>0.05	P<0.001
100+L v 200+L	P<0.001	P>0.05	P<0.001
100-L v 200-L	P<0.001	P>0.05	P<0.001
100-L v 200+L	P<0.001	P>0.05	P<0.001
200+L v 200-L	P>0.05	P>0.05	P>0.05

Fig. 2.8: Cage experiment 1, 1991. Mean and standard deviation of siphon lengths of second instar Ae. cantans larvae at different densities.



a = with leaf-litter

b = no leaf-litter

The variance to mean ratios for the Hotspot in 1989 indicate that the larval distribution in the pond was highly contagious at all instars (A variance to mean ratio of more than one indicates a clumped distribution). The ratio decreases from first through to fourth instar, indicating that the degree of contagion decreased, but despite this decrease, fourth instars were still highly aggregated.

The variance to mean ratios in Pond 9 in 1989 also indicate a contagious distribution although the ratios are not as high as those from the Hotspot, indicating that the larvae were not as clumped. As in the Hotspot, the degree of contagion decreases from first through to fourth instars.

The variance to mean ratios for both ponds in 1990 are not as high as in the previous years. In the Hotspot, the degree of contagion decreased from first to fourth instar so that, although firsts showed a contagious distribution, fourth instars did not. Similarly, in Pond 9, the degree of contagion was considerably lower. Pupae did not have a contagious distribution, but since they demonstrate an entirely different behaviour from larvae, it is not surprising that their distribution pattern is different.

The larval sampling data of all instars and pupae were non-normal in both 1989 and 1990 in both ponds. Although attempts were made to normalise the data by means of square root and log transformations, the only transformation that succeeded in normalising the data was Taylor's Power Law (Elliot, 1977). Attempts were then made to fit the normalised data to the negative binomial, the model which most accurately describes larval distributions. In the Hotspot in 1989, first and second instar data could not be fitted to this model, but both the third and fourth instars fitted the negative binomial. Pupae were not sampled in sufficient numbers. Similar results were obtained in Pond 9 in 1989, with first and second instars not fitting the distribution, but with third and fourth instars and pupae fitting the negative distribution.

Table 2.7: Variance to mean ratio for larvae sampled from Hotspot and Pond 9 in 1989 and 1990, calculated from the number of larvae per dip along the transect.

Year and site	Instar				
	I	II	III	IV	All
Hotspot	25.03	3.52	6.04	2.08	94.71
1989	88.07	18.21	30.63	18.30	23.92
	136.24	118.39	42.97	15.37	98.86
	40.01	25.20	7.56	10.19	136.24
	55.77	51.27	2.98	5.00	29.21
	130.50	118.46		2.98	121.47
	91.58	21.05			31.68
	142.53				127.56
	36.35				62.71
					91.71
					51.82
					9.86
					1.60
Pond 9	1.73	18.49	5.21	3.86	4.98
1989	15.13	16.50	4.55	1.52	2.15
	4.39	1.66	7.65		3.32
	38.29	40.48	10.29		1.12
	44.52	1.02	12.44		1.59
		6.65			1.57
					15.48
					17.49
					9.24
					3.60
					44.29
					41.08
					10.16
					11.68

Table 2.7 continued.

Year and site	Instar					
	I	II	III	IV	P	All
Hotspot	2.64	5.40	2.33	3.57		2.64
1990	4.00	6.71	4.06	4.05		4.00
	6.67	0.58	0.39	1.19		5.07
	0.93					1.23
						4.66
						3.75
						3.56
Pond 9	1.45	1.60	2.16	1.80	0.13	1.50
1990			1.84	2.01	0.39	1.55
				8.17	0.88	1.86
				4.20	1.05	1.95
						1.51
						5.89
						1.46

Note: Sufficient sample sizes of data for pupae were obtained for Pond 9 in 1990 only.

In 1990 in Pond 9, first and second instars were again not described by the negative binomial whilst third, fourth and pupae were. In the Hotspot in 1990 the results were different; the first and fourth instars were described by the negative binomial, but seconds and thirds were not.

Figure 2.9 shows the frequency distributions of larval sampling for Pond 9 in 1989 for each instar.

2.3.7 RELATIONSHIP OF LARVAE TO DEPTH AND TEMPERATURE

In 1989 in the Hotspot, there was a significant negative correlation ($r = -0.422$, $P<0.001$) and a significant negative regression between the larval number sampled at each transect point and the water depth (Fig. 2.10a).

$y = 16.9 - 0.0383x$

(T-ratio= -3.42, $P = 0.001$)

In 1989 in the Hotspot, there was also a significant positive correlation between water temperature and the number of larvae caught ($r = 0.693$, $P<0.001$). There was also a significant positive regression between temperature and numbers sampled (Fig. 2.10b).

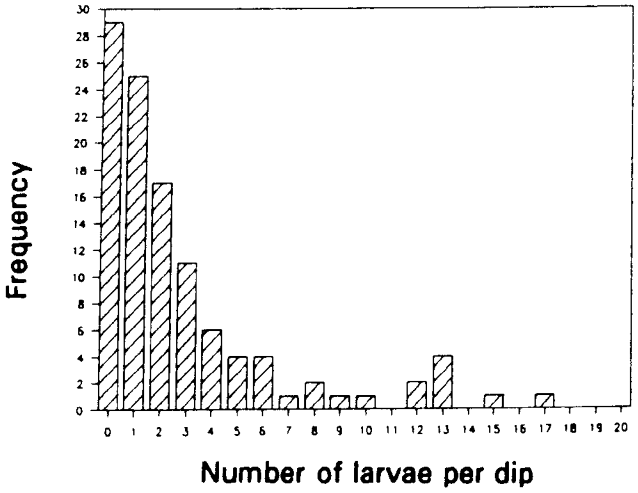
$y = -1089 + 132x$

(T-ratio= 4.8, $P=0.000$)

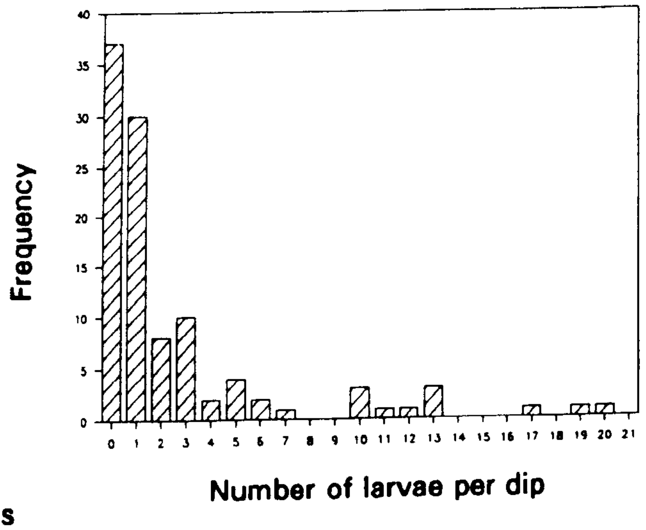
Therefore a combination of water depth and water temperature seems to influence larval distribution across the pond. No relationship between the number of larvae sampled and the pond depth or temperature could be found in 1990 and 1991, but since few larvae were present in those years, the failure to detect any relationship is not surprising.

Fig. 2.9: Frequency distribution of number of larvae and pupae per dip from Pond 9, 1989.

(a) First instars



(b) Second instars



(c) Third instars

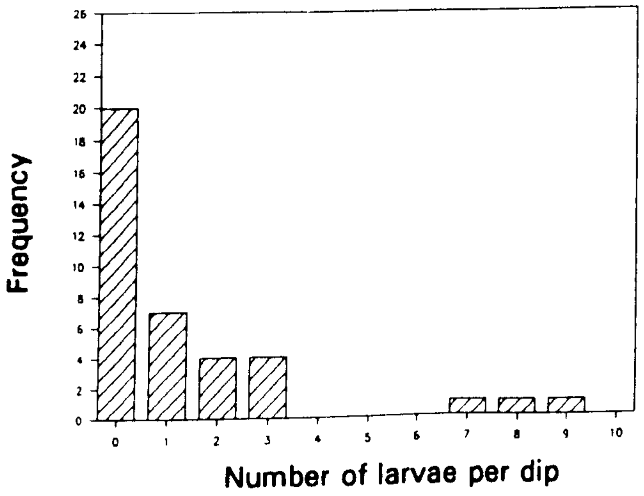
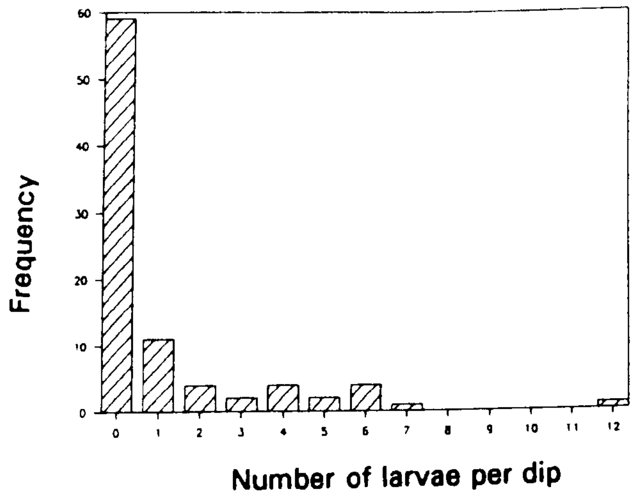


Fig. 2.9 continued

(d) Fourth instars



(e) Pupae

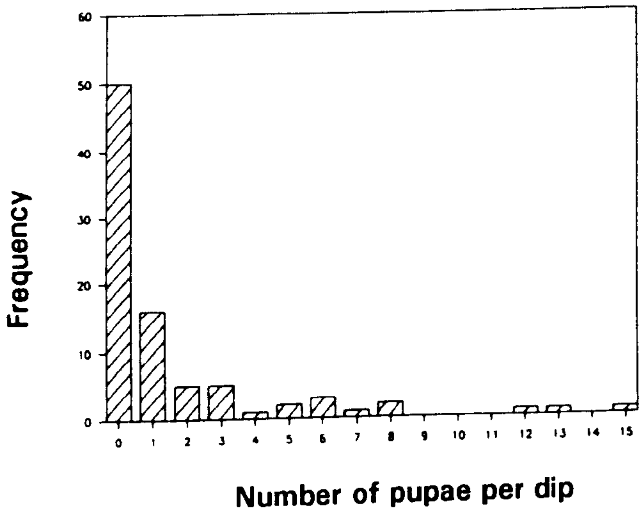
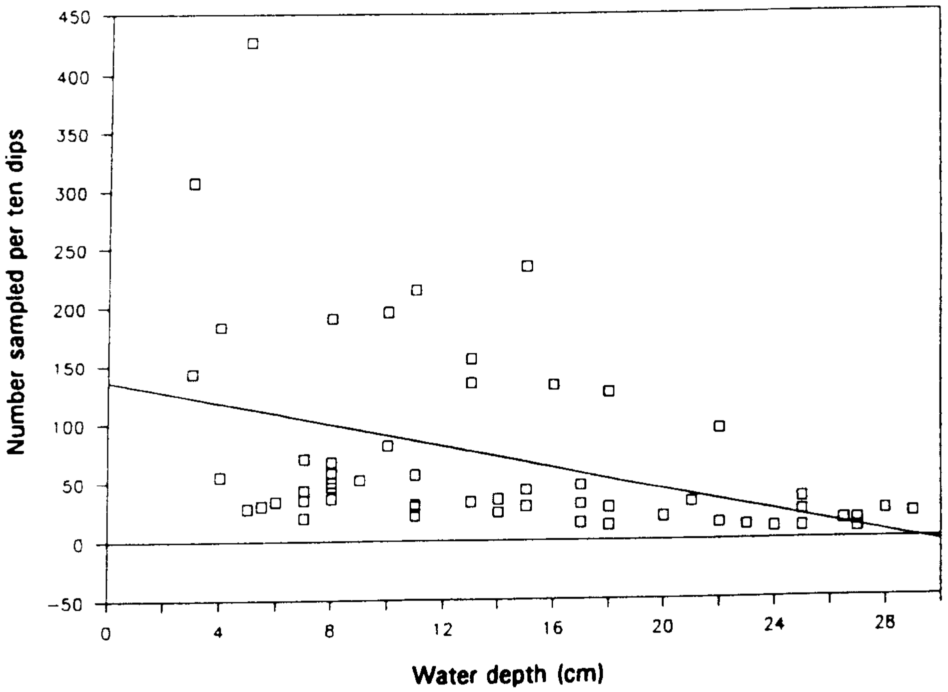
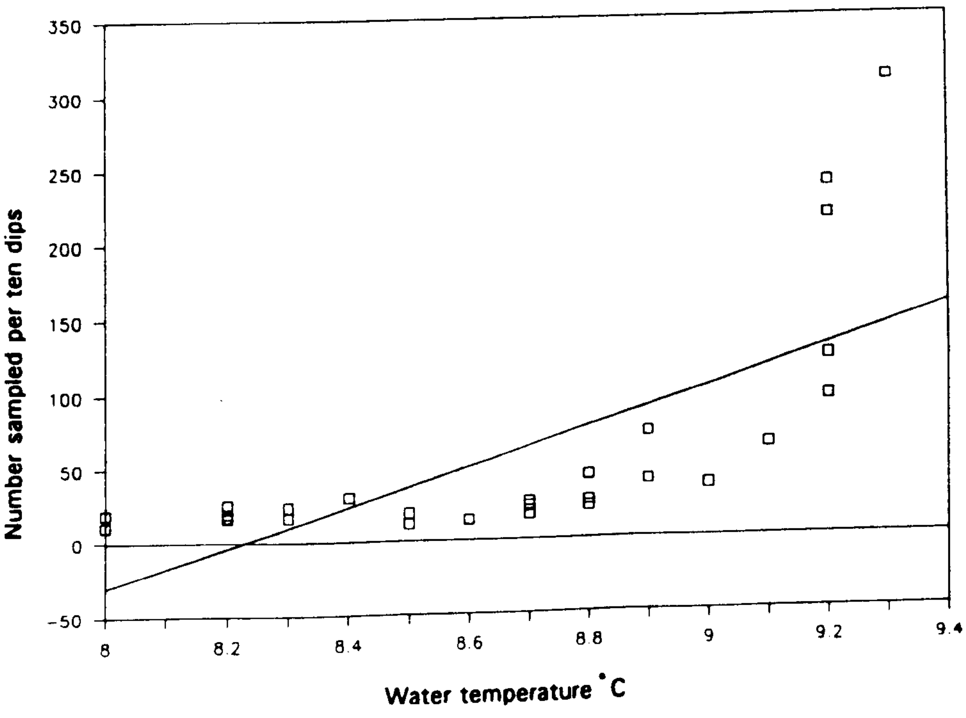


Fig. 2.10: Linear regression of number of larvae sampled from the Hotspot in 1989 against water depth and water temperature.

(a) Water depth



(b) Water temperature



2.3.8 LARVAL DENSITY IN THE FIELD

Figure 2.11 show the mean number of larvae caught per ten dips in both ponds in 1989 and 1990 over the entire sampling season. Significantly more larvae were caught per dip in the Hotspot in 1989 compared with Pond 9 (P=0.0001), but no significant difference was recorded between the Hotspot and Pond 9 in 1990 (P=0.31) (Table 2.8). The latter year was characterised by much lower numbers of larvae in both ponds. In 1989 the water in the Hotspot was 'black with larvae' and a maximum of 1,456 larvae were caught in ten dips at one point on the transect, compared to a maximum of 240 in Pond 9, and in 1990, a maximum of 214 in the Hotspot and 36 in Pond 9. Generally speaking, there was a very high larval density in 1989 in the Hotspot, but a fairly low larval density on all other occasions.

2.3.8.1 CYLINDERS

There was no significant difference in the number of larvae sampled per dip from the cylinders and those sampled across the transect in both ponds in 1990 and 1991. In addition, the number of each instar per dip were also similar, even when all the larvae were removed from the cylinders.

2.3.9 INSTAR MORTALITIES AND SURVIVORSHIP CURVES

2.3.9.1 MORTALITY IN THE FIELD

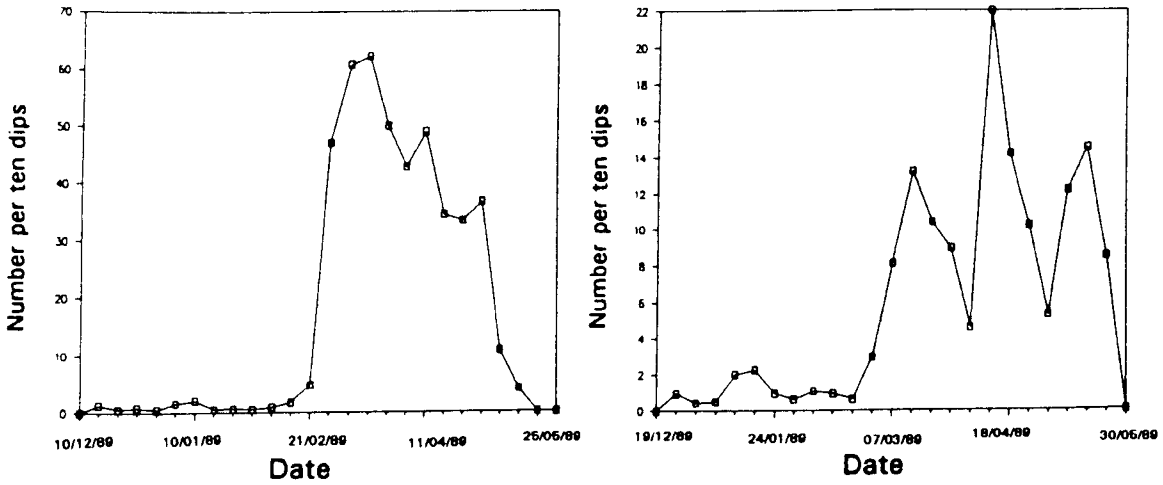
SURVIVORSHIP CURVES

Table 2.9 shows the instar durations for both the Hotspot and Pond 9 in 1989 and 1990, and for cage experiments in 1990 and 1991. At higher larval densities, the instar durations were longer than for larvae maintained at lower density.

Fig. 2.11: Mean number of larvae sampled per ten dips, using a 120 ml dipper, from the Hotspot and Pond 9 in the years 1989-1990.

(a) Hotspot, 1989

(b) Pond 9, 1989



(c) Hotspot, 1990

(d) Pond 9, 1990

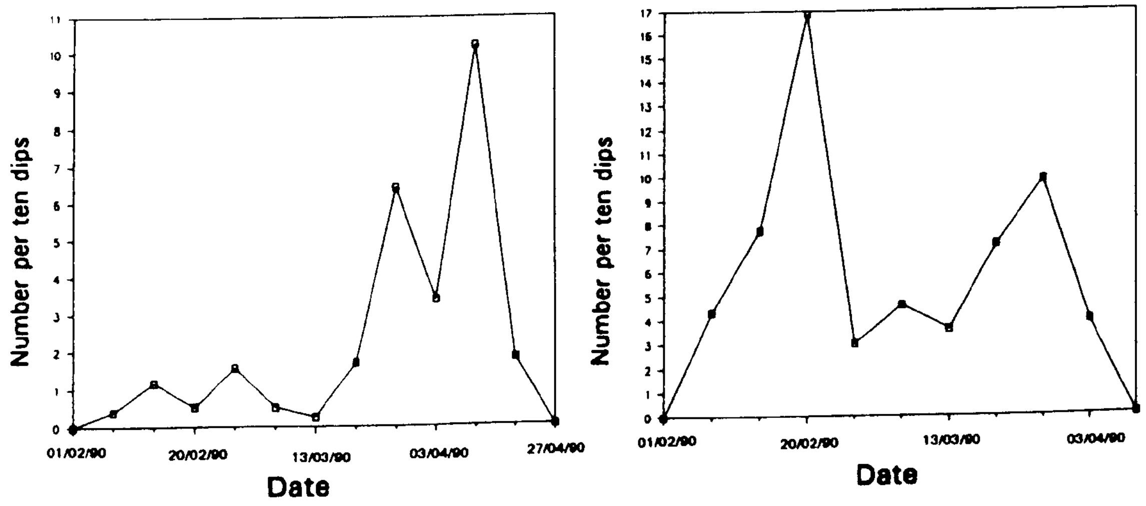


Table 2.8: Mean number of larvae sampled per ten dips, 1989 and 1990.

Year	Pond 9	Hotspot
1989	3.0	47.1
	8.1	60.8
	4.5	62.3
	9.0	50.0
	22.0	43.0
1990	4.3	1.7
	7.7	1.6
	16.9	6.4
	7.1	10.2
	4.0	1.8

Note: Data represent mean number of larvae per ten dips for five consecutive weeks of pond sampling in each year.

Table 2.9: Instar durations (in days) in natural ponds and cage experiments, 1989-1991.

(a) Ponds

	1989		1990	
Instar	Hotspot	Pond 9	Hotspot	Pond 9
I	15	15	12	13
II	15	15	12	13
III	13	14	13	13
IV	15	15	14	12
PUPAE	12	13	10	12

(b) Cage experiments

	1990	1991			
Instar	n=100	n=10	n=50	n=100	n=200
I	15	9	10	14	16
II	13	9	11	13	13
III	11	9	10	12	13
IV	13	9	11	12	12
PUPAE	10	8	10	11	11

Frequency histograms were constructed and as suggested by Ito (1961), a segmented survivorship curve rather than a smooth one was drawn through the midpoints.

Figure 2.12a illustrates the survivorship curves for Ae. cantans in the Hotspot in 1989 while Table 2.10a shows the instar mortalities. In the Hotspot in 1989, most larval mortality was in the early instars, with relatively little mortality in the third instars. The very high fourth instar and pupal mortality was due to desiccation of the habitat. The curve is of type IV of Slobodkin (Southwood, 1978), indicating that the heaviest mortality occurs in the younger stages.

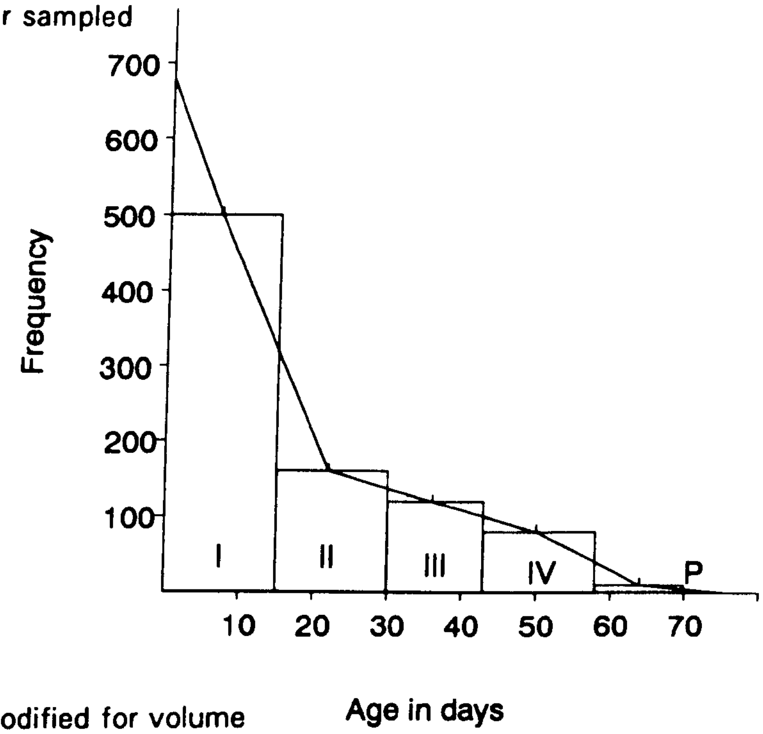
Figure 2.13a and Table 2.11a show the survivorship curve and age-specific life-table for Ae. cantans in Pond 9 in 1989. In this pond there was lower mortality in the earlier instars, and higher mortality in the later instars. The curve is of type I of Slobodkin, indicating that heaviest mortality was on later instars. Since more pupae were collected than fourth instars, fourths were undersampled and it is possible that this may take place in the other ponds in both years.

A major problem with the sampling data is that the volume of water in all the ponds in all three years changed considerably throughout the sampling period. Pond volume was at a maximum when first instars were present and at a minimum when the later instars and pupae were present. This will tend to underestimate first instars and overestimate later ones, and result in underestimating mortality. The data were therefore modified according to the number of samples taken and the pond volume. The survivorship curves were then redrawn and life-tables were recalculated.

Figure 2.12b and Table 2.10b show the redrawn survivorship curves and life-tables for the Hotspot in 1989. The pattern is similar to that observed in the earlier calculation, with mortality at a maximum in the first instar and low mortality in the third and fourth instars. The mortality is higher in the second instar than in the unmodified

Fig. 2.12: Histograms and Survivorship curves of larvae sampled from the Hotspot in 1989.

(a) Total number sampled



(b) From data modified for volume

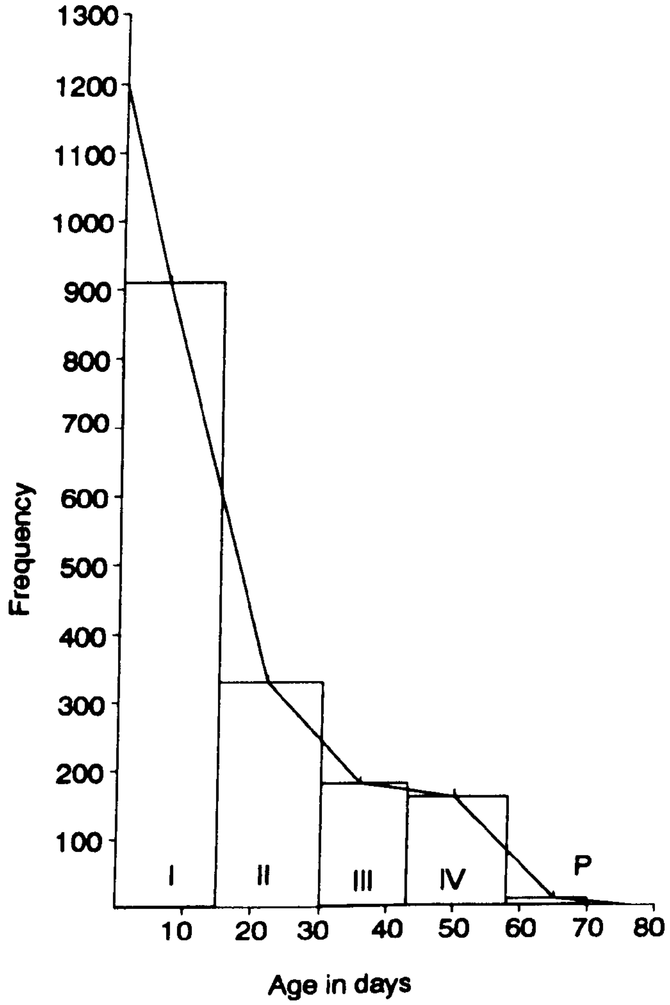


Table 2.10: _Ae_. _cantans_ instar mortalities in the Hotspot in 1989.

(a) Total number sampled.

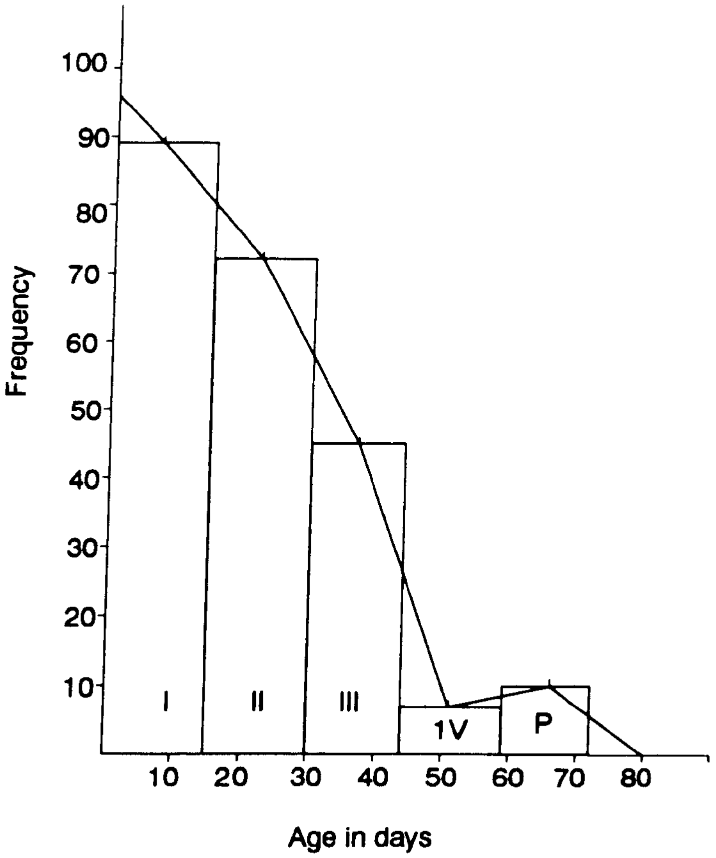
Instar	Age in days	Instar duration (days)	No. entering instar	Deaths in instar	Propor-tion dying	Propor-tion dying daily
I	0	15	680	360	0.529	0.056
II	15	15	320	180	0.563	0.055
III	30	13	140	35	0.250	0.022
IV	43	15	105	65	0.619	0.064
PUPAE	58	12	40	38	0.950	0.025
ADULT	70		2			

(b) Sampling data modified to account for changes in pond volume.

Instar	Age in days	Instar duration (days)	No. entering instar	Deaths in instar	Propor-tion dying	Propor-tion dying daily
I	0	15	12,000	5,800	0.483	0.044
II	15	15	6,200	3,500	0.565	0.055
III	30	13	2,700	900	0.333	0.031
IV	43	15	1,800	800	0.444	0.054
PUPAE	58	12	800	780	0.975	0.307
ADULT	70		20			

Fig. 2.13: Histograms and Survivorship curves for larvae sampled from Pond 9 in 1989.

(a) Total number sampled



(b) From data modified for pond volume

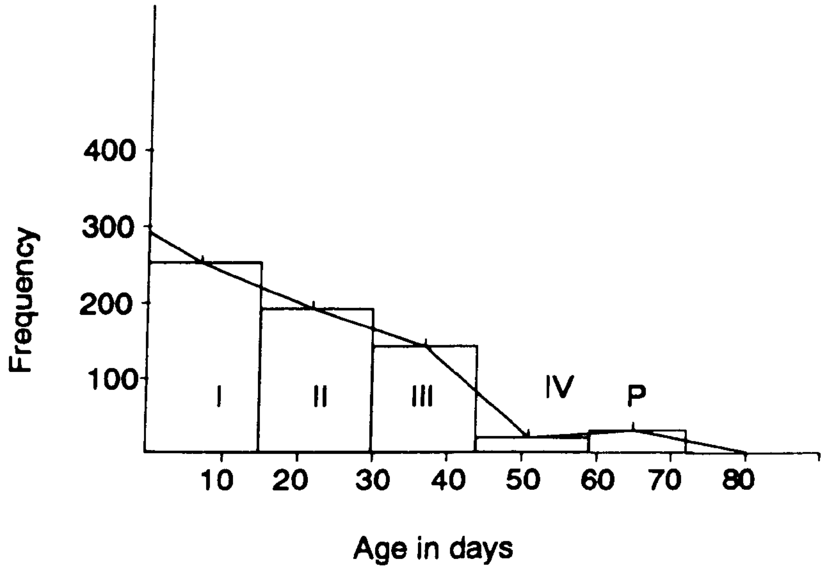


Table 2.11: *Ae. cantans* instar mortalities in Pond 9 in 1989 and 1990.

(a) **1989**, Total number sampled.

Instar	Age in days	Instar duration (days)	No. entering instar	Deaths in instar	Proportion dying	Proportion dying daily
I	0	15	96	16	0.167	0.012
II	15	15	80	23	0.288	0.023
III	30	14	57	31	0.544	0.056
IV	44	15	26	17	0.654	0.071
PUPAE	59	13	9			
ADULT	72		5.5			

(b) **1989**, Sampling data modified to account for changes in pond volume.

Instar	Age in days	Instar duration (days)	No. entering instar	Deaths in instar	Proportion dying	Proportion dying daily
I	0	15	285	60	0.211	0.016
II	15	15	225	60	0.267	0.021
III	30	14	165	85	0.515	0.052
IV	44	15	80	70	0.875	0.139
PUPAE	59	13	10			
ADULT	72					

Table 2.11: Continued

(c) **1990**, Sampling data modified to account for changes in pond volume.

Instar	Age in days	Instar duration (days)	No. entering instar	Deaths in instar	Propor-tion dying	Propor-tion dying daily
I	0	13	265	75	0.283	0.026
II	13	13	190	25	0.132	0.011
III	26	13	165	80	0.485	0.051
IV	39	12	85	60	0.706	0.102
PUPAE	51	12	25	15	0.600	0.076
ADULT	63		10			

data. Figure 2.13b and Table 2.11b show that the pattern of mortality was not altered by adjusting for volume in the calculation for Pond 9 in 1989.

Figure 2.14 shows the histogram of sampling data from the Hotspot in 1990. Since there were more larvae of the later instars sampled, it was not possible to draw a survivorship curve through these data. Modification of the data by allowing for the changes in volume did not make it any easier to fit a curve to the distribution.

Figure 2.15 and Table 2.11c show the survivorship curves and the life-tables for Pond 9 in 1990 from the modified data. It was not possible to draw a survivorship curve through the unmodified data. In this pond the larval population was relatively low and most mortality took place in the later instars, possibly as a result of the earlier onset of desiccation. Since the larval density in this pond in 1990 was low, it is unlikely that the carrying capacity of the habitat was reached, and so the larvae were unlikely to be stressed.

Table 2.12 shows the probability of an egg surviving to an adult. In 1989 in the Hotspot, this probability was very low with calculations varying, according to the method used, from 0.00078-0.00294. In Pond 9 in 1989, survivorship was higher, ranging from 0.0351-0.0573. In 1990, the probability of an egg surviving to an adult was also reasonably high at 0.0378.

LIFE-TABLES

Time-specific life-tables were constructed from the survivorship curves according to the methods of Service (1976).

The two most important columns are qx, the probability of a larva of age x dying before reaching age x+1 and ex, the mean expectation of further life of larvae that have attained age x.

The survivorship curves for the Hotspot in 1989 and Pond 9 in 1989 and 1990 are shown in Appendix 1.

Fig 2.14: Histogram of larvae sampled from the Hotspot in 1990.

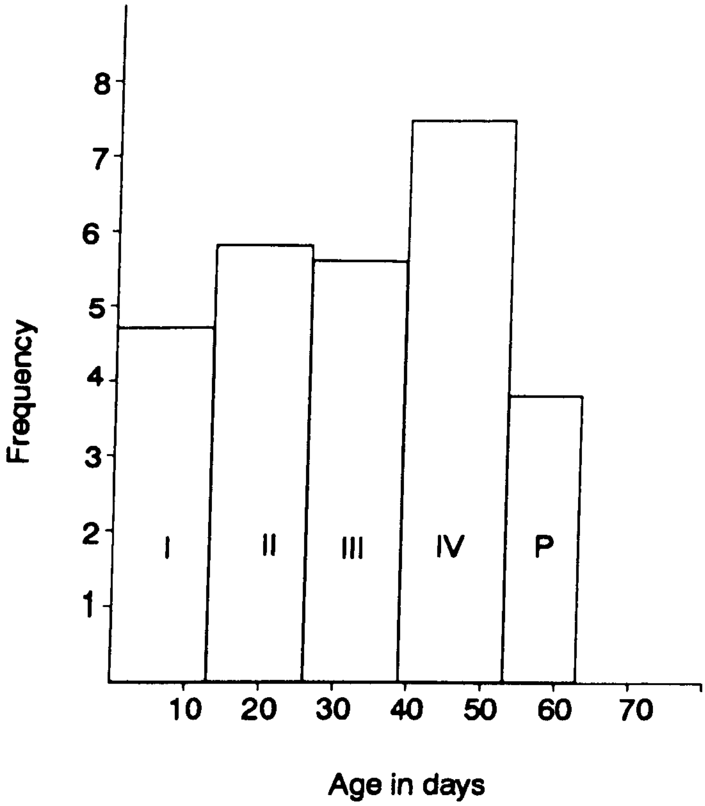
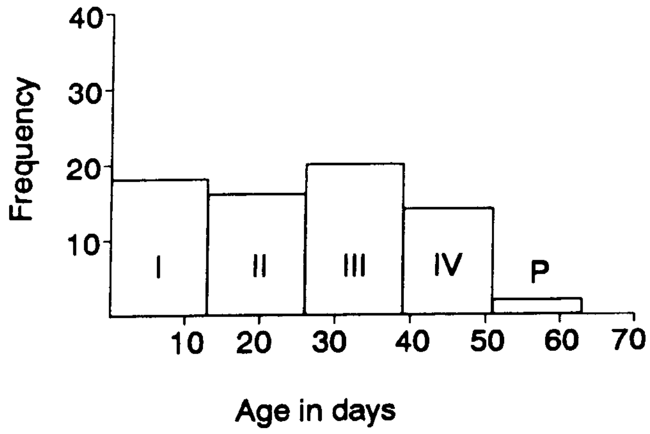


Fig. 2.15: Histograms and survivorship curves of larvae sampled from Pond 9 in 1990.

(a) Total number sampled



(b) From data modified for volume

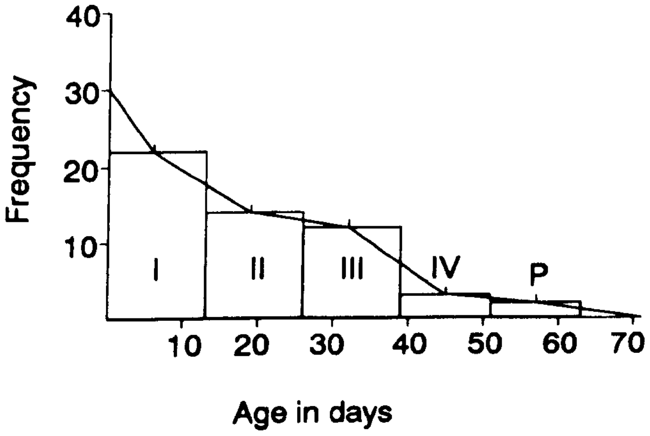


Table 2.12: Probability of an egg surviving to become an adult

(a) Field sampling

Year and Site		Probability of survival
Hotspot 1989	a	0.0294
	b	0.0008
Pond 9 1989	a	0.0573
	b	0.0351
Pond 9 1990	a	0.0378

Key: a=total numbers, b=numbers modified for pond volume changes.

(b) Cage experiments

Year	Larval density per 100 ml	Probability of survival
1990	100	0.139
1991	10	0.448
	50	0.380
	100	0.149
	200	0.081

2.3.9.2 MORTALITY IN CAGE EXPERIMENTS

Figure 2.16 and Table 2.13 show the survivorship curves and life-tables for the cage experiments carried out in 1990 and 1991.

In 1990, with a density of 100 first instar larvae per cage, substantial population loss occurred in the first instars, followed by second and then fourths, thirds and pupae; the proportion dying daily was highest in first instars, followed by seconds, pupae, thirds and fourths. The probability of an egg becoming an adult was S(62)/S(0) = 0.139.

In 1991, when larvae were raised at a density of 10 per cage, the highest proportion of larvae dying was in the first instar; however, the mortality was generally low for all stages and the probability of an egg surviving to the adult stage was S(44)/S(0) = 0.448.

When larvae were at a density of 50, the highest mortality was in the first instar, decreasing from seconds to pupae. The probability of an egg surviving to the adult stage was S(52)/S(0) = 0.35.

Mortality was highest in first instars, followed by seconds, fourths, thirds and pupae when larvae were raised at a density of 100. The probability of an egg surviving to the adult stage was similar to the result at the same density in 1990, S(62)/S(0) = 0.149

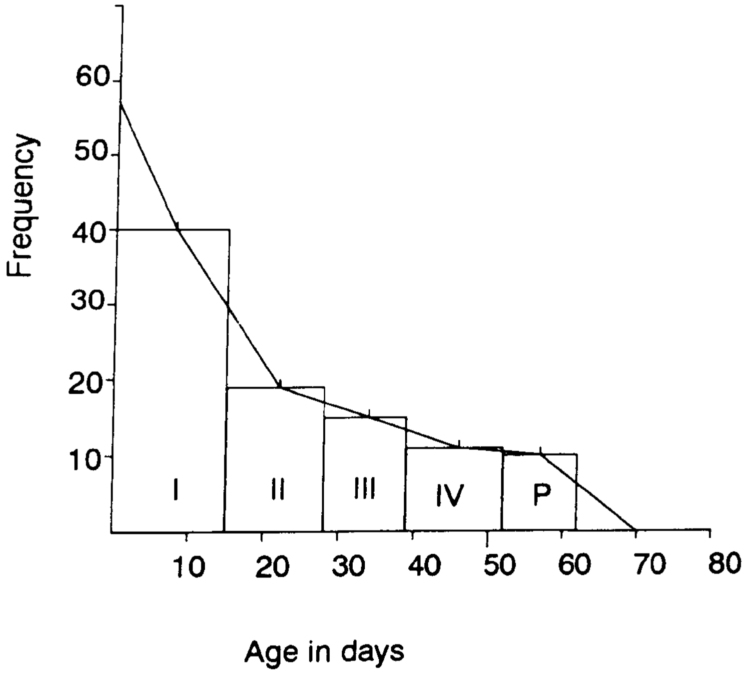
Finally, when larvae were raised at a density of 200, mortality was high at all stages, with first instars suffering the highest mortality. The probability of an egg surviving to the adult stage was very low, S(65)/S(0) = 0.081

LIFE TABLES

Time-specific life-tables constructed from the cage experiments in 1990 and 1991 are shown in Appendix 1.

Fig. 2.16: Histograms and survivorship curves from the cage experiments, 1990 and 1991.

(a) 1990 Density = 100



(b) 1991 (i)Density = 10

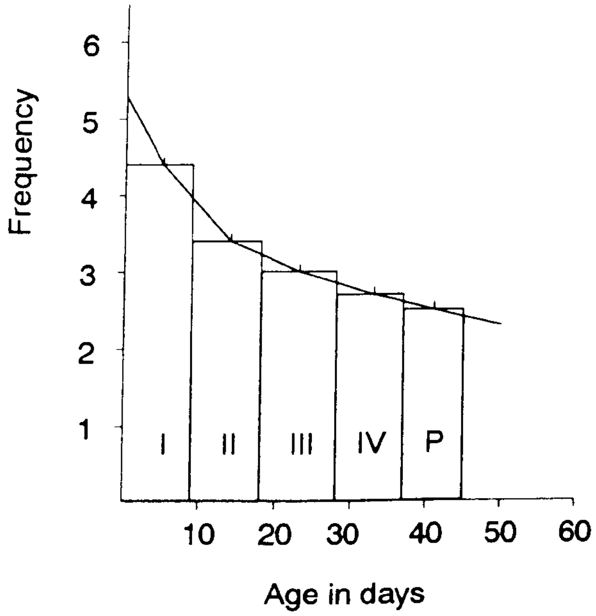


Fig. 2.16(b) continued

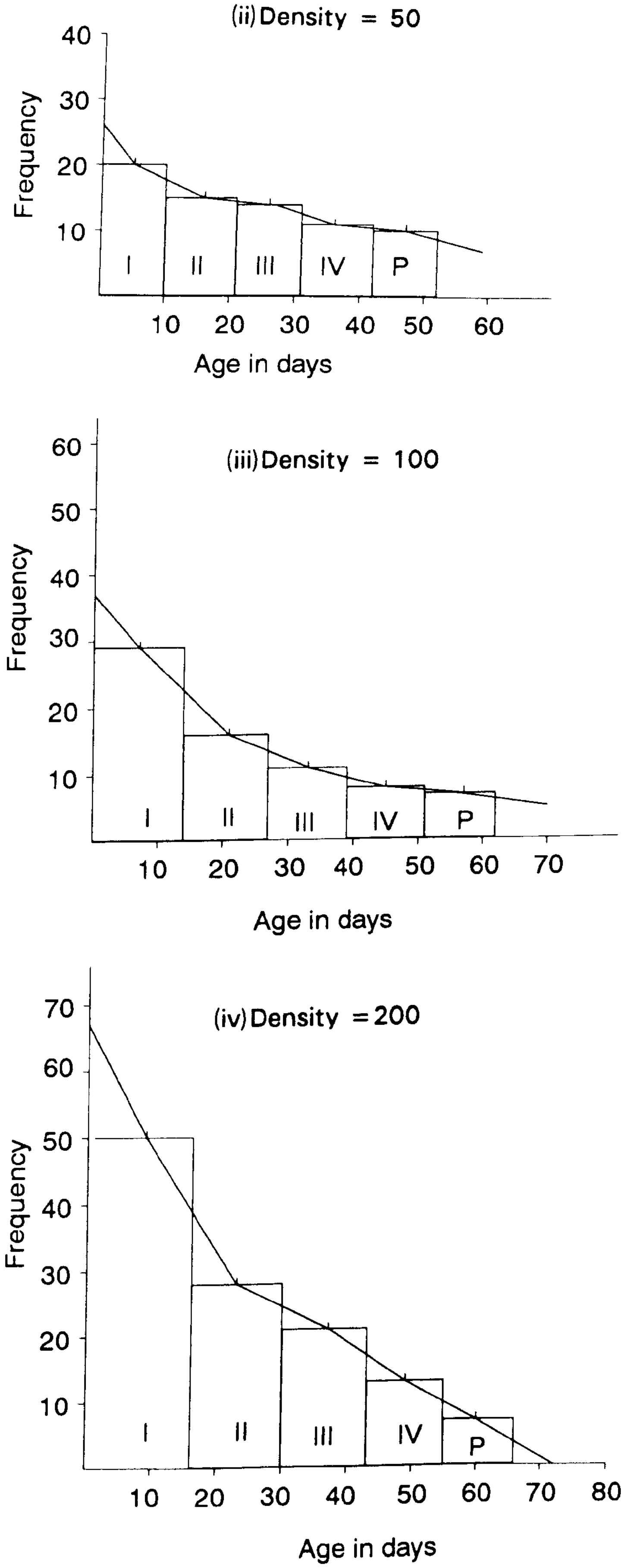


Table 2.13: Instar mortalities in cage experiments.

(a) **1990,** n=100

Instar	Age in days	No. entering instar	Deaths in instar	Proportion dying	Proportion dying daily
I	0	57.5	32.5	0.565	0.057
II	15	25	8.5	0.340	0.033
III	28	16.5	3.5	0.212	0.022
IV	39	13	3	0.231	0.020
PUPAE	52	10	2	0.200	0.023
ADULT	62	8			

(b) **1991,** n=10

Instar	Age in days	No. entering instar	Deaths in instar	Proportion dying	Proportion dying daily
I	0	5.25	1.40	0.267	0.034
II	9	3.85	0.65	0.169	0.021
III	18	3.20	0.35	0.109	0.013
IV	27	2.85	0.3	0.105	0.012
PUPAE	36	2.55	0.2	0.078	0.010
ADULT	44	2.35			

(c) **1991,** n=50

Instar	Age in days	No. entering instar	Deaths in instar	Proportion dying	Proportion dying daily
I	0	25	7.5	0.300	0.036
II	10	17.5	3.5	0.200	0.021
III	21	14	2.0	0.143	0.016
IV	31	12	1.5	0.387	0.012
PUPAE	42	10.5	1.0	0.250	0.010
ADULT	52	9.5			

Table 2.13 continued

(d) **1991,** n=100

Instar	Age in days	No. entering instar	Deaths in instar	Proportion dying	Proportion dying daily
I	0	37	17.0	0.460	0.045
II	14	20	6.5	0.325	0.031
III	27	13.5	3.5	0.259	0.025
IV	39	10	3.0	0.300	0.030
PUPAE	51	7	1.5	0.214	0.022
ADULT	62	5.5			

(e) **1991,** n=200

Instar	Age in days	No. entering instar	Deaths in instar	Proportion dying	Proportion dying daily
I	0	67.5	32.5	0.482	0.045
II	16	35	11.5	0.329	0.031
III	29	23.5	7.0	0.298	0.028
IV	42	16.5	7.0	0.424	0.047
PUPAE	54	9.5	4.0	0.421	0.051
ADULT	65	5.5			

2.3.10 PREDATION AND PARASITISM

Despite an extensive search for predators and parasites in all ponds, very few were found; in fact none was found in the Hotspot in any year. In Pond 9, three dytiscid larvae were collected in 1989, and two in 1990. When these were taken to the laboratory, they voraciously attacked Ae. cantans larvae; however, their numbers were so low that they could have had no significant effect on the population level of Ae. cantans larvae in the field. No parasites were observed in any larvae.

2.3.11 CANNIBALISM

Cannibalism in the early instars of Ae. cantans was initially observed by accident. First and second instar larvae were transported alive from the field in 3 X 1" tubes containing pond water, but no leaf-litter. Once in the laboratory, it was noted that there were many fewer larvae than were placed in the tubes and there was also an absence of corpses. Since this suggested the possibility of larval cannibalism, experiments were set up.

In direct observation of larvae in pots, two second instar larvae were observed predating on normally active and healthy-looking first instar Ae. cantans. One of these larvae was half eaten, the other was killed but was only partially consumed.

In the laboratory cannibalism experiments, the numbers of larvae in the pots without leaf-litter were reduced from 50 to an average of 20, after five days. An average of ten corpses per pot were observed; however, there was no sign of the other ten larvae, suggesting the possibility of cannibalism. It is difficult to state categorically that these larvae were predated upon because it is possible that they died from other causes and were then scavenged upon by the surviving larvae. In pots with leaf-litter, the average number of larvae surviving after five days was 33, with an average of five corpses per pot. Mortality was significantly lower in the pots with leaf-litter added ($\chi^2 = 3.97$, 1 degree of freedom, $P<0.05$).

In summary; it seems likely that cannibalism is a cause of larval mortality, however, further work is needed.

2.4 DISCUSSION

2.4.1 SEASONALITY

In 1988, Ae. cantans larvae hatched very early, on 15 December, in the Hotspot. During 1989-1990, hatching was considerably later, on 6 February, the same week as hatching took place in pots in my garden. In 1991, low rainfall levels delayed the onset of larval hatching until 25 February in the Hotspot and 12 March in Pond 9. Hatching of eggs immersed in water and maintained in the garden took place much earlier than this, on 8 February, indicating that the larval hatching was delayed by the lack of rainfall.

In the first year (1988-1989), the hatching rate of Ae. cantans was slow during December and January, but by mid-February to early March, hatching accelerated. In 1991 when the eggs were not soaked before the beginning of March, a high percentage hatched immediately.

2.4.2 LARVAL MORTALITY

Mortality of the larvae in the ponds, and in the cage experiments, can be summarised as follows.

In the Hotspot in 1989, most larval mortality occurred in the first two instars, and mortality in the third and fourth instars was low. As the season progressed, desiccation of the habitat took place and pupal mortality was high.

During the same year, larval mortality in Pond 9 was, by contrast, lowest in the earlier instars, and highest in the later instars as the pond started to dry up.

The sampling data in the Hotspot in 1990 made no sense since more later instars were collected in samples than earlier ones, and therefore no sensible conclusions can be drawn. In Pond 9 in 1990, most mortality was in the later instars. In 1991 there was complete larval mortality in both ponds because they dried up.

Larval mortality was low for all instars at a density of 10 larvae per cage, and the probability of an egg becoming an adult was high at 0.448. Similarly, at a density of 50 larvae per cage the probability of an egg becoming an adult was high at 0.35. Larval mortality was highest for first instars. At a density of 100 larvae per cage, the probability of an egg becoming an adult was reduced to 0.149 and the larval mortality was highest in the early instars. Finally, at a density of 200 larvae per cage, there was high instar mortality in all stages, but was at a maximum for first instars. The probability of an egg becoming an adult was 0.081.

The general pattern seems to be that when the larval population is at low density, as in Pond 9 in both 1989 and 1990 and in the Hotspot in 1990, larval mortality is higher in the later instars and this is probably caused by partial desiccation of the larval habitats, in other words it is density-independent. In the Hotspot in 1989, however, larval mortality was highest in the early stages and overall the probability of an egg surviving to an adult was extremely low. The density of larvae in this pond was extremely high and density-dependent mortality is likely to have been operating.

Service (1977a) found that mortality was more intense in the early stages of Ae. cantans, and the relatively few individuals that survived to the older age classes had a high expectation of pupation. Similarly, Lakhani and Service (1974) sampled Ae. cantans in a small woodland ditch in southern England and found a substantial population loss in the early stages. Mortality was more intense in the younger age classes in all years. The principal causes of mortality remained largely unknown. Service (1977a) suggested that the possible causes include a combination of competition for food and or space, and effects of toxic chemicals produced by larvae when they are overcrowded, that is overcrowding factors.

In assessing Ae. cantans larval mortality in 1980 and 1981 in ponds in Ness Woods Sulaiman (1982) found a marked difference between the pattern of mortality in the two years. In 1980 he found most mortality was in the later stages, with little in the earlier stages, whereas, in 1981 most mortality occurred in the earlier stages. In 1980 there was little rainfall and as a result of this the water level decreased and the water became de-oxygenated, but in 1981 however, there was more rainfall in the spring and mortality was predominantly in first and second instars, followed by pupae. Overall, in 1980, pre-adult mortality was 95.1% and in 1981, 79.7%.

2.4.3. CAUSES OF LARVAL MORTALITY

2.4.3.1 CANNIBALISM

In this investigation, cannibalism caused larval mortality in pots in the laboratory. In 1989, when there was a very high density of Ae. cantans in the Hotspot, and the water became 'black with larvae', it is possible that under such densities cannibalism took place in the field. The cannibalism observed in this investigation in the laboratory is different from that observed by others (e.g. Reisen and Emory, 1976) because the earlier, rather than later, instars were cannibalistic.

Although cannibalism has only been rarely observed in mosquito larvae, and especially so in filter feeders, several authors have suggested that cannibalism may be important in the regulation of larval populations. For example, Mogi (1978a) observed cannibalism in the typical filter feeders, *Cx. tritaeniorhynchus* and *An. sinensis*. Older larvae consumed as many as 800 firsts per day even when there was a sufficient amount of suitable food other than larvae. The occurence of cannibalism in these typical filter-feeders suggests that the phenomenon may not be uncommon in filter-feeding and browsing species, and may have a role in population regulation.

Reisen and Emory (1976) suggested the possible role of cannibalism in the population regulation of *An. stephensi* in Pakistan. In laboratory experiments, they found that when first instars entered into the effective range of water flow generated by the action of mouth brushes of fourths, they were drawn towards them and swept into the mouth-parts. Predation by fourth instars on seconds, and third instars on firsts were observed, although the efficiency was much lower in the latter. Cannibalism occurred even when a sufficient amount of food was present, although the predation rate was lower. Koenekoop and Livdahl (1986) examined cannibalism in the tree-hole species *Ae. triseriatus* in the USA. First instar larvae were cannibalised by fourth instars at both low and high density.

Surtees (1959) stated that filter feeders are species that strain out food particles from the surrounding medium, such particles being sufficiently small to pass directly into the digestive tract without undergoing any additional breakdown. Nevertheless, larvae of *Ae. cantans*, and other filter-feeding species, are clearly capable of consuming large objects.

2.4.3.2 DESICCATION

In all three years of this investigation, but especially in the final year, lack of rainfall leading to the desiccation of larval habitats was a major cause of larval mortality. In 1991, all the temporary pools in Wood A dried up by late March and consequently no mosquitoes emerged from them. Only some of the permanent ponds produced adults and the numbers caught in 1991 were significantly lower than in 1989 and 1990 (Chapter 3). In addition, the low rainfall levels in 1991 delayed hatching. Larvae in pots in my garden hatched more than three weeks before water filled Pond 9.

Desiccation has been observed as a major mortality factor in other studies. For example, both Sulaiman (1982) and Service (1973) identified habitat desiccation as a major cause of mortality in larval populations of Ae. cantans. Similarly, Yates (1979) found that in the British treehole mosquito Aedes geniculatus (Olivier) desiccation was the most important factor influencing larval survival.

Pritchard and Scholefield (1983) hatched Aedes spencerii (Theobald), Aedes cataphylla Dyar and Ae. vexans eggs by artificially flooding dry pond beds. Even though the water levels were maintained, survival to pupae was only 20% and mortality was higher in the early stages. They concluded that the survival rate may have depended on food and temperature, but that predation was unimportant. Normally the drying up of ponds caused high mortality.

Bradshaw and Holzapfel (1988) examined the effect of drought on tree-hole mosquitoes in Florida and found that it affected survivorship by the direct action of dehydration on developing larvae and pupae. Schoener (1983) and Connell (1983) felt that investigators of aquatic populations may be predisposed to select study sites that will persist for the duration of the study and therefore potentially compound bias towards documenting the importance of biotic over abiotic factors, especially drought.

2.4.3.3 FOOD

It is difficult to assess whether food shortages are leading to mortality in the field situation. Shortage of food is difficult to evaluate other than by ruling out other mortality factors. The fact that leaf-litter added to the cages in 1991 led to increased survival and also to larger larvae at a density of 50 per pot indicates that the larvae were competing for food. It is difficult to explain why the larvae at higher densities did not demonstrate this tendency. It is possible that at the higher densities larvae were so crowded that contact inhibition prevented larvae from feeding even when food was in plentiful supply. Alternatively, it is possible that at the higher densities cannibalism was more important, masking the effects of adding food.

Food shortages have been identified by several authors as leading to larval mortality in container-breeding mosquito species, but it is less likely that food shortages are as important in larger ground ponds (Service, 1985).

Carpenter (1983) examined Ae. triseriatus in a tree-hole ecosystem and found that the quality of the leaf litter strongly limited growth, survivorship and development rates. He considered that the food ration level was the most important factor in larval success. Bradshaw and Holzapfel (1986), working on several species of European tree-hole mosquitoes found that larvae in the field encounter severe resource depletion for food and intraspecific competition is important in tree-hole ecosystems. Farkas and Brust (1985) found that food limited the development of Wyeomyia smithii (Coquillett) breeding in pitcher plants. Larvae that were given a diet supplement pupated more synchronously and earlier. A shortage of food will greatly prolong the length of the larval stage (Nielsen and Haeger, 1954) and will therefore affect the estimation of development rates. In nature, food is often not abundant enough for rapid growth and for this reason, estimates should be restricted to particular situations.

Both Southwood et al. (1972) and Subra and Mouchet (1984) believed that food was the key-factor in regulating the numbers of Ae. aegypti breeding in village pots.

In Canada, Wagner and Newson (1975) proposed that mortality in the later instars of pond breeding Aedes fitchii (Felt and Young) was due to inadequate nutritional resources. It can be argued that inadequate resources immediately after flooding due to insufficient time to build up microbial populations would affect first stage larvae, while later larvae could experience food shortages as the area of the pool shrinks and larval density increases and severe competition for food becomes operative. Therefore, it appears that Aedes larvae in ponds in Alberta, as elsewhere (Service, 1973, 1977a), have a survival rate dependent upon food supply and temperature, as long as the pools contain water.

Mogi et al. (1984), in the Philippines found that adding food to rice fields shortened the fourth instar larval period of Culex vishnui Theobald or Anopheles peditaeniatus (Leicester) but did not influence their survival when reared in predator-free cages.

Aedes cantans larvae feed on a variety of microscopic aquatic organisms and browse on leaf litter. The nutritional requirements are not known and it seems unlikely that in this species food could be limited (Service, 1977a). However, it has been observed that high larval population densities result in the production of large numbers of small adults, whereas in years in which there is a small larval population this is followed by the production of fewer but larger adults (Service, 1977a). There may therefore be some competition for food and or space.

2.4.3.4. CONTACT INHIBITION

Broadie and Bradshaw (1991) examined the effect of high density on larvae of Aedes sierrensis (Ludlow) in the laboratory. They found that fourth instars inhibited each other in feeding when at densities of 64 larvae per ml, although addition of leaves decreased this feeding inhibition. They concluded that at densities above the population equilibrium, encounter competition would take place, especially in tree holes with few leaves. Among mosquitoes, intraspecific competition as a consequence of increased larval density and decreased food, leads to longer development time. Dye (1984) and Shannon and Putnam (1934) felt that competitive interference may occur through direct physical contact.

Sulaiman (1982) believed that competition for space could be the reason for the large population loss of Ae. cantans he recorded in Ness Woods. He thought that because Ae. cantans larvae aggregated in large numbers, and the earlier instars were found at the water's edge, competition for space could arise. I presume that such competition leads to intraspecific interference, and this can reduce food intake or lead to the utilisation of energy reserves through increased activity.

In my pot experiments at high density it is likely that contact inhibition occurred. In addition the high density of larvae observed in the Hotspot in 1989 is also likely to have led to contact inhibition.

2.4.3.5 PREDATION

In this investigation, both predation and parasitism had no discernible effect on the Ae. cantans populations in Pond 9 and the Hotspot. In fact, it was difficult to find any predators, except for occasional dytiscid larvae in Pond 9 in the first and second years.

Service (1977a) and Sulaiman (1982) also examined predation on Ae. cantans in its larval habitat. Although they found some predators, with C. crystallinus and larval dytiscids being the most important, they concluded that the numbers of predators were too small to cause much of a decrease in the mosquito populations. Mosquito larvae in temperate temporary ponds are much less exposed to aquatic predators than those in permanent water (James, 1966). If, as in this investigation, the ponds were completely dry prior to flooding, recolonisation by potential predators is slow and consequently, aquatic predators are more effective against later instars. Ground habitats range from temporary rain pools that appear mainly free from aquatic predators, to large natural more permanent ones such as swamps and marshes, and man-made ones such as rice fields, all of which usually contain a broad spectrum of predators.

Some authors have found that predation is important in regulating mosquito larval populations. Reisen *et al*. (1989) found that predation was the most important cause of larval mortality in *Culex tarsalis* Coquillett in stable and ephemeral breeding sites in California. Mogi *et al*. (1980) examined mortality in *Cx*. *tritaeniorhynchus* released in experimental cages in rice fields and found that emergence was low in cages with a natural complex of predators. They found that fishes and predaceous insects made a large contribution to larval mortality. Mogi *et al*.(1980) found that the mortality rate due to predation was density-independent and less influenced by weather conditions than mortality caused by drying up or poor water quality. They found that chemical control in the early season may lead to a population explosion in midsummer through predator elimination.

2.4.3.6 GRFS

Although no attempt was made to examine the effect of Growth Retardant Factors it is unlikely that they caused any appreciable effect on larval development in the field, especially considering the volume of the ponds. Laboratory studies have indicated that high larval densities are needed for chemical constraints to operate and there is very little evidence that they contribute to regulating natural mosquito populations (Dye, 1982; 1984).

2.4.4 LARVAL DISTRIBUTION

In the Hotspot in 1989, there was a significant positive correlation between the pond temperature and the number of larvae sampled, and a significant negative correlation between pond depth and the number of larvae caught in samples. Therefore it seems that the larvae prefer a combination of warmer and shallower waters. In this year, larvae in the Hotspot had a highly contagious distribution with a very large variance to mean ratio. Since it would seem disadvantageous for larvae to be crowded, some advantage must be gained from crowding together. Possibly larvae are adopting a strategy of safety in numbers as regards predation, but in this instance, predation was not important. Alternatively, it is possible that larvae are exploiting warmer temperatures, leading to quicker larval development, which is likely to be important in ephemeral habitats. However, clumping must also lead to greater competition for food and contact inhibition, but on the other hand aggregation in shallower water may lead to better access to leaf-litter. A contagious egg distribution creates a strongly contagious dispersion pattern of newly hatched larvae and possibly second instars, even in relatively homogenous habitats such as rice fields.

When larvae of Anopheles atroparvus Van Thiel are placed in water with a horizontal temperature gradient, most of them accumulate at a particular temperature by orthokinesis. This is achieved because larvae swim actively at temperatures above and below a preferred temperature, and when by chance they enter the preferred zone their swimming movements cease (Ivanova, 1940). Larvae of Ae. aegypti also show this behaviour (Omardeen, 1957). When subjected to a temperature gradient, larvae exhibited jerking movements until they finally entered a zone of temperature which had no effect on them. In the field, larvae of Aedes communis (De Geer) select the surface layer of water until the surface temperature rises above 16° C; then they aggregate at lower levels, except for occasional sorties to the surface (Haufe and Burgess, 1956).

2.4.5 GROWTH AND MEASUREMENTS

In 1989 larvae in the Hotspot were significantly smaller in all instars in terms of siphon length than larvae sampled from the much less crowded Pond 9. The density in the Hotspot in 1989 was extremely high and was significantly higher than in Pond 9. In 1990 the larval densities in both the Hotspot and Pond 9 were low and there was no significant difference in the size of larvae in these ponds in any instar. Correspondingly there was no significant difference in the size of adults produced by these two ponds in 1990, but in 1989, the adults emerging from the Hotspot were significantly smaller than those emerging from Pond 9 (Chapter 3).

These results indicate that the variations in size of larvae were density-independent at low densities but became density-dependent at higher densities. In conclusion, at a high larval density in a ground pond environment, density-dependent size variation and density-dependent mortality were proven.

In the cage experiments, larvae maintained at a low density are larger than those at an intermediate density which are in turn larger than those at a high density. There is a clear relationship whereby mortality is higher and the larvae are smaller at higher densities.

Mogi (1984) examined distribution and overcrowding in Aedes flavopictus miyarai in taro axils at high density. First and second Ae. flavopictus miyari showed no correlation between body size and larval density, whereas in older larvae and pupae, the body size was inversely correlated with density.

The use of larval siphons as a measure of larval size seems to have been very successful. Within Pond 9 in 1989; there was a clear separation into the instars, however, in the Hotspot the pattern was not quite as clear. Larvae that were fourth instars in the Hotspot were smaller than larvae that were only third instars in Pond 9.

This indicates how strong the effect of intraspecific competition was in the Hotspot in 1989. It must be noted that a larva cannot simply be taken at random and identified as a specific instar, the history and pattern of size distribution in the field needs to be known.

Reisen and Siddiqui (1979) found that when head capsule width of Cx. tritaeniorhynchus was measured, the four instars could be clearly distinguished. Similarly, Le Sueur and Sharp (1991) used head capsule width to identify the different instars of An. merus and found no overlap between instars in either winter or summer collections, despite a 276% increase in larval density in pools in the winter. They concluded that the head capsule width remains a reliable indicator of instar, irrespective of the density or nutritional status of the larvae. However, an inverse relationship was noted between both head capsule width and wing length, and seasonal fluctuations in air and water temperatures. Le Sueur and Sharp concluded that if absolute size characteristics are to be used for entomological identification purposes, then the annual temperature range should be taken into account. Increased variation and overlap of size between instars will occur in areas having a greater annual range of mean daily air temperature. The relationship between temperature and size in mosquitoes has been shown to affect morphometric measures (Clements, 1963).

2.4.6 DENSITY-DEPENDENCE

In this investigation I have shown that density-dependent mortality and density-dependent size variation take place in Ae. cantans breeding in ponds. Different authors have presented conflicting information on density effects in mosquito populations. Most information on density-dependent regulation of mosquito populations comes from artificial container breeding species.

Rajagopalan *et al*. (1976) studied natural breeding sites of *Cx*. *quinquefasciatus* in Delhi, and found the survival rate was density-dependent in some wells. However, the strength of the density-dependent effects varied from well to well and was related to the carrying capacity. In some wells, density-independent factors kept the population level low so that the density did not increase to a critical level. For example, the density in the wells was low in March, and the survival rate was high. In contrast, survival rates remained very low in all wells during periods of high density, indicating a strong density-dependent relationship between eggs deposited and survival rate. Variations in the wells were partly due to the chemical characteristics of the water.

Reisen *et al*. (1984) carried out laboratory experiments to evaluate the effects of temperature, food and density on *Cx*. *tarsalis*. They found that rearing stress decreased survival, altered development rates, distorted sex ratios and resulted in smaller adults. Immature developmental rate decreased and survival rate increased as the volume of food per larva increased. Immature stress induced by intraspecific competition apparently eliminated less fit individuals in pre-imaginal stages with little cost to the few more competitive ones which reached emergence. In nature, *Cx*. *tarsalis* frequently exploits unstable habitats, such as hoof prints and puddles and in these small, shallow, and often ephemeral, environments larvae may be stressed by inadequate food supply, crowding and high temperatures. Later, Reisen *et al*. (1989) studied the effects of abiotic and biotic factors on pre-imaginal survivorship and adult production of *Cx*. *tarsalis* in stable and ephemeral breeding sites in California. They found that catastrophic events, such as fluctuating water level and pollution, drastically reduced larval and pupal abundance. Mortality caused by lack of food was relatively low; however, low nutrient level at three sites lengthened the duration of the fourth instar, delayed pupation and led to smaller adults at emergence. Larval development in field water was slower than anticipated from laboratory experiments at comparable temperatures, and the addition of food appeared to shorten the duration of fourth instars.

In Pakistan, increasing the density of Cx. tritaeniorhynchus in floating cages along pond margins delayed development and decreased survivorship, and wings of adults emerging from crowded larval conditions were consistently shorter than those reared in uncrowded conditions (Reisen and Siddiqui, 1979). Intraspecific competition for food and space reduced adult body size at emergence. Mogi (1978) also studied Cx. tritaeniorhynchus in rice fields and found that second instars were well adapted to the habitat and mortality decreased from second instar onwards. He considered that there was a density- dependent increase in mortality.

Livdahl (1982) reared Ae. triseriatus in containers kept in the field, at different densities. He found that increasing density markedly reduced the mean mass of females, extended mean female development time and reduced larval survivorship. He also demonstrated intraspecific competition among larvae in the containers, and felt that it was likely that such competition occurred routinely in natural habitats, because the density levels in his experiments were chosen to mimic natural densities. Larvae most probably competed for food. In contrast, Hawley (1985b) found no evidence for density-dependent larval mortality in the tree-hole mosquito Aedes sinensis Chow, but pupal weights of females were inversely correlated with larval density, indicating density-dependent size variation.

Andis & Meek (1985) found that a three times increase in the larval density of Psorophora columbiae (Dyar & Knab) did not significantly affect survival. Since this increase in density was well above that encountered in natural populations they concluded that intraspecific competition in this species may not be important in population regulation.

In conclusion, it seems that at high densities intraspecific competition took place in the ponds in Ness Woods, and density-dependent regulation of the mosquito populations occurred. Causes of larval mortality are likely to be a combination of cannibalism and food shortages, possibly caused by contact inhibition. Predation, parasitism, growth retardant factors and temperature effects are unlikely to contribute very much to mortality in the field. The density-independent factor of rainfall caused very high levels of larval and pupal mortality, especially during the later years of the investigation.

CHAPTER THREE - ADULT STUDIES

3.1 INTRODUCTION

The main objectives of the adult studies discussed in this chapter were to examine the temporal emergence patterns and seasonal incidence of Ae. cantans, and also to investigate the effect of adult size on survivorship, parity and fecundity.

Adult Ae. cantans were studied during April to October in the summers of 1989 and 1990 and to a lesser extent in 1991. Emerging mosquitoes were trapped using bed-nets placed over ponds. Human-bait catches were used to examine the seasonal incidence of adults, and vegetation was sweep-netted to sample the resting population. Mark-release-recapture techniques were carried out on both newly emerged mosquitoes and those caught at bait (Chapter 5), and ELISA tests were undertaken to identify the main hosts of the mosquitoes (Chapter 4).

Service (1968a, 1968b, 1971c, 1971d, 1977a) has carried out extensive work on adult Ae. cantans mosquitoes in southern England. The ecology of Ae. cantans in Ness Woods has also been the subject of short MSc projects by the following students; Campbell (1986), Elmo (1987), Gavin (1986) and Majala (1987). Sulaiman (1982) also carried out a three-year investigation into Ae. cantans in Ness woods.

The rationale and background for the various studies discussed in Chapter 3 are presented below (3.1.1-3.1.9).

3.1.1 EMERGING MOSQUITOES

Aedes cantans adults start emerging in April (Marshall, 1938), and can be sampled by placing cages (Packer and Corbet, 1989a; Service, 1969, 1977a) or as in this study, bednets (Sulaiman, 1982) over their larval habitats. Emerging mosquitoes were counted, sexed and identified, wing lengths were measured, and the ovaries and spermathecae of some were dissected. Others were painted with 'Humbrol' paint for mark-release-recapture work (Chapter 5).

3.1.1.1 OVARIOLE DEVELOPMENT

Bowen (1991) stated that most female mosquitoes do not become host-responsive until several days after emergence. Service (1977a) observed that there was a minimum interval of 20 days from emergence of Ae. cantans to first appearance at human bait and postulated that this was the time it took for the ovaries to reach Stage II of development.

STAGES IN OVARIOLE DEVELOPMENT

Clements (1963) defined several stages of ovarian development based on the previous studies of Christophers (1960), Mer (1930) and Macan (1950).

Stage I is typical of newly emerged mosquitoes, and consists of Stage Ia where the follicle is spherical and the oocyte undifferentiated from the nurse cells, and Stage Ib where the follicle is oval and the oocyte and seven nurse cells are surrounded by a distinct follicular epithelium.

Stage II is also divided into different sections: at Stage IIa, there are a few fine yolk granules visible at high power (X200) and at Stage IIb the yolk is visible under medium power(X100), around the oocyte nucleus; the follicle is in the resting stage. Christophers (1960) stated that without a blood-meal the ovaries would not proceed past Stage II.

By **Stage IIIa** the yolk is visible under low power (X50) and the oocyte occupies half the follicle, and at Stage IIIb the yolk obscures the oocyte nucleus and the oocyte occupies more than two-thirds of the follicle.

At **Stage IVa** the egg follicle begins to elongate and by Stage IVb the follicle assumes the shape of the mature egg.

At **Stage V**, the ovaries contain fully developed ova.

Changes in the state of the ovaries from emergence to first appearance at bait will be examined in this study.

3.1.2 MOSQUITO SIZE

In this investigation wing length is used as a measure of mosquito body size. Service (1977a) found that there was good correlation between wing length and dry body weight of Ae. cantans (r=0.87 t=10.44 P<0.001). Similar relationships have been found in a variety of other mosquito species (Christophers, 1960; Fish, 1985; Landry et al., 1988; Packer and Corbet, 1989a). However, Nasci (1990) found that the relationship between wing length and dry weight at adult emergence differed among mosquito species and also between sexes, and may even differ within the same sex. It is therefore advisable to check the relationship between wing length and size in each instance.

Mosquito adult body size is controlled by genetic factors, but this relationship is modified by environmental ones (Christophers, 1960; Reisen et al., 1984; van den Huevel, 1963). For example, the size of emerging mosquitoes is influenced by larval conditions (Bock and Milby, 1981; Fish, 1985; Nayar, 1982), including density-dependent factors such as food availability, metabolite accumulation and competition for space as well as density-independent factors such as temperature (Chapter 2). Generally, rich nutrients and a low larval density will lead to larger adults, whereas overcrowding in the larval stages leads to competition for space and food and consequently smaller adults.

Considerable intraspecific variation in adult body size occurs commonly within and among natural mosquito populations and competition induces or exaggerates this. Small individuals are more affected by competition, which acts asymmetrically, and this leads to even greater discrepancies in size (Begon, 1982). Species using rapidly changing or ephemeral habitats, such as the temporary ponds studied in this investigation, display more variance in adult body size than those using more stable larval habitats (Nasci, 1987).

Adult size can influence a number of bionomic factors including fecundity and dispersal. Nasci (1987) concluded that body size in a mosquito population is a useful indicator of population quality and Bock and Milby (1981) felt that a predominance of small sized individuals indicated a population in decline. The larger the size of the mosquitoes, the greater the flight range (Bock and Milby, 1981), the greater the success at host location and blood-feeding (Packer and Corbet, 1989a) and the greater the fecundity (Washburn et al., 1989). Some authors have concluded that larger females are longer lived (Haramis, 1983; Nasci, 1986; Packer and Corbet, 1989a) whereas others have found no relationship between mosquito size and longevity (Landry et al.,1988; Mori, 1979). Nasci (1990) stated that large body size leads to increased survival and blood-feeding success and that this success has been identified in four different mosquito genera; Aedes, Anopheles, Culex and Psorophora.

In the present study, larval and adult sizes were compared. Adult size was monitored throughout the season to determine its association with longevity.

3.1.3 HUMAN-BAIT CATCHES

Seasonal incidence of adults was ascertained by performing human-bait catches. These have been used by Service (1977a) on Ae. cantans mosquitoes in Monk's Wood, Huntingdon and also by Sulaiman (1982) in Ness Woods. The advantages of human-bait catches are that there is little sampling bias, they are easy to perform and require very basic equipment. However, in studying the seasonal changes in population size, the attractiveness of man as a human-bait must not change over the sampling period. Such a change could result from fluctuations in the population size or availability of alternative hosts or from physiological or behavioural changes in the mosquitoes themselves (Service, 1976). Bait catches must be carried out at regular time of day and sites so that there can be comparisons throughout the season. Wind and rain will reduce the number of mosquitoes caught at bait and therefore it is important to record weather conditions prevailing during a bait catch.

Service (1969, 1971c, 1977a) found that large numbers of British mosquitoes were caught in sheltered sites where unfed females were resting during the day. Sulaiman (1982) carried out standardised human-bait catches during the day and felt that this was sufficient to measure variations in seasonal biting rates and differences between species numbers and composition. However, in this study, both afternoon and evening bait catches were carried out, so that both the mosquitoes resting in the woods during the day and those actively host-seeking in the evenings were sampled.

3.1.4 SWEEP-NETTING

Adult mosquitoes probably spend more time resting in vegetation than in flight (Service, 1976), and therefore sweep-netting of vegetation was undertaken to obtain samples of males, as well as unfed, blood-fed, half-gravid and gravid females. The age structure of mosquitoes caught in sweep-netting vegetation is more representative of the whole mosquito population than those caught at bait.

The hosts of blood-fed mosquitoes can be analysed using a variety of methods; in this study, ELISA tests were used (Chapter 4).

3.1.5 PARITY DISSECTIONS

The age of a mosquito is of particular importance in disease transmission. An aged population potentially contains more individuals that have had the opportunity to become infected and infective with a transmittable pathogen. Although disease transmission is not of concern in this study, it is still useful to be able to age a mosquito because physiology and behaviour may change with age. The most widely used techniques for aging mosquitoes are based on ovarian changes.

The simplest method of ovary dissection is based on the observations of Detinova (1962). She observed that the terminations of the tracheoles covering the ovaries are tightly coiled into skeins in unfed females which have never matured any eggs, that is, nulliparous ones. When the ovaries develop in a nulliparous mosquito, these skeins are stretched and become uncoiled; this is an irreversible change and after the mosquito has laid eggs the tracheoles remain uncoiled, and this denotes a parous female. Therefore, by using this technique, the mosquitoes can be classified as nulliparous, they have laid no eggs and have coiled skeins, or parous, they have laid one or more sets of eggs and have uncoiled tracheoles. This technique has been used more extensively than any other to age-grade a large variety of different mosquito species. Gavin (1986), Sulaiman (1982) and Service (1977a) have all used this technique to age-grade Ae. cantans with little difficulty.

Another, and much more difficult method for age-grading adults by means of ovary dissections is known as Polovodova's method (Detinova, 1962; Polovodova, 1949). Polovodova stated that when an egg passes from an ovariole into the oviduct it leaves a small sac-like dilatation at the site in the pedicel where the egg was originally formed. If additional eggs are laid, each one will leave a dilatation anterior to the dilatation of the previous egg. Thus, a dilatation represents each oviposition cycle and it is possible to determine how many sets of eggs have been laid. Hoc and Charlwood (1990), however, have argued that this is not true, and that a dilatation represents a degenerated egg, and that in any maturation of eggs some will always degenerate. However, this is a contentious issue and will be referred to later. Clements (1963) stated that the degeneration of a certain number of egg follicles in each gonotrophic cycle seems to be a normal occurrence in mosquitoes. The Polovodova method has been used on a variety of different species, including the British mosquito, Ae. punctor (Packer and Corbet, 1989b).

3.1.6 OVIPOSITION

In a wide range of insects, fecundity of the female is proportional to her weight (Southwood, 1978). Several authors have observed that larger adults lay larger egg clutches (Bock and Milby, 1981; Hawley, 1985b; Washburn et al, 1989). Some studies have found that smaller adult mosquitoes lay smaller eggs (Reisen, 1975), although Shannon and Hadjinicalao (1941) found that the size of the egg does not vary with the size of the female. Clements (1963) stated that in many mosquito species the number of eggs laid in each batch decreases with successive gonotrophic cycles. Bowen (1991) found that the threshold blood volume for distension inhibition is lower in chronologically old female mosquitoes.

3.1.7 WEATHER CONDITIONS

Weather can have a considerable effect on the biology and abundance of mosquitoes (Service, 1978). Temperature, wind speed and humidity are the most important factors affecting the activities of adult mosquitoes.

Perhaps the most important environmental parameter affecting population size and distribution of poikilothermic animals, including insects, is temperature. As temperatures increase, the rate of blood-digestion and egg development increases to a maximum and then decreases at still higher temperatures. The gonotrophic cycle of *Cs. annulata* is five to six days in summer but can be as long as 23 to 27 days in winter (Service, 1968*a*). Similarly, Service (1977*a*) found that in the laboratory, the length of the gonotrophic cycle of *Ae. cantans* decreased with increasing temperature. Therefore, with warmer temperatures, the greater the number of gonotrophic cycles an adult can complete in a season, and the greater the number of eggs laid.

Ambient temperature can also influence swarming, host-seeking and blood-feeding behaviours (Wright and Knight, 1966). Many insects have an individual temperature threshold (Bidlingmayer, 1985) and temperatures above and below these thresholds inhibit activity. For example, Corbet and Danks (1973) found that the flight of *Aedes nigripes* (Zetterstedt) and *Aedes impiger* (Walker) in Canada was inhibited by air temperatures below 5°C.

Wind speed influences the velocity and direction of insect dispersal. Several investigators have found *Ae. detritus* in Ness Woods (Majala, 1987; Sulaiman, 1982), which is some 1.5 km from the nearest possible saline larval habitats at Parkgate. Wind can also depress insect flight, and the biting activity of mosquitoes can be suppressed by windy weather. Bidlingmayer (1974) and Bidlingmayer *et al*. (1985) reported a decrease in the number of mosquitoes caught in suction traps on windy days, with an 8-23% decrease in the number of *Cx. nigripalpus* caught with each 0.1 mps increase in wind velocity. In central Alaska, Gjullin *et al*. (1961) found that winds of more than three km per hour reduced mosquito flight and at eight km per hour, flight ceased altogether. Wright and Knight (1966) found that winds over two miles per hour depressed both flight and biting activity in *Ae. vexans*.

Swarming in many mosquitoes, including *Ae. cantans*, will occur only at specific light intensities corresponding to dawn and dusk (Neilsen and Greve, 1950). Host-light intensities corresponding to dawn and dusk (Neilsen and Greve, 1950). Host-seeking in many species is also associated with particular light intensities. Service (1977a) found that Ae. cantans is an eocrepuscular species whose flight activity peaked at dawn and dusk. Flight activity of Ae. vexans and Ae. trivittatus can be shortened, delayed or postponed by manipulating light intensities in the laboratory or by abnormal conditions in the field such as extra cloud cover (Wright and Knight, 1966). Host-seeking by Ae. punctor is influenced by light intensity, with flight-activity being greatest when light intensity is within the range 495-1000 Lux (Taylor et al., 1979). Service (1977a) found that the numbers of Ae. cantans in suction traps placed at the edge of a wood in southern England was related to light intensity.

Heavy rainfall suppresses mosquito flight but adults can fly in lighter showers. Generally, rainfall is more important in the larval stages (Chapter 2).

It is difficult to study the effect of different weather conditions on mosquito populations in isolation from one another. In this study, certain weather conditions were recorded at Ness Gardens, 1.5 km from Ness Woods. In addition, a squirrel data-logger was used in Ness Woods to record water temperature, air temperature and humidity and attempts were made to relate these factors to mosquito behaviour.

3.1.8. COMPETITION

Availability of blood-meals and oviposition sites are very important for adult mosquitoes. If these are in short supply, individuals will have to compete for the limited resource and therefore the reproductive rate and survival of some individuals will be reduced. When the population size increases, the level of competition for these resources becomes more marked, density-dependent competition comes into effect and its influence on reproductive capacity can be important.

3.1.9 PARASITES

Adult mosquitoes can be parasitised by mites (Smith, 1988). The majority of mites parasitising mosquitoes are aquatic (Hydrachnellae) and belong to two families, Thyasidae and Arrenuridae. The four terrestrial mite families, Erythraeidae, Johnstonianidae, Trombellidae and Trombididae, also include species known to parasitise mosquitoes (Mullen, 1975). The presence of live aquatic mites on female mosquitoes are often a good indicator of nulliparity (Corbet, 1970). Some investigators have also found that mites can significantly reduce fecundity and possibly longevity, whereas, others have found that mites cause little or no harmful effects (Mullen, 1975). The presence of mites on Ae. cantans was therefore recorded in the present study.

3.2 METHODS AND MATERIALS

3.2.1 EMERGENCE

Emerging mosquitoes were sampled in all three years by placing double bednets over the ponds in April, as soon as pupae appeared, and sampling continued until the ponds had dried up. In 1989, double bednets (1.2 x 1.2 x 1.2m) were placed over the Hotspot and Pond 9. In 1990, double bednets were placed over the Hotspot, and ponds 9, 13, and 8 in Wood A. In 1991, the Hotspot and Pond 9 dried up more than a month before emergence normally takes place and so bednets were placed only over Ponds 8 and 13. In fact, desiccation was a major problem in all three years. In the Hotspot, in both 1989 and 1990, pupae were placed in buckets under the bednets as the pond began to dry up before emergence could begin. In addition, in 1990, water was pumped from more permanent ponds into the Hotspot and Ponds 8 and 9 because lack of rain was endangering the success of emergence (Chapter 2).

Mosquitoes were removed daily from the nets using an aspirator, counted, sexed and identified and then placed in cages. Five to ten percent were taken back to the laboratory for dissection and measurement, whilst others were painted for mark-release-recapture work (Chapter 5). All potential predators of adults, such as spiders, were removed from the nets.

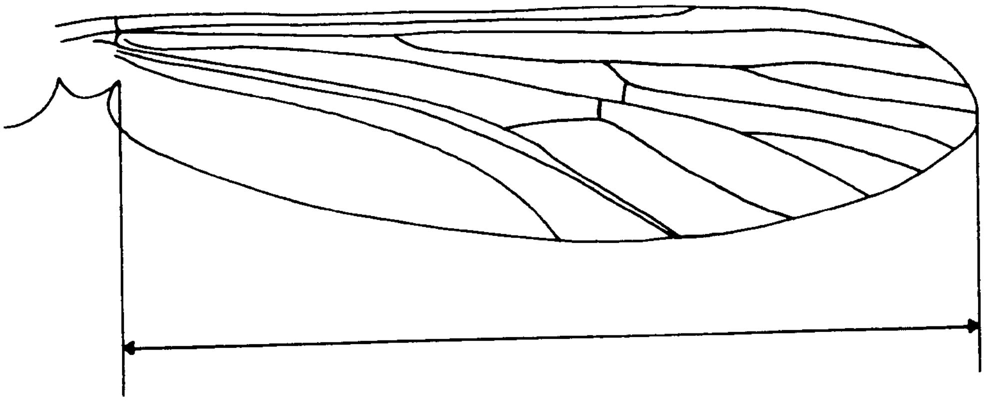
The ovaries of newly emerged Ae. cantans were dissected and their stage of development, according to Clements (1963), noted. Other mosquitoes that emerged at the same time were placed in cages and maintained on saturated sugar solution or water. Samples of these mosquitoes were dissected periodically over the next three weeks, and the ovarian stages recorded (The ovarian stage of mosquitoes when they first appeared at bait was also examined.). The longevity of mosquitoes in cages supplied with either sugar solution or water was also recorded.

The spermathecae of newly emerged mosquitoes that had been kept in one-foot square cages were examined for evidence of mating. Human blood-meals were regularly offered to the caged Ae. cantans from emergence onwards.

3.2.2 WING MEASUREMENTS

Wing measurements were used in this study to represent mosquito size. Figure 3.1a shows how the wing length was measured. After mosquitoes had been killed with chloroform, their wings were removed using fine forceps and were mounted on a glass slide in a drop of dilute salt solution. This kept the wings stuck to the slides. Mounted wings were measured to the nearest 0.01 mm using a binocular microscope with an eyepiece graticule. The microscope was recalibrated each time before use. Wing length was measured from the alular notch to the distal margin excluding the fringe scales. In 1989 only, the wing width was also measured, between the costa and

Fig. 3.1a: Wing of Ae. cantans showing the dimensions measured.



Approximate wing length = 5mm.

the fifth and sixth vein. Wing lengths of newly emerged mosquitoes, and those caught at human-bait and by sweep-netting vegetation were measured.

3.2.3 HUMAN BAIT CATCHES

The seasonal incidence of Ae. cantans was derived from human-bait catches, a procedure used by many authors to sample mosquito populations (Service, 1976). All such collections in this study were carried out by a single person only, usually myself, and most were stationary.

Bait catches lasted for one hour. I sat on the ground, usually leaning against a tree with my legs out-stretched. Any mosquito that landed on me was collected using either an aspirator or test-tubes, usually before the mosquito inserted its proboscis. Captured mosquitoes were identified at bait and were then placed in cages. Moving bait catches, which involved walking slowly through vegetation and periodically stopping to collect mosquitoes, were carried out from time to time when mosquitoes were needed in large numbers, usually for mark-release-recapture work (Chapter 5).

3.2.3.1 BAIT CATCH SITES AND TIMES

Bait catches were carried out daily at several different sites and times from April to September in 1989 and 1990, and occasionally in 1991. Both evening and afternoon bait catch sites were chosen. The times of day used in this investigation are GMT. Throughout this study, the times were not standardised to bear a constant relationship to sunset and therefore to the prevailing light intensity (e.g. Packer and Corbet, 1989a). This was because there was such a large variation in the number of mosquitoes caught at bait from day to day that it was decided that the differences in the sunset times as the year progressed would have little appreciable effect on the numbers of mosquitoes caught at bait in comparison to the effects of weather conditions on numbers caught.

1.Main Morning-Afternoon Bait Catches

i Wood B 1100-1200 hr.

ii Wood A Hotspot 1300-1400 hr.

iii Wood A Pond 9 1400-1500 hr.

2.Main Evening Bait Catches

i Wood A Pond 9 1730-1830 hr.

ii Wood A Hotspot 1830-1930 hr, 1930-2030 hr.

iii Leahurst Sheep Field (LSF) Moving catch 2030-2130 hr.

Bait catches were also carried out in fields 2-13 and the 4-udder sheep field (4USF) occasionally throughout the season to monitor movement of mosquitoes in other regions, and at different times (Fig 1.2). After identification, Ae. cantans caught in human-bait catches were checked for ectoparasites, and then either released in mark-release-recapture experiments (Chapter 5), or dissected and measured, or used in oviposition experiments.

In 1991, air temperatures were recorded at the site of the bait catches using the Squirrel Data Logger. The temperature probe was shaded from sunlight and suspended approximately 50 cm above ground level.

3.2.4 PARITY DISSECTIONS

Mosquitoes were age-graded in this study by means of ovary dissection techniques. The first and most simple method used was that of Detinova (1962), namely a dissection for nulliparity or parity. The ovaries were dissected in distilled water, by snipping the end of the abdomen and extracting the two ovaries, which were then placed on a glass slide and allowed to dry. Just before the ovaries were examined, the slides were squirted with distilled water and then shaken dry, making identification of parity easier. The ovaries were then examined under a stereo-microscope at 50X magnification. Each ovary was matched with a corresponding wing measurement from the same adult. Occasionally, towards the end of the season, some of the ovaries became cloudy in appearance and it was difficult to identify their parity state. This has also been observed in Ae. cantans in Ness Woods by Gavin (1986).

The second and more difficult method for age-grading adults, namely by counting the ovariole dilatations (Detinova, 1962; Polovodova, 1949), was used in 1990. This dissection is difficult and so initially the oil-injection technique perfected by Hoc and Charlwood (1990) on Ae. cantans collected in Ness Woods was attempted. This involved injecting light paraffin oil into the common oviduct to inflate the ovarioles. However, I found this particular technique extremely difficult because the oil was too viscous to fill a tube that was sufficiently thin to insert into the common oviduct. This method was therefore abandoned. The Polovodova dissection is particularly time-consuming. Firstly, the ovarian sheath is removed and then the ovarioles in the ovaries are teased out in saline using fine needles under a dissecting microscope at high magnification, so that the dilatations can be seen. Identification of one- and two-parous mosquitoes proved relatively easy, but it was too difficult to obtain results for the higher parities, because, in agreement with Hoc and Charlwood (1990), the number of ovarioles that act as reliable indicators of parity decreases with age, to less than five percent by the three-parous stage. This meant that all the ovarioles in the ovary had to be examined in order to obtain accurate results, since just one ovariole in the entire ovary may have given the true dilatation count. Only a few mosquitoes were dissected beyond the two-parous stage. Most Ae. cantans were identified as parous or nulliparous by the tracheolar method only.

3.2.5 SWEEP-NETTING VEGETATION

Resting mosquitoes were collected by sweep-netting the vegetation in all three woods and the surrounding fields once to twice weekly in the summer of 1989 and 1990. Blood-fed mosquitoes were squashed on filter paper and their blood source was analysed using the ELISA test (Chapter 4). Other sweep-netted mosquitoes were identified and sexed, and whenever possible females were dissected for parity and their reproductive condition noted. They were checked for parasites and for paint marks or coloured powders and some were used for wing measurements.

A 'univac' petrol-operated mechanical suction sampler was also used as an alternative for sweep-netting vegetation to collect resting mosquitoes, but this technique was unsatisfactory mainly because mosquitoes were heavily damaged in the process.

3.2.6 OVIPOSITION AND EGG LAYING

Oviposition experiments were set up for several reasons. Firstly, they provided an important supply of eggs for larval work. Secondly, they enabled the effect of adult size on clutch and egg size to be investigated, and also, the effect of parity on the number of eggs laid to be examined.

Captured mosquitoes were blood-fed, usually on Prof. M.W. Service, but ocassionally on mice. They were then left in one foot square cages until they were three-quarters gravid, to reduce mortality, after which they were placed in oviposition tubes. These consisted of 3 X 1 inch tubes, lined with damp filter paper, and with leaf litter placed on damp cotton wool at the bottom of the tube. Mosquitoes were carefully placed in the tubes using an aspirator and the tube was then covered with a nylon mesh, and the mosquitoes left to lay eggs. All the adults died within two days of oviposition. The eggs were counted, the adults dissected for retained eggs, and the wings measured. Occasionally the eggs were measured from anterior to posterior tips. Lids were placed on the tubes and they were placed outdoors in the shade under natural weather conditions but kept humid, until the following winter.

Blood-feeding experiments were also undertaken in 1991 to see whether very small Ae. cantans mosquitoes require more than one blood-meal to mature their eggs. This phenomenon has been noted in other species of mosquito (Feinsod and Spielman, 1980; Service, 1977a).

3.3 RESULTS

3.3.1 EMERGENCE

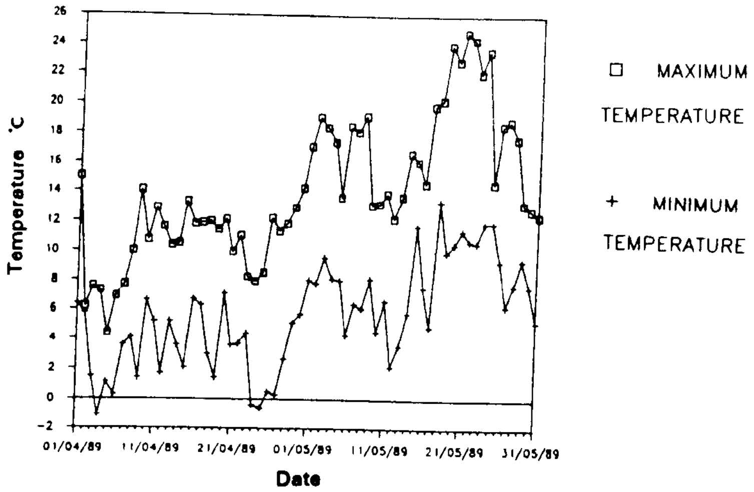
Figure 3.1 shows the air-temperature data in 1989, 1990 and 1991 for the period of adult emergence, recorded at Ness Gardens.

3.3.1.1 PATTERN OF EMERGENCE

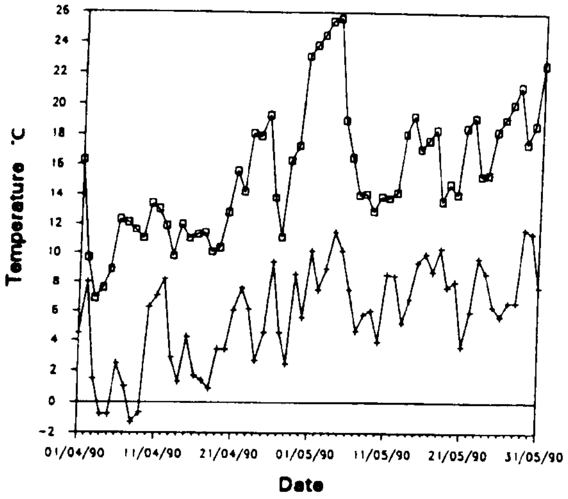
Figure 3.2 shows the emergence of Ae. cantans from the Hotspot and Pond 9 in 1989, while Fig.3.3 shows the percentage cumulative emergence from both habitats. In 1989, fewer mosquitoes emerged from the Hotspot (n=132) than Pond 9 (n=1,010). The Hotspot dried up early in 1989 before emergence could be completed, and this is the probable reason for the smaller number (Chapter 2). The plateau (from the 10 May-20 May) in the Pond 9 cumulative graph (Fig. 3.3) corresponds to a period of

Fig. 3.1: Air temperature for emergence period.

(a) 1989



(b) 1990



(c) 1991

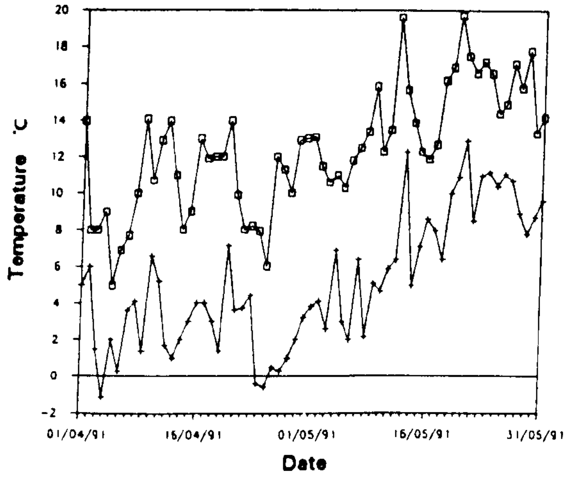
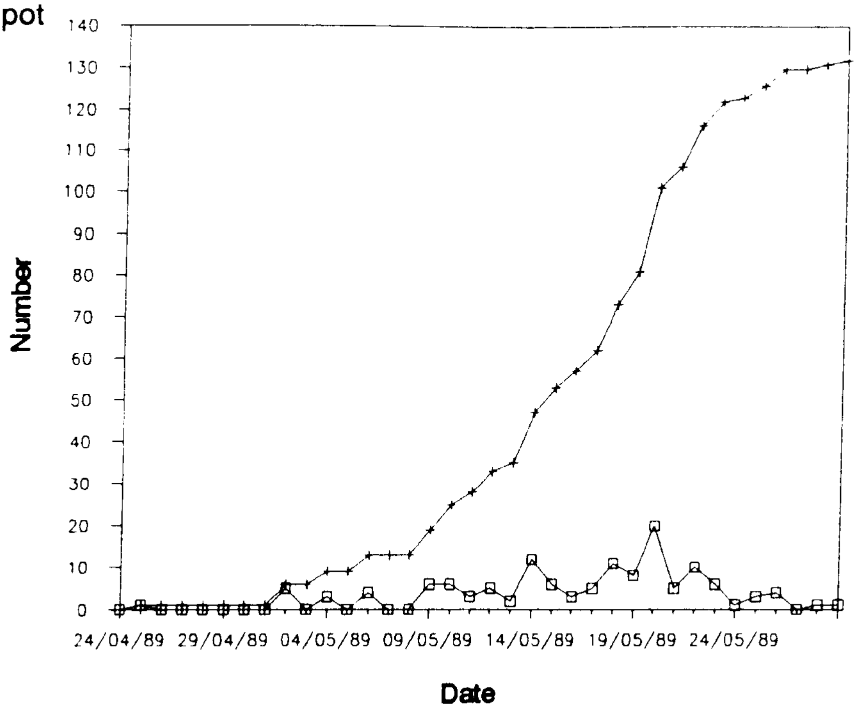


Fig. 3.2: Aedes cantans daily and cumulative emergence, 1989, males and females.

(a) Hotspot



(b) Pond 9

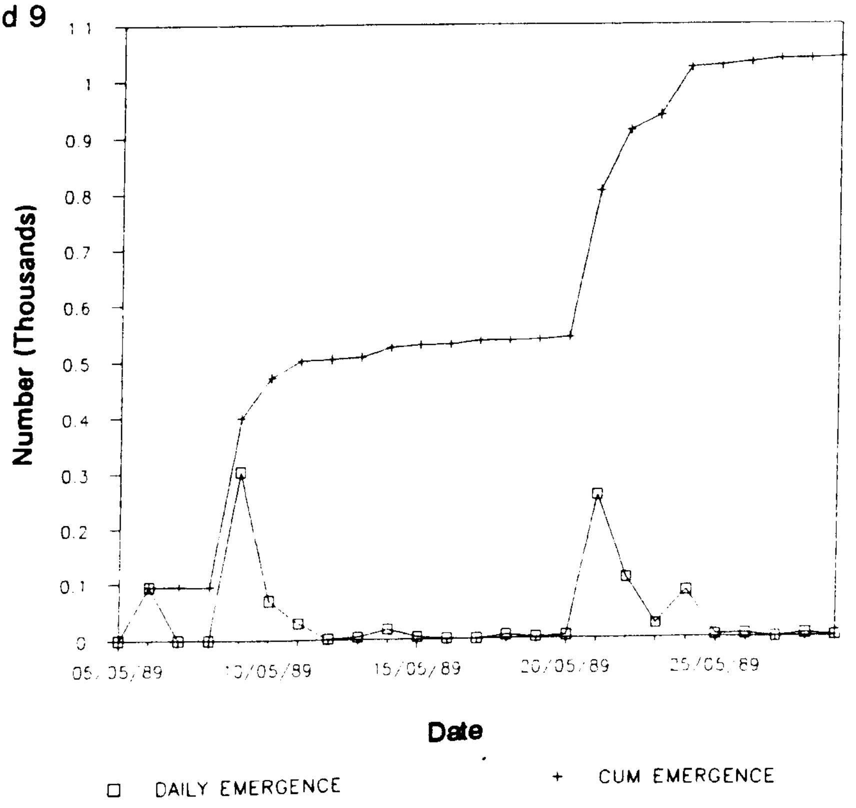
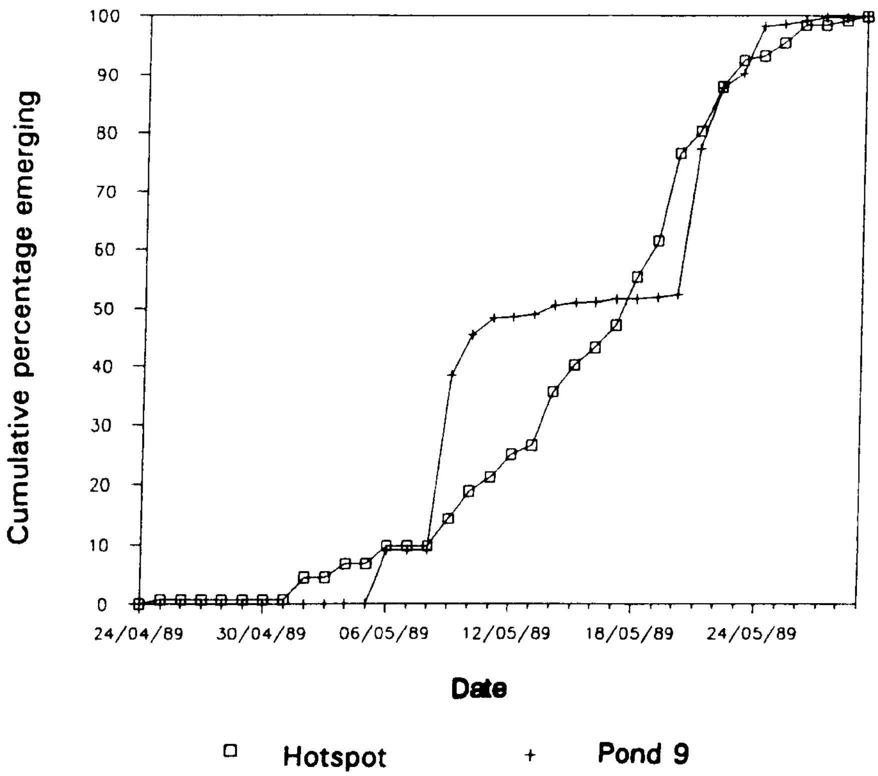


Fig. 3.3: Aedes cantans cumulative percentage emerging, 1989.



cooler temperature, when development of Ae. cantans would have been slowed down (Service, 1978).

Figure 3.4 shows the emergence of Ae. cantans from the Hotspot, Ponds 9, 8 and 13 in 1990, while Fig. 3.5 shows the percentage cumulative emergence for all four ponds in the same year.

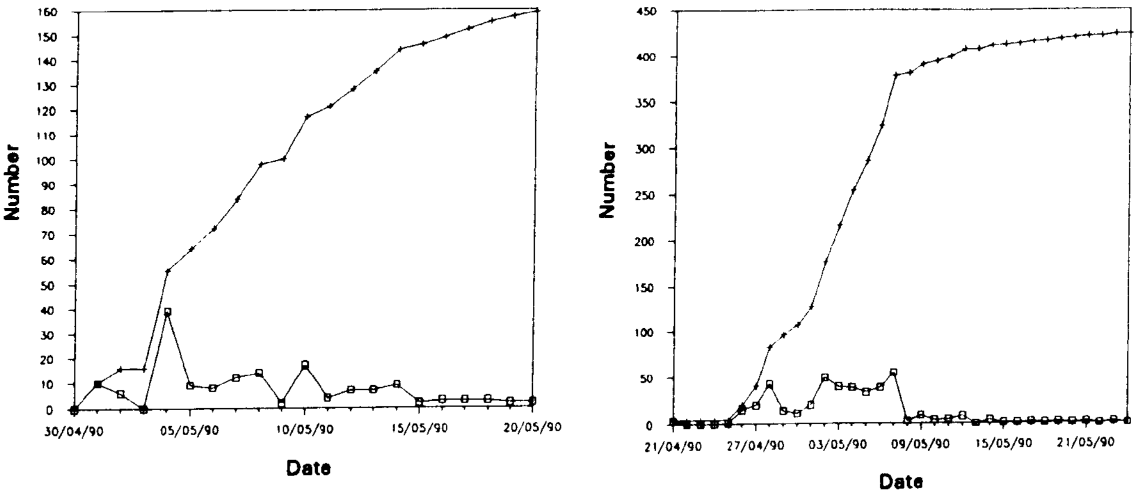
In 1991, both the Hotspot and Pond 9 dried up before emergence could begin (Chapter 2), so emergence patterns were studied in Ponds 8 and 13 (Fig. 3.6). Figure 3.7 shows the percentage cumulative emergence in these two ponds.

Table 3.1 shows the percentage (0, 10, 50, 100) of emergence (EM) of mosquitoes in all years at all sites. Comparison of the EM_{50} of the different ponds shows how emergence times can vary. For example, in 1989, the EM_{50} in the Hotspot was four days earlier than in Pond 9. In 1990, the EM_{50} of Pond 13 was one day earlier than Pond 9, five days earlier than Pond 8 and eight days earlier than in the Hotspot. In 1991, emergence was later than in the previous two years because the onset of the larval season had been delayed by low rainfall levels (Chapter 2). The EM_{50} of Pond 8 was three days earlier than in Pond 13. Overall, emergence was fairly rapid with a maximum of 36 days from the first to the last emergence in 1989, 34 days in 1990 and 24 days in 1991, in all the ponds over which nets were placed.

The EM_{50} of female and male Ae. cantans shows that males tend to emerge sooner (Table 3.1). In 1989, the EM_{50} of males was eight days earlier than females in Pond 9 and two days earlier in the Hotspot. In 1990, the EM_{50} of males was three days earlier in Pond 9, one day earlier in the Hotspot and Pond 8, but one day later in Pond 13. In 1991, the EM_{50} of males was three days earlier in Pond 8 and one day earlier in Pond 13. The overall pattern is for males to emerge earlier than females, with a mean difference of 2.38 ± 0.93 days.

Fig. 3.4: Aedes cantans daily and cumulative emergence, 1990. Males and females.

(a) Hotspot (b) Pond 9



(c) Pond 13 (d) Pond 8

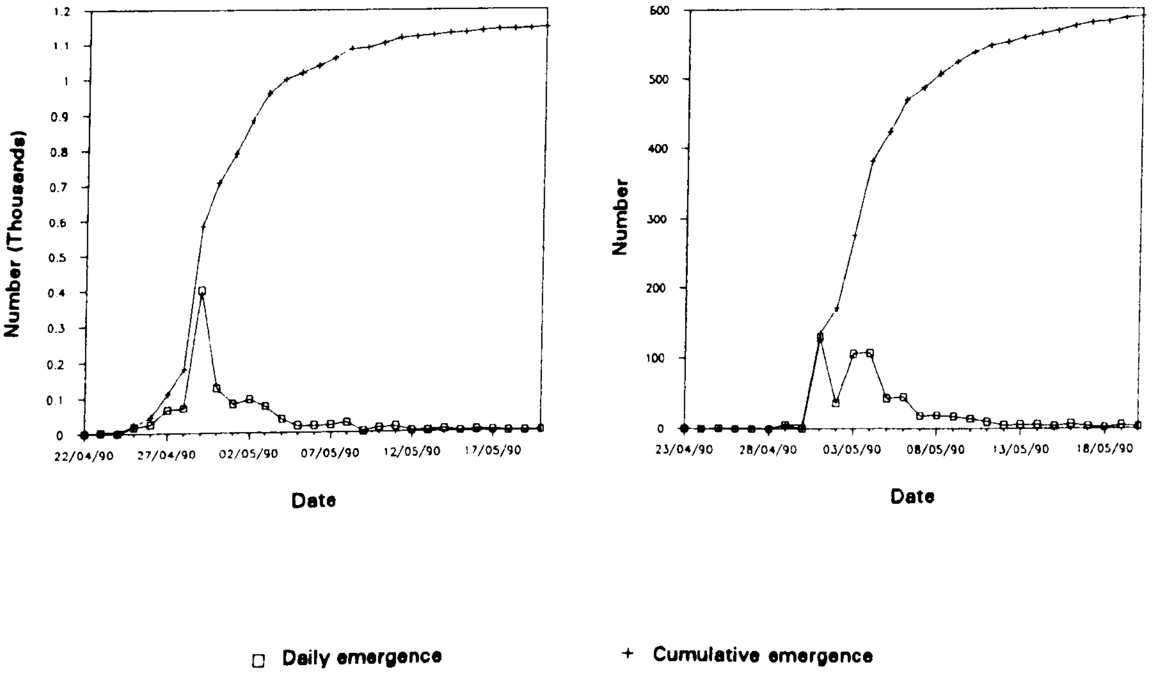


Fig. 3.5: Aedes cantans cumulative percentage emerging, 1990.

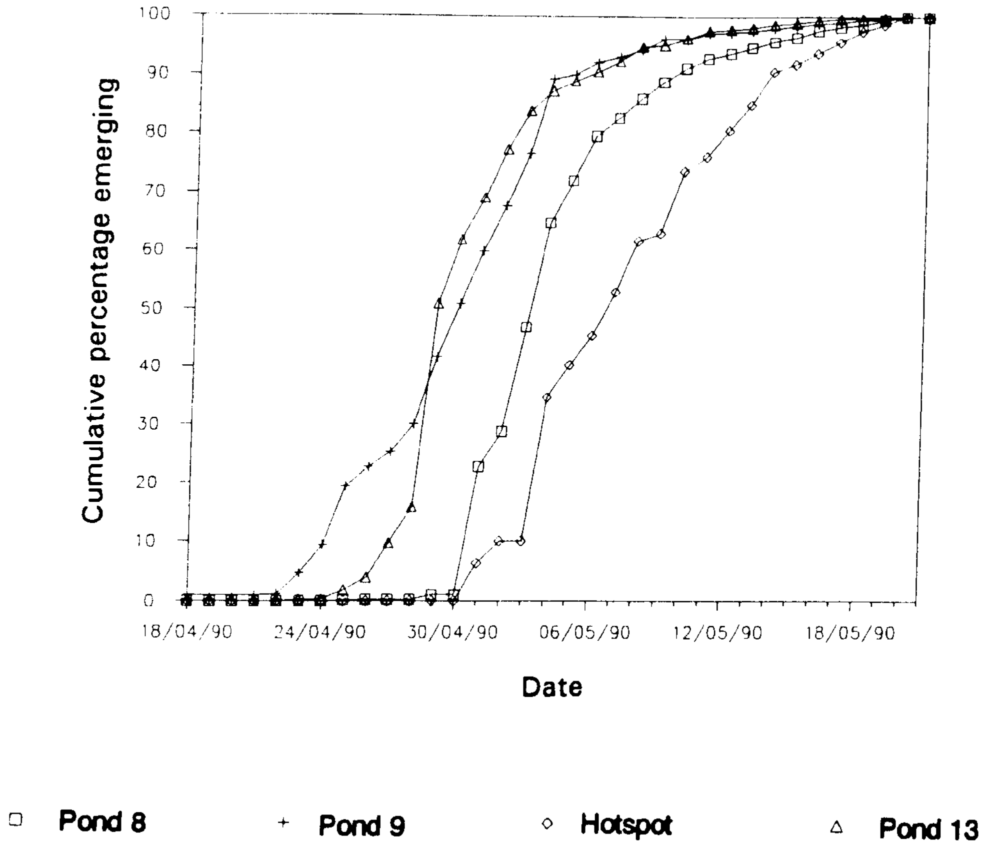
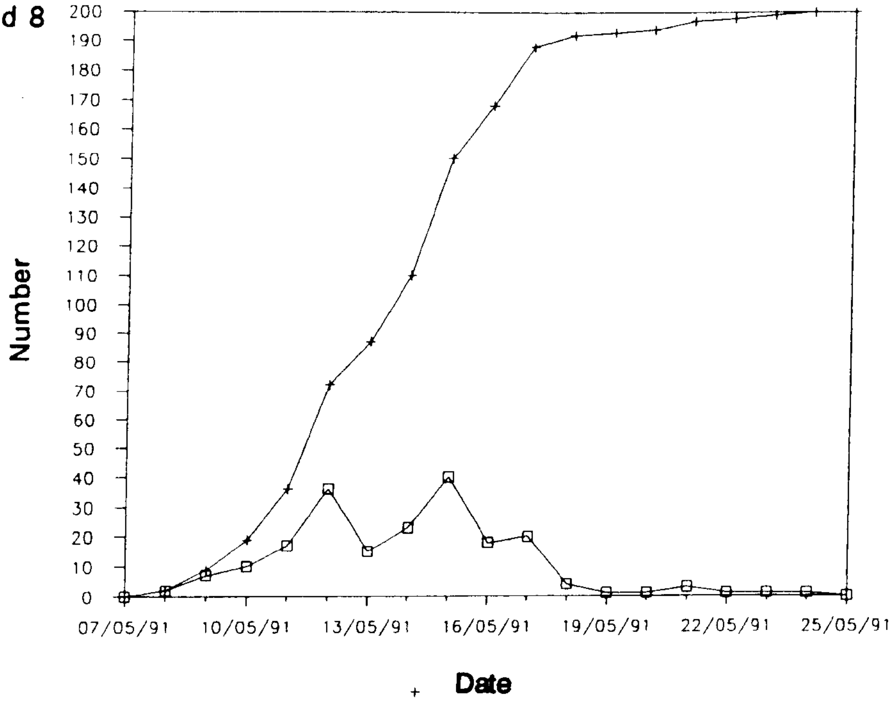


Fig. 3.6: Aedes cantans daily and cumulative emergence, 1991. Males and females.

(a) Pond 8



(b) Pond 13

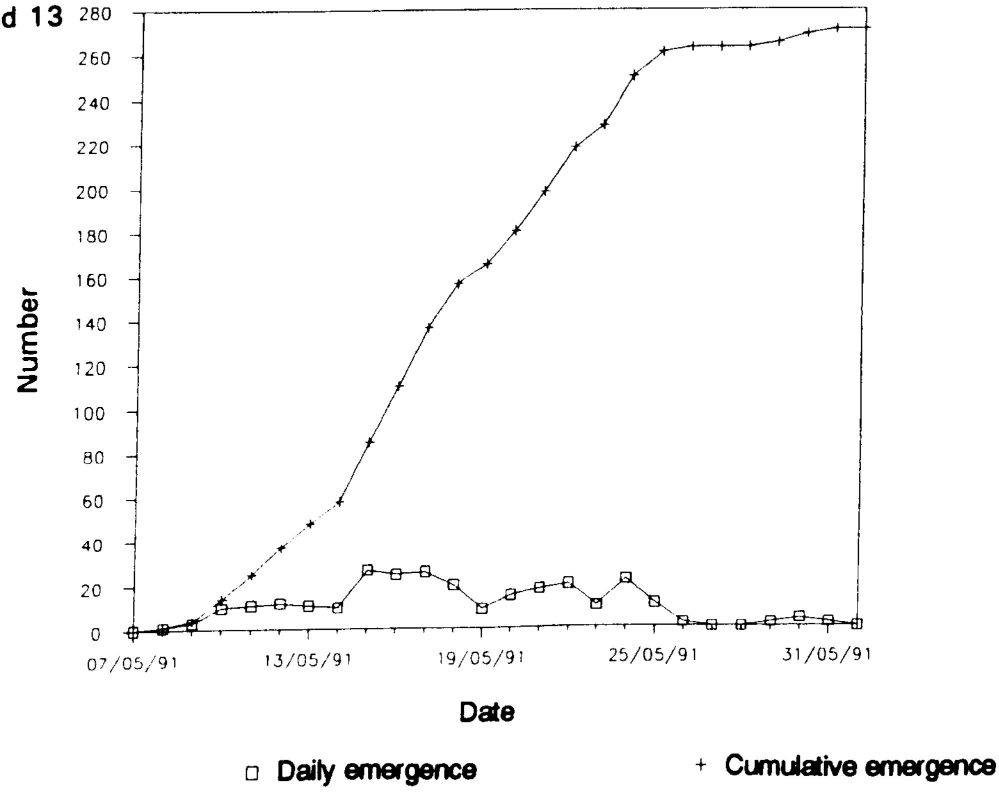


Fig. 3.7: Aedes cantans cumulative percentage emerging, 1991.

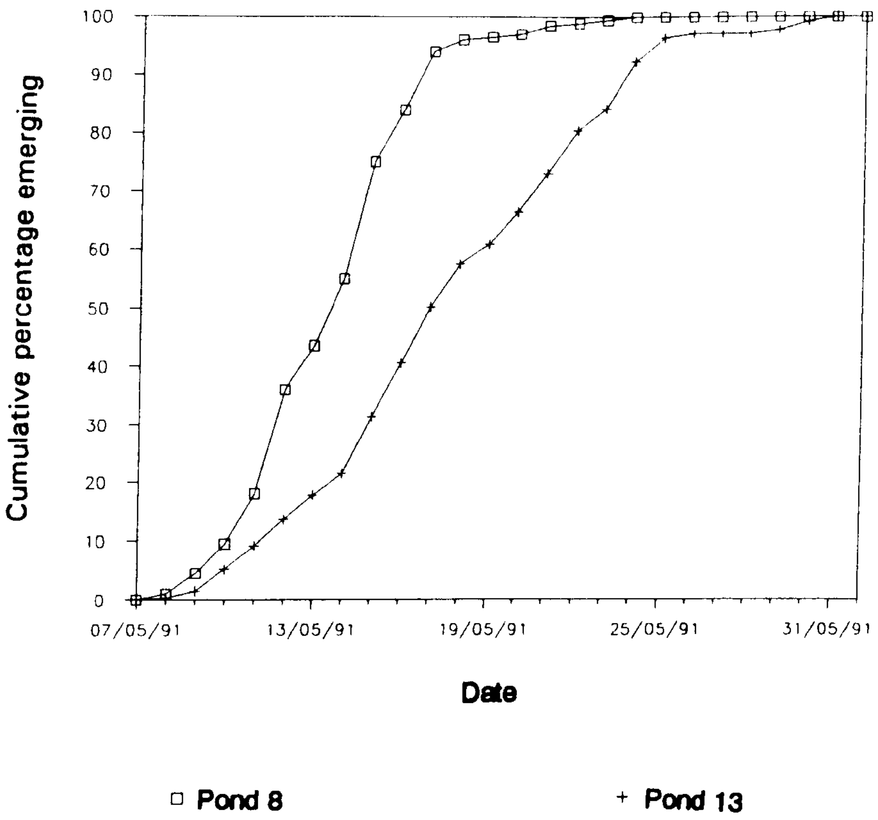


Table 3.1 : Percentage emergence dates 1989, 1990 and 1991.

Year + Site	EM_0	EM_{10}	EM_{50}	EM_{90}	EM_{100}
1989					
Pond 9	05.05	08.05	14.05	23.05	30.05
Hotspot	24.04	06.05	18.05	23.05	29.05
Males					
Pond 9	05.05	06.05	10.05	21.05	30.05
Hotspot	24.04	09.05	16.05	22.05	26.05
Females					
Pond 9	05.05	09.05	18.05	24.05	29.05
Hotspot	01.05	04.05	18.05	25.05	29.05
1990					
Pond 9	17.04	25.04	30.04	05.05	20.05
Hotspot	30.04	04.05	08.05	17.05	20.05
Pond 13	22.04	27.04	29.04	03.05	17.05
Pond 8	24.04	01.05	03.05	08.05	16.05
Males					
Pond 9	17.04	24.04	29.04	04.05	20.05
Hotspot	30.04	04.05	08.05	17.05	20.05
Pond 13	22.04	27.04	29.04	03.05	17.05
Pond 8	24.04	01.05	03.05	08.05	16.05
Females					
Pond 9	17.04	26.04	02.05	08.05	20.05
Hotspot	30.04	01.05	07.05	10.05	14.05
Pond 13	24.04	29.04	01.05	08.05	20.05
Pond 8	30.04	03.05	04.05	14.05	20.05
1991					
Pond 13	07.05	11.05	17.05	24.05	31.05
Pond 8	07.05	10.05	14.05	17.05	24.05
Males					
Pond 13	07.05	11.05	17.05	24.05	31.05
Pond 8	07.05	09.05	12.05	16.05	21.05
Females					
Pond 13	09.05	14.05	18.05	24.05	31.05
Pond 8	09.05	12.05	15.05	17.05	24.05

EM_{10-90} represents 80% of emergence. Emergence was briefest in 1991, with EM_{10-90} covering seven days in Pond 8 and 13 days in Pond 13. In 1990, EM_{10-90} covered 11 days in Pond 9, 13 days in the Hotspot, nine days in Pond 13 and ten days in Pond 8. In 1989, it was 16 days in Pond 9 and 18 days in the Hotspot. It is clear from these results that the main proportion of emergence is rapid, taking just 9-18 days.

Table 3.2 shows the numbers and sex ratio of males and females emerging from the ponds in all years. In 1989, there was no significant departure from an equal sex ratio in the Hotspot or Pond 9. Similarly, in 1990, Pond 9, the Hotspot and Pond 8 showed no significant departure from a 50:50 sex ratio, but in Pond 13 significantly more males emerged than females (χ^2 = 90.66, $P<0.05$). In 1991, there was no significant departure from an equal sex ratio in either Pond 8 or Pond 13.

Newly emerged male Ae. cantans had incomplete rotation of the terminalia, and it took two days for full rotation to be completed in the field.

The ovarian stage in females at emergence was Christopher's Stage I. The stages of development of females maintained on saturated sugar solution following emergence for up to four weeks are shown in Table 3.3. It is clear from this experiment that it takes between 8-15 days for the ovaries of newly emerged mosquitoes to reach Stage II when placed in cages in the field in late May (represented by the medium temperature scale), and maintained on sugar solution. These results compare well with the ovarian stages of females emerging in the field and those caught at bait. Females maintained on only water did not live for more than 15 days, and their ovaries never developed past Stage Ib. Females would not feed on human-hosts until their ovaries were in Stage IIb of development. These results on

Table 3.2 : Sex Ratio of emerging *Ae. cantans*.

Year + Site	M	F	Ratio Male:Female	Chi squared	P
1989					
Pond 9	532	508	51.2:48.9	0.3	P>0.05
Hotspot	73	59	55.3:44.7	0.7	P>0.05
1990					
Pond 9	252	223	53.1:46.9	0.9	P>0.05
Hotspot	96	63	60.4:39.6	3.5	P>0.05
Pond 13	857	390	68.9:31.1	90.7	P<0.05 **
Pond 8	307	282	52.1:47.9	0.5	P>0.05
1991					
Pond 13	132	138	48.9:51.1	0.07	P>0.05
Pond 8	95	105	46.0:54.0	0.06	P>0.05

M = Number of males F = Number of females P = Probability

Table 3.3 : Ovarian development of female *Aedes cantans* maintained on saturated sugar solution from emergence to blood-feeding.

Temp.	Days from Emergence	Ovarian Ia	Ib	Stage IIa	IIb	III
Low (April)	1	1	9	0	0	0
	5	0	10	0	0	0
	8	0	10	0	0	0
	10	0	10	0	0	0
	15	0	8	2	0	0
	20	0	1	8	1	0
	25	0	0	5	5	0
Medium (May)	1	0	10	0	0	0
	5	0	10	0	0	0
	8	0	9	1	0	0
	10	0	6	4	0	0
	15	0	0	9	1	0
	20	0	0	4	6	0
High (June)	1	0	10	0	0	0
	5	0	10	0	0	0
	8	0	6	4	0	0
	10	0	3	7	0	0
	15	0	1	6	3	0
	20	0	0	2	8	0

ovarian development and the time when females will feed compare well with field data. For example, in 1989, it took a minimum of eight days and a mean of 16.8 days for a marked mosquito painted at emergence to be caught at bait. Similarly, in 1990, there was a minimum of four days and a mean of 18 days between emergence and blood-feeding (Chapter 5). There were 18 days in 1989 between the first female caught in emergence traps to the capture of the first female at human-bait, but in 1990 the interval was just nine days.

Females kept in one-foot square cages with males in the field following emergence for 20 days were not inseminated, whereas females were inseminated after five days when they were kept in bednets (1.2m x 1.2m x 0.6m) in the field.

3.3.1.2 SIZE AT EMERGENCE

Figure 3.8 shows the size, as represented by wing length, of mosquitoes emerging from the Hotspot and Pond 9 in 1989. A Mann-Whitney test comparing the wing sizes of the mosquitoes emerging from the Hotspot and Pond 9 shows clearly that Pond 9 mosquitoes are significantly larger ($P<0.001$) (Table 3.4). The wing length distribution of adults emerging from the Hotspot shows a bimodal peak. The larger mosquitoes emerging are the ones which hatched as larvae before the main hatch in March and since they developed under uncrowded conditions, were larger than the majority of the adults which had been subject to overcrowding as first instars. There was no significant difference between the wing length of male and female mosquitoes emerging from the same sites and therefore the data are combined (Table 3.5).

Figure 3.9 shows the size of Ae. cantans emerging in 1990 from the Hotspot and Pond 9, and, Ponds 8 and 13. There was no significant difference between the size of Ae. cantans emerging from the Hotspot and Pond 9 ($t = 1.8$, 123 degrees of freedom, $P>0.05$) (Table 3.4), neither was there any significant difference between the

Fig. 3.8: Wing length distribution of *Aedes cantans* males and females at emergence, from the Hotspot and Pond 9, 1989.

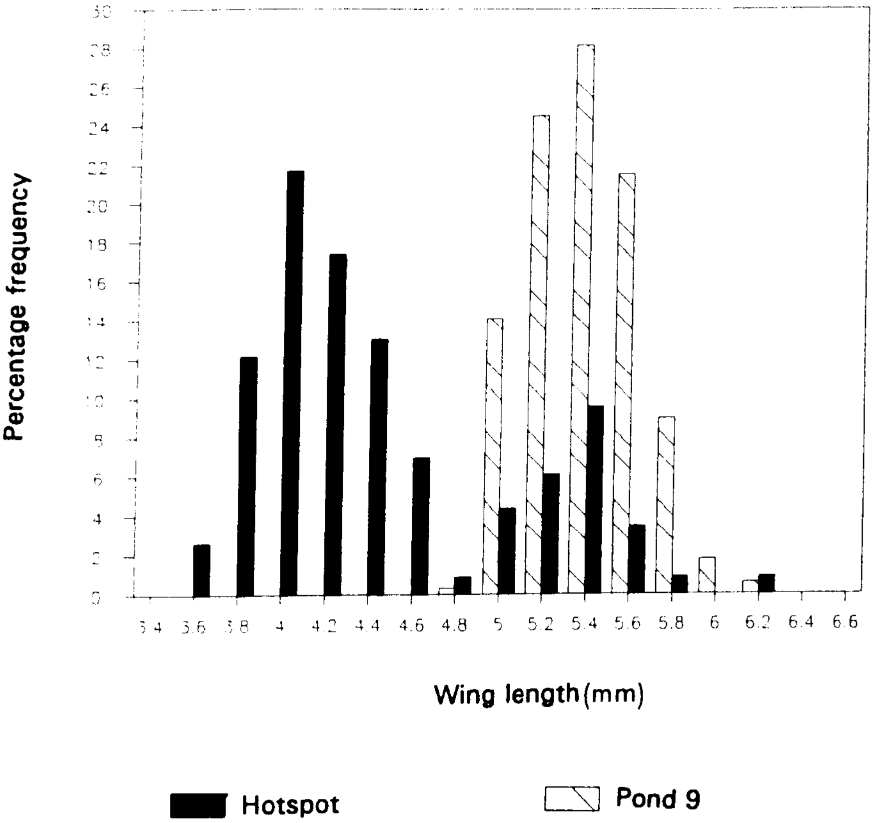


Table 3.4: Size differences of adult *Aedes cantans* emerging from Pond 9 and the Hotspot in 1989 and 1990.

(a) 1989. Mann-Whitney test.

Site	Number	Median
Pond 9	381	5.30
Hotspot	116	4.10

Point estimate for ETA1-ETA2 is 1.1000
95.0% CI for ETA1-ETA2 is (0.9999, 1.1700)
W = 46255.5
P = 0.0000

(b) 1990. Two-sample T-test.

Site	Number	Mean	St. dev	SE Mean
Pond 9	68	5.25	0.261	0.032
Hotspot	64	5.16	0.311	0.039

95% CI for MuP9-MuHS (-0.009, 0.19)
T-test MuP9-MuHS (vs NE) T=1.8 P=0.074 DF = 123.2

Table 3.5: Comparison of wing lengths of male and female *Ae. cantans* emerging from the same pond in the same year.

(a) 1989 and 1990. Mann-Whitney tests.

Site	Point est. ETA1-ETA2	95.1 pct ETA1-ETA2	P
1989			
HST1	-0.03	(-0.2, 0.1)	P=0.5166
Pond 9	0.0	(-0.1, 0.00)	P=0.06
1990			
HST1	0.09	(-0.04, 0.2)	P=0.23
Pond 9	0.1	(-0.06, 0.29)	P=0.2033
Pond 8	-0.00	(-0.13, 0.09)	P=0.7524
Pond 13	-0.11	(-0.0001, 0.3)	P=0.0748

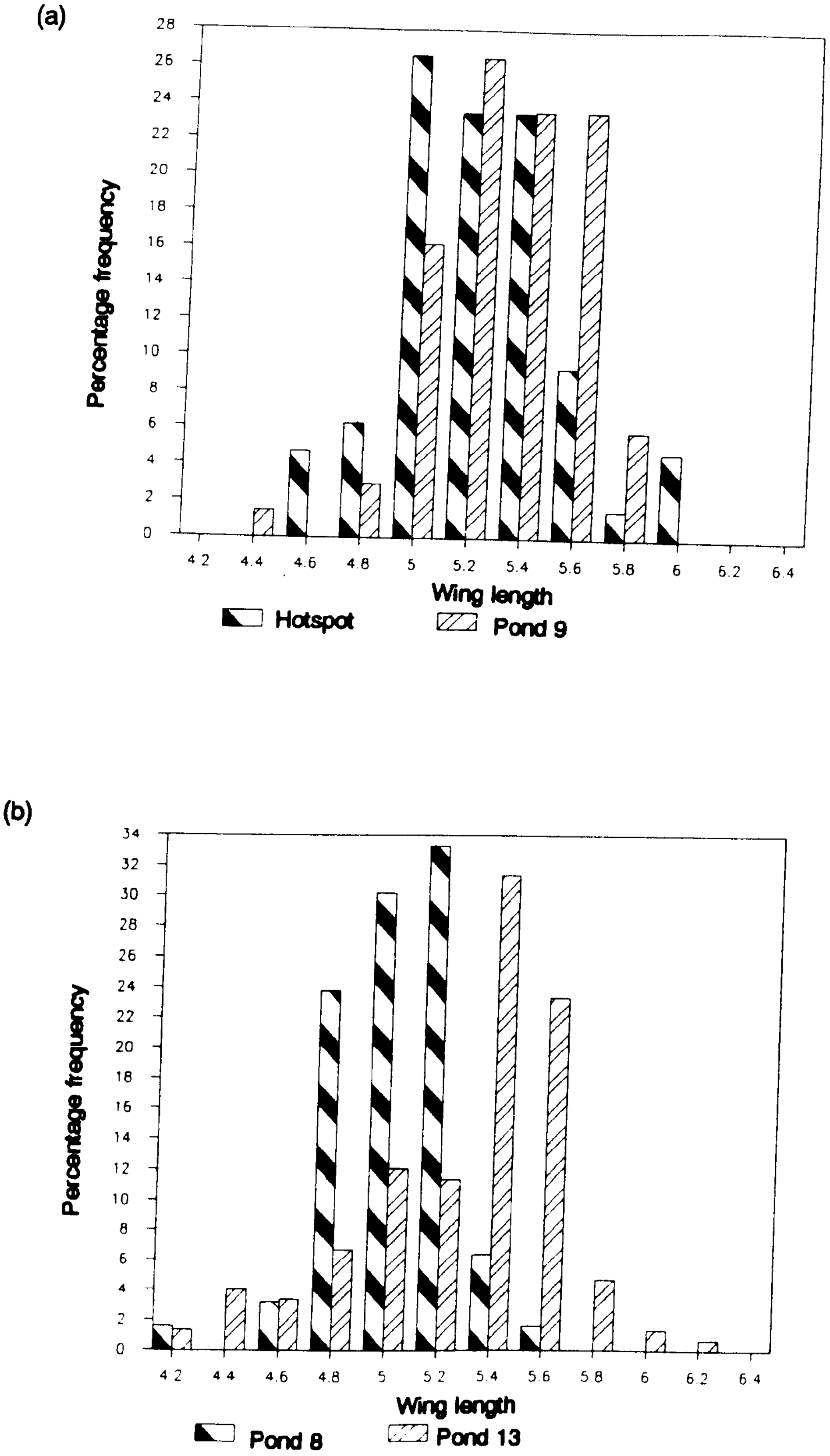
P = Probability

(b) 1991. T-Tests.

Site	95% CI	T	P	DF
Pond 8	(-0.214, 0.162)	-0.28	P=0.78	28
Pond 13	(-0.196, 0.02)	-1.66	P=0.11	28

T = Students t-test P = Probability
DF = Degrees of Freedom.

Fig. 3.9: Wing length distribution of <u>Aedes cantans</u> males and females at emergence, 1990.



size of mosquitoes emerging from Ponds 13 and 9 and the Hotspot ($P>0.05$). However, mosquitoes emerging from Pond 8 were significantly smaller than those from the other three ponds ($P<0.05$). There was again no significant difference between the size of males and females emerging from the same ponds in 1990 (Table 3.5).

Figure 3.10 shows the wing length distribution of Ae. cantans emerging from Ponds 8 and 13 in 1991. The mosquitoes emerging from Pond 13 were significantly larger than those emerging from Pond 8 ($P<0.05$). As in the previous two years, there was no significant difference between the sizes of male and female mosquitoes emerging from the same pond (Table 3.5).

Figures 3.11-3.13 show the decrease in size of emerging mosquitoes with time in all ponds in all years. The regression line equations for this data are given in Table 3.6. along with some relevant data. In all the ponds and with both sexes there is a significant linear negative regression in the size of the individuals as emergence progressed. It is clear from these results that the size of Ae. cantans at emergence decreases with time. In all ponds in all three years, there was no significant difference in the regression coefficients of male and female size with time emerging from the same pond.

3.3.2 SIZE AND SURVIVORSHIP

In 1989 only, the wing widths and the wing lengths were measured. Wing width and wing length are well correlated ($r=0.43$, $n=1,156$, $P<0.05$). There was also a significant positive regression between wing length and wing width ($y = 3.1+1.47x$, $P=0.00$). Consequently, only measurements of wing length were used to study variation in adult size, no further measurements of wing width being made. There was no significant difference between length of the right and left wings of the same

Fig. 3.10: Wing length distribution of *Aedes cantans* males and females emerging from Pond 13 and Pond 8, in 1991.

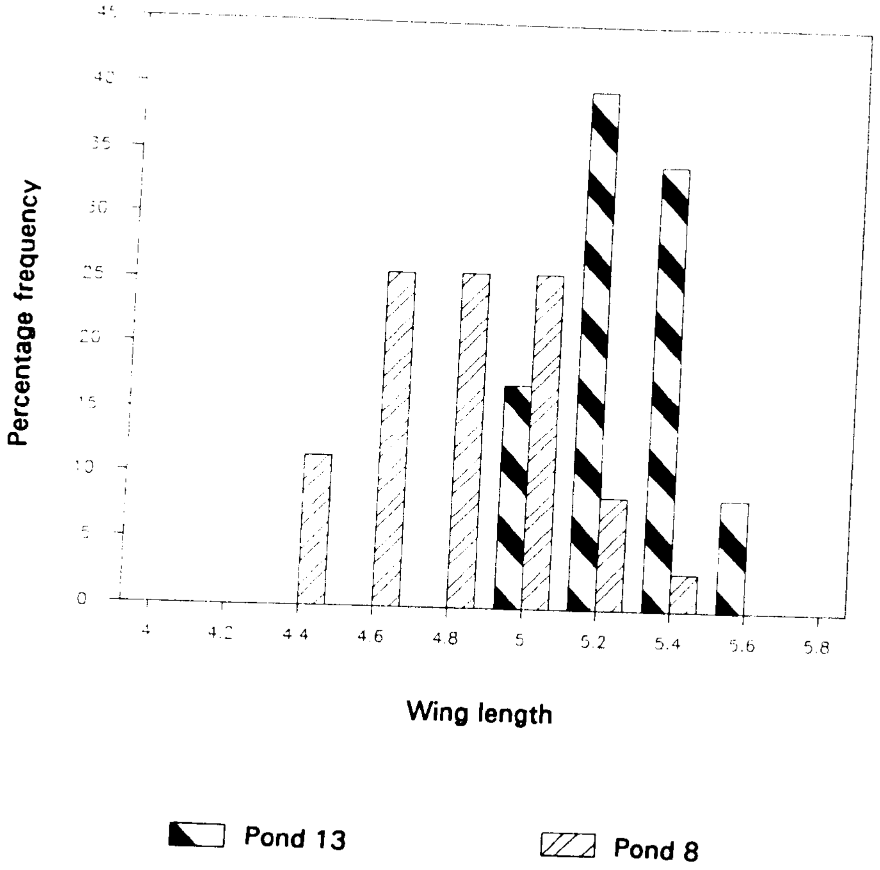
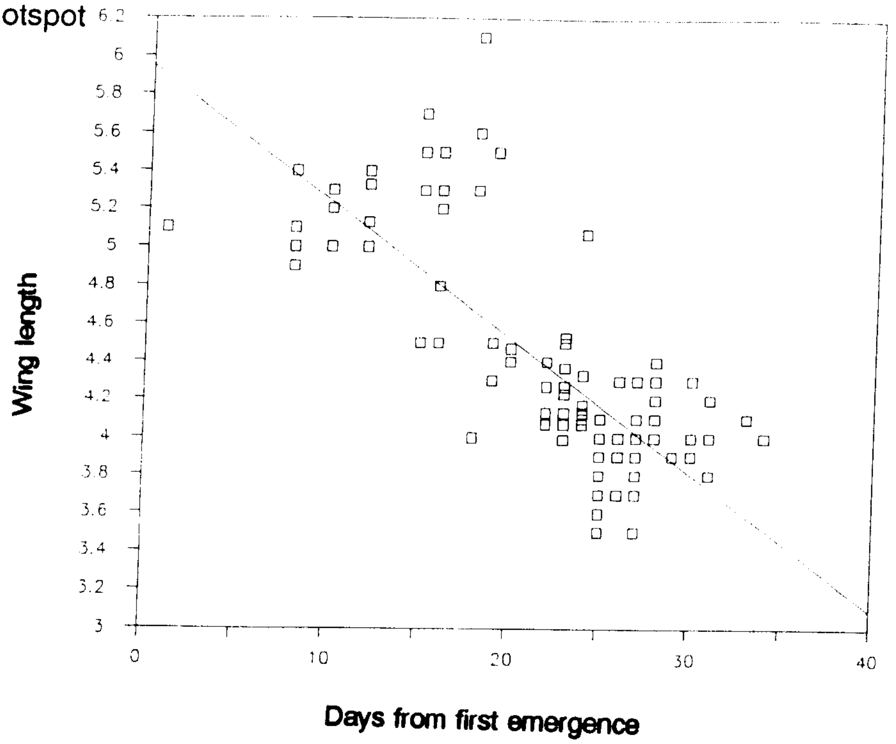


Fig. 3.11: Linear regression of wing length of male and female Aedes cantans against emergence date for each site sampled, 1989.

(a) Hotspot



(b) Pond 9

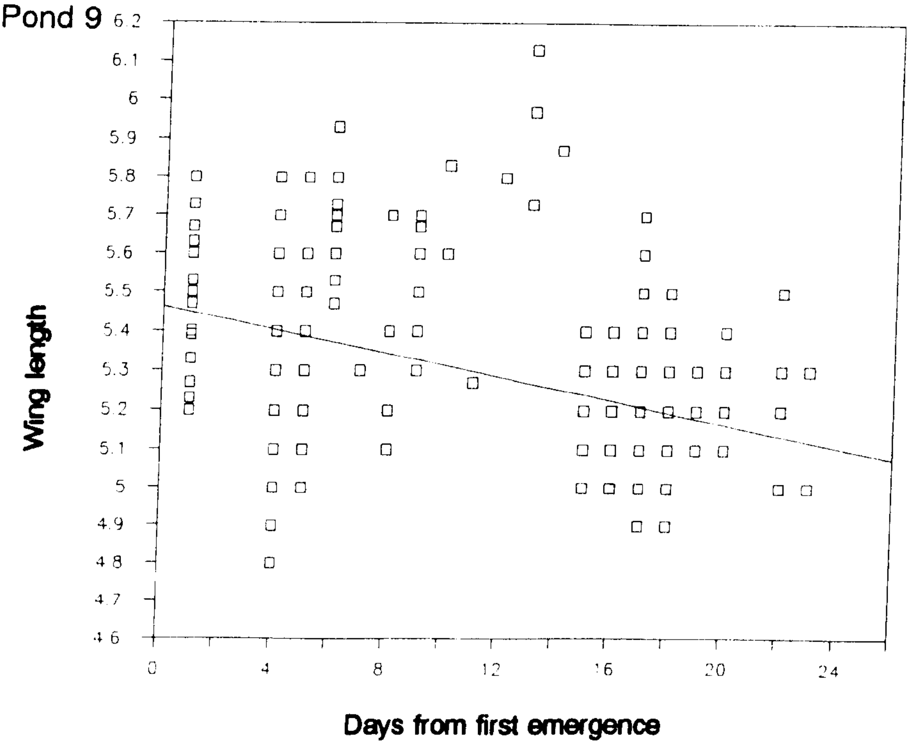
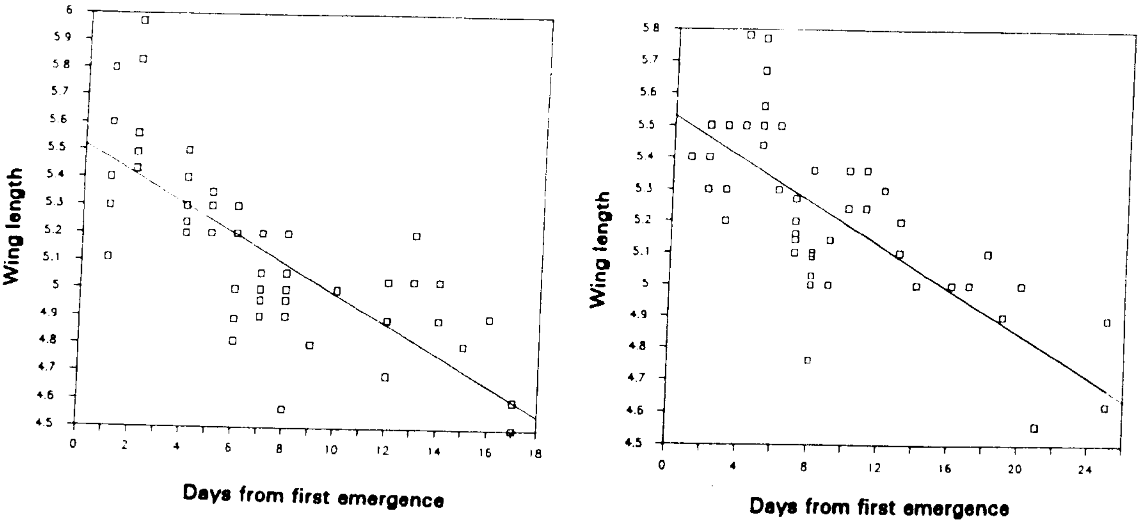
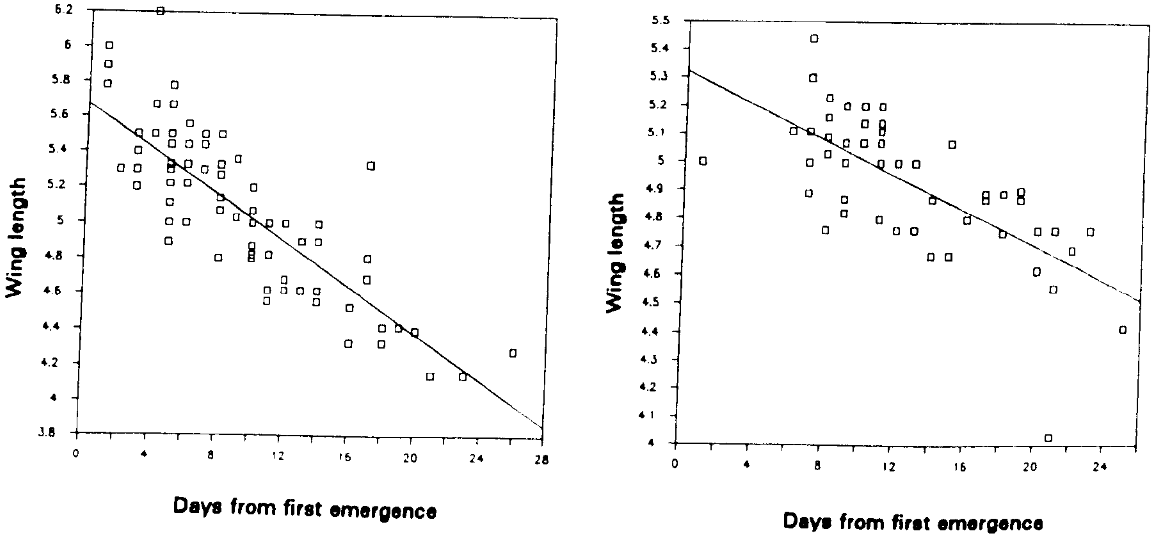


Fig. 3.12: Linear regression of wing length of male and female Aedes cantans against emergence date for each site sampled, 1990.



(a) Hotspot

(b) Pond 9

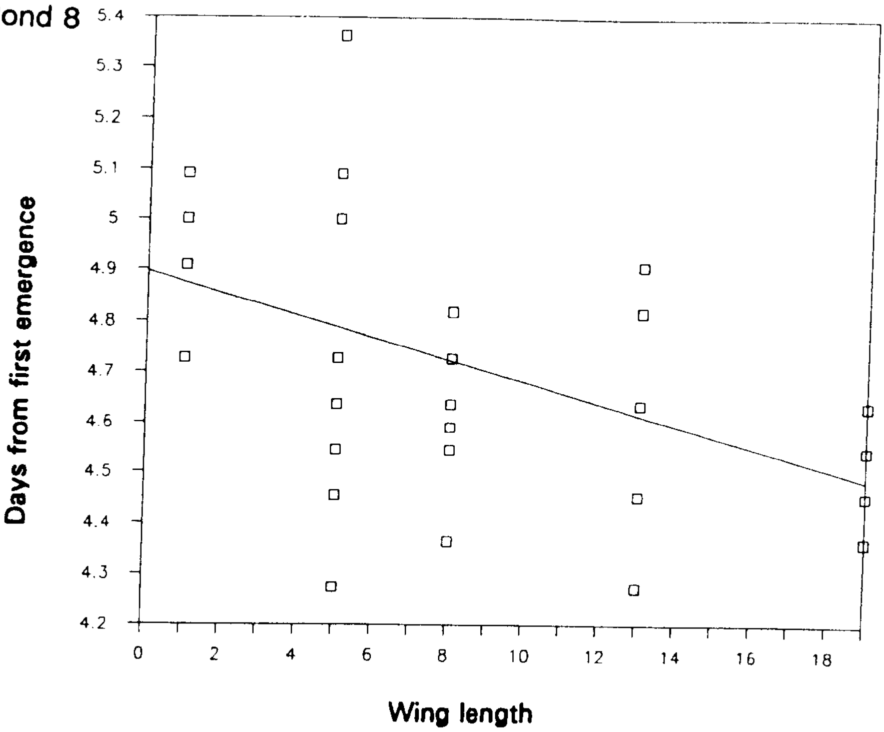


(c) Pond 13

(d) Pond 8

Fig. 3.13: Linear regression of wing length of male and female Aedes cantans against emergence date for each site sampled, 1991.

(a) Pond 8



(b) Pond 13

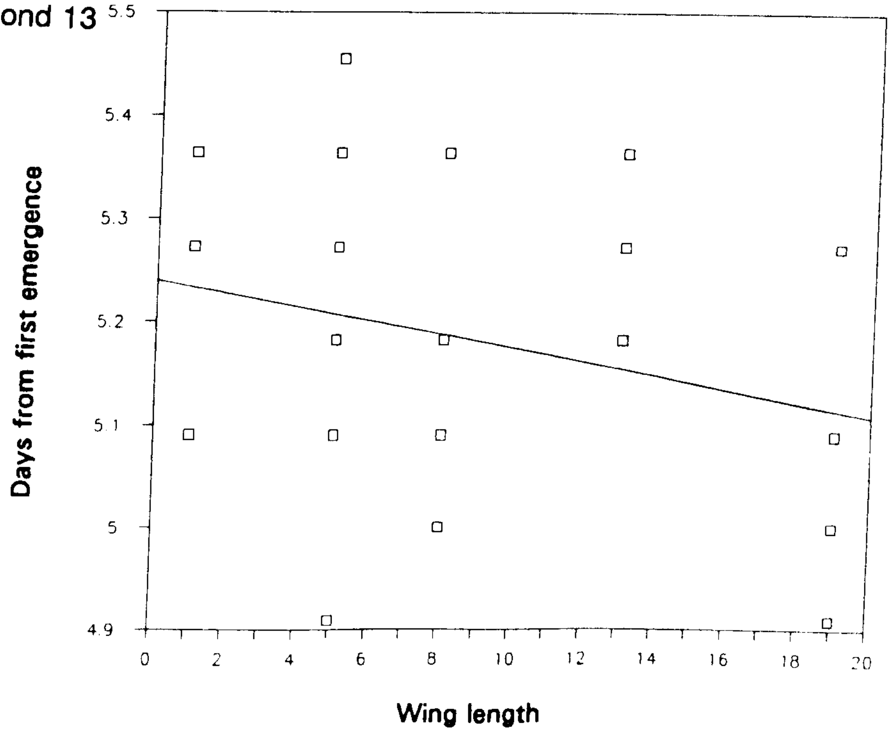


Table 3.6 : Decrease in wing length of emerging <u>Ae. cantans</u> with time- regression equations.

Site and Year	Equation	DF	T-Rat	Prob.	R Squared
1989					
Pond 9	y= 5.46-0.01484x	381	-9.05	0.000	17.5
Males	y= 5.42-0.019x	205	-10.6	0.000	35.1
Females	y= 5.55-0.0144x	174	-5.93	0.000	16.3
Hotspot	y= 5.95-0.0715x	113	-12.39	0.000	57.2
Males	y= 6.18-0.0812x	66	-10.30	0.000	61.1
Females	y= 5.37-0.0594x	46	-8.45	0.000	60.5
1990					
Pond 9	y= 5.53-0.034x	66	-8.35	0.000	50.6
Males	y= 5.5-0.0288x	25	-3.28	0.000	30.1
Females	y= 5.52-0.037x	39	-6.5	0.000	65.5
Hotspot	y= 5.52-0.052x	62	-9.15	0.000	56.8
Males	y= 5.56-0.069x	26	-6.67	0.000	61.7
Females	y= 5.51-0.05x	34	-6.5	0.000	54.1
Pond 13	y= 5.66-0.06x	148	-17.84	0.000	68.1
Males	y= 5.66-0.065x	89	-10.63	0.000	55.7
Females	y= 5.66-0.068x	59	-12.88	0.000	73.7
Pond 8	y= 5.36-0.029x	62	-7.48	0.000	47.4
Males	y= 5.27-0.028x	31	-4.38	0.000	36.9
Females	y= 5.28-0.037x	31	-9.87	0.000	75.7
1991					
Pond 13	y= 5.33-0.022x	34	-4.8	0.000	39.4
Male	y= 5.38-0.064x	14	-4.34	0.001	56.1
Female	y= 5.39-0.028x	20	-3.34	0.004	34.8
Pond 8	y= 4.9-0.0216x	34	-3.4	0.002	23.7
Male	y= 4.86-0.026x	15	-2.19	0.04	20.1
Female	y= 5.02-0.028x	18	-3.97	0.001	45.1

DF = Degrees of freedom   T-Rat = T-Ratio individual (r=0.99, n=50, P<0.001), whenever possible the right wing only was measured.

SIZE AND PARITY

Table 3.7 shows the results of the Mann-Whitney analysis between the size of nulliparous and parous Ae. cantans in 1989 and 1990. In 1989, parous mosquitoes were significantly larger than nulliparous ones (P<0.05). In 1989, dissections for ovarian dilatations were not made, but they were in the following year. In 1990, two-plus parous Ae. cantans were significantly larger than one-parous females (P<0.001) which in turn were significantly larger than nulliparous ones (P<0.01). Figures 3.14-3.15 show the wing size distributions of these females caught at human-bait in 1989 and 1990.

Table 3.8 gives the regression and correlation data of wing length of bait caught mosquitoes with time in 1989 and 1990. In 1989, the size distribution of nulliparous mosquitoes decreased significantly as the season progressed. The size of parous mosquitoes showed no regression or correlation with time; Figure 3.16 illustrates this point. In 1990, the wing length of one-parous and also two-parous plus mosquitoes, as well as nulliparous ones, showed a significant negative regression with time (Figs. 3.17).

The overall pattern therefore was that the individuals in the population of the later, parous, stages of mosquitoes were larger in terms of wing length, and therefore body size, than the earlier nulliparous stages, in both years.

In 1989, there was no significant difference between the size of female Ae. cantans caught in human-bait catches in Wood A and the Leahurst sheep field (Fig. 1.2), suggesting that they belonged to the same population.

Table 3.7 : Differences in the wing length of *Ae. cantans* caught at bait of nulliparous and parous mosquitoes (Mann-Whitney analysis).

State	N.	Med.	Point est. ETA1-ETA2	100.0 PCTCI ETA1-ETA2	P.
1989					
Nulls.	596	5.2	-0.02	(-1.74, 1.74)	P=0.047
Pars.	592	5.25			
1990					
Nulls.	573	5.0	-0.06	(-1.87, 1.73)	P=0.011
Par-1	715	5.07			
Nulls.	573	5.0	-0.2	(-1.87, 1.53)	P=0.00
Par-2+	195	5.2			
Par-1	715	5.07	-0.14	(-1.6, 1.4)	P=0.00
Par-2+	195	5.2			

N. = Number Med. = Median P = Probability.

Fig. 3.14: Wing length distribution of *Aedes cantans* females caught at human bait, 1989.

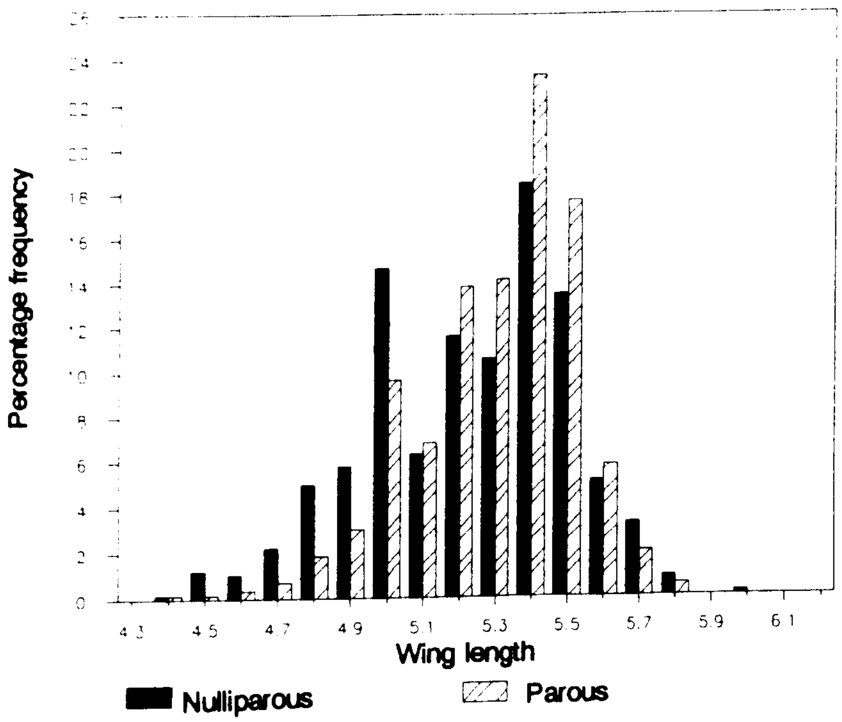


Fig. 3.15: Wing length distribution of *Aedes cantans* females caught at human bait, 1990.

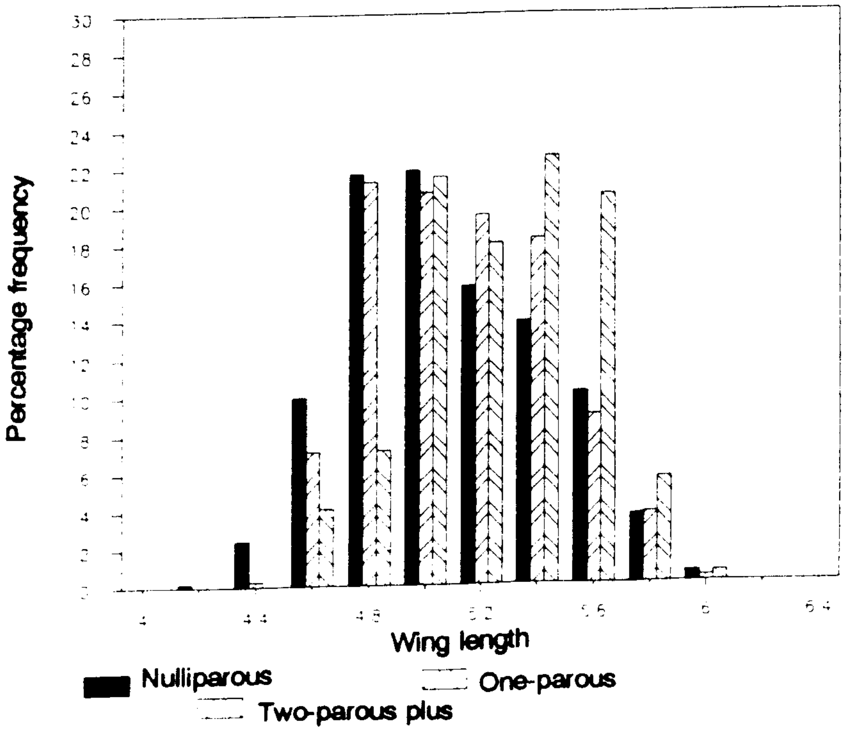


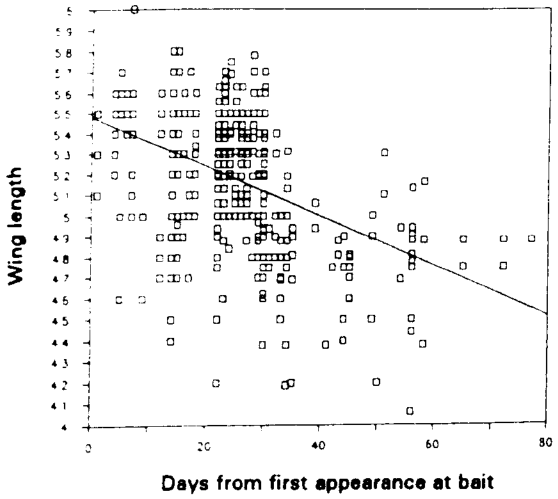
Table 3.8 : Decrease in wing length of bait catch *Ae. cantans* with time- regression equations.

Site and Year	Equation	T-Ratio	Prob.	R Squared
1989				
All wl with time	y= 5.28-0.021x	-4.33	0.000	1.5%
Null. wl with time	y= 5.48-0.012x	-12.88	0.000	21.7%
Par. wl with time	y= 5.19+0.067x	0.78	0.433	0.0%
1990				
All wl with time	y= 5.12-0.018x	-3.46	0.001	0.7%
Nulls. wl with time	y= 5.23-0.015x	-11.69	0.000	19.2%
Par. wl with time	y= 5.34-0.0078x	-7.21	0.000	6.7%
Par-2+ wl with time	y= 5.9-0.0134x	-6.9	0.000	19.4%

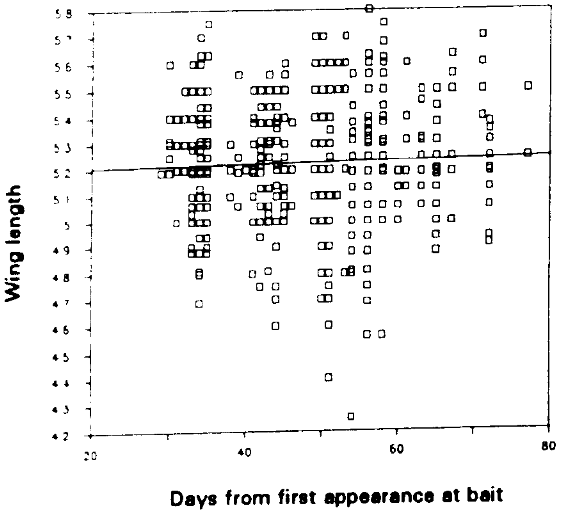
Null. = Nulliparous Par. = Parous wl= Wing Length

Fig. 3.16: Linear regression of wing length of female *Aedes cantans* caught at human-bait with time, 1989.

(a) Nulliparous mosquitoes



(b) Parous mosquitoes



(c) All mosquitoes

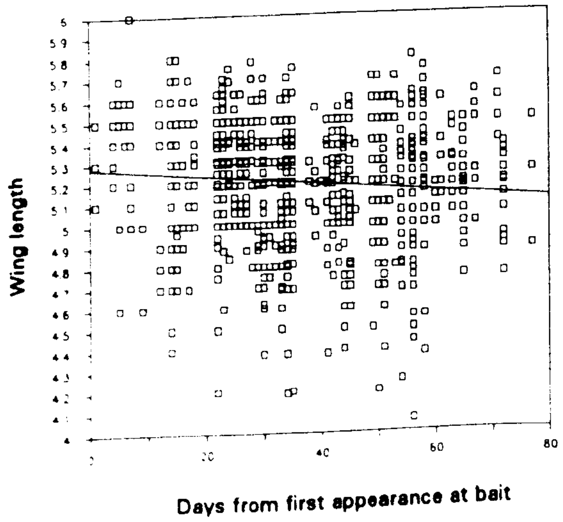
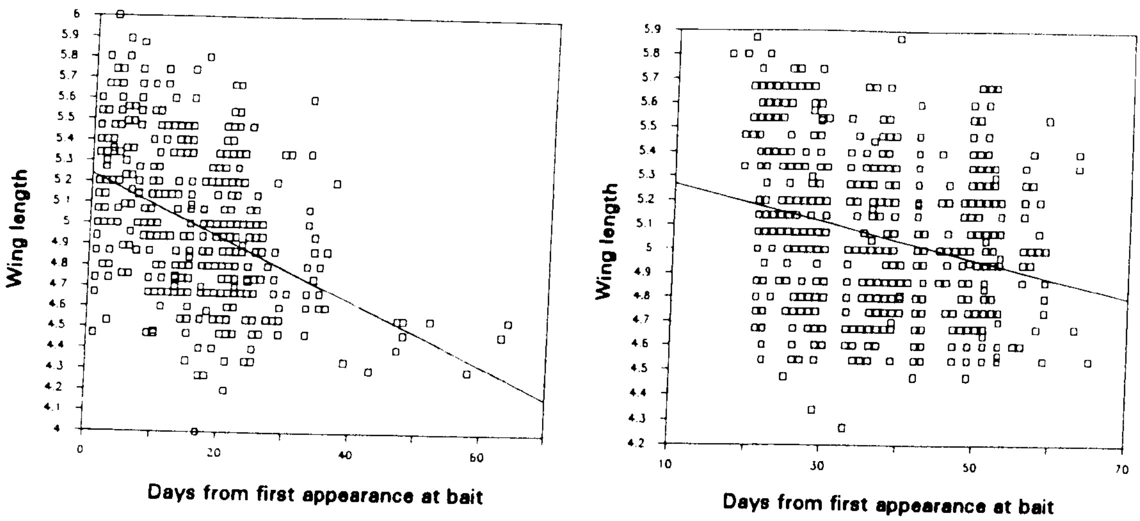


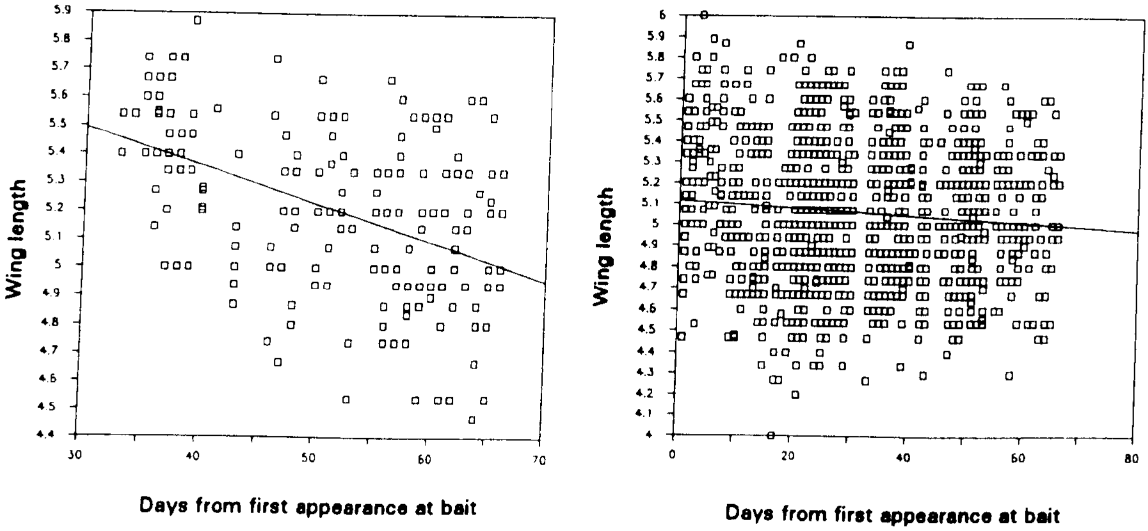
Fig. 3.17: Linear regression of wing length of female Aedes cantans caught at human-bait with time, 1990.


(a) Nulliparous mosquitoes

(b) One-parous mosquitoes

(c) Two-parous plus mosquitoes

(d) All mosquitoes



SIZE AT EMERGENCE AND BAIT

In 1989, the Ae. cantans females emerging from Pond 9 were significantly larger than those caught throughout the year in bait catches ($P<0.001$). In 1990, the mosquitoes emerging from Ponds 8, 9 and 13, and the Hotspot were significantly larger than those arriving at bait as nullipars ($P<0.001$) or one-pars ($P<0.001$). There was, however, no significant difference in the size of the two-parous plus mosquitoes caught biting and the emerging ones ($P>0.05$).

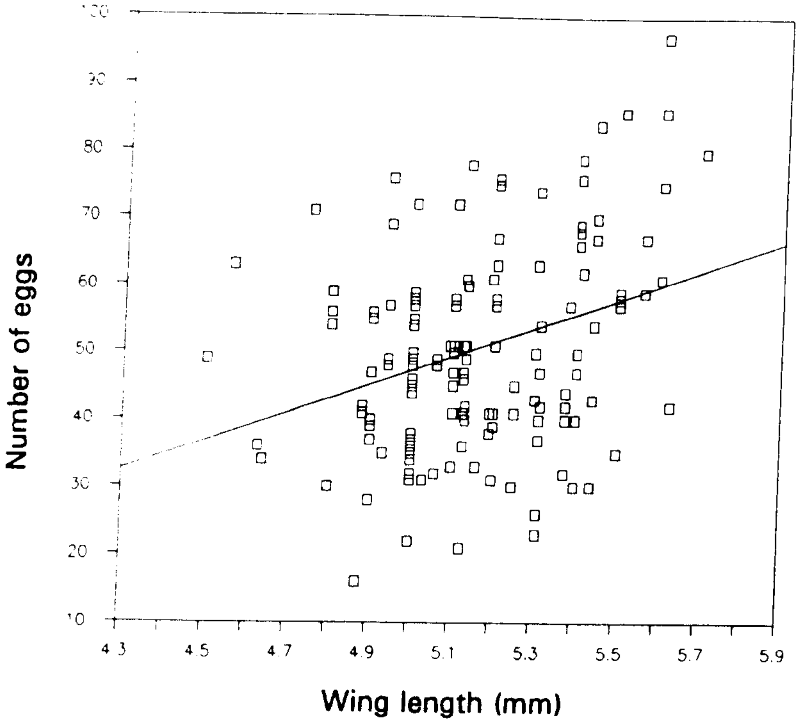
3.3.3 OVIPOSITION

Figure 3.18 shows the regression lines of wing length of Ae. cantans against the egg number laid for females collected in 1989 and 1990 respectively, while Table 3.9 shows the regression equations and some relevant data. In all cases wing length and the number of eggs laid are significantly correlated, that is larger adults lay more eggs. However, there is no correlation between wing length and the size of eggs laid (Table 3.10). Therefore it is fair to say that larger adults do not produce larger eggs. In 1989, there was no significant difference in the number of eggs laid by nulliparous and parous mosquitoes ($P>0.05$). Similarly, in 1990, there was no significant difference in the number of eggs laid by nulliparous, one-parous and two-parous-plus females ($P>0.05$). The ovaries were examined after egg-laying to check the parity.

In 1991, a large proportion of females caught at bait in late May and early June required two blood-meals before eggs could be fully matured. Out of 50 Ae. cantans that were caught at bait over this period, and blood-fed on M. W. Service, only three (5%) laid eggs. Twenty were dissected and these had their ovaries in Stage IIIa-b only. The remaining 20 were offered a second blood-meal and all of them completed oviposition. Seven died in the laboratory. Mosquitoes caught later in the season

Fig. 3.18: Linear regression of number of eggs laid in the laboratory against female wing length in 1989 and 1990.

(a) 1989



(b) 1990

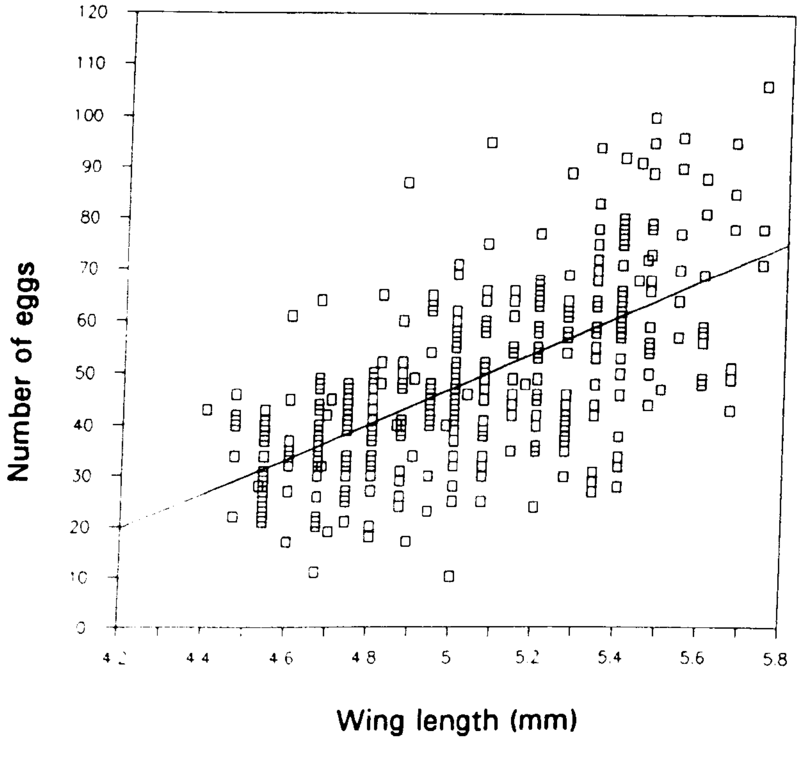


Table 3.9: Regression of female wing length and egg number in 1989 and 1990.

Year	Regression equation	DF	T-ratio	P.	R squared
1989	y= -125+34.6x	370	16.0	P=0.00	40.8
1990	y= -58.6+21.2x	151	4.09	P=0.00	9.5

DF = Degrees of freedom. P. = Probability.

Table 3.10: Regression analysis of wing length and egg size.

Regression equation	DF	T-Ratio	P	R Squared
y = -0.94+0.243x	25	1.18	P=0.278	4.6

DF = Degrees of freedom. P. = Probability showed a decreasing requirement for two-blood-meals. No similar observations were made in the earlier years.

3.3.4 HUMAN-BAIT CATCHES

3.3.4.1 SEASONAL INCIDENCE

More than 99.9% of the mosquitoes caught at human-bait were Ae. cantans. The remaining species were An. plumbeus, An. claviger, Ae. punctor, Ae. geniculatus and Cx. pipiens.

In 1989 a total of 4,061 Ae. cantans females were caught from 140 one-hour bait catches, averaging 29.0 per hour. In 1990, 11,120 Ae. cantans were caught from a total of 308 one-hour human bait catches, averaging 36.1 mosquitoes per hour. In 1991, 3,019 Ae. cantans were caught from 145 one-hour human-bait catches, averaging 20.8 mosquitoes per hour. Chisquared analysis showed that there was a significant difference in the hourly bait catch totals in all three years ($\chi^2 = 29.45$, two degrees of freedom, $P<0.001$), with significantly more in 1990, followed by 1989 and then 1991.

There was no significant difference between the numbers of mosquitoes caught in Wood A during the afternoon and evening, and in the LSF in the evening in both 1989 and 1990 ($P>0.05$).

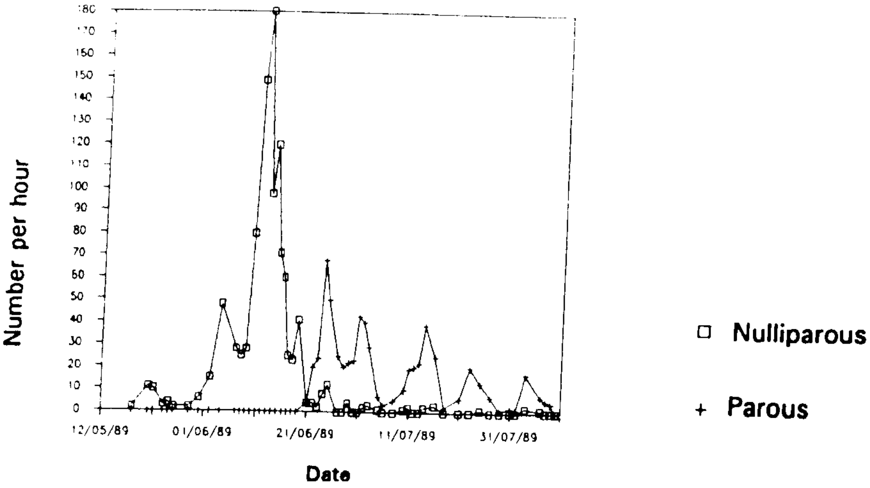
In 1989, the first Ae. cantans came to bait on 18 May, 18 days after the first emergence, in 1990 on 25 April, only eight days after the first emergence, and in 1991 on 21 May, 15 days after the first emergence. In 1989, the last Ae. cantans to come to bait was on 10 August, 102 days after the first emergence, in 1990 on the 30 August, 136 days from first emergence and in 1991 on 25 September, 144 days after first emergence. In the first year, the first parous mosquito was caught on 20 June, 34 days after the first appearance at bait, in the second year on 7 June, 44 days after the first appearance at bait and in the third year on 24 June, 35 days after first appearance at bait. The last nulliparous mosquito caught at bait in the first year was on the 25 July, 86 days after the first emergence, 69 days after the first appearance at bait and 35 days after the first parous mosquito. In 1990, the last nulliparous mosquito caught at bait was on the 24 July, 99 days after the first emergence, 60 days after first appearance at bait and 47 days after the first parous mosquito. In 1991, the last nulliparous mosquito came to bait on 5 August, 89 days after first emergence, 78 days after first appearance at bait and 44 days after the first parous mosquito.

Figures 3.19-3.21 show the numbers of Ae. cantans caught at bait per hour at various sites and at different times of the day in all three years.

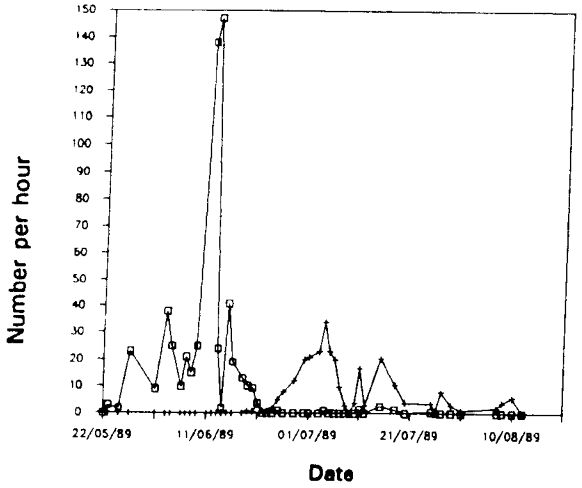
In the 1989 evening bait catches (1830-2030 hr.) in Wood A, although the first (nulliparous) Ae. cantans was caught on 23 May, adults were caught only in reasonable numbers after 1 June, peaking on the 11 June with a catch of 180 per hour (Fig. 3.19a). There was an unexpectedly rapid switch from nulliparous to one-parous mosquitoes on 21 June. Figure 3.19 shows the pattern of the peaks, suggesting that the one-parous mosquitoes declined in number until the 8 July, when it is likely that two-parous mosquitoes were present in the biting population. This, however, cannot be confirmed because age-grading by dilatations was not undertaken in 1989. Mark-release-recapture data (Chapter 5) nevertheless support this supposition. The pattern of the peaks suggests that the two-parous mosquitoes were present from 8 July until 15 July and then three-parous mosquitoes were present from 16 July until 31 July. There was therefore a period of 20 days from the nulliparous peak to the one-parous peak, and this represents the duration of the gonotrophic cycle (Chapter 5). Similarly,

Fig. 3.19: Seasonal incidence of *Aedes cantans* caught at human-bait, 1989.

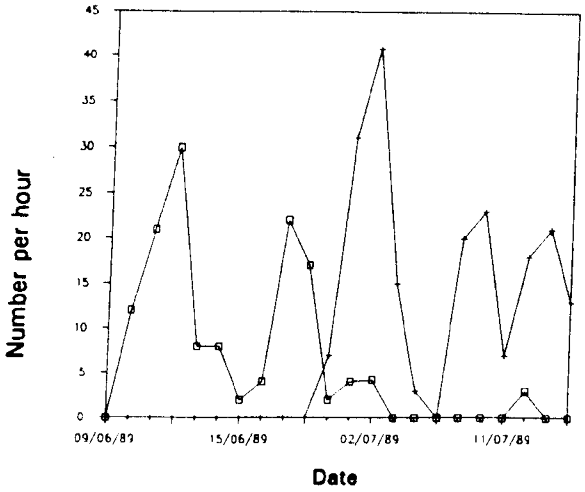
(a) Evening bait catches, Wood A.



(b) Afternoon bait catches, Wood A.



(c) Evening bait catches, LSF.



the pattern of the peaks suggests that it took 20 days for the first-parous mosquitoes to become two-parous. The size of the peaks declines from nulliparous onwards and this may be an indication of a decline in the overall population size.

Fewer mosquitoes were captured in the afternoon bait catches (1200-1400 hr.) in Wood A in 1989 than in evening bait catches (Fig. 3.19b), especially later in the season when parous mosquitoes predominated. The first nulliparous female was caught on 23 May, and a peak of 149 per hour occurred on the 13 June. The parous peaks are not clear and are difficult to interpret.

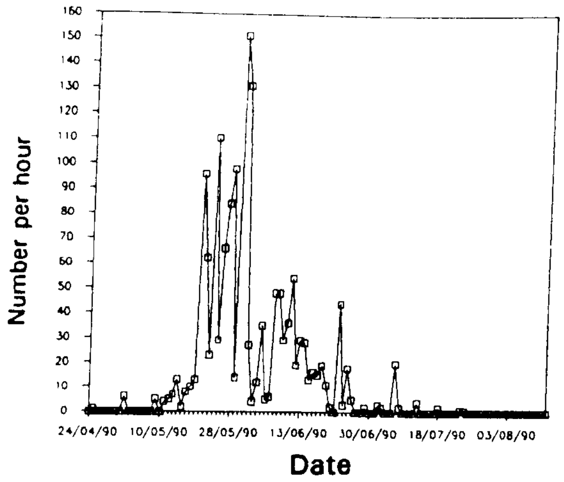
The 1989 pattern of evening human-bait catches in the Leahurst sheep field is confused (Fig 3.19c). The first nulliparous Ae. cantans were caught on 9 June and the last on 4 July. Parous mosquitoes were caught from 19 June until 8 August. Although nulliparous mosquitoes were caught in the evening bait catches in Wood A from 23 May, they were not caught hunting in the fields until 17 days later. In addition, the parous peak is greater than the nulliparous peak, which is unexpected since more nulliparous mosquitoes must have been present in the population originally.

During evening bait-catches in Wood A in 1990, the first nulliparous mosquitoes appeared on 24 April, but were caught in numbers only from 11 May to 26 June, peaking on 30 May, with 151 per hour (Fig. 3.20a). The first parous mosquitoes were caught from 9 June and continued to be caught in reasonable numbers until 13 July, with a maximum of 119 caught on 23 June. The change-over from nulliparous to one-parous was not as rapid as in 1989. The first two-parous mosquitoes (as determined by dissection) were caught on 20 June with numbers peaking on 7 July (121 mosquitoes per hour). The length of time from when more than ten nulliparous mosquitoes were captured per hour, until the first parous mosquitoes was 30 days, which represents the length of the gonotrophic cycle. The length of time from the first

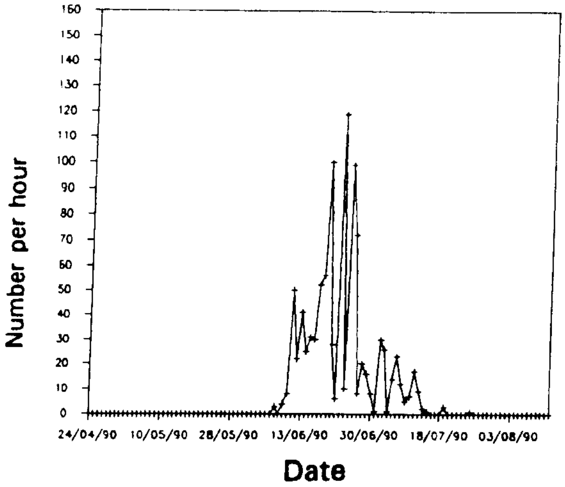
Fig. 3.20: Seasonal incidence of Aedes cantans caught at human-bait, 1990.

(a) Evening bait catches, Wood A.

Nulliparous



One-parous



Two-parous plus

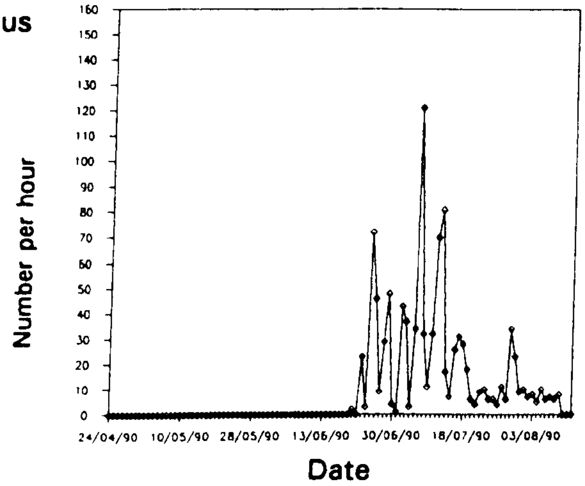
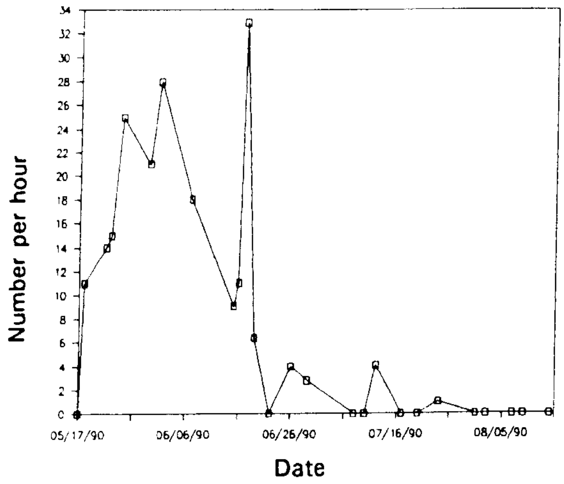


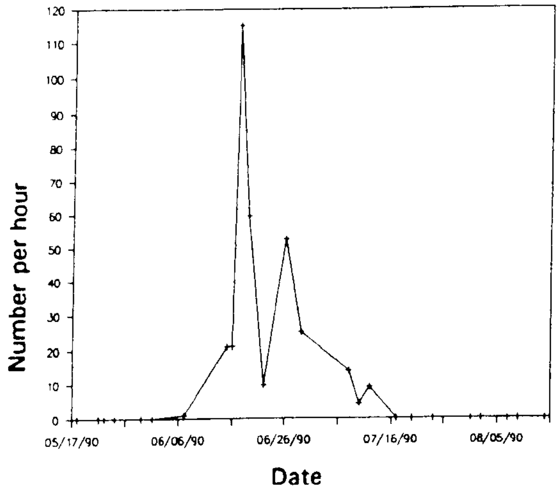
Fig. 3.20 continued

(b) Afternoon bait catches, Wood A.

Nulliparous



One-parous



Two-parous

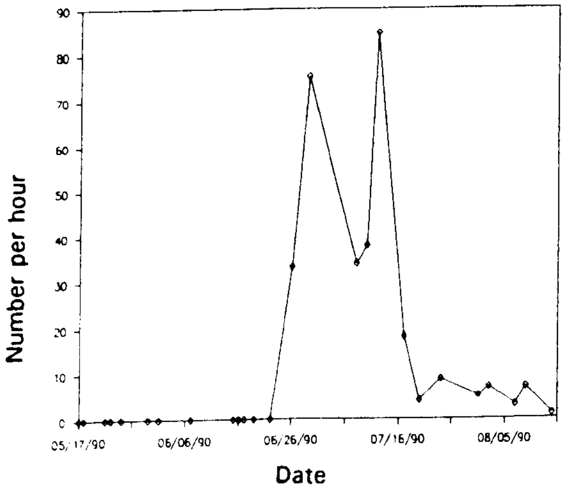
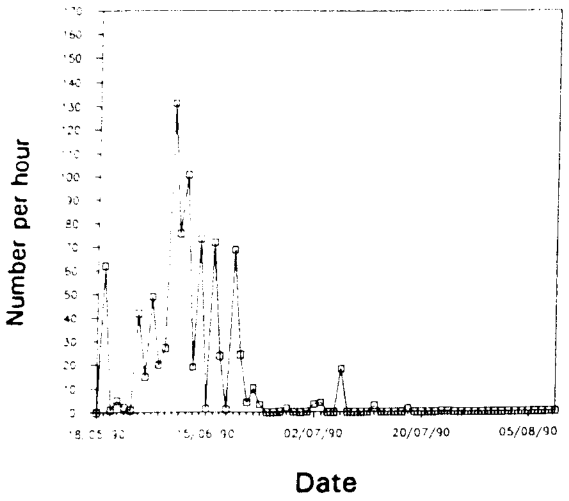


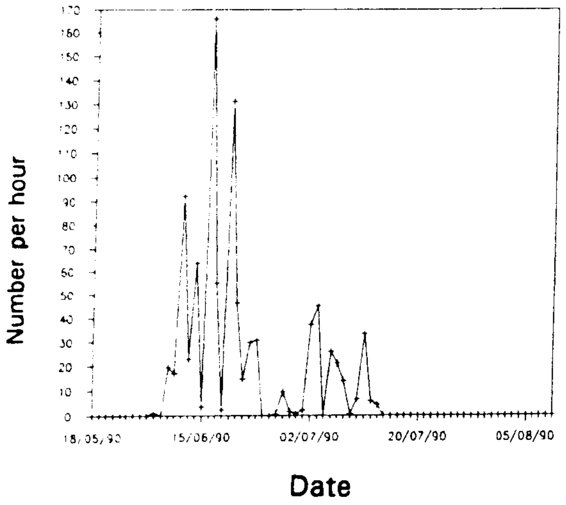
Fig. 3.20 continued

(c) Evening bait catches, LSF.

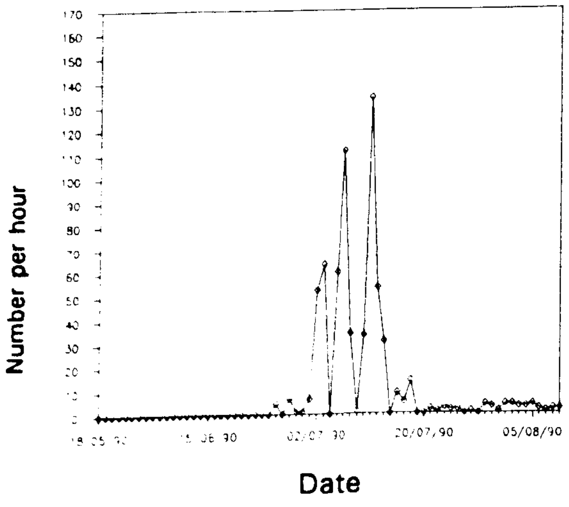
Nulliparous



One-parous



Two-parous



one-parous mosquito to the first two-parous mosquito was just 11 days. This compares well with the duration of the gonotrophic cycle of 13 days based on caged mosquitoes blood-fed on 13 June and kept in the field (Chapter 5).

The afternoon bait catches in 1990 present a very confused pattern, with more two-parous females caught than one-parous, and very few nulliparous mosquitoes caught at all (Fig. 3.20b). Similarly in the LSF (Fig. 3.20c) more one-parous females were caught than nulliparous ones. The drop in mosquito numbers from 16 June to 29 June was due to windy weather (personal observation) which prevented the mosquitoes from flying out of the woods to feed.

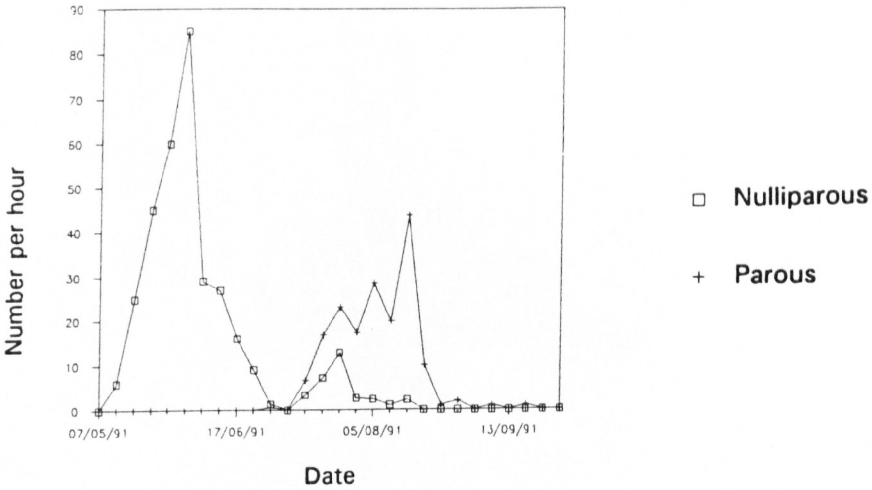
In 1991, fewer mosquitoes were caught at bait, especially in late June. Mosquitoes were caught late in the season, in September. In the evening bait catches in Wood A (Fig. 3.21a), nulliparous mosquitoes first appeared at bait on 29 May and biting peaked on 1 June with 85 per hour and then numbers dropped rapidly. The first parous mosquito appeared at bait on 9 July, with a maximum number of 48 on 16 August.

The first nulliparous mosquito caught at bait in afternoon bait catches in 1991 (Fig. 3.21b) was on 21 May. The nulliparous mosquitoes peaked rapidly and then as weather conditions deteriorated in June, few mosquitoes were caught at bait. The first parous mosquito was caught on 24 June and females were still caught well into September. The LSF evening bait catches (Fig. 3.21c) were most affected by the weather conditions in June, with very few mosquitoes being caught.

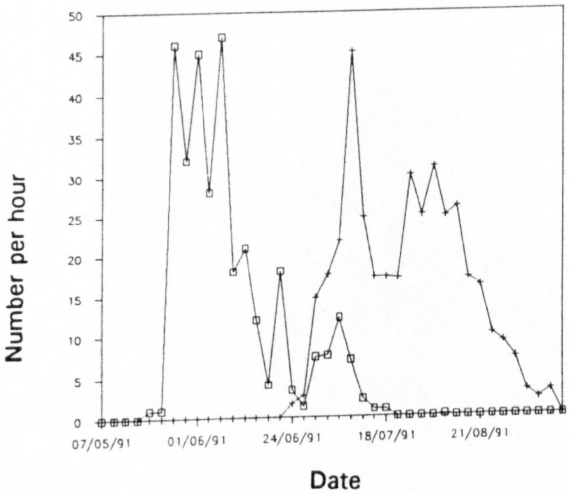
Fig. 3.22 shows that the mean monthly numbers caught in hourly bait collections were similar for all sites in 1989 and 1990 but different in 1991. In both 1989 and 1990 Ae. cantans first arrived at bait in May, maximum biting was in June and the population then declined until mosquitoes were no longer caught in

Fig. 3.21: Seasonal incidence of <u>Aedes</u> <u>cantans</u> caught at human-bait, 1991.

(a) Evening bait catches, Wood A.



(b) Afternoon bait catches, Wood A.



(c) Evening bait catches, LSF.

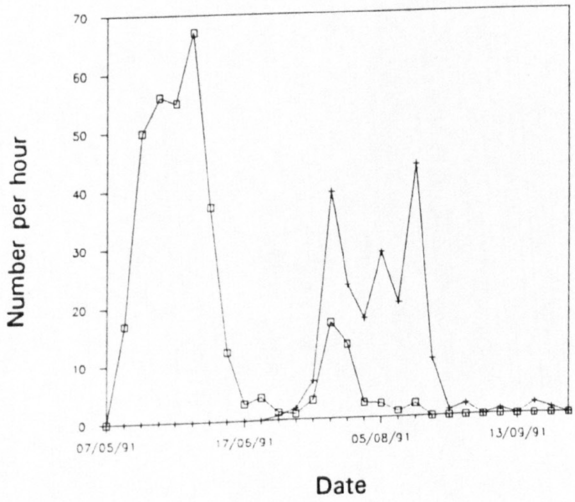
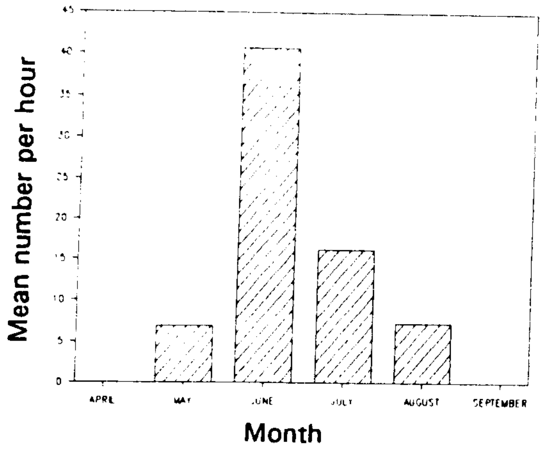
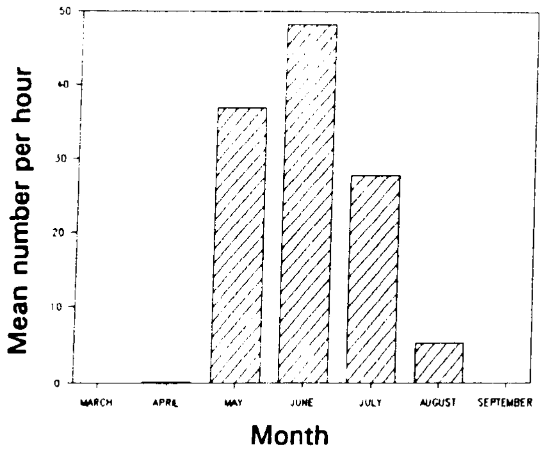


Fig. 3.22: Monthly mean numbers of adults caught in bait catches, 1989-1991.

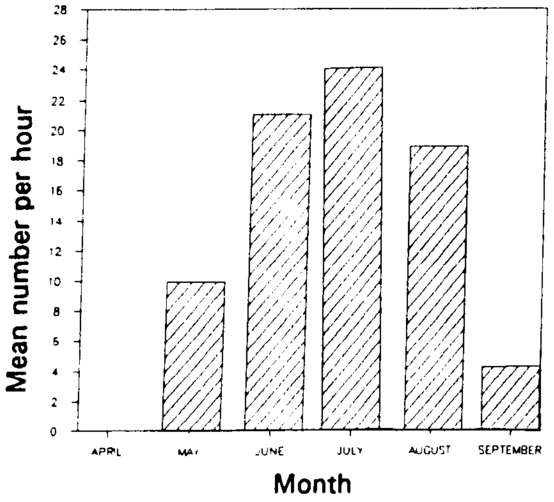
(a) 1989



(b) 1990



(c) 1991



September. The precise dates of these phenomena, however, varied by a week or two according to years and location. In 1991, mosquitoes arrived at bait later in the season due to the later onset of emergence. Poor weather conditions in June limited the numbers caught at bait and maximum biting was in July. Numbers declined more gradually than in the previous two years.

The numbers of one-parous mosquitoes coming to bait in comparison to the number of nulliparous mosquitoes can give some indication of the survivorship and mortality rates throughout the season. In 1990, the number of one-parous divided by nulliparous mosquitoes was 71.19%, which gives an indication of survivorship from the nulliparous to the one-parous stage. This method assumes that all the mosquitoes that were sampled when they were nulliparous will also be sampled when they are parous.

The parous rates were calculated by dividing the total number of all parous mosquitoes caught by the total number of all mosquitoes caught in each year. In 1989, the parous rate was at its lowest at 0.378, in 1990, it was 0.584 and in 1991, 0.798.

3.3.4.2 RESTING AND HOST-HUNTING BEHAVIOUR

Certain aspects of mosquito flight and resting behaviour can be seen from bait catches. Aedes cantans females were captured at human-bait at all times of the day in Wood A, whereas in contrast, very few were captured in Wood B either during the afternoon or evening. In addition, despite numerous bait catches during the mornings and afternoons in the LSF throughout the year, and during the afternoons in fields, 2-13, no mosquitoes were caught. This shows that during the daytime, Ae. cantans are resting amongst vegetation in the woods and do not fly out to feed in open areas. In contrast, during the evening, there are active host-seeking flights and adults are caught at human-bait in the fields.

3.3.4.3 FIVE MINUTE INTERVALS

The numbers of Ae. cantans caught in afternoon hourly bait catches during all three years were divided into five-minute intervals so that the pattern of arrival at bait could be determined (Table 3.11). Clearly the greatest number of mosquitoes are attracted to the host during the first five minutes. In 1989, 17.8% of the mosquitoes that came to bait were caught in the first five minutes, in 1990, 16.42% and in 1991, 23.16% (Fig. 3.23).

It was also noted that Ae. cantans tended to come to bait in waves, that is several hungry females often arrived almost simultaneously, followed by short periods of few or no mosquitoes arriving.

3.3.4.4 INFLUENCE OF WEATHER CONDITIONS ON BITING

In the first two years of this study, when the greatest number of bait catches were carried out, the summers were particularly good, with warm temperatures and low rainfall. On the occasions when it did rain heavily, mosquitoes were not caught at bait, although light rainfall did not seem to have any appreciable affect on biting numbers either in the woods or the fields. On particularly windy days, mosquitoes were still caught in the Woods, though in lower numbers, but even fewer were caught in the more exposed fields.

In 1991, the temperatures at the actual site of the bait catches were recorded using a Squirrel Data logger. There was a significant positive correlation between the mean temperature during the bait catch and the number of mosquitoes caught ($r=0.62$, $n=29$, $P<0.05$) (Fig.3.24). Regression analysis showed a positive linear regression between these two factors (T-ratio = 4.11, $P = 0.000$). 1991 was the only year in which the weather conditions were bad and had an obvious effect on the numbers of

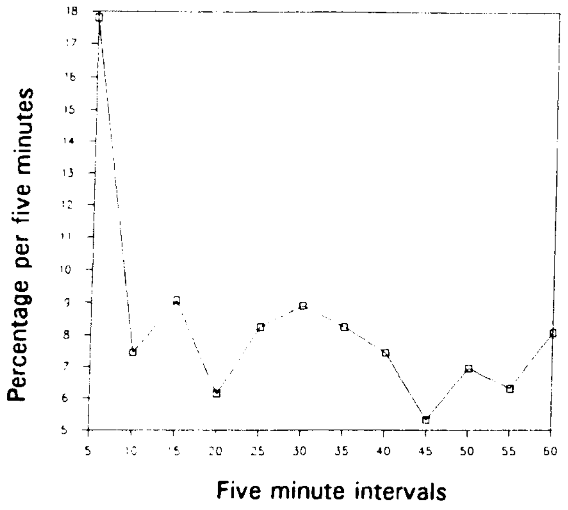
Table 3.11: Afternoon bait catches divided into five minute intervals.

Mins.	1989		1990		1991	
	T.	%	T	%	T	%
5	110	17.8	991	16.42	371	23.16
10	46	7.44	577	9.56	159	9.93
15	56	9.06	543	9.0	146	9.11
20	38	6.15	540	8.95	115	7.18
25	51	8.25	486	8.05	116	7.24
30	55	8.91	450	7.46	89	5.56
35	51	8.25	428	7.09	106	6.62
40	46	7.44	405	6.72	114	7.12
45	33	5.34	398	6.6	102	6.36
50	43	6.96	367	6.08	89	5.56
55	39	6.31	423	7.01	90	5.61
60	50	8.09	426	7.06	105	6.55
Total	618		6034		1602	

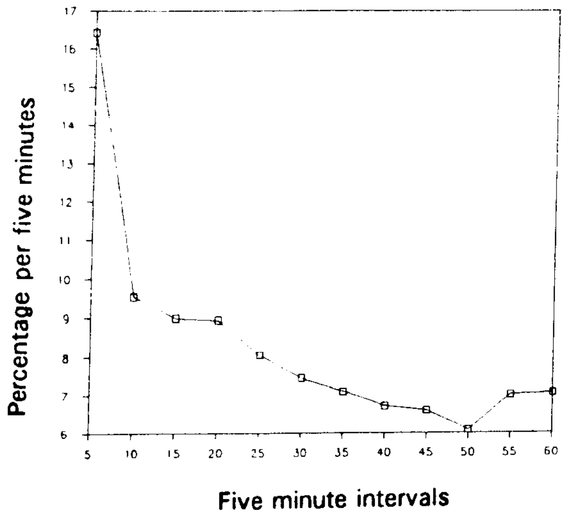
T = Total

Fig. 3.23: Afternoon bait-catches divided into five minute intervals.

(a) 1989



(b) 1990



(c) 1991

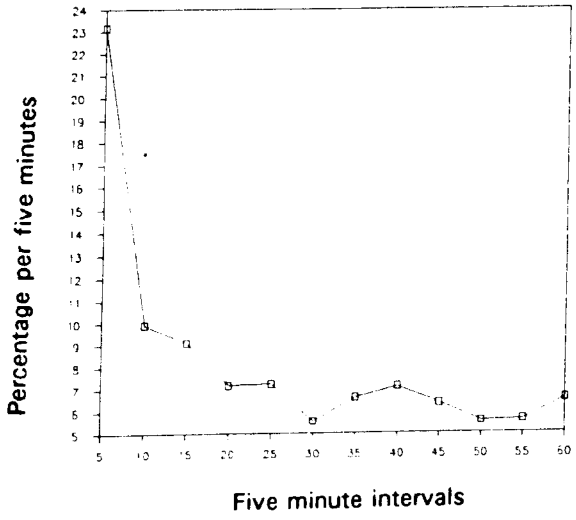
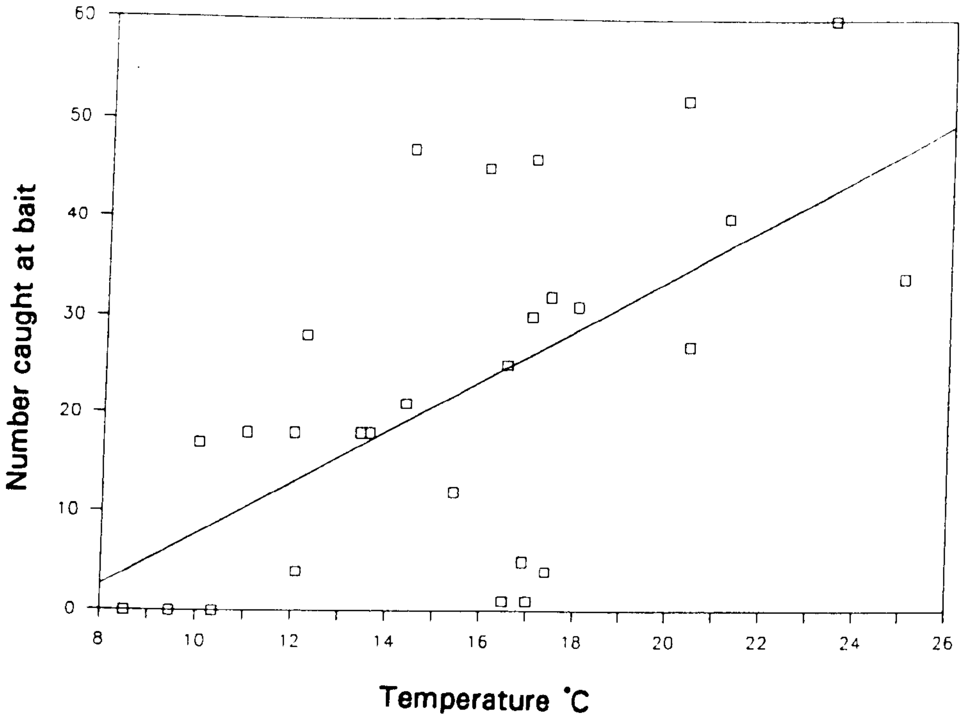


Fig. 3.24: Linear regression between temperature (recorded from a squirrel data logger) and the number of *Aedes cantans* caught at bait in 1991, Wood A.



mosquitoes coming in to bait. The weather was especially bad during June when peak biting normally occurrs.

In all three years, there was no significant correlation between the number of mosquitoes caught at bait and the mean daily temperature recorded at the Ness Gardens Weather station. Regression analysis also showed that there was no relationship between these two factors in all three years.

3.3.4.5 LONGEVITY

The mean life-expectancy of male Ae. cantans placed in cages following emergence and maintained on saturated sugar solution was 54 days, while the maximum was 93 days. The mean life-expectancy of females was longer than for males, being 69 days, with a maximum of 129 days. Males and females maintained on water had only a short life-span, with a mean of 10 days for males and 12 days for females.

3.3.4.6 GONOTROPHIC STATE AT BAIT

Generally, females arrived at bait as unfed individuals (with the ovaries in Stage IIb of development), but on rare occasions (<0.01%), adults were partially blood-fed. This happened in stationary bait catches in Wood A during both 1989 and 1990, and also in both years moving bait catches in the LSF and the 4USF.

3.3.4.7 ECTOPARASITES

All mosquitoes arriving at bait were checked for water mites but none was found until Ae. cantans had undergone more than one oviposition cycle, in other words water mites were found only on parous mosquitoes. All the water mites belonged to the genus Thyas (identification courtesy Dr.R. Wiles). The numbers attached ranged from one to seven per female. The first Ae. cantans arriving at bait having water mites were caught on 19 June in 1989 and 22 June in 1990. Less than one percent of all mosquitoes caught at bait had water mites attached. Most water mites were attached ventrally to the thorax. Although an attempt was made to identify the scars left by detatched water mites, none were found.

3.3.5 PARITY

3.3.5.1 PARITY CHANGES WITH TIME

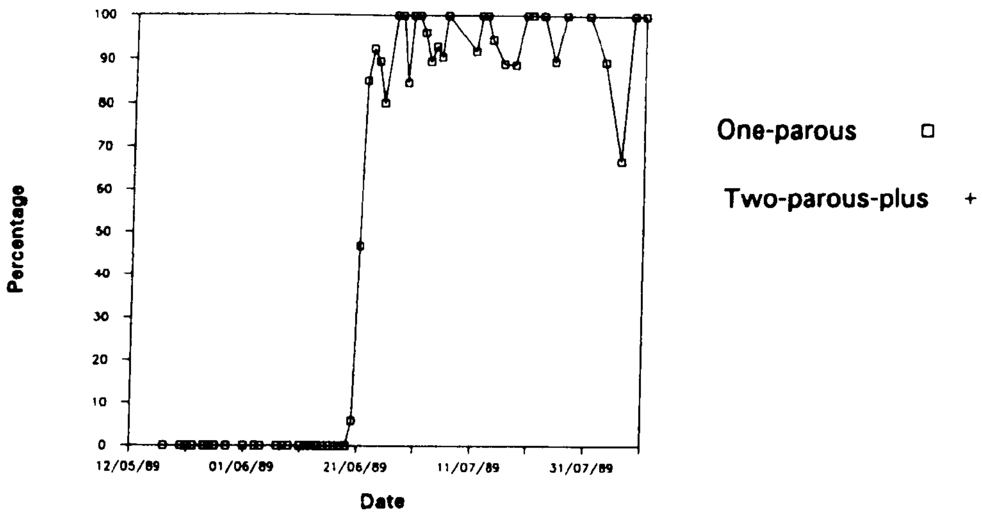
In all three years, there was a slow increase in the number of nulliparous mosquitoes caught at bait at all sites, in other words, recruitment to the biting population was initially slow. This may reflect the emergence pattern, with mosquitoes emerging at different times from different ponds.

In 1989, there was a rapid change-over from nulliparous to parous mosquitoes (Fig. 3.25). The first parous mosquitoes were caught biting on 20 June yet just two days later, 85% of the mosquitoes caught at bait were parous, and after three days, 92.3% were parous. This change-over was not as rapid in 1990 when it took eight days from the appearance of the first parous mosquito at bait until 70% of the population was parous, and 12 days for 90% of the population to become parous (Fig. 3.25). It took seven days from recording the first two-parous mosquito at bait for 70% of the biting population to become two-parous. The time elapsed between one and two parous is similar to the interval between nulliparity and parity noted above.

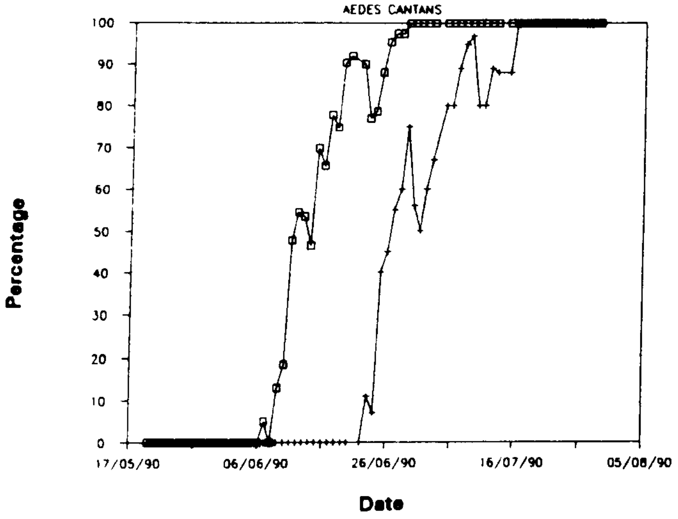
In 1991, the change from nulliparous to parous was the slowest of all three years, taking more than 12 days from the first parous female caught at bait until more than 80% of the population became parous.

Fig. 3.25: Change in parity with time.

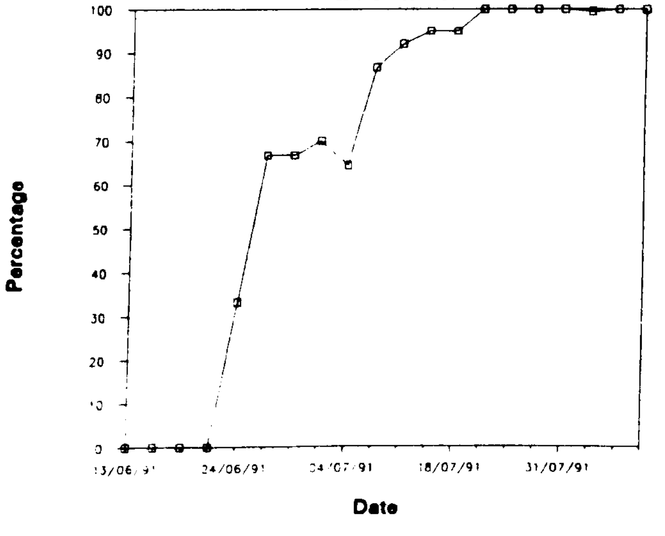
(a) 1989



(b) 1990



(c) 1991



3.3.5.2 MAXIMUM PARITY

In 1990, the greatest number of dilatations, five, was found in a female caught biting on 28 July. From emergence data this specimen was probably no older than 102 days, suggesting that the average duration of the gonotrophic cycles was 20.4 days.

The pattern of recaptures of mosquitoes marked at bait in 1989 suggests that female Ae. cantans can become three-parous by 16 July (Chapter 5), giving an average of 25.7 days per gonotrophic cycle. In 1990, when dissections for dilatations were undertaken, females reached the three-parous stage by 8 July (average gonotrophic cycle of 27.3 days) whereas the pattern of mark-release-recapture suggests that at least one mosquito reached the six parous stage by 15 August.

3.3.5.3 LATE NULLIPAROUS MOSQUITOES IN THE FIELD

In all three years, nulliparous mosquitoes were still arriving at bait late in the season, more than 60 days after mosquitoes were first caught biting and more than 26 days after the first parous mosquitoes were caught. These late nulliparous mosquitoes were always exceptionally small individuals with a wing length less than 4.6 mm long, compared with parous mosquitoes caught at the same time which had an average wing length of 5.2 mm. This suggests that, for some reason, small individuals are less sucessful in obtaining blood-meals than larger ones, although they can survive for many weeks.

3.3.6 SWEEP-NETTING

In 1989, sweep-netting vegetation was initially carried out in a wide variety of areas, including exposed and sheltered sites; however, no mosquitoes were captured by sweep-netting exposed sites such as the fields and grass verges along the roadsides. Consequently, sweep-netting was restricted to Woods A, B and C.

More than 99% of the mosquitoes caught by sweep-netting vegetation were Ae. cantans. The remaining species were Cs. morsitans, Cs. annulata, Cx. pipiens and Ae. punctor. Towards the end of the season, the numbers of other species, especially Cx. pipiens and Cs. annulata, increased slightly. Culex pipiens females with fat deposits were caught as early as August, indicating that they were ready to begin hibernation.

Most of the blood-fed mosquitoes were captured in Wood B, especially in 1989, and very few were caught in Wood A (Chapter 5). Table 3.12 shows the numbers and reproductive state of some of the Ae. cantans caught in sweep-netting vegetation. Woods B and C had significantly higher numbers of mosquitoes that were fully blood-fed to three-quarters gravid ($P<0.05$), whilst Wood A had the highest proportion of gravid individuals ($P<0.05$). All woods had a high number of resting unfed mosquitoes that were apparently ready to blood-feed. Figure 3.26 shows the percentages of adult mosquitoes caught at the various sites in 1989 and 1990, classified as unfed, blood-fed or gravid.

At the start of the season, in May, in both 1989 and 1990, Ae. cantans showed a 50:50 male:female sex ratio (Table 3.13). However, by July in both years, the number of females exceeds the number of males by more than four to one. Despite this, some males appear to be long lived, since the last male captured in 1989 was on 2 August and in 1990 on 15 September.

In agreement with results from bait catches, the greatest numbers of mosquitoes in both 1989 and 1990 were caught by sweep-netting vegetation in June, but, there was also a large number of females caught in May, despite few being caught at bait in that month, reinforcing the belief that there is a delay between emergence and blood-feeding. More Ae. cantans females were captured in sweep-

Table 3.12 : Number and percentages of blood-feds and gravids caught by sweep-netting vegetation.

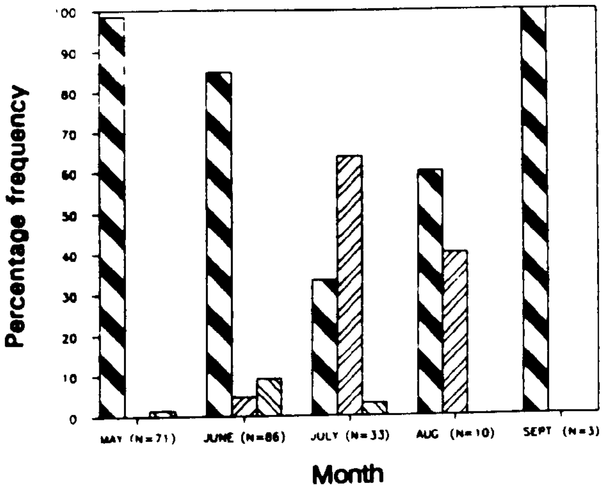
Year and Site	Total *Ae. cantans*	B-F N. (%)	Gravids N. (%)	Unfeds N. (%)	No. sns + mean n. per 45 min.
1989					
Wood A	203	10 (4.93)	29 (14.5)	164 (80.57)	14 14.5
Wood B	279	68 (24.37)	2 (0.7)	197 (74.93)	14 19.93
1990					
Wood A	332	23 (6.93)	28 (8.43)	295 (84.64)	14 23.71
Wood B	604	221 (30.3)	3 (0.5)	380 (62.91)	20 36.59
Wood C	242	87 (35.95)	2 (0.83)	153 (63.22)	13 18.62

B-F = Blood-feds to 3/4 gravid. N = Number SNS = Sweep nettings

Fig. 3.26: Percentage frequencies of adult _Ae_. _cantans_ caught by sweep-netting vegetation

(a) 1989

(i) Wood A



(ii) Wood B

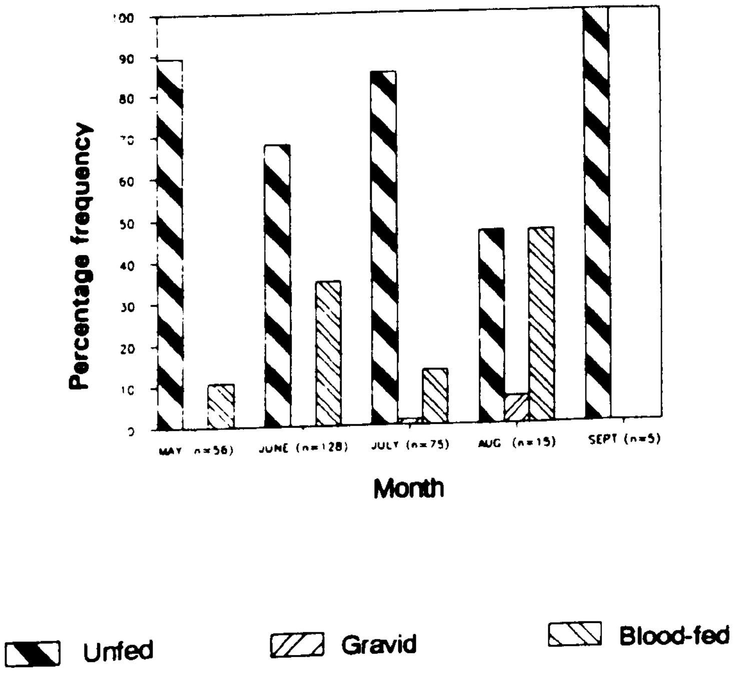
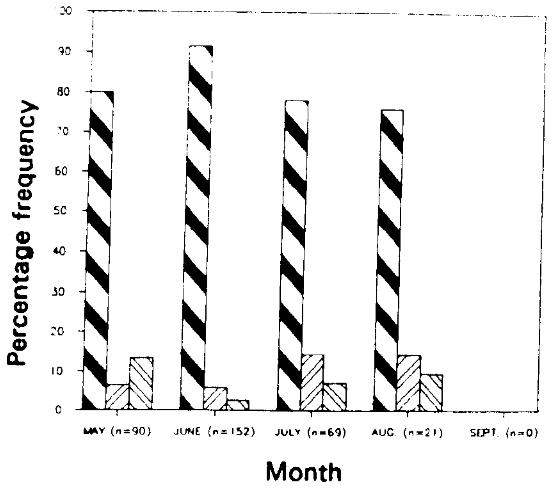


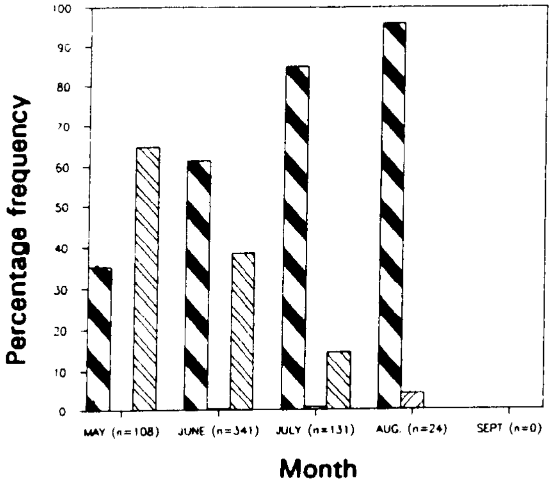
Fig. 3.26: continued

(b) 1990

(i) Wood A



(ii) Wood B



(iii) Wood C

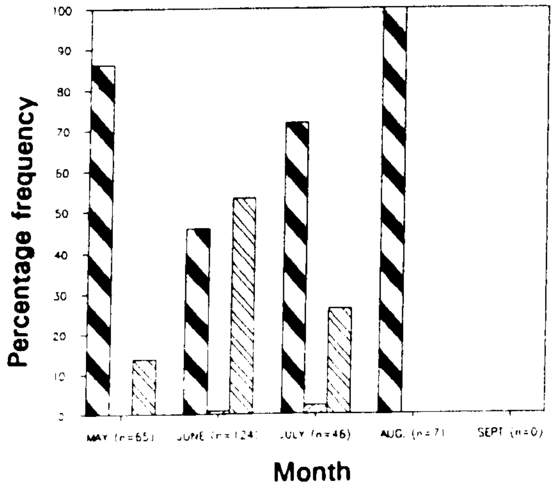


Table 3.13 : Numbers of male and female mosquitoes caught by sweep-netting vegetation.

Month	Number of Males	Number of Females
1989		
May	130	127
June	199	214
July	39	108
August	6	25
September	0	8
1990		
May	264	263
June	165	617
July	22	246
August	23	52
September	1	0

netting vegetation in 1990 than in 1989, confirming results of bait catches that the population of Ae. cantans was greater in the latter year.

Aedes cantans were checked for mites from the beginning of the season in May, and, the first ones were found on 4 July in 1989 and on 22 June 1990. These were identified as water mites belonging to the genus Thyas, and, as with adults caught at bait, they were found only on parous mosquitoes. Less than one percent of the mosquitoes were found to have these mites. After sweep-netting, the mites soon dropped off of the mosquitoes and therefore some mite-infested adults may have been missed. Numbers of mites per mosquito ranged from one to six, and most were attached ventrally around the anterior of the thorax. No male mosquitoes were caught with water-mites attatched.

3.4 DISCUSSION

3.4.1 EMERGENCE

3.4.1.1 PATTERN OF EMERGENCE

Emergence of Ae. cantans began in late April to early May in all three years and continued on average for 28 days. The last emergence was usually accompanied by the ponds drying up (Chapter 2). Bednets covered the whole of the Hotspot and Pond 9 in both 1989 and 1990 because the ponds began to dry up fairly early in the season. Consequently most of the adults emerging from these ponds would have been caught.

No adults emerged from the Hotspot and Pond 9 in 1991 because the ponds dried up early. Desiccation is a major cause of larval mortality in these and similar habitats in Ness Woods (Chapter 2).

In all three years in all four ponds sampled, male *Ae. cantans* emerged on average 2.4 days earlier than females, corroborating the findings of Service (1977*a*). Several authors have reported male mosquitoes emerging before females. For example, Washburn *et al.* (1989) found that the mean emergence time for males of the tree hole mosquito *Ae. sierrensis* in California preceded that of females by 20 days. Similarly, in Canada, Corbet and Danks (1973) found that male *Ae. impiger* and *Ae. nigripes* usually emerged one to three days before females, and in a given pond, females emerged over a longer period. In Scotland, Packer and Corbet (1989*b*) found that male *Ae. punctor* emerged two to six days before females.

There seems to be a general rule that males emerge before females, partly as a result of female larvae taking longer than male larvae to develop into pupae. In some species, such as *Ae. triseriatus*, male hatching from eggs has been found to precede that of females (Shroyer and Craig, 1981), and this could also lead to males emerging earlier. When male *Ae. cantans* emerge it takes two days or more for the terminalia to become rotated (Service, 1977*a*). Males are therefore at least two days old before they can mate, but as they emerge on average two days before females, they are ready to inseminate emerging females. Males therefore gain a competitive advantage by emerging before females, and natural selection would favour this difference in emergence pattern between males and females.

The seasonal timing of emergence varied between years, and also between ponds. There was an earlier emergence in 1990 (by 6-18 days) than in 1989, which in turn was earlier than in 1991. In 1990, for example, emergence began 14 days earlier in Pond 9 than in the Hotspot. These differences are due primarily to the effects of temperature. Pond temperatures in 1989 were significantly lower than in 1990, and temperatures in Pond 9 were higher than in the Hotspot (Chapter 2). The later emergence seen in the ponds in 1991 was partly due to lack of rainfall which meant that the ponds did not fill with water until March (Chapter 2), thus delaying egg hatching.

Packer and Corbet (1989*b*) similarly found that emergence of *Ae*. *punctor* occurred earlier, was more highly synchronised and finished sooner at warmer sites. Corbet and Danks (1973) found that in Canada the pond to pond differences in the timing of emergence of *Ae*. *impiger* and *Ae*. *nigripes* were usually related to water temperatures - development in warmer pools being faster.

There was an equal sex ratio of emerging *Ae*. *cantans* in all three years, except in Pond 8 in 1990, where significantly more males emerged. Distorted sex ratios have frequently been encountered in other studies. Washburn *et al*. (1989), working on *Ae*. *sierrensis* in California, found that the sex ratio from different tree holes varied significantly and was affected by tree holes drying up before emergence was completed. Since males emerge first, if a pond or tree hole dries up, it is likely that there will be more males than females emerging. This is the most probable explanation for the situation in Pond 8 in 1990 as this pond dried up before emergence was completed and so more males emerged than females.

Snow (1987*b*) working in southern England also found that more males of *Ae*. *cantans* and *Ae*. *punctor* emerged than females. Snow believed that mortality may operate in favour of males because females spend longer in the aquatic stages and so should have higher pre-emergence mortality.

Packer and Corbet (1989b) found that in Scotland there was an excess of female Ae. punctor, in 1984 (55.4-63.3%) and in 1985 (61.8-66%). They considered that this might have been due to female larvae being more viable than males. Alternatively, they felt that it may have been due to a sampling bias. Males emerged before females when the ponds were larger, and, because the cages covered only part of the ponds, it is possible that not all emerging males were trapped. As the season progressed the habitat dried up increasing the concentration of females under the emergence cages, and therefore higher numbers were collected (Packer and Corbet; 1989b). Danks and Corbet (1973) compared the sex ratios at emergence of Ae. impiger and Ae. nigripes from temporary and permanent ponds in Canada. They found that there was a predominance of females emerging and this may have been due to higher mortality of male larvae.

Generally, a 50:50 male to female sex ratio is the most stable in evolutionary terms and therefore departures from 50:50 are likely to be the result of external factors rather than reflecting genetic factors.

In Monks Wood, near Cambridge, Service (1977a) found that female Ae. cantans were not caught at bait until 20 days after emergence. In this study, it took a minimum of only four days for an individual marked at emergence to come to bait, but the mean was considerably greater at 17 days (Chapter 5). Service found that the ovaries of newly emerged females were in Stage I of Christophers at emergence, as were the ovaries in newly emerged mosquitoes in this investigation from both the laboratory and the field. Ovaries in Stage I need to develop to Stage II before the female will start host-seeking (Christophers, 1960). The first Ae. cantans females to arrive at bait had their ovaries in Stage IIb of development. Similarly, it was only at

Stage IIb that mosquitoes maintained on sugar solution in cages following emergence would accept a blood-meal. O'Meara and Haeger (1985) found that Aedes salt marsh mosquitoes were reluctant blood-feeders while their ovaries were in pre Stage II of development. Service (1977a) also found that it was difficult to get newly emerged Ae. cantans to feed in the laboratory and that a proportion require two blood-meals for egg development. In my experiments Ae. cantans that were maintained in cages with sugar solution in the field, took 15 days to mature their ovaries from Stage I to IIb, and this compares well to the mark-release-recapture period. The rate of development seems to be related to temperature, with a faster development to Stage II at warmer temperatures, and also size of the mosquito, with larger mosquitoes developing faster.

3.4.1.2 SIZE AT EMERGENCE

The wing size of emerging Ae. cantans corresponds extremely well with the larval siphon measurement data from the Hotspot and Pond 9 in both 1989 and 1990. In the first year, larval siphons in Pond 9 were significantly larger than in the Hotspot (Chapter 2) and a similar size difference was found with the adult wing measurements, that is, Pond 9 adults were significantly larger. Similarly, in 1990, there was no significant difference in the siphon lengths of Ae. cantans between the Hotspot and Pond 9 and no significant difference between the wing length of adults emerging from these ponds. The size differences seen between larvae from different ponds were therefore also seen in the adults emerging from these ponds.

There was no significant difference between the size of males and females emerging from the Hotspot and Pond 9 in all three years. Similarly, Washburn et al. (1989) found that the wing lengths of male and female Ae. sierrensis emerging from the same tree hole were highly correlated with each other. They also found that there was a positive correlation between the maximum volume of a tree hole and the number of adults emerging which is not surprising since volume is a measure of habitat size and larger habitats generally support more animals. However, in the present study there was no significant correlation between pond volume and size of adults emerging from it, and therefore another factor is involved in determining adult size (Chapter 2).

The large variation in size seen in Ae. cantans emerging from the ponds (min wl = 3.5mm , max wl = 6.2mm) has been observed in other species. McCombs (Haramis, 1983) found that container breeding mosquitoes vary markedly in size and that variation in diet can produce adults that differ seven times in dry weight. Fish and Carpenter (1982) found that Ae. triseriatus collected in the field were often smaller than those reared in the laboratory, and thought this probably resulted from competition for limited nutrients in tree holes.

In all three years there was a highly significant decrease in the wing lengths of emerging Ae. cantans as time progressed. This is most likely due to seasonal increases in pond temperature. As the pond becomes smaller and warmer, development is speeded up and the emerging mosquitoes are smaller. Similar seasonal decreases have been reported in many other mosquito species. For example, Bock and Milby (1981) observed that the weekly mean wing length of Cx. tarsalis emerging in California was smallest when temperatures were highest, and there was a significant negative correlation with temperature. They stated that higher temperatures accelerate larval metamorphosis so that the capacity to form tissue is inhibited. Packer and Corbet (1989a) found that the wing length of Ae. punctor decreased as emergence progressed in one year but not in another. Brust (1967) noted that in Ae. vexans, Ae. nigromaculis and Cs. inornata, larval development time decreased with increasing rearing temperature. Similarly, in South Africa, Le Sueur and Sharp (1991) found that wing length and larval head capsule width was negatively related to temperature in An. merus. They believed that metabolic requirements at higher temperatures may exceed the rate at which food can be gathered by the larvae. Temperature therefore appears to be a major factor in determining size of mosquitoes and the speed of larval development and metamorphosis.

Males which pupate first will be capable of inseminating the earliest emerging females and since larger females pupate earlier and produce more eggs than smaller females, these earlier males will have a potentially higher reproductive contribution compared to males emerging later.

The density-dependent size variation which I have observed between larvae in different ponds (Chapter 2) is reflected in the size of adult mosquitioes emerging from these ponds. It is reasonable to conclude that the size of emerging adults is influenced by a combination of density-dependent factors (e.g. food supply) and density-independent factors (e.g. temperature).

3.4.2 SIZE AND SURVIVORSHIP

Nasci (1990) compared mosquito wing length to adult body weight. He found that the proportional relationship of wing length to adult weight is highly variable and that unique regression formulae are required for each mosquito species, and moreover for each sex. Also, the relationship between wing length and weight differed within a species among specimens reared in the laboratory versus those collected from the field. In this investigation, as with that of Service (1977a), the wing length and body weights of Ae. cantans from both the laboratory and the field were well correlated. There was no significant difference in the regression formulae for males and females in this investigation.

In both 1989 and 1990, the size of Ae. cantans females coming to bait increased as the season progressed. In 1989, parous mosquitoes were significantly larger than nulliparous mosquitoes and in 1990, two-parous-plus mosquitoes were significantly larger than one-parous mosquitoes which were in turn significantly larger than nulliparous females. This observation is particularly important because it suggests that larger mosquitoes are longer lived than smaller ones, and therefore, size is positively related to survivorship and also ability to blood-feed.

Both laboratory and field studies have shown that for several mosquito species increased survivorship is positively correlated with size. On the other hand, some investigations have failed to demonstrate any such relationship. Examples of both situations are given below.

Haramis (1983) caught Ae. triseriatus adults and, using wing length as a measure of body size, divided them into three size classes; small, medium and large. The percentage of nullipars and parous mosquitoes in each size-class was then calculated. If large females have a higher survival rate than smaller females, or are more successful at blood-feeding, then large females should have an increased probability of being parous or gravid. In the Haramis investigation significantly more large females were gravid or parous than those in the two smaller size classes, thus indicating increased success. Laboratory experiments also showed that longevity increases with size.

Nasci (1987) examined wing length and parity in Aedes sollicitans (Walker), Aedes taeniorhynchus (Wiedemann) and Anopheles crucians Wiedemann in Louisiana.

He found that larger body size in Ae. taeniorhynchus and An. crucians led to increased survival and increased blood-feeding success, and concluded that body size in a population is a useful indicator of the population quality. Aedes sollicitans, however, showed opposite results, but Nasci (1987) considered that possibly the large mosquitoes may have dispersed further than the smaller ones, and were therefore under caught.

Packer and Corbet (1989b), working on Ae. punctor in Scotland found that larger females were more successful at locating hosts, developed more egg clutches and generally lived longer than did smaller ones. This suggests that larger females enjoy greater reproductive success than smaller females. They also found a decrease in the size of mosquitoes in May and June but an increase in July. As in the Ness Woods investigation, the initial decrease in May and June was related to the reduction in size of females observed as emergence progressed. The increase in size in July reflects the higher survival among larger females. Packer and Corbet (1989b) reported that survival among the largest classes was lower than for slightly smaller individuals. This may have been due to increased risks of predation, as many predators show preferences for larger prey (Begon et al., 1980). Also it is possible that larger individuals are more prone to emigration as larger individuals are generally stronger fliers (Nayar, 1969).

In Florida, Lounibos et al. (1990), found that parous rates of Mansonia dyari were correlated with wing length, indicating size-dependent survivorship. Also in the USA, Nasci (1986) examined body size and parity in Psorophora columbiae (Dyar and Knab), Aedes atlanticus Dyar and Knab, Ae. vexans and Culex salinarius Coquillett. He found that parous Cx. salinarius and Ae. vexans caught at bait had significantly longer wings than nulliparous females, but found no significant difference between parous and nulliparous mosquitoes in the other two species.

In contrast to these observations that size and parity are positively related, several workers have failed to find any such evidence. For example, Mori (1979) found no difference in the probability of daily survival between small and large male and female Aedes albopictus (Skuse) however smaller ones were caught biting in lower numbers. Similarly, Walker et al. (1987) reared large and normal sized adults of Ae. triseriatus and Ae. hendersoni and, by mark-release-recapture found no difference in the probability of daily survival for either sex or species, although in Ae. triseriatus females normal sized individuals were captured in smaller numbers. Washburn et al. (1989) found no correlation between wing size and longevity in Ae. sierrensis breeding in Californian tree holes. Similarly, although Landry et al. (1988) found that there was good correlation between wing length and body weight of Ae.triseriatus in southern Wisconsin, there was no good evidence that increased size led to increased survival. They concluded that survivorship was not affected by the size of the adult female.

The obvious question to ask is; Why should larger females survive longer than smaller females? Haramis (1985) found that larger female Ae. triseriatus lived longer when exposed to desiccation than smaller ones. Smaller mosquitoes also emerge with smaller fat reserves than larger ones and so may be less able to withstand the effects of starvation (Steinwasher, 1982). Larger mosquitoes may initiate host-seeking sooner, locate hosts more effectively and have a greater chance of securing a blood-meal after attacking the host, especially since smaller females have a decreased flight range and are weaker fliers (Nayar, 1969). Klowden et al. (1988) found that suboptimal larval nutrition resulting in smaller adult mosquitoes can significantly affect the ability of females to find a host. Larger females may also be more successful in producing a clutch of eggs because of increased reserves at emergence, and have the ability to take a bigger blood-meal. Atkinson and Begon (1988) stated that there was an unequivocal disadvantage for insects to be small and late.

The greater survivorship of larger females will increase the probability of their realising their reproductive potential.

In contrast to the many studies on females, few studies have examined the relationship between size and survival in males, but Reisen et al. (1984) found that survival of Cx. tarsalis males increases with increasing body size.

Aedes cantans females emerging from Ponds 8, 9 and 13 and the Hotspot (Fig. 1.2) were significantly larger than those caught biting. This seems to indicate that these ponds are producing larger mosquitoes than the others in the woods, such as Ponds 7, 11 and 12, which are also contributing to the population. This may be due to the fact that these temporary ponds are more suited to production of Ae. cantans and therefore the adults emerging from them are larger, but sufficient numbers of smaller individuals are emerging from the other ponds to reduce the overall size of the population.

3.4.3 OVIPOSITION

3.4.3.1 WING LENGTH AND NUMBER OF EGGS

This study substantiates the findings of Service (1977a) in southern England that there is a good correlation between wing length and the number of eggs laid. Similarly, Elmo (1987), found that there was a positive correlation between the wing length and the number of ovarioles in nulliparous Ae.cantans caught in Ness Woods (N=38, r=0.82).

Similar relationships between wing length and egg number have been reported for several different mosquito species. For example, Bock and Milby (1981) found that there was a strong positive correlation between the wing length of Cx. tarsalis and the number of eggs laid. Small females imbibed less blood and produced fewer eggs than larger ones. In California, Washburn et al. (1989) also observed a significant correlation between female wing length of Ae. sierrensis and number of eggs in the first clutch. Packer and Corbet (1989a), found that wing length of Ae. punctor correlated well with potential fecundity, namely the number of ovarioles, but less well with the actual fecundity, namely the number of eggs laid. Mokry (1984) found that fecundity was positively correlated with wing length in Ae. canadensis, Ae. punctor, Ae. absErratus and Ae. communis collected in Newfoundland. Colless and Chellapah (1960), working in the laboratory on Ae. aegypti, found that there was a positive correlation between body weight and both the weight of blood ingested and the number of eggs laid. Barr et al. (1986) found that egg rafts of Culiseta incidens (Thomson) collected from the field in the spring in southern California contained more eggs than rafts laid at other times of the year. Females emerging in spring had developed at lower temperatures, from less crowded larvae and were therefore larger than females emerging at other times of the year.

Not all authors, however, have reported a relationship between body size and number of eggs. For example, neither Roy (1936) nor Christophers (1960) found any correlation between body weight and number of eggs in Ae. aegypti.

3.4.3.2 WING LENGTH AND EGG SIZE

The size of eggs and the wing length of the females are not well correlated suggesting that larger Ae. cantans females do not lay larger eggs. In contrast, Reisen (1975), found that smaller An. stephensi females laid smaller eggs.

In addition, eggs from larger Ae. cantans did not produce significantly larger larvae than eggs from smaller adults, when these were hatched and reared at different densities.

3.4.3.3 GONOTROPHIC CYCLES AND NUMBER OF EGGS

In the present study, there was no significant reduction in the mean number of eggs laid by parous Ae. cantans compared with nulliparous females. However, it should be noted that the females caught later in the season were larger and therefore the number of eggs laid could actually be decreasing but the effect may be masked by the fact that the larger females are laying more eggs than the overall smaller nulliparous ones.

Because all Ae. cantans died in the laboratory after they had laid one set of eggs, it was not possible to observe any decrease in the number of eggs laid with increasing parity in individual mosquitoes. Service (1977a) observed that in the laboratory most adult Ae. cantans died after the first oviposition, but was able to conclude on the few that did not that there was a significant reduction in the numbers of eggs laid in later batches. The decrease in the number of eggs with increasing parity results from the increase in the number of follicles which degenerate during the later gonotrophic cycles. Putnam and Shannon (1934) found that in Ae. aegypti, each successive egg batch contained 15% fewer eggs than the one which preceded it. Clements (1963) stated that the degeneration of follicles with increasing parity eliminates the relationship between body weight and size of the egg batch in An. messeae.

Aedes cantans females have the capacity to produce several clutches of eggs in their lifetime. The potential and actual fecundity per clutch are positively correlated with female size. Blood-source, blood-meal size and reproductive age are also important (Clements, 1963) in determining the number of eggs laid per clutch. Since larger females are longer lived, they have the chance to produce more egg clutches and since they also produce larger clutches, the expected lifetime fecundity will increase with increasing female size.

3.4.4 BAIT CATCHES

3.4.4.1 SEASONAL INCIDENCE

Mosquitoes first came to human bait in late April to early May. In the first two years, *Ae. cantans* were caught in their highest numbers in June, after which, the population declined until September when no more adults were caught. In 1991, *Ae. cantans* were caught in their highest numbers in July. Peak biting was delayed until July by a combination of the late onset of emergence and also by poor weather conditions in June. Similarly, Sulaiman (1982) found that maximum numbers of *Ae. cantans* caught biting per hour in Ness Woods, was in June in his first two years (79.0 and 34.8) while in the third year most were caught in July (25.4). He also found that the smallest population was in September, and adults died off during this month.

The largest biting catches of *Ae. cantans* were in 1990, with 11,120 mosquitoes caught in 308 bait catches, giving an average of 36.0 per hour, followed by 1989 when 4,061 mosquitoes were caught in 140 hourly bait catches, with a mean of 29.0 per hour. In 1991, 3019 *Ae. cantans* were caught in 145 bait catches, averaging 20.8 mosquitoes per hour. These differences were also reflected in the numbers caught by sweep-netting vegetation. In addition, the population estimates from mark-release-recaptures in 1990 were much higher than those in 1989 (Chapter 5). It seems that relatively simple human-bait collections can give reliable comparative indices of population size.

The lowest parous rate of the three years was recorded in 1989, followed by 1990 and then 1991. This suggests that either there was a higher mortality in 1989 between the nulliparous and parous stage, or that the mosquitoes were having difficulty in obtaining a blood-meal and so were unable to become parous. The high rate in 1991 may be due to the poor weather conditions early in the bait catch season which may have limited the number of nulliparous mosquitoes caught at bait. The large number of parous mosquitoes caught suggests that although the weather conditions may have delayed the cycle, many mosquitoes still managed to obtain a blood-meal and oviposit.

In 1989, the first *Ae. cantans* caught at bait was on 18 May and the last was caught 87 days later. In 1990 the first *Ae. cantans* at bait arrived on 25 April, and the last one 124 days later. The length of the biting season compares well with the 106, 114 and 99 days recorded in the years 1979-1981 by Sulaiman (1982).

The life expectancy of male *Ae. cantans* maintained on saturated sugar solution and maintained in cages outside was a maximum of 93 days and a mean of 54 days. The longest lived female mosquito survived 129 days, whilst the mean age was 93 days. These compare reasonably well with the survival of females in the field estimated by mark-release-recapture studies, where the longest lived recaptured mosquito was 72 days old. Service, (1977*a*) found that life expectancy in the laboratory was a mean of 66-68 days and a maximum of 119 to 126 days.

Unfed mosquitoes predominated at bait and were usually the only category caught, although occasionally blood-fed mosquitoes did come to bait. All the Ae. cantans females caught at bait that were dissected for parity were found to be inseminated. Sulaiman (1982) found that females caught were largely unfed individuals but in his three year investigation, he caught a single gravid Ae. cantans at bait.

3.4.4.2 RESTING AND FEEDING BEHAVIOUR

Aedes cantans were caught biting in large numbers during the day in Wood A, but none was caught in any of the fields outside the woods, whether grazing animals were present or not, even when the weather conditions were warm and still. During the early evening, mosquitoes were caught in large numbers in bait catches performed in both Wood A and the surrounding fields. This is a pattern of behaviour observed in British mosquitoes by Campbell (1986), Service (1969, 1971b) and Sulaiman (1982).

Service (1971b), working in Poole in Dorset, found that there was little movement of resting mosquito populations during the day, unless a suitable bait entered the area, or resting sites became exposed to the sun. During the day, very few mosquitoes were attracted to human bait in exposed areas, but at night, substantial numbers of hungry females were caught in these sites. In Sweden, Nielsen and Greve (1950) found that Ae. cantans spent the day resting in sheltered vegetation.

In Ness Woods the mosquitoes resting amongst vegetation during the day, although not actively involved in host-seeking, would readily feed if a host was in the immediate area. Such behaviour is known as opportunistic host-feeding. Service (1971b) noted that resting mosquitoes were attracted to bait within a range of seven to ten metres. In my woods, adults actively flew out of the woods at night in search of hosts and were consequently encountered biting in both sheltered and exposed sites. Service (1969, 1971b) also observed this behaviour in southern England and felt that night-time hunting was under the influence of an endogenous biting rhythm. McCrae et al. (1976) observed similar behaviour in Anopheles implexus Theobald in Uganda. They found unfed females sheltering during the day in vegetation exhibited a passive or opportunistic attack when activated by the close proximity of a suitable host. During the evening this behaviour changed to an active, hunting flight, mainly outside the forest. Similarly, Jaenson (1988) found the daytime activity of Ae. cinereus, Ae. communis and Ae. punctor in Sweden was low or absent in exposed fields, because of their preference to rest in dense vegetation, which provides relatively stable micro-climates.

3.4.4.3. FIVE MINUTE INTERVALS

During afternoon bait catches the greatest proportion of Ae. cantans was caught in the first five minutes, a phenomenon that has also been observed by Sulaiman (1982) and Service (1977a). A shift of only a few yards can result in another high catch (Gillett, 1967, Service, 1969). Service (1977a) and Sulaiman (1982) believed that the high initial catch comprised mosquitoes from the immediate vicinity of the bait, after which there was a depletion of the large resting population of unfed hungry females and only those arriving from further afield were caught. Similar observations were made by Jaenson (1988) in Sweden.

I believe that the high initial catch was due to mosquitoes which followed me from my entry in the wood to the bait catch site. As I walked through the vegetation, large numbers of mosquitoes flew up and, instead of resettling, followed me to the bait site. In support of this I observed that when I was accompanied to the field and left a colleague to perform a bait catch half-way between the entrance to the wood and my bait catch site, my initial five-minute catch was significantly reduced.

Roberts and Scanlon (1975) also observed a depletion effect in *Aedes atlanticus* Dyar and Knab, *Aedes tormentor* Dyar and Knab and *Psorophora ferox* (Von Humboldt), finding that the initial attack rate was high and then decreased exponentially. They felt that this was due to a sudden response to host movement followed by a depletion once host movement had ceased. Nishimura (1982) also found high initial catches of *Ae. albopictus* and *Aedes japonicus* (Theobald) during the first ten minutes of human bait catches in Japan. He felt that these mosquitoes must be actively host-seeking, and that mosquitoes were attracted at distances of up to four to nine metres.

Adults also arrived at bait in waves. This may be due to changes in the drift of host odour causing the simultaneous stimulation of small groups of resting mosquitoes which then arrive at bait more or less together. Ahmadi and McClelland (1985) also observed mosquitoes arriving in waves at bait and suggested that this may be due to air currents, kairomones or feeding mosquitoes assembling.

3.4.4.4.BAIT CATCHES AND WEATHER CONDITIONS

Both 1989 and 1990 were characterised by good weather conditions, with little rain or wind and generally high temperatures. There was no correlation between the mean temperature recorded at Ness Gardens and the number of mosquitoes caught at bait in either 1989 or 1990. In 1991, however, a period of cold weather in early June had a large effect on the number of mosquitoes caught at bait, drastically reducing the numbers caught in all sites and at all times. The combination of cold temperatures and windy and rainy conditions reduced the catch to practically zero, especially in the more exposed fields, at a time when the population was peaking in terms of numbers caught per hour in the previous two years. In 1991, when the temperatures were recorded at the bait catch site using a squirrel data logger, there was a significant correlation between the numbers of mosquitoes caught at bait and the mean temperature. There was also a positive linear regression between these two factors. There was no correlation or regression between the mean temperature recorded at Ness Gardens and the bait catch numbers in 1991, and perhaps this suggests that the temperature data from Ness are either too general since they provide only the daily maximum and minimum temperature, or alternatively, the temperatures are more localised in the woods.

A combination of cool temperatures, windy conditions and heavy rainfall all have the effect of reducing the number of mosquitoes caught at bait. In this investigation, adult behaviour was not especially affected by adverse weather conditions, and it was larvae that were most affected by three successively dry winters (Chapter 2).

Gavin (1986), also found that there was no significant correlation between the numbers of Ae. cantans biting in Ness Woods and wind speed, temperature and hours of sunshine recorded at Ness Gardens. On the other hand, Majala (1987) when working on Ae. cantans in Ness Woods found that as temperature increased, more mosquitoes were caught at bait and this number increased above 13°C, below which very few were collected. He felt that Ae. cantans had a lower temperature threshold of 13°C below which the adults did not fly. M.H.Birley et al. (unpublished) working in the same woods stated that there was a correlation between sample size and mean temperature ($R=0.53$, $n=35$, $P<0.05$). They also believed that there was a log-linear relationship between the total sample per evening and mean temperature. However, the year in which they worked in the woods was characterised by poor weather conditions and this may have exaggerated the relationship.

Packer and Corbet (1989b) reported that the prevailing weather conditions had a marked effect on the numbers of Ae. punctor caught biting, and that adverse weather such as wind and rain accounted for small samples. Similarly, Jaenson (1988) found that wind and low temperatures were associated with reduced or no biting activity in Sweden.

3.4.4.5 MOVING BAIT CATCHES

When moving bait catches were carried out during the day within Wood A, large numbers of Ae. cantans were caught. In fact they were undertaken when mosquitoes were needed in large numbers for mark-release recapture work. This behaviour contrasts with the findings of Service (1971c) who found that mosquitoes were not caught by slowly walking through vegetation during the day. He believed that a moving bait does not stimulate the resting population in time for them to fly out, locate and settle on the collector.

3.4.5 PARITY

3.4.5.1 RATE OF CHANGE FROM NULLIPAROUS TO PAROUS MOSQUITOES

In all three years, there was a slow recruitment of nulliparous mosquitoes to the biting population, reflecting the emergence pattern. In 1989, the change from nulliparous to parous mosquitoes was extremely rapid, taking only three days from the first parous mosquito caught at bait until more than 90% of the population was parous. In 1990 and 1991, this change-over was not as rapid and took more than seven days. Similarly, the change-over from one-parous mosquitoes to two-parous mosquitoes in 1990 was slower, but mirrored the earlier change from nulliparity. The very rapid change from nulliparous to parous Ae. cantans in 1989 may reflect the rapid phase of the nulliparous increase, suggesting that the mosquitoes that were first caught nulliparous were not the first ones to be caught parous (in other words they were not sampled).

The fact that the change from nullipars to one-pars, and one-pars to two-pars, in 1990, was similar indicates that the mosquitoes were not having difficulty in obtaining a blood-meal, and also that the population was well synchronised. In contrast, Packer and Corbet (1989b) found that the recruitment of nulliparous Ae. punctor to the biting population was rapid but that the change from nulliparous to parous was slower. They considered that this may reflect mortality of nulliparous mosquitoes and/or difficulty in obtaining a blood-meal.

In 1990, it took 44 days from the first nulliparous mosquito to the first one-parous mosquito to be caught, but only 20 days from the first one-parous to the first two-parous mosquito, indicating that the duration of the oviposition and gonotrophic cycle shortened as the season progressed. This could be due to increasing temperatures speeding up blood digestion, but possibly to females becoming more efficient at getting a blood-meal and therefore returning to bait quicker. In addition, oviposition experiments in 1991 revealed that many of the Ae. cantans females which came in to bait at the start of the season required two blood-meals to lay eggs. This would cause a delay in the recruitment of the first parous mosquitoes. Nevertheless, even 20 days is a long time between refeeding, because Service (1977a), by carrying out oviposition experiments in the laboratory at different temperatures, has found that in the summer, Ae. cantans should be able to feed every seven to eight days. Something else is therefore extending the length of the cycle. Most adults have to fly out of the woods at night to feed on livestock, and whereas sheep and cows are plentiful in some fields, if the host-seeking adults flew out in the 'wrong direction' they would enter fields without livestock. It is possible that there are several nightly excursions in which Ae. cantans fails to find hosts. It is hard to believe that the extended period between feeding is due to difficulties in finding oviposition sites, as within the wood there are plenty of suitable larval habitats.

3.4.5.2 LATE NULLIPAROUS MOSQUITOES IN THE FIELD

In all three years there were significant numbers of late nulliparous mosquitoes appearing at bait and these were significantly smaller than the average size of other mosquitoes being caught at the same time. In 1989, nulliparous mosquitoes were caught up to 35 days after the first parous mosquitoes, in 1990, up to 47 days after the first parous mosquito and in 1991, up to 41 days. Similarly, Sulaiman (1982) found nulliparous Ae. cantans in Ness Woods up to 55 days after the last emergence. There were therefore some female Ae. cantans remaining nulliparous without blood-feeding for up to 55 days.

Since all the late nulliparous mosquitoes were small, it is possible that they were having difficulty in obtaining a blood-meal. Packer and Corbet (1989a) suggested that larger mosquitoes initiated host-seeking sooner, located hosts more effectively and had a greater chance of obtaining a blood-meal after attacking the host. Small females may therefore take longer to successfully blood-feed and so the change from nulliparous to parous will be delayed. Moreover, since oviposition experiments suggest that nulliparous Ae. cantans require two blood-meals before they can lay eggs, and if the smaller mosquitoes are weaker fliers and less efficient at locating hosts, the requirement for two blood-meals will delay them even further from becoming parous. Furthermore, because the ovaries of larger mosquitoes develop to Stage IIb more quickly than smaller ones, they will appear at bait earlier in the first place and thus get even more of a 'head start'. The requirement for two blood-meals for the maturation of the first batch of eggs has been found in several other aedine mosquitoes (e.g. Feinsod and Spielman, 1980) and some years ago for Ae. cantans (Service, 1977a).

Packer and Corbet (1989a) found that nulliparous Ae. punctor persisted in the field long after emergence had ended, and they felt that this was because blood-meals were difficult to obtain, in other words host availability was the limiting factor.

3.4.6 AGE-DETERMINATION

The dilatation results in this study agree with the findings of Hoc and Charlwood (1990) who also worked on Ae. cantans in Ness Woods. They believed that dilatations only form from follicles that degenerate at a very early stage in the gonotrophic cycle. The number of ovarioles that have follicles that degenerate each gonotrophic cycle decreases with increasing parity. It is assumed that follicles only degenerate once in each cycle and when Ae. cantans required two blood-meals to mature eggs in the oviposition experiments, no degenerations were observed after the first blood-meal. In my study, the number of ovarioles per ovary that could be read for parity were similar to those found by Hoc and Charlwood (1990). These proportions, with those found by Hoc and Charlwood (1990) in brackets, are as follows; 27% (21%) for the first cycle, 8% (8%) for the second, 6% (5%) for the third and <1% (<1%) for the fourth cycle. These results tend to support their theory that dilatations form only as a result of degenerating follicles. Moreover, it seems unlikely that when an egg passes down the oviduct, the oviduct should maintain the dilatations left from previous ovipositions (since this would be a traumatic event).

The greatest number of dilatations found in any one mosquito was five (28 July 1990) and this was found in only one ovariole. Mark-release-recapture studies (Chapter 5) suggest that many mosquitoes reached the three-parous stage in both 1989 and 1990, and it appeared that one mosquito could have been six-parous. Hoc and Charlwood (1990) found one Ae. cantans that was at least four-parous, but found that the mean age of the population was 2.03 parous.

3.4.7 SWEEP-NETTING VEGETATION

In agreement with human-bait-catches, more Ae. cantans were captured by sweep-netting vegetation in 1990 than in 1989, reflecting a higher population in the second year. Also in both years, most adults were caught in June.

The lack of mosquitoes caught in the fields surrounding the woods shows that mosquitoes do not rest in exposed sites during the day, corroborating the findings of Service (1971b) in southern England. Sulaiman (1982) found no significant difference between the number of Ae. cantans caught by sweep-netting ferns, brambles and grasses in Ness Woods, but he caught markedly fewer Ae. cantans resting on tree-trunks. Similarly, in this study, there were no differences in the numbers of mosquitoes caught resting on ferns or brambles.

A possible explanation for the large number of blood-fed mosquitoes found in Wood B and the large number of gravids collected in Wood A, is as follows. Mosquitoes came out of Wood A at night to hunt in the fields of Leahurst, where there were large numbers of grazing animals, and after blood-feding, rested in the sheltered vegetation of Woods B and C. After having digested their blood-meals they returned to Wood A, which had the largest number of ponds, to oviposit (Fig.1.2).

The rate of decline in the numbers of males caught by sweep-netting vegetation from May to September in both 1989 and 1990 suggests that male mortality is much higher than that of females. Sulaiman (1982), during his three-year study in Ness Woods, found that the last male Ae. cantans were caught six to ten weeks before the last females, again suggesting that male longevity was considerably less. He also found that the sex ratio decreased from 1:1 in May to more than 4 to 1 females to males in June, July and August. However, some males do survive for the whole season, since in the present study a few males were caught in late September.

Sweep-netting vegetation has the advantage of sampling the non-biting population, including blood-fed females and males. It also samples mosquitoes that are not attracted to human bait, such as the ornithophagic Culiseta morsitans. The main disadvantage of sweep-netting is that mosquitoes are frequently damaged and therefore they cannot be used for other work such as mark-release-recapture studies or for oviposition experiments. However, sweep-netting caused less damage than the 'univac' suction sampler which destroyed mosquitoes almost beyond recognition. Another disadvantage is that it is difficult to standardise sampling, except possibly in terms of time or number of sweeps.

3.4.8. ECTOPARASITES

Mites of the genus Thyas, comprising the so-called jumping water-mites, were found only on parous Ae. cantans and tended to cluster on the thorax towards the head. Mites as indicators of nulliparity or parity have been reported several times. Gillett (1957), for example found that mites were mainly found on nulliparous Mansonia africana, and could be used as a general indicator of mosquito age. Graham (1969) also found water-mites were good indicators of nulliparity, but in his experience they parasitised mosquitoes rarely. Corbet (1970) considered that mites indicating nulliparity are only useful for age-grading when they have a fairly high incidence. Mullen (1977) found that Thyas barbigera and Thyasides sphagnorum commonly parasitise mosquitoes in the USA. Thyasides sphagnorum attach to adult mosquitoes as they emerge and therefore indicate nulliparity, whereas Thyas barbigera attach to ovipositing females and indicate parity. In East Africa, thyasid mites were found on both nulliparous and parous Mansonia pseudoconopas (Corbet, 1963). The Thyas mites in this study attach to the mosquitoes when they are ovipositing, and in fact all the mite-infested adults were proved to be parous by ovarian dissection. However, in the present study, as less than one percent of all Ae. cantans had Thyas mites, they are too infrequent to be used effectively for age-grading.

Unidentified mites were recorded on Ae. cantans adults collected in Ness Woods by Sulaiman (1982). Sixteen infested Ae. cantans were parous, while nine infected adults were nulliparous. It would seem that Sulaiman (1982) was finding two types of mites on mosquitoes, one indicating nulliparity and the others indicative of parity.

CHAPTER 4 - HOST PREFERENCES

4.1 INTRODUCTION

It is especially important in ecological and epidemiological studies to be able to identify the hosts of blood-sucking insects, particularly when they are of medical or veterinary importance. Several tests are available for the identification of blood-meals and these are usually immunological techniques. These have been reviewed by Pant et al. (1987), Tempelis (1975) and Washino and Tempelis (1983).

4.1.1 THE PRECIPITIN TEST

The precipitin test makes use of the precipitation that occurs between the serum in a blood-meal and the specific antibodies of the serum raised in a laboratory animal. The precipitin ring test, an adaptation of the precipitin test, has until recently been the most widely used procedure. It is relatively simple to perform but is expensive because of the relatively large consumption of antisera, and is also rather an insensitive test. Another adaptation of the test, the double gel diffusion test is easier to perform, is very economical to use, and is also more sensitive. Generally the precipitin test is neither a very sensitive test nor always a species-specific test system (Washino and Tempelis, 1983).

4.1.2 COUNTER-CURRENT IMMUNOELECTROPHORESIS

Counter-current immunoelectrophoresis has been found to be useful in the detection of small blood-meals but requires expensive electrophoretic equipment and is therefore not easy to perform in the field.

4.1.3 HAEMAGGLUTINATION ASSAYS

Haemagglutination tests are highly sensitive but are much more complicated than precipitin techniques. The cost of materials is low but the labour costs are high. This test is used to distinguish blood-meals from closely related host species, and has been used mostly for the study of host preferences of tsetse flies.

4.1.4 LATEX-AGGLUTINATION TEST

The latex agglutination test is simple, rapid and requires no equipment. It is not widely used because of the instability of the latex particles coated with antibodies used in the test.

4.1.5 COMPLEMENT FIXATION TEST

The complement fixation test is hard to perform and requires supplies of sheep red blood cells, complement and a refrigerator. It is not a sensitive test.

4.1.6 ENZYME LINKED IMMUNOSORBENCE ASSAYS

The enzyme-linked immunosorbent assay (ELISA) has been successfully used in the identification of blood-meals in mosquitoes (Campbell, 1986; Service et al., 1986). Direct ELISA employs microplates coated with the elutes of blood-smears. The indirect ELISA set-up, developed by Service et al. (1986), is a much cleaner method and does not require any blocking agents such as gelatin or caesein, and is the one used in this investigation. The ELISA tests are relatively simple to use, are sensitive and the results can be read by the naked eye. They are also relatively cheap, compact and easy to operate (Washino and Tempelis, 1983).

The indirect ELISA tests work on a double antibody sandwich principle (Fig.4.1). Polyvinyl-chloride plates are coated with the appropriate antibody of various host species. A solution of the blood-meal, the antigen, is then placed on the plate and incubated for an hour so that any IgG in the blood-meal will bind with the homologous anti-IgG on the plate. The plate is then washed, after which it is incubated with an enzyme-labelled specific antibody, the conjugate. This will attach to any IgG captured in the previous step. Any unbound conjugate is then washed off. Finally, enzyme substrate, which changes from colourless to yellow-brown where enzyme conjugate is present, is added.

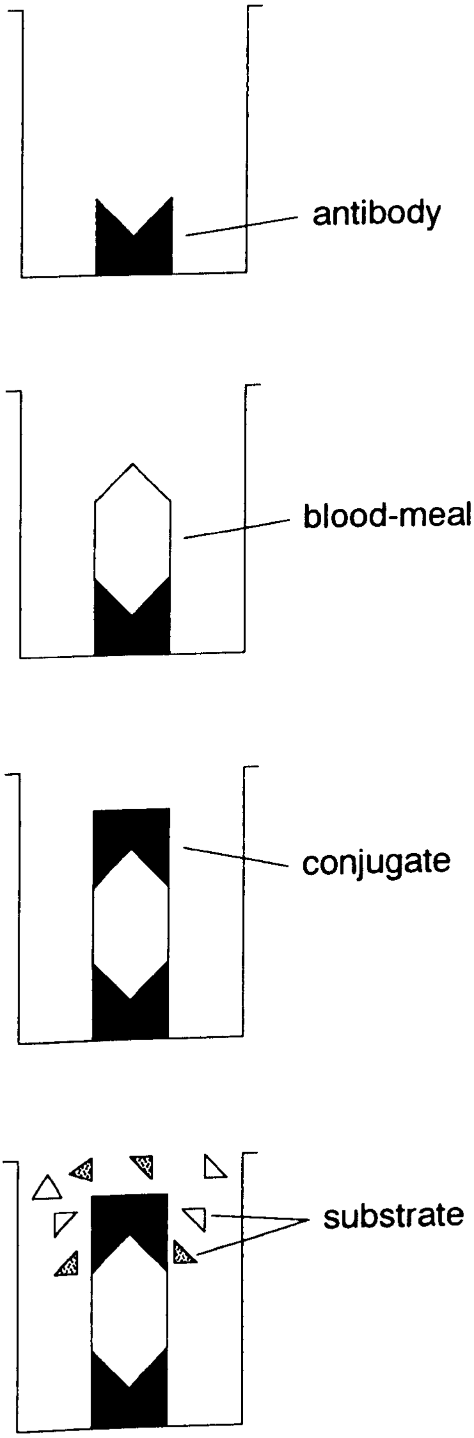
The test gives a strongly positive response and because of the lack of cross-reactions which result in the control well on the plate remaining colourless a plate reader is not necesary and the results can be read by eye.

4.1.7 FEEDING INDEX

The feeding index or forage ratio was used in this investigation to analyse host-feeding patterns. Kay et al. (1979) defined the feeding index as "the observed proportion of feeds on one host with respect to another, divided by the expected comparative proportion of feeds on these two hosts, based on factors affecting feeding". There are, however, several disadvantages to this technique, for example it neglects the ecological and behavioural differences between hosts (Edman, 1971). It is also difficult to undertake a host census for some animals, such as birds and small woodland animals.

A value of one indicates equal feeding on both hosts. A value of less than one indicates that there is a decrease in feeding on the first host, and a value of more than one indicates that there is increased feeding on the first host.

Figure 4.1 : ELISA Double antibody sandwich technique for identification of vector blood-meals



Antibody (e.g. human-anti-IgG) absorbed to polyvinyl plate

Wash off excess antibody

Add eluted blood smear (= antigen)

Wash off unbound blood-meal

Add enzyme-labelled specific antibody (= conjugate) e.g. peroxidase labelled human antibody.

Leave one hour

Wash off unbound conjugate

Add enzyme substrate (OPD) (Protect from light, leave 10-20 mins)

Positive reactions (e.g. human feeds) identified as yellow-brown coloured wells

Stop reactions with HCL

4.2 METHODS

4.2.1 SWEEP-NETTING

Mosquitoes were collected by sweep-netting the vegetation in all three woods and the surrounding fields. Sweep-netting was carried out once to twice weekly as soon as the mosquitoes began to emerge until there were no longer any present in the field, generally from May to October, in 1989 and 1990. The abdomens of the mosquitoes that were bloodfed, half-gravid, or even three-quarters gravid were squashed on filter paper and stored in a desiccator for up to five months.

4.2.2 HOSTS

In 1989 the hosts; cattle, horse, sheep, human and chicken were tested for, in 1990; blood-meals were in addition tested against dog/fox, mouse and rabbit. This covers the mammals grazing in the surrounding fields and also some of the smaller mammals seen in the woods.

In 1989, having only 78 smears, all were tested for all hosts, but in 1990, with more than 300 smears, it was too expensive to do this, so it was decided to test all the smears against the hosts cow, horse and sheep, whilst bird, human, mouse, dog and rabbit were only tested on the smears that were negative for the three main hosts. This, however, could have resulted in some double feeds being missed.

Throughout the spring and summer of both years, a rough count was made of the numbers and species of mammals in the surrounding fields, and the mammal and avian species seen in the wood, to give an idea of host abundance.

4.2.3 ELISA TESTS

Pre-prepared plates for the above hosts were obtained from A.Voller and D.Bidwell at the Zoological Society, London. These consisted of polyvinyl-chloride plates coated with rabbit antisera of the appropriate host.

For the tests, the mosquito gut smears were cut from the filter paper and eluted in 1.0 ml of PBS\Tween 20 and incubated at room temperature for 60 minutes. 100 µl of each eluted gut smear was then added to one well of the relevant plate. A negative control of PBS only and a positive control of the appropriate reference serum (10 µl of the serum in 5 ml PBS), were also included. The plates were then covered and placed on wet towelling and left for an hour. The appropriate enzyme-labelled specific antibodies (conjugates) were then prepared. 20 µl of each conjugate were added to 10 ml of the appropriate diluent, for use in all the wells of a plate. The diluents represent a balanced system of antigens that prevent cross reactions. The plates were then washed four times with PBS\T20 and dried. 100 µl of the diluted conjugate was then added to each well. The plates were again placed on wet paper for an hour.

Ten minutes before the end of the incubation period, the substrate solution was prepared. Four tablets of ortho-phenylenediamine (OPD) were placed in 12 ml of distilled water, and 5 µl of hydrogen peroxide solution were added. At the end of 60 minutes, the plates were again washed in PBS\T20 and 100 µl of substrate added to each well. The plates were placed in the dark for ten to twenty minutes. Positive reactions were identified by yellow-brown colouration in the wells. The reactions were halted by adding one drop of 2.5 molar HCL. The reactions were strongly positive and the results could be read using the naked eye. Figure 4.1 summarises this test.

In the first year, the dipstick method developed by Dr. K. Snowden at the Liverpool School of Tropical Medicine was also used. This test works on a similar principle to the plate method but uses a dipstick 9 x 0.8 cm in size. Dipsticks were prepared by Dr. Snowden with dots of rabbit antisera against bovine, sheep, chicken, horse and human hosts. These dipsticks were incubated in the mosquito eluate at the same time as the plate experiment was carried out. After 60 minutes, the dipsticks were washed in PBS, placed in peroxidase conjugated antisera and then washed in PBS again. The substrate 3,3' diaminobenzidine tetrahydrochloride (DAB, Sigma) was used to produce brown coloured positive dots on the dipstick. A positive result in the appropriate position on the dipstick identified the appropriate blood-meal host.

4.3 RESULTS

4.3.1 SWEEP-NETTING

In 1989, 91.03% of the mosquitoes caught by sweep-netting vegetation were Ae. cantans and in 1990 95.78% of those caught were Ae. cantans. The other species were a mixture of Ae. punctor, Cs. morsitans, Cs. annulata and Cx. pipiens. In 1989 the majority of the blood-fed mosquitoes were found in Wood B, with only a few found in Wood A. Wood C was not sweep-netted in the first year. A similar pattern arose in 1990, with the majority of the mosquitoes found in Wood B, followed by Wood C and then Wood A. The species caught, their hosts, and their capture sites are shown in Table 4.1.

4.3.2 ELISA TEST RESULTS

The results of the ELISA tests from 1989 and 1990 are shown in Figs. 4.2 and 4.3.

The two major hosts in both years were sheep and cattle. Only one mosquito, an Ae. cantans, in both years tested positive for bird blood. In 1989, there was a high

Table 4.1. KEY:

CA = *Aedes cantans*
CP = *Culex pipiens*
PU = *Aedes punctor*
RS = *Aedes rusticus*
CM = *Culiseta morsitans*
Cul.A= *Culiseta annulata*

Table 4.1a: Identification of blood meals for all species caught at all sites in 1989.

Year and site	Mosquito species	Host									
		Sheep	Cattle	Horse	Human	Sheep/ Cattle	Cattle/ Horse	Sheep/ Horse	Poor	Neg.	Total
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n
Wood A	CA	1	2	1	0	0	0	0	0	6	10
		(1.28)	(2.56)	(1.28)	(0)	(0)	(0)	(0)	(0)	(7.60)	
Wood B	CP	0	0	0	0	0	0	0	0	1	1
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1.28)	
	PU	2	0	1	0	1	0	0	0	1	5
		(2.56)	(0)	(1.28)	(0)	(1.28)	(0)	(0)	(0)	(1.28)	
	RS	0	1	0	0	0	0	0	0	0	1
		(0)	(1.28)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	
	CM	2	0	0	0	0	0	0	0	0	2
		(2.56)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	
	CA	24	13	6	2	0	6	3	4	4	59
		(30.77)	(16.67)	(7.69)	(2.56)	(0)	(7.69)	(3.85)	(5.13)	(5.13)	
	TOTAL	29	16	8	2	1	6	3	1	12	78
		(37.17)	(20.51)	(10.25)	(2.56)	(1.28)	(7.69)	(3.85)	(1.28)	(15.38)	

All samples tested negative for avian hosts. Rabbit was not tested for in 1989.

Table 4.1b: Identification of blood meals for all species caught at all sites in 1990.

Year and site	Mosquito species	Host									
		Sheep	Cattle	Human	Bird	Rabbit	Sheep/ Cattle	Cattle/ Horse	Poor	Neg.	Total
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n
Wood A	CA	4	4	1	0	3	4	0	2	2	20
		(1.21)	(1.21)	(0.30)	(0)	(0.91)	(1.21)	(0)	(0.60)	(0.60)	
	Cul.A	0	1	0	0	0	1	0	0	0	2
		(0)	(0.30)	(0)	(0)	(0)	(0.30)	(0)	(0)	(0)	
Wood B	CA	76	64	0	1	3	44	1	10	14	213
		(22.96)	(19.34)	(0)	(0.30)	(0.91)	(13.29)	(0.30)	(3.02)	(4.23)	
	PU	2	0	0	0	0	5	0	0	0	7
		(0.60)	(0)	(0)	(0)	(0)	(1.51)	(0)	(0)	(0)	
Wood C	CA	29	26	0	0	3	14	0	8	4	84
		(8.76)	(7.86)	(0)	(0)	(0.91)	(4.23)	(0)	(2.42)	(1.21)	
	Cul.A	0	1	0	0	0	1	0	0	0	2
		(0)	(0.30)	(0)	(0)	(0)	(0.30)	(0)	(0)	(0)	
	TOTAL	112	97	1	1	10	69	1	20	20	331
		(33.84)	(29.31)	(0.30)	(0.30)	(3.02)	(20.85)	(0.30)	(6.04)	(6.04)	

Note: 1 Ae. punctor (wood A) tested positive for Rabbit, 1 Cs. morsitans (wood B) tested positive for Cattle, and 1 (wood C) for Sheep.

Fig. 4.2 : Pie chart showing host-preferences of blood-fed mosquitoes caught by sweep-netting vegetation in 1989, identified by the indirect Elisa method.

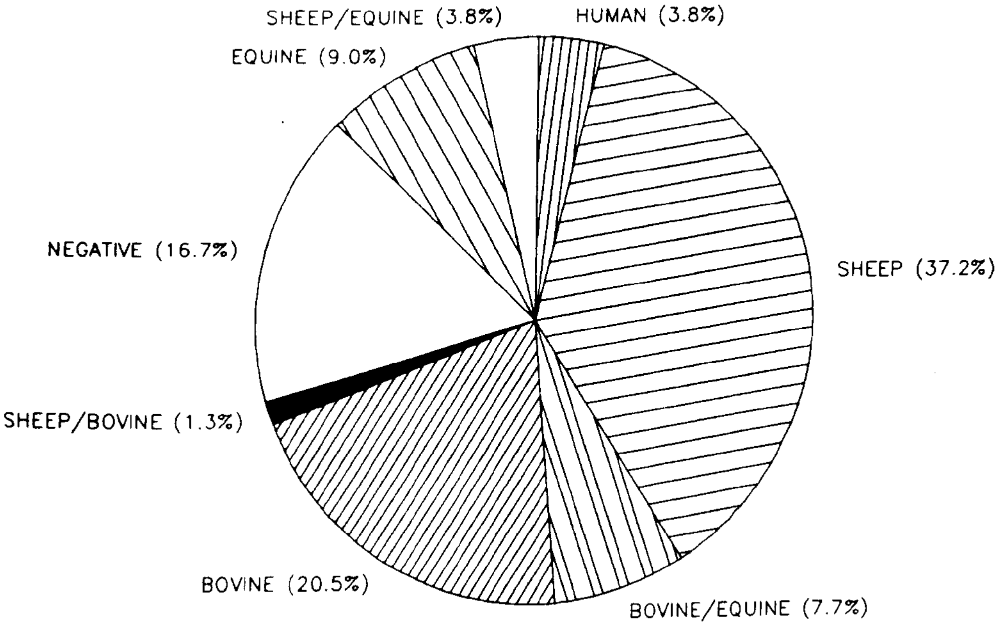
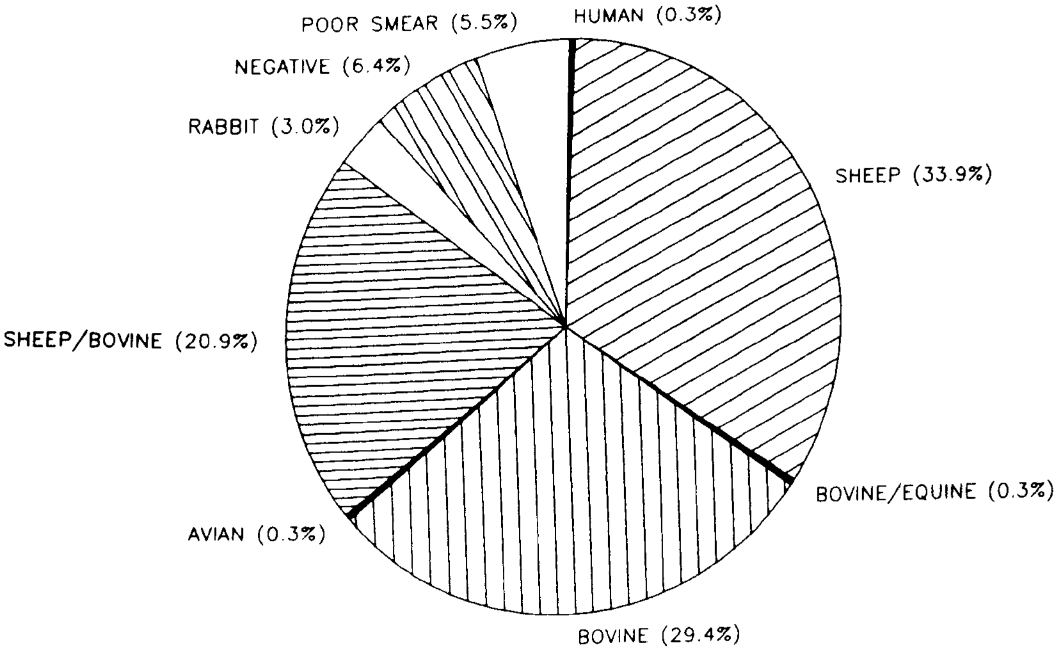


Fig. 4.3 : Pie chart showing host-preferences of blood-fed mosquitoes caught by sweep-netting vegetation in 1990, identified by the indirect Elisa method.



percentage (21.5%) of horse blood-meals, but in 1990 there was only one positive for horse, in a smear that was also positive for cattle, that is a double feed (0.3%).

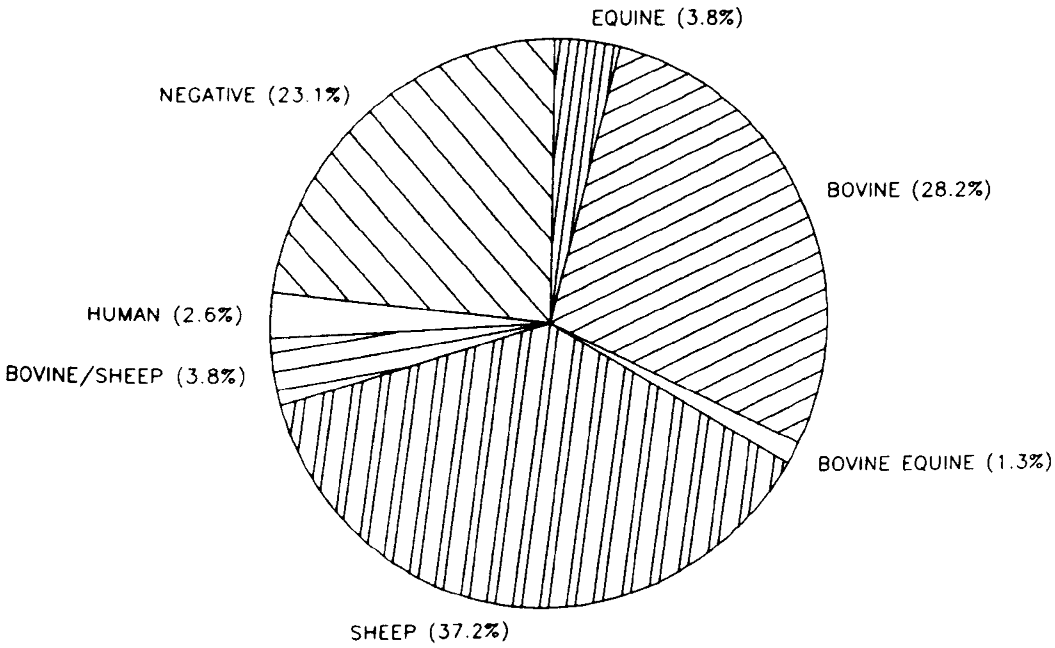
In the second year, dog/fox and mouse were tested for, but the results were negative in all single feeds and they can therefore be discounted as important hosts. There was only a small number of positive human feeds in both years, with 3.8% in 1989, and just 0.3% in 1990. The rabbit test revealed that 3.2% of the mosquitoes in the second year had fed on rabbits. In 1989, 16.7% of the tests were negative for all hosts, and 12.1% in 1990. Some of these can be discounted as poor smears, where the blood-meal was too far digested before the mosquitoes were squashed. In 1989, 6% of the negatives were due to poor smears, leaving 10.7% unidentified hosts, and in the second year, 5.5% of the negatives were due to poor smears, leaving 6.4% unidentified hosts.

There is a significant difference between the hosts of the mosquitoes caught in Wood B in 1989 and 1990 ($\chi^2 = 53.6$ $P<0.005$). This is mainly due to the large increase in the number of double feeds of sheep and cows in the second year (Table 4.1). There are too few mosquitoes collected at the other sites to analyse the data statistically.

The hosts of the mosquitoes tested in 1990 showed no significant difference between the three collecting sites ($P>0.05$). Again, there were too few catches in 1989 to analyse statistically.

The results of the dipstick method are shown in Fig. 4.4. Although there were some contradictions, generally, there was good agreement between the two tests used in 1989, suggesting that the dipstick method will provide a useful alternative test in the future. There was no significant difference in the numbers of each host identified between the two methods ($\chi^2 = 5.93$, $P>0.05$).

Fig. 4.4 : Pie chart showing host-preferences of blood-fed mosquitoes caught by sweep-netting vegetation in 1989, identified by the dipstick method.



4.3.3 FEEDING INDEX

Table 4.2 shows the feeding index between, sheep and cattle, sheep and horse, and cattle and horse. The feeding index was calculated for the larger grazing mammals because it is easy to make accurate population estimates for these species, and they represented the major hosts in both years. The index was not calculated for the other mammals because it is difficult to estimate the population of the smaller woodland mammals without performing time-consuming survey work, and in addition, there were generally low returns of positive feeds on these hosts.

The size of the hosts was taken into account using a rough approximation; it was decided that there was no significant difference in the size of cattle and horses but that the sheep were half the size.

It can be seen from Table 4.2 that there seems to be equal feeding on sheep and cattle with values of 1.02 in 1989 and 1.07 in 1990. In 1989, the mosquitoes showed a strong preference for horses over sheep and cattle, with values of 0.06 and 0.059 but in 1990, with only one horse remaining in the field, this preference was reversed and the mosquitoes clearly preferred cattle and sheep.

4.4 DISCUSSION

In both years the results were as one would expect with the two most common hosts being sheep and cows, corresponding to the two most common animal species grazing in the fields around the woods.

In 1989 there was a high percentage of both single and double horse feeds (16.7%), however, in 1990, there was only one positive double equine feed and no single feeds. In the former year, there were two horses grazing in the fields adjacent to the woods; however, one horse died of a heart attack towards the end of the summer.

Table 4.2: Feeding index between the larger grazing animals in 1989 and 1990.

	Feeding Index		
Year	Cattle + Sheep	Cattle + Horse	Sheep + Horse
1989	1.02	0.06	0.05
1990	1.07	2.28	1.29

The fact that this horse was old and sick may have made it easier for mosquitoes to feed on it. The remaining horse was fit and well, and seemed to have a good mosquito avoidance behaviour because although many mosquitoes were seen swarming around it in the evening, none was seen to feed. On several occasions the horse was seen to gallop around the field, swish its tail, shake its head and generally interrupt mosquito feeding.

In 1989 none of the blood smears was positive for bird blood, and only one in 1990. This indicates that Ae. cantans does not feed largely on bird species even though they are in plentiful supply in the woods. In Huntingdon, Service (1971b) found that Ae. cantans did not feed in the trees but tended to feed close to the ground, and this could be part of the reason that there were low numbers of bird smears in the present study. There is a poultry farm 500 m from the wood, but, it is not known whether the mosquitoes have any access to the birds in this farm.

In 1990, attempts to identify the source of blood-meals that were not from the major hosts were not entirely successful because tests with dog/fox, and mouse antisera were negative for all single smears. There were very few positive human smears in both years. The 3.8% positive rabbit smears in 1990 indicate that these are occasional hosts, but it seems unlikely that rabbit feeds could have accounted for all the 16.7% negative smears found in 1989. There still remain 6% good smears that tested negative in 1990. Other mammals seen in the wood were voles, moles, squirrels, rats and badgers and it is possible that these species make up the remaining negatives.

The feeding index results (Table 4.2) indicate that in both years there was equal feeding on sheep and cattle, with values of 1.02 in 1989 and 1.07 in 1990. In 1989, the values of 0.06 for cattle against horse and 0.059 for sheep against horse, show that the mosquitoes showed a strong preference for the horse, this has already been explained in terms of one of the horses being ill and having a poor host avoidance behaviour. In

1990, this pattern was altered and the mosquitoes showed a strong preference for sheep and cattle over the remaining horse which had a good host-avoidance behaviour.

The results of the ELISA tests in 1989 and 1990 are similar to those found when the blood-meals of Ae. cantans have been examined in other studies.

Service (1968a) found that the two Ae. cantans he collected on Brownsea Island, Dorset, had fed on humans. Sulaiman (1982) found that Ae. cantans collected in Wood A fed mainly on cow (81.3%), with only 3.5% feeding on sheep, 0.5% on horse, 1.0% on rabbit and 1.0% on man. Campbell (1986) also investigated feeding of mosquitoes in Woods A and B as well as in the surrounding fields. He found that 15% of Ae. cantans fed on birds, 40% on cattle, 9% on horse, 16% on human and 20% on sheep. The percentage of positive bird feeds in 1986 seems to be particularly high. Service et al. (1986) found that Ae. cantans collected in Wood A fed mainly on sheep (72.97%) with only 4.05% feeding on cows and 4.05% feeding on rabbits. The tests were negative for bird, mouse, and horse, and 6.76% of the smears were negative for all the hosts tested. It seems that Ae. cantans feeds on the most readily available hosts in the fields and is largely influenced by the rotation of animal stock in the fields around the woods. Although birds are available, they do not seem to be favoured hosts.

Shemanchuk et al. (1963), working mainly on Aedes species in Canada, considered that although mosquitoes have host preferences, it is the availability of hosts that is the most important factor in mosquito bloodfeeding. Similarly, Edman and Downe (1964) found little evidence of host specificity when they examined the hosts of Aedes, Anopheles, Culex, Culiseta and Psorophora mosquitoes in Kansas, U.S.A. They found that it was the larger and more abundant animals that were attacked most often, and in their case, as in this present study, sheep and cows were the principal hosts. They also noticed that there was a high incidence of multiple-feeding and that hosts which are seldom attacked in single feedings were much more abundant in multiple feedings. It is therefore possible that in 1990, some of the mouse, dog, bird, rabbit and human feeds were missed, as these were not tested for when positive reactions were obtained for sheep, cow or horse. It is thought that because smaller animals can scratch most of the regions of their bodies, they have a better host avoidance mechanism (Edman and Downe, 1964).

In the present study, mosquitoes were observed feeding on cows, over the whole of their bodies, and around the testes and faces of male sheep, during the evenings of spring and summer. Neither of these species appeared to exhibit much host-avoidance behaviour, and the mosquitoes appeared to have little difficulty in engorging. However, when I was a human-bait in the field with these animals, mosquitoes which were partly blood-fed sometimes came to feed on me, indicating that some bloodfeeding was interrupted. This was more apparent in the second year than the first. In addition, there were 12.8% double feeds in 1989 and 21.2% in 1990. Day and Edman (1984) discovered that large animals such as cows are not as defensive as small animals, but a high mosquito density can nevertheless elicit anti-feeding behaviour in hosts that are not normally defensive. Defensive behaviour increases as mosquito density increases. They found that mosquitoes feed primarily on hosts offering the least behavioural resistance. In laboratory experiments, chickens, hamsters, housemice and deer mice were either restrained or unrestrained and mosquitoes were introduced into cages containing these hosts. All the mosquito species tested (*Ae. aegypti*, *An. stephensi*, *An. quadrimaculatus*, *Cx. nigripalpus* and *Cx. quinquefasciatus*) fed successfully on all the restrained hosts but very poorly on the unrestrained hosts; in fact, many were captured and eaten by the unrestrained animals. The day-active species fed most successfully on animals that rested during daylight hours while night-active species fed better on animals that rested at night.

Klowden and Lea (1979), carried out experiments on tethered and unrestrained rabbits and found that the amount of blood ingested from the unrestrained defensive hosts was significantly reduced. Edman *et al*. (1974) found that when mosquitoes were offered the choice of restrained and unrestrained hosts they fed preferentially on the restrained hosts. Perhaps the reason for the large increase in the double feeds in the second year of my study, is because higher density of mosquitoes encountered in 1990 (Chapter 3) elicited greater defensive behaviour in the sheep and cows, and hence the mosquitoes had more difficulty in completing their blood-meal.

Service (1971*b*) examined feeding behaviour in British mosquitoes in Poole, Dorset and Monk's Wood, Huntingdon. He found that *Ae. cantans* took a long time engorging, with an average of 189 seconds, and also in 37.1% of feedings the human host was alerted to the bite of the mosquito. All the *Aedes* species fed on a broad spectrum of hosts. *Aedes cantans* fed primarily on cattle (76.9%); however, when cattle were not present and rabbits were common they accounted for 69.4% of the blood-feeds. Minority hosts for this species included, man (7.9%), sheep or goat (3.5%), pig (0.95%), avian (2.6%) and a double feed of avian and bovid (0.9%).

In the USA, Nasci (1984) examined the blood-feeding of *Ae. vexans* and *Ae. trivittatus*. He found that *Ae. trivittatus* was more opportunistic and fed on a wider variety of hosts, but mainly on the larger ones.

Double-feeding is of important epidemiological and epizootological significance in the transmission of vector-borne diseases, especially when the two hosts are of the same species. Except in man, it is impossible to discern between these double feeds within species and single feeds using the indirect ELISA method, so it is difficult to estimate the actual incidence of double feeds. It is possible to distinguish between feeds on different people by means of the ABO blood-groups (Bryan and Smalley, 1978) or haptoglobins (Boreham *et al*., 1978). It has been suggested that DNA fingerprinting might offer a method for the identification of individual human host blood up to ten hours post-feeding (Coulson *et al*., 1990). Double feeds are more likely to occur between the same species than between different species when animals of a single species are kept together in large numbers. If a mosquito is disturbed it is then more likely to feed on an animal close by rather than fly into another field, where there may be different host species.

It is difficult to determine whether the *Ae. cantans* are feeding on the hosts that are most readily available or are making a choice. Work in Czechoslovakia has shown that the number of eggs produced by *Ae. cantans* is related to the source of the blood-meal (Gardos and Smek, 1987). It was found that *Ae. cantans* feeding on guinea pigs and rabbits produced more eggs than those that fed on pigeons, which in turn produced more than those that fed on man. How much choice is exercised will depend on the relative benefits of obtaining a blood-meal from a specific host weighed against the cost of searching for alternative hosts and the dangers associated with taking a meal from different hosts. What determines the choice of host is probably a combination of availability and innate host preference. My results seem to indicate that the *Ae. cantans* mosquitoes are feeding on the larger more easily available hosts, which exhibit less vigorous host avoidance behaviour, namely cattle and sheep.

CHAPTER 5 - MARK-RELEASE-RECAPTURE

5.1 INTRODUCTION

Dispersal, survival, gonotrophic cycle duration and population size are all important parameters in the study of the ecology and population dynamics of mosquitoes. Mark-release-recapture techniques are principally designed to estimate population size but have often been used in mosquito work to study dispersal (Service, 1976). They can also be used to determine the duration of the gonotrophic cycles of mosquitoes as well as their survival rates. Since the survival rate of a mosquito determines its longevity and also its net reproductive rate (Holmes and Birley, 1987), the mark-release-recapture technique can give considerable insight into mosquito population dynamics. The technique of mark-release-recapture was used to investigate the dispersal and gonotrophic cycles of Ae. cantans in Ness Woods and the surrounding fields.

Mark-release-recapture experiments were carried out in the first two years of this investigation on emerging adults and those caught in human-bait catches. They were designed to examine the general movement of mosquitoes between Woods A, B and C, and the surrounding fields, to investigate the duration of the gonotrophic cycle, the survival rate of females and their population size.

5.1.1 MARK-RELEASE-RECAPTURE CRITERIA

There are several important criteria that have to be met before mark-release-recapture techniques can be used. Southwood (1978) has outlined these criteria and they will be discussed only briefly. It is important that marking and handling techniques do not affect the longevity or behaviour of the animals being marked. It is also important to confirm that the pigments used are non-toxic, long-lasting and durable. Compliance with these criteria can be tested by carrying out laboratory experiments.

It is also important that the marked animals become completely mixed within the population. The probability of capturing a marked animal must be the same as that of capturing any member of the population, i.e. all the individuals must be equally available to capture irrespective of their position in the habitat. The pigments should not appreciably increase predation. This is one of the most difficult criteria to assess.

5.1.2 ESTIMATES OF POPULATION SIZE

There are many different statistical models for the estimation of population size. These are reviewed by Service (1976) and Southwood (1978). There are two main types of model: deterministic ones such as the Lincoln index which assume that the survival rate is constant, and the stochastic models such as Jolly's method, which take account of the survival rate (White, 1985).

The data collected in this study are not suitable for mark-release-recapture population estimates because they do not fulfil one of the main criteria, namely that a marked individual should have an equal chance of being recaptured. In this study, when blood-fed mosquitoes are marked and released, they will disperse but they will not return to feed until they have digested their blood and laid eggs, and so they are essentially lost from the population. Similarly when non-blood-fed mosquitoes are released, as soon as they have obtained a blood-meal they are also lost from the biting population (Sheppard et al., 1969). The sampling of the resting population by sweep-netting vegetation (Chapter 3) is not sufficiently efficient to sample the resting population thoroughly.

In this investigation an approximate estimate of population size of nulliparous mosquitoes is attempted using Lincoln's index. This index gives only an inexact population estimate because it is based on a closed system, and assumes that there is no immigration or emigration and neither births nor deaths; it is an oversimplification. An attempt is also made to estimate the population size of nulliparous adults in 1990 using the Fisher-Ford method (Service, 1976). In this investigation therefore, only the less sophisticated deterministic methods are used.

5.1.3 DISPERSAL

Mosquito dispersal may be over relatively short distances such as movement from breeding places to host aggregations. Alternatively it may involve much greater distances, such as the migratory flight of Aedes taeniorhynchus (Wiedemann) (Haeger, 1960; Harden and Chubb, 1960) and Anopheles pharoensis Theobald (Garrett-Jones, 1962).

Many mosquito flights are described as appetitive, in other words, a suitable stimulus leads to the cessation of flight. Others, such as the migratory flight of Ae. taeniorhynchus (Provost, 1960), are termed non-appetitive: they seem to serve no purpose and are mostly wind-borne.

Taylor et al. (1973) summed up insect migration as follows. "Insect migration is a system for searching for new territories; linear flight, area, and perhaps distance covered are all relevant parameters, and the particular flight mechanism employed is species specific; it uses the same spatial co-ordinates within which the species lives at other times. Flight becomes linear when released from the inhibitions that maintain social cohesion; food, sex, territory and synchronised activity become secondary distractions".

Dispersal has been studied by the capture of adults at various distances from the natural breeding place, but this is not a very satisfactory method, because it is usually difficult to determine the habitats from which the adults originated. It is easier with coastal species because these are restricted to saline habitats.

Knowledge of the flight range of mosquitoes is of paramount importance in control programmes. In this study it is useful to determine whether the mosquitoes in the different woods form separate or the same populations. It is also useful to know how far mosquitoes will fly in order to find a suitable host for a blood-meal.

5.1.4 GONOTROPHIC CYCLE

The gonotrophic cycle has been variously defined. Gillies and Wilkes (1963) described it as the interval between blood-feeding and the time of the next blood-meal, while Hitchcock (1968) described it as the period just prior to a blood-meal to just prior to the next blood-meal. Alternatively it has been described as the duration between blood-feeding and egg laying and this is the definition which was used in this investigation.It is assumed that the period between oviposition and re-feeding is small. By examining the time difference between release and recapture, it is possible to gain information on the duration of the interval between feeding and refeeding and to compare these with durations estimated by laboratory and cage experiments. It may be possible to determine whether something is delaying the cycle, such as host scarcity. Information from mark-release-recapture is best used in conjunction with other information obtained from ovary dissections and bait catches (Chapter 3). Weather data are also very important. Temperature affects the rate of blood digestion in mosquitoes (Service, 1977a), with faster digestion and hence shorter oviposition cycles at higher temperatures. Low temperatures also affect flight behaviour, mosquitoes are less active at lower temperatures and this can affect the sucess of blood-feeding and also recapture at bait (Service, 1978; M. H. Birley et al., unpublished).

5.1.5 SURVIVAL RATES

Mark-release-recapture work can give indications of mosquito survivorship, which is important as it may have a bearing upon fecundity.

In this investigation, survivorship was estimated by regressing the number of recaptured mosquitoes transformed into ln($\underline{x}$+1) as a function of time in days after release (Charlwood et al. 1986; Gillies, 1961; Reisen and Aslamkhan, 1979; Reisen et al. 1980). The daily survival rate was then calculated from the antilog of the regression co-efficient.

5.2 METHODS AND MATERIALS

5.2.1 MARKING METHODS

One of the main problems in marking mosquitoes is that the handling techniques may affect the insect's survival. There may also be biological side-effects resulting from the marking pigments which are used. In this investigation, mosquitoes were marked using paints and powders.

5.2.1.1 PAINTS

Many different types of paint have been used to mark insects, including oil paints on tsetse flies and poster paints on mosquitoes (Southwood, 1978). 'Humbrol' enamel paints have proved particularly useful in marking mosquito populations (Mcdonald, 1977a; Service, 1976, 1977a; Trpis, 1973) and were used in this study. 'Humbrol' is suitable because it is non-toxic and available in a large range of colours.

Painting is particularly useful for small scale studies and a careful blending of colours allows a large number of different releases. It is possible, however, that in paint application, handling and the use of anaesthetic has an effect on longevity.

MARKING EMERGING MOSQUITOES

Emerging mosquitoes were trapped by placing bednets over Pond 9, the Hotspot and the Ditch in 1989 and Pond 9, Pond 13, Pond 8 and the Hotspot in 1990 (Fig. 1.3). All mosquitoes were removed from the nets using an aspirator, identified, sexed and counted. They were then placed into plastic 500 ml capacity cups with no more than 75 individuals per cup to reduce possible damage. Five to ten percent of the mosquitoes were taken back to the laboratory for measurement of wings and dissection of ovaries (Chapter 3). The remaining mosquitoes were prepared for marking and release. They were lightly anaesthetised with ether in the field and then emptied onto a sheet of paper and painted. A different colour and position on the thorax was used for each day's release. The mosquitoes were marked as lightly as possible by dipping a pair of size four watchmaker's fine forceps in the paint, or alternatively, a sharpened pencil or a fine glass pipette drawn to a point. They were then placed carefully onto clean paper and allowed to recover. This took between 5-30 minutes, and individuals were observed until all had dispersed. Any that were damaged or over-painted in the process were not allowed to escape and were not counted.

The mosquitoes were marked and released in the field near the pond from which they had emerged, but because of the large number of newly emerged mosquitoes released and the time-consuming nature of the process, mosquitoes from different ponds were not distinguished by different paint spot positions or colours. Mosquitoes were recaptured by sweep-netting vegetation, and by human bait catches throughout the remainder of the summer, until no adults were present.

In 1989, 355 female and 404 male Ae. cantans, caught in emergence traps were painted and released. In 1990, 825 females and 1307 males were marked and released (Tables 5.1 and 5.2).

Table 5.1 : Release and recapture of painted emerging *Aedes cantans* in 1989.

Date	Colour	*Aedes cantans* released Female	 Male	Female Recapture	% Recaptured
06.05.89	Yellow	1	45	0	0%
09.05.89	Blue	90	105	2	2.2%
10.05.89	Green	40	4	2	5%
11.05.89	Orange	16	4	1	6.3%
14.05.89	White	10	6	2	20%
21.05.89	Red	53	207	1	1.9%
22.05.89	Pink	81	28	0	0%
23.05.89	Mauve	10	2	0	0%
24.05.89	Purple	50	3	0	0%
25.05.89	Brown	4	0	0	0%
Total		355	404	8	2.25%

Table 5.2 : Release and recaptures of painted emerging *Ae. cantans* in 1990.

Date	Colour	*Aedes cantans* released Female	Male	Female Recapture	% Recapture
23.04.90	Grey f	1	10	1	100%
24.04.90	Grey b	2	12	0	0%
25.04.90	Red f	14	40	1	7.1%
26.04.90	Red b	7	28	0	0%
27.04.90	Orange f	2	69	0	0%
28.04.90	Orange b	9	74	2	22.2%
29.04.90	Blue f	73	320	6	8.2%
30.04.90	Blue b	93	143	2	2.2%
01.05.90	Yellow f	73	167	1	1.4%
02.05.90	Yellow b	57	88	3	5.3%
03.05.90	Green f	105	94	6	5.7%
04.05.90	Green b	168	111	4	2.4%
05.05.90	Purple f	33	24	0	0%
06.05.90	Purple b	27	33	2	7.41%
07.05.90	Pink f	22	22	4	18.2%
08.05.90	Pink b	26	22	8	30.8%
09.05.90	Brown f	9	13	5	55.5%
10.05.90	Brown b	23	13	0	0%
11.05.90	Red-brown	17	13	0	0%
12.05.90	Cream f	6	1	0	0%
13.05.90	Cream b	11	3	1	9.1%
14.05.90	Mauve f	15	2	0	0%
15.05.90	Mauve b	3	1	1	33.3%
16.05.90	White f	10	2	3	30%
17.05.90	White b	6	2	1	16.7%
18.05.90	Black f	2	0	1	50%
19.05.90	Black b	7	0	0	0%
20.05.90	Sky blue	4	0	0	0%
Total		825	1307	52	6.3%

f= front of thorax, b= back.

Any effect that painting might have on survivorship was examined by placing 20 painted mosquitoes in a cage and recording daily mortality for two weeks, with a control of 20 unpainted mosquitoes from the same batch in another cage.

5.2.1.2 POWDERS

Mosquitoes were collected in human bait catches throughout the spring and summer of both 1989 and 1990 in both Woods A and B and the surrounding fields, and at various times of day (Chapter 3).

Captured mosquitoes were identified to species and placed into cages. Release dates, sites and colours for both years are shown in Tables 5.3 and 5.4. When the releases comprised non-blood-fed individuals, the mosquitoes were placed in cups at densities of no more than 75 per cup to prevent damage. These were then marked using fluorescent powders puffed into the cups using syringes (capacity = 5ml), and then released at different sites within and outside the wood. Other mosquitoes were released as blood-feds after feeding on either mice in the laboratory or more often, on humans, usually M.W.Service. As far as possible individual mosquitoes were blood-fed within two hours of each other. They were then marked using fluorescent powders at the release sites. All mosquitoes which did not disperse within 30 minutes were retained and their number was subtracted from the total release number. However, over 99% of the marked mosquitoes were successfully released on each release day. The mosquitoes were recaptured throughout the spring and summer of both years using the human bait catch technique and sweep-netting vegetation, until no more mosquitoes were caught. Although it is usually possible to identify the powdered mosquitoes by naked eye, the mosquitoes were nevertheless screened using a portable battery-operated UV light.

Fluorescent dusts are used for several reasons. They are generally reported as having little or no effect on mosquito survivorship. For example, Ivanova and Ipatov (1987) found that fluorescent powders had no adverse effect on the survival rate and bloodsucking capacity of Ae. cantans. Another advantage of marking mosquitoes with fluorescent powders is that they can be examined without the use of solvents and therefore the mosquitoes do not have to be killed (Service, 1976).

Nevertheless, to check whether powders used in the present experiment had any detrimental effect on survivorsip, 30 powdered mosquitoes were retained in a cage in the field. A separate cage containing 30 unmarked mosquitoes was used as a control. Mortality in both cages was recorded for two weeks. In addition, feeding experiments were undertaken in the laboratory to determine whether marking with fluorescent powders affected feeding behaviour.

Mosquitoes were marked using "Fiesta" daylight fluorescent colours. The colours used together with their commercial codings in parentheses are as follows; blue (A60), lunar yellow (A27), blaze (A5), stellar green (A8), rocket red (A3), nova red (A2) and cerise (a15) provided by the company Swada Ltd, London. Because it was necessary to release adults on more days than there were available colours, different coloured powders were mixed to produce dark green, salmon-pink, purple, brown and lighter brown. All these colours could be easily distinguished from each other with the naked eye. Bennett et al. (1981) were able to produce more than forty unique marks by using coded combinations of only six fluorescent powders and screening using a light fluorescent microscope.

In 1989, 1,872 female Ae. cantans were powdered and released, and in 1990, 2,415 females were powdered and released.

5.3 RESULTS

5.3.1 PERCENTAGE RECAPTURE

Tables 5.1 and 5.2 show the release and percentage recaptures of painted mosquitoes in 1989 and 1990, respectively. The majority of mosquitoes were captured by the human bait catch technique and so males were not frequently encountered. In fact, no marked males were recaptured in either year. The mean recapture rate of painted mosquitoes in 1989 (2.25%) is lower than in 1990 (6.3%). In 1989 the recapture success of painted mosquitoes ranged from 0-20% and in 1990 from 0-100% depending on the release day.

Tables 5.3 and 5.4 show the percentage recaptures of powdered mosquitoes in 1989 and 1990. The mean recapture rate in 1989 (3.66%) is again lower than in 1990 (6.25%). The overall recapture rate in 1989 of powdered mosquitoes ranged from 0.4-6.92% and in 1990 from 2.22-12.88% depending on the release day.

The recapture rate in 1989 was significantly lower than the recapture rate in 1990 ($\chi^2 = 94.54$, 3 degrees of freedom, $P<0.05$). There was no significant difference between the total recapture of painted and powdered mosquitoes ($\chi^2 = 0.017$, 1 degree of freedom, $P>0.05$).

5.3.2 EMERGENCE TO BLOOD-FEEDING

In 1989, it took a minimum of eight days for a mosquito painted at emergence to come to bait, with a mean of 16.8 days. In 1990, it took a minimum of only four days, but the mean was 18.0 days.

All 60 painted females recaptured in both years were inseminated.

Table 5.3 : Release and recaptures of powdered *Aedes cantans* in 1989

Release Date + Colour	N.	Release Site + Condition	N. Recaptured	% Recapture
09.06.89 Blue (A60)	130	Pond 9 Unfed	9	6.9%
12.06.89 Yellow (A27)	285	Wood A Blood-fed	10	3.5%
12.06.89 Green (A8)	340	LSF Unfed	18	5.3%
13.06.89 Pink (a15)	210	LSF Unfed	6	2.9%
14.06.89 Orange (A5)	160	Pond 9 Unfed	8	5%
15.06.89 Purple	127	Field Unfed	3	2.4%
16.06.89 Salmon-pink	130	Wood B Unfed	8	6.2%
19.06.89 Light-brown	240	LSf Unfed	2	0.8%
22.06.89 Dark-green	250	Wood B Unfed	1	0.4%
Total	1872		65	3.7%

LSF = Leahurst Sheep Field N = Number

Table 5.4 : Release and recaptures of powdered *Aedes cantans* in 1990

Release Date + Colour	N.	Release Site + Condition	N. Recaptured	% Recapture
22.05.9 Orange (A5)	68	Hotspot Blood-fed	5	7.4%
24.05.90 Yellow (A27)	222	Hotspot Blood-fed	7	3.2%
25.05.90 Blue (A60)	201	Pond 9 Unfed	6	3%
27.05.90 Pink (a15)	261	Wood B Unfed	23	8.8%
01.06.90 Purple	240	Road Unfed	10	4.2%
01.06.90 Green (A8)	355	Hotspot Blood-fed	15	4.2%
10.06.90 Salmon-pink	200	LSF Unfed	21	10.5%
11.06.90 Brown	255	Wood A Unfed	14	5.5%
13.06.90 Mauve	180	Chicken farm Unfed	4	2.2%
13.06.90 Crimson	233	Hotspot Blood-fed	30	12.9%
28.06.90 Light-green	200	Road Unfed	14	7%
Total	2415		149	6.25%

LSF = Leahurst Sheep Field   N = Number

5.3.3 EFFECT OF MARKING ON SURVIVAL AND BEHAVIOUR

Table 5.5 shows the results of the two experiments designed to test the effect of painting on survival. There is no significant difference between the mosquitoes surviving in the painted and control cages in both years ($\chi^2 = 0.068$, $P>0.05$).

Tables 5.6 and 5.7 show the results of the powdering experiments designed to study the effect of powdering on survival and feeding behaviour. There was no significant difference in mosquito survival between the marked and the control cages ($\chi^2 = 0.02\text{-}0.16$, $P>0.05$).

When the effect of powdering on feeding behavior was examined no significant difference in the feeding behaviour of the lightly to medium powdered mosquitoes and the control was found ($\chi^2 = 0.57\text{-}0.22$, $P>0.05$). The light powdering most closely represents the strength of marking used in the mark-release-recapture experiments. The heavily powdered mosquitoes were still grooming themselves when the mice were introduced for feeding, and there was a significant reduction in the number of adults taking a blood-meal, especially on day one ($\chi^2 = 15.45$, $P<0.001$).

5.3.4 POPULATION ESTIMATES

The population of nulliparous Ae. cantans (P), and its variance (V), mainly in Wood A, was estimated as follows using the Lincoln index-

$$P = \frac{an}{r}$$

$$V = \frac{a^3 X\, n(n-1)}{r^3}$$

where, *a* = number marked, *n* = number of one-parous females and *r* = number of recaptured marked parous mosquitoes.

Table 5.5 : Effect of painting on *Aedes cantans* survivorship in cages.

Year	State	Number Surviving after 3 weeks.	Chi Square	Probability
1989	Control	28	0.000	P>0.05
	Powdered	28		
1990	Control	27	0.077	P>0.05
	Powdered	25		

Initial density = 30.

Table 5.6 : Effect of powdering on *Aedes cantans* survivorship in cages.

Year	State	Number Surviving after 3 weeks	Chi Square	Probability
1989	Control	27	0.019	P>0.05
	Powdered	26		
1990	Control	29	0.164	P>0.05
	Powdered	26		

Initial density = 30.

Table 5.7 : Effect of powdering on *Aedes cantans* feeding behaviour.

Powdering	State	Number feeding on mouse host daily Day 1	Day 2	Day 3	Chi Square	Prob.
Heavy	Control	19	7	3	15.45	$P<0.05$
	Powdered	3	10	10		
Medium	Control	21	6	3	0.57	$P>0.05$
	Powdered	19	8	2		
Light	Control	20	7	3	0.22	$P>0.05$
	Powdered	20	6	4		

Initial density = 30.

1989

$$P = \frac{1872 \times 1135}{28} = 75,883$$

$V =$ 200,576,975.

1990

$$P = \frac{3307 \times 2415}{73} = 109,403$$

$V =$ 159,002,457

Both these results are the estimates of the population of nulliparous mosquitoes. It should be noted that the variances calculated are extremely high which suggests that the population size estimates are not necessarily reliable.

Table 5.8 shows the results of the Fisher-Ford population estimates for 1990 according to the method used by Service (1976). This method is based on a series of recaptures and is essentially a series of Lincoln indices working backwards from the time when releases were made. A constant survival rate is calculated by a trial and error process. The Fisher-Ford method gives population estimates (N(t)) ranging from 2,750 on the 27 June 1990 to 117,268 on the 25 June 1990. The size calculated with the Lincoln index falls within this range, though admittedly this is not surprising since the range is so large.

$$N(t) = \frac{n_t a_i 0_{i-t}}{r_{ti}}$$

Where, N(t) = population estimate, n_t = total sample at time t, a_i = total marked animals released at time *i*, $ø_{i-t}$ = survival rate over period and r_{ti} = recaptures at time t of animals marked at time *i*

The estimated daily survival rate used in this method was 0.931.

Table 5.8 : Fisher-Ford population estimate 1990 of nulliparous *Ae. cantans*.

Date 1990	Fisher/Ford Population Estimate
19	3,144.6
18	7,237.7
12	110,160.9
11 JULY	67,387.1
9	2,754.8
8	28,931.9
7	55,795.8
3	24,442.7
2	116,629.3
29	13,496.6
28	8,865.0
27	2,750.2
26 JUNE	47,211.6
25	117.268.1
23	42,980.6
20	47,794.7
19	28,713.9
18	40,188.1
17	90,549.4
15	78,756.9
14	42,763.4
13	14,363.0
12	39,309.7
11	90,900.9
9	87,549.9
7	41,192.6
6	7,948.3
5	16,221.0
3	36,444.0
1	63,652.8
31	57,153.3
30	77,262.4
27 MAY	37,680.9
26	30,991.2

The present mark-release-recapture study was designed primarily to look at dispersal and the length of the gonotrophic cycle rather than obtain estimates of the population level. Nevertheless, it is an added bonus if an estimate, no matter how approximate can be made. The Fisher-Ford method has been adapted by some authors to take account of the availability of mosquitoes for recapture at bait. For example, Conway et al. (1974), included an availability proportion based on the duration of the gonotrophic cycle, in the calculations of the population estimates of Ae. aegypti in Tanzania. However,. this was not done in the present investigation because of the variability in the duration of the cycle.

5.3.5 DISPERSAL

Figure 5.1 shows the release sites and recaptures of Ae. cantans for 1989, while Fig.5.2 shows the release and recaptures for 1990. Recaptures were from both human-bait catches and sweep-netting vegetation. Each different coloured circle represents a different release. The circles with letters represent the release points whilst the circles with numbers represent the number of recaptures of that particular colour caught in that particular site over the whole of the summer.

The results in both years suggest that the mosquitoes move freely between the two woods and form part of the same single population (Figs 5.3 and 5.4). The arrows indicate the direction of mosquito movement from release site to recapture. Mosquitoes released at increasing distances from the woods still returned in varying numbers to bait catch sites around the field. This is well illustrated by some of the releases in 1990. When 240 purple mosquitoes, caught at bait at the Hotspot, were released 400 m south of the Hotspot, five mosquitoes returned to the Hotspot and two flew the 400 m to the Wood B bait catch site. Similarly, when 180 mauve mosquitoes were released

Fig. 5.1 and 5.2.

Note: Figs. 5.1 and 5.2 show release and recapture sites of powdered Aedes cantans in 1989 and 1990 respectively.
Coloured circles containing letters represent release sites, whilst circles containing numbers represent number of mosquitoes recaptured at various sites throughout the summer.

Figs. 5.3 and 5.4.

Note: Figs 5.3 and 5.4 illustrate the patterns of movement of the powdered mosquitoes between release and recapture sites.

Fig. 5.3: Pattern of marked released mosquito movements, 1989.

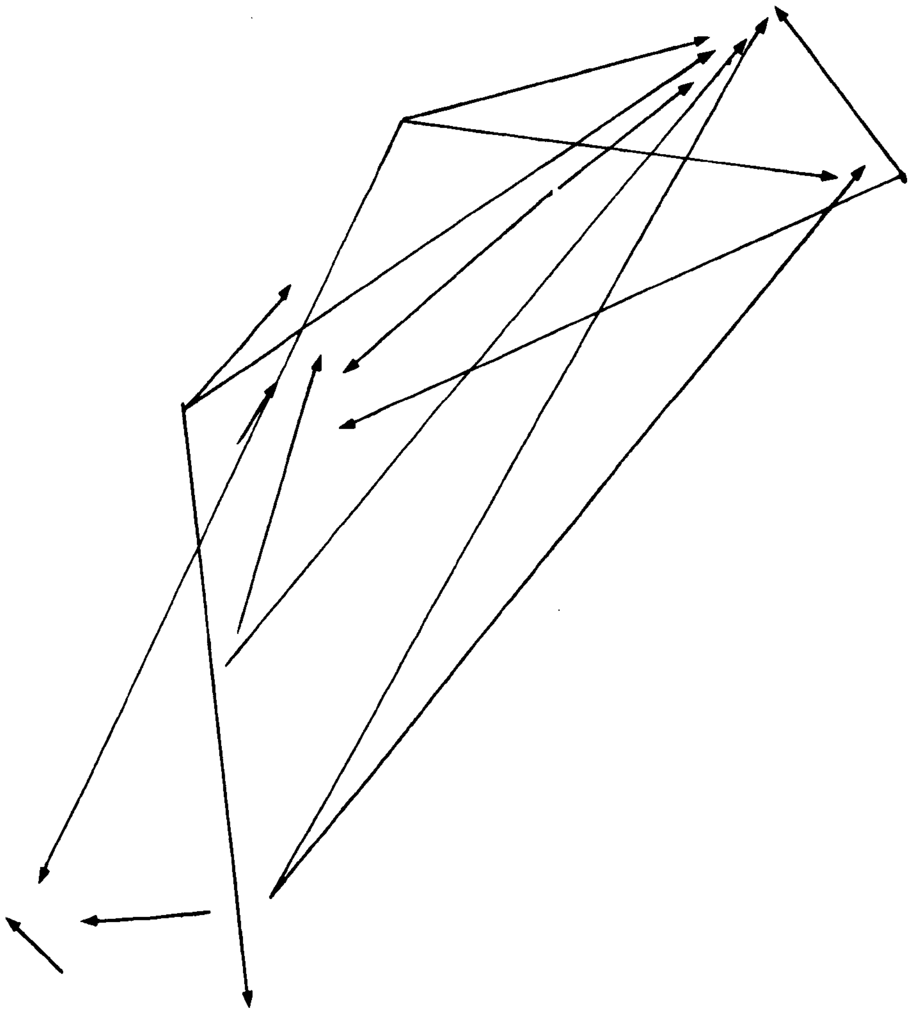


Fig. 5.1: Release and recapture sites, 1989.

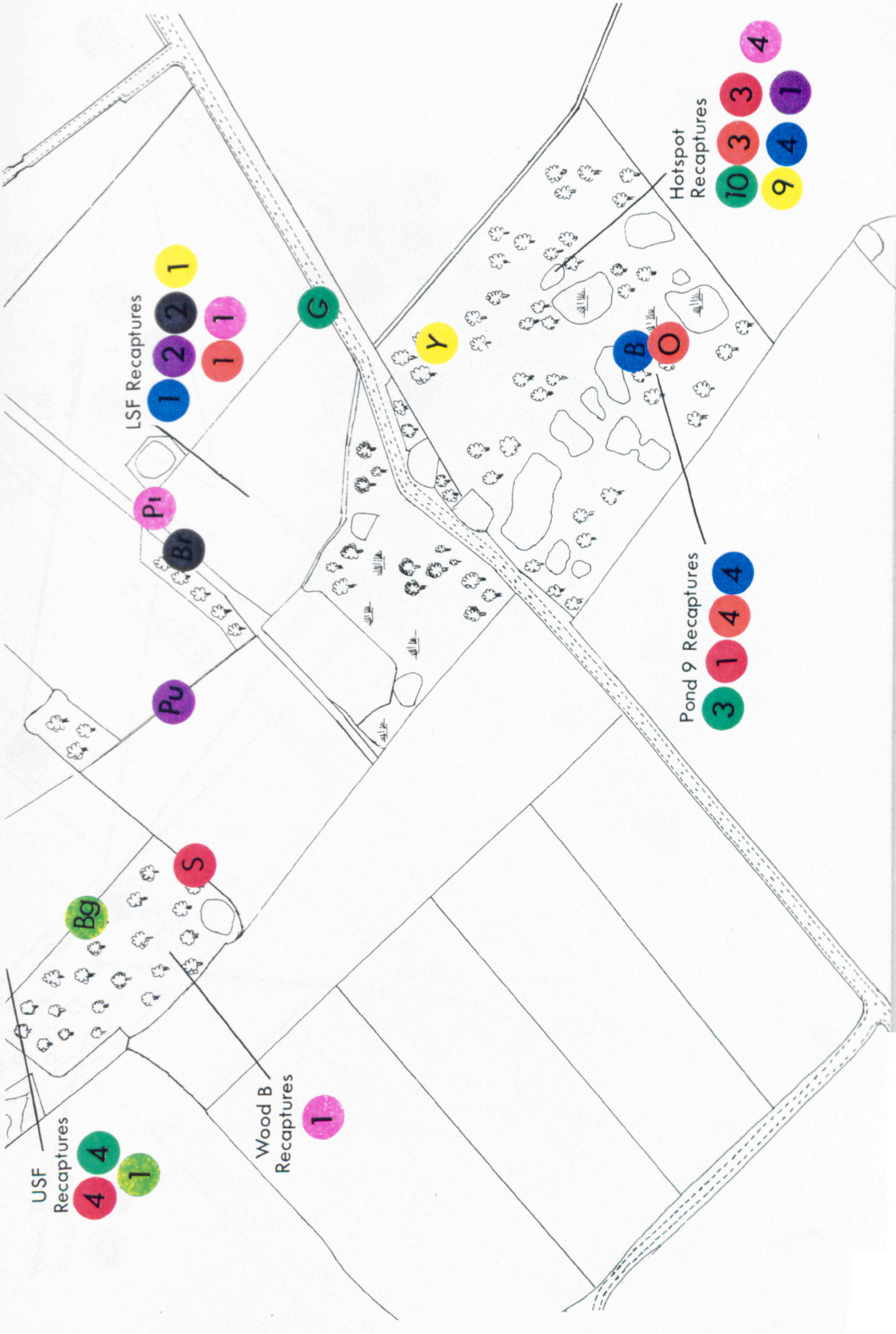


Fig. 5.3: Pattern of marked released mosquito movements, 1989.

Fig. 5.1: Release and recapture sites, 1989.

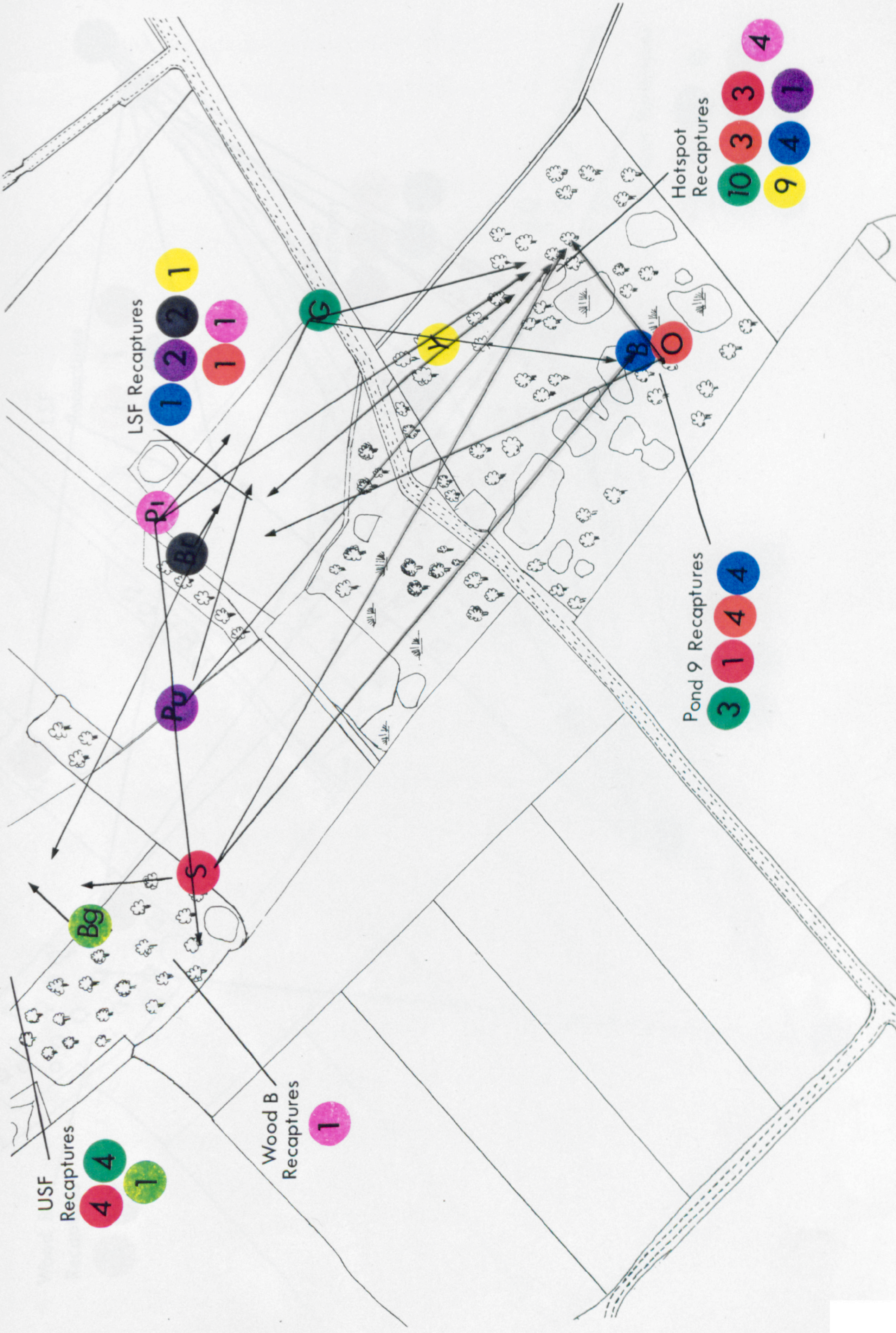


Fig. 5.4: Pattern of marked released mosquito movements, 1990.

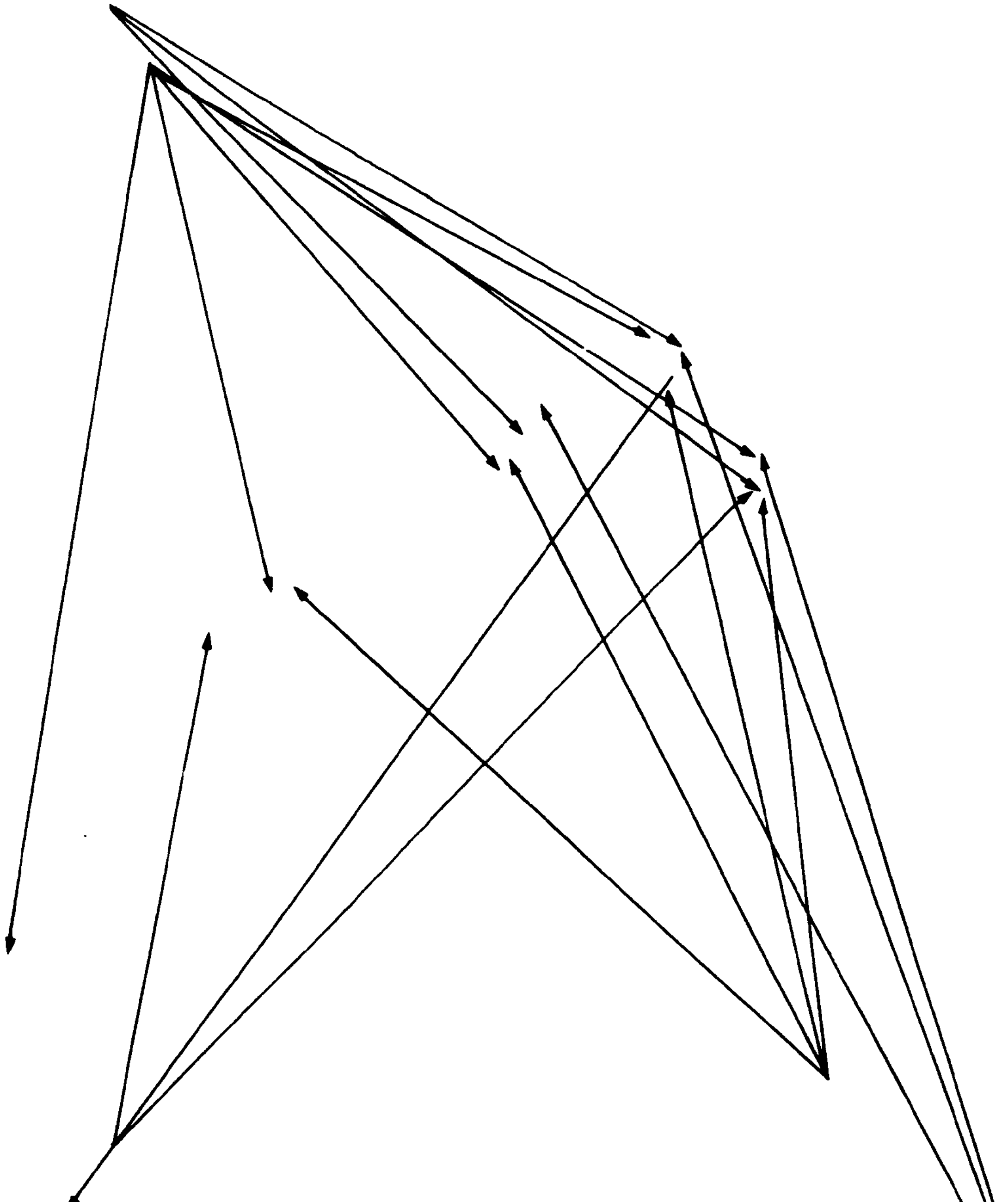


Fig. 5.2: Release and recapture sites, 1990.

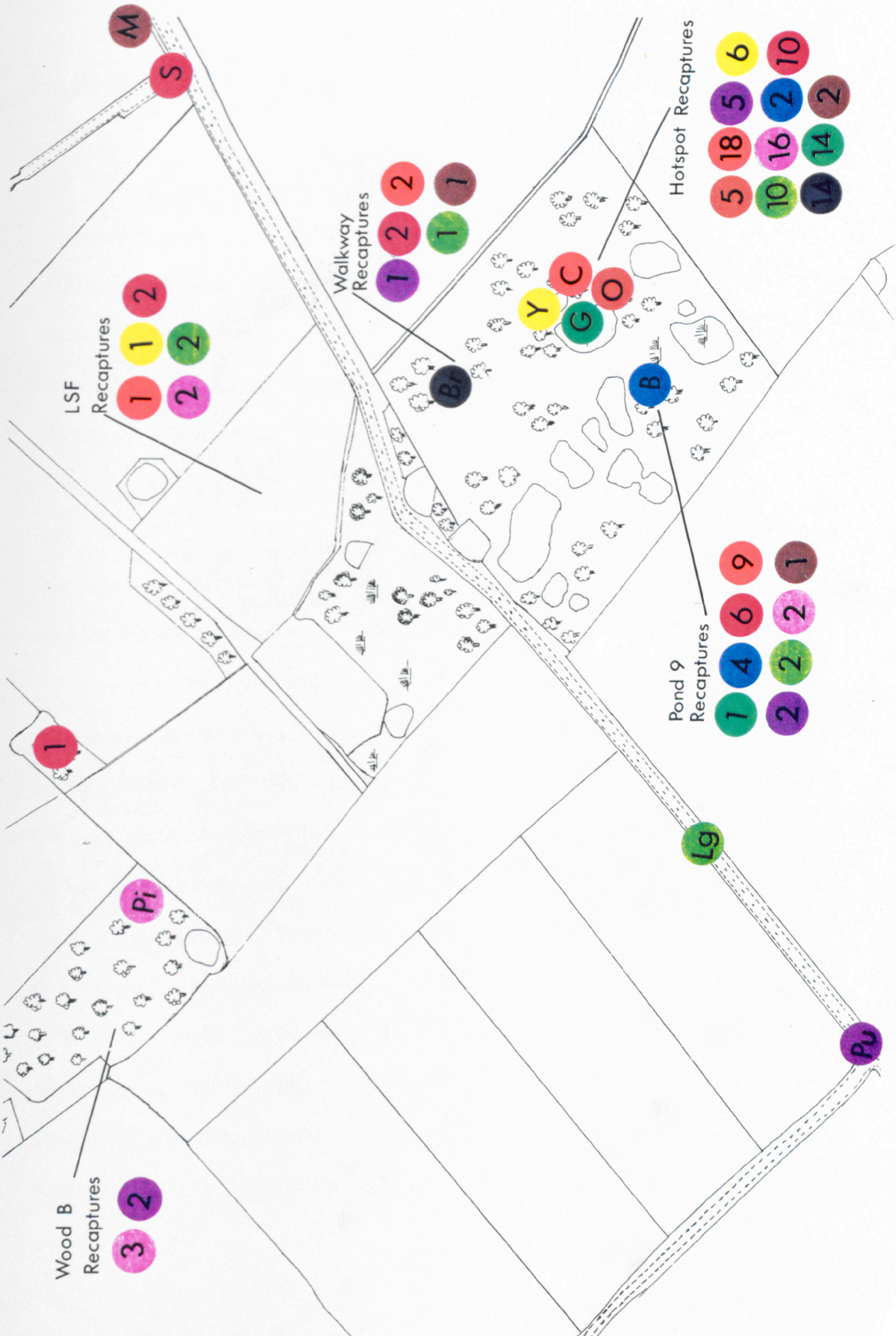
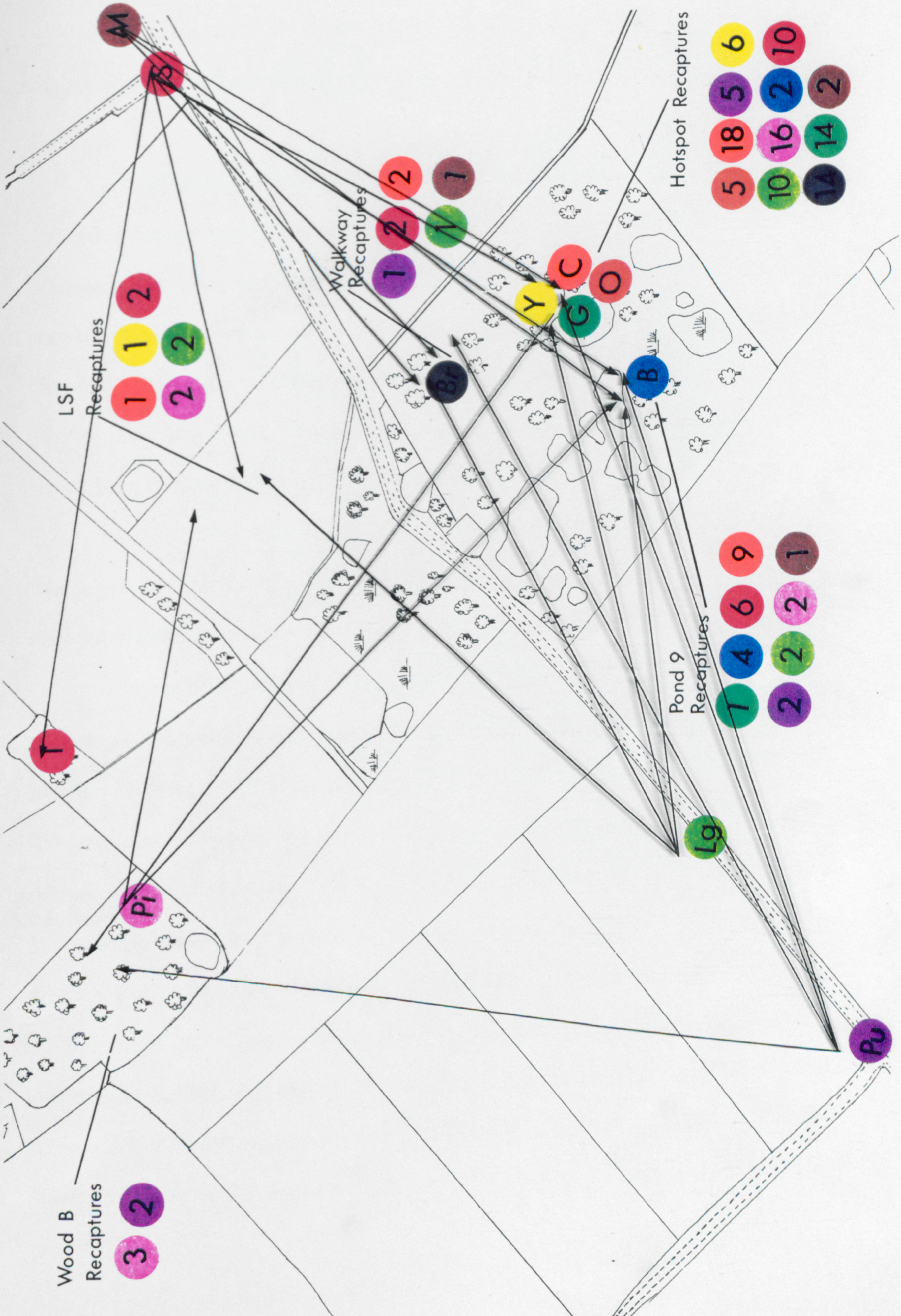


Fig. 5.4: Pattern of marked released mosquito movements, 1990.

Fig. 5.2: Release and recapture sites, 1990.



400 m to the north of the Hotspot, one mosquito returned 475 m to Pond 9 and 2 returned 400 m to the Hotspot.

It is unlikely that wind is responsible for these movements because mosquitoes are coming from different directions, both north and south of the wood (Figs.5.3 and 5.4).

It is possible that the mosquitoes are displaying a homing behaviour, or that they have a large home range covering the two woods and the surrounding fields. It is obvious that the mosquitoes form one large population and that they move freely between the two woods. When 261 pink mosquitoes captured in Wood A were released in Wood B, two recaptures were caught in the Leahurst Sheep Field (LSF - Fig.1.2) and also 18 at Pond 9 and in the Hotspot, clearly showing that the mosquitoes found their way back to the original site of capture.

In 1990, there was no significant difference between the recapture rate of mosquitoes released in Woods A and B and those released more than 200 m away from the Hotspot in Wood A ($\chi^2 = 0.335$, 1 degree of freedom, $P>0.05$).

It could be argued that mosquitoes were dispersing in all directions, but only movement between the two woods was observed because of the recapture sites chosen. However, in 1989 and 1990, human bait catches were carried out in some of the fields surrounding the woods. In 1989, Fields 8, 11 and 12 (Fig.1.2) were chosen and at each site one-hour human bait catches were carried out on six different occasions in the evenings throughout the summer. In all these bait catches, no marked or unmarked mosquitoes were captured. Similarly, in 1990, Fields 8, 10, 11, 12 and 13 were used as bait catch sites and again six one-hour human bait catches were carried out at each site with no returns. Bait catches were not carried out in the afternoons or mornings because mosquitoes were not caught at bait in any of the fields except during the evening (Chapter 3). The lack of mosquitoes in these bait catches suggests that there is limited movement away from the woods. There were no grazing animals in these fields in either year, and therefore there was no apparent reason for the mosquitoes to be moving away from the woods in this direction.

5.3.6 SURVIVAL

In 1989, two mosquitoes were recaptured 66 days after they were painted at emergence and released. The longest lived painted mosquito recaptured in 1990 was 72 days old. The longest lived powdered mosquito recaptured in 1989 was 68 days old, and in 1990, 69 days old.

There was a period of 32 days between the last marked and unmarked mosquitoes in 1989, and a period of eight days in 1990.

The regression of the numbers of powdered recaptured females with time is shown in Figs 5.5 and 5.6.

In 1989 the regression equation was

$\ln(\underline{x}+1) = 1.55 - 0.0434\underline{x}$

Regression coefficient = -0.0434

Regression coefficient T-value = -4.66

(With 34 degrees of freedom, $P<0.001$)

Correlation coefficient = 0.0389

(With 34 degrees of freedom, $P<0.05$)

Daily survival rate = **0.957**

Percentage daily mortality = **4.3%**

In 1990 the regression equation was

$\ln(\underline{x}+1) = 1.96 - 0.0382\underline{x}$

Regression coefficient = -0.0382

Fig. 5.5: Regression of powdered recaptured females with time, 1989.

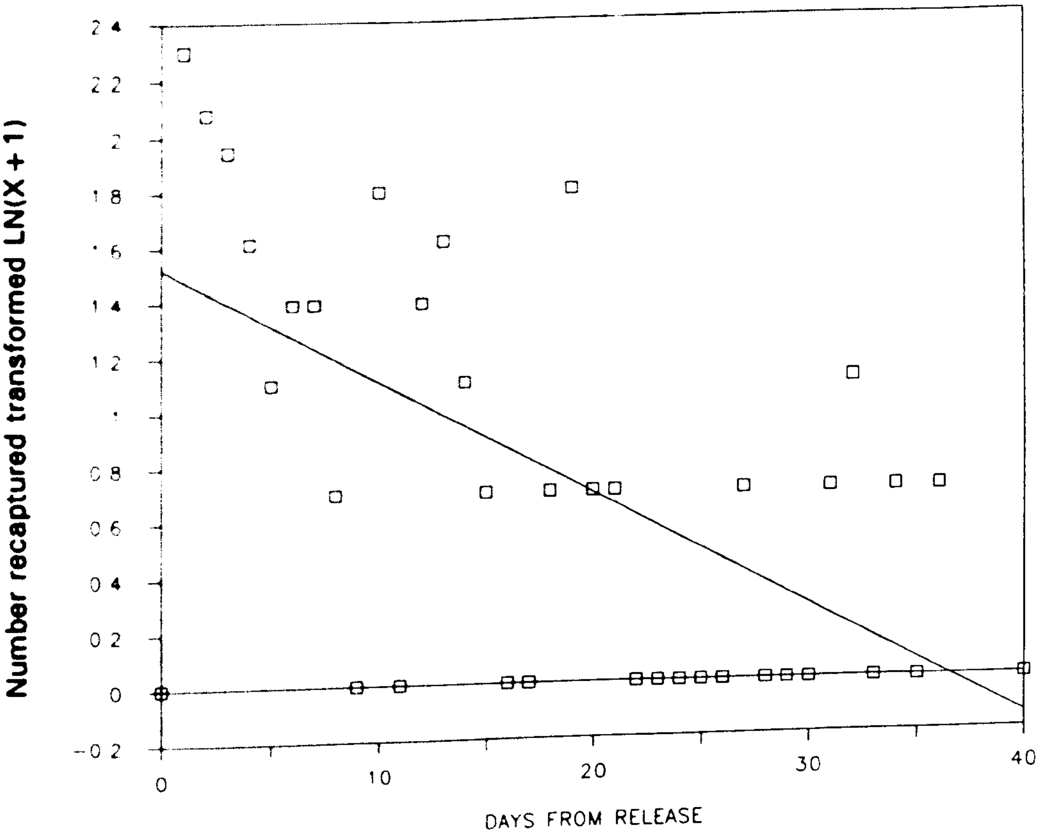
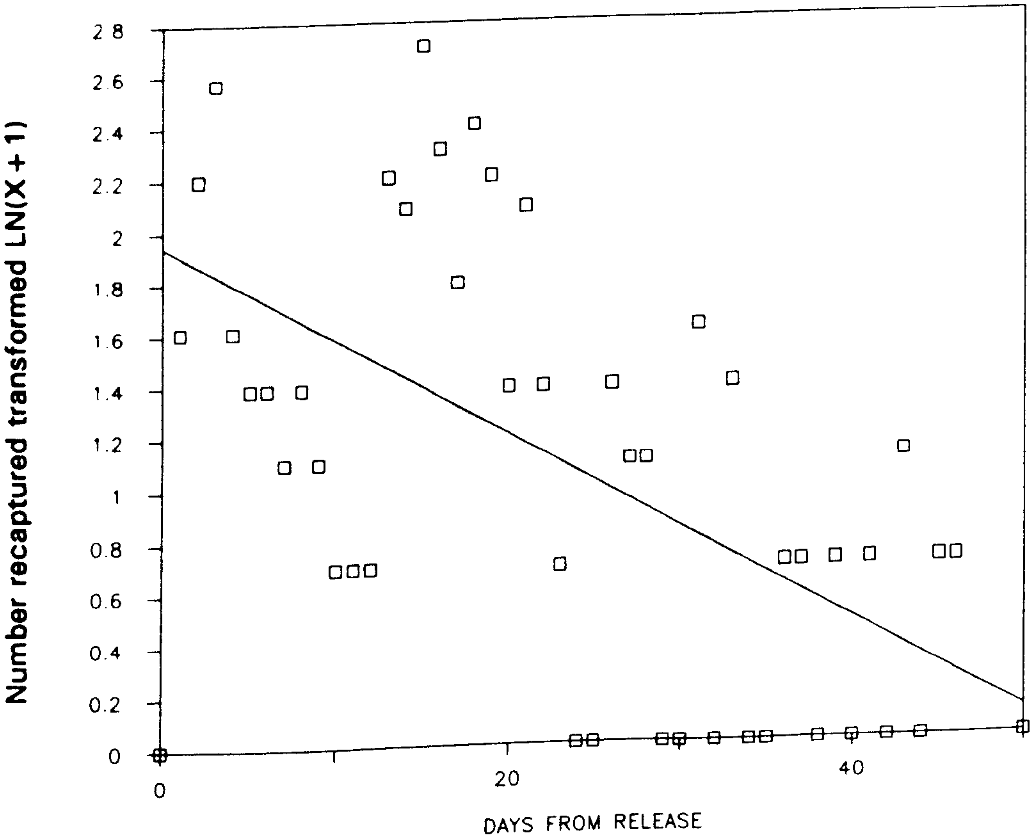


Fig. 5.6: Regression of powdered recaptured females with time, 1990.



Regression coefficient T-value = -6.16

(With 48 degrees of freedom, P<0.001)

Correlation coefficient = 0.43

(With 48 degrees of freedom, P<0.05)

Daily survival rate = **0.963**

Percentage daily mortality = **3.7%**

The survival was estimated using the ratio of recaptured two-parous marked to one-parous recaptured mosquitoes, the theory being that the ratio represents the loss of mosquitoes between the one-parous and two-parous stage.

In 1989 this represents 8:16 = 0.5

In 1990 this represents 28:73 = 0.38

5.3.7 GONOTROPHIC CYCLE

Table 5.9 shows the duration of the gonotrophic cycles of powdered mosquitoes recaptured in 1989, while Table 5.10 shows the duration of the gonotrophic cycles of powdered mosquitoes recaptured in 1990. The gonotrophic cycle is the period between blood-feeding and oviposition.

The results from 1990 are the more accurate because the number of cycles was confirmed by using the ovariole dilatation technique of Detinova (1962) (Chapter 3).

Figures 5.7 and 5.8 show the returns of both blood-fed and non-blood-fed mosquitoes in days after their release. Several patterns can be seen. Nulliparous mosquitoes returned to bait from day 1 to day 8 in 1989, and from day 1 to 13 in 1990 after marking and release. The average returns in 1989 are on day 13 for one-parous mosquitoes and on day 21 for two-parous mosquitoes, representing a second cycle

Table 5.9 : Estimation of the duration of the gonotrophic cycle from recaptures in 1989.

Release Date	Colour	1-Parous Date + L of 1st cycle	2-Parous Date + L of 2nd cycle	3-Parous Date + L of 3rd cycle
09.06.89	Blue	NC	NC	NC
12.06.89	Yellow *	24.06.89 13 days	01.07.89 8 days	16.07.89 16 days
12.06.90	Green	22.06.89 11 days	01.06.89 10 days	14.07.89 14 days
13.06.89	Pink	23.06.89 11 days	02.07.89 10 days	14.07.89 13 days
14.06.89	Orange	NC	NC	NC
15.06.90	Purple	29.06.89 15 days	NC	NC
16.06.89	Salmon-pink	01.07.89 16 days	NC	NC
19.06.89	Orange-brown	03.07.89 15 days	NC	NC
26.06.89	Blue green	NC	NC	NC

L = Length
* = Blood-fed NC = None Captured

Fig. 5.7: Recaptures of marked mosquitoes, 1989.

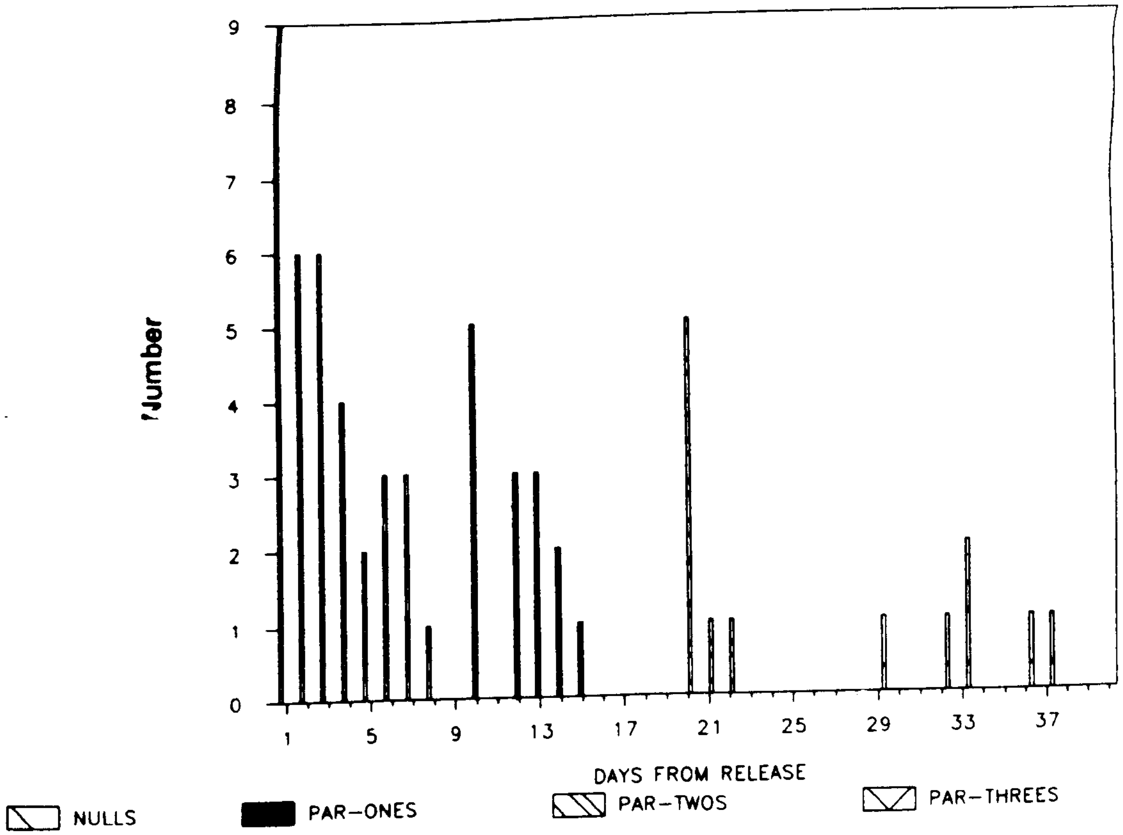
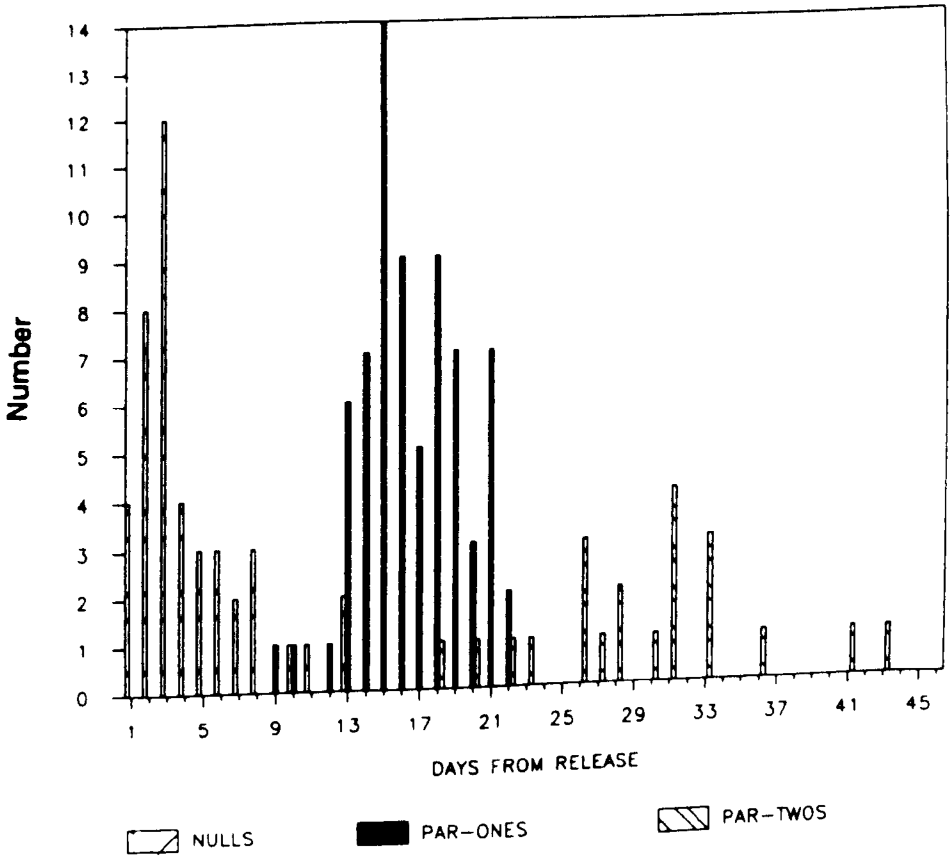


Fig. 5.8: Recaptures of marked mosquitoes, 1990.



of approximately 9 days. In 1990, one-parous mosquitoes showed a mean return on day 16 and on day 31 for two-parous mosquitoes, representing a second cycle of approximately 16 days.

The first gonotrophic cycle in 1990 averaged 14.9 days, the second one was 13.2 days and the third one was 11.33 days (Calculated from data in Table 5.10). In other words, the length of the cycle gets shorter as time progresses. Pearson's rank correlation coefficient comparing the lengths of the first cycles in 1989 with the average temperatures over the period of the cycle gives, $r = -0.907$, and in 1990 $r= -0.641$ (Table 5.11). This indicates that there is a significant negative correlation between the length of the cycle and temperature in both years (Table 5.10). Average temperatures were calculated by summing the daily maximum and minimum temperatures and calculating the mean. The weather data were obtained from Ness Gardens, some 1.5 km west of Ness Woods. Figures 5.9 and 5.10 show the mean daily temperature for the summers of 1989 and 1990.

The estimated duration of the gonotrophic cycle is very much dependent on the recaptures. The actual length of the cycle may be shorter than the estimated length, because some of the powdered mosquitoes are feeding elsewhere. It should be noted that the lengths of the cycles have been based on relatively low returns of marked mosquitoes.

The estimated duration of the cycles based on mark-recapture data compare well with the time taken for blood-fed mosquitoes placed in cages in the field to lay eggs. Blood-fed mosquitoes placed into cages on 24 May 1990 took 19 days before they laid their eggs, compared to a 20 day oviposition cycle in the yellow blood-fed releases on the same date. Similarly, it took 13 days for caged blood-fed mosquitoes to lay eggs when the same experiment was set up on 1 June 1990, which compares to 14 days for the dark green blood-fed releases. Finally, it took only 11 days for the

Table 5.10 : Estimation of the duration of the gonotrophic cycle from recaptures in 1990.

Release Date	Colour	1-Parous Date + L of 1st cycle	2-Parous Date + L of 2nd cycle	3-Parous Date + L of 3rd cycle
22.05.90	Orange *	11.06.90 21 days	NC	NC
24.05.90	Yellow *	12.06.90 20 days	23.06.90 12 days	NC
25.05.90	Blue	NC	NC	NC
27.05.90	Pink	12.06.90 17 days	29.06.90 18 days	11.07.90 13 days
01.06.90	Purple	15.06.90 15 days	29.06.90 14 days	09.07.90 11 days
01.06.90	Dark-green *	14.06.90 14 days	27.06.90 14 days	NC
10.06.90	Salmon-Pink	20.06.90 10 days	29.06.90 9 days	08.07.90 10 days
11.06.90	Brown	23.06.90 13 days	03.07.90 11 days	NC
13.06.90	Mauve *	25.06.90 13 days	NC	NC
13.06.90	Crimson	23.06.90 11 days	03.07.90 11 days	NC
28.06.90	Light-green	12.07.90 15 days	28.07.90 17 days	NC

L = Length
* = Blood-fed   NC = None Captured

Table 5.11 : Correlation between length of the gonotrophic cycle and temperature.

1989 Days of first cycle	Mean Temperature
16	15.98
15	15.53
15	16.4
13	17.5
11	17.55
11	17.95

Pearson's Rank Correlation = -0.907 $P<0.005$

1990 Days of first cycle	Mean Temperature.
21	12.91
20	13.13
17	12.51
15	13.03
15	15.09
14	12.43
13	13.88
13	13.5
11	14.12
10	15.19

Pearson's Rank Correlation = -0.641 $P<0.05$

Fig. 5.9: Mean daily temperature during the mark-release-recapture period, 1989.

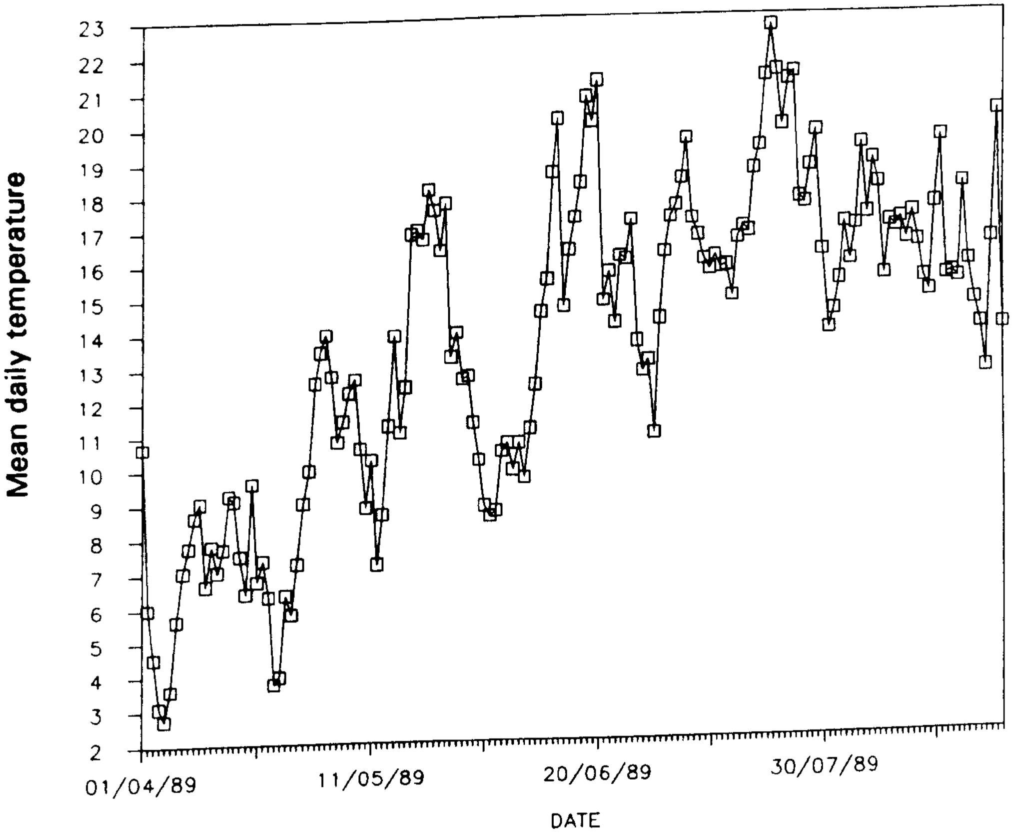
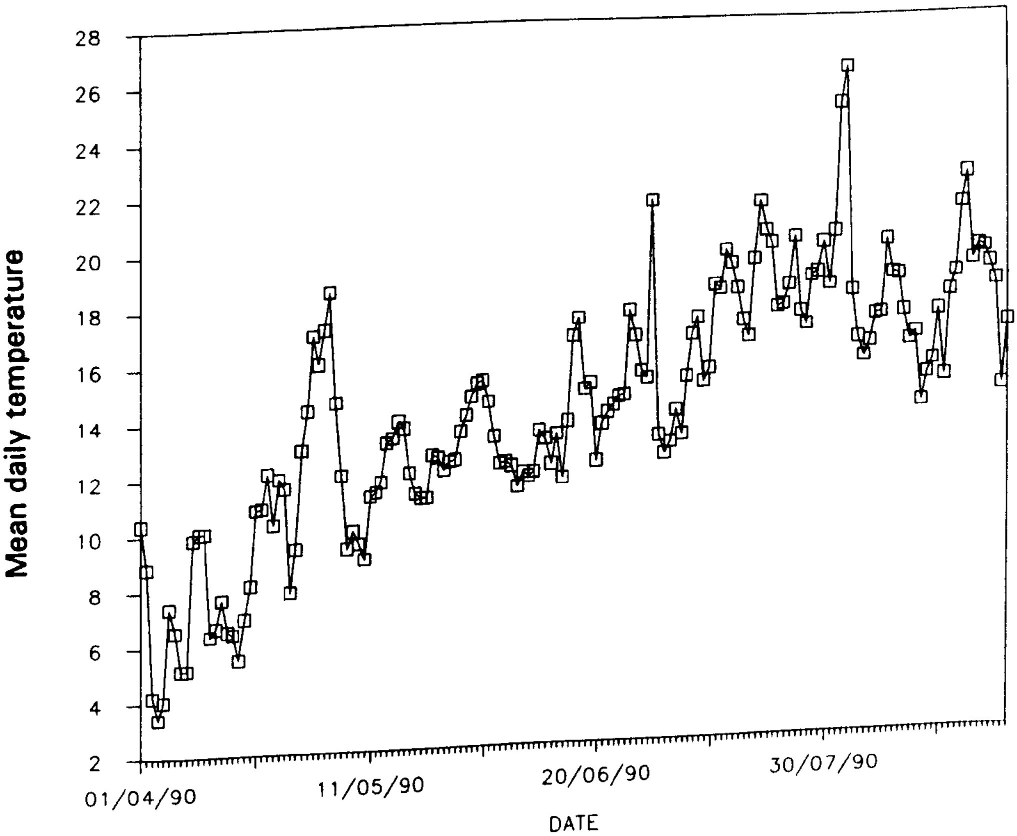


Fig. 5.10: Mean daily temperature during the mark-release-recapture period, 1990.



caged mosquitoes to lay eggs in the experiment set up on 13 June 1990, which again compares well to the 13 days for Ae. cantans released on the same date.

One of the most interesting discoveries is that there is no significant difference between the time taken for females that were released blood-fed and unfed to return to human baits after the completion of the cycle. For example, with the mosquitoes released on 1 June 1990, it took 14 days for the first formerly blood-fed marked mosquito to return, whilst it took mosquitoes that were released unfed only 15 days to return. Similarly, the blood-fed mosquitoes released on 13 June 1990 took 11 days to return whilst individuals that were released unfed took only 13 days. However, in 1989 blood-fed mosquitoes released on 12 June 1989 took longer than those that were released unfed to return, 13 days compared to 11 days. It should be noted that the blood-fed mosquitoes will have a "head start" in blood digestion.

5.4 DISCUSSION

5.4.1 PERCENTAGE RECAPTURE

The lower recapture rate in 1989 may be due to weather conditions, the weather was colder and windier in 1989 and this may have affected recaptures (Chapter 3). The overall recapture rates in both years compare well to previous studies carried out on Ae. cantans. Sulaiman (1982) had a particularly low rate and recaptured only one female out of 655 painted mosquitoes released at emergence in Ness Woods. At Monk's Wood, Service (1977a) recaptured 3.5-4.5% of the mosquitoes he released. Gavin (1986) recaptured 3.5-17.5% of the Ae. cantans mosquitoes released at the woods at Ness. There tends to be a large range of recapture success and Service (1976) tabulated some recapture rates of various mosquito species in different studies.

The recapture rates in this investigation are reasonably high. Low recapture rates can result from a high dispersion or mortality. Recovery attempts were made within 400 m of the hotspot, and it is possible that mosquitoes are dispersing much further than this. It is also possible that the large number of alternative hosts in the surrounding fields could decrease the number coming to a human bait, especially if Ae. cantans exhibits preference for feeding on non-human hosts. (Chapter 4).

5.4.2 EMERGENCE TO BLOOD-FEEDING

The short period of time, 4 to 8 days, from emergence to first blood-feeding is surprising since this contradicts the results of Service (1977a) working in Monk's Wood, Huntingdon. Service found that a minimum period of 20 days elapsed between emergence and the the first blood-meal taken by Ae. cantans. He postulated that this was the time taken for the ovaries to progress to Stage IIb. Sulaiman (1982) found a similar situation in Ness Woods in 1981, with a period of 28 days from the first recorded emergence of Ae. cantans to the first appearance at human bait. Similarly, Bowen (1991) stated that most female mosquitoes do not become host-responsive until several days after emergence. This, however, was not the case in my investigation in Ness Woods, and it is possible that it took at least part of this interval of four to eight days for the Ae. cantans to find a mate before host-seeking. The mean duration between emergence and blood-feeding found in this study compares better with the minimum calculated by Service (1977a). It is conceivable that it is the exception rather than the rule for Ae. cantans to blood-feed so quickly after emergence.

5.4.3 EFFECT OF MARKING ON SURVIVAL AND BEHAVIOUR

Reisen and Aslamkhan (1979) found that there was no significant difference between the mortalities of An. stephensi that were dusted with powder and control groups of undusted mosquitoes. On the other hand, Meek et al. (1988) considered that an inundative covering of pigment could interfere with the natural behaviour and flight performance of mosquitoes. Birley and Charlwood (1989) found that marked unfed Anopheles farauti Laveran in Papua New Guinea had a higher pre-release mortality than blood-fed females. The powdering experiments I carried out in the field and the laboratory showed, however, that there was no significant difference between the survival of powdered and control mosquitoes. Neither did light to medium powdering have any significant effect on the mosquitoes' ability to obtain a blood-meal from anaesthetised mice. Nevertheless heavily powdered mosquitoes did have their blood-feeding behaviour supressed, emphasising that care is required when marking mosquitoes to ensure that they receive only a light dusting. Gillies (1961) found that there was a slightly higher mortality in mosquitoes that had been painted in the first 24 hours following emergence, but there was no significant difference in survival between painted and non-painted mosquitoes after this period. The application of 'Humbrol' paint to the mosquitoes in this investigation was found to have no significant effect on their survival.

The marks on the mosquitoes were certainly durable, with painted mosquitoes being captured up to 72 days after release, and powdered mosquitoes up to 69 days after release.

It is still possible that marking mosquitoes with paints and powders could lead to an increase in predation, by increasing visibility.

5.4.4 POPULATION ESTIMATES

The population estimates using the Lincoln index are good from the point of view that the estimates are higher in 1990 (109,403) than in 1989 (75,883) in agreement with the bait catch results of both years (Chapter 3). It must be remembered that the estimate is that of the nulliparous mosquitoes probably within Wood A only and is not an estimate of the whole population.

The Fisher-Ford population estimates for 1990 show a wide difference in the population size on different days, ranging from 2,750 to 116,629. However, the size of the daily biting population depends considerably on weather conditions. For instance, on a warm still day the number of biting mosquitoes is high. It is difficult to say which population estimate is more accurate.

Many different population estimates have been made in mark-release-studies on mosquitoes. Using the Lincoln index, Gavin (1986) estimated the total nulliparous mosquito population in Ness Woods as 28,908. In Pakistan, Reisen and Aslamkhan (1979) used the Lincoln index method, modified to account for survivorship as suggested by Bailey (1951), and concluded that the Lincoln index gave meaningful estimates of population size of An. stephensi.

Many authors feel that the stochastic Jolly's method is the best one to use if the number of recaptures are high, but when there is a small recapture rate, as is the case in this study, the deterministic model of Fisher and Ford is a better method (Cook, 1985; Sheppard et al. 1969). Service (1977b) questioned whether anything is gained from using the more complex population estimates in preference to the simpler methods such as Lincoln's index since recapture rates are often low.

5.4.5 DISPERSAL

The Ae. cantans mosquitoes at my field site form one large population that seems to move freely between the Woods A and B and the surrounding fields. The movement of the mosquitoes seems to be non-random and they may well be showing a homing behaviour. This is indicated by the fact that mosquitoes released at increasing distances from the woods are recaptured in reasonable numbers within the woods.

Charlwood et al. (1988) working in Papua New Guinea postulated that An. farauti has a memorised home range which facilitates directive flight between feeding and breeding sites. Birley and Charlwood (1988) released marked An. farauti females in an alien village and found that they had a longer oviposition cycle and dispersed more than those released in the village of capture. This seems to indicate that mosquitoes are "aware of their surroundings". Many insect species show evidence of homing behaviour, including moths, butterflies, grasshoppers and dragonflies (Baker, 1982) and the above assumption would not appear to be unreasonable. Mosquitoes are more likely to gain a competitive advantage from having a homing behaviour, especially since they have to fly out of the woods in the evening to find hosts and then return to a suitable area to rest and later lay their eggs.

Sinsko and Craig (1979) looked at movement of both male and female Ae. triseriatus between two woodlots 300 m apart in Indiana and found that movement was more or less random within a woodlot, but there was no movement between the woodlots and therefore each woodlot could be considered as an ecological island. The main woodlot studied was 10.1 ha compared to the 6 ha of Wood A in the present study. Movement within a woodlot was not completely random, however, because the environment itself was not homogenous, and mosquitoes congregated according to the influence of wind, moisture, cover and the presence of blood-meals. This is clearly not the situation in Ness Woods where there is a definite movement between the two woods.

Most studies on mosquito dispersal have shown that mosquitoes do not tend to disperse far. In Kenya Mcdonald (1977b) found that Ae. aegypti mosquitoes have a very limited dispersal and he recaptured most of the mosquitoes within 20 m of the release site. Nayar et al. (1980) found that 82.4% of Culex nigripalpus were recaptured within 0.4 km of the release site. In Pakistan, Reisen and Aslamkhan (1979) found that the total number of marked male and female An. stephensi resting decreased significantly as a function of distance from the release site. They reported that males and females do not disperse evenly. The mean female dispersal distance increased significantly as a linear function of time in days after release, however, male recaptures were too infrequent and too variable for any conclusions to be made.

Information on the dispersal of Ae. cantans is varied. In the USSR, Petruchuk (1973) found that marked Ae. cantans dispersed to a maximum of 20 km. The distance the mosquito dispersed was related to topography, with mosquitoes dispersing further over rugged terrain. Service (1977a), working in southern England, concluded that female Ae. cantans had a rather limited flight range because he caught most marked mosquitoes within 25 m of the release site and all the recaptures within 125 m. Campbell (1986) found that in Wood A, Ae. cantans dispersed further than this, as females were recaptured 230 m from the release site. He thought this may have been due to the fact that mosquitoes in Ness Woods tend to fly out of the woods to find hosts, whereas those studied in southern England by Service (1977a) fed on rabbits within the boundaries of the wood. My study seems to indicate that Ae. cantans has quite a large flight range. This would certainly be an advantage when it comes to finding hosts and oviposition sites. It would be advantageous for a mosquito to have a recognised home range and to exhibit homing behaviour.

When investigating dispersal of mosquitoes the decrease in recaptures with increasing distance from the release site can be due in part to their numbers being dispersed over an ever-increasing area, which results in lower densities. It is advisable to increase the number of catching stations further away from the release point. This, however, involves a lot of time, often with little or no return and it is usually not practical to do this. In this study, bait catches were carried out in fields 8, 10, 11, 12 and 13 (Fig.1.2) in an attempt to observe mosquito movement away from the woods, however, no mosquitoes, marked or unmarked, were caught. The lack of grazing animals as potential hosts in these fields means that the mosquitoes have no apparent reason for flying out of the woods

5.4.6 SURVIVAL

Service (1977a) found that Ae. cantans survived for more than 100 days in the laboratory, and some Ae. cantans in Ness Woods are clearly living for more than two months.

The daily survival rates for both 1989 and 1990 were high, being 0.959 and 0.963, respectively. These survival rates compare well to those found in other studies. Reisen and Aslamkhan (1979) estimated the survivorship of female An. stephensi to be 0.808 to 0.865, while Hii and Vun (1985) estimated the survivorship of An. balabacensis to be from 0.719 to 0.787. Service (1976) reviewed some of the studies on estimation of survival rates and found that they varied from 0.49 for Cx. tritaeniorhynchus to 0.84 for An. gambiae.

5.4.7 GONOTROPHIC CYCLE

The duration of the gonotrophic cycle seems to be contracting with an increasing number of cycles. There are several possible reasons for this. Firstly and most likely, increasing temperatures could be accelerating the cycle, simply because blood-meal digestion is more rapid at higher temperatures (Service, 1977a). This explanation is supported by the Pearson's Rank Correlation Coefficient. Secondly, Ae. cantans may become more experienced at finding a blood-meal and/or an oviposition site, thus decreasing the search times, as time progresses.

The estimated lengths of the cycles compare well with those estimated by Gavin (1986) for Ae. cantans in Ness Woods, namely 14 days.

These results, which show that there is no significant difference in the length of the gonotrophic cycles of mosquitoes that were released blood-fed and unfed, are particularly interesting because they indicate that the mosquitoes are not having any difficulty in finding a host. This is not consistent with work carried out in Ness Woods by M.H. Birley et al.(unpublished) indicating that Ae. cantans mosquitoes at Ness Woods were suffering from temperature-dependent host scarcity. They found that mosquitoes released unfed had difficulty in obtaining a blood-meal, and that they were recaptured while still nulliparous after more than 25 days. Birley felt that the adults had difficulty in finding hosts because of adverse weather conditions, and that cold and windy conditions prevented mosquitoes from flying out to find hosts. In other words, there was temperature mediated host scarcity. This was not the case in the present study.

CHAPTER 6 - GENERAL SUMMARY

LARVAL WORK

Aedes cantans larvae in temporary ground ponds were used to investigate density-dependent and density-independent regulation of larval mosquito populations in the field.

Larvae from overcrowded conditions (eg. Hotspot, 1989) were significantly smaller in terms of siphon length than those sampled from less crowded conditions (eg. Pond 9, 1989) for all instars. Similarly, adults emerging from crowded ponds were significantly smaller than those emerging from uncrowded ponds. Larval mortality was much higher in crowded ponds, and this was confirmed by carrying out density experiments in cages placed in the ponds: larvae maintained at higher densities were significantly smaller and showed significantly higher mortality than those reared at lower densities. Therefore, both density-dependent size variation and density-dependent larval mortality were demonstrated in the Hotspot in 1989 as well as in cages placed in Pond 13 in 1990 and 1991. At high densities, larval mortality occurred mainly in the first instar.

Density-dependent competition for food and/or contact inhibition and also cannibalism were considered the main causes of larval mortality. Mortality due to predation, growth retardant factors and parasitism was discounted as being of major importance.

In all three years, the density-independent factor of desiccation led to high larval mortality. Lack of rainfall in 1991, resulted in 100% mortality in the temporary ponds and correspondingly, the numbers of adults caught at bait in that year were significantly lower than in the previous two years.

Larvae showed highly contagious distributions, with the degree of aggregation decreasing from first to fourth instar. Generally, first and second instars did not fit the negative binomial, but third and fourth instars as well as pupae did. The data were normalised using Taylor's Power Law. There was a positive correlation between the number of larvae sampled and the temperature along the transect, and also a significant negative correlation between the number of larvae sampled and water depth, indicating that the larvae aggregate in shallower, warmer water.

EMERGENCE

Males emerged before females in all ponds in all three years. The sex ratio of emerging *Ae. cantans* was 50:50 except for Pond 8 in 1990 when the pond dried up before emergence was completed, and in which more males emerged than females. There was no significant difference in the sizes of males and females emerging from the same pond in the same year. The size of adults decreased significantly in all ponds in all three years as water temperatures increased and emergence progressed.

MOSQUITOES CAUGHT AT BAIT

Females were not caught at bait until an average of 17 days after emergence, and this corresponds with the time taken for the ovaries to develop to Christophers Stage II.

The size of females arriving at bait in both 1989 and 1990 increased as the season progressed. In 1989, parous *Aedes cantans* were significantly larger than nulliparous ones and in 1990, two-parous mosquitoes were significantly larger than one-parous mosquitoes which were in turn significantly larger than nulliparous ones.

This suggests that larger mosquitoes are longer lived than smaller ones, and therefore size is positively related to survivorship and the ability to obtain a blood-meal. Some nulliparous mosquitoes were caught late in the season in all three years. These were all smaller than the average size of the biting population caught at the same time. It is likely that these small mosquitoes have difficulty in obtaining a blood-meal. Some nulliparous mosquitoes in the laboratory required two blood-meals before they could oviposit, and since the smaller mosquitoes were less successful at blood-feeing this would have delayed egg production even further.

Laboratory oviposition studies showed that larger females laid more eggs. There was good correlation between wing length and the number of eggs laid in both 1989 and 1990. The size of the eggs and wing length were not correlated. No relationship between the number of eggs and the number of gonotrophic cycles could be detected.

Aedes cantans first came to bait in late April to early May and biting peaked in June-July, after which numbers declined until September when no more mosquitoes were caught. In 1991 when the temporary ponds in Wood A dried up before emergence could take place, the numbers of mosquitoes caught in hourly bait-catches was significantly lower than in the previous two years.

Females were caught biting in large numbers in the woods during the day, but none was caught in the fields until the early evening. Mosquitoes rested amongst vegetation during the day, but if activated by the presence of a host, would blood-feed opportunistically. At night, females would start to hunt, flying out of the woods into the surrounding fields to feed on grazing animals. Cold and windy weather conditions in June 1991 reduced the number of mosquitoes caught at bait.

A significant positive correlation was detected between the number of mosquitoes caught at bait and the mean temperature recorded in the field.

The dissections carried out in this investigation indicated that dilatations only form from follicles that degenerate at an early stage in the gonotrophic cycle. In agreement with Hoc and Charlwood (1990), less than 1% of the ovarioles in three-parous mosquitoes could be read for parity.

Sweep-netting vegetation showed that although some male mosquitoes can survive for over two months, most die soon after emergence. Water mites indicating parity were found on females but were too few to act as reliable parity indicators.

The duration of the oviposition cycles decreased with female age, possibly due to a combination of higher temperatures and also increased "experience" of female mosquitoes in host location.

The most common hosts of the mosquitoes tested were sheep and cows, the two most common grazing animals in the fields surrounding the woods.

The mosquitoes studied appeared to form one large population that moved freely between Woods A and B and the surrounding fields. It is possible that the mosquitoes were capable of exhibiting homing behaviour and were able to return to Wood A. This is partially substantiated by the fact that mosquitoes released at distances over 400 m from Wood A were recaptured within the wood.

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APPENDIX 1. TIME-SPECIFIC LIFE TABLES FOR AEDES CANTANS IN CAGES AND PONDS.

1991 N=10								
AGE	Nx	lx	dx	Px	Qx	Lx	Tx	ex
0	5.25	1000	57.14	0.943	0.057	971.4	27126.7	27.13
1	4.95	942.9	47.62	0.949	0.051	919	26155.2	27.74
2	4.7	895.2	38.1	0.957	0.043	876.2	25236.2	28.19
3	4.5	857.1	19.05	0.978	0.022	847.6	24360	28.42
4	4.4	838.1	28.57	0.966	0.034	823.8	23512.4	28.05
5	4.25	809.5	19.05	0.976	0.024	800	22688.6	28.03
6	4.15	790.5	19.05	0.976	0.024	781	21888.6	27.69
7	4.05	771.4	28.57	0.963	0.037	757.1	21107.6	27.36
8	3.9	742.9	19.05	0.974	0.026	733.3	20350.5	27.39
9	3.8	723.8	9.524	0.987	0.013	719	19617.1	27.1
10	3.75	714.3	9.524	0.987	0.013	709.5	18898.1	26.46
11	3.7	704.8	19.05	0.973	0.027	695.2	18188.6	25.81
12	3.6	685.7	19.05	0.972	0.028	676.2	17493.3	25.51
13	3.5	666.7	9.524	0.986	0.014	661.9	16817.1	25.23
14	3.45	657.1	9.524	0.986	0.014	652.4	16155.2	24.58
15	3.4	647.6	19.05	0.971	0.029	638.1	15502.9	23.94
16	3.3	628.6	9.524	0.985	0.015	623.8	14864.8	23.65
17	3.25	619	9.524	0.985	0.015	614.3	14241	23
18	3.2	609.5	9.524	0.984	0.016	604.8	13626.7	22.36
19	3.15	600	9.524	0.984	0.016	595.2	13021.9	21.7
20	3.1	590.5	9.524	0.984	0.016	585.7	12426.7	21.05
21	3.05	581	9.524	0.984	0.016	576.2	11841	20.38
22	3	571.4	9.524	0.983	0.017	566.7	11264.8	19.71
23	2.95	561.9	5.714	0.99	0.01	559	10698.1	19.04
24	2.92	556.2	3.81	0.993	0.007	554.3	10139	18.23
25	2.9	552.4	5.714	0.99	0.01	549.5	9584.76	17.35
26	2.87	546.7	3.81	0.993	0.007	544.8	9035.24	16.53
27	2.85	542.9	9.524	0.982	0.018	538.1	8490.48	15.64
28	2.8	533.3	9.524	0.982	0.018	528.6	7952.38	14.91
29	2.75	523.8	9.524	0.982	0.018	519	7423.81	14.17
30	2.7	514.3	9.524	0.981	0.019	509.5	6904.76	13.43
31	2.65	504.8	5.714	0.989	0.011	501.9	6395.24	12.67

32	2.62	499	3.81	0.992	0.008	497.1	5893.33	11.81
33	2.6	495.2	0	1	0	495.2	5396.19	10.9
34	2.6	495.2	5.714	0.988	0.012	492.4	4900.95	9.896
35	2.57	489.5	3.81	0.992	0.008	487.6	4408.57	9.006
36	2.55	485.7	9.524	0.98	0.02	481	3920.95	8.073
37	2.5	476.2	3.81	0.992	0.008	474.3	3440	7.224
38	2.48	472.4	5.714	0.988	0.012	469.5	2965.71	6.278
39	2.45	466.7	9.524	0.98	0.02	461.9	2496.19	5.349
40	2.4	457.1	3.81	0.992	0.008	455.2	2034.29	4.45
41	2.38	453.3	0	1	0	453.3	1579.05	3.483
42	2.38	453.3	1.905	0.996	0.004	452.4	1125.71	2.483
43	2.37	451.4	3.81	0.992	0.008	449.5	673.333	1.492
44	2.35	447.6	447.6	0	1	223.8	223.81	0.5
1991 N=50								
AGE	**Nx**	**lx**	**dx**	**Px**	**Qx**	**Lx**	**Tx**	**ex**
0	25	1000	40	0.96	0.04	980	29088	29.09
1	24	960	60	0.938	0.063	930	28108	29.28
2	22.5	900	20	0.978	0.022	890	27178	30.2
3	22	880	40	0.955	0.045	860	26288	29.87
4	21	840	40	0.952	0.048	820	25428	30.27
5	20	800	20	0.975	0.025	790	24608	30.76
6	19.5	780	20	0.974	0.026	770	23818	30.54
7	19	760	20	0.974	0.026	750	23048	30.33
8	18.5	740	20	0.973	0.027	730	22298	30.13
9	18	720	20	0.972	0.028	710	21568	29.96
10	17.5	700	20	0.971	0.029	690	20858	29.8
11	17	680	20	0.971	0.029	670	20168	29.66
12	16.5	660	20	0.97	0.03	650	19498	29.54
13	16	640	10	0.984	0.016	635	18848	29.45
14	15.75	630	10	0.984	0.016	625	18213	28.91
15	15.5	620	20	0.968	0.032	610	17588	28.37
16	15	600	10	0.983	0.017	595	16978	28.3
17	14.75	590	10	0.983	0.017	585	16383	27.77

18	14.5	580	10	0.983	0.017	575	15798	27.24
19	14.25	570	6	0.989	0.011	567	15223	26.71
20	14.1	564	4	0.993	0.007	562	14656	25.99
21	14	560	20	0.964	0.036	550	14094	25.17
22	13.5	540	10	0.981	0.019	535	13544	25.08
23	13.25	530	10	0.981	0.019	525	13009	24.55
24	13	520	0	1	0	520	12484	24.01
25	13	520	8	0.985	0.015	516	11964	23.01
26	12.8	512	12	0.977	0.023	506	11448	22.36
27	12.5	500	10	0.98	0.02	495	10942	21.88
28	12.25	490	10	0.98	0.02	485	10447	21.32
29	12	480	0	1	0	480	9962	20.75
30	12	480	0	1	0	480	9482	19.75
31	12	480	20	0.958	0.042	470	9002	18.75
32	11.5	460	0	1	0	460	8532	18.55
33	11.5	460	10	0.978	0.022	455	8072	17.55
34	11.25	450	10	0.978	0.022	445	7617	16.93
35	11	440	0	1	0	440	7172	16.3
36	11	440	10	0.977	0.023	435	6732	15.3
37	10.75	430	0	1	0	430	6297	14.64
38	10.75	430	0	1	0	430	5867	13.64
39	10.75	430	0	1	0	430	5437	12.64
40	10.75	430	10	0.977	0.023	425	5007	11.64
41	10.5	420	0	1	0	420	4582	10.91
42	10.5	420	10	0.976	0.024	415	4162	9.91
43	10.25	410	0	1	0	410	3747	9.139
44	10.25	410	10	0.976	0.024	405	3337	8.139
45	10	400	0	1	0	400	2932	7.33
46	10	400	0	1	0	400	2532	6.33
47	10	400	6	0.985	0.015	397	2132	5.33
48	9.85	394	0	1	0	394	1735	4.404
49	9.85	394	12	0.97	0.03	388	1341	3.404
50	9.55	382	0	1	0	382	953	2.495
51	9.55	382	2	0.995	0.005	381	571	1.495
52	9.5	380	380	0	1	190	190	0.5

1991 N=100		27.03						
AGE	Nx	lx	dx	Px	Qx	Lx	Tx	ex
0	37	1000	54.05	0.946	0.054	973	24770.3	24.77
1	35	945.9	13.51	0.986	0.014	939.2	23797.3	25.16
2	34.5	932.4	54.05	0.942	0.058	905.4	22858.1	24.51
3	32.5	878.4	54.05	0.938	0.062	851.4	21952.7	24.99
4	30.5	824.3	27.03	0.967	0.033	810.8	21101.4	25.6
5	29.5	797.3	13.51	0.983	0.017	790.5	20290.5	25.45
6	29	783.8	40.54	0.948	0.052	763.5	19500	24.88
7	27.5	743.2	40.54	0.945	0.055	723	18736.5	25.21
8	26	702.7	27.03	0.962	0.038	689.2	18013.5	25.63
9	25	675.7	27.03	0.96	0.04	662.2	17324.3	25.64
10	24	648.6	27.03	0.958	0.042	635.1	16662.2	25.69
11	23	621.6	27.03	0.957	0.043	608.1	16027	25.78
12	22	594.6	27.03	0.955	0.045	581.1	15418.9	25.93
13	21	567.6	27.03	0.952	0.048	554.1	14837.8	26.14
14	20	540.5	13.51	0.975	0.025	533.8	14283.8	26.43
15	19.5	527	13.51	0.974	0.026	520.3	13750	26.09
16	19	513.5	27.03	0.947	0.053	500	13229.7	25.76
17	18	486.5	13.51	0.972	0.028	479.7	12729.7	26.17
18	17.5	473	13.51	0.971	0.029	466.2	12250	25.9
19	17	459.5	13.51	0.971	0.029	452.7	11783.8	25.65
20	16.5	445.9	13.51	0.97	0.03	439.2	11331.1	25.41
21	16	432.4	13.51	0.969	0.031	425.7	10891.9	25.19
22	15.5	418.9	13.51	0.968	0.032	412.2	10466.2	24.98
23	15	405.4	6.757	0.983	0.017	402	10054.1	24.8
24	14.75	398.6	6.757	0.983	0.017	395.3	9652.03	24.21
25	14.5	391.9	13.51	0.966	0.034	385.1	9256.76	23.62
26	14	378.4	13.51	0.964	0.036	371.6	8871.62	23.45
27	13.5	364.9	6.757	0.981	0.019	361.5	8500	23.3
28	13.25	358.1	6.757	0.981	0.019	354.7	8138.51	22.73
29	13	351.4	13.51	0.962	0.038	344.6	7783.78	22.15
30	12.5	337.8	6.757	0.98	0.02	334.5	7439.19	22.02
31	12.25	331.1	6.757	0.98	0.02	327.7	7104.73	21.46

32	12	324.3	6.757	0.979	0.021	320.9	6777.03	20.9
33	11.75	317.6	6.757	0.979	0.021	314.2	6456.08	20.33
34	11.5	310.8	13.51	0.957	0.043	304.1	6141.89	19.76
35	11	297.3	6.757	0.977	0.023	293.9	5837.84	19.64
36	10.75	290.5	0	1	0	290.5	5543.92	19.08
37	10.75	290.5	6.757	0.977	0.023	287.2	5253.38	18.08
38	10.5	283.8	13.51	0.952	0.048	277	4966.22	17.5
39	10	270.3	13.51	0.95	0.05	263.5	4689.19	17.35
40	9.5	256.8	0	1	0	256.8	4425.68	17.24
41	9.5	256.8	6.757	0.974	0.026	253.4	4168.92	16.24
42	9.25	250	6.757	0.973	0.027	246.6	3915.54	15.66
43	9	243.2	6.757	0.972	0.028	239.9	3668.92	15.08
44	8.75	236.5	6.757	0.971	0.029	233.1	3429.05	14.5
45	8.5	229.7	6.757	0.971	0.029	226.4	3195.95	13.91
46	8.25	223	6.757	0.97	0.03	219.6	2969.59	13.32
47	8	216.2	6.757	0.969	0.031	212.8	2750	12.72
48	7.75	209.5	6.757	0.968	0.032	206.1	2537.16	12.11
49	7.5	202.7	0	1	0	202.7	2331.08	11.5
50	7.5	202.7	13.51	0.933	0.067	195.9	2128.38	10.5
51	7	189.2	13.51	0.929	0.071	182.4	1932.43	10.21
52	6.5	175.7	0	1	0	175.7	1750	9.962
53	6.5	175.7	0	1	0	175.7	1574.32	8.962
54	6.5	175.7	0	1	0	175.7	1398.65	7.962
55	6.5	175.7	6.757	0.962	0.038	172.3	1222.97	6.962
56	6.25	168.9	0	1	0	168.9	1050.68	6.22
57	6.25	168.9	0	1	0	168.9	881.757	5.22
58	6.25	168.9	6.757	0.96	0.04	165.5	712.838	4.22
59	6	162.2	0	1	0	162.2	547.297	3.375
60	6	162.2	6.757	0.958	0.042	158.8	385.135	2.375
61	5.75	155.4	6.757	0.957	0.043	152	226.351	1.457
62	5.5	148.6	148.6	0	1	74.32	74.3243	0.5
1991 N=200			14.81					
AGE	**Nx**	**lx**	**dx**	**Px**	**Qx**	**Lx**	**Tx**	**ex**
0	67.5	1000	37.04	0.963	0.037	981.5	24600	24.6

1	65	963	44.44	0.954	0.046	940.7	23618.5	24.53
2	62	918.5	37.04	0.96	0.04	900	22677.8	24.69
3	59.5	881.5	44.44	0.95	0.05	859.3	21777.8	24.71
4	56.5	837	29.63	0.965	0.035	822.2	20918.5	24.99
5	54.5	807.4	29.63	0.963	0.037	792.6	20096.3	24.89
6	52.5	777.8	37.04	0.952	0.048	759.3	19303.7	24.82
7	50	740.7	22.22	0.97	0.03	729.6	18544.4	25.04
8	48.5	718.5	37.04	0.948	0.052	700	17814.8	24.79
9	46	681.5	22.22	0.967	0.033	670.4	17114.8	25.11
10	44.5	659.3	29.63	0.955	0.045	644.4	16444.4	24.94
11	42.5	629.6	22.22	0.965	0.035	618.5	15800	25.09
12	41	607.4	22.22	0.963	0.037	596.3	15181.5	24.99
13	39.5	585.2	14.81	0.975	0.025	577.8	14585.2	24.92
14	38.5	570.4	29.63	0.948	0.052	555.6	14007.4	24.56
15	36.5	540.7	22.22	0.959	0.041	529.6	13451.9	24.88
16	35	518.5	29.63	0.943	0.057	503.7	12922.2	24.92
17	33	488.9	7.407	0.985	0.015	485.2	12418.5	25.4
18	32.5	481.5	14.81	0.969	0.031	474.1	11933.3	24.78
19	31.5	466.7	14.81	0.968	0.032	459.3	11459.3	24.56
20	30.5	451.9	14.81	0.967	0.033	444.4	11000	24.34
21	29.5	437	14.81	0.966	0.034	429.6	10555.6	24.15
22	28.5	422.2	7.407	0.982	0.018	418.5	10125.9	23.98
23	28	414.8	14.81	0.964	0.036	407.4	9707.41	23.4
24	27	400	7.407	0.981	0.019	396.3	9300	23.25
25	26.5	392.6	7.407	0.981	0.019	388.9	8903.7	22.68
26	26	385.2	7.407	0.981	0.019	381.5	8514.81	22.11
27	25.5	377.8	14.81	0.961	0.039	370.4	8133.33	21.53
28	24.5	363	14.81	0.959	0.041	355.6	7762.96	21.39
29	23.5	348.1	7.407	0.979	0.021	344.4	7407.41	21.28
30	23	340.7	7.407	0.978	0.022	337	7062.96	20.73
31	22.5	333.3	7.407	0.978	0.022	329.6	6725.93	20.18
32	22	325.9	7.407	0.977	0.023	322.2	6396.3	19.63
33	21.5	318.5	7.407	0.977	0.023	314.8	6074.07	19.07
34	21	311.1	7.407	0.976	0.024	307.4	5759.26	18.51
35	20.5	303.7	7.407	0.976	0.024	300	5451.85	17.95

36	20	296.3	7.407	0.975	0.025	292.6	5151.85	17.39
37	19.5	288.9	7.407	0.974	0.026	285.2	4859.26	16.82
38	19	281.5	14.81	0.947	0.053	274.1	4574.07	16.25
39	18	266.7	14.81	0.944	0.056	259.3	4300	16.13
40	17	251.9	7.407	0.971	0.029	248.1	4040.74	16.04
41	16.5	244.4	7.407	0.97	0.03	240.7	3792.59	15.52
42	16	237	7.407	0.969	0.031	233.3	3551.85	14.98
43	15.5	229.6	7.407	0.968	0.032	225.9	3318.52	14.45
44	15	222.2	7.407	0.967	0.033	218.5	3092.59	13.92
45	14.5	214.8	7.407	0.966	0.034	211.1	2874.07	13.38
46	14	207.4	11.11	0.946	0.054	201.9	2662.96	12.84
47	13.25	196.3	3.704	0.981	0.019	194.4	2461.11	12.54
48	13	192.6	11.11	0.942	0.058	187	2266.67	11.77
49	12.25	181.5	7.407	0.959	0.041	177.8	2079.63	11.46
50	11.75	174.1	7.407	0.957	0.043	170.4	1901.85	10.93
51	11.25	166.7	11.11	0.933	0.067	161.1	1731.48	10.39
52	10.5	155.6	7.407	0.952	0.048	151.9	1570.37	10.1
53	10	148.1	7.407	0.95	0.05	144.4	1418.52	9.575
54	9.5	140.7	3.704	0.974	0.026	138.9	1274.07	9.053
55	9.25	137	3.704	0.973	0.027	135.2	1135.19	8.284
56	9	133.3	7.407	0.944	0.056	129.6	1000	7.5
57	8.5	125.9	7.407	0.941	0.059	122.2	870.37	6.912
58	8	118.5	7.407	0.938	0.063	114.8	748.148	6.313
59	7.5	111.1	3.704	0.967	0.033	109.3	633.333	5.7
60	7.25	107.4	3.704	0.966	0.034	105.6	524.074	4.879
61	7	103.7	3.704	0.964	0.036	101.9	418.519	4.036
62	6.75	100	0	1	0	100	316.667	3.167
63	6.75	100	14.81	0.852	0.148	92.59	216.667	2.167
64	5.75	85.19	3.704	0.957	0.043	83.33	124.074	1.457
65	5.5	81.48	81.48	0	1	40.74	40.7407	0.5
1989 HOTSPOT ALL		1.235						
AGE	**Nx**	**lx**	**dx**	**Px**	**Qx**	**Lx**	**Tx**	**ex**
0	810	1000	37.04	0.963	0.037	981.5	15990.1	15.99

1	780	963	74.07	0.923	0.077	925.9	15008.6	15.59
2	720	888.9	61.73	0.931	0.069	858	14082.7	15.84
3	670	827.2	61.73	0.925	0.075	796.3	13224.7	15.99
4	620	765.4	24.69	0.968	0.032	753.1	12428.4	16.24
5	600	740.7	61.73	0.917	0.083	709.9	11675.3	15.76
6	550	679	61.73	0.909	0.091	648.1	10965.4	16.15
7	500	617.3	49.38	0.92	0.08	592.6	10317.3	16.71
8	460	567.9	49.38	0.913	0.087	543.2	9724.69	17.12
9	420	518.5	24.69	0.952	0.048	506.2	9181.48	17.71
10	400	493.8	49.38	0.9	0.1	469.1	8675.31	17.57
11	360	444.4	37.04	0.917	0.083	425.9	8206.17	18.46
12	330	407.4	24.69	0.939	0.061	395.1	7780.25	19.1
13	310	382.7	37.04	0.903	0.097	364.2	7385.19	19.3
14	280	345.7	37.04	0.893	0.107	327.2	7020.99	20.31
15	250	308.6	12.35	0.96	0.04	302.5	6693.83	21.69
16	240	296.3	24.69	0.917	0.083	284	6391.36	21.57
17	220	271.6	18.52	0.932	0.068	262.3	6107.41	22.49
18	205	253.1	18.52	0.927	0.073	243.8	5845.06	23.1
19	190	234.6	12.35	0.947	0.053	228.4	5601.23	23.88
20	180	222.2	12.35	0.944	0.056	216	5372.84	24.18
21	170	209.9	12.35	0.941	0.059	203.7	5156.79	24.57
22	160	197.5	12.35	0.938	0.063	191.4	4953.09	25.08
23	150	185.2	6.173	0.967	0.033	182.1	4761.73	25.71
24	145	179	6.173	0.966	0.034	175.9	4579.63	25.58
25	140	172.8	12.35	0.929	0.071	166.7	4403.7	25.48
26	130	160.5	6.173	0.962	0.038	157.4	4237.04	26.4
27	125	154.3	3.086	0.98	0.02	152.8	4079.63	26.44
28	122.5	151.2	3.086	0.98	0.02	149.7	3926.85	25.97
29	120	148.1	6.173	0.958	0.042	145.1	3777.16	25.5
30	115	142	-3.09	1.022	-0.02	143.5	3632.1	25.58
31	117.5	145.1	9.259	0.936	0.064	140.4	3488.58	24.05
32	110	135.8	6.173	0.955	0.045	132.7	3348.15	24.65
33	105	129.6	3.086	0.976	0.024	128.1	3215.43	24.8
34	102.5	126.5	3.086	0.976	0.024	125	3087.35	24.4
35	100	123.5	0	1	0	123.5	2962.35	24

36	100	123.5	0	1	0	123.5	2838.89	23
37	100	123.5	0	1	0	123.5	2715.43	22
38	100	123.5	3.086	0.975	0.025	121.9	2591.98	21
39	97.5	120.4	3.086	0.974	0.026	118.8	2470.06	20.52
40	95	117.3	6.173	0.947	0.053	114.2	2351.23	20.05
41	90	111.1	0	1	0	111.1	2237.04	20.13
42	90	111.1	6.173	0.944	0.056	108	2125.93	19.13
43	85	104.9	0	1	0	104.9	2017.9	19.23
44	85	104.9	0	1	0	104.9	1912.96	18.23
45	85	104.9	6.173	0.941	0.059	101.9	1808.02	17.23
46	80	98.77	0	1	0	98.77	1706.17	17.28
47	80	98.77	0	1	0	98.77	1607.41	16.28
48	80	98.77	0	1	0	98.77	1508.64	15.28
49	80	98.77	0	1	0	98.77	1409.88	14.28
50	80	98.77	0	1	0	98.77	1311.11	13.28
51	80	98.77	0	1	0	98.77	1212.35	12.28
52	80	98.77	0	1	0	98.77	1113.58	11.28
53	80	98.77	0	1	0	98.77	1014.81	10.28
54	80	98.77	3.086	0.969	0.031	97.22	916.049	9.275
55	77.5	95.68	3.086	0.968	0.032	94.14	818.827	8.558
56	75	92.59	3.086	0.967	0.033	91.05	724.691	7.827
57	72.5	89.51	3.086	0.966	0.034	87.96	633.642	7.079
58	70	86.42	6.173	0.929	0.071	83.33	545.679	6.314
59	65	80.25	6.173	0.923	0.077	77.16	462.346	5.762
60	60	74.07	12.35	0.833	0.167	67.9	385.185	5.2
61	50	61.73	0	1	0	61.73	317.284	5.14
62	50	61.73	12.35	0.8	0.2	55.56	255.556	4.14
63	40	49.38	6.173	0.875	0.125	46.3	200	4.05
64	35	43.21	0	1	0	43.21	153.704	3.557
65	35	43.21	12.35	0.714	0.286	37.04	110.494	2.557
66	25	30.86	6.173	0.8	0.2	27.78	73.4568	2.38
67	20	24.69	6.173	0.75	0.25	21.6	45.679	1.85
68	15	18.52	6.173	0.667	0.333	15.43	24.0741	1.3
69	10	12.35	9.877	0.2	0.8	7.407	8.64198	0.7
70	2	2.469	2.469	0	1	1.235	1.23457	0.5

1989 POND 9 ALL		10.42						
AGE	Nx	lx	dx	Px	Qx	Lx	Tx	ex
0	96	1000	10.42	0.99	0.01	994.8	32770.3	32.77
1	95	989.6	10.42	0.989	0.011	984.4	31775.5	32.11
2	94	979.2	10.42	0.989	0.011	974	30791.1	31.45
3	93	968.8	10.42	0.989	0.011	963.5	29817.2	30.78
4	92	958.3	10.42	0.989	0.011	953.1	28853.6	30.11
5	91	947.9	10.42	0.989	0.011	942.7	27900.5	29.43
6	90	937.5	10.42	0.989	0.011	932.3	26957.8	28.76
7	89	927.1	10.42	0.989	0.011	921.9	26025.5	28.07
8	88	916.7	10.42	0.989	0.011	911.5	25103.6	27.39
9	87	906.3	10.42	0.989	0.011	901	24192.2	26.69
10	86	895.8	13.02	0.985	0.015	889.3	23291.1	26
11	84.75	882.8	13.02	0.985	0.015	876.3	22401.8	25.38
12	83.5	869.8	15.63	0.982	0.018	862	21525.5	24.75
13	82	854.2	10.42	0.988	0.012	849	20663.5	24.19
14	81	843.8	10.42	0.988	0.012	838.5	19814.6	23.48
15	80	833.3	10.42	0.988	0.013	828.1	18976	22.77
16	79	822.9	10.42	0.987	0.013	817.7	18147.9	22.05
17	78	812.5	10.42	0.987	0.013	807.3	17330.2	21.33
18	77	802.1	10.42	0.987	0.013	796.9	16522.9	20.6
19	76	791.7	10.42	0.987	0.013	786.5	15726	19.86
20	75	781.3	10.42	0.987	0.013	776	14939.6	19.12
21	74	770.8	10.42	0.986	0.014	765.6	14163.5	18.37
22	73	760.4	15.63	0.979	0.021	752.6	13397.9	17.62
23	71.5	744.8	15.63	0.979	0.021	737	12645.3	16.98
24	70	729.2	20.83	0.971	0.029	718.8	11908.3	16.33
25	68	708.3	10.42	0.985	0.015	703.1	11189.6	15.8
26	67	697.9	20.83	0.97	0.03	687.5	10486.5	15.03
27	65	677.1	31.25	0.954	0.046	661.5	9798.96	14.47
28	62	645.8	20.83	0.968	0.032	635.4	9137.5	14.15
29	60	625	31.25	0.95	0.05	609.4	8502.08	13.6
30	57	593.8	10.42	0.982	0.018	588.5	7892.71	13.29

31	56	583.3	10.42	0.982	0.018	578.1	7304.17	12.52
32	55	572.9	20.83	0.964	0.036	562.5	6726.04	11.74
33	53	552.1	31.25	0.943	0.057	536.5	6163.54	11.16
34	50	520.8	15.63	0.97	0.03	513	5627.08	10.8
35	48.5	505.2	15.63	0.969	0.031	497.4	5114.06	10.12
36	47	489.6	20.83	0.957	0.043	479.2	4616.67	9.43
37	45	468.8	52.08	0.889	0.111	442.7	4137.5	8.827
38	40	416.7	10.42	0.975	0.025	411.5	3694.79	8.868
39	39	406.3	31.25	0.923	0.077	390.6	3283.33	8.082
40	36	375	20.83	0.944	0.056	364.6	2892.71	7.714
41	34	354.2	20.83	0.941	0.059	343.8	2528.13	7.138
42	32	333.3	31.25	0.906	0.094	317.7	2184.38	6.553
43	29	302.1	31.25	0.897	0.103	286.5	1866.67	6.179
44	26	270.8	20.83	0.923	0.077	260.4	1580.21	5.835
45	24	250	31.25	0.875	0.125	234.4	1319.79	5.279
46	21	218.8	20.83	0.905	0.095	208.3	1085.42	4.962
47	19	197.9	31.25	0.842	0.158	182.3	877.083	4.432
48	16	166.7	31.25	0.813	0.188	151	694.792	4.169
49	13	135.4	20.83	0.846	0.154	125	543.75	4.015
50	11	114.6	31.25	0.727	0.273	98.96	418.75	3.655
51	8	83.33	11.46	0.863	0.138	77.6	319.792	3.838
52	6.9	71.88	9.375	0.87	0.13	67.19	242.188	3.37
53	6	62.5	10.42	0.833	0.167	57.29	175	2.8
54	5	52.08	12.5	0.76	0.24	45.83	117.708	2.26
55	3.8	39.58	8.333	0.789	0.211	35.42	71.875	1.816
56	3	31.25	10.42	0.667	0.333	26.04	36.4583	1.167
57	2	20.83	20.83	0	1	10.42	10.4167	0.5
1989 POND 9 MOD		3.509						
AGE	**Nx**	**lx**	**dx**	**Px**	**Qx**	**Lx**	**Tx**	**ex**
0	285	1000	17.54	0.982	0.018	991.2	31870.2	31.87
1	280	982.5	17.54	0.982	0.018	973.7	30878.9	31.43
2	275	964.9	10.53	0.989	0.011	959.6	29905.3	30.99
3	272	954.4	15.79	0.983	0.017	946.5	28945.6	30.33
4	267.5	938.6	8.772	0.991	0.009	934.2	27999.1	29.83

5	265	929.8	17.54	0.981	0.019	921.1	27064.9	29.11
6	260	912.3	17.54	0.981	0.019	903.5	26143.9	28.66
7	255	894.7	17.54	0.98	0.02	886	25240.4	28.21
8	250	877.2	17.54	0.98	0.02	868.4	24354.4	27.76
9	245	859.6	8.772	0.99	0.01	855.3	23486	27.32
10	242.5	850.9	8.772	0.99	0.01	846.5	22630.7	26.6
11	240	842.1	14.04	0.983	0.017	835.1	21784.2	25.87
12	236	828.1	3.509	0.996	0.004	826.3	20949.1	25.3
13	235	824.6	17.54	0.979	0.021	815.8	20122.8	24.4
14	230	807	17.54	0.978	0.022	798.2	19307	23.92
15	225	789.5	17.54	0.978	0.022	780.7	18508.8	23.44
16	220	771.9	8.772	0.989	0.011	767.5	17728.1	22.97
17	217.5	763.2	8.772	0.989	0.011	758.8	16960.5	22.22
18	215	754.4	17.54	0.977	0.023	745.6	16201.8	21.48
19	210	736.8	17.54	0.976	0.024	728.1	15456.1	20.98
20	205	719.3	10.53	0.985	0.015	714	14728.1	20.48
21	202	708.8	15.79	0.978	0.022	700.9	14014	19.77
22	197.5	693	8.772	0.987	0.013	688.6	13313.2	19.21
23	195	684.2	8.772	0.987	0.013	679.8	12624.6	18.45
24	192.5	675.4	19.3	0.971	0.029	665.8	11944.7	17.68
25	187	656.1	7.018	0.989	0.011	652.6	11278.9	17.19
26	185	649.1	17.54	0.973	0.027	640.4	10626.3	16.37
27	180	631.6	17.54	0.972	0.028	622.8	9985.96	15.81
28	175	614	17.54	0.971	0.029	605.3	9363.16	15.25
29	170	596.5	17.54	0.971	0.029	587.7	8757.89	14.68
30	165	578.9	17.54	0.97	0.03	570.2	8170.18	14.11
31	160	561.4	17.54	0.969	0.031	552.6	7600	13.54
32	155	543.9	10.53	0.981	0.019	538.6	7047.37	12.96
33	152	533.3	7.018	0.987	0.013	529.8	6508.77	12.2
34	150	526.3	17.54	0.967	0.033	517.5	5978.95	11.36
35	145	508.8	17.54	0.966	0.034	500	5461.4	10.73
36	140	491.2	8.772	0.982	0.018	486.8	4961.4	10.1
37	137.5	482.5	26.32	0.945	0.055	469.3	4474.56	9.275
38	130	456.1	35.09	0.923	0.077	438.6	4005.26	8.781
39	120	421.1	31.58	0.925	0.075	405.3	3566.67	8.471

40	111	389.5	21.05	0.946	0.054	378.9	3161.4	8.117
41	105	368.4	35.09	0.905	0.095	350.9	2782.46	7.552
42	95	333.3	17.54	0.947	0.053	324.6	2431.58	7.295
43	90	315.8	35.09	0.889	0.111	298.2	2107.02	6.672
44	80	280.7	35.09	0.875	0.125	263.2	1808.77	6.444
45	70	245.6	17.54	0.929	0.071	236.8	1545.61	6.293
46	65	228.1	35.09	0.846	0.154	210.5	1308.77	5.738
47	55	193	26.32	0.864	0.136	179.8	1098.25	5.691
48	47.5	166.7	26.32	0.842	0.158	153.5	918.421	5.511
49	40	140.4	8.772	0.938	0.063	136	764.912	5.45
50	37.5	131.6	43.86	0.667	0.333	109.6	628.947	4.78
51	25	87.72	17.54	0.8	0.2	78.95	519.298	5.92
52	20	70.18	0.702	0.99	0.01	69.82	440.351	6.275
53	19.8	69.47	16.84	0.758	0.242	61.05	370.526	5.333
54	15	52.63	7.018	0.867	0.133	49.12	309.474	5.88
55	13	45.61	5.263	0.885	0.115	42.98	260.351	5.708
56	11.5	40.35	5.263	0.87	0.13	37.72	217.368	5.387
57	10	35.09	3.509	0.9	0.1	33.33	179.649	5.12
58	9	31.58	3.509	0.889	0.111	29.82	146.316	4.633
59	8	28.07	2.105	0.925	0.075	27.02	116.491	4.15
60	7.4	25.96	3.158	0.878	0.122	24.39	89.4737	3.446
61	6.5	22.81	2.456	0.892	0.108	21.58	65.0877	2.854
62	5.8	20.35	2.807	0.862	0.138	18.95	43.5088	2.138
63	5	17.54	7.018	0.6	0.4	14.04	24.5614	1.4
64	3	10.53	5.263	0.5	0.5	7.895	10.5263	1
65	1.5	5.263	5.263	0	1	2.632	2.63158	0.5

1989 HOTSPOT MODIFIED			0.078					
AGE	Nx	lx	dx	Px	Qx	Lx	Tx	ex
0	12800	1000	31.25	0.969	0.031	984.4	19482.58	19.48
1	12400	968.8	31.25	0.968	0.032	953.1	18498.2	19.09
2	12000	937.5	35.16	0.963	0.038	919.9	17545.08	18.71
3	11550	902.3	27.34	0.97	0.03	888.7	16625.16	18.42
4	11200	875	31.25	0.964	0.036	859.4	15736.48	17.98
5	10800	843.8	39.06	0.954	0.046	824.2	14877.11	17.63
6	10300	804.7	62.5	0.922	0.078	773.4	14052.89	17.46
7	9500	742.2	39.06	0.947	0.053	722.7	13279.45	17.89
8	9000	703.1	46.88	0.933	0.067	679.7	12556.8	17.86
9	8400	656.3	62.5	0.905	0.095	625	11877.11	18.1
10	7600	593.8	23.44	0.961	0.039	582	11252.11	18.95
11	7300	570.3	39.06	0.932	0.068	550.8	10670.08	18.71
12	6800	531.3	39.06	0.926	0.074	511.7	10119.3	19.05
13	6300	492.2	23.44	0.952	0.048	480.5	9607.578	19.52
14	6000	468.8	39.06	0.917	0.083	449.2	9127.109	19.47
15	5500	429.7	39.06	0.909	0.091	410.2	8677.891	20.2
16	5000	390.6	23.44	0.94	0.06	378.9	8267.734	21.17
17	4700	367.2	15.63	0.957	0.043	359.4	7888.828	21.48
18	4500	351.6	23.44	0.933	0.067	339.8	7529.453	21.42
19	4200	328.1	23.44	0.929	0.071	316.4	7189.609	21.91
20	3900	304.7	23.44	0.923	0.077	293	6873.203	22.56
21	3600	281.3	15.63	0.944	0.056	273.4	6580.234	23.4
22	3400	265.6	15.63	0.941	0.059	257.8	6306.797	23.74
23	3200	250	7.813	0.969	0.031	246.1	6048.984	24.2
24	3100	242.2	7.813	0.968	0.032	238.3	5802.891	23.96
25	3000	234.4	7.813	0.967	0.033	230.5	5564.609	23.74
26	2900	226.6	7.813	0.966	0.034	222.7	5334.141	23.54
27	2800	218.8	7.813	0.964	0.036	214.8	5111.484	23.37
28	2700	210.9	7.813	0.963	0.037	207	4896.641	23.21
29	2600	203.1	7.813	0.962	0.038	199.2	4689.609	23.09
30	2500	195.3	7.813	0.96	0.04	191.4	4490.391	22.99
31	2400	187.5	7.813	0.958	0.042	183.6	4298.984	22.93
32	2300	179.7	3.906	0.978	0.022	177.7	4115.391	22.9

33	2250	175.8	3.906	0.978	0.022	173.8	3937.656	22.4
34	2200	171.9	3.906	0.977	0.023	169.9	3763.828	21.9
35	2150	168	3.906	0.977	0.023	166	3593.906	21.4
36	2100	164.1	7.813	0.952	0.048	160.2	3427.891	20.89
37	2000	156.3	1.797	0.989	0.012	155.4	3267.734	20.91
38	1977	154.5	2.109	0.986	0.014	153.4	3112.383	20.15
39	1950	152.3	3.906	0.974	0.026	150.4	2958.984	19.42
40	1900	148.4	1.953	0.987	0.013	147.5	2808.594	18.92
41	1875	146.5	3.906	0.973	0.027	144.5	2661.133	18.17
42	1825	142.6	1.953	0.986	0.014	141.6	2516.602	17.65
43	1800	140.6	1.953	0.986	0.014	139.6	2375	16.89
44	1775	138.7	3.906	0.972	0.028	136.7	2235.352	16.12
45	1725	134.8	1.953	0.986	0.014	133.8	2098.633	15.57
46	1700	132.8	3.906	0.971	0.029	130.9	1964.844	14.79
47	1650	128.9	3.906	0.97	0.03	127	1833.984	14.23
48	1600	125	3.125	0.975	0.025	123.4	1707.031	13.66
49	1560	121.9	2.344	0.981	0.019	120.7	1583.594	12.99
50	1530	119.5	2.344	0.98	0.02	118.4	1462.891	12.24
51	1500	117.2	3.906	0.967	0.033	115.2	1344.531	11.47
52	1450	113.3	3.906	0.966	0.034	111.3	1229.297	10.85
53	1400	109.4	3.906	0.964	0.036	107.4	1117.969	10.22
54	1350	105.5	3.906	0.963	0.037	103.5	1010.547	9.581
55	1300	101.6	3.906	0.962	0.038	99.61	907.0313	8.931
56	1250	97.66	3.906	0.96	0.04	95.7	807.4219	8.268
57	1200	93.75	7.813	0.917	0.083	89.84	711.7188	7.592
58	1100	85.94	3.906	0.955	0.045	83.98	621.875	7.236
59	1050	82.03	3.906	0.952	0.048	80.08	537.8906	6.557
60	1000	78.13	3.906	0.95	0.05	76.17	457.8125	5.86
61	950	74.22	3.906	0.947	0.053	72.27	381.6406	5.142
62	900	70.31	7.813	0.889	0.111	66.41	309.375	4.4
63	800	62.5	7.813	0.875	0.125	58.59	242.9688	3.888
64	700	54.69	7.813	0.857	0.143	50.78	184.375	3.371
65	600	46.88	7.813	0.833	0.167	42.97	133.5938	2.85
66	500	39.06	7.813	0.8	0.2	35.16	90.625	2.32
67	400	31.25	7.813	0.75	0.25	27.34	55.46875	1.775

68	300	23.44	7.813	0.667	0.333	19.53	28.125	1.2
69	200	15.63	14.84	0.05	0.95	8.203	8.59375	0.55
70	10	0.781	0.781	0	1	0.391	0.390625	0.5
1990 POND 9 MOD		3.333						
AGE	**Nx**	**lx**	**Px**	**dx**	**Qx**	**Lx**	**Tx**	**ex**
0	300	1000	0.95	50	0.05	975	23662.83	23.66
1	285	950	0.965	33.33	0.035	933.3	22687.83	23.88
2	275	916.7	0.964	33.33	0.036	900	21754.5	23.73
3	265	883.3	0.962	33.33	0.038	866.7	20854.5	23.61
4	255	850	0.971	25	0.029	837.5	19987.83	23.52
5	247.5	825	0.97	25	0.03	812.5	19150.33	23.21
6	240	800	0.958	33.33	0.042	783.3	18337.83	22.92
7	230	766.7	0.957	33.33	0.043	750	17554.5	22.9
8	220	733.3	0.966	25	0.034	720.8	16804.5	22.92
9	212.5	708.3	0.965	25	0.035	695.8	16083.67	22.71
10	205	683.3	0.976	16.67	0.024	675	15387.83	22.52
11	200	666.7	0.975	16.67	0.025	658.3	14712.83	22.07
12	195	650	0.949	33.33	0.051	633.3	14054.5	21.62
13	185	616.7	0.973	16.67	0.027	608.3	13421.17	21.76
14	180	600	0.958	25	0.042	587.5	12812.83	21.35
15	172.5	575	0.957	25	0.043	562.5	12225.33	21.26
16	165	550	0.97	16.67	0.03	541.7	11662.83	21.21
17	160	533.3	0.969	16.67	0.031	525	11121.17	20.85
18	155	516.7	0.968	16.67	0.032	508.3	10596.17	20.51
19	150	500	0.967	16.67	0.033	491.7	10087.83	20.18
20	145	483.3	0.983	8.333	0.017	479.2	9596.167	19.85
21	142.5	475	0.982	8.333	0.018	470.8	9117	19.19
22	140	466.7	0.993	3.333	0.007	465	8646.167	18.53
23	139	463.3	0.989	5	0.011	460.8	8181.167	17.66
24	137.5	458.3	0.983	7.667	0.017	454.5	7720.333	16.84
25	135.2	450.7	0.999	0.667	0.001	450.3	7265.833	16.12
26	135	450	0.999	0.667	0.001	449.7	6815.5	15.15
27	134.8	449.3	1	0	0	449.3	6365.833	14.17
28	134.8	449.3	1	0.167	4e-04	449.3	5916.5	13.17

29	134.75	449.2	0.998	0.833	0.002	448.8	5467.25	12.17
30	134.5	448.3	0.967	15	0.033	440.8	5018.5	11.19
31	130	433.3	1	0	0	433.3	4577.667	10.56
32	130	433.3	0.981	8.333	0.019	429.2	4144.333	9.564
33	127.5	425	0.98	8.333	0.02	420.8	3715.167	8.742
34	125	416.7	0.92	33.33	0.08	400	3294.333	7.906
35	115	383.3	0.87	50	0.13	358.3	2894.333	7.55
36	100	333.3	0.85	50	0.15	308.3	2536	7.608
37	85	283.3	0.882	33.33	0.118	266.7	2227.667	7.862
38	75	250	0.867	33.33	0.133	233.3	1961	7.844
39	65	216.7	0.923	16.67	0.077	208.3	1727.667	7.974
40	60	200	0.833	33.33	0.167	183.3	1519.333	7.597
41	50	166.7	0.9	16.67	0.1	158.3	1336	8.016
42	45	150	0.889	16.67	0.111	141.7	1177.667	7.851
43	40	133.3	0.875	16.67	0.125	125	1036	7.77
44	35	116.7	0.857	16.67	0.143	108.3	911	7.809
45	30	100	0.933	6.667	0.067	96.67	802.6667	8.027
46	28	93.33	0.964	3.333	0.036	91.67	706	7.564
47	27	90	0.963	3.333	0.037	88.33	614.3333	6.826
48	26	86.67	0.962	3.333	0.038	85	526	6.069
49	25	83.33	0.992	0.667	0.008	83	441	5.292
50	24.8	82.67	0.806	16	0.194	74.67	358	4.331
51	20	66.67	0.875	8.333	0.125	62.5	283.3333	4.25
52	17.5	58.33	0.714	16.67	0.286	50	220.8333	3.786
53	12.5	41.67	0.8	8.333	0.2	37.5	170.8333	4.1
54	10	33.33	0.9	3.333	0.1	31.67	133.3333	4
55	9	30	0.889	3.333	0.111	28.33	101.6667	3.389
56	8	26.67	0.75	6.667	0.25	23.33	73.33333	2.75
57	6	20	0.833	3.333	0.167	18.33	50	2.5
58	5	16.67	0.8	3.333	0.2	15	31.66667	1.9
59	4	13.33	0.5	6.667	0.5	10	16.66667	1.25
60	2	6.667	0.5	3.333	0.5	5	6.666667	1
61	1	3.333	0	3.333	1	1.667	1.666667	0.5