STUDIES ON THE ECOLOGY AND BIOLOGY OF *CULEX PIPIENS* L. AND *CULEX TORRENTIUM* MARTINI (DIPTERA : CULICIDAE) IN BRITAIN

by

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Abstract

Various aspects of the biology and ecology of the two sympatric sibling species of Culex, Cx. (Culex) pipiens L. and Cx. (Culex) torrentium Martini were studied from October 1977 to April 1980 at Silwood Park, Berkshire.

Taxonomic studies were undertaken to determine the reliability of using pre-alar scales to distinguish between the two species. Laboratory studies on the developmental pattern of the two species are described.

Studies of field populations in artificial containers and permanent ground water habitats are presented. Experiments are described which demonstrate differences in oviposition preferences between the adult females of the two species for these different habitats. An account is given of the abundance, seasonal occurrence and mortality of pre-adult mosquitoes as determined by regular population sampling in breeding sites. Identification of the natural predators of pre-adult mosquitoes was performed using serological techniques. The predation activity of some common predators of larval populations was assessed in the laboratory by functional response experiments.

Detailed population studies of overwintering adult Cx. pipiens were made in selected overwintering sites. The extent of movement into, out of and within sites was measured using entrance and exit traps and marked mosquitoes. The mortality factors acting during the course of hibernation were determined and evaluated. Attempts were made to locate the overwintering sites for Cx. torrentium.

Information is also presented on the seasonal occurrence of pre-adult and adult populations of Culiseta (Culiseta) annulata Schrank and Anopheles (Anopheles) claviger (Meigen) found in the same larval habitats and overwintering sites as the Culex species.

"I HATE THE WAY YOU LURCH OFF SIDEWAYS INTO THE AIR
HAVING READ MY THOUGHTS AGAINST YOU.

COME THEN, LET US PLAY AT UNAWARES,
AND SEE WHO WINS IN THIS SLY GAME OF BLUFF.
MAN OR MOSQUITO"

D. H. LAWRENCE, 1885-1930

CONTENTS

Page

ABSTRACT 2

LIST OF TABLES 10

LIST OF FIGURES 14

SECTION 1 INTRODUCTION 20

1.1 Distribution and economic importance of mosquitoes 20

1.2 British mosquitoes 24

1.3 Sibling species 30

1.4 Aims of the study 32

1.5 The study area 33

SECTION 2 THE TAXONOMIC CHARACTERISTICS OF PRE-ALAR SCALES IN CX. PIPIENS AND CX. TORRENTIUM 37

2.1 Introduction 37

2.2 Materials and methods 37

2.2.1 Collection and breeding of mosquitoes ... 37

2.2.2 Preparation of slide mounts of mosquitoes for microscopic examination .. 38

2.2.2.1 Male terminalia 38

2.2.2.2 Pre-alar scales 41

2.3 Results 43

2.4 Discussion 46

SECTION 3 COMPARATIVE STUDIES ON THE DEVELOPMENT PATTERN OF CX. PIPIENS AND CX. TORRENTIUM 49

3.1 Introduction 49

3.2 Experimental 49

3.2.1 Egg-batch size and fertility 49

3.2.2 Gonotrophic cycle and incubation period ... 51

CONTENTS (Continued) Page

3.2.3 Separation of larval instars based on body length 53

3.2.4 Larval and pupal development 56

(a) Duration of larval and pupal stages .. 57

(b) Larval and pupal mortality 63

(c) Sex ratio 63

3.3 Discussion 65

SECTION 4 SELECTION OF OVIPOSITION SITES BY CX. PIPIENS AND CX. TORRENTIUM 69

4.1 Introduction 69

4.2 Description of the experimental sites 69

4.2.1 Permanent ground water 69

4.2.1.1 Pond Field pond, Site A 69

4.2.1.2 Sandy Ride pond, Site B 72

4.2.2 Artificial containers 72

4.2.3 Tree holes 73

4.3 Occurrence of Cx. pipiens and Cx. torrentium in the different habitats 73

4.3.1 Method 73

4.3.2 Results 75

4.4. Factors influencing choice of habitat 75

4.4.1 Method 76

4.4.2 Results 78

4.5 Discussion 83

SECTION 5 STUDIES ON PRE-ADULT POPULATIONS OF MOSQUITOES.. 86

5.1 Methods of sampling larval populations: a review 86

5.1.1 Counting the entire population 87

CONTENTS (Continued) Page

5.1.2 Quadrats 87

5.1.3 Removal sampling 90

5.1.4 Mark-recapture method 91

5.1.5 Light traps 91

5.2 Seasonal occurrence of mosquitoes 94

5.2.1 Artificial container habitat 94

5.2.1.1 Description of the study sites 94

5.2.1.2 Sampling methods 97

(a) Assessment of the efficiency of sampling method 98

(b) Field sampling procedure ... 100

5.2.1. Results 101

5.2.2 Permanent ground water habitat 112

5.2.2.1 The study sites 112

5.2.2.2 Method of sampling 112

5.2.2.3 Results 114

(a) Cx. pipiens/torrentium 114

(b) An. claviger 120

(c) Cs. annulata 123

5.3 Duration of different instars of Cx. torrentium at different months 126

5.4 Mortalities of pre-adults of Cx. pipiens/torrentium 130

5.4.1 Permanent ground water habitat 130

5.4.2 Artificial container habitat 137

5.5 Discussion 146

SECTION 6 STUDIES ON THE NATURAL PREDATORS OF PRE-ADULT AND ADULT MOSQUITOES 152

6.1 Introduction 152

6.2 Identification of predators using serological techniques

6.2.1 Materials and methods 152

6.2.1.1 Collection of predators 152

CONTENTS (Continued) Page

6.2.1.2 Serological methods 153

(a) Preparation of antigen..... 153

(b) Preparation of antisera ... 154

(c) Specificity and sensitivity of antisera 154

(d) The precipitin test 156

6.2.1.3 Digestive time of predators following mosquito meal 159

6.2.2 Results 159

6.3 Predation rate of Agrion puella (Odonata : Zygoptera) and Sympetrum striolatum (Odonata : Anisoptera) on immature stages of mosquito 166

6.3.1 Materials and methods 167

6.3.2 Results 169

6.4 Discussion 174

SECTION 7 STUDIES ON ADULT OVERWINTERING POPULATION OF CX. PIPIENS 178

7.1 Introduction 178

7.2 Description of the study sites 179

7.3 Population fluctuation and distribution 181

7.3.1 Materials and method 181

7.3.2 Results 185

7.4 Movement of mosquitoes 199

7.5 Colonization and positive identification of species 203

7.6 The physiological condition of species 206

7.6.1 Parous rate and fertility 206

7.6.2 Sugar feeding 209

7.6.3 Estimation of fat and glycogen 210

7.7 Mortality factors 214

7.7.1 Exhaustion of food reserves 214

CONTENTS (Continued) Page

(a) Dead mosquitoes on the floor 215

(b) Mosquitoes in exit traps 216

7.7.2 Predators and parasites 217

7.7.3 Predation rate of spiders 218

7.8 Estimation of survival rate of mosquitoes 223

7.8.1 Method of study 223

7.8.2 Results 224

7.9 The hibernation site for Cx. torrentium 227

7.10 Discussion 229

GENERAL DISCUSSION 237

SUMMARY 242

ACKNOWLEDGEMENTS 245

REFERENCES 246

APPENDICES 270

LIST OF TABLES

Page

1.1 Geographical areas of the world and the numbers of different species of mosquitoes found within them 21

1.2 List of the mosquitoes of the United Kingdom and Ireland 26

2.1 Occurrence of pre-alar scale insertions in Culex pipiens and Cx. torrentium 43

3.1 Frequency distribution of the number of eggs per raft in Cx. pipiens and Cx. torrentium 50

3.2 Mean duration of gonotrophic cycle and incubation period in Cx. pipiens and Cx. torrentium 52

3.3 Body length (in mm) of the different larval instars of Cx. pipiens and Cx. torrentium reared in the insectary at 21 $\pm$ 1° C 55

3.4 Pattern of moulting in larvae and pupae of Cx. pipiens reared at 21 $\pm$ 1° C 58

3.5 Pattern of moulting in larvae and pupae of Cx. torrentium reared at 21 $\pm$ 1° C 59

3.6 Mean duration in days of larval instars and pupae in Cx. pipiens and Cx. torrentium reared at 21 $\pm$ 1° C 62

3.7 Larval and pupal mortality in Cx. pipiens and Cx. torrentium reared in the insectary at 21 $\pm$ 1° C 64

4.1a Identification (based on male terminalia and female pre-alar scales) of Culex species from various habitats 74

LIST OF TABLES (Continued) Page

4.1b Identification (based on male terminalia) of Culex species from the different habitats 74

4.2 Results of analysis of pond and rain water for certain chemicals (in ppm) present in them 79

4.3 Total number of egg-rafts of mosquitoes laid in the various artificial containers placed at sites in the field during the summer of 1979 80

4.4 Mean numbers of egg-rafts of Cs. annulata collected in the different containers 82

5.1 Temperature and rainfall data at Silwood Park (January 1978 - March 1980) 102

5.2 Field observation on the mean developmental time in days ($\pm$ SD) of egg and different instars of Cx. torrentium during different months, 1979 128

5.3 Larval and pupal mortality of Cx. torrentium reared in the field at different months, 1979 128

5.4 Mean developmental time ($\pm$ SD) in days of pupal stage of Cx. pipiens during different months 1979 ... 129

5.5 Weighted average instar duration, instar numbers and the derived age distribution of Cx. pipiens/torrentium collected from ground water habitats in 1979 132

5.6 Life table for Cx. pipiens/torrentium collected from Pond Field pond (Site A), 1979 134

5.7 Life table for Cx. pipiens/torrentium collected from Sandy Ride pond (Site B), 1979 135

5.8 Instar mortalities of Cx. pipiens/torrentium collected from ground water habitats in 1979 136

5.9 Weighted average instar duration, instar number and the derived age distribution of Cx. pipiens/torrentium collected from 5 artificial containers placed in Site C (Silwood Bottom), 1979 139

LIST OF TABLES (continued) Page

5.10 Weighted average instar duration, instar number and the derived age distribution of Cx. pipiens/torrentium collected from 5 artificial containers placed in Site D (Cannon Wood), 1979. 140

5.11 Mortalities of pre-adults of Cx. pipiens/torrentium breeding in artifícial containers placed at different sites in the field, 1979..... 145

6.1 Reciprocal of titres of antisera prepared against adults, pupae and larvae of Cx. pipiens/torrentium .. 155

6.2 Suspected predators of mosquitoes with numbers of gut smears tested and percentages giving a positive reaction with Cx. pipiens/torrentium antiserum 160

6.3 Digest time of a single larva of Cx. torrentium by predators collected from larval breeding sites 165

6.4 Estimates of attack coefficient a^1 (in Kg Units/h) and handling time T_h (in h) of Agrion puella and Sympetrum striolatum..... 173

7.1 Record of total population counts of mosquitoes and spiders in hibernation Sites F_1 and F_2 taken during peak population, 1977 - 1979 193

7.2 Record of number of mosquitoes enmeshed in spider-webs in Site F_2 at different times during hibernation, 1978 - 1980 193

7.3 Total number of Cx. pipiens (and Cs. annulata in parenthesis) caught at different times during hibernation in Entry and Exit traps fixed onto room G_1, 1978 - 1980 201

LIST OF TABLES (Contined) Page

7.4 Results of movement of Cx. pipiens in and out of hibernation sites as determined by mark-recapture method 202

7.5 Movement of Cx. pipiens as determined by marking the positions of mosquitoes in room G_{14}, 1979 - 1980 202

7.6 Success in feeding rate of hibernating Cx. pipiens exposed to prolonged illumination at 21 $\pm$ 1°C 205

7.7 Parous rates of Cx. pipiens collected from hibernation sites at different times during the winter period of 1977 - 1980 208

7.8 Insemination rate of mosquitoes collected from hibernation sites during the winter periods of 1977 - 1980 208

7.9 Mean quatity of fat in mg ($\pm$ SE) in dead mosquitoes collected at different times during hibernation, and compared with live mosquitoes using a t test, 1978 - 1980 215

7.10 Mean quantity of fat in mg ($\pm$ SE) in mosquitoes collected in exit trap at different times during hibernation and compared with those collected inside site using a t test, 1978 - 1980 216

7.11 List of suspected predators of hibernating adult Cx. pipiens with numbers of gut smears tested against anti-adult sera and percentage showing a positive reaction 218

LIST OF FIGURES

		Page
1.1	A sketch map of Silwood Park showing the study sites	34
2.1	Male terminalia of Cx. pipiens	39
2.2	Male terminalia of Cx. torrentium	40
2.3	Pre-alar knob of Cx. pipiens	42
2.4	Frequency distribution of the number of pre-alar scale insertions in Cx. pipiens (histogram) and Cx. torrentium (graph)	44
2.5	Frequency distribution of the number of pre-alar scale insertions in male (histogram) and female (graph) Cx. pipiens and Cx. torrentium	45
3.1	Pattern of adult emergence from pupae in male and female Cx. pipiens reared at 21 $\pm$ 1°C	60
3.2	Pattern of adult emergence from pupae in male and female Cx. torrentium reared at 21 $\pm$ 1°C	61
4.1	Pond Field pond, Site A	71
4.2	Sandy Ride pond, Site B	71
4.3	Plan of Pond Field pond (Site A) showing the arrangement of artificial containers around and within the pond	77
5.1	Part of North Gravel, Site E showing the arrangement of artificial containers along one of the transects ..	96
5.2	Quadrat sampling device used for absolute population estimates of pre-adult mosquitoes in the artificial containers	99
5.3	Mean number of egg-rafts of Cx. pipiens/torrentium per artificial container placed in Silwood Bottom, Site C in 1978 and 1979	104

LIST OF FIGURES (Continued) Page

5.4a Changes in population number of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom (Results for 1978) 105

5.4b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom (Results for 1978) 105

5.5a Changes in population number of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom (Results for 1979) 106

5.5b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom (Results for 1979) 106

5.6 Mean number of egg-rafts of Cx. pipiens/torrentium per container placed in Cannon Wood, Site D in 1978 and 1979 108

5.7a Changes in the population number of pre-adults of Cx. pipiens/torrentium per container in Site D, Cannon Wood (Results for 1978) 109

5.7b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site D, Cannon Wood. (Results for 1978) 109

5.8a Changes in the population number of pre-adults of Cx. pipiens/torrentium per container in Site D, Cannon Wood. (Results for 1979) 110

5.8b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site D, Cannon Wood. (Data for 1979) 110

5.9 Seasonal incidence of pre-adults of Cx.pipiens/torrentium along a transect in North Gravel (Site E) from open field into the woodland with light intensity readings 111

LIST OF FIGURES (Continued) Page

5.10a Changes in population number of pre-adults of *Cx. pipiens/torrentium* collected from Site A, Pond Field pond in 1978 115

5.10b Monthly proportions of pre-adults of *Cx. pipiens/torrentium* collected from Site A, Pond Field pond in 1978 115

5.11a Changes in population number of pre-adults of *Cx. pipiens/torrentium* collected from Site A, Pond Field pond in 1979 116

5.11b Monthly proportions of pre-adults of *Cx. pipiens/torrentium* collected from Site A, Pond Field pond in 1979 116

5.12a Changes in population number of pre-adults of *Cx. pipiens/torrentium* collected from Site B, Sandy Ride pond in 1978 118

5.12b Monthly proportions of pre-adults of *Cx. pipiens/torrentium* collected from Site B, Sandy Ride pond in 1978 118

5.13a Changes in population number of pre-adults of *Cx. pipiens/torrentium* collected from Site B, Sandy Ride pond in 1979 119

5.13b Monthly proportions of pre-adults of *Cx. pipiens/torrentium* collected from Site B, Sandy Ride pond in 1979 119

5.14 Monthly proportions of pre-adult stages of *An. claviger* collected from Pond Field pond (Site A) and Sandy Ride pond (Site B); June 1978 - March 1980 122

LIST OF FIGURES (Continued) Page

5.15 Monthly proportions of pre-adults stages of Cs. annulata collected from Pond Field pond (Site A) and Sandy Ride pond (Site B); June 1978 - March 1980 125

5.16 Age distribution and estimated survivorship curves for the immature stages of Cx. pipiens/ torrentium collected from Pond Field pond and Sandy Ride pond 133

5.17 Age distribution and estimated survivorship curves for the immature stages of Cx. pipiens/ torrentium collected from artificial containers 1 - 5 placed in Silwood Bottom (Site C)..... 142

5.18 Age distribution and estimated survivorship curves for the immature stages of Cx. pipiens/ torrentium collected from artificial containers 6 - 10 placed in Cannon Wood (Site D)..... 144

6.1 Illustration of precipitin tests showing a positive reaction between predator antigen and mosquito larval antiserum: (a) capillary ring test; (b) cross over electrophoresis..... 158

6.2 The functional response of Agrion puella to changes in prey density of third instar Cx. pipiens 171

6.3 The functional response of Sympetrum striolatum to changes in prey density of third instar Cx. pipiens 172

7.1 Plan of Site F, "The tunnel" (shaded area) and the adjoining parts of the basement 180

LIST OF FIGURES (Continued) Page

7.2 Sketch map of Site G, Basement Cellars 182

7.3 Temperature readings taken in the hibernation sites during the study period 187

7.4 Weekly relative humidity maxima and minima taken at Site F_2 during the study period, 1978 - 1980 188

7.5 Seasonal fluctuation in the number of Cx. pipiens and Cs. annulata in Site F_2 during the course of hibernation, 1977 - 1980 189

7.6 Seasonal fluctuation in the number of Cx. pipiens and Cs. annulata hibernating in Sites F_1 and G_1, 1977 - 1979 191

7.7 Hibernating mosquitoes resting on the walls of Site F_2 (Section 3) during peak population, November 1978 192

7.8 Pattern of distribution of hibernating Cx. pipiens along Site F_2 at different times during hibernation, 1977 - 1978 195

7.9 Pattern of distribution of hibernating Cx. pipiens along Site F_2 at different times during hibernation, 1978 - 1979 196

7.10 Pattern of distribution of hibernating Cx. pipiens along Site F_2 at different times during hibernation, 1979 - 1980 197

7.11 Distribution of Cx. pipiens on the roof, upper and lower sections of wall of hibernation Site F_2 at different times during hibernation, 1977 - 1980 198

7.12 Exit trap used to collect mosquitoes leaving the hibernation site 200

LIST OF FIGURES (Continued) Page

7.13 Reduction in mean dry weight and fat content of Cx. pipiens at different times during hibernation. (Results for 1977 - 1979) 212

7.14 Quantity of glycogen in an average Cx. pipiens collected at different periods during hibernation. (Results for 1977 - 1979) 213

7.15 The functional response of Amaurobius ferox to changes in prey density of adult Cx. pipiens 222

7.16 Population record of Cx. pipiens and spiders in room G_3 during the course of hibernation, 1979 - 1980 226

7.17 Battery operated suction device used to search rodent burrows and large tree holes for hibernating Cx. torrentium 228

SECTION 1

INTRODUCTION

1.1 Distribution and economic importance of mosquitoes

Mosquitoes have been a major pest for nearly a million years but it is only in recent years that man has begun to turn his attention towards them (Gillett, 1971). The remarkable discoveries in their life histories and their role as vectors of human diseases (Imms, 1925; Gutsevich et al., 1971) has lent an enormous stimulus to their study. The Culicidae are probably the most important blood-sucking arthropods known (James & Harwood, 1969). Their distribution is world-wide. Some have been found at altitudes of over 4,000 m while others occur in mines 1,000 m below the earth's surface. Species range in latitude northward from the tropics well into the Arctic regions and southward to the ends of the continents (Horsfall, 1972). They are absent only in the Antarctic.

There are about 3,100 species of mosquitoes belonging to over 30 genera (Stone et al., 1959; Knight & Stone, 1977), most of which occur in the tropics. "Not only are there many more genera and species in the tropics but there is also a marked ancient differentiation of the fauna which is reflected in the presence of endemic groups in each zoogeographical region in the tropics" (Gutsevich, 1971). A summary of the geographical areas of the world and the numbers of species of mosquitoes found therein, based on Gillett's (1971) list is given in Table 1.1.

Table 1.1 Geographical areas of the world and the numbers of different species of mosquitoes found within them.

Location	No. of Species
Australian Region	294
Nearctic Region	132
Afro-tropical	491
Malagasy	100
Neotropical Region	734
New Guinea	220
Oriental Region	734
Palaeartic Region	151
UK and Ireland	32

These figures do not include subspecies and varieties, and are based on Gillett's (1971) catalogue which excludes some species (see Knight & Stone, 1977).

Some species of mosquitoes show a much wider distribution than others. Aedes (Stegomyia) aegypti L., for instance, is found in much of the tropics both in the New and Old Worlds while Ae. (Aedimorphus) vexans Meigen is a species of most sub-tropical regions and is also present in the north and south temperate zones. Culex (Culex) pipiens L. is a more temperate species (Mattingly et al, 1951; Horsfall, 1972; Gilett, 1971) with subspecies and/or sibling species found in many parts of the world except in the extreme latitudes. Further information on mosquito zoogeography has been given by Mattingly (1962a) while Knight & Stone (1977) give an up-to-date catalogue of mosquito distribution throughout the world.

Mosquitoes are known to be the vectors of pathogens causing human filariasis, malaria, yellow fever, dengue, o'nyongnyong, chikunguya and a number of viral encephalitides.

Malaria and filariasis are the most prevalent mosquito-borne diseases of man. Malaria is wide spread throughout the temperate and tropical regions of the world, affecting many millions of people (Bellamy, 1955; Bruce-Chwatt, 1974). For example, in India alone before the nation-wide anti-malaria campaign went into effect, about seventy-five million persons suffered from, and another one million succumbed to, the direct ravages of malaria (James & Harwood, 1969).

Malaria was prevalent in the marshy areas of southern England in the middle ages but with improvements in hygiene and land drainage no indiginous malaria now exists. However, approximately 2,000 cases of malaria were imported into Britain in 1978 mainly from the Indian subcontinent and potential vectors of the disease exist(Boreham, pers. comm.). The English climate is not favourable for the development of sporozoites which require temperatures of at least 18°C for 12 days.

Human malaria is transmitted exclusively by species of Anopheles (Bellamy, 1955; Bruce-Chwatt et al., 1966; Mattingly, 1973). Some important vectors include An. (Anopheles) labranchiae Falleroni, An. (Ano.) sacharovi Favre in parts of the Mediterranean area; An. (Cellia) funestus Giles and members of the gambiae Giles complex in the Ethiopian Region; An. (Cel.) stephensi Liston in western Asia; An. (Cel.) culicifacies Giles in India and Sri-Lanka; An. (Cel.) maculatus Theobald in southern Asia; members of the An. punctulatus complex in Melanesia and An. (Ano.) pseudopunctipennis Theobald in central and South America. The natural vectors of bird malaria are ornithophilic species of Culex (Bellamy, 1955; Gutsevich et al., 1971).

The three species of the superfamily Filarioidea known to be transmitted to man by mosquitoes are those belonging to the genera Brugia (B. malayi and B. timori) and Wuchereria (W. bancrofti). B. malayi occurs in South and East Asia from the west coast of India to the Maluku Islands in Indonesia while B. timori is restricted to Timor, Flores and nearby islands. W. bancrofti is widely distributed in the tropical regions of the world (Partano et al., 1977; Sasa, 1976). Vectors of Bancroftian filariasis include several of the major malaria vectors and the highly domestic Cx. (Cux.) quinquefasciatus Say throughout the tropics and Aedes (Stg.) polynesiensis Marks in the Pacific area. Vectors of Brugian filariasis include Anopheles, Aedes and Mansonia species (Sasa, 1976).

The viral disease, yellow fever, dengue haemorrhagic fever remain common diseases of the tropical and subtropical regions. In 1941, an epidemic of yellow fever occurred in western Uganda during which deaths have been estimated at between 15,000 and 30,000 (Gillett, 1969). In the period 1960-62 a more dramatic outbreak of yellow fever occurred in Ethiopia resulting in about 200,000 cases, with 30,000 deaths (Haddow, 1968; Serie et al., 1968). In 1969, the first epidemic of yellow fever since 1953 occurred on the Jos Plateau in Nigeria during which an estimated 100,000 cases were reported (Carey et al., 1972). Between May 1978 and January 1979 an epidemic of yellow fever occurred in The Gambia resulting in an estimated 8,400 cases and 1,700 deaths (Monath et al., 1980). Ae. aegypti has been incriminated as the classical vector of urban yellow fever (Haddow, 1961) and the principal vector of dengue (Bellamy, 1955).

Many other arboviruses are transmitted by mosquitoes, a number of which cause disease. These include Venezuelan Equine Encephalitis (VEE), Ilheus virus in South America; Bwamba Fever, Rift Valley Fever, West Nile virus, Semliki Forest virus in Africa; Japanese B Encephalitis in Japan and adjacent areas of continental Asia; Eastern Equine Encephalomyelitis (EEE), Western Equine Encephalitis (WEE), St. Louis Encephalitis (SLE) and California Encephalitis in the USA (Bellamy, 1955) and Murray Valley Encephalitis and Ross River virus in Australia.

Mosquitoes are the principal vectors of myxomatosis of wild rabbit in Australia (Fenner & Ratcliffe, 1965) while in Britain several species of mosquitoes feed to some extent on wild rabbits and are therefore potential mechanical vectors (Service, 1971a). The principal vector, however, is the rat flea, Spilopsyllus cuniculi Dale (Andrews et al., 1959).

A general review of the role of mosquitoes in the transmission of viruses is given by Mattingly (1960), Reeves (1965), Hayes et al., (1961), Chamberlain & Sudia (1961).

1.2 British mosquitoes

Of the 3,100 mosquito species occurring throughout the world, about 33 are known to occur in Britain as well as other parts of the Palaearctic region (Marshall, 1938; Mattingly, 1950; Gillett, 1971).

Despite their number relatively little is known about their distribution, biology and ecology (Service, 1968a). Much of the

available information on British mosquitoes is due to the early work of Marshall (1938) who provided general information on most species. Killington (1946) gave a list of mosquitoes in Poole, Dorset and the surrounding area, while Mattingly (1950) provided a detailed taxonomic key for the identification of the various species. More recently, Service (1968a, 1968d, 1968e, 1970, 1971c, 1972a, 1977a) has provided a lot of information on some of the more common species found in southern England. A list of the mosquitoes occurring in the United Kingdom and Ireland with their distribution and prevalence is presented in Table 1.2.

British mosquitoes exploit a wide range of oviposition sites. For example, Ae. (Ochlerotatus) detritus (Haliday), Ae. (Och.) caspius (Pallas) and Ae. (Och.) dorsalis (Meigen) breed essentially in salt-marshes usually in the coastal areas (Ae. detritus has also been recorded in inland fresh water (Service, 1972, 1973a)) while An. (Ano.) plumbeus Stephens, Ae. (Aed.) geniculatus (Olivier) and Orthopodomyia pulchripalpis (Rondani) are species breeding almost exclusively in rain filled tree cavities. The remaining species breed in ponds, ditches, temporary pools, fresh water marshes and other collections of water. Whereas the larvae of other British mosquitoes swim freely in the water, those of Mansonia species attach themselves, by the modified structures on their siphons, to the roots of certain aquatic plants.

British mosquitoes differ greatly in their life histories and strategies of egg-laying. Females of Anopheles species (An. claviger and An. maculipennis complex) lay their eggs separately usually on

Table 1.2 List of mosquitoes of the United Kingdom and Ireland. (Generic and subgeneric abbreviations according to Reinert, 1975).

Species	Distribution and Prevalence
Anopheles (Anopheles) algeriensis Theobald	Rural; rare
An. (Ano.) claviger (Meigen)	Rural; widely distributed in Britain
An. (Ano.) atroparvus Van Thiel	Rural; locally common in southern Britain especially coastal; partly endophilic
An. (Ano.) messeae Falleroni	Rare
An. (Ano.) plumbeus Stephens	Arboreal; widely distributed in Britain
Mansonia (Coquillettidia) richiardii (Ficalbi)	Rural; widely distributed in England and Ireland
Orthopodomyia pulchripalpis (Rondani)	Arboreal; rare
Aedes (Ochlerotatus) annulipes (Meigen)	Rural
Ae. (Och.) cantans (Meigen)	Rural; widely distributed in England
Ae. (Och.) caspius (Pallas)	Saltmarsh; widely distributed, coastal
Ae. (Och.) communis (De Geer)	Rural; rare
Ae. (Och.) detritus (Haliday)	Saltmarsh; abundant at coasts, rare inland
Ae. (Och.) dorsalis (Meigen)	Saltmarsh; mainly coastal

Ae. (Och.) flavescen (Muller)	Rural; very rare
Ae. (Och.) leucomelas (Meigen)	Rural; very rare
Ae. (Och.) punctor (Kirby)	Rural; woodlands; widely distributed in Britain
Ae. (Och.) rusticus (Rossi)	Rural; widely distributed in Britain
Ae. (Och.) sticticus (Meigen)	Rural; very rare
Ae. (Finlaya) geniculatus (Olivier)	Arboreal; widely distributed in England
Ae. (Aedimorphus) vexans (Meigen)	Rural; localized in England and Wales
Ae. (Aed.) cinereus Meigen	Rural; widely distributed in Britain
Culiseta (Culiseta) alaskaensis (Ludlow)	Rural; localized in N. England & Scotland
Cs. (Cus.) annulata (Schrank)	Rural and partly endophilic; widely distributed in Britain
Cs. (Cus.) subochrea (Edwards)	Rare; England
Cs. (Culicella) fumipennis (Stephens)	Rural; widely distributed in Britain
Cs. (Cuc) morsitans (Theobald)	Rural; widely distributed in England, possibly Britain
Cs. (Climacura) litorea (Shute)	coastal
Cs. (Allotheobaldia) longiareolata (Macquart)	Very rare
Cx. (Barraudia) modestus Ficalbi	Very rare
Cx. (Neoculex) territans Walker	Very rare
Cx. (Cux.) pipiens Linnaeus	Abundant in urban & rural situations throughout British Isles
Cx. (Cux.) torrentium Martini	Abundant in urban & rural situations in southern Britain
Cx. (Cux.) molestus Forskal	Domestic; occasional outbreaks especially in cities

the surface of weedy and sometimes slow moving water while Culex and Culiseta species deposit their eggs in standing water usually as rafts. The number of eggs laid by an individual mosquito during one oviposition varies greatly depending on species, the size of the female and the size of the bloodmeal (Roy, 1936; Marshall, 1938; Service, 1968e). The largest numbers of eggs recorded for some British mosquitoes are 433 for Cx. pipiens, 343 for Cs. annulata, 220 for An. claviger and 128 for Ae. geniculatus (Marshall, 1938; Service, 1968e).

Production of fertile eggs by mosquitoes is generally a consequence of taking a blood meal which is a prerequisite for the maturation of ova. However, three British mosquitoes namely Cx. (Cux.) molestus Forskall and Cs. (Cus.) subochrea Edwards and Mansonia richiadii (Ficalbi) are autogenous, that is, able to produce the first batch of fertile eggs without a previous blood meal.

British mosquitoes exhibit a variety of overwintering strategies. Species such as Cx. pipiens and Cs. annulata pass the winter as inseminated adult females in cellars, attics, farm buildings and other hiding places. They leave their winter sites in the following spring after obtaining a blood meal to deposit their eggs on available collections of water. Eggs hatch after 2-4 days depending on the species, and adults may appear in a fortnight if climate conditions are favourable. These species are multivoltine producing a number of successive generations throughout the summer and autumn so long as suitable breeding sites and favourable temperatures are available.

In most Aedes species, eggs are deposted in hollows and low lying situations with temporarily dried up water or near water margins or above the surface level of an existing water. If, during the winter, water levels rise and the eggs become submerged, some of the eggs hatch into larvae which continue to develop very slowly for the rest of the winter (Marshall, 1938). Pupation and adult emergence take place in the spring. These species are univoltine. Eggs that do not become submerged remain dry and still retain their viability for many months and in some cases years.

Species such as An. claviger overwinter as larvae since their eggs cannot withstand drying. An. claviger produces more than one generation in a year. Cs. annulata and Cs. subochrea are the only two British species of mosquitoes that possess the unique peculiarity of overwintering in either the larval or adult form.

Mosquitoes do not generally present a biting problem in England although in some areas they may cause considerably annoyance (Service, 1968a). They generally feed on a variety of animals ranging from man to reptiles (Marshall, 1938; Service, 1968a, 1971d). Species such as Cs. subochrea and An. plumbeus feed on man and other animals and their bites can be painful in the case of the former and exceptionally severe in the case of the latter. Mansonia (Coquilletidia) richiardii (Ficalbi) and Cs. annulata feed on bovids, birds, man and a number of other animals. Cx. pipiens, Cx. (Cux.) torrentium Martini, Cs. (Culicella) morsitans (Theobald) are essentially avian feeders and have also

been recorded to feed on other animals (Service, 1971d).

1.3 Sibling species

Mayr (1963) has defined sibling species as "morphologically similar or identical natural populations that are reproductively isolated". When sibling species occupy the same geographical area they are said to be sympatric, but when they occupy mutually exclusive (but usually adjacent) geographical areas they are allopatric. Sibling species are important in biology as they permit the testing of the validity of the biological versus the morphological species concept (Mayr, 1963).

Sibling species occur in all groups of animals from mammals to the morphologically simple protozoan (eg. Paramecium), but they appear to be expecially common among insects. Among the Culicidae sibling species are represented by members of An. claviger, An. (Cel.) gambiae Giles, An. (Ano.) maculipennis Meigen, An. (Cel.) punctulatus Doenitz, Cx. pipiens and other mosquitoes (Davidson, 1977; White, 1979).

Sibling species have created a lot of problems to medical entomologists who have been unable to understand aspects of vector borne disease epidemiology. The case of An. maculipennis, a malaria vector in Europe is a good example. An. maculipennis was found to be abundant in certain parts of Europe where malaria was absent. In some areas it was found to feed on man while in others it fed on domestic animals. In some districts it bred in fresh water and in others, in brackish water. The famous problem of "anophelism

without malaria" was explained only when it was discovered that An. maculipennis was actually a group of six sibling species with different biological characteristics (Bates, 1940; Missiroli, 1939).

Studies carried out on the An. maculipennis complex and An. gambiae complex revealed some of the basic differences in the habits, ecology, morphology and physiology of sibling species. For example, among members of the An. gambiae complex, An. merus Dönitz and An. melas (Theobald) are salt water species while An. gambiae Giles, An. arabiensis Patton, An. quadrimaculatus Theobald and species D are fresh water species. Oviposition experiments have shown that whereas An. merus and An. melas prefer to oviposit on damp surfaces, An. gambiae and An. arabiensis prefer fresh water (Coluzzi, 1964; Giglioli, 1964). Members of the An. gambiae complex vary in their degree of anthropophily (White, 1975). In the laboratory some members of the complex require a large space for breeding (eurygamy) while others can breed in small cages (stenogamy).

Morphological characters have also been used to identify members of sibling species. Falleroni (1926) showed that the six palaearctic members of An. maculipennis can be distinguished by differences in the colour and floats of the eggs.

Hybridization experiments involving members of sibling species are important in demonstrating reproductive isolating mechanisms such as hybrid inviability and sterility. For example hybridization studies of An. maculipennis have revealed varying degrees of

genetic incompatibility among the members of the complex (Kitzmiller et al., 1967).

In Britain, sibling species are found in two groups of mosquitoes - An. maculipennis (messaea Falleroni and atroparvus Van Thiel) and Culex (pipiens, molestus and torrentium).

The members of the Culex species are wide spread in Britain and their biology is strikingly similar. The three species often co-exist in rural areas (Marshall,1938). Cx. molestus differs from both Cx. pipiens and Cx. torrentium in being man-biting, autogenous, stenogamous (the ability to mate in a confined space), and homodynamous (the inability to diapause) (Gutsevich et al., 1971). Cx. pipiens and Cx. torrentium are sympatric sibling species with few known differences in their ecology and biology. They are essentially ornithophilic and hibernate as adults. Hibernating Cx. pipiens are common in shelters associated with human dwelling whereas Cx. torrentium are rarely found in such situations (Service, 1968a, 1971a). There is, however, evidence to show that Cx. torrentium does hibernate since adult females with well developed fat reserves have occasionally been collected in light traps in the autumn (Service, 1968e). However, their hibernation sites are unknown.

1.4 Aims of the study

Cx. pipiens and Cx. torrentium are among the commonest species of mosquitoes in southern England and breed in artificial containers and permanent ground water.

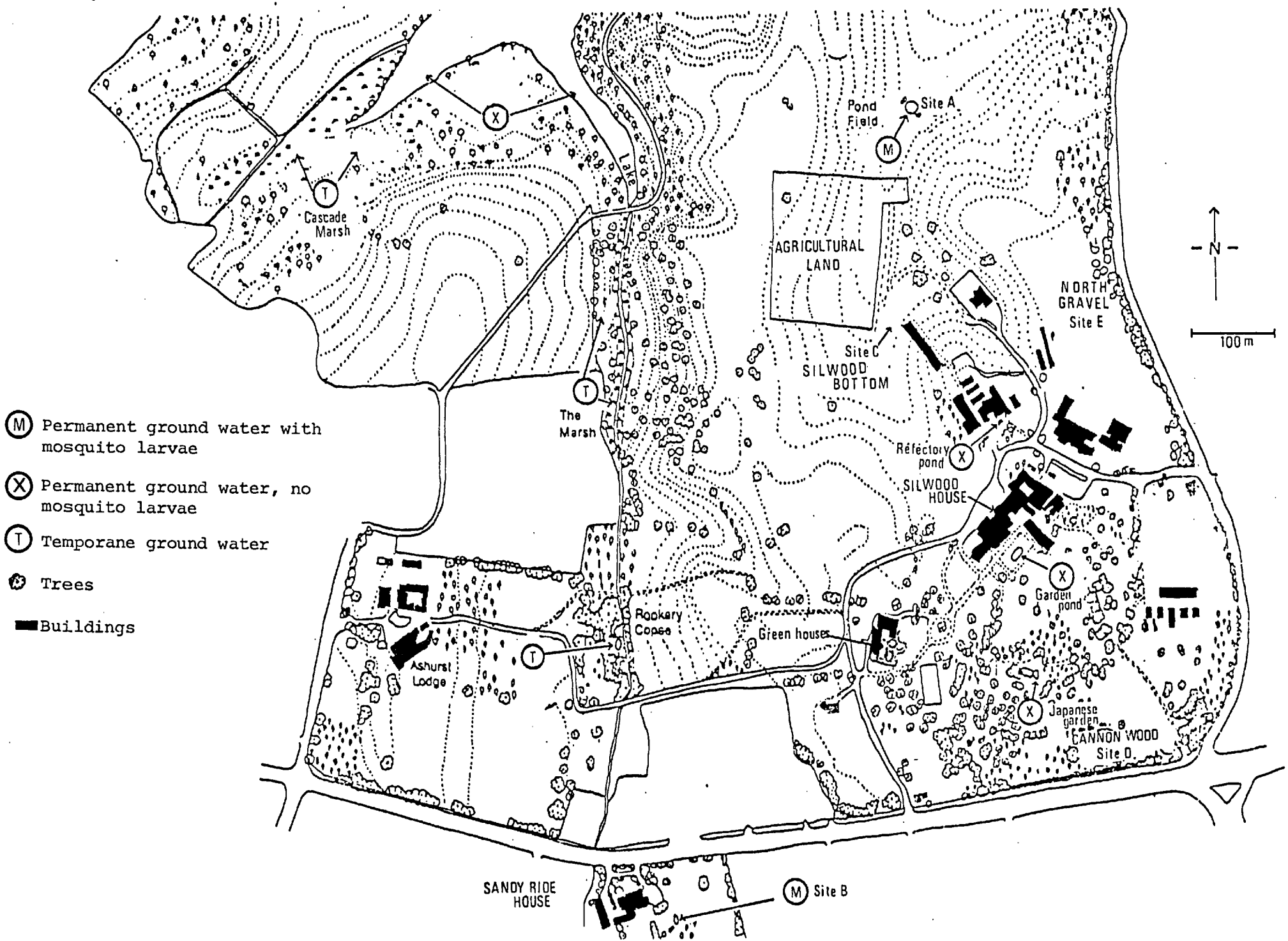
The aims of this study were to investigate aspects of the ecology and biology of these two sibling species with particular regard to their selection of oviposition sites, abundance, seasonal occurrence and mortality factors. Morphological studies were undertaken to determine the reliability of pre-alar scales to distinguish between the two species. Laboratory studies were undertaken to compare the developmental patterns of the two species in order to ascertain whether they could be differentiated on this bases. Detailed field studies were made to compare the selection of oviposition sites by adult females, seasonal fluctuations in larval populations and causes of larval mortality in the two species. Studies on the overwintering adult populations were carried out to investigate factors affecting distribution and mortality. At the same time this study was aimed at providing additional information on other mosquito species found in the same habitats as Culex species.

1.5 The study area

All studies were carried out in Silwood Park, Imperial College, Ascot, Berkshire, England (Fig.1.1). A general description of Silwood Park has been given by Waloff (1968).

There are several potential breeding sites for various species of mosquitoes within Silwood Park and the surrounding area. Within the Park itself are six areas of permanent ground water including the ornamental ponds in Silwood House gardens, the Silwood Lake, the Pond Field pond and the Sandy Ride ponds, all of which are suitable for pond-breeding species. There are a number of streams and ditches and

Fig 1.1 A sketch map of Silwood Park showing the study sites.


Permanent ground water with mosquito larvae
Permanent ground water, no mosquito larvae
Temporane ground water
Trees
Buildings
Cascade Marsh
Lake
Pond Field
Site A
AGRICULTURAL LAND
NORTH GRAVEL Site E
N
100 m
Site C
SILWOOD BOTTOM
The Marsh
Réfectory pond
SILWOOD HOUSE
Garden pond
Rookery Copse
Green houses
Ashurst Lodge
Japanese garden
CANNON WOOD Site D
SANDY RIDE HOUSE
Site B

also some temporary swamps which are potential breeding sites for species that breed in temporary woodland pools. Many types of tree are present including beech (Fagus sylvatica L.) mainly at the southeast corner of the Park, with cavities suitable for tree-hole breeding species. A number of artificial containers are to be found scattered around Silwood Park. These include small metal tanks in gardens, plastic buckets in gardens and around green houses, a few rain barrels, tin cans and several other objects which held water, all of which are potential breeding sites for some species of mosquitoes. The basement cellars of Silwood House and Sandy House plus some derelict buildings about provide good overwintering sites for some species.

About ten different species of mosquitoes have been recorded in Silwood Park. These include Ae. (Och.) cantans (Meigen), Ae. (Och.) punctor (Kirby), Ae. geniculatus, Ae. (Aed.) cinereus Meigen, Cx. pipiens, Cx. torrentium, Cs. annulata, An. plumbeus and An. claviger. Despite their number, only two studies of the mosquitoes have been undertaken in this area. These are by Gomez-Cova (1977) on the tree hole breeding Ae. geniculatus and by Turner (1978) on some aspects of the pre-adult stages of some species found in the Pond Field pond and other temporary collections of water.

SECTION 2

THE TAXONOMIC CHARACTERISTICS OF PRE-ALAR SCALES IN CX. PIPIENS AND CX. TORRENTIUM

2.1 Introduction

The pre-adult and adult stages of the sibling species of Culex - Cx. pipiens and Cx. torrentium very closely resemble each other and separation of the two can only be made reliably in the adult stage (Service, 1968c). The principal taxonomic character is the difference in the shape of the male terminalia of the two species (Mattingly, 1951a; Service, 1968c). One external taxonomic character which has been suggested to identify both adult male and female Cx. torrentium is the presence of a pre-alar patch of scales on the upper part of the sternopleuron, a character said to be lacking in Cx. pipiens

During the course of routine collections and identification of Cx. pipiens and Cx. torrentium in Silwood Park, it was observed that some adult mosquitoes bred from egg-rafts deposited by Cx. pipiens females possessed pre-alar scales. In each case the brood of progeny was reliably identified from the male terminalia. A detailed investigation was therefore undertaken to determine the reliability of using the presence or absence of these scales as a basis for distinguishing between Cx. pipiens and Cx. torrentium.

2.2 Materials and methods

2.2.1 Collection and breeding of mosquitoes

During the summer periods of 1978-79 egg-rafts of Cx. pipiens

and Cx. torrentium were collected from various breeding sites including artificial containers and permanent groundwater habitats. Egg-rafts were taken back to the insectary and placed separately in breeding pans to which a small amount of yeast had been added as a hatching stiumulus (Service, 1968e). Emerging larvae were fed on a mixture of brewers' yeast and powdered dog biscuits (Birtwisle, 1971) until pupation. Representative samples of pupae from each egg-raft were then transfered to a 16 x 16 x 16 cm emergence cage where the adults emerged. All rearing was carried out at 21 $\pm$ 1° C, 50% r.h. and 16 h light - 8 h dark photoperiod (McLintock, 1960; Miles, 1976; Sanburg & Larsen, 1973).

2.2.2 Preparation of slide mount of mosquitoes for microscopic examination

2.2.2.1 Male terminalia

Two male mosquitoes from each egg-raft were removed from the emergence cage with the aid of a glass aspirator and stunned by placing them in a freezing chamber. Their terminalia were dissected and placed in a test tube containing 10% sodium hydroxide. After heating for 5 - 10 minutes to clear (W. H. O. 1975) the specimen were dehydrated with alcohol and finally xylol and then mounted in Canada balsam. The slides were examined under a binocular microscope at 100X mangification and positively identified as either Cx. pipiens or Cx. torrentium based on the following differences: the dorsal arm of the phallosome in Cx. pipiens



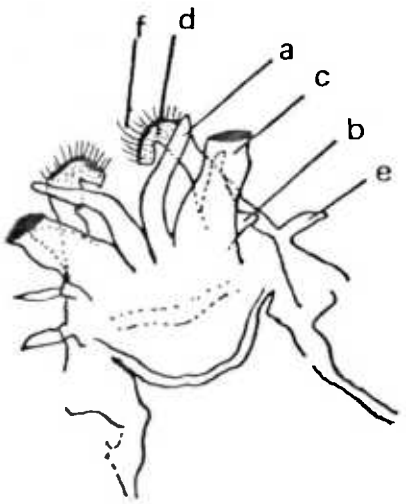


Fig. 2.1 Male terminalia of Cx. pipiens

a, b, c, ventral median and dorsal arms of phallosome; d, tip of paraproct; e, basal lateral projection of paraproct; f, spines of paraproct

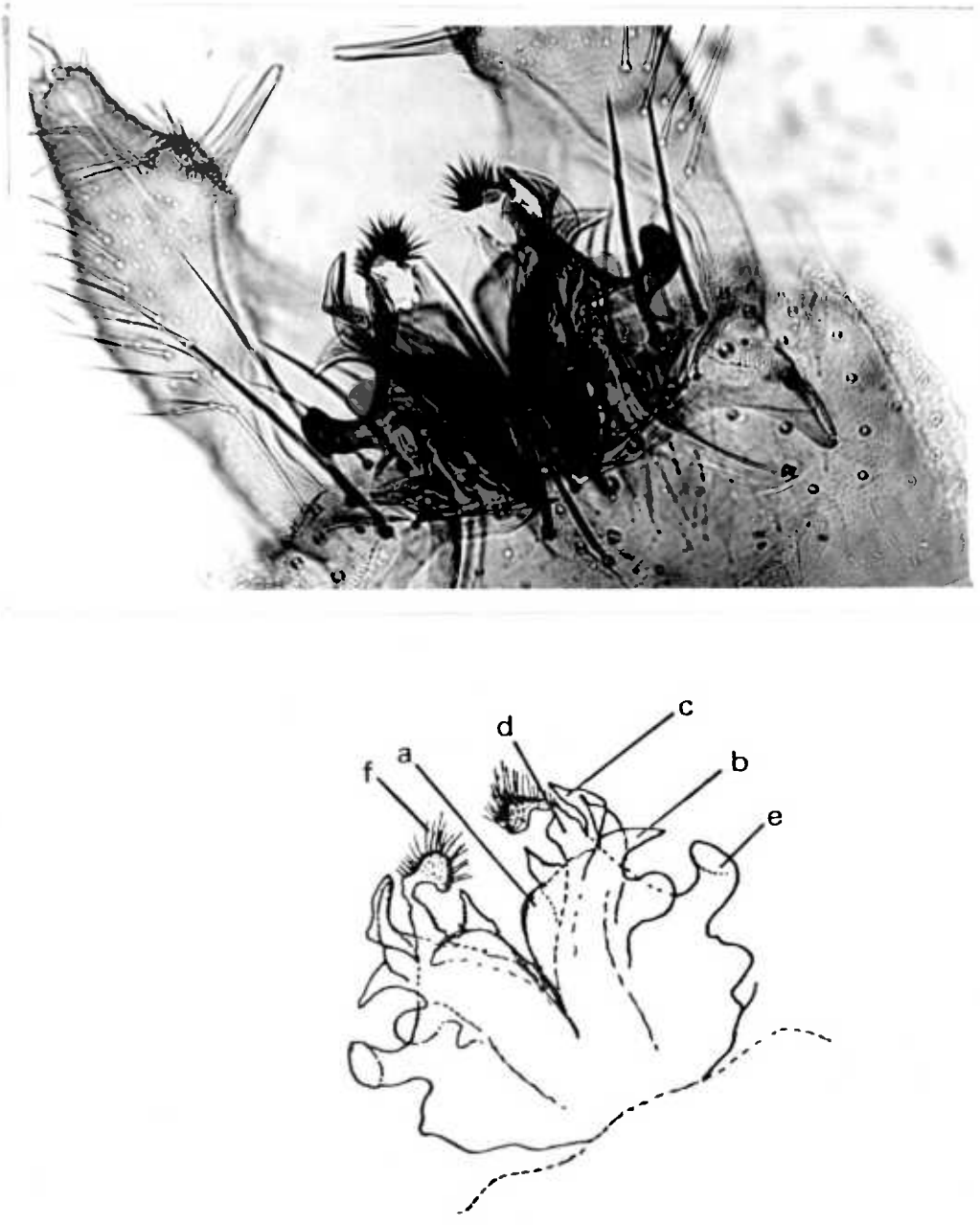


Fig. 2.2 Male terminalia of Cx. torrentium

a, b, c, ventral median and dorsal arms of phallosome; d, paraproct; e, basal lateral projection of paraproct; f, spines of paraproct

(Fig. 2·1)is tubular and does not project up to the level of the spines at the tip of the paraproct, whereas in Cx. torrentium (Fig. 2.2) the dorsal arm is pointed at the tip and projects to about the level of the spines; moreover, in Cx. torrentium, the basal process of the paraproct has a very well developed lateral projection which is vestigial in Cx. pipiens (Service 1968c).

2.2.2.2. Pre-alar scales

As the pre-alar scales easily become detached from mosquitoes (Service, 1968c), it was more appropriate to base this investigation on the presence or absence of the insertions of these scales which are readily visible in slide preparations. A slide mount was obtained by the following method (K. M. Harris, pers. comm.): The mosquito was killed and left for few days to dry - dried specimens were easier to prepare. The specimen was placed in a solid watch glass and wetted with absolute alcohol to eliminate air bubbles. Ten per cent potassium hydroxide was added to the specimen in absolute alcohol and the specimen was left for about 1 h to clear. The mosquito was then transferred to another watch glass containing glacial acetic acid and left for 15 minutes to neutralize the potassium hydroxide. The specimen was then mounted in Gum chloral and the slide dried in the oven at about 35° C. The coverslip was ringed round with Eupural and the preparation was then ready for examination under a binocular microscope at 100X.



Fig. 2.3 Pre-alar knob of Cx. pipiens

a, pre-alar scale; b, single scar of scale insertion point; c, pre-alar bristle insertion point (9 of them are seen).

Slide mounts were prepared of 24 adult mosquitoes (12 males and 12 females) from each of 45 egg-rafts of Cx. pipiens and Cx. torrentium. Since specimens were mounted on their sides it was not possible to see the scale insertions on both sides of the thorax of a single specimen. Therefore in each case the left thorax of 6 males and 6 females and the right of 6 males and 6 females were prepared. The presence or absence of pre-alar scale insertion scars which indicated the presence or absence of pre-alar scales was recorded.

2.3 Results

Fig 2.3 shows the pre-alar region of Cx. pipiens with one pre-alar scale and a single scale insertion. The proportions of Cx. pipiens and Cx. torrentium with or without pre-alar scale insertions are presented in Table 2.1.

Table2.1 Occurrence of pre-alar scale insertions in Cx. pipiens and Cx. torrentium.

Species	No. examined	No. with scale insertions	No. without scale insertions	% with scale insertions
Cx. pipiens	Male, 540	39	501	7.2
	Female, 540	60	480	11.1
Cx. torrentium	Male, 540	520	20	96.3
	Female, 540	525	15	97.2

Of the 1080 Cx. pipiens examined 99 (9.2%) were found to possess pre-alar scale insertions on either side of the thorax.

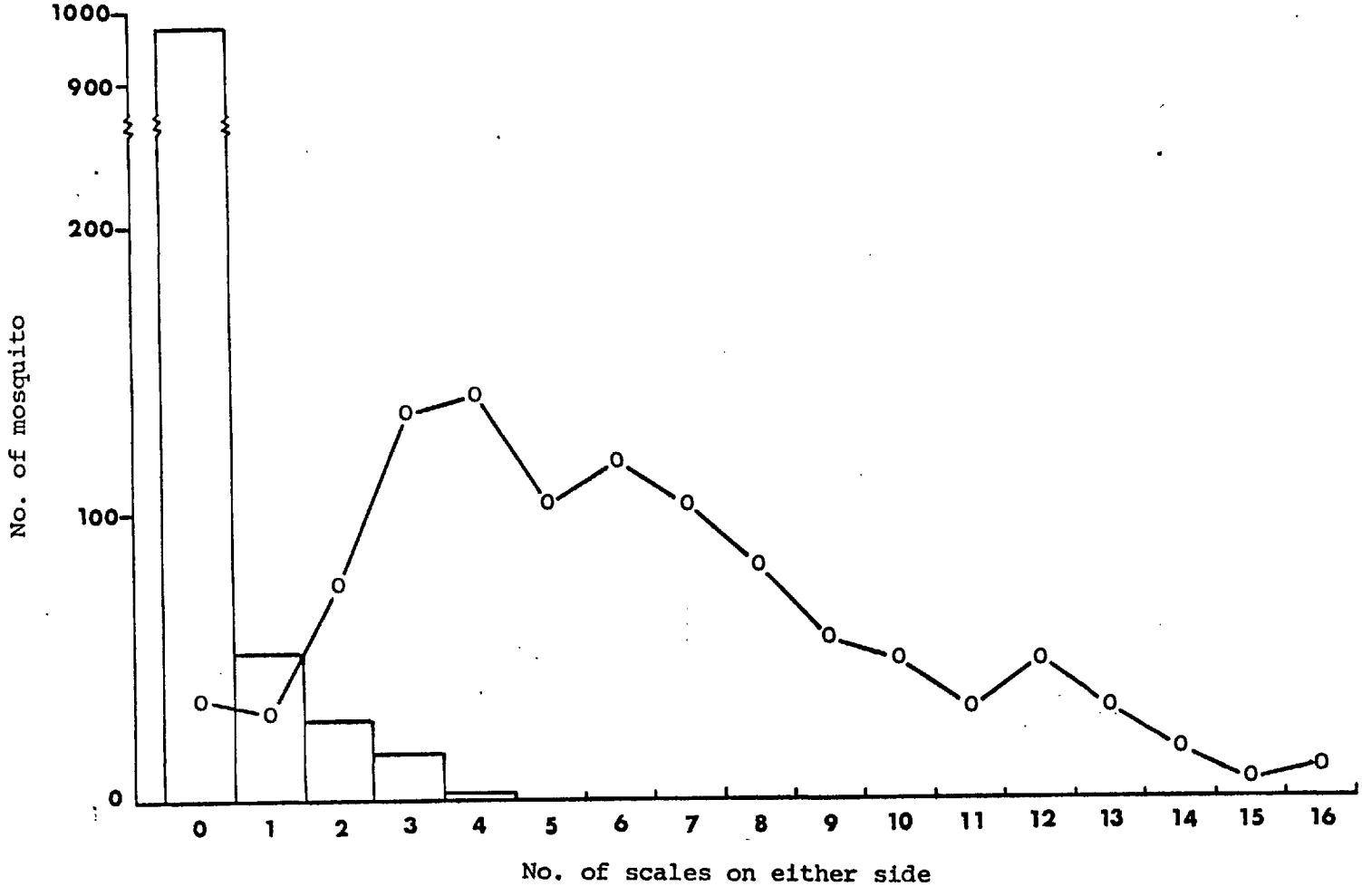


Fig. 2.4 Frequency distribution of the number of pre-alar scale insertions in Cx. pipiens (histogram) and Cx. torrentium (graph).

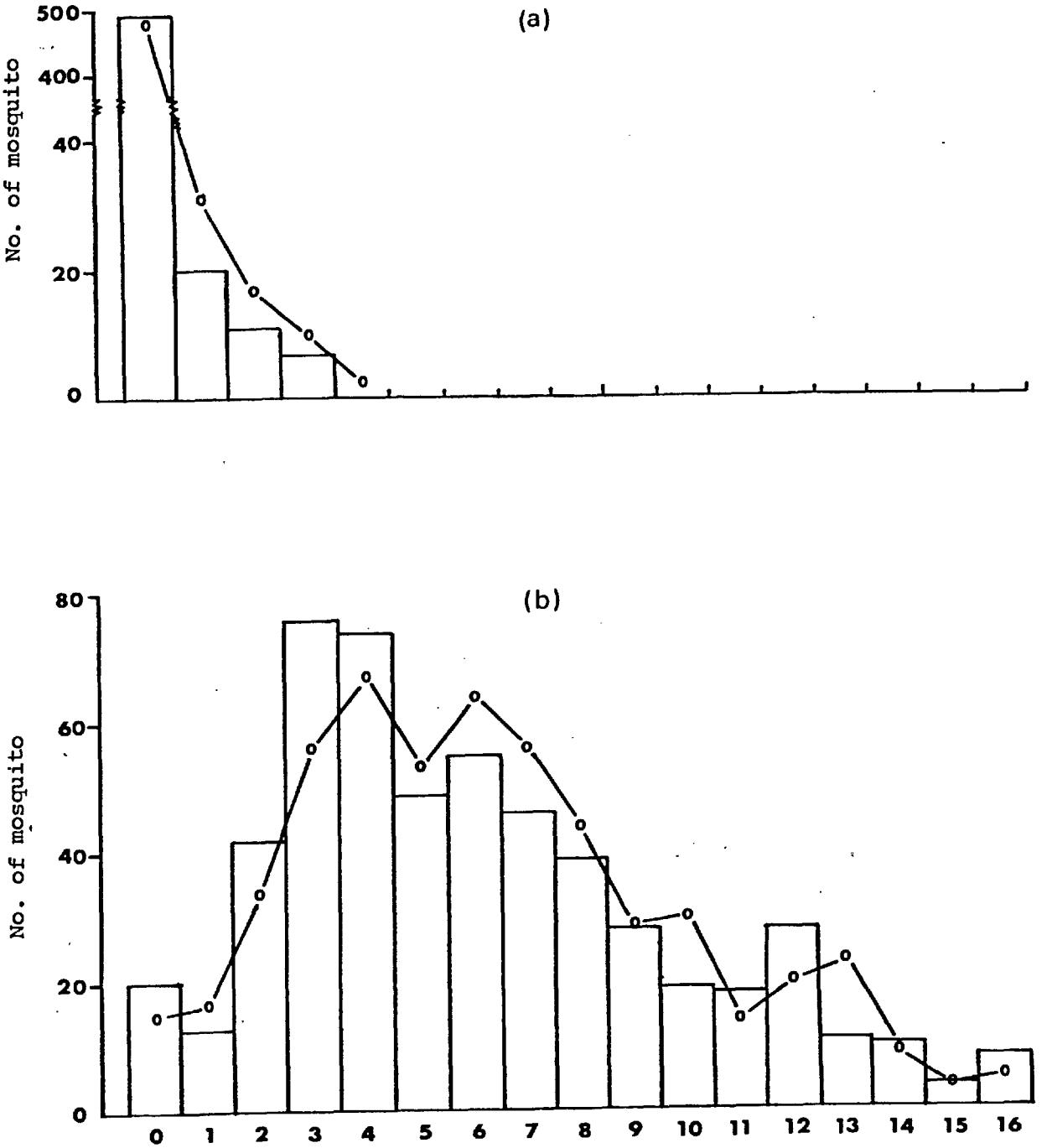


Fig. 2.5 Frequency distribution of the number of pre-alar scale insertions in male (histogram) and female (graph) (a) Cx. pipiens and (b) Cx. torrentium

In the same number of Cx. torrentium examined, 1045 (96.8) possessed scale insertions but in 35 (3.2%) individuals scale insertions were absent. Excluding all mosquitoes without scales, the number of scale insertions found in Cx. pipiens varied between 1 - 4 with a mean of 1.7, whereas in Cx. torrentium the number varied between 1 - 16 with a mean of 6.4 (Fig. 2.4).

Observation on the occurrence of pre-alar scale insertions in males and females of Cx. pipiens and Cx. torrentium showed that in Cx. pipiens more females possessed scales than males (Table 2.1). A X^2 test showed the difference to be significant ($P < 0.05$). The number of scales in this species range from 1 - 4 with a mean of 1.8 in females and 1 - 3 with a mean of 1.6 in males (Fig. 2.5a). There is no significant difference ($P \geq 0.05$) between the number of males and females of Cx. torrentium with scales. The numbers of scales range from 1 - 16 in both males and females (Fig. 2.5b). with a mean of 6.3 in males and 6.5 in females.

2.4 Discussion

A number of differences in the pre-adult and adult characters have been considered for distinguishing Cx. pipiens from Cx. torrentium. Among larval characters, Martini (1931) suggested differences in the siphonal index (length of siphon/width at its base) and in the colour of the two species. Callot (1957) and other workers used differences in the branching of the saddle hair and in the ratio of siphon/length of saddle. Callot (1957) also considered differences in the chaetotaxy of pupae in the two species.

Among adult characters, Natvig (1948) used differences in the wing venation to separate the females. He found that the length of the stem of vein R_1 was between 1/4 and 1/3 the length of its fork in Cx. pipiens and between 1/5 and 1/6 in Cx. torrentium. Moreover, the length of the radio-medial cross vein was 2/3 and 1/2 its distance from the medio-cubital cross vein in Cx. pipiens and Cx. torrentium, respectively. Mattingly (1951) observed that the dark scales on the abdominal sternite were more numerous in Cx. torrentium than in Cx. pipiens. In his studies on the taxomonic characteristics of the two species, Service (1968c) concluded that all these differences mentioned above could not be used with confidence to separate the two species.

Positive identification of the two species has been possible only in the adult stage using the obvious difference in the structure of their male terminalia. Service (1968c) found no pre-alar scales in Cx. pipiens he collected from Brownsea Island and Monks Wood and examined for this character. However, the present investigations have shown that in Silwood Park as much as 9.2% of Cx. pipiens population possessed scales. Mattingly (pers. comm.) collected two female Cx. pipiens from Gloucestershire in south-west England which he believed possessed scales. Service (1968c) found that in few Cx. torrentium also from Brownsea Island and Monks Wood pre-alar scales were absent and in others they were easily detached. He concluded therefore that absence of pre-alar scale could not always be taken as indicative of Cx. pipiens.

This investigation points out the degree of unreliability in using the presence or absence of pre-alar scales as a diagnostic feature to separate these sibling species of Culex. With reference to Fig.2.4 it can be said that when between 1 - 4 scales are present, identification using this character may not be applied with confidence, but when more than four scales are present the specimen can be safely identified as Cx. torrentium. As the pre-alar scales are easily detached, it is necessary to look for the insertions of these scales whenever there are doubts. When a male mosquito is involved it is possible to confirm its specific identity by examination of the terminalia. But when a female individual is involved it is recommended that, whenever possible, eggs are obtained and progeny reared so that F_1 males can be identified by means of the phallosome characteristics.

Throughout this study identification of sibling species has always been made by the male terminalia since this is the only foolproof method currently available.

SECTION 3

COMPARATIVE STUDIES ON THE DEVELOPMENT PATTERN OF CX. PIPIENS AND CX. TORRENTIUM

3.1 Introduction

There is little or no information on the comparative biology of the pre-adult stages of Cx. pipiens and Cx. torrentium apart from observation by Service (1968c) on aspects of the taxonomy and biology of the two species. In this study, a laboratory investigation was undertaken to determine whether there are differences in the biology of the two species as regards their gonotrophic cycle, egg-batch size and the duration of the different larval instars and pupae.

3.2 Experimental

3.2.1. Egg-batch size and fertility

Egg-rafts of Culex species taken from various field oviposition sites were taken back to the insectary at $21 \pm 1^{o}C$ and 50% r.h. and placed separately in plastic cups until they hatched. The first stage larvae were counted using a Pasteur pipette and hand counter and the number per raft recorded. It was found more convenient to count the larvae about a day or two after hatching when they had grown in size. Each raft was further left for about a week after which time any unhatched eggs were dissected to determine why they failed to hatch. Larvae were bred to adults and representative samples positively identified by the examination of the male terminalia.

Table 3.1 Frequency distribution of the number of eggs per raft in 132 and 112 egg-rafts of *Cx. pipiens* and *Cx. torrentium*, respectively.

Number of eggs	% no. of rafts	
	Cx. pipiens	*Cx. torrentium*
110 - 129	0	1.8
130 - 149	0	1.8
150 - 169	4.6	5.4
170 - 189	7.6	8.0
190 - 209	11.4	11.6
210 - 229	6.8	9.8
230 - 249	15.9	20.5
250 - 269	20.5	8.0
270 - 289	8.3	6.3
290 - 309	6.8	5.4
310 - 329	6.1	8.9
330 - 349	2.3	5.4
350 - 369	4.6	2.7
370 - 389	0.8	2.7
390 - 409	3.0	1.8
410 - 429	1.5	0
No. of egg-rafts examined	132	112

The mean egg-batch size in 132 egg-rafts of Cx. pipiens examined was 257.7±59.4 with a range of 156 - 415. In 112 egg-rafts of Cx. torrentium, the mean size was 250.0±61.7 with a range of 128 - 398. The percentage sterility was 0.5% for Cx. pipiens and 0.8% for Cx. torrentium. The students t test showed that there is no significant difference ($P > 0.1$) in the mean values of the egg-batch size in the two species. Table 3.1 shows the frequency distribution of the number of eggs per raft in the two species.

Of the 132 egg-rafts of Cx. pipiens examined 96 rafts gave 100% development and none of the remainder gave less than 94% development. Of the 112 egg-rafts of Cx. torrentium examined, 89 gave 100% development while only two rafts gave as low as 78% and 79% development. On dissection, all eggs that failed to hatch were sterile, and contained only a thick whitish fluid.

3.2.2 Gonotrophic cycle and incubation period

Adult mosquitoes which had been bred from egg-rafts collected from the field (Section 3.2.1) and whose identities were known were transferred to 45x45x45 cm colony cages and fed on 10% glucose for about 6 days. During this time mating took place. Female mosquitoes were removed with an aspirator and placed in a feeding cage the same size as the colony cage, and then starved for 24 h. A pigeon rendered relatively immobile by putting it in a sock and exposing only the head and feet (Kitzmiller & Micks, 1954) was introduced into the cage as a source of blood meal. Mosquitoes were left in the dark for 4h during which time blood feeding occurred. Blood-fed mosquitoes were removed with an aspirator and isolated individually in 16x16x16 cm oviposition cages. A piece of cotton wool soaked in water was placed on top of the cage while

a plastic bowl containing a small quantity of water was placed in the cage to receive the egg. Each day the cotton wool was replaced and mosquitoes checked to see the extent of blood digestion which was easily observed by examining the abdomen. The time in days between blood feeding and complete blood digestion, hereafter referred to as the gonotrophic cycle, was recorded. Daily observation of mosquitoes was continued until oviposition. On some occasions egg-rafts were obtained from individuals whose gonotrophic cycle was not recorded but the time of egg-laying was known. The period in days from deposition of egg-rafts to the appearance of first free-swimming larvae (De Meillon et al., 1967) was recorded as the incubation period. Eggs that did not hatch were discarded. All observations were carried out at $21\pm1^{o}C$, 50% r.h. and 16h day - 8h dark photoperiod.

A record of the gonotrophic cycle and incubation period in the two species is presented in Table 3.2

Table 3.2 Mean duration of gonotrophic cycle and incubation period in days ($\pm$SD) in Cx. pipiens and Cx. torrentium (numbers involved are shown in parenthesis).

Species	Gonotrophic cycle	Incubation period
Cx. pipiens	3.9 ± 0.5 (52)	2.3 ± 0.5 (68)
Cx. torrentium	4.1 ± 0.6 (49)	2.7 ± 0.7 (42)

A students t test showed that there is no significant difference ($P > 0.05$) in the mean values for gonotrophic cycle in the two species. There is, however, a significant difference ($P < 0.01$) in the mean values for incubation period.

3.2.3 Separation of larval instars based on body length

The total body length of different larval instars of mosquitoes was measured to ascertain if there are differences in the two species. Body length was also used to separate larval instars in field population of mosquitoes (see Section 5) whenever necessary.

Egg-rafts of Cx. pipiens and Cx. torrentium taken from the laboratory colony (Section 3.2.2) were placed separately in plastic cups (200ml capacity) containing a small quantity of water collected from the breeding sites of mosquito which were permanent ground water for Cx. pipiens and artificial container for Cx. torrentium. As soon as eggs hatched the first instar larvae of each species were placed in groups of about 30 in a set of plastic cups containing 100ml of water and 50mg of brewer's yeast. When moulting to second instar larvae commenced, moulted individuals were transferred daily to a second set of rearing cups so that those moulting on the first day of moult were placed separately from those moulting on the second and subsequent days. Individuals moulting to the third and fourth instar larvae were similarly treated. Samples of each stage of larval instar taken daily from various rearing cups starting from the beginning of the particular instar to the time moulting to the next stage commenced, were measured. By so doing it was believed that measurements of larvae were obtained at different times during the duration of each stage. All rearing of mosquito was done in an insectary at $21 \pm 1^{o}C$, 50 r.h. and 16h day -

8h dark photoperiod.

Larval measurement was done by placing each larva in a drop of water on a microscope slide with millimeter graph paper placed underneath. The total larval length was measured from the tip of the mouth to the posterior edge of 8th abdominal segment, using a stereoscopic microscope.

The results of examination of 38 - 40 individuals of the different larval instars of mosquitoes are presented in Table 3.3. It is interesting to note that according to size, the larvae of each species of mosquito are clearly separated into four instars whose ranges do not overlap. There are no differences in the ranges of the same larval instars in the two species. There is also no difference in the mean body length of first - third larval instars in the two species. There is however a significant difference (t - test : $P < 0.001$) in the mean body length of fourth instar larvae of mosquitoes. Since the ranges of the body length of fourth instar larvae are the same in the two Culex species and in view of the fact that larval size may depend on adequate food supply, the difference in the mean body length cannot be used to separate the two species.

The factors of increase of body length from instar to instar (mean body length of instar x / mean length of instar x - 1) are 2.23, 1.59 and 1.24, respectively with a mean of 1.69 for Cx. pipiens, and 2.33, 1.61 and 1.33 with a mean of 1.76 for Cx. torrentium.

Table 3.3 Body length (in mm) of different larval instars of Cx. pipiens and Cx. torrentium reared in the insectary at 21±1°C. (No. involved is shown in parenthesis).

Larval instars	Cx. pipiens		Cx. torrentium	
	Range	Mean±SD	Range	Mean±SD
I	0.8-1.8	1.3±0.36 (40)	0.8-1.5	1.2±0.22 (40)
II	2.2-3.6	2.9±0.52 (40)	2.1-3.4	2.8±0.33 (38)
III	4.0-5.0	4.6±0.38 (38)	3.9-5.0	4.5±0.41 (40)
IV	5.2-6.4	5.7±0.32 (36)	5.2-6.5	6.0±0.29 (40)

It seems that in both species, increase in body length is greatest in the second and third larval instars.

3.2.4 Larval and pupal development

Egg-rafts of Cx. pipiens and Cx. torrentium obtained from the laboratory colony (section 3.2.2) were used for this study. As soon as laid, egg-rafts were placed in a white plastic cup as above. As soon as the eggs hatched, 150 first instar larvae were removed individually using a Pasteur pipette and transferred to a white plastic rearing bowl 17cm in diameter and 6cm deep containing 500ml of water to which 0.2g brewers' yeast had been added. Everyday the water in the rearing bowl was changed and fresh yeast added to avoid a surface film developing, which would, if left, interfere with larval breeding and hence result in high mortality (Barbosa & Peters, 1973; Sanburg & Larsen, 1973). The bowl was observed daily and any dead larvae removed and recorded. As soon as the first instar larvae started to moult, a second rearing bowl was set up and any second instars appearing were transferred into it. When the larvae reached the third and fourth instars they were similarly transferred to the third and fourth rearing bowls respectively. Pupae were transferred to emergence cage until adults emerged. In each case the number of individuals moulting to the next stage and the time taken for moulting of all individuals to that stage were recorded. The development time of each stage, the larval and pupal survivals and sex ratios were calculated. The experiment was replicated five times for Cx. pipiens and Cx. torrentium, respectively.

The results obtained are presented as follows

(a) Duration of larval and pupal instars

Tables 3.4 and 3.5 show the pattern of moulting of different larval stages and pupae in a total of 750 recently hatched first instars (5 replicates of 150 larvae each) of Cx. pipiens and Cx. torrentium. If first instar larvae of Cx. pipiens hatched out on day 1, it will be seen from Table 3.4 that moulting to second instars proceeded from the 2nd - 5th day, to third instars from 4th - 8th day, to fourth instars from 6th - 12th day, to pupae from 9th - 17th day and to adults from 12th - 21st day with the duration for each stage overlapping into the next. In Cx. torrentium (Table 3.5) moulting from first to second instars lasted from 2nd - 5th day, to third instars from 4th - 9th day, to fourth instar from 7th - 13th day, to pupae from 10th - 18th day and to adult from 13th - 23rd day. Characters common to the two species are as follows. a) The period of time required for all the members of each stage to moult to the next stage was always longer as development proceeded. b) The longer the duration of moulting of any stage lasted the more it overlapped into the duration of moulting of the next stage. c) In both species the first male adult emerged a day or two before the first female. Fig.3.1 and 3.2 show the pattern of adult emergence in the two species.

The pattern of moulting in each of the five replicates for Cx. pipiens and Cx. torrentium is presented in Appendices 3.1 and 3.2.

Table 3.4 Pattern of moulting in larvae and pupae of Cx. pipiens reared at 21±1°C from an initial population of 750 first instar larvae (per cent values are obtained from the sum of 5 replicates of 150 larvae each).

Days	Per cent number entering stage on the day					
		Larval instars			Pupa	Adult
	I	II	III	IV		
1	750	-	-	-	-	-
2		3.9	-	-	-	-
3		41.3	-	-	-	-
4		45.3	12.4	-	-	-
5		9.5	18.3	-	-	-
6		-	27.6	4.4	-	-
7		-	26.6	10.1	-	-
8		-	15.0	18.3	-	-
9		-	-	22.3	0.9	-
10		-	-	24.7	5.7	-
11		-	-	13.2	12.6	-
12		-	-	7.1	14.0	0.9
13		-	-	-	16.9	1.8
14		-	-	-	15.6	8.5
15		-	-	-	17.9	13.8
16		-	-	-	9.1	13.8
17		-	-	-	7.2	18.5
19		-	-	-	-	11.8
20		-	-	-	-	12.3
21		-	-	-	-	2.6
Total Number moulting		746	732	705	680	660

Table 3.5 Pattern of moulting in larvae and pupae of Cx. torrentium reared at 21±1°C from an initial population of 750 first instar larvae (per cent values are obtained from the sum of 5 replicates of 150 larvae each).

Days		Per cent number entering stage on the day				
		Larval instars			Pupa	Adult
	I	II	III	IV		
1	750	-	-	-	-	-
2		5.9	-	-	-	-
3		39.0	-	-	-	-
4		46.8	2.7	-	-	-
5		8.2	30.7	-	-	-
6		-	30.9	-	-	-
7		-	22.0	11.4	-	-
8		-	12.0	17.9	-	-
9		-	1.6	25.9	-	-
10		-	-	16.7	4.5	-
11		-	-	17.5	9.1	-
12		-	-	3.8	14.5	-
13		-	-	6.9	18.6	1.6
14		-	-	-	17.1	4.0
15		-	-	-	14.8	11.0
16		-	-	-	14.0	16.3
17		-	-	-	5.0	18.7
18		-	-	-	2.5	19.4
19		-	-	-	-	13.2
20		-	-	-	-	10.3
21		-	-	-	-	4.6
22		-	-	-	-	0.6
23		-	-	-	-	0.4
Total Number moulting		743	732	714	695	681

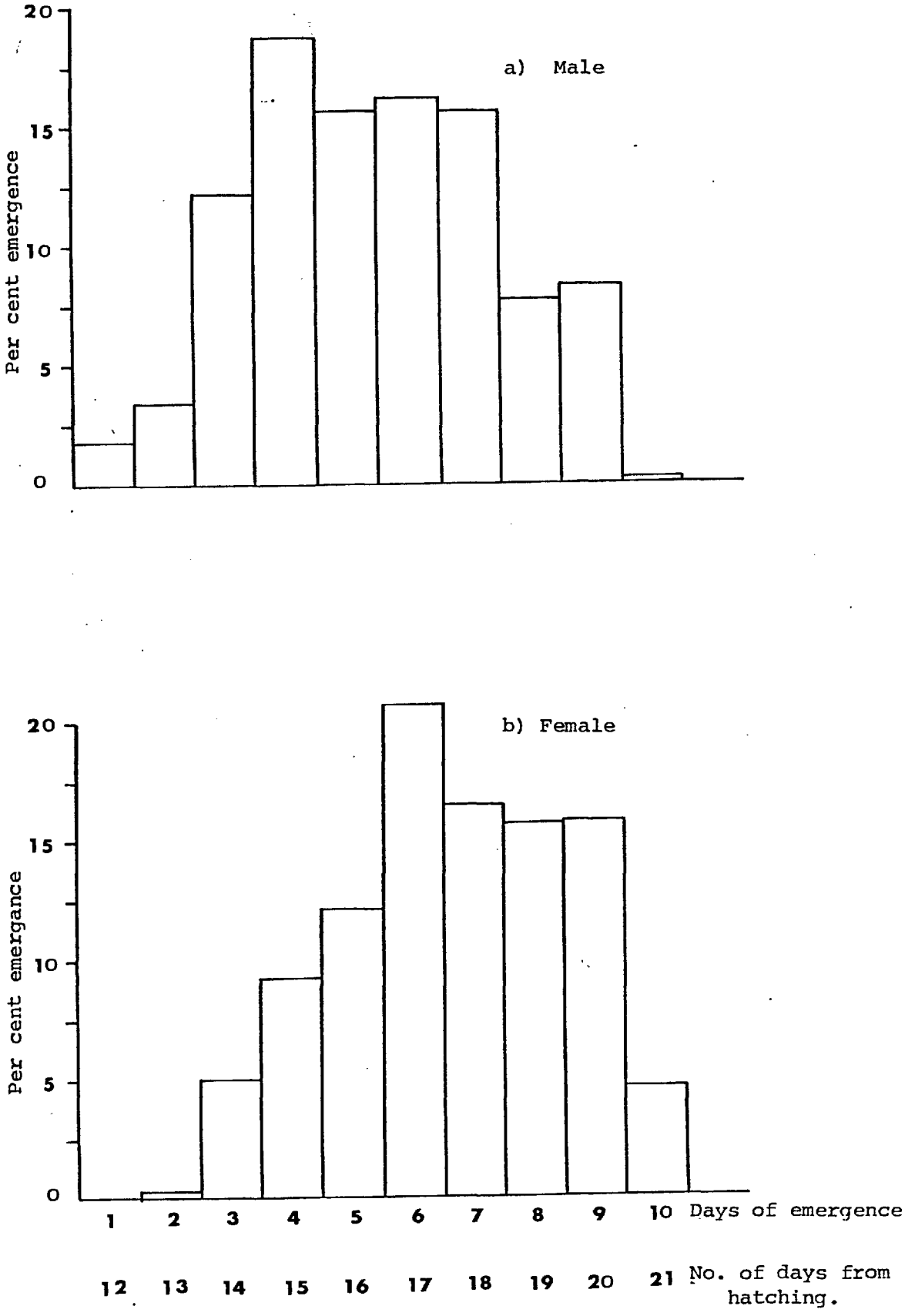


Fig 3.1 Pattern of adult emergence in Cx. pipiens reared at 21 ± 1°C.

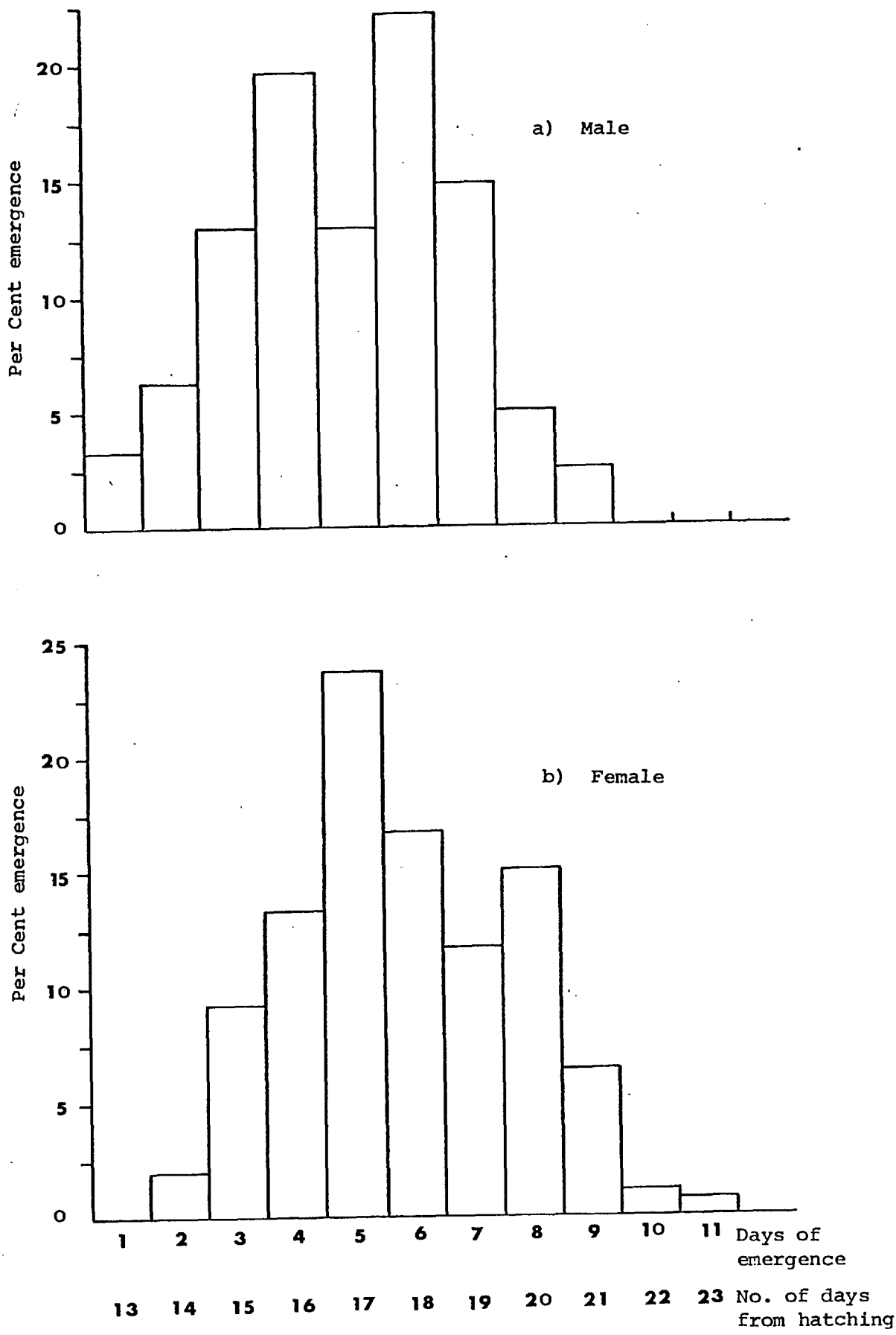


Fig 3.2 Pattern of adult emergence in Cx. torrentium reared at 21 ± 1° C.

Table 3.6 Mean duration in days (±SD) of larval instars and pupae of Cx. pipiens and Cx. torrentium reared at 21±1°C. (Five replicates of 150 larvae each).

Stage		Cx. pipiens	Cx. torrentium
Larval instars	I	2.6 ± 0.7	2.6 ± 0.7
	II	2.5 ± 1.2	2.6 ± 1.1
	III	3.1 ± 1.5	3.3 ± 1.6
	IV	4.3 ± 2.0	4.3 ± 2.0
Pupa	Male	3.1 ± 1.5	3.2 ± 1.5
	Female	4.1 ± 1.5	4.2 ± 1.4
Total duration of larval period		12.5 ± 2.0	12.8 ± 2.0

Several workers have calculated the developmental period of any stage of insects as the time required for 50% of the individuals to moult to the next stage. This is to overcome the problem arising from slow developing individuals of a population. In the present studies the duration of every individual of a particular stage has been taken into account and the duration of that stage has been calculated as the arithmetic mean of developmental times. Since development of Cx. pipiens and Cx. torrentium appears to be normally distributed (Tables 3.4 and 3.5) the use of this method is justified and considered accurate. The mean duration of pre-adults of the two species is presented in Table 3.6. There is no difference in the duration of the various stages of the two species.

b) Larval and pupal mortality

The percentage larval and pupal mortality in the two species was low (Table 3.7). In Cx. pipiens the mean larval mortality was 9.3% and that of pupae 2.3%. The overall mortality from first instar larva to the adult was 12.0%. In Cx. torrentium the mean larval mortality was 7.3% with mean pupal mortality of 2.1%. The overall mortality from first instar larva to adult was 9.2%.

c) Sex ratio

Observation made on the sex ratio of emergent adults showed that in the two Culex species there were less males than females. The female to male sex ratio was 1 : 0.95 in Cx. pipiens and 1 : 0.90 in Cx. torrentium. A x^2 test showed the difference to be not-significant ($P > 0.5$). This result agrees with that of

Table 3.7

Larval and pupal mortality of (a) Cx. pipiens and (b) Cx. torrentium reared in the insectary. Each replicate is based on the initial population of 150 first instar larvae.

(a)

Replicates	% larval mortality (No. of pupae)	% pupal mortality (No. of adults)	% total mortality
1	13.3 (130)	3.9 (125)	16.7
2	4.7 (143)	2.8 (139)	7.3
3	10.0 (135)	0.0 (135)	10.0
4	5.3 (142)	3.5 (137)	8.7
5	13.3 (130)	4.6 (124)	17.3
Mean (%) $\pm$ S.D.	9.3 ± 4.2	2.3 ± 1.8	12.0 ± 4.7

(b)

Replicates	% larval mortality (No. of pupae)	% pupal mortality (No. of adults)	% total mortality
1	7.3 (139)	0.7 (138)	8.0
2	6.0 (141)	1.4 (139)	7.3
3	4.7 (143)	2.1 (140)	6.7
4	5.3 (142)	0.7 (141)	6.0
5	13.3 (130)	5.4 (123)	18.0
Mean (%) $\pm$ S.D.	7.3 ± 3.5	2.1 ± 2.0	9.2 ± 5.0

Service (1968c) in which he recorded the female to male sex ratio to be 1 : 0.96 and 1 : 0.86 in Cx. pipiens and Cx. torrentium, respectively. Mclintock (1960) also found the female to make sex ratio in Cx. pipiens to be 1 : 0.94.

3.3 Discussion

"The duration of gonotrophic cycle is important in mosquito biology as it governs the number of times feeding occurs, the number of ovipositions and the total egg production" (Service, 1968e). In the present study the average gonotrophic period was found to be 3.9 days and 4.1 days in Cx. pipiens and Cx. torrentium, respectively, and the difference in the two species was not significant. The duration of gonotrophic cycle requires less time at higher temperature, as the development of egg is inversely proportional to temperature (Bates, 1949; Clements, 1963). Ilchenko (1974) reported that in Cx. pipiens digestion of blood and maturation of egg took 3 days at 27 - 29°C, 3.5 days at 21 - 25°C and 5.5 days at 18.2°C.

In the present study, the mean incubation period was found to be 2.3 days for Cx. pipiens and 2.7 days for Cx. torrentium, and the values were significantly different. The incubation period of the egg may be determined by temperature (De Meillon et al, 1967). Jobling (1938) carried out experiments on the effect of temperature on the duration of egg stage in Culex species. He showed that between the mean temperature of 22.6°C and 19.8°C the egg stage lasted 48h in Cx. pipiens and Cx. molestus and between 17°C and 15.4°C it lasted 72h. At

the mean temperature of 11.7°C the egg stage of Cx. pipiens lasted 120h. DeMeillon et al (1967) showed that at 28.1±0.7°C the mean incubation period was 27.11±0.57h for Cx. fatigans.

The number of eggs laid by mosquitoes varies between individuals of the same species. The maximum number of eggs per raft recorded in this study was 415 for Cx. pipiens and 398 for Cx. torrentium and the difference in number was not significant. Marshall (1938) recorded a maximum number of eggs for Cx. pipiens as 433, while Service (1968e) recorded maxima of 388 and 343 for Cx. pipiens and Cx. torrentium, respectively. The number of eggs laid by mosquitoes can be affected by the size and age of the individual, with smaller and older females laying fewer eggs, and also by the size of the blood meal taken (Clement, 1963; Service, 1968e). Further information on the egg size in British mosquitoes can be obtained from Marshall (1938) and Service (1968e).

Information on the duration of immature stages of mosquitoes is important from the point of view of control by larvicides (DeMeillon et al, 1967). The time taken in the different larval instars and pupae added together gives the complete pre-adult duration (Christophers, 1960). It was found in this study that the larval-pupal ecdysis (hence larval period) was 12.5 days for Cx. pipiens and 12.8 days for Cx. torrentium. The duration of pupal stage was 3 - 4 days in the two species with males having shorter duration than females. Möllring (1956) recorded larval duration of 10 days for Cx. pipiens at 23±1°C while Sanburg & Larsen (1973) recorded

12 days at 22±1°C for the same species. The duration of larval and pupal stages differ greatly in different species of mosquitoes. Senior-White (1952) observed that the average larval and pupal life was 8 days for An.(Nyssorhynchus) aquasalis Curry while De Meillon et al found it to be 118.4h for male and 135.3h for female Cx. fatigans. The pupal stage was 32.95h for male and 34.16h for female, all at 28.1±0.7°C. Gomez-Cova et al (1977) recorded an average larval period of 164.74±9.26h at 26°C for Cx. fatigans.

Observation made in this study showed that only 0.5 and 0.8% of the eggs of Cx. pipiens and Cx. torrentium, respectively, failed to hatch due to sterility. Service(1968e) recorded 0.1% egg sterility in Cx. pipiens and 0.3% in Cx. torrentium. Jobling (1938) observed a very low mortality of eggs of Cx. pipiens. De Meillon (1967) observed that the unhatched eggs of Cx. fatigans were made up of 1.5% dead embryos and 6.5% non-embryonated eggs. Gomez-Cova et al (1977) recorded as high as 18% egg mortality in the same species.

It was observed in this study that 90.7% of the larvae of Cx. pipiens pupated while 97.7% of pupae reached adult stage. In Cx. torrentium, 92.7% of larvae pupated while 97.9% of pupae reached the adult stage. Pre-adult mortality, though very low indeed, occurred mainly in the pre-pupal stages. McIntoch (1960) observed 96% larval and 99% pupal survival in Cx. pipiens reared in the laboratory. Gomez-Cova et al (1977) observed that 70.91% larvae of Cx. fatigans reached the pupal stage at 26±2°C, while 52.58% of the original eggs reached the adult stage at 25±1°C.

Males of several species of mosquitoes have been shown to spend less time in the pupal stage than females (Nielsen & Haeger, 1954; Haddow et al, 1959; Service, 1968c). This was found to be true with both Cx. pipiens and Cx. torrentium.

This section of the study has shown that the development pattern of Cx. pipiens and Cx. torrentium are identical in most respects; the only difference appears to be in the mean incubation period for the two species, which is slightly longer in Cx. torrentium.

SECTION 4

SELECTION OF OVIPOSITION SITES BY CX. PIPIENS AND CX. TORRENTIUM

4.1 Introduction

Selection of oviposition sites by gravid mosquitoes is considered to be the primary factor determining the distribution of larval habitats of a given species. The characteristics of the substrate which influence the mosquito in its choice of an oviposition site is of practical importance in the field from the point of view of where to find the eggs, and in the laboratory in an attempt to obtain the maximum number of eggs from fed females (Beckel, 1955). The larvae of Cx. pipiens and Cx. torrentium often occur together in the same general habitats (Service, 1968c) and little is known about the factors which influence their choice of oviposition sites. This section of the study was undertaken in an attempt to determine the degree of occurrence of these two Culex species in the different breeding habitats in Silwood Park and the factors influencing choice of oviposition sites by females.

4.2 Description of larval habitats

Three different habitats supporting Culex larvae were studied.

4.2.1 Permanent ground water habitat

4.2.1.1 Pond Field pond, Site A.

This is a roughly circular permanent ground water located in a mixed acid grassland area north of the Agricultural land in Silwood Bottom (Fig 1.1). The pond is about 10m in diameter and has a

surface area of approximately 80 sq.m. (Fig 4.1). Though shallow at the edges, the maximum depth may reach some 35-40 cm. Its source is drainage water from the surrounding land which collects and flows into the pond via a water pipe at its southern end. Excess water flows out through a drainage pipe at the northern end. As a result, most of the water is relatively rich in silt and organic matter resting on thick mud, and only the top 10 cm is clear water. Throughout the study period, the water level remained relatively constant.

A number of different plants grow around and within the pond providing cover over a good portion of it. The immediate surrounding of vegetation includes a medium sized oak (Quercus robur L.) 1.2 m from the edge of the pond on the southern end, and a large nettle bed (Urtica spp.)on the northern end. The pond vegetation consists mainly of clumps of Juncus effusus L. (Soft Rush) and an edge vegetation mainly of Epilobium spp. (E. hirsutum L. and E. adenocaulon Hausskn) and Carex spp. (C. paniculata L. and C. hirta L.). Also present are considerable amounts of Solanum spp., Agrostis spp., Ranunculus spp., Rumex acetosa L. Glyceria fluitans (L), Lycopus europaeus L. and Galium spp. The duckweed Lemna minor L. and mats of algae Spirogyra spp., cover an extensive area of the pond near the outlet.

The pond holds a large population of animal life. This includes mosquito larvae and pupae, dragonfly and damselfly nymphs (mainly Agrion puella L. and Ischnura elegans Lind. (Odonata: Zygoptera) and Sympetrum striolatum Charp (Odonata: Anisoptera)), larvae and pupae of chironomid and dixid midges (Diptera: Nematocera); and larvae and adults of dytiscid water beetles (mainly colembytinids



Fig 4.1 Pond Field pond, Site A.



Fig 4.2 Sandy Ride pond, Site B.

and hydroporinids). Adults of the small dungfly, Leptocera humida Haliday (Diptera: Sphaeroceridae) were found resting on the mats of algae and duckweed.

4.2.1.2 Sandy Ride pond, Site B.

This is another permanent ground water located in the gardens of Sandy Ride house (Fig 1.1). The site is made up of three ornamental ponds but only one of them, the upper pond, contained mosquitoes and was used for this study (Fig 4.2).

The pond has a surface area of about 1 sq m and a depth of about 0.3 m with a relatively high organic content settling at the bottom. A pond-weed, Potamogeton berchtodii Fieb., and a grass, Agrostis stolonifera L. growing around the edge, and a water lily, Nymphaea alba L. growing in the pond gives it a little shading. Further shading is provided by an overhanging ornamental maple tree and a thick hedge of Rhododendron spp. about 2 m from the pond. The pond harboured a large number of animal species including the larvae and pupae of midges (Diptera : Dixidae); dragonfly and damselfly nymphs (mainly Sympetrum striolatum (Odonata : Anisoptera), Agrion puella and Pyrrhosoma nymphula Sulz (Odonata : Zygoptera)), and dytiscid beetle larvae and adults (mainly hydroporinids). There were also many water snails (Limnaea stagnalis (L.)), juvenile common newts (Triturus vulgaris, L.) and a few pond-skaters (Gerris spp. (Hemiptera : Gerridae)).

4.2.2 Artificial containers

Several tin cans, plastic buckets, a few rain barrels and three metal tanks found lying about houses, gardens and greenhouses were good breeding sites for mosquitoes. In addition to these

containers, a number of 107-litre plastic dustbins (Cope Allman Plastics Ltd) put out at several different places both in open areas and in woods also proved to be good breeding places, all of which were used in the study. These dustbins will hereafter be referred to as standard artificial containers.

4.2.3 Tree holes

A number of old beech trees with rain-filled cavities were found to contain mosquito larvae. Some of these were marked and used for the study. They are located mainly at the south-east corner of Silwood Park.

4.3 Occurrence of Culex species in the different habitats

The aim of the preliminary observations was to determine the degree of occurrence of Cx. pipiens and Cx. torrentium in the three types of habitat.

4.3.1 Method

During the summers of 1978 and 1979, samples of mosquito larvae and pupae were taken from 35 artificial containers, 17 tree holes and the two permanent ground water habitats. These samples were then bred up to the adult stage in the laboratory. Initially species separation was on the basis of male terminalia and/or female pre-alar scales, but at a later date the potential confusion in the use of female pre-alar scales was discovered (see Section 2) and species separation was then done exclusively on the basis of male terminalia.

Table 4.1a Identification (based on male terminalia and female pre-alar scales) of Culex species from the various habitats

Habitats	No. examined	% Cx. pipiens	% Cx. torrentium
Artificial containers	2831	27.7	72.3
Permanent ground water	1067	65.1	34.9
Tree holes	73	0	100

Table 4.1b Identification (based on male terminalia) of Culex species from the different habitats.

Habitats	No. examined	% Cx. pipiens	% Cx. torrentium
Artificial containers	1092	23.3	76.7
Permanent ground water	414	67.4	32.6
Tree holes	52	0	100

4.3.2 Results

The results of the identification using the two different methods are given separately in Tables 4.1a and 4.1b. Subsequent analysis are based on the results from Table 4.1b.

Examination of the males from artificial containers showed 77% to be Cx. torrentium and 23% to be Cx. pipiens. The males from the permanent ground water sites were 33% Cx. torrentium and 67% Cx. pipiens. All Culex species collected from tree holes were Cx. torrentium (Table 4.1b). These data suggest that although the two species occur together in both artificial containers and permanent ground water, there is a significant difference in the proportions of the two species of mosquitoes in the two habitats. Cx. torrentium appears to prefer artificial containers while Cx. pipiens appears to prefer permanent ground water (x^2 test : $P < 0.001$). Cx. torrentium appears to exploit a wider variety of breeding sites than Cx. pipiens since it was present in the tree holes where the latter was entirely absent.

4.4 Factors influencing choice of habitat by Culex species

It is certain that mosquitoes choose specific types of water in which to lay eggs, but little is known of what finally influences their choice (Hudson, 1956). In this investigation, two factors that could influence the choice of either the permanent ground water or the artificial containers as oviposition sites by the two Culex species were considered. These are the effects of the physical nature of the habitat and the quality of water itself.

4.4.1 Method

The Pond Field pond (Fig 4.1) was chosen for this study because of its size and location. Eight standard artificial containers were placed around the upper half of the pond about 1 m from its edge and 2.5 - 3 m apart. Four contained rain water and alternated with four containing water taken from the pond. The rain water containers were put out 5 months before studies commenced so that rain water collected in them. Containers that had little water in them at the beginning of the study were supplemented with rain water taken from another container. Each container was held in position by placing it some 20 cm into the ground and supporting it with two stakes through its two handles. Four other containers (two with rain water and two with pond water) smaller in size than the standard artificial containers but having the same circumference, were sunk in the pond so that each was only 2 cm above the water level.

Two areas, each equal in circumference to any of the containers were marked off on the water surface with wire mesh held in position with bamboo sticks to prevent any egg-raft laid within them from drifting away. Fig 4.3 shows the arrangement of the containers around and within the pond. Six other standard artificial containers (three with rain and three with pond water) were placed 100 m away from the pond east of the Agricultural land (Fig 1.1).

Between April and September 1979, all egg-rafts laid in all the containers and the areas marked on the surface of the pond were collected every day. The egg-rafts were taken back to the laboratory and bred up to adult for positive identification as

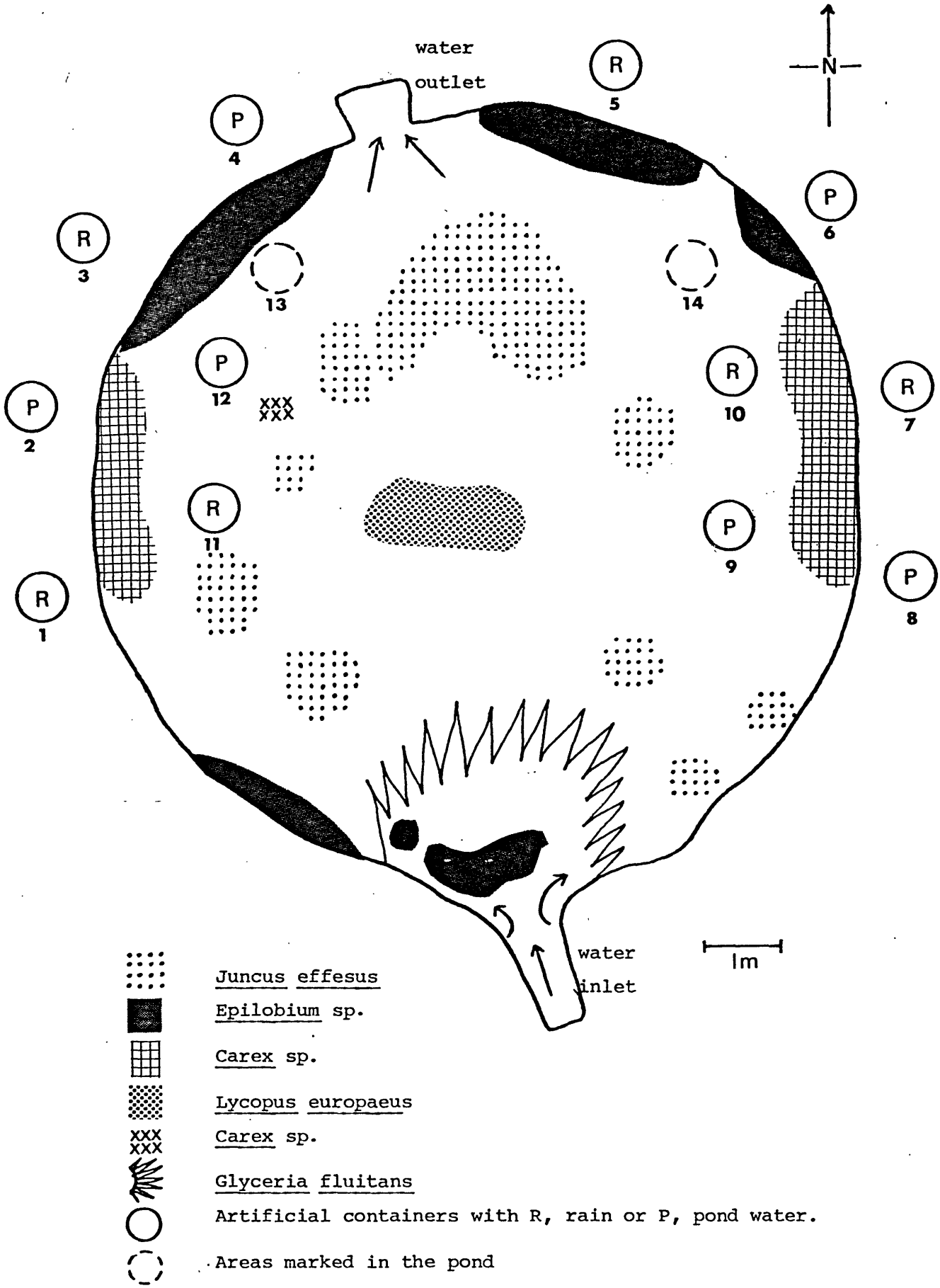


Fig 4.3 Plan of Pond Field pond (Site A) showing the arrangement of artificial containers around and within the pond.

either Cx. pipiens or Cx. torrentium on the basis of male terminalia. (Cs. annulata was identified in the larval stage). Water samples collected in July from the pond and three other containers with rain water were analysed for their chemical content using a Technicon Auto Analyser (Technicon Instruments Company, London, England) and Automatic Absorption Spectrophotometer (Instrumentation Laboratory Inc., Massachuttes, USA).

4.4.2. Results

The result of the chemical analysis of water from the breeding sites is shown in Table 4.2. The obvious differences between the chemical composition of the pond water and the rain water in the artificial containers are its high sodium, calcium and magnesium content.

Details of the numbers of egg-rafts laid by mosquitoes in the containers are presented in Table 4.3. When the egg-rafts laid in the containers surrounding the pond are considered (Table 4.3,a) a t test showed that the difference between the two Culex species as regards water preference for oviposition is not significant ($P > 0.05$). In both kinds of water there were highly significantly more Cx. torrentium eggs than Cx. pipiens (t test: $P < 0.01$ in each case).

A x^2 test applied to the numbers of egg-rafts collected in the containers sunk in the pond (Table 4.3,b) also revealed that neither Culex species showed a significant preference for either type of water ($P > 0.10$), when all the egg-rafts in these containers

Table 4.2 Results of analysis of pond and rain water for certain chemicals (in ppm) present in them.

	Pond water	Rain water	
		Mean	Range
Sulphate	NA	12.1	6.75 - 15.0
Nitrate	NA	0.29	0.05 - 1.0
Phosphate	1.0	0.24	0.01 - 0.65
Sodium	80.0	12.14	7.5 - 21.5
Potassium	ND	2.36	1.2 - 4.8
Zinc	0.03	0.21	0.10 - 0.26
Lead	0.01	0.04	0.02 - 0.07
Manganese	0.14	0.08	0.0 - 0.2
Calcium	65.0	7.8	6.2 - 9.4
Magnesium	43.0	0.93	0.60 - 1.2
pH	8.2	9.0	7.49 - 10.02

ND, not detectable
NA, no data available due to faulty machine

Table 4.3 Total number of egg-rafts of mosquitoes laid in the various artificial containers placed at sites in the field during the summer of 1979

	Cx. pipiens	Cx. torrentium	Total
a) Containers at the edge of Pond			
Containers with Rain Water			
Container No. 1	8	23	31
3	10	35	45
5	7	26	33
7	4	19	23
Total	29	103	132
Mean ($\pm$SD)/container	7.3$\pm$2.5	25.8$\pm$6.8	33$\pm$9.1
Containers with Pond Water			
Container No. 2	14	37	51
4	17	20	37
6	8	25	33
8	11	30	41
Total	50	112	162
Mean ($\pm$)/container	12.5$\pm$3.9	28.0$\pm$7.3	40.5$\pm$7.7
b) Containers sunk in the Pond			
Containers with Rain Water			
Container No. 10	7	3	10
11	13	4	17
Total	20	7	27
Mean	10.0	3.5	13.5
Containers with Pond Water			
Container No. 9	6	5	11
12	8	1	9
Total	14	6	20
Mean	7.0	3.0	10.0

Table 4.3 (continued)

		Cx. pipiens	Cx. torrentium	Total
c) Areas marked inside Pond				
Area No.	13	9	2	11
	14	17	8	25
	Total	26	10	36
	Mean	13.0	5.0	18.0
d) Containers placed 100m away from Pond				
Containers with Rain Water				
Container No.	1	9	27	36
	3	12	20	32
	5	14	18	32
	Total	35	65	100
	Mean	11.7	21.7	33.3
Containers with Pond Water				
Container No.	2	4	16	20
	4	19	28	47
	6	2	6	8
	Total	25	50	75
	Mean	8.3	16.7	25.0

Table 4.4 Mean numbers ($\pm$SD) of egg-rafts of Cs. annulata collected in the containers.

location	mean/ container
1. Containers at the edge of Pond	
Containers with Rain Water	6.8 $\pm$ 2.4
Containers with Pond Water	12.3 $\pm$ 6.2
2. Containers sunk in the Pond	
Containers with Rain Water	5.5 $\pm$ 0.7
Containers with Pond Water	6.5 $\pm$ 0.7
3. Areas marked inside Pond	5.5 $\pm$ 5.0
4. Containers placed 100m away from Pond	
Containers with Rain Water	5.0 $\pm$ 2.7
Containers with Pond Water	2.7 $\pm$ 3.8

were combined a x^2 test showed that there were significantly more Cx. pipiens in these containers than Cx. torrentium ($P < 0.001$). Similarly there were significantly more Cx. pipiens eggs (x^2 test : $P < 0.05$) than Cx. torrentium collected from the two areas marked on the surface of the pond (Table 4.3,c).

The two species show no preference ($P > 0.50$) for rain or pond water in the containers 100 m away from the pond (Table 4.3,d). Again, the values for the two types of water were therefore combined and a x^2 test showed that there were significantly more Cx. torrentium than Cx. pipiens ($P < 0.001$). It was thus concluded that the two species do show a preference as to choice of oviposition sites.

The mean numbers of egg-rafts of Cs. annulata collected in the containers during the study period are given in Table 4.4. Although the numbers are small there are no obvious differences between pond and rain water or between artificial containers and open water.

4.5 Discussion

In his studies on the pre-adult stages of some common mosquitoes in Silwood Park, Turner (1978) found that Cx. pipiens was absent from small artificial containers and tree holes while Cx. torrentium was present. Cx. pipiens was the dominant mosquito in permanent ground water where the two species occurred together. He concluded that the two species show a preference to oviposition sites. However, his observations were based on 90, 36 and 10

male mosquitoes collected as larvae and pupae from permanent ground water, artificial containers and tree holes, respectively. Marshall (1938) reported that Cx. pipiens has, on rare occasions, been recorded in tree holes in Britain. Service, (1968c) recorded for the first time in Britain, the occurrence of Cx. torrentium in sections of large bamboos designed to simulate rain-filled tree holes. Jupp (1967) observed that in South Africa Cx. pipiens prefers natural ground pools, river margins and irrigation furrows to artificial containers.

A number of factors have been found to influence selection of oviposition sites by gravid female mosquitoes. Most oviposition experiments, however, have been carried out in the laboratory where gravid mosquitoes were given choices between container water treated in different ways in which to lay eggs.

The process of oviposition by a mosquito may be divided into two behavioural strategies

a) Attraction to the oviposition site

b) The stimulus to oviposition

Many laboratory studies have been undertaken to elucidate these mechanisms and it has been shown that the dark appearance of the water (Kennedy, 1941), the reflective illuminance (Jobling, 1935; O'Gower, 1954) and the amount of organic material and vegetation (Buxton and Hopkins, 1927; Russel and Rao, 1942; Rozeboom and Hess, 1944) can all influence choice of site.

The chemical nature of the water especially the salinity will affect oviposition (Macfie 1915; Klingler and Theodor, 1925;

Cassio, 1931; De Buck et al 1932; Woodhill, 1941; Roubaud & Colas-Belcour, 1945; Senior White, 1952; Petersen, 1969).

Temperature of water may also affect the number of eggs laid. For example Hecht (1930) observed that more eggs of Ae. aegypt were laid between 20 and 30°C than at other temperatures.

It has been shown in this study that there appears to be different preferences for oviposition sites by Culex species. Whereas Cx. pipiens prefers permanent ground water, Cx. torrentium prefers artificial containers, irrespective of their location, and occasionally will breed in tree holes. Since the quality of water in the pond or container habitat did not seem to play any part in selection, it appears that what actually governs the choice of oviposition site by these species is the physical nature of the habitat. More detailed studies are required to determine whether factors such as illumination reflected from the surface of the habitat or the type and amount of vegetation present on the water surface are important. It may be that, like the members of the subgenus Stegomyia (O'Gower, 1954), selection of oviposition sites by Cx. pipiens and Cx. torrentium is guided more by visual and tactile stimuli than by olfactory stimuli.

SECTION 5

STUDIES ON PRE-ADULT POPULATIONS OF MOSQUITOES

5.1 Methods of sampling pre-adult populations: a review

A number of methods are available for sampling populations of blood-sucking insects and the technique chosen will depend upon the background of the researcher and the objectives of the sampling (Gillies, 1974). In his reappraisal of the various methods of sampling vectors, Muirhead-Thompson (1968) called for greater uniformity. The principles involved in sampling insect population densities have been reviewed by Morris (1960).

Andrewartha (1961) described two types of population densities : absolute density, in which an accurate estimate of the number of all the individuals in a habitat is made either by direct count or otherwise, and relative density, in which the size of a population is determined only as a ratio to the size of another population either in time or space. Estimate of absolute density is expensive in terms of time and money and therefore is not often made (Knight, 1964).

Estimates of the densities of mosquito pre-adult and adult populations have provided information both for research studies and for the assessment of the effectiveness of control programmes. The various methods used have been discussed by Knight (1964), Gillies (1974) and Service (1976). A summary of some of the more important method for larval surveys and their limitations is given below.

5.1.1 Counting the entire population

The most accurate and direct method of determining the absolute population density of animals in any particular habitat is to count them all (Andrewartha, 1961). This method could be applied for pre-adult mosquitoes where small container habitats are involved. The entire content of the container is tipped into a white bowl and the mosquitoes are counted. Surtees (1959) applied this method to determine the population of Ae. aegypti in a limited number of water pots and used the figures he obtained to calculate fluctuations in the larval population size. Mosquitoes breeding in small tree holes or water-filled cut sections of bamboo can be counted by pipetting or siphoning out the entire content of the container (Service, 1976) and counting the pre-adult stages present. The advantage of the small container habitats is that other associated fauna such as predators can also be counted (Service, 1976) and the containers can be moved to chosen sites (Service, 1965; Southwood et al, 1972). The major problem is mechanical damage during handling especially with small instars. Since it is not possible to count the entire population in a larger habitat, other methods of survey are adopted in which small proportions of the habitat are sampled and from these estimates the total population is calculated.

5.1.2 Quadrats

Quadrats are small areas randomly chosen from a larger area containing the entire population (Andrewartha, 1961). The different devices that have been used in collecting the population of a quadrat are either mobile (dippers and nets) or static.

Conventional dippers are the most commonly used mobile quadrat device for qualitative sampling of mosquito larvae and pupae from various habitats. These are usually made of cans, tins and other types of containers which may be of a small (Service, 1968b), medium (Schemanchuk, 1959) or large (Boyd, 1930) size depending on the area under survey. To sample a larval habitat a dipper may be gently submerged or swiftly skimmed at an angle through the water making sure that it is removed before it overflows. Attachment of handles of suitable lengths make it possible to reach any habitat without necessarily having to go so close as to disturb the inhabitants. The disadvantage of this type of dipper is that during use it tends to push larvae away and as a result fewer than the number present may be caught. It may therefore be necessary to replace the bottom of a dipper with a screen. In some cases different types of aquatic nets may be used.

Hess (1941) used a dipper with an opening 4 in. square and a screened back. Drawing the dipper through water surface for 1 yard sampled 1 square foot of water surface area, or 1/3 cubic foot of water if drawn submerged.

However, the disadvantage of dippers in general is that they are difficult and inaccurate to use in an area that is covered with dense stands of vegetation or large amounts of debris or algae (Goodwin & Eyles, 1942; Knight, 1964).

Static devices have also been used to evaluate mosquito larval population densities. These are principally open-ended metal cylinders or boxes placed at selected points in the area

under survey in such a way that they form enclosures in which organisms are trapped. The organisms can then be collected as they surface and counted. Cambournac (1939) used metal tins of this sort to assess the larval populations of Anopheles spp. in rice fields in Portugal. In Alabama, Bates (1941) similarly used petrol tins to sample An. maculipennis group while Horsfall (1946) used metal boxes to study population densities of larval mosquitoes in Arkansas rice fields. Godwin and Eyles (1942) used a wooden frame 15 - 20 cm deep which was floated on the water enclosing 0.5 - 1 m^2 of water surface. Anopheles larvae that were trapped within the frame were removed by dipping and counted. The limitation of this sampling device is some larvae would be lost by moving sideways out of the area while submerged (Knight, 1964). Bidlingmayer (1954) developed a trap appropriate for sampling the larvae of Mansonia species which affix themselves to the roots of aquatic plants by means of specially adapted siphon. The trap consisted of a galvanized cylinder with an opening of 1 ft^2. The cylinder was pressed firmly into the mud and all enclosed host plants were pulled up and thoroughly rinsed in the water within the cylinder. After allowing about 10 minutes for coarse material to settle an insert consisting of an inverted cone with a collecting chamber on the upper surface of the cone's apex was pushed down into the cylinder until the cone was just under water. The larvae having been washed off the roots surface are channelled through a small opening in the cone's apex into a collecting chamber. The water in the chamber was tipped in a white bowl and the larvae counted.

Most static quadrat collecting devices have the advantage over dippers of collecting most of the larvae that have been frightened

away by the approach of the collector (Knight, 1964). But one major problem that is encountered when quadrats are used to estimate the absolute density of a population is that of relating accurately the size of the quadrat to the whole area under survey (Knight, 1964). Some pools may be irregular in shape and a good portion of the waterline may be covered with vegetation making it difficult to estimate fairly adequately the area of the water surface.

5.1.3. Removal sampling

This method was developed principally for estimation of populations of small mammals in an area (Leslie, 1939) and is based on the principle that if individuals are removed from a population, the number caught during unit time is proportional to the number originally present (Zippen, 1950). When successive samples are taken and the individuals not returned there is a gradual decrease in the number caught due to the fact that the population is each time depleted. By applying Zippen's (1950) equation, the overall density of the population could be calculated. In Japan, Wada (1962a, 1962b) applied this method in the estimation of the populations of Cx (Cul.) pallens Coquillett and Ae. (Finlaya) togoi (Theobald) in fertilizer pits and tide-water rock-pools, respectively. The method is time-consuming and has not been used very often in mosquito larval surveys. Secondly, it does not take into account changes due to increased egg laying if carried over several days.

5.1.4 Mark-recapture method

Individuals are removed from a population and after marking are returned to the population. Sufficient time is allowed for complete mixing of population before a second sample is taken and the number of marked and unmarked individuals is recovered. The actual density of the population can be estimated on the assumption that the number of marked individuals in the second sample will have the same ratio to the total number of marked and unmarked individuals in this second sample as the total number originally marked would have to the entire population. Welch (1960) used the method to compare the densities of mosquito larvae in temporary and permanent pools. Similarly, Service (1968b) applied the method in the estimation of the populations of third and fourth instar larvae of Aedes detritus in a marsh. Service (1971b) compared estimates of population made in different habitats by mark-recapture and selective removal methods and found the former to be more reliable. Though quite easy to use in the field, the mark-recapture method has the following limitations: a large number of larvae should be marked; the duration of the experiment should be short and as many samples as possible must be taken (Knight, 1964).

5.1.5 Light trap

Sub-aquatic light traps have occasionally been used to collect mosquito larvae for qualitative studies. Husbands (1967) used a very simple light trap to collect mosquito larvae in rice fields in California. His trap is made of a glass jar and a translucent plastic funnel tightly fitted at the mouth of the jar and held in

position with rings. A tube was inserted through a tiny hole drilled at the back of the jar and inside this tube was passed a torch bulb connected to a power source. The trap was operated by suspending it in water and mosquito larvae attracted by light entered the trap through the funnel but were unable to leave again. The mosquito larvae caught in the trap were counted, after a fixed period of time.

Numerous modifications of Husband's trap have been devised for larval collections (see Hungerford et al, 1955; Bertram et al, 1970; Carlson, 1971).

Aquatic light traps have not always proved very useful for collecting mosquito larvae because different species of mosquitoes react differently to certain types of light. Moreover, this method cannot successfully be applied for absolute estimates of population densities.

A number of common problems are encountered whichever sampling method is used, and these become very obvious when information on the absolute population density is required. First, is the larval and pupal distribution and aggregation. It has been reported by many workers that the distribution of pre-adult stages of mosquito in a pool is not random, but is usually clumped with a tendency to prefer certain areas to others. Hocking (1953) observed that the larvae of Ae. (Och.) communis (De Geer) concentrated round the edge of a pool and a single dip with a ladle taken from such clumps contained as many as fifty times the number of larvae in another dip taken elsewhere.

Service (1968b) observed similar clumping of Ae. detritus around the edge of a marsh and also noticed that the degree of aggregation was more marked in some stages than others. Because of such uneven distribution of larvae which results in great variability between the numbers of individuals caught in different dips, there is great need to take many more samples if the mean number of individuals per dip should be used to estimate the population density (Service, 1976).

Furthermore, different species of mosquito and different instars of the same species may react differently to alarm stimuli such as the approach of a collector or the shadow cast over water during sampling and this may influence the number caught. Thomas (1950) observed that more younger larvae of Cx. fatigans dived to the bottom than did old larvae when a shadow was cast on the surface of water, and in the absence of such stimulus younger larvae remained submerged more than the older larvae. This may result in an underestimate of larvae by most sampling procedures (Service, 1977c).

Whenever embarking on any sampling method, it is always important to bear these problems in mind and to choose the most suitable procedure for each experimental situation.

In the present studies different methods have been used to estimate the absolute and relative population of pre-adult mosquitoes in the different habitats.

5.2 Seasonal occurrence of mosquitoes

Cx. pipiens and Cx. torrentium are the two main mosquito species that breed in artificial containers and permanent ground water habitats at Silwood Park. During the summers of 1978 and 1979 the pre-adult population fluctuations of the two species were studied in both types of habitat. Since these species cannot be separated in the larval forms the mixed populations have been referred to as Cx. pipiens/torrentium. At the same time a record of other species of mosquitoes found in these habitats was taken. A comparison of mosquitoes breeding in sets of artificial containers placed at different sites at Silwood Park was undertaken to determine whether mosquitoes preferred open or shaded areas for breeding.

Climatological data for the study period were obtained from the meteorological site located in front of Silwood House. Daily temperature readings were recorded from Stevenson screen records while rainfall readings were obtained from Dines Tilting Syphon recorder checked my M.O. gauge.

5.2.1 Artificial container habitat

All containers used in this study were the standard aritificial containers (see Section 4).

5.2.1.1 Description of the study sites

Three different sites were chosen for the study of container breeding species (Fig 1.1).

Silwood Bottom, Site C

This is an open area of land lying between the Agricultural land and building. It is a mixed acid grassland consisting mainly of willow herb (Epilobium spp.). Grasses such as Holcus spp., Agrostis spp. and Festuca spp. are also present. The site is boarded on the eastern edge by oak (Quercus robur) and Sycamore (Acer pseudoplatanus L.). Five artificial containers were placed in this site 15 m apart along a straight line. Regular sampling of mosquitoes breeding in these containers provided information on mosquito population fluctuation in a typical open area.

Cannon Wood, Site D

This is a mixed woodland containing a number of exotic conifers. Dominant in this area are Oak trees (Q. robur), Birch (Betula spp.) and Rowan (Sorbus spp.), the branches of which overhang to form a canopy producing a light shading underneath. There is, however, a large clearing in the middle with a number of Bramble (Rubus fruticosus L) and Foxglove (Digitalis purpurea L.). At the top end of the site are a number of Sycamore, and along the edge is a Holly (Ilex aquifolium L.) understory. Five artificial containers were placed here and used to provide information on mosquito population fluctuations in a typical wooded area.

North Gravel, Site E

This site is made up of a grassland strip and a wooded strip. The grassland is a typical mixed acid grassland dominated by Holcus spp. which form the ground cover, with Agrostis spp. in close association. The wooded area is dominated by Beech (F. sylvatica)



Fig 5.1 Part of North Gravel, Site E showing the arrangement of artificial containers along one of the transects.

and Oak (Q. robur) which form a canopy. Other trees common include Sallow (Salix spp.) and Sweet chestnut (Castanea spp.). Beneath the canopy is a scrub understory largely composed of Holly (I. aquifolium). This site was chosen so that the transition from open to shaded areas could be studied.

Twelve containers were placed here in three transects of four containers each so that in each transect the first container was placed in the open (grassland strip) 6 m from the edge of wood, the second container at the edge of wood and the third and fourth containers inside the wood, 6 and 16 m respectively from the edge (Fig 5.1). The transects were 76 m apart. The degree of shading produced by the trees along the transect in this site was determined by light intensity readings taken on 5th August 1978 at the positions of the containers.

At each site empty containers were put out in December each year so that they were naturally filled with rain water before the breeding season. If necessary this was supplemented with rain water taken from another container. Each container was held in position by placing it 20 cm into the ground and supporting it with two stakes through its handles. Leaves that fell into the containers were removed to minimise pollution of water through leaf decay.

5.2.1.2 Method of sampling

The efficiency of several sampling methods for the artifical containers including the use of dippers and sub-aquatic light traps was assessed in the laboratory but were found to be inaccurate. The number of mosquitoes sampled could not be related to the actual

population. Therefore a modified quadrat device which suits the structure of the larval habitat was decided upon despite its shortcomings (see Section 5.1).

The quadrat sampling device was constructed from a grey-coloured plastic rain water pipe (Osma, B.S. 4576), 6.4 cm in diameter and 22.86 cm long. One end of the pipe was covered with a fine netting through which first instar larvae could not pass. A strip of wire was attached to the other end to act as a handle to pull the sampler out of the container during sampling (Fig 5.2). The technique of sampling involved introducing three samplers into a container in a straight line covering half of the radius of the container. This was to account for the uneven larval distribution across the container. The samplers were left in position for 24 h and rapidly and simultaneously pulled out of the container. The contents were tipped into a white bowl and carefully washed with water and the mosquitoes counted. The volume of the core of water sampled by each sampler was calculated as the volume of water through which the sampler mesh was pulled. The population of mosquitoes in the container was then calculated on the assumption that the number caught would have the same ratio to the total number of mosquitoes in the container as the volume of water sampled would have to the volume of water in the container.

a) Assessment of the efficiency of sampling

The efficiency of the quadrat sampling device was assessed in the laboratory before it was used in the field. A known number of pre-adult stages of Cx. pipiens/torrentium were introduced into a container containing a known volume of water. The pre-adult

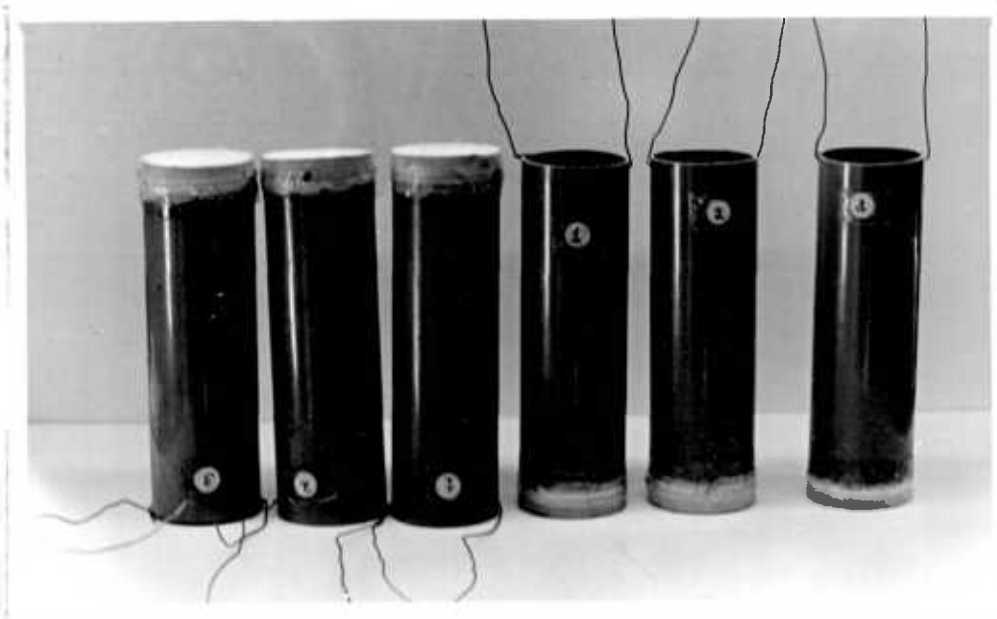


Fig 5.2 Quadrat sampling device used for absolute population estimates of pre-adult mosquitoes in the artificial containers.

mosquitoes were then sampled as described above and the population density was then compared with the known number of mosquitoes introduced into the container in order to determine the efficiency of this technique.

In the first experiment when only fourth instar larvae of Cx. pipiens/torrentium were used, the sampling method was found to be 90% efficient in predicting the population of mosquitoes. In other experiments with different immature stages the method was found to be 85, 88 and 83% efficient for younger (first and second) larvae, older (third and fourth) larvae and pupae, respectively. Details of this experiment are given in Appendix 5.1.

b) Field sampling procedure

When a count was to be made, three quadrat samplers were introduced into each container as described above and left overnight. The following morning the contents of the samplers were recovered and sorted into instars. It was easy to determine the different stages of pre-adults by eye after preliminary laboratory studies. Whenever there was doubt mosquitoes were taken back to the laboratory for microscopic examination using identification keys prepared by Marshall (1938), Mattingly (1950). All mosquitoes were then returned into the appropriate containers. The volume and height of water in the containers were measured with the aid of a calibrated dip-stick. The population of the different pre-adult stages was calculated as described above.

Samples were taken once a week in 1978 when a general survey of all available breeding sites in Silwood Park and the surrounding area was undertaken. The numbers of unhatched egg-rafts in the containers were also counted on each sampling occasion. However, in 1979 when a more detailed study was undertaken, samples were taken three times a week. After each count all egg-rafts in a container were collected and put into a plastic cup 12.5 cm in length and 4.4 cm in diameter. The bottom of the cup was replaced by coarse mesh. The cup was suspended inside each container in such a way that it was 3/4 submerged in water. All larvae hatching out of the eggs could thus move freely into the water. This ensured that all egg-rafts laid in the containers throughout the season were counted. The number of egg-rafts multiplied by 250 which was the mean egg-batch size of Cx. torrentium (Section 3.2.1). (the dominant species in the artificial containers) gave the estimates of the number of eggs in the habitat. This precise information on egg density was useful in the construction of life table for Cx. pipiens/torrentium.

5.2.1.3 Results

Data on temperature and rainfall obtained for the study period are presented in Table 5.1. The results of population sampling of egg-rafts and pre-adults of Cx. pipiens/torrentium in the artificial containers are presented graphically in Fig 5.3 - 5.8. Further details are shown in Appendices 5.2 - 5.5.

Table 5.1 Temperature and Rainfall data at Silwood Park (January 1978 - March 1980)

	MEAN TEMPERATURE (^{o}C)									TOTAL RAINFALL (MM)		
	1978			1979			1980			1978	1979	1980
MONTH	MAX	MIN	MEAN	MAX	MIN	MEAN	MAX	MIN	MEAN			
January	6.00	-0.30	3.15	2.97	-2.74	0.58	5.30	-0.70	2.50	75.90	55.40	37.90
February	5.70	0.60	2.90	3.94	-4.42	1.44	9.10	2.60	5.90	42.10	54.50	45.70
March	10.60	2.50	6.30	8.32	1.49	4.64	8.39	1.31	4.80	69.10	107.80	64.60
April	10.10	2.90	6.36	12.00	3.43	7.45				60.20	80.30	
May	16.70	6.04	11.34	15.20	5.71	10.23				76.40	94.00	
June	18.90	8.70	13.79	18.40	9.60	14.10				48.20	34.10	
July	19.48	10.80	15.14	21.50	11.30	16.80				51.60	15.00	
August	19.50	10.10	14.71	19.70	9.95	14.80				68.10	47.10	
September	18.68	9.10	13.99	18.40	7.60	13.10				19.70	12.30	
October	15.79	6.69	11.07	15.30	6.30	10.80				3.00	84.80	
November	11.54	4.63	8.23	9.80	2.60	6.60				14.10	63.70	
December	6.83	2.04	4.37	8.70	3.50	6.30				131.90	89.60	

Egg-rafts of mosquitoes were first found at Silwood Bottom (Site C) in the first week of April 1978 and the last week of the same month in 1979 (Fig 5.3). No larvae were sampled throughout this month each year showing that apparently the eggs failed to hatch due to cold weather at this time of the year. In both 1978 and 1979 first and second instar larvae appeared in the second week of May (Figs 5.4a and 5.5a). Adult emergence was delayed until late May. From late June onwards there was continuous breeding at high levels. In 1978 the peak population of first and second instars occurred in the second week of August. This was following by a steep fall in population which reached a very low level by the middle of September. In 1979, however, the highest numbers of first and second instars occurred in the third week of July earlier than in 1978 followed by a rapid decline until the last week of August.

The numbers of third and fourth instars followed the same pattern as the first and second instars. Pupal numbers remained low throughout the breeding season with a slight rise in July 1979. Eggs were last seen in the containers in the first week of October 1978 and the last week of September 1979 (Fig 5.3). It can be seen from Fig 5.4b and 5.5b that the proportions of the younger instars made up the bulk of the population from the beginning of May to August when eggs were constantly being laid. From September to the end of the breeding season the proportions of the older instars and pupae greatly increased.

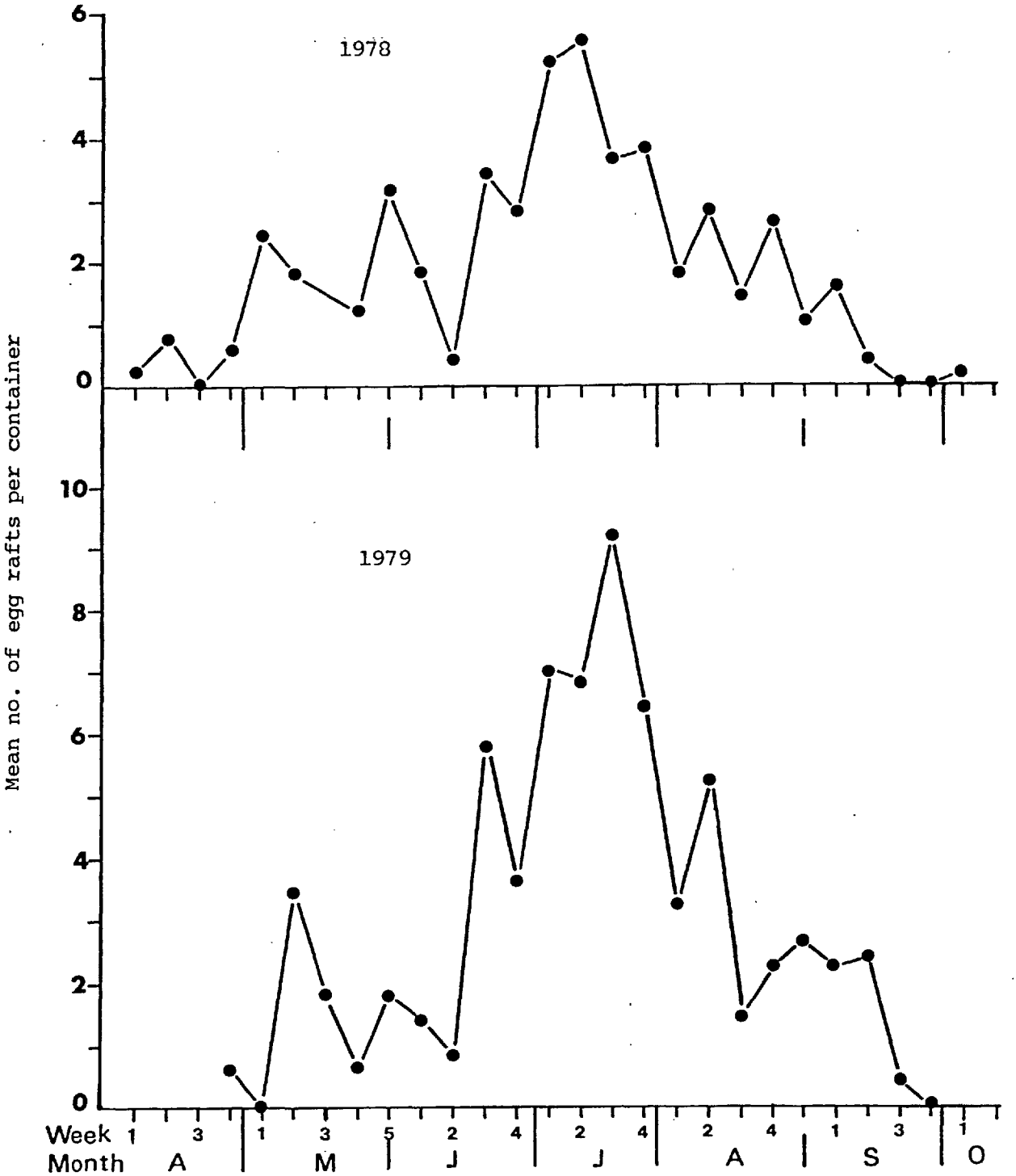


Fig 5.3 Mean number of egg-rafts of Cx. pipiens/torrentium per artificial container placed in Silwood Bottom, Site C in (a) 1978 and (b) 1979. Samples were taken once per week in 1978 and three times per week in 1979.

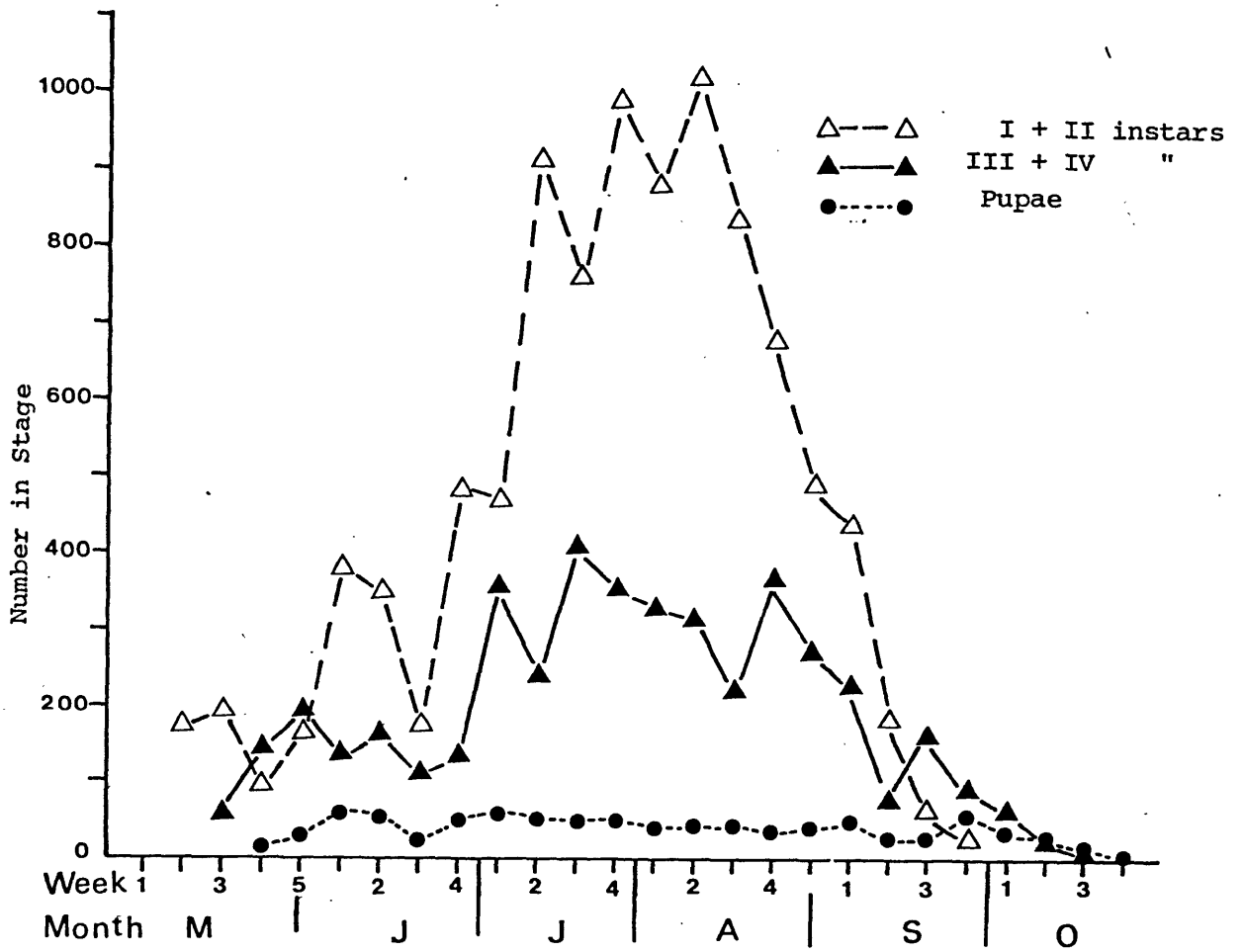


Fig 5.4a Changes in population number of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom. (Result for 1978)

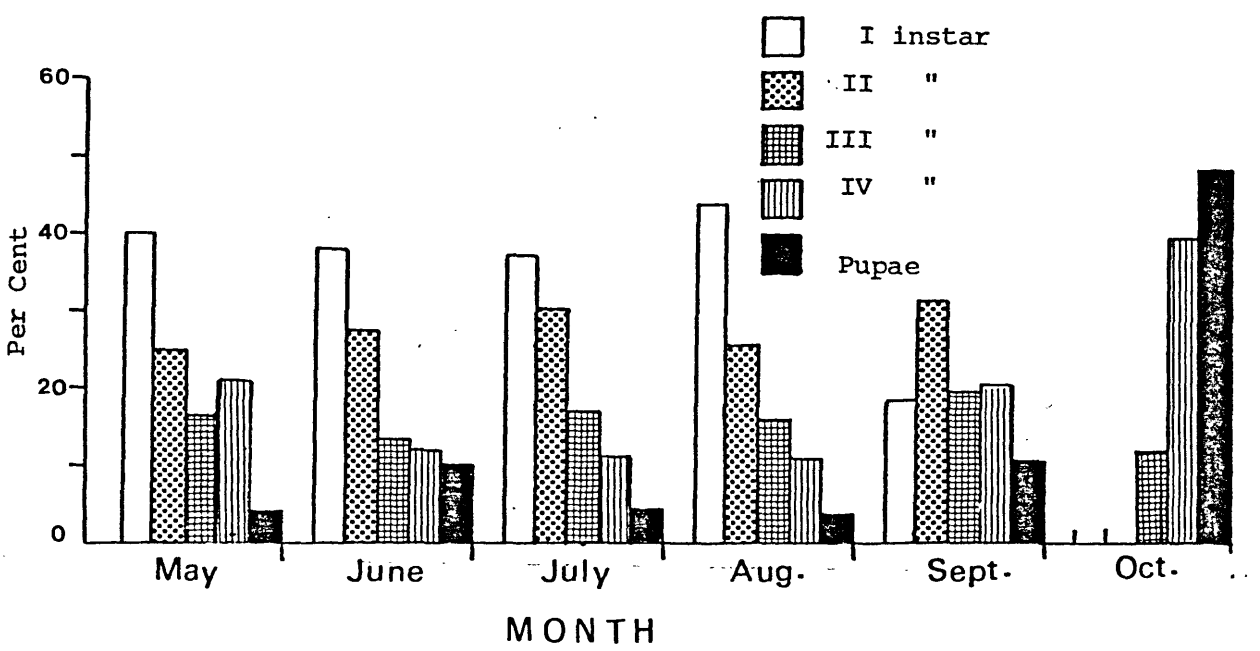


Fig 5.4b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom. (Result for 1978).

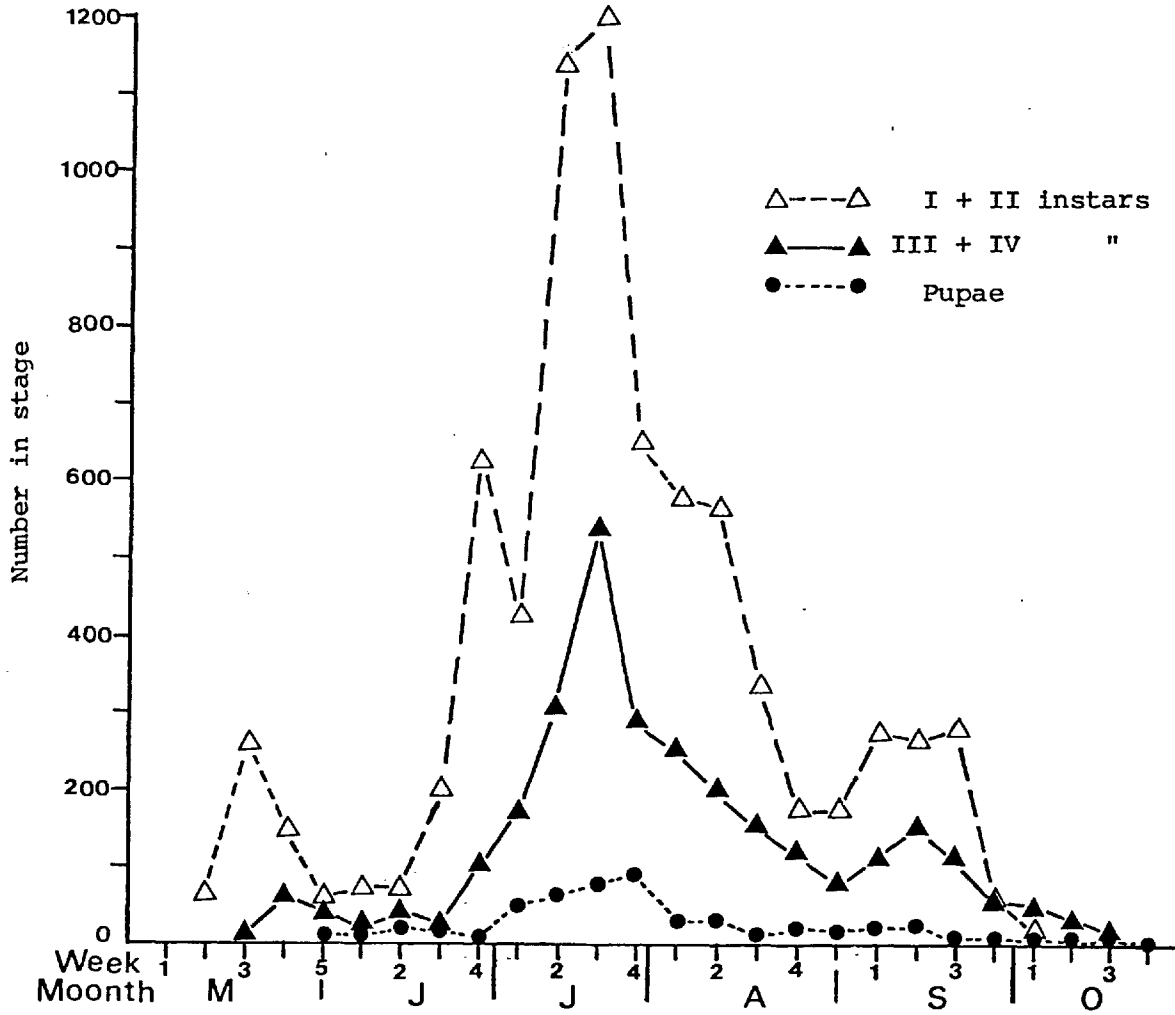


Fig 5.5a Changes in population number of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom (Result for 1979).

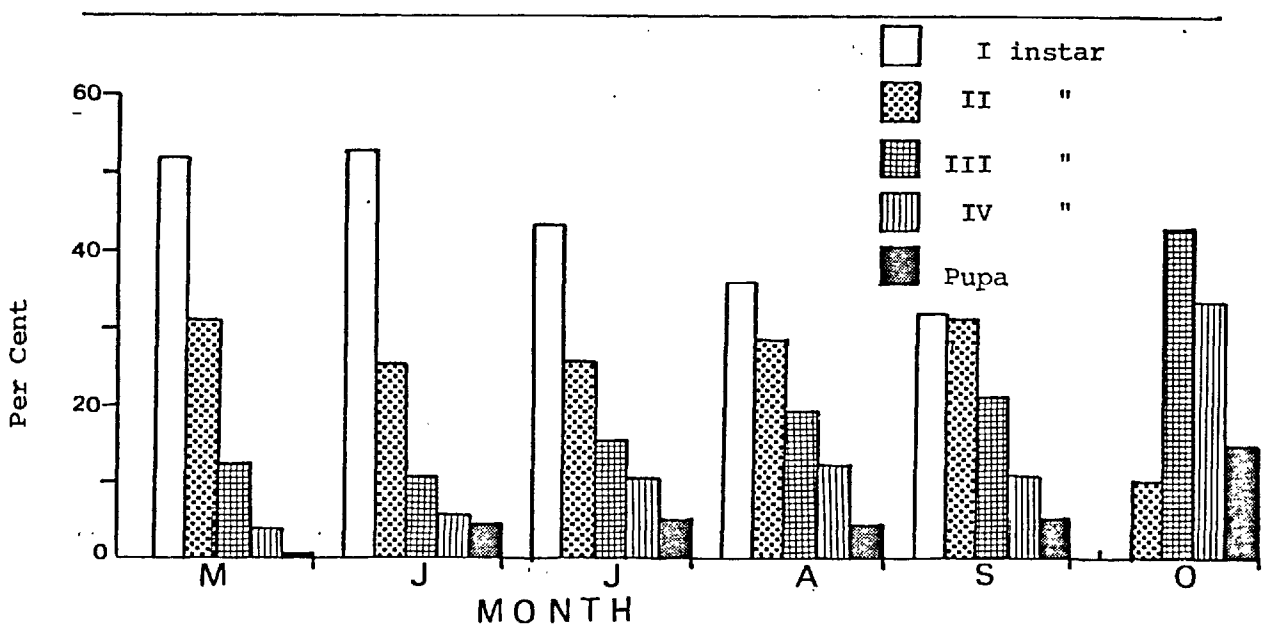


Fig 5.5b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site C, Silwood Bottom (Result for 1979)

In Cannon Wood (Site D) egg-rafts were first found in the containers in the third week of May each year (Fig 5.6). The first and second larval instars appeared in the fourth week of May (Fig 5.7a and 5.8a) about two weeks after they were first collected in Site C. Adults emerged in the second week of June. From late June onwards the numbers of first and second instars increased until a peak number was reached in the fourth week of July 1978 or the second week of July 1979. In both years the pupal numbers remained low throughout the breeding season with a slight increase in July 1979. Egg-rafts were last seen in the containers in the last week of September each year (Fig 5.6). Fig 5.7b and 5.8b show that the younger instars form the bulk of the population from May to August. In September and October there was a greater proportion of the older instars and papae.

The result of the transect studies at North Gravel (Site E) are presented in Fig 5.9. In the month of May, all four larval instars and pupae were collected from the containers placed in the open field and the edge of wood whereas in those placed 6 and 16 m inside the woodland only the first, second and third larval instars were collected. From June to September all five pre-adult stages were present in numbers in all the containers with higher densities in those in the open field and edge of wood. In October first and second instars were absent in the containers 16 m inside the wood. Details of population sampling of mosquitoes along the transects are presented in Appendices 5.6 - 5.9.

An. claviger and Cs. annulata were occasionally found in some of the containers. Their numbers were low and no record of them has been taken.

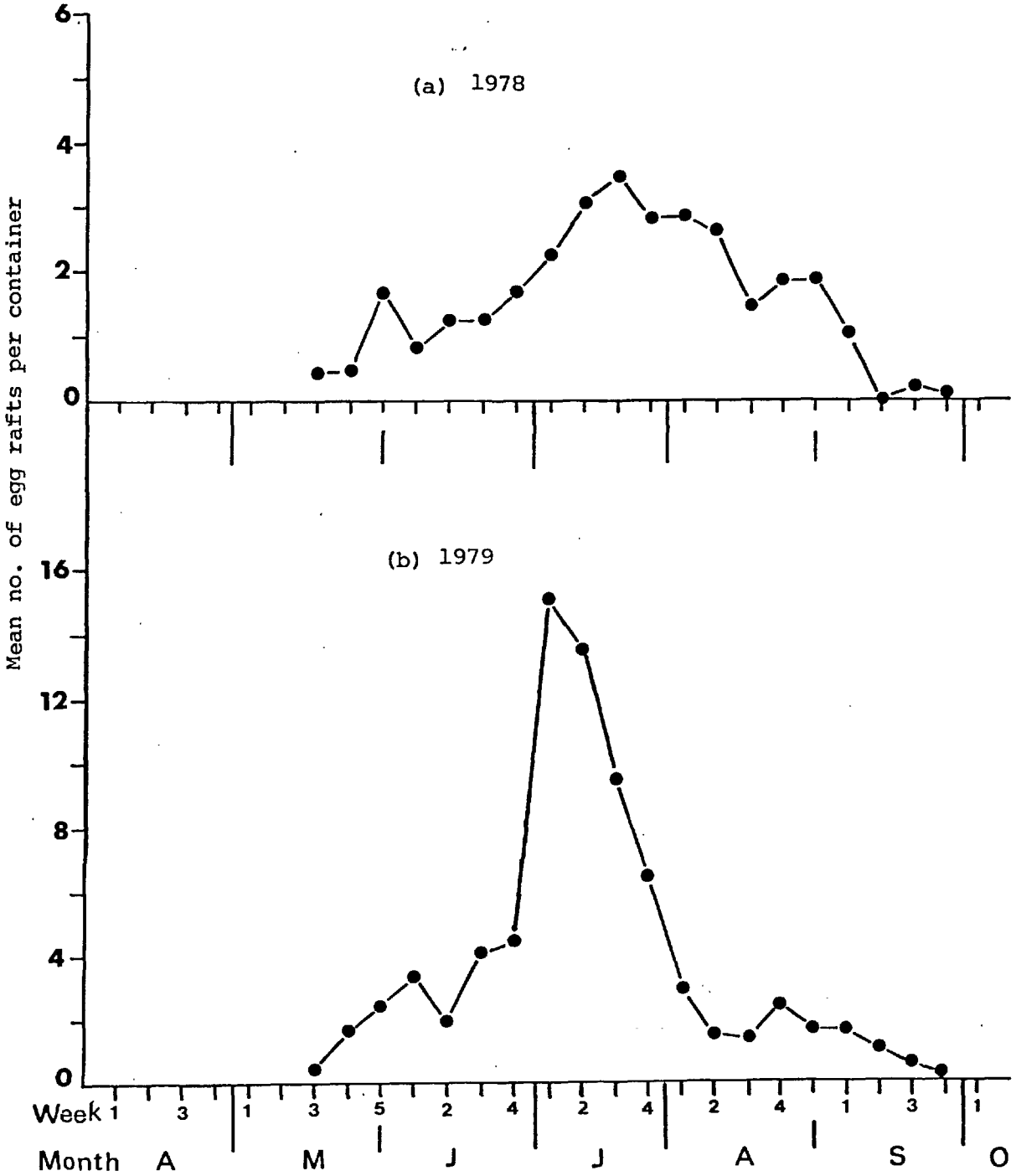


Fig 5.6 Mean number of egg-rafts of Cx. pipiens/torrentium per container placed in Cannon Wood, Site D in (a) 1978 and (b) 1979. Samples were taken once per week in 1978 and three times per week in 1979.

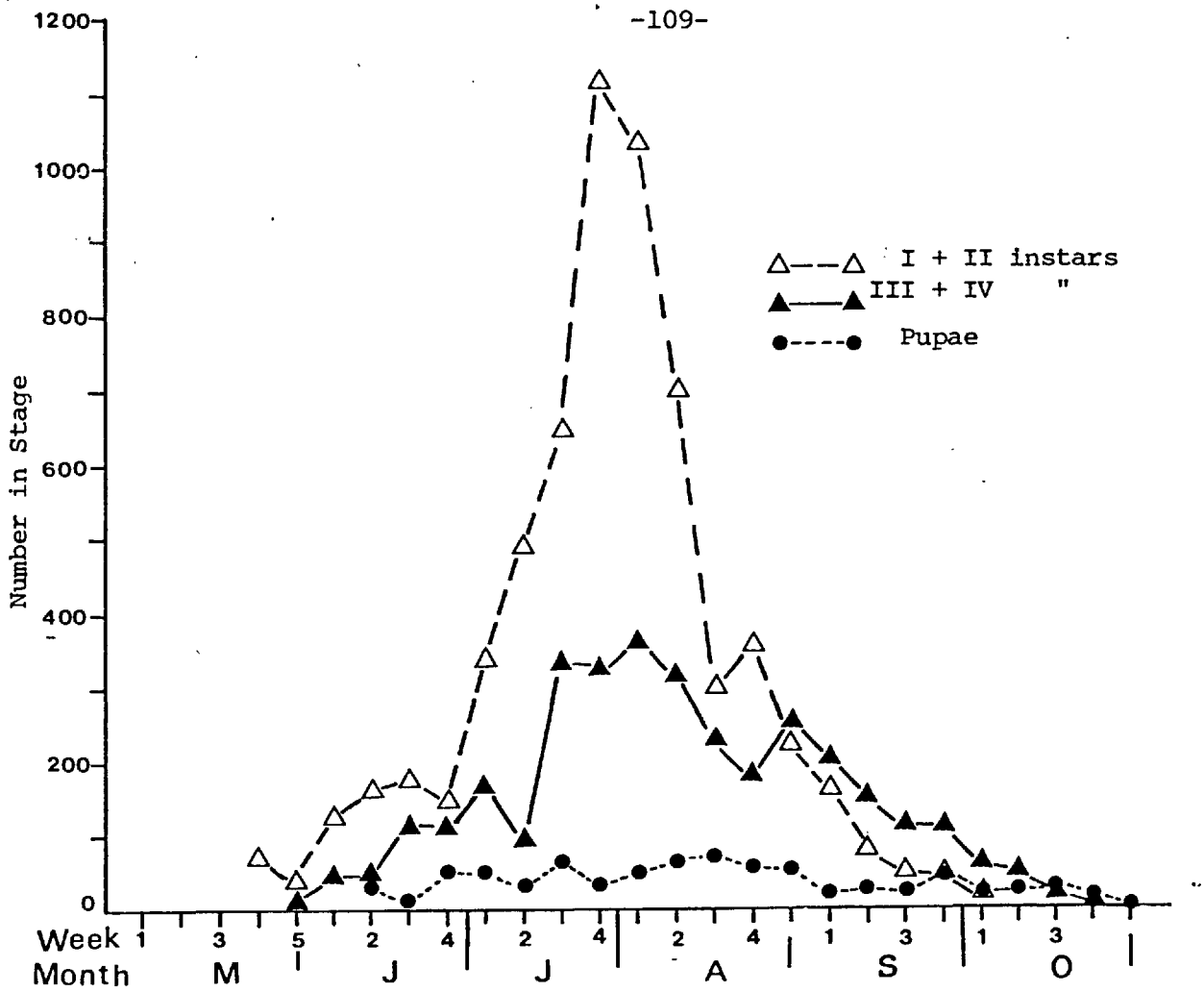


Fig 5.7a Changes in the population number of pre-adults of Cx. pipiens/torrentium per container in Site D, Cannon Wood (Results for 1978)

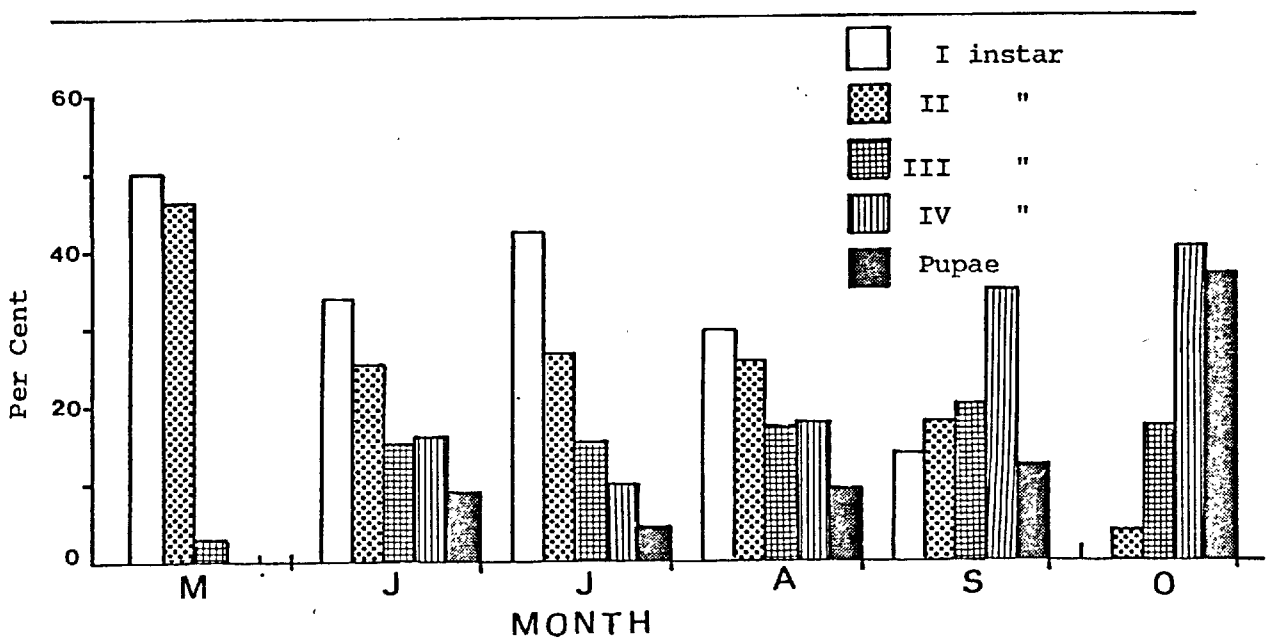


Fig 5.7b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site D, Cannon Wood. (Result for 1978).

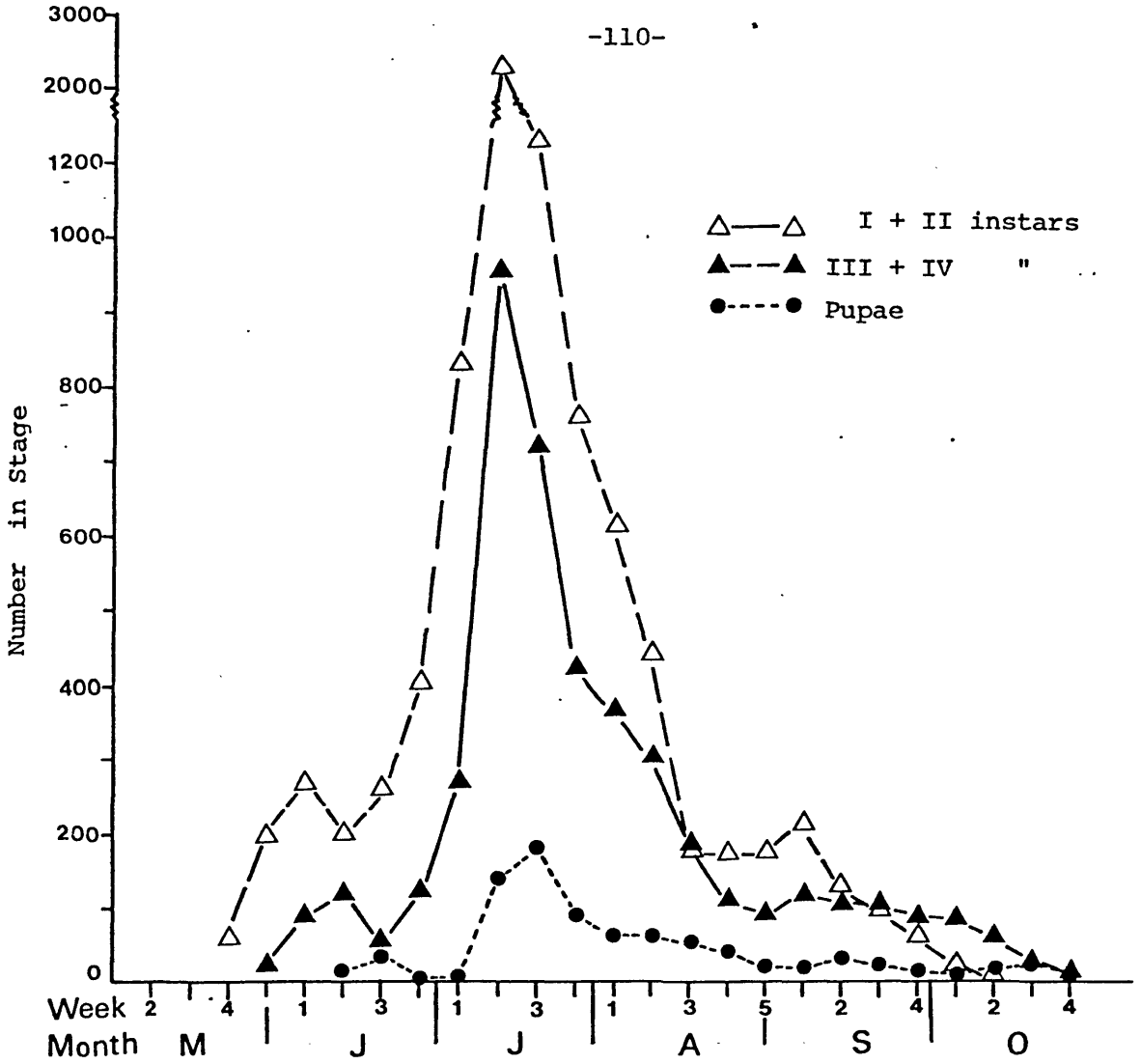


Fig 5.8a Changes in the population number of pre-adults of Cx. pipiens/torrentium per container in Site D,Cannon Wood. (Result for 1979).

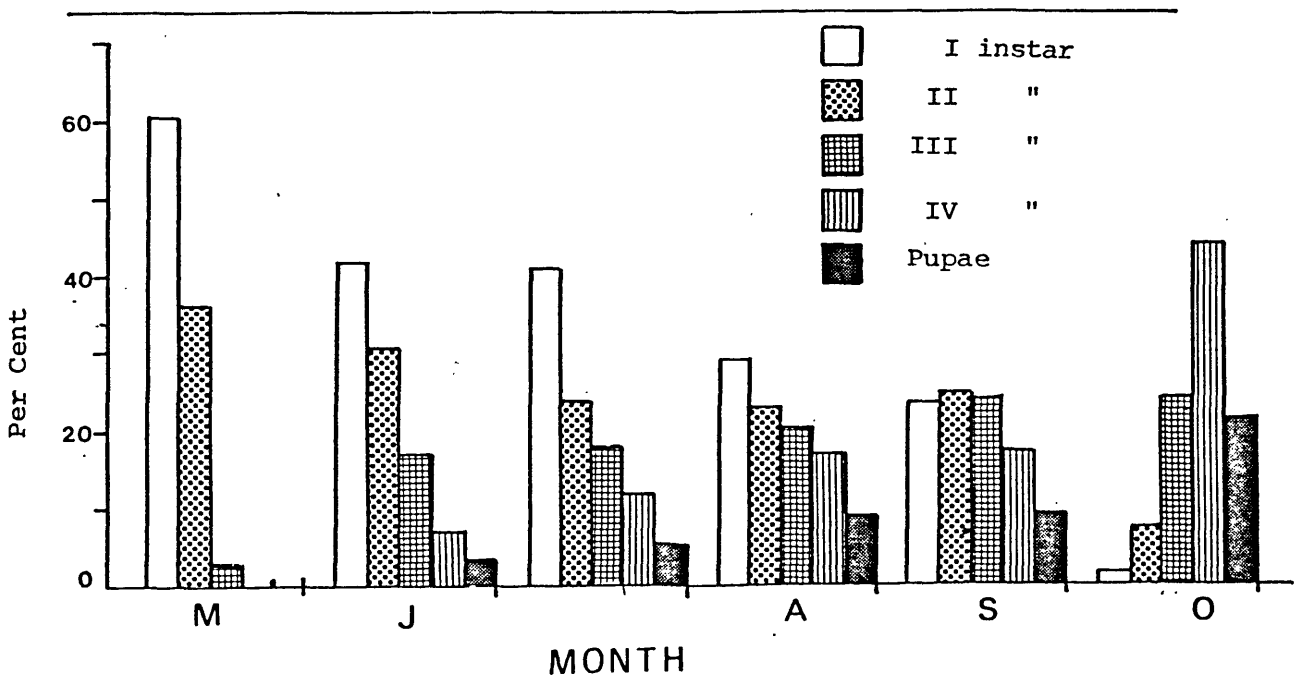


Fig 5.8b Monthly proportions of pre-adults of Cx. pipiens/torrentium per container in Site D,Cannon Wood. (Data for 1979).

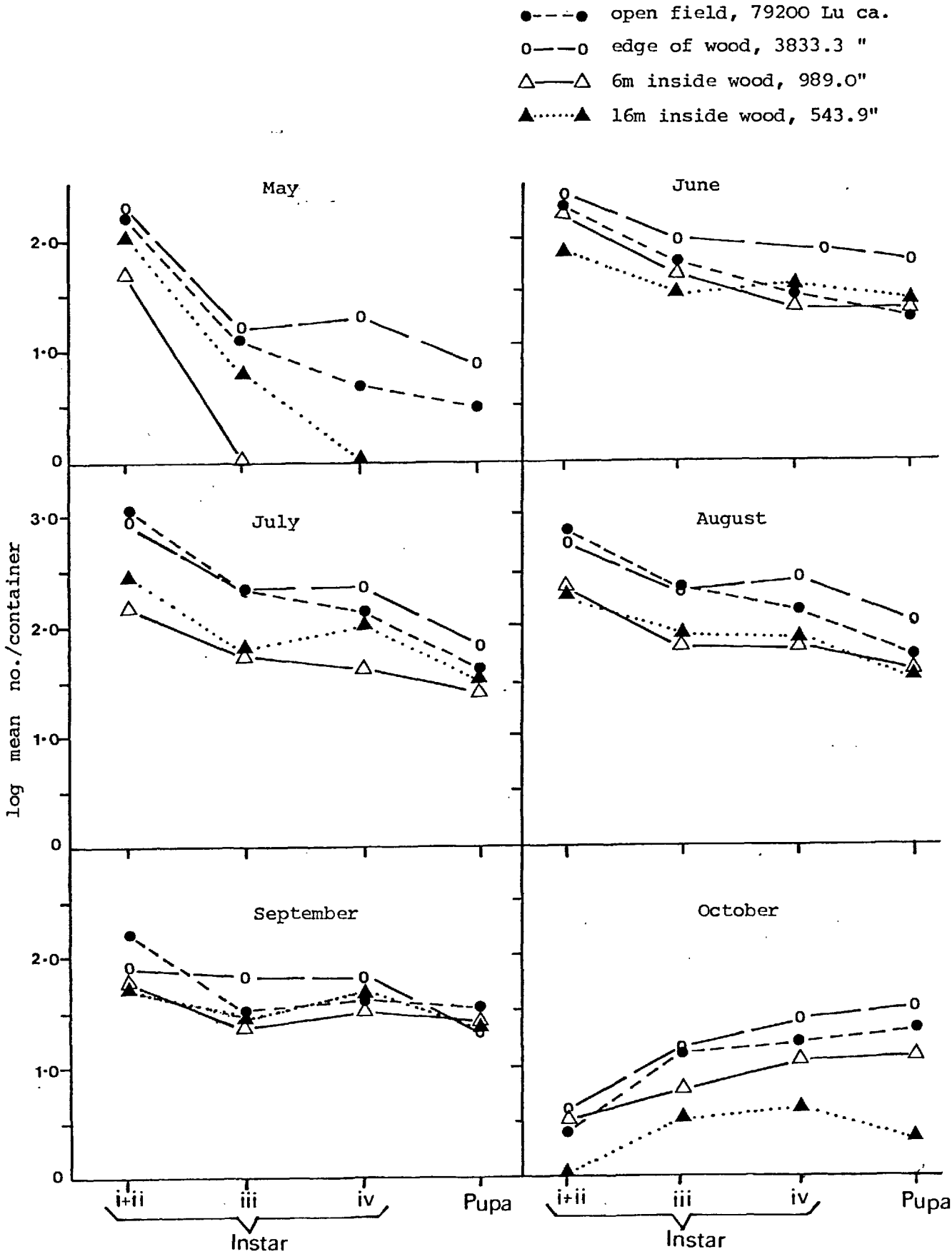


Fig 5.9 Seasonal incidence of pre-adults of Cx. pipiens/torrentium along a transect in North Gravel (Site E) from open field into the woodland, with light intensity readings. Each point represents the mean of three measurements. (Results for 1978).

5.2.2. Permanent ground water habitat

5.2.2.1 The study sites

The two permanent ground water habitats studied were Pond Field pond (Site A, Fig 4.1) and Sandy Ride pond (Site B, Fig 4.2). A detailed description of the ponds has been given in Section 4.2.1.

5.2.2.2 Method of sampling

Mosquitoes in the ground water habitats were sampled with a standard dipper. Since the purpose of sampling was to determine the changes in population distribution and number of mosquitoes in these habitats, sampling with a dipper which gives only the relative estimate of population, was considered sufficient. This method will sample most species of mosquito though with varying efficiencies (Service 1976) but will not sample the larvae of Mansonia sp. which attach themselves to the roots of aquatic plants.

In a preliminary survey of mosquitoes in these ponds, Turner (1978) observed that the distribution was not uniform but was highly aggregated and associated with the vegetation of the pond. It was necessary to bear this in mind when deciding on the sampling technique.

A total of twenty sampling sites were marked in Site A with bamboo sticks and samples were taken only from these points throughout the sampling period. Ten of the sampling sites were located around the edge of the pond about 1 m apart. The other sampling sites were located further inside the pond. In all, twelve of the chosen sites were associated with vegetation while

eight sites were open water. As time progressed, however, more vegetation grew in and around the pond thereby increasing the areas associated with vegetation. Therefore, on one or two occasions, the positions of two sampling sites which became covered with vegetation were moved a few cm away from the original points in order to maintain the number of sampling sites located in the open water at 8.

In the smaller Sandy Ride pond (Site B) only ten sampling sites were chosen from the edge and middle of the pond.

Dips were taken with a white 0.5 litre plastic bowl attached to a 1.5 m wooden handle. The technique of sampling involved immersing the dipper in water at an angle of about 45° and quickly removing it before it overflew (WHO, 1975). The contents were tipped into a white bowl.

In 1978 samples were taken from the two sites once a week but in 1979 this was increased to three times a week. Mosquitoes were identified in the field and returned to the sampling sites from where they had been taken. Whenever there were doubts about the identity of mosquitoes particularly with the younger instars they were taken back to the laboratory and identified with the aid of a microscope. Samples of pupae were occasionally removed and reared to adult in the laboratory in order to confirm the identity of the species. When no more Culex sp. were present in the sites sampling was continued once a week throughout the year in order to obtain information on the other mosquito species present in these habitats.

Observation was made on the possible presence of Mansonia sp. in these sites. On a number of occasions roots of plants taken from the sites were erersed in 5% sodium hydroxide (McDonald 1970). This would cause any larvae attached to the roots to detach.

5.2.2.3 Results

Four species of mosquitoes were recorded in the two ground water habitats. Mansonia sp. was absent.

a) Cx. pipiens/torrentium

Details of the population sample of Cx. pipiens/torrentium in the two sites are presented in Appendices 5.10 - 5.13 and represented graphically in Fig 5.10 - 5.13.

In 1978, first and second larval instars appeared in Site A in the fourth week of May (Fig 5.10a). The numbers of these stages remained low until the fourth week of June when there was a dramatic rise which reached a peak population in the second week of July. There was continuous breeding of mosquitoes at a high level until the third week of August when the numbers fell steeply reaching a very low level by the second week of September. By the first week of October no further first and second instars were collected. The numbers of third and fourth instars remained low from the beginning of the breeding season until the third week of July when it rose and fluctuated at fairly high level, dropping sharply in the first week of September. The pupal number was low throughout the season but showed a slight increase between middle August and early September.

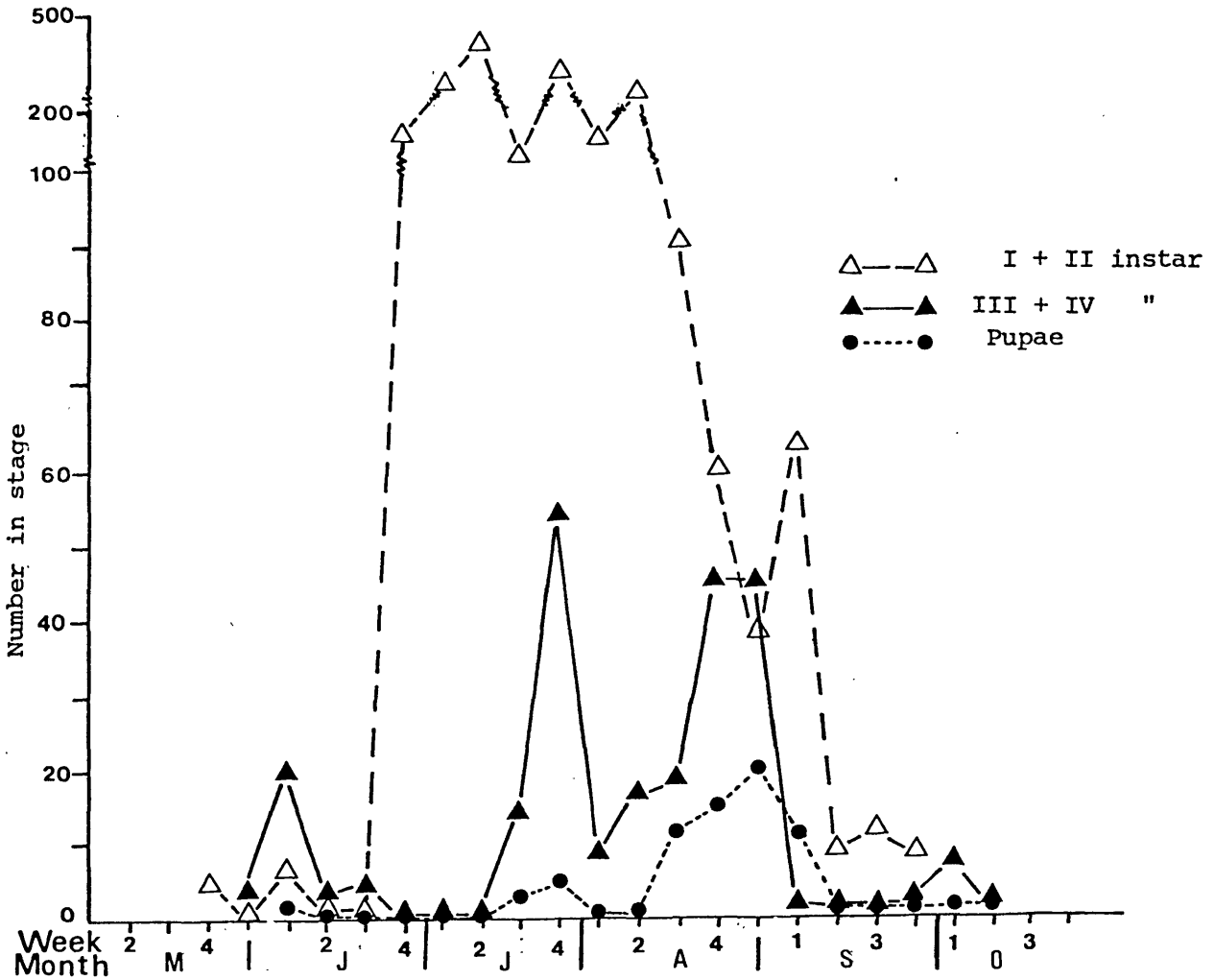


Fig 5.10a Changes in population number of pre-adults of Cx. pipiens/torrentium collected from Site A, Pond Field pond in 1978.

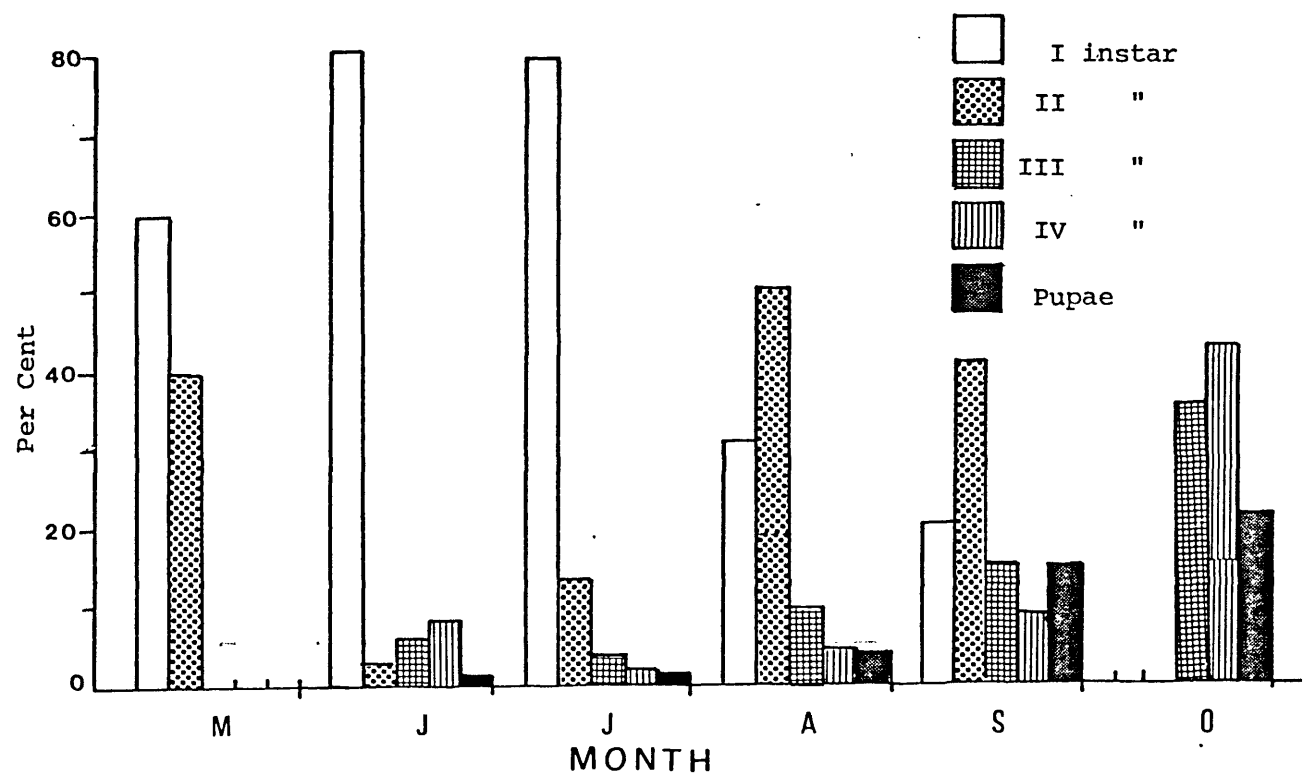


Fig 5.10b Monthly proportions of pre-adults of Cx. pipiens/torrentium collected from Site A, Pond Field pond in 1978.

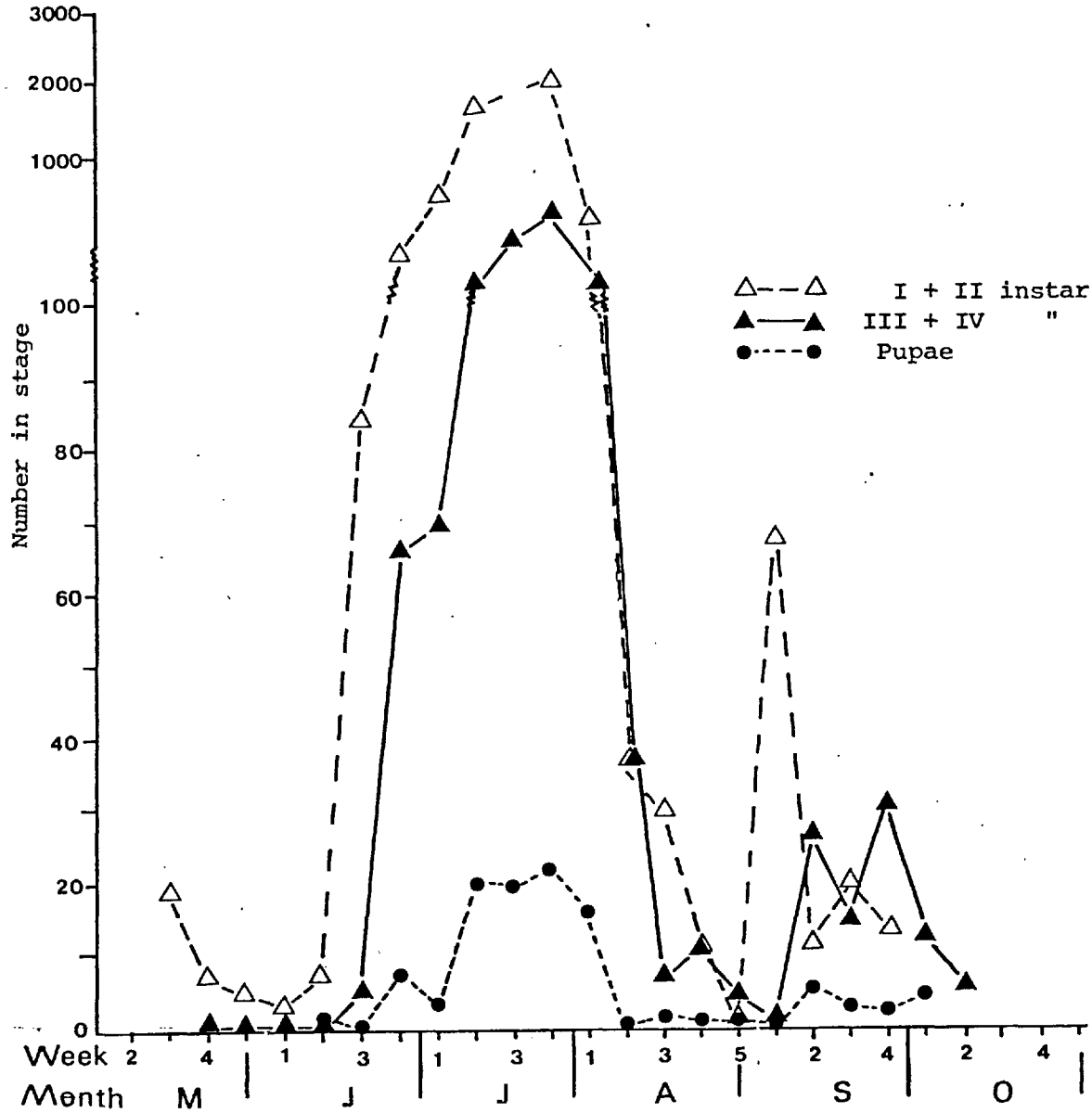


Fig 5.11a Changes in population number of pre-adults of Cx. pipiens/torrentium collected from Site A, Pond Field pond in 1979.

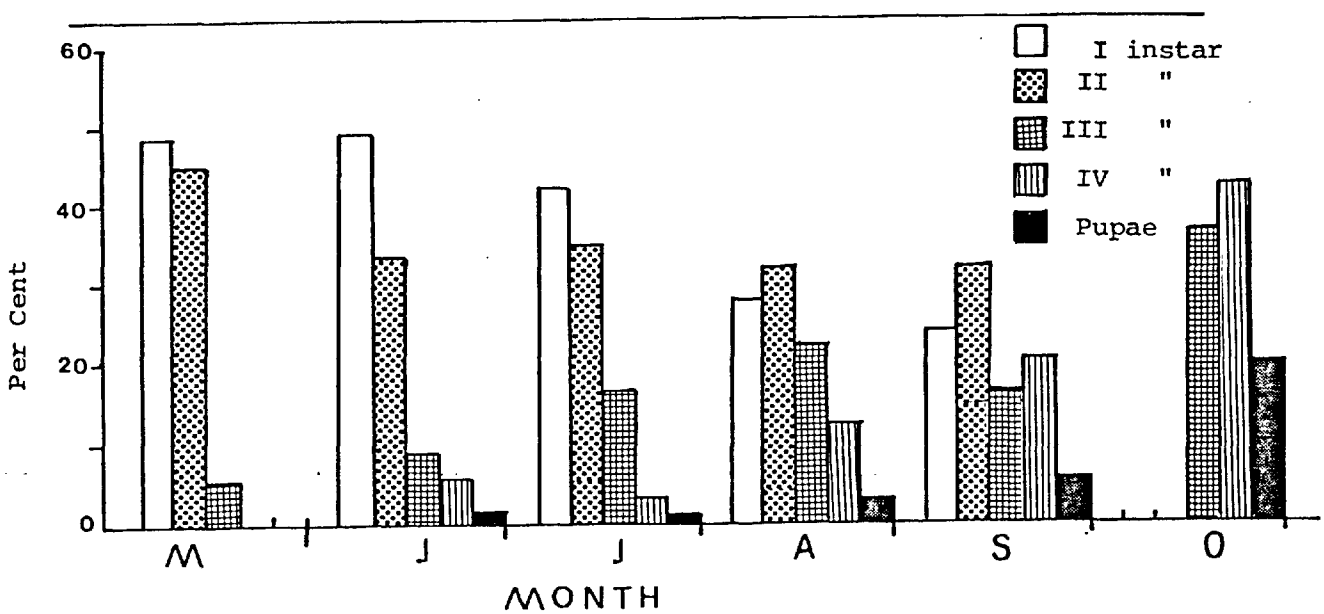


Fig 5.11b Monthly proportions of pre-adults of Cx. pipiens/torrentium collected from Site A, Pond Field pond in 1979.

In 1979 there was a similar rise in the number of the first and second instars (Fig 5.11a) which occurred in the third week of June, one week earlier than the previous year. Peak population occurred in the fourth week of July and then the population dropped sharply from the first week of August. There was a corresponding rise and fall in the numbers of the third and fourth larval instars. Pupal number remained relatively low throughout the breeding season with a rise in July. Fig 5.10b and 5.11b show that between May and August the bulk of the population of mosquitoes was composed of younger instars but in September and October there was an increase in the proportion of the older instars and pupae. A much greater proportion of first instars occurred from May to July in 1978 than in the following year.

In Sandy Ride pond (Site B) there was a dramatic rise in the numbers of first and second instars in the second week of July 1978 (Fig 5.12a) followed by a corresponding rise in the numbers of third and fourth instars. The population of larval instars dropped sharply in the first week of August and reached a very low level by the second week of September. Pupal numbers remained very low throughout the breeding season with slight rises in the third week of July and the second week of September. No first, second and third larval instars were collected in June and only older larvae and pupae were present in October (Fig 5.12b). In 1979 (Fig 5.13a) an increase in the numbers of first and second larval instars started in the last week of June and reached a peak in the fourth week of July. The population declined the following week and reached a very low level by the third week of August, earlier than in the previous year. The numbers of third and fourth instars rose in the third week of July and dropped again in the third week of August. Slight rises in pupal numbers was observed between

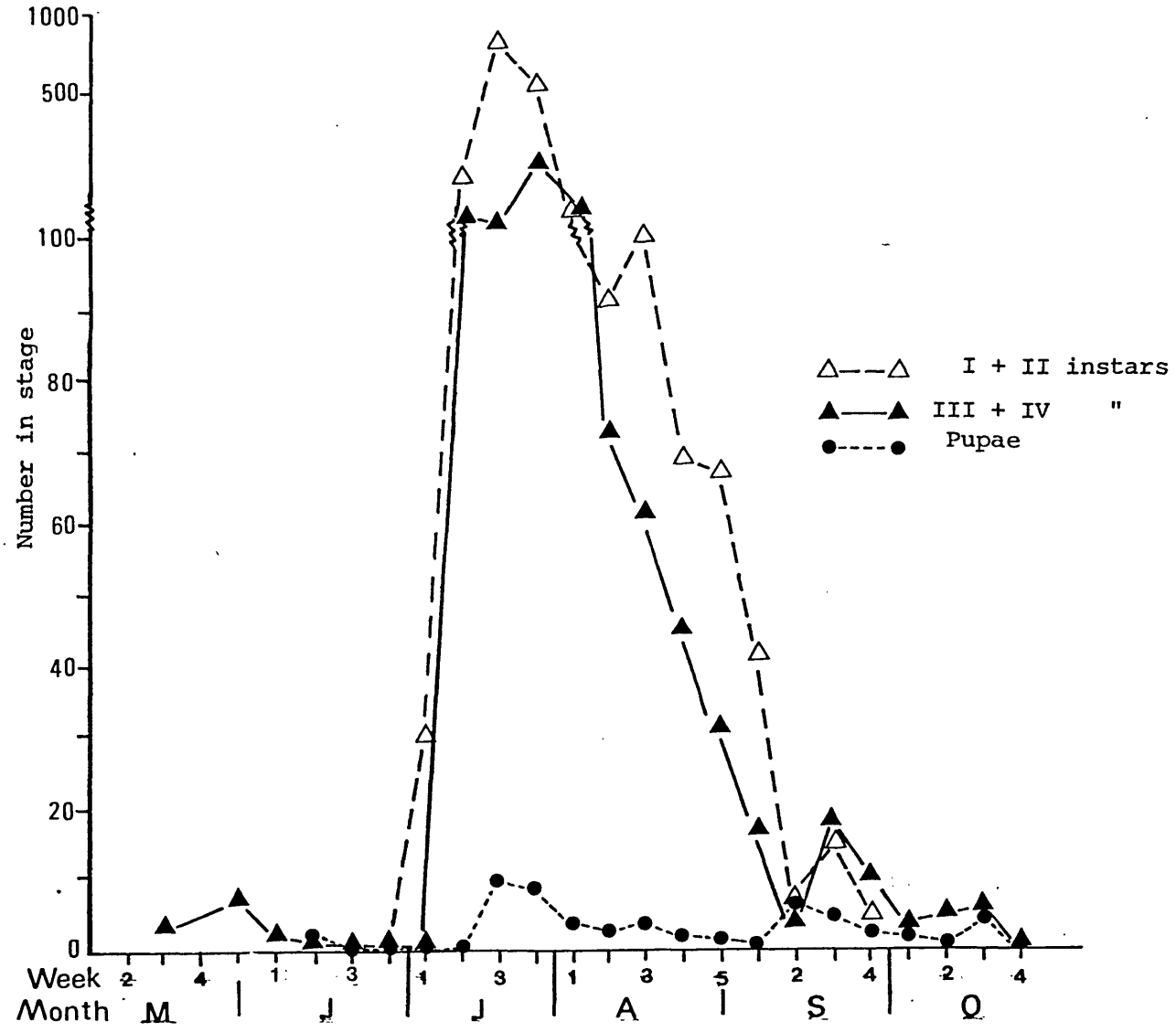


Fig 5.12a Changes in population number of pre-adults of Cx. pipiens/torrentium collected from Site B, Sandy Ride pond in 1978.

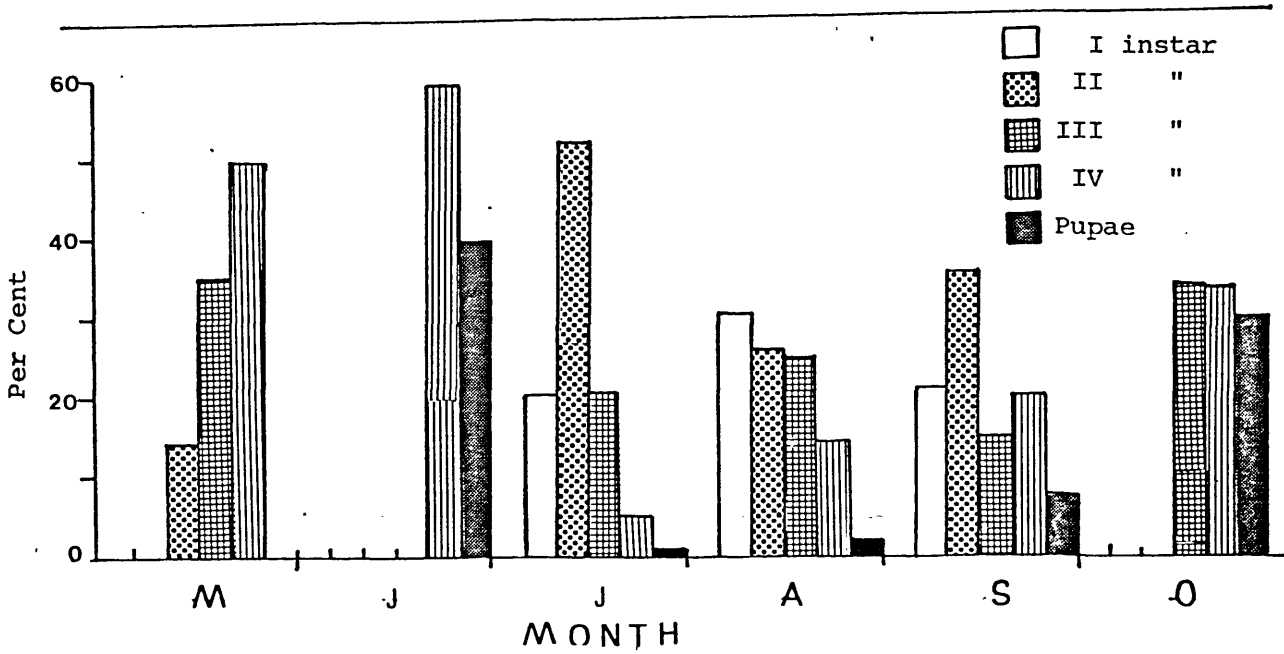


Fig 5.12b Monthly proportions of pre-adults of Cx. pipiens/torrentium collected from Site B, Sandy Ride pond in 1978.

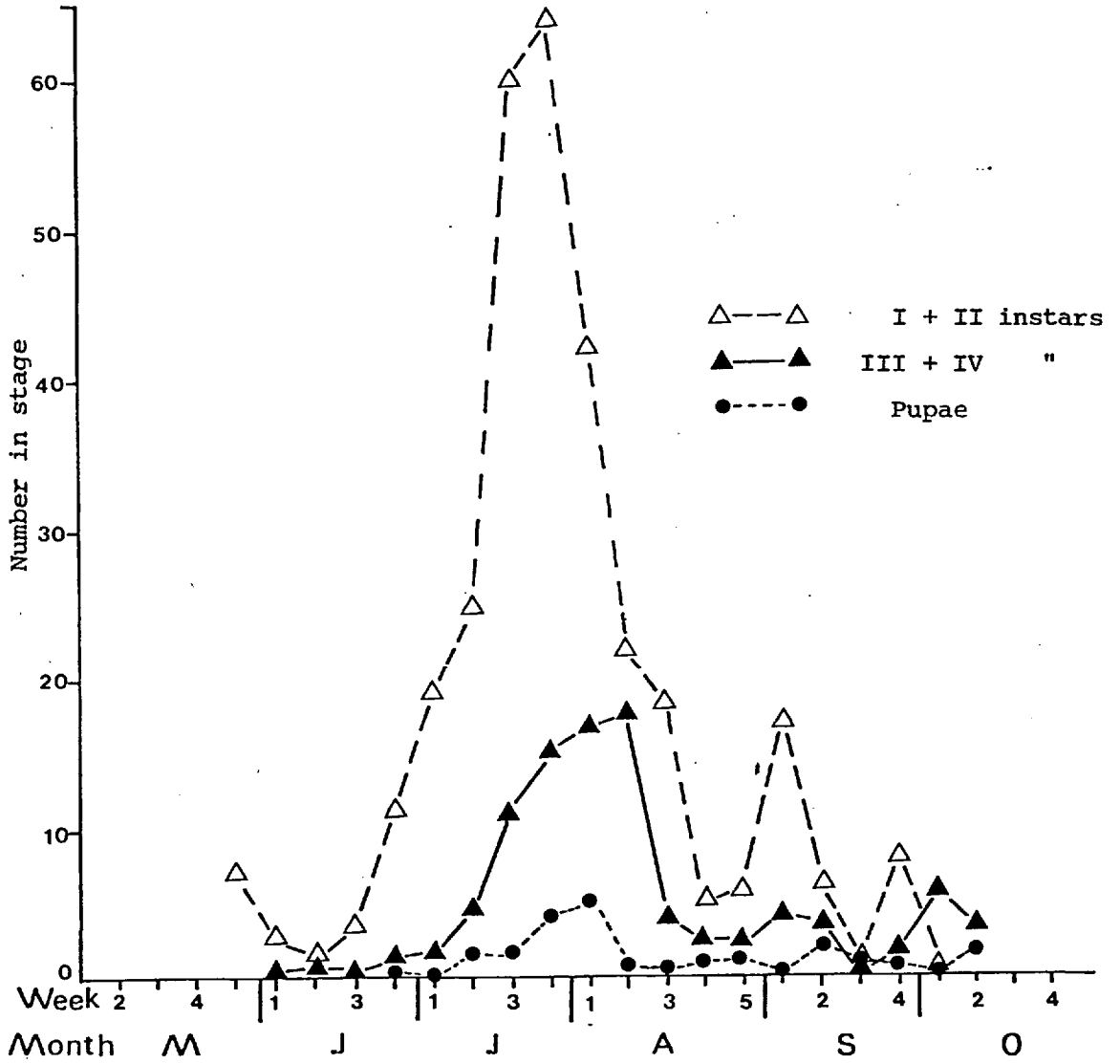


Fig. 5.13a Changes in population number of pre-adults of Cx. pipiens/torrentium collected from Site B, Sandy Ride pond in 1979.

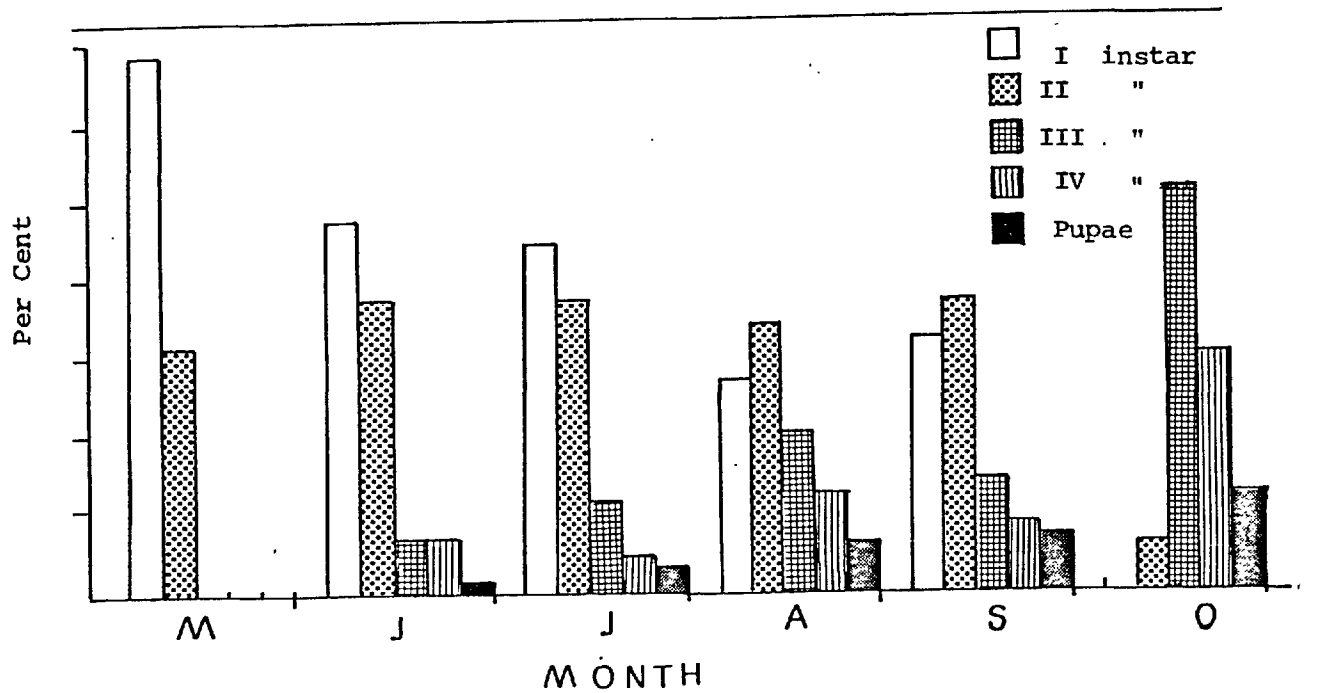


Fig 5.13b Monthly proportions of pre-adults of Cx. pipiens/torrentium collected from Site B, Sandy Ride pond in 1979.

the end of July and early August. From May to July the bulk of mosquito population was made up of younger larvae (Fig 5.13b). Towards the end of the breeding season the proportion of older larvae and pupae had greatly increased.

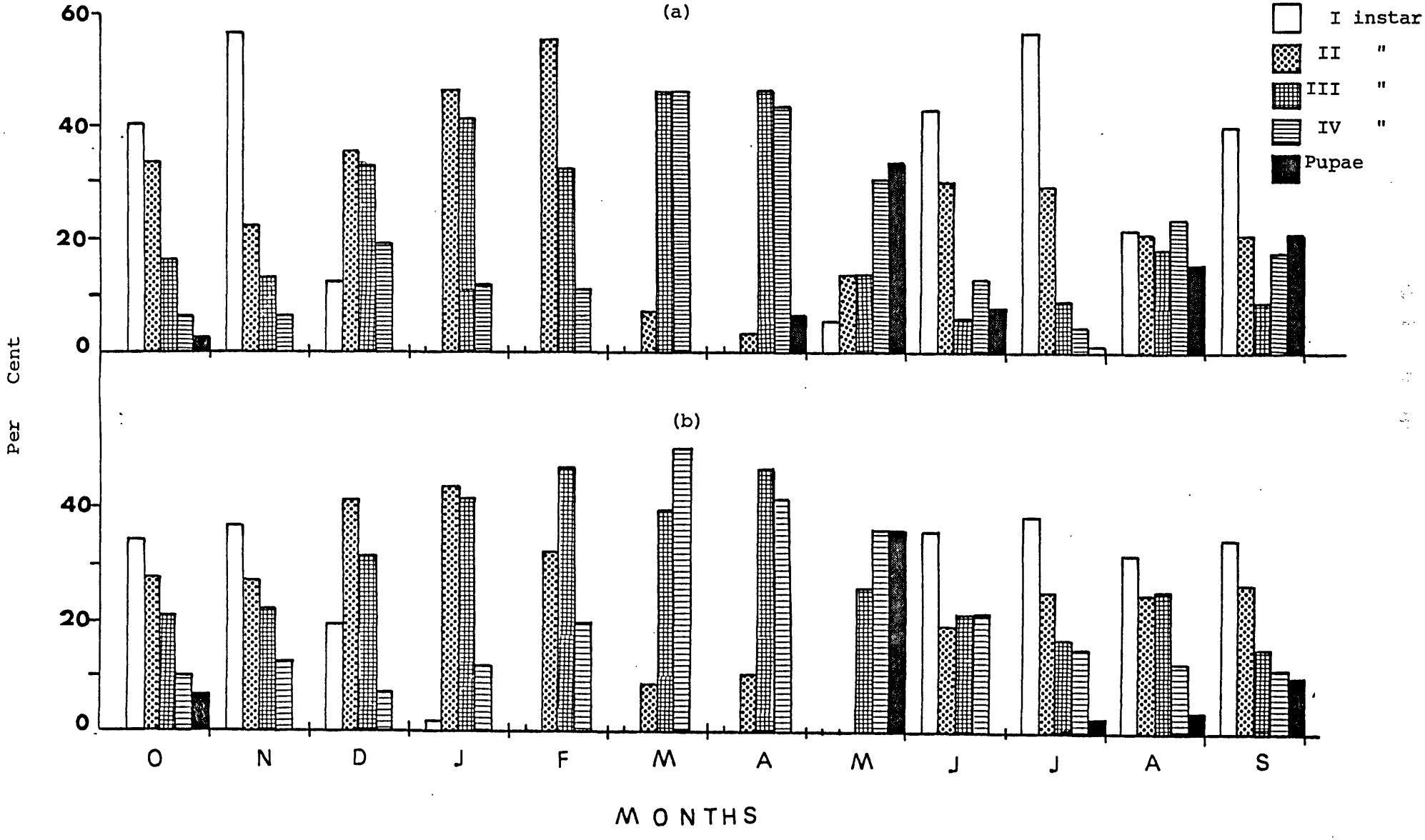
b) An. claviger

The monthly collections of pre-adults of this species made in 1978 - 1980 in each of the two larval habitats were combined and the numbers of different stages expressed as percentages of total monthly catches.

In Pond Field pond (Site A) a very high proportion of first instar was observed in November (Fig 5.14a). From late December to April inclusive, no first instar was sampled. During this time larvae gradually developed to later instars resulting in the appearance of pupae in late April. The highest proportion of pupae was observed in May and this gave rise to adults which were responsible for the reappearance of a new generation of first instar larvae at the end of the month. A high proportion of first instar larvae occurred in July and this in turn gave rise to a new generation of pupae which appeared from August to early October. These pupae produced adults which again were responsible for the first instars that appeared from September to early December.

A similar pattern of population changes was observed in Sandy Ride pond (Site B) with minor differences (Fig 5.14b). The first generation of pupae was observed in May only. These produced adults

Fig 5.14 Monthly proportions of pre-adult stages of *An. claviger* collected from (a) Pond Field pond (Site A) and (b) Sandy Ride pond (Site B); June 1978 - March 1980. Data for both years for each month were combined.


(a)
(b)
I instar
II "
III "
IV "
Pupae
60
40
20
0
Per Cent
O
N
D
J
F
M
A
M
J
J
A
S
MONTHS

responsible for first instar larvae that appeared in June and July. The next generation of pupae appeared first in late July, slowly building up until a higher proportion occurred in September. These pupae gave rise to adults which in turn produced the next generation of first instars present in the habitat until early January.

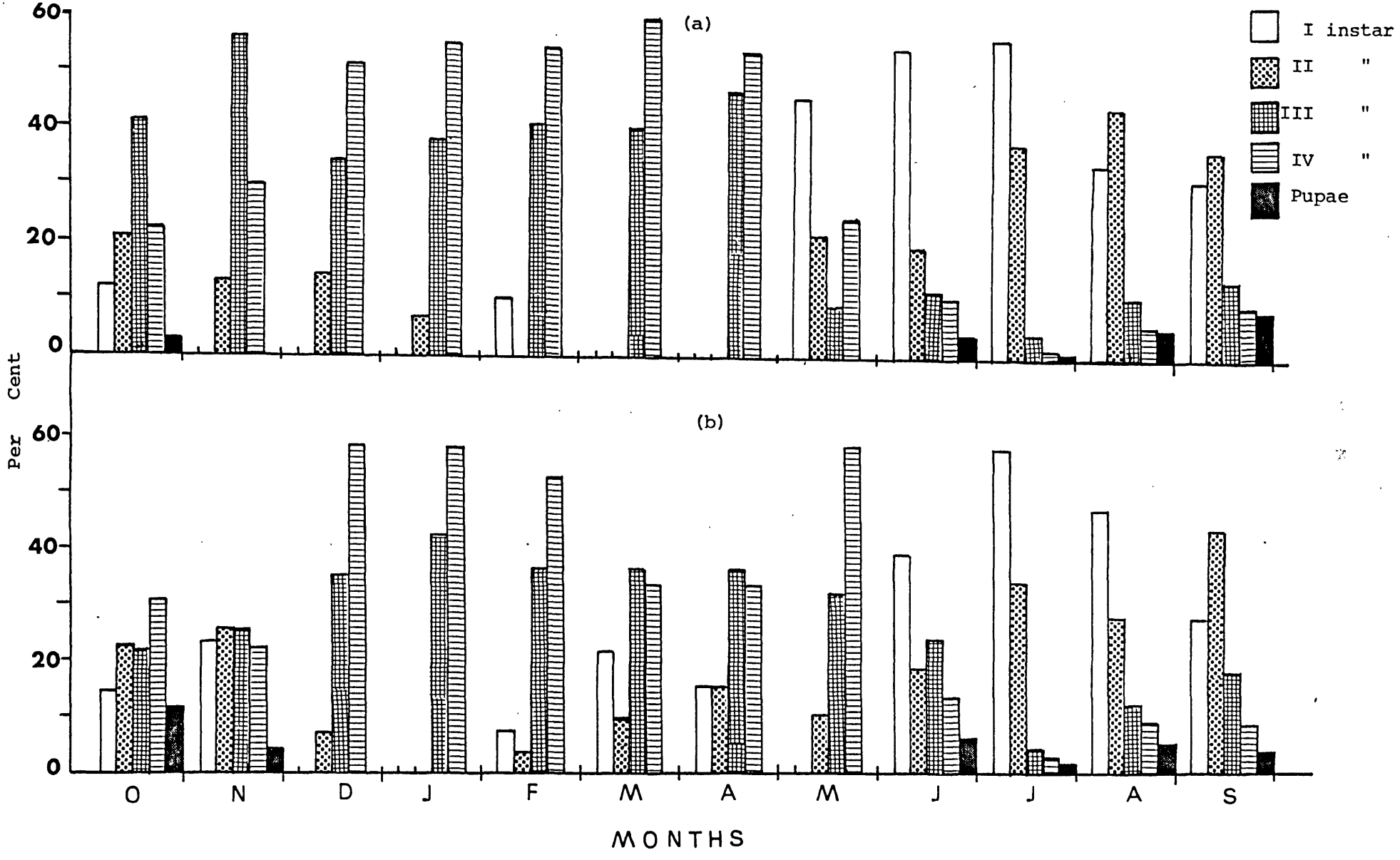
c) Cs. annulata

The monthly collections of pre-adults of Cs. annulata made during the study period in each of the two ground water habitats were combined and the numbers of different stages expressed as percentages of total monthly catches.

In Pond Field pond (Site A) first instar larvae were recorded in May and their proportion built up reaching the highest level in July (Fig 5.15a). Pupae were present in low proportions from June to October. From November to April the population existed mainly as third and fourth instar larvae. First instar larvae were recorded in February when no pupae was present in the habitat.

In Sandy Ride pond (Site B) first instar larvae were recorded in June with the highest proportion occurring in July (Fig 5.15b). Pupae were present from June to November. Between December and May no pupae were collected and the population existed mainly as third and fourth instar larvae. First instar larvae were, however, collected between February and April.

Fig 5.15 Monthly proportions of pre-adult stages of Cs. annulata collected from (a) Pond Field pond (Site A) and (b) Sandy Ride pond (Site B); June 1978 - March 1980. Data for both years for each month were combined.


(a)
(b)
I instar
II "
III "
IV "
Pupae
Per Cent
60
40
20
0
O
N
D
J
F
M
A
M
J
J
A
S
MONTHS

5.3 Duration of different instars of Cx. torrentium at different months

It has been shown by laboratory observation (Section 3.2.4) that there is no difference in the duration of the different instars of Cx. pipiens and Cx. torrentium. Field observations on the duration of the instars of Cx. torrentium at different months during the breeding season were made with a view to using the information for the construction of life tables for Cx. pipiens/torrentium breeding in field sites.

At the beginning of each month from May to September, some egg-rafts were obtained immediately they were laid from a colony of Cx. torrentium maintained in the insectary at $21 \pm 1^{o}C$ and 50% r.h. They were isolated in plastic cups containing a small quantity of water and taken to a field site where, apart from protection from direct sunlight, they were exposed to natural climatological factors. The duration of the egg stage was recorded. As soon as the eggs hatched, 150 first instar larvae were removed from the plastic cups and placed in rearing bowls which were also protected from direct sunlight. The duration of the various instars were determined as already described in Section 3.2.4. The experiment was replicated three times for each month.

Time was not available to obtain information on the duration of the instars of Cx. pipiens in the field except for the pupal stage. Pupae appearing from collections of larvae kept in the laboratory were isolated in glass vials and transferred to the field site, and the duration of the stage recorded at different months. This was

compared with the pupal duration for Cx. torrentium.

The results of these experiments for Cx. torrentium are presented in Table 5.2 Details of the observation are given in Appendix 5.14. The duration of the different instars were generally shortest in July and August (Table 5.2). This was when the mean monthly maximum and minimum temperatures were highest (Table 5.1). The duration of instars increased with decrease in temperature. The percentage larval and pupal mortality observed at the different months is presented in Table 5.3. The overall mortality of pre-adults was heaviest in May and September which were the coldest months during the study period.

There was no appreciable difference between the duration of pupal stage of Cx. pipiens (Table 5.4) and that of Cx. torrentium. In view of this it was assumed for subsequent analysis that the durations of larval instars at different months are approximately the same in the two species.

Table 5.2 Field observation on the mean developmental time in days (±SD) of egg and different instars of Cx. torrentium during different months, 1979

Month	Egg	Larval instars				Pupa
		I	II	III	IV	
May	4.7±0.6	4.7±1.9	5.0±1.9	6.7±2.6	6.5±2.3	5.0±2.5
June	3.0±0.0	3.3±1.5	3.2±1.8	3.8±1.7	5.3±1.6	4.3±1.7
July	2.8±0.4	3.2±1.3	2.8±1.6	3.1±1.5	4.8±1.9	3.6±1.7
Aug.	2.7±0.6	3.3±1.3	2.3±1.6	3.4±1.7	4.6±1.9	4.3±2.2
Sept.	3.0±0.0	4.4±1.5	3.6±1.8	6.0±1.9	5.2±2.3	4.7±1.9

Table 5.3 Larval and pupal mortality of Cx. torrentium reared in the field at different months in 1979. Values are obtained from the sum of three replicates of 150 larvae each.

Month	No. of first-instar larvae	% larval mortality	% pupal mortality	% total mortality
May	450	32.0	14.1	41.7
June	450	24.9	1.5	26.0
July	450	21.1	2.8	23.3
Aug.	450	25.7	3.6	28.2
Sept.	450	32.4	4.9	35.8

Table 5.4 Mean developmental time ($\pm$SD) in days of pupal stage of Cx. pipiens during different months, 1979.

Month	No. of specimens involved	Pupal Duration
June	10	4.5 ± 0.9
July	19	4.2 ± 0.6
Aug.	30	4.7 ± 0.8
Sept.	12	5.0 ± 0.9

5.4 Mortalities of pre-adults of Cx. pipiens/torrentium

Life table analysis has been used for the study of insects of agricultural and forestry importance (Southwood, 1966; Varley & Gradwell, 1970) and has only recently been applied to mosquito studies (Southwood et al, 1972; Service, 1973b, 1973c, 1977a; Lakhani & Service, 1974).

The mortalities of the immature stages of Cx. pipiens/torrentium in the artificial containers and permanent ground water habitats were estimated from the 1979 results of population sampling. (It was not possible to do the same for 1978 results since during this time samples were not taken often enough). Information on the duration of larval and pupal instars of Cx. torrentium (Table 5.2) was utilized in these estimates.

The method of mortality estimates by Service (1973c, 1977a) and Lakhani & Service (1974) was employed with some modifications. Analysis show mortality acting on different instars of mosquitoes over the entire breeding season.

5.4.1 Permanent ground water habitat

Lakhani & Service (1974) stated that "if the durations of larval instars are taken into consideration there is a relationship between the numbers collected in the different instars and their survivorship". Since the durations of the various instars of Cx. torrentium differed in the different months (Table 5.2) it was decided that

weighted average instar durations be used in the mortality estimates. These were calculated as the sum over the breeding season of

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

The use of weighted instar durations was considered appropriate since it takes into account the differences in the numbers of pre-adult stages of mosquitoes sampled each month.

The total numbers of the different pre-adult stages of Cx. pipiens/torrentium collected in each of the two ground water habitats (Pond Field pond, Site A and Sandy Ride pond, Site B) throughout the breeding season (May - October 1979) were divided by the appropriate weighted instar duration in days to give the age distribution of the pre-adult stages of mosquitoes (Table 5.5). These values were plotted against age in days of the immature stages and the resultant histograms give a graph of stage-specific age distribution. A smooth curve drawn through the graph gives an age-specific age distribution curve (Fig 5.16). This was taken to give the same shape as the survivorship curve assuming that the population was steady during the sampling period. The numbers of the immature stages of mosquitoes surviving to each age in days were read off from the curve to give the numbers of larvae surviving to age X (nx column of Tables 5.6 and 5.7). Life tables for pre-adult mosquitoes were then constructed starting with a convenient number of 1000 individuals (lx column, Tables 5.6 and 5.7). An overall mortality of 99.3 and 92.6% was observed among the pre-adults of mosquitoes in Sites A and B, respectively. From estimates of the probability of a larva of age X dying before reaching age X + 1 (qx column,

Table 5.5 Weighted average instar duration, instar numbers and the derived age distribution of Cx. pipiens/torrentium collected from ground water habitats in 1979

a) Pond Field pond, Site A

Instar	I	II	III	IV	Pupa
Weighted instar duration (days)	3.2	2.8	3.2	4.9	3.9
Total no. of instar collected	12585	9457	4962	1227	306
No. collected/weighted instar duration	3932.8	3377.5	1550.6	250.4	78.5

b) Sandy Ride pond, Site B

Instar	I	II	III	IV	Pupa
Weighted instar duration (days)	3.4	2.8	3.8	4.8	4.1
Total no. of instar collected	482	352	192	102	58
No. collected/weighted instar duration	141.8	125.8	50.5	21.3	14.1

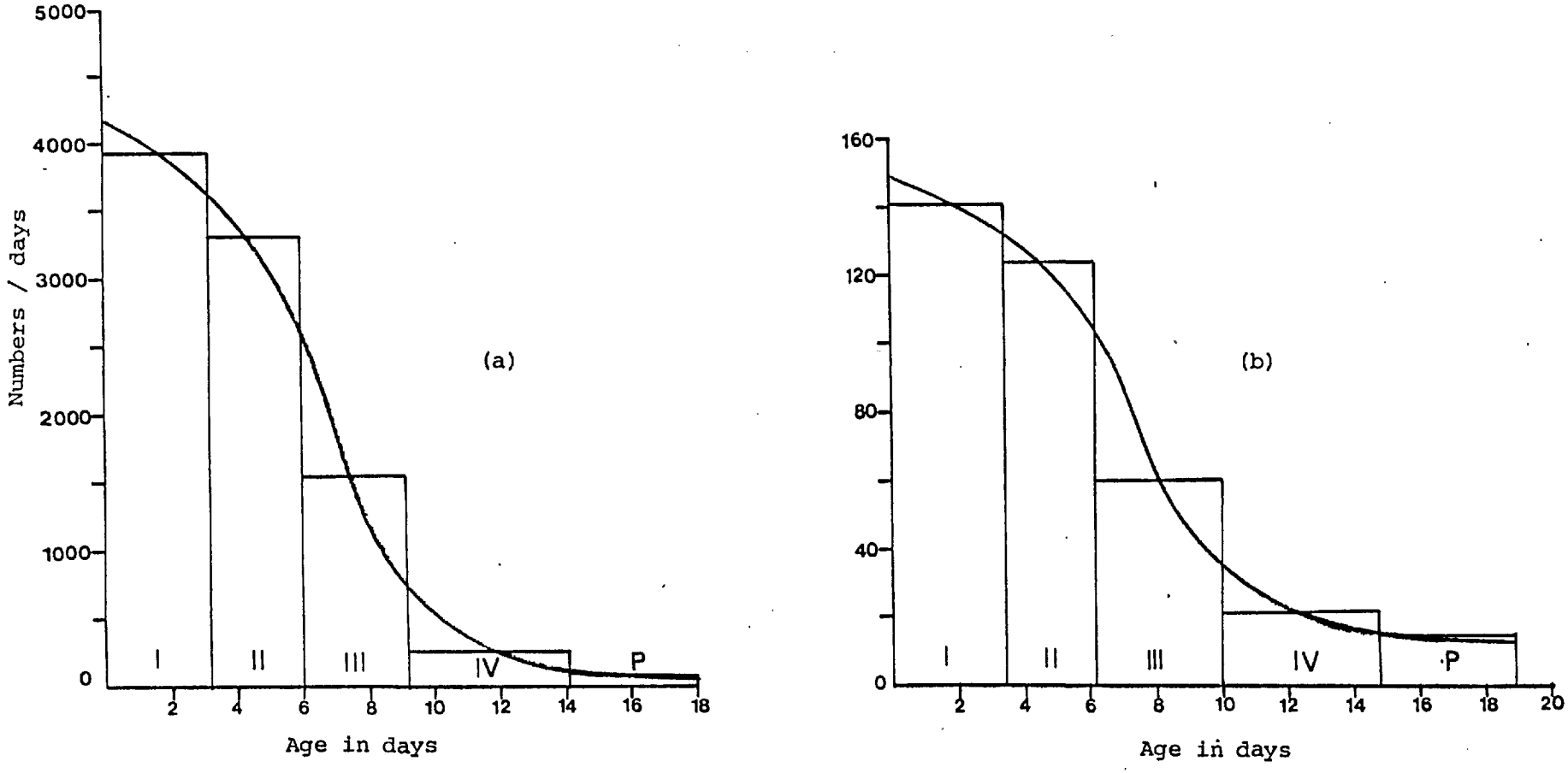


Fig 5.16 Age distribution and estimated survivorship curves for the immature stages of Cx. pipiens/torrentium collected from (a) Pond Field pond and (b) Sandy Ride pond; May - October 1979. Curves are fitted by eye.

Table 5.6 Life table for Cx. pipiens/torrentium collected from Pond Field pond, May - October 1979

x	nx	lx	dx	px	qx	lx
0	4150	1000	24	0.98	0.02	7.03
1	4050	976	36	0.97	0.04	6.19
2	3900	940	48	0.95	0.05	5.41
3	3700	892	60	0.93	0.07	4.68
4	3450	832	72	0.91	0.09	3.98
5	3150	760	97	0.87	0.13	3.31
6	2750	663	157	0.76	0.24	2.72
7	2100	506	169	0.67	0.33	2.40
8	1400	337	120	0.64	0.36	2.35
9	900	217	72	0.67	0.33	2.38
10	600	145	54	0.63	0.37	2.31
11	375	91	30	0.67	0.33	2.39
12	250	61	24	0.61	0.39	2.31
13	150	37	12	0.68	0.32	2.49
14	100	25	6	0.76	0.24	2.44
15	75	19	6	0.68	0.32	2.05
16	50	13	4	0.69	0.31	1.77
17	30	9	2	0.78	0.22	1.33
18	25	7				0.57

Key: x = age in days, nx = no. of larvae surviving to age x, lx = no. of 1000 larvae surviving to age x, dx = mortality between ages x and x+1, px = probability that a larva of age x survives to age x+1, qx = probability of a larva of age x dying before reaching age x+1, ex = expectation of life for individuals of age x.

Table 5.7 Life table for Cx. pipiens/torrentium collected from Sandy Ride pond, May - October 1979

x	nx	lx	dx	px	qx	ex
0	148	1000	20	0.98	0.02	8.27
1	145	980	34	0.96	0.04	7.43
2	140	946	34	0.96	0.04	6.68
3	135	912	47	0.95	0.05	5.91
4	128	865	54	0.94	0.06	5.21
5	120	811	81	0.90	0.10	4.52
6	108	730	122	0.83	0.17	3.96
7	90	608	176	0.71	0.29	3.66
8	64	432	115	0.73	0.27	3.95
9	47	317	74	0.77	0.23	4.20
10	36	243	47	0.81	0.19	4.32
11	29	196	41	0.79	0.21	4.24
12	23	155	27	0.83	0.17	4.22
13	19	128	20	0.84	0.16	4.00
14	16	108	13	0.88	0.12	3.65
15	14	95	7	0.93	0.07	3.07
16	13	88	7	0.92	0.08	2.27
17	12	81	7	0.91	0.09	1.42
18	11	84				0.50

Key: as in Table 5.6

Table 5.8 Instar mortalities of Cx. pipiens/torrentium collected from ground water habitats, May - October 1979

Instars (i)	Age in days at beginning of instar (t_{i-1})	No. entering instar (St_{i-1})	Deaths in instar (D_i)	Relative proportion dying in instar ($\frac{D_i}{St_{i-1}}$)	Proportion dying daily in instar $1-\left(\frac{St_i}{St_{i-1}}\right)^{1/d*}$	k values $\log_{10}\left(\frac{St_i}{St_{i-1}}\right)$
		a) Pond field pond				
I	0	4150	500	0.12	0.04	0.06
II	3.2	3650	900	0.25	0.10	0.12
III	6.0	2750	1950	0.71	0.32	0.54
IV	9.2	800	700	0.88	0.35	0.90
Pupa	14.1	100	75	0.75	0.30	0.60
Adult	18.0	25				K = 2.22
		b) Sandy Ride pond				
I	0	148	16	0.11	0.03	0.05
II	3.4	132	28	0.21	0.08	0.10
III	6.2	104	68	0.65	0.24	0.46
IV	10.0	36	21	0.58	0.17	0.38
Pupa	14.8	15	3	0.20	0.05	0.10
Adult	18.9	12				K = 1.9

d*, weighted instar duration

Tables 5.6 and 5.7) it was observed that there was a higher mortality of older than younger instars in the two habitats.

The numbers of pre-adults of Cx. pipiens/torrentium entering each instar and the instar mortalities were estimated from the survivorship curves (Fig 5.16) on the assumption that mortality rate was constant in any given instar. The results are presented in Table 5.8. From estimates of proportions dying daily in instar and the k-values (differences between successive values of the logarithms of numbers per stage (Varley and Gradwell, 1960)) (Table 5.8) it can be seen that mortality was most intense in the fourth larval instar and pupae in Pond Field pond (Site A) and in the third and fourth larval instars in Sandy Ride pond (Site B).

5.4.2 Artificial container habitat

In these artificial containers placed in Silwood Bottom (Site C) and Cannon Wood (Site D), it was possible to estimate fairly accurately the absolute numbers of eggs laid throughout the sampling period (Section 5.2.1.2b; Appendices 5.4 and 5.5). It was therefore possible to include this information in the mortality estimates.

Laboratory observations on the duration of the egg stage showed a significant difference in the mean values for Cx. pipiens and Cx. torrentium (Section 3.2.2). However, since about 77% of the population in artificial containers has been shown to be Cx. torrentium (Section 4.3.2) it was decided that the duration of the egg stage of Cx. torrentium at different months (Table 5.2) be used in the mortality estimates.

Due to the fact that the containers are of fairly large size estimates of mortalities were made for individual containers. Since the absolute numbers of eggs are known, the first step in these mortality estimates was to determine the approximate values of the total population of the various larval instars and pupae in the containers. This was done by the graphical method (Southwood, 1966) whereby successive estimates of the number of each instar collected at sampling intervals were plotted on a graph paper allowing one square per individual and per day. The points were joined up and the number of squares under the line were counted. This would give an estimate of the number of each instar that would have been sampled if sampling was done daily. The average weighted development times of the different instars were calculated as above using these estimates. The estimated values were then divided by the appropriate weighted development time to give values that approximate to the total population at the median age of each stage (Tables 5.9 and 5.10), assuming that mortality rate was constant within a given instar. The average weighted duration of the egg stage was also calculated using the known absolute numbers. Since eggs were placed in hatching cups during each sampling occasion (Section 5.2.1.2b) they were counted only once and therefore the numbers did not have to be divided by the average weighted duration as in the larval and pupal stages. The age-specific age distribution (hence the survivorship curve assuming that the population was steady during the sampling period) were plotted as in Section 5.4.1. for each container (Fig 5.17 and 5.18). It was considered more appropriate to draw the survivorship curves starting in each case from the point representing the number of eggs in each container since these were absolute estimates. From the survivorship curves the average daily mortalities of the different instars and the

Table 5.9 Weighted average instar duration, instar number and the derived age distribution of Cx. pipiens/torrentium collected from 5 artificial containers placed in Site C (Silwood Bottom), 1979

Instars		Egg	I	II	III	IV	Pupa
			Container No. 1				
Weighted duration of instar	(A)	3.1	3.4	2.8	3.9	4.9	4.0
Total no. of instar collected	(B)	12750	17930	12090	8210	4920	2400
No./instar duration	(C)		5273.5	4317.9	2105.1	1004.1	600
			Container No. 2				
	A	3.1	3.4	3.0	3.4	4.8	3.8
	B	16000	21610	13550	7645	4381	2050
	C		6355.9	4516.7	2248.5	912.5	539.5
			Container No. 3				
	A	3.0	3.4	2.8	3.3	4.7	4.2
	B	20000	37330	22440	7548	1670	730
	C		10979.4	8014.3	2287.3	355.3	173.8
			Container No. 4				
	A	3.0	3.5	3.0	3.9	4.9	4.0
	B	23000	46820	26630	18020	12710	5952
	C		13377.1	8876.7	4620.6	2593.9	1488
			Container No. 5				
	A	3.0	3.5	3.3	4.6	4.9	4.3
	B	15500	24890	16890	15010	6890	2290
	C		7111.4	5118.2	3263.0	1406.1	532.6

Table 5.10 Weighted average instar duration, instar number and the derived age distribution of Cx. pipiens/torrentium collected from 5 artificial containers placed in Site D (Cannon Wood)

Instars		Egg	I	II	III	IV	Pupa
			Container No. 6				
Weighted duration of instar	(A)	2.9	3.3	2.8	3.4	4.8	4.0
Total no. of instar collected	(B)	15750	22412	16200	10980	7460	3540
No./instar duration	(C)		6791.5	5785.7	3229.4	1554.2	885
			Container No. 7				
	A	3.0	3.3	2.9	3.8	4.9	4.0
	B	15500	26100	22390	14220	10960	5300
	C		7909.1	7320.7	3742.1	2236.7	1325
			Container No. 8				
	A	2.9	3.3	2.8	3.7	4.9	4.1
	B	21250	35189	19738	16090	9.40	4912
	C		10663.3	7049.3	4348.7	1865.3	1198.1
			Container No. 9				
	A	2.9	3.3	2.9	3.5	4.9	3.9
	B	23000	38880	25320	19680	13296	6160
	C		11781.8	8731.0	5622.9	2713.5	1579.5
			Container No. 10				
	A	3.0	3.4	3.0	3.5	4.9	3.8
	B	19000	31378	16527	10494	6674	2716
			9228.8	5509	2998.3	1362.0	714.7

Fig 5.17 Age distribution and estimated survivorship curves for the immature stages of Cx. pipiens/ torrentium collected from artificial containers 1 - 5 placed in Silwood Bottom (Site C); April - October 1979.

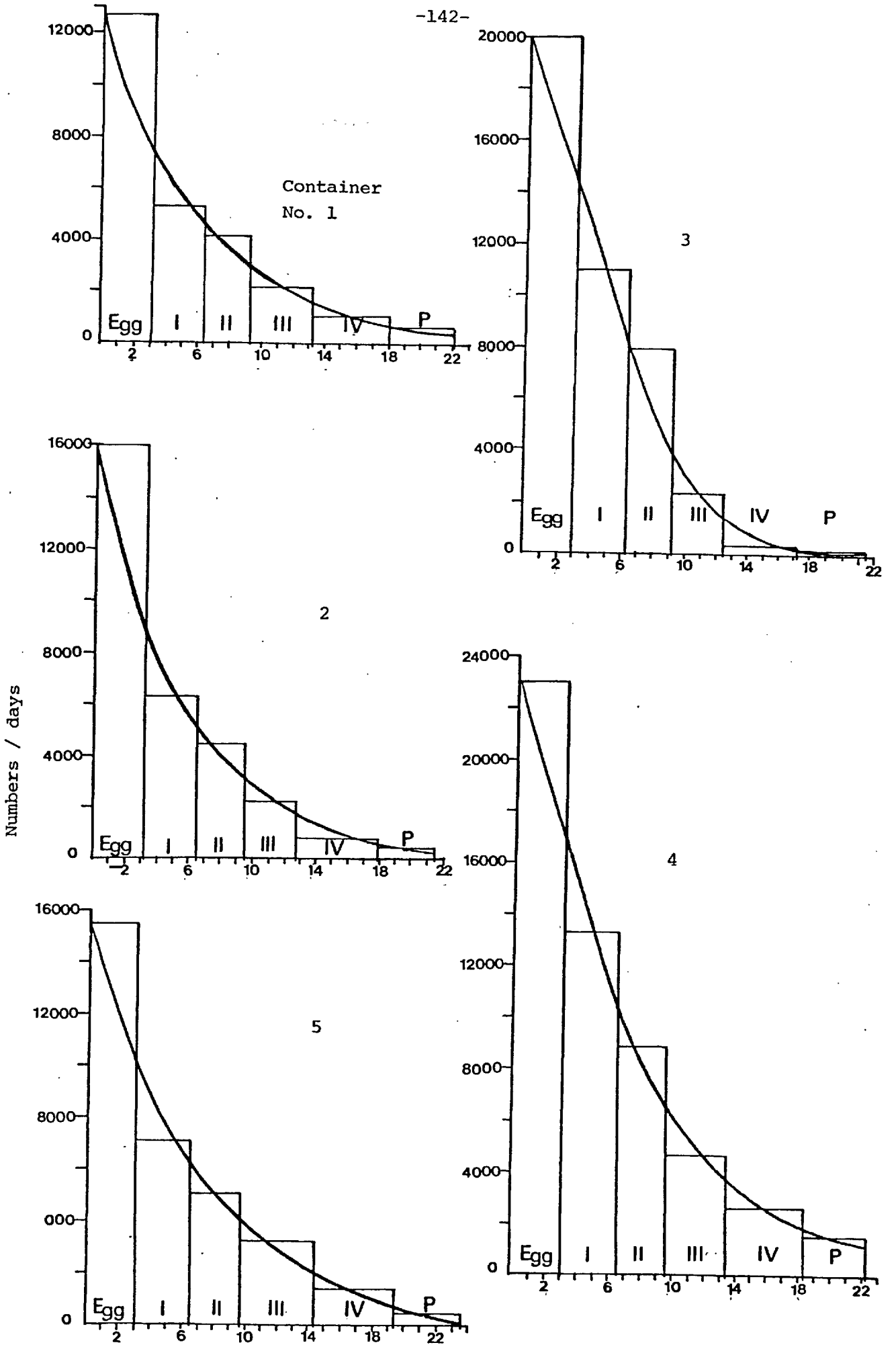
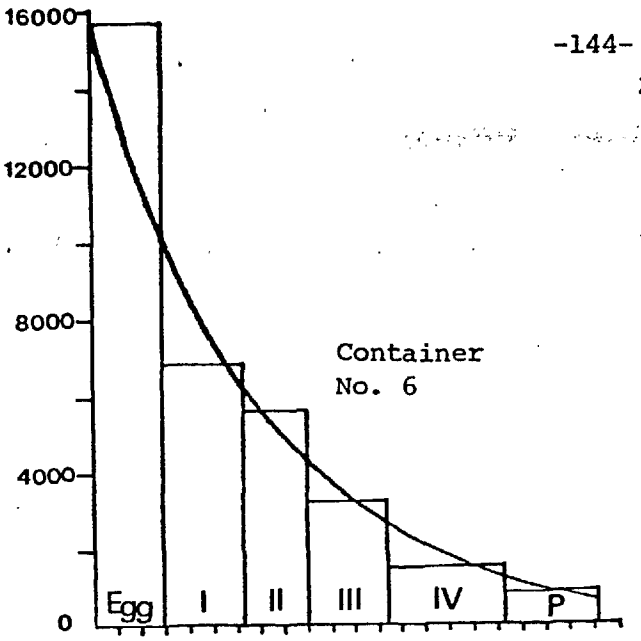
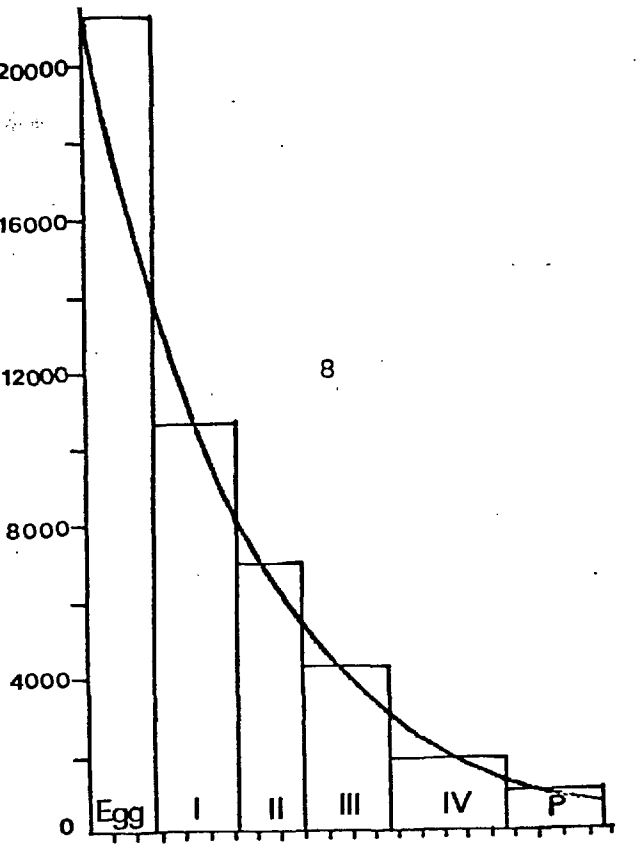

Container
No. 1
2
3
4
5
Egg
I
II
III
IV
P
Numbers / days
Age in days

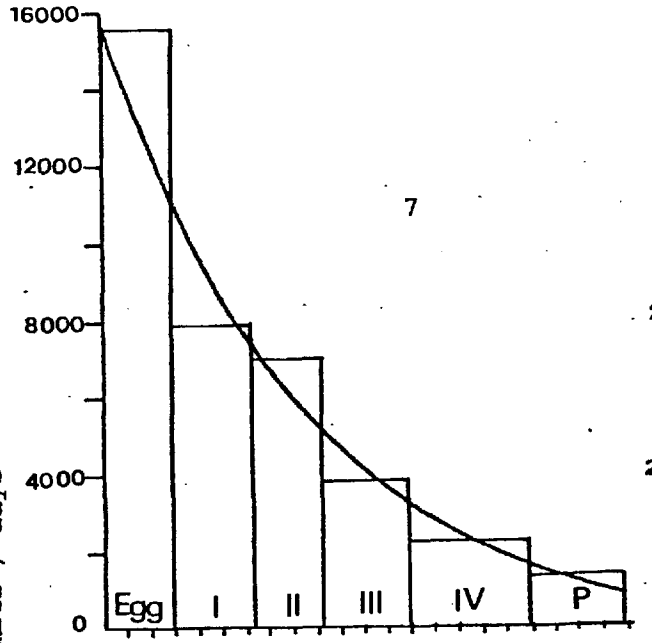
Fig 5.18 Age distribution and estimated survivorship curves for the immature stages of Cx. pipiens/torrentium collected from artificial containers 6 - 10 placed in Cannon Wood (Site D); May - Oct. 1979.



Container
No. 6

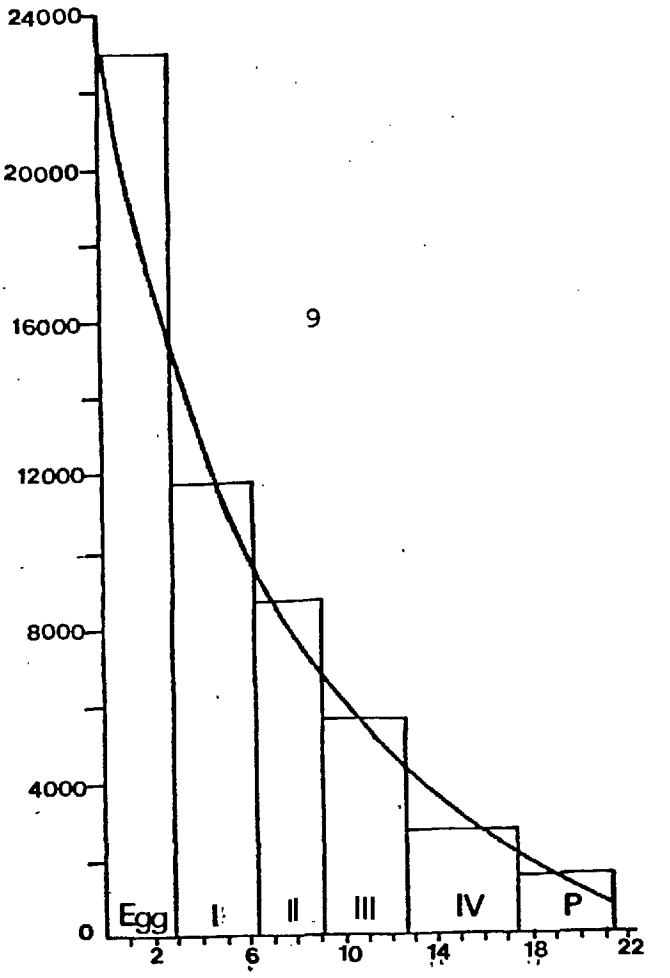


8

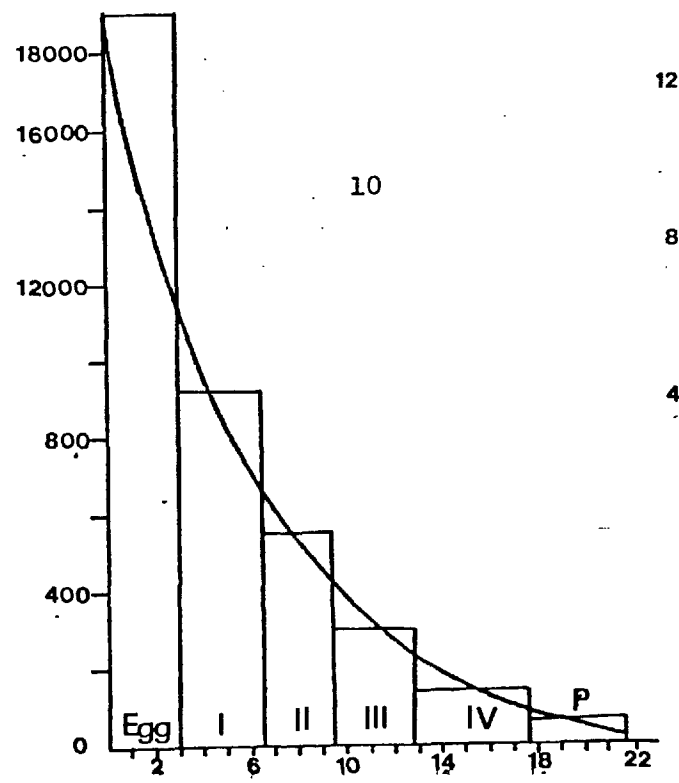


7

Number / days



9



10

Age in days

Table 5.11 Mortalities of pre-adults of Cx. pipiens/torrentium breeding in artificial containers placed at different sites in the field, 1979.

Stage	k - values						K
		Larval instars					
	Egg	I	II	III	IV	Pupa	
Site C, Silwood Bottom							
Container No. 1	0.23	0.22	0.19	0.27	0.36	0.24	1.51
2	0.26	0.23	0.21	0.25	0.48	0.30	1.73
3	0.14	0.25	0.34	0.47	0.81	0.40	2.41
4	0.13	0.21	0.20	0.25	0.33	0.18	1.30
5	0.18	0.21	0.19	0.29	0.42	0.60	1.89
Mean	0.19	0.22	0.23	0.31	0.48	0.34	1.78
SD	0.06	0.12	0.06	0.09	0.19	0.17	0.42
Site D, Cannon Wood							
Container No. 6	0.19	0.22	0.16	0.23	0.32	0.30	1.42
7	0.15	0.17	0.17	0.22	0.32	0.22	1.25
8	0.19	0.22	0.18	0.26	0.36	0.21	1.42
9	0.19	0.19	0.15	0.20	0.32	0.37	1.42
10	0.23	0.15	0.27	0.26	0.41	0.35	1.67
Mean	0.19	0.19	0.19	0.23	0.35	0.29	1.44
SD	0.03	0.03	0.05	0.03	0.04	0.07	0.15

k-values were estimated.

The estimated k-values for the different containers are presented in Table 5.11. Details of numbers entering instar read from the survivorship curves and the calculated instar mortalities are presented in Appendices 5.15 and 5.16.

It will be seen from the k-values (Table 5.11) that in all the containers placed in both sites mortality was generally heaviest in the older (third and fourth) larval instars and pupae. The overall k-value was 1.78 and 1.44 in sites C and D, respectively, suggesting that mortality of pre-adults was more in site C than in site D. The difference in the k-values is, however, not significant (t - test : $P > 0.10$).

5.5 Discussion

Marshall (1938) observed that the breeding season of Cx. pipiens "is mainly included in the period April to August; but egg-rafts are not infrequently found in September and larvae as late as November". In this species, a number of generations occur a year and they overlap to such an extent that no differentiation into broods is observable (Horsfall, 1955). The biology of the immature stages of Cx. pipiens and Cx. torrentium are essentially similar (Service, 1968c; and the present study)and therefore the same will apply to Cx. torrentium.

With both Cx. pipiens and Cx. torrentium the overwintering stage is the hibernating female which start to leave the hibernation sites in April (see Section 7) to deposit egg-rafts in any suitable collections of water after taking a blood meal. During the study period the first

egg-rafts laid by mosquitoes at the beginning of the breeding season were found in the first week of April in the containers placed in Silwood Bottom. No first instar larvae were sampled in that month showing that eggs failed to hatch presumably due to the cold weather experienced at that time of the year. Many more eggs were laid in late April and hatching of eggs occurred in the second week of May. In that week each year the mean daily temperatures were above 12°C for several days for the first time. Egg-rafts were first found in Cannon Wood in the third week of May and first instar larvae in the fourth week. The occurrence of younger instars in Silwood Bottom in the second week of May some two weeks before they were found in Cannon Wood suggests that because the containers in Silwood Bottom were placed in the open field, they were more readily located by gravid mosquitoes at the beginning of the breeding season than those placed in the wooded area. This observation was further substantiated by the fact that in North Gravel all four larval instars and pupae were collected in May in the containers placed in the open and edge of wood when only the first three instar larvae were sampled from those 6 and 16 m inside.

A number of factors may be considered to be responsible for the dramatic rise in larval population of Cx. pipiens/torrentium generally observed from late June to August in both artificial containers and permanent ground water habitats (Fig 5.4 - 5.8 and 5.10 - 5.13). Mosquitoes that leave the hibernation sites in late April and early May require some time to obtain a blood meal and more time is still needed for the maturation of egg and egg-laying and hatching. Since the developmental time of eggs is inversely proportional to temperature (Bates 1949; Clements, 1963) the cold weather in April and May may

have been unfavourable to egg development. An increase in temperature above 18°C experienced from June to September (Table 5.1) may have resulted in the population increase in both artificial containers and ground water habitats. Secondly, the rain that accumulated over the past months may have resulted in an increase in breeding sites so that the present generation of mosquitoes would find better oviposition sites under better conditions than the earlier generation. Jobling (1938) observed that many more egg-rafts of Cx. pipiens were laid immediately after prolonged rain followed by very warm calm weather. He stated that "a very heavy precipitation usually increases the number of breeding placed either by changing the conditions in the already existing water by dilution or by filling up the dried ditches and depressions in the ground". Moreover an increase in the amount of vegetation growing in and around the permanent ground water habitat observed at this time may have resulted in an increase in the number of suitable oviposition sites for mosquitoes thereby giving rise to dramatic population rise.

Very few observations have been made on the instar mortalities of mosquitoes (Service 1973c; Lakhani & Service 1974). Southwood et al (1972) studying the life budget of Ae. aegypti breeding in water jars and ant traps in Thailand observed that the greatest mortality occurred in the early (first and second) and last (fourth) instar larvae. In his studies on mortalities of larval population of An. gambiae in Kenya, Service (1973c) observed that mortality was most intense on the fourth instar larvae and pupae. Service (1977a) and Lakhani & Service (1973c) noted that in Ae. cantans breeding in woodland ditch in southern England, mortality was heaviest in the first and second instar larvae. Observa-

tions made in the present study show that in Pond Field pond mortality of the pre-adults of Cx. pipiens/torrentium was heaviest in the fourth instar larvae and pupae while in Sandy Ride pond it was heaviest in the third and fourth instar larvae. In the artificial containers mortality appears to be heaviest in the fourth larval instar. It should, however, be pointed out that the present analysis shows estimated mortalities of pre-adult stages of mosquitoes throughout the entire breeding season and has not shown changes in mortalities at different times during the season.

A number of factors were considered responsible for the heavy mortalities of pre-adults observed in the present study. The predators particularly naiads (Odonata) and larvae and adult Coleoptera present in these habitats (Section 6) may have had a significant effect on larval mortality. The overcrowding of larvae particularly at the peak population and possible competition for limited food supply may also have been important. It is also possible that toxic chemicals produced by mosquito larvae in crowded conditions may constitute a mortality factor. Ikeshoji & Mulla (1970) observed that in crowded conditions (5 - 7 larvae/ml) third instar larvae of Cx.(Cul.) quinquefasciatus Say produced chemical factors highly toxic to first instar larvae. Furthermore the extent to which water of breeding sites was polluted, particularly the permanent ground water habitat may also be important. Jobling (1938) observed that in highly polluted water there was a very high mortality among the last stage larvae and pupae of Cx. pipiens due to unsuitable food supply.

No infection by nematode parasites was observed in 100 fourth-instar larvae of Cx. pipiens/torrentium taken from the ground water

habitat and examined.

However, the very high mortality of pre-adults observed in the present study may be subject to certain errors: a) estimates have been made on the assumption that mortality was constant in any given instar; b) the accuracy of the mortality estimate using the present method depends on the subjective judgement required to fit a smooth curve through the observed stage-specific age distribution (Lakhani & Service, 1974). This is particularly important when the curve does not pass through the midpoints of the stage-specific age distribution histograms as in Fig 5.17 and 5.18. A curve not accurately fitted will result in errors in mortality estimates. For instance if the absolute numbers of egg in each container have not been shown in Fig 5.17 and 5.18, any attempt to predict these numbers by extending the survivorship curves would probably have given different values from the known values. c) The accuracy of the sampling method is very important. If the sampling methods failed to give an accurate indication of the proportions of the various age classes of mosquito throughout the study period misleading mortality estimates would result.

An. claviger and Cs. annulata were observed to be breeding in numbers in the ground water habitats. An. claviger has been described as multivoltine in Eastern Europe (Rettich, 1971) and as bivoltine in southern England (Service, 1973b). Observations made in the present study show two distinct generations in the year (Fig 5.14). Greater numbers of this species (and in fact all species of mosquitoes) were collected per sample in Pond Field pond than in Sandy Ride pond. Many factors such as predators, water conditions and differences in the size of the two ground water habitats may affect the population density

of mosquitoes breeding in them.

Rettich (1971) also described Cs. annulata as multivoltine. Marshall (1938) reported tbat in Britain all four larval instars are found throughout the year. In the present study younger instars of this species were recorded in the ground water habitat throughout the year showing that eggs were constantly laid. The observation that younger instar larvae were present during the winter period shows that females of this species leave the hibernation sites to deposit egg-rafts on suitable collections of water when ever climatic and other conditions are favourable.

SECTION 6

STUDIES ON THE NATURAL PREDATORS OF PRE-ADULT AND EMERGING ADULT MOSQUITOES

6.1 Introduction

Many arthropods were found in the artificial containers and ground water habitats which could be potential predators of mosquitoes. Predators were identified using serological techniques to determine whether mosquito antigens were present in their guts (Boreham & Ohiagu, 1978; Boreham, 1979). Laboratory observations were also made on the predation rate of two of the commonest predators found in these habitats with a view to determining how important they might be in regulating the larval population of mosquitoes.

6.2 Identification of predators using serological methods

6.2.1 Materials and methods

6.2.1.1. Collection of predators

Suspected predators were collected from the artificial containers and ground water habitats during routine sampling with the same sampling devices used to collect pre-adult mosquitoes (see Section 5.2). Potential predators of larval and pupal mosquito populations consisted mainly of naiads (Odonata) and larval and adult Coleoptera. Adult Diptera and Spiders found on the water surface were suspected of preying on emerging adults.

Predators were taken back to the laboratory where they were

immediately identified using the identification keys prepared by Balfour-Brown (1940, 1950, 1958); Lucas (1930); Locket (1951) and Locket et al (1974). The identification of representative samples of predators was checked by the staff of the British Museum (Natural History), London. The abdominal contents of larger predators and whole individuals of smaller ones were smeared onto filter papers and stored separately in a deep-freeze at -40°C until testing.

Before testing smears were soaked in 0.5 ml saline in separate 2ml plastic tubes and the tubes kept overnight at 4°C.

6.2.1.2 Serological methods

a) Preparation of Antigen

The method of antigen preparation was based on that described by Boreham & Ohiagu (1978). Larvae, pupae and adults of Cx. pipiens and Cx. torrentium were collected separately from field sites and from laboratory cultures. Larvae and adults were starved for 24h and the three stages stored separately at -40°C until sufficient material has been collected.

The antigen extract was prepared by taking 2g of material and grinding it in 10ml 0.85% sodium chloride solution. The mixture was transferred to a beaker and placed on a mechanical stirrer for 24h at 4°C. After centrifuging at 1,000g for 5 minutes the extract was filtered through a Seitz EKS filter pad, freeze-dried and stored at -40°C.

b) Preparation of Antisera

The freeze-dried material was reconstituted with 3ml sterile distilled water. One ml of the antigen was mixed with an equal volume of Freund's complete adjuvant (Difco) and aliquots injected into each of the two axillary and inguinal lymph nodes of a New Zealand rabbit (Boreham and Gill, 1973). The rabbit was then given an injection of oxytetracycline (4mg/kg) intramuscularly. A second injection was given seven days later using the same quantity of antigen. Fourteen days after this injection 5ml of blood was taken from the marginal ear vein of the rabbit and the titre of the antiserum determined by the capillary ring test using adult pupal and larval extracts as antigens (Weitz, 1952; Boreham & Ohiagu, 1978).

The titre of the adult mosquito antiserum prepared was 1/2560 (that is, the antiserum reacted to homologous antigen diluted in saline to 1 : 2560), while the larval and pupal antisera each had a titre of 1/1280. The titre of the adult antiserum was considered sufficient and 50ml of blood was taken, the serum separated and stored in aliquots at -40°C. A third injection was given to each of the two remaining rabbits and on subsequent testing after a further period of fourteen days the titre had risen to 1/2560. The rabbits were bled and the antisera similarly stored.

c) Specificity and sensitivity of antisera

The specificity of the three antisera was determined against homologous larval, pupal and adult antigens. No appreciable difference

was found in the three antisera (Table 6.1).

The sensitivity of the larval antisera was determined by testing it against serial two-fold dilutions (extracted in 0.2ml saline) of a single fourth-instar larva, pupa or adult Cx. pipiens and Cx. torrentium. Positive reactions occurred with dilutions of 1/80 of larva and pupa and with a dilution of 1/40 of adult of each species of mosquito.

Table 6.1 Reciprocal of titre of antisera prepared against adult, pupae and larvae of Cx. pipiens/torrentium

Antisera	Antigens		
	Larvae	Pupae	Adult
Larvae	2560	2560	1280
Pupae	2560	2560	1280
Adult	1280	1280	2560

Since no difference was found in the titre of the three antisera prepared, the larval antiserum was used in all the tests involving the predators of mosquito larvae and pupae. The specificity of the larval antiserum was tested against serial two-fold dilutions (after extracting in 0.2ml saline) of Cs. annulata, An. claviger; Chironomus luridus Strenzke and Psectrotanypus varius Fabricius (Chironomidae); all of which were present in the habitats under study. A positive reaction occurred with a dilution of 1/40 of Cs. annulata, 1/20 of An. claviger and 1/10 of C. luridus and P. varius. It is possible to remove such cross-reactions from the antiserum by the process of absorption (Weitz, 1952; Dempster, 1960; Boreham, 1975;

Service, 1976). A small amount of Cs. annulata antigen was added to a small quantity of the antiserum and the mixture stored at 4°C for 48h. After centrifuging the antiserum was retested. The process was repeated as necessary until the most specific antiserum was obtained by adding 0.01ml antigen to 5ml antiserum. This resulted in an antiserum which reacted with 1/40 of Cx. torrentium and 1/10 of Cs. annulata Although the antiserum was now more specific it has lost some of its sensitivity.

The unabsorbed antiserum was tested against extracts of whole individuals of all suspected predators after they had been starved for 24h. No positive reaction occurred.

d) The precipitin test

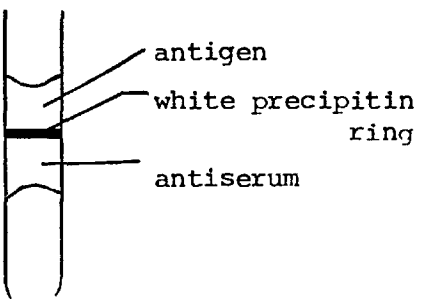
Three methods involving precipitation reactions have been employed during this study. These are (i) the capillary ring test using the automatic serological dispenser (Weitz, 1957), (ii) Cross over electrophoresis (COE) in agar gel (Laurell, 1965), (iii) Agar gel double diffusion (Ouchterlony, 1948). Full details of the methods are given in Boreham & Ohiagu (1978).

About 1/3 of all gut smears prepared were tested by the capillary ring test but since this involved filteration of the antigen which could result in loss of material, COE was substituted. The sensitivities of the different tests have previously been compared (Boreham, 1979) and COE is satisfactory for most purposes. Agar gel double diffusion was occasionally used to check results but has a lower sensitivity.

Fig 6.1 Illustration of precipitin tests showing a positive reaction between predator antigen and mosquito larval antiserum.

a) Capillary ring test

A positive reaction is indicated by a white precipitin ring at the interface of antigen and antiserum as shown in this diagram.

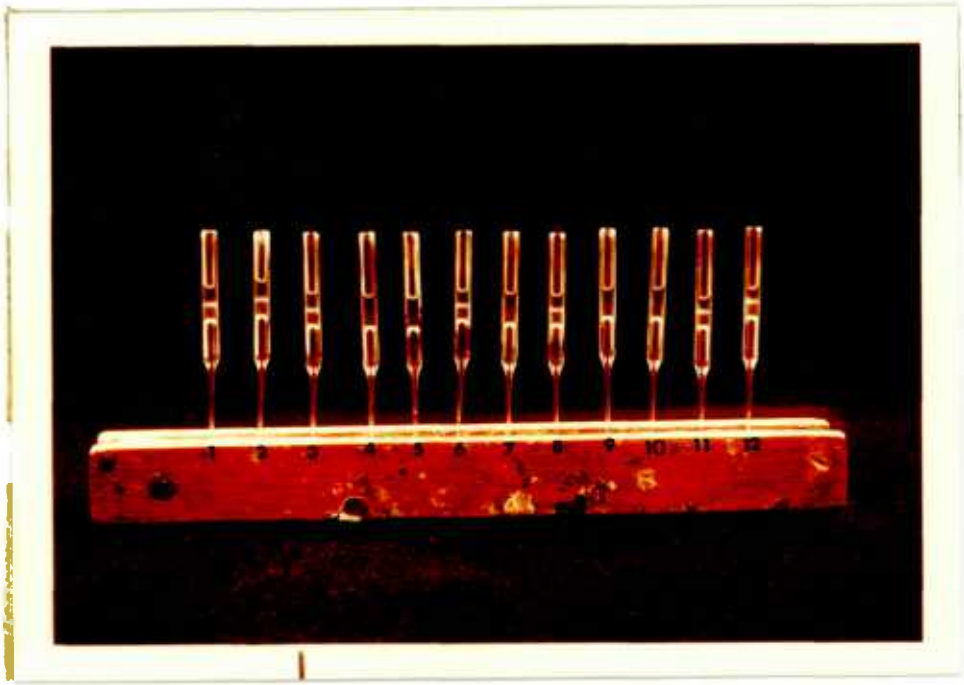


In this figure, therefore, a positive reaction was recorded from pipettes 1, 2, 3, 6, 8, 11 and 12. The rest were negative.

b) Cross over electrophoresis

This method allows testing several antigens with a single antiserum at the same time. The antiserum was placed in the well made in agar (5) and the antigens around it (1 - 4). A white precipitin band (6) shows a positive reaction between 5 and 3.

(a)



(b)

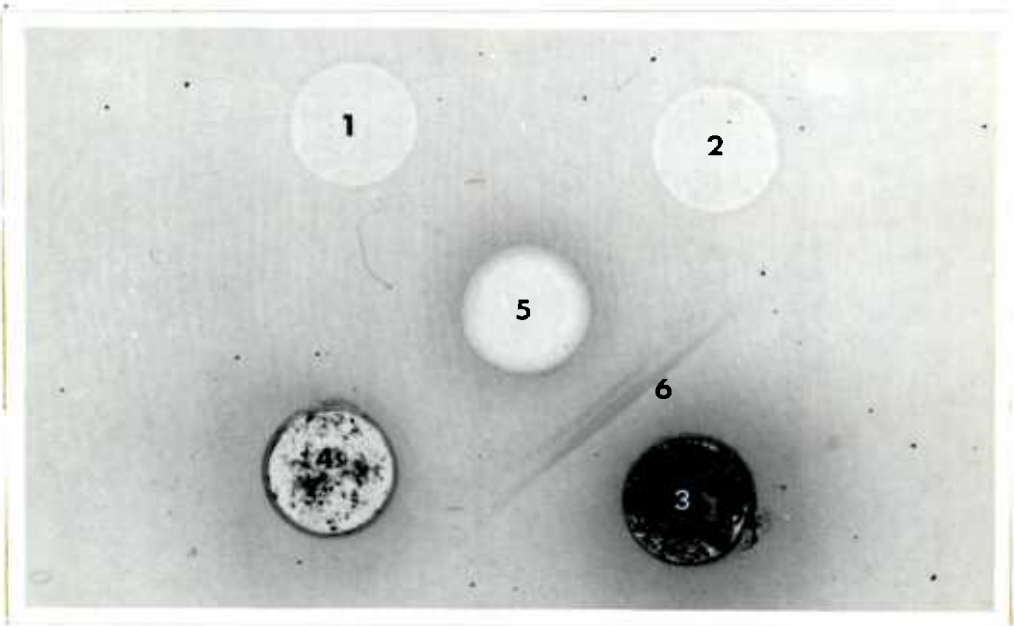


Fig 6.1 shows positive tests obtained by capillary ring test and COE.

6.2.1.3 Digestive time of predators following mosquito meal

Observations were made to determine the length of time a mosquito meal was detectable in the guts of common predators present in the different habitats.

Predators to be tested were collected from the larval habitats and taken back to the insectary at $21\pm1^{o}C$ and 50% r.h, where they were starved for 24h to empty their guts. They were then allowed to feed on a single fourth-instar larva of Cx. torrentium. At 4h interval between 4-48h after feeding, predators were killed and series of gut contents of each species made in 0.2ml saline were tested against Cx. pipiens/torrentium larval antiserum.

6.2.2 Results

A list of suspected predators collected from the different larval habitats and the percentages showing a positive reaction with the anti Cx. pipiens/torrentium serum is presented in Table 6.2.

A total of 1098 gut smears of potential predators of pre-adult and adult mosquitoes were tested, of which 487 (44.4%) gave positive results. Of the 453 naiads tested, 51.9% gave a positive reaction (55.1% of the 325 from Pond Field pond and 43.8% of the 128 collected in Sandy Ride pond). These predators were found to be abundant in the two ground water habitats, but absent in the artificial containers. On five sampling occasions between May and August 1979, during which counts of predators were taken, naiads were found to make up 72% of all predators collected. Agrion puella and Sympetrum striolatum

Table 6.2 Suspected predators of mosquitoes with numbers of gut smears tested and percentages giving positive reaction with Cx. pipiens/torrentium antiserum (l = larvae, a = adult)

Species	Artificial containers		Pond Field pond		Sandy Ride pond		Total	
	No. tested	% positive	No. tested	% positive	No. tested	% positive	No. tested	% positive
Predators of larval and pupal population								
Odonata (nymphs)								
Anisoptera								
Sympetrum striolatum	0	0	61	68.9	33	48.5	94	61.7
Libellula depressa L.	0	0	76	51.3	10	40.0	86	50.0
Zygoptera								
Agrion puella	0	0	64	70.3	20	45.0	84	64.3
A. mercuriale Charp	0	0	57	31.6	16	43.8	73	34.3
Ischnura elegans	0	0	26	53.9	30	46.7	56	48.2
Pyrrhosoma nymphula	0	0	41	51.2	19	36.8	60	46.7

Coleoptera								
Dytiscidae								
Agabus bipustulatus L.	(l)	76	72.4	30	53.3	16	43.8	122	63.9
A. bipustulatus	(a)	22	36.4	21	14.3	2	0	45	24.4
Dytiscus marginalis L.	(l)	67	56.7	36	33.3	14	50.0	117	48.7
D. marginalis	(a)	16	37.5	25	24.0	5	20.0	46	28.3
Colymbetes fuscus L.	(l)	0	0	22	59.1	0	0	22	59.1
Hydroporus memnonius Nic	(a)	11	63.6	2	0	0	0	13	53.9
Hydroporus sp.	(l)	7	57.1	8	12.5	0	0	15	33.3
Hydroporus sp.	(a)	12	25.0	5	40.0	0	0	17	29.4
Hygrotus sp.	(a)	0	0	6	16.7	0	0	6	16.7
Hyphydrus ovatus L.	(a)	4	25.0	16	37.5	8	25.0	28	32.1
Rantus sp.	(l)	0	0	1	100.0	0	0	1	100.0
Haliplidae									
Peltodytes sp.	(l)	0	0	20	15.0	0	0	20	15.0

Continued

Table 6.2 (Continued)

Species	Artificial containers No. tested	Artificial containers % positive	Pond Field pond No. tested	Pond Field pond % positive	Sandy Ride pond No. tested	Sandy Ride pond % positive	Total No. tested	Total % positive
Hydrophilidae								
Helophorus aquaticus L. (1)	13	23.1	0	0	0	0	13	23.1
Hemiptera								
Gerridae								
Gerris gibbifer Schmmel	0	0	0	0	15	13.3	15	13.3
G. lacustris L.	0	0	0	0	15	6.7	15	6.7
Notonectidae								
Notonecta glauca? L.	0	0	0	0	16	0	16	0
Amphibia								
Triturus vulgaris (newt)	0	0	0	0	40	75.0	40	75.0

Predators of emerging adults								
Diptera (adults)								
Asilidae								
Asilus sp.	0	0	10	0	0	0	10	0
Amphididae								
Hilara interstincta Fallen	2	0	7	28.6	0	0	9	22.2
Hilara sp.	1	0	4	0	1	0	6	0
Araneae								
Argiopidae								
Meta segmentata (Clerk)	4	25.0	27	18.5	11	18.2	42	16.7
Lycosidae								
Pirata piscatorius (Clerk)	0	0	13	23.1	4	0	17	17.7
Theridiidae								
Theridion ovatum (Clerk)	0	0	8	12.5	2	0	10	10.0
Total predators tested	235	53.6	586	43.4	277	38.6	1098	44.4

were the most numerous of all Odonata species in the two ground water habitats. Of the 84 A. puella and 94 S. striolatum collected from Pond Field pond and Sandy Ride pond respectively, 63.3% and 61.7 gave a positive reaction with the antiserum.

Nine species of Coleoptera were collected from both the artificial containers and the ground water habitats. Of the 465 individuals tested, 44.3% gave a positive reaction. Three species of Hemiptera were collected from Sandy Ride pond but not from Pond Field pond or artificial containers. Forty-six individuals were tested and only 6.5% gave a positive reaction. Triturus vulgaris (Amphibia) was also present in numbers in Sandy Ride pond but absent from the other habitats. Out of 40 individuals tested, 75.0% gave a positive reaction.

Predators of emerging adults included Diptera and Araneae, the commonest of which have been collected. Of the 25 and 69 smears made from the various Diptera and Araneae respectively, 8.0% and 15.9% gave a positive reaction.

The results of the predators tested and the time when a single meal of mosquito larvae remained detectable in their guts are shown in Table 6.3. The time in all predators tested ranged between 12 - 24h except for T. vulgaris which was only 8h.

Table 6.3 Digestive time of a single larva of Cx. torrentium by predators collected from Pond Field pond, Sandy Ride pond and artificial containers. The time given is the last test period at which the meal was detected (i.e. the meal was not detected at the next period four hours later).

Predator species	Time (in h)
Odonata (naiads)	
Sympetrum striolatum	16
Libellula depressa	16
Agrion puella	16
A. mercuriale	20
Ischnura elegans	24
Pyrrhosoma nymphula	16
Coleoptera	
Agabus bipustulatus (larva)	20
A. bipustulatus (adult)	16
Dytiscus marginalis (larva)	16
D. marginalis (adult)	12
Araneae	
Meta segmentata	12
Amphibia	
Triturus vulgaris	8

6.3 Predation rate of Agrion puella (Odonata : Zygoptera) and Sympatrum striolatum (Odonata : Anisoptera) on immature stages of Cx. pipiens.

A. puella and S. striolatum were the two predators most frequently encountered in the two ground water habitats. Examination of their gut smears (Table 6.2) showed that a high proportion of them had mosquito larvae included in their meal. Since naiads have relatively long generation times (Johnson et al, 1975) they may be very important in regulating the larval population of mosquitoes in these habitats. The purpose of these laboratory observations was to compare the predation activities of A. puella and S. striolatum by comparing their functional responses. It was believed that this will give an insight into their potential levels of predation in the field and hence their importance in regulating mosquito populations.

Solomon (1949) used the term "functional response" to describe changes in the number of attacks made by a predator (or parasite) as prey (or host) density changes. This is represented in the following Holling's (1959a, 1959b) functional response equation commonly known as the "disc equation"

$$Na = \frac{a^1TN}{1 + a^1T_hN}$$

where Na = the number of prey attacked

a^1 = the coefficient of attack

T = the total time available

T_h = the handling time (the interval between a predator first encountering a prey and search being resumed)

N = the initial number of prey

The form of functional response predicted from the disc equation depends on a^1 and T_h. The value of a^1 determines the slope of the functional response curve, that is how rapidly the curve rises to the upper asymptote, while T_h determines the maximum number of prey that is eaten in a particular time.

Various components contribute to the values of a^1 and T_h. Holling (1963, 1965) considers a^1 to be a function of the maximum distance at which a predator will react by attacking a prey, the speed of movement of predator and prey and the capture success of predator, while T_h depends on the time spent pursuing and subduing a prey, the time spent eating and then digesting the prey.

6.3.1 Materials and methods

All experiments were carried out at $21 \pm 1^{o}C$, 50% r.h. and 14h light - 10h dark photoperiod.

Larvae of A. puella and S. striolatum were collected from the ground water habitats in separate vials to prevent cannibalism. They were fed on mosquito larvae until they moulted. Only individuals that have moulted 2 - 5 days previously were used in these experiments. The minimum period of 2 days was chosen to ensure that predators had recovered from moulting and settled down to a steady feeding rate (Thompson, 1978). The exact instar stages of the predators were not known, though, based on wet weight it was believed that the same stage of each predator was

used in all observations. The mean weights of A. puella and S. striolatum were 0.08g (range 0.07 - 0.10g) and 0.26g (range 0.25 - 0.28g), respectively.

When sufficient moulted predators had been collected they were provided with an excess of mosquito larvae for 24h. They were then starved for a further period of 24h in order to stimulate feeding responses (Nelson 1977). Third instar Cx. pipiens larvae obtained from a laboratory colony were accurately counted and placed in a plastic bucket containing 5 litres of pond water. Densities of 4, 8, 16, 32, 64, 128 and 200 larvae were employed, and a small amount of ground yeast was added into each bucket as food for the mosquito larvae. A small stick was also added as a "fishing" site (Thompson, 1978), since naiads are generally ambush feeders, detecting their prey by the use of tactile cues (Corbet, 1962; Richard, 1970). The predators were transferred to the buckets and the tops secured with a netting. Controls consisting of mosquito larvae and no predator were also set up at each prey density.

After 24h predators were removed and placed in a second set of identical buckets at the same prey density. This was repeated after 48, 72 and 96h. Each time predators were transferred the number of live and dead mosquitoes were counted. It was assumed that any defecit could be accounted for by consumption by predators. It was observed that some of the dead mosquitoes were not eaten at all while others were partially eaten by the predators ("wasteful killing"). This phenomemon is common amongst naiads (Thompson et al,1975). The number of mosquitoes killed but not consumed at all was small in the present study and so prey which had been killed, partially consumed

and completely eaten were grouped together.

6.3.2 Results

The results of the numbers of mosquito larvae killed at indicated prey densities and time are presented in Appendices 6.1 and 6.2, while the functional response curves produced from the results are shown in Fig. 6.2 and 6.3. Means, 95% confidence limits and fitted curves have been shown. Curves have been fitted using estimates of a^1 and T_h (Table 6.4). These estimates were obtained by transforming the disc equation to a linear regression enabling a^1 and T_h to be calculated from the slope and the intercept, respectively (Rogers, 1972).

The curves are of Holling's (1959a) type II functional response, frequently found in arthropods. During the first 24h the predation rate of A. puella and S. striolatum was high, but this decreased progressively as the experiment proceeded. This is illustrated by the fall in the asymptote indicating a decrease in the maximum number of prey eaten per day. Table 6.4 shows that in the four successive days during which prey were exposed to predators, each predator showed a gradual decrease in a^1 and an increase in T_h. When the two predators were compared, it was immediately obvious that at all prey densities, S. striolatum has a higher voracity than A. puella. Moreover, throughout the study period S. striolatum has a higher a^1 than A. puella indicating that more prey were killed by S. striolatum than A. puella.

Fig 6.2 The functional response of A. puella to changes in prey density of third-instar Cx. pipiens. (Means, 95% confidence limit and fitted curves are plotted).

Fig 6.3 The functional response of S. striolatum to changes in prey density of third-instar Cx. pipiens. (Means, 95% confidence limit and fitted curves are plotted).

Fig 6.2

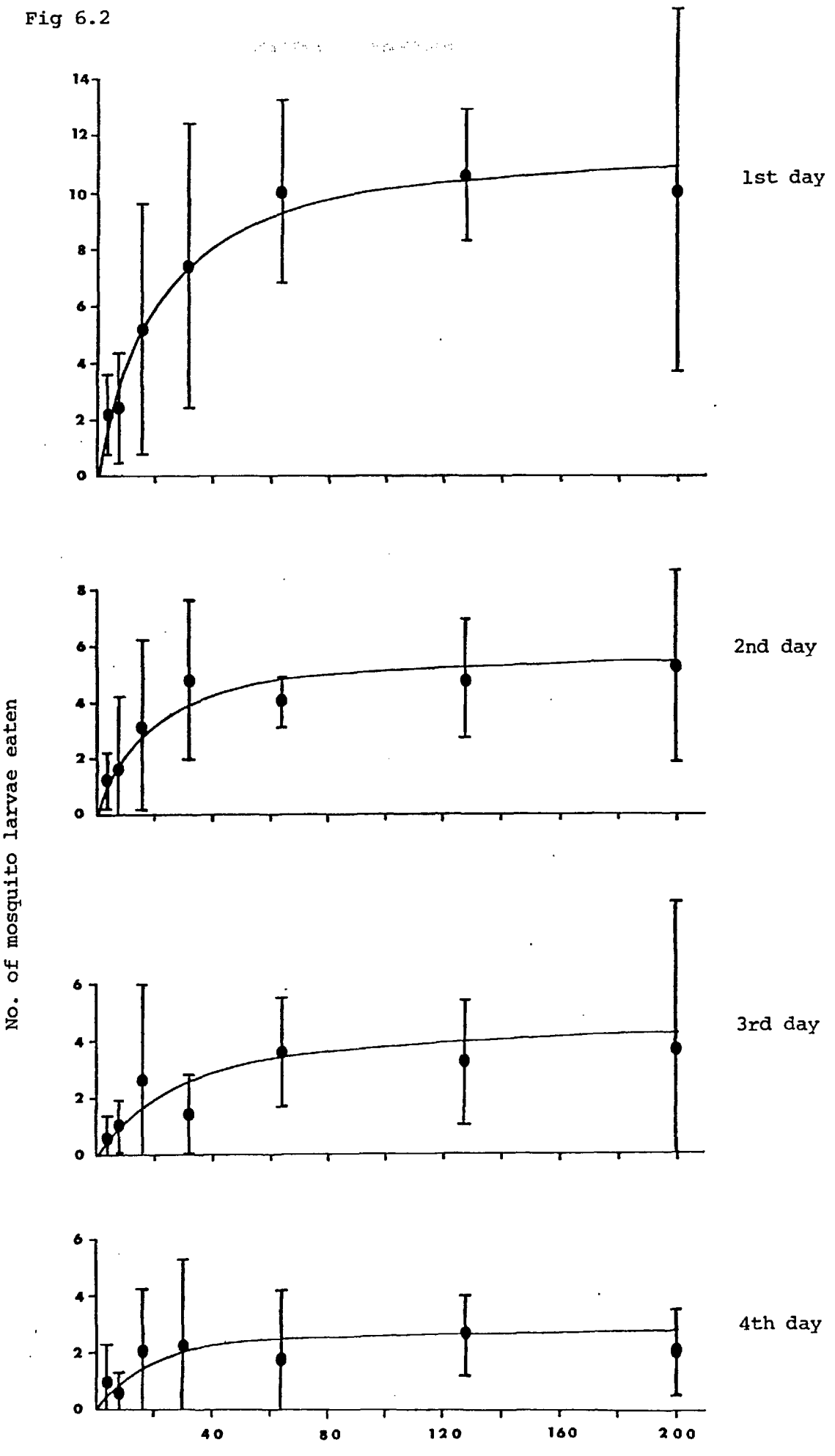

1st day
2nd day
3rd day
4th day
No. of mosquito larvae eaten
Prey density
0
2
4
6
8
10
12
14
40
80
120
160
200

Fig 6.3

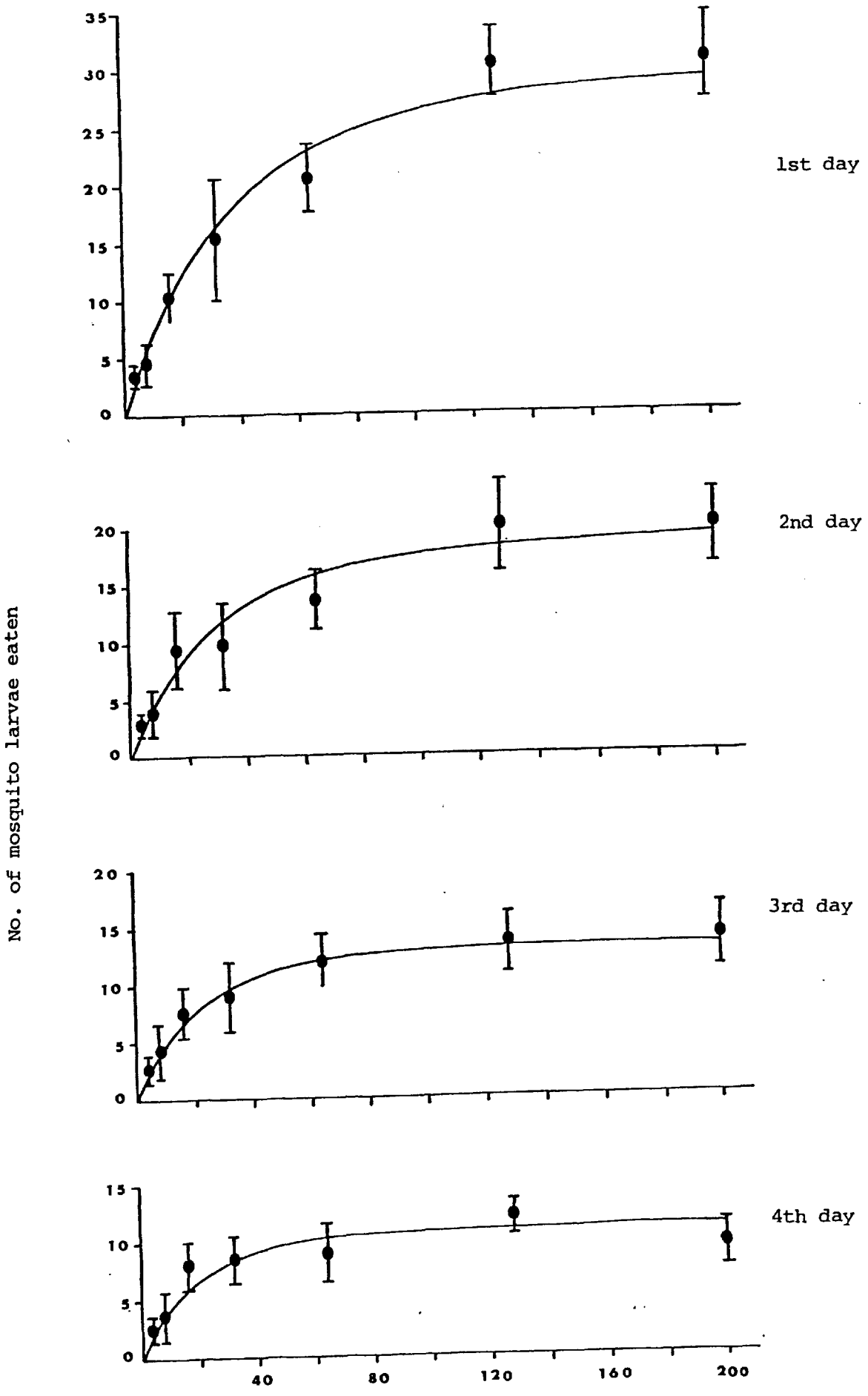

1st day
2nd day
3rd day
4th day
No. of mosquito larvae eaten
Prey density
0
5
10
15
20
25
30
35
40
80
120
160
200

Table 6.4 Estimates of attack coefficient, a^1 (in 5 litre units/h) and handling time, T_h (in h) of A. puella and S. striolatum.

Species		Time (in h)			
		24	48	72	96
A. puella	a^1	0.0298	0.0162	0.007	0.009
	T_h	2.0280	4.0553	4.8699	8.0631
S. striolatum	a^1	0.0635	0.0464	0.0540	0.0458
	T_h	0.7303	1.1182	1.6926	1.9740

Furthermore, there is a greater variability in individual A. puella than in S. striolatum as seen by the confidence limits for the two species.

6.4 Discussion

As early as 1946, Brook & Proske demonstrated the possibility of using precipitin test to identify the natural predators of mosquito larvae but the usefulness of this method in medical entomology was not recognised until recently. Service (1973c, 1973d, 1973e) applied it to identify the predators of larvae and adults of An. gambiae in Kenya and Ae. cantans in southern England.

Serological techniques have a number of advantages in the study of predator-prey relationships. Firstly, they offer a means of determining the extent of predation occurring in the field under natural conditions (Service, 1973e) and the possible effects of a given predator on a given prey population. Secondly, due to the sensitivity of the tests small amounts of antigens in the gut of a predator, even as little as 10^{-6} g/ml of fluid, could be detected (Titova, 1970). Thirdly, gut smears of predators properly made on a filter paper can be tested after several months without loosing their sensitivity. There are also a number of disadvantages which have been discussed by Boreham & Ohiagu (1978) and Boreham (1979). These include the problems of cross reactivity which may be overcome by absorbing the antisera, the difficulties of quantification and the failure to be able to distinguish between active predation and carrion feeding.

Several workers have shown by field and laboratory observations that members of the following taxa can be important and active predators of mosquitoes: Dytiscidae (Service 1973c, 1977a; Roberts et al, 1967; Twin, 1931), Hydrophilidae (Nielsen & Nielsen, 1953; Notestine, 1971), Hemiptera (James, 1965), Muscidae (Service 1971b), Empididae (Frohne, 1952; Service, 1973e), Amphibia (tadpoles) (Service, 1970).

In the present study, naiads, larval and adult Dytiscidae and the Amphibian, Triturus vulgaris were numerous in some or all the habitats studied and appear to be very important in regulating the larval population of mosquitoes. Odonata were present in the two ground water habitats but completely absent in the artificial containers while larval and adult Dytiscidae mainly A. bipustulatus and D. marginalis were present in all habitats. T. vulgaris was present in numbers only in Sandy Ride pond where 75% had fed on mosquito larvae (Table 6.2).

The impact of predators on reducing the size of prey population depends on the predator number and the number of prey they are able to find and consume (Service 1973e; Hassell 1978). The number of prey eaten will depend on the population size of both the prey (Jackson 1953) and the predator. Sailer (1954) showed by laboratory experiments that one larva of Agabus sp. consumed 80 Aedes larvae in 20 days. Predacious mosquito larvae can also be important, for example, Jackson (1953) observed that Cx. (Lutzia) tigripes Grandpre & Charmoy ate 88 larvae of Ae. aegypti in 3 days in the laboratory.

Muspratt (1951) showed that a single larva of Toxorhynchites (=Megarhinus) brevipalpis Theobald, another predacious mosquito, could kill from 100 - 200 or more larvae of Ae. aegypti during its normal life of 11 - 20 days. Service (1973e) observed from laboratory experiment that a single larvae of A. bipustulatus could eat about 2 - 4 larvae of Ae. cantans every 24h, although he further remarked that laboratory observations on predation rates cannot be related to what actually happens in the field without difficulties.

The overall efficiency of predation of a number of predators have been assessed and compared by laboratory experiments on functional responses, during which different prey densities have been offered to predators and the numbers eaten over chosen time intervals noted. It was possible to determine from such experiments which predators have greater potential in regulating prey populations. Nelson (1977) using this method found that the larvae of Dytiscus marginalis was consistently more effective than that of Hydrophilus triangularis (Say) (Coleoptera: Hydrophilidae) in regulating larval population of Cx. quinquefasciatus. Robert et al (1967) also compared the predation potential of two adult Dytiscidae, Laccophilus fasciatus Aubé and L. maculosus. Say, on various instars of mosquito, Cx. pipiens and midge, Glyptotendipes barbipes (Staeger) larvae. He found that the predation potential of the two beetle species were the same for third and fourth instars of Cx. pipiens, but that L. fasciatus appeared to be a more efficient predator of G. barbipes for both sizes of larva.

In the present study, observations on the functional response of A. puella and S. striolatum have shown that their predation rate was high in the first 24h of experiment. This could be interpreted as a direct effect of starving the predators for 24h prior to the experiment. During the successive 24h periods, the predation rate decreased and remained fairly constant for the later part of the observation. The predation rate observed at this time represents what may actually happen in the natural habitat where the predators are constantly surrounded by their prey. The predators' attack coefficient a^1 and handling time T_h will contribute to their effectiveness in regulating prey populations. The obvious difference in a^1 and T_h observed in A. puella and S. striolatum clearly indicates that S. striolatum was consistently more effective than A. puella. This may be due to the difference in the sizes of the two species. S. striolatum might be more important in reducing the larval population of Cx. pipiens/torrentium in the ground water habitats. As biological control agents, S. striolatum is more promising than A. puella.

Among invertebrates, larval and adult Dytiscidae and Hydrophilidae (Coleoptera) and all stages of Notonectidae (Hemiptera) have received the most attention as biological control agents of mosquitoes (Notestine, 1971). Different species of Odonata are also effective predators of mosquitoes (Twin, 1931; Laird, 1947) and are therefore potential biological agents. One of the problems of using these and other predators in mosquito control in the field is that no suitable method has so far been devised to rear the potential biological agents economically and in large numbers (Nelson, 1977).

SECTION 7

STUDIES ON ADULT OVERWINTERING POPULATIONS OF CX. PIPIENS

7.1 Introduction

Inseminated adult females of Cx. pipiens pass the cold winter months in cellars, attics and other hiding places in a state of complete hibernation (Marshall, 1938; Service, 1968a). At the approach of autumn the physiological behaviour of the hibernating population changes. In this species blood feeding ceases and mosquitoes feed on plant secretions and develop heavy accumulation of fat and glycogen in the fat body cells (Marshall 1938; Clements 1963; Service 1968a). The fat accumulates as globules, partly beneath the body wall and partly around the alimentary canal (Marshall, 1938). It acts as a food reserve and is probably involved in resistance to cold (Buffington & Zar, 1968). Mosquitoes leave their hibernation sites in April the following year and after successfully obtaining a blood meal deposit egg-rafts in suitable collections of water (Marshall, 1938).

This section of the study was undertaken in an effort to provide more information on the overwintering habits of Cx. pipiens including the population fluctuations during the course of hibernation, the physiological condition of hibernating individuals and the possible causes of mortality. Information was also collected for Cs. annulata which hibernates in association with Cx. pipiens. Similar studies could not be made on Cx.

torrentium because of the difficulty in finding their hibernation sites (see Section 7.9).

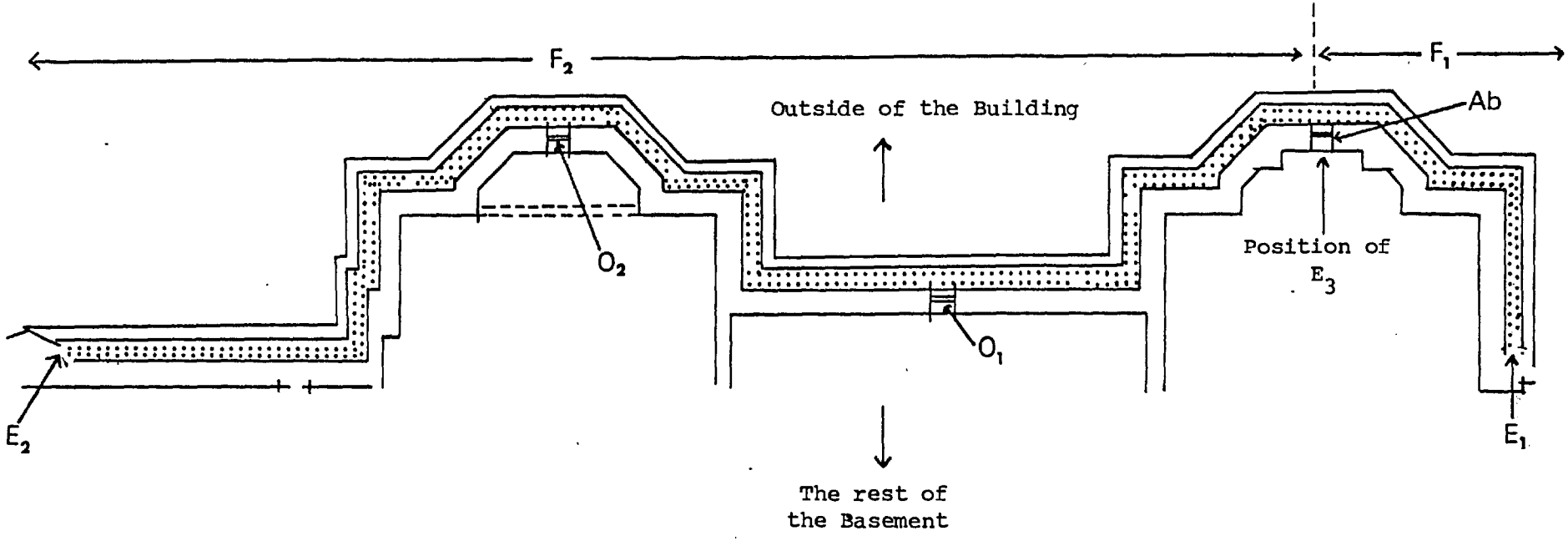
7.2 Description of the study sites

The hibernation site studied is in the basement of Silwood House (Fig 1.1). The site was divided into two main areas for convenience.

Site F, The tunnel

This site designated as "The tunnel" is in fact a "drainage cavity" commonly found in Victorian Houses. Its primary function is to drain the water that escapes from the house thereby preventing dampness in the foundation walls of the building. The tunnel runs along one side of the building starting from the south end (Fig 7.1) and measures 47.5 m in length with an average height and width of 1.5 and 0.6 m, respectively. The roof which forms part of the foundation of the building is about 0.3 m below ground level.

There are three main entrances to the tunnel: entrances E_1 and E_2 are located at the two ends of the tunnel and are accessible from the outside of the building. The third entrance E_3 accessible only from the basement is located at a distance of 7.3 m from the southern end of the tunnel. Other openings into the tunnel are an air brick (Ab) 35.6 cm^2 with five small openings each 7.6 cm in diameter, and located above E_3; and O_1 and O_2 which are



E_1, E_2 Entrances from Outside

E_3 Entrance from Basement

Ab Air brick

O_1, O_2 Air openings

Scale 10m

N

Fig 7.1 Plan of Site F, "The Tunnel" (shaded area)

and the adjoining parts of the basement.

simply internal air openings about 30 cm in diameter, leading into the adjoining parts of the basement.

It was assumed that hibernating mosquitoes entered this site through entrances E_1, E_2 and Ab.

Site G, basement cellars

This is the basement cellars located on the western side of the building and separated from Site F by experimental Controlled Temperature rooms (Fig 7.2). Seventeen individual rooms (Rooms G_1 - G_{17}) make up this site. The entire site is accessible only internally through a door at the main entrance leading from the basement into room G_6. Air bricks located on rooms G_1, G_3, G_9, G_{11} and G_{13} provide access to the outside.

It was assumed that hibernating mosquitoes entered the site through these air bricks.

Other sites

Collections of mosquitoes used for laboratory experiments were also made from Sandy Ride House basement (Fig 1.1). This site is made up of several rooms with a number of air bricks and is similar to Site G, although harbouring fewer hibernating population.

7.3 Population fluctuation and distribution

7.3.1 Materials and method

Between September and April 1977 - 78, 1978 - 79 and 1979 - 80

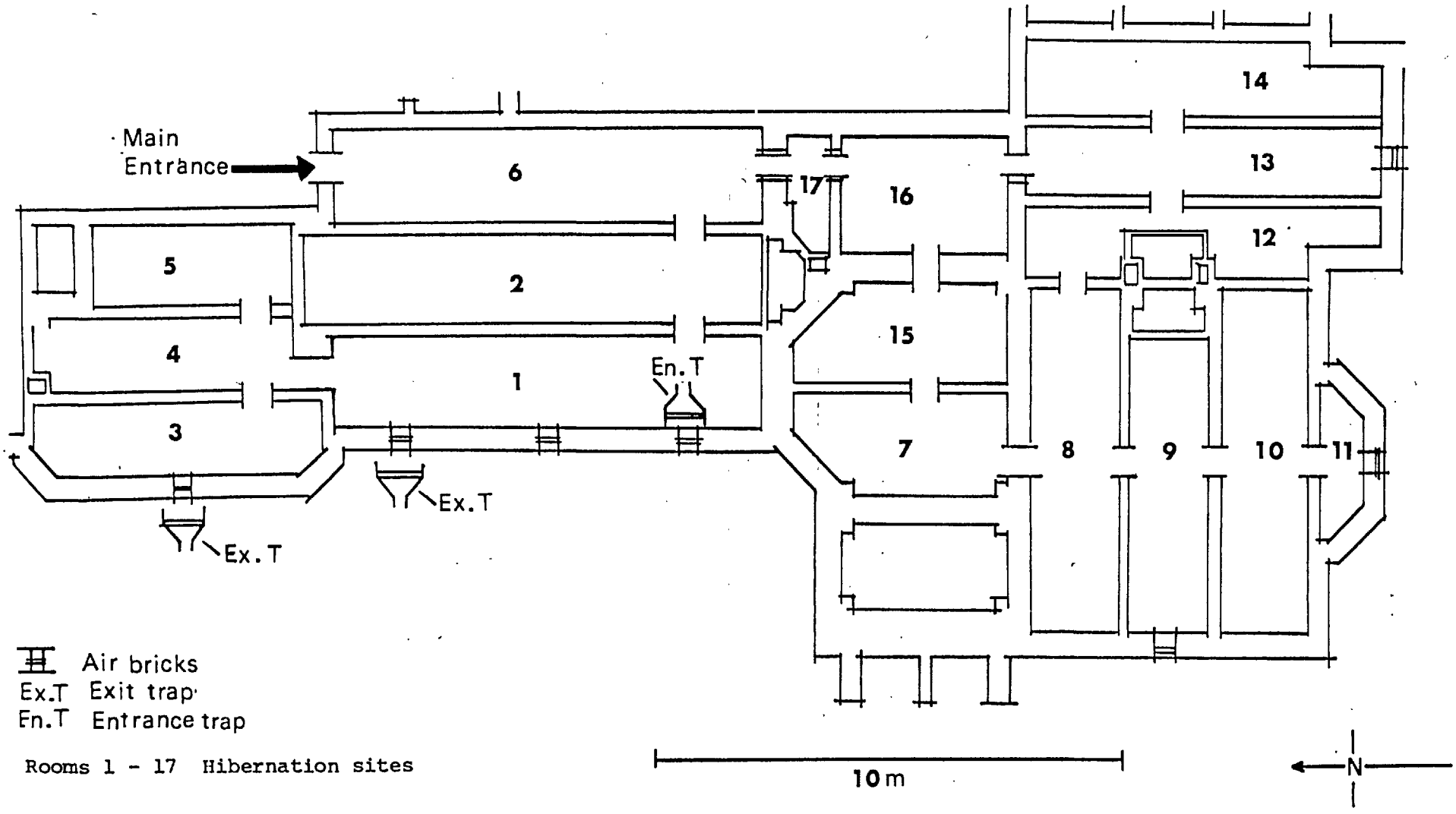


Fig 7.2 Sketch map of Site G, Basement Cellars

regular population counts of hibernating Cx. pipiens and Cs. annulata were made in selected areas in the hibernation sites. In the first winter counts were started in October 1977 when apparently most of the population had entered sites. In subsequent winters, however, population studies were started from the time the first hibernating mosquito was found in the site.

In Site G (Fig 7.2) the total number of mosquitoes resting on the walls and roof of room G_1 was counted. In Site F (Fig 7.1) it was not possible to count all the mosquitoes due to the size of the tunnel. The site was therefore divided into two - F_1 and F_2. Site F_1 is 7.3 m long and starts from entrance E_1 to E_3. Here the total population count of mosquitoes was taken. Site F_2 is the rest of the tunnel 40.2 m long starting from the entrance E_3 to enctrance E_2. Fourteen sampling sites were established beginning at a distance of 0.6 m from entrance E_3, each being 0.6 m wide and 2 m apart. Each of these sections was further divided into three consisting of the lower and upper portions of the wall each measuring approximately 0.6 x 0.8 m and an equal area on the roof of the tunnel above this area. Records of population count of mosquitoes resting on the 14 sections of the wall and roof was used to obtain information on the population fluctuations and distribution along the entire tunnel.

Throughout the study period population counts were made between 9.00 and 11 a.m. every Monday at site F_2. In sites F_1 and G_1 counts were taken only for two winters of 1977 - 78

and 1978 - 79. In the first winter counts were made weekly as in F_1 but thereafter this was reduced to two counts per month usually at the middle and end of each month. Sites were entered only during population counts and care was taken not to brush the walls (particularly in the case of the narrow Tunnel) thereby causing unnecessary disturbance to hibernating mosquitoes.

In the first week of November each year, during the peak population, a total count of all the mosquitoes and spiders found in the entire site F_1 and F_2 was taken. Accuracy of mosquito population counts was estimated at $\pm$ 2% in recounts of several samples. In 1978 - 80 the number of mosquitoes enmeshed in 17 selected spiderwebs and later eaten by spiders was recorded throughout this period. Since captured mosquitoes are partially eaten by spiders it was possible to record fairly accurately the number of mosquitoes disappearing in webs. Measurements of the webs were taken and the total surface area was calculated.

Changes in temperature and relative humidity were recorded regularly throughout this study at Site F_2 using thermometers at different positions in the tunnel and by means of a hygrometer. On a number of occasions temperature and humidity readings using a swing hygrometer were taken at Site G. Since no appreciable difference was found in the readings between Sites F_2 and G the former have been used to represent the temperature and humidity changes in the entire site.

7.3.2 Results

The results of temperature and humidity readings are presented in Fig 7.3 and 7.4. The temperature in the site ranged between 23 - 4°C while relative humidity ranged between 78 - 31%.

In 1978 and 1979 hibernating Cx. pipiens first entered the sites on the 8th and 16th August, respectively. From then onwards, there was a rapid build-up of population reaching a peak in late October or early November each year (Fig 7.5 and 7.6). An impression of the distribution of mosquitoes on the walls of Site F_2 during peak population is given in Fig 7.7. From December onwards there was a gradual reduction in the number of mosquitoes until in late April no mosquitoes were found in the study areas (Fig 7.5 and 7.6). Hibernating mosquitoes were last seen in the entire sites on the 10th, 12th and 5th May 1978, 1979 and 1980 respectively. The results of total population cound of mosquitoes at the time when maximum numbers were present in early November and spiders present at the same time are given in Table 7.1. The number of spiders counted does not give the actual population size since some remain in the holes and cracks on the walls and roof. The number of mosquitoes enmeshed in spiderwebs is presented in Table 7.2.

A small number of Cs. annulata was found in the sites between October and May and their population fluctuated without any clearly defined peak (Fig 7.6 and 7.7). Details of actual numbers of mosquitoes counted in the sites are presented in Appendices 7.1 - 7.5.

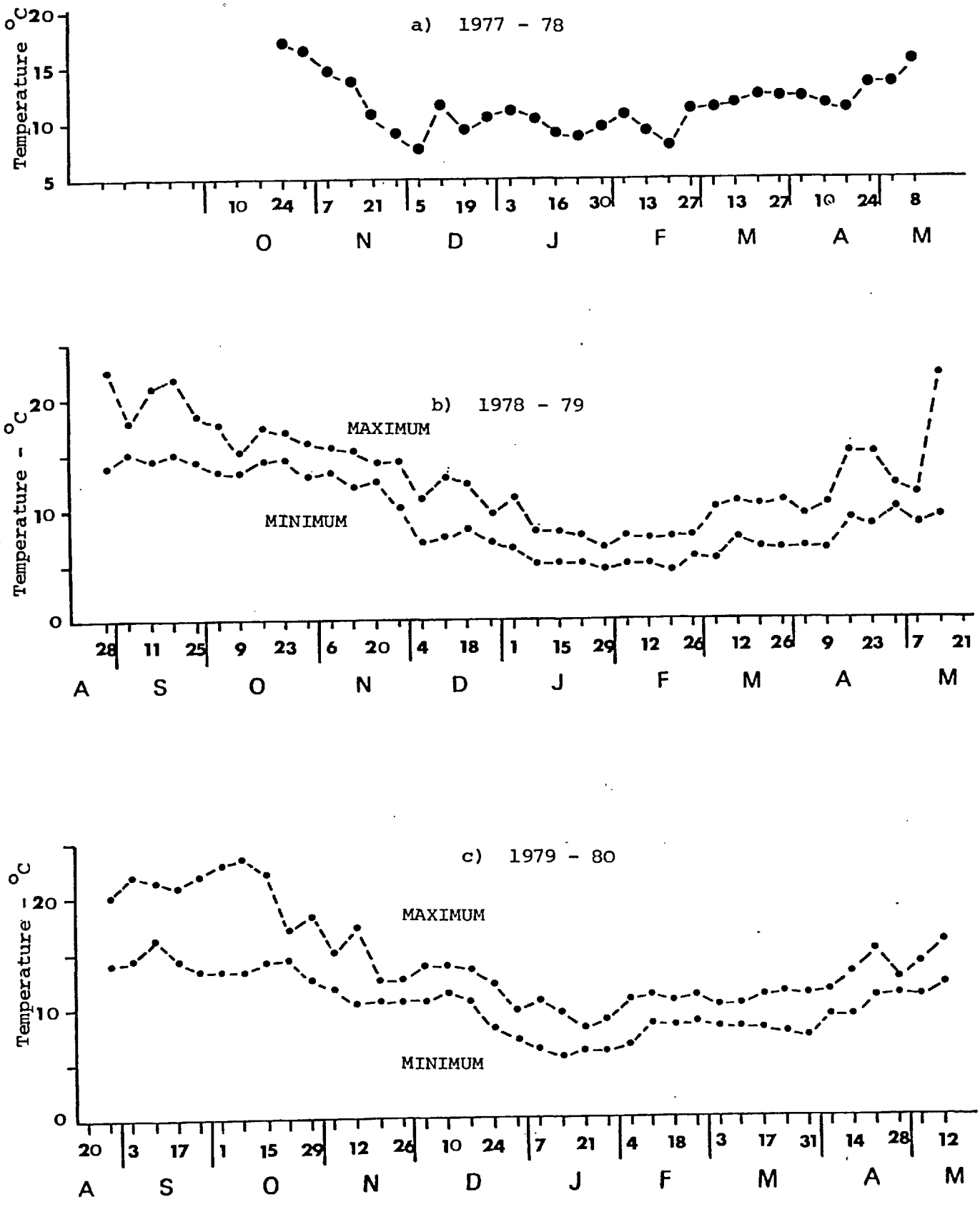
Fig 7.3 Temperature readings taken in the hibernation site during the study period.

a) Weekly temperature reading at Site F_2 measured with a standard thermometer suspended from the ceiling at three points on the days counts were made; 1977-78

b) Weekly temperature maxima and minima at Site F_2 measured by a maximum and minimum thermometer; 1978-79

c) Weekly temperature maxima and minima at Site F_2 measured by a maximum and minimum thermometer; 1979-80.

Fig 7.3


a) 1977 - 78
Temperature °C
20
15
10
5
10 24 7 21 5 19 3 16 30 13 27 13 27 10 24 8
O N D J F M A M
b) 1978 - 79
Temperature - °C
MAXIMUM
MINIMUM
20
10
0
28 11 25 9 23 6 20 4 18 1 15 29 12 26 12 26 9 23 7 21
A S O N D J F M A M
c) 1979 - 80
Temperature - °C
MAXIMUM
MINIMUM
20
10
0
20 3 17 1 15 29 12 26 10 24 7 21 4 18 3 17 31 14 28 12
A S O N D J F M A M
MONTHS

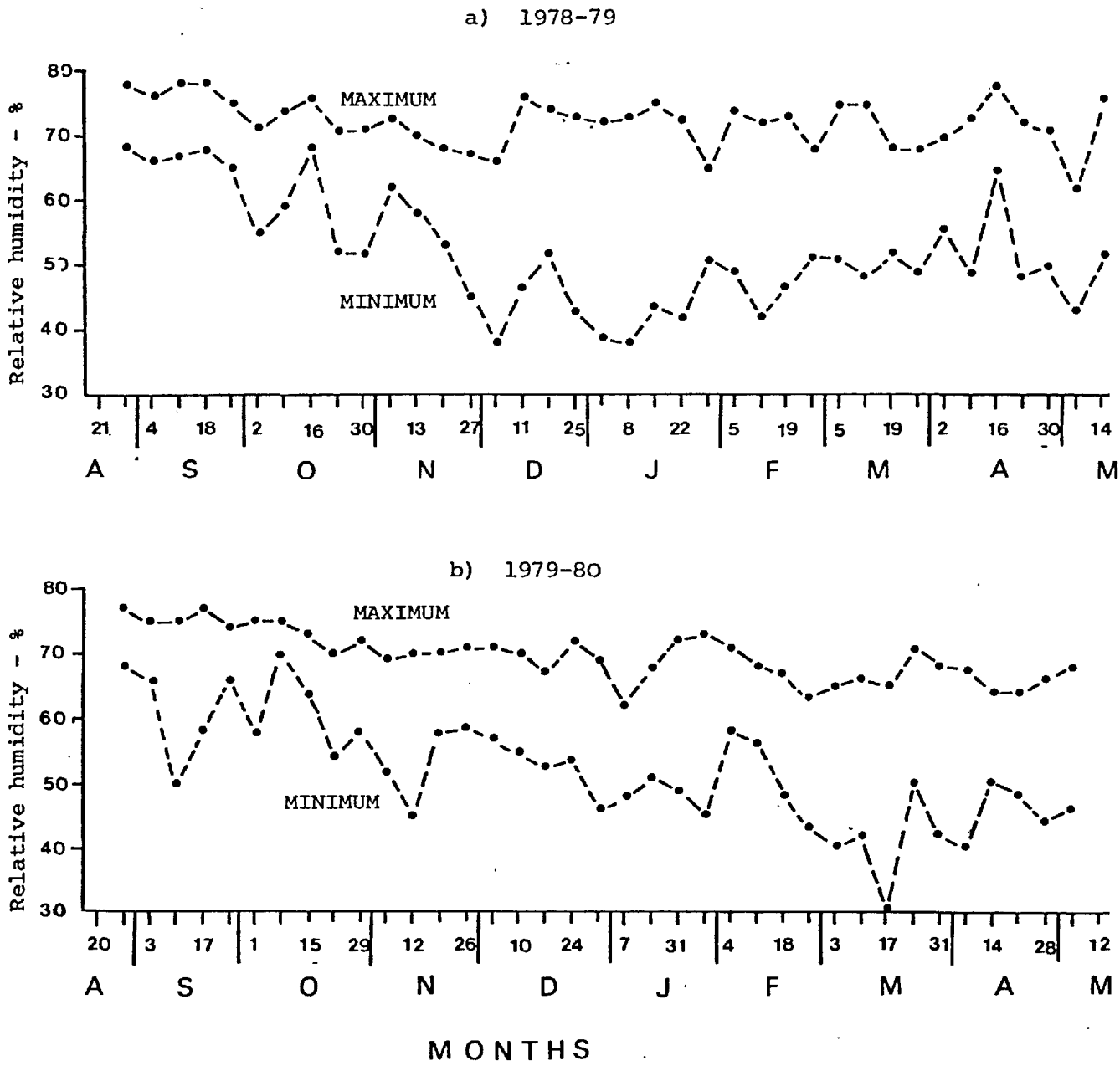


Fig 7.4 Weekly relative humidity maxima and minima at Site F_2,

(a) 1978-79, (b) 1979-80.

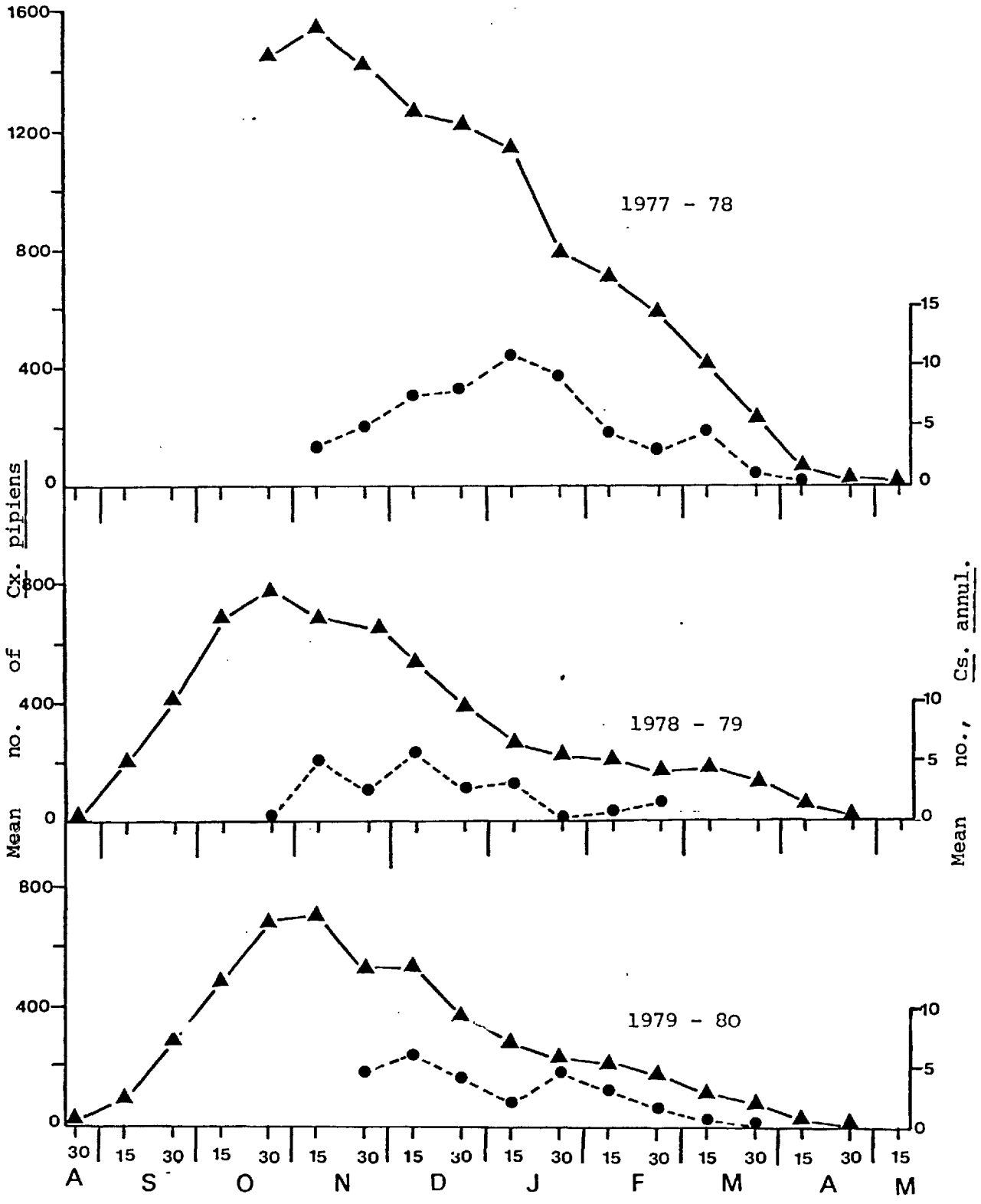


Fig 7.5 Seasonal fluctuation in the number of Cx. pipiens (△—△) and Cs. annulata (●----●) in Site F_2 during the course of hibernation. The figures '15' and '30' are used to represent the first and second halves of each month. (Results for 1977-1980).

Fig 7.6 Seasonal fluctuation in the number of Cx. pipiens (Δ—Δ) and Cs. annulata (●----●) recorded in (a - b) Site F_1 and (c - d) Site G_1. The figures 15 and 30 represent first and second halves of each month (1977 - 1979). (Record was taken weekly in 1977 - 78 but in 1978 - 79 this was reduced to twice per month usually at the middle and end of each month).

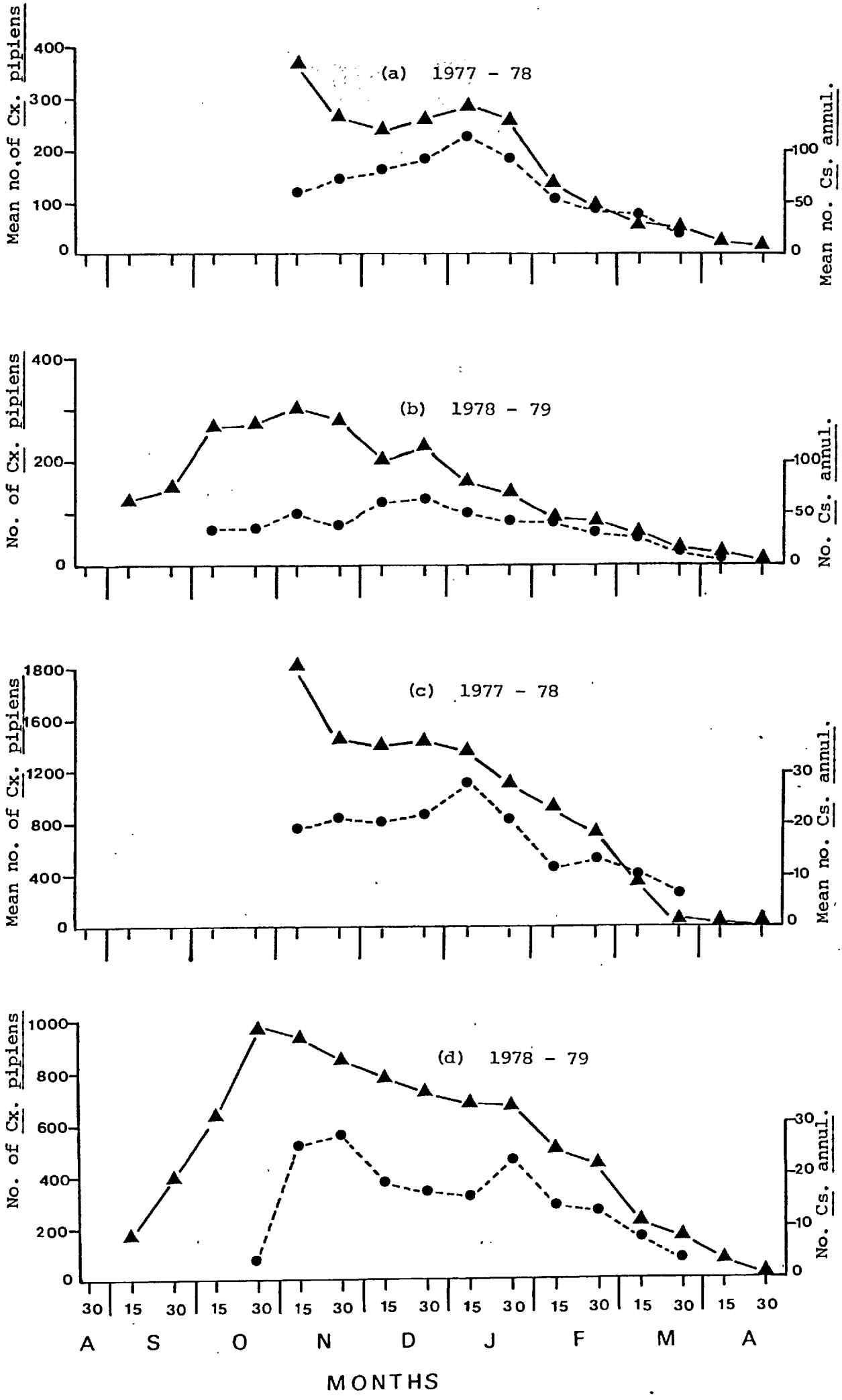

(a) 1977 - 78
Mean no.of Cx. pipiens
Mean no. Cs. annul.
400
300
200
100
0
100
50
0
(b) 1978 - 79
No. of Cx. pipiens
No. Cs. annul.
400
200
0
100
50
0
(c) 1977 - 78
Mean no. of Cx. pipiens
Mean no. Cs. annul.
1800
1600
1200
800
400
0
30
20
10
0
(d) 1978 - 79
No. of Cx. pipiens
No. Cs. annul.
1000
800
600
400
200
0
30
20
10
0
30 15 30 15 30 15 30 15 30 15 30 15 30 15 30 15 30
A S O N D J F M A
MONTHS



Fig 7.7 Hibernating mosquitoes resting on the walls of Site F_2 (Section 3) during peak population, November 1978.

Table 7.1 Record of total population counts of mosquitoes and spiders in hibernation Sites F_1 and F_2 taken during peak population, 1977 - 1979.

Date	Nov. 7, 1977	Nov. 6, 1978	Nov. 5, 1979
Cx. pipiens	8004	5424	5008
Cs. annulata	121	73	104
Spiders	109	89	71

Table 7.2 Record of number of mosquitoes enmeshed in spiderwebs in Site F_2 at different times during hibernation, 1978 - 1980.

	Approx. surface area of web	No. of mosquitoes caught in web						
		Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1978-79	2361.5 sq.cm	-	21	38	7	6	0	0
1979-80	2066.7 sq.cm	5	17	22	14	3	4	1

Further analysis of the results of population count of Cx. pipiens in Site F_2 shows the pattern of population distribution in the tunnel. The numbers of mosquitoes counted per month in each of the fourteen sections of the wall were expressed as percentages of total monthly counts and the results are presented in Fig 7.8 - 7.10. It was observed that throughout the hibernation period mosquitoes tended to avoid the areas around the centre of the tunnel while at the same time they tended to concentrate more at the areas towards the entrances. Moreover, there were persistently more mosquitoes in Section No. 3 throughout the hibernation period than in any other section of the wall (Fig 7.8 - 7.10). Possible reasons for the variability in the distribution of mosquitoes in this site is discussed in Section 7.10.

Fig 7.11 shows the proportions of Cx. pipiens counted on the lower and upper sections of wall and roof of Site F_2. Throughout the hibernation period, more mosquitoes rested on the lower section of the wall than the upper section and the roof. More mosquitoes were found on the upper section of the wall at the onset of hibernation than on the ceiling. However, this was reversed towards the end of hibernation.

Very few male Cx. pipiens were found in the hibernation sites and all these were present at the beginning of hibernation. The latest time male mosquitoes were seen was October 22nd in 1979.

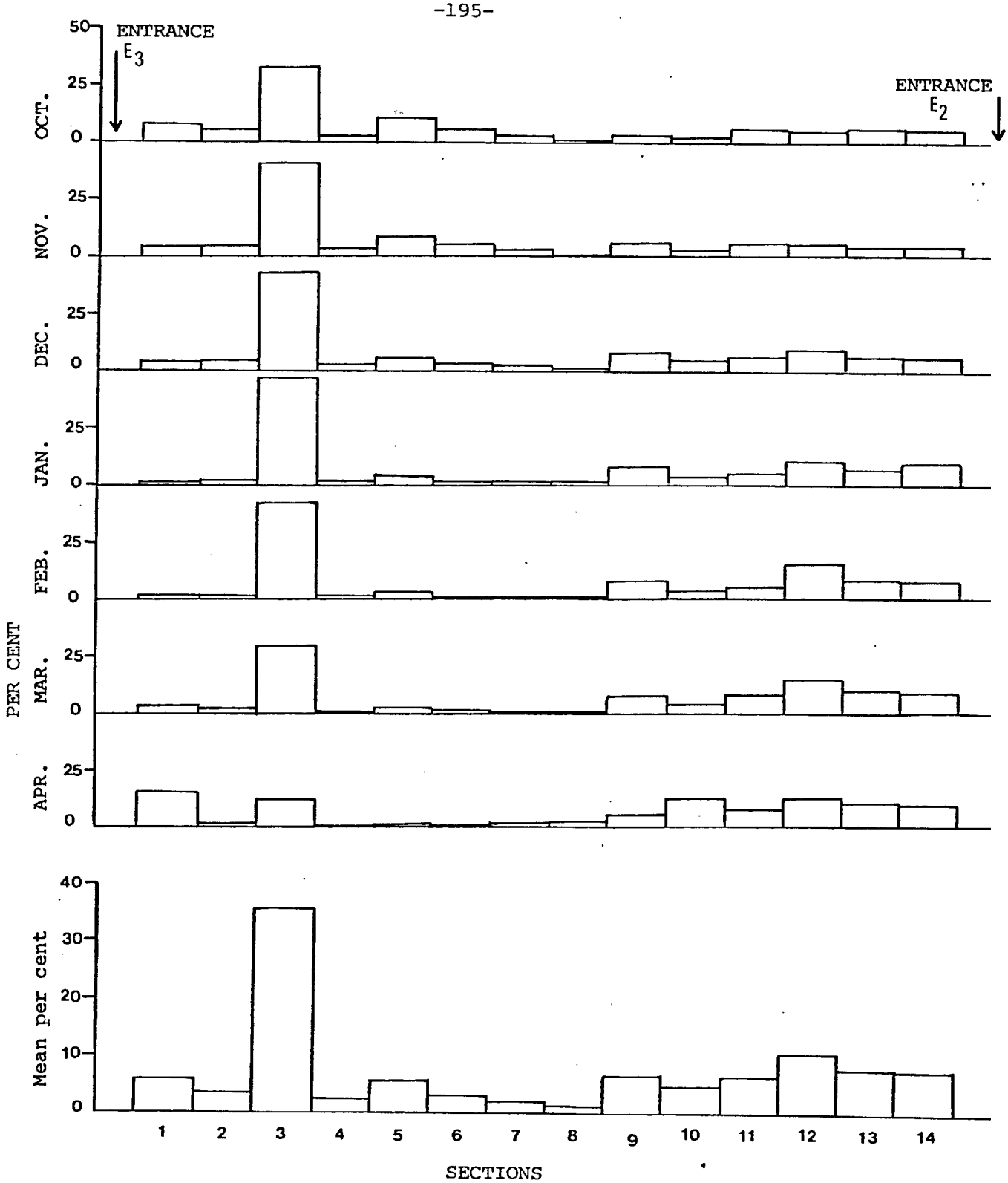


Fig 7.8 Pattern of distribution of hibernating Cx. pipiens along Site F_2, at diferent times during hibernation, 1977 - 78.

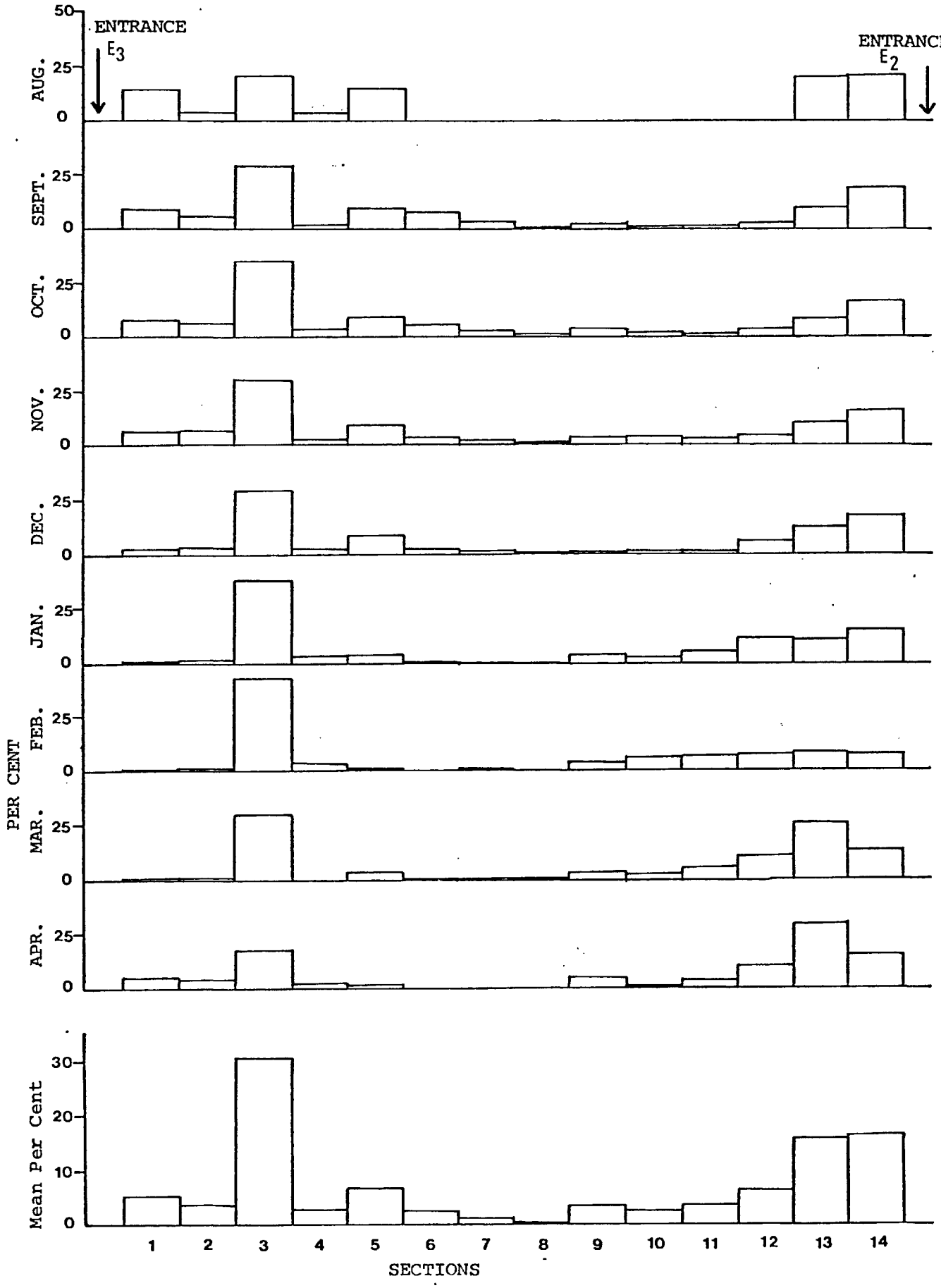


Fig 7.9 Pattern of distribution of hibernating Cx. pipiens along Site F_2, at diferent times during hibernation, 1978 - 1979.

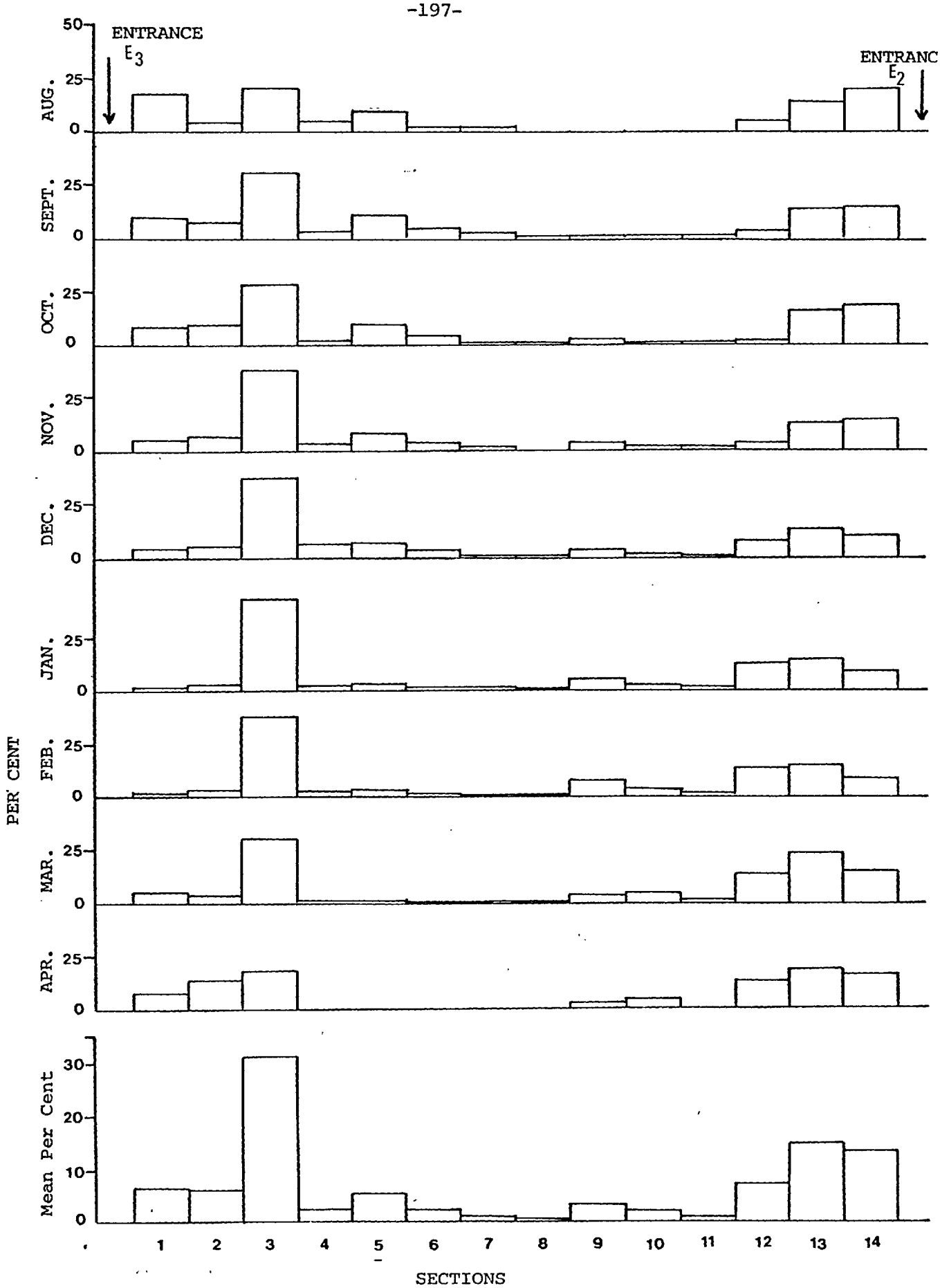


Fig 7.10 Pattern of distribution of hibernating Cx. pipiens along Site F_2, at diferent times during hibernation, 1979 - 80.

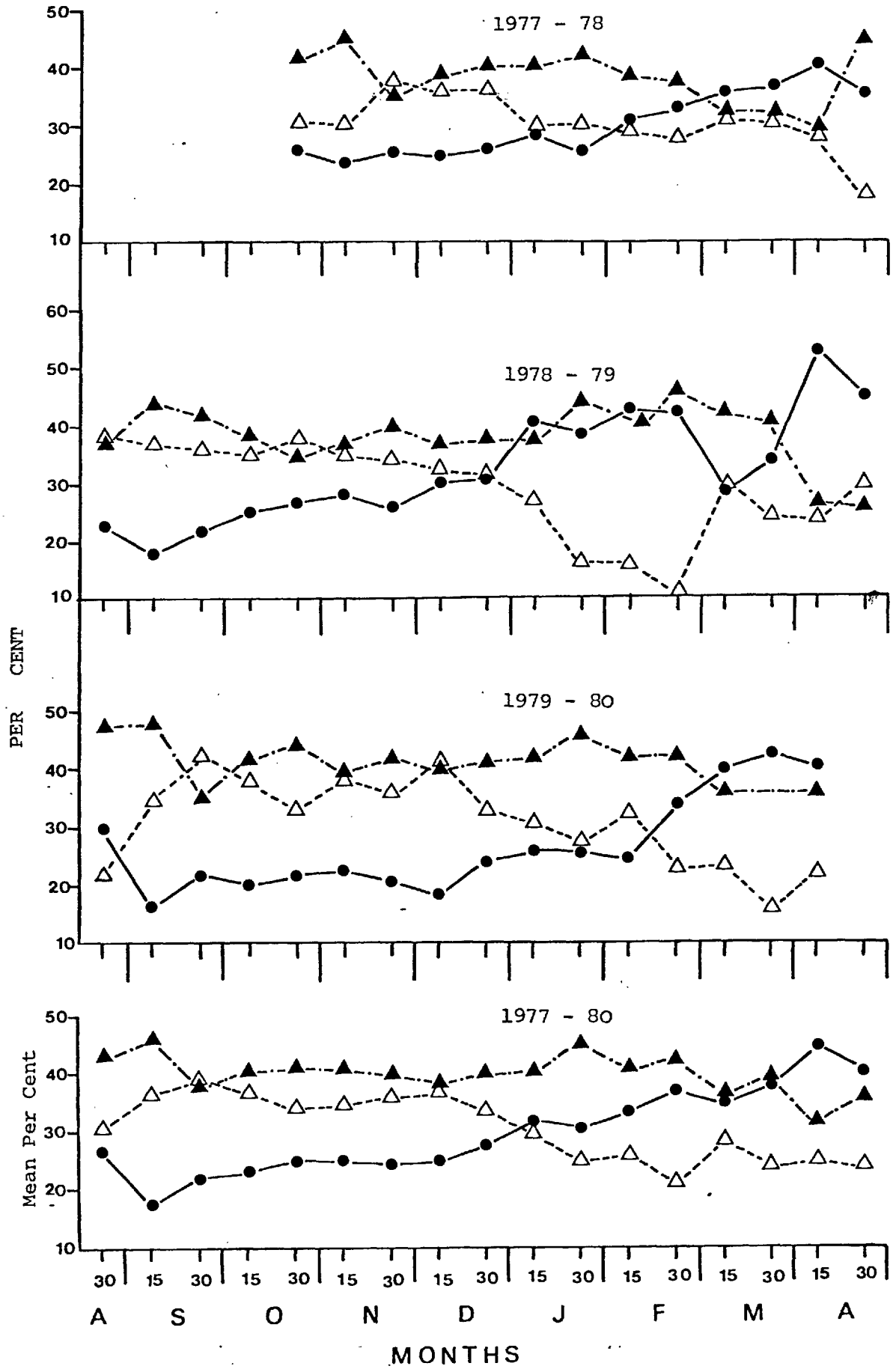


Fig 7.11 Distribution of Cx. pipiens on the roof (●—●), upper section (△---△) and lower section (▲-·-▲) of wall, of hibernation site F_2 at different times during hibernation. The figures '15' and '30' represent first and second halves of each month. (Results for 1977 - 1980).

7.4 Movement of mosquitoes

The rate at which mosquitoes entered or left hibernation sites was recorded with entrance and exit traps fixed onto the air bricks at room G_1. Each trap (Fig 7.12) was similar to Worth's (1953) window trap. They consist of a conical transparent plastic funnel 48.3 cm long and 30.5 cm in diameter which has been stapled onto a plywood board 34.3 cm^2 with an opening 30.5 cm in diameter cut from it to accommodate the funnel. The plywood was screwed onto the air brick and a screen cage 34.3 cm^3 supported on a stand was placed a few cm from the air brick so that the narrow apex of the funnel was inserted into it. The top of the cage was sheltered to prevent contamination by pigeon droppings and rain. Mosquitoes were aspirated from the cages 2 - 3 times a week.

Some of the mosquitoes collected in the exit trap at different times during hibernation were marked as soon as they were collected with fluorescent powder and released outside at the point of collection. One or two days after releasing marked mosquitoes the entire hibernation sites were searched thoroughly with a UV lamp to see whether any marked mosquito had re-entered the sites.

Further indication of movement during hibernation was recorded by marking the positions of some of the resting mosquitoes on the walls of room G_{14} on several occasions and recording the numbers that left their positions at pre-determined time interval.



Fig 7.12 Exit trap used to collect mosquitoes leaving the hibernation site.

Table 7.3 Total number of C. pipiens (and Cs. annulata in parenthesis) caught at different times during hibernation in Entry and Exit traps fixed onto room G_1, 1977 - 1980

		Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
	Entrance Trap	-	-	0	3	1	9	5	0	0
1977-78				(0)	(1)	(0)	(2)	(1)	(0)	(0)
	Exit Trap	-	-	13	43	30	52	93	8	0
				(1)	(2)	(2)	(3)	(1)	(0)	(0)
	Entrance Trap	45	9	6	0	7	0	0	0	0
1978-79		(0)	(0)	(4)	(0)	(2)	(0)	(0)	(0)	(0)
	Exit Trap	1	15	27	24	14	21	20	13	2
		(0)	(0)	(3)	(1)	(0)	(2)	(0)	(0)	(0)
	Entrance Trap	17	33	7	19	6	0	0	0	0
1979-80		(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
	Exit Trap	14	16	-	-	-	31	14	9	0
		(0)	(0)				(0)	(0)	(0)	(0)

Dashes, no record taken

Table 7.4 Movement of Cx. pipiens in and out of hibernation sites at different times during hibernation as determined by mark-recapture method.

Date mosquito marked	No. marked	Powder	No. of marked mosquitoes re-entering site (Site of collection in Parenthesis)
Oct.	5	Orange MS	2 (1 in Entry trap, 1 in Room G_1)
Nov.	8	Yellow LS	0
Dec.	20	Red LS	4 (1 in Entry trap, 3 in Room G_1)
Jan.	7	Magenta DS	0
Feb.	29	Blue LS	1 (Room G_9)
Mar.	12	Orange MS	0
Apr.	18	Yellow LS	0

Table 7.5 Movement of hibernating Cx. pipiens as determined by marking the positions of mosquitoes in Room G_{14}

Date (1979-80)	No. marked	No. of mosquitoes moved on days after positions were marked					
		1	2	3	4	5	6
Oct.	20	3	4	6	10	10	11
Nov.	15	0	3	9	9	9	10
Dec.	15	0	0	5	5	6	6
Jan.	13	1	3	4	4	4	6
Feb.	7	1	1	2	3	3	3

The results obtained in these studies are presented in Tables 7.3 - 7.5. It was observed that throughout the course of hibernation mosquitoes constantly moved out of the site (Table 7.3). Since no Cx. pipiens were observed breeding in the field at this time all mosquitoes collected in the entry trap must have been ones that had recently left the hibernation site. This was substantiated by the marking experiments (Table 7.4). Between October and February about 10% of the mosquitoes that left sites re-entered sites. There would probably be a high mortality of these mosquitoes leaving the sites due to adverse weather conditions. Substantial movement occurred among mosquitoes already in the site as shown by marking the resting sites (Table 7.5). Free-flying mosquitoes were often seen in the sites throughout the study period especially during any warmer weather.

7.5 Colonization and positive identification of species

Investigations were carried out to determine the maximum time required to break diapause in hibernating mosquitoes collected at different times during the hibernation period. Such information is necessary in laboratory work involving colonization of over-wintering mosquitoes. Egg-rafts laid by these mosquitoes, after successfully taking a blood meal, were used for species identification (see Section 2.2).

Mosquitoes were collected from rooms G_8, G_9 and G_{10} of the hibernation site by aspiration and transferred to a holding tube (WHO 1970) with a maximum of 10 per container. Care was taken

in handling them to avoid any possible damage which could produce misleading results. The tubes were warmly wrapped in a woollen scarf and quickly taken to the insectary at $21 \pm 1^{o}C$ and 50% r.h. They were then left standing for about 10 minutes after which any damaged mosquitoes were removed. Between 15 and 20 mosquitoes were placed in a colony cage 45 cm^3 in which was placed a restrained pigeon (see Section 3.2.2) as a source of blood meal. The mosquitoes were left in the dark for 4 h. The number of fed mosquitoes was recorded. The mosquitoes were supplied with a water source and then exposed to constant illumination provided by Philips 65/80 watt fluorescent tube. The feeding process was repeated every 72 h for 18 days having removed the water 24 h before each feeding. Dead mosquitoes were removed and counted. Blood fed mosquitoes were removed with an aspirator and placed in separate 16 cm^3 oviposition cages provided with plastic bowls containing a small quantity of water to receive the egg-rafts. Some of the rafts were bred up to adult and the species positively identified by means of male phallosome characteristics (see Section 2.2.2).

The results obtained from observations made on a total of 15 - 55 mosquitoes collected each month during hibernation are presented in Table 7.6

In the early months of hibernation (August - October) exposure to constant light for a maximum of 12 days was required before mosquitoes would take a blood meal. As hibernation proceeded, however, less time was needed and towards the end of hibernation

Table 7.6 Success in feeding rate of hibernating Cx. pipiens exposed to prolonged illumination.

Month	No. collected	No. fed	No. unfed	No. dead	% Number feeding on days after exposure to light						
					0	3	6	9	12	15	18
Aug.	15	7	5	3	0	0	0	0	8.3	0	50.0
Sept.	40	25	7	8	0	-	0	3.1	9.4	34.4	31.3
Oct.	55	38	11	6	-	-	0	0	26.5	36.7	14.3
Nov.	35	27	8	0	0	0	0	0	34.3	42.9	0
Dec.	34	20	7	7	-	0	0	33.3	63.0	3.7	0
Jan.	39	34	3	2	0	0	5.7	40.0	22.9	28.6	0
Feb.	35	30	2	3	0	0	25.0	46.9	9.4	12.5	0
Mar.	38	35	3	0	0	7.9	47.4	26.3	10.5	0	0
Apr.	30	28	2	0	0	53.3	13.3	23.3	3.3	0	0

Dashes - no feeding test

(March - April) mosquitoes fed readily after 3 - 6 days of exposure to light. Exposure to prolonged illumination has an activating influence on hibernating mosquitoes and stiumulates gorging (Tate & Vincent, 1932, 1936).

Representative samples of adults bred from a total of 219 egg-rafts were positively identified as Cx. pipiens. No Cx. torrentium was observed.

7.6 The physiological condition of species

7.6.1 Parous rates and fertility

Most of the mosquitoes used for age-grading were collected from the basement cellars of Sandy Ride House (Fig 1.1) which like Site F and G harboured a high population of hibernating species. Only a few samples were taken from Site G (rooms 8 - 10) to avoid depletion of the population.

At the beginning of each month from August to April hibernating mosquitoes were collected with an aspirator from the walls and ceiling of hibernation sites. They were transferred to holding tubes and taken to the laboratory where they were immobilized by placing them in a freezing chamber. Dissections were carried out either immediately or after storage at $-20^{\circ}C$ for a maximum period of 3 days.

Parity in mosquitoes collected during the winter period of 1977 - 78 was determined by Detinova's (1945, 1962) method of ovarian tracheation. Ovaries were examined for the nature of tracheolar skeins: tightly coiled tracheoles indicate nulliparity while uncoiled tracheoles indicate parity. As a check on these

results the stage of development of the ovarian follicles in the second half of the ovaries was determined using Christopher's (1911) method of gonotrophic condition.

During 1978 - 79 and 1979 - 80 the physiological age of mosquitoes was determined by Polovodova's method (in Detinova 1962). Ovaries were dissected out in physiological saline and the stalks straightened out thoroughly and examined for the presence of dilatations (Gillies & Wilkes, 1965; and pers. comm.). The number of dilatations on the follicular tube indicated the sites of previously ovulated follicles and hence the parous rate.

In order to obtain information on the rate of insemination in mosquitoes the spermothecae were dissected out in physiological saline and transferred to another drop of saline. They were gently crushed by the application of little pressure to a cover slip and then examined under a binocular microscope at 400X magnification, for motile sperm.

The results of the parous rates of mosquitoes examined during 1977 - 1980 are presented in Table 7.7.

All 202 mosquitoes dissected in 1977 - 78 were nulliparous. However, in 1978 - 79 and 1979 - 80 a very small number of 1-parous mosquitoes were collected in August and September. From October until the end of hibernation only nulliparous mosquitoes were collected. Examination of the ovarioles for yolk deposition in 200 mosquitoes collected in 1977 - 78 showed that they all had

Table 7.7 Parous rates of Cx. pipiens collected from hibernation sites at different times during the winter periods of 1977-1980.

Month	1977 - 78		1978 - 79		1979 - 80		Total	
	No. dissected	parous	No. dissected	parous	No. dissected	parous	No. dissected	parous
Aug.	0	0	23	0	45	2	68	2 (2.9%)
Sept.	0	0	40	2	36	1	76	3 (4.0%)
Oct.	40	0	41	0	38	0	119	0
Nov.	40	0	37	0	26	0	103	0
Dec.	39	0	28	0	25	0	92	0
Jan.	23	0	25	0	27	0	75	0
Feb.	30	0	23	0	18	0	71	0
Mar.	25	0	17	0	14	0	56	0
Apr.	5	0	9	0	10	0	24	0
Total	202	0	243	2	239	3	684	5
		0%		0.8%		1.3%		0.7%

Table 7.8 Insemination rate of mosquitoes collected from hibernation sites during the winter periods of 1977 - 1980.

Period	No. dissected	No. inseminated
1977/1978	90	85 (94.4%)
1978/1979	183	161 (88.0%)
1979/1980	206	188 (91.3%)
Total	479	434 (90.6%)

their ovarioles in Stage II of Christophers (1911).

Ninety per cent of all mosquitoes dissected for insemination contained sperm in the spermothecae (Table 7.8) and this did not vary significantly in the three years of this study.

On 14th March 1978 during the routine collection of mosquitoes for laboratory studies, one blood fed Cx. pipiens was collected from room G_{11}. Analysis of blood meal by precipitin test (Boreham, pers. comm). showed that it had fed on a passerine.

7.6.2 Sugar feeding

Floral nectars consist of about 50% fructose which may be present as free fructose or as the fructose component of sucrose (Van Handel et al, 1972) both of which can be detected in mosquitoes with a single reagent (Van Handel, 1967).

In the present studies mosquitoes were removed from the entry trap and walls of hibernation site at the onset of hibernation and tested for the presence of sugar in their guts using anthrone test (Van Handel 1972).

None of the 79 mosquitoes tested between August and early October gave a positive reaction. Since sugars converted into fat and glycogen cannot be determined (Bidlingmayer & Hem 1973) this observation shows complete conversion had taken place before mosquitoes came into hibernation sites.

7.6.3 Estimation of fat and glycogen

Studies on the food reserves of hibernating mosquitoes have been undertaken by Buxton (1935) but very little is known about changes which may occur during the hibernation period. In the present study fat and glycogen were extracted from individual mosquitoes during the course of hibernation in order to access their nutritional status.

Mosquitoes were collected twice a month from the walls of rooms G_{7-13} with a glass aspirator and transferred to a plastic holding tube. Much care was taken in handling the mosquitoes to avoid any damage. The tubes were taken to the laboratory and left standing for 5 minutes and any damaged specimens discarded. Mosquitoes were stunned by putting them in a freezing chamber and then stored at -40°C until assay for fats and glycogen were undertaken.

Twenty five mosquitoes were used for each extraction whenever population densities permitted.

Extraction of fat was carried out by the method of Bursell (1959) with slight modification (P. A. Langley, pers. comm.). Mosquitoes were placed separately in a shallow partitioned agglutination tray and dried to constant weight by leaving them for 24 h in an oven at 67 ± 2°C. The tray was quickly removed and transferred into a desiccator containing silica gel from where each mosquito was picked up with a fine forceps and weighed on a Cahn Gram Electrobalance (Cahn Instruments, Paramount, California, U.S.A.)

The weighed mosquitoes were then placed individually in stoppered 3.5 x 2 mm glass vials containing 3 ml of a mixture of 2 : 1 chloroform - methanol and left standing for 48 h at room temperature. The chloroform - methanol mixture was changed approximately every 16 h in order to ensure complete extraction. The mosquitoes were then carefully removed from the tubes, dried again in an oven for ½ h and reweighed. The amount of chloroform - methanol extractable lipid substances, referred to as fat, was determined by subtraction (Folch et al, 1957; Bursell, 1959; Cockbain, 1960).

Glycogen was extracted from mosquitoes using the Montgomery method as described by MacInnis (1970). The phenol - sulphuric acid method is simple, rapid and highly sensitive (Montgomery, 1957).

Each mosquito was dried and weighed as above and homogenized in 2 ml distilled water. The sample was mixed with phenol and sulphuric acid. The resultant colour was read at 490 mμ in a spectrophotometer Model G (Beckman DB, Great Britain). The quantity of glycogen in each sample was then calculated from the standard curve using Oyster glycogen Type II (Sigma Chemical Comp. U.S.A.) as a standard.

The results of fat and glycogen carried out in the two winter periods of 1977 - 1979 are represented graphically in Fig 7.13 and 7.14. Details of extraction are presented in Appendices 7.6 and 7.7.

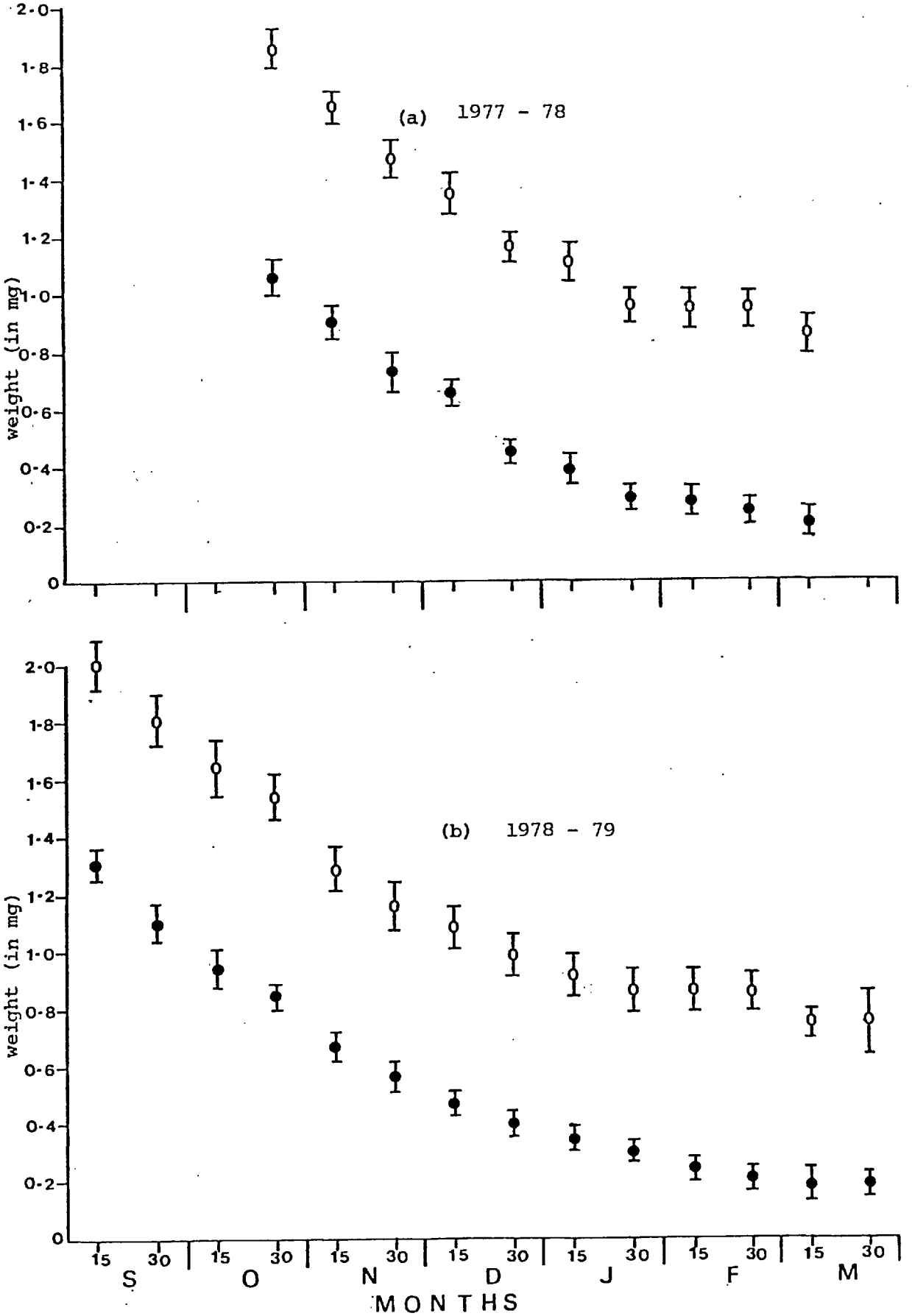


Fig 7.13 Reduction in the mean dry weight (O) and fat content (●) of Cx. pipiens at different times during hibernation (Vertical bars indicate $\pm$ SE; The numbers "15" and "30" indicate first and second halves of each month). (Results for 1977 - 79).

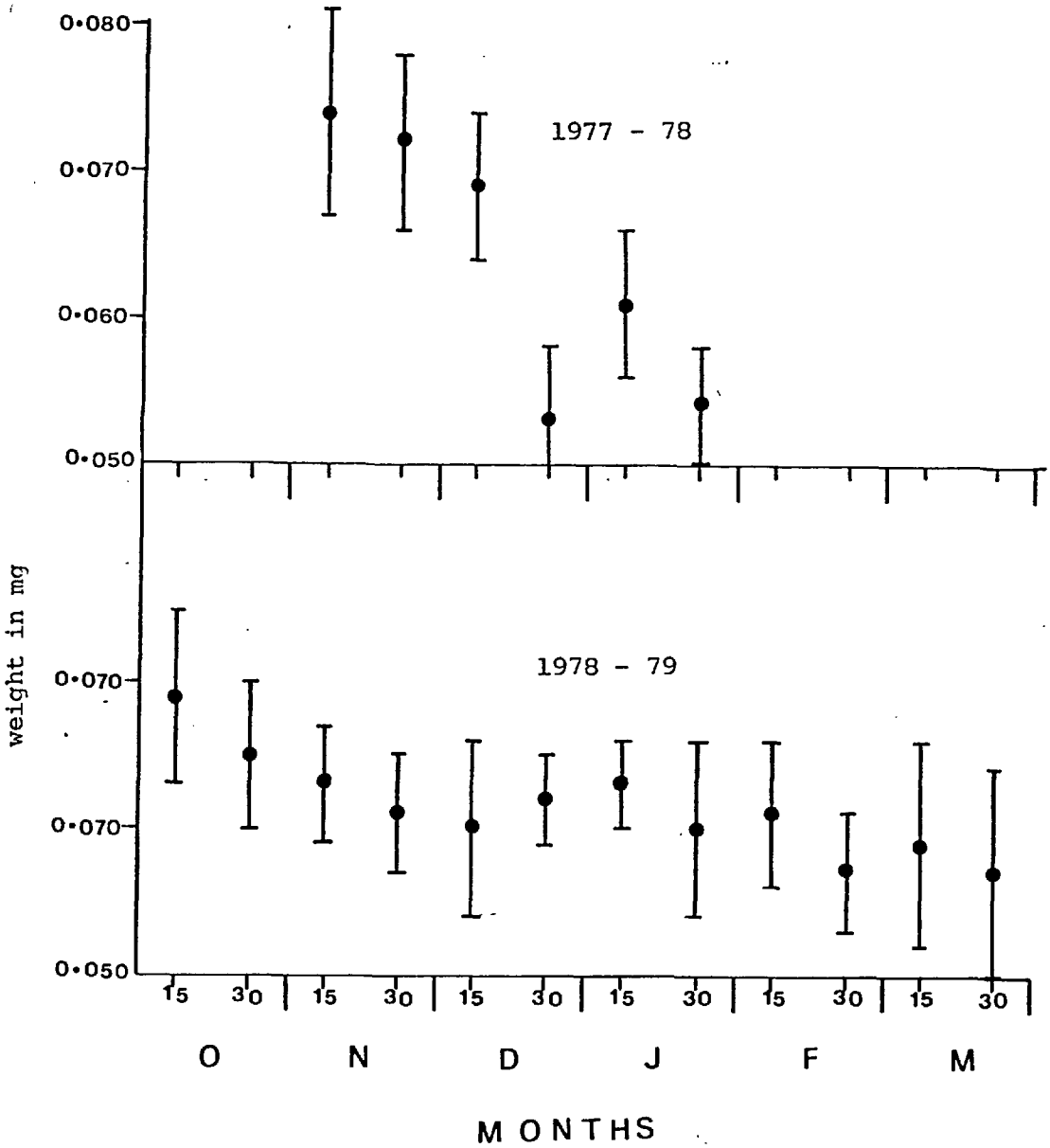


Fig 7.14 Quantity of glycogen in an average Cx. pipiens collected at different periods during hibernation (Vertical bars in dicate $\pm$ SE; The numbers '15' and '30' indicate first and second halves of each month. (Results for 1977 - 79).

There was a gradual reduction in the dry weight and fat content of mosquito as hibernation proceeded (Fig 7.13). During late October when most of the hibernating species have gone into sites the mean quantity of fat was found to be 1.06 and 0.84 mg in 1977 and 1978 respectively. In early March towards the end of hibernation the mean quantity of fat has dropped to 0.203 and 0.180 mg respectively, approximately 20% of the values for October. At the same time the mean dry weight of mosquito has reduced to less than a half. A t test shows that for each winter the difference in the dry weight and quantity of fat between samples of mosquitoes collected in late October and early March was significant ($P < 0.001$).

There was a small change in the quantity of glycogen in mosquitoes as hibernation progressed (Fig 7.14). However, a t test shows that there was a significant difference ($P < 0.05$) between samples of mosquito collected in early November and late January 1977 - 78. There was no significant difference in glycogen ($P > 0.05$) between samples taken at the same time in 1978 - 79.

7.7 Mortality factors

A number of factors could be affecting the mortality of the mosquitoes. An attempt to identify those which are the most important has been made.

7.7.1 Exhaustion of food reserves

The importance of depletion of fat reserves as a direct or indirect cause of death was investigated using dead mosquitoes found on the floor of the hibernation site and mosquitoes caught

in the exit trap.

a) dead mosquitoes on the floor

A white piece of paper towelling was spread on the floors of some of the rooms in Site G so that dead mosquitoes falling on the floor were easily collected. In December and January 1978 - 80 these rooms were visited every day and any dead mosquitoes collected. They were then stored in a deep freeze at -40°C after dried mosquitoes had been excluded. The fat content of these mosquitoes was estimated as described in Section 7.6.3. The results for each two-week period were compared with fat extracted from live mosquitoes collected at the same time.

From the results obtained (Table 7.9) it was found that the dead mosquitoes contained significantly less quantity of fat than live mosquitoes collected from the walls and ceiling of the same site.

Table 7.9 Mean quantity of fat in mg ($\pm$SE) in dead mosquitoes collected at different times during hibernation and compared with live mosquitoes using a t - test.

		Dead mosquitoes		Live mosquitoes	
	Date	No. collected	Quantity of fat (mg)	Quantity of fat (mg)	Level of significance
1978-79	Dec. 1-15	9	0.34 ± 0.04	0.47 ± 0.03	$P < 0.001$
	Dec.16-30	15	0.37 ± 0.03	0.40 ± 0.03	$P < 0.05$
1979-80	Dec.16-30	15	0.28 ± 0.03	0.40 ± 0.03	$P < 0.02$
	Jan. 1-15	8	0.26 ± 0.04	0.35 ± 0.03	$P < 0.001$

b) Mosquitoes in exit trap

Some of the mosquitoes caught in exit traps located on room G_1 and later room G_3 were collected daily and fat extracted from them. The mean quantity of fat in collections made every half month were compared with that extracted from live mosquitoes collected at the same time.

It was observed from the results of collections made between October and November (Table 7.10) that mosquitoes in exit traps generally had less fat than those within the sites.

Table 7.10 Mean quantity of fat in mg ($\pm$SE) in mosquitoes collected in exit trap at different times during hibernation and compared with those collected inside site using a t - test

	Date	Mosquitoes in exit trap		Mosquitoes inside site	Level of significance
		No. collected	Quantity of fat (mg)	Quantity of fat (mg)	
1978-79	Oct.16-30	11	0.56 ± 0.07	0.84 ± 0.03	$P< 0.001$
	Nov. 1-15	15	0.55 ± 0.04	0.67 ± 0.04	$P<0.05$
	Nov.16-30	10	0.45 ± 0.04	0.57 ± 0.04	$P>0.10$
1979-80	Sep.16-30	14	0.64 ± 0.08	1.19 ± 0.05	$P<0.001$
	Oct. 1-15	8	0.49 ± 0.09	1.01 ± 0.06	$P<0.001$
	Oct.16-30	8	0.60 ± 0.11	0.74 ± 0.05	$P>0.10$
	Nov. 1-15	25	0.54 ± 0.04	0.74 ± 0.05	$P<0.01$

7.7.2 Predators and parasites

Three species of spiders were found in numbers in the hibernation sites and on several occasions some spiders were observed feeding on mosquitoes. Mosquitoes were also found enmeshed in spiderwebs and later some of their parts were found missing, apparently consumed by the spiders. A few Reduviid bugs (Empicoris vagabundus L.) were occasionally seen in the site.

On a number of occasions during the study period these potential predators of mosquitoes were removed from the walls of hibernation site. They were immediately taken to the laboratory and their gut contents extracted as described in Section 6. These were tested with anti-adult mosquito serum to see whether they have fed on mosquitoes. The results obtained are presented in Table 7.11.

Of the 89 spiders tested, 43 (48.3%) gave a positive reaction.

Some of the dead mosquitoes collected from the floor of the hibernation sites were examined under the microscope to see whether any fungal infection was the cause of death. Of the twenty-three mosquitoes examined no fungal infection was observed.

A few dead mosquitoes found on the floor of hibernation sites have distended abdomen rather suggestive of protozoan infection. No protozoan were observed in three specimens dissected and observed under a microscope. One such specimen was sent to Unit of Vertebrate Virology, Oxford for screening. No virus was present. However, chains of a large rod-like bacterium with associated bacterial spores were present in fair concentration (L.K. Spencer,

Table 7.11 List of suspected predators of hibernating adult Cx. pipiens with numbers of gut smears tested against anti-adult sera and percentage showing positive [a] reaction.

Species	No. tests	% positive
Araneae		
Amaurobiidae		
Amaurobius ferox (Walckenaer)	28	64.3
Scytodidae		
Scytodes thoracica Latreille	39	30.7
Agelenidae		
Tegenaria domestica (Clerk)	22	59.1
Hemiptera		
Reduviidae		
Empicoris vagabundus	5	0

pers. comm.).

Some of the dead mosquitoes found either on the walls of site or enmeshed in spiderwebs were covered with saprophytic Penicillium, P. brevicompactum Dierckx (A. H. S. Onions, pers. comm.).

7.7.3 Predation rate of Spiders

The predation rate of spiders was assessed by a functional response experiment in which different densities of mosquitoes were offered to the spider and the number eaten over a time interval recorded. It was hoped that this information would be useful in assessing the possible role of predatory spiders in reducing the population number of mosquitoes during the winter period. The procedure is as follows:

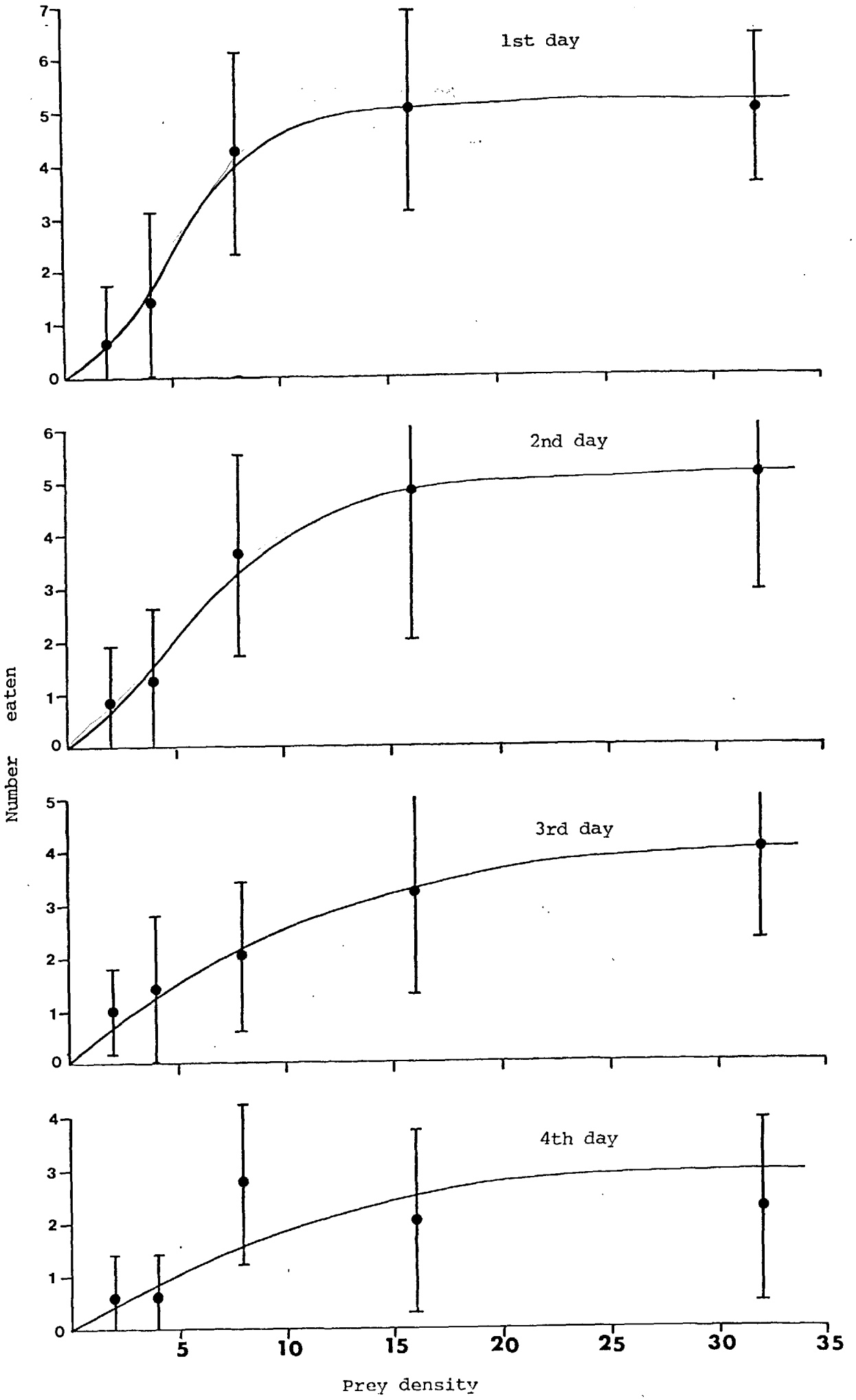
Amaurobius ferox identified by the precipitin test to feed on mosquitoes more than the other two common species (Table 7.11) were collected from the walls of the hibernation site and placed seperately in 16 x 16 x 16 cm screen cages. They were fed for two days with excess mosquitoes which were collected and introduced into the cages with the aid of a glass aspirator. Then they were starved for 48 h in order to stimulate feeding responses. During this period of captivity webs were spun in the cages. Different densities of hibernating mosquitoes were introduced into the cages with a glass aspirator taking care not to get them entangled in the web prematurely. Densities of 2, 4, 8, 16 and 32 were employed with five replicates of each. Controls consisting of mosquitoes and no predators were set up at each density. After 24 h the number of live and dead mosquitoes in each cage was carefully counted. Missing mosquitoes were assumed to have been consumed by the spiders.

The number of mosquito in each cage was brought up to the original number and the observation repeated after 48, 72 and 96 h. Most of the dead mosquitoes recorded have been partially eaten and in many of them only the abdominal aspect was missing. It seems likely that the spiders prefer the softer parts of their prey leaving the harder head and thoracic regions. In the present study, however, all mosquitoes killed, partially consumed and completely eaten were grouped together. All observations were carried out in room G_1 between October - December 1979.

Details of the results obtained are presented in Appendix 7.8 while Fig 7.15 shows the functional response curves. On the first and second days of the experiment the functional response curves obtained are of Holling's (1959a) sigmoid type III. (Type III response was thought to be characteristic of vertebrate predators but is now known to occur in arthropod predators (Murdoch & Oaten, 1975; Hassell et al, 1977)). During this time, an increasing proportion of mosquitoes are killed by spiders until the response begins to level off due to the combined effects of satiation and handling time. On the third day of the experiment the predators may be getting satiated and the curve begins to resemble Holling's (1959a) type II functional response. On the fourth day, feeding has been depressed due to satiation. Variability in spiders tended to increase with time.

The handling time (T_h) calculated from the upper level of the curves shows the values of 4.8, 4.8, 6 and 6.3 h for the first, second, third and fourth days, respectively suggesting a gradual increase in T_h as the experiment progressed. It was

Fig 7.15 The functional response of Amaurobius ferox to changes in prey density of adult Cx. pipiens. Means, and 95% confidence limit are plotted. Curves have been fitted by eye.


1st day
2nd day
3rd day
4th day
Number eaten
Prey density
0
1
2
3
4
5
6
7
10
15
20
25
30
35

observed from the experiment that a starved spider that is surrounded by its prey may feed on a maximum of five mosquitoes each day for the first two days. On the third and fourth day, however, this number may be reduced to about 4 and 3, respectively.

7.8 Estimation of survival rate of mosquitoes

It has been established above that movement of mosquitoes out of hibernation sites, predation by spiders and death apparently due to starvation are all factors which may account for the substantial reduction in numbers of hibernating mosquitoes. An attempt was made to determine the impact of these factors on a mosquito population of known size.

7.8.1 Method of study

The investigation was carried out in room G_1 (Fig 7.2).

On October 29th 1979 when most of the hibernating mosquitoes have gone into sites all openings on walls leading from this room to other rooms of site G were carefully covered with netting. This prevented any movement of mosquitoes in and out of this room while at the same time maintaining air circulation. All dead mosquitoes found on the spiderwebs and on the walls of the site were carefully removed. An exit trap was fitted onto the only Airbrick leading from the room to the outside. A white sheet of paper towelling was spread over the entire floor of the room so that dead mosquitoes falling onto the floor were easily found. The population count of mosquitoes and spiders resting on the walls and roof was carefully taken once every week usually on Mondays. Accuracy of

population count was estimated at $\pm$2% in recounts of several samples. Mosquitoes enmeshed in spiderwebs and later killed by spiders, as well as dead mosquitoes found on the walls and floor were counted and removed to avoid being counted twice. All dead mosquitoes caught in the exit trap were regularly removed and recorded. One problem encountered during the course of this study was the fact that a good portion of the walls of this room were covered with webs which were flat on the rough surface of the walls. Sometimes it was difficult to ascertain whether any dead mosquito on the wall was killed by a spider and not eaten or just died and could have fallen on the floor if not held by webs. Therefore some mosquitoes killed by spiders might have been recorded as "dead on the wall" and vice versa.

7.8.2 Results

The results are represented graphically in Fig 7.16. Details are presented in Appendix 7.9.

There was a gradual reduction in the population size of Cx. pipiens as hibernation progressed until the first week of May when no more mosquitoes were left in the site. The population of spiders remained fairly constant until towards the end of hibernation when their number also decreased (Fig 7.16a). At the beginning of the study period many mosquitoes were found enmeshed in spiderwebs. This number decreased as hibernation progressed (Fig 7.16b). The number of dead mosquitoes on the wall and floor increased towards the end of hibernation (Fig 7.16b). Throughout

Fig 7.16 Population record of Cx. pipiens and spiders in room G_3

(a) Total population count of mosquitoes (●——●) and spiders (0---0) taken weekly during the course of hibernation, 1979.

(b) Total number of mosquitoes enmeshed in web and later killed by spider (●——●) and number found dead on the wall and roof (0---0) of site during the course of hibernation, 1979 - 80.

(c) Number of mosquitoes caught in eixt trap at different times during hibernation, 1979 - 80.

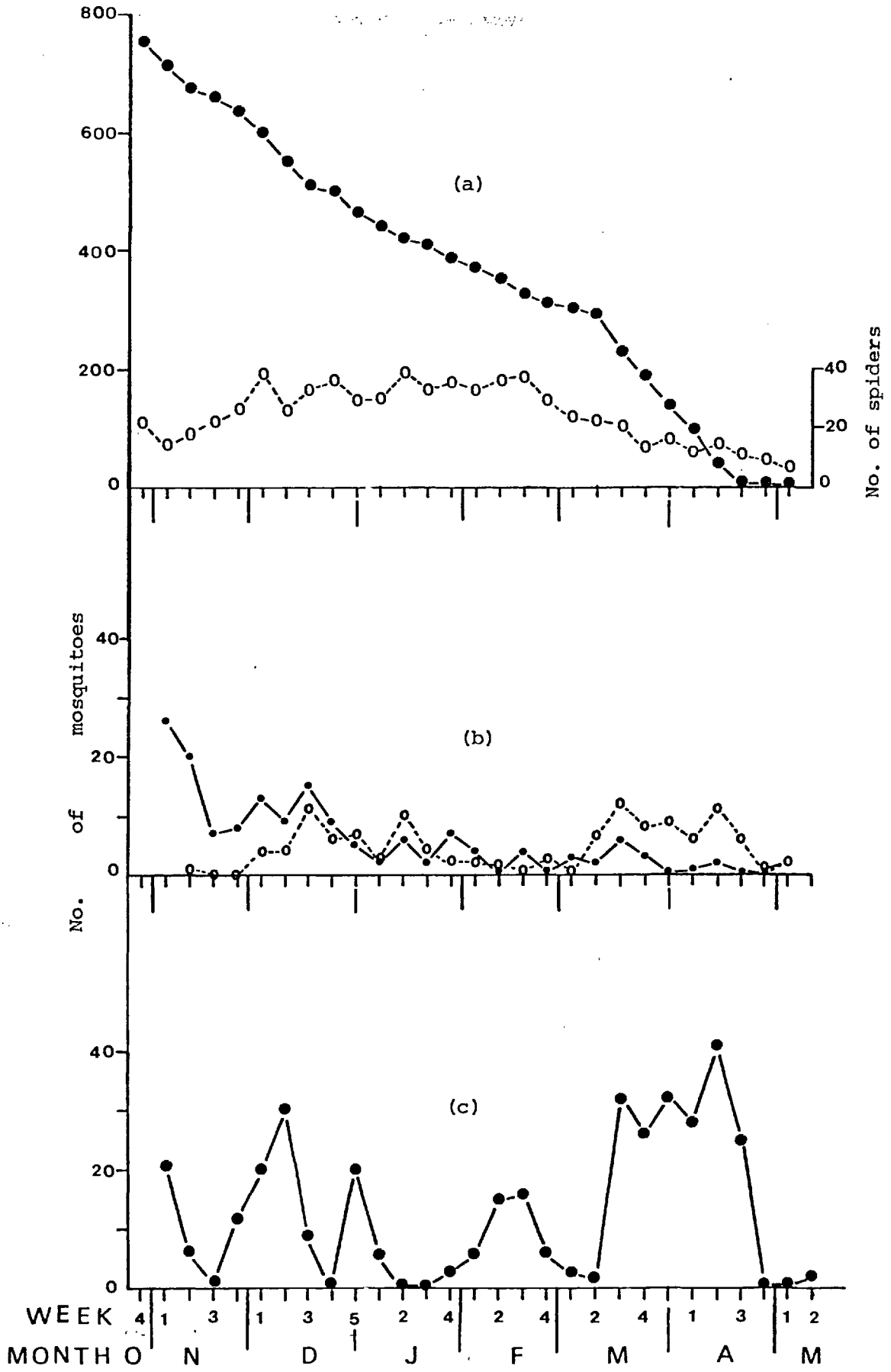

800
600
400
200
0
(a)
40
20
0
No. of spiders
40
20
0
No. of mosquitoes
(b)
40
20
0
(c)
WEEK 4 1 3 1 3 5 2 4 2 4 2 4 1 3 1 2
MONTH O N D J F M A M

Fig 7.16

the study period mosquitoes were constantly caught in the exit trap. More mosquitoes were caught in December and towards the end of hibernation than at any other month (Fig 7.16c). It was observed that throughout the study period a total of 154 (20.3%) mosquitoes out of the original 759 counted in the room were caught in webs, while 118 (15.6%) were found dead on the wall and floor of site. A total of 120 (15.8%) mosquitoes could not be accounted for.

Supposing that hibernating mosquitoes started leaving sites finally (that is, without re-entering site) at the beginning of April, it was observed in the present study that by the end of March 256 (33.7%) mosquitoes had left the site. If, as estimated from Section 7.4 (Table 7.4) about 8.6% of this number re-entered site elsewhere, then 234 (30.8%) may not have succeeded in re-entering site and would not have survived the cold temperature outside.

Very few Cs.annulata were present in this site at the beginning of the study. By the third week of February no more of this species was present (Appendix 7.9).

7.9 The hibernation site for Cx. torrentium

It was observed from the results of colonization experiment (Section 7.5) that Cx.torrentium was absent in Silwood house basement (Sites F_1, F_2 and G; Fig 7.1 and 7.2) which harboured a high population of hibernating Cx. pipiens. An attempt was made to find the possible hibernation site for this species particularly as it was observed to be abundant in the field during the summer (Section 5).



Fig 7.17 Battery-operated suction device used to search rodent burrows and large tree holes for hibernating Cx. torrentium

Samples of hibernating mosquitoes collected from Green-houses located at Silwood Park and Ashurst lodge and also from two derelict buildings in Sandy Ride (Fig 1.1) were first observed for the presence of pre-alar scales and then some were colonized as described in Section 7.5. The progeny from eggs obtained were reared and the F_1 generation identified by means of phallosome characteristics. None of the 17 individuals colonized were Cx. torrentium. It was concluded from this observation and that of Section 7.5 that Cx. torrentium was absent in shelters where Cx. pipiens was present. The search was therefore extended further into the field.

Fifteen rabbit burrows and 11 tree holes located in the areas of Cannon Wood, North Gravel and Rookery Corpse (Fig 1.1) were selected for the observation. Starting from 4th September 1979 about two weeks after hibernating mosquitoes were seen in Silwood House basement (Fig 7.1 and 7.2) these areas were searched once a week usually between 9 a.m. - 12 noon with a battery-operated suction device (Fig 7.17). The suction apparatus was pushed down the rodent burrow and large holes in fallen trees after a stick was used to dislodge any resting mosquitoes. Smaller tree holes were thoroughly searched with a smaller pipe fitted onto the end of the suction equipment. Two piles of wood located in Cannon Wood were searched once every fortnight. No Cx. torrentium were found, neither were any other species of mosquitoes. Searching was discontinued on 18 December 1979.

7.10 Discussion

Hibernating adult females of British mosquitoes pass the

unfavourable winter period in suitable hiding places either in a "torpid condition" of complete hibernation or in a less inactive state of partial hibernation (Marshall, 1938). In the former case (e.g. Cx. pipiens) hibernating populations live entirely on fat reserves which they have accumulated at the onset of hibernation. In the latter case (e.g. Cs. annulata) mosquitoes supplement their food reserves by leaving their hibernation quarters to obtain a blood meal during favourable weather conditions.

Initiation of hibernation responses in Cx. pipiens is reported to be due to a combination of short photoperiod and cool temperature in autumn which acts as a phenological control altering the physiological response of mosquitoes (Danilevskii & Glinyanaya, 1958; Vinogradova, 1960; Whang, 1961; Beck, 1968; Eldridge, 1968). This results in cessation of ovarian development and species consume carbohydrates, develop fat and enters hibernation sites (Vinogradova, 1960; Spielman & Wong, 1973b; Sanburg & Larsen, 1973). The photosensitive period occurs during the aquatic stage (Oda 1968) and more specifically the pupa has been identified as the critical photosensitive stage (Eldridge, 1968; Spielman & Wong, 1973a).

Cx. pipiens utilizes a wide variety of shelters as hibernation sites. There is, however, little information about their natural hibernation sites apart from the fact that they occur in caves (Service, 1971c).

Studies on overwintering populations of Cx. species by various workers show that there is a marked population fluctuation of

hibernating individuals during the winter period. Service (1968a) observed a rapid population build-up in this species at the beginning of hibernation which reached a peak in November and then dropped sharply by December. Similar observations were made in the present study.

The initial choice of site by hibernating mosquitoes is affected by a number of factors. Buffington (1972) observed that the initial choice of hibernation by Cx. pipiens in abandoned mines in Illinois is governed to an extent of 22.7% by light while distance from the entrance, texture and dampness of the wall appear unimportant. Shimogama & Takatsuki (1967) observed that the initial choice of site in caves near Nagasaki City by Cx. pallens were most frequently 10 - 15 m from the entrance where light was 0.3 - 0.0 Lux. They interpret this behaviour as a response to temperature and humidity which were different away from the entrance. The apparent concentration of mosquitoes near the entrances observed in Site F_2 (Fig 7.8 - 7.10) in the present study may be due to indirect illumination from the entrances. It was further observed that throughout the study period there were persistently more mosquitoes at Section No. 3 of Site F_2 (Fig 7.8 - 7.10) than in any other place. This particular area in a corner was damper than the rest of the walls of the site due to water dripping from the foot of the wall into a drain on the floor. Humidity of the surface of the wall may therefore influence distribution of mosquitoes. It was further observed that mosquitoes tended to prefer rough areas of the wall to smooth surfaces. Again texture of the wall may be important to distribution though to a lesser degree.

Extensive movement of mosquitoes during the course of winter has been reported by several workers. Buffington (1972) observed that more than 75% of Cx. pipiens in an abandoned mine in Illinois changed their hibernacula during the period of hibernation. Berg and Lang (1948) noted that in Massachusetts hibernating Cx. pipiens exhibit extensive movement with no mosquito occupying the same location for more than two weeks. Similar findings were reported by Lomax (1967) and Service (1968a). Movement in this species may be in response to temperature so that mosquitoes distribute themselves to remain in optimal temperature conditions (Muirhead-Thompson, 1938; Lomax, 1968).

Based on the information available in Table 7.5, it can be seen that about 50% of hibernating mosquitoes changed their location during the course of hibernation. It is considered that the extensive movement of mosquitoes within the sites observed in the present studies may be in response to small termperature variations. Movement of mosquitoes out of the sites as determined with exit traps may be in response to occasional spells of comparatively warm weather experienced during the winter period. The fact that room G_3 was covered thereby preventing free movement of mosquito within the various rooms of the site may also have contributed to the high number of mosquitoes caught in the exit trap (see Section 7.8) which was the only exit available to mosquitoes.

Less than 1% of the mosquitoes dissected had deposited eggs before coming into hibernation. Service (1968a) did not observe

any parous Cx. pipiens from 72 specimens he collected from Brownsea. Slaff and Crans (1977) however, observed that 13 of the 940 (1.4%) Cx. pipiens they dissected in two consecutive winters in New Jersey were parous. The fact that the five parous mosquitoes dissected in the present study were collected in August and September suggests that it is likely that parous mosquitoes may come into hibernation sites but are unlikely to survive the winter. The single blood fed mosquito collected on March 14, 1978 suggests that towards the end of hibernation, Cx. pipiens that left the sites too early during a spell of warm weather may successfully obtain a blood meal and may re-enter the site or hide in a fairly warm shelter rather than succumb to the unfavourable temperature conditions in the field.

Insemination in hibernating populations is an important phenomenon by which the continuity of the individuals is ensured. Service (1968a) observed that 5.4% of hibernating Cx. pipiens contained no sperm in their spermothecae. In the present study, 9.4% of the mosquitoes were not inseminated. This is considered rather high and may affect the overall survival rate of hibernating population since uninseminated mosquitoes will not produce new individuals even when they have survived the winter.

The food reserves of hibernating Cx. pipiens is in the form of fat which is gradually used up as hibernation progresses. Buxton (1935) observed that up to 85% of the fat reserves of Cx. pipiens are exhausted during the course of hibernation, whereas Teckle (1960) demonstrated a loss of more than 60% of

fat reserves in 6 weeks of induced hibernation of Cx. pipiens. In the present study it was observed that about 80% of the initial fat reserves of mosquitoes are exhausted during hibernation. A very slight reduction in the quantity of glycogen was also seen. Since glycogen is utilized for flight (Clements, 1955; Rowley, 1970; Nayar & Van Handel, 1971) this slight loss is interpreted as being due to extensive movement of mosquitoes observed in the course of this study during which glycogen was used.

A number of mortality factors are responsible for the reduction in the population of hibernating mosquitoes. These include spiders (Service, 1968a; Jumars et al, 1969), centipedes and phalangids (Buffington, 1972) fungi Cephalosporium sp. and Entomophthera sp. (Service, 1968a) but the most important factors appear to be variations in temperature and relative humidity (Jumars et al, 1969). However, Service (1968a) concluded that the decrease in Cx. pipiens population was mainly due to an exodus of hibernating individuals.

In the present study it was observed that exodus of hibernating mosquitoes contributed to more than 30% reduction in population size during the course of hibernation (Section 7.8). Another important factor is predation by spiders which as observed in site G_3 (Section 7.8) accounted for about 20% mortality. Predation by spiders was highest at the onset of hibernation when the population of mosquitoes was greatest. Dead mosquitoes collected from the wall and roof of the site contained less food reserves than live

mosquitoes collected at the same time (Table 7.9). Death in these mosquitoes cannot be attributed directly to the depletion of food reserves since mosquitoes that survived the entire winter often had less fat by the end of hibernation (Appendices 7.6 and 7.7) than the dead ones collected in the middle of hibernation period. In addition to their role in energy storage fats are probably involved also in the maintenance of cold hardiness (Buffington & Zar, 1968). It is considered possible that the small quantity of fat observed in the dead mosquitoes may have made them more susceptible to minor temperature and humidity changes in the sites or even to any infection. It is also possible that movement in hibernating mosquitoes is greater in those individuals with insufficient food reserved than in those fully laden with enough reserves, hence the difference in the fat content of mosquitoes in exit trap and those inside site (Table 7.10).

The absence of hibernating Cx. torrentium in all shelters where Cx. pipiens are present demonstrates a difference in the behaviour of these two species. Service (pers. comm.) did not find hibernating Cx. torrentium during his studies in the Poole area of Dorset and in collections over several years at Monks Wood. The absence of Cx. torrentium in rodent burrows and treeholes cannot be taken to mean that they do not hibernate in such natural sites. One problem with such natural sites is that despite the fact that they are numerous they may be difficult to locate and therefore mosquitoes resting in them are likely to be at low densities (Service, pers. comm.). Secondly because the rodent burrows are exposed any mosquitoes resting in them are likely to be found far down in the burrow and therefore may not

be easily picked up with a suction device. A detailed search of these natural sites using emergence traps is necessary before absence of Cx. torrentium in them is finally established.

GENERAL DISCUSSION

Prior to the discovery in Britain of Cx. torrentium (Mattingly, 1951) many studies had been made on Cx. pipiens sensu lato in various localities in the British Isles and in other countries. Marshall (1938) summarized information on Cx. pipiens and described the morphology and biology of Cx. molestus, which he recognized as a distinct species in the British fauna. Since the realization that Cx. torrentium was widespread and abundant in southern England (Service,1968c), it has become necessary to reconsider the status and biology of Cx. pipiens as compared with Cx. torrentium. The comparative rarity of Cx. molestus makes this third British species of the group less important to consider. This thesis represents the first detailed studies of sympatric populations of Cx. pipiens and Cx. torrentium in Britain.

The major problem for studies of sibling species such as Cx. pipiens and Cx. torrentium is to identify accurately the individual specimens. These two Culex species can be reliably identified only in the adult stage by means of male terminalia, and no other morphological characters have been found to distinguish them without error. Characters such as the differences in the wing venation (Natvig, 1948) and in the colour of the scales on the abdominal sternite (Mattingly, 1951) have been suggested, but were found to be inconsistent and therefore cannot be used with any measure of confidence (Service, 1968c). The present studies have further revealed a degree of unreliability in using the pre-alar patch of scales at the upper aspect of the sternopleuron to distinguish between the two species. The character of the male terminalia (Mattingly, 1951; Service, 1968c; and the present studies) therefore remains the only foolproof

method of identification currently available. It is obviously due to the similarity in the two Culex species that the presence of Cx. torrentium in Britain was not discovered until 1951, despite its widespread distribution.

A number of biological contrasts have been detected between Cx. pipiens and Cx. torrentium during the course of this study. The main behavioural divergence concerns the different overwintering habits which cannot yet be fully explained. The most accessible adult populations of Cx. pipiens s.l. are found in the hibernation sites. It might have been assumed that both Cx. pipiens and Cx. torrentium would be found together in suitable hibernation places. The results of this study are therefore surprising because none of the overwintering females studied were found to be Cx. torrentium on the bases of the morphology of male terminalia of their progeny. This raises the outstanding question of where Cx. torrentium hibernates during the winter, which has already been discussed in Section 7.10. The other chief distinction concerns oviposition site which, of course, determines the place where the immature stages can develop. It was found that in general Cx. pipiens prefers permanent ground water habitats whereas Cx. torrentium breeds essentially in artificial container habitats and also in tree holes. It follows that Cx. torrentium tends to be common in urban situations, due to the availability of numerous artificial flooded containers, etc. in which it breeds. Mattingly (1967) suggested that Cx. torrentium has become widespread in Britain and seems to be replacing Cx. pipiens to some extent. Mattingly also made the suggestion (cited in Service, 1968c) that in southern England Cx. torrentium has in recent years largely

replaced Cx. pipiens in peridomestic larval habitats. Observations made in the present studies seem to suggest that based on the population of pre-adult stages Cx. torrentium is the most common species of mosquito in Silwood Park, utilizing many more breeding sites than Cx. pipiens. However, since the overwintering populations of Culex found in all hibernation sites are Cx. pipiens it is then not clear which of the two species is more closely associated with man. The differences in the larval and adult habits may help to explain how the two ecologically similar species can coexist. In other aspects of development such as the duration of gonotrophic cycle and the duration of pre-adult stages, there are no appreciable differences in the two species.

Studies on the bionomics of Cx. pipiens and Cx. torrentium in the breeding places at Silwood Park suffer from the ambiguity that mixed populations of the two species were involved. Therefore it is only possible to discuss the dynamics of pre-adult populations irrespective of the species involved.

A number of methods have been employed in sampling the pre-adult populations of mosquitoes (see Section 5.1). A major problem often encountered is the difficulty of obtaining an absolute population estimate using the methods currently available. In his discussion of attempts to obtain quantitative estimates of population density, Service (1976) stated that "there is an overdue need for the development of new and better sampling procedures." The dipping method of sampling remains a technique most frequently employed in mosquito population surveys. Service (1973c) and Lakhani & Service (1974) estimated mortalities of pre-adults of An. claviger and Ae. cantans respectively,

using a dipping method of population sampling. It is difficult to obtain an absolute population estimate using this method, in situations where the population of mosquitoes are strongly aggregated. This problem would be obvious when looking at the complex nature of the permanent ground water habitats (especially Pond Field pond, Site A) sampled during the present study. However, in the case of artificial containers a technique using modified quadrat device was developed in this study, which gives a fairly accurate absolute estimate of population.

Among the possible population limiting factors (see Section 5.5) predation on the pre-adult populations of mosquitoes was considered to be important. Naiads were particularly abundant in the ground water habitats and were likely to be of special importance in reducing the larval populations of mosquito. It was found that Sympetrum striolatum was a more effective predator than Agrion puella. An overall mortality of pre-adult stages of more than 90% was recorded in the two ground water habitats.

Studies made on the hibernating population of Cx. pipiens showed that exodus of mosquitoes from hibernation sites was responsible for about 30% reduction in population size of hibernating individuals. Predation by spiders was also a major mortality factor responsible for about 20% reduction in population size while death apparently due to depletion of fat reserves and other unknown causes resulted to about 16% mortality. A further 16% of the population could not be accounted for (see Section 7.8). Assuming that this loss was also due to mortality it follows that less than 20% of the original population of hibernating individuals

would survive the winter.

Judging from the way that the first broods of Cx. torrentium and Cx. pipiens develop almost synchronously and at similar densities in the Spring, it might be expected that Cx. torrentium females pass the winter in similar numbers to the hibernating population of Cx. pipiens. This makes it all the more disappointing that the hibernation sites of Cx. torrentium were not discovered despite every effort.

SUMMARY

1 Laboratory and field studies were undertaken from October 1977 to April 1980 on the ecology and biology of the two sympatric sibling species of Culex, Cx. pipiens and Cx. torrentium. These were the commonest species of mosquitoes breeding in artificial containers and permanent ground water habitats in Silwood Park. Cs. annulata and An. claviger were also present in numbers in the ground water habitats.

2 Morphological studies of the Culex species show that about 9% of Cx. pipiens population possess pre-alar scales, a character thought to be lacking in this species. Also, about 3% of Cx. torrentium population were found to lack these scales. Separation of the two species can be made reliable by means of male phallosome characteristics which is the only foolproof method currently available.

3 There is no significant difference in the mean egg batch size, duration of gonotrophic cycle and the duration of larval instars and pupae in Cx. pipiens and Cx. torrentium. There is however a significant difference in the incubation period in the two species, this being slightly longer in Cx. torrentium. The total pre-adult durations (time taken in the different larval instars and pupa added together) was about 16 days in each species at $21 \pm 1^{o}C$.

4 Cx. pipiens and Cx. torrentium often occur together in the same field breeding sites but there is a well defined preference to choice of oviposition sites. Cx. pipiens prefer permanent ground water habitat while Cx. torrentium prefer artificial

containers. Cx. torrentium appears to prefer a wider variety of oviposition sites since pre-adult stages were present in tree holes where Cx. pipiens were absent. The physical nature of the habitat rather than the quality of water appears to govern the choice of oviposition sites by the two species.

5 The breeding season of Cx. pipiens and Cx. torrentium was between May and October. Egg-rafts laid by the population from overwintering sites were first found as early as the first week of April in the artificial containers placed in the open field. Peak population of pre-adult stages was observed in July and August in both artificial containers and permanent ground water habitats.

6 An estimate of mortalities acting on the pre-adult stages of Cx. pipiens/torrentium throughout the breeding season showed that in both artificial containers and permanent ground water habitats mortality was heaviest in the older (third and fourth) larval instars and pupae. Naiads, larval and adult Dytiscidae and the amphibian Triturus vulgaris were numerous in some or all the habitats studied and were important predators of pre-adult populations of mosquitoes. A functional response experiment in the laboratory showed that Sympetrum striolatum was a more effective predator than Agrion puella and therefore might be more important in regulating the pre-adult population of mosquitoes in their natural habitat.

7 The population fluctuations of hibernating Cx. pipiens were studied in selected overwintering sites. There was a rapid build-up in the numbers of mosquito at the beginning of hibernation and

a peak population occurred between late October and early November. By middle May all mosquitoes had left hibernation sites. There was an extensive movement of hibernating mosquitoes in and out and within sites; movement may be in response to small temperature variations. Movement of mosquitoes out of the sites contributed to a substantial reduction in population size of hibernating individuals.

8 Hibernating Cx. pipiens can be colonized by exposure to constant illumination for about 3 - 12 days at $21 \pm 1^{\circ}C$. This has the activating influence on mosquitoes and stimulates gorging.

9 Very few Cx. pipiens (less than 1%) had deposited eggs before entering hibernation sites. Parous mosquitoes may enter sites but are unlikely to survive the winter. About 10% of hibernating mosquitoes were not inseminated.

10 About 80% of fat reserves of hibernating Cx. pipiens was exhausted during the course of hibernation. There was a little change in the quantity of glycogen in mosquitoes at this time. Predation by spiders resulted in high mortality of hibernating individuals.

11 Hibernating Cx. torrentium were absent in all sites where Cx. pipiens were present. The hibernation sites for Cx.torrentium was not found.

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Appendix 3.1 Pattern of moulting to different Larval stages, pupae and adult in Cx. pipiens, starting each replicate with 150 first instar larvae that hatched out of a single egg-raft on day 1.

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Replicate 1	I instar	150																						
	II		14	61	75																			
	III				17	28	47	39	14															
	IV						16	30	26	41	23													
	Pupa										11	33	34	16	15	12	9							
	Adult Male													5	19	31	12	7	2					
	Adult Female														9	15	14	0	7	4				
Replicate 2	I instar	150																						
	II		2	73	75																			
	III				45	56	22	11	13															
	IV						15	20	43	31	24	14												
	Pupa									6	28	36	32	20	14	6	1							
	Adult Male												6	6	15	9	10	9	9	5				
	Adult Female													1	8	16	16	8	8	5	8			
Replicate 3	I instar	150																						
	II			19	58	71																		
	III						50	49	47															
	IV								24	28	38	21	29											
	Pupa												14	27	23	30	19	22						
	Adult Male														3	8	11	9	10	0	14	1		
	Adult Female																7	21	10	21	13	7		

Appendix 3.1 (continued)

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Replicate 4	I instar	150																						
	II		8	66	76																			
	III				15	36	43	40	16															
	IV							21	30	38	40	18												
	Pupa											17	15	27	31	38	11	3						
	Adult Male														2	12	14	19	16	-	4			
	Adult Female																4	21	5	18	20	2		
Replicate 5	I instar	150																						
	II		5	89	54																			
	III				14	14	40	56	20															
	IV								6	19	49	40	21											
	Pupa													25	23	36	22	24						
	Adult Male																3	8	13	20	9			
	Adult Female																	20	26	5	13	7		
Replicate	I instar																							
	II																							
	III																							
	IV																							
	Pupa																							
	Adult Male																							
	Adult Female																							

Appendix 3.2 Pattern of moulting to different larval stages, pupae and adults in Cx. torrentium starting each replicate with 150 first instars that hatched out from a single egg-raft.

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Replicate 1	I instar	150																						
	II		15	78	57																			
	III				12	63	52	8	12															
	IV							50	30	51	11													
	Pupa										31	33	29	26	18	-	2							
	Adult Male													11	9	12	16	3	9					
	Adult Female														7	33	6	23	7	2				
Replicate 2	I instar	150																						
	II		29	48	68	5																		
	III				8	59	50	29	2															
	IV							31	36	43	13	20												
	Pupa											21	35	26	22	20	17							
	Adult Male														7	15	10	12	12	2				
	Adult Female																23	26	20		8	4		
Replicate 3	I instar	150																						
	II			70	66	13																		
	III					44	35	43	20	7														
	IV								16	29	38	41	10	15										
	Pupa												21	34	15	28	30	15	0					
	Adult Male															3	16	8	16	20	-	8		
	Adult Female																	16	13	12	14	7	4	3

Appendix 3.2 (continued)

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Replicate 4	I instar	150																						
	II			53	71	24																		
	III					29	31	38	49															
	IV								26	28	24	25	13	28										
	Pupa												8	19	34	19	25	20	17					
	Adult Male																10	6	20	18	14			
	Adult Female																	9	0	25	27	12		
Replicate 5	I instar	150																						
	II			41	86	19																		
	III					30	58	43	5	5														
	IV								20	34	33	39	4	6										
	Pupa											9	8	24	30	36	23							
	Adult Male														4	12	11	13	15	8	2			
	Adult Female																19	11	20	3	5			
Replicate	I instar																							
	II																							
	III																							
	IV																							
	Pupa																							
	Adult Male																							
	Adult Female																							

Appendix 5.1 Assessment of the efficiency of larval and pupal sampling method

The volume of water sampled with the quadrat device was calculated as follows:

Volume of water in the container = 55 litres (known)

Height of water in the container = 31 cm

Actual volume of water sampled when each sampler was pulled out of water = $\pi r^2 h$ = 3.14 x 3.2^2 x 21 (where h = height of water in the container)

Total volume of water sampled by the 3 samplers = 3.14 x 3.2^2 x 31 x 3 = 2.99 litres

Experiment I (10 replicates).

No. of larvae introduced in the container = 1000 instar

	No. sampled/sampler No.			
	1	2	3	Total
Replicate 1	22	10	4	36
2	27	11	10	48
3	16	18	19	53
4	24	8	14	46
5	29	20	9	58
6	30	15	8	63
7	20	9	13	42
8	23	16	10	49
9	28	9	7	44
10	19	20	12	51
Mean				49.0

(Sampler No. 1 was at the side of the container, followed by 2 and then 3)

Appendix 5.1 (Continued)

Calculation

In 2.99 litres of water were 49.0 larvae

$\therefore$ in 55 litres of water there would be $\frac{55 \times 49}{2.99}$ = 901.3 larvae

But 1000 larvae were in the container

$\therefore$ % efficiency of sampling method = $\frac{901.3 \times 100}{1000}$ = 90.1%

Experiment II (5 replicates)

Total number of larvae introduced in the container:

Younger larvae = 400

Older larvae = 400

Pupae = 200

Total number of larvae sampled by the 3 samplers:

	Younger larvae	Older larvae	Pupae
Replicate 1	20	21	26
2	25	17	12
3	17	16	9
4	11	30	11
5	19	12	7
Mean	18.4	19.2	9.0

% efficiency of

sampling method = 84.6 88.3 82.8

(calculated as above).

Appendix 5.2 Mean number of pre-adults of Cx. pipien/torrentium per container placed in Site C, Silwood Bottom in 1978. (Actual number of egg-rafts counted in all 5 containers are shown in parenthesis).

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
3.4	(1)	0	0	0	0	0	0
10.4	(4)	0	0	0	0	0	0
17.4	(0)	0	0	0	0	0	0
24.9	(3)	0	0	0	0	0	0
1.5	(12)	0	0	0	0	0	0
8.5	(9)	87.1	87.6	0	0	0	174.7
15.5	(0)	84.5	110.5	55.3	3.3	0	253.6
22.5	(6)	74.6	19.5	22.8	120.3	13.0	250.2
29.5	(16)	116.7	48.7	96.9	100.7	29.1	392.1
5.6	(9)	228.1	151.8	76.2	59.9	59.5	575.5
12.6	(2)	142.2	205.0	94.9	67.0	56.7	565.8
19.6	(17)	75.7	100.7	56.7	57.3	25.2	315.6
26.6	(14)	361.0	125.9	59.7	75.5	47.2	669.3
3.7	(26)	308.5	161.1	270.6	88.0	59.8	888.0
10.7	(28)	378.6	532.0	148.1	91.5	53.5	1203.7
17.7	(18)	406.8	352.9	211.2	198.4	50.4	1219.7
24.7	(19)	592.0	399.8	198.6	154.5	50.4	1395.3
31.7	(9)	518.3	359.9	185.9	143.5	45.7	1253.3
7.8	(14)	616.7	404.8	200.4	117.3	42.3	1381.5
14.8	(7)	631.5	204.5	114.0	107.4	42.2	1099.6
21.8	(13)	371.2	309.3	231.2	136.8	35.8	1084.3
28.8	(5)	294.6	195.3	153.5	122.5	38.6	804.5
4.9	(8)	179.6	256.4	120.4	110.8	39.7	706.9
11.9	(2)	52.0	132.8	51.8	29.1	26.9	292.6

Appendix 5.2 (Continued)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
18.9	(0)	32.5	32.2	77.6	90.5	25.6	258.4
25.9	(0)	0	22.8	32.2	57.7	54.7	167.4
2.10	(1)	0	0	22.8	35.7	32.4	90.9
9.10	(0)	0	0	0	22.8	32.3	55.1
16.10	(0)	0	0	0	16.3	16.3	32.6
23.10	(0)	0	0	0	0	9.8	9.8
30.10	(0)	0	0	0	0	0	0

Appendix 5.3 Mean number of pre-adults of Cx. pipiens/torrentium per container placed in Site D, Cannon Wood in 1978. (Actual number of egg-rafts counted in all 5 containers are shown in parenthesis).

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
3.4	(0)	0	0	0	0	0	0
10.4	(0)	0	0	0	0	0	0
17.4	(0)	0	0	0	0	0	0
24.4	(0)	0	0	0	0	0	0
1.5	(0)	0	0	0	0	0	0
8.5	(0)	0	0	0	0	0	0
15.5	(2)	0	0	0	0	0	0
22.5	(2)	38.9	25.9	0	0	0	64.8
29.5	(8)	13.1	22.3	3.2	0	0	38.6
5.6	(4)	79.1	44.0	0	42.3	0	165.4
12.6	(6)	124.4	34.7	40.0	6.4	25.8	232.0
19.6	(6)	69.6	102.3	60.1	50.3	12.6	294.9
26.6	(8)	69.8	76.1	51.2	60.3	50.7	308.1
3.7	(11)	283.3	53.8	69.8	98.0	53.8	558.7
10.7	(15)	272.6	212.7	35.4	57.2	35.0	612.9
17.7	(17)	349.1	291.5	196.8	133.2	66.6	1037.2
24.7	(14)	720.6	392.7	188.3	141.6	31.4	1474.6
31.7	(14)	577.1	452.6	288.7	77.4	51.4	1472.2
7.8	(13)	381.1	309.4	171.9	144.9	65.1	1072.4
14.8	(7)	154.0	140.6	105.6	124.2	71.9	596.3
21.8	(9)	219.0	135.9	96.2	83.0	59.2	593.3
28.8	(9)	81.6	140.2	104.3	146.5	58.6	531.2
4.9	(5)	71.7	88.0	71.7	132.6	22.8	386.8
11.9	(0)	32.5	45.6	51.9	97.6	29.4	257.0

Appendix 5.3 (Continued)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
18.9	(2)	22.4	26.0	48.8	61.4	26.2	184.8
25.9	(1)	19.4	29.1	39.1	71.5	48.8	207.9
2.10	(0)	0	9.6	15.9	45.6	19.2	90.3
9.10	(0)	0	0	15.9	38.7	22.7	77.3
16.10	(0)	0	0	12.7	9.5	29.3	51.5
23.10	(0)	0	0	0	9.5	16.1	25.6
30.10	(0)	0	0	0	0	6.4	6.4

Appendix 5.4 Mean no. of eggs and pre-adults of Cx. pipiens/torrentium per container placed in Site C, Silwood Bottom in 1979. (Actual no. of egg-rafts counted in all 5 containers are shown in parenthesis).

Date 1979	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
23.4	50 (1)	0	0	0	0	0	0
25.4	100 (2)	0	0	0	0	0	0
7.5	100 (2)	0	0	0	0	0	0
9.5	200 (4)	97.8	0	0	0	0	97.8
11.5	550 (11)	81.5	0	0	0	0	81.5
14.5	200 (4)	100.2	22.8	0	0	0	123.0
16.5	200 (4)	247.9	103.7	0	0	0	351.6
18.5	50 (1)	217.7	72.9	41.8	0	0	332.4
21.5	100 (2)	71.3	120.1	48.6	19.8	0	259.8
23.5	(0)	52.1	96.4	40.8	6.5	0	195.8
25.5	50 (1)	19.5	79.8	32.1	19.6	0	151.0
28.5	150 (3)	16.3	45.4	35.5	9.8	3.3	110.3
30.5	200 (4)	19.6	9.8	22.8	19.6	0	71.8
1.6	100 (2)	71.7	16.2	39.0	16.3	6.5	149.7
4.6	150 (3)	45.6	19.6	3.3	16.3	3.3	88.1
6.6	50 (1)	61.8	16.4	6.6	9.8	6.5	101.1
8.6	150 (3)	39.1	39.0	29.5	3.3	9.8	120.7
11.6	(0)	75.6	35.9	29.4	16.5	13.1	170.5
13.6	100 (2)	26.0	19.5	29.1	21.5	19.6	115.7
15.6	100 (2)	26.5	32.7	19.5	13.1	32.7	124.5
18.6	450 (9)	23.2	19.6	6.5	32.9	13.2	95.4
20.6	350 (7)	172.9	19.6	16.4	16.4	29.6	254.9
22.6	650 (13)	290.0	68.4	0	13.1	15.3	386.8
27.6	200 (4)	319.2	208.4	107.3	32.8	0	667.7

Appendix 5.4 (Continued)

Date 1979	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
29.6	350 (7)	356.4	250.0	107.3	39.0	29.3	782.0
2.7	100 (2)	230.9	103.9	116.7	65.1	55.3	571.9
4.7	700 (14)	261.2	198.5	117.4	58.7	42.4	678.2
6.7	950 (19)	297.3	186.2	104.6	58.9	46.0	693.0
9.7	600 (12)	942.1	376.2	194.8	81.5	32.5	1627.1
11.9	400 (8)	679.7	461.1	206.0	155.2	110.5	1612.5
13.7	700 (14)	586.2	364.6	195.6	84.7	45.6	1276.7
16.7	1050 (21)	716.3	526.0	204.8	159.6	71.6	1678.3
18.7	850 (17)	689.5	594.7	425.6	250.4	94.4	2054.6
20.7	400 (8)	643.1	417.4	294.1	278.5	64.6	1697.7
23.7	650 (13)	430.7	193.9	138.3	171.4	64.8	999.1
25.7	550 (11)	477.3	194.5	152.4	129.4	80.8	1034.4
27.7	400 (8)	428.8	217.4	155.4	127.9	89.8	1019.3
30.7	300 (6)	513.9	246.1	106.7	61.5	25.9	954.1
1.8	100 (2)	266.5	204.6	191.5	123.6	42.3	828.5
3.8	400 (8)	309.5	175.8	123.6	146.4	16.3	771.6
6.8	600 (12)	363.2	291.1	198.3	45.9	35.9	934.4
8.8	500 (10)	264.2	229.2	125.4	32.5	29.2	680.5
10.8	200 (4)	341.7	198.1	120.0	78.7	22.9	761.4
13.8	150 (3)	254.6	217.8	45.9	22.8	9.8	550.9
15.8	50 (1)	152.8	204.8	87.2	61.8	16.4	523.0
17.8	150 (3)	38.6	120.5	153.0	91.3	22.8	426.2
20.8	450 (9)	201.4	64.7	65.2	62.0	22.8	416.1
22.8	(0)	110.8	69.4	55.2	42.4	16.4	294.2

Appendix 5.4 (Continued)

Date 1979	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
24.8	100 (2)	16.3	61.5	93.5	42.1	16.4	229.8
27.8	250 (5)	6.5	25.7	38.9	64.5	22.6	158.2
29.8	200 (4)	191.3	77.3	19.7	29.4	22.9	340.6
31.8	200 (4)	80.6	135.1	70.4	29.0	6.6	321.7
3.9	300 (6)	129.0	125.0	19.4	25.7	16.2	315.3
5.9	- (0)	165.2	171.2	90.4	19.4	19.3	465.5
7.9	250 (5)	1)7.7	110.6	123.7	62.1	25.9	430.0
10.9	200 (4)	223.5	98.2	52.2	29.4	19.6	422.9
12.9	100 (2)	123.7	153.4	117.1	65.4	22.9	482.5
14.9	300 (6)	84.2	90.2	115.5	71.0	22.5	383.4
17.9	50 (1)	276.9	97.6	44.6	25.9	19.5	438.6
19.9	50 (1)	141.9	166.4	106.4	35.6	9.7	460.0
21.9	(0)	25.6	116.1	73.8	55.0	12.9	283.4
24.9	(0)	6.4	64.4	38.7	28.8	6.5	144.8
26.9	(0)	6.4	61.3	22.5	12.7	22.4	125.3
28.9	(0)	0	29.0	51.4	3.1	6.4	89.9
1.10	(0)	0	16.1	32.2	19.2	6.5	74.0
3.10	(0)	0	22.6	38.7	22.5	9.7	93.5
5.10	(0)	0	0	32.3	9.6	9.6	51.5
8.10	(0)	0	0	36.0	3.2	6.4	45.6
10.10	(0)	0	0	19.7	13.2	9.9	42.8
12.10	(0)	0	0	6.6	29.7	6.6	42.9
15.10	(0)	0	0	0	6.6	0	6.6
17.10	(0)	0	0	0	13.2	6.6	19.8
19.10	(0)	0	0	0	13.2	0	13.2
22.10	(0)	0	0	0	0	3.3	3.3

Appendix 5.5 Mean no. of eggs and pre-adults of Cx. pipiens/torrentium per container placed in Site D, Cannon Wood in 1979. (Actual no. of egg-rafts counted in all 5 containers are given in parenthesis).

Date 1979	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
7.5	(0)	0	0	0	0	0	0
9.5	(0)	0	0	0	0	0	0
11.5	(0)	0	0	0	0	0	0
14.5	(0)	0	0	0	0	0	0
16.5	50 (1)	0	0	0	0	0	0
18.5	50 (1)	0	0	0	0	0	0
21.5	100 (2)	26.3	0	0	0	0	26.3
23.5	200 (4)	32.9	0	0	0	0	32.9
25.5	100 (2)	108.0	13.2	0	0	0	121.2
28.5	150 (3)	85.1	98.4	0	0	0	183.5
30.5	300 (6)	80.8	87.7	16.4	0	0	184.9
1.6	200 (4)	129.8	105.4	42.8	6.6	0	284.6
4.6	200 (4)	122.4	139.5	52.6	29.6	0	343.1
6.6	300 (6)	156.3	90.4	91.9	6.6	0	345.2
8.6	300 (6)	153.9	139.4	74.4	19.7	0	387.4
11.6	100 (2)	106.7	129.5	78.0	16.2	13.2	330.4
13.6	200 (4)	103½2	94.5	94.0	32.3	9.9	333.9
15½6	200 (4)	86.4	74.5	64.8	74.0	13.2	312.9
18.6	350 (7)	80.1	22.7	58.6	51.6	35.7	248.7
20.6	300 (6)	222.5	92.8	0	12.7	45.1	373.1
22.6	350 (7)	231.1	128.1	38.6	6.4	19.3	423.5
25.6	200 (4)	230.4	175.9	63.9	28.7	6.4	505.3
27.6	500 (10)	245.7	149.1	126.4	12.8	3.2	537.2
29.6	400 (8)	221.6	182.2	74.2	68.6	0	546.6

Appendix 5.5 (Continued)

Date 1979	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
2.7	800 (16)	250.1	190.1	58.5	84.8	13.2	596.7
4.7	1400 (28)	519.7	229.6	173.4	80.3	0	1003.0
6.7	1550 (31)	899.4	402.6	265.5	168.9	0	1736.4
9.7	1350 (27)	1728.0	766.2	660.6	299.8	123.9	3578.5
11.7	1100 (22)	1429.4	916.4	615.6	349.9	126.5	3437.8
13.7	850 (17)	1131.5	751.3	519.1	445.4	178.2	3025.5
16.7	800 (16)	1156.3	784.2	431.8	353.1	239.6	2965.0
18.7	850 (17)	814.9	438.8	649.4	280.7	181.3	2365.1
20.7	650 (13)	618.4	491.1	397.3	231.7	120.6	1859.1
23.7	850 (17)	745.6	382.6	353.3	301.3	154.7	1937.5
25.7	200 (4)	415.0	293.9	180.6	101.6	79.4	1070.5
27.7	550 (11)	243.6	185.4	201.4	146,7	54.4	831.7
30.7	50 (1)	467.2	248.2	209.7	198.9	86.5	1210.5
1.8	450 (9)	324.9	193.6	213.0	141.8	58.2	931.5
3.8	200 (4)	346.4	252.6	186.3	166.1	54.8	1006.2
6.8	250 (5)	285.6	209.6	173.2	137.3	95.9	901.6
8.8	100 (2)	214.3	221.5	191.2	122.4	58.0	807.4
10.8	100 (2)	227.6	162.1	157.3	147.6	48.2	742.8
13.8	50 (1)	125.9	120.1	130.1	104.2	55.2	535.5
15.8	50 (1)	72.9	103.6	75.9	99.9	65.9	535.5
17.8	250 (5)	44.2	66.6	73.7	59.4	51.7	295.6
20.8	250 (5)	116.1	41.5	60.6	57.9	51.5	327.6
22.8	50 (1)	120.4	93.5	64.5	51.1	35.6	365.1
24.8	300 (6)	85.7	59.1	48.3	58.2	35.4	286.7

Appendix 5.5 (Continued)

Date 1979	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
27.8	100 (2)	136.6	91.9	45.7	41.9	22.5	338.6
29.8	0 (0)	90.8	120.1	59.1	52.6	19.5	342.1
31.8	300 (6)	3.2	77.6	48.1	39.0	22.7	190.6
3.9	250 (5)	123.2	55.2	74.4	51.4	16.2	320.4
5.9	100 (2)	107.1	65.2	74.5	70.9	22.6	340.3
7.9	50 (1)	154.3	145.2	52.0	38.8	25.8	416.1
10.9	100 (2)	51.3	122.2	58.7	51.8	42.0	326.0
12.9	150 (3)	44.9	61.2	54.5	64.1	41.9	266.6
14.9	0 (0)	74.8	35.8	76.9	45.2	19.5	252.2
17.9	100 (2)	51.5	51.5	83.9	48.2	25.7	260.8
19.9	0 (0)	29.4	61.7	58.3	42.2	32.5	224.1
21.9	50 (1)	19.6	81.3	64.9	39.0	19.5	224.3
24.9	50 (1)	26.1	35.7	68.3	29.3	16.3	175.7
26.9	(0)	26.2	55.3	55.1	55.1	9.7	201.4
28.9	(0)	39.0	13.1	55.3	16.5	22.7	146.6
1.10	(0)	9.9	22.7	48.7	45.4	16.2	142.9
3.10	(0)	6.6	19.7	48.6	58.5	9.7	143.1
5.10	(0)	0	9.8	39.0	42.4	19.6	110.8
8.10	(0)	0	6.5	32.5	45.6	16.3	100.9
10.10	(0)	0	6.5	19.5	45.6	16.4	88.0
12.10	(0)	0	3.3	19.7	39.0	26.3	88.3
15.10	(0)	0	0	6.6	42.5	26.2	75.3
17.10	(0)	0	0	0	29.1	16.2	45.3
19.10	(0)	0	0	0	16.1	13.1	29.2

Appendix 5.5 (Continued)

Date 1979	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
22.10	(0)	0	0	0	13.0	6.4	19.4
24.10	(0)	0	0	0	9.8	13.1	22.9
26.10	(0)	0	0	0	3.3	9.8	13.1

Appendix 5.6 Mean no. of pre-adult stages of Cx. pipiens/torrentium per container placed in the open field at Site E, North Gravel in 1978. (Actual number of egg-rafts counted in all 3 containers is given in parenthesis)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
3.4	(0)	0	0	0	0	0	0
10.4	(0)	0	0	0	0	0	0
17.4	(0)	0	0	0	0	0	0
24.4	(2)	0	0	0	0	0	0
1.5	(0)	0	0	0	0	0	0
8.5	(2)	0	0	0	0	0	0
15.5	(1)	73.2	0	0	0	0	73.2
22.5	(2)	157.8	40.5	0	0	0	198.3
29.5	(2)	111.7	127.0	36.8	15.9	9.6	301.0
5.6	(3)	183.2	90.0	30.6	37.3	5.2	346.3
12.6	(6)	129.3	41.9	59.5	20.8	37.7	289.2
19.6	(4)	63.2	73.9	81.1	25.3	10.6	254.1
26.6	(10)	178.4	36.6	62.8	50.6	25.3	353.7
3.7	(4)	131.3	146.6	47.3	36.6	15.7	377.5
10.7	(15)	352.1	120.2	175.9	45.3	10.6	704.1
17.7	(23)	628.4	204.6	220.1	93.2	69.4	607.85
24.7	(8)	1239.9	773.4	215.1	196.3	42.4	2467.1
31.7	(7)	538.4	455.0	388.7	291.5	72.0	1745.6
7.8	(8)	274.2	202.8	370.2	253.4	55.2	1155.8
14.8	(10)	448.7	240.3	128.8	69.7	26.2	913.7
21.8	(4)	473.9	380.2	300.2	103.1	66.3	1323.2
28.8	(6)	169.8	52.6	36.8	57.6	36.9	353.7
4.9	(2)	107.3	26.6	31.5	31.6	42.2	239.2
11.9	(0)	48.4	55.5	14.2	46.3	29.8	194.2

Appendix 5.6 (Continued)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
18.9	(2)	126.4	89.3	47.0	46.9	0	309.6
25.9	(0)	131.8	63.9	35.7	30.8	50.4	312.6
21.10	(0)	0	10.4	35.2	20.7	25.7	92.0
9.10	(0)	0	0	18.9	20.3	20.7	59.9
16.10	(0)	0	0	0	25.7	18.8	44.5
23.10	0	0	0	0	0	10.3	10.3

Appendix 5.7 Mean no. of pre-adult stages of Cx. pipiens/torrentium per container placed at the edge of wood in Site E, North Gravel in 1978. (Actual number of egg rafts counted in all 3 containers is given in parenthesis)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
3.4	(0)	0	0	0	0	0	0
10.4	(2)	0	0	0	0	0	0
17.4	(0)	0	0	0	0	0	0
24.4	(0)	0	0	0	0	0	0
1.5	(1)	0	0	0	0	0	0
8.5	(1)	0	0	0	0	0	0
15.5	(4)	111.3	63.6	0	0	0	174.9
22.5	(6)	129.6	48.7	0	0	0	178.3
29.5	(4)	207.9	85.8	51.6	58.1	25.7	429.1
5.6	(2)	145.2	106.3	78.8	73.5	90.3	494.1
12.6	(2)	270.0	188.5	112.4	57.8	111.5	740.2
19.6	(3)	10.8	113.8	102.3	37.3	10.4	274.6
26.6	(4)	140.8	113.8	123.0	148.8	32.3	558.7
3.7	(16)	136.1	102.1	176.0	186.1	79.6	679.8
10.7	(12)	468.2	234.7	110.7	37.7	26.8	878.1
17.7	(10)	373.8	390.6	283.1	141.1	86.1	1274.7
24.7	(17)	537.4	317.0	216.0	337.3	47.7	1455.4
31.7	(12)	999.4	390.1	221.1	360.0	90.6	2061.2
7.8	(10)	410.3	462.3	346.3	264.5	82.3	1565.7
14.8	(4)	314.8	510.4	238.0	216.2	136.2	1415.6
21.8	(5)	203.9	106.3	231.2	342.1	98.3	981.8
28.8	(0)	148.8	31.7	32.0	69.6	110.6	392.7
4.9	(5)	70.9	93.6	79.6	74.3	26.4	344.8
11.9	(1)	26.7	75.8	61.1	37.4	15.8	216.8

Appendix 5.7 (Continued)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
18.9	(2)	22.5	25.3	81.2	86.4	21.1	236.5
25.9	(0)	0	20.9	14.7	80.9	18.9	135.4
21.10	(0)	0	16.3	22.9	36.5	27.8	103.5
9.10	(0)	0	0	24.3	44.2	40.3	108.8
16.10	(0)	0	0	0	29.6	44.2	73.8
23.10	(0)	0	0	0	0	20.1	20.1

Appendix 5.8 Mean numbers of pre-adult stages of Cx. pipiens/torrentium per container placed 6 m inside wood in Site E, North Gravel in 1978. (Actual number of egg-rafts counted in all 3 containers is given in parenthesis).

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
3.4	(0)	0	0	0	0	0	0
10.4	(0)	0	0	0	0	0	0
17.4	(0)	0	0	0	0	0	0
2.44	(0)	0	0	0	0	0	0
1.5	(0)	0	0	0	0	0	0
8.5	(0)	0	0	0	0	0	0
15.5	(1)	0	0	0	0	0	0
22.5	(5)	21.8	51.8	0	0	0	73.6
29.5	(2)	53.7	32.5		0	0	86.2
5.6	(0)	113.6	26.2	53.9	22.2	5.4	221.3
12.6	(6)	125.6	65.0	32.5	32.4	75.2	330.7
19.6	(2)	216.2	121.6	21.6	15.4	16.2	391.0
26.6	(3)	32.4	137.8	99.6	20.8	10.6	301.2
3.7	(3)	65.5	0	26.7	21.8	21.3	135.3
10.7	(9)	96.0	127.2	21.8	16.4	21.8	283.2
17.7	(4)	120.1	46.8	78.3	63.2	47.0	355.4
24.7	(2)	53.7	62.7	80.7	15.4	15.4	227.9
31.7	(3)	71.6	43.3	58.4	59.4	27.1	259.8
7.8	(6)	152.6	114.2	59.7	107.5	64.2	498.2
14.8	(1)	138.3	110.9	32.6	54.3	54.3	390.4
21.8	(1)	156.9	120.3	99.6	42.8	32.1	451.7
28.8	(3)	21.8	20.4	48.3	46.3	16.3	153.1
4.9	(4)	92.1	0	10.8	51.4	21.1	175.4
11.9	(1)	37.3	64.4	58.4	43.8	37.1	241.0

Appendix 5.8 (Continued)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
18.9	(2)	15.4	16.3	0	62.6	31.8	126.1
25.9	(0)	0	20.9	25.8	10.4	20.4	77.5
21.10	(0)	0	13.1	11.6	34.3	17.0	76.0
9.10	(0)	0	0	12.4	16.9	16.4	45.7
16.10	(0)	0	0	0	5.5	16.4	21.9
23.10	(0)	0	0	0	0	4.3	4.3

Appendix 5.9 Mean number of pre-adult stages of Cx. pipiens/torrentium per container placed 16 m inside wood in Site E, North Gravel in 1978. Actual number of egg-rafts counted in all 3 containers is given in parenthesis).

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
3.4	(0)	0	0	0	0	0	0
10.4	(0)	0	0	0	0	0	0
17.4	(0)	0	0	0	0	0	0
24.4	(0)	0	0	0	0	0	0
1.5	(1)	0	0	0	0	0	0
8.5	(2)	0	0	0	0	0	0
15.5	(0)	68.3	15.8	0	0	0	84.1
22.5	(3)	100.0	15.6	0	0	0	115.6
29.5	(2)	36.7	70.0	20.4	0	0	127.1
5.6	(0)	0	21.2	46.9	76.0	20.8	164.9
12.6	0	0	15.9	26.0	41.3	10.3	93.5
19.6	(1)	192.3	0	0	25.3	61.9	279.5
26.6	(0)	0	70.9	46.3	0	16.3	133.5
3.7	(7)	0	0	0	56.8	26.6	83.4
10.7	(8)	186.9	16.1	0	0	36.1	239.1
17.7	(6)	99.3	200.1	5.9	0	0	305.3
24.7	(4)	234.8	209.9	83.3	193.0	0	721.0
31.7	(3)	144.9	159.7	178.9	268.3	137.2	889.0
7.8	(3)	141.7	48.5	109.3	75.3	32.2	407.0
14.8	(1)	21.6	117.6	82.3	89.0	58.9	369.4
21.8	(0)	210.4	38.6	83.9	66.4	61.3	460.6
28.8	(0)	32.3	160.1	69.4	79.2	15.9	356.9
4.9	(1)	27.1	75.7	35.5	69.7	53.5	261.5
11.9	0	40.8	59.1	59.0	73.6	26.7	259.2

Appendix 5.9 (Continued)

Date 1978	Eggs	Larval instars				Pupa	Total pre adult stages
		I	II	III	IV		
18.9	0	0	21.5	17.2	31.0	21.7	91.4
25.9	0	0	0	5.3	21.4	0	26.7
21.10		0	0	12.9	16.1	8.3	37.3

Appendix 5.10 Number of pre-adult stages of Cx. pipiens/torrentium collected weekly from Site A, Pond Field pond in 1978

Date 1978	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
17.5	0	0	0	0	0	0
26.5	3	2	0	0	0	5
2.6	0	0	2	2	0	4
9.6	2	5	8	12	2	29
16.6	0	1	2	2	0	5
24.6	0	0	1	3	1	5
28.6	177	0	0	0	0	177
11.7	421	8	0	0	0	429
21.7	107	17	8	7	3	142
28.7	260	108	31	23	5	427
6.8	59	114	9	0	0	182
12.8	83	120	17	0	0	220
18.8	40	52	13	6	12	123
24.8	18	42	24	22	15	121
1.9	18	20	28	18	20	104
6.9	25	38	2	0	11	76
15.9	0	9	0	0	0	9
20.9	0	12	0	0	0	12
26.9	0	9	2	1	1	13
4.10	0	0	3	5	1	9
11.10	0	0	2	1	2	5
17.10	0	0	0	0	0	0
24.10	0	0	0	0	0	0

Appendix 5.11 Number of pre-adult stages of Cx. pipiens/ torrentium collected weekly from Site B, Sandy Ride pond in 1978.

Date 1978	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
17.5	0	2	3	1	0	6
26.5	0	0	2	6	0	8
2.6	0	0	0	3	0	3
9.6	0	0	0	0	2	0
16.6	0	0	0	0	0	0
24.6	0	0	0	0	0	0
28.6	0	0	0	0	0	0
6.7	30	0	0	0	0	30
13.7	104	161	147	0	0	412
22.7	200	641	93	45	10	989
28.7	125	395	242	70	9	841
6.8	100	60	63	49	4	276
12.8	49	42	51	22	3	167
18.8	39	66	40	21	4	170
24.8	43	26	30	15	3	117
1.9	31	36	16	16	2	101
6.9	15	26	10	7	1	59
15.9	4	3	0	4	7	18
20.9	0	15	6	13	5	39
26.9	0	5	3	7	2	17
4.10	0	0	2	2	2	6
11.10	0	0	3	2	1	6
17.10	0	0	3	4	4	11
24.10	0	0	0	0	0	0

Appendix 5.12 Number of pre-adult stages of Cx. pipiens/ torrentium collected from Site A, Pond Field pond in 1979

Date 1979	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
15.5	0	7	0	0	0	7
17.5	23	6	0	0	0	29
19.5	11	10	0	0	0	21
22.5	4	4	3	0	0	11
24.5	3	2	0	0	0	5
26.5	0	10	1	0	0	11
29.5	0	1	1	0	0	2
31.5	4	2	0	0	0	6
2.6	6	2	1	0	0	9
5.6	0	5	0	1	0	6
7.6	2	0	0	0	0	2
9.6	0	4	1	2	0	7
12.6	8	8	0	2	1	19
14.6	2	1	0	1	2	6
16.6	3	1	0	0	0	4
19.6	5	3	0	0	0	8
21.6	139	0	15	1	1	156
23.6	100	7	0	0	0	107
26.6	227	27	0	20	0	274
28.6	156	223	0	0	10	389
30.6	119	243	120	60	12	554
3.7	344	127	26	2	0	499
5.7	693	154	68	11	0	926
7.7	806	162	76	29	11	1084

Appendix 5.12 (Continued)

Date 1979	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
10.7	1099	205	132	36	28	1500
12.7	1563	623	211	46	21	2464
14.7	1117	529	197	33	11	1887
17.7	1212	253	127	60	16	1668
19.7	1400	1079	1183	42	8	3712
21.7	609	1190	66	113	35	2013
24.7	963	1101	123	104	36	2327
26.7	523	1076	1011	203	21	2834
28.7	642	779	617	60	11	2109
31.7	339	1054	603	105	29	2130
2.8	107	133	77	74	9	400
4.8	79	123	51	8	12	273
7.8	29	17	47	10	0	103
9.8	15	22	18	12	1	68
11.8	17	11	14	11	0	53
14.8	20	9	5	0	5	39
16.8	15	18	3	0	1	37
18.8	11	17	14	2	0	44
21.8	17	7	9	3	2	38
23.8	6	8	11	4	2	31
25.8	0	0	3	3	0	6
28.8	0	0	2	4	0	6
30.8	0	0	0	0	4	4
1.9	0	0	0	11	0	11

Appendix 5.12 (Continued)

Date 1979	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
4.9	36	10	5	0	0	51
6.9	50	82	0	0	0	132
8.9	15	8	0	0	0	23
11.9	12	9	32	0	0	53
13.9	6	4	4	13	6	33
15.9	0	4	3	29	11	47
18.9	12	7	0	0	0	19
20.9	4	18	12	0	0	34
22.9	9	11	4	30	9	63
25.9	3	32	19	17	4	75
27.9	0	5	17	16	3	41
29.9	0	4	4	19	0	27
2.10	0	0	15	4	10	29
4.10	0	0	2	13	4	19
6.10	0	0	1	3	0	4
9.10	0	0	8	10	0	18
11.10	0	0	0	0	0	0
13.10	0	0	0	0	0	0
16.10	0	0	0	0	0	0

Appendix 5.13 Number of pre-adult stages of Cx. pipiens/torrentium collected from Site B, Sandy Ride pond in 1979

Date 1979	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
15.5	0	0	0	0	0	0
17.5	0	0	0	0	0	0
19.5	0	0	0	0	0	0
22.5	0	0	0	0	0	0
24.5	0	0	0	0	0	0
26.5	0	0	0	0	0	0
29.5	9	0	0	0	0	9
31.5	2	5	0	0	0	7
2.6	3	2	0	0	0	5
5.6	0	7	0	0	0	7
7.6	0	1	0	1	0	2
9.6	0	0	0	0	0	0
12.6	2	2	0	0	0	4
14.6	1	0	1	0	0	2
16.6	0	0	1	1	0	2
19.6	0	0	0	0	0	0
21.6	4	2	0	1	0	7
23.6	3	1	1	0	0	5
26.6	3	4	2	2	1	12
28.6	10	5	0	0	0	15
30.6	8	3	0	0	0	11
3.7	11	3	0	0	0	14
5.7	14	6	0	2	0	22
7.7	9	5	3	0	0	17

Appendix 5.13 (Continued)

Date 1979	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
10.7	8	12	0	0	0	20
12.7	14	11	3	3	4	35
14.7	21	9	5	2	1	38
17.7	18	8	0	0	0	26
19.7	56	17	8	5	0	86
21.7	33	19	15	7	4	78
24.7	53	22	20	8	0	103
26.7	24	25	13	1	10	73
28.7	17	12	3	0	2	34
31.7	22	6	9	2	1	40
2.8	25	19	13	8	3	68
4.8	9	26	8	10	12	65
7.8	10	12	30	2	0	54
9.8	15	13	1	13	0	42
11.8	9	7	4	3	2	25
14.8	14	2	2	1	0	19
16.8	6	28	4	0	1	39
18.8	3	2	3	2	0	10
21.8	0	2	0	0	0	2
23.8	0	2	4	0	1	7
25.8	3	8	2	2	1	16
28.8	4	3	2	1	0	10
30.8	0	0	0	4	2	6
1.9	8	2	0	0	0	10

Appendix 5.13 (Continued)

Date 1979	Larval instars				Pupa	Total pre adult stages
	I	II	III	IV		
4.9	4	14	10	0	0	28
6.9	2	25	0	0	0	27
8.9	6	0	3	0	0	9
11.9	4	2	0	9	0	15
13.9	0	0	0	2	0	2
15.9	10	3	0	0	6	19
18.9	2	0	0	0	3	5
20.9	0	1	0	0	0	1
22.9	0	0	0	0	0	0
25.9	5	3	0	0	1	9
27.9	8	4	2	0	0	14
29.9	0	5	3	0	0	8
2.10	0	2	4	2	0	8
4.10	0	0	7	0	0	7
6.10	0	0	2	2	0	4
9.10	0	0	4	6	1	11
11.10	0	0	0	0	3	3
13.10	0	0	0	0	0	0
16.10	0	0	0	0	0	0

Appendix 5.14 Development times of different instars of Cx. torrentium at different months of the year. In each case 150 first instar larvae that hatched out on day 1 were bred to adult

May

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		
Replicate	I instar	150																																	
	II			27	31	29	17	14	8	14																									
	III							4	14	22	20	24	34	5	6																				
	IV													8	9	9	20	17	19	14	15	9													
	Pupa																					13	8	20	27	18	17	2							
	Adult Male																									8	10	0	15	4	0	4			
	Adult Female																											3	9	12	13	5	2	5	
Replicate	I instar	150																																	
	II				17	22	30	27	20	9	7																								
	III								14	21	11	17	18	7	9	11																		
	IV															2	4	4	21	18	7	14	15	5	5										
	Pupa																							7	5	21	16	13	30	3					
	Adult Male																												1	9	9	6	13	5	
	Adult Female																													6	0	4	9	7	10
Replicate	I instar	150																																	
	II			21	36	23	26	17	6	10																									
	III							8	14	20	28	16	20	26																					
	IV													21	14	20	17	23	16	8															
	Pupa																			5	4	18	29	31	9	10									
	Adult Male																								3	8	4	7	11	0	0	3			
	Adult Female																										12	12	9	17	5	0	3		

Appendix 5.14 (Continued)

July

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Replicate	I instar	150																						
	II		20	52	50	9	6	5																
	III				21	31	26	25	21	4														
	IV						8	12	19	38	25	7	9	5										
	Pupa											19	15	34	29	8	4	5	0	1				
	Adult Male															5	16	17	6	0	1			
	Adult Female																	14	32	8	3	10		
Replicate	I instar	150																						
	II			40	59	30	8	8	1															
	III					10	23	9	30	60	8													
	IV								12	14	40	44	20	0	5									
	Pupa													21	20	49	21	7	5	4	6			
	Adult Male																8	13	15	4	2	4		
	Adult Female																		21	26	20	7	7	6
Replicate	I instar	150																						
	II			21	44	25	14	20																
	III					14	23	34	30	9	2													
	IV								6	19	27	40	13	3	4									
	Pupa													5	20	8	30	28	9	7				
	Adult Male																7	17	3	8	8	12	6	
	Adult Female																		18	19	0	4	1	

Appendix 5.14 (Continued)

June

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Replicate	I instar	150																										
	II			31	45	30	17	10	6	1																		
	III						25	24	16	28	11	6	-	3														
	IV									8	12	20	33	22	4	4												
	Pupa														9	39	26	10	9	0	4							
	Adult Male																		10	16	27	0	0	3	2			
	Adult Female																				10	16	6	1				
Replicate	I instar	150																										
	II			29	66	20	15	6	0	0	5																	
	III						17	35	18	24	20	8	-	8														
	IV										19	42	19	18	15	8	4	5										
	Pupa														2	4	9	28	20	29	30	1				1		
	Adult Male																				5	19	17	7	1	2		
	Adult Female																					17	22	12	14	6	2	5
Replicate	I instar	150																										
	II			22	49	30	21	10	3																			
	III					18	33	41	20	3	10	3	2															
	IV									24	35	23	9	20	3	7												
	Pupa														9	29	30	11	30	9								
	Adult Male																		10	4	4	18	2	7	0	6		
	Adult Female																			14	17	23	0	1	4	3		

Appendix 5.14 (Continued)

August

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Replicate	I instar	150																									
	II			49	39	26	20	6	8																		
	III					24	33	21	40	8	15	4															
	IV								12	38	31	43	7	10													
	Pupa													20	36	27	17	30	9								
	Adult Male																	8	29	16	12	5	8				
	Adult Female																		20	24	5	5	0	3			
Replicate	I instar	150																									
	II			62	45	14	8	7	=	3																	
	III						34	36	24	28	4	3	1														
	IV								6	20	10	26	38	22	4												
	Pupa													19	32	46	18	8									
	Adult Male																		5	8	9	13	6	8			
	Adult Female																	18	22	27	0	0	5				
Replicate	I instar	150																									
	II			24	44	46	10	2																			
	III						15	14	19	24	20	3															
	IV										18	22	20	8	0	8											
	Pupa														9	15	4	12	20	13							
	Adult Male																			8	10	6	7	2	6		
	Adult Female																						11	11	2	4	

Appendix 5.14 (Continued)

September

	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Replicate	I instar	150																															
	II			21	66	15	22	5	0	0	2																						
	III						19	32	19	30	6	10	7																				
	IV											14	20	33	26	11	9	6														
	Pupa																7	16	15	26	17	7	19	10								
	Adult Male																					9	8	14	9	12.	5	3	2				
	Adult Female																						7	25	4	6	5	1	4				
Replicate	I instar	150																															
	II				16	22	27	29	19	6																							
	III							19	24	17	22	14	14	1																			
	IV										4	8	8	36	18	23	5	3														
	Pupa																4	15	14	21	36	9	0	4								
	Adult Male																						3	3	4	25	0	2	0	3			
	Adult Female																								13	20	21	3	1	5			
Replicate	I instar	150																															
	II			23	51	30	7	17																									
	III						8	19	11	36	15	0	8	1																			
	IV												13	21	8	15	0	4	3													
	Pupa																	5	6	21	10	11	21	10								
	Adult Male																						9	14	15	2	1	4				
	Adult Female																								8	14	3	0	2				

Appendix 5.15 Instar mortalities of Cx. pipiens/torrentium collected from artificial containers in Silwood Bottom

State/ instar	Age in days of beginning of stage	No. entering instar	Death in instar	Relative proportion dying in instar	Proportion dying daily in instar	k-values
Container No. 1						
Egg	0	12750	5150	0.40	0.15	0.23
I-instar	3.1	7600	3000	0.40	0.14	0.22
II	6.5	4600	1600	0.35	0.14	0.19
III	9.3	3000	1400	0.47	0.15	0.27
IV	13.2	1600	900	0.56	0.15	0.36
Pupa	18.1	700	300	0.43	0.13	0.24
Adult	22.1	400				K = 1.51
Container No.2						
Egg	0	16000	7100	0.44	0.17	0.26
I-instar	3.1	8900	3700	0.42	0.15	0.23
II	6.5	5200	2000	0.39	0.15	0.21
III	9.5	3200	1400	0.44	0.16	0.25
IV	12.9	1800	1200	0.67	0.21	0.48
Pupa	17.7	600	300	0.50	0.17	0.30
Adult	21.5	300				K = 1.73
Container No.3						
Egg	0	20000	5400	0.27	0.10	0.14
I-instar	3.0	14600	6300	0.43	0.15	0.25
II	6.4	8300	4500	0.54	0.24	0.34
III	9.2	3800	2500	0.66	0.28	0.47
IV	12.5	1300	1100	0.85	0.33	0.81
Pupa	17.2	200	120	0.60		0.40
Adult	21.4	80				K = 2.41

Appendix 5.15 (Continued)

State/ instar	Age in days of beginning of stage	No. entering instar	Death in instar	Relative proportion dying in instar	Proportion dying daily in instar	k-values
Container No. 4						
Egg	0	23000	6000	0.26	0.10	0.13
I-instar	3.0	17000	6500	0.38	0.13	0.21
II	6.5	10500	3800	0.36	0.14	0.20
III	9.5	6700	2900	0.43	0.14	0.25
IV	13.4	3800	2000	0.53	0.14	0.33
Pupa	18.3	1800	600	0.33	0.10	0.18
Adult	22.3	1200				K = 1.30
Container No. 5						
Egg	0	15000	5000	0.33	0.13	0.18
I-instar	3.0	10000	3800	0.38	0.13	0.21
II	6.5	6200	2100	0.34	0.12	0.19
III	9.8	4100	2000	0.49	0.14	0.29
IV	14.4	2100	1300	0.62	0.18	0.42
Pupa	19.3	800	600	0.75	0.28	0.60
Adult	23.6	200				K = 1.89

Appendix 5.16 Instar mortalities of Cx. pipiens/torrentium collected from artificial containers in Cannon Wood.

State/ instar	Age in days of beginning of stage	No. entering instar	Death in instar	Relative proportion dying in instar	Proportion dying daily in instar	k-values
Container No. 6						
Egg	0	15750	5650	0.36	0.14	0.19
I-instar	2.9	10100	4000	0.40	0.14	0.22
II	6.2	6100	1900	0.31	0.13	0.16
III	9.0	4200	1700	0.41	0.14	0.23
IV	12.4	2500	1300	0.52	0.14	0.32
Pupa	17.2	1200	600	0.51	0.16	0.30
Adult	21.2	600				K = 1.42
Container No. 7						
Egg	0	15500	4500	0.29	0.11	0.15
I-instar	3	11000	3500	0.32	0.11	0.17
II	6.3	7500	2400	0.32	0.13	0.17
III	9.2	5100	2000	0.39	0.12	0.22
IV	13.0	3100	1600	0.52	0.14	0.32
Pupa	17.9	1500	600	0.40	0.12	0.22
Adult	21.9	900				K = 1.25
Container No. 8						
Egg	0	21250	7450	0.35	0.14	0.19
I-instar	2.9	13800	5500	0.40	0.14	0.22
II	6.2	8300	2800	0.34	0.14	0.18
III	9.0	5500	2500	0.46	0.15	0.26
IV	12.7	3000	1700	0.57	0.16	0.36
Pupa	17.6	1300	500	0.38	0.11	0.21
Adult	21.7	800				K = 1.42

Appendix 5.16 (Continued)

State/ instar	Age in days of beginning of stage	No. entering instar	Death in instar	Relative proportion dying in instar	Proportion dying daily in instar	k-values
Container No.9						
Egg	0	23000	8000	0.35	0.14	0.19
I-instar	2.9	1500	5200	0.35	0.12	0.19
II	6.2	9800	2800	0.29	0.11	0.15
III	9.1	7000	2600	0.37	0.12	0.20
IV	12.6	4400	2300	0.52	0.14	0.32
Pupa	17.5	2100	1200	0.57	0.20	0.37
Adult	21.4	900				K = 1.42
Container No. 10						
Egg	0	19000	7900	0.42	0.16	0.23
I-instar	3	11100	3300	0.30	0.10	0.15
II	6.4	7800	3600	0.46	0.19	0.27
III	9.4	4200	1900	0.45	0.16	0.26
IV	12.9	2300	1400	0.61	0.17	0.41
Pupa	17.8	900	500	0.56	0.19	0.35
Adult	21.6	400				K = 1.67

Appendix 6.1 Number of third instar larvae of Cx. pipiens killed by A. puella at indicated prey densities and time

Prey density	4				8				16				32				64				128				200			
Time (in h)	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Replicate 1	3	1	1	1	2	2	1	1	3	4	0	2	7	5	1	0	9	3	5	0	8	3	6	4	2	9	3	9
" 2	3	1	1	2	2	0	1	1	6	2	1	5	6	5	2	0	7	4	2	1	11	4	2	3	13	4	11	3
" 3	1	0	1	0	2	5	0	0	2	2	2	2	3	1	0	2	10	5	2	1	10	7	2	1	12	2	0	2
" 4	1	2	0	0	1	0	2	1	4	1	3	1	14	7	1	4	14	4	5	5	11	4	2	3	8	7	2	3
" 5	3	2	0	2	5	1	1	0	11	7	7	0	7	6	3	3	10	4	4	2	13	6	4	2	15	4	2	0

Appendix 6.2 Number of third instar larvae of Cx. pipiens killed by S. striolatum at indicated prey densities and time

Prey density	4				8				16				32				64				128				200			
Time (in h)	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Replicate 1	4	3	3	2	7	4	2	1	9	6	8	7	17	8	8	8	23	15	13	9	31	21	15	11	31	21	15	10
" 2	3	3	2	2	4	2	4	3	12	13	7	10	10	7	9	7	22	13	9	8	33	21	13	14	29	20	12	7
" 3	3	2	2	2	5	3	4	4	9	8	8	6	15	9	8	8	20	11	11	10	29	24	14	11	36	18	11	9
" 4	3	2	4	3	3	5	7	4	12	11	9	9	13	10	7	8	17	14	13	9	27	19	10	12	28	23	15	11
" 5	4	4	3	4	5	6	4	5	10	9	7	8	21	15	13	11	20	16	13	10	31	15	15	12	30	17	16	10

Appendix 7.1 Record of population count of hibernating Cx. pipiens (and Cs. annulata in parenthesis) resting on the walls and roof of Site F_2, 1977-78.

Date	Lower half of wall	Upper half of wall	Roof	Total
24.10.77	602	453	369	1424
31.10.77	641	444	408	1494
7.11.77	715 (1)	485 (1)	401 (1)	1603 (3)
14.11.77	703 (2)	465 (1)	342 (1)	1510 (4)
21.11.77	529 (4)	556 (2)	378 (1)	1463 (7)
28.11.77	483	536 (2)	350 (1)	1359 (3)
5.12.77	484 (1)	435 (3)	319 (3)	1238 (7)
12.12.77	502 (1)	468 (6)	307 (1)	1277 (8)
19.12.77	499 (2)	471 (6)	306	1276 (8)
26.12.77	500	427 (7)	341 (1)	1268 (8)
3. 1.78	494	365 (5)	340 (5)	1199 (10)
9. 1.78	439 (2)	319 (8)	320 (2)	1078 (12)
16. 1.78	381 (3)	288 (10)	275 (1)	944 (14)
23. 1.78	298 (2)	210 (3)	172 (2)	680 (7)
30. 1.78	337 (2)	235 (1)	175 (3)	747 (6)
6. 2.78	290 (1)	232 (2)	222 (2)	744 (5)
13. 2.78	260	180 (2)	221 (2)	661 (4)
20. 2.78	235	144 (1)	224 (2)	603 (4)
27. 2.78	202	180	162 (3)	544 (3)
6. 3.78	157	152 (3)	154 (2)	463 (5)
13. 3.78	104	102 (1)	139 (3)	345 (4)
20. 3.78	90 (2)	83	120	293 (2)
27. 3.78	56	56	47	159

Cont....

Appendix 7.1 Continued

Date	Lower half of wall	Upper half of wall	Roof	Total
3. 4.78	25	28	33	86
10. 4.78	18	12	24	54
17. 4.78	14	4	14	32
24. 4.78	8	5	3	16
1. 5.78	3	2	1	6
8. 5.78	o	o	o	o

Appendix 7.2 Record of population count of hibernating Cx. pipiens (and Cs. annulata in parenthesis) resting on the walls and roof of Site F_2, 1978-79.

Date	Lower half of wall	Upper half of wall	Roof	Total
21. 8.78	3	2	0	5
28. 8.78	7	8	6	21
4. 9.78	48	64	24	136
11. 9.78	132	89	50	271
18. 9.78	139	118	63	320
25. 9.78	212	182	119	513
2.10.78	255	162	136	553
9.10.78	276	324	214	814
16.10.78	308	337	193	838
23.10.78	292	310	243	845
30.10.78	227	257 (1)	205	689
6.11.78	275 (3)	284 (2)	213 (1)	772 (5)
13.11.78	227 (3)	201 (1)	178	606 (4)
20.11.78	275	263	167	705
27.11.78	245 (3)	188 (1)	172 (1)	605 (5)
4.12.78	206 (3)	146 (1)	161	513 (4)
11.12.78	190 (4)	205 (3)	163	558 (7)
18.12.78	199	149 (1)	134	482 (1)
25.12.78	98 (2)	95 (2)	110	303 (4)
1. 1.79	108	81 (2)	108	297 (2)
8. 1.79	105 (1)	81 (3)	91 (1)	277 (5)
15. 1.79	90	60	127 (2)	277 (2)
22.1.79	103	48	78	229

Cont.....

Appendix 7.2 Continued

Date	Lower half of wall	Upper half of wall	Roof	Total
29. 1.79	95	24	94	213
5. 2.79	80	40 (1)	85	205 (1)
12. 2.79	88	26	95	209
19. 2.79	78	21 (1)	69	168
26. 2.79	75 (1)	17	73	165 (2)
5. 3.79	69	41	61	171
12. 3.79	80	65	39	184
19. 3.79	64	28	44	136
26. 3.79	43	36	44	123
2. 4.79	12	19	30	63
9. 4.79	14	4	25	41
16. 4.79	7	7	11	25
23. 4.79	0	2	3	5
30. 4.79	1	0	0	1

Appendix 7.3 Record of population count of hibernating Cx. pipens (and Cs. annulata) in parenthesis (resting on the walls and roof of Site F_2, 1979-80.

Date	Lower half of wall	Upper half of wall	Roof	Total
20. 8.79	6	2	3	11
27. 8.79	15	8	10	33
3. 9.79	30	34	10	74
10. 9.79	62	33	22	117
17. 9.79	101	130	59	290
24. 9.79	115	131	75	321
1.10.79	163	167	105	435
8.10.79	239	204	105	548
15.10.79	283	254	119	656
22.10.79	299	238	160	697
29.10.79	319	226	147	692
5.11.79	299	284	151	734
12.11.79	247	255	163	665
19.11.79	220 (2)	194 (2)	114 (2)	528 (6)
26.11.79	230 (2)	194 (1)	114	538 (2)
3.12.79	256	230 (2)	104 (3)	590 (5)
10.12.79	192 (2)	237 (3)	105 (2)	534 (7)
17.12.79	160 (1)	144 (1)	113 (2)	417 (4)
24. 1.79	130 91)	96	91 (2)	317 (3)
31.12.79	166	132 (5)	79	377 (5)
7. 1.80	143 (1)	121 (2)	76	340 (3)
14. 1.80	112	66 (1)	80	258 (1)

Cont...

Appendix 7.3 Continued

Date	Lower half of wall	Upper half of wall	Roof	Total
21. 1.80	109 (1)	48 (2)	68 (2)	225 (5)
28. 1.80	107 (2)	82 (2)	52	241 (4)
4. 2.80	97 (3)	91 (2)	57	245 (5)
11. 2.80	96	59 (1)	59	214 (1)
18. 2.80	92	48	59	199 (2)
25. 2.80	66 (1)	38	71	175 (1)
3. 3.80	49	29 (1)	54	132 (1)
10. 3.80	34	25	39	98
17. 3.80	36	18	50	104
24. 3.80	32	7	33	72 (1)
31. 3.80	33	15	23	71
7. 4.80	14	10	18	42
14. 4.80	7	3	6	16
21. 4.80	1	1	0	2
28. 4.80	0	2	0	2

Appendix 7.4 Record of total population count of hibernating *Cx. pipiens* (and *Cs. annulata* in parenthesis) in Site F_1.

1977-78		1978-79	
Date	No. counted	Date	No. counted
November 2	522 (37)	August 15	0
8	380 (78)	30	00
15	231 (75)	September 15	127
22	280 (77)	30	156
29	240 (70)	October 15	269 (35)
December 6	238 (86)	30	273 (37)
13	245 (78)	November 15	304 (53)
20	243 (83)	30	289 (44)
27	279 (101)	December 15	207 (62)
January 3	309 (115)	30	234 (68)
10	289 (117)	January 15	166 (54)
17	278 (121)	30	154 (46)
24	195 (89)	February 14	98 (42)
31	207 (83)	28	88 (33)
February 7	159 (74)	March 15	54 (35)
15	118 (40)	30	34 (19)
21	107 (35)	April 15	21 (8)
28	87 (61)	30	6 (0)
March 7	70 (56)	May 15	0
14	63 (40)		
21	54 (47)		

Cont...

Appendix 7.4 Continued

1977-78

Date		No. counted
March	28	58 (7)
April	4	33
	11	20
	18	25
	25	5
May	2	0

Appendix 7.5 Record of total population count of Cx. pipiens (and Cs. annulata in parenthesis) in room G_1 of hibernation Site G.

1977-78		1978-79	
Date	No. counted	Date	No. counted
November 3	2001 (18)	August 15	0
8	1811 (21)	30	18
15	1647 (18)	September 15	183
22	1484 (22)	30	402
28	1451 (20)	October 15	643
December 6	1466 (21)	30	983 (4)
13	1445 (20)	November 15	933 (26)
20	1551 (23)	30	850 (28)
27	1483 (21)	December 15	791 (19)
January 3	1440 (27)	30	720 (17)
10	1398 (29)	January 15	680 (16)
17	1370 (26)	30	664 (23)
24	1003 (20)	February 14	509 (14)
31	989 (16)	28	442 (13)
February 7	901 (13)	March 15	204 (8)
14	933 (10)	30	187 (6)
21	951 (12)	April 15	73
28	600 (15)	30	25
March 7	451 (12)	May 15	0
14	298 (8)		
21	120 (10)		
28	36 (3)		

Cont....

Appendix 7.5 Continued

1977-78

	Date	No. counted
April	4	17
	11	6
	18	6
	25	0

Appendix 7.6 The mean weight in mg (±SE) of various substances in female Cx. pipiens collected at different times during hibernation

1977 - 78

Date	No. of mosquitoes collected	Dry weight	Fat	Solids not fat (by difference)
Oct.30	25	1.85±0.06	1.06±0.04	0.79
Nov.15	25	1.65±0.04	0.90±0.04	0.75
Nov.30	25	1.46±0.05	0.73±0.05	0.73
Dec.15	25	1.35±0.06	0.66±0.03	0.69
Dec.30	25	1.16±0.03	0.45±0.02	0.71
Jan.15	25	1.10±0.04	0.39±0.03	0.71
Jan.30	25	0.95±0.04	0.29±0.02	0.66
Feb.15	25	0.94±0.05	0.28±0.03	0.66
Feb.28	25	0.94±0.03	0.25±0.02	0.69
Mar.15	25	0.85±0.04	0.20±0.03	0.65

Cont....

Appendix 7.6 Continued

1978 - 79

Date	No. of mosquitoes collected	Live weight	Dry weight	Water	Fat	Solids not fat (by difference)
Sept.15	25	4.00±0.13	2.00±0.06	2.0	1.31±0.04	0.69
Sept.30	25	4.40±0.17	1.80±0.06	2.6	1.10±0.05	0.70
Oct. 15	25	3.86±0.18	1.64±0.07	2.22	0.94±0.05	0.70
Oct. 30	25	3.78±0.14	1.54±0.06	2.24	0.84±0.03	0.70
Nov. 15	25	3.40±0.13	1.28±0.06	2.12	0.67±0.04	0.61
Nov. 30	25	3.36±0.17	1.16±0.06	2.20	0.57±0.04	0.59
Dec. 15	25	3.34±0.18	1.08±0.05	2.26	0.47±0.03	0.61
Dec. 30	25	3.23±0.15	0.99±0.05	2.24	0.40±0.03	0.59
Jan. 15	25	2.92±0.12	0.91±0.05	2.01	0.35±0.03	0.56
Jan. 30	25	2.83±0.15	0.86±0.05	1.97	0.30±0.03	0.56
Feb. 15	19	2.69±0.13	0.86±0.05	1.83	0.24±0.03	0.62
Feb. 28	13	2.50±0.14	0.87±0.04	1.63	0.21±0.03	0.66
Mar. 15	8	2.50±0.30	0.75±0.04	1.75	0.18±0.04	0.57
Mar. 30	5	2.10±0.40	0.75±0.09	1.35	0.19±0.03	0.56

Appendix 7.7 The dry weight and the quantity of Glycogers in mg ($\pm$SE) in an average female Cx. pipiens collected at different periods during hibernation.

	1977 - 78			1978 - 79		
Month	No. Collected	Dry weight	Glycogen	No. Collected	Dry weight	Glycogen (mg)
Oct.15		-	-	25	1.76$\pm$0.06	0.069$\pm$0.006
Oct.30		-	-	25	1.50$\pm$0.044	0.065$\pm$0.005
Nov.15	25	1.56$\pm$0.08	0.074$\pm$0.007	25	1.24$\pm$0.05	0.063$\pm$0.004
Nov.30	25	1.30$\pm$0.05	0.072$\pm$0.006	25	1.14$\pm$0.04	0.061$\pm$0.004
Dec.15	25	1.36$\pm$0.06	0.069$\pm$0.005	25	1.07$\pm$0.06	0.060$\pm$0.006
Dec.30	25	1.20$\pm$0.05	0.053$\pm$0.005	25	0.98$\pm$0.05	0.062$\pm$0.003
Jan.15	25	1.14$\pm$0.06	0.061$\pm$0.005	25	0.92$\pm$0.038	0.063$\pm$0.003
Jan.30	25	0.96$\pm$0.04	0.054$\pm$0.004	25	0.88$\pm$0.033	0.060$\pm$0.006
Feb.15		-	-	20	0.77$\pm$0.04	0.061$\pm$0.005
Feb.28		-	-	11	0.76$\pm$0.04	0.057$\pm$0.004
Mar.15		-	-	10	0.72$\pm$0.06	0.059$\pm$0.007
Mar.30		-	-	5	0.65$\pm$0.05	0.057$\pm$0.007

Dashes, no collection made.

Appendix 7.8 Number of adult Cx. pipiens killed by spider, Amaurobius ferox at indicated prey densities and time

Prey density	2				4				8				16				32			
Time (in h)	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Replicate 1	0	1	1	1	0	0	3	0	4	4	3	2	6	6	4	1	4	4	3	2
" 2	2	0	2	0	2	2	2	1	6	2	1	4	7	8	1	1	4	5	4	4
" 3	1	2	1	1	0	2	0	0	5	3	2	4	3	4	4	4	5	9	6	1
" 4	0	0	1	1	3	2	1	1	2	3	3	2	5	2	2	2	6	6	4	3
" 5	0	1	0	0	2	0	1	1	4	6	1	2	4	4	5	2	6	2	3	1

Appendix 7.9 Weekly record on population fluctuation of hibernating Cx. pipiens (and Cs. annulata in parenthesis) in room G_3 of Site G, 1979-1980.

Date	No. of mosquitoes	Spider population	Number of mosquitoes			
			caught in web	dead on wall & floor	caught in Exit trap	Not accounted for
Oct.29	759 (13)	22				
Nov. 5	710 (12)	15	26	0	21 (1)	2
12	678 (10)	18	20	1	6 (2)	5
19	664 (10)	23	7	0	1	6
26	642 (10)	27	8	0	12	2
Dec. 3	601 (7)	39	13	4	20 (3)	4
10	551 (7)	26	9	4	30	5
17	516 (7)	33	15	11	9	0
24	501 (4)	36	9	6	0 (1)	0 (2)
31	463 (4)	29	5	7	20	4
Jan. 7	446 (4)	30	2	2	6	7
14	422 (3)	38	6	10	0	8 (1)
28	396 (3)	36	7	2	3	2
Feb. 4	375 (1)	33	4	2	6 (1)	9 (1)
11	357 (0)	36	0 (1)	1	15	2
18	330	38	4	0	16	7
25	317	29	0	3	6	4
Mar. 3	305	24	3	0	3	6
10	291	22	2	7	2	3
17	231	20	6	12	32	10
24	189	13	3	8	26	5
31	140	16	0	9	32	8

Cont...

Appendix 7.9 Continued

Date	No. of mosquitoes	Spider population	Number of mosquitoes			
			caught in web	dead on wall & floor	caught in Exit trap	Not accounted for
Apr. 7	98	12	1	6	28	7
14	41	15	2	11	41	3
21	8	11	0	6	25	2
28	7	13	0	0	0	1
May 5	4	9	0	2	1	0
12	0	6	0	0	2	2