THE INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON OVARIAN DEVELOPMENT IN *CULEX TARSALIS* AND *CULISETA INORNATA* (DIPTERA: CULICIDAE), IN SOUTHERN MANITOBA

A Thesis

Submitted to the Faculty

of

Graduate Studies

by

Andrew James Mackay

In Partial Fulfilment of the

Requirements for the Degree

of

Master of Science

Department of Entomology

University of Manitoba

May 1996

THE UNIVERSITY OF MANITOBA

FACUTY OF GRADUATE STUDIES

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BY

ANDREW JAMES MACKAY

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Dedicated to my wife Denine

ACKNOWLEDGEMENTS

I would like to sincerely thank the members of my examining committee, Dr. Terry Galloway, Dr. Roger Evans, and my advisor Dr. Reinhart Brust. Their guidance and patience throughout the development of this thesis is greatly appreciated. I would especially like to thank Dr. Brust. Over the past several years his insight, wisdom and strength of character have been a great inspiration.

I would like to thank Daryl Wright and Dave Holder for their technical assistance throught this project. I would also like to thank Andy Fox for his many valuable suggestions, and my parents Jim and Margaret Mackay for their support.

This thesis might not have been possible without the assistance of my wife Denine. Her unflagging support, encouragement and understanding helped me endure many a difficult time.

Financial support for this research was provided by a National Research Council of Canada grant (No. A2545) awarded to Dr. Brust.

ABSTRACT

Ovarian follicle development in *Cx. tarsalis* Coquillett, and ovarian follicle development and blood meal utilization in *Cs. inornata* (Williston), were examined under laboratory and field conditions. In the laboratory, photoperiod (L:D 18:6, 14:10 and 12:12) had a significant influence on the number of *Cx. tarsalis* entering diapause at two temperatures (19°C and 22°C). Temperature did not have an influence on the proportion of *Cx. tarsalis* in diapause at 18:6 or 12:12 (L:D). In 1992, *Cx. tarsalis* emerging under natural conditions did not begin to enter diapause until after the middle of August. At two temperatures (16°C and 19°C), *Cs. inornata* reared in the laboratory entered diapause at 14:10 and 12:12 (L:D), but many females terminated diapause within 34 days after emergence. In 1993, *Cs. inornata* emerging under natural conditions in late August were in diapause, and maintained diapause for at least eighteen days.

Females emerging in a state of reproductive diapause do not seek a blood meal, and therefore would not vector a pathogen such as Western Equine Encephalomyelitis virus (WEEV). Should another outbreak of WEEV occur in southern Manitoba, the ability to predict the seasonal timing of diapause induction in *Cx. tarsalis* would be an important factor in evaluating the transmission potential of a pathogen to new hosts.

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CHAPTER I

GENERAL INTRODUCTION

Insects must be able to synchronize growth and development with the temporal distribution of favourable environmental conditions. Some insects achieve this by arresting development in a diapause state prior to the onset of adverse conditions. In cool-temperate climates, mosquitoes of the genera *Culex*, *Culiseta* and *Anopheles* overwinter as inseminated adults in a state of reproductive diapause (Washino 1977, Eldridge 1987, Mitchell 1988).

The ability of a disease vector to acquire and transmit a pathogen may be influenced by the seasonal timing of diapause induction. From a public health viewpoint, the most important characteristic of reproductive diapause in *Culex tarsalis*, *Culex pipiens* and *Culiseta inornata* is the absence of host-seeking behaviour (Hudson 1977b, Mitchell 1981, Bowen *et al.* 1986). Overwintering females do not normally seek a blood meal in nature (Bellamy and Reeves 1963, Hudson 1979, Mitchell 1979), and are unlikely to be involved in the transmission of pathogens. Therefore, the ability to predict the onset of reproductive diapause is advantageous when assessing the risk of transmission posed by females emerging late in the season.

The Western Equine Encephalomyelitis virus (WEEV) is endemic to much of western Canada and the United States, including southern Manitoba. The virus is maintained in a primary enzootic cycle involving the primary vector, *Cx. tarsalis* (Sekla *et al.* 1980) and several wild bird species. Other mosquito

species, such as *Cs. inornata*, may play a minor role in the enzootic cycling of WEEV during the early spring, or in epizootic transmission to equines in the late summer (Spalatin *et al.* 1963, McLintock *et al.* 1970). Epidemic transmission of the virus (WEEV) may occur late in the season when infective *Cx. tarsalis* shift from feeding on the avian reservoir hosts to feeding on mammalian hosts (Tempelis *et al.* 1965). When periodic outbreaks occur in Manitoba, emergency efforts to control the vector are often extended late into the summer (Brust and Ellis 1976, Ellis 1982).

Cx. tarsalis occupies an extensive range across the United States and western Canada, closely coinciding with the geographical distribution of WEEV. However, much of the research devoted to diapause in *Cx. tarsalis* has been limited to the southwestern United States. In southern California, diapause in *Cx. tarsalis* is often weakly expressed and of a brief duration (Bellamy and Reeves 1963, Nelson 1971, Reisen *et al.* 1986b, Reisen *et al.* 1995). Assumptions on the vectorial capacity and seasonal patterns of reproductive activity of *Cx. tarsalis*, based on data collected from these populations, may not be representative of populations in southern Manitoba. To develop practical epidemiological models which may be used in making vector control decisions, phenological data on reproductive diapause in Manitoba populations of *Cx. tarsalis* must be available. The primary objectives of this research project were to investigate the influence of temperature and photoperiod on diapause induction in local (southern Manitoba) strains of *Cx. tarsalis* and *Cs. inornata*, and to

determine the seasonal onset of reproductive diapause in the two species, under field conditions in southern Manitoba.

CHAPTER II

LITERATURE REVIEW

Oogenesis in the Mosquito

Mosquito ovaries consist of a number of ovarioles. Each ovariole consists of a germarium, which contains the germinal tissue, and a polytrophic ovarian follicle, containing a single oocyte and seven trophocytes (Mitchell 1988). Maturation of the oocyte, a process referred to as oogenesis, produces a single unfertilized egg. The primary follicles are the first set to develop, with subsequent follicles designated as secondary, tertiary, etc (Meola and Readio 1989).

In gonoactive (i.e. non-diapausing) females, ovarian development begins immediately after adult emergence. Initially the follicle consists of eight undifferentiated cells (Hagedorn *et al.* 1977). The oocyte becomes discernable from the trophocytes as juvenile hormone (JH) (Readio *et al.* 1988), synthesized and secreted by the corpora allata (CA), stimulates follicle development (Gwadz and Spielman 1973). In anautogenous females, growth continues over three to four days (Sanburg and Larsen 1973), ending in what is referred to as the resting stage. The period of ovarian development from emergence to the resting stage is called previtellogenesis (PVG).

The second phase of ovarian development, vitellogenesis (VG), is initiated once a blood meal is acquired by the mosquito. Either a neural

mechanism (Larsen and Bodenstein 1959), a hormonal mechanism (Van Handel and Lea 1984), or some combination of the two (Uchida *et al.* 1992), stimulates the release of enzymes for digestion of the blood meal. It appears that a signal from the blood, possibly the amino acids liberated during digestion (Uchida *et al.* 1992), initiates egg development. A general overview of vitellogenesis made be found in Hagedorn (1994).

Ovarian Diapause

In diapausing mosquitoes, ovarian development is arrested prior to reaching the resting stage. This was first observed by Mer (1936). He reported that previtellogenic development was impaired in *Anopheles elutus* Edwards, when the aquatic stages of the mosquito were subjected to diapause inducing conditions. Danilevskii and Glinyanaya (1958) later found that in *Culex pipiens pipiens* Linnaeus, the ovarian follicles of non-diapausing females were one and a half times greater in size than those of diapausing females. Gwadz and Spielman (1973) established that PVG is dependent on the influence of JH. Spielman (1974) later demonstrated that removal of the CA in non-diapausing *Cx. pipiens* caused an arrest of ovarian development, similar to that seen in diapausing mosquitoes. He also found that exposure to synthetic JH restored development in diapausing females, and concluded that diapause in this species was controlled by the secretion of JH from the CA. The regulation of adult diapause by changes in JH levels has been documented in many other insect

species (Denlinger 1985).

Clements and Boocock (1984) have listed five stages (developmental gates) at which ovarian development may be arrested in the absence of an appropriate stimulus. Some authors have used the term 'ovarian diapause' to describe any arrest in ovarian development (Spielman 1957, 1974). In the present paper, ovarian diapause refers exclusively to the condition of the ovaries in diapausing adult mosquitoes ('stage I gate' of Clements and Boocock 1984).

Recognition of Diapause In Adult Culex spp. and Culiseta inornata

There are significant physiological and behavioral changes associated with diapause. Some characteristics of diapause in these species include: (i) premature arrest of ovarian development, (ii) cessation of host-seeking behaviour, (iii) an increase in size and functional capacity of the fat body, (iv) a reduction in metabolic rate, and (v) increased cold-hardiness (Hudson 1977a, Eldridge 1987).

Initial recognition of the adult diapause condition began with observations on the activities of field and laboratory populations of anopheline mosquitoes, noting such things as the lack of oviposition after blood feeding and larger fat body associated with the winter season (Tate and Vincent 1936). However, most authors have defined the diapause condition based on the morphology of the ovarian follicles. In the earliest studies, Mer (1936) used the classification system introduced by Christophers (1911) to evaluate follicle development.

Christophers' (1911) system has been modified by other authors (Kawai 1969, Watts and Smith 1978, Clements and Boocock 1984), but the classification of diapause-stage follicles is unchanged. By simple definition, ovarian follicles of diapausing females are distinguished by the absence of visible lipid droplets at 200x magnification (Watts and Smith 1978). This is designated as Christophers' stage I by Wilton and Smith (1985), Arntfield *et al.* (1982) and Clements and Boocock (1984), and by stage N of Kawai (Oda *et al.* 1978).

Although descriptive follicle stages are useful, some authors have preferred to use quantitative methods to evaluate ovarian development. Most often the mean primary follicle length (FL) (Oda and Wada 1972a), or the mean primary follicle length to mean germarium length (F:G) ratio (Spielman and Wong 1973a) is used. When the F:G ratio method is used, preliminary experiments are usually done to determine the mean F:G ratios of females that have been classified previously as diapausing or nondiapausing, based on their stage of follicular development. A somewhat arbitrary value is then determined that best separates the F:G ratios of the two groups.

Blood Feeding and Diapause in Culex spp. and Culiseta inornata

Many authors have applied the term 'gonotrophic dissociation' (GTD) when discussing blood feeding in diapausing *Culex* mosquitoes (Tekle 1960, Takahashi 1970, Oda and Wada 1972, Oda and Kuhlow 1976, Mitchell 1981, Arntfield *et al.* 1982). GTD, first described by Swellengrebel (1929), is a

phenomenon observed in certain *Anopheles* spp. that continue blood feeding throughout the overwintering period (Danilevskii and Glinyanaya 1958, Whang 1961), and use the blood to develop fat body rather than eggs (Washino 1977). Eldridge (1987) has cautioned against the use of this term in reference to blood feeding by diapausing *Culex* mosquitoes. For the "true" definition of GTD to be appropriate, the behavioral (blood feeding during diapause) and physiological (products of blood digestion not used for oogenesis) components must be present.

Host-seeking Behaviour

In mosquitoes, the acquisition of a blood meal is dependent on a complex sequence of behaviours, beginning with host-seeking (Friend and Smith 1977). Host-seeking behaviour may be defined as the inflight orientation of the avid female to a potential host (Bowen 1991). Once the appropriate host cue is detected, a programmed behavioral response is triggered in females primed to receive the signal. Each behaviour is mediated by different stimuli detected by different sets of receptors (Bowen 1991). For long range orientation towards a potential host, mosquitoes rely primarily on olfactory signals, such as carbon dioxide and lactic acid (Bowen 1991).

In the first study to differentiate host-seeking behaviour from blood feeding behaviour in diapausing *Cx. tarsalis*, Mitchell (1981) examined the blood feeding frequency in different sized cages. Mitchell induced diapausing females

to feed by placing them in close proximity to the host, eliminating the need for host-seeking behaviour to be expressed. This method has been used to induce blood feeding in diapausing *Cx. pipiens* (Eldridge 1968) and diapausing *Cs. inornata* (Kalpage 1970). Mitchell (1981) concluded that host-seeking behaviour and blood-feeding behaviour are mutually exclusive events, controlled by independent mechanisms. Thus when host-seeking behaviour is bypassed, inadvertent contact with the host allows blood feeding behaviour to be expressed.

Diapausing *Cx. pipiens* females do not respond to certain host-related, olfactory stimuli, even when deprived of food (Bowen 1992). Bowen *et al.* (1986) evaluated host-seeking behaviour in *Cx. pipiens* by measuring flight and probing activity in response to human breath. The authors reported a strong diel periodicity of host-seeking behaviour in gonoactive females, but did not observe any response in diapausing females. Bowen *et al.* (1988) associated the absence of host-seeking behaviour in diapausing *Cx. pipiens* with a decrease in the number of highly sensitive peripheral receptors on the antennae that are responsible for the detection of lactic acid (LA). Bowen (1990) noted an increase in the number of highly sensitive LA receptors in *Cx. pipiens* after diapause had been terminated. Bowen (1990, 1991) suspected that the state of the peripheral sensory system in diapausing *Cx. pipiens* may represent a condition of arrested development, akin to the state of diapausing ovaries. Host-seeking behaviour, like ovarian development, is restored in diapausing *Culex* mosquitoes after

exposure to JH (Meola and Petralia 1980). Conversely, JH deficiency apparently results in cessation of host-seeking behaviour (Meola and Readio 1989).

Blood Meal Utilization

The acquisition of a blood meal initiates vitellogenesis in non-diapausing, anautogenous mosquitoes. In diapausing females however, the status of the reproductive and hormonal systems is very different. Hudson (1977b) reported that diapausing *Cs. inornata* took smaller blood meals and seldom developed eggs. Mitchell (1981) found that the majority of diapausing *Cx. tarsalis* maintained under winter conditions failed to initiate vitellogenesis after given a blood meal. These results were contrary to earlier observations on diapausing *Cs. inornata* (Kalpage 1970) and *Cx. pipiens* (Oda and Wada 1972, Sanburg and Larsen 1973), where a much higher rate of egg development followed blood feeding. Hudson (1977b) suggested that this discrepancy was due to larger blood meal volumes in the earlier studies. In non-diapausing mosquitoes, threshold blood meal volumes for initiating egg development (Volozina 1967, Lea *et al.* 1978) and protease activity (Briegel and Lea 1975) have been reported. However, Hudson (1979) was unable to detect trypsin activity in the midgut of diapausing females. He speculated that diapausing *Cs. inornata* were physiologically incapable of digesting blood. Hudson associated the lack of trypsin activity with premature blood meal ejection from the midgut of diapausing females. However, Mitchell and Briegel (1989b) observed that blood digestion

was reduced, not absent, in diapausing *Cx. pipiens*. The authors speculated that the threshold-blood meal volume for blood digestion may be higher in diapausing females, due to slow or inactive endocrine mechanisms. Mitchell and Briegel (1989a) confirmed that diapausing females initiating vitellogenesis consumed significantly larger blood meals than diapausing females that did not initiate vitellogenesis.

Although blood feeding can be induced in diapausing *Culiseta inornata* and *Culex* spp. without subsequent egg development, no evidence has been presented to suggest that blood is used for fat body development. When Mitchell and Briegel (1989a) compared the total lipid content of blood fed vs. sugar fed diapausing *Cx. pipiens*, the lipid content of the blood fed females was substantially lower. It seems unlikely that a prehibernation blood meal would provide sufficient stores of energy for overwintering.

Field Evidence of Blood Feeding By Overwintering Females

In *Culex* mosquitoes, the only convincing evidence for blood feeding in overwintering females was reported by Bailey *et al.* (1982). They collected a single, blood-engorged *Cx. pipiens* female from a resting site in early November. After a two week period under diapause conditions, the ovaries of the blood fed female were undeveloped and there was no indication of parity, suggesting that it was in diapause. Although this is a clear example of blood feeding in an overwintering *Cx. pipiens* female, this is the only documented case and probably

represents a very rare phenomenon in *Culex*. Whang (1961) looked at over five thousand hibernating *Cx. pipiens* in Korea and failed to find any blood fed females. Similarly, Slaff and Crans (1977) examined 940 female *Cx. pipiens* in a New Jersey overwintering site and were unable to locate any blood engorged mosquitoes. None of the 87 *Cx. territans* Walker collected by Hudson (1978) from natural overwintering sites in central Alberta were blood fed or parous. Of the 487 *Cx. pipiens*, 160 *Cx. torrentium* Martini and 54 *Cx. territans* collected by Jaenson (1987) over an entire overwintering period (August to May), there was no visible blood found in any of the mosquitoes and only three parous females (*Cx. torrentium*) were collected after October. Jaenson (1987) noted that the absence of hosts and the cool, moist conditions in the overwintering site did not facilitate blood feeding.

Some circumstantial evidence has been forwarded to support the notion that prehibernation blood meals are common in *Cs. inornata*. In the study by Shemanchuk and Morgante (1968) in central and southern Alberta, a number of blood-engorged *Cs. inornata* and *Cx. tarsalis* were collected from animal burrows during the middle and late part of August. However, they did not attempt to determine the physiological status. Hudson (1977a) observed the presence of gonoactive *Cs. inornata* in central Alberta during late August. It is possible that the blood fed mosquitoes collected by Shemanchuk and Morgante (1968) were part of a previous, non-diapausing generation not destined to overwinter. Dow *et al.* (1976) were of the opinion that in Colorado, nearly all *Cs. inornata* take a

blood meal before overwintering. They based this conclusion on the parity status of females collected after the middle of March. However, the mean weekly air temperature reported in this study was above freezing for over a month before the first parous female was collected. The mean blood digestion rate under these conditions was calculated to be 10.4 days (Dow *et al.* 1976). Females could have blood fed and completed a gonotrophic cycle prior to the first collection of parous females in late March. Hudson (1977a) observed the same phenomenon in central Alberta. He noted that overwintering *Cs. inornata* collected in calf-baited traps were mostly parous or gravid, and did not appear until about seven weeks after snowmelt, about five weeks later than most other species examined. He suggested that an undetected period of blood feeding may have occurred soon after snowmelt. Overwintering females may have fed on cattle at some time in the day (diel) when trapping was not done. It is apparent from the literature that there is simply insufficient evidence to support the hypothesis that diapausing *Cs. inornata* normally blood feed prior to overwintering.

Induction of Diapause

In the Northern Hemisphere, female *Culex* overwinter as inseminated adults (Eldridge 1987), as does *Cs. inornata* (Shemanchuk 1965). Like most mosquito species that hibernate as adults (Mitchell 1988), *Cs. inornata* and most *Culex* spp. are multivoltine, with diapause facultatively expressed. Prior to the

onset of unfavourable conditions, environmental cues experienced by the sensitive stage(s) predetermine the physiological status of the adult female (i.e. females cannot enter diapause once reproductive development is complete).

Culex tarsalis

Culex tarsalis is widely distributed throughout western North America, from northern Alberta and Manitoba, south to Mexico (Wood *et al.* 1979). In populations situated close to the southern limits of the range, diapause may only be weakly expressed or even absent (Nelson 1971). Most of the research on overwintering in *Cx. tarsalis* has been restricted to populations from the southern regions of the Canadian prairies and the western United States, primarily Colorado and California.

The photosensitive stage in *Cx. tarsalis* is controversial. Harwood and Halfhill (1964) inferred that photosensitivity was occurring during the larval stage. Reisen (1986a, 1986b) later suggested that *Cx. tarsalis* was sensitive to photoperiod and temperature during the larval and early pupal stages. Reisen (1986b) transferred females from 25° C and 16 hours light, to 18° C and 10 hours light, at different stages of the life cycle. Very few females entered diapause when transferred as pupae, but a high percentage entered diapause when transferred as fourth instar larvae. In the former group however, females were transferred in this experiment as late as 48 hours after pupation. As Brust (1991) pointed out, 25° C and 16 hours light may have initiated ovarian development in

the late fourth instar larvae and young pupae, negating the effect of short photoperiod in the latter portion of the pupal stage. When Brust (1991) conducted a similar experiment, examining the effects of photoperiod under a constant temperature of 21° C, the pupae were photosensitive.

In *Cx. tarsalis*, the diapause induction response to photoperiod is of the 'long-day' type (i.e. diapause induction occurs only at short daylengths). Insects which exhibit a long-day photoperiodic response typically have a narrow range between inductive and non-inductive photoperiods (Danks 1987). However, Eldridge (1987) claims that *Culex* spp. exhibit a more 'graded' diapause induction response to photoperiod. He reported a gradual increase in the proportion of *Cx. tarsalis* females entering diapause between 17L:7D and 11L:13D. As supporting evidence, Eldridge (1987) cited similar studies with *Cx. pipiens* (Vinogradova 1960, Sanburg and Larsen 1973) and *Cx. peus* Speiser (Skultab and Eldridge 1985). However, Sanburg and Larsen (1973) reported that FL was not significantly different between females reared at 9, 10, 11 and 12 hours light, nor between females reared at 13 and 14 hours light. The FL of females reared at ≤12 hours light, and the FL of females reared at 13 and 14 hours light was consistent with the FL's of diapausing and non-diapausing *Cx. pipiens*, respectively (Eldridge and Bailey 1979). Similarly, Reisen *et al.* (1989) reported that the FL of *Cx. tarsalis* was consistent with the diapause condition at 8 and 10 hours light, consistent with the non-diapause condition at 14 and 16 hours light, and intermediate at 12 hours light (Reisen *et al.* 1989).

The effect of temperature on diapause induction is not clear. Buth *et al.* (1990) suggested that temperature has very little influence on the proportion of females entering diapause at a specific daylength. At 8L:16D, the incidence of diapause at 15, 20 or 25° C was consistently about 65 per cent. However, Reisen *et al.* (1989) suggested that higher temperatures may inhibit diapause induction. At 10L:14D, diapause was induced in *Cx. tarsalis* at 18° C, but not at 26° C. As well, Harwood and Halfhill (1964) reported that ovarian and fat body development were influenced by short photoperiod, but not to the extent seen in nature. Low temperatures may have enhanced the response to short photoperiod. This was substantiated by Reisen *et al.* (1986a). When *Cx. tarsalis* was reared at about 11 hours light, diapause was induced more uniformly at 16° C than at higher temperatures. By delaying preimaginal development, lower temperatures may extend the length of the photosensitive stage and permit the females to experience a greater number of short daylength cycles. In other insects, the number of short days experienced during the photosensitive stage determines whether the insect enters diapause (Denlinger and Bradfield 1981, Saunders 1981). However, it is not clear why Buth *et al.* (1990) were unable to demonstrate an effect of temperature on diapause induction. It may be that 8 hours light is sufficiently short to nullify the effects of higher temperatures (i.e. at 8 hours light, the maximum potential for diapause induction is reached, regardless of temperature). Sanburg and Larsen (1973) reported that the influence of temperature on diapause induction is reduced at shorter

photoperiods, in *Cx. pipiens*. Although only about 65 per cent of the females examined by Buth *et al.* (1990) entered diapause, the strain of *Cx. tarsalis* used in this experiment had been colonized for many years, and Reisen *et al.* (1986a) have suggested that colonization may select against the ability to enter diapause.

In the northern range of *Cx. tarsalis*, observations on the environmental control of diapause in field populations have failed to generate a clear understanding of the mechanisms involved. Shemanchuk and Morgante (1968) reported that overwintering *Cx. tarsalis* entered hibernation in southern Alberta when the temperature at night dropped to 6° C, and the daylength decreased by three hours from maximum daylength. The collection sites were located at approximately 50° N latitude, so the mosquitoes observed in this study would have entered hibernation when the daylength was about 13.5 hours light (Danks 1987). However, they only observed hibernation behaviour, which may not relate to the timing of physiological events. Hudson (1977a) collected diapausing females in Edmonton, Alberta, beginning in late August. However, Hudson (1977a) had only collected six females, and thus this sample may not be representative of the reproductive status of the field population as a whole. Other authors have reported diapause in *Cx. tarsalis* to occur much earlier than suggested from laboratory experiments. In Winnipeg, Buth *et al.* (1990) found that the incidence of diapause in field-reared females rose from zero in mid-July, to about 70% by the middle of August. The mean natural air temperature was

relatively constant at about 20° C from mid-July to mid-August, and the daylength in Winnipeg (50° N latitude) was under 16 hours light (15.7), during the middle of August (Danks 1987). Based on observations from laboratory experiments, fewer females should be entering diapause under these conditions. Buth *et al.* (1990) had reported that less than 20 per cent of the *Cx. tarsalis* females reared at 15° C and 16 hours light initiated diapause in the laboratory. It appears that some natural influence on diapause induction was not replicated in the laboratory. A similar phenomenon has been noted in *Anopheles maculipennis* Falleroni. Danilevskii and Glinyanaya (1958) found that this species began diapause induction in Leningrad when the natural daylength was about 19 hours light. The authors suggested that diapause induction under a very long daylength may be characteristic of northern populations.

In the southern range of *Cx. tarsalis*, workers have been more successful interpreting the influence of environmental factors in promoting diapause induction in field populations. In a study in Kern County (35° N latitude), California, Bellamy and Reeves (1963) observed a decline in the number of blood-engorged females in resting sites during the late fall. The authors speculated that the seasonal decline in blood feeding was not due simply to flight inhibition during cool temperatures, but rather it was a facultative diapause condition. Although diapause was initiated primarily by declining daylength, a concurrent drop in temperature was recorded. Reisen *et al.* (1983) later associated declining autumnal temperatures with a cessation in blood feeding. In

Kern County, the decline in the relative abundance of host-seeking females occurred as the mean air temperature dropped from about 28° C in early September to less than 20° C in early October, and the daylength decreased from about 12.5 to 11.5 hours light. There was an increase in the number of overwintering females collected in resting sites subsequent to the drop in temperature. Reisen *et al.* (1986a) contributed additional evidence to relate the decline in daylength and temperature with diapause induction in the field. *Culex tarsalis* were reared in Kern County, under natural conditions. During the preimaginal development of the experimental females, the weekly mean temperature dropped from approximately 23° C in late October to 19° C in early November, while daylength declined from about 12 to 10.5 hours light. This coincided with the laboratory observations on this population. Reisen (1986a) had found that, in the laboratory, the progeny of females collected from Kern County initiated diapause at 16° C and a constant photophase of 10 hours light. Although the weekly mean air temperature was above 16° C in the field experiment, the weekly mean minimum temperature during pupation was approximately 15-16° C. Cool temperatures during the scotophase may enhance the response to short photophase (Danks 1987). It is possible that diapause induction in southern populations of *Cx. tarsalis* does not occur until the late autumn temperature falls below a specific threshold.

Culiseta inornata

Cs. inornata is widely distributed throughout most of North America, extending from Mackenzie Bay in the Yukon, down to Florida and northern Mexico (Wood *et al.* 1979). There has been limited research to examine the influence of environmental factors on diapause in *Cs. inornata*.

In the southern range of *Cs. inornata*, this species is reproductively active throughout the winter and aestivates in the summer (Barnard and Mulla 1978). Ovarian development in aestivating females is not under arrest, as it is in diapausing females (Reisen 1987). In the field, aestivation is exhibited by parous, gravid and nulliparous females (Reisen *et al.* 1989).

In colder regions, *Cs. inornata* is active during the warmer months and hibernates in a diapause condition during the winter. The diapause induction mechanism in *Cs. inornata* is very different from that observed in *Culex* species. Development under a constant short photoperiod is not sufficient to arrest ovarian development in this species, regardless of the temperature. The first evidence of this was seen in work done by Kalpage (1970). Blood feeding rates were examined in females reared under a number of different constant temperature and photophase combinations. Low temperature and/or short photoperiod reduced the number of females that would accept blood. However, all blood-fed females initiated egg development, regardless of the conditions under which they were reared. Females reared under short photoperiod and/or low temperatures in this experiment may not have been in diapause. This was

substantiated by Hudson (1977b), who reported that two week old females, reared as larvae under a constant photophase of 12 hours light and 10° C, developed resting stage follicles and had a mean F:G ratio indicative of the nondiapause condition. He also found that when females were initially reared at 20° C and 16 hours light, and then transferred as fourth instar larvae to 10°C and 12 hours light, the F:G ratio and follicular development of two week old, adult females were indicative of diapause. The assumption that *Cs. inornata* perceives a change in daylength, rather than an absolute daylength, was supported by the observation that the blood feeding rate was reduced under the transfer conditions, and none of the females that fed developed eggs. Hudson found that the transfer from long day to short day conditions was only effective in promoting diapause when applied during the fourth larval instar or larval-pupal ecdysis.

Contrary to the results of Hudson (1977b), Buth *et al.* (1990) have reported that *Cs. inornata* will enter diapause under constant conditions. In their study, *Cs. inornata* was subjected to constant conditions and the F:G ratios were calculated two weeks after adult emergence. Under a constant 8L:16D, the proportion of females in diapause was approximately 80% at 15° C, 60% at 20° C, and 60% at 25° C. The characteristic used to define diapause females was a F:G ratio equal to or less than 2.5:1. Hudson (1977a) noted that the mean F:G ratios of diapausing stage follicles and resting stage follicles were 1.35 and 2.73 respectively. Hudson (1977b) defined diapausing females as having a F:G ratio

of equal to or less than 1.5:1, which is consistent with diapause studies on *Culex* species (Spielman and Wong 1973a). Under the criteria used by Buth *et al.* (1990), the females reared by Hudson (1979) under constant long day/high temperature (L:D 18:6 and 20°C) would have been incorrectly classified as diapausing. Therefore, Buth *et al.* (1990) may have overestimated the rate of diapause induction.

A reduction in temperature alone is not sufficient to induce diapause in *Cs. inornata*. Hudson (1977b) found that under 16 hours light, females transferred from 20° C to 10° C at pupation had F:G ratios indicative of gonoactivity, and most successfully developed eggs after blood feeding. In the reverse experiment, females were maintained at a constant temperature of 20° C, but transferred from 16 to 12 hours light as pupae. The F:G ratio of females in this experiment was indicative of females in diapause, but most of the females accepted blood and subsequently developed eggs. However, this does not conclusively establish that a decline in temperature is required for diapause induction. It may be that a transfer from 16 to 12 hours light at a constant temperature of 10° C would be sufficient to induce diapause. Unfortunately, the author did not examine *Cs. inornata* under these conditions.

Tauber *et al.* (1986) described long day/short day photoperiodic induction as a response mechanism that requires a change in daylength across a critical photoperiod in order to maintain or induce diapause. This definition is appropriate for *Cs. inornata*, though a critical photoperiod has not been

identified. Hudson (1977a) suggested that a critical daylength may not exist. He examined the seasonal appearance of diapausing females over a two year period at George Lake, Alberta, and found that diapause induction in both years began in early August, and peaked by late August, with approximately 90% of the females collected in diapause. Hudson conducted several experiments in the laboratory to assess developmental rates under various conditions. Based on the environmental parameters recorded at George Lake, he estimated that the preimaginal stages of the diapausing generation would have experienced a decrease in daylength from about 18 hours light to about 16 hours light, and a drop in mean water temperature from approximately 20° C to 13° C. This led Hudson (1977a) to suggest that, under natural conditions, *Cs. inornata* does not require a decrease in daylength across a specific threshold, as diapause had been induced in the laboratory by a transfer from 16 to 12 hours light. The photoperiodic response mechanism may only operate either when the decrease in daylength occurs below a certain threshold daylength, or when the rate of the decrease in daylength is representative of late summer. Either restriction would prevent the first generations developing after the summer solstice (i.e. the date when daylength begins to decline) from entering diapause. To date, there has been no laboratory experimentation to support either hypothesis.

The manner in which diapause is terminated in *Cs. inornata* is also not clearly understood. Hudson (1979) found that diapause could be artificially terminated in field-collected, diapausing females, by exposing them to a

photophase of 16 hours light at 20° C for 7 days. However, Hudson also found that diapause may be terminated spontaneously under a simulated hibernation period. After subjecting field-collected, diapausing females for 2 to 3 months at 5° C and 12 hours light, the mean F:G ratio of surviving females was greater than 1.5:1, and egg development occurred in females that accepted blood. This may be similar to the gradual reactivation of ovarian development observed in hibernating *Cx. tarsalis* (Mitchell 1979, Eldridge 1987). Shemanchuk (1965) has shown that, in Alberta, the emergence of both *Cx. tarsalis* and *Cs. inornata* from overwintering sites is associated with spring soil temperature inversions. Restoration of reproductive activity, prior to spring emergence, may explain the rapid response of both species to sudden warming periods in the spring.

Autogeny In Culex tarsalis

In some hematophagous species, individuals can develop their first egg batch without acquiring a blood meal. The term autogeny is used to define development of the ovarian follicles beyond the resting stage without a blood meal (Spielman 1957). In autogenous females, precursors for vitellogenin synthesis are derived primarily from nutrient stores accumulated during larval development (Roubaud 1932). In *Cx. tarsalis*, autogenous females are able to complete egg development without interruption (i.e. without a resting stage), ovipositing much sooner than anautogenous females (Reisen and Milby 1987).

In *Cx. tarsalis*, autogeny is a genetically inherited trait (Eberle and Reisen 1986). In genetically autogenous females, the facultative expression of autogeny is influenced by environmental factors such as nutrition, larval crowding, temperature and photoperiod (Harwood 1966, Reisen *et al.* 1984, Reisen 1986a). Maximum autogeny expression has been observed in this species when reared at high temperatures and long photoperiods (Reisen *et al.* 1989). Brust (1991) reported that short photoperiod suppressed autogeny expression at temperatures below 28°C (but not at higher temperatures). He also reported that at temperatures below 21°C, autogeny rates were reduced, even under long photoperiod. Under diapause inducing conditions, autogeny is not expressed in genetically autogenous females (Reisen 1986a).

CHAPTER III

THE INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON OVARIAN DEVELOPMENT IN *CULEX TARSALIS* (DIPTERA: CULICIDAE), IN SOUTHERN MANITOBA

ABSTRACT

Ovarian diapause in *Culex tarsalis* Coquillett was examined using the mean primary ovarian follicle length (FL) of five age classes of anautogenous females as a measure of reproductive development. In the laboratory, significant differences ($P<0.05$) in mean FL were detected among three photoperiods (L:D 18:6, 14:10 & 12:12), at two temperatures (19°C & 22°C). Estimates of the proportion of laboratory-reared females in reproductive diapause at 18:6, 14:10 and 12:12 (L:D) were 0, 32.5 and 92 per cent at 19°C, and 0, 25 and 92 per cent at 22°C. In the field, mean FL declined in successive experiments. From July to September, 1992, the estimated proportion of diapausing females in each field experiment increased from 2 to 44 per cent at 19°C and natural daylength, and 0 to 100 per cent under natural temperature and daylength. In 1992, diapause did not occur in field-reared females emerging in Winnipeg (49° 54' N, 97° 9' W; Fullard and Willett 1979) before the middle of August.

In the laboratory, there were significant differences ($P<0.05$) in the proportions of females expressing autogeny under three photoperiods (L:D 18:6, 14:10 & 12:12), at two temperatures (19°C & 22°C). Autogeny ranged from 0 per

cent (L:D 12:12 & 19°C) to 25.8 per cent (L:D 18:6 & 22°C). In the field, the maximum autogeny was much higher. From July to September, 1992, the proportion of autogenous females declined from 69.3 to 3.3 per cent at 19°C and natural daylength. Under natural temperature and daylength, autogeny was 29.8, 47.4 and 0.3 per cent in three successive experiments from July to September, 1992.

INTRODUCTION

Culex tarsalis Coquillett, the primary vector of the Western Equine Encephalomyelitis (WEE) virus, overwinters in a state of reproductive diapause in southern Manitoba. Ovarian development in overwintering females is arrested in a premature state in response to environmental cues they received as pupae and late instar larvae (Reisen 1986b). Diapausing females do not seek a host (Mitchell 1981), and consequently do not play a role in the epidemiology of the WEE virus. Thus the seasonal timing of diapause induction in *Cx. tarsalis* populations is an important consideration in a vector control program.

In California, the influence of environmental factors on diapause induction has been studied extensively. In Kern County, *Cx. tarsalis* females emerging in late autumn arrest ovarian development and blood feeding behavior in response to a decline in temperature and daylength (Bellamy and Reeves 1963, Reisen *et*

al. 1983). In southern Manitoba, the specific environmental conditions responsible for arresting ovarian development in field populations have not been adequately examined.

Autogenous egg development is another aspect of the reproductive biology of *Cx. tarsalis* which influences vector competence (Hardy and Reeves 1973). Females expressing autogeny do not normally blood feed during the first gonotrophic cycle (Bellamy and Corbet 1973), delaying the opportunity for acquisition of the WEE virus. Though Brust (1991) has described the influence of environmental factors on autogeny, the relationship between autogeny expression and diapause induction has not been described in *Cx. tarsalis*, in southern Manitoba.

The principle objective of this study was to determine the influence of temperature and daylength on the onset of reproductive diapause in a wild population of *Cx. tarsalis* from southern Manitoba. In this study, diapause induction and autogenous egg development in *Cx. tarsalis* were examined under controlled, natural and semi-natural conditions.

MATERIALS AND METHODS

Rearing and Handling

Culex tarsalis egg rafts were collected from Glenlea, Manitoba, using the method described by Brust (1990). The larvae were pooled after reaching the second instar, then placed in polypropylene pans (21 x 32 x 7 cm) containing approximately 2 L of dechlorinated tap water, at a density of 400 larvae per pan. A water slurry containing approximately 300-500 mg of finely ground, bovine liver powder (ICN Biochemicals, Inc.) was added to each pan daily. A foam pad was drawn across each pan twice daily to remove the surface film. Pupae were transferred to cups enclosed in a 16 x 16 x 16 cm Plexiglas® cage. To obtain females of known age, the pupal cups were transferred to a fresh cage at 24 hour intervals. Adults were provided with a 10% sucrose solution for the duration of the experiment.

Experimental Conditions

In the laboratory, mosquitoes were maintained in controlled-environment chambers (Model 1-35 VL, Percival Manufacturing Company, Boone, Iowa), at 2 temperatures (19 and 22°C) and 3 photoperiods (L:D 12:12, 14:10 and 18:6). In each chamber, the water temperature of a sealed pan was recorded twice daily, once at the end of the scotophase, and then 4-8 hours after the photophase had begun. Adjustments were made to maintain the water temperature in the sealed pan within ±0.2°C of the desired temperature.

In the field, larvae were reared under natural conditions of temperature and daylength, in pans partially submerged in a 1 m^2 x 30 cm deep artificial pool (Fig. 1). Pupae were transferred to emergence cages (2 L, clear plastic containers) immersed in the pool. Adults were collected daily from the emergence cages, and transferred to 16 x 16 x 16 cm Plexiglas cages housed in a shaded, outdoor insectary. Maximum and minimum water temperatures were recorded daily using a max/min mercury thermometer (Stortz MMT-15, PSG Industries Inc., Perkasie, PA) situated 1-2 cm below the water surface. The water temperature in the larval pans and pupal containers approximated those of the pool. Air temperature in the insectary was determined by placing a max/min thermometer in an empty cage.

A controlled temperature experiment was also conducted in the field. Larvae were reared in Plexiglas pans (21 x 21 x 6.5 cm) partially submerged in an enclosed, outdoor water bath (140 L capacity, Fig. 2 and 3), at the same larval density as in previous experiments. Adults were maintained in cages placed approximately 15 cm above the water surface. The maximum and minimum air and water temperatures were recorded daily using max/min thermometers. Four thermostatically controlled, infrared bulbs (125 W) situated beneath the bath, and a partially submerged refrigeration coil, maintained the water temperature at 19 ±0.5°C. Air temperature in the sleeve cages was within ±3.0°C of the water temperature. Indirect sunlight entered the bath through a colourless, Plexiglas top.

Assessment of Ovarian Development

In the diapause experiments, mean primary follicle length was used as a measure of ovarian development. Females were dissected at 6, 12, 18, 26 and 34 days after adult emergence. Ten females from each age group were dissected in each experiment.

Each female was immobilized, then transferred to a drop of physiological saline on a glass slide (Hagedorn *et al.* 1977). A minuten pin embedded in the end of a wooden swab stick was used to remove the terminal abdominal segments and free the ovaries. The ovaries were then transferred to a fresh drop of saline. To separate the ovarioles, a minuten pin was inserted into the ovary and gently vibrated. A glass cover slip, which was supported around the circumference by No. 2 coverslip fragments, was placed over the drop. The ovarioles were examined by phase contrast microscopy at 200x magnification.

The lengths of fifteen randomly selected, primary follicles were measured in each female. Follicle length (FL) was measured along the longitudinal axis of the follicle, from the base of the pedicel to the constriction linking the secondary follicle. Females with one or more follicles at or beyond stage IIIa (Watts and Smith 1978) were considered to be autogenous and not examined. Follicles exhibiting signs of degeneration or previous resorption were not measured. In each treatment, the FL's of all age classes (6, 12, 18, 26, and 34 days post-emergence) were pooled, and statistical comparisons were made among the overall treatment means using a Tukey HSD multiple comparison test (Wilkinson

1990). Within each treatment, statistical comparisons between mean FL's were also made among the five age classes.

Autogenous development was also examined in each group. Adults were frozen at a minimum of 8 days after emergence. Females were dissected in distilled water to which a minute quantity of dish detergent had been added. Using the ranking system described by Watts and Smith (1978), the stage of development of the most advanced ovarian follicle was used to classify each female. As with follicle measurements, females with one or more follicles at or beyond stage IIIa were considered to be autogenous. Statistical comparisons among treatments were made by transforming the percentage of autogenous females from each replicate to the arcsine, as described by Brust (1991).

RESULTS

Ovarian Development under Controlled Conditions

Significant differences ($P<0.05$) in overall mean FL were apparent among the three photoperiods, at each temperature (Fig. 4). At 12:12 and 18:6 (L:D), the majority of follicles examined in anautogenous females were in stage I and stage II, respectively. The influence of temperature was not as clear. At both 18:6 or 12:12 (L:D), the overall mean FL's of females reared at 19°C were not statistically different from those of females reared under the same light regime at

22°C. At 14:10 (L:D), the overall mean FL of females reared at 22°C was significantly higher than the overall mean FL of females reared at 19°C. Significant differences in mean FL were also detected between specific age groups, within treatments.

There is a clear separation in the frequencies of mean FL's of individual females (Fig. 5 and 6) between 12:12 and 18:6 (L:D) at both temperatures. At 14:10 (19 and 22°C), the distributions of individual mean FL's overlapped 12:12 and 18:6. The distribution of individual mean FL's among treatments was consistent among the five age classes. The variation in FL within individual females was relatively small.

Ovarian Development under Natural Daylength and Constant Temperature

When mosquitoes were reared at a constant temperature of 19°C, under natural daylength, a successive decline in overall mean FL was observed over the season (Fig. 7). There was a gradual shift in the distribution of mean FL's of individual females for each experiment as overall mean FL declined (Fig. 8). By mid-August, a significant decrease in overall mean FL was detected (Fig. 7). Natural daylength in the field at the end of August had declined to approximately 14.5 hours light (Fig. 11).

Ovarian Development under Natural Conditions

When mosquitoes were reared under field conditions, a significant decrease in mean FL was observed in females emerging in September (Fig. 9). In early September, there was no significant difference in overall mean FL between females emerging from the outdoor pool (Fig. 9), and females emerging in the outdoor water bath (Fig. 7). The mean daily air and water temperatures recorded in this experiment are shown in Figure 11.

Autogeny

In the laboratory, both temperature and photoperiod had a significant effect on the expression of autogeny (Table 1). The highest percentage of autogenous females was observed at 22°C, at 18:6 and 14:10 (L:D). At 12:12 (L:D), few of the females at 22°C, and none of the females at 19°C, expressed autogeny.

In the water bath, the expression of autogeny was suppressed as daylength declined. The percentage of autogenous females was highest at 69.3% in mid to late July, declining to 3.3 % in September (Table 2). The least mean number of eggs produced per autogenous female (13.3 eggs/female) occurred in September.

Under field conditions (Table 3), the proportion of females expressing autogeny was 29.8% in the first experiment (mid July to early August), 47.4% in the second experiment (early to mid August), and 0.3% in the third experiment

(late August to mid September). Similarly, mean daily water temperature during pupation, and mean daily air temperature for the first 8 days after the last adult emergence, were highest during the second field experiment, and lowest during the last experiment (Table 3, Fig. 11). In the third experiment, none of the females scored as autogenous had developed follicles to stage five.

DISCUSSION

Criteria that have been used to distinguish reproductive diapause in *Culex* include: 1) fat body development (Bennington *et al.* 1958, Harwood and Halfhill 1964), 2) blood feeding behaviour (Blackmore and Dow 1962), 3) egg maturation (or lack of) subsequent to blood feeding (Mitchell 1981), and 4) the size and/or stage of development of the primary ovarian follicles.

Morphometric assessments of follicle development are frequently used to differentiate diapausing and nondiapausing *Culex* females. Resting stage follicles (i.e. anautogenous, gonoactive females) are generally about one and a half times larger than the follicles of females in reproductive diapause (Danilevskii and Glinyanaya 1958). Follicle development may be determined by measuring the mean primary follicle length (FL) (Oda and Wada 1972, Sanburg and Larsen 1973), or the mean primary follicle length to germarium length ratio (F:G) (Spielman and Wong 1973a,1973b). In *Cx. tarsalis*, mean FL and the

mean F:G ratio respond very similarly to photoperiod and temperature (Reisen *et al.* 1986a, Reisen *et al.* 1995). In the present study, mean FL was used to describe the ovarian development of *Cx. tarsalis* females reared under various conditions.

Individual females (*Culex* spp.for e.g.) may be classified as diapausing or nondiapausing based on a predetermined FL value or range of FL values which most reliably define the two states of reproductive developmental arrest (Bowen *et al.* 1988). The range of mean FL's in diapausing females has been defined as <50 μm for *Cx. pipiens* (Spielman and Wong (1973b, Eldridge and Bailey 1979, Oda and Nuorteva 1987) and *Cx. tarsalis* (Reisen *et al.* 1995), and <60 μm for *Cx. peus* (Skultab and Eldridge 1985).

Ovarian Development under Controlled Conditions

Photoperiod was the primary factor influencing diapause induction in this study. The inability to detect a significant effect of temperature at 12:12 and 18:6 (L:D) may mean that the influence of a relatively small temperature difference (3°C) is diminished at extreme photoperiods. In contrast, Spielman and Wong(1973a) compared *Cx. pipiens* reared at 8:16 (L:D), and two temperatures (18 and 22°C), and noted that significantly fewer females entered diapause at the higher temperature. At 25°C, *Cx. tarsalis* females from California and Oregon did not enter a diapause condition, even under a short photoperiod (Reisen *et al.* 1989, Eldridge 1987).

At both temperatures (Fig. 4), overall mean FL's of females reared under 12:12 and 18:6 (L:D) were consistent with values reported for diapausing and non-diapausing *Cx. tarsalis* (Reisen *et al.* 1986a), respectively. Under 14:10 (L:D), the overall mean FL's of females reared at 19 and 22°C were intermediate between these values. If mean FL's of less than 50 μm are assumed to be representative of females in reproductive diapause, the proportions of females in diapause at 18:6, 14:10 and 12:12 (L:D) are 0, 32.5 and 92 per cent at 19°C (Fig. 5), and 1, 25 and 92 per cent at 22°C (Fig. 6). This is similar to the 0, 60 and 90 per cent diapause at 18°C and 17:7, 14:10 and 12:12 (L:D), respectively, reported for a *Cx. tarsalis* strain originating from Benton County, Oregon (Eldridge 1987).

In females reared under long photoperiod (L:D 18:6), degeneration was more common in larger follicles (late stage IIb), resulting in a gradual decline in mean FL over the 34 day period (Fig. 4). Sanburg and Larsen (1973) observed a similar decline in the mean FL of *Cx. pipiens* approximately twenty days after emergence at 22°C and 15.2 hours light. This may be an artifact produced by a constant photoperiod (Eldridge 1987). In *Cx. tritaeniorhynchus*, the incidence of follicle degeneration in females reared under long photoperiod can be reduced significantly by transferring them to a shorter photoperiod (Oda *et al.* 1978). However it has been suggested that in most *Culex* spp., 'resting stage' follicles are not dormant, the ultimate follicles are continually resorbed and replaced by the penultimate follicle (Rosay 1969, Nayar and Knight 1981). In the current

study, follicle degeneration was more common in laboratory-reared females than in the field-reared females.

Bowen *et al.* (1988) reported a gradual increase in the mean FL of diapausing *Cx. pipiens* maintained at 22°C and 10:14 (L:D) for 36 days. However, Eldridge (1987) reported that follicle degeneration in *Cx. pipiens* maintained at 25°C and 8:16 (L:D), caused mean FL to decrease 5 to 7 days after emergence. In the present study, a significant decrease in mean FL was observed in *Cx. tarsalis* females maintained at 12:12 (L:D) and 19°C for 34 days (Fig. 4). No significant change in mean FL was detected in females maintained at 12:12 (L:D) and 22°C for 34 days. In overwintering *Cx. tarsalis*, evidence of resorption (dilatations) is rarely observed (Reisen *et al.* 1986b).

Ovarian Development under Natural Conditions

Very little information is known about the timing of diapause induction in *Cx. tarsalis* on the Canadian prairies. In southern Alberta, overwintering *Cx. tarsalis* entered hibernacula when the evening low dropped to about 6° C, and the daylength declined to approximately 13.5 hours light (Shemanchuk and Morgante 1968). In central Alberta, diapausing females first appear in late August (Hudson 1977a). In southern Saskatchewan, from comparisons of light trap catches and sentinel flock trap catches, McLintock *et al.* (unpublished) suggested that diapausing *Cx. tarsalis* first appear in the field in mid to late August. In the present study, the overall mean FL's of females emerging under

natural temperature and daylength on 25 July and 14 August (Fig. 9) were not significantly different from the overall mean FL of females reared under long day (L:D 18:6) conditions in the laboratory (Fig. 4). None of the females examined had a mean FL of <50μm (Fig. 10); therefore *Cx. tarsalis* emerging under field conditions in Winnipeg did not enter reproductive diapause before the middle of August, during 1992.

In an earlier Winnipeg study, it was reported that 20-30 per cent of females emerging 27 July, and 70-75 per cent of females emerging 9 August, were in diapause (Buth *et al.* 1990). However the author had used a strain of *Cx. tarsalis* that had been colonized in the laboratory for at least seven years. It has been shown that the ability of *Cx. tarsalis* to respond to diapause induction cues is altered after several generations of selection in the laboratory (Reisen *et al.* 1986a). Buth *et al.* (1990) defined diapausing females as having a mean F:G ratio less than 2.5:1, much higher than the value reported previously for diapausing *Cx. tarsalis* (Arntfield *et al.* 1982, Reisen 1986a). It is likely that using the criteria of Buth *et al.* (1990) the proportion of females in diapause was overestimated.

In Kern County, California, the timing of diapause induction in the field is usually associated with a decrease in temperature (Reisen *et al.* 1983). In the present study, lower temperatures in the outdoor pool (Fig. 10 and 11; Table 3), relative to the water bath (19°C), likely enhanced the influence of the shorter daylengths experienced by females emerging in September. In the outdoor pool,

more than two thirds (67.5 %) of the females emerging on 11 September, and all of the females emerging on 26 September, were in diapause (mean FL <50 μm, Fig. 10), compared to only 44 per cent in the 19°C water bath in mid September (Fig. 8).

In the present study, caution is needed when describing the physiological condition of individual lab reared *Cx. tarsalis* females based on follicle size. In this study and others (Eldridge 1987, Reisen *et al.* 1989), under an intermediate photoperiod in the laboratory (i.e. between inductive and non-inductive photoperiods), the overall mean FL of *Cx. tarsalis* is significantly different from the mean FL of females reared under short and long daylengths. Under an intermediate photoperiod, one might expect a bifurcated distribution of individual mean FL's as females sort into diapausing and non-diapausing groups. In the current study however, the distribution of females was consistent with the normal curve, situated between the distributions of females reared at 12:12 and 18:6 (L:D) at both temperatures (Figs. 5, 6). The mean FL's of the majority of females developing under an intermediate photoperiod are not consistent with the range of mean FL's observed in diapausing females reared under short photoperiod, or the range of mean FL's observed in non-diapausing females reared under long photoperiod. Ovarian developmental arrest in *Cx. tarsalis* is therefore not limited to two distinct states based on follicle size. Reisen *et al.* (1986a) reported *Cx. tarsalis* females reared under autumn conditions with diapause stage follicles (stage I of Kawai 1969), but with mean FL's (and F:G ratios) significantly higher

than observed in diapausing females reared in the laboratory under short daylength. Using criteria of mean FL <50 μm (or corresponding F:G ratio) may underestimate the proportion of females in diapause, and should be used in conjunction with a second criterion (e.g. follicle stages of development).

Under natural daylength of approximately 14 hours light (Fig. 8 and 10), the distribution of FL's was more consistent with the assumption of two distinct states of reproductive developmental arrest (i.e. diapause and resting stage follicles), than at the same photoperiod in the laboratory (Figs. 5 and 6). Under natural conditions, the shift from 0 to 67.5 per cent diapause (Fig. 10) occurred when daylength declined from a range of 16.1-15.2 hours light (during pupal and early adult development, Fig. 11, Table 3), to a range of 14.9-13.5 hours light. Much cooler temperatures in late August and early September appear to have accelerated the transformation from non-inductive to inductive photoperiods, leading to diapause in the field.

Autogeny

High temperatures and long daylengths favour the expression of autogeny in *Cx. tarsalis* females (Reisen *et al.* 1989, Brust 1991). In this study, a 3°C increase in temperature significantly increased the proportion of autogenous females at all three photoperiods (Table 1). At 22°C, responses at 18:6 and 14:10 (L:D) were not significantly different. Brust (1991) reported that at a rearing temperature of 24°C, autogeny was not suppressed at 14:10 (L:D).

The percentage of *Cx. tarsalis* females in a field population developing their first batch of eggs autogenously declined with the seasonal reduction in temperature and daylength (Spadoni *et al.* 1974). Brust (1991) reported a decline in autogeny, from 54 to 0 per cent in *Cx. tarsalis* reared in consecutive field experiments in Winnipeg, from the middle of July to the middle of September. In the present study, similar results were achieved when females were reared at 19°C and natural daylength (69.3% to 3.3%, Table 2). Under natural temperature and daylength (Table 3), the highest mean per cent autogeny (47.4 %) was observed in females beginning pupation on August 8th, though it was not significantly different from the mean per cent autogeny of females pupating earlier in the season. Presumably the higher mean daily air and water temperatures experienced by females pupating in early August (Table 3, Fig. 11) were sufficient to overcome the inhibitory influence of a shorter daylength on autogeny.

High autogeny rates in a *Cx. tarsalis* population may suppress the transmission of WEEV (Hardy and Reeves 1973). Females expressing autogeny are unlikely to seek a blood meal during the first gonotrophic cycle (Nelson and Milby 1982), delaying the first opportunity for virus acquisition.

In southern Manitoba, isolations of WEEV from *Cx. tarsalis* were highest in July and August (Sekla and Stackiw 1982). Maximum autogeny rates under field conditions in Winnipeg were observed from mid July to early August (Brust 1991, current study). Similarily, Spadoni *et al.* (1974) found that the seasonal

occurrence of high autogeny rates (>85%) in *Cx. tarsalis* field populations from southern California coincided with maximum WEEV activity. Spadoni *et al.* (1974) interpreted this to mean that autogeny did not significantly inhibit WEEV transmission. However, further research would be necessary before the influence of autogeny on WEEV transmission could be determined.

Under cool temperatures and short daylengths, the expression of autogeny in *Cx. tarsalis* is inhibited and follicle development is arrested in a diapause state (Reisen 1986a). In this study, the mean rates of autogeny under diapause-inducing conditions (L:D 12:12) in the laboratory were 0 and 1 per cent at 19 and 22°C, respectively (Table 1). In the field, an abrupt reduction in the mean per cent autogeny (Table 3) in late summer coincided with a significant increase in the proportion of females in reproductive diapause (mean FL <50 μm, Fig. 10).

Influence of Diapause on Pathogen Transmission by Cx. tarsalis in Manitoba

Known epidemics of Western Equine Encephalomyelitis virus (WEEV) have occurred sporadically in Manitoba (Sekla 1982). Control programs directed against *Culex tarsalis*, the primary vector of WEEV (Sekla *et al.* 1980), were initiated on a province wide basis during some of these epidemics. In 1981, aerial applications of insecticide were applied from 30 July to 26 August to reduce populations of *Cx. tarsalis* and other potential vector species (Ellis 1982).

Estimates of the date on which natural populations of *Cx. tarsalis* in

southern Manitoba enter diapause are of value to predictive models of epidemic WEEV transmission risk. Should there be an impending outbreak of WEEV in the future, the timing of reproductive diapause induction may be an important consideration when making vector control decisions during the late summer in Manitoba.

In southern Manitoba, *Cx. tarsalis* collections at New Jersey light traps and sentinal chicken flock traps (Brust 1982) begin to decline rapidly after the middle of August, possibly due to an increase in the proportion of females in diapause, and/or a decline in the abundance and activity of gonoactive females. The cessation of epidemic WEEV transmission has been associated with declining vector populations and a reduction in blood feeding activity in the late summer and early fall due to a decrease in mean daily temperatures (Hess and Hayes 1967, Morgante *et al.* 1968, Fraser and Brust 1976). However the duration of WEE epidemics in Winnipeg may be extended into early autumn under favourable weather conditions (Sekla *et al.* 1991).

In the current study, *Cx. tarsalis* emerging under natural conditions in Winnipeg began entering diapause after the middle of August in 1992. By the end of August, approximately 2/3 of newly emerged females were in reproductive diapause. Hayles *et al.* (1972) reported a minimum extrinsic incubation period for WEEV in a Saskatchewan population of *Cx. tarsalis* of about 4 days at constant temperatures between approximately 20.5 and 23.8°C (69 and 75°F). At a constant temperature of 24°C, the minimum extrinsic incubation period of a

Manitoba isolate of WEEV in a Manitoba strain of *Cx. tarsalis* was also 4 days (Henderson *et al.* 1979). In southern California, estimates of the interval between emergence and blood feeding, and the length of the gonotrophic cycle were 6 and 4 days, respectively, at high (30°C) mean daily temperatures (Reisen *et al.* 1983). Based on these values, *Cx. tarsalis* females emerging in the first half of August, in the presence of an infected reservoir host and under favourable weather conditions, could potentially acquire and later transmit WEEV to human or equine hosts in the late summer or early autumn. This affirms the importance of late season monitoring and control of vector populations during epidemic outbreaks of WEEV. To associate the late season abundance of *Cx. tarsalis* with the actual risk of WEEV transmission, attention should be given to the reproductive status and age structure of females collected in late August so that nulliparous (diapausing and nondiapausing) females may be distinguished from the 'potentially-infective', parous females.

Figure 1. Outdoor pool used to rear *Cx. tarsalis* and *Cs. inornata* under natural conditions in 1992 and 1993, respectively. Rearing pan is shown in the left foreground. Three pupal containers are visible in the background. A Max/Min mercury thermometer for recording water temperature is located in the center of the pool, just below the surface.

Figure 2. Interior of waterbath used to rear *Cx. tarsalis* at 19°C and natural daylength in 1992, and *Cs. inornata* at 16°C and natural daylength in 1993 (Plexiglas lid removed). Larval rearing pans (foreground), pupal containers (right) and adult sleeve cages (center) are shown. Two small fans helped maintain the air temperature by circulating air across a partially submerged refrigeration coil.

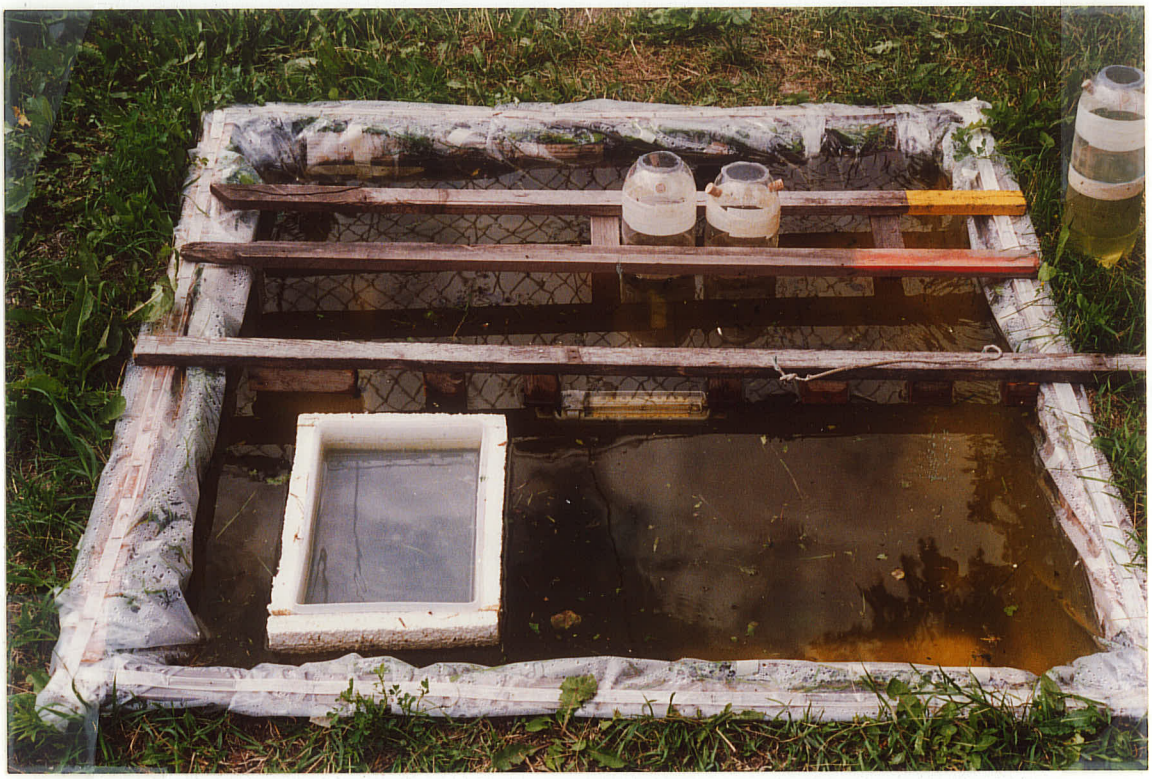


Figure 3. Waterbath used to rear *Cx. tarsalis* at 19°C and natural daylength in 1992, and *Cs. inornata* at 16°C and natural daylength in 1993. Wooden frame covered with black polypropylene (foreground) was used to shield the bath from direct sunlight.

Figure 4. Mean primary follicle length (±s.e.) of *Culex tarsalis* females reared in the laboratory at 19 and 22°C, under three photoperiods. Each point represents the mean of 20 to 30 females. Means within treatments followed by the same symbol (* or **) indicate a significant ($P<0.05$) difference between age classes. Treatments followed by the same letter indicate no significant difference between overall (pooled) treatment means at the same temperature.

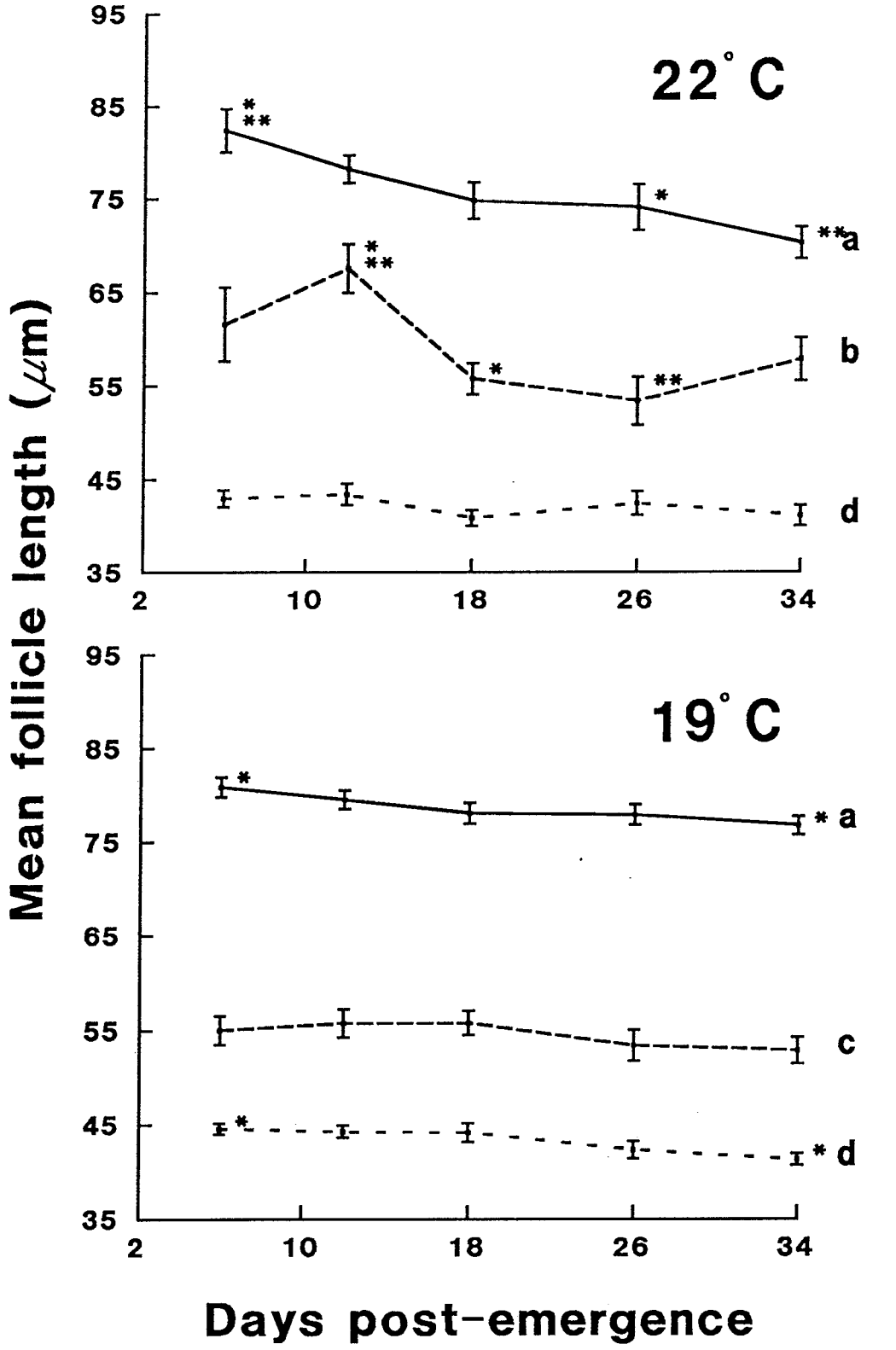

22° C
19° C
Mean follicle length (μm)
95
85
75
65
55
45
35
2
10
18
26
34
a
b
d
c
Days post-emergence
L:D 18 : 6
L:D 14 : 10
L:D 12 : 12

Figure 5. Frequency histograms of mean primary follicle lengths of *Culex tarsalis* reared in the laboratory at 19°C, under three photoperiods (6, 12, 18, 26 and 34 day old females pooled, Fig. 4). Vertical bars depict both the proportion of females dissected (left axis), and number of females dissected (right axis), in each follicle length class (class width=2.5 μm). A curved line is used to represent a normal distribution calculated from the sample mean and standard deviation.

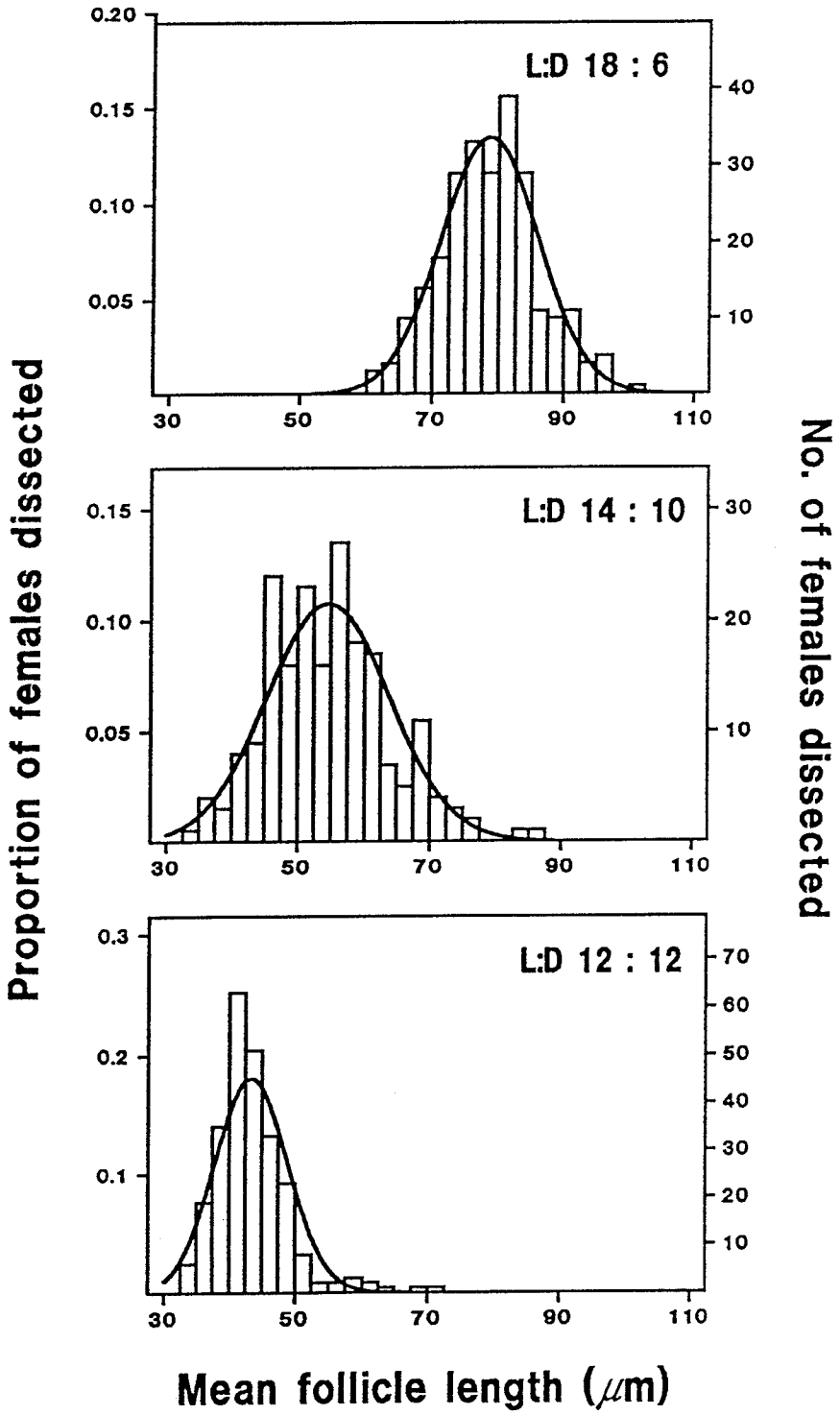

L:D 18 : 6
L:D 14 : 10
L:D 12 : 12
Proportion of females dissected
No. of females dissected
Mean follicle length (μm)
0.20
0.15
0.10
0.05
0.3
0.2
0.1
40
30
20
10
70
60
50
30
20
10
30
50
70
90
110

Figure 6. Frequency histograms of mean primary follicle lengths of *Culex tarsalis* reared in the laboratory at 22°C, under three photoperiods (6, 12, 18, 26 and 34 day old females pooled, Fig. 4). Vertical bars depict both the proportion of females dissected (left axis), and number of females dissected (right axis), in each follicle length class (class width=2.5 μm). A curved line is used to represent a normal distribution calculated from the sample mean and standard deviation.

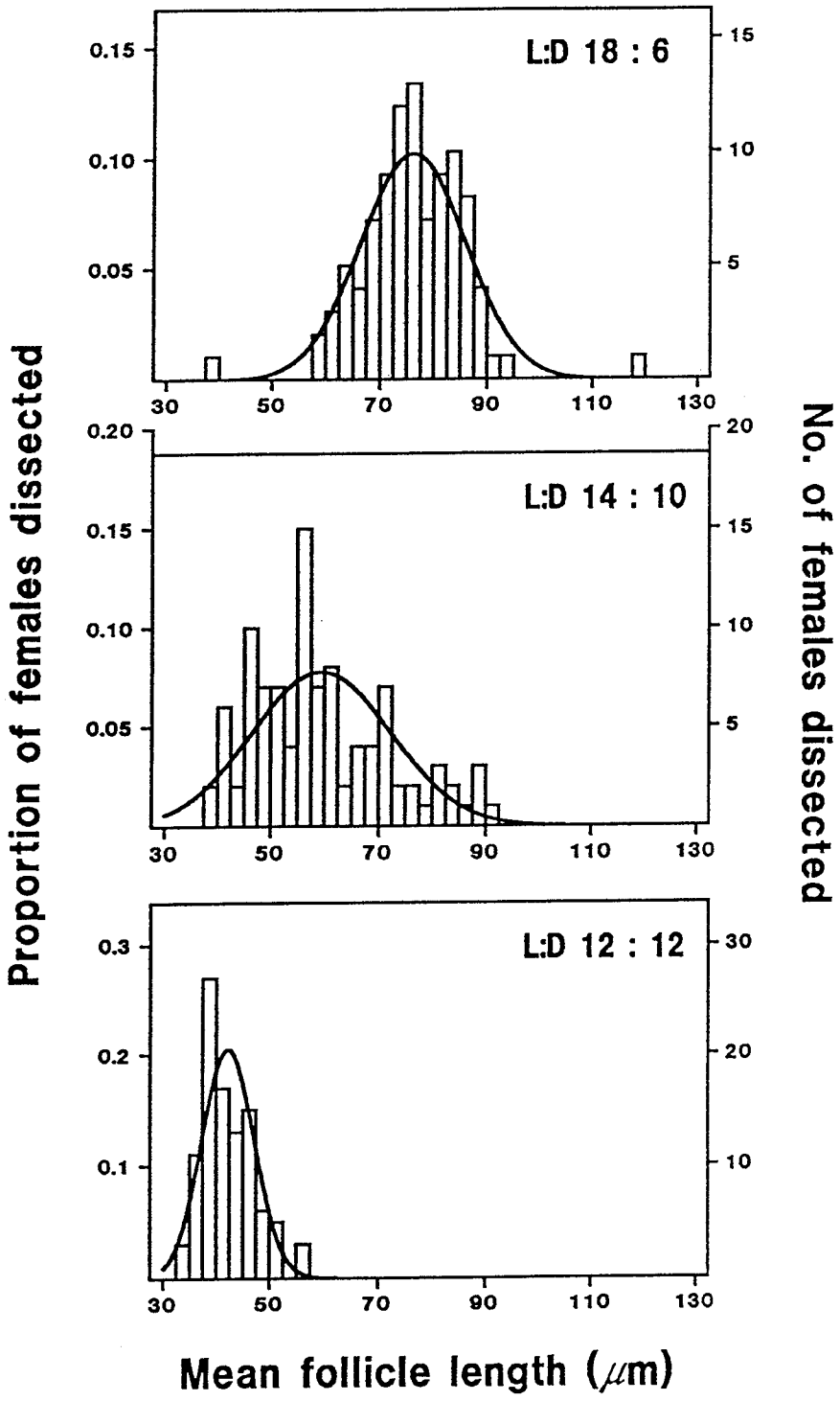

L:D 18 : 6
L:D 14 : 10
L:D 12 : 12
Proportion of females dissected
No. of females dissected
Mean follicle length (μm)
0.15
0.10
0.05
0.20
0.3
0.2
0.1
15
10
5
20
30
30
50
70
90
110
130

Figure 7. Mean primary follicle length (±s.e.) of *Culex tarsalis* females reared in an outdoor waterbath at 19°C, under natural daylength. Each point represents the mean of 10 females. Means within treatments followed by the same symbol (* or **) indicate a significant ($P<0.05$) difference between age classes. Treatments followed by the same letter indicate no significant difference between overall (pooled) treatment means.

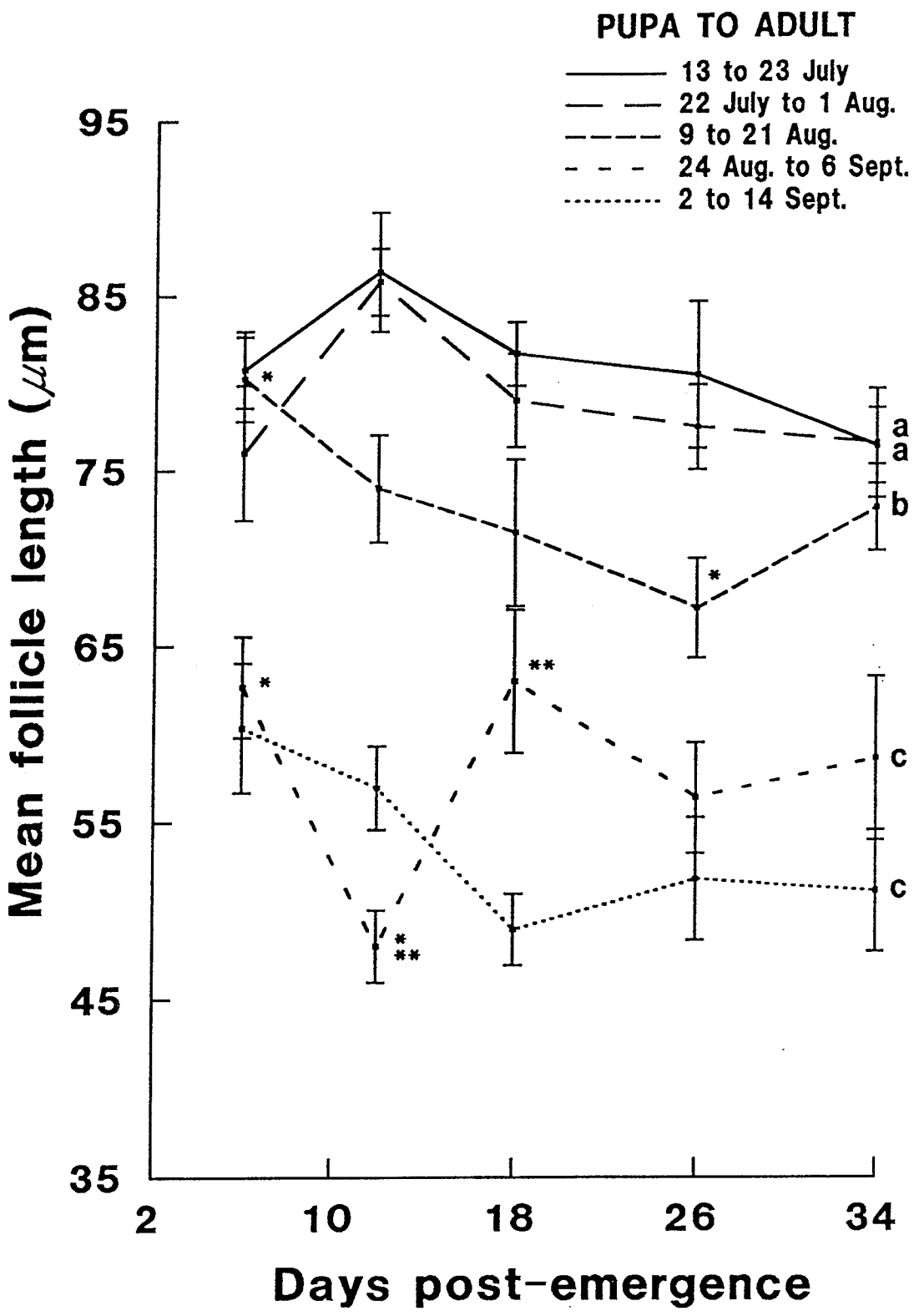

PUPA TO ADULT
13 to 23 July
22 July to 1 Aug.
9 to 21 Aug.
24 Aug. to 6 Sept.
2 to 14 Sept.
Mean follicle length (μm)
95
85
75
65
55
45
35
2
10
18
26
34
Days post-emergence
a
a
b
c
c
*
**

Figure 8. Frequency histograms of mean primary follicle lengths of *Culex tarsalis* reared in an outdoor waterbath at 19°C, under natural daylength (6, 12, 18, 26 and 34 day old females pooled, Fig. 7). The interval from the beginning of pupation to emergence of the youngest female, is presented for each experiment. Vertical bars depict both the proportion of females dissected (left axis), and number of females dissected (right axis), in each follicle length class (class width=2.5 μm). A curved line is used to represent a normal distribution calculated from the sample mean and standard deviation.

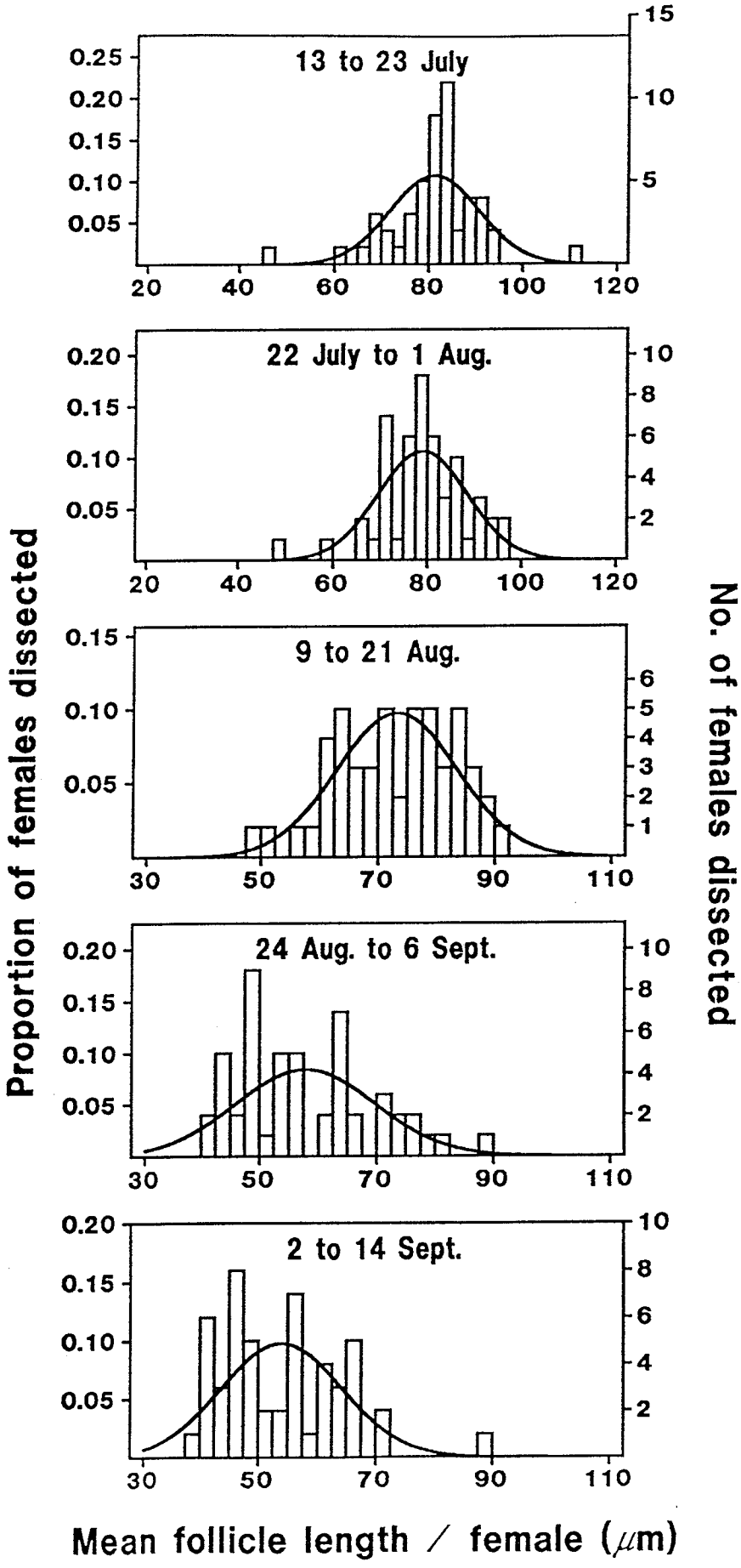

13 to 23 July
22 July to 1 Aug.
9 to 21 Aug.
24 Aug. to 6 Sept.
2 to 14 Sept.
Proportion of females dissected
No. of females dissected
Mean follicle length / female (μm)

Figure 9. Mean primary follicle length (±s.e.) of *Culex tarsalis* females reared in an outdoor pool under field conditions in 1992. Each point represents the mean of 10 females. Means within treatments followed by the same symbol (* or **) indicate a significant ($P<0.05$) difference between age classes. Treatments followed by the same letter indicate no significant difference between overall (pooled) treatment means.

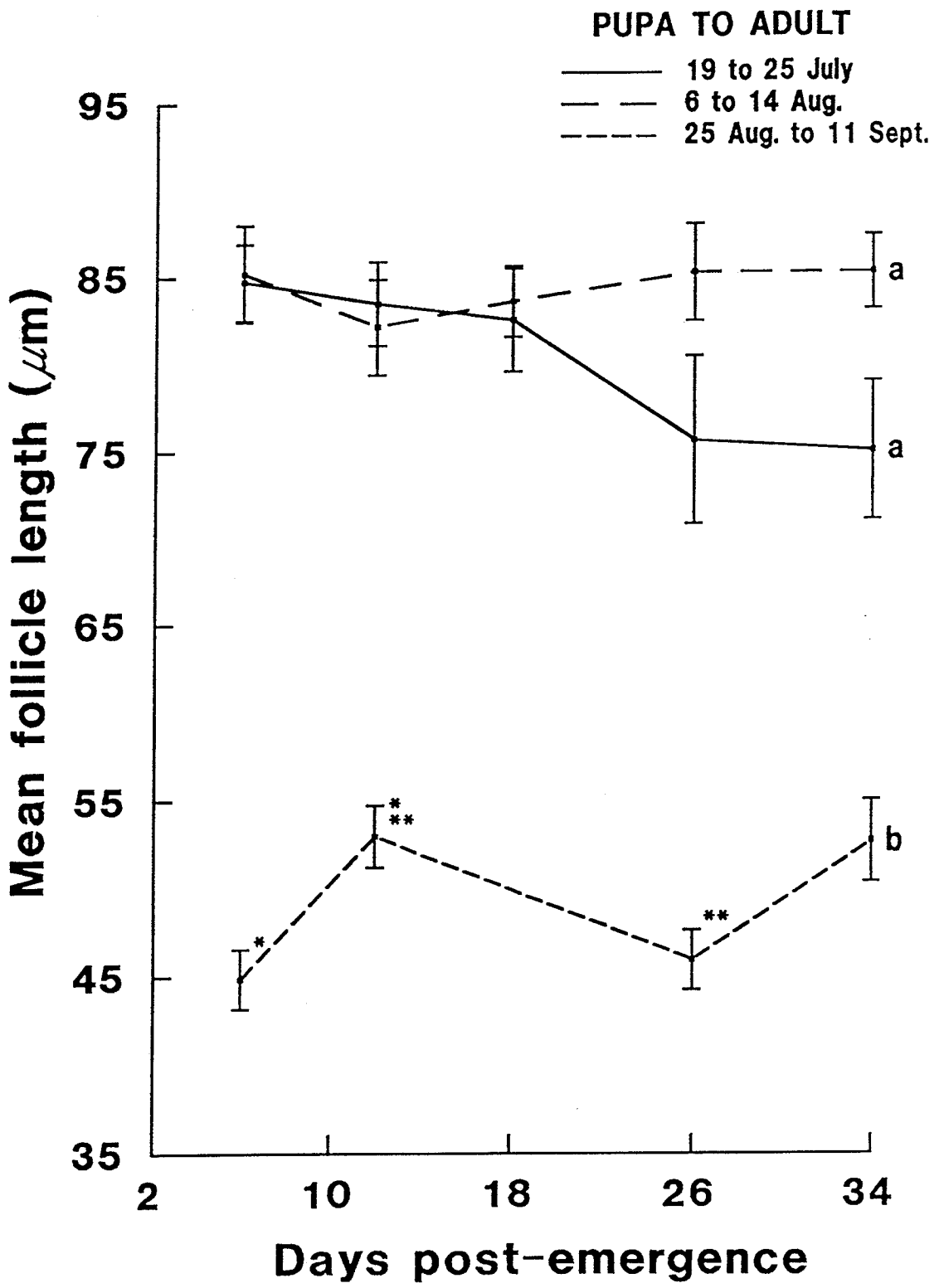

PUPA TO ADULT
19 to 25 July
6 to 14 Aug.
25 Aug. to 11 Sept.
Mean follicle length (μm)
95
85
75
65
55
45
35
2
10
18
26
34
Days post-emergence
a
a
b
*
**
*
**

Figure 10. Frequency histograms of mean primary follicle lengths of *Culex tarsalis* reared in an outdoor pool, under field conditions (6, 12, 18, 26 and 34 day old females pooled, Fig. 9). Vertical bars depict both the proportion of females dissected (left axis), and number of females dissected (right axis), in each follicle length class (class width=2.5 μm). A curved line is used to represent a normal distribution calculated from the sample mean and standard deviation. The interval from the beginning of pupation to emergence of the youngest female, is presented for each experiment. In the fourth experiment (6-26 September), high mortality due to low temperature, limited ovarian dissections to 6 days after emergence (n=10). In Winnipeg, the mean (± s.e.) daily maximum and minimum air temperatures from 6 to 26 September were 16.9±1.0°C and 4.9±0.9°C, respectively (Source: air temperatures recorded at Winnipeg International Airport, from Environment Canada Monthly Meteorological Survey, September 1992), and daylength ranged from 14.7 to 13.0 hours light (Source: United States Naval Observatory data, from Beck 1980).

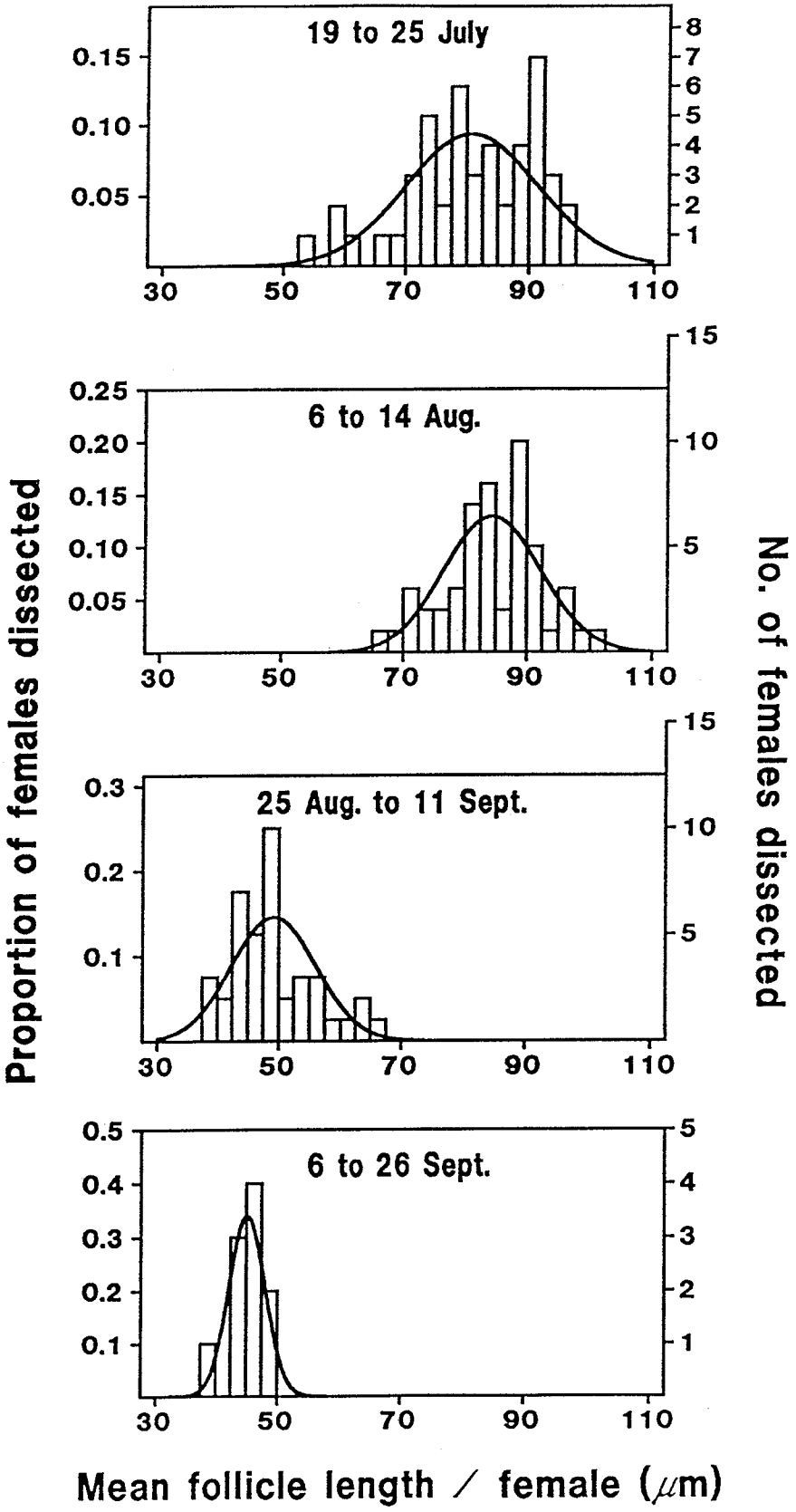
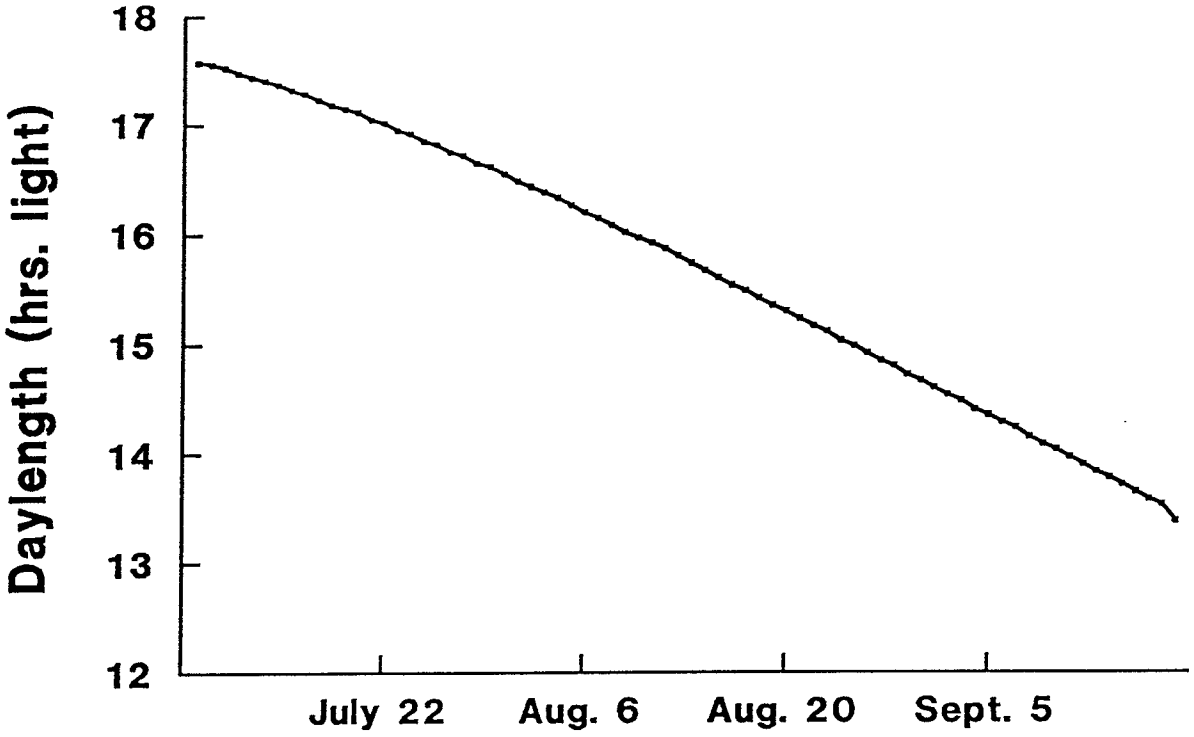

19 to 25 July
6 to 14 Aug.
25 Aug. to 11 Sept.
6 to 26 Sept.
Proportion of females dissected
No. of females dissected
Mean follicle length / female (μm)
30
50
70
90
110

Figure 11. Field conditions, 8 July to 20 September, 1992. Daylength = hours light + (2 × civil twilight) (Source: United States Naval Observatory data, from Beck 1980). Mean daily water temperature was measured 1-2 cm below the pool surface. Mean daily air temperature was recorded in the outdoor insectary.


Daylength (hrs. light)
18
17
16
15
14
13
12
July 22
Aug. 6
Aug. 20
Sept. 5

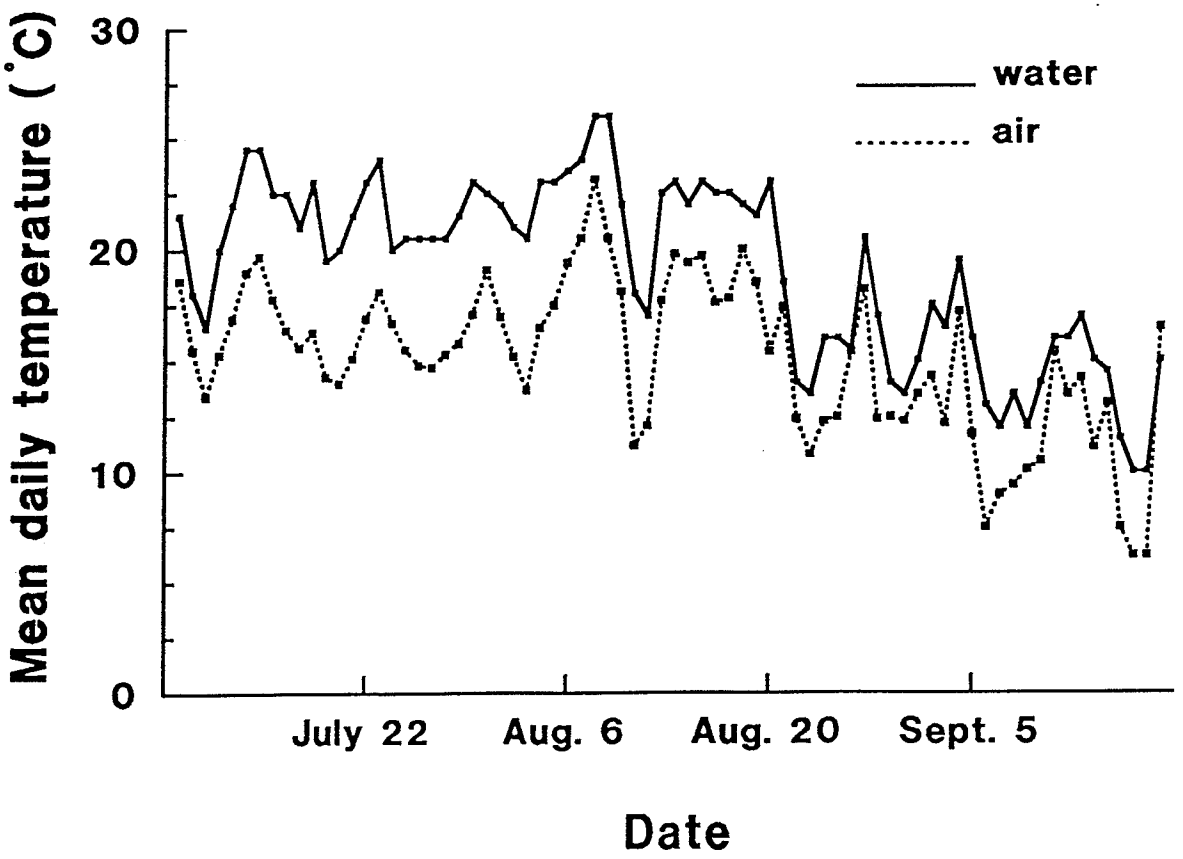

Mean daily temperature (°C)
30
20
10
0
water
air
July 22
Aug. 6
Aug. 20
Sept. 5
Date

Table 1. Autogenous development in *Culex tarsalis* females reared under controlled conditions.

Temp (°C)	19			22		
Photo. (L:D)	18:6	14:10	12:12	18:6	14:10	12:12
No. dissected (No. rep.'s[a])	1223 (15)	1016 (12)	1132 (15)	339 (3)	280 (3)	310 (3)
Mean % autogeny[b]	9.7 b (6.7-12.7)[c]	1.8 c (1.0-2.6)	0.0 d	25.8 a (20.8-31.1)	23.7 ab (6.5-40.1)	1.0 c (0.1-1.2)
Mean no. eggs/♀ ± s.e.[d]	31.7 ±1.4	24.6 ±4.0	-	35.2 ±1.4	39.98 ±1.8	31.5 ±14.5

[a] Approximately 100 females dissected per replicate. Adults maintained under treatment conditions for a minimum of 8 days.
[b] Means followed by same letter indicate no significant difference ($P \geq 0.05$).
[c] 95% confidence limits.
[d] Mean calculated from females with at least one follicle in stage V.

Table 2. Autogenous development in *Culex tarsalis* females reared at 19°C, under natural daylength.

Pupa to adult[a]	13-23 July	22 July - 1 Aug.	9-21 Aug.	24 Aug. - 6 Sept.	2-14 Sept.
Daylength[b]	17.3-16.6	17.0-16.0	16.0-14.8	15.0-13.8	14.4-13.3
No.dissected [c]	268	306	93	220	300
Mean % Autogeny[d]	69.3 a (61.5-76.3)[e]	34.4 b (18.0-49.8)	12.6 c (3.5-21.5)	11.6 cd (0.0-26.2)	3.3 d (0.0-7.5)
Mean No. Eggs/♀ ± s.e.[f]	36.3 ±1.2	39.2 ±1.2	30.1 ±2.9	30.9 ±3.7	13.3 ±2.5

[a] Dates for beginning of pupation, and emergence of youngest female, respectively.
[b] Sunrise to sunset and 2 x civil twilight (United States Naval Observatory data, from Beck 1980), from beginning of pupation to 8 days after emergence of youngest female, respectively.
[c] Approximately 100 females dissected per replicate (3 pans per experiment). Adults maintained under treatment conditions for a minimum of 8 days.
[d] Means followed by same letter indicate no significant difference ($P \geq 0.05$).
[e] 95% confidence limits.
[f] Mean calculated from females with at least one follicle in stage V.

Table 3. Autogenous development in *Culex tarsalis* females reared under natural conditions.

Pupa to adult[a]	19-25 July	6-14 Aug.	25 Aug. - 11 Sept.
Daylength[b]	17.1-16.4	16.1-15.2	14.9-13.5
Temp.(°C):			
Water[c]	21.2±0.6	22.4±1.0	15.4±0.6
Air[d]	16.3±0.4	17.3±0.8	11.4±0.7
No.Dissected[e]	305	116	315
Mean %[f] Autogeny	29.8a (18.6-40.7)[g]	47.4a (27.0-58.2)	0.3b (0-1.1)
Mean No. Eggs/♀ ± s.e.[h]	43.4 ±2.6	47.56 ±2.0	0.0

[a] Dates for beginning of pupation, and emergence of youngest female, respectively.
[b] Sunrise to sunset and 2 x civil twilight (United States Naval Observatory data, from Beck 1980), at the start of pupation, and eight days after emergence of youngest female, respectively.
[c] Mean daily water temperature during pupation ± S.E.
[d] Mean daily air temperature from first adult emergence to 8 days after emergence of youngest female.
[e] Approximately 100 females dissected per replicate (3 pans per experiment). Adults maintained under treatment conditions for a minimum of 8 days.
[f] Means followed by same letter indicate no significant difference ($P \geq 0.05$).
[g] 95% confidence limits.
[h] Mean calculated from females with at least one follicle in stage V.

CHAPTER IV

INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON OVARIAN DEVELOPMENT AND BLOOD MEAL UTILIZATION, IN *CULISETA INORNATA* (DIPTERA: CULICIDAE), IN SOUTHERN MANITOBA.

ABSTRACT

Ovarian diapause in *Culiseta inornata* (Williston) was investigated under laboratory and field conditions, using primary ovarian follicle length (FL) and stage of follicle development (FS) after blood feeding as indicators of physiological status. Comparisons were made with a control group of wild, prehibernating females collected from resting sites in early October, 1993.. Diapause was not maintained under controlled conditions. There was a significant increase in mean FL within 34 days of adult emergence at two temperatures (16°C & 19°C) and at two photoperiods (L:D 12:12 & 14:10). At 19°C, 30.8 and 46.1 per cent of females reared at 14:10 and 12:12 (L:D), respectively, failed to develop follicles past the resting stage (follicle stage II) after receiving a blood meal at 6 days of age. When blood was offered at 18 days of age, only one female (L:D 12:12) failed to develop follicles past the resting stage. At 25°C, preimaginal survival and adult body size were reduced. There were no significant differences in overall mean FL; therefore *Cs. inornata* does not enter diapause at short photoperiods when maintained at ≥25°C.

Under natural conditions, there was a significant decline in overall mean

FL in consecutive experiments from late June to late August. The overall mean FL of females arising from individuals that pupated from 22 to 27 August was consistent with the diapause condition. Only 11.1 and 50 per cent of the females receiving a blood meal 6 and 18 days after emergence, developed follicles past the resting stage. None of the prehibernating females initiated egg development after blood feeding.

INTRODUCTION

Culiseta inornata (Williston) is widely distributed throughout North America. Its geographical range extends from Mackenzie Bay in the Yukon, to Florida and northern Mexico (Wood *et al.* 1979). Within this range, this species exhibits a great plasticity in life history (Reisen 1987). In the southern United States, females are reproductively active throughout the cool seasons, and aestivate during the summer (Washino *et al.* 1962, Chew and Gunstream 1970, Barnard and Mulla 1978). In the northern United States and western Canada, including southern Manitoba, *Cs. inornata* overwinters in a diapause state (Dow *et al.* 1976, Hayles *et al.* 1979). This condition is generally characterized by a premature arrest of ovarian development (Hudson 1977b), and an inability to digest and assimilate a blood meal for egg production (Hudson 1979).

There is evidence to suggest that the photoperiodic response of *Cs.*

inornata differs from that of other mosquito species that undergo reproductive diapause in response to short photoperiods and low temperatures. Kalpage (1970) reported that most females reared under a constant short photoperiod (L:D 8:16 and 12:12) in the laboratory initiated egg development (vitellogenesis) after blood feeding, even at low temperatures (10°C). Hudson (1977b) suggested that with regard to ovarian development, the photoperiodic response in *Cs. inornata* is of the 'long day-short day' type, not the typical 'long day' response observed in *Culex* spp. (Eldridge 1987). Only females transferred from long day (L:D 16:8) to short day (L:D 12:12) conditions during preimaginal and early adult development entered diapause. However Buth *et al.* (1990) reported that more than 70 per cent of females reared in the laboratory at 15°C and a constant photoperiod of 8:16 (L:D), entered diapause. This apparent contradiction regarding the photoperiodic control of reproductive diapause in *Cs. inornata* has not been resolved.

In this study, the principle objectives were (i) to examine the influence of a constant photoperiod and temperature on ovarian development and blood meal utilization in the laboratory and (ii) to identify when ovarian development was arrested under natural conditions in southern Manitoba. Comparisons of field-reared and laboratory-reared females were made with a group of wild-caught, prehibernating (diapausing) females collected from the field in early October, 1993.

MATERIALS AND METHODS

Rearing and Handling

Egg rafts of *Culiseta inornata* were collected at Glenlea, Manitoba, using the method described by Brust (1990). Larvae were reared using the methods described previously for *Culex tarsalis*. Unlike *Cx. tarsalis*, *Cs. inornata* colonies established from local (southern Manitoba) field populations will mate in 16 x 16 x 16 cm cages, in the F1 generation. As mating influences reproductive development (O'Meara and Evans 1977, Klowden and Chambers 1991) and the volume of blood imbibed (Adlakha and Pillai 1976) in other species, a uniform ratio of males to females was maintained among cages of known-age females. In each treatment, the first males to emerge were kept separate until the end of the emergence period, then evenly distributed among the cages of known-age females.

Experimental Conditions

In the laboratory, mosquitoes were maintained in controlled-environment chambers (Model 1-35 VL, Percival Manufacturing Company, Boone, Iowa), at three different temperatures (16, 19 and 25°C) and three different photoperiods (L:D 12:12, 14:10 and 18:6). Temperature was maintained within ±0.2°C.

In the field, mosquitoes were reared in a 1 m^2 x 30 cm deep outdoor pool (Chapter III, Fig. 1), and maintained as adults in an outdoor insectary, as

described for *Cx. tarsalis.* Daily minimum and maximum air and water temperatures were recorded throughout the study. In September, a single experiment was also performed in an outdoor waterbath (Chapter III, Fig. 2 and 3) maintained at a constant temperature of 16°C.

Wild-Caught, Prehibernating Adults

Ovarian development was examined in a group of wild-caught, diapausing females. In early October, 1993, adult females were aspirated from diurnal resting sites at the Fort Garry Campus, University of Manitoba. Females not dissected immediately after collection were either offered a blood meal, or maintained in the outdoor waterbath for 12 days at 16°C under natural daylength.

Blood Meal Utilization

Ovarian development was also examined in blood-fed females. Females reared under controlled (19°C / L:D 12:12, 14:10 & 18:6) and natural conditions (late August) were given a blood meal 6 and 18 days after eclosion. Wild-caught, prehibernating females were offered blood within 24 hours after capture, then maintained in an unheated building under natural daylength.

In all treatments, the sucrose solution was replaced with tap water 3 days prior to blood feeding. A maximum of 50 females was placed in a 16 x 3 x 2.5 cm Plexiglas® cage (nylon screen on two sides), and held on the author's forearm

for 30 minutes. In each experiment (except prehibernating females), females were repeatedly offered blood until at least one third of the exposed females from each treatment had acquired a minimum volume of blood. The criteria used to discriminate minimum blood meal volume was as follows: (i) *if the crop did not contain fluid*, the blood meal was considered to be acceptable only if the entire length of the midgut was full of blood, and the abdomen was sufficiently distended to expose the pleural membranes, or (ii) *if the crop was full of fluid* (sucrose solution or water), the blood meal was considered to be acceptable only if it occupied at least 1/3 of the length of the abdomen, and the abdomen was prominently distended, producing a conspicuous curvature along the outer margins of the pleural membranes. Females with blood meals that did not meet these criteria were not examined. Mosquitoes were given access to sucrose solution after blood feeding.

The proportion of females voiding their entire blood meal was noted at 3 and 4 days after the first exposure to a host, in the laboratory and field reared/collected females, respectively. In both groups, females were dissected and ovarian development of each female assessed at 8 and 10 days, respectively after the last exposure to a host.

Simulated Hibernation

Ovarian development and blood meal utilization were examined under simulated hibernaculum conditions. Adult mosquitoes were transferred to a

controlled environment chamber set at 5°C, L:D 0:24, for 60 days. Blood was offered 24 hours before the transfer to 5°C. Sucrose solution was replaced with water 12 hours before the transfer. Blood-fed and non-blood-fed females reared at 19°C were transferred at 15 to 18 days of age, and blood-fed females reared at 16°C were transferred at 7 days of age. Under natural conditions, blood-fed and non-blood-fed females with a mean emergence date of 12 July and 15 August, were transferred at 15-18 and 6-7 days after emergence, respectively. All surviving females were dissected after 60 days, and ovarian development assessed.

Assessment of Ovarian Development

Ovarian dissections were performed as described in the previous study for *Cx. tarsalis*. In each experiment, mean primary follicle length was used as a measure of ovarian development. In each treatment, the follicle lengths at all ages examined were pooled, and statistical comparisons were made among the overall treatment means using a Tukey HSD multiple comparison test (Wilkinson 1990). In all experiments, the number of females unsuitable for follicle measurement (i.e. autogenous females, and females with fewer than 15 intact primary follicles) was recorded.

In the wild-caught, prehibernating adults, and in the blood meal utilization and simulated hibernation experiments, the stage of development of the primary follicles was evaluated (Watts and Smith 1978). Females were classified based

on the most advanced follicle stage observed. Follicle stages I and II were considered representative of the diapause and resting stage (non-diapause) conditions, respectively. Follicle stages III to V were associated with vitellogenesis (egg development).

To confirm that mating had occurred, several females were examined for the presence of sperm. The spermathecae were removed and placed into a drop of physiological saline on a glass slide. A cover slip was placed over the drop and lightly pressed to crack the spermathecae and release their contents. Motile sperm could then be identified by phase contrast microscopy, at 200x magnification.

RESULTS

Ovarian Development under Controlled Conditions

There were statistically significant differences ($P<0.05$) in overall mean FL's among females reared under 18:6, and females reared under 14:10 or 12:12 (L:D), at both 16 and 19°C (Fig. 1). At 16°C, overall mean FL of females reared under 12:12 (L:D) was significantly higher than in females reared under 14:10 (L:D). No significant differences in overall mean FL were evident between the two temperatures. The proportion of females inseminated always exceeded 90 %.

Ovarian development was not arrested at 14:10 or 12:12 (L:D). At 19°C, there were significant increases in mean FL between 12 and 26 days after eclosion at 12:12, and between 12 and 34 days after eclosion at 14:10 (L:D). At 16°C and both 14:10 and 12:12 (L:D), there were significant differences in mean FL between 12 and 18 days after eclosion. At 14:10 and 12:12 (L:D), a significant proportion of 12 to 34 day old females had less than 15 intact primary follicles (Fig. 2).

At 25°C, no significant difference in overall mean FL was observed among the three photoperiods (Fig. 3). High mortality during preimaginal development limited the number of adult females available for dissection.

Ovarian Development under Natural Conditions

When females were reared under field conditions, a successive decline in overall mean FL was observed over the season (Fig. 4). The overall mean FL of females pupating in the outdoor waterbath (16°C) in late September was significantly higher than the overall mean FL of females pupating under natural temperatures in late August. The mean daily air and water temperatures recorded in this study are shown in Figure 5. In all field experiments, the proportion of females in each age class with fewer than 15 intact primary follicles was low (0 to 17%). Very few degenerated follicles were observed in the females developing in late August.

Ovarian Development in Pre-hibernating Females

There was a significant difference ($P<0.05$) in mean FL between the wild-caught females dissected immediately after collection (48.0 μm) and the wild-caught females held for 12 days at 16°C under natural daylength (57.0 μm). All of the thirteen wild-caught adults dissected immediately after capture had follicles in stage I. Six of the ten females examined after 12 days had follicles in stage I, and four had developed follicles to stage II. All females dissected immediately after collection were inseminated.

Ovarian Development after Blood Feeding

a) Controlled conditions

When blood was offered to 6-day old females reared at 19°C, two (15.4%) of the females reared at 14:10, and four (30.8%) of the females reared at 12:12 (L:D), had follicle development arrested in stage I (Table 1). Two (15.4%) of the females in both treatments had follicles in stage II. Most of the females that did not retain the blood meal more than three days after feeding did not develop follicles beyond stage II. All females reared at 18:6 (L:D) retained the blood meal and developed stage V follicles.

When blood was offered at 18 days after eclosion, all of the females reared at 19°C and both 18:6 and 14:10 (L:D), and most (96.8%) of the females reared at 12:12 (L:D), developed follicles beyond stage II. All females reared at 18:6 and 14:10 (L:D), and 28 (90.3%) of the females reared at 12:12 (L:D),

retained the blood meal three days after feeding.

b) Natural conditions

When 6-day old females emerging under natural conditions in late August were given a blood meal, most (77.8%) did not develop follicles beyond stage I (Table 2), and only one (11.1%) of the females had developed follicles beyond stage II. The majority of blood-fed females (66.7%) voided their entire meal within 4 days after feeding. When blood was offered to 18-day old females, eight (50.0%) of the females had follicles greater than stage II. Only two (12.5%) had follicle development arrested in stage I.

c) Wild-caught females

None of the wild-caught females receiving blood developed follicles beyond stage II (Table 3). Twenty-eight (75.7%) of the females examined had stage I follicles. Over half (54.1%) voided the entire blood meal within 4 days of feeding.

DISCUSSION

Hudson (1977b) stated that *Cs. inornata* did not enter diapause under a constant short photoperiod. Similarly, Kalpage (1970) reported that the majority of *Cs. inornata* reared under constant short photoperiods (L:D 8:16 and 12:12) initiated vitellogenesis (egg development) after blood feeding, regardless of

temperature. In the present study, a constant photoperiod of 14:10 or 12:12 (L:D) was sufficient to arrest ovarian follicle development in *Cs. inornata* in a diapause state, at 16 and 19°C. The mean FL's of 6 and 12 day old females reared under these conditions (Fig. 1) were slightly higher than the mean FL of wild, prehibernating females (48 μm), but lower than the mean length of stage I (diapause stage) follicles (66 μm) reported by Hudson (1977a). However, *Cs. inornata* did not maintain diapause under a constant short photoperiod. There was a significant increase in mean FL within 34 days after emergence (Fig. 1), as ovarian development continued past stage I. At 19°C, none of the females reared at 14:10, and only one female (3.2%) reared at 12:!2 (L:D), failed to initiate vitellogenesis after receiving a blood meal at 18 days of age (Table 1). Buth *et al.* (1990) reported reproductive diapause in *Cs. inornata* under similar rearing conditions, two weeks after adult emergence. However, as can be seen from Figure 1, a significant change in mean FL (and therefore mean F:G ratio) occurred after 14 days. Had Buth *et al.* (1990) assessed follicle development in older females, the results would undoubtedly have been quite different.

When *Cs. inornata* is reared at a temperature ≥25°C, preimaginal survival and adult body size are greatly reduced (McLintock 1952, Hanec and Brust 1967, Shelton 1973). A significant reduction in adult body size was observed at 25°C in the current study, which may explain the lower mean FL at 18:6 (L:D), relative to the other two temperatures (Fig. 1). In the laboratory, short photoperiods are not sufficient to induce diapause in *Cx. pipiens* (Eldridge 1966,

Spielman and Wong 1973a) and *Cx. tarsalis* (Reisen *et al.* 1989) at 25°C. Buth *et al.* (1990) reported that more than 50 per cent of *Cs. inornata* females reared at 25°C and 8:16 (L:D) were in diapause. In the current study, there were no significant differences in mean FL among the three photoperiods tested, indicating a high temperature threshold for diapause induction of ≤25°C. The F:G ratio used by Buth *et al.* (1990) to define the diapause condition (mean F:G<2.5) was high, relative to the mean F:G value of diapausing females reared at 20°C (mean F:G<2.0, from Hudson 1977b), and may have led the authors to mistake gonoactive females with small follicles (due to the influence of a high temperature) for diapausing females.

Low juvenile hormone (JH) levels are characteristic of adult diapause in most insects (Mitchell 1988). In gonoactive mosquitoes, the release of JH during previtellogenic development stimulates ovarian follicle growth to the resting stage (Gwadz and Spielman 1973, Hagedorn *et al.* 1977). Topical applications of a JH analogue have been used to terminate diapause in *Cx. tarsalis* (Mitchell 1981) and *Cx. pipiens* (Spielman 1974). JH is also required to maintain the ultimate ovarian follicles at the resting stage, preventing follicle degeneration (Gwadz and Spielman 1973). In the current study, a high proportion of 12 to 34 day old females had degenerating follicles (Fig. 2). Perhaps the circulating levels of JH in females reared at 14:10 and 12:12 (L:D) were high enough to terminate diapause and initiate follicle growth, but were not sufficient to maintain resting stage follicles.

Hudson (1977b) found that diapause was maintained in 16 to 17 day old females when the larvae were reared at 20°C and 16:8, then transferred at the larval-pupal ecdysis to 10°C and 12:12 (L:D). Diapause was not maintained when all stages were reared under constant conditions (10°C and L:D 12:12, or 20°C and L:D 16:8). Hudson (1977b) indicated that this species exhibits a long day/short day photoperiodic response, i.e. a change in daylength, but not necessarily across a critical value, is required to maintain or induce diapause (type (d) of Tauber *et.al.* 1986). Hudson (1977b) attempted to determine the photosensitive stages of development for the transfer (from long day to short day) and found that females would also enter diapause if the transfer was made 0 to 24 hours after the 3rd to 4th larval instar ecdysis, or 0 to 24 hours after adult emergence. The author found that the earlier in development the transfer was made, the smaller the F:G ratio, thus the influence of short photoperiod during these developmental stages may be cumulative. In the current study, there was some evidence that diapause could be maintained in females reared under constant conditions if they are exposed to a reduction in daylength and temperature before the end of previtellogenic development (to stage II) (Appendix II). In this experiment, females reared at 16°C and constant photoperiod (L:D 18:6, 14:10 and 12:12) were given a blood meal 6 days after adult emergence, held at 10°C for 24 hours, then transferred to simulated hibernaculum conditions (5°C and L:D 0:24) for 60 days. Follicle development was arrested in the diapause (stage I) state in 0 (0/6), 75 (12/16) and 29.4 (5/17)

per cent of the surviving females reared at 18:6, 14:10 and 12:12 (L:D), respectively. Reproductive development in non-diapausing females (L:D 18:6) was not inhibited at 5°C and 0:24 (L:D). The proportions of surviving females reared at 18:6, 14:10 and 12:12 that initiated vitellogenesis were 100, 0 and 17.6 per cent. Thus a decrease in daylength (and/or possibly a decline in temperature) 7 days after adult emergence can influence diapause maintenance.

In a two year study in central Alberta, diapause rates in field collected *Cs. inornata* were very low (5-10%) in early August, and rose to 80 to 90 per cent by the end of August (Hudson 1977a). Hudson (1977b) estimated that females that entered diapause in the field in mid-August experienced a decline in mean water temperature during preimaginal development temperature from approximately 20 to 13°C, and a reduction in daylength from about 18 to 16 hours light. As Hudson (1977b) had shown that diapause was maintained by a transfer from 16:8 to 12:12 (L:D) in the laboratory, he speculated that *Cs. inornata* does not require a decrease in daylength across a specific (critical) photoperiod. However, based on data from Hudson's (1977b) study, Tauber *et al.* (1986) have disagreed, classifying the long day/short day photoperiodic response in *Cs. inornata* as type (b); defined as one which requires a change in daylength across a specific photoperiod. However, the authors did not provide any arguments or additional data to support this interpretation.

In the current study, females emerging under natural conditions in late August were in diapause, as indicated by low mean FL (Fig. 4) and the relatively

low proportion (11%) of 6-day old females initiating vitellogenic development after blood feeding (Table 2). The declines in the mean daily water temperature and daylength, from the date when the 2nd instar larvae were placed under field conditions (16 August) to the date when the last adult emerged (1 September), were approximately 22 to 17°C and 15.6 to 14.6 hours light, respectively (Fig.5). However a much higher rate (50%) of vitellogenic development was observed when 18-day old females were offered a blood meal (Table 2). In the laboratory, Hudson (1977b) found that when *Cs. inornata* was transferred from 20°C and 16:8, to 15°C and 12:12 (L:D) (at the larval-pupal ecdysis) the mean F:G ratio of females was indicative of diapause, but blood feeding and egg maturation (after a blood meal) rates were significantly higher than when transferred to 10°C. In the outdoor pool in the current study, a higher mean temperature during preimaginal development may have interfered with diapause maintenance in some females pupating in late August.

Culiseta inornata exposed to natural daylength did not maintain diapause at constant temperatures ≥16°C. In the outdoor waterbath, females experienced a decline of 14.2 to 13.0 hours light from 2nd larval instar (7 September) to last adult emergence (26 September) and temperature was kept constant at 16°C, yet they clearly did not maintain diapause (Fig. 4). Similarly, 40 per cent (4/10) of the wild, diapausing (prehibernating) females maintained in the 16°C water bath for 12 days in early October had terminated diapause. Assuming *Cs. inornata* does not require a decline in daylength across a specific daylength

(Hudson 1977b), the decline in natural daylength should have been sufficient to maintain diapause in these two experiments. In both cases, females were exposed to a decline in natural daylength within three weeks of an equinox (22 September), the time of the season when the rate of change in natural daylength is highest. Although the mean daily water temperature in the outdoor pool was consistently above 16°C from 16 August to 1 September, the overall mean air temperature and the mean daily minimum air temperatures (±s.e.) from 1 to 7 September (i.e. 0 to 6 days after adult emergence) were 13.5 ±0.5°C and 9.1 ±1.2°C, respectively (Fig. 5). The cool, night air temperatures experienced during the first six days after adult emergence may have been sufficient to overcome the relatively high mean water temperature experienced during preimaginal development. Cool temperatures during the scotophase can enhance the influence of a short photoperiod (Danks 1987).

In the present study, ovarian development after blood feeding was used as a criterion for evaluating the physiological status of females reared under controlled and natural conditions. Hudson (1979) found that diapausing *Cs. inornata* will accept blood, but do not necessarily produce eggs. It was noted that trypsin levels in diapausing females were significantly lower, consequently the blood meal was not digested, and was usually voided within 24 hours after feeding. In the current study, diapausing females often took smaller blood meals and voided the blood shortly after feeding (Tables 1, 2 and 3). The blood defecated by diapausing females was bright red, very different from the dark

brown faeces of non-diapausing females observed after blood digestion. In some cases, trace amounts of blood were retained in the midgut after defecation, similar to the observations of Hudson (1979). In the current study, females which retained their blood meal for 3 (Tables 1 and 2) or 4 (Table 3) days were more likely to develop follicles beyond stage I.

Diapausing *Cs. inornata* females are more reluctant to blood feed and they imbibe trace blood meal volumes more frequently (Hudson 1977b); therefore, diapausing females were less likely to take a blood meal consistent with the minimum blood meal volume criteria used in this study, possibly leading to an underestimation of the proportion of diapausing females in each sample. Only 29 per cent (42/145) of the prehibernating females accepted a suitable blood meal volume after three opportunities to feed (30 minutes each). In the laboratory, between 1/3-1/2 of the females reared at 14:10 or 12:12 (L:D) blood fed. Hudson (1979) argued that measuring follicle size is a more accurate method of describing the physiological status of a female than evaluating blood meal utilization. Some of these problems may have been overcome by introducing a premeasured volume of blood directly into the midgut of every female in a sample by rectal enema (Mitchell and Briegel 1989b).

In some females, follicle development was arrested in stage II after blood feeding (Tables 1, 2 and 3). In many of the 6-day old females exhibiting this phenomenon, the blood meal probably did not influence ovarian development; i.e. diapause was terminated for other reasons (after defecation). In others, the

blood meal itself may have been a stimulus for diapause termination. Although they were maintained at cool temperatures (mean air temperature was <10°C), almost half (8/17)of the prehibernating females that retained the blood meal 4 days after feeding developed follicles to the resting stage (Table 3). In *Cx. pipiens*, diapausing females will terminate diapause and develop eggs if a sufficient volume of blood is acquired (Sanburg and Larsen 1973, Oda and Wada 1972), however the minimum volume of blood required to stimulate digestion and egg development is probably higher (than in nondiapausing females) due to slow or inactive endocrine mechanisms (Mitchell and Briegel 1989a,1989b). In the current study, when less than a third of the females blood fed, the females with sufficient blood meal volumes were removed, and females with little or no blood were then given the opportunity to feed again several hours later. Previously fed females were frequently observed engorging on the 2nd or 3rd exposure. Assuming that most of the blood was excreted within 24 hours after feeding, as reported by Hudson (1979), diapausing females taking 2 or 3 small blood meals over a 12 to 24 hour period could have blood present in the midgut longer than diapausing females taking one large meal, although the volume retained in the midgut at any one time may be smaller. Mitchell and Briegel (1989b) correlated ovarian development after blood feeding in diapausing *Cx. pipiens* with the volume of blood taken, and volume of blood meal retained at the end of an overnight feeding period (Mitchell and Briegel 1989b). Multiple feedings may have artificially extended the retention time without

providing enough protein for vitellogenic development. However, it is not clear how a blood meal could trigger the release of JH so that previtellogenic development may proceed. JH production in gonoactive *Cx. pipiens* actually decreases just after blood feeding and does not return to normal levels for several days (Readio *et al.* 1988).

As a cool season species, *Cs. inornata* is most abundant in Winnipeg during the late spring and late summer when temperatures are cooler. Buth *et al.* (1990) found that oviposition by *Cs. inornata* in artificial oviposition pools in Winnipeg continued until early to mid-September. Larvae have been observed in the field in mid to late September (McLintock 1944). In the current study, there was an increase in the proportion of females in diapause throughout the month of August, with most of the females emerging at the end of August in diapause, a few weeks before the reported disappearance of gonoactive females in previous field studies. This compares well with field data from central Alberta, where diapausing *Cs. inornata* began appearing in early August, dominated field collections by late August (Hudson 1977a), and were collected from overwintering sites from late August to early September (Shemanchuk and Morgante 1968). In central Alberta, parous and gravid females have been collected in mid September, and larvae have been observed in late September (Hudson 1977a). In the present study, however, *Cs. inornata* did not enter diapause at a constant temperature ≥25°C, and temperatures ≥16°C inhibited diapause maintenance. In Winnipeg, the mean daily maximum air temperature

for August, 1993 was 22.9°C, two degrees lower than the average maximum air temperature for August (24.9°C) (from: Environment Canada 1993). In an average year, higher daytime temperatures in August may delay the induction of diapause in field populations until later in the summer.

Although it was determined in this study that diapause induction in *Cs. inornata* does occur under a constant short photoperiod, contrary to the findings of Hudson (1977b), the environmental factors responsible for diapause maintenance have not been conclusively demonstrated. Further studies are needed to clarify whether this species requires a change in photoperiod and/or temperature during preimaginal development (and/or early adulthood) to maintain a state of reproductive diapause.

Figure 1. Mean primary follicle length (±s.e.) of *Culiseta inornata* females reared in the laboratory at 16 and 19°C, under three photoperiods. Each point represents the mean of 10 to 20 females. Treatments with no significant difference ($P \geq 0.05$) between overall (pooled) mean FL's are followed by the same letter.

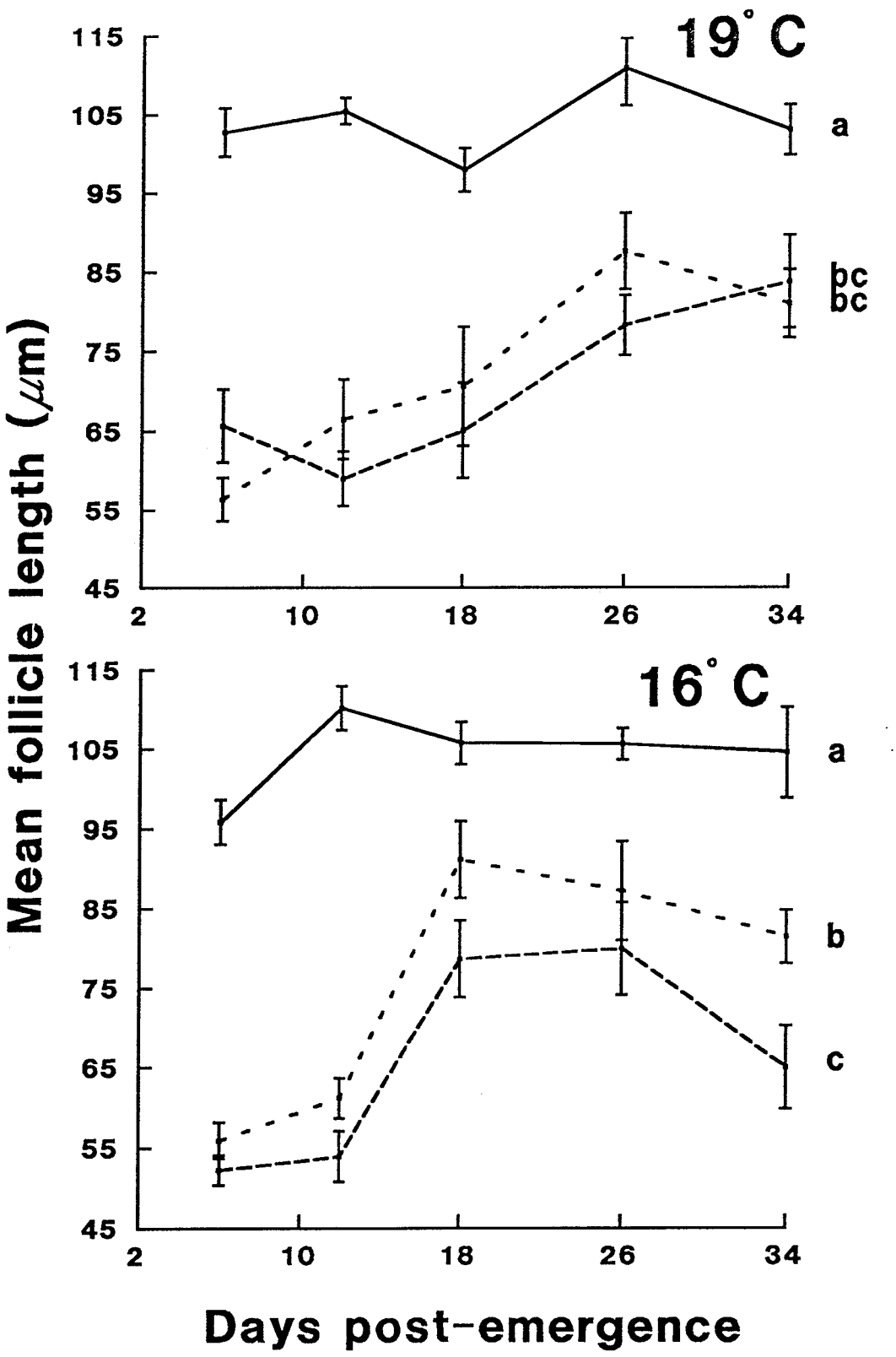

19° C
16° C
a
bc
bc
a
b
c
115
105
95
85
75
65
55
45
2
10
18
26
34
Mean follicle length (μm)
Days post-emergence
L:D 18 : 6
L:D 14 : 10
L:D 12 : 12

Figure 2. Primary follicle degeneration in *Culiseta inornata* females reared in the laboratory at 16 and 19°C under three photoperiods (FL's of non-degenerated females shown in Fig.1). Females with fewer than fifteen intact primary follicles remaining were classified as having degenerated follicles. Follicles were not considered intact if the cellular membranes of the oocyte or trophocytes had ruptured. The primary follicle of an ovariole was assumed to have undergone resorption if there was a dilatation present on the pedicel. For each treatment, a minimum of 10 to 20 females was dissected in each age class.

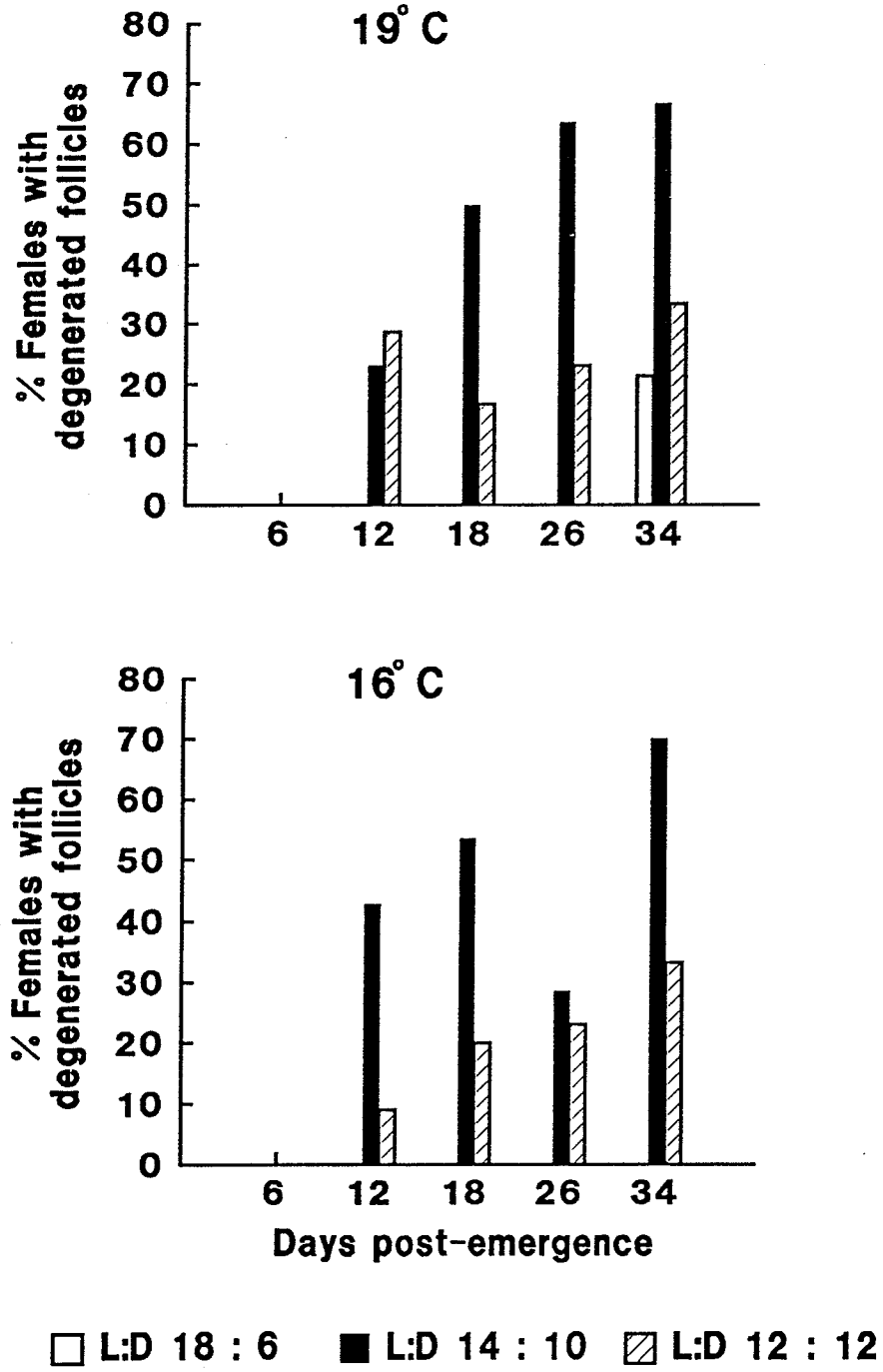

19° C
16° C
% Females with degenerated follicles
80
70
60
50
40
30
20
10
0
6
12
18
26
34
Days post-emergence
L:D 18 : 6
L:D 14 : 10
L:D 12 : 12

Figure 3. Mean primary follicle length (±s.e.) of *Culiseta inornata* females reared in the laboratory at 25°C, under three photoperiods. Each point represents the mean of 8 to 10 females. Overall (pooled) mean FL's were not significantly different ($P \geq 0.05$).

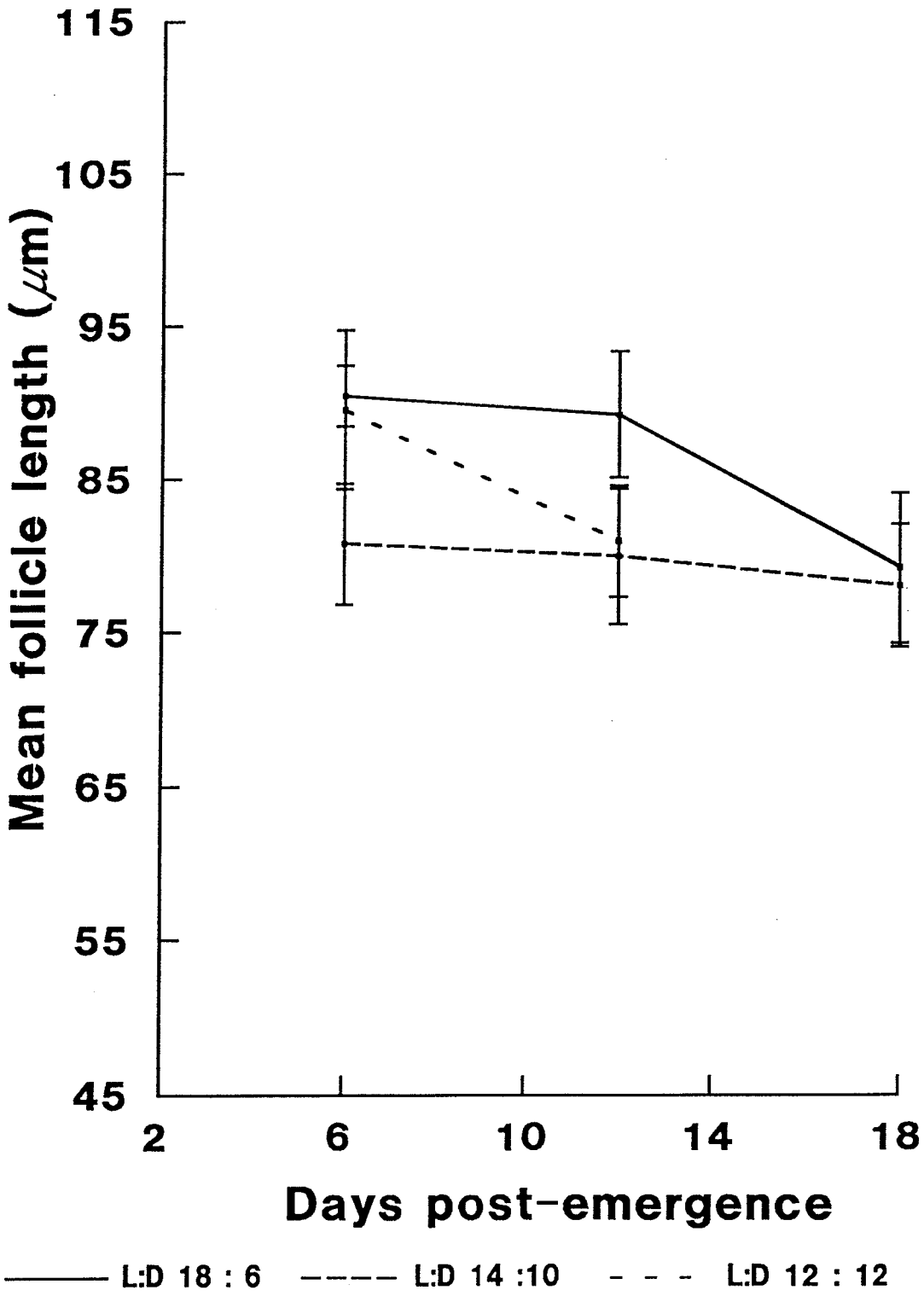

115
105
95
85
75
65
55
45
Mean follicle length (μm)
2
6
10
14
18
Days post-emergence
L:D 18 : 6
L:D 14 :10
L:D 12 : 12

Figure 4. Mean primary follicle length (±s.e.) of *Culiseta inornata* females reared in an outdoor pool under field conditions (except for one exception shown below). Each point represents the mean of ten females. The interval between the appearance of the first and last pupa is shown for each experiment. The fifth experiment (pupated 18 to 23 September) was conducted in the outdoor water bath at a constant temperature of 16°C. Treatments with no significant difference ($P \geq 0.05$) between overall (pooled) mean FL's are followed by the same letter.

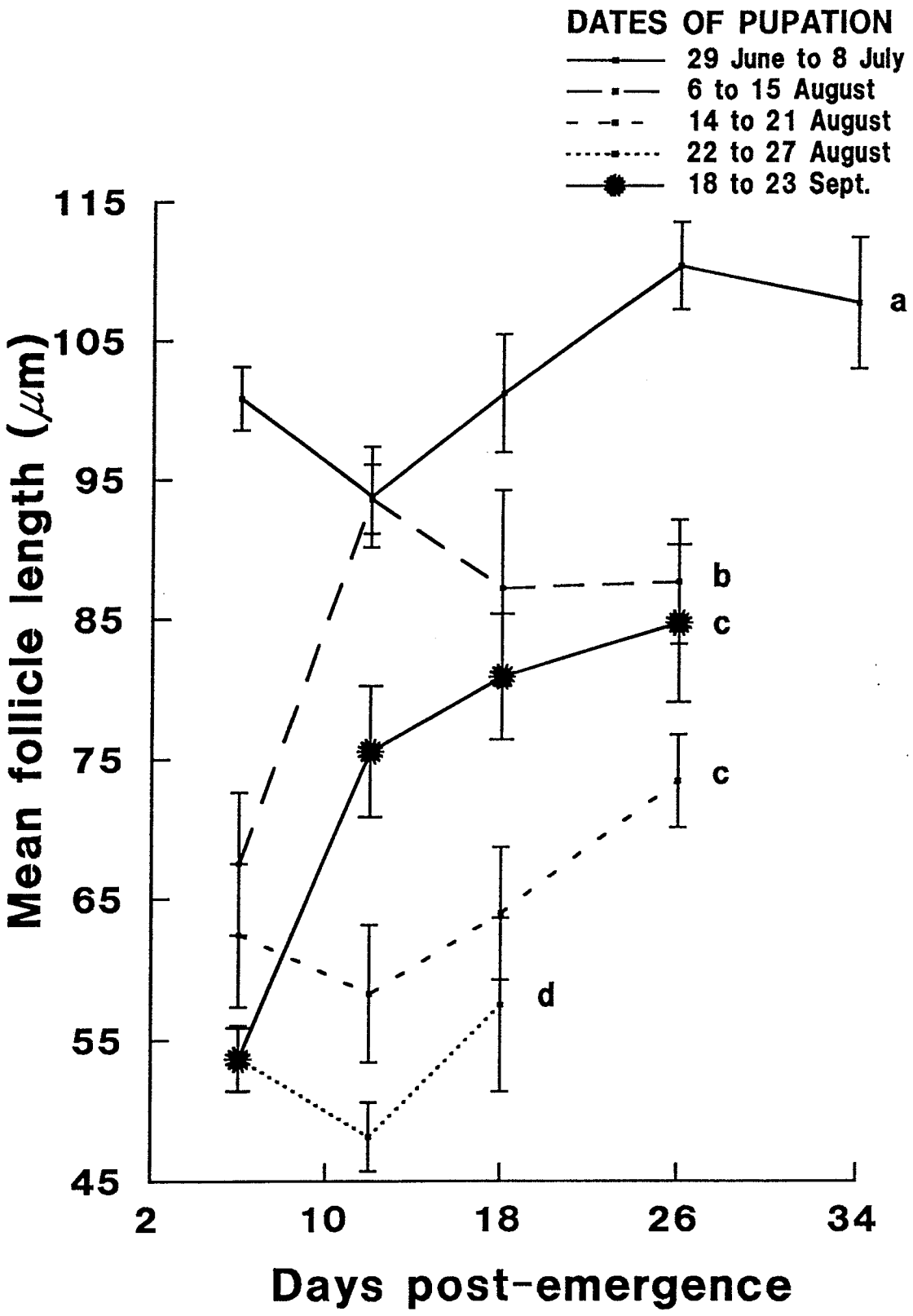
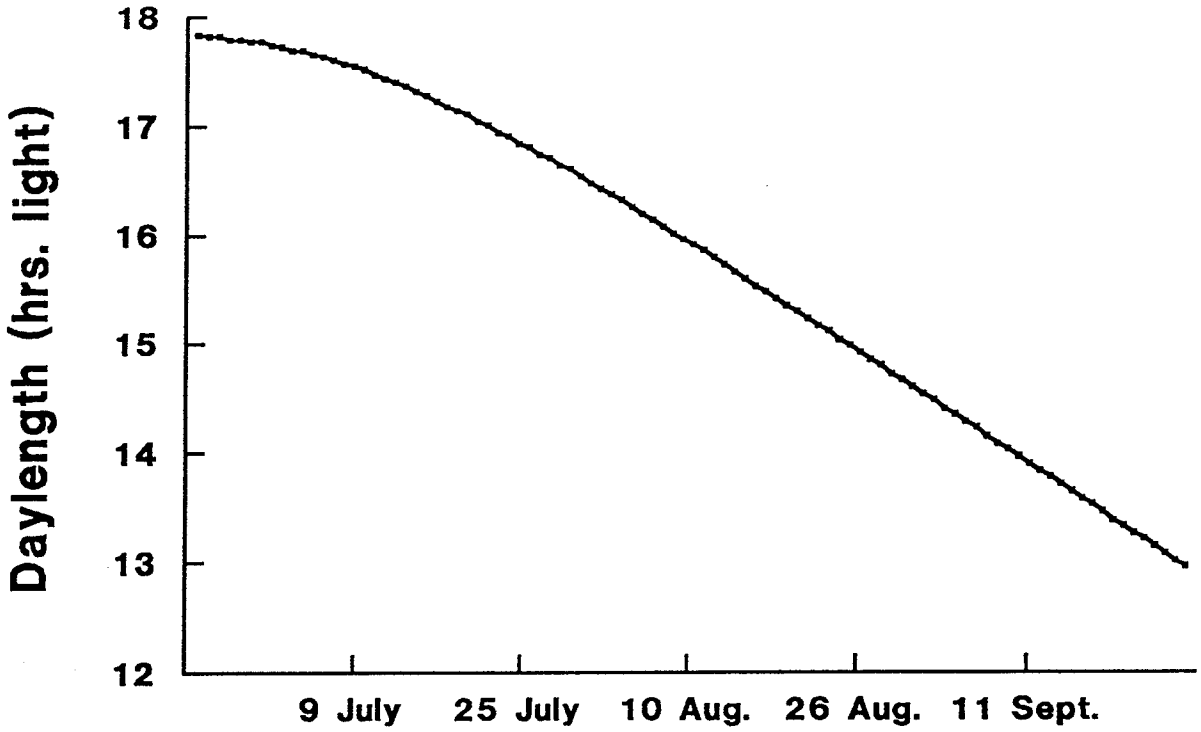

DATES OF PUPATION
29 June to 8 July
6 to 15 August
14 to 21 August
22 to 27 August
18 to 23 Sept.
Mean follicle length (μm)
115
105
95
85
75
65
55
45
2
10
18
26
34
Days post-emergence
a
b
c
c
d

Figure 5. Field conditions, 23 June to 27 September, 1993. Daylength = hours light + (2 × civil twilight) (United States Naval Observatory data, from Beck 1980). Mean daily water temperature was measured 1-2 cm below the pool surface. Mean daily air temperature was recorded in the outdoor insectary. Relative light intensities under various field conditions are shown in Appendix V.


Daylength (hrs. light)
18
17
16
15
14
13
12
9 July
25 July
10 Aug.
26 Aug.
11 Sept.

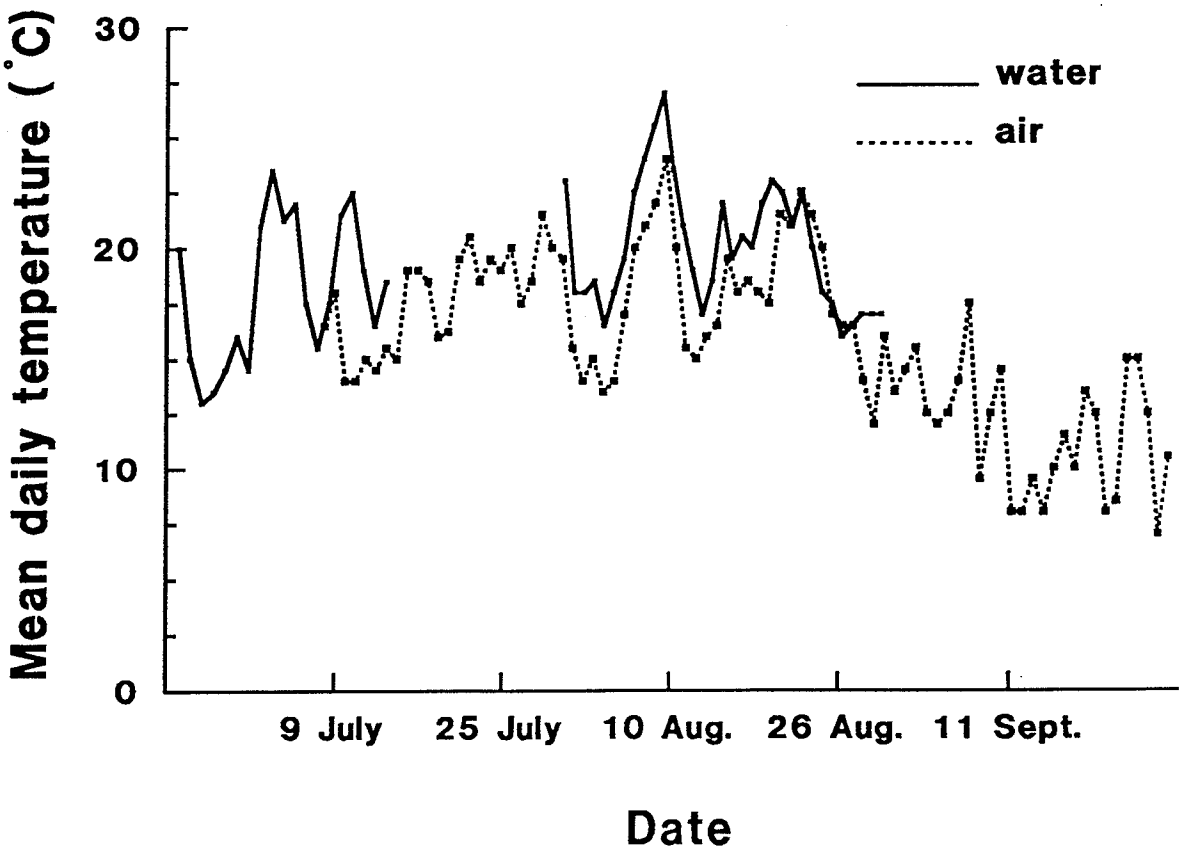

Mean daily temperature (°C)
30
20
10
0
water
air
9 July
25 July
10 Aug.
26 Aug.
11 Sept.
Date

Table 1. Ovarian follicle development and blood-meal utilization in *Culiseta inornata* reared at 19°C.

Age at B.F.[a]	Photo-period (L:D)	Blood meal[b]	No. dissected[c]	F.S.[d]				% >F.S. II[e]	Mean F.L.±s.e. (μm)[f]
				I	II	III-IV	V		
6	18:6	Retained[g]	10	0	0	0	10	100	-
	14:10	Retained	10	1	1	0	8	80	90±27.2
		Voided	3	1	1	0	1	33	79±13.7
	12:12	Retained	10	1	2	0	7	70	67±7.1
		Voided	3	3	0	0	0	0	58±7.9
18	18:6	Retained[g]	14	0	0	1	13	100	-
	14:10	Retained[g]	8	0	0	0	8	100	-
	12:12	Retained	28	0	0	1	27	100	-
		Voided	3	0	1	1	1	67	77

[a] Age (days after adult emergence) when blood was offered.
[b] Assessed 3 days after blood feeding.
[c] Females were dissected 8 days after feeding.
[d] Number of females with follicles in each stage of development.
[e] Percentage of females in each treatment which developed follicles beyond stage II.
[f] Mean primary follicle length of females which did not develop follicles beyond stage II. Sample size was too small for statistical comparison.
[g] None of the females voided the blood meal.

Table 2. Ovarian follicle development and blood-meal utilization in *Culiseta inornata* reared under natural conditions in late August[a].

Age at B.F.[b]	Blood meal[c]	No. dissected[d]	F.S.[e]				% >F.S. II[f]	Mean F.L.±s.e. (μm)[g]
			I	II	III-IV	V		
6	Retained	3	2	0	1	0	33	57±1.2
	Voided	6	5	1	0	0	0	50±1.0
18	Retained	8	0	0	8	0	100	-
	Voided	8	2	6	0	0	0	78±1.6

[a] Pupation occurred from 22 to 27 August, 1993.
[b] Age (days after adult emergence) of females when blood meal was offered.
[c] Assessed 4 days after blood feeding.
[d] Females were dissected 8-10 days after feeding.
[e] Number of females with follicles in each stage of development.
[f] Percentage of females in each treatment which developed follicles beyond stage II.
[g] Mean primary follicle length of females which did not develop follicles beyond stage II. Sample size was too small for statistical comparison.

Table 3. Ovarian follicle development and blood-meal utilization in wild-caught, prehibernating *Culiseta inornata*[a].

Blood meal[b]	No. dissected[c]	F.S.[d]				% >F.S. II[e]	Mean F.L.±s.e. (μm)[f]
		I	II	III-IV	V		
Retained	17	9	8	0	0	0	67±3.6 a
Voided	20	19	1	0	0	0	53±1.2 b

[a] Adult females collected from resting sites, 5 October, 1993. Females were offered blood immediately after collection. Blood-fed females were maintained for ten days in an unheated building, under natural daylength. The mean daily maximum and minimum air temperatures recorded over the ten day period were 10.25°C and 3.75°C, respectively.

[b] Assessed 4 days after blood feeding.

[c] Females were dissected 10 days after blood feeding.

[d] Number of females with follicles in each stage of development.

[e] Percentage of females which developed follicles beyond stage II.

[f] Mean primary follicle lengths followed by same letter not significantly different ($P \geq 0.05$).

CHAPTER V

GENERAL DISCUSSION

Reproductive Diapause

Cx. tarsalis and *Cs. inornata* occupy a similar geographical range, and often exploit the same larval and overwintering habitats (McLintock 1944, Shemanchuk 1965), yet they utilize very different mechanisms for determining seasonal position.

The fundamental relationship observed between environmental factors (temperature and photoperiod) and diapause induction in *Culex tarsalis* was consistent with previous studies on reproductive diapause in this species (Harwood and Halfhill 1964, Reisen 1986b), as well as *Cx. pipiens* (Sanburg and Larsen 1973, Spielman and Wong 1973a), *Cx. restuans* (Eldridge *et al.* 1976, Madder *et al.* 1983) and *Cx. peus* (Skultab and Eldridge 1985). These species all exhibit a long-day photoperiodic response, i.e. females arrest reproductive development in response to short photoperiods.

In the laboratory, ovarian development in *Cx. tarsalis* was most sensitive to temperature at the intermediate photoperiod (L:D 14:10), and diapause was uniformly expressed only at the short photoperiod (L:D 12:12). These findings are highly relevant to the mechanisms of diapause induction observed in the field in southern Manitoba. In Winnipeg (49° 54' N), natural daylength does not decline to 12 hours light until the beginning of October. Under field conditions,

Ct. tarsalis females began entering diapause when natural daylength at adult emergence had declined to approximately 15 hours light (mid August). When natural daylength declined to about 13 hours light (mid-September), all newly emerged females were in diapause. This late season shift from gonoactivity to reproductive diapause in field-reared *Cx. tarsalis* was mediated by a drop in mean daily temperature. Hence it can be predicted, that below normal August temperatures in southern Manitoba would result in a greater proportion of females emerging in reproductive diapause. Similarly, above normal August temperatures would result in a smaller proportion of emerging females in reproductive diapause.

The effects of temperature and photoperiod on reproductive diapause in *Cs. inornata* are not clear. In the current study, diapause was not maintained in females reared at 16 and 19°C, and constant short photoperiod (L:D 14:10 and 12:12) in the laboratory, nor was diapause maintained in females pupating at 16°C and natural daylength in late September. Diapause was terminated in prehibernating females kept at 16° and natural daylength in early October. Diapause was only maintained in females pupating under natural conditions in late August, and females transferred from 19°C and 14:10 to 5°C and 0:24 (L:D), at 7 days after emergence. Therefore, it can be concluded that diapause maintenance in *Cs. inornata* requires a temperature below 16°C, and may require a reduction in daylength during development (as proposed by Hudson 1977b).

Several insect species have been shown to enter diapause in response to a change in daylength during development (Tauber and Tauber 1975, Butler *et al.* 1978). In the tobacco hornworm, *Manduca sexta* (L.), the duration of pupal diapause is much shorter in insects reared under constant short photoperiod than in insects transferred from long day to short day as older larvae (Denlinger and Bradfield 1981). In the seed bug, *Neacoryphus bicrucis* (Say), it was suggested that a long day/short day photoperiodic response insures seasonal synchronization at a wide range of latitudes, and may be important in insects with substantial dispersal capabilities (Solbreck 1979). Long range dispersal has not been reported in *Cs. inornata*.

Very little is known about overwintering and diapause in other members of the genus *Culiseta*. Reisen (1987) reviewed the overwintering strategies of the North American species and, based on field observations of the seasonal abundance of adults and preimaginal stages, determined that only two other species might overwinter as adults in a facultative reproductive diapause, *Cs. minnesotae* and *Cs. incidens*. However, the mechanisms of diapause induction and maintenance in both species have not been investigated.

Culiseta inornata is a cool-season species. In the southern United States, *Cs. inornata* is active during the cooler months of the year, and adults aestivate during the summer (Meyer and Washino 1976, Barnard and Mulla 1978). This species is not as tolerant of warm temperatures as many *Culex* spp. (including *Cx. tarsalis*) (Shelton 1973, Brust 1991). *Culiseta inornata* cannot develop at

constant temperatures ≥29°C, and does poorly at constant temperatures ≥23°C (Hanec and Brust 1967, Shelton 1973). The optimal rearing temperature is about 21°C, and larvae are able to develop normally at 10°C (Hanec and Brust 1967). At 15°C, *Cs. inornata* develops much faster than *Cx. tarsalis* (Buth *et al.* 1990). Preimaginal survival in *Cx. tarsalis* is greatly reduced at constant temperatures ≤15°C (Buth *et al.* 1990).

In southern Manitoba, one the most important factors influencing the relative abundance of *Cx. tarsalis* is temperature; high mean daily temperatures encourage the production of high numbers of this species (McLintock 1948, Raddatz 1986). Peak abundance of adult *Cx. tarsalis* generally occurs in late July to early August (Brust 1982, Sekla *et al.* 1991). In contrast, the inability of *Cs. inornata* to tolerate high temperatures may produce a mid-summer depression in oviposition activity during the hottest part of the summer (Gallaway 1983, Buth *et al.* 1990). Peak *Cs. inornata* abundance occurs earlier in the season than *Cx. tarsalis* (Spalatin *et al.* 1963), but oviposition activity in *Cs. inornata* extends later in the season (Buth *et al.* 1990). This preference of *Cs. inornata* for cooler temperatures probably accounts for the reduced high-temperature threshold (<16°C) for diapause maintenance observed in the present study. It would be advantageous for this species to remain reproductively active in the late summer and early autumn while field temperatures remain suitable for preimaginal development.

Autogeny

Brust (1991) reported a significant difference in the rate of autogeny between *Cx. tarsalis* females reared under comparable conditions in the laboratory and the field. Maximum autogeny (82%) in the field occurred when the mean daily air temperature and natural daylength in the field were 23°C and 17 hours light (Brust 1991). In the laboratory, maximum autogeny (51%) occurred at 21°C and 16:8 (L:D). In the current study, both *Cx. tarsalis* and *Cs. inornata* exhibited a much higher rate of autogeny under field conditions than in the laboratory. Maximum autogeny rates in *Cx. tarsalis* in the laboratory (L:D 18:6 and 22°C), water bath and outdoor pool were 25.8, 69.3 and 47.4 per cent, respectively (Chapter III, Tables 1, 2 and 3). Maximum autogeny rates in *Cs. inornata* in the laboratory (L:D 18:6 and 16°C) and outdoor pool were 4.8 and 15.0 per cent, respectively (Appendix IV, Tables 1 and 2). In the field (water bath and outdoor pool), females were exposed to shorter daylengths and/or cooler temperatures, and visible light intensity was higher in the laboratory than in the bath (Appendix V).

Larval nutrition has a significant influence on the rate of autogeny in *Cx. tarsalis* (Reisen *et al.* 1984). In the present study, mosquitoes were fed an exclusive diet of bovine liver powder. However, pool-reared females received additional organic inputs, such as leaves, grass clippings, dead insects, etc., that inadvertantly fell into the rearing pans. Under natural light, profuse algal blooms developed in the rearing pans, in both the water bath and the outdoor

pool. In the current study, essential nutrients associated with autogenous egg production may have been limited in the artificial larval diet. In the water bath and outdoor pool, supplementary dietary sources present in the larval habitat may have overcome any limitations on egg production imposed in the laboratory by an exclusive diet of bovine liver powder. Differences between laboratory-reared and wild *Cx. tarsalis* females, in the levels of fatty acids essential for flight activity, have been reported (Dadd *et al.* 1988). This indicates that both abiotic and biotic factors must be considered when interpreting differences between autogeny rates in the field and laboratory.

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Appendix 1. Ovarian follicle development in *Culiseta inornata* reared at 19°C, then transferred to simulated hibernaculum conditions (5°C/L:D 0:24) for 60 days, 15-18 days postemergence.

Photo-period (L:D)	Status[a]	No. Transferred (%Survival)[b]	No. Dissected	F.S.[c]				No. De-generated[d]	Mean F.L.±s.e. (μm)[e]
				I	II	III-IV	V		
18:6	Blood-fed	26 (0)	0	-	-	-	-	-	-
	Unfed	46 (9)	4	0	4	0	0	0	95±1.2
14:10	Blood-fed	25 (24)	6	0	1	1	2	2	111±2.8
	Unfed	53 (28)	12	3	9	0	0	0	80±1.5
12:12	Blood-fed	22 (68)	15	0	1	0	12	2	78±1.8
	Unfed	38 (71)	13	1	9	0	0	3	92±1.3

[a] Blood fed females were offered a blood meal 24 hours prior to transfer.
[b] Proportion of females surviving 60 days at 5°C, L:D 0:24.
[c] Number of females with follicles in each stage of development.
[d] Females with fewer than fifteen intact primary follicles were classified as having degenerated ovarioles. Follicles were not considered intact if the cellular membranes of the oocyte or trophocytes had ruptured. The primary follicle of an ovariole was assumed to have undergone resorption if there was a dilatation present on the pedicel.
[e] Mean primary follicle length of females which did not develop follicles beyond stage II.

Appendix II. Ovarian follicle development in blood-fed[a] *Culiseta inornata* reared at 16°C, then transferred to simulated hibernaculum conditions (5°C/L:D 0:24) for 60 days, 7 days postemergence.

Photoperiod (L:D)	No. Transferred (%Survival[b])	No. Dissected	F.S.[c]				No. Degenerated[d]	Mean F.L.±s.e. (μm)[e]
			I	II	III-IV	V		
18:6	24 (25)	6	0	0	0	6	0	-
14:10	59 (27)	16	12	0	0	0	4	57±0.6
12:12	39 (44)	17	5	9	1	2	0	74±1.3

[a] All females received a blood meal 24 hours prior to transfer.
[b] Proportion of females surviving 60 days at 5°C, L:D 0:24.
[c] Number of females with follicles in each stage of development.
[d] Females with fewer than fifteen intact primary follicles were classified as having degenerated ovarioles. Follicles were not considered intact if the cellular membranes of the oocyte or trophocytes had ruptured. The primary follicle of an ovariole was assumed to have undergone resorption if there was a dilatation present on the pedicel.
[e] Mean primary follicle length of females which did not develop follicles beyond stage II.

Appendix III. Ovarian follicle development in *Culiseta inornata* reared under natural conditions, then transferred to simulated hibernaculum conditions (5°C/L:D 0:24) for 60 days.

Pupation Date (Age at Transfer)	Status[a]	No. Transferred (%Survival)[b]	No. Dissected	F.S.[c]				No. Degenerated[d]	Mean F.L.±S.E. (μm)[e]
				I	II	III-IV	V		
2 to 8 July (15-18d)	Blood-fed	25 (8)	2	0	0	0	2	0	-
	Unfed	30 (43)	11	2	8	0	0	1	87±1.2
6 to 15 Aug. (6-7d)	Blood-fed	36 (31)	11	1	3	0	7	0	81±2.6
	Unfed	29 (10)	3	0	2	0	0	1	65±0.9

[a] Blood fed females were offered a blood meal 24 hours prior to transfer.
[b] Proportion of females surviving 60 days at 5°C, L:D 0:24.
[c] Number of females with follicles in each stage of development.
[d] Females with fewer than fifteen intact primary follicles were classified as having degenerated ovarioles. Follicles were not considered intact if the cellular membranes of the oocyte or trophocytes had ruptured. The primary follicle of an ovariole was assumed to have undergone resorption if there was a dilatation present on the pedicel.
[e] Mean primary follicle length of females which did not develop follicles beyond stage II.

Appendix IV: Autogeny in *Culiseta inornata*.

Table 1. Autogeny in *Culiseta inornata* under controlled conditions

Temperature (°C)	Photoperiod (L:D)	No. Dissected	% Autogeny[a]
16	18:6	84	4.8
	14:10	107	0.9
	12:12	95	0.0
19	18:6	76	3.9
	14:10	100	1.0
	12:12	88	0.0

[a] Sample sizes were too small for statistical comparison.

Table 2. Autogeny in *Culiseta inornata* under natural conditions.

Date of pupation	No. Dissected	% Autogeny[a]
29 June to 8 July	60	15.0
6 to 15 Aug.	45	6.7
14 to 21 Aug.	45	2.2
22 to 27 Aug.	28	0.0

[a] Sample sizes were too small for statistical comparison.

Appendix V: Relative light intensity under various field conditions.

Date	Time[a]	% Cloud Cover[b]	Light Intensity (lux × 10^3) Water Bath	Outdoor Pool	Adult Cage
23 June	15:30	<10	2.35	120.0	-
27 June	13:00	100	2.2	18.5	-
2 July	11:30	0	1.2	115.0	0.1

[a] Time of day when measurements were taken.
[b] Approximate proportion of visible sky covered by clouds.

Light intensity was measured using a photometer (Type LI-185, Lambda Instrument Corporation) and photocell (Type LI-210S, Lambda Instrument Corporation). Light intensity was also measured at five separate locations, in each of seven Percival® incubators used in the study. Overall mean light intensity (±s.e.) in the incubators was 2.0 ±0.9 × 10^3 lux.