AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECT OF PHOTOPERIOD AND TEMPERATURE ON

OVARIAN DEVELOPMENT AND FAT PRODUCTION IN *CULEX PEUS*

SPEISER (DIPTERA: CULICIDAE)

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Bruce F. Eldridge

The effect of photoperiod and temperature on ovarian follicle development and fat production was studied in a colonized population of *Culex peus* Speiser from Philomath, Oregon.

Females were subjected to simulated fall conditions of photoperiod and temperature. Under a combination of short photoperiod and low temperature, there were various effects on their physiological activities such as the retardation of follicular development, a reduction in the blood-feeding rate and the occurrence of hypertrophic fat. In the laboratory, conditions of a short day length photoperiod (8hL:16hD) and cool temperatures (15°C) to which females were subjected from the pupal stage to eight days after emergence influenced the development of follicles, and resulted in the ovaries remaining in a diapause condition. Under conditions of

16 hour photophases and 25°C, females showed an increase in follicle size over time. Females exhibited a marked reduction of blood-feeding activity in response to a combination of short photophases (8 hours) and cool temperatures (15°C). Blood-fed females held under simulated fall conditions developed a considerable amount of fat reserve while non-blood-fed females, maintained under the same conditions, and females taking a blood-meal at warmer temperatures had significantly less fat.

It was concluded that daylength is an important factor controlling the follicular development of females of *C. peus*. Pupae and adults were exposed to combinations of 12 photoperiods (photophases of 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5 and 15 hours) and a temperature of 18°C. Follicle size gradually increased as photophase was lengthened. At photophases between 9.5 and 12.5 hours the follicles remained small and the sharp increase was seen at photophases of 13 hours or more. Experimental study showed that less than 13 hours of light per day stimulated the entire population to enter ovarian diapause.

Field collections of larvae made in 1981 showed that adult activity decreased in September. With the retardation of follicle development, suppression of blood-feeding drive and formation of hypertrophic fat in response to simulated fall conditions, it was concluded

that the northern population of *C. peus* undergoes ovarian diapause each fall as inseminated adult females.

The Effect of Photoperiod and Temperature on Ovarian
Development and Fat Production in *Culex peus*
Speiser (Diptera: Culicidae)

by

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Finally, thank you to all friends for giving me merry and joy during these past years.
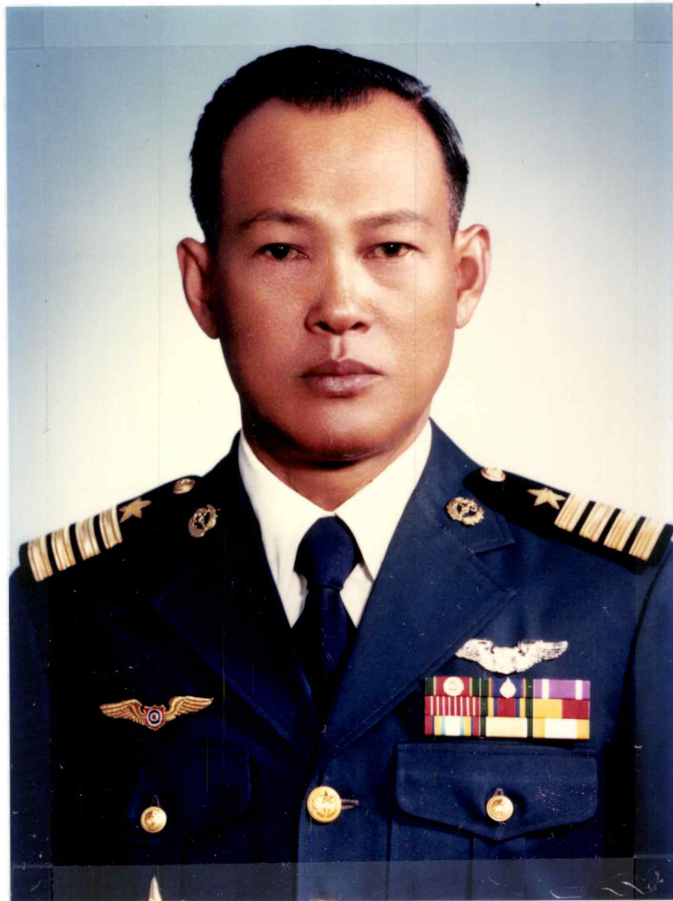
This thesis is dedicated to my father dearest, Group Captain Virach Skultab M.D., for giving me the inspiration, motivation, confidence and love that has kept me going to reach this goal.

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INTRODUCTION

Culex (Culex) peus Speiser is a common mosquito occurring in western parts of North, Central and South America. It ranges from Colombia and Venezuela north to the southwestern part of the state of Washington (Knight and Stone, 1977; Darsie and Ward, 1981). The status of the species as a pest and as a human and animal disease vector is largely unknown. However, considerable information is available concerning control of the species. The common name of *C. peus,* the banded foul-water mosquito, stems from its appearance and the fact that larvae are often found in relatively polluted water. *C. peus* shares the characteristic of a white-banded proboscis with *Culex tarsalis* Coquillett, but the two species can be distinguished from each other by examination of the ventral surface of the abdomen and the outer surface of the rear femora and tibiae.

The mosquitoes of the genus *Culex* are known to survive winters in cooler portions of the temperate zone as inactive adult females, assumed to be in a state of diapause. Diapausing mosquitoes normally show reduction in blood-feeding drive and cessation of ovarian

follicle development, and restrict their feeding to a carbohydrate diet, resulting in accumulation of body fat which will be depleted gradually as hibernation proceeds. These physiological alterations occur in several *Culex* species, which undergo a true diapause as reproductive adult females (Eldridge,1968; Sanburg and Larsen, 1973; Eldridge et al., 1976, 1979b). The role of environmental factors in triggering and terminating diapause in *Culex* mosquitoes is unclear. Several studies have been done to investigate the influence of photoperiod and temperature on ovarian development in *Culex* mosquitoes based on either behavioral or physiological aspects or both. Most results indicate that a combination of short daily photophases and cool temperatures induce ovarian diapause. However, this phenomenon is not evident in all species of *Culex* mosquitoes.

Among vector species, the fact that the adult female overwinters has raised the possibility of them serving as overwinter hosts of disease pathogens. The role of *Culex* mosquitoes as overwinter carriers of pathogenic viruses for man and animals has been discussed in several studies. Although hibernating mosquitoes have a lower rate of metabolism than active ones, an energy source is nevertheless needed for overwinter survival. They obtain this energy from fat stored in

the form of hypertrophied abdominal fat bodies. Ordin-
arily, blood-fed female mosquitoes do not develop fat
bodies and it has been assumed that cessation of blood-
feeding is necessary to hibernation. Furthermore, it
is the immature stages which are sensitive to short
photophases, and once the adult form emerges, the physi-
ological status of hibernation is established. Such
females would not serve as overwinter virus reservoirs,
since they would not take prehibernation blood-meals.
Gonotrophic dissociation (whereby a prehibernation
blood-meal results in fat production rather than egg
production) has been demonstrated to occur in the genus
Culex (Eldridge, 1966, 1968), and the possibility thus
exists that diapausing mosquitoes may function as a
reservoir for arboviruses throughout the winter. Such
female mosquitoes would become infected following a
blood-meal containing an arbovirus and undergo gono-
trophic dissociation. The energy derived from the
blood-meal would be used in fat production rather than
ovarian development. The isolation of St. Louis en-
cephalitis virus from overwintering *Culex pipiens* is
evidence of this possibility (Bailey et al., 1978).

Many studies have been done on species of great
importance, i.e. *C. tarsalis*, well-known as a primary
vector of viral diseases of man and animals (Hender-
son et al., 1979; Walters and Smith, 1980). The less

significant species, *C. peus,* was the choice of this study because of its possible role as a secondary vector of viral diseases and because of the scant knowledge of its winter biology. Existing information concerning *C. peus* is mostly about its systematics, distribution and control; not many details are available about its biology and almost nothing concerning its overwintering habits. Although little is known about the vector competence of *C. peus* for arboviruses, females of this species have been shown to be infected with western equine encephalitis (WEE) and St. Louis encephalitis (SLE) in nature. Thus this species is a potential overwinter host for these viruses. Very few collections of *C. peus* have been made during winter, but they are assumed to overwinter as adult females (Bohart and Washino, 1978).

Since so little is known about the winter biology of *C. peus,* I chose to study various aspects of its phenology in both field and laboratory populations. To determine when populations were active in nature, I studied collection records of larvae and adults made from log ponds near Corvallis. I also studied adult eclosion rates and blood-feeding activities in laboratory populations under different conditions of photoperiod and temperature. Retardation of development of the ovaries and body fat production are phenomena

associated with hibernation; therefore, I conducted experimental studies of ovarian follicle growth and fat formation under various combinations of photoperiod and temperature to determine if *C. peus* undergoes reproductive diapause and can survive through simulated winter conditions.

The specific objectives of these studies were:

(1) To observe the effect of photoperiod and temperature on ovarian development in *C. peus* and, specifically, to determine whether or not the combination of short photophase and cool temperature induces ovarian diapause in this species.

(2) To determine, if *C. peus* proves to be photoperiod sensitive, the photoperiod required to induce ovarian diapause at a selected temperature in non-blood-fed female mosquitoes.

(3) To determine the development of body fat in response to those combinations of light and temperature in both blood-fed and sugar-fed female mosquitoes in the laboratory.

I hope these studies will contribute to the eventual understanding of the bionomics of this species.

REVIEW OF LITERATURE

Systematics and Biology of *Culex peus*

The name *Culex peus* Speiser replaced the previous
Culex stigmatosoma Dyar (Stone, 1958). Eldridge (1979a)
proposed a guide to the pronunciation of the name *peus* as
'pe-us, in which the first syllable should be pronounced
nearly as the word "pay" and the following syllable as
"use."

C. peus ranges from northwestern United States to
Mexico, Central America and northern South America
(Freeborn and Bohart, 1951; Carpenter and La Casse, 1955).
In the United States (Figure 1), it occurs from the
southwestern half of Washington, the western part of
Oregon, throughout California except in the high Sierra
(Bohart and Washino, 1978), south through Arizona and
Texas, and extends east to Nevada and Oklahoma (Darsie
and Ward, 1981). It was found in Utah but in less abund-
ance (Dyar, 1922). No collection has been made in Ida-
ho (Darsie and Ward, 1981).

This species is a medium-sized brown mosquito
(Figure 2). The adult female is similar to *Culex tarsalis*
Coquillett sharing the same characteristic of the broad
median white band around the proboscis. These two
species can be distinguished from each other by the lack
of white scales in a line on the rear femora and tibiae
and the presence of a dark oval spot on each sternite
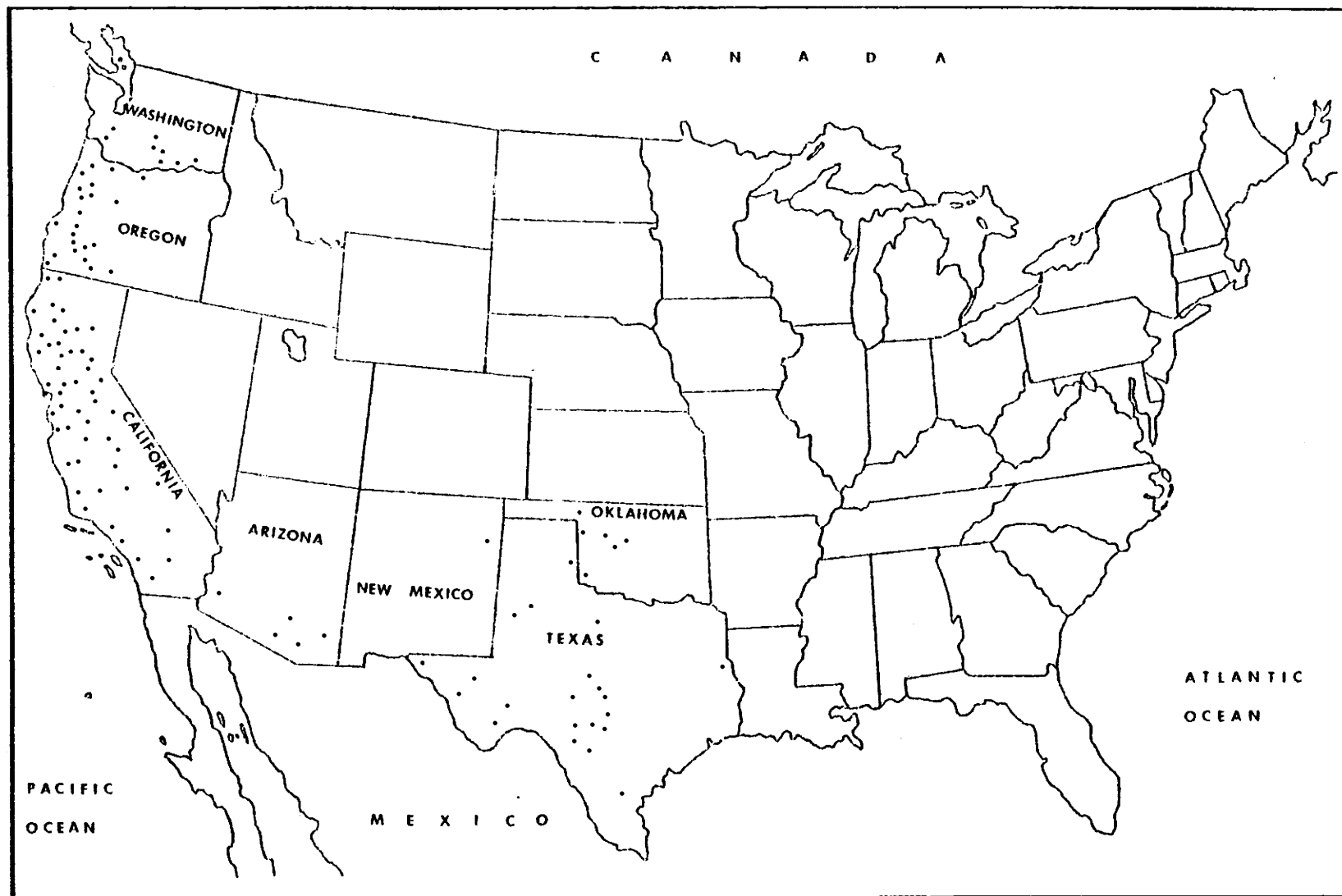
Figure 1. Distribution of *Culex peus* in North America, north of Mexico
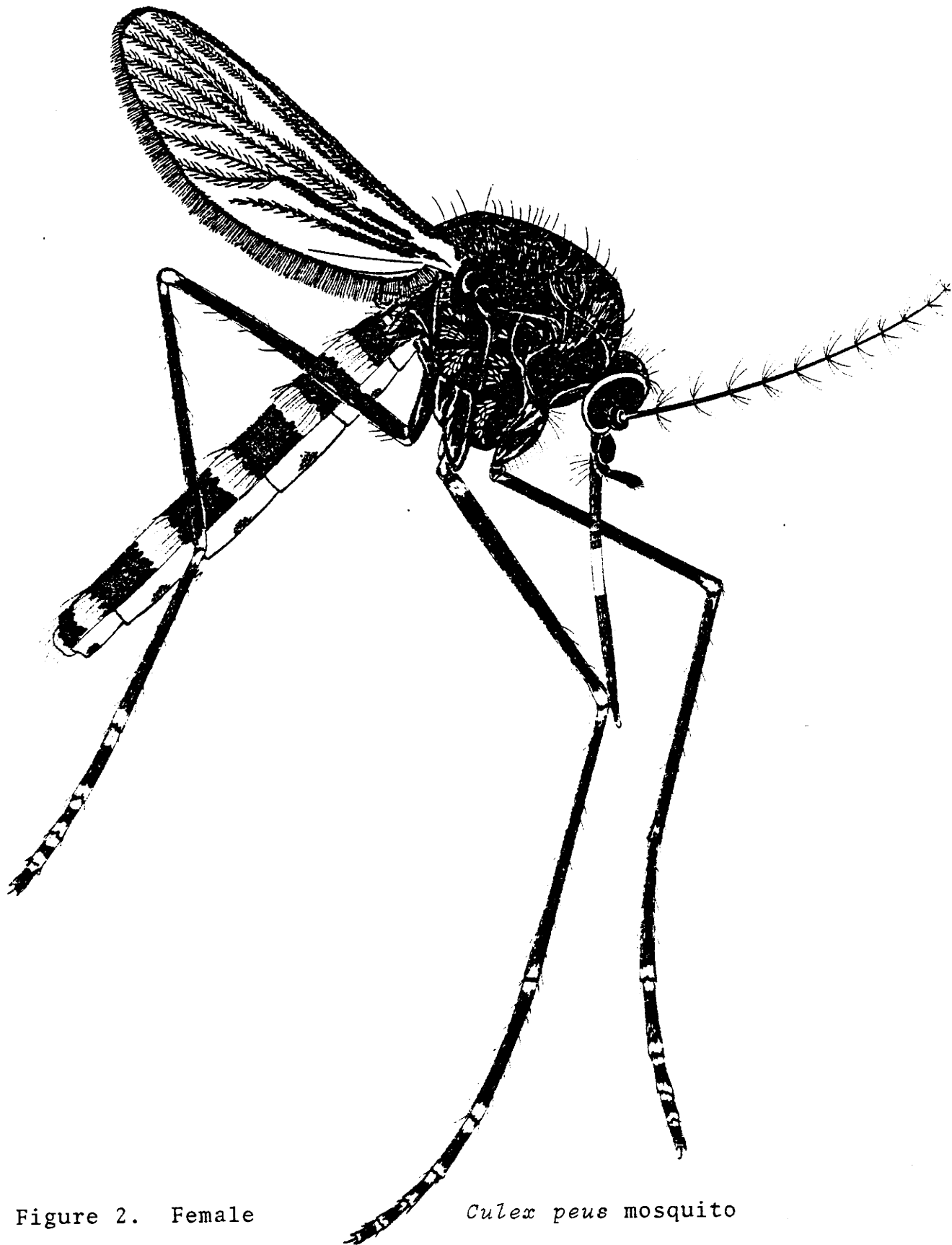
Figure 2. Female   *Culex peus* mosquito

of the abdomen in *C. peus*. The adult male terminalia
of *C. peus* is close in appearance to that of *Culex
thriambus* Dyar but some difference is found in the sub-
apical lobe. *C. peus* has a short slender hooked spine
which is absent in *C. thriambus*. The fourth stage
larva has the eighth segment as long as wide and the
comb scales on this segment are in a patch of 28 to 44
(Myers, 1964). The air tube has about 5 pairs of hair
tufts with the subapical ones smaller than the others
and slightly out of line. However, the position and
number of tufts on the air tube vary considerably
(Breland, 1957).

The larvae develop in large numbers in log ponds
and are generally found in natural permanent ponds,
oxidation ponds, stagnant, foul water at sewage plants,
street drains, polluted water on farms or around dairies
and can be found occasionally in rather clean water.
They also occur in artificial containers or ground pools
and were found in fountains, and water troughs for
horses (Dyar, 1922). Freeborn and Bohart (1951) stated
that this species is prominent in sewer farms in the
Sacramento Valley and that adults can be seen in numer-
ous swarms. The females rarely bite man under natural
conditions but can be induced to feed on chickens,
guinea pigs, mice, and even human beings in the labor-
atory (Carpenter and La Casse, 1955; Bohart and Washino,

1978). Although autogeny (egg development without prior blood-meal) was found to occur in *C. peus* (Washino and Shed-del, 1969), it exists at very low levels. Tempelis and Reeves (1964) reported that *C. peus* showed evidence of greatest feeding on birds. Most specimens collected in Oregon by Gjullin and Eddy (1972) were found to be very unwilling to feed on white mice, chickens and frogs. They commented that this reluctance was common-ly expressed in strains initially brought into the la-boratory.

Medical Importance and Control Techniques

Culex peus is not usually considered to be a seri-ous pest of man and domestic animals; and furthermore it is considered to be far less significant as a pri-mary vector of human viral disease agents because the females of this species rarely bite man in nature and because of its lower population density in many en-demic areas. However, there are some documents related to its involvement as a carrier of viral diseases. Western equine encephalitis (WEE) was isolated from wild-caught female *C. peus* in nature (Hammon et al., 1945; Stage et al., 1952). Ferguson (1954) reported that this species was able to harbor the viruses of St. Louis encephalitis (SLE). Reeves et al., (1954) also demonstrated that it can be easily infected with

avian plasmodia; besides, it has shown the capability of transmitting local strains of *Plasmodium relictum* to canaries (Rosen and Reeves, 1954).

Among the bloodsucking arthropods, mosquitoes are known to be the most conspicuous insects that disturb man, other mammals and birds. They trouble our normal living, particularly outside dwellings; and also are able to transmit serious pathogens of man and animals in many parts of the world. Although, *C. peus* is not considered to be a serious pest, many workers have conducted research on the effective control of this species. Numerous documents on its biological control are available; for example, using of planaria (Yu and Legner, 1976), *Notonecta unifasciata* (Hazelrigg, 1976, and the bacterial pathogen *Bacillus thuringiensis* H-14 (Mulla et al., 1980; Eldridge and Callicrate,1982). Moreover, some chemical insecticides and growth regulators have been tested as effective techniques against *C. peus* (Mulla and Darwazeh, 1975; Georghiou et al., 1975).

Overwintering in the Genus *Culex*

It is quite difficult to find an appropriate word to describe the overwintering of adult female mosquitoes. Mansingh (1971) proposed a physiological classification of dormancy in insects. He defined the word "hibernation" as a physiological condition of arrested growth or growth

retardation of insects due to temperature which is lower than the optimum. He also explained the word "diapause", which represents a sequence of evolutionary adaptations, as one system of dormancy to overcome extreme and long term conditions of seasonal climatic changes. Based on his definitions, these two words could be used to describe the overwintering of adult female mosquitoes but the word "diapause" would seem to have more specific meaning.

Extreme and severe conditions would normally require a specific stage for overwintering, generally a non-feeding or a resting stage, i.e. eggs, or adults in reproductive diapause (Danks, 1978). Mosquitoes undergo diapause as eggs, larvae or adult females, depending on the species. In Culicinae, overwintering in cold climates mostly occurs as diapausing eggs in *Aedes* mosquitoes, i.e. *Aedes sierrensis* (Ludlow)(Jordan, 1980), *Aedes triseriatus* (Shroyer and Craig, 1980); and as inseminated diapausing female adults in *Culex* mosquitoes, i.e. *Culex pipiens* complex (Jakob et al., 1980), *Culex restuans* Theobald (Madder, 1981), *Culex salinarius* Coquillett (Slaff and Crans, 1981), *Culex tarsalis* Coquillett (Arntfield et al., 1982), *Culex territans* Walker (Hudson, 1978). The diapausing females prepare for hibernation by reducing blood-feeding, undergoing fat body hypertrophy and inactivity of the reproductive system (Harwood and James, 1979; Wang, 1979; Arntfield et al., 1982). However, not all

Culex mosquitoes hibernate as adult females. *Culex
erythrothorax* Dyar was reported to overwinter as larvae
in Nevada (Chapman, 1959). Besides, Eldridge (1968)
concluded in his study that *Culex quinquefasciatus* Say,
the southern house mosquito, does not hibernate. Al-
though they showed fat development on a sugar diet, in
experimental hibernation studies, they died with con-
siderable fat remaining. Even though this species has
not been shown to hibernate, Jakob and his colleagues
(1980) reported that small proportion of the overwinter-
ing *C. pipiens* complex populations collected in Memphis,
Tennessee were *quinquefasciatus*-like. *C. quinquefascia-
tus*, however, was considered to overwinter in a gono-
active stage in Pakistan (Suleman and Reisen, 1979).
The differences in geographical area and local climate
seem to have some effects on the ability of this species
to overwinter. In species having broad geographic
ranges (i.e. *C. tarsalis*), it is probable that the over-
wintering mechanism differs among populations depending
upon the severity of the climate involved (Eldridge,
1981).

Environmental Factors Affecting Ovarian Diapause

Diapause in which the diapausing female mosquito
fails to enlarge the reproductive organs is referred
to as reproductive diapause (Beck, 1980). The term

"ovarian diapause" is widely used by many workers to
describe the reproductive cessation or arrested growth
of ovaries in non-blood-fed females (Eldridge and Bailey,
1979; Spielman and Wong, 1973b). Not all species of
Culex mosquitoes express ovarian diapause; but those
which have been known to overwinter as diapausing female
adults generally exhibit this phenomenon (Eldridge et al.,
1976, 1979b; Harwood and Halfhill, 1964; Madder, 1981;
Spielman and Wong, 1973b; Wang, 1979). It has been shown
that photoperiod is the major environmental factor in-
fluencing the onset of and emergence from diapause in
most insect species, particularly short photophases or
long-scotophases (Beck, 1980; Saunders, 1976). Spiel-
man and Wong (1973a) stated that the ability to enter
ovarian diapause in mosquitoes is genetically determined
as it is present only in anautogenous populations. Eld-
ridge and his coworkers (1976) studied *Culex salinarius*
Coquillett and reported that the mid-Atlantic populations
of this species do not undergo ovarian diapause in re-
sponse to the simulated conditions of autumn photo-
period. The cool temperature that affected ovarian
development was considered to retard rather than to ar-
rest the follicle growth. Their report was supported
by a recent study on the activity and physiological
status of this species. Slaff and Crans (1981) moni-
tored pre- and post-hibernating populations of *C.*

salinarius in New Jersey and found that they remained
active and looking for hosts throughout the autumn.
Thus it seems certain that at least one species of
temperate zone *Culex* does not exhibit ovarian diapause
in response to short daylengths.

The diapause inducing stimulus of photoperiod alone,
or in combination with temperature, has been studied in
several species of *Culex* mosquitoes (Eldridge et al.,
1976; Madder, 1981; Spielman and Wong, 1973b; Wang,
1979). Short daily photophases and cooling temperature
of late summer and early fall are the environmental cues
for female mosquitoes to prepare themselves for hiber-
nation. The effects of these two factors in influenc-
ing female adult mosquitoes to enter diapause have been
observed to manifest themselves by the reduction of
blood-feeding (Arntfield et al., 1982), cessation of
ovarian follicle growth (Madder, 1981) and the develop-
ment of body fat (Wang, 1979).

Spielman and Wong (1973b) reported that photoperiods
of less than 12 hours of light stimulated *Culex pipiens*
L. populations to enter ovarian diapause and the ovaries
of diapausing females would resume development after
exposure to 16 hours of light per day. They also stated
that higher temperatures reduce the trend to enter dia-
pause in this species. A recent experimental study of
C. pipiens L. by Eldridge and Bailey (1979) confirmed

that under conditions of short daily photophase (9 hours
per day) and cool temperature (15°C.), the ovaries of
the tested females were in diapause. The follicles
gradually increased in size when transfered from 15°C.
to 25°C. *C. restuans* is another species which under-
goes a true diapause in response to short photoperiod
and low temperature. They showed a marked reduction of
blood-feeding under eight hours of light per day at
15°C. (Eldridge et al., 1972, 1976). Madder's experi-
mental study (1981) reported that percentage of dia-
pausing female *C. restuans* increased as the daily
photophase decreased. Wang (1979) observed the in-
fluence of photoperiod on diapause of *Culex pipiens
pallens* Coquillett. He found that short daylengths
of 13.5 hours of light per day at 20-22°C. caused ces-
sation of growth and reproduction, and thus induced
hibernation of newly emerged adults.

Physiological Characteristics of Diapause

Mosquitoes have been considered in diapause when
they express altered physiological characteristics
under endocrine control in response to environmental
adversity (Eldridge et al., 1972). The action of photo-
period on insect behavior and development is thought to
be on the neurosecretory activity of the brain (Adkisson,

1966; Mansingh, 1971; Beck, 1980). When exposed to appropriate photoperiods, the neurosecretory cells do not release the brain hormone, growth and development are arrested and, thus, diapause occurs (Williams and Adkisson, 1964).

There are two stages to ovarian development: previtellogenic and vitellogenic. The previtellogenic stage, which extends from the "preresting" to the "resting" stage, has no behavioral component. The vitellogenic stage, which extends from the "resting" stage to full development of eggs will not proceed in non-blood-fed females (except in autogenous strains), and thus has a behavioral component. It is the previtellogenic stage which is suspended in diapausing females.

Follicles of non-diapausing non-blood-fed females were found to be in the resting stage (I-II of Kawai, 1969) and the follicle length was about 75µ or more while those of diapausing female were in the pre-resting stage (No-2 of Kawai, 1969) and the follicle size was about 50µ (Spielman and Wong, 1973a,b; Eldridge and Bailey, 1979).

The corpora allata of mosquitoes are connected to the brain by axonal pathways (Burgess and Rempel, 1966; Larsen and Broadbent, 1968), and the action of the brain hormone is believed to mediate through the corpora allata (Larsen and Bodenstein, 1959; Gillett, 1971).

Gwadz and Spielman (1973) suggested that development of ovarian follicles from early stage to the pre-vitello-genesis stage is controlled by juvenile hormone which is secreted from the corpora allata. Thus, suppression of corpus allatum function in newly emerged female *C. pipiens*, resulting from seasonal changes in photoperiod and temperature in fall, induces diapause. However, if female mosquitoes which had been held under diapause conditions were fed or topically treated with synthetic juvenile hormone, the ovarian follicles resumed development and diapause was disrupted (Spielman, 1974). Meola and Petralia (1980) also reported the role of natural or synthetic juvenile hormone in influencing the ovarian follicle size and in inducing biting behavior in females pre-conditioned for diapause. Under non-diapause conditions, this hormone initiates pre-vitellogenic follicular development (from pre-resting to resting stage, No-2 to I-II, of Kawai, 1969)(Meola and Petralia, 1980). The follicles will develop further to fully developed eggs, following a blood-meal, by the influence of a second brain hormone, egg development neurosecretory hormone (EDNH), which is stored in the corpus cardiacum (Lea, 1972).

Mosquitoes require accumulation of extensive nutritional reserves to sustain them throughout the hibernation. This phenomenon is a conspicuous physiological

characteristic and has been demonstrated for species of overwintering mosquitoes in California (Shaffer and Washino, 1974). Before overwintering, adult female mosquitoes require carbohydrates or both carbohydrate and a blood-meal which will be depleted during hibernation (Teckle, 1960). Gonotrophic dissociation is a phenomenon in which prehibernation blood-meals are turned into large amounts of fat reserve instead of being utilized for the development of eggs. It is known to occur in *Culex* mosquitoes, i.e. *Culex pipiens pipiens* L. (Eldridge and Bailey, 1979), *C. restuans* (Eldridge, Johnson and Bailey, 1976), and *C. tarsalis* (Arntfield et al., 1982).

Male longevity or survival is dependent only on nectar as they lack functional mouthparts to pierce and suck blood. Male mosquitoes do not overwinter. However, females are known to feed on flower nectar also (Harwood and James, 1979; Magnarelli, 1979; Patterson et al., 1969). Tate and Vincent (1936) observed that hibernating *C. pipiens* females did not require a blood-meal for the formation of fat. Reeves et al., (1958) also found that most overwintering *C. tarsalis* females had no trace of blood in their guts and, yet, no sign of ovarian development. They commented that those mosquitoes may have obtained only nectar as their energy source. *C. restuans* could develop fat bodies without

taking a blood-meal (Wallis, 1959). In a recent study on *C. pipiens pallens*, Wang (1979) found that they consumed sugar-water and developed a remarkable amount of fat. Francy et al., (1981) also indicated that overwintering females which have ingested a carbohydrate meal rather than a blood meal develop fat reserves and seem to be better prepared for hibernation survival.

The effect of photoperiod and temperature upon the degree of fat body develpment has been observed. Shelton (1973) showed that the lower the temperature, the greater was the amount of fat and body weight. *C. restuans* developed fat in response to a combination of short photophase (eight hours) and cool temperatures (15 °C and 20°C)(Eldridge et al., 1972, 1976).

Hibernacula of *Culex* Mosquitoes

There are extensive reports in the literature of collections of some species of *Culex* mosquitoes during winter. *Culex tarsalis* (Keener, 1952; Rush et al., 1958; Rush, 1962), *Culex pipiens* (Buxton, 1935) have been collected repeatedly. Very few collections of overwintering females of other species have been made. I was unable to find any references to overwinter collections of *Culex peus*.

MATERIALS AND METHODS

Mosquito Colony

Culex peus utilized in these experiments were colonized from larvae collected from log ponds in Philomath, Oregon.

In the laboratory, stock colony adults were held in a screened cage (60cm. x 60cm. x 60cm.) under a 16hL:8hD photoperiod provided by fluorescent and incandescent lamps, controlled by an electronic timer, at a temperature of approximately 20°C. The timer provided a dawn and dusk period of about one hour each. Blood was offered periodically by placing a shaved baby chick in the adult mosquito cage. Subsequently, Japanese quail were used instead of the chickens because of ease of handling the smaller quail. Both chickens and quail were obtained from the Department of Poultry Science. Sugar-water was available to adults at all times as a source of energy by placing a wad of cotton soaked with 10% sucrose solution in the cage. Later, absorptive cotton rolls, 15 cm. long, in a flask filled with 10% sucrose solution were found to be much more convenient. A bowl of fresh tap water was also provided in the cage for an oviposition site.

Each egg raft obtained from the stock colony was placed in a round glass bowl (16 cm. diameter and 6 cm.

high) filled with one liter of tap water. The bowl was
covered with a square glass plate, 30 cm. x 30 cm. The
eggs hatched within two days after placing in the bowls.
The larvae were fed daily with TRY diet (Tetramin[R]-Pur
ina Rat Chow[R]- brewer's yeast in the proportion of 4:4:1
by weight and blended to a fine powder in a household
blender). The food was administered at the rate of
approximately 0.1 mg. per egg raft per day. The larvae
were maintained under 16 hours of light per day and at
25°C until they pupated. The average time to pupation
was 7.5 days.

Experimental Treatments

Four low temperature incubators, "Freas Model
815" manufactured by GCA Corporation, were used for all
experiments to produce various combinations of temper-
ature and photoperiod. Each incubator contained a
Westinghouse 15-Watt, 125-Volt incandescent lamp placed
60 cm. above the experimental samples as the illumin-
ation source. To maintain constant temperature, a
wire-wound resistor provided heating equivalent to the
lamps when the latter were not on. A pan of fresh water
was placed on the incubator floor providing a relative
humidity of about 60%, to avoid dessication of the
mosquitoes (Figure 3). Photoperiods were controlled
by a 24-hour cycle industrial time switch.

The experiments were divided into two sections. The first section concerned the effect of photoperiod and temperature on ovarian development. The second one dealt with production of fat under various combinations of light and temperature. Experimental conditions employed in these experiments were briefly summarized as follows:

Section I

    Experiment Ia 15°C and 25°C under 8hL:16hD and
                16hL:8hD

    Experiment Ib 18°C under 9.5, 10, 10.5, 11, 11.5,
                12, 12.5, 13, 13.5, 14, 14.5 and
                15 hour photophase

    Experiment Ic 15°C and 25°C under 8hL:16hD and
                16hL:8hD

Section II

    Experiment II 15°C and 25°C under 8hL:16hD and
                16hL:8hD

The temperatures, 15°C and 25°C, were chosen in most experiments of this study because they are sublimital and supralimital temperature, respectively, for phenomenon under study of *Culex* mosquitoes such as *Culex pipiens* (Eldridge and Bailey, 1979). The experimental treatments were designed to simulate natural conditions of daylength and temperature in late summer and early fall. Therefore, duration of 8 hours of light per day was selected and using 16 hour photophase for comparison. In experiment Ib, 18°C was employed to simulate natural late summer temperature.
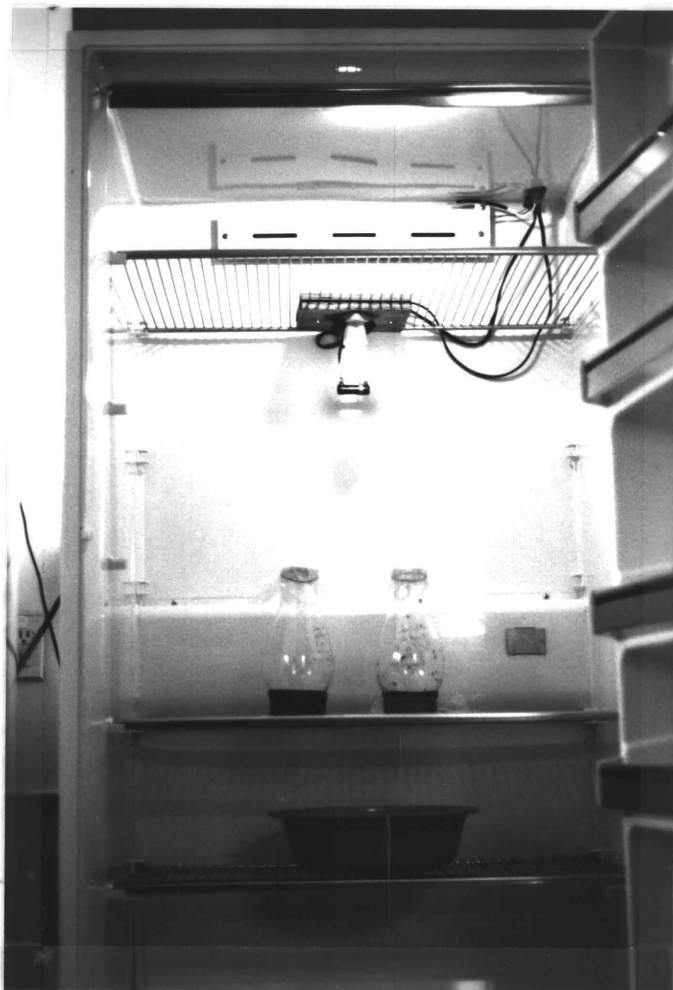
Figure 3. Inside a programmed incubator showing an
           illumination source, a wire-wound resistor,
           a pan of fresh water and two experimental
           globes
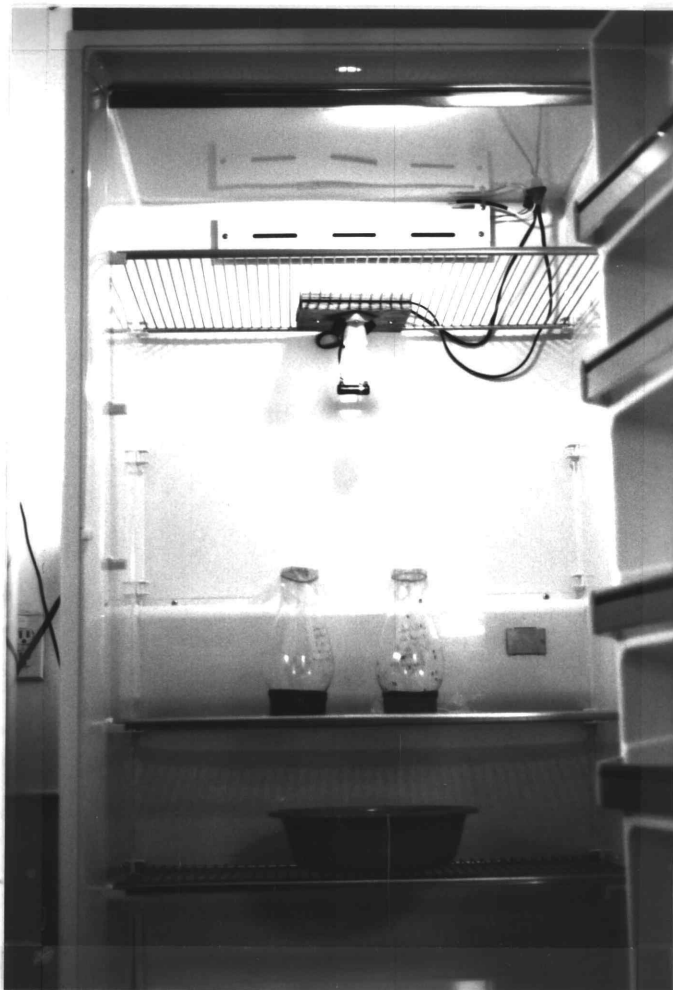
Figure 3.   Inside a programmed incubator showing an
            illumination source, a wire-wound resistor,
            a pan of fresh water and two experimental
            globes

Fat Extraction

Individual mosquitoes were dried in an oven at 40°C
and were weighed repeatedly on a Cahn Electrobalance[R]
until their weight was constant. To determine the amount
of fat contained in individual mosquitoes, a soxhlet ex-
traction apparatus was used. It consisted of a fat ex-
traction flask filled with petroleum ether; a soxhlet
extractor containing 10 marked thimbles, each of which
contained a single pre-dried mosquito sample and was
plugged with a small cotton ball; and a condenser.
Petroleum ether vaporized through the side arm of the
extractor, condensed and dripped down into the extract-
or. The solvent, now containing soluble fat from the
sample mosquitoes, drained automatically through the
siphon arm and was reused. After a four hour-extrac-
tion period, the mosquito samples were re-dried and
re-weighed. The difference in weight between before
and after extraction was considered to represent the
extracted lipids. Both blood-fed and non-blood-fed
mosquitoes were extracted using the same procedure.

Experimental Procedures

All experiments were started at the time of pupa-
tion by randomly transferring 50 pupae from larval rear-
ing bowls to a small container which was then placed

under a screened-top lantern globe. A cotton ball soaked
with 10% sucrose solution was placed on the top provid-
ing sugar diet for the emerging adult mosquitoes. The
globes were subjected to various experimental treat-
ments within programmed incubators.

For the experiments designed to determine follicu-
lar development and fat production after blood-feeding,
a blood-meal was offered overnight on the eighth day
after the peak of the emergence of adults. Because
pupae were divided and maintained at different temper-
atures, time of adult ecdysis varied. Therefore, blood-
feeding trials were not conducted simultaneously for
all treatments. Treatments at 25°C were offered the
blood-meals eleven days post-pupation. Females held
at 15°C under long photoperiod were offered a blood-
meal twelve days post-pupation and a day later under
short photophase conditions. The mosquitoes were held
at 20°C during blood-feeding trials for all treatments,
but photoperiods were the same as those provided dur-
ing their pre-feeding conditions. The next morning,
all blood-fed females were segregated from non-blood-
fed females and were returned to their pre-feeding
treatment temperatures. After the blood-meal was com-
pletely digested, which was about eight days after blood-
feeding trials, the mosquitoes were dissected for fol-
licular measurement and were extracted with petroleum

ether to determine the amount of fat formation in both blood-fed and non-blood-fed females.

Examination of Follicles

Female mosquitoes were removed from each treatment by an aspirator and were immobilized by placing them into a household freezer for a few minutes. Each female was then placed on a clean microscope slide and the ovaries were dissected in a drop of saline solution under a stereoscopic microscope at 40X magnification. After teasing ovaries apart with dissecting needles and covering them with a cover slip, the slide was transferred to a compound microscope. The follicles were measured at 40X magnification by mean of a squared reticle, a scale unit of which was 0.0024 mm., contained in the eyepiece. Five follicles from each ovary were selected at random for measurement of length of follicle and its germarium.

Females were considered in diapause if they were found to have a follicle: germarium length ratio of no more than 1.5:1.0. This ratio has been chosen to separate diapausing from non-diapausing females and is based on the value suggested by Spielman and Wong (1973b). The developmental stages of follicles were classified as follows using the scheme of Kawai (1969):

Stage

No-1   The follicle of newly emerged females is included within the germarium.

No-2   The first follicle becomes distinguishable from the germarium by the constriction.

N   Eight undifferentiated cells and a completed epithelium are present.

Ia   One oocyte and seven nurse cells are differentiated, but the nucleus of the oocyte is not clearly visible.

Ib   The nucleus of the oocyte becomes visible.

I-II   A few yolk granules appear around the nucleus of the oocyte.

IIa   The yolk granules are slightly deposited around the nucleus of the oocyte.

IIb   The oocyte occupies 1/4 to 1/2 of the follicle. The nucleus of the oocyte is hardly visible because the yolk granules are densely deposited all over the oocyte.

IIIa   The oocyte occupies 1/2 to 3/4 of the follicle.

IIIb   The oocyte occupies 3/4 to 4/5 of the follicle.

IV   The oocyte occupies nearly all parts of the follicle. The nurse cells are pushing upwards. The follicle becomes long and oval.

Va   The follicle reaches its maximum size and be-
gins to produce a micropilar apparatus and a
chorion.

Vb   The follicle becomes a full grown egg with
a completed micropilar apparatus and a
chorion.

RESULTS

Effect of Photoperiod and Temperature on Ovarian Development

The approximate relationship between stage of development, follicle size and germarium : follicle length ratio for *Culex peus* is shown in Table 1.

Experiment Ia

Eldridge and Bailey (1979) stated that ovarian diapause is characterized by the presence of pre-resting stages at seven days post-adult-emergence. To observe if ovarian diapause so defined is evident in *Culex peus*, a preliminary experiment was conducted. Four combinations of photoperiod and temperature, 8hL : 16hD and 16hL : 8hD; 15°C and 25°C, were programmed for this experiment. Beginning with the pupal stage, first-day pupae were randomly divided into four groups and were placed into the programmed incubators. After adults emerged, females were held at the same experimental conditions as the pupae and were provided only 10% sucrose solution. After eight days at these conditions, they were removed, immobilized in a freezer, and dissected in physiological saline for measurement of ovarian follicle length. Ten females were removed from each treatment. Both ovaries of each female were examined and five follicles of each ovary were classified to

TABLE 1. Approximate relationship between stage of development, follicle size and germarium: follicle ratio in *Culex peus*[1]

Stage (Kawai 1969)	Mean Size (mm.)	Germarium:Follicle ratio
No-1		
No-2	0.034 (0.026 - 0.038)[2]	1 : 1.1
N	0.060 (0.053 - 0.072)	1 : 1.4
Ia	0.083 (0.077 - 0.086)	1 : 1.8
Ib	0.098 (0.089 - 0.106)	1 : 1.8
I-II	0.107 (0.098 - 0.115)	1 : 2.0
IIa	0.107 (0.098 - 0.130)	1 : 2.2
IIb	0.117 (0.106 - 0.204)	1 : 2.4
IIIa	-	-
IIIb	-	-
IV	-	-
Va	0.520 (0.469 - 0.611)	-
Vb	0.560 (0.523 - 0.585)	-

[1]Reared under 16hL:8hD at 25°C

[2]Range

stage and measured. A mean value of follicle length
for each female was recorded.

The results are shown in Table 2 and 3. The
short-photophase females held under warm temperature
(25°C) had ovaries with a mean follicle length of
0.079 mm., while females from the long-photophase group
maintained at the same temperature had ovaries with
follicles which had developed to a mean of 0.102 mm.
At cooler temperature (15°C), the mean follicle lengths
of the short-photophase and the long-photophase females
were 0.033 and 0.046 mm., respectively. The Christo-
pher's stages were No-2 to N for cool-temperature groups,
representing pre-resting stages, and I-II to IIa for the
warm-temperature groups which indicated resting stages.
Temperature differences were significant at both photo-
periods (t=11.18**& 15.01**, 18 & 18 df) and photoperiod
differences were significant at both temperatures (t=
4.47** & 6.01**, 18 & 18 df).

In order to study follicle growth over time, an ex-
periment was conducted to determine the rate of ovarian
development of non-blood-fed female *C. peus* held under
various conditions for a period of several weeks. First-
day pupae were randomly divided into four groups and were
subjected to the following four conditions: 25°C/16:8LD,
25°C/8:16LD, 15°C/16:8LD and 15°C/8:16LD. Adult eclosion
rates were also observed under these conditions. The
result of the adult eclosion rates are shown in Table 4 and

TABLE 2. Ovarian follicle measurements of non-blood-fed
         female *Culex peus* maintained under four differ-
         ent combinations of photoperiod and temperature

Treatment Conditions	Sample Size	Range (mm.)	Length of Follicle* (mm.)
25°C and:			
16 Hours Photoperiod	10	0.087-0.112	0.102 ± 0.026
8 Hours Photoperiod	10	0.059-0.099	0.079 ± 0.036
15°C and:			
16 Hours Photoperiod	10	0.034-0.062	0.046 ± 0.029
8 Hours Photoperiod	10	0.028-0.038	0.033 ± 0.018

*Mean ± standard error

TABLE 3. Group comparison by t-test method of non-blood-
         fed female *Culex peus* held under different con-
         ditions of photoperiod and temperature

Variation Source	df	t
Temperatures at		
16 Hours Photoperiod	18	11.18**
8 Hours Photoperiod	18	15.01**
Photoperiods at		
25°C	18	4.47**
15°C	18	6.01**

**Indicates significance at 1% probability level.

Figure 4.

When incubated at 25°C, adults emerged within two days of pupation and 50% of adult eclosion occurred by day 2.5 post-pupation for both short and long photoperiods. At 15°C incubation, however, emergence was retarded under short photophase conditions. For long photophase conditions, 50% of eclosion occurred by day 3.5, whereas, under short photophase conditions it occurred a day later.

After adults emerged, females were held for three weeks at the same experimental conditions as the pupae and were provided only sugar-water. Females from each treatment were removed every fourth day, frozen, and later dissected for ovarian follicle measurement. Both ovaries of each female were examined and five follicles of each ovary were measured and classified to stage.

The results of the dissections of sugar-fed (without a blood-meal) females is shown in Table 5 and Figure 5, 6, 7, 8, and 9. Follicle development proceeded at about the same rate for 25°C/8:16LD and 15°C/16:8LD while it proceeded at a higher rate at 25°C under long photophase and more slowly at 15°C under short photophase conditions. By day 21 post-adult-emergence, ovarian follicles of females held under conditions of 16 hours photoperiod and 25°C developed rapidly to a mean of 0.117 mm. and to Christopher's stage IIb (of Kawai, 1969)

TABLE 4. Rate of adult eclosion for *Culex peus* under different conditions of photoperiod and temperature[*]

Treatment Conditions	Days Post-pupation							
	0	1	2	3	4	5	6	7
25°C and:								
16hL:8hD	0	0	10	74	16			
8hL:16hD	0	2	6	76	15			
15°C and:								
16hL:8hD	0	0	3	19	39	25	10	
8hL:16hD	0	0	0	2	18	55	16	8

[*] Sum of two determinations with 50 pupae per determination
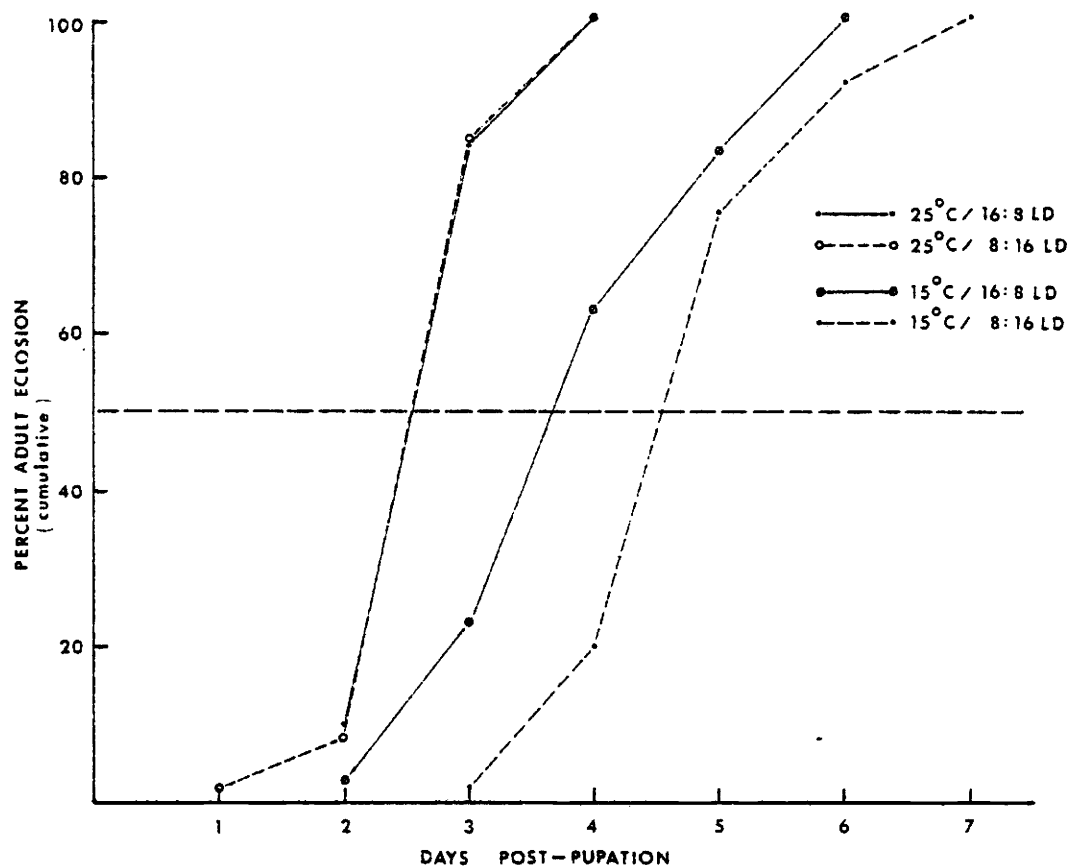
Figure 4. Rate of adult eclosion for *Culex peus* at indicated photoperiods and temperatures

TABLE 5. Effect of photoperiod and temperature on rate
of ovarian follicle development for *Culex peus*
at various time periods post-adult-emergence

| Treatment Conditions | Days Post-adult-emergence | | | | | |
	1	5	9	13	17	21
25°C and:						
16hL:8hD	0.034	0.079 (1.6)*	0.088 (1.8)	0.095 (2.0)	0.107 (2.2)	0.117 (2.3)
8hL:16hD	0.031	0.058 (1.3)	0.071 (1.8)	0.093 (1.9)	0.080 (1.9)	0.088 (1.9)
15°C and:						
16hL:8hD	0.030	0.045 (1.2)	0.066 (1.5)	0.088 (1.6)	0.098 (1.7)	0.094 (1.7)
8hL:16hD	0.027	0.037 (1.2)	0.047 (1.2)	0.049 (1.2)	0.053 (1.1)	0.055 (1.1)

*Ratio of follicle: germarium length
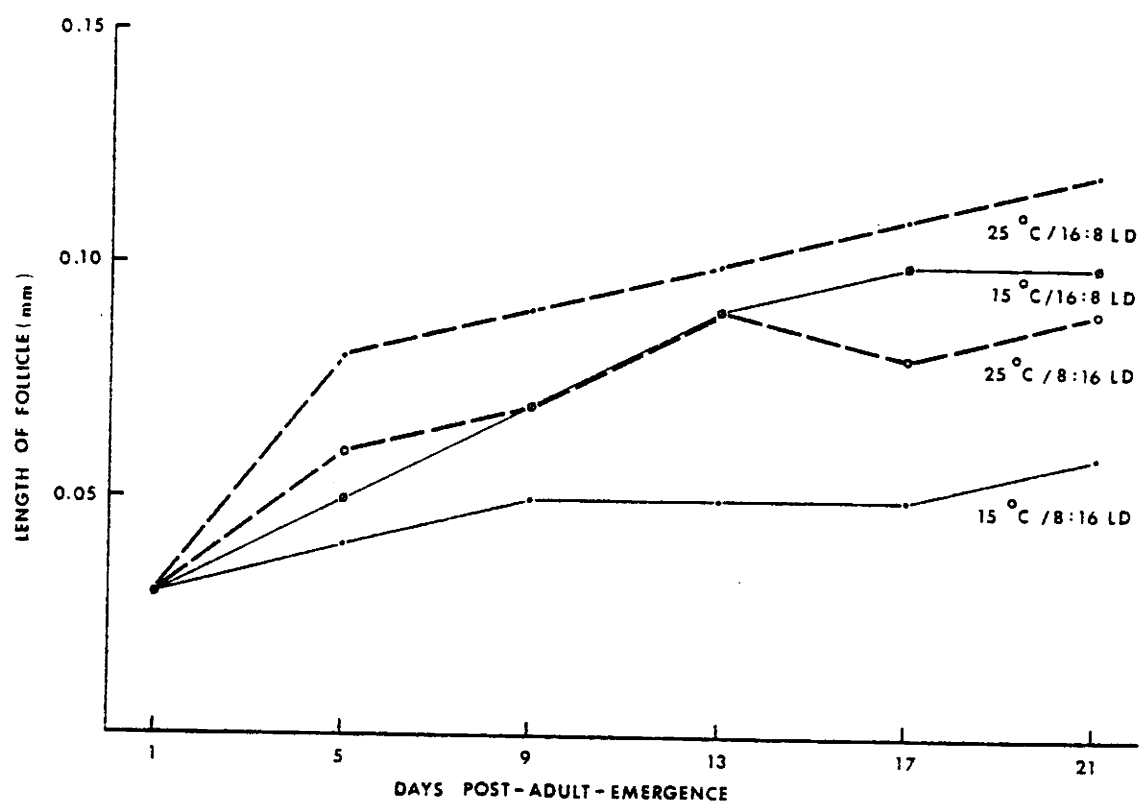
Figure 5. Rate of ovarian follicle development for
Culex peus at indicated photoperiods and
temperatures
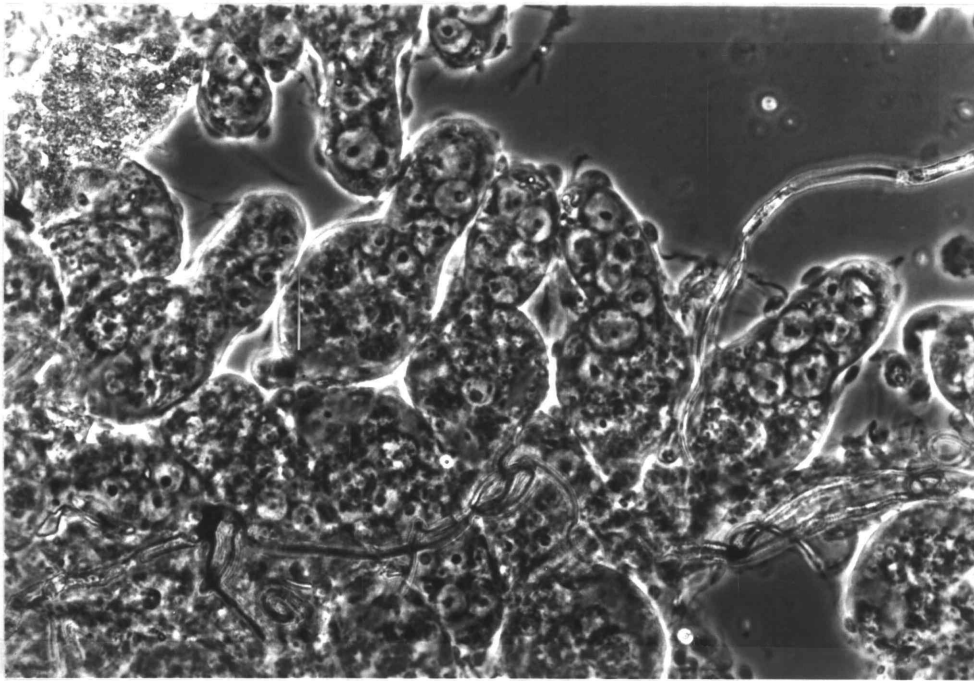
Figure 6. Follicles of *Culex peus* mosquito reared at
15°C under 8hL:16hD, 17 days post-adult-
emergence (at 40X magnification)
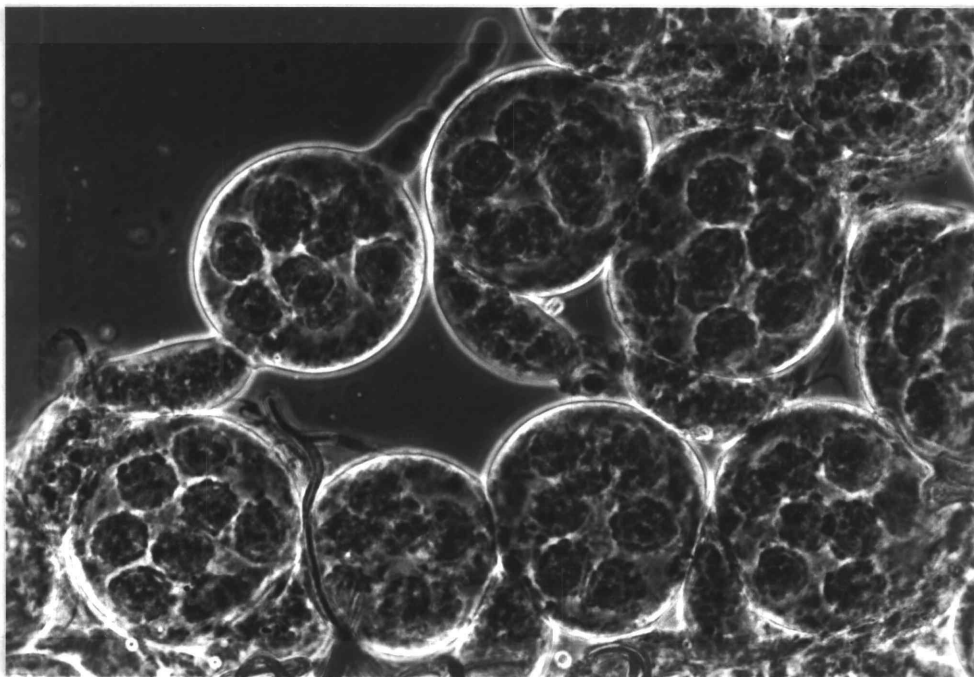
Figure 7. Follicles of *Culex peus* mosquito reared at
15°C under 16hL:8hD, 17 days post-adult-
emergence (at 40X magnification)
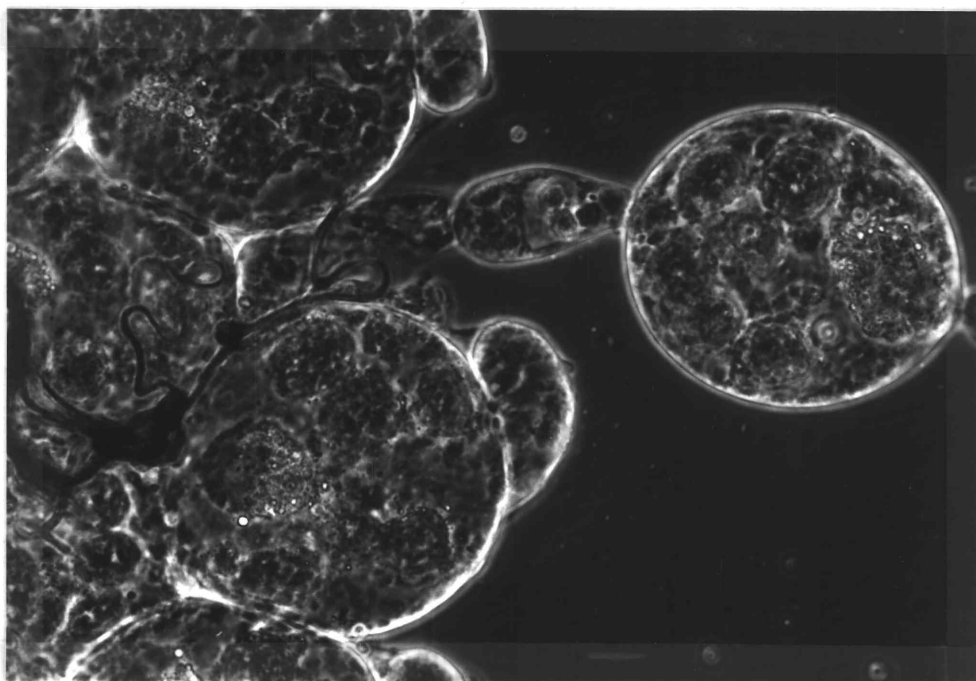
Figure 8.   Follicles of *Culex peus* mosquito reared at
             25°C under 8hL:16hD, 17 days post-adult-
             emergence (at 40X magnification)
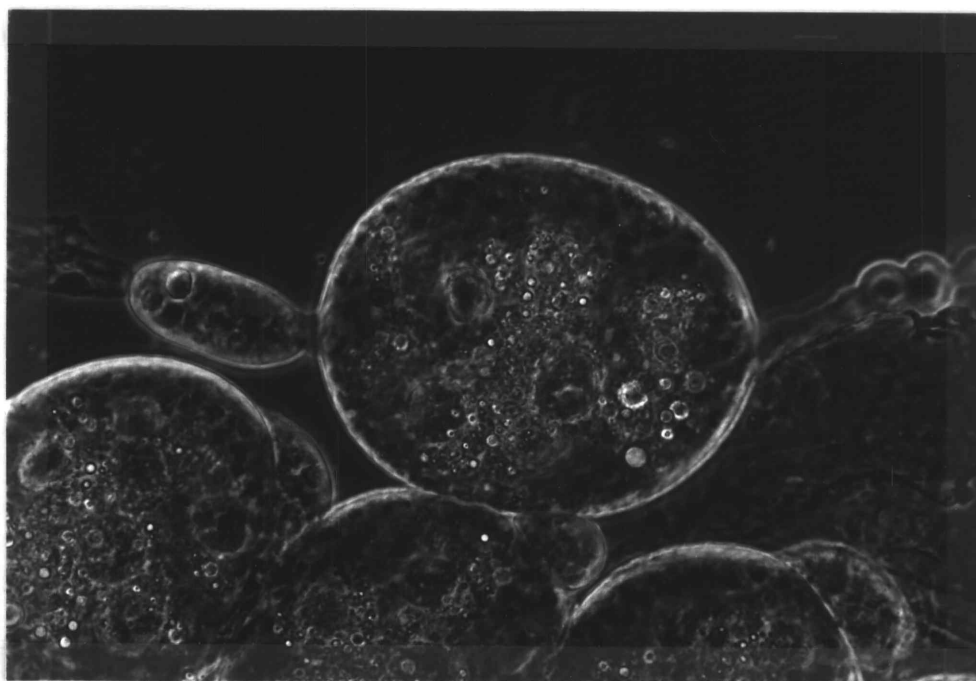
Figure 9.   Follicles of *Culex peus* mosquito reared at
             25°C under 16hL:8hD, 17 days post-adult-
             emergence (at 40X magnification)

while follicles of females maintained at 25°C and eight hours photoperiod developed to stage I-II with a mean follicle length of 0.088 mm. The ovarian follicle length was slightly longer under conditions of 15°C/16:8LD than under 25°C/8:16LD conditions, the average follicle length and Christopher's stage were 0.094 mm. and IIa, respectively. Under short photoperiod and cool temperature conditions, the follicles had developed slightly to a mean of 0.055 mm. but never exceeded stage N (pre-resting stage). The correlation between follicle size and Christopher's stage was quite high (r=0.91).

When expressed as follicle:germarium ratio, the -data agree closely with those expressed as follicle length (Table 5). The correlation between follicle size and follicle:germarium length ratio was 0.92.

Experiment Ib

An experiment was conducted to determine the duration of light required to induce ovarian diapause at 18°C (i.e. to see if a "critical" photophase could be determined). Pupae were subjected to 12 different photoperiods (9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, and 15 hours of light per day). Only four treatment combinations could be programmed simultaneously. Each treatment was tested twice. The second batches of pupae were placed into the incubators

two days after the first groups. Emerged adult females were provided only 10% sucrose solution. On the eighth day after the peak of the emergence of adults, females from each treatment were removed, frozen and dissected for measurement of length of ovarian follicle and associated germarium. The method of dissection was the same as in the previous experiment. This procedure was repeated until all 12 treatments had been done.

The result of the dissection is shown in Table 6. Follicle sizes increased with the increasing duration of light per day. Females exposed to 13 hour or greater photophases had ovaries with a mean follicle length of greater than 0.075 mm. and a follicle:germarium length ratio over 1.5:1.0. On the other hand, females subjected to less than 13 hour photophases had a mean follicle length of less than 0.075 mm. and ratio of follicle:germarium length not exceed 1.5:1.0. The range and mean values of ovarian follicle lengths and of ratios are shown graphically in Figure 10 and 11. Table 7 shows the percentages of female having diapause-stage ovaries under different photoperiod conditions at 18°C. Probit analysis performed on a microcomputer (Figure 12) indicated that 50% of the populations entered diapause when held under 13.051 hours of light per day (95% confidence limits were 13.432 and 12.687 respectively).

TABLE 6. Mean ratio of follicle : germarium length and mean ovarian follicle length of female *Culex peus* raised in laboratory at 18°C and 12 different photoperiods

Photoperiod (hL:hD)	Sample Size	Length of Follicle (mm.)	s.e.[1]	Ratio[2]	s.e.[1]
9.5:14.5	10	0.059 (0.052-0.091)[3]	0.034	1.13 (0.99-1.44)[3]	0.118
10:14	10	0.050 (0.046-0.056)	0.019	1.00 (0.92-1.12)	0.080
10:5:13.5	10	0.059 (0.049-0.072)	0.026	1.20 (1.01-1.38)	0.107
11:13	10	0.060 (0.050-0.076)	0.028	1.32 (1.18-1.53)	0.110
11:5:12.5	10	0.054 (0.048-0.077)	0.029	1.20 (1.11-1.50)	0.107
12:12	10	0.072 (0.053-0.089)	0.034	1.38 (1.16-1.66)	0.120
12.5:11.5	10	0.057 (0.042-0.065)	0.029	1.17 (0.94-1.30)	0.108
13:11	10	0.078 (0.059-0.093)	0.035	1.57 (1.16-1.81)	0.140
13.5:10.5	10	0.082 (0.062-0.116)	0.047	1.66 (1.36-2.14)	0.179
14:10	10	0.106 (0.088-0.131)	0.037	1.97 (1.64-2.72)	0.182

[1]Standard error, [2]Length of follicle : length of germarium, [3]Range

TABLE 6 - CONTINUED

Photoperiod (hL:hD)	Sample Size	Length of Follicle (mm.)	s.e.[1]	Ratio[2]	s.e.[1]
14.5:9.5	10	0.089 (0.065-0.134)[3]	0.047	1.86 (1.38-2.49)	0.186
15:9	10	0.088 (0.059-0.122)	0.047	1.82 (1.40-2.30)	0.170

[1]Standard error

[2]Length of follicle : length of germarium

[3]Range
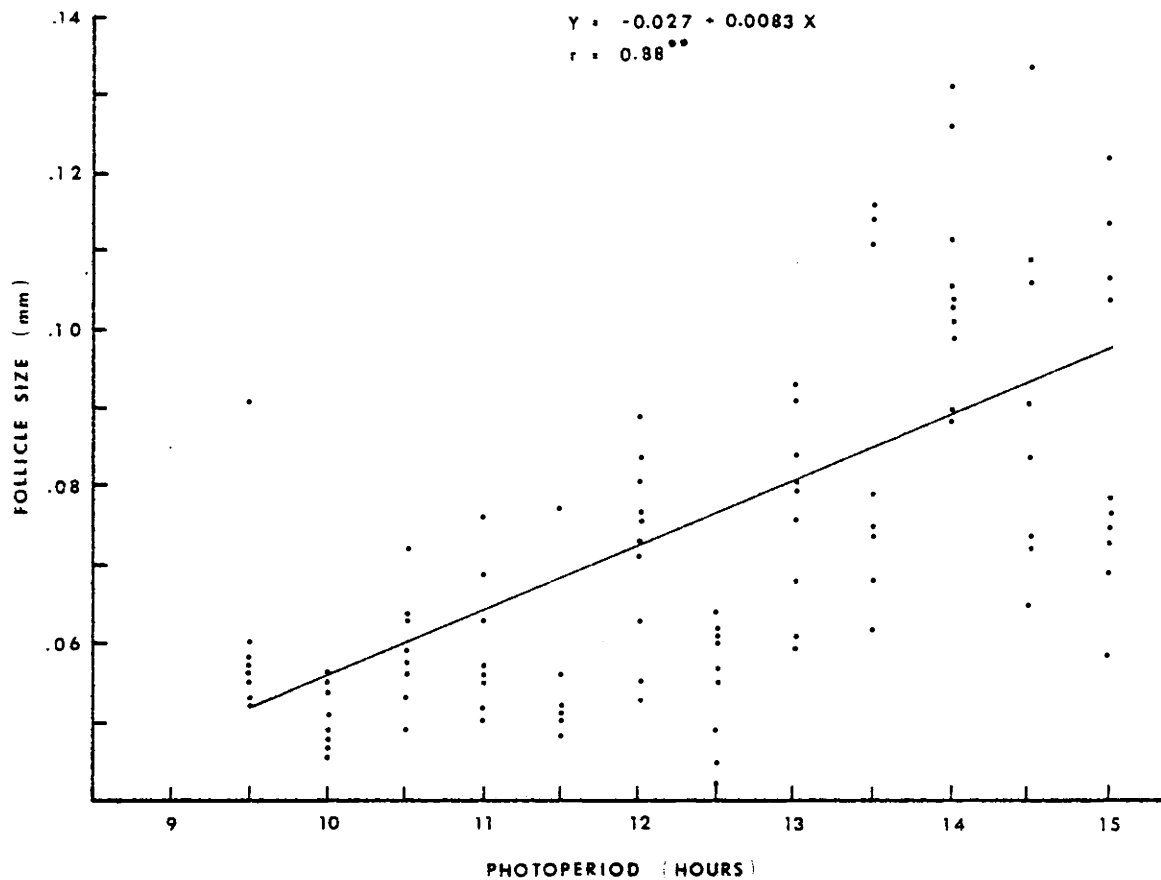
Figure 10.   Ovarian follicle length of *Culex peus*
maintained at 18°C and 12 different
photoperiods

$$Y = -0.56 + 0.164 X$$
$$r = 0.91^{**}$$
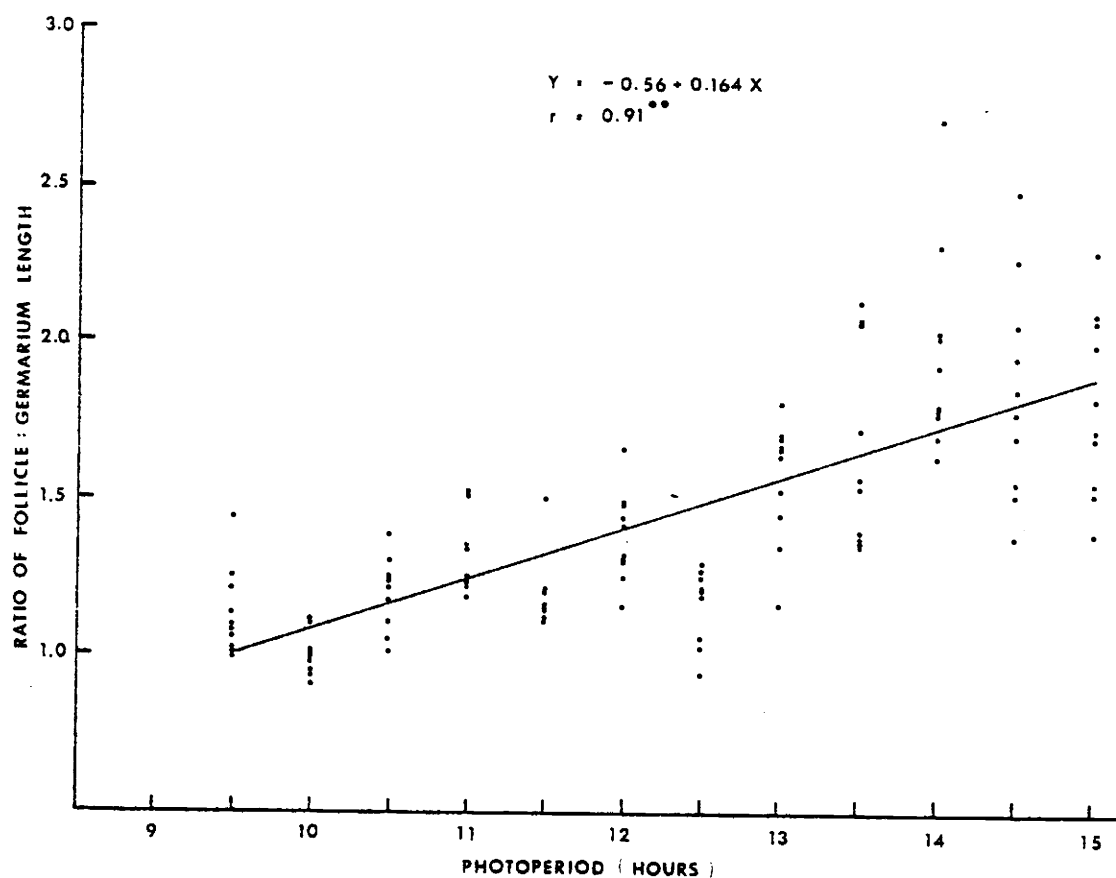
** Indicates significance at 1% probability level.

Figure 11.   Ratio of follicle : germarium length of
Culex peus maintained at 18°C and 12 dif-
ferent photoperiods

TABLE 7. Percentage of female *Culex peus* with diapause-stage primary ovarian follicles when maintained at 18°C and with 12 different hours of light per day

Duration of Light Per Day	No. Females	% Female with[1] Diapause-stage Follicles
9 h 30 min	10	100
10 h	10	100
10 h 30 min	10	100
11 h	10	80
11 h 30 min	10	100
12 h	10	100
12 h 30 min	10	100
13 h	10	30
13 h 30 min	10	40
14 h	10	0
14 h 30 min	10	10
15 h	10	10

[1]Determinations based on Spielman and Wong (1973b); the follicle was determined in diapause if follicle length: its germarium length ratio was 1.5 or less
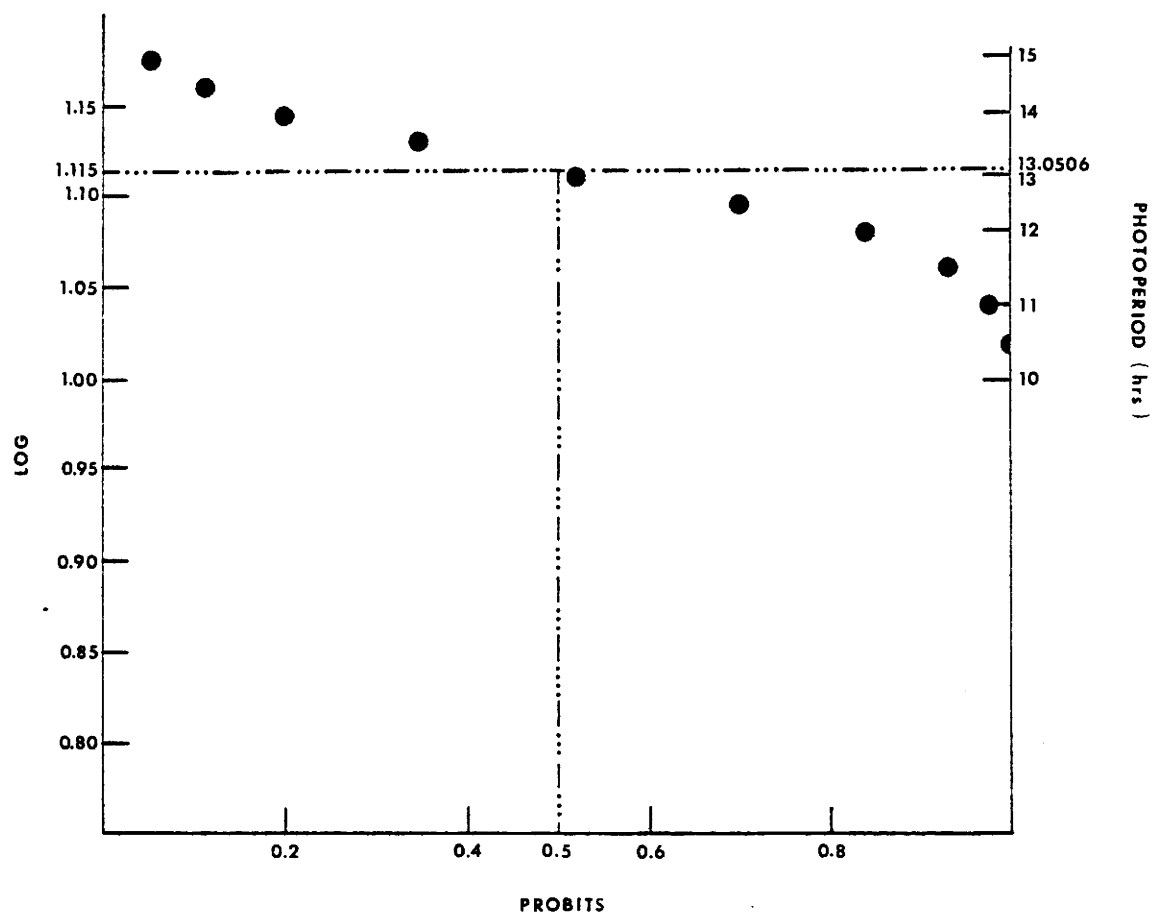
Figure 12. Probit analysis showing a critical photo-
            period of female *Culex peus* maintained at
            18°C and 12 different photoperiods

Experiment Ic

The general pattern of ovarian development in mos-
quitoes consists of two periods. The first period of
oocyte growth called previtellogenic development occurs
prior to yolk deposition and extends from the "pre-
resting" to the "resting" stage. The second period
called vitellogenic development extends from the
"resting" stage to full development of eggs. General-
ly, follicles of non-blood-fed females will not develop
to mature eggs (except in autogenous strains). This
experiment was conducted to observe the development of
the follicle of *Culex peus* after a blood-feeding under
four combinations of photoperiod (8 and 16 hours) and
temperature (15°C and 25°C). Each treatment was run
twice. Females from each conditioning incubator were
placed into a small screened cage and were fed on a
Japanese quail on the eighth day after their emergence.
All blood-feeding trials were conducted overnight at
20°C in a separate room. Blood-fed and non-blood-fed
females were segregated from each other and were held
in their respective incubators. When digestion had been
completed, which last about eight days, both blood-fed
and non-blood-fed females were dissected for determin-
ation of follicular development. The results are shown
in Table 8.

TABLE 8. Blood-feeding and stages of follicular development in *Culex peus* exposed to various combinations of photoperiod and temperature

Treatment Conditions	Percent Fed	Number Dissected	Stage of the Follicle[*]					
			N	I-II	IIa	IIb	Va	Vb
15°C/8:16LD	0	11	11	0	0	0	0	0
15°C/16:8LD	28.85	9	0	0	0	0	5	4
	Non-fed	8	0	2	6	0	0	0
25°C/8:16LD	58.62	10	0	0	0	0	4	6
	Non-fed	12	0	1	10	1	0	0
25°C/16:8LD	68.42	14	0	0	0	0	0	14
	Non-fed	9	0	0	4	5	0	0

[*]Determination eight days after blood-feeding trials

Females subjected to short photophase at 15°C did not feed at all even after four consecutive blood-feeding trials. The follicles were in stage N and never developed to stage I. Seventy-five percent of females held under long photophase at 15°C had follicles developed to stage IIa. At 25°C, 8% and 56% of females under short and long photophases, respectively, had the ovaries with some follicles developed to stage IIb. Mature eggs were formed in all females taking a blood-meal.

Effect of Photoperiod and Temperature
on Fat Production

Experiment II

The purpose of this experiment was to observe the
effect of photoperiod and temperature on the development
of fat produced by female mosquitoes. Four combinations
of photoperiod (8 hours and 16 hours) and temperature
(15°C and 25°C) were tested. Two replicate groups were
conducted for each treatment. The second group of fe-
males was placed into the incubators three days after
the first group. A blood meal was offered on the eighth
day after the peak of the emergence of adults. Females
from each treatment were removed from the conditioning
incubator and were placed into a screened cage measuring
30 cm. x 30 cm. x 30 cm. in which a quail had been placed
as a blood-meal source. All cages were held at 20°C
in a separate room. Blood-feeding trials were conducted
overnight. Females which took a full blood-meal were
segregated from non-fed females and were returned to
incubate at their pre-feeding temperature and photo-
period conditions. Number of blood-fed and non-blood-
fed females were observed and recorded. The results of
blood-feeding trials are shown in Table 9. The data
within columns were analyzed by X^2. Differences between
temperature were found to be significant at the 1% prob-
ability level. Percentages of females taking a blood-meal

TABLE 9. Blood-feeding of *Culex peus* under different
conditions of photoperiod and temperature

Treatment Conditions[*]	No. Tested	No. Feeding	Percent[**]
25°C and:			
16 Hours Photoperiod	11	6	54.5a
8 Hours Photoperiod	12	8	66.7a
15°C and:			
16 Hours Photoperiod	14	4	28.6b
8 Hours Photoperiod	13	1	7.7c

[*]For 25°C vs. 15°C, $X^2 = 9.28$[**]

[**]Percentage differences followed by different letters
are significant at the 1% probability level by X^2.
Difference between percentages followed by the same
letter are not significant

were lower at the lower temperature. At 25°C, more than

50% of the females tested took a blood-meal under both

photoperiod conditions. There was no significant dif-

ference between photoperiods at the higher temperature,

whereas, at the lower temperature (15°C), the percentage

of blood-feeding among females held under short photo-

phases was significantly lower than that observed for

long photophase females.

After the blood-meal had been completely digested,

the females were processed for determination of fat

production by extraction with petroleum ether. They

were oven-dried at 40°C, weighed, ether-extracted,

re-dried and re-weighed as discussed in the "Materials
and Methods" section. The difference between pre- and
post-extraction weights represented the extracted fat
of the mosquitoes. The mean dry weights, the mean
weights of extracted fat and the mean percentages of
fat for each of the eight groups are shown in Table 10.
The differences among groups in dry weight were sig-
nificant at the 1% probability level (F=4.86**). The
differences in amount and percentages of fat between
blood-fed and non-blood-fed females were not significant
at the 1% probability level except for a significantly
greater amount of fat under conditions of eight hours
photoperiod at cool temperature. At 15°C, however,
females which took a blood-meal under 16 hours photo-
phase showed a significantly lower amount and percent-
age of fat formation than females which took a blood-
meal under 8 hours photoperiod. Non-blood-fed females
held at 15°C also showed a significant difference in
percentage of fat between the groups exposed to short
and long photoperiods at the 1% probability level.

Field Collections of Mosquitoes

Field collections of mosquito larvae were made
from a log pond in Philomath, Oregon to determine the
natural occurrence of *Culex peus*. The results of the

TABLE 10. Dry weight and fat content of blood-fed and non-blood-fed female *Culex peus* maintained under different conditions of photoperiod and temperature

Group	Number Tested	Dry Weight* (micrograms)	Fat* (micrograms)	Percent*
25°C, 16hL:8hD				
Non-blood-fed	5	1.151 ± 0.299	0.117 ± 0.105	10.1 ± 0.8 a
Blood-fed	6	1.591 ± 0.307	0.160 ± 0.110	9.8 ± 0.7 a
25°C, 8hL:16hD				
Non-blood-fed	4	1.029 ± 0.208	0.163 ± 0.116	15.6 ± 0.9 a
Blood-fed	8	1.915 ± 0.203	0.254 ± 0.092	13.5 ± 0.7 a
15°C, 16hL:8hD				
Non-blood-fed	10	1.404 ± 0.139	0.292 ± 0.075	20.9 ± 0.6 b
Blood-fed	4	1.668 ± 0.221	0.358 ± 0.124	21.4 ± 0.8 b
15°C, 8hL:16hD				
Non-blood-fed	12	1.223 ± 0.168	0.378 ± 0.099	30.7 ± 0.6 c
Blood-fed	1	1.874	1.024	54.6 d

*Mean ± standard error
Differences between percentages followed by different letters are significant at the 1% probability level by t-test method. Difference between percentages followed by the same letter are not significant.

field collections including the duration of natural daylight plus civil twilight are shown in Figure 13. *C. peus* larvae first appeared in late April. They reached peak abundance in August. Larvae had disappeared from the log pond by Mid-November.
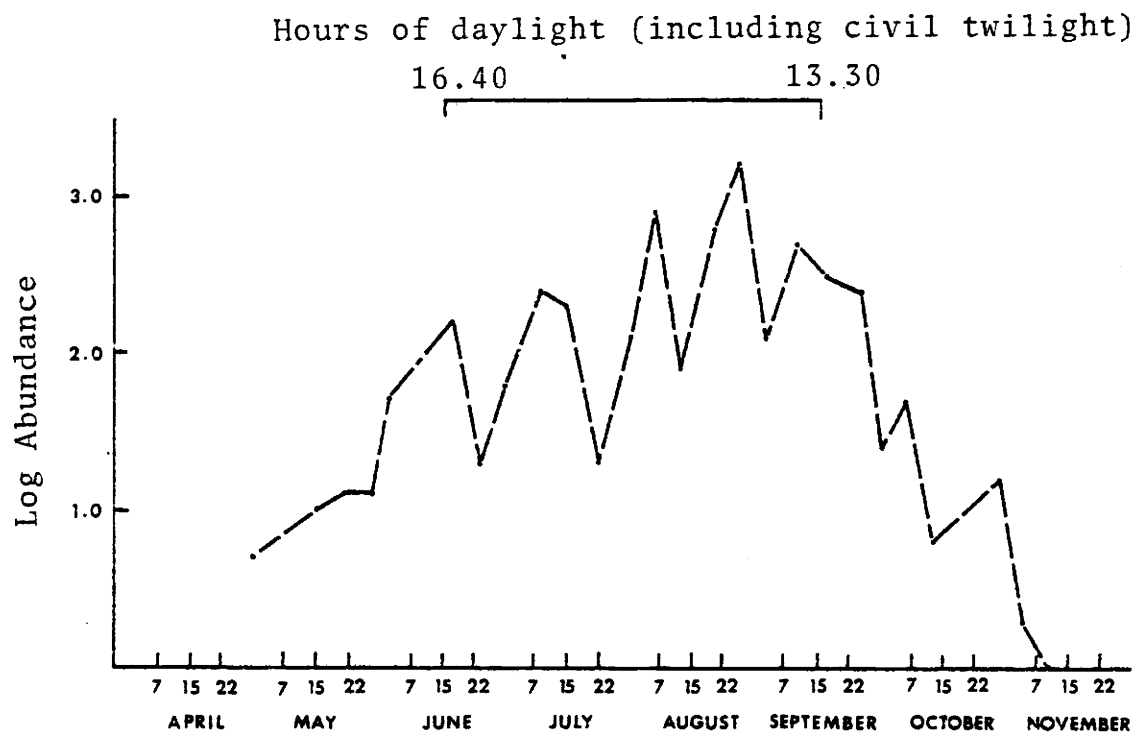
Figure 13. Abundance of adult *Culex peus* reared from
collections at Philomath log pond, Oregon,
April-November, 1981

DISCUSSION

The primary objective of this research was to de-
velop some details of the winter biology of *Culex peus*
since the knowledge about hibernation of this species
is scant. The studies were designed to observe the ef-
fects of photoperiod and temperature on the development
of ovarian follicle and of body fat.

Photoperiod and temperature have proven to have
an effect on ovarian development in several species of
Culex mosquitoes, for example, *Culex pipiens pallens*
(Wang, 1979), *Culex restuans* and *Culex pipiens* (Eldridge
et al., 1976; Madder, 1981). Eldridge (1966, 1968)
found that ovarian development in *C. pipiens* was sup-
pressed by a combination of low temperature and short
photoperiod. The results here show that a combination
of 15°C and eight hour photophases resulted in the
ovaries of *C. peus* remaining in a diapause condition.
Diapause seems likely to be induced by short photophases
and modified by cool temperature since the effect of
temperature on follicle size differed significantly
under photoperiod conditions of 8hL:16hD; follicles
of females maintained at a temperature of 25°C were
twice as long as those of females maintained at 15°C.
Danielevskii (1961) pointed out that diapausing females
during late summer and fall resulted from the response
to shortening daily light that was enhanced by cooler

temperature. Sanburg and Larsen (1973) also reported
in their study of *C. pipiens pipiens* that at 22°C, fol-
licle size increased when females were reared under
15 hour photophases but not under those of 10 hours.
In this experiment, follicle size increased over time
at 25°C, regardless of photoperiod. At 15°C, however,
photoperiod influences on ovarian follicle development
were evident. The follicles of females exposed to
16 hour photophases developed slower and were smaller
after the first nine days of adult life but they de-
veloped to the resting stage (stage I-II) after 21
days. On the other hand, follicles of females held
under eight hour photophases never developed beyond
stage N and remained in the diapause stage. Therefore,
follicle development of *C. peus* appeared to be arrested
by a combination of 15°C and eight hours photoperiod.

Experiments on the effect of photoperiod and
temperature on rate of adult eclosion revealed that
both photoperiod and temperature to which pupae were
subjected appeared to influence the timing of emergence
of adults. Since temperature is known to directly af-
fect rate of eclosion, the differences observed be-
tween the two photoperiod treatments at 15°C suggest
subtle differences in temperature even though the ex-
perimental treatments are designed to avoid this. In-
candescent lamps used in the incubators provided a more
natural illumination source than fluorescent lamps

even though the latter are cooler and freer from com-
plicating secondary heat effects. However, to avoid
the fluctuation of temperature, wire-wound power heat
compensating resistors were installed in the incubators.
Another possible factor which cannot be ruled out is
the disruption of circadian rhythms established under
insectary lighting conditions. Since the larvae were
reared under 16 hour photophases, when the pupae were
moved to the 8hL:16hD treatment conditions, they may have
required some time to adapt to the new rhythm, and
thus, activities such as eclosion in the eight hour
photophase groups were delayed. The results of the
experiment reported here are similar to those reported
by Eldridge et al., (1976) in *C. restuans* and *Culex*
salinarius. They found that at a temperature of 25°C
under 16 hours of light per day, both species under-
went 50% of adult eclosion by day two post-pupation,
while in this study those of *C. peus* underwent eclosion
by day 2.5 under both short and long photophases. At
15°C, however, adult eclosion occurred later in the
three species. Under 16 hour photophases, 50% of
eclosion of *C. restuans* had occurred by the same day
as that of *C. peus* (day 3.5), whereas, in *C. peus* ex-
posed to 15°C and eight hour photophases it occurred
a day later (day 4.5). Photoperiod is believed to in-
sert its action on the brain (Adkisson, 1966; Mansingh,

1971; Beck, 1980). It is possible that ecdysone which is secreted from the prothoracic gland and is necessary for differentiation to the adult stage was suppressed by an inappropriate photoperiod of short daily light. This physiological event needs more investigation.

The methods used to determine diapausing follicles in these experiments were based on the follicle length along with the ratio of follicle:germarium length. The use of these ratios to distinguish diapausing from non-diapausing females varies from worker to worker. Spielman and Wong (1973b) proposed the ratio of 1.5:1.0 or less in classifying diapausing follicle. Madder (1981), on the other hand, used the ratio of no more than 2.0:1.0. However, he commented that the difference in the ratio value may be due to the inclusion of the connecting channel between follicle and its germarium in the measurement of the germarium by Spielman, thus reducing the ratio. Both the follicle length and the ratio are not definitely reliable in considering diapausing and non-diapausing status because some non-diapausing females have been reported to have a ratio of less than 2.0:1.0 and vice versa (Madder, 1981). Also, there is no reason to believe that ratios for one species will necessarily hold for other species of *Culex*. I found that in *C. peus*, some resting stage follicles (stage I-II of Kawai, 1969) had a ratio of

1.2:1.0. Determination of stage of follicular develop-
ment along with follicular measurement is suggested.
Individual difference and uneven size of follicles,
called "mosaic" as reported by Danielevskii (cited in
Eldridge, 1968), were also observed in *C. peus* as
follicles of different stage of development were found
at the same time and within the same ovary. This
uneven growth was seldom found but it is apparently
more commonly seen in vitellogenic stage of develop-
ment.

The pupal stage of *C. pipiens* (Eldridge, 1968)
and *C. tarsalis* (Harwood and Halfhill, 1964) was identi-
fied to be sensitive to photoperiod. The author could
not confirm this observation in *C. peus* since none of
the experiments were designed to test this, but it is
likely to be so. My initial results suggested only
that *C. peus* was sensitive to photoperiod and tempera-
ture. Attempting to determine a critical photoperiod
required subjecting females of this species to a
series of photoperiods at a single temperature (18°C).
The response of follicle size to photoperiod was not
linear. Also, graphical results show a wide range of
follicle lengths and ratios at almost every photoperiod
tested. The difference may be due to individual vari-
ation since many other factors were uniform in each
treatment. Rearing techniques, like crowding, food

rations, and salinity of media, which are known to affect growth, rhythm and synchrony of pupation and emergence (Nayar, 1968; Nayar and Sauerman, 1970) might have some effect on variation of individual responses. However, pupae were randomly divided so that such variation should have been confused among groups. It is obvious that follicle lengths increased as photophases increased. The correlation between photoperiod and follicle size is 0.88. Again, the ratio of follicle: germarium length in addition to follicle length was used to consider diapause status in the mosquitoes. The correlation between photoperiod and the ratio is quite high (r=0.91). The data indicated that at 18°C, diapause in *C. peus* was induced by the duration of less than 13 hours of light per day. This observation agrees closely with the findings of Spielman and Wong (1973b) that at 18°C, nearly all female *C. pipiens* entered diapause when subjected to no more than 12 hour photophases.

A linear regression fitted in Figure 6 expressed the photo-periodic response of *C. peus*. The shape of the response curve here was similar to that studied by Sanburg and Larsen (1973) in *C. pipiens pipiens*, namely, follicle size was larger at longer photophases. The critical photoperiod of *C. peus*, based on the data obtained from this experiment and analyzed by a probit

analysis, was 13.051 hours of light per day. Spielman
and Wong (1973b) found that at 18°C, the critical
photoperiod of *C. pipiens* was a 13 hour photophase.
Variation in critical photoperiod is not uncommon
since Bradshaw (1976, 1977) reported that critical
photoperiod was closely correlated with latitude
and altitude. That is, critical photoperiod will
be lengthened at the higher latitudes. The colonies
of *C. pipiens* studied by Spielman and Wong (1973b)
and of *C. peus* studied by me were collected at
latitudes of approximately 42°N and 44°N, respective-
ly.

Prehibernating female mosquitoes of several *Culex*
species showed a reduction of blood-feeding activity
in response to conditioning by short photoperiod and
low temperature (Eldridge, 1965, 1972, 1979; Oda, 1971).
Reduction of blood-feeding drive is a characteristic
used as a criterion for diapause in many *Culex* mos-
quitoes. *C. pipiens* and *C. restuans* were reported to
have suppression of blood-feeding under simulated fall
conditions of short photoperiod and cool temperature,
and ovaries of females which happened to take blood-
meals would remain in a diapause state if the post-
feeding temperature was still low (Eldridge et al.,
1972, 1979). Eldridge (1965) demonstrated the effect
of crowding of adult females on blood-feeding in

C. pipiens where by blood-feeding decreased with an increase in density. This factor did not occur in these blood-feeding trials in *C. peus* due to small number of females in the feeding cages. Also, the factor of age was eliminated by using females of the same age. However, they varied in size. The author could not confirm whether carbohydrate diet had an influence on blood-feeding response in *C. peus* as it did in *C. pipiens* and *C. quinquefasciatus* (Eldridge, 1965). Adult female *C. peus* were provided with 10% sucrose solution from the first day of emergence in all treatments and throughout the experiment. On the eighth day post-adult-emergence, blood-feeding trials were conducted overnight. Under simulated fall conditions of short photophase and cool temperature, females were reluctant to take blood and exhibited a preference for feeding on sugar-water. The concentration of sugar-water was not considered to affect the rate of blood-feeding since Eldridge (1965) demonstrated that in *C. pipiens* and *C. quinquefasciatus* blood-feeding rate did not vary after feeding on a series of sucrose solutions ranging from 5% to 50%. A factor that might cause reduction in blood-feeding is the duration of maintenance of females on sugar-water, even though female *C. pipiens* (Eldridge, 1965) and *C. p. pallens* (Hosoi, 1954) with dilated abdomens

containing sucrose solution were observed to take full blood-meals. Other possible sources of variation include the size of the test cage and defensiveness of the quail hosts. However, the results reported here indicate that a combination of low temperature and short photoperiod suppressed the blood-feeding response of *C. peus*. Nevertheless, one female showed evidence of taking blood under a combination of 15°C and eight hour photophases. This suggests the possibility that this species may take blood-meals, at least at low frequencies, in the fall in nature. Females which took a blood-meal at warmer temperature and at cool temperature but under long photophases, either fully or partially, developed mature eggs after the blood-meal had been completely digested.

Wallis (1959) reported that blood is not necessary for fat formation and hibernation in *C. restuans*, since females preferred feeding upon sucrose solutions late in the summer. However, *C. restuans* took blood and exhibited gonotrophic dissociation (the phenomenon whereby a blood-meal results in fat body production rather than ovarian development) in response to fall conditions of photoperiod and temperature. Gonotrophic dissociation could not be demonstrated in *C. peus*. The female that took blood after conditioning under eight hour photophases and at 15°C formed a considerable amount of

fat reserve but was not examined for follicular develop-
ment. However, sucrose-fed females, maintained under
the same conditions, and which had not taken a blood-
meal previously developed less body fat. It appears
evident that the increased amount of fat was derived
from the blood-meal taken. This is strong circumstan-
tial evidence of gonotrophic dissociation, but more
blood-fed females which have been held under short
photophase and low temperature conditions need to be
examined. At warm temperatures (25°C), percentages
of fat extracted were slightly higher in non-blood-
fed females than in blood-fed ones and follicle ex-
amination revealed that females which took a blood-
meal developed their follicles to mature eggs or at
least to stage Va (of Kawai, 1969), while the follicles
of non-blood-fed females did not develop past stage
IIb (of Kawai, 1969).

The results of this study indicate that *C. peus*
exhibits ovarian diapause in response to a combination
of short daily photophases and low temperature. It
is interesting to compare these results with the works
of Eldridge et al., (1968, 1976) on the effect of tem-
perature and photoperiod on ovarian development in *C.
quinquefasciatus* and *C. salinarius*. The approximate
northernmost limits of *C. quinquefasciatus*, *C. peus*
and *C. salinarius* are 42°N, 47°N and 48°N latitude,

respectively. Eldridge and his coworkers performed their studies of *C. quinquefasciatus* on a laboratory colony colonized from larvae collected in Florida and of *C. salinarius* colonized from Maryland, both of which are below 40°N latitude. I conducted these studies of *C. peus* with materials collected at about 44.6°N latitude. Although the three species have basically southern ranges, the important distinction is that *C. quinquefasciatus* and *C. salinarius* do not show ovarian diapause in response to fall photoperiod conditions. Presumably, this difference is due to the geographic variation as discussed above and variation among and within species, since *Culex taraslis* reared in the laboratory from females collected near Corvallis under-go ovarian diapause, whereas, those from two California areas do not (Eldridge 1983, personal communication). Thus it seems likely that ovarian diapause would only be evident in the northern populations of the range of *C. peus* but this needs further investigation.

Direct evidence of hibernation of *C. peus* in nature is still lacking. Although females of *C. peus* were collected in Planada and Berkelcy, California in late Janaury (Freeborn and Bohart, 1951), it can not be de-termined whether this species actually survived an en-tire winter at these locations. To confirm how suc-cessfully *C. peus* can utilize their fat reserves and

hibernate, the survival period after feeding activity
should be ovserved and, especially, the interactions
between photoperiod and thermoperiod should be studied.
Beck (1983) reported that the close relationship of
thermophase temperatures which occur during photophase
and cryophase temperatures during scotophase are of
importance in the determination of diapause, since
the incidence of diapause in several insect species
was influenced by the cryophase temperatures coincid-
ing with the scotophase.

Field collections of larvae showed that adult
activity of *C. peus* declined in September coinciding
with the shortening of day light and decreasing of tem-
perature. Their first appearance of larval populations
in April suggest that at this latitude (44°N) this
species hibernates here, but overwintering *C. peus* have
never been recovered in Oregon. The results of this
study suggest that the physiological response to en-
vironmental factors are consistent with a species which
overwinters as inseminated adult females.

The question of the possibility of *C. peus* serving
as an overwinter host for arboviruses of medical or
veterinary importance remains unresolved. Evidence of
infected blood-meals by other *Culex* mosquitoes during
fall season was reported in several documents (Kokernot
et al., 1969; Dalrymple et al., 1972). Bailey et al.,
(1978) reported that two strains of St. Louis encephalitis

virus (SLE) were isolated from hibernating *C. pipiens* females during winter. Japanese encephalitis (JE) has also been isolated from *Culex tritaeniorhynchus* and WEE from *C. tarsalis* (Eldridge, 1981). This evidence suggests that such females would take viremic blood-meals in fall and that the virus would persist in the mosquitoes, or, alternatively that the overwinter generation of mosquitoes would become infected transovarially from the summer, parental generation. There is also evidence which points to the possibility of prehibernation blood-meals in *C. pipiens*. In the laboratory, an increase in temperature from 15°C to 25°C for 72-84 hours resulted in blood-feeding of previously hibernating females *C. pipiens* and a proportion of those females taking blood had undeveloped ovaries (Eldridge and Bailey, 1979). This finding suggests the possibility of prehibernating *C. pipiens* females harboring viral diseases while overwintering as adult females. More research is needed on blood-feeding habits of *C. peus* in nature i.e. seasonal feeding patterns and possible seasonal shifts in host range. Under laboratory conditions, *C. tarsalis,* a vector of WEE and SLE viruses, fed both on birds and on mammals, while *C. peus* exhibited a strong preference of feeding on avian hosts (Nelson et al., 1976). It is interesting to wonder whether *C. peus* would feed on mammals

when birds are scarce as hosts, as they would be in
late fall, since Nelson and his colleagues conducted
their experiments using double-feedings (baits with
a jackrabbit and either a chicken or a pheasant).
Wild-caught *C. peus* females have yielded isolations
of WEE virus in nature (Hammon et al., 1945; Stage
et al., 1952). Ferguson (1954) also reported the abil-
ity of this species to harbor the virus of St. Louis
encephalitis (SLE). Since *C. peus* showed evidence
of some blood-feeding under simulated fall conditions
in the laboratory, questions about prehibernating fe-
males taking a blood-meal in nature and the possibility
of viruses surviving in hibernating mosquito vectors
of this species are of interest.

CONCLUSIONS

1. Three characteristics were considered in determin-
    ation of reproductive diapause in *Culex peus*.
    They are failure of ovarian development, retard-
    ation of blood-feeding drive and formation of hy-
    pertrophic fat in response to short photoperiod
    and low temperature. *C. peus* expresses ovarian
    diapause after exposure of pupal stage to the
    eighth day of adult life to a combination of eight
    hour photophases and cool (15°C) temperatures.

2. Diapausing females have ovarian follicles in the
    pre-resting stage (stage N of Kawai, 1969) with
    a mean follicle length of 0.055 mm. and a ratio
    of follicle:germarium length 1.1 : 1.0.

3. At 18°C, at least 13 hours of light per day are
    required for normal follicle development, while
    a shorter duration of daily light stimulates
    females to enter ovarian diapause.

4. Simulated fall conditions not only retard fol-
    licular development but also delay adult eclosion,
    suppress blood-feeding and result in the accumu-
    lation of body fat.

5. Gonotrophic dissociation may exist in *C. peus*
    since an increased amount of fat was shown to
    derive from a blood-meal in female held under

diapause-inducing conditions. More confirmation is needed to prove failure of ovarian follicle development following a blood-meal.

6.   In these experiments, the correlation between the length of follicle and that of its associated germarium was relatively high ($r=0.96$). To use the follicle : germarium length ratio in determining ovarian diapause is a better way compared to the use of follicle size since the correlation between photoperiod and the ratio ($r=0.91$) was higher than that between photoperiod and follicle size ($r=0.88$).

7.   The role of this species as a vector of viral diseases is still unknown but it should be considered since it shows the possibility of taking blood-meals under simulated fall conditions and exhibiting ovarian diapause.

8.   Future research should be undertaken to understand the ability of this species in taking infected blood-meals, remaining in diapause condition, hibernating successfully and transmitting viral agents.

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