Induction of Embryonic and Larval Diapause in *Aedes togoi* (Theobald) (Diptera:Culicidae).

by

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ABSTRACT

Aedes togoi (Theobald) from Vancouver, B.C. may enter diapause in either the egg or the larval stage. Larval diapause occurred in the 4th instar, when immatures were maintained at 16°C or less, at photoperiods shorter than 12h light/day. The critical photoperiod was calculated as LD 10.9:13.1 at 16°C. Larvae which did not pupate after 40 days at the experimental conditions were assessed as being in diapause. Non-diapause larvae reached the pupal stage in 17-20 days at 16°C. At 14°C, 100% of the larvae diapaused at LD 10:14. Temperatures of 18°C or higher, at LD 10:14, resulted in no larval diapause.

Embryonic diapause resulted when immatures, adults and eggs were maintained at temperatures ranging from 22 to 28°C, at photoperiods shorter than 15h light/day. The critical photoperiod was calculated as LD 15.3:8.7 at 25°C, and LD 14.4:9.6 at 22°C. There was no diapause when the population was maintained at 24°C LD 16:8.

An experiment was conducted to determine if a simulated dawn/dusk of 90 minutes/day would increase mating success. After three days at the experimental conditions, there was no difference in mating success between populations of males and females maintained with a dawn/dusk period and those maintained at the same photoperiod where the light/dark change was abrupt.

FORWARD

The format followed for this thesis is that of the paper style thesis. No material from this thesis has yet been published but chapters III and IV will be submitted for publication at a later date.

Chapter I

Introduction

Aedes (Finlaya) togoi (Theobald) is native to the Pacific east coast of Asia from the southeastern seaboard of Siberia, USSR, to Malaysia (Ramalingam 1969). This mosquito breeds in coastal rock pools above the high tide level, but has also adapted to using artificial containers which may contain fresh water or water that is more salty than sea water (Omori 1962, Petrishcheva 1948). The first Canadian record of this species is believed to be from the late 1940's (Belton 1980). This species is currently established near Vancouver, B.C. and in Washington State, U.S.A.

Some studies have been carried out to determine the influence of photoperiod and temperature on egg and larval diapause in Asian strains of Ae. togoi (Vinogradova 1965, Mogi 1981). Vinogradova (1965) reported that the daylength condition of the female parent determines the diapause status of the egg. Short photoperiods induced diapause while long photoperiods promote development without interruption. Mogi (1981) noted that with a 2h increase in photoperiod, from LD 10:14 to 12:12, at 15°C, larvae progress without delay and produce adults which lay diapausing eggs. Shorter photophases produced larvae which enter a 4th instar diapause. Resultant females laid non-diapausing eggs.

Literature on the ecology of the North American strain of this species is limited to only a few papers. In my study of the Vancouver, B.C. population of Ae. togoi, I reared larvae, pupae and adults at preselected daylengths and temperatures to determine under what conditions maximum frequency of egg diapause would occur and if there was a larval diapause. I also wanted to determine how overwintering strategies influenced this mosquito's ability to survive at temperatures and daylengths typical of Vancouver, B.C. (49°N Lat).

Chapter II

Literature Review

DISTRIBUTION

Ae. togoi (Theobald) was first described by Theobald in 1907. Type females come from Osaka, Japan (Knight and Stone 1977). Later records indicate that this species occurs in Siberia (Yamada 1927), China (Feng 1933), Formosa (Yamada 1927), Korea (Yamada 1927), Japan (Yamada 1927, LaCasse and Yamaguti 1950, Sasa et al. 1952), and Vietnam (Gould et al. 1968, Ramalingam 1969). Other habitats for this species include the following islands: Bonin Island (Bohart 1956), Cheju-do Island (Seo 1978), Danjo Island (Miyagi 1973), Goerhand Island (Edeson 1962), Marcus Island (Knight and Stone 1977), Ryukyu-Retto (Knight and Stone 1977) and Taishan Island (Dzyan-Chan 1960). In Japan, LaCasse and Yamaguti (1950) found this species at practically all locations checked including Kyushu, Shikoku, Honshu and Hokkaido. In China, Ho (1931) reported finding Ae. togoi as far inland as Beijing (=Peiking=Peiping). Petrishcheva (1948) observed that Ae. togoi may occur several hundred miles inland from the coasts of Manchuria and Korea, breeding in man-made habitats such as containers and outhouses.

Ae. togoi has also been found on the west coast of North America. Canadian collection sites are rock pools near

Vancouver and Victoria, British Columbia (Belton 1980, Meredith and Phillips 1973, Trimble and Wellington 1979b). Belton (1980) has also found this species in rock pools of coastal Washington State, U.S.A. All of the known North American locales for Ae. togoi lie within 10 km of commercial ports or ferry terminals. It is therefore likely that Ae. togoi came to North America across the Pacific Ocean from China or Japan by ship.

MEDICAL IMPORTANCE OF AE. TOGOI

Filariasis

The most widespread human microfilariae in Asia are Wuchereria bancrofti Cobbold and Brugia malayi (Brug). In Japan, Yamada (1927) reported that Ae. togoi is a suitable host for the development of Filaria (Wuchereria) bancrofti Cobbold larvae. Filaria larvae of B. malayi can also reach maturity and attain high infection levels in Japanese strains of Ae. togoi (Omori 1962). Bancroftian filariasis was widely distributed in Japan, displaying high endemicity in southern Japan where Ae. togoi and Culex pipiens pallens Coquillett were responsible for transmission of the disease (Omori 1962). More recent findings indicate that the incidence of bancroftian filariasis in Japan has declined due to vigorous mosquito control measures (Hawking 1976). Studies in the Nagasaki Prefecture of Japan indicated a high incidence of bancroftian filariasis in the human population

examined, where C.p. pallens is believed to be a more important vector than Ae. togoi (Nagatomo 1960a, 1960b, Wada 1966). Malaysian filariasis occurs in Japan only on the islet of Hachijo-Koshima, where Ae. togoi vectors B. malayi (Edeson 1962, Sasa et al. 1952).

In China, Ae. togoi has been found to support natural and artificial infections of W. bancrofti and Wuchereria (Brugia) malayi and is an important vector of these nematodes along coastal China, including Choushan Island and the Goerhand Islands (Huei-Han 1959, Edeson 1962).

Four species similar to B. malayi, including B. pahangi (Buckley and Edeson), B. patei (Buckley, Nelson and Hiesch), B. tupiae Orihel and B. timori (David and Edeson) have all been shown to survive in mature Ae. togoi (Laurence and Pester 1967, Manning et al. 1972, Partono et al. 1977, Purnomo et al. 1976, Ramachandran et al. 1963). Ramachandran et al. (1963) report that the following filariae would also develop in Ae. togoi: Dirofilaria immitis (Leidy), a Breinlia species from the Malayan slow loris, and a Setaria species from the Malayan mouse-deer. Breinlia booliati (Singh and Ho) may also survive in Ae. togoi (Ho et al. 1973).

Japanese Viral Encephalitis

Japanese Encephalitis (JE) virus is one of the most important mosquito - borne encephalitides in terms of human morbidity and mortality. The disease has been recorded from the southeastern parts of the Soviet Union and southern Japan, south to Indonesia and west to India (Pant 1979). Cases of JE have been recorded from as far east as Guam, with major outbreaks having occurred in Japan, Korea, China, Thailand and India (Pant 1979). Ae. togoi is suspected as a vector of JE virus in Japan (Pant 1979) and in the Primorye region of the USSR where Ae. togoi comprised 78% of 19 species of mosquitoes found in that region (Shestakov and Mikheeva 1966). Japanese encephalitis occurred for the first time in the USSR near Khasan in 1938, when it appeared in epidemic form (Grascenkov 1964). Identical encephalitides were later found in North and South Manchuria and North Korea (Grascenkov 1964).

Most epidemiologic studies of JE virus have been conducted in northern temperate areas, where the mosquito is not active during the winter (Rosen et al. 1978). The incidence of human disease is usually higher at higher latitudes (Rosen et al. 1978). Transovarial transmission of JE virus does occur in Ae. togoi and may be why the virus reappears in the same locality year after year (Rosen et al. 1978).

Petrishcheva (1948) reported that the most dangerous period for the transmission of JE virus, in the Soviet Far East, is from mid-July to early September when the temperature is highest, favouring mosquito and viral activity. According to Fedder and Reznik (1971), females of Ae. togoi may be most dangerous as vectors of JE virus at the age of 14-16 days, after 2 gonotrophic cycles. They reported that among attacking mosquitoes such females constituted 12% of the population in the Khasan area of the Primorye region, USSR.

Concern for the introduction of JE virus into North America was prompted when 7 species representing 3 genera of Western North American mosquitoes were demonstrated to be vectors of JE virus under laboratory conditions (Reeves and Hammon 1946). McLintock and Iversen (1975) report that the presence of Ae. togoi in North America could lead to an introduction of JE virus into North America. If the virus was transferred to birds, pigs, or bats, it could then become available to other potential vectors such as Culex pipiens L., Cx. tarsalis Coquillett, Ae. dorsalis (Meigen), Culiseta incidens (Thomson) or Cs. inornata (Williston) (McLintock and Iversen 1975). These species are all present in southwestern British Columbia.

Ae. togoi has also been found to be susceptible to the following viruses under laboratory conditions: Semliki Forest (Nye and Lien 1960), Sindbis (Bidwell 1976), Chikungunya (Bidwell 1976), and West Nile (Bidwell 1976).

BIOLOGY OF AEDES TOGOI

Larval Habitats

Ae. togoi occurs along rocky coastlines of east Asia (Knight and Stone 1977) and southwestern British Columbia (Belton 1980). The larvae develop in rock pools above the high tide mark. These pools receive salt water from ocean spray and fresh water from runoff, seepage and rain. Larvae can tolerate salt concentrations 3X greater than seawater but also develop successfully in rainwater or water of low salt (0.046% chlorides) concentration (Jackson 1938, Lien 1960, Omori 1962).

Numerous studies concerning the salt water adaptation of this mosquito have been carried out (Asakura 1978, 1980, 1982, McGinnis and Brust 1983, Meredith and Phillips 1973, Trimble and Wellington 1979a). The anal papillae seem to be important in regulating ionic osmosis in the larvae (Meredith and Phillips 1973). The gastric caecum may play a similar role (Asakura 1982).

Ae. togoi may also occupy a variety of containers holding fresh or salt water (Edwards 1921). The density of larvae in containers has been reported to increase with proximity to the sea (Omori and Fujii 1953). In Russia, larvae were observed in artificial water containers, but most of the breeding occurred in natural sites (Petrishcheva 1948). She suggested Ae. togoi might spread inland from coastal areas

of Russia as the settlement of uncultivated areas progresses.

Larvae have also been found in cisterns (Bohart 1956), and in tubs and kettles filled with sea water used for processing dried sardines (Omori 1962). When the tubs are left unused for a week or more larvae may become numerous, especially if these tubs are located close to rocky coastal shores (Omori 1962). Ae. togoi appears to be scarce in inland Japanese villages (Omori 1962). This may be due to a preference for saline habitats.

Host Preferences

Reports about the feeding preferences of Ae. togoi are conflicting. Chang et al. (1957) reported that females feed more on fowl or horses than man, but Edeson (1962) stated that Ae. togoi readily bites man. Adult females have been reported to enter habitations to feed at night and can become a serious pest when present in sufficient numbers (Bohart 1956). Petrishcheva (1948) found this mosquito to be synanthropic, given the opportunity, and females will enter human dwellings to feed. Some workers have found Ae. togoi to be a vigorous human biter, which readily enters human dwellings (Jackson 1938, Sasa et al. 1952), while others reported that the female was not a troublesome biter (Miyagi 1973).

Sea birds have also been considered as possible hosts for Ae. togoi. Mammalian species were reported rare on the Danjo Islands but wild birds were reported to be abundant (Miyagi 1973). Similar reports were made for the Maritime Province of the USSR, where vast coastal areas are inaccessible to man but are inhabited by sea birds (Petrishcheva 1948).

Feeding studies of Ae. togoi indicated that it is typically a nocturnal feeder. Omori and Fujii (1953) reported that females show a primary feeding peak 1-4 hours after sunset and a secondary peak just after sunrise. Wada (1966) reported a peak in feeding activity 2-3 hours after midnight.

Overwintering Mechanisms

Ae. togoi overwinters in the egg or larval stage. Petrishcheva (1948) reported that hibernation occurred in the egg stage along coastal areas of Russia. Larvae and pupae were present from mid-April until the beginning of November. Larvae were reported surviving in water which was frozen to three-quarters of its depth (Petrishcheva 1948). Eggs of this strain are highly tolerant of cold temperatures and can withstand frozen water environments for several months (Chagin and Savoyskiy 1943, Shestakov 1962). Vinogradova (1965) stated that Ae. togoi overwintered in the egg stage under the natural conditions of the Soviet

Maritime Provinces and found that the length of the photoperiod during the development of parent females determined whether or not diapausing eggs were laid. Under short day conditions (LD 12:12) most females laid diapausing eggs. Under long day conditions eggs hatched without interruption. Vinogradova (1965) also reported that decreasing the temperature from 18°C to 15°C under short day conditions caused an increase in the frequency of egg diapause, but diapausing eggs were reactivated by cold treatment (Vinogradova 1965).

Ishii et al. (1954) and Mogi (1981) stated that Ae. togoi overwinters in the egg and the larval stages in Japan. Mogi (1981) reported that larvae entered diapause in the 4th instar at 15°C when photoperiods were between LD 10:14 and 12:12. He found 1st to 4th instar larvae, pupae and emerging adults in a Nagasaki rock pool as late as 26 November. McGinnis (1982) reported 3rd and 4th instar larvae, and pupae, in coastal rock pools near Vancouver, B.C. from 15 December to 26 March. She also reported 1st and 2nd instar larvae in rock pools during the March collection.

Chapter III

The Effect of Temperature and Photoperiod on the Induction of Larval Diapause in *Aedes togoi* (Theobald) (Diptera:Culicidae).

INTRODUCTION

Aedes togoi (Theobald) is a multivoltine rock pool mosquito and occurs along the Pacific coast of Asia, Canada and the United States (Belton 1980, Ramalingam 1969, Wood *et al*. 1979).

Populations of *Ae. togoi* along the Soviet eastern seaboard overwinter exclusively in the egg stage (Vinogradova 1965). The overwintering egg contains a fully-formed larva and is able to survive freezing conditions for several months (Chagin and Savoyskiy 1943, Shestakov 1962).

In Japan, *Ae. togoi* overwinters in both the egg and the larval stages. A Nagasaki population of this species enters a 4th instar diapause in response to conditions of short daylength and low temperatures (Mogi 1981).

In Vancouver, British Columbia, 3rd and 4th instar larvae of *Ae. togoi* may be collected throughout the winter (McGinnis 1982).

The present study was undertaken to determine:

1. if a larval diapause exists in the Vancouver population of Ae. togoi
2. the critical photoperiod for larval diapause induction
3. the highest temperature for inducing and maintaining larval diapause.

MATERIALS AND METHODS

Colony Maintainance

Ae. togoi for these experiments came from a colony established by K.M. McGinnis in 1981. The original collection was made from rock pools near North Vancouver (49°N 15'). The colony was reared through 5 generations at 23.0 ± 0.5°C, 70 ± 5% RH, and LD 17.5:6.5 photoperiod before experimentation was begun. Adults were maintained in 30 X 30 X 30 cm clear plastic cages. Paper towel soaked in 10% sucrose solution provided the adult carbohydrate source. Blood meals were taken from laboratory mice. Eggs were deposited on moist paper towel and allowed to embryonate. Embryonated eggs were hatched in a 1 g/L solution of nutrient broth (Bacto Nutrient Broth®, Difco Laboratories Detroit, MI.) using dechlorinated tap water. Larvae were maintained in 22 X 33 X 6.5 cm plastic pans containing 1.5l of a 10 g/l commercial sea salt (Instant Ocean® , Aquarium Systems, Mentor, OH.) solution. Larvae were fed a liver powder (ICN Pharmaceuticals, Cleveland, OH.) slurry daily,

in excess of consumption. The surface film was removed from the larval medium daily. Survival from 1st instar larvae to adults was greater than 90%.

Experimental Design

Eggs from blood-fed adults were hatched in a 1 g/l solution of nutrient broth using dechlorinated tap water. Larvae were removed after 6 hours and transferred to plastic pans (22 X 33 X 6.5 cm) containing 1.5l of a 10 g/l commercial sea salt solution, at a density of 100 larvae per pan. A total of 3 replicates (100 1st instar larvae per replicate) were placed in constant temperature environmental chambers at 5 temperatures ranging from 14 to 28°C ± 0.5°C. Photoperiods ranged from a maximum of LD 18:6 to a minimum of 10:14.

Larvae were fed a liver powder slurry daily, in excess of consumption. The surface film was removed from the larval medium daily. Photoperiod was manipulated in the incubators (Percival model 135 LVL) which contained 4 GE Cool-White® 40W fluorescent tubes. Light to subject distance ranged from 10 to 15 cm. Light intensity was 2850 ± 50 lux. Pan lids were applied to prevent water loss through evaporation The number of individuals entering 4th instar or pupating were recorded each day. Trials lasted a maximum length of 40 days.

Critical photoperiod (CP), defined as the number of hours of light per day which initiates and maintains 50% diapause in the test population, was determined by two methods. First, the development times to 4th instar and pupation were used to assess if diapause was initiated. The first point of reference was the number of days that elapsed before 50% of the surviving population reached the 4th instar (i.e. T50 4th instar). The second point of reference was when 50% of the surviving larvae pupated (i.e. T50 pupa). The difference, T50 pupa - T50 4th instar, was used to determine if diapause was initiated. Interpolation was necessary since 50% levels were not exactly achieved in larval or pupal populations. Percent diapause was calculated as non-pupating larvae divided by total survivors. Second, the CP was calculated by plotting percent diapause as a function of hours light per day and taking the 50% intercept. All CP values were interpolated since no photoperiod tested elicited exactly 50% diapause in any trial.

RESULTS

The Vancouver population of *Ae. togoi* undergoes a 4th instar larval diapause under certain conditions of temperature and photoperiod. A photoperiod of LD 10.5:13.5 or less was found to induce larval diapause in the 4th instar at 16°C. Photoperiods between LD 10.5:13.5 and 13:11, at 16°C, resulted in a decrease in the frequency of larval diapause (Fig. 1). Photoperiods of LD 13:11 or

longer resulted in no larval diapause after 40 days (Fig. 1). At 16°C, it took 39 days, under a short day cycle (LD 10:14), for 75% of the larvae to reach the 4th instar. Under a long day cycle (LD 18:6), it took only 12 days for 75% of the larvae to reach 4th instar. It took 40 days under the short day condition (LD 10:14), and 19 days under the long day condition (LD 18:6), for 30% of the larvae to develop to pupae. Therefore it took 3 times as long for larvae to develop to 4th instar and twice as long for 4th instar larvae to pupate when larvae were maintained at the short photoperiod as compared to the long photoperiod. At photoperiods greater than LD 10.5:13.5 (16°C) there was no difference in the length of the 4th instar stage (Duncan's multiple range test (DMRT)).

Temperature also influenced diapause. At 14°C (LD 10:14), all of the surviving larvae were in diapause on day 40. At 16°C (LD 10:14), 75% of the surviving larvae were in diapause on day 40. When the photoperiod was increased to LD 10.5:13.5, at 16°C, the incidence of diapause increased to 85% (Fig. 1). Photoperiods greater than LD 10.5:13.5, at 16°C, resulted in a marked decrease in larval diapause (Fig. 1). At 17°C (LD 10:14), approximately 50% of the larvae were in diapause on day 40 (Fig. 2). Photoperiods of LD 12:12 or greater, at 17°C, resulted in no larval diapause. Temperatures of 18°C and higher produced no diapause at photoperiods ranging from LD 10:14 to 18:6 (Appendix I).

Nearly all of the mortality encountered was during the larval-pupal transition. Mortality was less than 10% in any replicate with the exception of the 28°C trial. This temperature approaches the upper maintainance limit of Ae. togoi from Vancouver, B.C.

DISCUSSION

Larval diapause occurs infrequently among North American mosquitoes. Only 3 aedine species in North America, Ae. triseriatus (Say), Ae. hendersoni Cockerell and Ae. sierrensis (Ludlow) have a larval diapause (Gallaway 1985, Love and Welchel 1955, Sims 1982, Jordan and Bradshaw 1978). Each of these species has the capability of overwintering in the egg stage, and does so exclusively in the northern parts of their range in the U.S.A. and in Canada. At more southern and coastal regions, the treehole larval habitat of Ae. sierrensis and Ae. triseriatus remains unfrozen and larvae could survive throughout the winter (Holzapfel and Bradshaw 1981, Jordan and Bradshaw 1978).

The larval habitat of Ae. togoi at Vancouver, B.C. remains unfrozen during the winter months (Table 1). This permits larvae to live throughout the winter, if they should hatch and develop slowly, or if they are maintained in a diapause state in response to environmental cues.

Populations of Ae. triseriatus from Ohio and Indiana (U.S.A.) undergo larval diapause if maintained at 16°C and

short day photoperiods of LD 10:14 or 11:13 (Clay and Venard 1972, Sims 1982). At 40°N, which is the approximate latitude of the Ohio and Indiana populations, photoperiods of LD 11:13 or shorter occur between 1 November and 1 February (daylength plus civil twilight, Beck 1968). Monthly mean daily air temperatures for Indianapolis (40°N), IN. range from +5.4°C (November) to -2.3°C (January) (Table 2). These temperatures would likely kill diapausing larvae. Egg diapause occurs at longer photoperiods (LD 14:10 and LD 15:9) and at higher temperatures (i.e. 21°C) in the Indiana population (Shroyer and Craig 1980). This means that eggs from the Indiana population laid under natural conditions after 1 August (daylength approximately LD 15:9 at 40°N) should undergo embryonic diapause, and resist hatching, provided daily temperatures remained below 21°C (Shroyer and Craig 1980). In practice, the percentage of diapausing eggs would probably increase gradually between 1 August and September, depending upon the daily temperature in that region. During most years, at 40°N, Ae. triseriatus eggs are unlikely to hatch after September. Any larvae that would occur in treeholes before 1 November at Indianapolis, should not be in diapause, because the critical photoperiod is greater than LD 11:13 prior to 1 November. However, nondiapausing larvae may face lethal temperatures beyond 1 November at this latitude (Table 2).

RESULTS

Mating Trials

A temperature of 18°C and a long photoperiod (LD 18:6) appeared to be most conducive for mating. Under these conditions, 53% of the females were successfully mated (Table 1). The incidence of mating was significantly reduced at both 18 and 20°C at LD 12:12, and at 20°C and LD 17.5:6.5 and 18:6. At the latter conditions, mating resulted in 5-6% of the females (Table 1). There was no significant difference in the number of females mated when a dimming cycle was incorporated into the photoperiod (Table 1).

Both temperature and photoperiod affect mating in *Ae. togoi*. Regression analysis shows temperature and temperature-photoperiod interaction significantly ($P < 0.0001$) influence mating. Photoperiod alone was also significant ($P < 0.0207$).

Induction of Embryonic Diapause

The Vancouver population of *Ae. togoi* experienced embryonic diapause when all life stages were maintained at short day conditions (Table 2). The critical photoperiod was calculated as LD 14.4:9.6 at 22°C and calculated as LD 15.3:8.7 at 25°C (Figs. 1,2). The frequency of diapause at 22°C, LD 18:6, was unusually variable. One experiment, out

of five, gave an aberrant result. I am unable to explain the high % diapause in the 6 replicates of the first experiment at this condition. All subsequent experiments (24 replicates) resulted in low % diapause (Fig. 1, Appendix II).

Photoperiods greater than 15h light/day resulted in a lower incidence of diapause at all temperatures between 22 and 28°C. Photoperiods below 14h light/day resulted in a greater incidence of diapause at all temperatures between 22 and 28°C (Table 2, Appendix II). The incidence of diapause was lower at 25°C under short day conditions (< 14h light/day), than at 22°C (Figs. 1,2). At 28°C, the incidence of diapause under short day conditions was further reduced; at LD 10:14, 36% of the embryos were in diapause versus 75% and 86% at 25 and 22°C respectively (Table 2).

Egg viability was affected by the temperature regimes under which all stages or pupae, adults and eggs were maintained (Tables 2, 3). Presumably this was due to the lack of mating under certain conditions. Preliminary trials showed that almost no eggs were viable when all stages, or when pupae, adults and eggs were maintained at 30°C. However, when Ae. togoi was maintained at 24°C, and only eggs were placed at 30°C for 10-28 days, viability ranged from 30-92%, considerably above average for any condition (Table 4).

Short day conditions (LD 10:14) gave rise to embryonic diapause in a significant number of embryos at all temperatures tested when larvae were maintained at 24°C LD 16:8 and pupae, adults and eggs were kept at temperatures ranging from 18 to 28°C (Table 3). At these temperatures the % diapause ranged from 100 to 22%.

The least amount of diapause (3%) occurred at 24°C and LD 16:8 (Table 2), so this was used as the 'non-diapause' condition. When pupae, adults and eggs were maintained at these conditions and eggs were maintained at low temperatures and short day conditions, the frequency of diapause increased (Table 4). Under long day conditions at 25°C, and at both long and short day conditions at 28 and 30°C, the incidence of diapause was below 13%. Under short day conditions at 25°C, and at both long and short day conditions from 22 to 15°C, the incidence of diapause increased from 21 to 97%. The lower the temperature, the greater the % diapause, when identical photoperiods were compared.

When photoperiod was held constant at LD 10:14, embryonic diapause increased as temperature decreased (Table 5). When temperature was varied from 18 to 25°C for pupae, adults and eggs, the incidence of diapause was 100% at 18, 20, and 22°C. At 25°C, 66% of the embryos were in diapause. When eggs were maintained at LD 10:14, and pupae and adults were maintained at LD 16:8, 24°C, the incidence of diapause

decreased as temperatures were increased from 18 to 25°C (Table 5). It can be seen that diapause results when only the egg stage was subjected to a diapause photoperiod. When pupae and adults, as well as eggs, were subjected to LD 10:14, the incidence of diapause was greater at each temperature than if only the egg stage was subjected to short day conditions. The effect of temperature on embryonic diapause was shown again, when all stages were kept at LD 10:14. The incidence of diapause was greater at 22 than at 25°C (Table 5).

Regression analysis was used to determine which conditions influenced egg diapause and egg viability. Only the extreme photoperiods (LD 10:14, LD 18:6) were tested at each stage, since these were most frequently used throughout the temperature range. In all trials, pupal and adult conditions were identical, hence the effects of the treatments on pupae and on adults could not be analyzed independently. Factors which significantly ($P < 0.0002$) influenced egg diapause included: photoperiod of larval, pupal-adult, and egg stages; temperature of the pupal-adult and egg stages; larval temperature and photoperiod interaction; larval and pupal-adult temperature interaction; and larval photoperiod and pupal-adult temperature interaction.

Those factors which significantly ($P < 0.0001$) influenced egg viability included: temperature and photoperiod of the larval stage, and photoperiod of the pupal-adult stage.

Under some conditions, the time spent in the egg stage also affected egg diapause. When all stages were maintained at 24°C LD 18:6 with eggs at experimental conditions for one, two or three weeks, 2.9%, 7.2% and 9.6% diapause occurred respectively (Table 2). The results of the two and three week trials did not differ significantly from each other, but differed from the trial lasting one week (Table 2). There was no difference in the frequency of diapause in trials lasting 14 or 21 days at 24 or 25°C (Tables 2,3,4). Trial duration also had no affect on diapause, after 7 or 19 days, when pupae, adults and eggs were reared at 28°C LD 10:14 (Table 3). When all stages except the egg stage were maintained at 24°C LD 16:8, trial duration had no affect on the frequency of diapause among eggs under the same conditions (Table 4). Trials lasting 14 and 21 days in the egg stage did not differ significantly from each other, but differed from the trial lasting one week (DMRT). The length of the trial did not influence egg viability.

DISCUSSION

Mating Trials

The mating behaviour of *Ae. togoi*, under laboratory conditions, has not been examined by other workers. From my results, I was unable to conclude how temperature and/or photoperiod affected mating in *Ae. togoi*. Only two temperatures and three photoperiod conditions were tested,

and the frequency of mating was low in four out of five conditions. Extending the trial duration, and providing more conditions might have provided more conclusive results. During the three day trial, the presence or absence of a 90 minute twilight period had no effect on mating.

Trimble and Wellington (1979b, 1980) did not simulate twilight in their experiments but used a photoperiod of LD 17.5:6.5 for laboratory maintenance of Ae. togoi. Vinogradova (1965) used natural twilight to stimulate mating in Ae. togoi, however she did not examine if mating was more successful under these conditions.

Induction of Embryonic Diapause

Diapause is a developmental arrest which may be induced by environmental conditions well in advance of prolonged, adverse conditions. In all instances of facultative (non-obligatory) diapause, environmental cues are perceived by stages preceeding and/or including the diapausing stage. In Ae. togoi, eggs are induced to diapause as a result of certain photoperiod and temperature cues being perceived by stages preceeding, and including, the egg stage. Certain stimuli are also required to terminate embryonic diapause, such as prolonged exposure of eggs to high or low temperatures, or exposure to long photoperiods. Lowered oxygen tension of the habitat surrounding the eggs causes hatching of non-diapausing eggs under natural conditions

(Judson et al. 1965). All of the eggs in my experiments were flooded once with a nutrient broth solution, which induced hatching in some of the eggs. The hatching stimulus was applied only once since the majority of non-diapausing eggs have been shown to hatch after one flooding, however repeated floodings may result in increased hatching (McGinnis 1982). Any eggs which hatched upon two or more floodings would not have been in diapause since no prolonged temperature or photoperiod condition was required to terminate diapause. McGinnis (1982) showed that increased hatching, but still less than 100%, may occur after as many as 10 floodings. From her results, it appeared that some viable embryos failed to hatch regardless of the number of hatching stimuli that were applied. Because of this problem, I chose to use only a single hatching stimulus. In addition to some viable eggs failing to hatch under 'non-diapause' conditions, 'diapause' conditions do not result in all eggs failing to hatch. In nature, the former condition would bring about delayed or sporadic hatching of some of the eggs; the latter condition would mean some eggs hatch under adverse conditions, and larvae would need to enter diapause to prevent adult emergence.

A critical photoperiod (CP) of approximately 14.4h light/day was found to induce embryonic diapause in the majority of eggs, when all stages (larvae, pupa, adult, egg) were maintained at 22°C (Fig. 1). I am unable to explain

the high % diapause in the 6 replicates of the first experiment at this condition. All subsequent experiments (24 replicates) resulted in a low % diapause. At 25°C, the CP was calculated as 15.3h light/day (Fig. 2). When all stages were maintained at 22°C or 25°C, at photoperiods of LD 15:9 or greater, the incidence of diapause rapidly declined (Fig. 1,2 Table 2). Whereas Mogi (1981) found that rearing larvae, adults and eggs at 25°C LD 16:8 produced little diapause in eggs, I found that these conditions produced a mean of 38% embryonic diapause in the Vancouver population (Table 2).

The CP for embryonic diapause in another rock pool mosquito, Ae. atropalpus (Coquillett), appears to be similar to that for Ae. togoi. Ae. atropalpus from Belleville (44°N 15') Ontario was found to have a CP between LD 14:10 and 15:9 at 23°C (Kalpage and Brust 1974). Similarly, the CP for embryonic diapause in Ae. triseriatus (Say), which may experience either embryonic or larval diapause depending upon temperature and photoperiod, from St. Joseph Co., IN. (41°N 15') was between LD 14:10 and 15:9 (Shroyer and Craig 1980).

Photoperiods less than LD 15:9 occur at Vancouver (49°N 15') B.C. between 15 August and 15 April (daylength plus civil twilight, Beck 1968). Mean daily air temperatures (30 year average) at Vancouver, B.C. between 15 August and 15 April ranged from 17.3°C (15 August) to 2.1°C (6 January)

(Climate Services, Environment Canada). Selected pool temperatures at Lighthouse Park Vancouver, B.C. during this period ranged from 16.1°C (26/8/77) to 5.6°C (16/2/78) (Table 1, Chapter 3). A high percentage of the eggs in nature, that would be laid between 15 August and the termination of oviposition for the season, would be in diapause (85 ± 5% were in diapause at these conditions in the laboratory, Fig. 1). Between 15 April and 15 August photoperiods are long enough and temperatures high enough that development should be continuous. Selected pool temperatures during this time ranged from 12.1°C (21/4/78) to 17.2°C (14/6/78) (Table 1, Chapter 3), and these are suitable for development. Mean daily air temperatures (30 year average) ranged from 9.3°C to 15.1°C during this period (Climate Services, Environment Canada).

Embryonic diapause in Ae. togoi is influenced by photoperiod and temperature of the egg stage and larval, pupal and adult stages immediately preceeding the egg stage. When all stages were maintained under identical conditions, long photoperiods (e.g. LD 18:6) resulted in a significant reduction, but not elimination, of diapause at temperatures ranging from 22°C to 28°C. Photoperiod was demonstrated to have a significant effect on diapause at temperatures as high as 28°C.

Photoperiods shorter than LD 15:9, at 22 and 25°C, caused a significant increase in embryonic diapause (Table 2). My

data suggest that stages influencing egg diapause are more affected by photoperiod and less so by temperature, and that a proportion of the egg population may resist hatching even when high temperatures and long photoperiods predominate. The fact that some eggs may not respond to the first hatching stimulus is an advantage for this species, where ambient conditions quickly become intolerable for larval development. This could also be beneficial in continental locations where temperature variations are usually greater within shorter periods of time. McGinnis (1982) has shown that eggs that did not hatch during the first hatching stimulus, may hatch after the 2nd, 3rd or subsequent stimuli, however hatching was usually greatest after the first hatching stimulus.

In my experiments I observed a wide variation in egg diapause under certain conditions. I speculate that this variation may be caused by multiple alleles governoring egg diapause. McGinnis (1982) also suggested this may be the reason for the variable hatching in diapausing eggs of Ae. togoi.

Regression analysis, and the results in Table 5, show that photoperiod of all stages, and temperature of pupal-adult and egg stages influence egg diapause and egg viability. Since pupal and adult conditions were always identical, the separate effect of the pupal and adult stages on diapause and viability cannot be analyzed.

Vinogradova (1965) noted that an increase in hatching occurs where the temperature of the larval stage of the parent females is higher than that of the pupal or adult stages as compared to where all three stages are kept at a constant temperature but the larval photoperiod is longer. However, her results may be regarded cautiously since she admits asynchronous hatching is probably related to genetic heterogeneity of the eggs, with the hatching response differing amongst the eggs laid per female. The eggs I used were not segregated by individual batches as laid by a single female, but were chosen from eggs laid by a large number of females. This should have eliminated any genetic bias produced by selecting particular egg batches.

Mogi (1981) demonstrated that conditions of the adult and egg stages influence embryonic diapause. However, not enough trials were done to determine how photoperiod or temperature influenced embryonic diapause. Other studies have confirmed that photoperiod of the adult and/or egg stages influence diapause. These include Ae. atropalpus (Anderson 1968), Ae. vexans (Meigen) (Taylor 1981) and Psorophora ferox (Von Humboldt) (Pinger and Eldridge 1977). Taylor (1981) also found that photoperiod during the pupal stage influences embryonic diapause when all stages are kept at 23°C, and when larvae are kept at short photoperiod (LD 12:12) only. No difference in diapause was detected where larvae were maintained at long photoperiod and adults and

eggs at short photoperiod. His results suggest that the photoperiod of the larval stage interacts with the pupal stage which then influences embryonic diapause. Unfortunately a regression analysis modeling instar, pupal, adult and egg photoperiod and temperature interactions was not done. Anderson (1968) noted that the sensitive stages for light reception, which determines embryonic diapause in Ae. atropalpus, are the 4th larval instar and pupal stages of the maternal generation. No distinction was made as to what stage(s) of the female parent and what conditions of that stage(s) influenced embryonic diapause in Ps. ferox (Pinger and Eldridge 1977).

In order to properly assess under what conditions embryonic diapause occurs, egg viability must be considered. Vinogradova (1965) and Mogi (1981) reported variable hatch in eggs of Ae. togoi under certain conditions, however these authors did not consider variation in egg viability in their trials. It is possible that some eggs were not embryonated and these may have been included in their results. The results of my experiments are based on viable embryos only. Further, I found that a number of factors influence egg viability. These include photoperiod and temperature of the larval stage, and photoperiod of the pupal-adult stage.

Table 1. Effect of temperature and photoperiod on mating in Aedes togoi (Theobald).

24 C	18 C		20 C			
LD 18:6	LD 12:12	LD 18:6	LD 12:12	LD 17.5:6.5[1]	LD 18:6	% Mated[2,3] Mean ± S.D.
L	P,A[4]					16 ± 2.9[a]
L		P,A				53 ± 6.6[b]
L			P,A			27 ± 8.8[a]
L				P,A		5 ± 2.5[b]
L					P,A	6 ± 1.4[b]

[1] Dimmer used to simulate 45 minute dusk and 45 minute dawn.

[2] Common letter following column indicates no significant difference at 5% level among percentage mated according to Duncan's multiple range test.

[3] Duncan's multiple range test was performed only on groups where pupal and adult temperatures were the same.

[4] L= Larvae, P= Pupae, A= Adults

Table 2. Egg viability and the effect of temperature and photoperiod on embryonic diapause, when all developmental stages were maintained at experimental conditions.

Experimental Condition	Replicates	Duration of Egg Condition (days)	% Diapause[1] Mean ± S.D	% Egg Viability Mean ± S.D
22 C LD 10:14	6	21	86 ± 3.4 [a]	70 ± 6.2
22 C LD 12:12	6	21	77 ± 4.7 [a]	76 ± 7.0
22 C LD 13:11	6	21	91 ± 2.6 [a]	77 ± 4.5
22 C LD 14:10	6	21	81 ± 4.1 [a]	81 ± 3.0
22 C LD 15:9	6	21	4 ± 3.7 [b]	33 ± 5.9
22 C LD 18:6	30	21	13 ± 22.9 [b]	79 ± 14.1
23 C LD 10:14	6	21	67 ± 15.5 [a]	41 ± 7.2
23 C LD 18:6	6	21	3 ± 2.5 [b]	82 ± 6.9
24 C LD 10:14	6	21	82 ± 3.4 [a]	56 ± 4.2
24 C LD 16:8	6	21	3 ± 2.6 [b]	61 ± 5.8
24 C LD 18:6	6	7	3 ± 2.6 [b]	79 ± 6.8
24 C LD 18:6	6	14	7 ± 3.5 [a]	74 ± 6.1
24 C LD 18:6	6	21	10 ± 3.1 [a]	73 ± 6.2
25 C LD 10:14	9	21	75 ± 7.0 [a]	56 ± 22.5
25 C LD 12:12	6	21	76 ± 7.7 [a]	46 ± 4.7
25 C LD 13:11	6	21	79 ± 8.3 [a]	75 ± 7.5
25 C LD 14:10	6	21	69 ± 8.4 [a]	50 ± 8.5
25 C LD 15:9	12	21	49 ± 17.9 [b]	50 ± 12.0
25 C LD 16:8	6	21	38 ± 12.6 [b]	33 ± 2.6
25 C LD 18:6	6	21	3 ± 1.5 [c]	89 ± 7.2
28 C LD 10:14	12	21	36 ± 13.2 [a]	29 ± 12.1
28 C LD 18:6	12	21	5 ± 3.6 [b]	37 ± 11.7

[1] Common letter following column indicates no significant difference at the 5% level among percentage diapause using Duncan's multiple range test at selected conditions.

Table 3. Egg viability and the effect of temperature and photoperiod on embryonic diapause, when pupae, adults and eggs were maintained at experimental conditions, and larvae were maintained at 24 C LD 16:8.

Experimental Conditions P A E[1]	Replicates	Duration of Egg Condition (Days)	% Diapause[2] Mean ± S.D.	% Egg Viability Mean ± S.D.
18 C LD 10:14	6	21	100 ± 0.0	32 ± 11.0
20 C LD 10:14	6	21	100 ± 0.0	15 ± 5.6
22 C LD 10:14	6	21	100 ± 0.0	57 ± 13.6
25 C LD 10:14	3	14	79 ± 4.2[a]	49 ± 6.2
25 C LD 10:14	6	21	66 ± 37.8[a]	23 ± 7.4
28 C LD 10:14	3	9	27 ± 8.1[a]	47 ± 4.9
28 C LD 10:14	3	17	22 ± 5.9[a]	37 ± 8.1

[1] P= Pupae, A= Adults, E= Eggs

[2] Common letter following column indicates no significant difference at the 5% level among percentage diapause using Duncan's multiple range test at selected conditions.

Table 4. Egg viability and the effect of temperature and photoperiod on embryonic diapause when larvae, pupae and adults were maintained at 24 C LD 16:8, and egg conditions were varied.

Egg Condition	Replicates	Duration of Egg Condition (Days)	% Diapause[1] Mean ± S.D.	% Egg Viability Mean ± S.D.
15C LD 10:14	3	14	97 ± 3.6	64 ± 5.0
18C LD 10:14	6	21	97 ± 2.4	72 ± 10.5
20C LD 10:14	3	14	72 ± 8.7[a]	40 ± 3.1
20C LD 10:14	6	21	74 ± 7.0[a]	78 ± 8.4
20C LD 10:14	3	28	69 ± 11.0[a]	72 ± 1.5
22C LD 10:14	6	21	56 ± 11.5[a]	74 ± 8.8
22C LD 18:6	6	21	21 ± 8.0[b]	23 ± 5.3
25C LD 10:14	6	14	24 ± 9.2[ab]	73 ± 7.5
25C LD 10:14	6	21	38 ± 25.7[a]	48 ± 15.2
25C LD 18:6	6	14	8 ± 7.9[b]	84 ± 9.2
25C LD 18:6	3	21	13 ± 7.8[b]	74 ± 9.0
28C LD 10:14	3	5	9 ± 5.0[a]	43 ± 6.5
28C LD 18:6	3	3	6 ± 5.9[a]	56 ± 4.0
28C LD 18:6	3	5	4 ± 3.6[a]	56 ± 0.6
30C LD 10:14	3	10	11 ± 6.2[a]	30 ± 8.1
30C LD 10:14	3	14	11 ± 2.0[a]	92 ± 4.5
30C LD 10:14	3	28	3 ± 2.9[a]	75 ± 5.5

[1]Common letter following column indicates no significant difference at the 5% level among percentage diapause using Duncan's multiple range test at selected conditions.

Table 5. Effect of temperature on embryonic diapause when predetermined stages were moved from long day to short day conditions.

	Photoperiod LD 10:14				
24C LD 16:8	18 C	20 C	22 C	25 C	% Diapause[2,3] Mean ± S.D.
L	P,A,E[1]				100 ± 0.0[a]
L		P,A,E			100 ± 0.0[a]
L			P,A,E		100 ± 0.0[a]
L				P,A,E	66 ±37.8[b]
L,P,A	E				97 ± 2.4[a]
L,P,A		E			74 ± 7.0[b]
L,P,A			E		56 ±11.5[b]
L,P,A				E	38 ±25.7[c]
			L,P,A,E		86 ± 3.4[a]
				L,P,A,E	75 ± 7.0[b]

[1] L= Larvae, P= Pupae, A= Adults, E= Eggs.

[2] Common letter following column indicates no significant difference at 5% level among percentage diapause according to Duncan's multiple range test.

[3] Duncan's multiple range test was performed only on groups where the same developmental stages were subjected to different temperatures.

Figure Caption

Fig.1 Effect of photoperiod on egg hatch of *Aedes togoi* at 22°C.

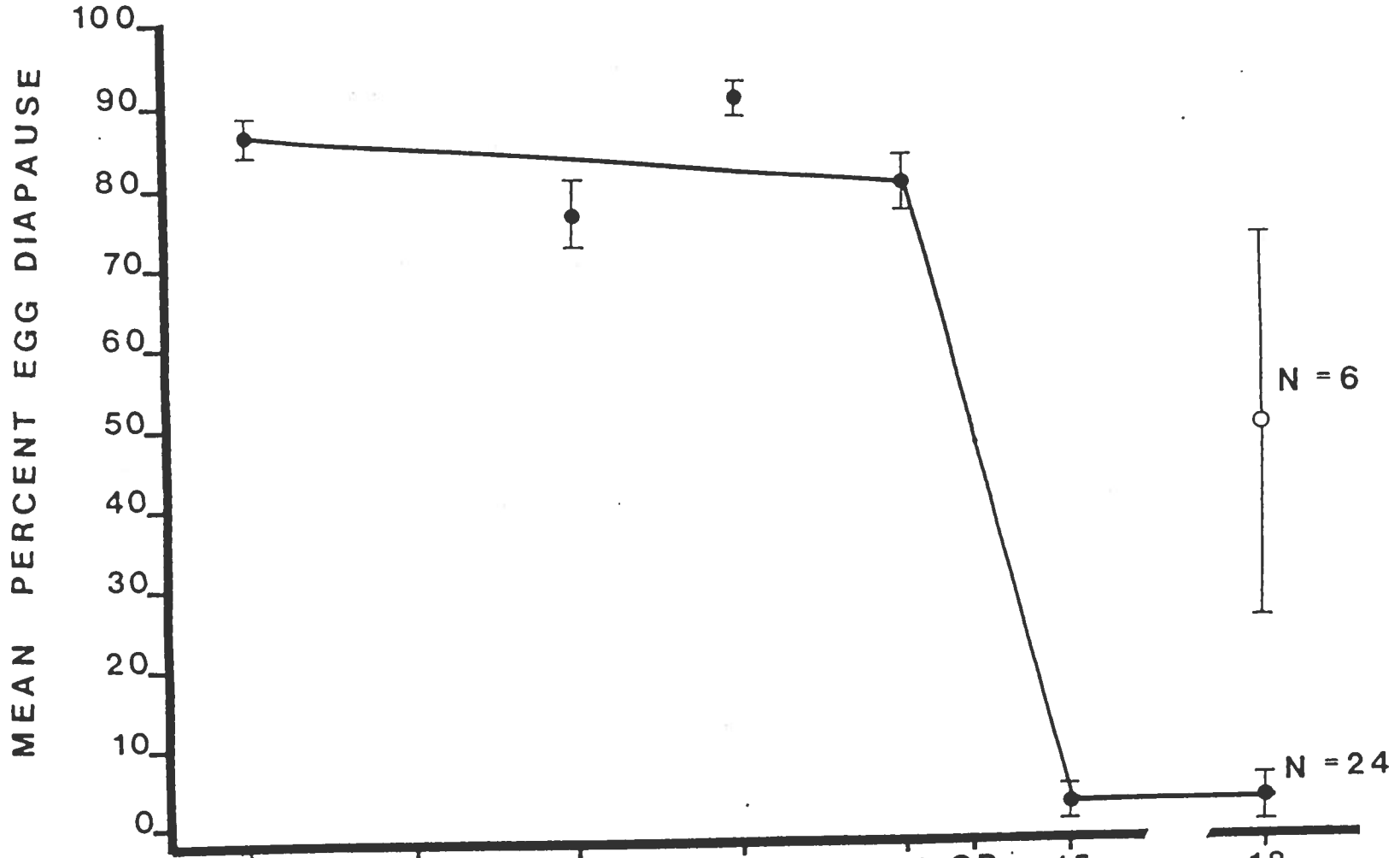
MEAN PERCENT EGG DIAPAUSE
100
90
80
70
60
50
40
30
20
10
0
N = 6
N = 24
10
11
12
13
14 CP
15
18
PHOTOPERIOD (HOURS)

FIG. 1

Figure Caption

Fig.2 Effect of photoperiod on egg hatch of *Aedes togoi* at 25°C.

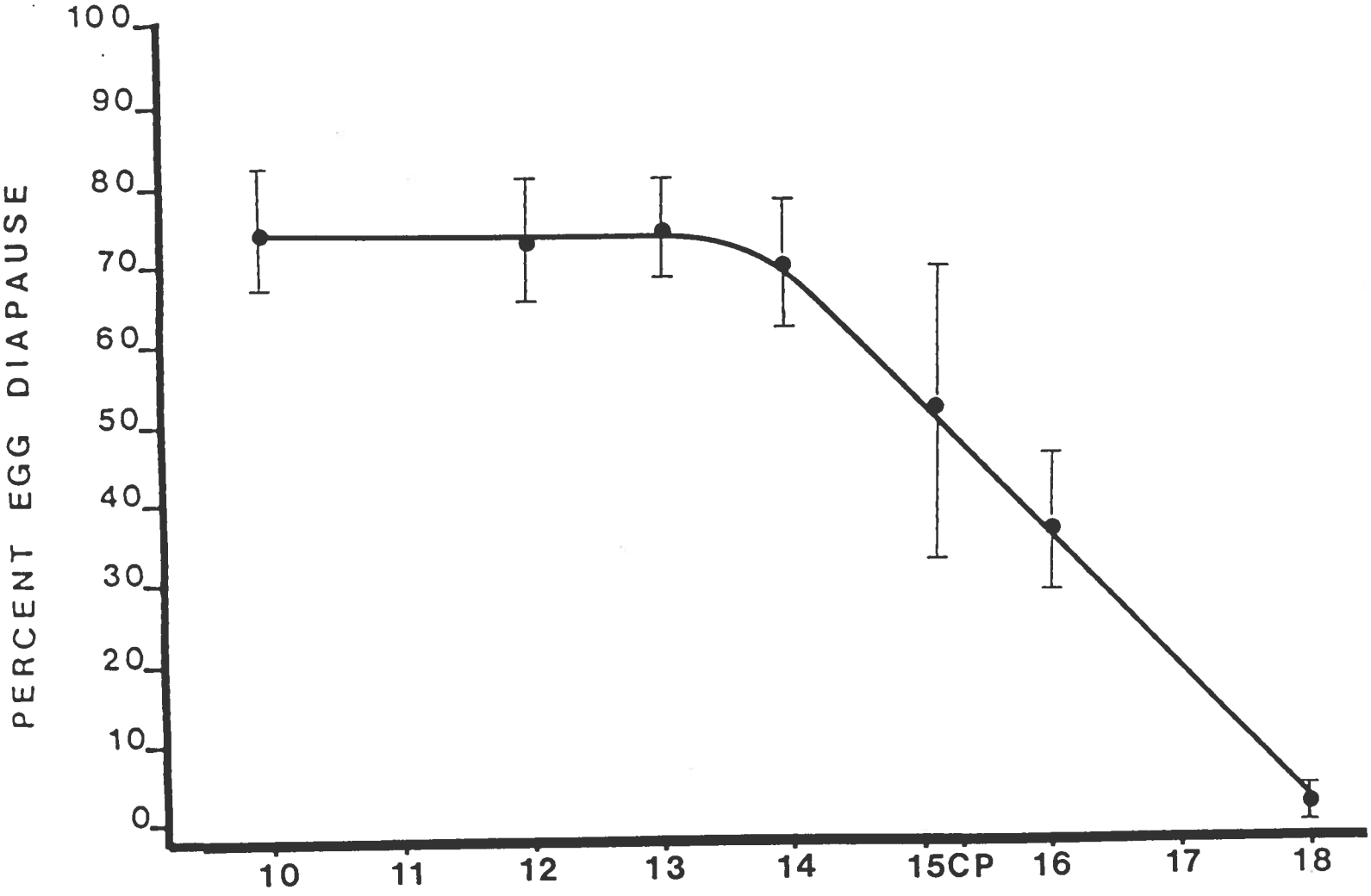
PERCENT EGG DIAPAUSE
100
90
80
70
60
50
40
30
20
10
0
10
11
12
13
14
15CP
16
17
18
PHOTOPERIOD (HOURS)

FIG. 2

Chapter V

General Discussion

Aedine mosquitoes typically possess one overwintering strategy, embryonic diapause. A few aedine species (e.g. Aedes triseriatus, Ae. sierrensis) possess a second strategy for coping with extreme and prolonged adverse conditions. In these species the last larval instar may undergo diapause. Treeholes are the typical overwintering sites for these species. These sites may remain unfrozen throughout the year in southern and coastal regions of the United States, enabling larvae of Ae. sierrensis and Ae. triseriatus to survive through the winter (Holzpfel and Bradshaw 1981, Jordan and Bradshaw 1978).

In more northern and continental locations, such as Indianapolis, IN., the climate may be more severe. Winter temperatures in Indianapolis are low enough to kill overwintering larvae of Ae. triseriatus. Egg diapause then becomes the only overwintering strategy for this species. Induction of egg diapause occurs at long photoperiods (LD 15:9 or less) and at moderate temperatures (21°C) in Indiana populations of Ae. triseriatus (Shroyer and Craig 1980). This means that eggs of the Indiana population should undergo diapause, and resist hatching after 1 August. By September, nearly all of the eggs laid should be in diapause. Larvae occurring in treeholes for the next month

should not be in diapause because the photoperiod is greater than LD 11:13, which is the critical photoperiod for larval diapause induction for Ae. triseriatus (Clay and Venard 1972, Sims 1982). Larvae occurring in treeholes after 1 November could be killed by low winter temperatures.

Ae. togoi is also capable of larval or egg diapause, depending upon the temperature and photoperiod experienced by stages preceeding the diapausing stage. At LD 11:13 or less, and 16°C, larvae diapause in the 4th instar. Temperatures higher than 17°C override the photoperiod cues and larval development proceeds despite short photoperiods. The majority of eggs should be in diapause at temperatures less than 22°C, when photoperiods of LD 15:9 or less predominate. Hence, temperatures may be high enough for larvae to develop without delay, but at the same time eggs may be in diapause, and maintained in this state by low temperature and a short photoperiod. Eggs which are not in diapause would likely hatch and larvae should develop without delay unless temperatures were less than 16°C or short photoperiods predominated at 16°C. Larvae are able to survive the winter in rock pools near Vancouver (49°N 15') B.C. (McGinnis 1982). Larval diapause could be induced in the population between 1 November and 15 February due to the short photophase occurring during this period, provided pool temperatures are high enough for development to proceed to the 4th instar. Selected pool temperatures for this period

ranged from 8.4°C (4/11/77) to 5.6°C (16/2/78) (Table 1, Chapter 3). The calculated threshold for larval development is 10°C, so unless pool temperatures exceed 10°C for part of each day, or for a certain number of days from November to February, development would not proceed. Larval diapause therefore, does not appear to be very beneficial for survival. However, when conditions become favourable for larvae to resume development in the spring, the first spring generation could result from overwintering larvae. Larval development from eggs hatching in spring would be expected to be prolonged on the other hand, due to low pool temperatures. Therefore, by undergoing larval diapause, Ae. togoi may have one more summer generation, than if this species experienced only egg diapause.

Eggs of Ae. togoi are induced to undergo diapause at photoperiods of LD 15:9 or less and moderate temperatures (22°C). This means that most eggs (calculated 85±5%) from the Vancouver population of Ae. togoi laid after 15 August should undergo diapause and resist hatching. Any 4th instar larvae that would occur in rock pools prior to 1 November should not be in diapause, since the photoperiod prior to this time is longer than the critical photoperiod for larval diapause induction (i.e. LD 11:13). Larval development of all instars should progress slowly from 1 November to 15 February not only because of short photoperiod but because of low temperatures. However,

unlike larvae of Ae. triseriatus in Indiana, Ae. togoi larvae are not likely to freeze during the winter (McGinnis 1982; Table 1, Chapter 3).

The change in the larval diapause CP as a function of latitude is unknown for Ae. togoi. In species such as Ae. sierrensis, the CP governoring the induction of larval diapause increases 1h/4.8 degree increase in latitude (Jordan and Bradshaw 1978). In Wyeomyia smithii, the larval diapause CP increases 1h/5.4 degree increase in latitude (Bradshaw and Lounibos 1977). However in Ae. triseriatus, larval diapause CP changes less than 1.3h over 20 degrees of latitude (Sims 1982). Mogi (1981) found that larval diapause in Ae. togoi was initiated between LD 10:14 and LD 12:12, at 15°C, at Nagasaki (32°N), Japan. The CP for the Vancouver (49°N 15') population is LD 10.9:13.1. It appears that over 18 degrees of latitude (Nagasaki to Vancouver) there may be only one hour in the variation of the CP.

In conclusion, Ae. togoi is well adapted to overwintering under a wide range of temperatures and photoperiods, enabling the species to survive over a broad geographic range.

Chapter VI

Summary and Conclusions

Aedes togoi may undergo larval or embryonic diapause in response to ambient conditions. The majority of larvae entered diapause at photoperiods of LD 10.9:13.1, or less, at 16°C. Photoperiods greater than LD 10.9:13.1, at 16°C, caused a large decline in the frequency of larval diapause. At temperatures of 18°C and higher, photoperiod had no effect on diapause induction.

Mating of Ae. togoi was influenced by temperature and temperature -photoperiod interaction. The greatest incidence of mating occurred at 18°C LD 18:6 (average of 53% mating). In contrast, higher temperature (20°C) at the same photoperiod (LD 18:6) produced the lowest incidence of mating (average of 5% mating). The use of a dimmer to simulate 90 minute dusk/dawn at 20°C LD 18:6 had no effect on mating as compared to trials which lacked a dimming cycle. The frequency of mating was significantly higher at 18°C LD 12:12 and 20°C LD 12:12, increasing to 16% and 27% respectively.

Eggs of Ae. togoi were found to enter diapause at approximately LD 14.4:9.6 where all stages were maintained at 22°C. Shorter photoperiods resulted in a significantly higher incidence of diapause, while longer photoperiods produced a low incidence of diapause. At 25°C, eggs entered

diapause at approximately LD 15.3:8.7. Photoperiods longer than LD 14:10, at 25°C, resulted in a gradual decrease in the frequency of diapause as compared to similar photoperiods at 22°C.

A small proportion (< 11%) of the eggs were always in diapause even at temperatures as high as 30°C. When pupae, adults and eggs were maintained at temperatures of 22°C or lower, LD 10:14, all of the eggs in the experimental populations entered diapause. Photoperiod of all stages and temperature of pupal-adult, and egg stages influenced egg diapause and egg viability.

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Appendix I

Larval Diapause Data Calculations

APPENDIX I

LARVAL DIAPAUSE DATA

TEMP	LIGHT	MEANPUP[2]	MEANT_50[3]	MEANDIFF[4]
14.0	10.0	.	40.31	.
16.0	10.0	.	27.63	.
16.0	10.0	.	24.81	.
16.0	10.5	.	30.56	.
16.0	10.5	.	29.35	.
16.0	11.0	20.09	11.51	8.58
16.0	11.5	18.79	11.33	7.45
16.0	12.0	17.44	10.71	6.72
16.0	13.0	19.64	12.65	6.99
16.0	14.0	20.41	12.90	7.50
16.0	15.0	20.72	12.80	7.92
16.0	18.0	20.07	11.41	8.66
17.0	10.0	25.41	13.18	12.75
17.0	10.0	.	20.45	.
17.0	12.0	17.02	10.39	6.63
17.0	14.0	17.44	10.52	6.92
17.0	15.0	18.73	10.48	8.25
17.0	16.0	19.33	10.68	8.65
17.0	18.0	17.40	9.82	7.57
18.0	10.0	13.52	8.17	5.35
18.0	10.5	14.18	8.72	5.46
18.0	11.0	14.55	8.94	5.61
18.0	11.5	14.71	9.05	5.65
18.0	12.0	16.05	10.03	6.02
18.0	14.0	15.78	9.52	6.25
18.0	16.0	17.68	10.24	7.44
18.0	18.0	17.65	10.27	7.38
28.0	10.0	9.06	6.11	2.95

[1] Missing values indicate that 50% level was not achieved in the pupal stage, therefore the difference between 50% levels of pupal and larval stages could not be determined.

[2] Number of days to reach 50% pupation.

[3] Number of days to reach 50% 4th instar larvae.

[4] Difference (days) between 50% pupation and 50% 4th instar.

Appendix II

Embryonic Diapause Data Calculations

APPENDIX II

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
24C 16L	24C 16L	15C 10L	14	63	1.00000
24C 16L	24C 16L	15C 10L	14	69	0.92754
24C 16L	24C 16L	15C 10L	14	59	0.98305
24C 16L	24C 16L	18C 10L	21	87	0.98851
24C 16L	24C 16L	18C 10L	21	80	0.98750
24C 16L	24C 16L	18C 10L	21	76	0.94737
24C 16L	24C 16L	18C 10L	21	65	0.95385
24C 16L	24C 16L	18C 10L	21	67	0.94030
24C 16L	24C 16L	18C 10L	21	59	1.00000
24C 16L	24C 16L	20C 10L	14	37	0.62162
24C 16L	24C 16L	20C 10L	14	39	0.74359
24C 16L	24C 16L	20C 10L	14	43	0.79070
24C 16L	24C 16L	20C 10L	28	74	0.81081
24C 16L	24C 16L	20C 10L	28	72	0.59722
24C 16L	24C 16L	20C 10L	28	71	0.64789
24C 16L	24C 16L	20C 10L	21	81	0.76543
24C 16L	24C 16L	20C 10L	21	85	0.81176
24C 16L	24C 16L	20C 10L	21	86	0.74419
24C 16L	24C 16L	20C 10L	21	77	0.63636
24C 16L	24C 16L	20C 10L	21	64	0.79688
24C 16L	24C 16L	20C 10L	21	72	0.66667
24C 16L	24C 16L	22C 10L	21	89	0.40449
24C 16L	24C 16L	22C 10L	21	76	0.53947
24C 16L	24C 16L	22C 10L	21	71	0.46479
24C 16L	24C 16L	22C 10L	21	76	0.64474
24C 16L	24C 16L	22C 10L	21	64	0.68750
24C 16L	24C 16L	22C 10L	21	67	0.64179
24C 16L	24C 16L	22C 18L	21	16	0.18750
24C 16L	24C 16L	22C 18L	21	21	0.33333
24C 16L	24C 16L	22C 18L	21	28	0.25000
24C 16L	24C 16L	22C 18L	21	29	0.13793
24C 16L	24C 16L	22C 18L	21	18	0.11111
24C 16L	24C 16L	22C 18L	21	25	0.24000
24C 16L	24C 16L	25C 10L	14	78	0.29487
24C 16L	24C 16L	25C 10L	14	77	0.36364
24C 16L	24C 16L	25C 10L	14	74	0.31081

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
24C 16L	24C 16L	25C 10L	14	79	0.15190
24C 16L	24C 16L	25C 10L	14	59	0.13559
24C 16L	24C 16L	25C 10L	14	70	0.18571
24C 16L	24C 16L	25C 10L	21	44	0.13636
24C 16L	24C 16L	25C 10L	21	73	0.13699
24C 16L	24C 16L	25C 10L	21	59	0.15254
24C 16L	24C 16L	25C 10L	21	37	0.62162
24C 16L	24C 16L	25C 10L	21	32	0.65625
24C 16L	24C 16L	25C 10L	21	44	0.56818
24C 16L	24C 16L	25C 18L	14	80	0.22500
24C 16L	24C 16L	25C 18L	14	100	0.06000
24C 16L	24C 16L	25C 18L	14	88	0.10227
24C 16L	24C 16L	25C 18L	14	85	0.05882
24C 16L	24C 16L	25C 18L	14	77	0.01299
24C 16L	24C 16L	25C 18L	14	75	0.02667
24C 16L	24C 16L	25C 18L	21	65	0.21538
24C 16L	24C 16L	25C 18L	21	74	0.09459
24C 16L	24C 16L	25C 18L	21	83	0.08434
24C 16L	24C 16L	28C 10L	5	37	0.08108
24C 16L	24C 16L	28C 10L	5	50	0.04000
24C 16L	24C 16L	28C 10L	5	43	0.139535
24C 16L	24C 16L	28C 18L	3	60	0.133333
24C 16L	24C 16L	28C 18L	3	57	0.035088
24C 16L	24C 16L	28C 18L	3	52	0.019231
24C 16L	24C 16L	28C 18L	5	55	0.072727
24C 16L	24C 16L	28C 18L	5	56	0.053571
24C 16L	24C 16L	28C 18L	5	56	0.000000
24C 16L	24C 16L	30C 10L	14	87	0.114943
24C 16L	24C 16L	30C 10L	14	92	0.119565
24C 16L	24C 16L	30C 10L	14	96	0.083333
24C 16L	24C 16L	30C 10L	28	80	0.062500
24C 16L	24C 16L	30C 10L	28	75	0.013333
24C 16L	24C 16L	30C 10L	28	69	0.014493
24C 16L	24C 16L	30C 10L	10	25	0.160000
24C 16L	24C 16L	30C 10L	10	25	0.040000
24C 16L	24C 16L	30C 10L	10	39	0.128205

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
24C 16L	18C 10L	18C 10L	21	21	1.00000
24C 16L	18C 10L	18C 10L	21	22	1.00000
24C 16L	18C 10L	18C 10L	21	27	1.00000
24C 16L	18C 10L	18C 10L	21	50	1.00000
24C 16L	18C 10L	18C 10L	21	35	1.00000
24C 16L	18C 10L	18C 10L	21	37	1.00000
24C 16L	20C 10L	20C 10L	21	11	1.00000
24C 16L	20C 10L	20C 10L	21	12	1.00000
24C 16L	20C 10L	20C 10L	21	10	1.00000
24C 16L	20C 10L	20C 10L	21	16	1.00000
24C 16L	20C 10L	20C 10L	21	17	1.00000
24C 16L	20C 10L	20C 10L	21	25	1.00000
24C 16L	22C 10L	22C 10L	21	40	1.00000
24C 16L	22C 10L	22C 10L	21	53	1.00000
24C 16L	22C 10L	22C 10L	21	44	1.00000
24C 16L	22C 10L	22C 10L	21	71	1.00000
24C 16L	22C 10L	22C 10L	21	63	1.00000
24C 16L	22C 10L	22C 10L	21	72	1.00000
24C 16L	25C 10L	25C 10L	14	47	0.74468
24C 16L	25C 10L	25C 10L	14	56	0.82143
24C 16L	25C 10L	25C 10L	14	44	0.79545
24C 16L	25C 10L	25C 10L	21	19	1.00000
24C 16L	25C 10L	25C 10L	21	21	1.00000
24C 16L	25C 10L	25C 10L	21	14	1.00000
24C 16L	25C 10L	25C 10L	21	21	0.19048
24C 16L	25C 10L	25C 10L	21	36	0.36111
24C 16L	25C 10L	25C 10L	21	24	0.41667
24C 16L	25C 18L	25C 18L	21	39	0.20513
24C 16L	25C 18L	25C 18L	21	40	0.30000
24C 16L	25C 18L	25C 18L	21	37	0.27027
24C 16L	25C 18L	25C 18L	21	72	0.16667
24C 16L	25C 18L	25C 18L	21	61	0.39344
24C 16L	25C 18L	25C 18L	21	72	0.25000
24C 16L	28C 10L	28C 10L	9	45	0.35556
24C 16L	28C 10L	28C 10L	9	53	0.20755
24C 16L	28C 10L	28C 10L	9	44	0.22727
24C 10L	28C 10L	28C 10L	17	31	0.29032
24C 10L	28C 10L	28C 10L	17	46	0.19565
24C 10L	28C 10L	28C 10L	17	33	0.18182
24C 16L	30C 10L	30C 10L	21	3	1.00000
24C 16L	30C 10L	30C 10L	21	6	0.66667
24C 16L	30C 10L	30C 10L	21	4	0.75000
24C 16L	30C 10L	30C 10L	21	7	0.57143
24C 16L	30C 10L	30C 10L	21	8	0.87500
24C 16L	30C 10L	30C 10L	21	7	0.71429

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
22C 10L	22C 10L	22C 10L	21	79	0.886076
22C 10L	22C 10L	22C 10L	21	71	0.873239
22C 10L	22C 10L	22C 10L	21	69	0.826087
22C 10L	22C 10L	22C 10L	21	60	0.916667
22C 10L	22C 10L	22C 10L	21	73	0.835616
22C 10L	22C 10L	22C 10L	21	70	0.842857
22C 12L	22C 12L	22C 12L	21	75	0.733333
22C 12L	22C 12L	22C 12L	21	74	0.783784
22C 12L	22C 12L	22C 12L	21	83	0.759036
22C 12L	22C 12L	22C 12L	21	68	0.838235
22C 12L	22C 12L	22C 12L	21	86	0.709302
22C 12L	22C 12L	22C 12L	21	71	0.802817
22C 13L	22C 13L	22C 13L	21	74	0.972973
22C 13L	22C 13L	22C 13L	21	80	0.937500
22C 13L	22C 13L	22C 13L	21	75	0.866667
22C 13L	22C 13L	22C 13L	21	74	0.932432
22C 13L	22C 13L	22C 13L	21	84	0.916667
22C 13L	22C 13L	22C 13L	21	72	0.888889
22C 14L	22C 14L	22C 14L	21	82	0.817073
22C 14L	22C 14L	22C 14L	21	78	0.743590
22C 14L	22C 14L	22C 14L	21	78	0.846154
22C 14L	22C 14L	22C 14L	21	86	0.802326
22C 14L	22C 14L	22C 14L	21	81	0.777778
22C 14L	22C 14L	22C 14L	21	81	0.839506
22C 15L	22C 15L	22C 15L	21	22	0.090909
22C 15L	22C 15L	22C 15L	21	35	0.000000
22C 15L	22C 15L	22C 15L	21	39	0.025641
22C 15L	22C 15L	22C 15L	21	35	0.000000
22C 15L	22C 15L	22C 15L	21	30	0.066667
22C 15L	22C 15L	22C 15L	21	34	0.029412
22C 18L	22C 18L	22C 18L	21	78	0.230769
22C 18L	22C 18L	22C 18L	21	52	0.288462
22C 18L	22C 18L	22C 18L	21	66	0.515152
22C 18L	22C 18L	22C 18L	21	60	0.800000
22C 18L	22C 18L	22C 18L	21	67	0.507463
22C 18L	22C 18L	22C 18L	21	68	0.808824

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
22C 18L	22C 18L	22C 18L	21	99	0.000000
22C 18L	22C 18L	22C 18L	21	99	0.010101
22C 18L	22C 18L	22C 18L	21	99	0.030303
22C 18L	22C 18L	22C 18L	21	99	0.000000
22C 18L	22C 18L	22C 18L	21	89	0.044944
22C 18L	22C 18L	22C 18L	21	85	0.011765
22C 18L	22C 18L	22C 18L	21	77	0.000000
22C 18L	22C 18L	22C 18L	21	92	0.000000
22C 18L	22C 18L	22C 18L	21	84	0.023810
22C 18L	22C 18L	22C 18L	21	82	0.036585
22C 18L	22C 18L	22C 18L	21	71	0.042254
22C 18L	22C 18L	22C 18L	21	80	0.012500
22C 18L	22C 18L	22C 18L	21	81	0.185185
22C 18L	22C 18L	22C 18L	21	85	0.023529
22C 18L	22C 18L	22C 18L	21	98	0.020408
22C 18L	22C 18L	22C 18L	21	81	0.000000
22C 18L	22C 18L	22C 18L	21	97	0.041237
22C 18L	22C 18L	22C 18L	21	86	0.034884
22C 18L	22C 18L	22C 18L	21	56	0.035714
22C 18L	22C 18L	22C 18L	21	75	0.000000
22C 18L	22C 18L	22C 18L	21	66	0.075758
22C 18L	22C 18L	22C 18L	21	61	0.032787
22C 18L	22C 18L	22C 18L	21	74	0.067568
22C 18L	22C 18L	22C 18L	21	60	0.000000
23C 10L	23C 10L	23C 10L	21	43	0.860465
23C 10L	23C 10L	23C 10L	21	47	0.765957
23C 10L	23C 10L	23C 10L	21	44	0.772727
23C 10L	23C 10L	23C 10L	21	45	0.466667
23C 10L	23C 10L	23C 10L	21	27	0.518519
23C 10L	23C 10L	23C 10L	21	40	0.625000
23C 18L	23C 18L	23C 18L	21	88	0.000000
23C 18L	23C 18L	23C 18L	21	72	0.027778
23C 18L	23C 18L	23C 18L	21	83	0.000000
23C 18L	23C 18L	23C 18L	21	76	0.052632
23C 18L	23C 18L	23C 18L	21	90	0.055556
23C 18L	23C 18L	23C 18L	21	80	0.025000

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
24C 10L	24C 10L	24C 10L	21	55	0.781818
24C 10L	24C 10L	24C 10L	21	59	0.813559
24C 10L	24C 10L	24C 10L	21	59	0.779661
24C 10L	24C 10L	24C 10L	21	48	0.854167
24C 10L	24C 10L	24C 10L	21	58	0.862069
24C 10L	24C 10L	24C 10L	21	57	0.807018
24C 16L	24C 16L	24C 16L	21	68	0.014706
24C 16L	24C 16L	24C 16L	21	66	0.000000
24C 16L	24C 16L	24C 16L	21	54	0.037037
24C 16L	24C 16L	24C 16L	21	55	0.054545
24C 16L	24C 16L	24C 16L	21	59	0.067797
24C 16L	24C 16L	24C 16L	21	63	0.031746
24C 18L	24C 18L	24C 18L	7	82	0.073171
24C 18L	24C 18L	24C 18L	7	77	0.012987
24C 18L	24C 18L	24C 18L	7	85	0.000000
24C 18L	24C 18L	24C 18L	7	88	0.034091
24C 18L	24C 18L	24C 18L	7	71	0.014085
24C 18L	24C 18L	24C 18L	7	73	0.041096
24C 18L	24C 18L	24C 18L	14	76	0.105263
24C 18L	24C 18L	24C 18L	14	84	0.047619
24C 18L	24C 18L	24C 18L	14	73	0.123288
24C 18L	24C 18L	24C 18L	14	70	0.028571
24C 18L	24C 18L	24C 18L	14	66	0.060606
24C 18L	24C 18L	24C 18L	14	73	0.068493
24C 18L	24C 18L	24C 18L	21	78	0.115385
24C 18L	24C 18L	24C 18L	21	75	0.146667
24C 18L	24C 18L	24C 18L	21	66	0.075758
24C 18L	24C 18L	24C 18L	21	71	0.098592
24C 18L	24C 18L	24C 18L	21	80	0.050000
24C 18L	24C 18L	24C 18L	21	65	0.061538
25C 10L	25C 10L	25C 10L	21	35	0.800000
25C 10L	25C 10L	25C 10L	21	27	0.777778
25C 10L	25C 10L	25C 10L	21	23	0.913043
25C 10L	25C 10L	25C 10L	21	54	0.703704
25C 10L	25C 10L	25C 10L	21	67	0.671642
25C 10L	25C 10L	25C 10L	21	59	0.694915

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
25C 10L	25C 10L	25C 10L	21	78	0.769231
25C 10L	25C 10L	25C 10L	21	78	0.717949
25C 10L	25C 10L	25C 10L	21	80	0.750000
25C 12L	25C 12L	25C 12L	21	52	0.711538
25C 12L	25C 12L	25C 12L	21	43	0.813953
25C 12L	25C 12L	25C 12L	21	44	0.886364
25C 12L	25C 12L	25C 12L	21	42	0.714286
25C 12L	25C 12L	25C 12L	21	52	0.692308
25C 12L	25C 12L	25C 12L	21	43	0.744186
25C 13L	25C 13L	25C 13L	21	84	0.869048
25C 13L	25C 13L	25C 13L	21	72	0.763889
25C 13L	25C 13L	25C 13L	21	62	0.758065
25C 13L	25C 13L	25C 13L	21	78	0.679487
25C 13L	25C 13L	25C 13L	21	76	0.736842
25C 13L	25C 13L	25C 13L	21	79	0.898734
25C 14L	25C 14L	25C 14L	21	41	0.609756
25C 14L	25C 14L	25C 14L	21	60	0.716667
25C 14L	25C 14L	25C 14L	21	46	0.804348
25C 14L	25C 14L	25C 14L	21	42	0.619048
25C 14L	25C 14L	25C 14L	21	49	0.632653
25C 14L	25C 14L	25C 14L	21	60	0.783333
25C 15L	25C 15L	25C 15L	21	39	0.256410
25C 15L	25C 15L	25C 15L	21	38	0.289474
25C 15L	25C 15L	25C 15L	21	43	0.232558
25C 15L	25C 15L	25C 15L	21	34	0.470588
25C 15L	25C 15L	25C 15L	21	45	0.333333
25C 15L	25C 15L	25C 15L	21	37	0.486486
25C 15L	25C 15L	25C 15L	21	66	0.621212
25C 15L	25C 15L	25C 15L	21	59	0.677966
25C 15L	25C 15L	25C 15L	21	61	0.573770
25C 15L	25C 15L	25C 15L	21	62	0.774194
25C 15L	25C 15L	25C 15L	21	62	0.645161
25C 15L	25C 15L	25C 15L	21	59	0.525424
25C 16L	25C 16L	25C 16L	21	34	0.558824
25C 16L	25C 16L	25C 16L	21	37	0.270270
25C 16L	25C 16L	25C 16L	21	33	0.454545
25C 16L	25C 16L	25C 16L	21	29	0.379310
25C 16L	25C 16L	25C 16L	21	34	0.205882
25C 16L	25C 16L	25C 16L	21	33	0.424242

EMBRYONIC DIAPAUSE DATA

LARVA	PUPA-ADULT	EGG	DAYS	VIAB	DIAPT (1.0 = 100%)
25C 18L	25C 18L	25C 18L	21	85	0.023529
25C 18L	25C 18L	25C 18L	21	98	0.051020
25C 18L	25C 18L	25C 18L	21	85	0.011765
25C 18L	25C 18L	25C 18L	21	97	0.030928
25C 18L	25C 18L	25C 18L	21	80	0.025000
25C 18L	25C 18L	25C 18L	21	90	0.011111
28C 10L	28C 10L	28C 10L	21	35	0.514286
28C 10L	28C 10L	28C 10L	21	38	0.236842
28C 10L	28C 10L	28C 10L	21	42	0.285714
28C 10L	28C 10L	28C 10L	21	49	0.448980
28C 10L	28C 10L	28C 10L	21	35	0.457143
28C 10L	28C 10L	28C 10L	21	40	0.475000
28C 10L	28C 10L	28C 10L	21	24	0.500000
28C 10L	28C 10L	28C 10L	21	22	0.272727
28C 10L	28C 10L	28C 10L	21	22	0.272727
28C 10L	28C 10L	28C 10L	21	14	0.214286
28C 10L	28C 10L	28C 10L	21	17	0.176471
28C 10L	28C 10L	28C 10L	21	12	0.500000
28C 18L	28C 18L	28C 18L	21	27	0.074074
28C 18L	28C 18L	28C 18L	21	34	0.000000
28C 18L	28C 18L	28C 18L	21	29	0.103448
28C 18L	28C 18L	28C 18L	21	23	0.04348
28C 18L	28C 18L	28C 18L	21	29	0.03448
28C 18L	28C 18L	28C 18L	21	17	0.00000
28C 18L	28C 18L	28C 18L	21	42	0.04762
28C 18L	28C 18L	28C 18L	21	49	0.02041
28C 18L	28C 18L	28C 18L	21	44	0.06818
28C 18L	28C 18L	28C 18L	21	56	0.10714
28C 18L	28C 18L	28C 18L	21	43	0.06977
28C 18L	28C 18L	28C 18L	21	46	0.02174
30C 10L	30C 10L	30C 10L	21	2	0.50000
30C 10L	30C 10L	30C 10L	21	2	0.50000
30C 10L	30C 10L	30C 10L	21	0	.
30C 10L	30C 10L	30C 10L	21	2	1.00000
30C 10L	30C 10L	30C 10L	21	1	0.00000
30C 10L	30C 10L	30C 10L	21	2	1.00000